

LSM 880 Introduction and Application

蔡司革新共聚焦成像技术在生命科学中的应用



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Microscopy Division

Life Science Segment Application Specialist
Zhang huayi

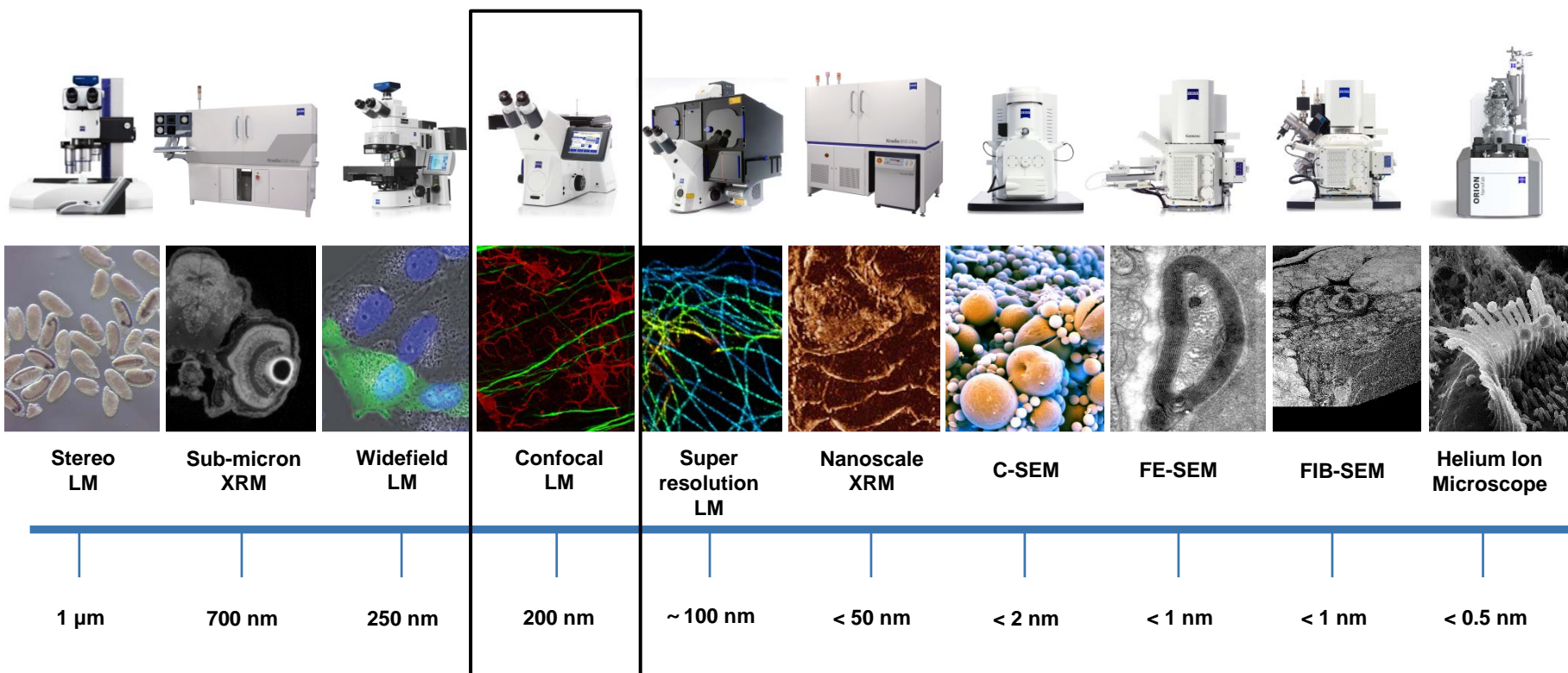
LSM 880 seen from the outside

Revolutionize Your Confocal Imaging



ZEISS: 显微成像领域领导者

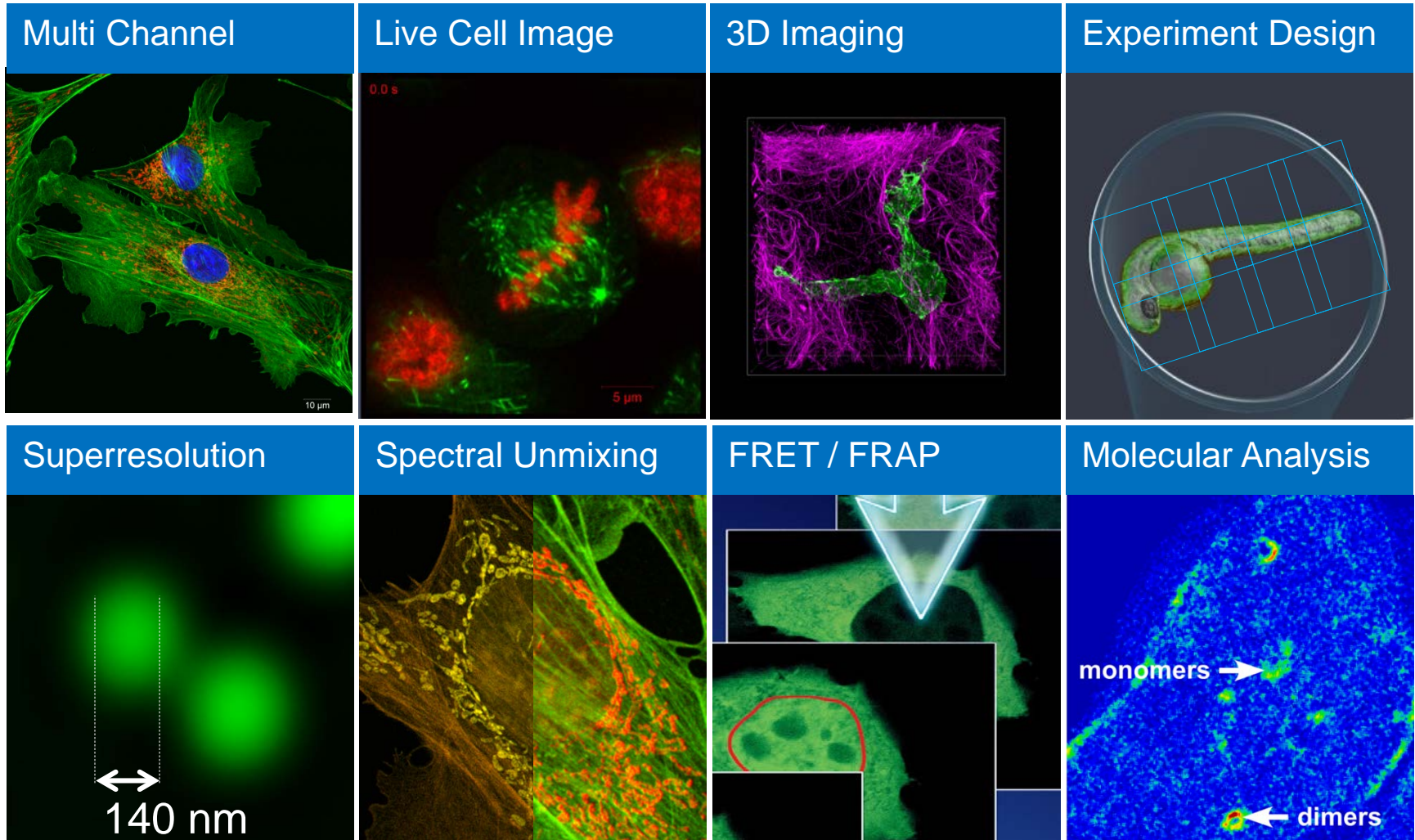
完整的显微成像解决方案



光学显微镜 - X射线显微镜 - 电子显微镜 - 离子显微镜 从宏观到纳米世界.....

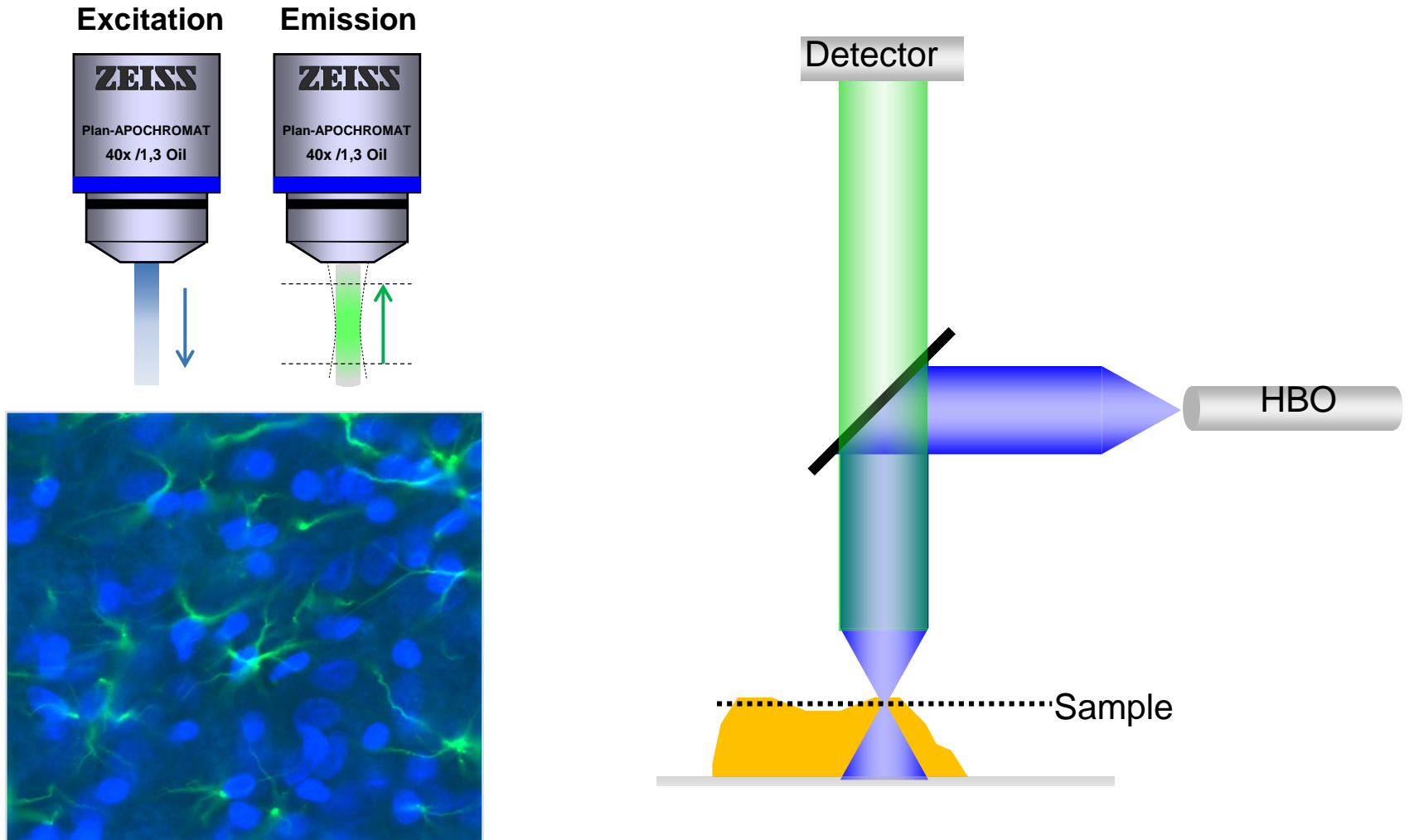
LSM 880 with Airyscan

Enter A New World of Confocal



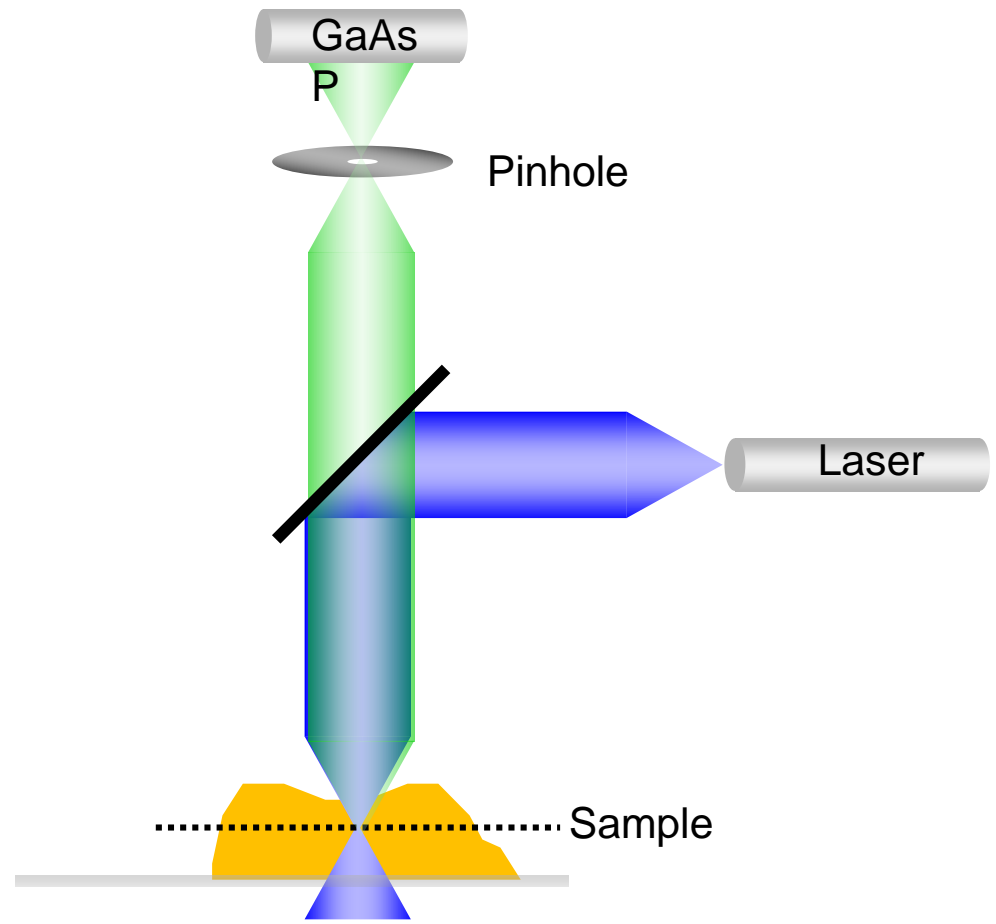
At the heart of the LSM: The Confocal Principle

Rejection of out-of-focus light at the pinhole and 3D Imaging



At the heart of the LSM: The Confocal Principle

Rejection of out-of-focus light at the pinhole and 3D Imaging

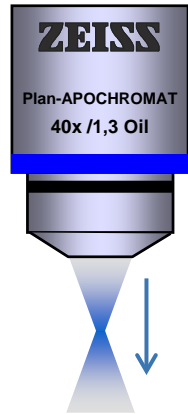


At the heart of the LSM: The Confocal Principle

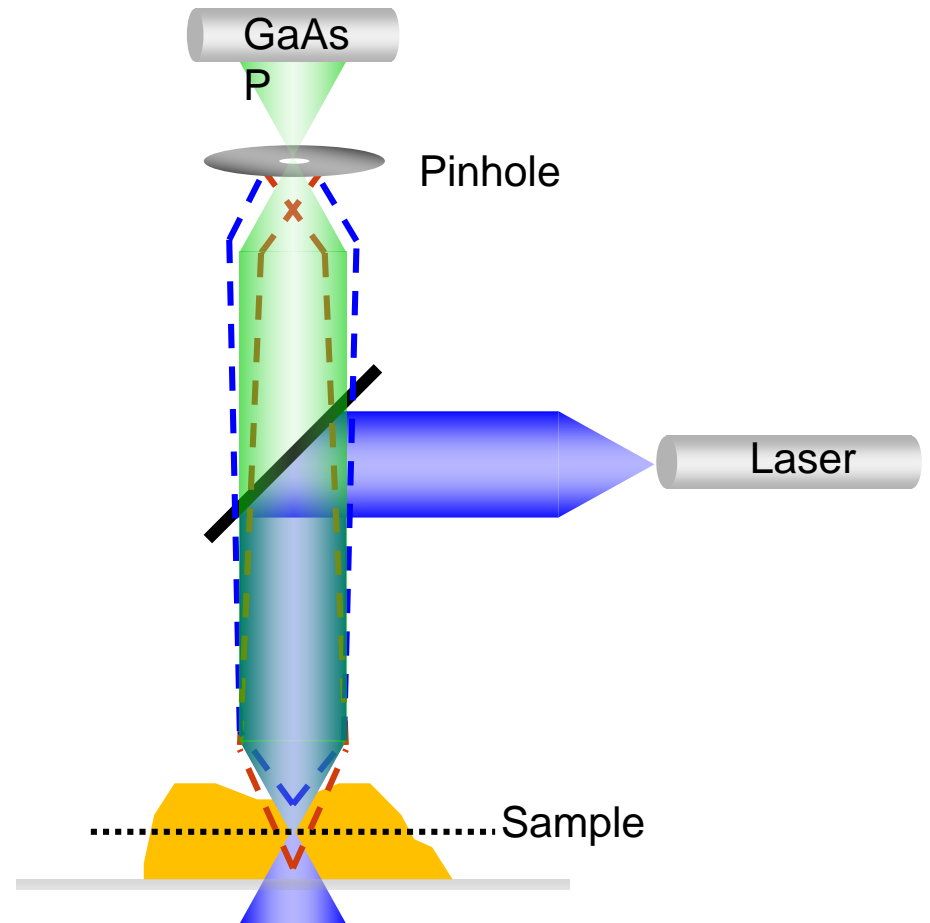
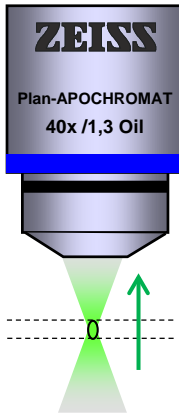
Rejection of out-of-focus light at the pinhole and 3D Imaging



Excitation



Emission



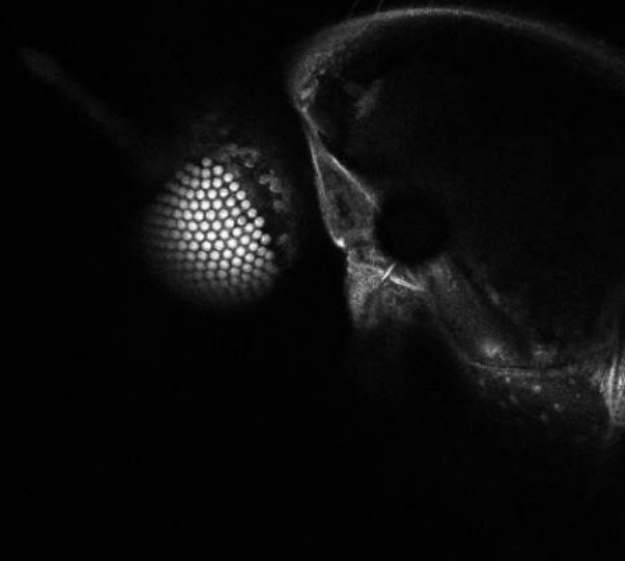
At the heart of the LSM: The Confocal Principle

Rejection of out-of-focus light at the pinhole and 3D Imaging



Wide Field

(out-of-focus light blurs the image)

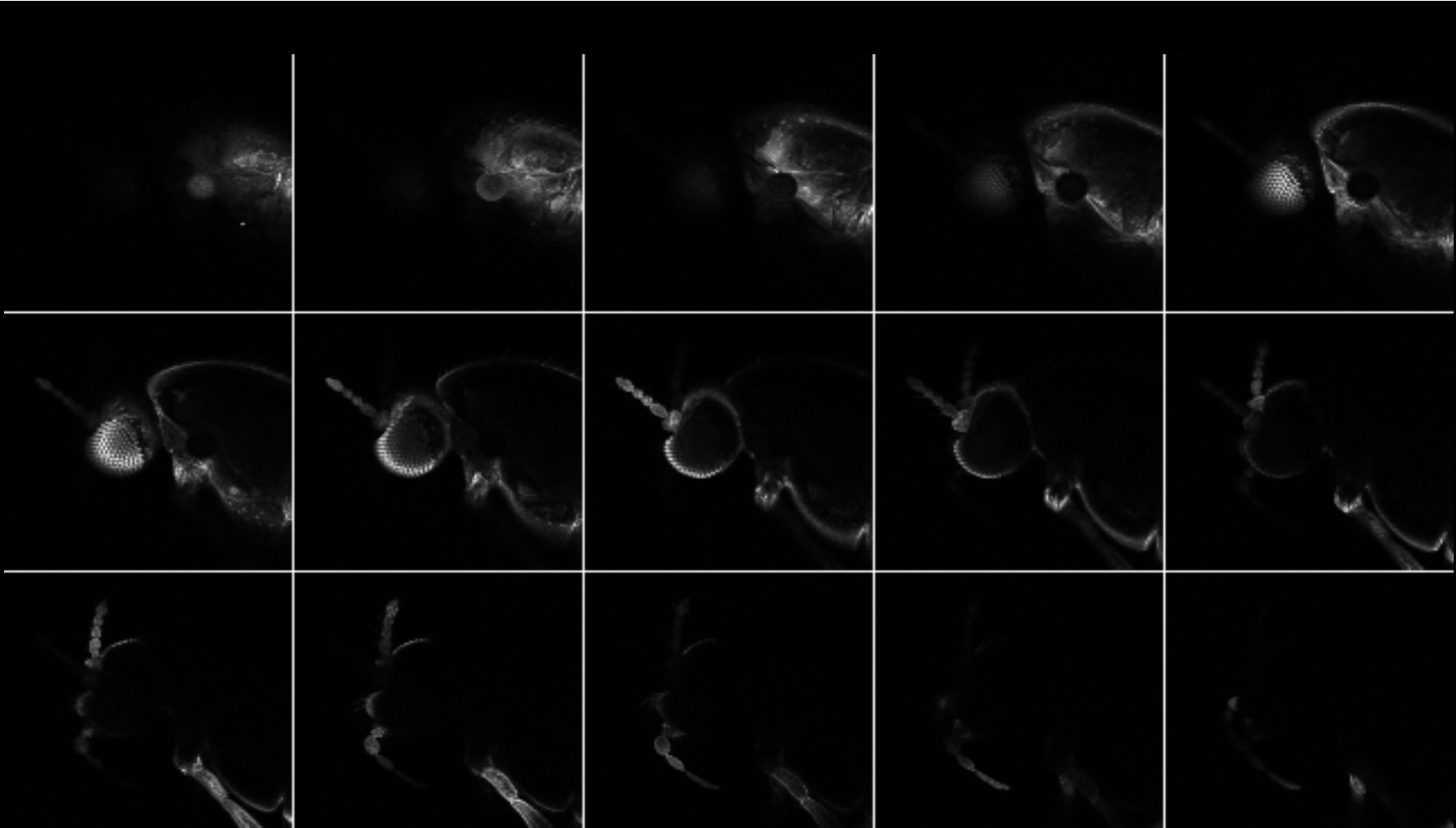


Confocal

(optical sectioning rejects out-of-focus light)

At the heart of the LSM: The Confocal Principle

Rejection of out-of-focus light at the pinhole and 3D Imaging



At the heart of the LSM: The Confocal Principle

Rejection of out-of-focus light at the pinhole and 3D Imaging



Wide Field

(out-of-focus light blurs the image)



Confocal

(optical sectioning rejects out-of-focus light)

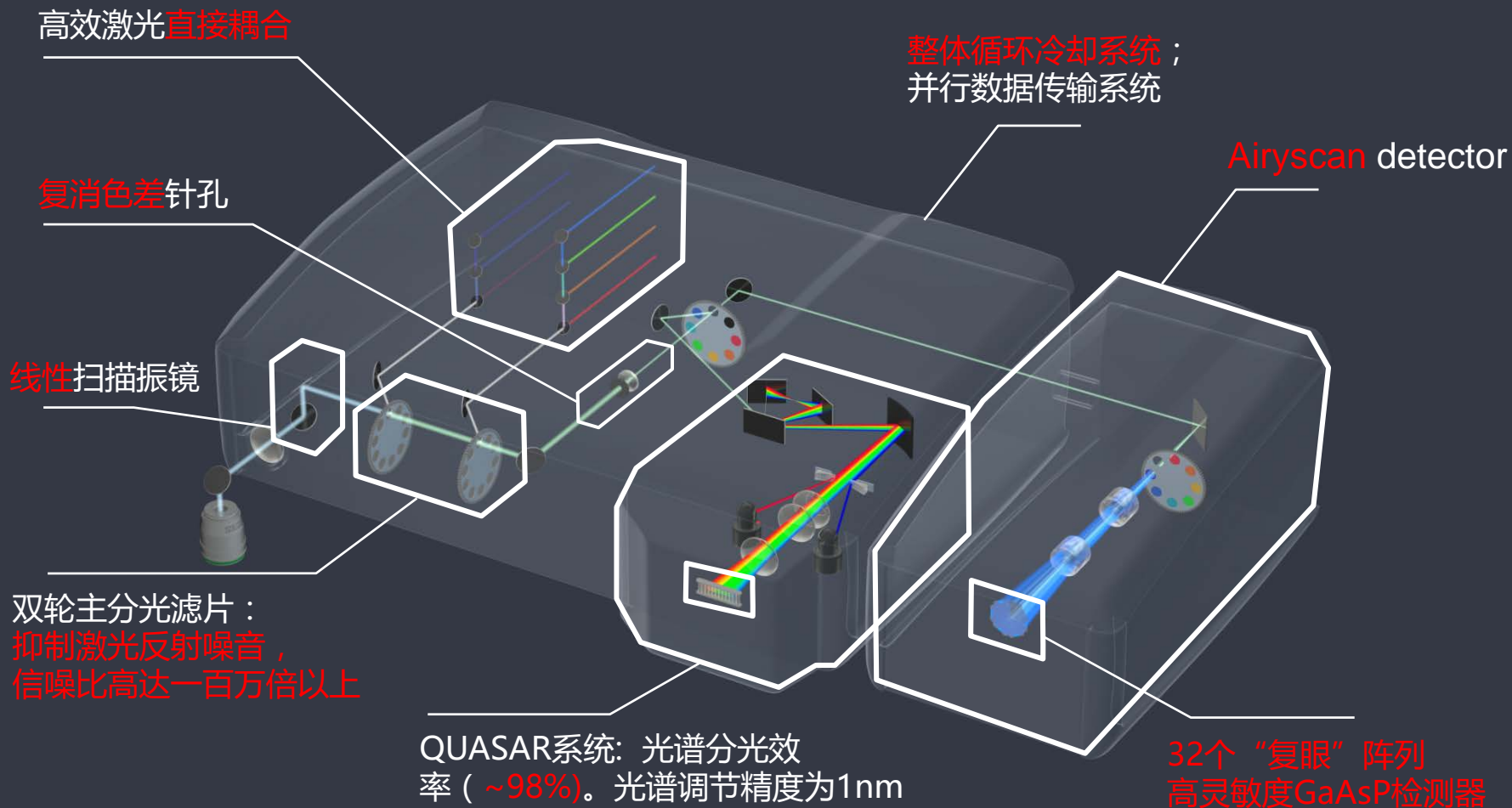
LSM 880 – the core news



LSM880的扫描检测单元

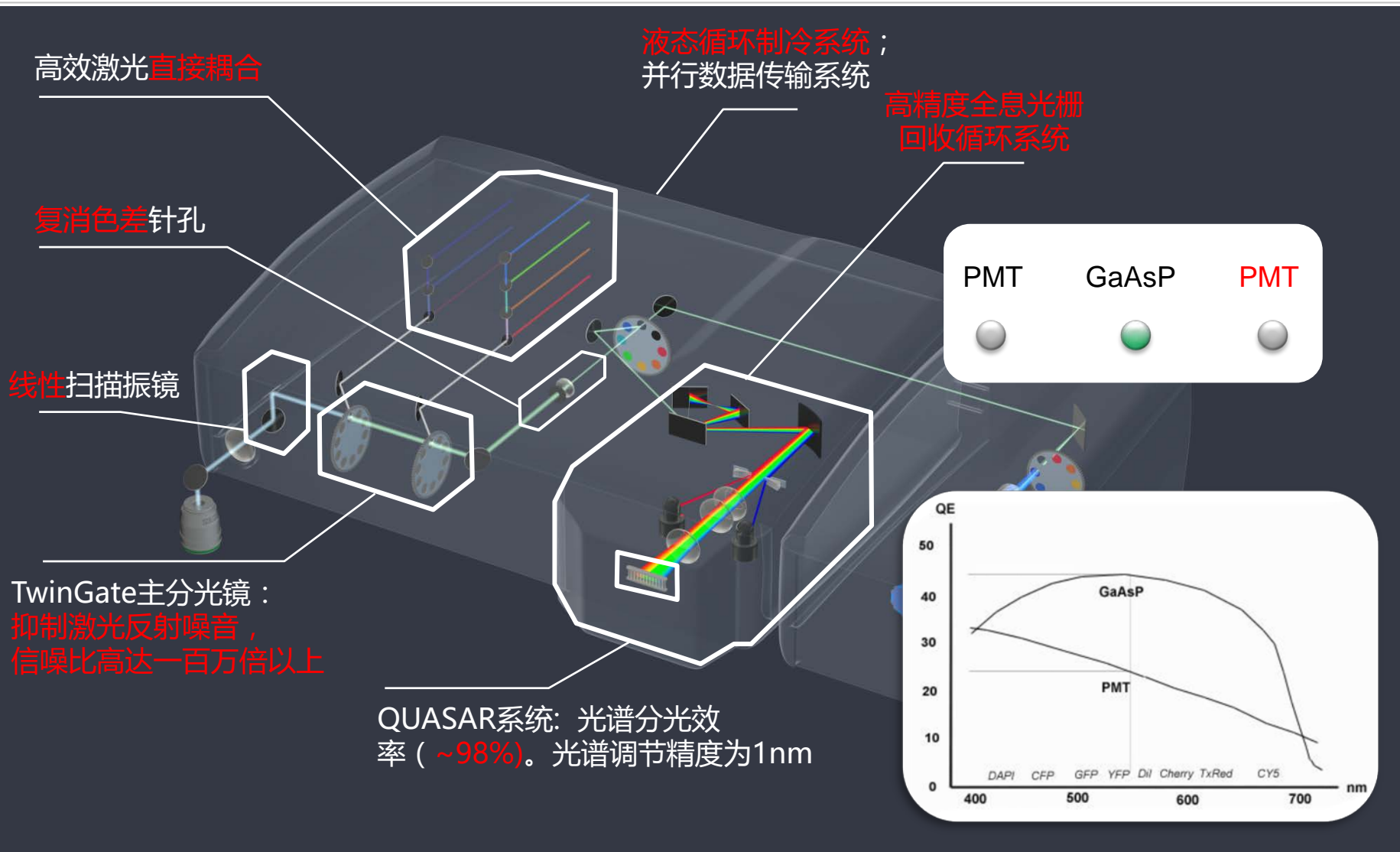


——集成众多专利技术



LSM 880 扫描检测单元

集成众多蔡司创新技术



Zeiss 高性能物镜

—— 大孔径，高分辨率，大视野



Plan-Apochromat
10x/0.45
工作距离2mm

荧光效果好，分辨率高，大视野

适于观察**斑马鱼、全脑、果蝇**等大范围样品



Plan-Apochromat
20x/0.8 DIC
工作距离高达 0.55mm

工作距离大，全波段荧光通透性高，适合通用样品的荧光样品观察

适于观察**斑马鱼、脑片、免疫组化切片**等较大范围样品



Plan-Apochromat
40x/1.3
工作距离0.21mm

荧光效果好，分辨率高，大视野

适于观察**斑马鱼、脑片、免疫组化切片**等较大范围样品



Plan-Apochromat
63x/1.4
工作距离达190 μm

高分辨率，荧光观察效果极好，工作距离大

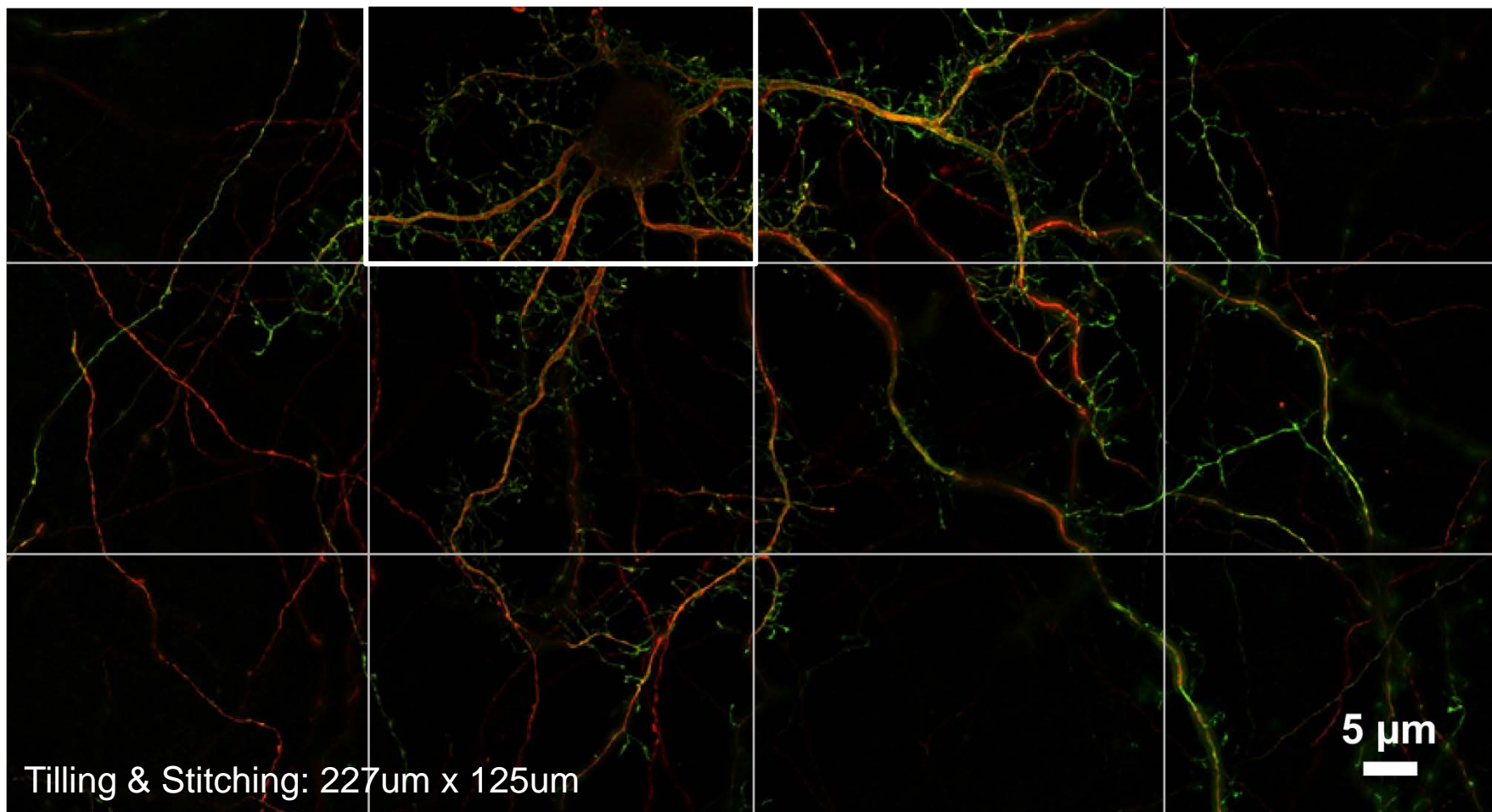
尤其适合**玻片样品、培养皿中活细胞**的高分辨率拍照弱荧光信号的采集

LSM 880

large FOVs with tiles imaging

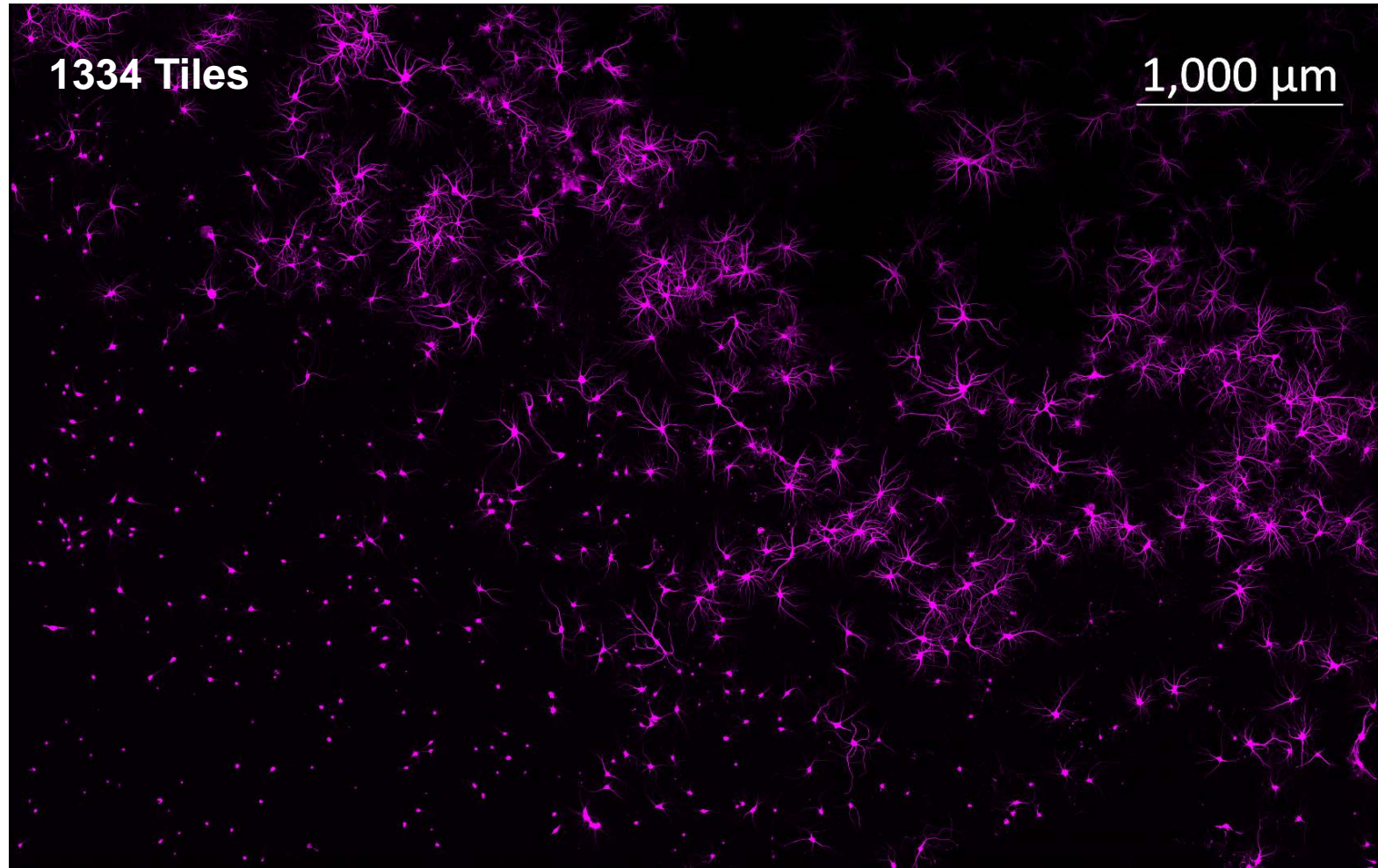
大视野三维结构

视野的扩展



LSM 800 System Workflows

Acquire large FOVs with tiling



Cultured Rat Hippocampal Neuron; AlexaFluor 488-Anti Beta III Tubulin and Cy3-Anti MAP2 (HM-2). Sample courtesy of Drs. Stefanie Kaech and Gary Banker.

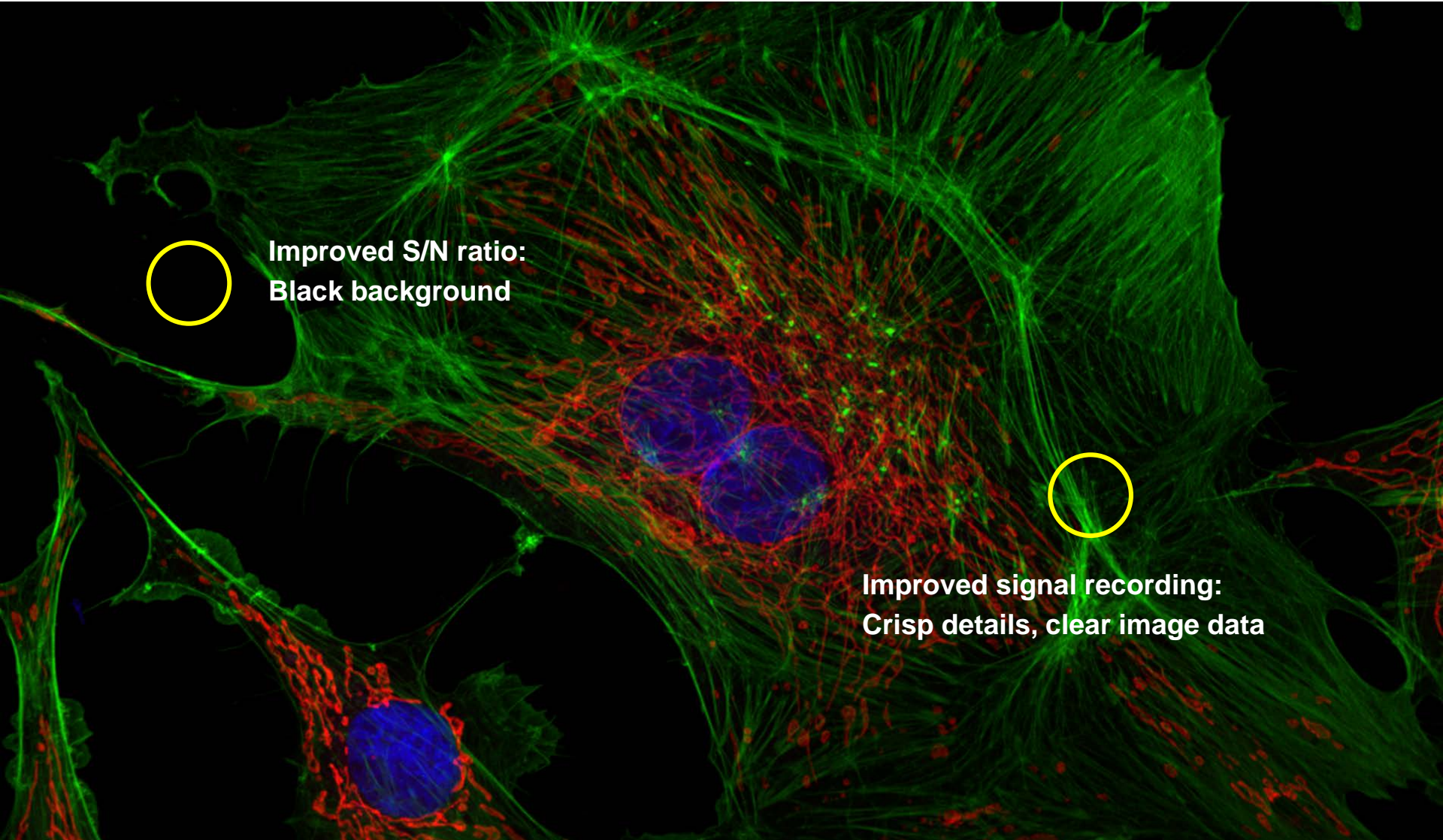
LSM 880

multichannel imaging

- ***No limitation multiple colors***
- ***Photo counting mode for weak FL signal***
- ***High image contrast***

LSM 880– High Image Sensitivity

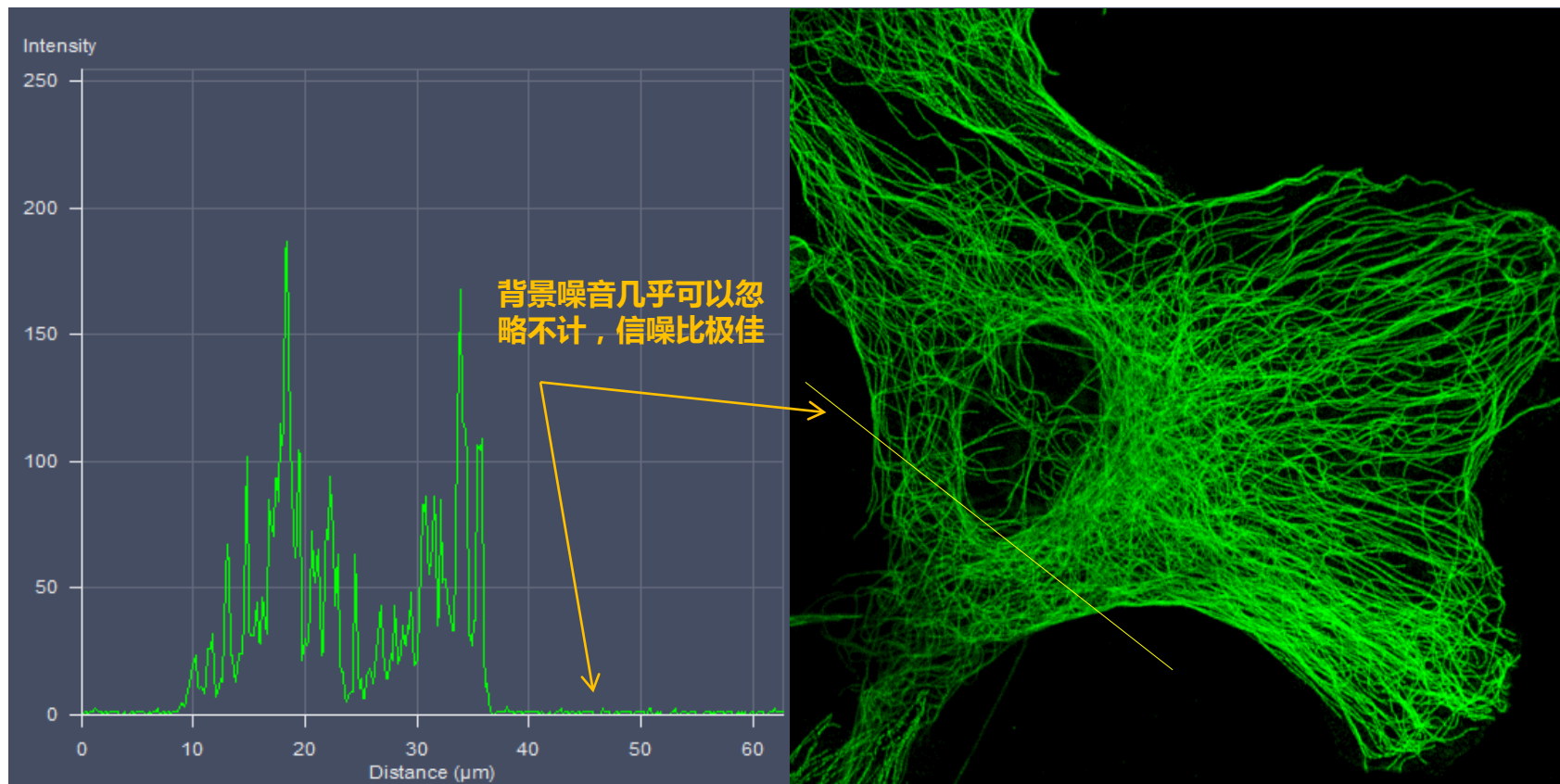
+ LSM 880 provide high contrast image



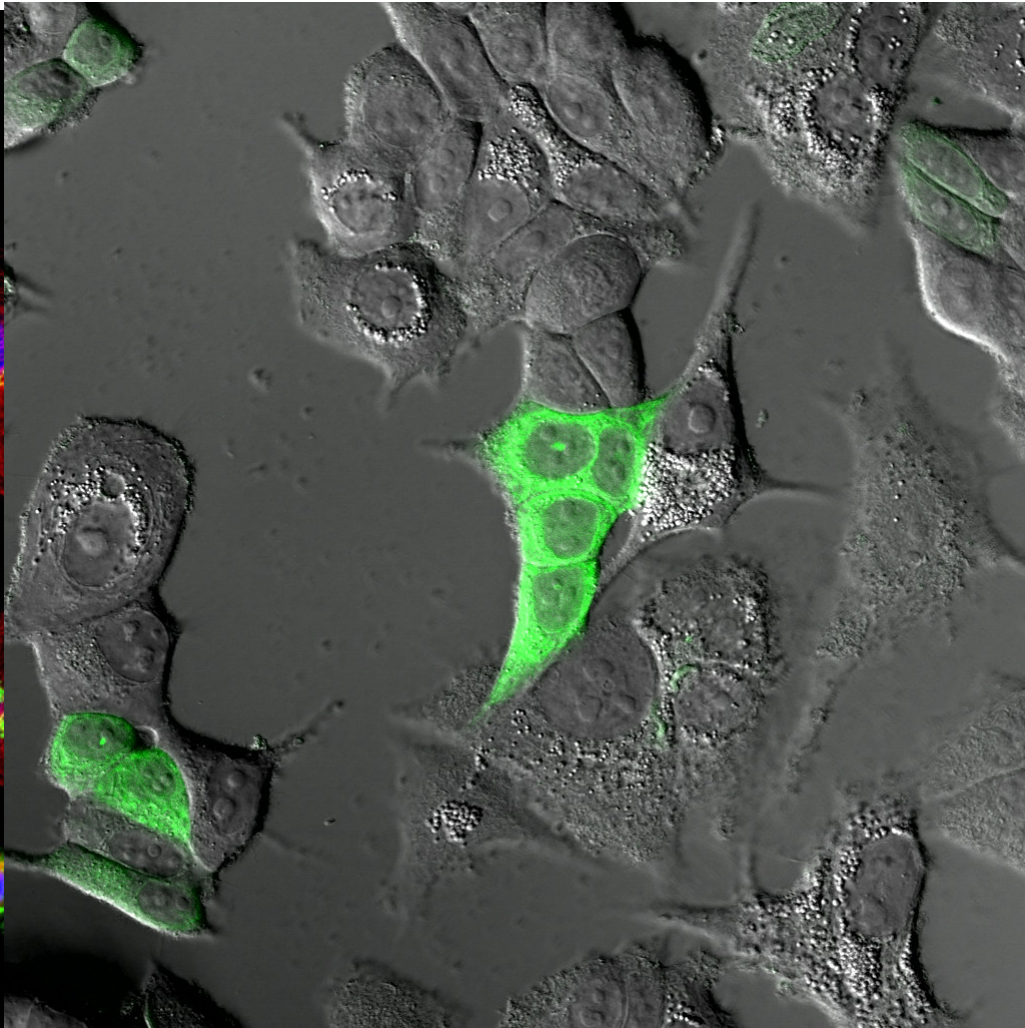
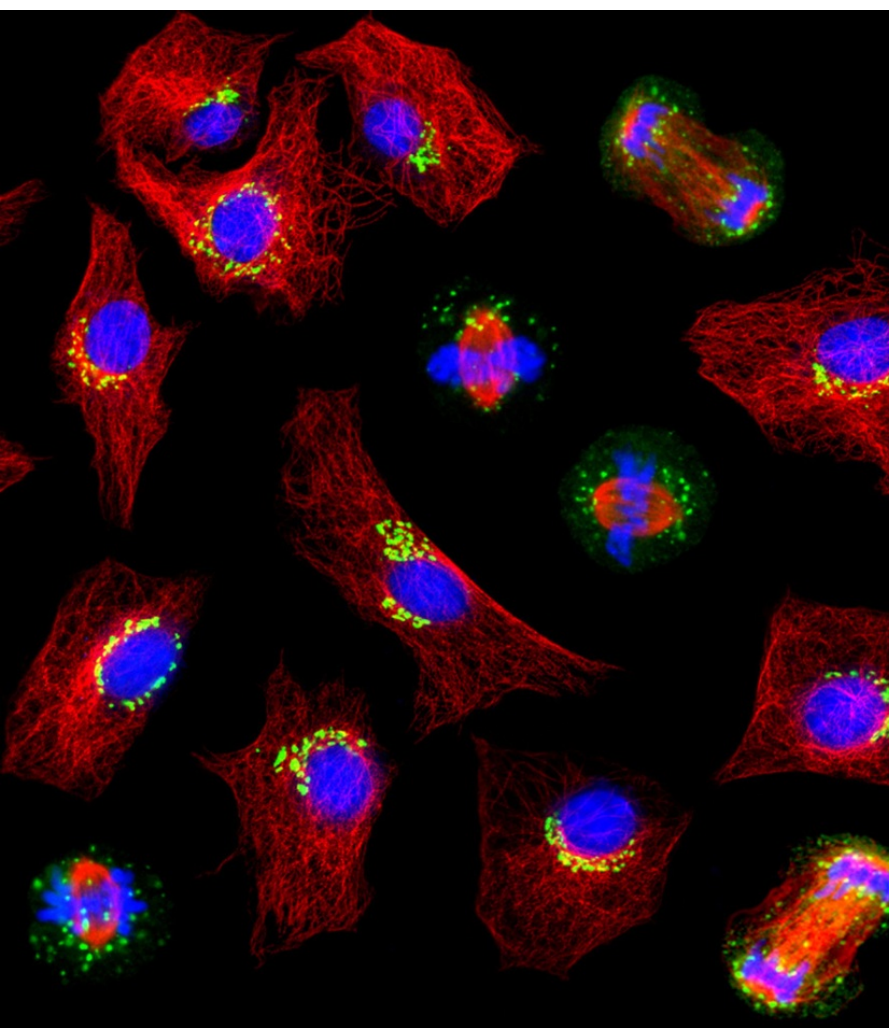
Improved S/N ratio:
Black background

Improved signal recording:
Crisp details, clear image data

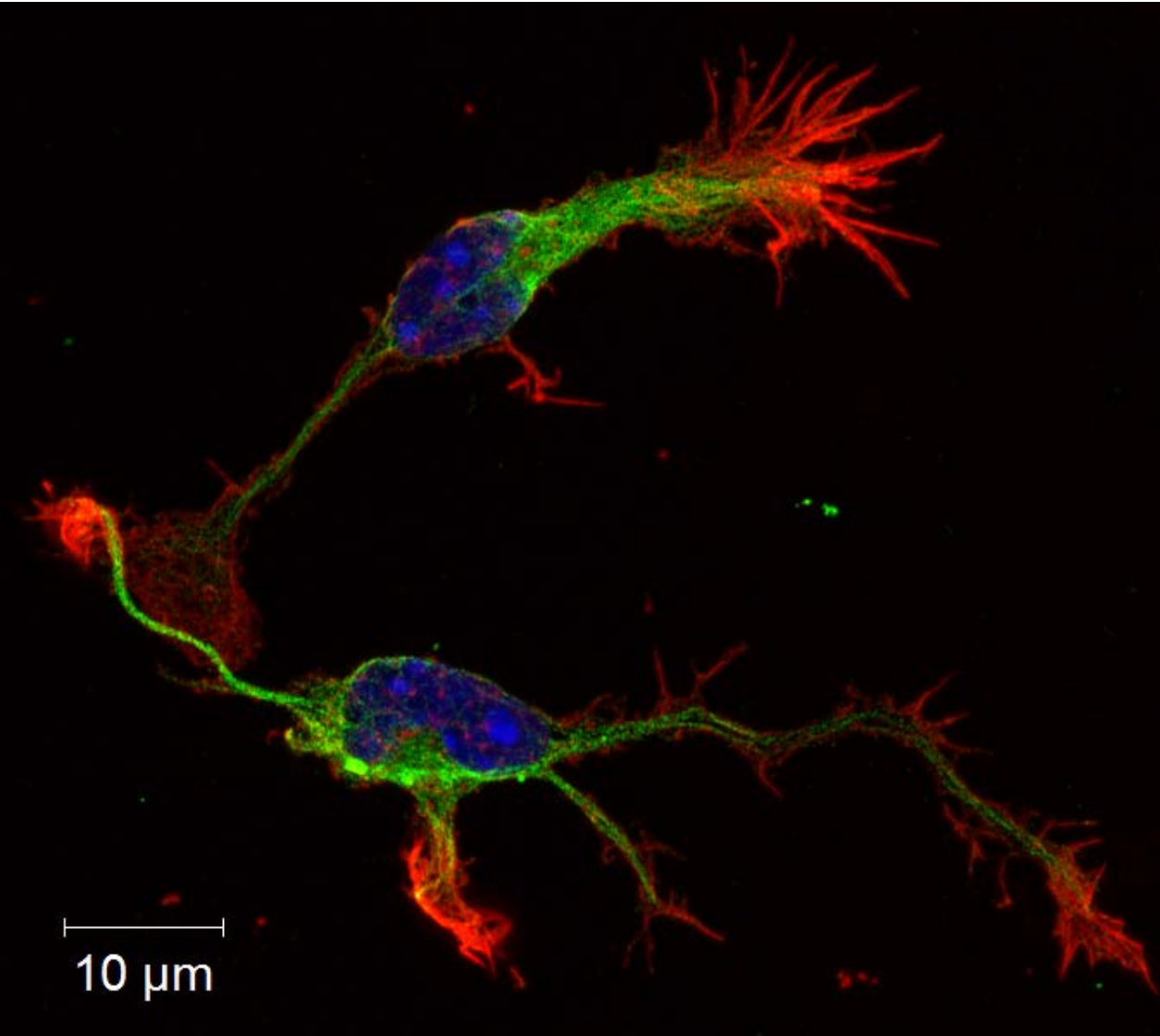
LSM 880 大幅提高图像质量 (信噪比提高)



LSM 880 大幅提高图像质量



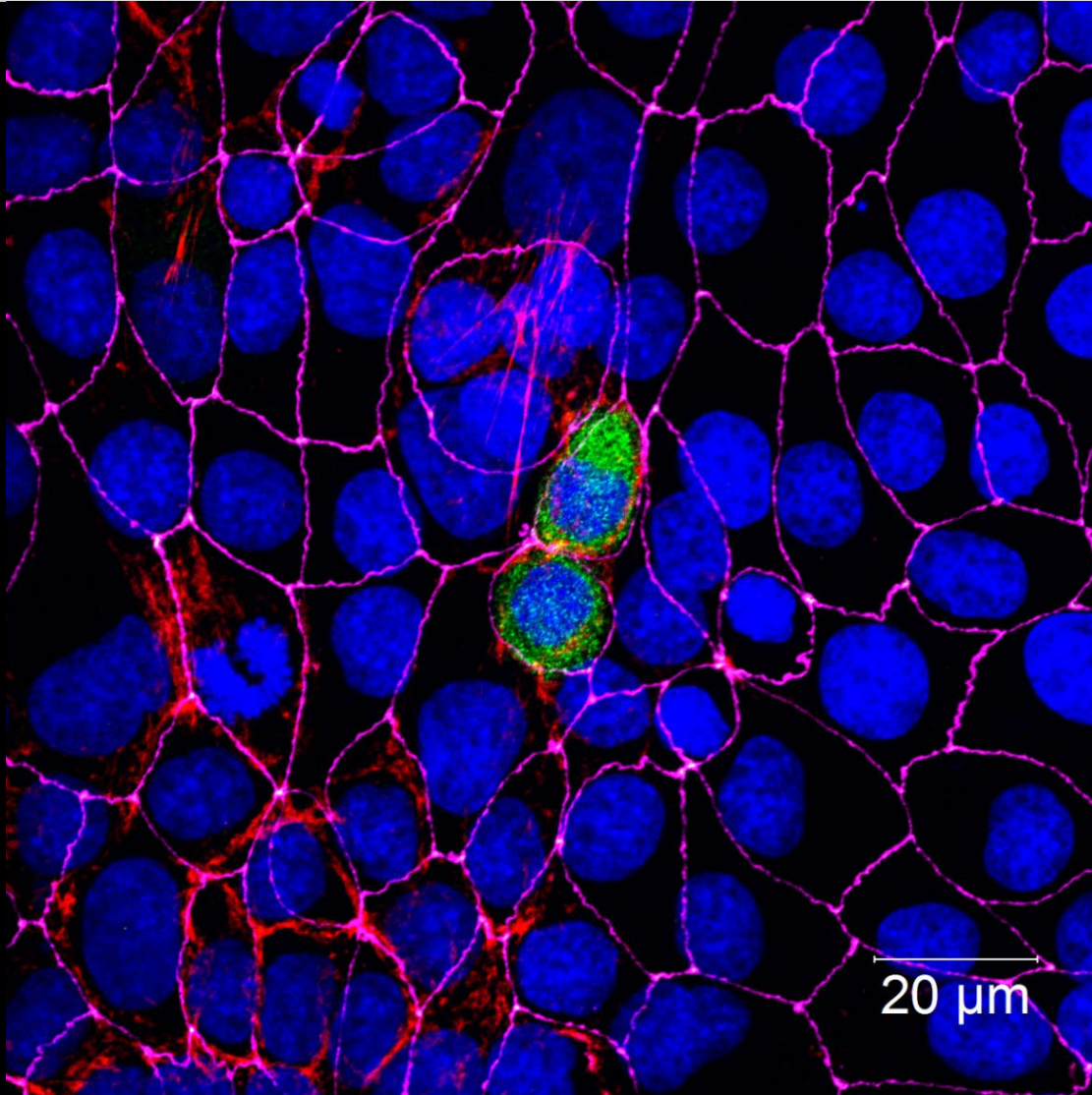
Very Nice Neuron



- Objective: Plan-Apo 63x/1.4
- Maximum intensity projection

Sample from and snapped by Li Qianru,
Shanghai Institute of Materia Medica.

LSM 880 – 多色荧光成像



**Madin-Darby canine
kidney (MDCK)
马犬肾上皮细胞**

**Blue: DAPI
Green: EGFP
Red: AF555
Magenta: AF647**

Squid, a model for neurodegenerative diseases in humans, examined with LSM 880



Sample

Longfin inshore squid embryo
(*Loligo pealei*)

Labels

F-actin (red; phalloidin), acetylated tubulin
(green), and DAPI (blue; nuclei)



Image courtesy of

MBL, Woods Hole, US, and The Node,
Company of Biologists, Cambridge, UK

Squid, a model for neurodegenerative diseases in humans, examined with LSM 880



Sample

Longfin inshore squid embryo
(*Loligo pealei*)

Labels

F-actin (green; phalloidin), acetylated tubulin (red), anti-HRP (yellow), and DAPI (blue; nuclei)

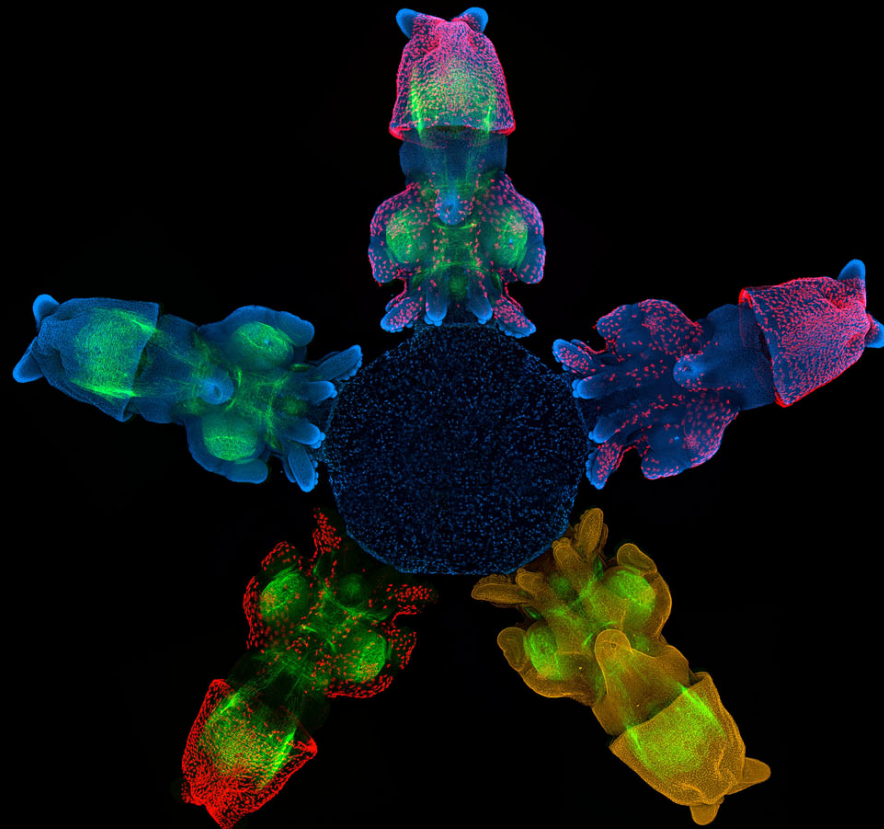
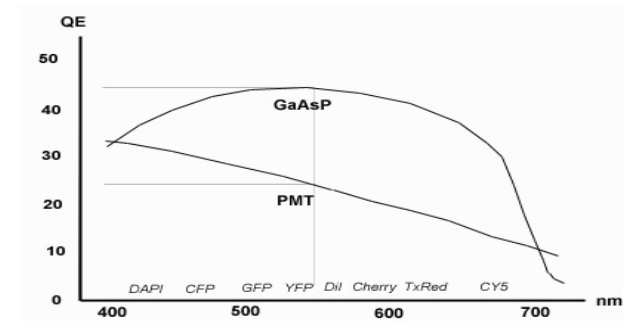
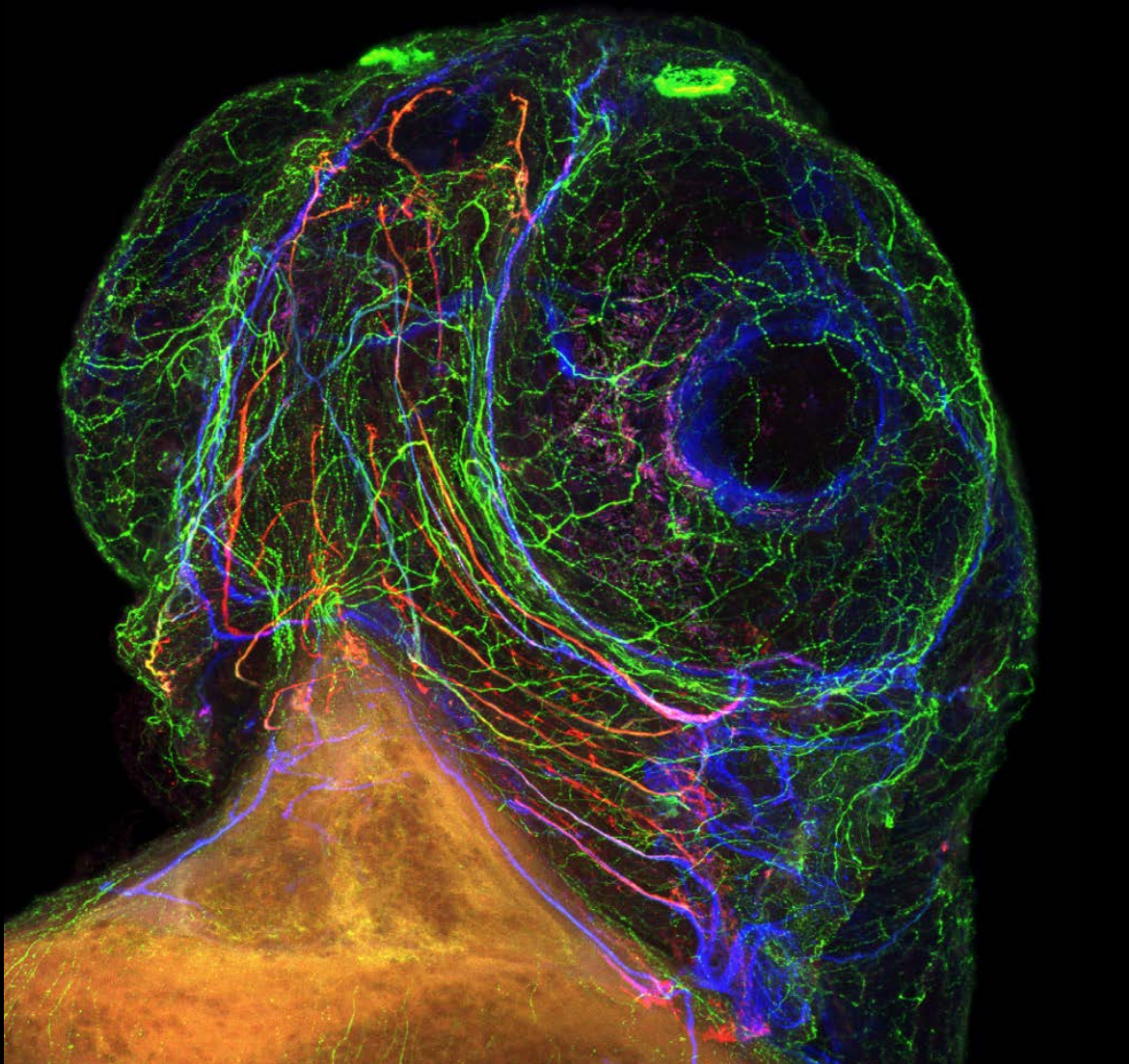


Image courtesy of

MBL, Woods Hole, US, and The Node,
Company of Biologists, Cambridge, UK

LSM 880

+ Photon count for weak FL Signal



High Sensitivity GaAsP Detectos

Sample: Zebrafish

Objective: 20x/0.8

Laser: 0.2%

GaAsP detector

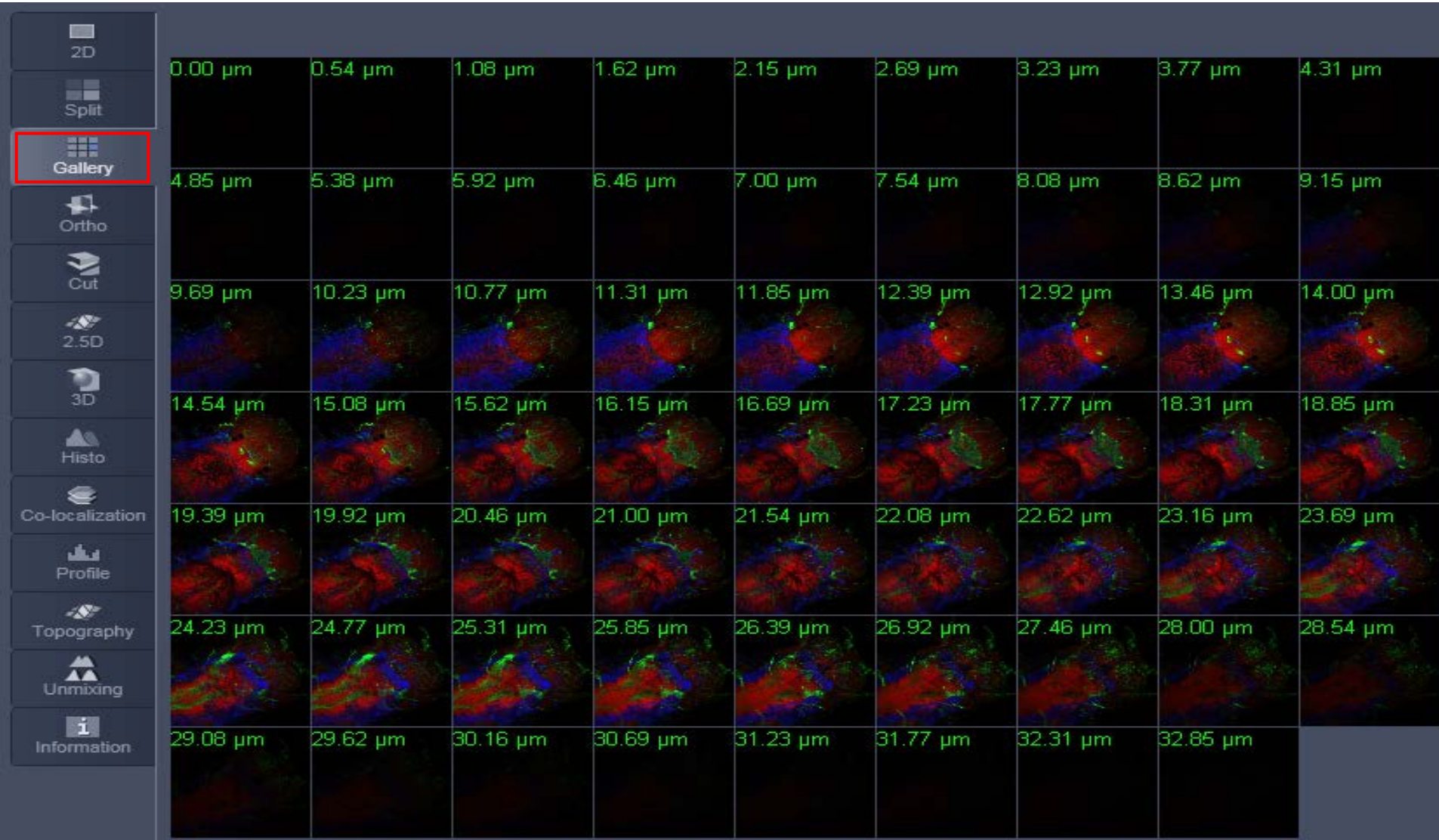
LSM 880

3D Z stack imaging

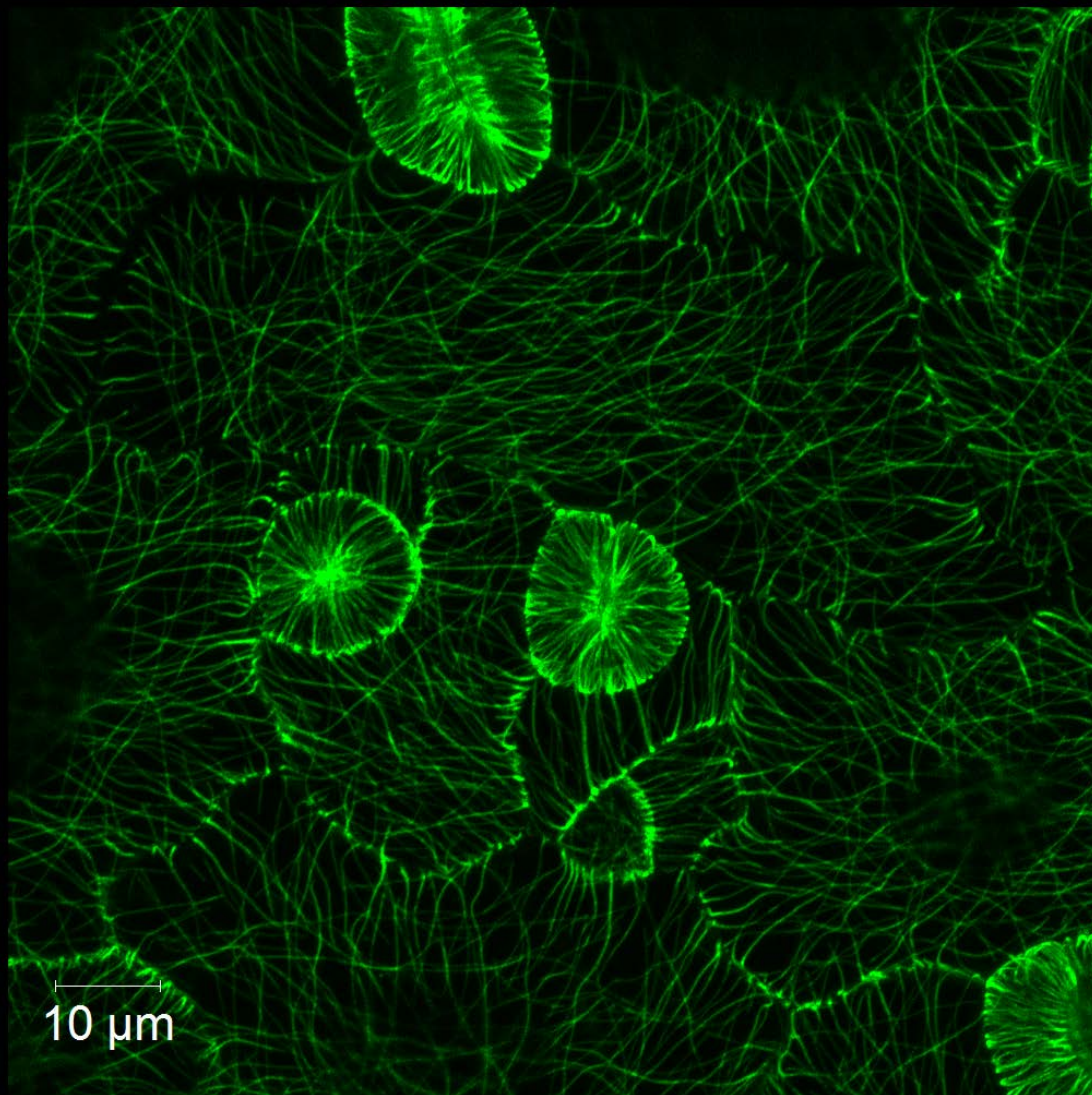
- *Z drive down to 10nm/step*
- *Extremely fast Z driver for fast Z stack imaging*

LSM 880 Z stack image

3D reconstruction display



LSM 880 – z轴层扫 最大强度投影



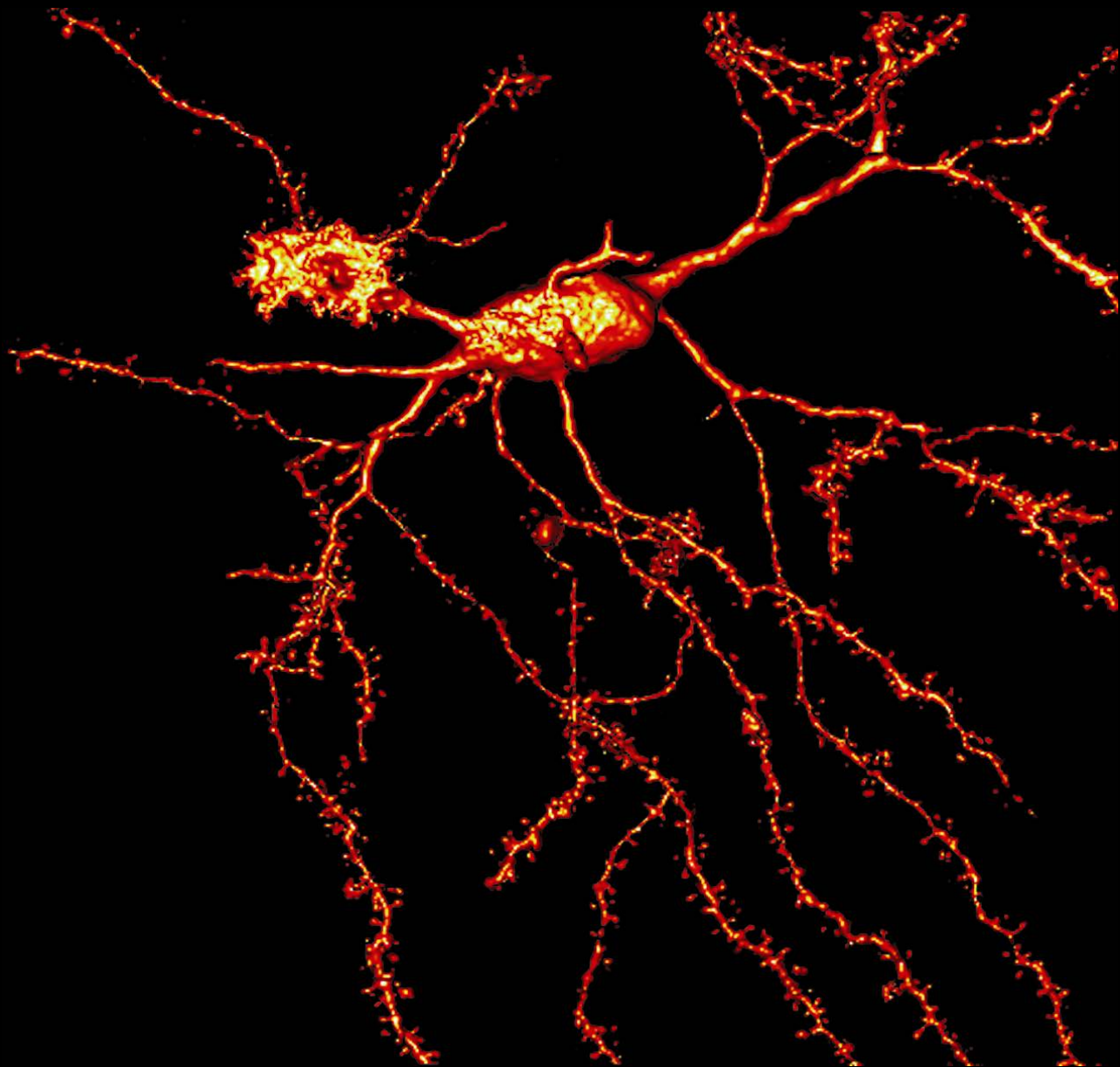
融合GFP的微管蛋白

激光: 488nm, 1%

物镜: C-Apo 40x/1.2 Water

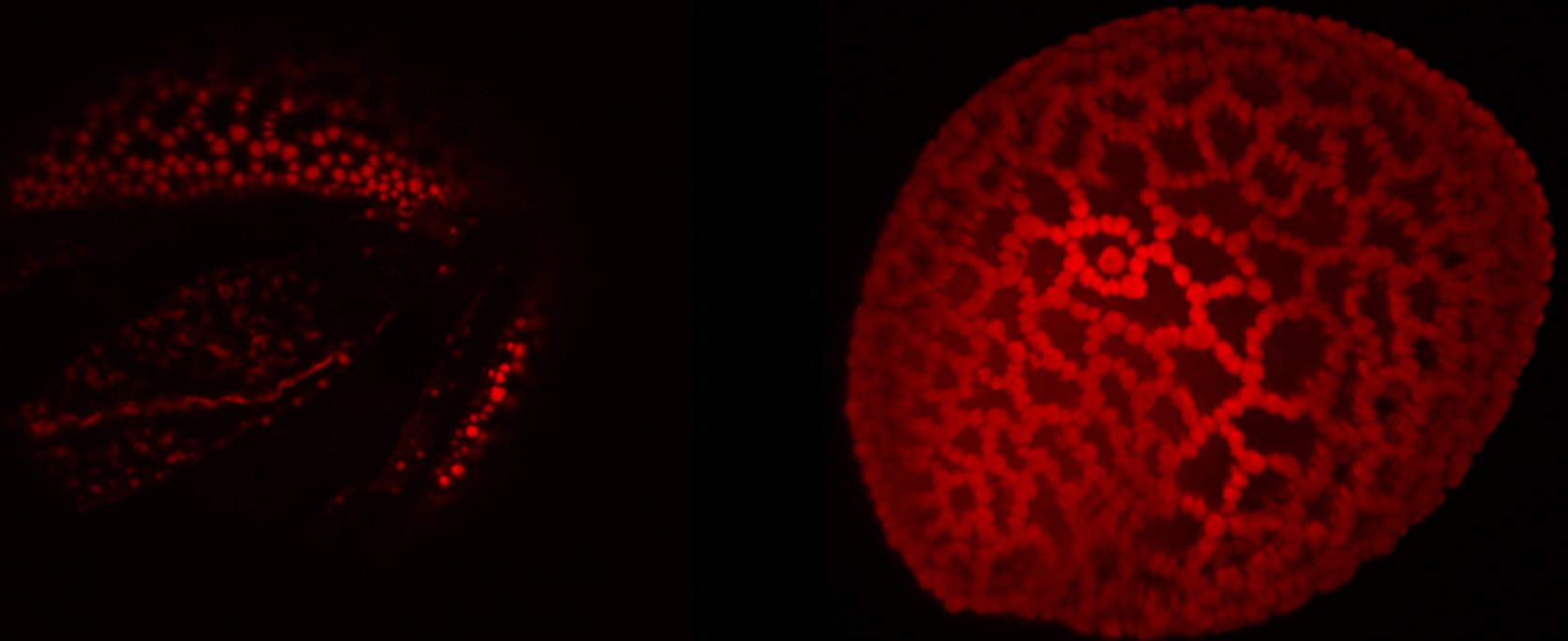
x: 106.17 μ m, y: 106.17 μ m,

z: 12.01 μ m



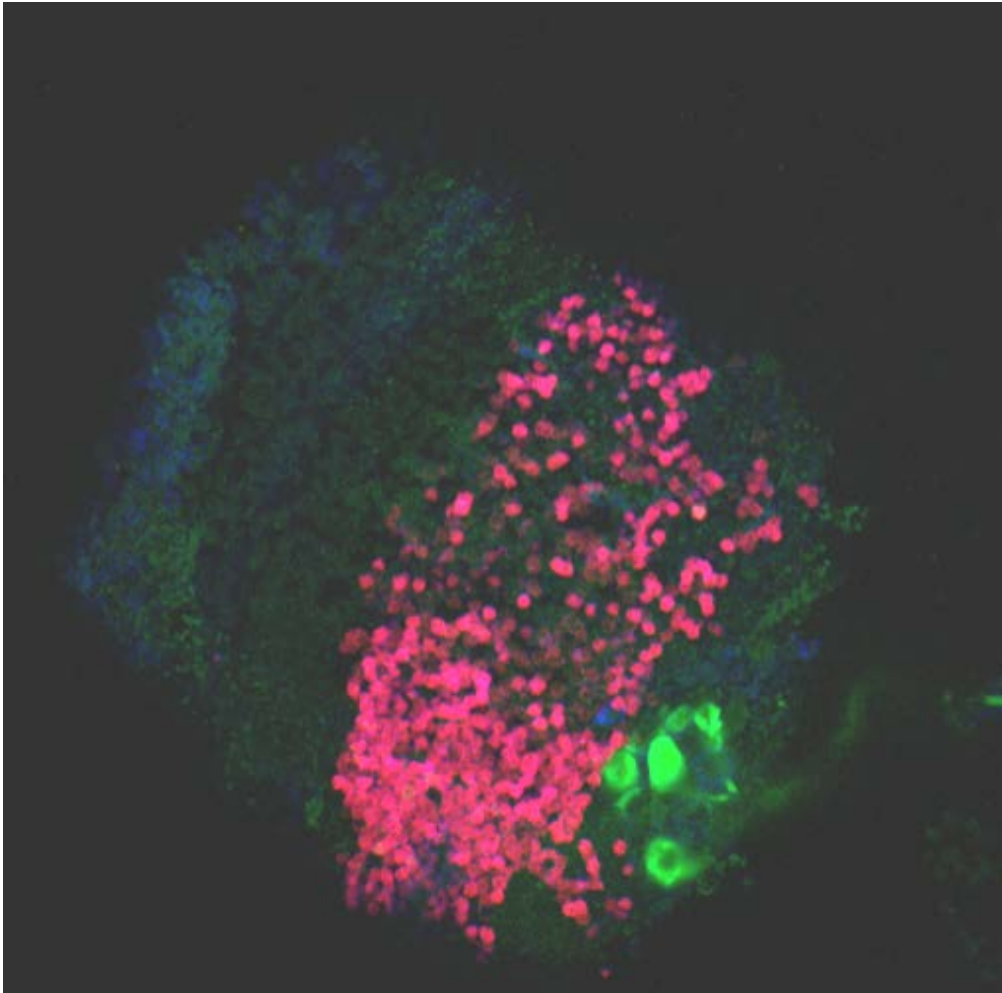
LSM 880– Z stack

+ *Z stack and 3D reconstruction*



LSM 880 3D Imaging

Neuron populations in Drosophila brain



Clear color separation
with 3 GaAsP detectors:

Sensitive imaging of red
and far red signal.

Alexa 488

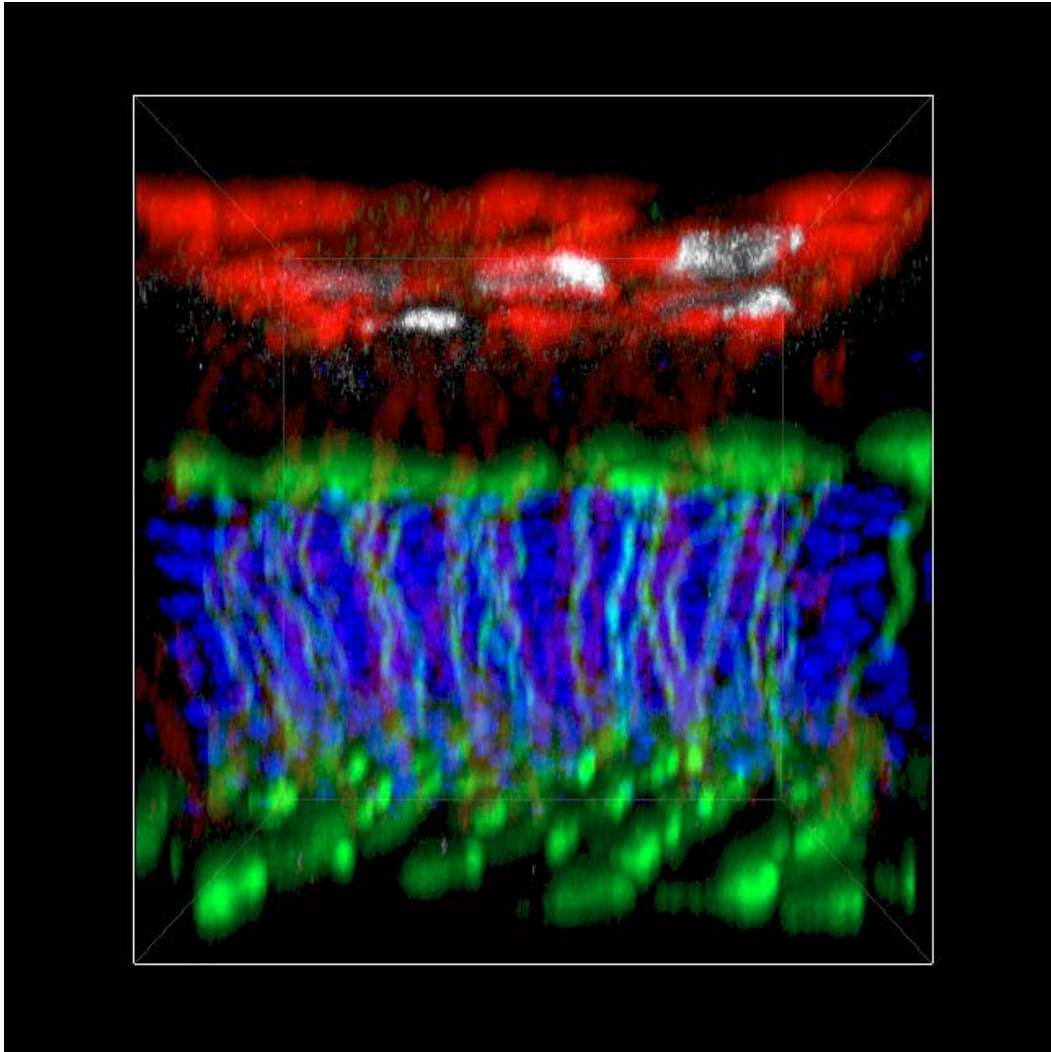
Alexa 568

Alexa 633

Sample courtesy of D. Reiff, Institute
of Biology, Albert-Ludwigs-University
Freiburg, Germany

LSM 880 System Sensitivity

Gentle Imaging for Demanding Samples



Acquisition of large volumes, even in sensitive samples.

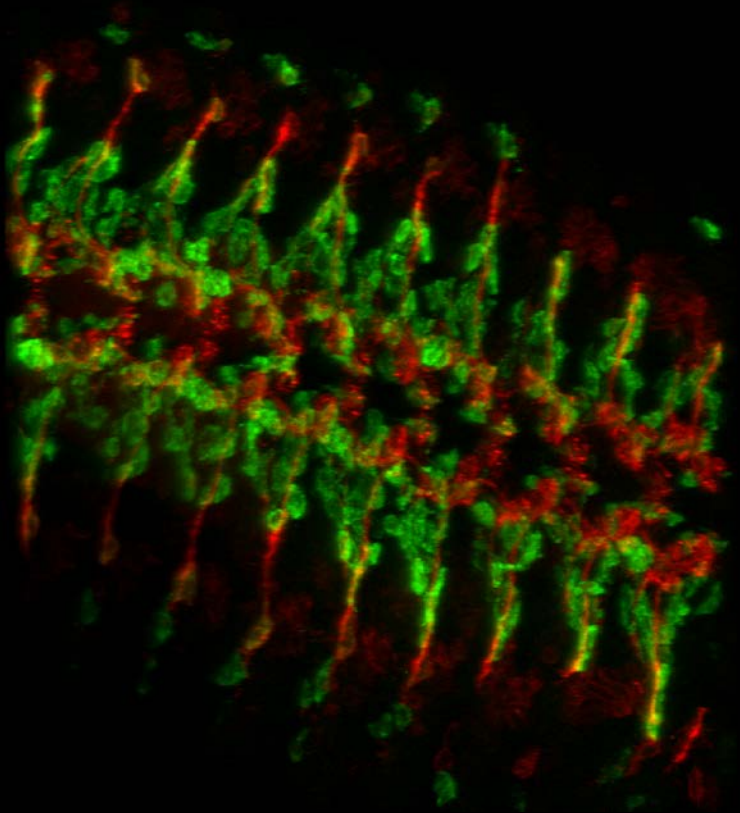
4 Channels
Z-stack
Mouse retina

Sample: courtesy of B. Roska, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

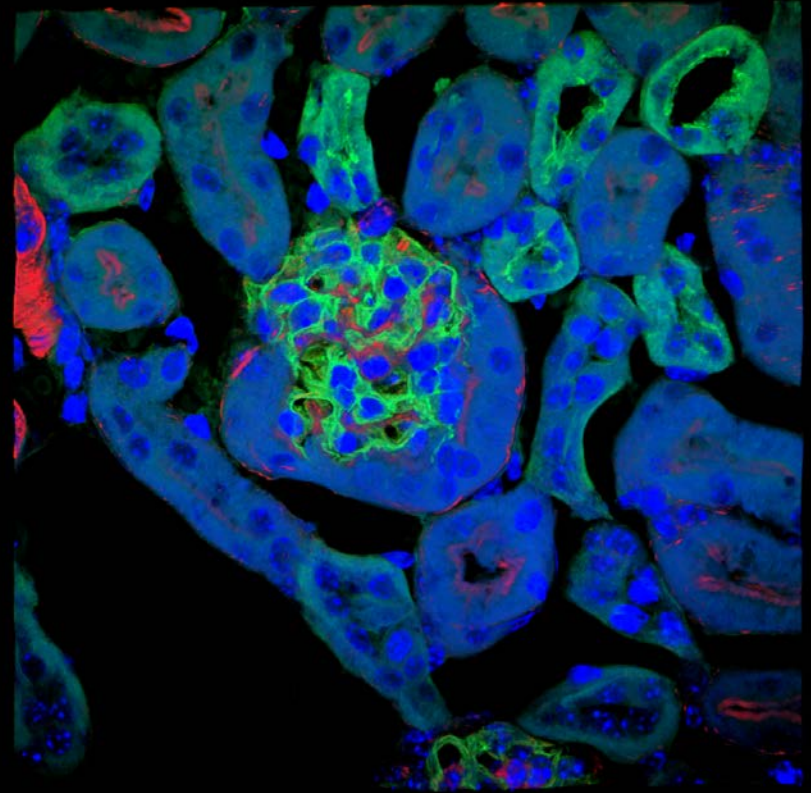
LSM 880 – 三维重构 z-stack



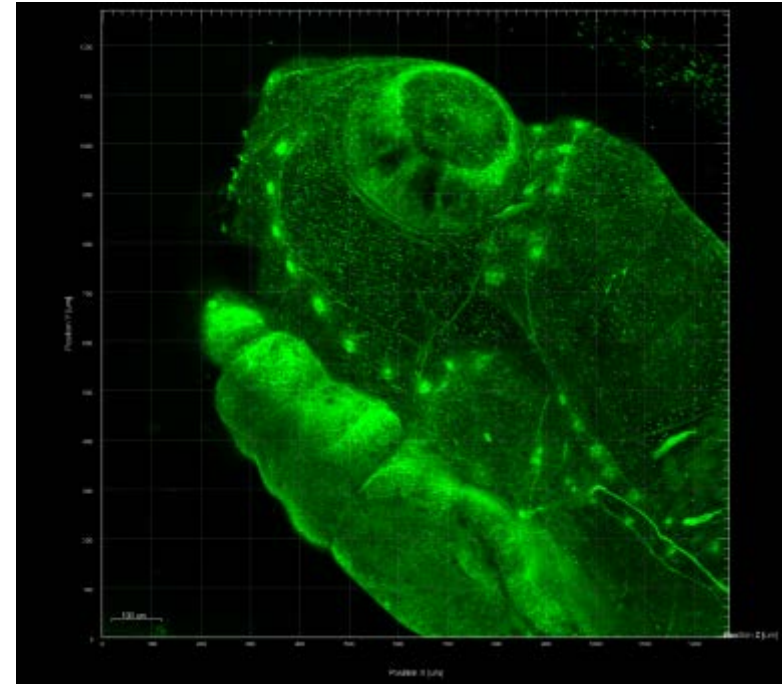
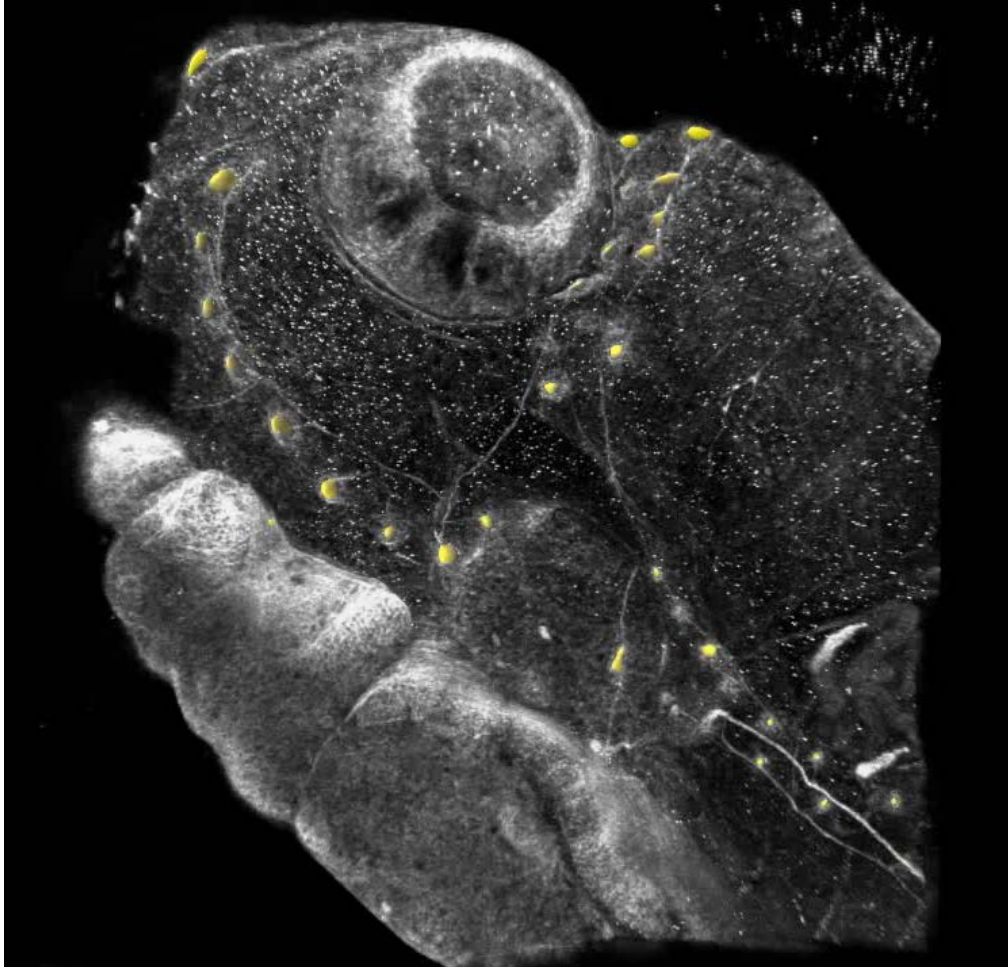
果蝇胚胎



大鼠肾小球



3D展示：

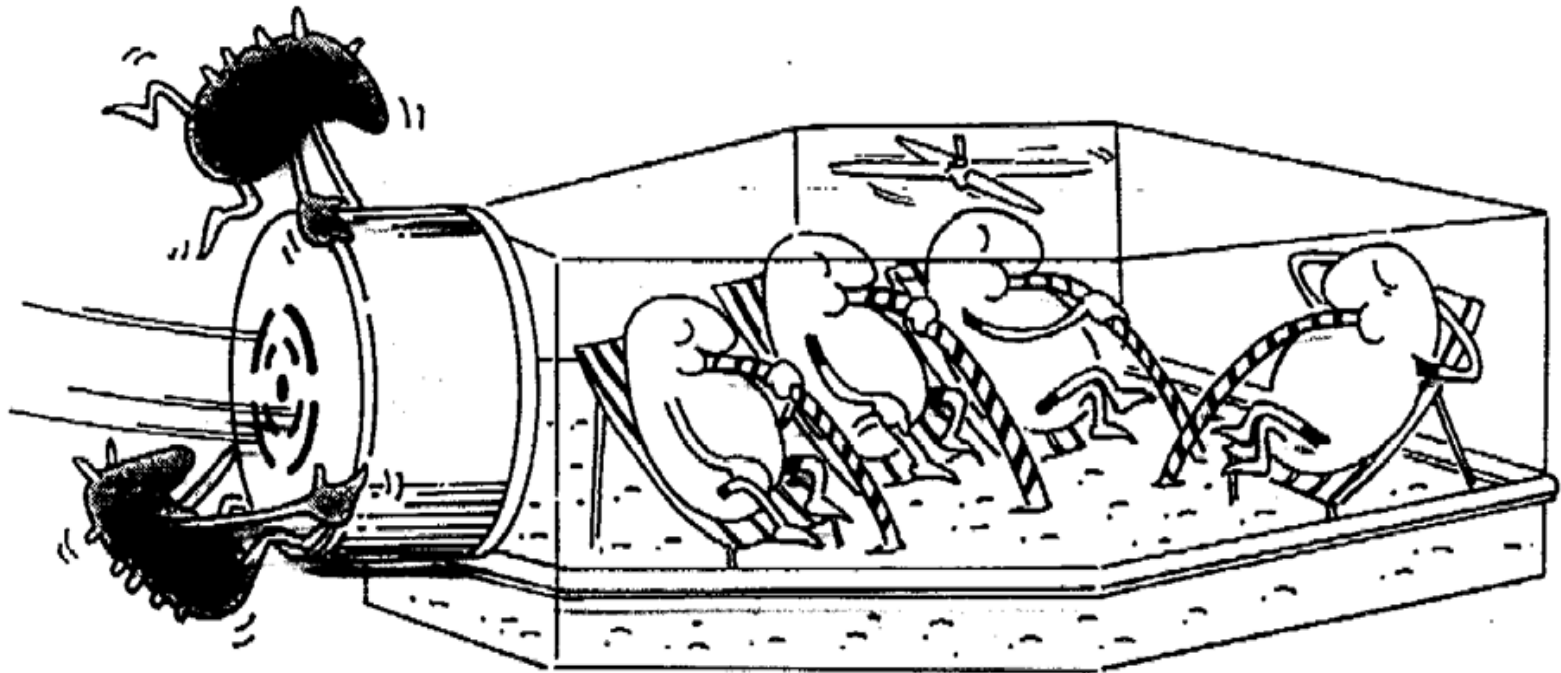


LSM 880

Live Cell Imaging

- *Linear scanning system improve image quality*
- *13fps @ 512 x 512*
- *Gentle Imaging with minimal laser power*

Copy in-vivo conditions „to make your cells happy“!



LSM 880 Time Series



File Acquisition Maintain Macro Tools View Window Help

Workspace Zoom: [Reset]

Workspace Configuration [Save] [Close]

Ocular Acquisition Processing Maintain

Configuration [Save] [Close]

Smart Setup [Show manual tools]

New Auto Exposure Live Continuous Snap

Z-Stack 14 Slice(s)
 Time Series 100 Image(s)
 Bleaching
 Regions

350.00 MB

Start Experiment

Online Acquisition

- Acquisition Mode [Show all]
- Channels [Show all]
- Focus [Show all]

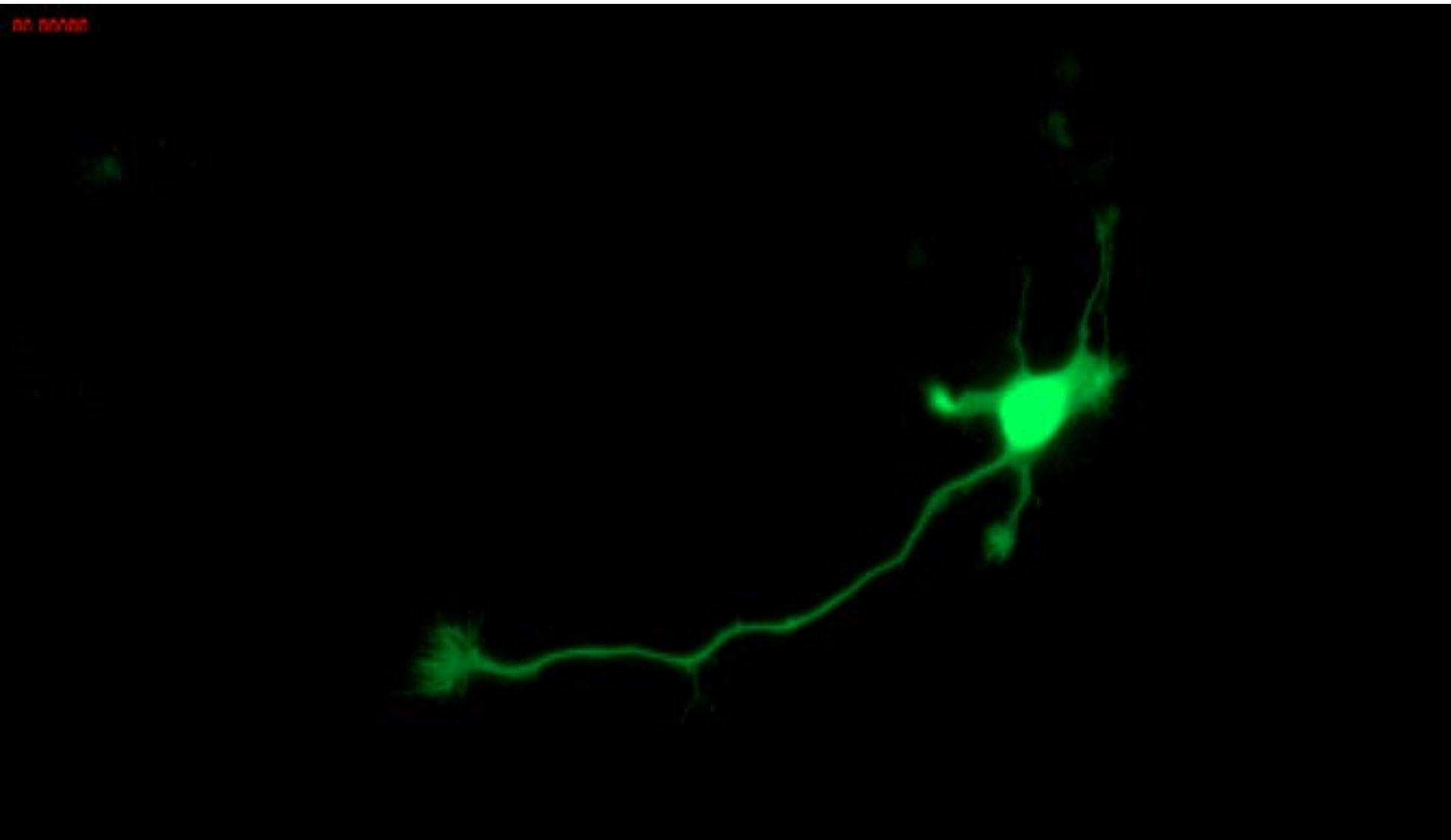
Multidimensional Acquisition

- Z-Stack [Show all]
- Time Series [Show all]
- Information On Experiment [Show all]

2D
Gallery
Ortho
Cut
2.5D
3D
Histo
Profile
Topography
Mean ROI
FRAP
RICS
Information



应用实例 - 培养神经元神经锥生长 时间序列，过夜实验 >16小时



LSM 880 System Sensitivity

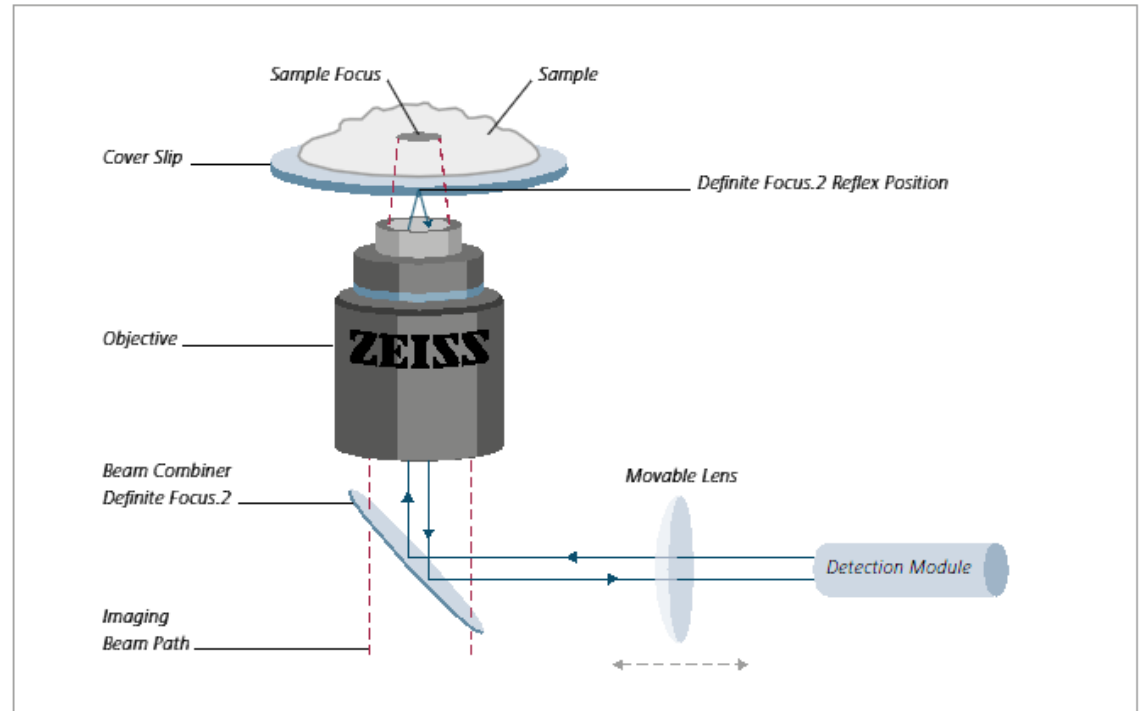
Gentle Live Cell Imaging



Fox lung cells are observed for 24 hours while dividing.

第二代精确聚焦系统 Definite Focus.2

稳定的焦距



新特点:

→ 高速、稳定聚焦；实时成像

→ 多点成像时可设定不同的offset距离；保证每个位置的聚焦准确性

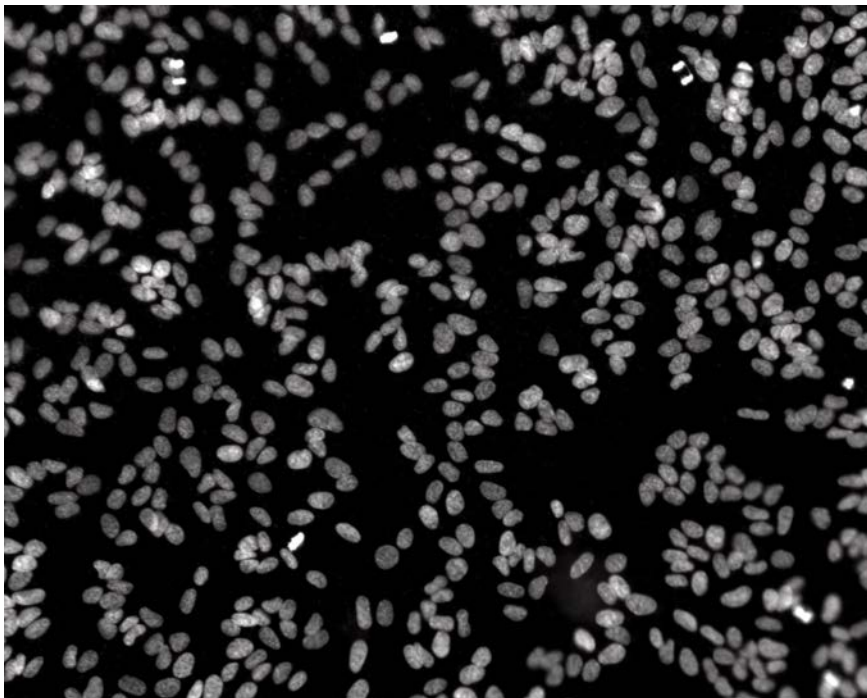
活细胞工作站 - 长时间活细胞成像

稳定快速的焦距控制 - **Definite Focus.2**



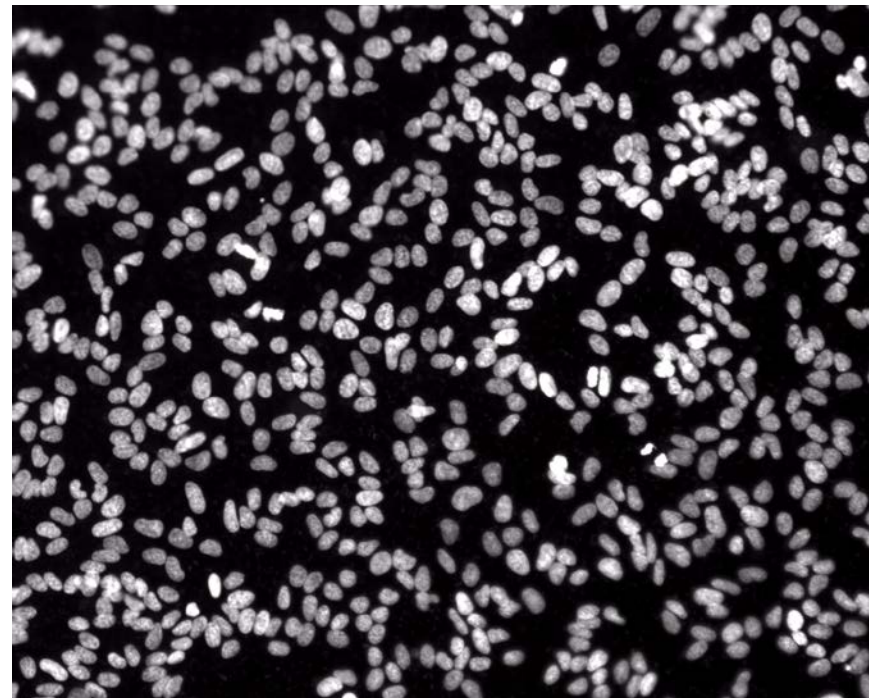
“焦距锁定” 关闭

培养板底不平状态下，
不同孔位切换时，样品离焦

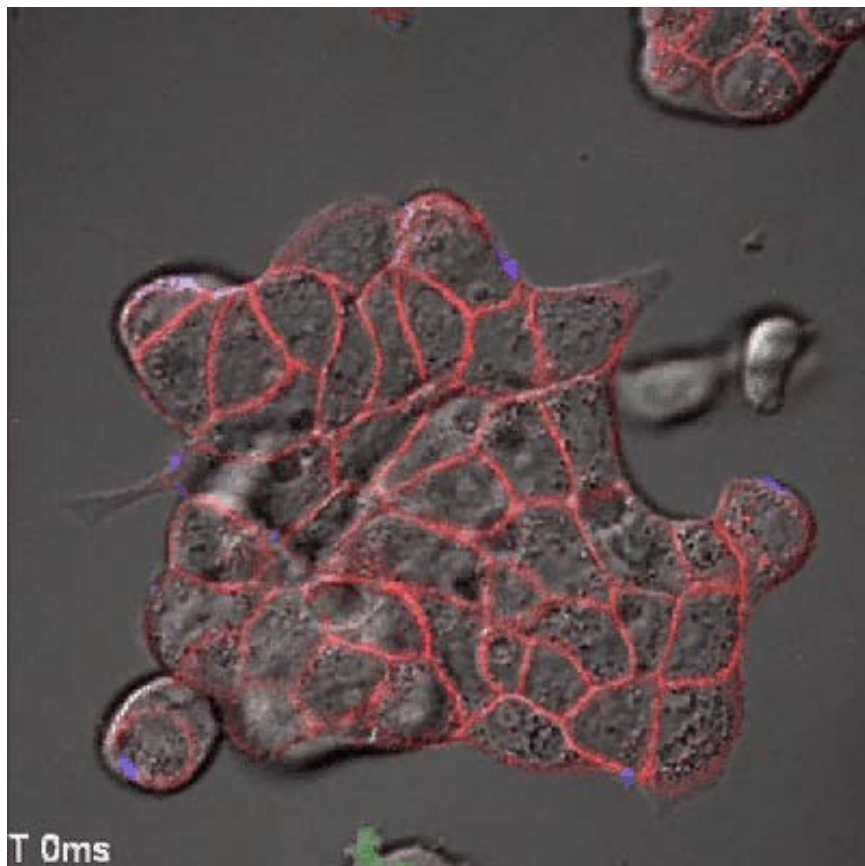


“焦距锁定” 开启

培养板底不平状态下，
不同孔位切换时，聚焦依然清楚

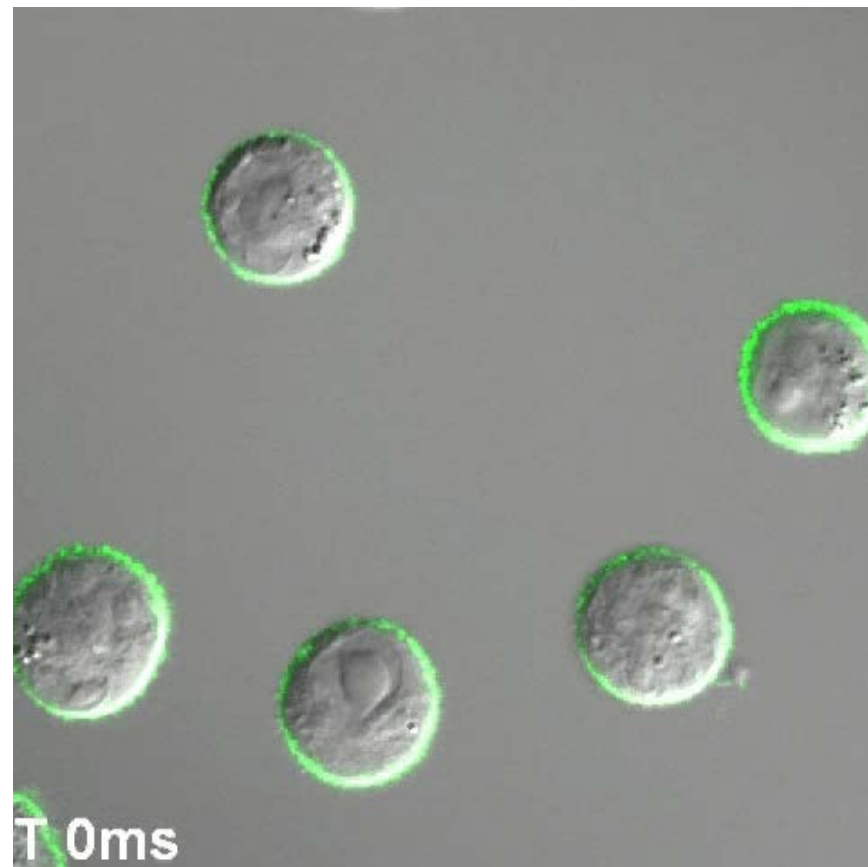


全新防焦点漂移系统 高速自动寻焦；连续补偿



ADCC

- Cetuximab: Alexa Fluor 647, 抗体药物 (red)
- Nature Killer cells: ZsGreen (green)
- Detection of dead cells: DAPI (blue)



CDC

Green: antibody drug, Red: PI

一体化整合的活细胞工作站



CO₂的湿度、温度控制

内层培养装置



活细胞培养装置

一体化整合的活细胞工作站

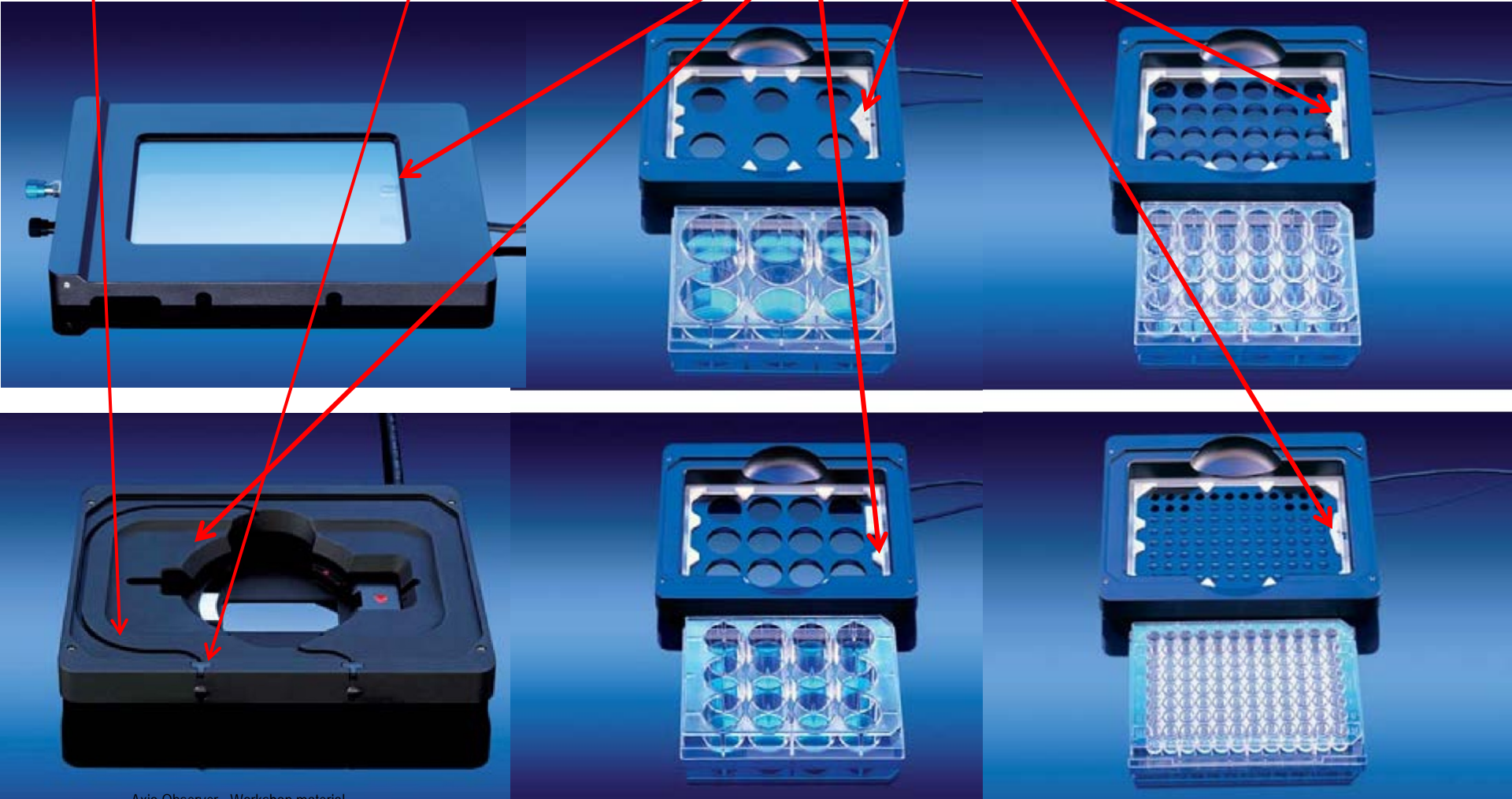


- 四通道温度精确控制，范围室温至 60° C，精度：0.01° C
- CO₂浓度设定范围：0-8%，精度：0.01

加药预热槽

管道夹口

Temperature Sensor



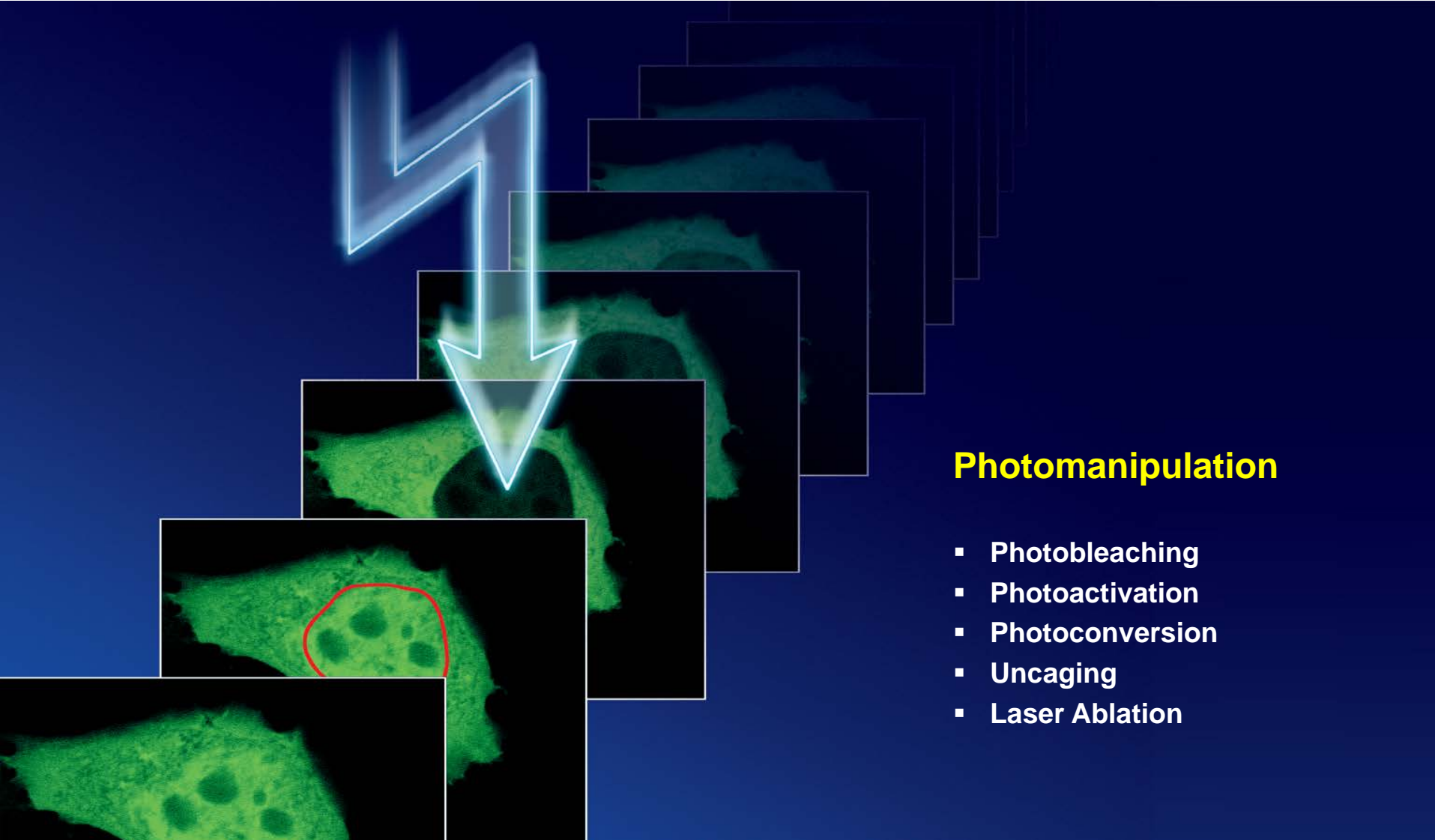
LSM 880

Data quantity analysis

- ***Linear scan system for precise quantity analysis***
- ***Physiology; FRAP; FRET; image analysis***

LSM 880 - Quantitative

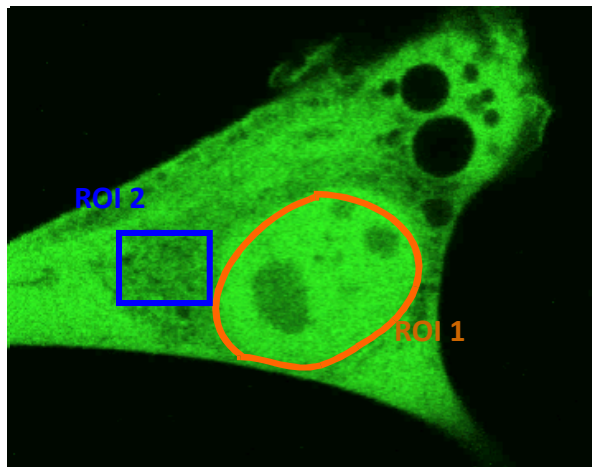
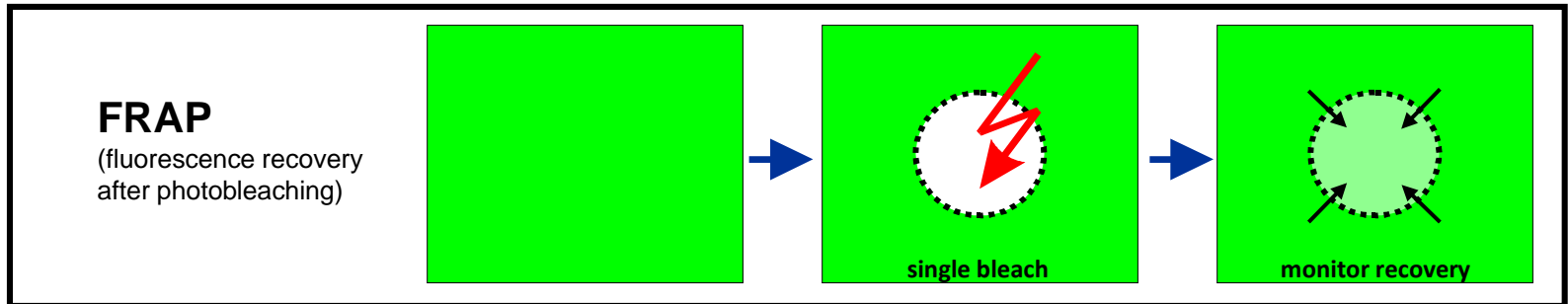
Understanding dynamic processes: photomanipulation



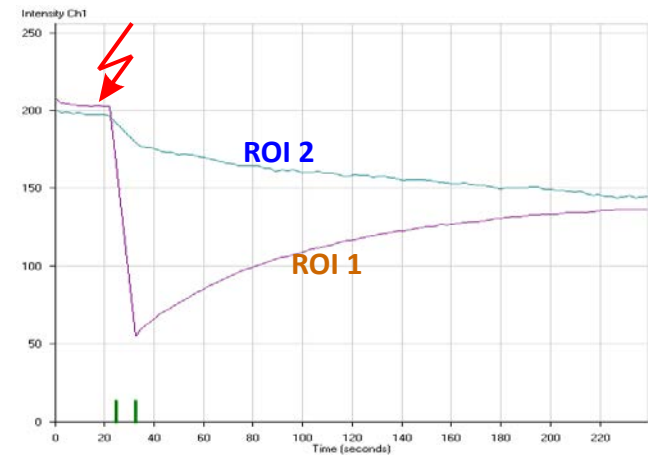
Photomanipulation

- Photobleaching
- Photoactivation
- Photoconversion
- Uncaging
- Laser Ablation

LSM 880 - Quantitative FRAP



online

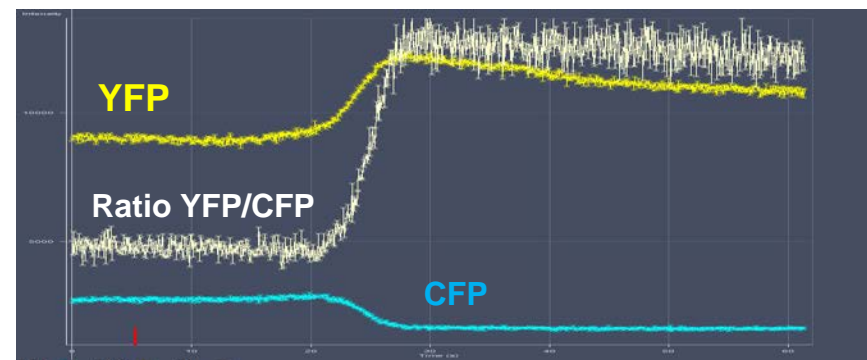
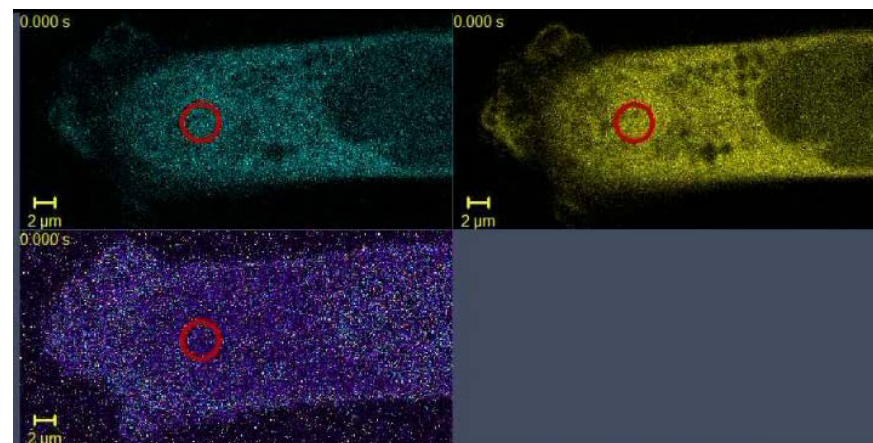
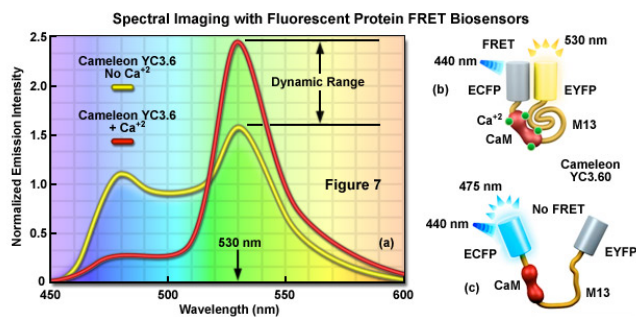


A. Sporbert, MDC Berlin, Germany

• Why will Fast Linear Scanning improve my imaging?

- **Fast Dynamics**

- New scanners on LSM 800 enable faster scanning of large FOVs at Nyquist sampling for highest temporal and spatial resolution
 - FRAP, Calcium Imaging, FRET etc
- Linear Scanning approach yields efficient use of scan time for higher frame rates



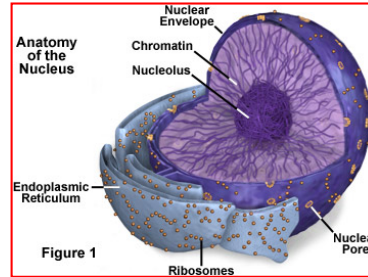
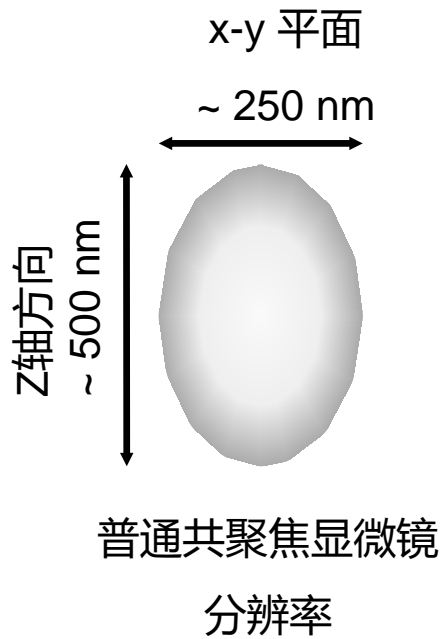
20 fps Calcium Imaging – Cameleon YC3.6 and Ionomycin

LSM 880 Airyscan

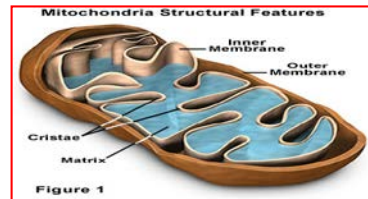


Superresolution – Seeing beyond diffraction limit

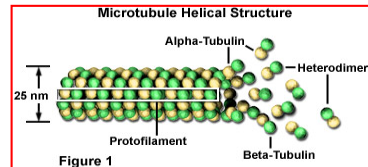
超高分辨率显微镜系统 — 超越光学分辨率极限



细胞 Confocal (200-250nm)



亚细胞结构 Airyscan
SR-SIM (50-150nm)
STED



分子水平 PALM/dSTORM (<20nm)

“阿贝魔咒”

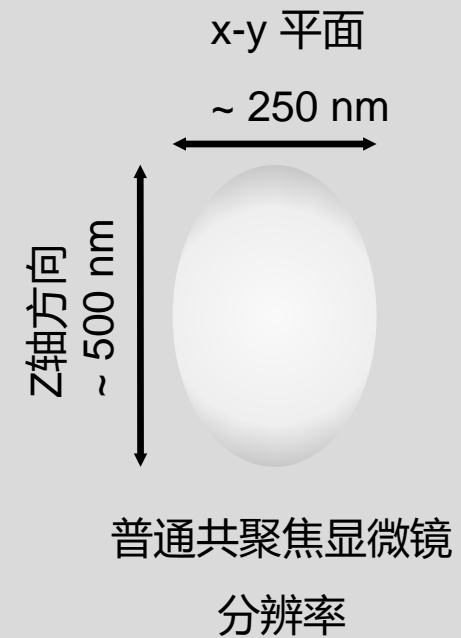
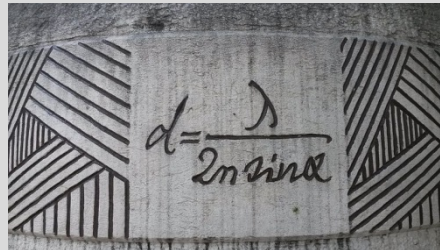


Ernst Karl Abbe

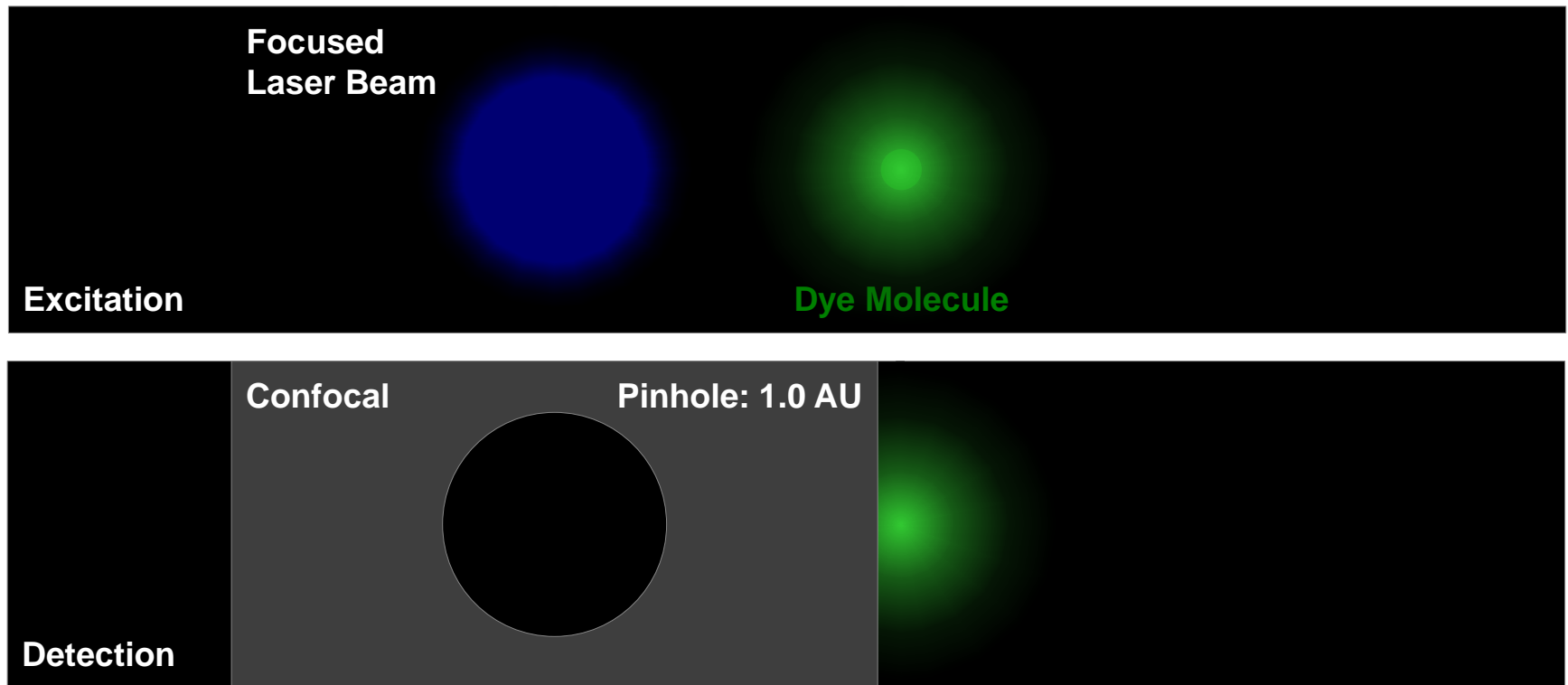
蔡司创始人之一
恩斯特 阿贝教授

光学衍射极限理论

1873



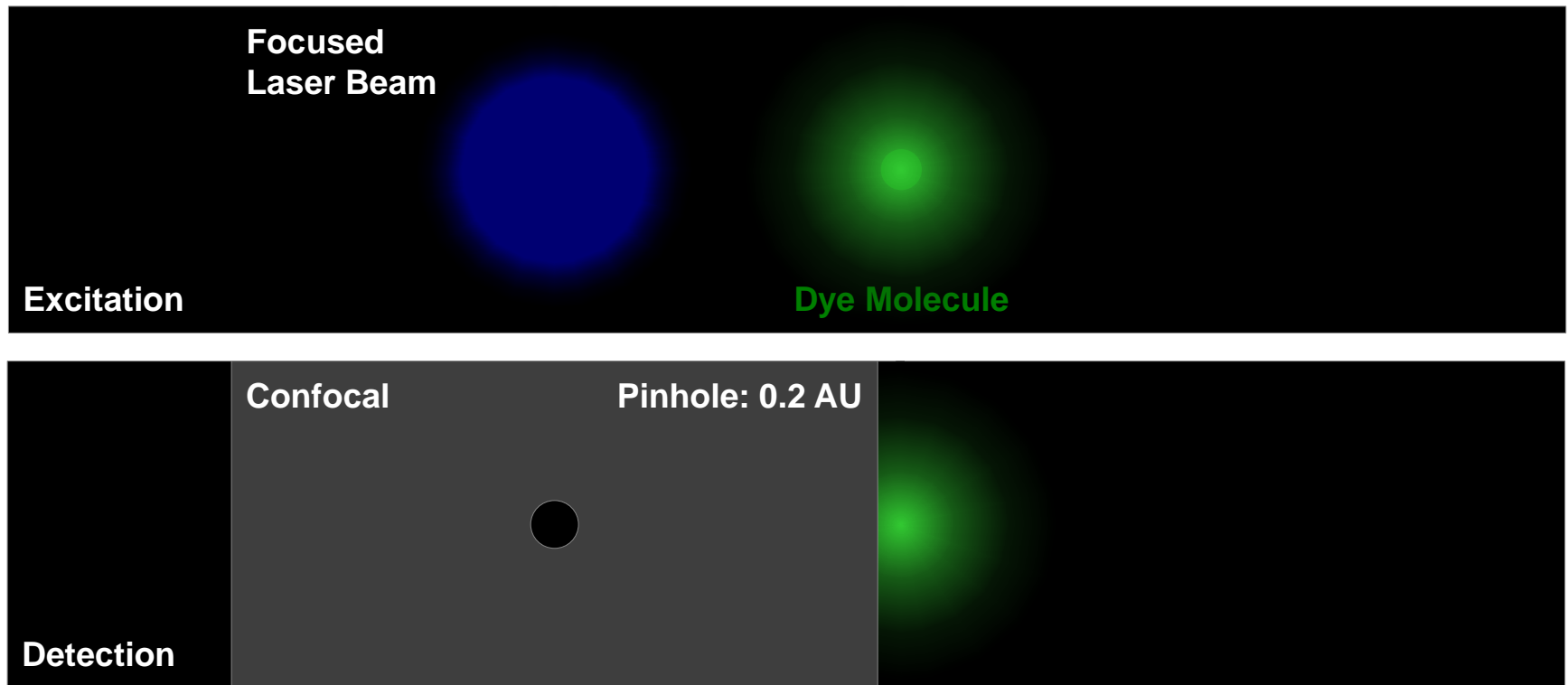
共焦针孔大小对成像效果的影响



When Pinhole = 1 A.U

- *Resolution: 250nm@XY*
- *S/N is good enough*

共焦针孔大小对成像效果的影响

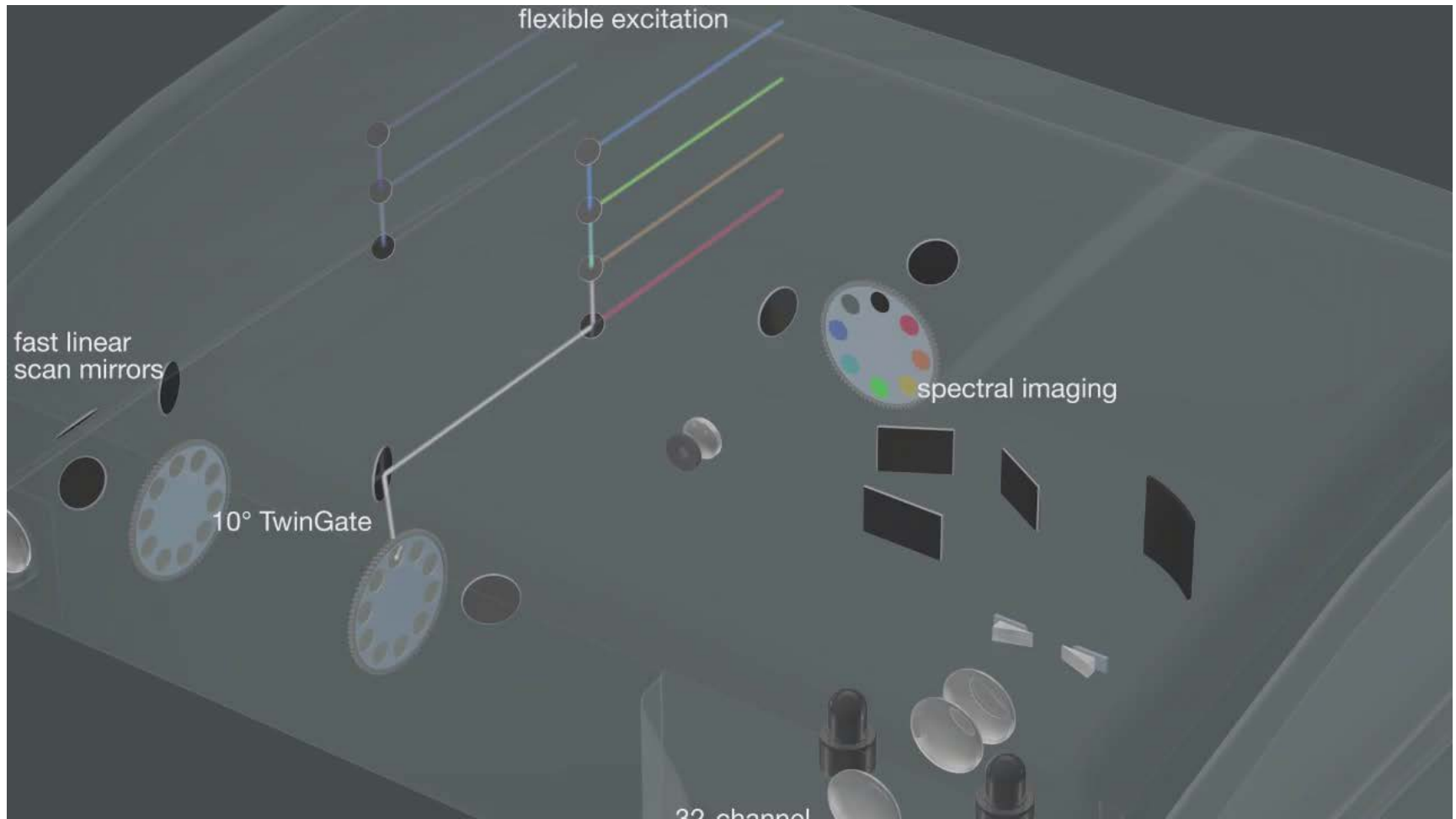


~~When Pinhole = 0.2 A.U~~

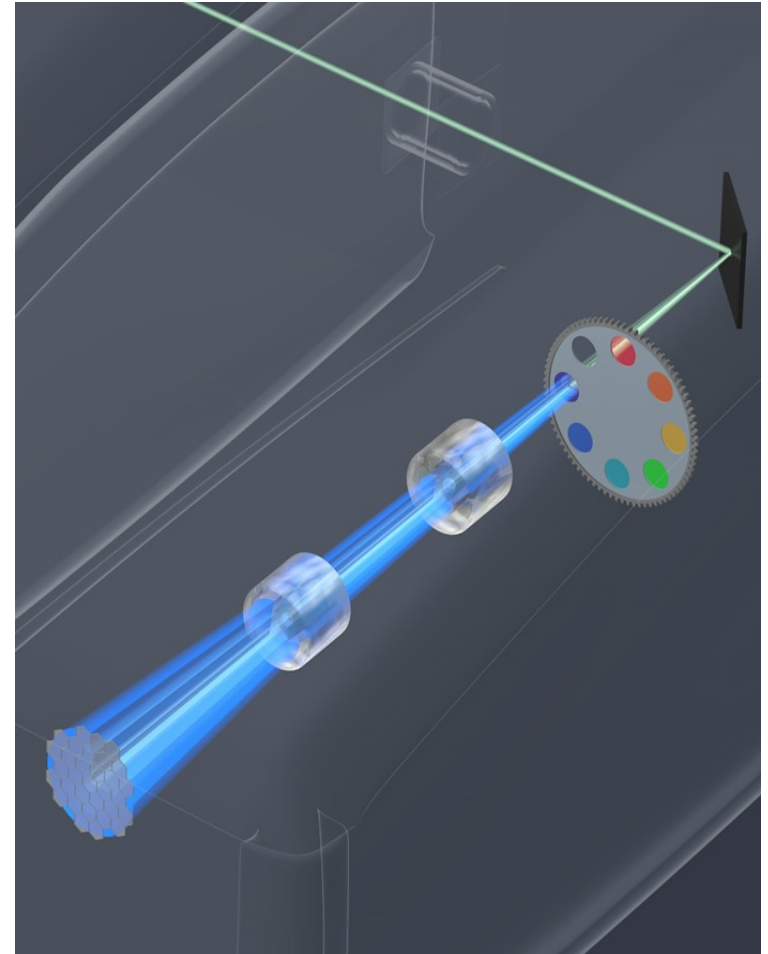
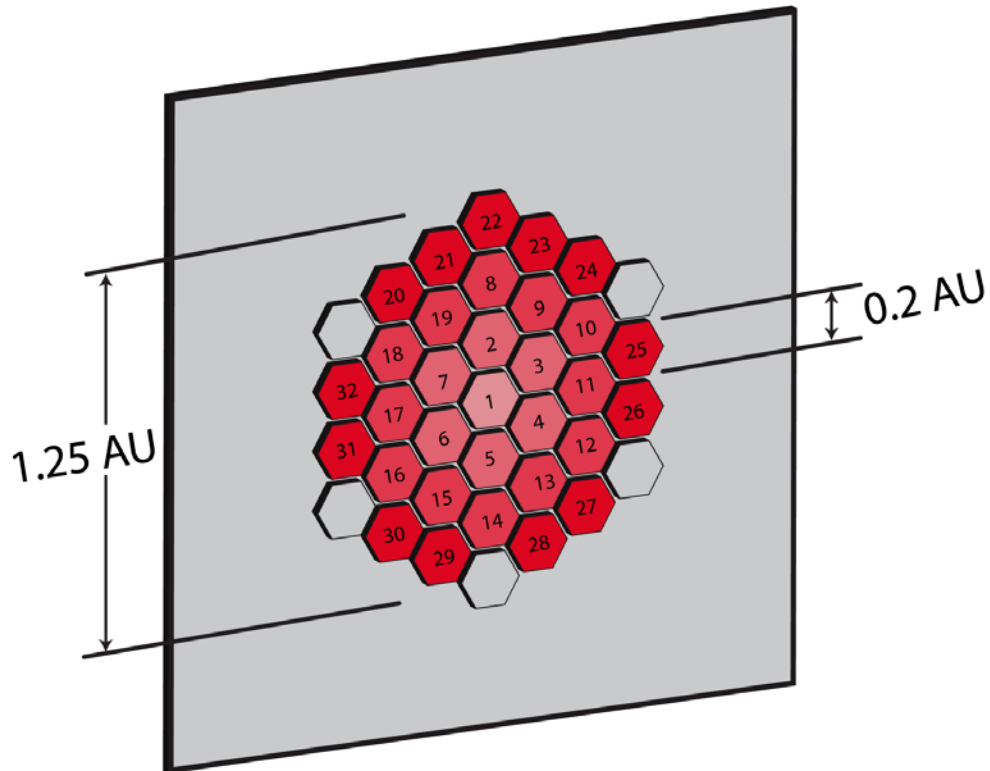
- ~~• Resolution: 140nm@XY~~
- ~~• S/N reduces 80% compare to 1A.U confocal~~

LSM 880 with Airyscan

Your New Standard for Fast and Gentle Confocal Imaging

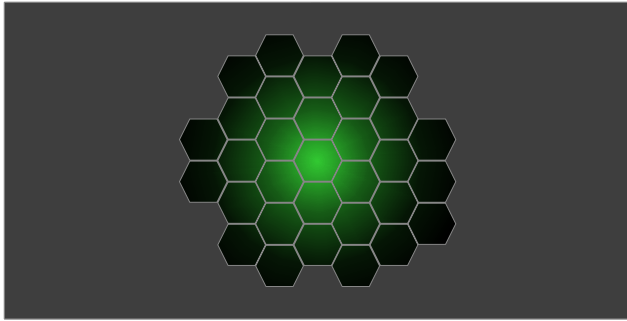


Airyscan将最高的光效率和小针孔的高分辨率完美的结合

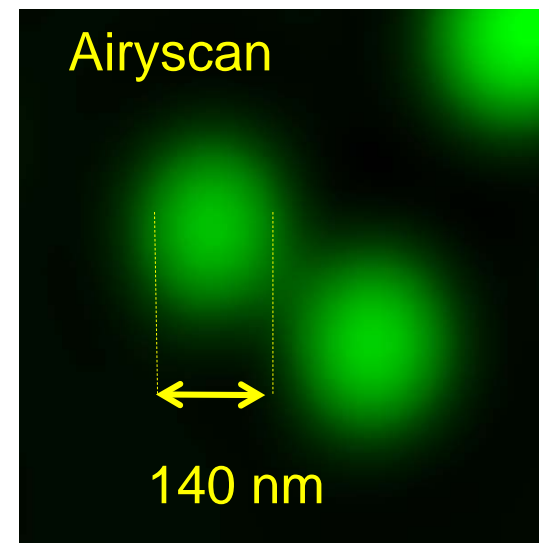
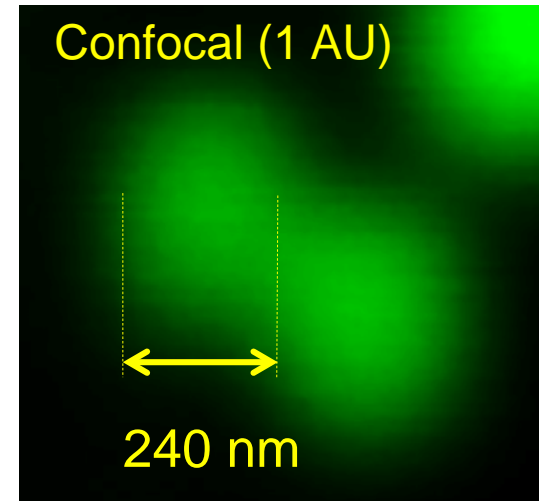


- 32 GaAsP-PMT “复眼” 阵列
- 每个元件直径0.2 AU
- 阵列收集几乎所有信号

Airyscan将最高的光效率和小针孔的高分辨率完美的结合

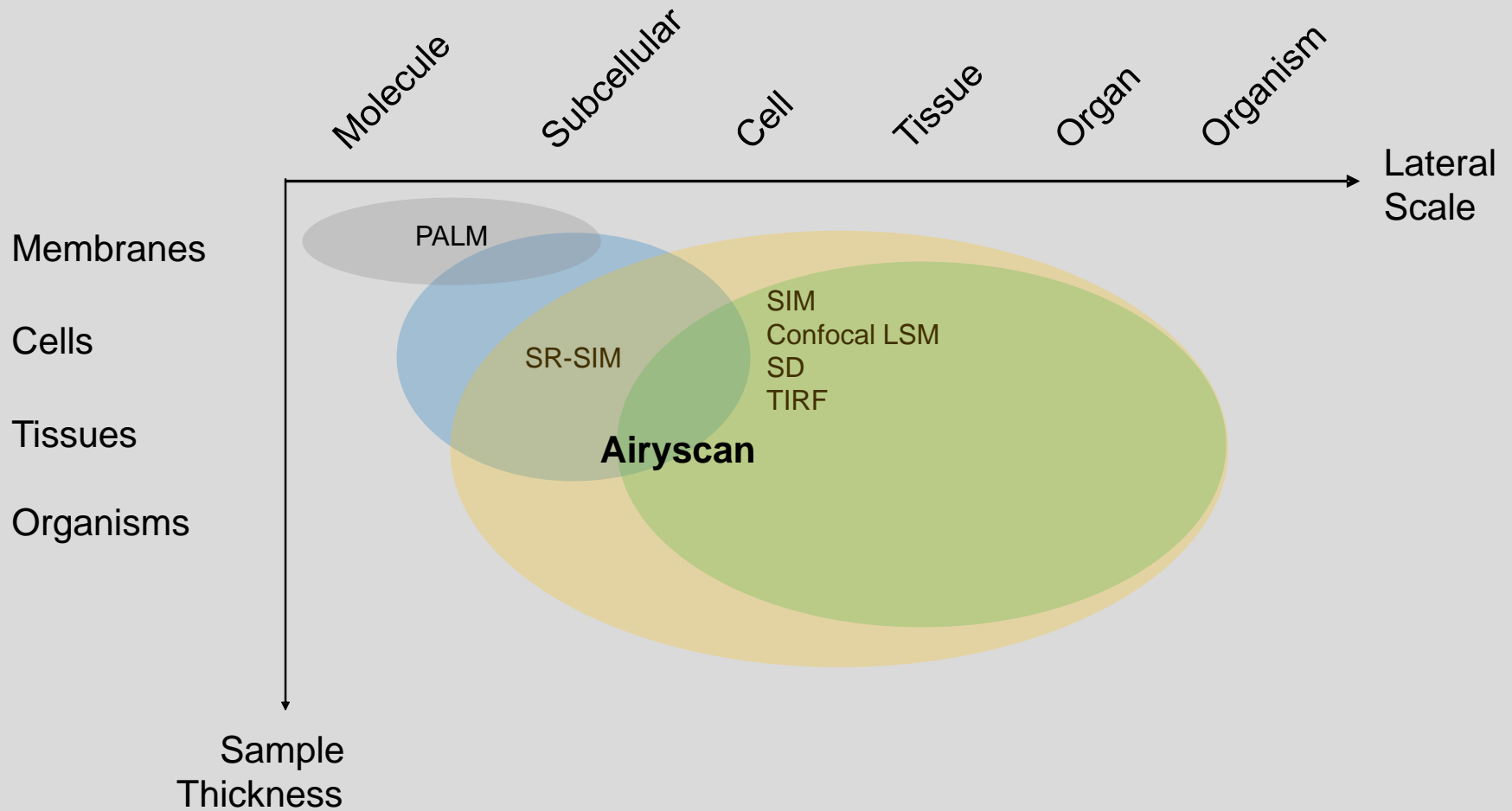


- 32 GaAsP-PMT “复眼” 阵列
- 每个元件直径0.2 AU
- 阵列收集几乎所有信号
- **同时提升分辨率与信噪比！**



样本与成像方式

拓展生物成像的界限



Airyscan的应用特点

- **高信噪比的图像质量，超越传统共聚焦分辨率极限**
- **没有任何样品限制，适用于所有共聚焦成像常用样品和荧光染料. (而样品限制是其他超高技术最大的问题)**
- **在厚样品中也能有非常好的成像效果；第一款能在双光子上实现超分辨率的产品**
- **可以超分辨率下进行活细胞高速运动的视频采集**
- **可以在超分辨率模式下实现光谱扫描成像和线性拆分 (LSM880 + Airyscan)**
- **线性数据处理模式，数据准确可靠，可以用于对比实验和定量分析.**

更高的分辨率，更好的灵敏度及更快的成像速度！

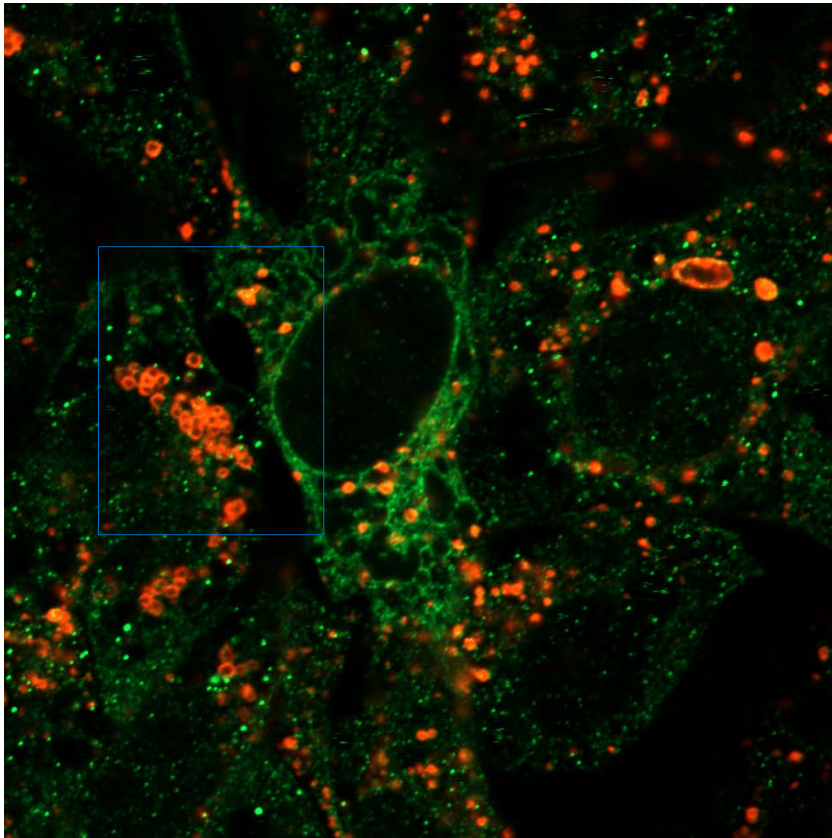
Sample Name: 小鼠上皮细胞

Plan-Apo 63x Oil Objective, Airyscan

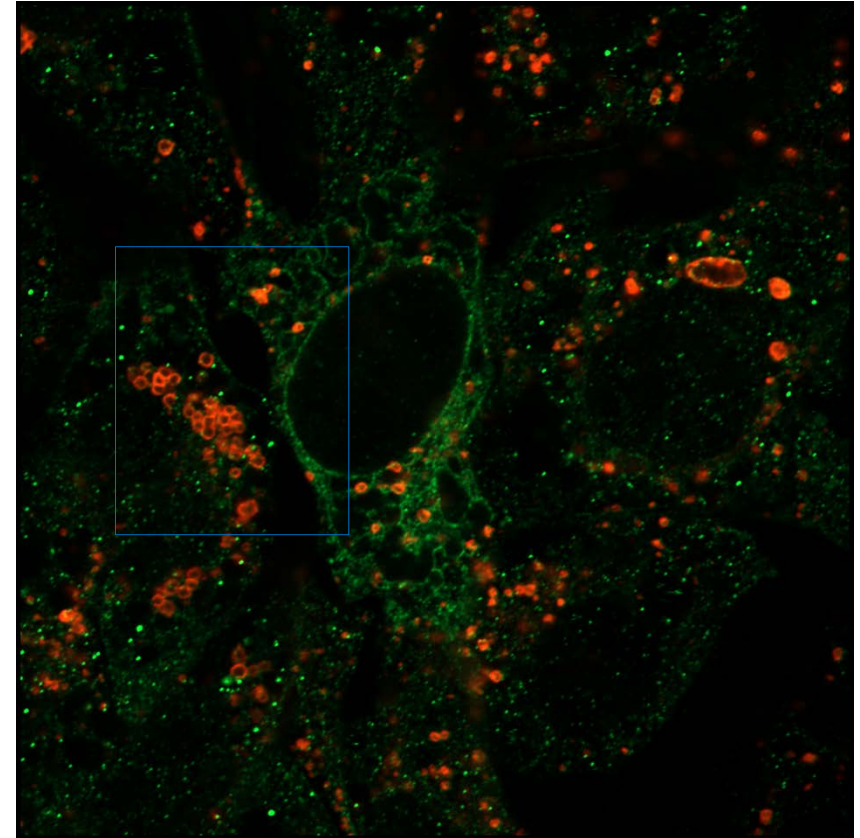


图像：红色溶酶体：AF568
绿色：AF488

红色溶酶体的结构在Airyscan拍摄下无论信噪比和分辨率都更胜一筹

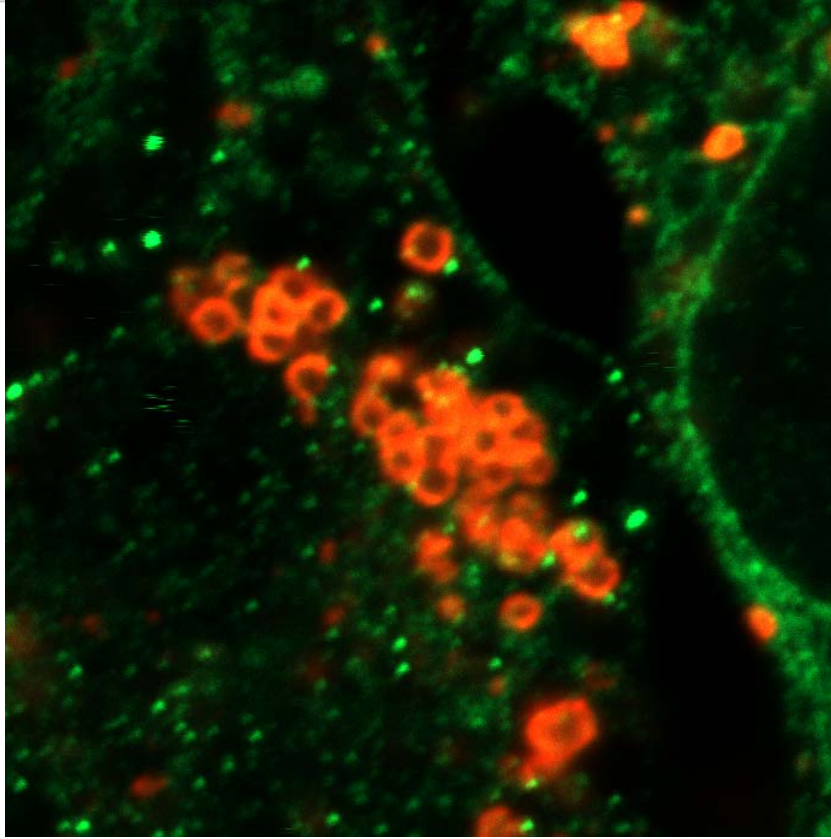


63X Confocal

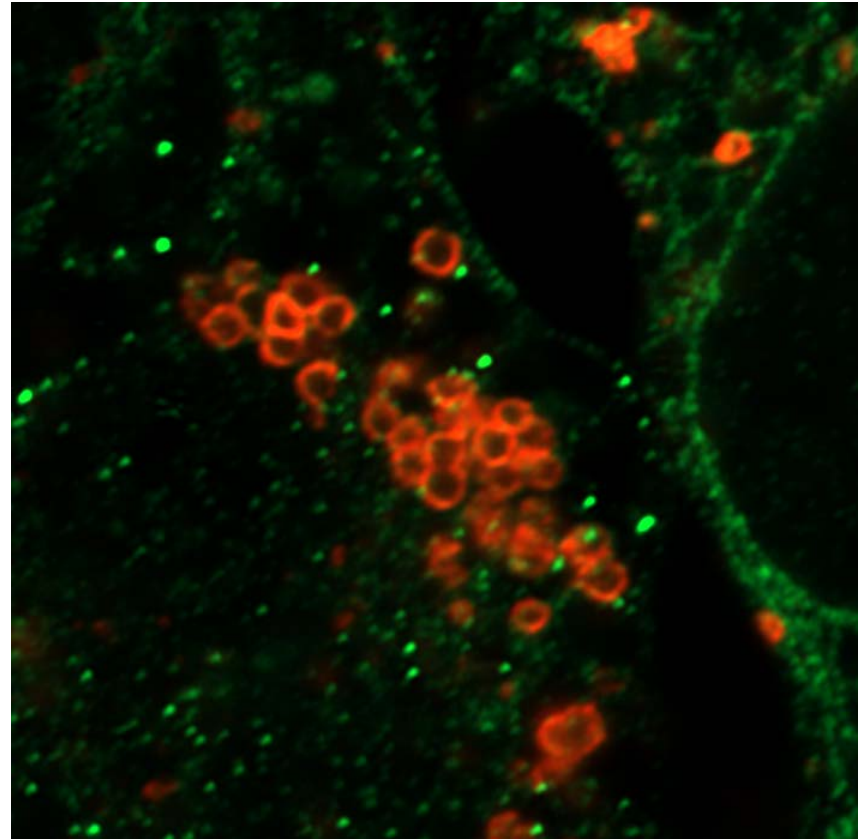


63X Airyscan

对相同感兴趣区域进行放大后的效果



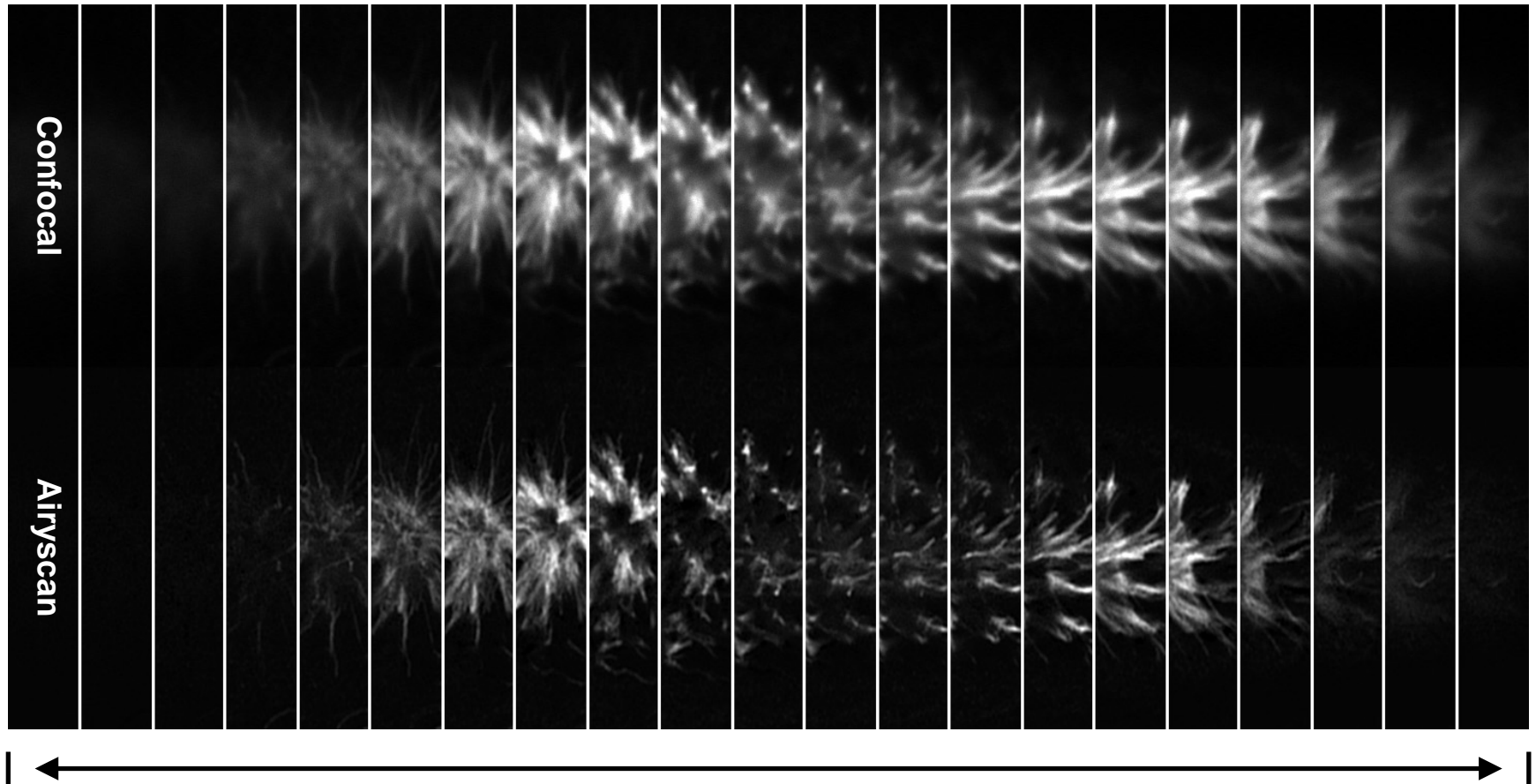
Confocal



Airyscan

Seeing beyond diffraction limit: Application Case IV

超越光学分辨率极限：肿瘤细胞 微管蛋白

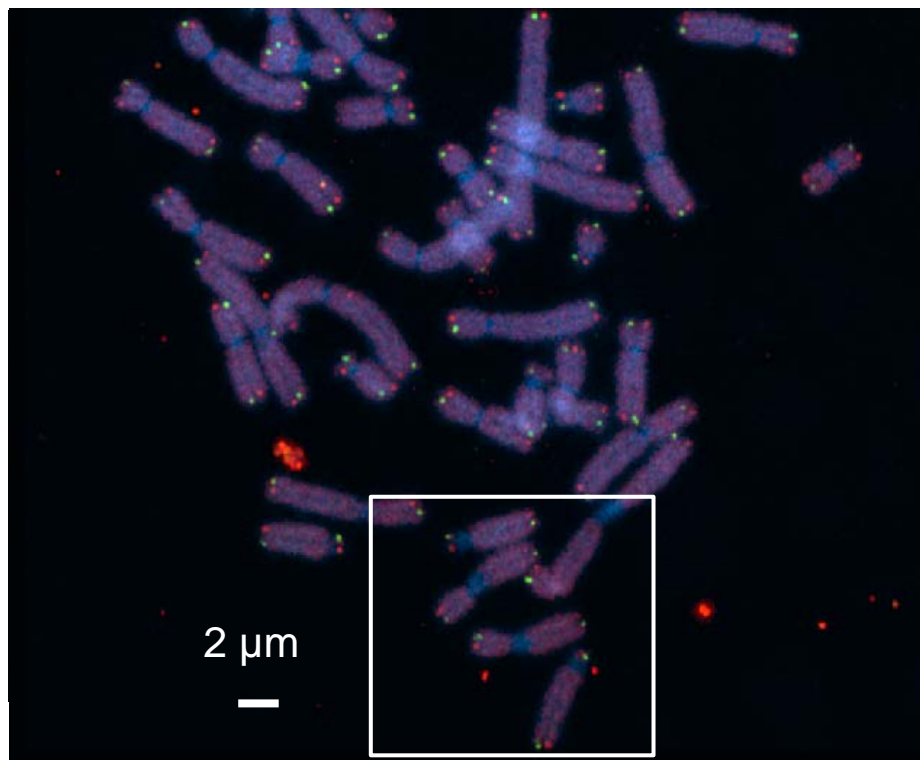
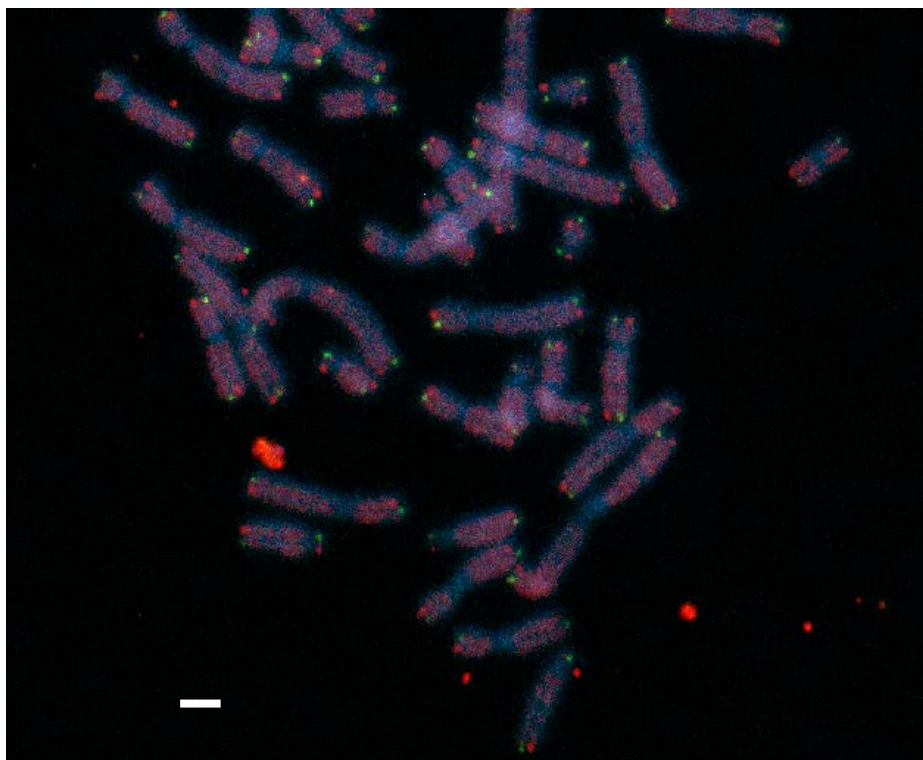


Z-stack: 3.98 μm ; increment: 200 nm (Plan-Apochromat 63x/1.4 Oil)

Cultivated mitotic cells stained for tubulin

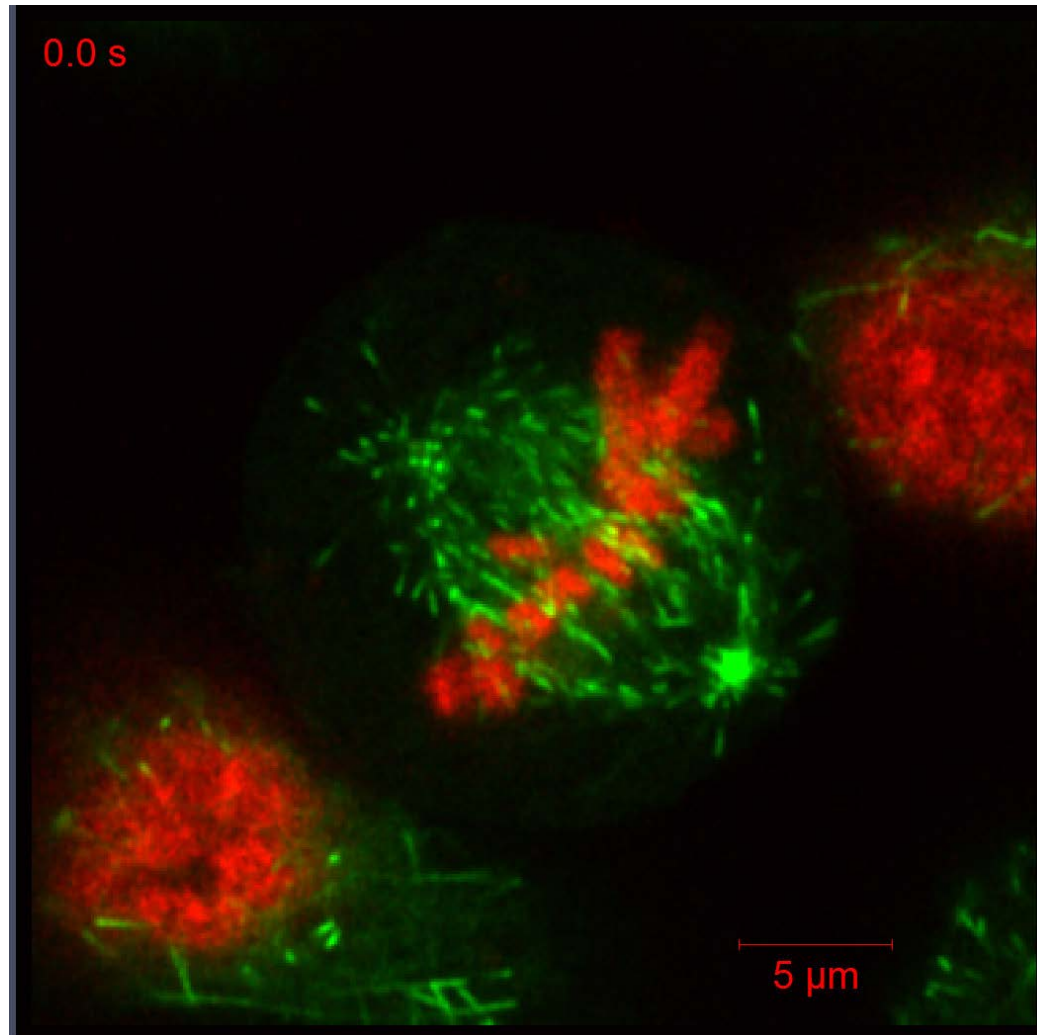
High signal to noise ratio: Application Case I

高信噪比：IMR90 人肺成纤维细胞 端粒



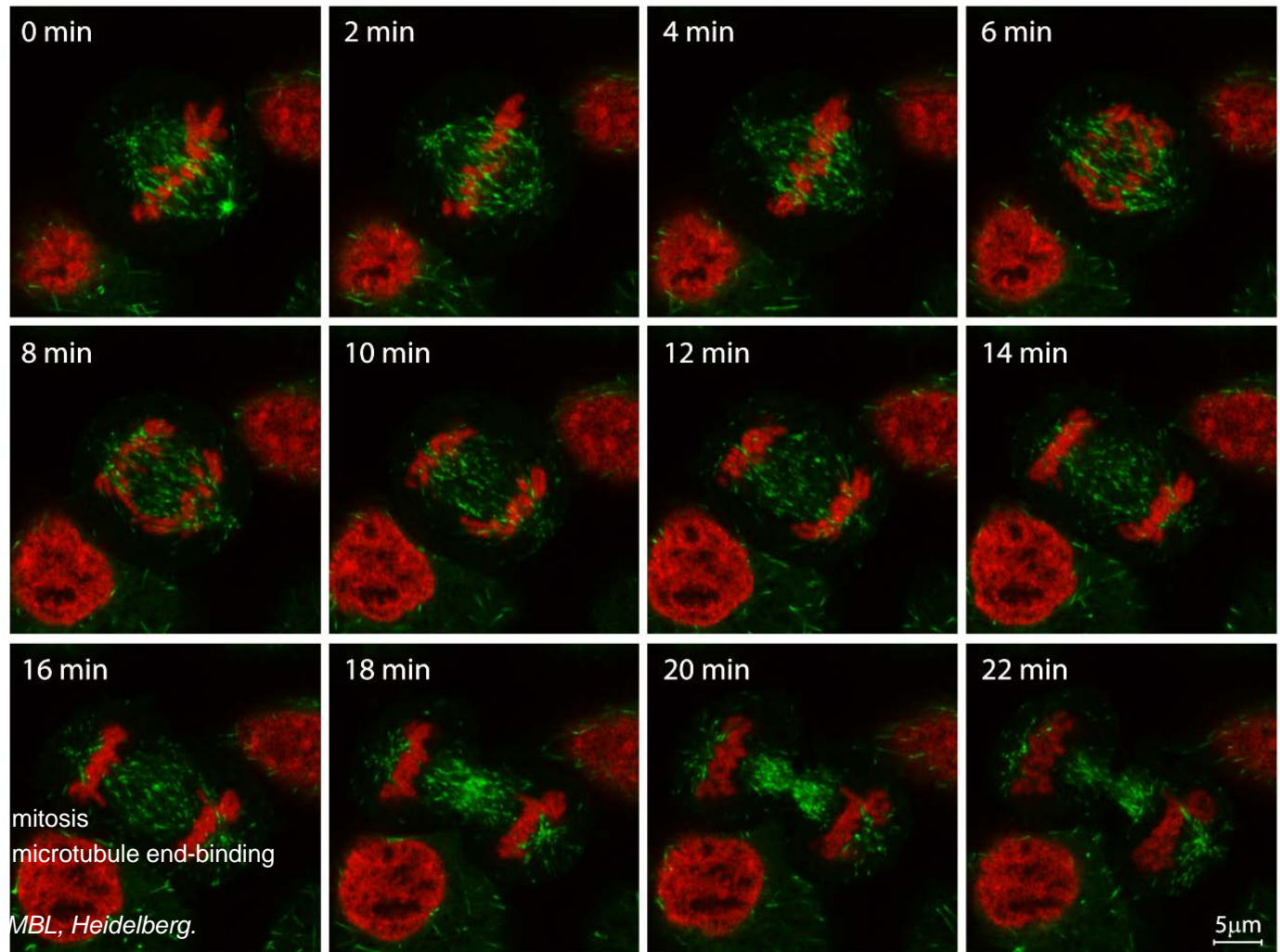
Airyscan for Live cell imaging: Application Case III

超高分辨率活细胞成像 HeLa-Kyoto细胞系 有丝分裂



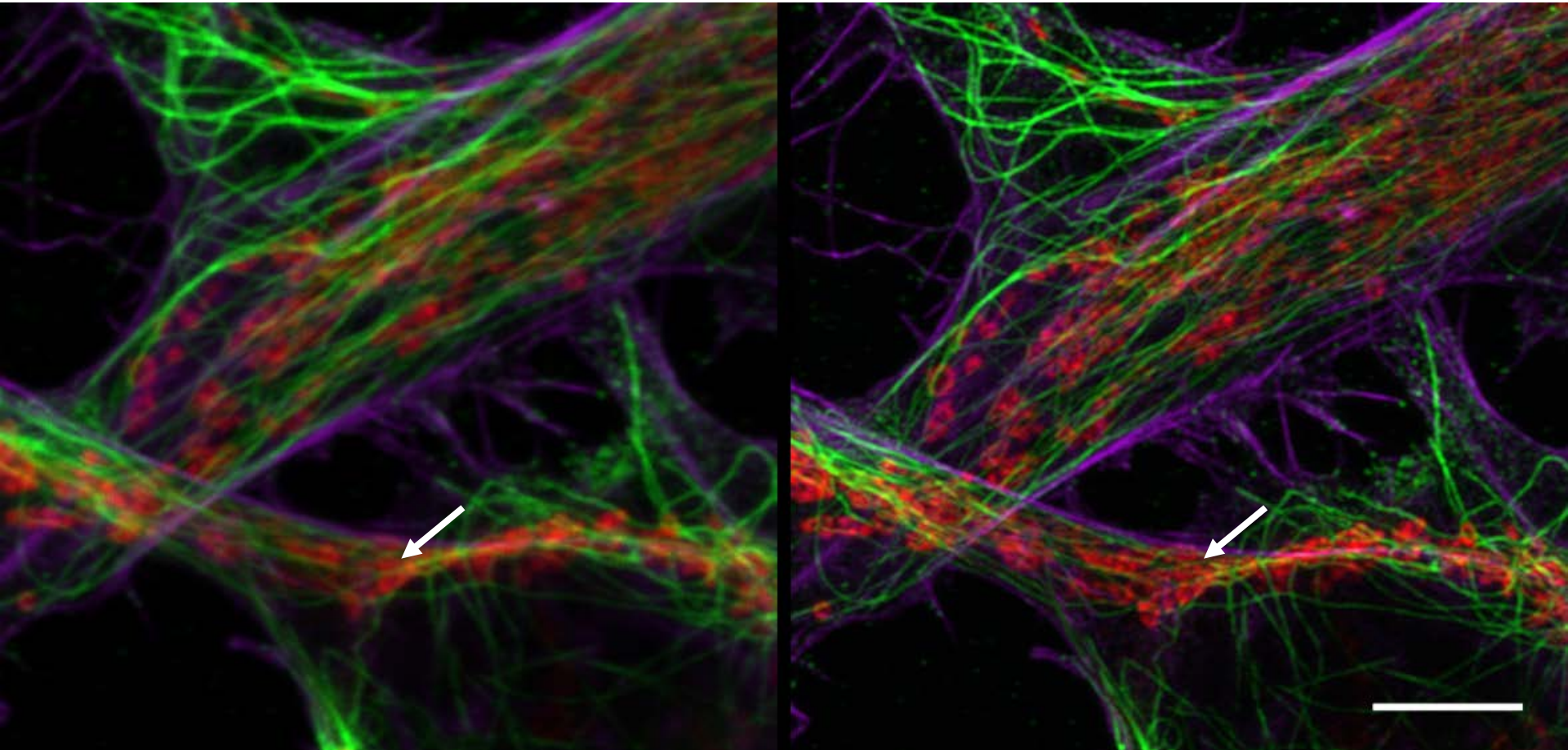
Airyscan for Live cell imaging: Application Case III

超高分辨率活细胞成像 HeLa-Kyoto细胞系 有丝分裂



Airyscan Cell Biology Application Case

细胞生物学 : Hela 细胞



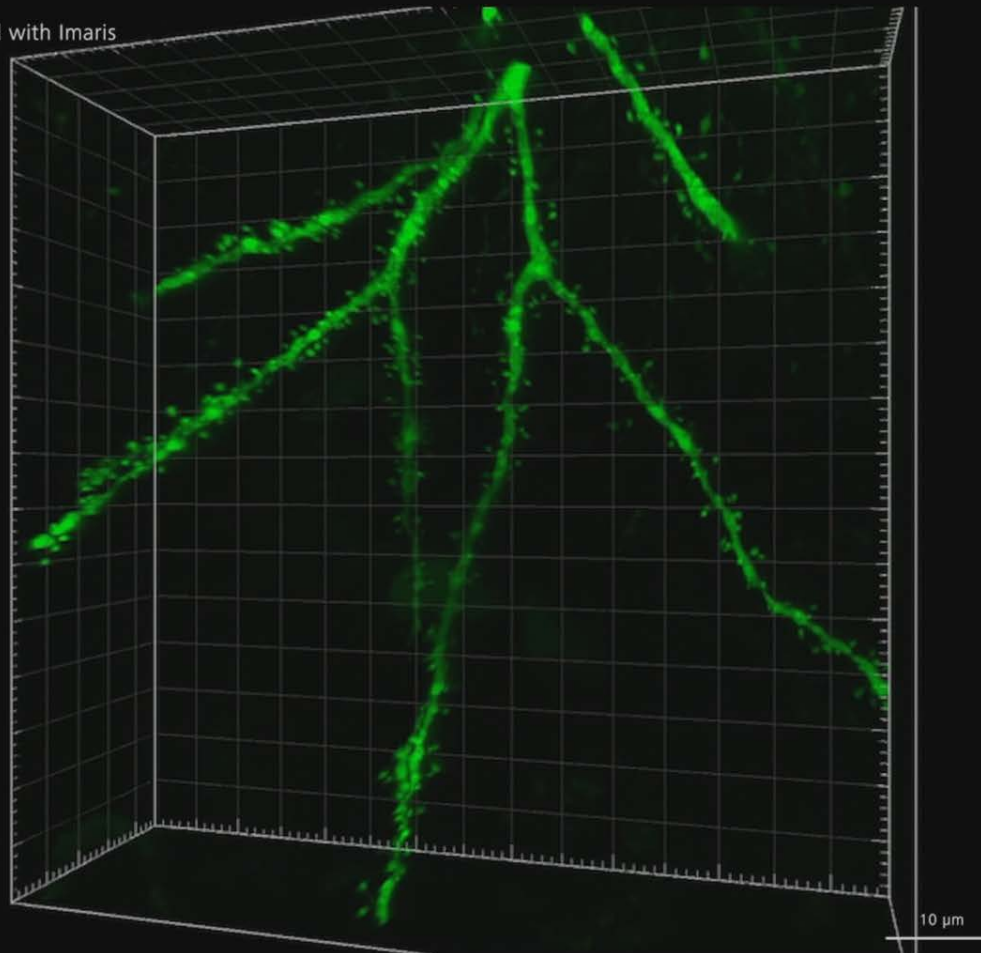
Airyscan Neuroscience Application Case

神经生物学 Parietal Cortex 顶叶皮层



Rendered with Imaris

Movie



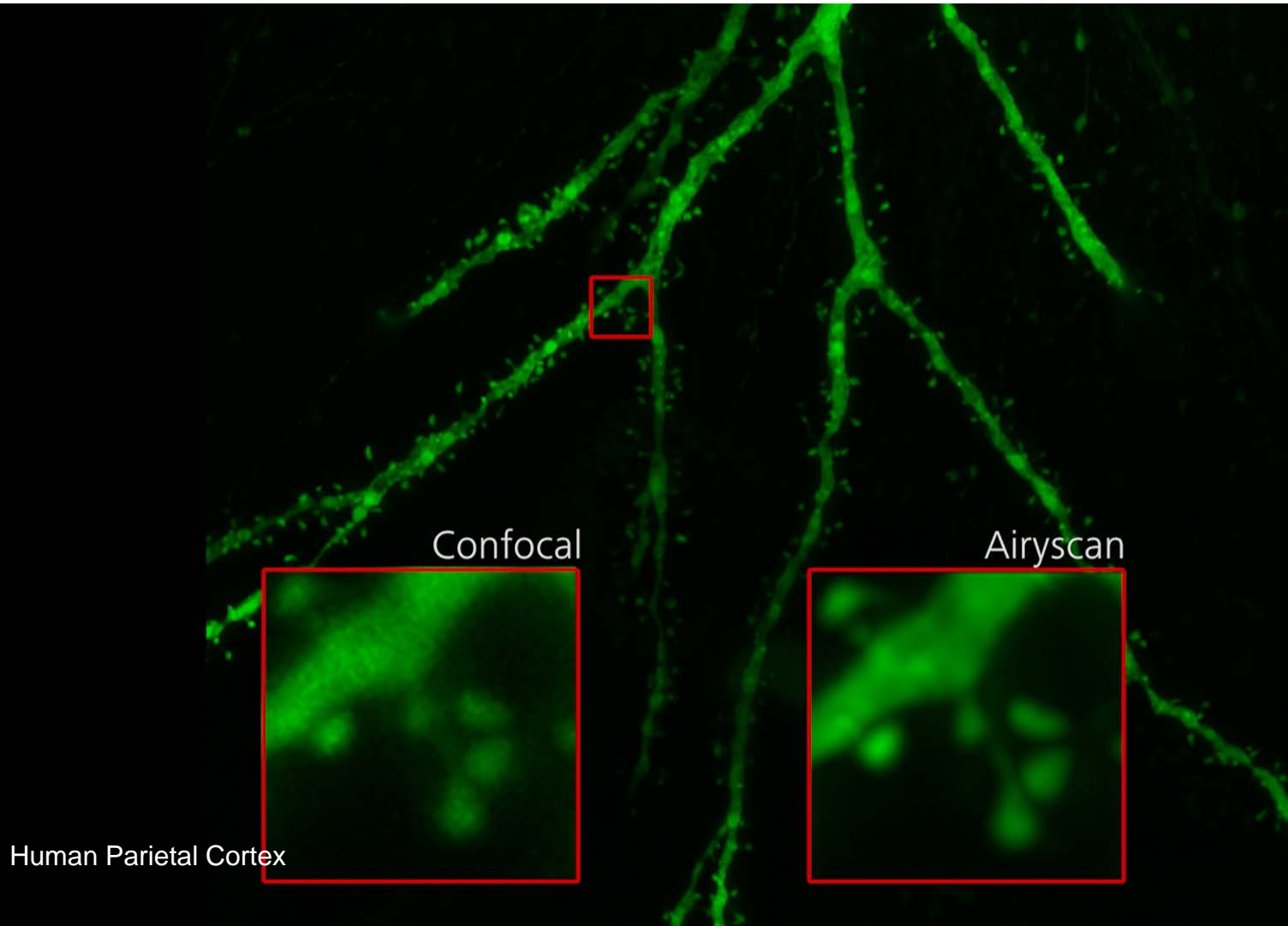
Human Parietal Cortex

Airyscan Neuroscience Application Case

神经生物学 : Parietal Cortex 顶叶皮层



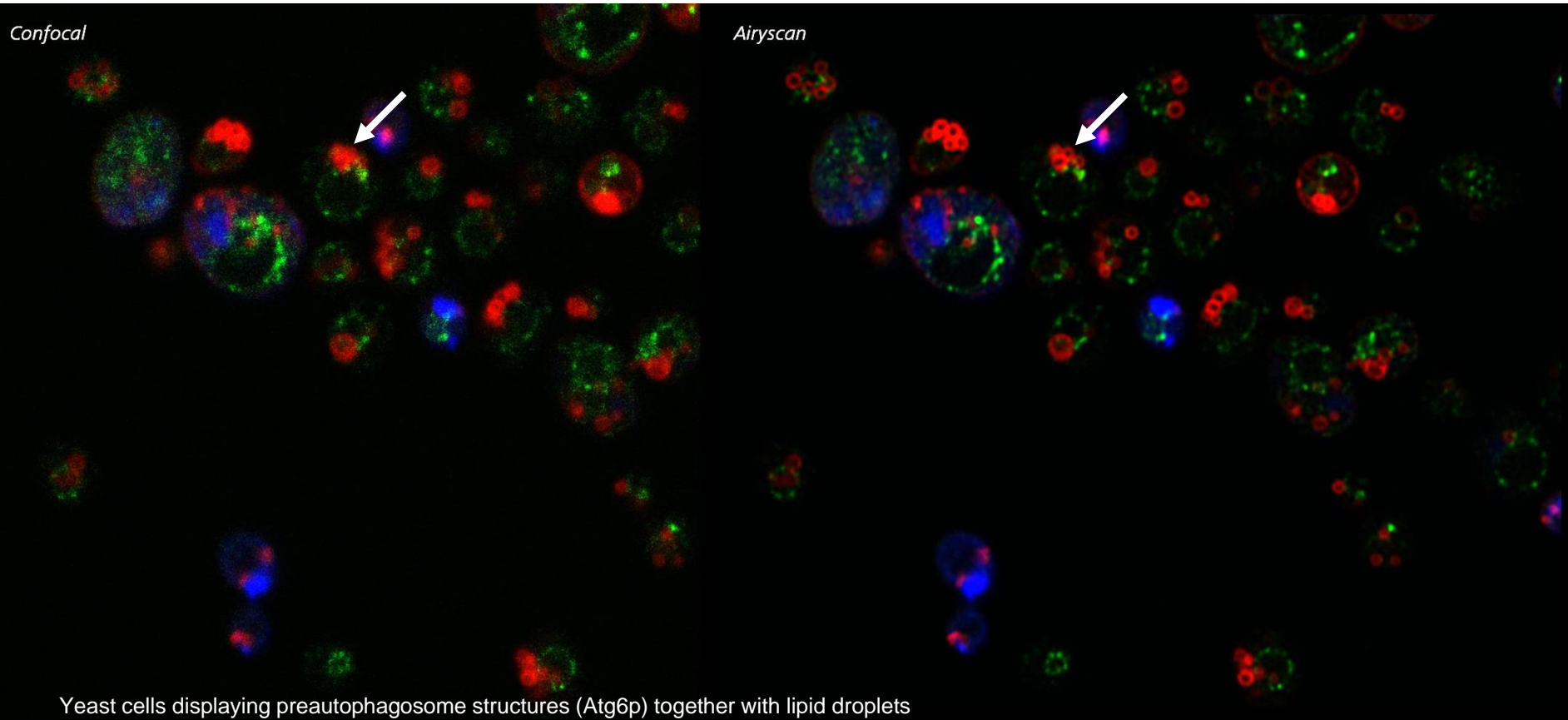
Movie



Human Parietal Cortex

Microbiology – Application Case

微生物学：酵母细胞



Yeast cells displaying preautophagosome structures (Atg6p) together with lipid droplets

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Thank you for your attention!



We make it visible.