

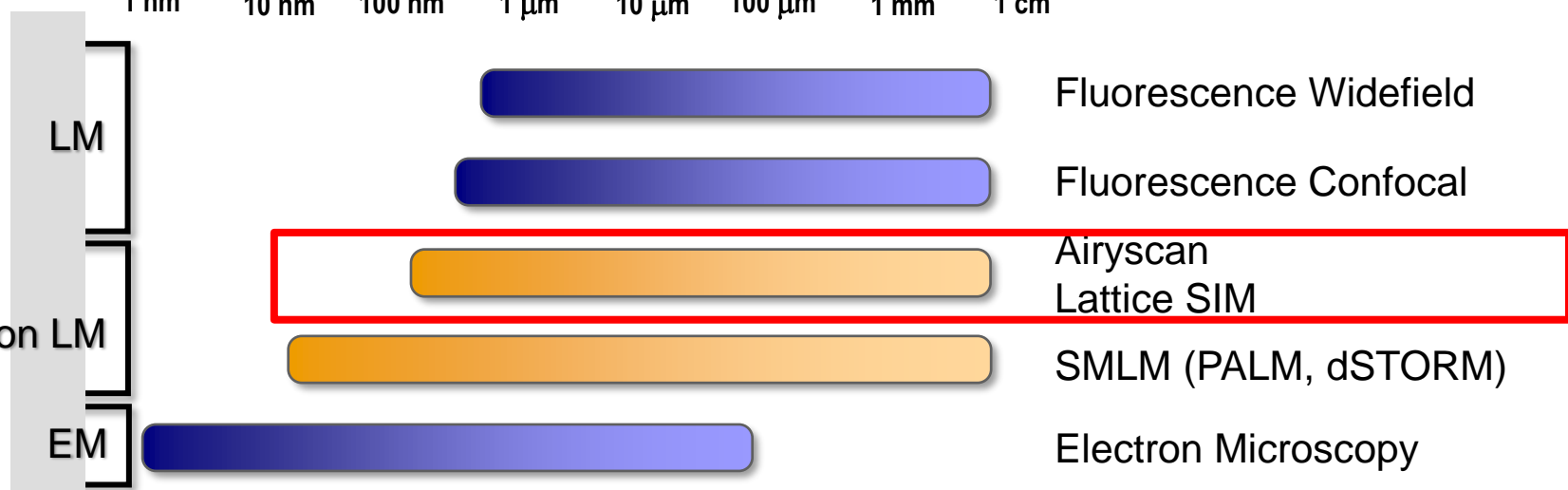
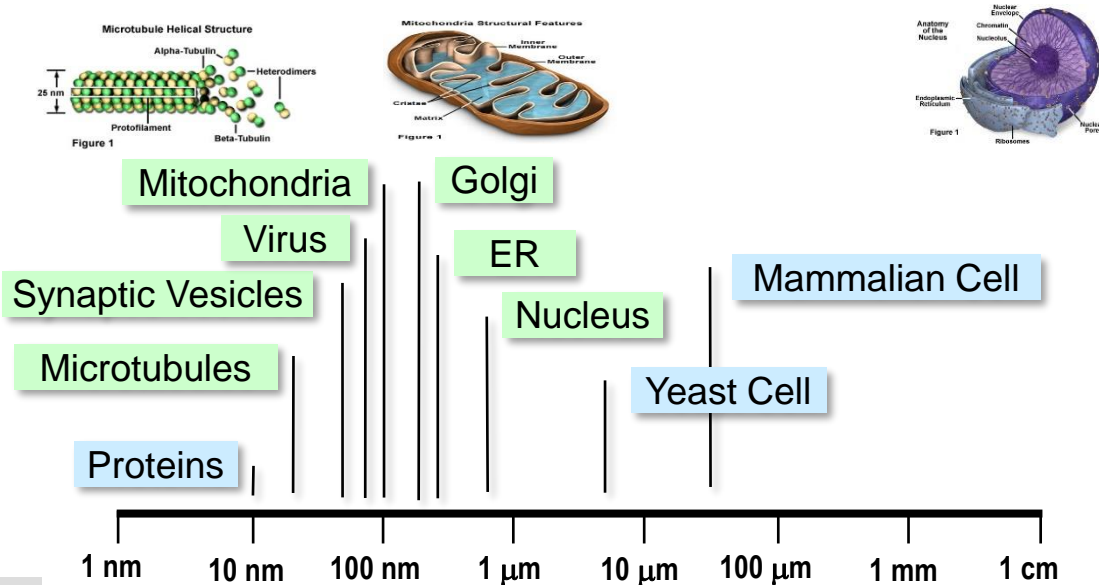
# ZEISS LSM 980 with Airyscan Revolutionize Your Confocal Imaging



Chris Power  
3D Imaging Specialist - UK  
Carl Zeiss Microscopy GmbH

# LSM 880 – Airyscan

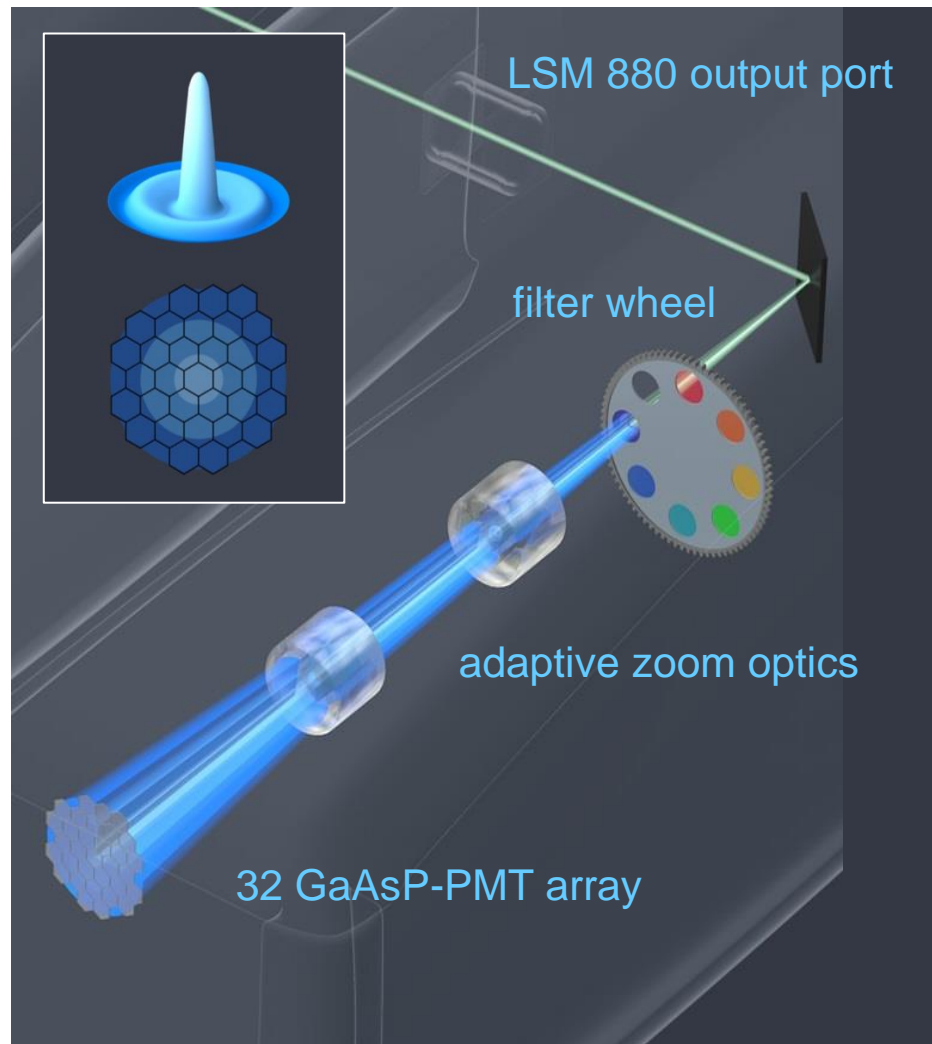
## Samples and Imaging Modalities



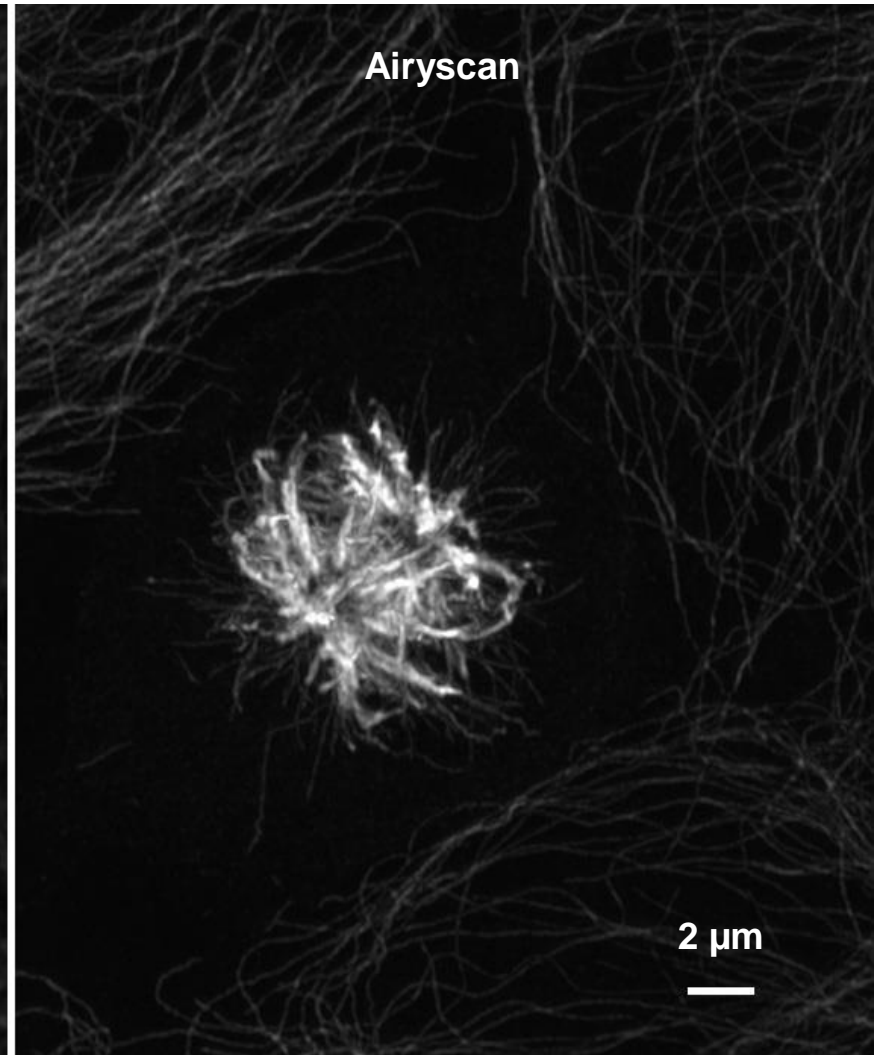
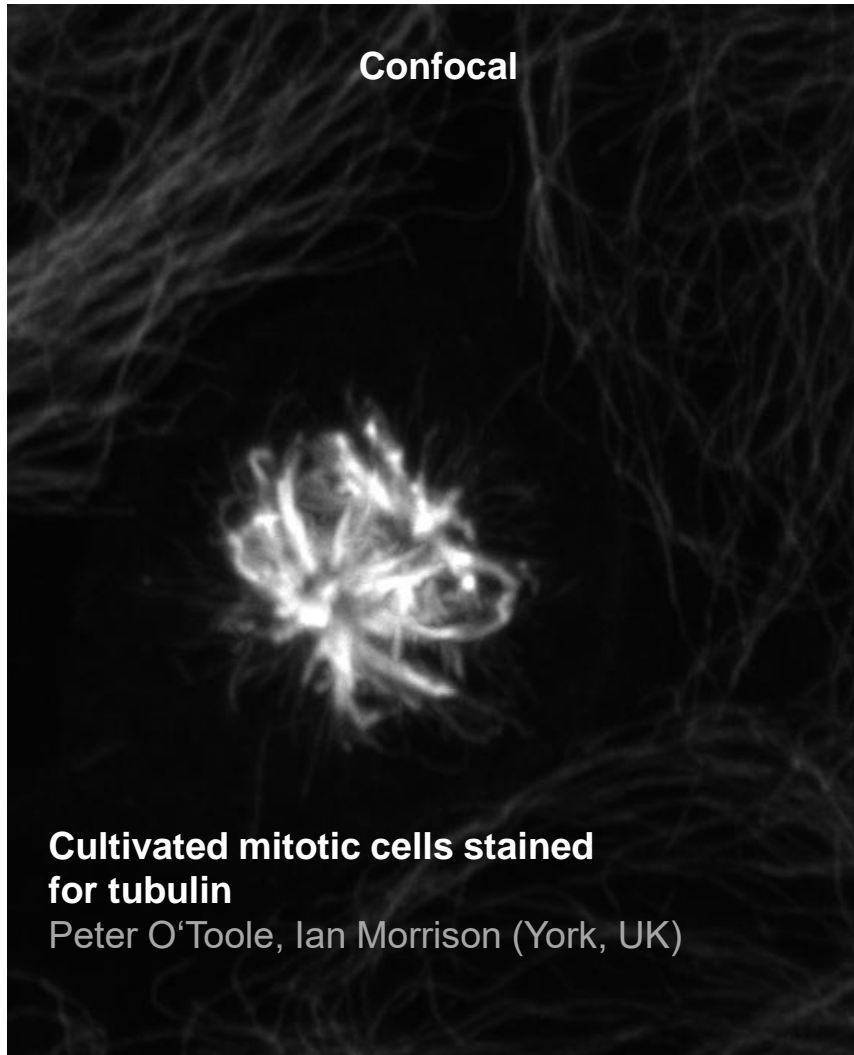
Modified after Fernández-Suárez and Ting, Nature Rev. Mol. Cell Biol. 9, 929 (2008)

# LSM 880 – Airyscan Detection

*Zoom optics and a unique 32 GaAsP-PMT design*



# AiryScan reveals more details in your samples by increasing the resolution of LSM up to 1.7-fold



# Airyscan reveals more details in your samples by increasing the resolution of LSM up to 1.7-fold



Confocal

Airyscan

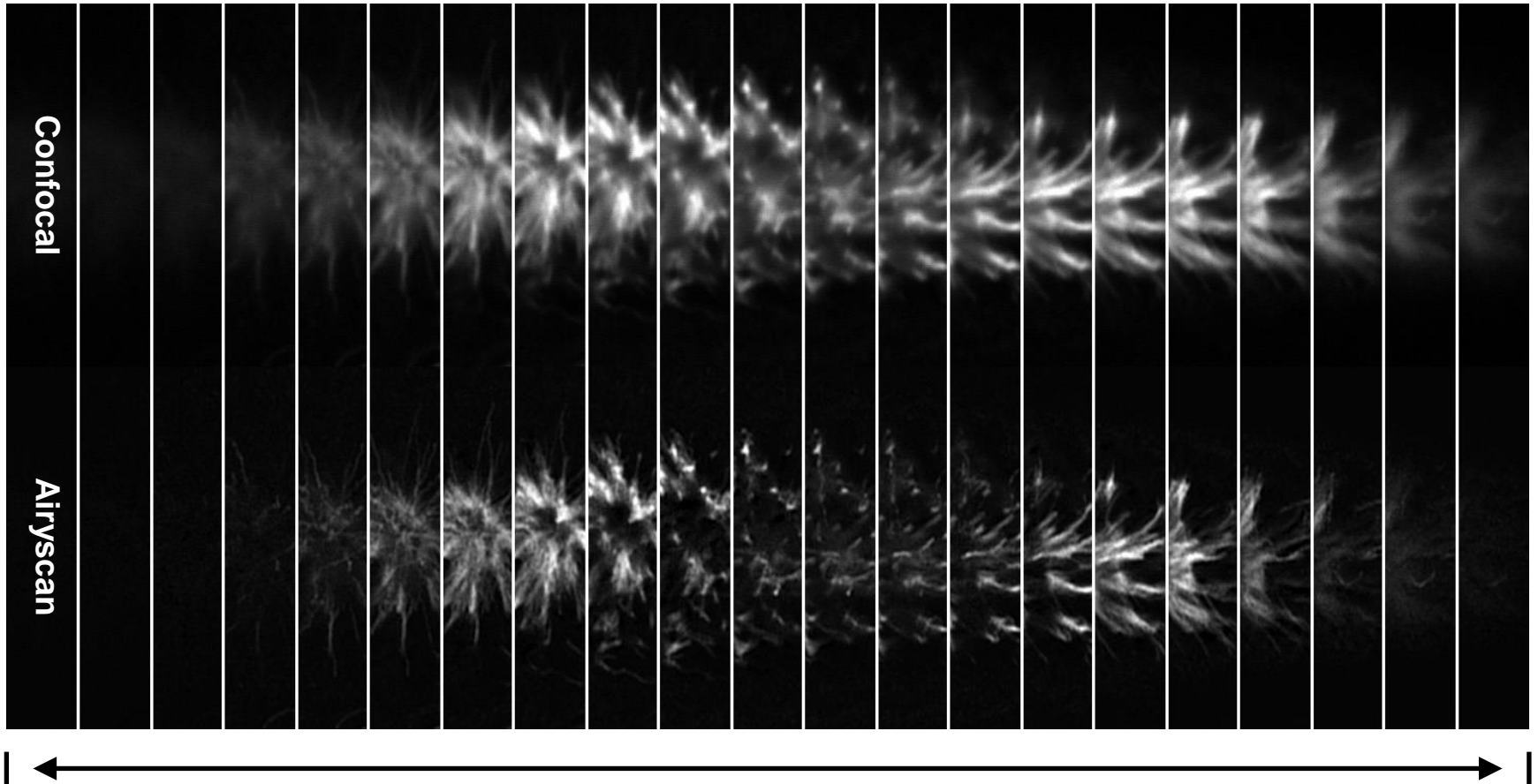
**Cultivated mitotic cells stained  
for tubulin**

Peter O'Toole, Ian Morrison (Univ. of York, UK)

2  $\mu$ m



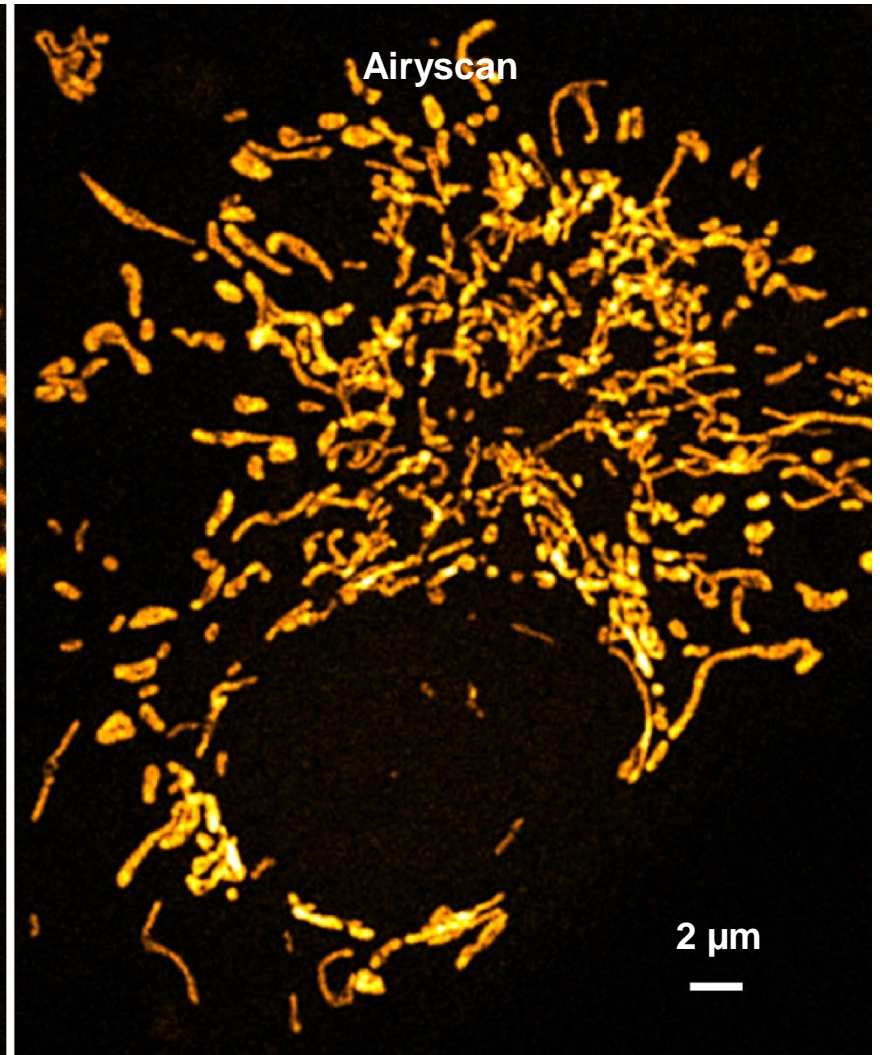
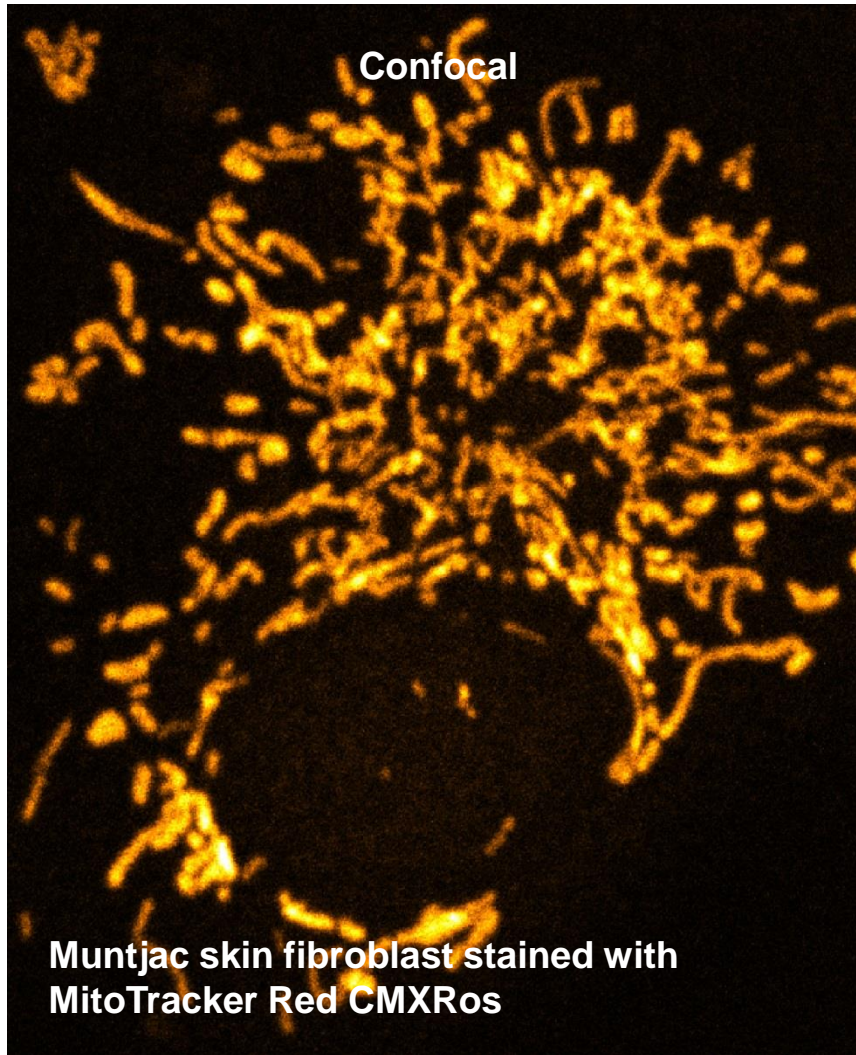
# Airyscan improves not only the lateral but also the axial resolution by the same factor



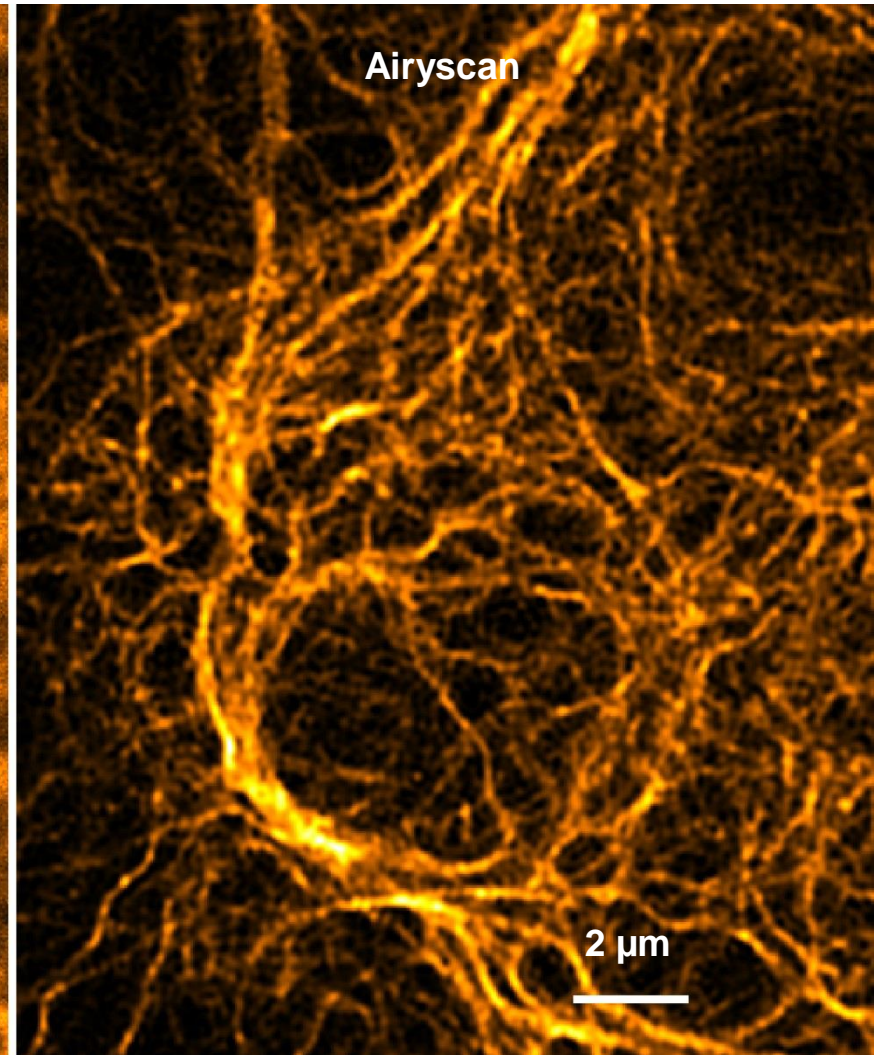
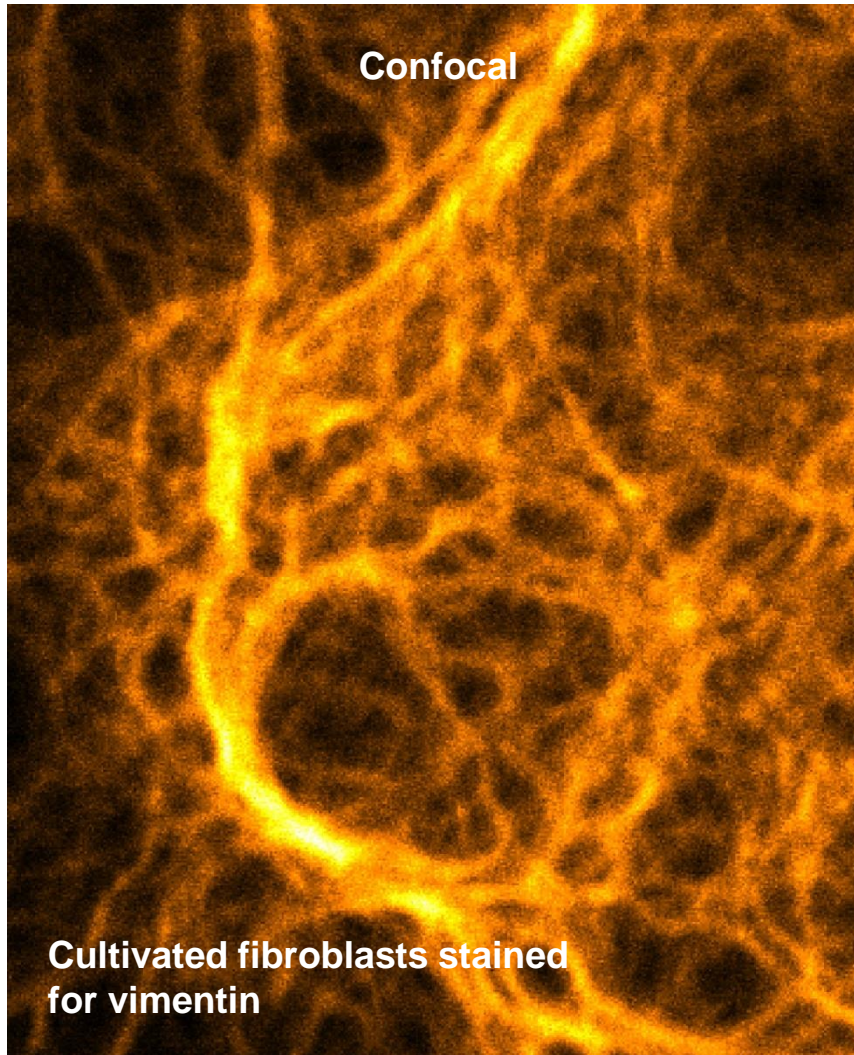
Z-stack: 3.98  $\mu\text{m}$ ; increment: 200 nm (Plan-Apochromat 63x/1.4 Oil)

Cultivated mitotic cells stained for tubulin

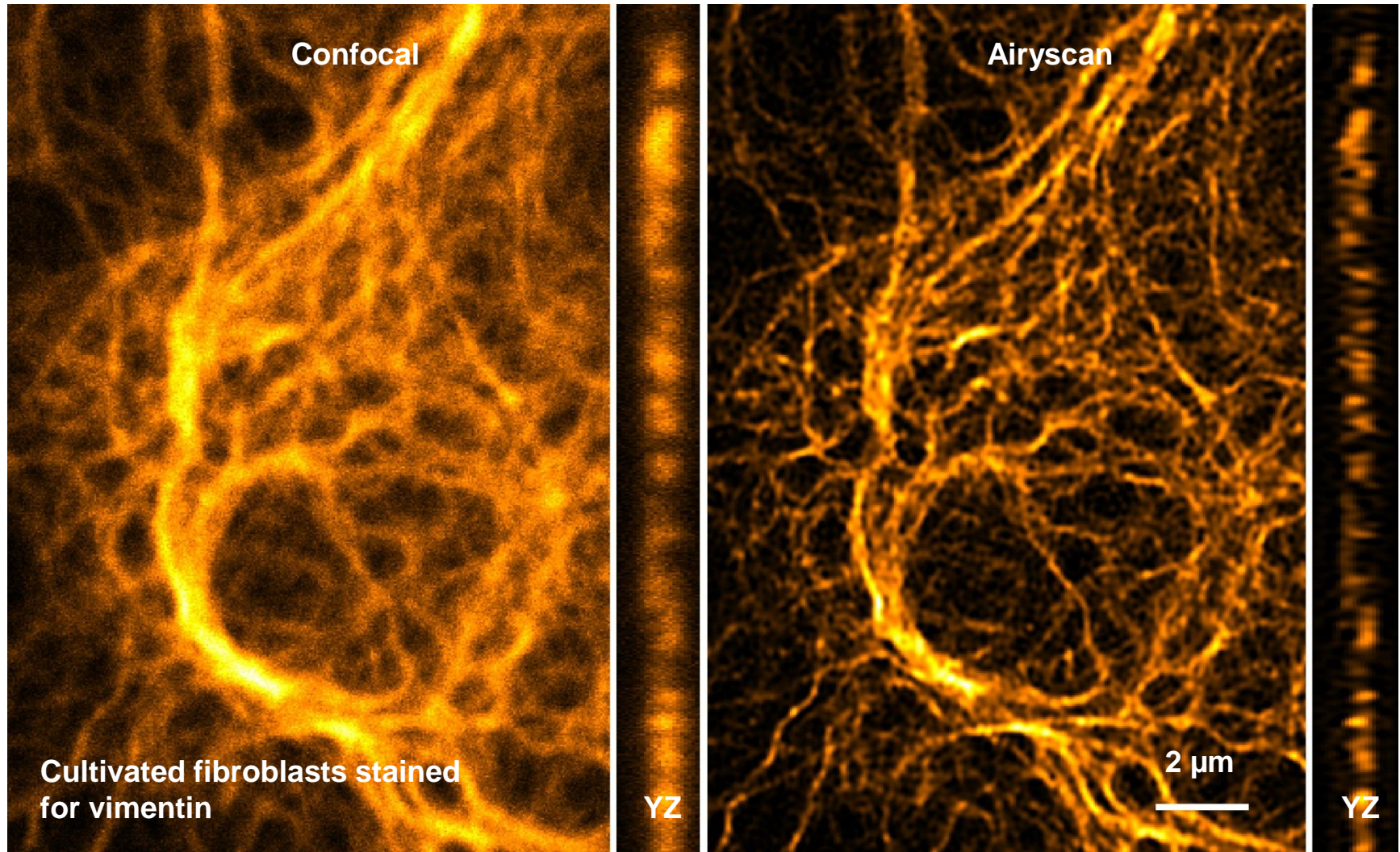
# Airyscan reveals more details in your samples by increasing the resolution of LSM up to 1.7-fold



# Airyscan reveals more details in your samples by increasing the resolution of LSM up to 1.7-fold



# Airyscan improves not only the lateral but also the axial resolution by the same factor



# Scanning confocal and the 1 Airy unit pinhole “limit”.

*At  $PH < 1$  A.u. signal loss is larger than gain in resolution.*



The potential to increase resolution by closing the pinhole (PH) is not a new idea (red)

One finds this idea described in many places, including:

“The Handbook of Confocal Microscopy” by Jim Pawley

However, there is a second constraint on the choice of pinhole size. Because almost all of the light originating from the plane of focus will pass through a properly-aligned pinhole, 1 Airy unit in size, one might expect that there could be no reason for ever wanting to use any other aperture size. This might be the case if the diameter of the pinhole did not also affect the spatial resolution of the microscope in both the  $xy$ -plane and, to a lesser extent, in  $z$ . If the pinhole is made very small ( $<0.1$  Airy units), the  $xy$ -resolution of the instrument is improved by  $-40\%$  over that set by the Abbe limit, but only at the cost of reducing the signal level by  $95\%$ . As the pinhole is made larger, it begins to accept more light while the  $xy$ -resolution is reduced. When it equals 1 Airy unit,  $80\%$  of the light originating from the focus plane is accepted, while a  $10\%$  resolution gain is still being realized. On the other hand, when the pinhole is opened still more, any extra light that it accepts must be that originating from either above or below the focus plane, and this reduces the optical sectioning effect as well as providing more photons.

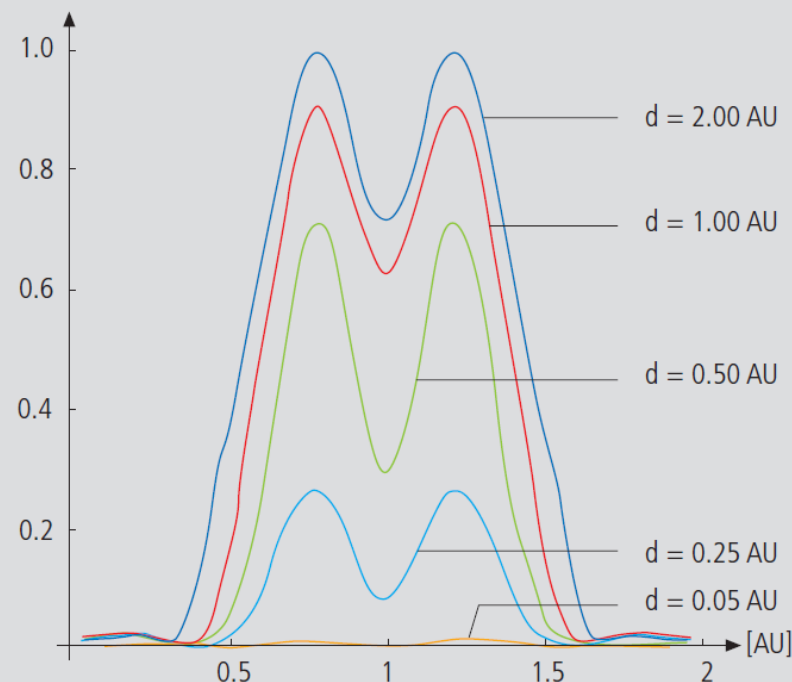
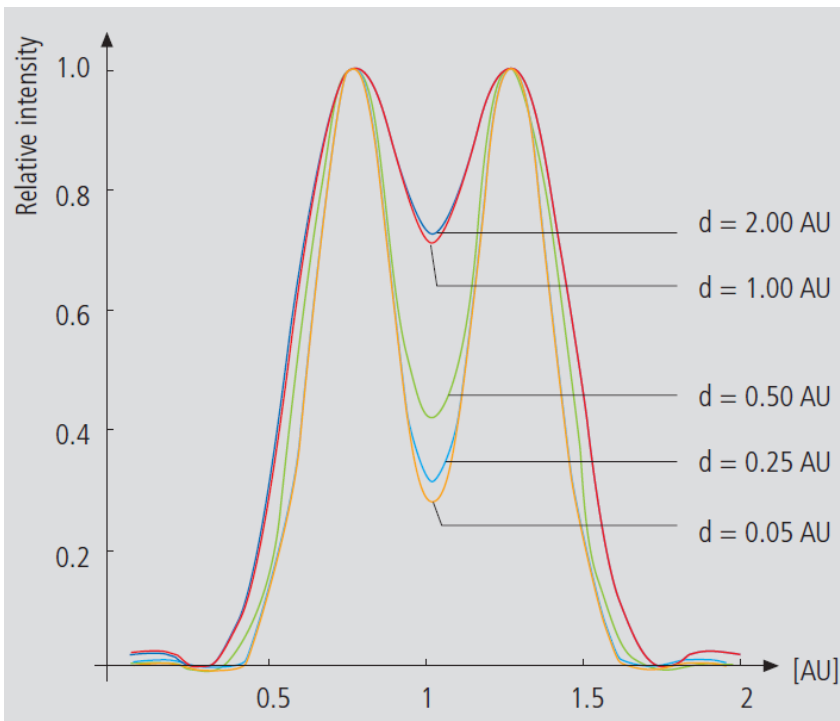
The price to pay, however, is sacrificing  $95\%$  of the signal (green).

The famous 1 A.u. setting is more of a practical barrier than a theoretical limit.

Fortunately, now there is a clever workaround with Airyscan...

# Scanning confocal and the 1 Airy unit pinhole “limit”.

*At  $PH < 1$  A.u. signal loss is larger than gain in resolution.*



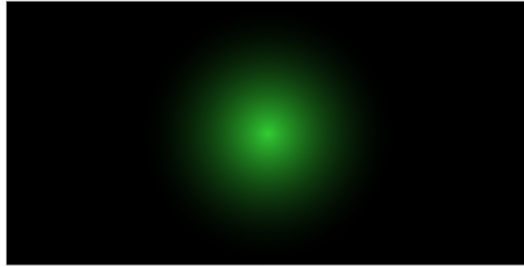
**Left = Individually normalised graphs.**

**Right = Normalised to 2AU intensity.**

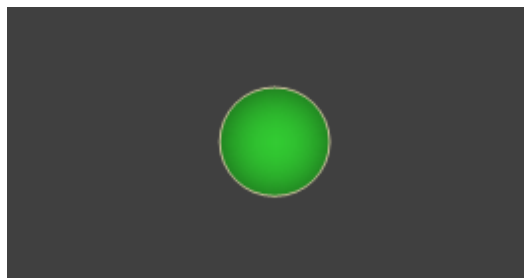
Small pinhole diameters lead to improved resolution. A smaller FWHM leads to deeper dips.

Constricting the pinhole is connected with a drastic reduction in signal level. The drop in intensity is significant from  $PH < 1$  AU

# Airyscan将光效率和超分辨率完美的结合

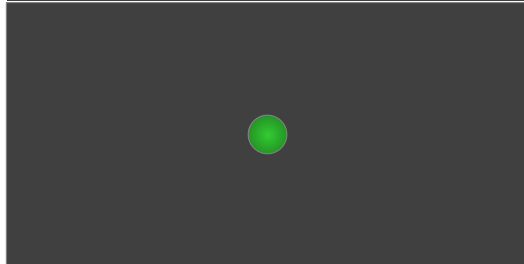


信号光斑



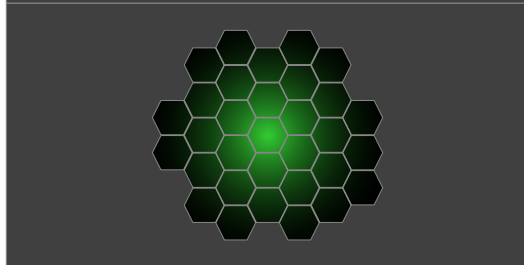
共聚焦检测

(pinhole: 1.0 AU)  
为达到最佳光学切面效果



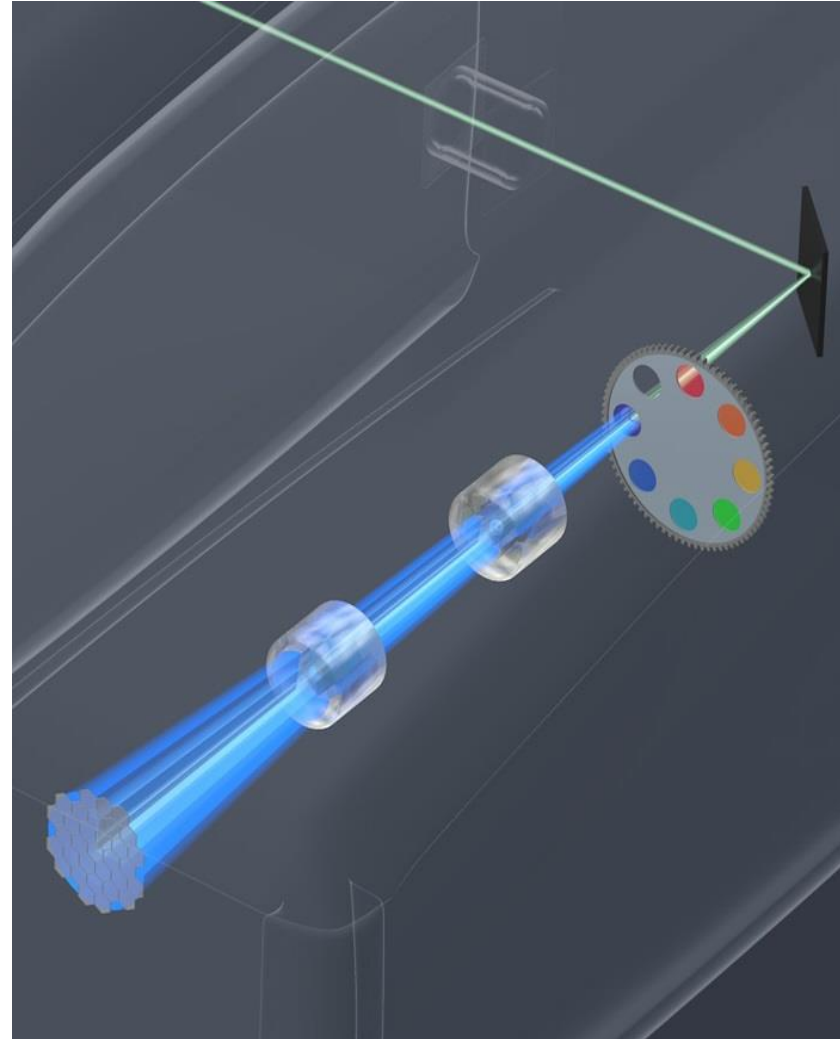
共聚焦检测

为达到最佳分辨率,  
(pinhole: 0.2 AU)  
摒弃绝大多数信号



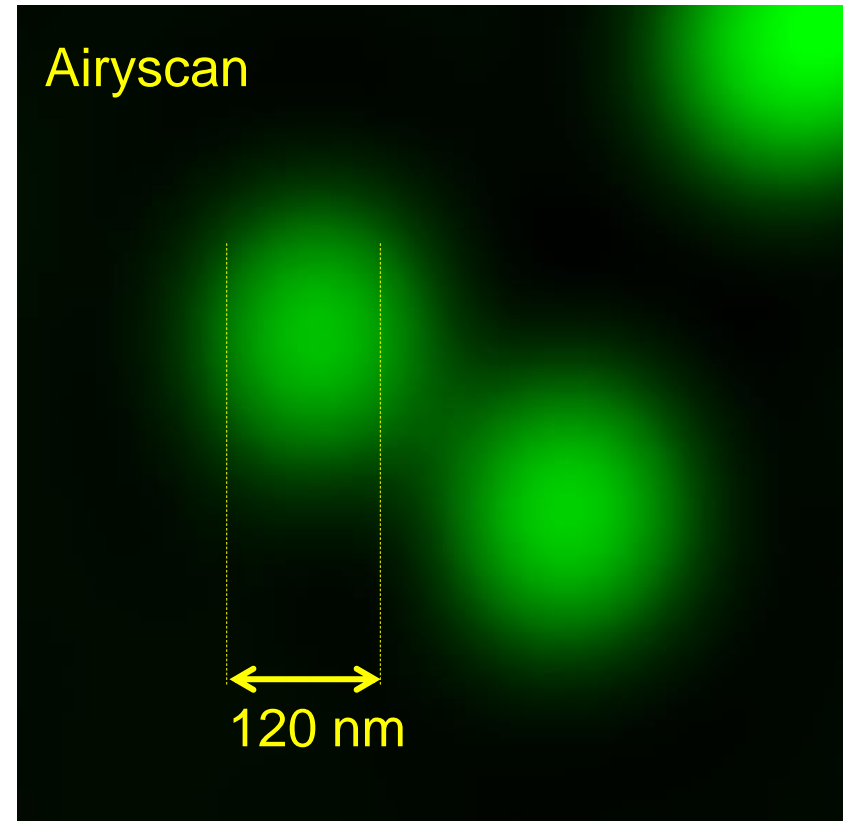
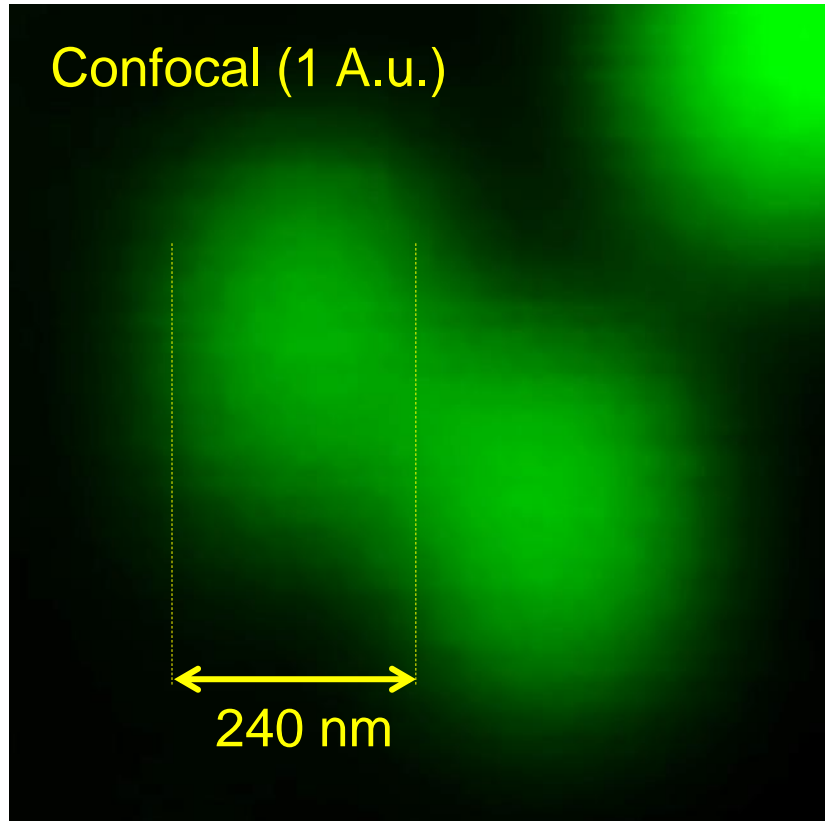
复眼阵列检测

- 每个检测器直径为0.2 AU.
- 收集几乎所有信号



# Airyscan enhances resolution up to 1.7 fold

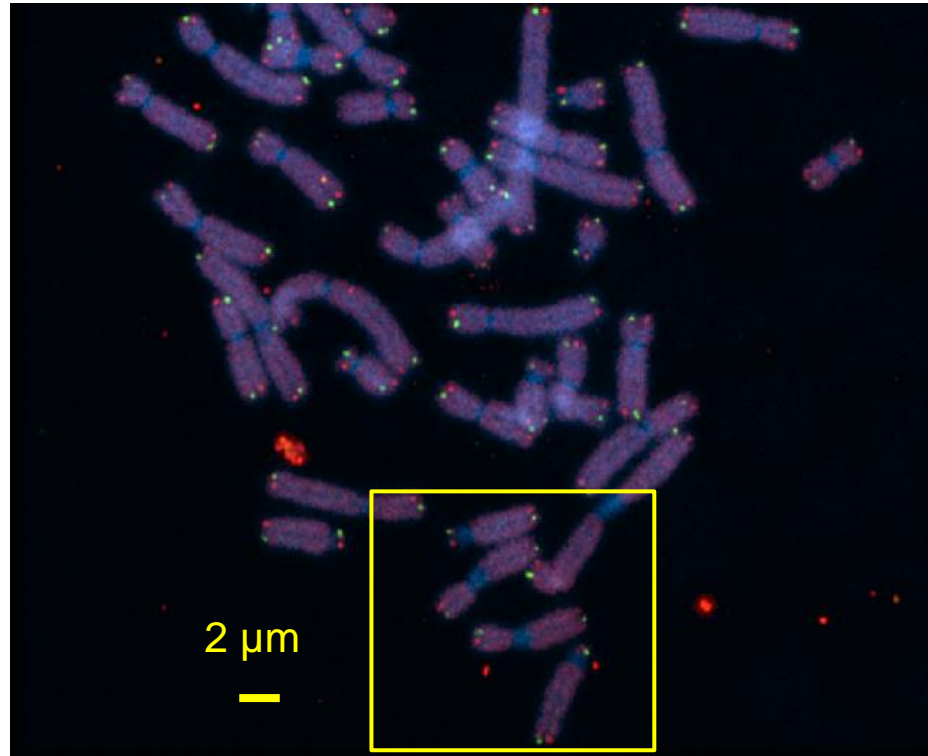
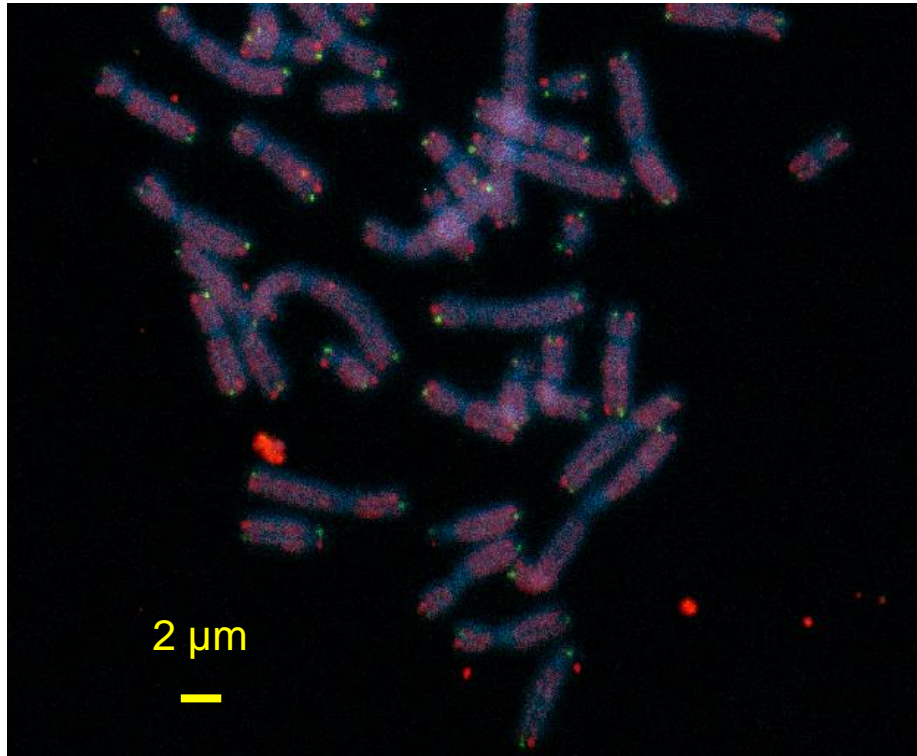
*The comparison is made with confocal using PH = 1 A.u.*



Improvement 1,7x: Measured using 40 nm beads (excitation at 488 nm)  
Same acquisition time, same laser power.

# LSM 880 – Airyscan enhances resolution, boosts SNR

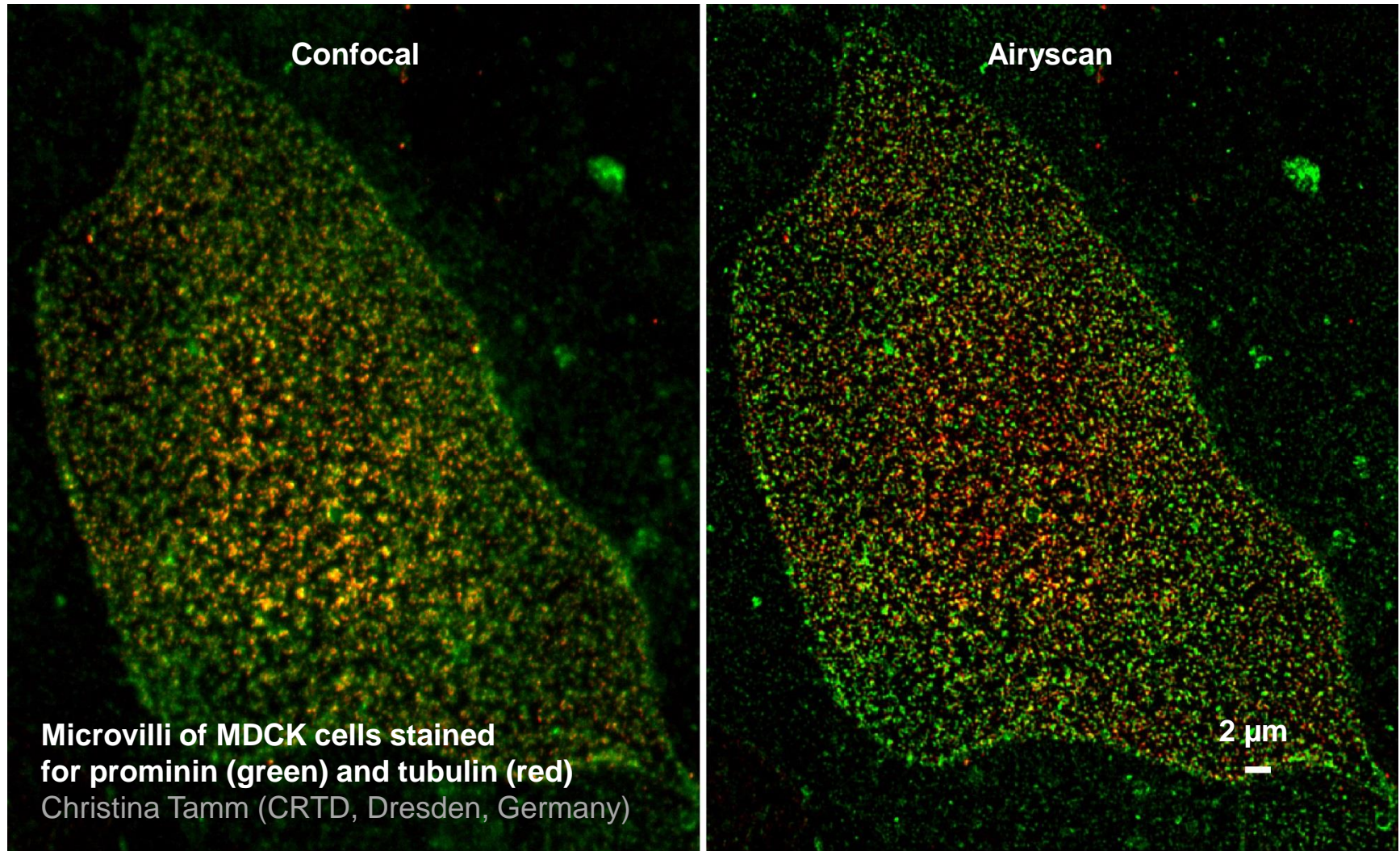
*“...thereby allowing for a much more accurate quantification”  
(Karlseder and Fitzpatrick, The Salk Institute, La Jolla, CA, USA)*



Telomere replication without RTEL1: Stalled forks and telomere breakage visualized as doubled dots using Airyscan. Resolution is meaningless without good SNR.

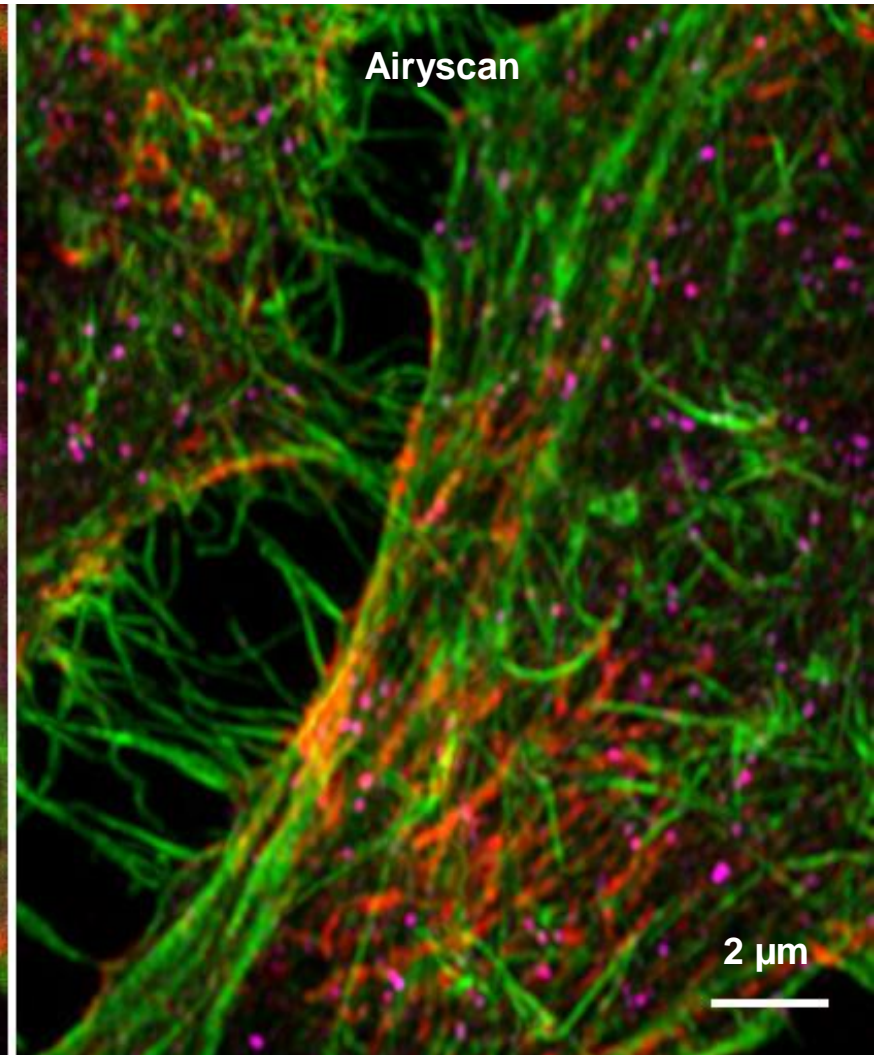
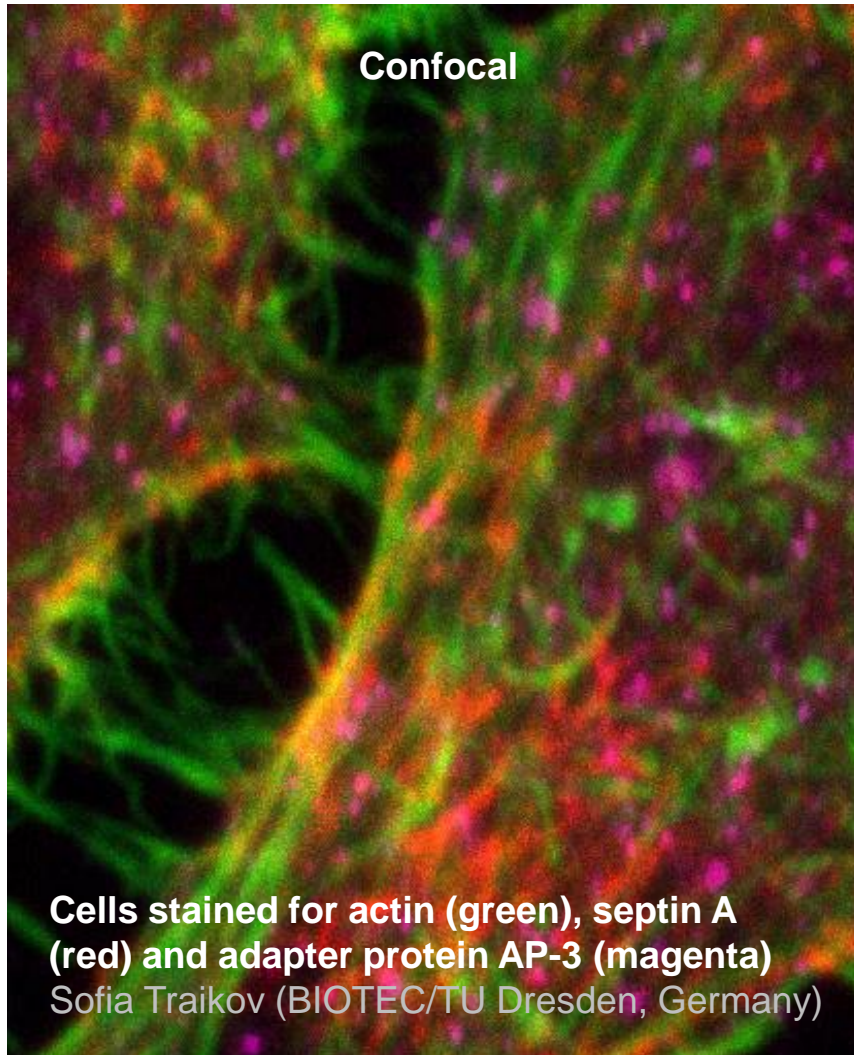
**Courtesy: J. Karlseder Ph.D. (Molecular and Cell Biology Laboratory) and J. Fitzpatrick Ph.D. (Director, Waitt Advanced Biophotonics Core), The Salk Institute, La Jolla, USA.**

**Airyscan is very robust, easy to use, compatible with standard samples, and works with all common dyes**



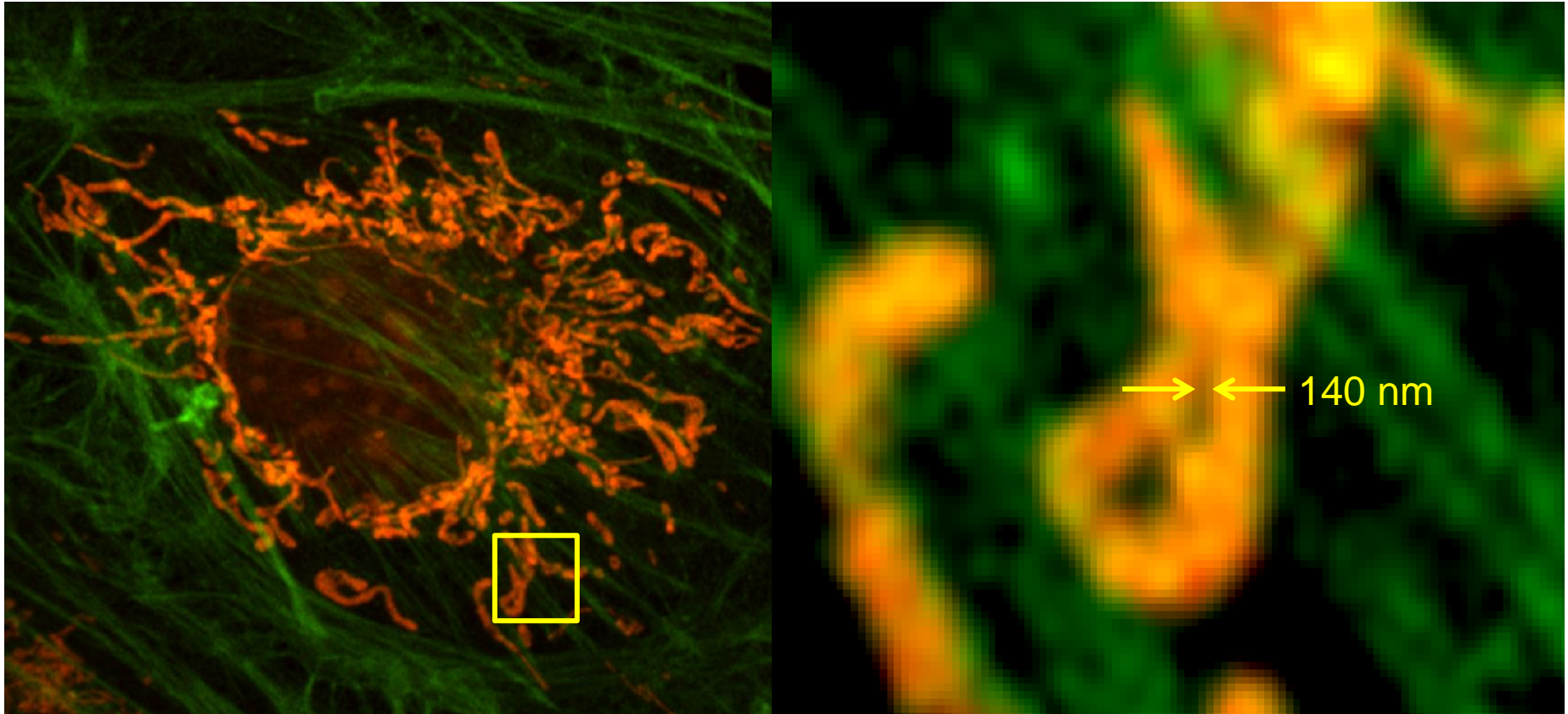
# LSM 880 – Airyscan: Multi-color imaging of samples

*8 position filter wheel, multiple-bandpass possible*



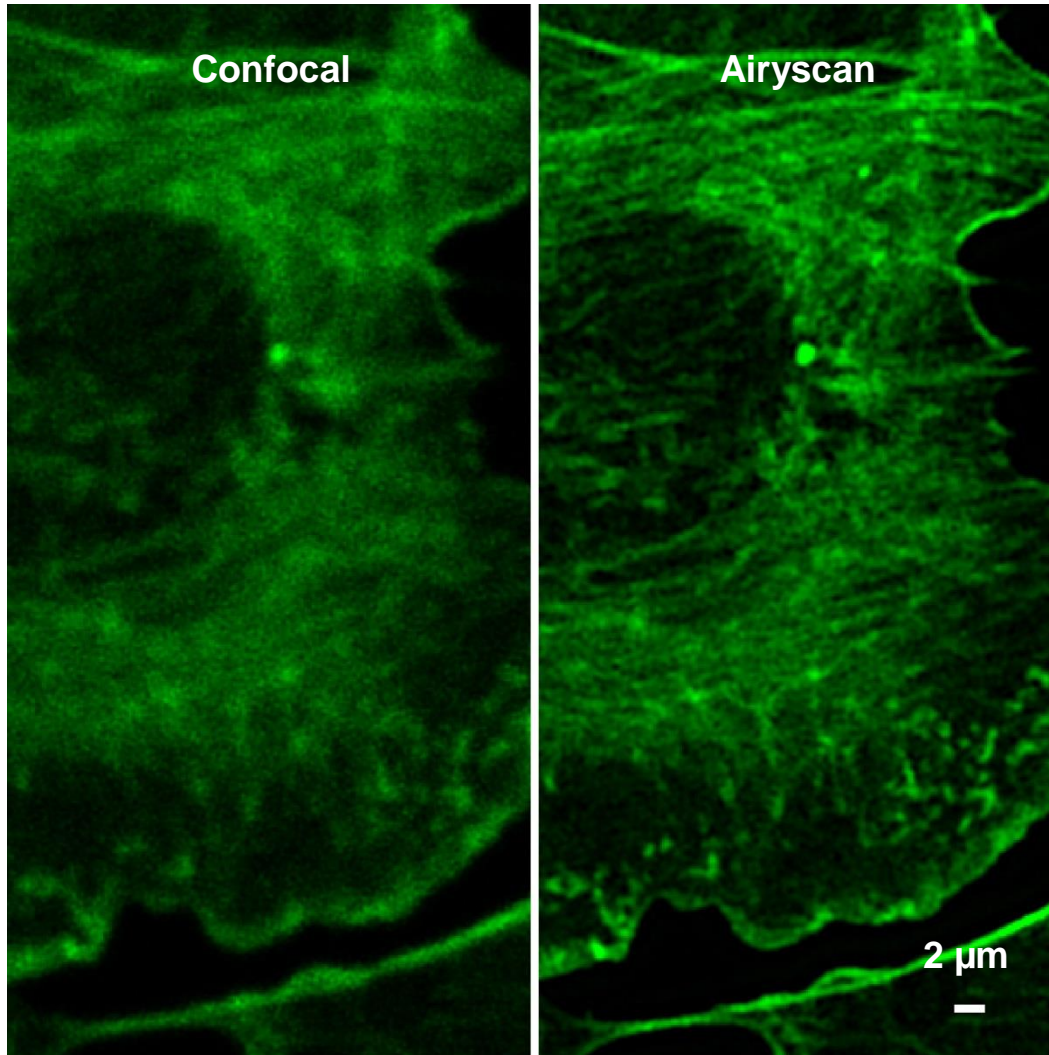
# LSM 880 – Airyscan: Resolution, SNR, and Flexibility

*Choose zoom-in ROI's, orientations, laser wavelengths, etc.*



Use Airyscan just as an additional detector. Scan speeds, ROIs, field orientations, laser wavelengths, multitracking, z-stacks, time series etc. are setup as always. Sample handling and fluorophore selection don't need rethinking

# When imaging tissue samples, Airyscan resolves finer details – even when using lenses with a long WD!

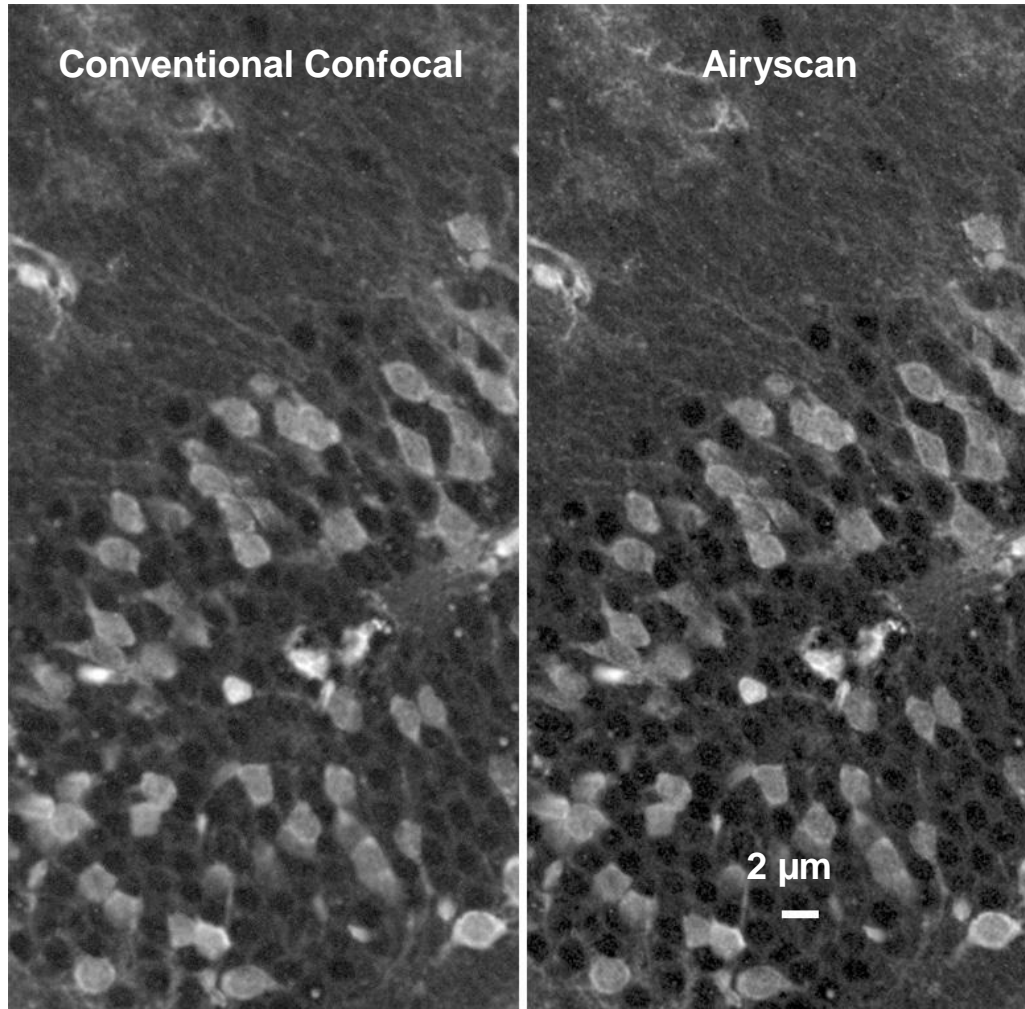


**Muntjac skin fibroblast  
stained for actin**

**Acquisition:  
10x objective**



# When imaging tissue samples, Airyscan resolves finer details – even when using lenses with a long WD!



**Mouse brain slice**

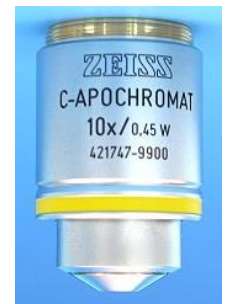
Antibody staining for CNPase

**Author:**

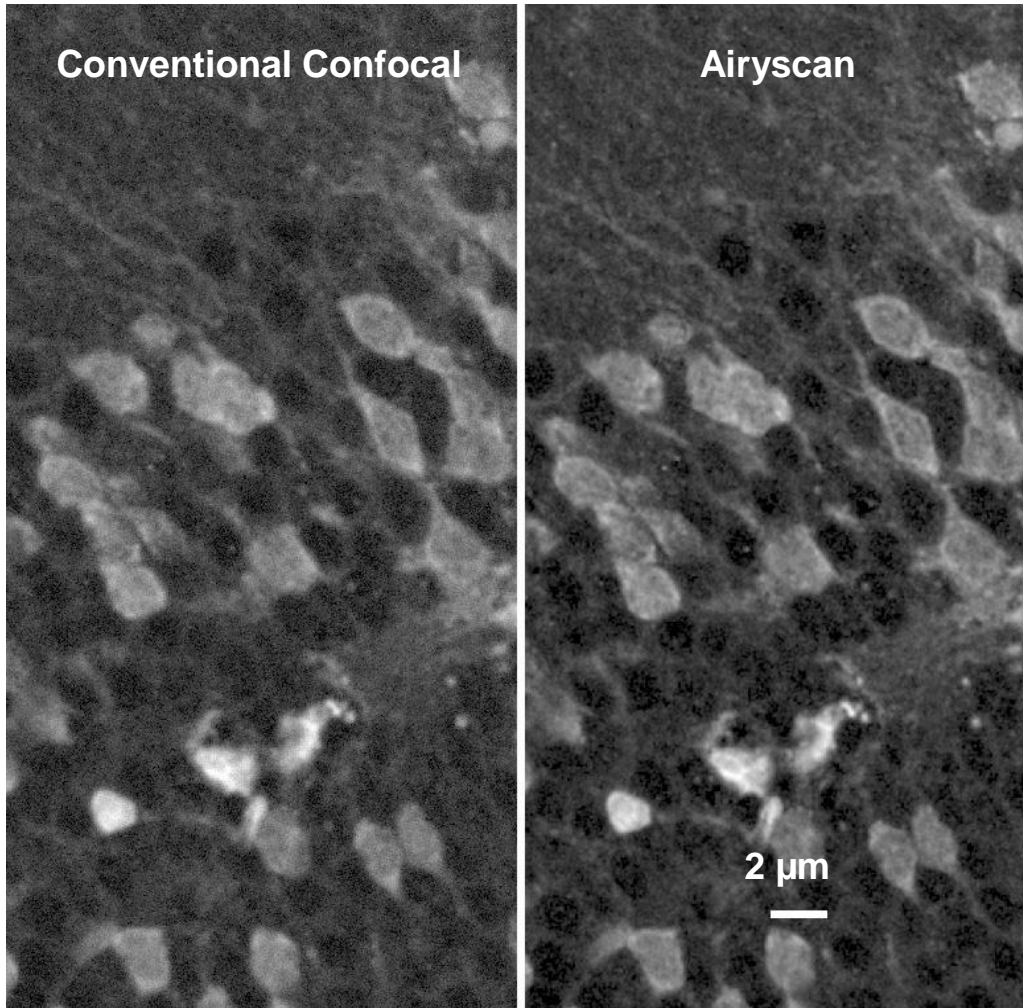
Carsten Dornblut  
(FLI Jena, Germany)

**Acquisition:**

**10x objective!**



# When imaging tissue samples, Airyscan resolves finer details – even when using lenses with a long WD!



**Mouse brain slice**

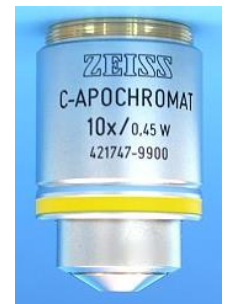
Antibody staining for CNPase

**Author:**

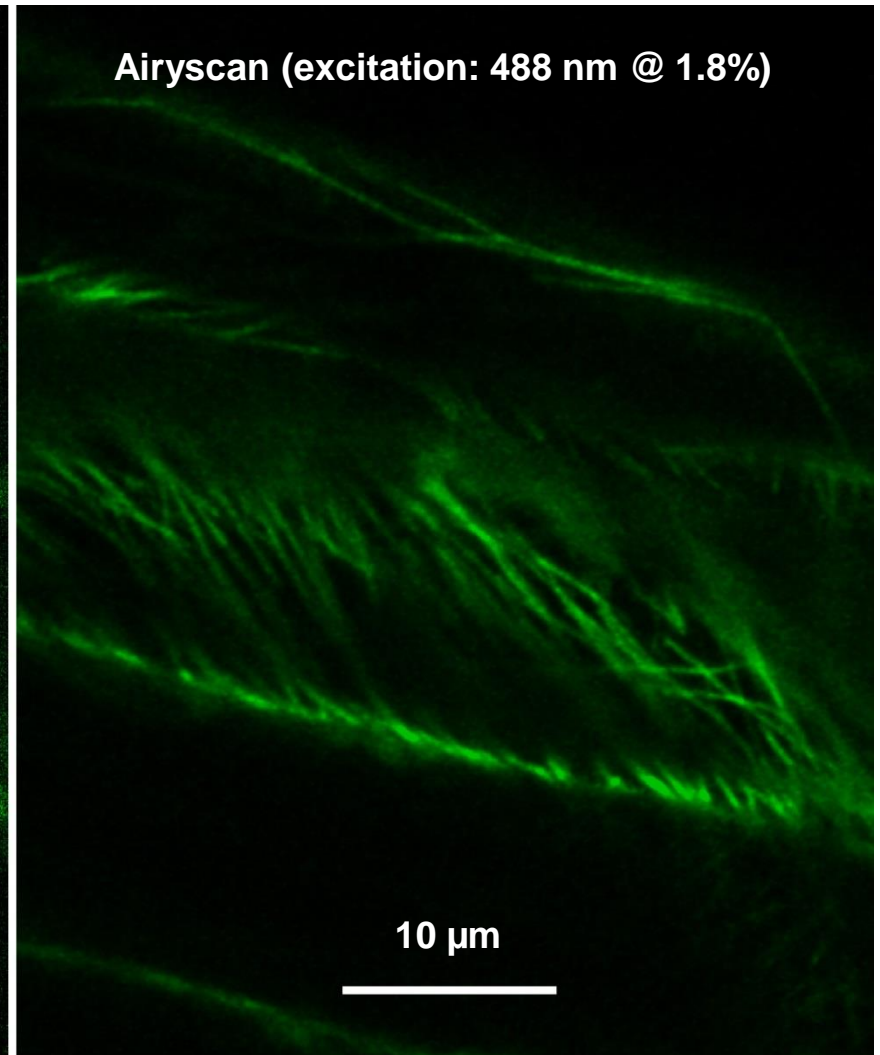
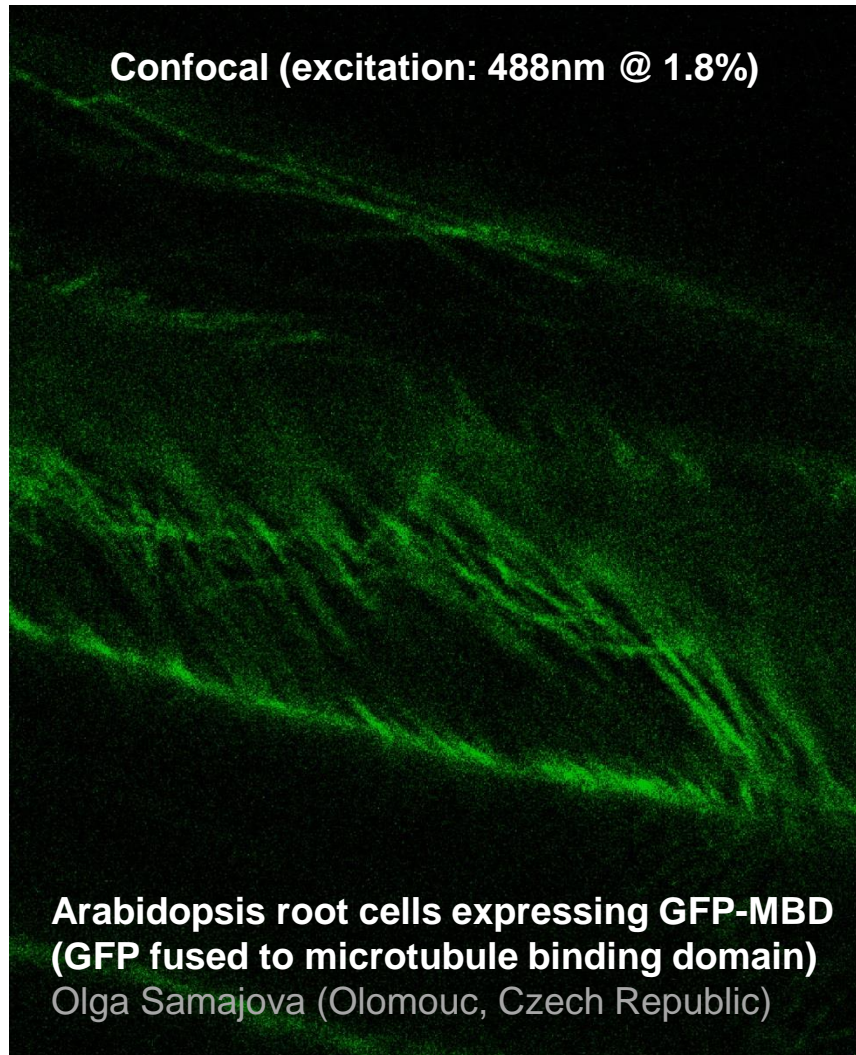
Carsten Dornblut  
(FLI Jena, Germany)

**Acquisition:**

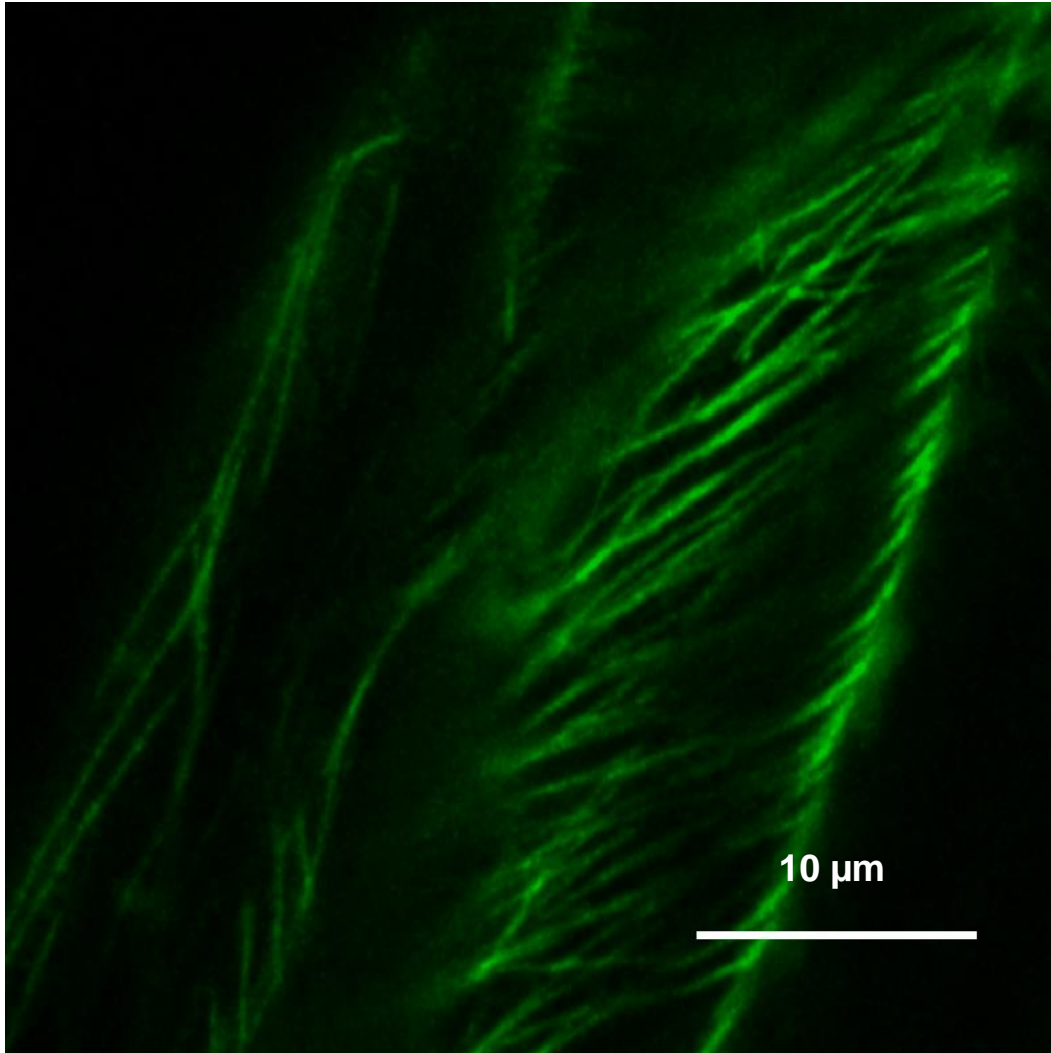
**10x objective!**



# With its drastically improved SNR, Airyscan delivers quality images previously impossible with LSMs



# Airyscan delivers exceptional data of live samples using the same laser power than in confocal imaging



## Cytoskeleton dynamics of Arabidopsis root cells expressing GFP-MBD

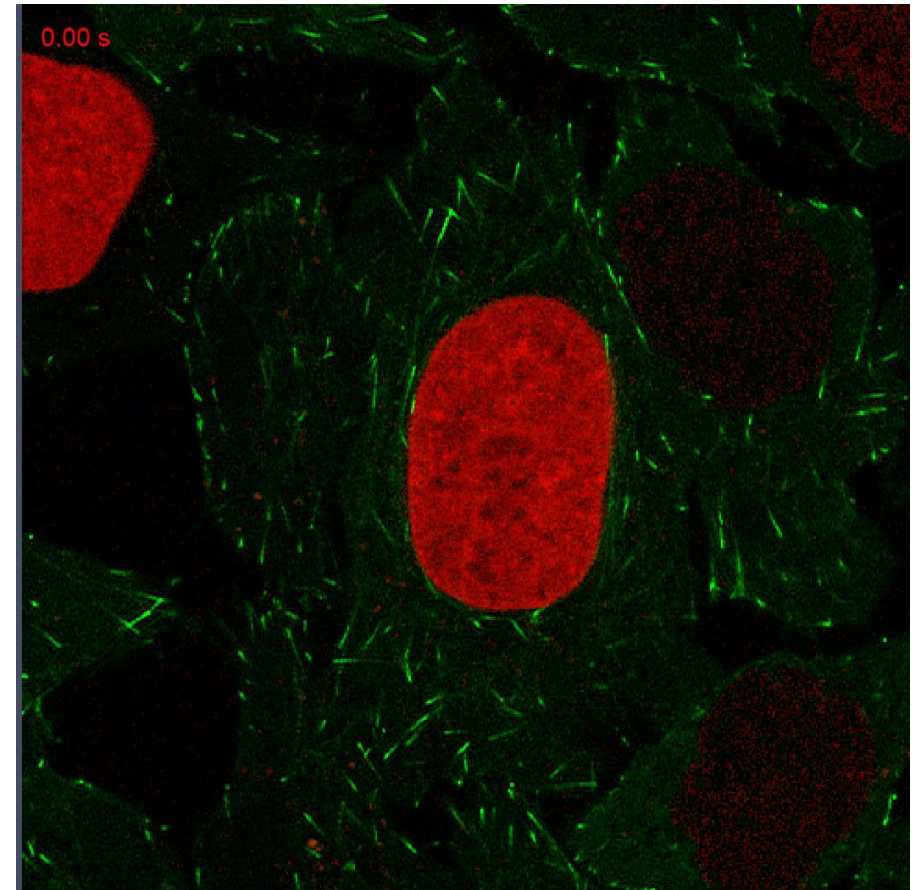
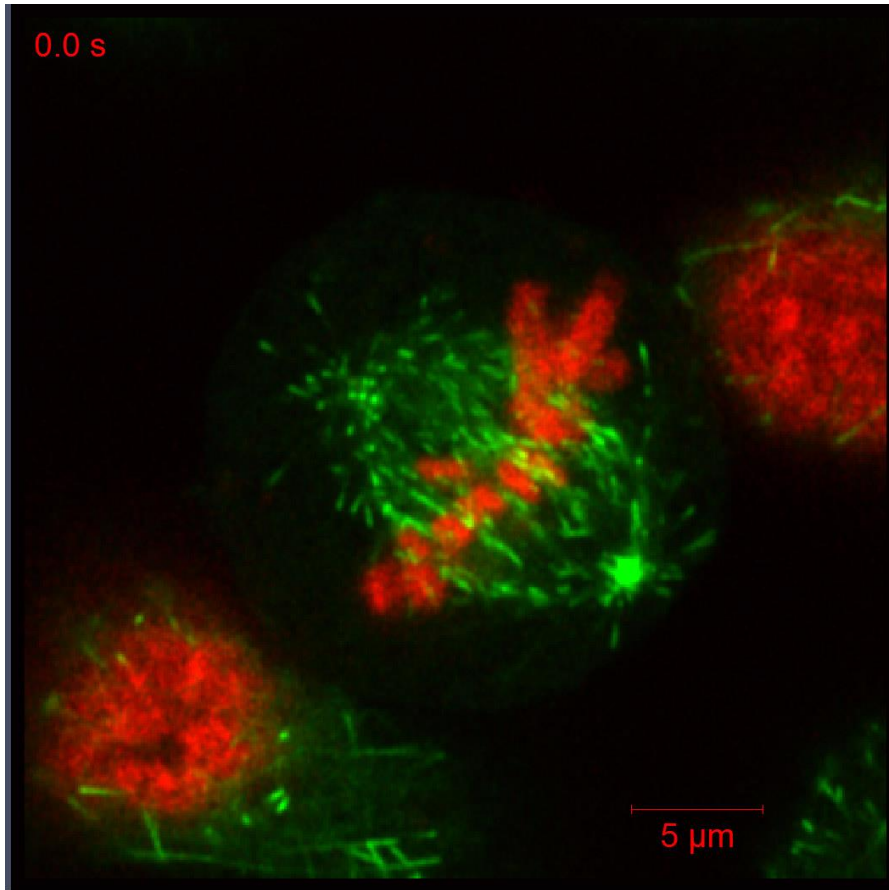
(GFP fused to microtubule binding domain)

**Sample courtesy of:**  
Olga Samajova  
(Olomouc, Czech Republic)

**Acquisition settings:**  
Excitation: 488 nm @ 1.8%  
Z-stacks (levels): 5  
Z-stack (increment): 0.3 μm  
Interval: 19.5 sec.

# LSM 880 – Airyscan: Resolution, SNR, and Flexibility

*Intrinsically compatible with live cell imaging*



I. Beyond Diffraction Limit \ 超越光学分辨率极限

II. High Signal to Noise Ratio \ 高信噪比

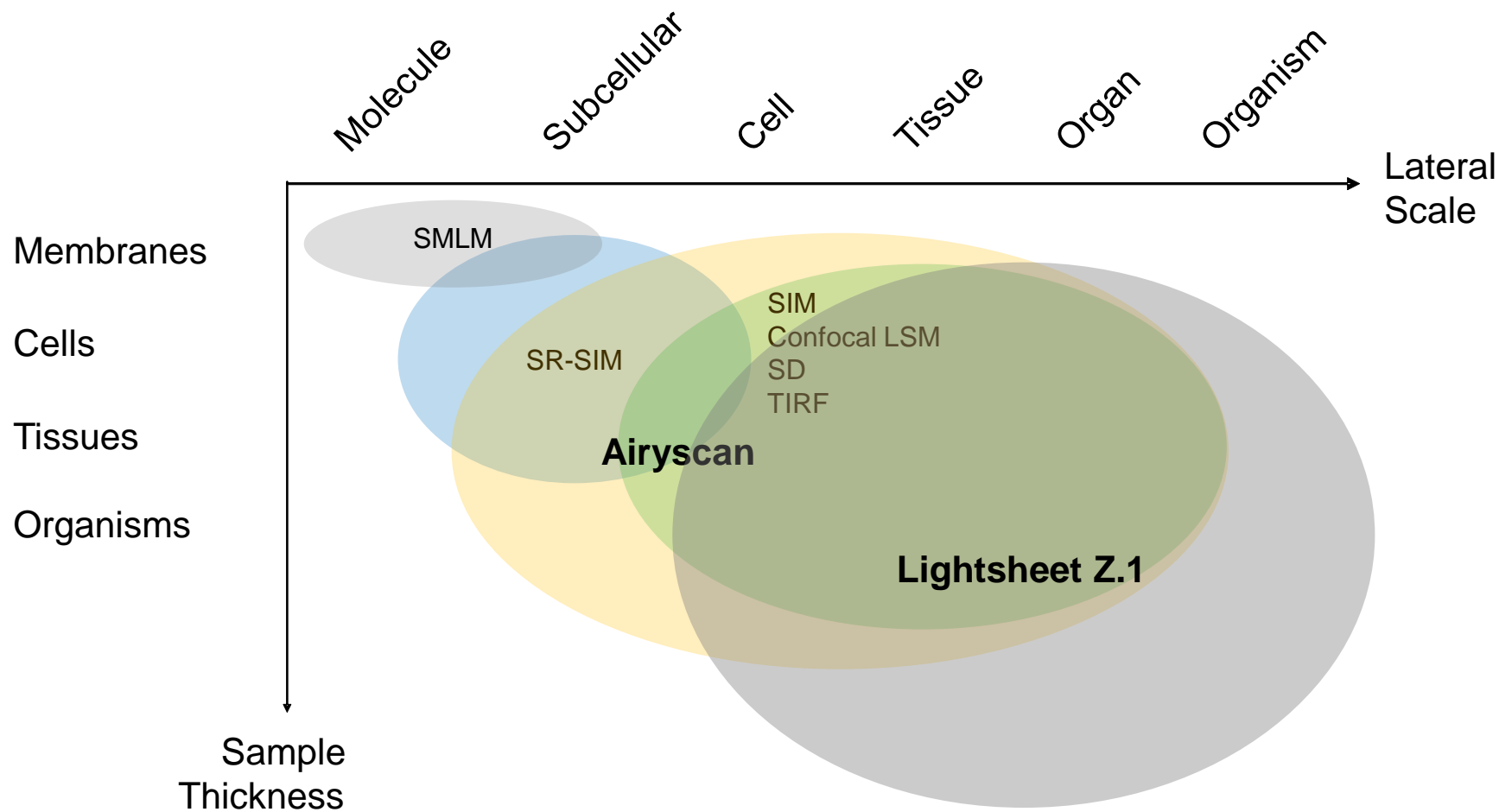
III. Depth of Image \ 超高分辨率深度成像

IV. Live Cell Image \ 超高分辨率活细胞成像

V. Low Magnification Obj. \ 使用低倍物镜获得高分辨率图像

# Samples and Imaging Modalities

Pushing the boundaries of biological imaging



# ZEISS 关联显微技术

## 提供跨尺度多维度成像解决方案



Connecting platforms...

### Light Microscope



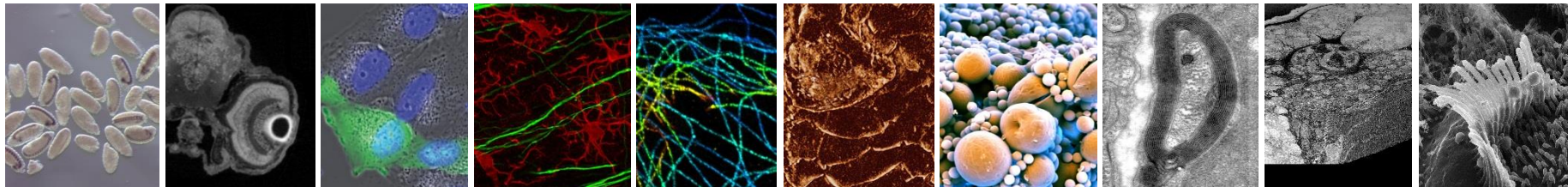
### X-ray



### EM



### Ion Microscope



Stereo LM	Sub-micron XRM	Widefield LM	Confocal LM	Super resolution LM	Nanoscale XRM	C-SEM	FE-SEM	FIB-SEM	Helium Ion Microscope
体式显微镜	X射线显微镜	宽场显微镜	共聚焦/双光子显微镜	超分辨率显微镜	X射线显微镜	扫描电镜		聚焦离子束显微镜	氦离子显微镜

1  $\mu$ m      700 nm      250 nm      200 nm      120-20 nm      < 50 nm      < 2 nm      < 1 nm      < 1 nm      < 0.5 nm

...to enable multi-dimensional research

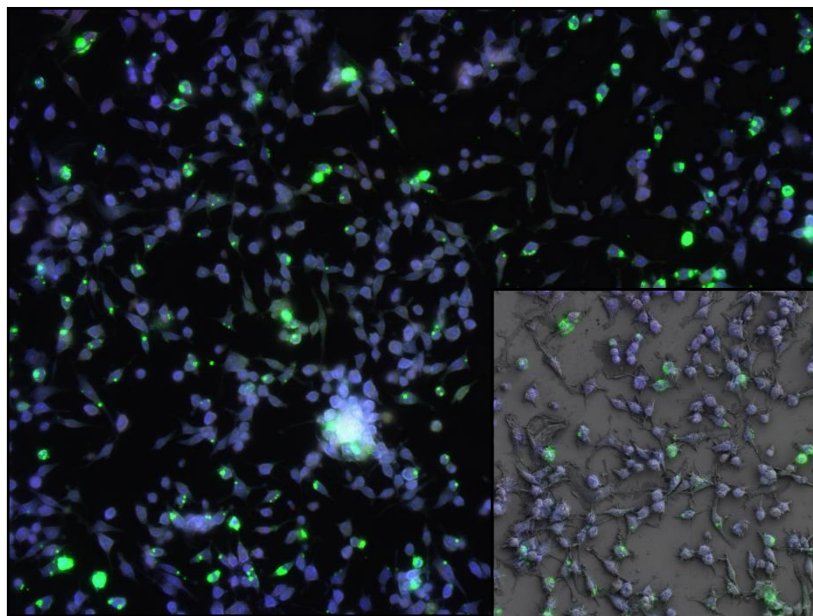
# ZEISS 关联显微技术

## 提供跨尺度多维度成像解决方案

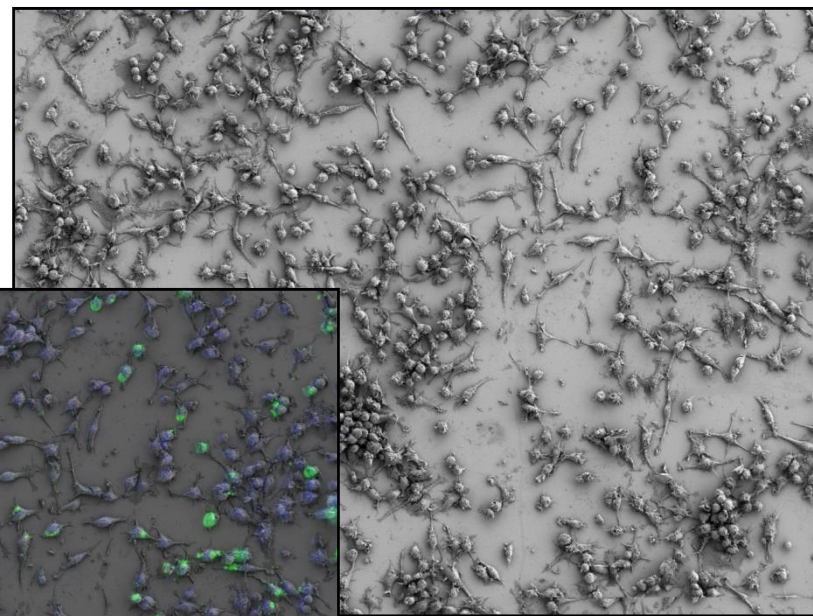


# 关联显微成像应用实例

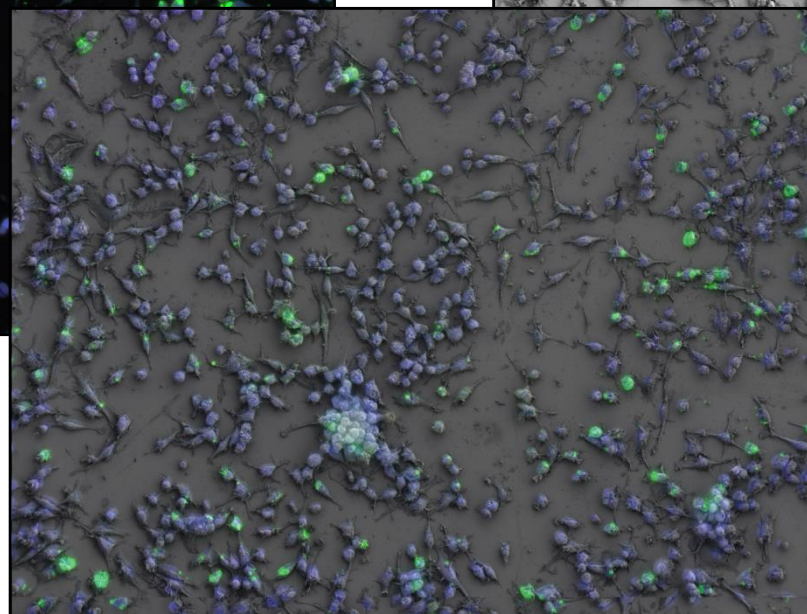
## 发现新现象、研究新问题



LM image



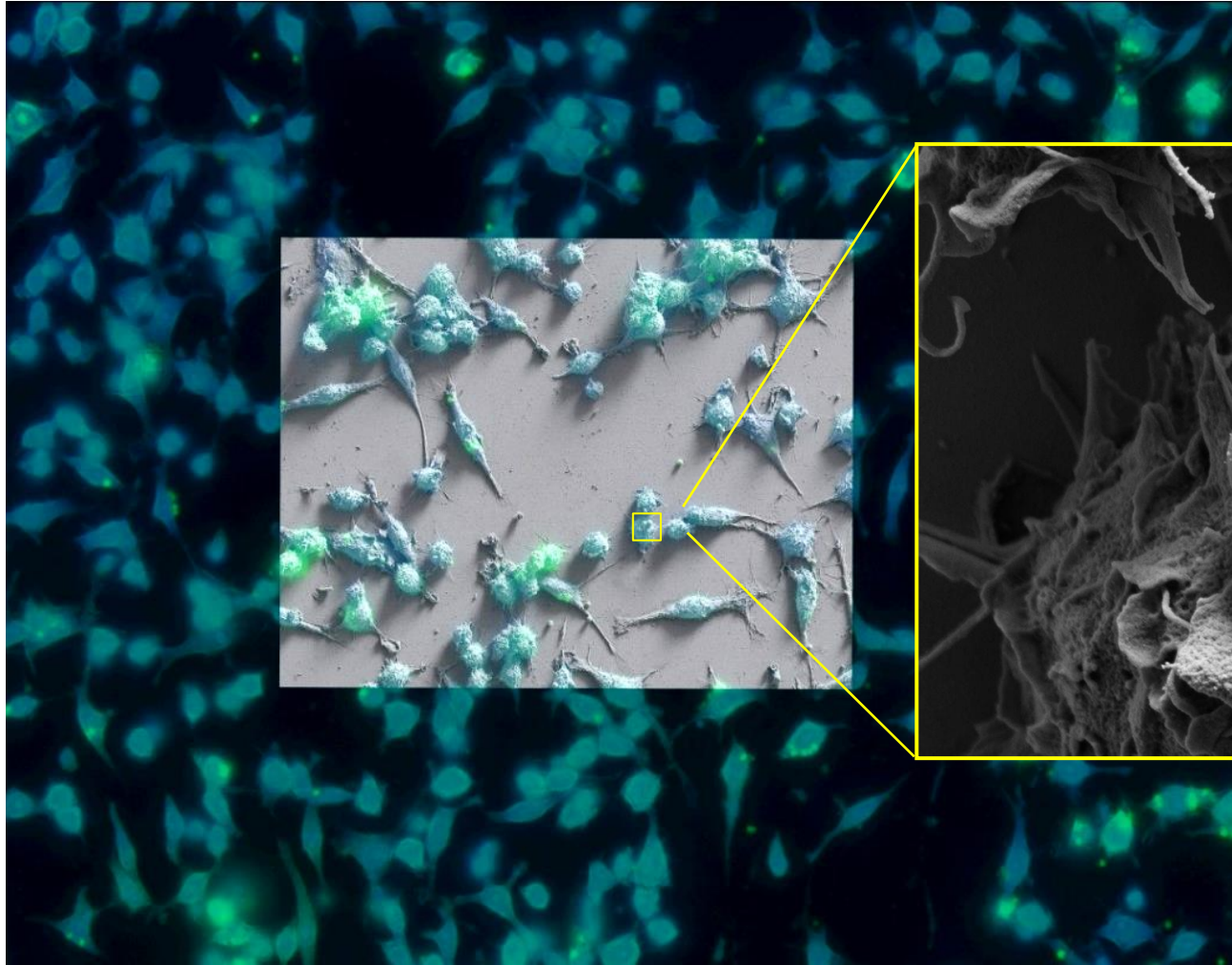
SEM image



Correlated LM + SEM image

# 关联显微成像应用实例

## 发现新现象、研究新问题



将光镜结果和电镜结果重合能清晰的展示出吞噬过程



扫描电镜 能够提供超高分辨下的细节信息.



Seeing beyond