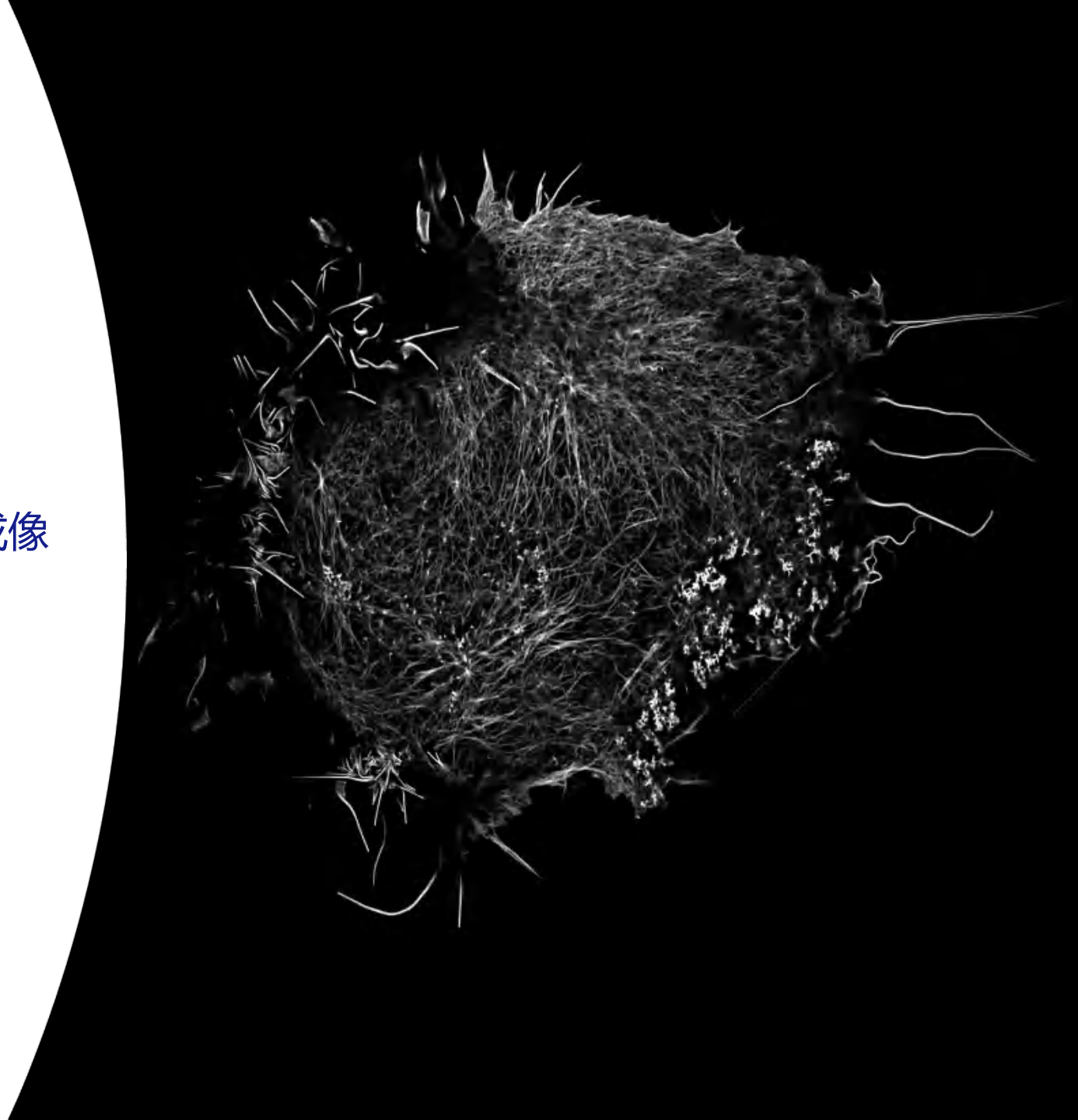
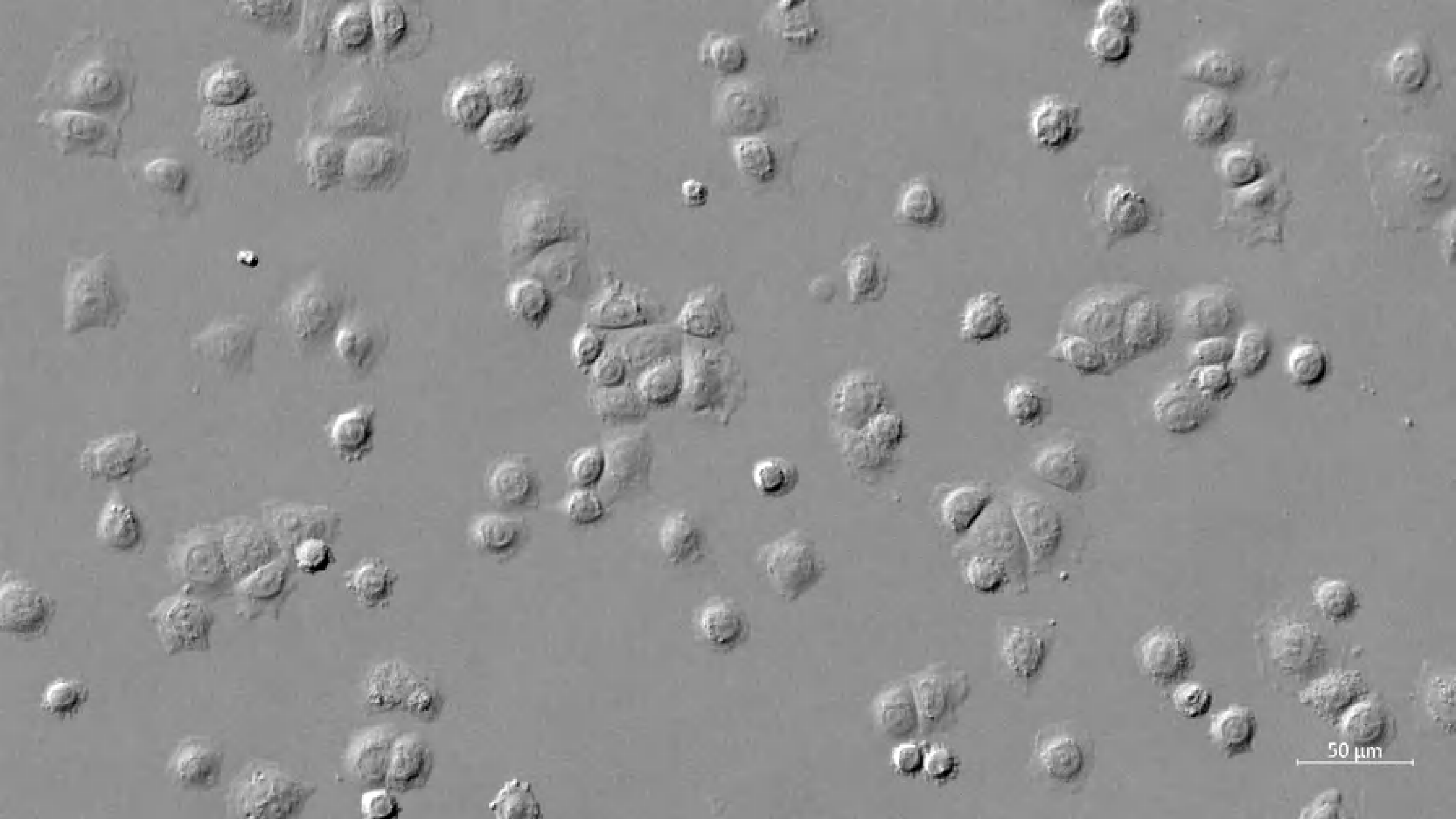
结构光超分辨显微镜 Multi-SIM

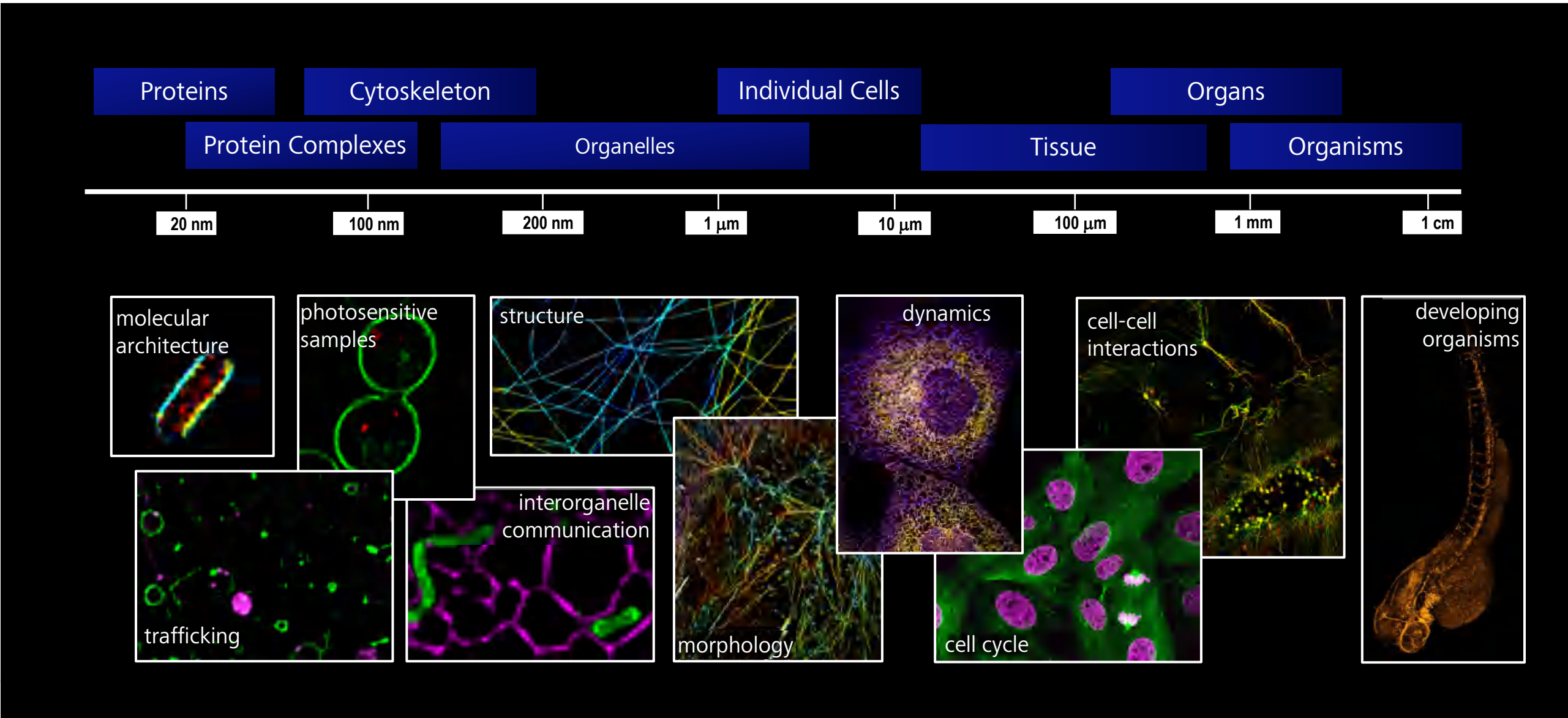
实现活细胞高速超分辨温和成像

# New possibilities and explorations




50 µm

# 荧光显微镜发展趋势：Faster，sharper，and deeper

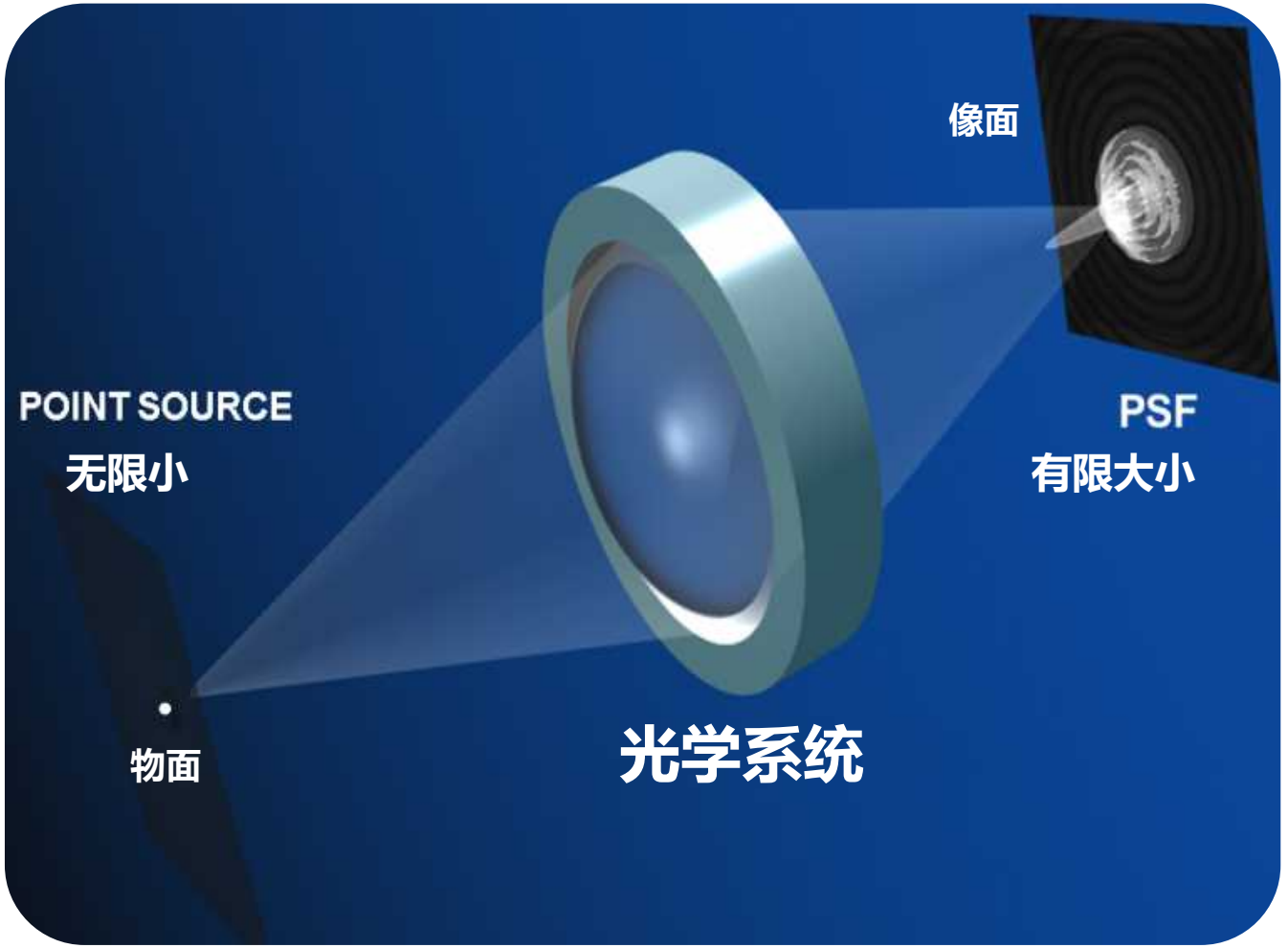


# Because the resolution of a microscope is limited!

## 显微镜的分辨率是有极限的!

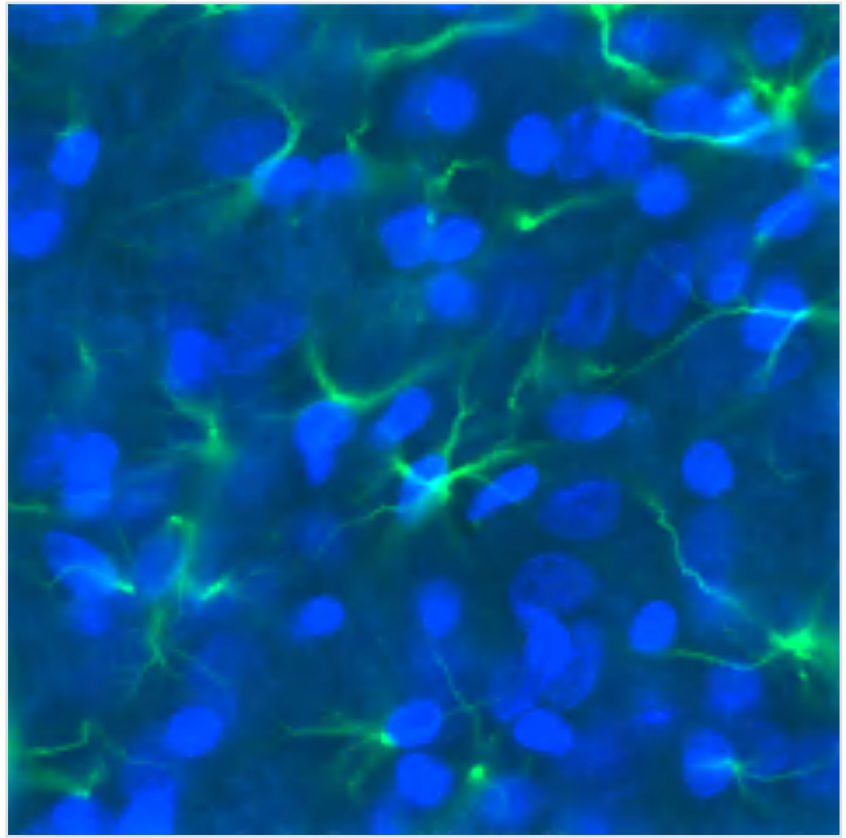
**The resolution of light microscopy is limited because light is a wave and is subject to diffraction.**

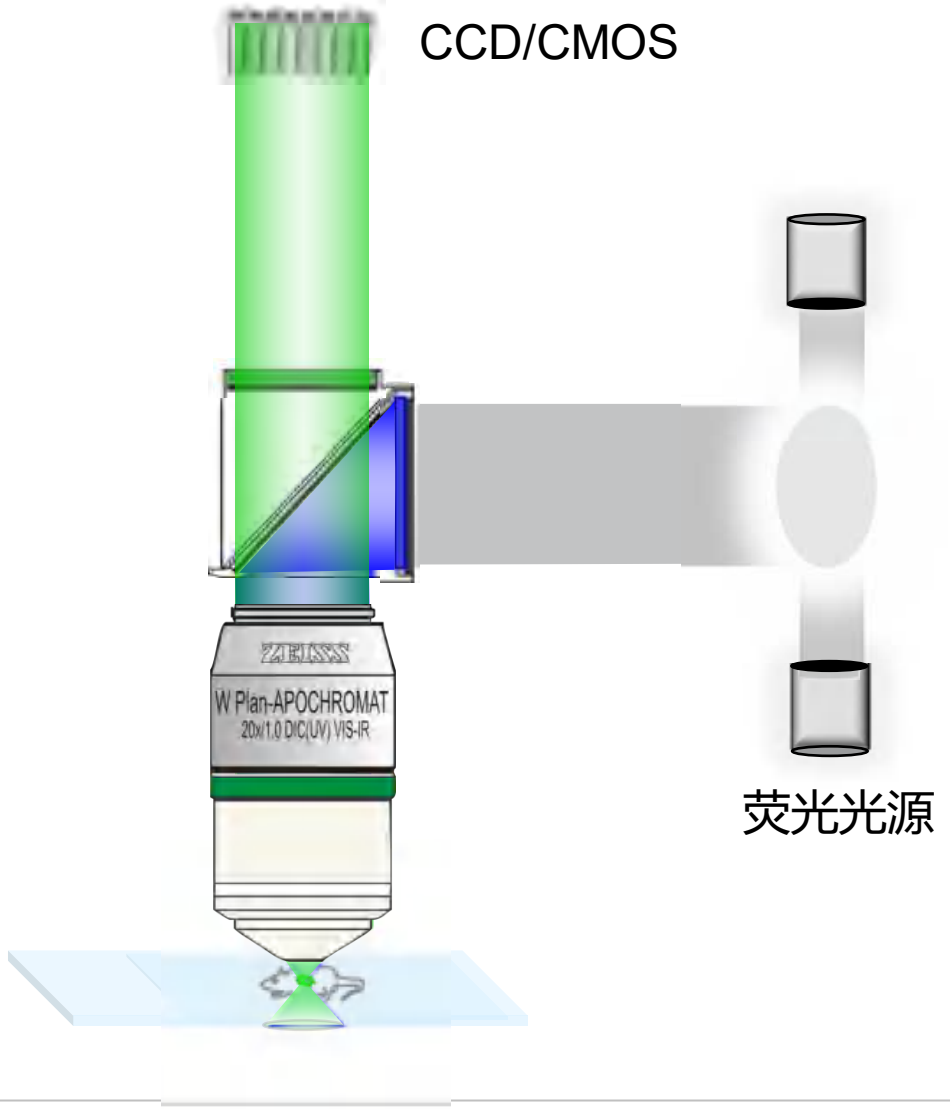
***由于光是一种波，并受制于衍射的因素，显微镜的分辨率是有限的。***



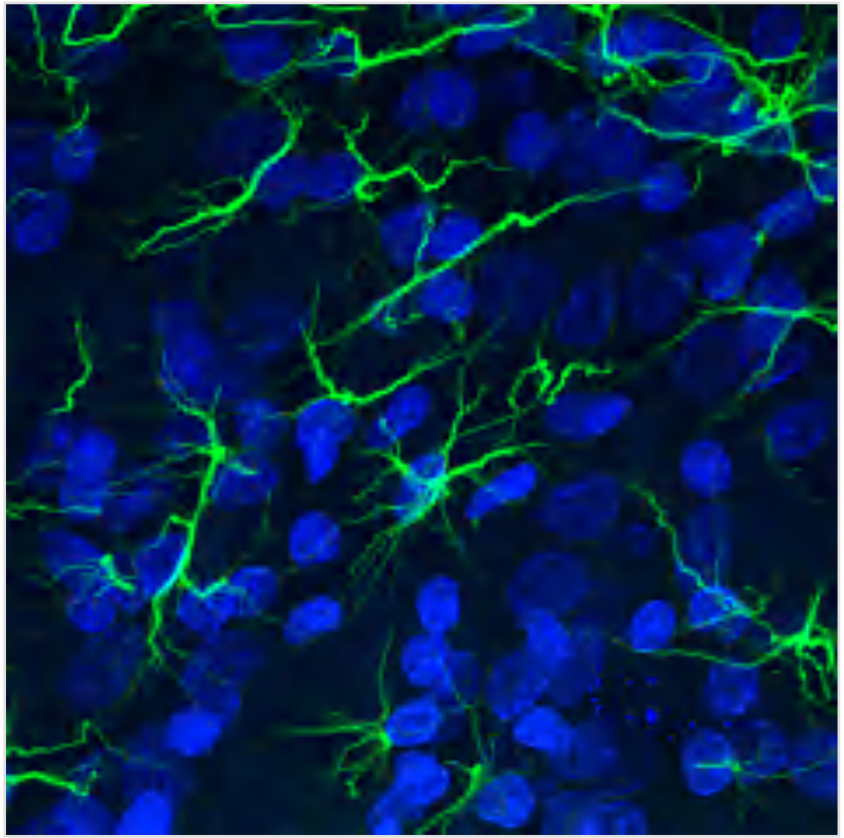
# 宽场显微镜成像原理

## 物镜的景深是有限的!



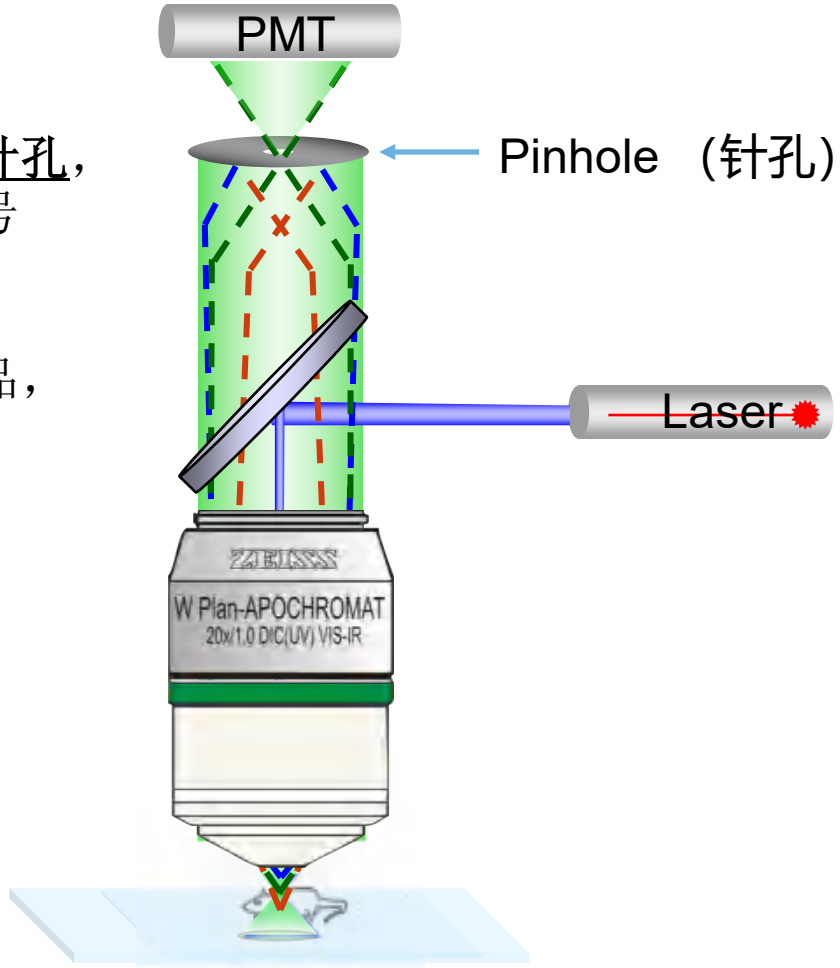


# 共聚焦显微镜成像原理



在共轭平面增加**针孔**，
屏蔽非焦平面信号

不用手动切薄样品，
实现**光学切片**



# 共聚焦显微镜成像原理

**光学切片的厚度**

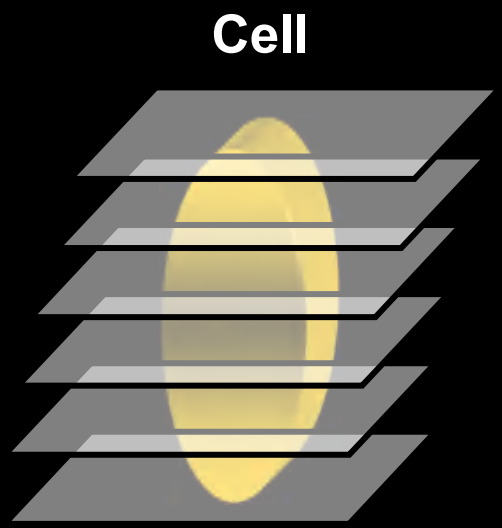
$d = Pn\lambda / (NA)^2$

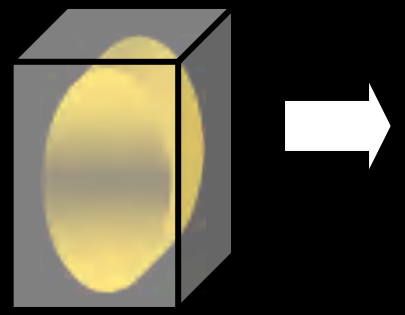
P：针孔直径

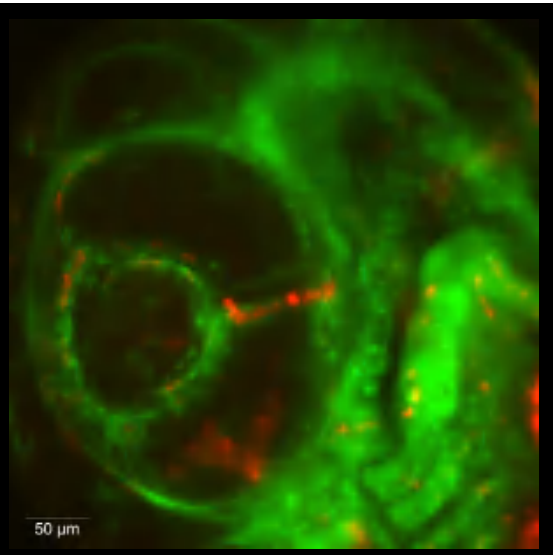
λ：光的波长

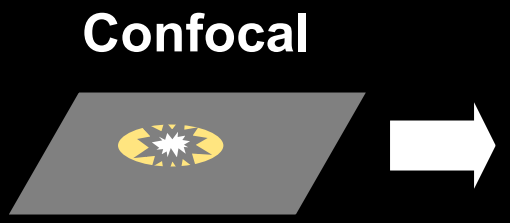
n：介质折射率

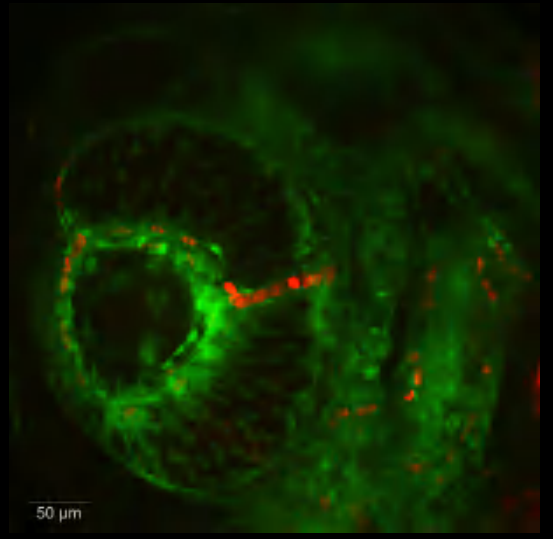
NA：物镜数值孔径



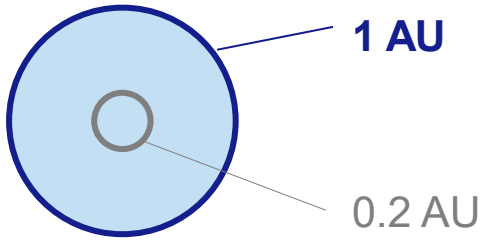



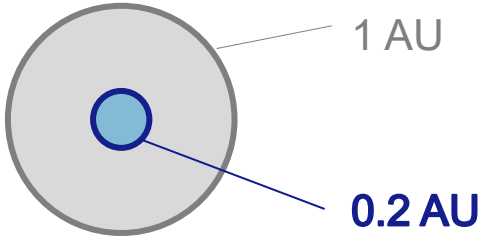



# Airyscan 成像原理

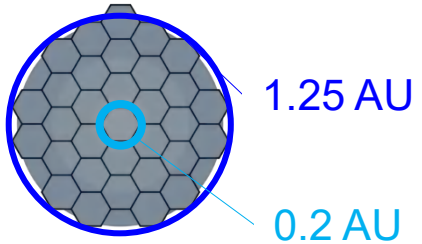
LSM 1 AU:

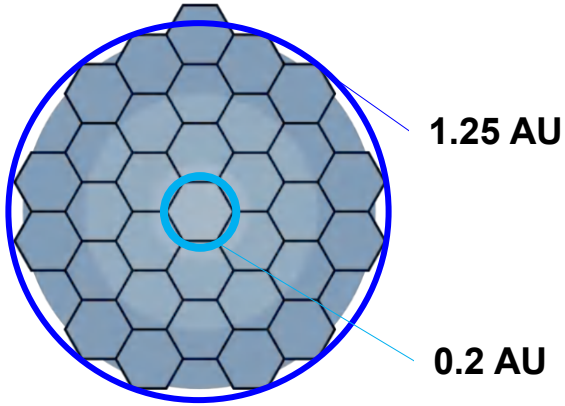


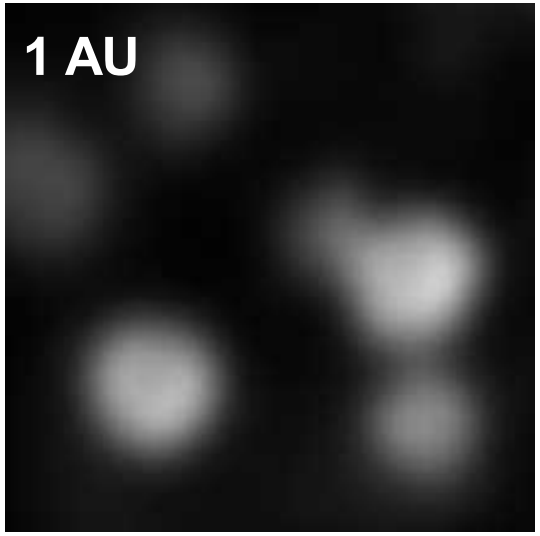

LSM 0.2 AU:

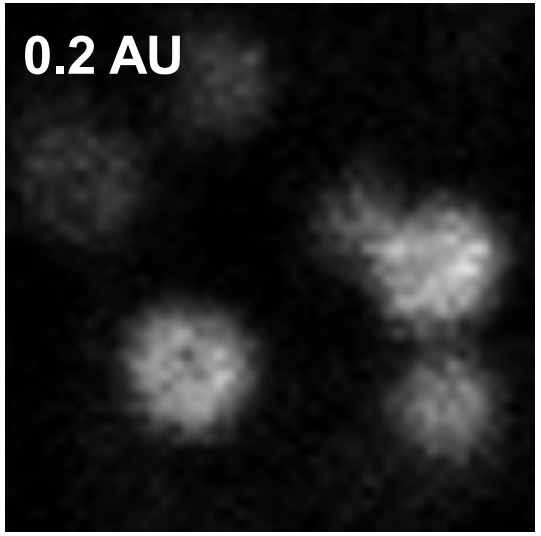


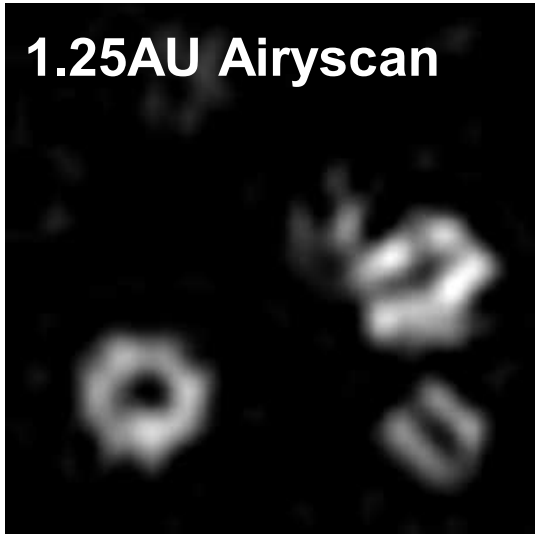

Airyscan :
32 channel area detector



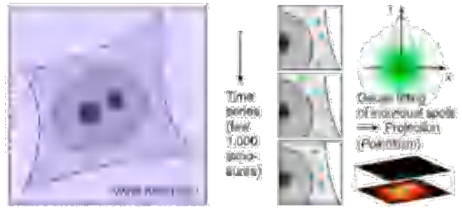
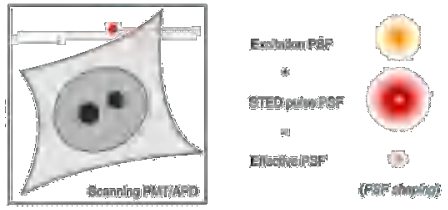
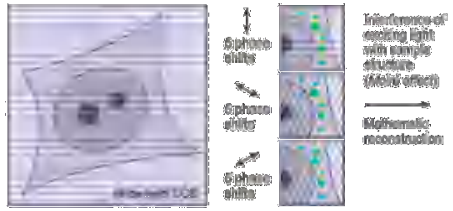




**分辨率 2 倍**

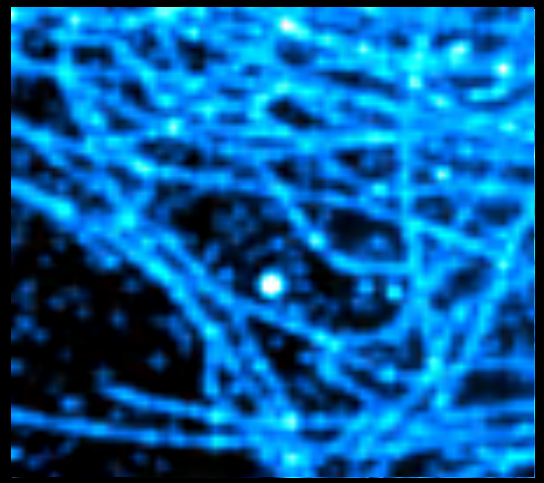
**信噪比4-8倍**

# 超分辨显微技术的机遇与挑战


| | | 分辨率 | 光强(W/cm²) | 采集时间 |
|---|---|---|---|---|
| 单分子定位 (PALM/STORM) |  | 20 nm | $10^3$ - $10^4$ | > 10 sec |
| 受激辐射耗尽 (STED) |  | 30 nm | $10^4$ - $10^7$ | > 10 sec |
| 结构光照明 (SIM) |  | 60 nm | 10 – $10^2$ | > 100 FPS |

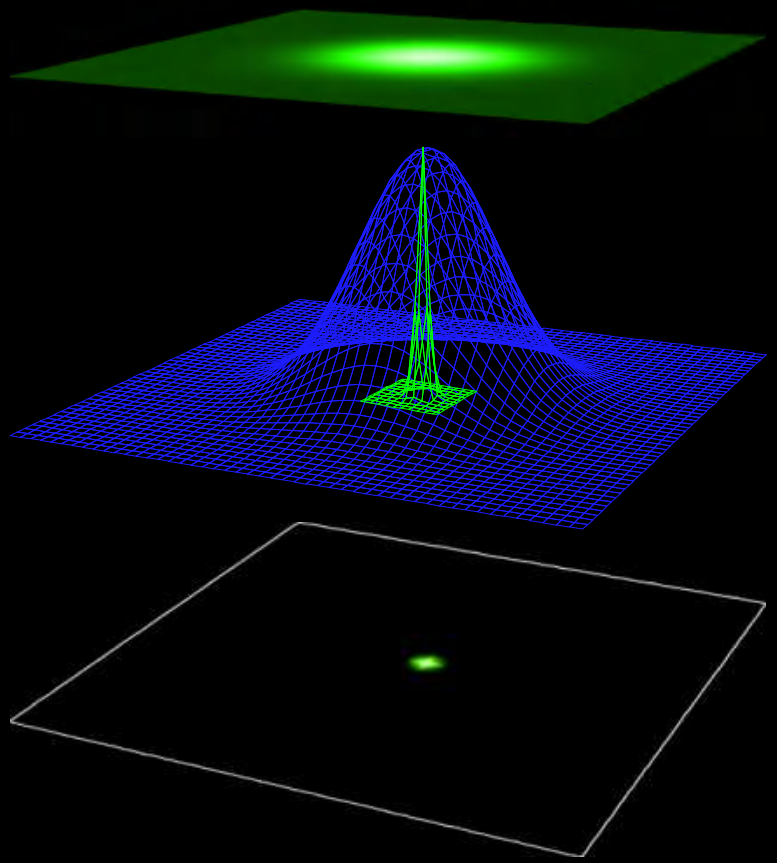
# 单分子定位显微技术原理

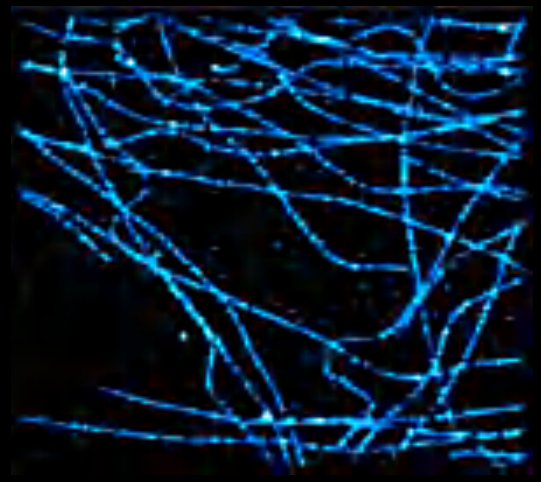
## Single Molecular Localization Microscopy



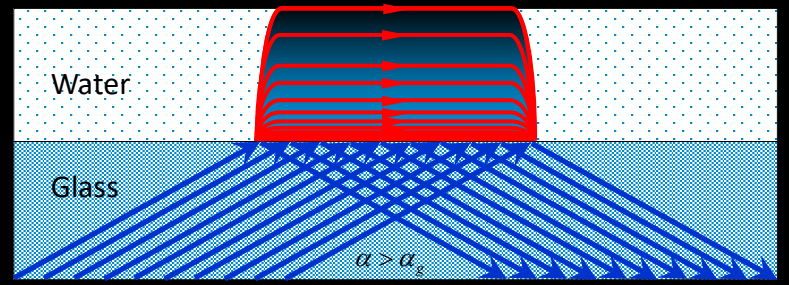
d = 240 nm

10x





d = 20 nm




TIRF 全内反射

**PAL-M**

Photoactivation Localization Microscopy

**dSTORM**

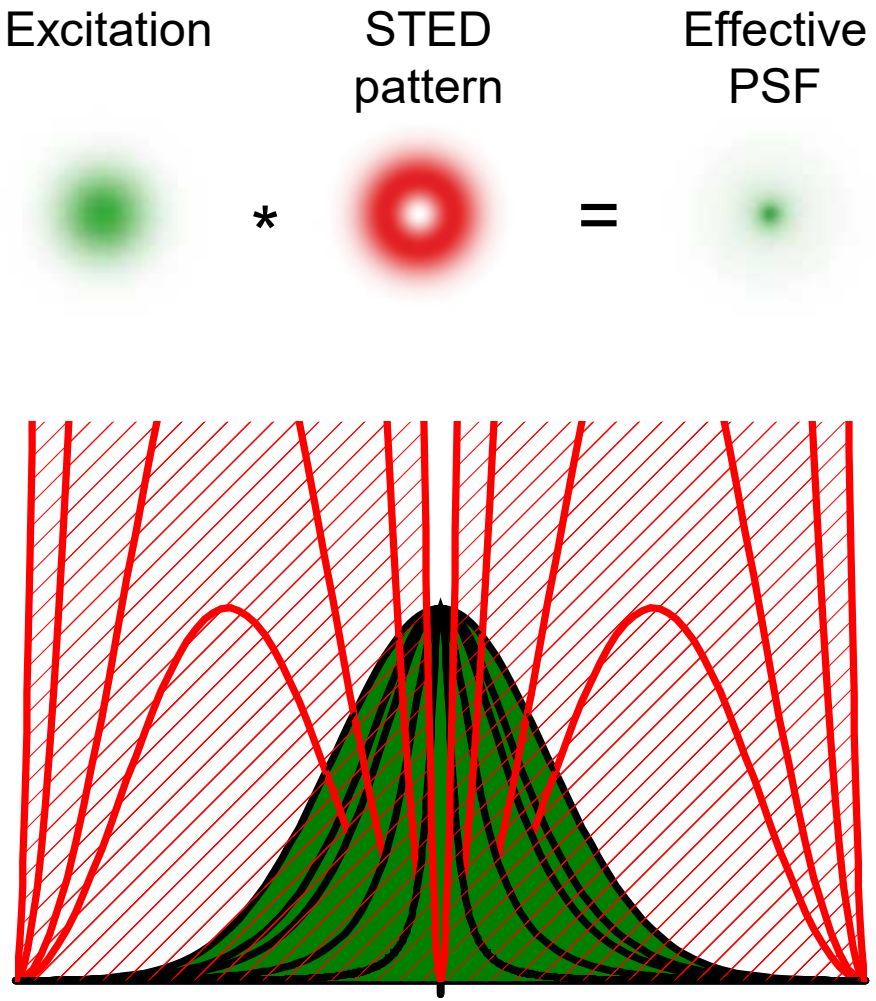
direct stochastic optical reconstruction microscopy

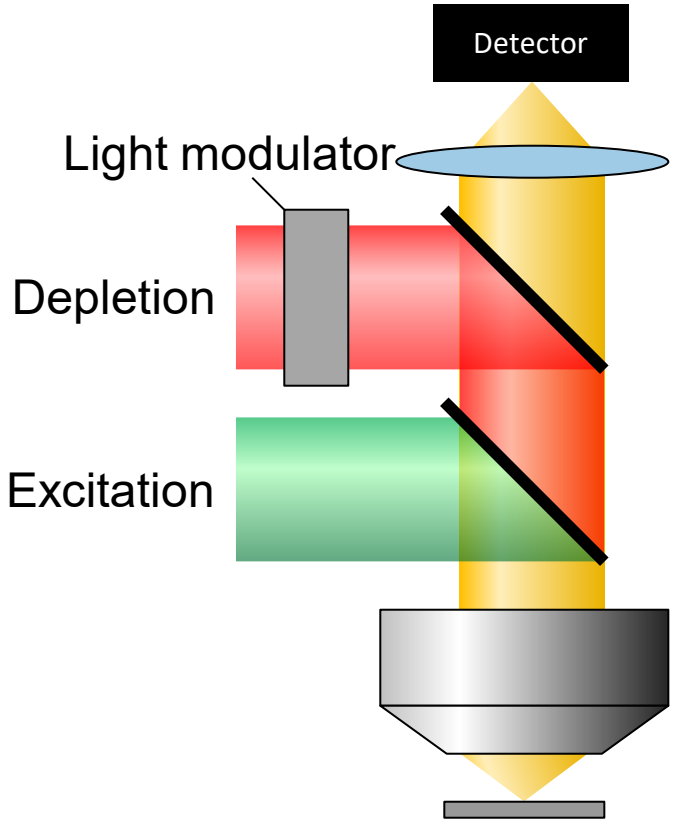
**PAINT**

Point Accumulation for Imaging in Nanoscale Topography

# 受激发射耗散原理

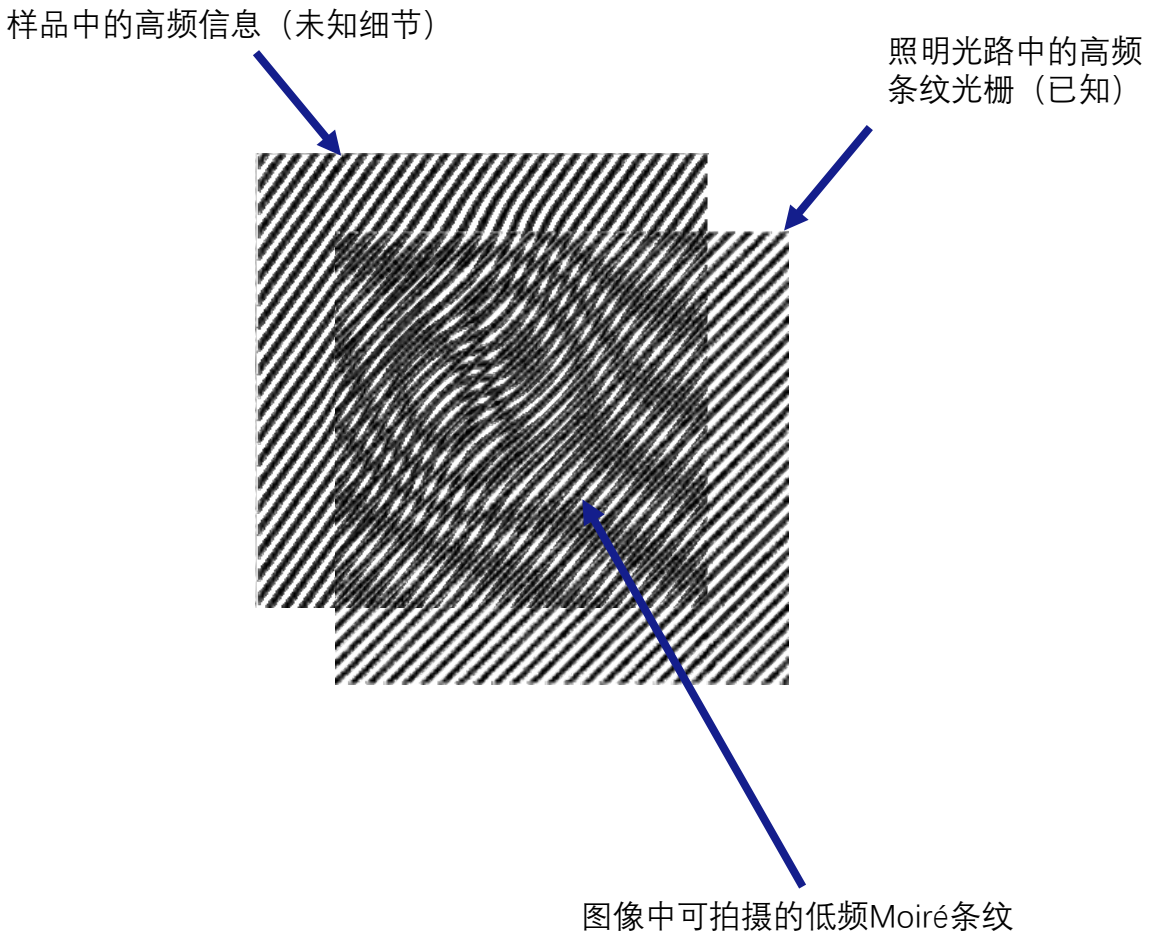
## Stimulated Emission Depletion Microscopy



# SIM工作原理

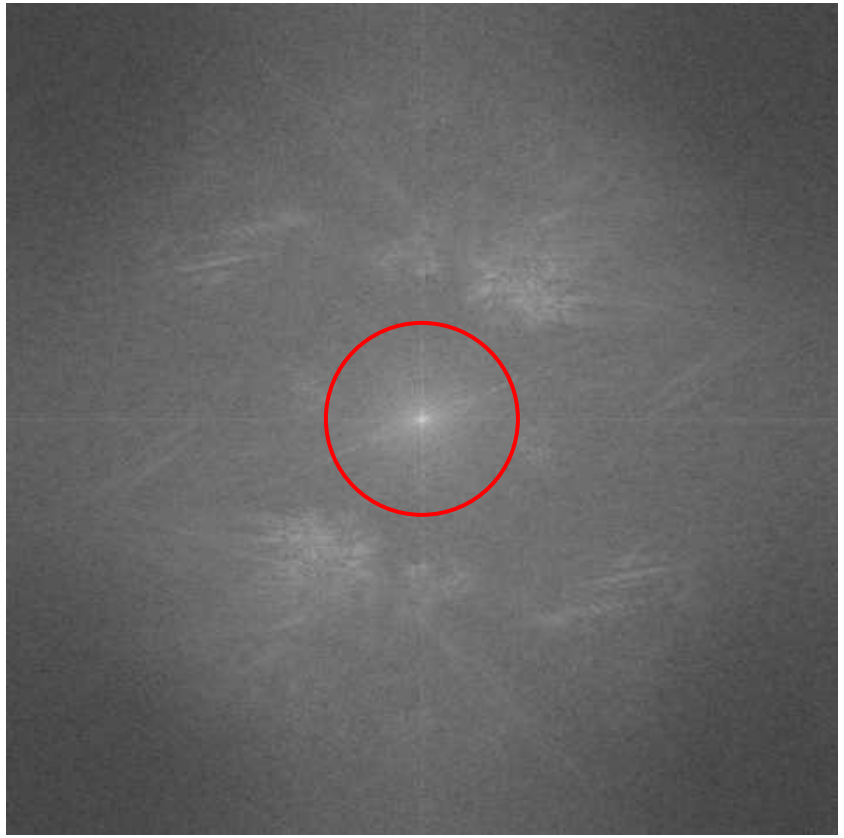
## 莫尔条纹



# SIM工作原理

## 傅里叶变换

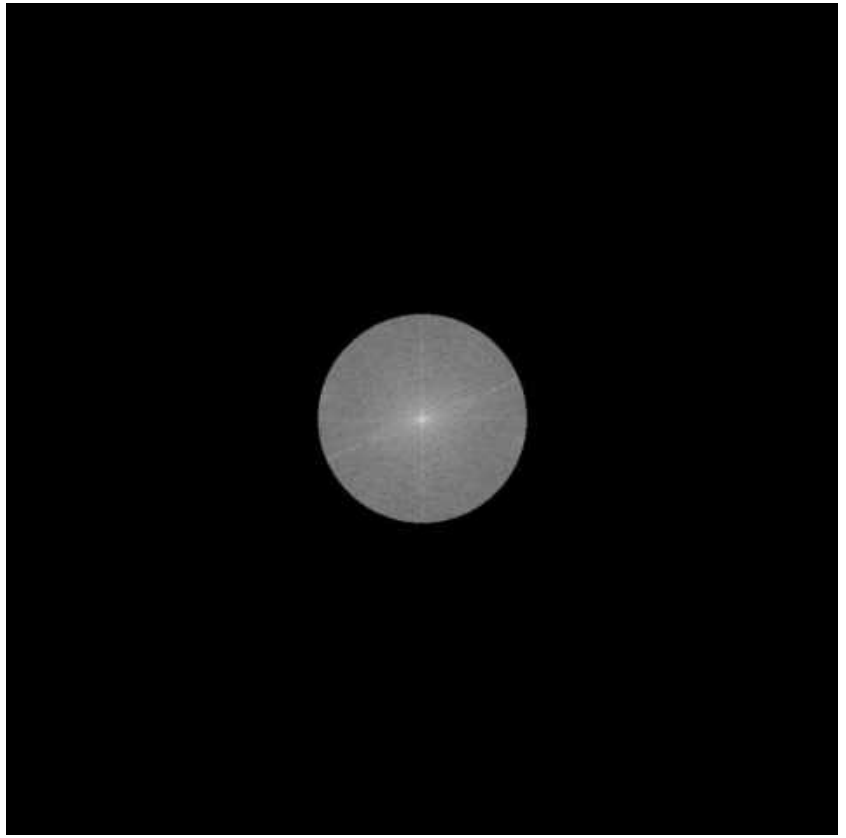
That is how Mr. Fourier looks like in Fourier space



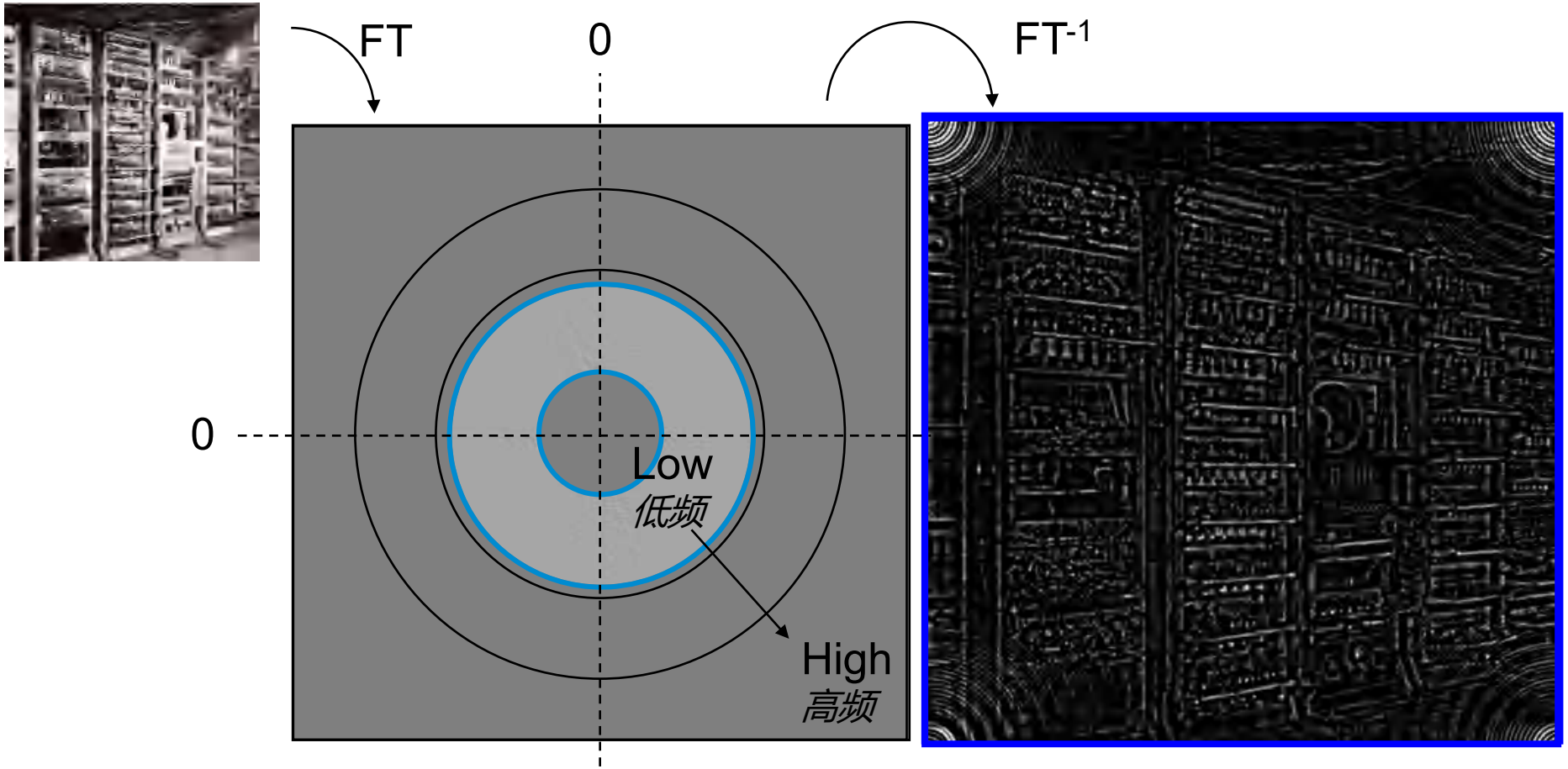
# SIM工作原理

## 傅里叶变换

Blurry Mr. Fourier

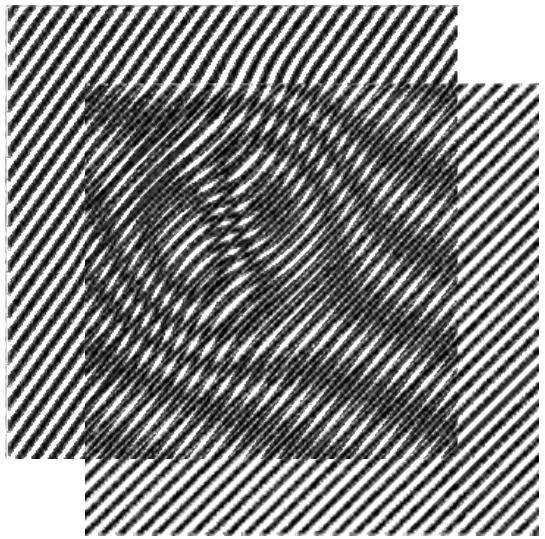


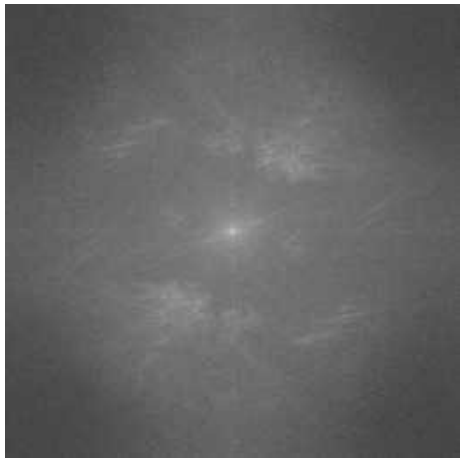
# 傅里叶频域与图像



# SIM工作原理

## 解方程——XY=Z





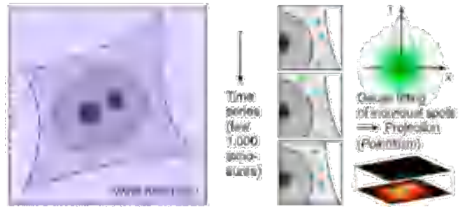
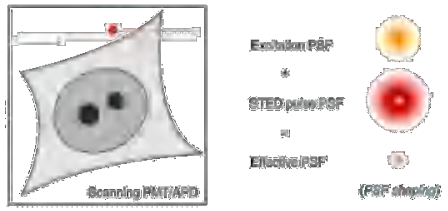
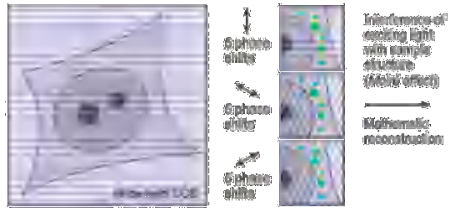
样品高频信息 + 已知高频光栅 → 编码 → 低频Moiré条纹 → 相机成像 → 傅里叶变换 → 相位频域图像 → 解码 → 样品细节

# 超分辨显微技术的机遇与挑战

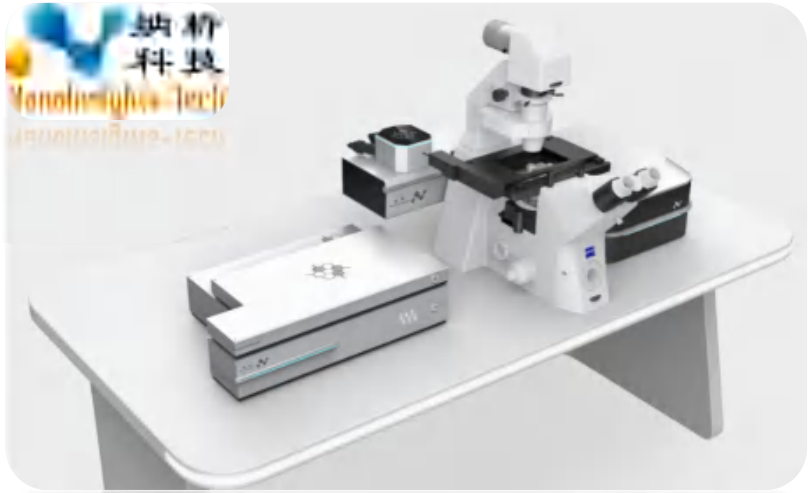

- 牺牲 **时间分辨率/低光毒性** 换取 **空间分辨率** 提高

↓

- 长时程高速超分辨**活细胞**成像难以实现

| | | 分辨率 | 光强(W/cm²) | 采集时间 |
|---|---|---|---|---|
| 单分子定位 (PALM/STORM) |  | 20 nm | $10^3$ - $10^4$ | > 10 sec |
| 受激辐射耗尽 (STED) |  | 30 nm | $10^4$ - $10^7$ | > 10 sec |
| 结构光照明 (SIM) |  | 60 nm | 10 – $10^2$ | > 100 FPS |

# 蔡司，自主高端显微技术推动者与参与者



## 中国科学院生物物理研究所李栋团队发展

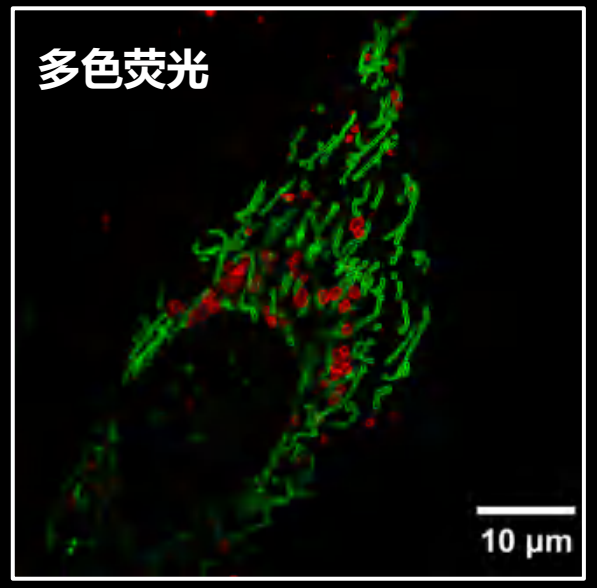
- 2015年《科学》杂志封面文章
- 2018年科技部“中国科学十大进展”

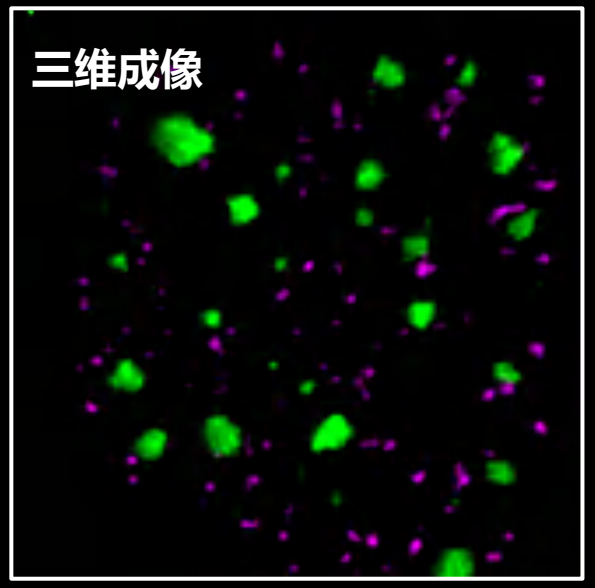
## 纳析科技 & 蔡司 联合设计、制造

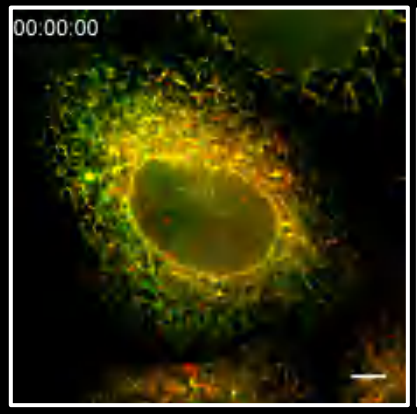
- 纳析科技继Multi-SIM后的新一代SIM产品
- 2023年6月全新发布上市

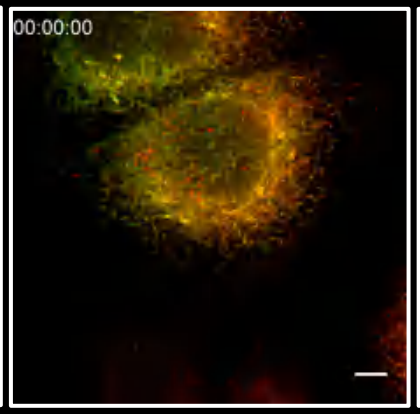
# Multi-SIM应用

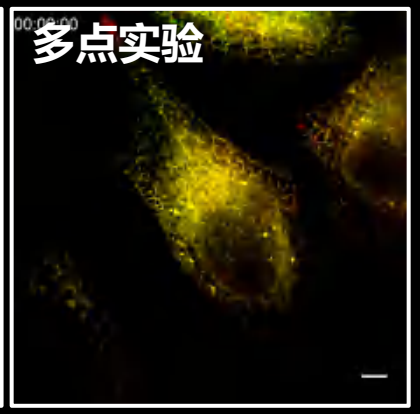
## 超高分辨率实现多色、三维、时间序列等多维实验

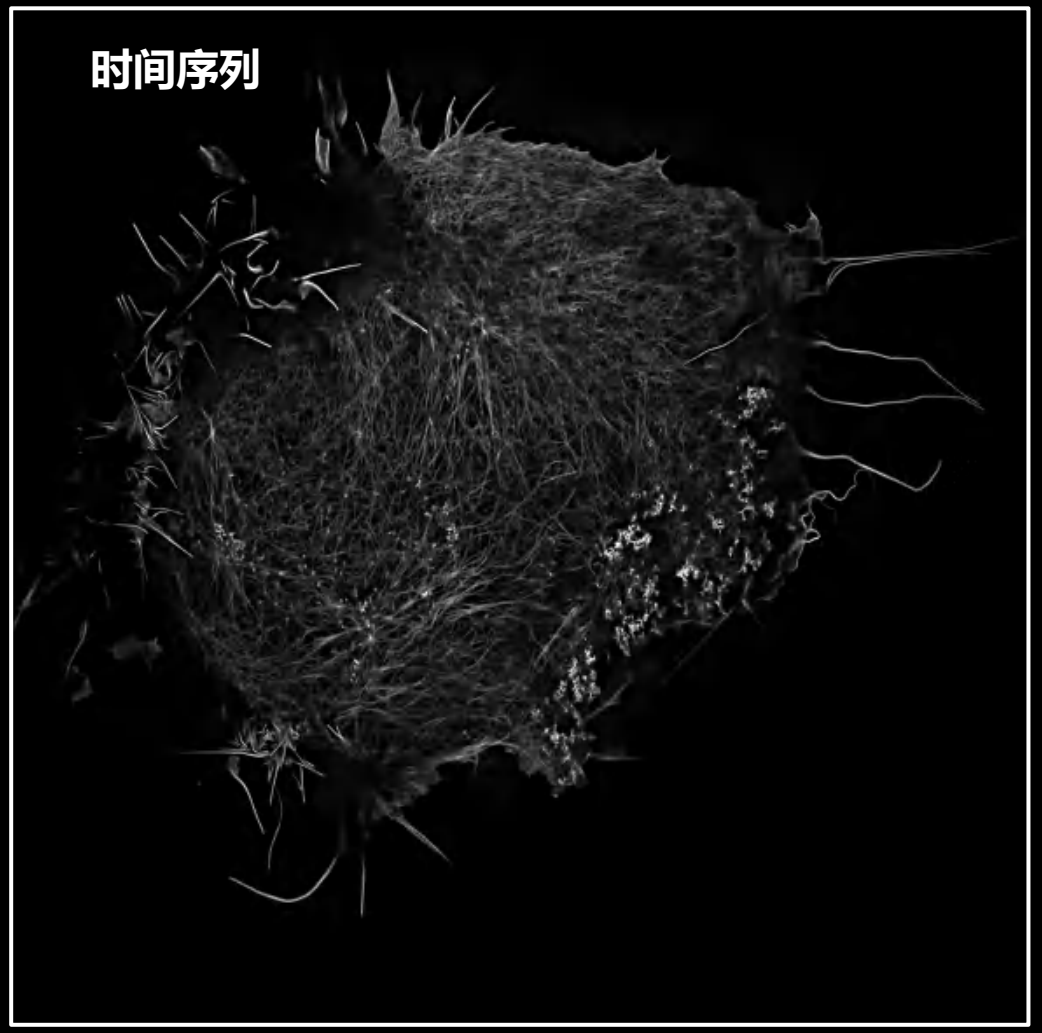


# 多模态

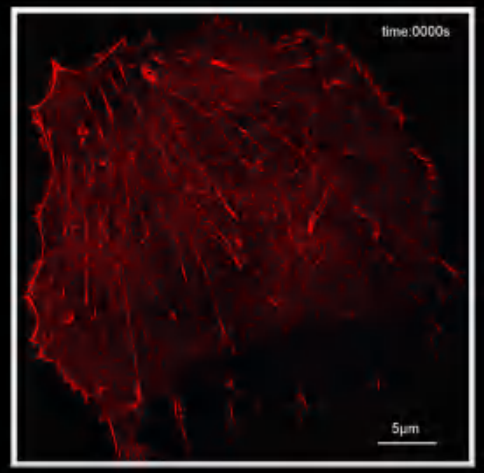
以GI-SIM为代表的高质量模态

多模态扩展SIM通用性

低光毒性延长成像时程

# 多模态SIM

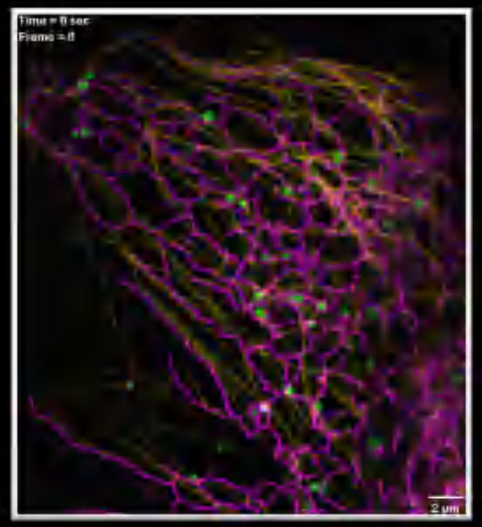
针对不同生命过程在体内定位和分布选择最优成像模态，扩展SIM通用性




TIRF-SIM

## GI-SIM→TIRF-SIM

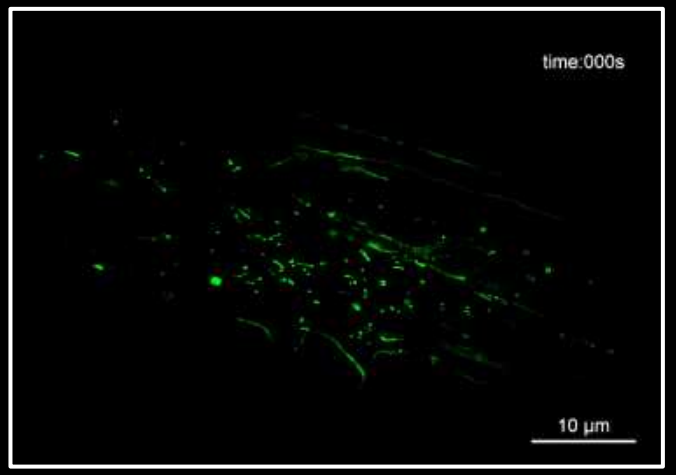
**精确调节成像厚度→ 降低光毒性→优化信噪比→延长成像时程**



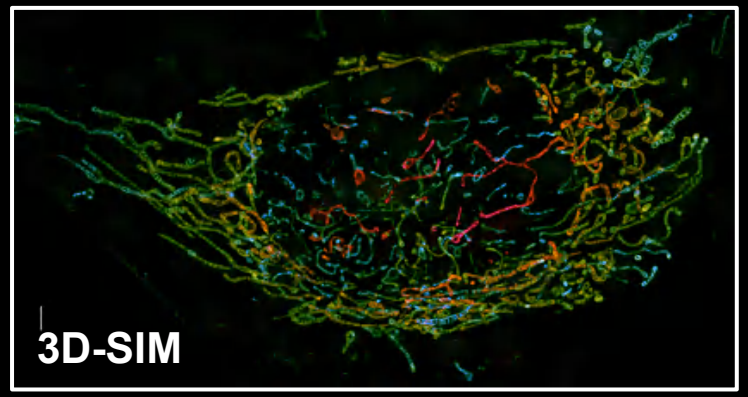

GI-SIM

## 3D-SIM→Stacked Slices-SIM→Single Slice-SIM

**减少无用信息采集→ 降低光毒性→延长成像时程**



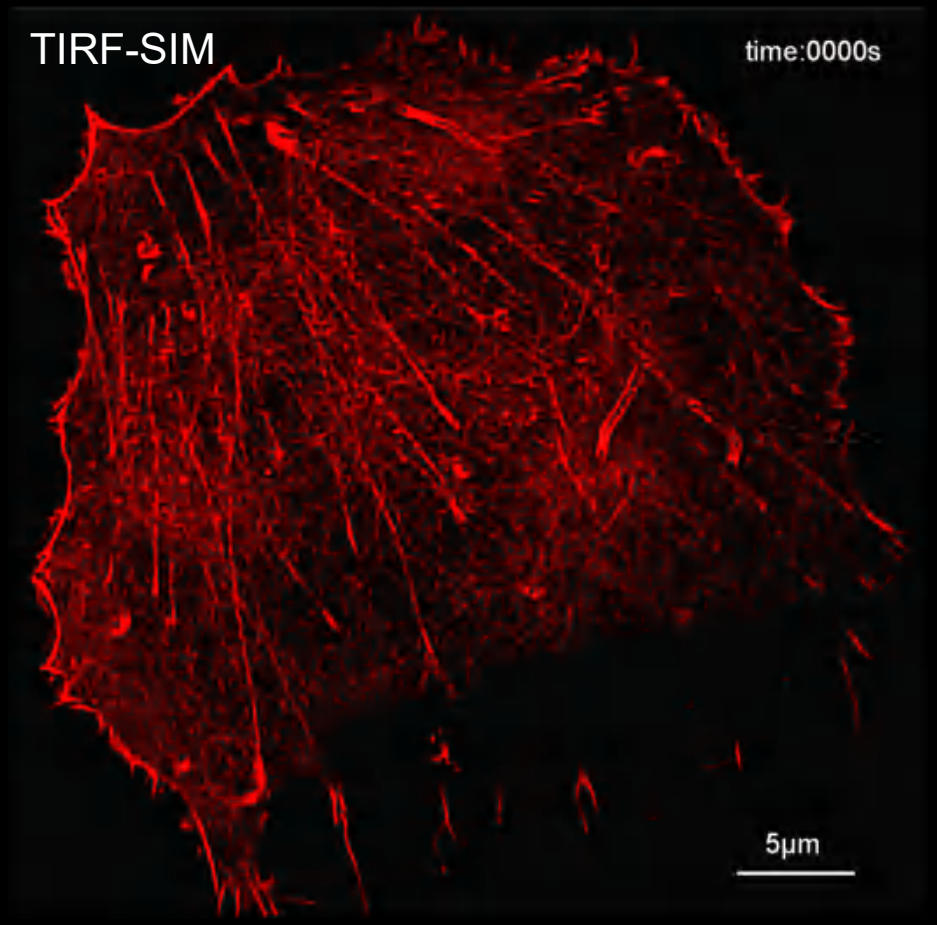

Single Slice-SIM

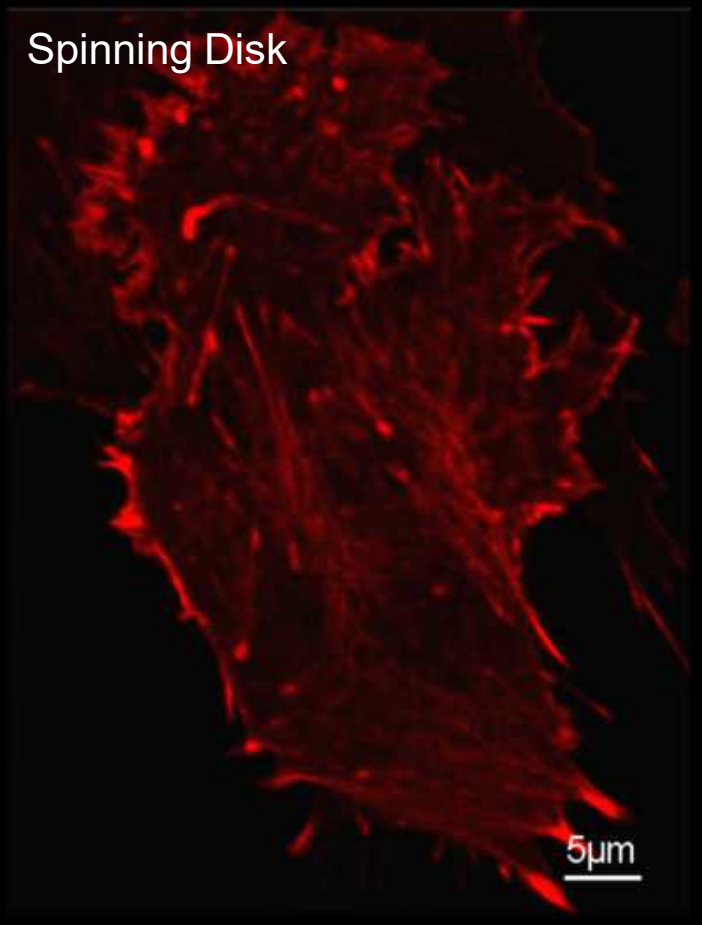


# TIRF-SIM

## 低光毒性研究细胞质膜附近生命过程

首次观察到病毒在细胞内呈桶状结构

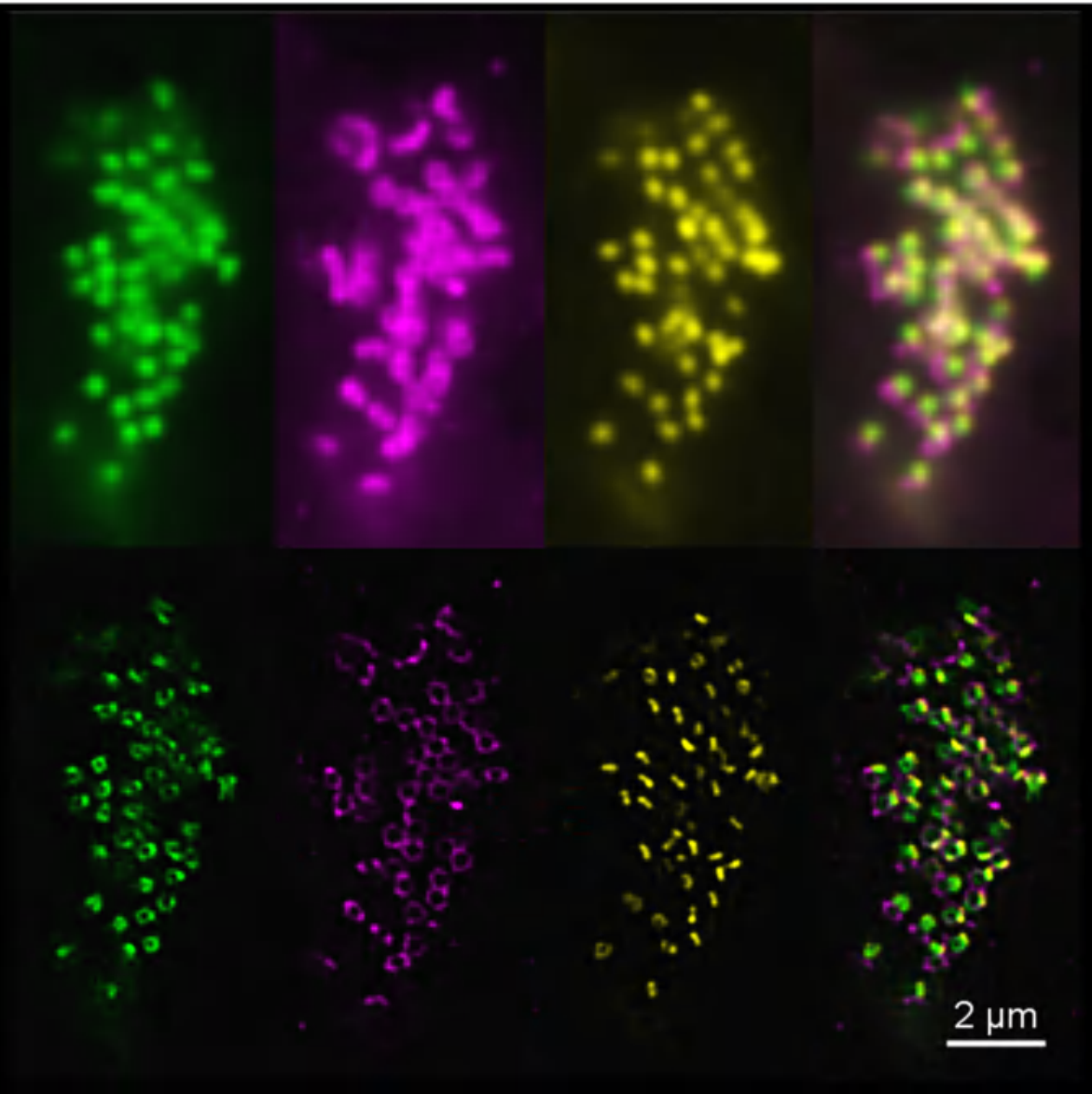



样本由复旦医学院刘老师课题组提供

# TIRF-SIM

## 低光毒性研究细胞质膜附近生命过程

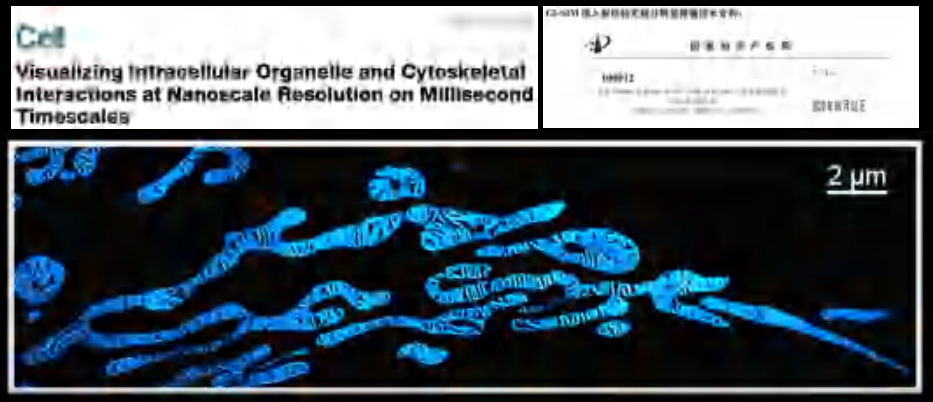



小鼠组织上纤毛结构

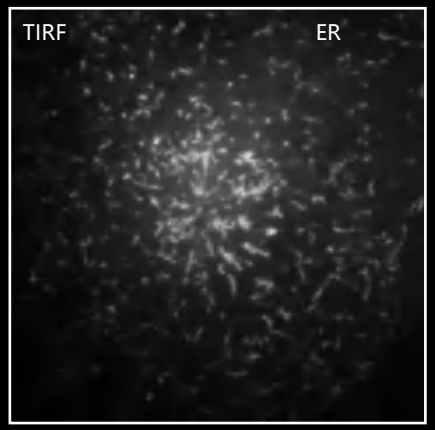
样本由生化所朱老师课题组提供

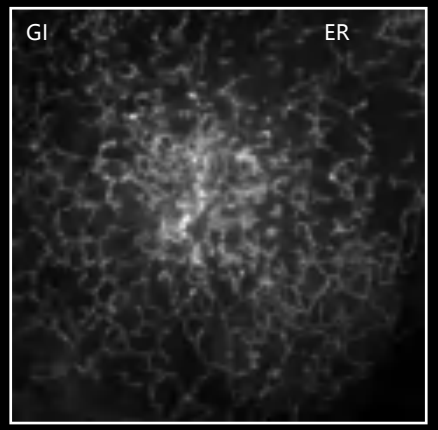
# GI-SIM

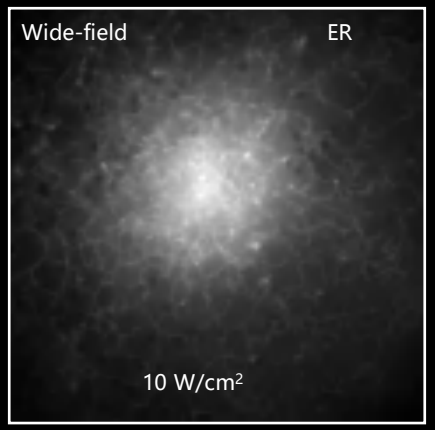
## 细胞器成像利器



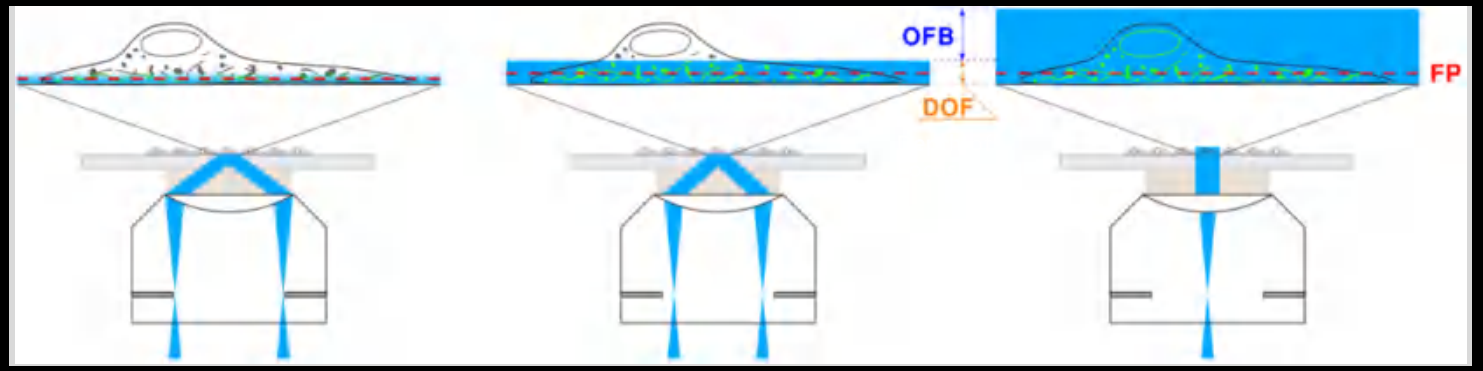

**入选2018年度 科技部“中国科学十大进展”**




成像深度: 全内反射 ~0.1 μm　　掠入射 ~1 μm

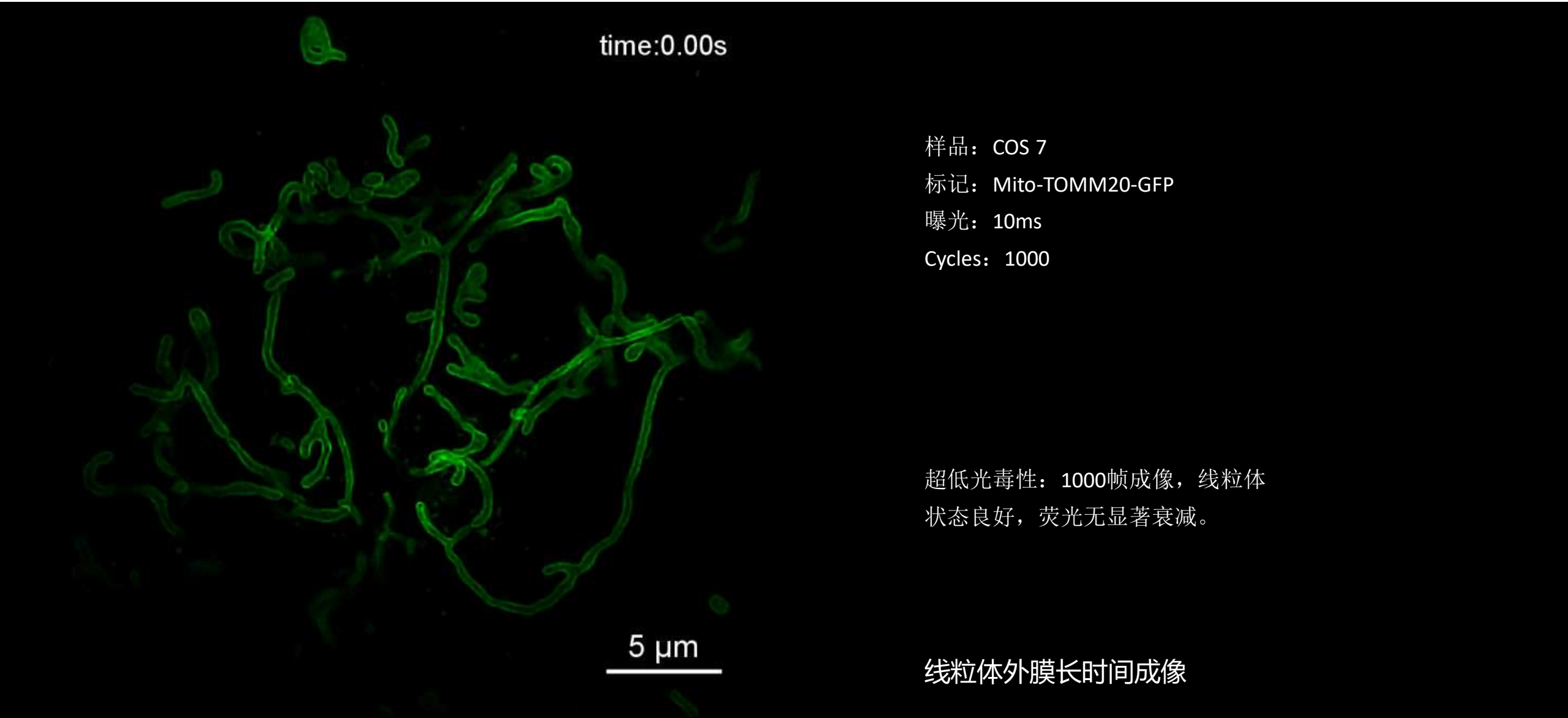


# GI-SIM

## 细胞器成像利器

# GI-SIM

## 细胞器成像利器




样品：COS 7

标记：Mito-TOMM20-GFP

曝光：10ms

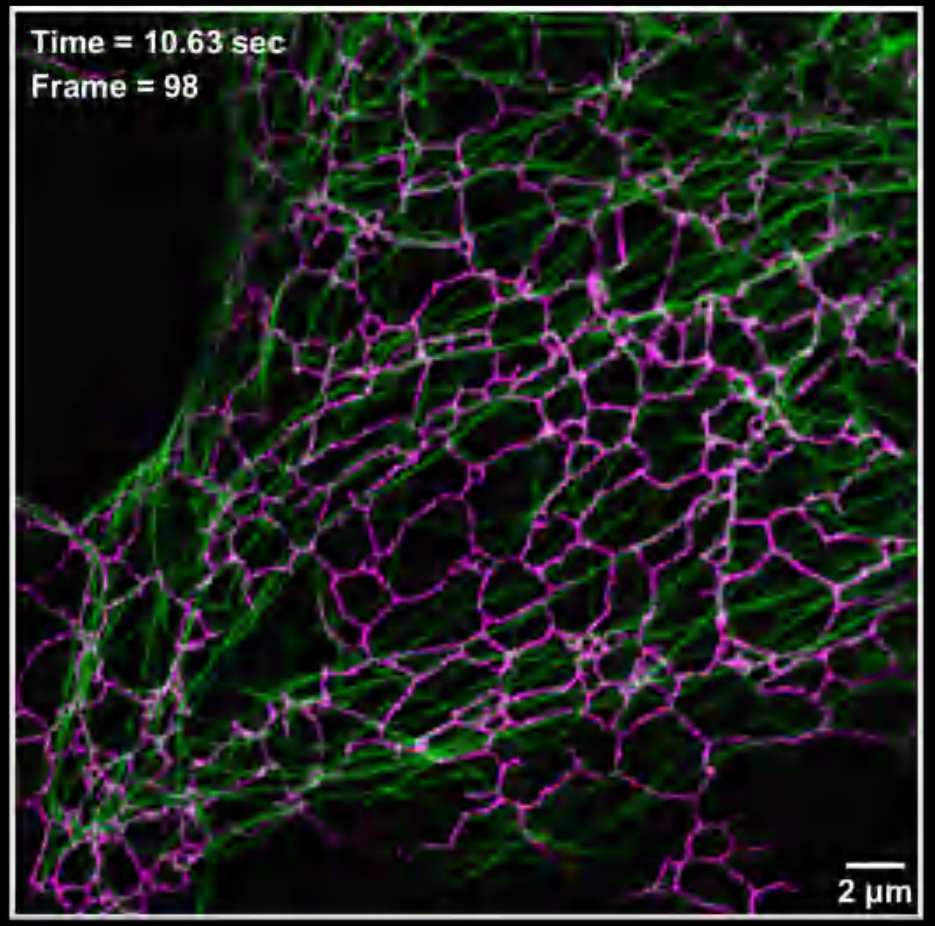
Cycles：1000

超低光毒性：1000帧成像，线粒体状态良好，荧光无显著衰减。

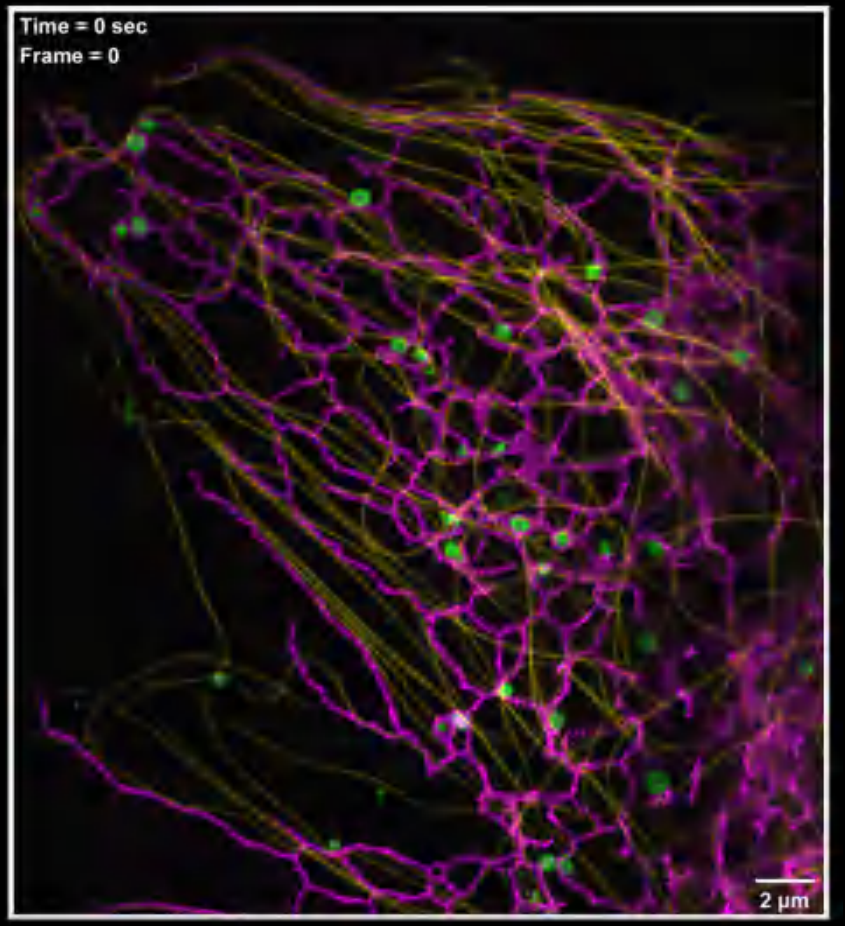
线粒体外膜长时间成像

# GI-SIM

## 细胞器成像利器



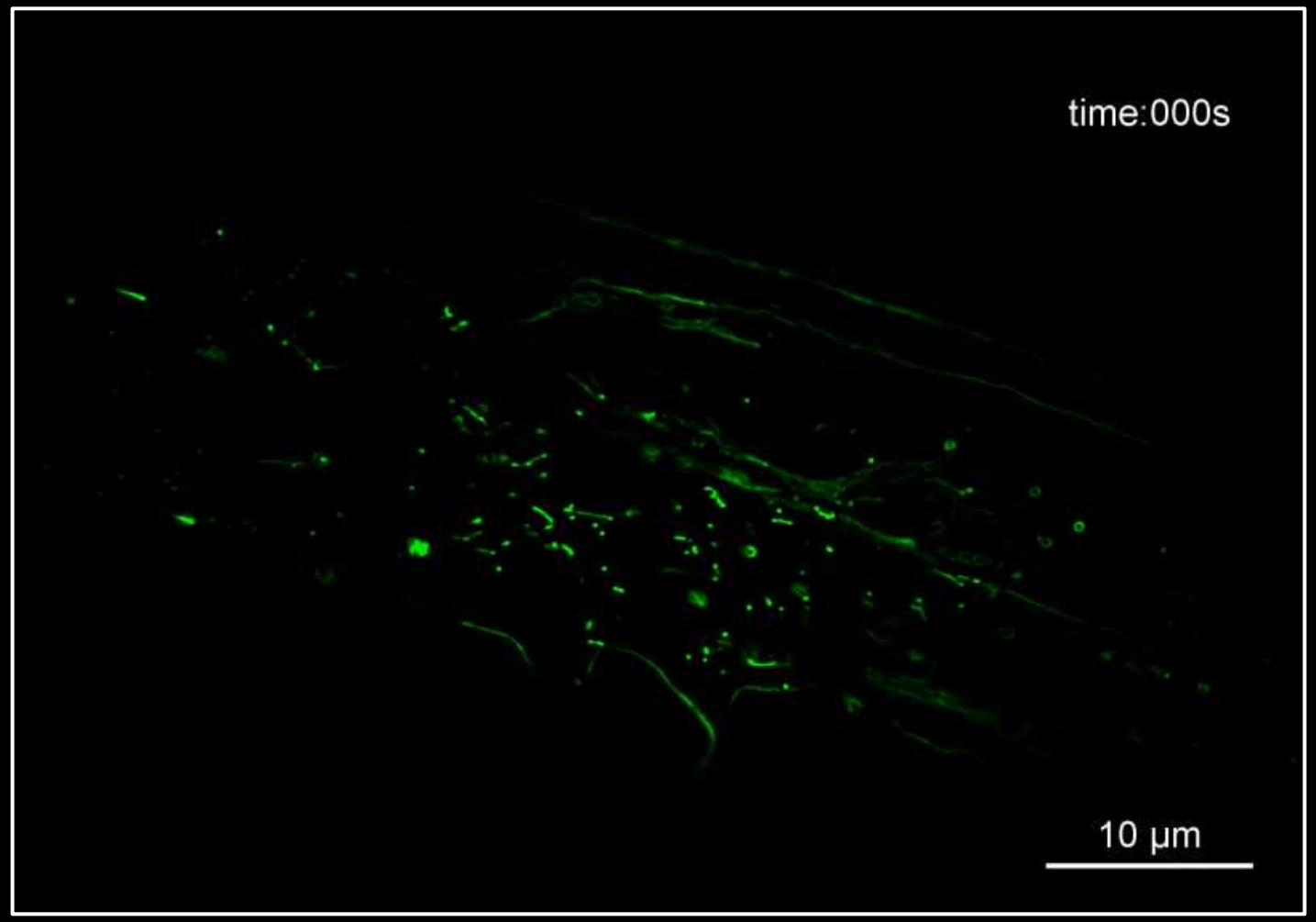

内质网的动态变化与微管具有高度相关性



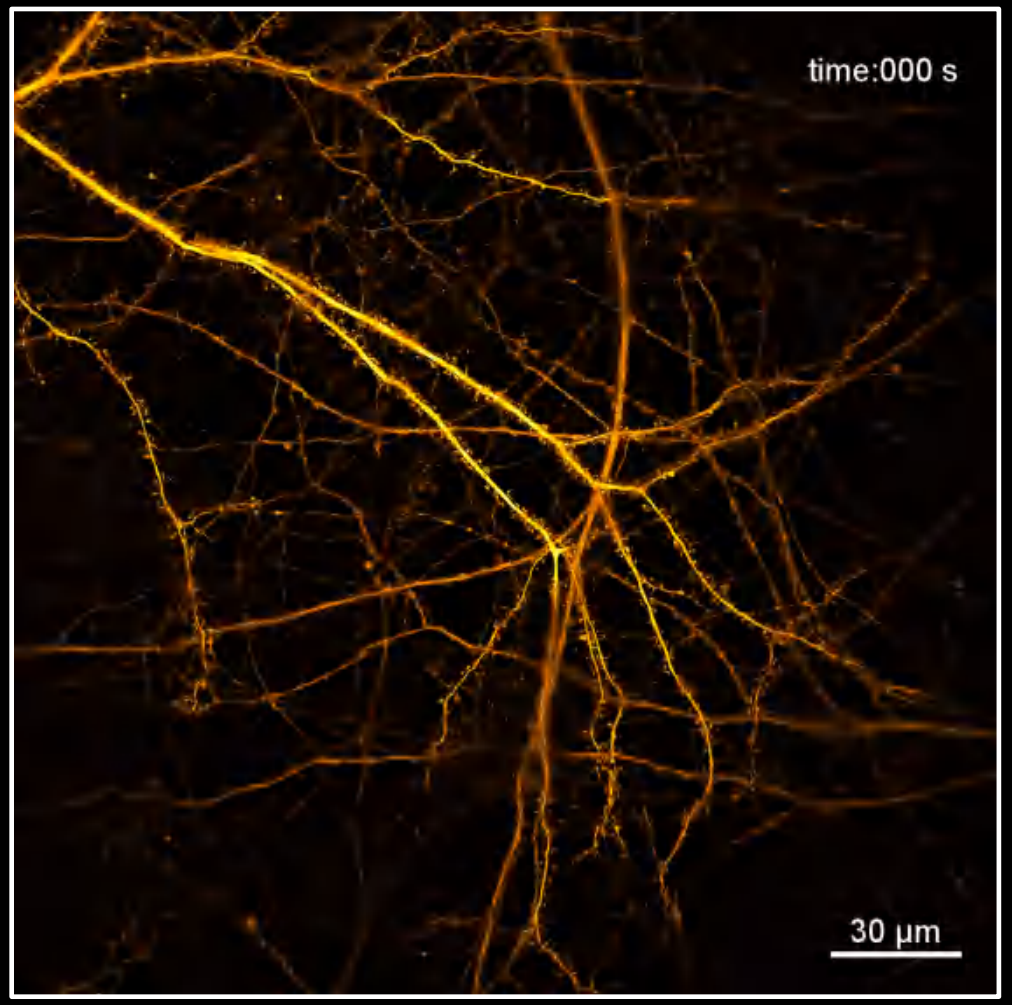

内质网与溶酶体互作调节溶酶体分布与运输

# Single Slice-SIM

## 厚样品深度成像



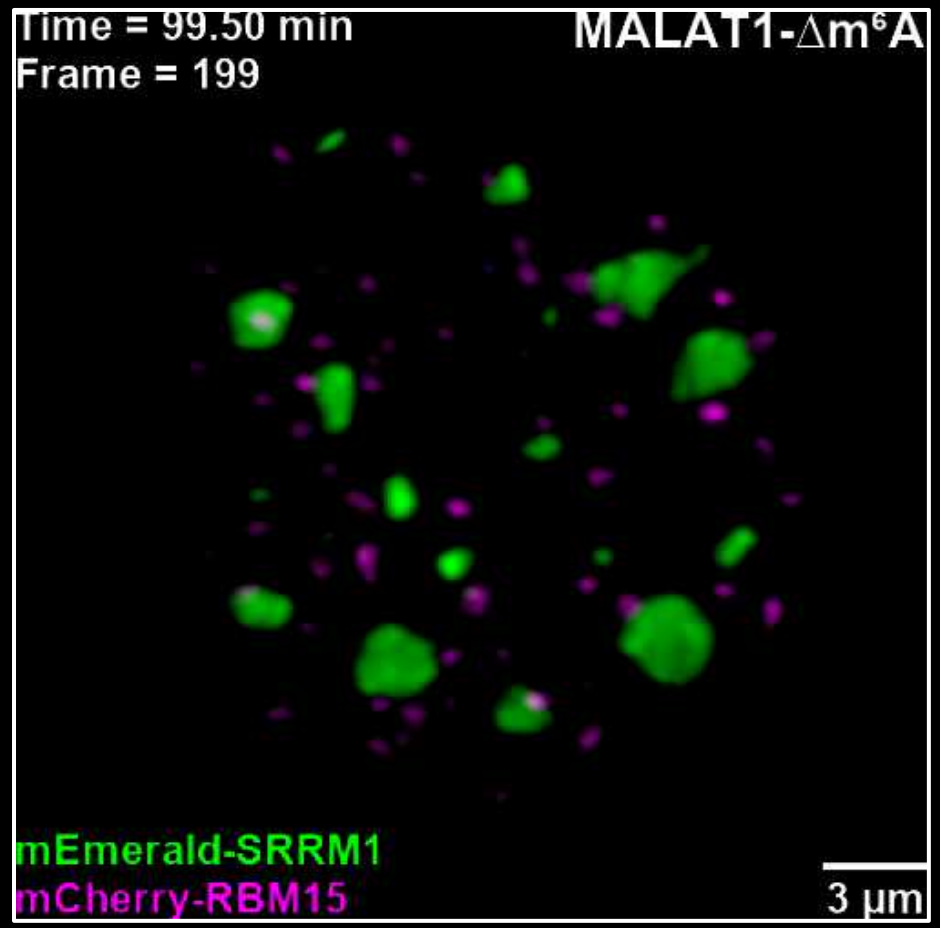

线虫体内囊泡运输



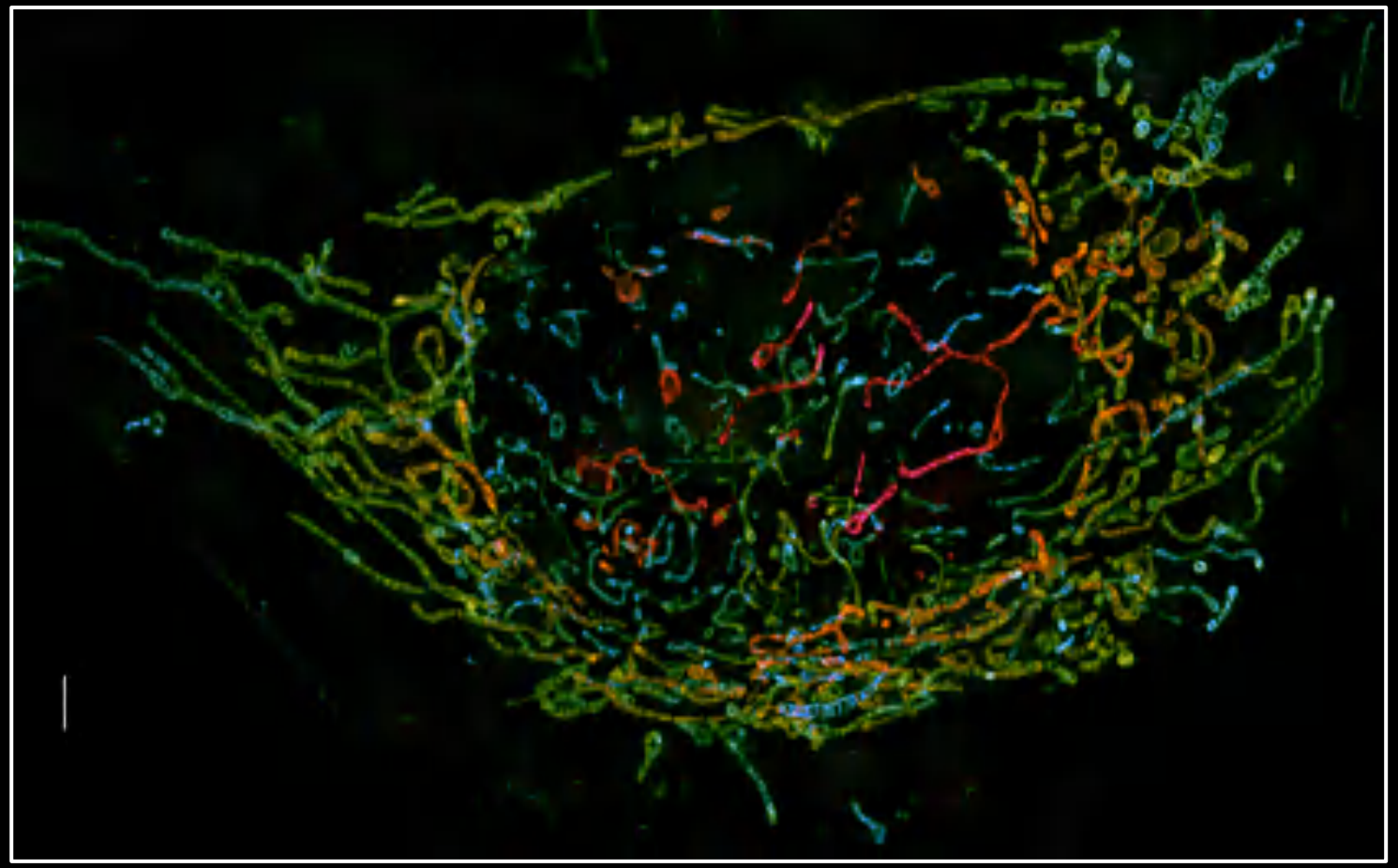

大视野神经元成像

# 3D-SIM

## 三维超高分辨率成像




核斑相关蛋白三维共定位及时空动态



线粒体内嵴的三维超分辨动态成像

# 速度和效率

高速活细胞成像 687 fps

大视野四色同步成像

# 687 fps活细胞高速成像

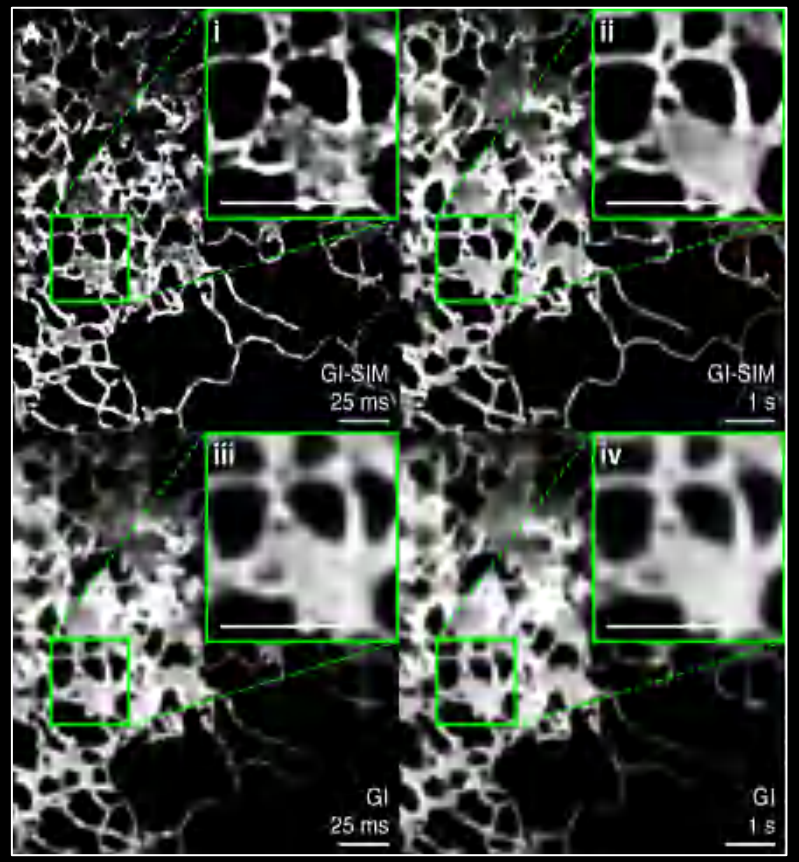
## 捕获高速动态样品变化

GI-SIM
40 Hz
97 nm

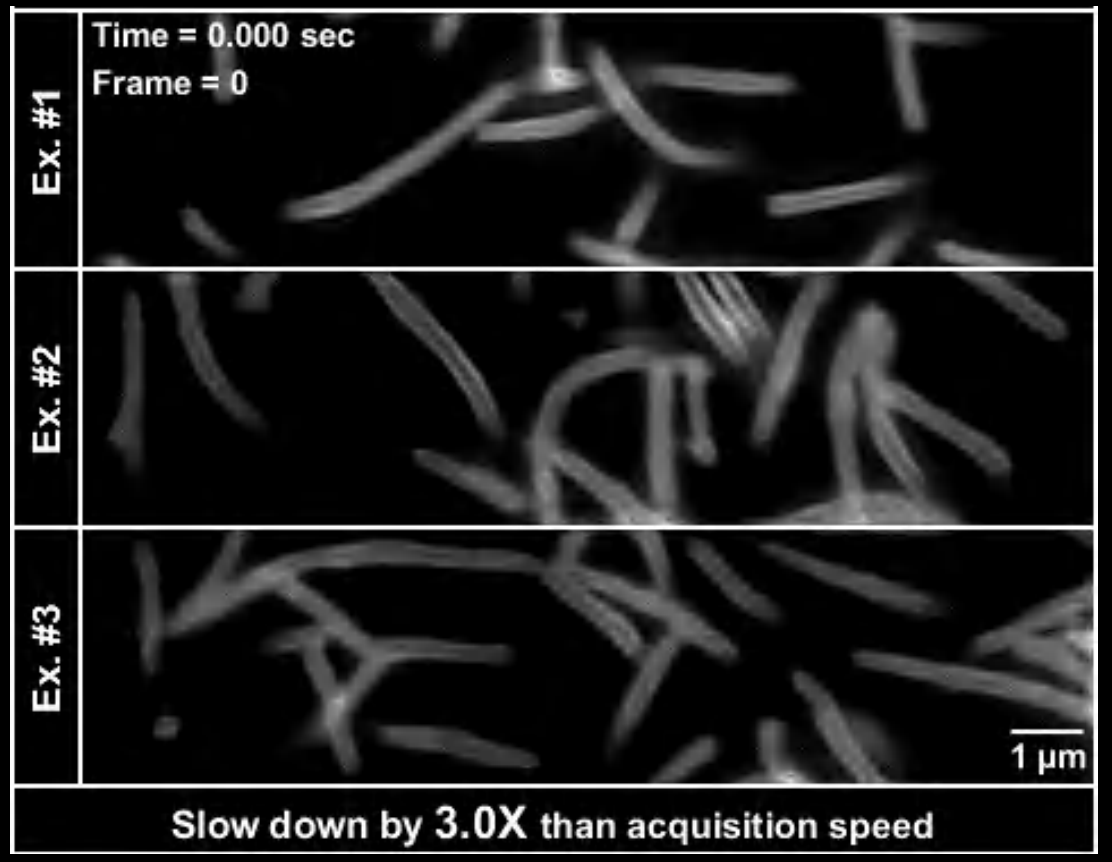
GI-SIM
1 Hz
97 nm

GI
40 Hz
220 nm

GI
1 Hz
220 nm




高时空分辨率观察片状ER孔洞结构
(*Science, 2016*)




高速摆动纤毛快速成像

**684Hz**成像速率进行了**60,000**时间点的连续超分辨观测，无明显光漂白或细胞活性损伤

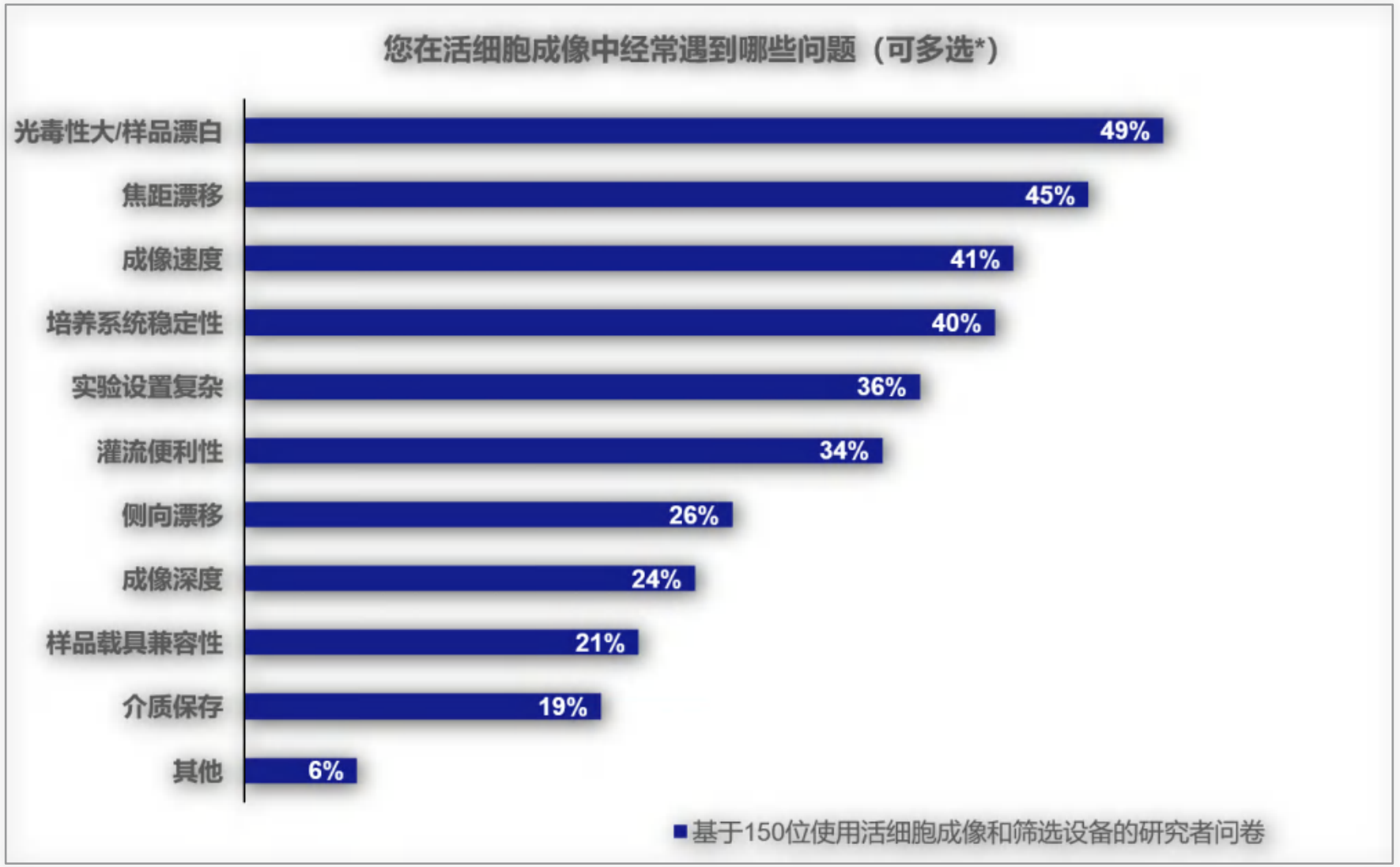
# 可靠性

蔡司光学加持的硬件配置

简单易用的软件模块

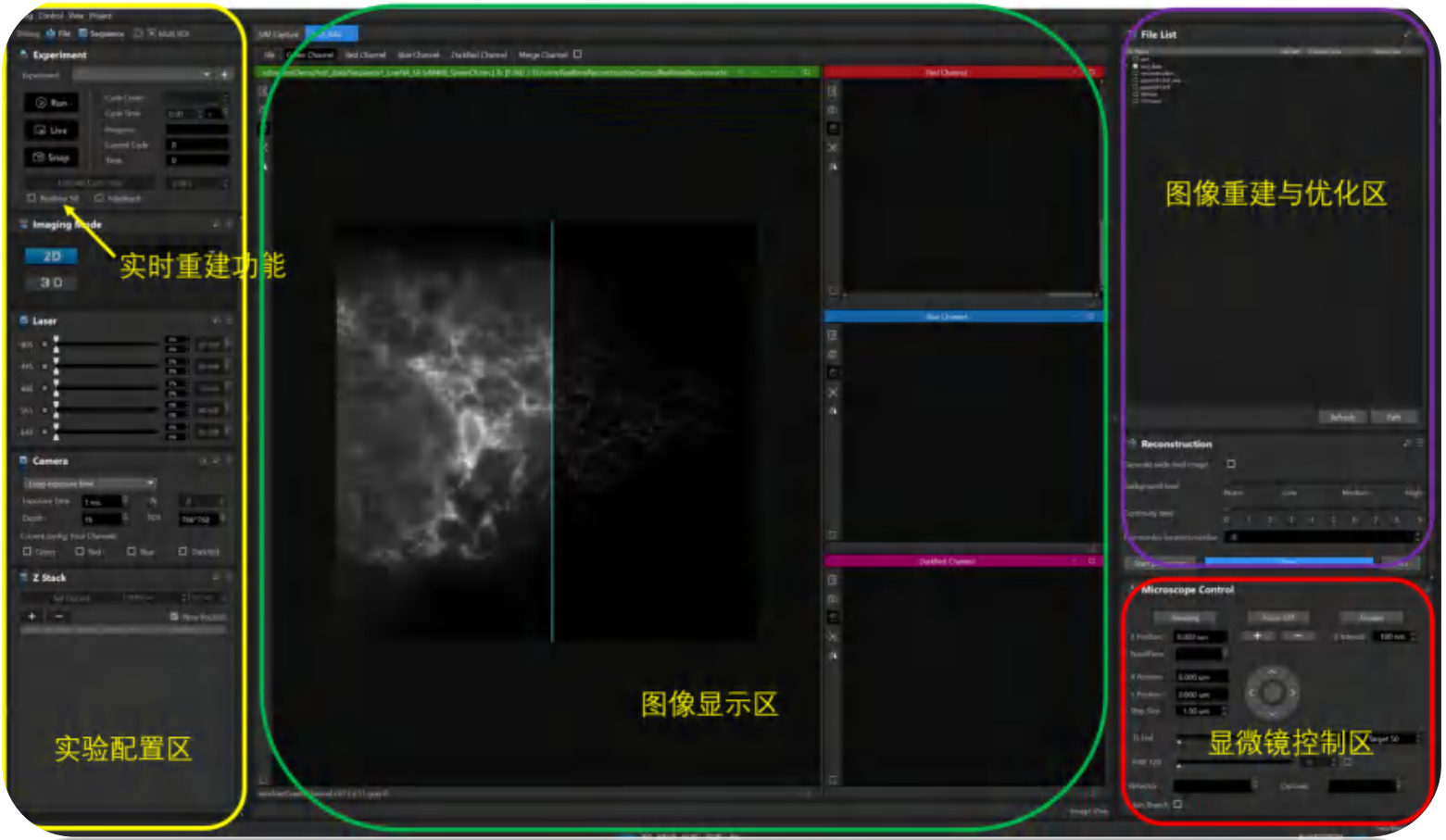
智能算法体系

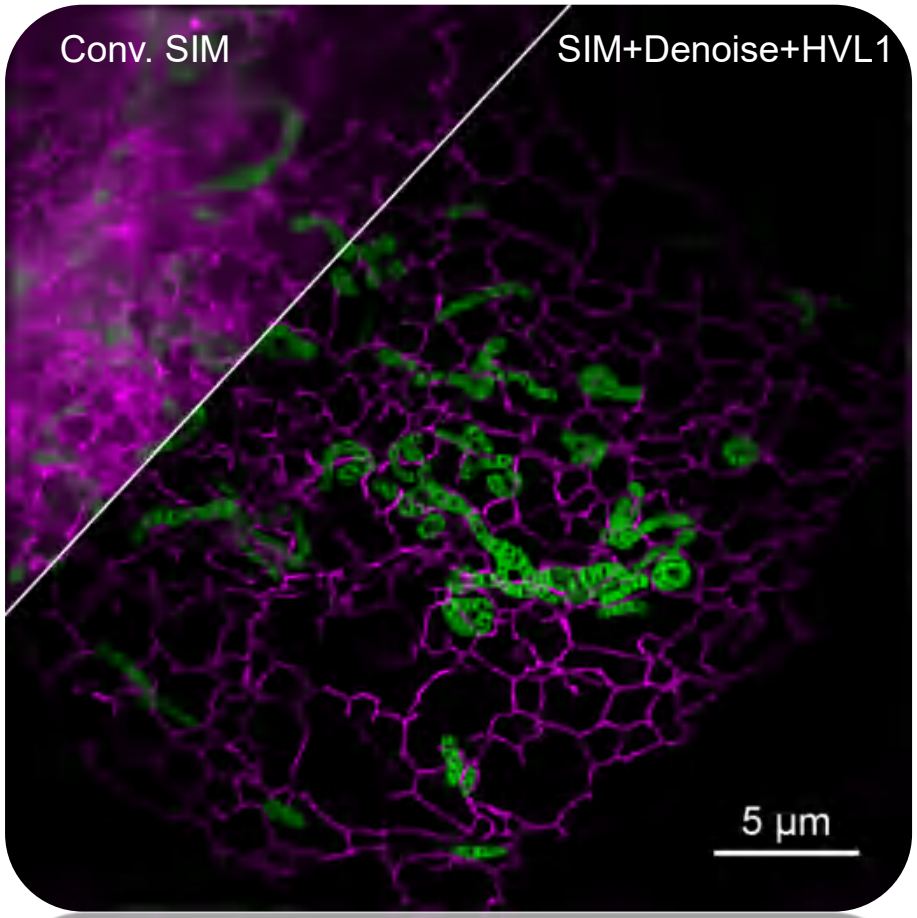
# 活细胞成像常见问题


您在活细胞成像中经常遇到哪些问题（可多选*）
光毒性大/样品漂白
49%
焦距漂移
45%
成像速度
41%
培养系统稳定性
40%
实验设置复杂
36%
灌流便利性
34%
侧向漂移
26%
成像深度
24%
样品载具兼容性
21%
介质保存
19%
其他
6%
基于150位使用活细胞成像和筛选设备的研究者问卷

# 简单易用的软件模块

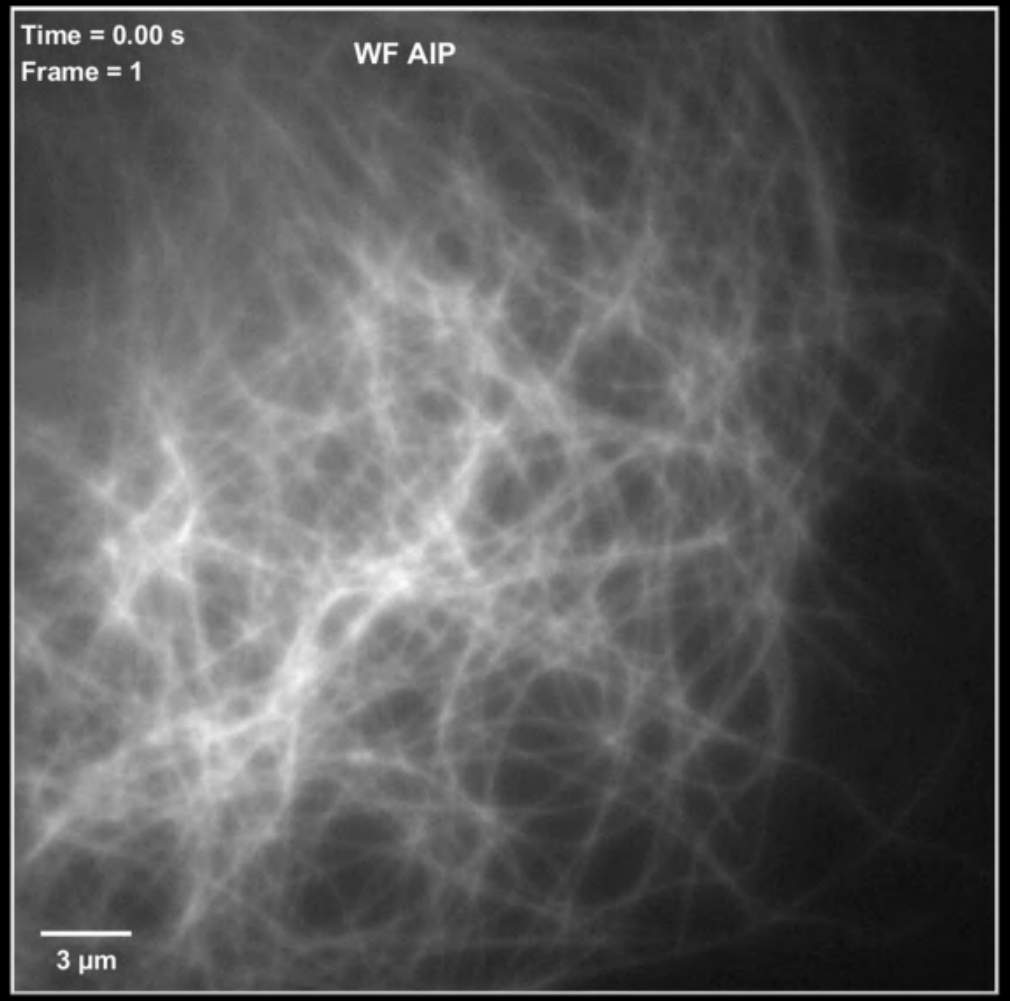
## 重建参数一键调节



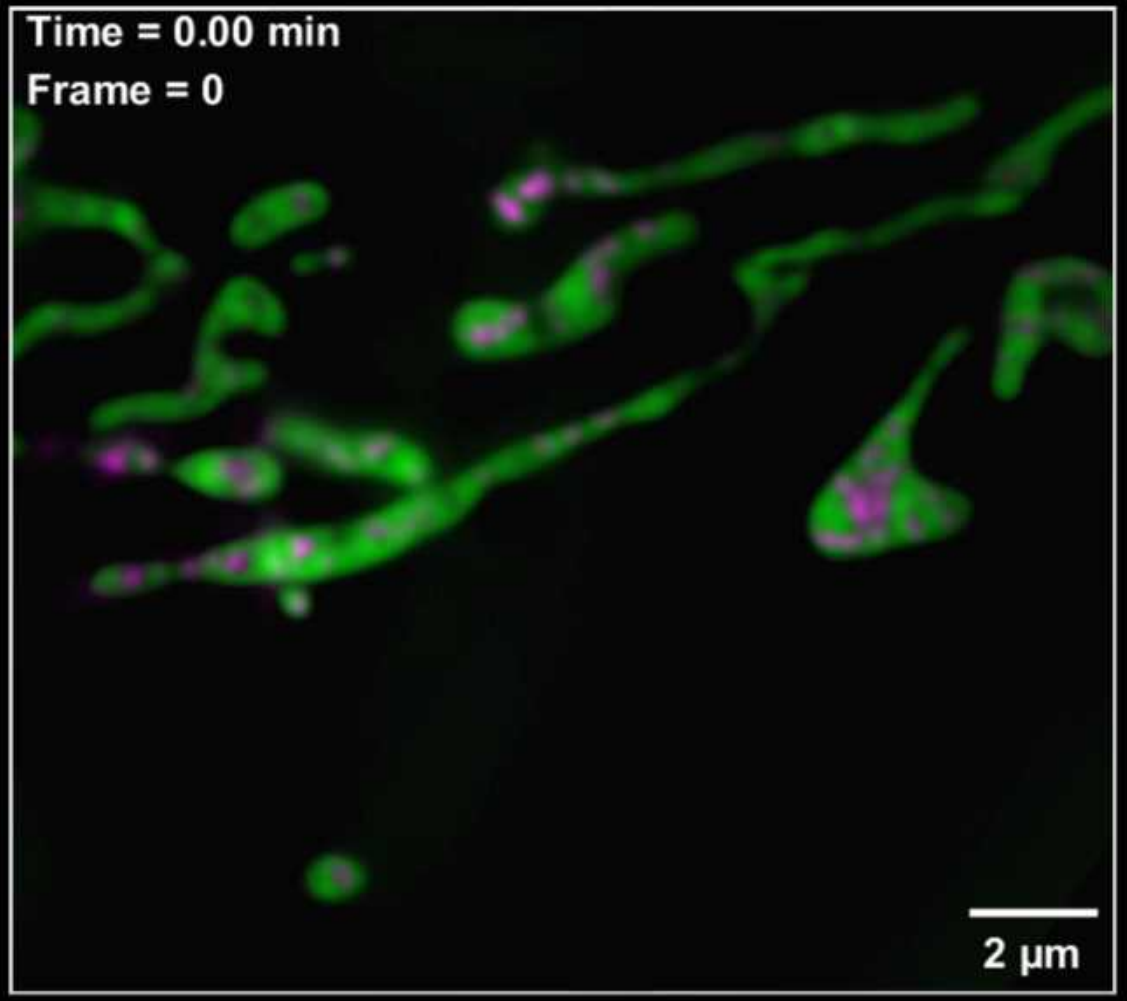



# DFGAN超分辨算法

## 弱荧光原始数据到高信噪比超分辨



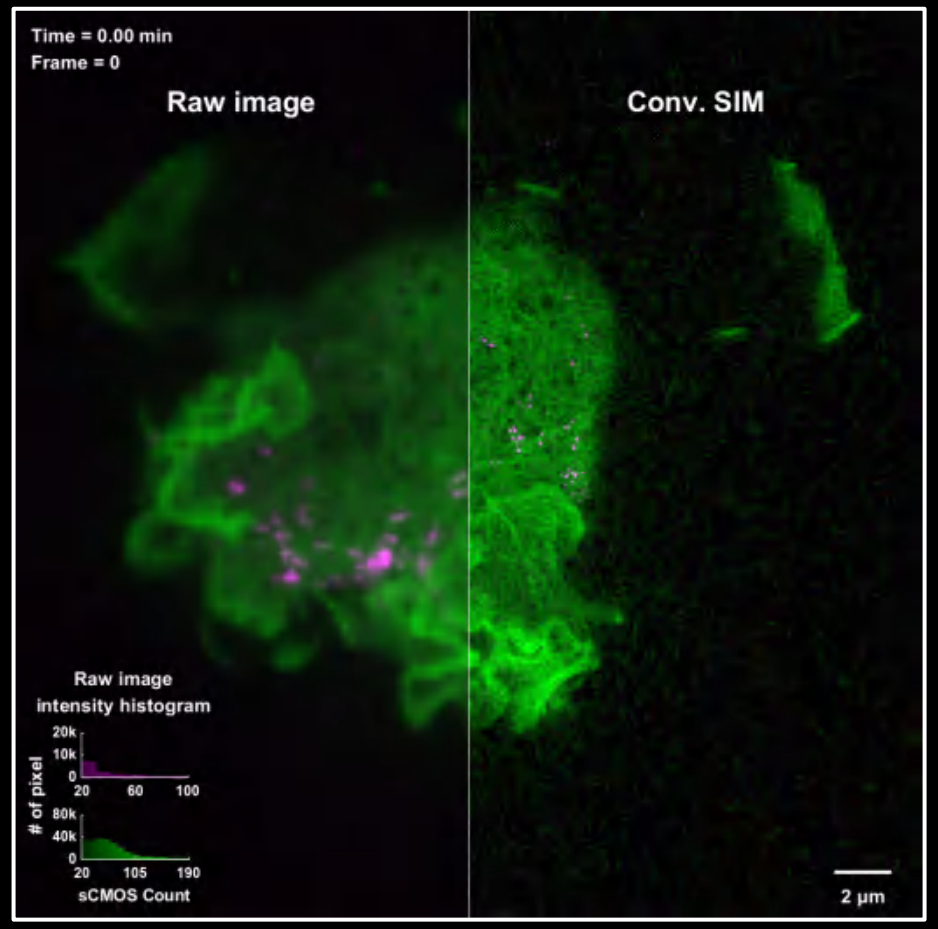

微管及溶酶体DFGAN超分辨成像




线粒体DFGAN超分辨成像

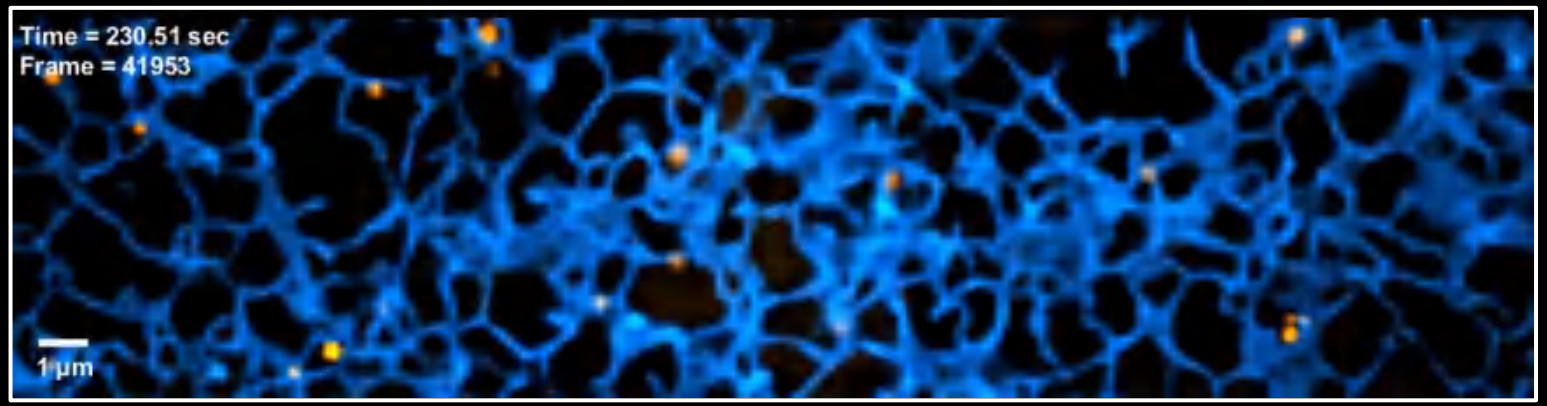
# rDL智能去噪算法

## 10倍以上去噪性能，30倍以上成像时程提升




U2OS细胞动态

高分辨记录F-actin和肌球蛋白myosin-IIA在长达一小时的沉降过程中的重塑动力学

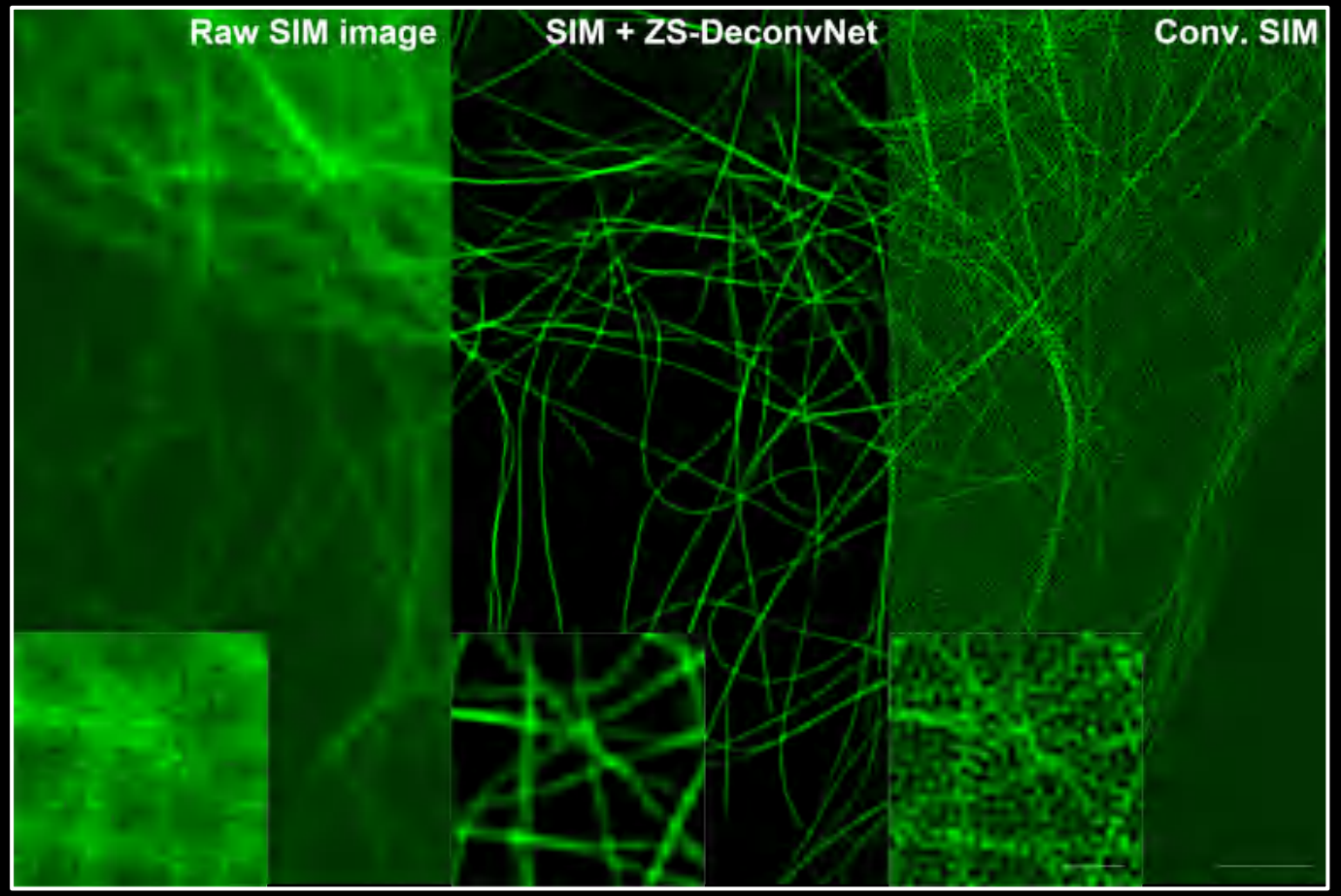



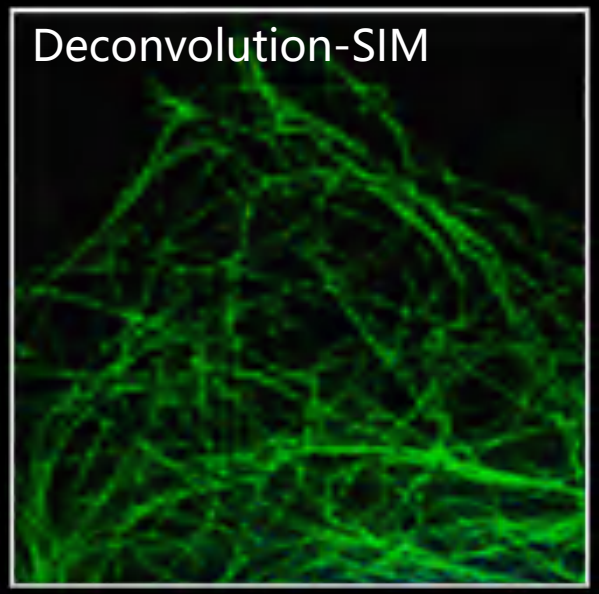

COS 7细胞ER和PO动态交互

mEmerald-KDEL (blue) and mCherry-SKL (orange)，45,000时间点

# 深度学习解卷积算法

## 稳定将显微图像信噪比提升10倍以上、分辨率提升1.5倍以上



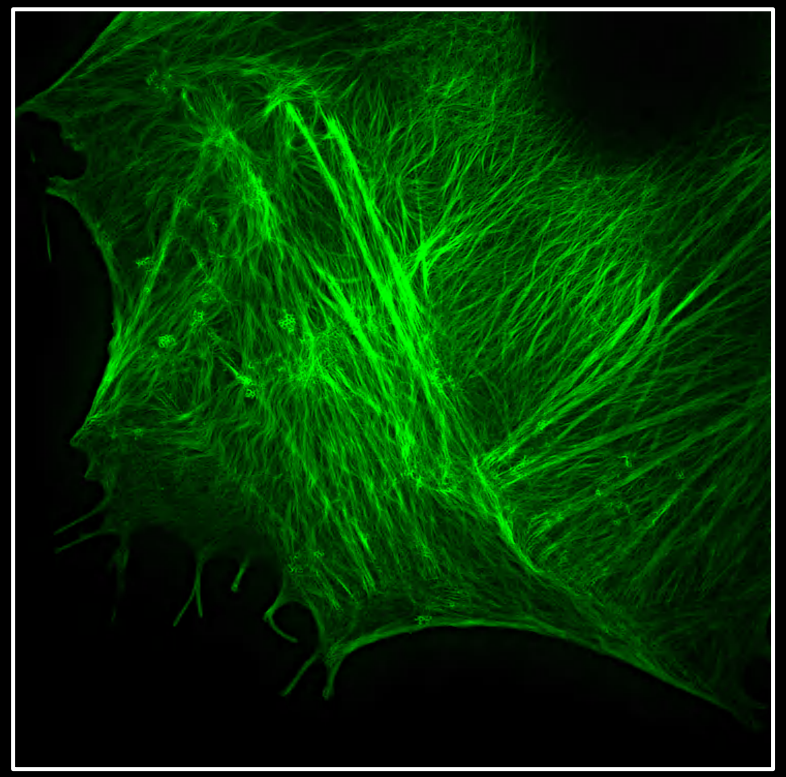




大量去卷积使弱信号遗失，
微管出现断续失真

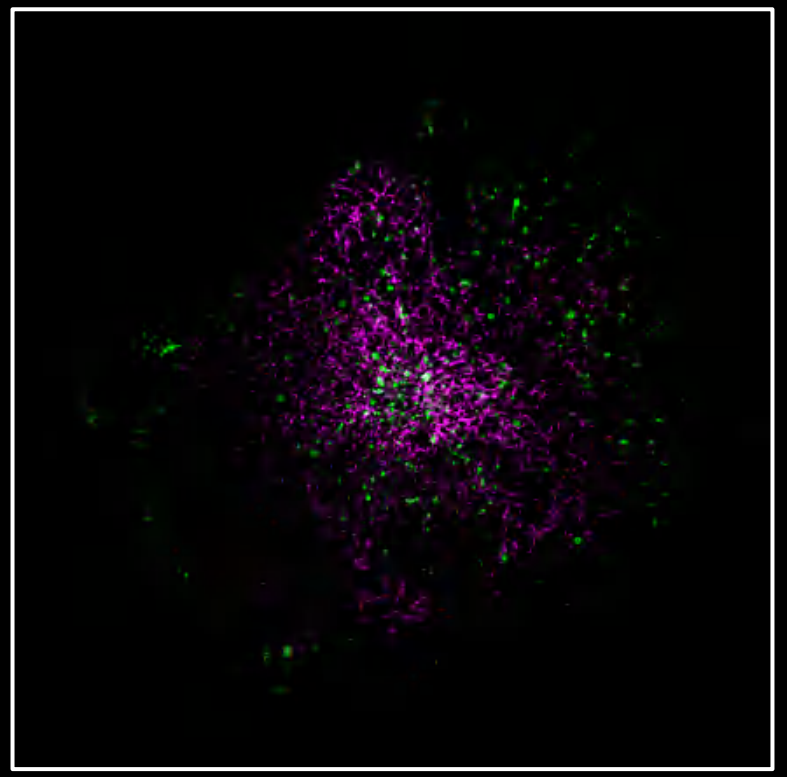
# 应用特点总结

## 活细胞高分辨、高速、低光毒性成像

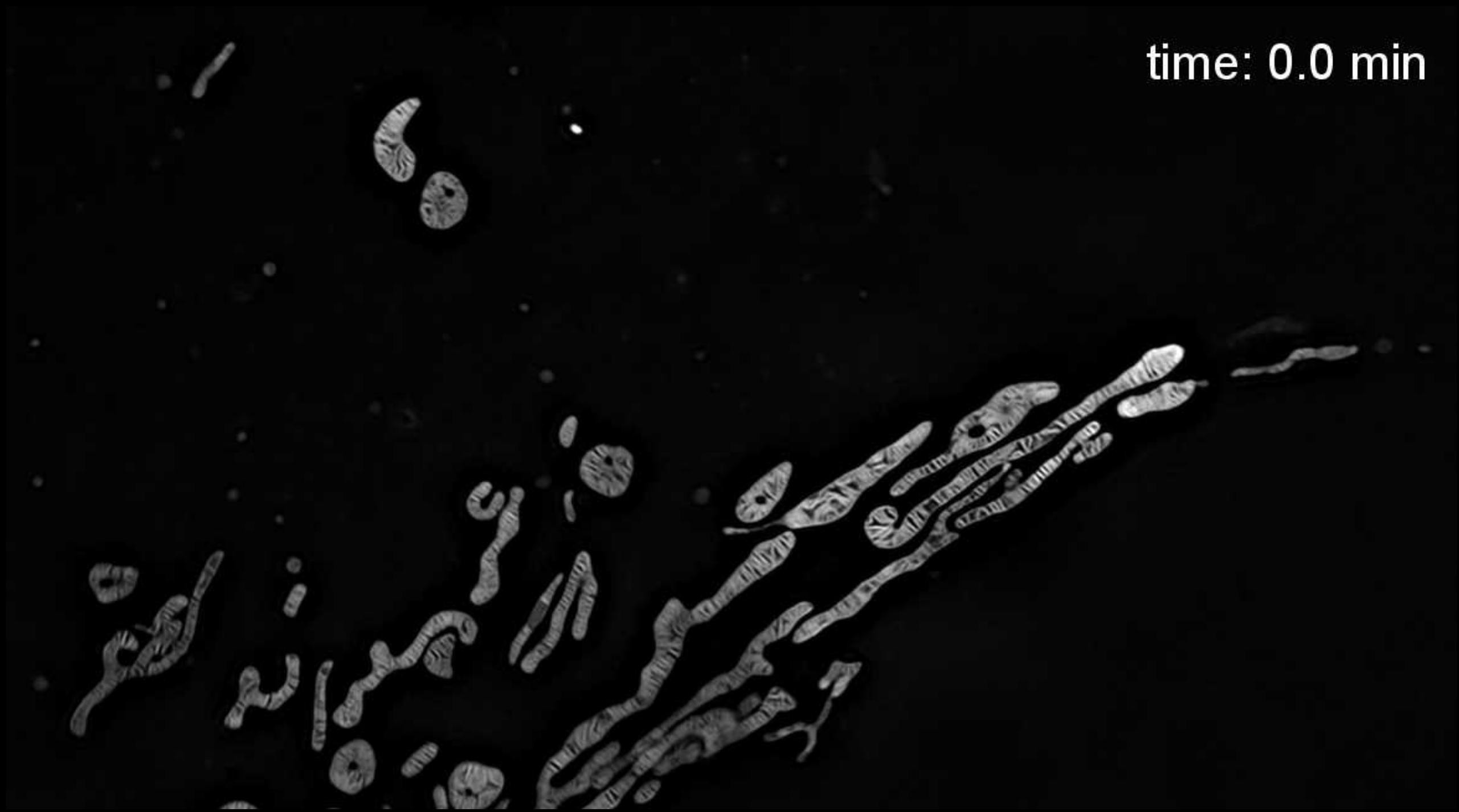
60 nm 分辨率



687 fps高速大视野四色



低光毒性


time: 0.0 min


Seeing beyond