计算光学成像与光信息处理技术前沿

（第14讲）

左 超，叶 燃，钱佳铭

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Nanjing University of Science and Technology,
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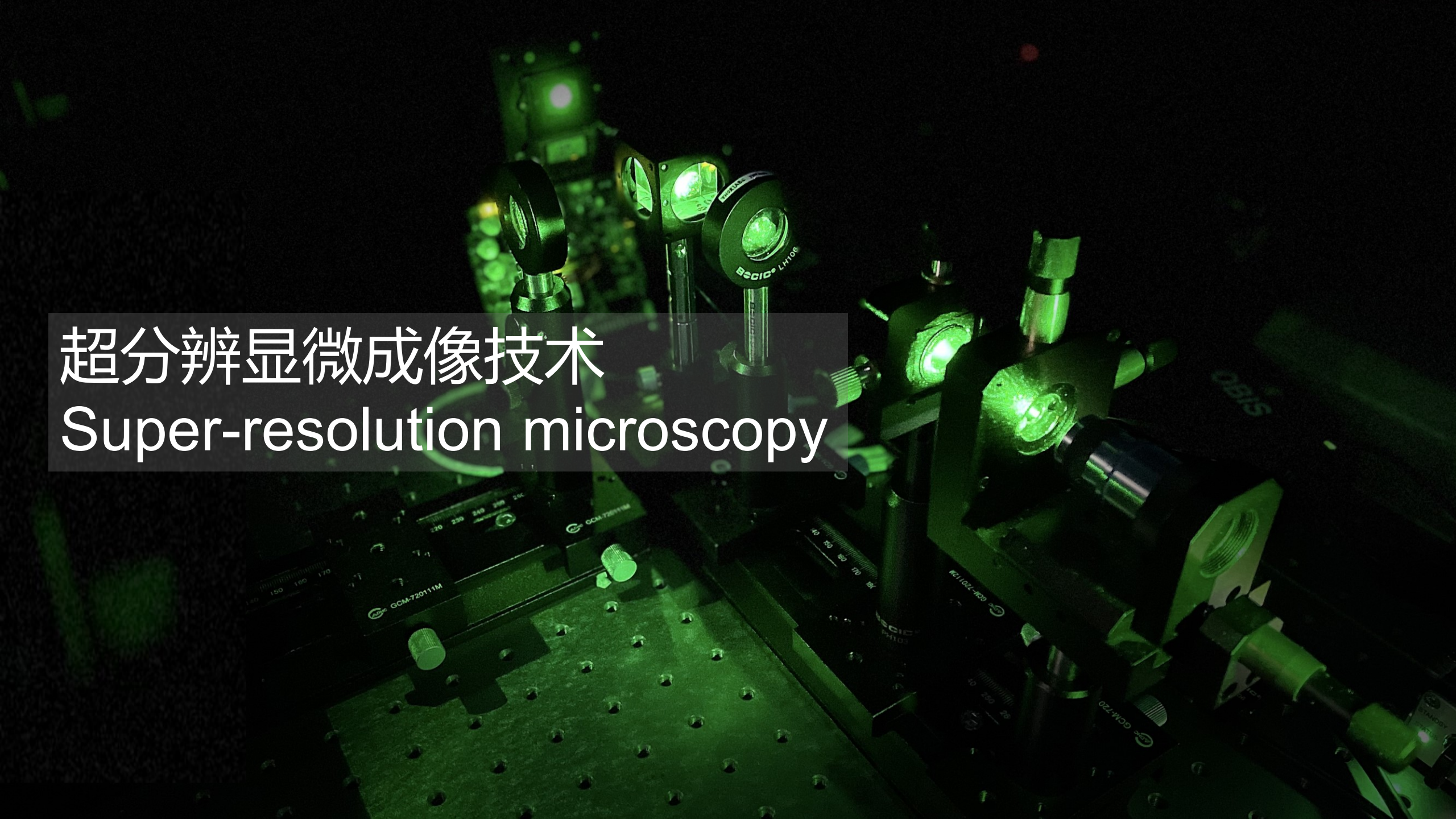
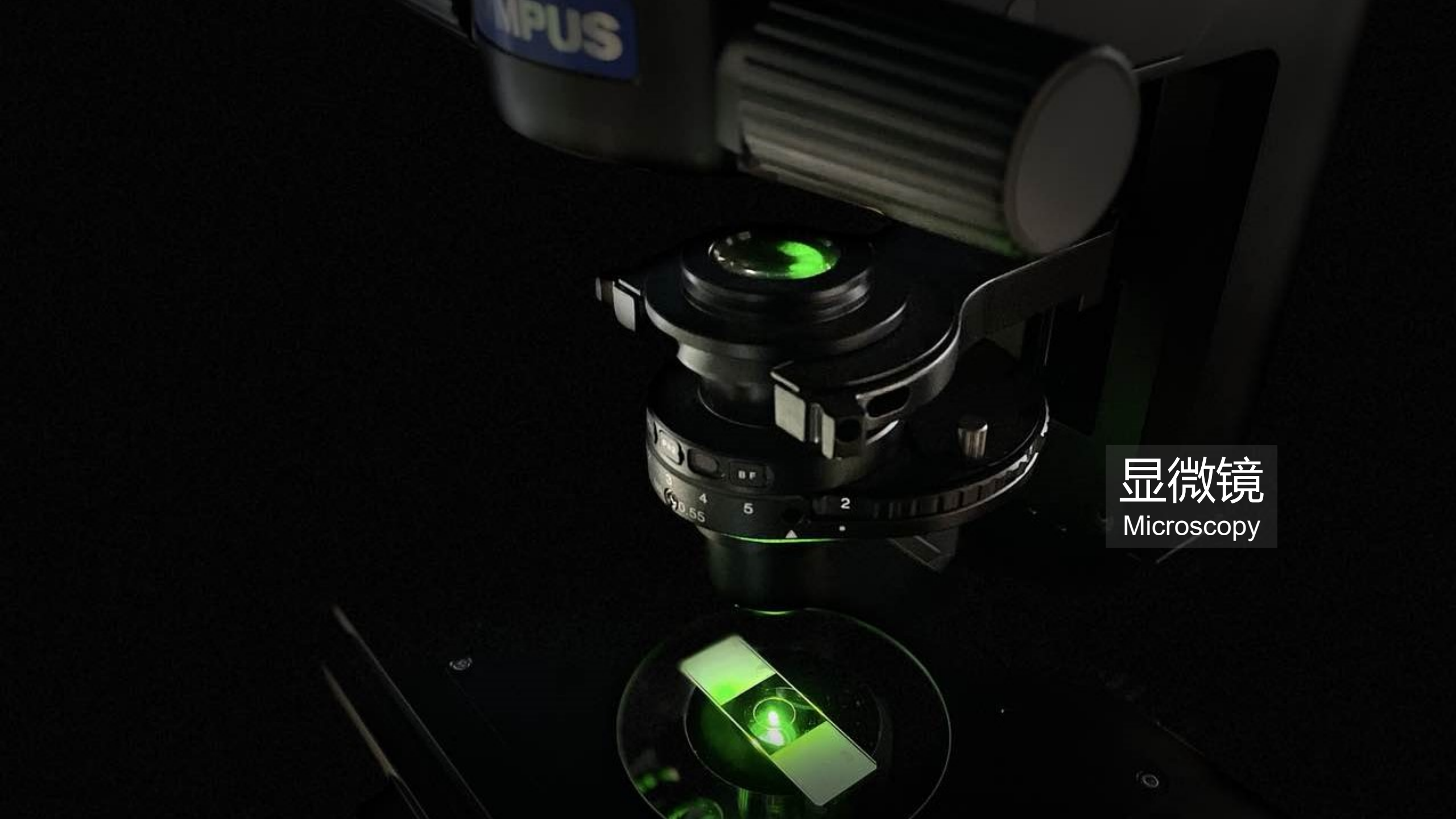

超分辨显微成像技术
Super-resolution microscopy

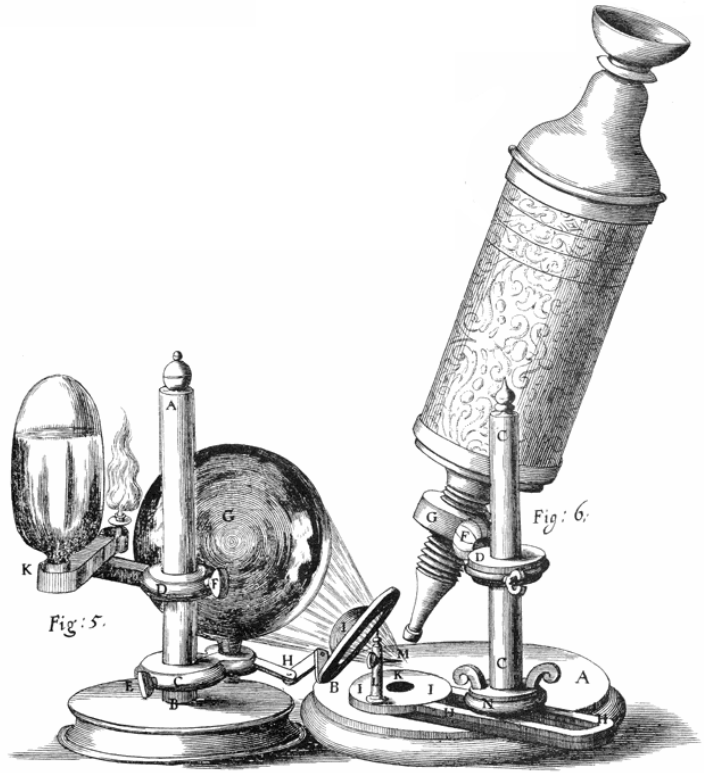

显微镜
Microscopy

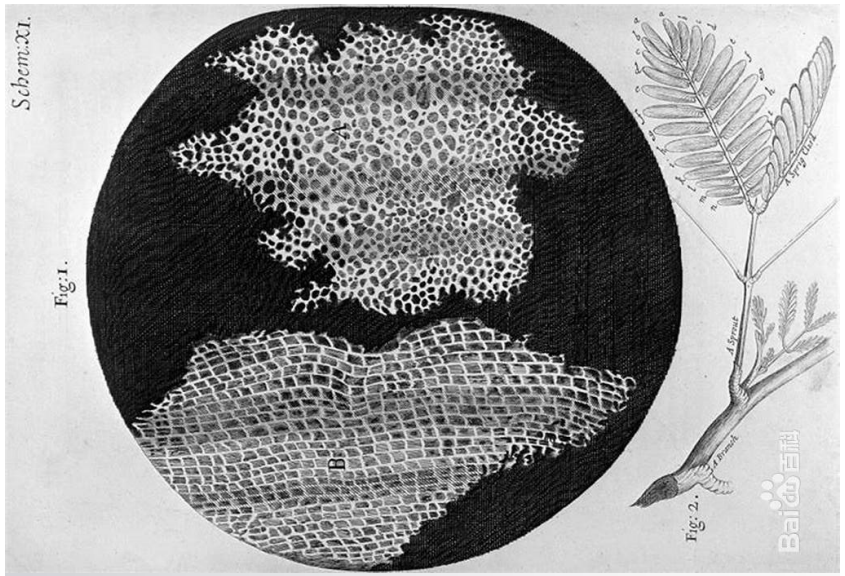
亚斯·詹森 | 荷兰　　1590

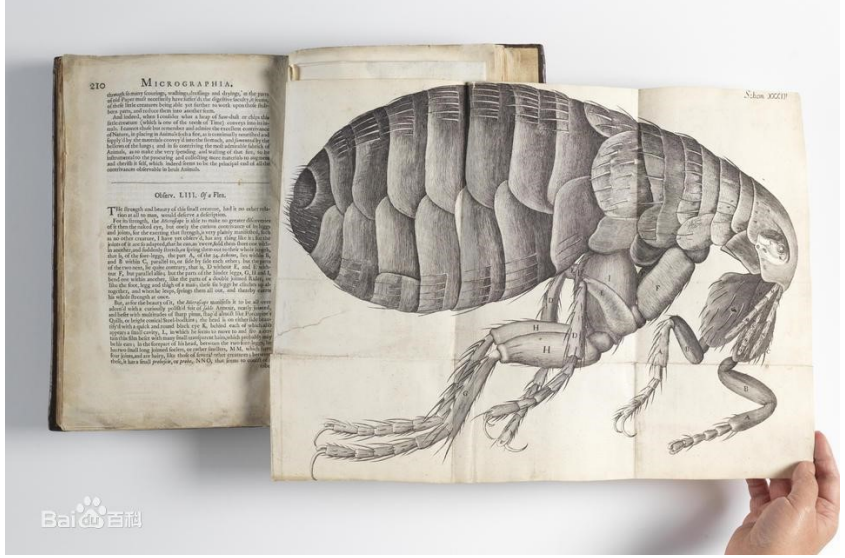
米德尔堡科学协会|1604

cell

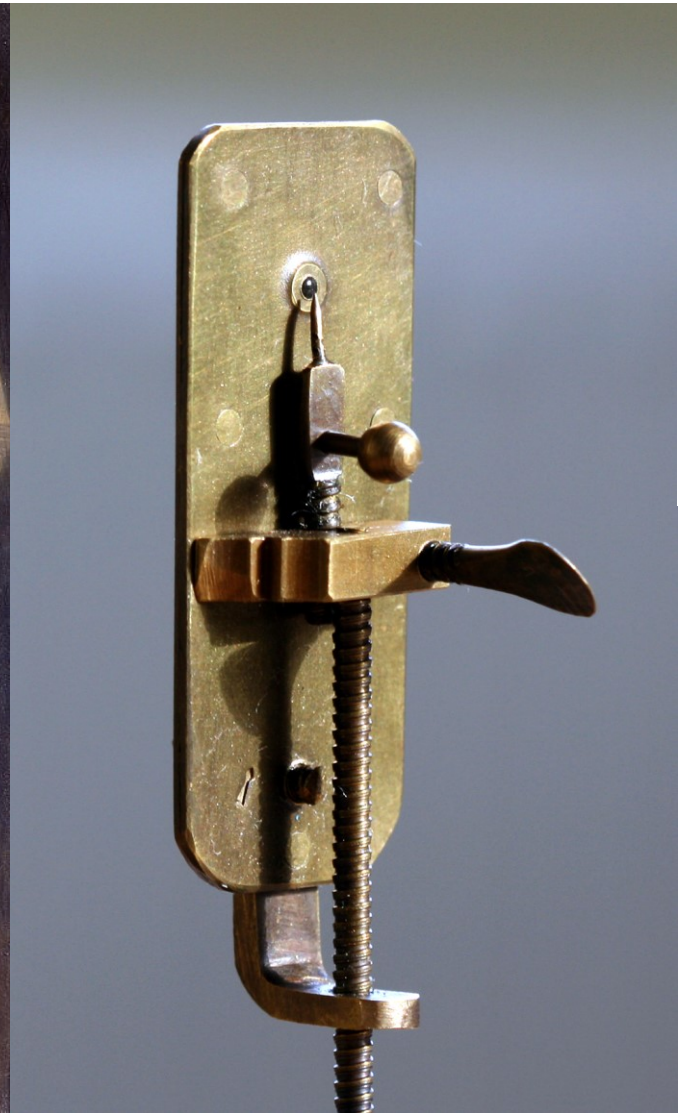
罗伯特·胡克| 英国　1665

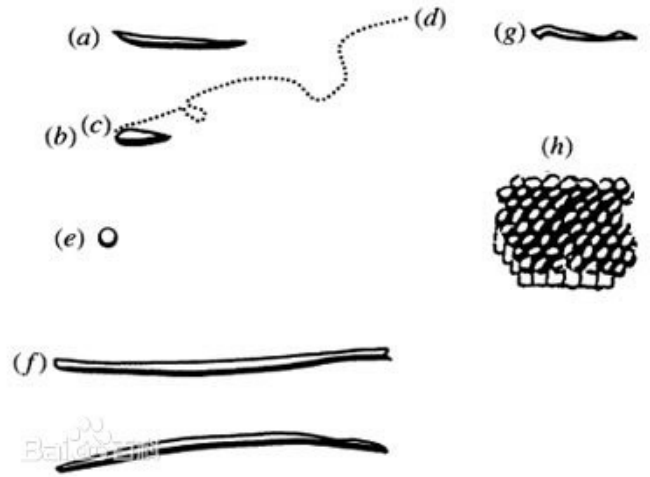


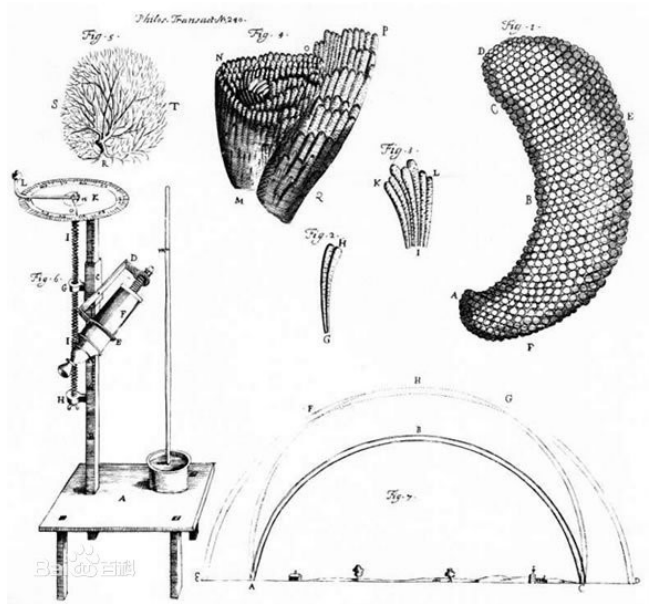


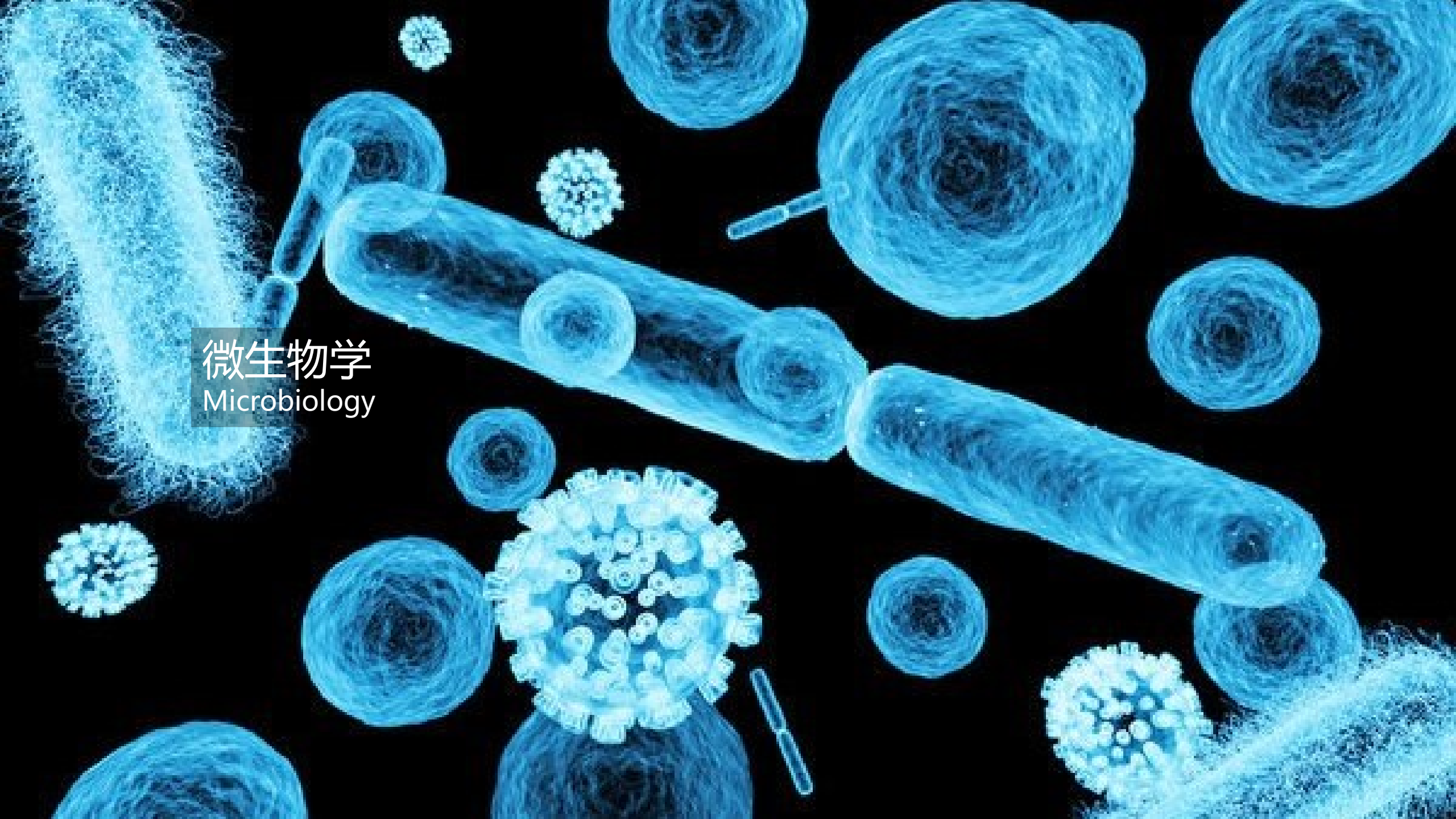


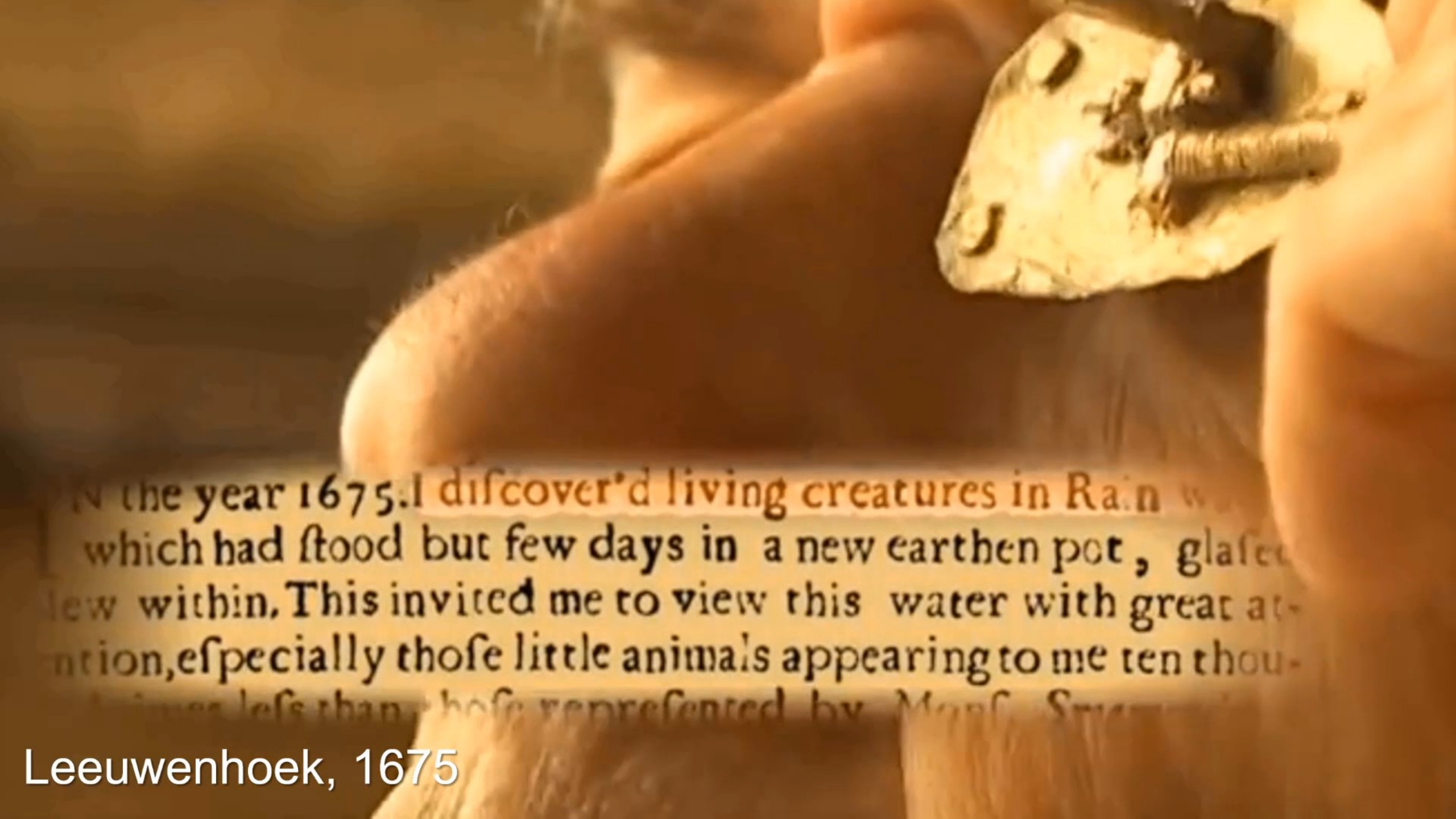
列文虎克| 荷兰　1673



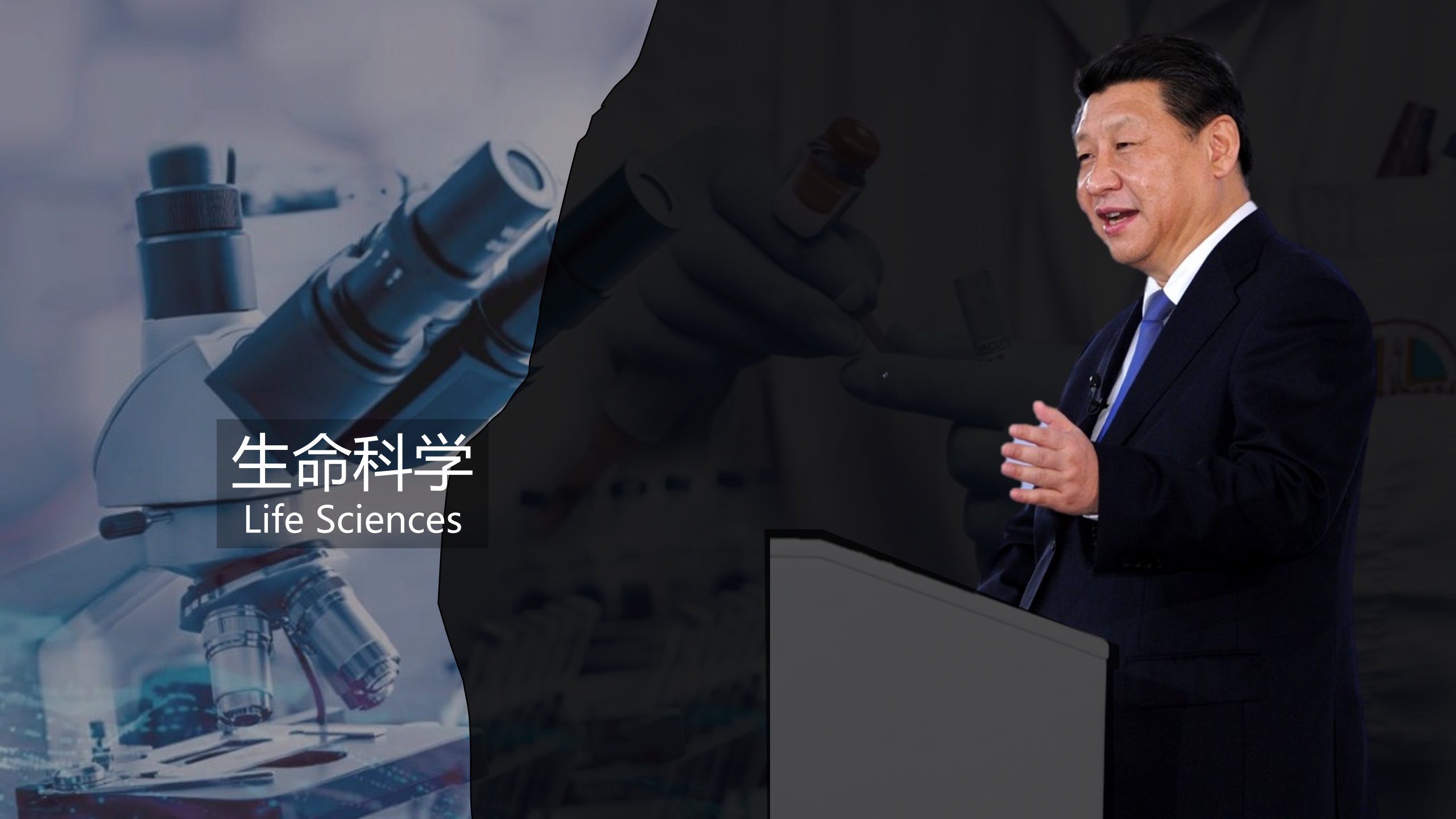


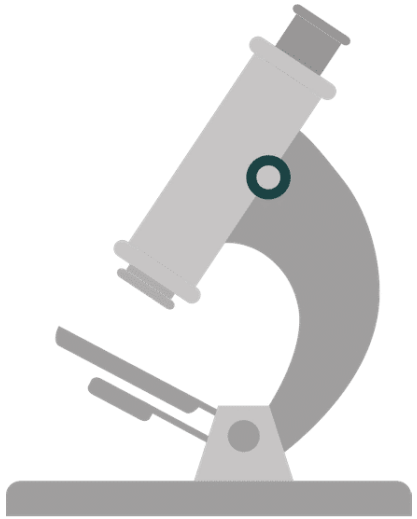


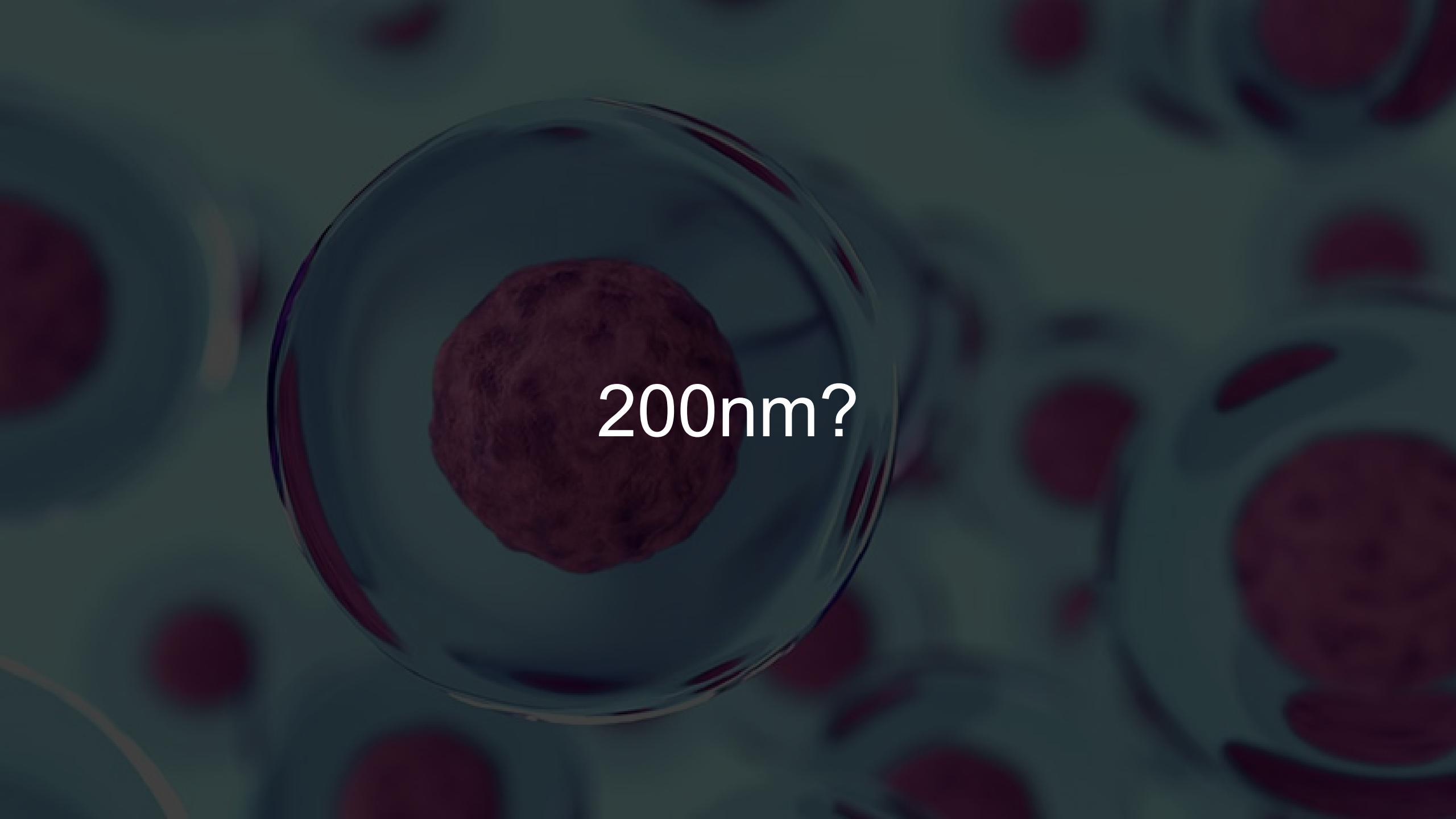

微生物学
Microbiology



Leeuwenhoek, 1675


生命科学
Life Sciences

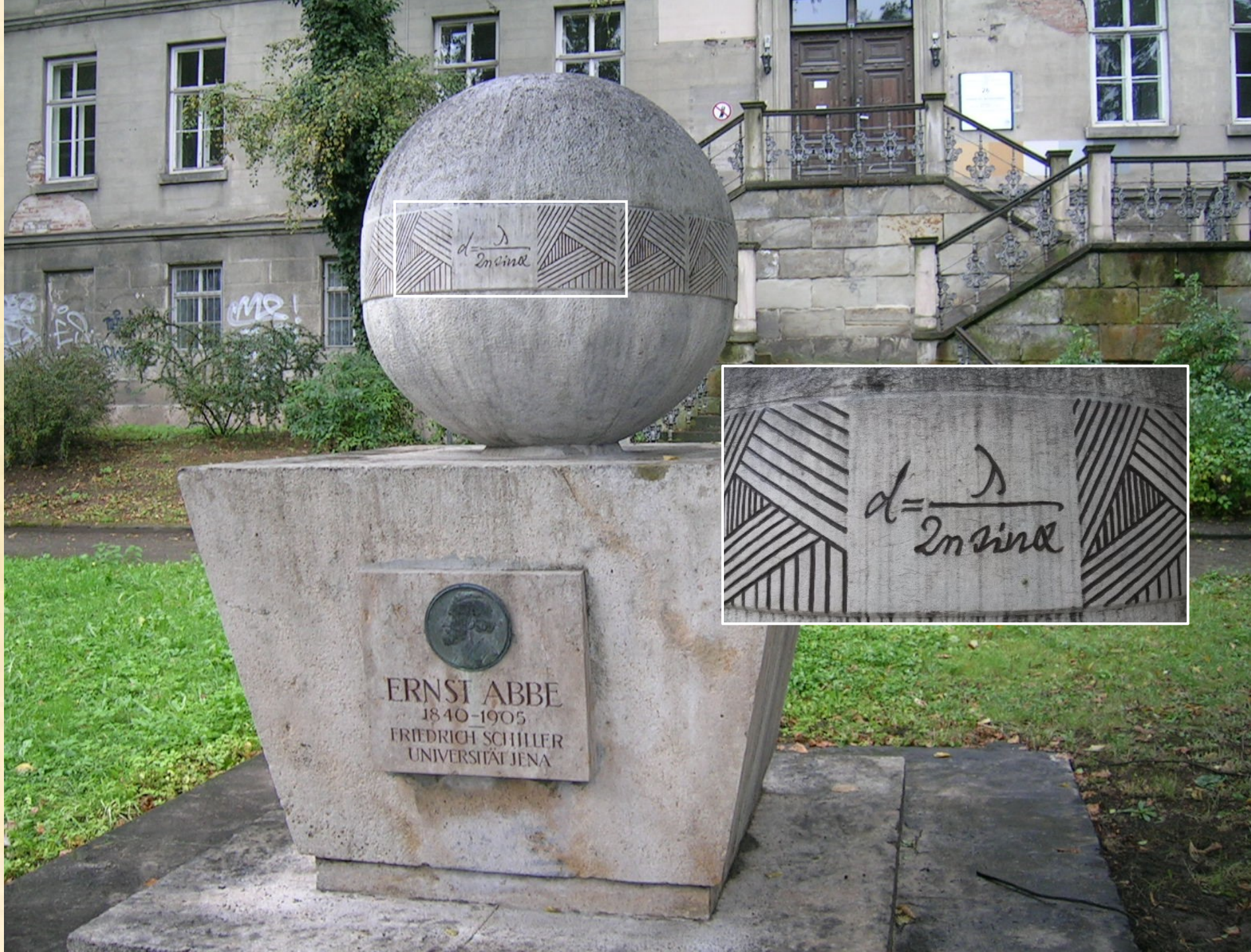



200nm?

ERNST ABBE.

D. 8. 1866

1873

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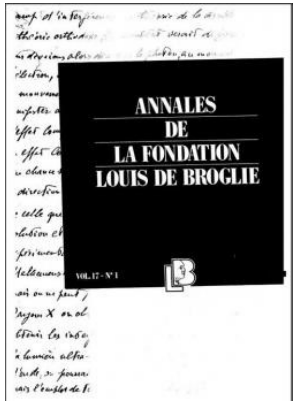
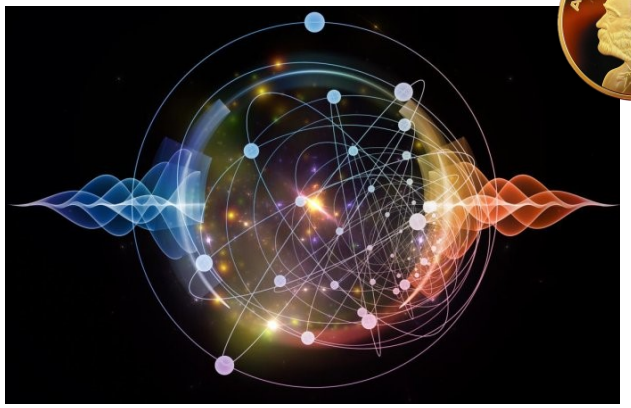
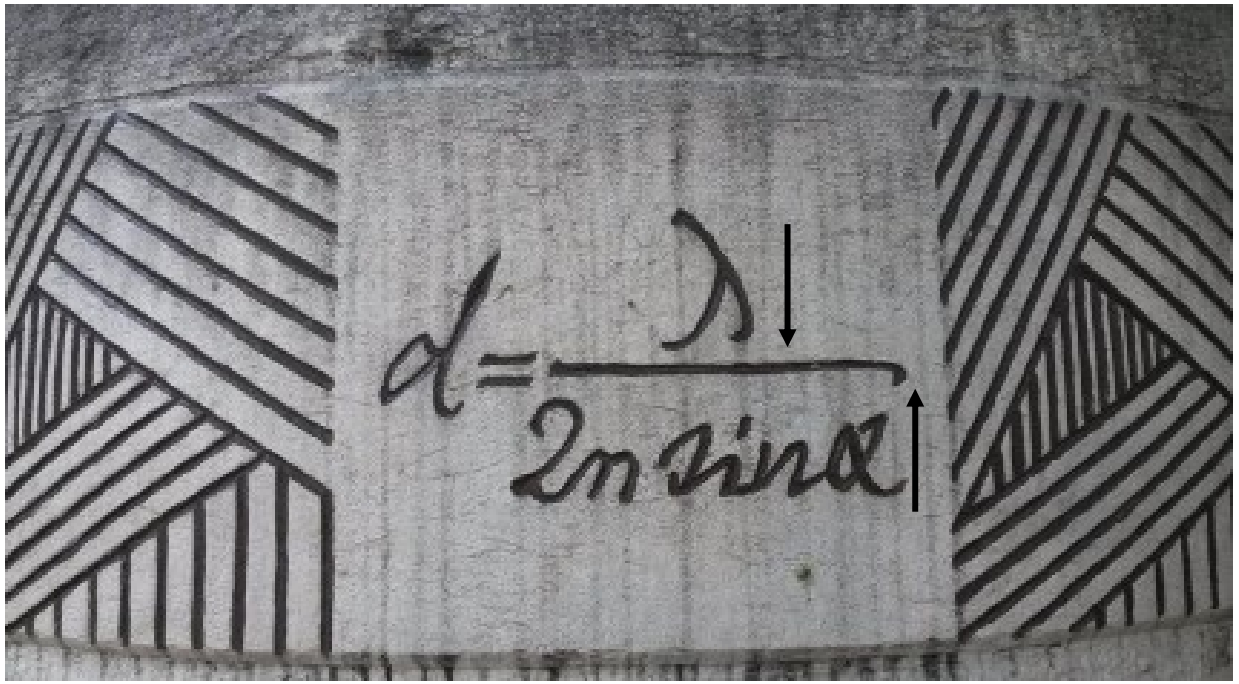

ANNALES
DE
LA FONDATION
LOUIS DE BROGLIE

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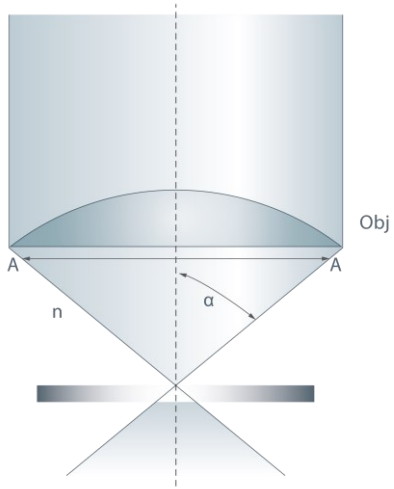
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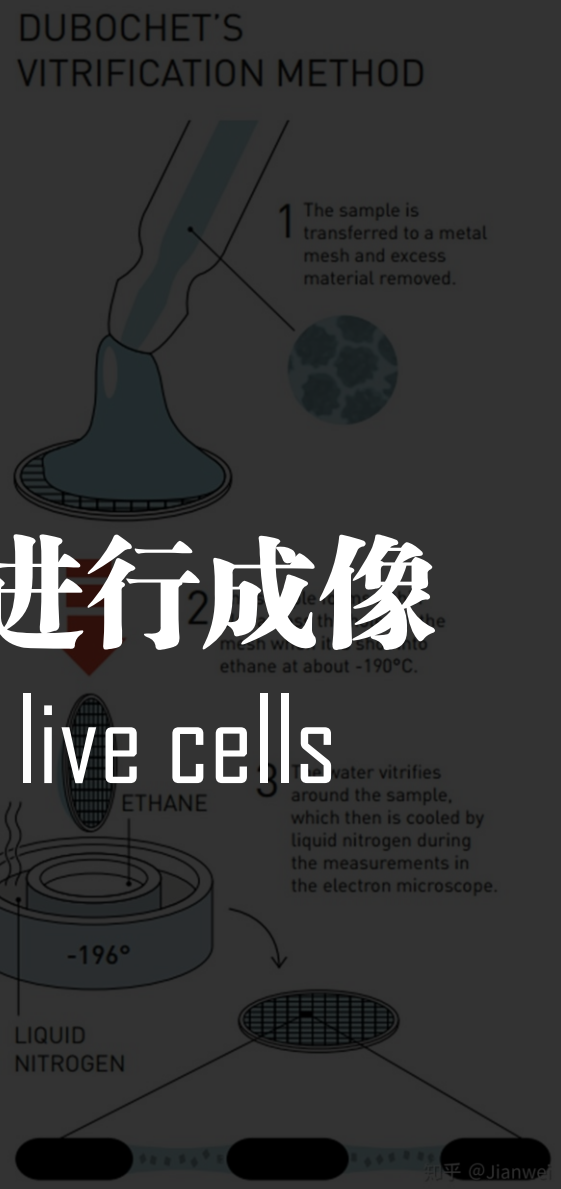
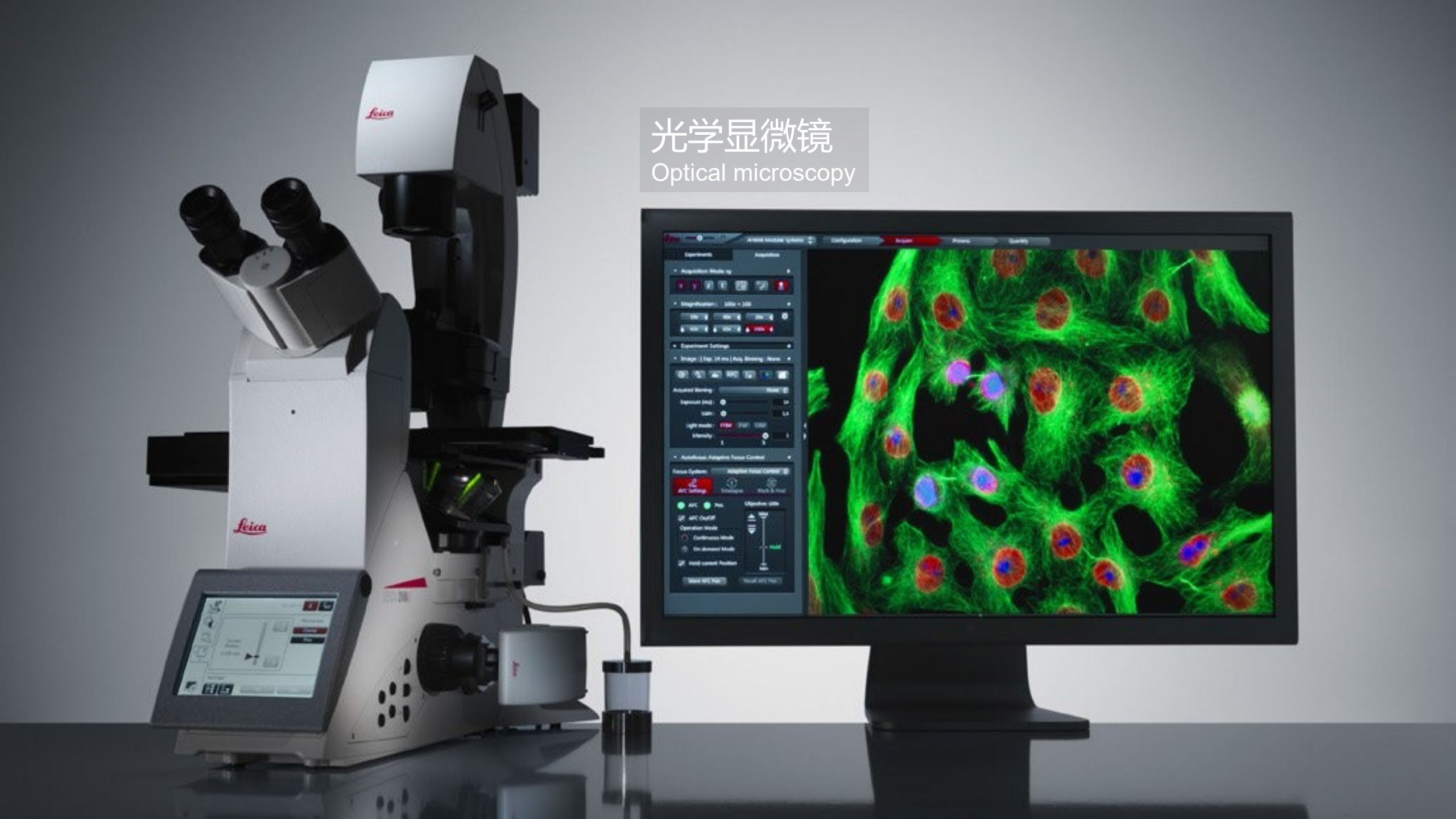
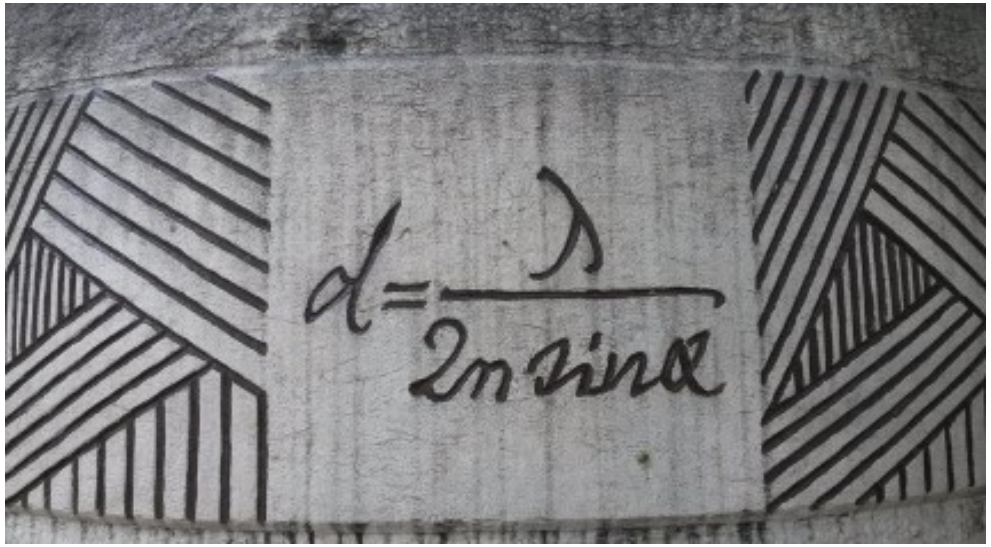


无法对活细胞进行成像

Inability to image live cells

光学显微镜
Optical microscopy

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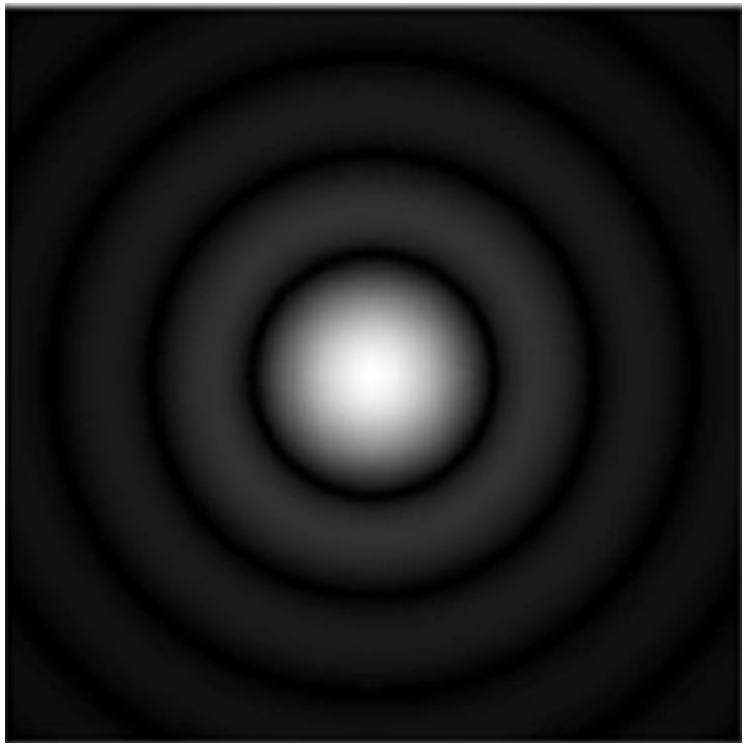

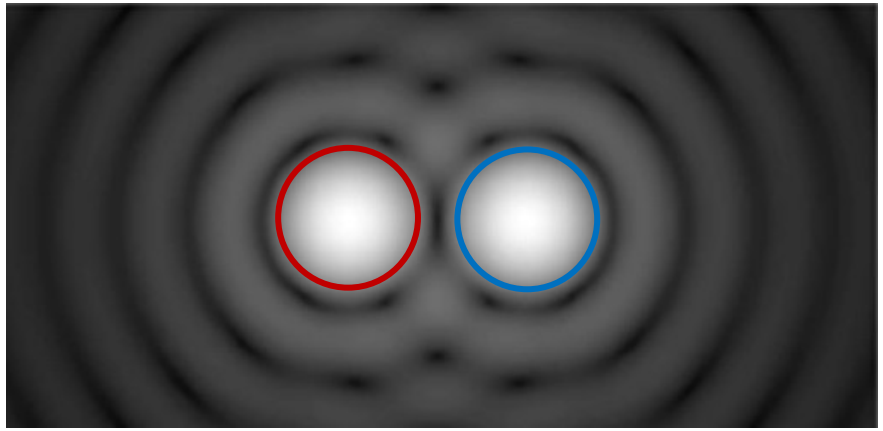

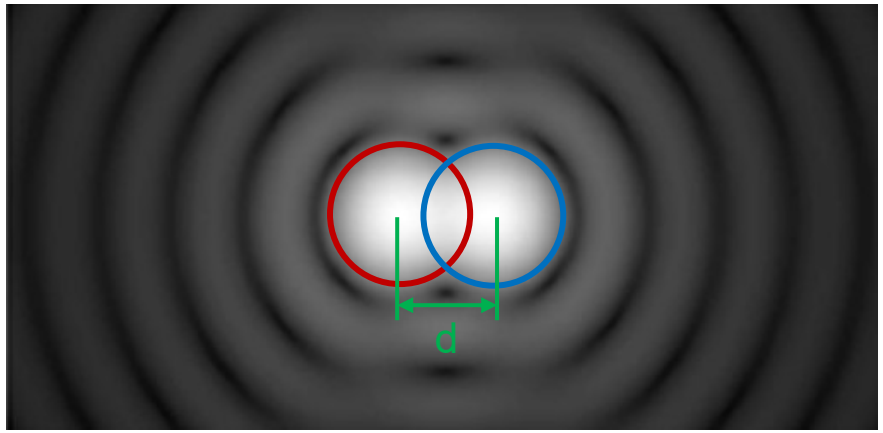
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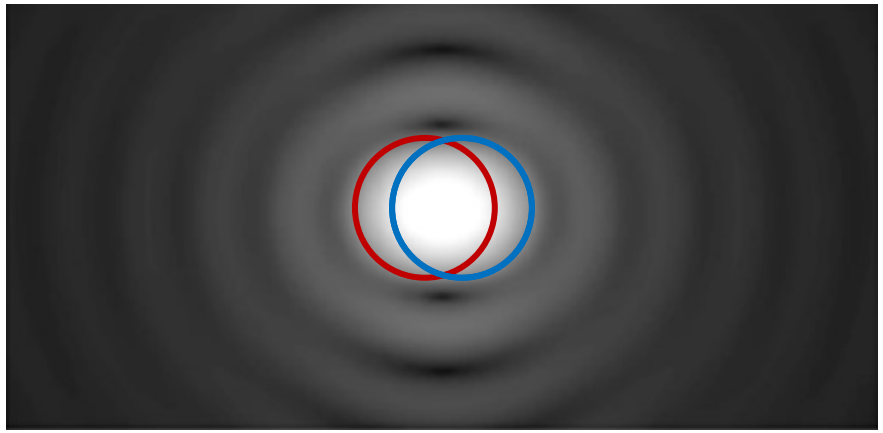
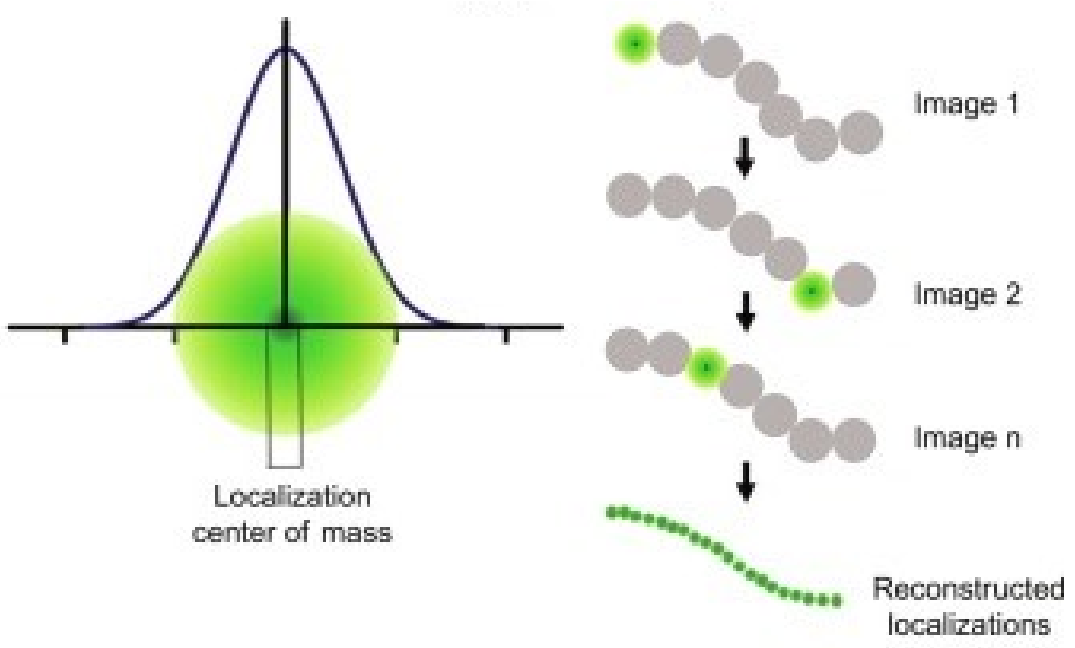
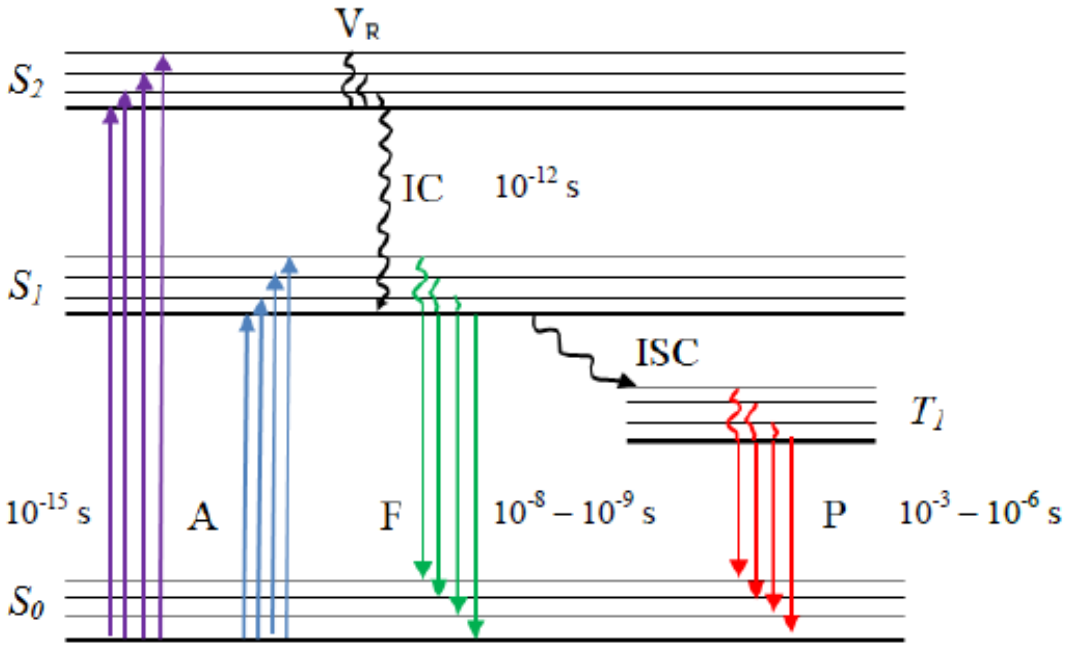
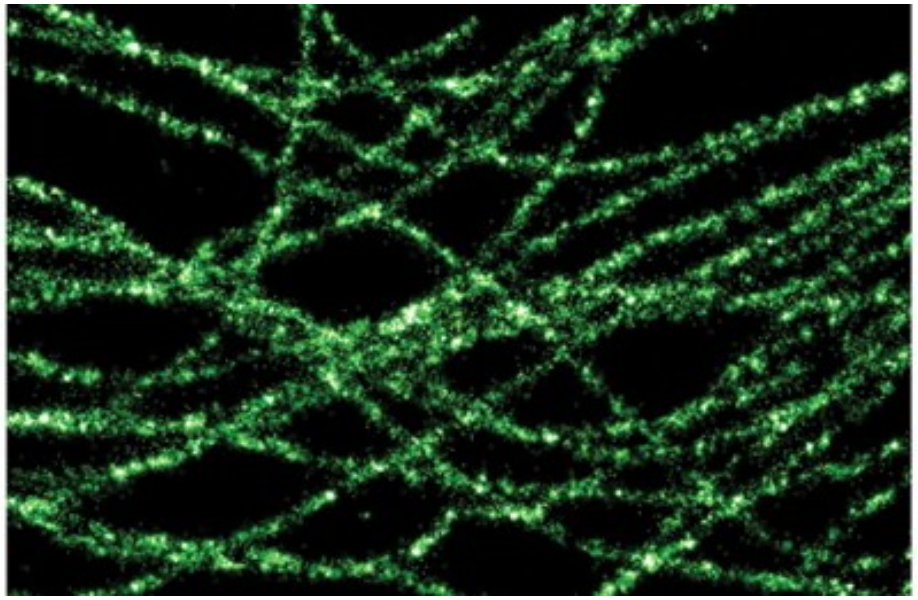


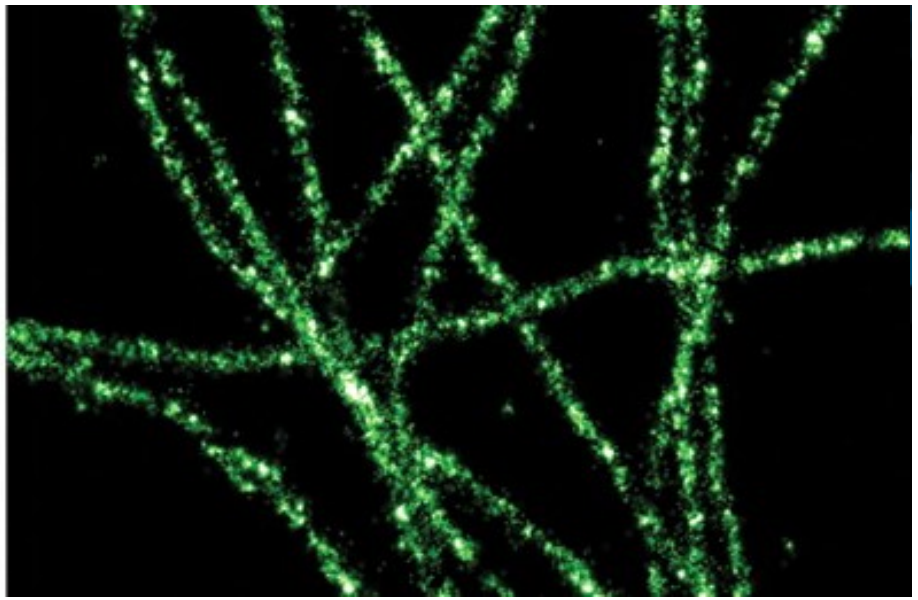
PALM（光活化定位显微术）
STORM（随机光学重构显微术）

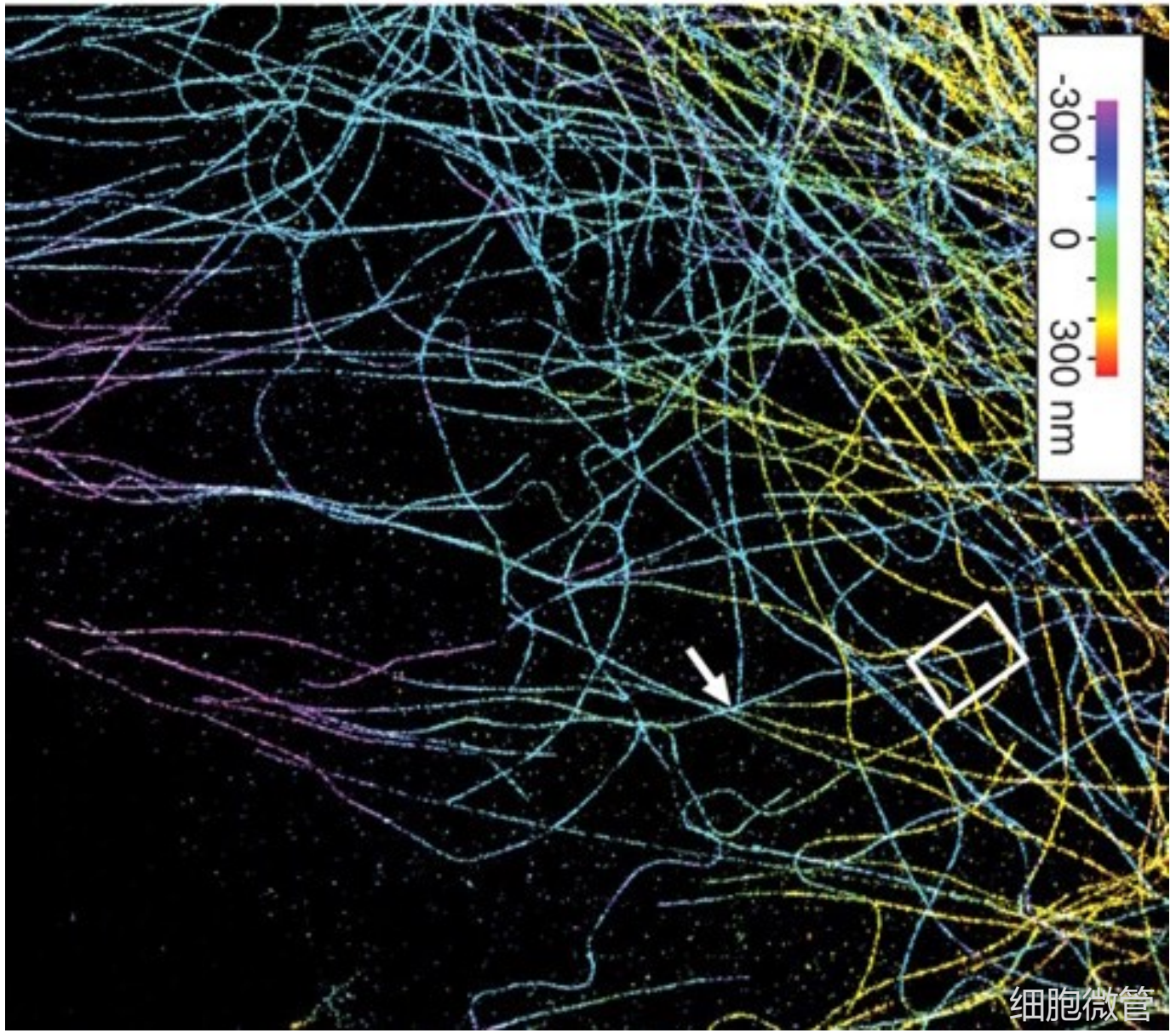


荧光成像

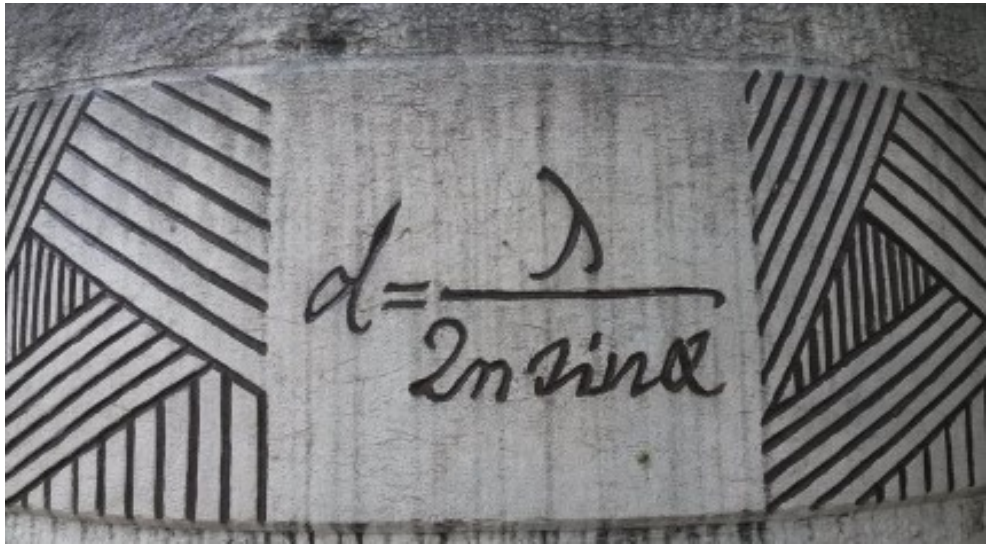


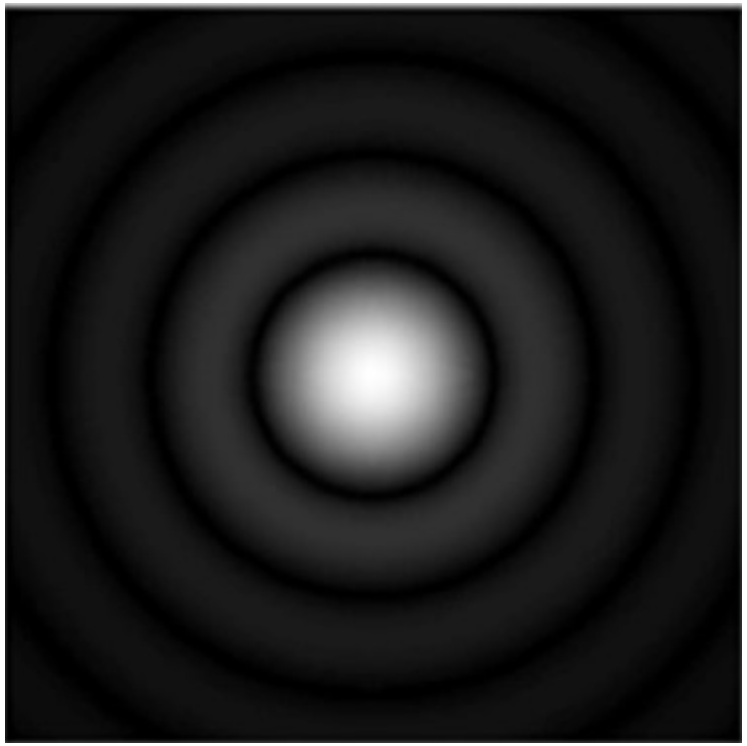


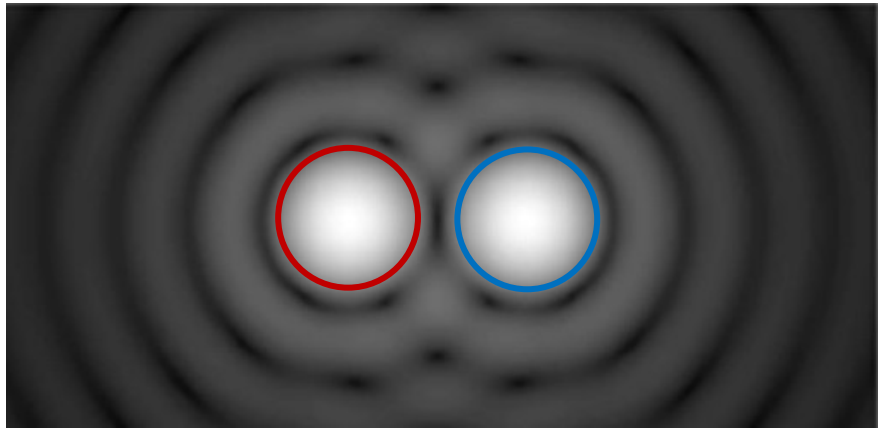


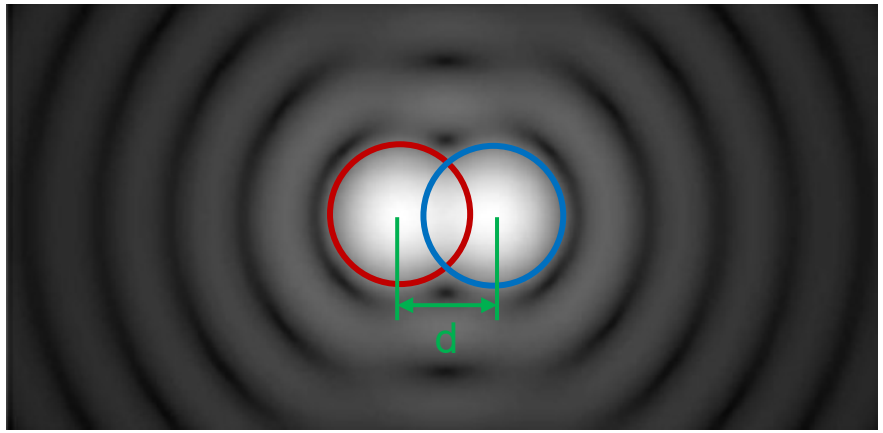


细胞微管


$d = \frac{\lambda}{2n \sin\alpha}$



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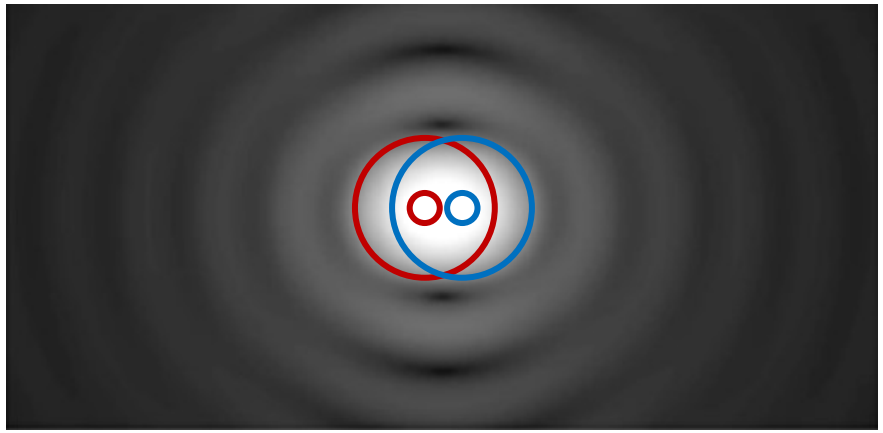
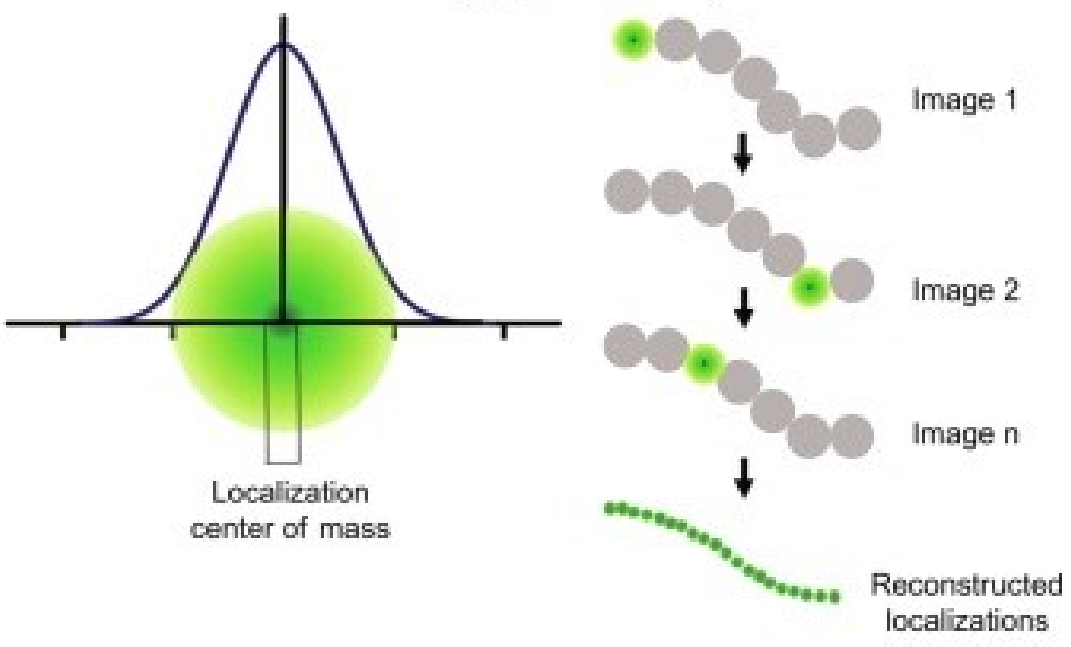
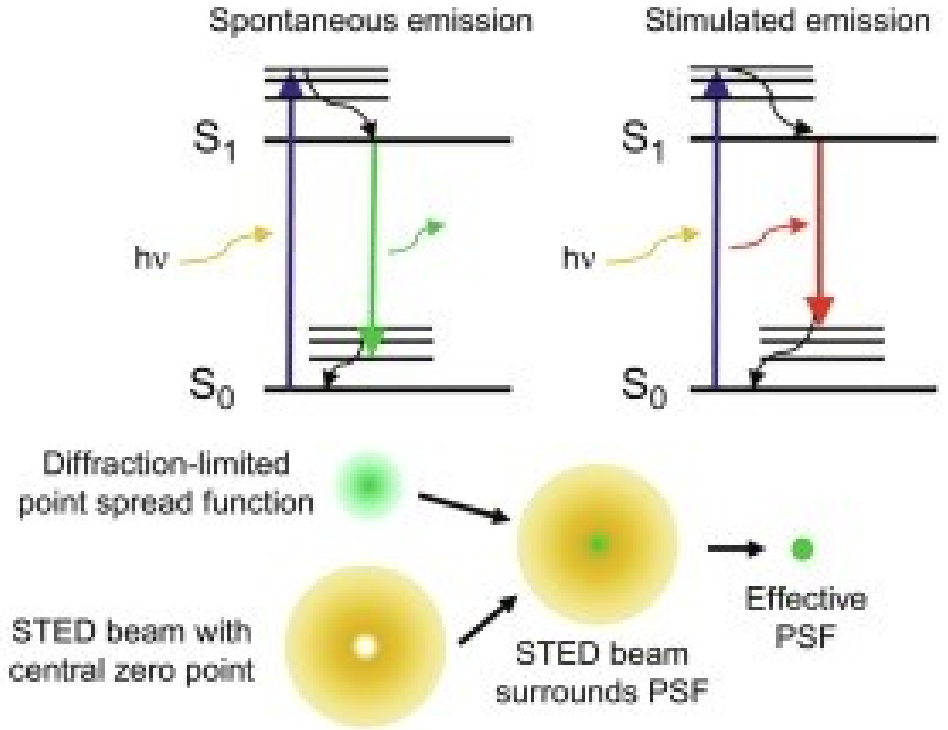
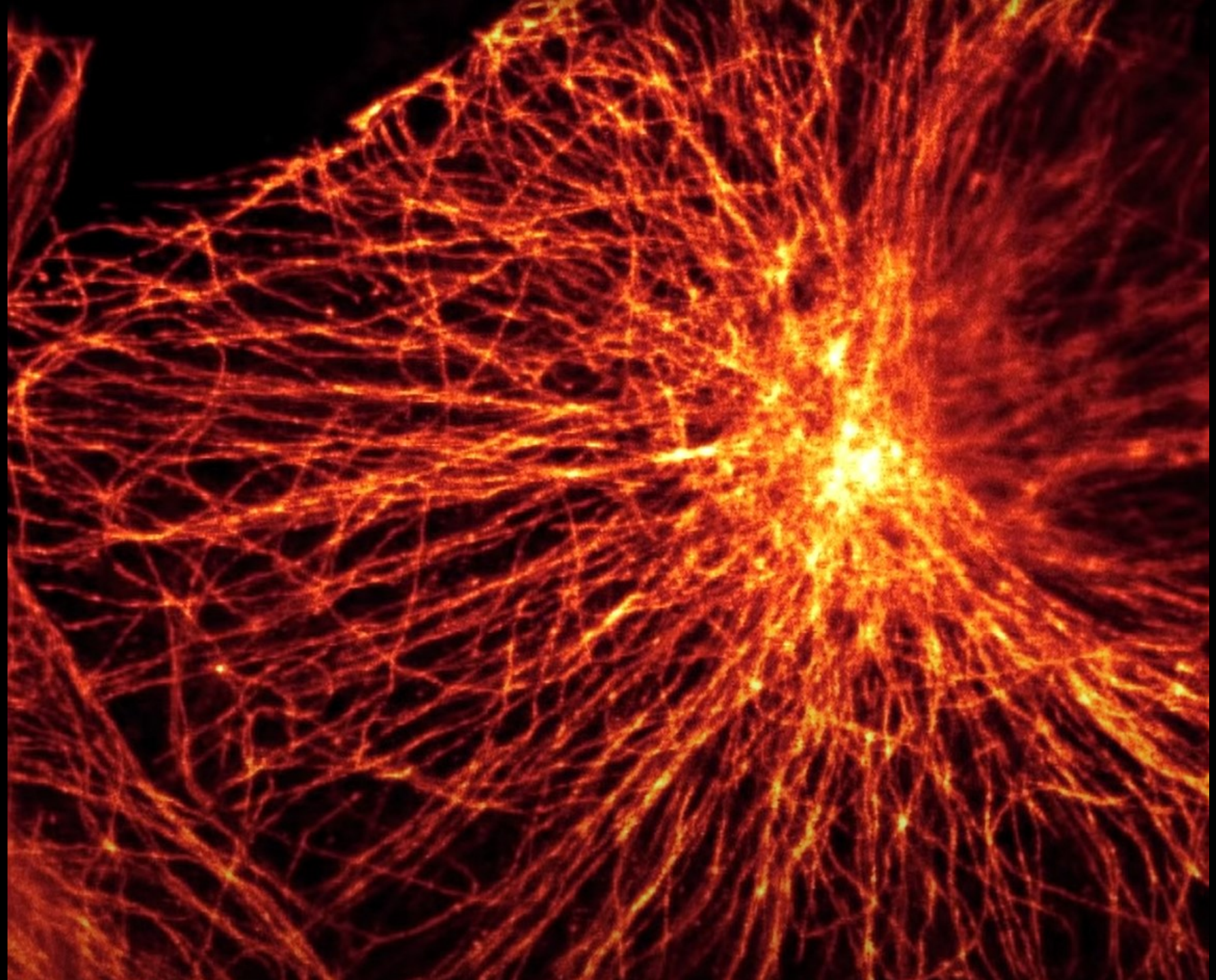


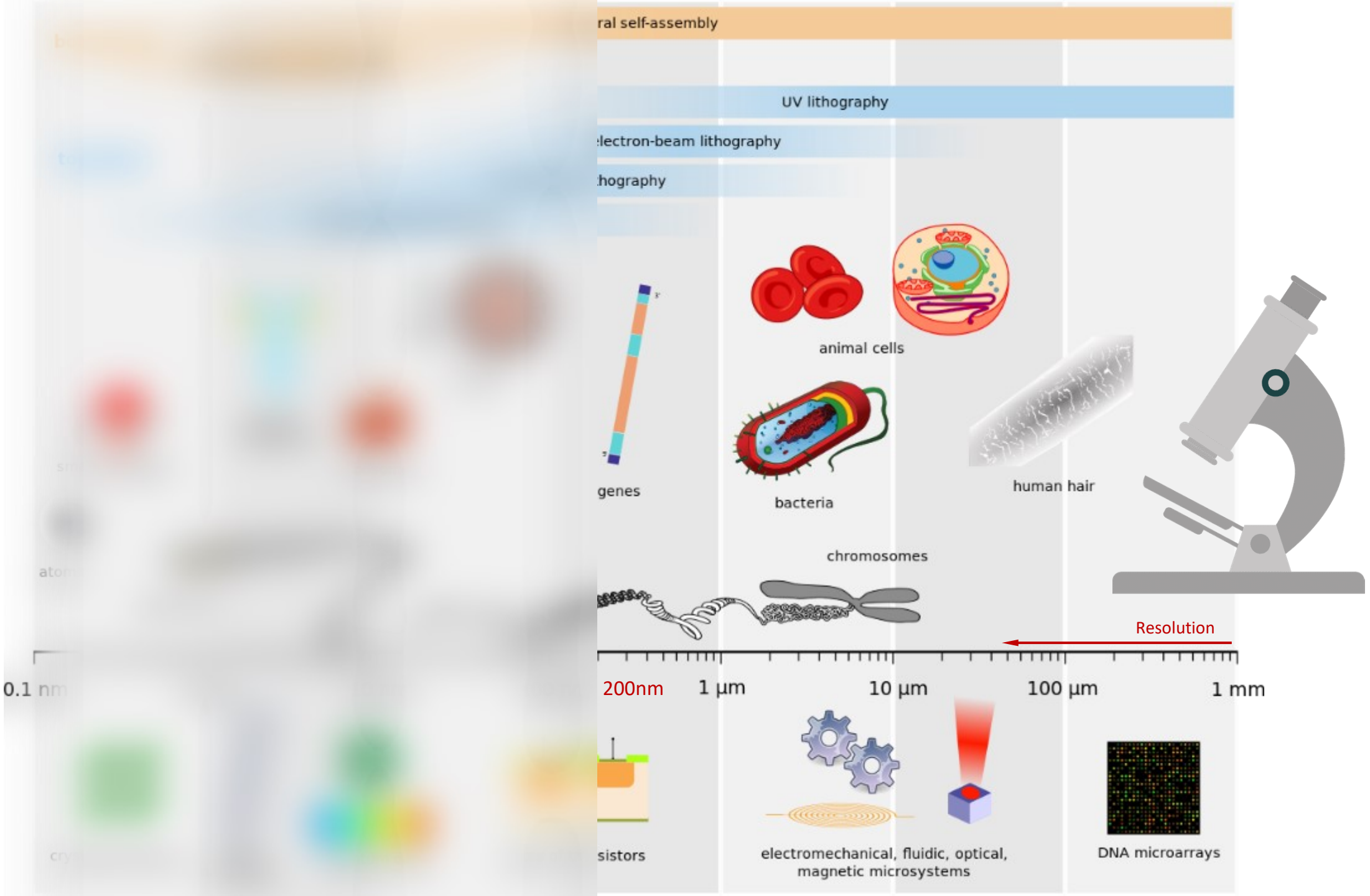
PALM（光活化定位显微术）
STORM（随机光学重构显微术）



STED（受激发射损耗显微术）

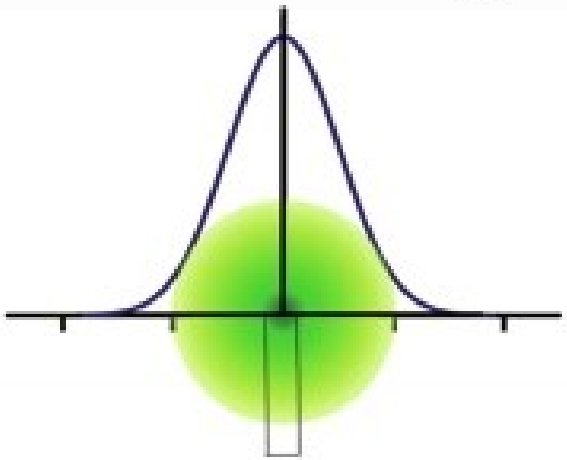


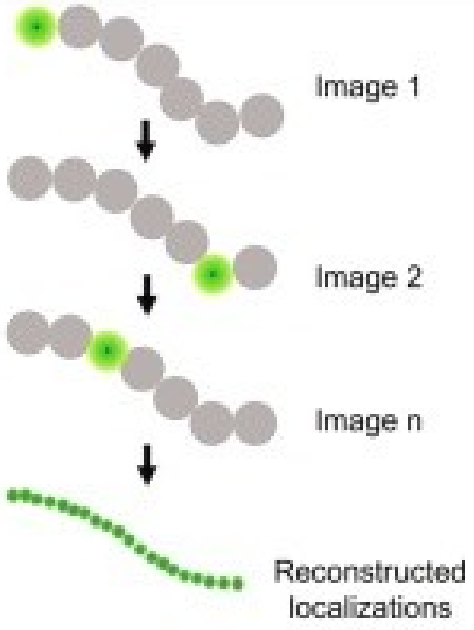



ral self-assembly
UV lithography
lectron-beam lithography
hography
animal cells
genes
bacteria
human hair
chromosomes
Resolution
0.1 nm
200nm
1 µm
10 µm
100 µm
1 mm
sistors
electromechanical, fluidic, optical,
magnetic microsystems
DNA microarrays

2014年·诺贝尔奖

PALM（光活化定位显微术）
STORM（随机光学重构显微术）





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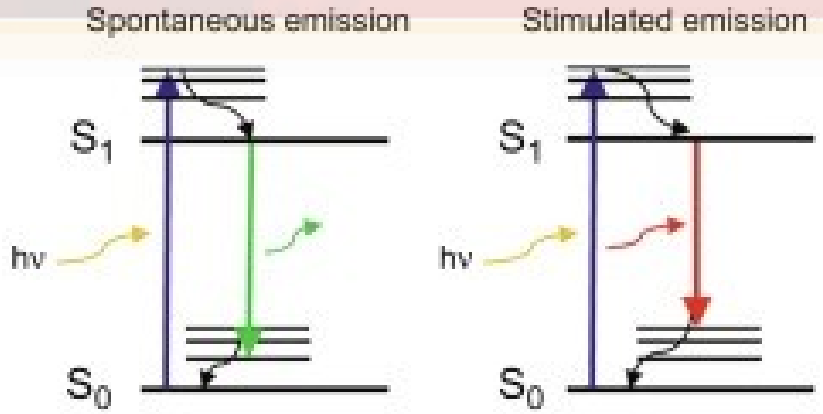
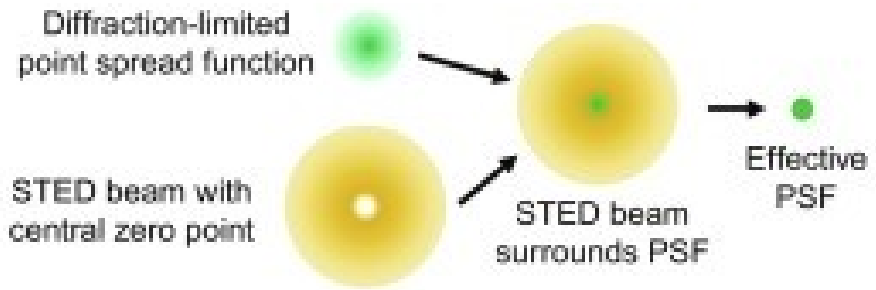
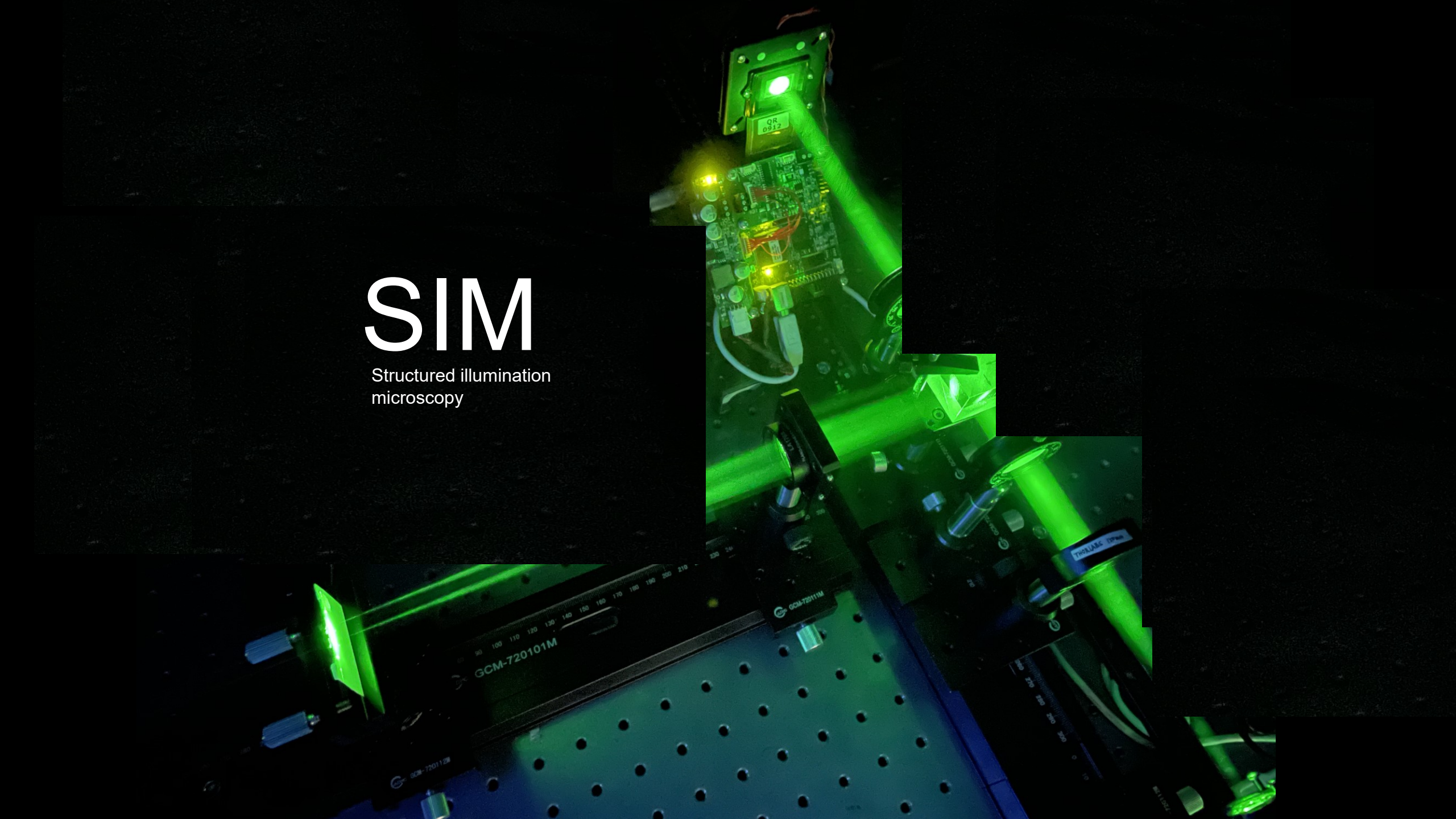
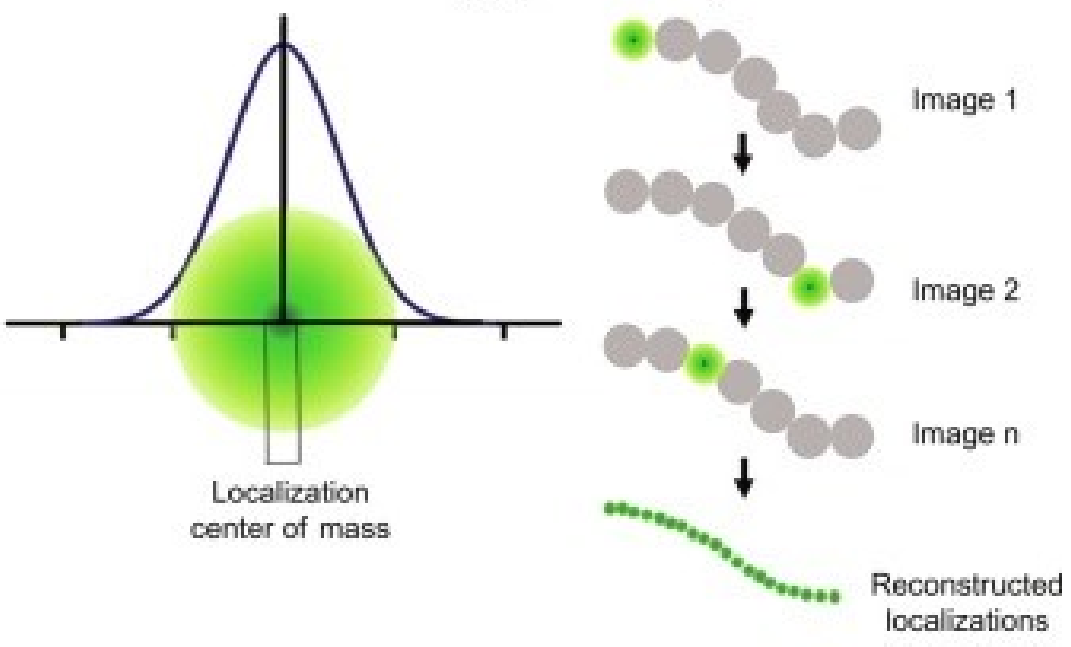


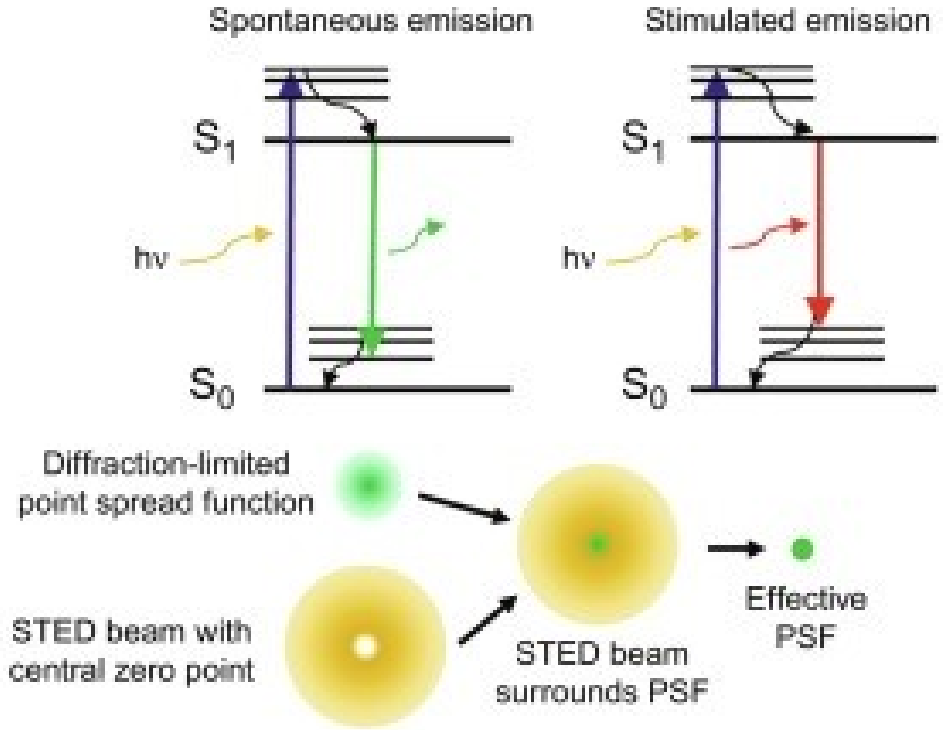



SIM
Structured illumination
microscopy
GCM-720101M

PALM（光活化定位显微术）
STORM（随机光学重构显微术）



STED（受激发射损耗显微术）



PALM（光活化定位显微术）
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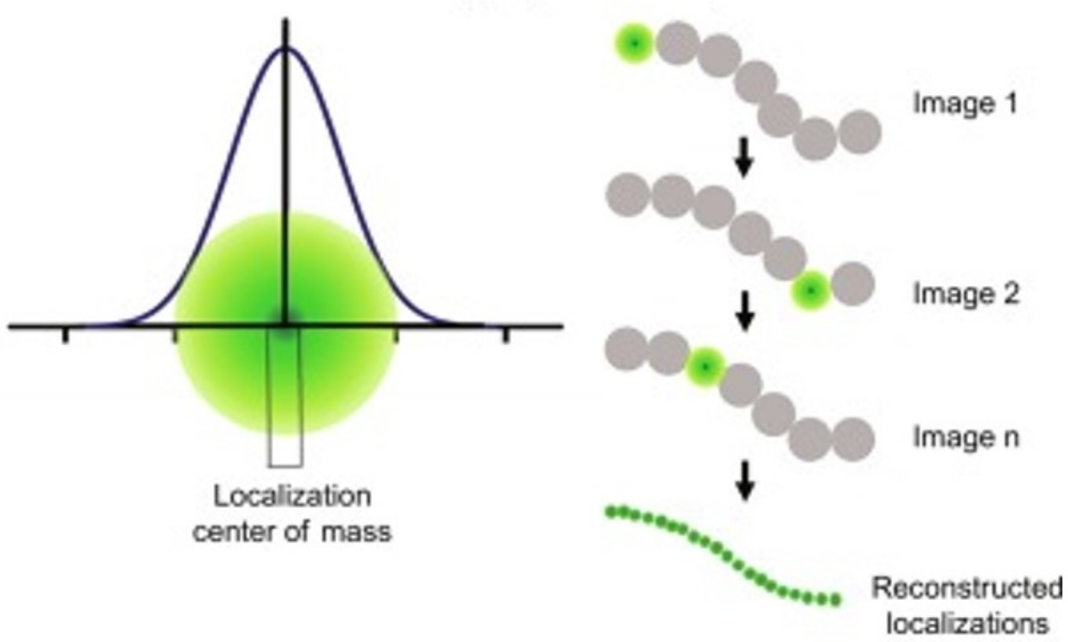
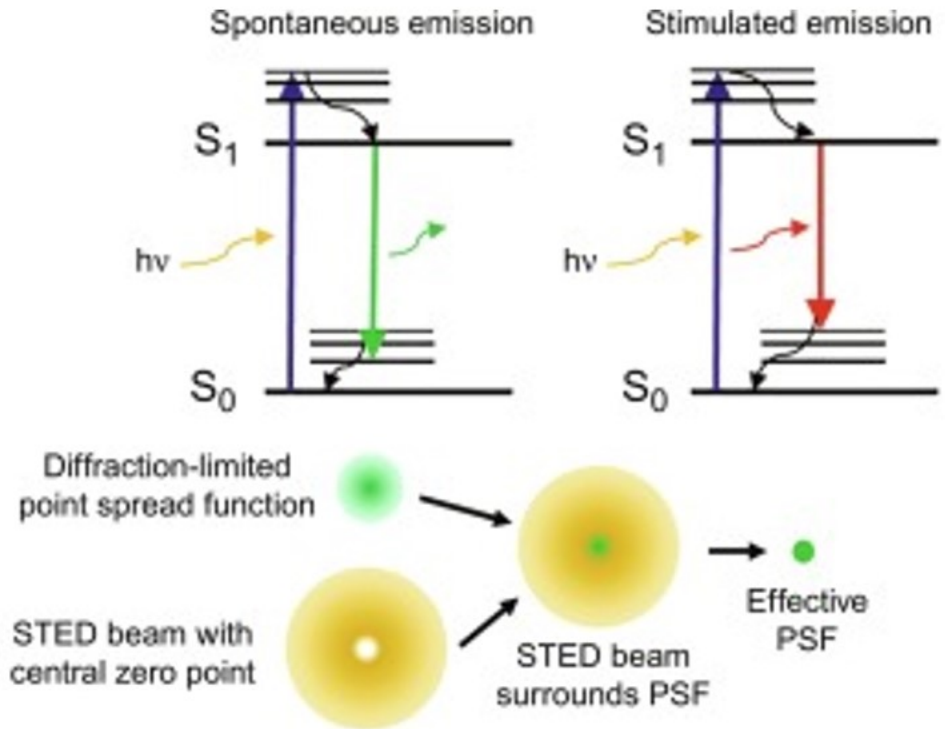
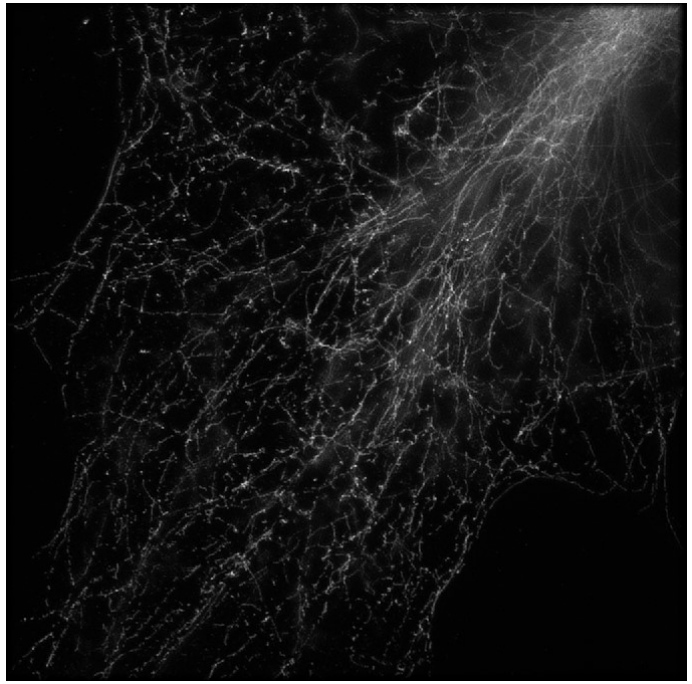


STED（受激发射损耗显微术）

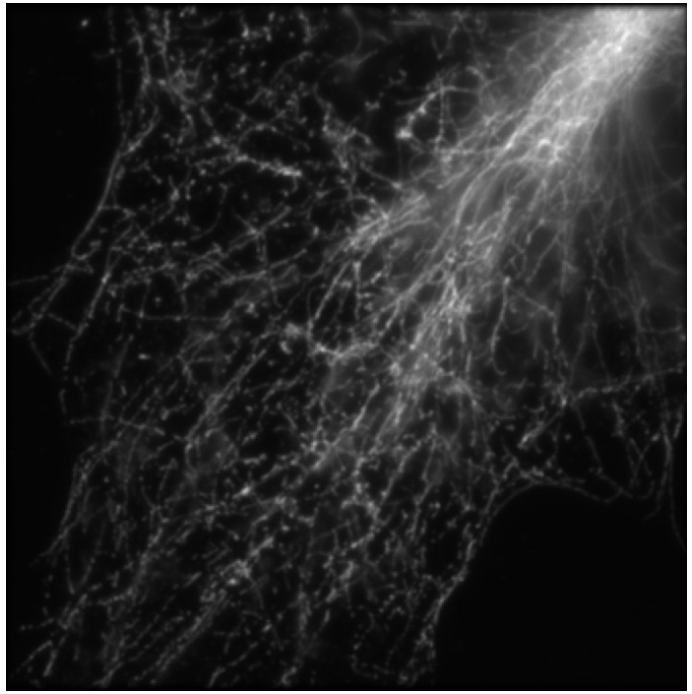


$S(r)$

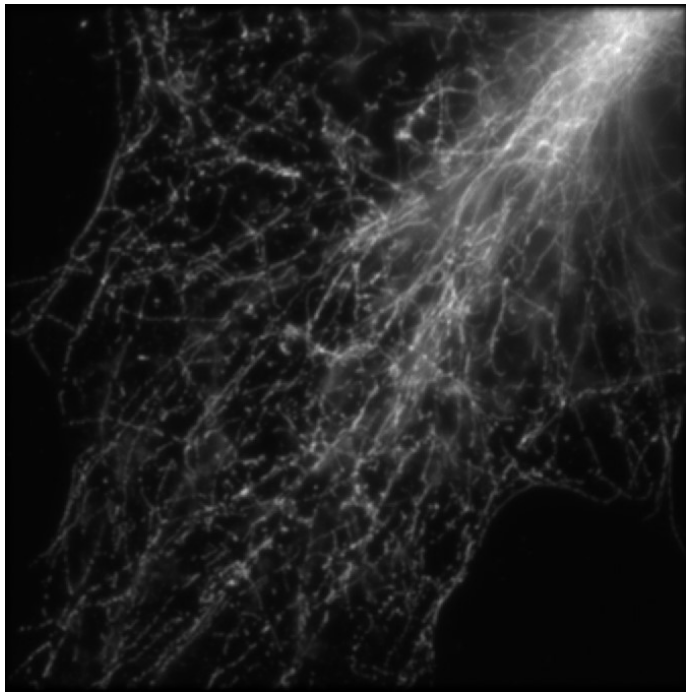


$PSF(r)$

$D(r)=S(r)\otimes PSF(r)$

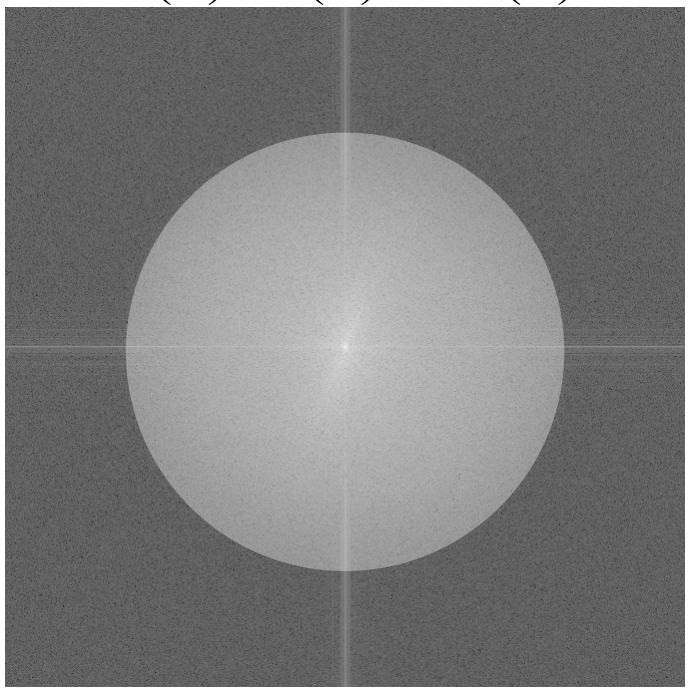


$$D(r)=S(r)\otimes PSF(r)$$

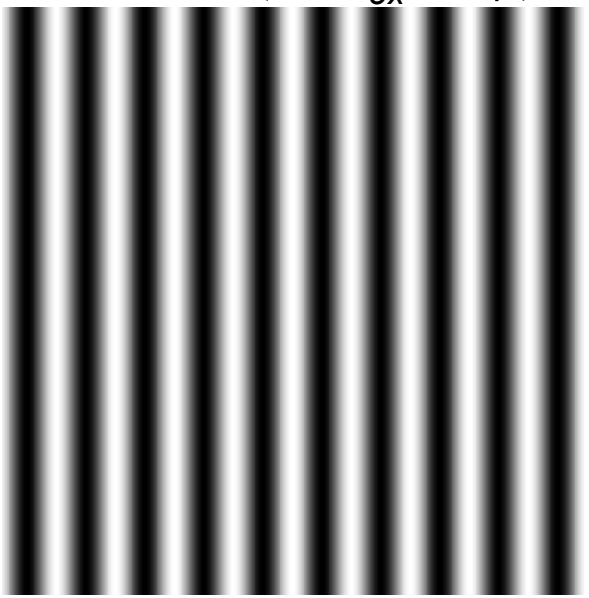


FFT

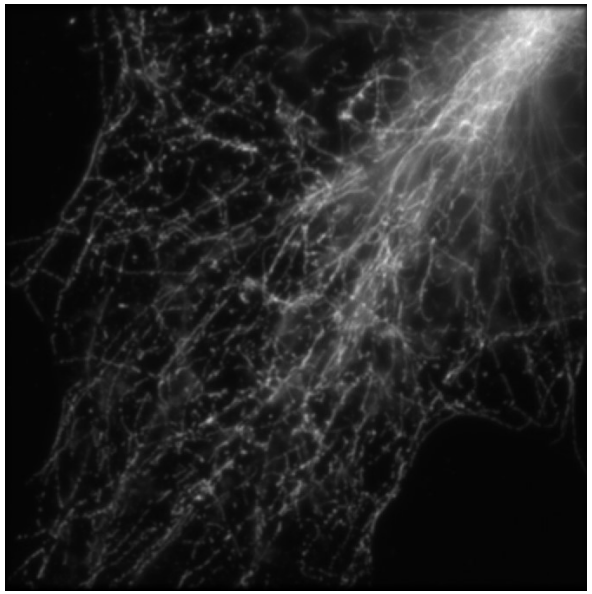
$$\tilde{D}(k)=\tilde{S}(k)OTF(k)$$



$$1+m\cos(2\pi k_{ex}r+\phi)$$



$$D(r)=S(r)\otimes PSF(r)$$



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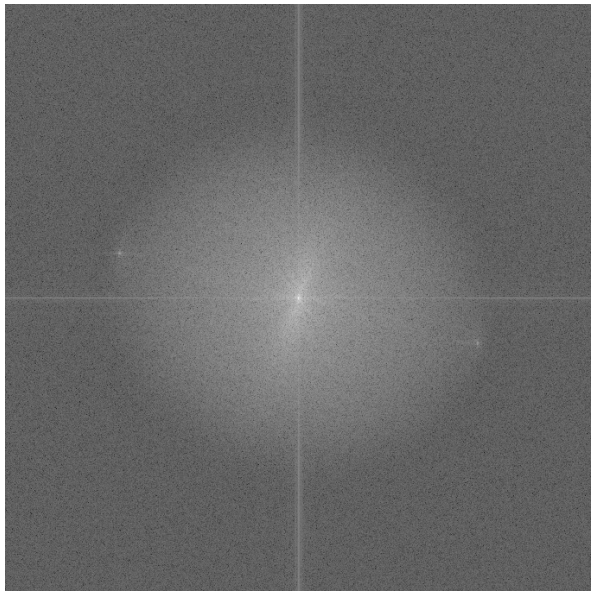
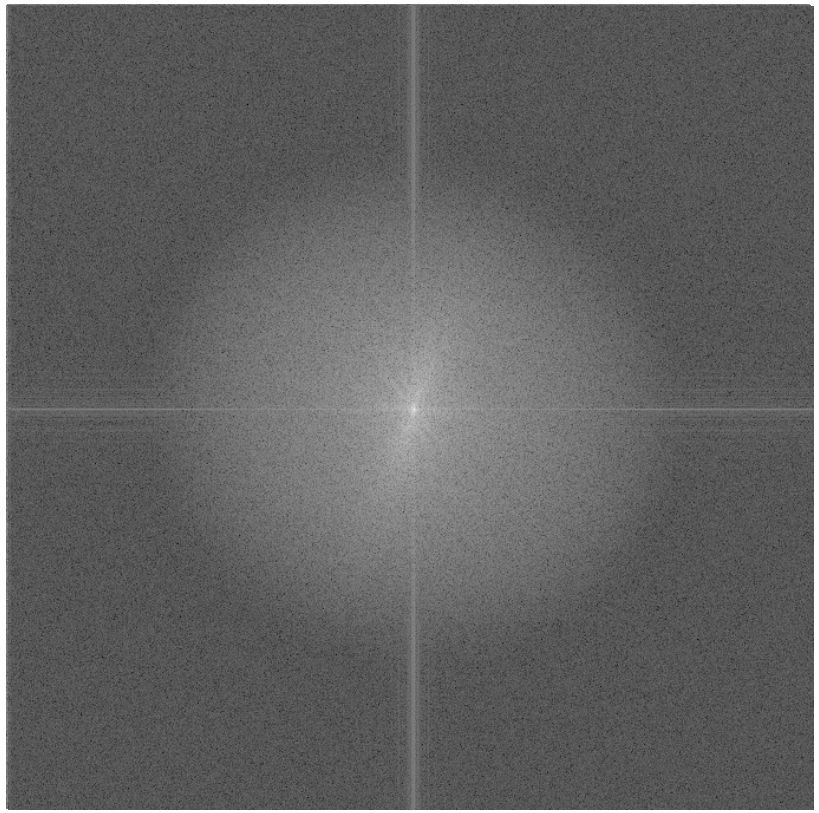
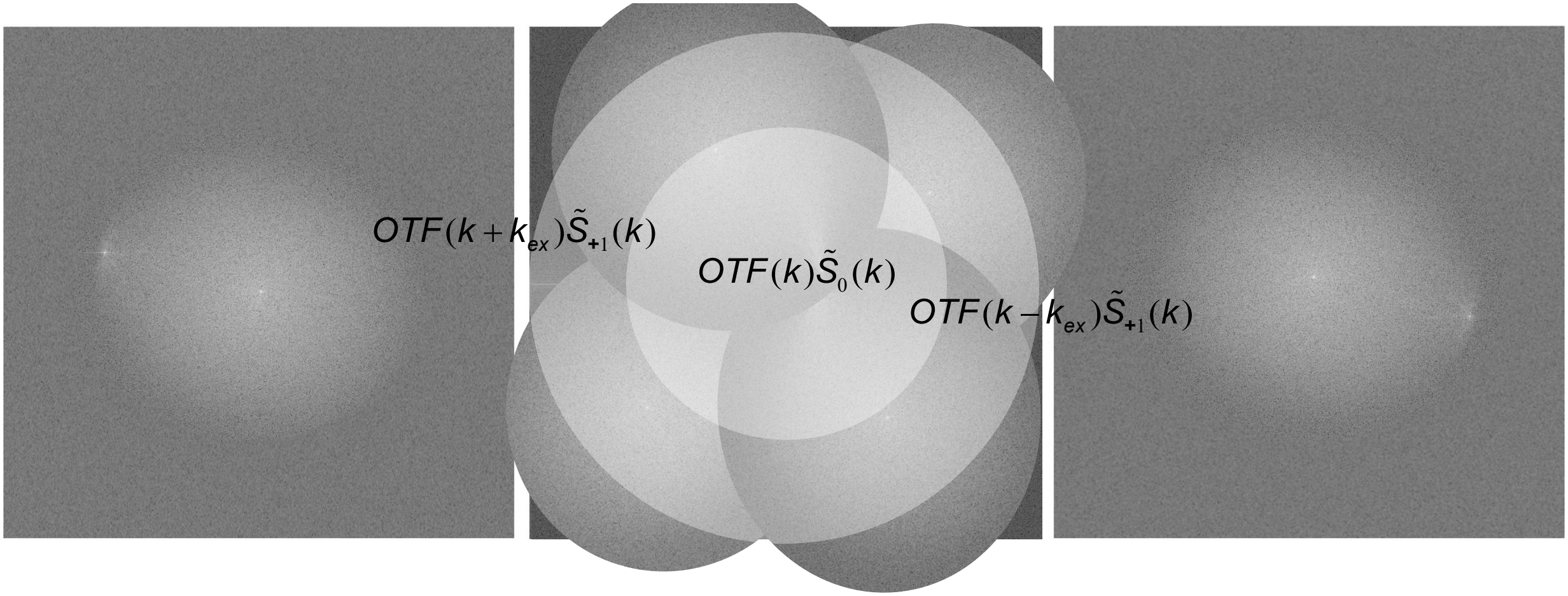
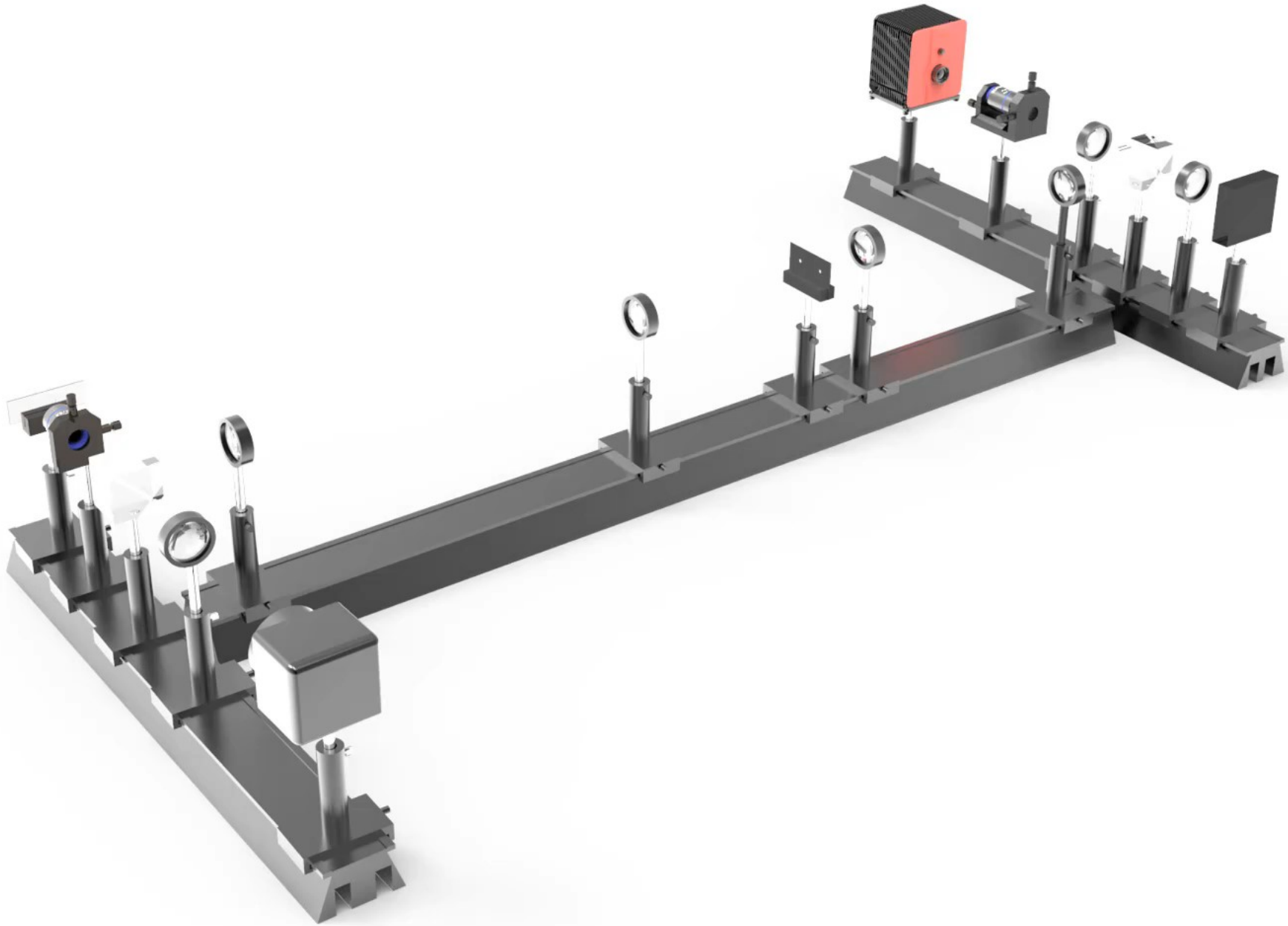


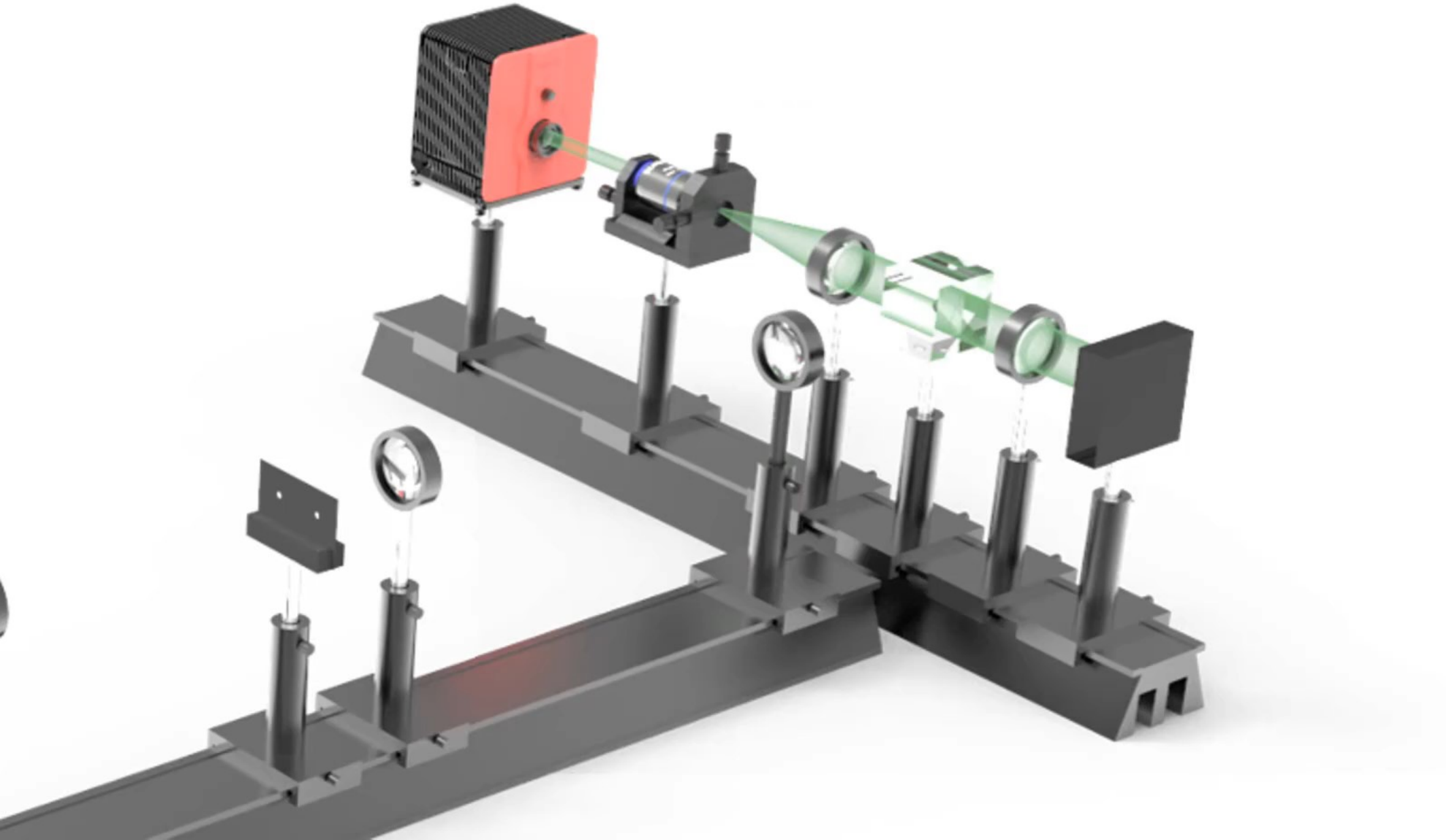
$$\tilde{D}(k)=[\frac{m}{2}\tilde{S}_{+1}(k-k_{ex})e^{j\phi}+\tilde{S}_0(k)+\frac{m}{2}\tilde{S}_{-1}(k+k_{ex})e^{-j\phi}]OTF(k)$$

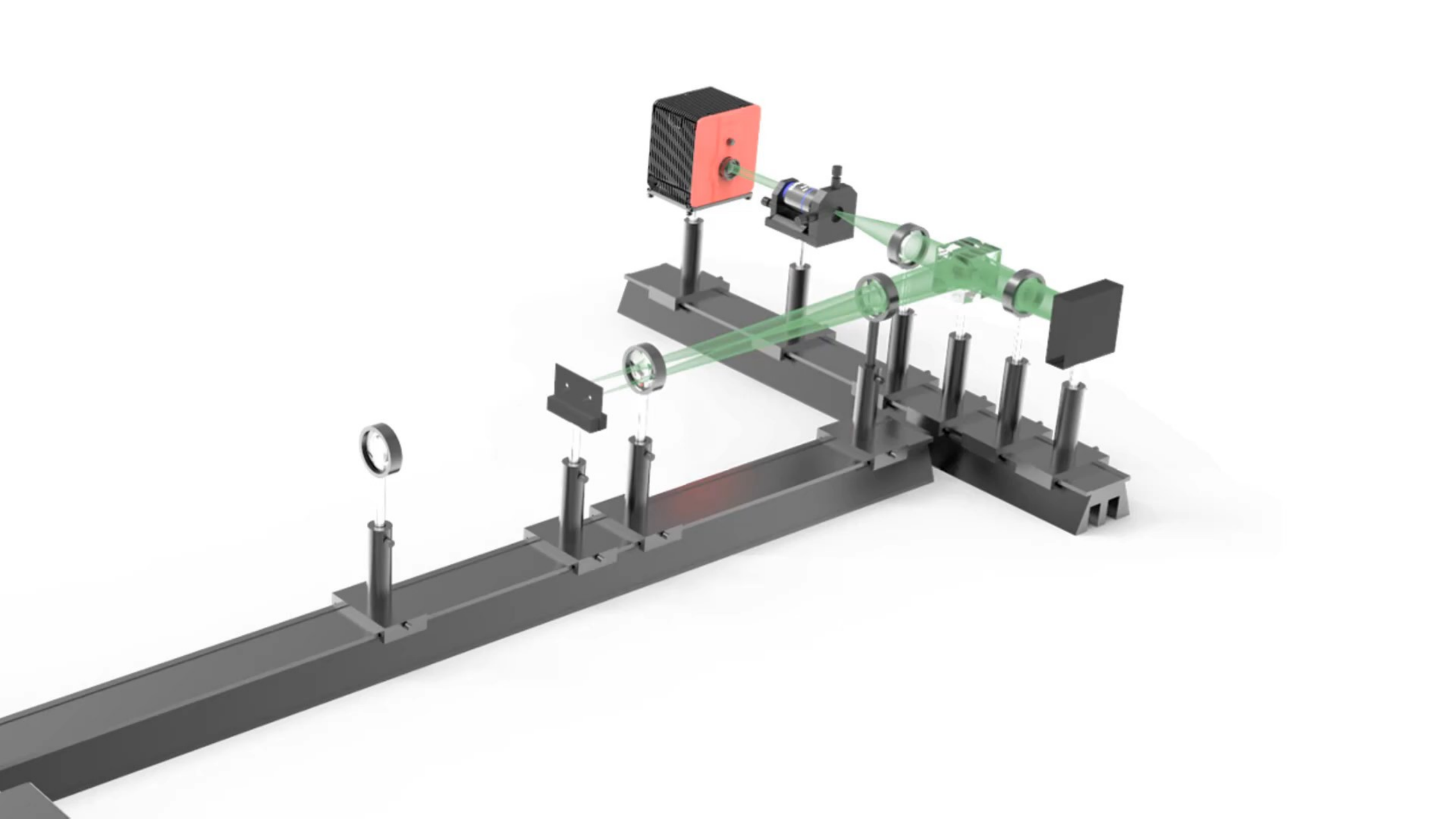
$OTF(k)\tilde{S}_{-1}(k+k_{ex})$ $OTF(k)\tilde{S}_{0}(k)$ $OTF(k)\tilde{S}_{+1}(k-k_{ex})$

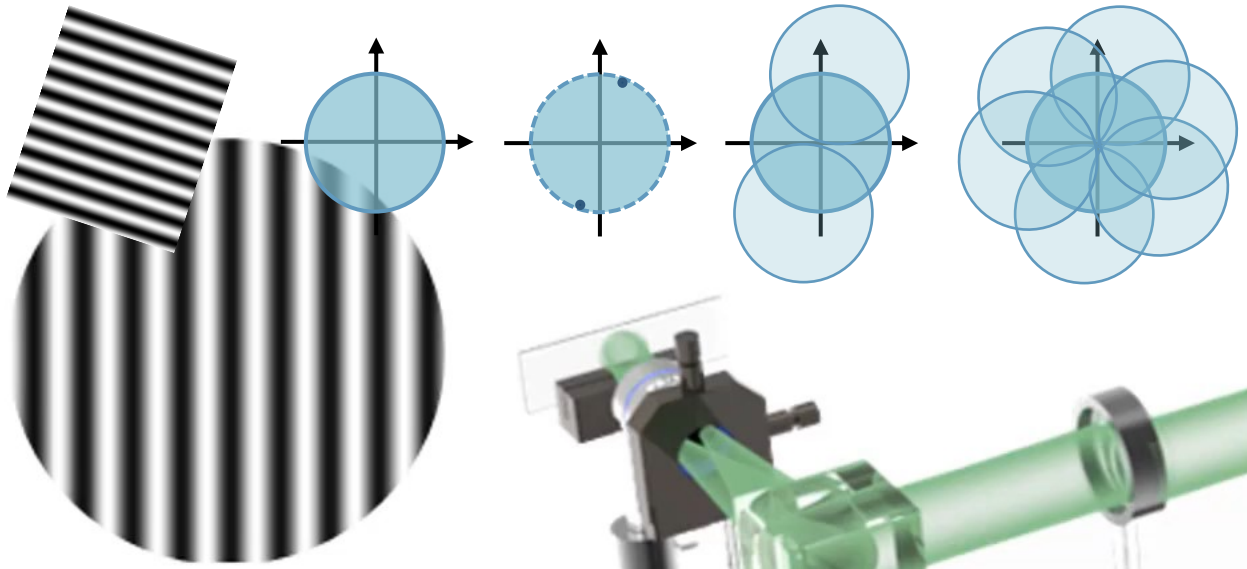


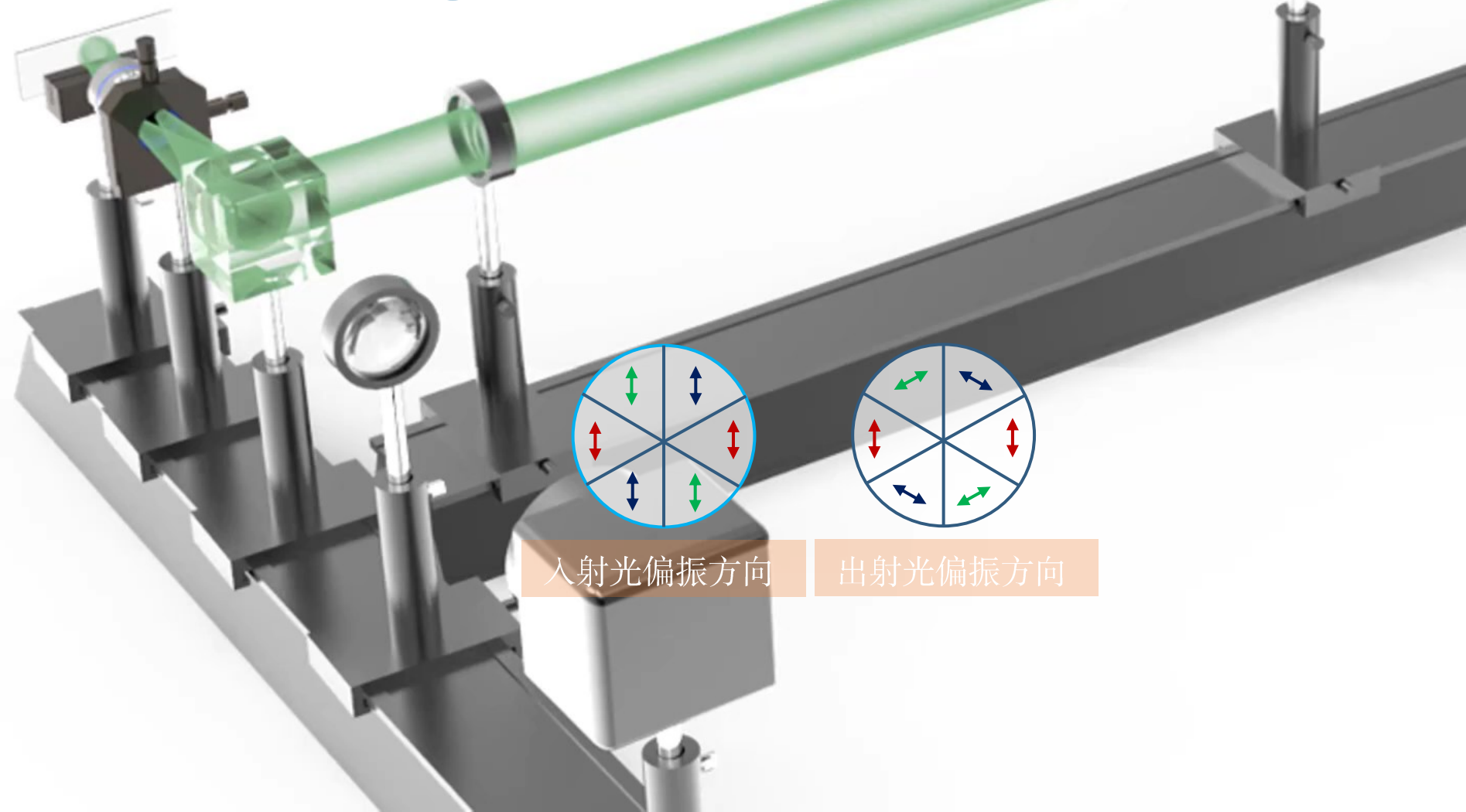

$OTF(k)\tilde{S}_{-1}(k+k_{ex})$
$OTF(k)\tilde{S}_{0}(k)$
$OTF(k)\tilde{S}_{+1}(k-k_{ex})$
$OTF(k+k_{ex})\tilde{S}_{+1}(k)$
$OTF(k)\tilde{S}_{0}(k)$
$OTF(k-k_{ex})\tilde{S}_{+1}(k)$





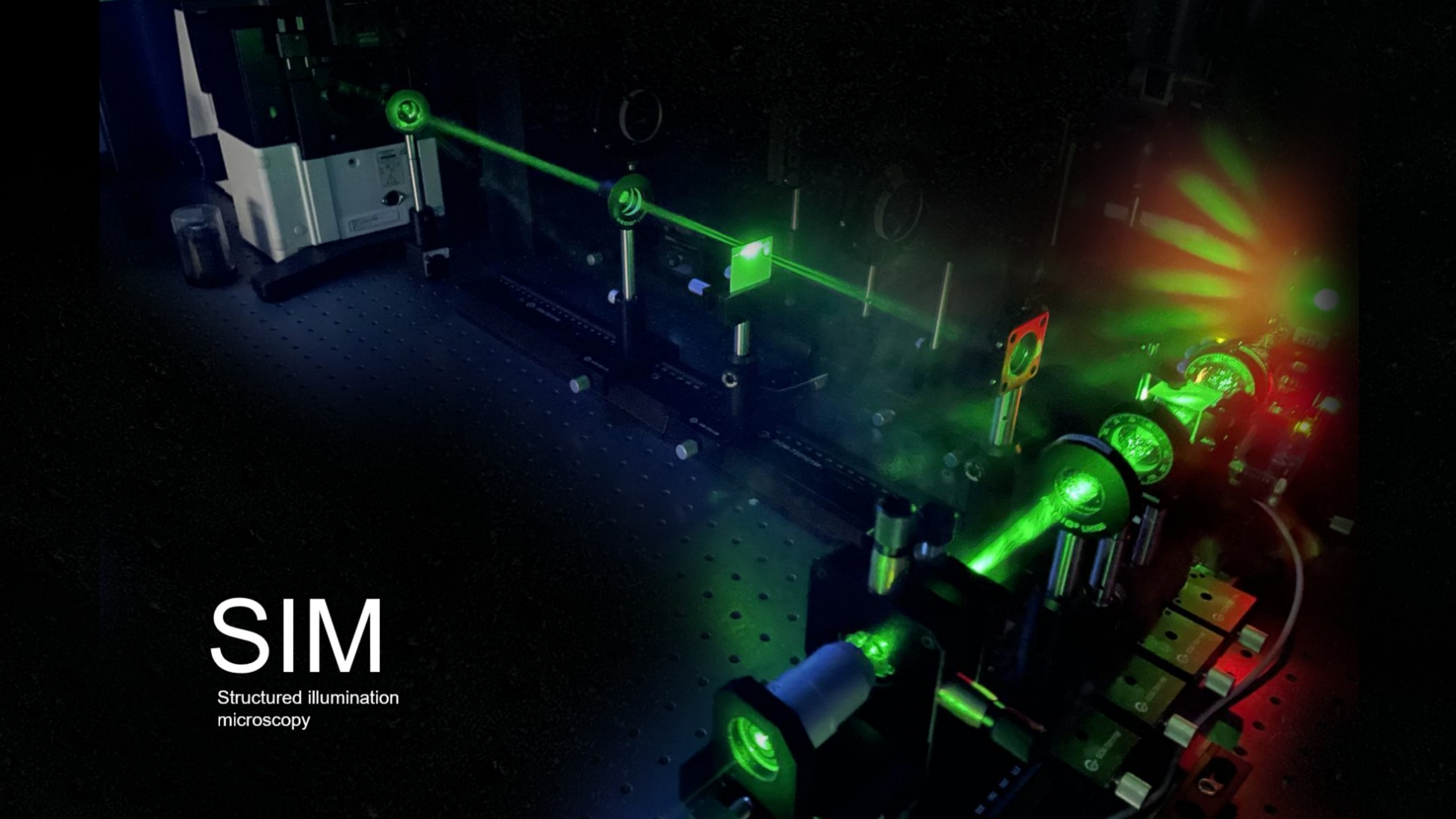


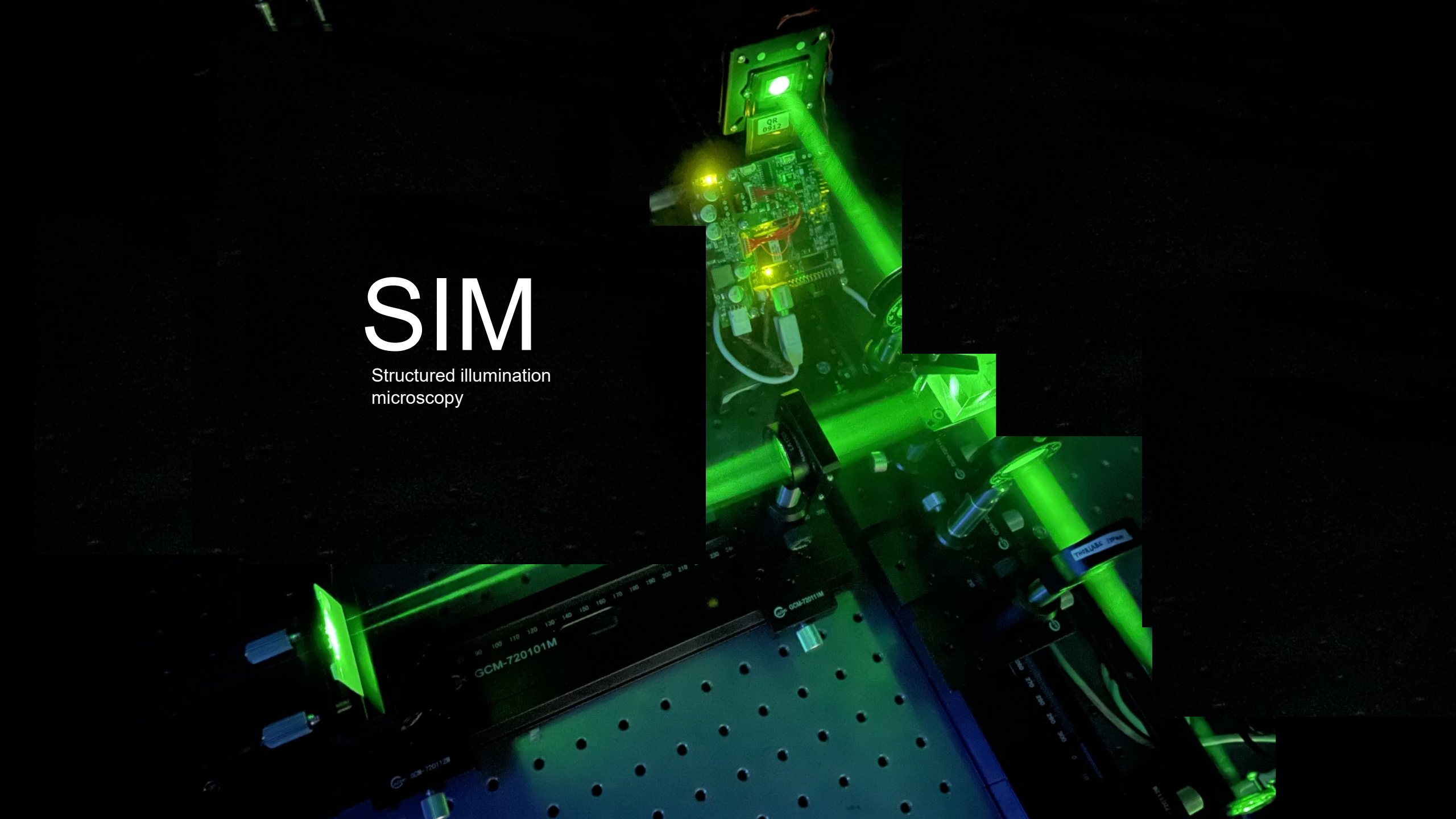




入射光偏振方向

出射光偏振方向


SIM
Structured illumination
microscopy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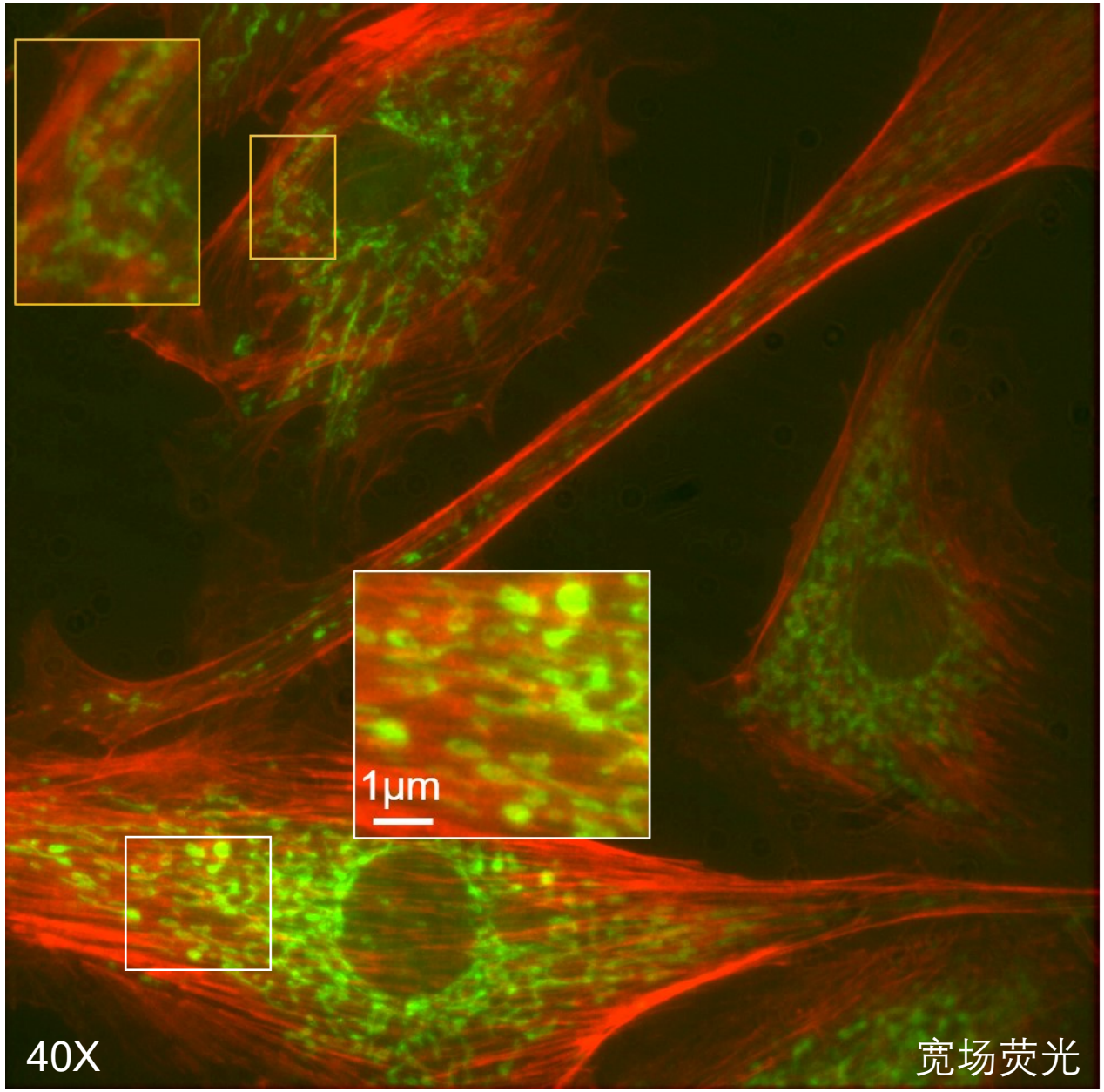
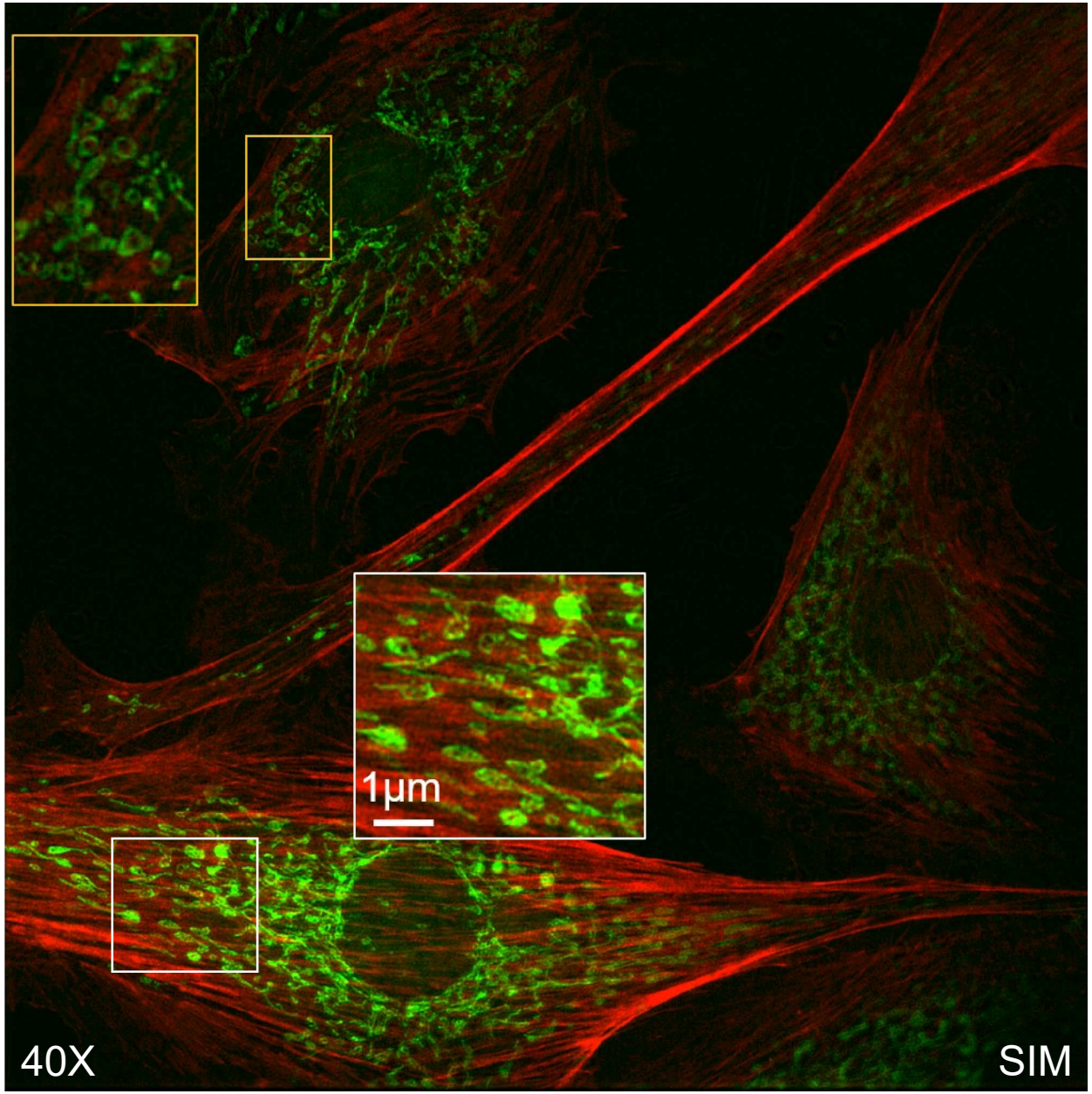
microscopy
GCM-720101M


1μm
40X
宽场荧光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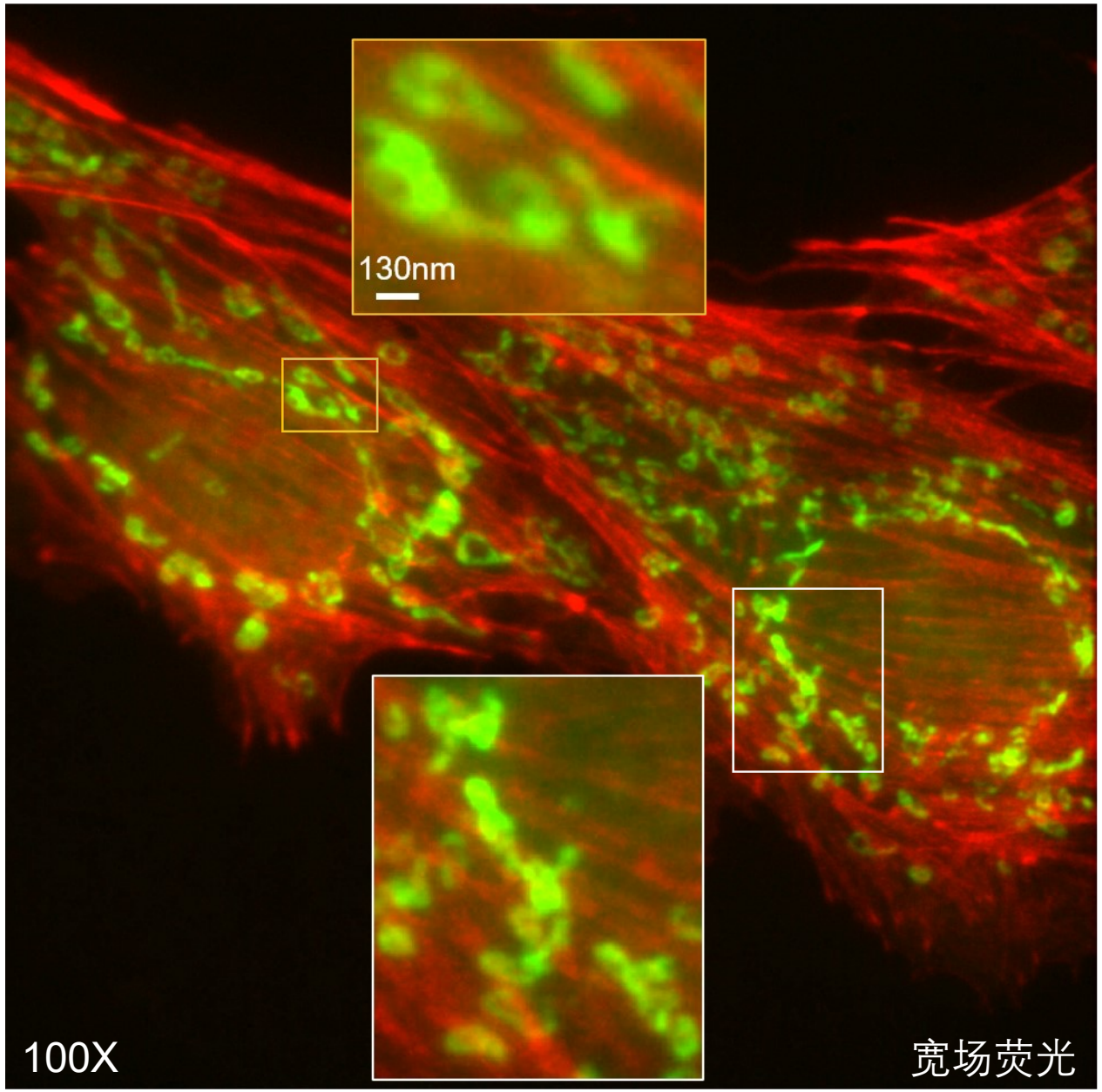
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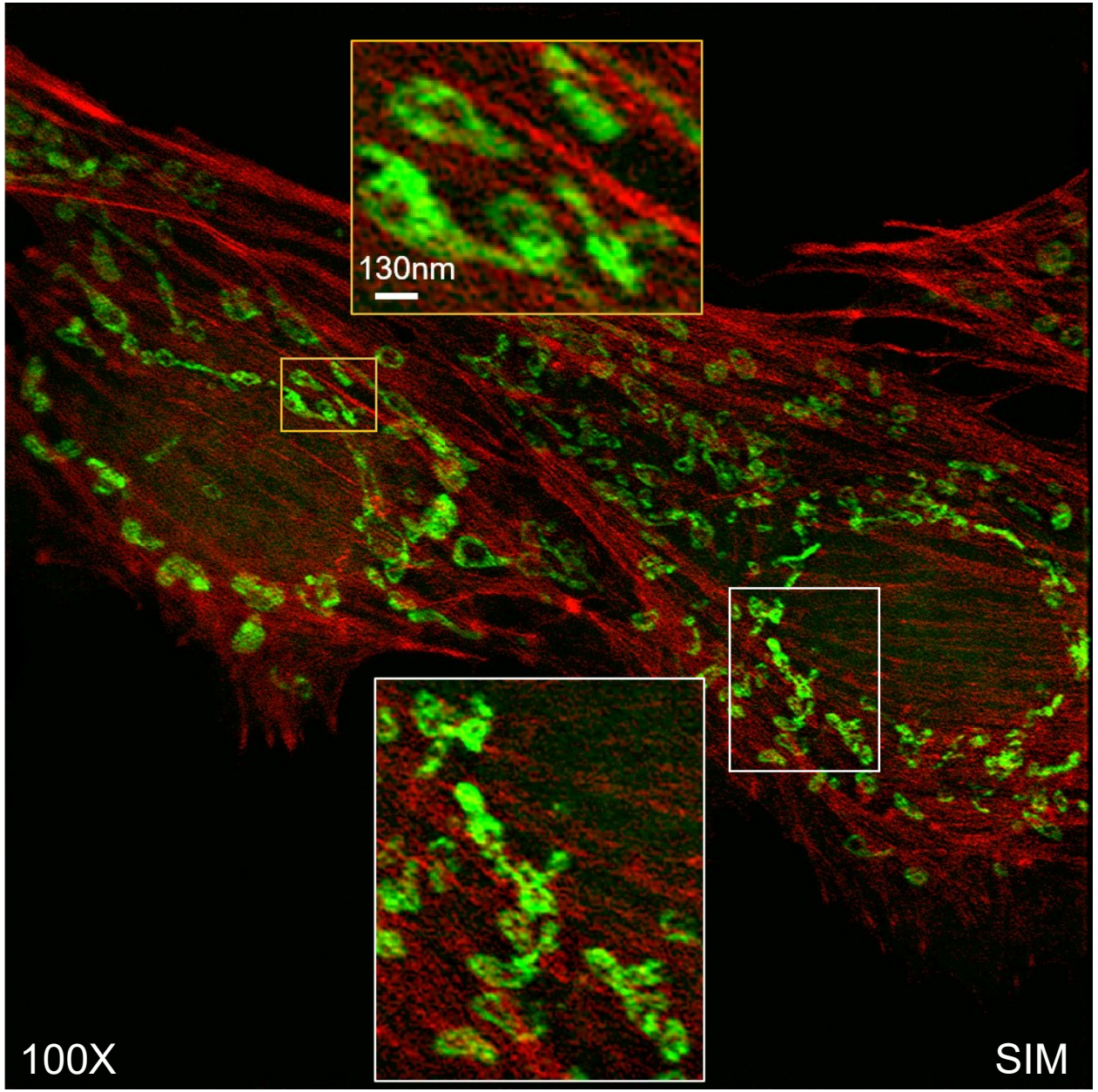
100X
宽场荧光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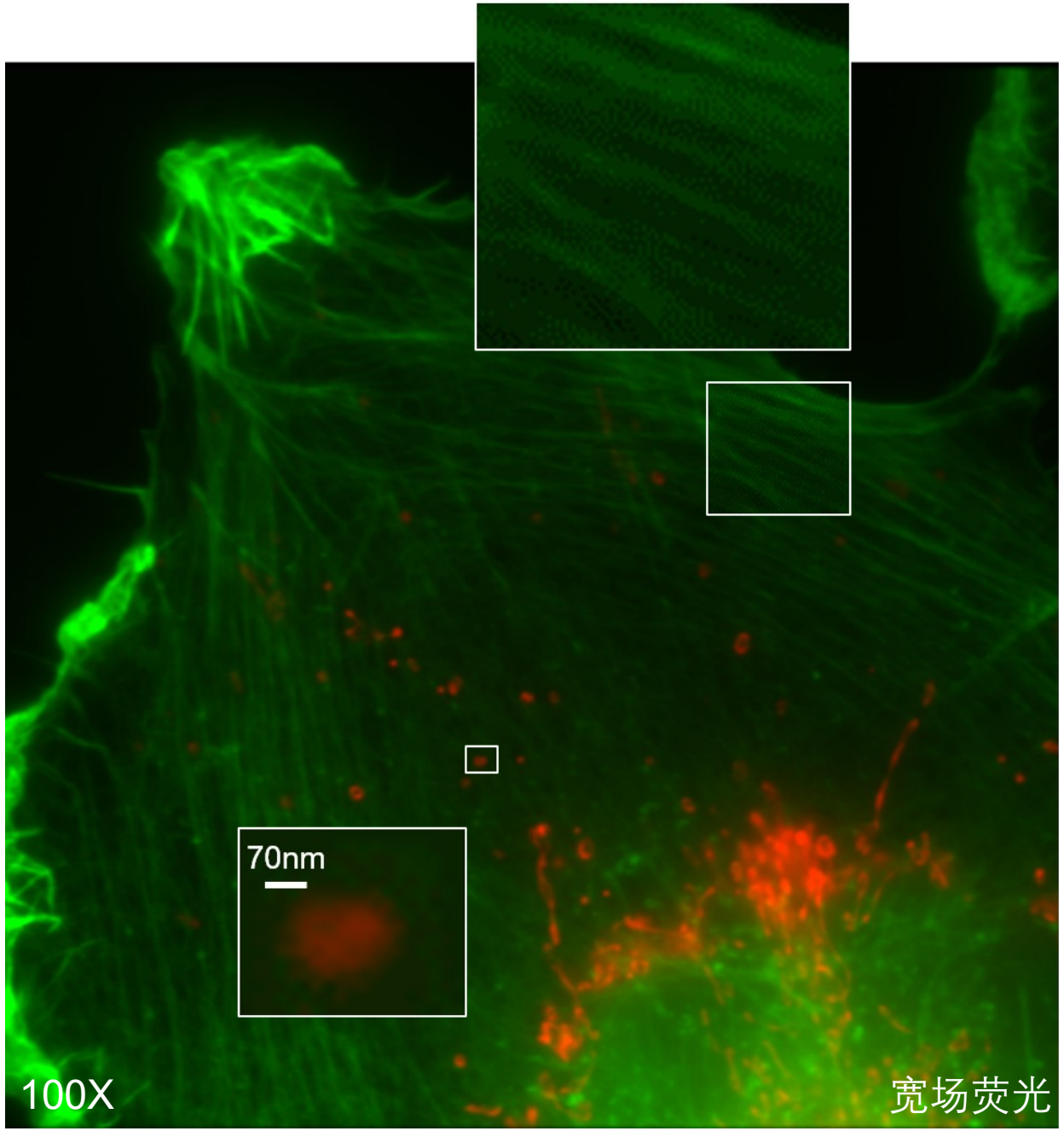
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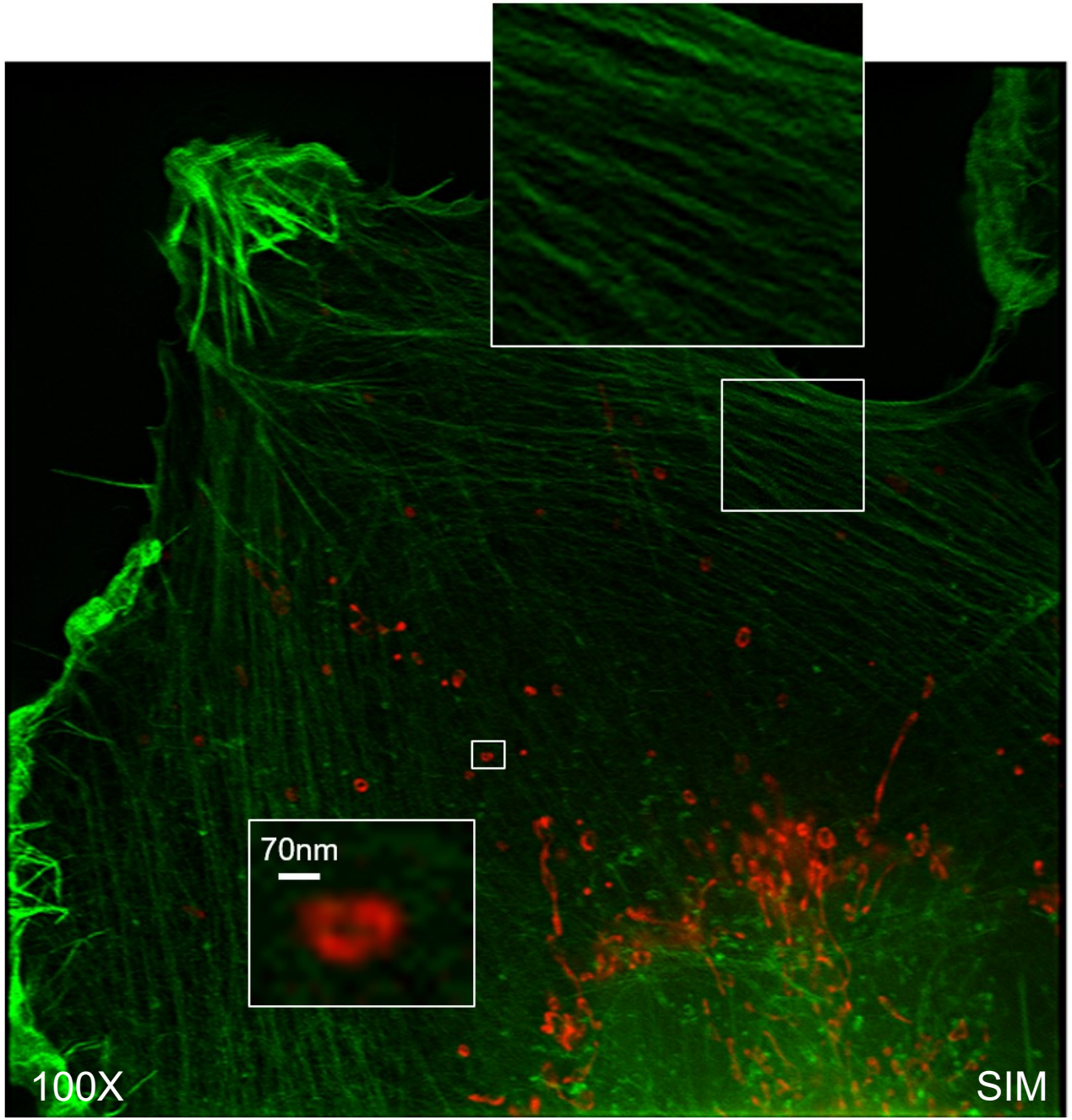
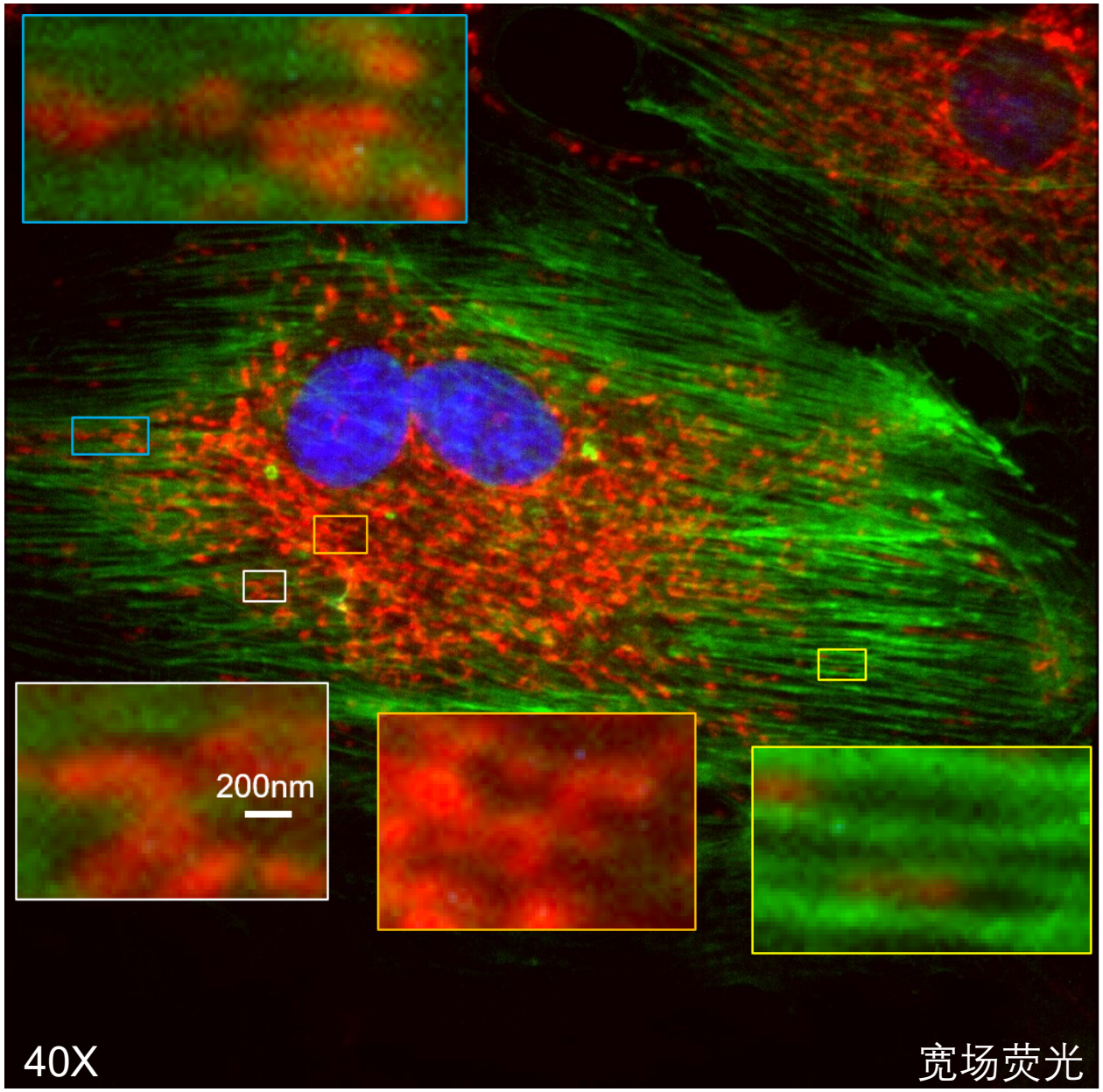
100X
宽场荧光


70nm
100X
SIM

SIM

Structured illumination microscopy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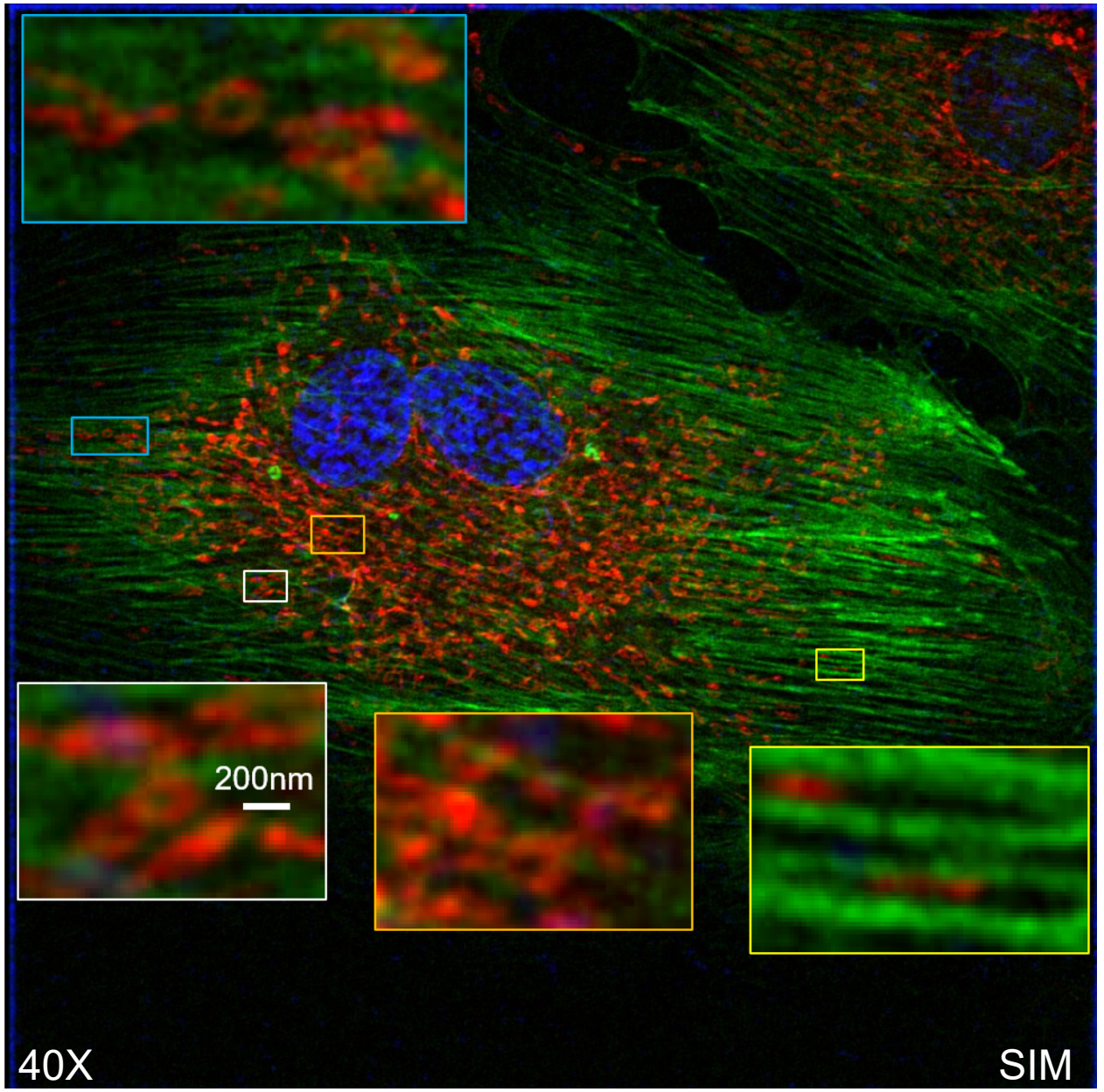
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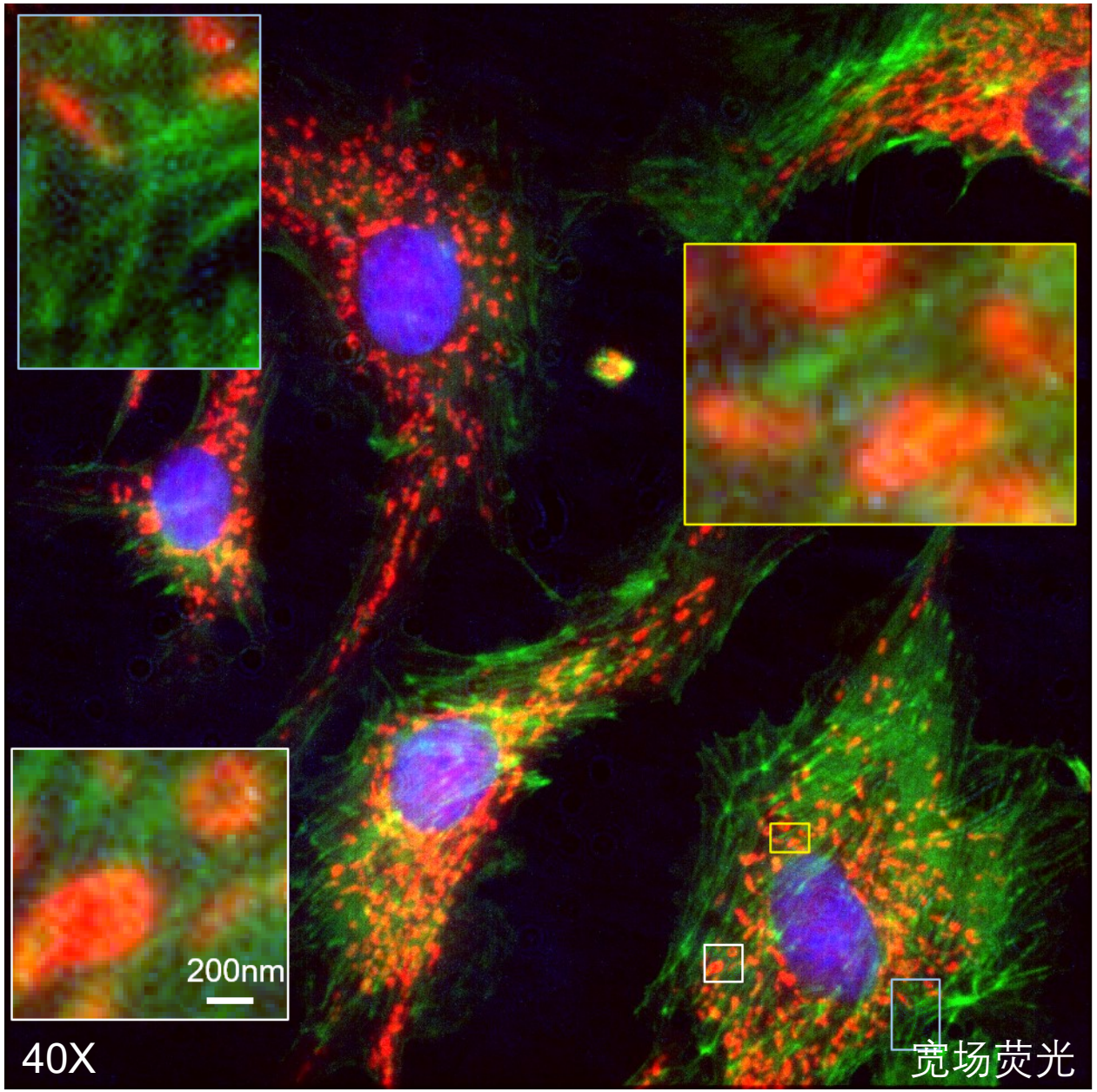
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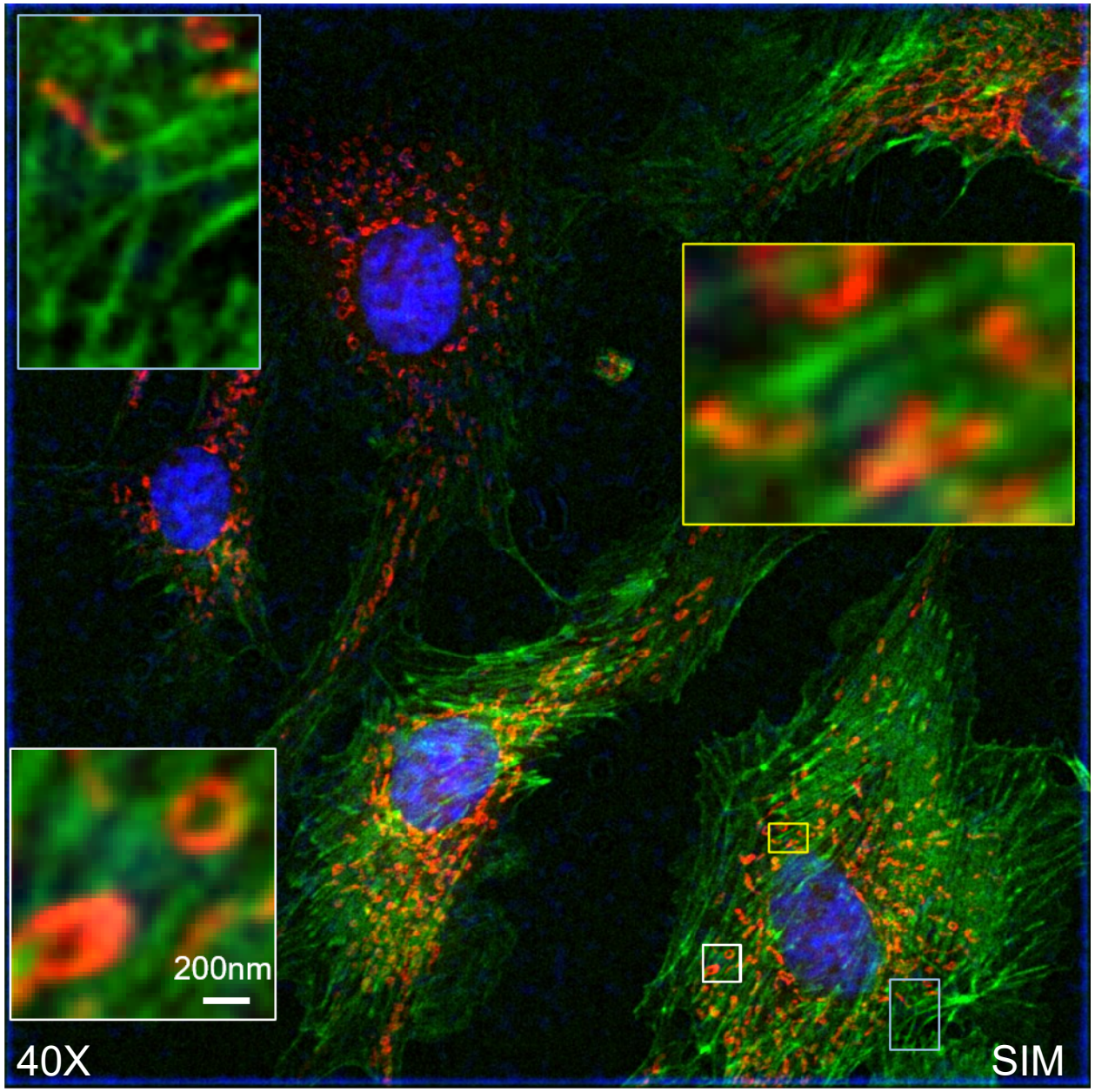
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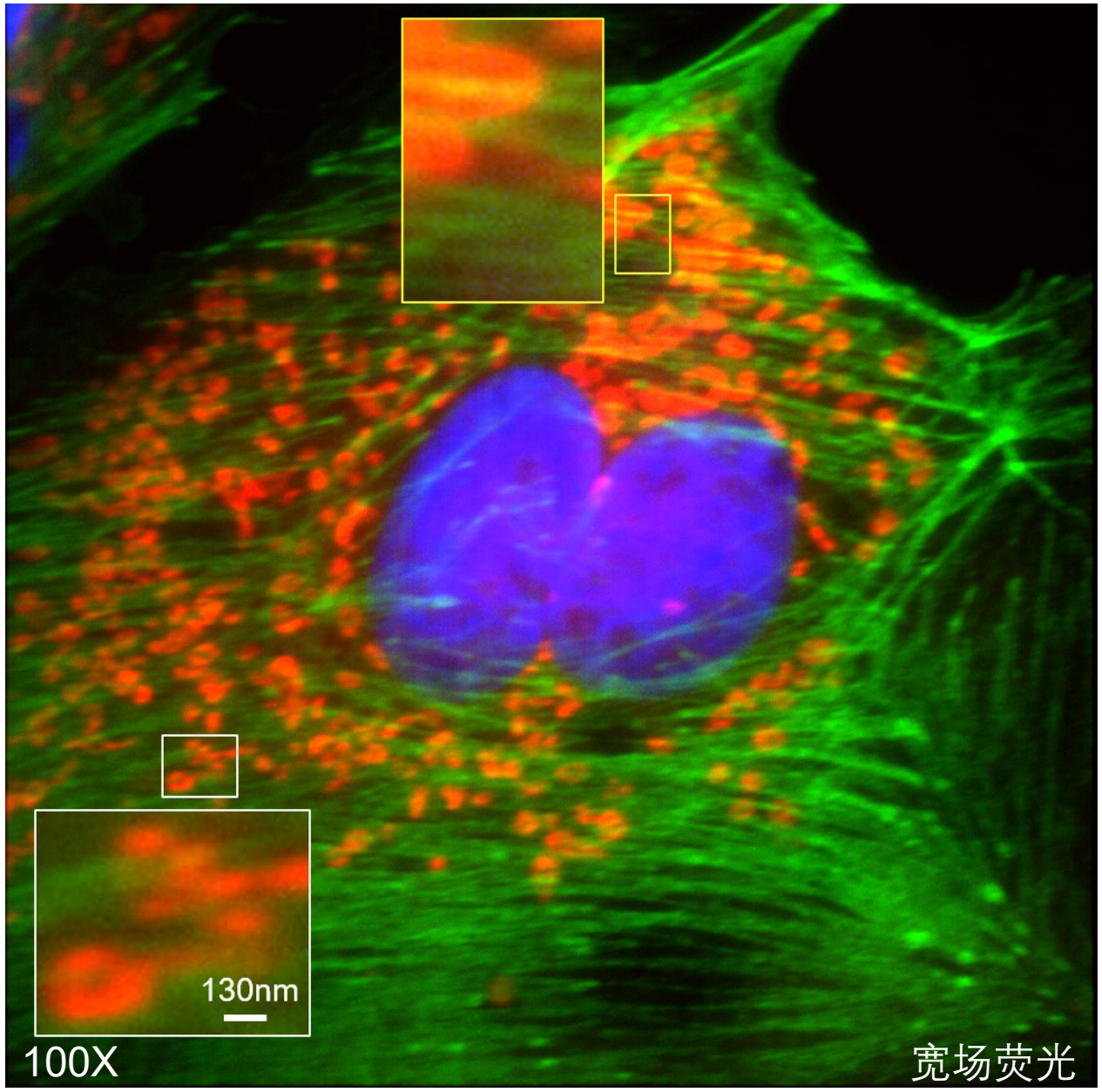
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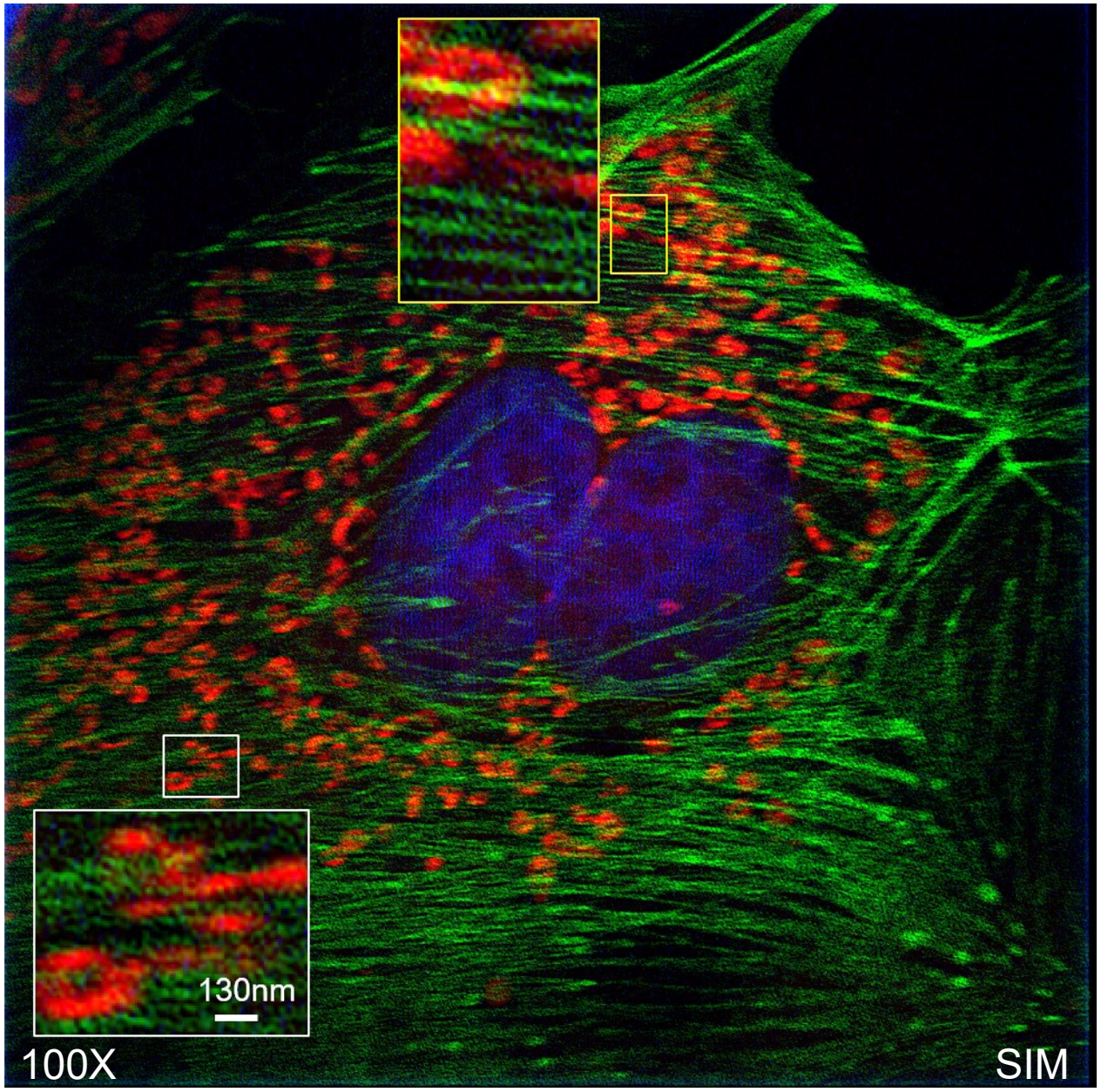
宽场荧光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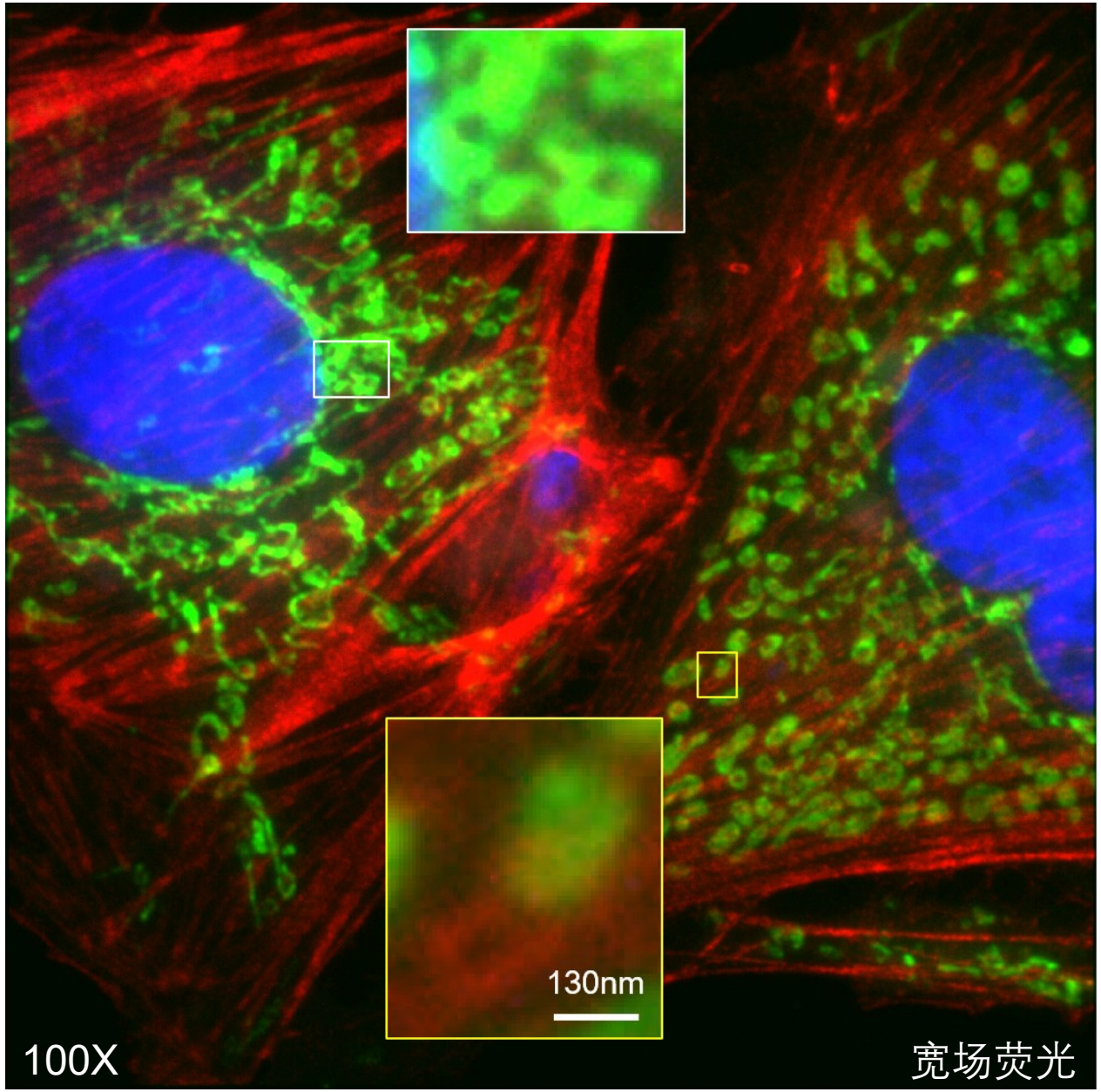
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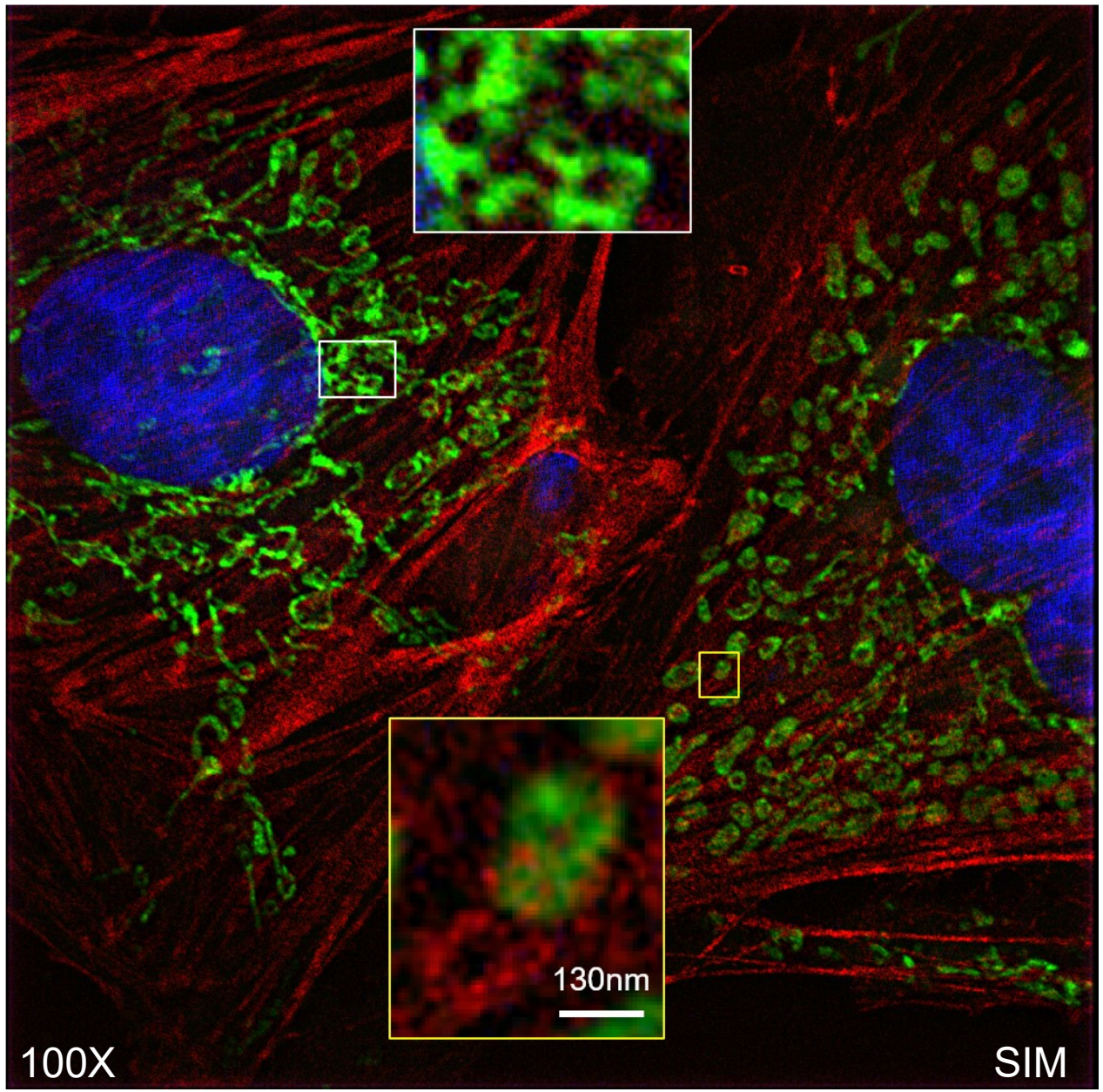
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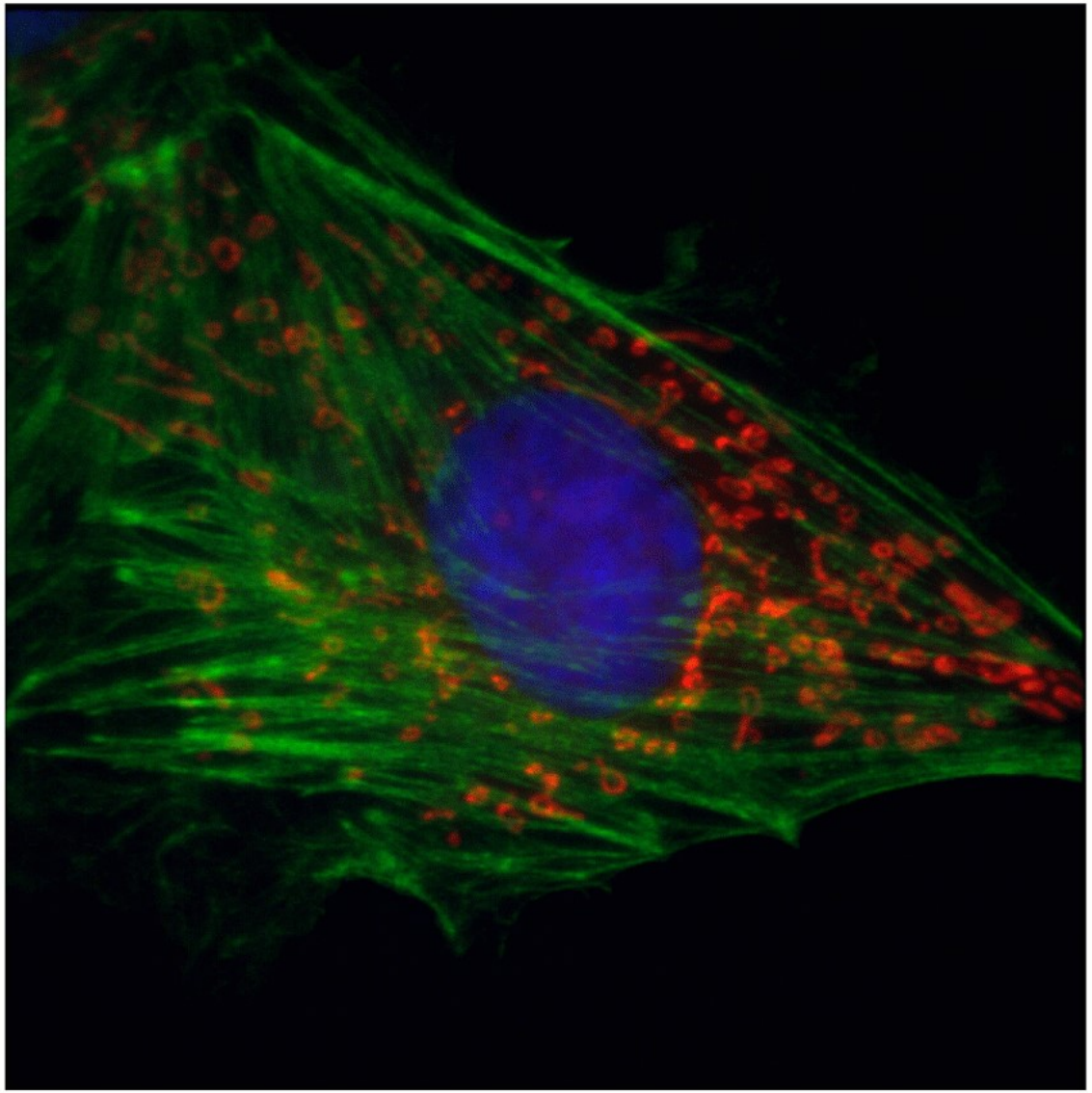

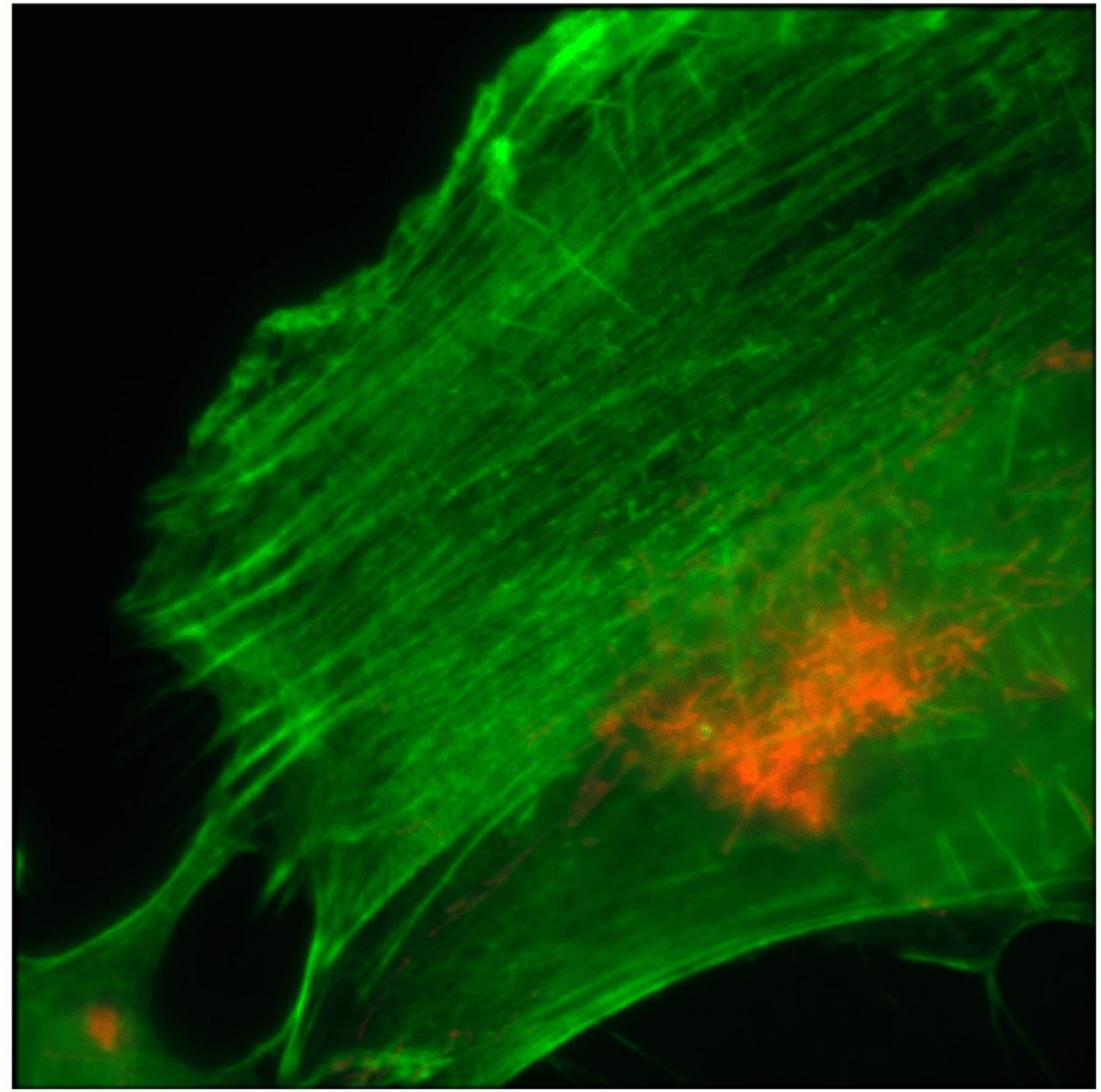

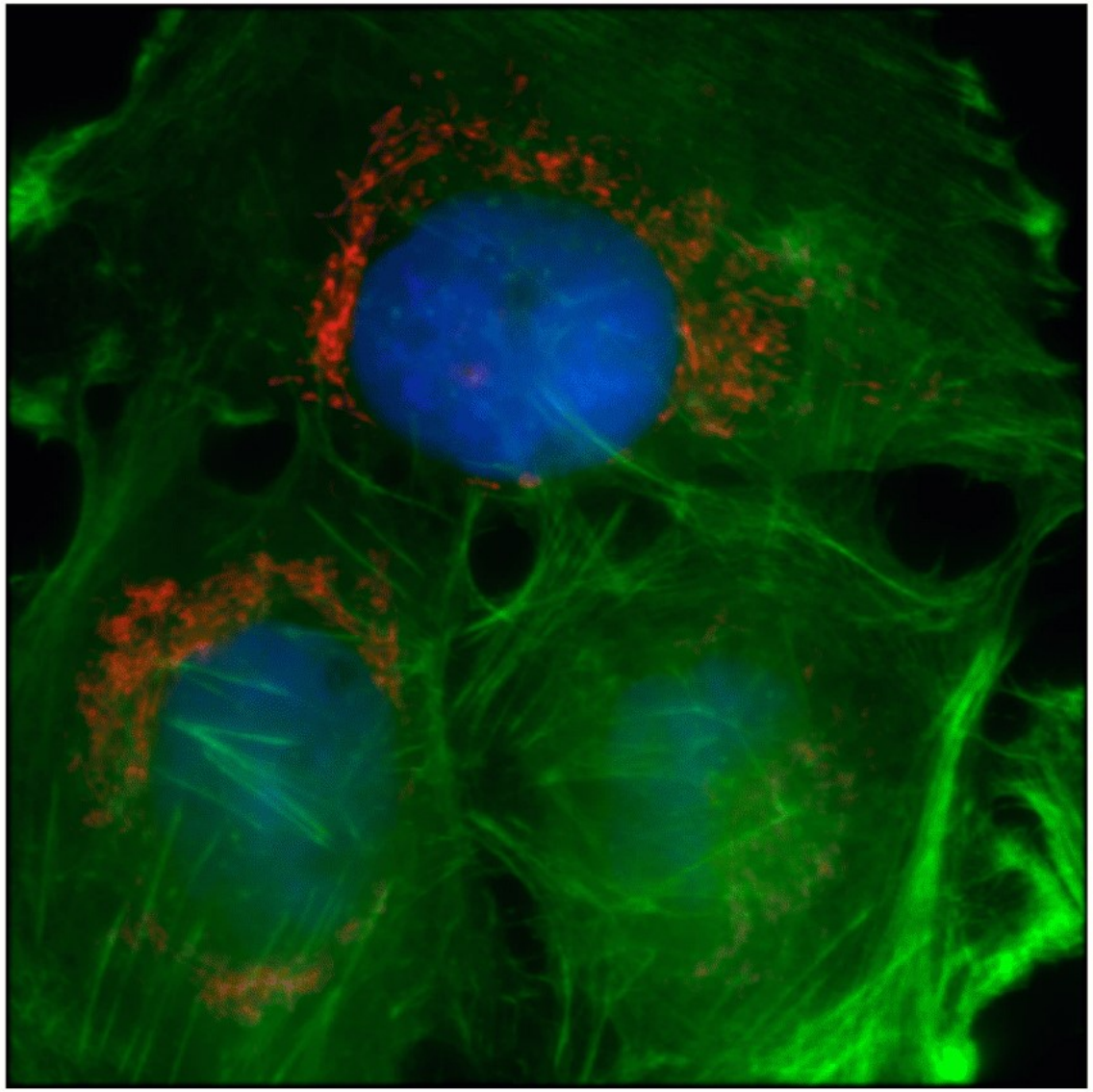

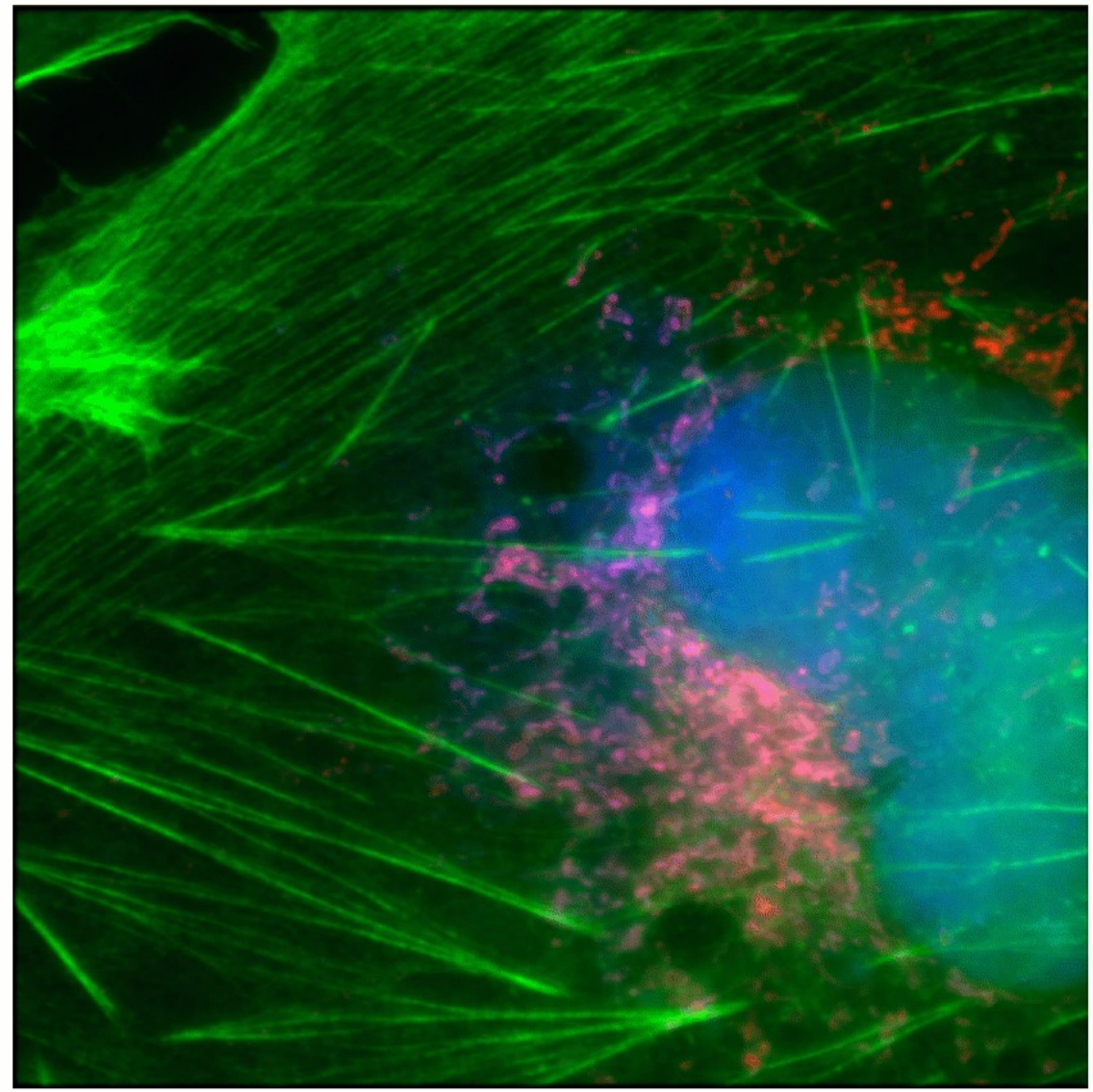

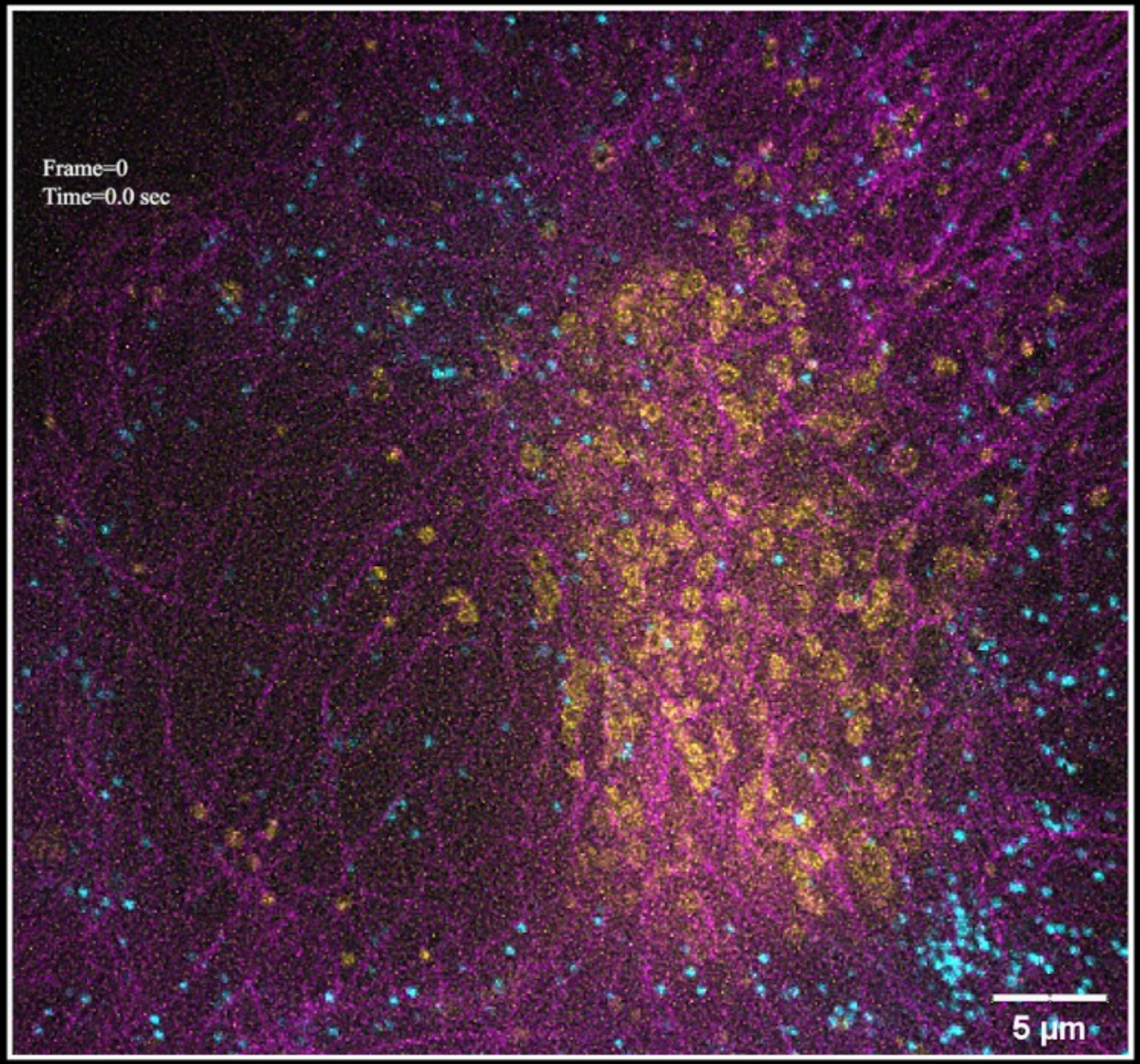
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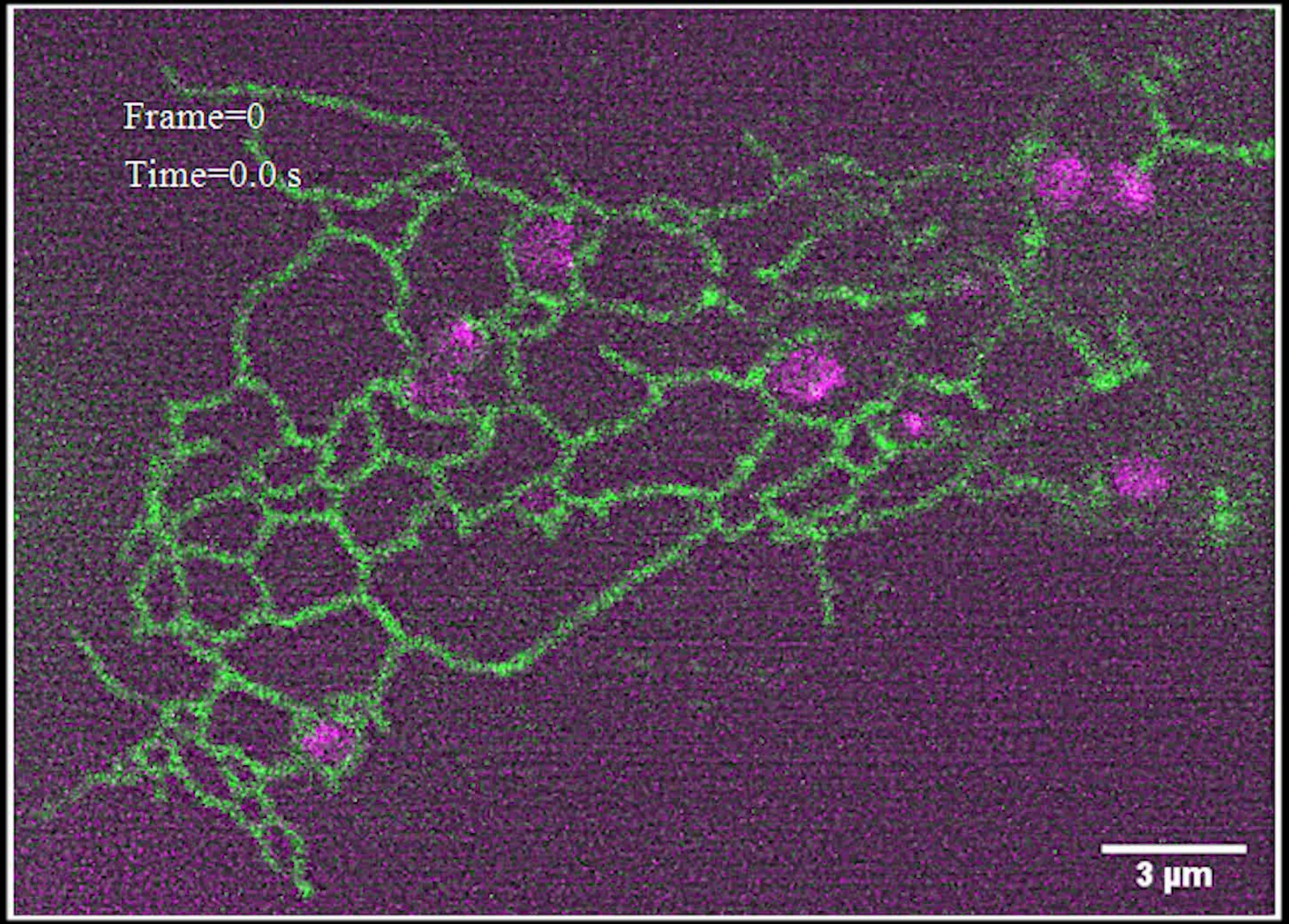
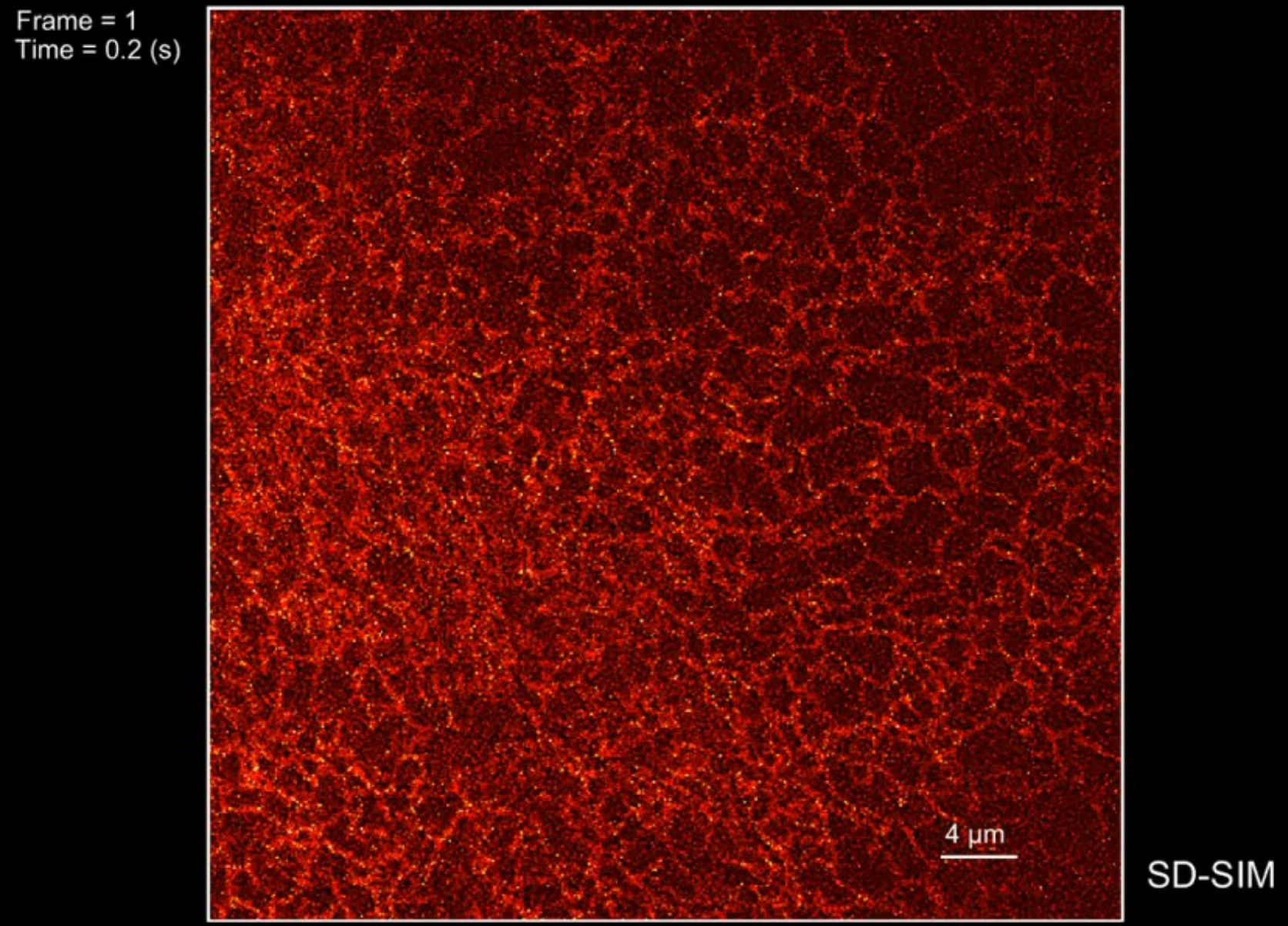
Time=0.0 sec
5 µm





细胞外围的管状内质网

PNAS

Nonlinear structured-illumination microscopy: Wide-field fluorescence imaging with theoretically unlimited resolution

Mats G. L. Gustafsson*

Department of Physiology and Program in Bioengineering, University of California, San Francisco, CA 94143-2532

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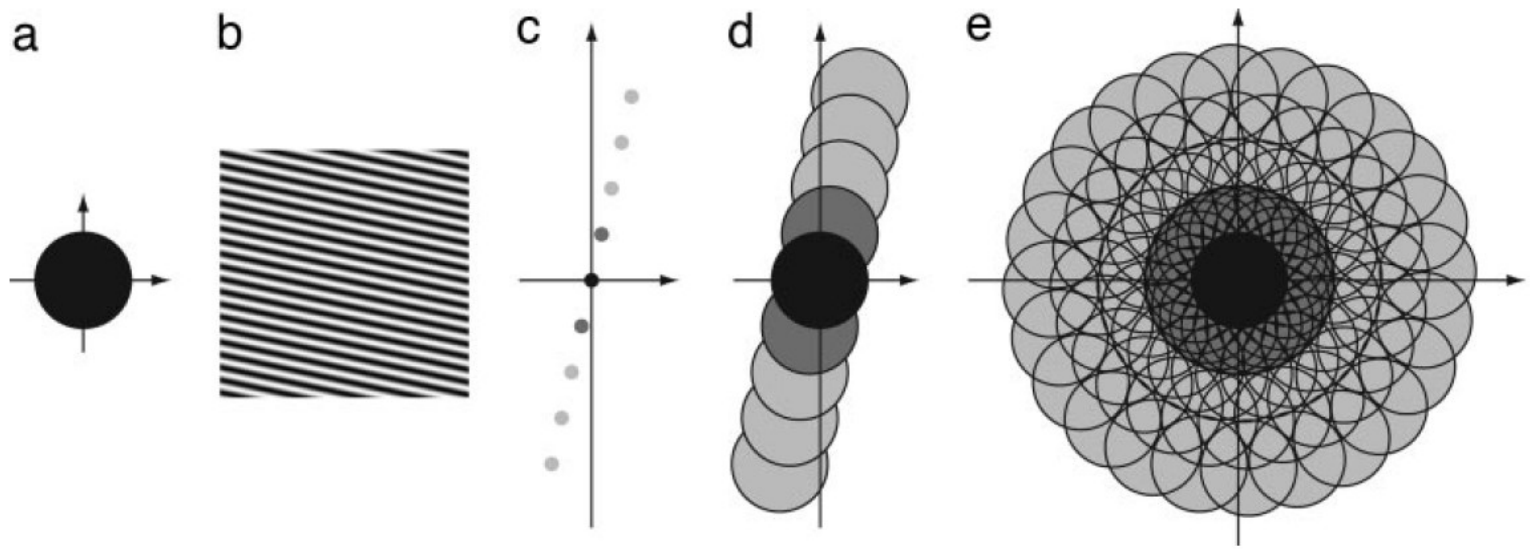
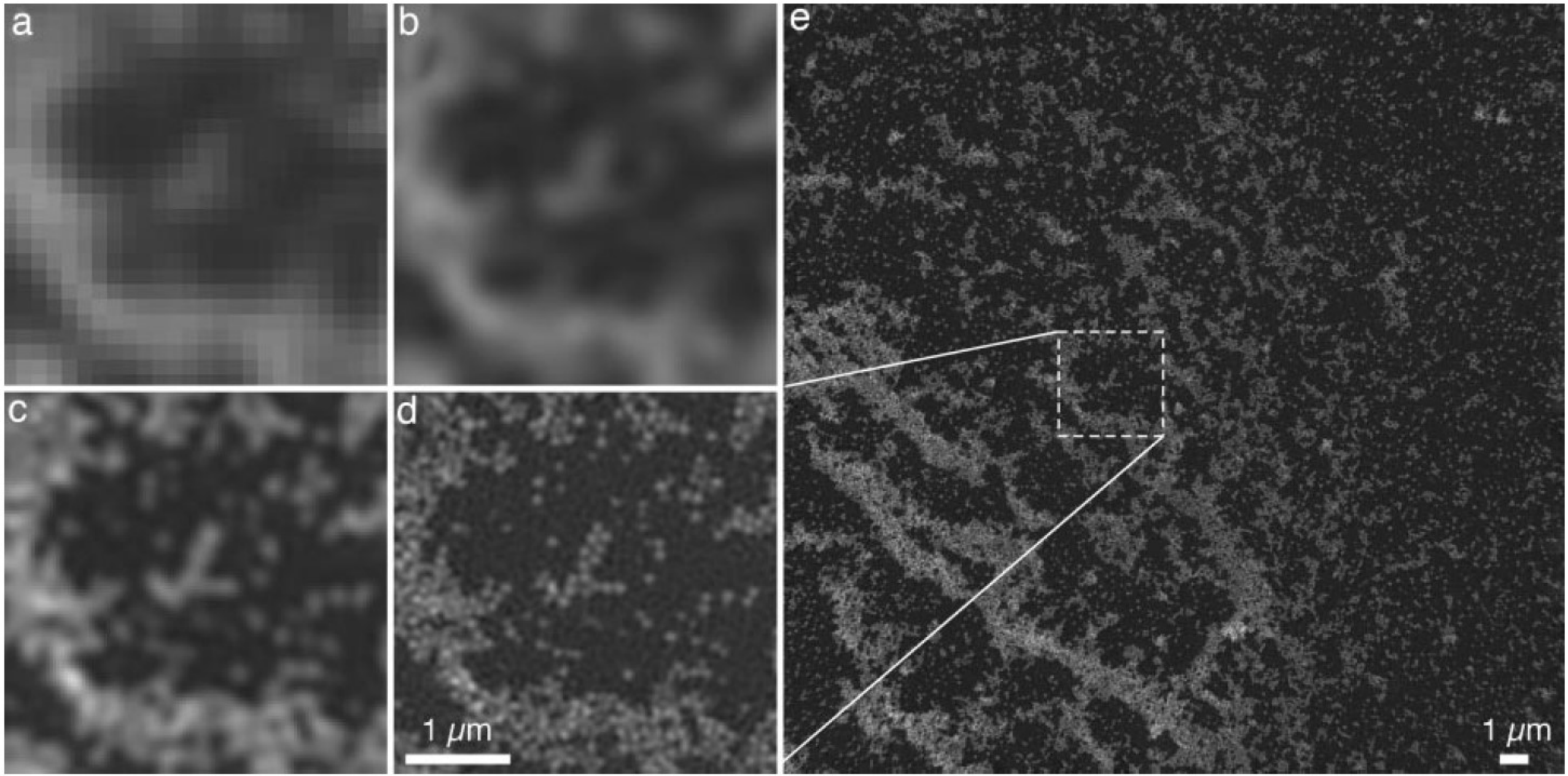
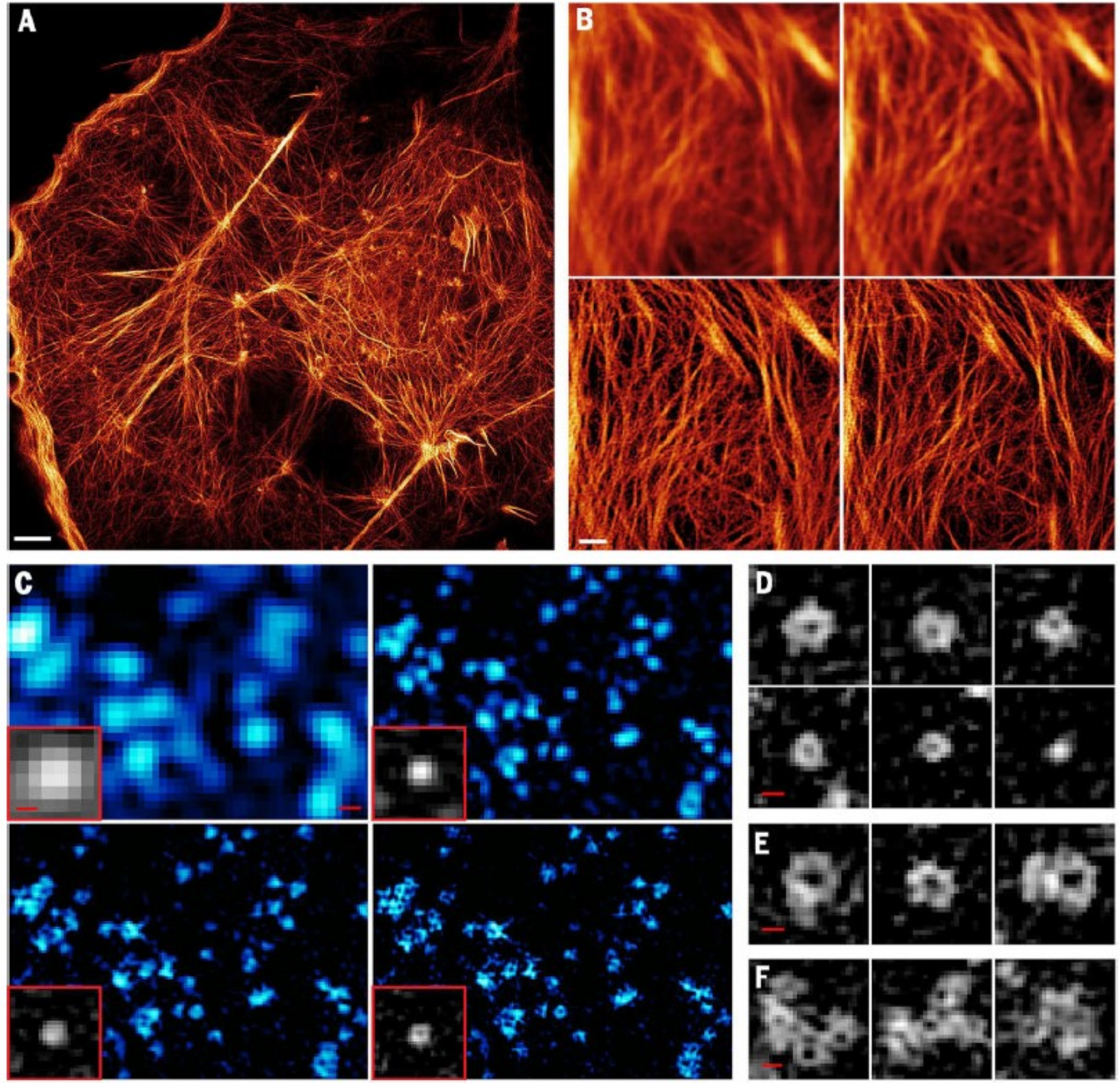
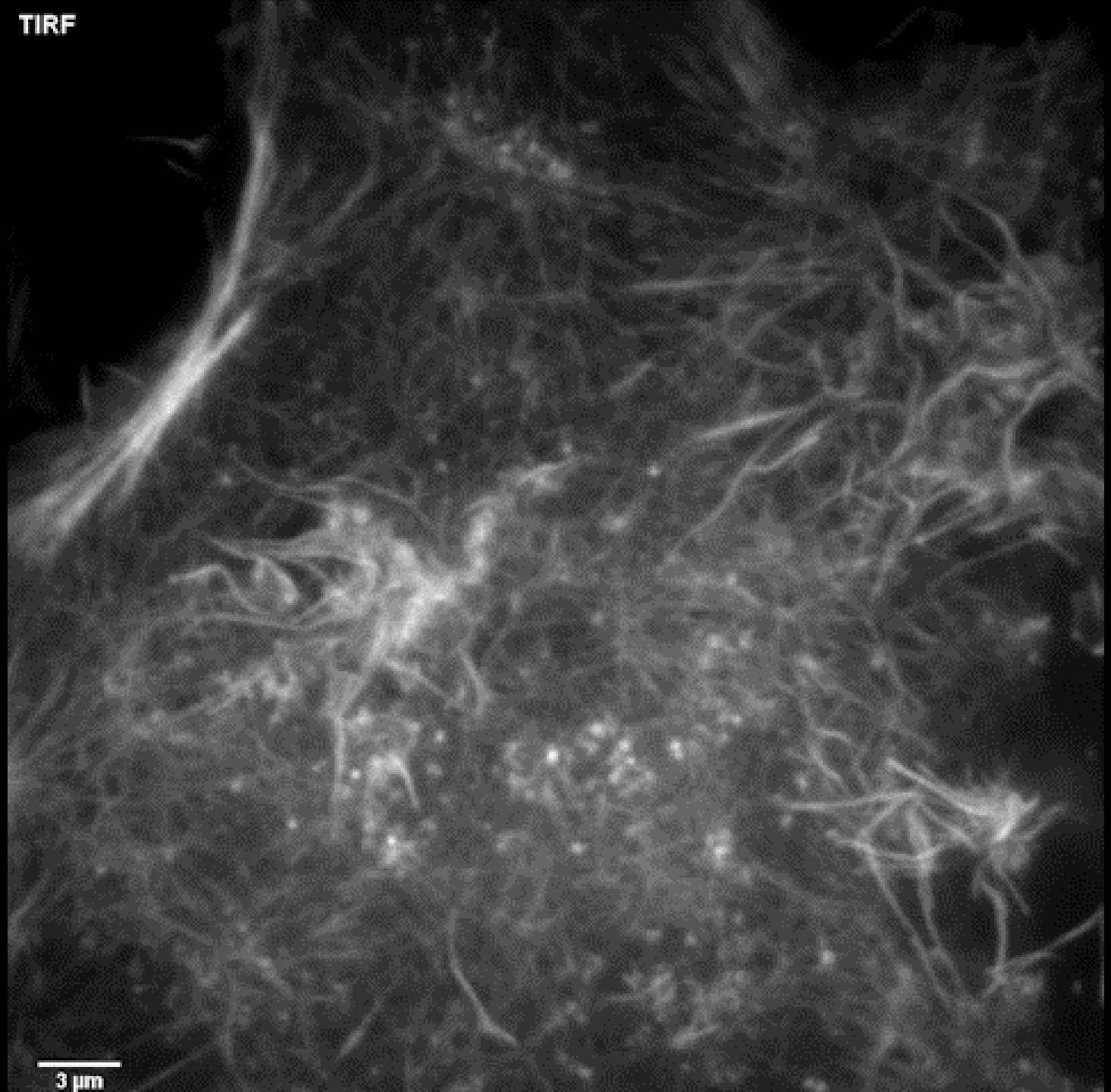
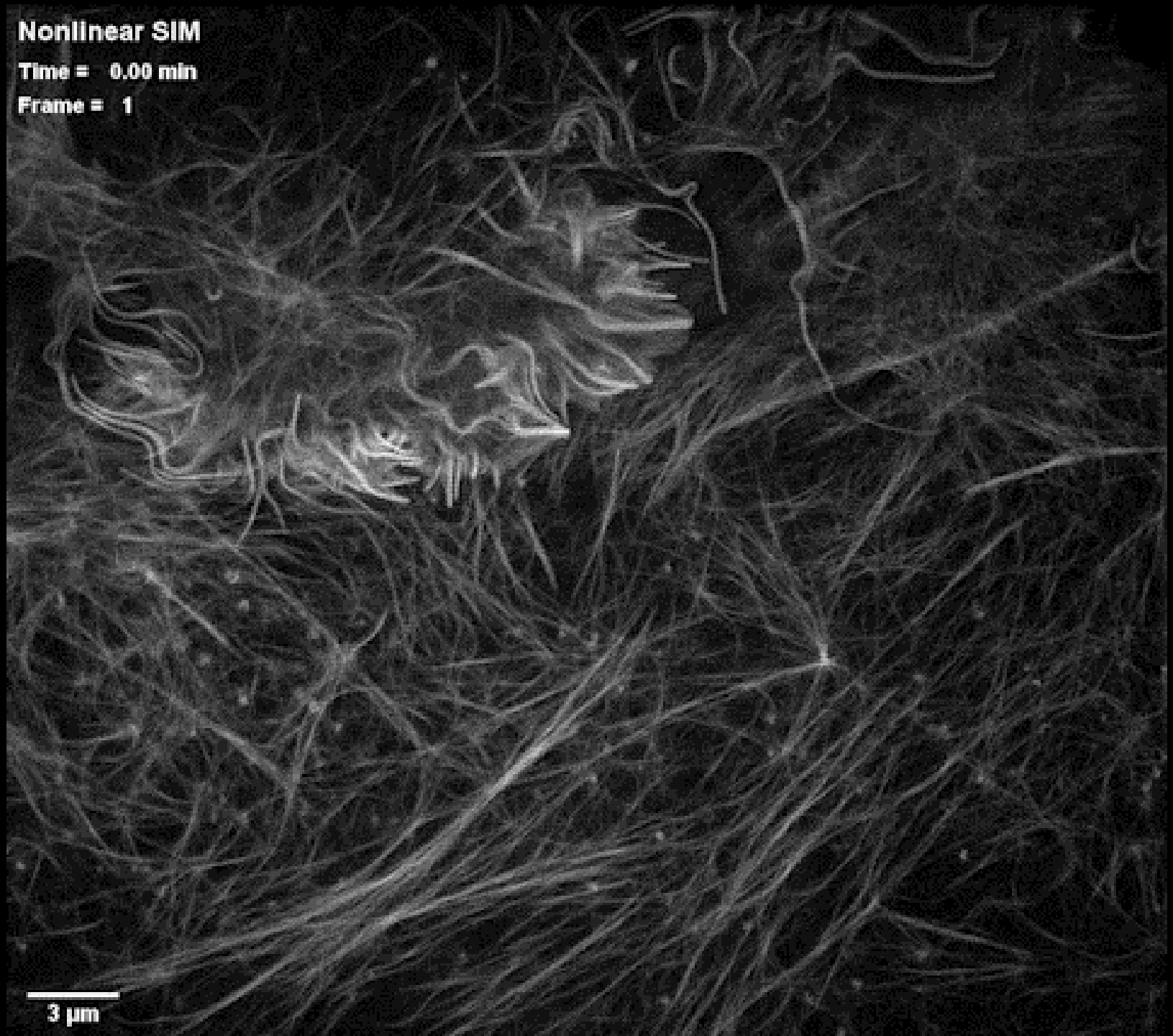


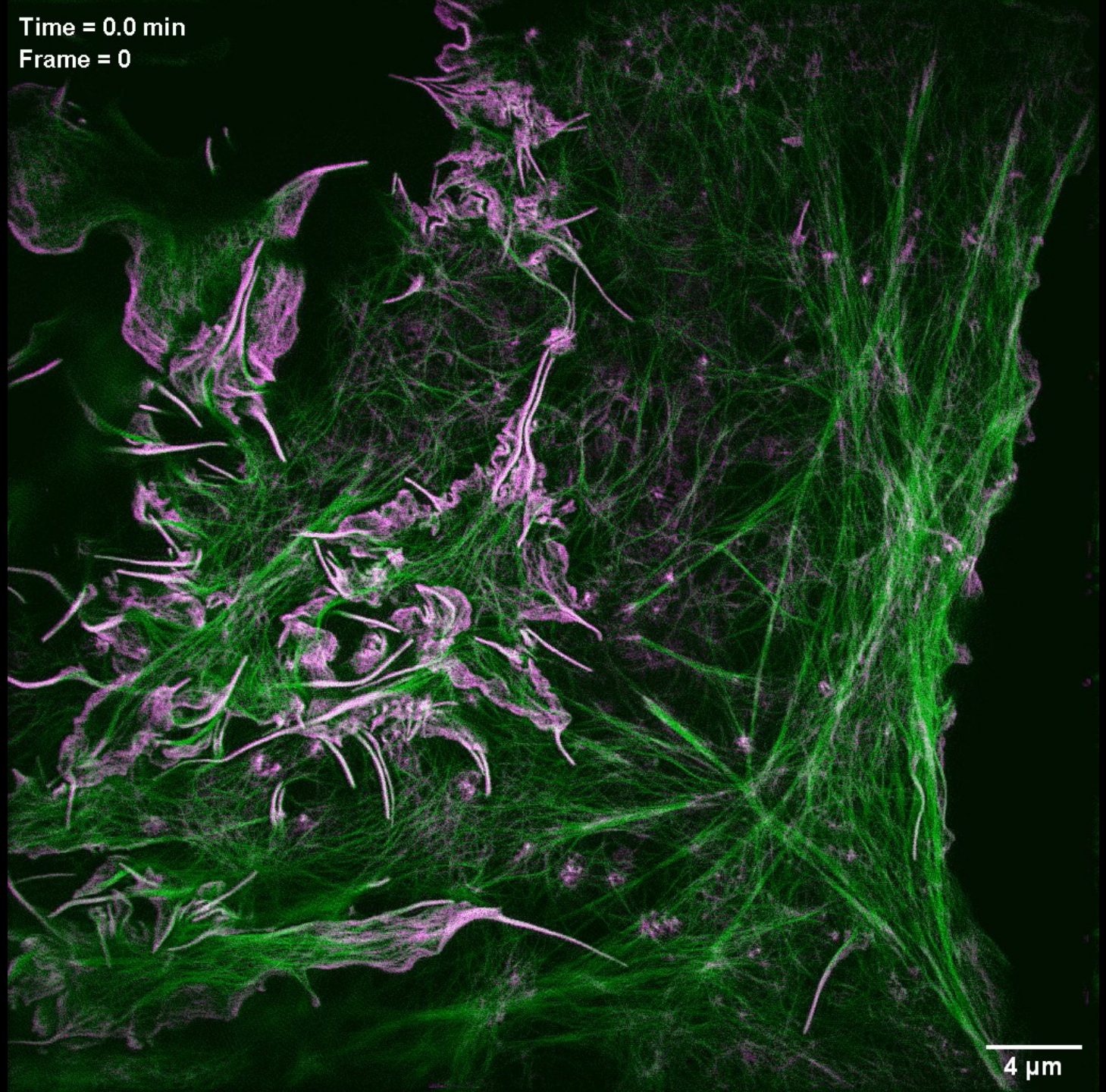

a
b
c
d
e
1 μm
1 μm


A
B
C
D
E
F


TIRF
3 µm



COS-7细胞肌动蛋白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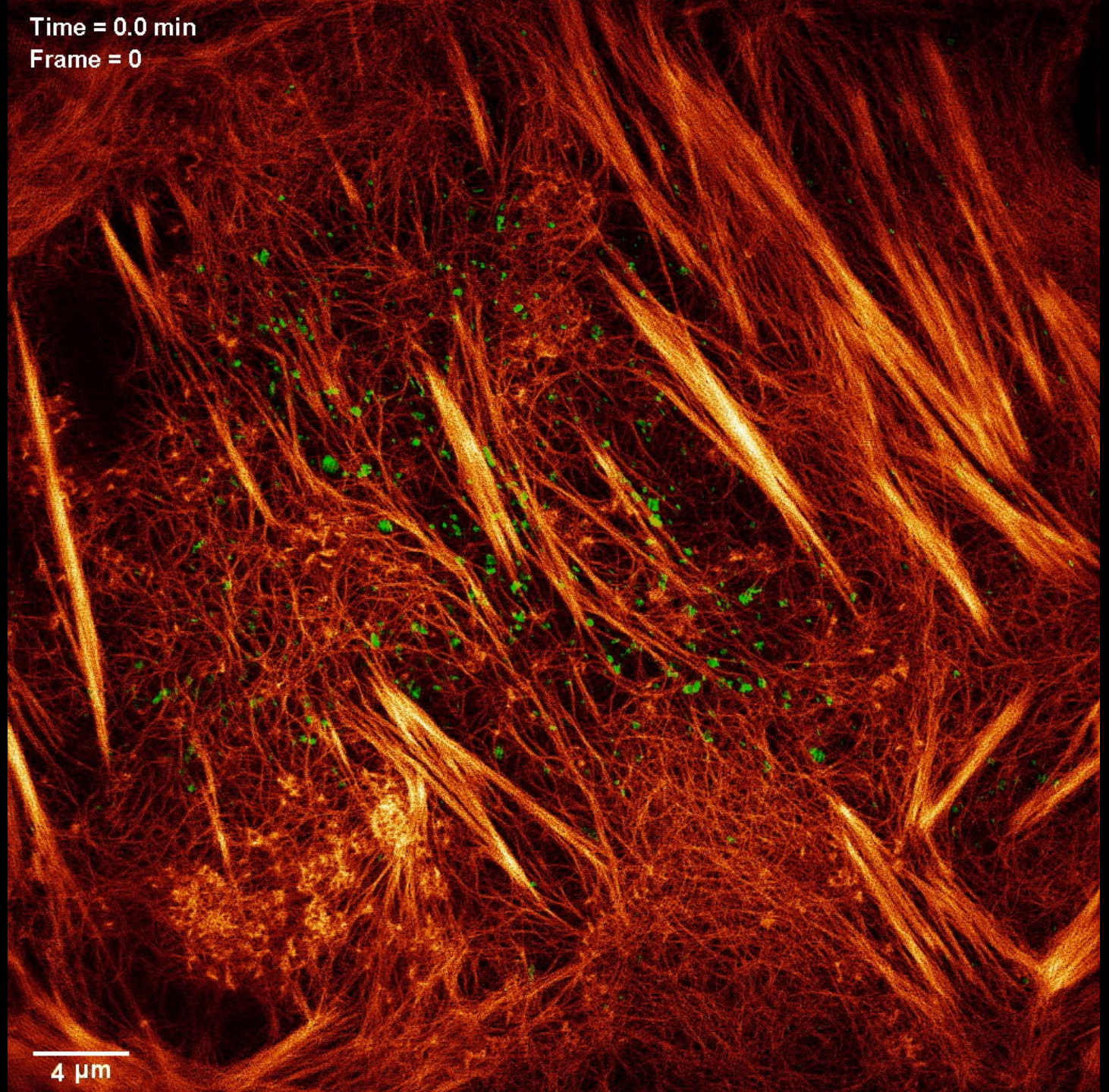
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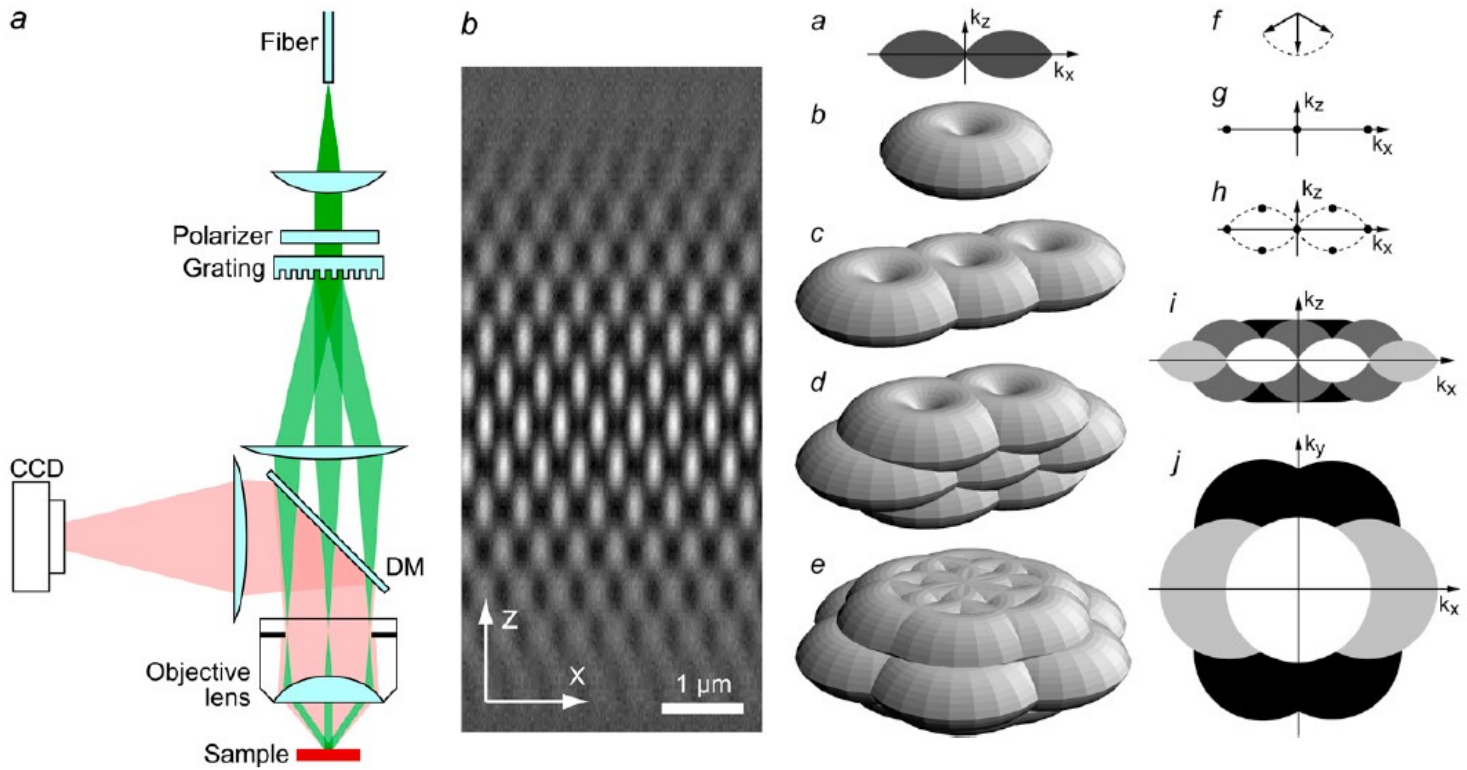
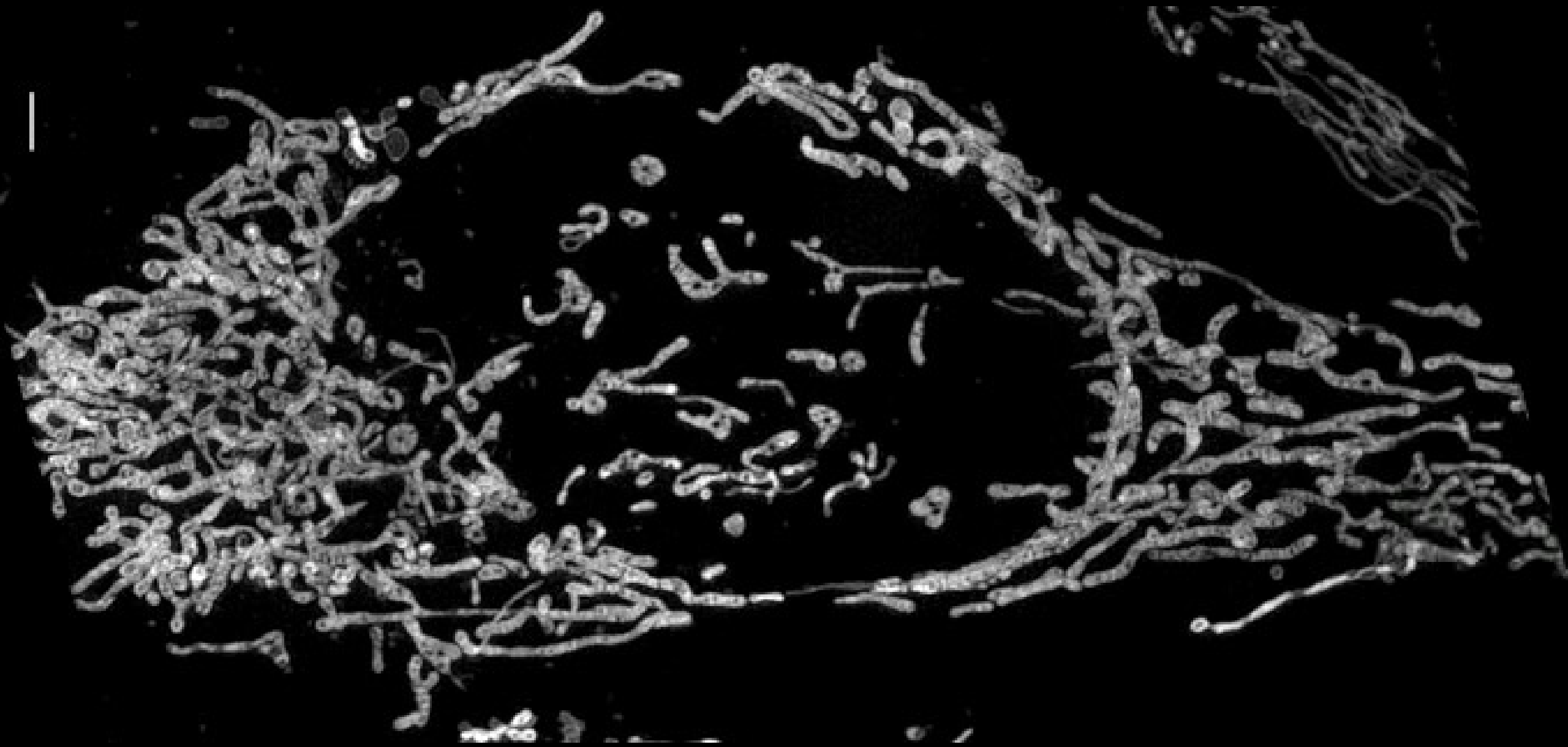
Biophysical Journal Volume 94 June 2008 4957–4970

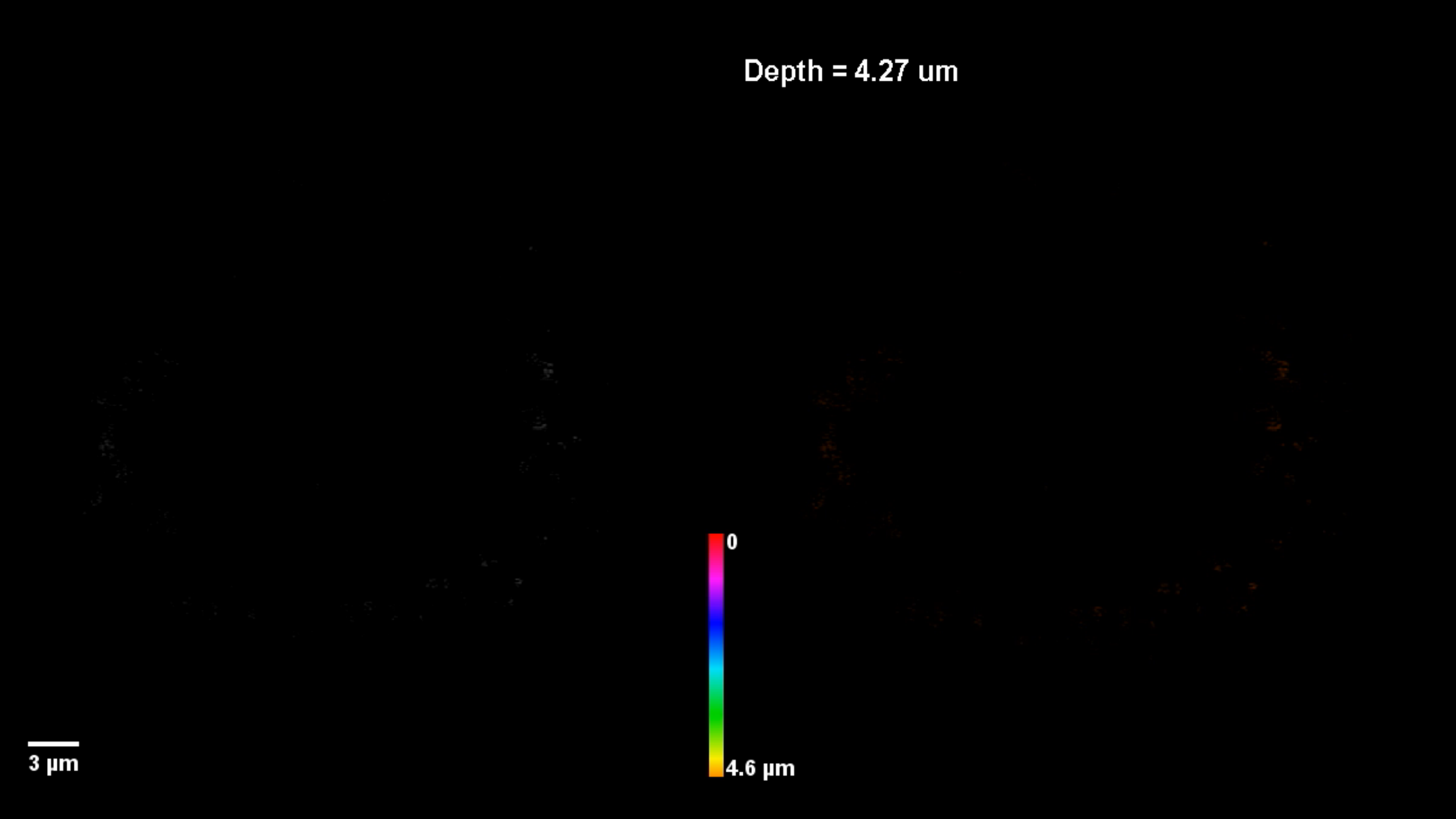
Three-Dimensional Resolution Doubling in Wide-Field Fluorescence Microscopy by Structured Illumination

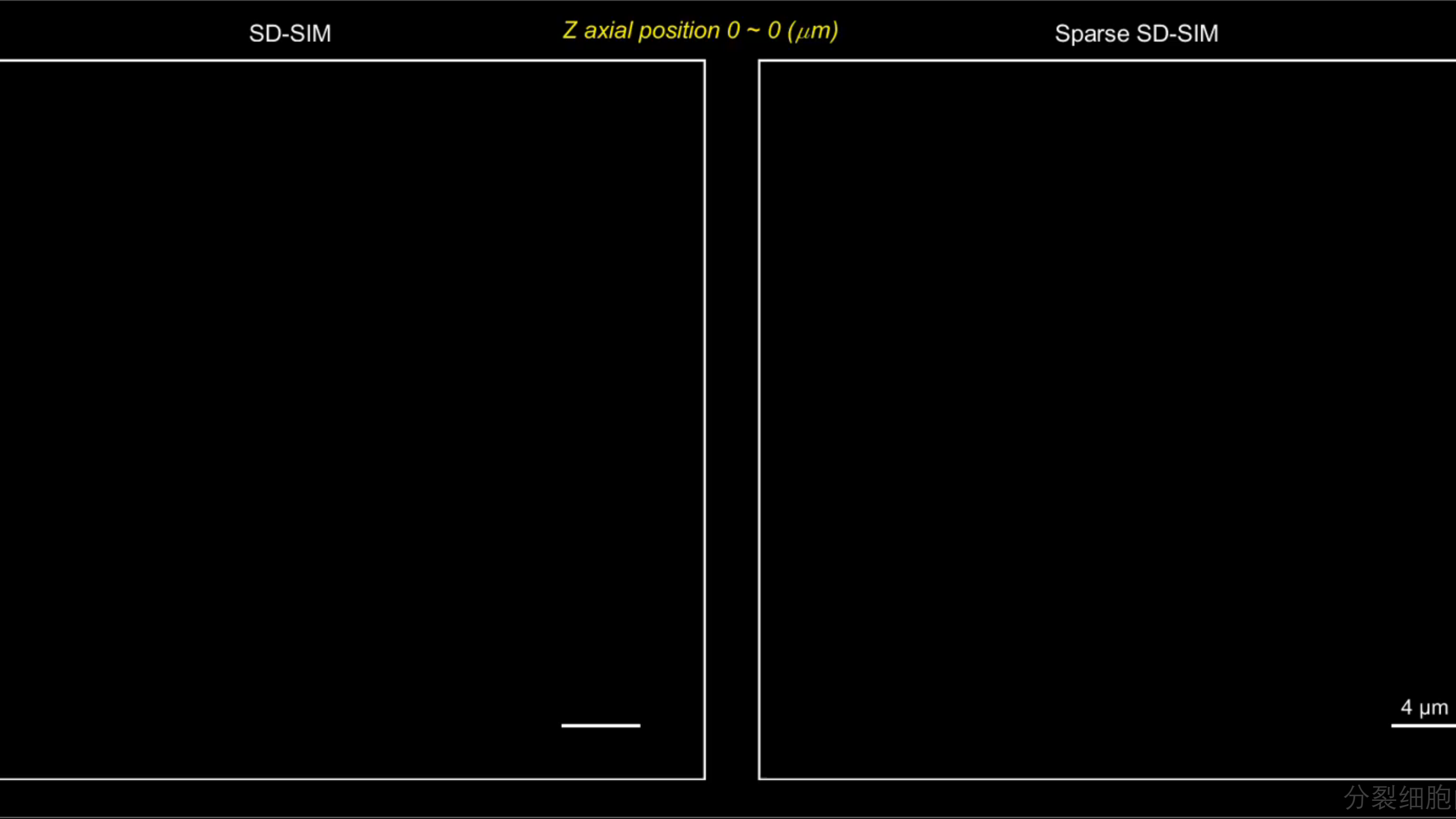
Mats G. L. Gustafsson,* Lin Shao,† Peter M. Carlton,† C. J. Rachel Wang,‡ Inna N. Golubovskaya,‡ W. Zacheus Cande,‡ David A. Agard,†¶ and John W. Sedat†

*Department of Physiology and Program in Bioengineering, †The Keck Advanced Microscopy Laboratory and the Department of Biochemistry and Biophysics, University of California, San Francisco, California; ‡Department of Molecular & Cell Biology, University of California, Berkeley, California; and ¶Howard Hughes Medical Institute

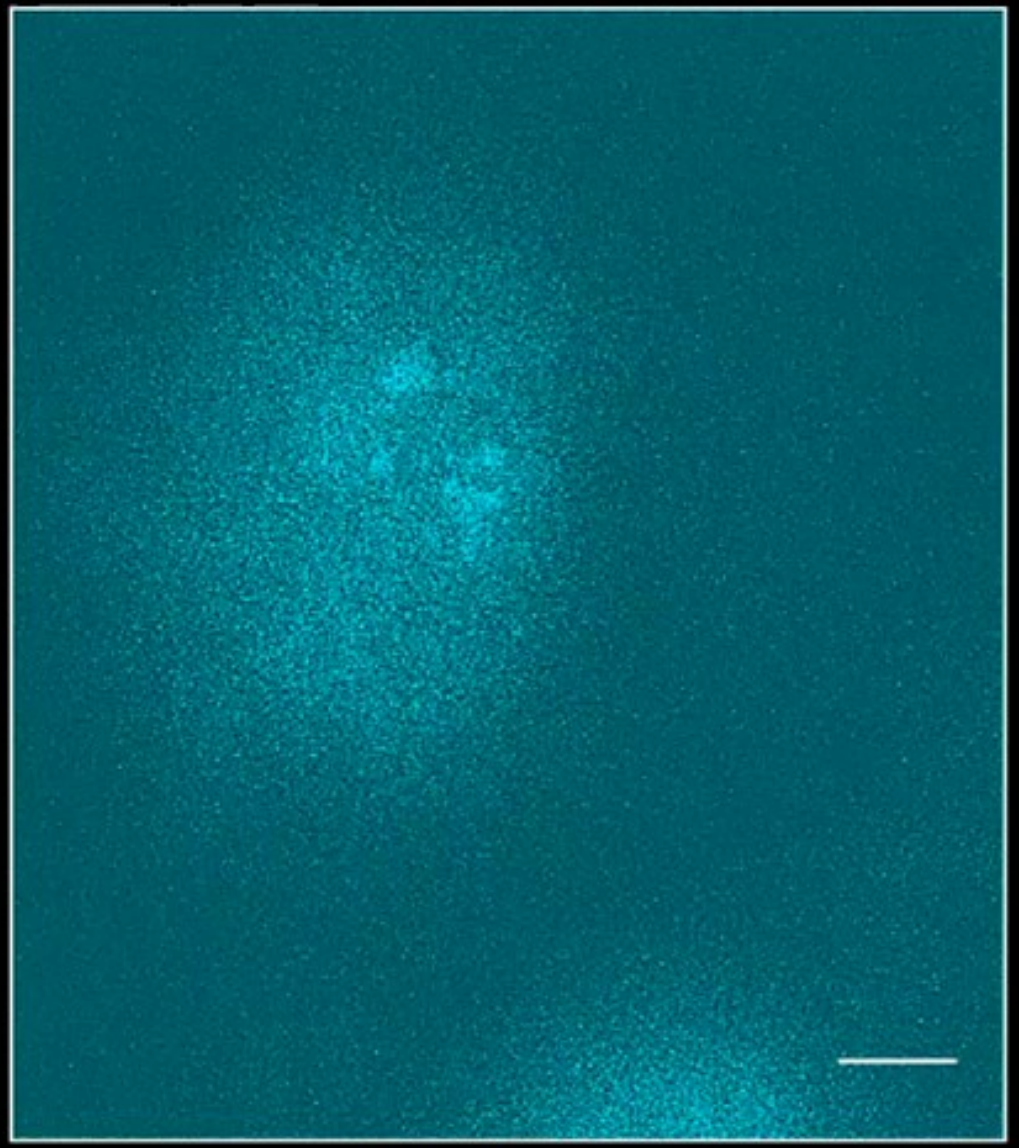




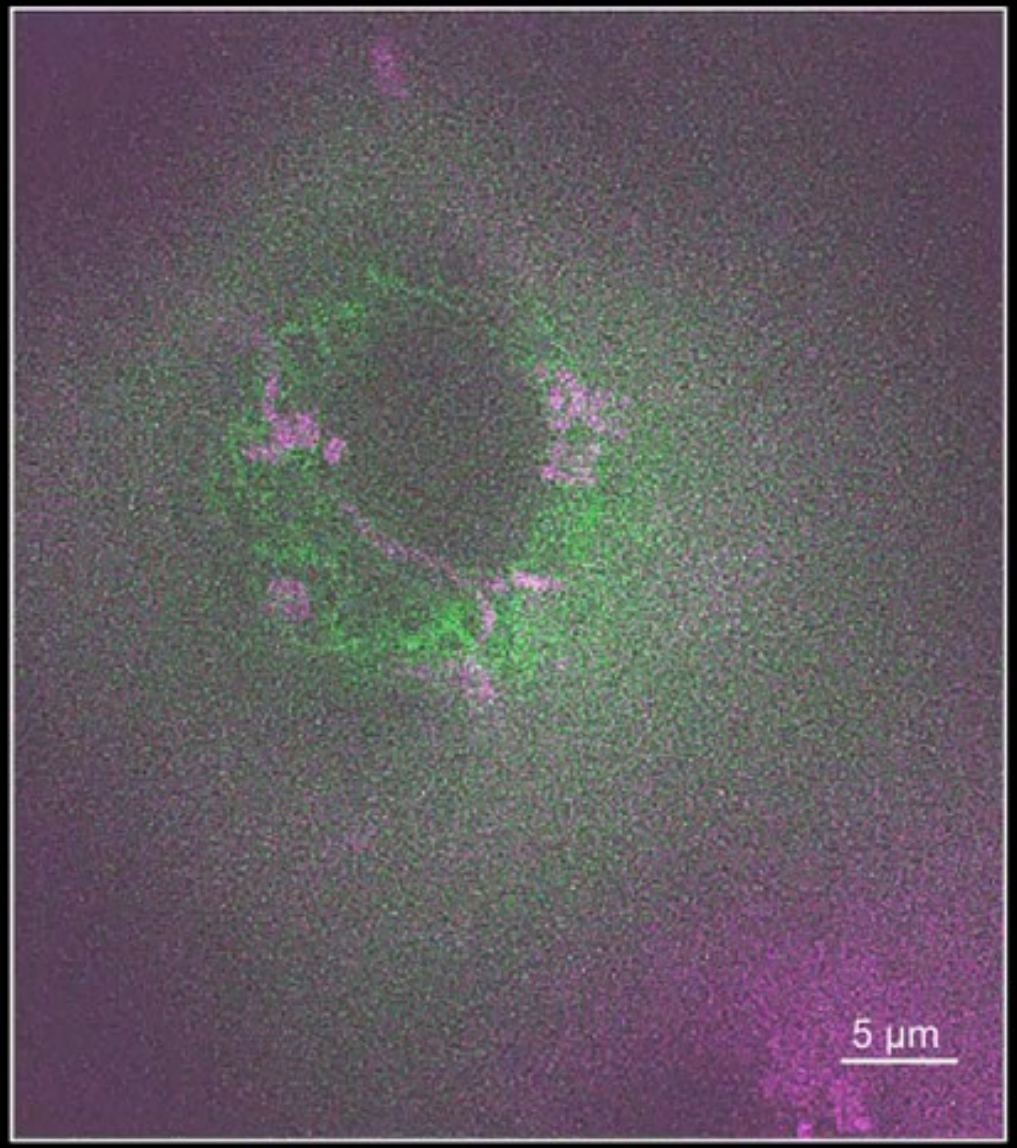

Depth = 4.27 um
0
4.6 µm
3 µm


SD-SIM
Z axial position 0 ~ 0 (μm)
Sparse SD-SIM
4 μm

Z axial position = 0 (μm)



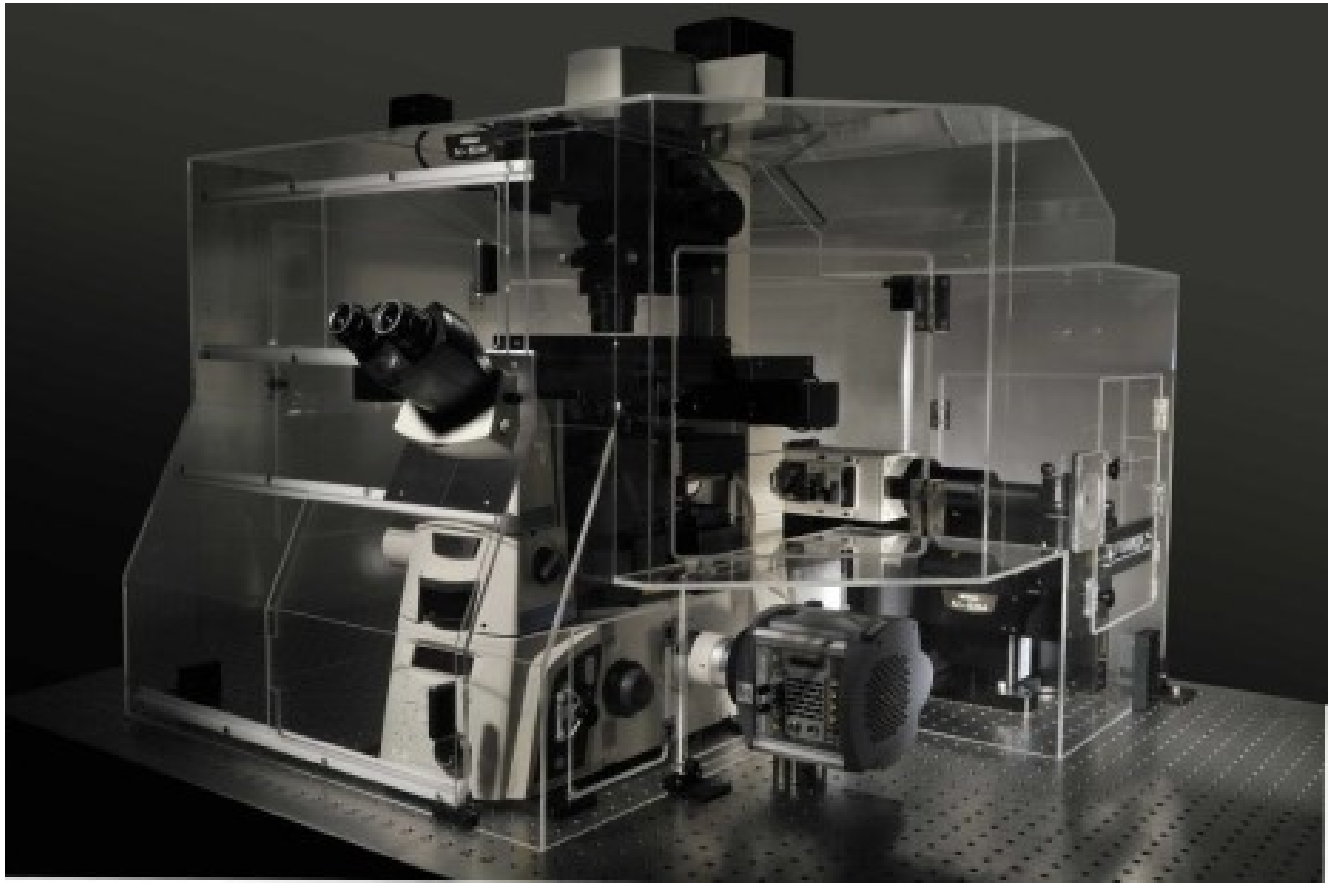
Nucleus



Mitochondria + Tubulin

SD-SIM

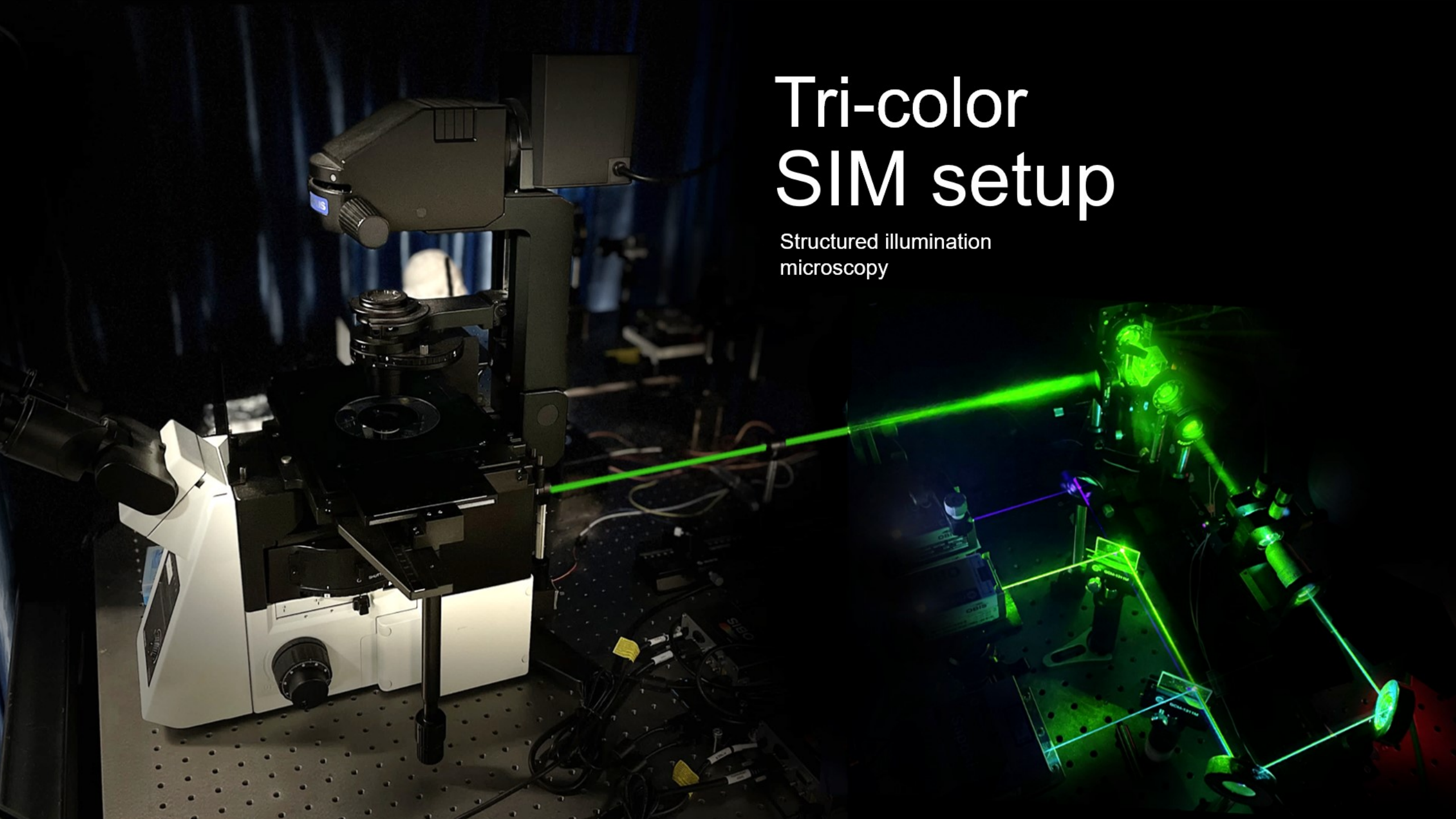
Nikon



ZEISS

Tri-color SIM setup

Structured illumination microscopy

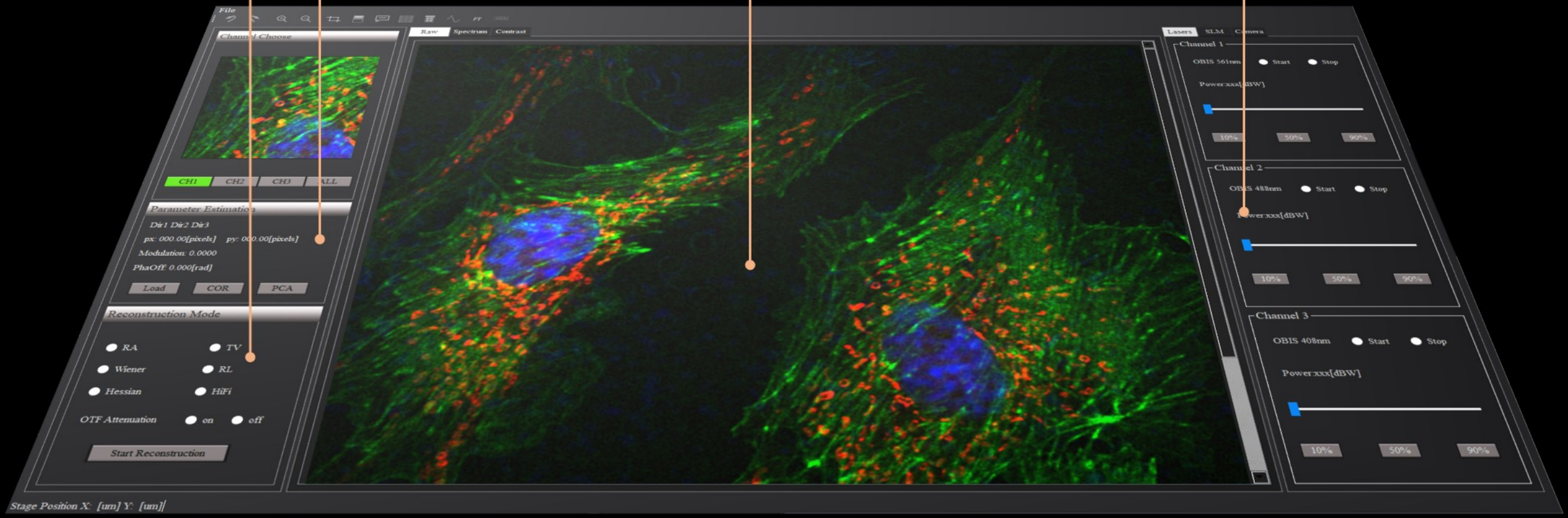


成像操作区

采集显示区

照明控制区

数据重建区



谢谢