



Nikon Corporation
Instruments Company

超分辨率显微技术 在生命科学中的应用

N-SIM/N-STORM

2016年9月



从宏观到微观，尼康的显微世界

发育生物学

显微成像技术领域有着数不尽的研究方向，所需的设备也是多种多样。作为领先的设备制造商，尼康依靠最尖端的技术与丰富的经验全力支持并参与着生命科学的研究工作。

神经科学

个体

肿瘤研究

器官

宏观共焦显微镜

组织

完整结构的观察

超分辨率显微镜

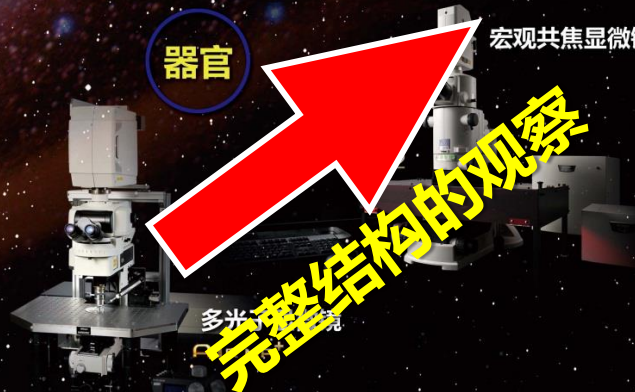


免疫学



细胞生物学

细胞



多光子显微镜

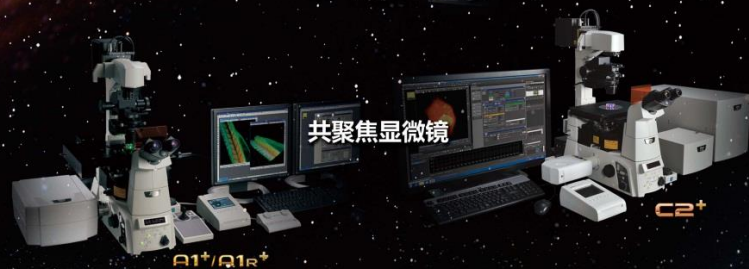
分子生物学

细胞器

共聚焦显微镜

分子

超微结构的观察



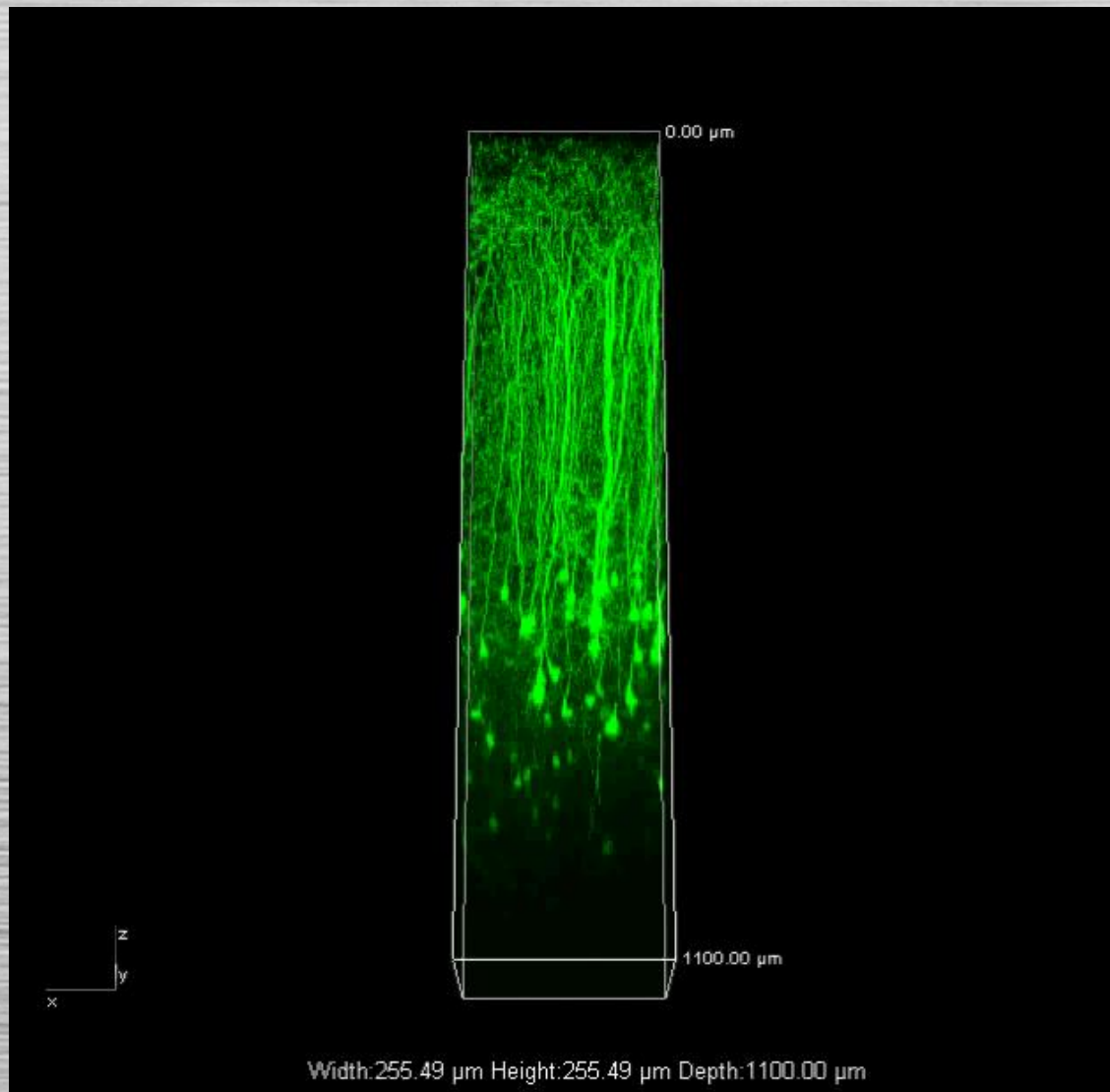
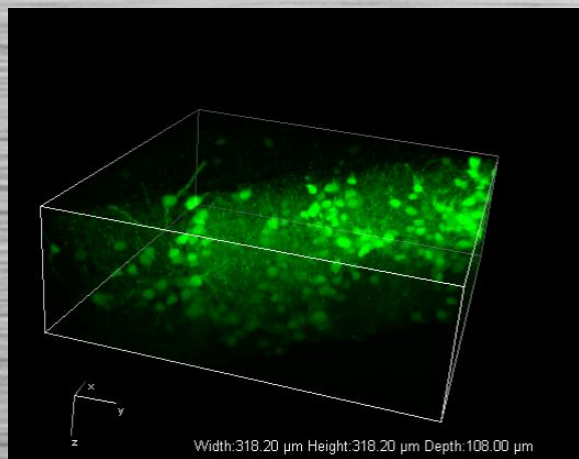
A1+/Q1R+

C2+

高品质的光学与制造技术能让研究者从研究样本中获得最大量的信息，同时NIS-Elements软件作为显微镜系统的中心将研究者对于研究样品的好奇心转化为实际的操作步骤。



双光子解决深度的问题！



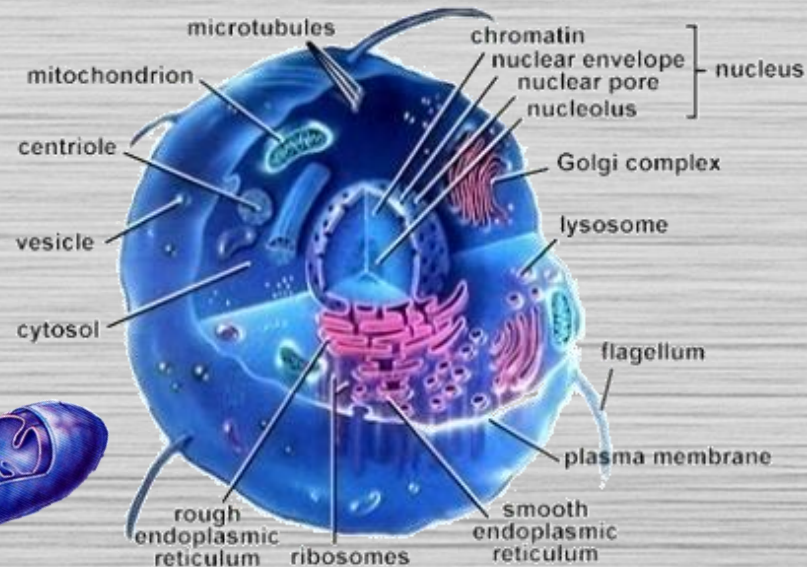
单光子 488nm GFP

只能看到100 μm 左右的深度

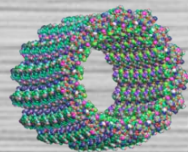
能看到1000 μm 以上的深度



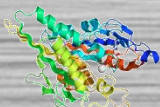
显微镜的观察范围



线粒体



微管



驱动蛋白



氨基酸

1Å

1nm

10nm

100nm

1μm

10μm

0.1mm

原子

分子

亚细胞

细胞

电子显微镜

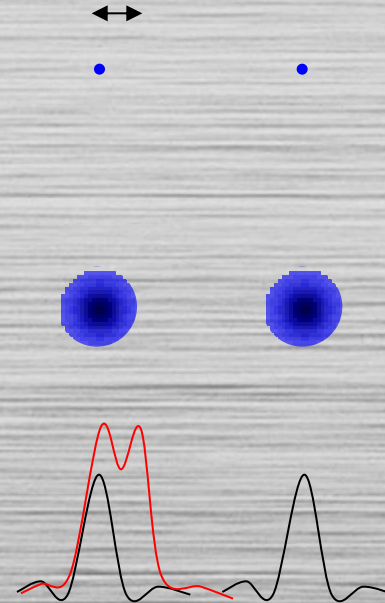
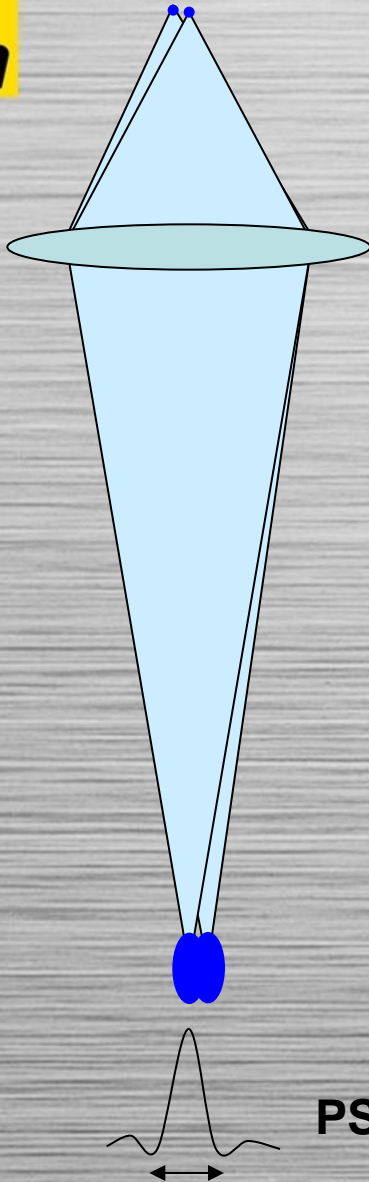
超分辨率

传统光学显微镜



为什么会有分辨率极限？

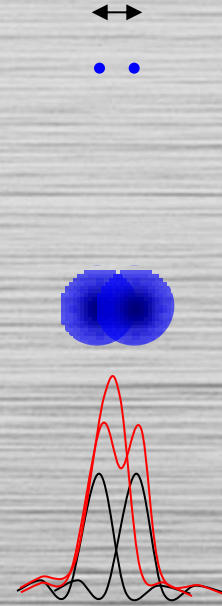
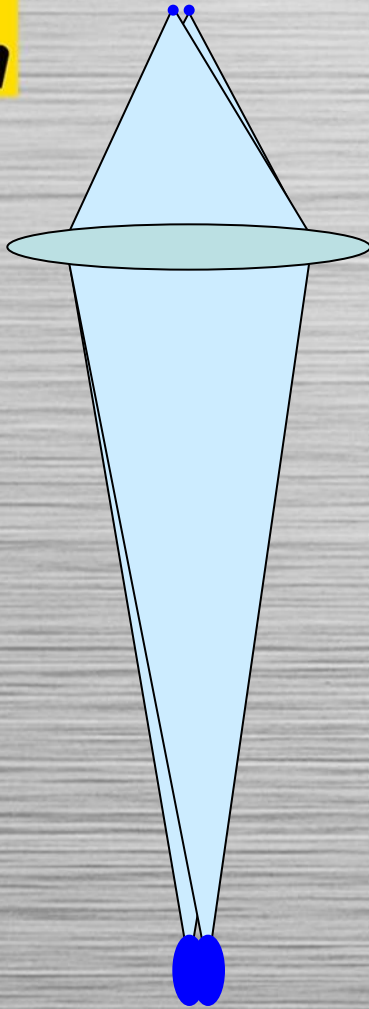
Rayleigh's limit = resolution = $0.61 \times \text{NA} / \lambda$



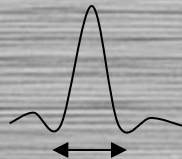
$$\text{PSF} = 1.22 \frac{\lambda}{\text{NA}}$$



Rayleigh's limit = resolution = $0.61 \times NA / \lambda$



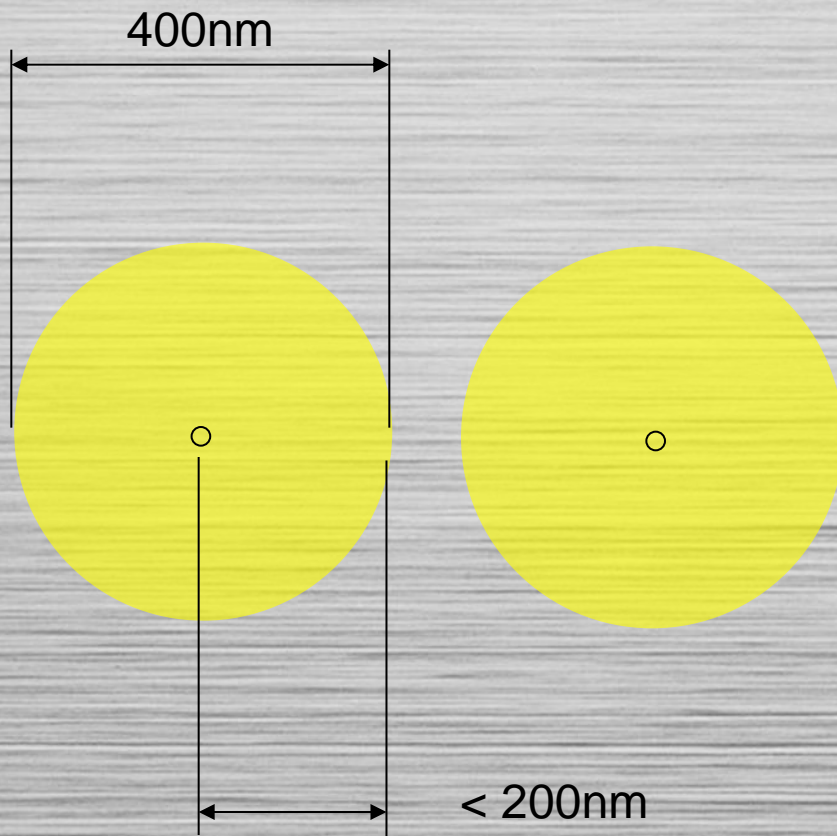
Un-resolvable



$$PSF = 1.22 \frac{\lambda}{NA}$$

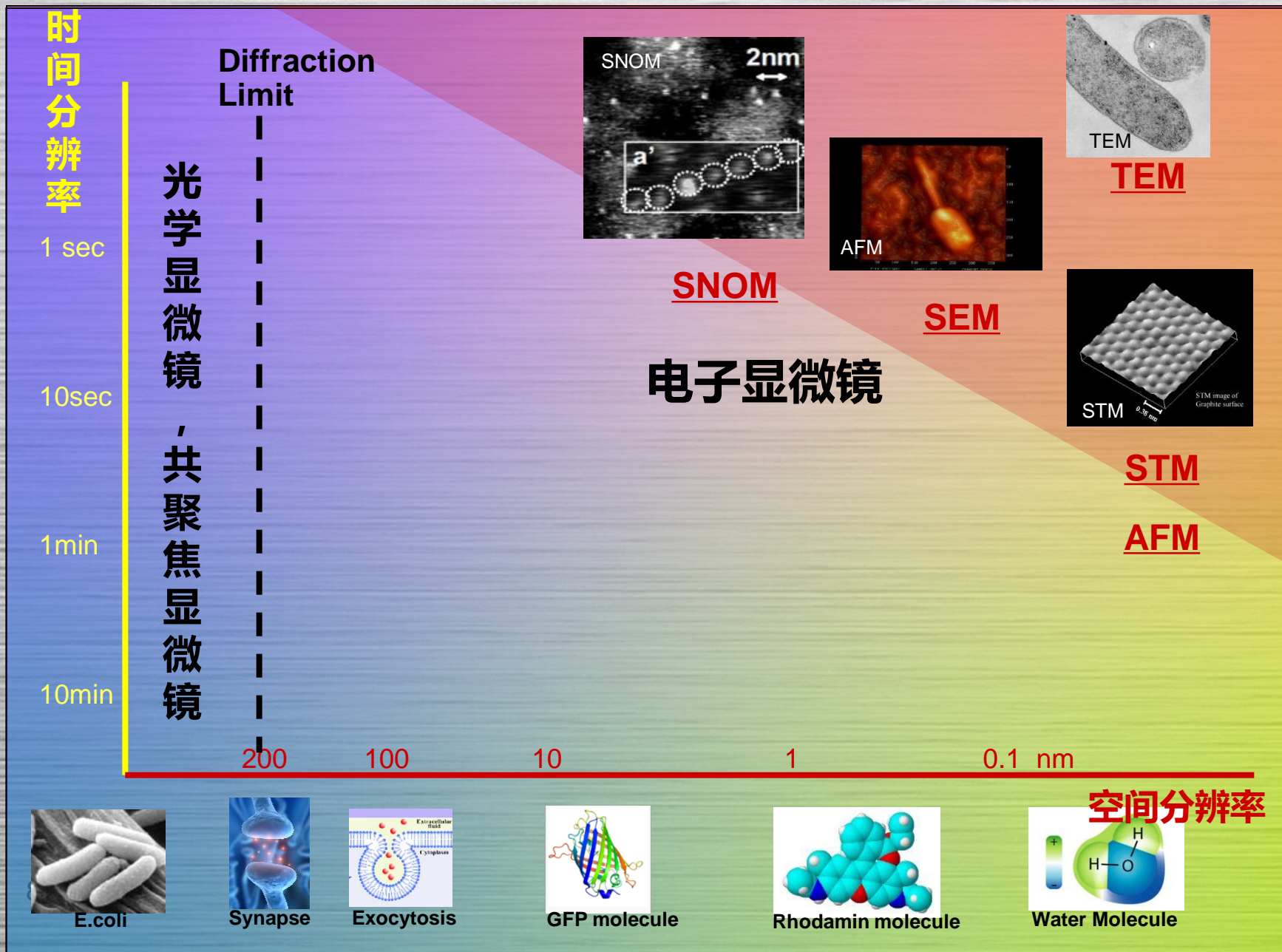


分辨率极限



$$\text{Rayleigh's criterion} = 0.61 \frac{\lambda}{NA}$$

显微技术图



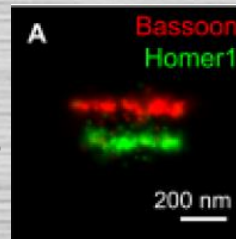


Smaller

生物学综述很多都集中在以下领域

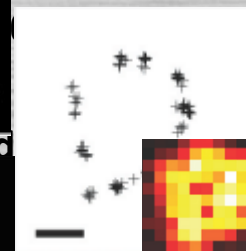
Signal molecules

Dani A, et al.



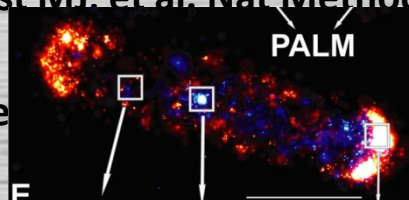
DNA molecules

Rust MJ, et al. Nat Methods



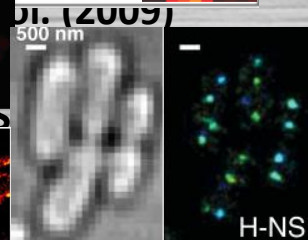
Receptors

Gre



Chromosome

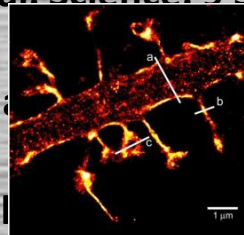
Wang W, et al. Science. 9 S



~10nm

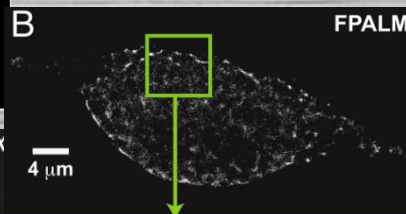
Cytoskeleton

Izeddin I, et al

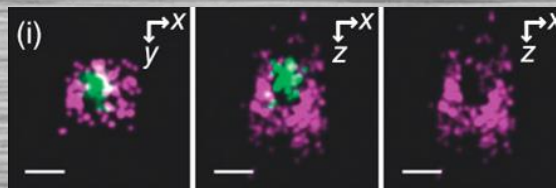


Lipid rafts

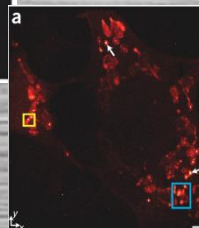
Hess ST, et al



Vesicles



Membranous organelles
(Mitochondria, ER, etc)



~200nm

Bigger



The Nobel Prize in Chemistry 2014



Photo: Matt Staley/HHMI

Eric Betzig

Prize share: 1/3



© Bernd Schuller, Max-Planck-Institut

Stefan W. Hell

Prize share: 1/3



Photo: K. Lowder via Wikimedia Commons, CC-BY-SA-3.0

William E. Moerner

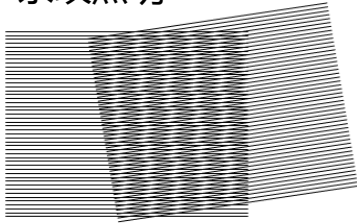
Prize share: 1/3

The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner *"for the development of **super-resolved fluorescence microscopy**".*



SRM有些什么种类？

条纹照明



- **SIM：构造化照明法**

用SIM捕捉以条纹的模式照射样本后得到的“条纹状图像”，通过图像演算恢复细微构造的方法

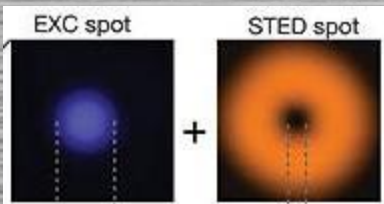
可活化探针



- **STORM/PALM：随机活化法**

使用活性荧光色素以微弱的光照射样本，零星地使荧光分子得到活性化，再以激发光照射，在荧光中心标示位置一边进行点扫描一边进行图像构筑的方法

改变 PSF



- **STED：诱导发射损耗法**

在STED状态下，超短脉冲激发光照射后马上“诱导”脉冲光进行照射，使样本的荧光产生的范围变小的方法



Nikon 的超分辨产品

1. N-SIM (方便, 对染料样品无限制)

1. 应用「构造化照明」理论。
2. 获取从前显微镜约2倍的分辨率
(XY:100nm,Z:250nm)。
3. 最快以0.6秒一张的速度获取图像。

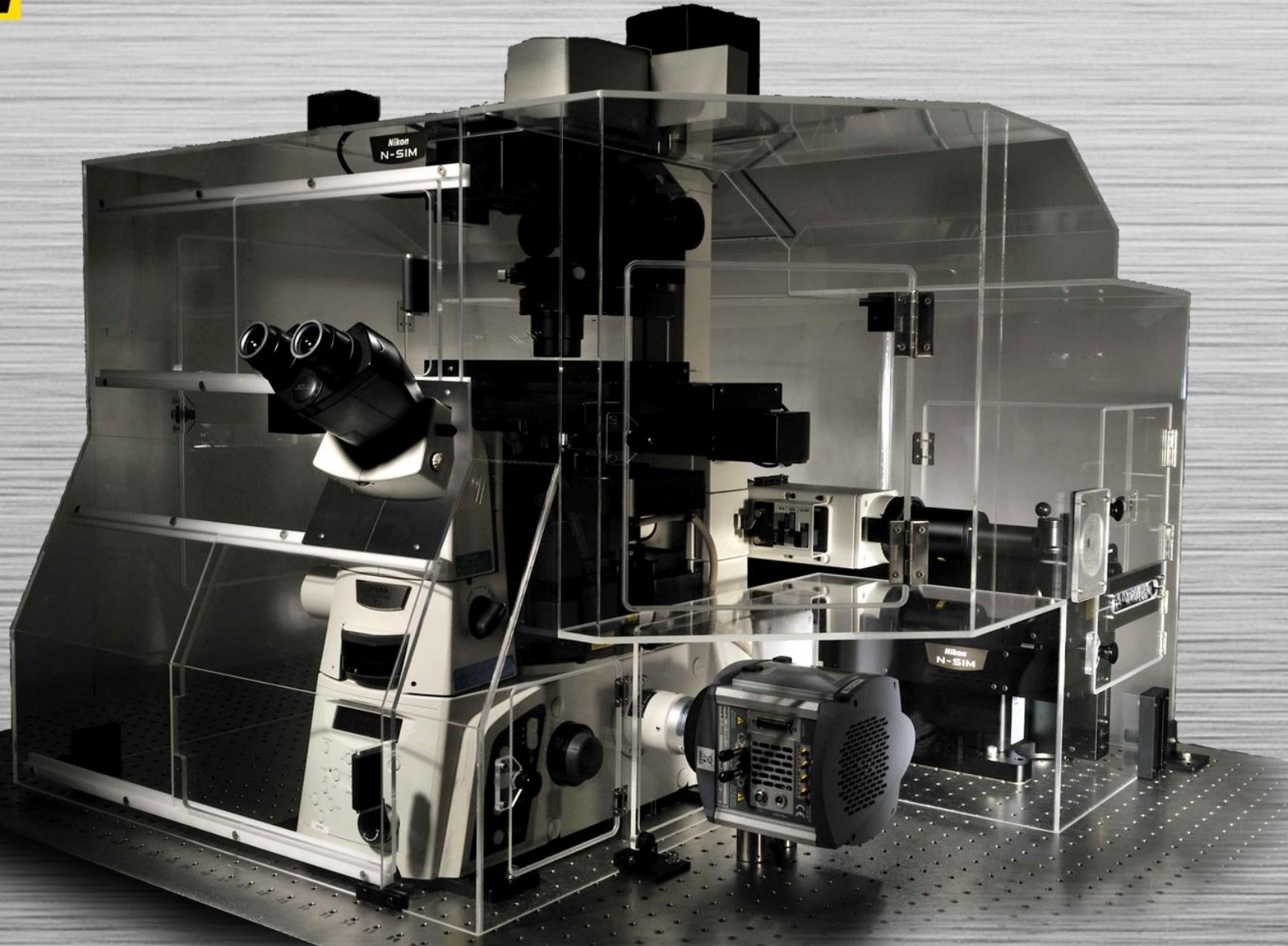
2. N-STORM (极高空间分辨率)

1. 利用定位法原理。
2. 获取从前显微镜约10倍的分辨率
(XY:20nm,Z:50nm)。
3. 获取时间需要几十秒到几分钟。



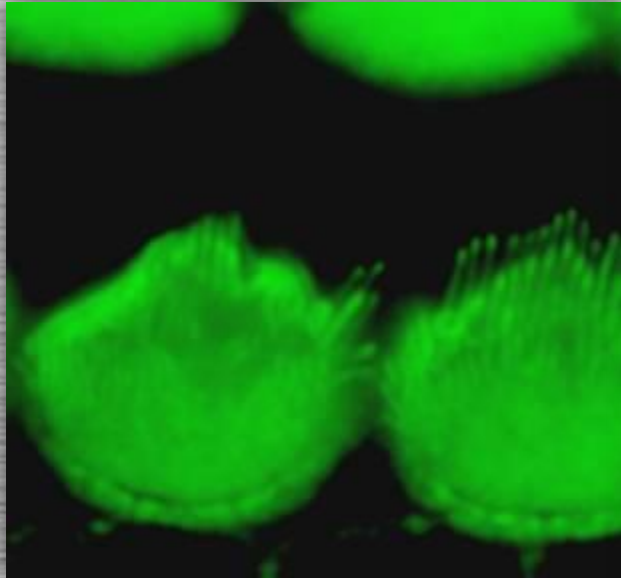
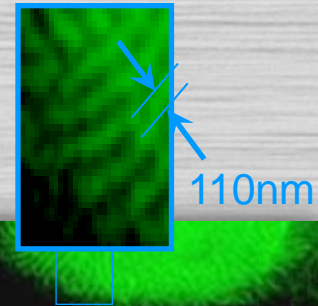
N-SIM — 超分辨率显微镜

Nikon-Structured Illumination Microscopy



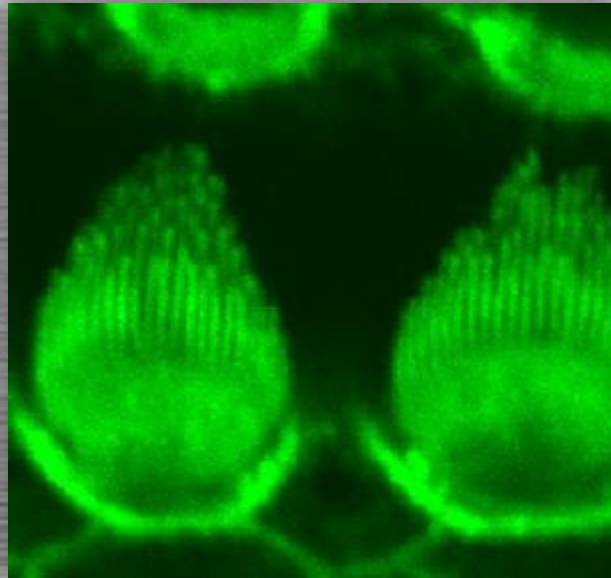


两倍于传统显微镜分辨率



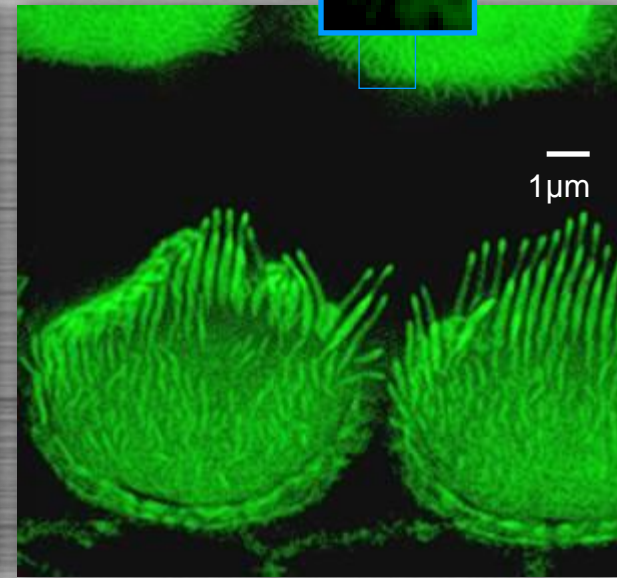
Conventional

ApoTIRF 100x/NA1.49



Confocal

ApoTIRF 100x/NA1.49
Ti-E+A1R Ex488nm



SIM

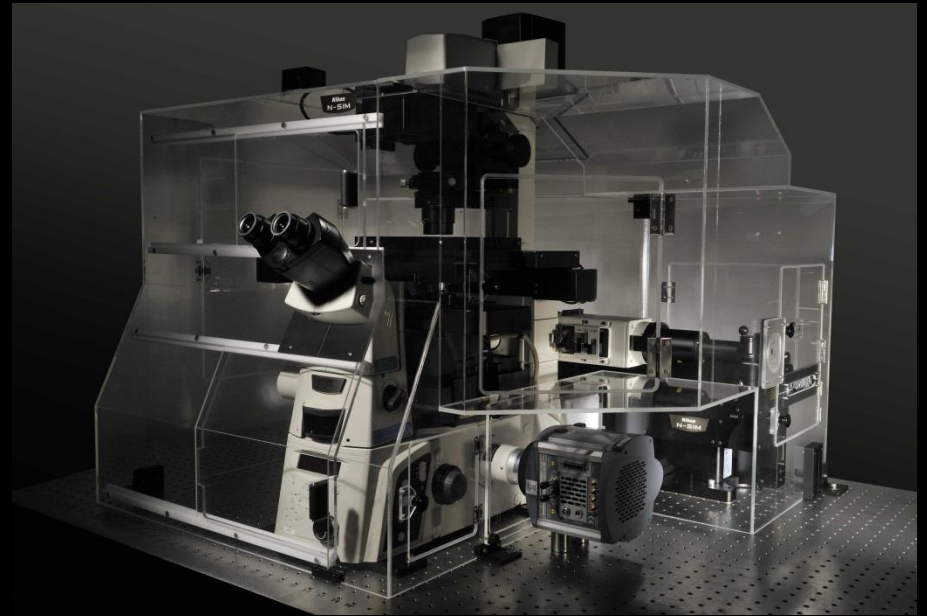
ApoTIRF 100x/NA1.49
N-SIM Ex488nm



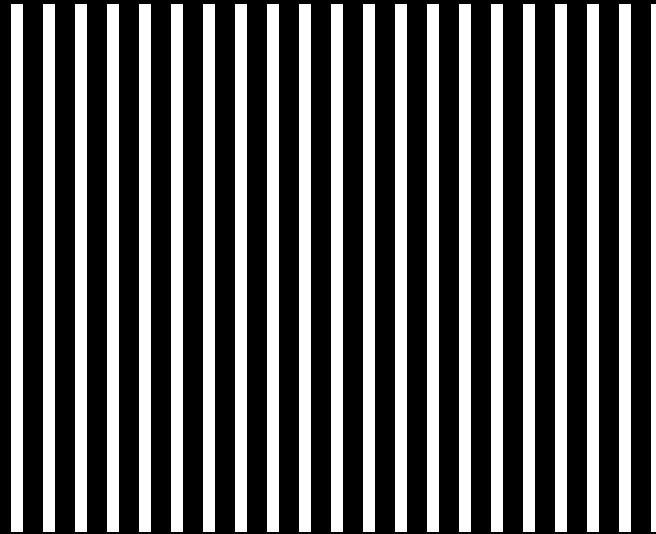
Sample courtesy of: Kanoko Kominami, Hideru Togashi, Yoshimi Takai
Division of Molecular and Cellular Biology Kobe University Graduate School of Medicine/Faculty of Medicine
Luminal surface of the organ of Corti at postnatal day 1. (Mouse) Green, F-actin.



N-SIM 的原理



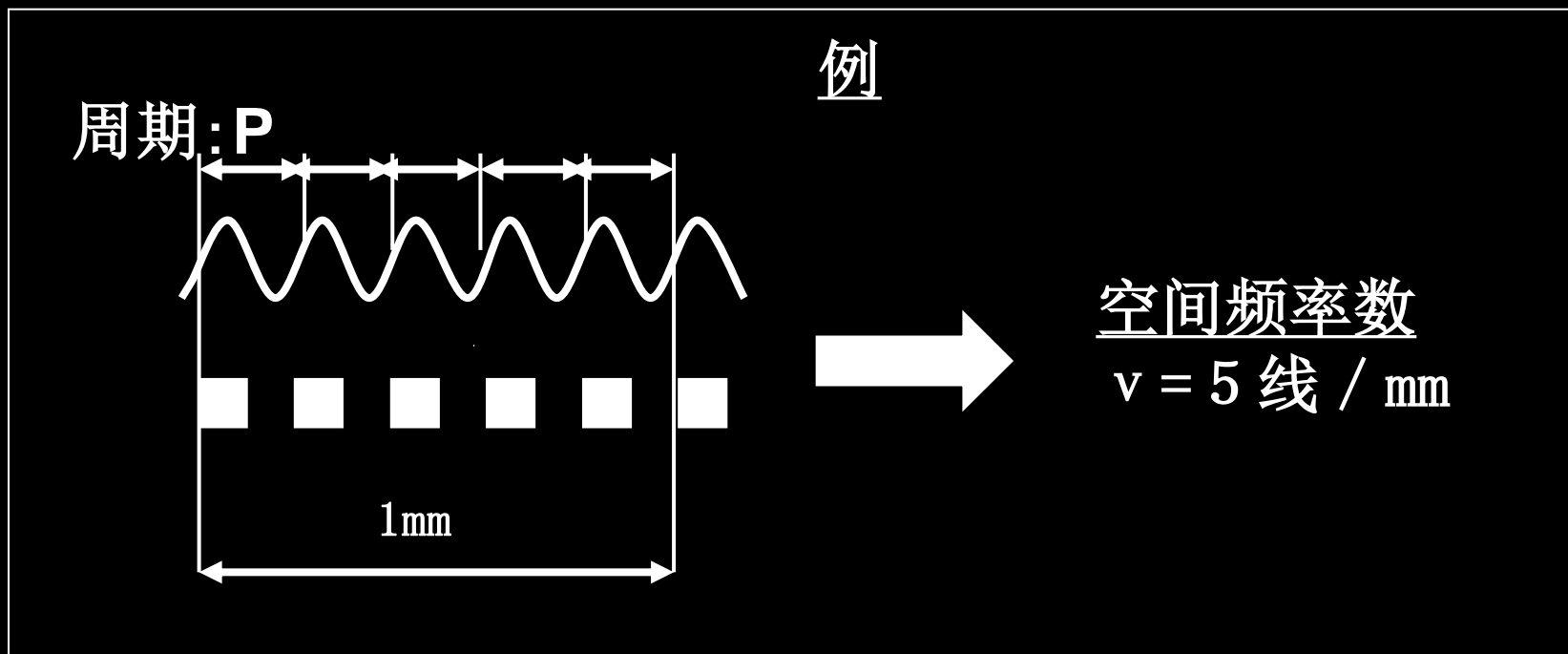
什么是空间频率？



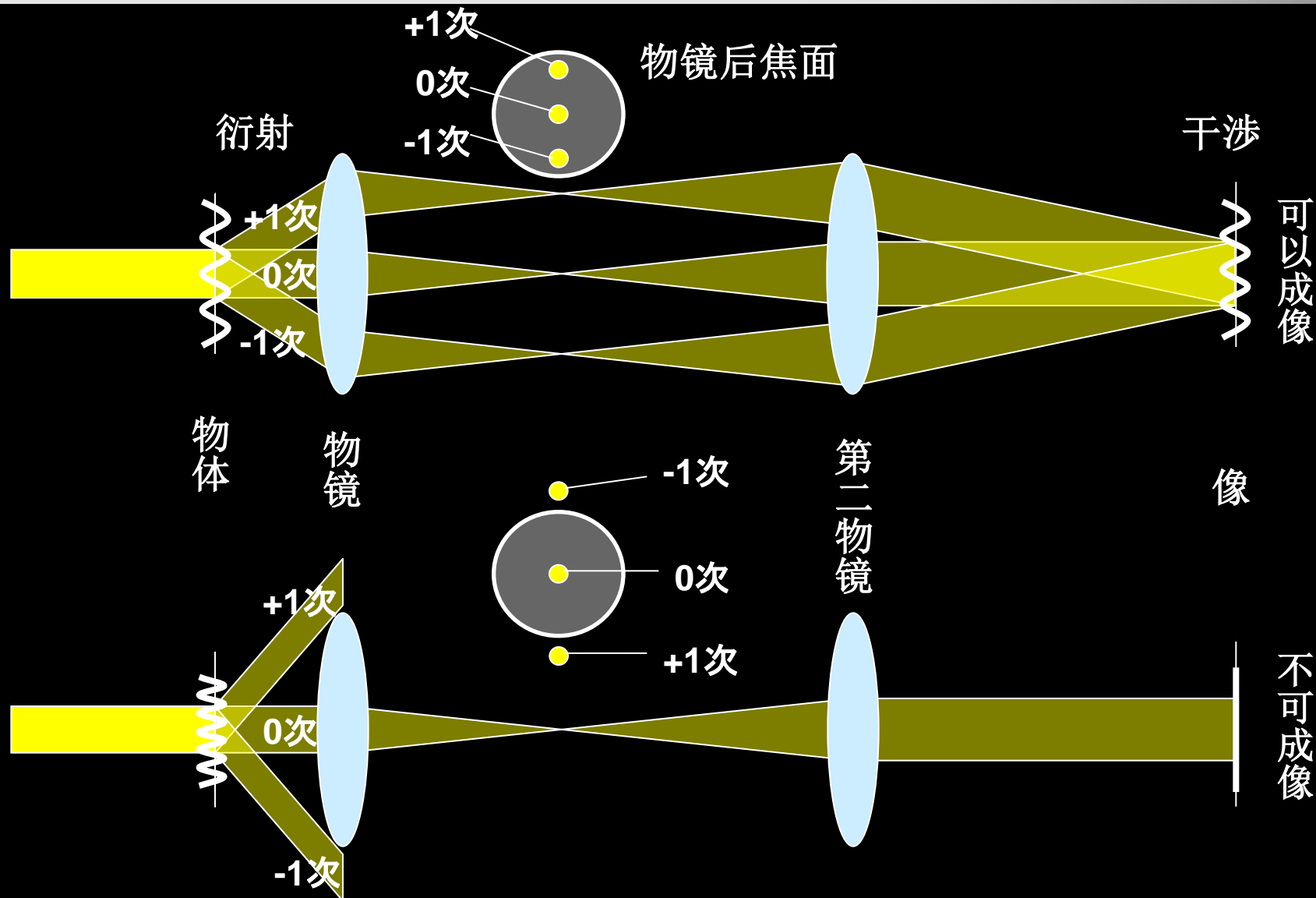
空间频率： ν (线 / mm)

指单位长度中包含的结构周期的多少

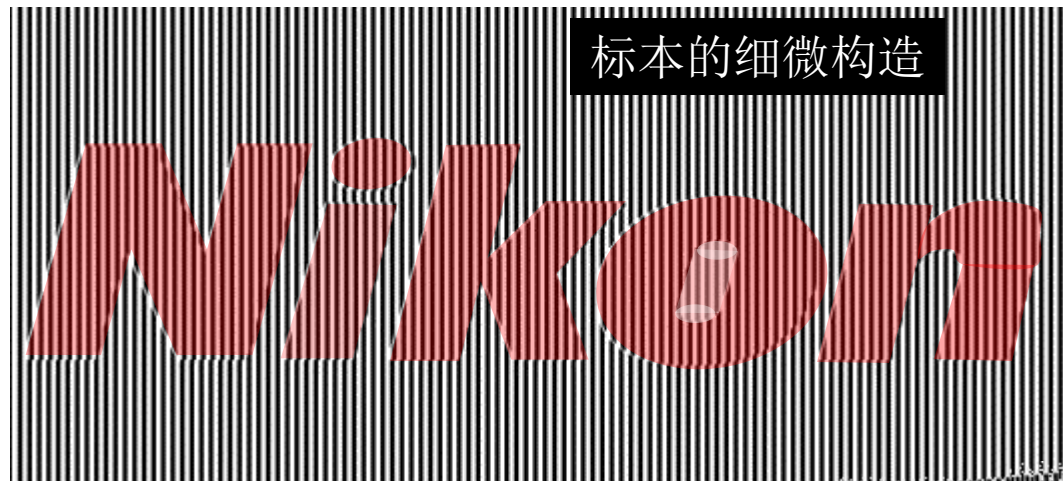
在光学领域，是用来表示1mm内包含多少条周期结构的光学系统分辨率的指标



N-SIM原理：成像理论



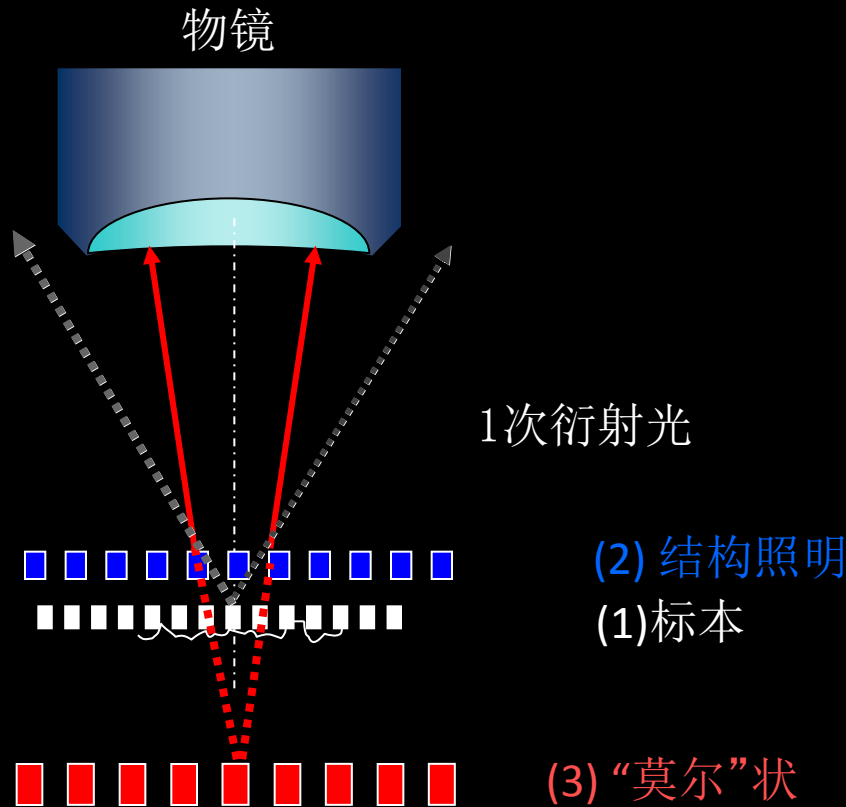
成像是指：0次光和+/- 1次衍射光在像面上的干涉条纹



莫尔纹
低空间频率数

莫尔纹的空间频率数(粗) = 标本体空间频率数 - (结构照明模块的空间频率数)

结构照明模块



v_1 : 标本的空间频率数

v_2 : 结构照明的空间频率数

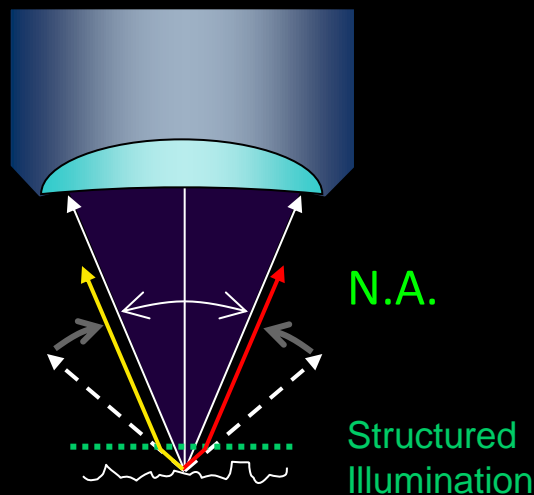
v_3 : 莫尔的空间频率数

$$v_3 = v_1 - v_2$$

$$5000 = v_1 - 5000$$

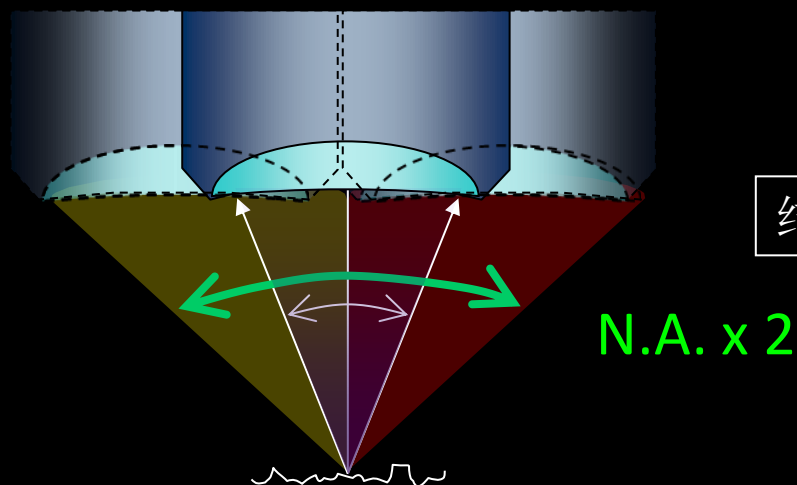
- 通过进行结构照明，一次衍射光发生「衍射」
- 通过读取莫尔纹，获得一次衍射光的超分辨率信息
- 进行重建，得到超分辨率图像

N-SIM原理：衍射效应



分辨率由数值孔径N. A. 决定。
如果细微结构发出的衍射光的角度太大，
就无法被物镜捕捉到。

条纹（结构照明）被照射后，大角度的折射光
作为莫尔纹，被物镜读取。



结果产生了相当于传统显微镜分辨率两倍的图像。

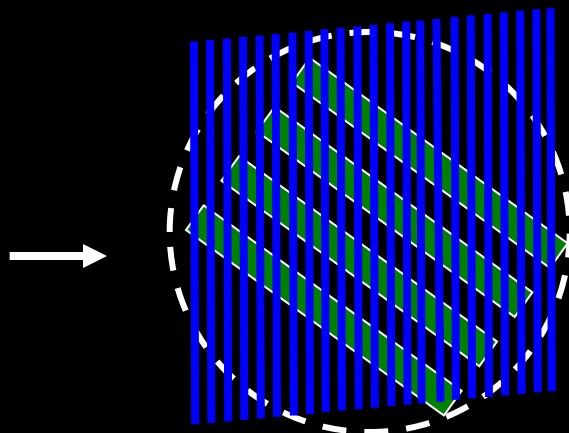
N-SIM原理：高频成分的分离

结构照明发生移动时

- 低频成分（原来的图像成分）不动

结构照明

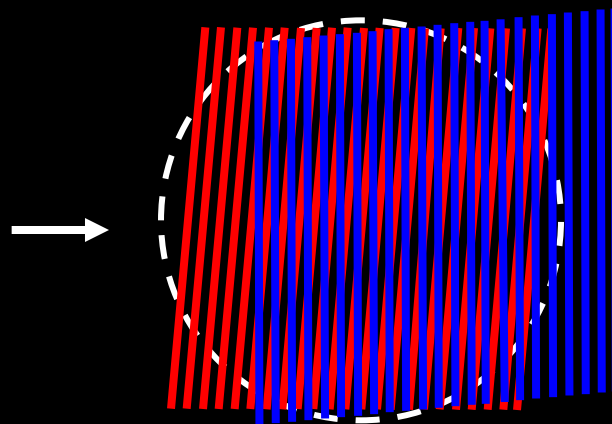
标本



- 高频成分（超分辨率成分=莫尔）同照明模块一起移动

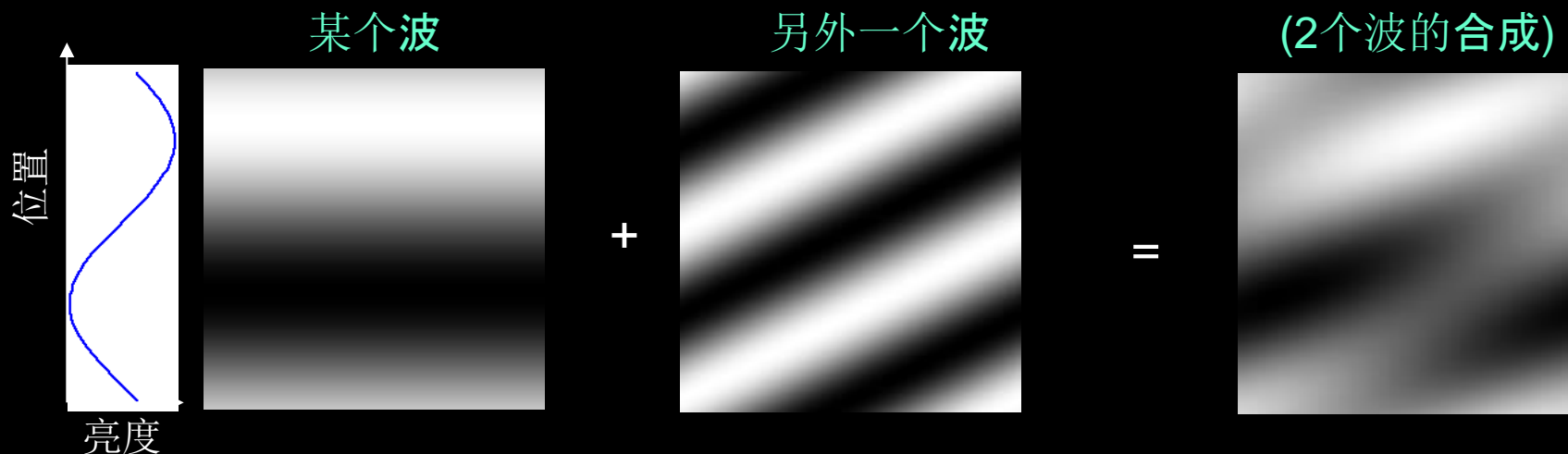
结构照明

标本



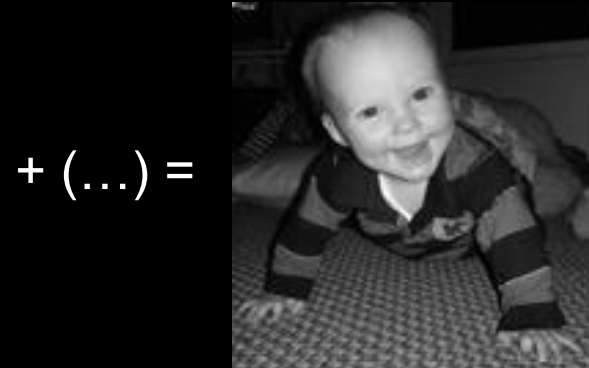
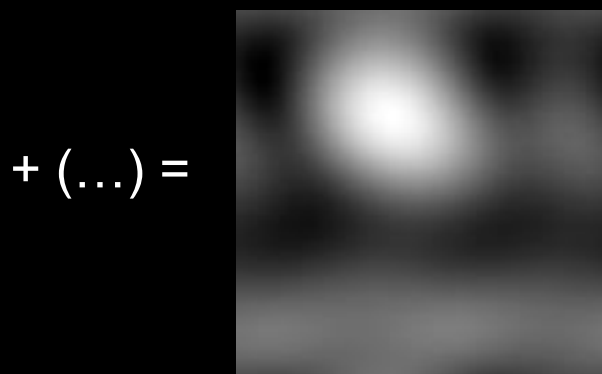
N-SIM原理：图像是各种波的合成

「各种波通过合成可以生成图像」
或「图像是不同的空间频率成分的合成」

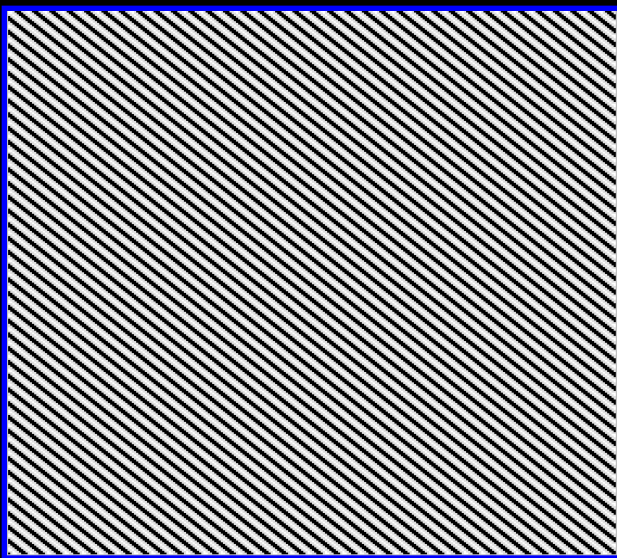


(25的波)

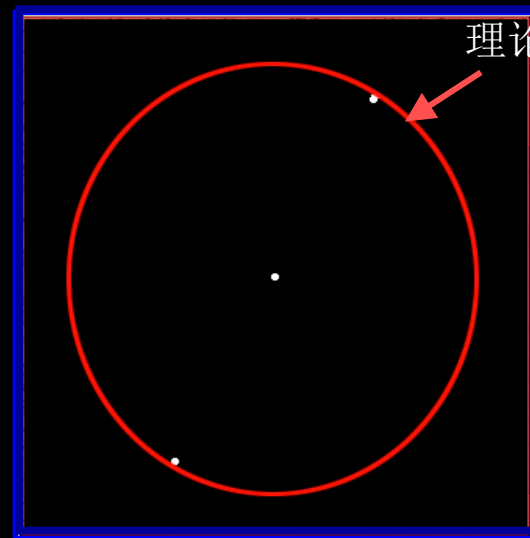
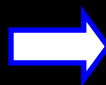
(9855 的波)



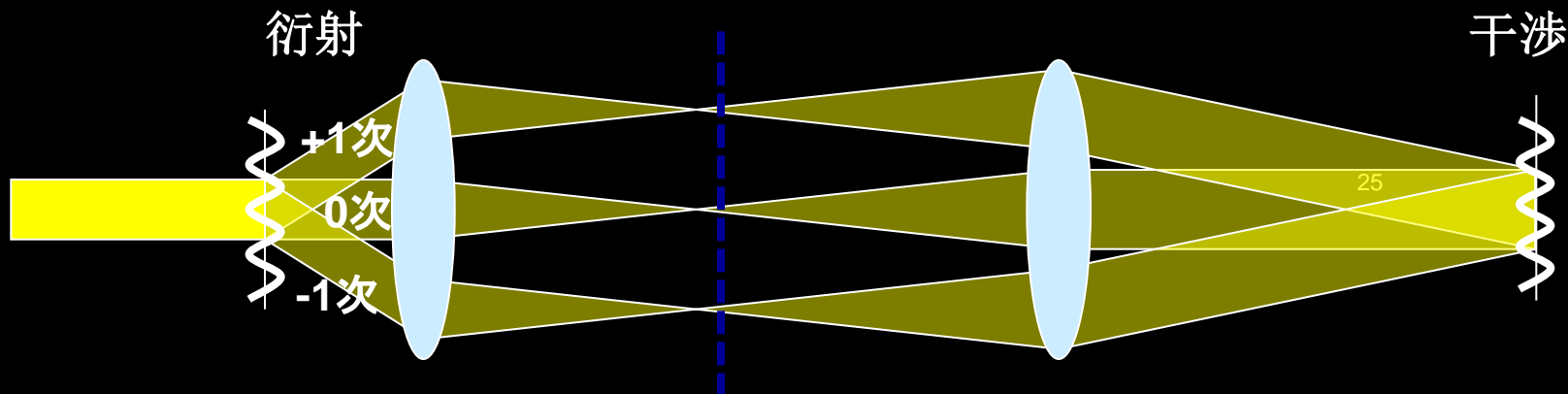
物镜后焦面的图像（傅里叶变换）



实际空间的图像

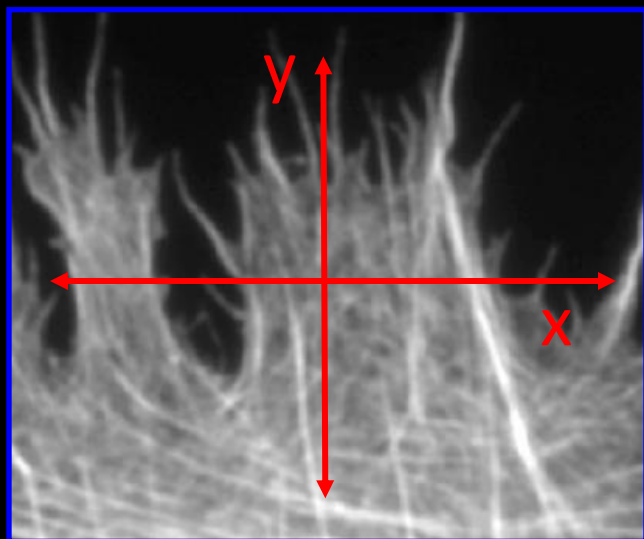


进行傅立叶变换后的频率空间



N-SIM原理：实际空间和频率空间

实际空间

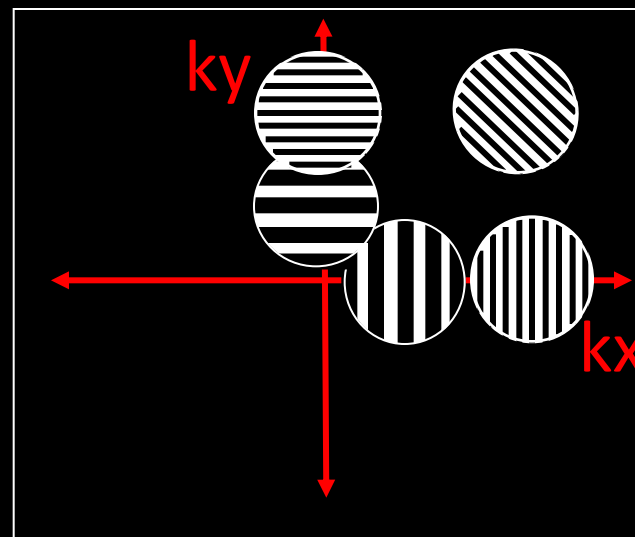


轴表示空间的距离。
(数越大距离越远)

傅立叶变换

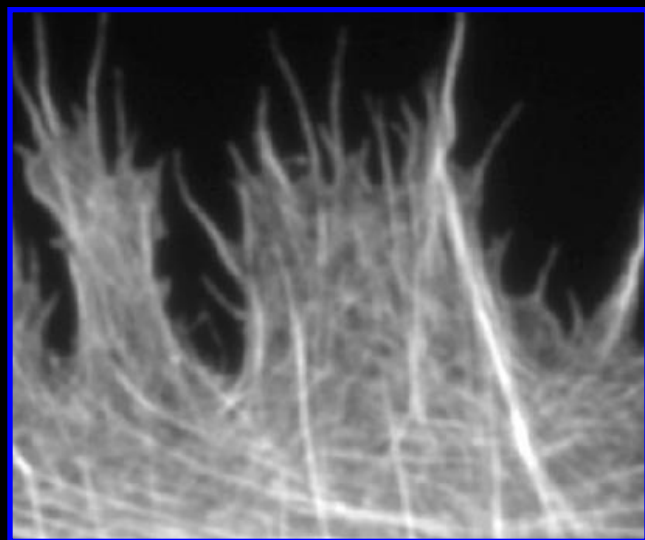


频率空间
(Fourier Space)

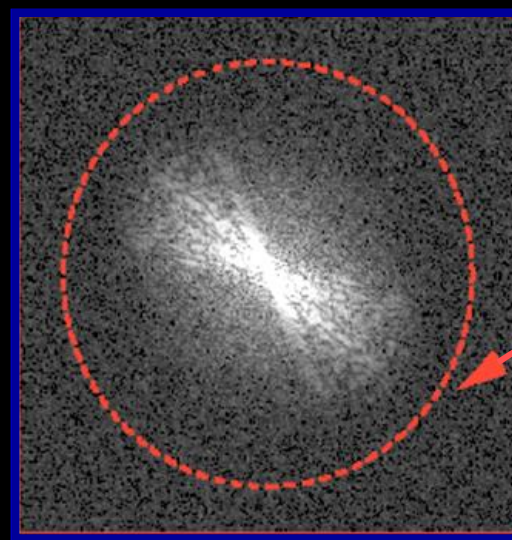
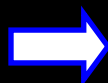


轴表示空间频率。
(数越大频率越高)

 = 1 Hz



实际空间的图像



理论上的分辨极限

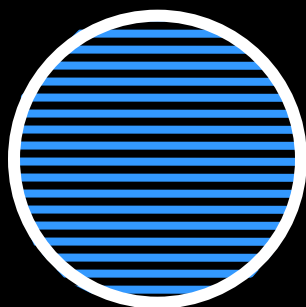
进行傅立叶变换后的频率空间

N-SIM原理：使用结构照明获取图像

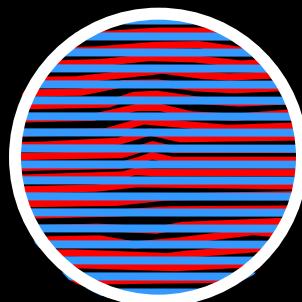
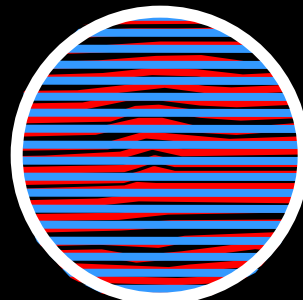
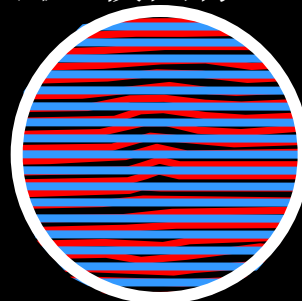
标本



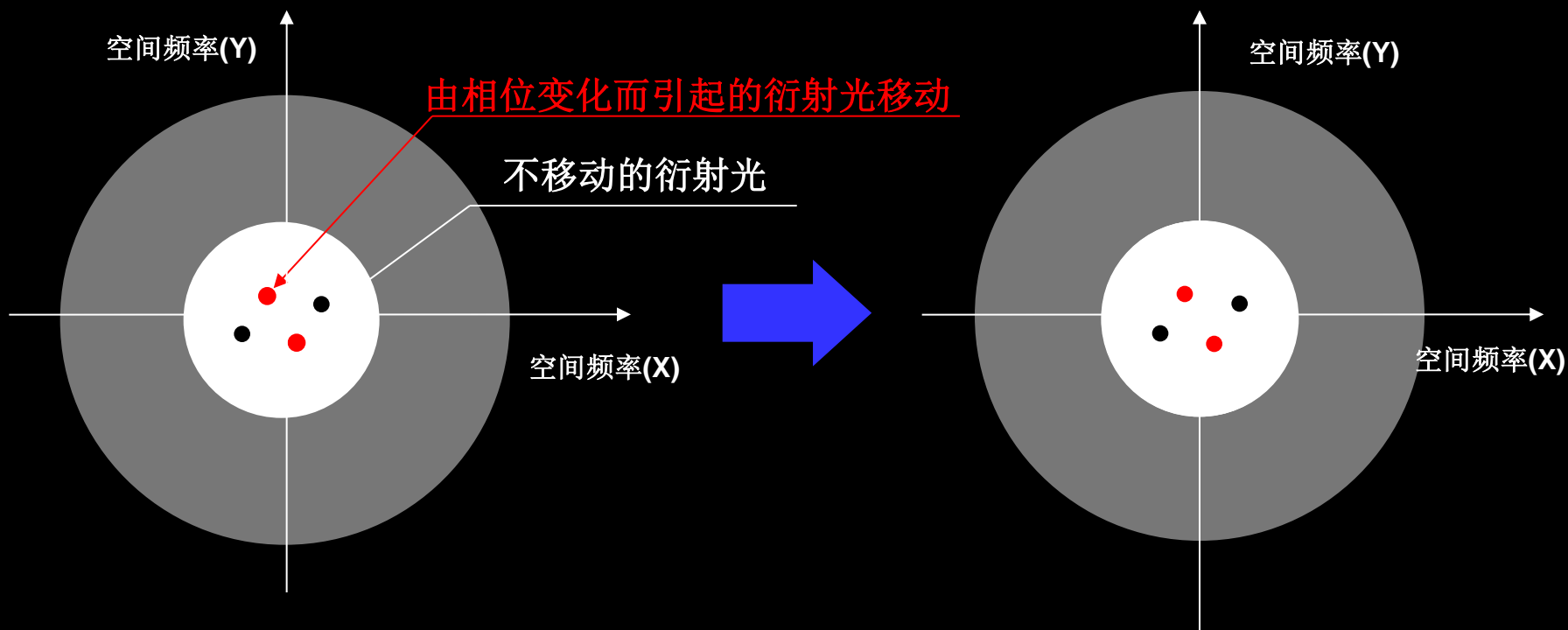
结构照明



变换条纹的相位
获取3枚图像



y 方向的超分辨率



由于结构照明的位置变化，移动的衍射成分与非移动的衍射成分相互区分。然后将移动的衍射成分重新恢复到应有的位置就获得了超分辨率信息。



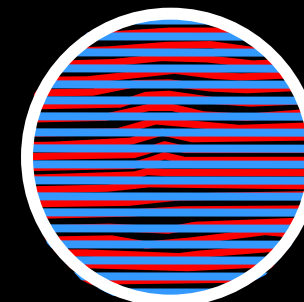
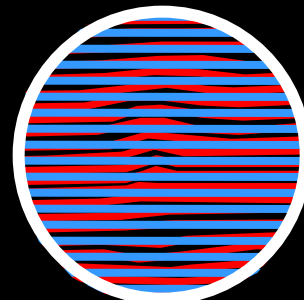
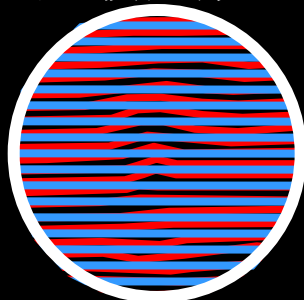
等同于获得了更大的数值孔径

N-SIM原理：使用结构照明获取图像

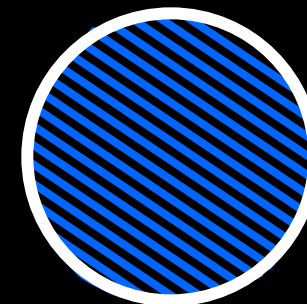
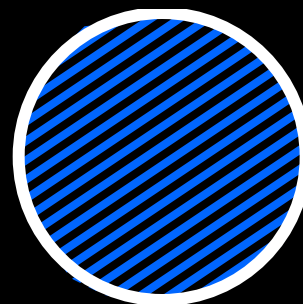
标本



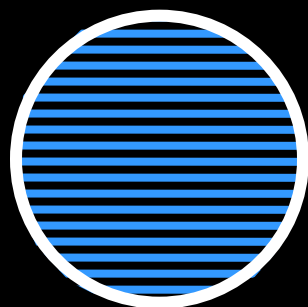
变换条纹的相位
获取3枚图像



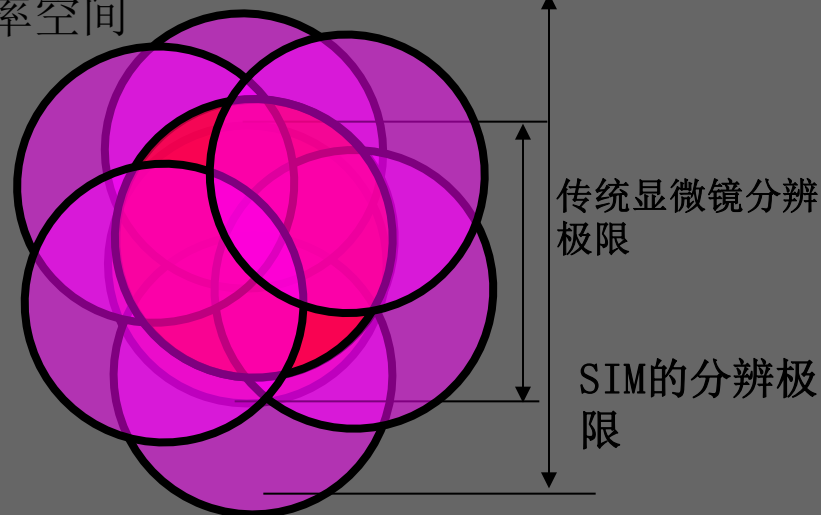
改变条纹方向
各拍摄3个相位图像



结构照明



频率空间



y 方向的超分辨率

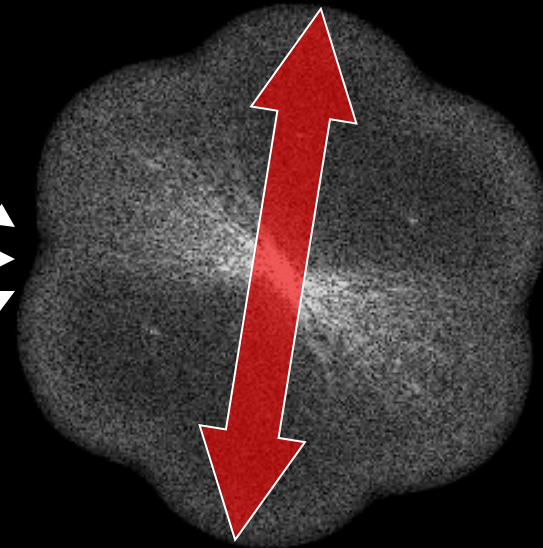
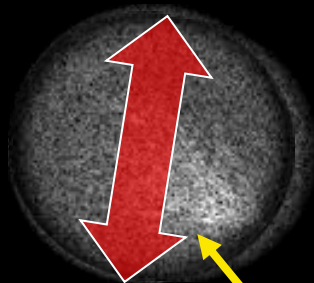
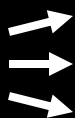
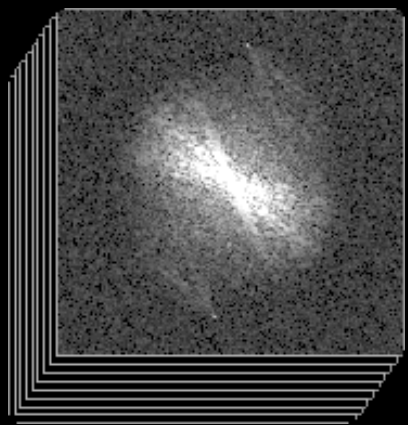
2维的超分辨率（分辨率约2倍）

N-SIM原理：在频率空间进行成分分离和还原

高频成分同低频成分分离后再进行
还原

合成

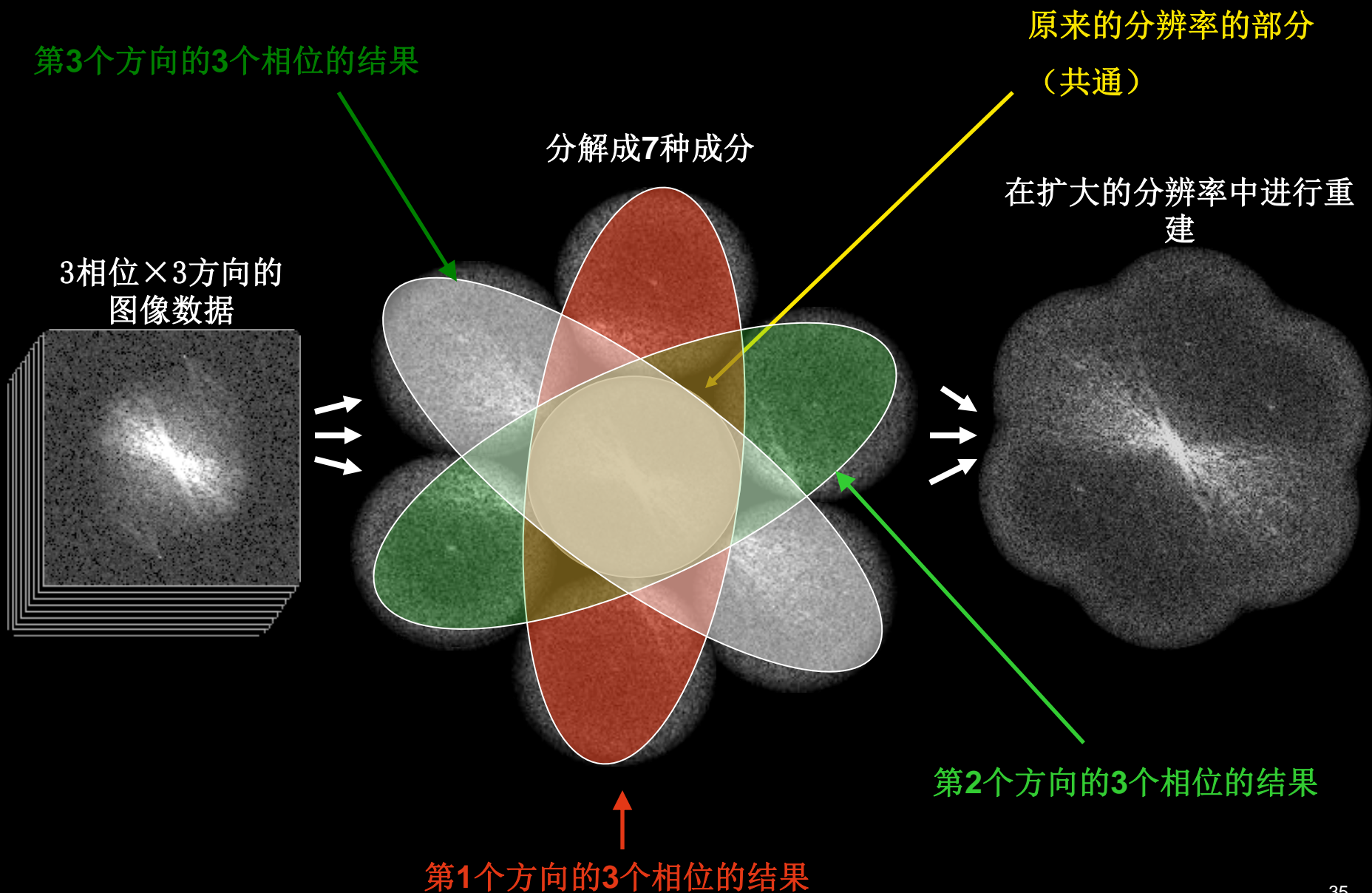
3(5)相位×3方向
的图像数据



超分辨率图像信息
(高频成分)

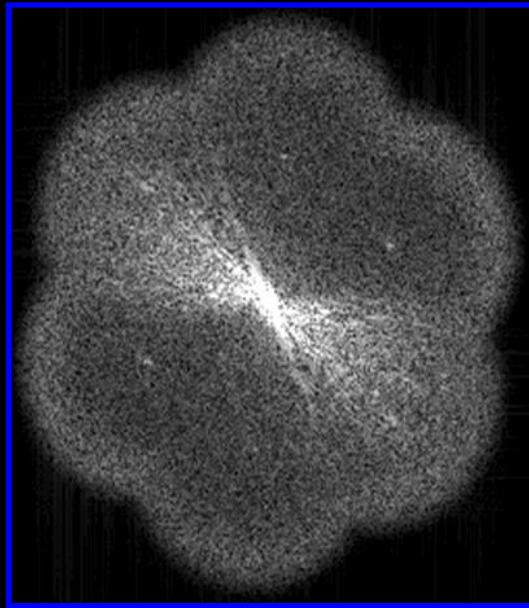
普通显微镜的信息
(低频成分)

N-SIM原理：在频率空间进行重建

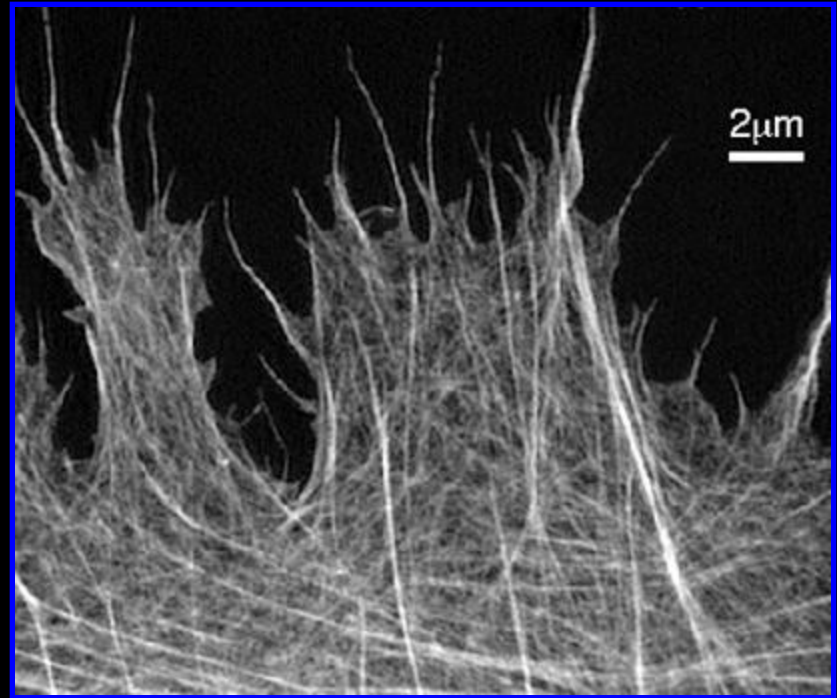


提高分辨率后的SIM图像

在频率空间进行分辨率扩大的图片通过反向傅立叶变换，重建实际空间的图像。



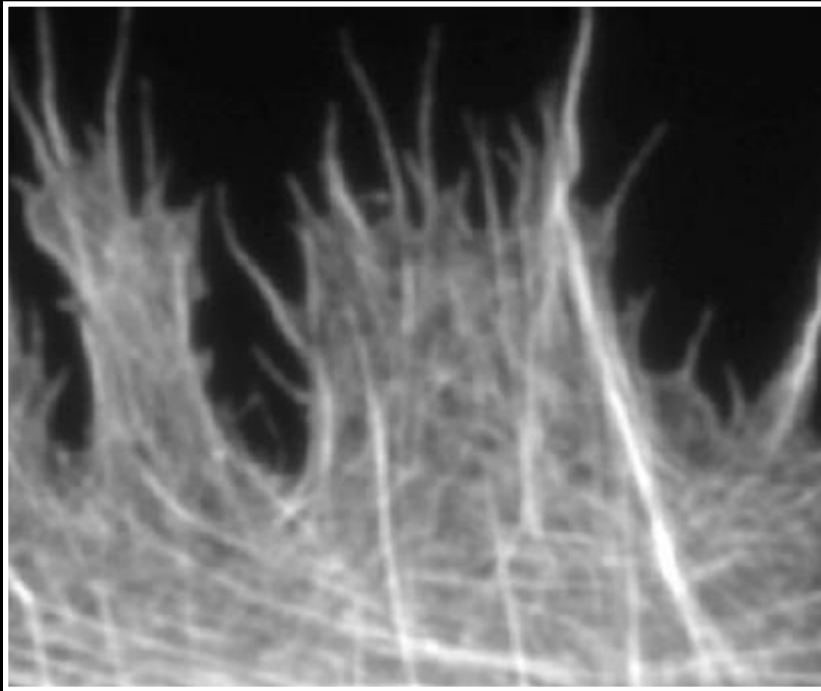
频率空间



实际空间

传统图像和N-SIM图像的比较

Conventional Epi-FL Microscopy



N-SIM

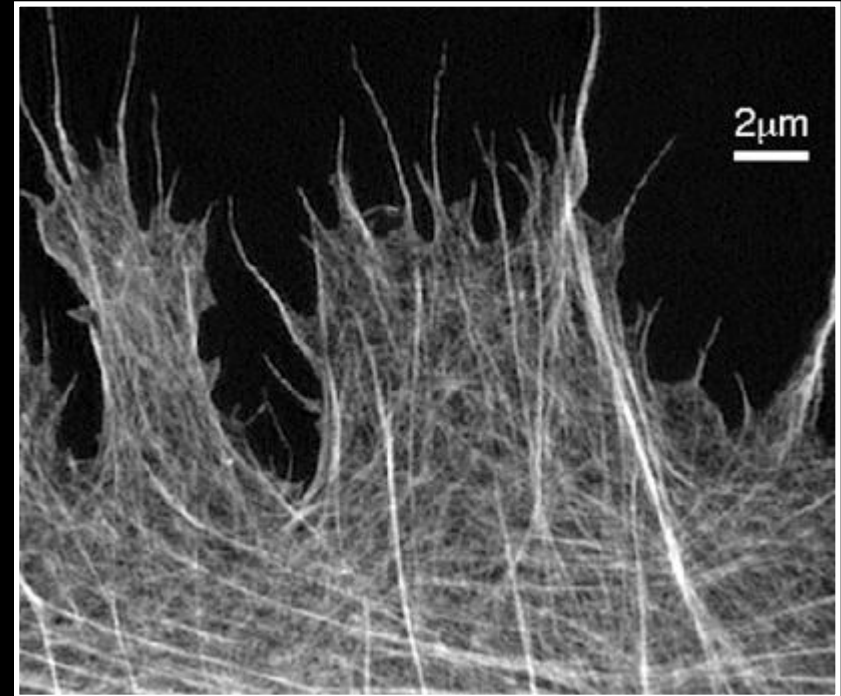


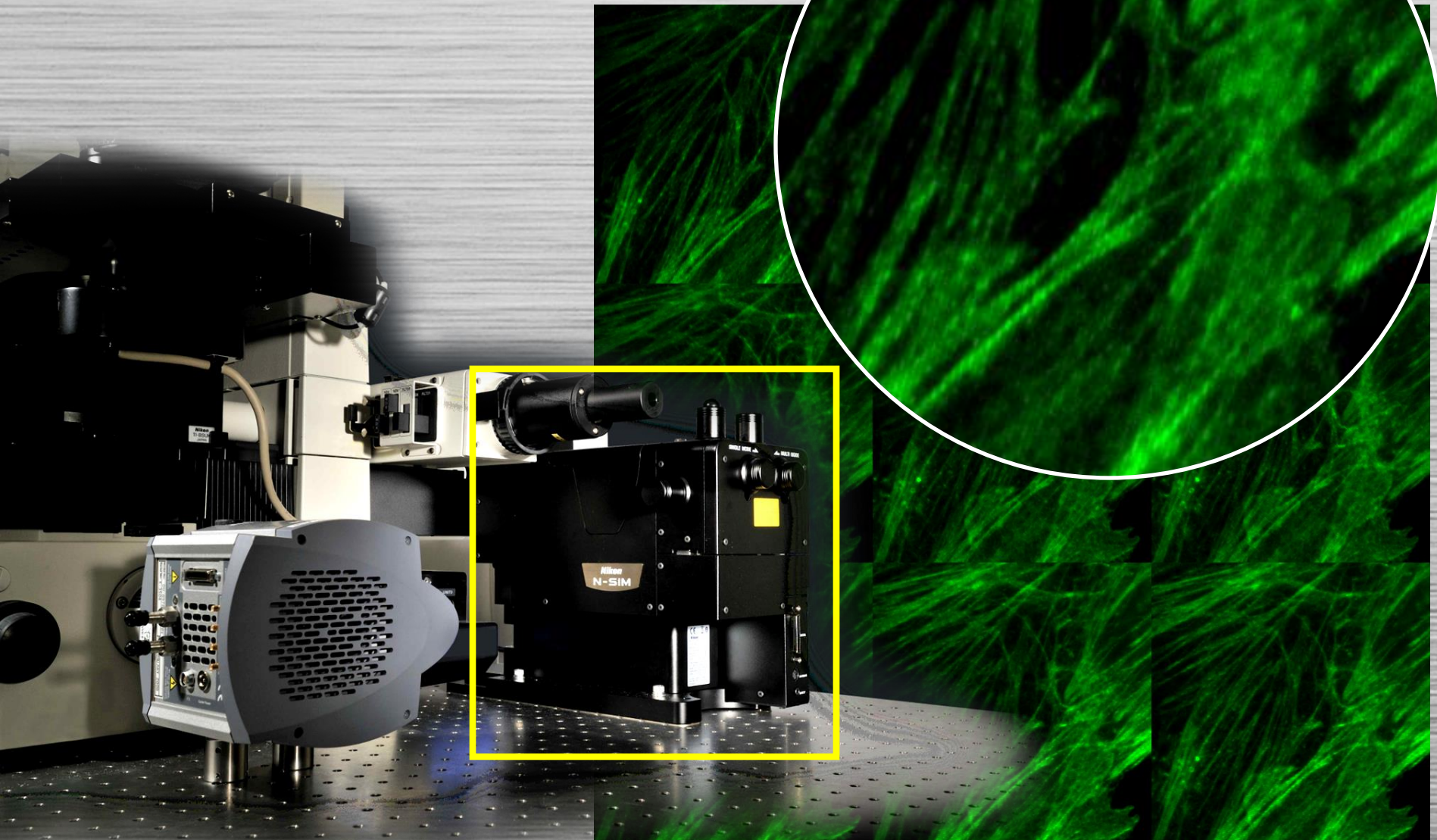
Image From - Mats Gustafsson - UCSF

N-SIM实现传统显微镜2倍的分辨率

1. 把带有已知空间频率的**结构照明**（条纹模块）照射在标本上。
2. 获取**包含莫尔纹的图像**。
3. 把图像在3位相 \times 3方向（或者5相位 \times 3方向）反复操作。
4. 把得到的图像从实际空间到频率空间**进行傅立叶变换**。
5. 在频率空间把低频成分（原有的图像成分）和**高频成分（超分辨率成分）进行分离**。
6. 在频率空间对高频成分进行**还原**。
7. 再进行**反向傅立叶变换，生成实际的图像**。

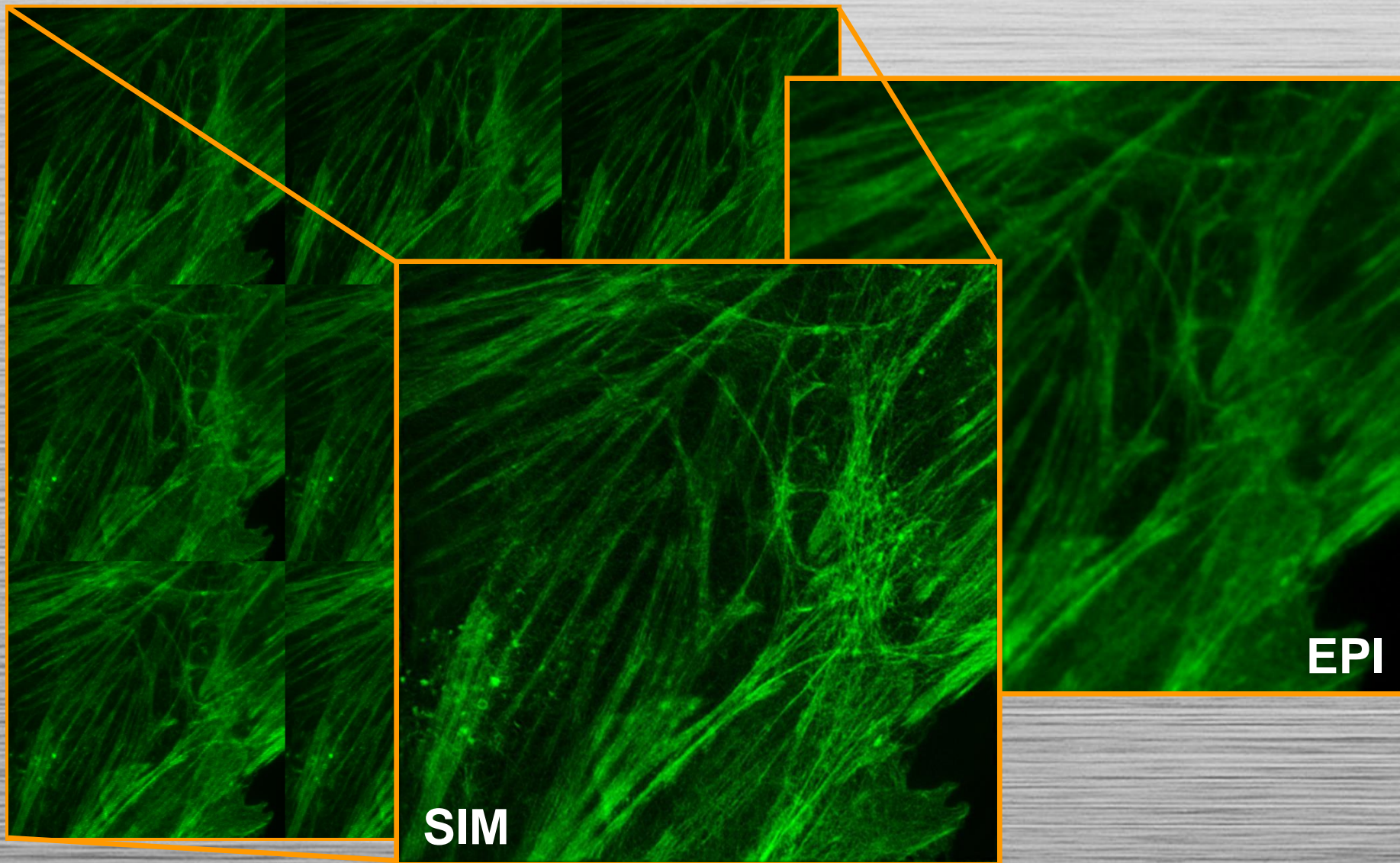


核心—— 独特的结构化照明装置



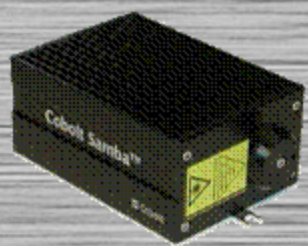
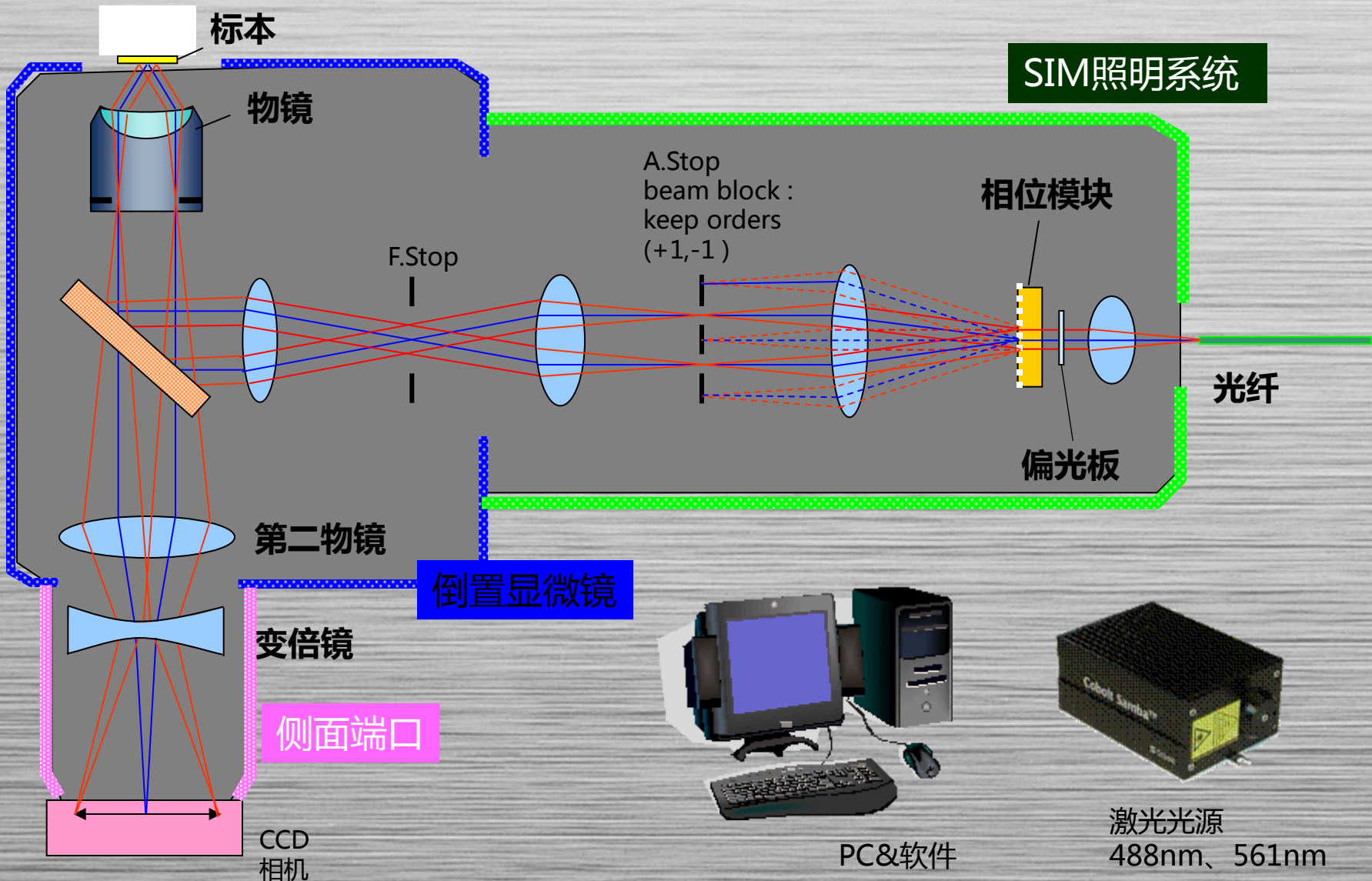


9 张图像合成一张超分辨率图像





N-SIM光学 · 设备构成



激光光源
488nm、561nm

PC&软件



N-SIM 外观图

防风罩

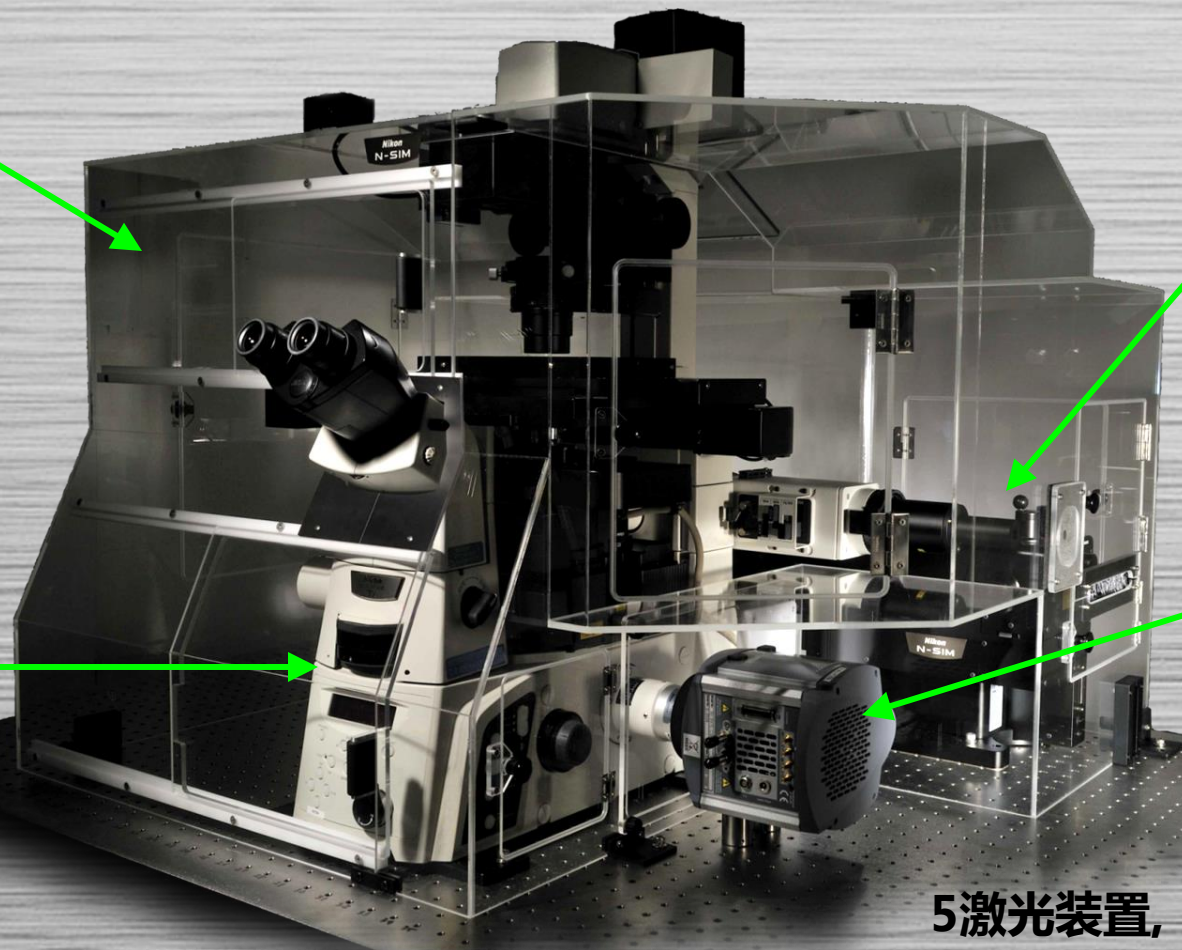
SIM照明装置

倒置显微镜

EMCCD相机

5激光装置, PC&Software

可同尼康共聚焦显微镜 A1共同使用





专用超分辨率物镜

精挑细选最小色差、相差

兼顾深度

最佳分辨率



CFI SR Plan Apochromat IR 60x WI

CFI SR Apochromat TIRF 100x oil



N-SIM激光照明

激光选择

标准配置：

405nm (20mW)
488nm (70mW)
561nm (70mW)
640nm (40mW)

选配：

445nm (20mW)
514nm (20mW)

皆为光纤出口功率

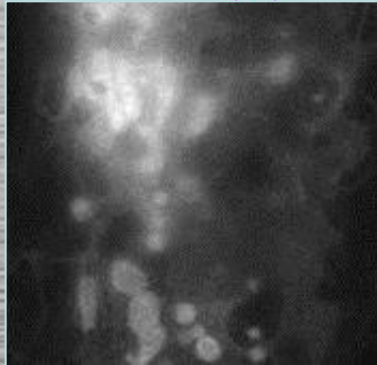
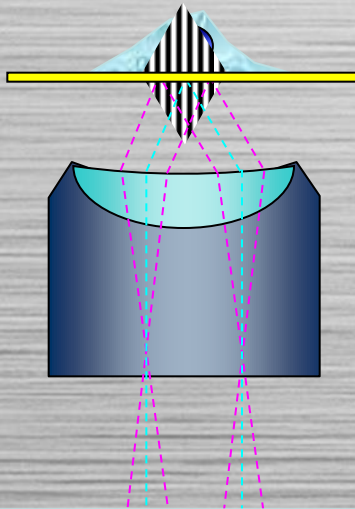


■ 常规共聚焦所使用的染料都可适用！



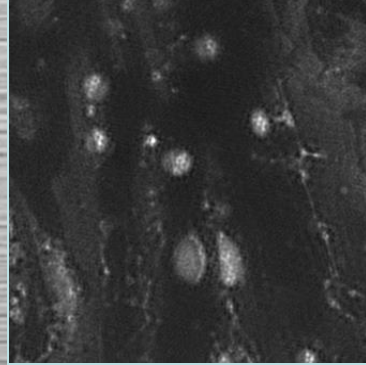
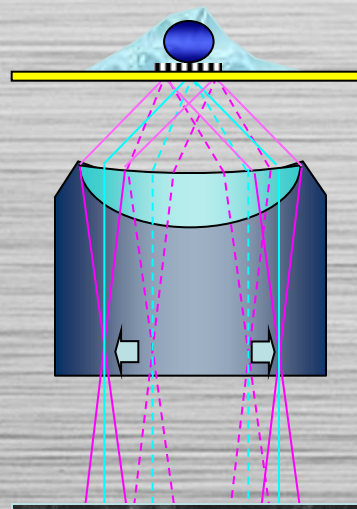
N-SIM 的多种模式

2D-SIM



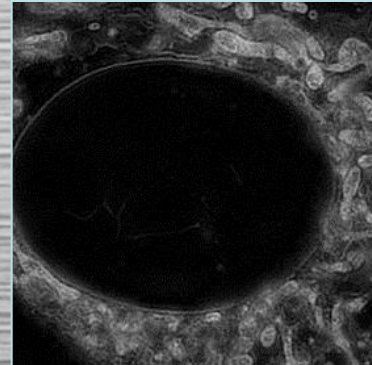
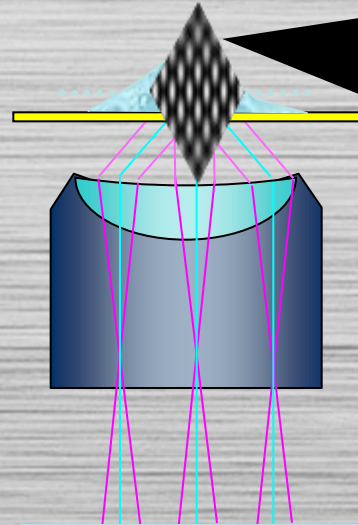
XY:100nm

TIRF-SIM

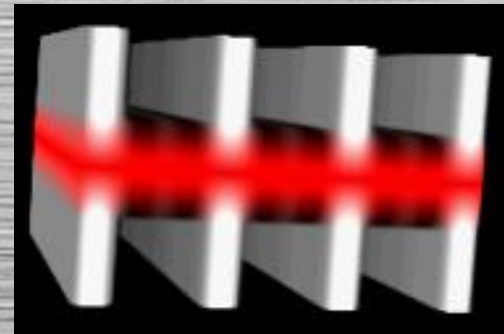
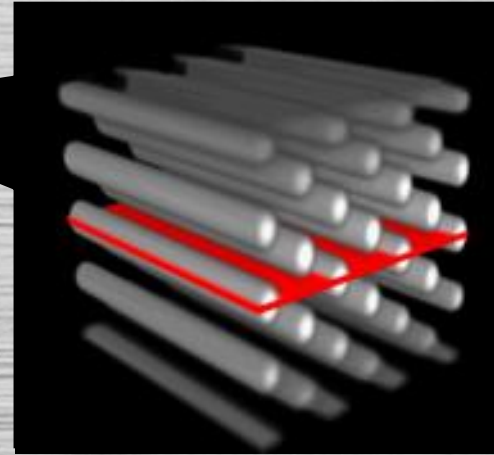


TIRF超分辨率

3D-SIM



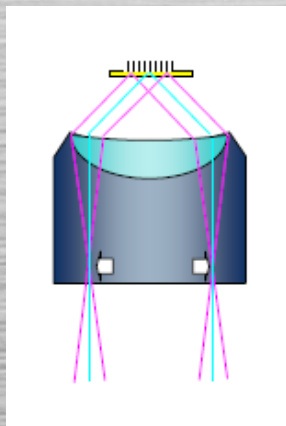
XYZ超分辨率(250nm)





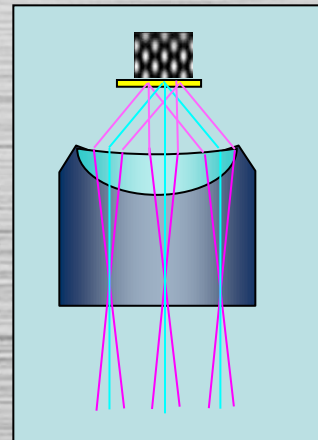
SIM种类：TIRF/2D-SIM & 3D-SIM

TIRF/2D-SIM型



照明方法：2束光干涉
画像取得：3相位、3方向
取得時間：最短0.6秒左右
表示時間：1~2秒/枚
超分辨率：只在XY方向
观察深度：距盖玻片3 μ m

3D-SIM型

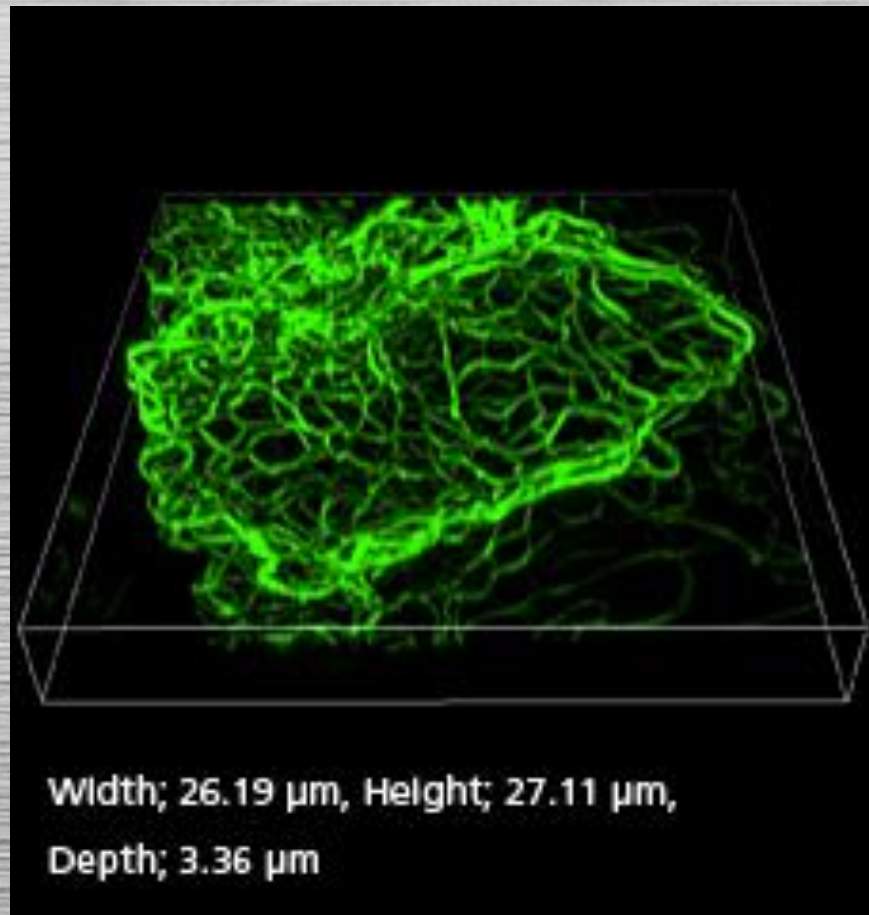
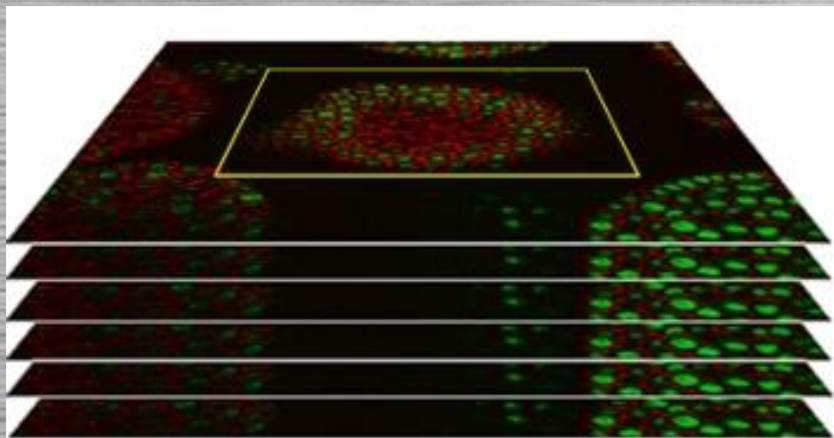


照明方法：3束光干涉
画像取得：5相位、3方向
取得時間：最短1秒左右
表示時間：2~3秒/枚
超分辨率：XY及Z方向
观察标本厚：20 μ m



Stack 3D-SIM 模式

基于三维片层的运算法

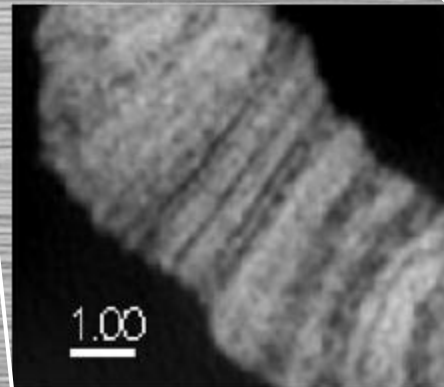
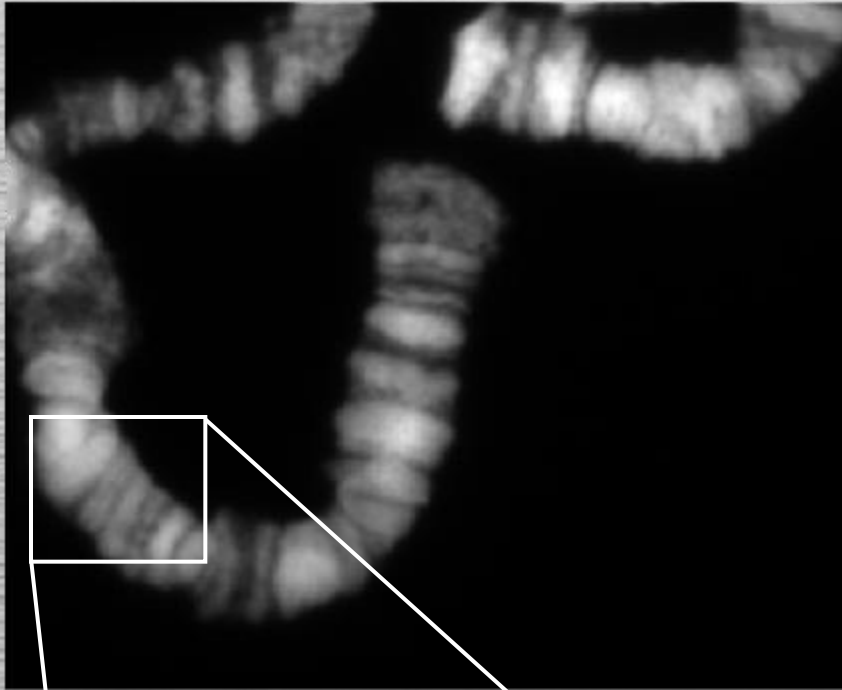




Drosophila polytene chromosome squash

Conventional microscopy

Structured illumination



Data by Harry Saumweber

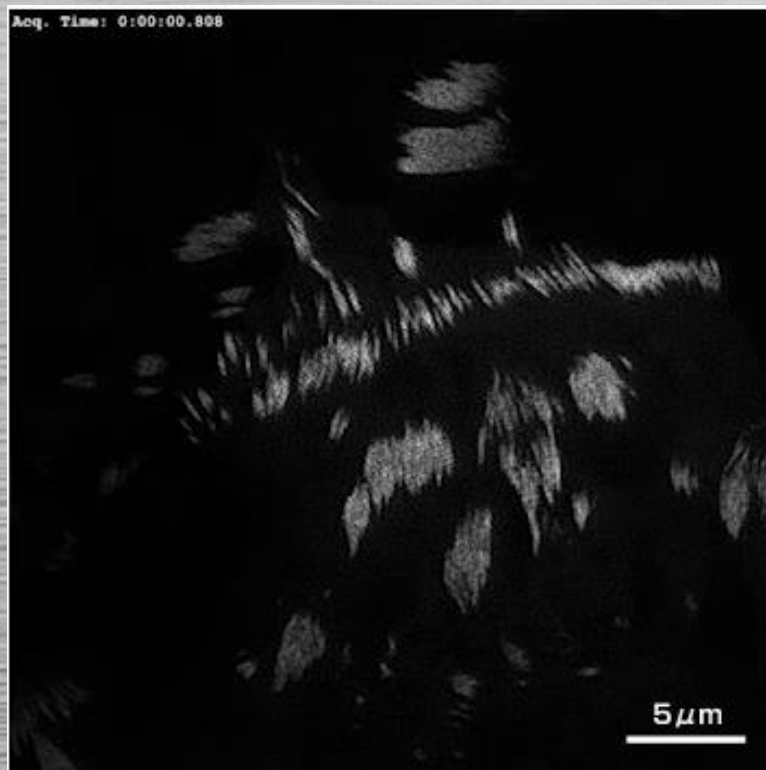
Image From - Mats Gustafsson - UCSF



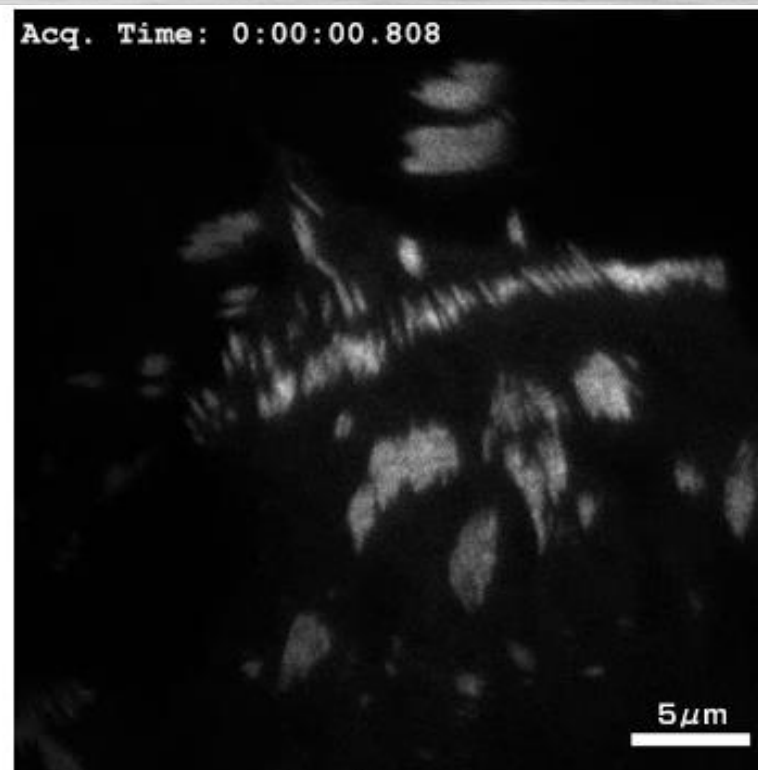
TIRF-SIM

FoLu (fox lung) cells eGFP Vinculin

TIRF-SIM



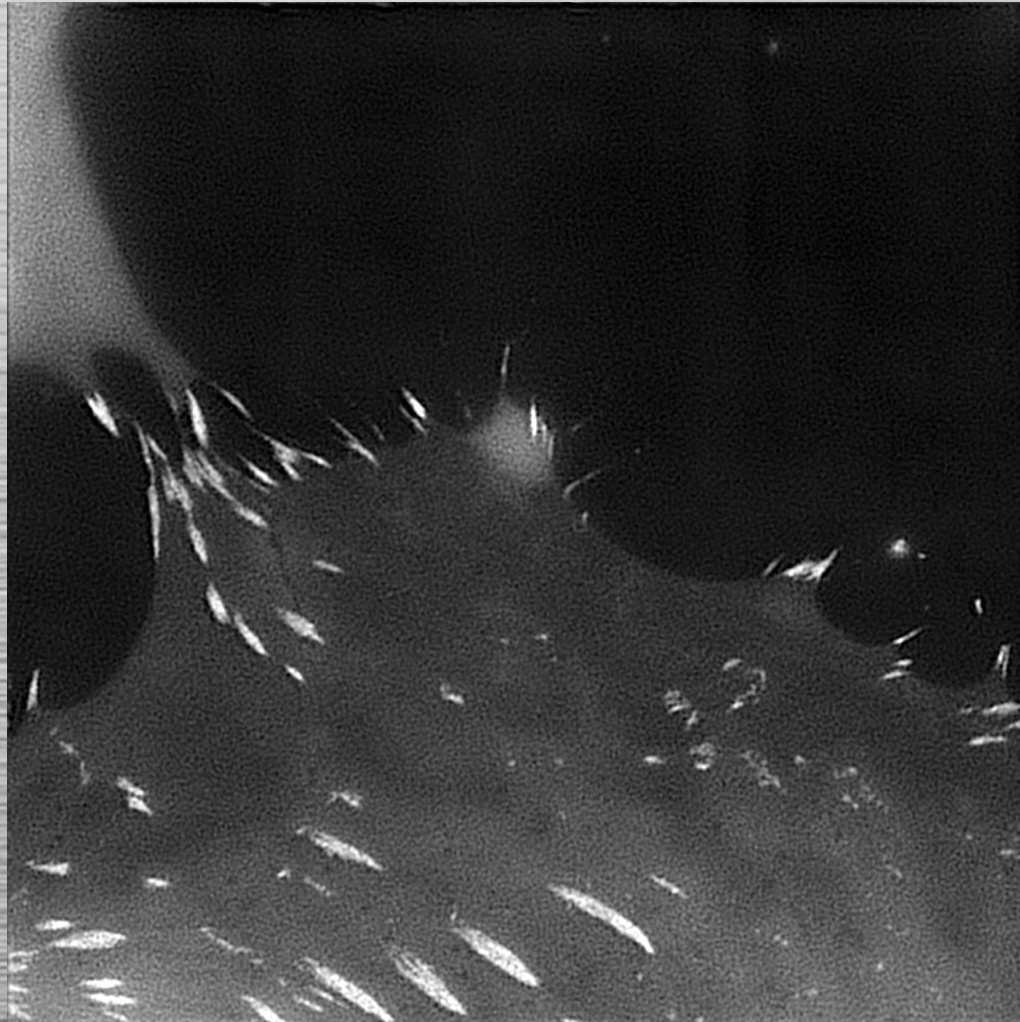
Conventional TIRF





TIRF-SIM

Vinculin





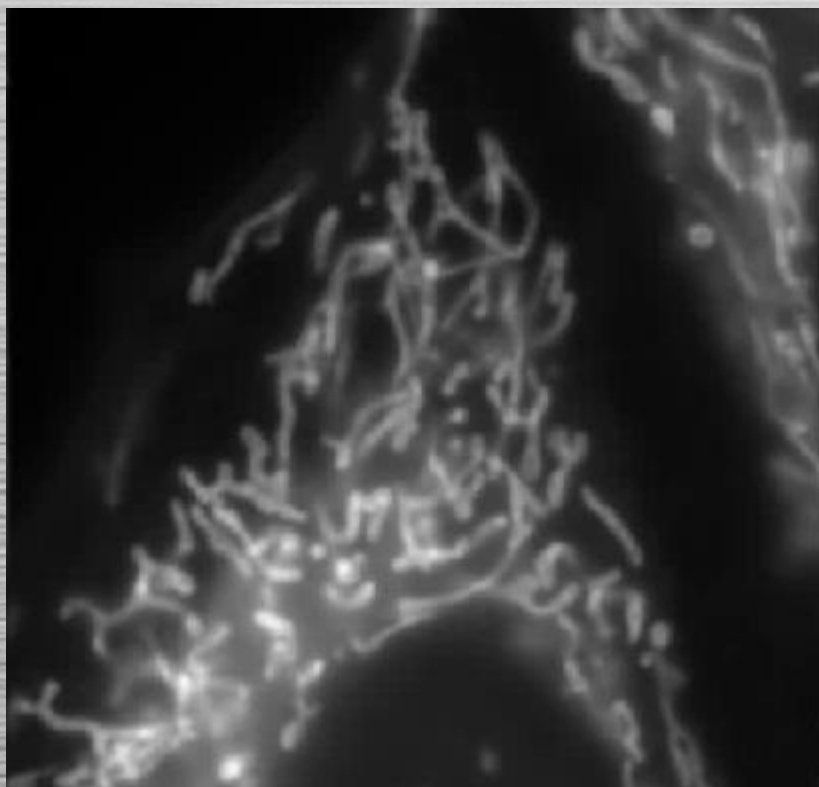
N-SIM 时间序列成像



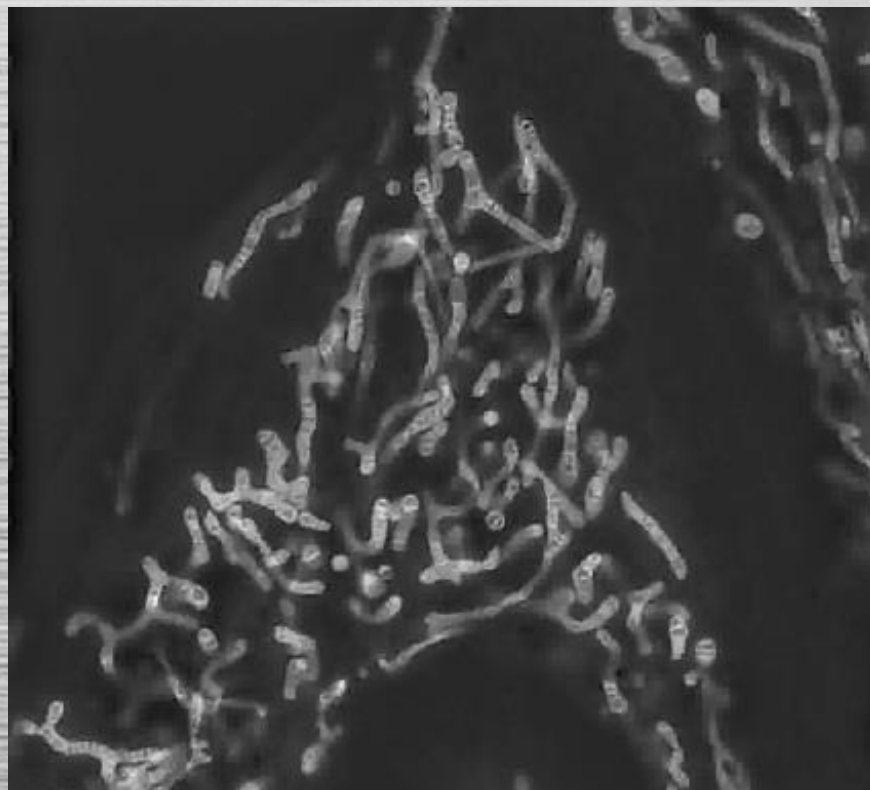
0.6秒/帧 超快超分辨率成像

Exposure: 64ms, 15 images. Total acq. time: 1.8s. 5-cycle timelapse, 1s interval

活细胞- NIH3T3 Mitochondria -MitoTracker Red-时间序列成像

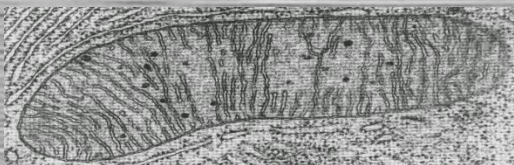


Conventional

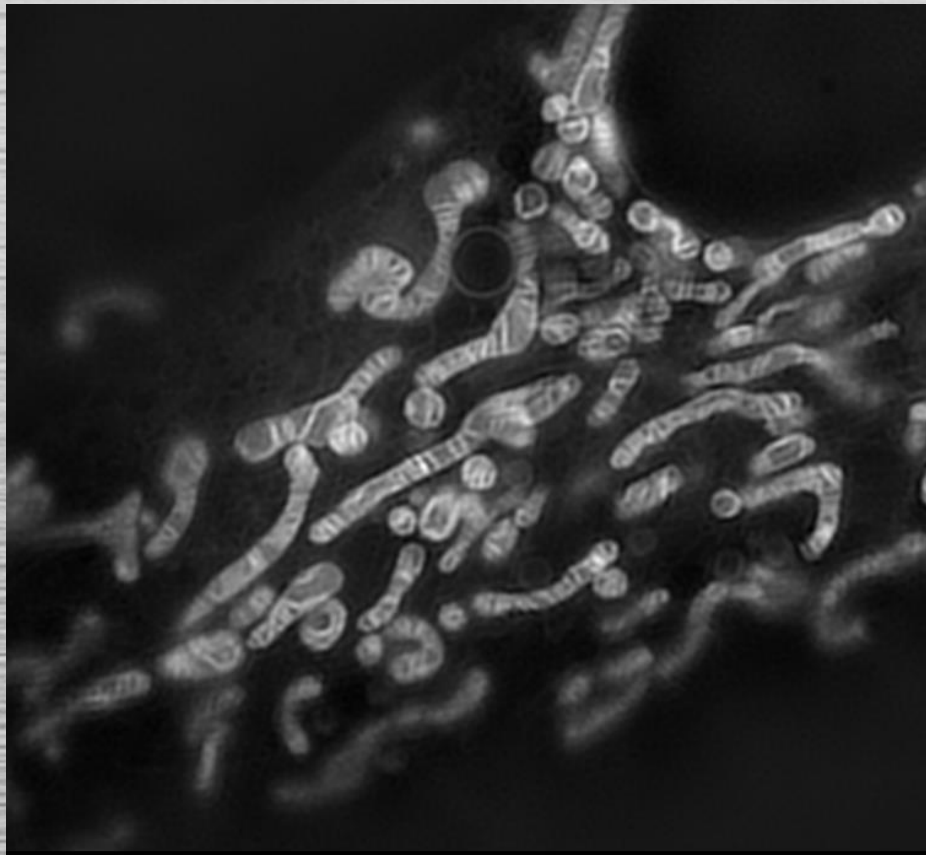


N-SIM

样品厚度达到20 μ m



线粒体脊可以分辨

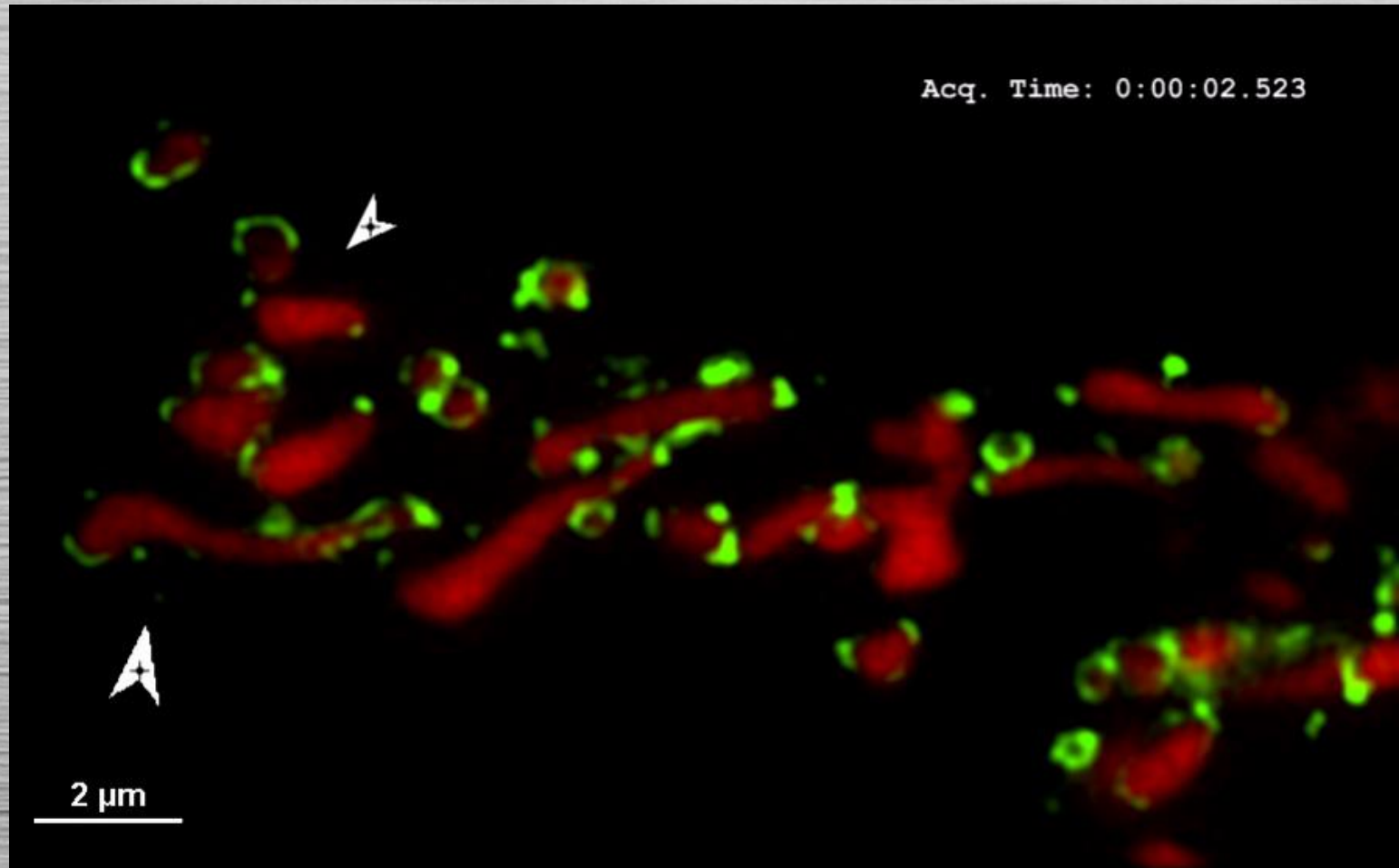


线粒体脊清晰可辨



用户SIM影像

浙江大学 生科院--小鼠胚胎成纤维细胞线粒体与X蛋白





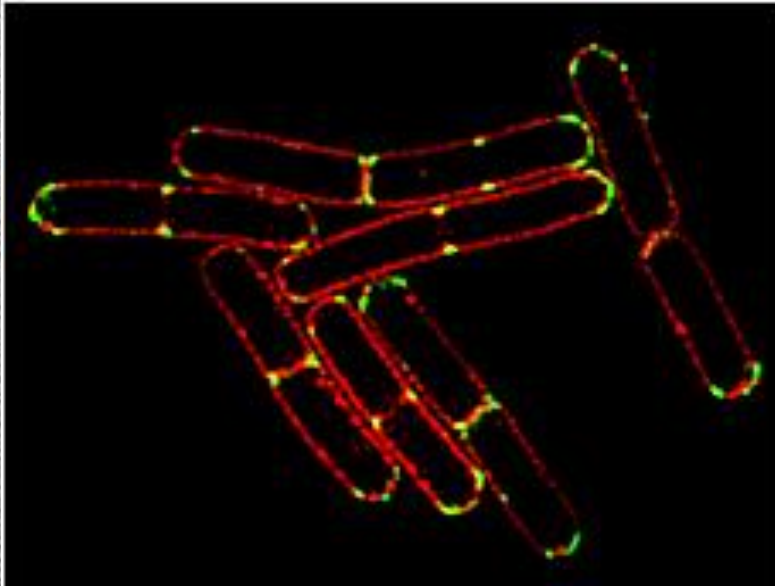
N-SIM 多色成像



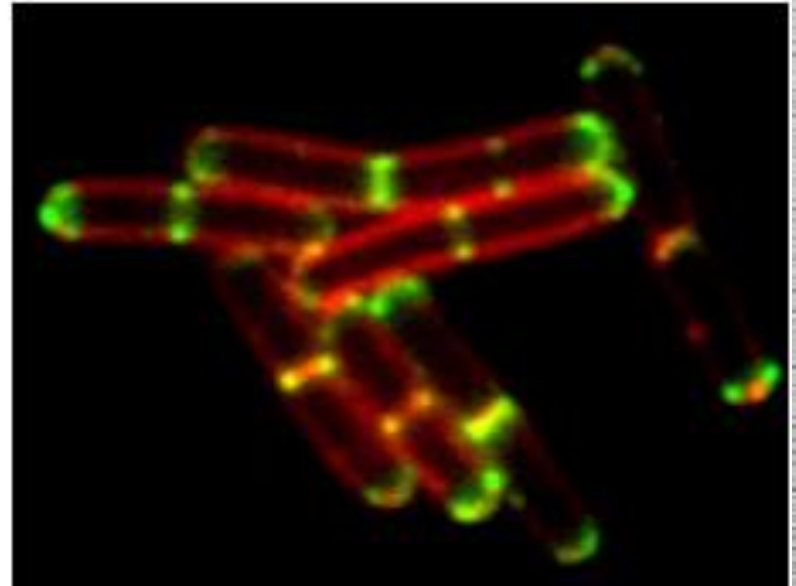
N-SIM 多色样品图像

Bacillus subtilis bacterium stained with membrane dye Nile Red (red), and expressing the cell division protein DivIVA fused to GFP (green).

3D-SIM



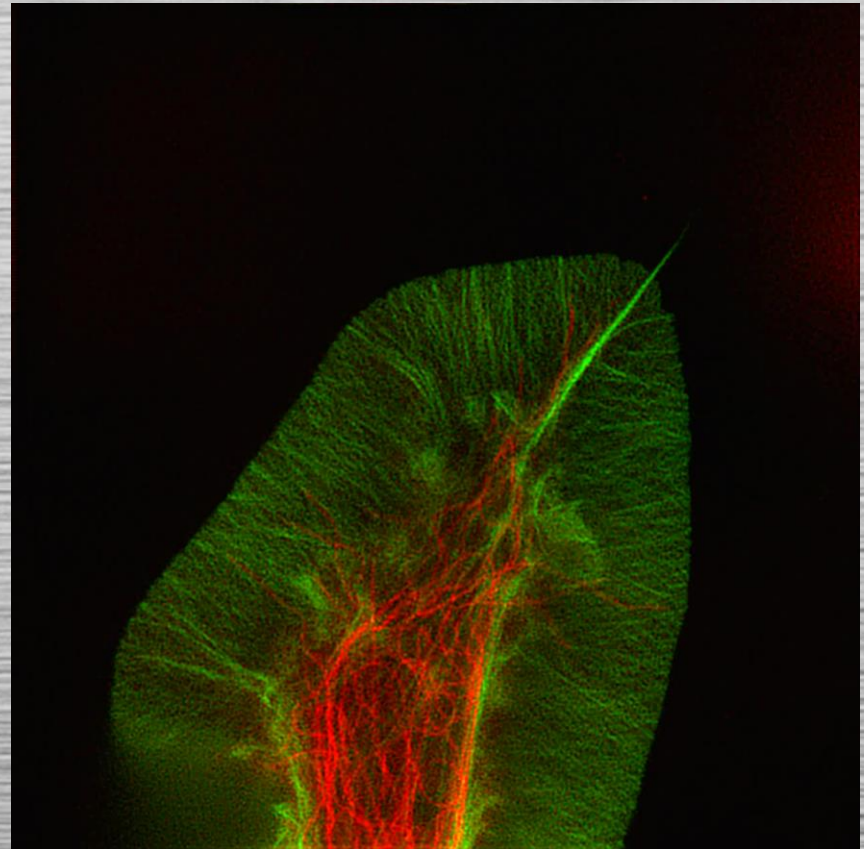
Conventional





双色同时成像

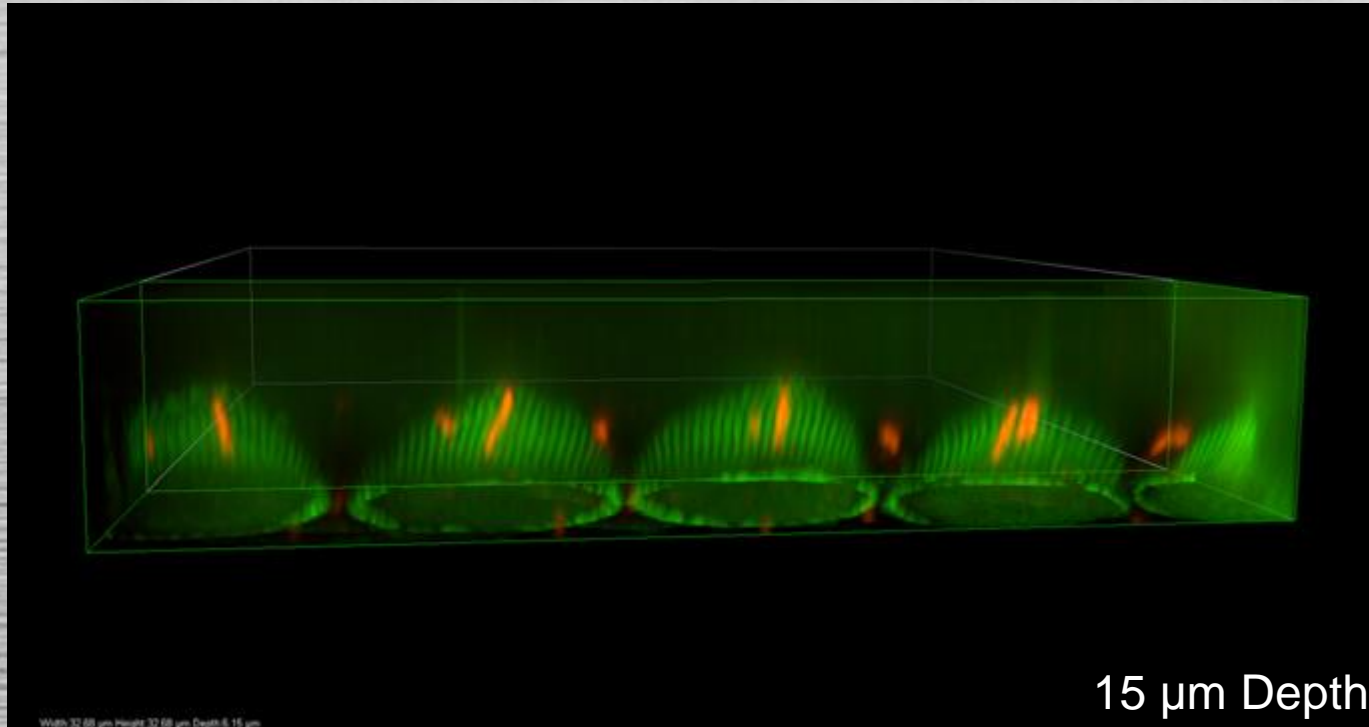
Growth cone of NG108 cell expressing GFP-LifeAct (F-actin, green) and mCherry-tubulin (microtubules, red)





3D-SIM 三维图像

内耳螺旋器内腔表面超分辨率三维成像



Luminal surface of the organ of Corti at postnatal day 1. (Mouse)

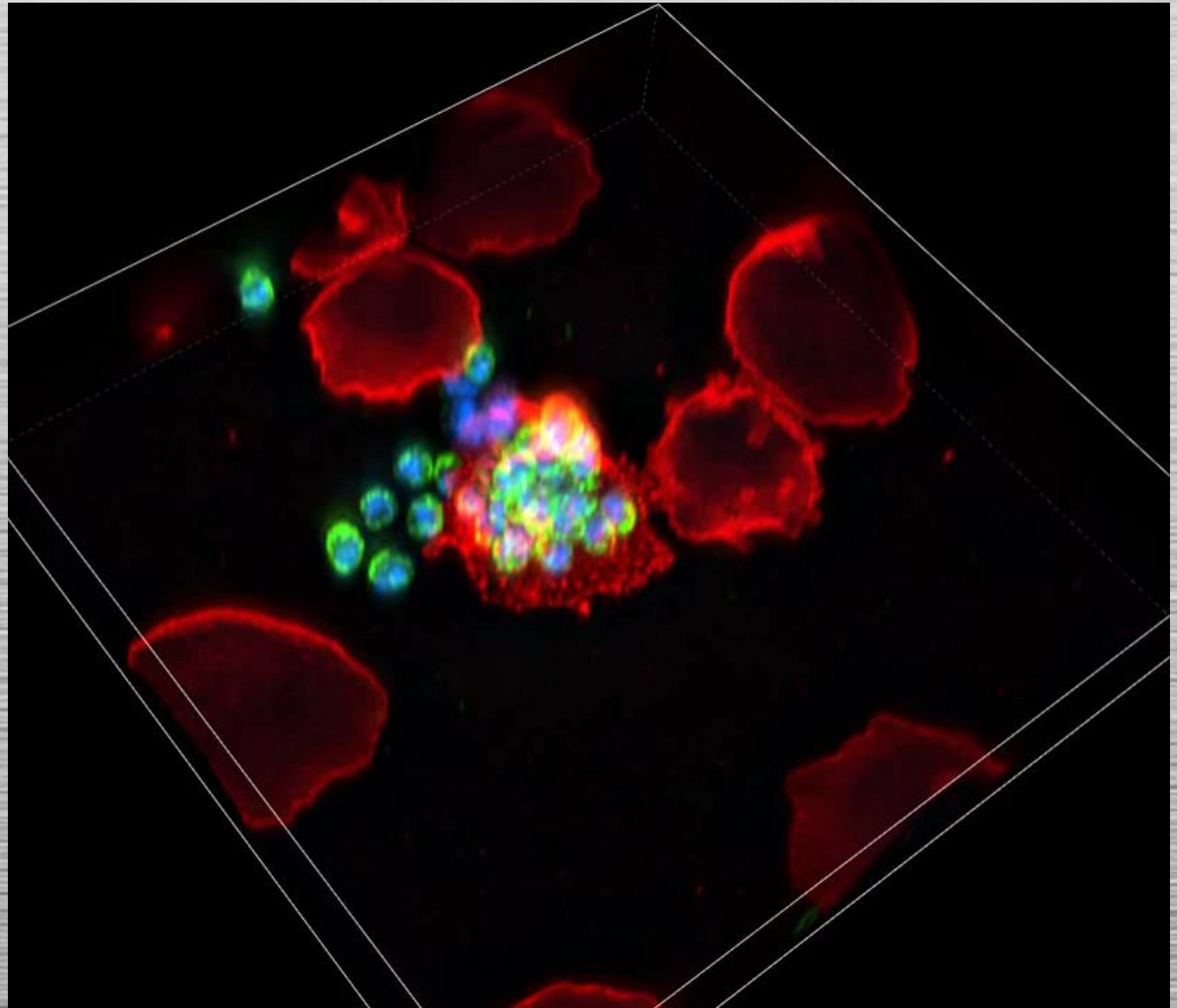
Green: F-actin.

Red: Acetylated Tubulin

Specimen courtesy of: Ms. Kanoko Kominami, Dr. Hideru Togashi and Prof. Yoshimi Takai, Division of Molecular and Cellular Biology, Kobe University Graduate School of Medicine



3D-SIM 三维图像



疟原虫侵入血红细胞

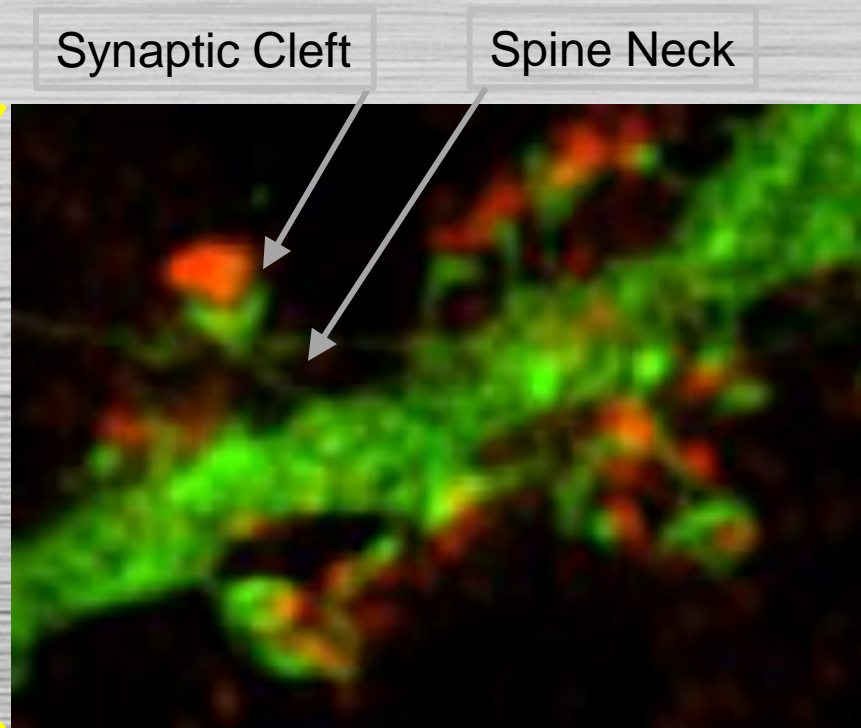
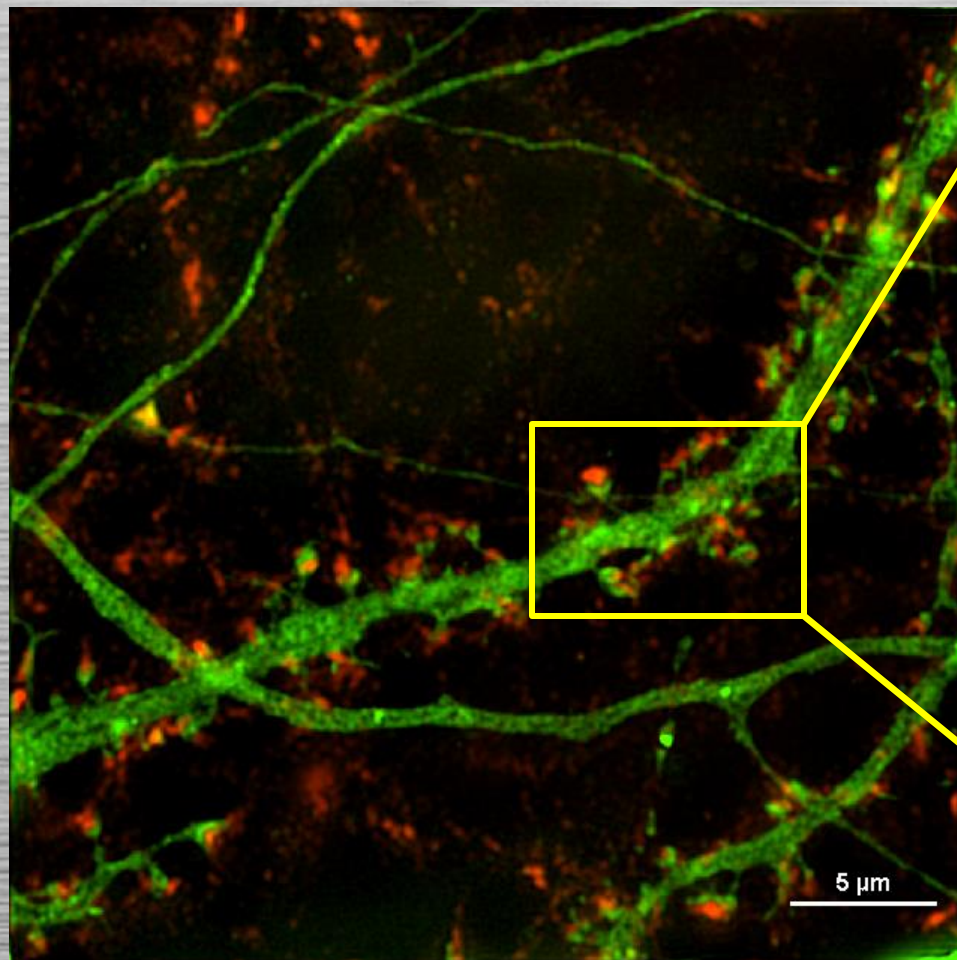
Photo courtesy of: Drs. Masayuki Morita ect. Proteo-Science Center, Ehime University



N-SIM应用举例



3D-SIM Image : 培养神经细胞

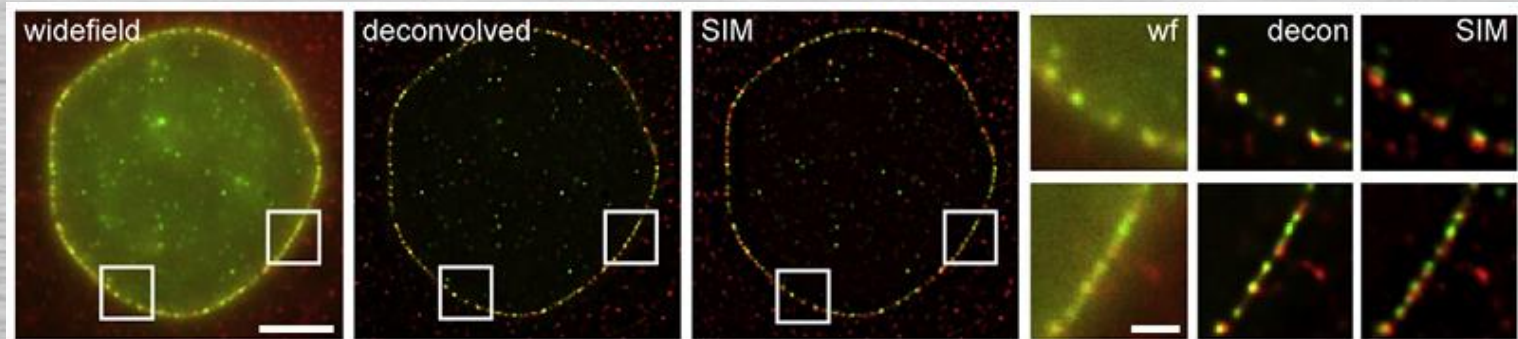


小鼠海馬培养神经元树状与棘突(GFP标记、DIV21)GFP及vGlut1(vesicular glutamate transporter-1)免疫组化使用Alexa488(GFP)及Alexa568(vGlut1)做荧光标记。

财团法人东京医学综合研究所 神经可塑性项目 安田 新、山形 要人



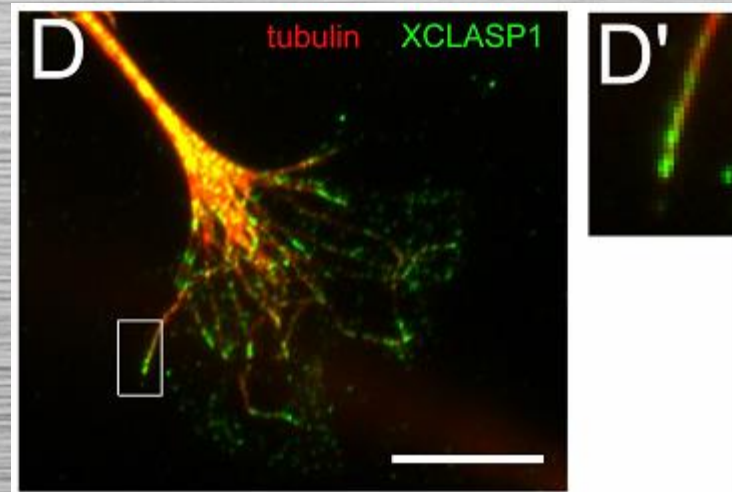
发表文章举例



核孔复合体

Journal of Structural Biology 177 (2012) 81–89

生长锥



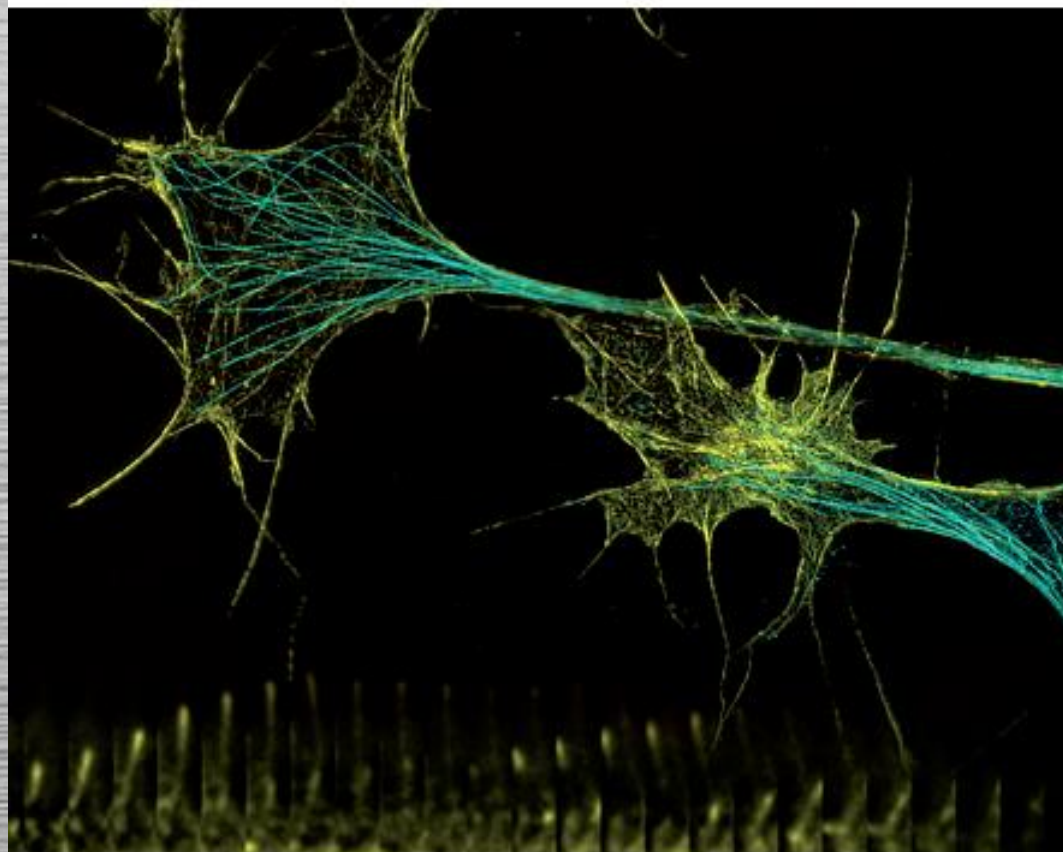
Volume 24 May 15, 2013 MBoC



THE AMERICAN SOCIETY
FOR CELL BIOLOGY

MBoC

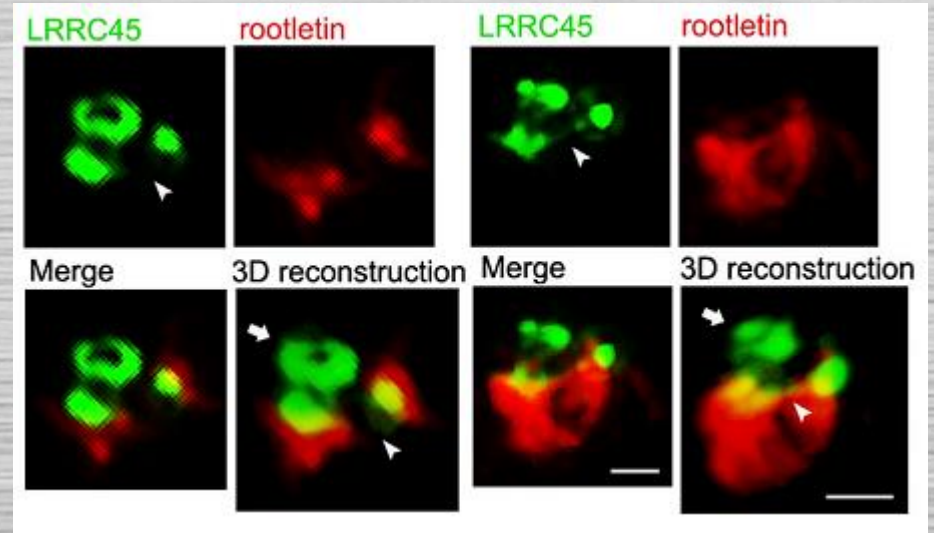
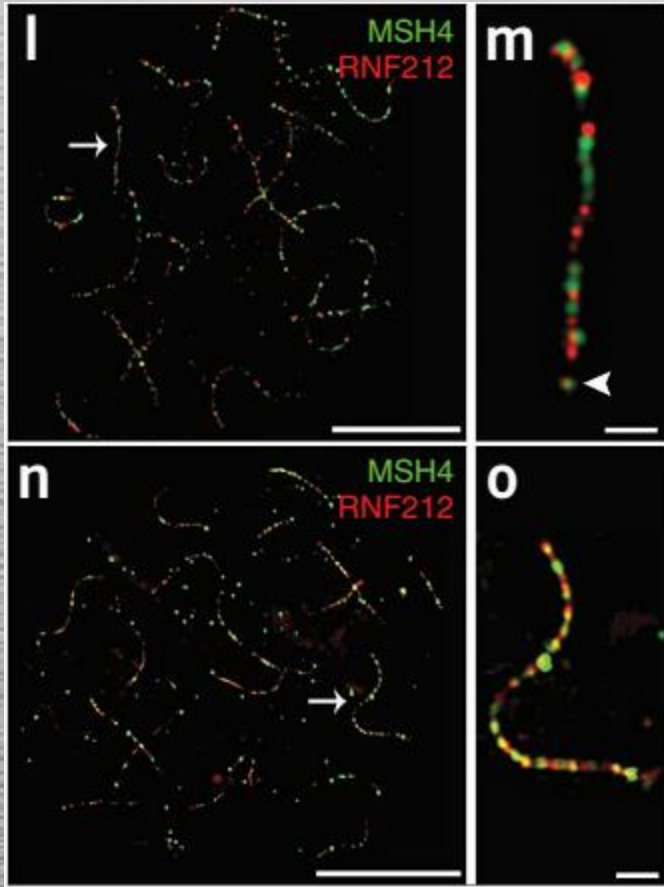
MOLECULAR BIOLOGY OF THE CELL



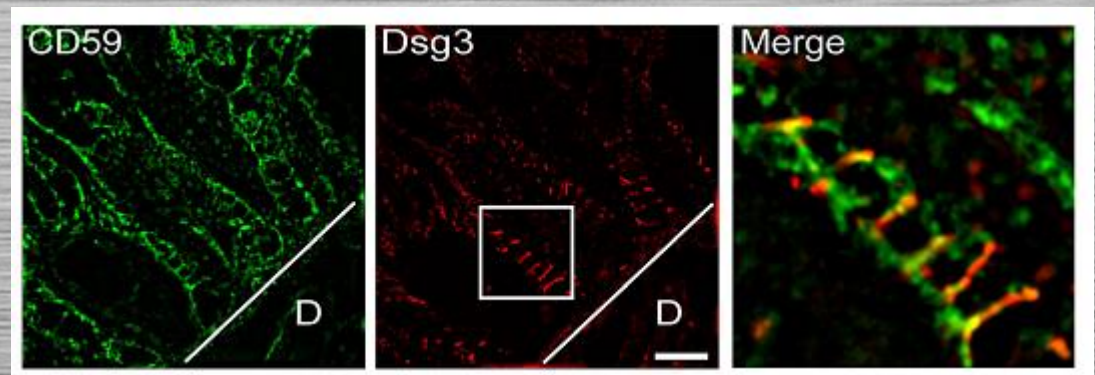
VOLUME 24 • NUMBER 10 • MAY 15, 2013



Cell Reports 4, 1–8, September 26, 2013



Plos One January 2014 Volume 9





国内实际应用举例

尼康N-SIM文章部分列表 (国内)

Year	Author	Journal	Title	
2016	SX Zhang, LH Duan, H Qian, X Yu	Neuroscience bulletin	Actin Aggregations Mark the Sites of Neurite Initiation	北京中科院
2016	Haining Zhou,1 Tianning Wang,1 Tao Zheng,1,2 Junlin Teng,a,1 and Jianguo Chenb,1,3	Nat Commun.	Cep57 is a Mis12-interacting kinetochore protein involved in kinetochore targeting of Mad1–Mad2	清华
2015	Chong Wang,1,4,* Wanqing Du,1,4,* Qian Peter Su,2,3,* Mingli Zhu,1,4 Peiyuan Feng,	Cell Res	Dynamic tubulation of mitochondria drives mitochondrial network formation	清华
2015	Yuhua Fu,1 Pin Lv,1 Guoquan Yan,1,2 Hui Fan,1 Lu Cheng,1 Feng Zhang,3 Yongjun Dang,1 Hao Wu,4 and Bo Wena,1,3	Sci Rep.	MacroH2A1 associates with nuclear lamina and maintains chromatin architecture in mouse liver cells	复旦
2015	Na Mi, Yang Chen, Shuai Wang	Nature Cell Biology	CapZ regulates autophagosomal membrane shaping by promoting actin assembly inside the isolation membrane	清华大学
2015	L Jiafeng, X Fu, Z Chang	Scientific reports	Hypoionic shock treatment enables aminoglycosides antibiotics to eradicate bacterial persisters	北大
2015	LS Havel, ER Kline, AM Salgueiro, AI Marcus	Oncogene	Vimentin regulates lung cancer cell adhesion through a VAV2–Rac1 pathway to control focal adhesion kinase activity	北大
2015	H Mirendil, EA Thomas, C De Looze, K Okada	Translational Psychiatry	LPA signaling initiates schizophrenia-like brain and behavioral changes in a mouse model of prenatal brain hemorrhage	苏州大学
2015	B Wu, S Geng, Y Bi, H Liu, Y Hu, X Li	Clinical and Vaccine Immunology	Herpes Simplex Virus-1 Suppresses the Function of Lung Dendritic Cells via Caveolin-1	复旦
2015	SS Siller, MC Burke, FQ Li, KI Takemaru	Cell Cycle	Chibby functions to preserve normal ciliary morphology through the regulation of intraflagellar transport in airway ciliated cells	清华
2015	K Marno, L Al'Zoubi, M Pearson, M Posch	Methods	The evolution of structured illumination microscopy in studies of HIV	中国中科院理化技术研究所
2015	N Hans, U Relan, N Dubey, D Gaur	Molecular and Biochemical Parasitology	Identification and localization of a Novel Invasin of Plasmodium falciparum	北大
2015	Y Li, Z Liu, Y Zhang, QP Su, B Xue	Molecular Oral Microbiology	Live-cell and super-resolution imaging reveal that the distribution of wall-associated protein A is correlated with the cell chain integrity of Streptococcus mutans	北大
2015	L Sun, R Tan, J Xu, J LaFace, Y Gao, Y Xiao	Nucleic acids Research	Targeted DNA damage at individual telomeres disrupts their integrity and triggers cell death	清华
2015	B Zhang, T Zhang, G Wang, G Wang, W Chi, Q Jiang	PLOS Biology	GSK3β-Dzip1-Rab8 Cascade Regulates Ciliogenesis after Mitosis	北大
2015	Y Li, Z Liu, Y Zhang, QP Su, B Xue	Molecular Oral Microbiology	Live-cell and super-resolution imaging reveal that the distribution of wall-associated protein A is correlated with the cell chain integrity of Streptococcus mutans	北大
2015	Q Lu, C Insinna, C Ott, J Stauffer, PA Pintado, J Rahajeng, U Baxa, V Walia, A Cuenca, Y-S	Nature Cell Biology	Early steps in primary cilium assembly require EHD1/EHD3-dependent ciliary vesicle formation	北大



Prof. Peng Xi, Department of Biomedical
Engineering, College of Engineering,
Peking University

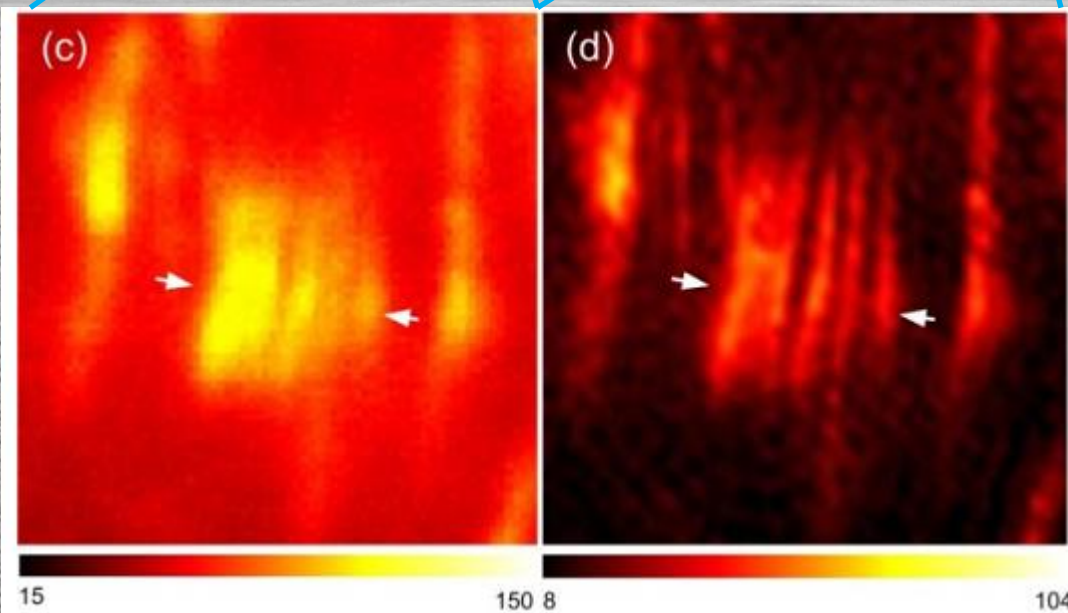
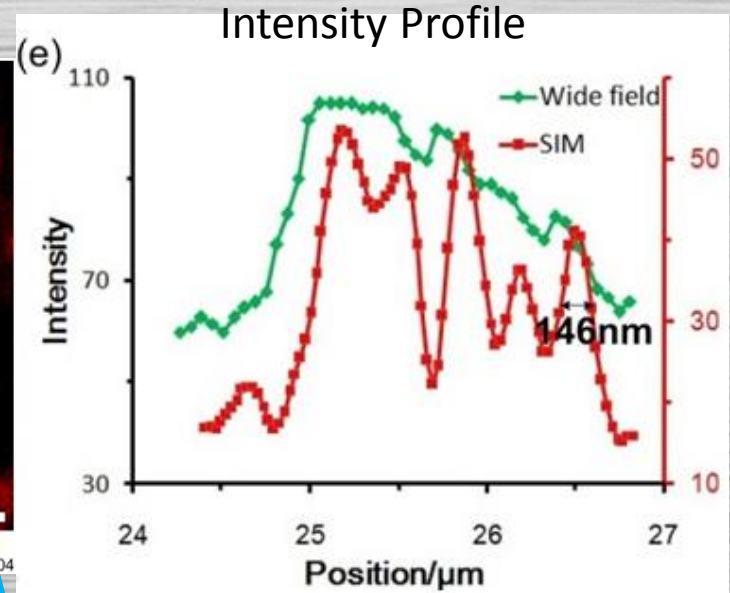
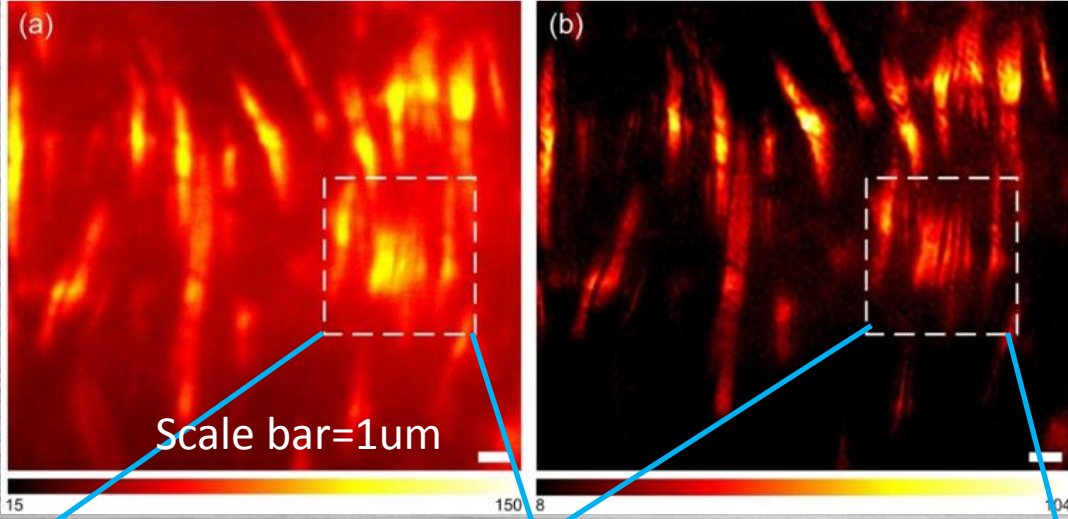
Yang, X.; Tzeng, Y.-K.; Zhu, Z.; Huang, Z.; Chen, X.; Liu, Y.; Chang, H.-C.; Huang, L.; Li, W.-D.; Xi, P., Sub-diffraction imaging of nitrogen-vacancy centers in diamond by stimulated emission depletion and structured illumination. *Rsc Advances* 2014, 4 (22), 11305-11310.

Article link: <http://pubs.rsc.org/en/content/articlelanding/2014/ra/c3ra47240j>

Sample: cryostat section of mouse kidney

Wide Field

SIM



White arrows In (C) and (D)

Nikon's CFI Apo TIRF 100X N.A. 1.49

Dye: Alexa Fluor 568 phalloidin

Excitation: 561 nm

Research object: filamentous actin in the Brush boarder of the mouse kidney



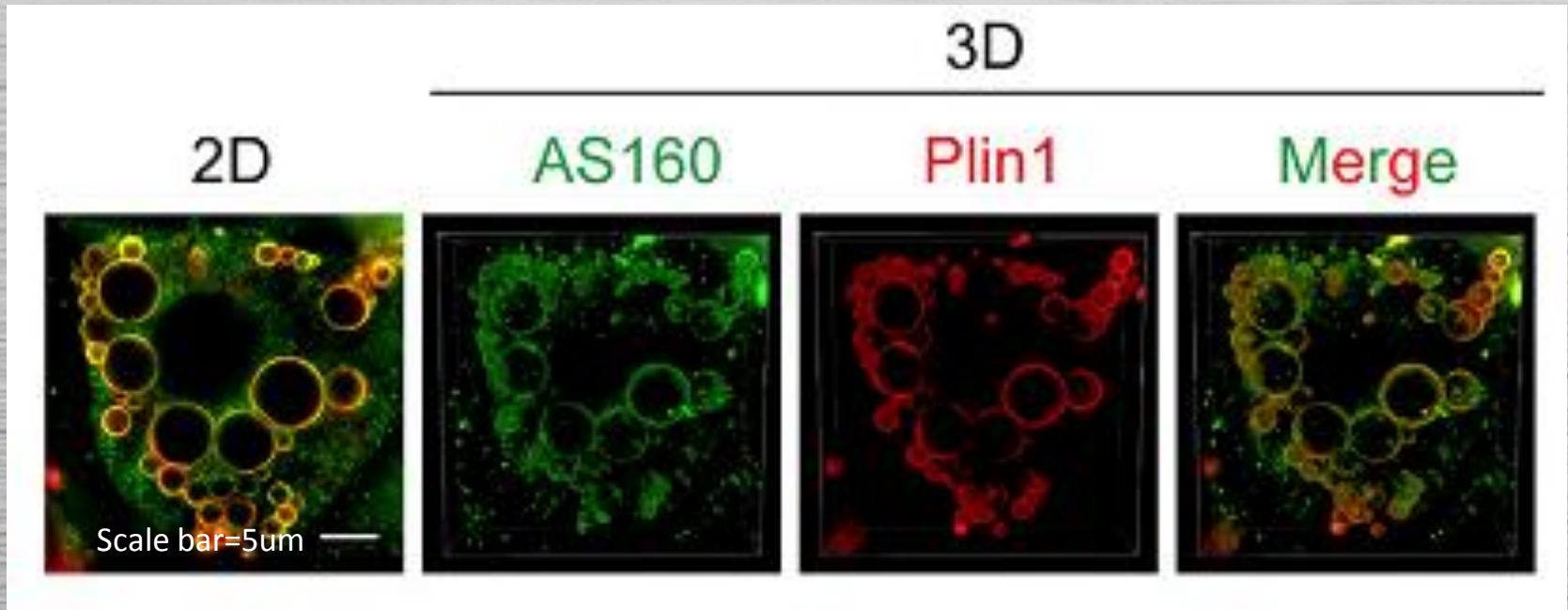
Prof. Li Peng, MOE Key Laboratory of Bioinformatics and Tsinghua-Peking Center for Life Sciences, School of Life Sciences, Tsinghua University

Wu, L.; Xu, D.; Zhou, L.; Xie, B.; Yu, L.; Yang, H.; Huang, L.; Ye, J.; Deng, H.; Yuan, Y. A.; Chen, S.; Li, P., Rab8a-AS160-MSS4 Regulatory Circuit Controls Lipid Droplet Fusion and Growth. *Developmental Cell* 2014, 30 (4), 378-393.

Article link: <http://www.sciencedirect.com/science/article/pii/S153458071400447X>

Sample: 3T3-L1 adipocytes (脂肪细胞) associated with LDs (Lipid droplets).

Nikon



AS160: a protein, GFP; Plin1 (perilipin):mcherry

3D-images were acquired on an N-SIM using a X100 oil-immersion lens (numerical aperture 1.49) by Z-stack scanning at 0.2 μm intervals.

Endogenous AS160 was associated with LDs in mature adipocytes and in preadipocytes expressing Fsp27.



谢谢！



清华图像中心N-SIM超分辨率显微镜

