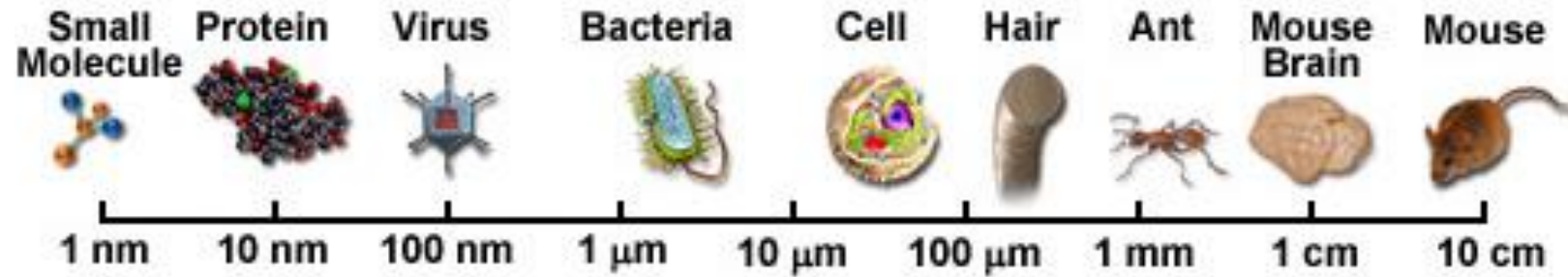


# Superando el limite de la difracción Microscopia de superresolucion a nanoescala

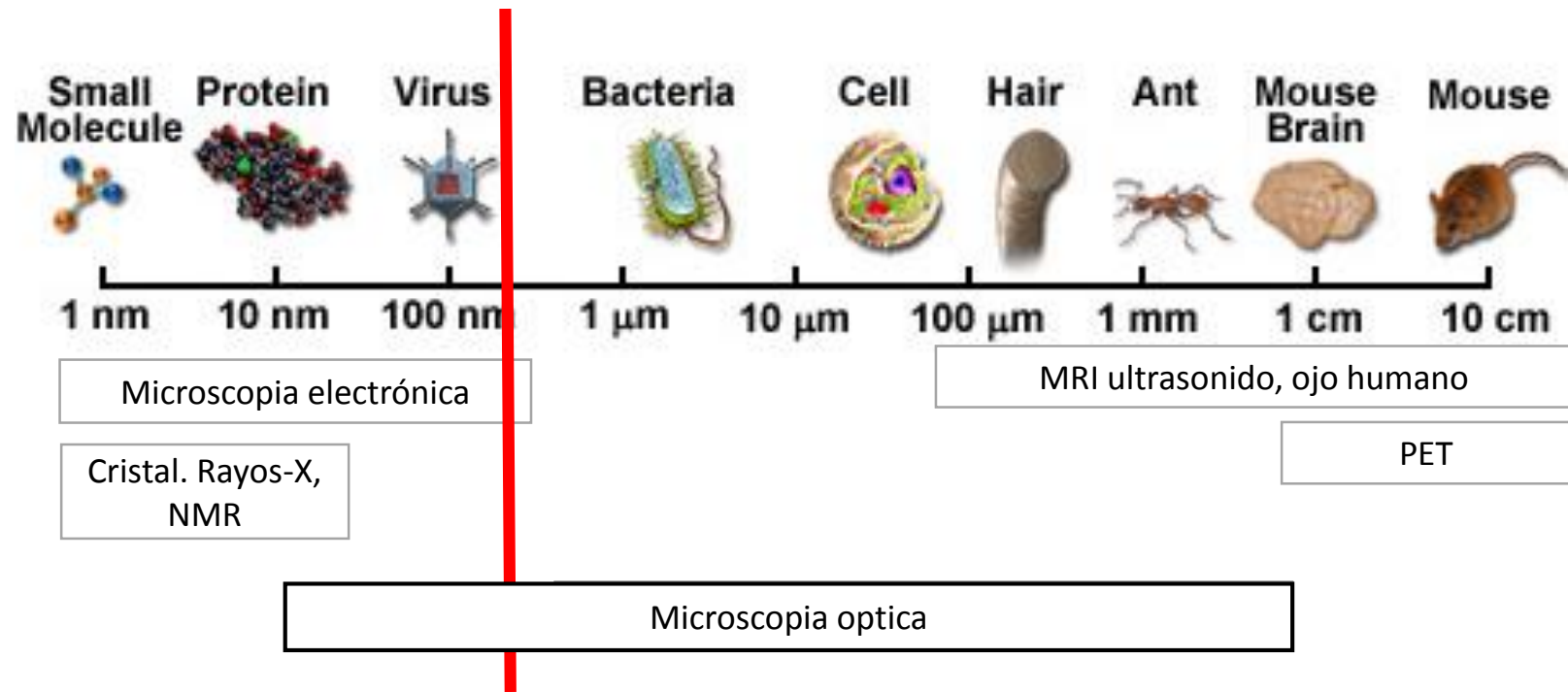
**Jorge Toledo, PHD(c)**

Laboratory of Scientific Image Analysis (SCIAN-Lab)  
Biomedical Neuroscience Institute (BNI)  
Anatomy and Developmental Biology Program,  
Facultad de Medicina; Universidad de Chile

# Resolución espacial de técnicas de imágenes biológicas

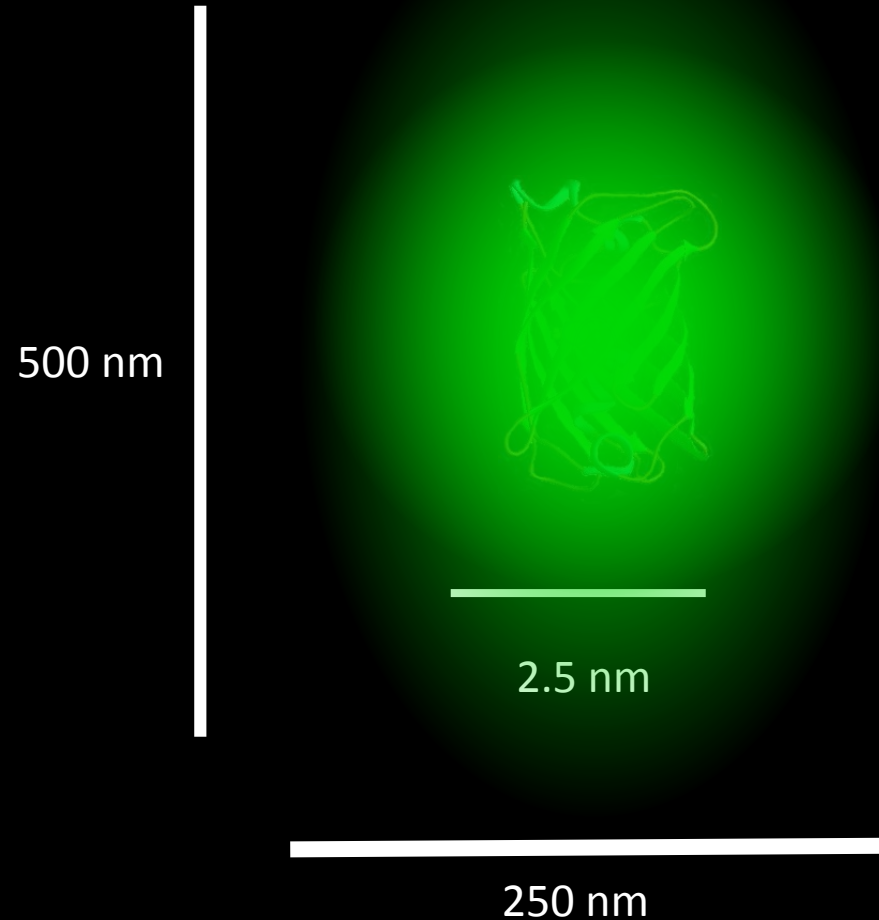


# Resolución espacial de técnicas de imágenes biológicas



Limite de la resolución

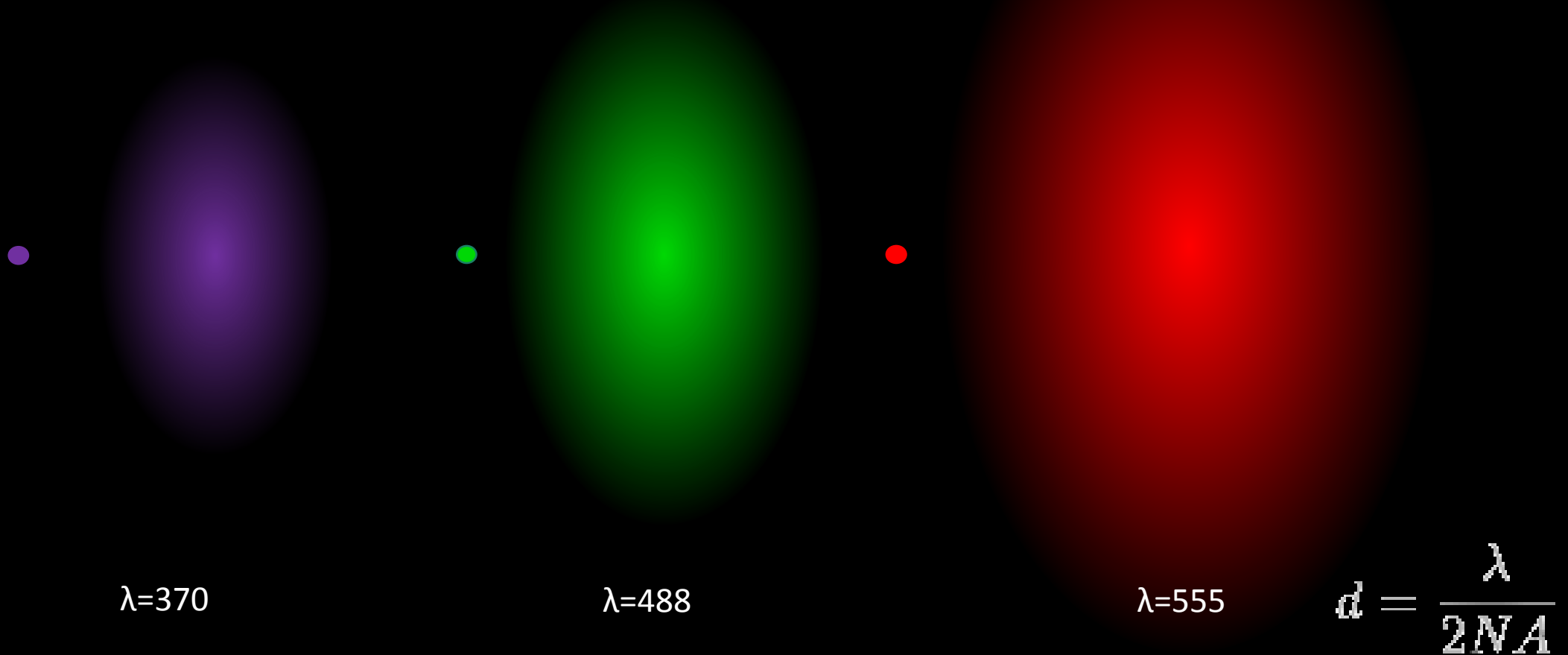
# Limite de difraccion



PSF  
Función de dispersión de la luz  
(*Point spread function*)

$$d = \frac{\lambda}{2NA}$$

# Limite de difracción



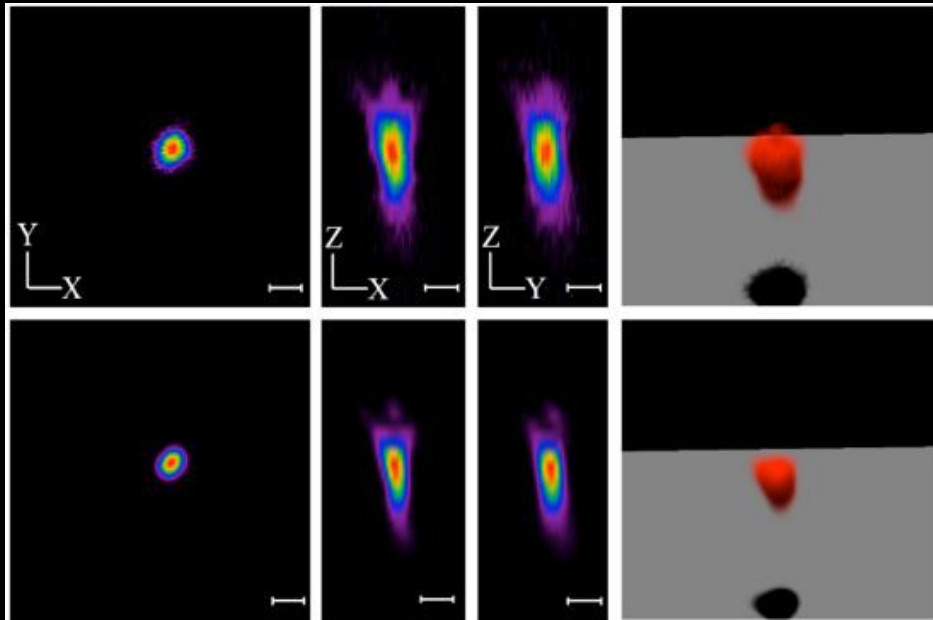
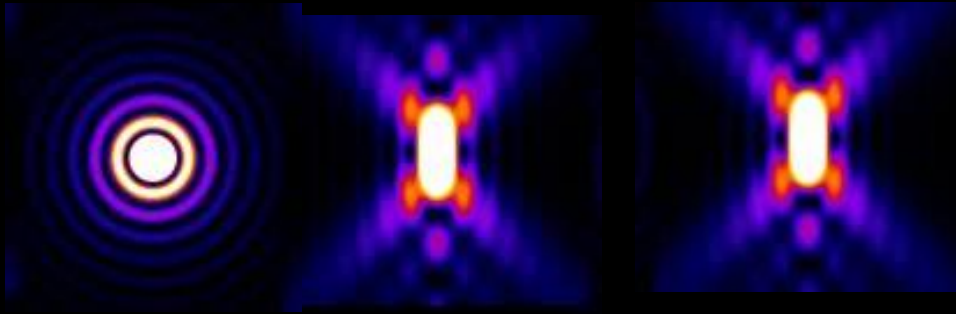
$\lambda=370$

$\lambda=488$

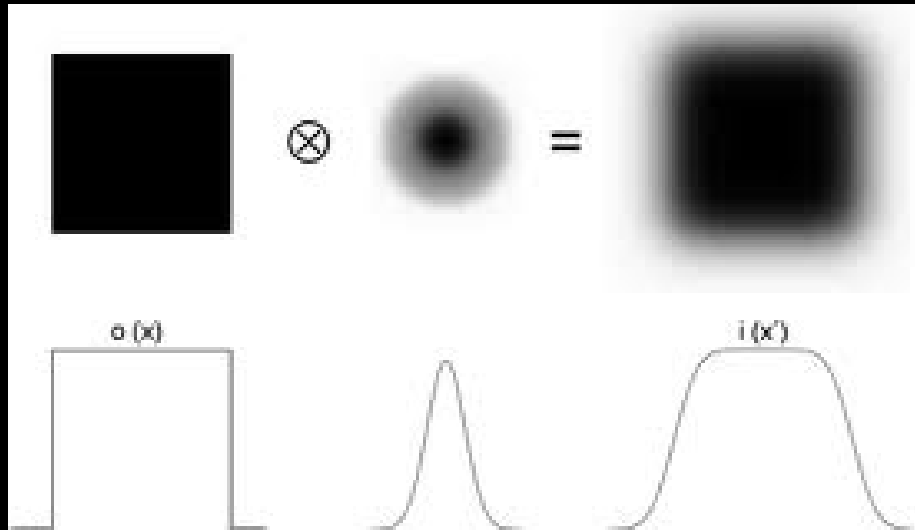
$\lambda=555$

$$d = \frac{\lambda}{2NA}$$

# Deconvolucion

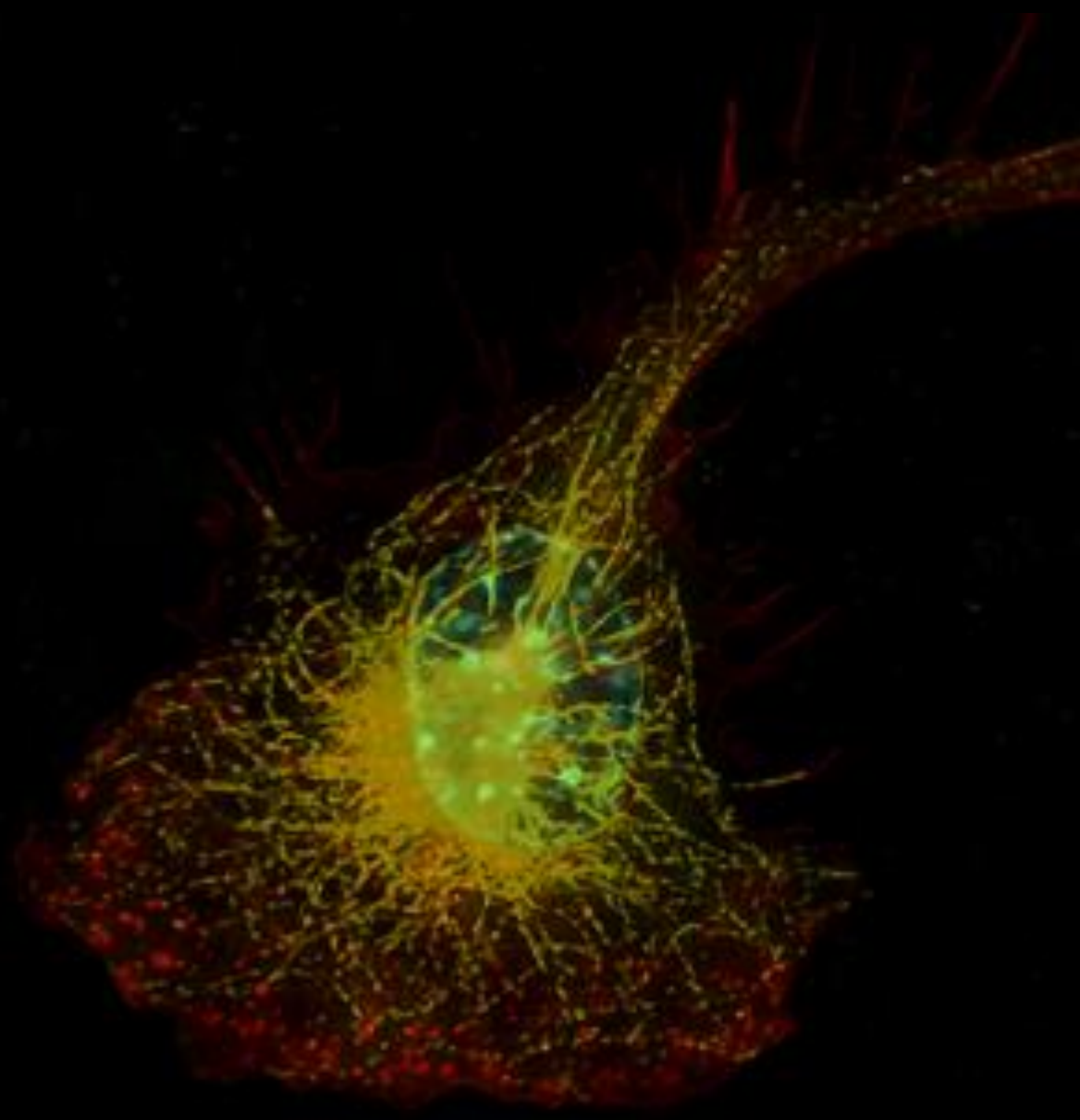
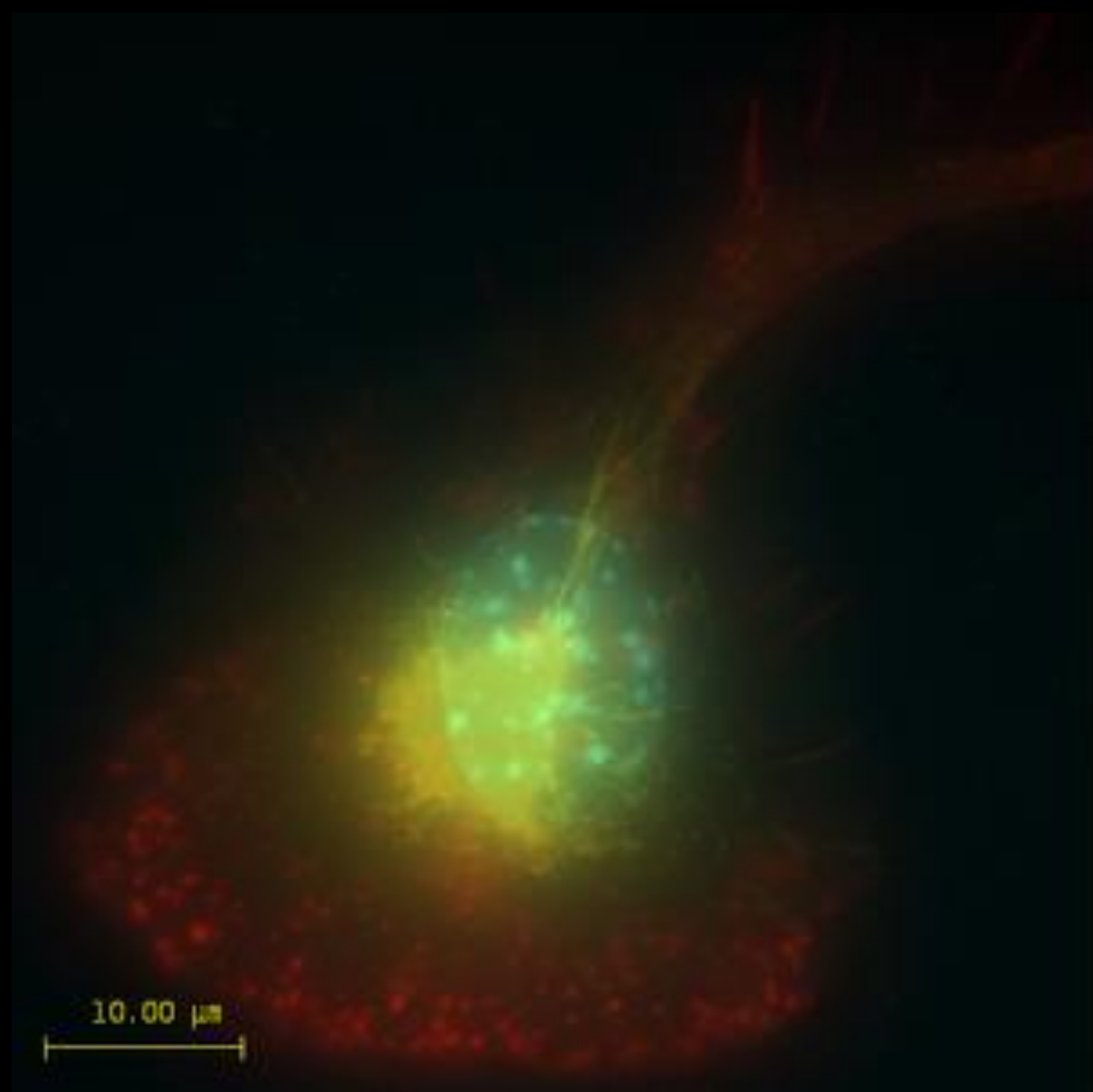


# Deconvolucion



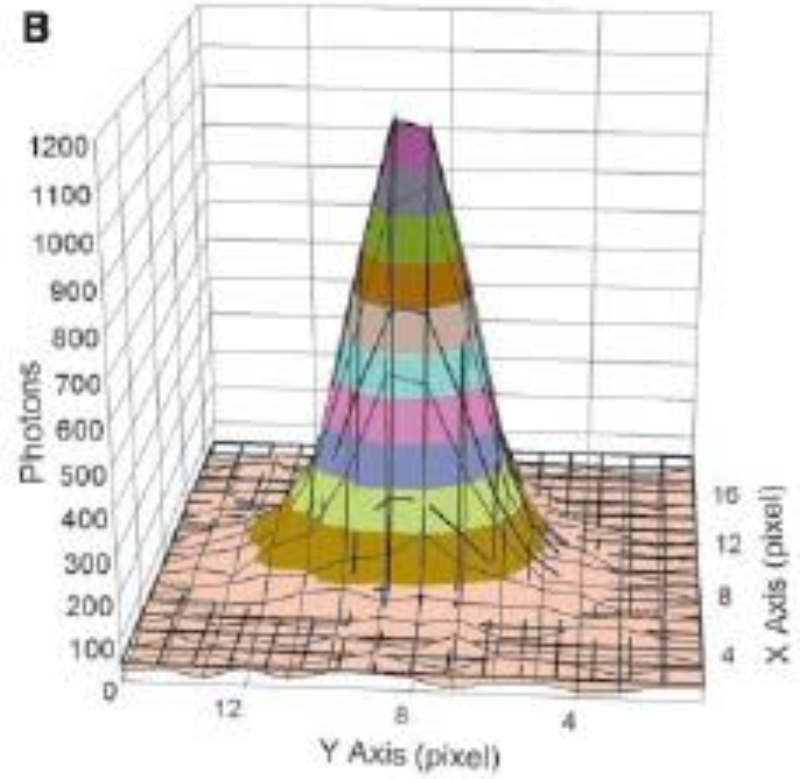
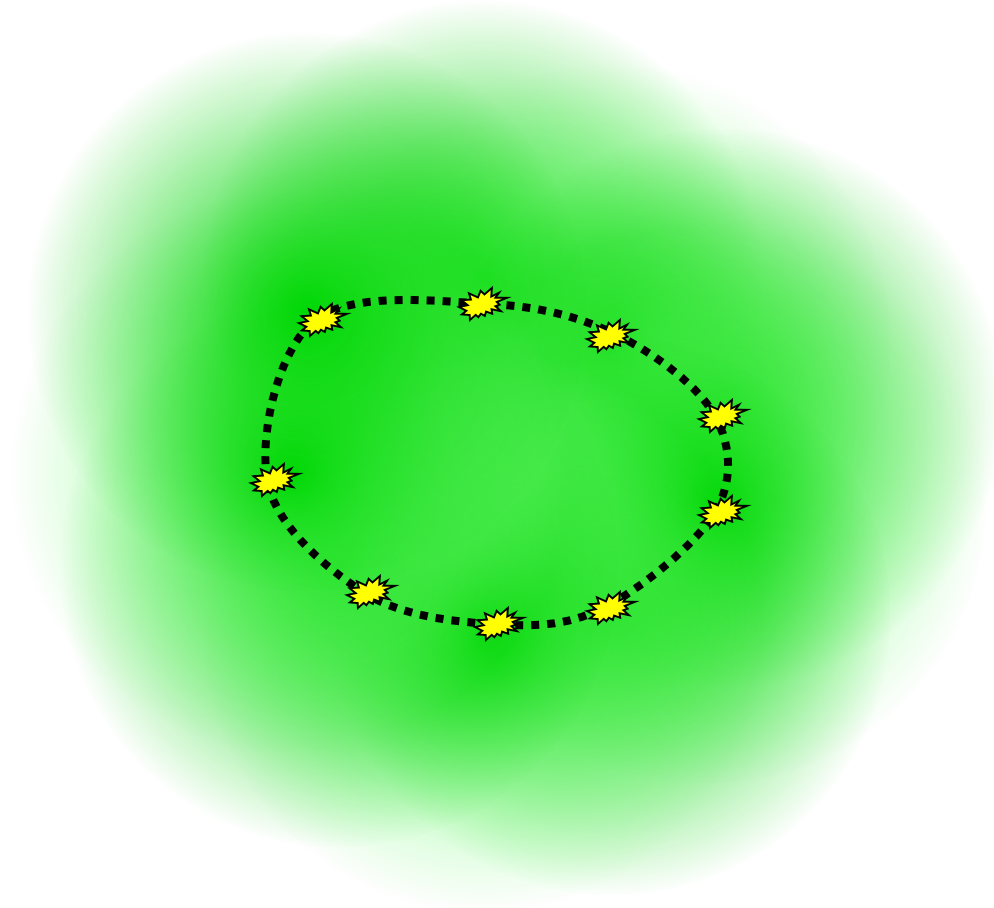
   
Convolucion



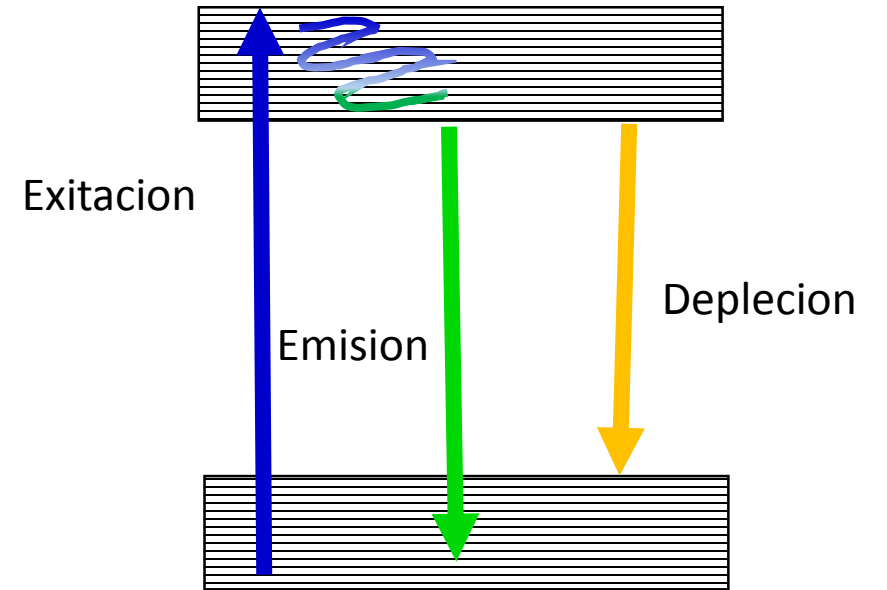
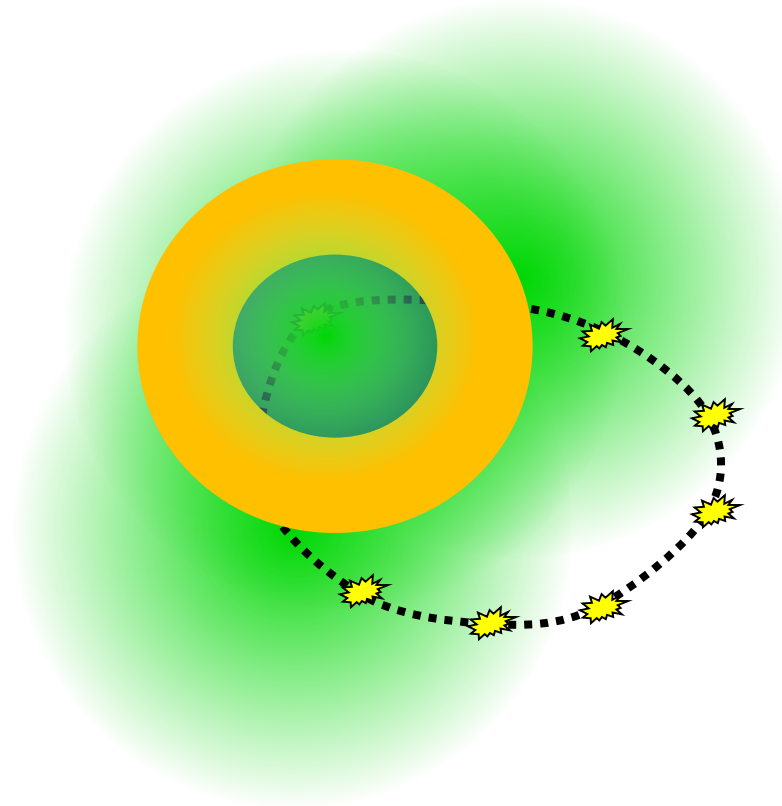




# Resolucion



# STimulated Emission Depletion STED



Hell SW and Wichman J (1994) Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion-microscopy. Opt. Lett. 19:780-782

Hell SW and Kroug M (1995) Ground-state depletion fluorescence microscopy, a concept for breaking the diffraction resolution limit. Appl. Phys. B. 60:495-497.

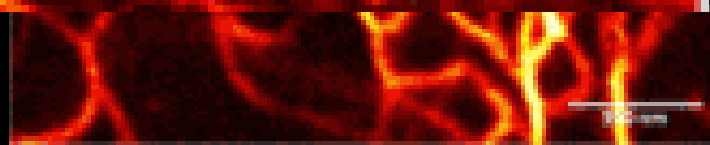
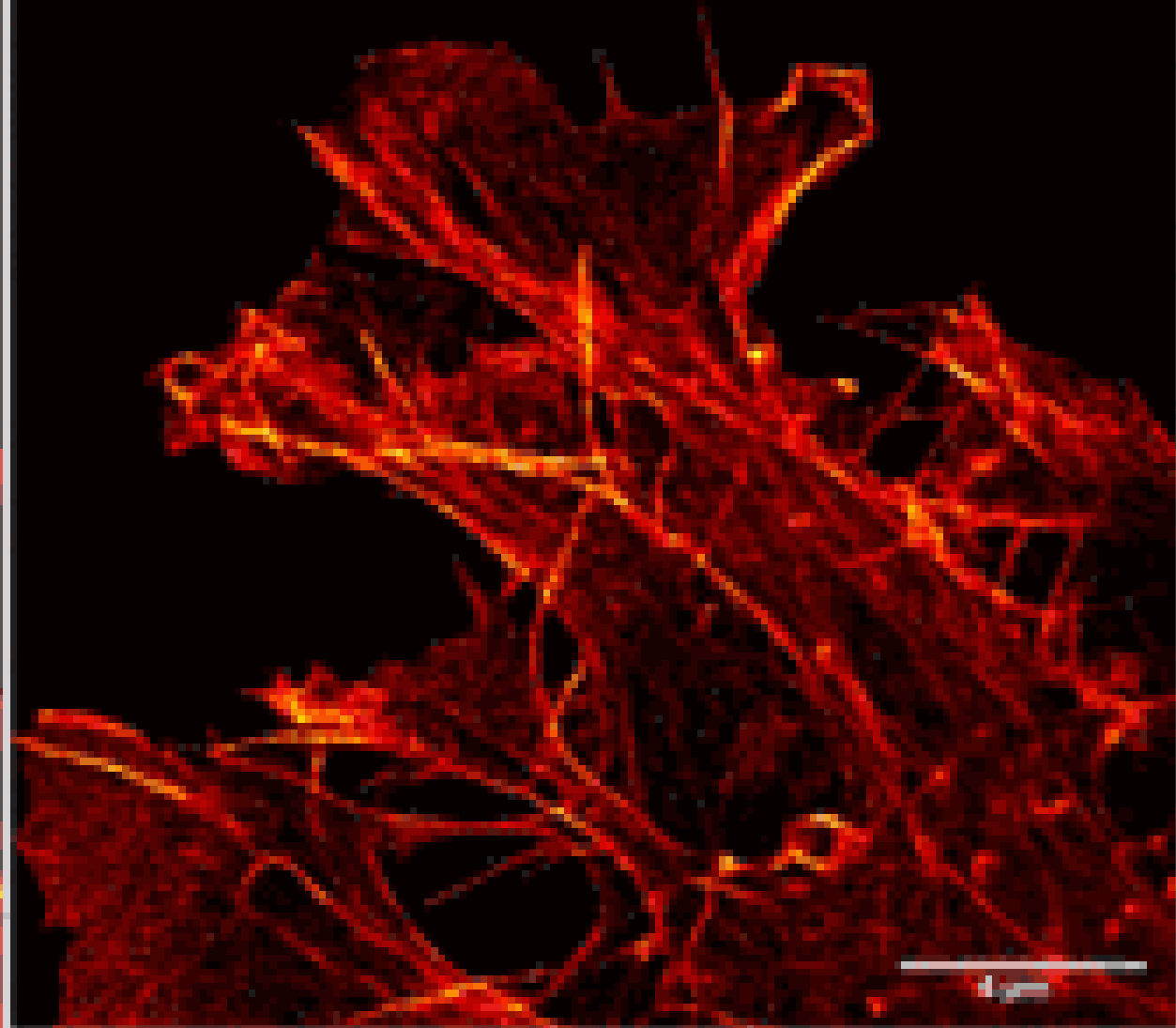
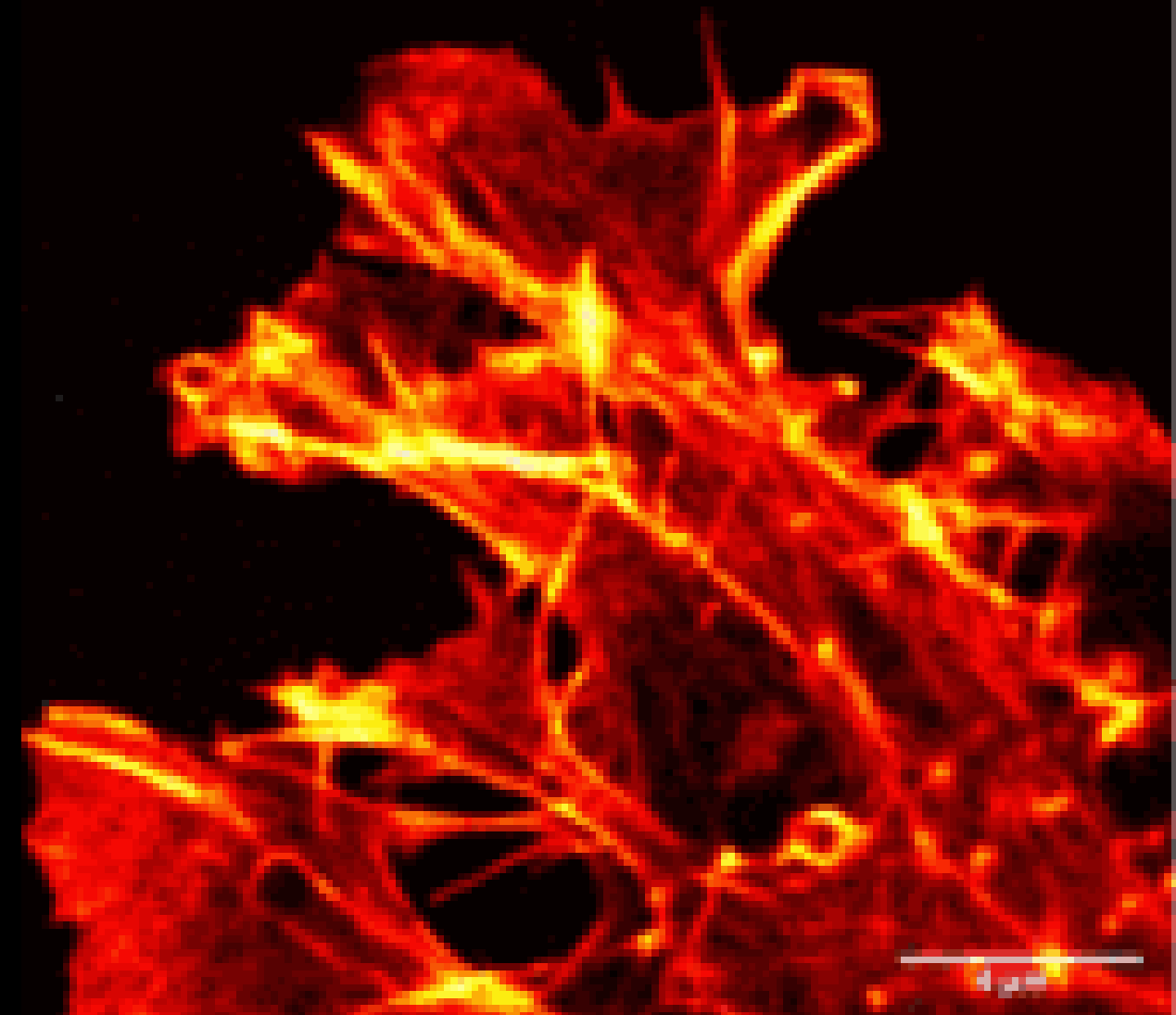
Klar TA, Jakobs S, Dyba M, Egner A and Hell SW (2000) Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission. Proc. Natl. Acad. Sci. USA. 97: 8206-8210

Confocal

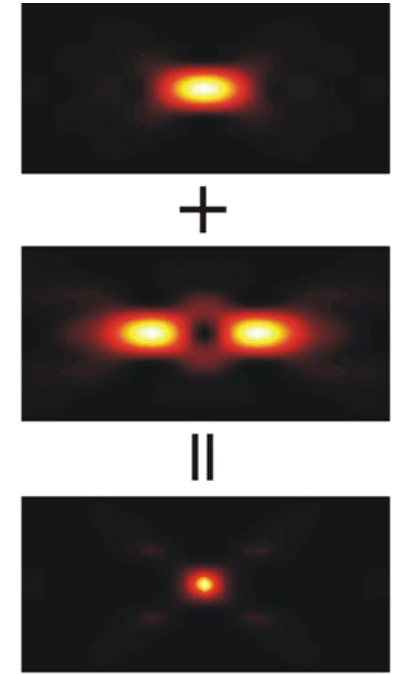
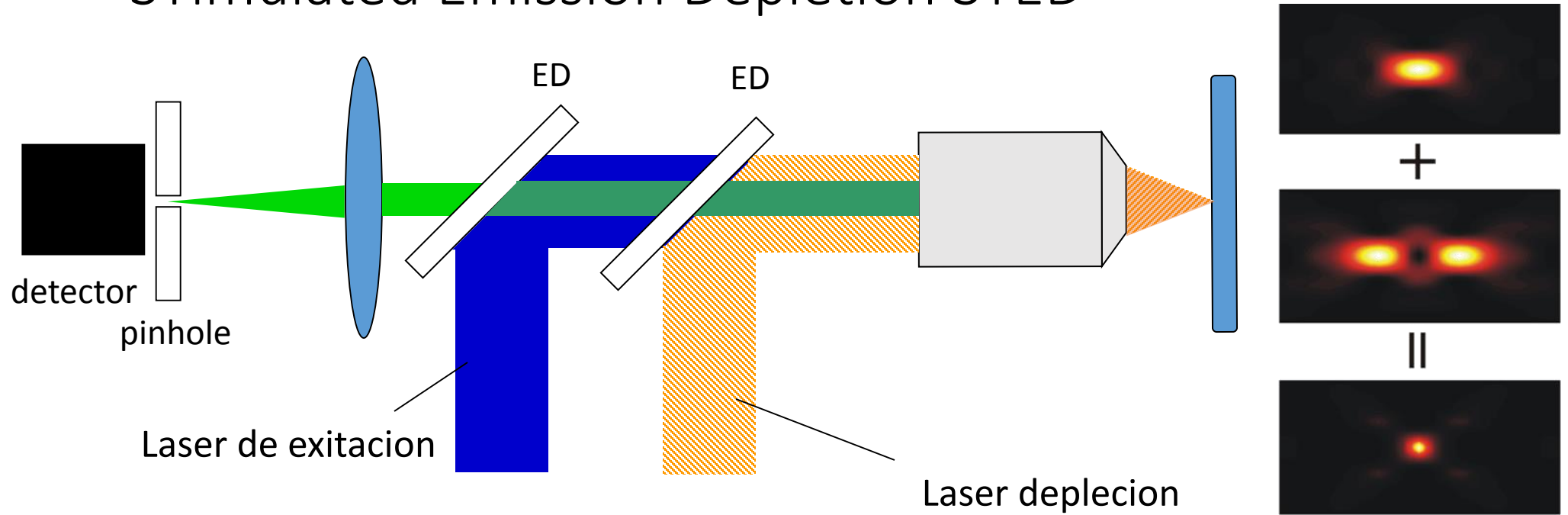
STED

Confocal

STED



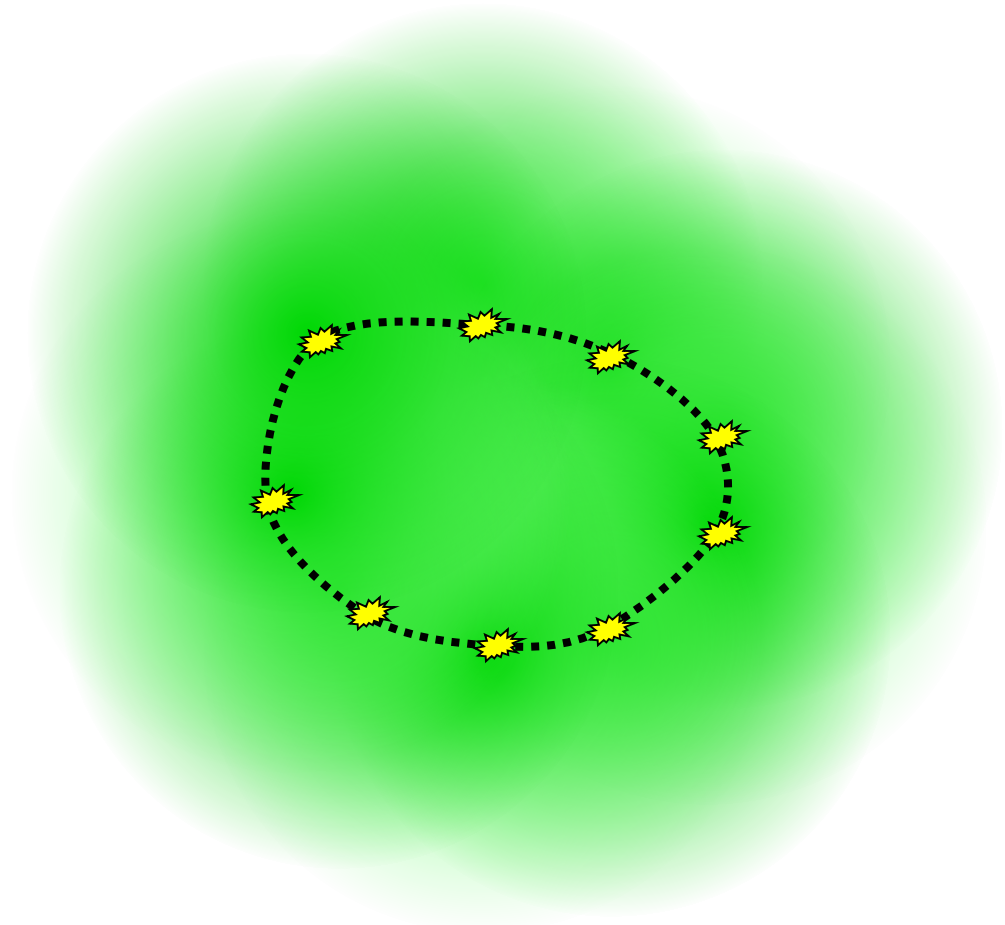
# STimulated Emission Depletion STED



Intensidad de la deplecion incrementa la resolucion

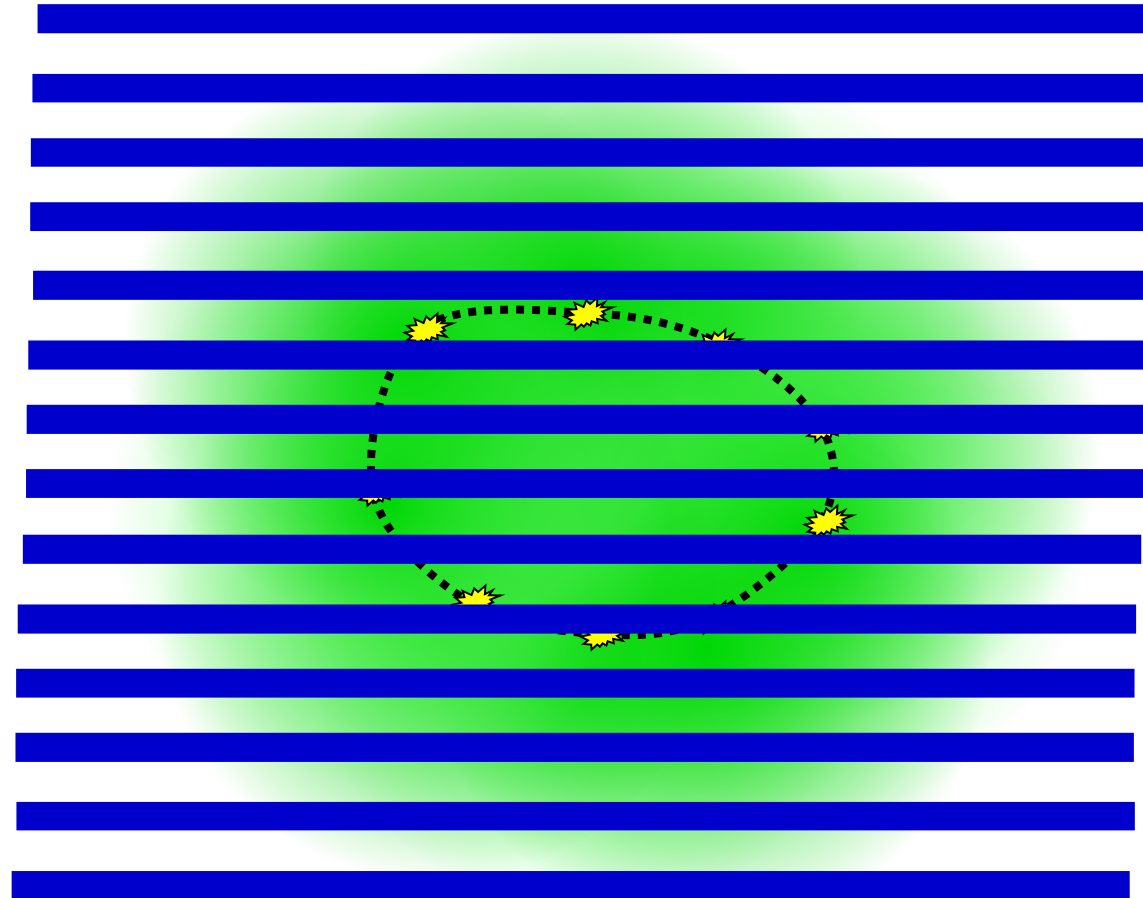
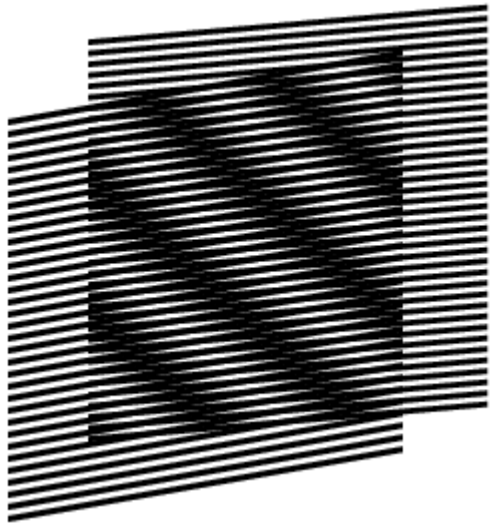


# Resolucion

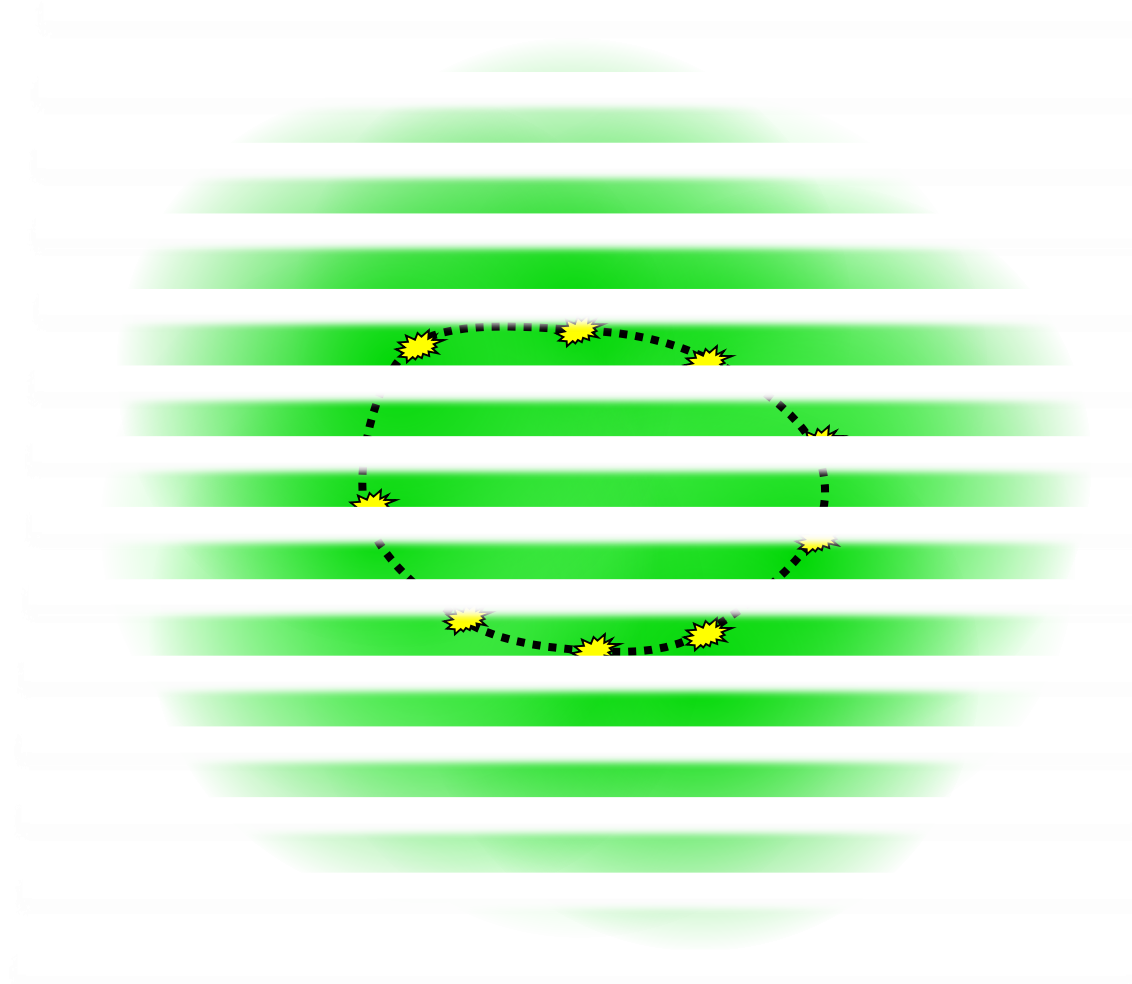


# Iluminacion estructurada (SIM)

Moiré patterns



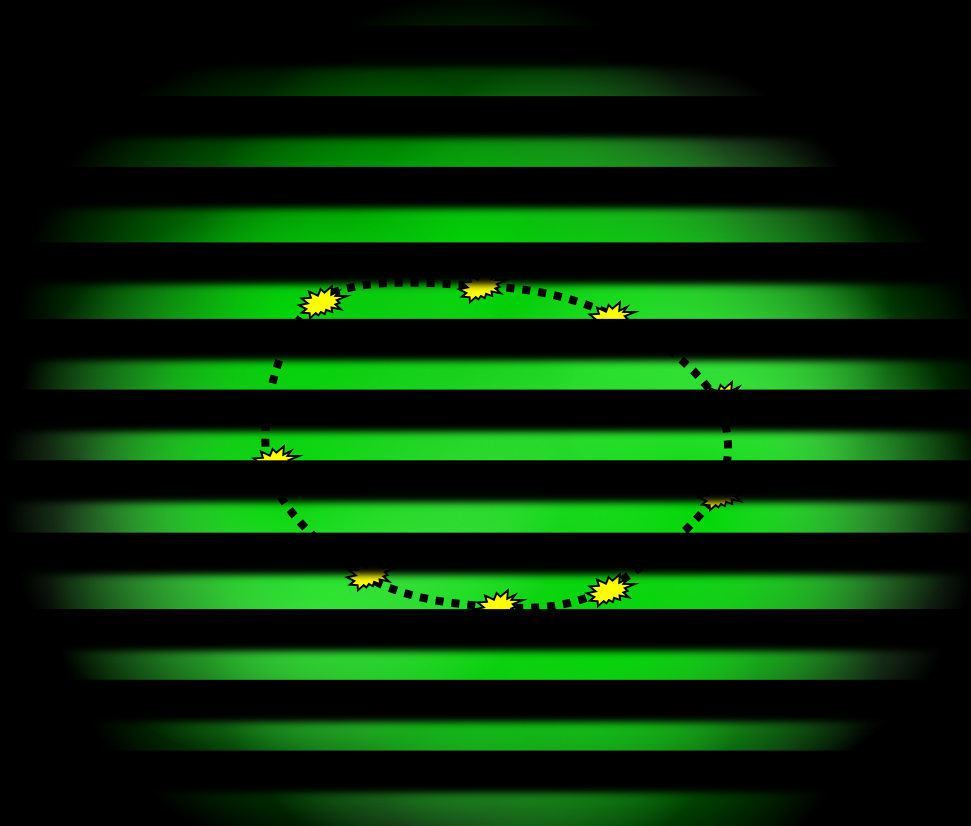
# Iluminacion estructurada



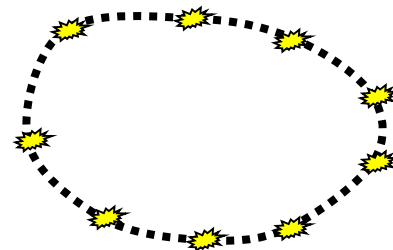
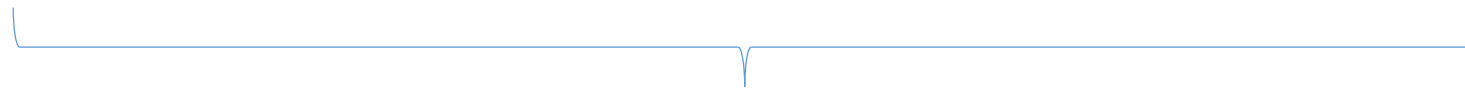
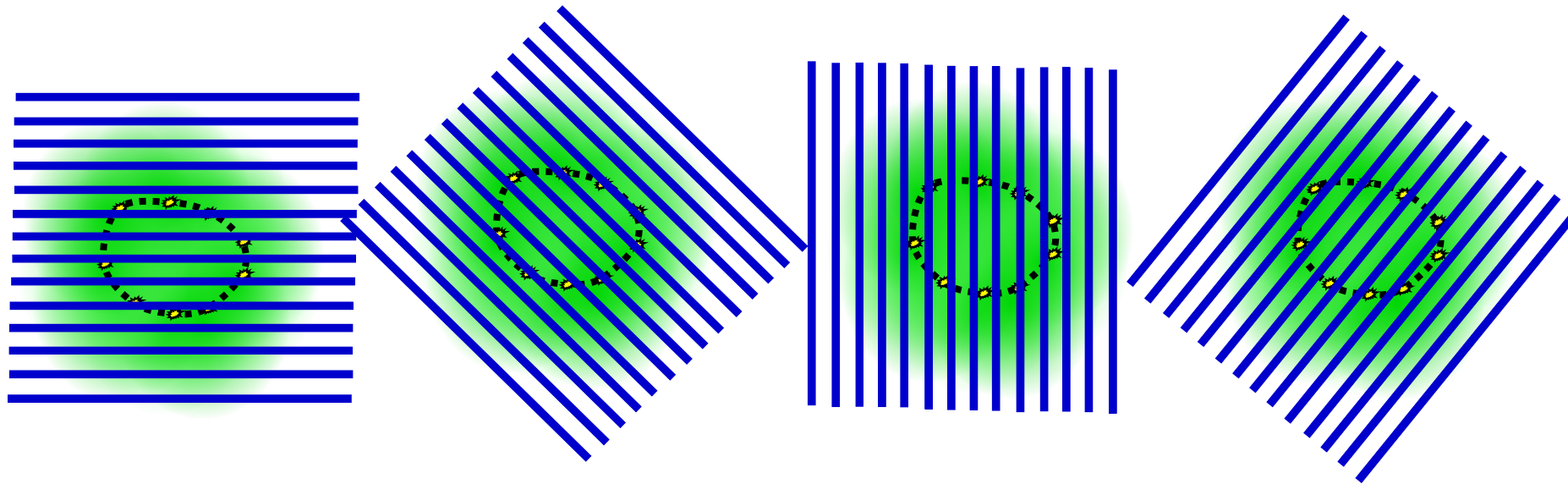
Gustafsson MG (2000)



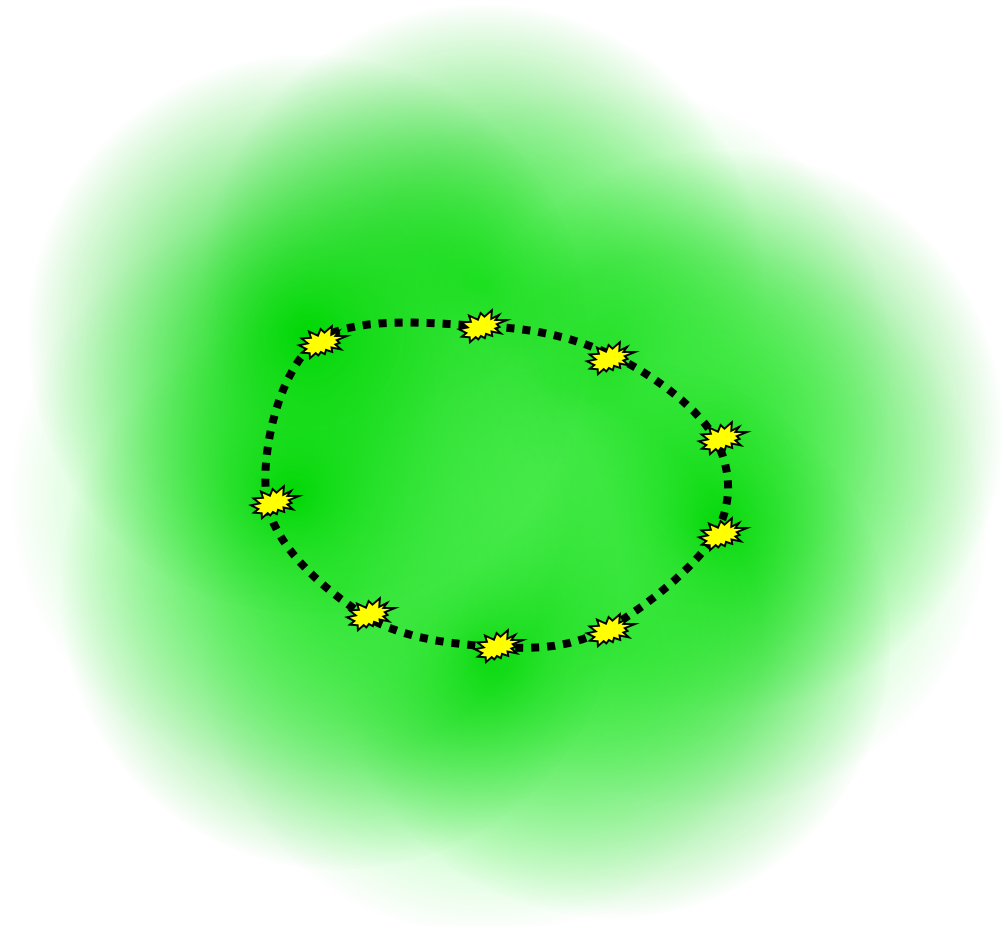
# Iluminacion estructurada



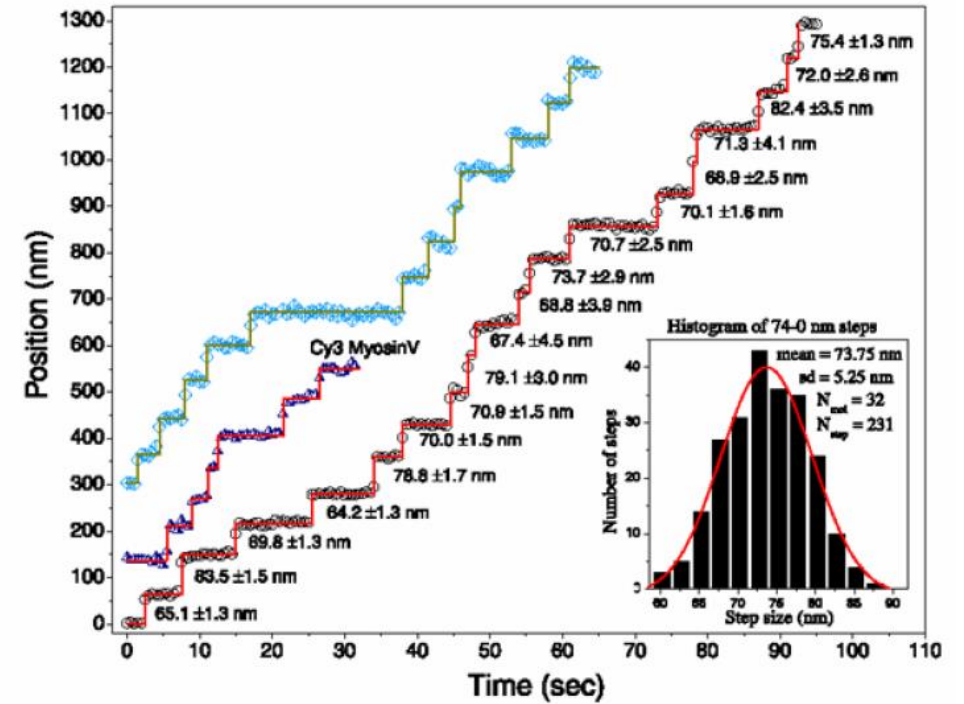
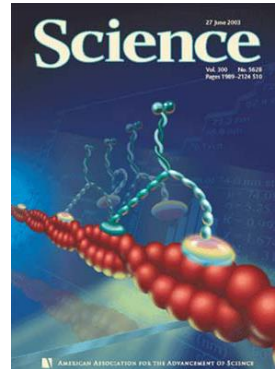
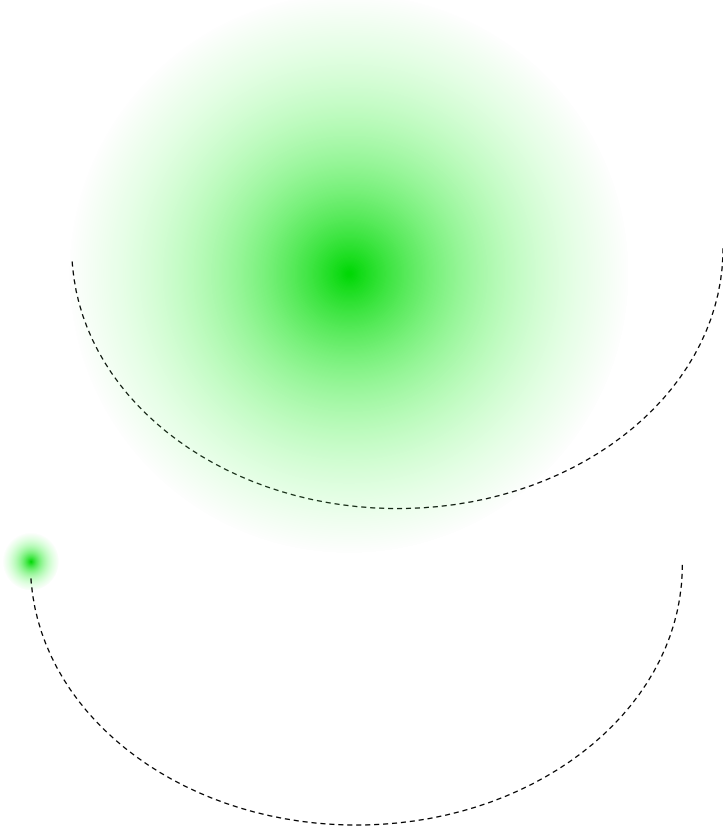
# Iluminacion estructurada



# Resolucion



# Seguimiento de una unica molecula

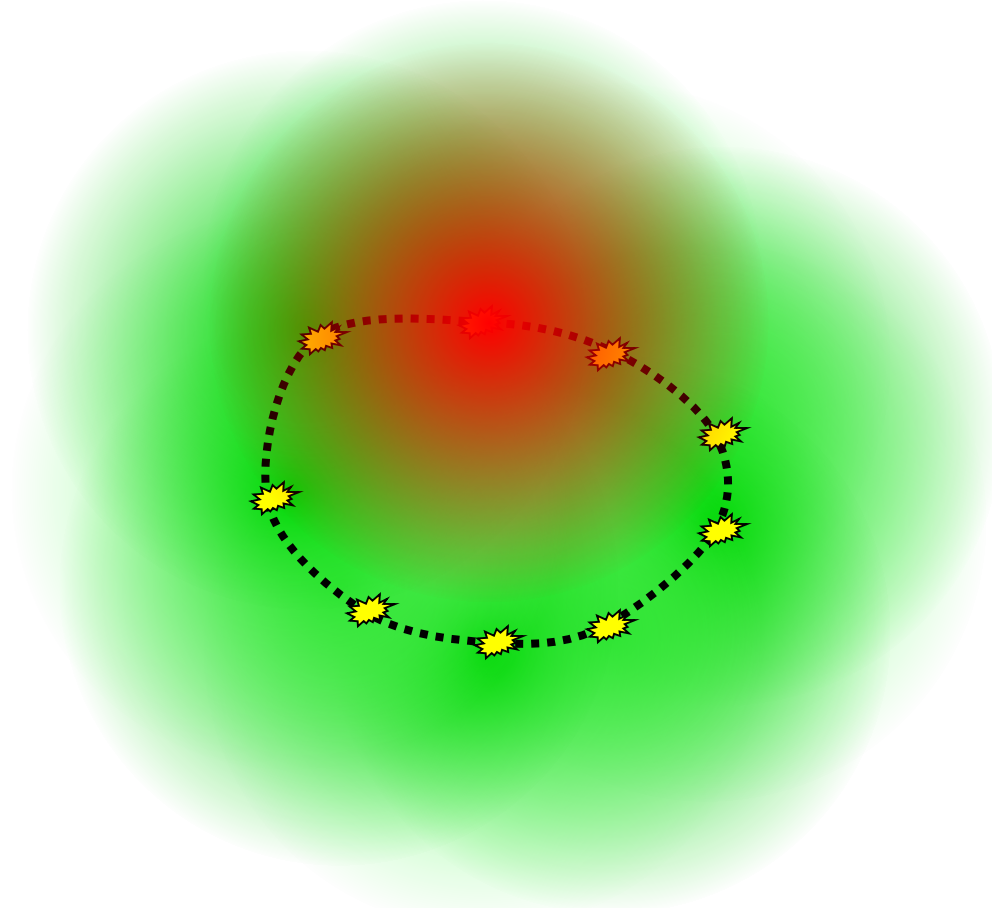


Velocidad de una miosina sobre un filament de actina

# Microscopia de super resolución mediante Localización de moléculas únicas

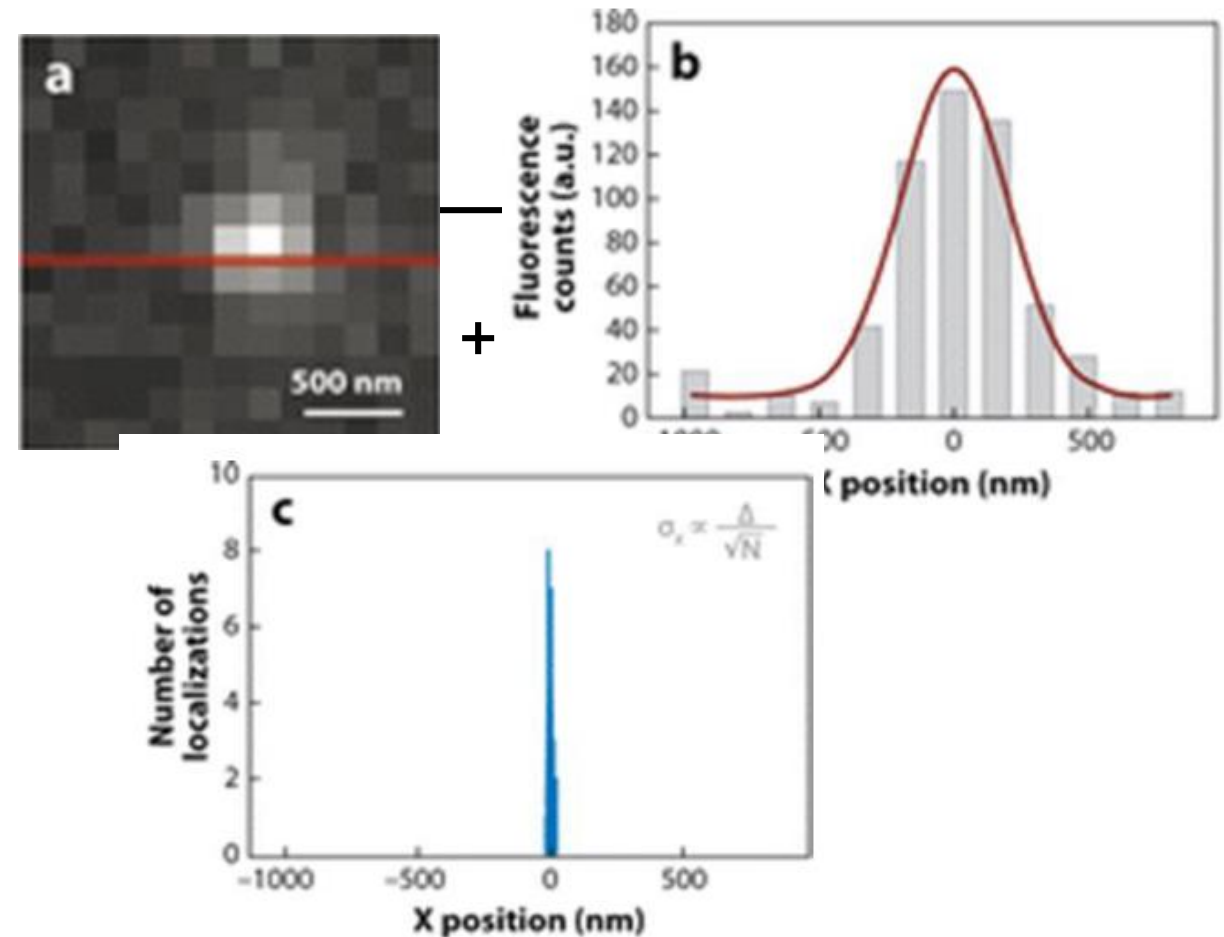
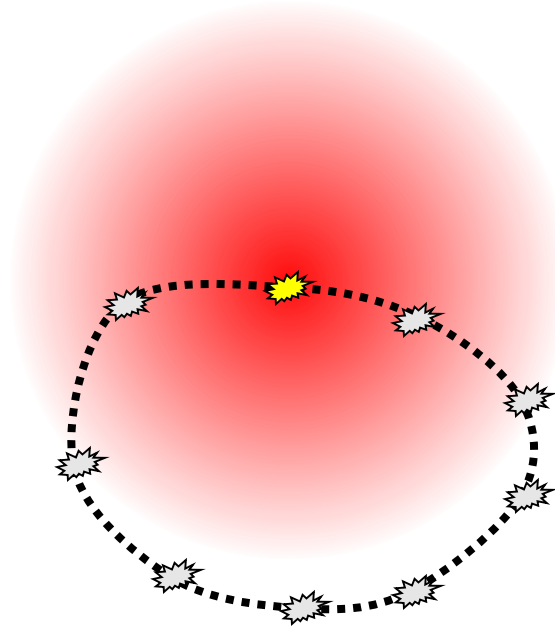
**Photo-activated localization microscopy (PALM)**

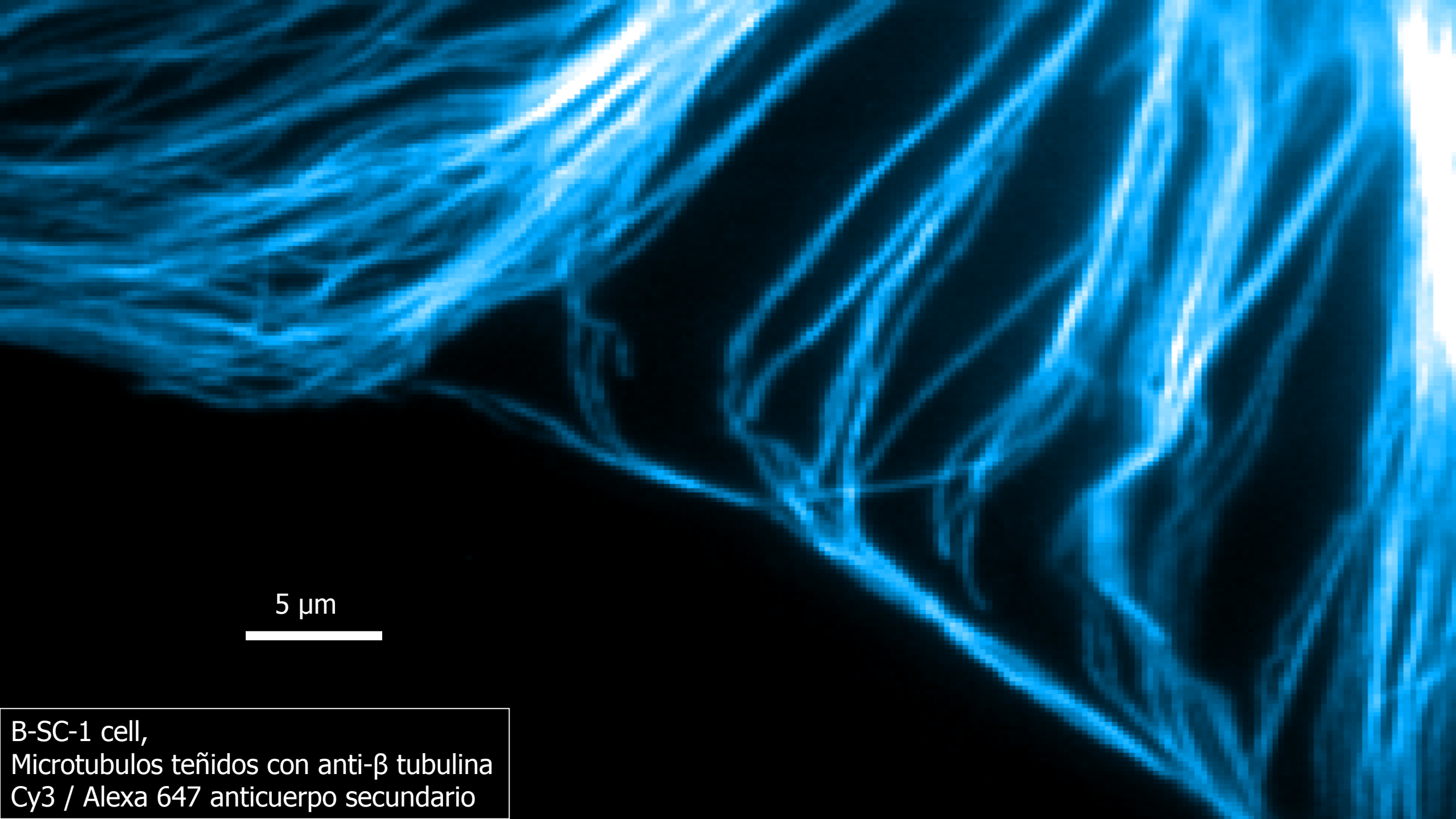
stochastic optical reconstruction microscopy (STORM)



photoactivatable GFP (**PA-GF Kaede**), (Patterson and Lippincott-Schwartz, 2002)

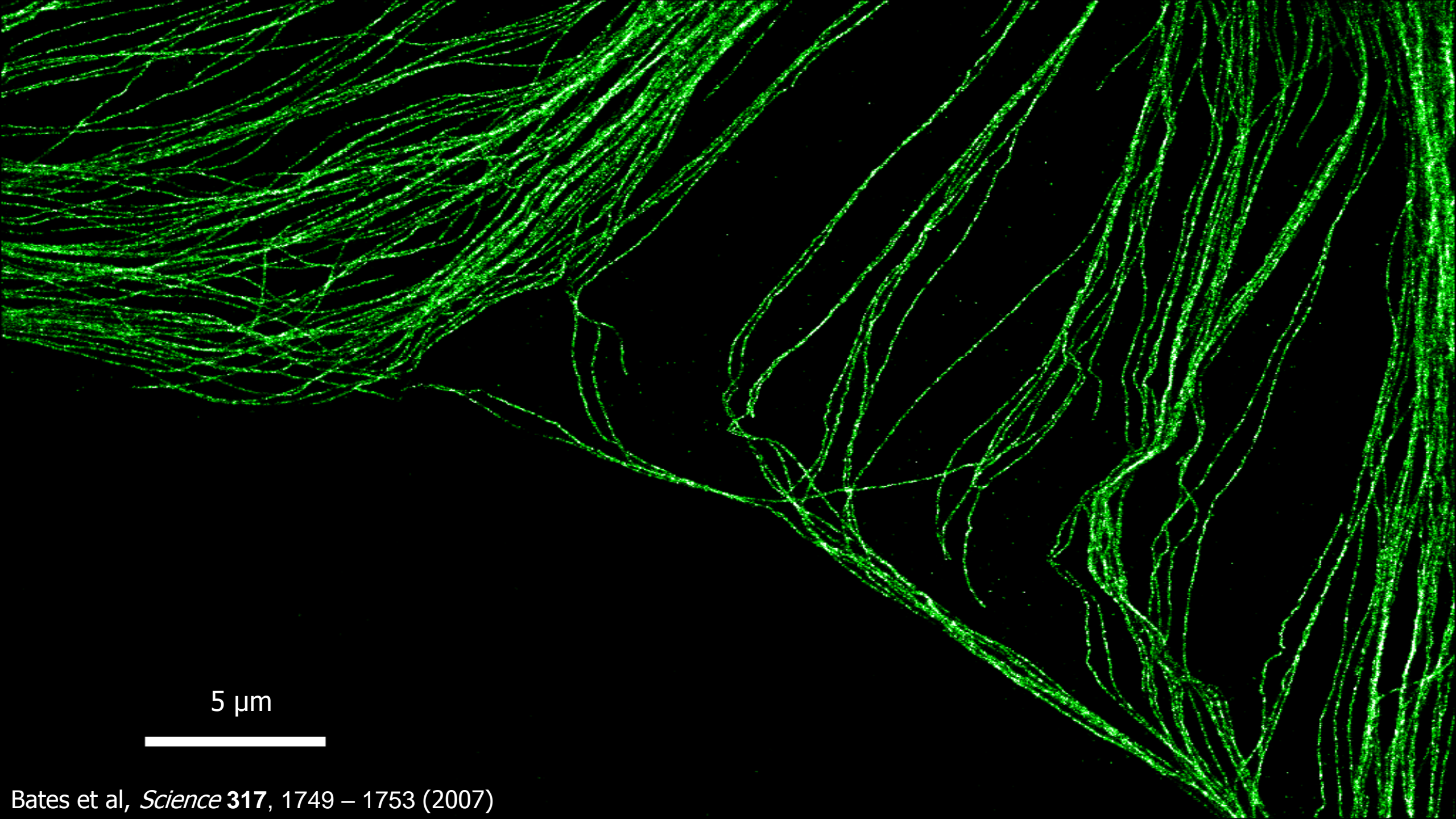
# Microscopia de super resolución mediante Localización de moléculas únicas





5  $\mu\text{m}$

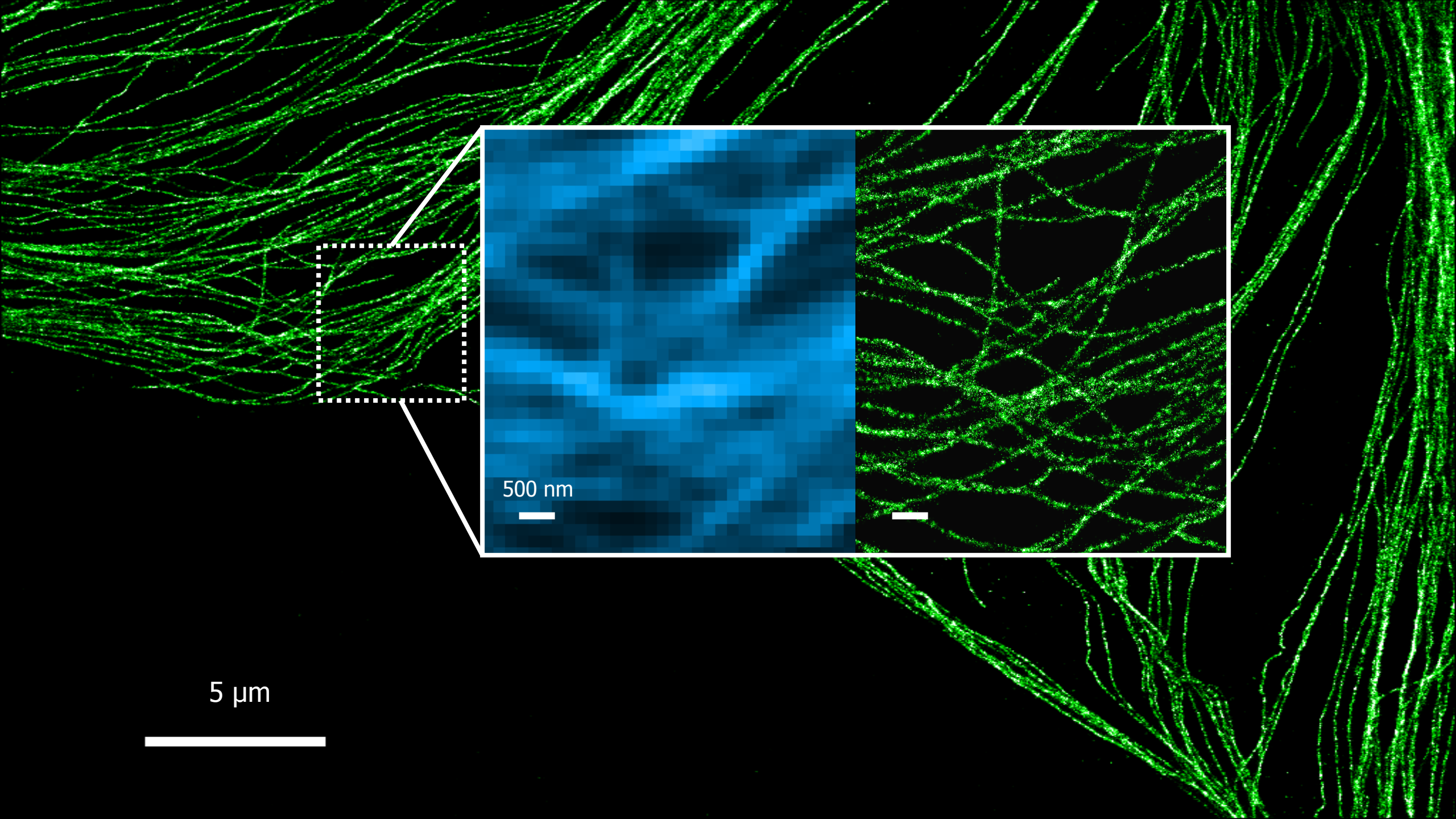
B-SC-1 cell,  
Microtubulos teñidos con anti- $\beta$  tubulina  
Cy3 / Alexa 647 anticuerpo secundario

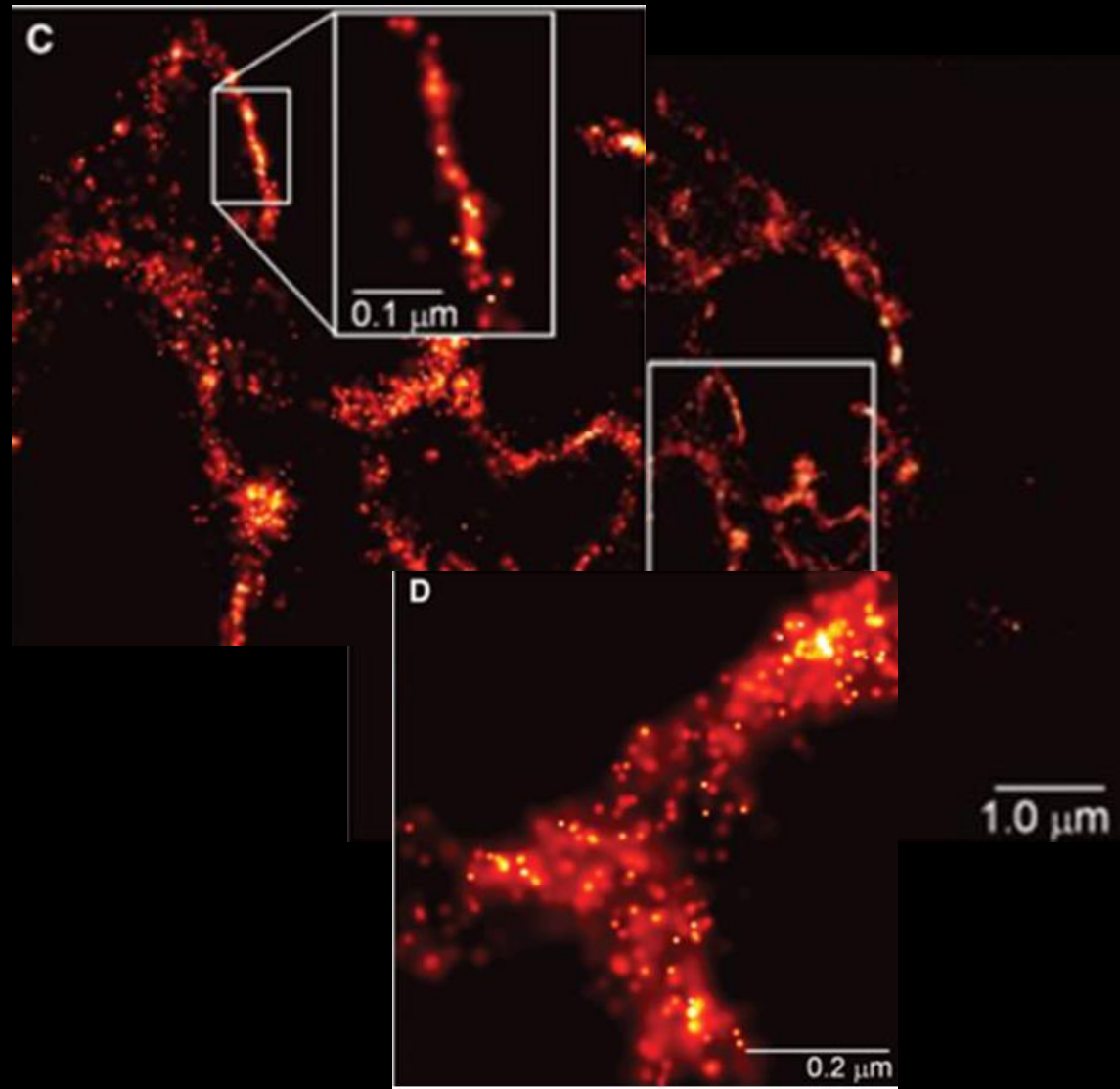
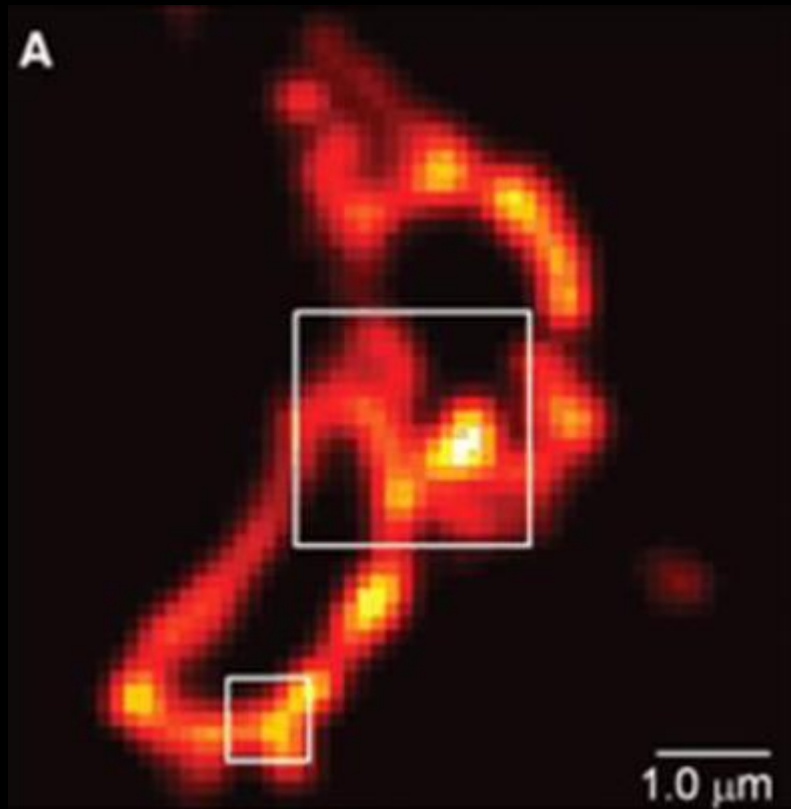


5  $\mu\text{m}$

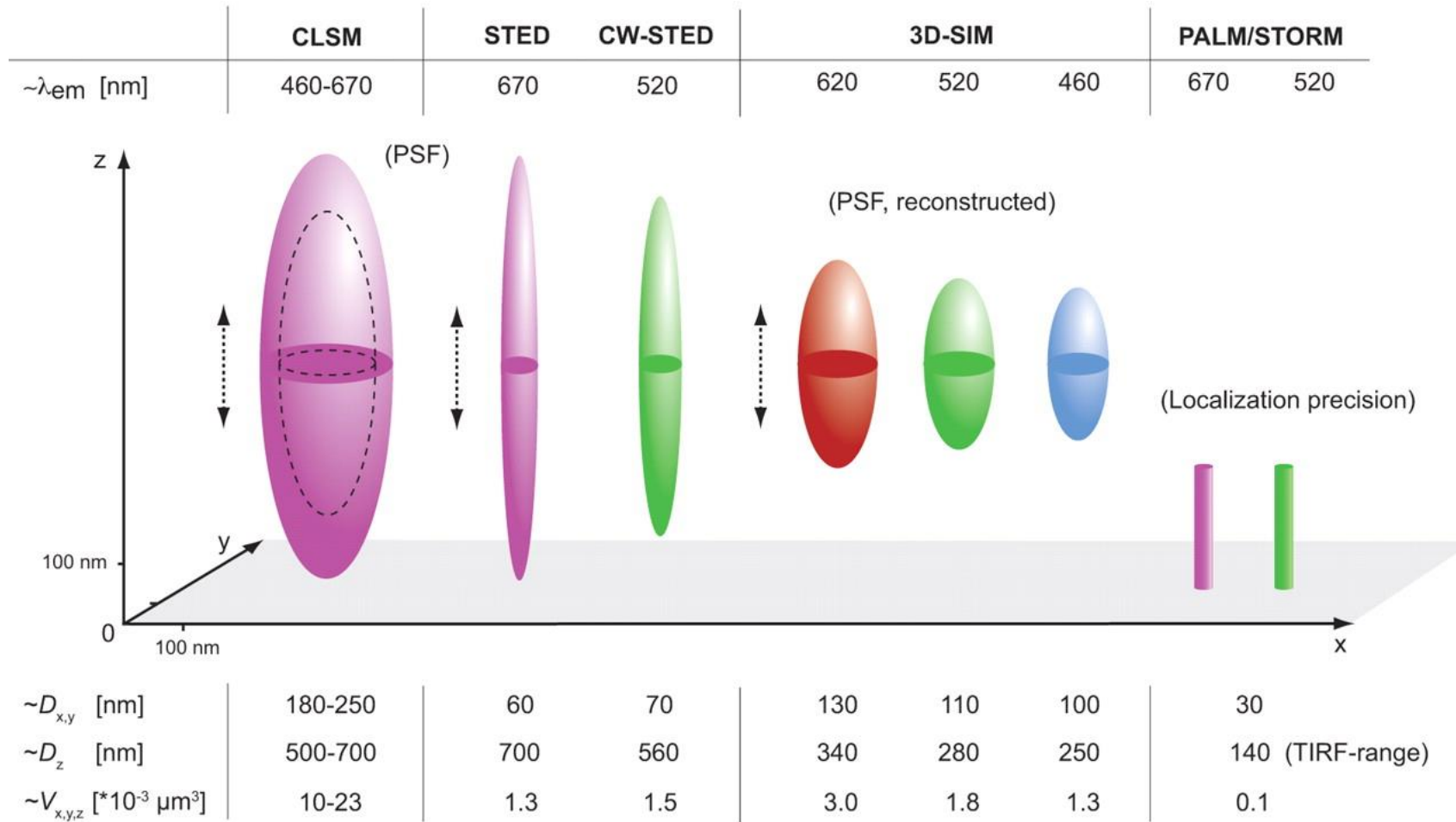






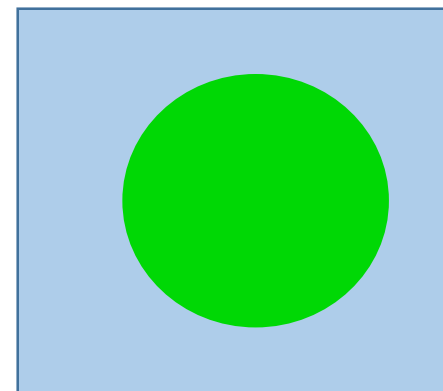
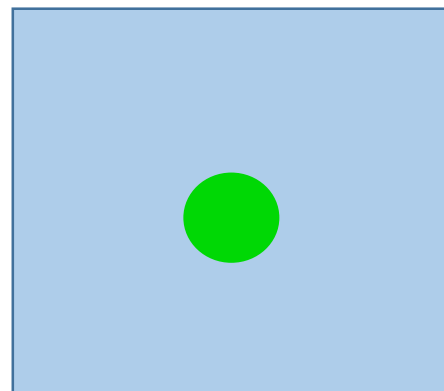
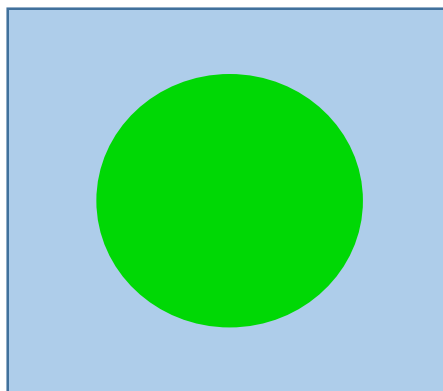
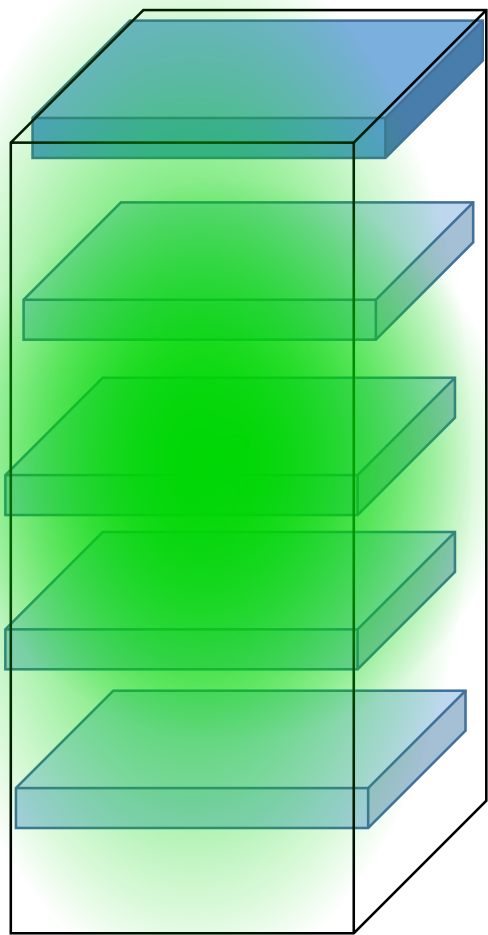


# Volumenes de la PSF con diferentes sistemas de superresolución

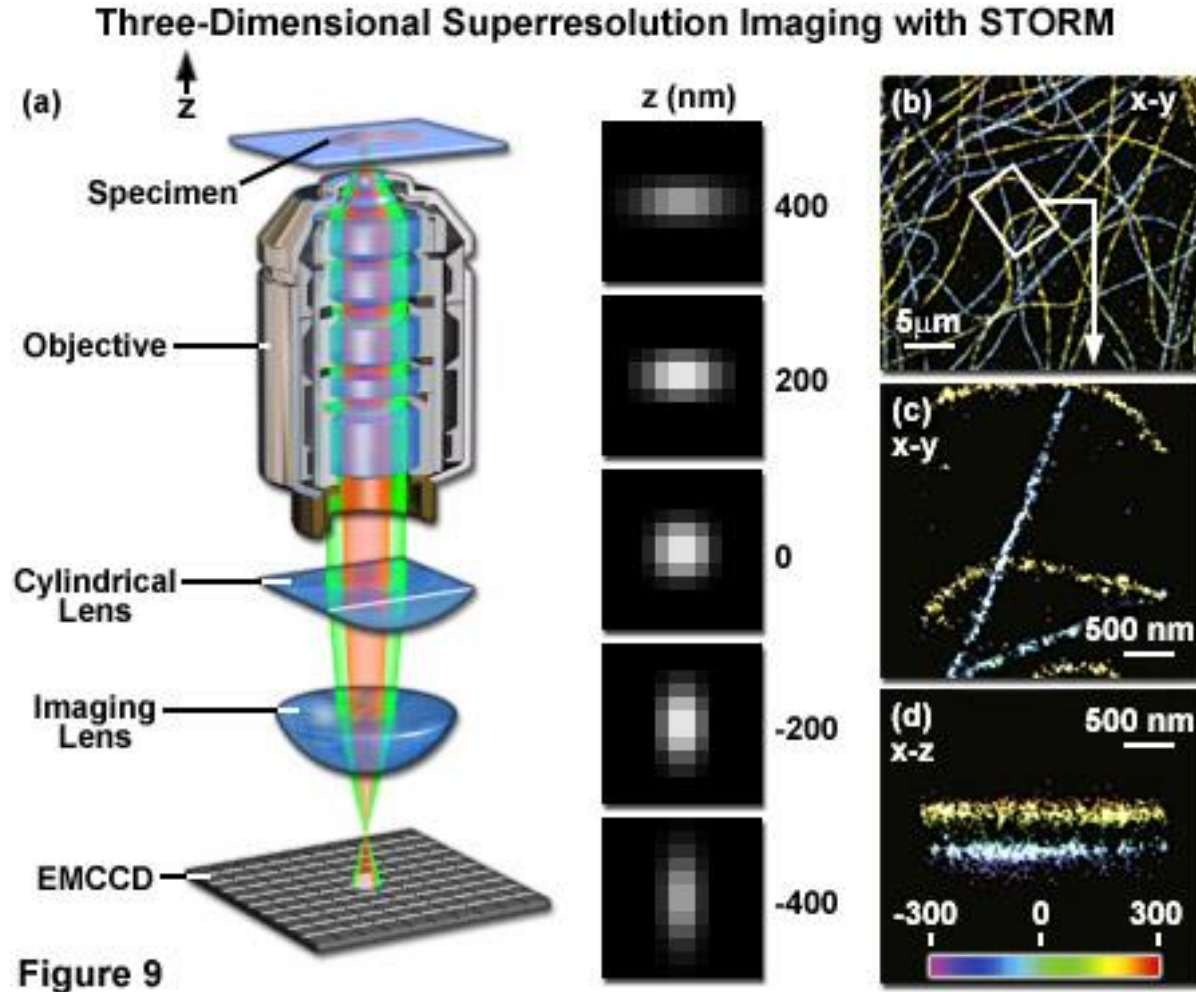


Schermelleh, Lothar, Rainer Heintzmann, and Heinrich Leonhardt. 2010. "A guide to super-resolution fluorescence microscopy." *The Journal of Cell Biology* 190 (2) (July 26): 165 -175. doi:10.1083/jcb.201002018.

# Resolucion 3D



# Superresolucion 3D



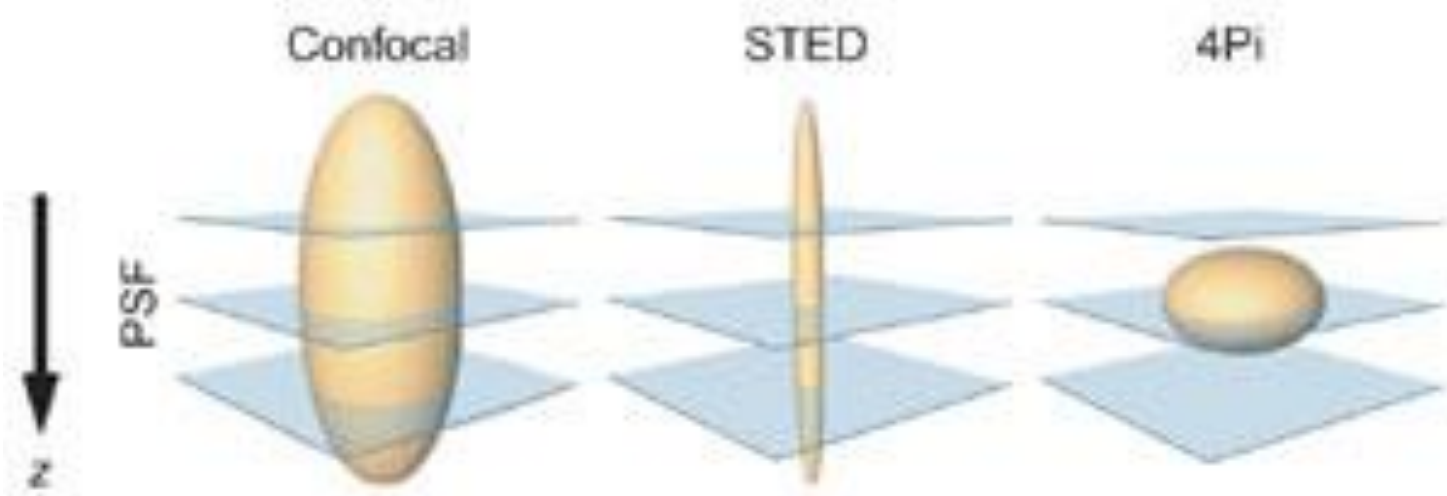
resumen

$$\frac{\lambda}{2n \sin \Theta \sqrt{1 + I_{STED} / I_{sat}}}$$

$$\Delta x \approx \frac{\lambda}{2NA\sqrt{m}}$$

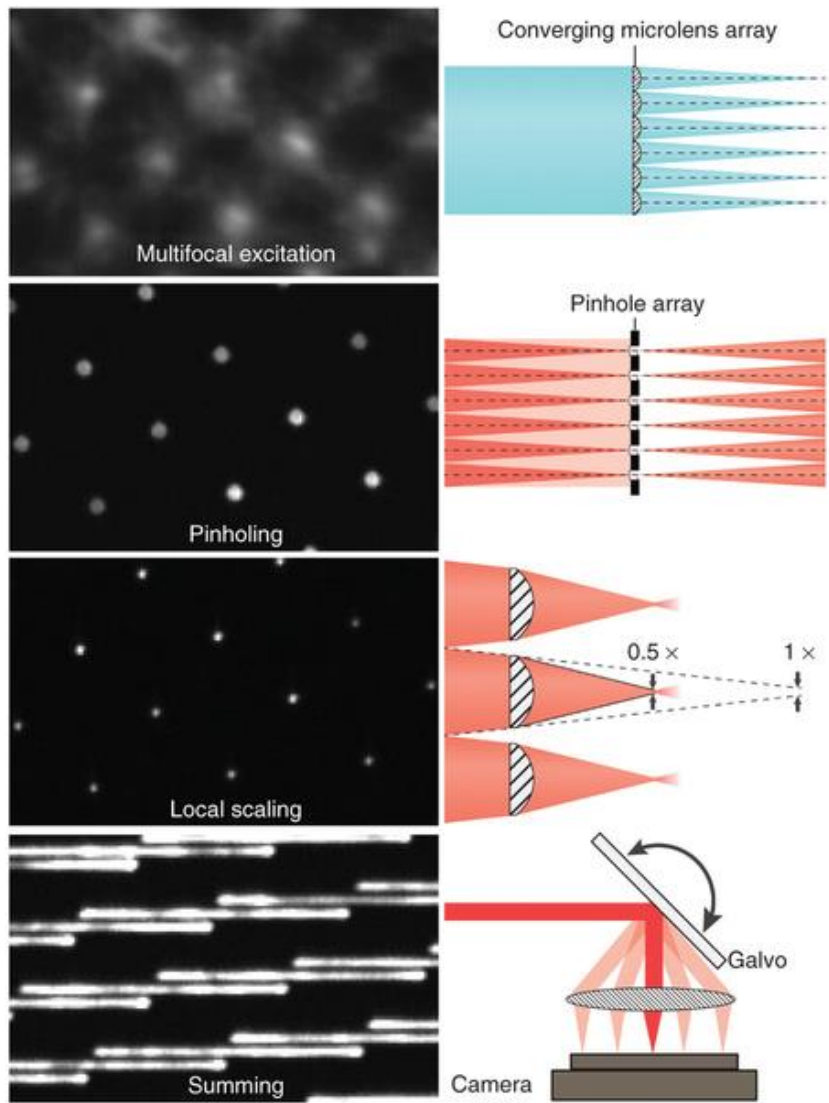
# Fin

Gustafsson MG (2000) Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. *J. Microsc.* 198:82-87.

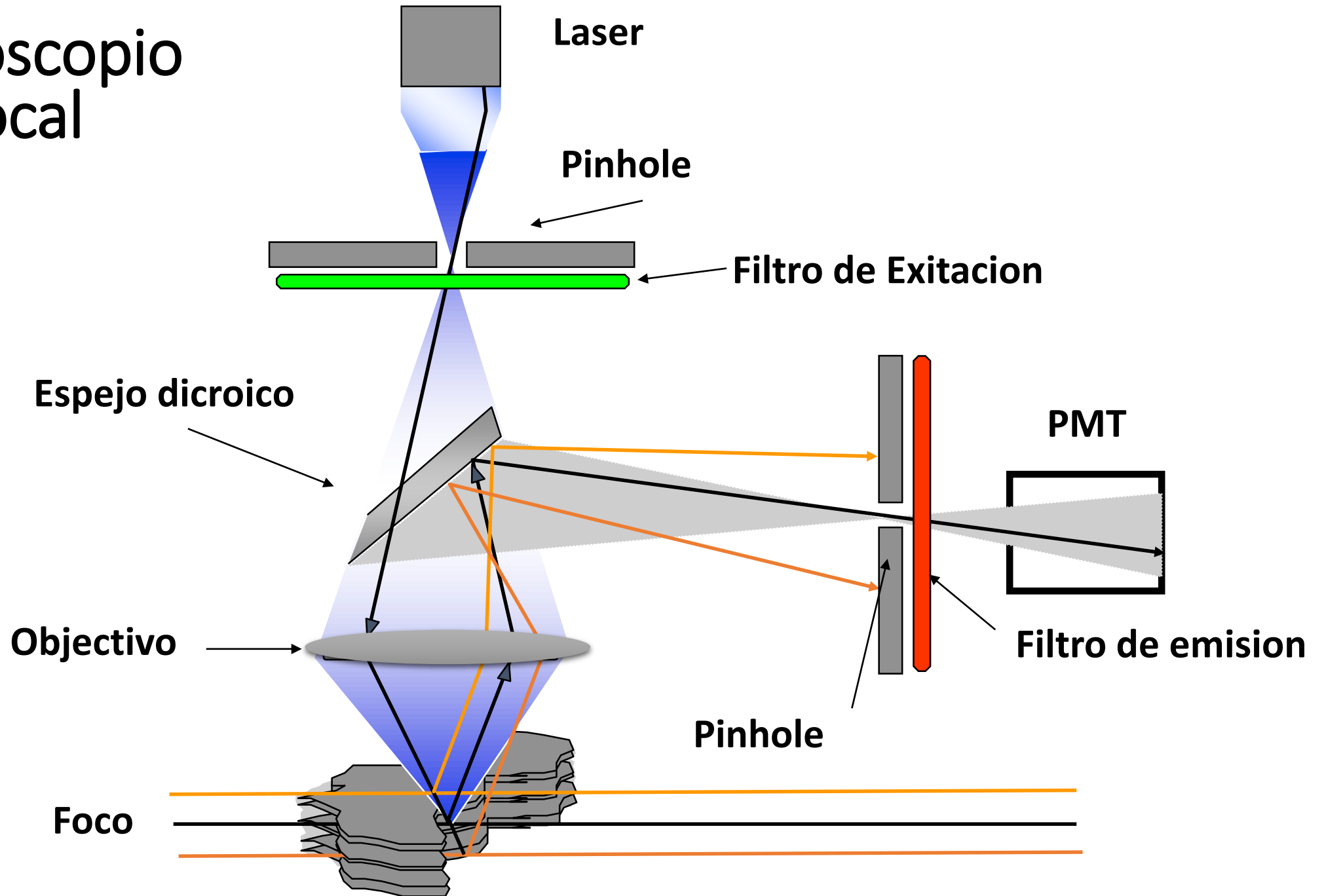


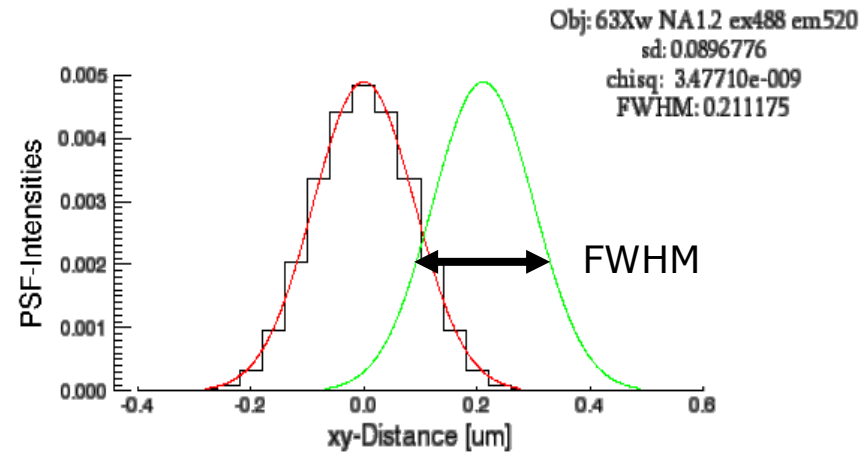
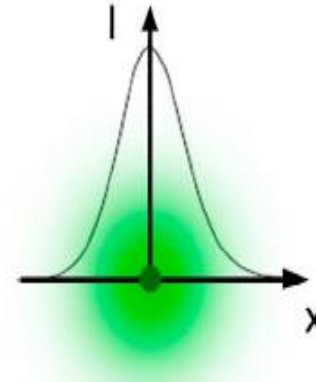
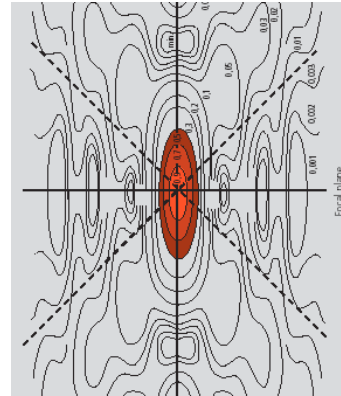
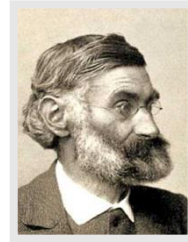


# SD-SIM



# Microscopio Confocal





# Light Microscopy outside Chile

M Goeppert-Mayer  
1906-1972



M Gustafson  
1960-2011



S Hell  
MPI Göttingen  
BIOQUANT Hdg



E Betzig  
Janelia Farm



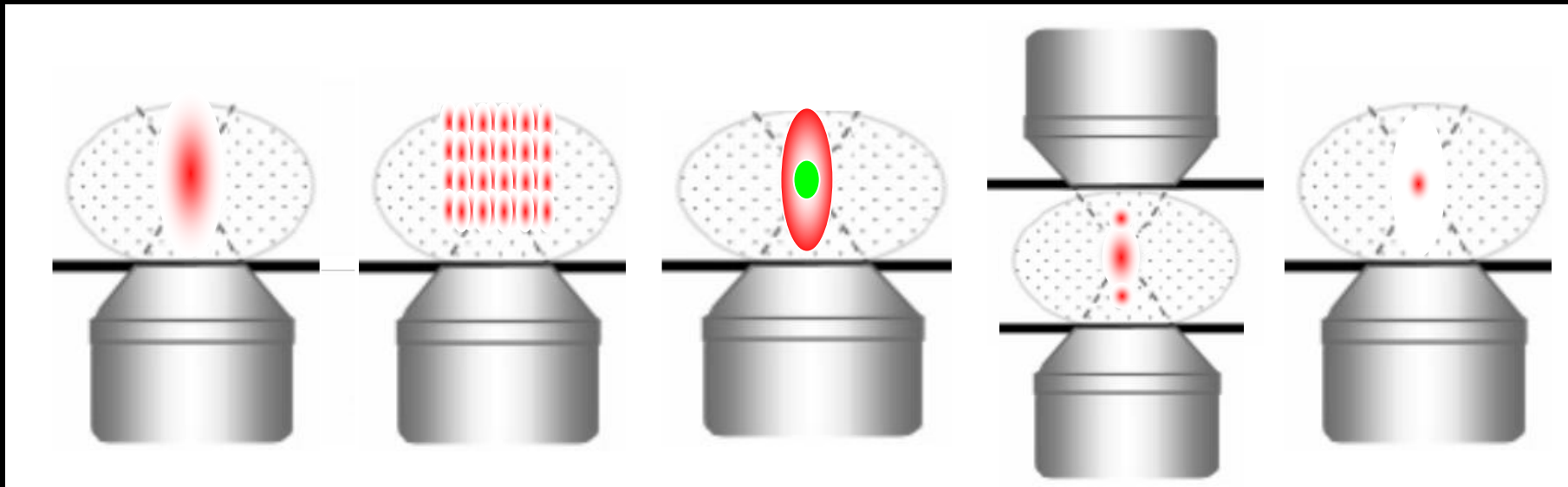
FWHM(xy)  $\sim \lambda/2$

$\sim \lambda/4$

$\sim \lambda/\infty$

$\sim \lambda/4$

$\sim \lambda/100$



2-photon

SIM

STED

4- $\pi$

PALM