Laboratory for SCIAN Scientific Image Analysis

BIOMEDICAL NEUROSCIENCE INSTITUTE

Advanced Course

BNI

Procesamiento de Imágenes y Bioseñales I & II

Math omics

Primavera, 2012

Escuela de Postgrado, Facultad de Medicina, U-Chile

SCIAN

OVERVIEW

Part I

:. Biological and biomedical imaging acquisition

:. Methods and techniques of signal and image processing

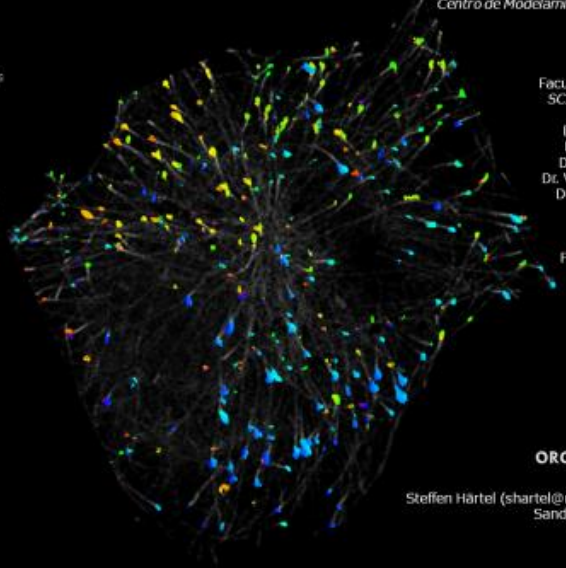
:. Analysis of biological structures in digital images

Part II

:. Interpretation of morphological, topological and dynamical information in biological and biomedical images

:. High throughput microscopy

:. Super-resolution microscopy



PROFESSORS

Universidad de Chile
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Takeshi Asahi

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SCIAN-Lab / CEDAI

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Dr. Enzo Brunetti
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Dr. Mauricio Cerda
Dr. Jarno Ralli
Jorge Jara
Luis Briones
Felipe Santibañez

ACTIVITIES

:. Lectures

:. Bibliographic Seminars

:. Hands-on Microscopy

:. Hands-on Computational Analysis

ORGANIZATION

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Sandra de la Fuente
Pamela Weber

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Escuela de Postgrado
Facultad de Medicina
Universidad de Chile

More Information @
www.scian.cl/mim

fcfm · Facultad de Medicina · cgr · CMM Center for Mathematical Modeling · CBM · Universität Heidelberg · DAAD

Laboratory for SCIAN
Scientific Image Analysis

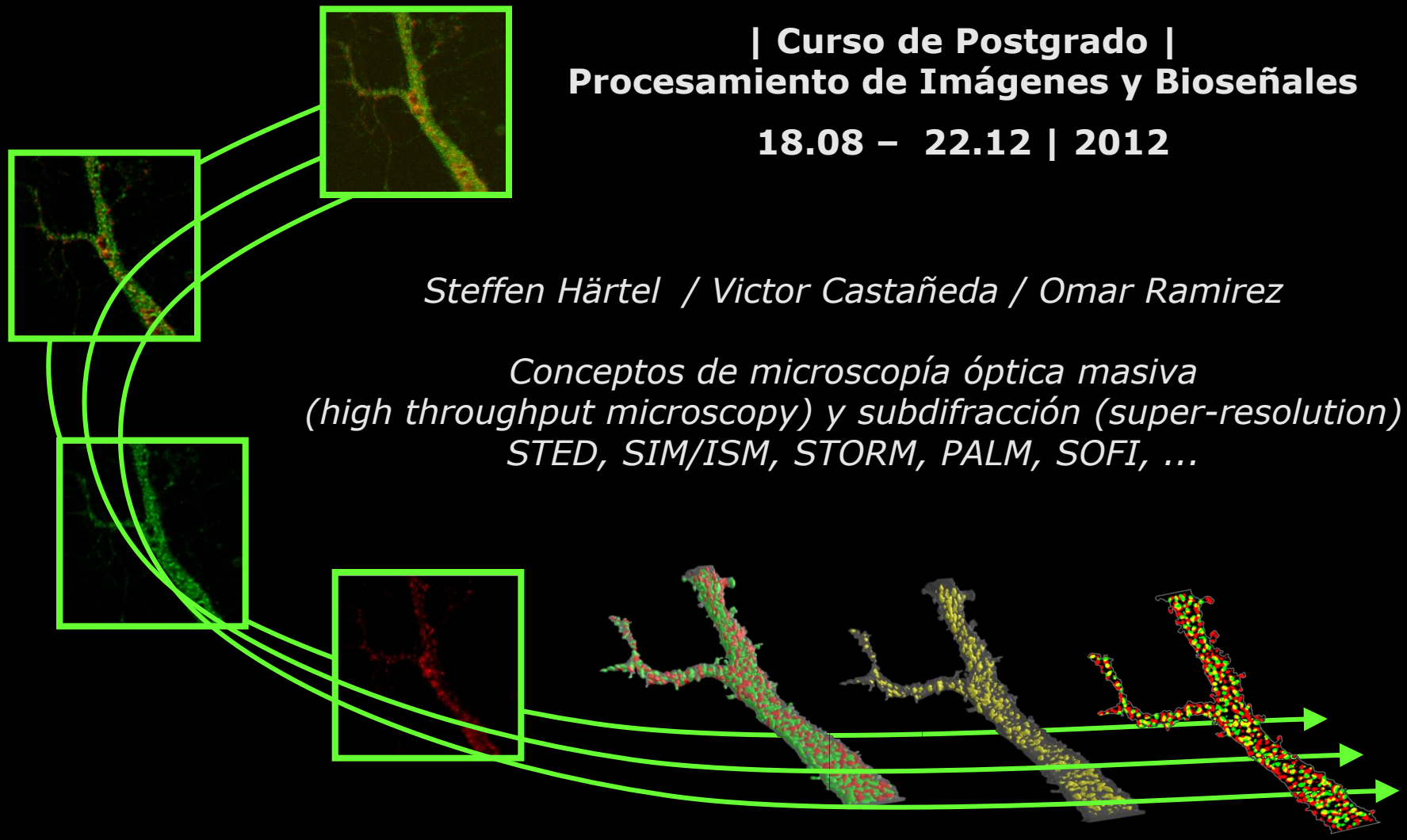
BIOMEDICAL NEUROSCIENCE INSTITUTE

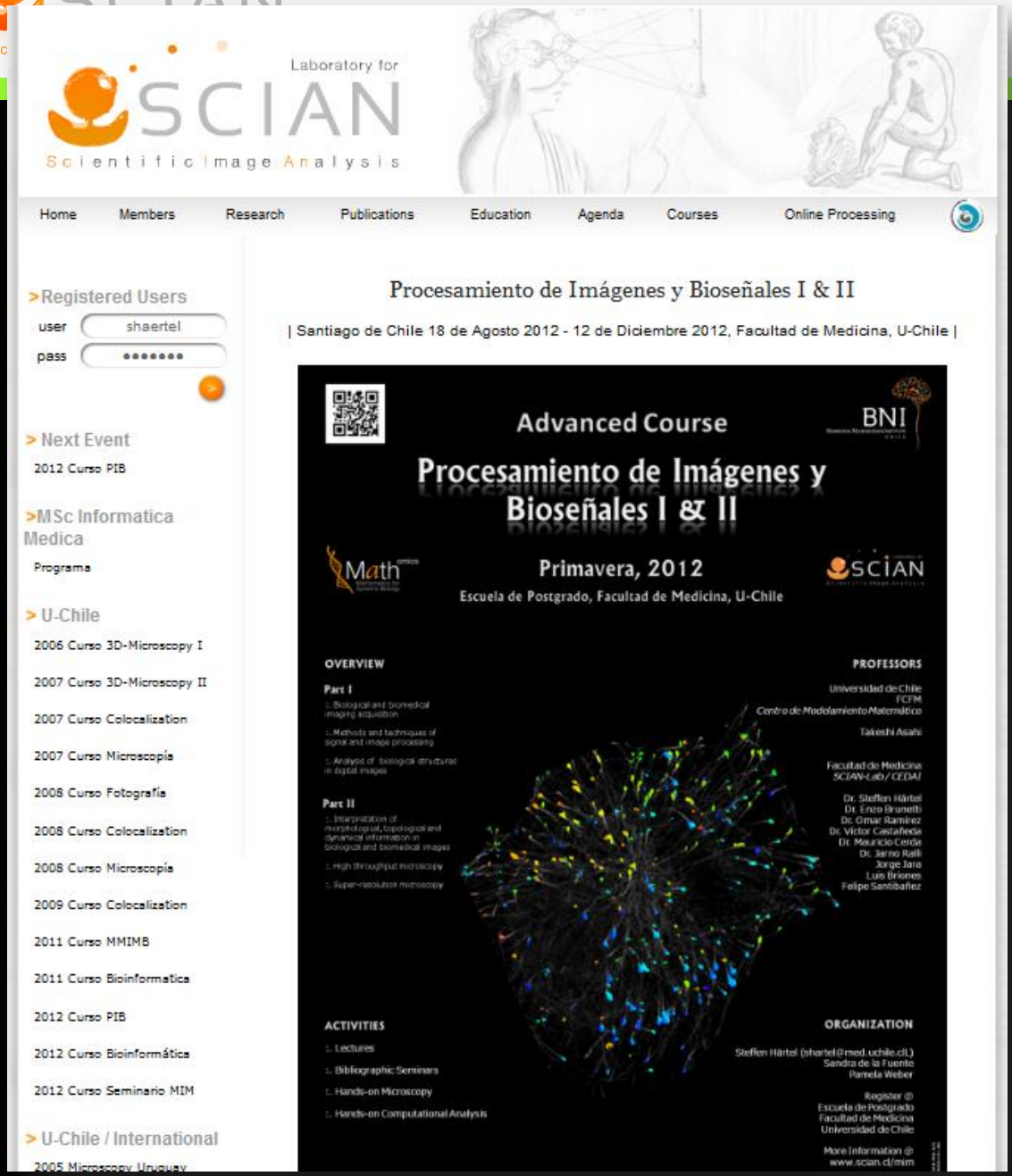
| Curso de Postgrado |
Procesamiento de Imágenes y Bioseñales

18.08 – 22.12 | 2012

Steffen Härtel / Victor Castañeda / Omar Ramirez

Conceptos de microscopía óptica masiva
(high throughput microscopy) y subdifracción (super-resolution)
STED, SIM/ISM, STORM, PALM, SOFI, ...





Informaciones en:

-> www.scian.cl

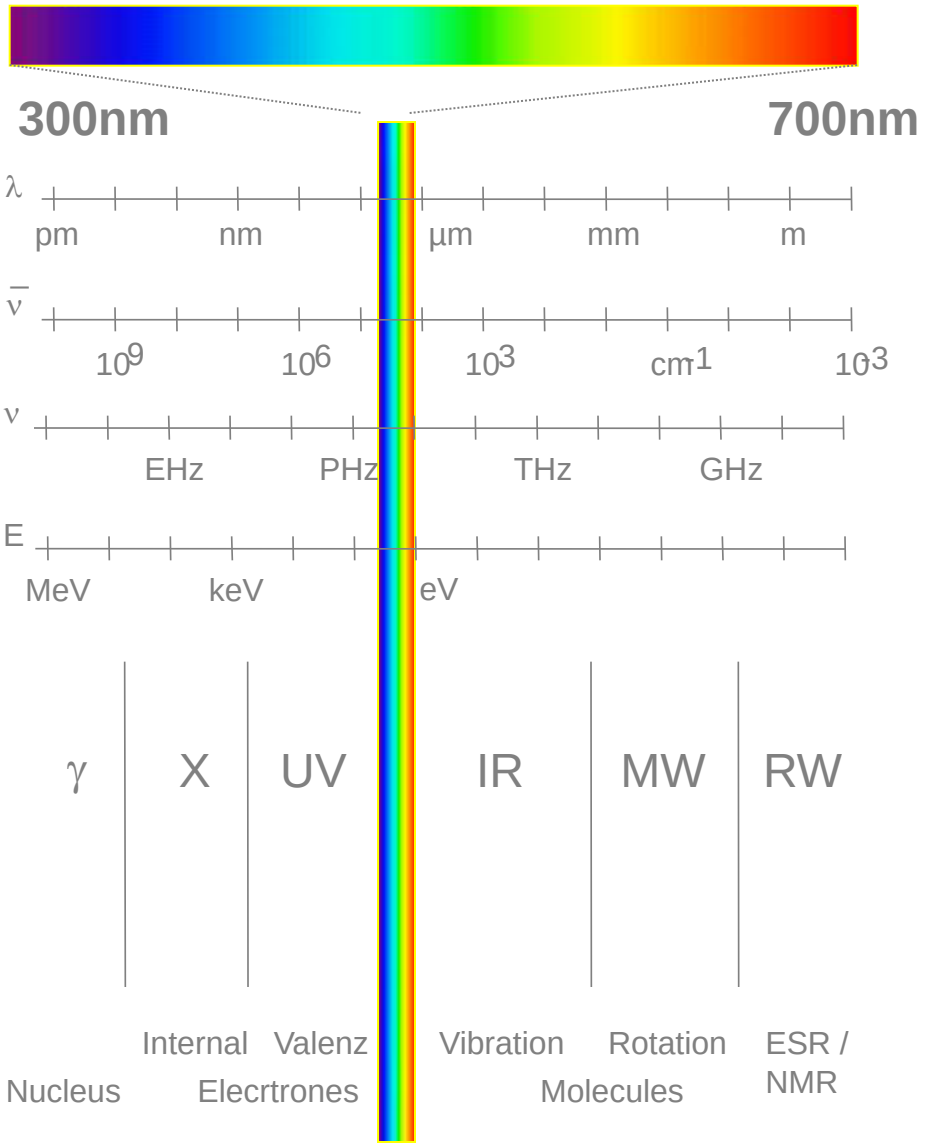
-> www.scian.cl/mim

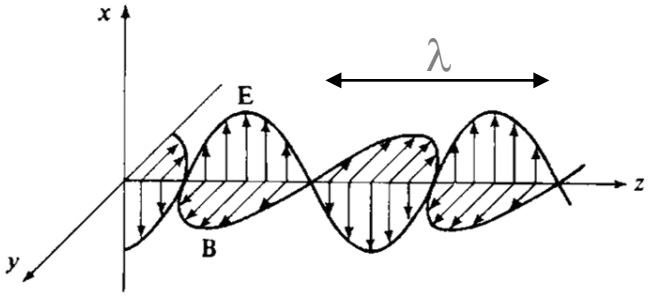
Notas:

Prácticos (50%)

Examen Final (50%)

|-> Fluorescencia II





Energía de un fotón: (~1-5eV)

$E = h\nu = hc\lambda^{-1} \mid c = \nu\lambda$

ν, frecuencia [s^{-1}]
h, constante de Planck [$6.626\ 10^{-34}\ Js^{-1}$]
λ, longitud de onda [m]
c, velocidad de luz [$\sim 3\ 10^{8}\ ms^{-1}$]

Energía molecular:

$E = E_{rot} + E_{vib} + E_{el}$

$1 : 10^3 : 50 \cdot 10^3$

Energía térmica:

$E = k\,T$ (~$2.5\ 10^{-2}$ eV, T ~20°C)

k = Constante de Bolzmann ($0.86\ 10^{-4}$ eV/K)

|-> Jablonski Diagram

Transiciones: → con radiación

~ sin radiación |

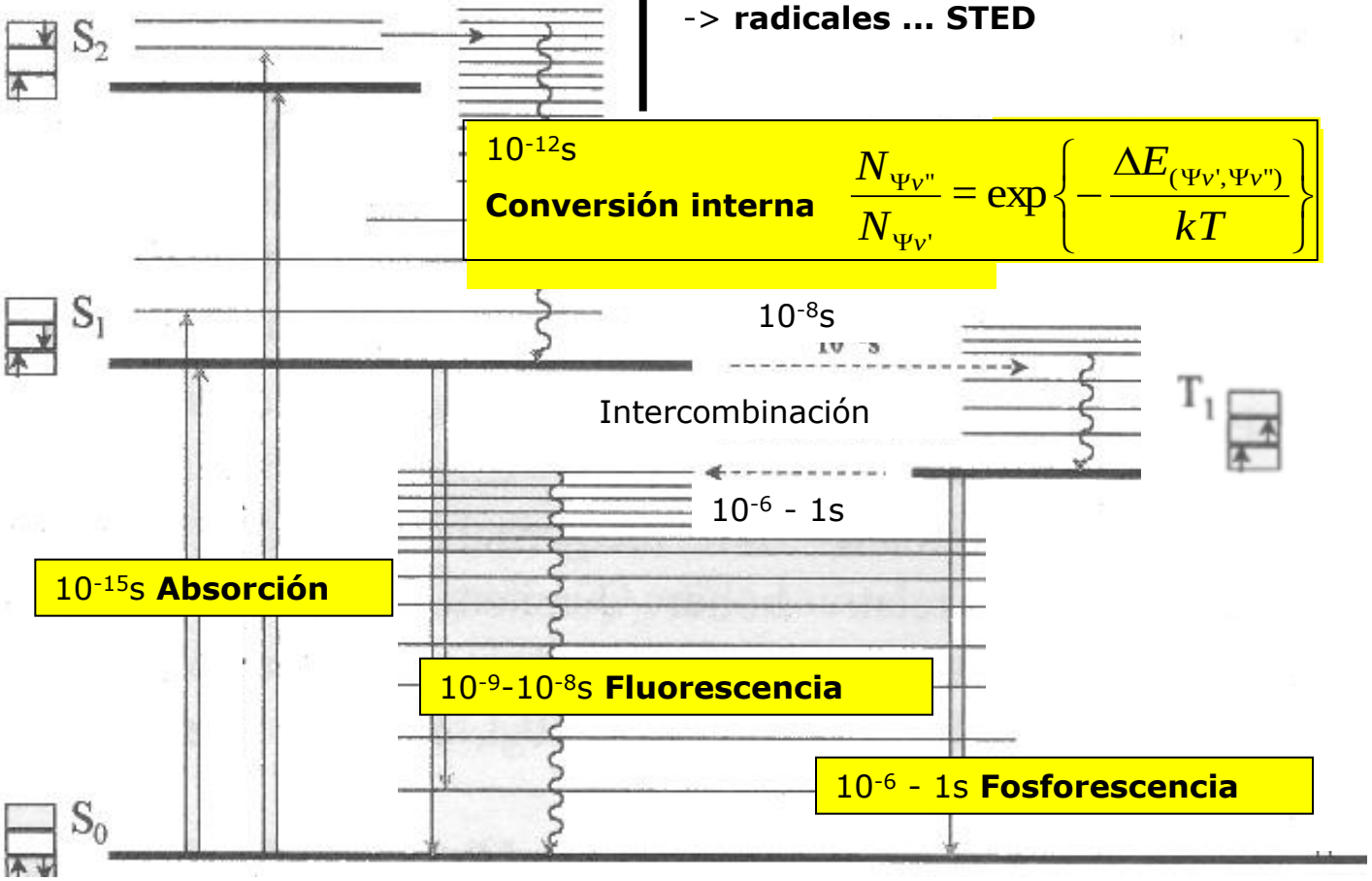
Niveles: —— vibracionales

▬ electrónicos

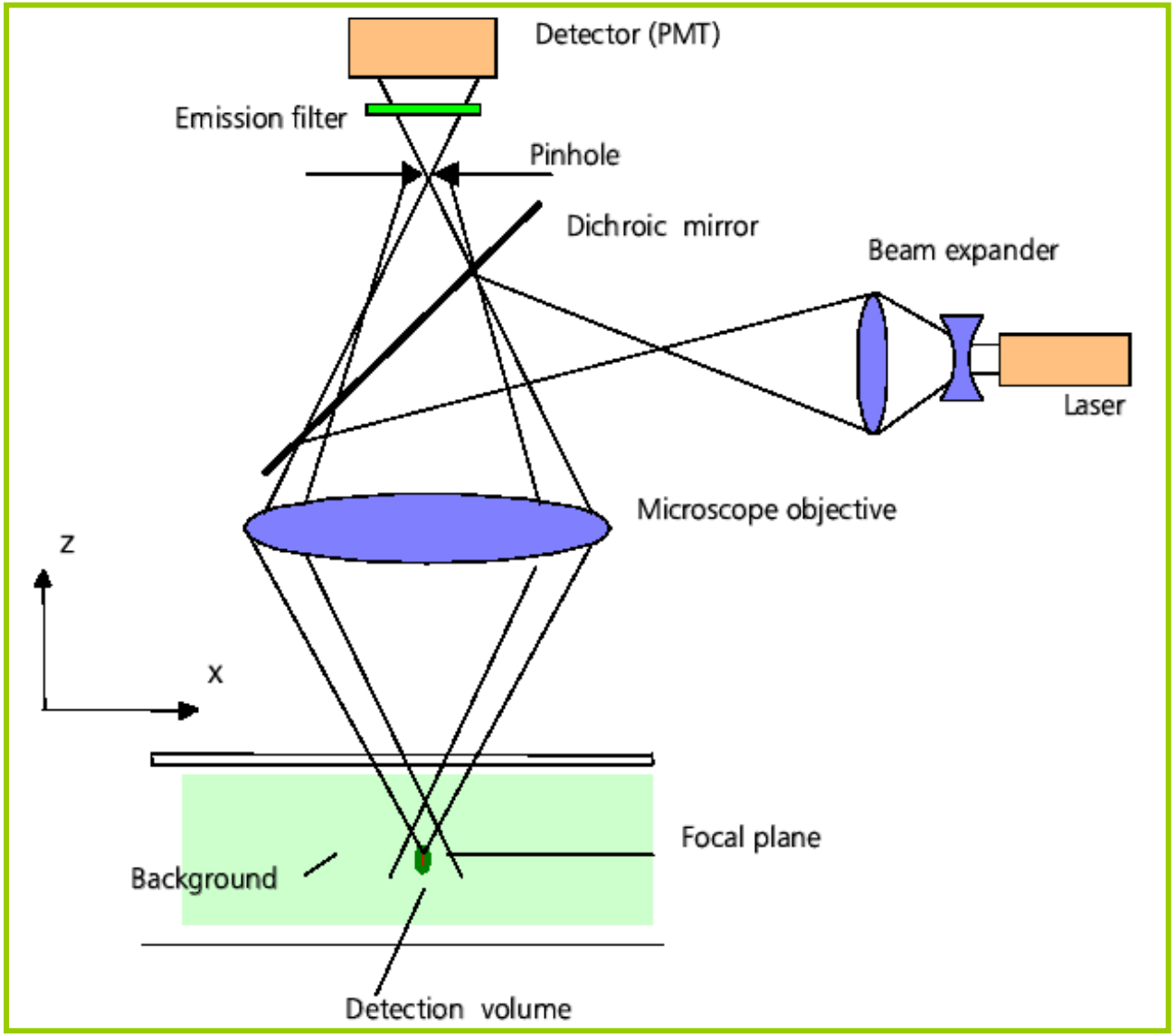
-> **calor**

-> **transferencia de energía (FRET)**

-> **radicales … STED**

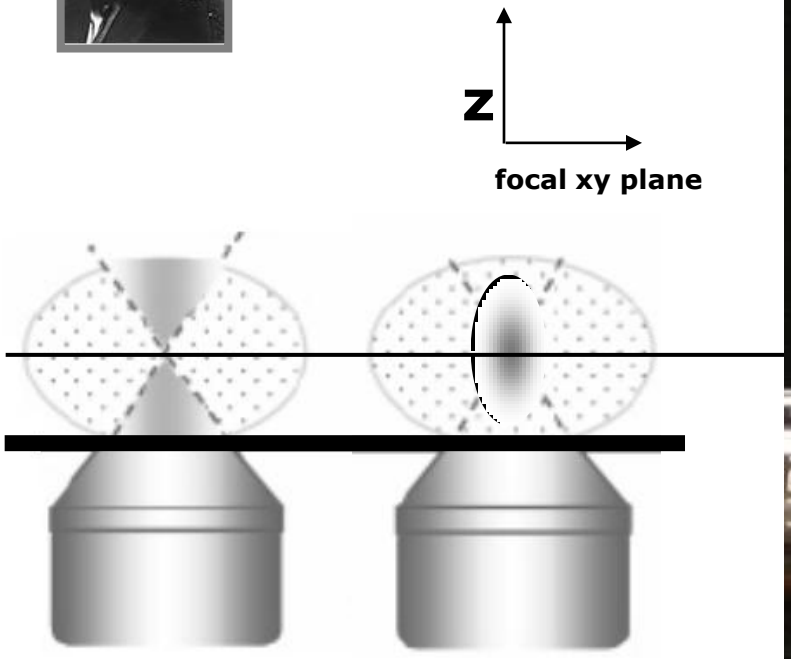


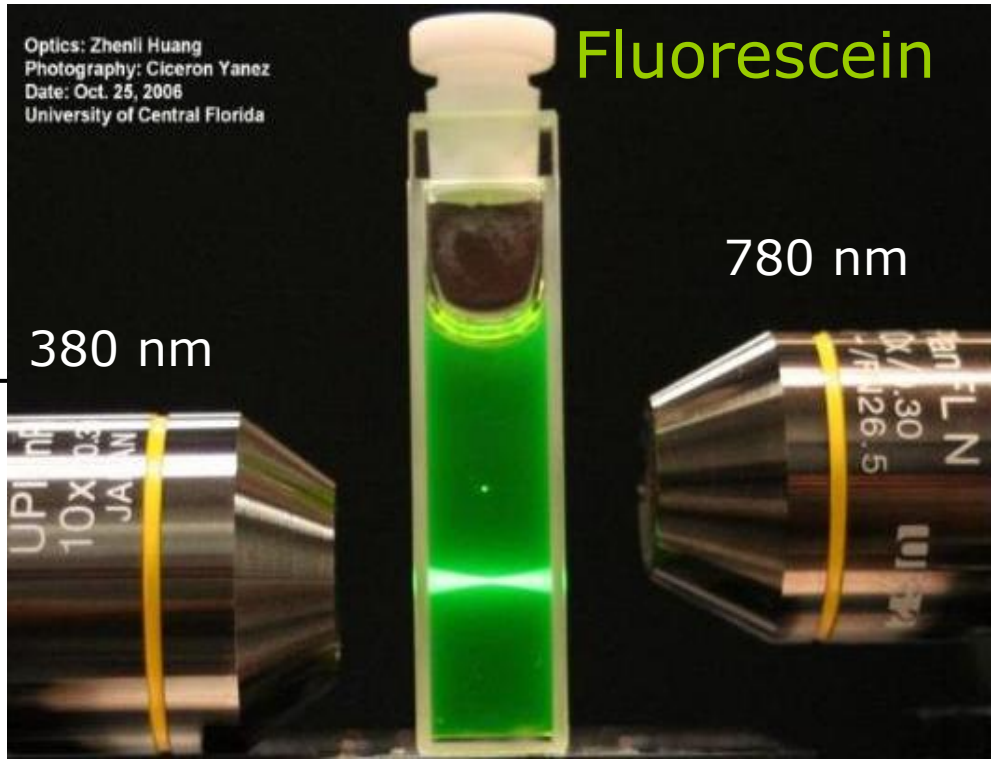
|-> Diffraction limited Microscopy



|-> Diffraction limited Microscopy

| Best localization: confocal microscopy





|-> Diffraction limited Microscopy

Óptica no-geométrica /

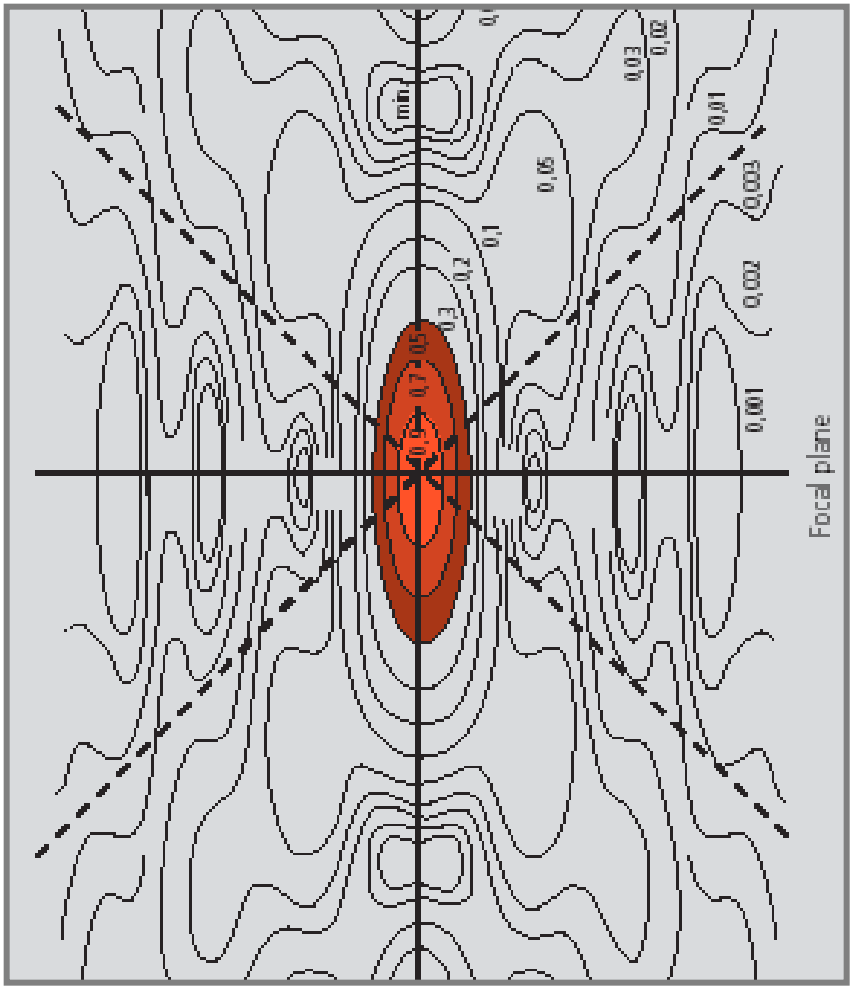
Teoría de difracción

PSF = $|U|^2 = f(J_0)$

U, Integral de Difracción de Kirhoff

J_0, Serie de funciones de Bessel

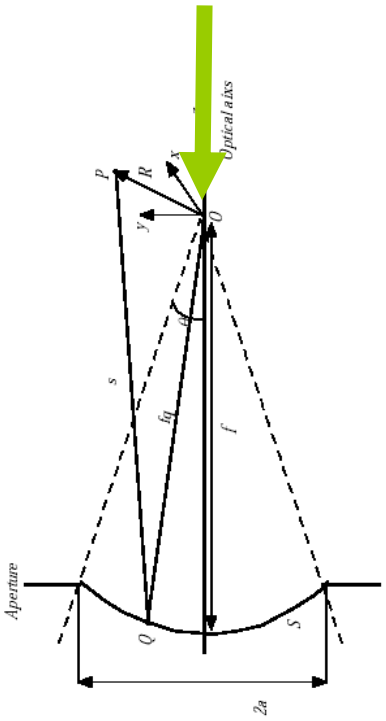
(Born & Wolf, Priniples of Optics, 6th edition 1988, Pergamon Press)

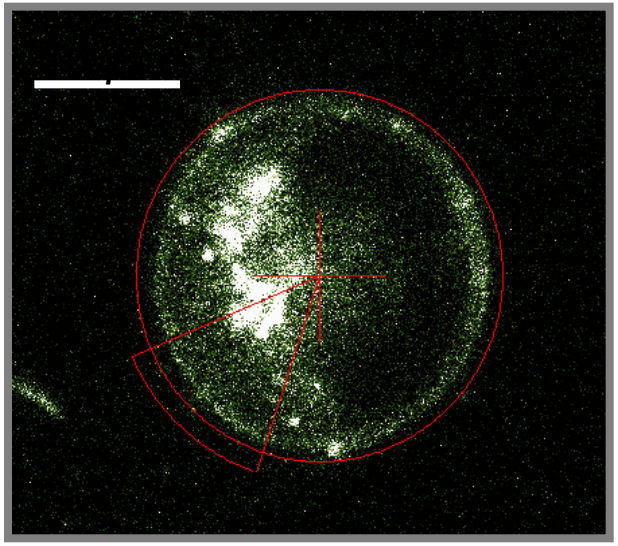


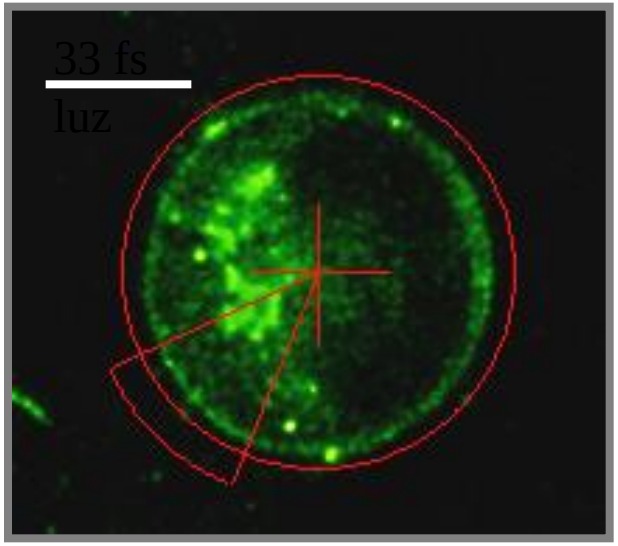
Confocal Microscopy | From Geometric Optics to Diffraction Theory

Diffraction: The deviation of an electromagnetic wavefront from the path predicted by geometric optics when the wavefront interacts with a physical object such as an opening or an edge.

Object → **OTF** → **O**bject**I**mage → **OTF^{-1}** → **O**bject**M**odel





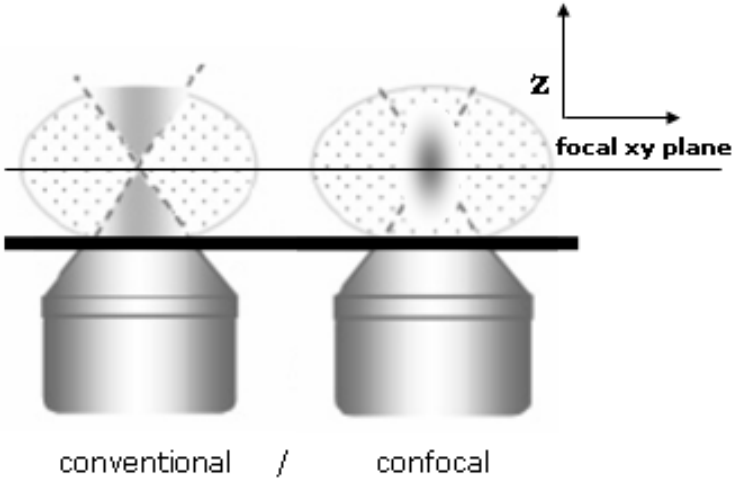


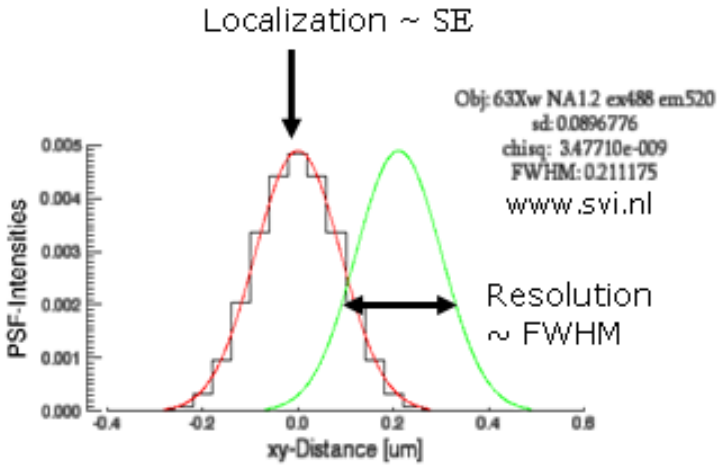
| Diffraction limited microscopy

E. Abbe († 1905)

$\lambda / 2 \cdot NA \sim \lambda / 2$ Resolution (Full Width at Halft Maximum, FWHM)

$FWHM / N^{1/2}$ Localization, N number of photons





|-> Beyond diffraction

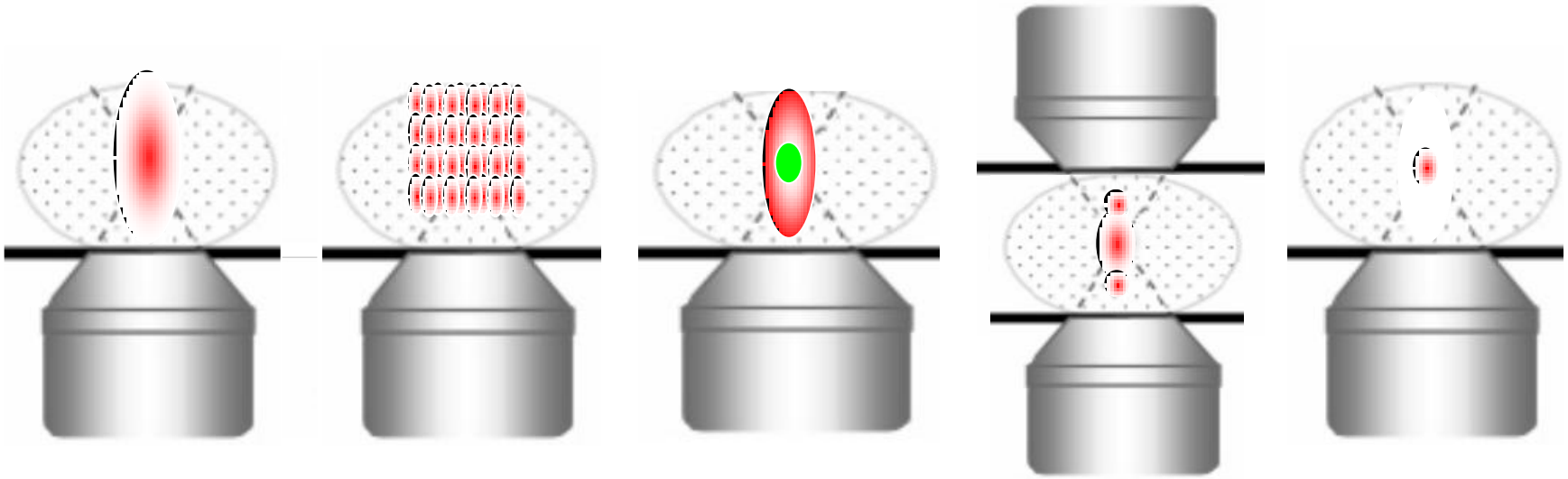
M Goeppert-Mayer
1906-1972

M Gustafson
1960-2011

S Hell
MPI Göttingen
BIOQUANT Hdg

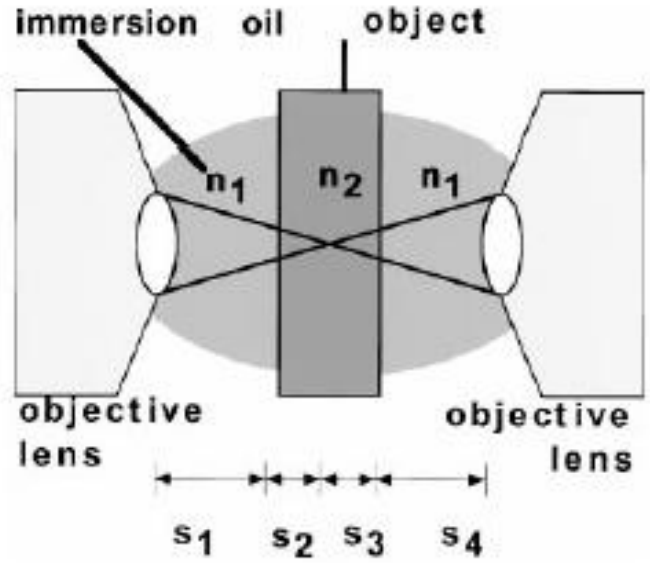
E Betzig
Janelia Farm

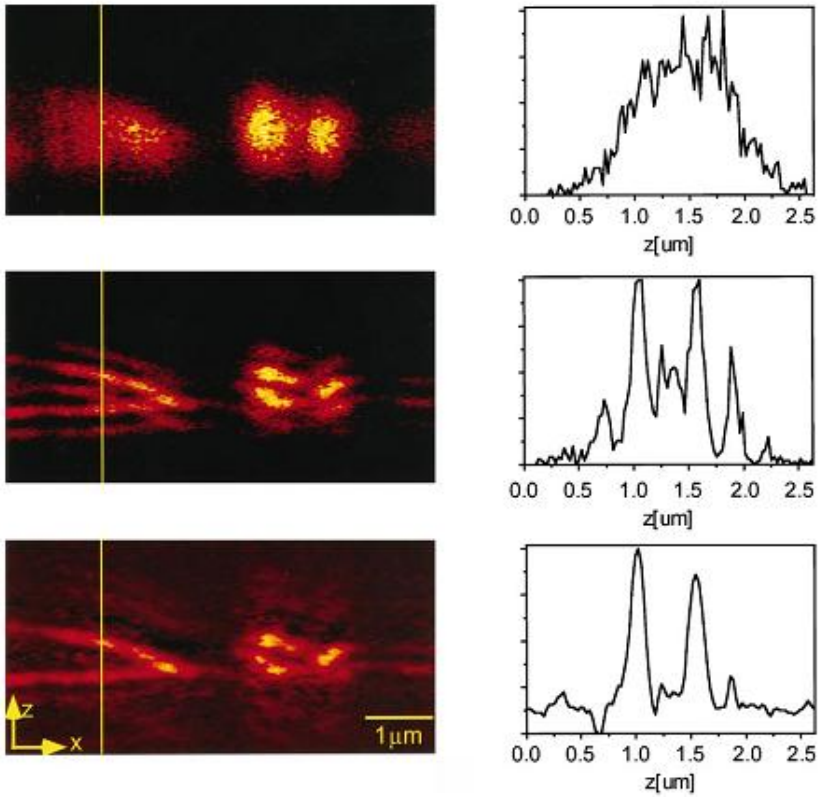
FWHM(xy) ~ λ/2 ~ λ/4 ~ λ/∞ ~ λ/4 ~ λ/100



2-photon SIM STED 4-π PALM

|-> 4π Microscopy





4Pi-Confocal Imaging in Fixed Biological Specimens

Martin Schrader,* Karsten Bahlmann,* Günter Giese,# and Stefan W. Hell*

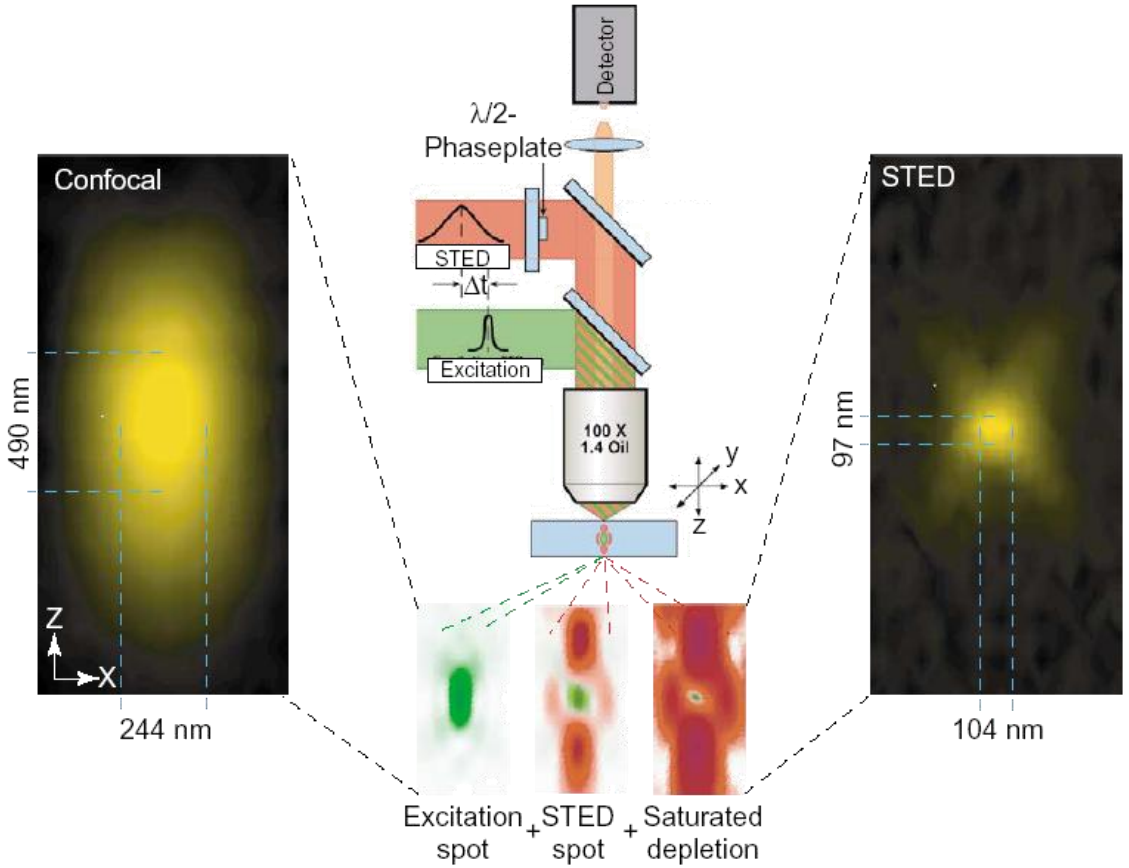
Biophysical Journal Volume 75 October 1998 1659–1668

STED Microscopy

Stimulated

Emission

Depletion

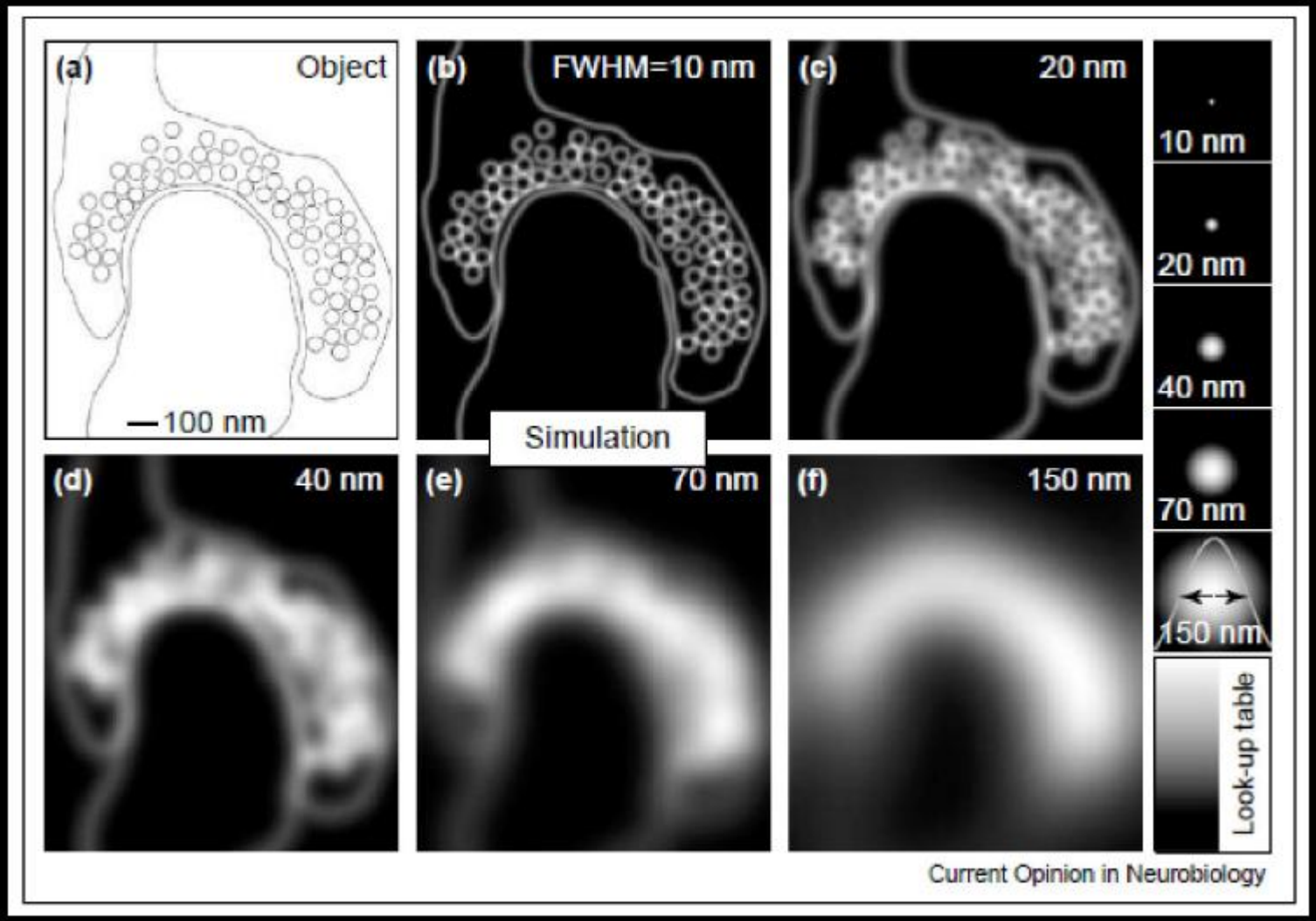


Concepts for nanoscale resolution in fluorescence microscopy

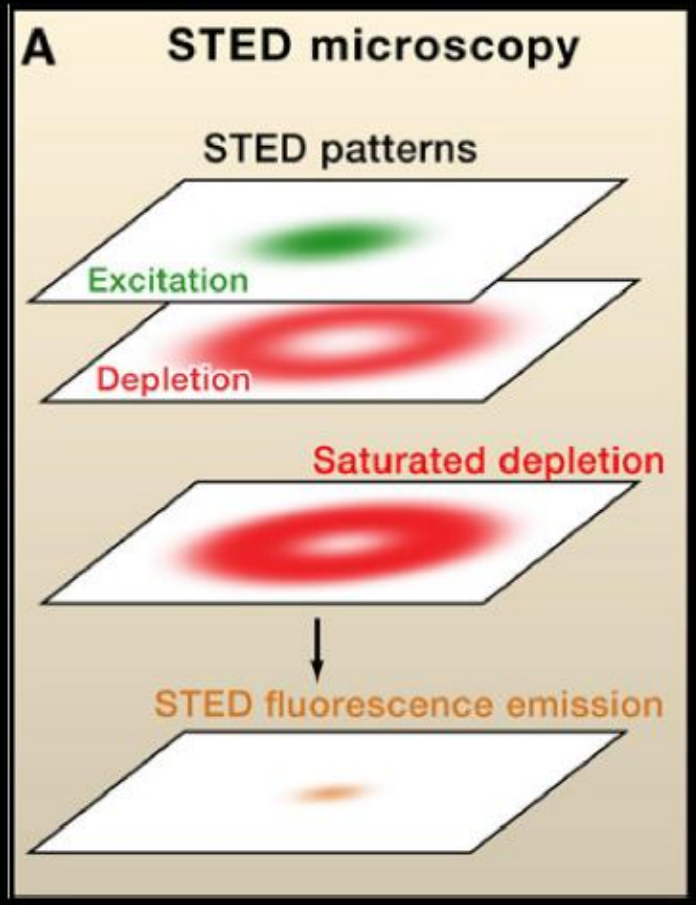
Stefan W Hell*, Marcus Dyba[1] and Stefan Jakobs[2]

Current Opinion in Neurobiology 2004, **14**:599–609

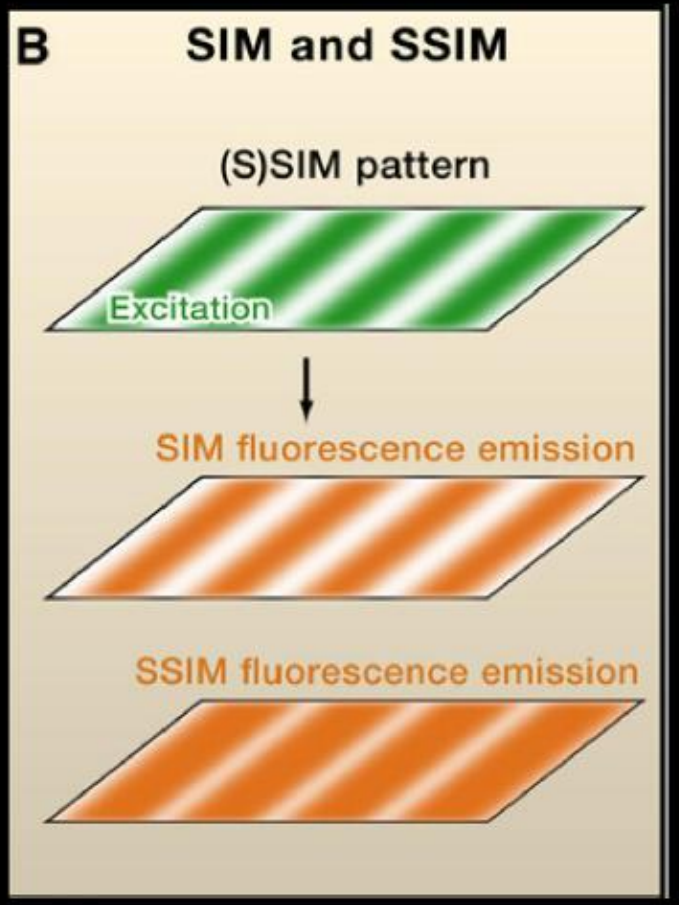
|-> STED


(a)
Object
100 nm
(b)
FWHM=10 nm
(c)
20 nm
Simulation
(d)
40 nm
(e)
70 nm
(f)
150 nm
10 nm
20 nm
40 nm
70 nm
150 nm
Look-up table
Current Opinion in Neurobiology

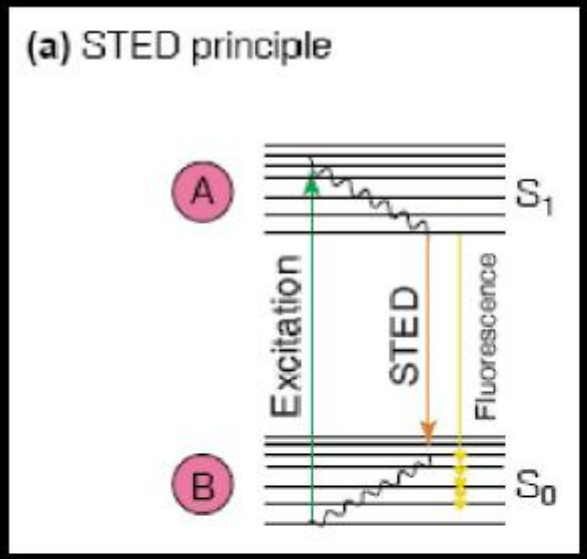
- Negative patterning – STED

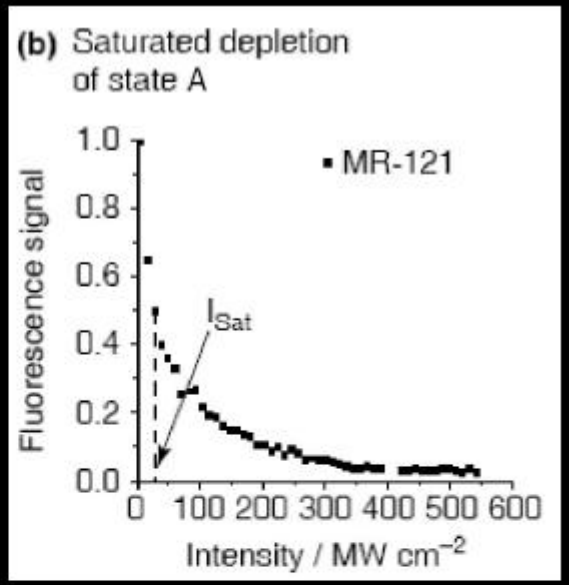


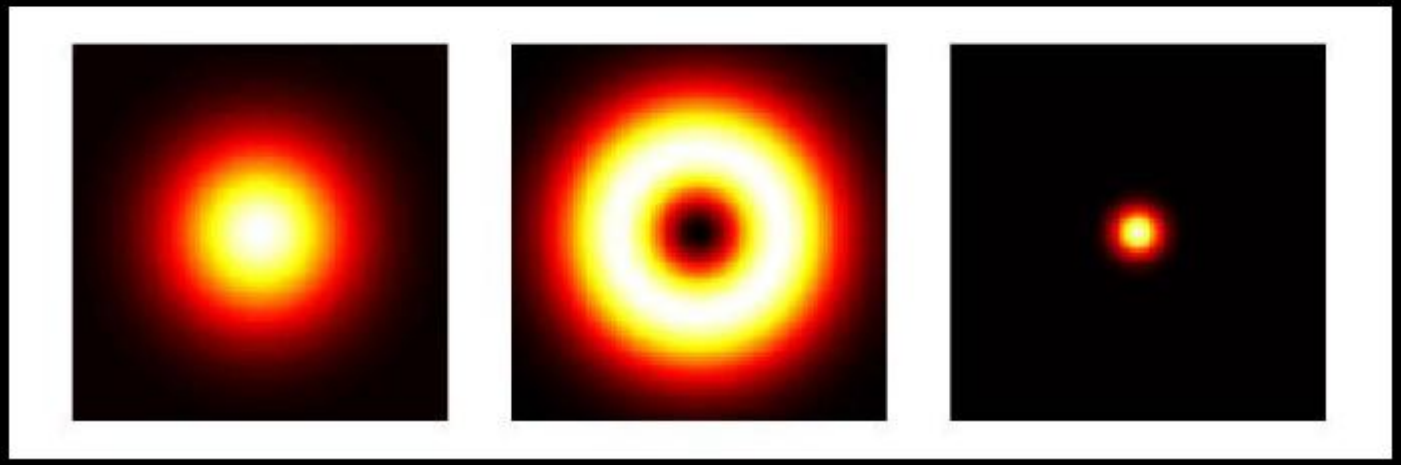
- Positive patterning – SIM



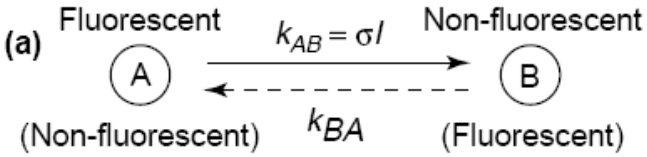
|-> STED


(a) STED principle
A
B
S_1
S_0
Excitation
STED
Fluorescence


(b) Saturated depletion of state A
MR-121
I_{Sat}
Fluorescence signal
1.0
0.8
0.6
0.4
0.2
0.0
0
100 200 300 400 500 600
Intensity / MW cm^{-2}



|-> STED

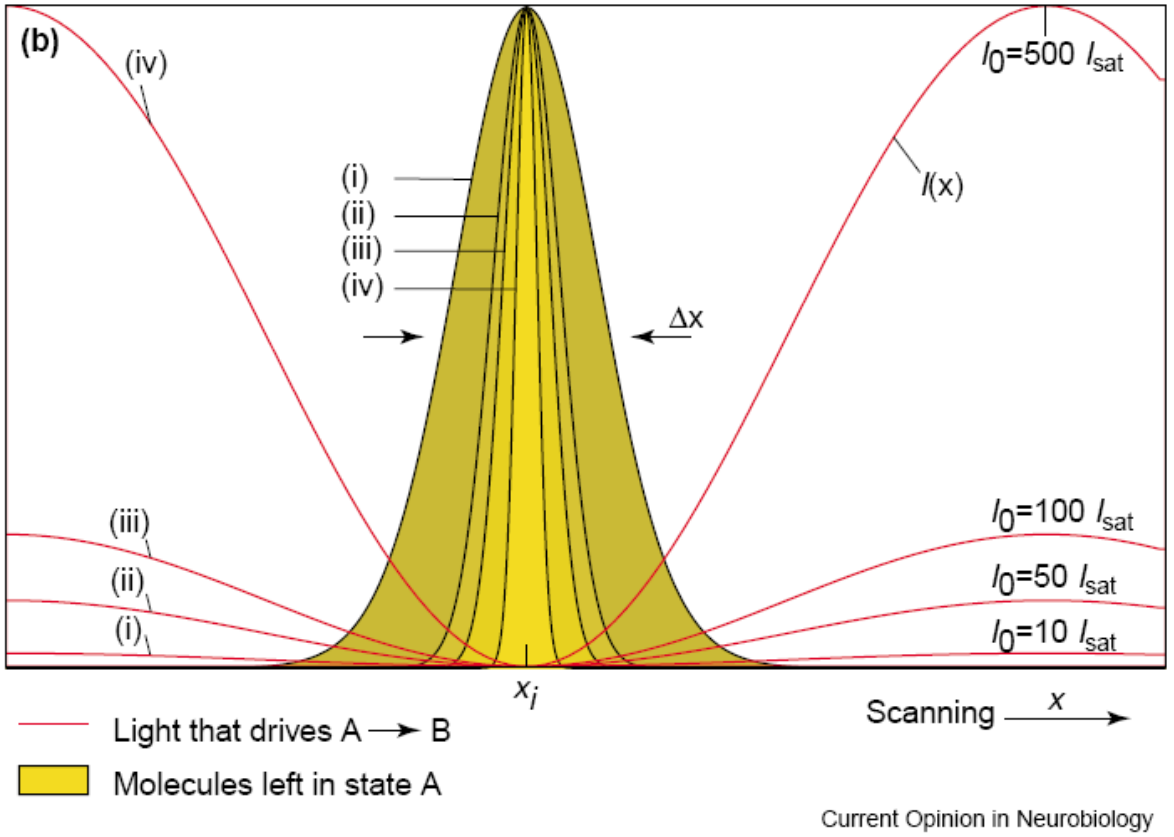


$$\frac{dN_A}{dt} = -\frac{dN_B}{dt} = k_{BA}N_B - k_{AB}N_A$$

$N_{A,B}$ = normalized population probability

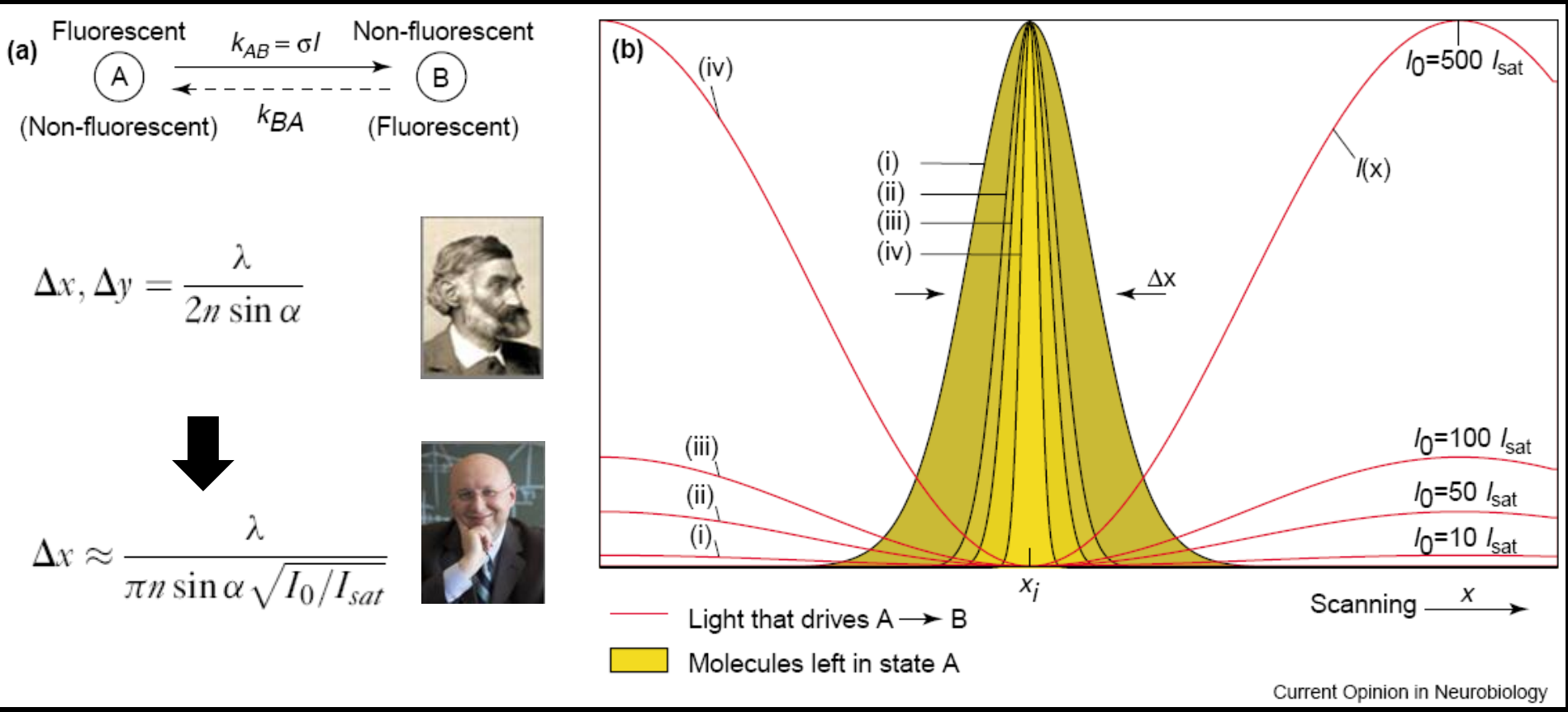
We define 'Saturation Intensity'

$$I_{SAT} = \frac{k_{AB}}{\sigma_{AB}} \rightarrow N_A^{\infty} = 1/2$$

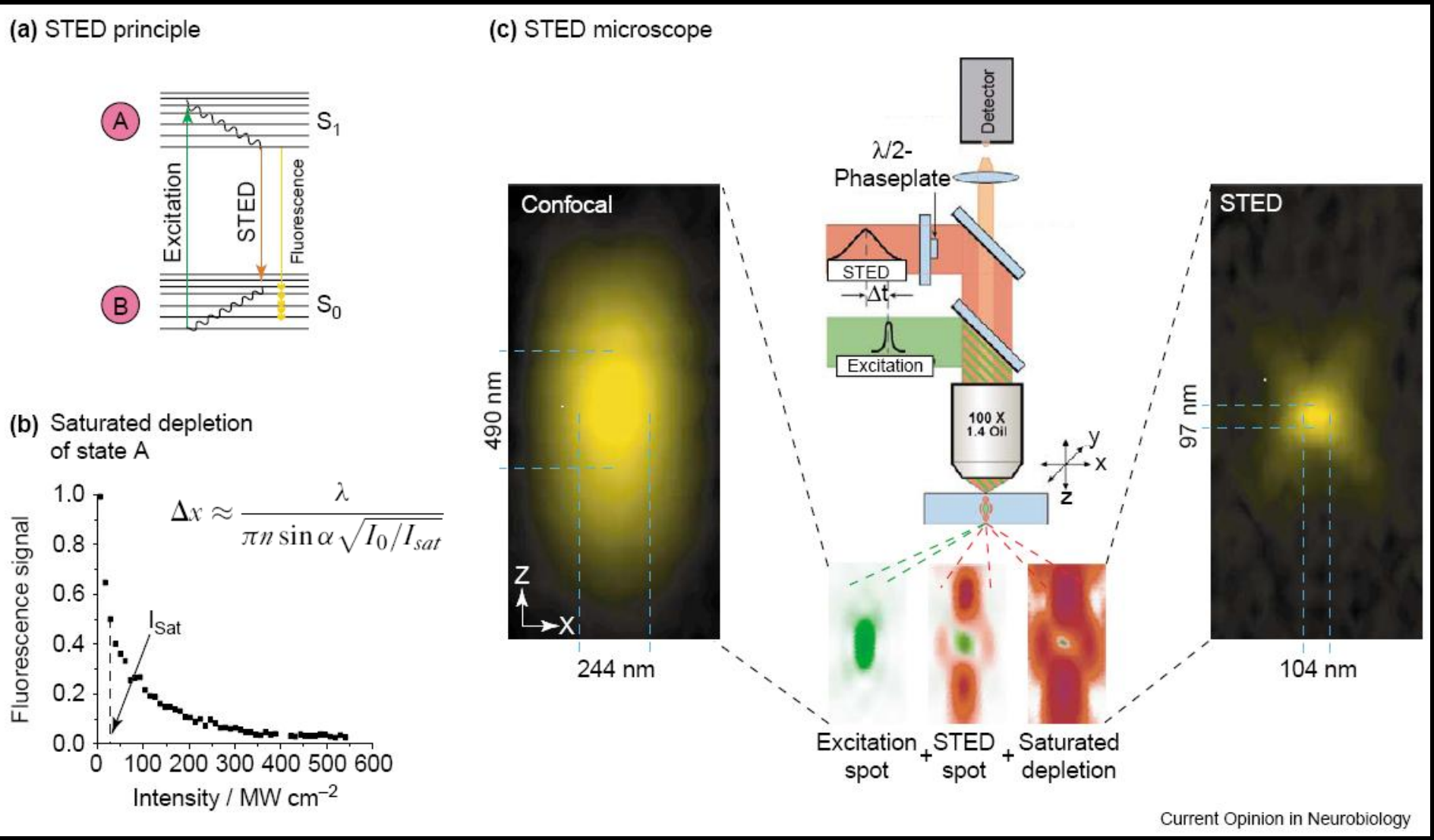


$$I >> I_{SAT} \Rightarrow k_{AB} >> k_{BA} \Rightarrow N_A^{\infty} \rightarrow 0$$

|-> STED


(a)
Fluorescent
Non-fluorescent
$k_{AB} = \sigma I$
A
B
(Non-fluorescent)
k_{BA}
(Fluorescent)
$\Delta x, \Delta y = \frac{\lambda}{2n \sin \alpha}$
$\Delta x \approx \frac{\lambda}{\pi n \sin \alpha \sqrt{I_0 / I_{sat}}}$
(b)
(iv)
(i)
(ii)
(iii)
(iv)
I_0=500 I_{sat}
I(x)
Δx
(iii)
(ii)
(i)
I_0=100 I_{sat}
I_0=50 I_{sat}
I_0=10 I_{sat}
x_i
Scanning x
Light that drives A → B
Molecules left in state A
Current Opinion in Neurobiology

|-> STED


(a) STED principle
A
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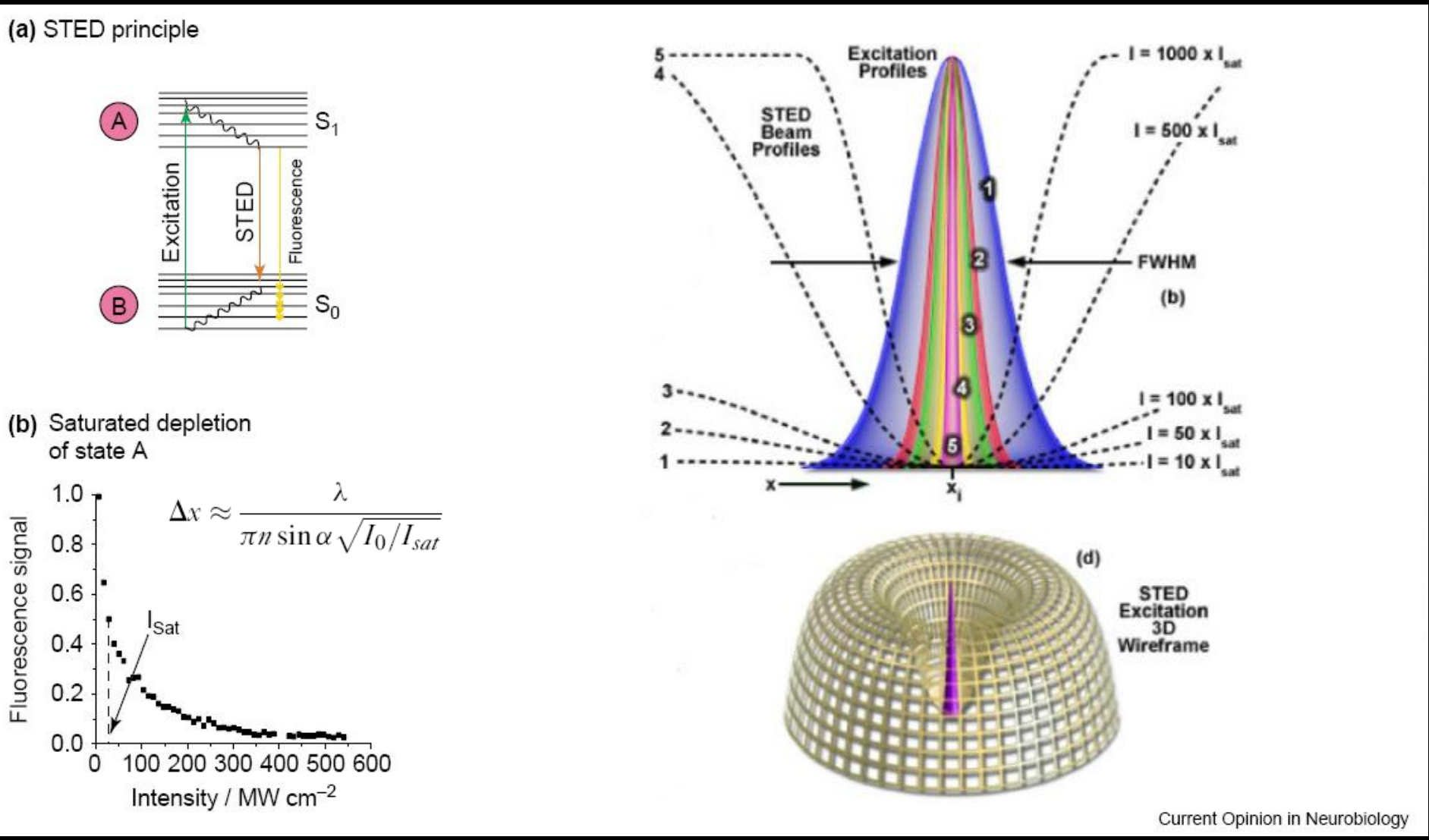
S_1
S_0
Excitation
STED
Fluorescence
(b) Saturated depletion of state A
$\Delta x \approx \frac{\lambda}{\pi n \sin\alpha \sqrt{I_0/I_{sat}}}$
Fluorescence signal
1.0
0.8
0.6
0.4
0.2
0.0
I_{Sat}
0 100 200 300 400 500 600
Intensity / MW cm^{-2}
(c) STED microscope
Detector
λ/2-Phaseplate
STED
Δt
Excitation
100 X 1.4 Oil
y
x
z
Confocal
490 nm
Z
X
244 nm
STED
97 nm
104 nm
Excitation spot + STED spot + Saturated depletion
Current Opinion in Neurobiology

|-> STED


(a) STED principle
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B
S_1
S_0
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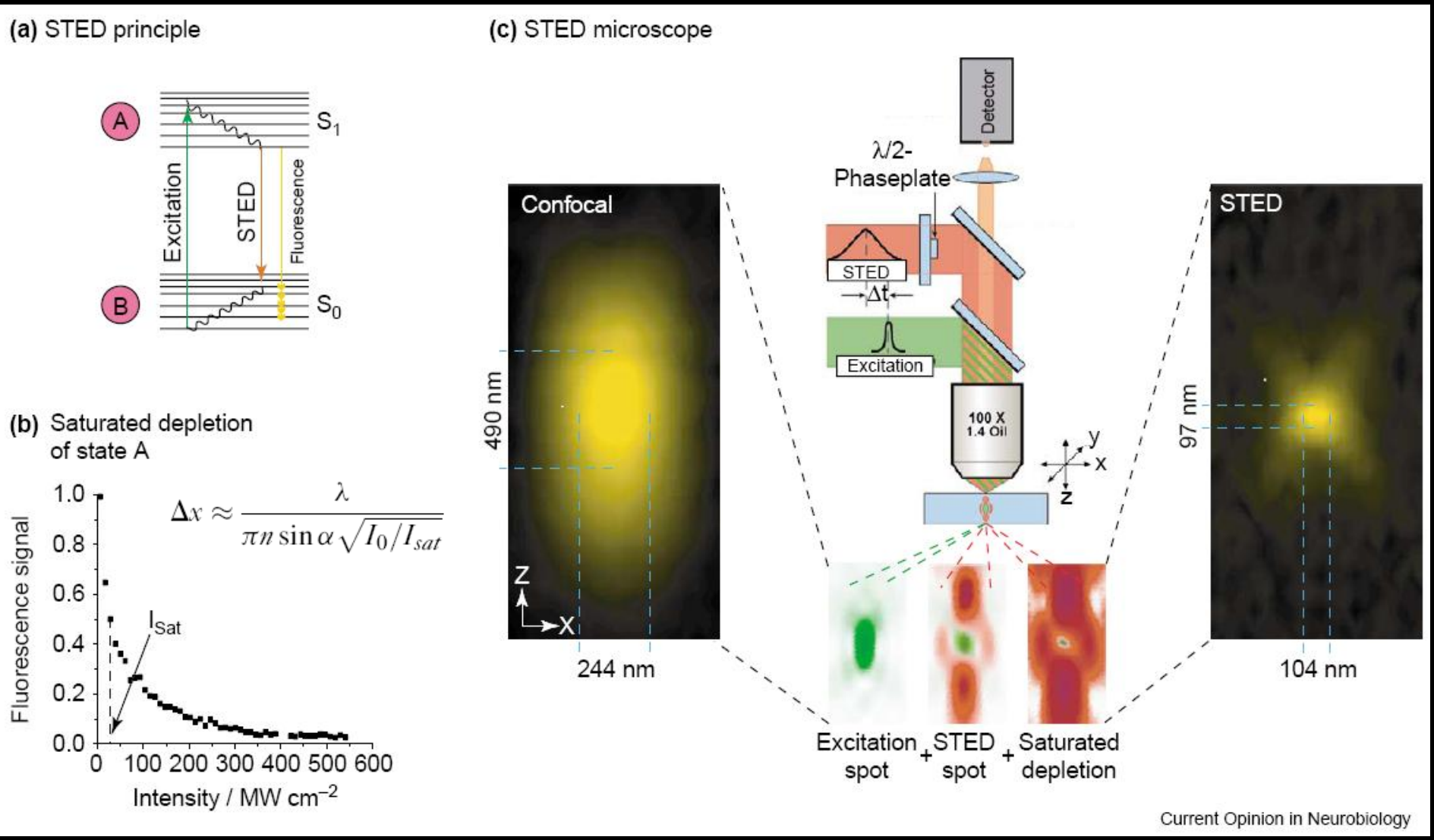
STED
Fluorescence
(b) Saturated depletion of state A
$\Delta x \approx \frac{\lambda}{\pi n \sin\alpha \sqrt{I_0/I_{sat}}}$
Fluorescence signal
I_{Sat}
1.0
0.8
0.6
0.4
0.2
0.0
0 100 200 300 400 500 600
Intensity / MW cm^{-2}
Excitation Profiles
STED Beam Profiles
I = 1000 x I_{sat}
I = 500 x I_{sat}
FWHM
(b)
I = 100 x I_{sat}
I = 50 x I_{sat}
I = 10 x I_{sat}
x
x_I
(d)
STED Excitation 3D Wireframe
Current Opinion in Neurobiology

|-> STED


(a) STED principle
A
B
S_1
S_0
Excitation
STED
Fluorescence
(b) Saturated depletion of state A
$\Delta x \approx \frac{\lambda}{\pi n \sin\alpha \sqrt{I_0/I_{sat}}}$
Fluorescence signal
1.0
0.8
0.6
0.4
0.2
0.0
I_{Sat}
0 100 200 300 400 500 600
Intensity / MW cm^{-2}
(c) STED microscope
Detector
λ/2-Phaseplate
STED
Δt
Excitation
100 X 1.4 Oil
y
x
z
Confocal
490 nm
Z
X
244 nm
STED
97 nm
104 nm
Excitation spot + STED spot + Saturated depletion
Current Opinion in Neurobiology

Laboratory for SCIAN Scientific Image Analysis

|-> STED

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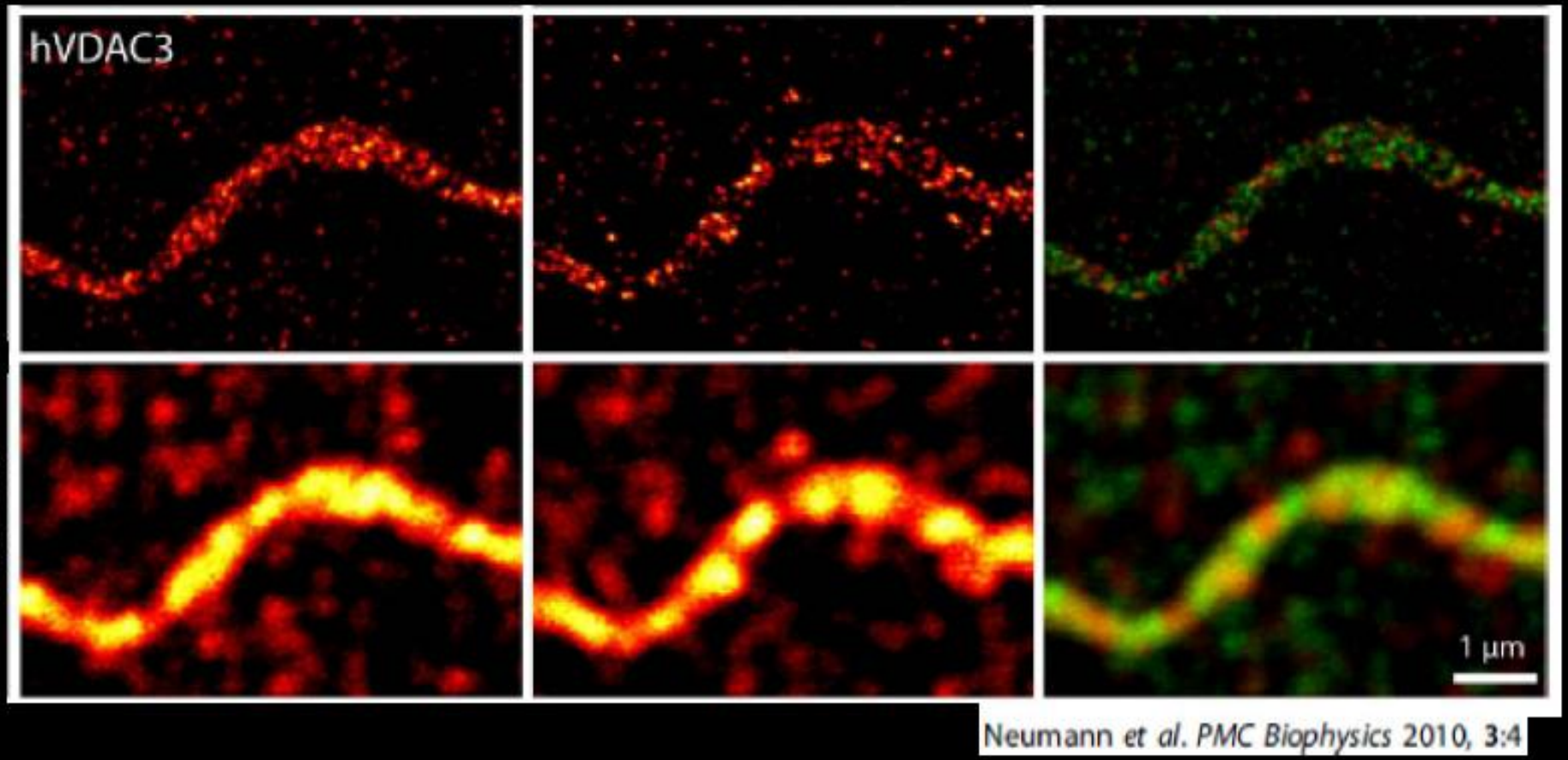
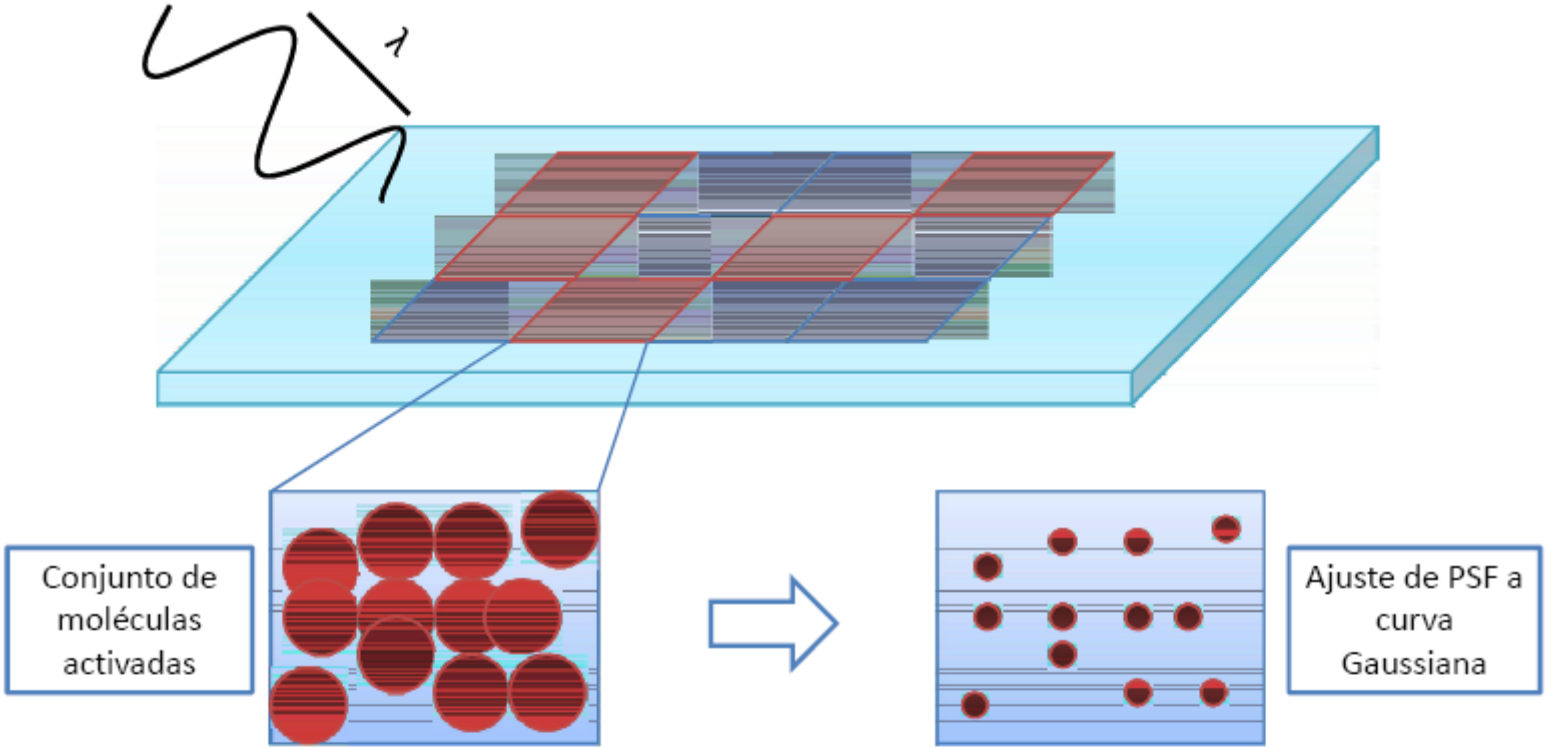


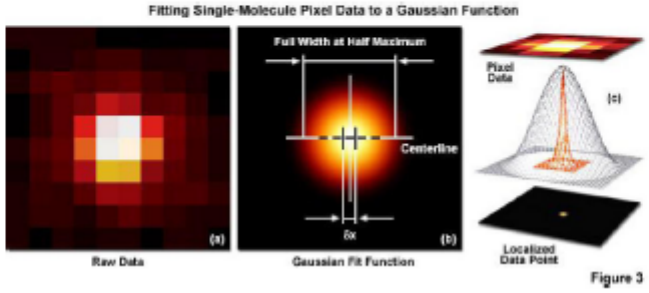
Neumann *et al. PMC Biophysics* 2010, **3**:4

Bibliography

- "Superresolution Microscopy References" http://zeiss-campus.magnet.fsu.edu/referencelibrary/superresolution.html
- "Concepts for nanoscale resolution in fluorescence microscopy" S. Hell, Marcus Dyba and Stefan Jakobs – Current Opinion in Neurobiology 2004, 14:599-609
- "Breaking The Diffraction Barrier: Super-Resolution Imaging of Cells" Bo Huang, Hazen Babcock and Xiowei Zhuang, Cell 143: 1047-1058
- "Microscopy and its focal switch" Stefan W. Hell, Nature Methods Jan. 2009, Vol. 6 N° 1, 24-32
- "Review: Far-Field Optical Nanoscopy" – S. Hell, Science May 2007, Vol 316:1153-1158
- "Microscopy: ever-increasing resolution" – Kelly Rae Chi, Nature 462: 675-678, Dec 2009

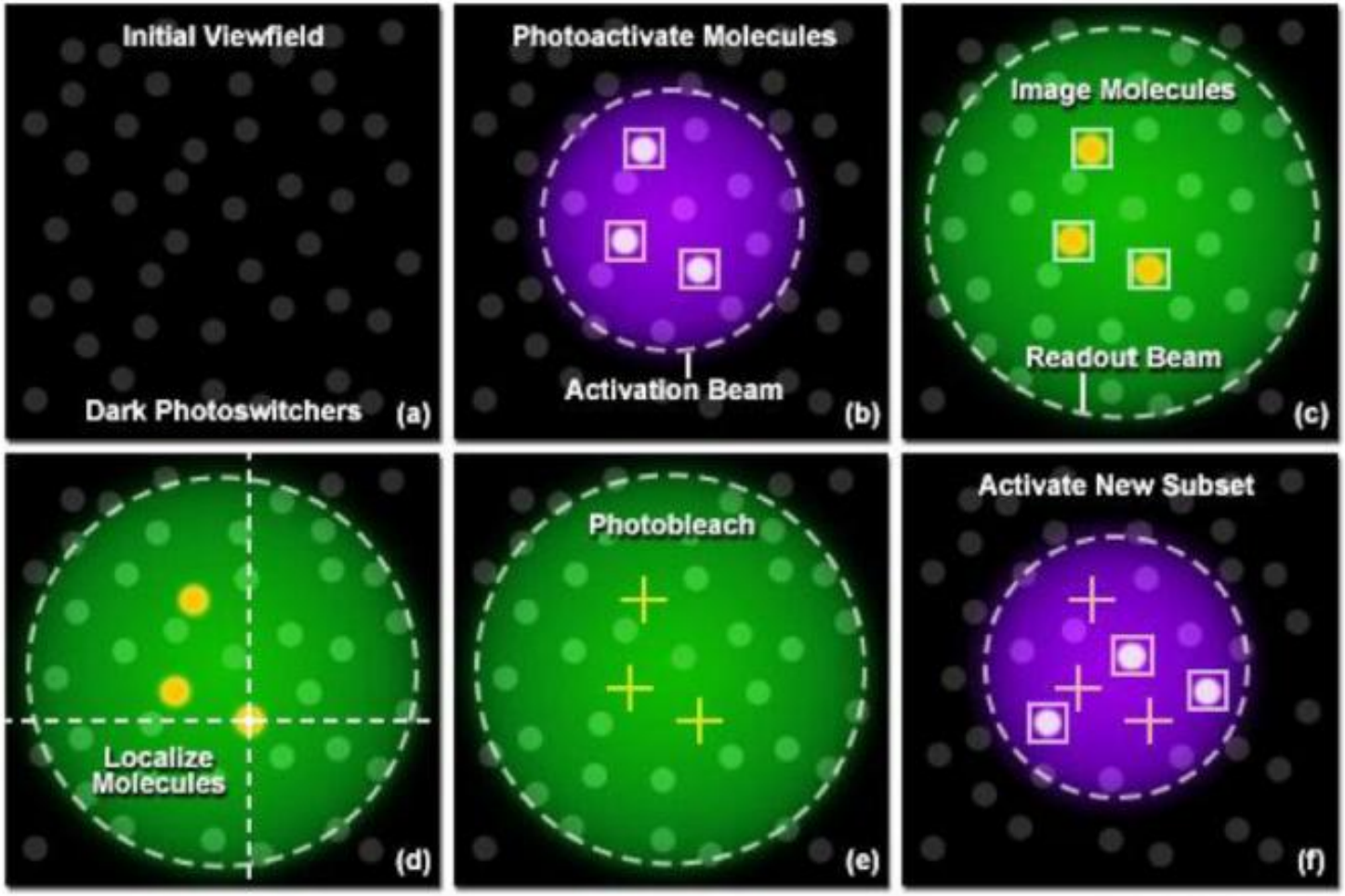
Photo-activated localization microscopy PALM



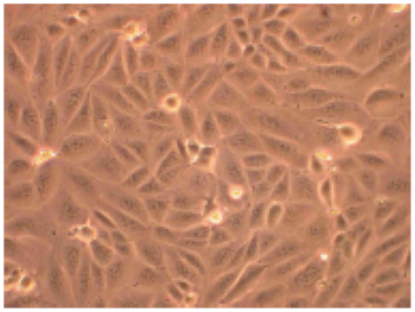


|-> STED

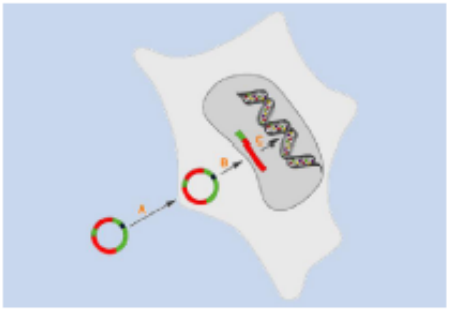
http://zeiss-campus.magnet.fsu.edu/tutorials/superresolution/palmbasics/index.html



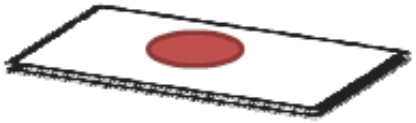
|-> PALM



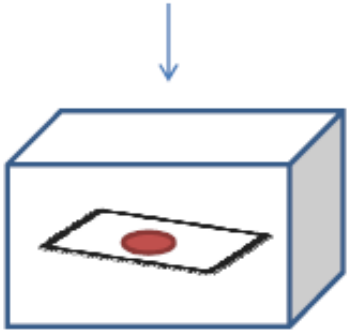
Células de Mamíferos



Transfectar



Sub-cultivo en cover´s ya sea como células individuales o cortes finos de tejido y fijar.



Camara que impide el "drift" térmico y mecánico.

Excitación breve a 405 nm

Longitud de onda de excitación 561 nm

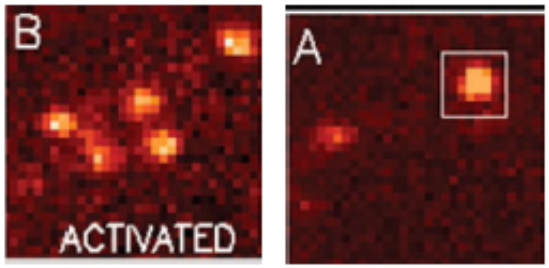
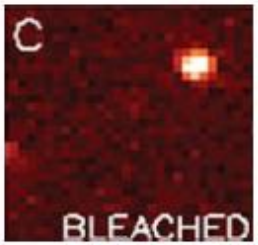


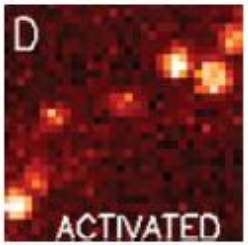
Imagen adquirida mediante TIRF y una camara capaz de captar de a un fotón (EMCCD)

Betzig et al, 2006 Science.

|-> PALM

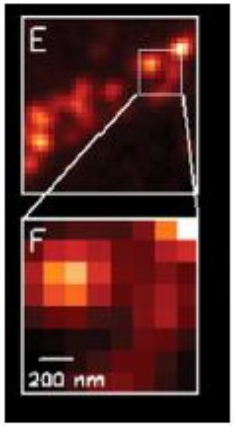


La excitación a 561 nm continua (despues de la adquisición de imágenes) hasta que ocurre el fotoblanqueamiento de la mayoría de las molécula YA activadas.



Nuevamente se entrega un pulso de 405 nm para fotoactivar las moléculas restantes.

El proceso de foto activación – adquisición y fotoblanqueamiento se repite "n" veces, hasta que todas las moléculas que fueron fotoactivadas fueran depletadas por fotoblanqueamiento.



Suma de los stacks en XY en el tiempo, sigue dando una imagen limitada por la difracción

Algoritmo no lineal de mínimos cuadrados

$$(\sigma_{x,y}^{\,2})_m \approx \frac{s^2 + a^2/12}{N_m} + \frac{4\sqrt{\pi}\, s^3 b_m^{\,2}}{a N_m^{\,2}}$$

Ajuste a una Gaussiana de la PSF en dos dimensiones.

N is the number of photons gathered,
a is the pixel size of the imaging CCD detector,
b is the standard deviation of the background (which includes background fluorescence emission combined with detector noise), and
$\mathbf{S_i}$ is the standard deviation or width of the distribution

Betzig et al, 2006 Science.

|-> STED

Localization Resolution and Molecular Density in PALM

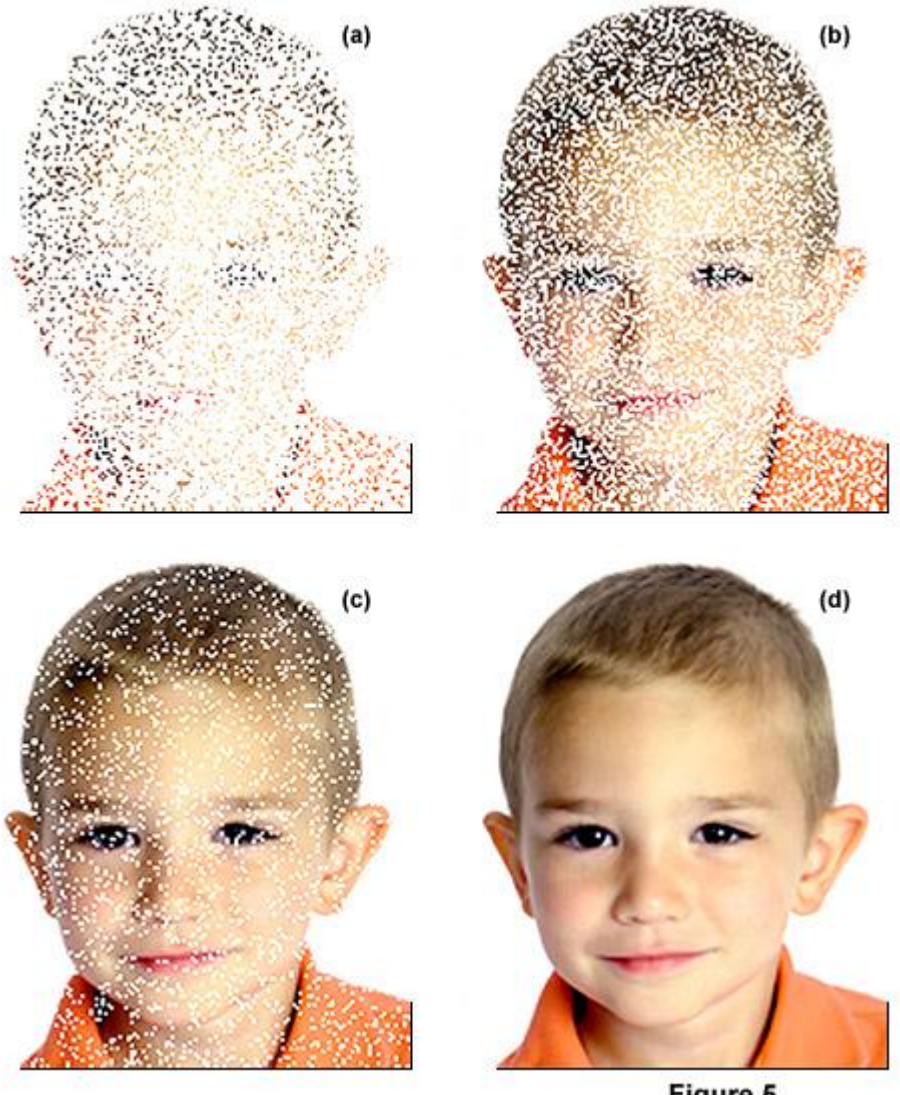
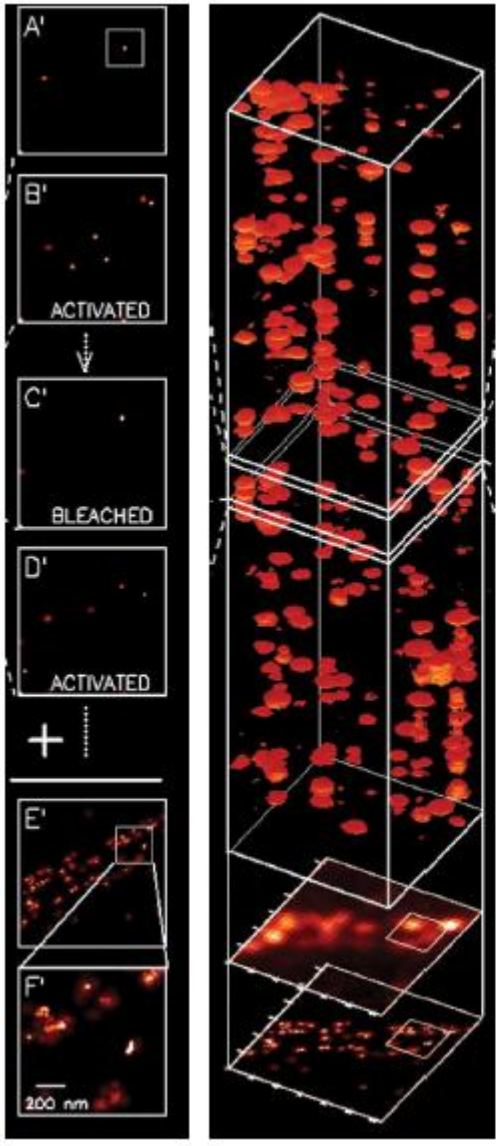
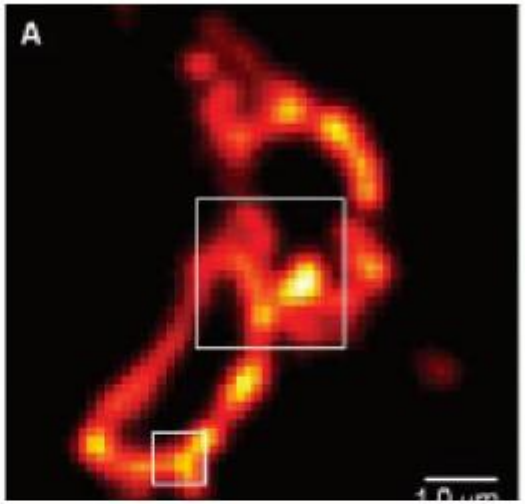


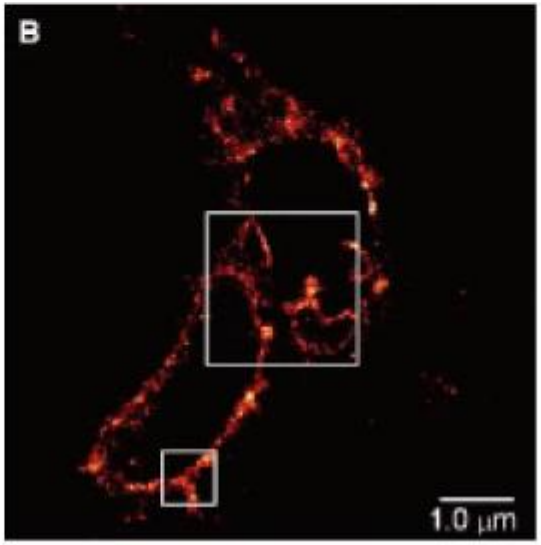
Figure 5

|-> PALM



Betzig et al, 2006 Science.





Células Cos-7 que expresan l a proteína de transmembrana lisosomal CD 63 marcada con Kaede

|-> PALM

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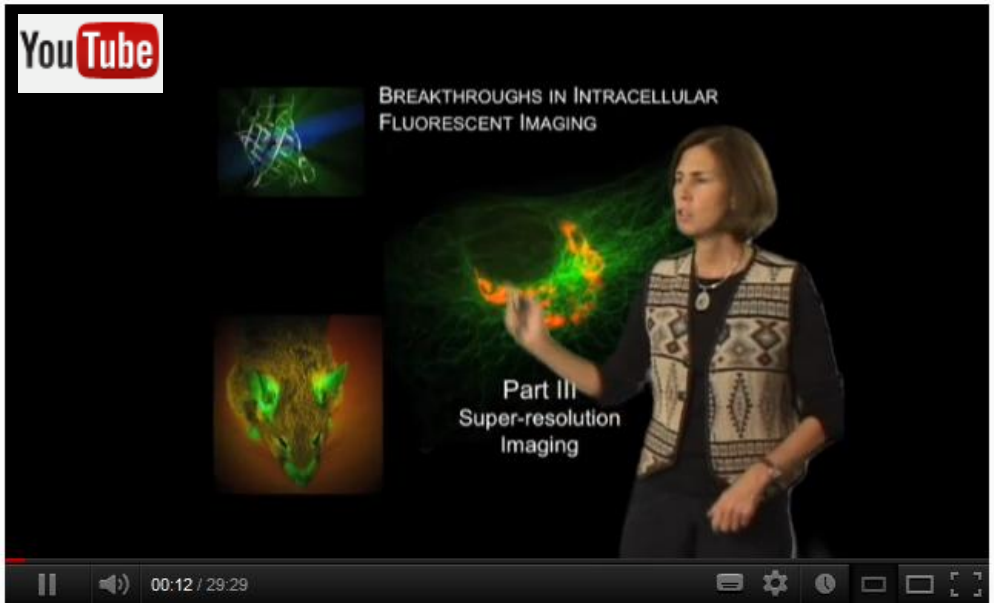
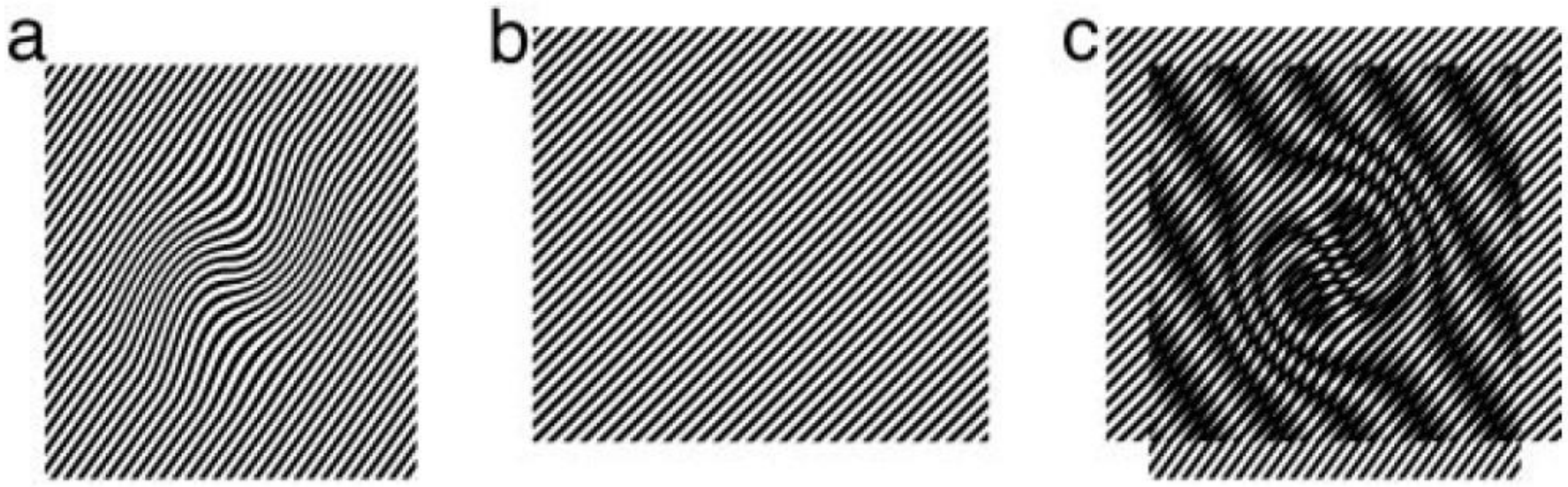


Jennifer Lippincott-Schwartz

Super Resolution Imaging

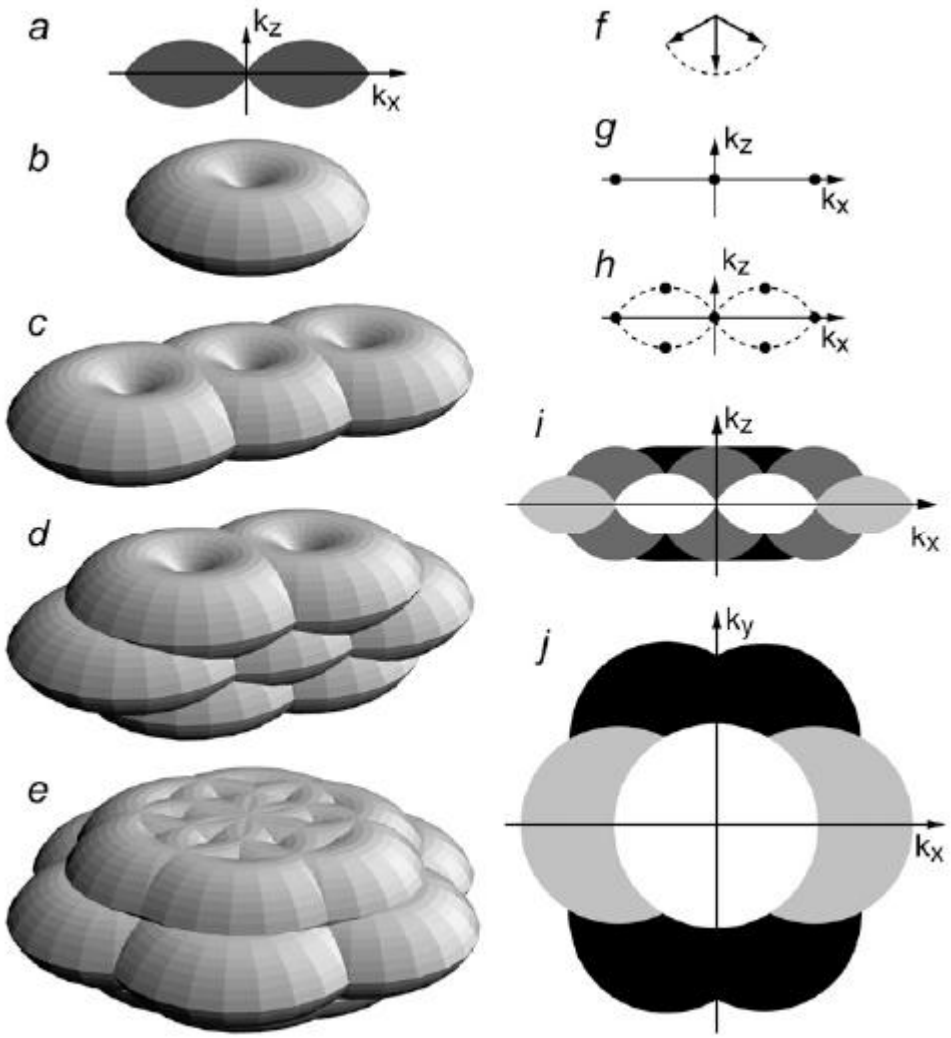
Nature Methods: Method of the Year 2008

http://www.youtube.com/watch?v=UCJ6oQSdxN0&feature=related

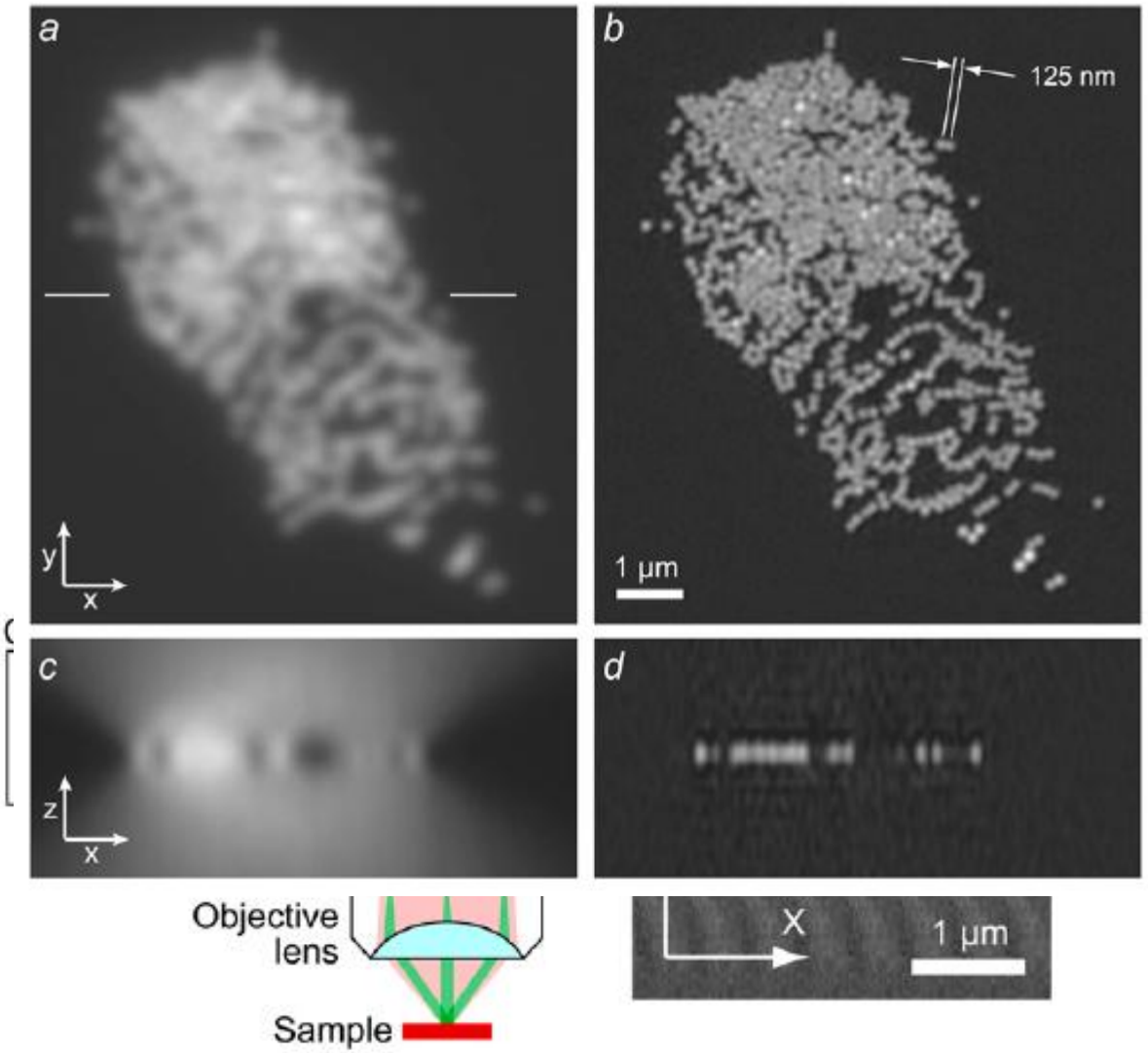


http://www.youtube.com/watch?v=EM5YuOZiAII&feature=related

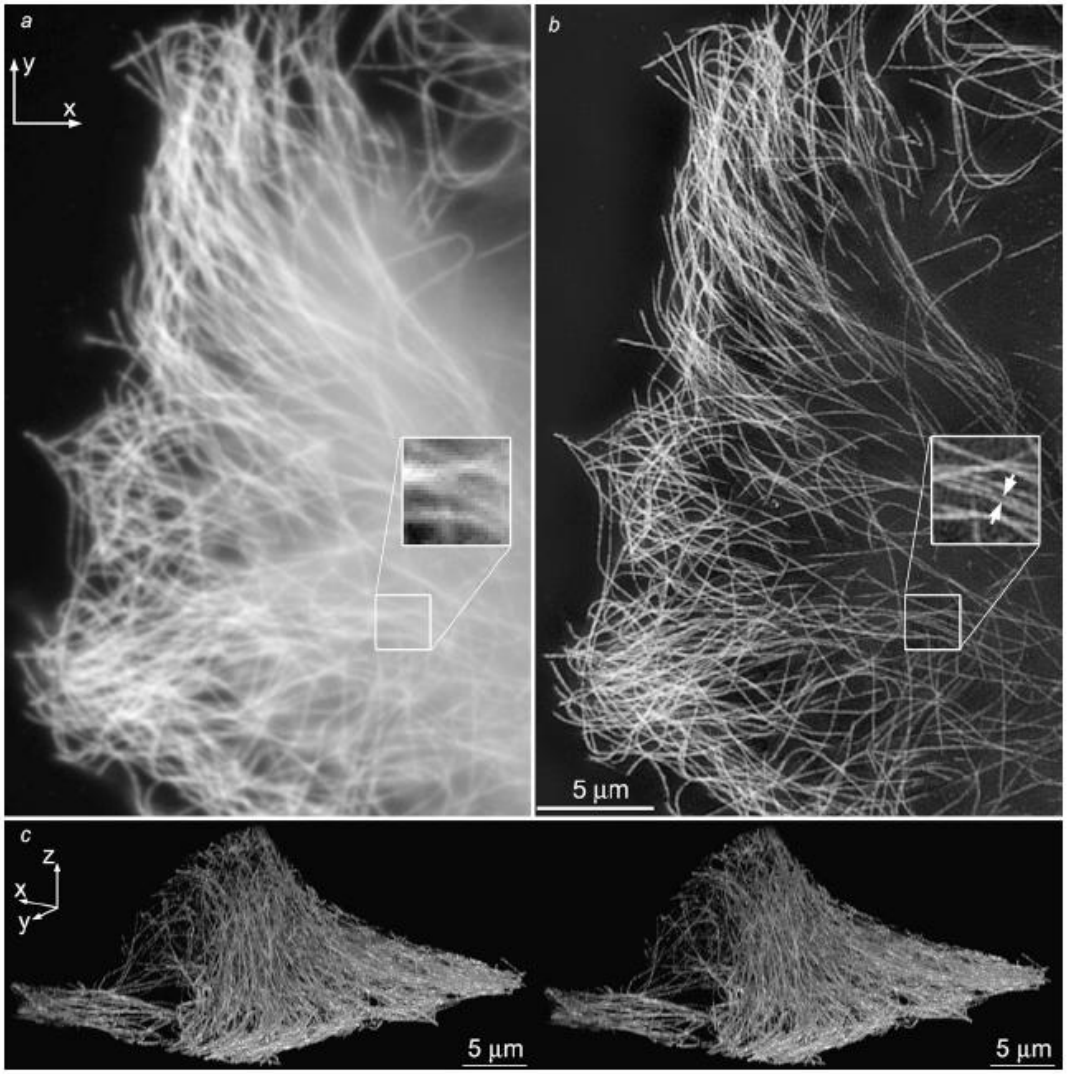
|-> STED



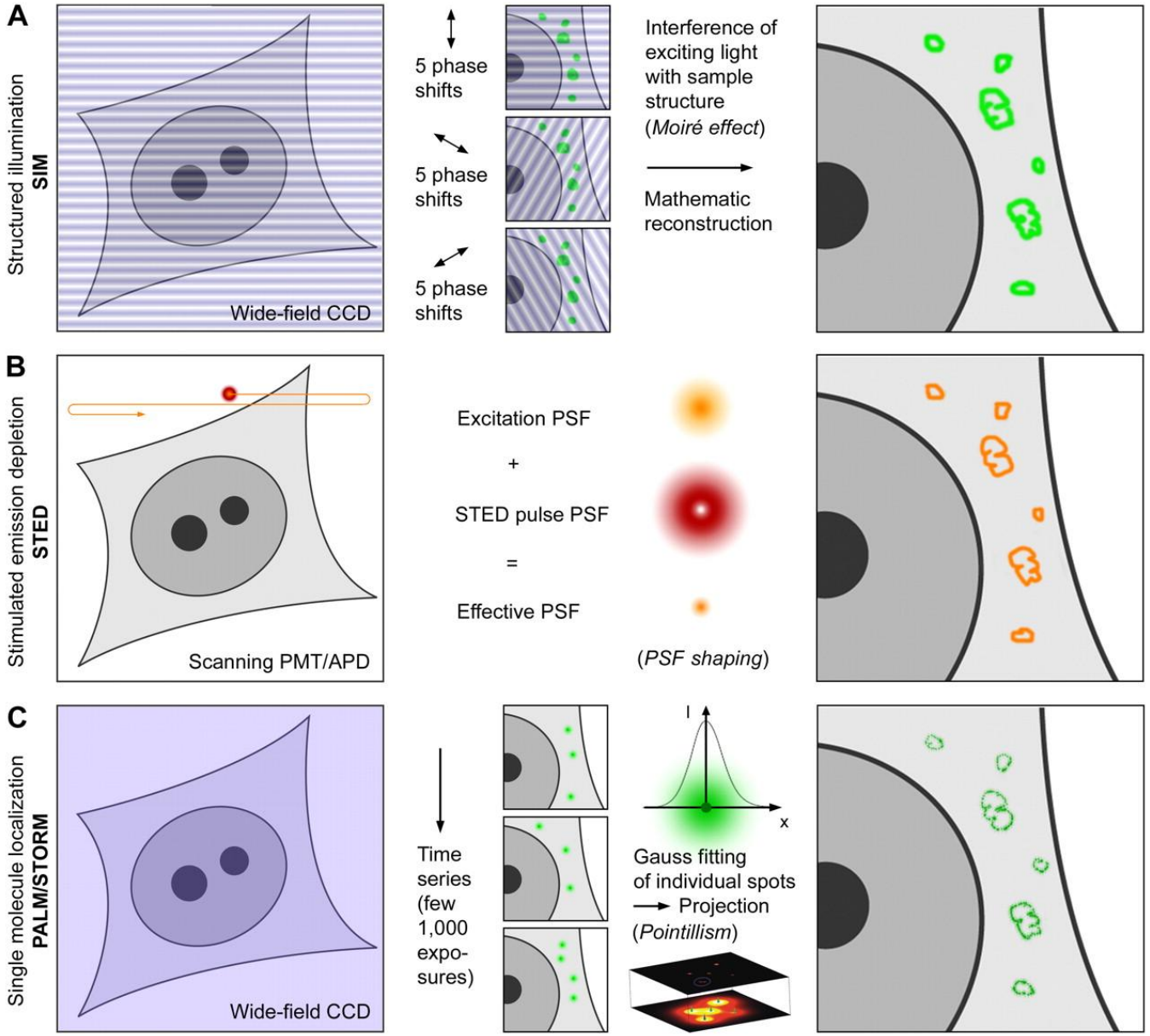
|-> SIM



|-> STED



|-> SIM STED PALM



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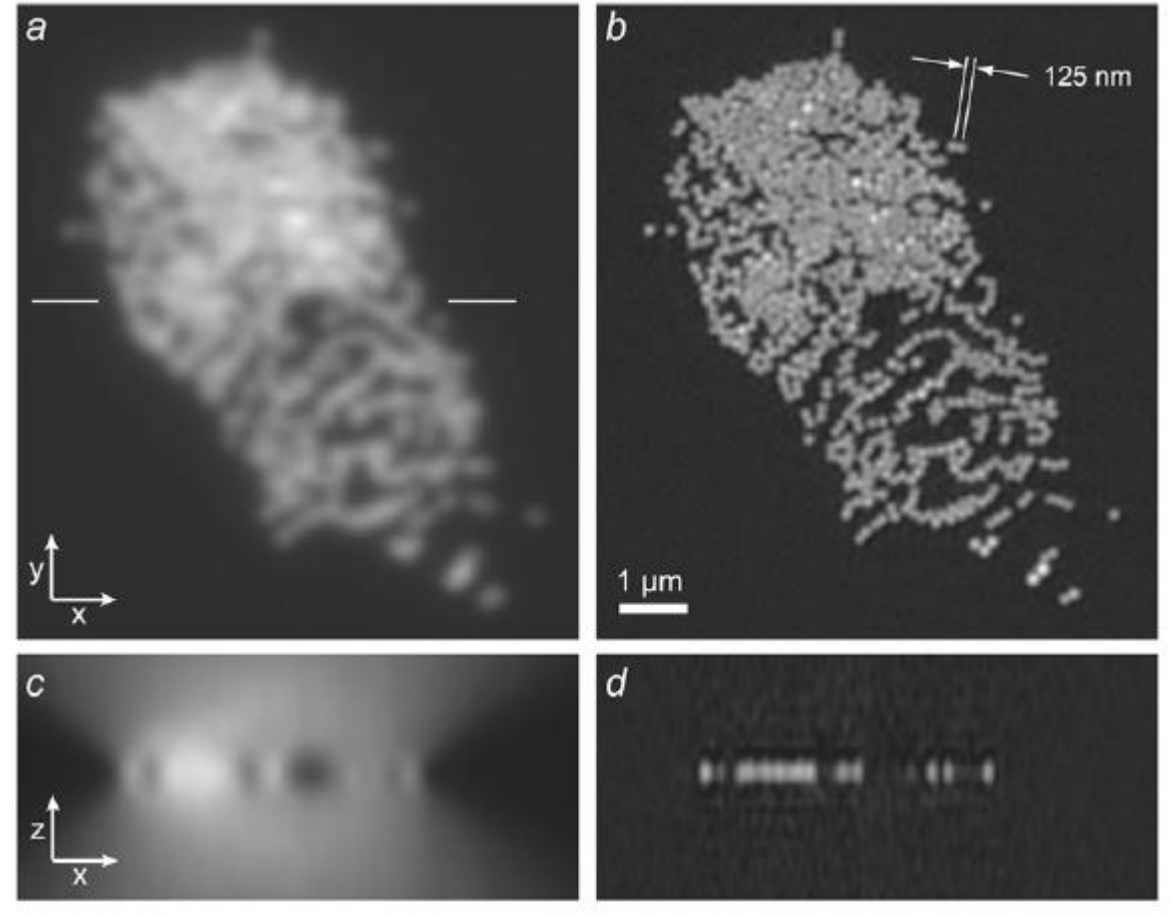
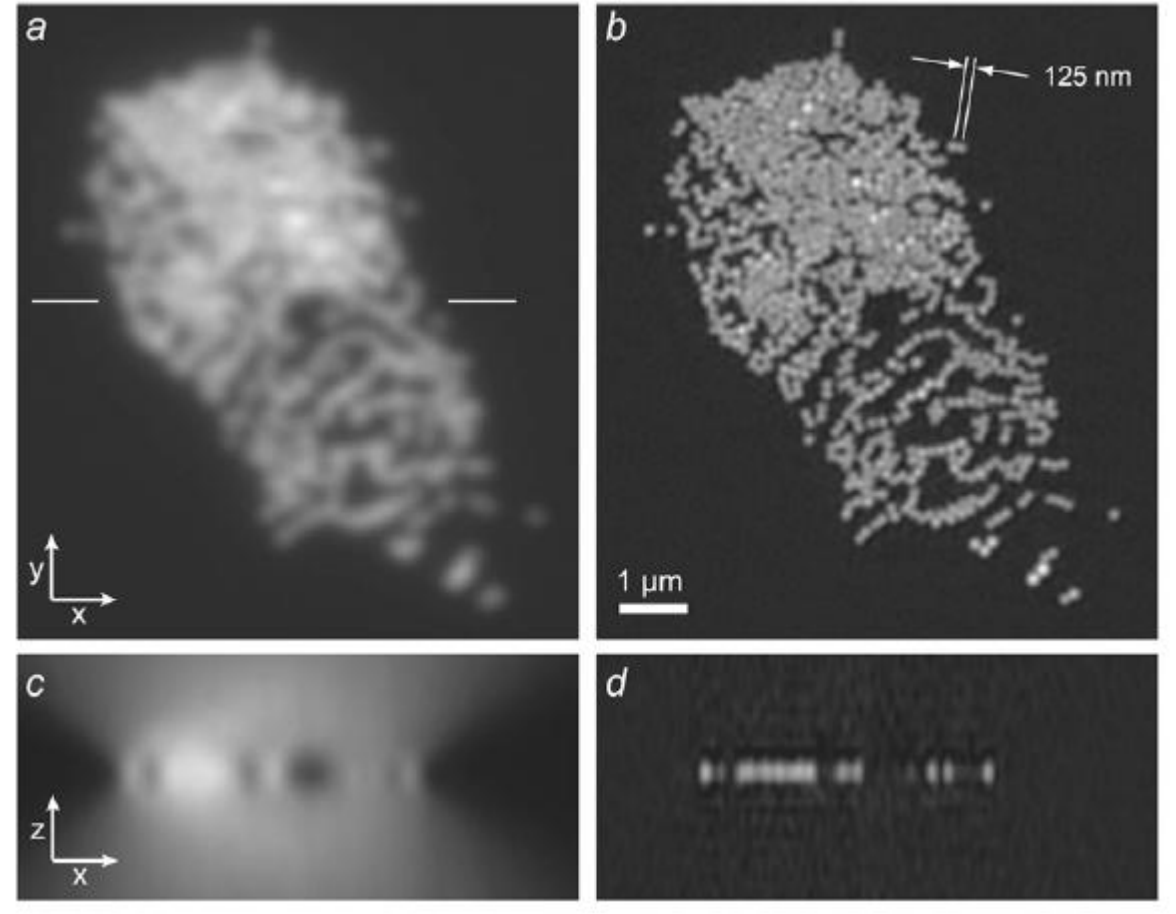
|-> STED