Laboratory for SCIAN Scientific Image Analysis

Welcome

Biomedical Neuroscience Institute

CIMT Centro de Informatica Medica y Telemedicina

MIM 2024: Procesamiento de Imágenes y Bioseñales I & II

MIM Magister en Informatica Medica

Procesamiento de Imágenes y Bioseñales I

Prof. Dr. Steffen Härtel / Dr. Jorge Jara: www.scian.cl / www.cimt.cl / www.cens.cl

Dir Laboratory of Scientific Image Analysis (SCIAN-Lab)
Dir Centro de Informática Medica y Telemedicina (CIMT)
Centro Nacional en Sistemas de Información en Salúd (CENS)
Biomedical Neuroscience Institute (BNI)
Innovation @ Institute of Biomedical Sciences (ICBM)
Cento de Modelamiento Matematico (CMM)
Faculty of Medicine, University of Chile
Red de Salud Digital de las Universidades del Estado (RSDUE)

Facultad de Medicina Universidad de Chile

Temas para Seminarios Curso I-II

Patología digital/microscopía virtual, Tissue Scanner *(Francisca Valdés)*
Diego Ormeño y Michelle Pacheco

Microscopía y Microbiología de Expansión, Publicación de Métodos (Dante Castagnini, Steffen Hartel)
Proteus mirabilis biofilm expansion microscopy for preparation as Methods Paper: Journal of Visualized Experiments. www.jove.com
Aníbal Molina y Felipe Carrasco

Radiología & IA, CIMT/HCUCH (Constanza Vásquez), Cristóbal Pineda y Javiera León / Magdalena Sanhueza y Tatiana Boza

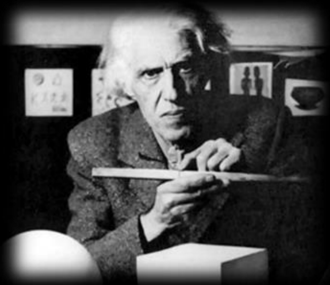
ALPACA I: SCIAN-Drop & SCIAN-Force, estimación de fuerzas y modelos de contornos celulares/droplets (Jorge Jara, Karina Palma, Steffen Hartel)
Muriel Ponce y Rolando Vernal

ALPACA II: segmentación y modelos de contorno (Jorge Jara, Mauricio Cerda)
Iván Roa y Pablo Cabello

Colocalización de marcadores de DAMPS en células del sistema inmune (Fermín González, Karina Palma, Steffen Hartel)
Alfredo Torres, Fabián Tempio & estudiantes

Tatiana Boza	MIM	The Good, the Bad and the Ugly		29. Aug
Pablo Cabello	MIM	Proteus mirabilis biofilm expansion microscopy yields over 4-fold magnification for super-resolution of biofilm structure and subcellular DNA organization		23. Sep
Felipe Carrasco	MIM	4.1.6. Steady-state and Time Resolved Fluorescence	1.7 Steady-state and Time	10. Sep
Javiera León	MIM	Coelho *et al.* (2009) Descriptores de Similaridad/Calidad en Segmentación		08. Okt
Anibal Molina	MIM	4.1.2. Jablonski Diagram	1.2 Jablonski Diagram	05. Sep
Diego Ormeño	PhD(c), M. Cáceres	4.1.4. Fluorescence Anisotropy	1.5 Fluorescence Anisotropy	23. Sep
Michelle Pacheco	MIM	4.1.1. Phenomena of Fluorescence	1.1 Phenomena of Fluorescence	05. Sep
Cristóbal Pineda	MIM	Cap. 3: Histogramas		07. Okt
Muriel Ponce	MIM	4.1.4. Fluorescence Lifetimes	1.4 Fluorescence Lifetimes and Quantum Yields	23. Sep
Iván Roa	MIM	Cap. 7: Chain codes		08. Okt
Magdalena Sanhueza	MIM	4.1.3. Fluorescence Emission	1.3 Characteristics of Fluorescence Emission	05. Sep
Rolando Vernal	Odonto	Cap. 2/4: Convolución, Filtros basados en convolución		07. Okt
Alfredo Torres Fabián Tempio Estudiantes	Fermín G.	Seeing is believing? A beginners' guide to practical pitfalls in image acquisition. Alison J. North. 2006 The Journal of Cell Biology, 172(1):9-18		29. Aug

Welcome

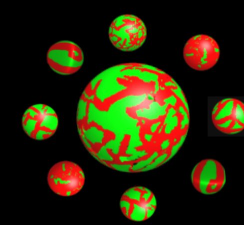


Joaquín Torres García 1874-1949

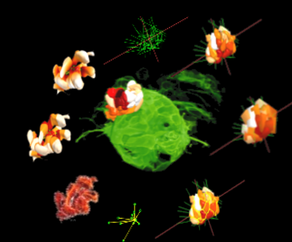
Richard Feynman (1918-1988)

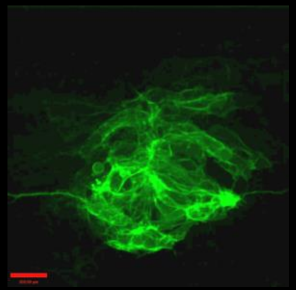
Maria Goeppert-Mayer 1906-1972

René Descartes (1596-1650)



Mats Gustafson 2006 - 2011



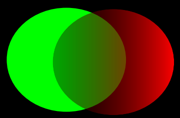


Ernst Abbe 1840- 2005

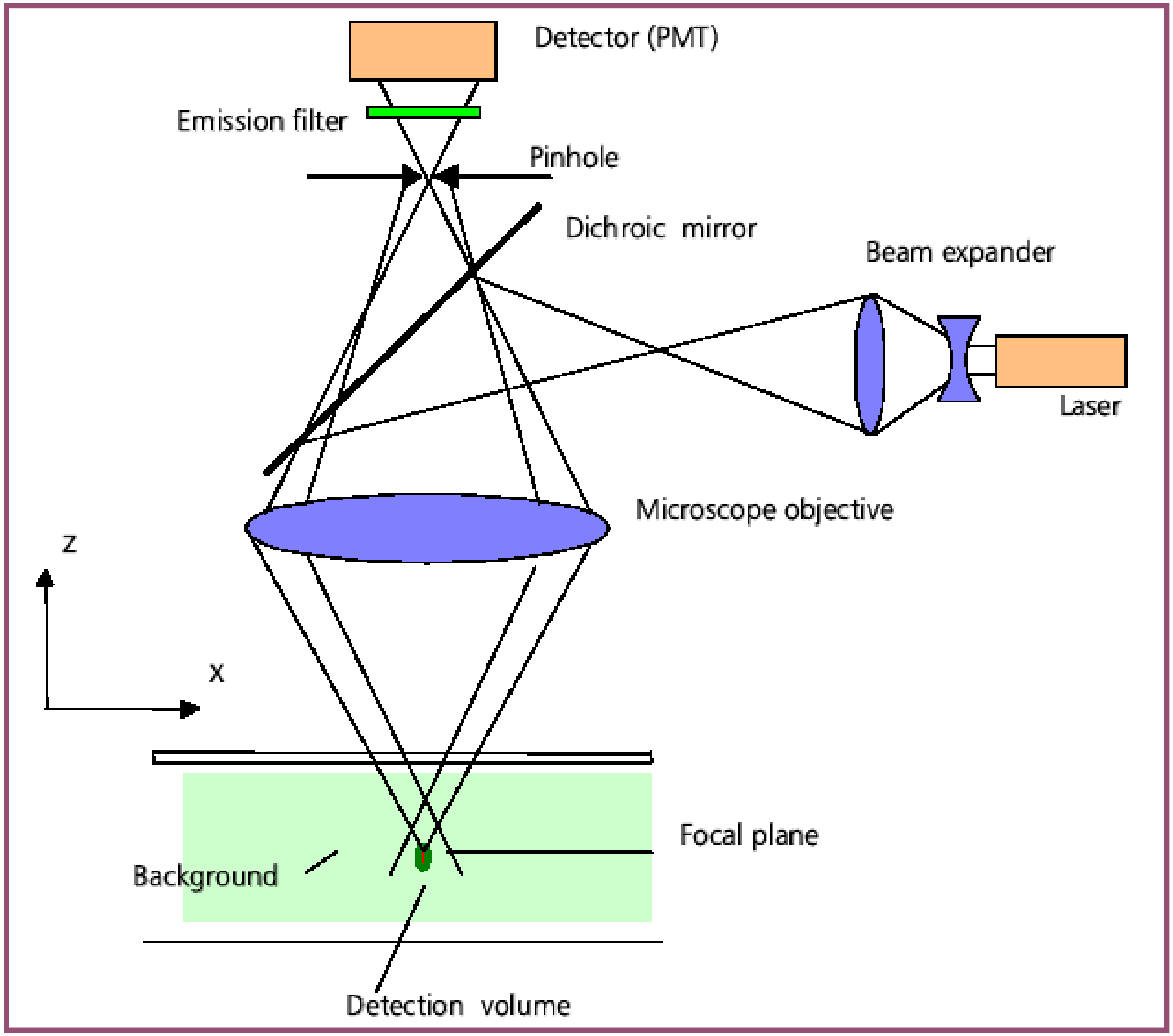
E Betzig

S Hell

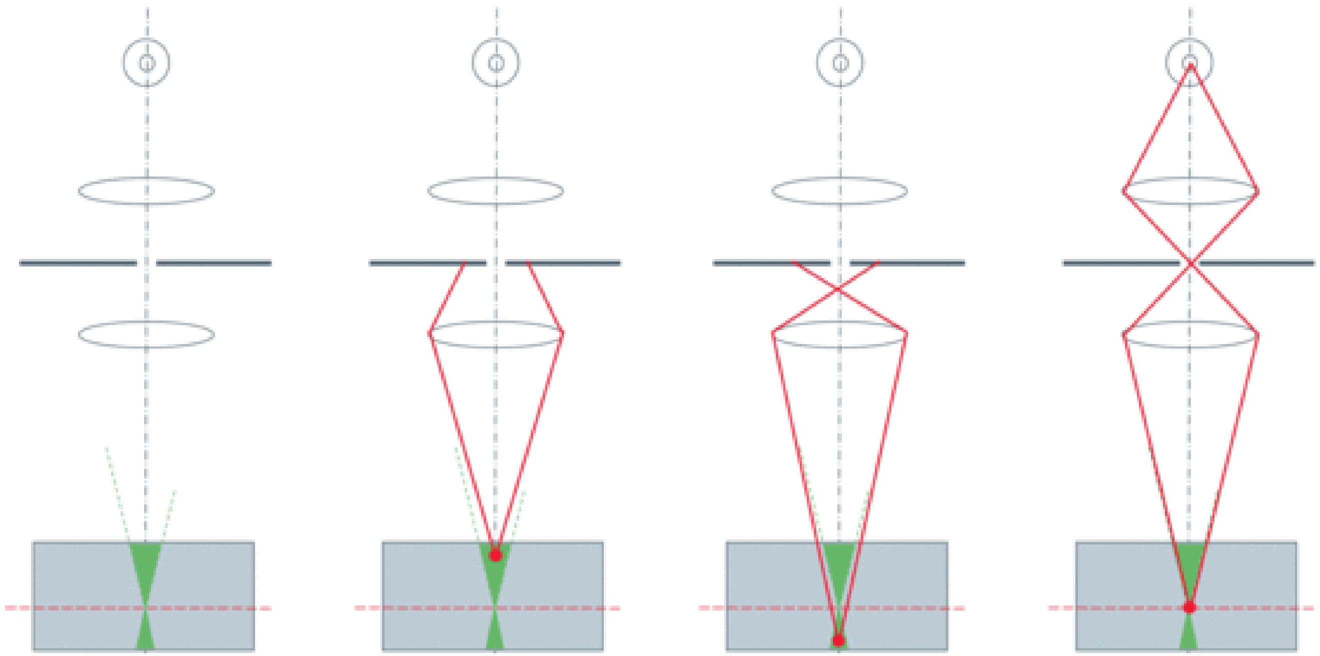
W Moerner



|-> Diffraction limited Microscopy



|-> Diffraction limited Microscopy



Rebanada óptica en µm, modificable según Airy units del pinhole

http://www.leica-microsystems.com/science-lab/confocal-microscopy-optical-path/

|-> Diffraction limited 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.

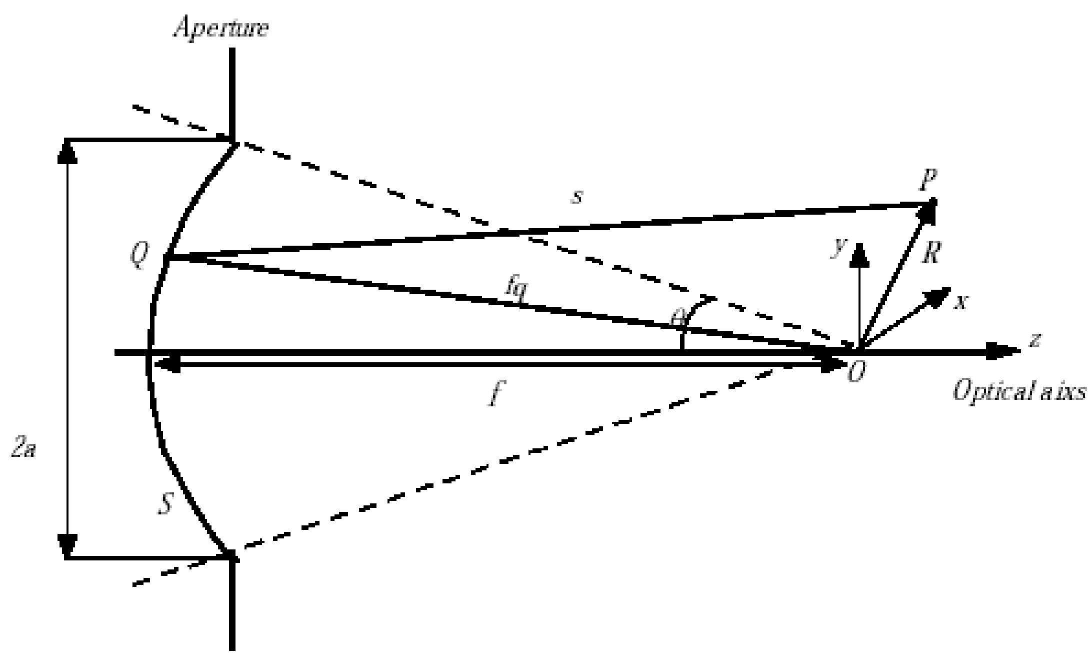


Figure 2.1 Diffraction of a converging spherical wave at a circular aperture

|-> Diffraction limited Microscopy

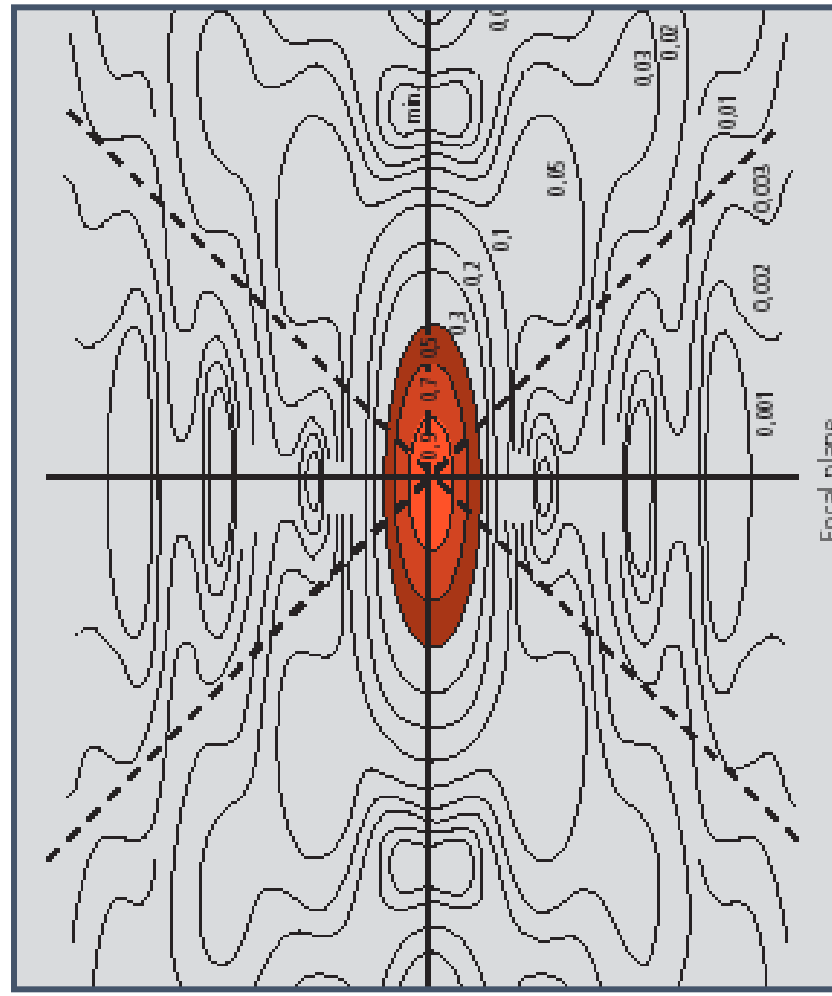
Óptica no-geométrica /

Teoría de difracción

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

U, Integral de Difracción de Kirhoff

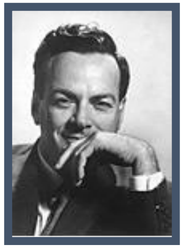
J_0, Serie de funciones de Bessel

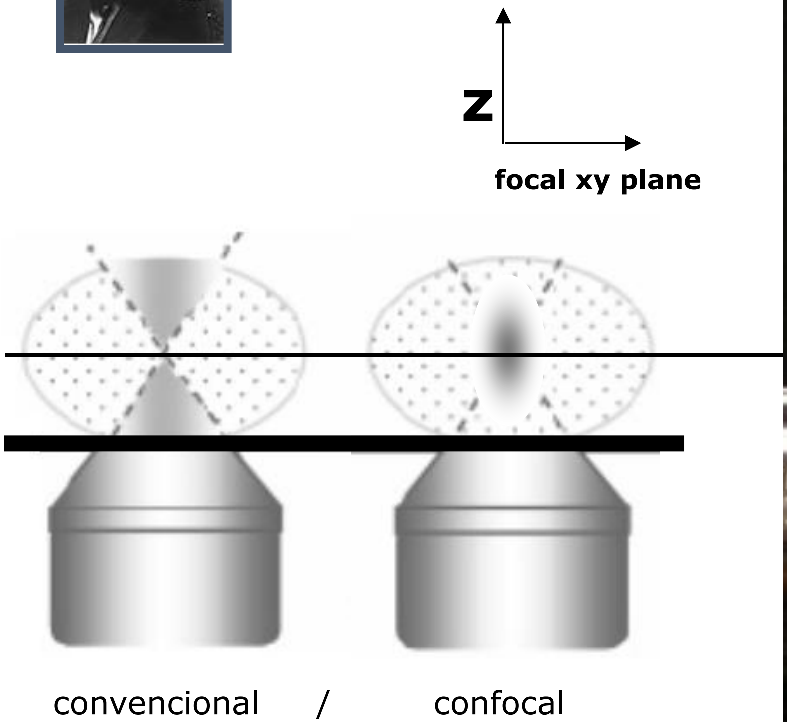


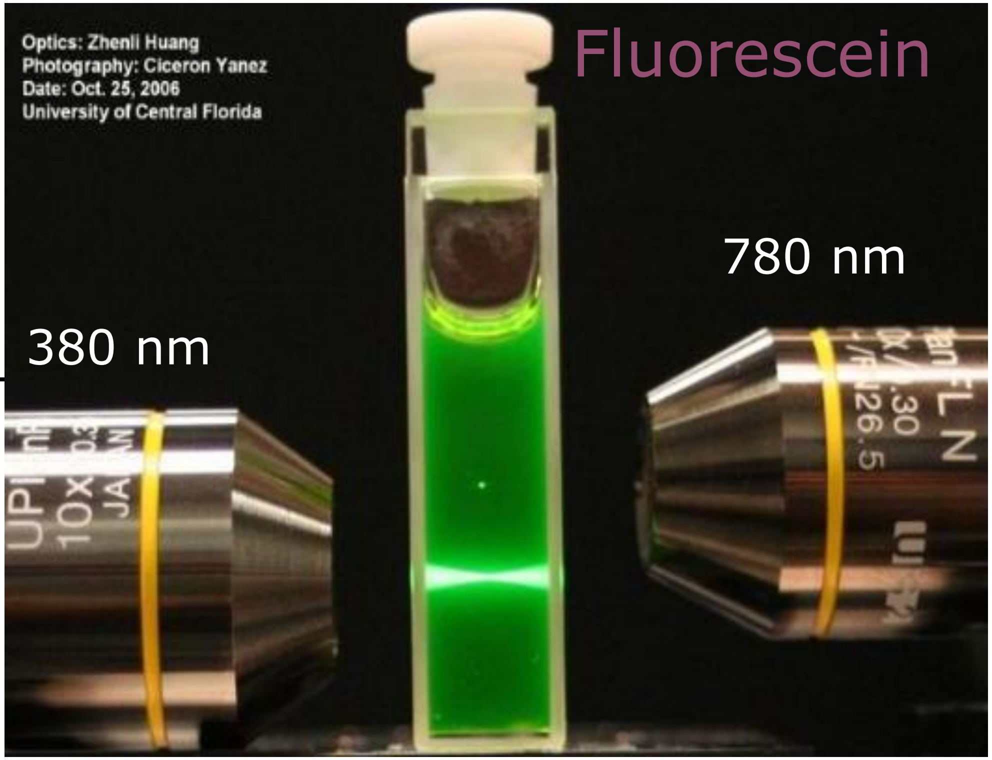
(Born & Wolf, Priniples of Optics, 6th edition 1988, Pergamon Press)

|-> Diffraction limited Microscopy

| Best localization: confocal microscopy



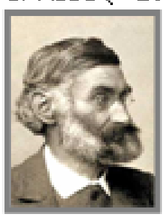




|-> Diffraction limited Microscopy

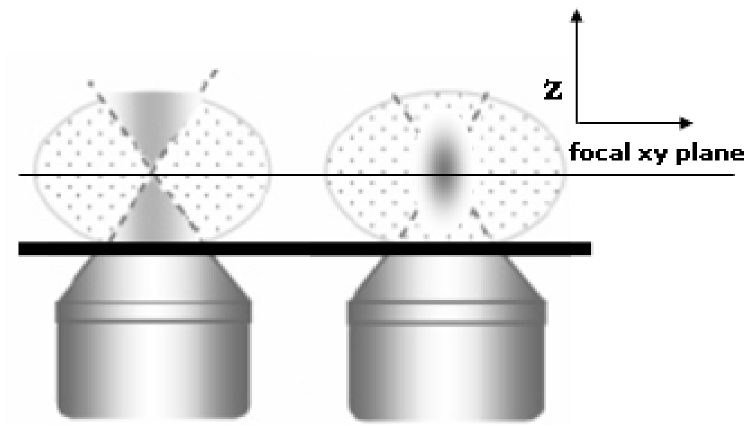
| Diffraction limited microscopy

E. Abbe († 1905)

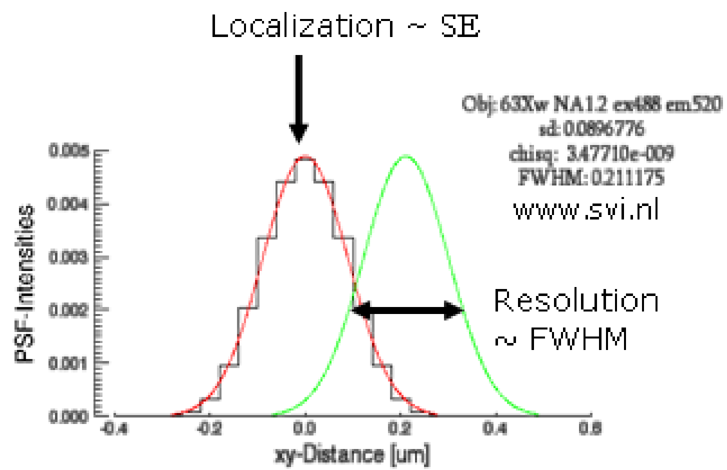


$\lambda / 2 \cdot NA \sim \lambda / 2$ Resolution (Full Width at Halft Maximum, FWHM)

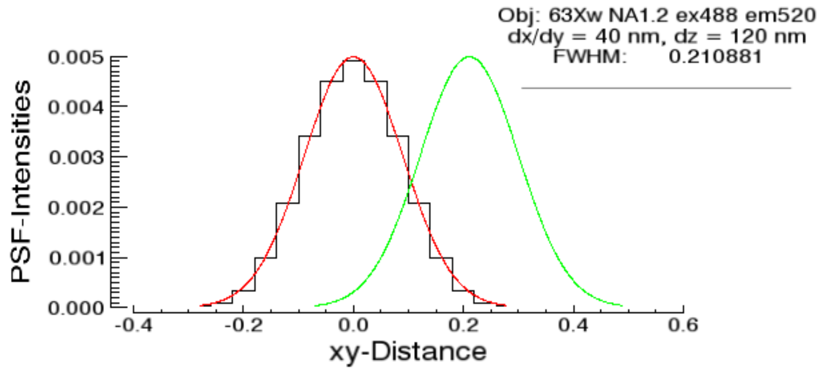
$FWHM / N^{1/2}$ Localization, N number of photons

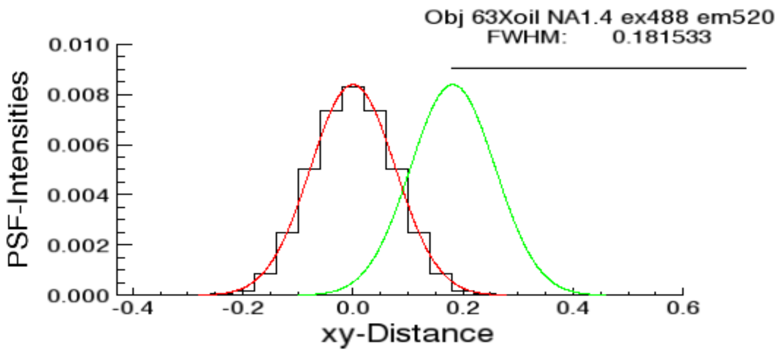


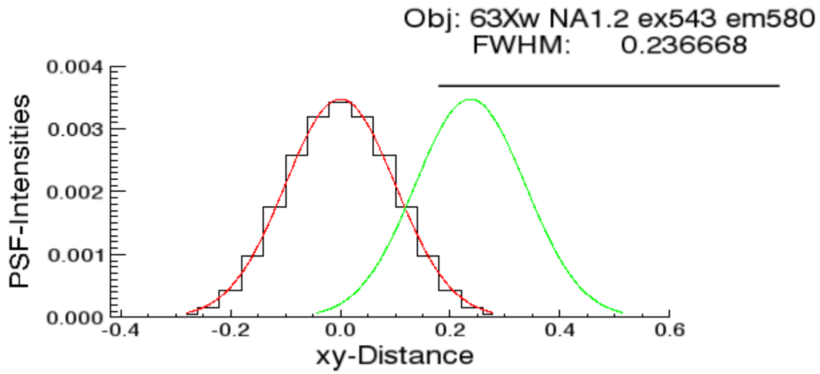
conventional / confocal

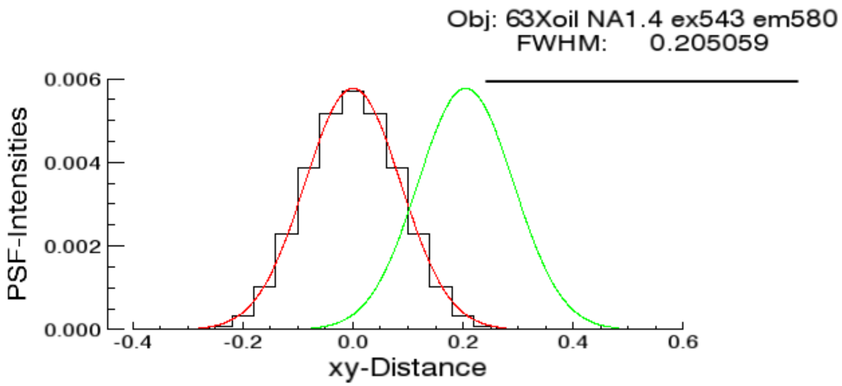


|-> Diffraction limited Microscopy

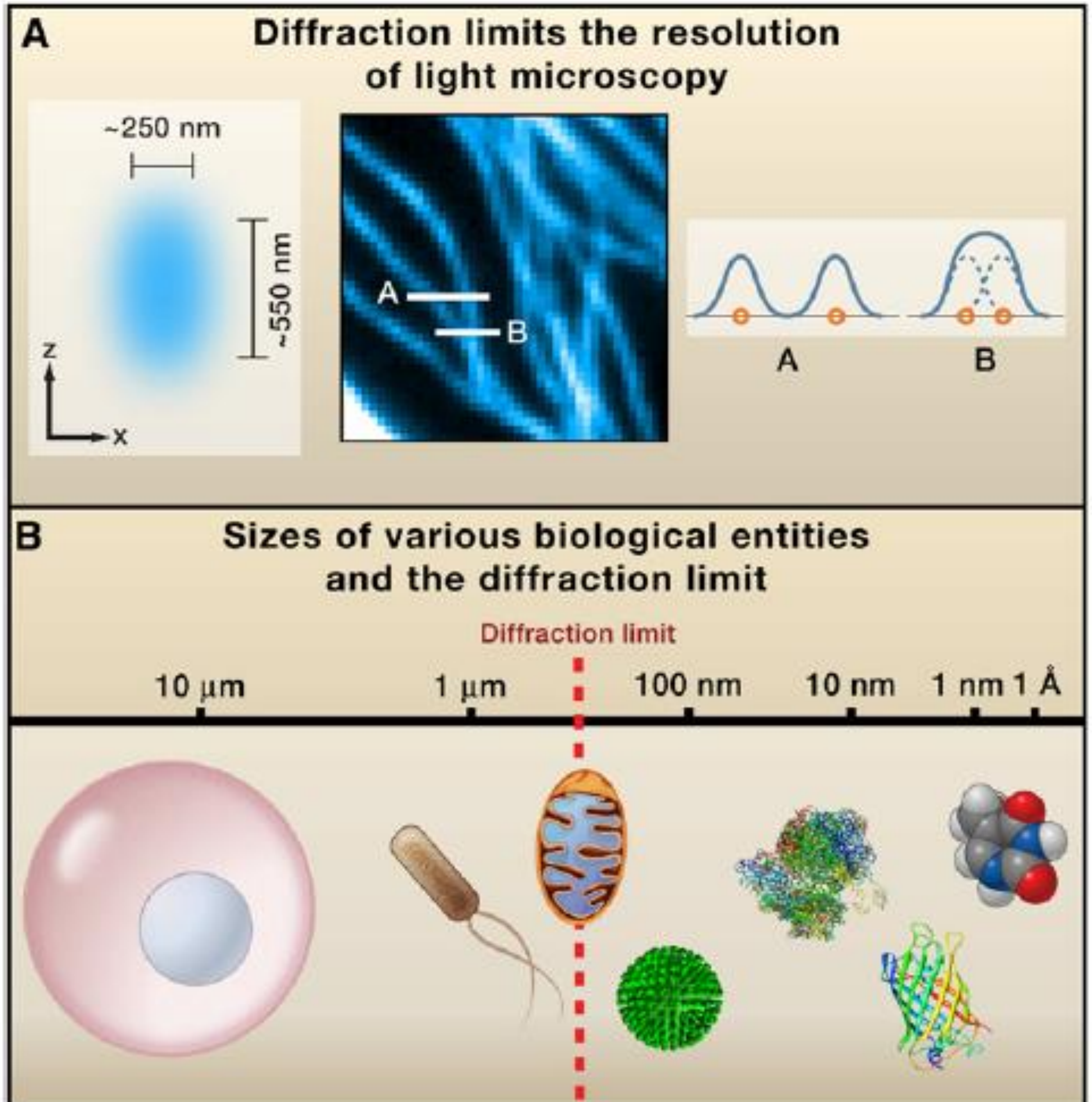








|-> Diffraction limited Microscopy



Laboratory for SCIAN

Scientific Image Analysis

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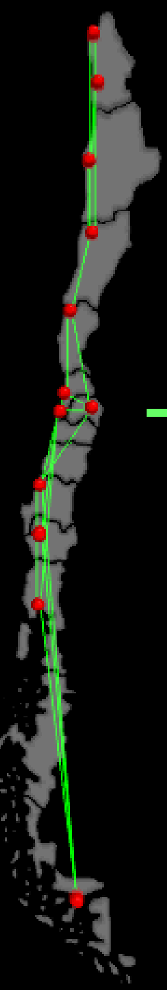
Very Large Telescope (VLT), 4 Telescopes, 8m, 2600 m

Atacama Large Millimeter/submillimeter Array (ALMA), 66 Antenna, 5000 m

E-ELT European Extremely Large Telescope, 39 m, 3000 m



Extraterrestrial Monster Science produces:
TeraB, PetaB, ExaB, ZettaB, YottaB

… Tera is a unit prefix in the metric system denoting multiplication by 10E12 or 1.000.000.000.000 !

… Tera is derived from Greek τέρας (teras), meaning “monster”.

… Tera was confirmed for use in the SI in 1960…. Wiki !

Metric prefixes

Prefix	Symbol	1000^m	10^n	Decimal	English word[n 1]	Since[n 2]
yotta	Y	1000^8	10^{24}	1 000 000 000 000 000 000 000 000	septillion	1991
zetta	Z	1000^7	10^{21}	1 000 000 000 000 000 000 000	sextillion	1991
exa	E	1000^6	10^{18}	1 000 000 000 000 000 000	quintillion	1975
peta	P	1000^5	10^{15}	1 000 000 000 000 000	quadrillion	1975
tera	T	1000^4	10^{12}	1 000 000 000 000	trillion	1960
giga	G	1000^3	10^9	1 000 000 000	billion	1960
mega	M	1000^2	10^6	1 000 000	million	1960
kilo	k	1000^1	10^3	1 000	thousand	1795
hecto	h	$1000^{2/3}$	10^2	100	hundred	1795
deca	da	$1000^{1/3}$	10^1	10	ten	1795
		1000^0	10^0	1	one	–
deci	d	$1000^{-1/3}$	10^{-1}	0.1	tenth	1795
centi	c	$1000^{-2/3}$	10^{-2}	0.01	hundredth	1795
milli	m	1000^{-1}	10^{-3}	0.001	thousandth	1795
micro	µ	1000^{-2}	10^{-6}	0.000 001	millionth	1960
nano	n	1000^{-3}	10^{-9}	0.000 000 001	billionth	1960
pico	p	1000^{-4}	10^{-12}	0.000 000 000 001	trillionth	1960
femto	f	1000^{-5}	10^{-15}	0.000 000 000 000 001	quadrillionth	1964
atto	a	1000^{-6}	10^{-18}	0.000 000 000 000 000 001	quintillionth	1964
zepto	z	1000^{-7}	10^{-21}	0.000 000 000 000 000 000 001	sextillionth	1991
yocto	y	1000^{-8}	10^{-24}	0.000 000 000 000 000 000 000 001	septillionth	1991

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"It is very easy to answer many of these fundamental biological questions. You just look at the thing !

Make microscopes a hundred times more powerful and many problems of biology would be made very much easier."

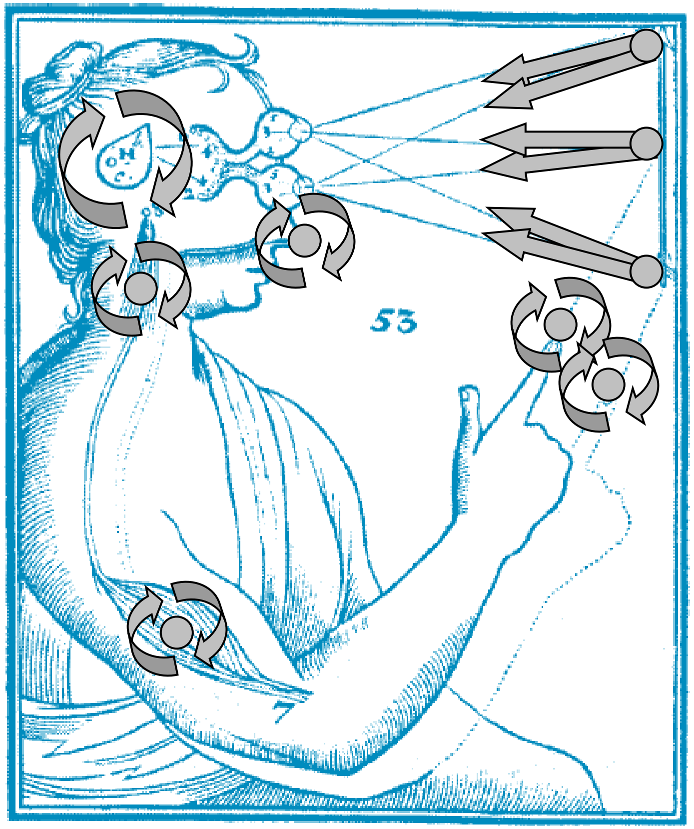
Richard Feynman (1918-1988)

... just look at the thing ...
¿ Human visual perception ?

***Treatise of man* (~ 1637)**

***Passions of the soul* (~ 1649)**

René Descartes (1596-1650)



***glandula pinealis* / pineal organ**

A combination of ...

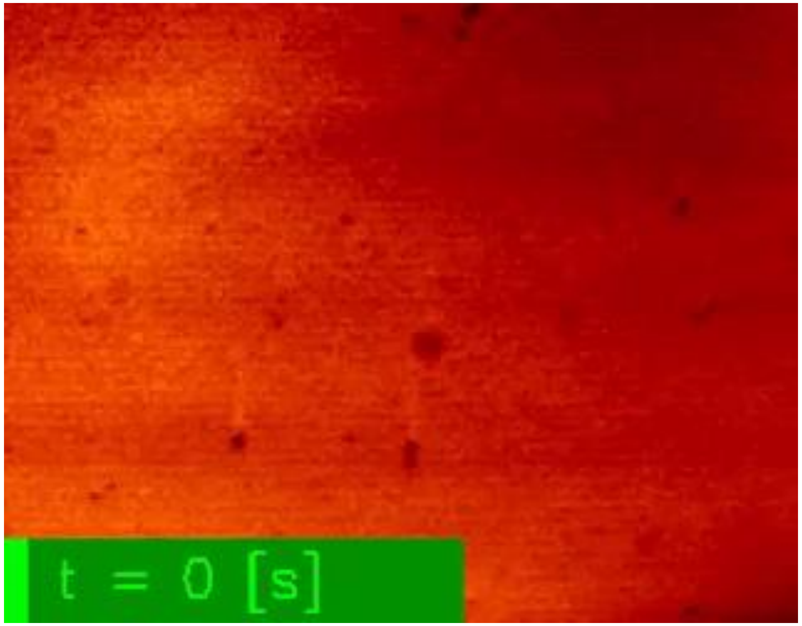
1| direct signals ...

2| signals from other senses ...

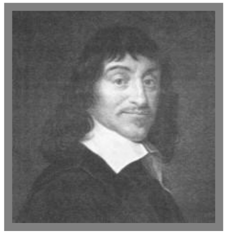
3| feedback loops ...

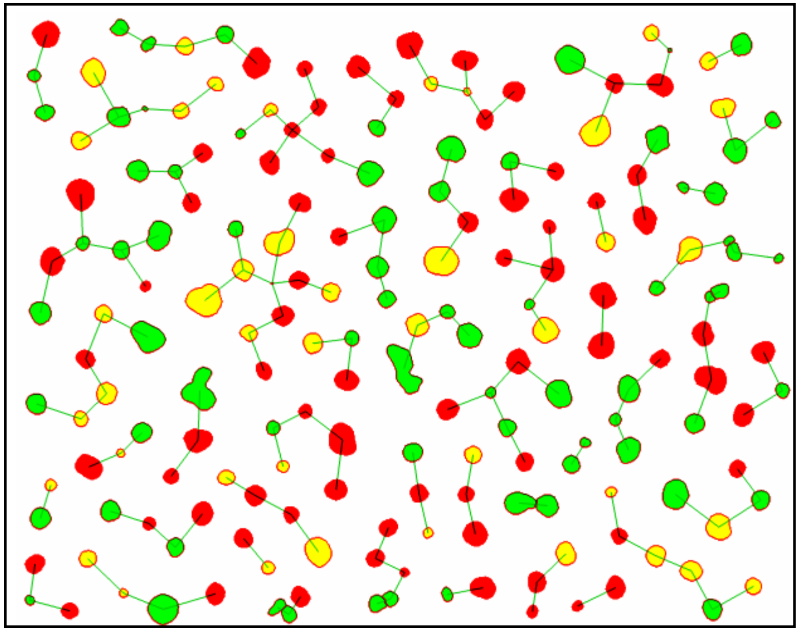
... produce a symbolic representation of an object.

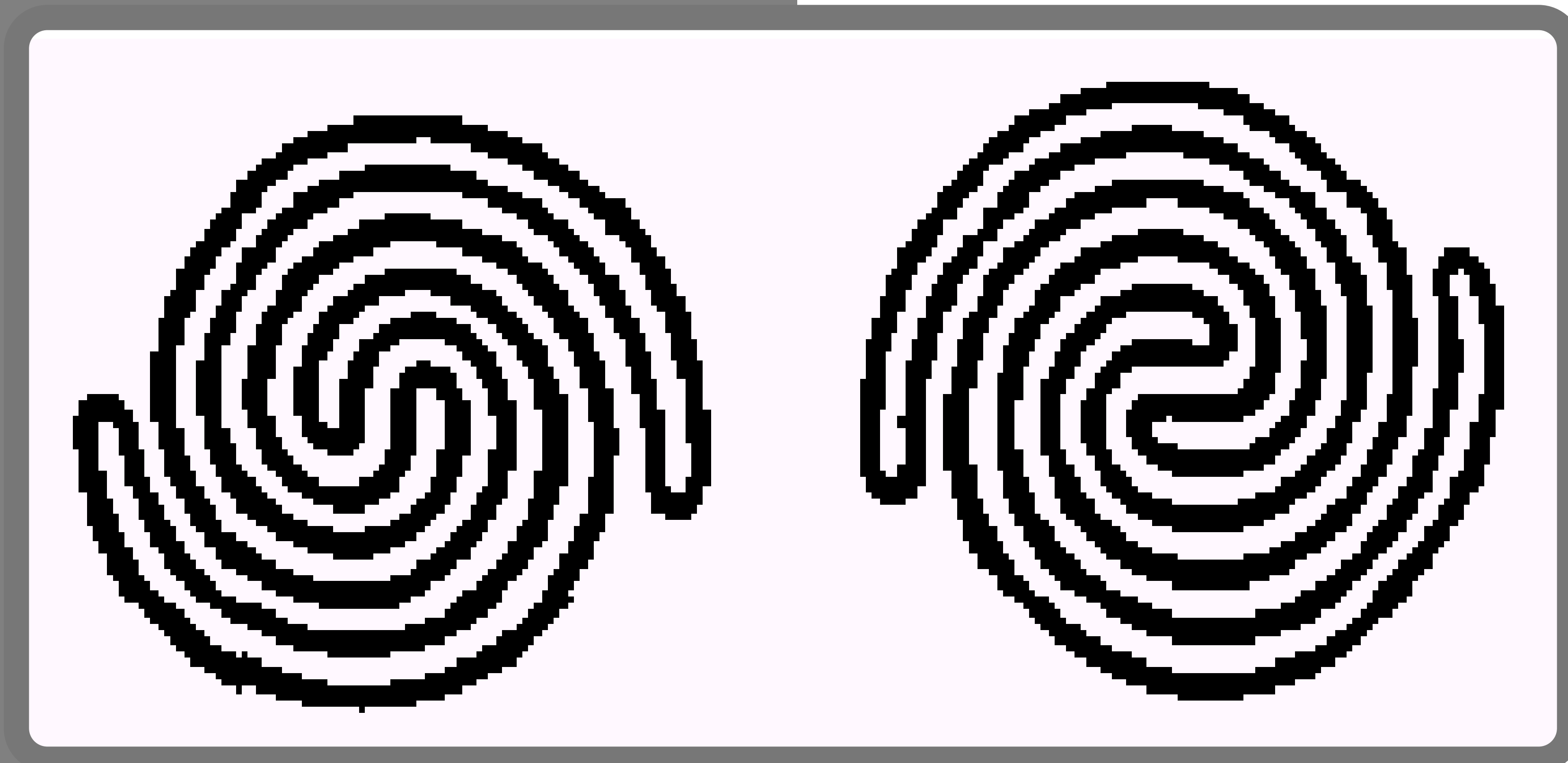
| Best resolution in t & x ...



|| Humas vs machine vision





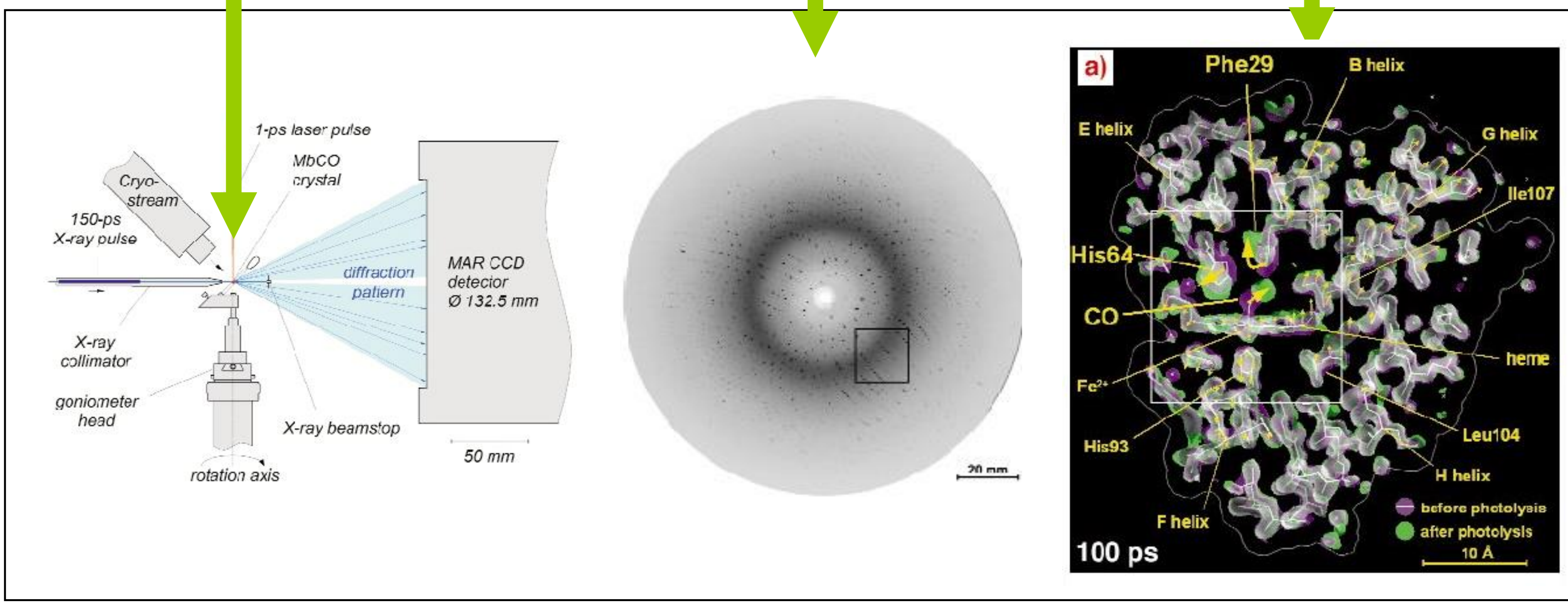


OTF: Object/Optical Transfer Function

Myoglobin in Action |Picosecond Laue Crystallography Diffraction Data

Schotte et al (2003) Science

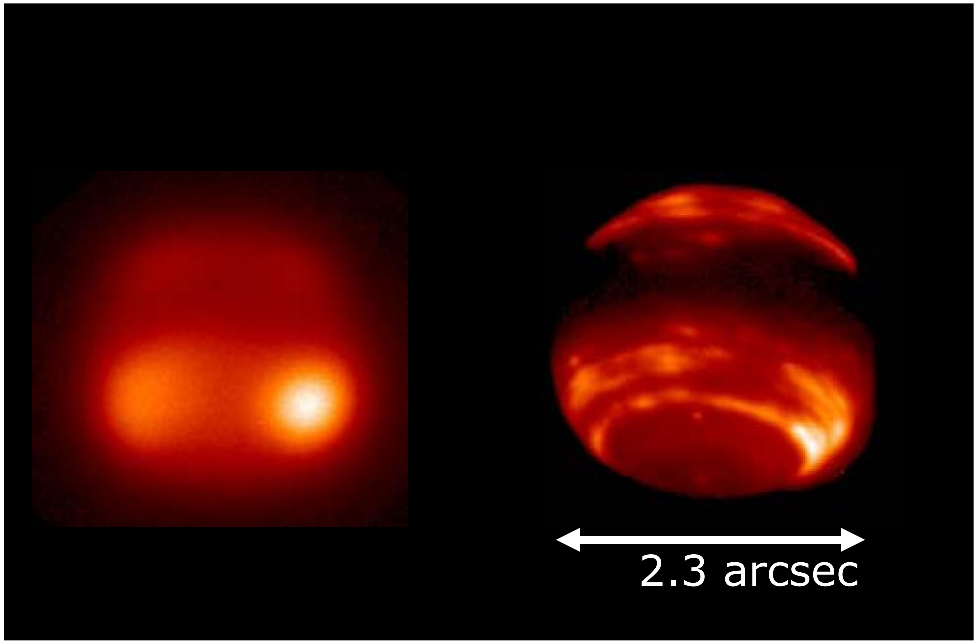
O_{bject} **OTF** → $O_{bject}I_{mage}$ **OTF^{-1}** → $O_{bject}M_{odel}$



http://www.youtube.com/watch?v=lnKIBZYarzM

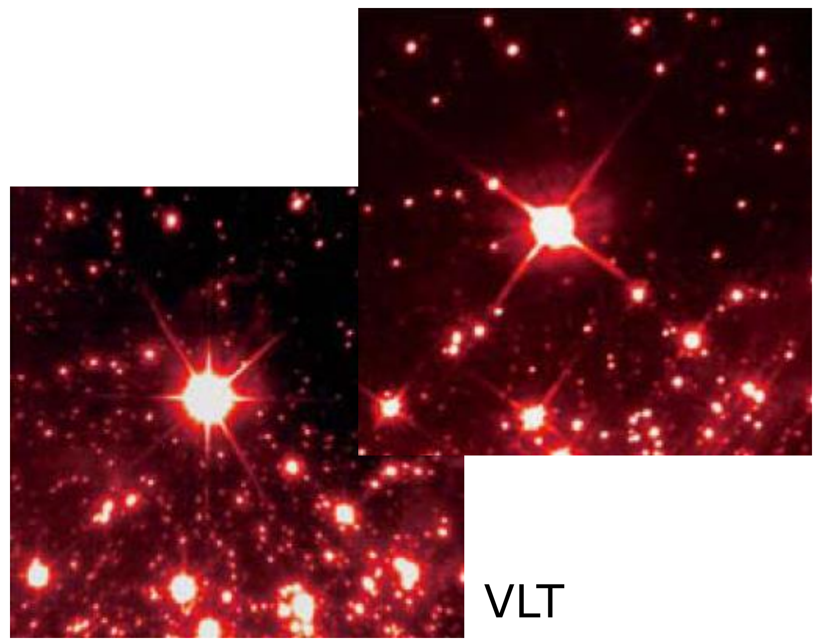
Diffraction Limited Resulution for a 10m telescope ~ λ/D ~ 0.01 arcsec
is limited to ~ 0.5 arcsec by the turbulent atmosphere.
NAOS creates an artificial star at 90 km altitude in the Earth´s mesosphere.
The Laser Guide Star is used to correct atmospheric effects

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



Diffraction Limited Resulution for a 10m telescope ~ λ/D ~ 0.01 arcsec is limited to ~ 0.5 arcsec by the turbulent atmosphere.

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



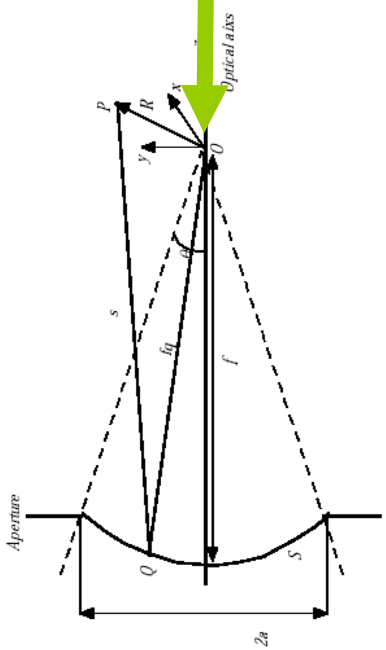
Hubble

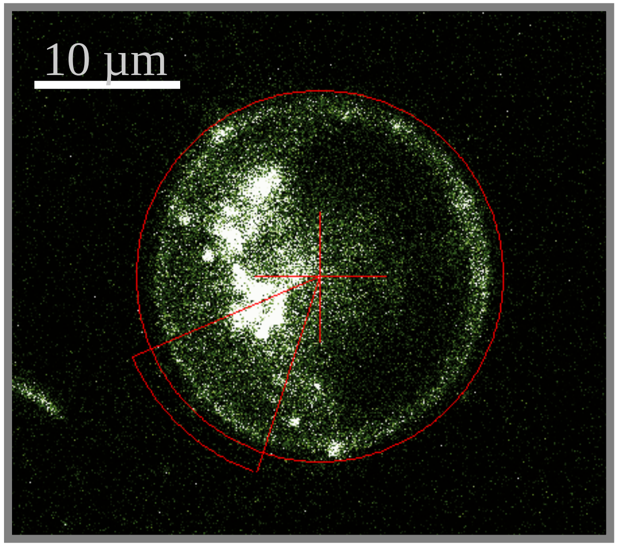
VLT

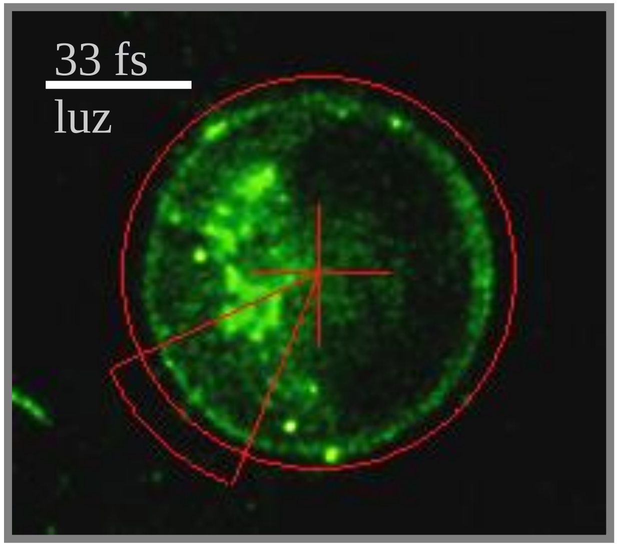
Confocal Microscopy | From Geometric Optics to Diffraction Theory

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.

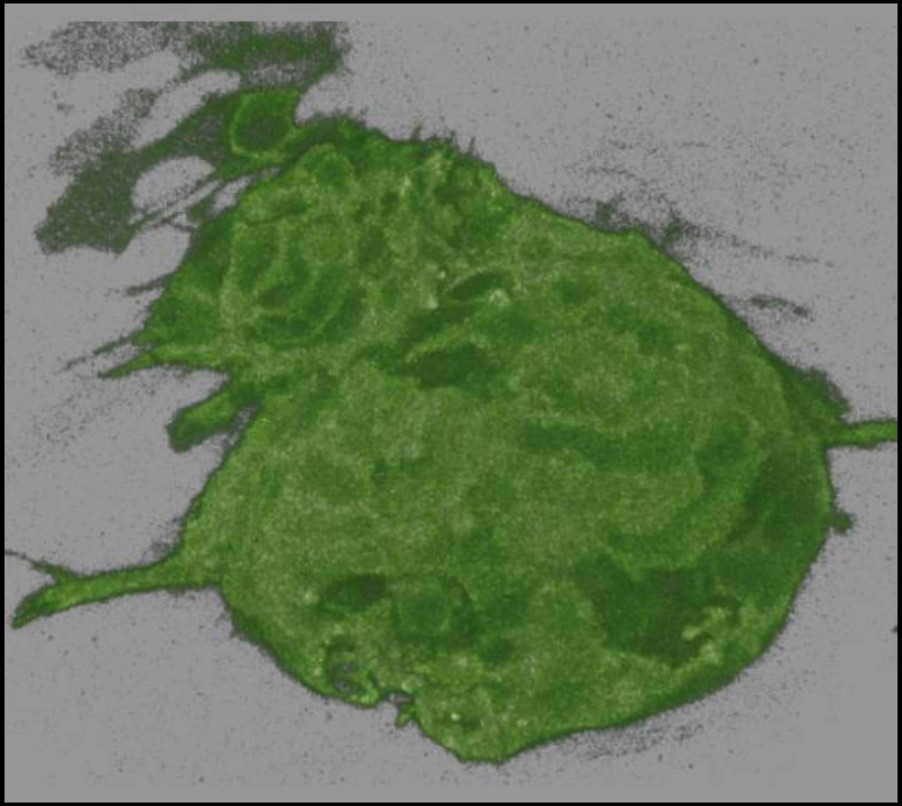
Object → **OTF** → **O**bject**I**mage → **OTF^{-1}** → **O**bject**M**odel

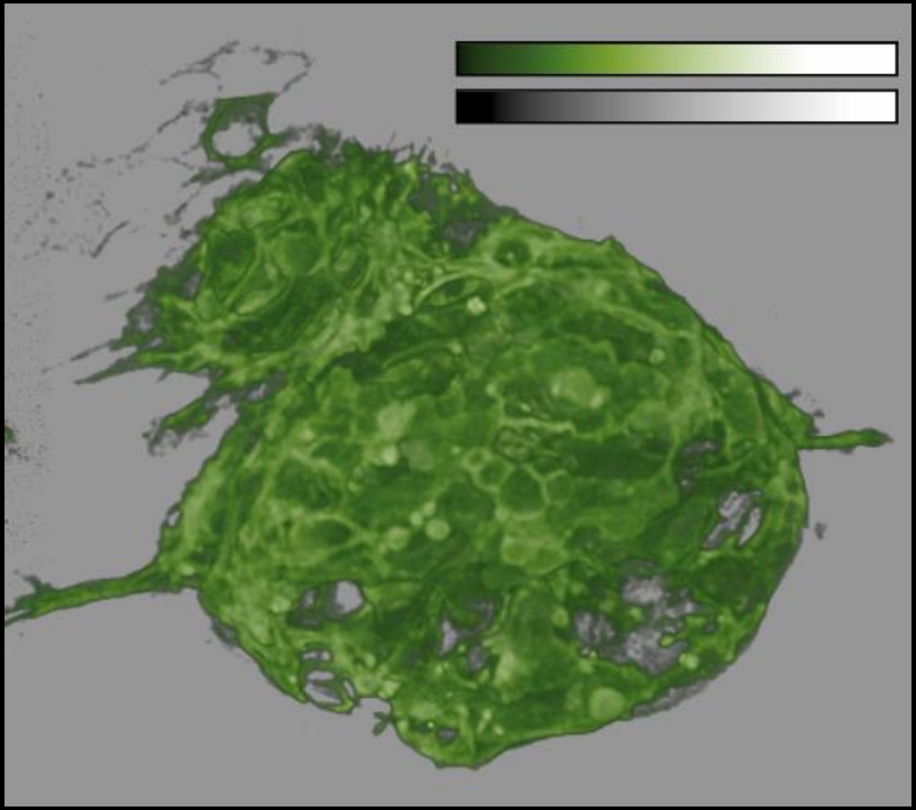






|-> Deconvolution





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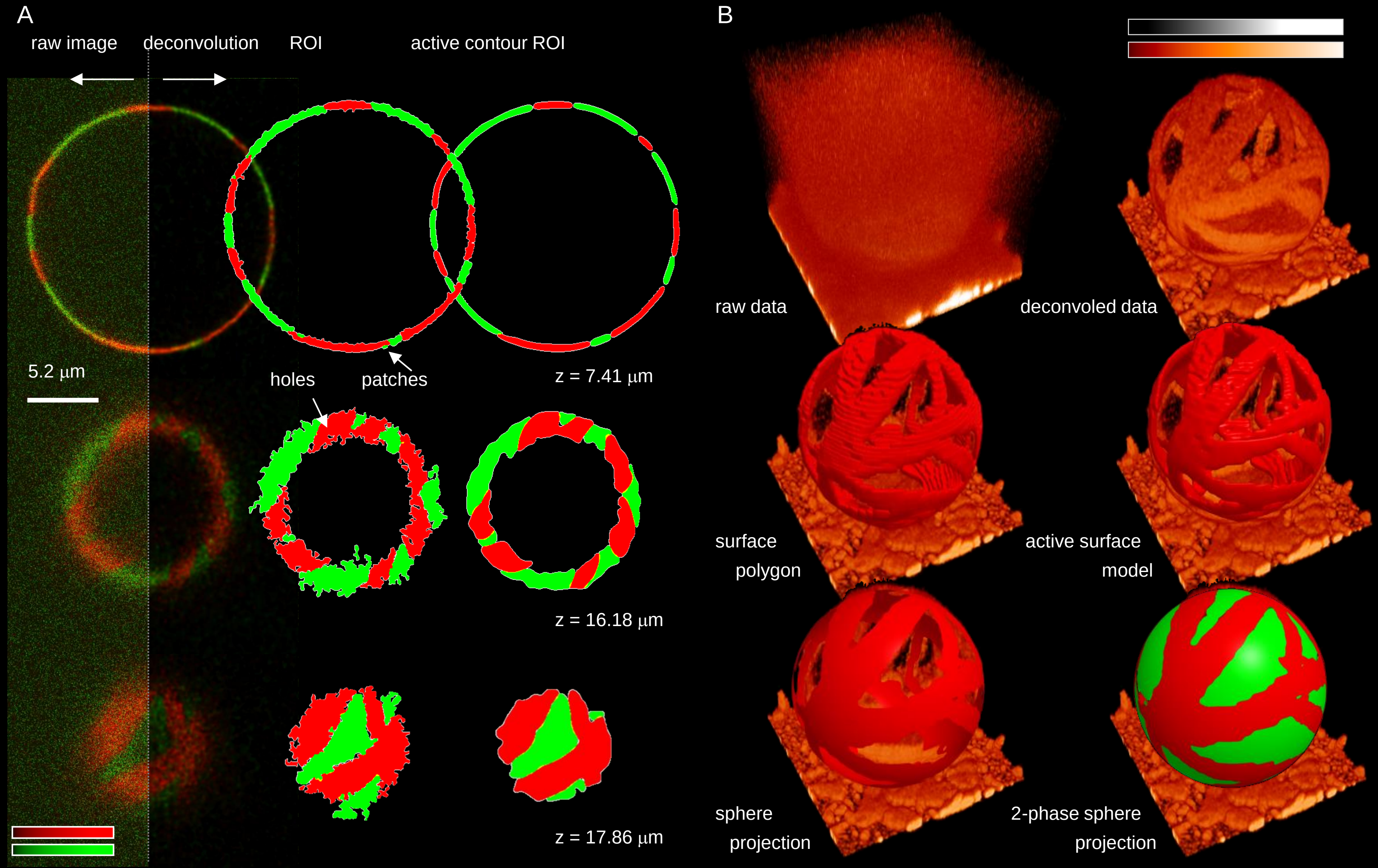
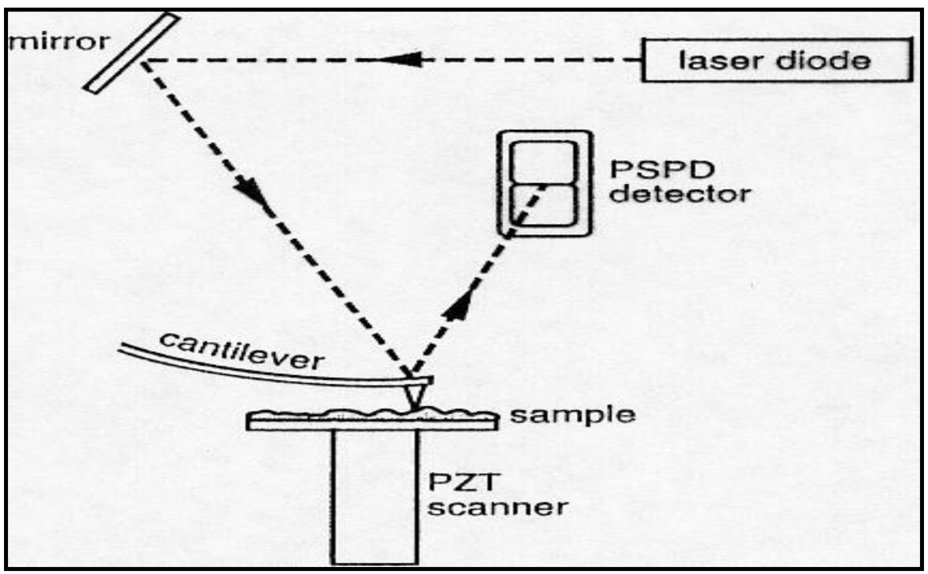
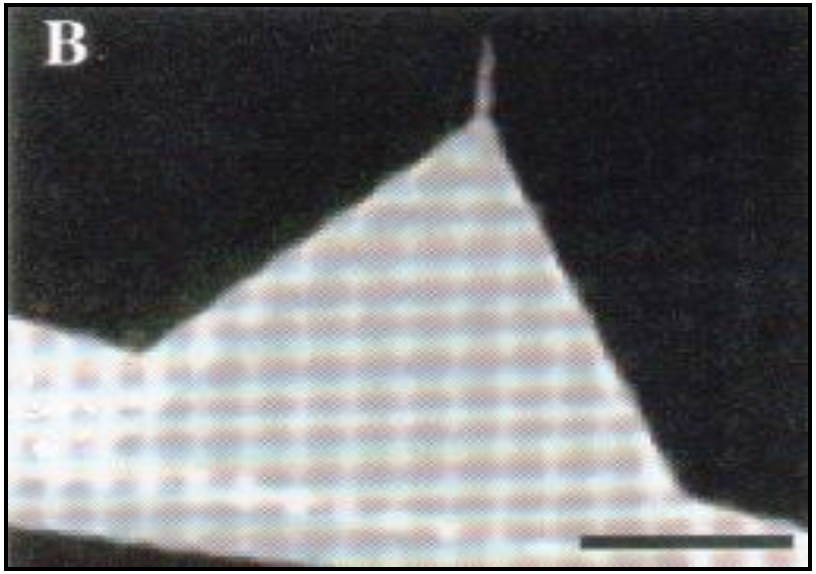


|-> Atomic Force Microscopy

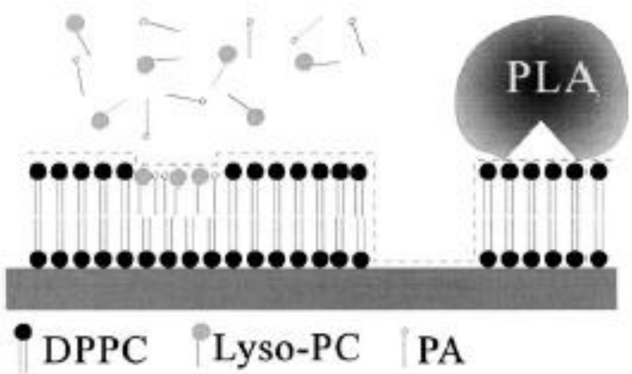
AFM allows the investigation of structural and functional properties of biomolecules in liquid environments, by a unique combination of :

- ***subnanometer*** spatial resolution
- ***millisecond*** temporal resolution
- ***piconewton*** force sensitivity

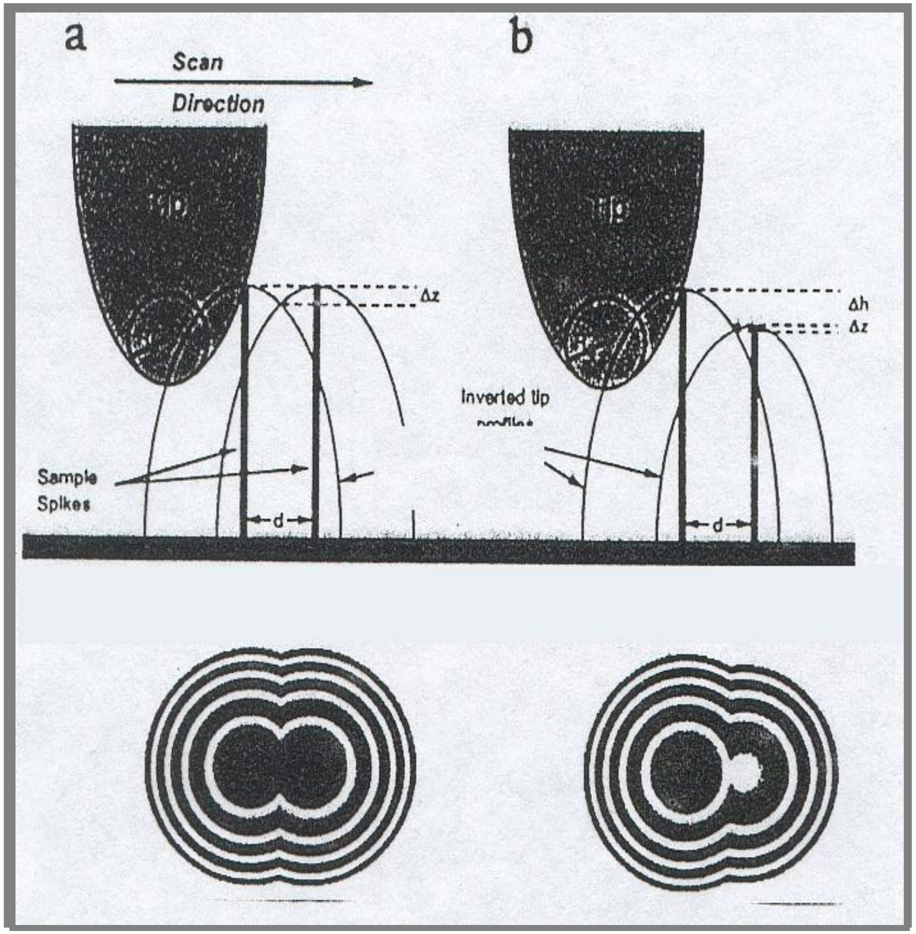




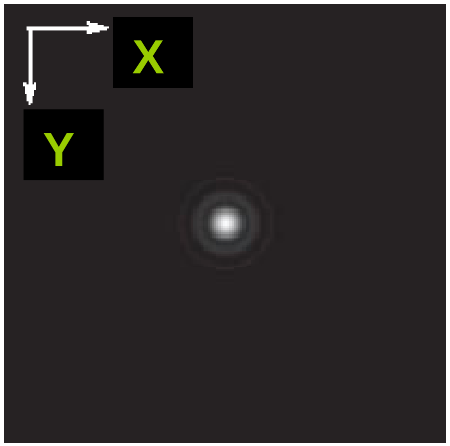
|-> Atomic Force Microscopy

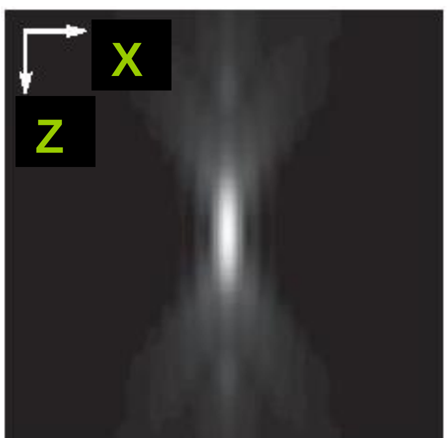


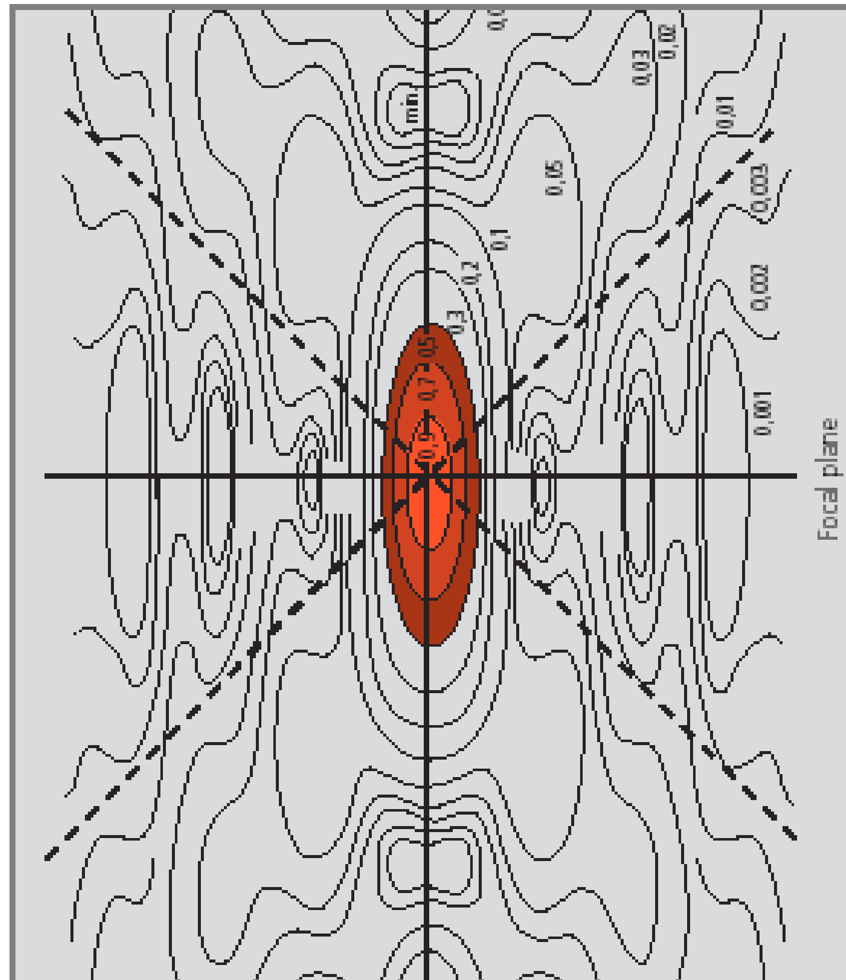
M Grandbois et al. (1998) Biophys J.



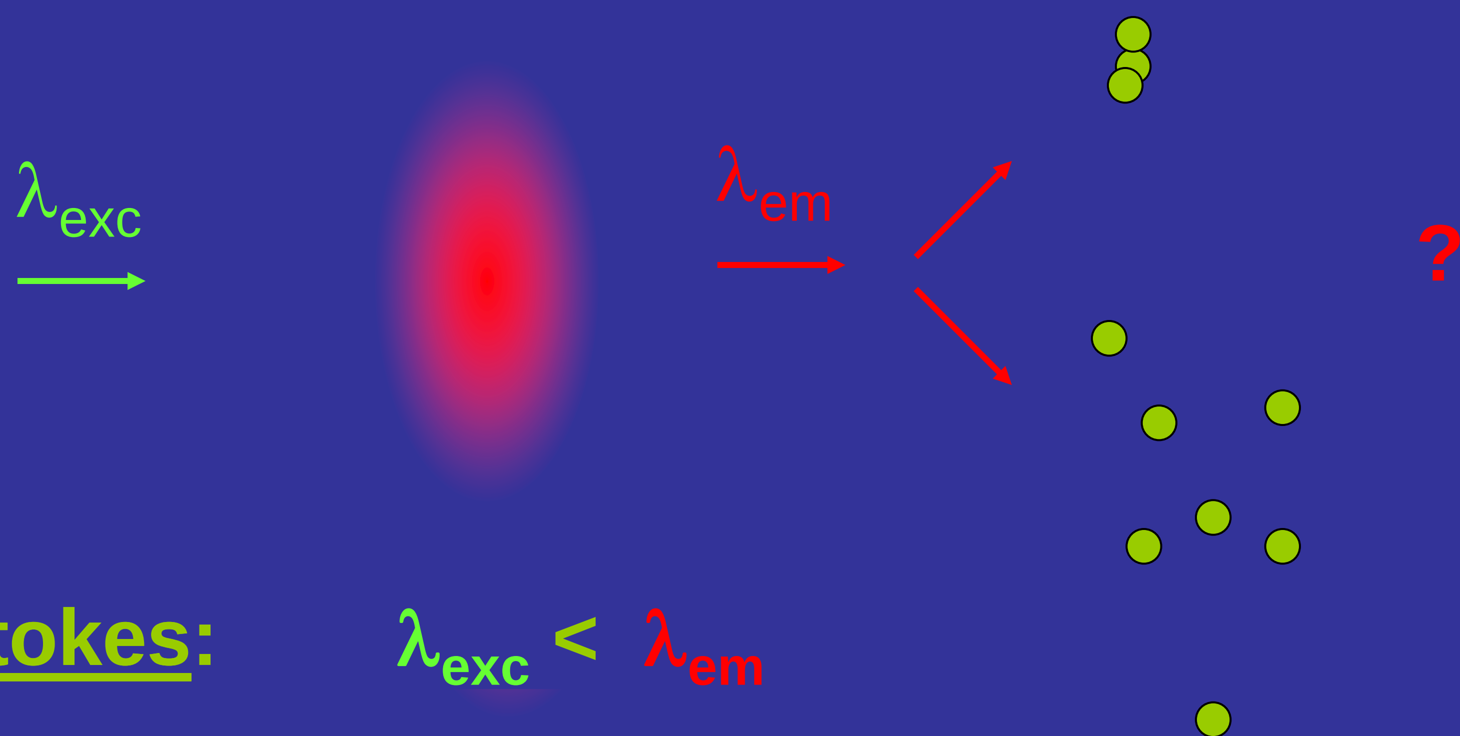
|-> PSF







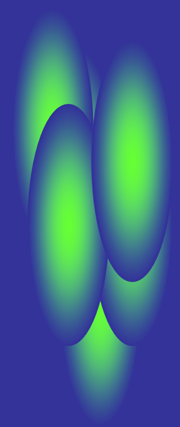
|-> Convolution



Stokes: $\lambda_{exc} < \lambda_{em}$

|-> Convolution

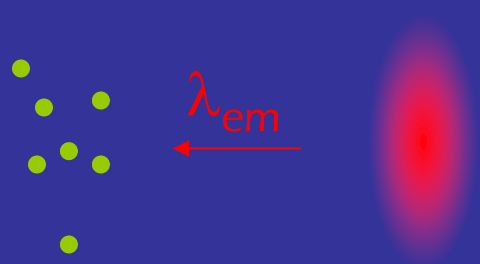
λ_{exc}



Stokes: $\lambda_{exc} < \lambda_{em}$

$n(\lambda_{exc}) > n(\lambda_{em})$

λ_{em}



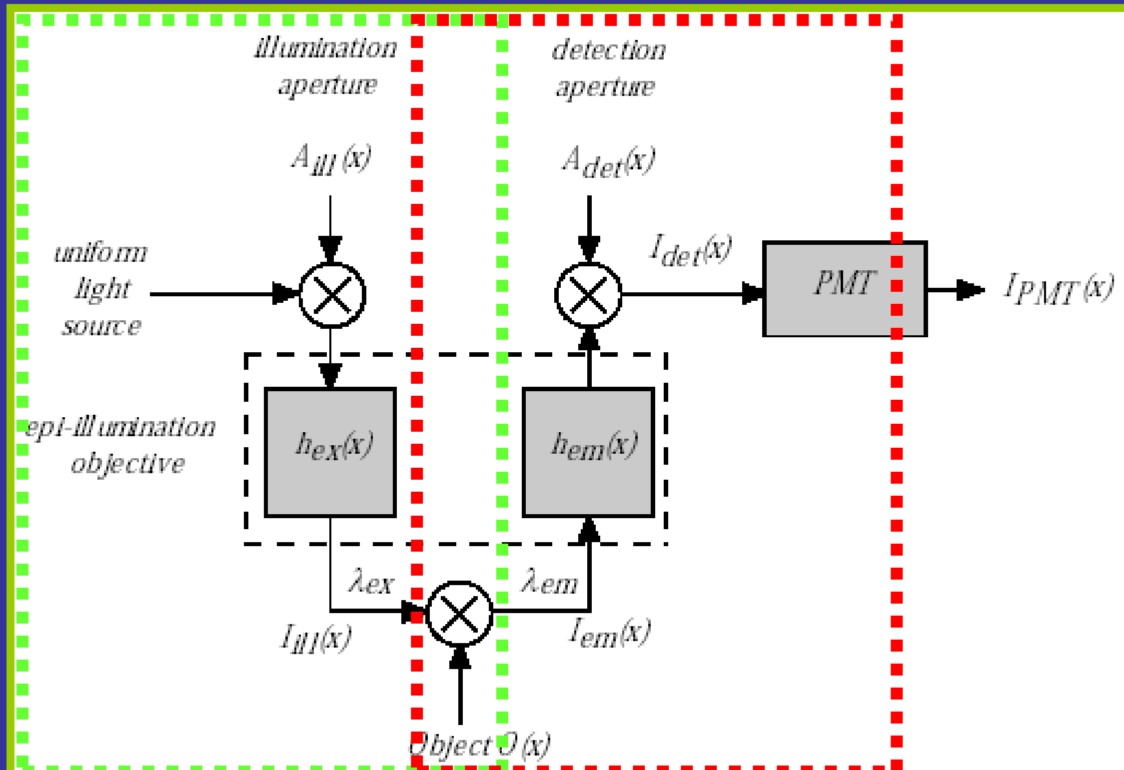
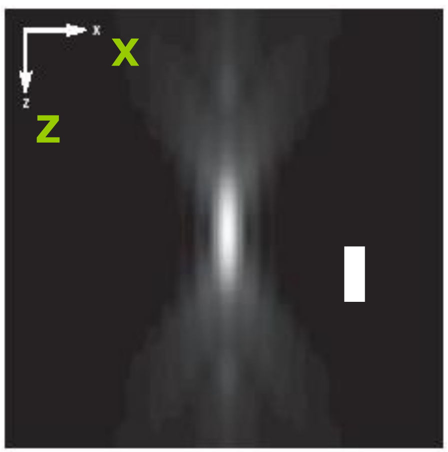
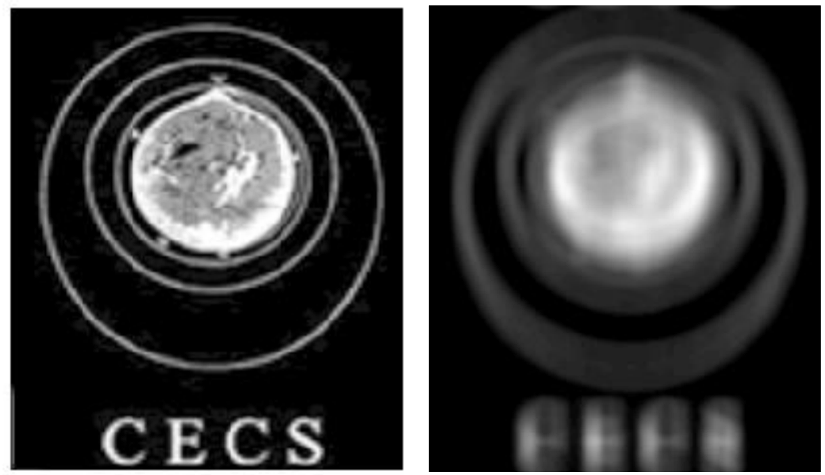
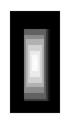


Figure 2.5 System diagram of a confocal epi-fluorescence microscope.

|-> Convolution







PSF:
Δxy ~ 500 nm | Δz ~ 1500 nm

|-> Deconvolution

PSF: Point Spread Function

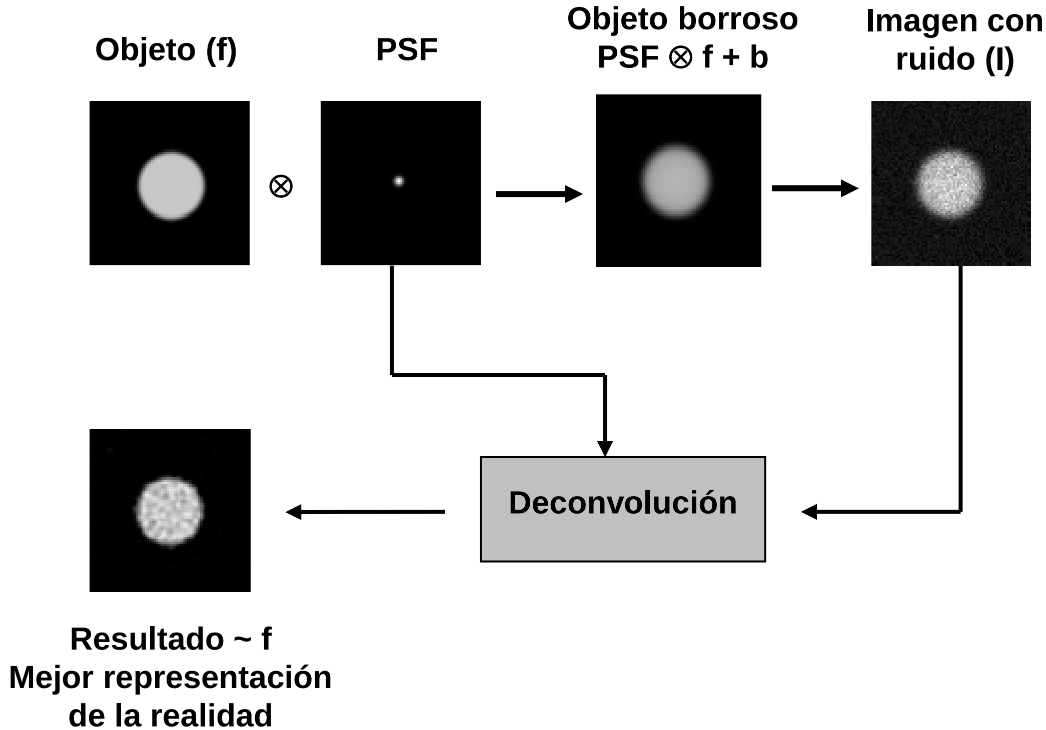
f: Object Function

b: Offset Function

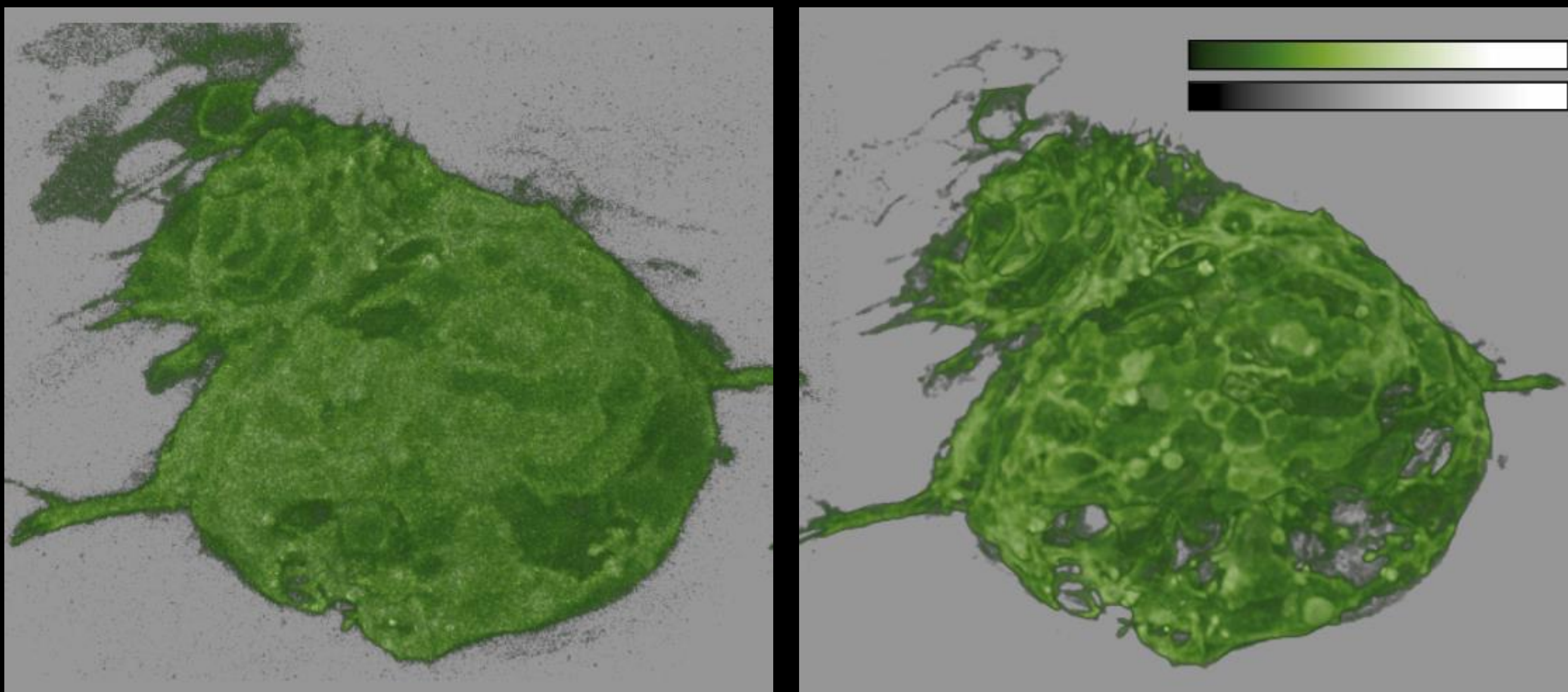
I: Image Matrix

N: Noise Function

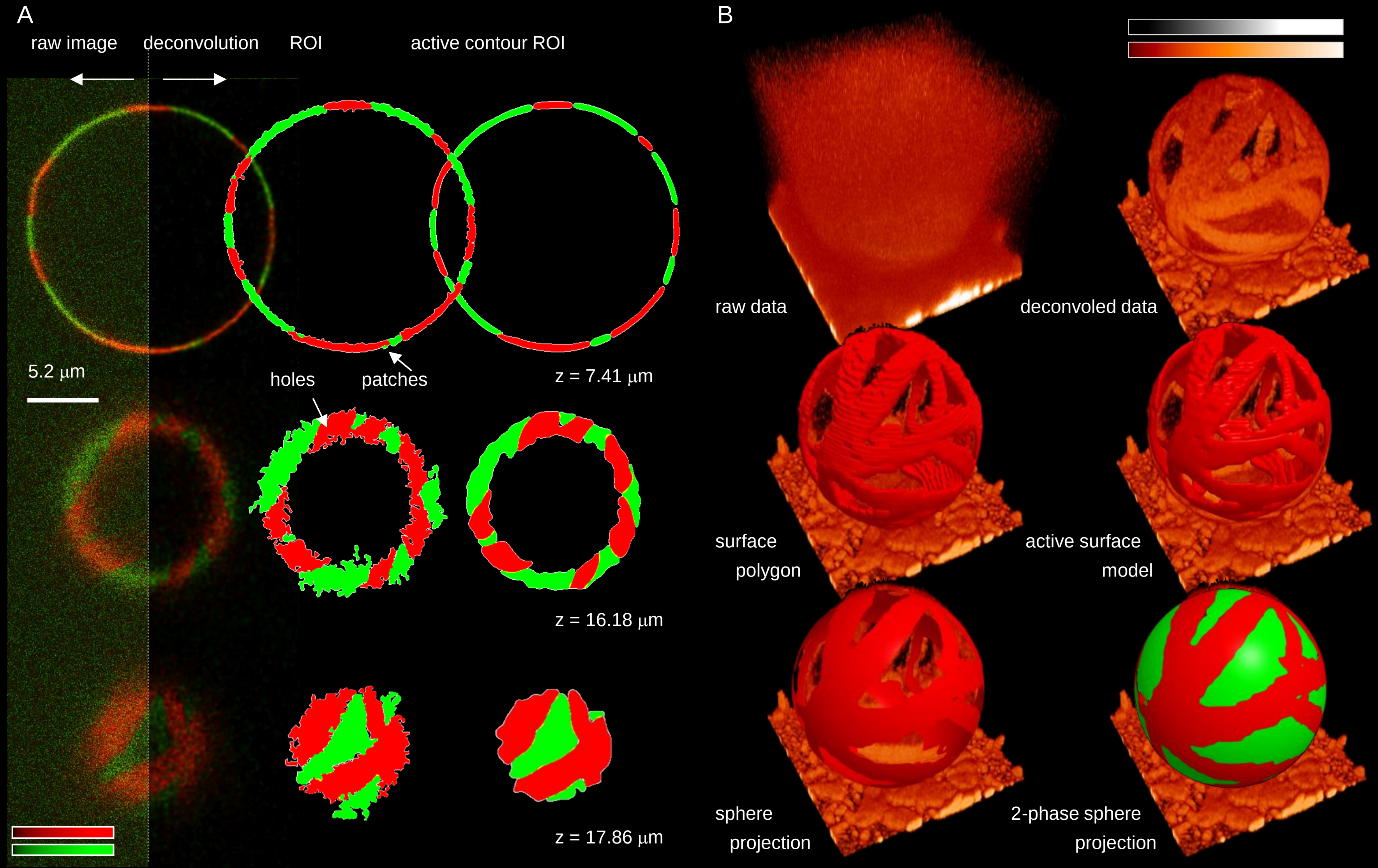
$$N(PSF(x, y, z) \otimes f(x, y, z) + b(x, y, z)) = I(x, y, z)$$



|-> Deconvolution



|-> Deconvolution



|-> Deconvolution

PSF: Point Spread Function

f: Object Function

b: Offset Function

I: Image Matrix

N: Noise Function

$$N(PSF(x, y, z) \otimes f(x, y, z) + b(x, y, z)) = I(x, y, z)$$

Calculator

- (selected) confocal
- widefield
- nipkow
- 4Pi

Select one

Numerical aperture	1.3	
Excitation wavelength	488	(nm)
Emission wavelength	520	(nm)
Number of excitation photons	1	
→ Backprojected pinhole radius	250	(nm)
B.P. distance between pinholes	2.53	Only for Nipkow disks (µm)
Lens medium refractive index	1.515	
Specimen medium refractive index	1.45	
Acquisition depth	0	(µm)

☐ Calculate also PSF

http://support.svi.nl/wiki/NyquistCalculator

|-> Pinhole

$$N(PSF(x, y, z) \otimes f(x, y, z) + b(x, y, z)) = I(x, y, z)$$

PSF: Point Spread Function

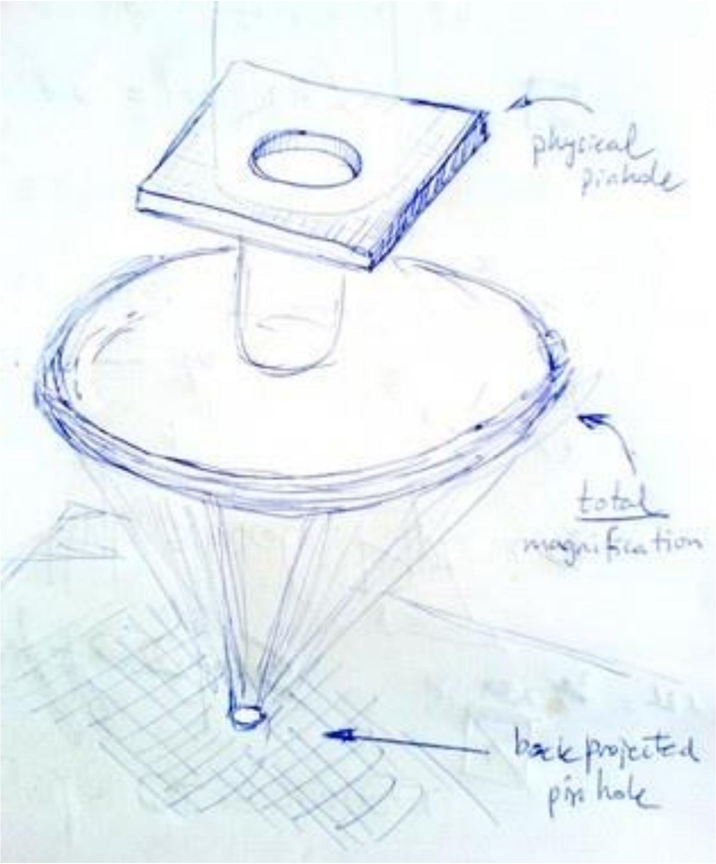
f: Object Function

b: Offset Function

I: Image Matrix

N: Noise Function

Backprojected confocal pinhole



http://support.svi.nl/wiki/NyquistCalculator

|-> Pinhole

PSF: Point Spread Function

f: Object Function

b: Offset Function

I: Image Matrix

N: Noise Function

$$N(PSF(x, y, z) \otimes f(x, y, z) + b(x, y, z)) = I(x, y, z)$$

Biorad

- Biorad MRC 500, 600 and 1024
- Biorad Radiance

Leica

- Leica confocals TCS 4d, SP1 and NT
- Leica confocal SP2
- Leica confocal SP5

Nikon

- TE2000-E with the C1 scanning head

Olympus

- Olympus FV300
- Olympus FV500
- Olympus FV1000

Zeiss

- Zeiss LSM410 inverted
- Zeiss LSM510

http://support.svi.nl/wiki/NyquistCalculator

|-> Noise

Literature: eg. Noise Theory and Application to Physics: Philippe Réfrégier, Springer

|-> Noise

PSF: Point Spread Function

f: Object Function

b: Offset Function

I: Image Matrix

N: Noise Function

- *Black Body Irrdiation (Poisson)*
- *Detector Noise (Gauss)*

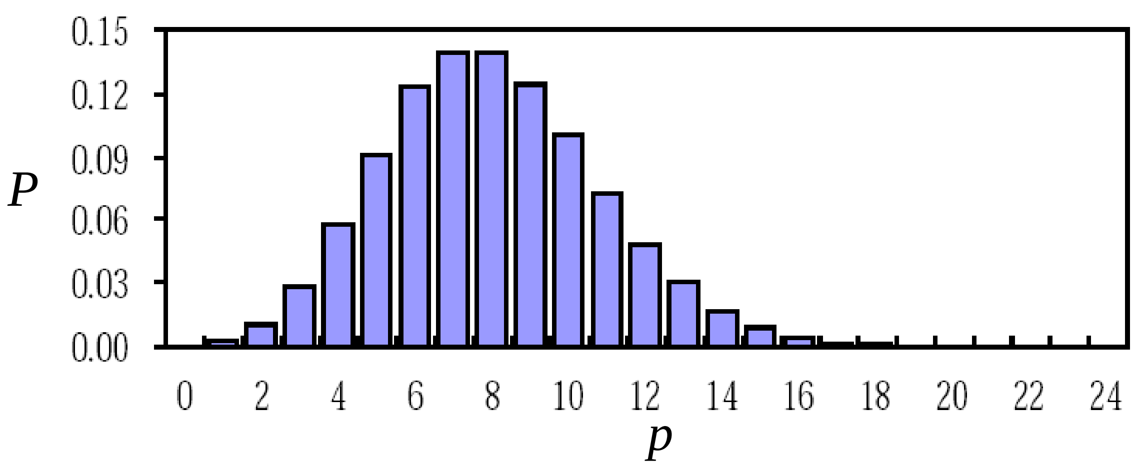
$$N(PSF(x,y,z) \otimes f(x,y,z) + b(x,y,z)) = I(x,y,z)$$

$$P(p,\mu) = \frac{\mu^p}{p!} \cdot e^{-\mu}$$

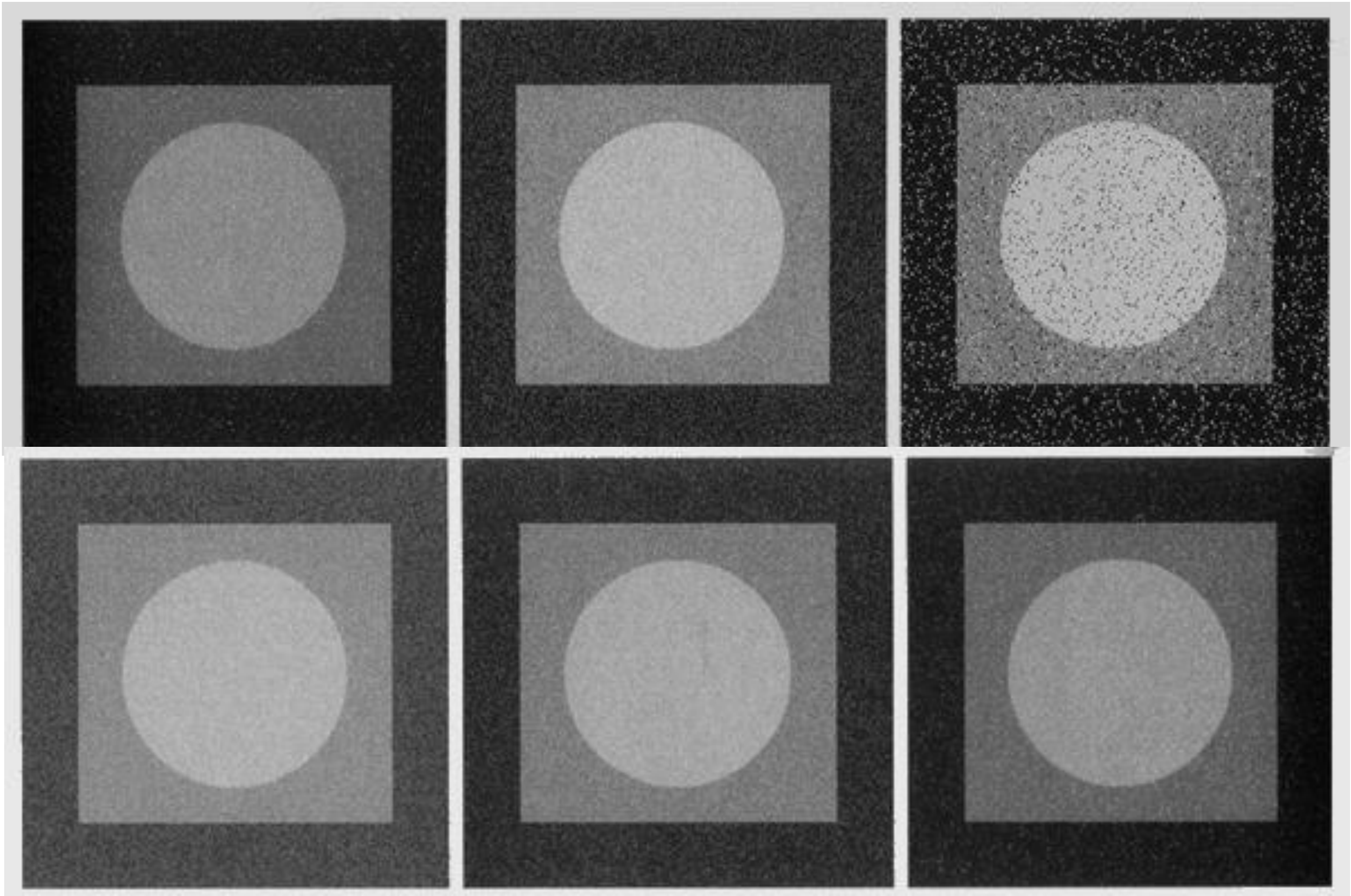
$$1.\ \bar{p} = \mu = \sigma^2,\ sd = \sigma = \sqrt{\bar{p}} = \sqrt{\mu}$$

$$2.\ counting:\ \bar{p} \pm \sqrt{\bar{p}}$$

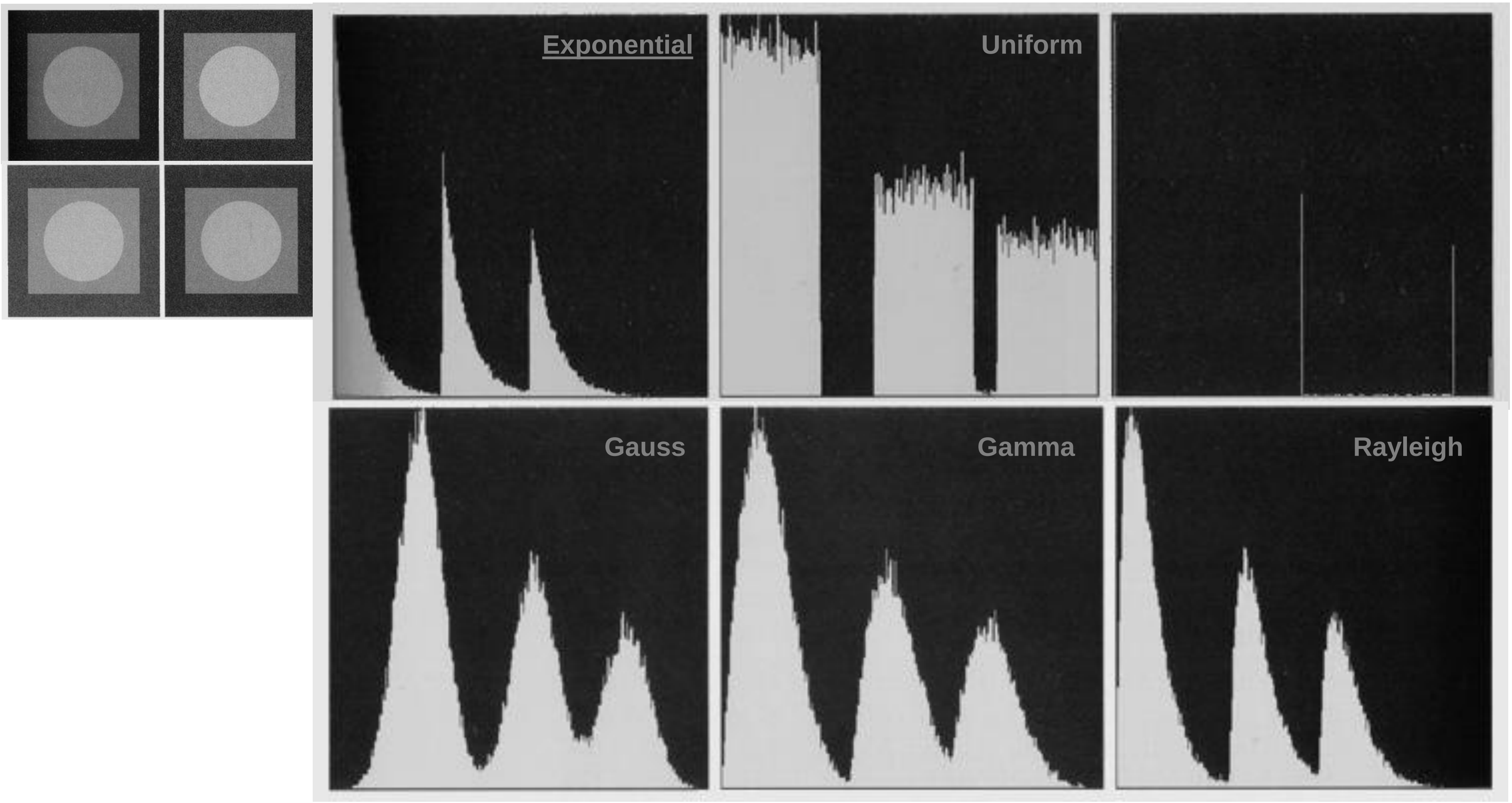
$$3.\ Poisson(discrete) \rightarrow Gauss(continuous): \mu \rightarrow \infty$$



|-> Noise



|-> Noise



|-> Noise

The Signal to Noise ratio (SN) is a number not always easy to estimate. The easiest way to obtain some fi gure is to look at the textures of bright areas in your object image. In the fi gure at left you see examples of such textures obtained from originally the same object image to which various levels of poisson noise were added.

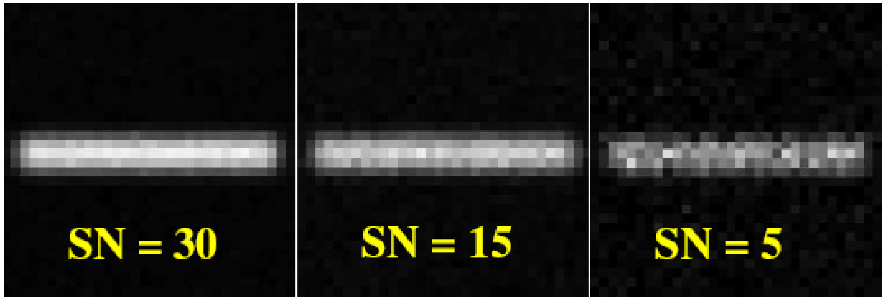
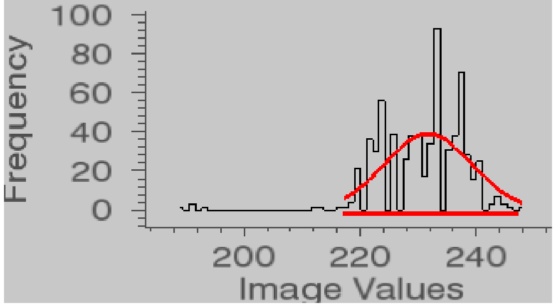
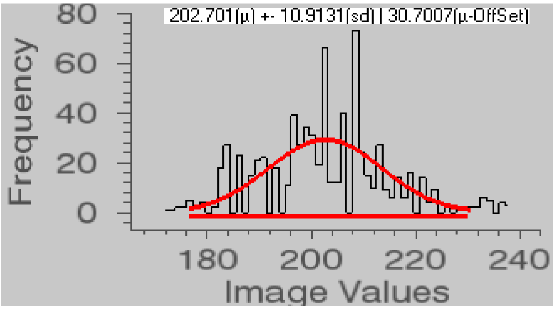
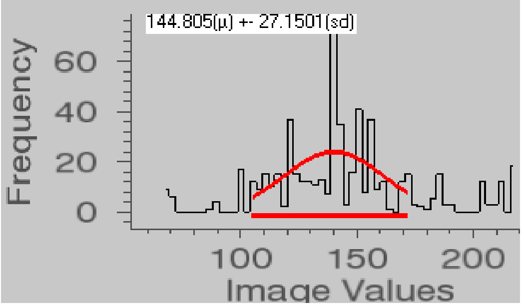


Figure 9. Images with different generated noise levels





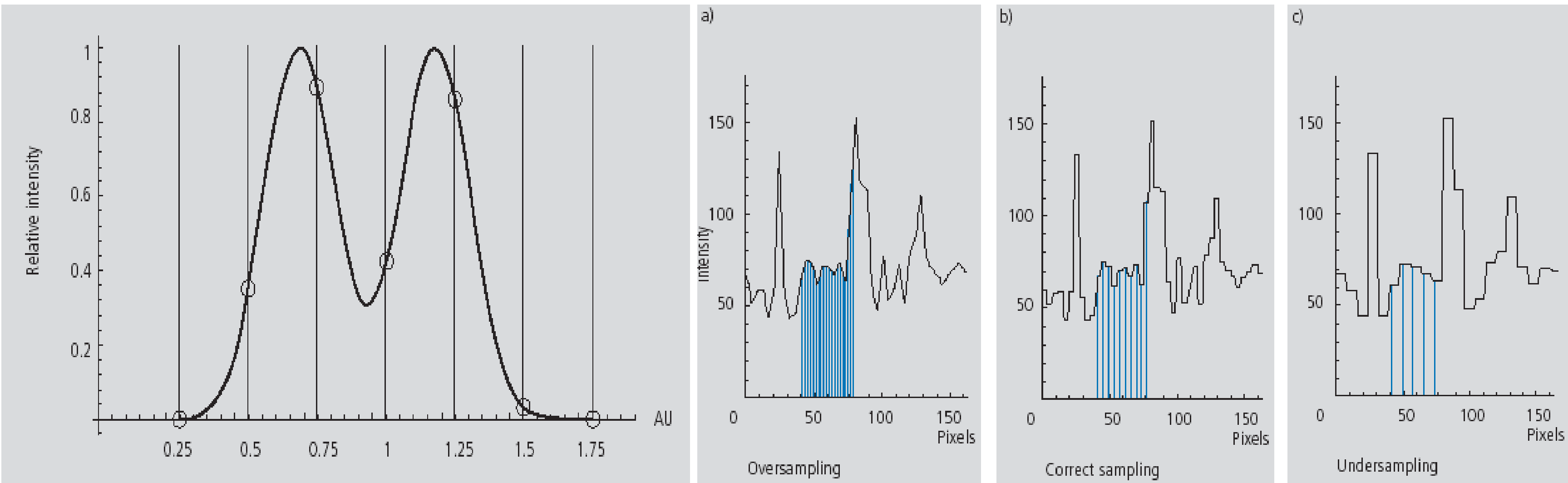


$$SNR = \frac{\bar{I}}{\sigma} = \frac{\bar{I}}{\sqrt{\sigma^2}} = \frac{229}{7.5}$$

$$SNR = \frac{\bar{I}}{\sigma} = \frac{\bar{I}}{\sqrt{\sigma^2}} = \frac{200}{10}$$

$$SNR = \frac{\bar{I}}{\sigma} = \frac{\bar{I}}{\sqrt{\sigma^2}} = \frac{139}{27}$$

|-> Nyquist /Shannon Theorem



- Undersampling looses structures.
- Oversampling waists memory/computation time.

The 'Nyquist /Shannon Theorem' or 'Sampling Theorem' for the digital sampling of analogue signals suggests a Nyquist rate NR ≥ 2ν ?

! Diffraction theory calculates lateral NR ~ 20 pixel/µm(~50 nm/pixel) !
... laxial NR ~ (~150 nm/pixel)

|-> PSF calculator

PSF: Point Spread Function

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b: Offset Function

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N: Noise Function

$$N(PSF(x, y, z) \otimes f(x, y, z) + b(x, y, z)) = I(x, y, z)$$

Calculator

- (•) confocal
- ( ) widefield
- ( ) nipkow
- ( ) 4Pi

Select one

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Lens medium refractive index	1.515	
Specimen medium refractive index	1.45	
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☐ Calculate also PSF

http://support.svi.nl/wiki/NyquistCalculator

|-> PSF calculator

PSF: Point Spread Function

f: Object Function

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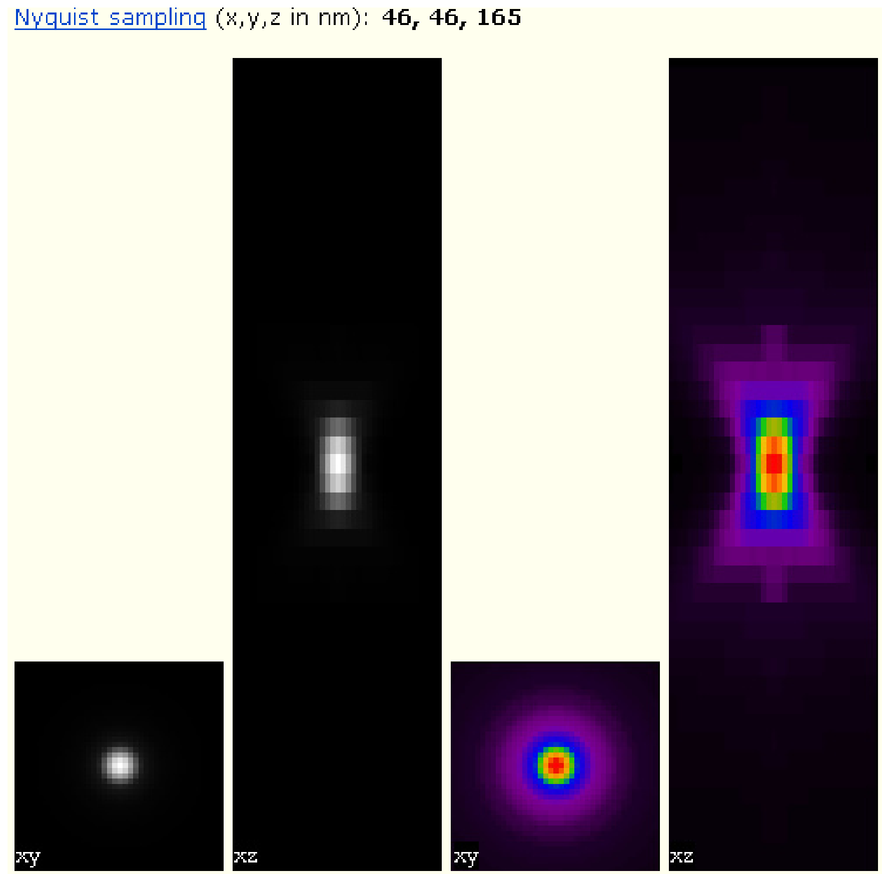
I: Image Matrix

N: Noise Function

$$N(PSF(x, y, z) \otimes f(x, y, z) + b(x, y, z)) = I(x, y, z)$$



http://support.svi.nl/wiki/NyquistCalculator

|-> Snell's Law

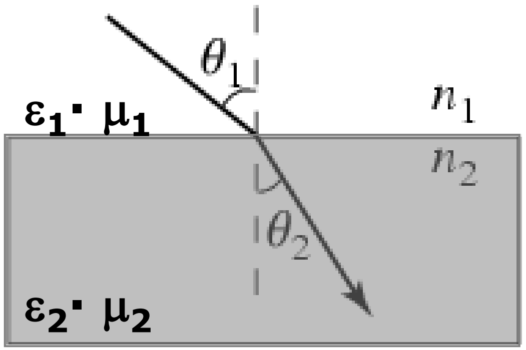
Index of refraction: $n = (\varepsilon \cdot \mu)^{1/2} = c/v$,

ε electric permittivity and μ magnetic permeability.

Snell's Law:

$\sin\Theta_1 \, n_1 = \sin \Theta_2 \, n_2$

- **1.518 [Zeiss Oil]**
- **1.33 [Water]**
- **1.0008 [Air]**



|-> Refractive index

Refractive Index:

$RI = n_1/n_2 = v_2/v_1$

Snell's Law:

$\sin\Theta_1 \, n_1 = \sin \Theta_2 \, n_2$

$n = n(\lambda)$!

- 1.518 [Zeiss]
- 1.33 [Water]
- 1.0008 [Air]

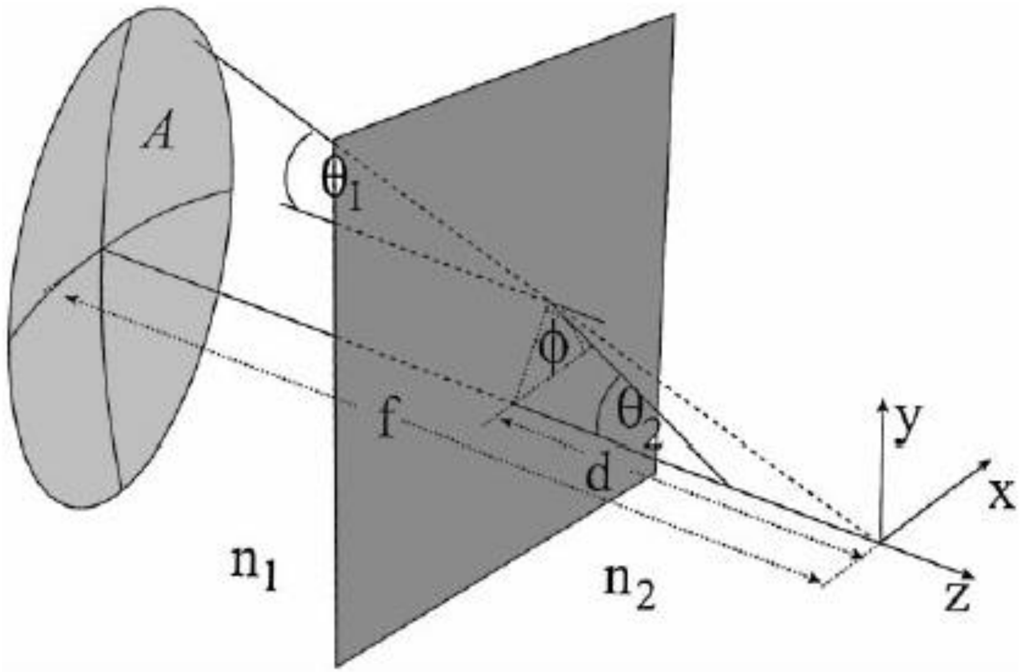
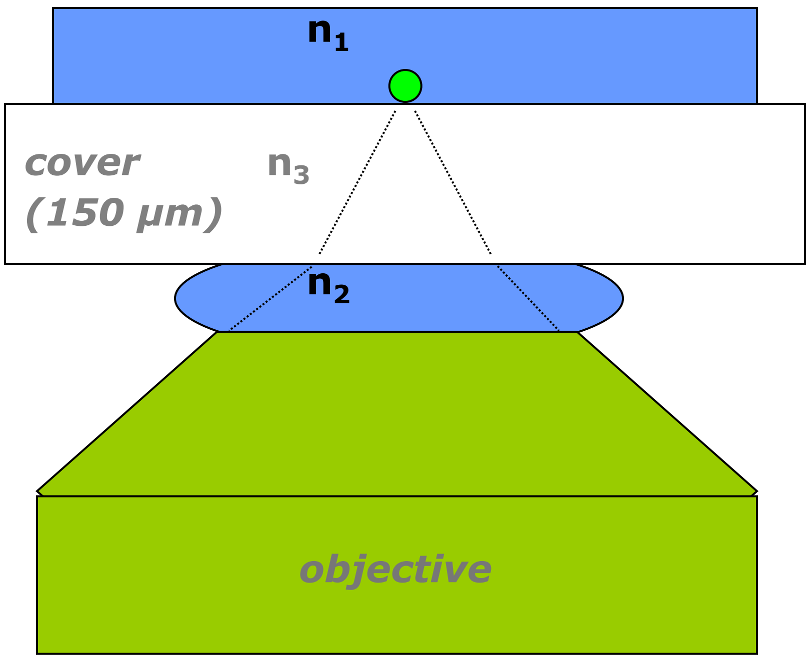


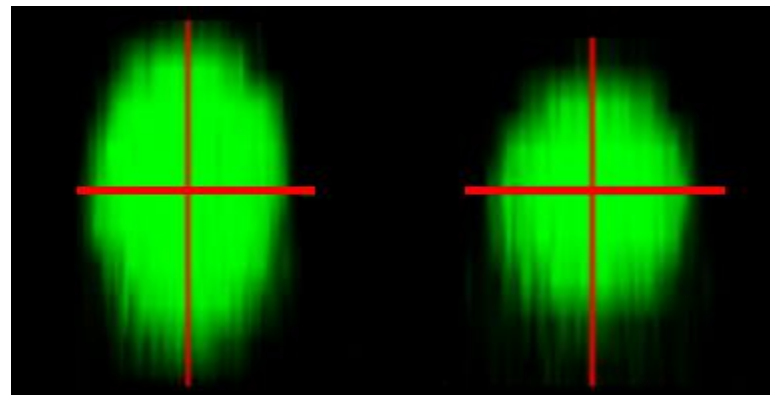
Fig. 1. Geometry when focusing with refractive index mismatch.

(Egner et al 1998)

|-> Refractive index

● **Micro-esfera:** ∅ = 6 µm





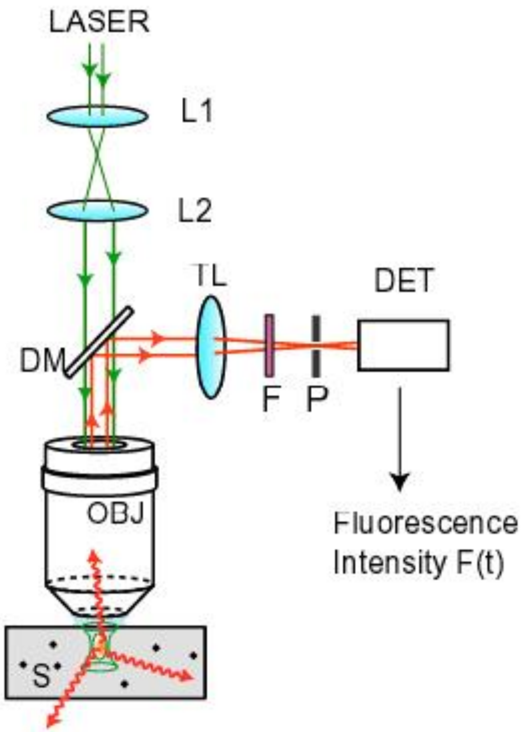
agua/aceite -- *aceite/aceite*

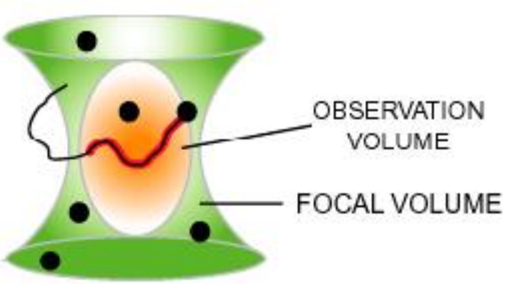
$n_1 \neq n_2$ $n_1 = n_2$

Ley de Snell: $n_i \cdot \sin\Theta_i = n_k \cdot \sin\Theta_k$

$n = n(\lambda)$!

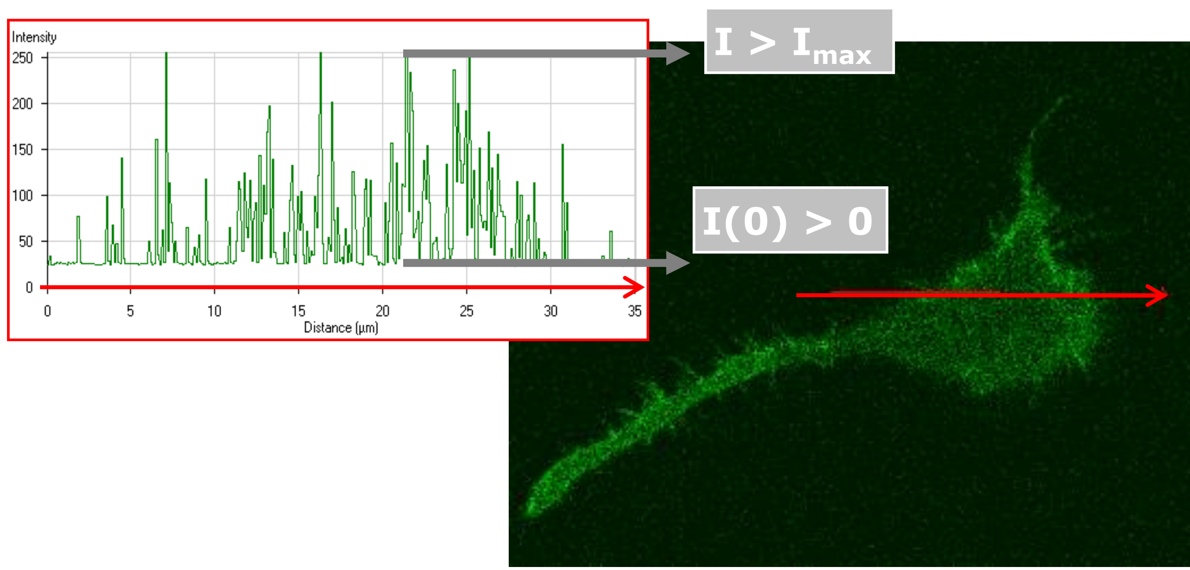
|-> Resumen I



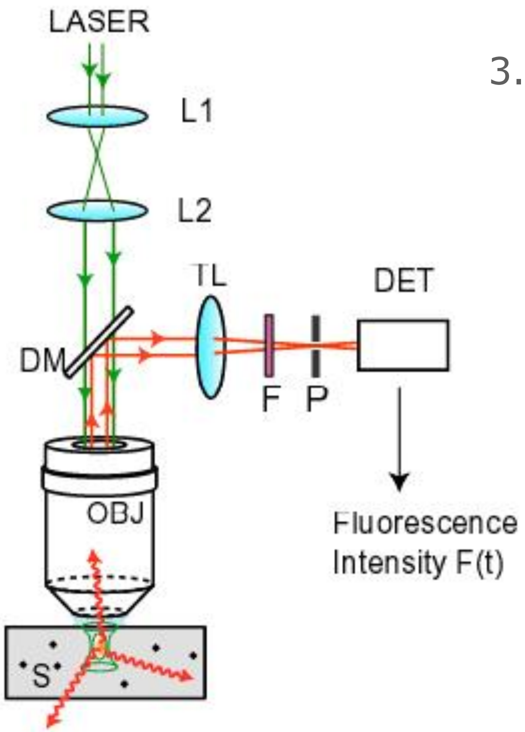


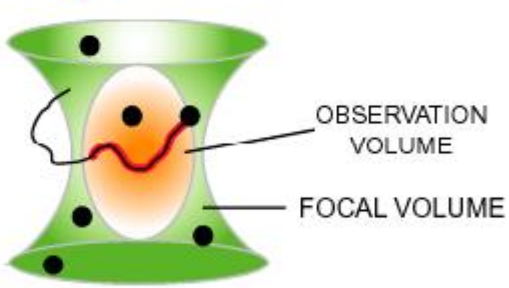
The observation volume (femtoliter) defined by the Point Spread Function must be considered as a <u>mini-sprectrofluorimeter</u>.

1. You need to consider the Offset I(0) in order to calibrate your signal I(0) ≥ 0 !
2. Never saturate the signal: $I \leq I_{max}$ (255 for 8 bit) !

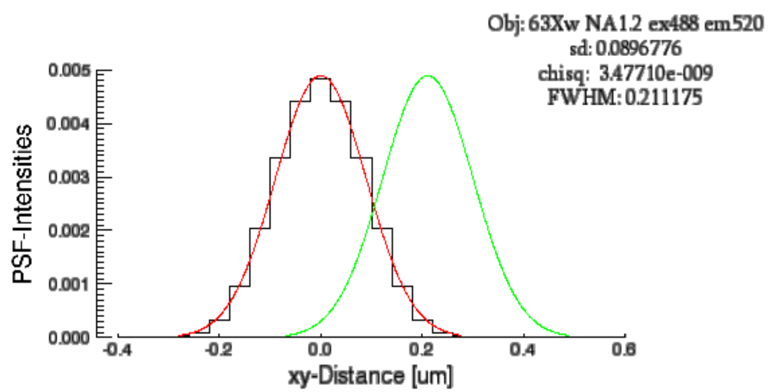


|-> Resumen II

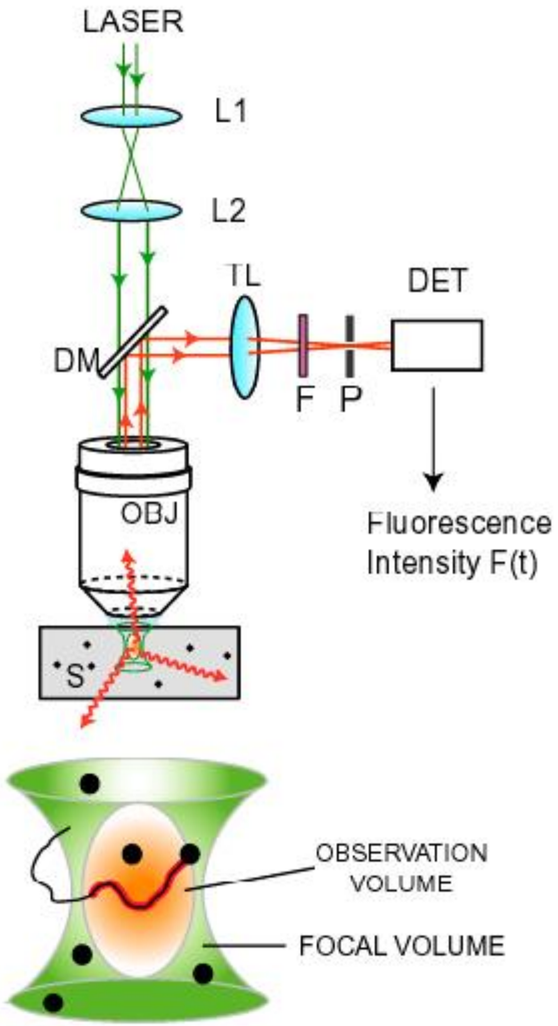




3. You need to consider sampling distances in Δx and Δy $\approx$ 50 nm and Δz $\approx$ 150-300 nm for later deconvolution, or calculate the explicit sample distances @ http://support.svi.nl/wiki/NyquistCalculator



|-> Resumen III



4. Use the right inmersion setup !

   n1 = n2 !

   Keep refractive index / index of refraction constant !