

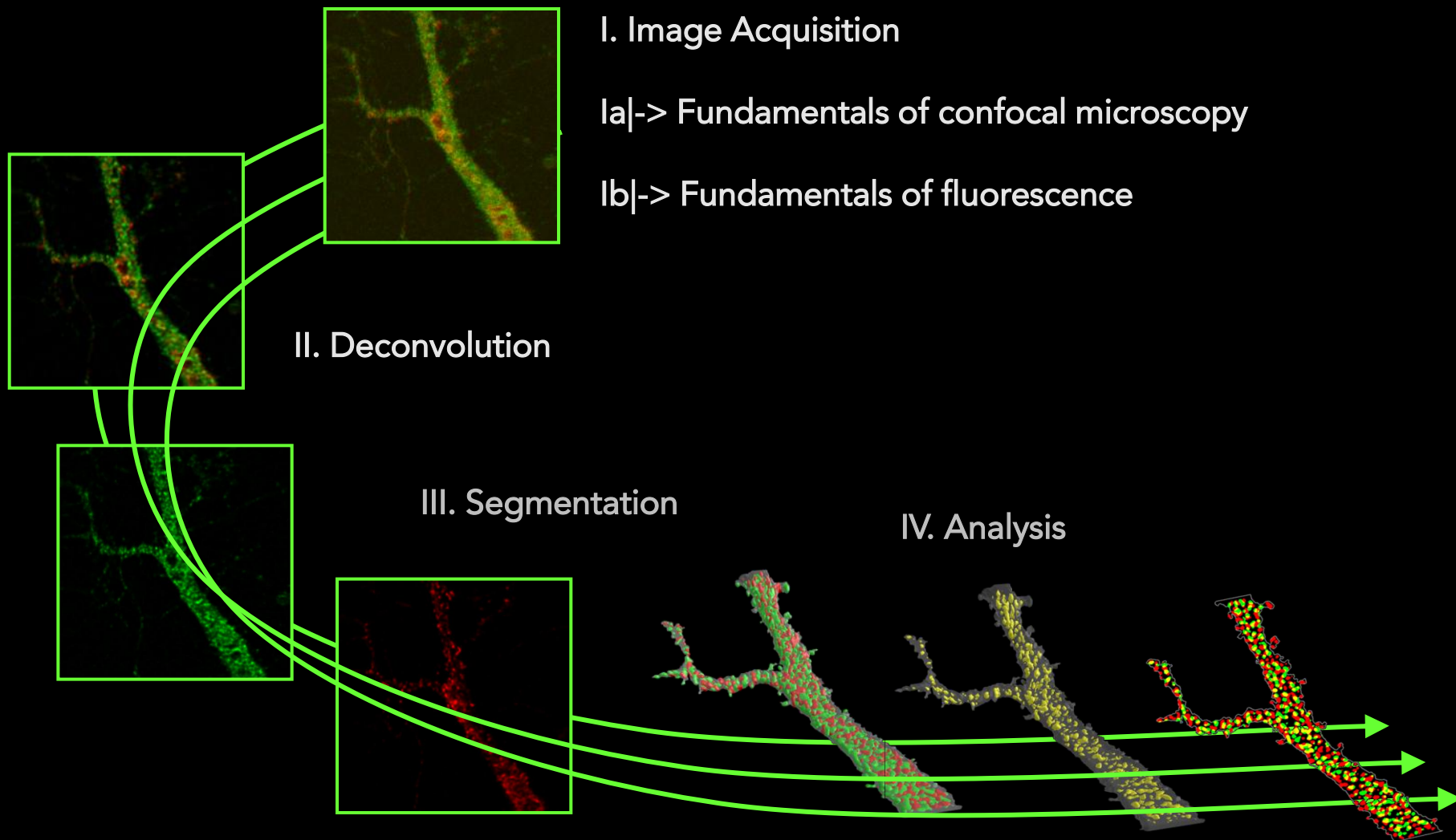


Prof. Dr. Steffen Härtel

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Laboratory for Scientific Image Analysis (SCIAN-Lab)
Centro de Informática Médica y Telemedicina (CIMT)
Centro Nacional en Sistemas de Información en Salud (CENS)
Biomedical Neuroscience Institute (BNI)
Red de Salud Digital de Universidades del Estado (RSDUE)
Institute of Biomedical Sciences (ICBM)
Núcleo Milenio de AutoOrganización y Mecánica de Tejidos (SELFO)

Facultad de Medicina, Universidad de Chile



- Principles of Fluorescence Spectroscopy, Joseph R. Lakowicz 4.1 Introduction to Fluorescence

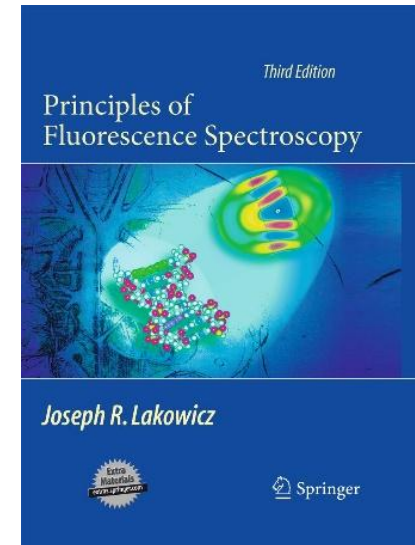
| -> Quenching, Bleaching ...

| -> Polarisation ...

| -> Steady-State and Time-Resolved Fluorescence...

| -> Förster Resonance Energy Transfer ...

- Photomultiplier www.olympus-lifescience.com/en/microscope-resource/primer/digitalimaging/concepts/photomultipliers
- Chrome Spectra Viewer www.chroma.com/spectra-viewer
- Thermo Fisher Spectra www.thermofisher.com/order/spectra-viewer



Luminescence :



- *Fluorescence* $\Delta t \sim 10^{-8}s$
- *Phosphorescence* $\Delta t \sim 10^{-3}-10^0s$

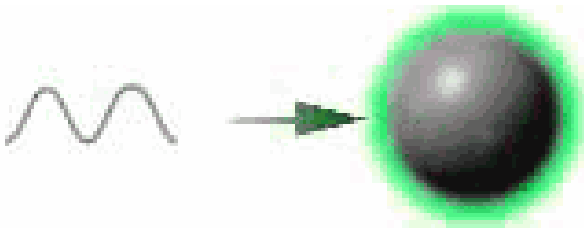
Interactions ...

- intra- and intermolecular...

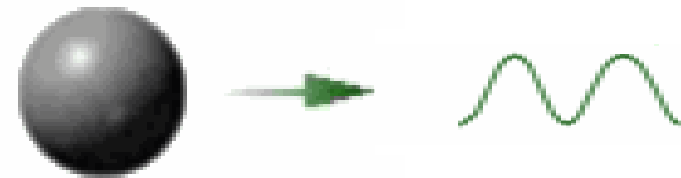
produce changes ...

- spectral
- lifetimes
- polarization
- intensity ...

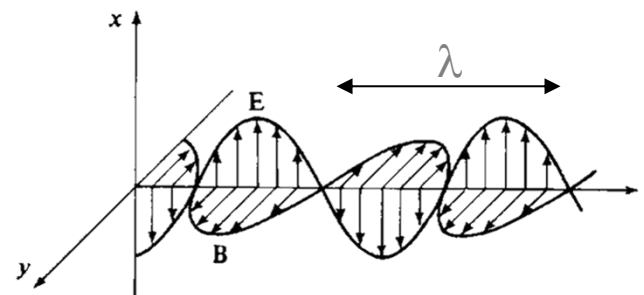
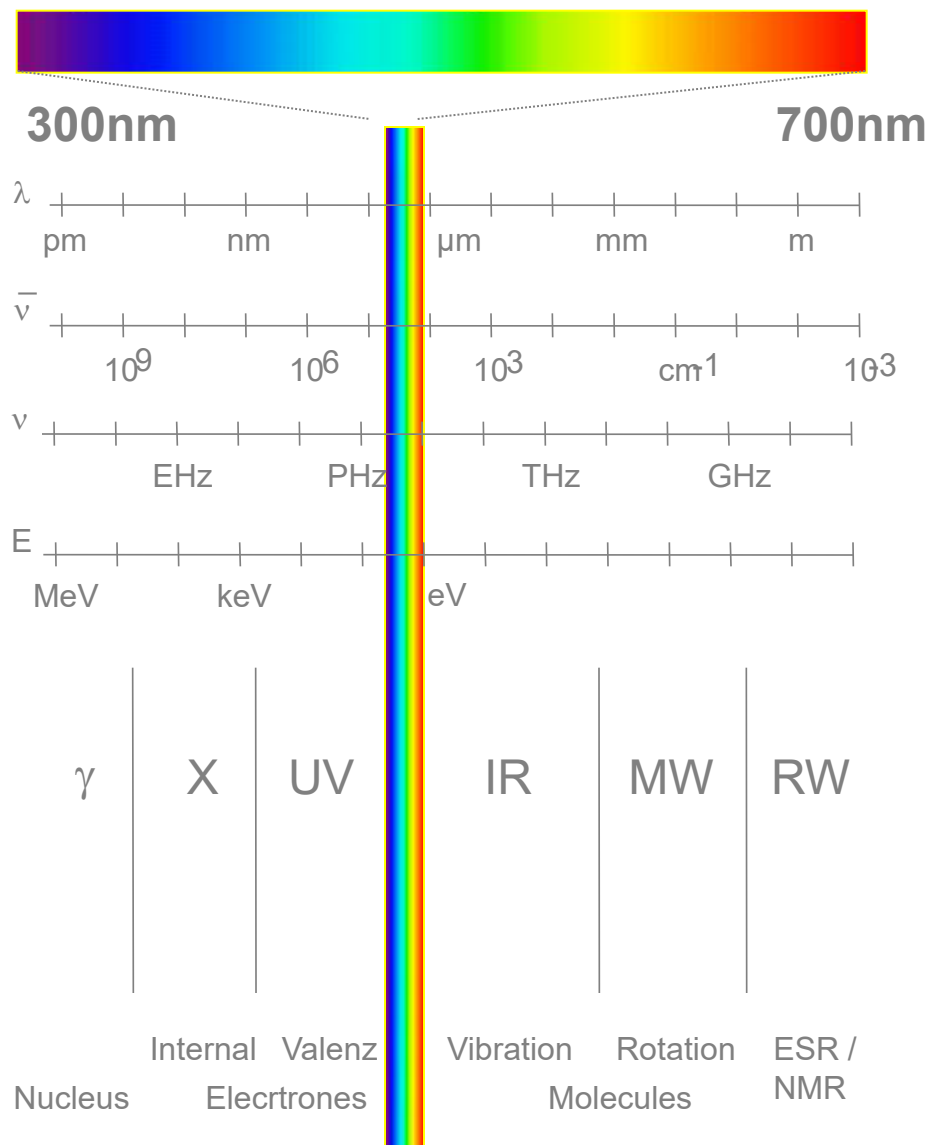
← Δt →



Absorption / Excitation



Emission



Energy of a photon : (~1-5eV)

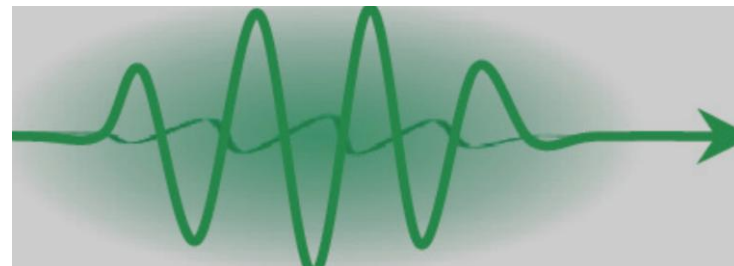
$$E = h \nu = hc\lambda^{-1} \quad | \quad c = \nu \lambda$$

ν , frequency [s^{-1}]

h , Planck's constant [$6.626 \cdot 10^{-34} \text{ Js}^{-1}$]

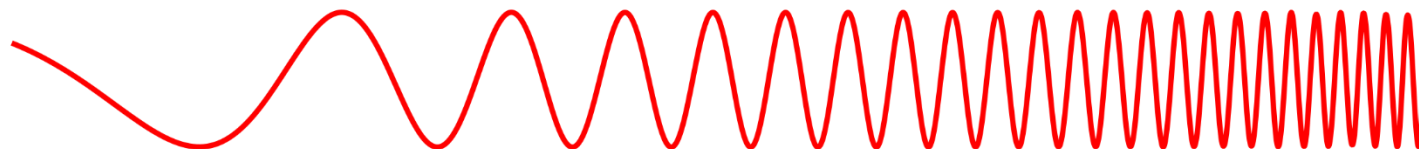
λ , wavelength [m]

c , speed of light [$\sim 3 \cdot 10^8 \text{ ms}^{-1}$]



|-> Fluorescence

Penetrates Earth's Atmosphere?



Radiation Type
Wavelength (m)

Radio
 10^3

Microwave
 10^{-2}

Infrared
 10^{-5}

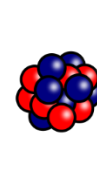
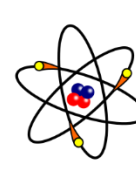
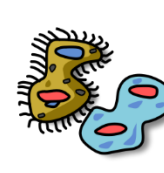
Visible
 0.5×10^{-6}

Ultraviolet
 10^{-8}

X-ray
 10^{-10}

Gamma ray
 10^{-12}

Approximate Scale
of Wavelength



Buildings

Humans

Butterflies

Needle Point

Protozoans

Molecules

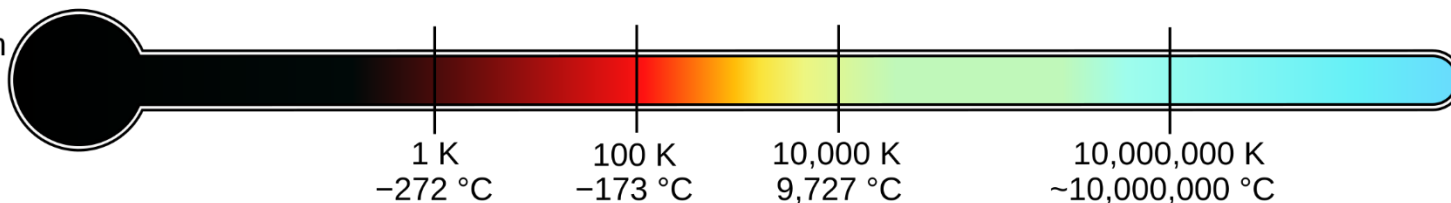
Atoms

Atomic Nuclei

Frequency (Hz)



Temperature of
objects at which
this radiation is the
most intense
wavelength emitted



|-> Jablonski Diagram

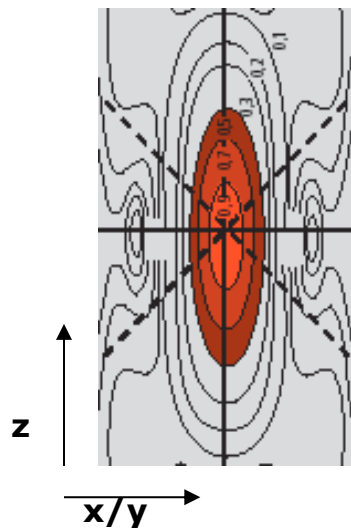
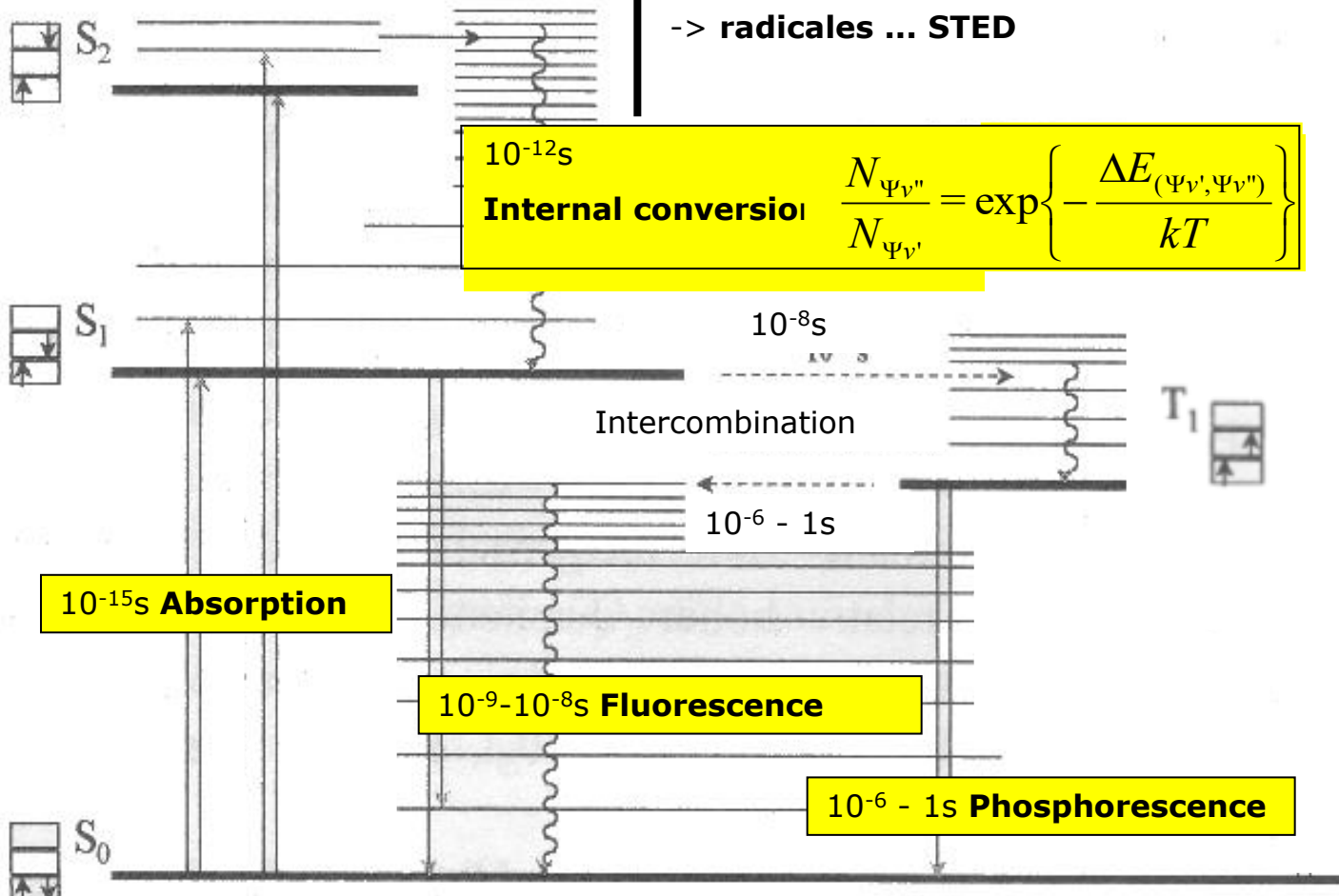
Transitions :

—→ w radiación
 ~→ w/o radiación

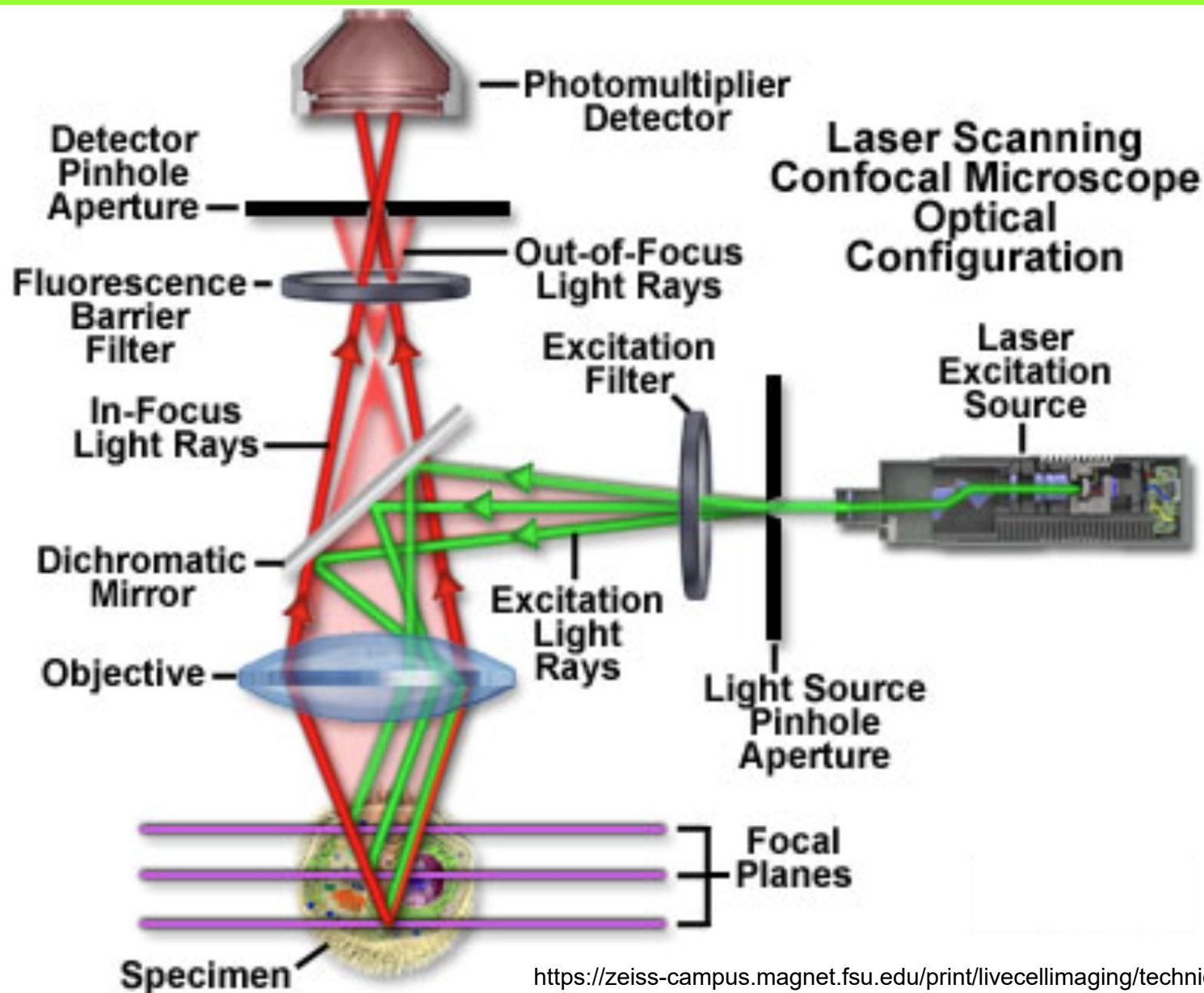
Levels:

— vibrational
 — electronic

-> **heat**
 -> **energy transfer (FRET)**
 -> **radicales ... STED**



| -> 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.

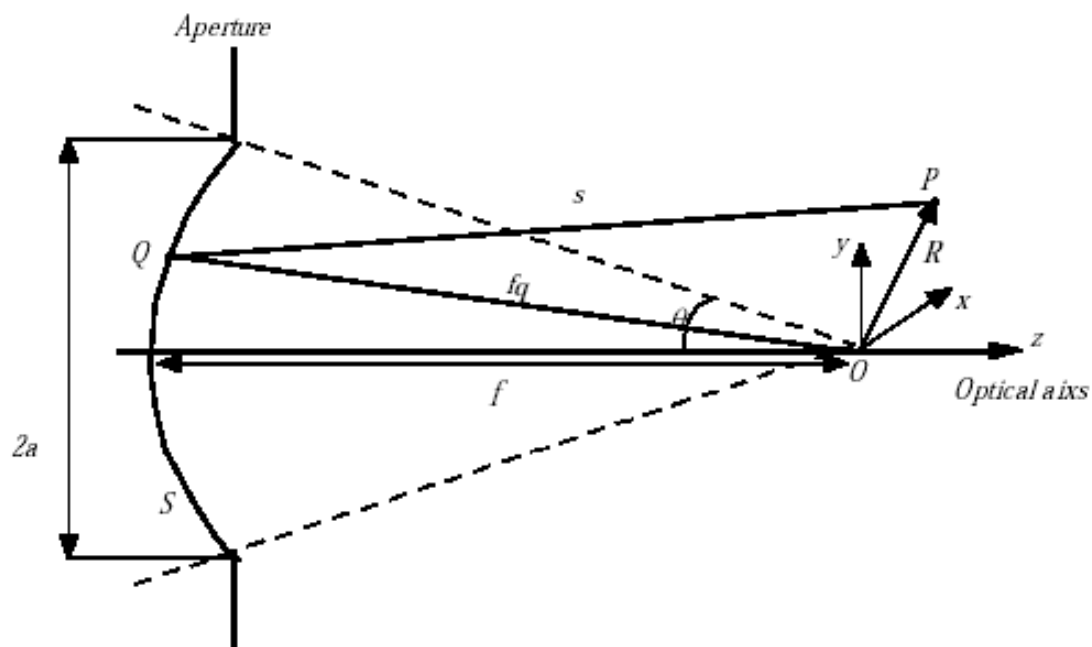


Figure 2.1 Diffraction of a converging spherical wave at a circular aperture

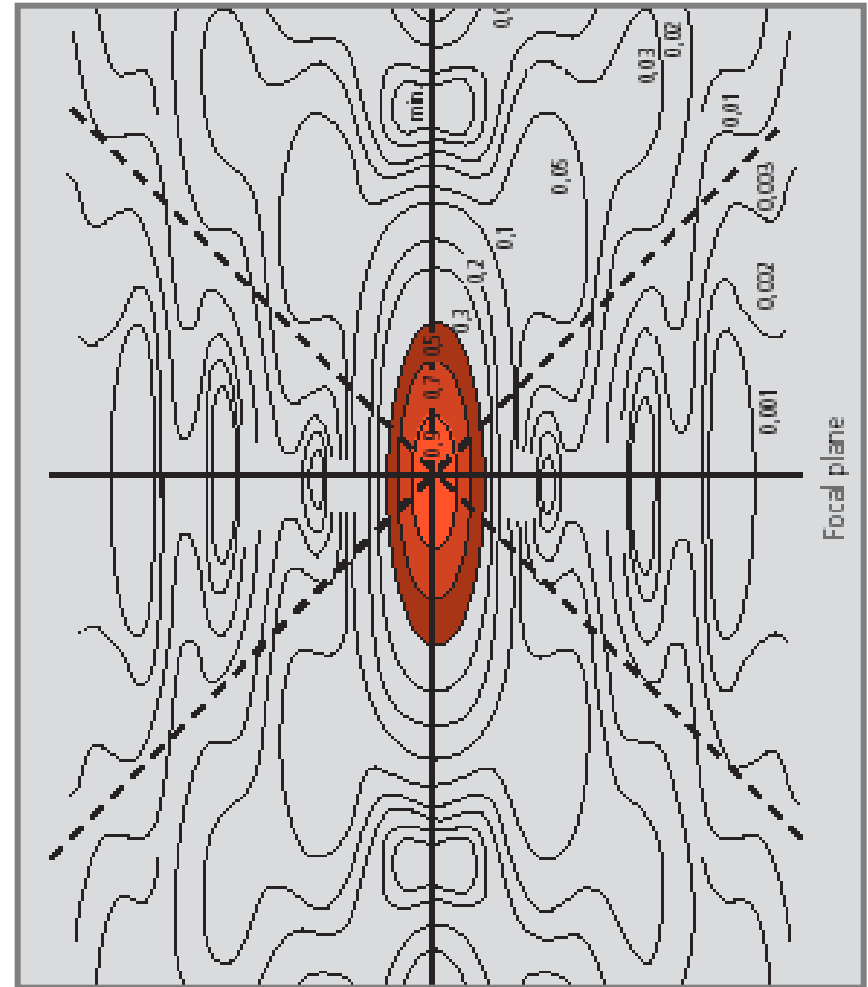
Non-geometric optics / Diffraction theory

$$\text{PSF} = |U|^2 = f(J_0)$$

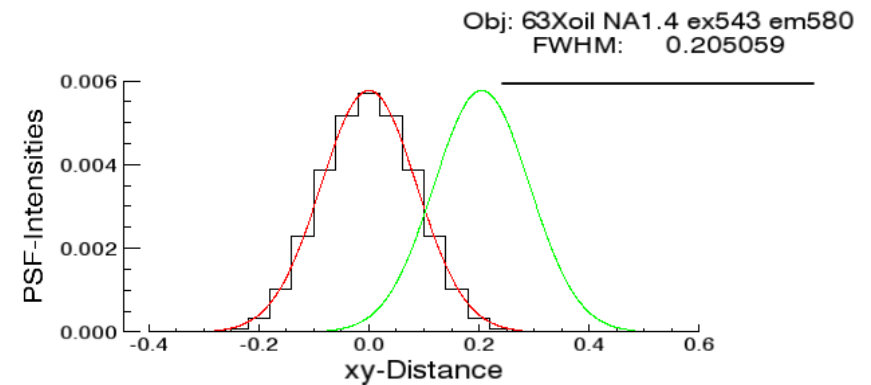
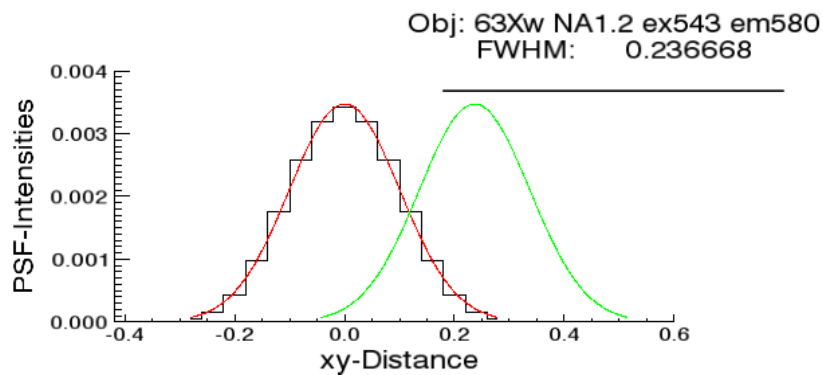
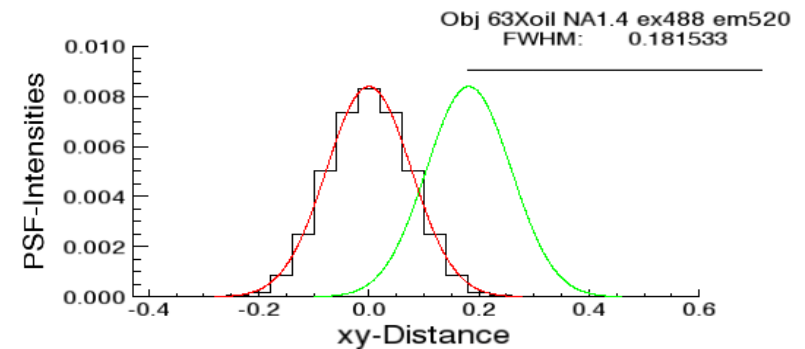
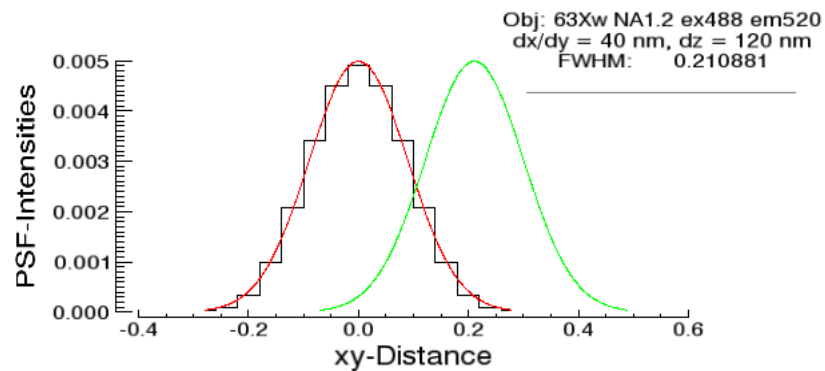
U , Kirhoff's Diffraction Integral

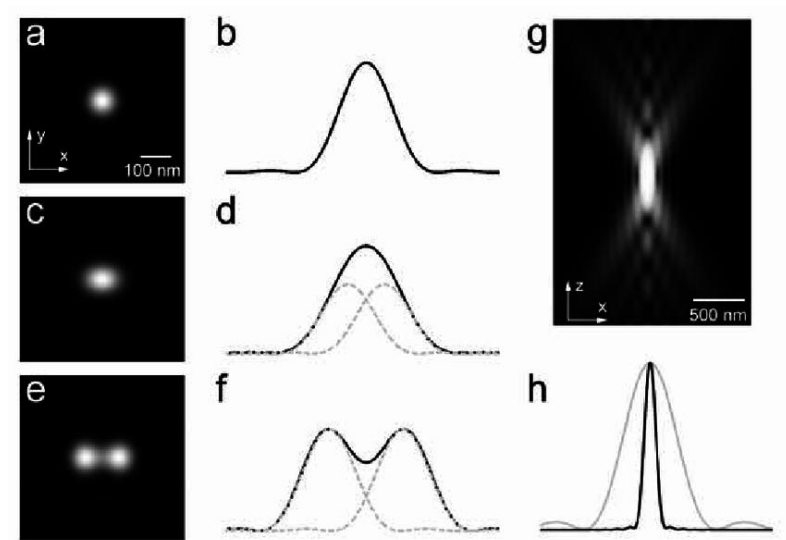
J_0 , Bessel function series

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



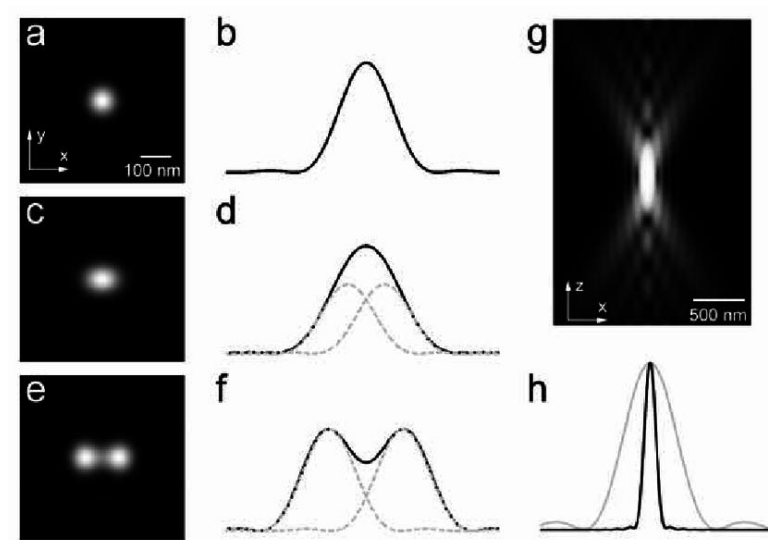
| -> Diffraction limited Microscopy





Bertocchi, Cristina & Goh, Wah & Zhang, Zhen &
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 reviews in biomedical engineering. 41. 281-308.
 10.1615/CritRevBiomedEng.2014010448.

| -> Diffraction limited Microscopy



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