Single Molecule Spectroscopy

Nobel Prize 2014 to Betzig, Hell, Moerner

What is it, why is it interesting?

Class of methods that allow the detection and study of single, isolated molecules, usually on surfaces

It is interesting because:
Fundamental molecular dynamics
(avoid averaging over 10^{23} particles)
Biological applications
Chemical reactions on surfaces

Difficulties

A molecule is usually very small.
A single molecule does not absorb much light, so conventional absorption spectroscopy can not be performed
Molecules on surfaces normally are mobile
One needs a level of sensitivity of the order $1/N_A$

Homogeneous line broadening

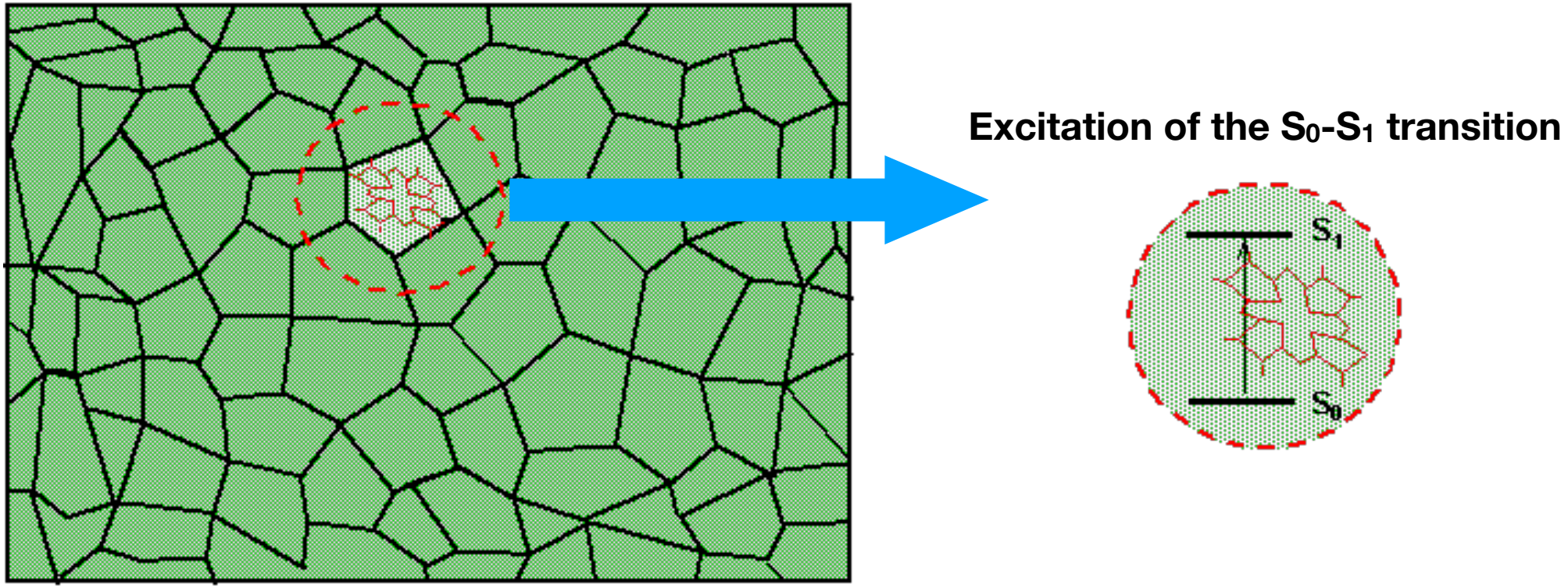
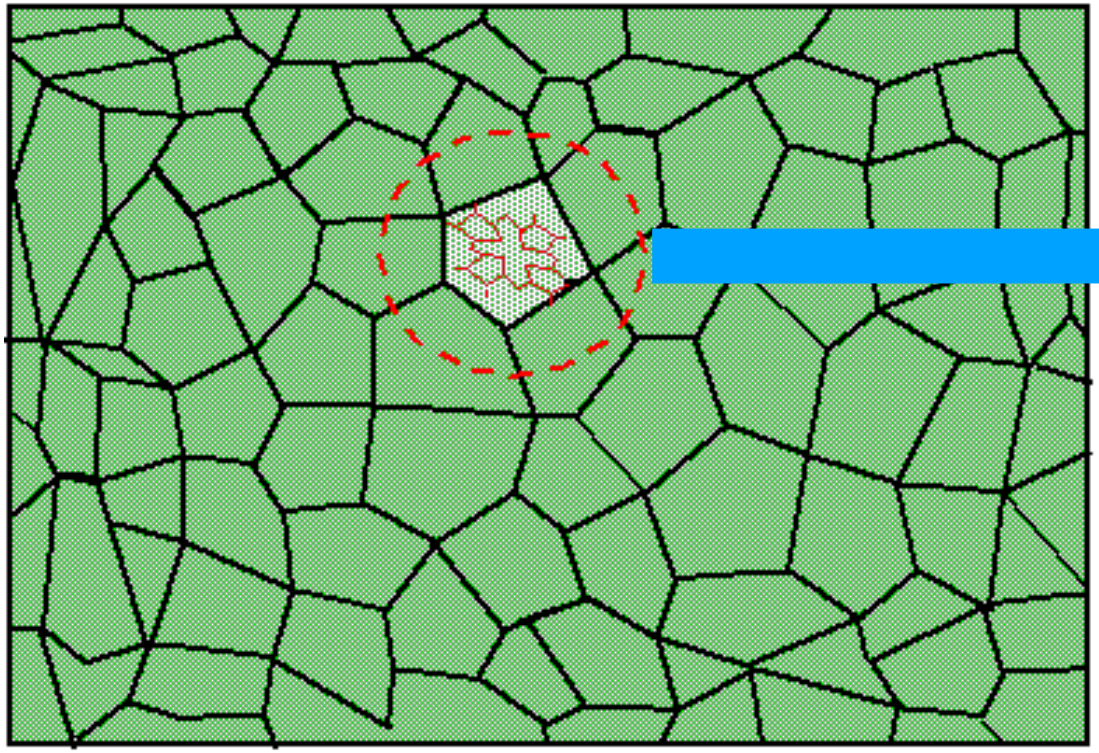
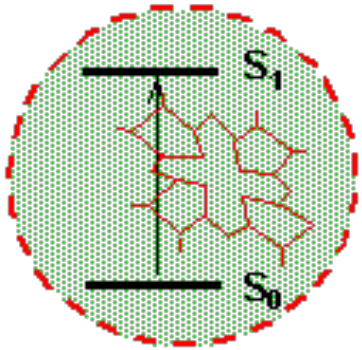


Figure from
http://www.physics.montana.edu/arebane/research/tutorials/hole_burning/index.html

Homogeneous line broadening



Excitation of the S_0-S_1 transition



But:
The matrix itself can also be excited!
Collective lattice vibrations of the surrounding crystal are called phonons, they can also be excited

Figure from
http://www.physics.montana.edu/arebane/research/tutorials/hole_burning/index.html

Phonons and zero-phonon lines

Vibrations of the crystal lattice

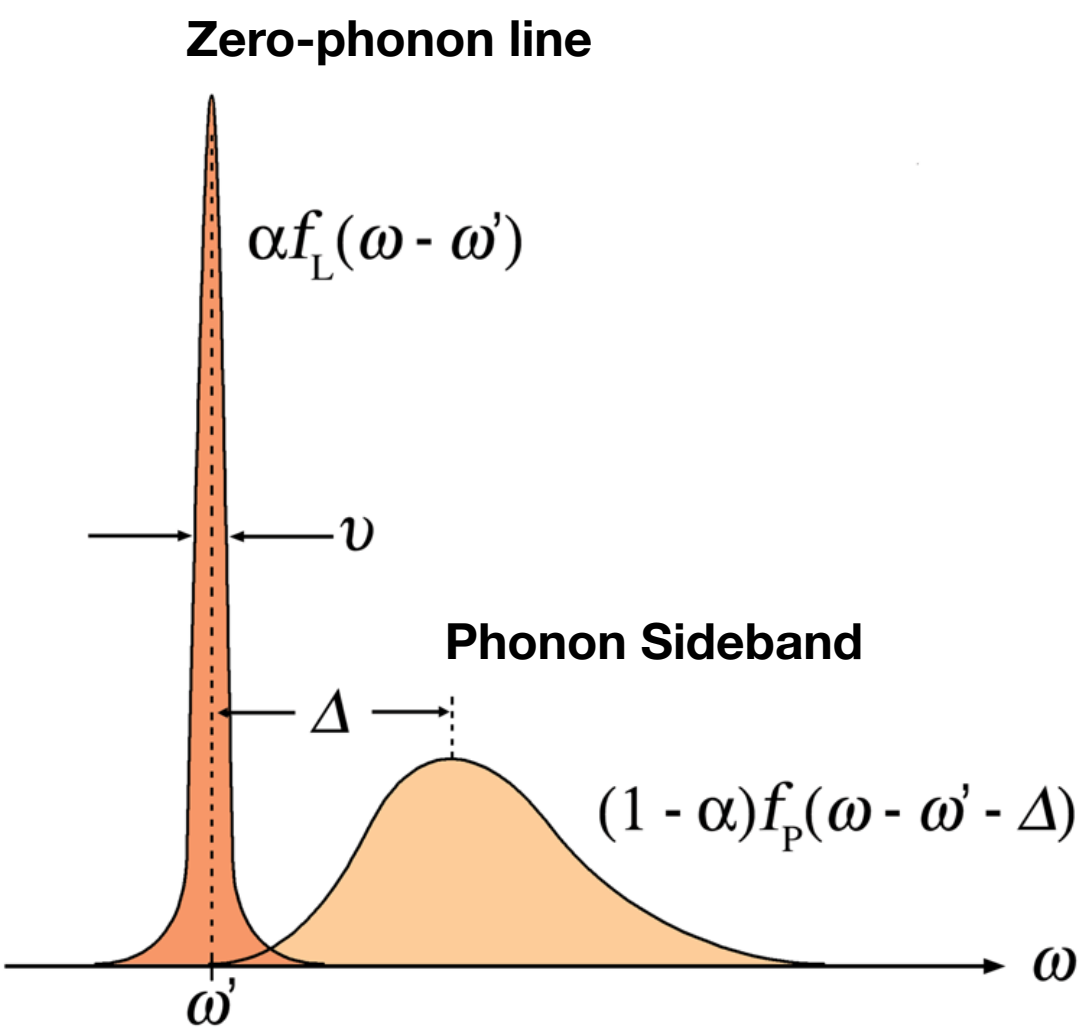
$$\mathcal{H} = \sum_{i=1}^{N} \frac{p_i^2}{2m} + \frac{1}{2} m\omega^2 \sum_{\{ij\}(\mathrm{nn})} \left(x_i - x_j\right)^2$$

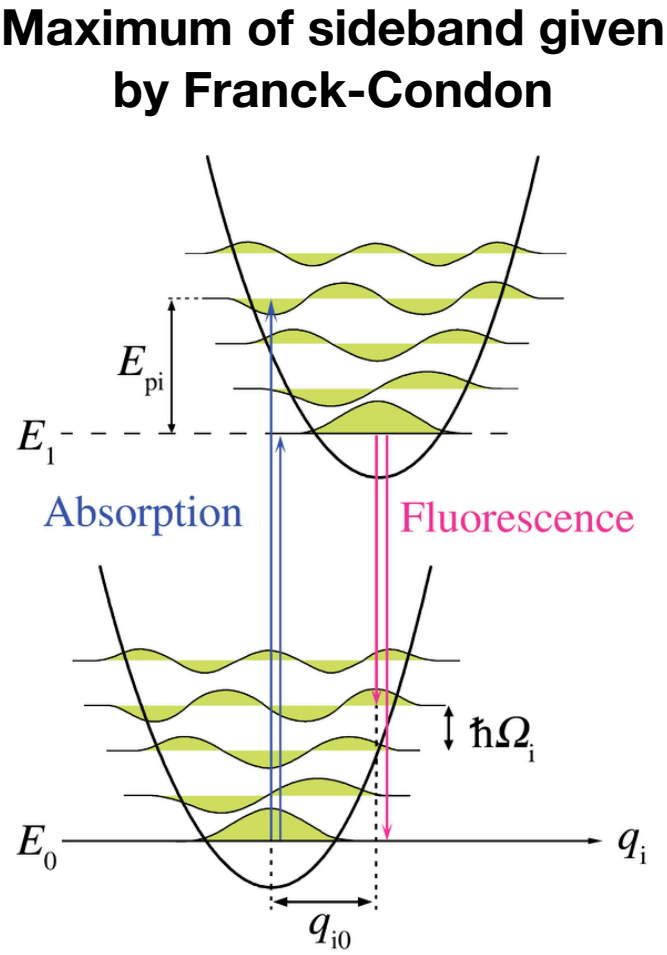
Energies:

$$E_n = \left(\frac{1}{2} + n\right)\hbar\omega_k \quad n = 0, 1, 2, 3\ldots$$

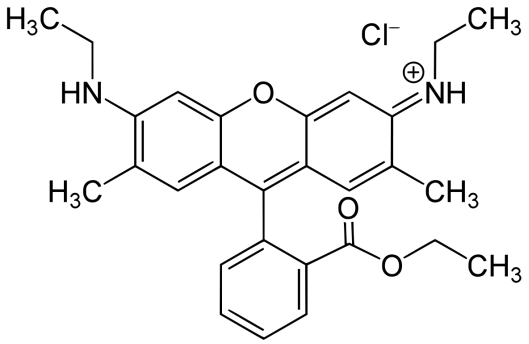
Harmonic Oscillators!
The frequencies usually lie in the IR

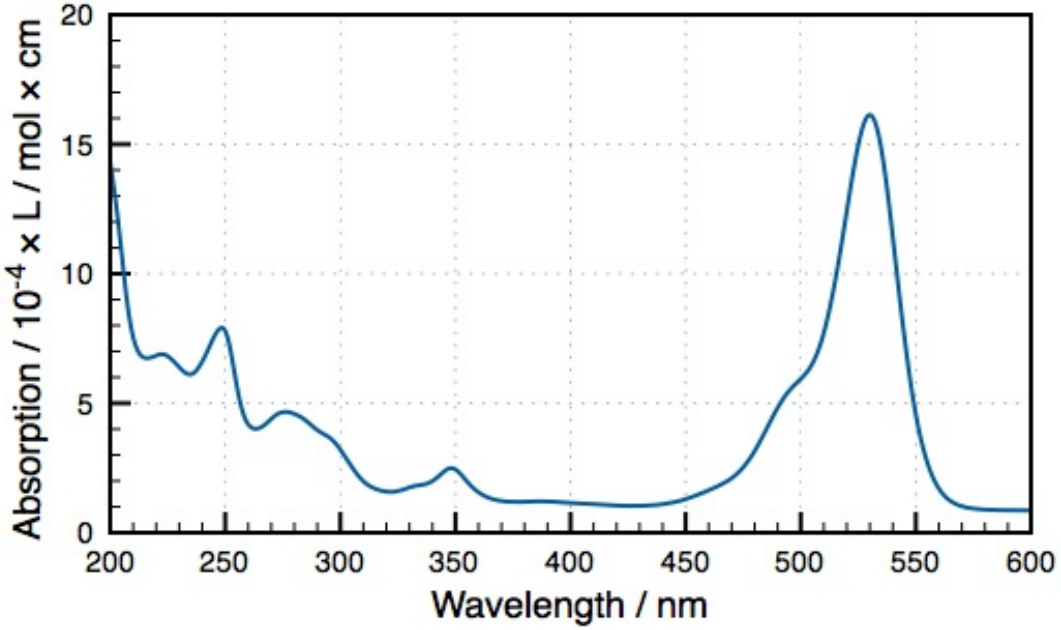
Phonons


Zero-phonon line
$\alpha f_L(\omega - \omega')$
υ
Phonon Sideband
Δ
$(1 - \alpha) f_P(\omega - \omega' - \Delta)$
ω
ω'


Maximum of sideband given by Franck-Condon
E_{pi}
E_1
Absorption
Fluorescence
$\hbar\Omega_i$
E_0
q_i
q_{i0}

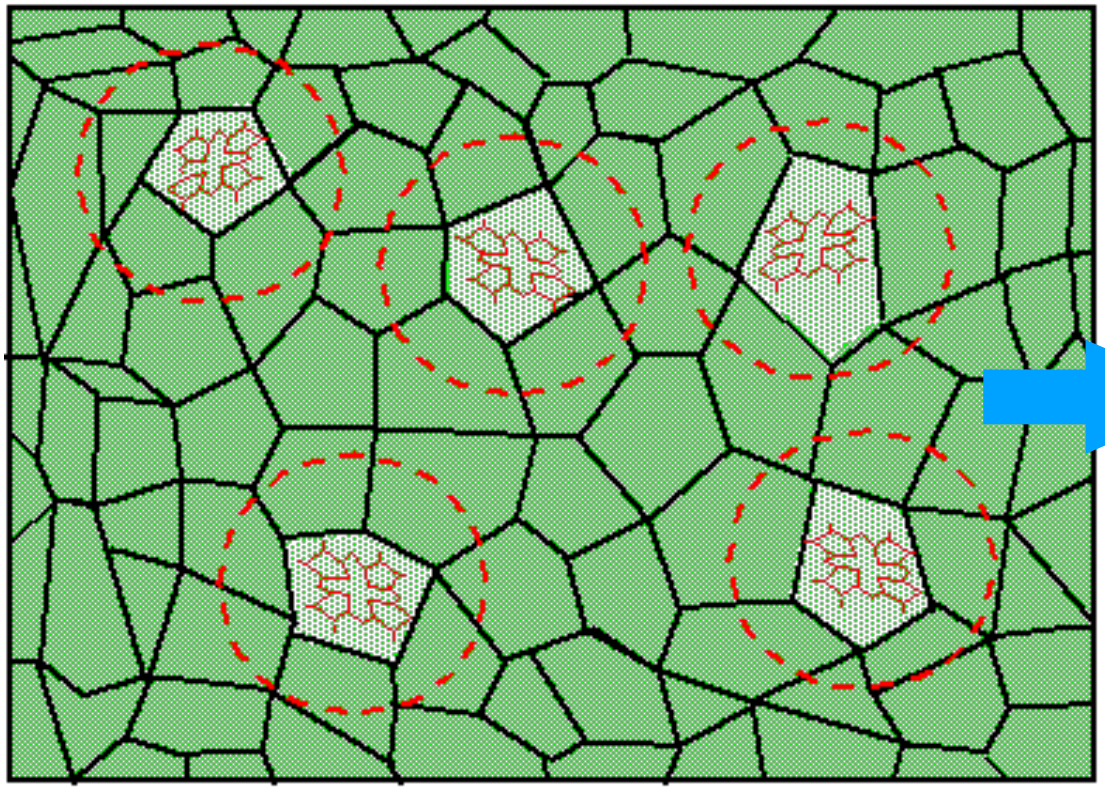
Rhodamine 6G



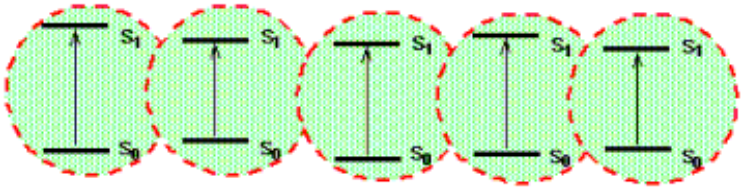


How is this compatible with what you learned in your spectroscopy class?

Inhomogeneous line broadening



Excitation of the S_0-S_1 transitions



Frequency-shift depending on surroundings (unlike gas-phase!)

Figure from
http://www.physics.montana.edu/arebane/research/tutorials/hole_burning/index.html

Spectral Hole burning

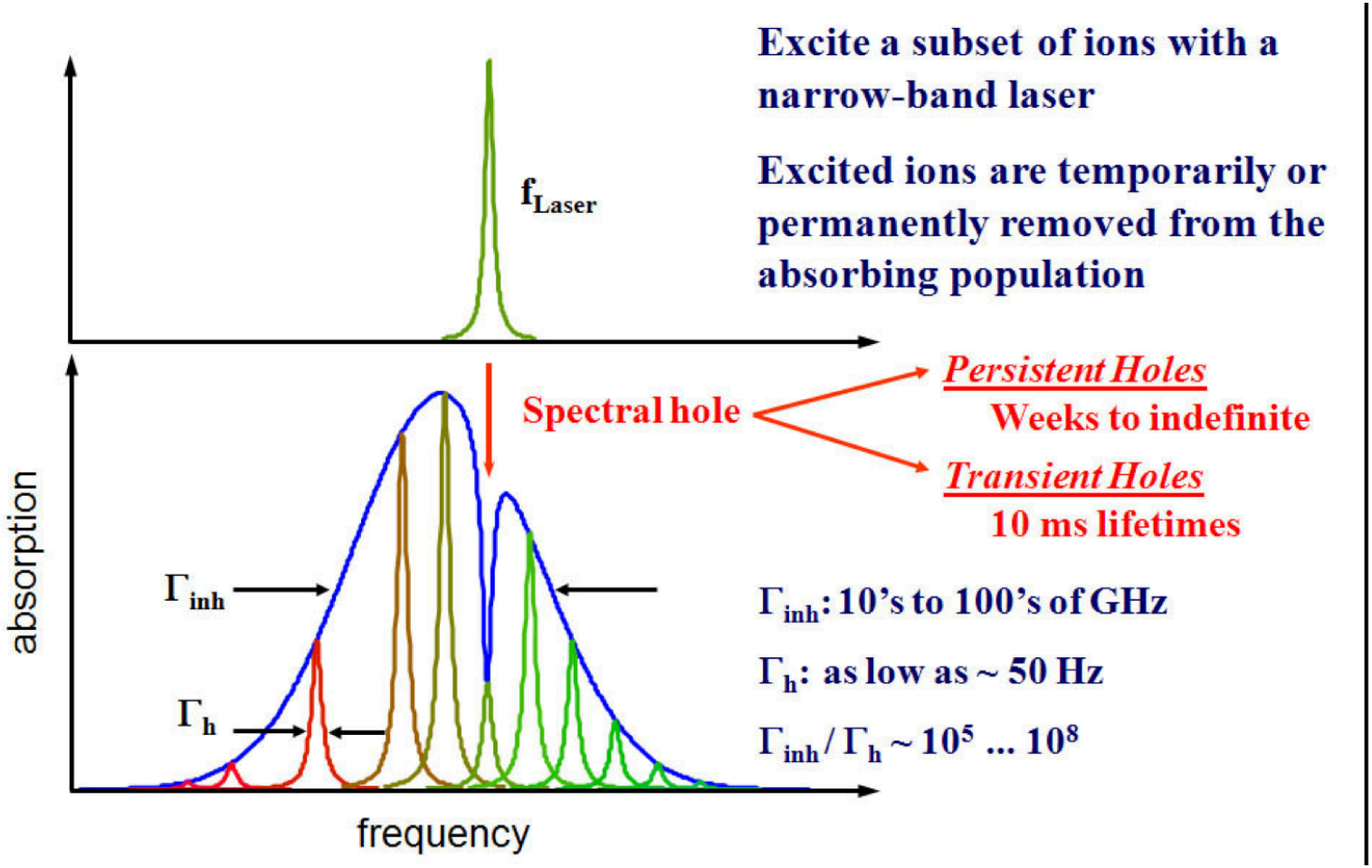


Image from:
Advances in Photonics of Quantum Computing, Memory, and Communication V, edited by Zameer Ul Hasan et al., Proc. of SPIE Vol. 8272

Spectral Hole burning

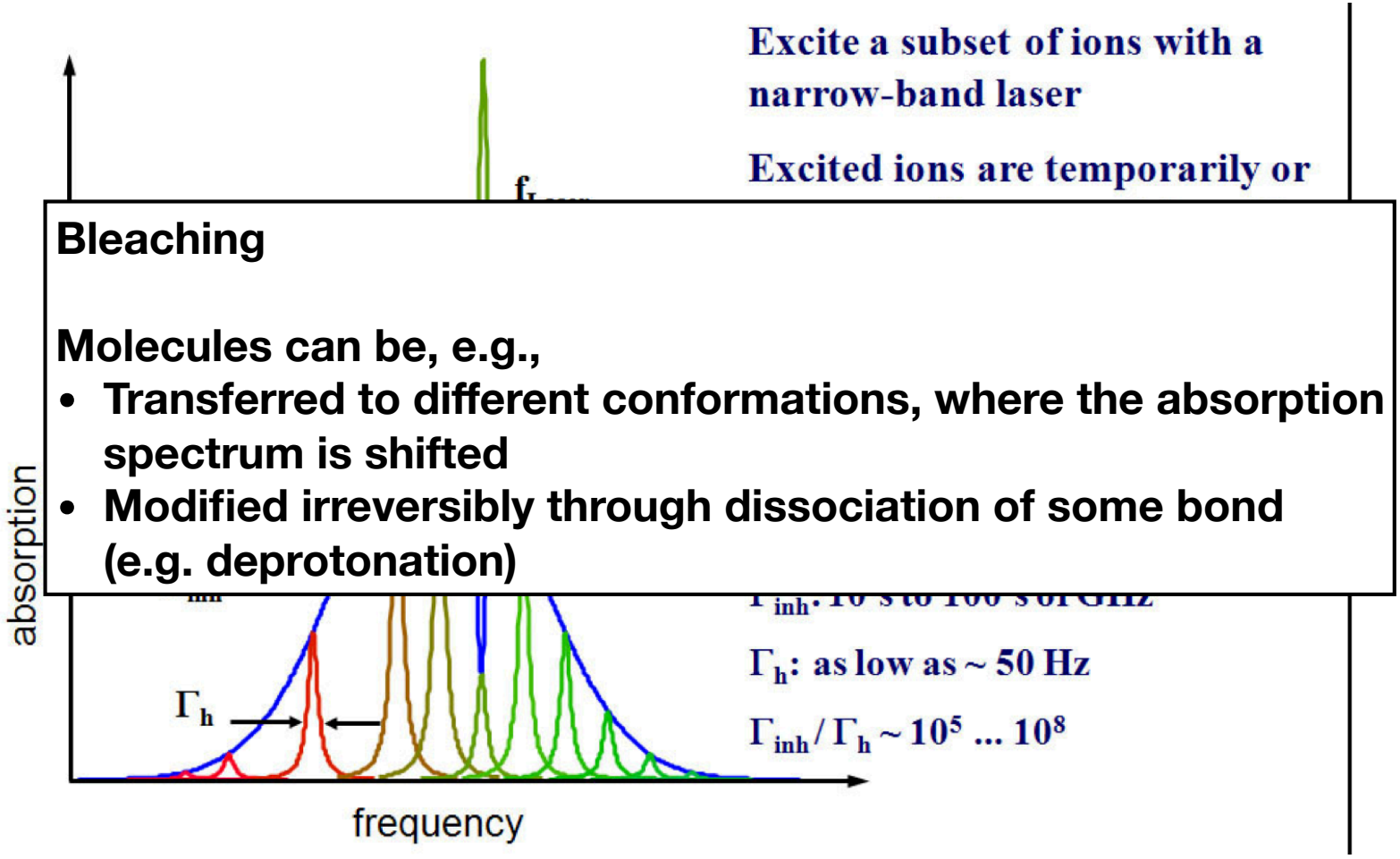
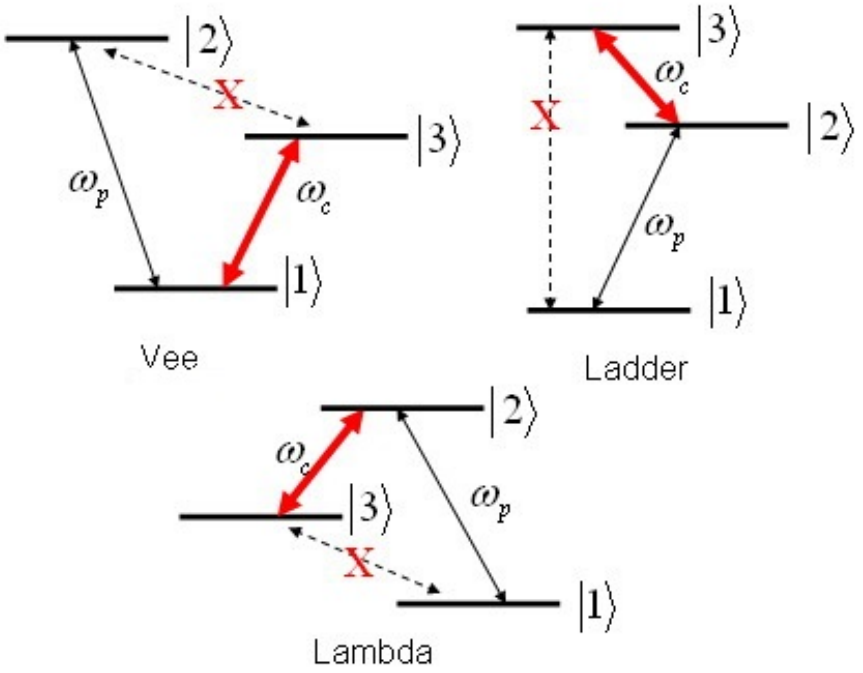


Image from:
Advances in Photonics of Quantum Computing, Memory, and Communication V, edited by Zameer Ul Hasan et al., Proc. of SPIE Vol. 8272

Spectral Hole burning

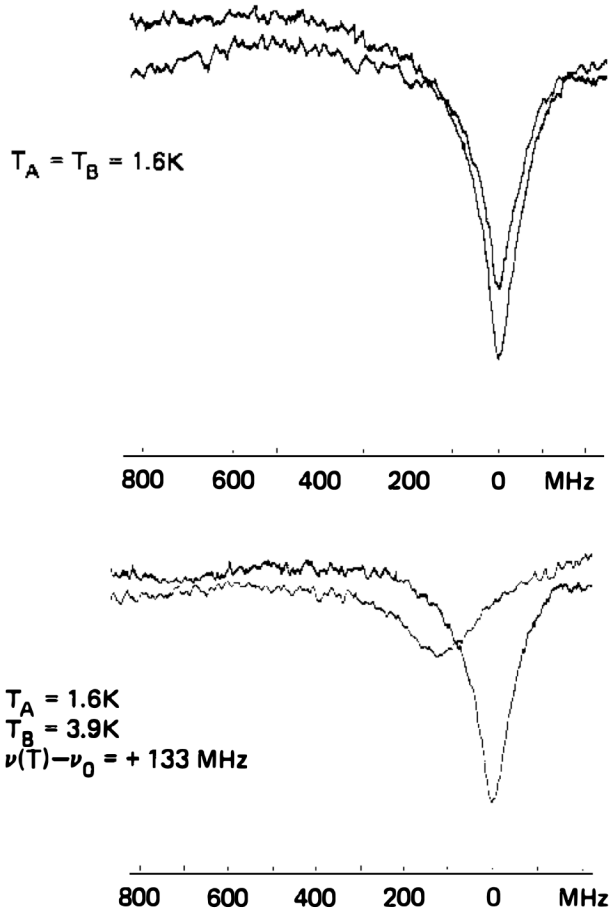
Note:
This is an incoherent process that leads to a loss of absorption at a particular wavelength.

There are also coherent processes that lead to similar effects: Electromagnetically Induced Transparency (EIT)



Transfer of population from |1> to |3> will reduce the absorption on the |1>-|2> transition

Spectral Hole Burning



Example:

Frequency shift and broadening of a hole burnt in the B1 site of the 0-0 transition of free-base porphin (H2P) in n-octane (n-Cs).

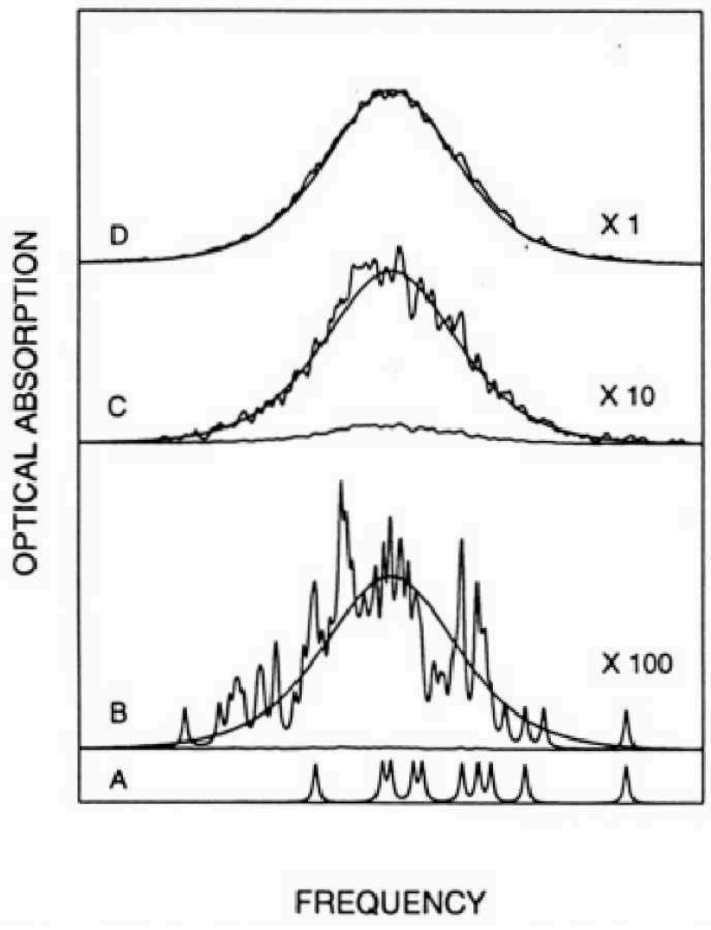
Top: Excitation spectra of two holes burnt at the same frequency at 1.6 K in identical samples in two cryostats, A and B.

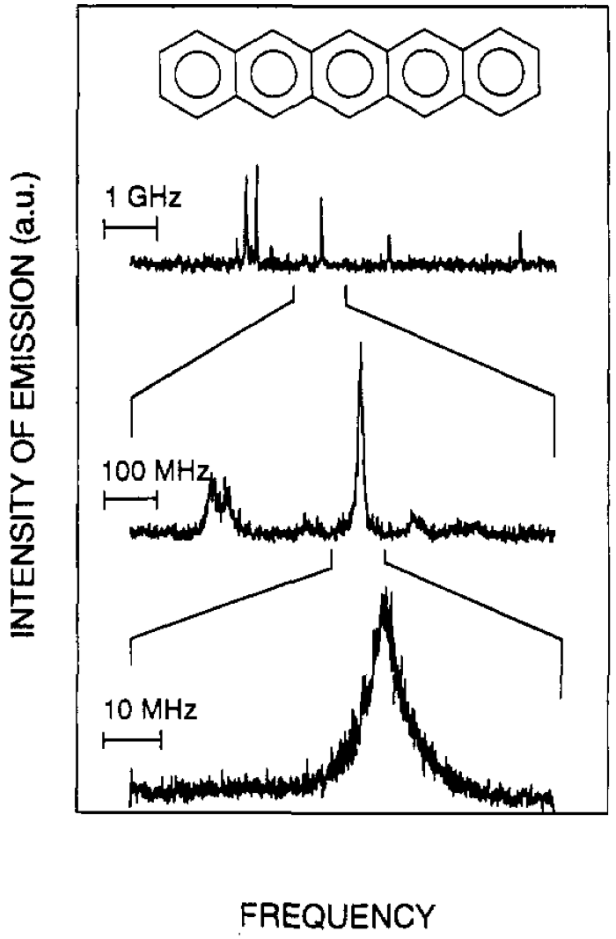
Bottom: Excitation spectra of the same holes after raising the temperature of cryostat B to 3.9 K. The temperature behavior of the hole is reversible.

Annu. Rev. Phys. Chem. 1989. 40: 499-530

Fluorescence spectroscopy

statistical fine structure

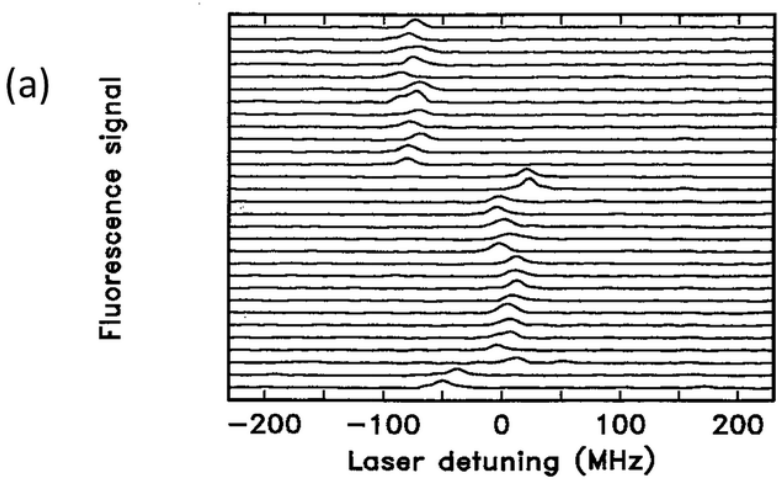


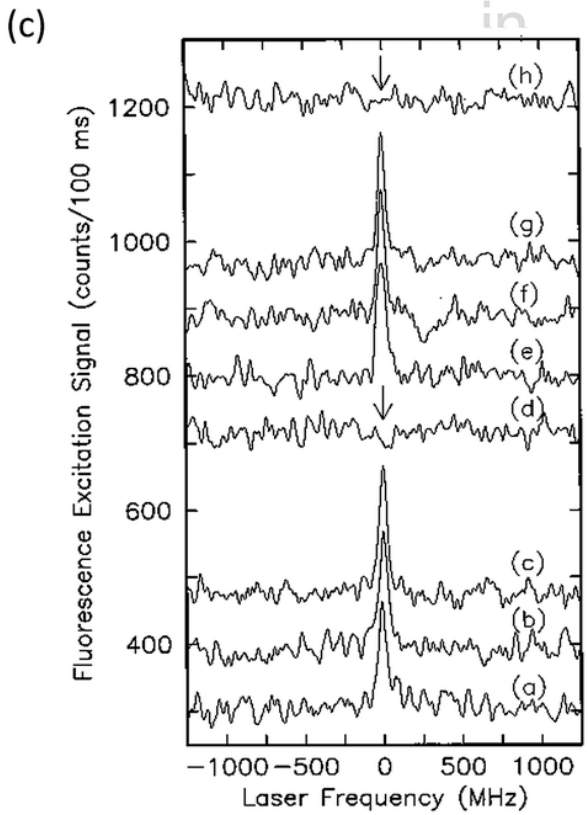


crystal of p-terphenyl doped with pentacene at 1.8 K

Journal of Physical Chemistry 97, 10256(1993)

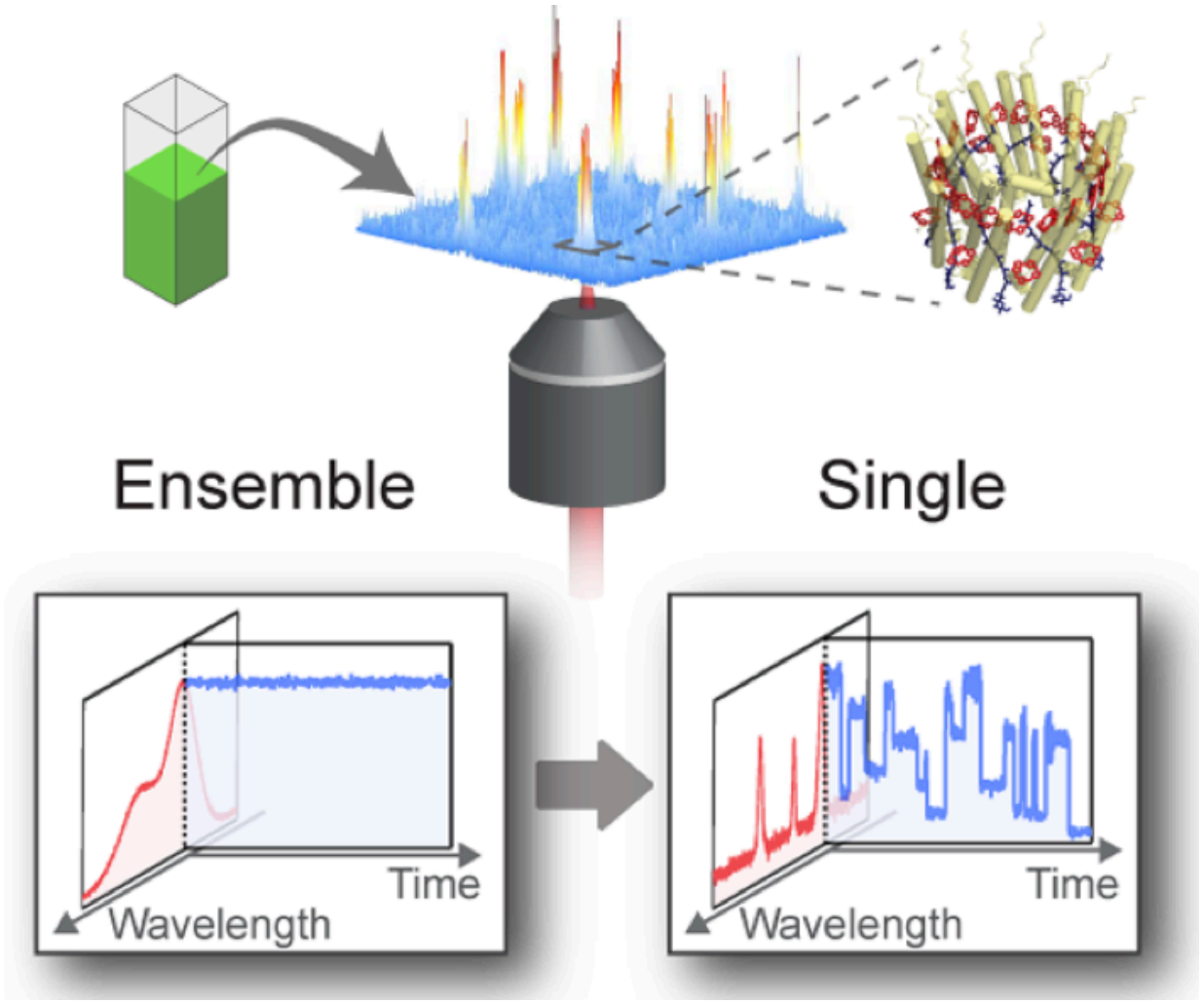
Spectral diffusion





J. Chem. Phys. 95, 7150 (1991)

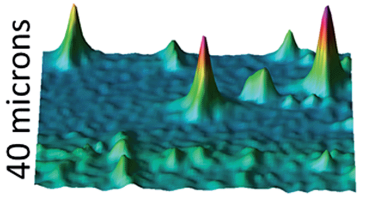
Temporal evolution

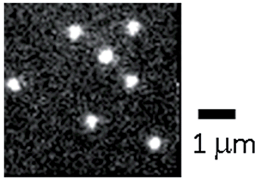


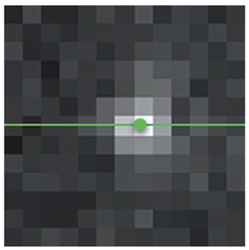
Chem. Rev. 2017, 117, 86

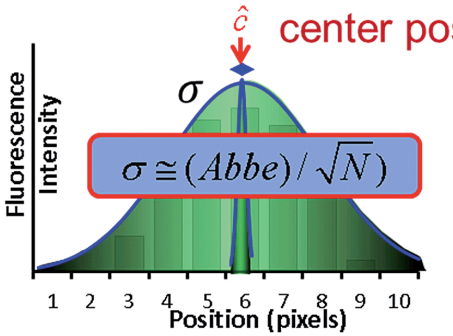
Super resolution spectroscopy

Key Idea #1: Super-localization





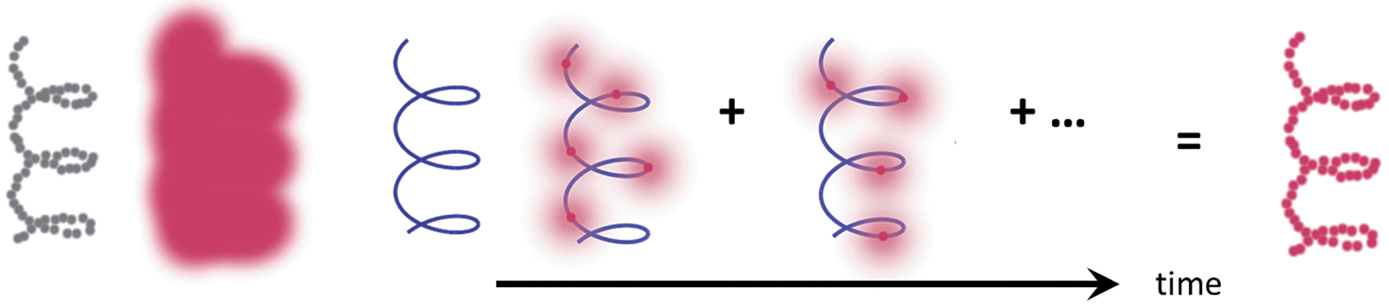




10^4 photons: 2 nm prec.

Key Idea #2: Active control of emitting concentration, sequential imaging

B (off) ⇄ **A (on)**

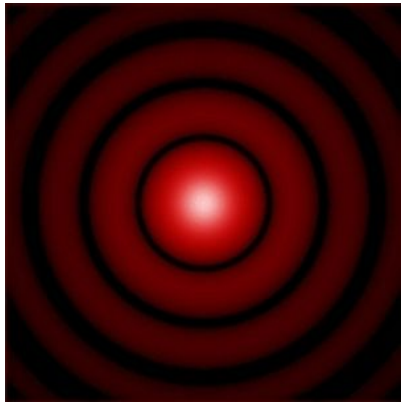


Near-field methods

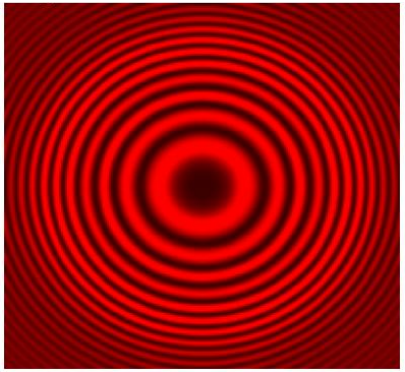
Far-field vs near-field

Far-field:
Far away from the emitter (e.g. an antenna)
Waves behave as regular EM fields
No interference

Near-field:
Inside or close to emitters
Strong interferences



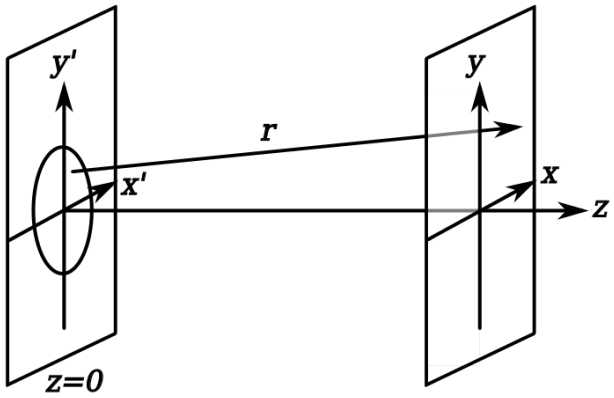
Fraunhofer diffraction (far-field)



Fresnel diffraction (near-field)

Near-field methods

Far-field vs near-field



Aperture **Screen**

Calculate the intensity pattern on the screen

$$E(x,y,z) = \frac{1}{i\lambda}\iint_{-\infty}^{+\infty} E\left(x',y',0\right)\frac{e^{ikr}}{r}\cos(\theta)dx'dy'$$

No analytical solution

Near-field methods

Far-field vs near-field

$$E(x,y,z) = \frac{1}{i\lambda}\iint_{-\infty}^{+\infty} E\left(x',y',0\right)\frac{e^{ikr}}{r}\cos(\theta)dx'dy'$$

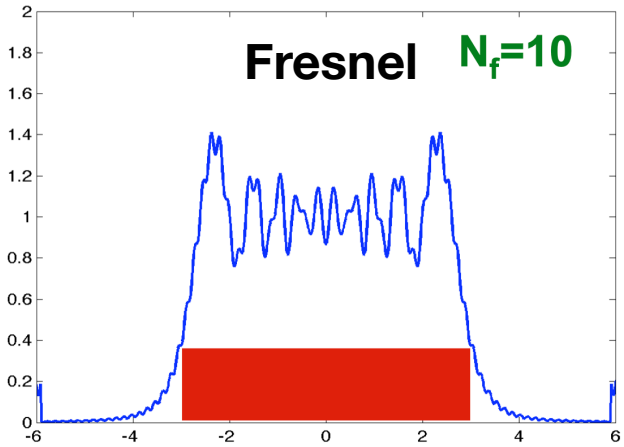
No analytical solution

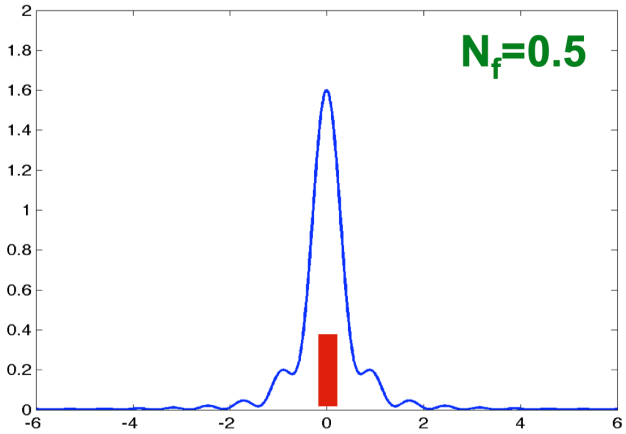
Fresnel/Fraunhofer Approximations are valid for large and small Fresnel numbers, respectively.

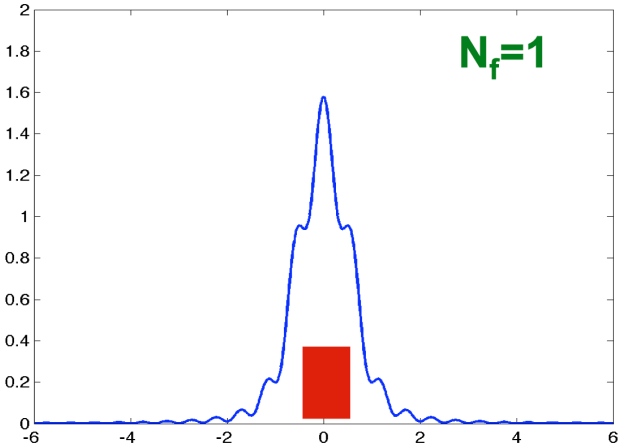
$$N_F = \rho^2/(\lambda z)$$

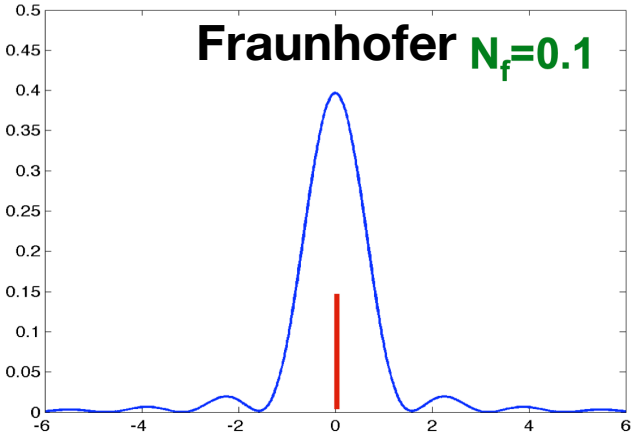
Near-field methods

Far-field vs near-field



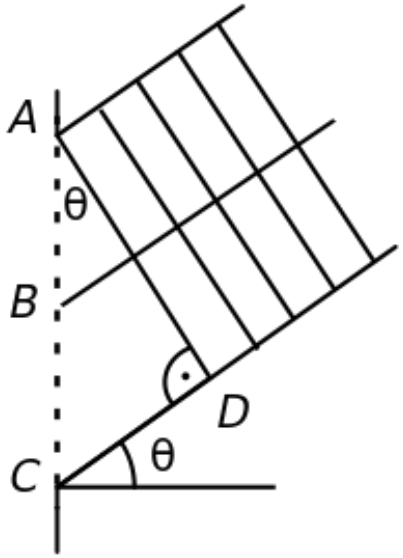


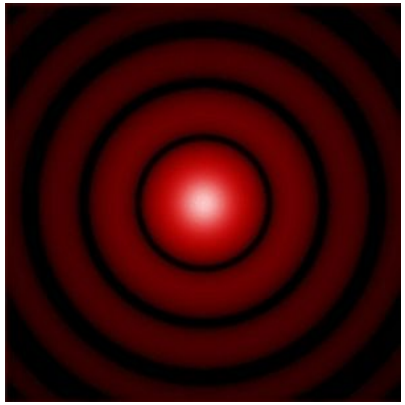




Near-field methods

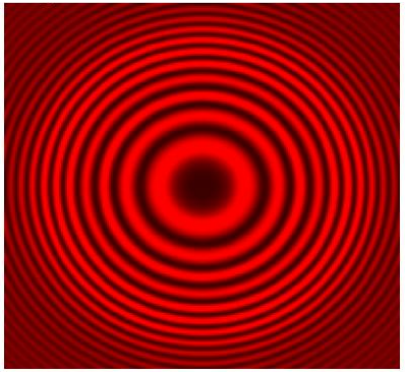
Far-field vs near-field





Fraunhofer diffraction (far-field)

Fresnel diffraction takes the curved wave fronts into account



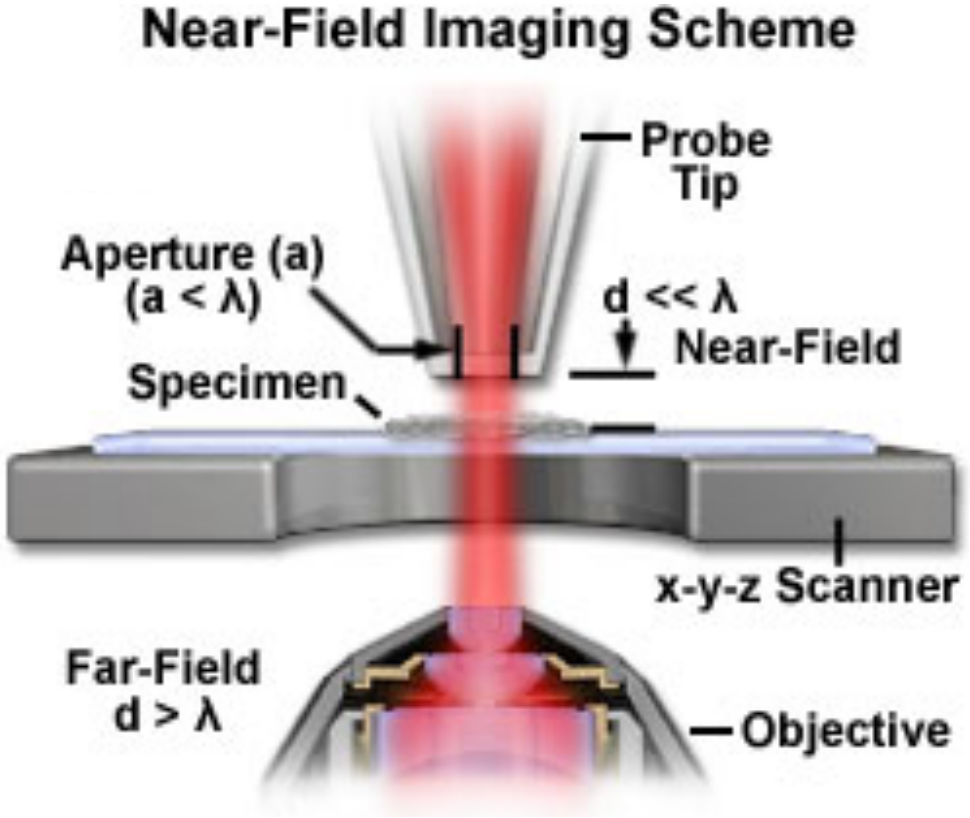
Fresnel diffraction (near-field)

Near-field methods

Far-field vs near-field

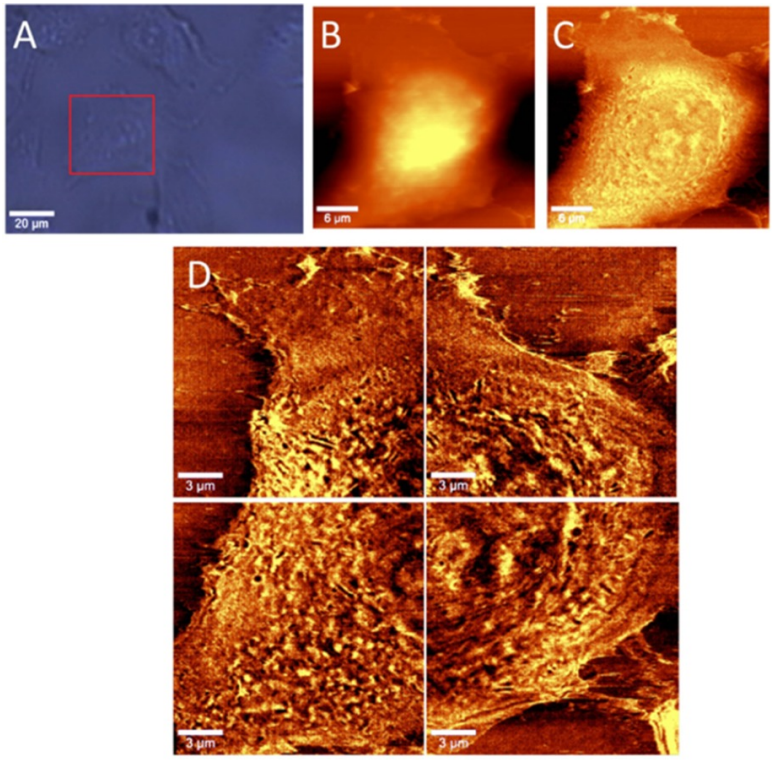
The Abbe limit is valid only in the far-field

Scanning Near-field Optical Microscopy
Uses very sharp tips that are scanned across a surface



Source: Olympus

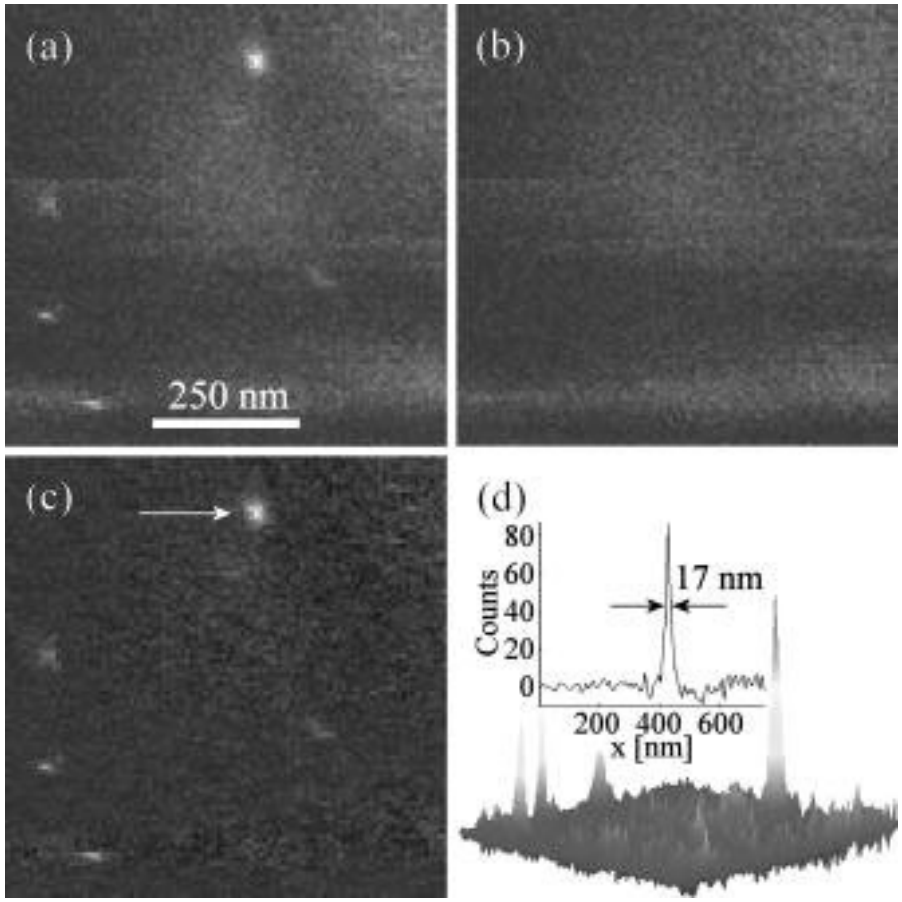
SNOM (example)



Human Lung Microvascular Endothelial Cell

J. Biophotonics **10**, 928–938 (2017)

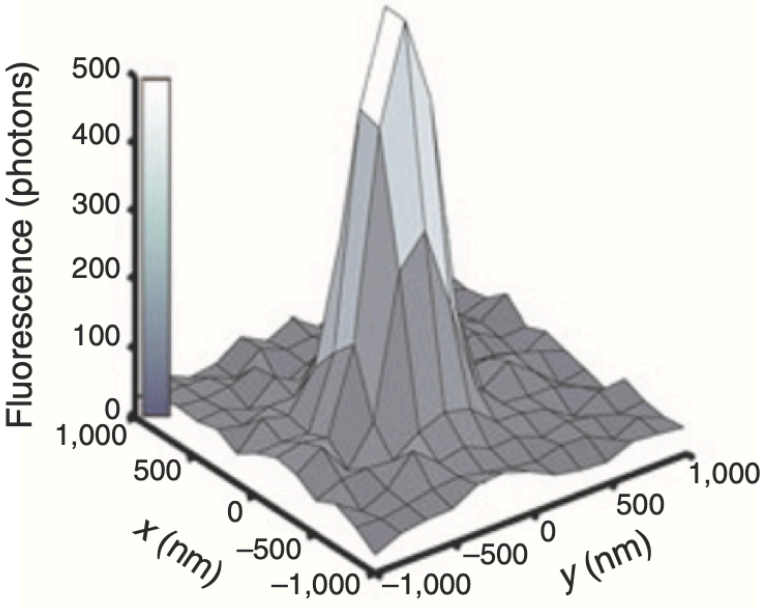
Tip-enhanced single molecule fluorescence near-field microscopy in aqueous environment



SNOM image (BIM) of individual ATTO-740 molecules in water attached to a glass surface. (a) Fluorescence near-field image of single molecules with a diffuse background resulting from the far-field illumination. (b) Fluorescence far-field image. It shows only the background. (c) Background corrected near-field image. (d) 3d image of (c) and a cross-section through a single dye image with a FWHM of 17 nm.

Applied Physics Letters **94**, 241116(2009)

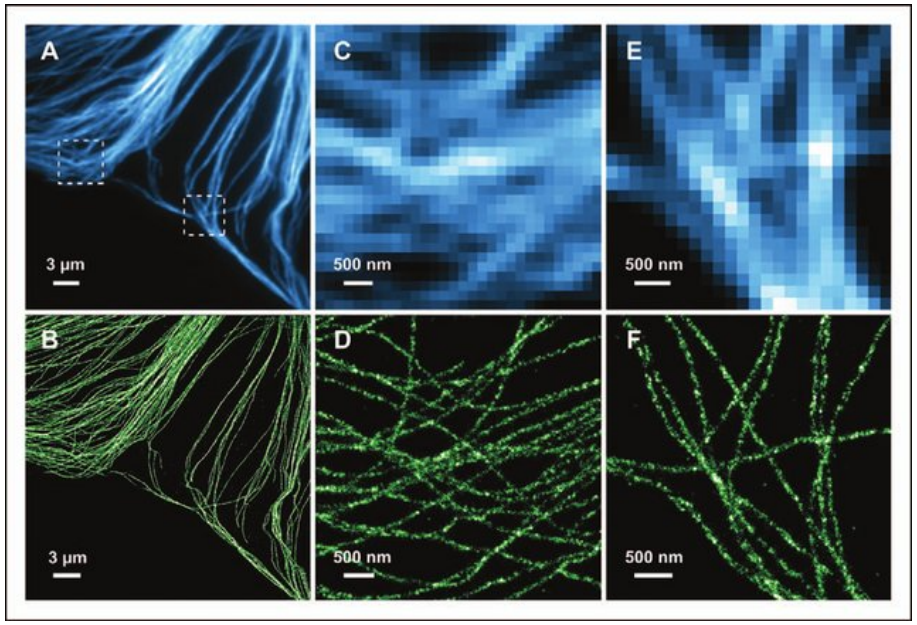
STORM
Stochastic optical reconstruction microscopy



Idea:
Many closely-spaced emitters will produce a broad spot and they can not be separated.
Single molecules produce diffraction-limited spots, but the center of these spots can be determined with much higher accuracy

Nature Methods **3**, 793 (2006)

STORM
Stochastic optical reconstruction microscopy

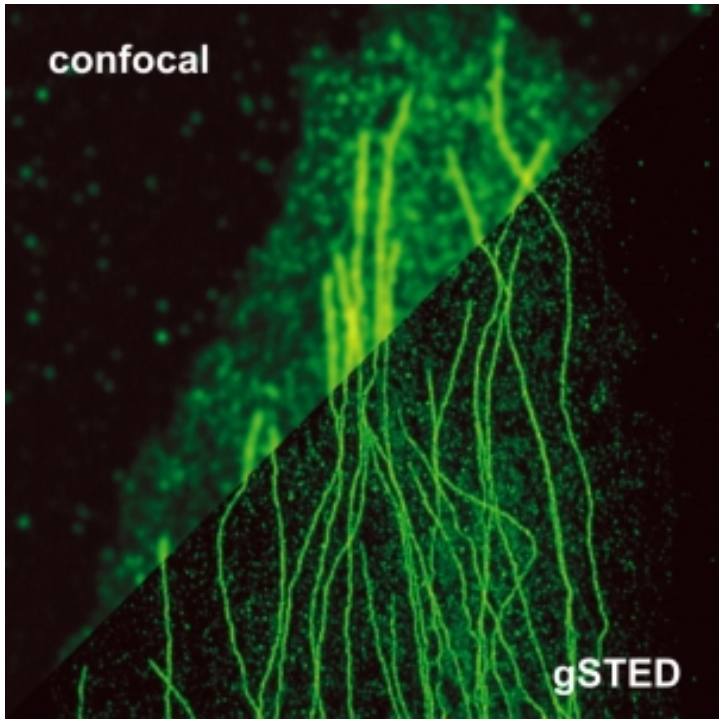


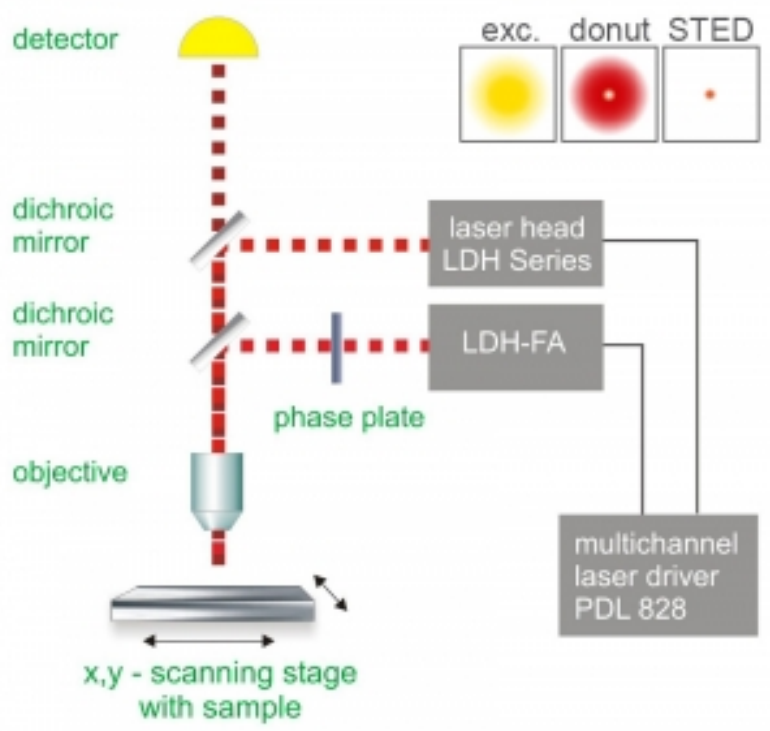
B, D, F) STORM and (A, C, E) conventional fluorescence imaging of microtubules in a mammalian cell.

Idea:
Many closely-spaced emitters will produce a broad spot and they can not be separated.
Single molecules produce diffraction-limited spots, but the center of these spots can be determined with much higher accuracy

Nature Methods **3**, 793 (2006)

STED
Stimulated Emission Depletion Microscopy





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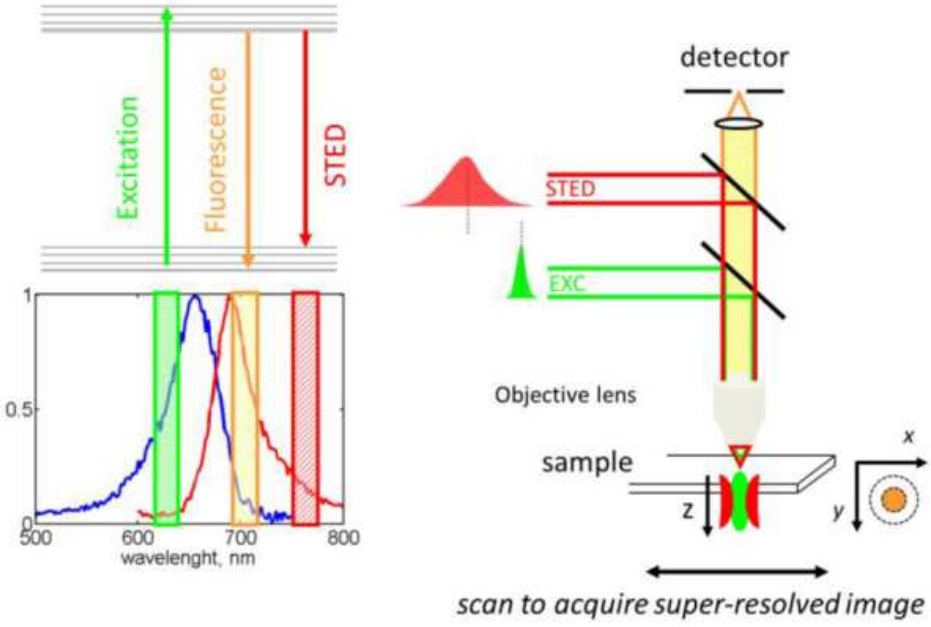
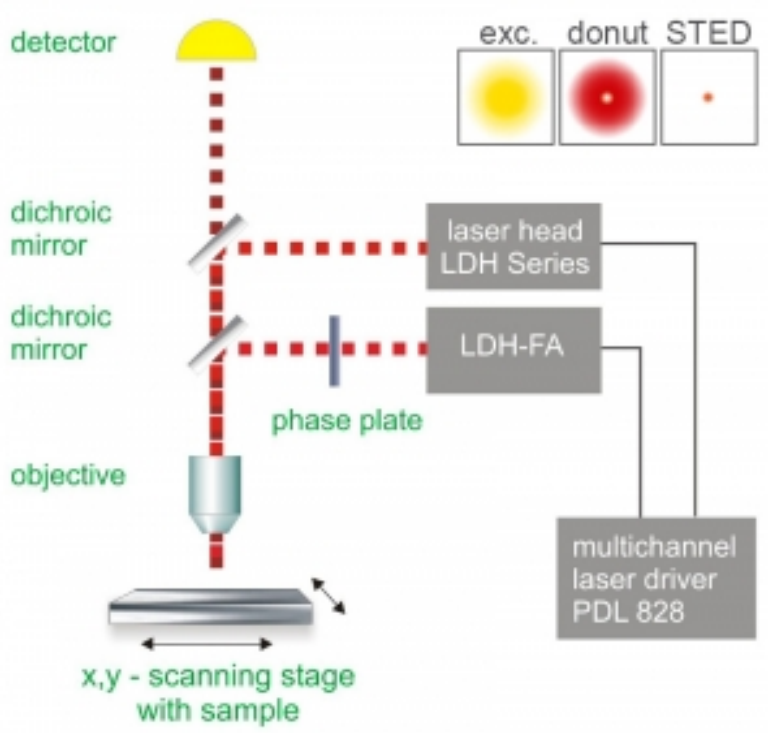


Schematic of STED microscopy



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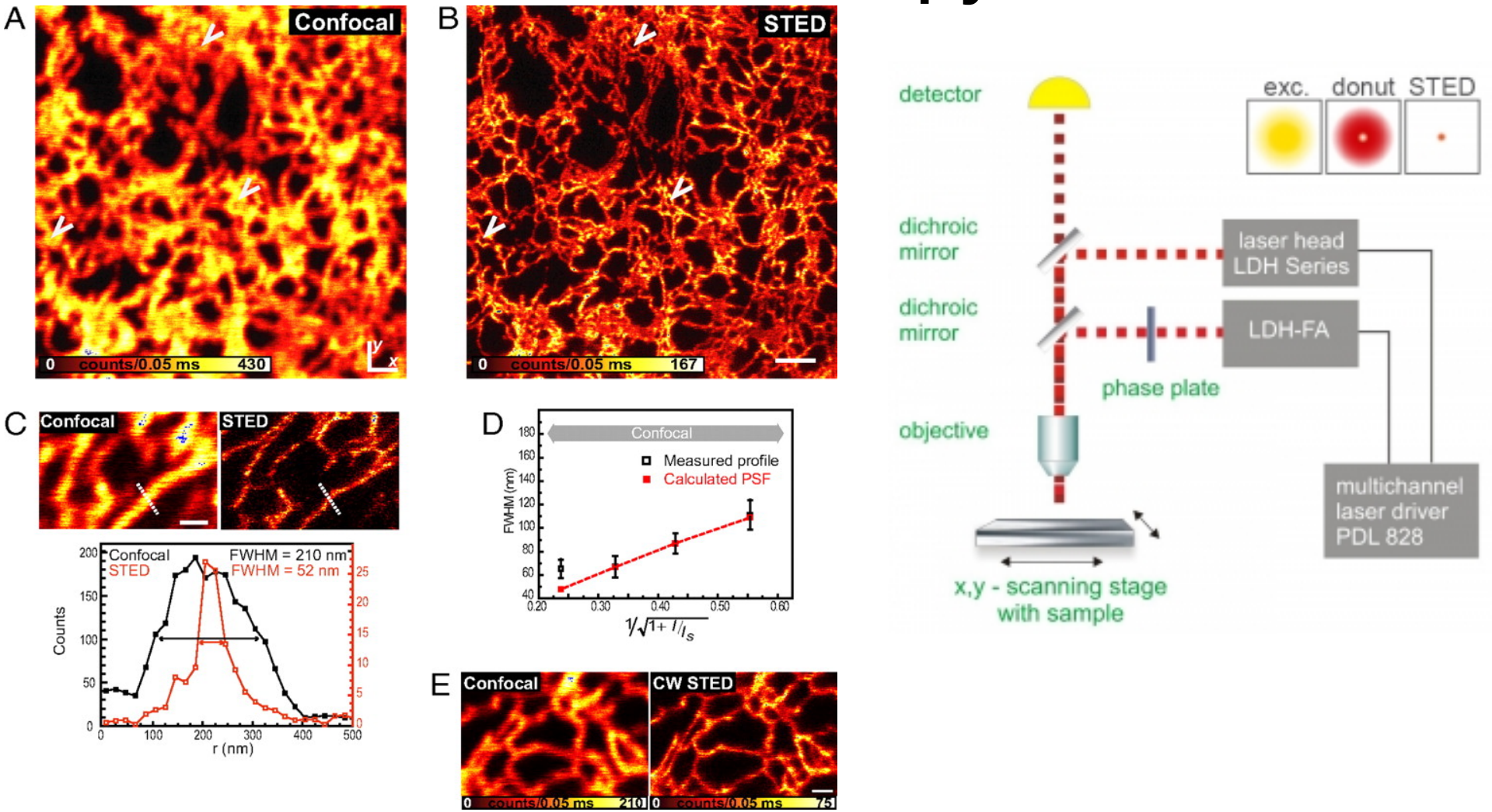

Subdiffraction-resolution imaging of the ER in a living mammalian cell

Hell et al.,PNAS **105**, 14271 (2008)