Introduction to

Optics and Spectroscopy

beyond the diffraction limit

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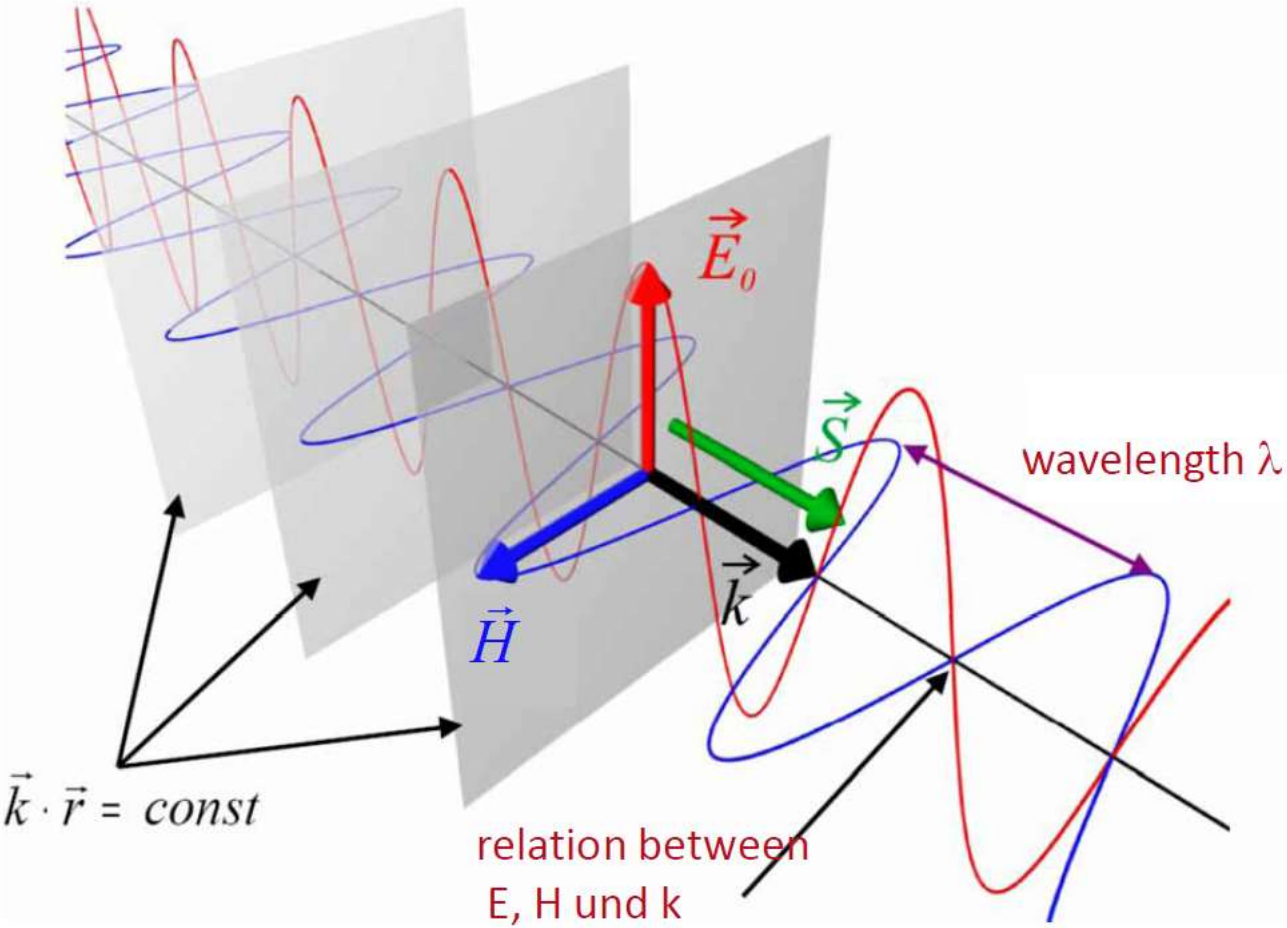
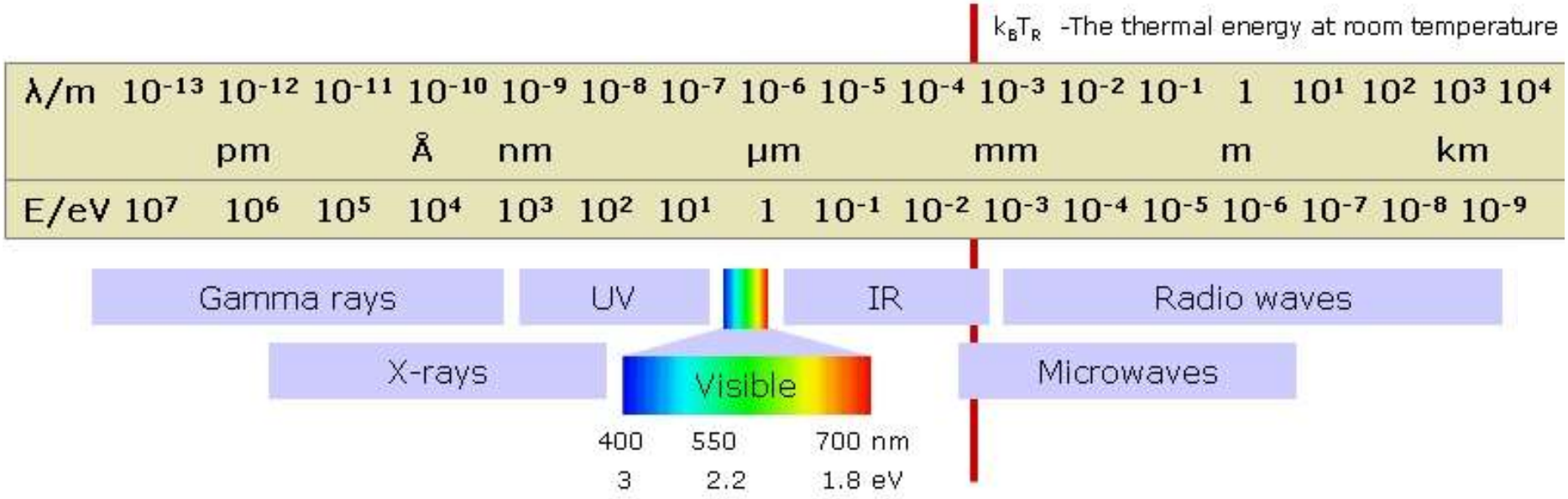
2015Apr09

Light and Optics

Light as Wave

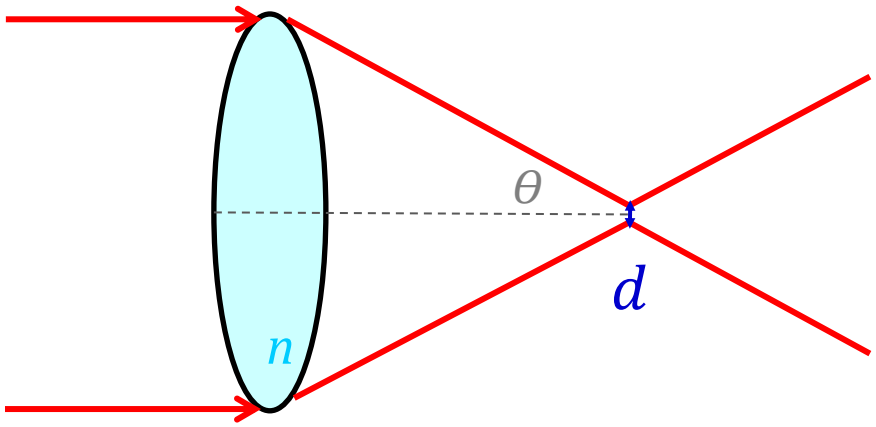


Electromagnetic Spectrum



Diffraction Limit

- **Abbe diffraction limit** --- by Ernst Abbe, 1873



$$d = \frac{\lambda}{2n \sin\theta}$$

$$= \frac{\lambda}{2N.A.}$$

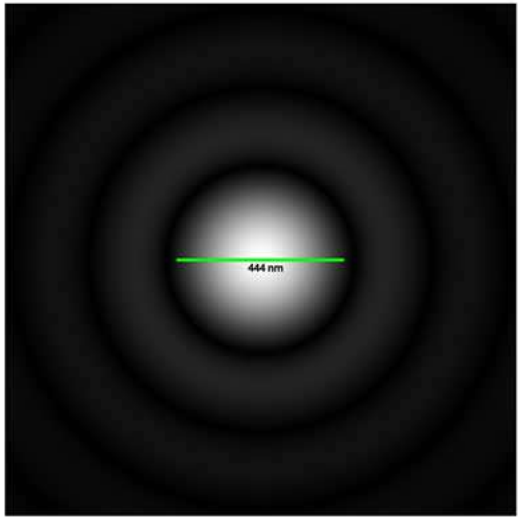
(Numerical Aperture)

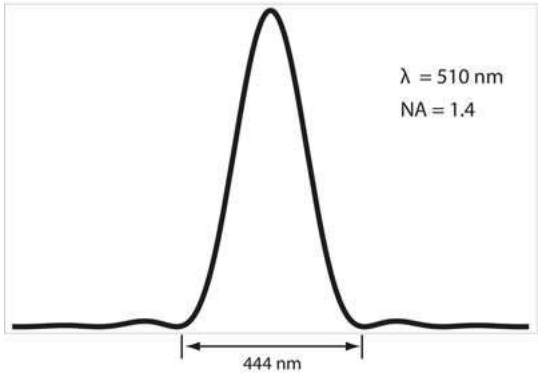
High N.A. Objectives

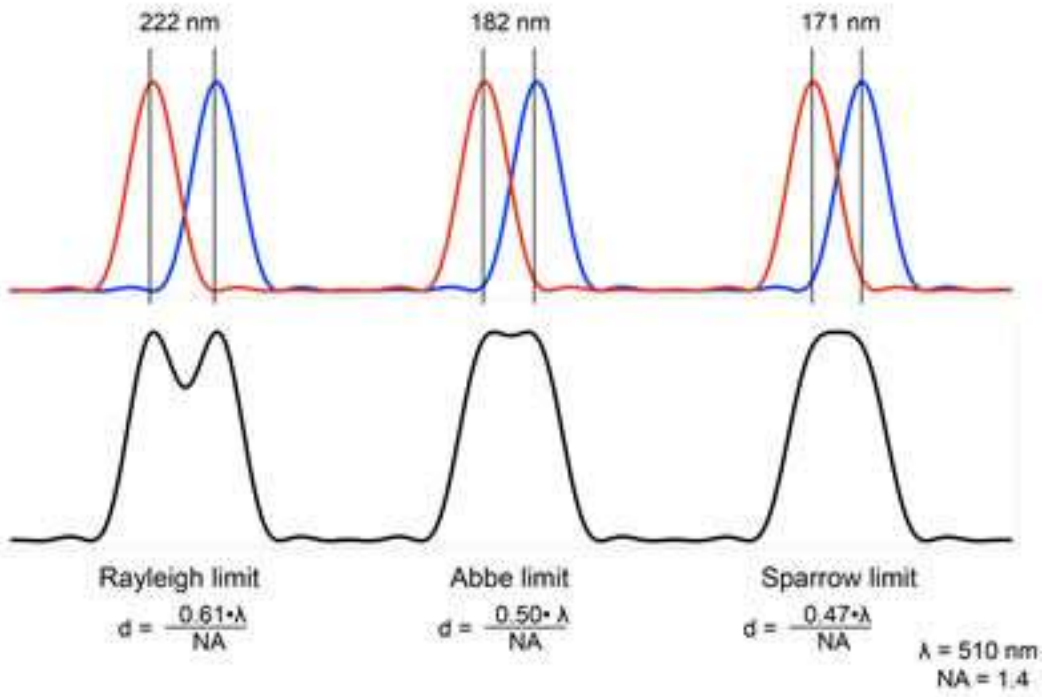
$$d = \frac{\lambda}{2N.A.} = \frac{532nm}{3} \sim 180\text{nm}$$

- n is usually around 1.5-1.52 for immersion oils, so $\theta \rightarrow 90^\circ$

Resolution Limits





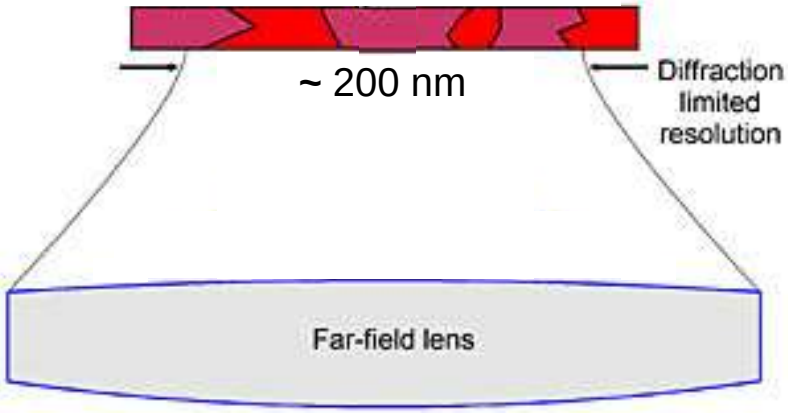


http://advanced-microscopy.utah.edu/education/super-res/

Methods to go beyond the diffraction limit - 1

Near Field Optics

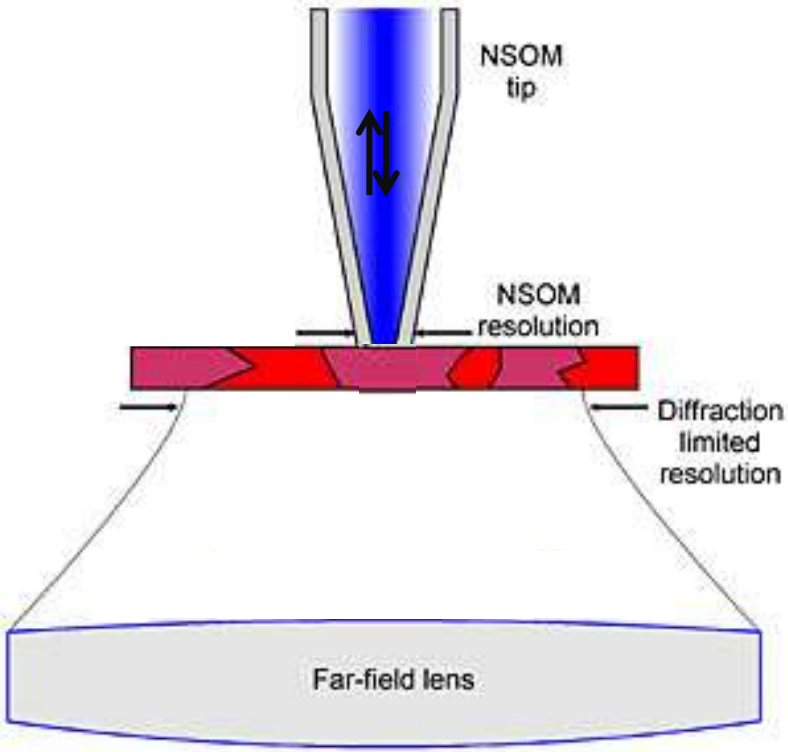
Far Field Optics



Far-Field (Diffraction Limited)

$$d = \frac{\lambda}{2N.A.}$$

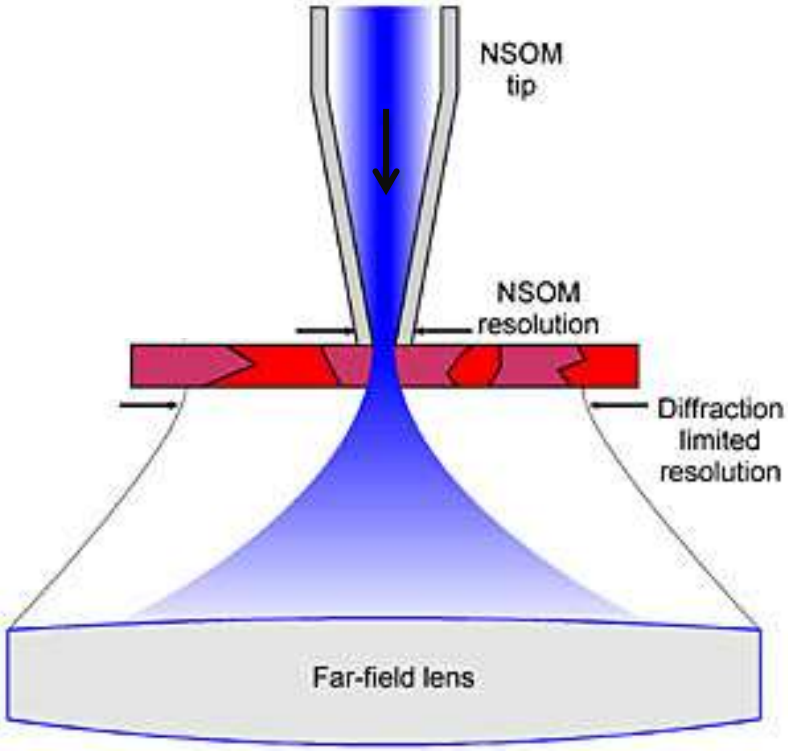
Near Field Optics

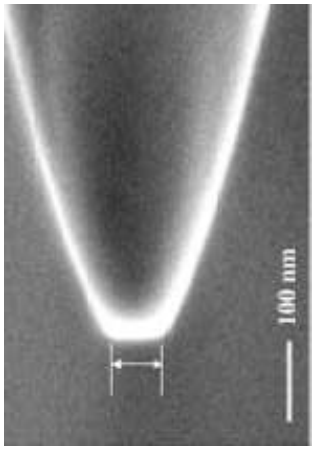


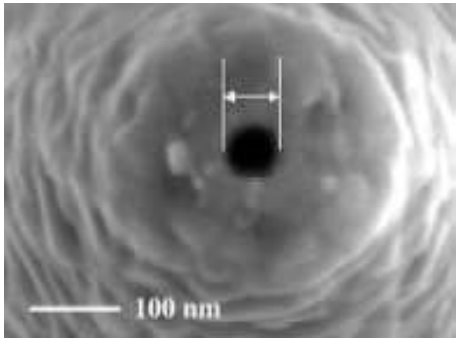
SNOM:
Scanning Nearfield Optical Microscopy.

Aperture SNOM:
Clear aperture for light transmission.

Aperture SNOM

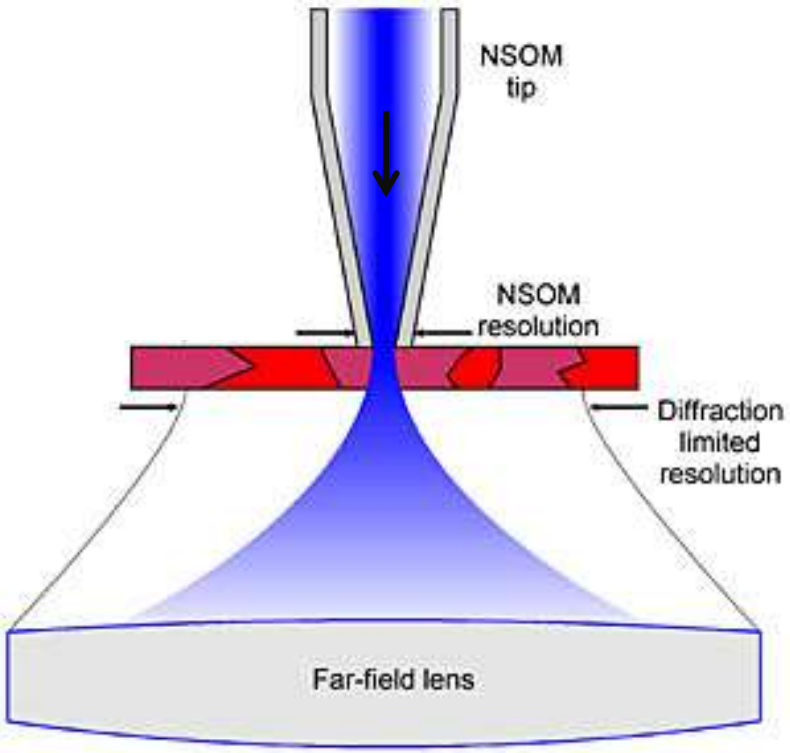


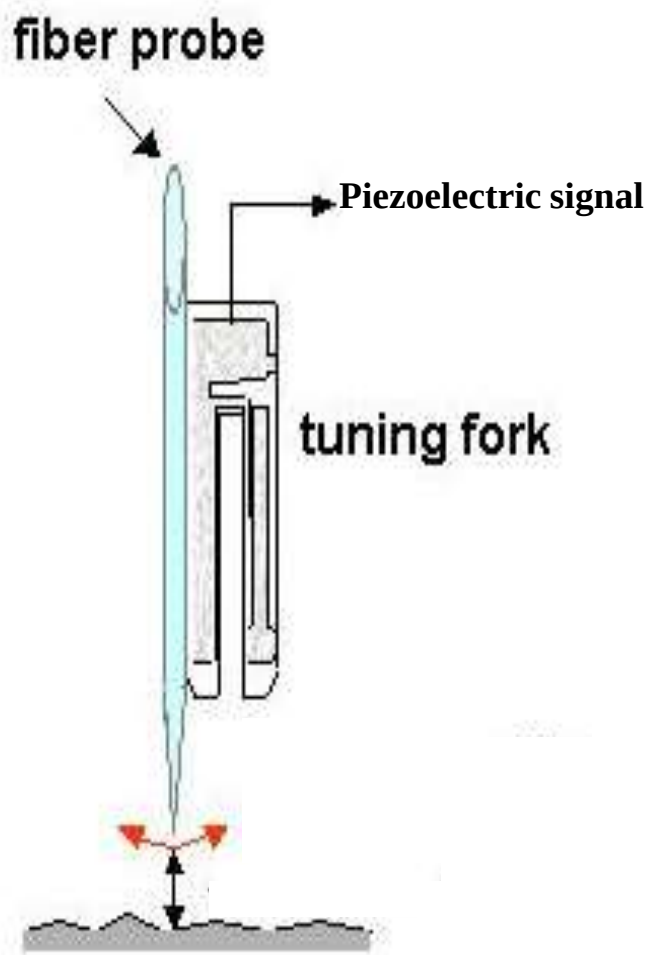




- Typical aperture size: 30-100 nm.

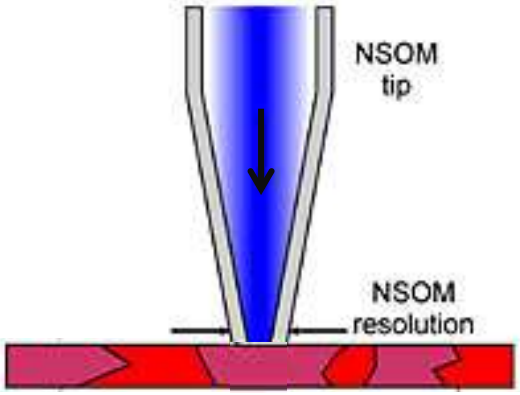
Aperture SNOM





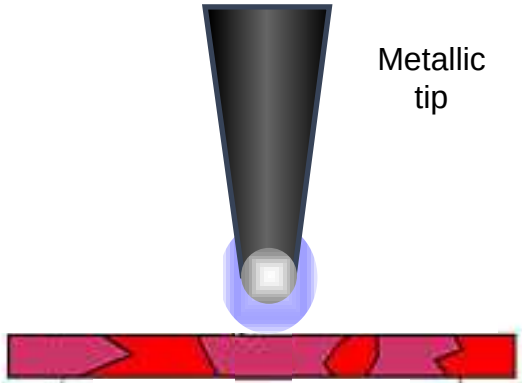
- Typical aperture size: 50-100 nm.

Apertureless SNOM



Aperture SNOM

Resolution: > 30- 50 nm

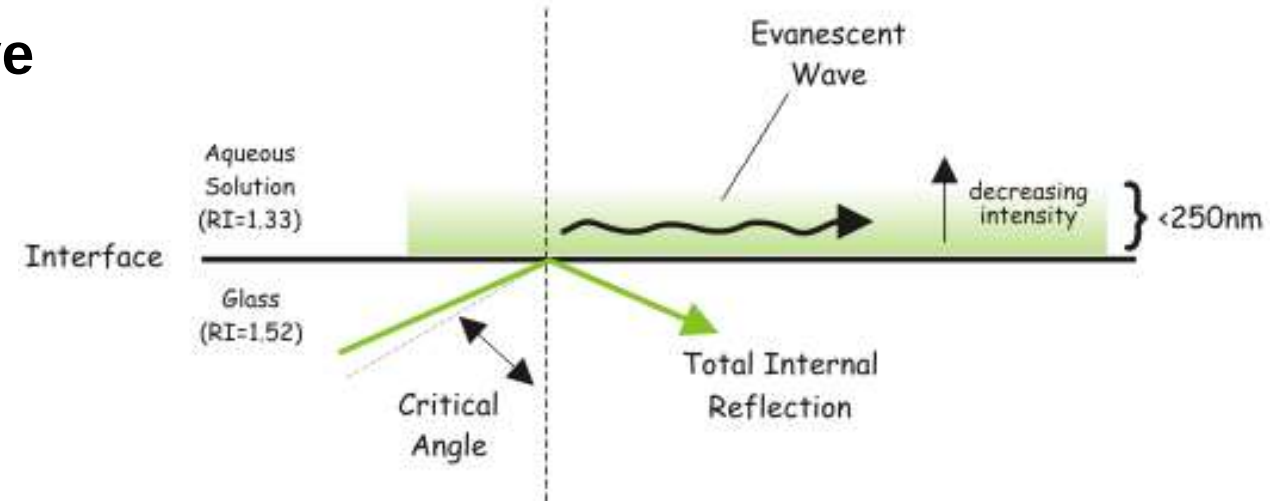


Apertureless SNOM
(Scattering SNOM)
(Tip enhanced SNOM)

Resolution: < 10- 20 nm

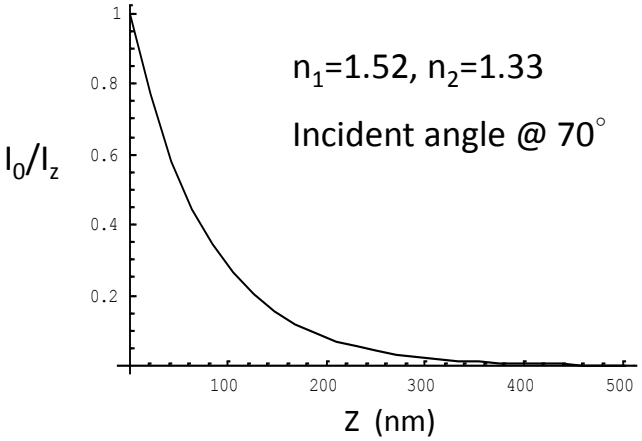
How Near is Near Field ?

- **Evanescent wave**



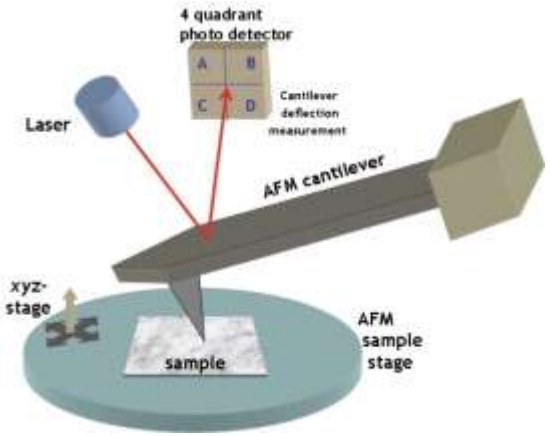
- Intensity decay $I_z = I_0 e^{-z/d}$

$$d = \lambda / 4\pi\sqrt{n_1^2 \sin^2\theta_1 - n_2^2}$$



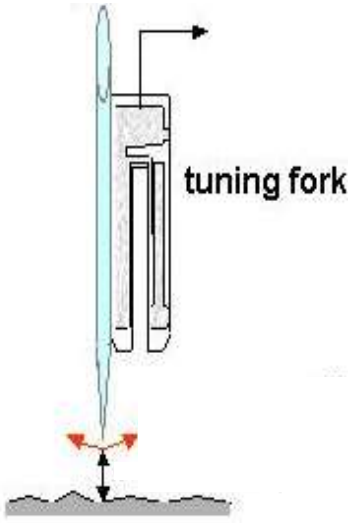
Typical SPM Working Range

Cantilever AFM



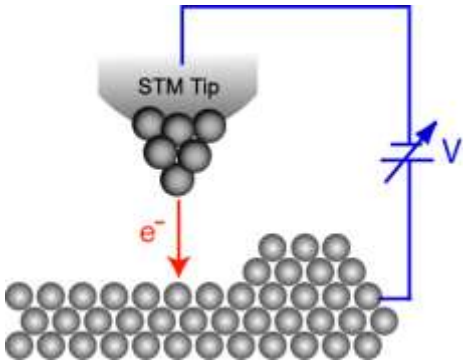
0.5-50nm

Tuning fork AFM



0.5-5 nm

STM

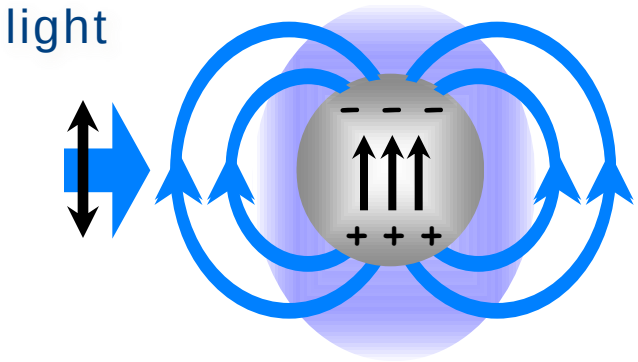


0.5-1 nm

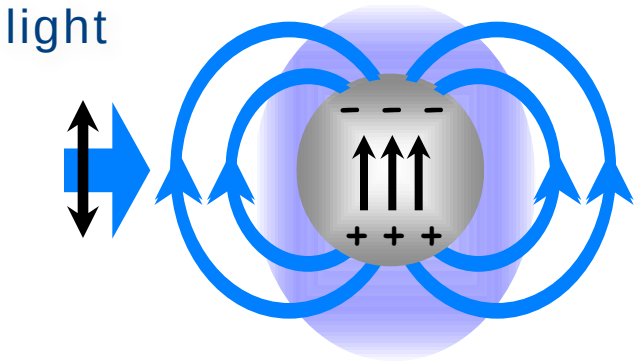
Confine Photon into Nanoscale

Localized Surface Plasmon

- Collective oscillation of free electrons on a nanoparticle.

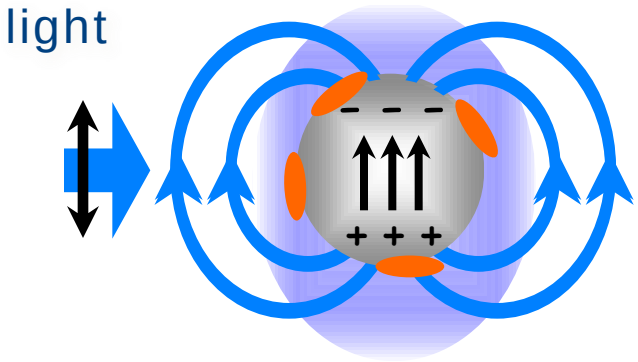


Surface Enhancement



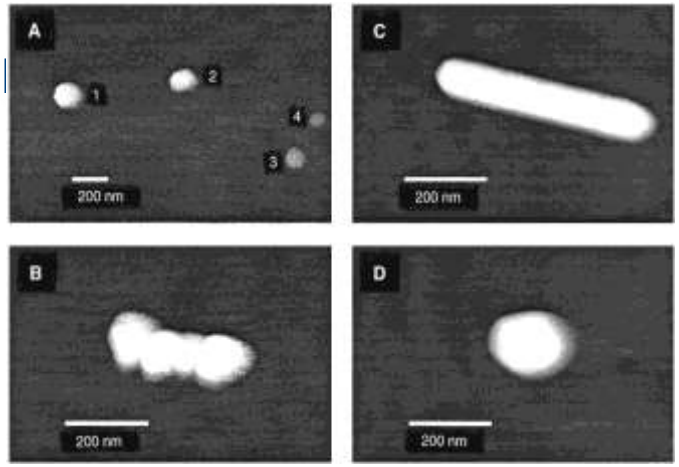
- LSP results in enhancement of EM field.

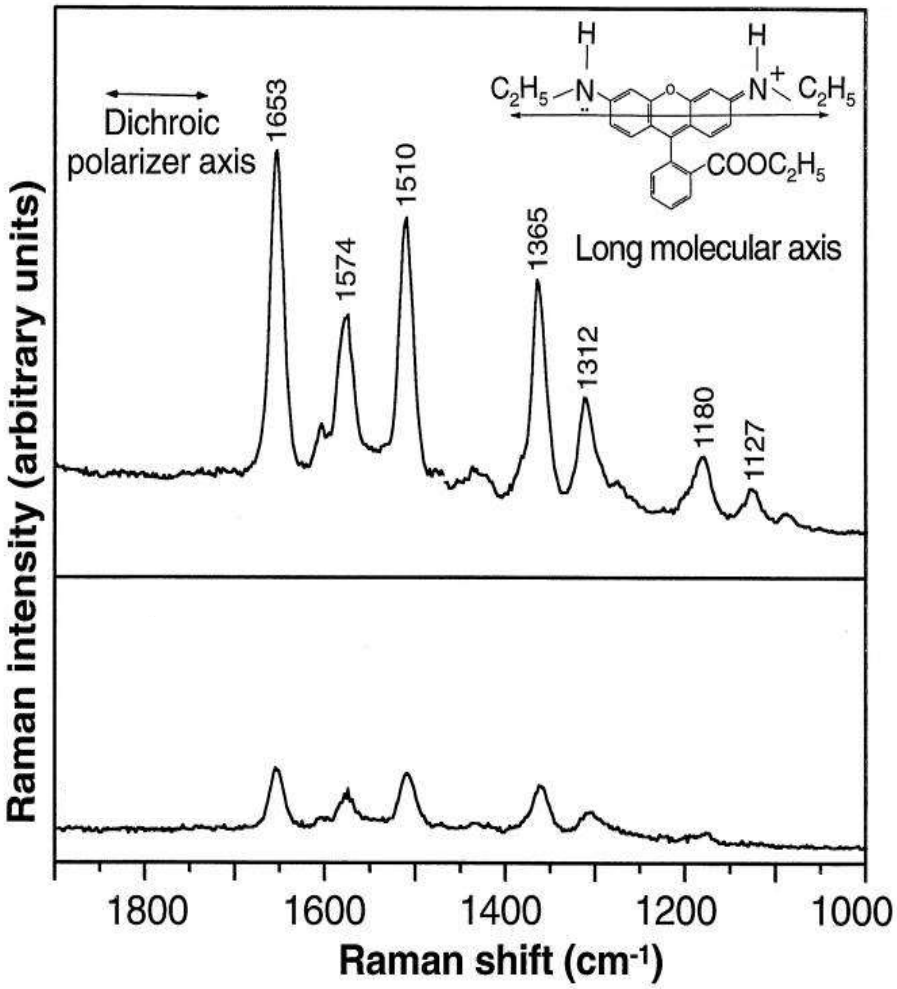
Surface Enhancement



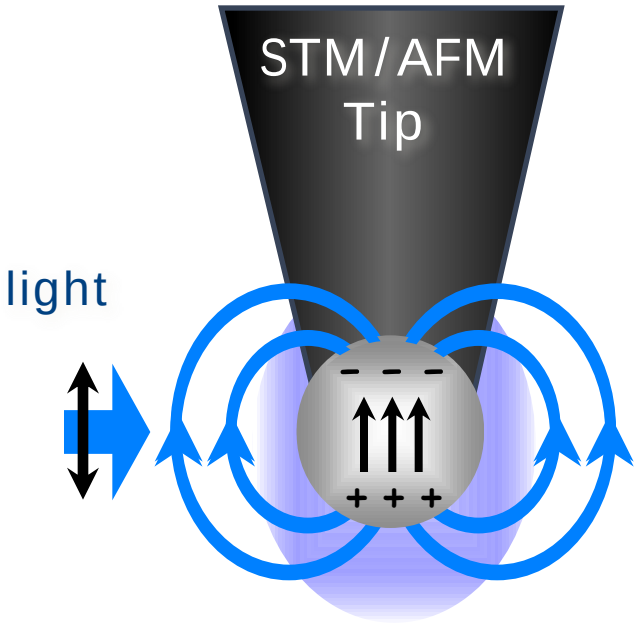
- LSP results in enhancement of EM field.

Surface Enhancement



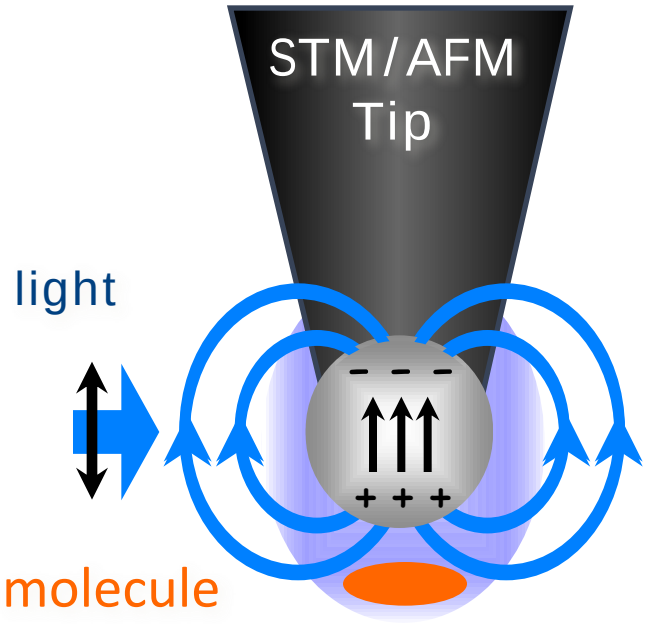


Tip Enhancement



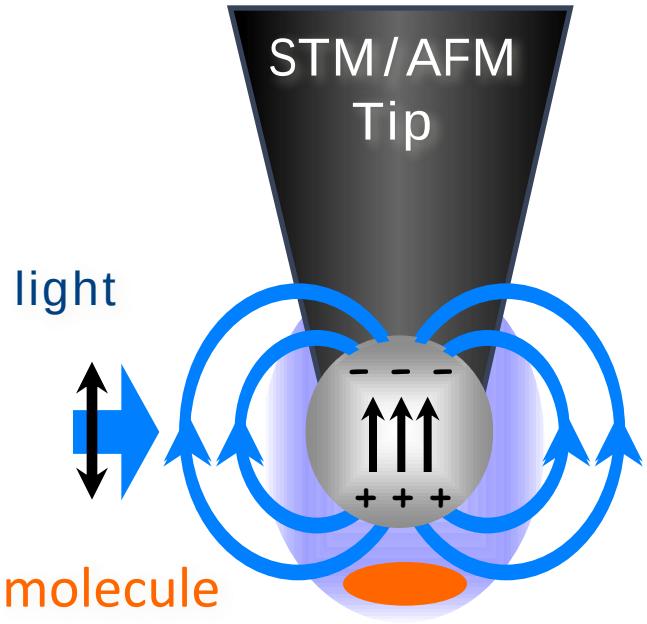
- LSP results in enhancement of EM field.

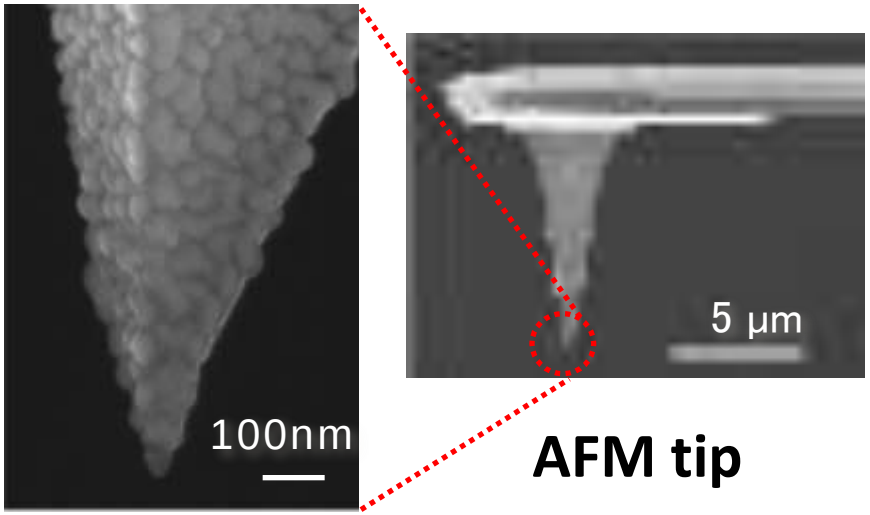
Tip Enhancement

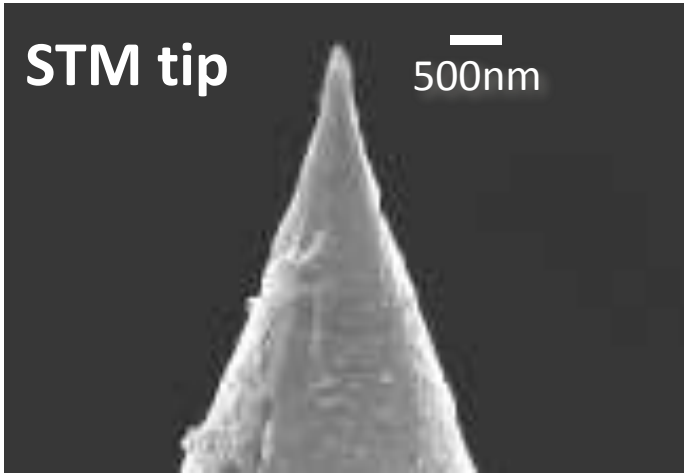


- LSP results in enhancement of EM field.
- Similar to surface enhanced Raman scattering (SERS)
- Provides location dependent tip enhanced Raman spectrum.

Tip Enhancement

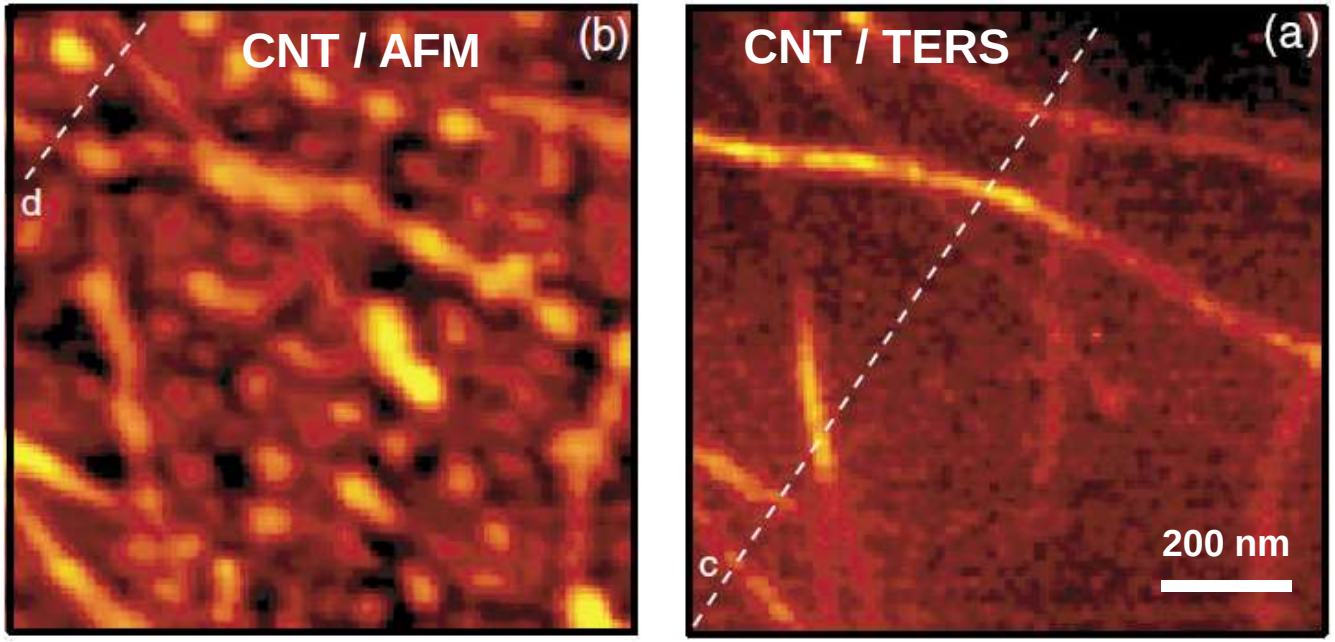






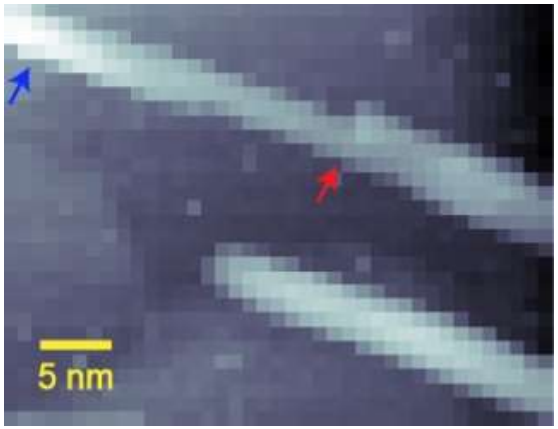
Tip Enhanced Raman Imaging

- Near field Raman (tip enhanced Raman).
- *Optical resolution < 20 nm.*

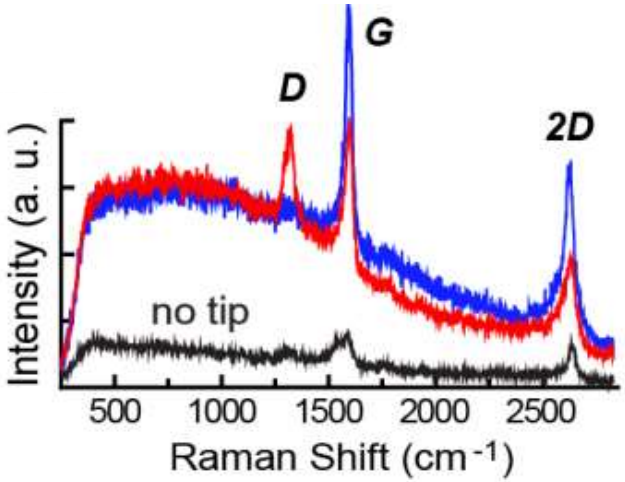


A. Hartschuh *et al.*, *Phys. Rev. Lett.* **90**, 95503 (2003)

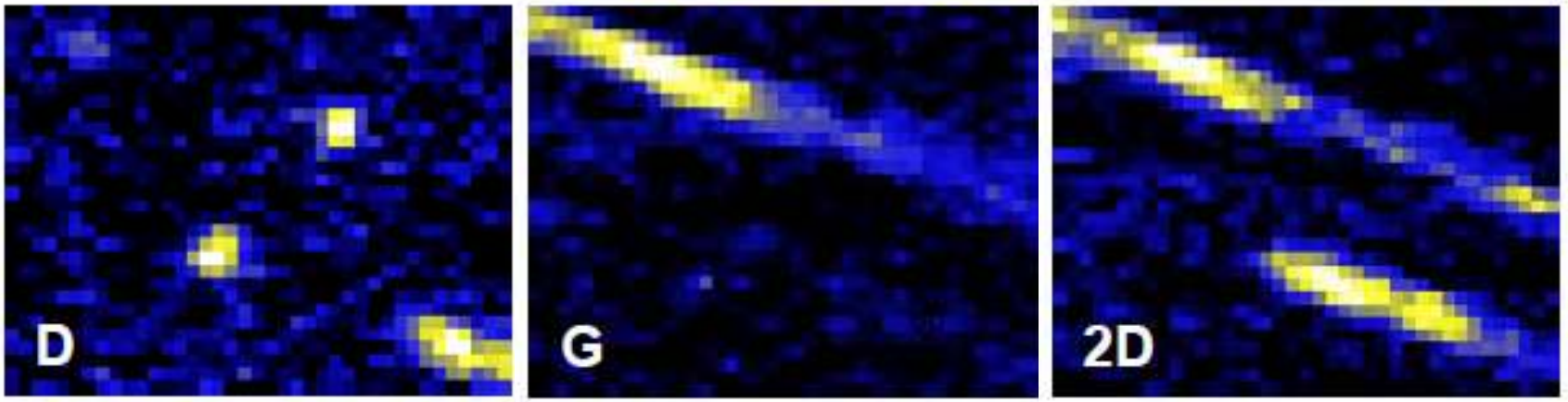
STM TERS Imaging of CNT



40 x30 px

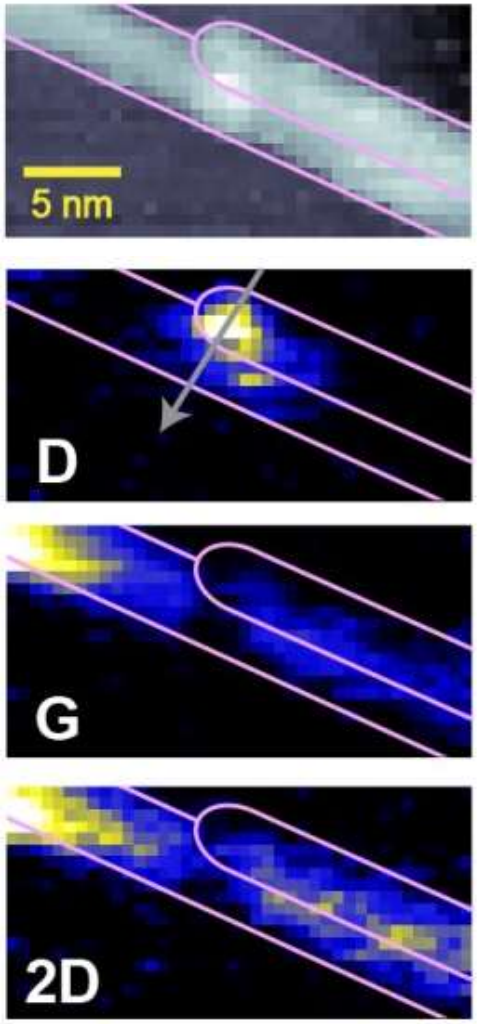


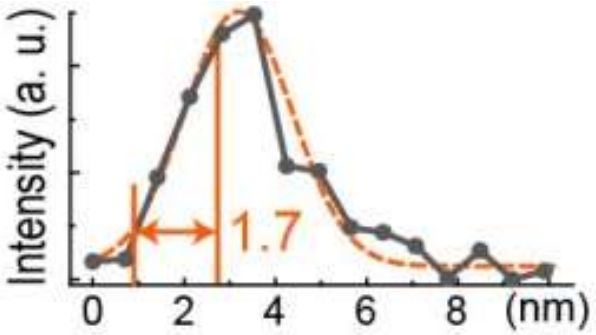
Simultaneous STM and Raman imaging.



TERS Imaging @ 1nm/pixel

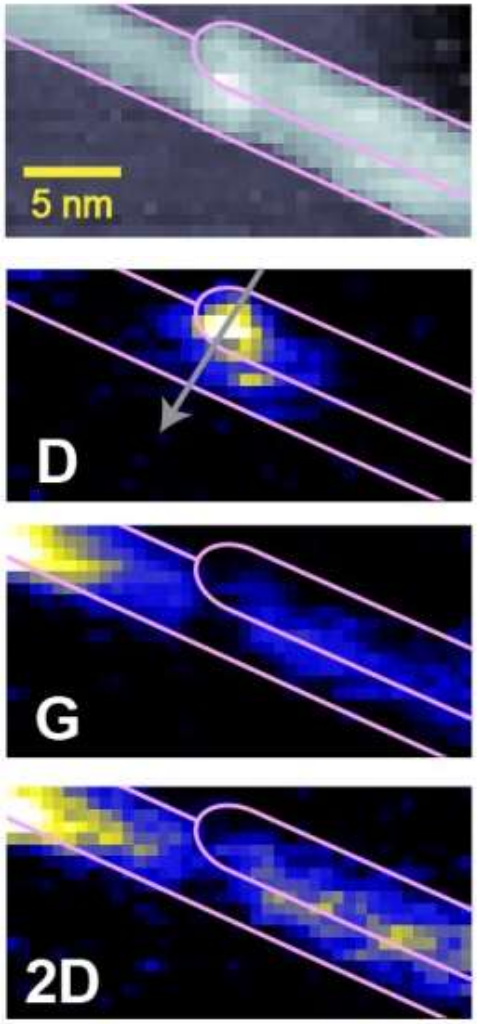
1.7 nm Optical Resolution

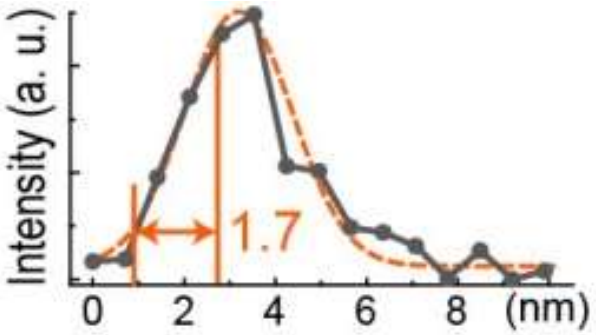




C. Chen, N. Hayazawa, and S. Kawata, *Nature Comm*. **5**, 3312 (2014)

1.7 nm Optical Resolution



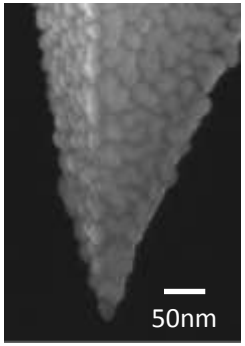


World best spatial resolution in optics, spectroscopy, and chemical analysis in the ambient condition.

C. Chen, N. Hayazawa, and S. Kawata, *Nature Comm*. **5**, 3312 (2014)

Near Field Method

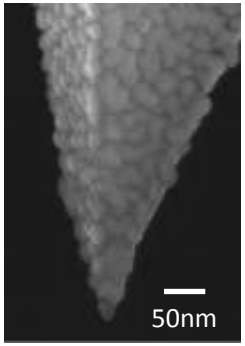
- Scanning Probe Microscope
- Sharp tips or probes



- Ultrahigh spatial resolution ~ 1nm.
- Various spectroscopic information.
- 2D mapping for samples on surface.
- Not yet successful in liquid.

Near Field Method

- Scanning Probe Microscope
- Sharp tips or probes



Far Field Method

- Inverted Microscope
- High speed imaging CCD

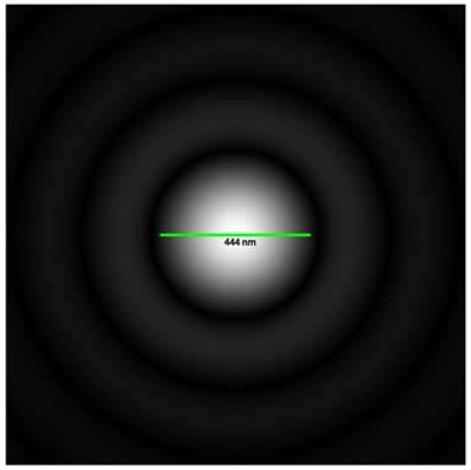
- Ultrahigh spatial resolution ~ 2nm.
- Various spectroscopic information.
- 2D mapping for samples on surface.
- Not yet successful in liquid.

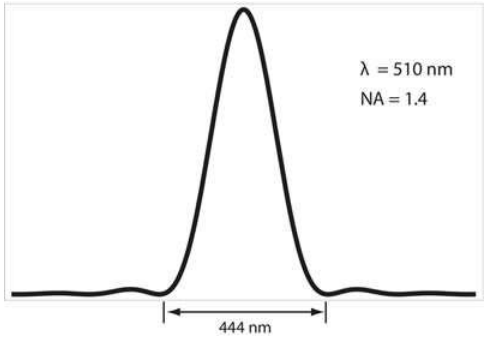
Methods to go beyond the diffraction limit - 2

Fear Field Optics

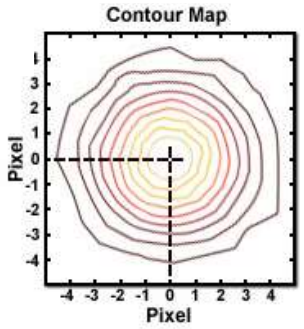
Far Field Super Resolution

- Airy disk (point spread function)





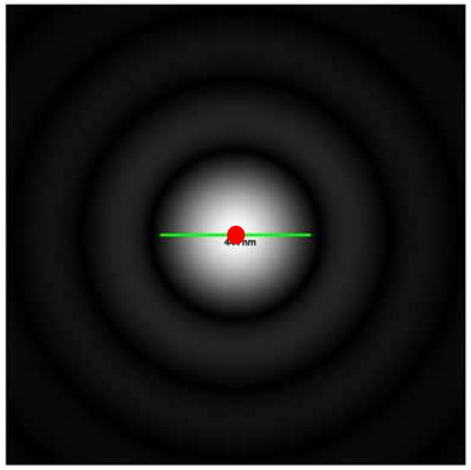
- From a single emitter. (Fluorescence protein or dye)
- Localize the center of PSF.

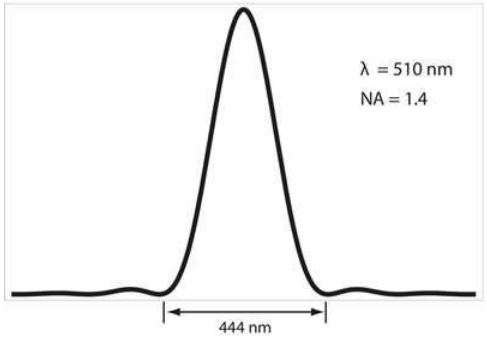


http://advanced-microscopy.utah.edu/education/super-res/

Far Field Super Resolution

- Airy disk (point spread function)

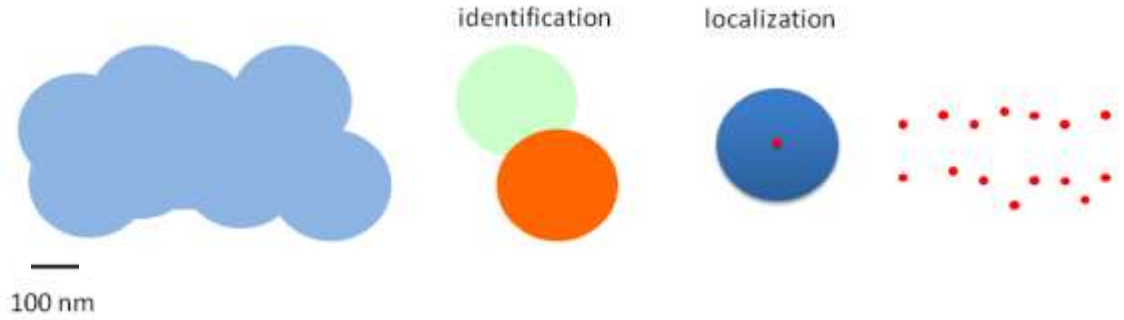


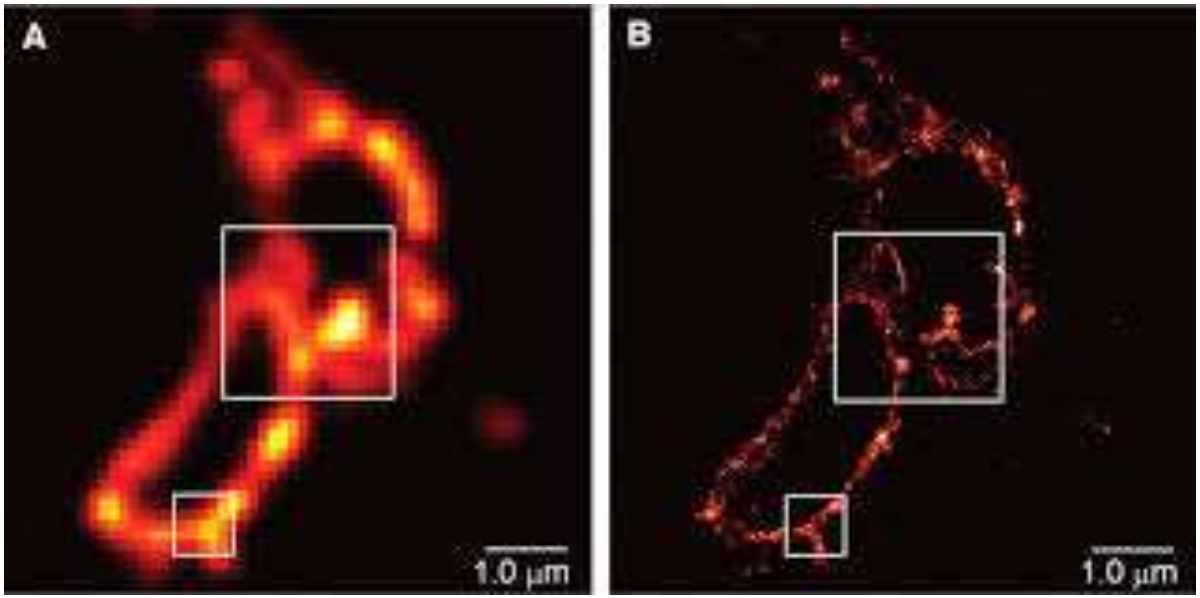


- From a single emitter. (Fluorescence protein or dye)
- Localize the center of PSF.
- Resolution > 20nm due to molecular diffusion and motions

http://advanced-microscopy.utah.edu/education/super-res/

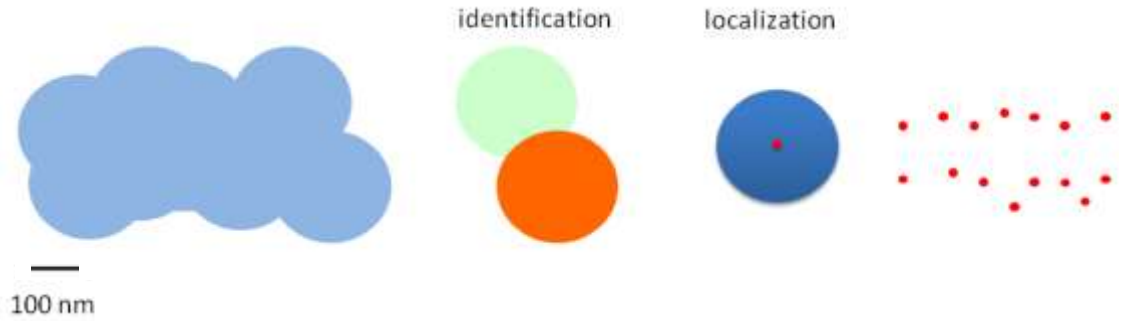
Localization - PALM

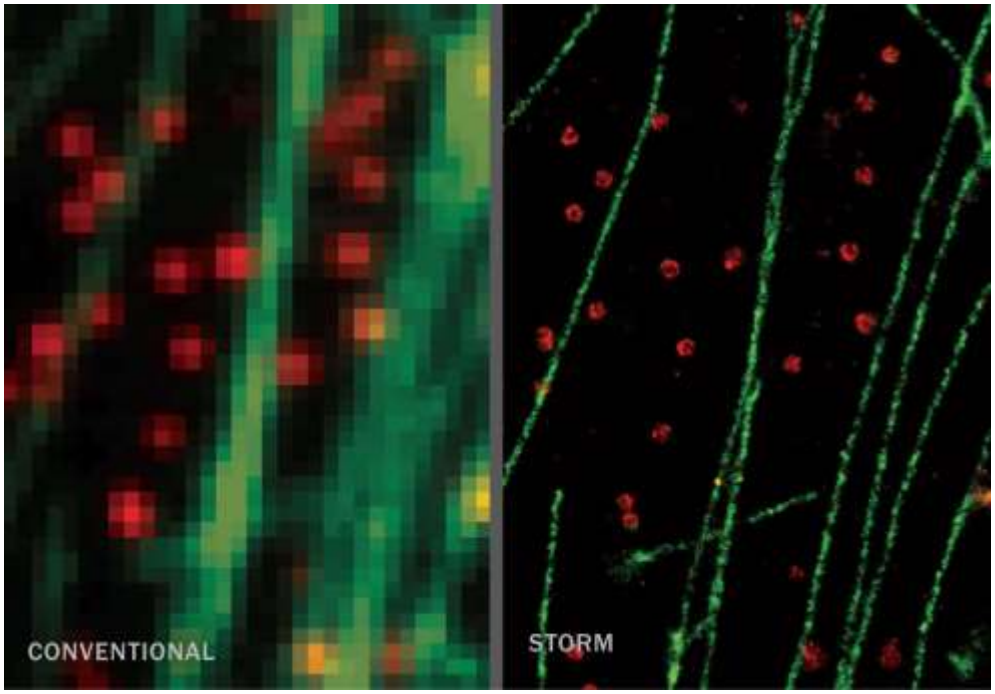




E. Betzig et al., 313, 1642 (2006).

Localization - STORM

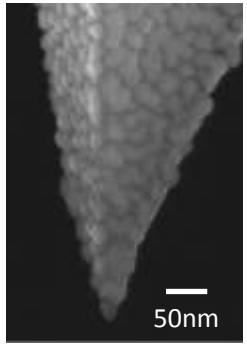




M. Bates, B. Huang, G. T. Dempsey, X. Zhuang, Science 317, 1749-1753 (2007)

Near Field Method

- Scanning Probe Microscope
- Sharp tips or probes



- Ultrahigh spatial resolution ~ 1nm.
- Various spectroscopic information.
- 2D mapping for samples on surface.
- Not yet successful in liquid.

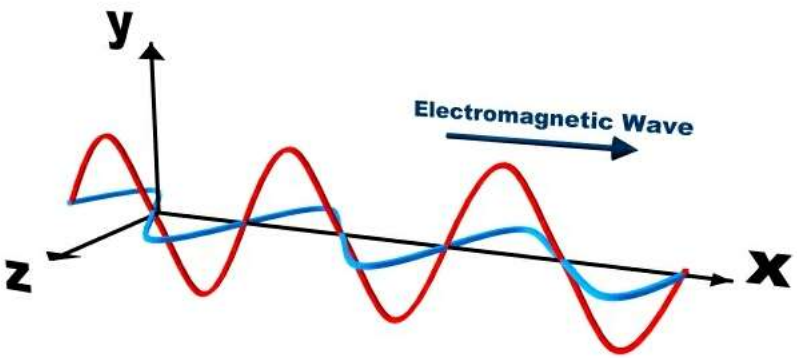
Far Field Method

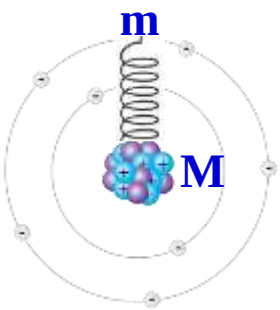
- Inverted Microscope
- High speed imaging CCD

- Good for biological samples.
- Pseudo 3D imaging.
- Resolution > 20 nm.
- Need huge amount of computation.
- Fluorescence label needed.

Absorption and Emission

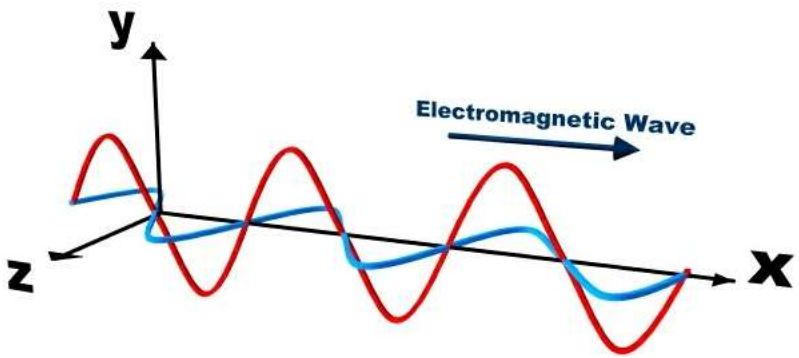
Interaction of Electron and E Field

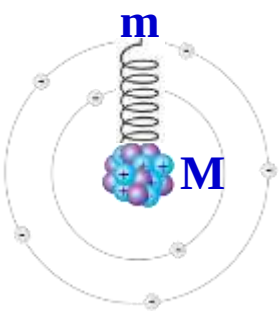


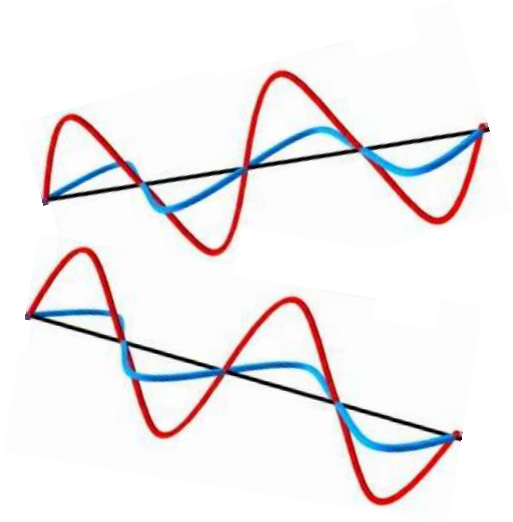


- Electrons response to the E field.
- Electrons ⇨ Polarizability ⇨ Susceptibility ⇨ Dielectric function ⇨ Refraction index.
- Resonance will result in absorption and dispersion

Re-emission and Resonance

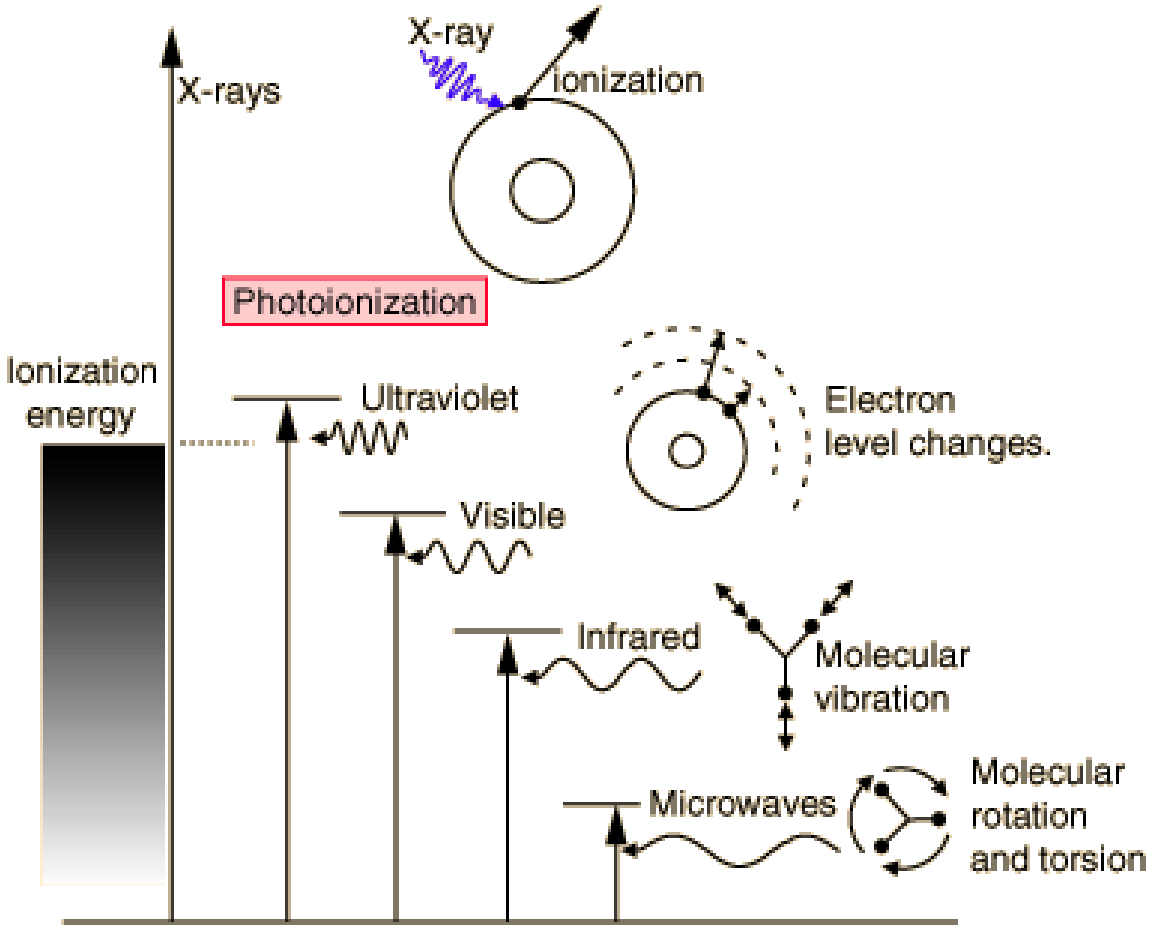




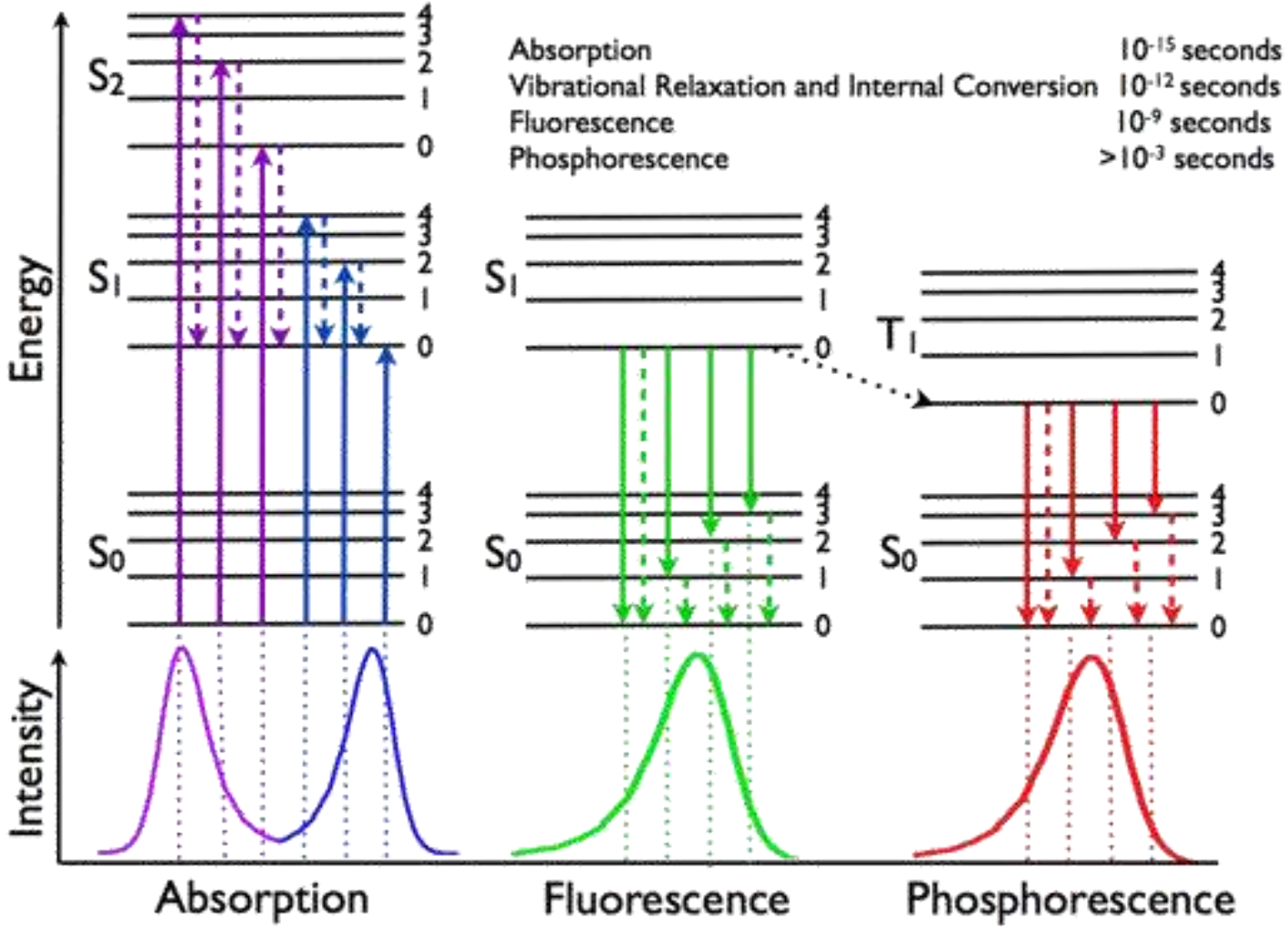


- Nonresonance $\xrightarrow{1\text{ -}10\text{ fs}}$ re-emission **(scattering)**
- Resonance : absorption $\xrightarrow[0.1\text{ -}100\text{ ns}]{}$ re-emission **(photoluminescence)**

Resonance

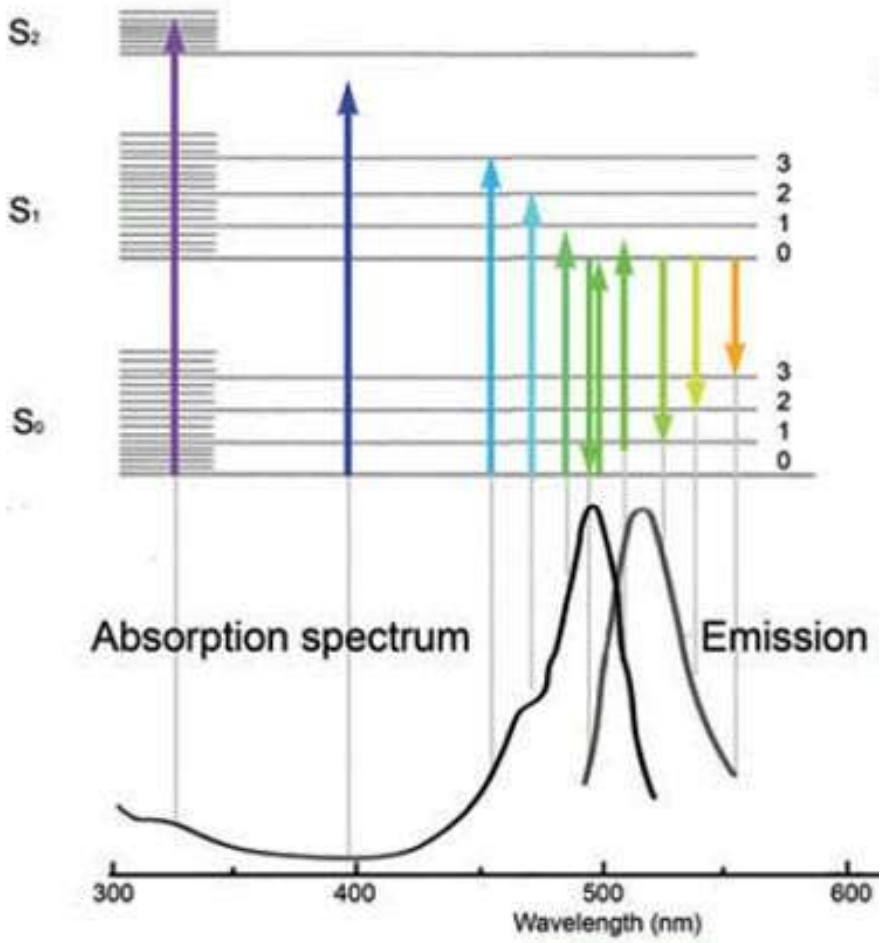

X-rays
X-ray
Ionization
Photoionization
Ionization energy
Ultraviolet
Electron level changes.
Visible
Infrared
Molecular vibration
Microwaves
Molecular rotation and torsion

Energy Diagram



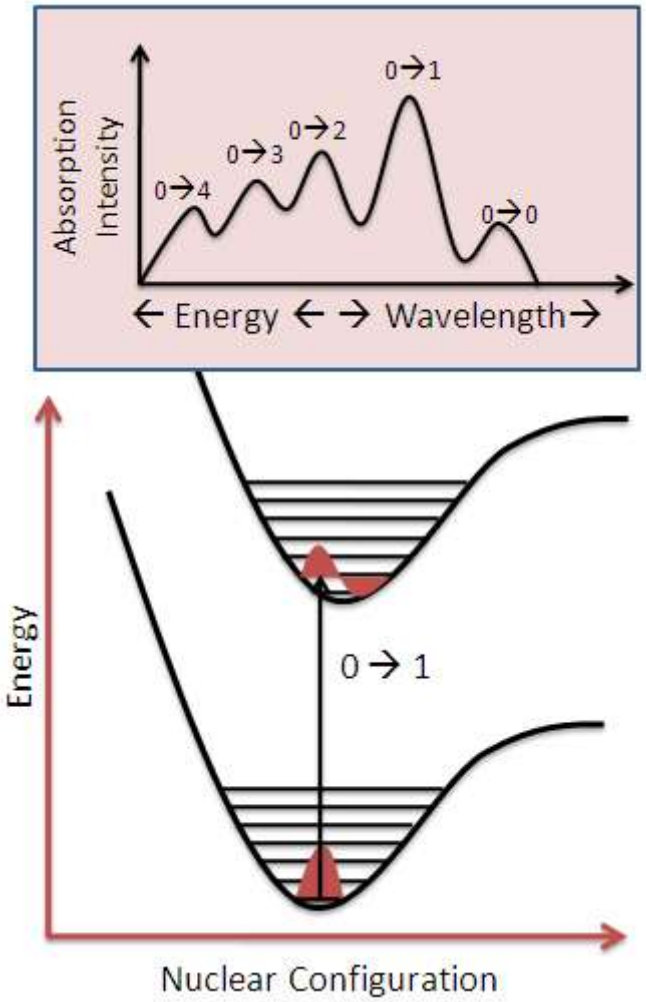
Fluorescence

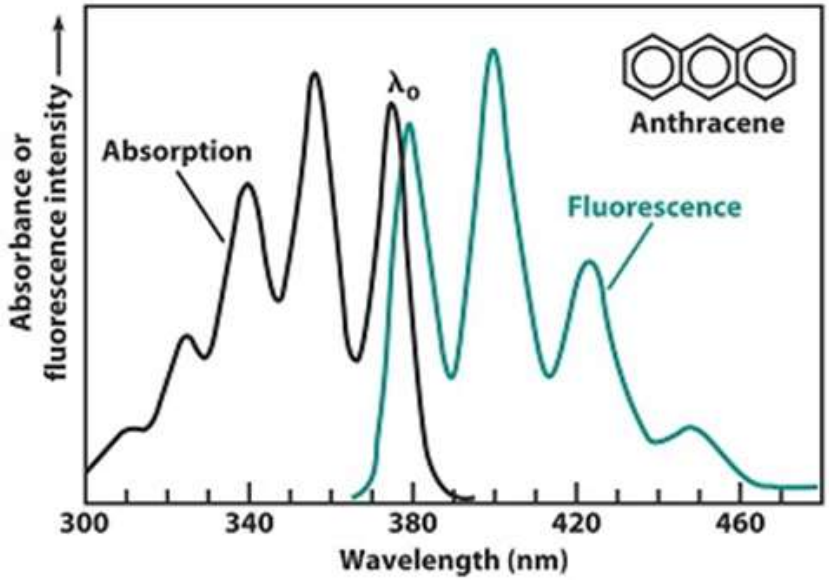
Stokes Shift



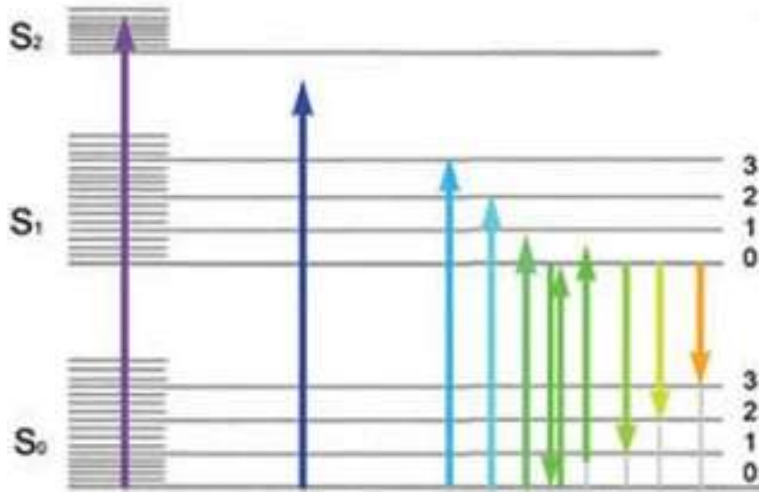
- The ***Stokes shift*** is the gap between the maximum of the absorption band and the maximum of the spectrum.
- Loss of vibrational energy (relaxation) in the excited state is dissipated as heat by collision with solvent.

Vibronic Transition

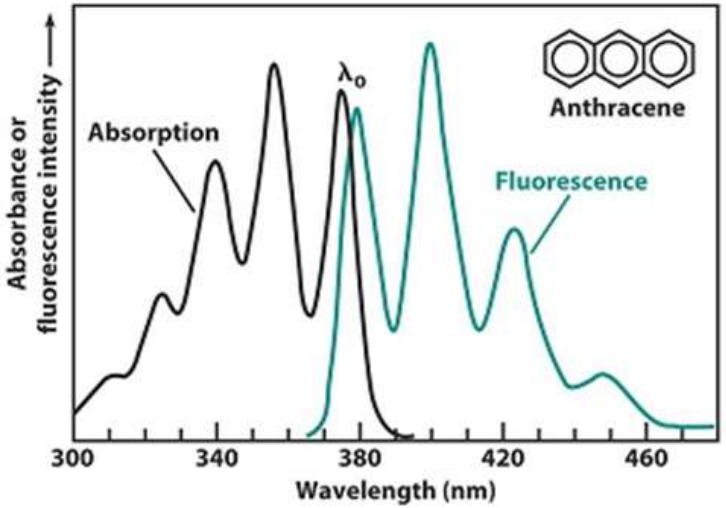




Mirror Image of Abs and Fl

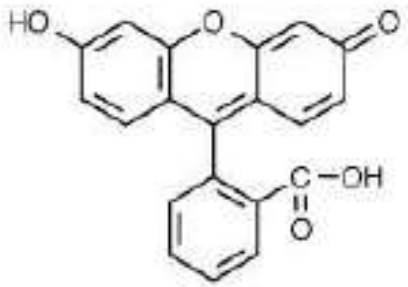


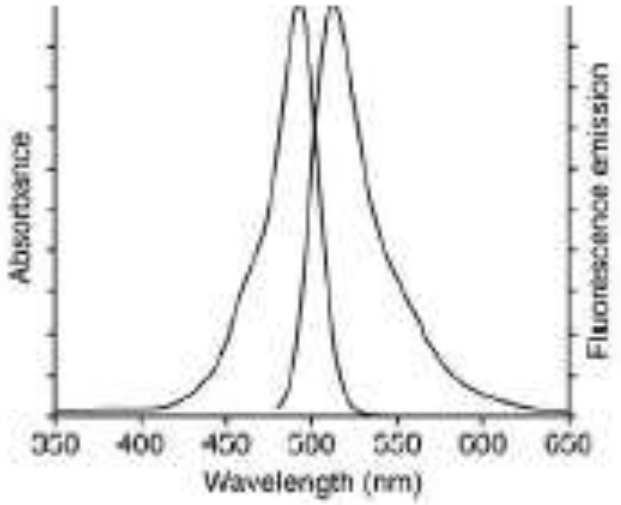
- Absorption spectrum ⇨ vibrational levels of the electronically excited state
- Emission spectrum ⇨ vibrational levels of the electronic ground state
- Fluorescence spectrum is mirror image of absorption spectrum



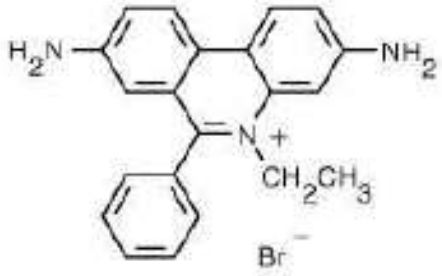
Example of Fluorescent Dyes

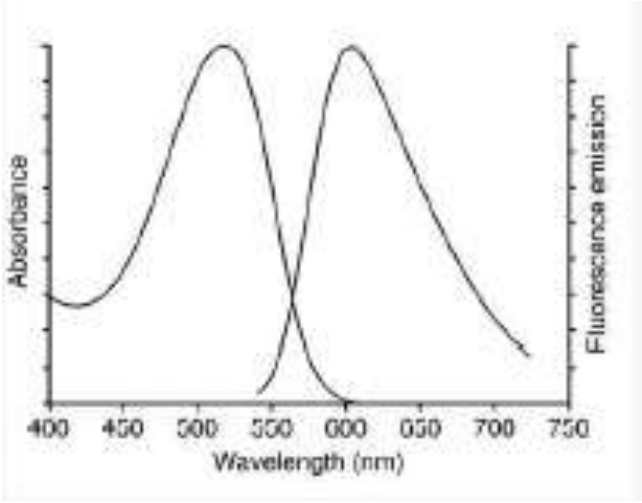
fluorescein





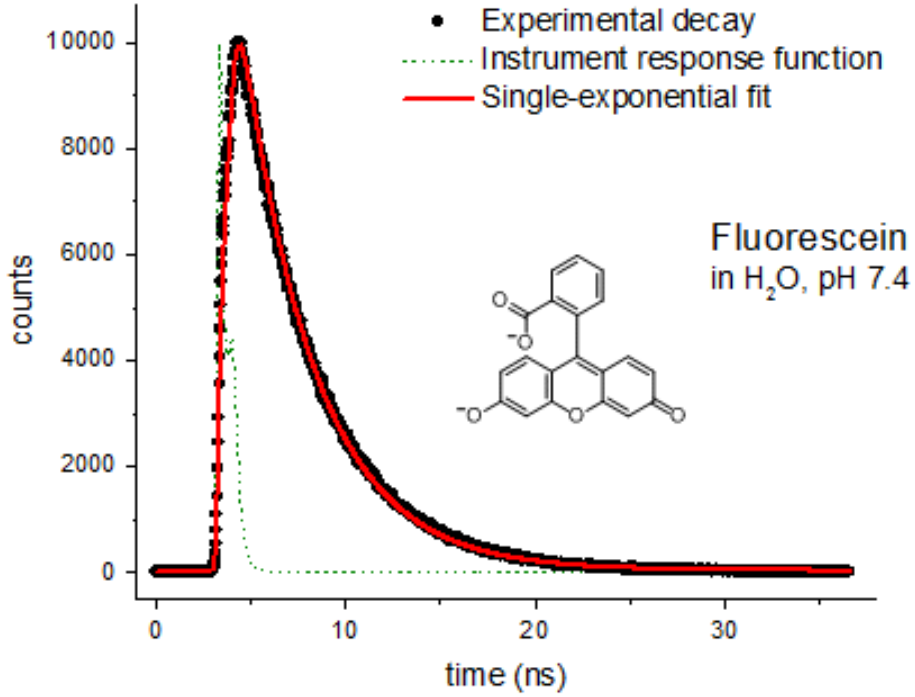
ethidium bromide bound to DNA.





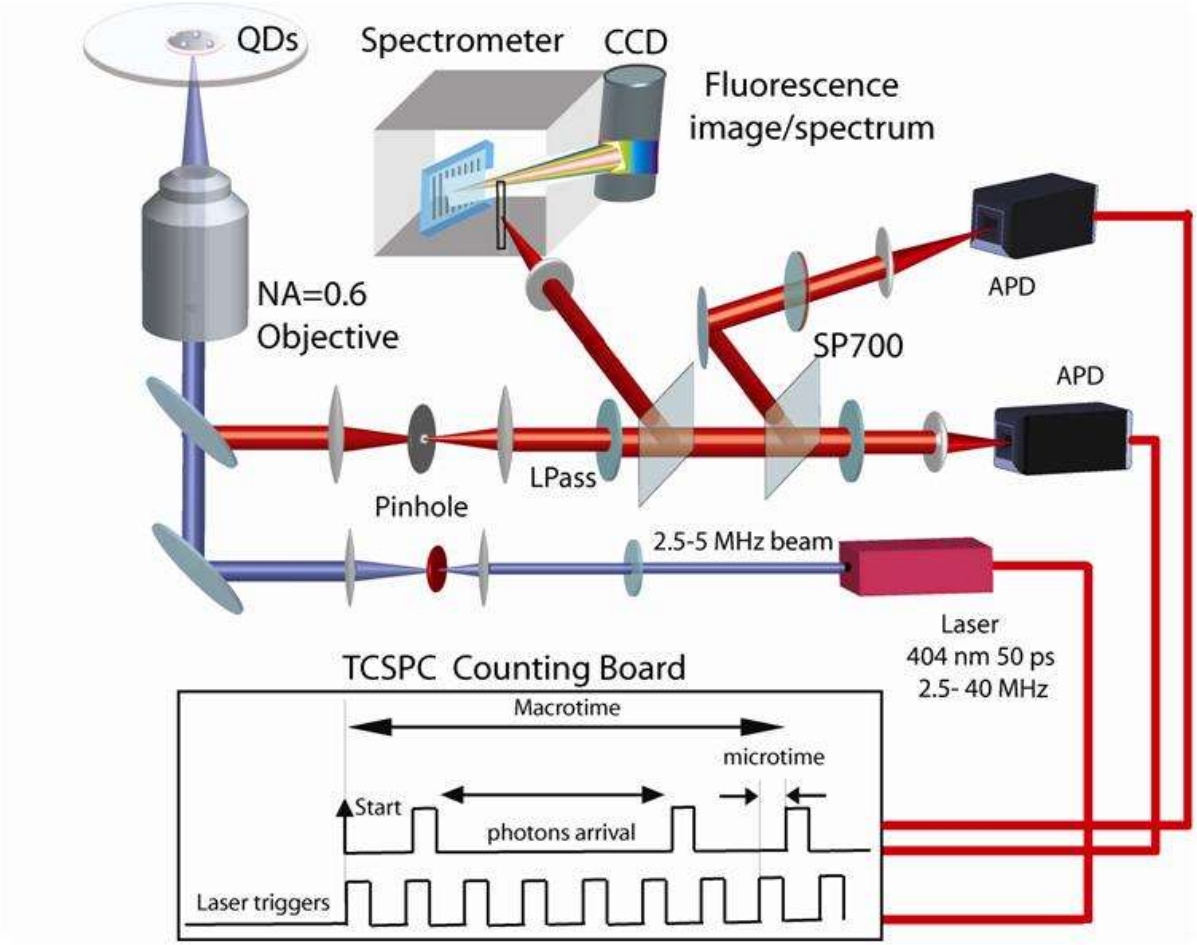
Fluorescence Lifetime

- The lifetime (τ) of the lowest excited singlet state.
- The average time the molecule spends in the excited state prior to return to the ground state.
- Generally, fluorescence lifetimes are around 10 nsec.



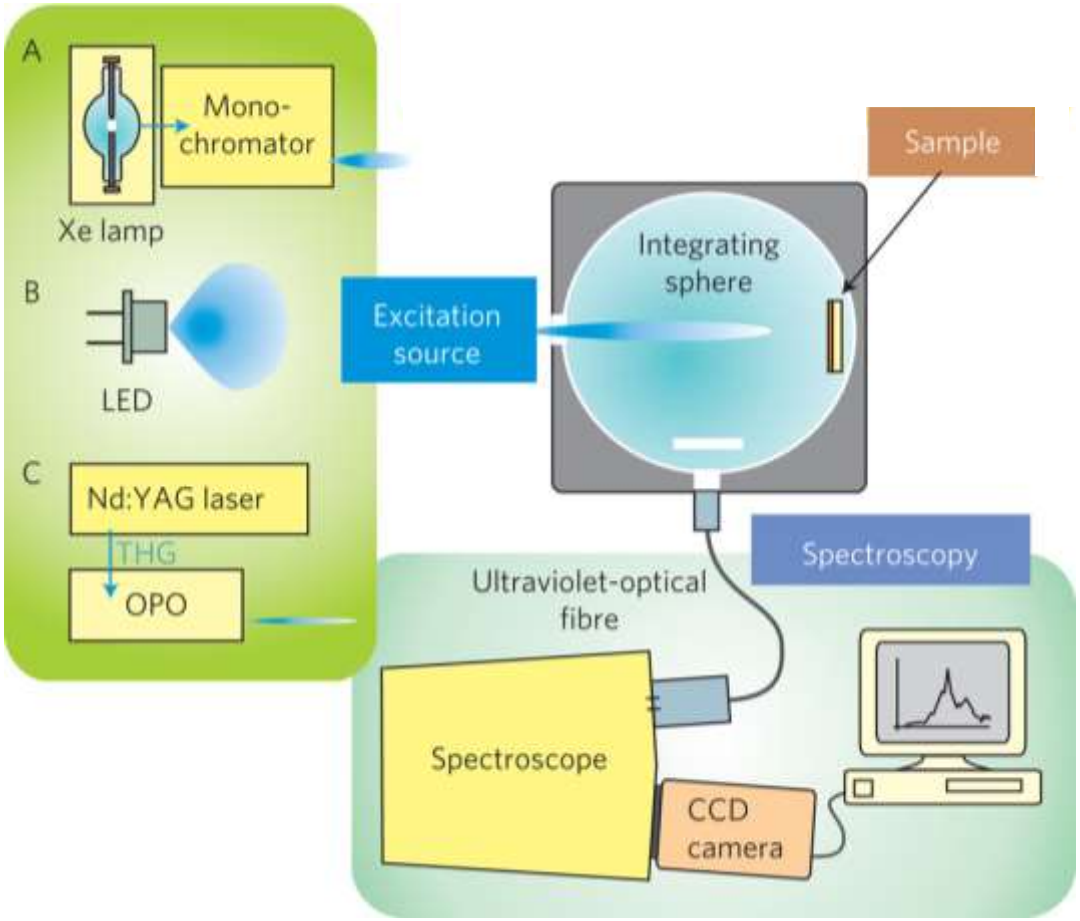
Fluorescence Lifetime

- Time correlated single photon counting.

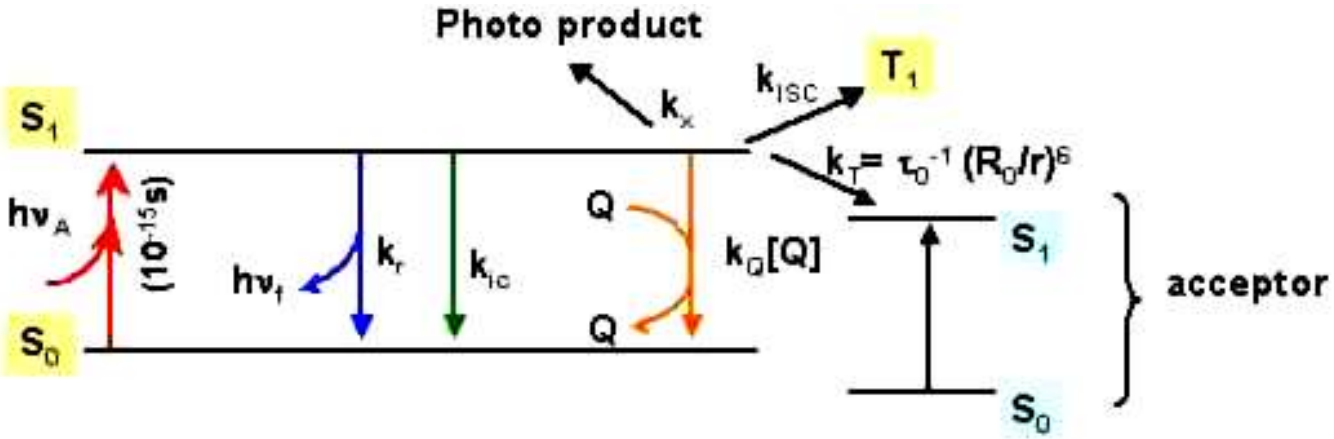


Quantum Yield

- **Quantum Yield** $\Phi_f = \dfrac{\text{number of photons emitted}}{\text{number of photons absorbed}}$



Quantum Yield



k_r = fluorescence radiative rate

k_{ic} = internal conversion (collision with solvent + internal vibrational modes) → increases with T

k_Q = quenching (collision with solute molecules, such as O_2)

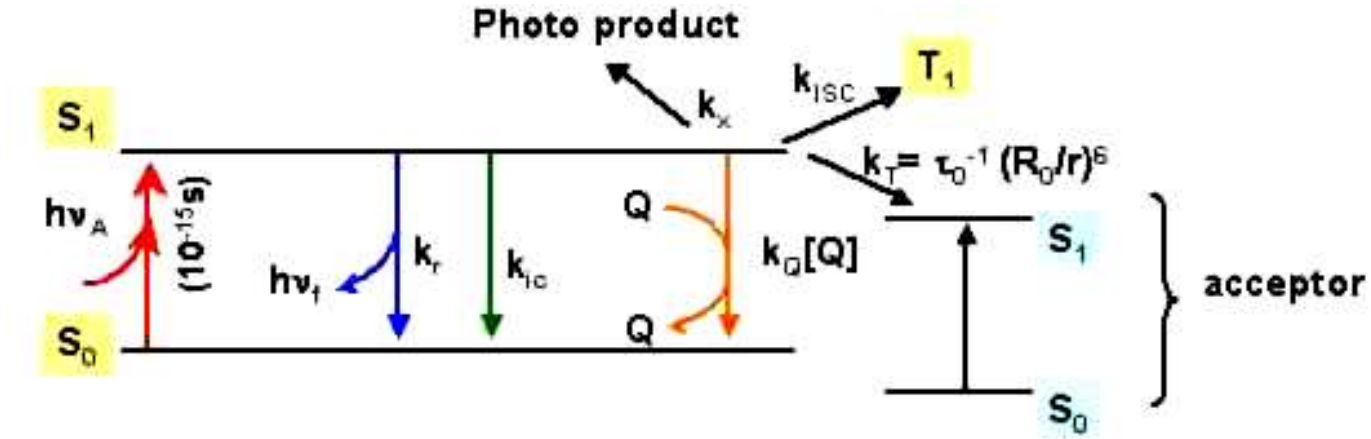
k_T = energy transfer (FRET)

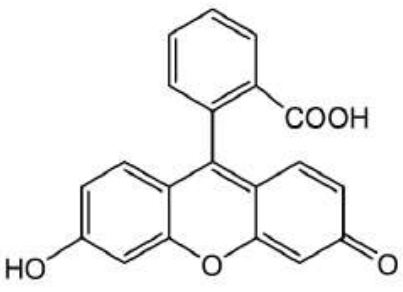
k_x = reaction from excited state

k_{ISC} = intersystem crossing (forbidden)

$$k = (k_r + k_{IC} + k_{ISC} + k_x + k_q[Q] + k_{FRET} \ldots)$$

Quantum Yield and Molecular Structure

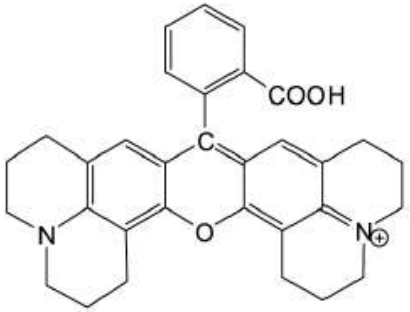




fluorescein

Φ = 0.79

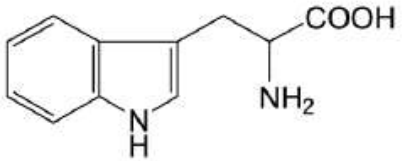
At pH 12



rhodamine 101

Φ = ~1.00

EtOH + HCl



tryptophan

Φ = 0.14

In solution

In proteins?

Φ = 0.00 - 0.3

Raman Scattering

Scattering of Light

True solution
(No scattering)

Colloidal solution
(Scattering of light)

Scattering of Light

Remitted photon without energy resonance.

Colloidal solution
(Scattering of light)

Scattering of Light

Remitted photon without energy resonance.

99.99% of scattered photon keeps the same energy as incident photon.

→ **Rayleigh scattering.**

Scattering of Light

99.99% of scattered photon keeps the same energy as incident photon.

→ **Rayleigh scattering.**

< 0.01 % of scattered photon exchanges energy with the media molecules.

→ **Raman scattering.**

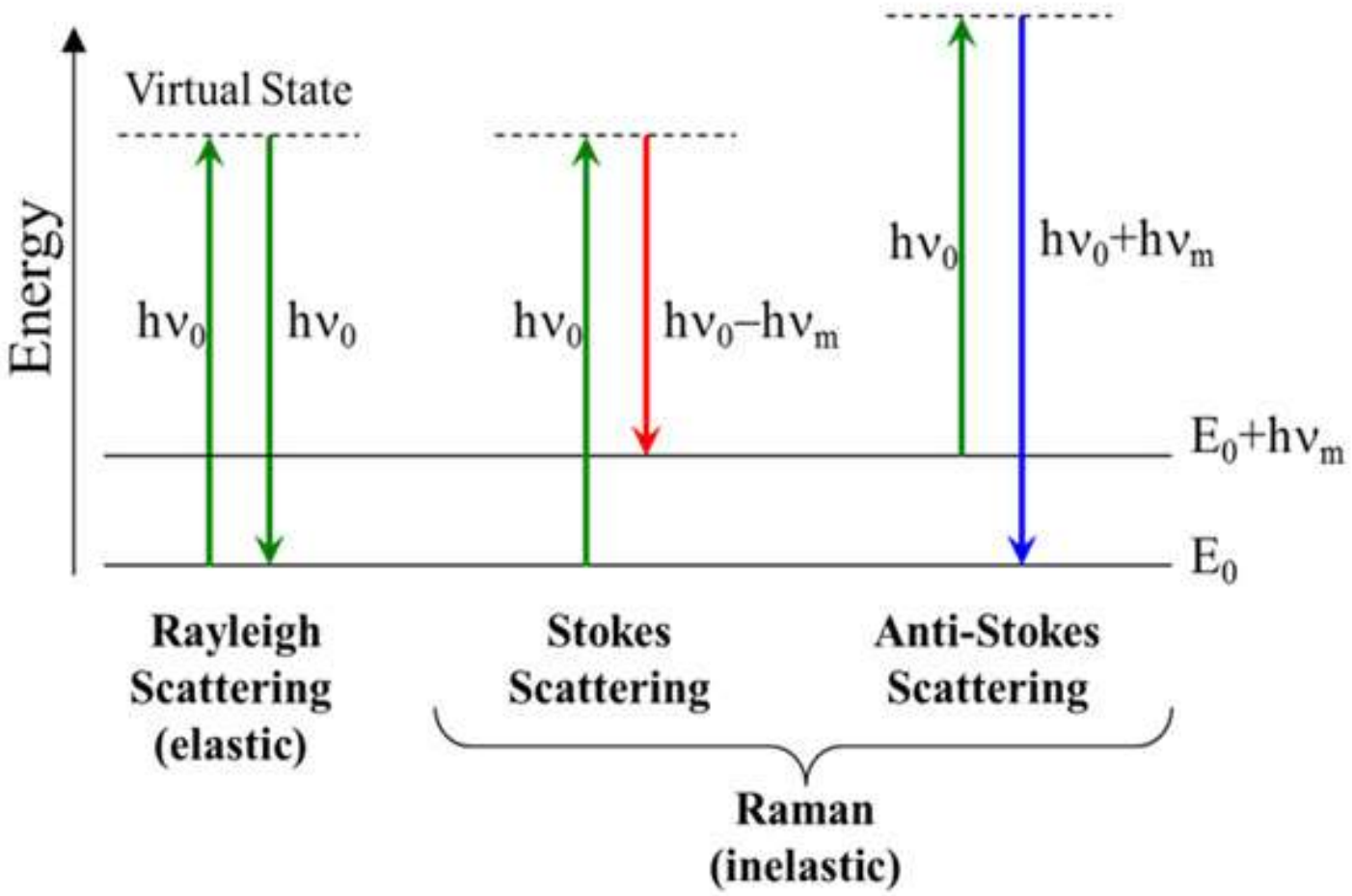
Discovery of Raman Scattering

C.V. Raman (India)

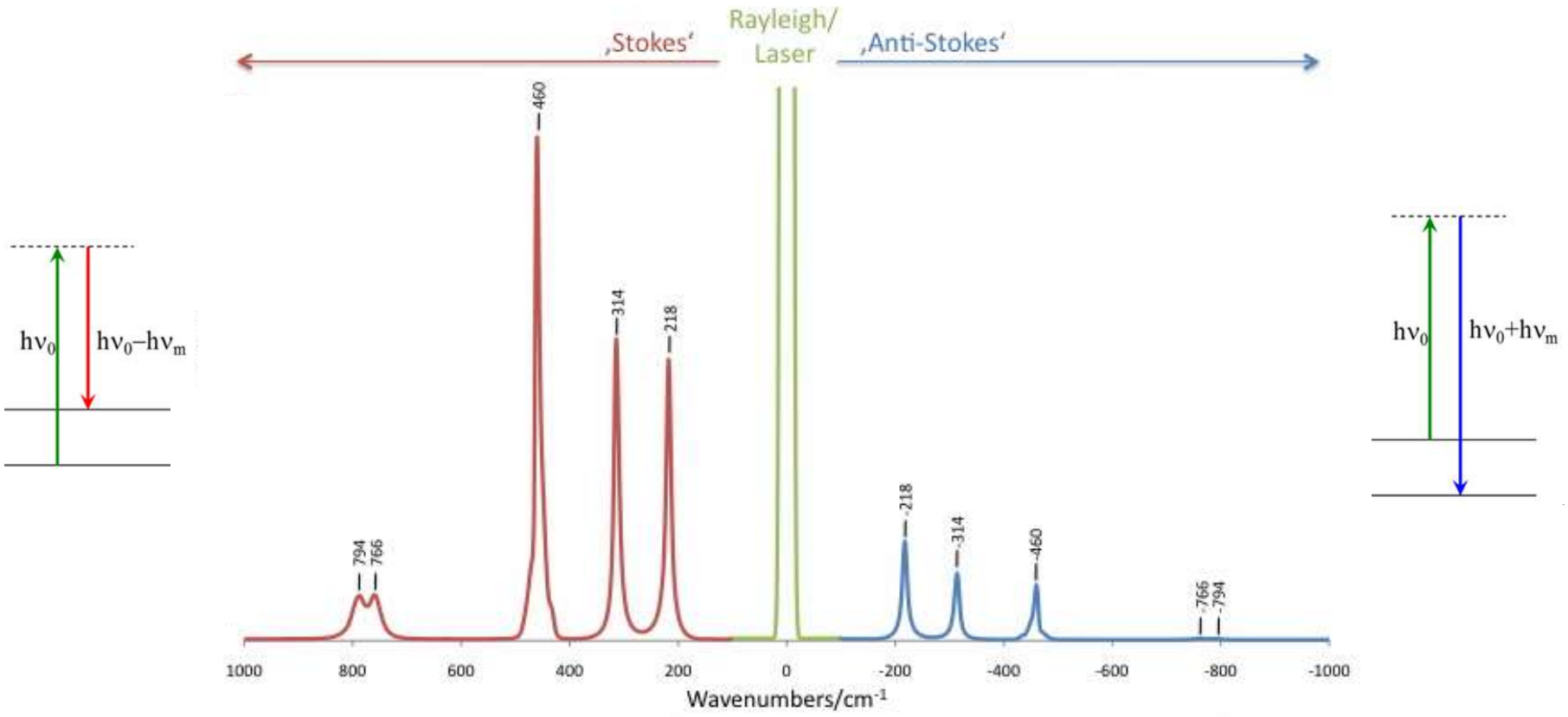
1928 - Discovery of the inelastic scattering of photon.

1930 - Nobel prize in physics.

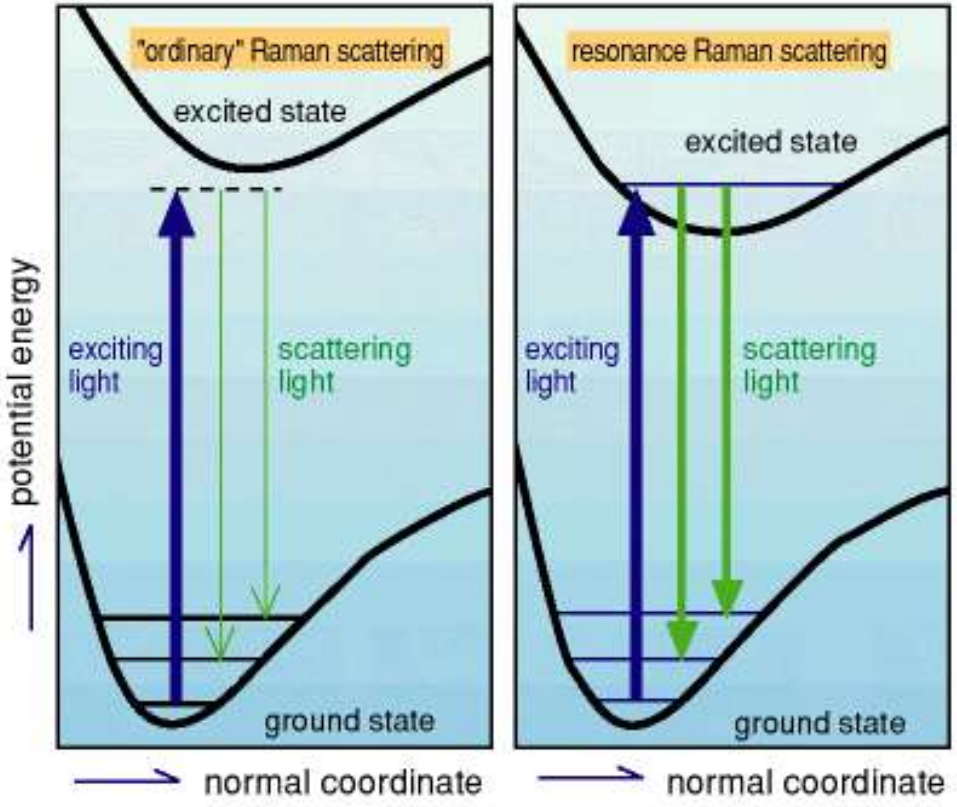
Raman and Rayleigh Scattering



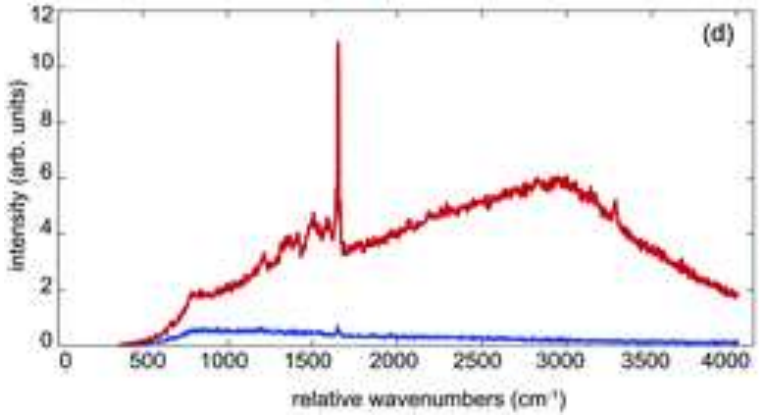
Raman and Rayleigh Scattering



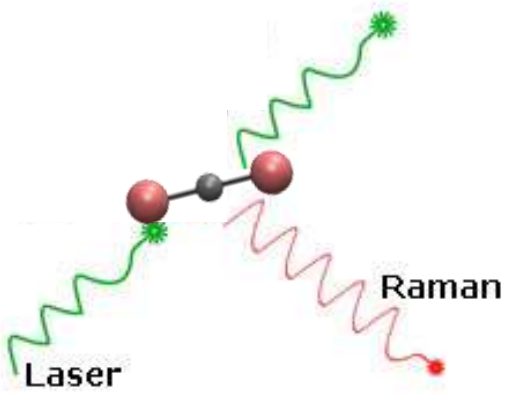
Resonance Raman

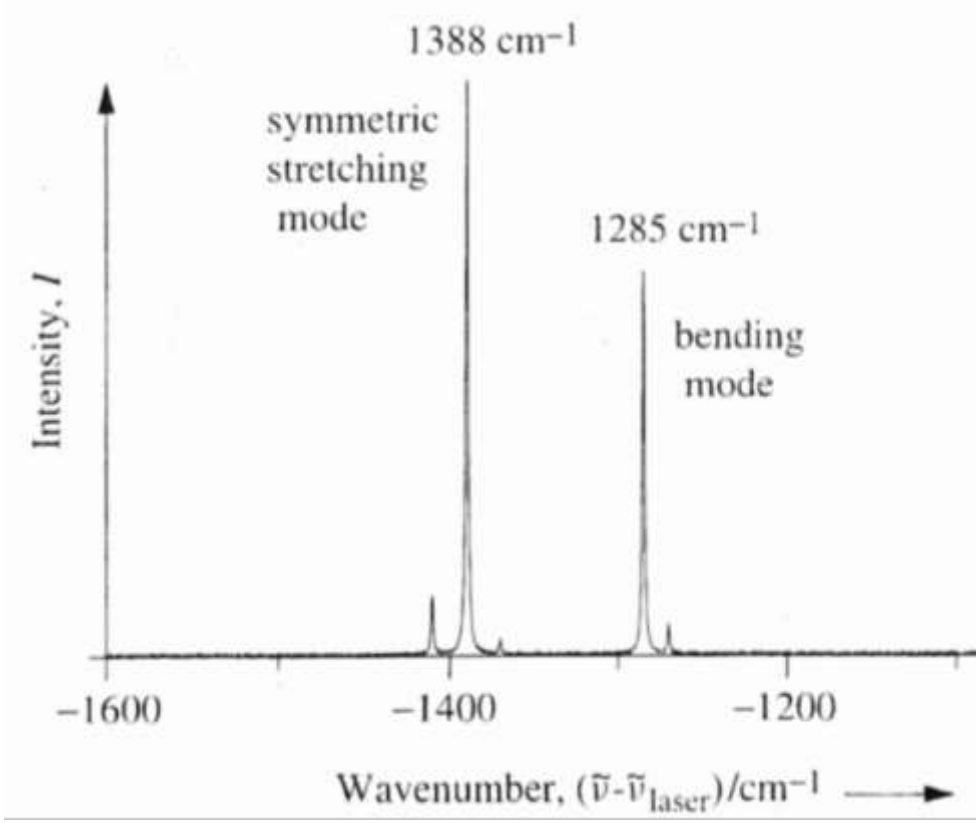


- Stronger Raman signal due to electronic resonance .
- May accompany with fluorescence.

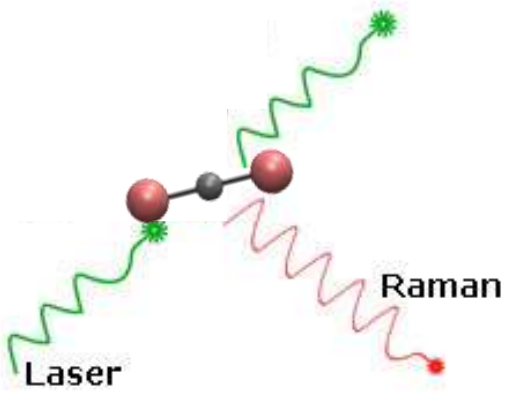


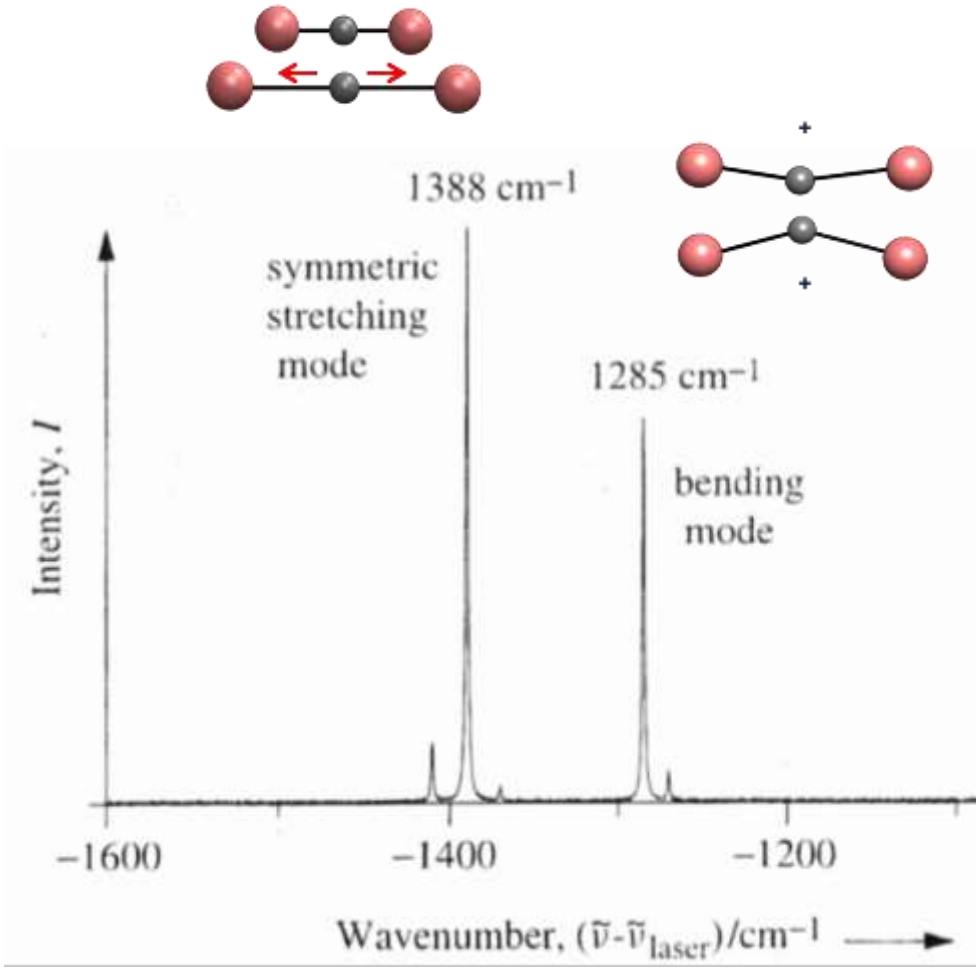
Raman and Molecular Vibration





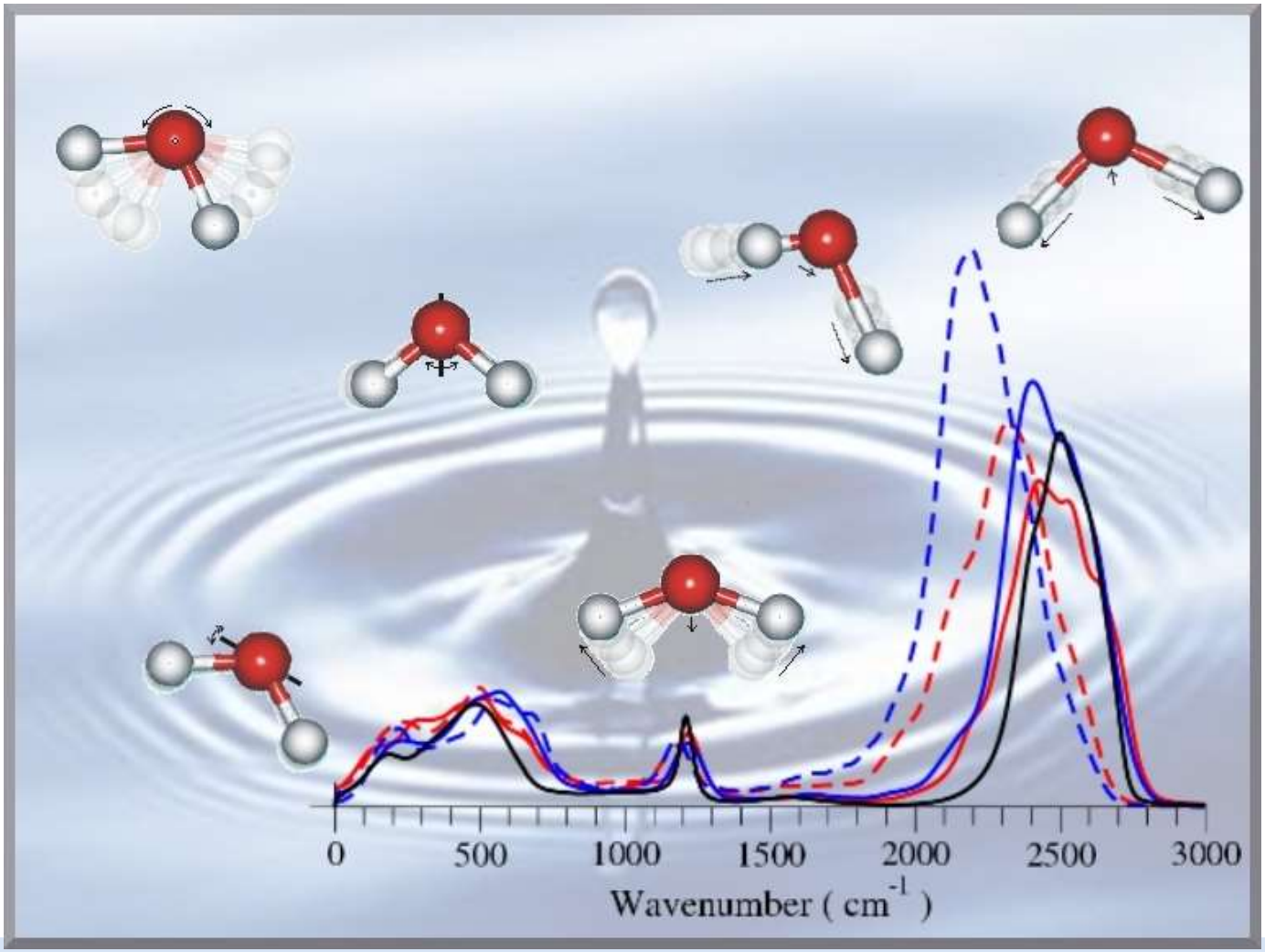
Raman and Molecular Vibration



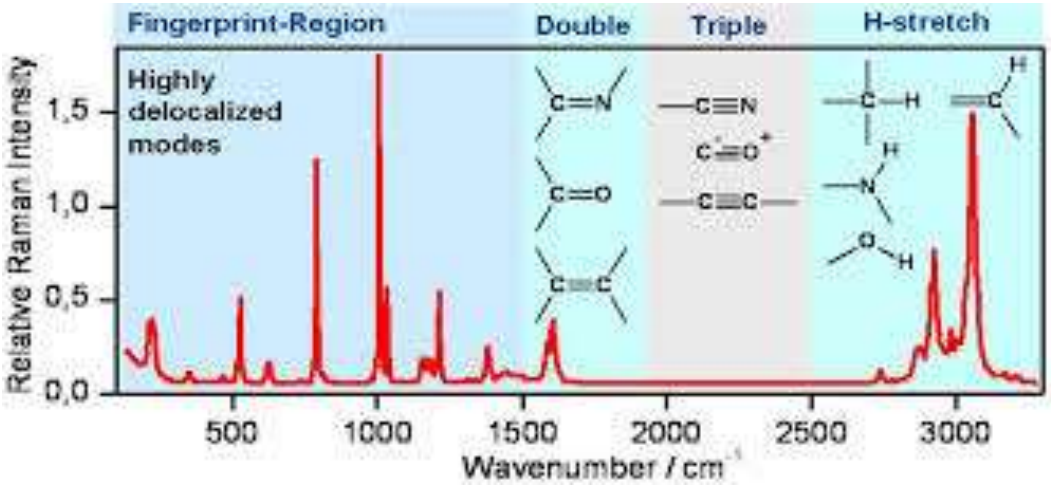


Gymnastics of Water Molecule

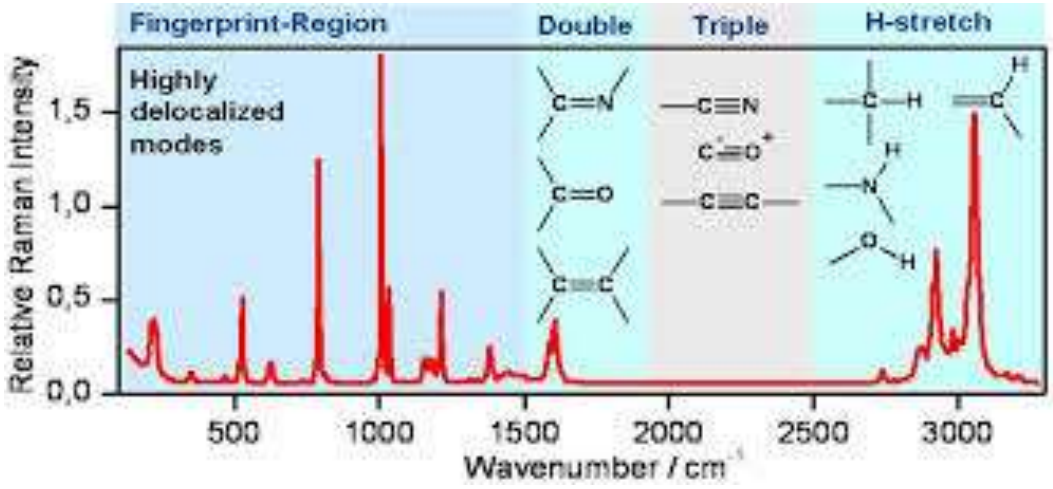
Rotation **Bending** **Stretching**

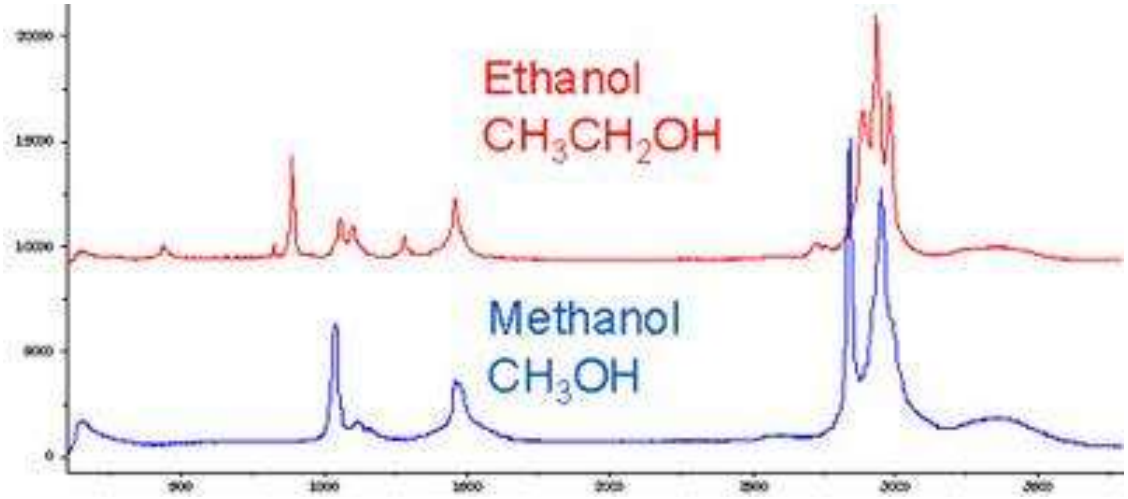


Raman Spectroscopy for Molecular Fignerprint

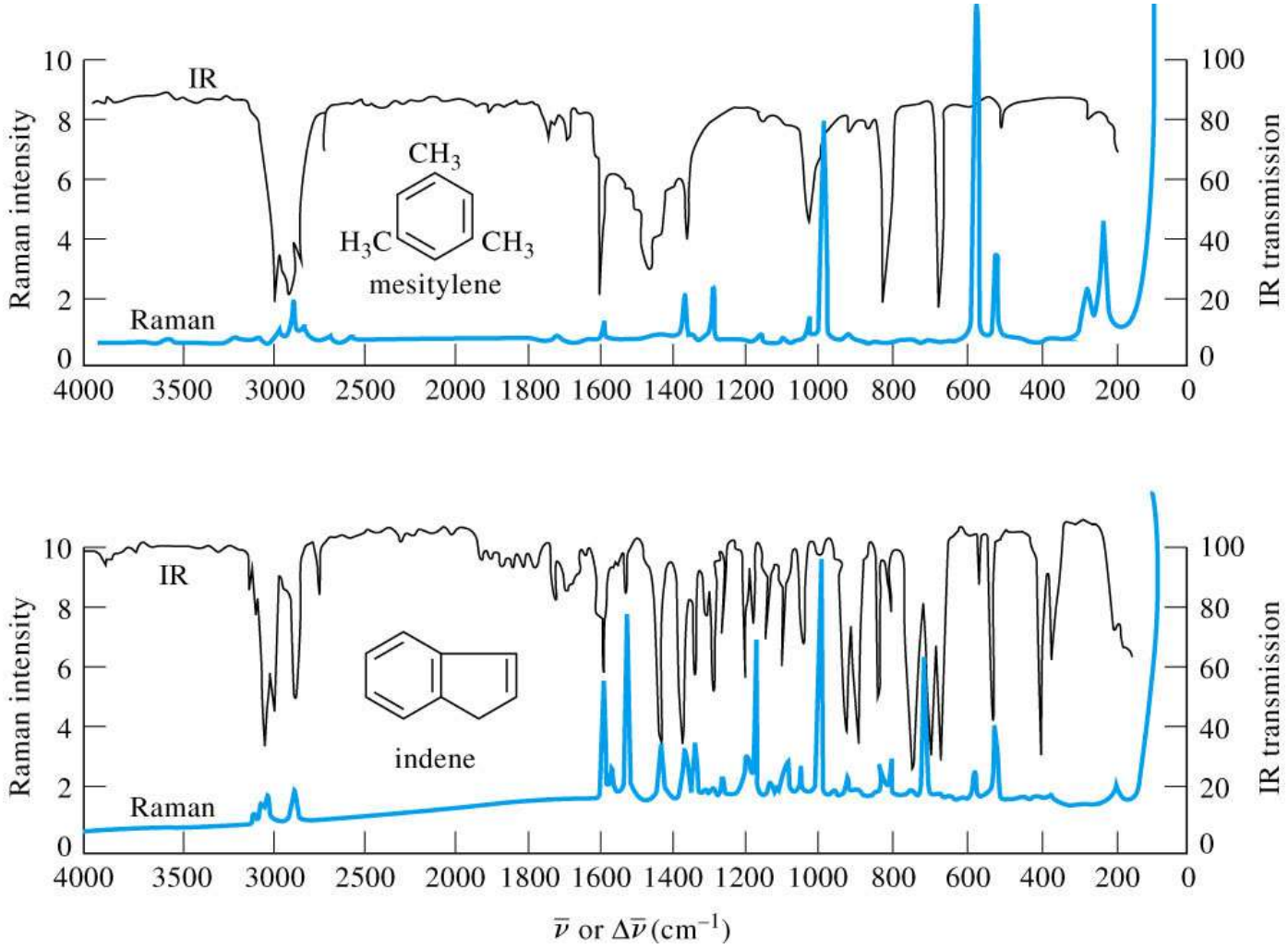


Raman Spectroscopy for Molecular Fignerprint

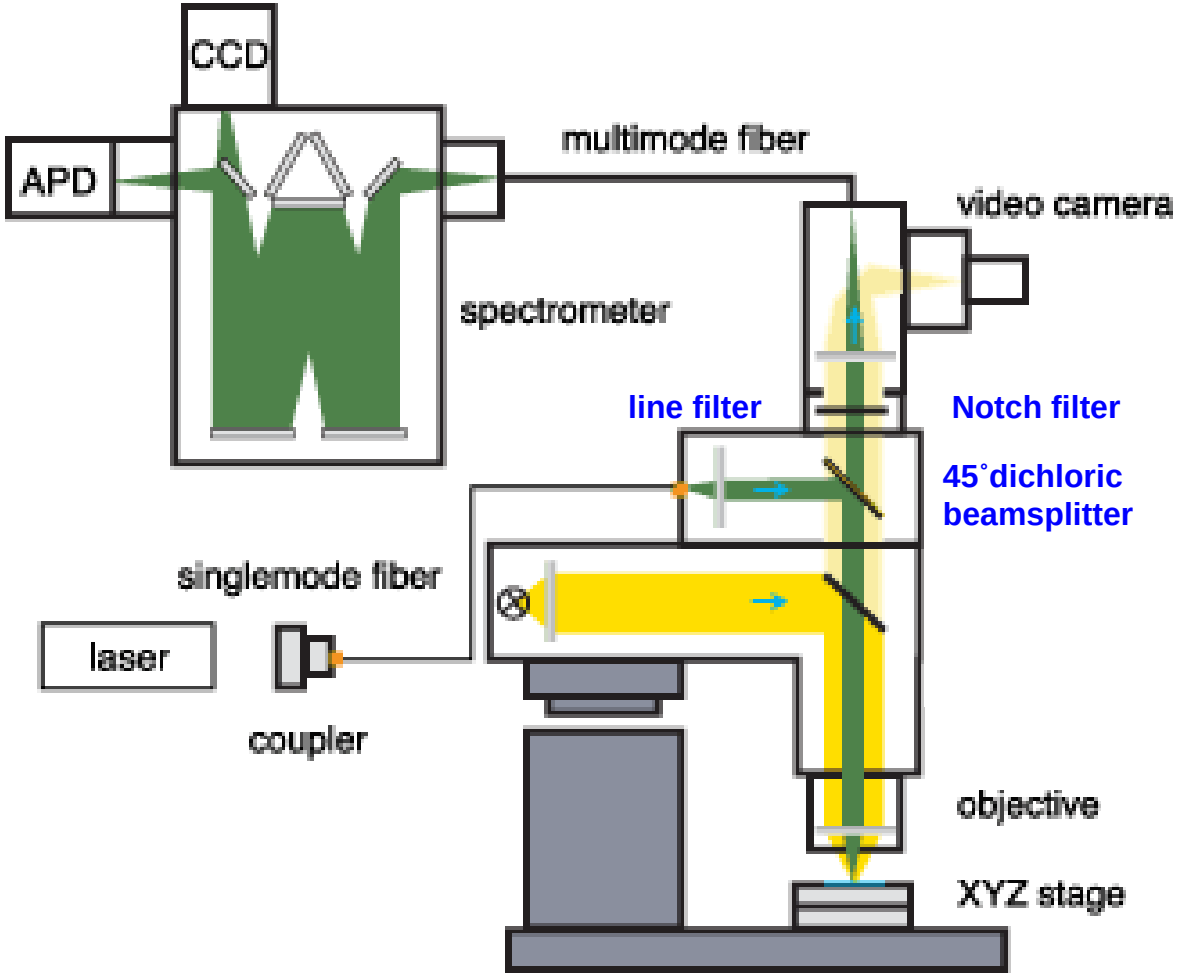




Vibration Spectroscopy: Raman v. s. IR

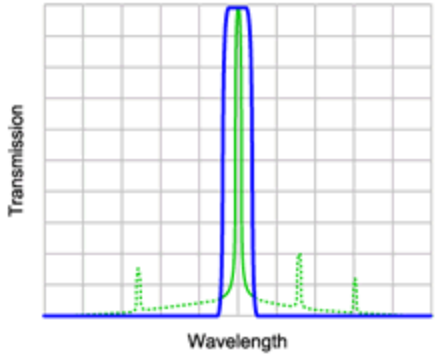


Experimental Setup

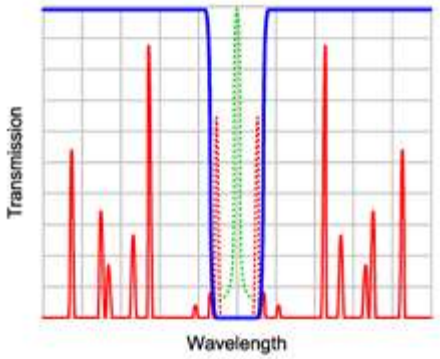


Extremely Expensive Filters

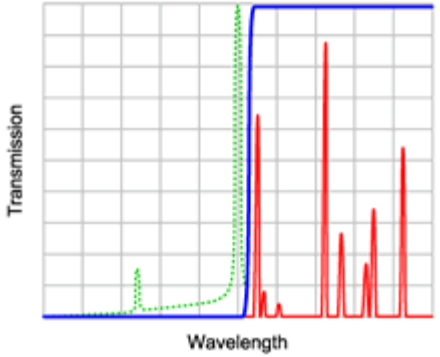
Laser line cleanup filter



Notch filter

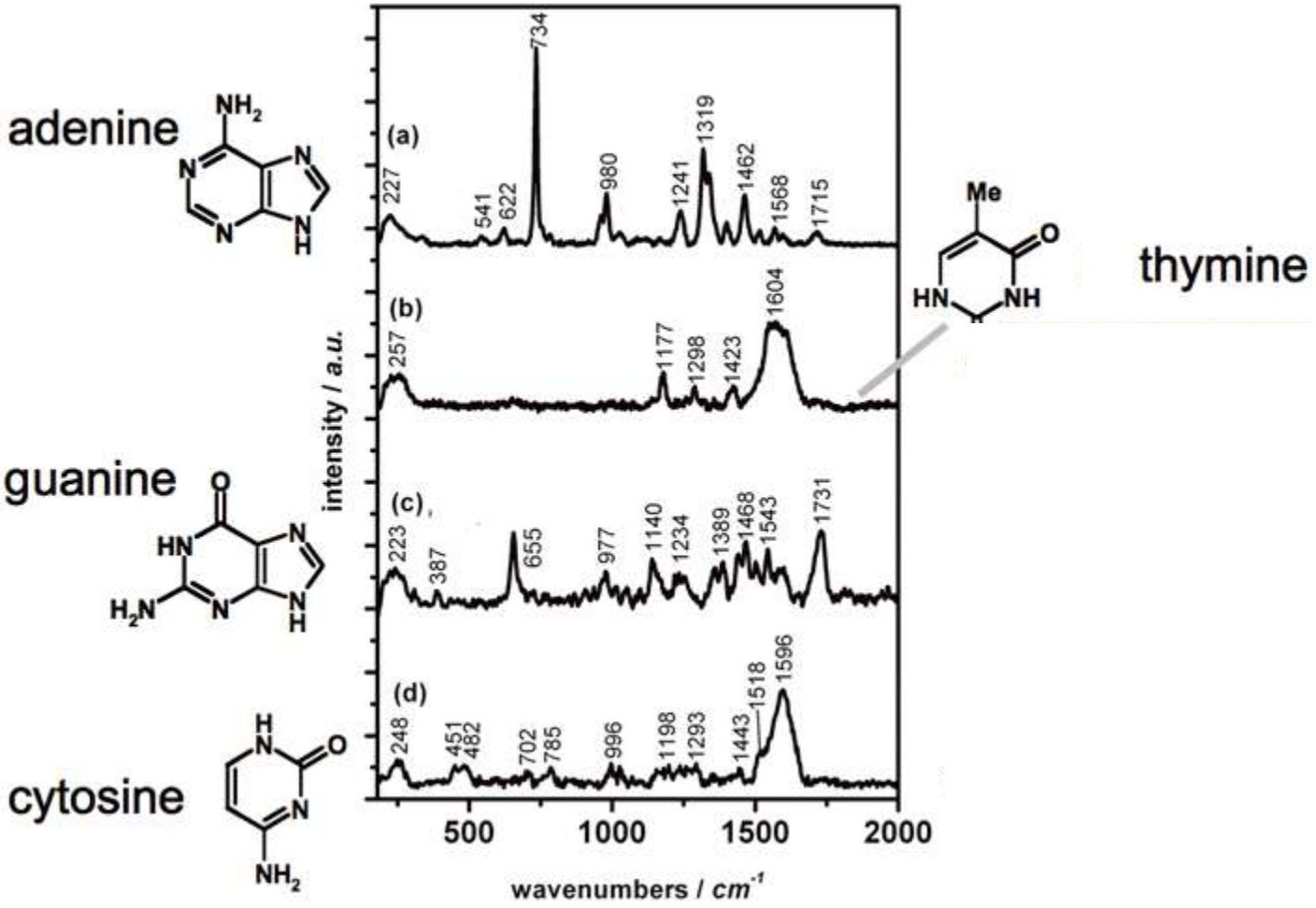


45˚dichloric beamsplitter



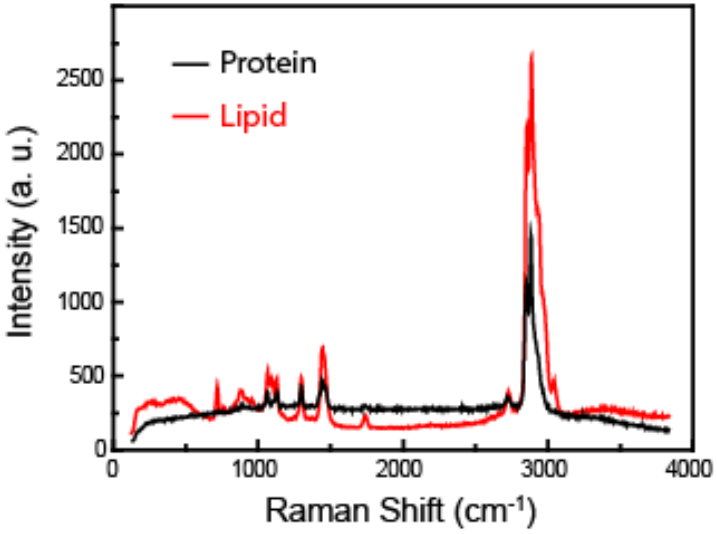
Raman for Bio Molecules

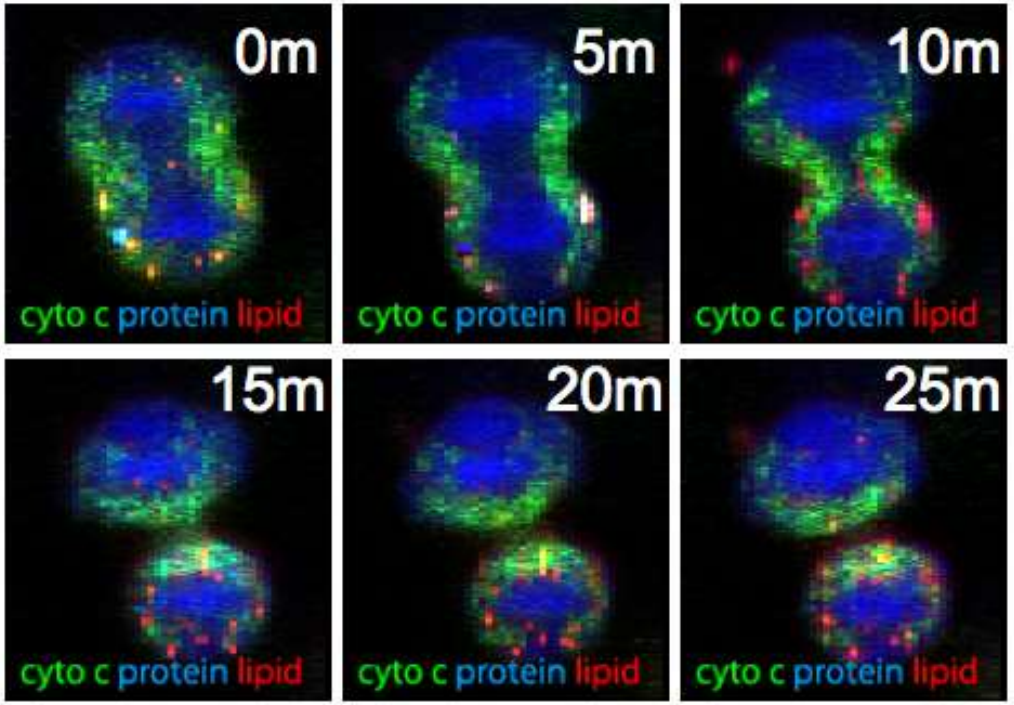
- DNA



Raman for Bio Molecules

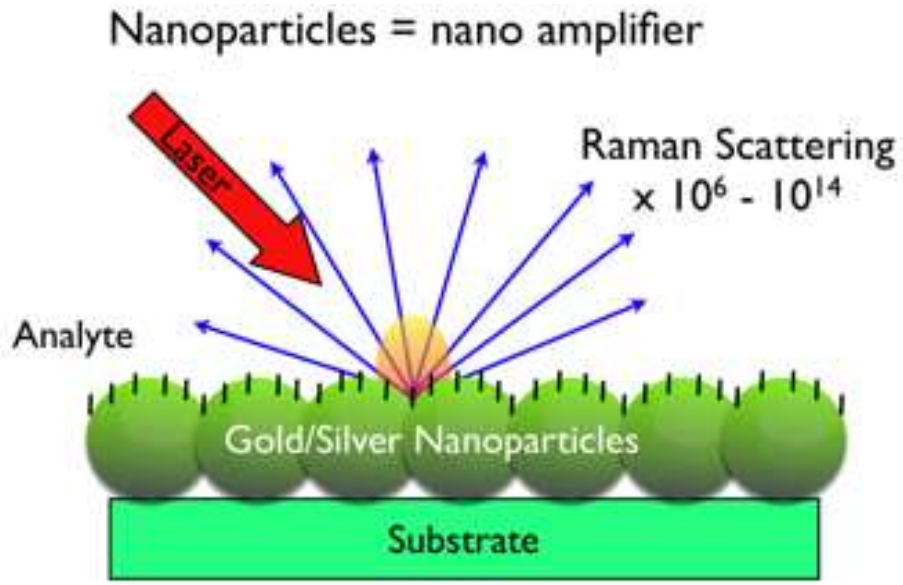
- Label-free imaging for different cellular components





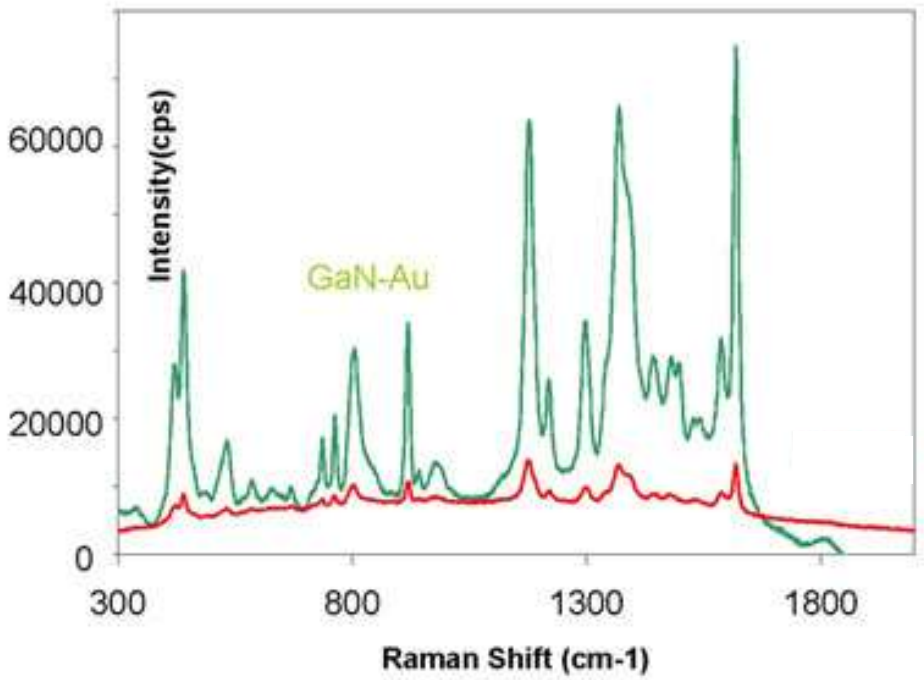
SERS

Surface Enhanced Raman Scattering



- SERS is a surface sensitive technique.
- Huge enhancement on rough metal surfaces.
- The enhancement factor can be as much as $10^{14} – 10^{15}$.
- High sensitivity possibly for single molecule detection.

SERS



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