Single molecule spectroscopy 1:

Why is it useful?
How does it work?
How do you do it ?

Mike Barnes
Department of Chemistry, and Department of Physics
University of Massachusetts
Amherst, MA 01003
mdbarnes@chem.umass.edu
258 Goessmann Hall

UMassAmherst

What is "*chemical microscopy*" ? …

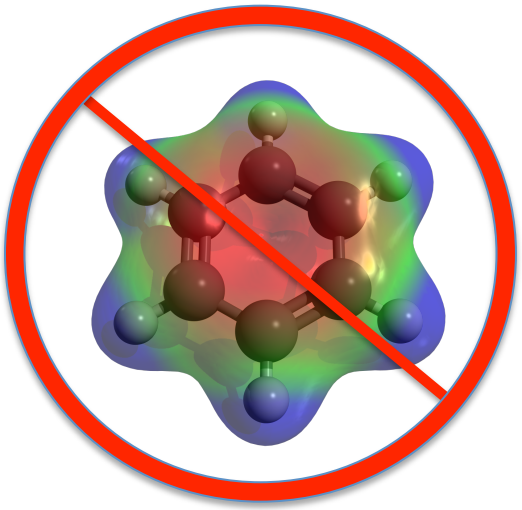
- What we'd like to do:

- *is to look at the molecules!*

- obtain structural,chemical, morphological information with the *convenience* of an optical probe

- derive structure/photophysical property relations at nanoscopic level

Taking pictures of single-molecules



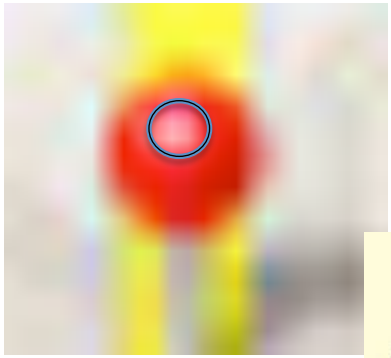
- Motivations
  - -every molecule is special!
  - - connection between structure and photophysics

- Similarities and differences with conventional photography?
  - -Not quite the same as conventional photography
  - -Interpreting molecular photographs is not so simple...

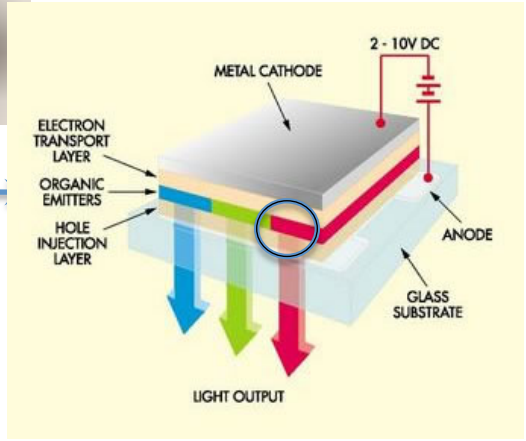
- Some cool examples
  - -SMS as a molecular 'ruler' (sm-FRET)
  - -molecular motors
  - -'super-resolution' techniques applied to complex molecular structures

Polymer optoelectronics and the iPhone display

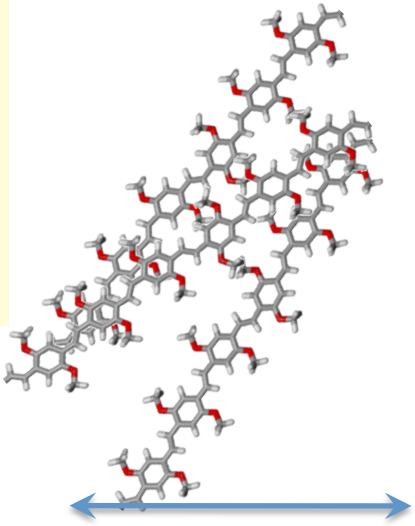
50 mm



1 mm



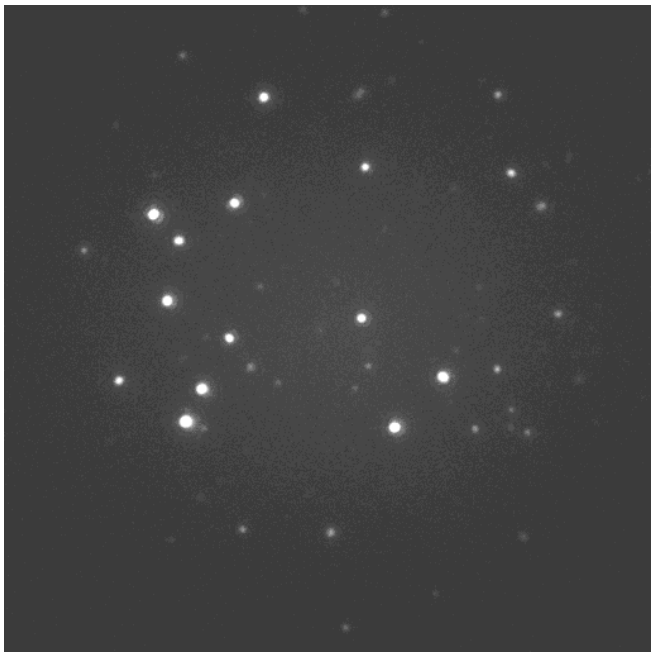
0.02 mm



0.00001 mm

- molecular heterogeneity?
- morphology and optoelectronic function?
- molecular packing and charge-transport efficiency?

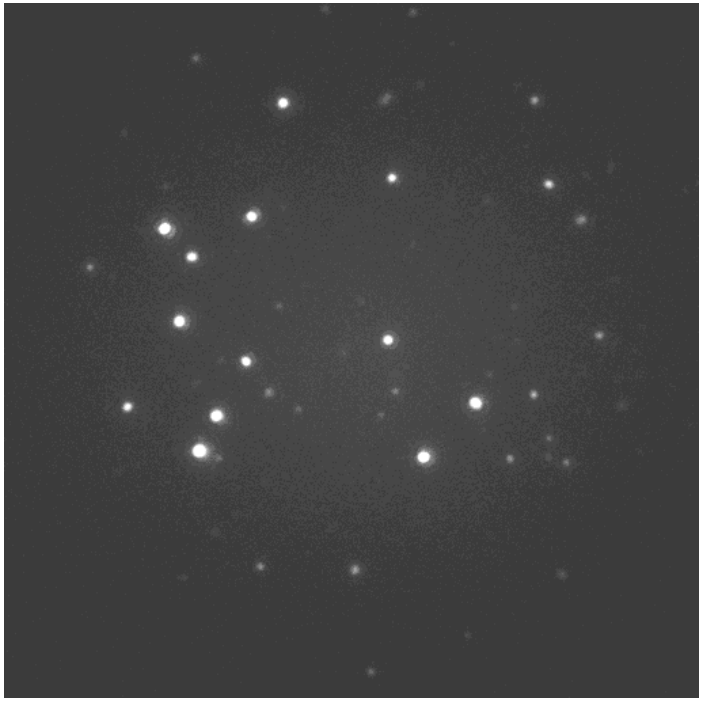
Molecular photography: Just take a picture?



- Imaging "***big***" things is straightforward – dynamical/environmental information is carried in contextual clues
- Imaging "small" (< 300 nm) not so straightforward….

Molecular imaging and astronomy…

Google images: Hubble Space Telescope



- *like stars, we need the molecules to make their own light!*

- *How to interpret the image?*

Basic ingredients of a single-molecule experiment

1. Need strong transition dipole $< e \mid \mu \mid g >$, with fluorescence energy in visible range (400 – 850 nm); this is where CCD cameras, APDs are most efficient

2. Need molecule to be ≈ immobilized (at least not moving very fast!)

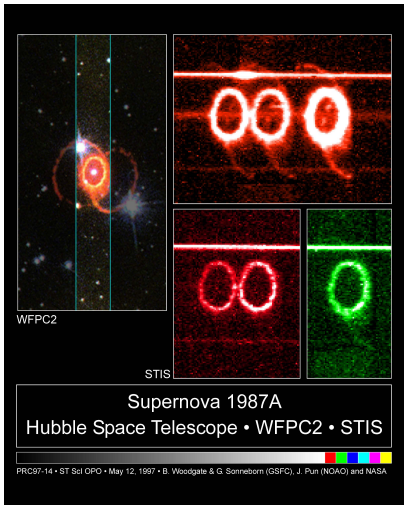
3. Low background (solvent Raman, impurities); low fluorophore density

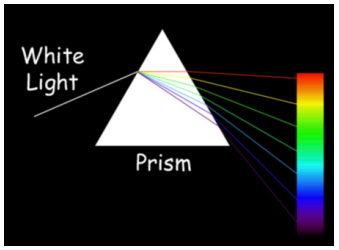
4. Need to excite molecules efficiently ($t_{excite} \approx 0$); high-flux lasers matched to excitation (S0- S1) energy

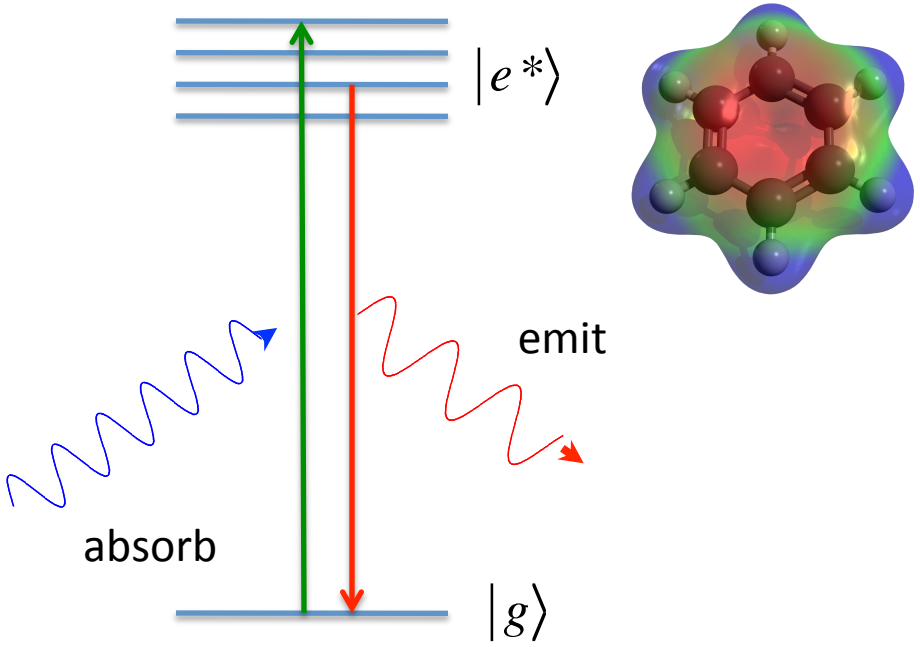
5. Need to have very efficient light collection/detection system (capture as many photons as possible)

6. Useful (but not necessarily required) to have good short-time (no blinking) and long-time (low photobleaching quantum yield) stability

Chemical information is in the photons!!





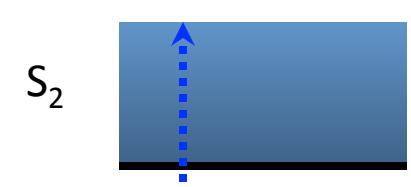


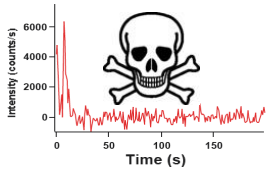
... light spectrum is characteristic of particular atoms/molecules!!

... every 'photon' carries a few *bits* of information (wavelength, polarization, correlations in time...)

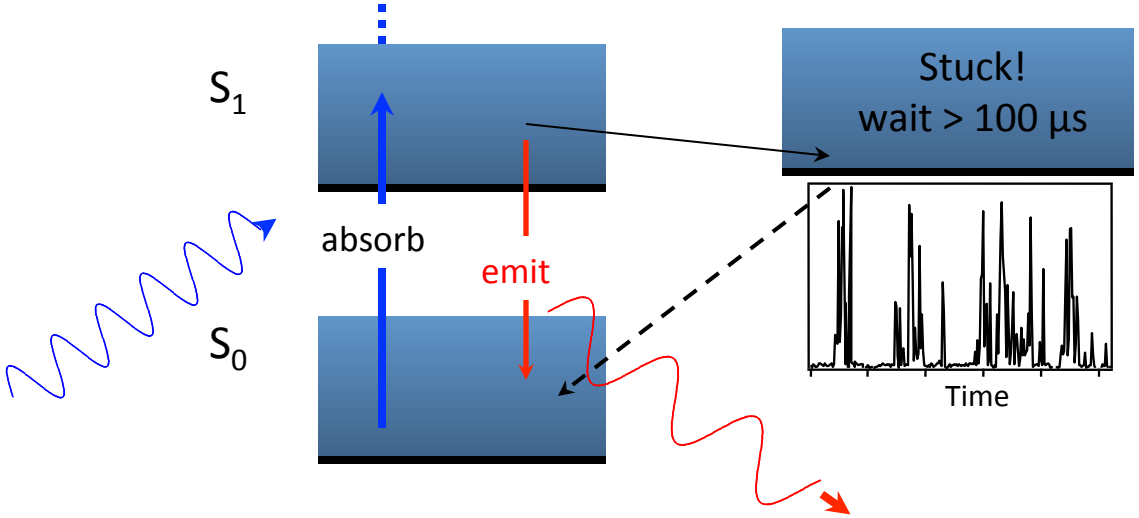
More photons = More Information !

- like light-bulbs, molecules *'burn out'*





Excited state absorption (Photobleaching)

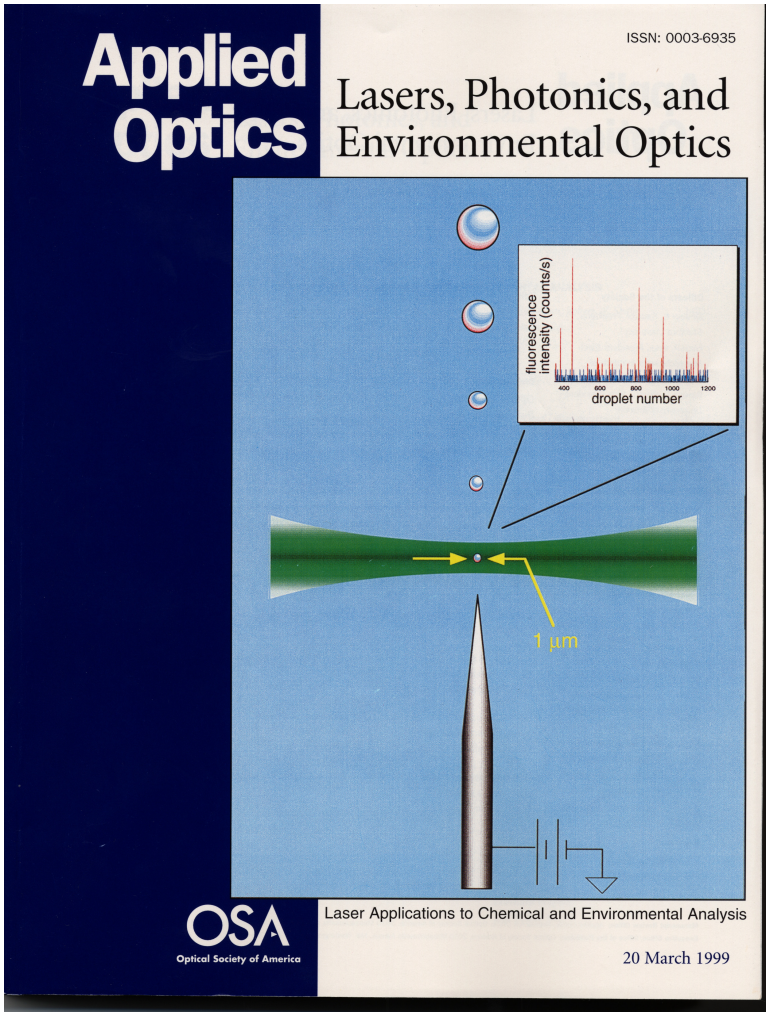


- limited at long times by photobleaching ($N \approx 10^6$ - 10^8)

- *Unlike* light bulbs, stream of fluorescence photons isn't continuous!

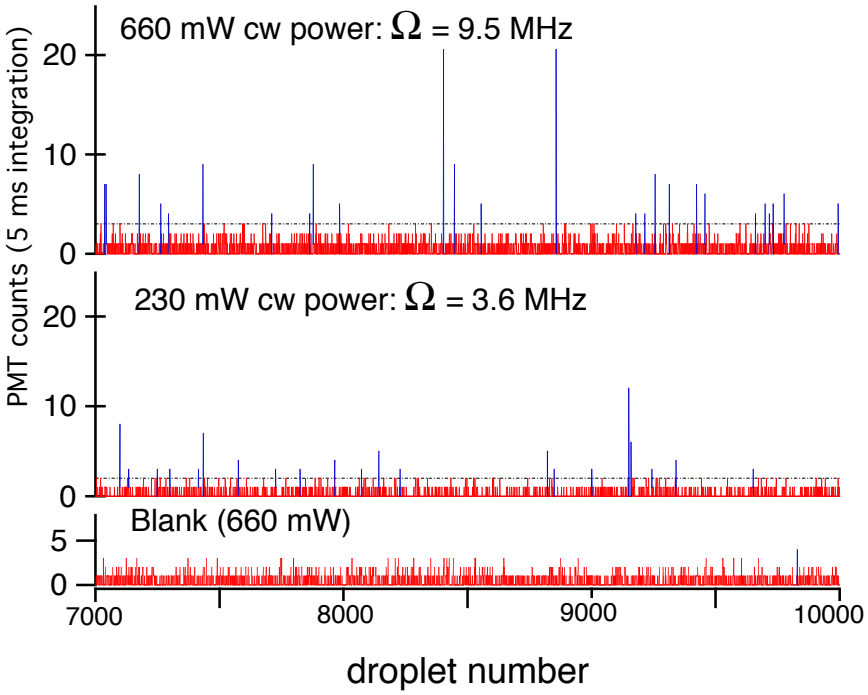
- limited at short times by triplet shelving (*dark state*), "blinking"

Probing single molecules in microdroplets (1999)



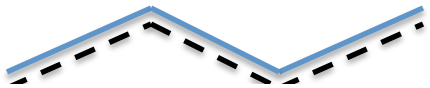
C-Y. Kung, et al. *Appl. Optics* **38**, 1481 (1999)

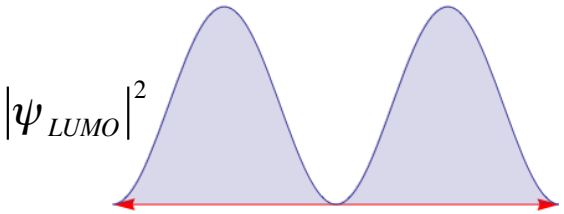
(note intensity scale!!)



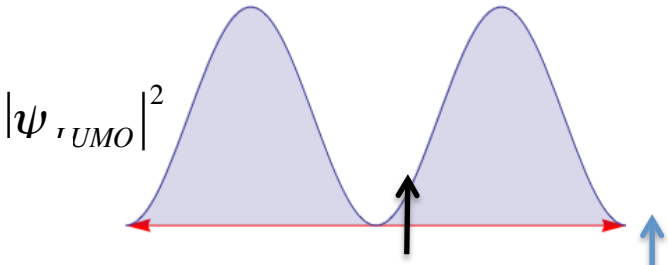
Blue *denotes single-molecule events detected with >99% statistical confidence*

How does an atom/molecule absorb (emit) light ?

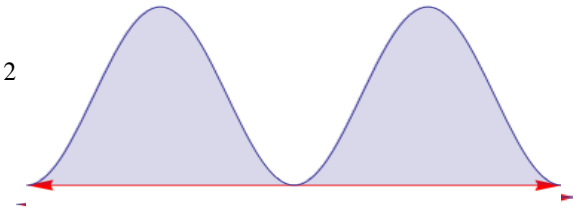




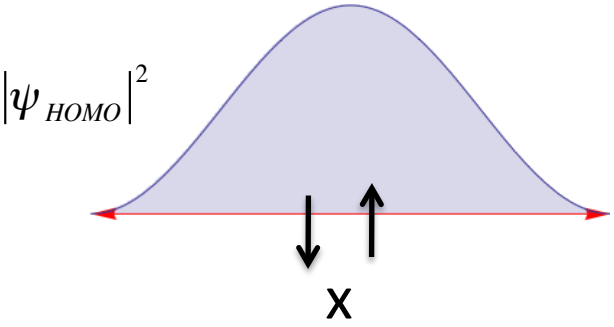
"LUMO"
(**L**owest **U**noccupied
Molecular **O**rbital)



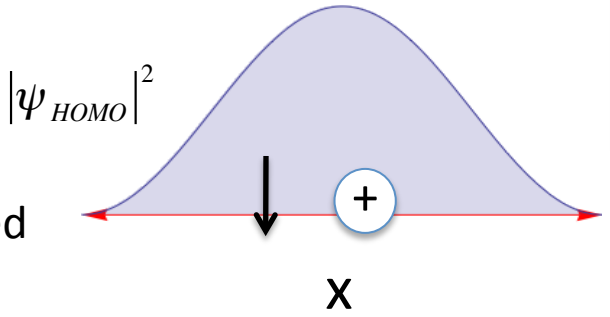
$$|\psi(t)|^2 = |c_1(t)\psi_{HOMO} + c_2(t)\psi_{HOMO}|^2$$



energy



"HOMO" (**H**ighest **O**ccupied
Molecular **O**rbital)

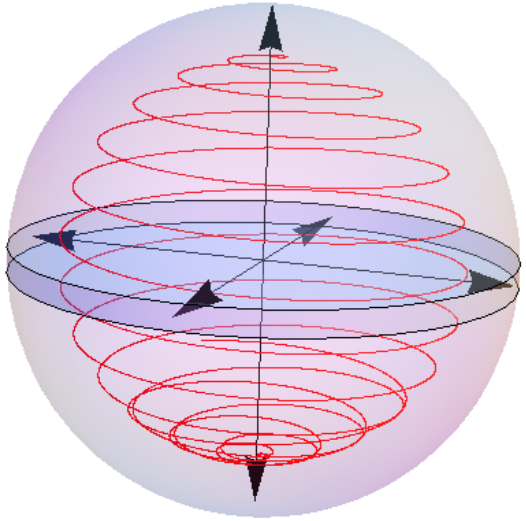


How does an atom/molecule absorb (emit) light ? Bloch vector description

$$\vec{P} \rightarrow \hat{i}S_x + \hat{j}S_y + \hat{k}S_z$$

$S_z \equiv$ *state population*

$S_{x,y} \equiv$ *coherences*



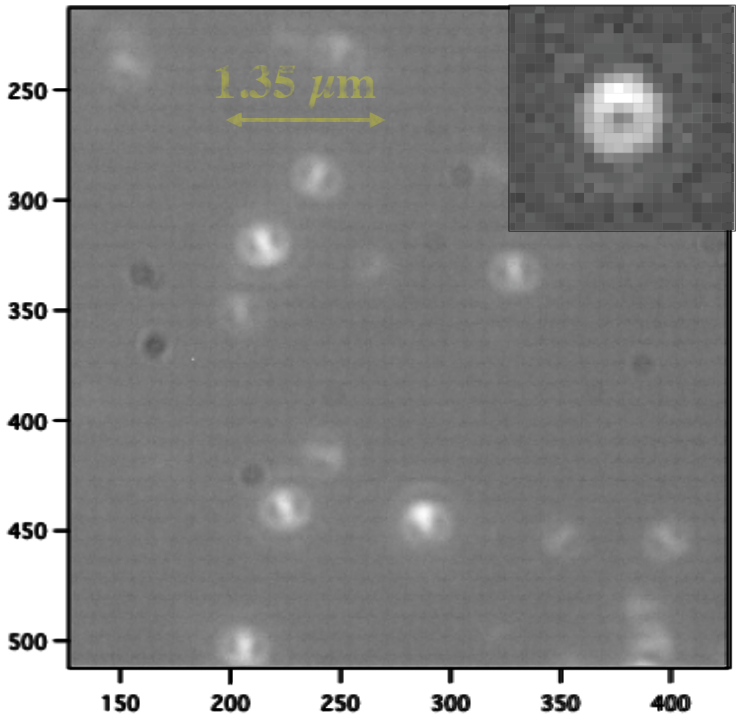
$\vec{P} \rightarrow \{0,0,1\}$ (system is in excited state)

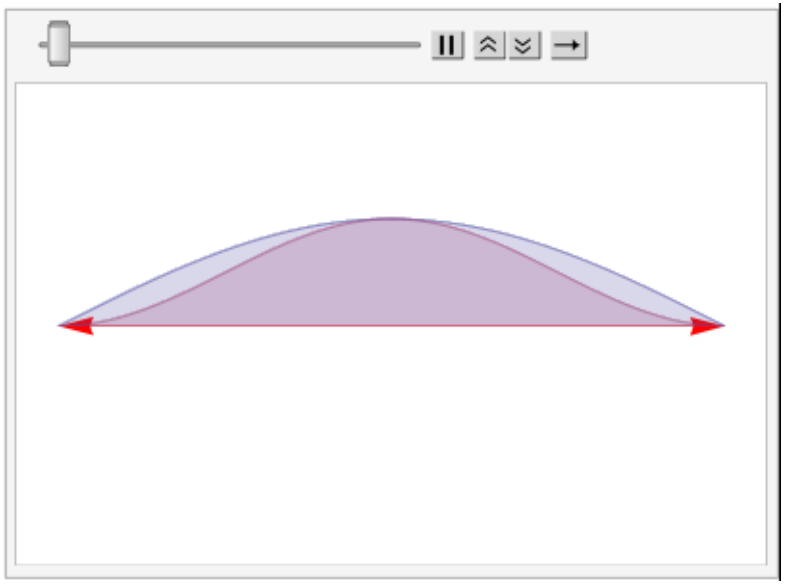
$\vec{P} \rightarrow \{0,0,-1\}$ (system is in ground state)

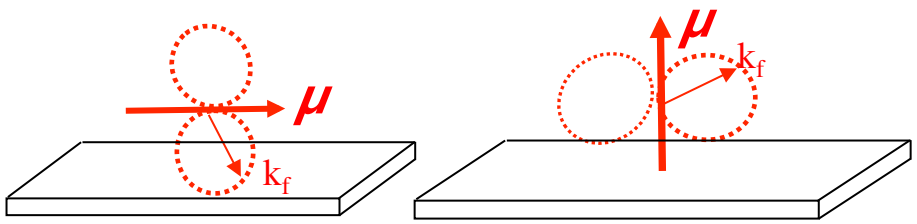
$\vec{P} \rightarrow \{a,\mathbf{i}b,0\}$ (system is in coherent mixture of excited and ground state)

- In NMR, "rotate" Bloch vector from {0,0,1} to {a, **i**b, 0} via timed applied field; turn OFF transverse field, detect decay of $P_{x,y}$
- In spontaneous radiation (fluorescence), prepare {0,0,1} via excitation pulse; interaction with *vacuum electric* field drives system downward, accompanied by emission of a *photon*

Charge 'sloshing' and polarization properties of single molecules



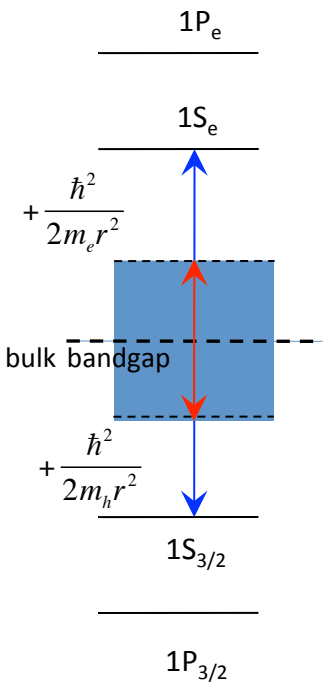




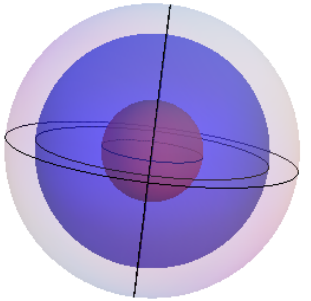
- "sloshing" of charge density in 1D gives rise to antenna behavior for single molecules!

- Patterns are *distinct for linear dipoles (randomly oriented in x-y) -> extract molecular orientation (and dynamics!) from single molecules*

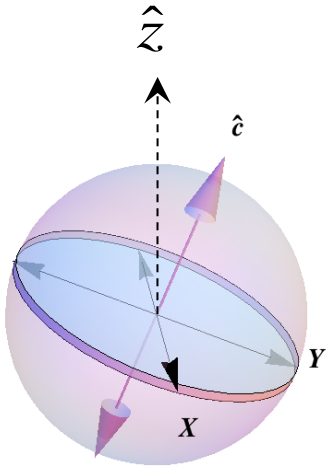
Fluorescence Properties of Single Quantum Dots

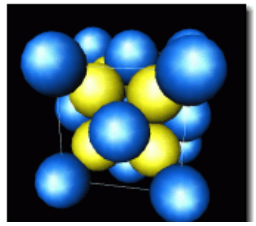


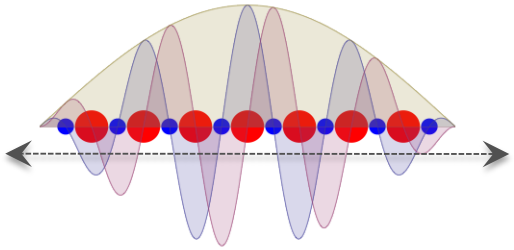
nano-confinement gives rise to size-dependent transition energies



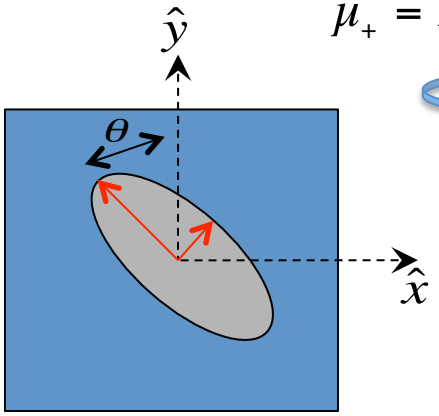
≈ 4-6 nm





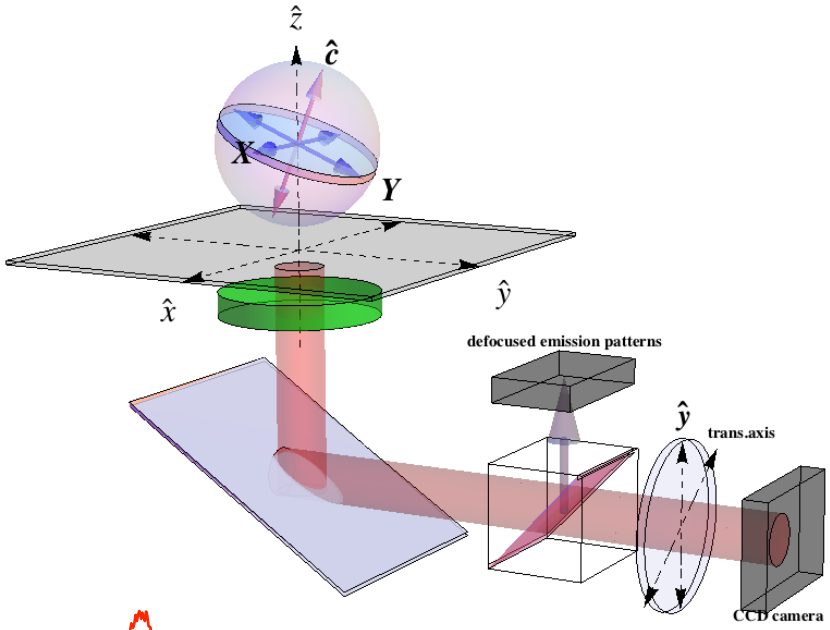


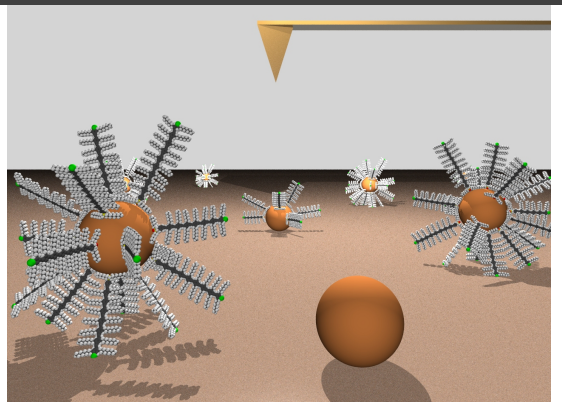
$\mu_+ = X + iY$ $\mu_- = X - iY$

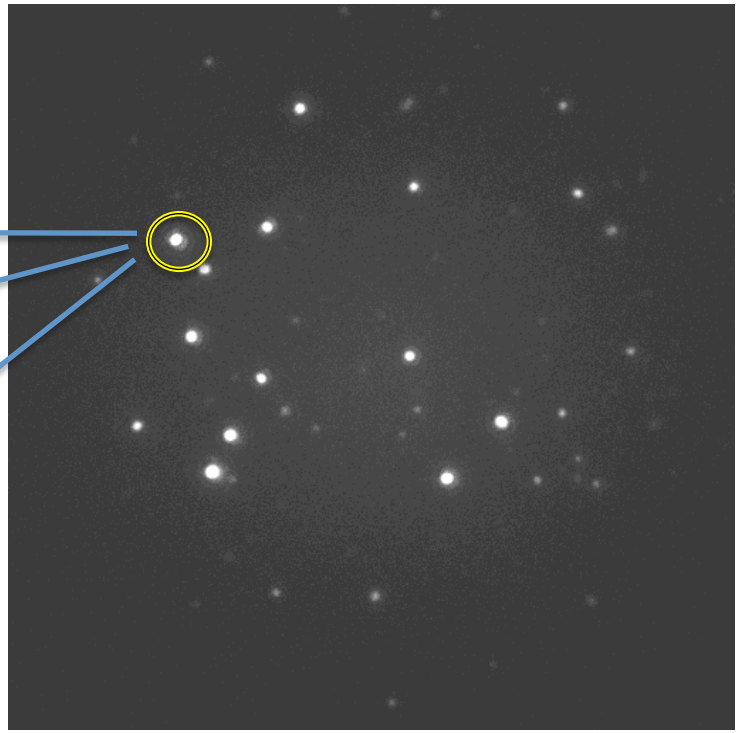


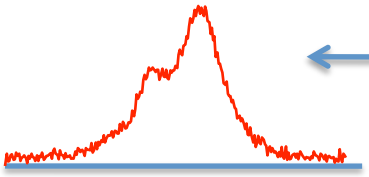
Charge density oscillates on a ring!

Barnes Lab Single-Molecule Photography Studio

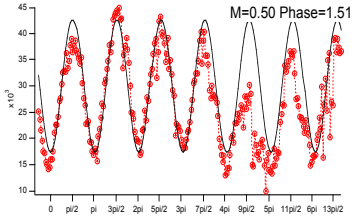




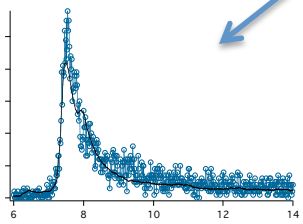




Wavelength (nm)

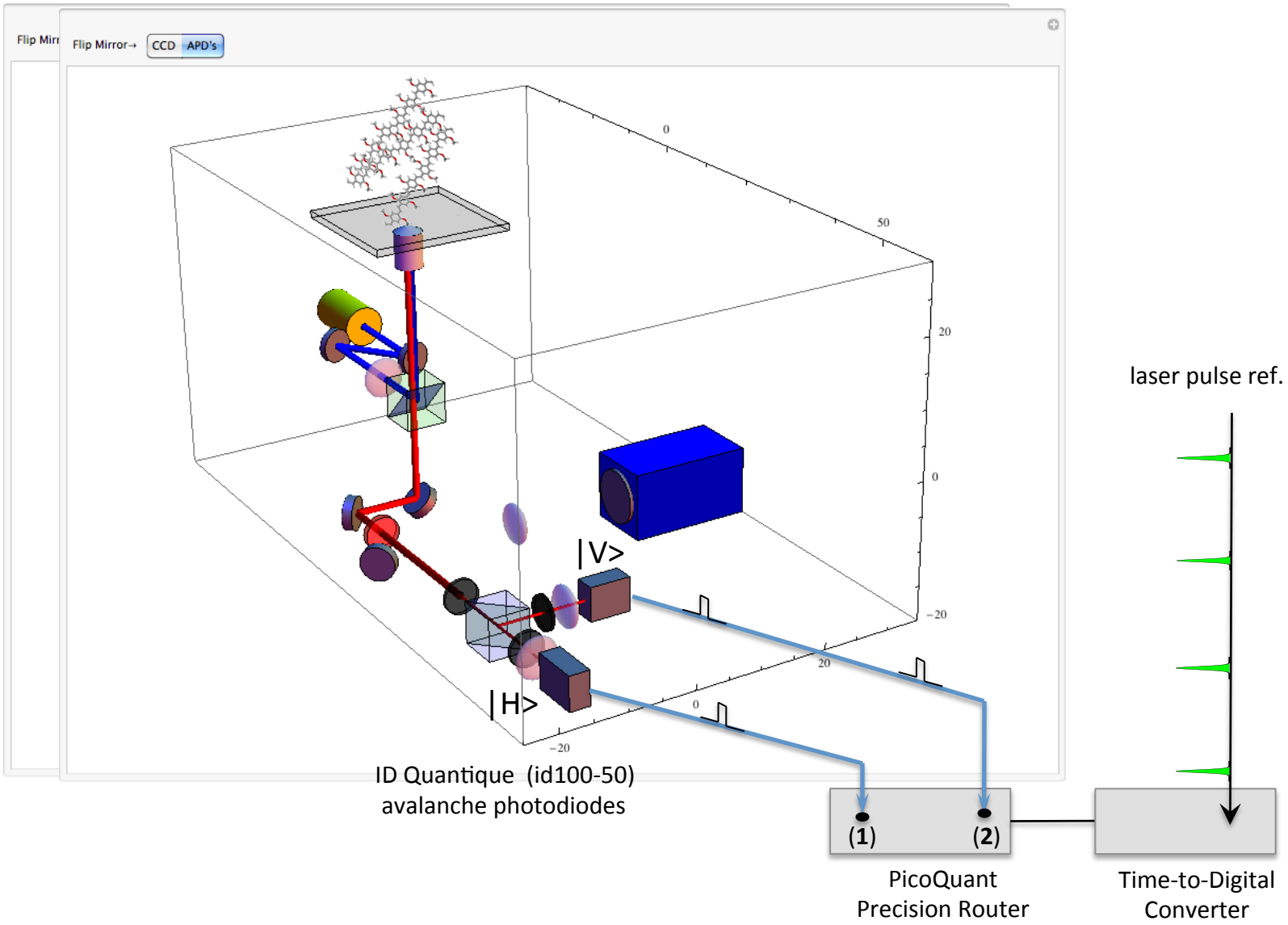


Linear polarizer angle

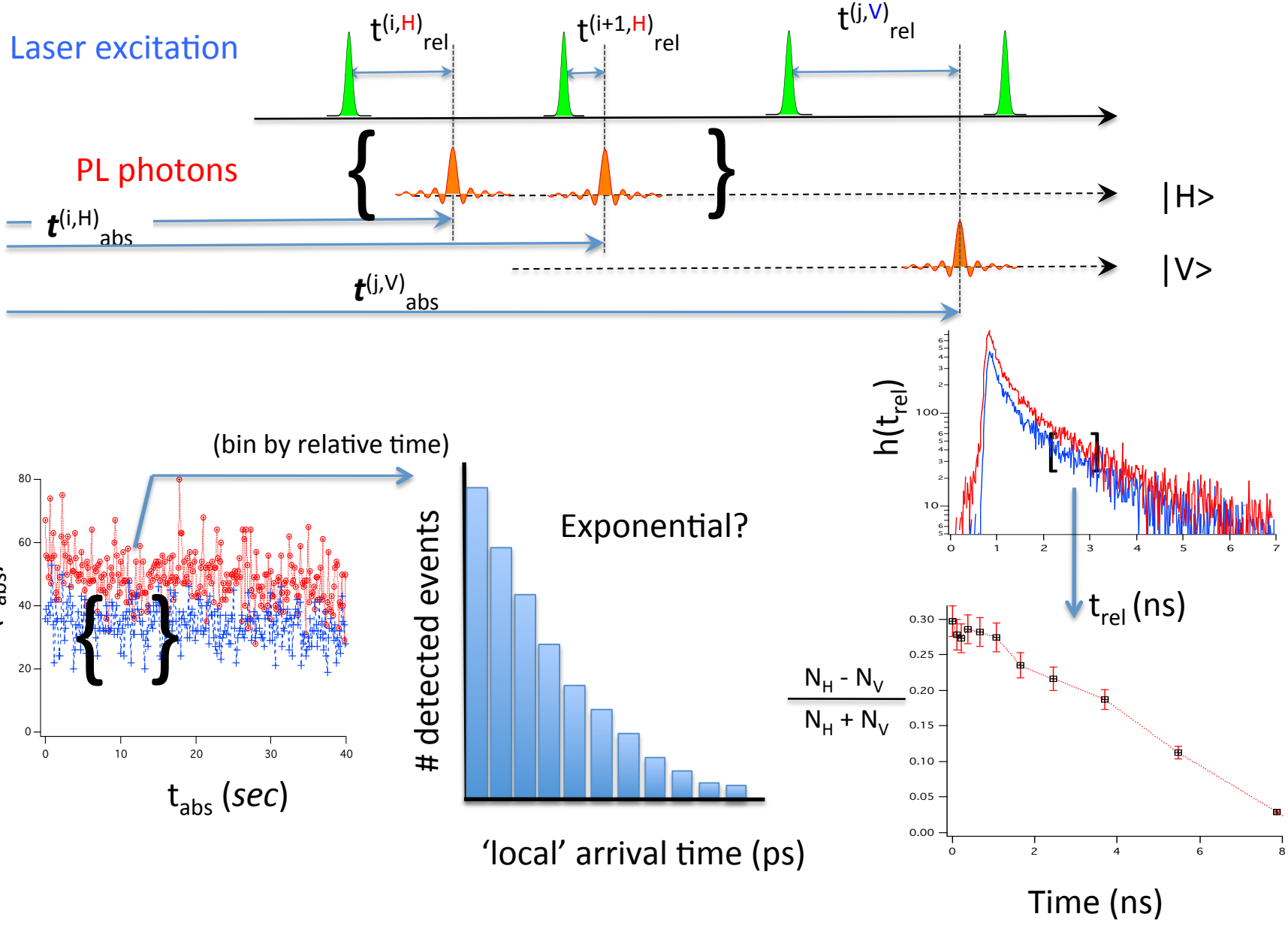


Photon arrival time (ps)

Probing lifetime and polarization properties on single molecules (photon by photon….)



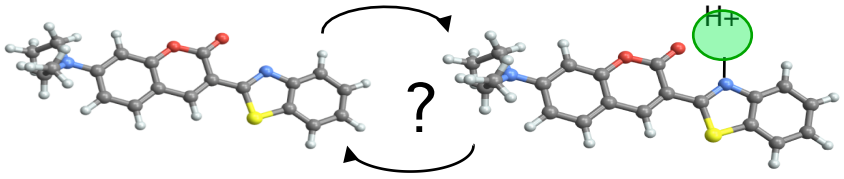
rT3r: time and polarization resolved luminescence from single molecules/np's

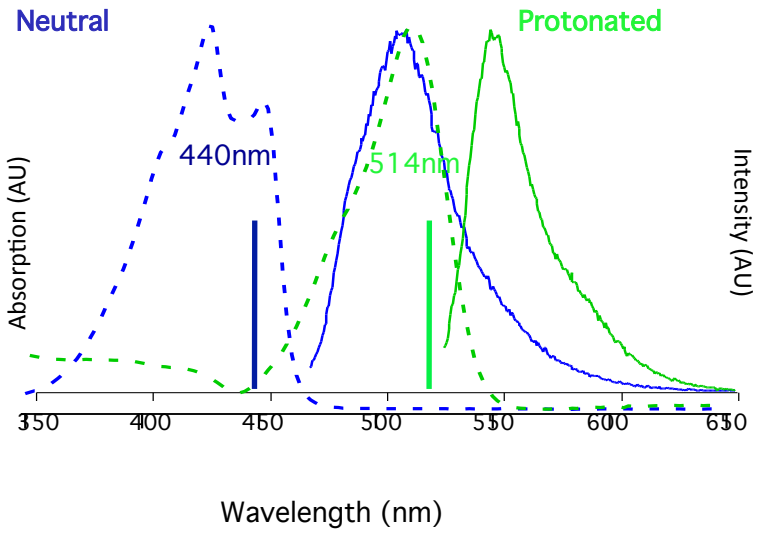


Single-Molecule Probes of Proton Attachment Kinetics

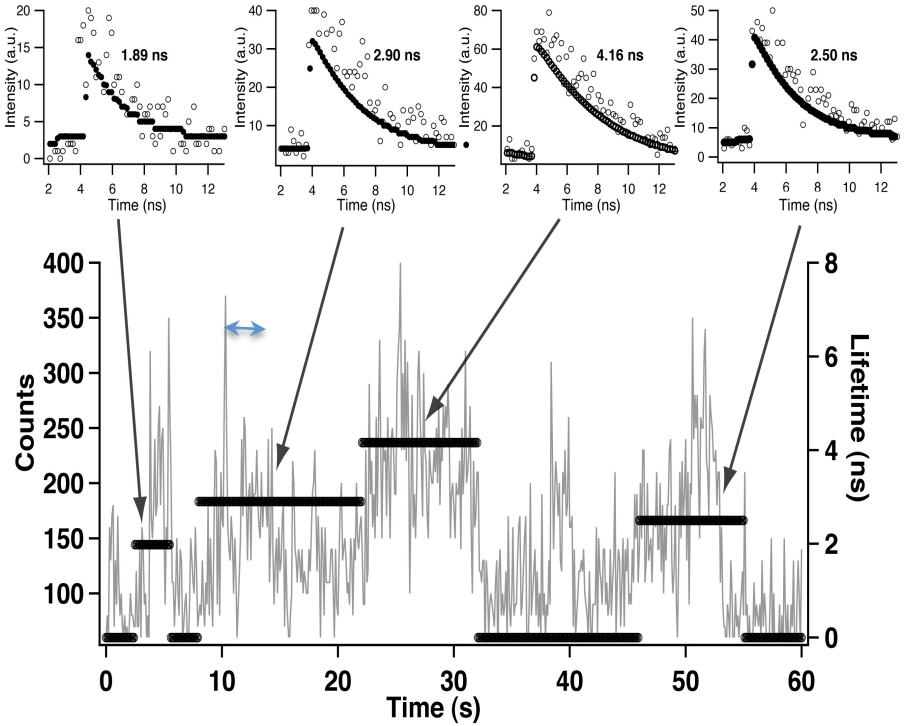
(M. Y. Odoi, J. Labastide, S. Ghosh, J. Hardy, and M. D. Barnes)

• Do neutral and protonated molecules have different fluorescence lifetimes?

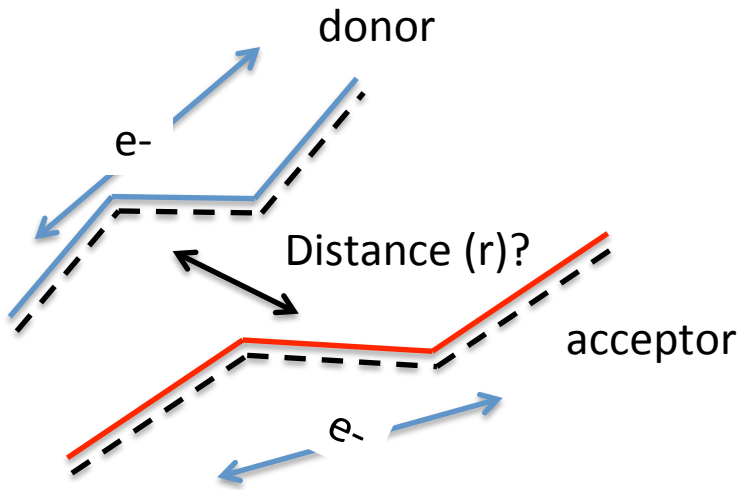




Lifetime fluctuations associated with C6 <-> C6+ interconversion

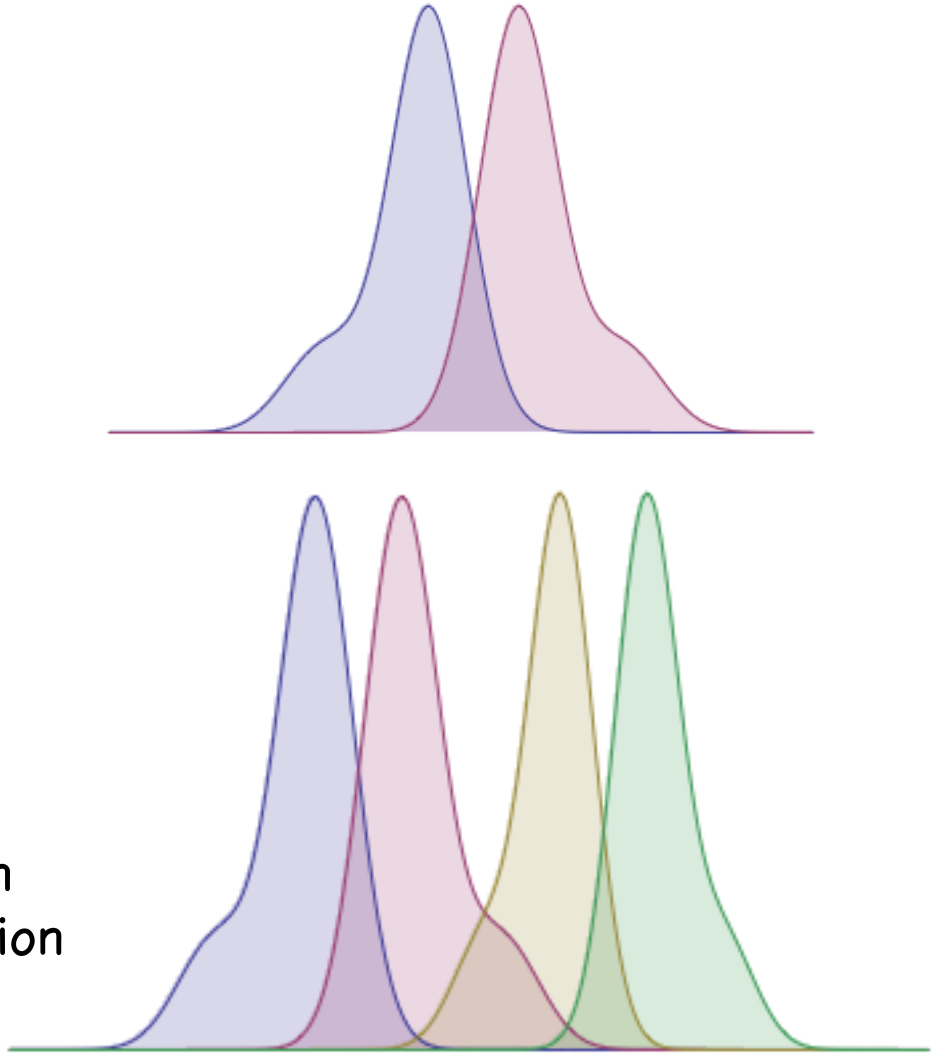


SM-FRET: Fluorescence as a molecular 'ruler'



"sloshing-induced sloshing"

Efficiency related to overlap between
Donor emission and Acceptor absorption



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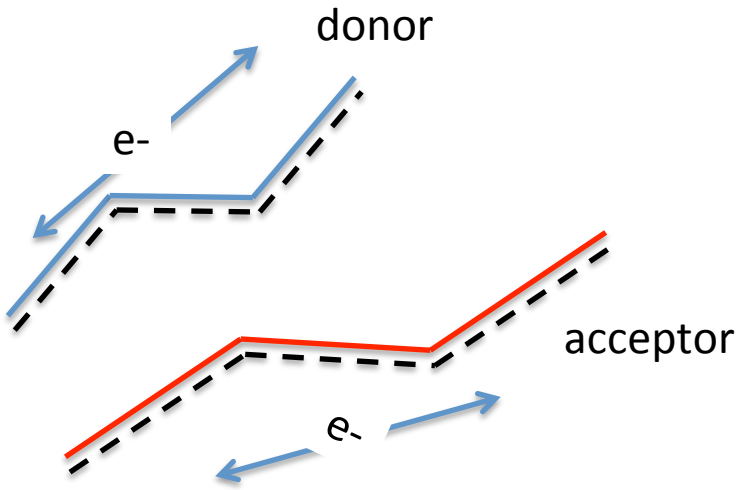


"sloshing-induced sloshing" occurs over very short distance scale

$$E(r) = \frac{1}{1 + \left(\frac{r}{R_0}\right)^6}$$

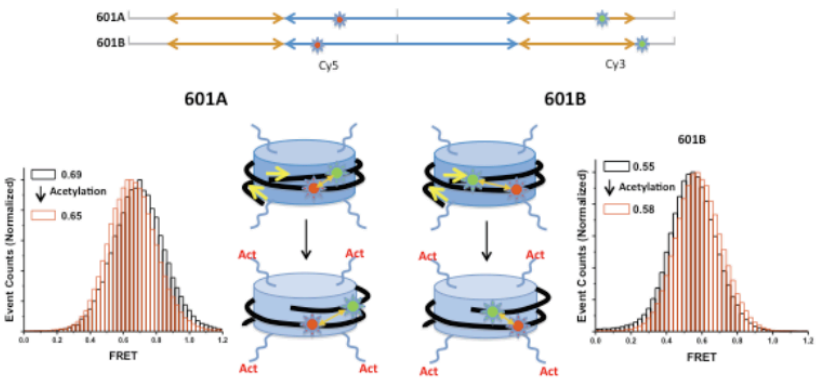
$$R_0 = C \cdot \kappa^2 \cdot \Phi_D \cdot \int I_D(\lambda)\, \varepsilon_A(\lambda) \cdot \lambda^4 d\lambda$$

(Forster Radius)

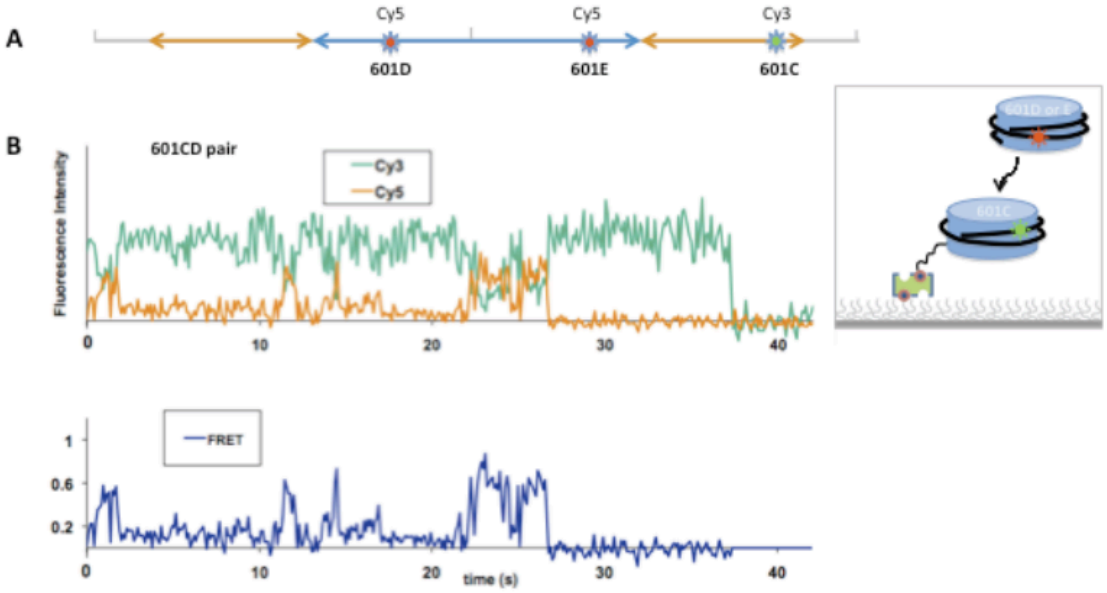
Determine r from donor/acceptor fluorescence intensities:

$$r = \left(\left(\frac{I_D}{\Phi_D} \right) \left(\frac{\Phi_A}{I_A} \right) \right)^{1/6} R_0$$

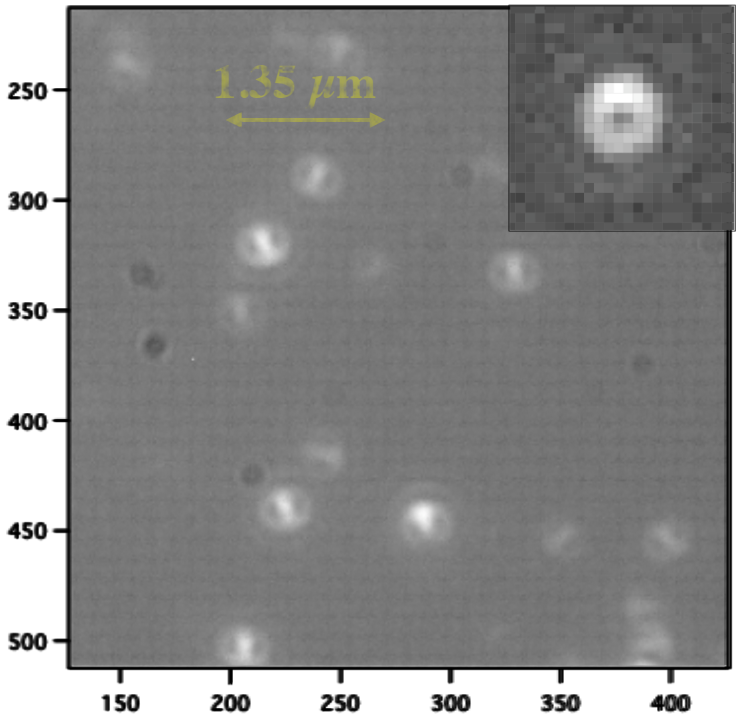
SM-FRET: Fluorescence as a molecular ‘ruler’

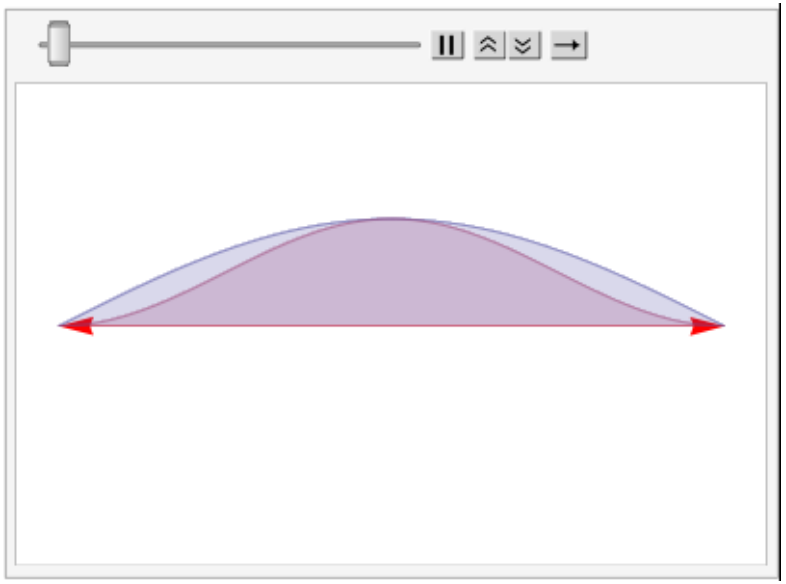


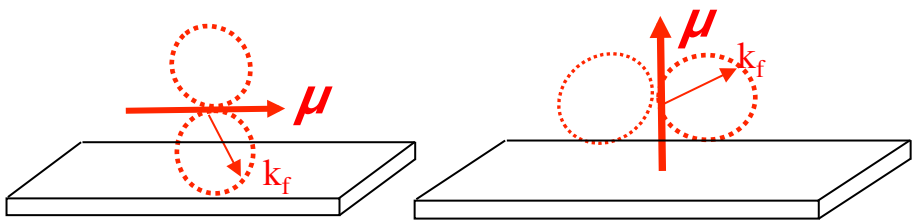
Probing structure and dynamics of nucleosomes upon DNA methylation usind sm-FRET (T-H. Lee, PSU Chemistry)



Charge 'sloshing' and polarization properties of single molecules







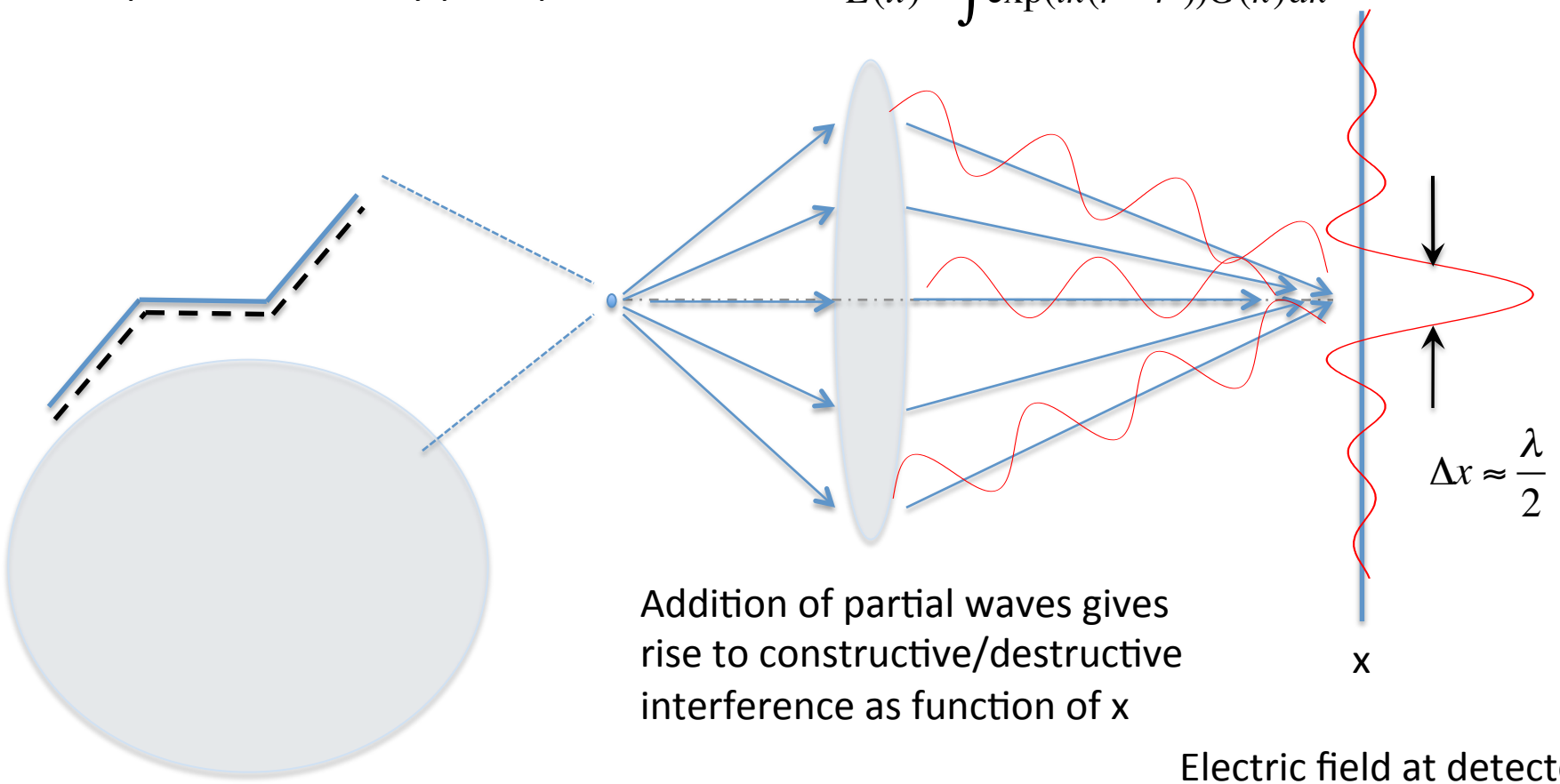
- "sloshing" of charge density in 1D gives rise to antenna behavior for single molecules!

- Patterns are *distinct for linear dipoles (randomly oriented in x-y) -> extract molecular orientation (and dynamics!) from single molecules*

Why doesn't a single-molecule fluorescence image look like a molecule???

Optical uncertainty principle:

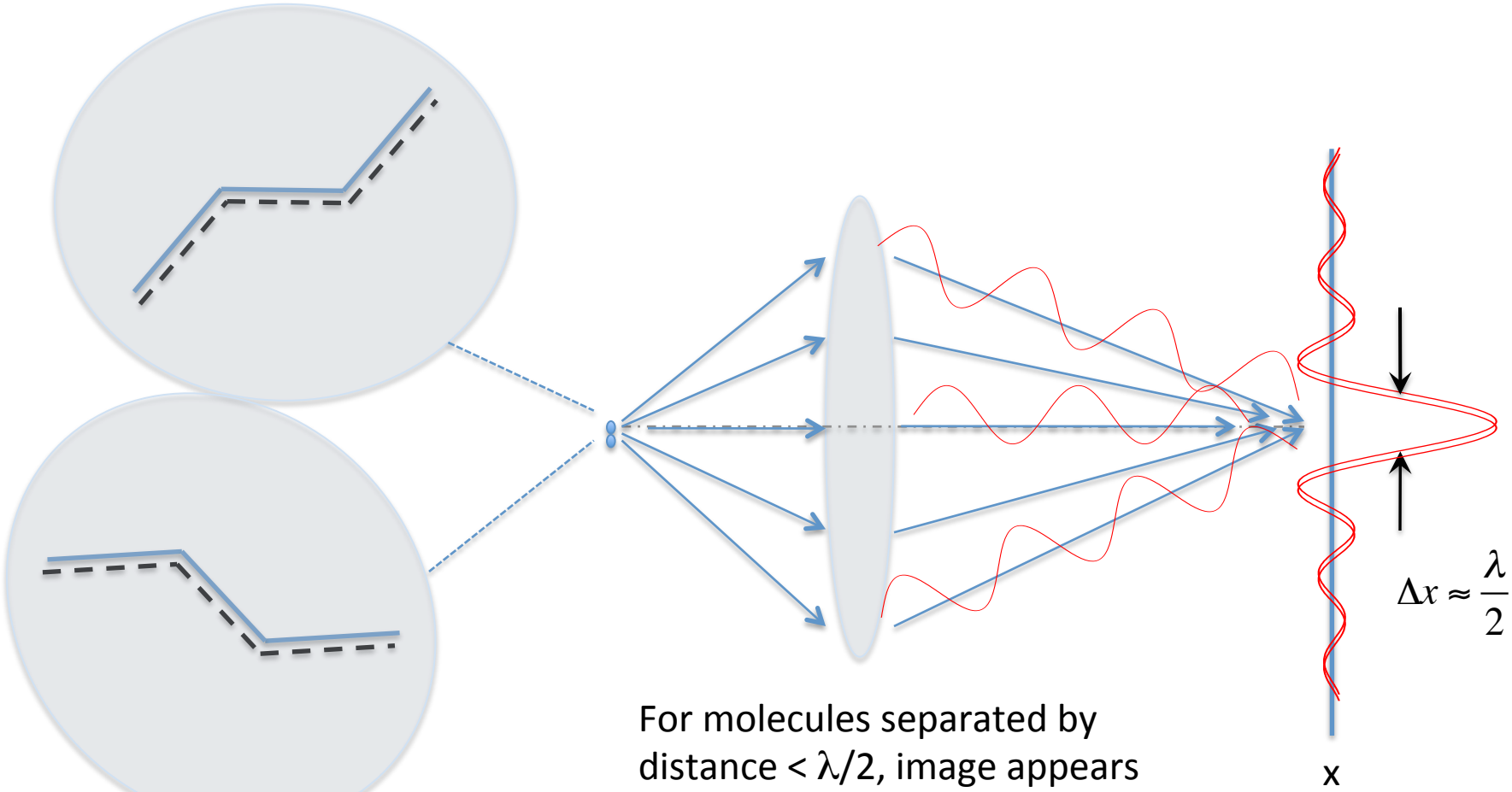
$$E(x) = \int \exp(ik(r - r'))G(k)\,dk$$



Addition of partial waves gives rise to constructive/destructive interference as function of x

Electric field at detector

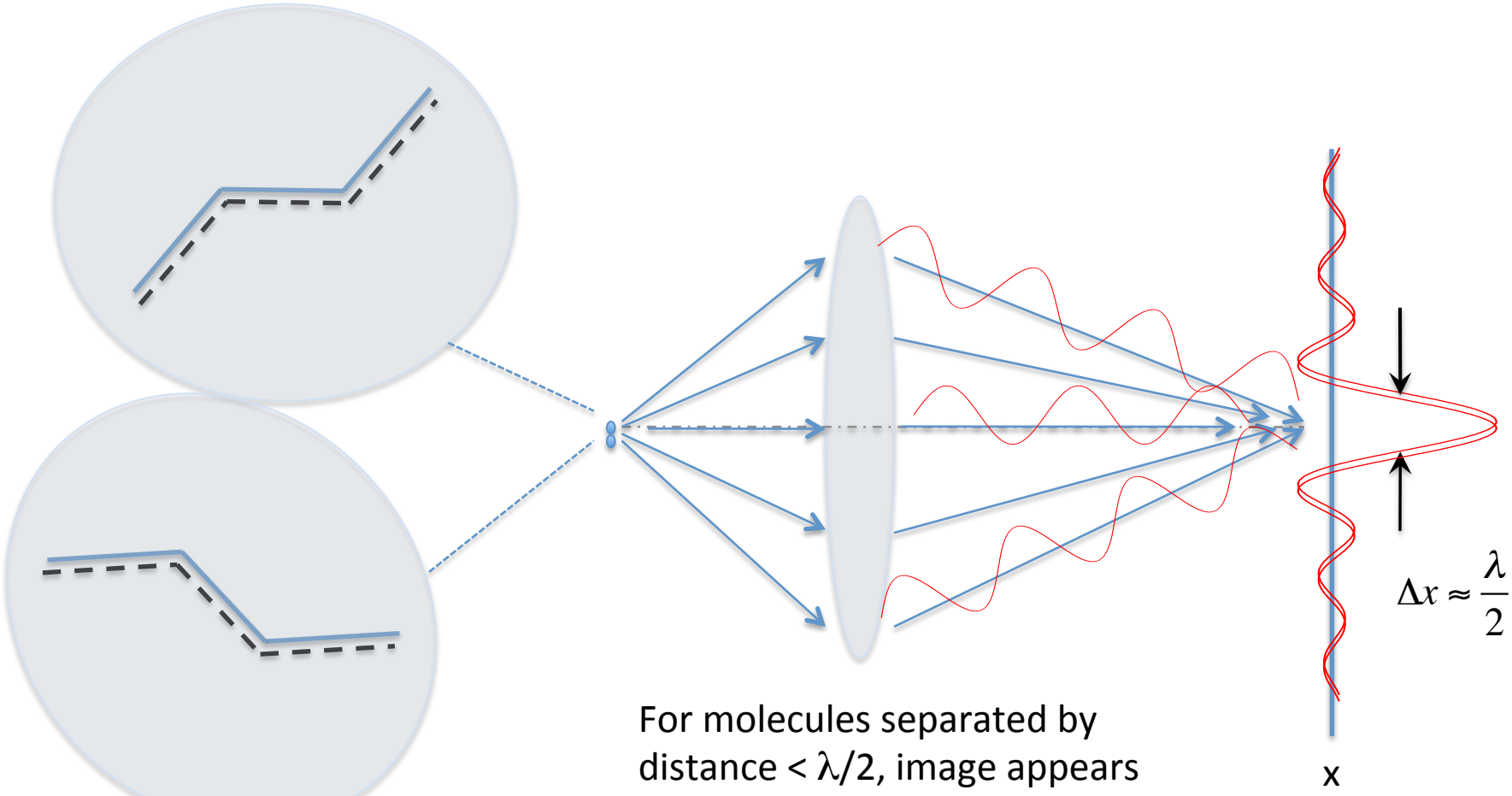
Spatial resolution in optical imaging of SM's



For molecules separated by distance < λ/2, image appears like one molecule

Electric field at detector adds incoherently for different molecules

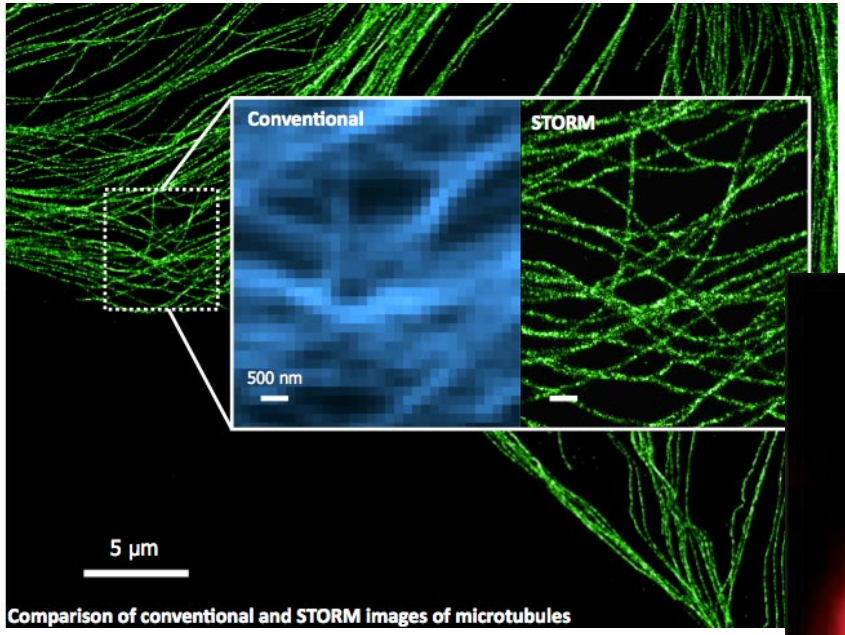
How could you tell if you had "one" or "two"?



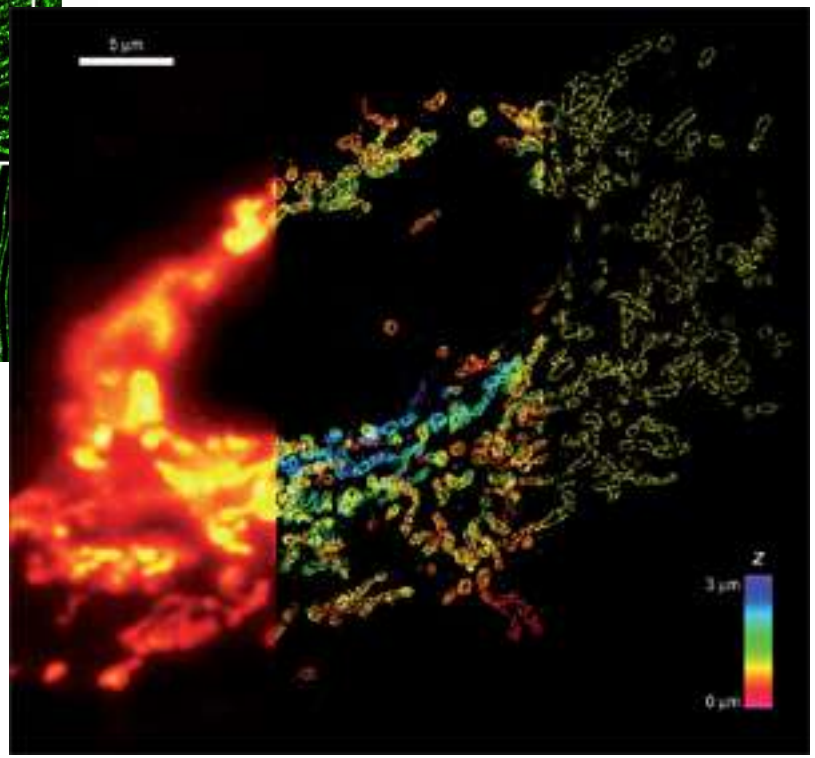
For molecules separated by distance < λ/2, image appears like one molecule

Electric field at detector adds incoherently for different molecules

STORM super-resolution images of biological structures

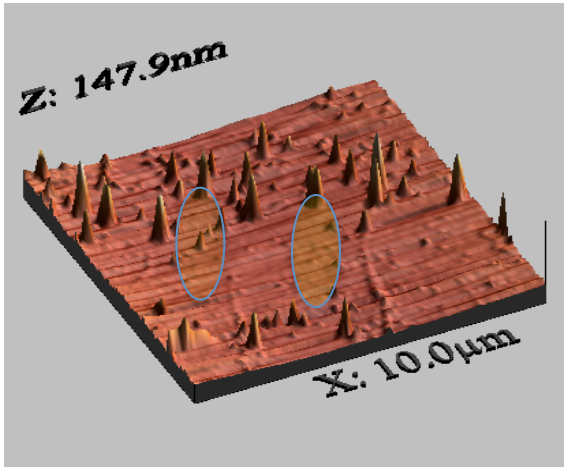


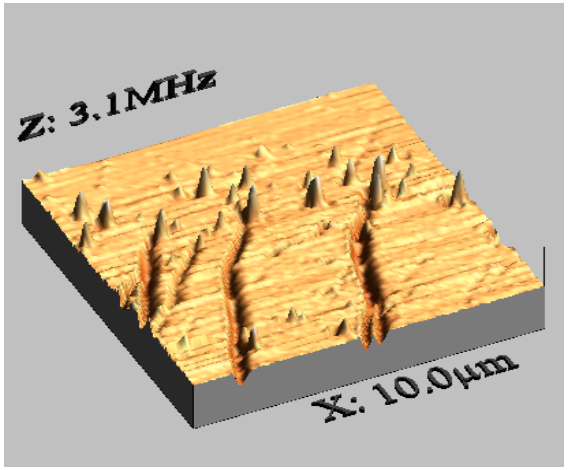
Comparison of conventional and STORM images of microtubules

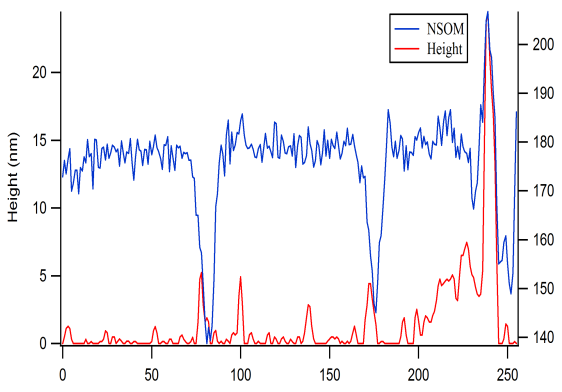


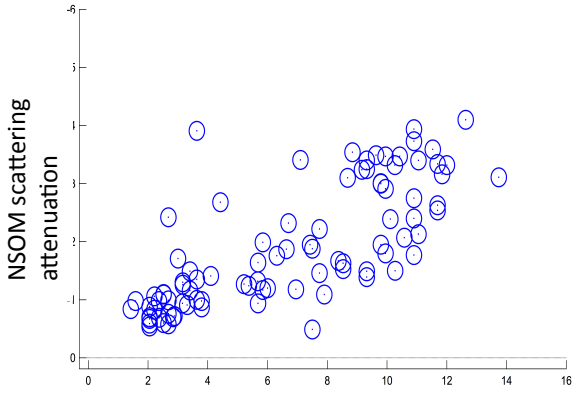
Near-field optical probes of conjugated polymer nanoparticle thin-film structure

Near-field *absorption* probes of nonluminescent polymer nanostructures









- Scan near-field excitation (532 nm), detect Rayleigh scattering (180°) in far-field
- Absorption is signaled by 'dip' in scattering intensity – nanoparticles show enhanced scattering and absorption; fibrils appear to show only absorption
- Scattering attenuation scales linearly with surface height
- Currently developing multiple-wavelength scanning capability to probe local differences in structure (i.e. amorphous vs. crystalline) or chemical composition

M. Baghgar, et al. to be published

Collaborators: Hayward, Emrick