Today's Announcements

1. HW due Wednesday, 4/4/12.
2. 1st discussion about Individual Projects.
   - Due next Monday: Res. Article + Gen Art. + ½ pg discussion.
3. Last ½ hr: A tour of my lab.

Today's take-home lessons

(i.e. what you should be able to answer at end of lecture)

1. How to do a Physics 498Bio Individual topic.
2. Accuracy and Resolution—what are they.
3. Total Internal Reflection Fluorescence (TIRF)- near surface; confocal.
4. STORM, PALM, STED.
5. 1-Photon vs. 2-Photon microscopy.

How to go about finding research article

Idea: what it takes to understand an original research article

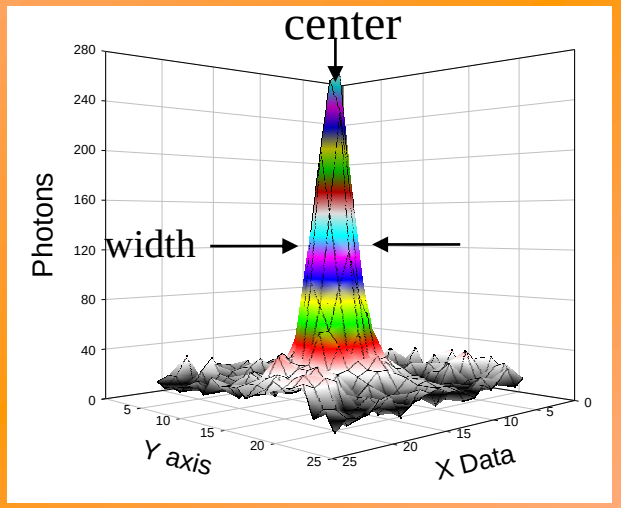
Good places to start: Library on course web site, Google, Biology/Biochemistry textbook.

1. I “ask” : how does molecular motors move? i.e. hand-over-hand vs. inchworm?
2. Find Yildiz et al., Science, 2003: primary research article.
3. I need to understand myosin V vs. other molecular motors.
    1. Find general/review article on molecular motors.– review article cited (e.g. Vale, Science, 2002; Veigel, Nat. Cell Bio, 2002). Google.
    2. Molecular motor chapter in general Biology/Biochemistry textbook.

Super-Accuracy
(Accuracy << 250 nm: 1.5 nm, 1-500 ms)

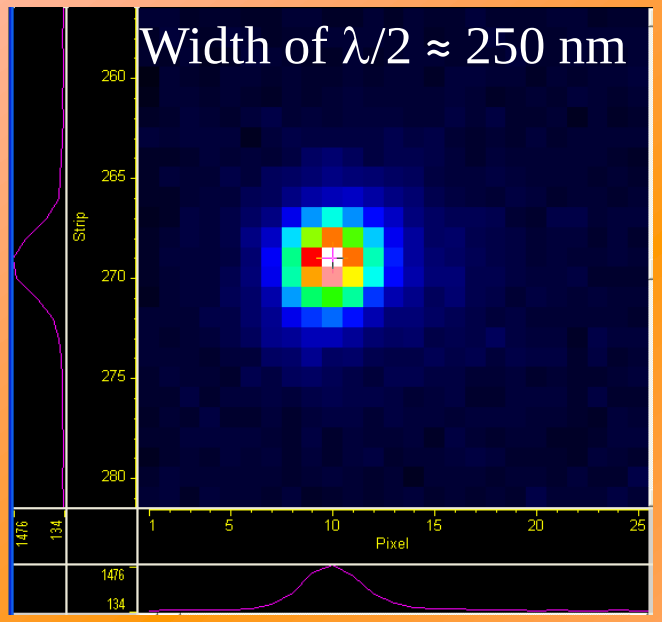
FIONA

Fluorescence **I**maging with **O**ne **N**anometer **A**ccuracy



Center can be found much more accurately than width

S/N ≈ width /√N ≈ 250/√10^4 ≈ 1.3 nm

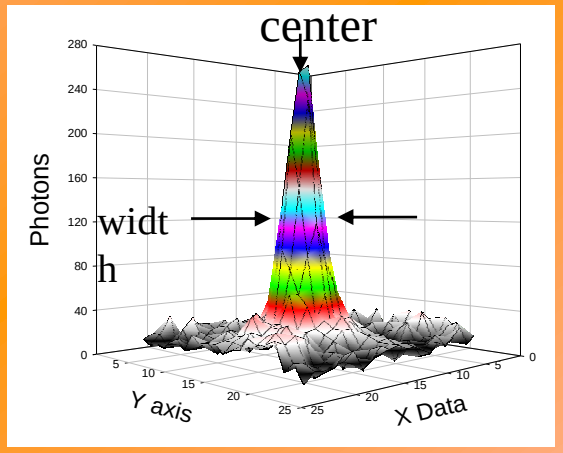


Super-Accuracy: Nanometer Distances

FIONA

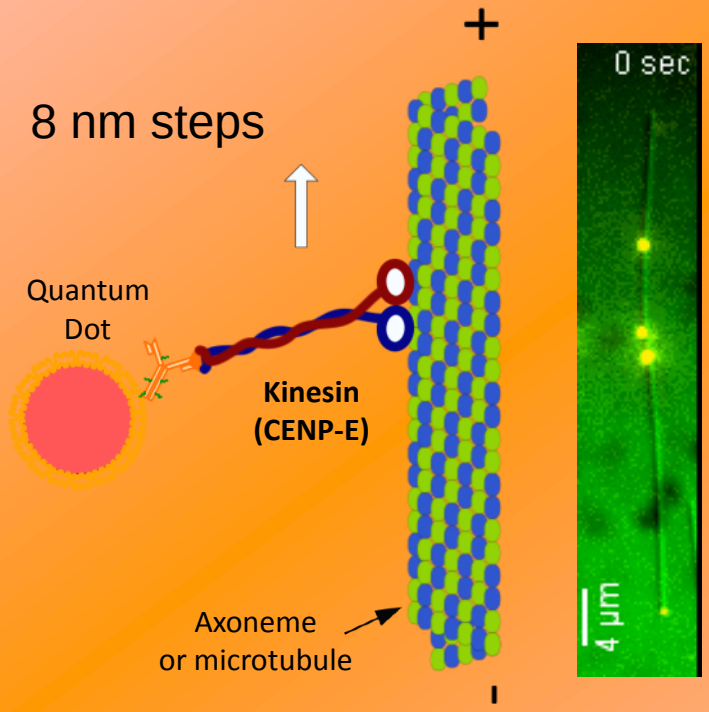
Fluorescence **I**maging with **O**ne **N**anometer **A**ccuracy

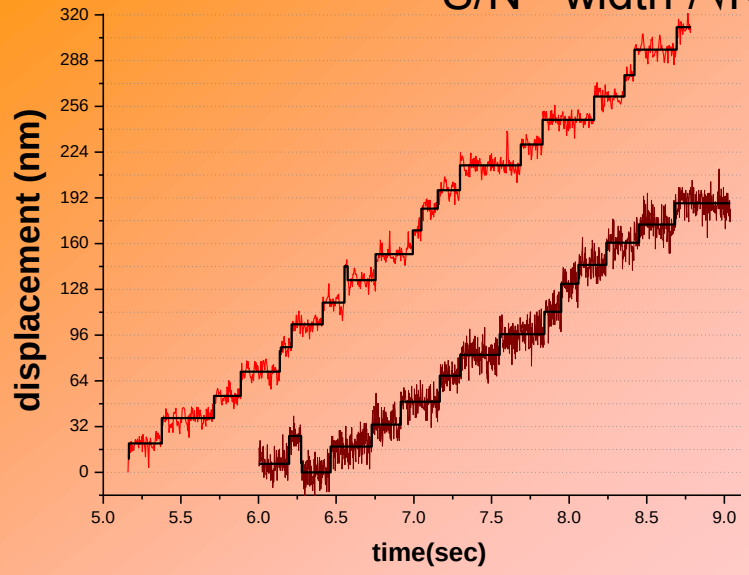
Very good accuracy:
1.5 nm, 1-500 msec



Center can be found much more accurately than width

S/N= width /√N





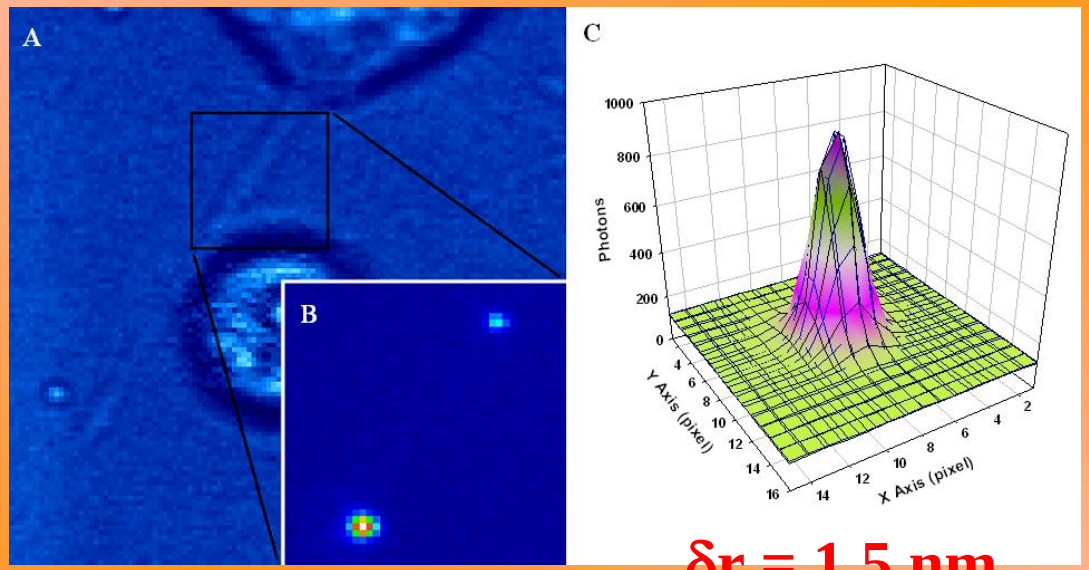
We have great x-y accuracy *in vitro* with fluorescent dyes and quantum dots…

Can we get this accuracy *in vivo*?

Yes…in Drosophilia cells,

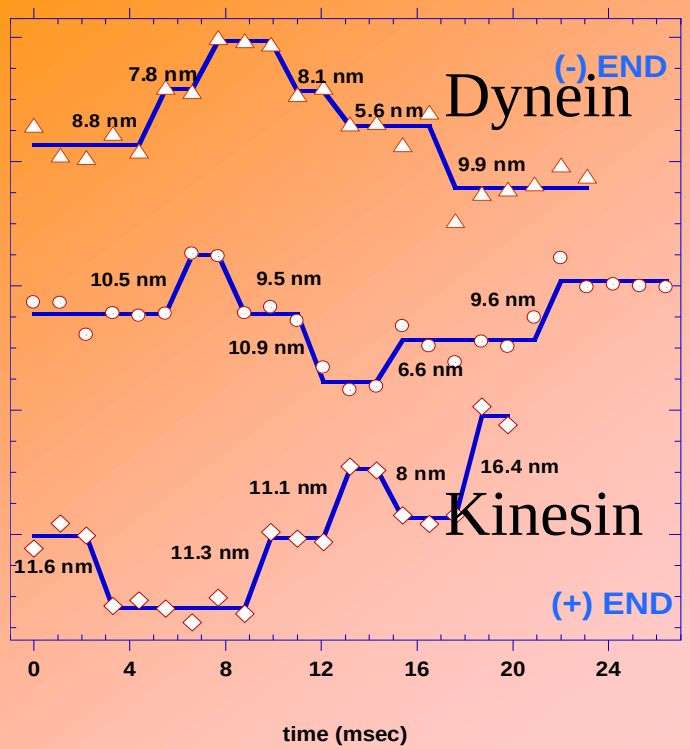
individual kinesin & dynein moving cooperatively

(Kural, Science, 2005)



$\delta r = 1.5$ nm

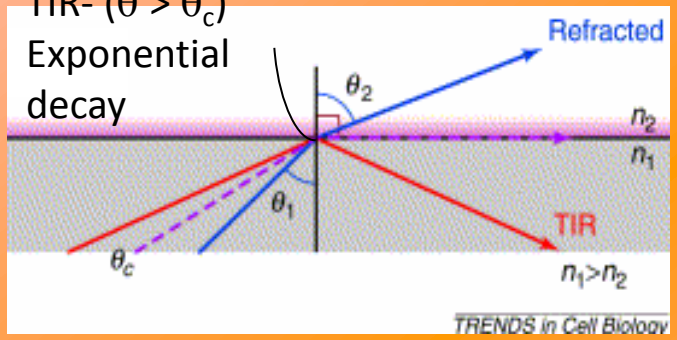
$\delta t = 1.1$ msec

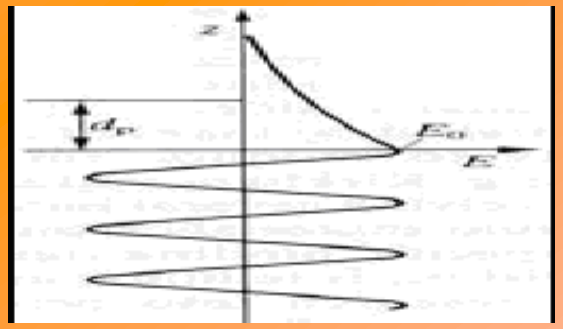


Imaging (Single Molecules) with very good S/N
(at the cost of seeing only a thin section very near the surface)

Total Internal Reflection (TIR) Microscopy

TIR- ($\theta > \theta_c$)
Exponential
decay





$$d_p = (\lambda/4\pi)[n_1^2 \sin^2\theta_i) - n^2_2]^{-1/2}$$

For glass (n=1.5), water (n=1.33):

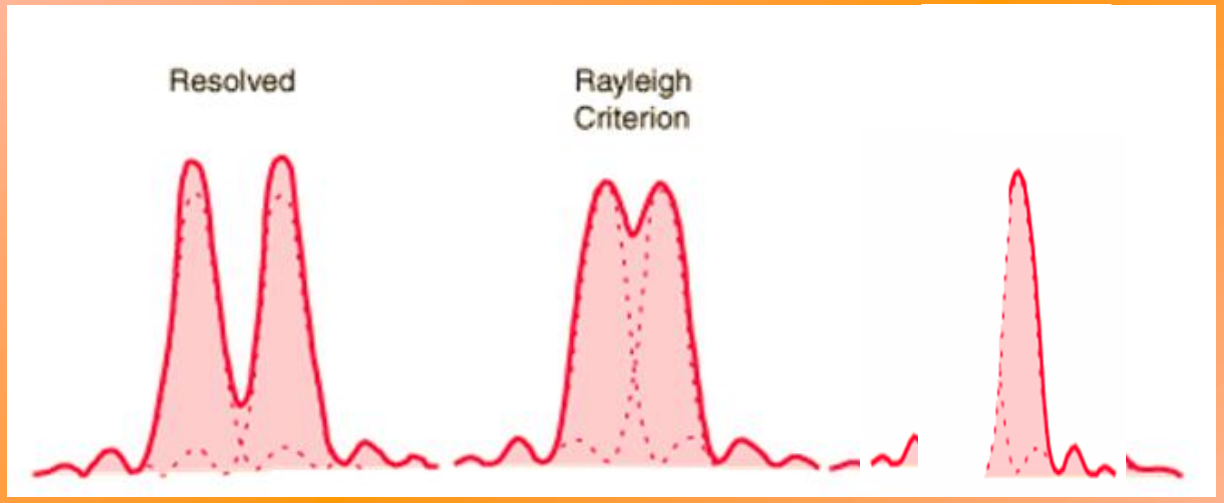
TIR angle = >57°

Penetration depth = d_p = 58 nm

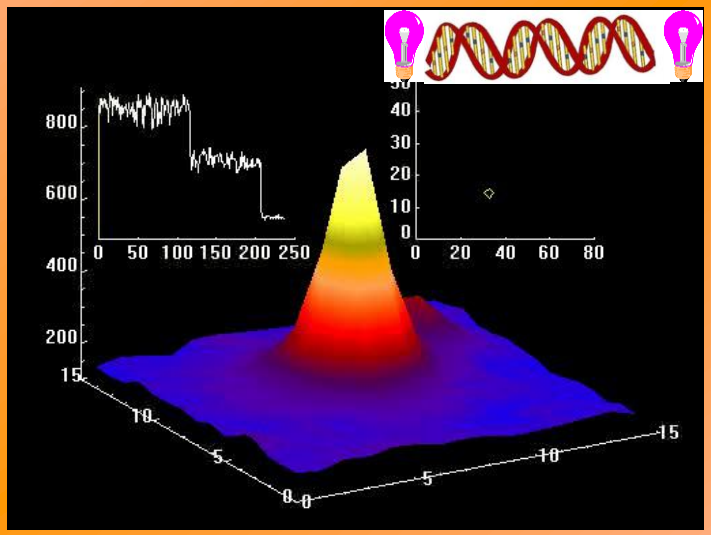
With d_p = 58 nm , can excite sample and not much background.

Super-Resolution:

Nanometer Distances between two (or more) dyes



SHRImP Super High Resolution IMaging with Photobleaching



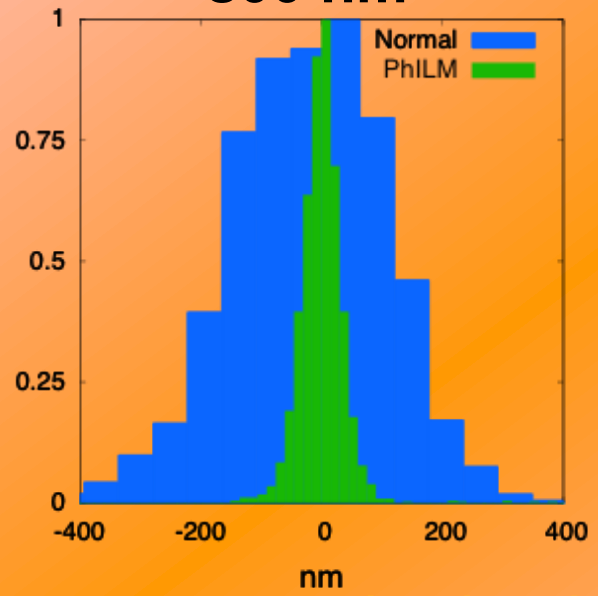
Distance can be found much more accurately than width (250 nm)
Resolution now:
Between 2-5 molecules: <10 nm
(Gordon et al.; Qu et al, PNAS, 2004)

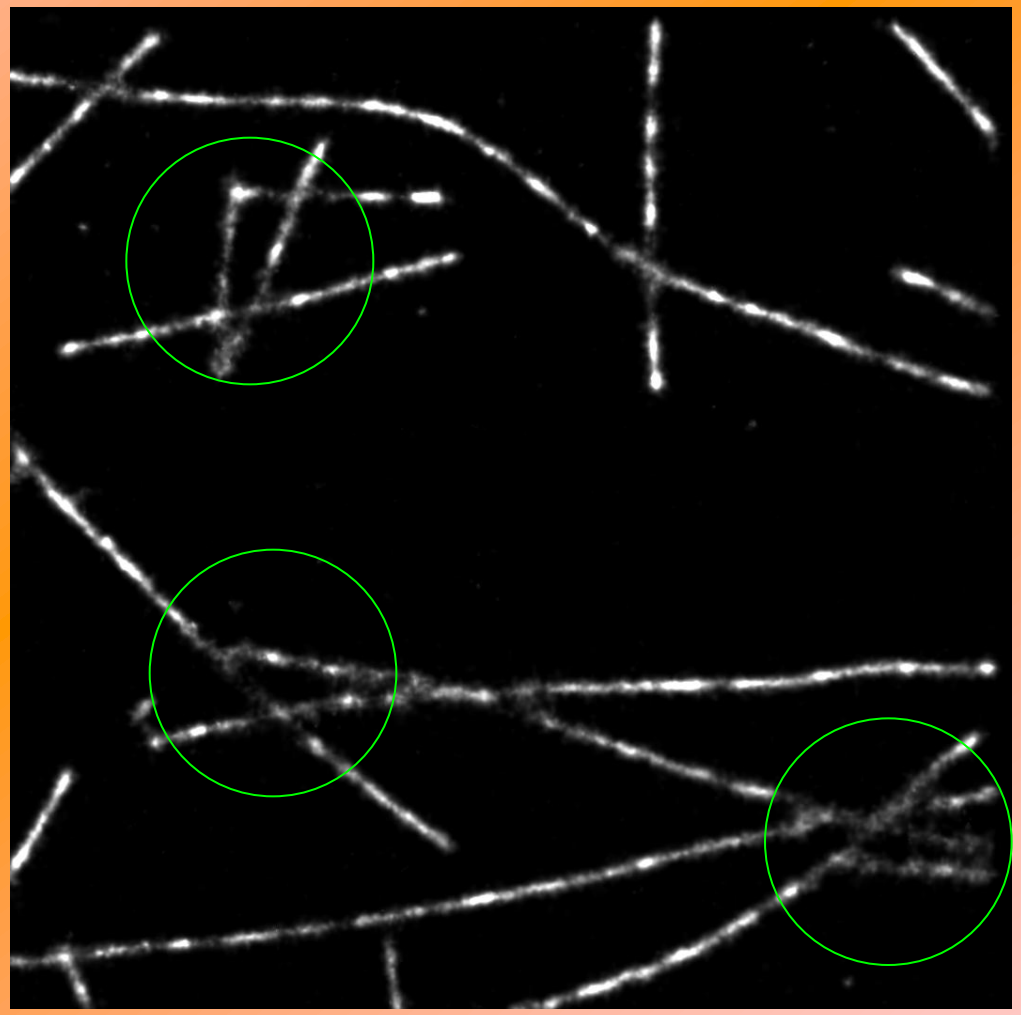
Next slides
gSHRIMP: > 5-40 molecules
~ 20-100 nm
Via 2-photon: ~ 35 nm (next time)

Regular Microtubules (In vitro) Image

- Take regular Image.
- Then one fluorophore photobleaches.
- Subtract off, get high resolution, repeat.

Imaging resolution 300 nm

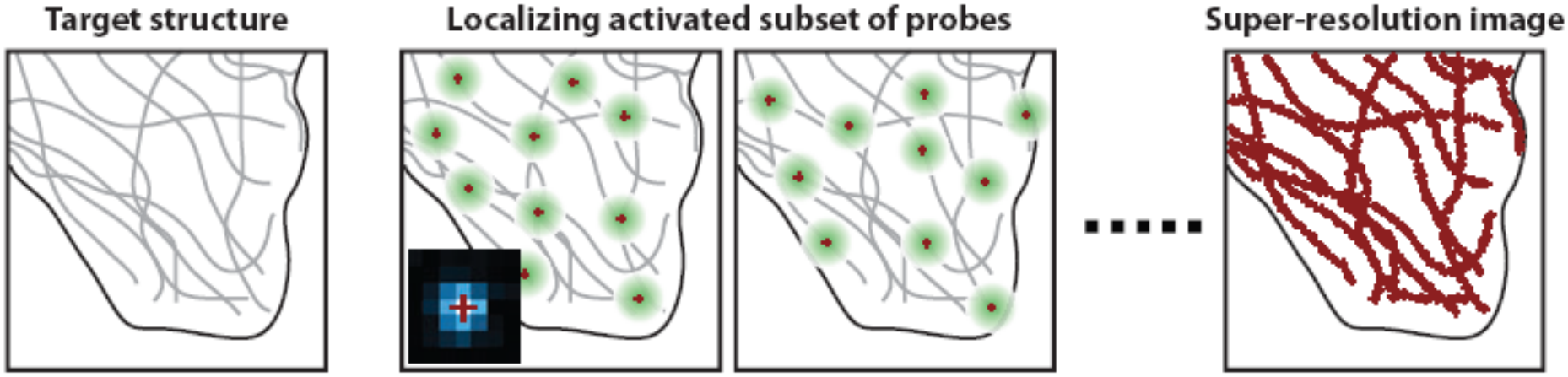




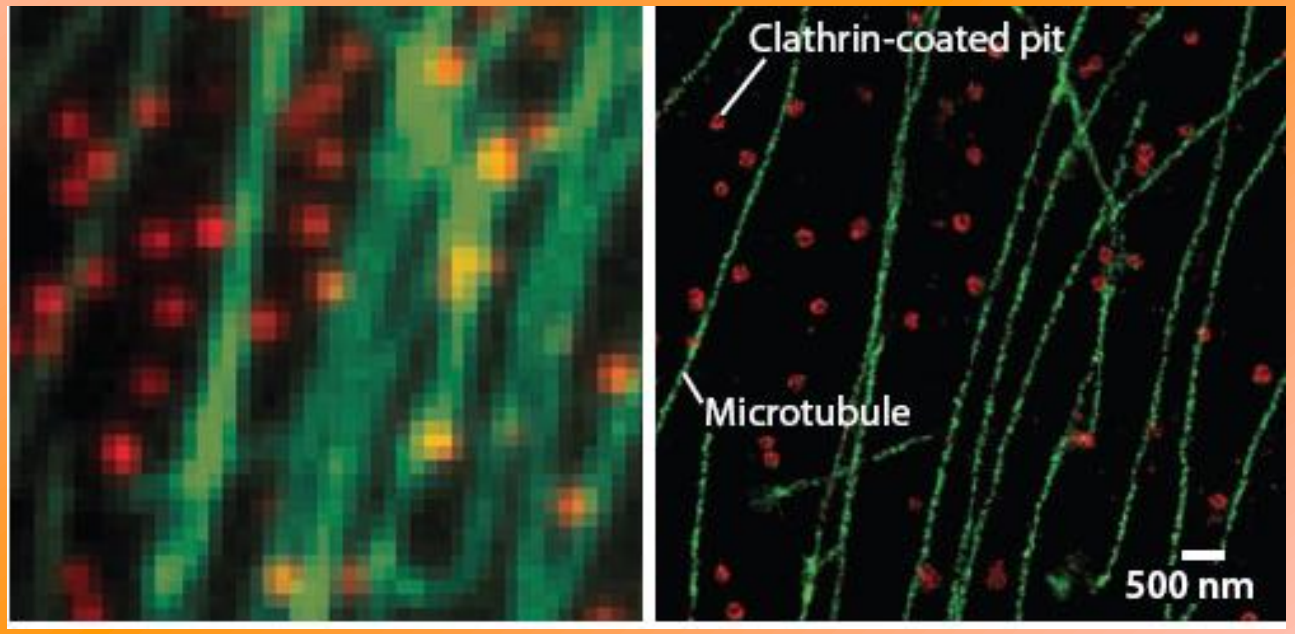
Rhodamine-labeled microtubules, TIR

Actual 24 nm; Measured 60 nm

Most Super-Resolution Microscopy Inherently a single-molecule technique



Huang, Annu. Rev. Biochem, 2009



STORM

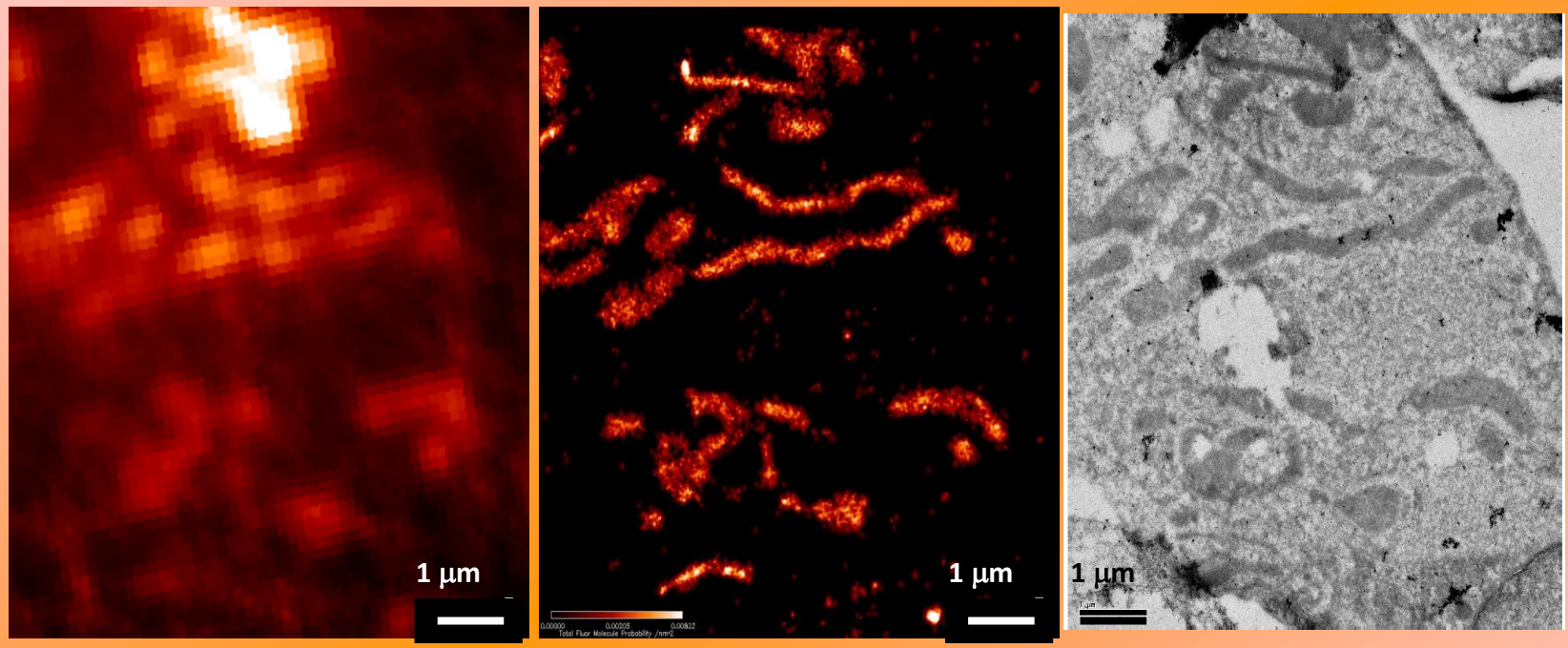
STochastic Optical Reconstruction Microscopy

PALM

PhotoActivation Localization Microscopy (Photoactivatable GFP)

Bates, 2007 Science

PhotoActivation Localization Microscopy (F)PALM (Photoactivatable GFP)



TIRF PALM TEM

Mitochondrial targeting sequence tagged with mEOS

Patterson et al., Science 2002