Today's Announcements

1. HW due Wednesday, 4/11/12.
2. Due next Monday: Res. Article + Gen Art. + ½ pg discussion.

Today's take-home lessons

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

1. How to get 3D resolution: confocal.
2. STED.
3. 1-Photon vs. 2-Photon microscopy.
4. Fluorescence Polarization.

How to go about finding research article

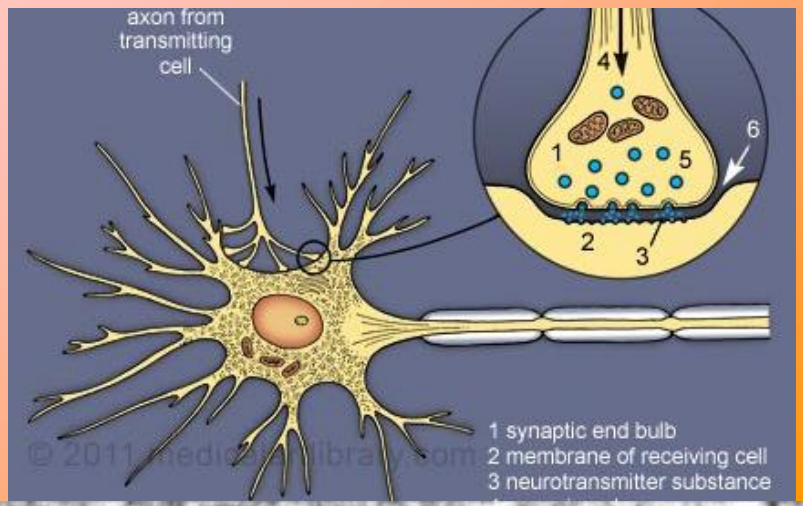
Idea: what it takes to understand an original research article

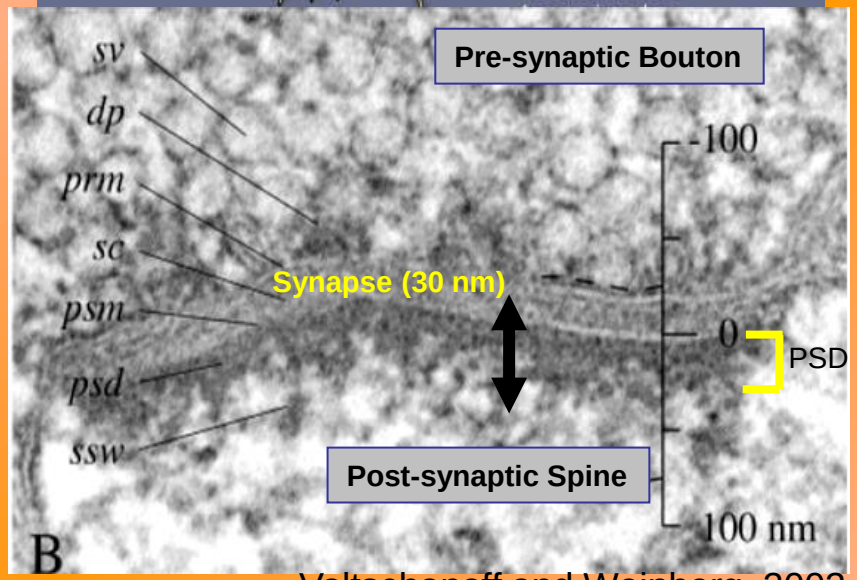
Good places to start: Library on course web site, Google, Biology/Biochemistry textbook.

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.

Your Brain: 100 billion Nerves; 100 trillion Synapses

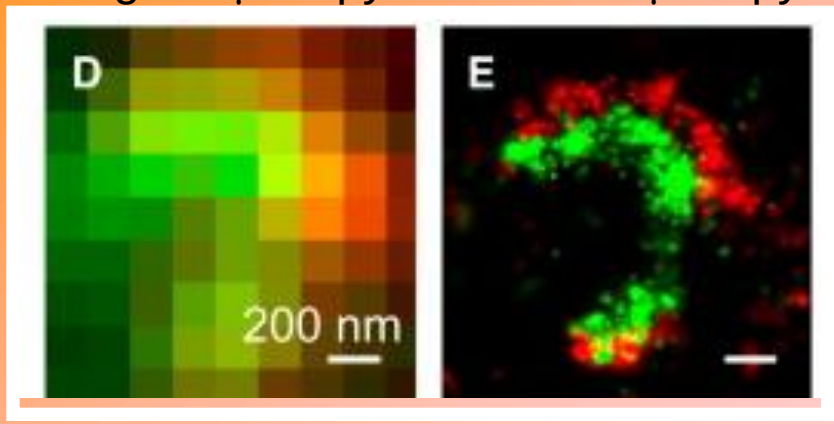
How you remember, learn; effect of stroke





Valtschanoff and Weinberg, 2003

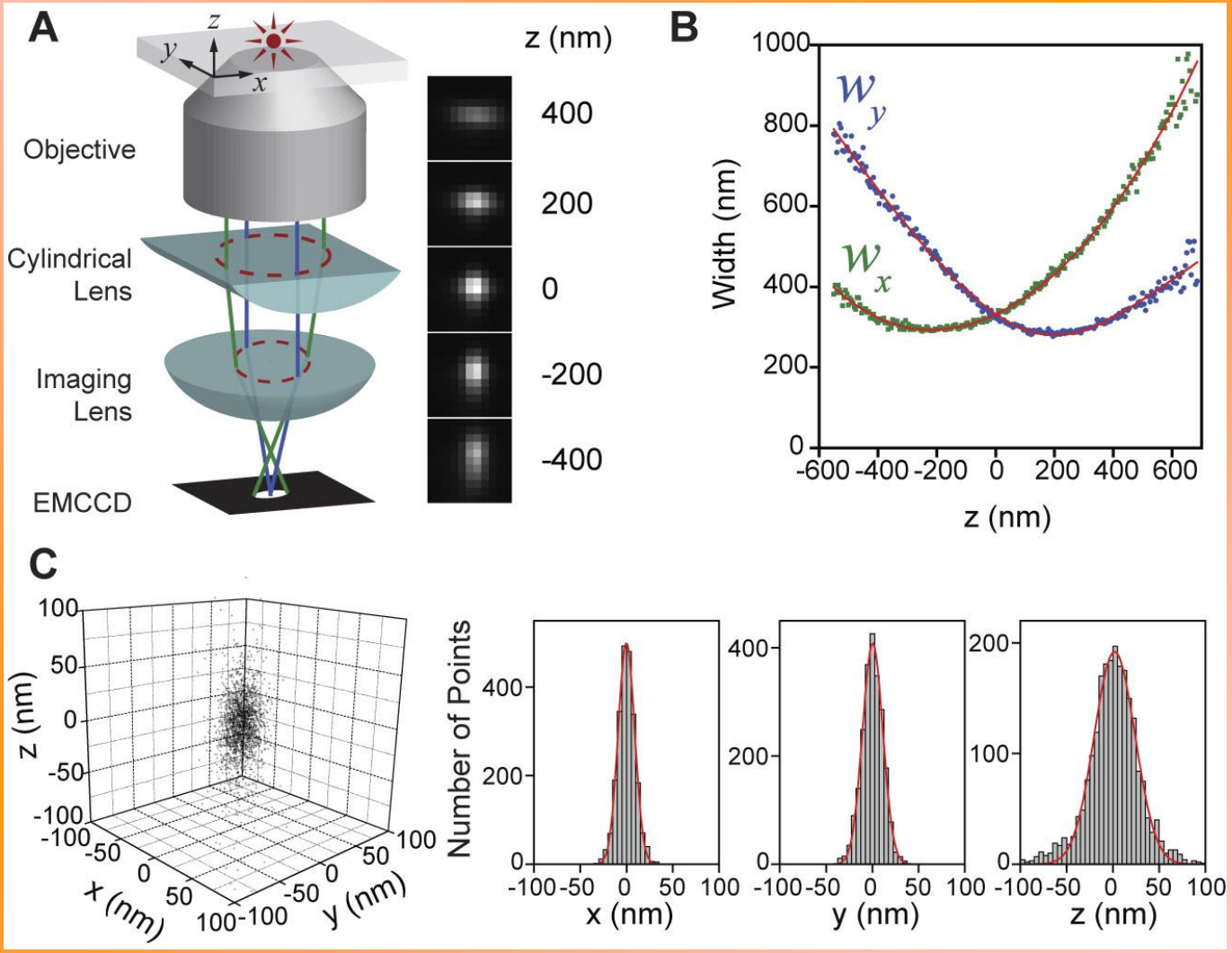
Regular μscopy STORM μscopy



Zhuang, Neuron, 2010

Need 3-D fluorescence, small probes, photostable, live cells

3-D (z) resolution

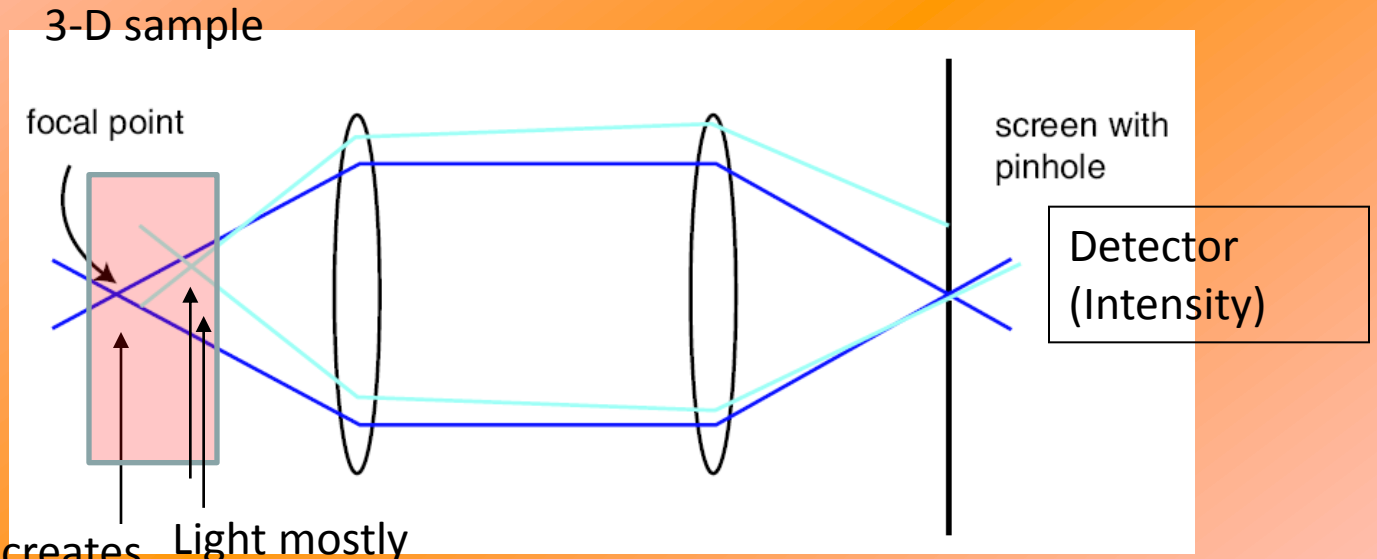


(Regular microscopy) Confocal Detection

Sample is 3-D. Detectors are 2-D.

How do you get z-axis sectioning with Microscopy?

A pinhole allows only in-focus light through



Focused Light creates fluorescence which gets to detector

Light mostly gets rejected

Smaller the pinhole, better out-of-focus discrimination but lose more signal.

Scan sample in x, y, z and reconstruct entire image

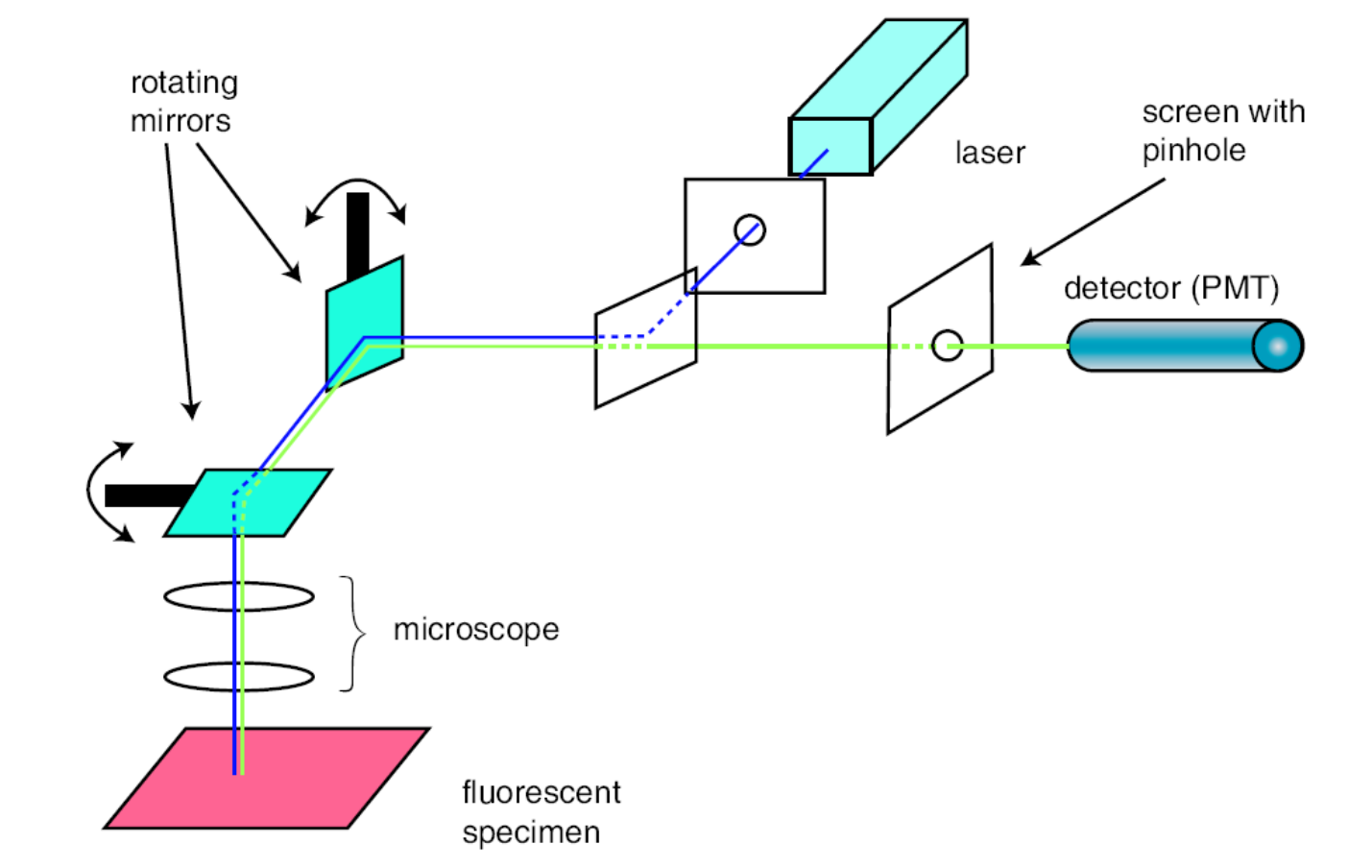
Confocal Microscopy

Lots of different ways of arranging to get fast scanning:

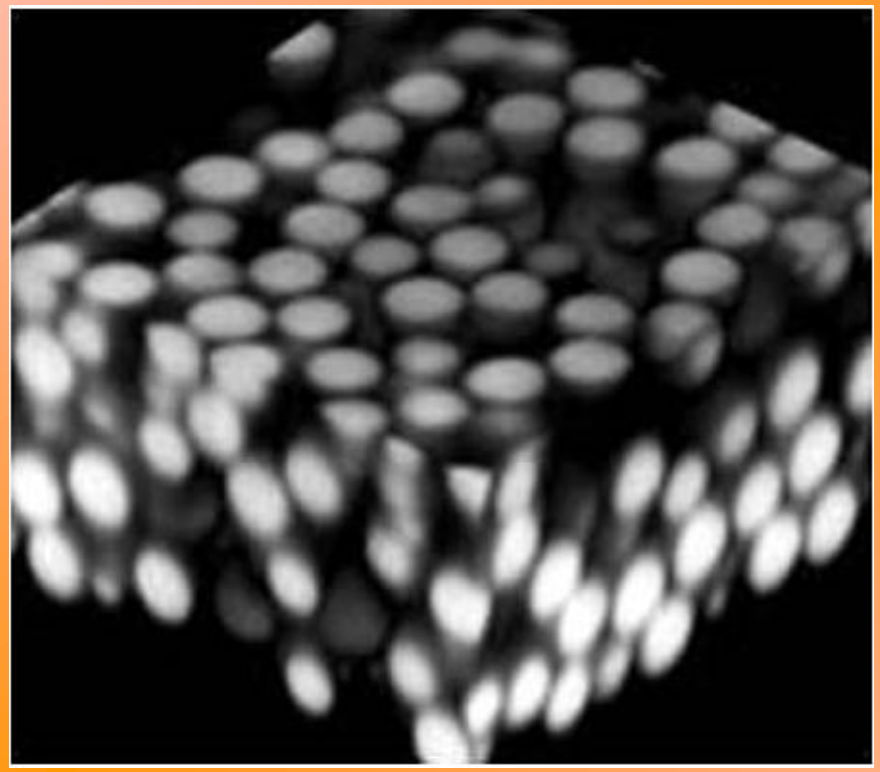
Moveable mirrors –moveable spot excitation

(only have to move sample in z-direction)

Other more sophisticated methods… Nipow disk



3-D sectioning with Confocal



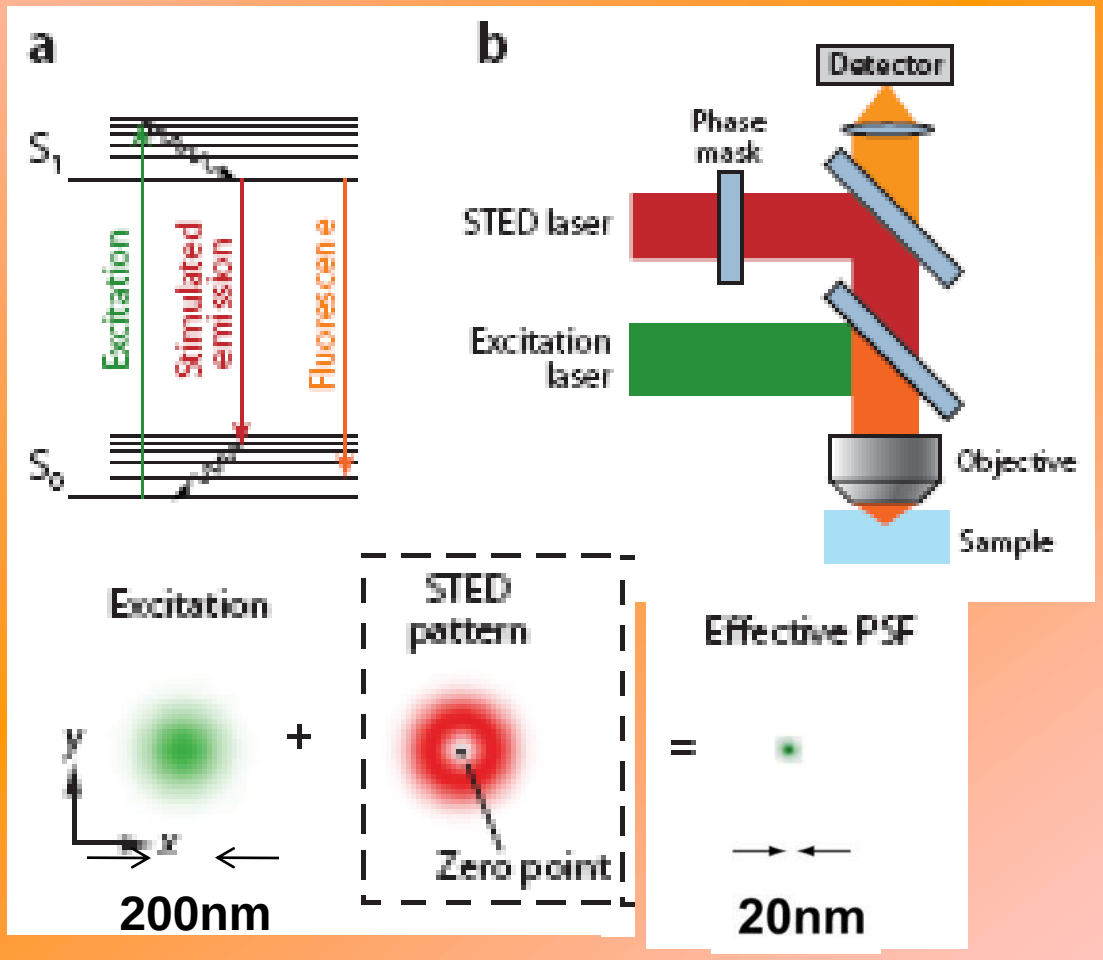
Three-dimensional reconstruction of a series of 2D images of PMMA spheres

STimulated Emission Depletion (STED)

Recent development in super-resolution microscopy S. Hell

Net result is a smaller Point Spread Function

Sharpen the fluorescence focal spot is to selectively inhibit the fluorescence at its outer part.

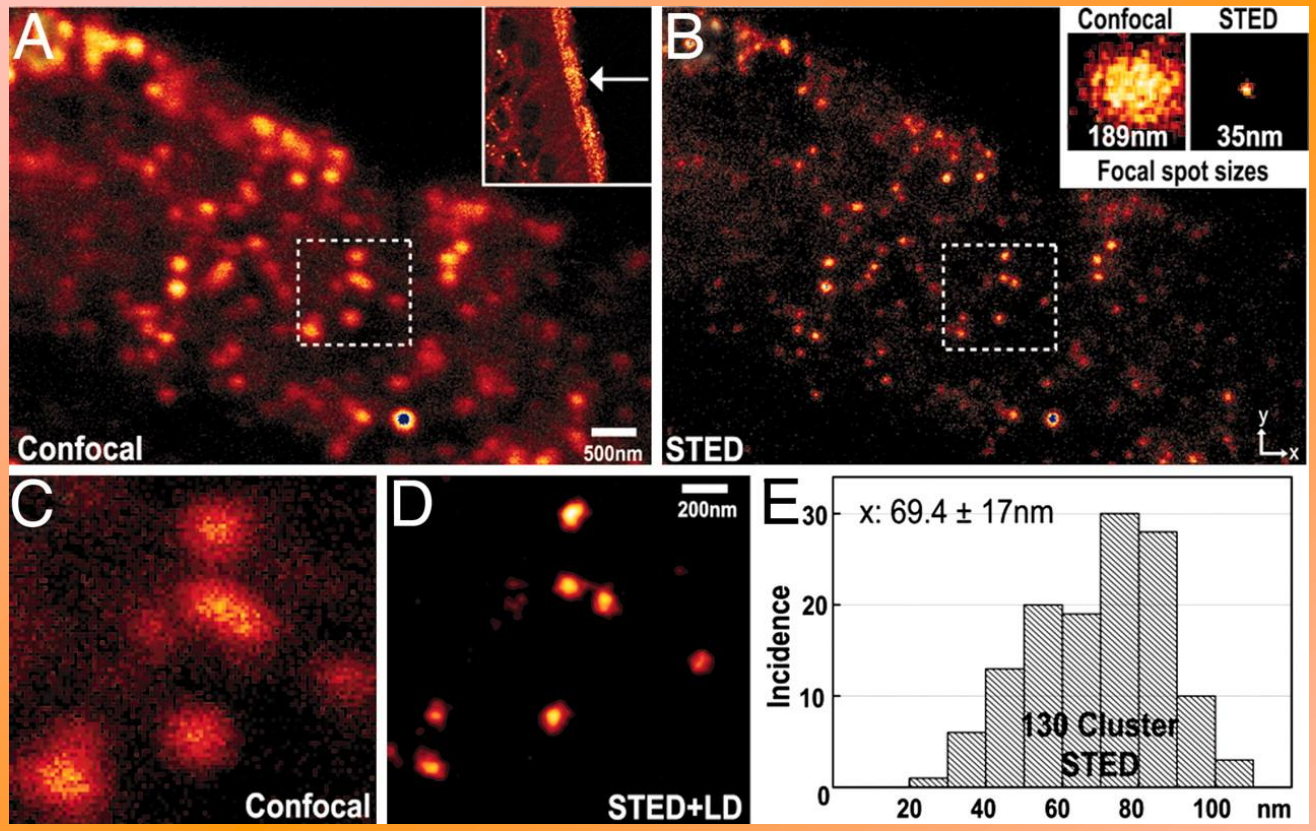


Huang, Annu. Rev. Biochem, 2009

http://www.mpibpc.gwdg.de/groups/hell/

Biological Example of STED

The transient receptor potential channel M5



Analysis of spot size for **Confocal** (*A*) and **STED** (*B*) images of TRPM5 immunofluorescence layer of the olfactory epithelium. (*A, C Inset*) Confocal image at a lower (higher; box) magnification taken with a confocal microscope. (*B*) STED image. Effective point-spread function in the **confocal (189 nm)** and **STED (35 nm)** imaging modes.

Hell, PNAS, 2007

Two-Photon Microscopy

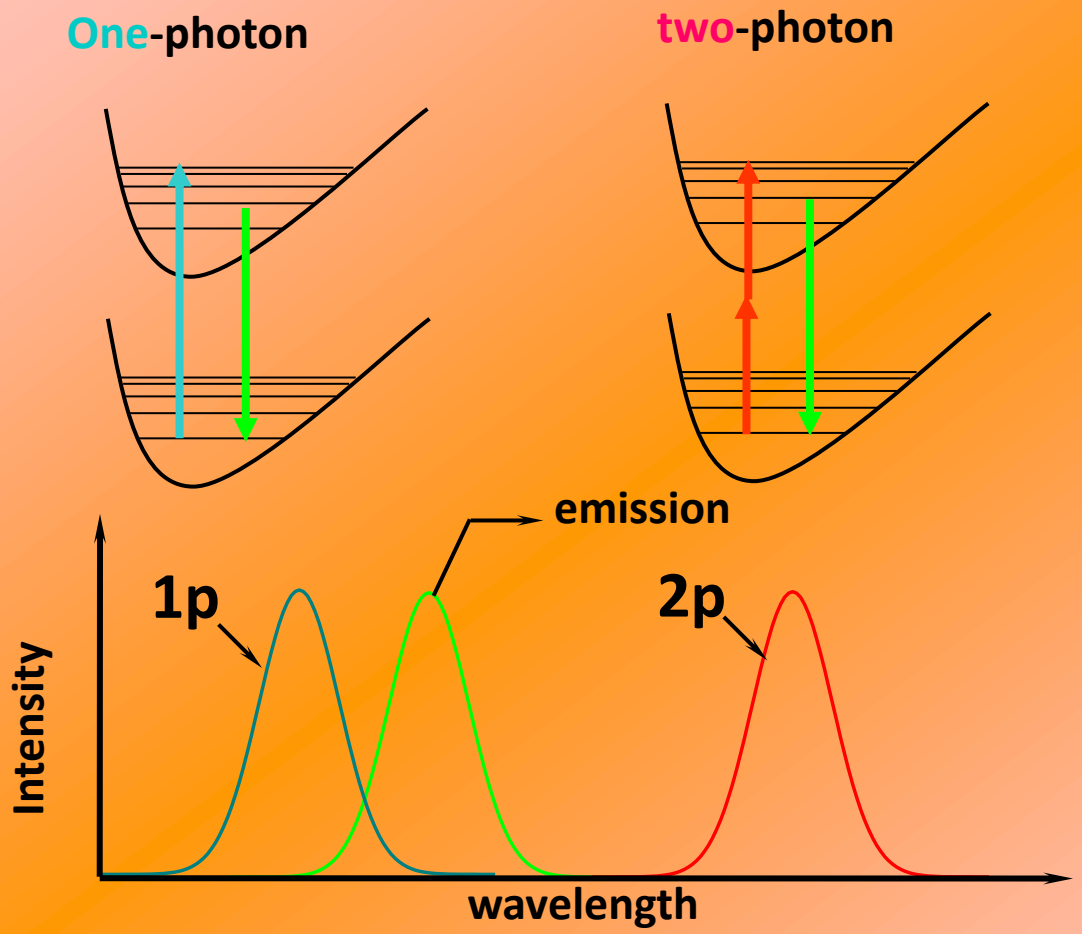
(Watt Webb, Science, 2003)

You get automatic confocal detection with 2-photon microscopy

…plus other advantages

Two-Photon Microscopy

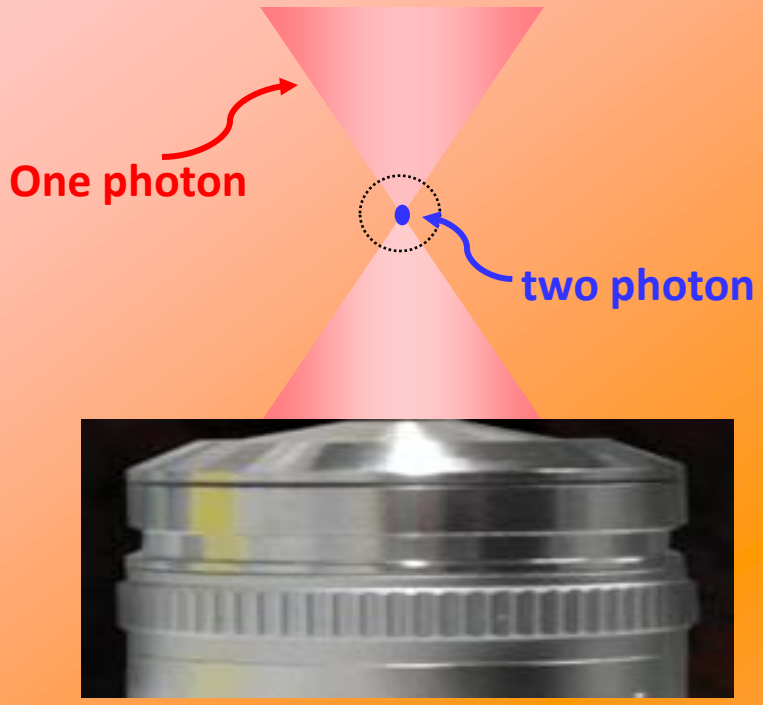
Inherently confocal, long wavelength (less scattering)



Simultaneous absorption of two photons

Reasonable power if use pulsed laser

(Dis-)Advantages of 2-Photon Excitation



Inherent spatial (z-) resolution

Low light scattering (scattering like λ^{-4})

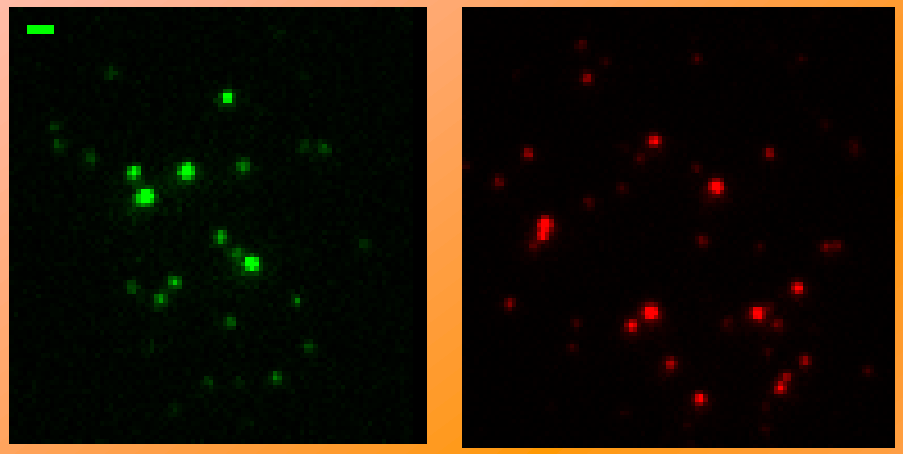
Single-color excitation with multiple emission colors

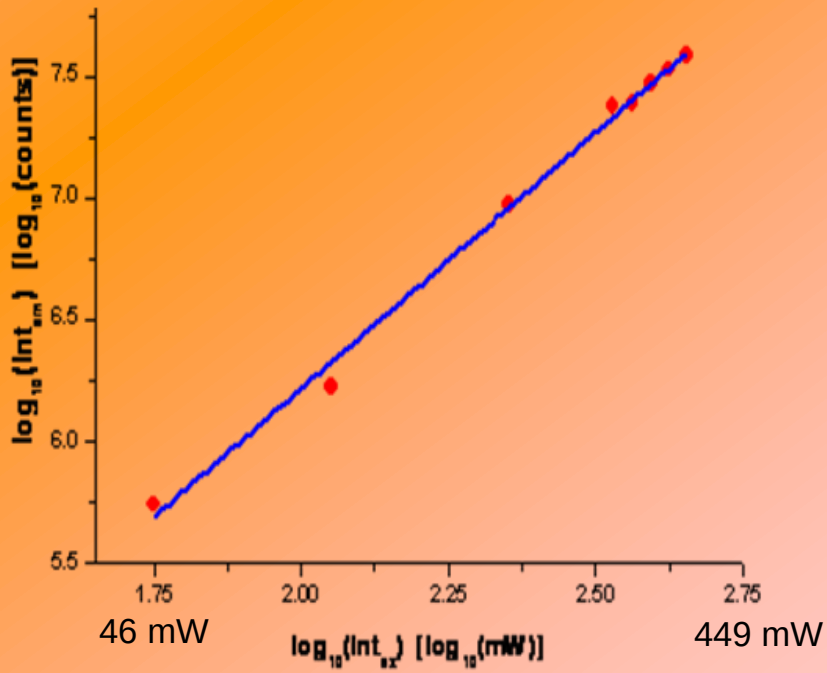
Disadvantage: Huge Excitation Powers:

must use photostable dyes (e.g. quantum dots)

2-Photon Widefield Excitation of Single Quantum Dot

- Blinking and emission intensity – laser power plot prove that it is single Qdots and 2-photon excitation



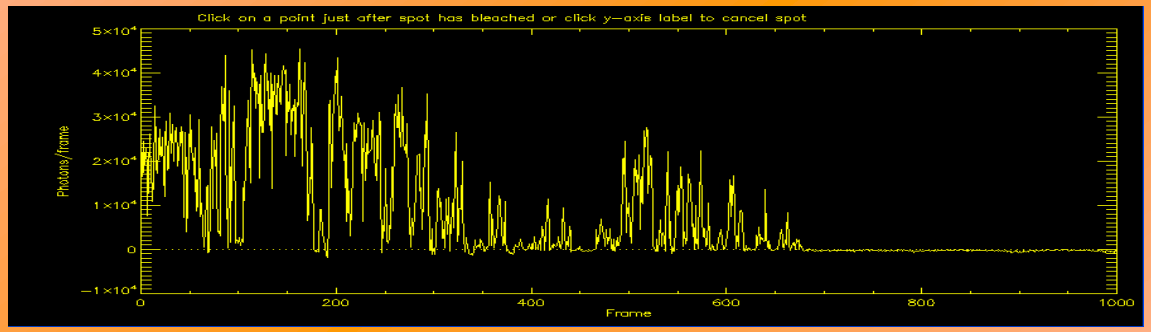


Qdot585, 655 in PBS buffer, no reductants (no deoxygenation) <P> = ~150 W/cm^2 , 30 msec/frame, scale bar 1 um. 160 nm effective pixel size

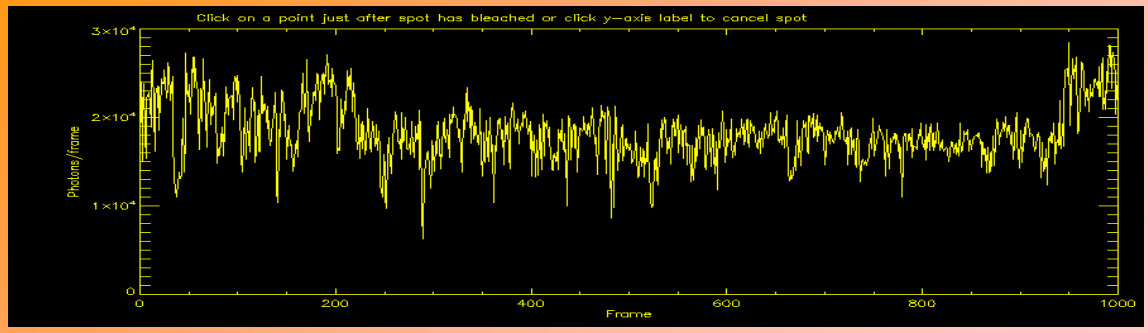
50x lower power with Single Quantum Dot than with single fluorophores

Suppression of Blinking and Photobleaching by Thiol-group Containing Reductants

- Similar with under 1-photon excitation, small thiol-group containing reductants, such as DTT and BME, can sufficiently, though not completely, suppress Qdot's blinking
- Large thiol-containing molecule like glutathione, carboxylic reductant like TCEP and Trolox do not work well
- Thiol-containing ligands may help passivate the Qdot surface



100 mM DTT



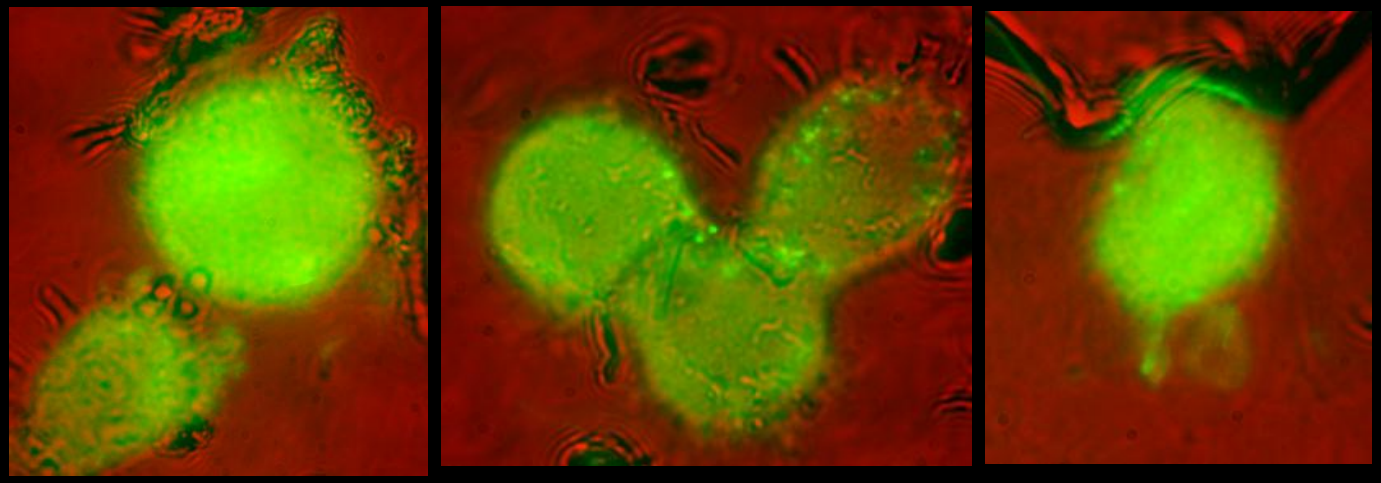
Qdot655, 1800 W/cm^2, 30 msec/frame, 30 sec

Individual EGF Receptors in Single Breast Cancer Cells

Overlay of cells' brightfield images (red) and fluorescence (green)

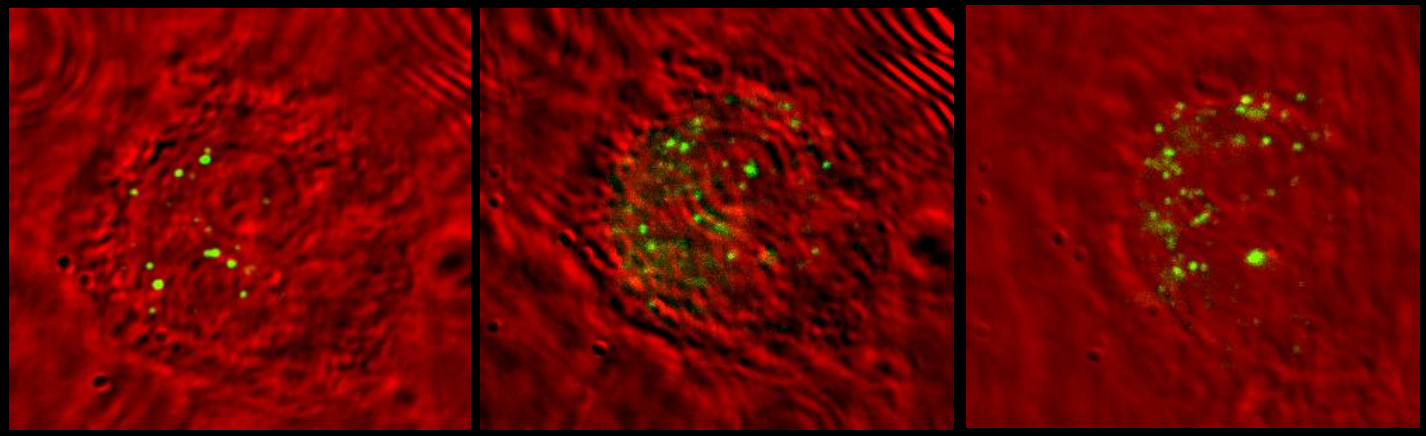
REGULAR FLUORESCENCE MICROSCOPY

A lot of autofluorescence



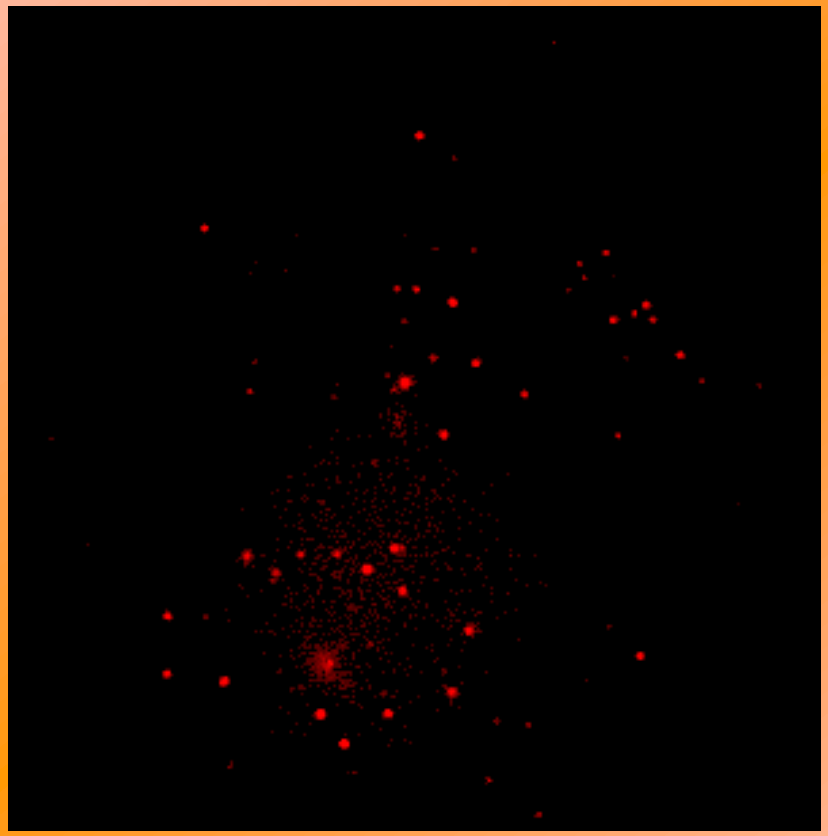
With tissues, have a lot less autofluorescence with two-photon microscopy

TWO-PHOTON Q-DOT EXCITED FLUORESCENCE MICROSCOPY

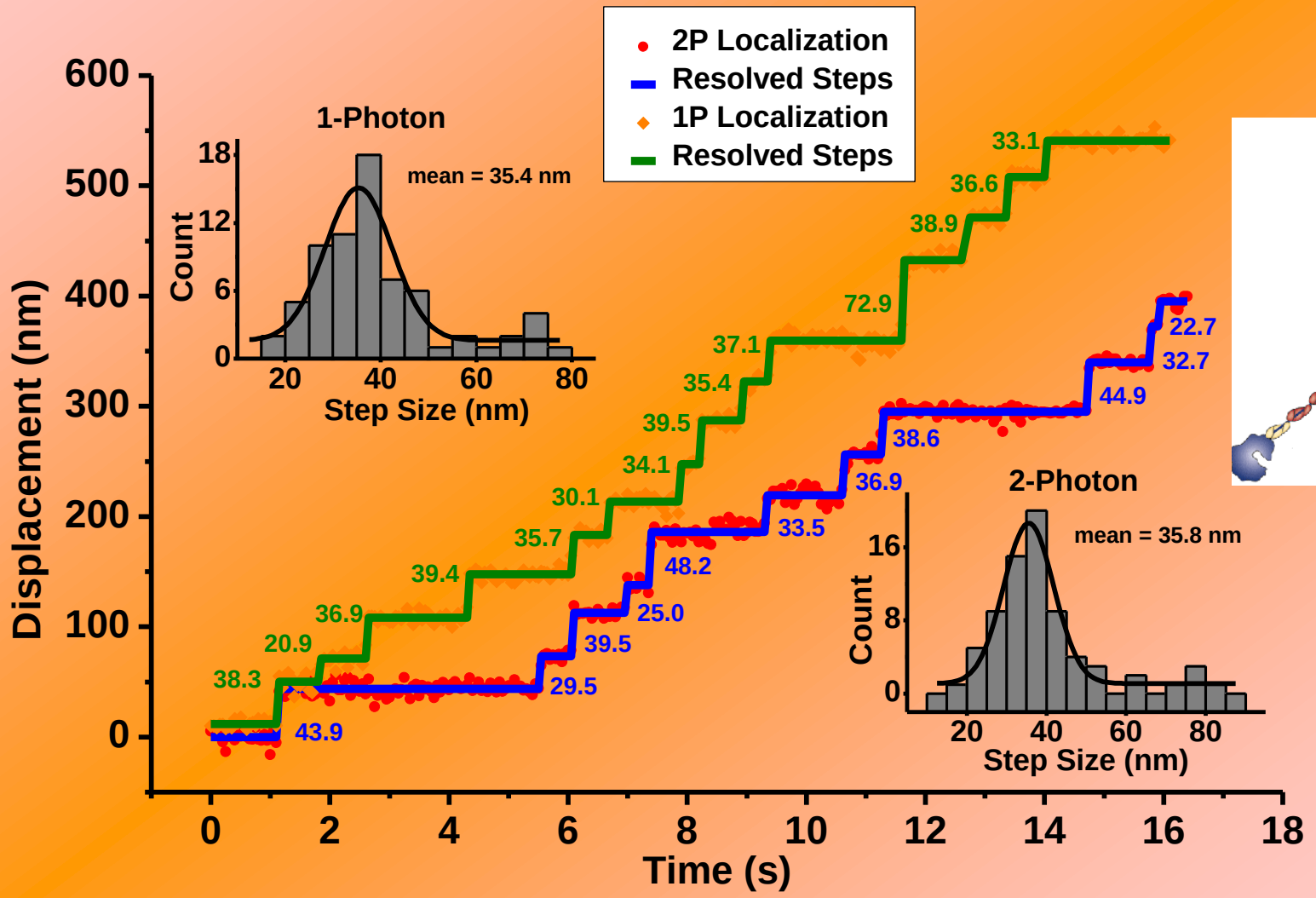


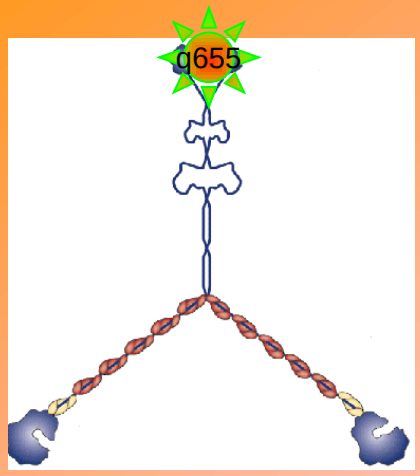
Eli Rothenberg at UIUC; Tony Ng and Gilbert Fruhwirth @ King's College School of Medicine & Dentistry, London

3D FIONA Super-Accuracy Imaging

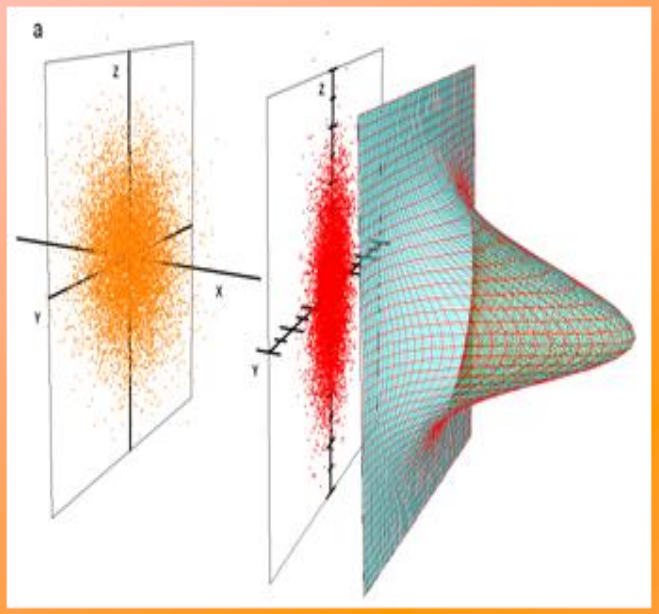


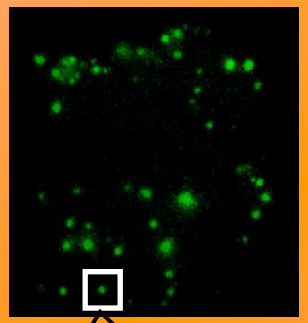
Myosin V walking
1P and 2P widefield excitation
Cargo binding domain walks 36 nm

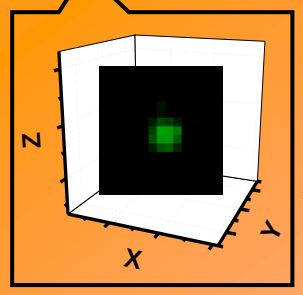


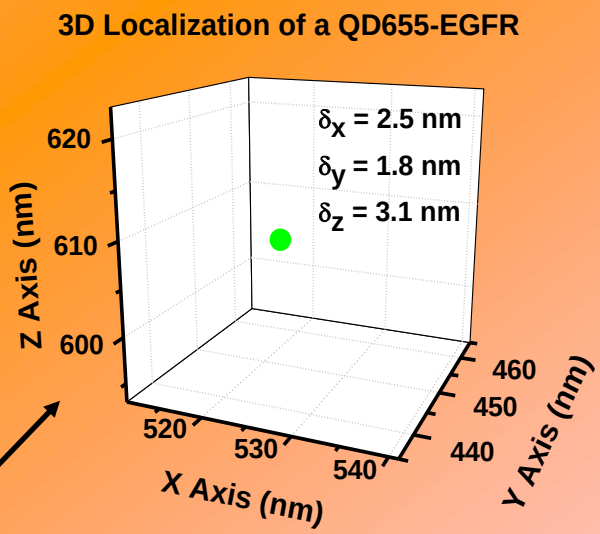


Get 3-D FIONA on quantum dots—nanometer accuracy
2 nm x-y and 3 nm in z









"Normal" FIONA is *x-y* with *z* fixed (with 1-2 nm accuracy).
The *z*-dimension, is *x-z* with *y*-fixed,
or *y-z* with *x*-fixed (with 3 nm accuracy)

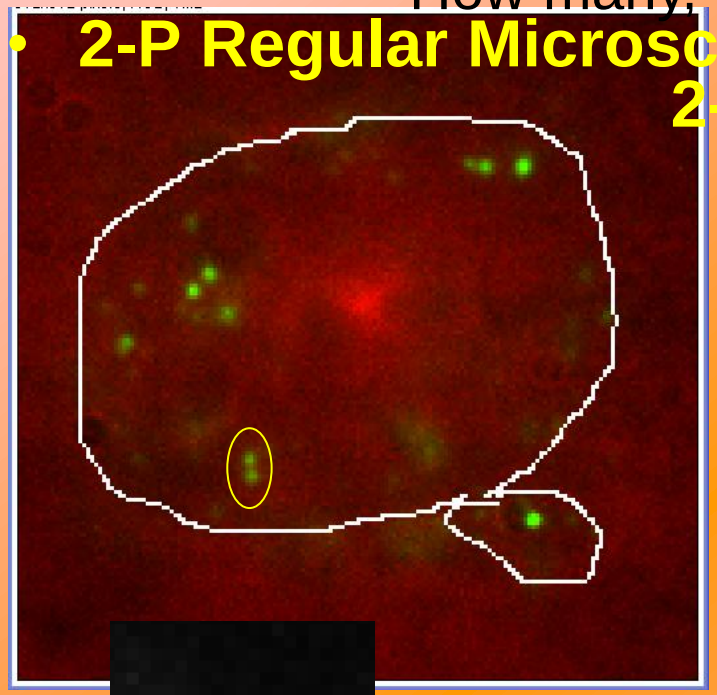
Super-Resolution: 2-P gSHRImP on q-dots

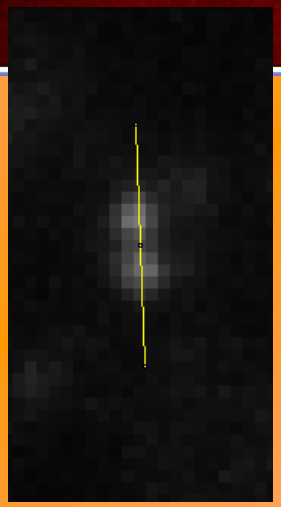
Why only 14% of breast cancer patients helped by EGFR Inhibitors?

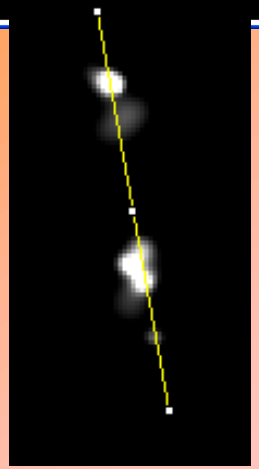
EGF & related membrane proteins get internalized in <150 nm vesicles.
How many, and how far apart?

- 2-P Regular Microscopy

2-P gSHRImP

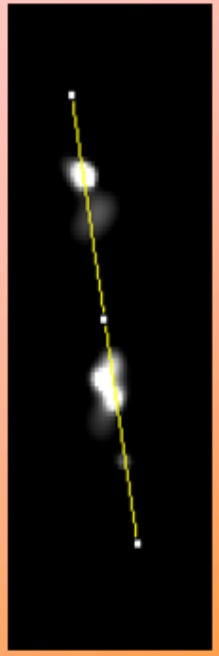


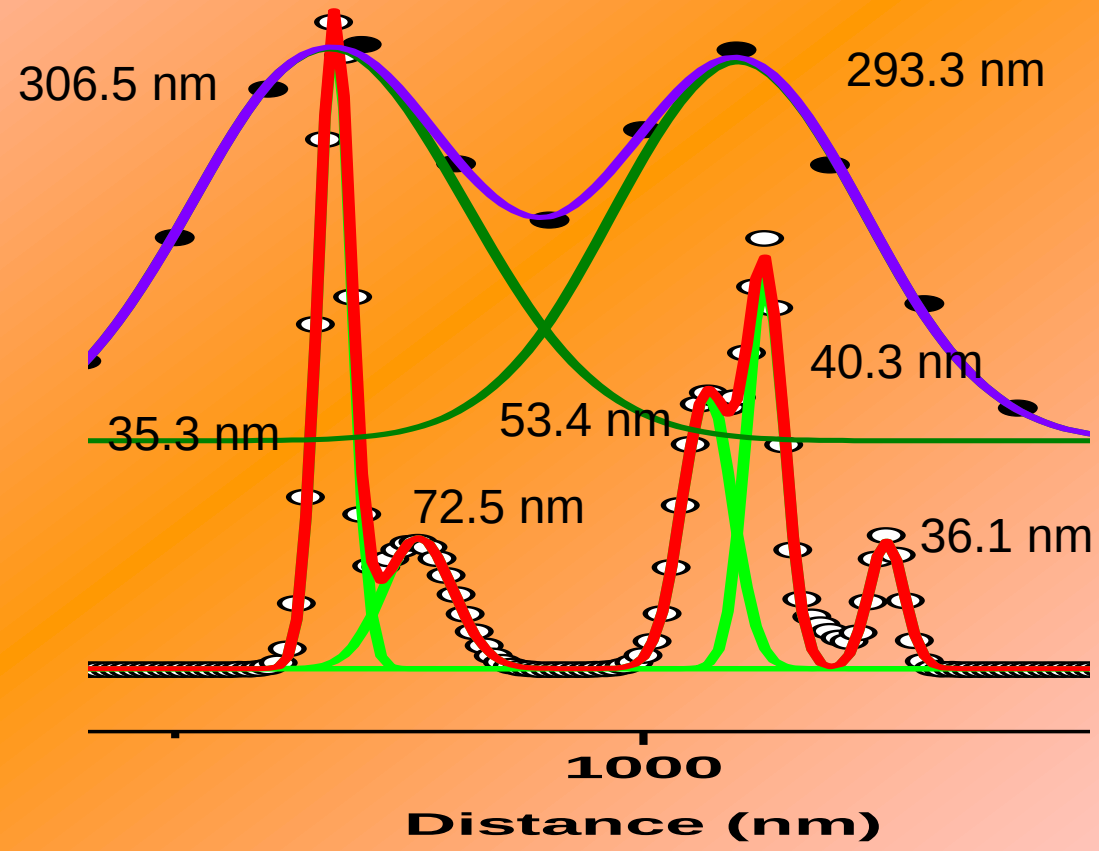




EGF-QD605
Middle of cell
EGF endocytosed

Super-Resolution: with gSHRImP + 2-Photons





35 nm x-y resolution; z-resolution?

Sample: EGF-QD605 4nM in in Breast Cancer Cells

Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

3. Related to today's subject, what would you like to know more about?

4. Any helpful comments.

Answer, and turn in at the end of class.