- Outline
  - Introduction
  - Results from our LHC experiment
  - Conclusions

Don’t show your outline!

However, it can be useful to make one for yourself.

title slide

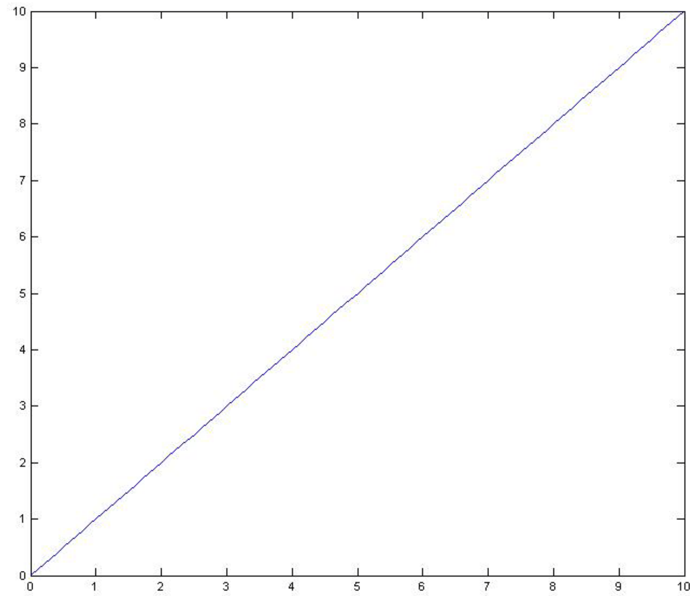
1. Intro 1- brief history of metal working shops
2. Intro 2- overview of shop tools
3. Intro 3- Importance of the Mill and Lathe in modern shops
4. Detailed description of the mill 1-cutting tools, speeds, oils
5. Detailed description of the mill 2-part alignment and measurement
6. Detailed description of the lathe 1-cutting tools, speeds, oils
7. Detailed description of the lathe 2-part alignment and measurement
8. Description of desired part
9. Detailed description of mill work.
10. Detailed description of lathe work.
11. Show Finished piece and comparison of tolerances to final measurements
12. Discussion of process- successful and areas to be improved

Acknowledgement slide

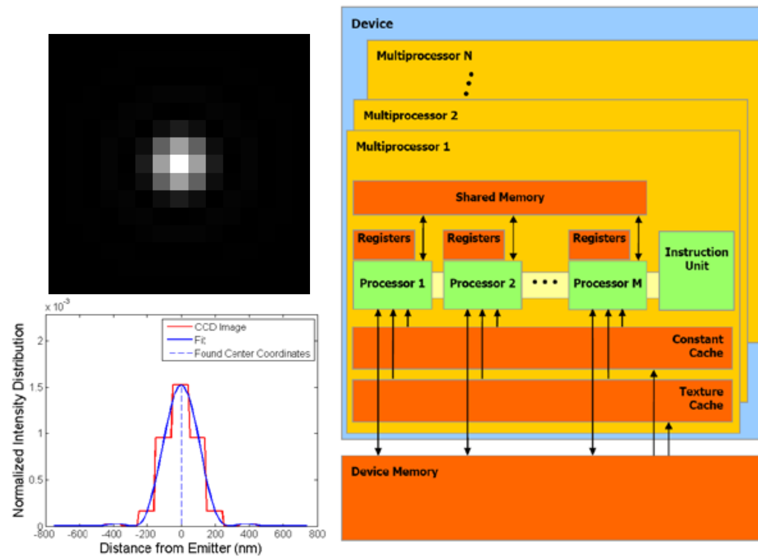
General Rule: 1 Slide or less per minute.

- Writing a bunch of text that the audience has to read is one of the worst things that you can do when giving a presentation.
- This can be made even more annoying when you have a bad color scheme such as that used in this slide.
- Just because powerpoint can easily make bullets doesn’t mean that you should use them.
- You are probably going to make all of these comments when discussing your experiment or results, so its better to just give that information orally when you are talking about that material.
- However, sometimes in conclusions, etc, a list can be helpful. Just keep it as short as possible.

Results!



This agrees with Smith's previous paper.



Single Molecule Localization

Fitting Model: 2D finite pixel Gaussian PSF

$$\mathrm{PSF}(x,y) = \frac{1}{2\pi\sigma^2} e^{\frac{-(x-\theta_x)^2-(y-\theta_y)^2}{2\sigma^2}}$$

$$\mu_k(x,y) = \theta_{I_0} \iint_{A_k} \mathrm{PSF}(u,v)\,du\,dv + \theta_{bg}$$

$$L(\vec{x}|\theta) = \Pi_k \frac{\mu_k(x,y)^{x_k} e^{-\mu_k(x,y)}}{x_k!}$$

θ_x: x-position

θ_y: y-position

θ_{I0}: emission rate

θ_{bg}: background count rate

Newton's method iterative update: achieves CRLB

$$\theta_i \to \theta_i + \left[\sum_k \frac{\partial \mu_k(x,y)}{\partial \theta_i}\left(\frac{x_k}{\mu_k(x,y)} - 1\right)\right] \times \left[\sum_k \frac{\partial^2 \mu_k(x,y)}{\partial \theta_i^2}\left(\frac{x_k}{\mu_k(x,y)} - 1\right) - \frac{\partial \mu_k(x,y)}{\partial \theta_i}^2 \frac{x_k}{\mu_k(x,y)^2}\right]^{-1}$$

Cramér-Rao Lower Bound (CRLB)

$$I(\theta)_{i,j} = \sum_k \frac{1}{\mu_k(x,y)} \frac{\partial \mu_k(x,y)}{\partial \theta_i} \frac{\partial \mu_k(x,y)}{\partial \theta_j}$$

$$var(\hat{\theta}) \geq I(\theta)^{-1}$$

Use CRLB to estimate localization accuracy

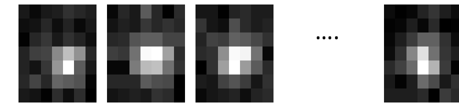
CRLB: Minimum variance of an estimated parameter

Every position is found with theoretically best precision

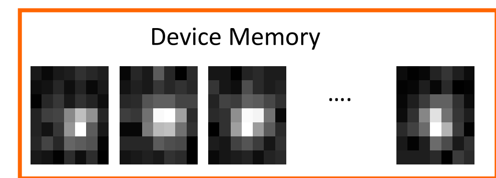
Every position is returned with a fit precision

Single Molecule Localization on the GPU

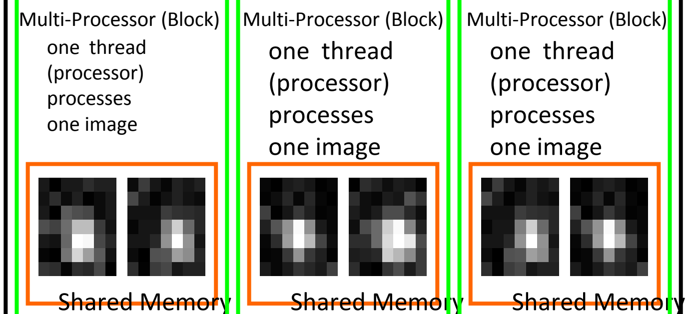
Package all sub-regions together (~10^6 sub regions)



GPU



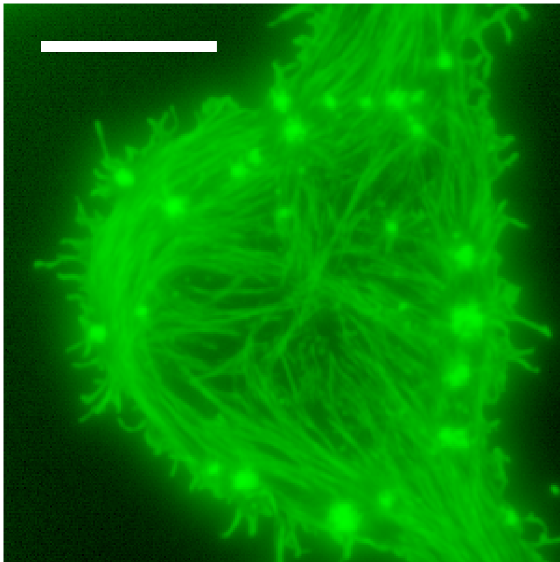
fill shared memory in each Block (Multi-Processor)

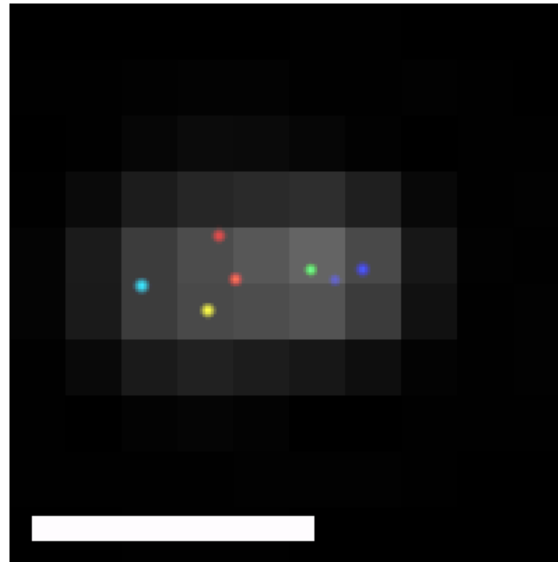


Single Molecule Fluorescence for Localizing and Tracking Proteins in Cells

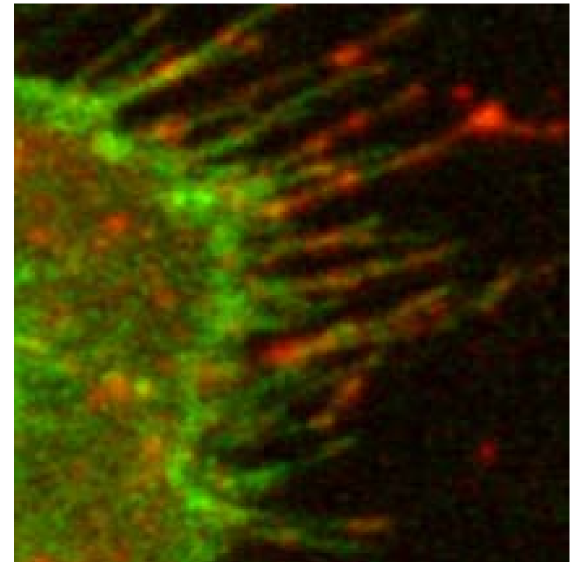
Keith Lidke
Department of Physics and Astronomy
University of New Mexico

10 μm



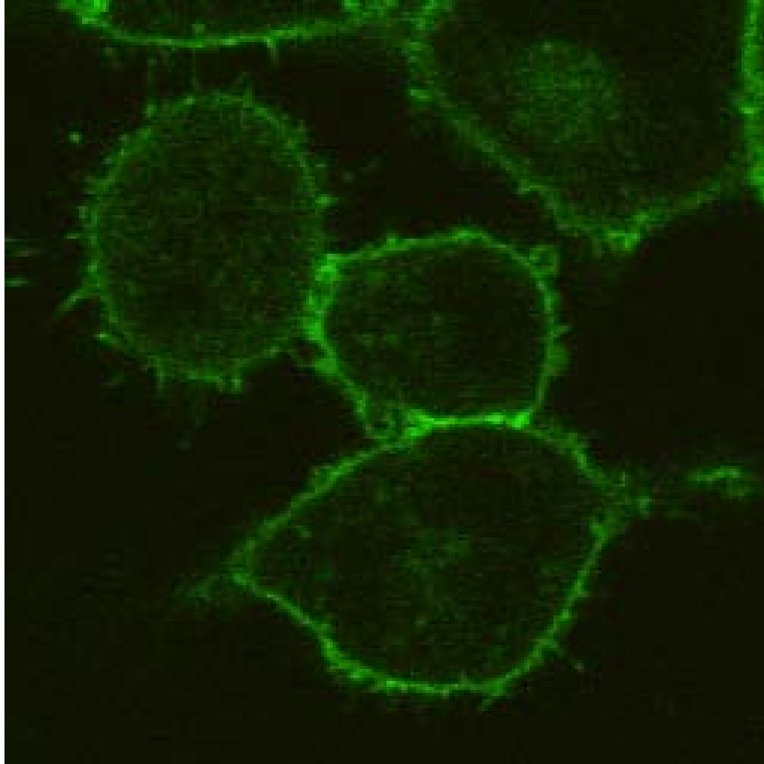


500 nm

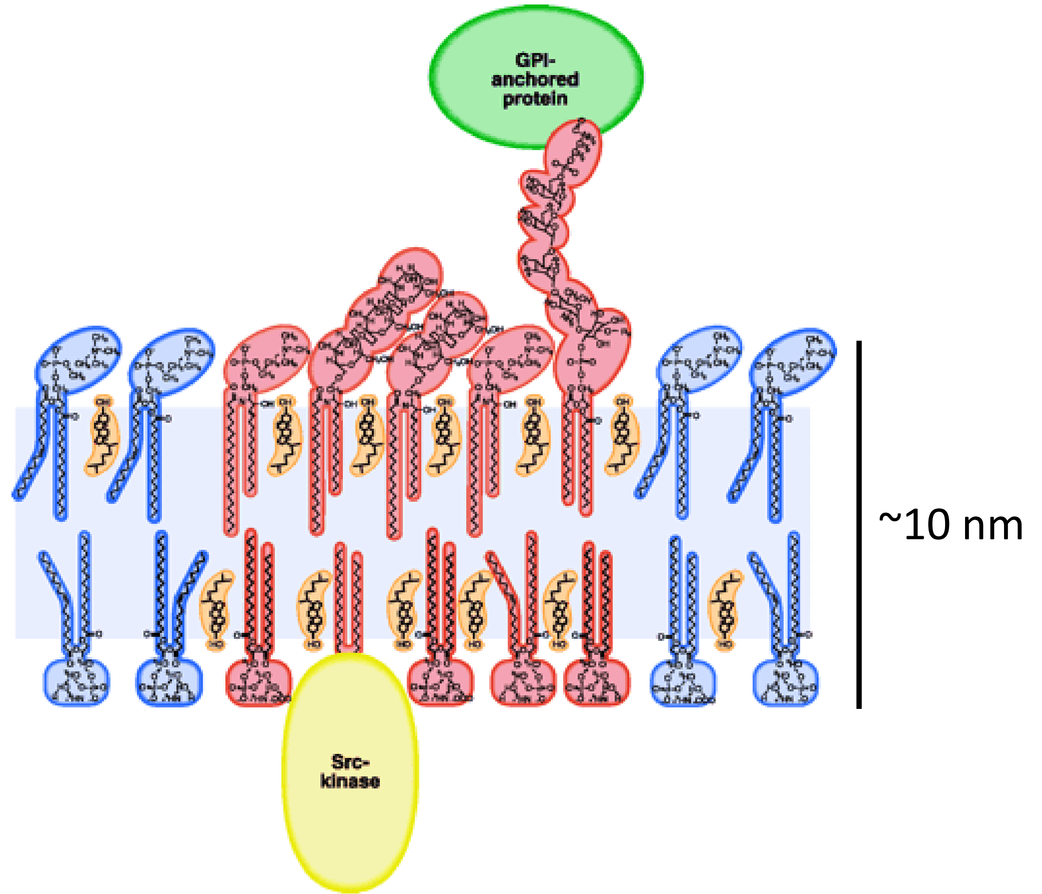


~10 μm

Cell Membrane Basics



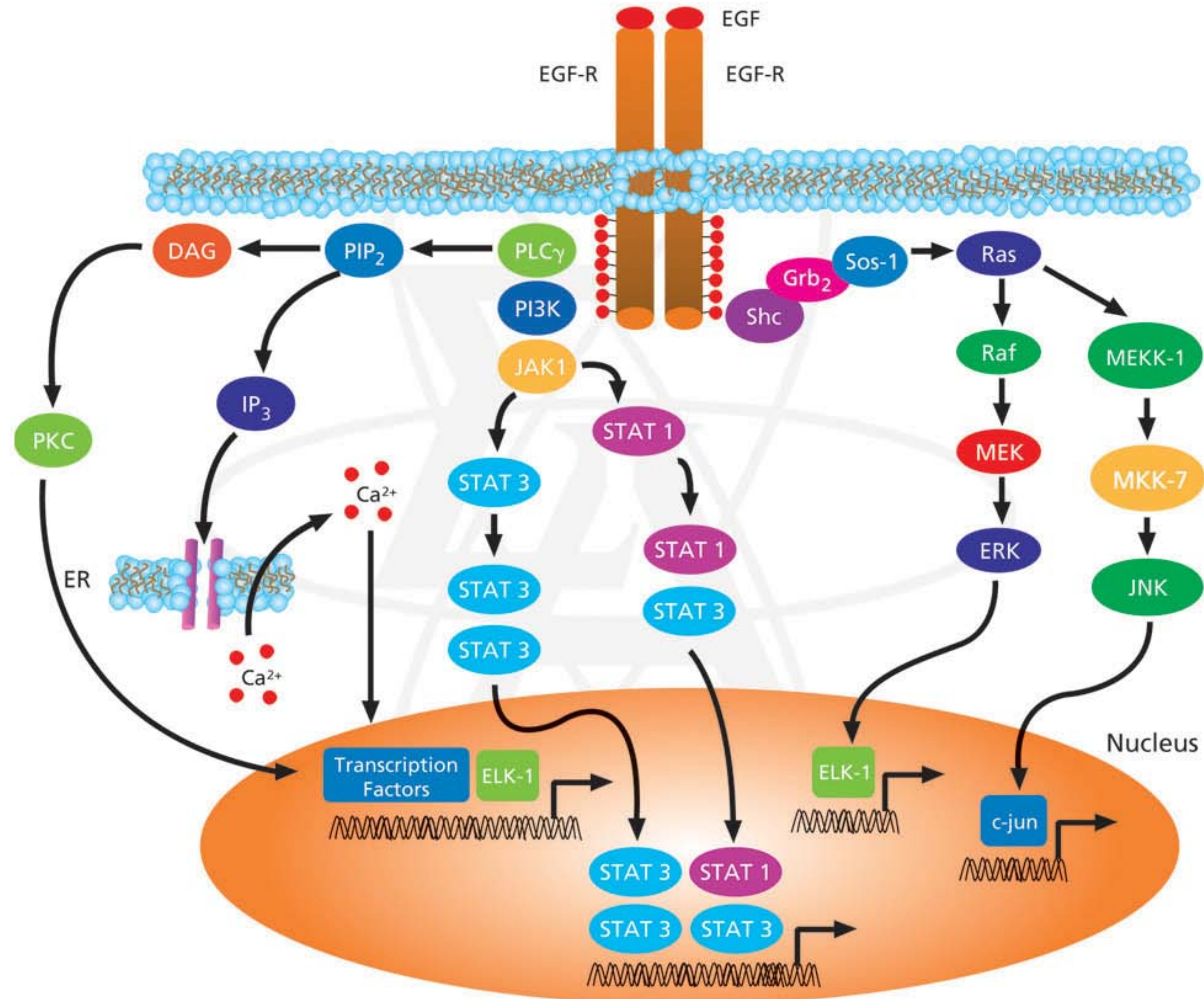
~ 30 μm



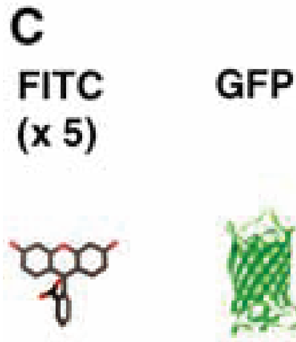
Simons, *Science* **290** 1721 (2000)

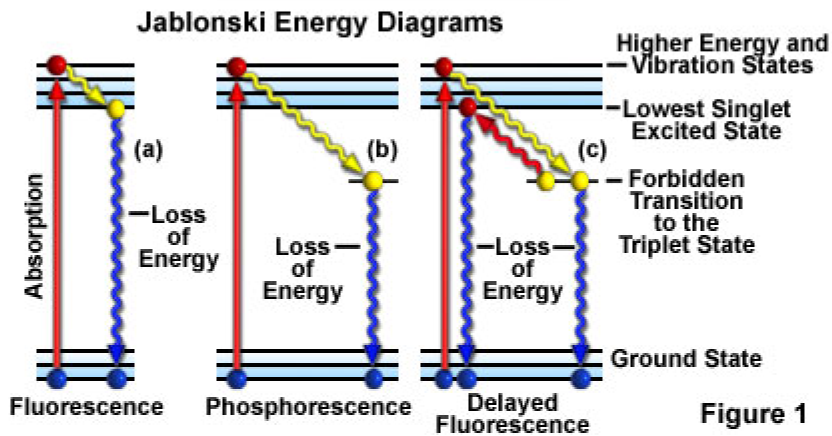
EGF Receptor Signal Transduction Pathway

SIGMA-ALDRICH

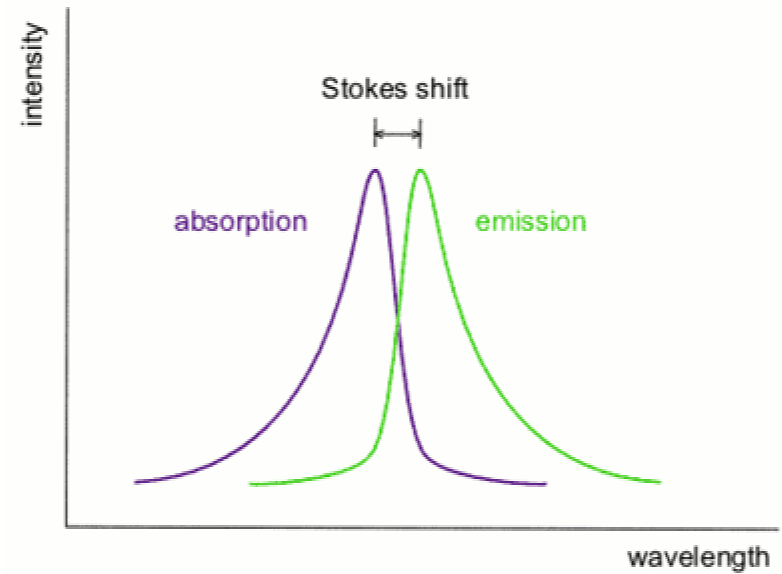


Stokes shift allow emission light to be separated from excitation light

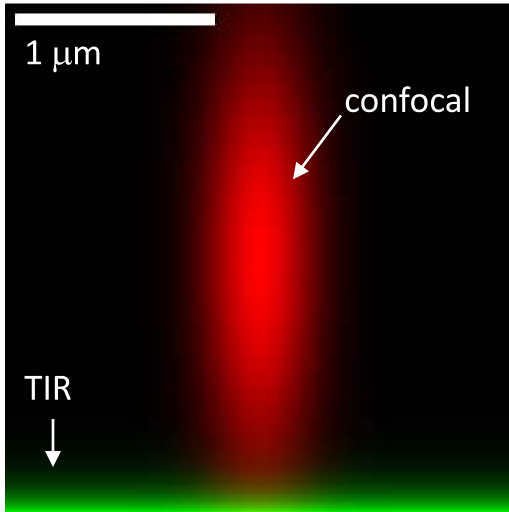




http://micro.magnet.fsu.edu/primer/java/jablonski/lightandcolor/index.html



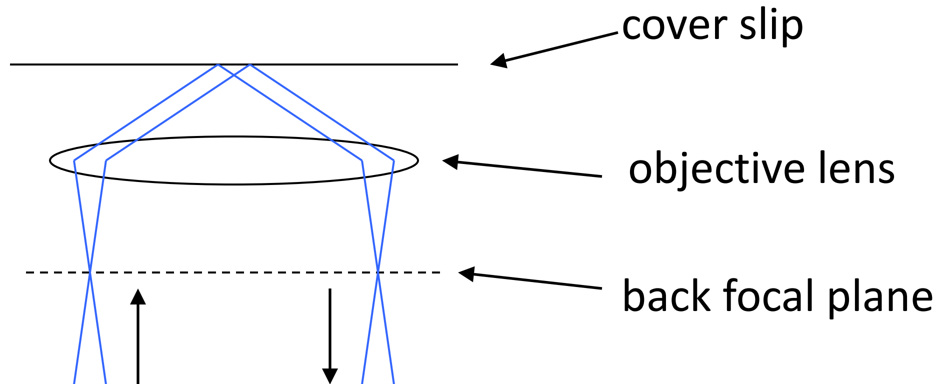
http://en.wikipedia.org/wiki/Stokes_shift



$$I(z) = I(0)e^{-\beta}$$

$$\beta = \frac{\lambda}{4\pi\sqrt{n_1{}^2 \sin(\alpha)^2 - n_2{}^2}}$$

λ: wavelength of light
α: incident angle
n_1: index of water (1.33)
n_2: index of cover slip (1.52)



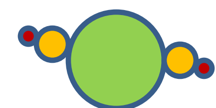
$$NA = n\sin(\alpha) = 1.45$$

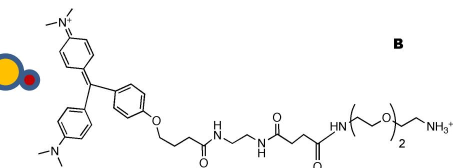
β_{min} ~ 70 nm

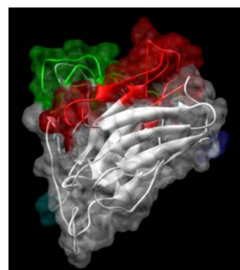
Fluorophores for Single Molecule Superresolution

Fluorogen Activating Peptide (FAP) Concept

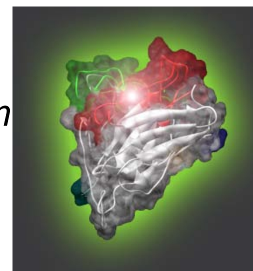
Equilibrium
Localization
Microscopy







Equilibrium



ELM

Actin

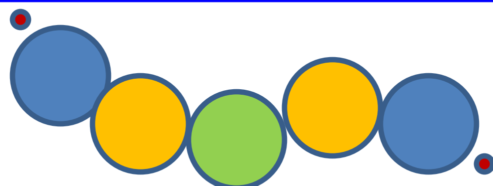
FAP

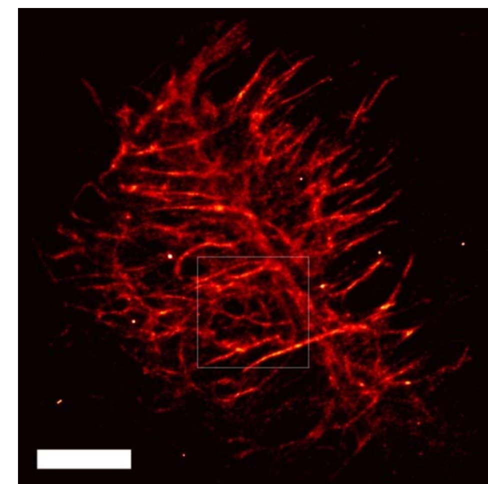
Dye

Non-fluorescent dyes

Expressible protein modules

Sam Schwartz - lightning talk





STORM

Actin

Primary Anti-body

Secondary

Cy5

Requires 50 mM MEA
+ Oxygen Scavenging System

Real Time Analysis of SM-SR Data

Time for Fitting and Reconstruction

Processor	Total Time	Segmentation	ROI collection	Fitting	Reconstruction
	10^4 frames, 10^5 localizations				
GPU	8.8 s	90 %	1 %	8.5 %	0.5 %
CPU	41 s	19.4 %	0.2 %	80.3 %	0.1 %
	10^4 frames, 10^6 localizations				
GPU	14 s	57.8 %	2.7 %	38.9 %	0.6 %
CPU	300 s	2.8 %	0.1 %	97 %	0.1 %

Analysis Time

10,000 Frames
128 x 128 Pixels
7 x 7 Fitting ROI
σ_{PSF} = 1 (Pixel)

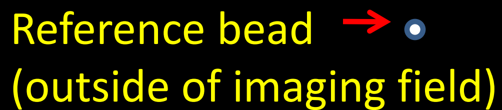
Fastest EMCCD is ~ 500 Frames/s @ 128 x 128 Pixels

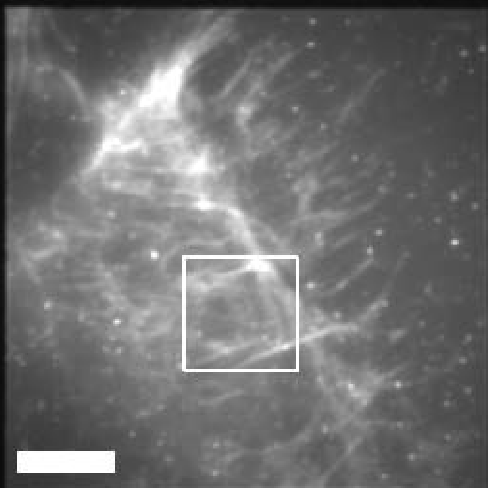
10,000 Frame / (500 Frames/s) = 20 s

10 MHz Read-out rate limited
Data Collection Time

Smith et al, Nature Methods 2010

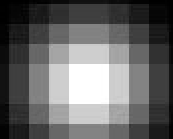
Active Stage Stabilization



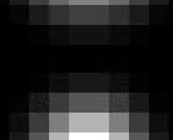


Defocus in z (nm)

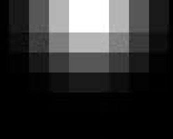
500



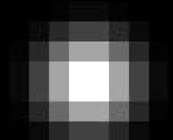
250



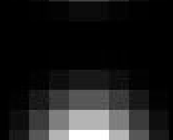
0



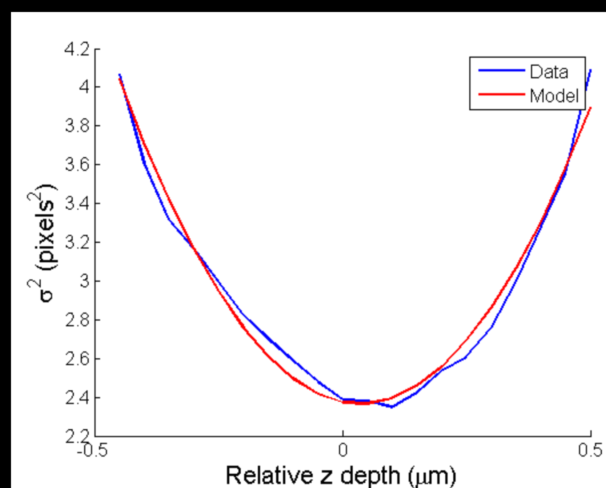
-250

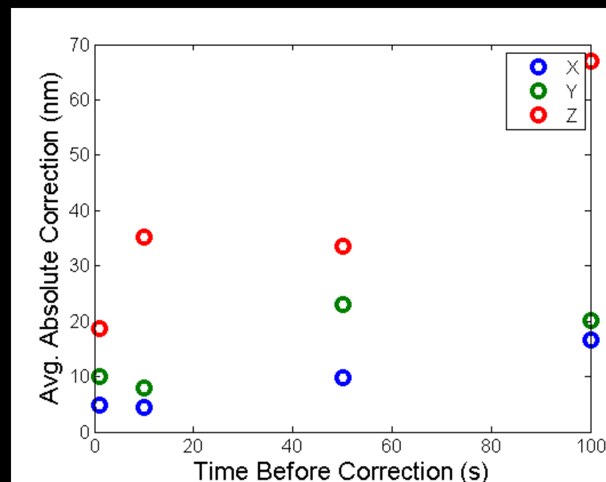


-500



$\sigma^2=\sigma_0^2(1+(z\text{-offset})^2/d^2)$





Before Imaging find bead position.

Periodically move stage to bead, align stage, then move back