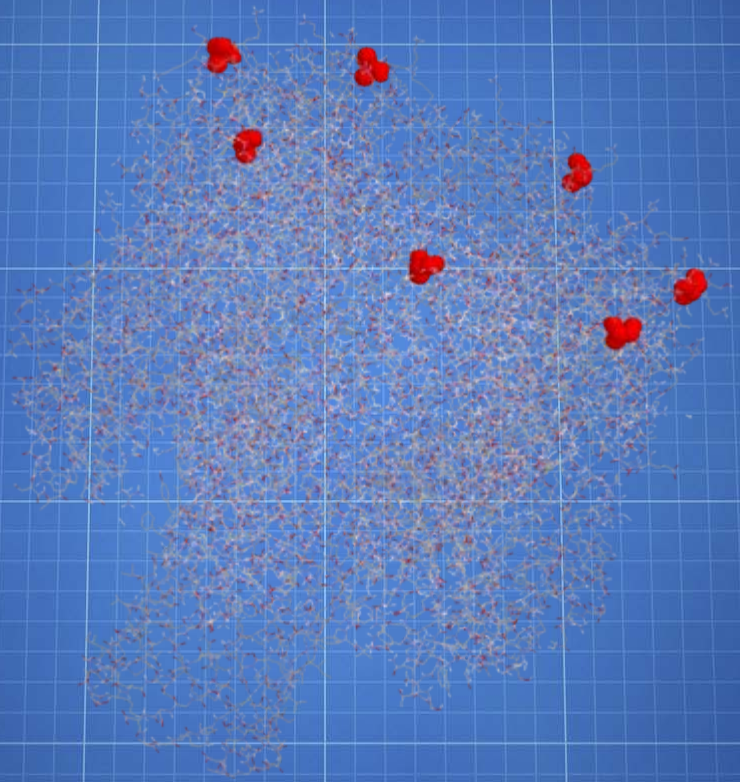
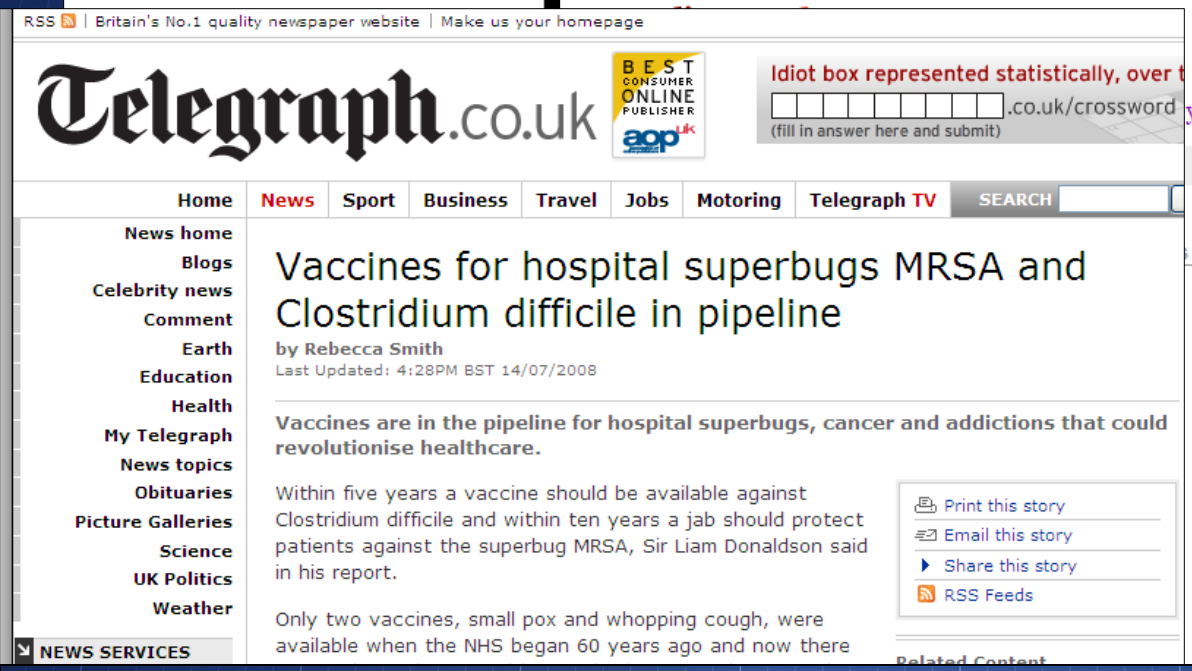
Single Molecule Tracking of Fluorescently Derivatized α-Hemolysin



Jared Kindt and Derek Bailey

With Dr. Daniel Burden



MRSA infections occur in approximately 94,000 persons each year and are associated with approximately 19,000 deaths.

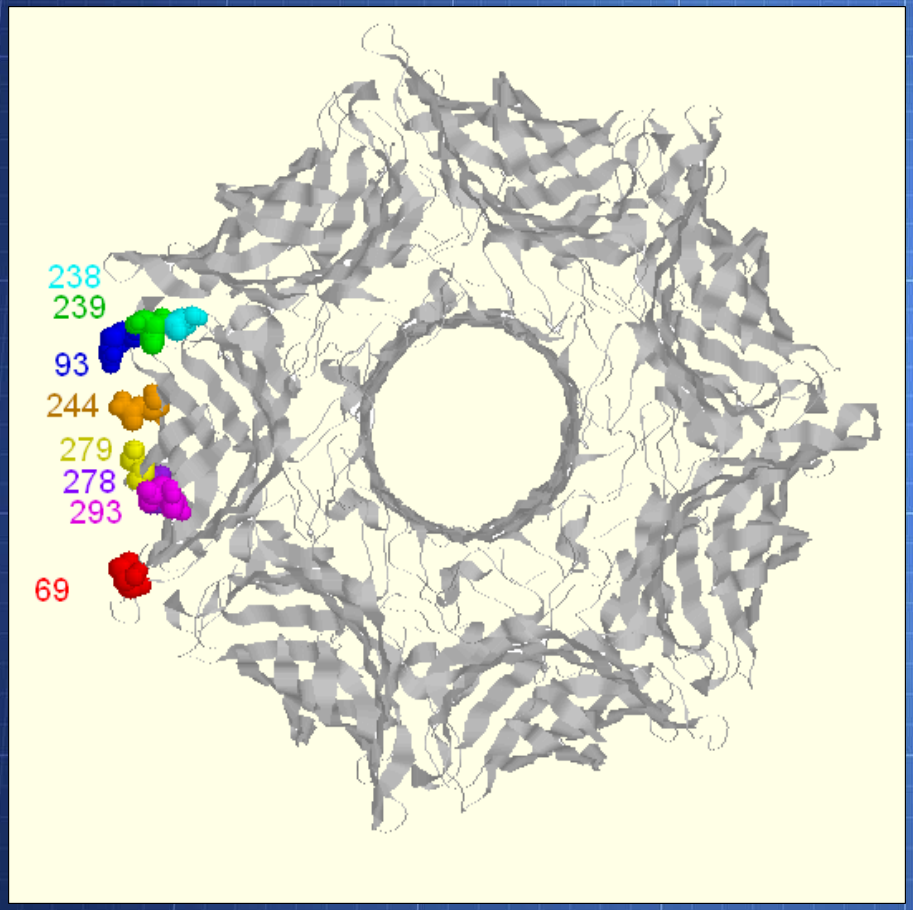
Of these infections

- 86% are healthcare-associated
- 14% are community-associated.

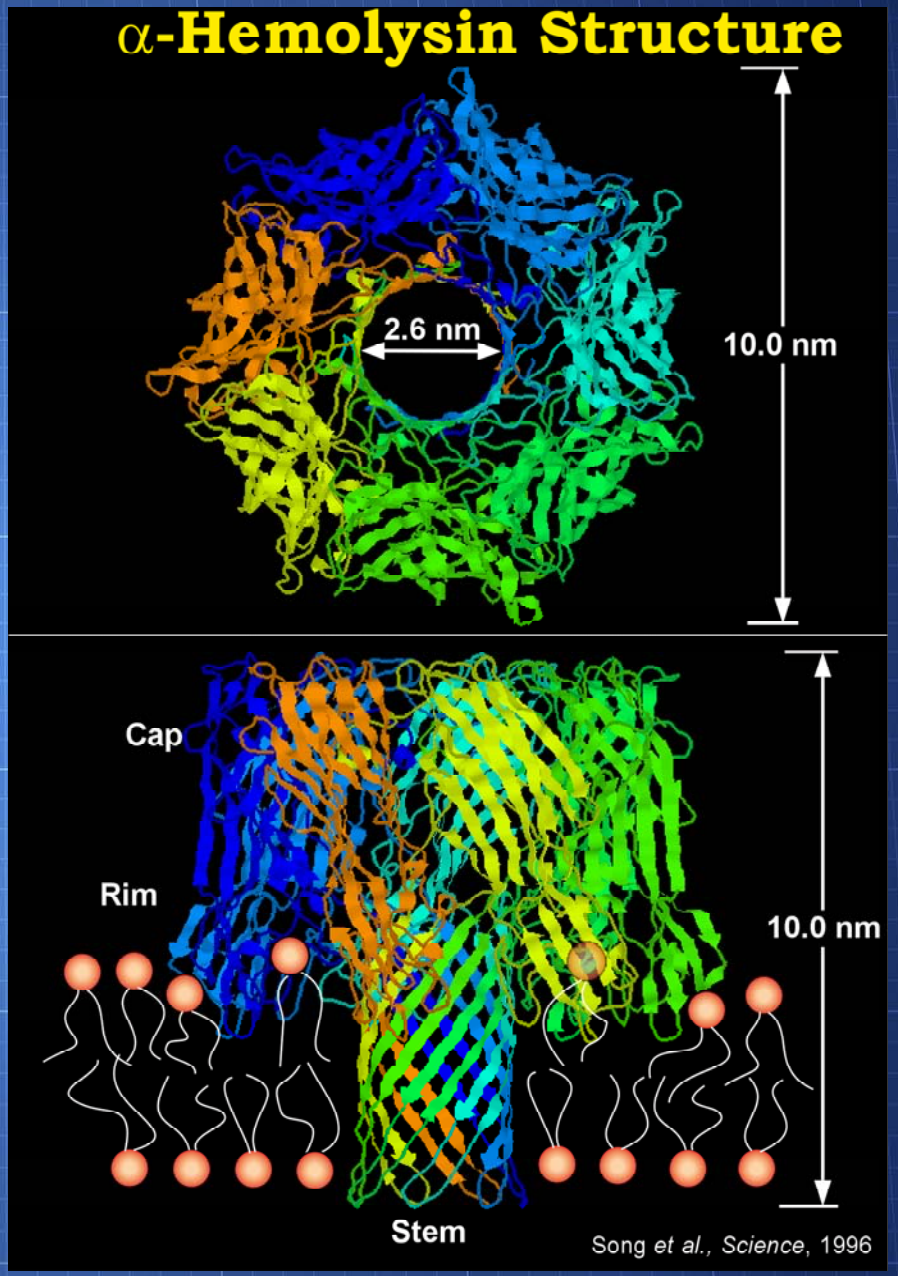
Source:

Klevens et al. Journal of the American Medical Association 2007;298(15):1763-1771

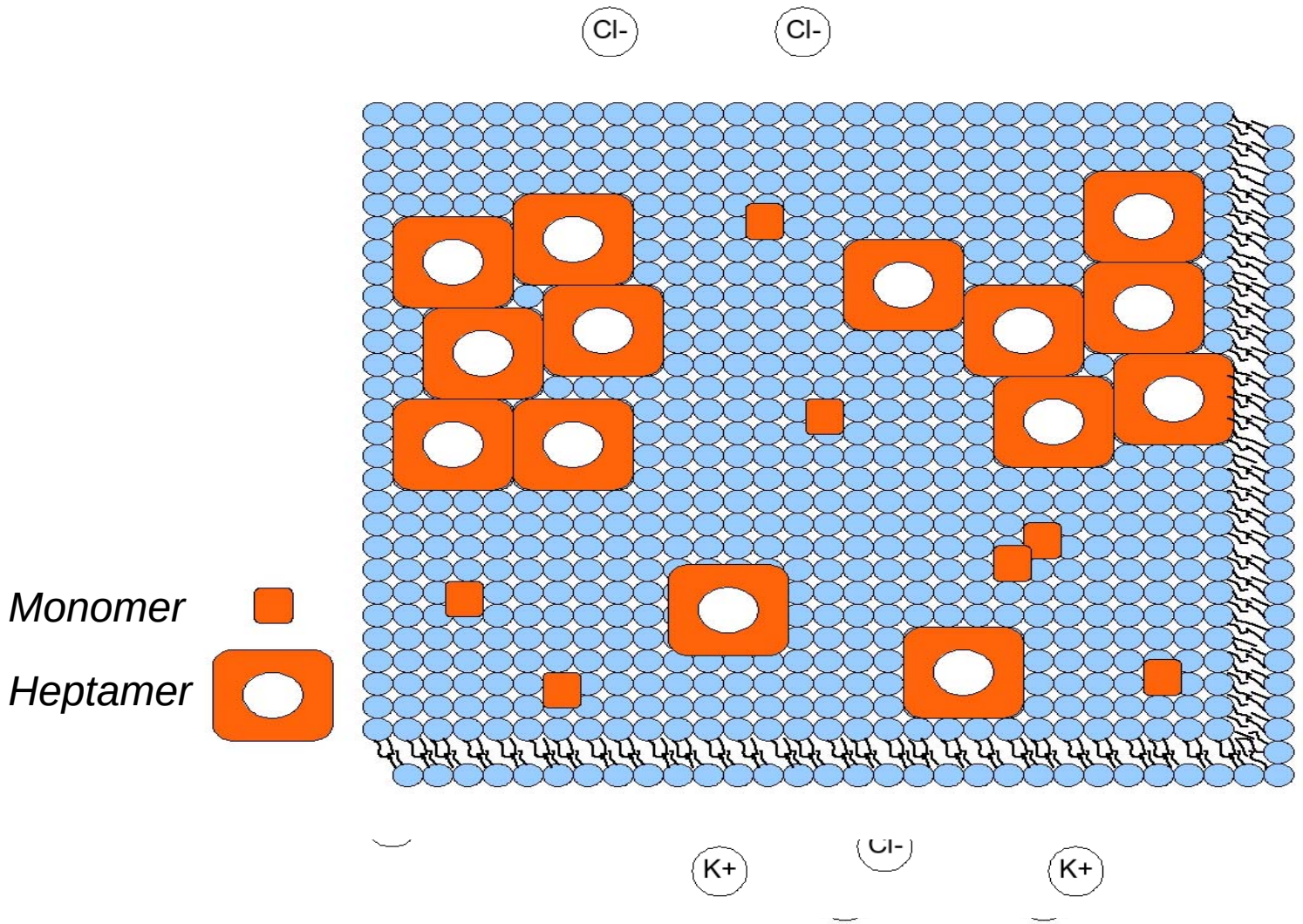
α-Hemolysin Structure and Labeling

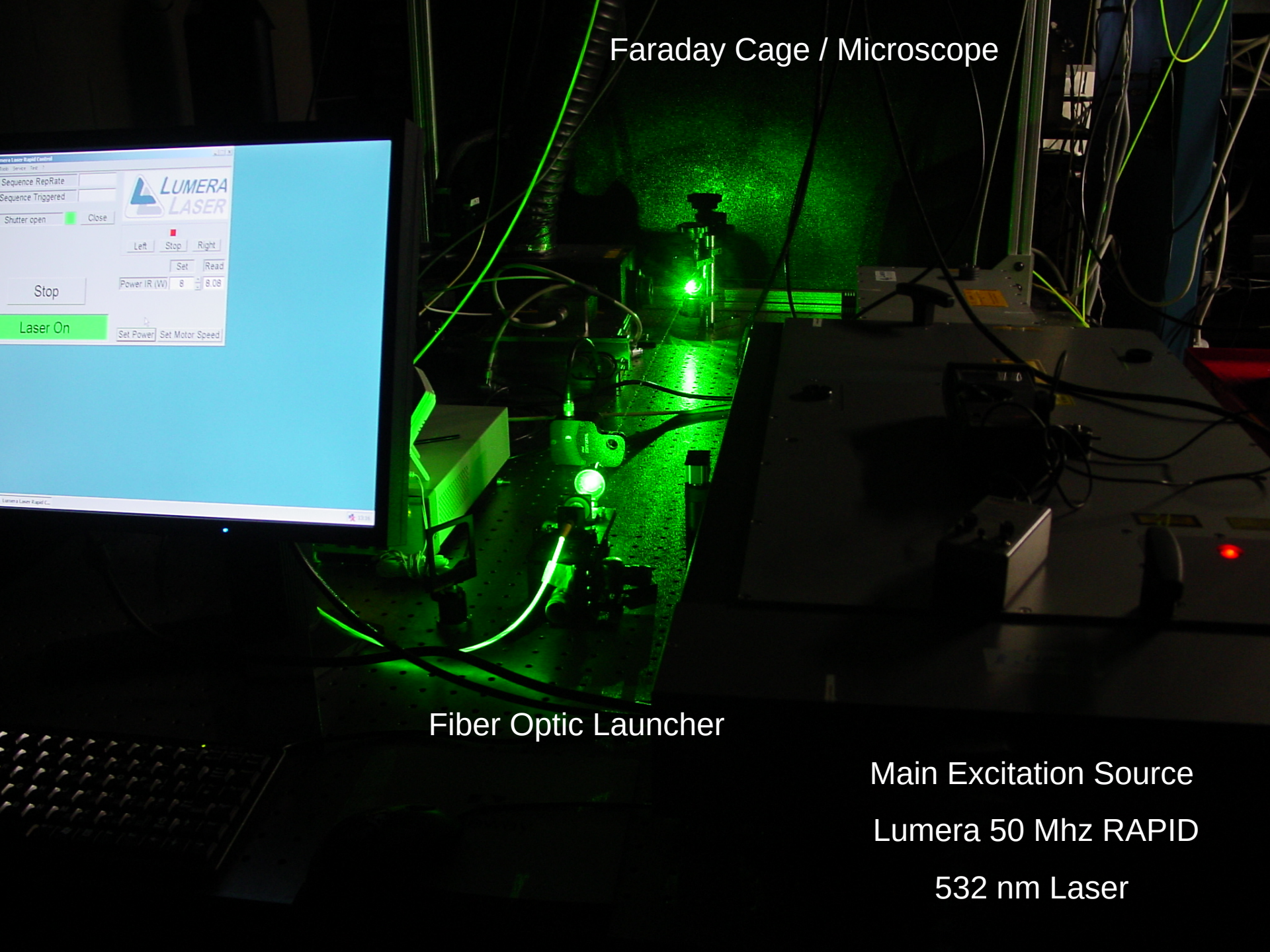


Side-View



Proposed Assembly Mechanism and Dynamics

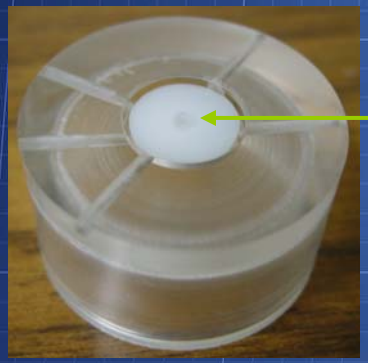



Faraday Cage / Microscope
Sequence RepRate
Sequence Triggered
LUMERA LASER
Shutter open
Close
Left
Stop
Right
Set
Read
Stop
Power IR (W)
8
8.08
Laser On
Set Power
Set Motor Speed
Fiber Optic Launcher
Main Excitation Source
Lumera 50 Mhz RAPID
532 nm Laser

Microscope Setup

Electron Multiplying Charge Couple Device

Chamber Location/Objectives



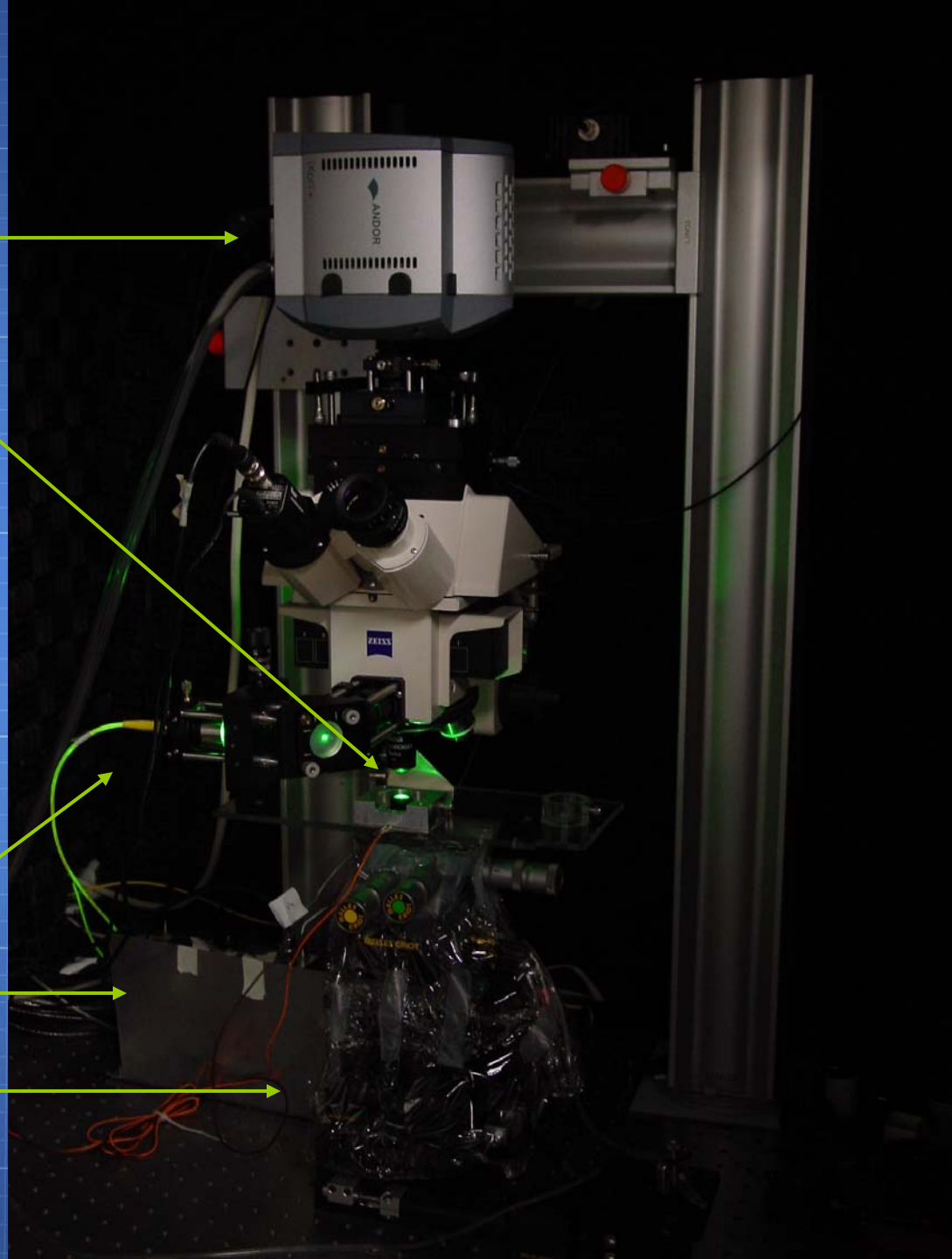
Chamber

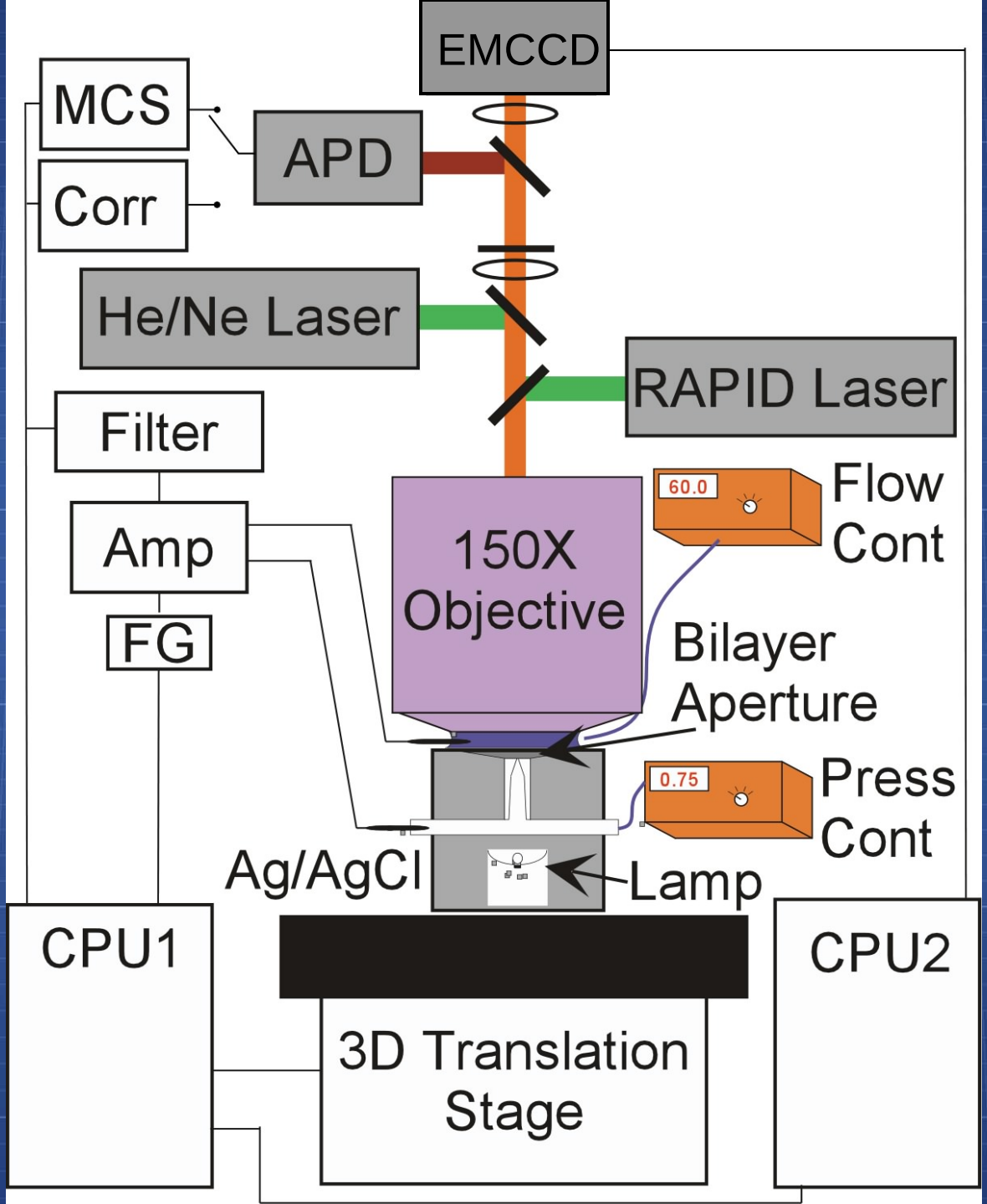
Teflon Aperture

Laser Light (532 nm)

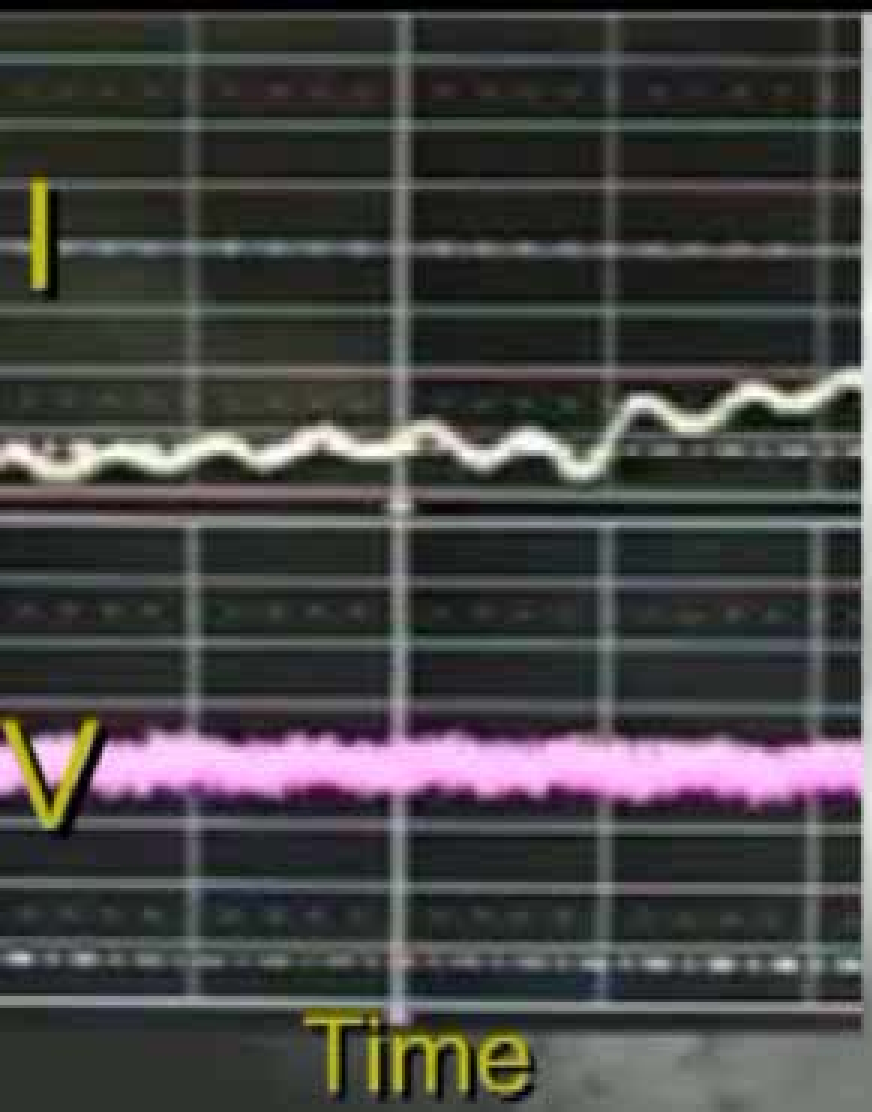
Amplifier

Positional Stage

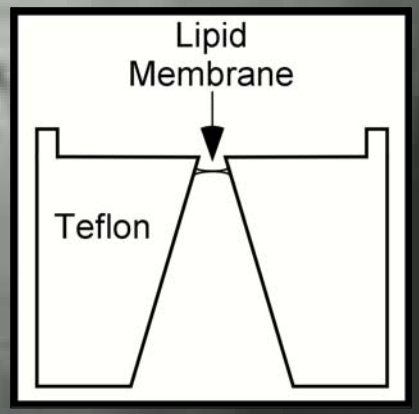



EMCCD
MCS
APD
Corr
He/Ne Laser
RAPID Laser
Filter
Amp
FG
150X
Objective
60.0
Flow
Cont
Bilayer
Aperture
0.75
Press
Cont
Ag/AgCl
Lamp
CPU1
CPU2
3D Translation
Stage

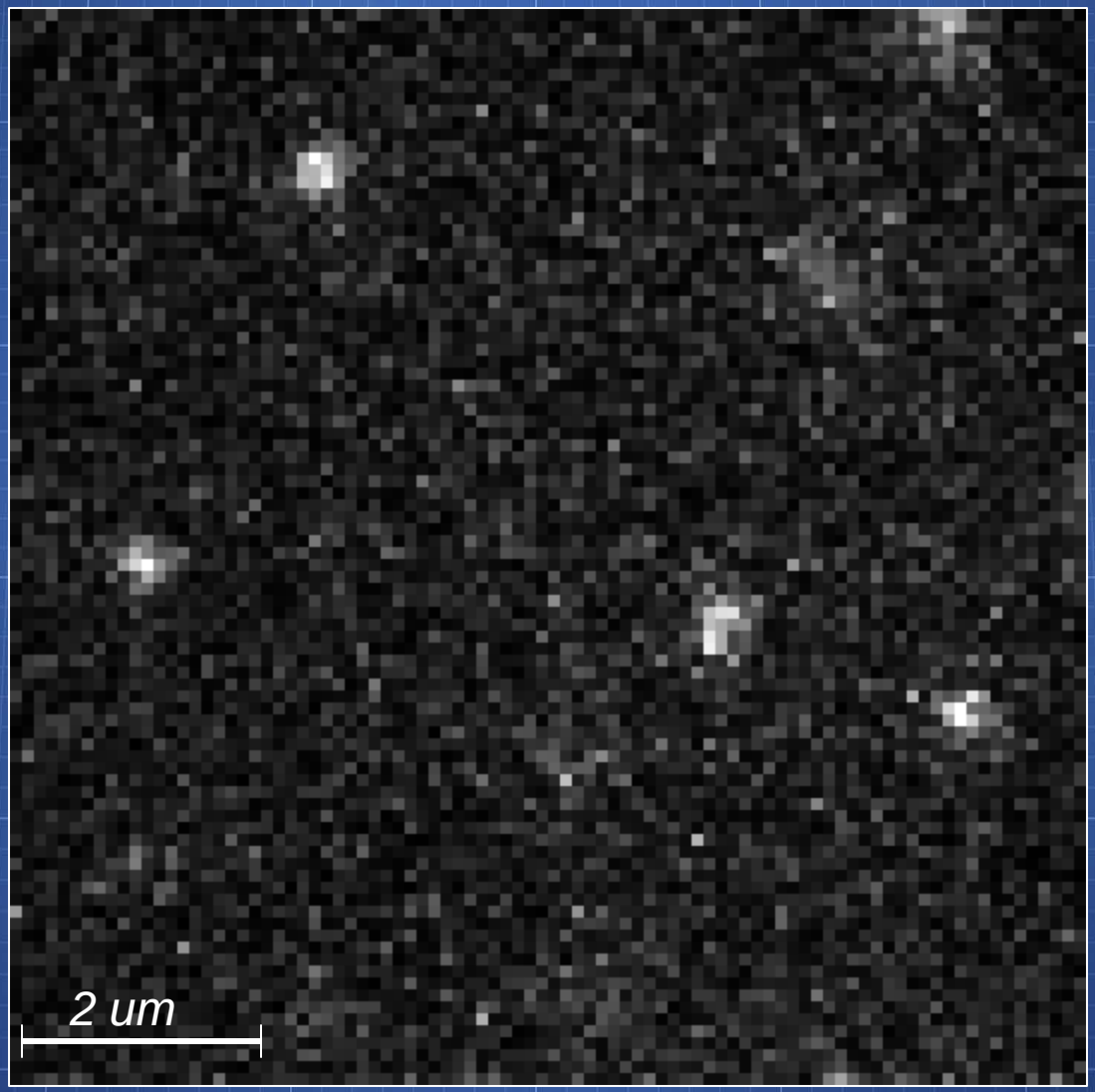
Creating a Membrane



Membrane aperture ~50 µm

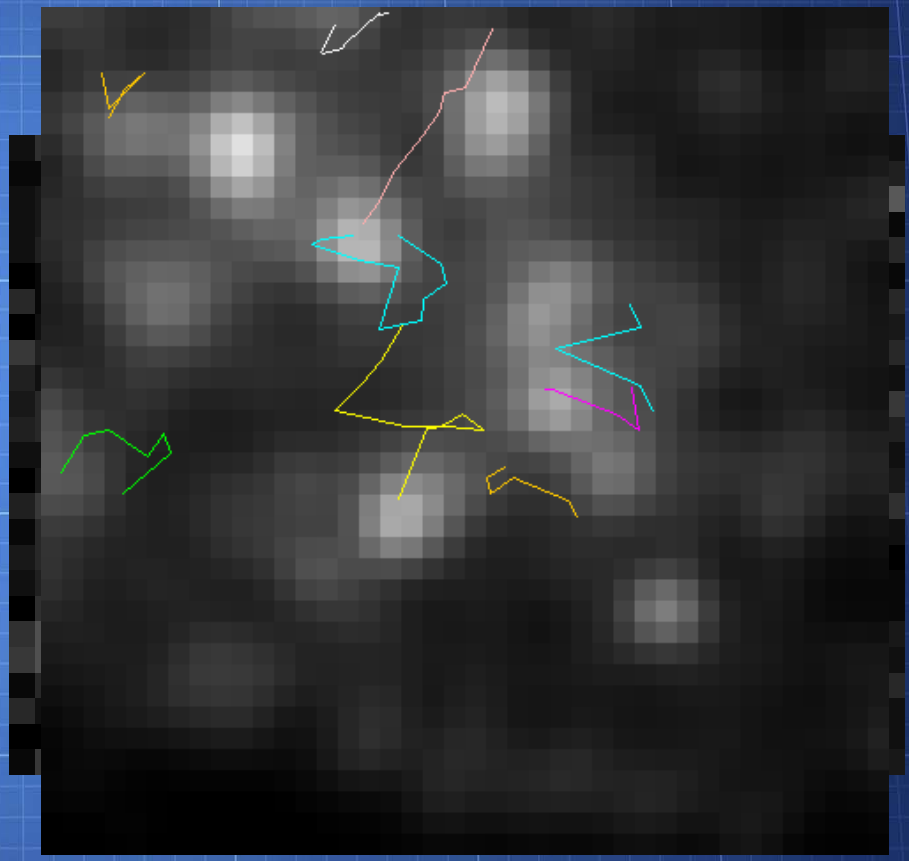


Single Molecule Fluorescence



Particle Tracking

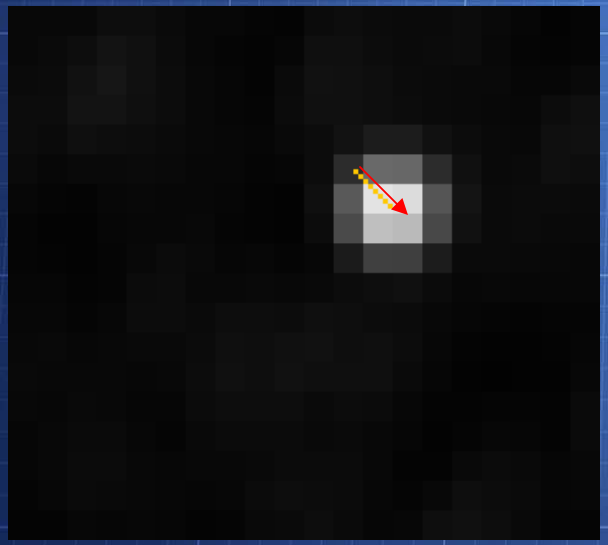
- *Tracking algorithm uses size, intensity, and relative position to trace particles from frame to frame*
- *Input variables such as threshold level, frame filters and maximum displacement remove background noise and unusable data*
- *Allows differentiation between species by physical observables such as diffusion, fluorescence intensity, and time to photobleaching*



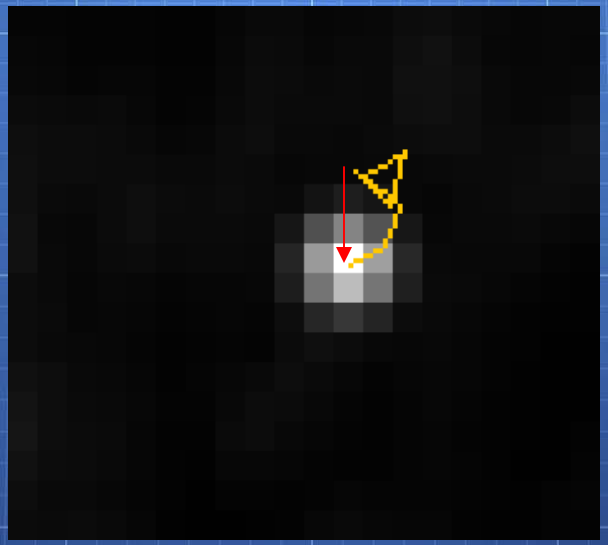
Two Dimensional Diffusion

$$MSD(t_{lag}) = \frac{1}{\sum_{t_i - t_j = t_{lag}}} \sum_{t_i - t_j = t_{lag}} (x(t_i) - x(t_j))^2$$

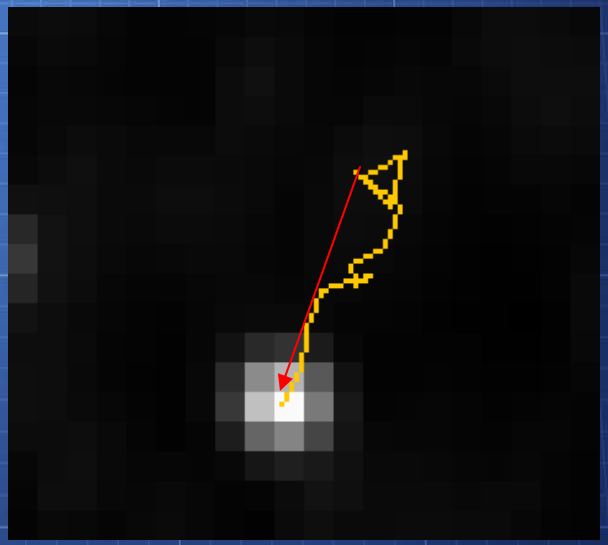
$$MSD(t_{lag}) = 4D_{lat} \cdot t_{lag}$$



1 Frame

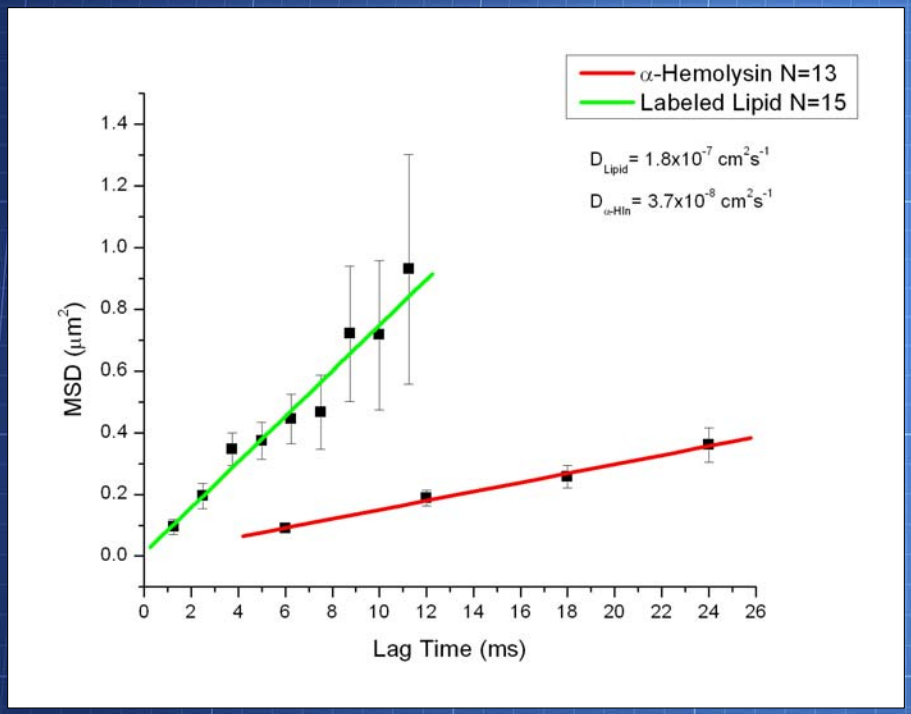


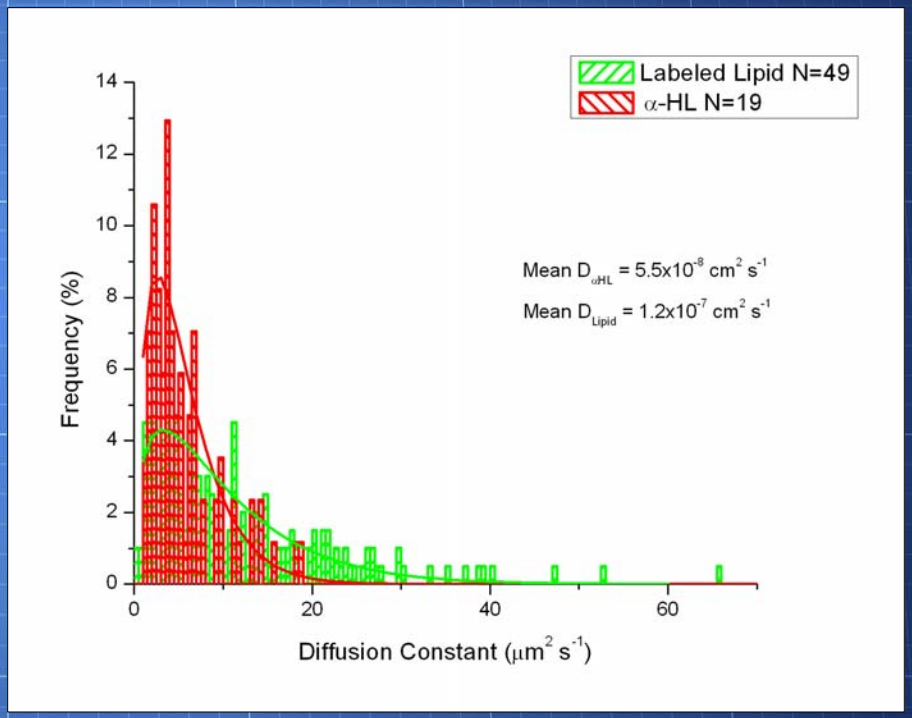
6 Frames



12 Frames

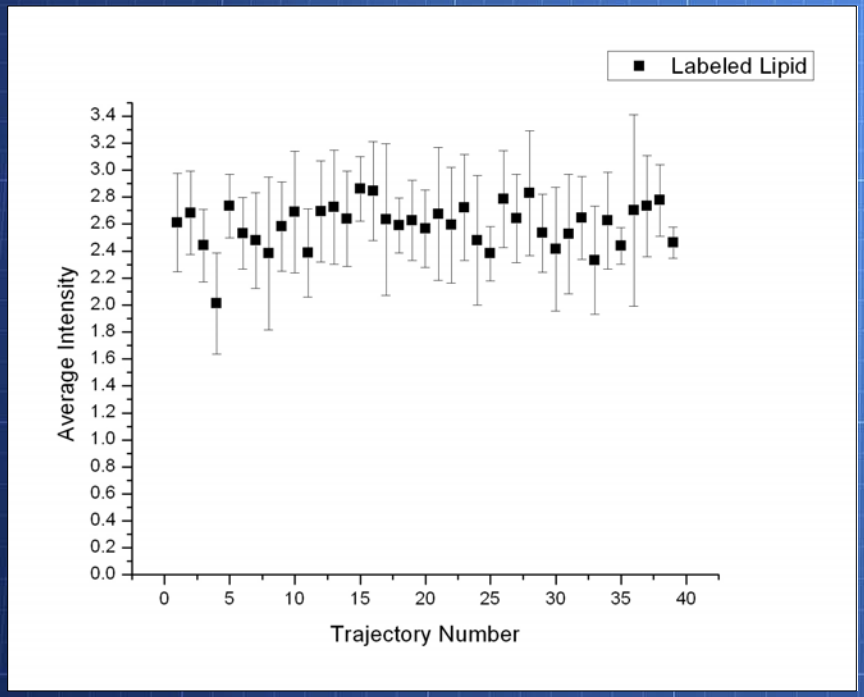
Diffusion Results

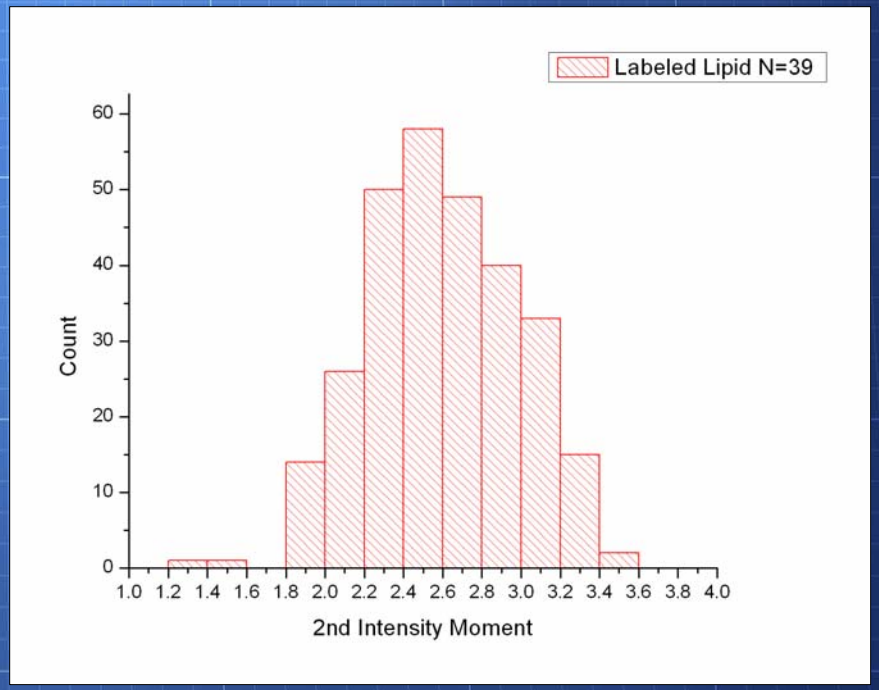




Fluorescence Intensity

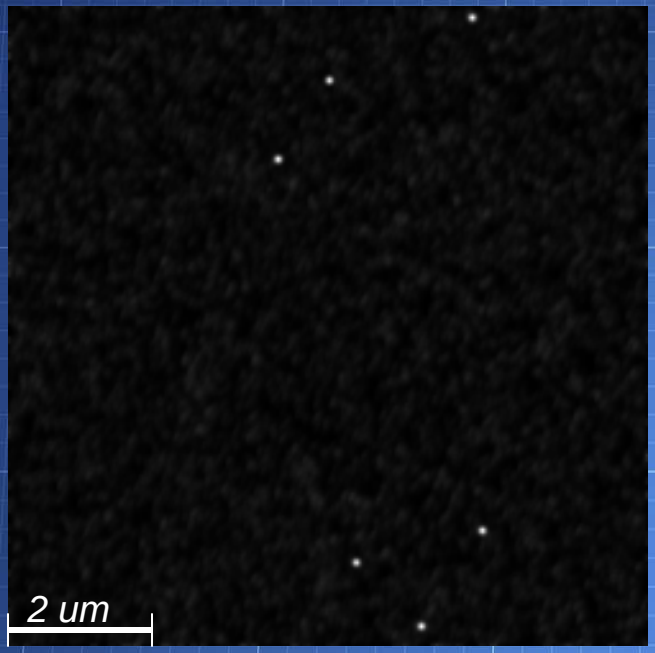
- Different types of fluorescing molecules should be distinguishable in a lipid membrane based on their unique intensity and standard deviation





One of these things is not like the other

1

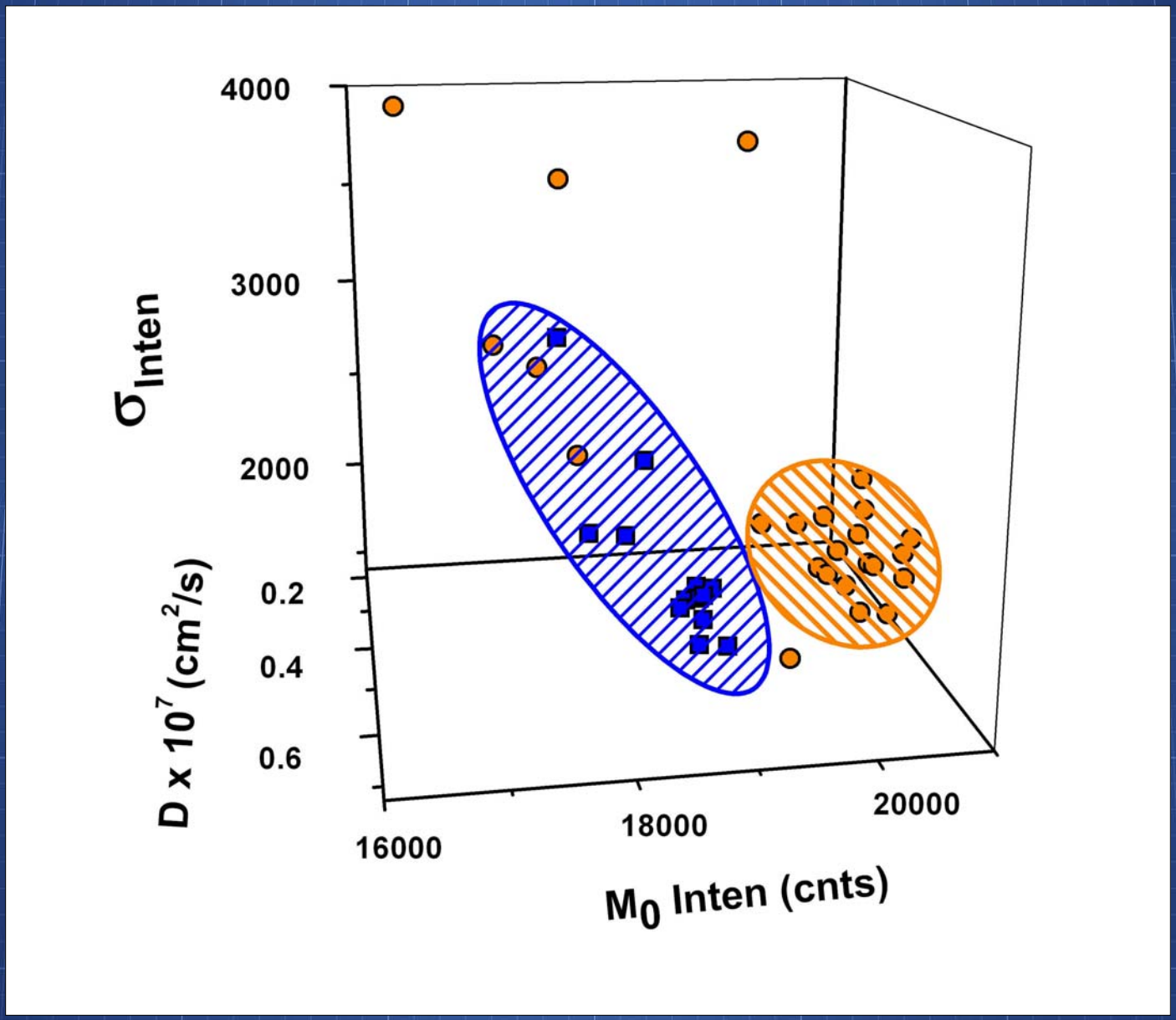


2

3

4

Pattern Recognition Technique



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