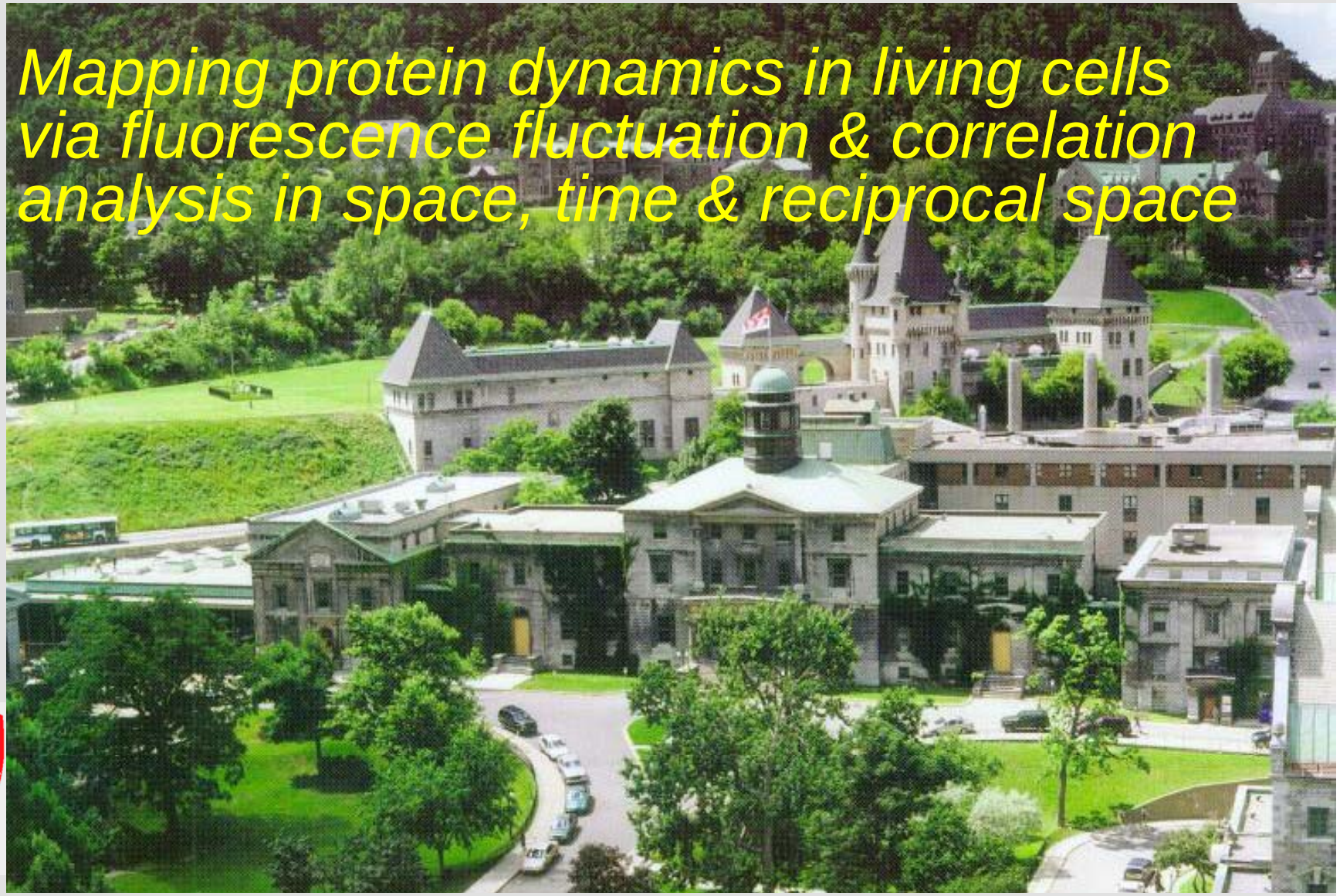
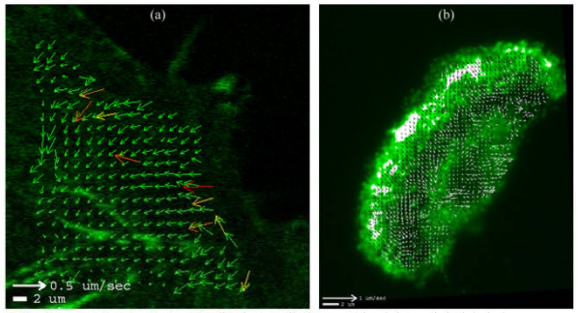
Mapping protein dynamics in living cells via fluorescence fluctuation & correlation analysis in space, time & reciprocal space

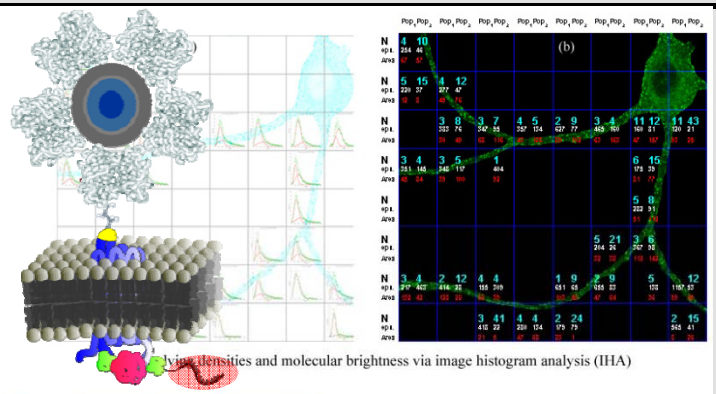


Paul W. Wiseman McGill University
Dept. of Physics, Dept. of Chemistry

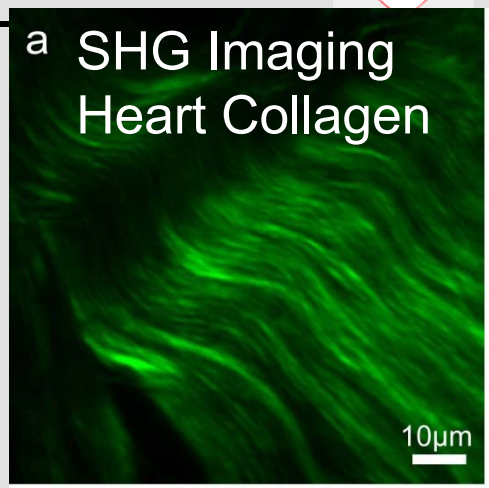
Wiseman Lab Biophysical Research McGill

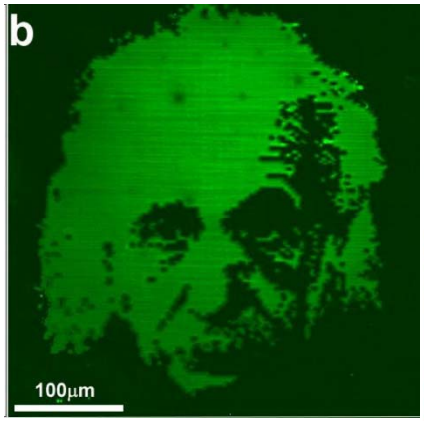


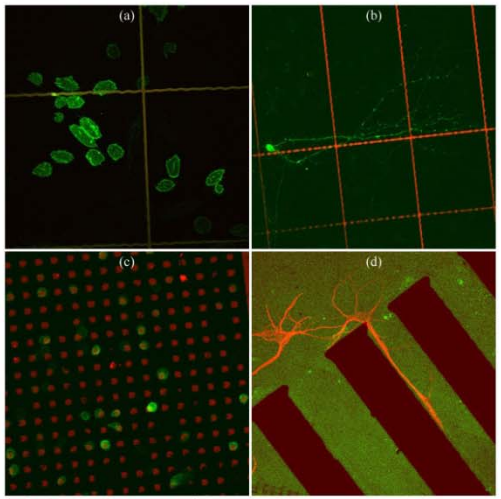
Velocity mapping analysis: (a) adhesion mediating protein alpha-actinin labeled with GFP in a retracting protrusion from a CHO cell; (b) quantum dots in a migrating keratocyte cell (cell movie courtesy of Dr. Julie Theriot Stanford University)

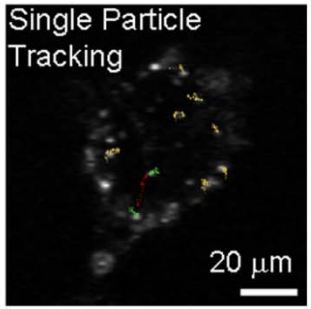


lying densities and molecular brightness via image histogram analysis (IHA)

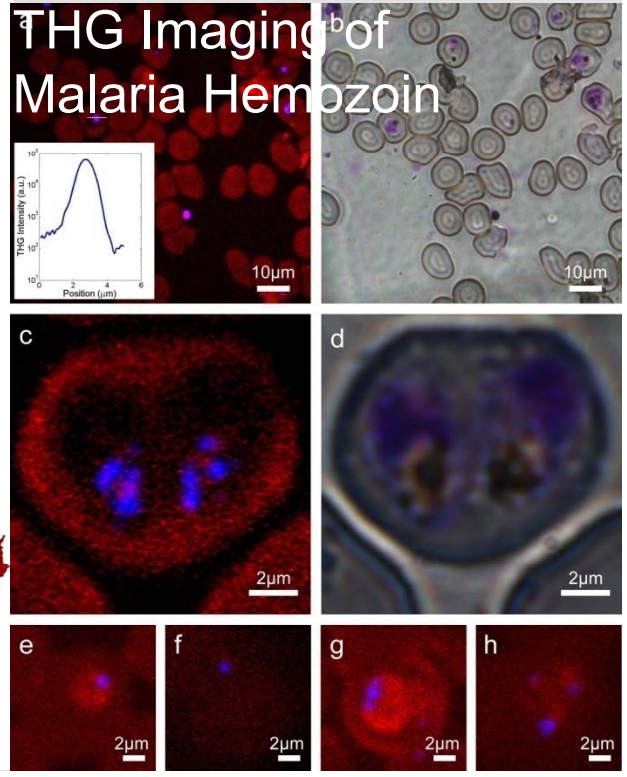




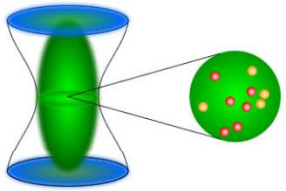


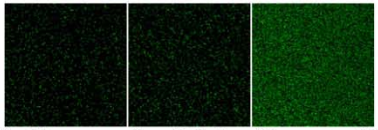


Single particle tracking (SPT) of quantum dots attached to EGF receptors in an NIH 3T3 fibroblast cell

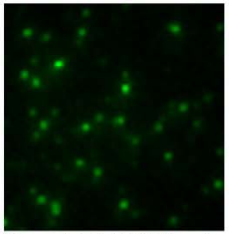


Two-color CLSM images of cells on red fluorescent grids:
(a) CHO cells expressing EGFP/α5-integrin; (b) rat hippocampal neuron (12 DIV) transfected with GFP; (c) CHO cells expressing α5-integrin/EGFP fusion constructs plated on a patterned substrate with a mixture of fibronectin and Alexa633 labeled human fibrinogen; (d) pattern of poly-D-lysine/EGFP on a glass substrate, allowing rat hippocampal neurons (immunostained for MAP2) to grow their neurites in specific corridors.

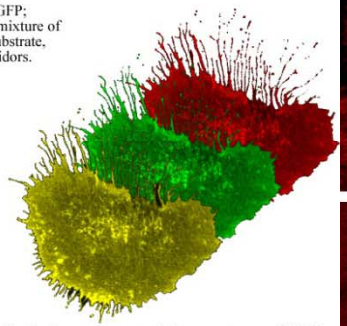




Resolving monomer-dimer distributions of fluorescent particles via high order moment analysis

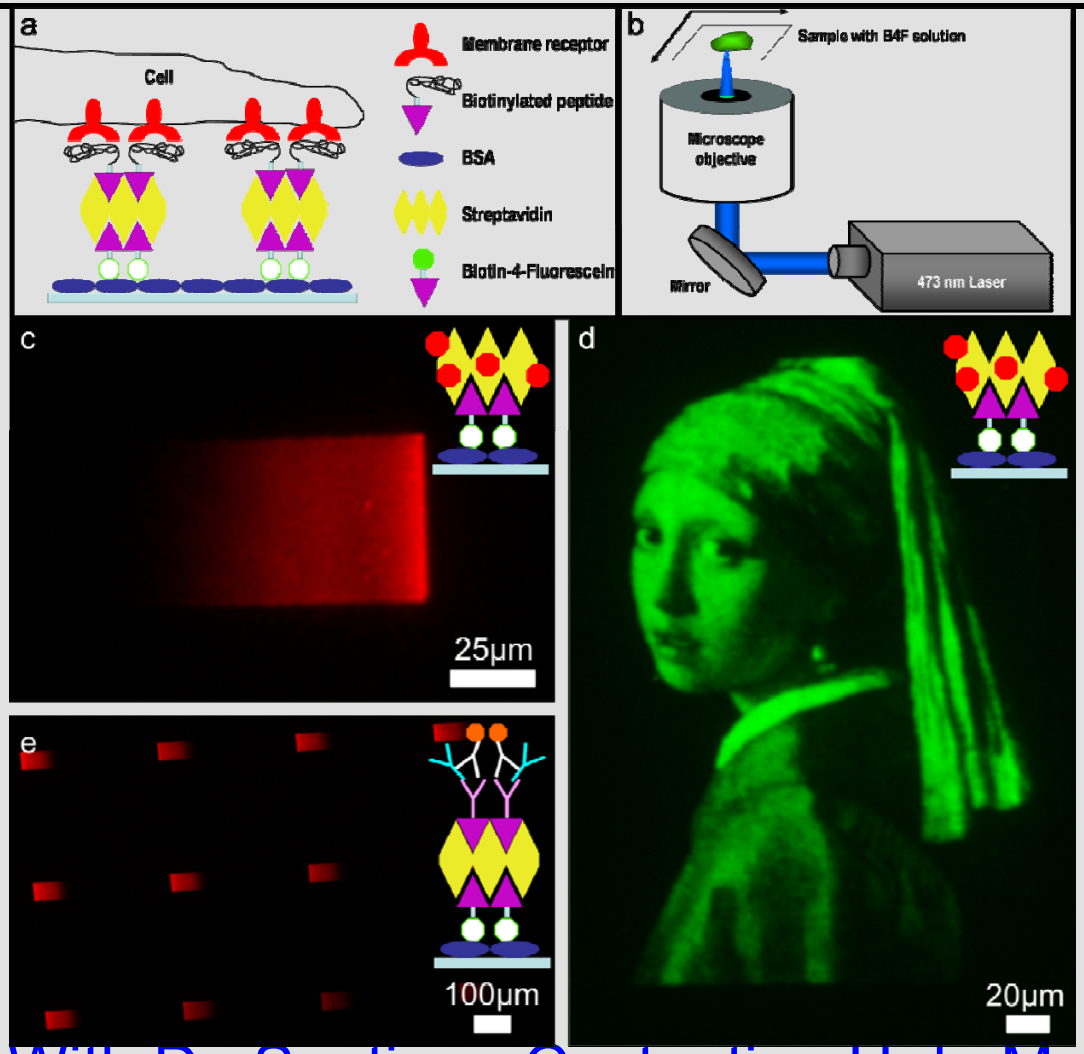


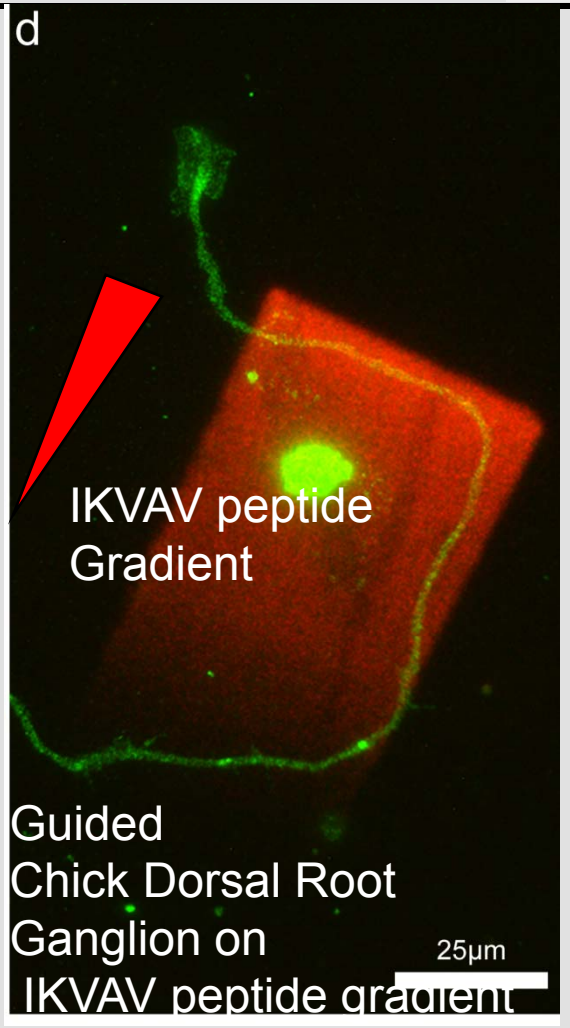
TIRF image of single CsSe/ZnS quantum dots imobilized on a glass substrate.



Colocalization studies by image cross correlation spectroscopy(ICCS)

Guiding Neurons on Patterned Protein Gradients





With Dr. Santiago Costantino U de Montreal
Dr. Tim Kennedy Montreal Neurological Institute
Belisle et al. *Lab on a Chip* in press

Goal: Measure the Biomolecular Dance

What are rules of the game within the Living Cell?

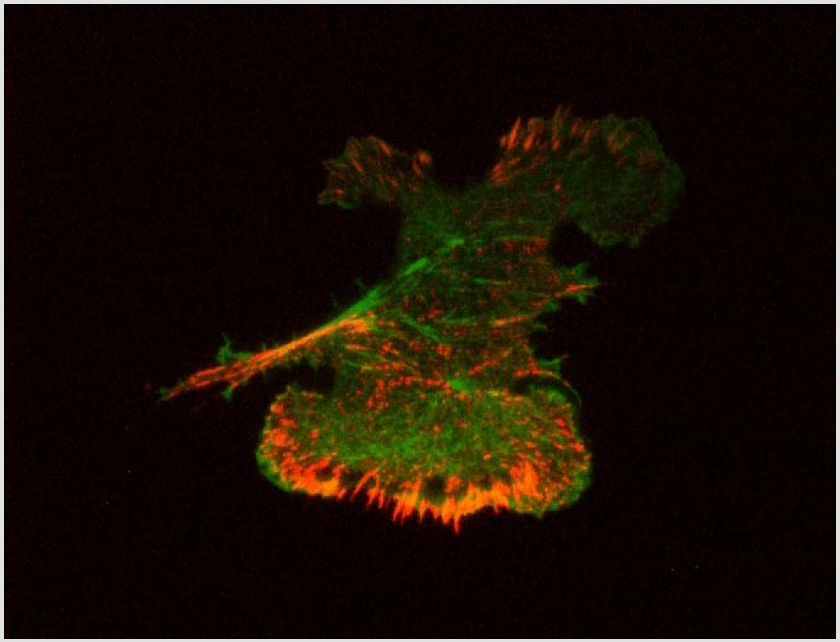
Physico-chemical Understanding

New Techniques are needed…

i) Spatio-temporal Image Correlation Spectroscopy (STICS)

ii) k-space Image Correlation Spectroscopy (kICS)

170 μm

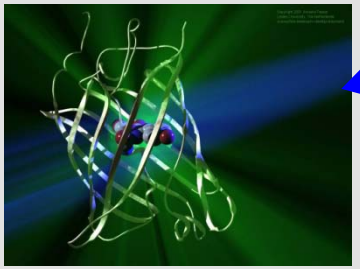


Paxillin-dsRed (red) &
α-actinin GFP (green)
in CHO Cell
TIRF Microscopy
Total time = 50 min δt =15 s

How do We Observe the Dance? Fluorescence

Green Fluorescent Protein genetic fusion construct to create a Protein with a fluorescent protein attached

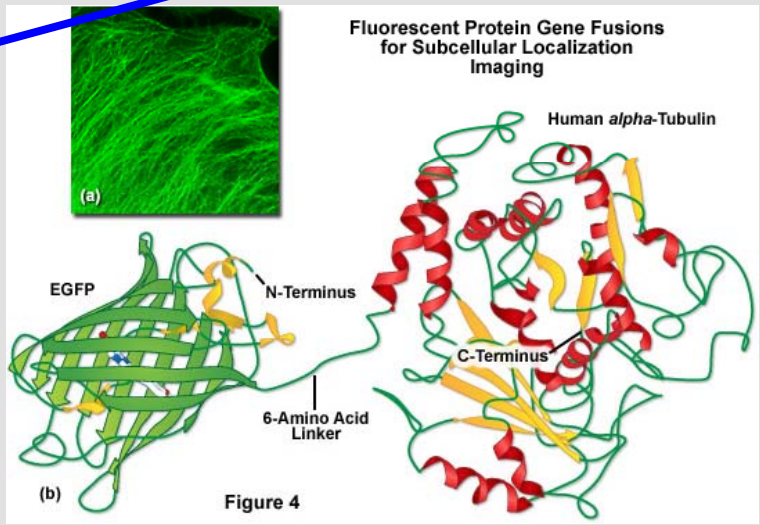
Excite GFP with focused laser light…Collect Fluorescence photons



~ 3 nm

λ_{ex} 488 nm

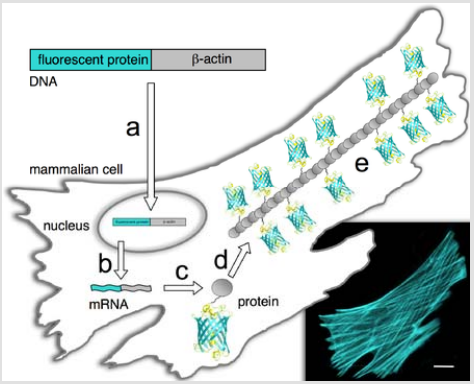
λ_{em} 509 nm

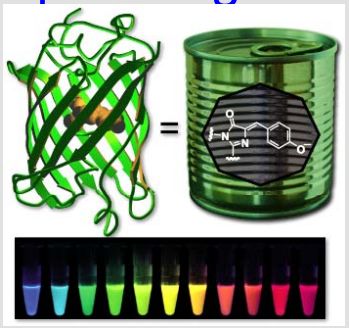


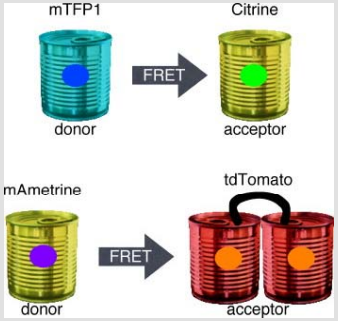
Emitting

Photobleached

From http://zeiss-campus.magnet.fsu.edu/articles/probes/fpintroduction.html





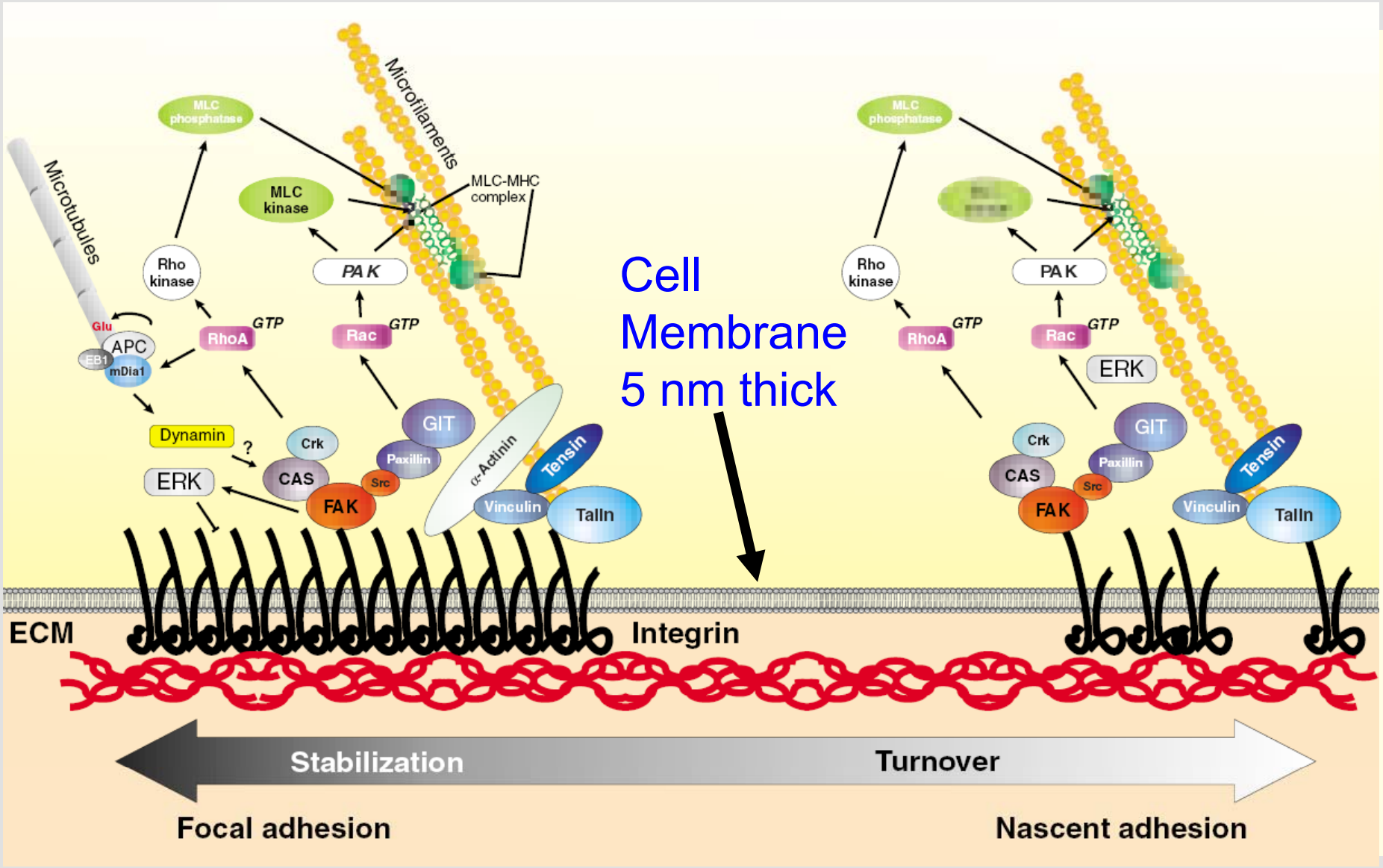


Many Color Variants

Dr. R. Campbell U of Alberta

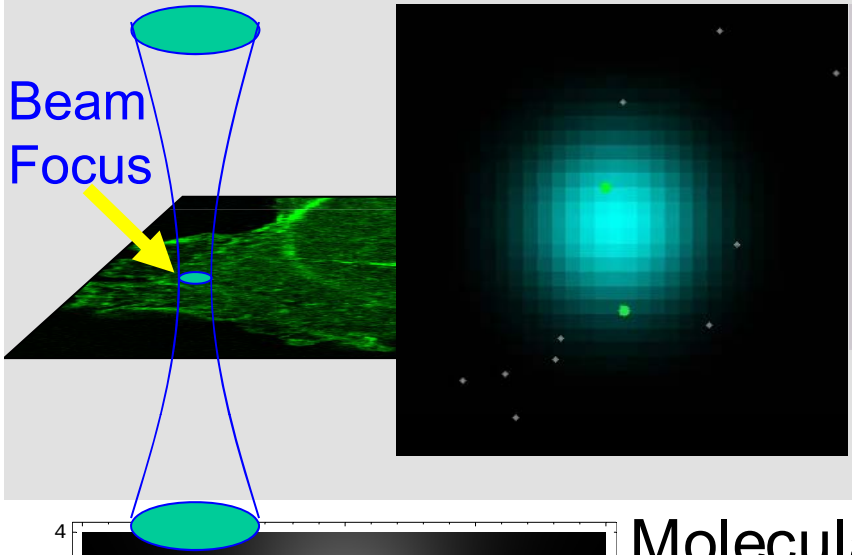
See: Fluorescent Proteins By Dr. R. Campbell (Scolarpedia)

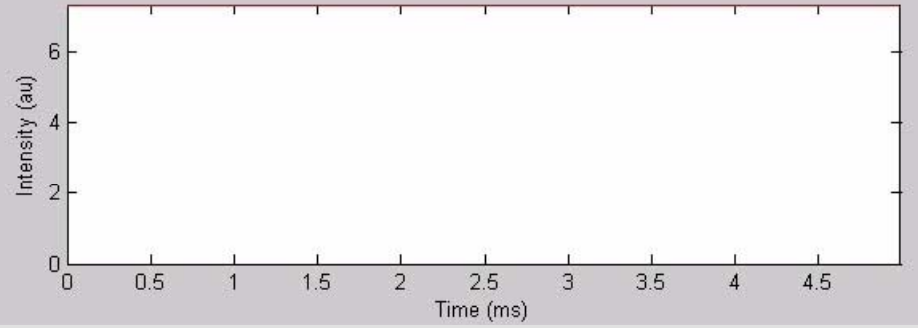
Focal Adhesion Proteins…Molecular Clutch

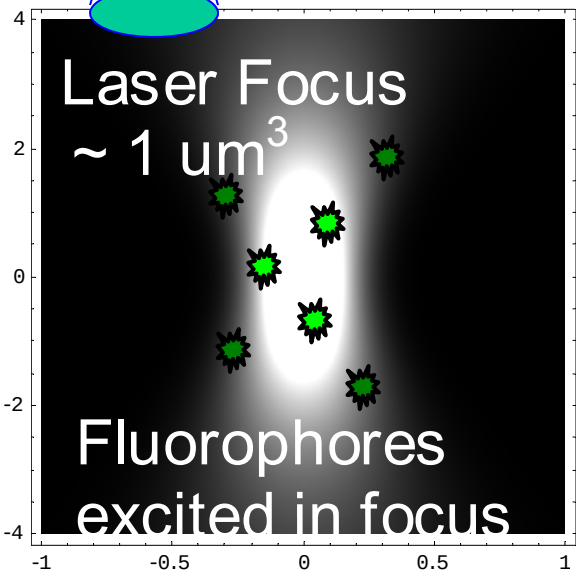


Vicente-Manzanares, M. et al. JCS (2005)

Fluorescence Correlation Spectroscopy

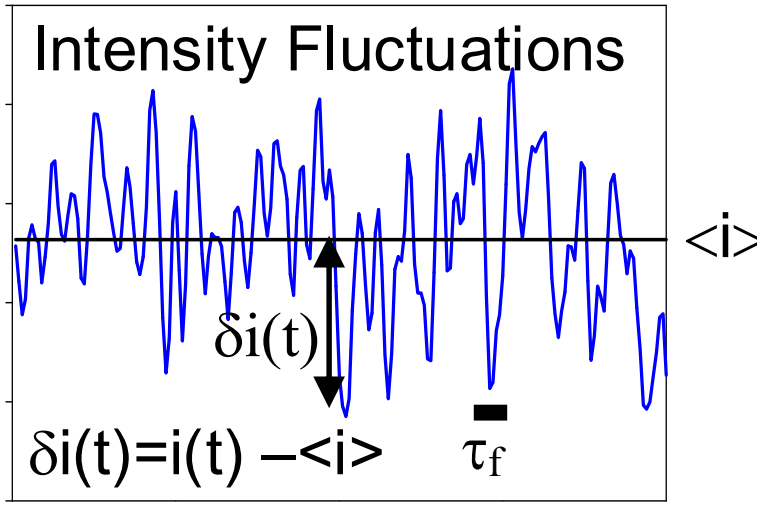




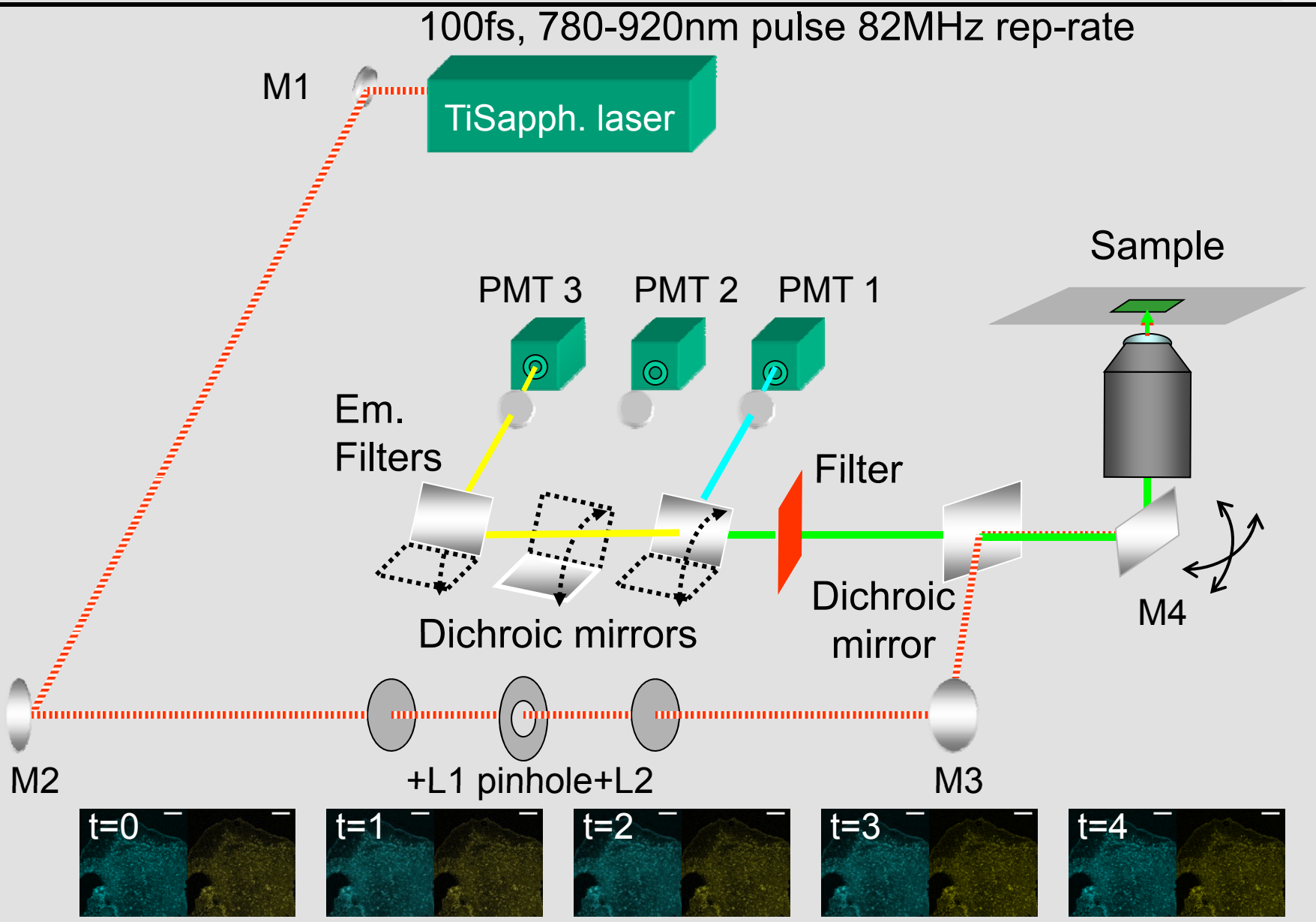


Molecular Dynamics

Number in the
Focus fluctuates

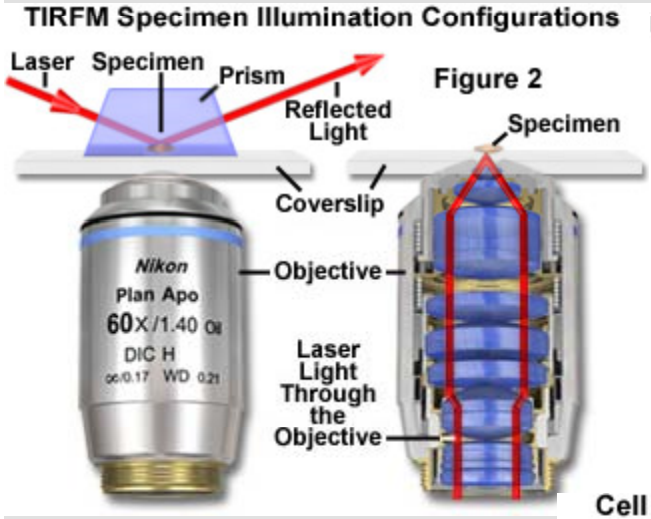


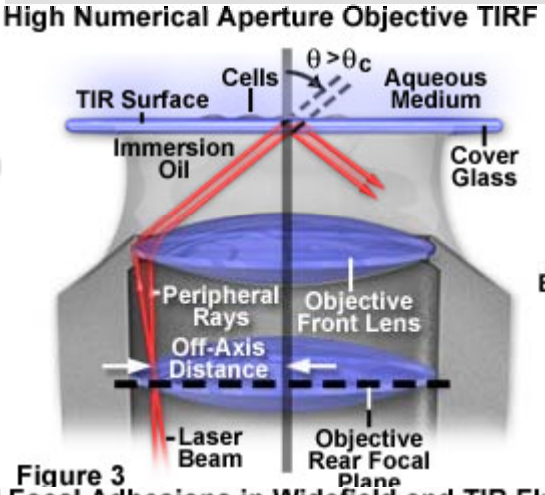
Laser-Scanning Microscopy…Imaging

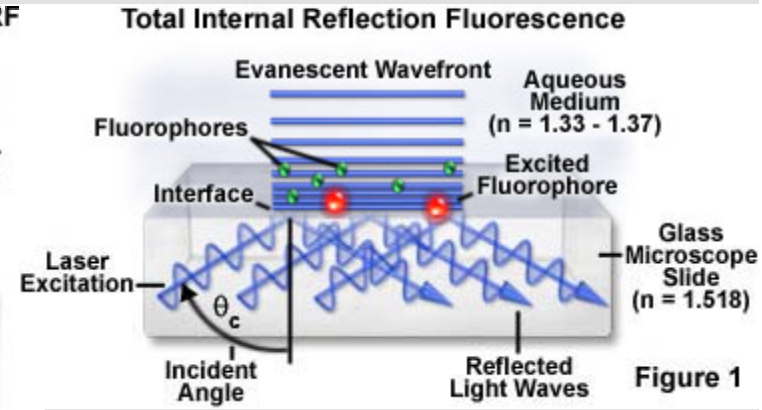


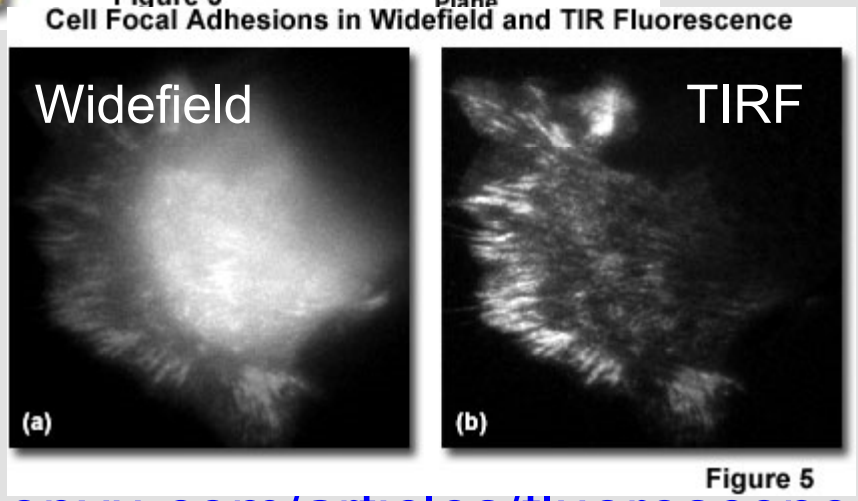
Evanescent Wave Fluorescence Excitation

- Total Internal Reflection Fluorescence Microscopy (TIRF Microscopy)





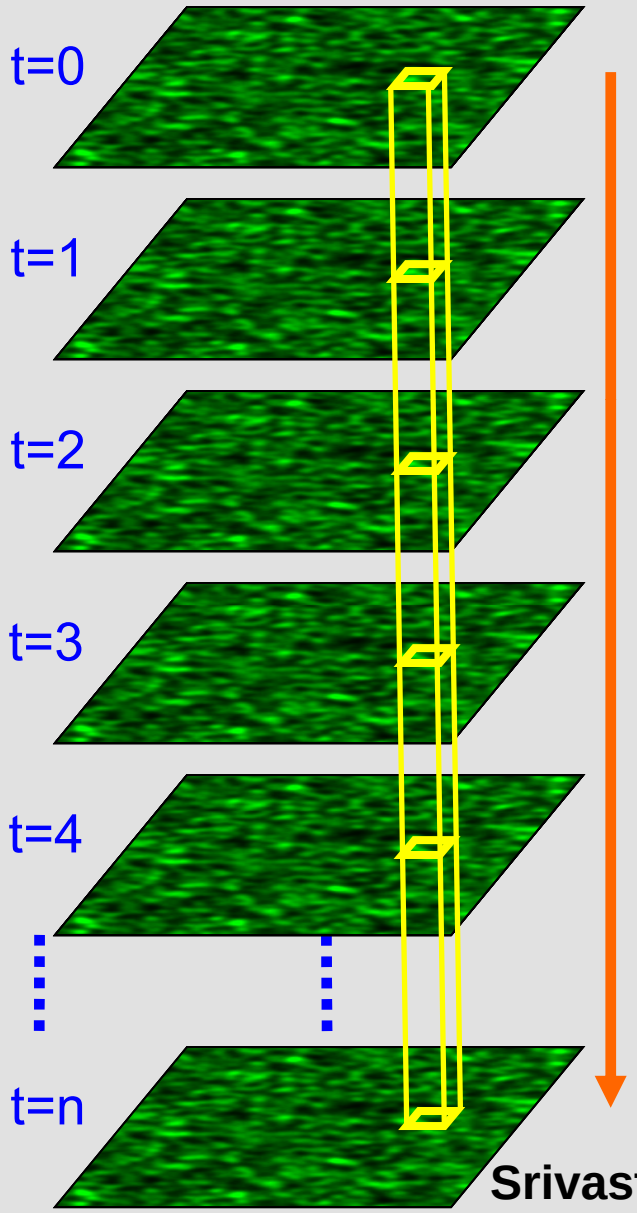




Z-Depth of field ~ 100 nm at the boundary

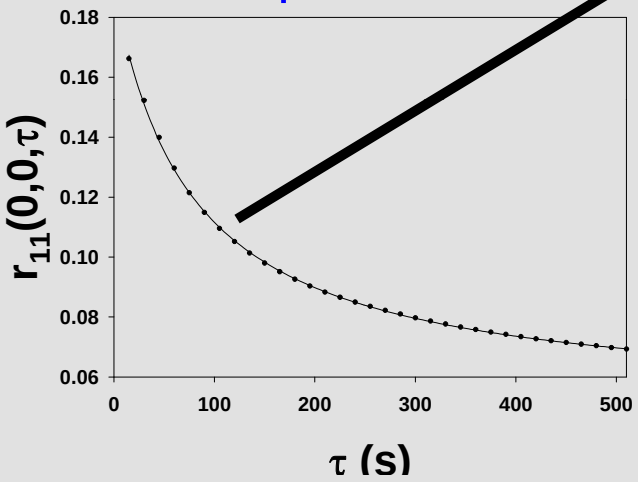
http://www.microscopyu.com/articles/fluorescence/tirf/tirfintro.html

Temporal Image Correlation Spectroscopy (TICS)



Temporal Autocorrelation
of $\delta i(x,y,t) = i(x,y,t) - \langle i \rangle$
Through Time Series

Temporal ACF



Decay
SlowTransport
Dynamics
Diffusion Coeff.
&
Flow Speeds

Offset
Immobile
Population

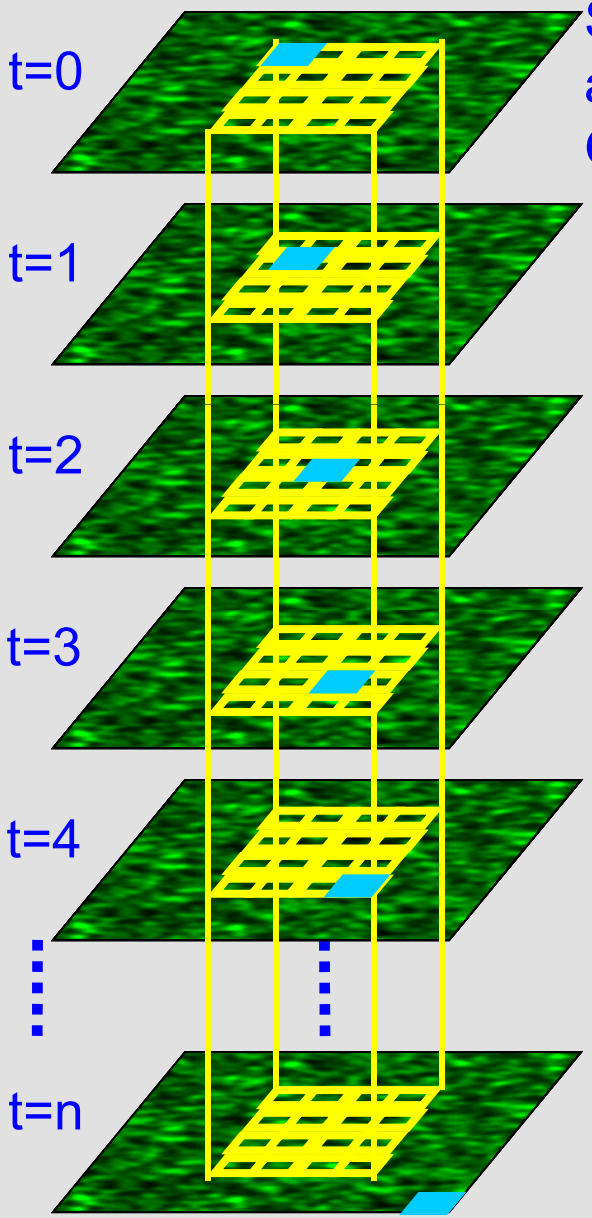
$$r_{11}(0,0,\tau) = \frac{\langle \delta i(x,y,t)\, \delta i(x,y,t+\tau) \rangle}{\langle i \rangle_t \langle i \rangle_{t+\tau}}$$

Srivastava and Petersen *Methods Cell Sci.* 18, 47-54 (1996)

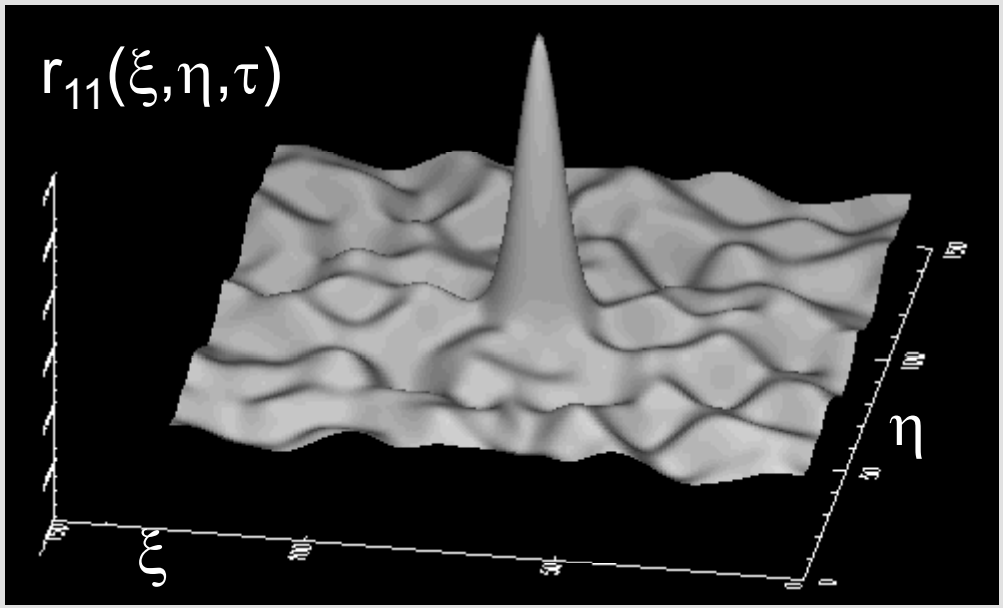
Overview for Talk

- Spatio-Temporal Image Correlation Spectroscopy
- k Reciprocal Space Image Correlation Spectroscopy (kICS)

Spatio-Temporal Image Correlation Spectroscopy

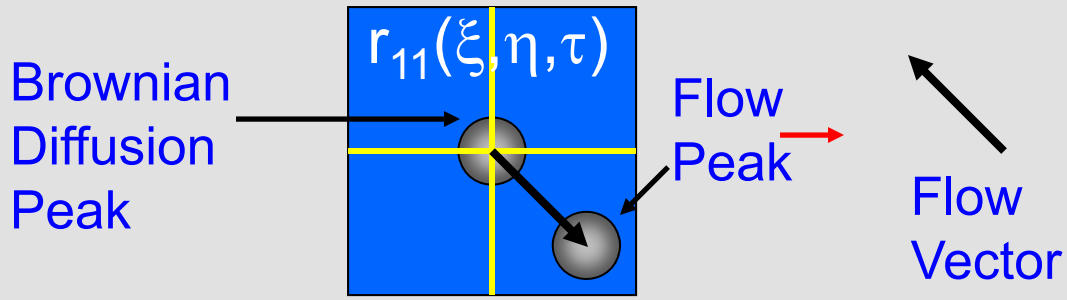


Spatial Correlation as a function Of Time

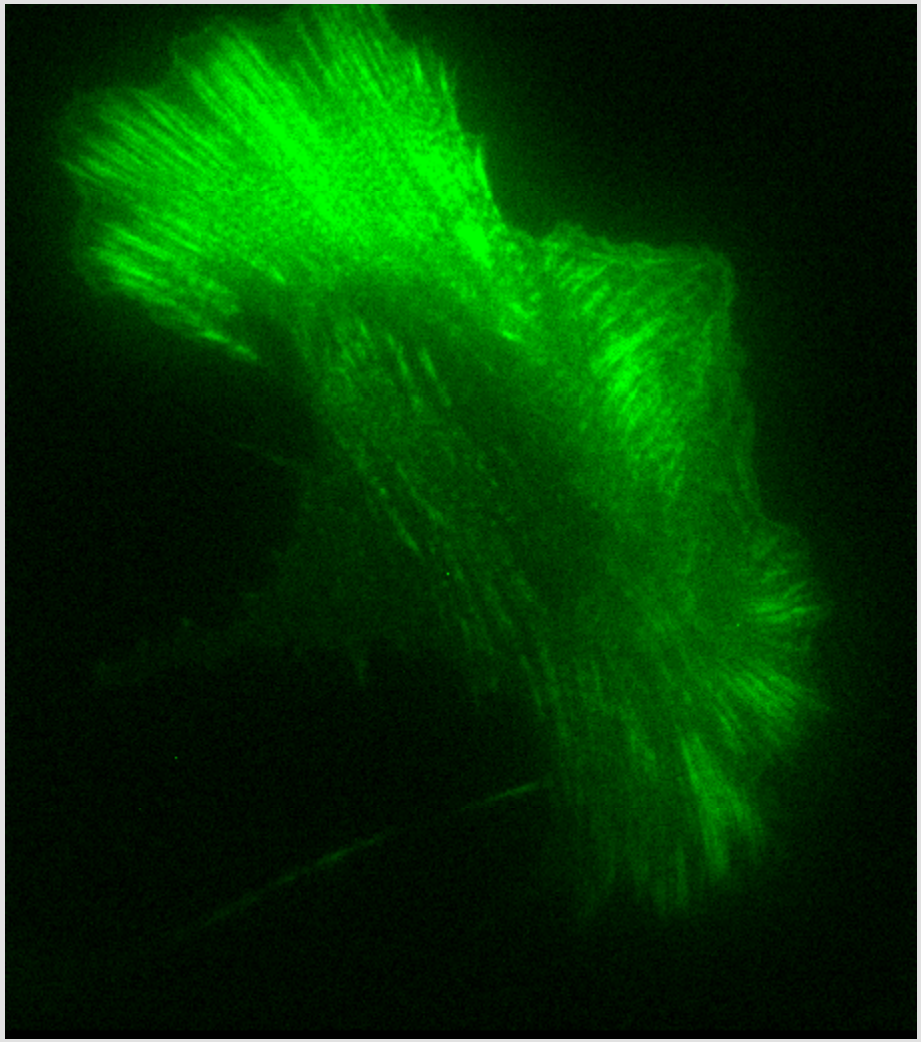


$$r_{11}(\xi,\eta,\tau)=\frac{\langle \delta i(x,y,t)\,\delta i(x+\xi,y+\eta,t+\tau)\rangle}{\langle i\rangle_t\,\langle i\rangle_{t+\tau}}$$

Hebert et al. Biophys. J. 88-3601 (2005)

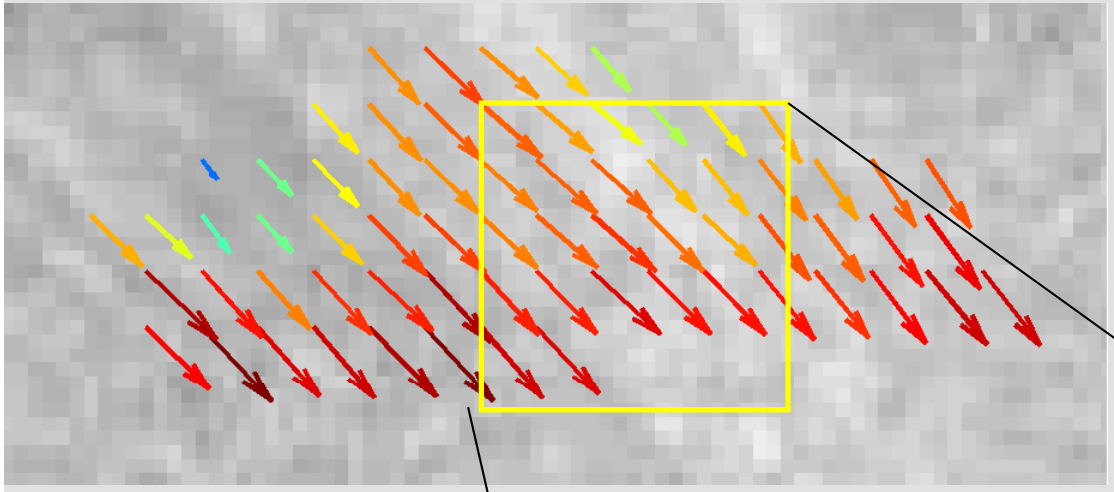


Vector Maps of α-actinin MEF Cell



TIRF Microscopy Time 100 s with Images sampled at 0.1 Hz
Dr. Claire Brown and Ben Hebert

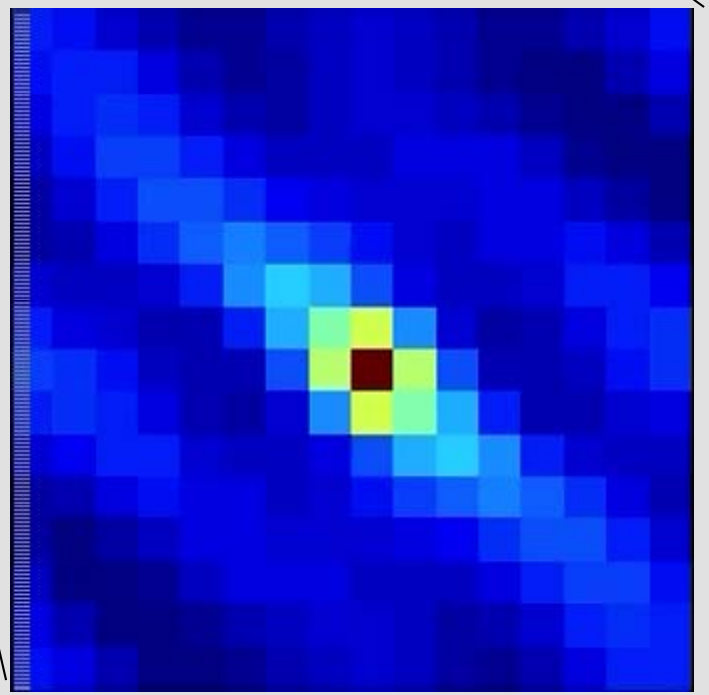
Vector Maps of α-actinin



α-actinin/EGFP in MEF Cell
TIRF Microscopy

$r(\xi,\eta,\tau)$
Immobile
Filtered

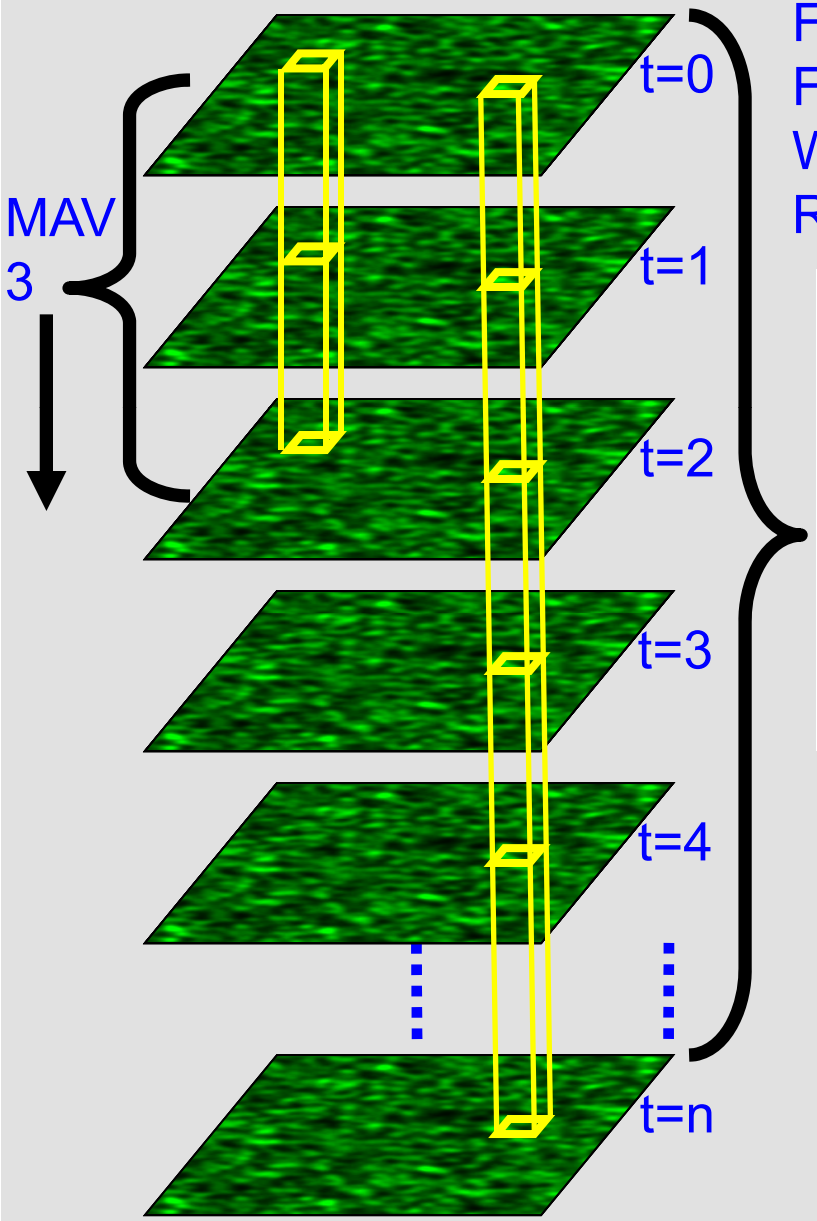
3.4 μm



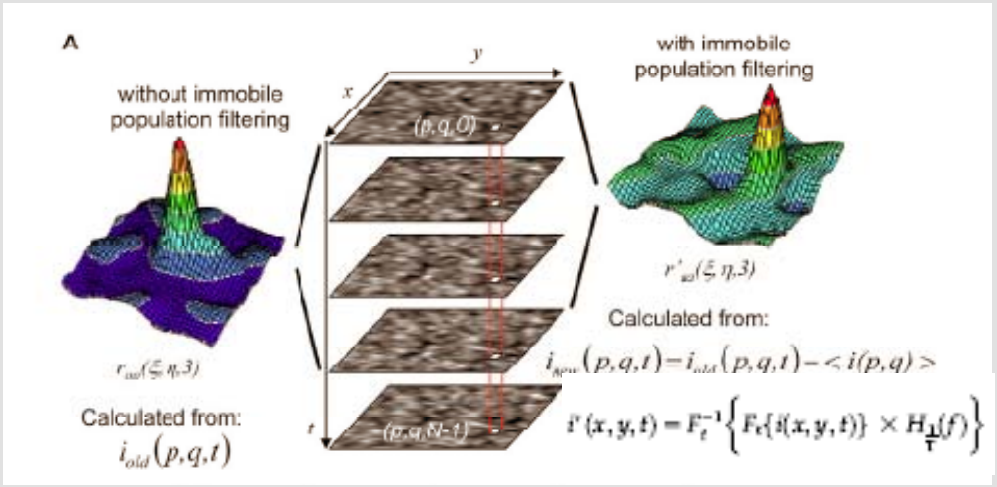
Accelerated
40 times faster
than
Real-time

$\tau = 0$ s ➔ 200 s

Correcting for Immobile or Slowly Moving Species



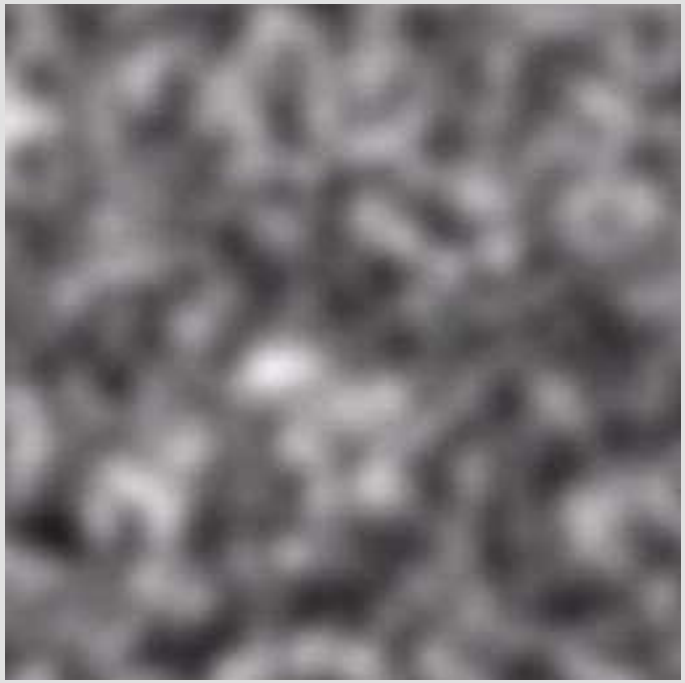
Fourier Filtering (Full Time Stack)
Filter pixel stacks in temporal frequency domain
With Heaviside Function before STICS analysis
Removes Immobile Features



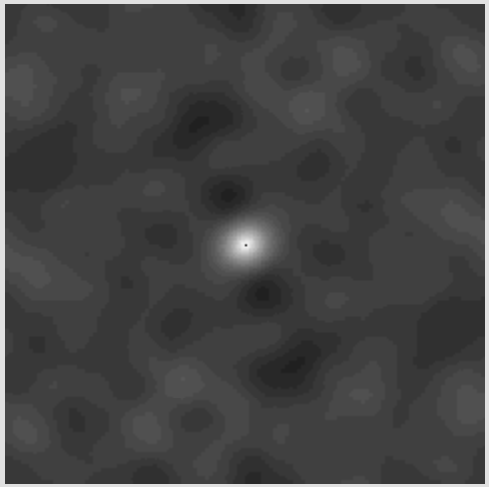
Moving Average Filter Odd # Frames
Applies Immobile Filtering over Shorter
Time Window

Space Time Correlation for Mobile Fraction

- Filter Immobile in Frequency Space → $r(\xi,\eta,\tau)$ Mobile Fraction



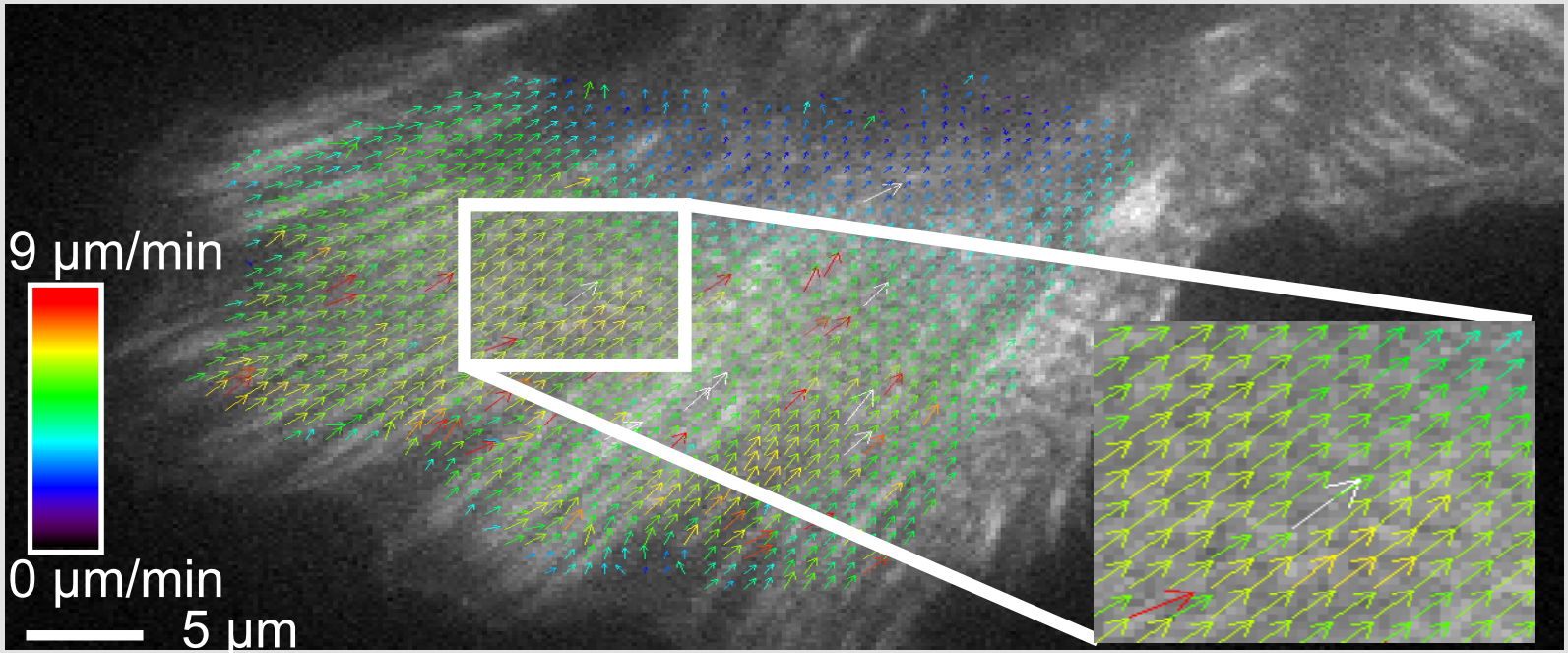
- Simulation:
  90% immobile,
  10% with flow + diffusion
  in x and y

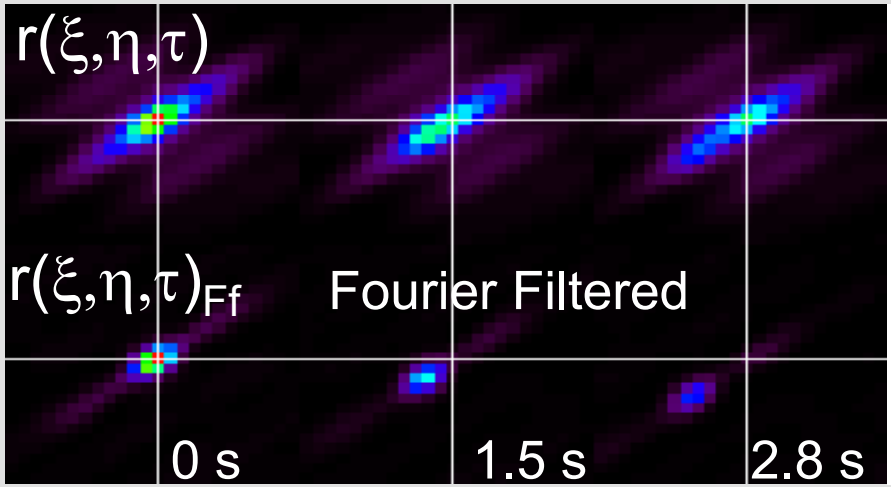


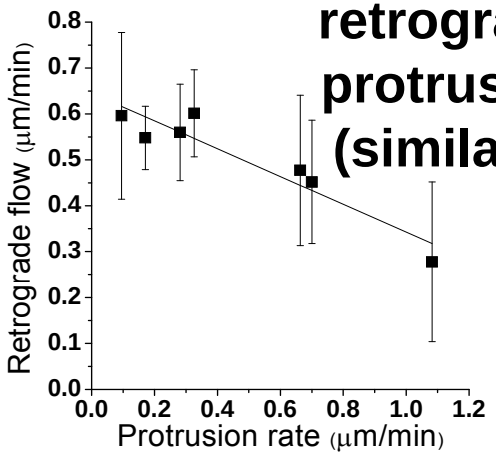
2D Grey Scale Contour Map
Spatial Correlation Function(τ)

$r(\xi,\eta,\tau)$ vs. τ
For Mobile Fraction

Vector Maps of α-actinin

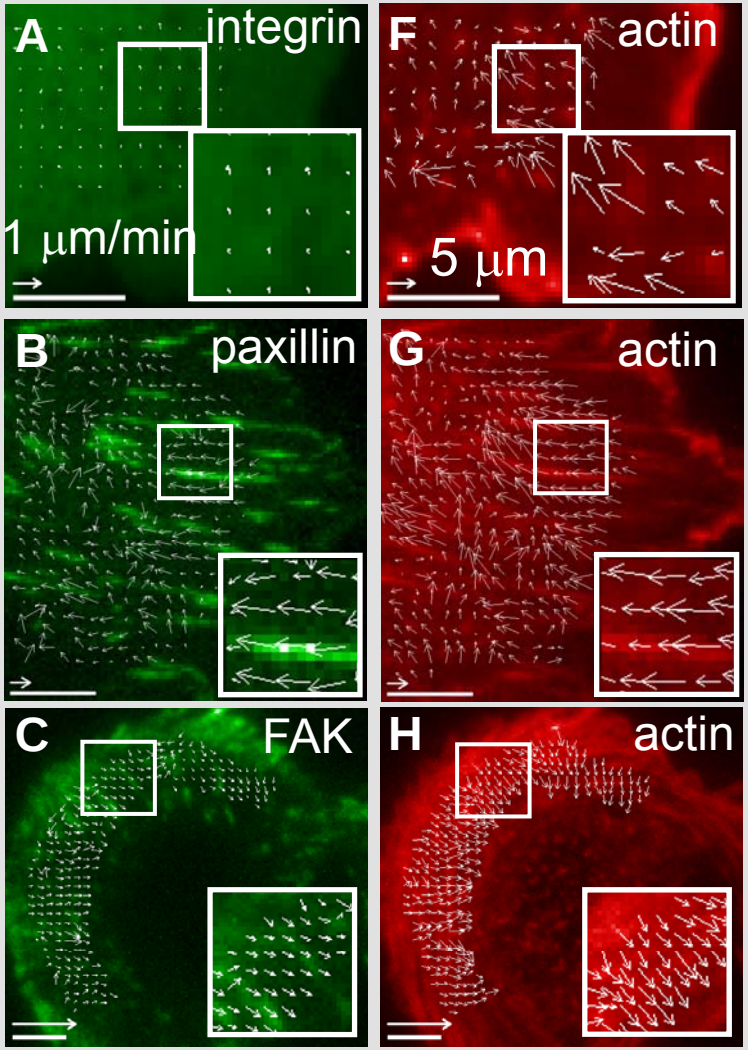


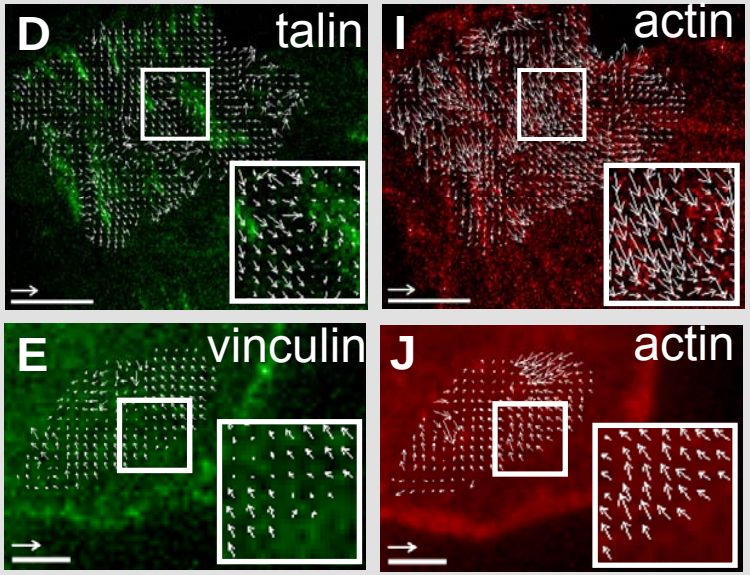


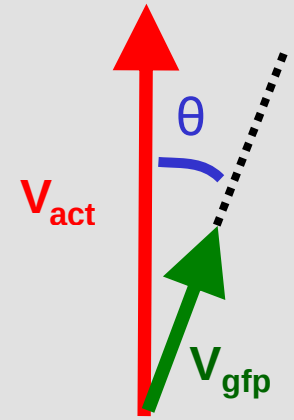


Inverse relationship b/w retrograde flow & protrusion speed (similar to actin)

Co-Transport of Adhesion Molecules & Actin

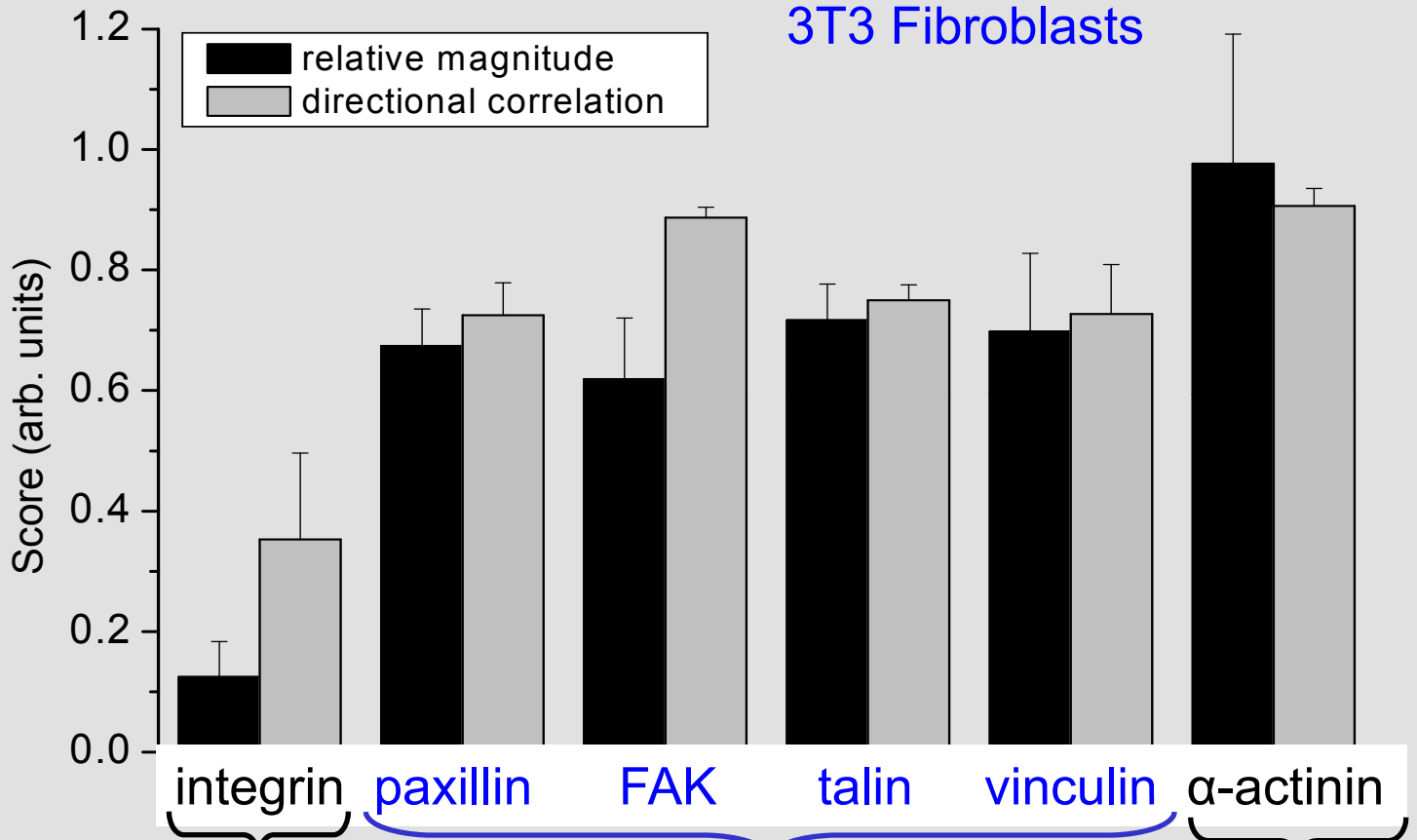






Compare every
Vector in Ch1 & Ch2
For magnitude &
Direction correlation

Correlated Transport Properties



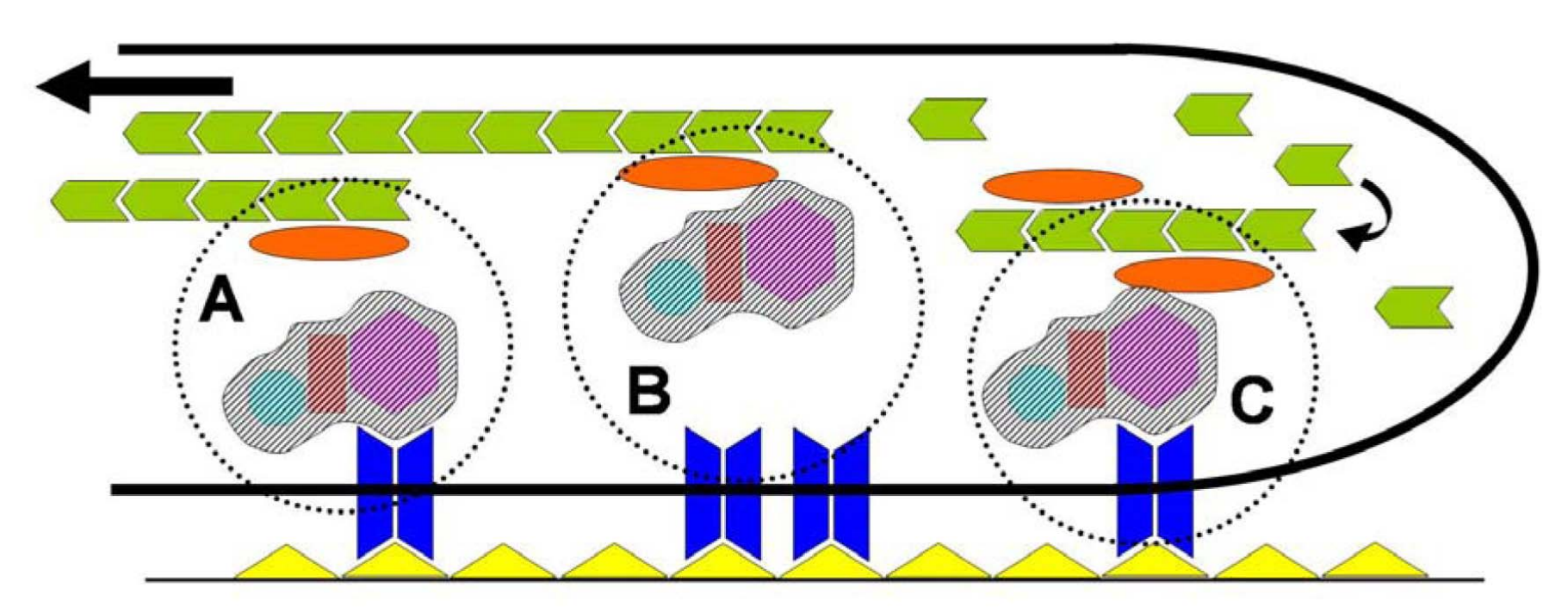
very low magnitude & low directional correlation

Intermediate ~ 70% level

high magnitude & directional correlation

→ pax-FAK-talin-vinculin are part of a distinct linkage complex

Emerging picture of linkage



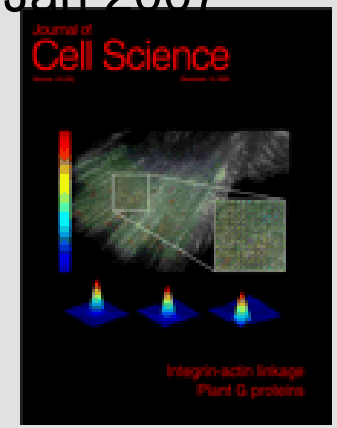
Brown et al. JCS (2006) 119: 5204-5214
Faculty of 1000 Selection Jan 2007

⟨-actinin

actin

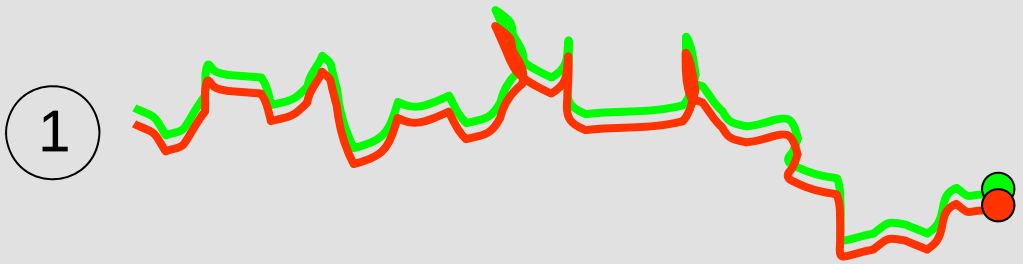
integrin

ECM

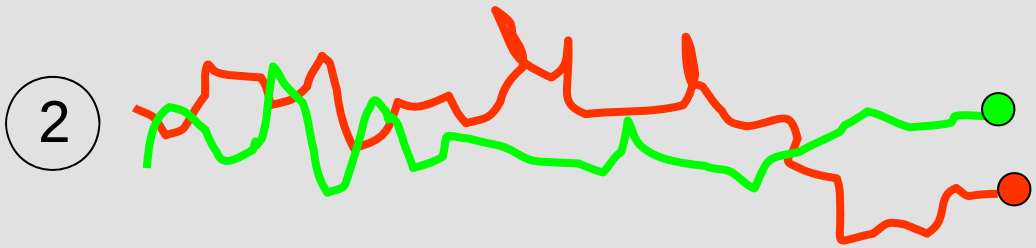


See Also Ke Hu, et al.*Science* 5 January 2007: 111-115

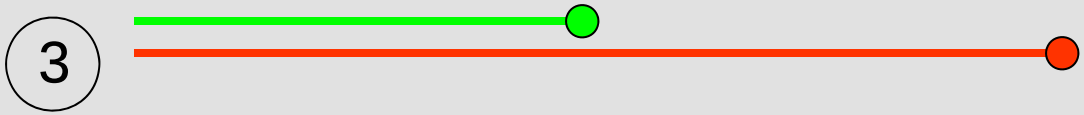
Microscopic basis for similar flow fields



Always co-localized



Same speed and direction,
not co-localized

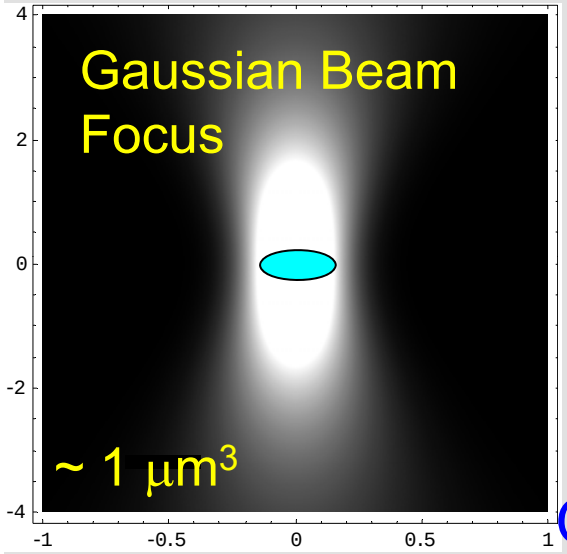


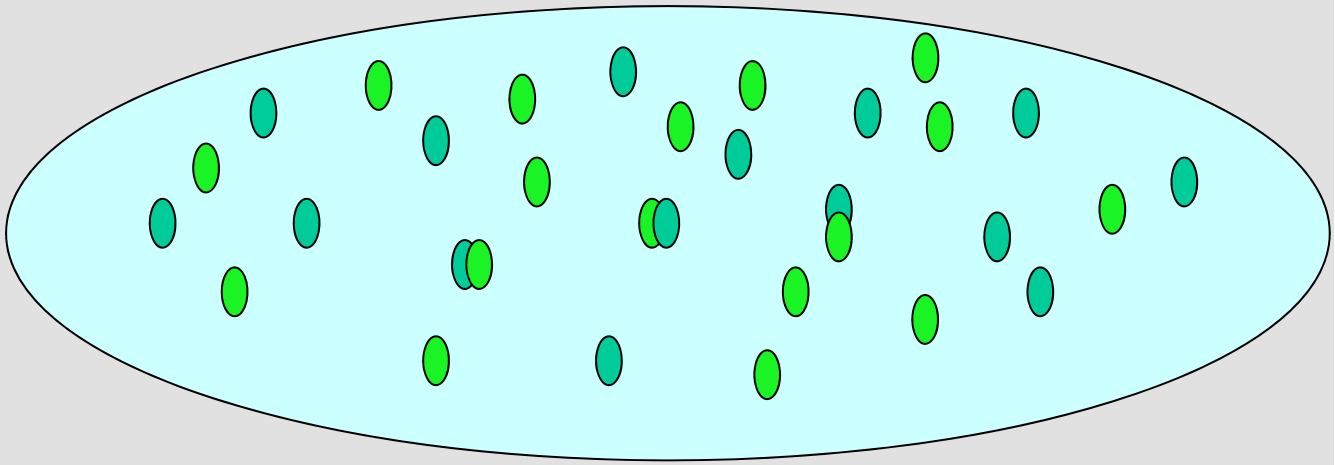
Same direction,
different speed

Transient binding/unbinding
and co-transport

Mapping the Dance Partners…

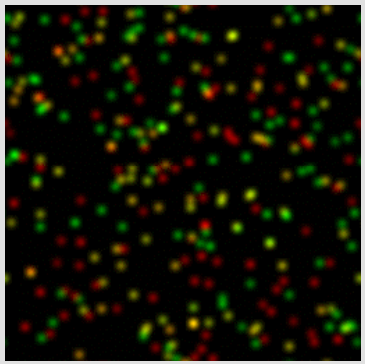
Challenge
Trying to Resolve the Molecular Dance Partners



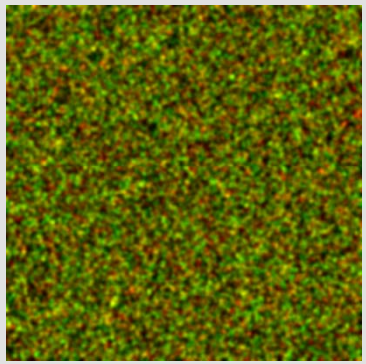


Optical Resolution ~ λ/2 Macromolecules ~ λ/50

λ = Wavelength ~ colour of the light



vs.

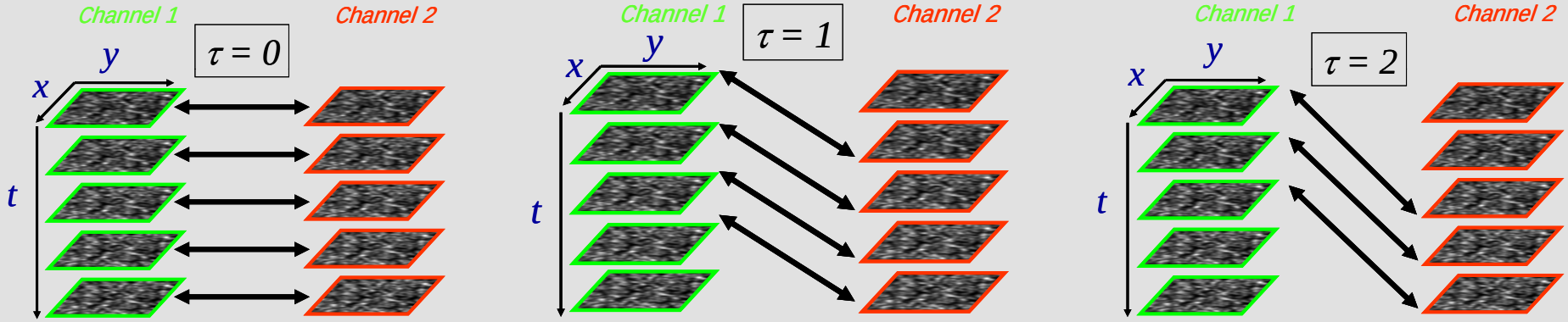


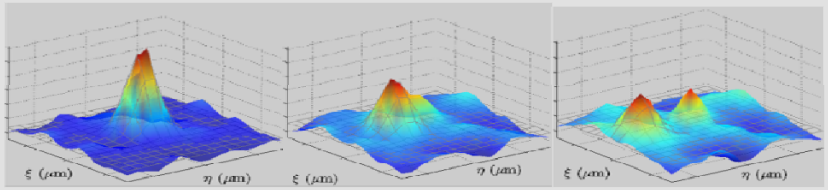
Cross-correlation

Leafs Habs

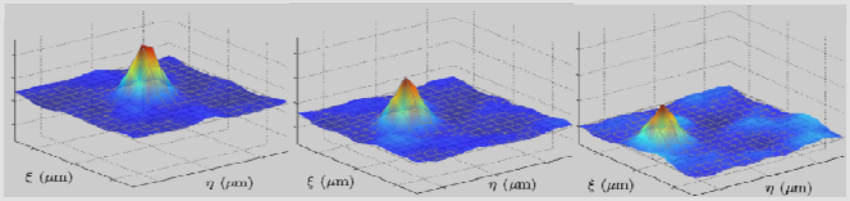
STICCS: two color cross-correlation

$$r_{12}(\xi,\eta,\tau) = \frac{\langle \delta i_1(x,y,t)\,\delta i_2(x+\xi,y+\eta,t+\tau)\rangle}{\langle i_1\rangle_t \langle i_2\rangle_{t+\tau}}$$





With Immobile Filtering

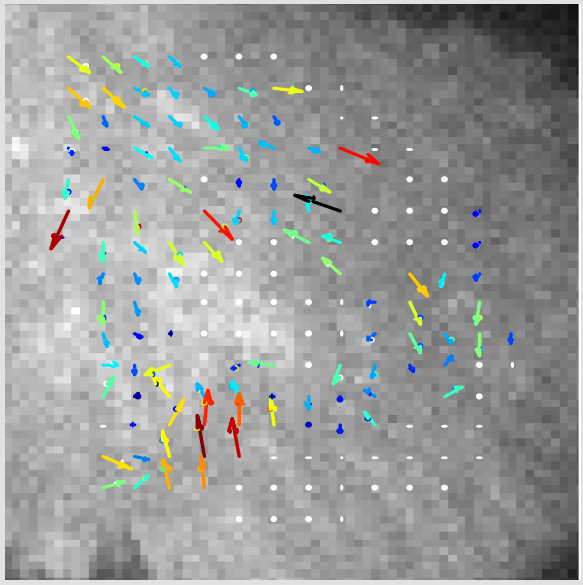


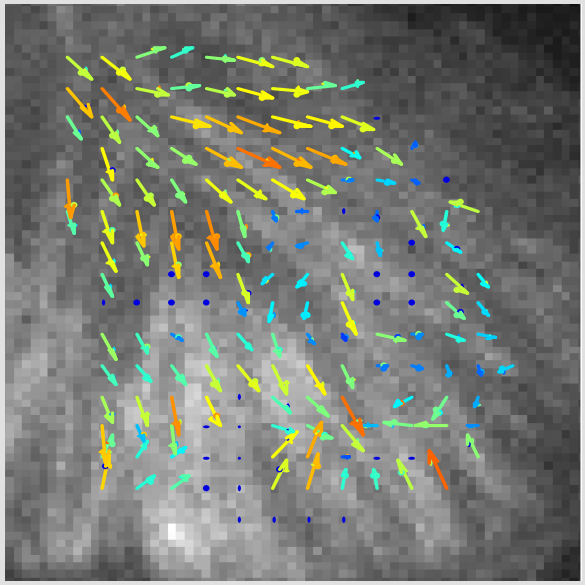
No Cross-Correlation between integrin & actin

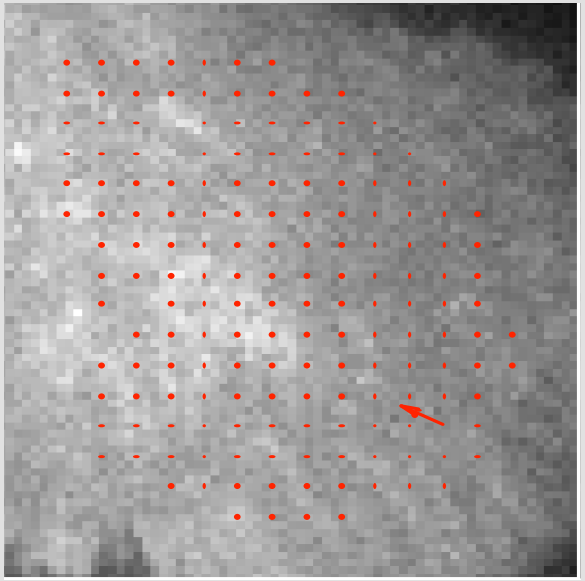
α5-integrin EGFP

mRFP-actin

Cross Correlation







v = 0.14 μm/min

v = 0.18 μm/min

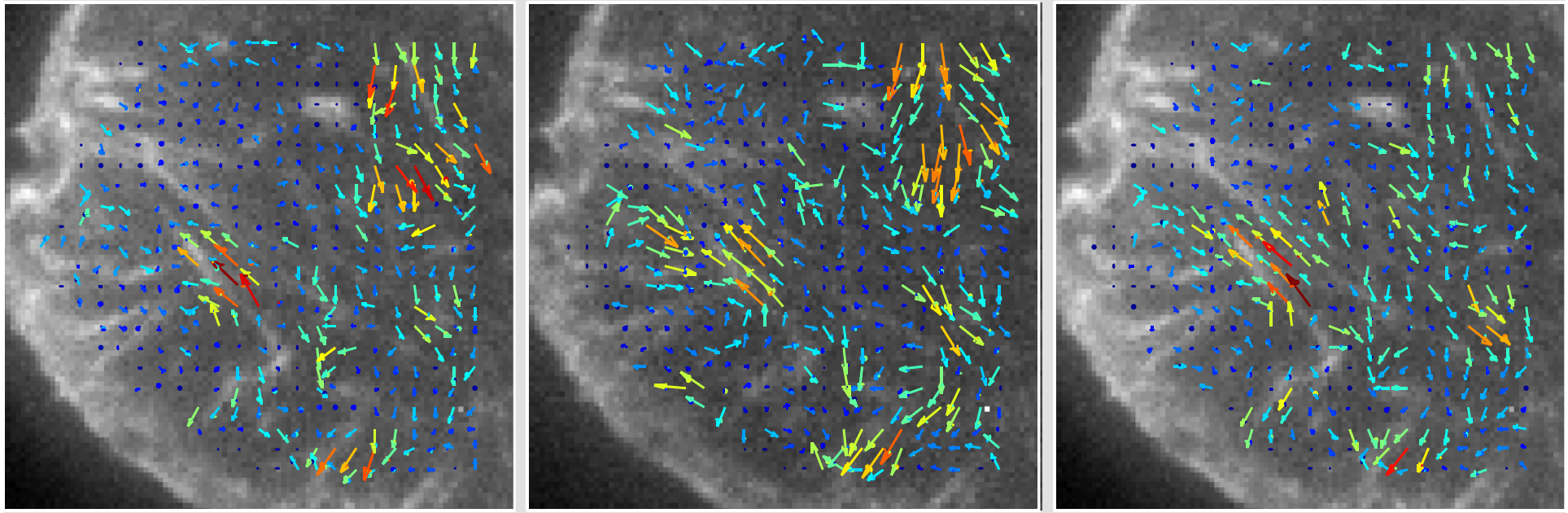
Positive Control

Actin-GFP, actin-mRFP MEF cell

GFP Autocorrelation

"RFP" Autocorrelation

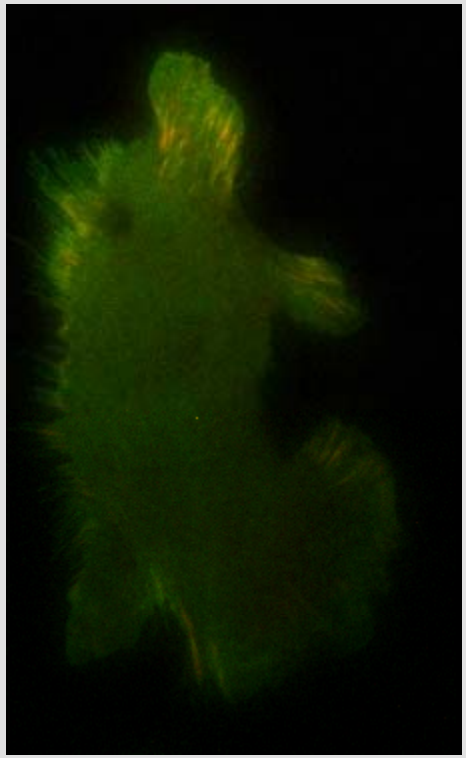
Cross-correlation



Strong, consistent GFP, RFP autocorrelations, and cross-correlation

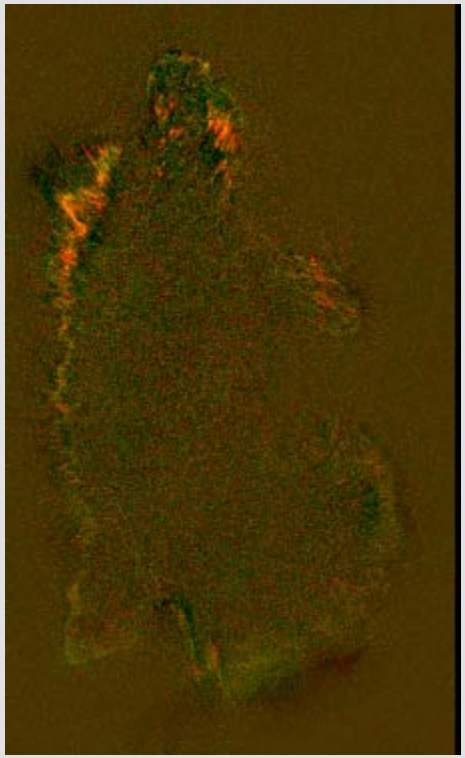
STICCS CHO α6β1-gfp + Paxillin-mcherry

Unfiltered



48 μm

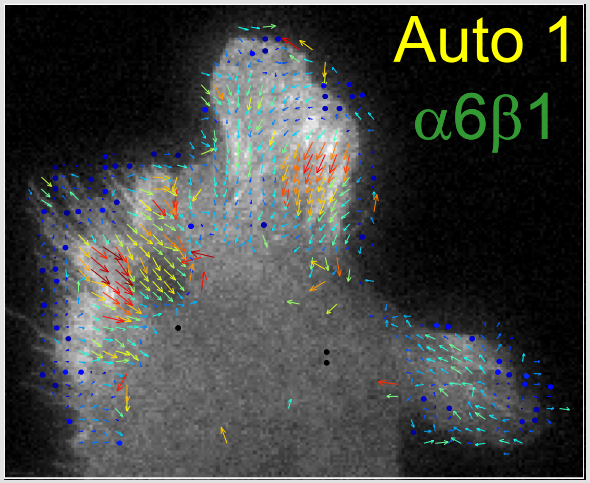
Fourier filtered

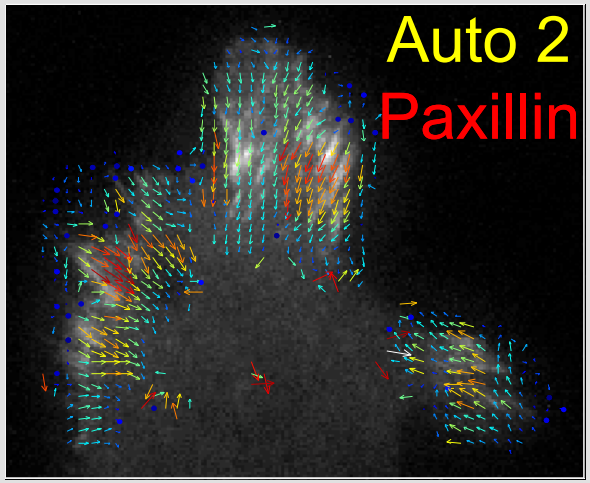


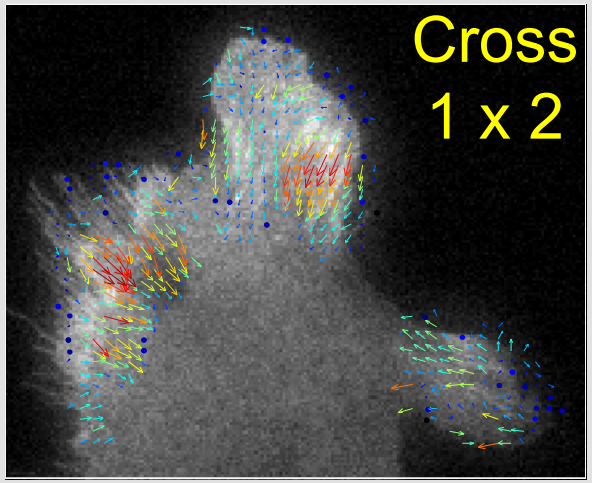
48 μm

Video length: 100 seconds

STICCS CHO α6β1-gfp + Paxillin-mcherry



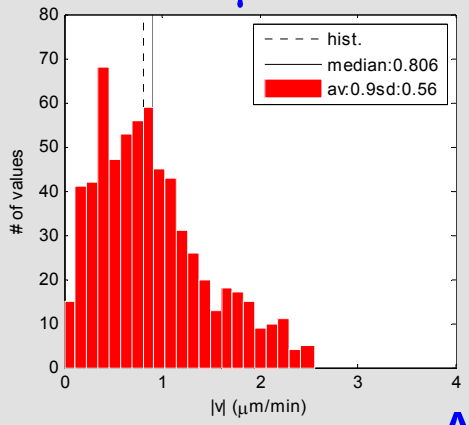




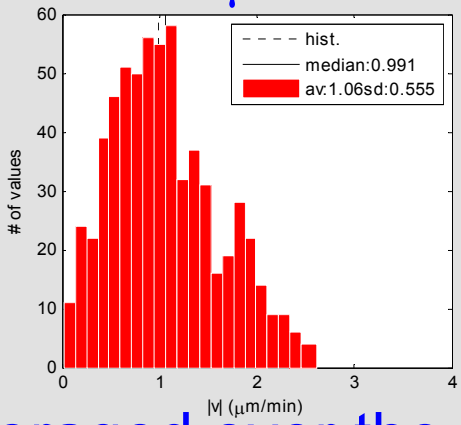
Fourier Filter Frames 51-100

Pixel size 0.21 μm
Δt 2 s

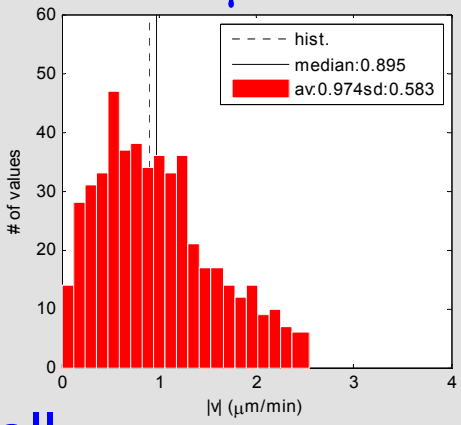
Median 0.81 μm/min



0.99 μm/min



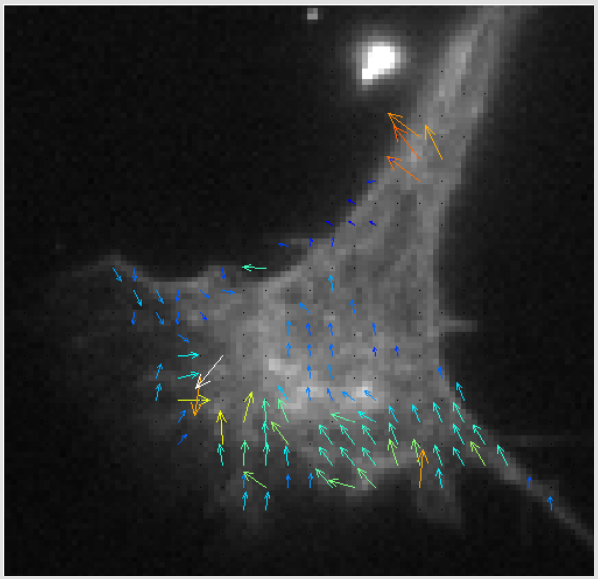
0.90 μm/min

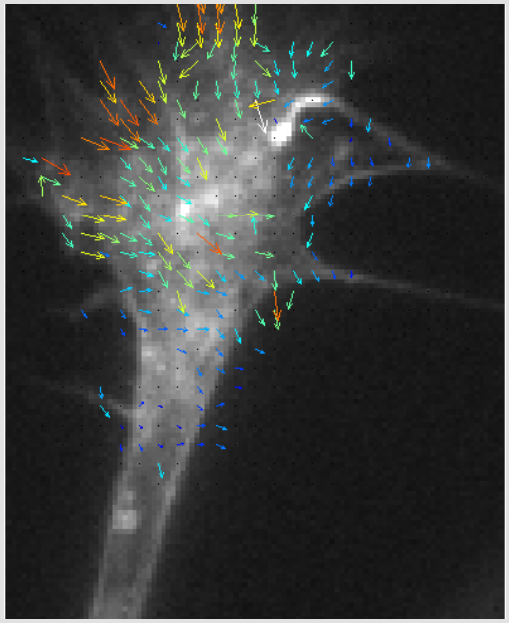


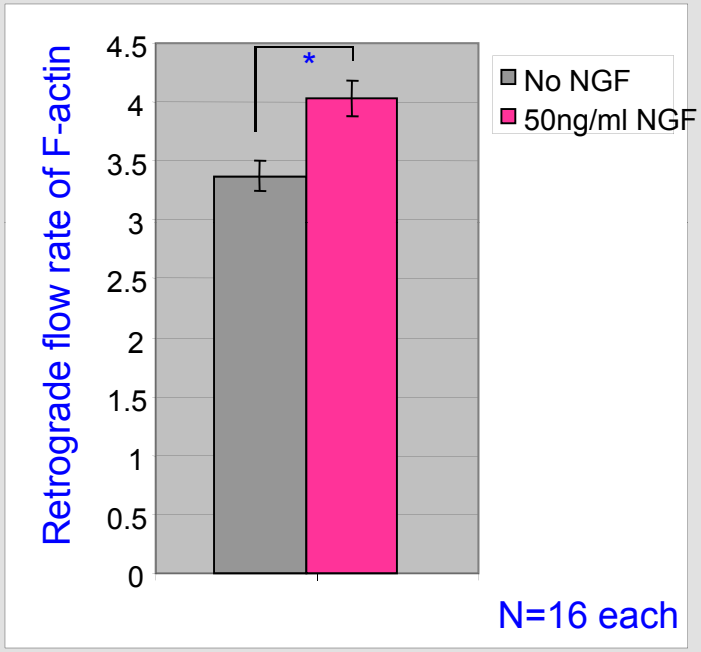
Averaged over the Cell

Neuroscience Applications: Growth Cones

- Pathfinding of Chick DRG Neuron Axon Growth Cones
- Cytoskeletal Dynamics (both Actin and Microtubules)







Utrophin
marker of f actin
<v> = 3.5 μm/min

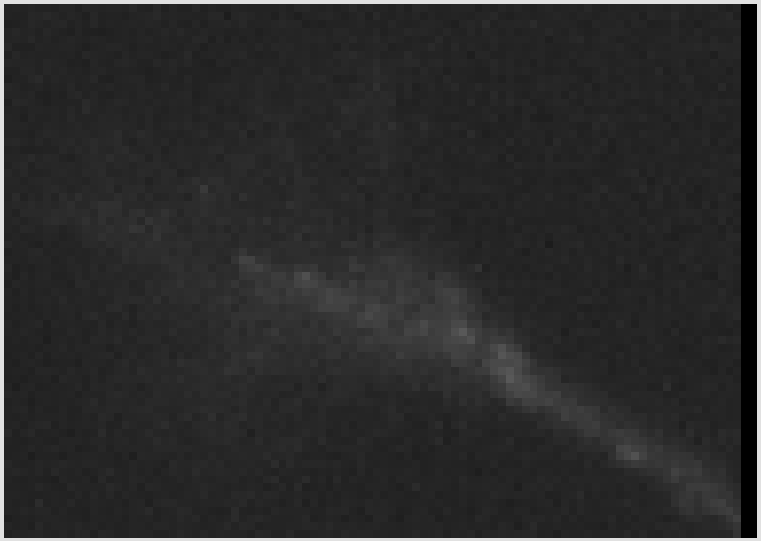
Disrupt Actin with
blebbistatin

+ 50 ng/mL NGF
Overnight

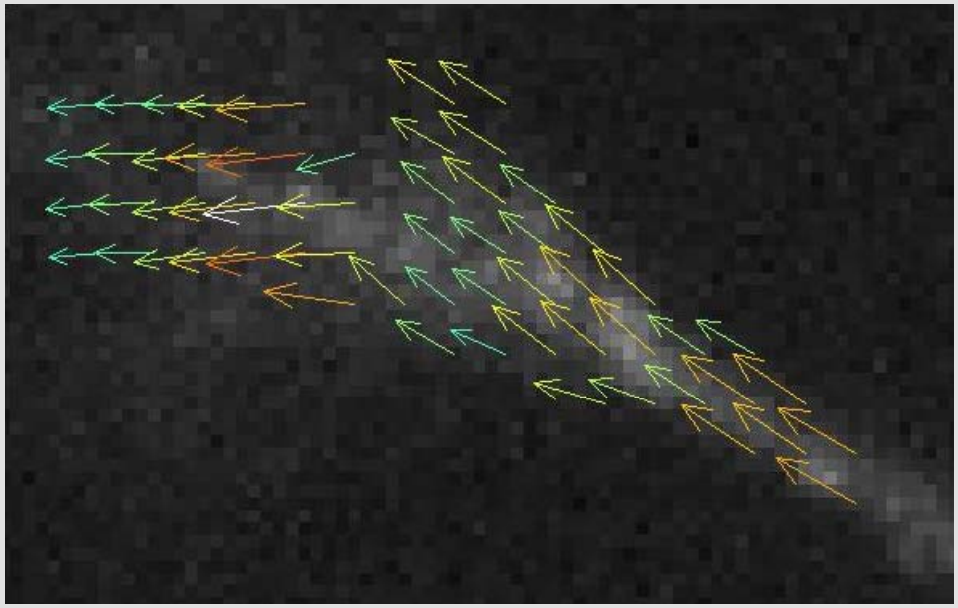
Collaboration with Dr. Tadayuki Shimada
Prof. Alyson Fournier MNI, McGill

Neuroscience Applications: Growth Cones

- Pathfinding of Chick DRG Neuron Axon Growth Cones
- Cytoskeletal Dynamics (both Actin and Microtubules)



EB3 (3.4 images/s)
marker of microtubule tips



<v> = 11.4 μm/min

We are looking at turning gradients as NGF is applied on one side of the growth cone

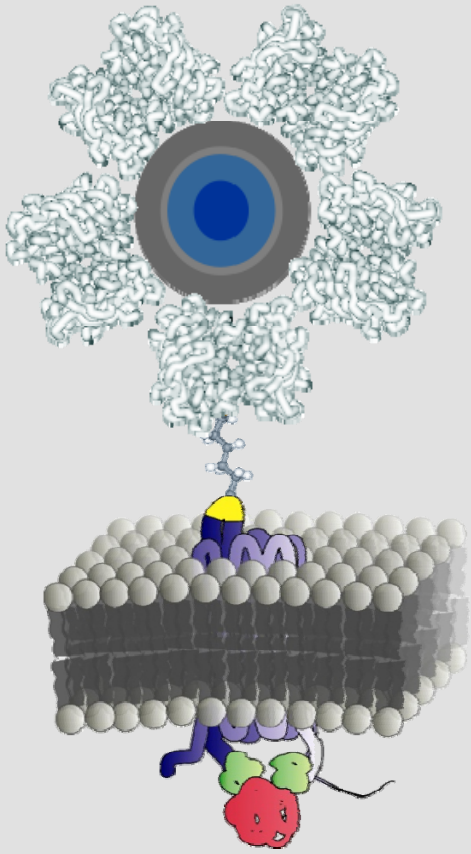
Collaboration with Dr. Tadayuki Shimada
Prof. Alyson Fournier MNI, McGill

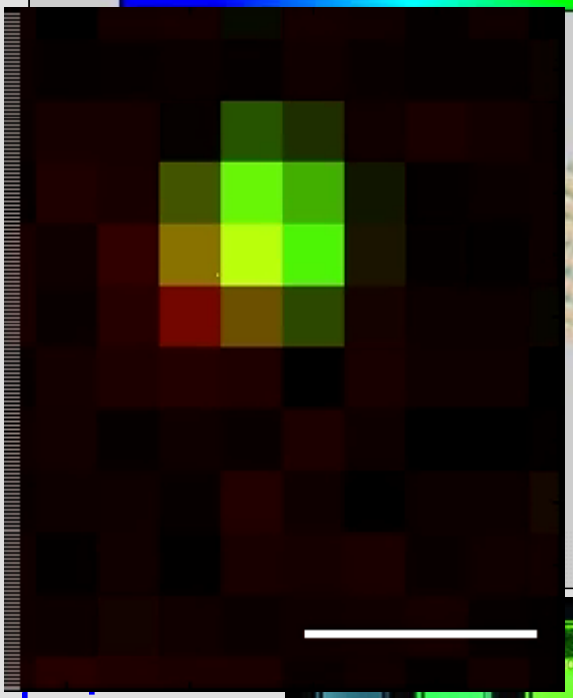
Overview for Tutorial

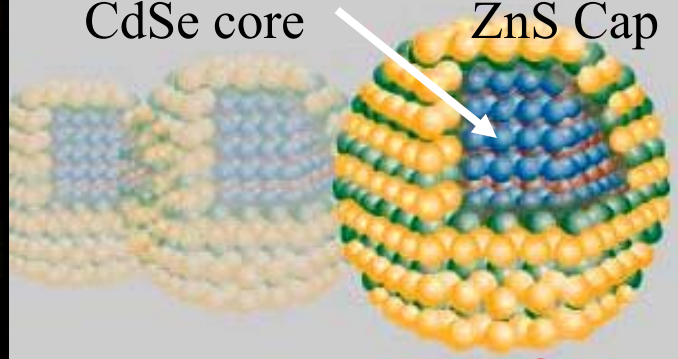
- Spatio-Temporal Image Correlation Spectroscopy
- k Reciprocal Space Image Correlation Spectroscopy (kICS)

Quantum Dots as Biomolecular Labels

Semiconductor Quantum Dots…Luminescent Nanoparticles





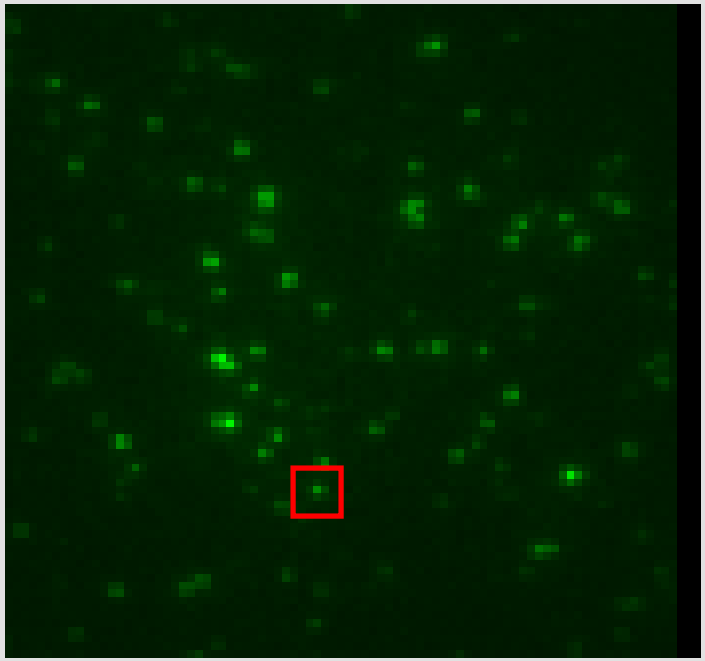


Larger QD…
reder emission

Photostable…Different sizes…

Different Colors, Track proteins

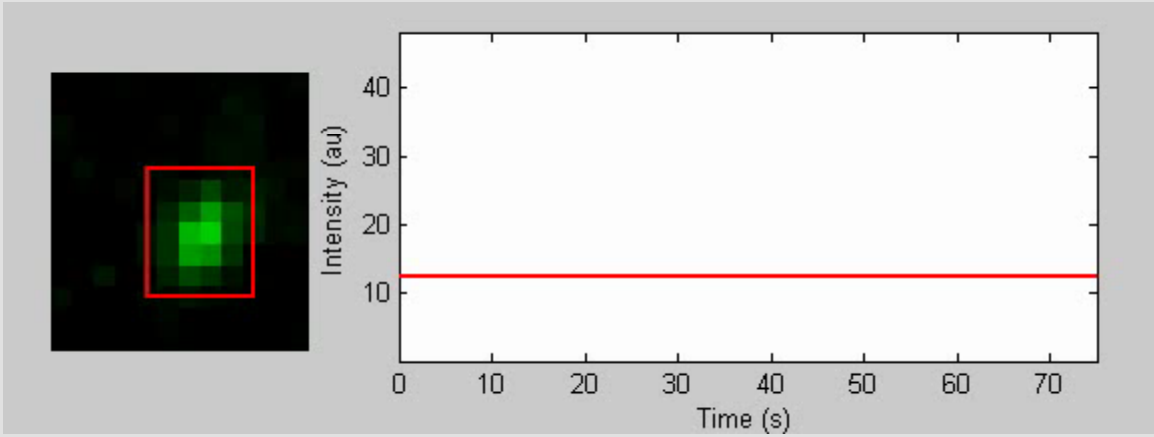
Fluctuation Spectroscopy on QDs



(CdSe)ZnS – Streptavidin (QD605)
TIRF Illumination CCD Detection
50ms Integration Time
2000 Frames

See Bachir et al. JAP 99 (2006)
Perturbs fluctuation measurements

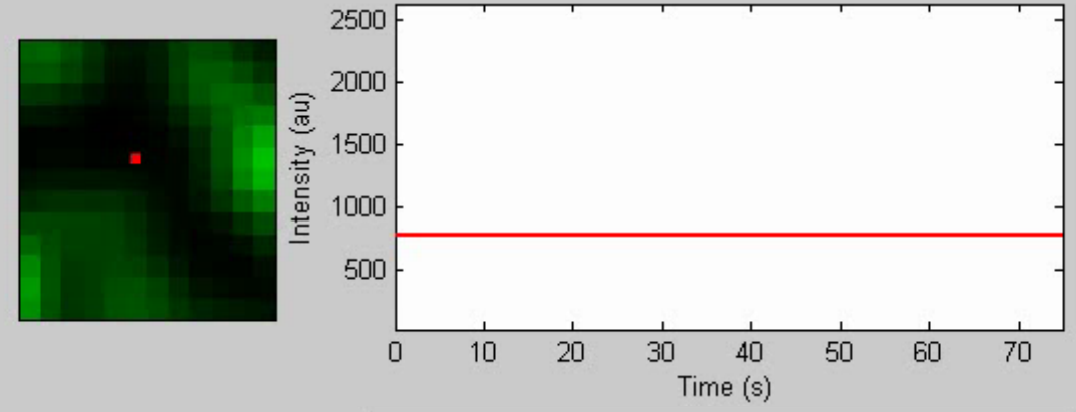
Single Dot i(t) trace



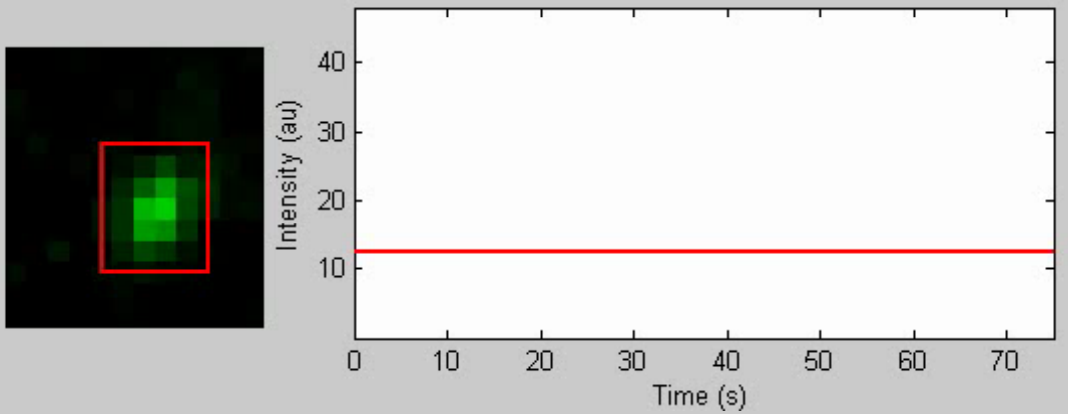
Nirmal et al. Nature(London) (1996)

Fluctuation Spectroscopy on QDs

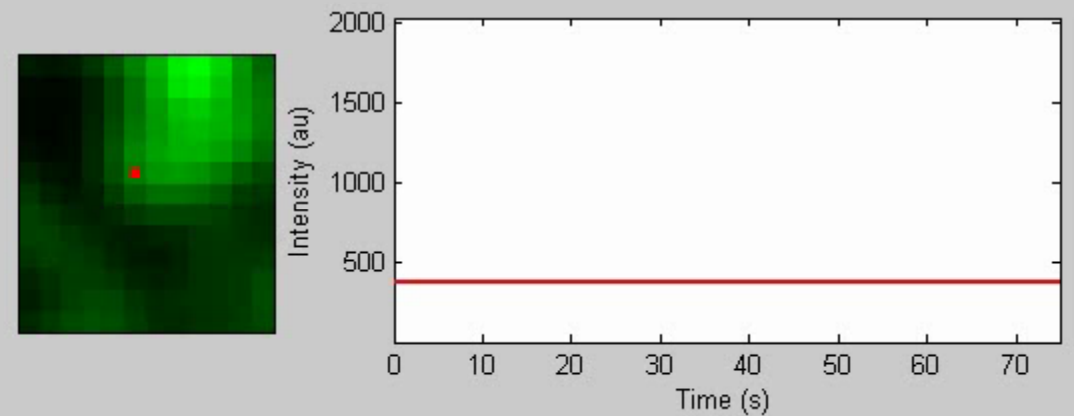
Diffusion only



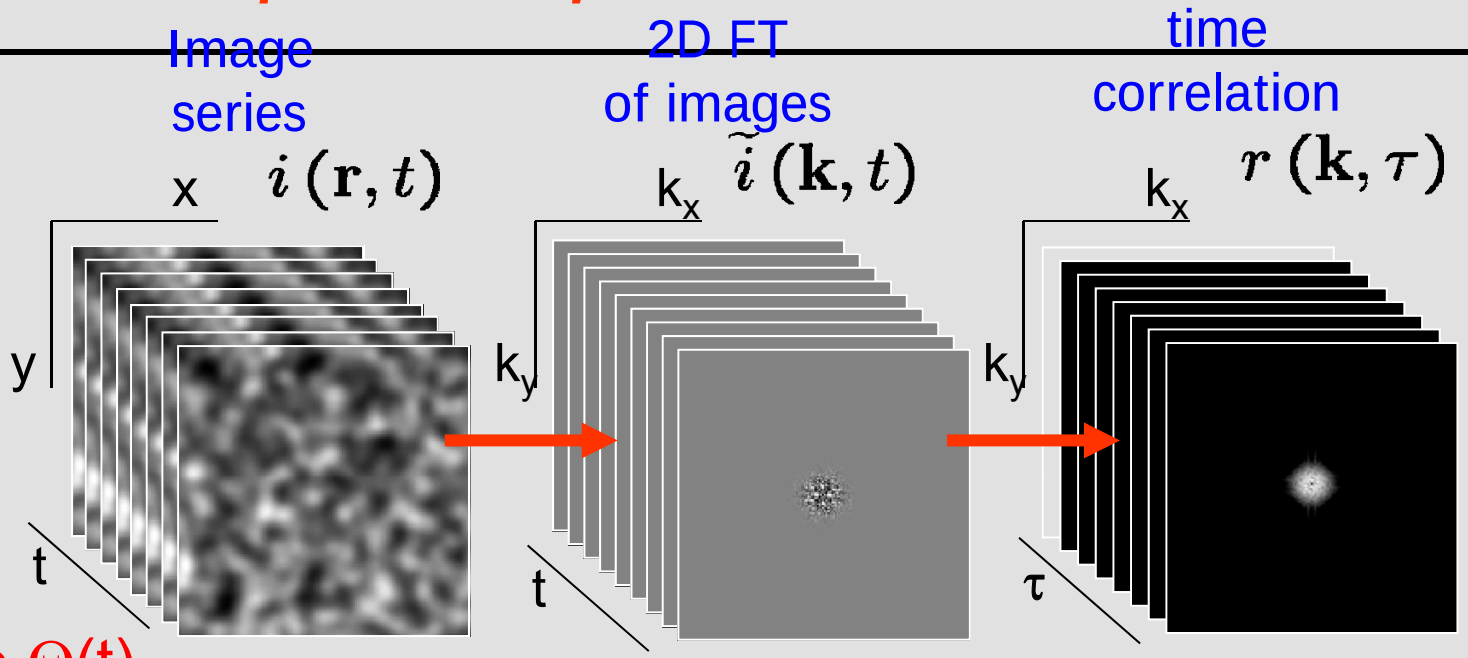
QD Blinking



QD Blinking
and diffusion

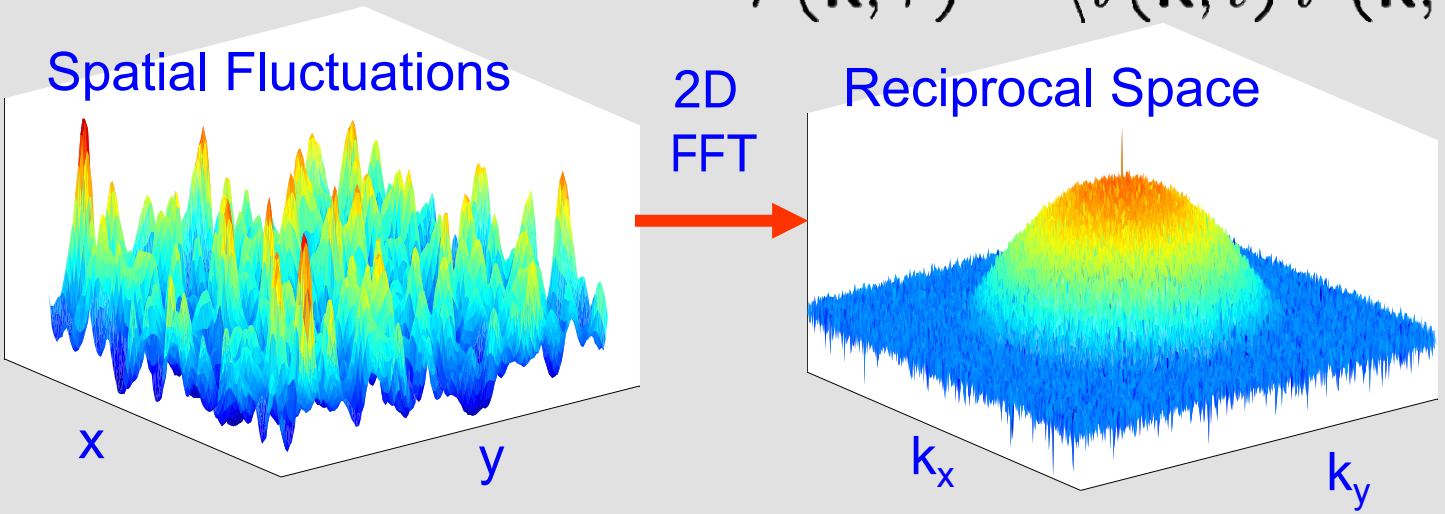


kICS: Reciprocal Space Time Correlation



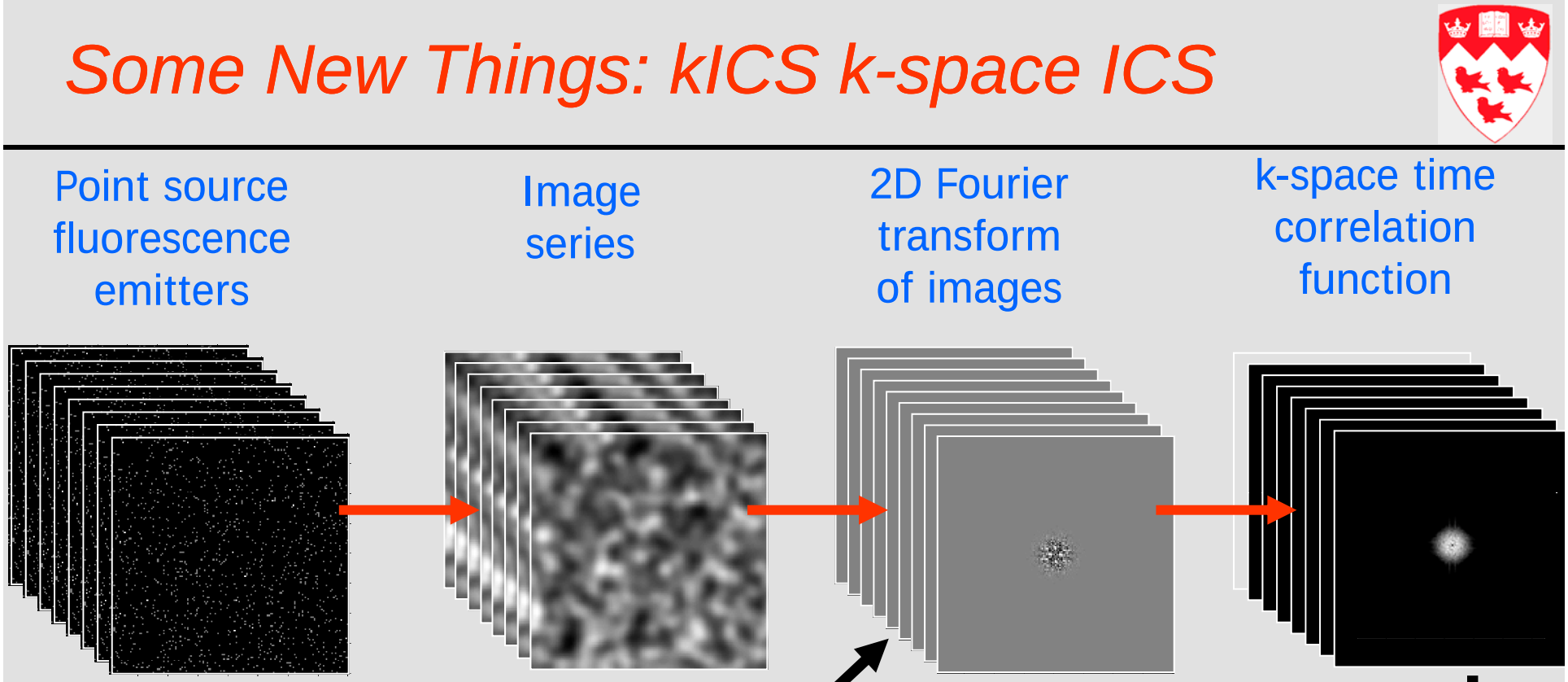
Include Θ(t) (1or 0)

$$r(\mathbf{k},\tau) = \langle \tilde{i}(\mathbf{k},t)\,\tilde{i}^{*}(\mathbf{k},t+\tau)\rangle_t$$



See Kolin et al. Biophysical Journal 91 3061-3075 (2006)

Some New Things: kICS k-space ICS



$$i(\vec{r},t) = q\, I(\vec{r}) * \rho(\vec{r},t)$$

$$\tilde{i}(\vec{k},t) = \frac{qI_o\omega_o^2\pi}{2}\sum_{i=1}^{N}\Theta_i(t)\exp\left[i\vec{k}\cdot\vec{r} - \frac{\omega_o|\vec{k}|^2}{8}\right]$$

q = instrumental constant

$I(\vec{r})$ = Point Spread Function

$\rho(\vec{r},t)$ = microscopic number density

$$r(\vec{k},\tau) = \left\langle \tilde{i}(\vec{k},t)\,\tilde{i}^{*}(\vec{k},t+\tau)\right\rangle =$$

k - space time correlation funtion

See Kolin et al. Biophysical Journal 91 3061-3075 (2006)

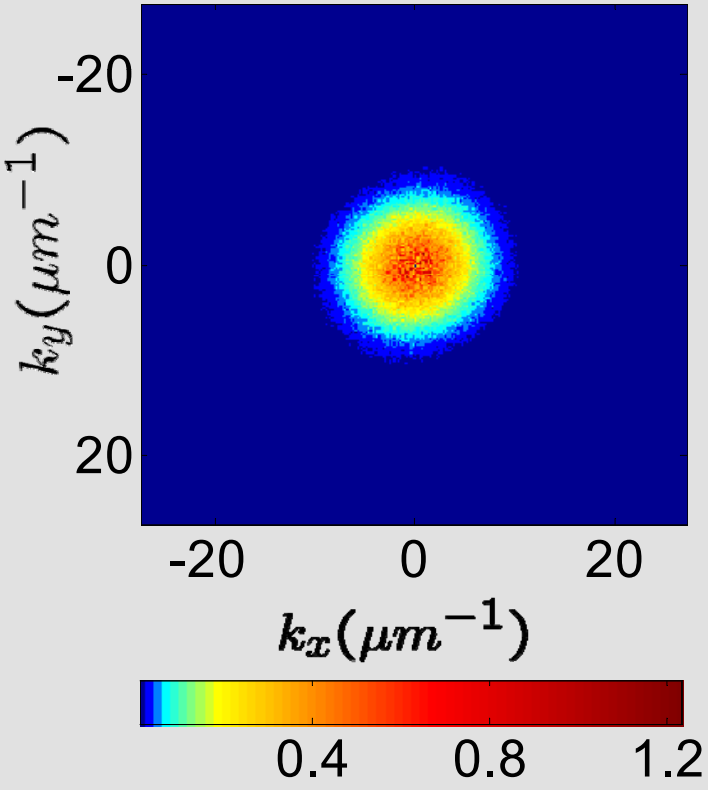
kICS...separates photophysics & transport

$$\ln\left[\phi_d\left(|\mathbf{k}|^2,\tau\right)\right] = \ln\left[N\langle\ominus(t)\ominus(t+\tau)\rangle q^2\frac{I_0^2\omega_0^4\pi^2}{4}\right] - \left(\frac{\omega_0^2}{4}+D\tau\right)|\mathbf{k}|^2$$

Intercept: Photo-Physics

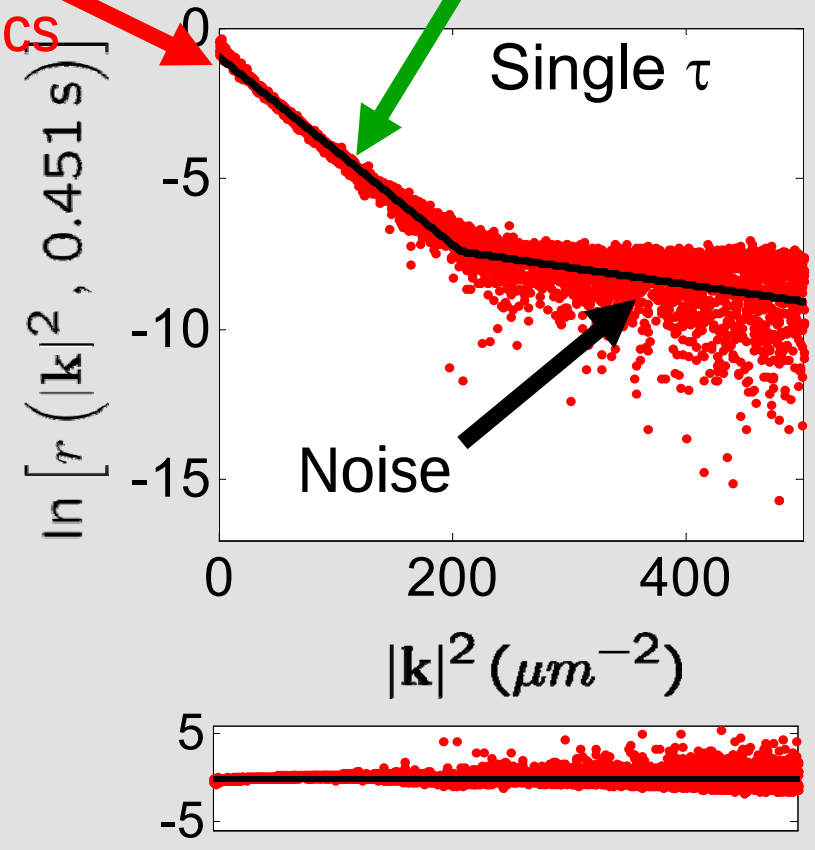
Slope

For a given τ_i:

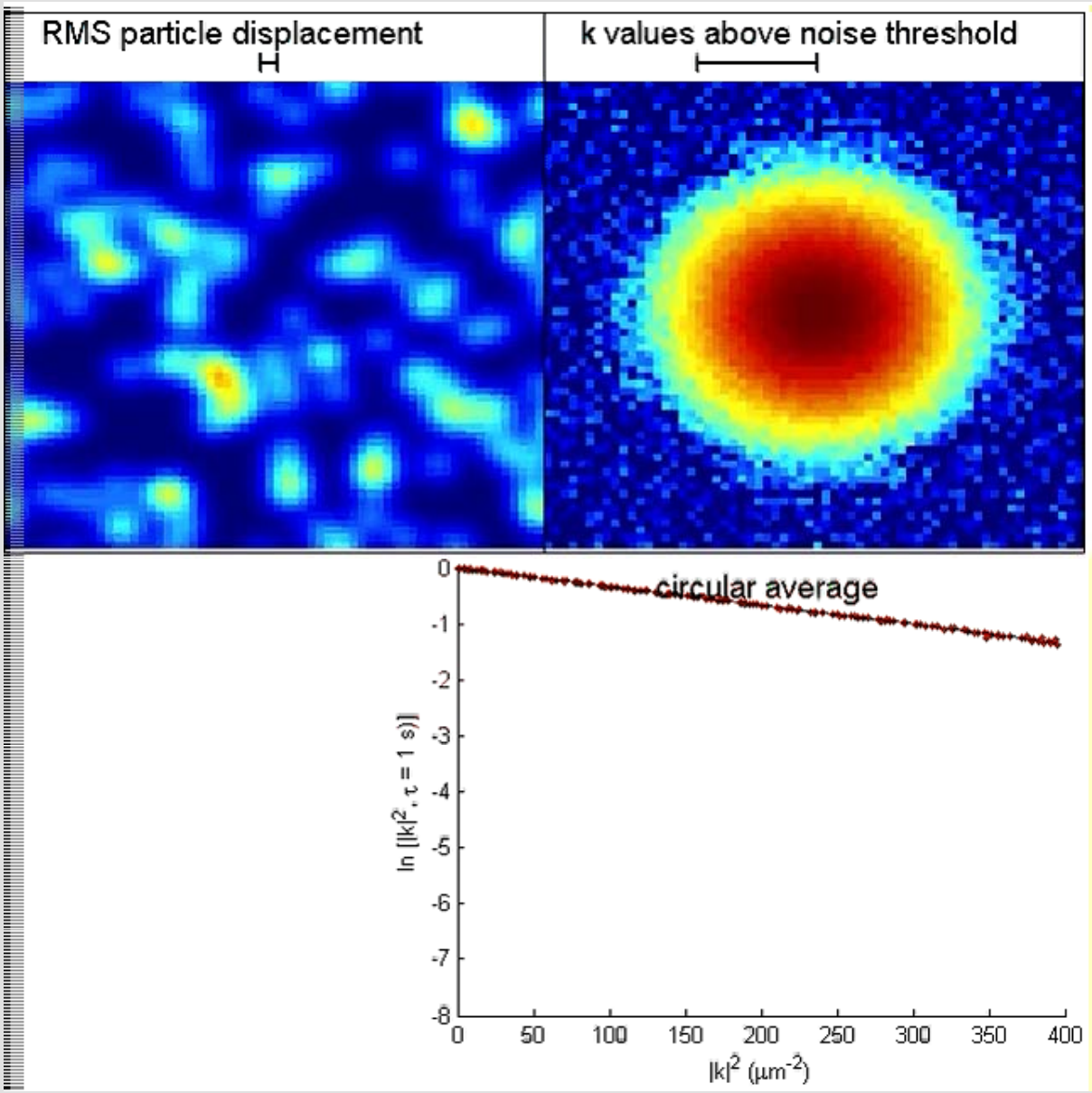


Circularly average

and log transform



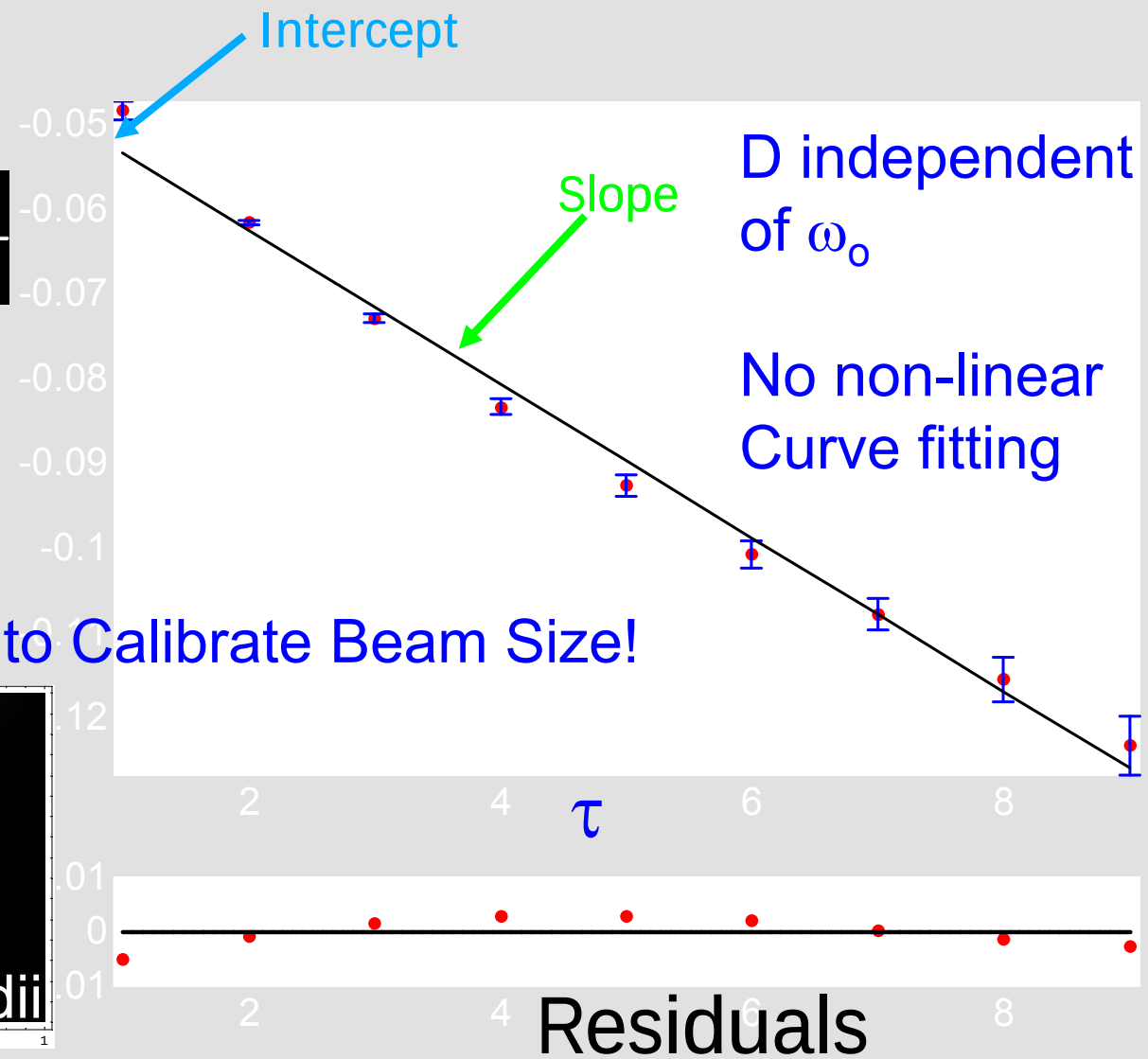
kICS: Reciprocal Space Time Correlation



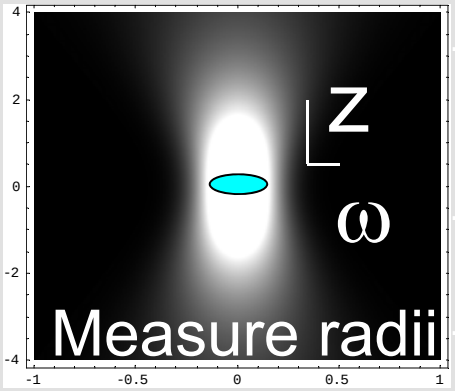
kICS: Reciprocal Space Time Correlation

Determine the slopes for each value of τ, plot them as a function of τ:

$$-\frac{\omega_0^2}{4} - D\tau$$

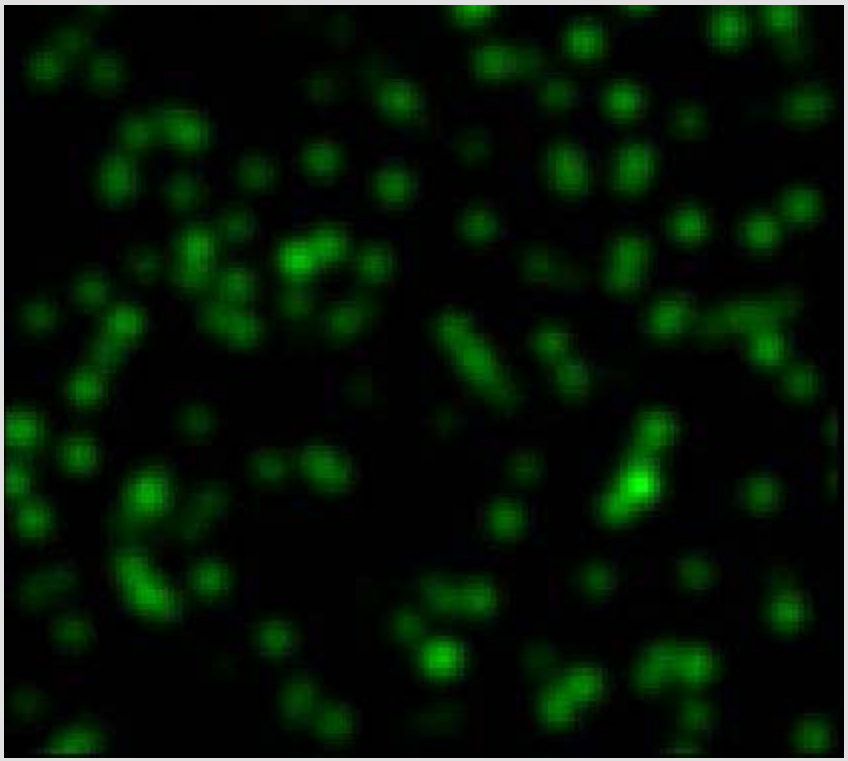


Do Not need to Calibrate Beam Size!



kICS: Reciprocal Space Time Correlation

Computer Simulations



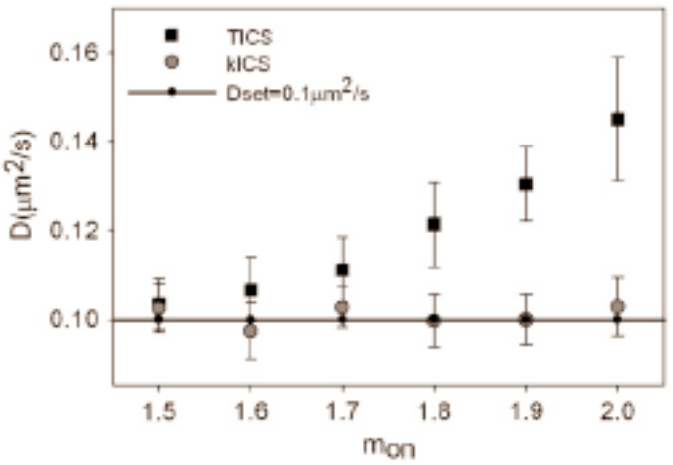
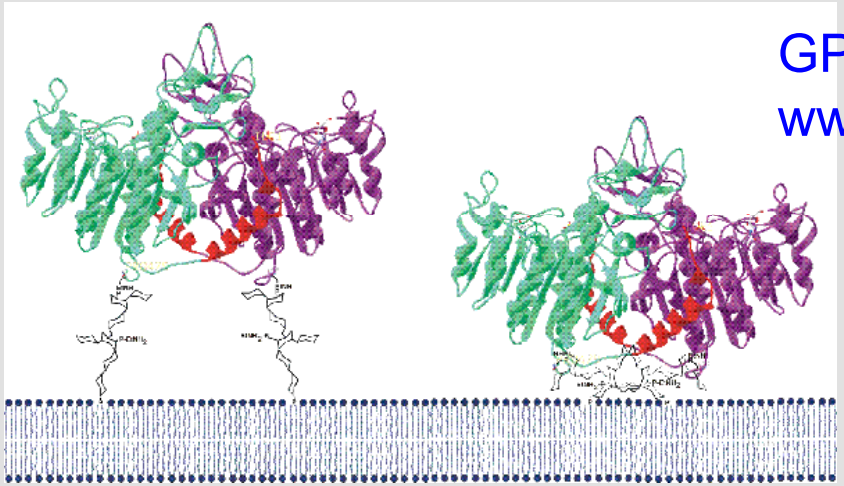


FIGURE 5 Diffusion coefficients calculated from TICS analysis of combined blinking and diffusion simulations of point emitters with varying "on" time PDF exponents and an "off" time PDF exponent set to 1.5 (*solid squares*). kICS results do not change with "on" time PDF exponent (*shaded circles*). Parameters in simulations were set to mimic experimental conditions in model systems that did not contain a static population of QDs. Each image time series was 2000-frames long, with an area of 64 × 64 pixels, time lag of 60 ms between images, and ~250 QDs per frame. The diffusion coefficient was set to 10×10^{-2} $\mu m^2/s$. Each value is an average from 20 simulations. Error bars are standard deviations.

Simlulation Parameters
128x128 pixels 500 frames
0.1 s/frame, 0.1μm/pix, 0.4 μm PSF,
D=0.1 $\mu m^2/s$, $m_{on}=2$, $m_{off}=1.5$

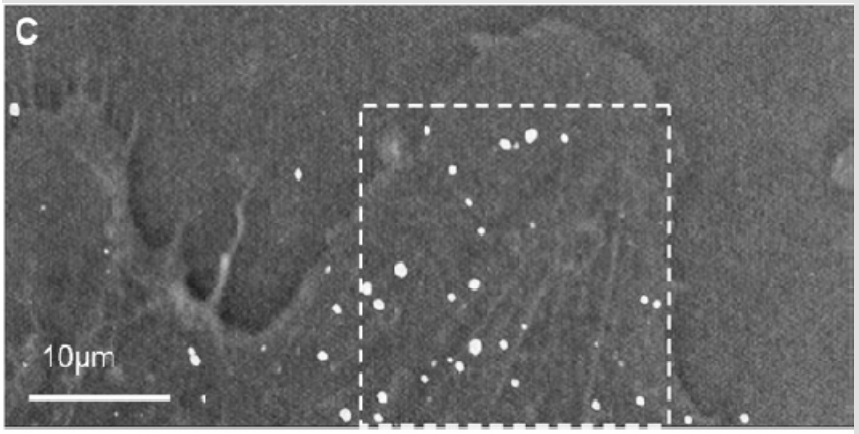
Durisic et al. Biophys. J. 93-1338 (2007)

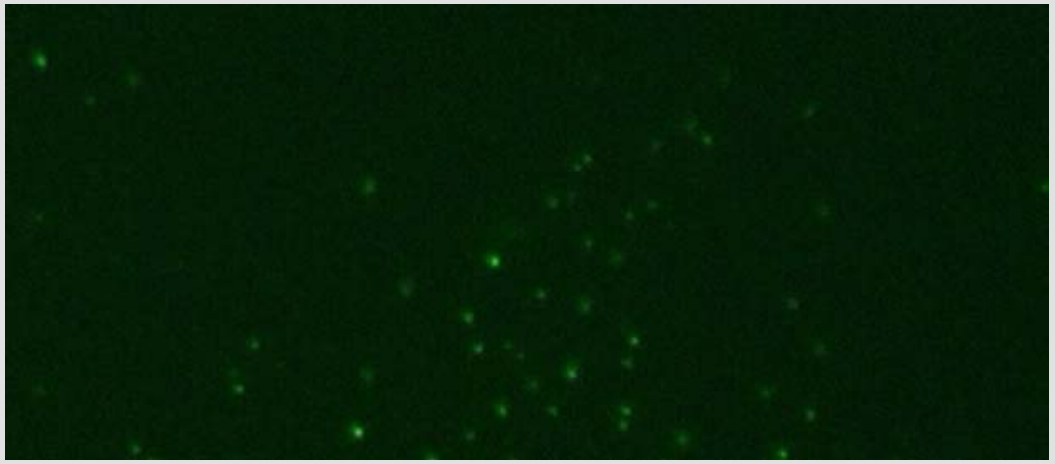
QD Labeled CD73 GPI Anchored Protein In Cells



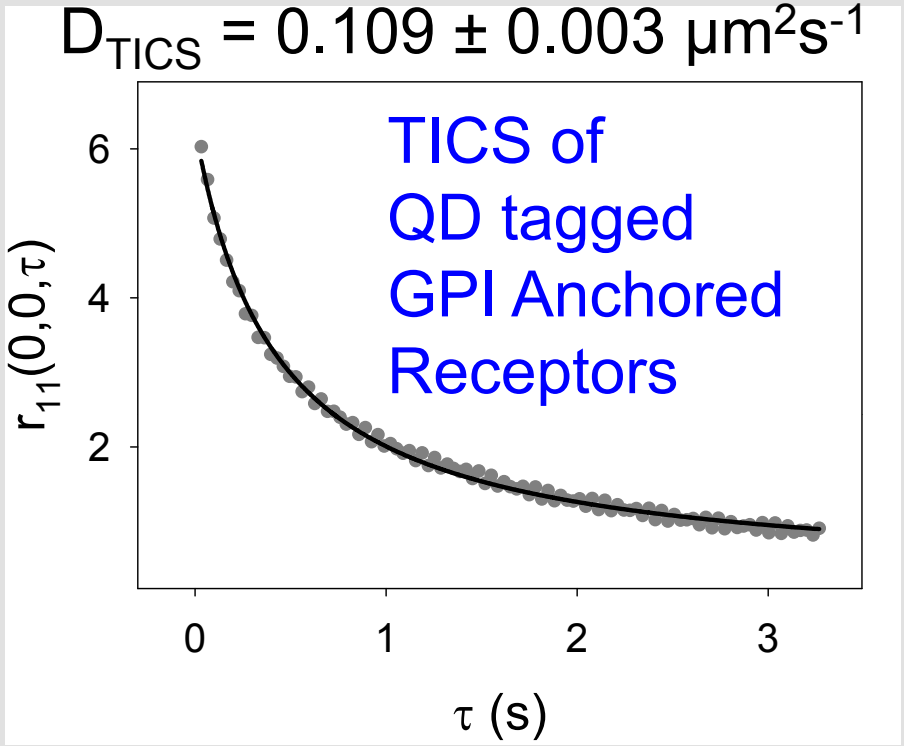
GPI Lipid Anchored Proteins
www.uoguelph.ca/~fsharom/research/gpi.html

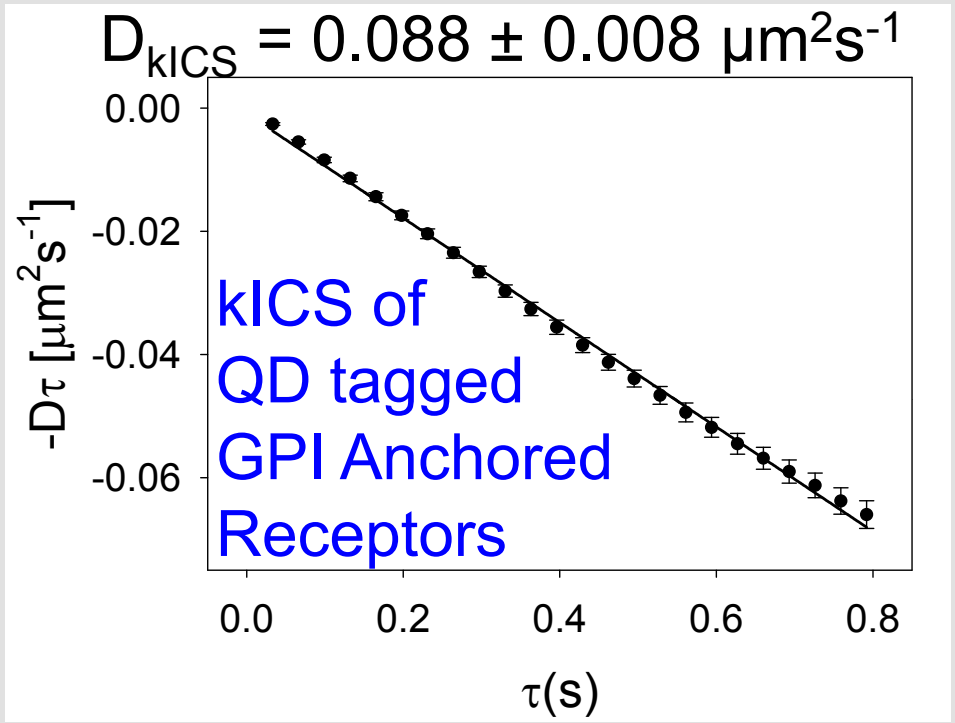
IMR-90 Fibroblast Cells
CD73 GPI Anchored Proteins Tagged with QDs
Imaged at Video Rate (30 fps) (Chris Lagerholm UNC Chapel Hill)

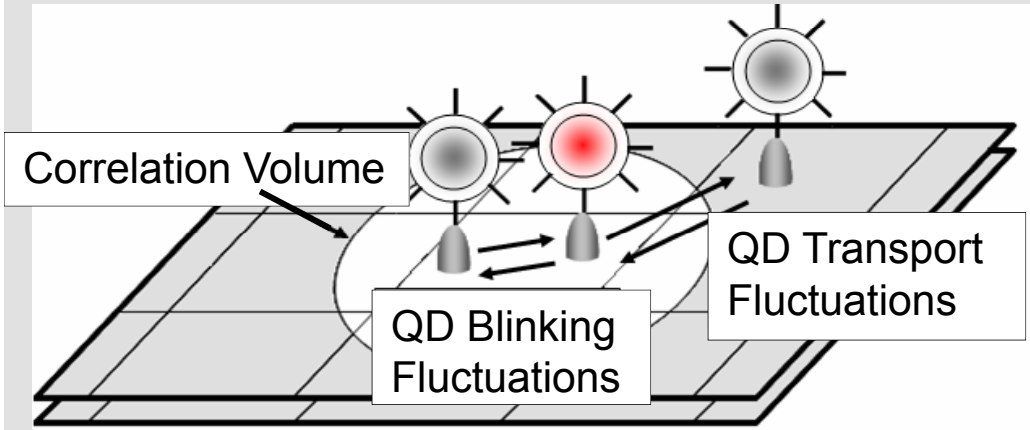




QD Blinking Does Affect Temporal Image Correlation…kICS eliminates the problem



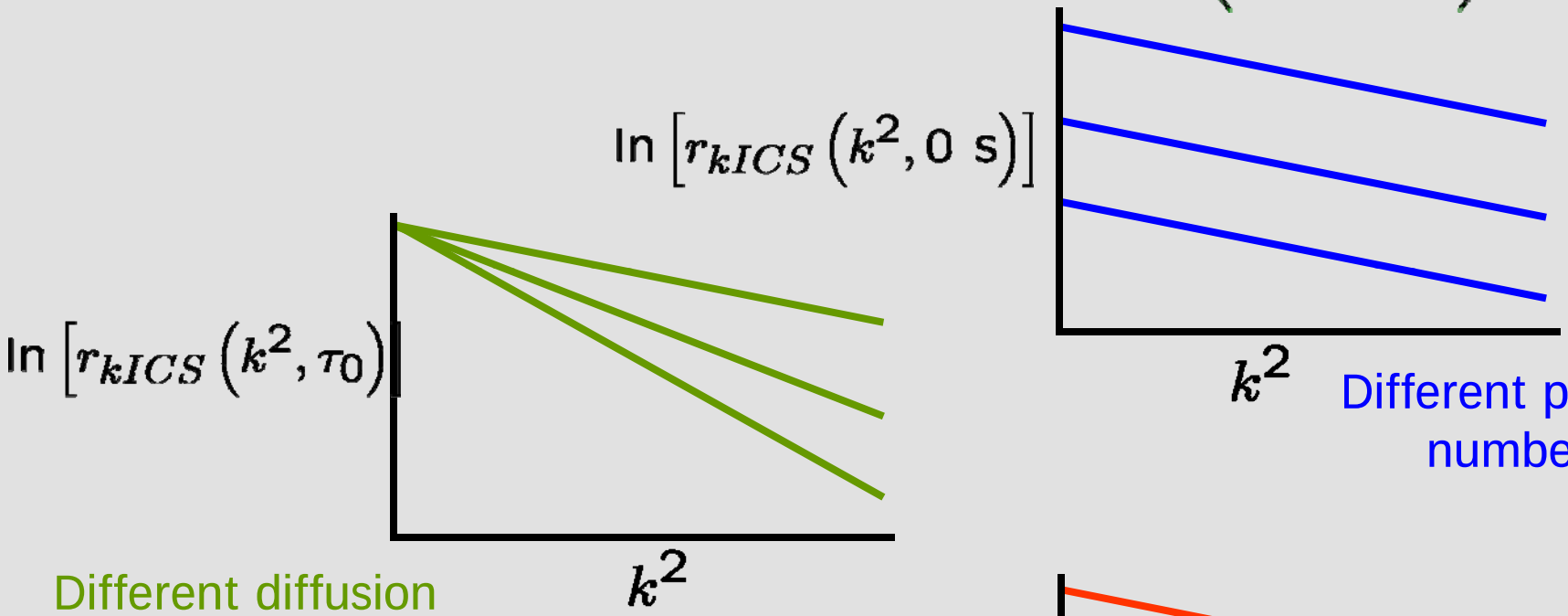




Durisic et al. Biophys. J. 93-1338 (2007)
Quantum dot labeled
GPI Anchored Receptors

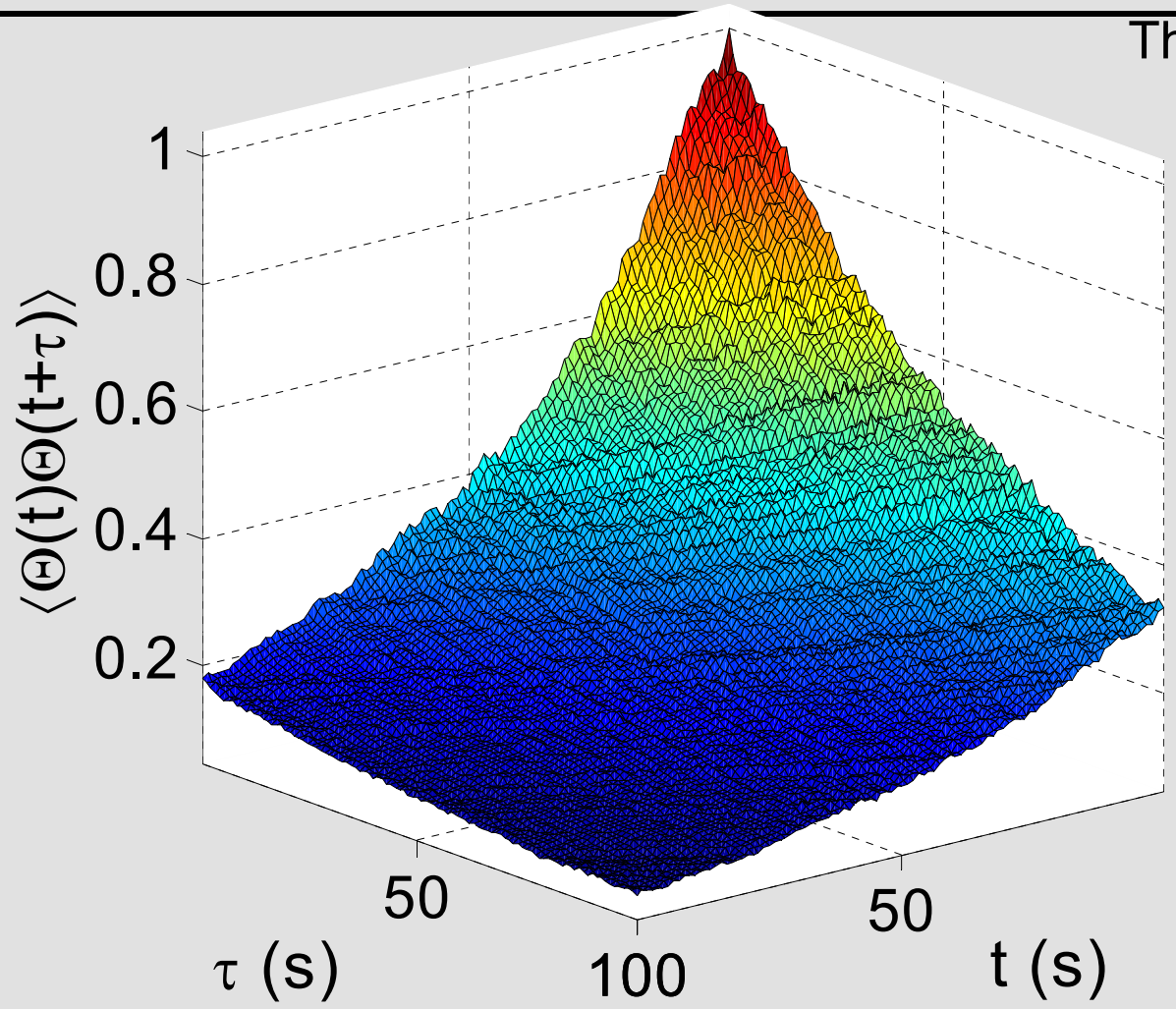
kICS separates photo-physics & transport

$$\ln\left[r\left(|\mathbf{k}|^2,\tau\right)\right] = \ln\left[Nq\langle\ominus(t)\ominus(t+\tau)\rangle\right] - \left(\frac{\omega_0^2}{4} + D\tau\right)|\mathbf{k}|^2$$



Kolin, *et al.,* Biophys. J. 2006 91: 3061-3075.

kICS can measure Changes in Blinking



GFP-adhesion protein
CHO cell

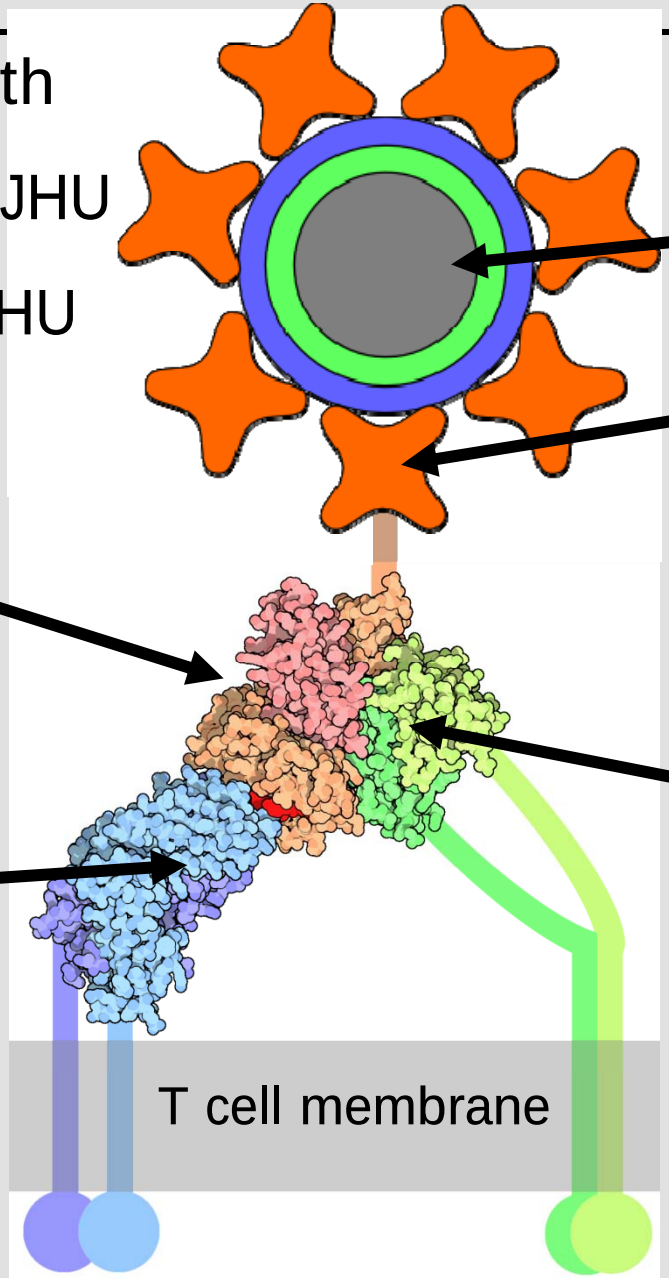
GFP mono-exponential bleaching

Kolin, *et al.,* Biophys. J. 2006 91: 3061-3075.

QD labelling of T cell receptors

Collaboration with

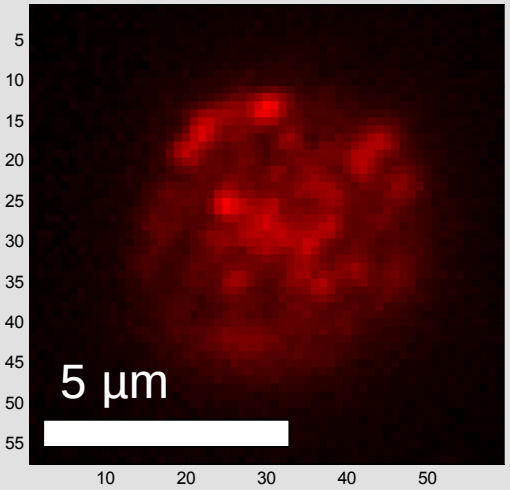
- Michael Edidin JHU
- Jon Schneck JHU

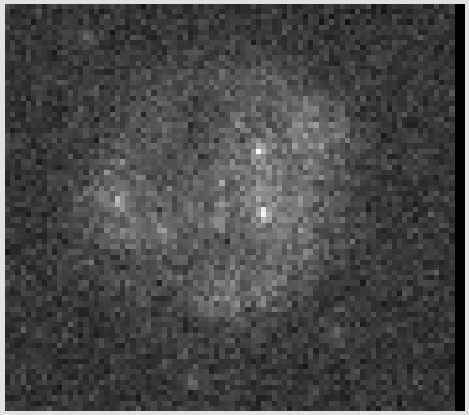


Drawing by David S. Goodsell

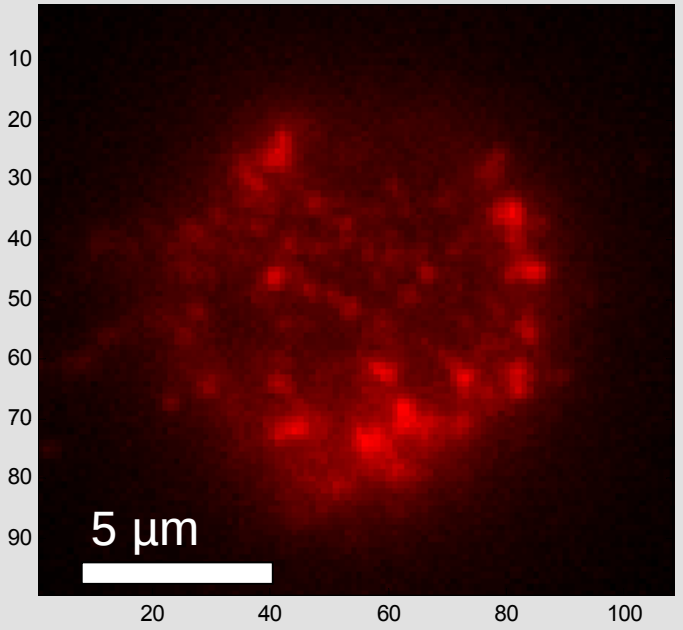
Qualitative Differences

- Change in blinking of QDs?
- Cell size
- Change in distribution of QDs?
- Can these be quantified?





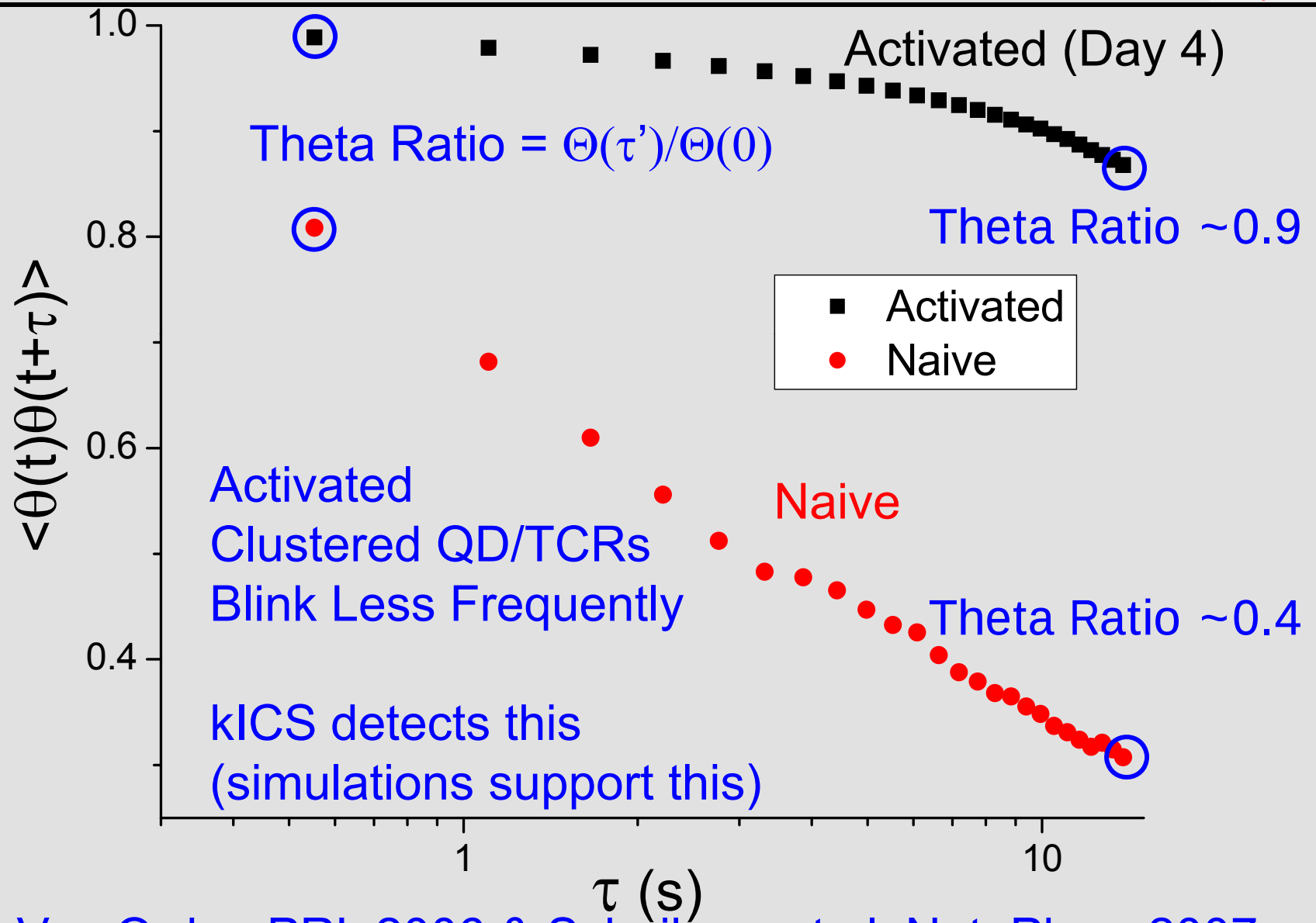
Naïve cell Day 0



Activated cell – Day 4
TCRs are more clustered

Epi-fluorescence captured on an EM-CCD Camera
Results are for 300-500 image time series averaged over many cells (~20 cells/day)

kICS detects blinking changes in T Cells



Yu & Van Orden PRL 2006 & Scheibner et al. Nat. Phys. 2007

Conclusions

- Spatio-Temporal Image Correlation Spectroscopy

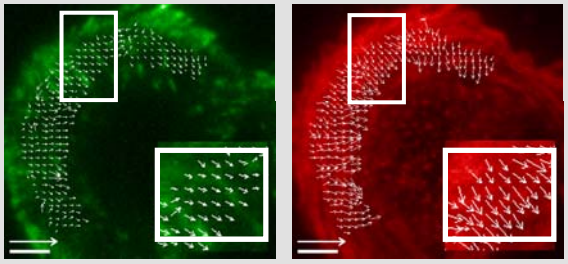
  Transport maps of FP labeled proteins in cells

- k Reciprocal Space Image Correlation

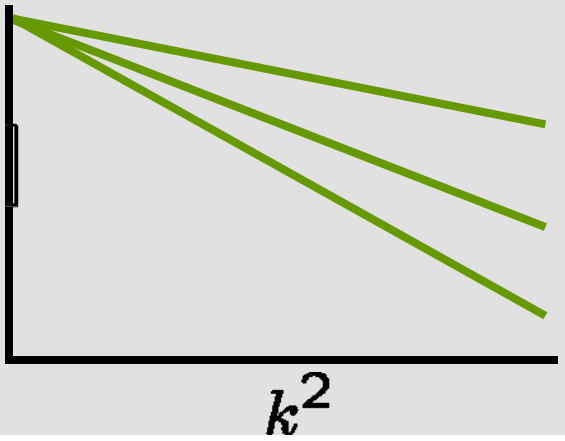
  Spectroscopy (kICS)

  Separates photo-physics and transport fluctuations

- Data mining the images (there's gold in those hills (fluctuations))



$$\ln\left[r_{kICS}\left(k^2,\tau_0\right)\right]$$



Acknowledgements

STICS Molecular Clutch

- Allan “Rick” Horwitz UVa
- Claire Brown UVa & McGill University
- Ben Hebert
- David Kolin
- Tim Toplak
- Tadayuki Shimada (MNI)
- Alyson Fournier (MNI)

Johns Hopkins University

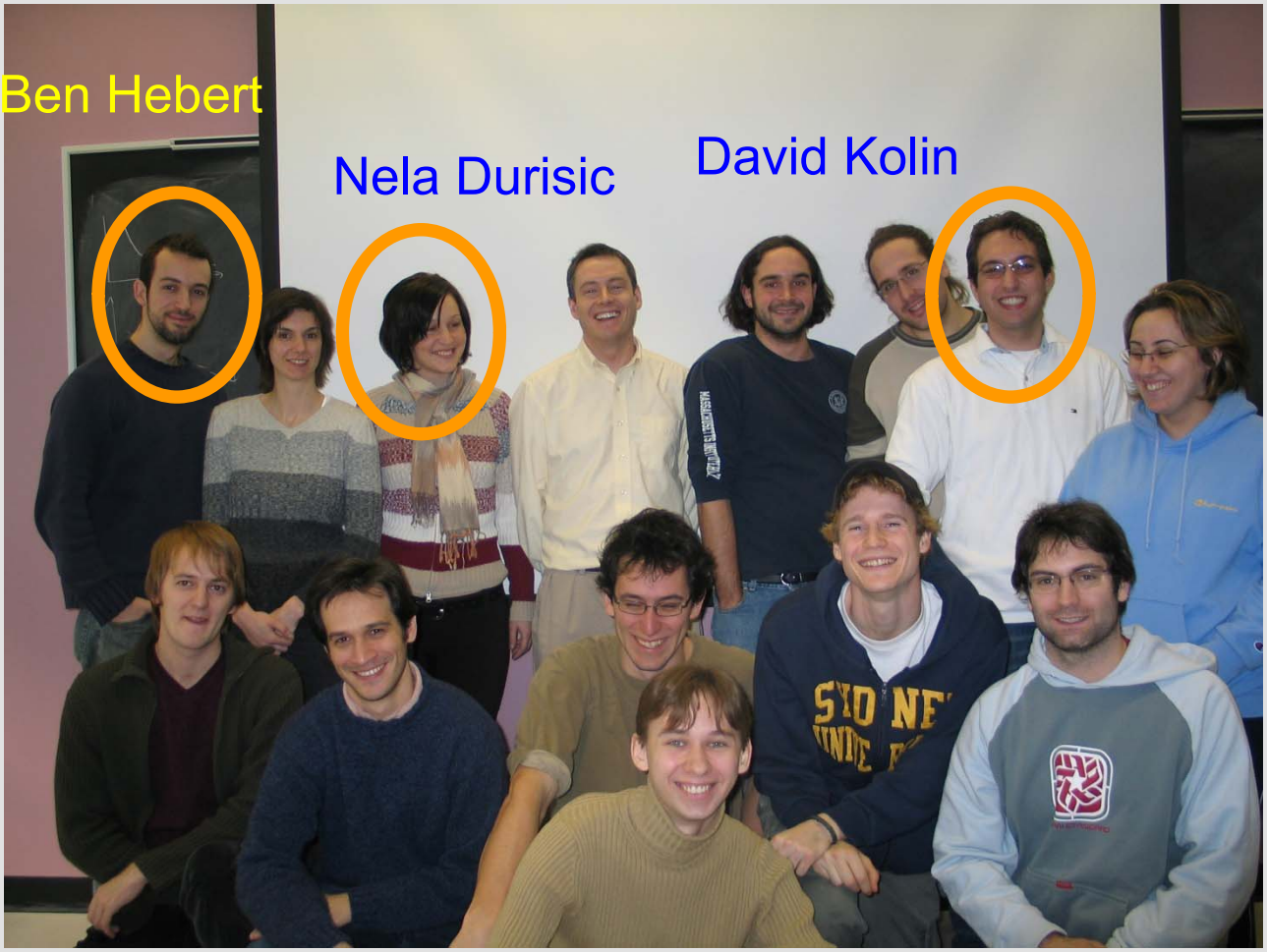
NSERC CRSNG

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

kICS

- Michael Edidin JHU
- Jon Schneck JHU
- The Schneck lab
- David Kolin
- David Ronis
- Nela Durisic
- Chris Lagerholm UNC
- Jeremy Schwartzentruber

Thanks to the Group!



Thanks to Prof Rick Horwitz UVa & Dr. Claire Brown McGill

Thanks to the Group!

Tim Toplak

& Jeremy Schwartzentruber, Dominique Guillet
Sebastien Cote, Benoit Vallaincourt

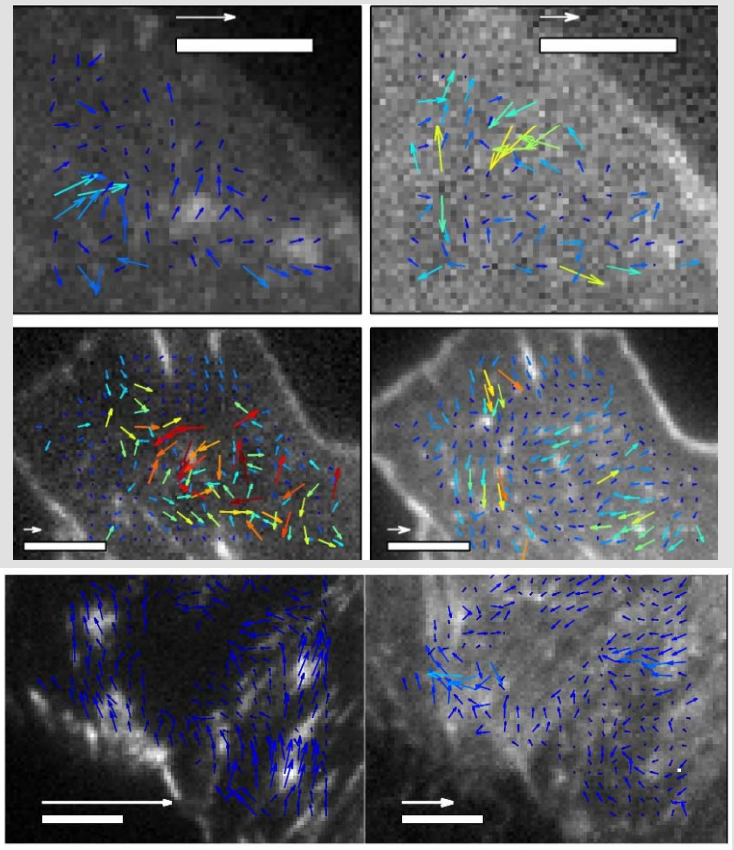
Correlated Transport Depends on [ECM]

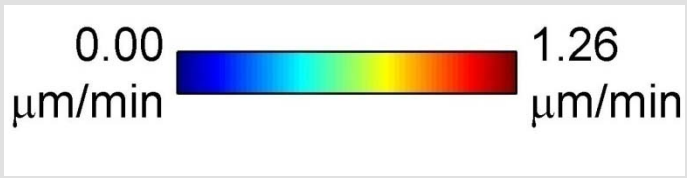
EGFP Protein mRFP-Actin

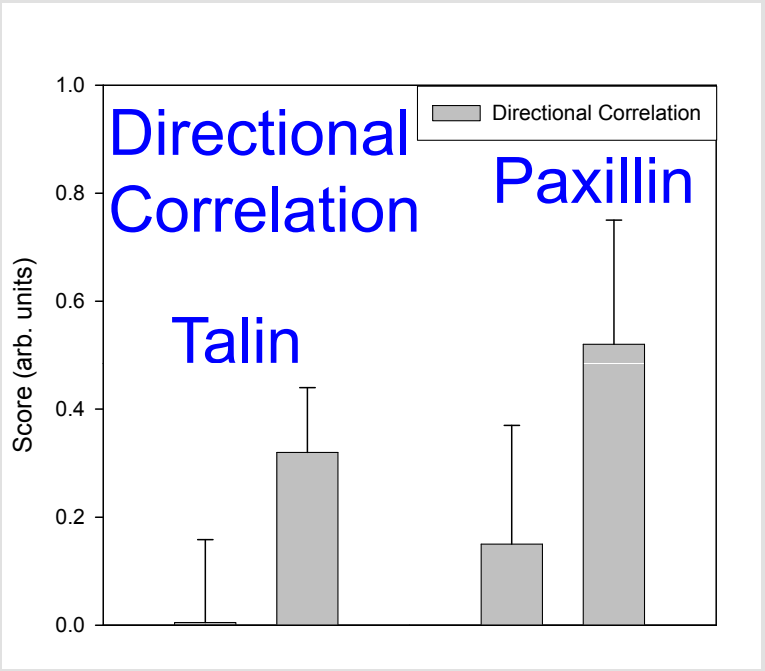
2 μg/ml Fibronectin Talin

5 μg/ml Fibronectin Talin

5 μg/ml Fibronectin Paxillin

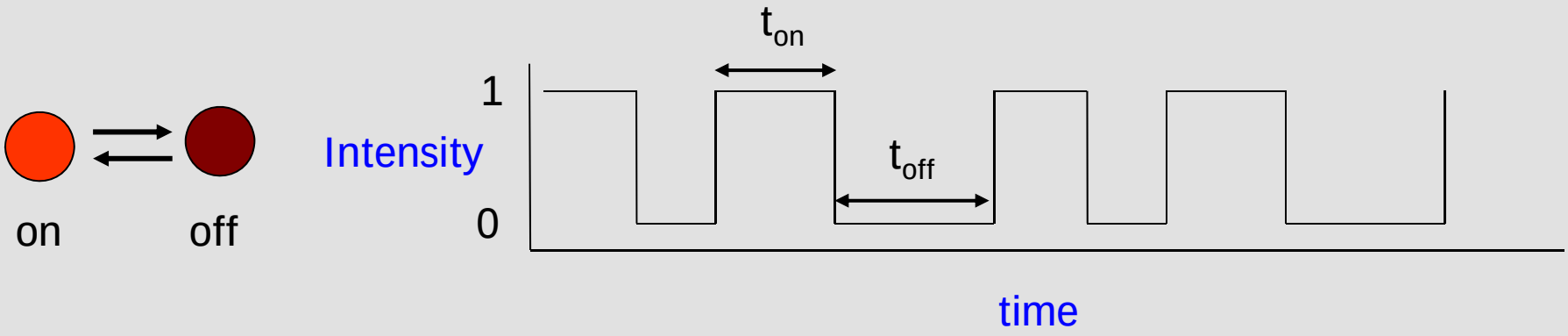






[FN] Low High Low High

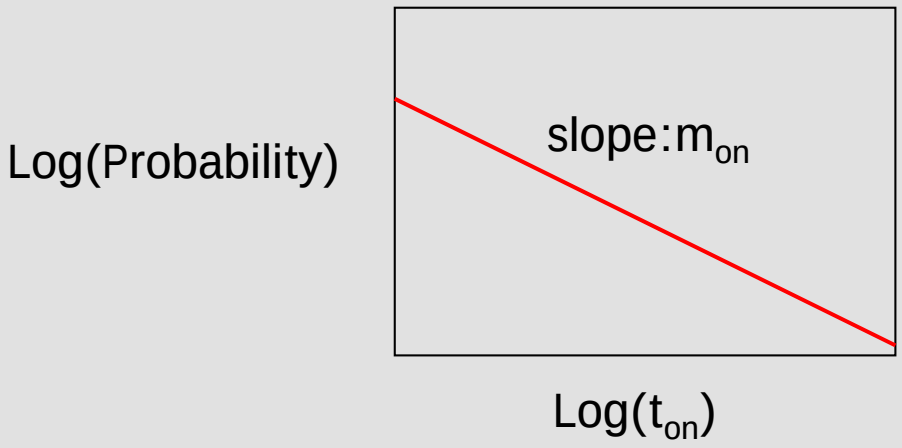
Quantum dot blinking



On and off time distributions have “power-law” decays

- Fractal
- Occur on all timescales
- t_{on} and t_{off} have power law distribution

$$P(t_{On/Off}) \propto \frac{1}{t_{On/Off}^{m_{On/Off}}}.$$

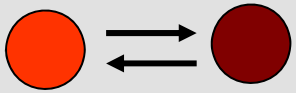


m_{on} depends on:

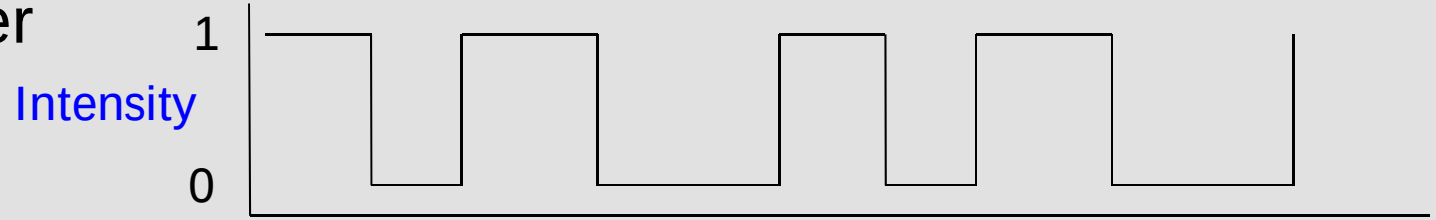
- Core
- Shell
- Surface functionalization
- Excitation intensity
- Ligands in solution (e.g., pH)
- Manufacturer/Batch of QDs

Three blinking simulations

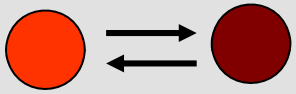
"slow" blinker



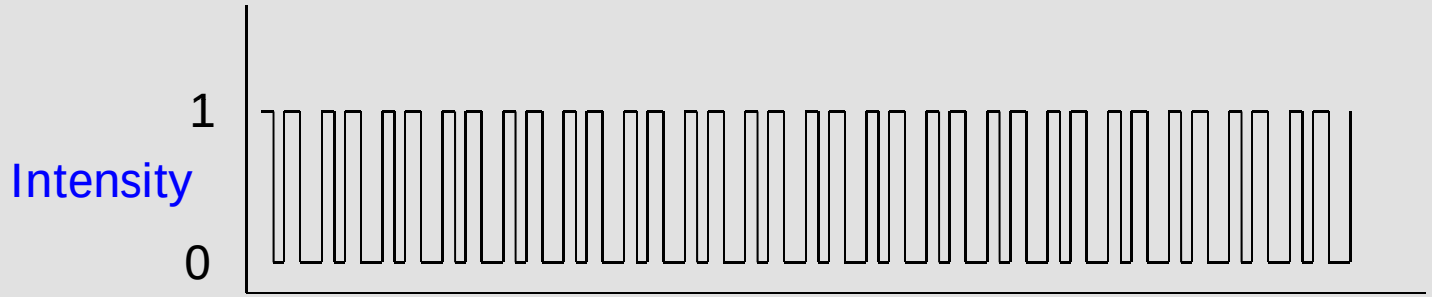
on off



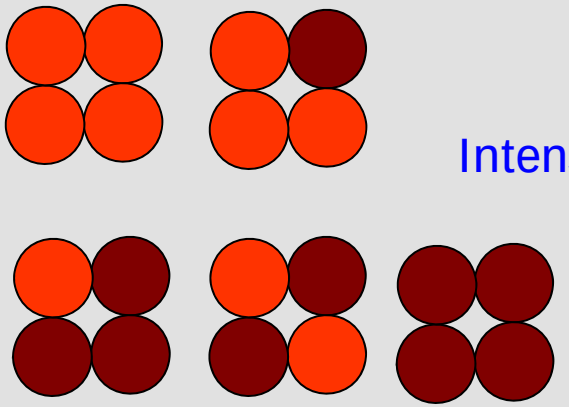
"fast" blinker

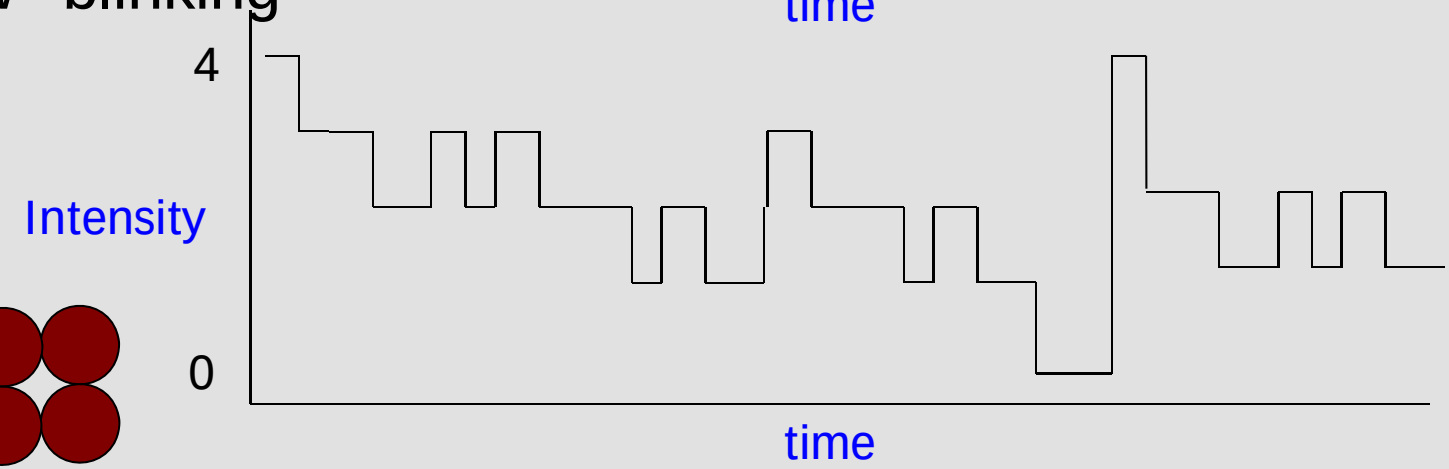


on off

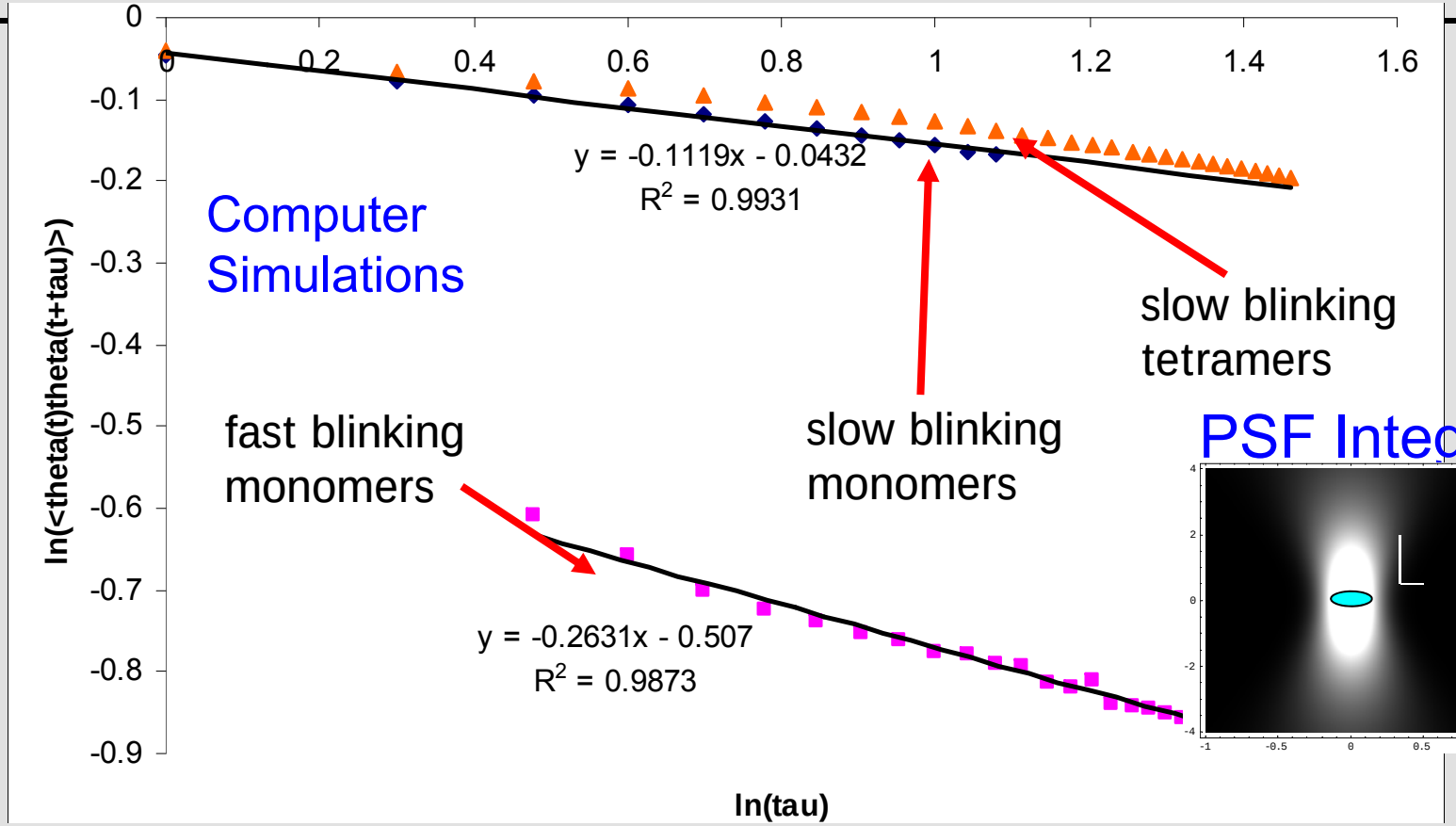


tetramer "slow" blinking



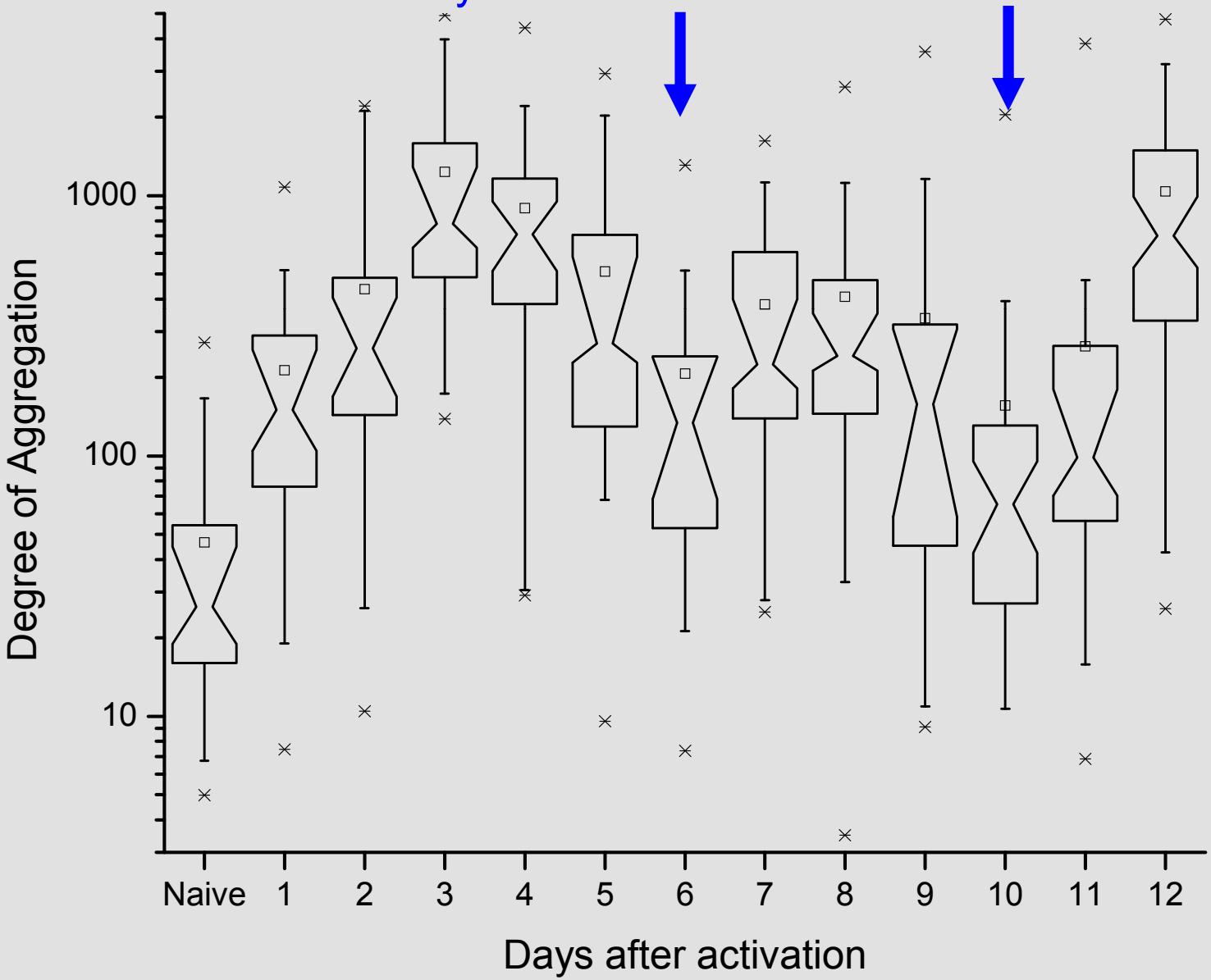


kICS can measure blinking within clusters

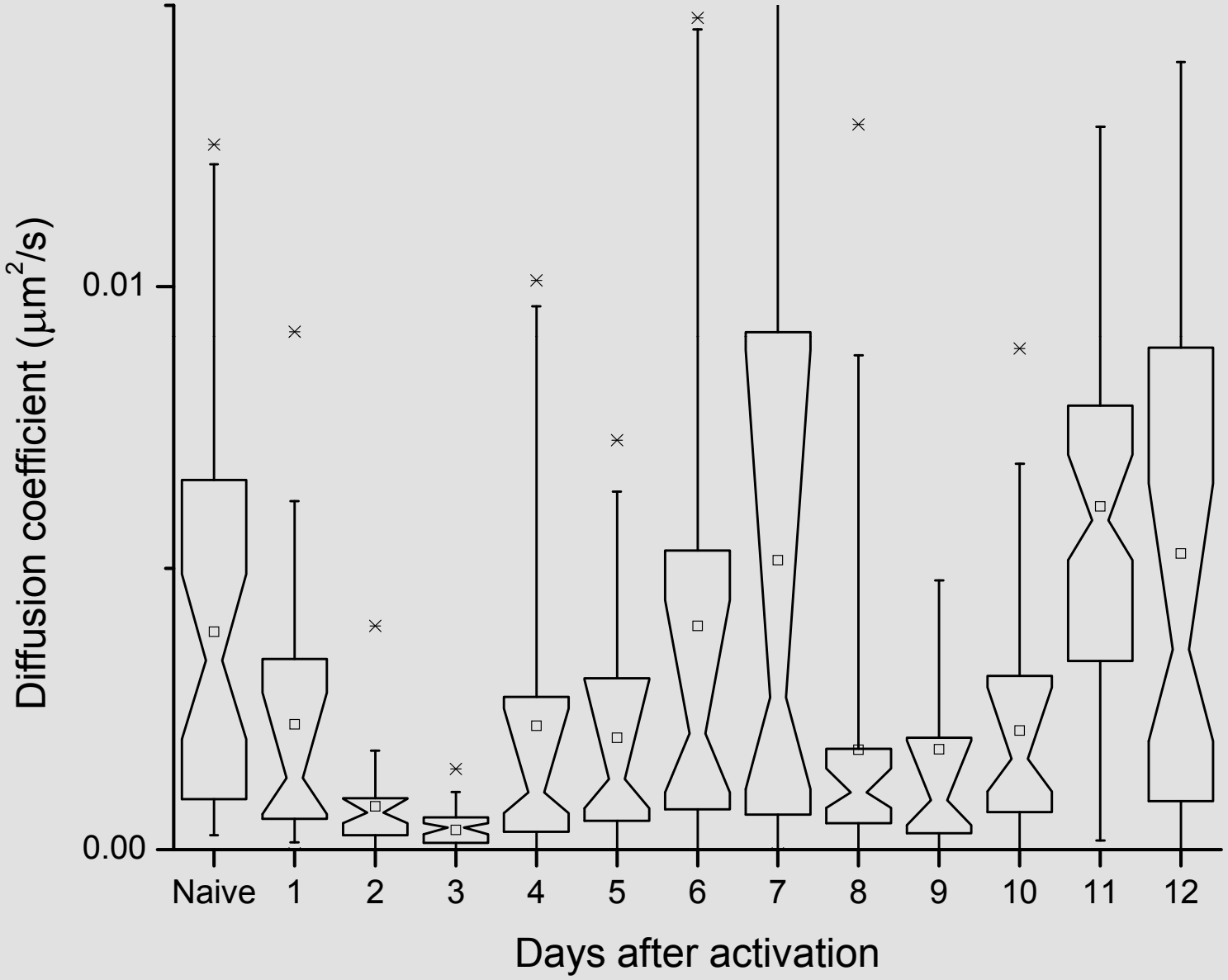


Degree of aggregation

Add T Cell Growth Factor Days 6 & 10…activation echo



Diffusion coefficient



QD clusters blink differently than single QDs

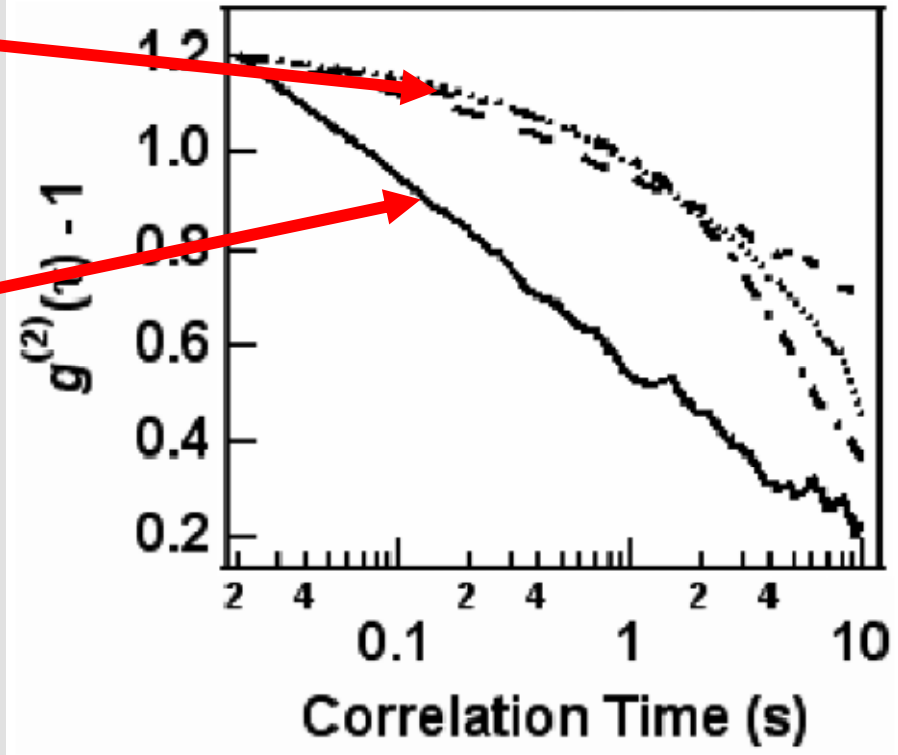
PRL 97, 237402 (2006) PHYSICAL REVIEW LETTERS week ending 8 DECEMBER 2006

Enhanced Fluorescence Intermittency of CdSe-ZnS Quantum-Dot Clusters

Ming Yu and Alan Van Orden
Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523, USA
(Received 23 June 2006; published 8 December 2006)

Single QD

QD Cluster



As well Scheibner et al. Nature Physics 3:106–110, 2007