Three dimensional tracking of individual quantum dots

(plus some other stuff, time permitting)

Jim Werner
Center for Integrated Nanotechnologies
Los Alamos National Laboratories
Quantum dots as fluorescent labels for tagging biomolecules

“Quantum dots for live cells, in vivo imaging and diagnostics,” Michalet, Piinaud, Bentolila, Tsay, Doose, Sendaressan, Wu, Gambhir, and Weiss

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Why track a single, small particle? examples from one and two dimensions

Myosin V Walks Hand-Over-Hand: Single Fluorophore Imaging with 1.5-nm Localization
Ahmet Yildiz,1 Joseph N. Forkey,2 Sean A. McKinney,1,2 Taekjip Ha,1,2 Yale E. Goldman,3 Paul R. Selvin1,2*

Potential for ~nm spatial precision

Can follow dynamic, stochastic processes

Phospholipids undergo hop diffusion in compartmentalized cell membrane
Fujiwara, Ritchie, Murakoshi, Jacobson, Kusumi

The Journal of Cell Biology
Volume 157, Number 6, 2002
We’re not living in flatland:

Diffusive Motion of a protein
- cytoplasm $\sim 4 \, \mu m^2/s$ to $20 \, \mu m^2/s$
- membrane $\sim 0.01$ to $1 \, \mu m^2/s$

Directed Motion
- kinesins $\sim 0.02$-2 $\mu m/s$
- myosins $\sim 0.2$ to $60 \, \mu m/s$
For 3D tracking, start with confocal microscopy as a base:
Simulating our microscope:
Using a few things we might know

Yields # of photons absorbed per second,
Yields # of photons emitted per second

Yields fraction of light for a dipole at X, Y, Z that makes it through an aperture in the image plane

“How three dimensional tracking of fluorescent particles” Lessard, Goodwin, Werner
Simulation, quantum dot
D=1.0 um^2/s; NO TRACKING
Tracking simulation, quantum dot
\[ D = 1.0 \, \text{um}^2/\text{s} \]
The tracking apparatus (Hardware):

Equipment:

- A Fast closed loop XYZ Piezo stage (PI-733-3DD)
- SPC 630 (not used for tracking)
- Four SPADs
- Pulsed semiconductor diode laser
- 60x, 1.2 NA water immersion objective
- LabView REALTIME
Experimental Data:
Glycerol/water mixture, D~ 1 um^2/s
Randomly selected 3-D trajectories

2 μm scale bar
More Randomly selected 3-D trajectories
How do you know you’re tracking a single qdot?

1. Count rate is what you’d expect from a single quantum dot.

2. The mean squared displacement of the measured trajectories reflects particle size:

\[ D = \frac{KT}{6\pi\eta R} \]

From 3D trajectories:

\[ R_H = 16 \text{ nm} \]

From FCS:

\[ R_H = 15 \text{ nm} \]

“Three dimensional tracking of individual quantum dots”
Lessard, Goodwin, Werner
(submitted)
Future directions: 3D trajectories in cells, over-lapped with structure
Time-resolved spectroscopy while tracking

Raw Photons:
ANY analysis method

Fluorescence lifetime measurements:
Proximity to a FRET partner
Conformation of molecule

Not limited by camera “frame rate”

Window on cellular process spanning 100 ps to 10 seconds!
Conclusions: 3D tracking

We can track single quantum dots in 3D at rates faster than many intracellular transport processes

Next Steps

Into the cell
Rates of motion
Spatial accuracy
 Measure CEF
Protein folding

Unfolded states

Native state

Astronomical number of different conformations

\[ \sim 10^{30} \]
Yeast cytochrome c labeled with TRITC

TRITC

Dansyl label (lifetime only)

Cytochrome c

Mapping the Cytochrome c Folding Landscape
Julia G. Lyubovitsky, Harry B. Gray,* and Jay R. Winkler*

Contribution from the Beckman Institute, California Institute of Technology, Pasadena, California 91125

Received October 26, 2001
Fluorescence Correlation Spectroscopy (FCS) to measure gross conformation and fluctuations in fluorescence intensity.

\[ G(\tau) = \frac{\langle \delta I(t) \cdot \delta I(t+\tau) \rangle}{\langle I(t) \rangle^2} \]

\[ \tau_c = \frac{\omega_0^2}{4D} \]
Fluorescence correlation spectroscopy of cyt c-TMR

Site-specific distance distributions measured by FRET via TCSPC

\[ k = k_{sp} + k_{NR} \]

\[ k_{TR} \sim |\langle d^a | M | d^a^* \rangle|^2 = \frac{1}{\tau} \frac{R_0^6}{R^6} \]

\[ k = k_{sp} + k_{NR} + k_{TR} \quad R_0 \sim 40 \text{ Å} \]

\[ I(t) = \int p(k) e^{-kt} \, dk \]

How do we extract this?

Maximum Entropy Methods

Minimize \( \chi^2 \)

Maximize \( S = \sum_i p_i \cdot \log(p_i) \)
P(k) distribution from TCSPC MEM

Structural characterization of folding intermediates in cytochrome c by H-exchange labelling and proton NMR

Heinrich Roder, GÜlnur A. Elöve & S. Walter Englander

Nature 335, 700 - 704 (20 October 1988)
Correlation between FCS and TCSPC

cyt c folding conclusions

Combination of methods reveals details that can’t be easily discerned by either independently.

Not only “2D” static view of landscape

Possible use of 3D tracking.
Single Molecule Studies of Antigen-Antibody Binding: Why

Scientific Goals:

1. Explore “conformational memory” effects
2. Correlate average affinity with deviation from average
3. Examine reasons for loss of affinity due to surface immobilization
4. Observe single molecule dynamics for decades of timescales

“Antibody Multispecificity Mediated by Conformational Diversity”
L. C. James, P. Roversi, and D. S. Tawfik
Wide-field imaging by total internal reflection microscopy

- Single fluorescent molecule or quantum dot

“Surface-immobilized antibody-antigen binding affinity studies by single molecule fluorescence imaging”
Antigen-Antibody Conclusions

Progress thus far:

Surface Chemistry:
  “Clean” enough for single molecule detection
  Prevents non-specific binding to levels needed for single molecule studies
  Preserves antibody activity

Data Acquisition:
  Image and data analysis software written

Future:

Need to distinguish binding from blinking
Learn how to account for it in the data
Switch to a different fluorescent reporter
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Antigen-Antibody Binding
Jamshid Temirov
Andrew Bradbury
Jim Werner