Tracking single quantum dots in 3 dimensions

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Quantum dots as fluorescent indicators for labeling biomolecules

“Quantum dots for live cells, in vivo imaging and diagnostics,” Michalet, Piinaud, Bentolila, Tsay, Doose, Sendaresan, 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, Joseph N. Forkey, Sean A. McKinney, Taekjip Ha, Yale E. Goldman, Paul R. Selvin

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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:

Fibers are 50 μm core 2.5 μm cladding

\[ \frac{1}{S_i} + \frac{1}{S_o} = \frac{1}{f} \]
Simulating our microscope: Using a few things we might know

“How three dimensional tracking of fluorescent particles” Lessard, Goodwin, Werner
Simulation, quantum dot

$D = 1.0 \text{ \mu m}^2/\text{s}; \text{ NO TRACKING}$
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, $D \sim 1 \text{um}^2/\text{s}$
Randomly selected 3-D trajectories

2 μm scale bar
More Randomly selected 3-D trajectories

2 μm scale bar
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:

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)
How do you REALLY know you are following a single quantum dot?

Anti-bunching curve shows we have single quantum emitters (NOT TRACKING)
Future directions: 3D trajectories in cells, over-lapped with structure
Particular problems of interest

Bridget Wilson and Diane Lidke
UNM Cell Pathology Department

IgE-FcεRI

Human Allergic response

Diffusion on surface ~ 0.1 um^2/s
Recruitment to pit ~ 0.1 um/sec
Internalization ~ 0.5 um/sec
Know thine enemy……

Enderlein’s Method

Other confocal methods

Haw Yang (UC Berkeley)- Gold 80 nm diameter

Enrico Gratton (UC Irvine)
Beads ~ 10000 FITC equivalent

Hideo Mabuchi (Stanford)

CCD based approaches:

Moerner/Dickson (TIR- Nature 96)

Ramund Ober (Texas Medical Center)

Paul Selvin (U. Illinois Urbana-Champaign)

Thomas Schmidt (Leiden)
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 & Acknowledgements

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

One obvious next step:

Goodwin, Lessard, Werner

Funding:
Laboratory Directed Research & Development
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National Nanotechnology Enterprise Development Center
What is the limit?

Need to integrate a few ms to collect enough photons to decide which way to move (Shot noise on the source)

Blinking “off” of the quantum dots limits the time we can continuously follow it. (simulations suggest ability to re-acquire a dot)

Photobleaching of other reporters (GFP) or organic dyes could limit the length of the measured trajectories for those fluorophores

Close to stage limit in response time (1 msec) and in travel length (30x30x10 um)
Statistics of 400 trajectories