Lecture 13: Particle Tracking

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6th LFD Workshop
October 24–28, 2011 - Irvine
Outline

• Introduction / Motivation
• Particle Tracking Basics
• Real-Time 3D-Particle Tracking
• Examples
• Modulation Tracking
• Summary / Literature
Moving Particles

Single-Molecule (U1-snRNPs) tracking observed in a living eukaryotic cell nucleus

Siebrasse et al. Anal Bioanal Chem 2007

Single-virus tracking: how actin cortex might propel viruses towards neighbouring cells during egress

Moving Particles

Complex 3D trafficking itinerary of a QD-IgG molecule undergoing endocytosis

Ram et al, Biophys J 2008

Retrograde axonal transport of nerve growth factor (NGF)

Cui et al, PNAS 2007
Moving Particles

Mapping Local Matrix Remodeling Induced by a Migrating Tumor Cell Using 3D-Multiple-Particle Tracking.

*Bloom et al, Biophys J 2008*

Particle Tracking of single functional Qdots in tumors of mice from a capillary vessel to cancer cells in living animals

*Tada et al, Cancer Res 2007*
Why single molecule experiments?

Orders of magnitude (for 1 μM solution, small molecule, water)

<table>
<thead>
<tr>
<th>Volume</th>
<th>Device</th>
<th>Size(μm)</th>
<th>Molecules</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>milliliter</td>
<td>cuvette</td>
<td>10000</td>
<td>6x10^{14}</td>
<td>10^4</td>
</tr>
</tbody>
</table>

What information are we missing in "cuvette" assays?

e.g. conformational state of an enzyme by FRET
Bulk vs. single molecule measurements

Limitations of bulk measurements:
- molecules are not synchronized
- only average properties

Single molecule/particle assays
- Temporal evolution of each molecule/particle
- No need to synchronize processes
- Detection of different populations

Disadvantages
- More expensive equipments
- Statistics!
Measuring motion in cells:

• FRAP (fluorescence recovery after photobleaching)
  – Photodamage
  – Photobleaching is not always irreversible

• FCS (fluorescence correlation spectroscopy)
  – Low laser power
  – Low concentration of fluorescent molecules

• Single particle tracking – WHY?
  – It is hard to study complex processes (changes of motion mechanism, multiple populations, etc.)
Position of a particle as a function of time

- mechanism of motion:
  - interactions
  - populations
  - switching (no synchronization)
Image-based tracking techniques

Examples:
- FIONA (fluorescence imaging with one nanometer accuracy)
- Pattern-recognition tracking methods

δx_{cm} \approx 1.5 \text{nm}

\delta = \sum_{i,j} \sqrt{(I_{\text{image}}(i, j) - I_{\text{pattern}}(i, j) - B)^2 \cdot w(i, j)}

\sim 300 \text{nm}

\text{Yildiz et al, Science 2003}

\text{Levi et al, Biophys J 2006}
Improving SPT: Error in the particle position

\[
\langle (\Delta x)^2 \rangle = \frac{s^2}{N} + \frac{a^2/12}{N} + \frac{8\pi s^4 B^2}{a^2 N^2}
\]

\(\Delta x\) = error in the particle position
\(s\) = standard deviation of the PSF
\(N\) = number of photons detected
\(a\) = pixel size
\(B\) = background noise

- Photon noise
- Background noise
- Pixelization noise

\(B\) increases
\(a\) increases
\(a >\) diffraction limit

Improving SPT: Tracking error

\[
\langle (\Delta x)^2 \rangle = \frac{s^2}{N} + \frac{a^2}{12N} + \frac{8\pi s^4 B^2}{a^2 N^2}
\]

- photon noise
- background noise
- pixelization noise

\[
\begin{align*}
B (\text{photons}) &= 0, 1, 10 \\
N &= 10^4 \\
\end{align*}
\]

\[
\begin{align*}
\Delta x (\text{nm}) \quad N (\text{photons}) \\
10^0 &\quad 10^1 &\quad 10^2 &\quad 10^3 &\quad 10^4 &\quad 10^5 \\
\end{align*}
\]

\[
\begin{align*}
\Delta x (\text{nm}) \quad a/s \\
0 &\quad 2 &\quad 4 &\quad 6 &\quad 8 &\quad 10 \\
\end{align*}
\]
Obtaining quantitative information from trajectories analysis

- In the three cases the trajectories look extremely different: how to quantify?

- The distance traveled by the particle after a given time period is different for the different motion regimes!

Mean Square Displacement (MSD)

\[ \text{MSD}(\tau) = \left\langle [x(t) - x(t + \tau)]^2 + [y(t) - y(t + \tau)]^2 \right\rangle_\tau \]

Now the ‘shape’ of the trajectory is quantified through the MSD vs \( \tau \) relationship.
What information can we obtain from MSD?
Determination of the mechanism of motion

$$(\nu \tau)^2 \quad \text{ballistic motion} \quad (\Delta \vec{r} = \vec{v} \tau)$$

$6D \tau \quad \text{random diffusion}$

$6D \tau^\alpha \quad \text{anomalous subdiffusion}$

$\text{constrained diffusion}$

in 3-dimensions
What’s in a Trajectory?

Heterogeneities become visible directly!

Single point-FCS, for example, cannot discern between these cases:

- **Populations of velocities**
- **Transient diffusion zones**
- **Non-random motion**
- **Barrier to diffusion**
Probes for SPT experiments

In SPT experiments, probes may:

1) delay the motion of the particle
2) change the properties of the tracked molecule

<table>
<thead>
<tr>
<th>Probe</th>
<th>Size (nm)</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>fluorescent beads</td>
<td>10-1000</td>
<td>brightness</td>
<td>size</td>
</tr>
<tr>
<td>quantum dots (CdSe)</td>
<td>10-20</td>
<td>brightness, spectra</td>
<td>blinking</td>
</tr>
<tr>
<td>fluorescent proteins</td>
<td>2-3</td>
<td>endogenously expressed</td>
<td>low brightness, high concentration, bleaching</td>
</tr>
<tr>
<td>single dye molecules</td>
<td>0.5-1</td>
<td>size</td>
<td>low brightness, bleaching (&lt;10s)</td>
</tr>
<tr>
<td>nanoparticles (Si,SiO$_2$)</td>
<td>2-50</td>
<td>last ‘forever’</td>
<td>low signal/noise</td>
</tr>
</tbody>
</table>
How to track in 3D?

- Using two image planes: limited to movements in axial direction of 1–2 μm (without additional feedback)

- Fast 3D stack (e.g. spinning disk): limited in time to ~1 sec

→ Feedback approaches: follow the particle in real-time, particle always at center

Ram et al, Biophys J 2008
Real-Time 3D single particle tracking

• ‘Tetrahedral’ tracking (4 detectors)


• Orbital scan tracking

  Levi et al Biophys J 2005

Kis-Petikova & Gratton. Micro Res Tech 2004

2D → 3D
Orbital scanning: mathematical details

\[ AC = b_1 = \frac{1}{\pi} \int_{-\pi}^{\pi} \cos \theta \cdot F_{01} \times \exp \left[ -\frac{4}{w_0^2} \left( A^2 + r_1^2 - 2Ar_1 \cos \theta \right) \right] d\theta \]

\[ DC = a_0 = \frac{1}{2\pi} \int_{-\pi}^{\pi} F_{01} \exp \left[ -\frac{4}{w_0^2} \left( A^2 + r_1^2 - 2Ar_1 \cos \theta \right) \right] \times d\theta = F_{01} \exp \left[ -\frac{4}{w_0^2} \left( A^2 + r_1^2 \right) \right] \cdot I_0 \left( \frac{8Ar}{w_0^2} \right) \]

\[ \Delta d_{\text{min}} = \frac{0.85}{w_0} \sqrt{F_{01}} \]

AC/DC:

\[ MOD = 2 \cdot I_1 \left( \frac{8Ar}{w_0^2} \right) / I_0 \left( \frac{8Ar}{w_0^2} \right) \]

\[ F(t) = \frac{2F_0}{\pi} / \left[ 1 + \frac{A^2 \left( z_t - z_{i(t)} \right)^2}{w_0^2 \pi^2} \right] \times \exp \left[ \frac{-2 \left( \left( x_t - x_i(t) \right)^2 + \left( y_t - y_i(t) \right)^2 \right)}{w_0^2 + \frac{A^2 \left( z_t - z_{i(t)} \right)^2}{w_0^2 \pi^2}} \right] + B_i \]
Remember: the PSF

- The intensity at each scanned point is a function of the distance from the particle:

\[ I(\vec{r}) \propto PSF(\vec{r} - \vec{r}_p) \]

\[ \vec{r}_p = \vec{r}_{center} \rightarrow I(\vec{r}) = \text{const} \]
To locate the particle we need to know: **Angle**, distance and height from center.

\[
\text{FFT}[I(t)] \rightarrow \text{DC and AC}
\]

\[
\begin{align*}
\text{DC} & \rightarrow < \text{PSF} (\vec{r} - \vec{r}_P) > \\
\text{AC} & \rightarrow \Delta \text{PSF} (\vec{r} - \vec{r}_P)
\end{align*}
\]

**Average of the function along the orbit**

**Variation of the function along the orbit**
Estimate Position from Orbit

To locate the particle we need to know:
Angle, **distance** and height from center

\[ DC \rightarrow < PSF(\vec{r} - \vec{r}_p) > \]
\[ AC \rightarrow \Delta PSF(\vec{r} - \vec{r}_p) \]
To locate the particle we need to know:
Angle, distance and **height** from center

Estimate Position from Orbit

\[ h = f \left( \text{MOD} \right) \]

\[ \text{MOD} = \frac{2 \left( I_{\text{top}} - I_{\text{bottom}} \right)}{I_{\text{top}} + I_{\text{bottom}}} \]
Tracking Routine

Initial position for the scanner \((x_s, y_s, z_s)_{t=0}\)

New scanner position \((x_s, y_s, z_s)_t = (x_p, y_p, z_p)_{t-1}\)

Intensity profile

FFT

Particle position \((x_p, y_p, z_p)\)

Intensity < threshold

Increase orbit radius
Basic Microscope Setup

Two-photon or confocal microscope

X-Y scanning mirrors

Objective

Z-nano positioner

Dichroic mirror

Specimen

Laser

Detector

PMT

Amplifier/discriminator

Computer
Testing the Machine

• Particle tracking along pre-defined 1D trajectory

FFT tracking with < 20 nm resolution
Testing the Machine

- Works, of course, also in 2D and 3D.
Precision and accuracy of the method

\[ \propto \frac{1}{\sqrt{N}} \]
Examples

• Melanosome transport along microtubules, after stimulation of aggregation or dispersion

• The melanosome position was recovered every 10 ms with 2-nm precision

• Melanosome velocity depends linearly on the number of active motors
Examples

Movement of a particle in the cell nucleus:

Trajectory in jump regions is not random!

Levi et al Biophys J 2005
Examples:
Nanoimaging of collagen fibers using Au nanoparticles

Particle moving fast along the fiber

3D trajectory of a slower particle exploring the collagen fiber

Chen et al Biomed Opt Exp 2011
Information on top of particles

- Lifetime
- Spectrum
- A
- B
- Multiple particles
- Int
- \frac{d}{d\chi}
- Shape and Positions
- Scanning FCS, RICS, …
- Spectrum
- Polarization
- Lifetime
- d/d\chi
- Int
- 0° 180° 360°
Information Contained in Orbits

Every line is the average of 32 orbits.

On this time scale the particle is always at center.

The “in-between” steps can be recovered fitting all the orbits in the carpet.

Particle can move while scanner still in the same position (same ‘cycle’).

1 ms/orbit

individual orbits
Spatio-Temporal Resolution

May be possible: 4 ms = 8 orbits, 0.5 ms
More Information in Orbits

Round shape (e.g. bead, molecule)

Oblong shape (e.g. aggregates, organelles)

This is: Distance measurement in the 50-300 nm regime. Complements FRET
Can we use tracking to perform imaging?

Instead of following a predetermined pattern, use a feedback approach to adapt the scan to the shape of sparse or moving objects.

We need to track the shape of the object in real time at each section.
The Modulation Tracking (MT) Method

Oscillating the radius of a certain amount $\Delta r$ produces a modulation of the intensity which is a function of the distance between the laser spot and the surface.

For a Gaussian PSF the modulation increases linearly with the distance.
Application to Microvilli in epithelial cells

We can track the motion of microvilli and obtain 3D images of microvilli expressing different proteins.
**Direct comparison**

<table>
<thead>
<tr>
<th>SPT from Images</th>
<th>Lock-in SPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Established, straightforward</td>
<td>Very high 3D and $t$ resolution.</td>
</tr>
<tr>
<td>Many particles simultaneously</td>
<td>Other info from recorded orbit.</td>
</tr>
<tr>
<td>Visible features, obstacles, etc.</td>
<td>On-the-fly measurements.</td>
</tr>
<tr>
<td>Temporal vs. spatial resolution</td>
<td>Selected particles per measurement</td>
</tr>
<tr>
<td>Depth ($z$) resolution problem</td>
<td></td>
</tr>
</tbody>
</table>
Summary

The key to “on-the-fly” 3D particle tracking is estimating the particle’s position fast. This is accomplished by combining circular scanning and FFT in a feedback loop.

In addition to obtaining the particle’s 3D trajectory in a large 3D volume (several μm in x, y and z), this approach provides a series of interesting possibilities such as:

- High spatial and temporal resolution (nm, ms)
- Obtain additional information from the particle (shape, spectrum, lifetime…)
- Possibility to interact with the particle (e.g. by changing excitation intensity)
- Limiting “photodamage” on the specimen (only surrounding particle)
- Possibility to track multiple particles simultaneously
Some useful literature

“On-the-fly” 3D SPT
K. Kis-Petikova and E. Gratton Microscopy Res. & Tech., 2004, 63, 34.

Other 3D SPT Techniques
GA Lessard, PM Goodwin, JH Werner Appl Phys Lett 2007 (Tetrahedral tracking)
Ram et al, Biophys J 2008 (Multifocal Plane Microscopy)

Trajectory Analysis

Biophysical Applications

Mathematical Stuff (Error Estimation, Fourier Transforms)
Various Authors, “Fourier Transform” Wikipedia: The Free Encyclopedia