Exploring the Membrane Dynamics of Receptors & Integrins with image Mean Square Displacement (iMSD)

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Membrane Dynamics: an excellent biological system for iMSD analysis

- How do proteins move around in lipid membranes?
- Molecular diffusion, interaction, and signal transduction in the plasma membrane.
- The plasma membrane is a heterogeneous entity, containing diverse structures on nano-meso-scales (2–200 nm) with a variety of lifetimes, where certain membrane molecules stay together for limited durations.
- Prior work has utilized FRAP, spFCS, and single molecule tracking, each of these has their drawbacks
What is iMSD and what are its advantages?

Generally, the MSD is obtained in single particle tracking experiments.

Similar to Spatiotemporal Image Correlation Spectroscopy (STICS) but it works with fast cameras.

We can resolve motions at
- very fast (D=10µm²/s) and
- very slow (D=0.001µm²/s) time scales
- very large (microns) and
- very small (nanometers) spatial scales

- 3D fast Fourier transform
- Fit using Gaussian
- Initialized using central moments
- The algorithm evaluates the first and second order moments
- The second order non-symmetric moments give the flow size and direction
The iMSD correlation function

\[ G(\xi, \chi, \tau) = \frac{\langle I(x, y, t) \cdot I(x + \xi, y + \chi, \tau + t) \rangle}{\langle I(x, y, t) \rangle^2} - 1 \]

Spatial and temporal correlation (the same of STICS)

The calculation of the correlation function is done using the 3D FFT algorithm. So always collect data that are power of 2 in all dimensions!

\[ G(\xi, \chi, \tau) = g_0 \cdot p(\xi, \chi, \tau) \otimes W(\xi, \chi) \]

Go is the usual G(0) and it depends on the number of particle p depends on the physics of the motion W is the point spread function (approximated by a Gaussian)

Fick's law

\[ p(\xi, \chi, \tau) = \frac{1}{\pi 4D \tau} \exp \left( -\frac{\xi^2 + \chi^2}{4D \tau} \right) \]

The iMSD simplification

\[ G(\xi, \chi, \tau) = g(\tau) \cdot \exp \left( -\frac{\xi^2 + \chi^2}{\sigma^2(\tau)} \right) + g_\infty(\tau) \]

temporal correlation function iMSD function Constant offset
Illustration of the iMSD functions

Diffusion

Confinement

Transient confinement
Schematic illustration of the iMSD analysis
Effect of the “size” of the particle

- The particle size can be obtained with very high precision to 0.01 \( \mu \text{m} \)
- Note that if the sampling is “slow” a small particle will appear big
- This is important in the determination of cluster size by ICS
The diffusion coefficient can be obtained from the iMSD and the $1/g(\tau)$.
Data Acquisition for iMSD

• Total Internal Reflection Fluorescence (TIRF)
  – Uses a camera to acquire a snapshot of the entire image
  – Our system can collect up to 100 frames/sec

• Raster Confocal Laser Scanning Microscopy
  – Uses a laser to scan lines in the carpet representation
Total Internal Reflection Fluorescence (TIRF) Microscopy
TIRF is superior for visualizing membrane dynamics

GFP - Actin

GFP - paxillin
Integrin alpha 5

- A member of integrin alpha chain family.
- A heterodimeric integral membrane protein.
- Complexes are composed of an alpha chain and a beta chain.
- Alpha chain 5 joins with beta 1 to form a fibronectin receptor.
- In addition to adhesion, integrins are known to participate in cell-surface mediated signalling.

Integrin connects the extracellular matrix with the actin cytoskeleton inside the cell.

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Example 2:

Fast spatiotemporal correlation spectroscopy to determine protein lateral diffusion laws in live cell membranes utilizing TIRF and iMSD

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Carmine Di Rienzo, Enrico Gratton, Fabio Beltram, Francesco Cardarelli
Prior work: Transmembrane transferrin receptor explored with SPT by Kusumi et al. 2009

The authors described the dynamics of single particle tracking to address how the membrane skeleton is confining the transferrin receptor.
Transferrin receptor (TfR) movement is transiently confined.
Treatment with Latrunculin-B (an actin-perturbing agent) releases the confinement
Transient confinement if TfR is due to an Arrhenius barrier
iMSD can also be used as a line scan.
Conclusions

• iMSD is a great new analysis tool that will work with TIRF camera images as well as raster LSM confocal images that makes it possible to investigate proteins movement in the plasma membrane

• Allows a way to investigate diffusive properties of a population of molecules, as opposed to single molecule tracking

• This was shown to work on fibronectin activated integrins (example I) and to reveal the barriers in the membrane based on the movement of the transmembrane receptor TfR (example II)
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