ONI Resources

Imaging guide

Single Particle Tracking: Fluorophores to dye for

What is single-particle tracking?

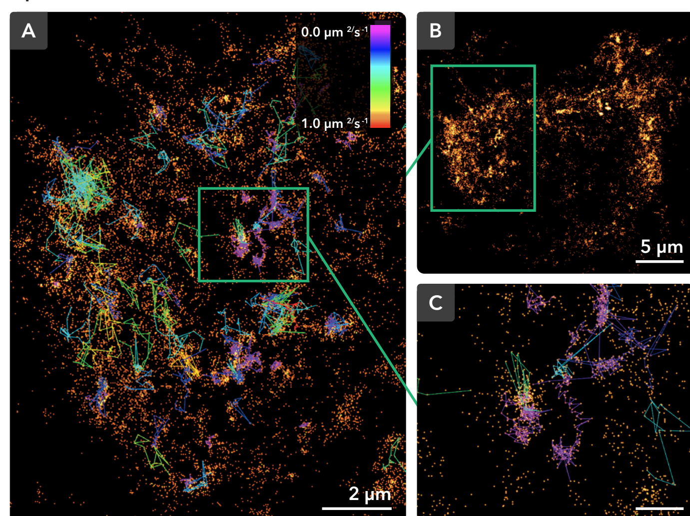
Understanding the dynamics, trajectories and interactions of single molecules and vesicles in cells presents a fundamental challenge in cell biology. Single-particle tracking (SPT) is a powerful microscopy technique that allows the motion of individual fluorescently-labeled particles (molecules, vesicles, virions or other molecular complexes) to be followed within a medium, or in living cells to investigate behaviour.

SPT provides valuable data concerning the positions, paths and interactions of molecules that are highly dynamic or require imaging over extended periods of time. Using sophisticated image processing algorithms, trails of motile particles can be determined with nanometer-precision, and trajectories resolved with millisecond time resolution. This enables track length and diffusion coefficient data to be analysed quantitatively, applicable for a wide variety of experimental investigations. Moreover, SPT also enables powerful analysis of particle concentration and size distribution for quality control.

To clearly track a protein that has a low abundance in a sample, simply labeling it with a bright and photostable fluorophore is sufficient. However, in order to be able to meet the single-molecule criteria for a protein that is highly expressed, either the concentration of the fluorescent ligand (if you use a tag) needs to be adjusted accordingly, or it is possible to use photoactivatable/convertible proteins, and in turn modulate the intensity of the activation laser to ensure only a subset of molecules are activated simultaneously. This is known as SPT-PALM (Photo-Activated Localisation Microscopy). See our application note on PALM for further details on how this technique works.

Measure real-time assembly of viruses

With SPT it is possible to study vesicular transport pathways, protein-protein interactions and transcriptional events as well as the diffusion profiles of external factors such as drugs. The ability to examine the movement of particles across cellular and subcellular compartments can elucidate important structure-function relationships and help generate accurate localization maps, such as the assembly of viruses. For instance, SPT of influenza virus demonstrated significant differences during real-time assembly, as particles of higher velocities were noted on the basal membrane compared to that observed at apical membrane (Figure 1).



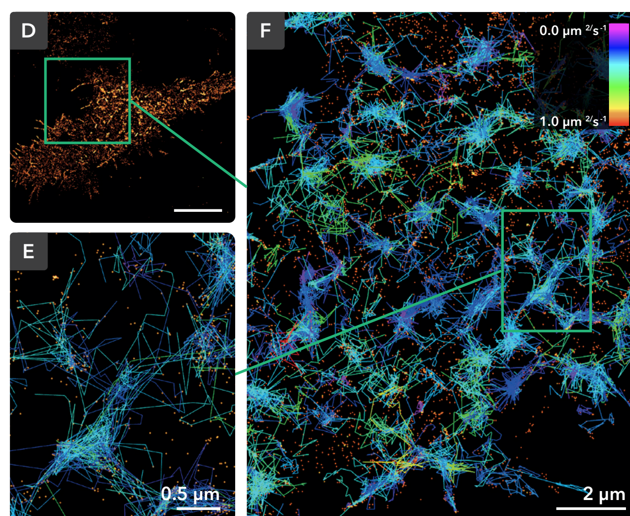


Figure 1 | Single-particle tracking Influenza HA (HA-Dendra) HEK293T cells. Virus particles show a greater motility on apical membrane of the cell (A-C), in comparison to the basal membrane (D-F), suggesting differences in viral mechanisms or interactions. *Prof, Salvo Chianti from Potsdam University.*

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Choosing a suitable SPT fluorophore

Many factors need to be considered when choosing the optimal labelling strategy for any microscopy experiment, and the additional temporal dimension in SPT presents further challenges. Whilst any fluorophore that is compatible with the Nanoimager configuration can be used for SPT, we recommend using synthetic fluorescent dyes to be able to meet the single-molecule criteria. This is due to live-cell applications requiring maximum photostability and fluorophore brightness for the fast tracking and localization precision associated with protein tags coupled to synthetic dyes, such as SNAP-, FLAG-, CLIP- and HaloTag®. Please see our HaloTag® application note for further details.

During SPT, long-term laser excitation may induce phototoxicity to the cells and in the case of SPT-PALM, the additional use of activation light, often near-UV light, can enhance toxicity. Furthermore, labelling and extensive washing of unbound synthetic ligands may also complicate applications that are sensitive to fluctuations in environmental conditions. However, these challenges can be overcome by considering a dye such as whose fluorescence increases upon conjugation to a protein tag, thereby minimising the required excitation power and reducing exposure to wash buffers for the removal of unconjugated dye.

Popular fluorophores for SPT

Name	Excitation max (nm)	Emission max (nm)	Comments
Standard SPT labels			
GFP	395	509	Commonly chosen fluorescent protein for the green range, but suffers from low photon count, and poor resistance to photobleaching.
mCherry	587	610	Low photon count, and poor resistance to photobleaching.
mOrange	549	565	Low photon count, and poor resistance to photobleaching.
TMR	552	578	Compatible with HaloTag®
Oregon Green®	492	520	Compatible with HaloTag®
diAcFAM	492	521	Compatible with HaloTag®
Alexa fluor® 488	499	518	Cell impermeable (membrane marker). Compatible with HaloTag®
Alexa fluor® 660	654	690	Cell impermeable (membrane marker). Compatible with HaloTag®
Janelia Fluor® 549	549	571	Twice as bright as TMR. Excellent cell permeability and requires less washing steps. Compatible with HaloTag®
Janelia Fluor® 646	649	664	
SPT-PALM labels			
PA-GFP	504 (UV-405 or 488 activation)	517	Offers less flexibility and complexity in experimental design than others.
PA-TagRFP	562 (UV-405 activation)	595	Bright and photostable.
PA-mCherry1	570 (UV-405 activation)	579	Low photon count.
PA-mKate	586 (UV-405 activation)	628	Higher pH and photo stability than PA-mCherry.
Dendra2	490 (G), 553 (R) (UV-405 or 488 activation)	507 (G), 753 (R)	Blue light activation and reliable performance makes Dendra2 a good candidate. Primed photoconversion or point mutations reduce phototoxicity.
PA-Janelia Fluor® 549	549 (UV-405 activation)	571	Photoactivatable versions: good for high-speed and dual-colour live-cell SPT. Compatible with HaloTag®
PA-Janelia Fluor® 646	646 (UV-405 activation)	664	

Solution with the Nanoimager

ONI is focused on advancing the scientific tools to empower researchers to continue to explore and learn the microscopic details of life.

For more information on single-molecule localization microscopy and SPT in your research, please visit www.oni.bio

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