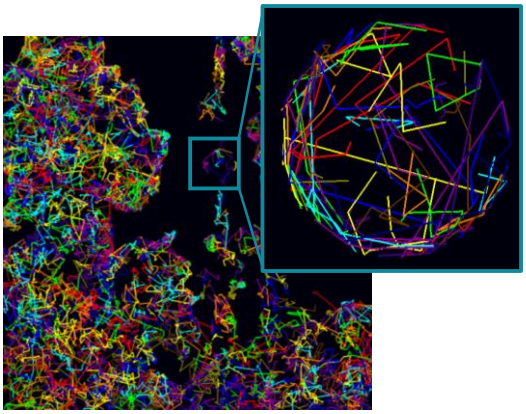
How to track single molecules in living cells

In fluorescence microscopy, the principle of tracking is to follow the motion of a fluorescent entity over time in living cells. This entity can be a group of molecules, a full structure or, in the case of single particle tracking (SPT), a single molecule. SPT is compatible with most single molecule techniques (see our note on SMLM techniques). The most commonly-used is SPT-PALM where single proteins fused to photoactivatable/convertible proteins are tracked.



Sample provided by Dr. Chiatunetti, University of Geneva, Switzerland

In practice

What do I need to do SPT?

- Living cells or mobile structures
- A fluorescent probe compatible with SMLM in living cells
  - Photoactivatable or photoconvertible proteins (mEos, PA-mCherry, PA-GFP, mMaple, Dendra2,…):
  - Photoswitchable dyes compatible with transparent culture media (TMR, Janelia Farm dyes, SiR647,…).
- A SMLM microscope
- A software for single molecule localization and tracking

How can I acquire SPT data?

SPT raw data are similar as standard single molecule acquisitions. The main differences are the exposure time - which needs to be short to follow fast dynamics but long enough to guarantee sufficient signal – and the laser power – high enough for single molecule imaging but not too high to avoid photobleaching and phototoxicity.
The main challenge in SPT is to analyze these data to reconstruct tracks.

How can I analyze SPT data?

The goal of an SPT algorithm is to connect the dots from frame to frame. The algorithm takes all the tracks at frame *t* and all the dots at frame *t+1* and calculates the probability of assigning each track to each dot. Afterwards, it chooses the solution that maximizes the probability. These probabilities can be calculated based on a number of factors, including distance and motion speed.

How do I know if a molecule is the same or its neighbor? How fast are my molecules moving? How long are my tracks? How bright are the molecules? These are essential questions in SPT that are challenging to address in post-processing as well as during the live acquisition.

Abbelight SAFe modules and **NEO software** offer features covering:

Acquisition:

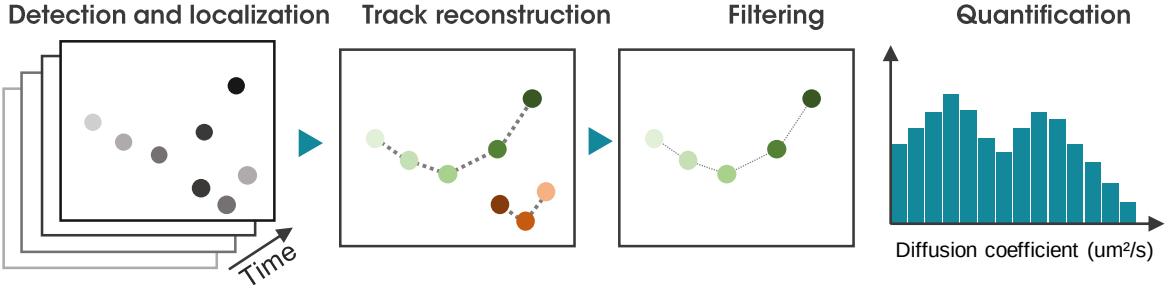
- Control of exposure time down to a few ms
- Control of laser acquisition (continuous or pulsed)
- Real-time feedback on track reconstruction (number of tracks, duration, number of photons per track,…)

Reconstruction:

- SPT algorithm based on Jaqaman *et al.* 2008 and Sergé *et al.* 2008 where distance and speed are considered
- Gap closing included
- Track visualization

Quantification:

- Number of tracks
- Track duration
- Track intensity
- Diffusion coefficient based on Mean Square Displacement (MSD)



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