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Robust classification of particle tracks for characterization of diffusion and dynamics in fluorescence microscopy

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The characterization of molecule dynamics in living cells is of paramount interest in quantitative microscopy. This challenge is usually addressed in fluorescent video-microscopy from particle trajectories computed by tracking algorithms. However, classifying individual trajectories into three diffusion groups –subdiffusion, free diffusion (or Brownian motion) and superdiffusion –is a difficult task. To overcome this problem, we have developed a two stage approach based on statistical measure of diffusion and requiring the setting of only one parameter corresponding to a p-value. In the first stage, the procedure is related to a statistical test with the Brownian motion as the null hypothesis and the subdiffusion and super diffusion as the alternative hypothesis. The testing procedure is well grounded in statistics, robust to different lengths of trajectories and low signal-to-noise ratios. However, it is known that applying multiple times a test without care leads to a high number of false positives.

Accordingly, in the second stage, we modified the results of the first stage to address this problem. We considered the multiple testing framework to reduce the number of trajectories wrongly classified as superdiffusion or sub diffusion. This approach has been especially developed to process individual trajectories provided by particle tracking algorithms and 2D+t and 3D+t images acquired with standard microscopy methods such as wide-field or confocal microscopy or with super-resolution microscopy such as (Single Particle Tracking) SPT-PALM. We demonstrate that the proposed approach is more robust than previous techniques, including the Mean Square Displacement (MSD) method.

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Track Classification: 11 : Using tracks