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An advanced method of tracking temporarily invisible particles in video imaging

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1. Introduction

Single-particle Tracking (SPT) is one of the common tools to investigate the transport processes in confined liquids and living cells. Thereby the analysis of the obtained trajectories reveals the motion characteristics of the observed particles/molecules and as well the local rheological properties of the surrounding liquid. Especially in the case of tracking single molecules, the usage of dye molecules and quantum dots poses a problem due to their photophysics. Hereby blinking, bleaching and missed detections in the presence of noise lead to temporary invisibility in the “eyes” of any detection algorithm. In consequence the re-linking of the particle positions results in only short trajectories.

We want to present an advanced tracking algorithm which is able to re-link particle paths even after some time of invisibility. We test our algorithm in comparison with the one from Sbalzarini and Koumoputsakos [1] in number and quality of the obtained trajectories and their speed.

2. Method

The analysis of the temporal behaviour of molecular motion is in need of long trajectories to identify time-dependent changes in the local diffusion coefficient. Contrarily to this the usually used fluorescent dye-molecules and quantum dots show blinking effects, which hinder their continuous observation. Nevertheless the Brownian motion of these particles can be described by the Fokker-Planck-Equation and a generalized propagator can be used to predict the most probable path of a molecule during its invisibility.

The first step in tracking is the identification and refinement of all visible spots throughout the microscopic video. This can be done by any of the methods described for example by Cheezum et al. [2]. With this set of time discrete spot positions the particle trajectories have to be re-linked. The usual way is to define a cost functional which is the negative logarithm of the probability of a set of links. Thus the minimization of this cost functional gives the optimal set of links between two consecutive video frames.

By defining a new cost functional which is based on the mean squared displacement of all identified trajectories in a given memory range and the usage of “dummy” points in cases when a particle is invisible we are able to re-link trajectories also in that cases. The minimization procedure itself is nearly equal to that described in [1] except of some further optimization steps which partly compensate the additional computation cost due to calculation of the new cost functional. So our algorithm is nearly as fast as the original one. In cases of many particles to track (>= 40) and a long memory range (>= 4 frames) our algorithm is even able to outperform the one of Sbalzarini.

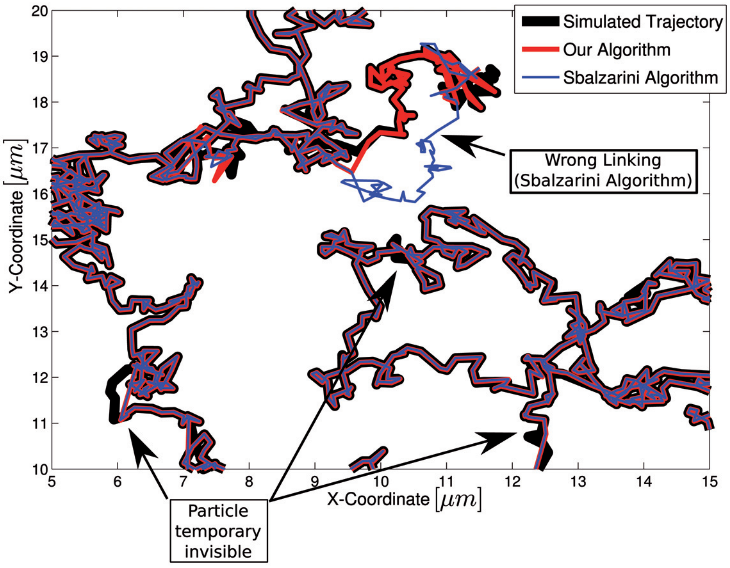

Fig 1: Comparison of trajectories

Finally by testing both algorithms on artificial videos showing randomly blinking diffusing spots, we can show that our algorithm produces the higher number of long trajectories. And when comparing the obtained trajectories with the simulated ones (see figure 1), our algorithm also shows less false links.

3. Conclusion

The usage of our algorithm when tracking diffusing and randomly blinking particles increases the number of obtainable long trajectories and reduces the error produced by false links. Consequently the analysis of these trajectories is more reliable and with help of time-series-analysis also time-dependent features of the motion could be revealed. Thereby the additional computational effort is limited.

References

[1] I.F. Sbalzarini, P. Koumoutsakos, J. Struct. Bio. 151 (2005) 182-195.

[2] M.K. Cheezum, W.F. Walker, W.H Guilford, Biophys. J. 81 (2001) 2378-2388