SINGLE-MOLECULE INSTANTANEOUS VELOCITY MEASUREMENT WITH AN ULTRAFAST DOUBLE-EXPOSURE sCMOS CAMERA

Mirella V. Koleva, Gerhard Holst, Simon M. Ameer-Beg
Centre for Cancer Studies
King's College London
New Hunt's House, Guy's Campus, London SE1 1UL, United Kingdom
E-mail: mirella.koleva@kcl.ac.uk

KEY WORDS: Live-cell imaging, TIRF, EGFR, quantum dots, SPT, high-speed imaging.

Precise tracking of individual molecules in living cells enables the unravelling of the biochemical reactions that underlie complex cellular functions. Deep understanding of mechanisms such as actin remodelling (which underpins cellular locomotion) could be leveraged to exert control over cell migration and obstruct cancerous metastatic invasion. Strategies for establishing the occurrence of molecular interactions include super-resolved Single-Particle Tracking (SPT) (*e.g.*, sptPALM) [1] and high-speed SPT [2]. However, the time-consuming data-gathering protocol of pointillistic super-resolution reconstruction forces sptPALM images to be compromised in either spatial or temporal resolution to a certain extent. High-speed SPT, on the other hand, requires very expensive cameras which are outside of the budget of most research groups.

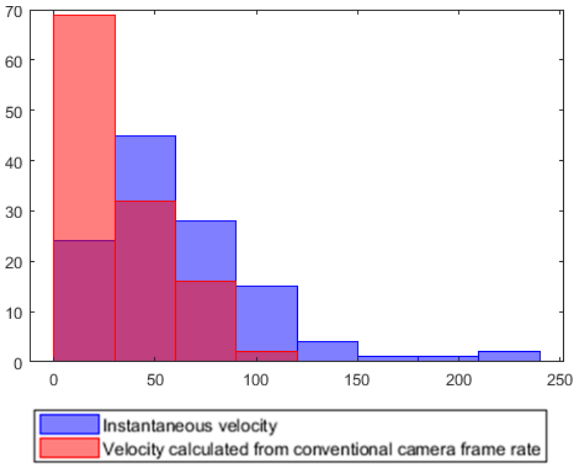

Figure 1. Histogram of the speed (in μm/s) of individual EGFR molecules. Imaging at a low frame rate tends to underestimate the molecular speed.

In this work, we take a method originally developed for the automotive industry, called particle tracking velocimetry (PTV), and adapt it to single-molecule cell-biological fluorescence imaging. As a proof of concept, we apply the technique to determine the instantaneous velocity of individual epidermal growth factor receptor (EGFR) molecules in live cells and attempt to infer the nature and properties of the diffusion that they undergo.

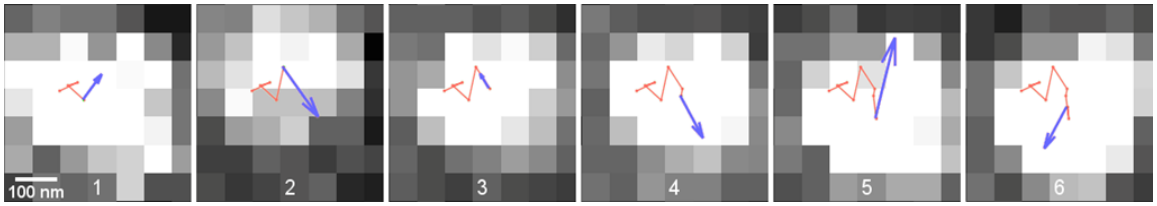

Figure 2. At low frame rates, linking the positions of a molecule in consecutive frames (last pair of points on red line) traces out a trajectory that differs from the instantaneous velocity vector as determined by PTV (blue arrow) in both direction and magnitude.

[1] S. Manley, J. M. Gillette, G. H. Patterson, H. Shroff, H. F. Hess, E. Betzig, and J. Lippincott-Schwartz, "High-density mapping of single-molecule trajectories with photoactivated localization microscopy," *Nat. Methods*, **5**, 155–157 (2008).
[2] A. Kusumi, Y. Sako, and M. Yamamoto, "Confined Lateral Diffusion of Membrane Receptors as Studied by Single Particle Tracking (Nanovid Microscopy). Effects of Calcium-induced Differentiation in Cultured Epithelial Cells," *Biophys. J.*, **65**, 2021–2040 (1993).