

High-Speed Bio-imaging Project

Life is four dimensional. Techniques used to study the molecules making up life have developed rapidly over the years. While single molecule techniques can be routinely applied to tracking molecular motions at the 1 nm level, the temporal resolution is currently limited to the millisecond or sub-millisecond level, mainly due to the lack of ultra high-speed cameras. We have recently set up a high-speed Differential-Interference-Contrast (DIC) microscope and achieved a temporal resolution of 10 μ s and a spatial resolution of 2.7 nm for tracking single gold colloid particles. By using our high-speed DIC microscope, we studied the dynamics of a transmembrane protein (TfR1) on B tumor cells expressing high levels of endogenous hTfR at 25 μ s resolution. Fig. 1 shows the tracking of a 40 nm gold colloid linked to TfR1 on the cell surface with a frame rate of 40 kHz. Under these controlled conditions, we are able to acquire data sets of 0.25 s time spans. The position of a single gold colloid is shown as a function of time (in pseudocolored scale to highlight the temporal behavior of tracked particles). Experiments are now underway to understand the motion of TfR1 and other cell surface proteins under different conditions and in different cells. The dynamics of TfR1 on the surface of these cells would provide not only insights into the intrinsic behavior of the protein under physiological conditions but also insights into how the cellular environment, particularly in the context of a tumor cell line, could influence the behavior of transmembrane proteins. This powerful method can be used to dissect the dynamics of member associated proteins. Of interest to us are the dynamics of transmembrane protein localization, oligomerization, and diffusion (whether passive or active) on the cell surface. Cellular events such as cell adhesion, synapse formation, vesicle release and signaling events all depend in part on the selective localization and movement of cell-surface membrane associated proteins, which can be studied at high temporal resolution. We have been applying this high-speed DIC microscope to studying a wide range of biological systems including the dynamics of transmembrane proteins, the motion of motor proteins and rotary motors.

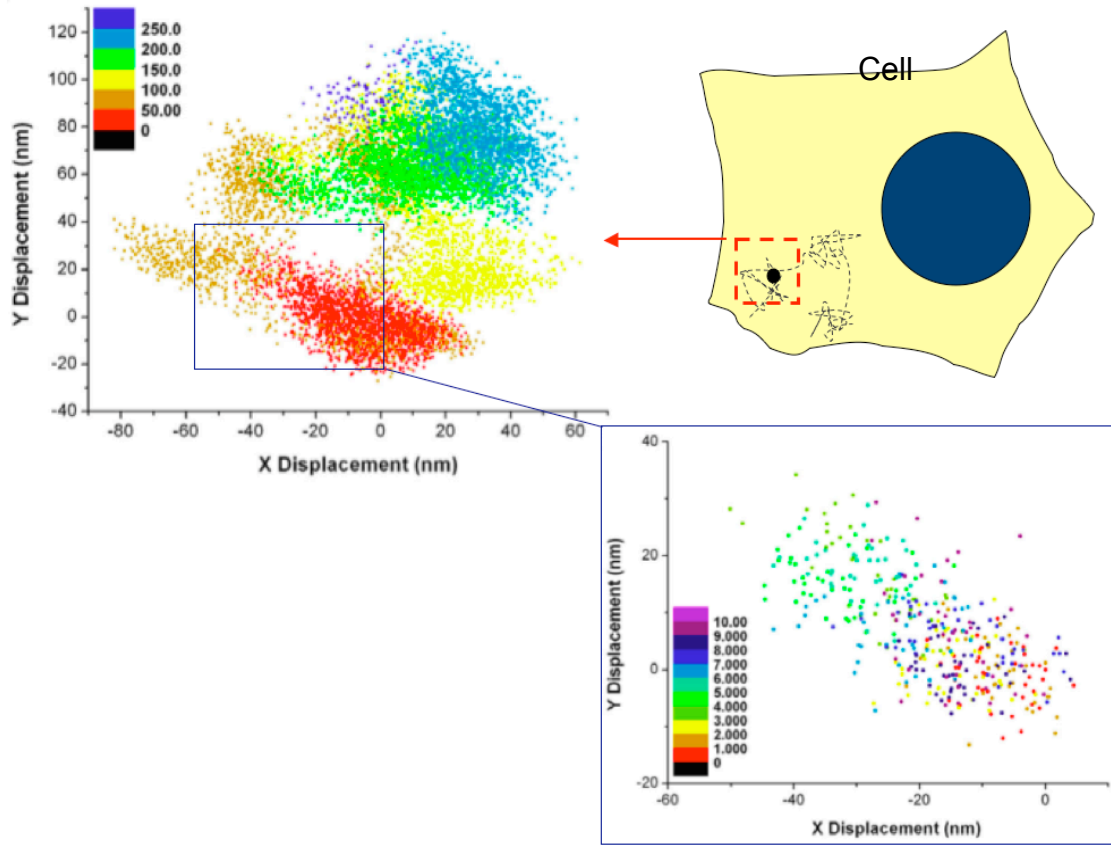


Fig. 1 Tracking of a 40 nm gold colloid (black circle on cell) non-covalently linked to TfR1 on the cell surface using DIC illumination with a frame rate of 40 kHz (*i.e.* 25 μ s temporal resolution).