Nanoscopic protein motion on a live cell membrane

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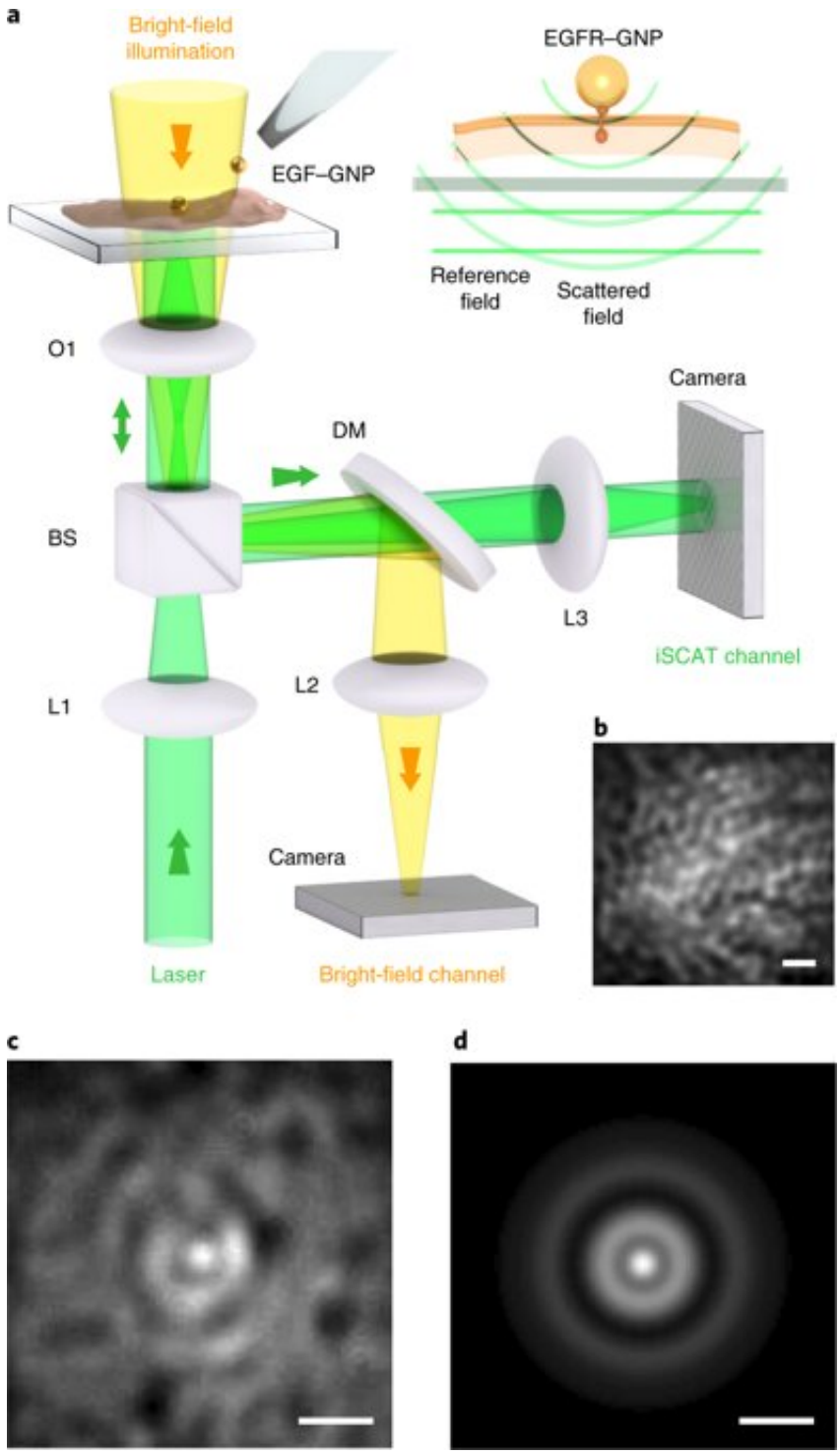


iSCAT microscopy on live cells. a, Experimental arrangement of the iSCAT microscope for live-cell imaging. Cells are plated in a glass-bottomed dish under Leibowitz medium. (a) micropipette delivers the EGF–GNP probes directly onto the cell culture, where they specifically target the EGFR protein in the cell membrane. The bright-field illumination channel from above assists in

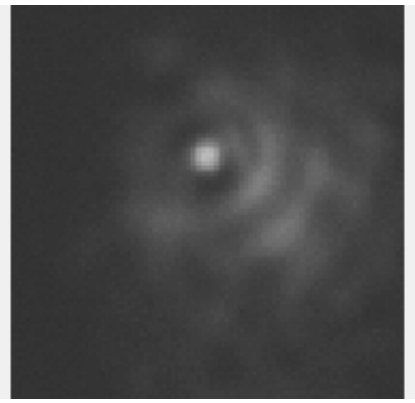
inspecting the culture but is not required for iSCAT imaging. L1–L3, lenses; O1, ×100 objective; BS, 90:10 beam splitter; DM, 590 nm short-pass dichroic mirror. iSCAT imaging was performed with illumination intensities of 1–8 kW cm−2, which are known to be viable for HeLa at the wavelength of interest. Inset, wavefronts of the fields contributing to the iSCAT signal. (b), A section of the membrane of the HeLa cell before labelling, viewed via reflection iSCAT. (c), iSCAT image of the cell membrane including a bound EGF–GNP probe. (d), The PSF extracted from c. Scale bars in b–d are 1 μm. Credit: *Nature Photonics*, doi: 10.1038/s41566-019-0414-6

Cellular functions are dictated by the intricate motion of proteins in membranes that span across a scale of nanometers to micrometers, within a time-frame of microseconds to minutes. However, this rich parameter of space is inaccessible using fluorescence microscopy, although it is within reach of interferometric scattering (iSCAT) particle tracking. The new iSCAT technique is, however, highly sensitive to single and unlabelled proteins, thereby causing non-specific background staining as a substantial challenge during cellular imaging.

In a recent study, Richard W. Taylor and colleagues at the interdisciplinary departments of Physics and Biology in Germany developed a new image processing approach to overcome this difficulty. They used the method to track the transmembrane epidermal growth factor receptor (EGFR) with nanometer scale precision in three dimensions (3-D). The technique allowed imaging across microseconds to minutes. The scientists provided examples of nanoscale motion and confinement using the method to image ubiquitous processes such as diffusion in plasma membranes, transport in filopodia and rotational motion during endocytosis. The results are now published in *Nature Photonics*.

While steady progress in fluorescence microscopy has allowed scientists

to monitor cellular events at the nanometer scale, a great deal still remains to be accomplished with advanced imaging systems. The challenges of fluorescence microscopy occurred due to the finite emission rate of a fluorescent source (dye molecule or semiconductor quantum dot), where too few photon emissions during a very small time-frame prevented effective or prolonged imaging. The central difficulty of scattering-based microscopy is relative to the nanoscopic probe, which competes against the background noise and a low signal-to-noise ratio (SNR); limiting the potential of imaging to only a few nanometers in high speed tracking experiments.

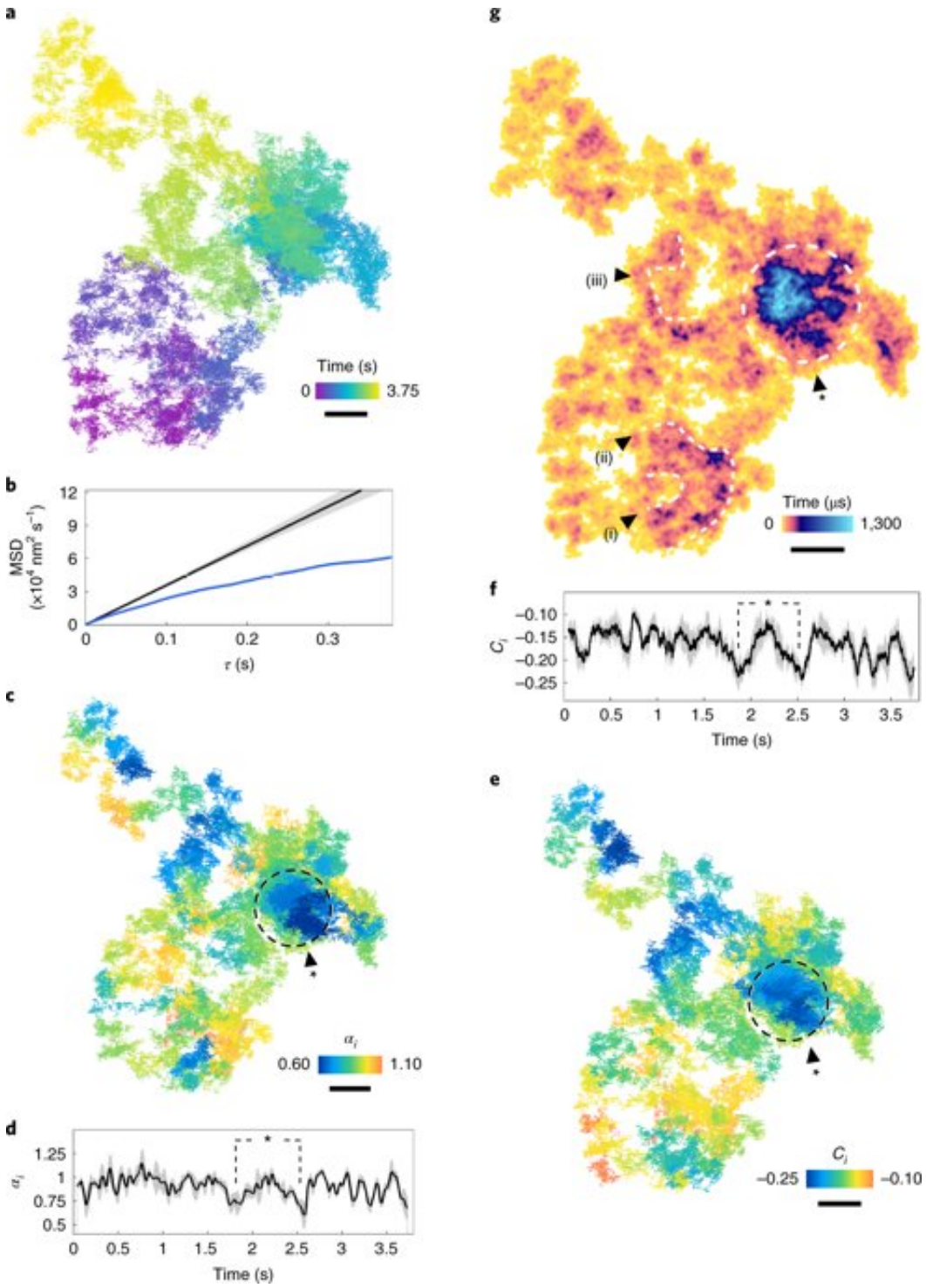


Raw video of an epidermal growth factor-gold nanoparticle (EGFR–GNP) diffusing on a HeLa cell membrane. Credit: *Nature Photonics*, doi: 10.1038/s41566-019-0414-6

In the present work, Taylor et al. used interferometric scattering (iSCAT) microscopy to track protein in live cell membranes. The method could visualize probe-cell interactions to understand the dynamics between diffusion and local topology. During the experiments, the scientists used gold nanoparticles (GNPs) to label epidermal growth factor receptors (EGFRs) in HeLa cells. The EGFRs are type I transmembrane proteins that can sense and respond to extracellular

signals, whose aberrant signaling is linked to a variety of disease. Taylor et al. showed the GNP-labelled protein as a 'nano-rover' that mapped the nano-topology of cellular features such as membrane terrains, filopodia and clathrin structures. They provided examples of subdiffusion and nanoscopic confinement motion of a protein in 3-D at high temporal resolution and long time-points.

In the experiments, Taylor et al. introduced the epidermal growth factor-gold nanoparticle (EGF-GNP) probes to the sample chamber of the microscope using a micropipette to label the EGFRs (epidermal growth factor receptors) on HeLa cells and verified that the probes stimulated the EGFRs. Previous studies had already indicated that the probe size could influence rates of lipid diffusion in synthetic membranes, although they did not affect the mode of diffusion. Additionally, in live cells, molecular crowding was negligible for particles equal to or smaller than 50 nm.



Diffusion on the plasma membrane. (a), A lateral diffusional trajectory (17.5 μs exposure time, see color scale for chronology). (b), MSD (mean square displacement) versus τ. The blue curve shows the MSD of a. The black curve is simulated normal diffusion (α= 1), with the grey envelope indicating the uncertainty. (c), The diffusional exponent of rolling windows (color scale) over

the trajectory. Regions of subdiffusion (α