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## How to Get an AFM Tip into a Living Cell?

J.K.H. Hoerber

HH Wills Physics Laboratory, Tyndall Avenue,  
Bristol BS8 1TL, UK

Conventional scanning probe microscopes with their mechanically attached tips have serious geometrical restriction to investigate 3-D structures. These restrictions allow only investigations on reasonably flat and well-oriented surfaces. To investigate complicated topologies, especially of living cells, the tip has to be controlled by other means. One possible alternative is the focus of a laser beam, which can be used to manipulate micro- to nanometer-sized particles in liquids, as Ashkin demonstrated in 1986, the same year the first AFM was built. The trapping potential of a laser focus obeys in first approximation Hooke's law and, therefore, with an appropriate 3-D detection, the position of the probe with respect to the focus can be used like the bending of a cantilever to measure forces acting on the particle. The force range accessible for such a microscope complements a conventional AFM at the lower force range providing freedom for measurements and manipulations even on internal structures of cells and inside many other optical transparent structures in solutions.

The Photonic Force Microscope (PFM), as we called it, can be used like an AFM for imaging and to do force spectroscopy with glass or latex spheres as tips. The 3-D detection system that we developed is the essential step from just an optical trap to a force microscope. With the nanometer spatial and microsecond time resolution possible, it enables the use of the thermally driven position fluctuations of the sphere to characterize its interaction with the environment. E.g. in the case of a sphere tethered by a single molecule to a surface, the thermal fluctuation measurements can be transformed into 3-D energy profiles using Boltzmann's equation. Energy profiles and their changes are both accessible with a resolution of one tenth of the thermal energy. From such profiles, force versus extension or stiffness versus extension profiles can be calculated along arbitrary paths to characterize the mechanical properties of the molecular structure. In a similar way, surface potentials for different types of interactions can be mapped in a solutions.

The PFM proofed from the very beginning to be a powerful tool to study at the nano-meter scale lipid vesicles as well as the plasma membrane of intact cells. In the latter case, the diffusion of membrane components and their interactions can be observed over minutes with the instrument's high spatial and time resolution. PFM measurements on intact cells for the

first time gave protein diffusion coefficients comparable to values reported for artificial lipid films. These experiments demonstrated that only on the nanometer scale the properties of the lipid component dominate the diffusion within the membrane and proved for the first time the physical existence of membrane rafts. The possibility to characterize also larger membrane aggregates and their diffusion opened up new ways to study membrane functions. Furthermore, the technique also provides information about membrane elasticity and the interactions of membrane components with the cytoskeleton.

The development of a field called nano-optics during recent years and the knowledge acquired about the interaction of light with nano-structures provides now the great opportunity for a major step in the development of the PFM that will extend the sensing capabilities of the particle acting as a tip. Depending on size and shape, metal nano-structures have Plasmon resonances at distinct frequencies. With a tunable laser adjusted to this resonance the light scattering cross-section increases significantly allowing the detection of 5-50 nm beads with our detection system. Furthermore, metal particles can be used as chemical sensors using the surface enhanced Raman (SERS) effect, and they can behave like “nano-lenses”. Due to the electric field enhancement at their surface fluorophores close by are excited with a much enhanced but quickly with distance decaying probability. This gives the same advantage of very low background fluorescence inside a cell, as total internal reflection fluorescence (TIRF) excitation close to a surface, and allows to determine where and when certain fluorescent labeled molecules come close to the particle. With pulsed laser sources, also a two-photon excitation scheme is possible, having the advantage of a much less damaging infrared-light excitation-wavelength. The introduction of such metal nano-sensors as tips in a PFM creates a tool for future cell-biology studies on regulation and transport processes within living cells providing the information of where and when molecular interactions take place.