VERTICAL PROBES IN LATERAL MOLECULAR FORCE MICROSCOPY (LMFM)

Vertically orientated probes can be used to overcome the limitations of conventional atomic force microscopy (AFM).

Atomic force microscopy (AFM) has proven to be a powerful tool for imaging at the nanoscale. A simplified schematic of an AFM setup is shown in Fig. 1 (a).

Conventionally, a pyramidal, tetrahedral or conical tip, positioned at the end of a horizontally mounted cantilever, is raster-scanned across the sample to be investigated. A laser beam focused onto the surface of the cantilever is reflected onto a photodetector. The position of the laser spot on the photodetector shifts as the tip is scanned across the surface because of the vertical deflection of the cantilever, owing to the change in force between the tip and sample. An image of the surface is built from the position shift of the laser spot on the photodetector, as shown on the computer screen in Figure 1. The colour bar represents the height of the features.

Positioning the cantilever almost parallel to the sample surface leads to some limitations. Firstly, the cantilever must be sufficiently stiff to extend horizontally over the sample surface, which limits how low its spring constant can be and thus, its sensitivity. Secondly, when the tip approaches the surface, the ensuing shorter-range attractive forces experienced by the tip often become larger than the spring constant of the cantilever. This may result in the tip jumping into contact with the sample, called a "jump-to-contact" event, which can cause damage to soft or sensitive samples. Thirdly, since the deflection of the cantilever in the vertical direction is being measured, it is not sensitive to lateral and shear forces.

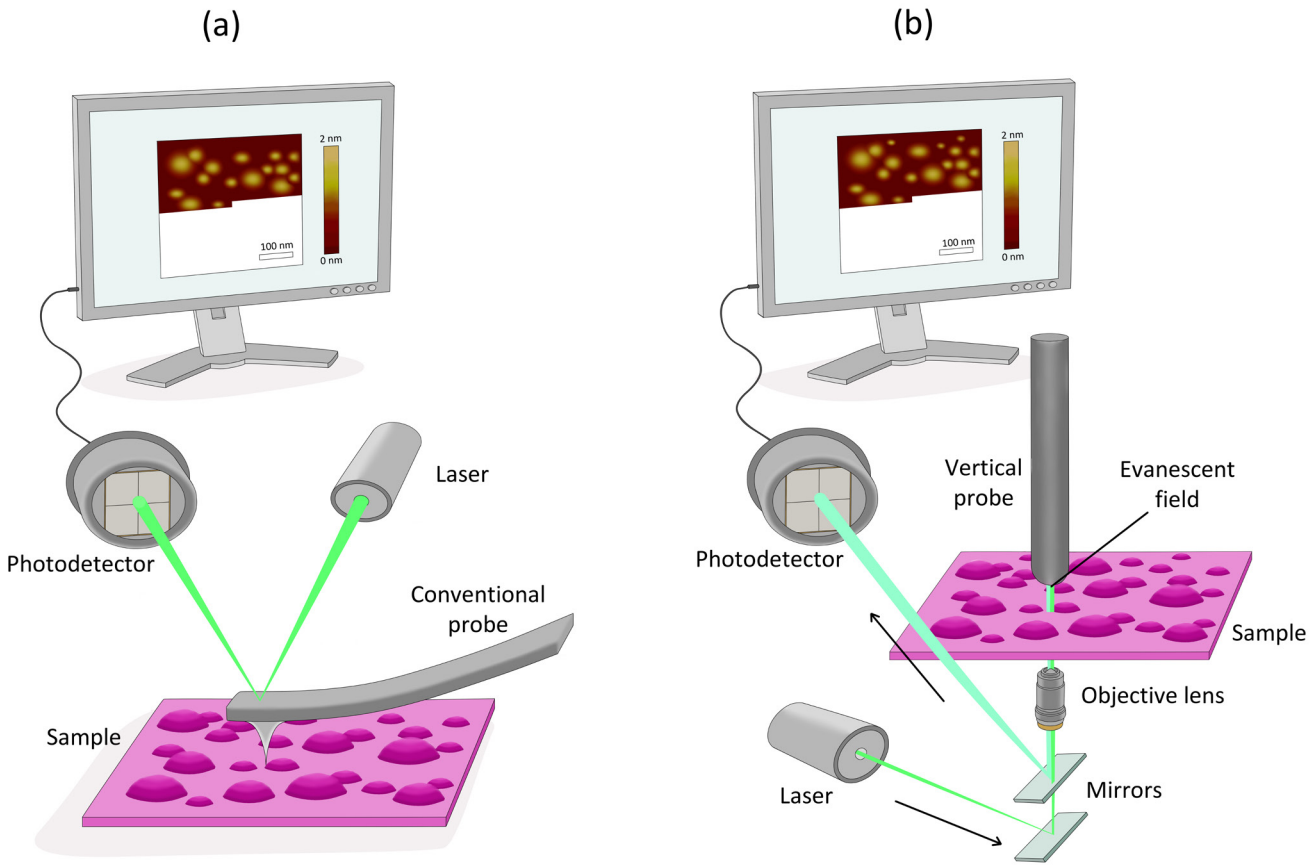

Figure 1: Simplified schematic of an (a) AFM and (b) LMFM system.

To overcome these limitations, a new technique called Lateral Molecular Force Microscopy (LMFM) has been established by Dr. Massimo Antognozzi at the University of Bristol [1], which allows for sensing via vertically-orientated cantilevers [2]. Naturally, the LMFM exploits a different detection system to conventional AFM, known as "scattering evanescent wave" detection, as shown in Fig. 1 (b). An evanescent electromagnetic field is generated just above the sample surface using a total internal reflected laser beam from an objective lens below the sample. When the tip of the cantilever enters the evanescent field, the electromagnetic radiation is scattered. This scattered radiation is collected with the objective lens and projected onto the photodetector, which detects the position of the tip.

Since the cantilever is vertically orientated, it can be tip-less with the free end of the cantilever entering the evanescent field. The actual movement of the tip is sensed, rather than the movement inferred from the deflection of the cantilever. The shape of the cantilever is not so crucial and metal coating of the cantilever to increase light reflectivity is not required because a laser beam does not need to be reflected off the back of it. Only the last few tens of nanometres of the free end of the cantilever are interacting with the evanescent field, therefore, the cantilever

can be extremely small. The vertical orientation of the cantilever means that there is no "jump-to-contact" effects and therefore, it is suited for the study of very soft samples. The cantilevers do not need to be as stiff and therefore, can have very low spring constants, making them highly sensitive. NuNano have produced tip-less, ultra-soft probes with spring constants in the range 0.003 – 6000 pN/nm, which are particularly suited for use in the described LMFM technique.

LMFM has been used in a variety of applications, which makes use of its ability to measure lateral and shear forces with nanometer resolution and femto-Newton sensitivity. These include:

- Imaging the motion of the biomolecular motor, Kinesin in the lateral force direction (2011) [3]
- Studying the mechanical properties of the adhesion protein, UspA1 when expressed on the cell surface of the bacterium, Moraxella catarrhalis (2011) [4]
- Shear force imaging of DNA in liquid (2012) [5]
- Imaging of self-assembled cages formed from mixing coiled coil peptide modules (2013) [6]
- Studying the viscoelastic properties of membrane-free peptide/nucleotide protocells with small-molecule uptake (2013) [7]
- Observing directly the extraordinary optical momentum and force directed perpendicular to the wavevector, and proportional to the optical spin (2016) [8]
- Tracking the evolution of hydration layers during metal nucleation in real-time (2017) [9]

This article was prepared in collaboration with Dr Massimo Antognozzi, University of Bristol.

References

[1] M. Antognozzi, A. Ulcinas, L. Picco, S. H. Simpson, P. J. Heard, M. D. Szczelkun, B. Brenner, and M. J. Miles, "A new detection system for extremely small vertically mounted cantilevers," Nanotechnology, 19(38), 384002, 2008.

[2] J. A. Vicary, A. Ulcinas, J. K. H. Hörber, and M. Antognozzi, "Micro-fabricated mechanical sensors for lateral molecular-force microscopy," Ultramicroscopy, 111(11), pp. 1547-1552, 2011.

[3] T. Scholz, J. A. Vicary, G. M. Jeppesen, A. Ulcinas, J. K. H. Hörber, and M. Antognozzi,"Processive behaviour of kinesin observed using micro-fabricated cantilevers," Nanotechnology, 22(9), 095707, 2011

[4] C. Agnew, E. Borodina, N. R. Zaccaia, R. Conners, N. M. Burton, J. A. Vicary, D. K. Cole, M. Antognozzi, M. Virjib, and R. L. Bradya, "Correlation of in situ mechanosensitive responses of the Moraxella catarrhalis adhesin UspA1 with fibronectin and receptor CEACAM1 binding," PNAS, 108(37), pp. 15174–15178, 2011.

[5] R. L. Harniman, J. A. Vicary, J. K. H. Hörber, L. M. Picco, M. J. Miles, and M. Antognozzi, "Methods for imaging DNA in liquid with lateral molecular-force microscopy," Nanotechnology, 23(8), 085703, 2012.

[6] J. M. Fletcher, R. L. Harniman, F. R. H. Barnes, A. L. Boyle, A. Collins, J. Mantell, T. H. Sharp, M. Antognozzi, P. J. Booth, N. Linden, M. J. Miles, R. B. Sessions, P. Verkade, and D. N. Woolfson, "Self-Assembling Cages from Coiled-Coil Peptide Modules," Science, 340(6132), pp. 595-599, 2013.

[7] T.-Y. Dora Tang, M. Antognozzi, J. A. Vicary, A. W. Perriman, and S. Mann, "Small-molecule uptake in membrane-free peptide/nucleotide protocells," Soft matter, 9(31), pp. 7647–7656, 2013.

[8] M. Antognozzi, C. R. Bermingham, R. L. Harniman, S. Simpson, J. Senior, R. Hayward, H. Hörber, M. R. Dennis, A. Y. Bekshaev, K. Y. Bliokh, and F. Nori, "Direct measurements of the extraordinary optical momentum and transverse spin-dependent force using a nano-cantilever," Nature Physics, 12(8), pp. 731–735, 2016.

[9] R. L. Harniman, D. Plana, G. H. Carter, K. A. Bradley, M. J. Miles, and D. J. Fermín, "Real-time tracking of metal nucleation via local perturbation of hydration layers," Nature communications, 8, 971, 2017.