Invited Perspective (Nanotechnology IOP)

Designer cantilevers for even more accurate quantitative measurements of biological systems with multifrequency AFM

S Contera

Oxford Martin Programme on Nanotechnology , Clarendon Laboratory, Physics Department, University of Oxford, Parks Road, Oxford, OX1 3PU, UK

E-mail: sonia.antoranzcontera@physics.ox.ac.uk

Abstract: Multifrequency excitation/monitoring of cantilevers has make it possible both to achieve fast, relatively simple, nm-resolution quantitative mapping of mechanical of biological systems in solution using atomic force microscopy (AFM), and single molecule resolution detection by nanomechanical biosensors. A recent paper by Penedo *et al.* has made a significant contribution by developing simple methods to improve the signal to noise ratio in liquid environments, by selectively enhancing cantilever modes, which will lead to even more accurate quantitative measurements.

Advances in atomic force microscopy (AFM) based on the simultaneous monitoring and/or excitation of different harmonics and/or modes or the cantilever, the so-called "Multifrequency AFM" [1], are realising a long coveted aspiration: fast, simple, quantitative mapping of the mechanical properties of materials with nm resolution. Progress has been particularly significant in the context of living biological systems in solution; it has been shown that multifrequency AFM based on widely used amplitude-modulation AFM can achieve a fast, nm-resolution quantitative mapping of mechanical properties of living cells [2]. Recent advances have improved the speed even further by using the AFM feedback on the cantilever deflection while exciting other harmonics/eigenmodes [3]. It is safe to predict that these new techniques will be widely adopted in the near future because the advances are mainly theoretical and can easily be implemented in most commercial AFMs.

The sensitivity of multifrequency methods in solution depend on a good signal to noise ratio of the observables resulting from exciting and measuring the cantilever response at multiple eigenmodes and harmonics, where quality factors (Q) of the cantilevers are low. A recent paper by Mónica Luna's group makes a significant technological development to this rapidly expanding field [4]. They report on a simple method to enhance the oscillation amplitudes of cantilever eigenmodes in liquid environments that results in a clear improvement of the signal to noise ratio of multifrequency techniques. Penedo *et al.* show that by using a simple theoretical calculation of the transfer function it is possible to design a cantilever coating that selectively enhances the excitation efficiency of the required mode [4]. The design is then put into practice by etching specific areas of the cantilever utilising a focused ion bean or alternatively by implanting Ga^+ ions following the pattern predicted by theory. The results are experimentally demonstrated for commercial cantilevers using magnetostrictive excitation, which is the most efficient technique for higher eigenmode excitation when using soft cantilevers in liquid media [5]. In average, the cantilever oscillation amplitude is 52% higher for the second mode, and 119% higher for the third mode, after the selective etching. The method is also applicable to other techniques that require cantilever coating such as magnetic torque or photothermal excitation.

Additionally the authors demonstrate the wide applicability of their approach by showing that selective etching can be used for enhancing the torsional modes of the cantilever in liquid environment; torsional modes can also be used to measure the mechanical properties of biological systems [6] and to detect biomolecular interactions with great accuracy [7]. The torsional oscillation amplitude increases an average of 3.2 times after the selective etching method.

Importantly, these advances are not only applicable to AFM imaging but are also useful to boost the performance of nanomechanical biosensors [8]. Multifrequency techniques have recently been used to measure -in real time and with molecular resolution- the spatial distribution of mass within an individual analyte when molecules adsorb onto a nanomechanical resonator [9].

As multifrequency techniques become established and more extensively utilised in AFM imaging, advanced force and interaction measurement experiments as well as in novel nanomechanical biosensors, the need of cantilevers that are especially designed to enhance specific modes will arise; this paper paves the way for an easy technical solution to the task.

Interestingly our knowledge and technology to measure forces, and mechanical properties and to convert chemistry and binding into biosensing using microcantilevers is developing in parallel to our understanding of how biology uses forces, mechanical properties and thermal fluctuations to generate the complexity of biological function. Force and mechanics are key to biology, e.g. from the complex mechanical design to the inner ear, to stem cell differentiation, tumour growth and the infiltration of metastatic cells. In fact, biology uses nanoscale mechanics and thermal fluctuation for signalling, converting chemistry into mechanics and the other way around, as exemplified by the function of mechanosensitive channels inserted in biological lipid membranes [10] or the rotation of the ubiquitous ATP synthases [11,12].

Both fields converge and should feed from each other: biophysicists already use cantilevers in creative ways to unravel the mechanical and even the chemical and electrical aspects biology at the molecular and cellular scales. Perhaps soon cantilever designers will use inspiration from biological systems to achieve even more sensitivity and more applications. In the meantime the work of Luna's group developing technologies for smart designer nanomechanical sensors takes us a step further to a stimulatingly dynamic future.

References

[1] Garcia R and Herruzo E T 2012 The emergence of multifrequency force microscopy *Nat. Nanotechnol.* **7** 217–26

[2] Raman A, Trigueros S, Cartagena A, Stevenson A P Z, Susilo M, Nauman E and Contera S A 2011 Mapping nanomechanical properties of live cells using multi-harmonic atomic force microscopy *Nat. Nanotechnol.* **6** 809–14

[3] Cartagena-Rivera A X, Wang W-H, Geahlen R L and Raman A 2015 Fast, multi-frequency, and quantitative nanomechanical mapping of live cells using the atomic force microscope *Sci. Rep.* **5** 11692.

[4] Penedo M, Hormeño S, Prieto P, Alvaro R, Anguita J, Briones F and Luna M 2015 Selective enhancement of individual cantilever high resonance modes *Nanotechnology* **26** 485706

[5] Penedo M, Raman A, Hormeño S, Fernández-Martínez I, Luna M and Briones F 2014 Enhanced efficiency in the excitation of higher modes for atomic force microscopy and mechanical sensors operated in liquids *Appl. Phys. Lett.* **105** 173102

[6] Dong M, Husale S and Sahin O 2009 Determination of protein structural flexibility by microsecond force spectroscopy *Nat. Nanotechnol.* **4** 514–7

[7] Husale S, Persson H H J and Sahin O 2009 DNA nanomechanics allows direct digital detection of complementary DNA and microRNA targets *Nature* **462** 1075–8

[8] Arlett J L, Myers E B and Roukes M L 2011 Comparative advantages of mechanical biosensors *Nat. Nanotechnol.* **6** 203–15

[9] Hanay M S, Kelber S I, O'Connell C D, Mulvaney P, Sader J E and Roukes M L 2015 Inertial imaging with nanomechanical systems *Nat. Nanotechnol.* **10** 339–44

[10] Dong Y Y, Pike A C W, Mackenzie A, McClenaghan C, Aryal P, Dong L, Quigley A, Grieben M, Goubin S, Mukhopadhyay S, Ruda G F, Clausen M V, Cao L, Brennan P E, Burgess-Brown N A, Sansom M S P, Tucker S J and Carpenter E P 2015 K2P channel gating mechanisms revealed by structures of TREK-2 and a complex with Prozac *Science* **347** 1256–9

[11] Uchihashi T, Iino R, Ando T and Noji H 2011 High-Speed Atomic Force Microscopy Reveals Rotary Catalysis of Rotorless F1-ATPase *Science* **333** 755–8

[12] Czub J and Grubmüller H 2011 Torsional elasticity and energetics of F1-ATPase *Proc. Natl. Acad. Sci.* **108** 7408–13