SUPUVIR Microscopy and Sensing Workshop, Cambridge UK, November 2018

Atomic Force Microscopy (AFM)

Contact: Ioanna Mela im337@cam.ac.uk

Research topics – Mechanical properties of materials, imaging of a super-resolution chip

Introduction:

Since the introduction of the Nobel Prize-winning scanning tunneling microscope (STM) and the invention of the atomic force microscopy (AFM), the field of scanning probe microscopy has developed beyond its primary use of imaging sample topography on the nanometer scale. We now have the ability to extract information on a wide range of properties of materials, including stiffness, friction, electrical forces, capacitance, magnetic forces, conductivity, viscoelasticity, surface potential, and resistance. The latest advance in Atomic Force Microscopy is the ability to couple an AFM with a variety of fluorescence microscopes, including STED, FLIM and SIM.

In the default AFM mode, a very thin tip (~20 nm radius) is scanned (rastered) over the sample. The tip is attached to a spring-like cantilever, which is deflected whenever it encounters a topological feature. The movement of the cantilever is tracked using a so called optical lever. The optical lever operates by reflecting a laser beam off the cantilever and the displacements of the reflected laser beam are monitored via a position-sensitive, four-segment photo-detector. The differences between the segments of photo-detector of signals indicate the position of the laser spot on the detector and thus the angular deflections of the cantilever. A short animation of the principle can be found here: https://www.youtube.com/watch?v=j6B-HYsvkvo

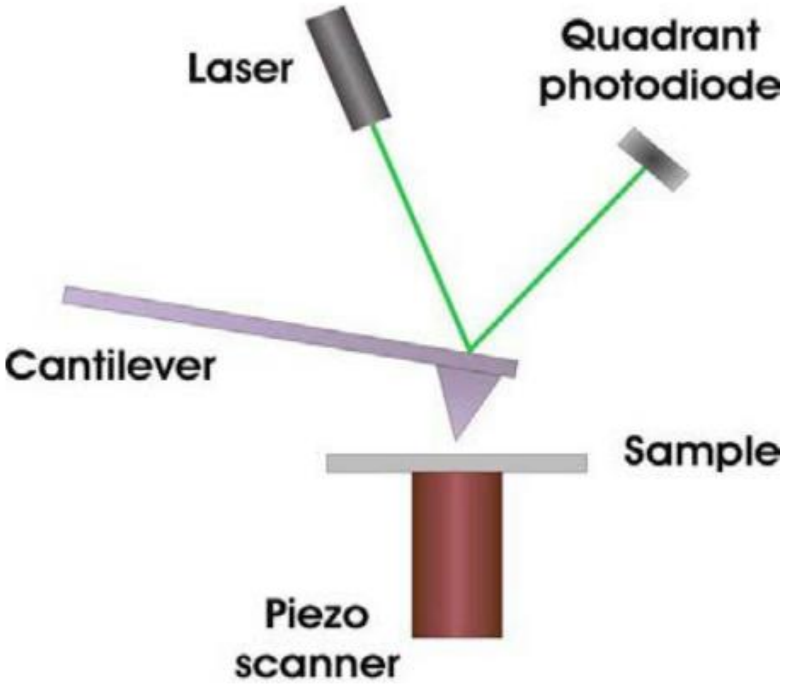

Fig.1: Basic operating mode of an AFM. The sample is scanned in a raster pattern, using a nanometer-thin tip. The movement of the tip is monitored by collecting the signal of the laser beam reflected off the back of the spring-like cantilever.

In our AFM set-up, we focus on the study of mechanical properties of materials and biological samples. We will work on extracting the Young's modulus of a photonic chip and observe changes in the deformation of the material. We will use this information to assess the quality of the material and relate this to fabrication parameters.

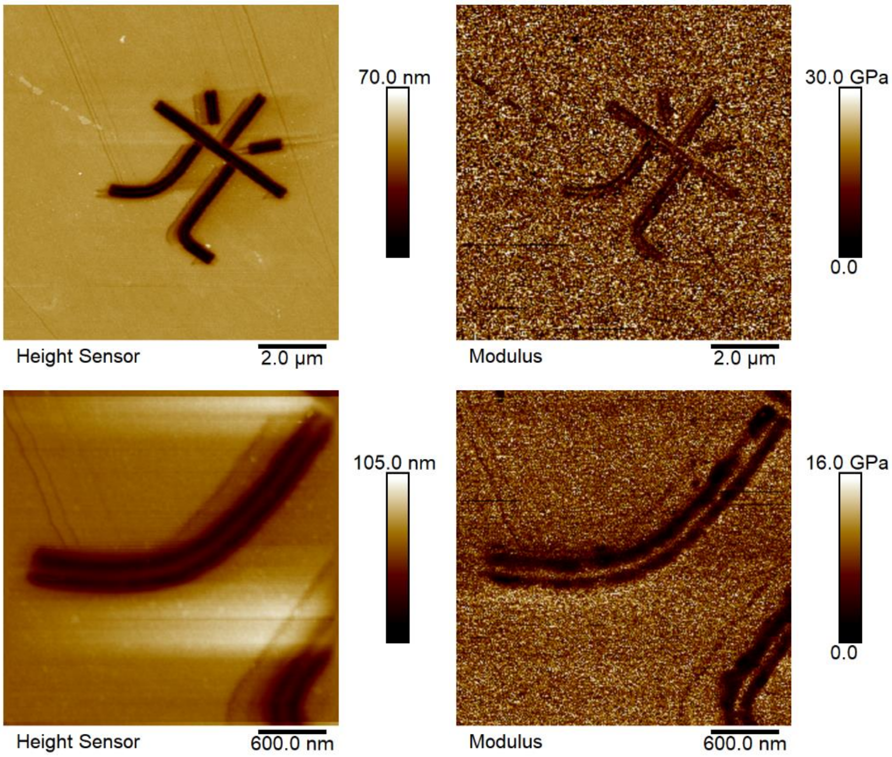

Fig.2: Representative images of etched super-resolution chip. On the left is the topography image and on the right the corresponding Young's modulus map. Sample courtesy of Professor Qing Yang, Zhejiang University.

For more information, you can find below a reference to the original article that introduced the AFM (1), three useful articles on force curve generation and interpretation (2-4 – please note, article 4 is written with living cells in mind as the reference sample, but the information on the different models is very relevant to any material) and an application on coupling AFM with STED (5), the latter a technique you will also learn about in this workshop.

1. Binnig, Quate, and Gerber, Atomic Force Microscope, Phys. Rev. Lett. 56, 930, March 1986
2. https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/SurfaceAnalysis/AFM/ApplicationNotes/AN128-RevB0-Quantitative_Mechanical_Property_Mapping_at_the_Nanoscale_with_PeakForceQNM-AppNote.pdf
3. Bouchonville N., Nicolas A. (2019) Quantification of the Elastic Properties of Soft and Sticky Materials Using AFM. In: Santos N., Carvalho F. (eds) Atomic Force Microscopy. Methods in Molecular Biology, vol 1886. Humana Press, New York, NY
4. Schillers H. (2019) Measuring the Elastic Properties of Living Cells. In: Santos N., Carvalho F. (eds) Atomic Force Microscopy. Methods in Molecular Biology, vol 1886. Humana Press, New York, NY
5. Curry N, Ghézali G, Kaminski Schierle GS, Rouach N, Kaminski CF, Correlative STED and Atomic Force Microscopy on Live Astrocytes Reveals Plasticity of Cytoskeletal Structure and Membrane Physical Properties during Polarized Migration, Front Cell Neurosci. 2017 Apr 19;11:104.

Aims of the practical:

- Develop an understanding of the operating principles of AFM
- Calibration of AFM probes for force measurements
- Characterisation of sample topography by AFM
- Principle of PeakForce Quantitative Nanomechanical Mapping (PFQNM) and use to characterize mechanical properties of the sample
- To explore different models that can be used to fit force curve data

Questions:

- Explain the basic features of an AFM force curve and their physical origin.
- What are the most commonly used models for force curve analysis?
- How would you choose a probe for accurate mechanical characterization of your sample?
- What are the basic steps to calibrate your probe?
- How can AFM be used in conjunction with optical super-resolution microscopy? Give an example of an application that benefits from such an application.
- What are the limitations of the technique?

Experimental details and procedures:

- Sample loading and immobilization
- Probe calibration:
    - Spring constant and calibration of deflection sensitivity on a sapphire sample
    - Tip radius qualification using a rough Ti sample
- Measurement of the topography and the mechanical properties of the sample using PFQNM operating mode
- Fitting of the data using an appropriate model

Special considerations:

- The AFM is a very expensive instrument and needs to be handled with care. If you have any questions or are unsure how to do something, ask!
- Take care when loading and unloading AFM probes. They are very fragile and very easy to drop.
- The “High Voltage” in the scanner head should always be disabled before the probe holder is loaded or unloaded.
- Always use the fluid probe holder for imaging in fluid!
- The AFM computer should always be on if the controllers are on. Never turn the computer off before the controllers and never turn the controllers on before the computer.