

A practical guide to AFM force spectroscopy and data analysis

"Fishing" for single molecules

The atomic force microscope (AFM) is best known for its high-resolution imaging capabilities, but it is also a powerful tool for sensitive force measurements. The AFM can be used to "fish" for single molecules and investigate their structure and biological function. The study of interaction forces with the AFM has led to deeper understanding of many biological and physical processes down to the single molecule level.

The AFM tip is able to probe an extremely small interaction area (using a tip radius in the range of 5-50 nanometers), and this gives it a high sensitivity to small forces. To make these forces accessible, the tip is suspended on an very soft spring, the AFM cantilever (images in Figure 1). These cantilevers are usually silicon or silicon-nitride beams, 100-200 microns long, and allow forces in the pico-Newton range to be measured. This corresponds to the order of magnitude of forces that are required to separate receptors from ligands or to break a single hydrogen bond.

In AFM imaging modes, the cantilever is usually scanned over the surface to produce a three dimensional image of the surface. In AFM "force spectroscopy" experiments, the cantilever and tip are moved directly towards the sample until they are in contact with it, and then retracted again, while the interaction between the tip and sample is measured. This may then be repeated at different locations to build up a map of the tip-surface interaction, or can be repeated at the same point to give a full statistical understanding of the interaction.



Fig 1. Scanning electron microscopy images of typical pyramidal (A) and cone-shaped (B) AFM tips and an optical microscope image of an AFM cantilever and tip (C).

The applications of these experiments range from nano-mechanical investigations of elastic properties to protein unfolding and investigations of single chemical bonds [1]. This technical report introduces the data collected in force curves and the standard steps that are used in converting the data for a quantitative force analysis.

Overview

The schematic diagram in Figure 2 shows the movement of the cantilever and tip during the force spectroscopy experiment – towards the sample (the approach part) and then away again (the retract part). The image series reflects different points in the approach and retract cycle, the lateral position of the cantilever is usually constant. A flexible software interface allows for waiting times at different points of the movement – for example at the sample surface to give time for molecules to adsorb to the cantilever tip or at the end of the retract movement, to give the surface time to recover.

Table 1 gives a summary of interactions that have been measured using the atomic force microscope, to give an idea of the experiments that are possible and the parts of the force curves where particular interactions are seen.



Fig. 2 Schematic diagram of the vertical tip movement during the approach and retract parts of a force spectroscopy experiment.

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Liyong Dong

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