VIDEO-RATE AFM OF BIOMOLECULAR STRUCTURES

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Introduction

The advantages of atomic force microscopy (AFM) for the study biomolecular structures are well-known:

- High-resolution 3D imaging;
- Imaging in aqueous environments;
- No need to stain to increase contrast;
- No radiation damage;
- Mapping of mechanical properties;

These factors mean that it is possible to follow biological processes occurring in the AFM in conditions close to physiological. In conventional AFM, only processes that occur on a timescale of minutes can be followed because of the relatively slow imaging rate. The slow imaging rate of the AFM brings the following disadvantages:

- Inability to follow processes occurring on the sub-second timescale;
- Inability to examine large areas of specimen in reasonable timescale;
- No real-time feedback to the microscope operator;

The principal reason for the limitation in the imaging speeds stems from the fact that scanning probe microscopy, and AFM in particular, are based on mechanical microscopes, and, as such, imaging speeds are limited by the inertia and resonances of the scanning systems and of the AFM cantilever probe. At high imaging speeds, the bandwidth of the feedback system (to maintain, for example, constant force or RMS tapping amplitude) also becomes a limiting factor.

A logical solution to the mechanical limitations is to decrease the mass and increase the stiffness of the scanning system and the cantilever in order to decrease their response times by reducing inertial effects and increasing resonant frequencies. Over the last ten years, such AFM systems have been under development in several laboratories worldwide. In this presentation, alternative methods of high-speed AFM imaging will be described and results shown for mostly biological specimens.

Alternative Approach to high-speed AFM

The solution to high-speed imaging presented here is different to the above method. Instead of avoiding resonance in the scanning system, resonance was used to scan the probe in the fast-scan direction. The original implementation was for scanning near-field optical microscopy[1], which achieved 120 frames per second. In the case of the high-speed AFM, the specimen is mounted on the resonant scanner and a conventional AFM cantilever probe is brought in to continuous contact with the specimen and the topographic structure derived for the deflection of the cantilever. The slow scan is provide by a piezo stack. The average deflection of the cantilever is used to provide a slow feedback. The damage to specimens resulting from this high-speed contact-mode imaging is surprisingly very considerably less than would be caused at normal speeds. The reason for this is now understood and will be described briefly in this presentation.

Movies illustrating some examples of imaging biological specimens in either air or in an aqueous environment will presented.

References

[1] ADL Humphris, JK Hobbs, & MJ Miles, *Applied Physics Letters* **83** (2003) 6-8.

[2] Humphris, MJ Miles, & JK Hobbs, *Applied Physics Letters* **86** (2005) art no. 034106.