Laser-Guided Atomic Force Microscopy

Precision Photonics Meets Nanotechnology

Application of Atomic Force Microscopy (AFM) to a broad range of research, and biological systems in particular, is hampered by two longstanding technical problems: mechanical drift and finding sparsely distributed samples. By adapting ideas from the optical-trapping community, we have made significant progress in addressing both of these issues, including a 100-fold improvement in the stability of AFM at ambient conditions.

The atomic force microscope (AFM) is an invaluable instrument in nanoscience research and development. AFM's broad use arises from its ability to probe and manipulate a wide array of materials in an equally wide variety of operating conditions. For instance, scientists have used AFMs to study nanometer-scale structures and dynamics in vacuum over five decades in temperature (0.01-1,000 K), to image and to mechanically unfold individual proteins at physiologically relevant conditions, and even to visualize soil and ice grains on another planet (NASA Phoenix Mars Lander).

Two long-standing problems in AFM are mechanical drift and finding sparsely distributed samples. Drift, particularly at room temperature, limits observation time because it is difficult to distinguish from the true signal. Additionally, it is time consuming to find desirable regions for detailed study because the samples are often randomly absorbed onto a substrate. This process can also degrade the mechanical and chemical integrity of the tip.

An Optically Based Reference Frame for AFM

We solved this pair of problems by integrating two additional highly stabilized lasers to supplement traditional force detection. Initially, the motivation was to detect and thereby control the three dimensional (3D) position of the tip and the substrate via scattered light, a strategy inspired by precision optical-trapping techniques [1-3]. In an artist's rendition of the central components (fig. 1), the tip-detection laser beam is red. A second beam (green) detects the position of the sample via scattering off a fiducial mark on the substrate. These two measurements are independent of and complementary to the traditional force measurement, based
These advances rely on detecting back-scattered light from a focused laser beam (fig. 2). Using a focused laser beam enables 3D determination of the object's location, in contrast to the 1D information derived in a traditional interferometer. Sub-Ångstrom sensitivity was achieved by minimizing a variety of optical noise sources.

We solved the first problem, drift, by referencing the position of the tip to the position of the sample via an optically based reference frame. The resulting optically stabilized AFM drifts by only a few Ångstroms over an hour operating at "real-world" conditions (in air at room temperature) [4]. This lateral stability arises because it is not the absolute stability of the lasers that matters but rather their differential-pointing stability.

We solved the second problem, locating regions of interest, by enhancing an existing label-free optical-imaging technique [5, 6]. In doing so, we employed the same laser used for tip stabilization. The resulting optical images of thin, transparent biological structures were registered to subsequent AFM images with nanometer-scale precision (<40 nm) because the tip is aligned to the optical axis of the imaging laser.

Long-Term Stability at Real-World Conditions

Unwanted mechanical drift between the tip and the sample places fundamental limits on AFM instrument performance and decreases utility. Drift can be minimized by operating in vacuum or under cryogenic conditions, but this strategy precludes many important investigations that require operating in air or fluid at room temperature such as imaging and probing the dynamics of biological assemblies. If drift were absent from an AFM, it would be possible to (i) enhance
the imaging signal by scanning slowly to average the Brownian motion of the cantilever, (ii) return the tip to a precise feature in an image (e.g., a protein domain), (iii) hover the tip over a feature to study local dynamics (e.g., conformational fluctuations), and (iv) control the 3D position of the tip when disengaged from the surface (e.g., force spectroscopy).

Our optically based, active feedback virtually eliminates drift at room temperature. We measure environmentally induced noise and then use active feedback via a pair of three-axis piezo-electric stages to mitigate its effect. Tip-sample stability is achieved because there is essentially no differential-pointing noise between these added lasers (0.2 Å, Δf = 0.1-50 Hz) [7]. Thus, a local, optically based reference frame minimizes the traditional need for a stiff "mechanical loop", the physical path through the frame of the instrument that connects the AFM tip to the substrate. The benefit of this stabilization method is immediately apparent from fig. 3. In the top panel, the onset of feedback is shown to essentially eliminate a substantial drift. Moreover, as shown in the bottom panel, feedback provides a means for arbitrary control of the tip position in two (or three) dimensions with sub-Ångstrom precision.

**An Optical "Tripod" for AFM**

Acquiring an AFM image is analogous to taking a photograph in low light conditions; both techniques suffer from low signal-to-noise ratio (fig. 4 (a and b). A photographer takes a longer exposure in low light, allowing more photons to impinge on the film or detector. In AFM, one would like to average the effect of Brownian motion by decreasing the tip scan rate. In both cases, a longer acquisition time typically leads to a distorted image due to motion between the "camera" and the subject. For photography, the remedy is well known: place the camera on a tripod (fig. 4c).

A similar strategy can now be applied using the optically stabilized AFM. As shown in figure 4d, slow stabilized scanning led to a clear improvement in image quality. More specifically, a fivefold enhancement of the signal-to-noise ratio was achieved while imaging 5-nm gold nanospheres. We expect that this technique will be especially valuable for future AFM imaging of delicate biological samples. Soft samples demand low imaging forces, where the cantilever deflection signal is hidden within the larger instantaneous Brownian motion.

**Optically Finding the Needle in the Haystack**

In many single-molecule AFM experiments, the regions of interest are sparsely distributed across the surface at random locations, resulting in a "needle in the haystack" type-search problem. To locate a region for study, the tip is typically
scanned rapidly across the surface until the desired structure is found. High-speed scanning over large areas can damage the tip and the sample. For instance, a tip coated with gold for covalent attachment can become contaminated during scanning. Hence, although AFM relies on interactions between the tip and the sample, it is advantageous to limit their interaction during this preliminary search process.

A tip-free method is ideal for locating biological assemblies for subsequent AFM studies. To achieve this end, we were inspired by a recently developed label-free imaging technique that is also based on back-scattered light [6]. Such imaging is quite simple; the sample is raster scanned through a stationary laser focus (fig. 5(a and b)). The improved laser stability and detector electronics we developed for AFM stabilization allowed us to image transparent biological structures [8], which had an inherent contrast 30-fold smaller than prior work [5]. In spite of this small signal, the images of individual ~5 nm thick membrane protein patches had a signal to noise ratio of 20, suggesting that objects which are smaller and/or have a lower inherent contrast can be successfully imaged.

Because the optical images are spatially aligned or registered to the subsequent AFM data with nanometer scale-precision (~40 nm; fig. 5c), one can locate a region of interest optically and then deterministically engage the tip at that desired location. This registration arises because optical imaging is done with the tip detection laser. More specifically, once the AFM tip is centered with respect to this optical axis, then the AFM and optical images are registered. The sequential combination of this optical imaging to identify regions of interest followed by registered tip engagement minimizes damage to the fragile tip as well as soft biological structures. Moreover, the tip's chemical functionality can be preserved by decreased scanning.

At a fundamental level, cantilever-deflection detection in AFM is a force-measuring technique. Advances in optical-trapping microscopy enable tracking the position of a bead in 3D. By integrating these techniques, we can now simultaneously and independently measure the conjugate variables of force and position. This integration has resulted in enhanced precision and expanded capacity. For instance, we have demonstrated ultrastable AFM imaging, real-time averaging to increase the signal-to-noise ratio, and optically locating sparse regions of interest. We anticipate that these capabilities will enable a number of additional applications, including hovering the tip over a protein to study its conformational dynamics over an extended period. More broadly, these additional capabilities should increase AFM's utility in biophysics and in a variety of other nanoscale studies and applications.
Acknowledgements
We thank Brad Baxley for figure 1. This work was supported by the Burroughs Wellcome Fund (CASI), the NSF (DBI-0923544), and NIST.

References

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