Bringing optics into the nanoscale – a double-scanner AFM brings advanced optical experiments within reach

Beyond the diffraction limit

The resolution of optical microscopy is generally limited by the diffraction limit described by Abbé, around 200 - 300 nm for visible light. Over recent years various strategies have been developed to resolve smaller structures, study single molecules and even perform spectroscopy on regions below the optical resolution "limit".

One approach is to use the special properties of light over distances much shorter than the wavelength. In this near-field regime, a tiny scattering or illuminating object near a surface can be used to probe the optical properties of the surface with resolution above the diffraction limit, and this technique is also capable of single-molecule detection [1]. The light is often collected in the far-field regime, where the light source or scattering object would still be seen as a diffraction-limited spot. The intensity, fluorescence, or polarisation of this spot will change, however, as the tip is scanned on the nanometre scale over the surface.

This type of imaging is often called scanning near-field optical microscopy (SNOM). When a small object is used to scatter the light in the near-field, rather than to directly illuminate the sample, it is known as apertureless or scattering SNOM [2,3]. An atomic force microscopy (AFM) tip can be used as the scattering object for scattering or apertureless SNOM. As the tip is scanned over the surface, it images the topography using the standard AFM modes, and can simultaneously generate high-resolution optical contrast.

This type of experimental setup also has a very interesting application to the field of Raman spectroscopy, since the cantilever tip can provide a very strong local enhancement of the Raman effect. This offers the potential for true chemical identification of the structures seen in the high-resolution AFM image.

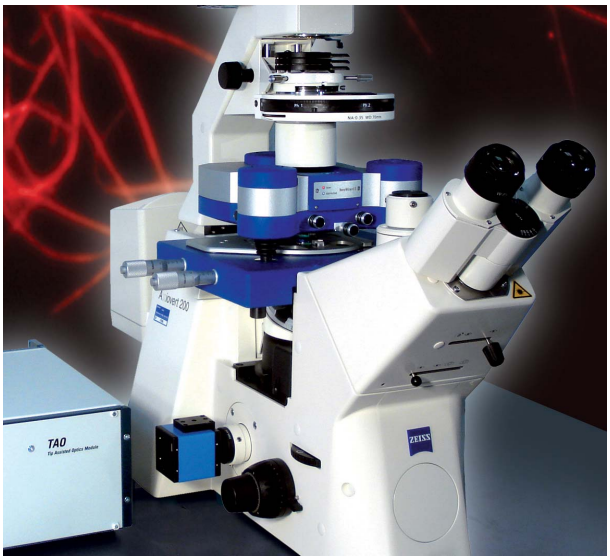
Imaging is not the only application of such a combined AFM and near-field optical setup. A wide variety of different experiments are possible where the AFM tip is used to create or modify nanostructures. Metal colloids, fluorescent proteins or quantum dots can be moved around the surface to create optically active nanostructures directly within the laser focus.

The force spectroscopy mode of the AFM also has great potential – force measurements can be made during protein unfolding to measure the internal structure. Combining these kinds of measurements with simultaneous single-molecule fluorescence adds the possibility of combined FRET (fluorescence resonance energy transfer) with AFM for full structural interpretation of the optical and AFM signals.

Tip-assisted optics (TAO)

Previously, experiments requiring alignment of the AFM tip or sample with a laser focus required specialist hardware knowledge and a large time investment to build a system or make significant modifications to commercial equipment and software. The NanoWizard® AFM is designed for the integration of bio-AFM with standard optical techniques. The Tip Assisted Optics (TAO™) module consists of an additional lateral scanner, integrated with the same software, so it allows both the tip and sample to be positioned and moved independently by the software. The availability of an off-the-shelf system to align tip and sample independently with an external laser beam allows users to start immediately with the optical experiments that are the focus of their research.

Using this combined system it is straightforward to position the AFM tip or sample in the optical focus [4]. The tip or sample is simply scanned through the focus to generate an image, the position is selected directly in the software, and control switches to the other stage. One scanning stage holds either the tip or sample fixed in the focus, and the other is available for imaging, manipulation, and all the normal AFM modes of operation.



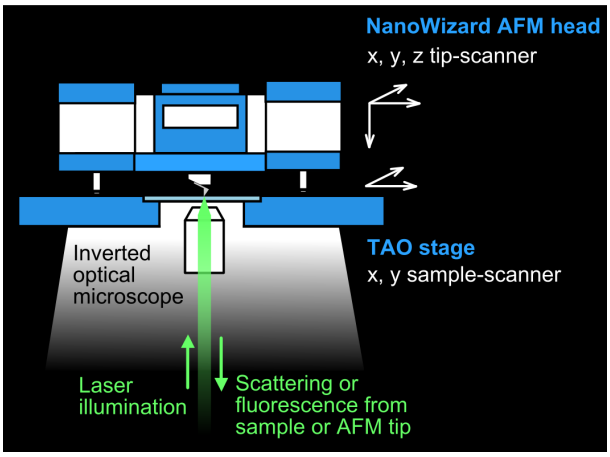


Fig. 1 TAO-NanoWizard® dual scanning system for Tip Assisted Optics experiments. The second scanner and controller are integrated into the AFM software, providing a 5-axis scanning system where the tip or sample can be independently scanned and positioned using linearized piezos.

Locating the focus through scanning is a straightforward method of finding the precise tip position that is made possible by piezo technology. Unfortunately, piezo material suffers from inherent problems of creep and nonlinearity. If the tip or sample is positioned at the centre of the laser focus, it is important that it stays there over the duration of the experiment, and this requires a piezo linearization system. The TAO module uses the same capacitive-sensor piezo linearization as the NanoWizard® AFM head. This closed-loop positioning system continuously measures and corrects for the piezo movement, so whether the tip or the sample is held in the laser focus, the problems of piezo creep or drift are eliminated.

In one range of applications, the tip can be positioned accurately in the centre of a laser focus for performing tip-enhanced optics, scattering SNOM, or overlaid AFM and optical imaging of single molecules using the sample scanner. Alternatively, single molecules, quantum dots or other sample features can be placed in the laser focus, leaving the tip scanner free for imaging, manipulation, or protein unfolding.

Protein structure from combined force spectroscopy and FRET

Protein structure can be investigated using the AFM by using the force spectroscopy mode. The protein molecule is stretched between the tip and the surface, and once a critical force is reached the higher-order structures of the molecule will unfold. Characteristic parts of the structure unfold in succession, converting the compact 3D structure of the native protein molecule to a linear unfolded amino acid chain. The force is measured during the process, and the various peaks in the plot of force against unfolding distance are characteristic of the structures being unfolded. This gives a structural "fingerprint" for a molecule and its different unfolding pathways, which can be studied under different environmental conditions [5].

FRET (fluorescence resonance energy transfer) is an optical technique that can also be used for monitoring protein structure. Special pairs of fluorescent molecules are attached to specific sites on the protein molecule, and the intensity of transferred radiation from one fluorophore to the other gives a very sensitive measurement of the separation distance. The measurements are usually done in bulk, where for instance the chemical conditions or temperature are changed to make the protein unfold, and the fluorescence signal can be used to measure the point at which those selected sites lose their connection.

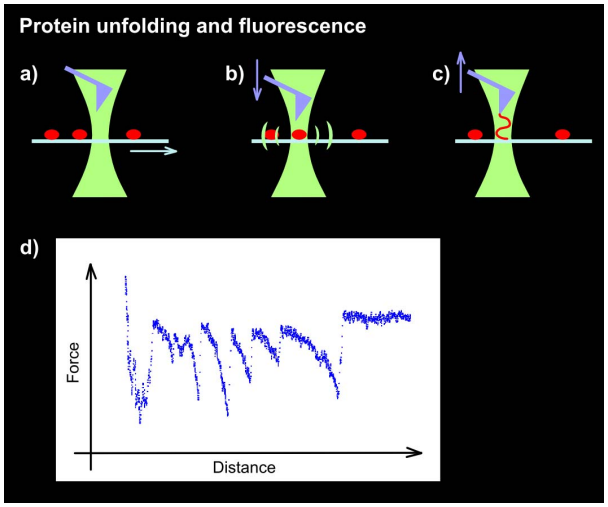


Fig. 2 Schematic of protein unfolding within a laser focus for combination with FRET. a) First the sample scanner is used to position the protein in the laser focus. b) The tip scanner moves the AFM tip over the protein. c) The tip is used to unfold the protein while the FRET signal is collected. d) Example of unfolding data from the membrane protein NhaA, courtesy of A. Kedrov, Prof. D. Muller, TU Dresden. Total extension length 140nm, force scale 150pN.

Creation of optically active nanostructures using AFM manipulation

The tip-sample force in the AFM can be controlled very sensitively. Usually the force is minimized during imaging to make sure that the surface is not disturbed. It is possible to deliberately increase the force, however, to move particles around on the surface. In the software for the NanoWizard® AFM system, manipulation paths can be drawn freehand or saved and imported as graphics files. This gives a very flexible control for creating patterns or nanostructures on the sample surface.

Metal colloids, fluorescent proteins and quantum dots are among the most interesting particles for combined manipulation and optics experiments. The particles can be easily deposited on transparent substrates and imaged. Using the sample scanner, a particular particle can be selected and moved into the laser focus. Intensity or spectroscopy measurements can be made, then the structure can be modified and the measurements repeated. The sample scanner holds the original particle reliably in the laser focus, while the tip scanner is free for AFM imaging to accurately characterize the nanostructure and manipulation, for example to bring other colloids or quantum dots nearby within the focus.

Combined nanostructures can be created directly within the laser focus, exactly where they are needed for investigation. The AFM imagng allows nanometre accuracy in the position and arrangement of the particles within the structures, and in the characterization of the sizes and distances.

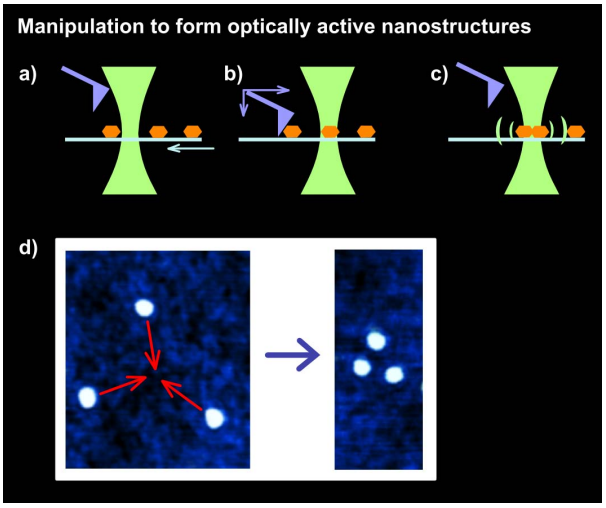


Fig. 3 Schematic of AFM manipulation to form nanostructures within the optical focus. a) First the sample scanner is used to locate a suitable particle and move it into the laser focus. b) The tip scanner is then used to image the surface and manipulate other particles. c) Particles can be moved together within the optical focus. d) AFM images of 3 quantum dots, before and after manipulation. Larger image side 500 nm in both cases, quantum dot size/height 5 nm.

Confocal optical imaging with aligned AFM and optics

If a pinhole is placed in the optical detection system, this setup can also be used as a sample-scanning confocal optical microscope using the AFM software. First the AFM tip is scanned through the optical field, and the optical signal can be localised where the tip is in the laser focus. The cantilever is held in this position using the tip scanner, while the sample scanner is free for imaging.

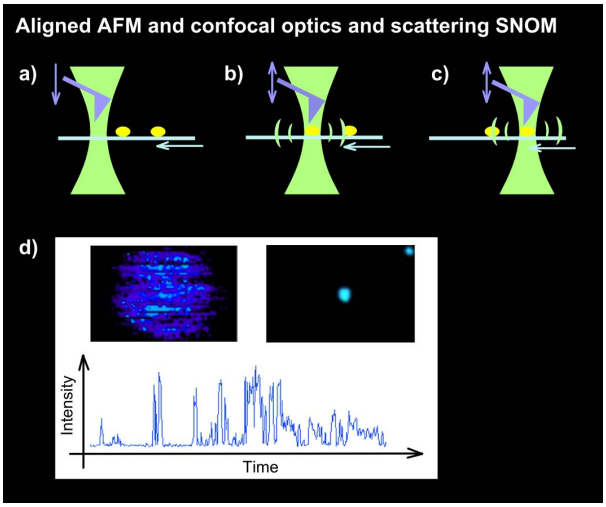


Fig. 4 Schematic of confocal optical microscopy with aligned AFM. a) First the tip scanner is used to align the AFM tip in the focus. b) and c) The sample scanner is then used for imaging, keeping the tip in the focus. d) Aligned optical and AFM images of a cluster of 3 quantum dots. Larger image side 1 micron in both cases. Also shown is a plot of intensity against time over the centre of the cluster. The characteristic quantum dot blinking is seen as different combinations of the 3 quantum dots are bright or dark.

The single molecule optical imaging is now aligned with the high-resolution AFM image, and the optical intensity at each point can be read into the AFM controller. The optical image is created as the sample is scanned, at the same time as the AFM image and appears in the AFM software. This system can be used for apertureless or scattering SNOM measurements, where the tip of the AFM cantilever is used as a nano-scattering object that can generate optical contrast with a resolution beyond the far-field Abbé limit.

Tip-enhanced Raman Spectroscopy, TERS

A confocal system set up as described above can also be combined with optical spectroscopy methods. All spectroscopy methods are possible, but Raman spectroscopy offers a particular advantage because of the surface enhancement effect. Certain metal particles (e.g. silver nano colloids) can give an enhancement of many orders of magnitude for the Raman signal of molecules nearby. This is called surface-enhanced Raman spectroscopy (SERS) [6,7]. When an AFM cantilever is metal coated, the metal grains at the end of the tip can act like the colloidal metal particles in SERS and provide a strong enhancement of the Raman signal from nearby molecules. This is known as TERS, or tip-enhanced Raman spectroscopy [8]. TERS can provide local enhancement of the Raman signal of several orders of magnitude, enabling true high-resolution contrast on the surface.

At any point in the AFM image, Raman spectra can be collected and compared, allowing true chemical identification of the structures seen in the high-resolution AFM image. Using the TAO system this technique has already been applied to DNA nucleotides and bacteria, for example [9,10].

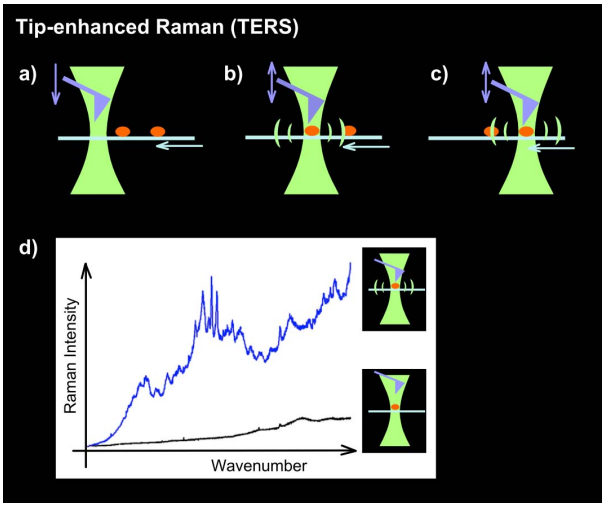


Fig. 5 Schematic of TERS. a) First the tip scanner is used to align the AFM tip in the focus. b) and c) The sample scanner is then used for imaging, keeping the tip in the focus. d) Comparison of Raman spectra of the background signal (black, tip away from surface) and enhanced signal (blue, tip on the surface). Data courtesy of V. Deckert, ISAS Dortmund. The general level is enhanced, and also particularly the sharp Raman peaks.

Conclusions

This combination of AFM with two separate scanners controlled by the same software interface facilitates the positioning of the AFM tip or sample features within the optical focus, allowing a wide range of combined AFM and optical experiments. The focus location through scanning

is an easy and robust method of finding the precise tip position, and the linearized piezos used throughout provide the stability so that the tip can stay within the focus for extended experiments. The tip only needs to be placed manually near the optical focus within the 100 x 100 µm scan range of either the tip or the sample. The two applications shown here are only small examples of the range of experiments that will benefit from this combined scanner

Literature

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