NanoManipulation with NanoWizard® Technology

Introduction
Nanotechnology, with the development of new tools and materials, is an exciting field which promises to enable increased performance of sensors, actuators and computers. It also adds another dimension to life science research as manipulation and imaging on the nanoscale allow new biological questions to be investigated. From the technical side, nanoparticles, such as quantum dots and nanowires, such as DNA, carbon nano tubes (CNT) or other polymer molecules, have a variety of potential applications, from data storage to single-electron electronics, nanophotonics and nanoelectromechanical systems (NEMS). Additionally, nanoparticles and nanostructures have had an increasing impact on basic research in biology (i.e. using quantum dots for labelling) as well as providing potentially new mechanisms for drug delivery, cancer therapy and medical imaging. In fact, the list of possible applications is continually growing in such diverse fields from biology to photonics research to molecular chemistry and physics. However, while using such nanoscale structures in research and for imaging is opening new doors in investigation, the construction of complex nanoscale devices is the ultimate goal of such research.

One of the biggest challenges in the advancement of nanotechnology is the controlled manipulation of components that have dimensions less than 100nm and are subjected to forces at the molecular level- such as Van der Waals, electrostatic, capillary and chemical forces. Precise positioning of individual nanoparticles is essential to assemble complex two- and three-dimensional structures. Regular, symmetric patterns of nanoparticles can be formed by self-assembly [1], yet such an approach is limiting as many of the possible applications require asymmetric shapes, or a mixture of heterogeneous components.

One promising approach for the construction of such nanostructures is assembly from single, molecular-sized components, requiring precise positioning on the nanometer scale. The Atomic Force Microscope (AFM) can be used as a manipulation tool to move and arrange nanoparticles and to build nanostructures. The imaging capacity of the AFM also means that manipulation and imaging can be combined to allow precise control over which structures are moved and characterisation of the structures at each step of manipulation or assembly.

AFM as an imaging tool
The basic working principle of an AFM is based on using a flexible cantilever, with a very sharp tip, at the end to scan the surface of the sample. As the tip is scanned across the surface there are interaction forces (in the order of piconewtons) between the tip and the molecules of the sample. These forces can lead to the deflection of the cantilever. Changes in surface topography lead to changes in cantilever deflection, which is monitored by shining a laser off the back of the cantilever and onto a detector. AFM imaging produces a nanometric 3 dimensional image of the scanned surface. Such imaging can be conducted in air, vacuum or liquid but most importantly is non-destructive and does not require pre-treatment of a sample or complicated sample preparation. Such characteristics have made AFM highly suitable for the surface analysis of a wide range of materials, from magnetic media and compact disks to metals, biomaterials, nanoparticles and ceramics. The mechanical nature of the AFM broadens the scope of this instrument from just imaging to use in characterising elasticity, measuring binding forces between biomolecules or whole cells and use of the tip for the controlled manipulation of structures.

AFM as an Nanomanipulation tool
Imaging nanometric structures with the AFM requires precise positioning of the nanosensor. This precise control is what enables the use of the instrument as a nanomanipulation tool. The cantilever tip can be used to cut, move, align or indent samples with extremely high precision. This controlled manipulation teamed with non-destructive imaging capacity makes the AFM a breakthrough tool for nanoscience. The one instrument can be used to image a sample, move components in a controlled fashion and then reimage the resulting
structures. Basically, the AFM sensor acts as a hand to perform manipulations under computer-controlled conditions. As such, the AFM has been used as a nanomanipulator in the fields of biology [2,3,4], genetics[5], photonics research [6,7] and nanorobotics[8].

**Experimental setup**

For the experiments in this report the NanoWizard® AFM head and Life Science stage were mounted on a Zeiss Axiovert 200 inverted optical microscope. The inverted microscope is the base for the AFM and allows acquisition of additional data i.e. optical images such as fluorescence or polarisation contrast simultaneously with AFM.

For imaging, force measurements and nanomanipulation applications the performance of the scanner is extremely important. JPK scanning stages are based on a different design principle than conventional scanning stages. To ensure the highest performance of the scanner unit in terms of resolution, z-response, linearity, creep, hysteresis, aging and range of motion JPK NanoWizard® scanners incorporate high-force piezo actuator drives, frictionless flexure guiding systems and absolute measuring capacitive position sensors. The high force of the solid-state piezo actuators allows for fast response times, in the millisecond range, plus high scanning frequencies. The XY system consists of only one moving part, a single module rather than two individual stacked modules, as is common in other XY systems. In other SPMs piezotubes are used as scanning units. Monolithic ceramic tubes are yet another form of piezo actuator. These tubes are silvered inside and out and operate on the transversal piezo effect, when an electric voltage is applied between the outer and inner side of a thin walled tube, the tube contracts axially and radially. Tube design SPMs have some disadvantages, these actuators are not designed to withstand large forces and have out of plane errors.

Next generation SPMs, like the NanoWizard®, use sensor controlled, flexure guided, parallel kinematics systems. This "parallel-kinematics" mechanical design is complemented by a parallel-motion metrology feedback system. With parallel-metrology, all sensors "look" at the moving platform "from the outside" and can "see" off-axis and run-out errors. The controller then eliminates unwanted motion in real-time. Conventional serial-metrology sensors (integrated in each axis) cannot detect off-axis errors. Parallel motion metrology allows for significantly higher overall XYZ precision. To ensure optimal z-response, the z-axis is specially decoupled from the xy axis and optimized for high scan rates.

To conduct accurate manipulation experiments, the user must be able to control all manipulation parameters in real-time. In the NanoWizard® software, the JPK manipulation mode allows the user to control tip velocity, distance, angle and all cantilever parameters like height, amplitude and phase signals. Additionally the user can import external signals. The graphical user interface (GUI) is easy to use for beginners while still appropriate for advanced users.

Experiments can be conducted with or without force feedback control. With force feedback control the tip moves with a preselected force, avoiding the destruction of fragile structures. The user can also access a real time oscilloscope widow, in which all signal channels can be monitored in real time, allowing more precise user control over the manipulation process. To select paths for the cantilever movement, the user selects the desired image.
and then can either draw a freehand path or import a predefined, scalable curve into the image.

**Application examples**

To demonstrate the ability of the NanoWizard® AFM to manipulate and image nano-scale structures, we have manipulated quantum dots, carbon nanotubes (CNT) in air and polymer nanowires and collagen type I fibrils in liquid.

1. **Nanomanipulation of Quantum dots**

A quantum dot can be a tiny island-like region in a semiconductor with an extension of only a few nm³, where a single electron-hole pair (exciton) can be quantum-mechanically confined in all three dimensions. Such a quantum dot can also be formed as particles by self-organization, if two semiconductors with significantly different lattice constants (e.g. CdSe and CdTe) are grown epitaxially on top of each other (‘self-assembled quantum dots’) [9]. Quantum dots have special electronic properties that, when excited, cause the emission of light at a single wavelength that is dependent on dot size.

There are many potential applications for quantum dots in nano optics, physics and biology research. To characterize the quantum mechanical properties in conjunction with electromagnetic radiation of such tiny particles, an experimental setup is required that can specifically manipulate the pattern and alignment of quantum dots, allow high resolution imaging of the respective patterns and the acquisition of fluorescent optical data. All of these features are provided by the JPK NanoWizard®.

![Fig. 2](image-url)

**Fig. 2** Sequence of images showing the arrangement of five single quantum dots into an arbitrary figure. Successive images were taken between movement of quantum dots on the cover glass by the AFM tip (imaging IC mode, manipulation contact mode).
Here we have moved quantum dots into close proximity on glass. The sample was prepared by spin coating a cleaned coverslip with quantum dots. In this case, the particles used were commercial CdSe quantum dots with a 5 nm diameter. Five successive images were taken, after the movement of each quantum dot (Figure 2).

The assembly of quantum dots into aggregates may lead to changes in optical and electrochemical properties. The use of the AFM to manipulate quantum dot organisation on a surface extends the study of collections of these particles from regular arrays, driven by self assembly, to the study of how these properties may change as the proximity of the quantum dots to each other is manipulated in situ.

2. Nanomanipulation of carbon nanotubes

The manipulation of nano-rods or nano tubes with the AFM tip is more complex than the controlled positioning of nanoparticles. The size scale of the nano-particle in relation to the AFM tip means that manipulation of nano-particles is usually only involves translation. The manipulation of nano-rods and nano tubes, on the other hand, can involve not only translation but also rotation, and kinking. The real-time interactive forces are used to update the AFM image in order to provide the operator with real-time visual feedback during nano-manipulation of nano-rods.

Here we have used the AFM tip to manipulate the structure and orientation of CNTs. CNTs have unique electrical properties, ranging from semiconducting to metallic depending on structure. The size, mechanical and electrical properties of CNTs make them interesting candidates for use in nano-device technology. However, there must be some way of manipulating CNTs in a controlled manner. The manipulation of CNTs does not merely depend on the nanotubes and the AFM tip. The interaction of the CNTs with the substrate will influence the possible manipulation. Initially, CNTs were fixed to a sputtered gold surface. At forces in the 500 pN range, the bundle was stable, with no movement observed. At higher manipulation forces (up to 200 nN) there was still no rolling or bending of the CNT, rather the application of this high force lead to the longitudinal shearing of the bundle (Figure 3).

![Fig. 3 A single bundle of CNTs is shown here in the height and phase channel.](image)

Obviously, a surface-CNT interaction that is strong enough to stabilise manipulated structures without being so strong that CNTs can not be moved without shearing is preferable. As such the CNTs were deposited on a silicon surface. In this case the surface interaction is strong enough to support energetically unfavourable structures, such as kinking, without inhibiting the movement of the bundles with the tip.
A number of different manipulations were conducted, with different tip movements inducing different changes in the CNT structure. By drawing the tip across the CNT at a right angle the CNT could be kinked (Figure 4 B). By moving the CNT from the side, shallow curves could be introduced into a previously straight nanotube, or a long segment of nanotube could be moved (Figure 4 C). The interaction between the CNTs and the silicon surface was not so strong that manipulation could not be conducted, however, after the movement of segments of the nanotube the interaction between the CNT and the surface was sufficient to hold the new structure in place.

3. Manipulating collagen fibres

While the previous examples have highlighted the use of AFM for moving and manipulating inorganic structures, the AFM can also be used to manipulate soft and fragile organic nanostructures like DNA, RNA, microtubules, or chromosomes. This manipulation can involve excising regions (for instance in chromosome research) or repatterning substrates. In the case of molecules such as collagen or microtubules (extracellular matrix or cytoskeletal components) the manipulation of structure could allow the investigation of the particular structural components recognised by other biomolecules.

Collagen type I will spontaneously form aligned fibrils if injected into buffer over a mica surface [2]. If the buffer used contains sufficient potassium ions then the collagen...
fibrils will display the characteristic 67 nm banding pattern observed in collagen from muscle tissue. Shortly after deposition, these fibrils can then be manipulated using the AFM tip (figure 5). The fibrils can be caused to change direction, to bundle or they can be separated from each other. Such manipulative capacity would allow investigation of the structural features of collagen that result in the directed motion of cells. Obviously, in the case of such biomolecules, manipulation and imaging must be conducted in fluid. In this case the collagen fibrils were imaged in buffer in intermittent contact mode and manipulated in buffer in constant contact mode.

4. Nanomanipulation of polymer nanowires
The AFM can also be used to excise specific regions of polymeric samples, rather than just moving structures.

We took a rigid polymeric network, CNTs coated with biomolecules fixed to glass. The network was imaged in intermittent contact mode and then small regions excised and moved away from the polymer. The precision of the instrument and local application of force via the sharp tip of the AFM sensor means that small pieces can be removed without disturbing the rest of the network. Successive images are show below (Figure 6) of two small regions being cut away from the nano wire and shifted away from the wire itself. The imaging capacity of the AFM can easily distinguish the region from which the segment was removed.

Conclusions
The NanoWizard® AFM is an advanced but easy to use tool for nanomanipulation applications. Important parameters like the force, velocity, and the bending on the cantilever during the manipulation process can easily be monitored and controlled. Additionally, the software interface makes precise and dynamic fine positioning of the cantilever straightforward using the JPK manipulation mode. Manipulation patterns can be input by freehand path drawing, directly in the image viewer in the JPK software. Alternatively, manipulation data can easily be imported into the control interface from a saved file in SVG format. Multiple paths can be defined for a single manipulation run, allowing multiple components of a sample to be moved before re-imaging.

Fig. 6 Manipulation of nanowires. A series of topographic images shows the excision of specific nanowire segments.
The combination of manipulation and non-destructive testing makes AFM an optimal technique for the manipulation and monitoring of nanometric structures. The other major advantage of working with JPK NanoWizard® is the extension of imaging capabilities to include high end optical microscopy techniques (laser scanning confocal imaging, FRET, SERS, DIC) by virtue of the installation of the AFM on an inverted light microscope.

Microscopy techniques that can resolve structures at the nanoscale opened our eyes to a new world. The fact that the AFM sensor can be used as a manipulator means that we can now interface with and manipulate this nano-world in a controlled fashion. The manipulation and imaging (AFM and optical) capabilities of our setup have applications in a broad spectrum of fields, in both basic research and in the development of new technology.

The high end precision of the mechanical setup, the easy to use software and the experimental freedom to use advanced optics simultaneously makes the NanoWizard® a powerful tool for nanomanipulation.

**Literature**


