

# Micro/Nano Motion Control for Biological Specimen Handling

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## Abstract

*We present our recent research on the development of a sensor and manipulation system for precise and controlled handling and manipulation of biological cells, molecules and tissues, down to the DNA level. Our system centers on a Scanning Probe Microscope robotic design specifically tailored for investigations of biological samples. First SPM experiments, which focus on manipulations of DNA, are reported. Motion control designs, performed separately, which focus on adaptive learning closed loop architectures integrated on a chip are also discussed. The outcome of this research will enable biologists and medical scientists to perform precise and controlled transport, positioning, insertion into and site-specific modification of bio-cells and related samples.*

## 1 Introduction

We present our recent research on the development of a sensor and manipulation system for controlled handling and manipulation of biological cells, molecules and tissues, down to the DNA level. Our system centers on a Scanning Probe Microscope (SPM) robotic design which is specifically tailored for investigations of biological samples. The research seeks to understand and exploit the interaction forces between nano-probing mechanism, the bio-samples, and their environment. Within this system, we are designing and incorporating novel sensor based processing and control mechanisms, integrated on a chip. The outcome of this research will enable biologists and medical scientists to perform precise and controlled transport, positioning, insertion into and site-specific modification of bio-cells and related samples.

## 1.1 Previous Work in Scanning Probe Microscopies for Imaging and Manipulation

The family of scanning probe microscope (SPM) techniques has revolutionized studies of micro and nano objects. SPM is based on piezoelectric sample positioning which can achieve nanometer lateral accuracy and angstrom vertical accuracy in imaging mode. Its potential for use in biological studies was immediately apparent, and research efforts began shortly after the invention of the scanning tunneling microscope (STM) in the mid-1980's. STM is not wholly suited to imaging of largely nonconductive biological samples and, for biological investigations, it was quickly superseded by the invention of the atomic force microscope (AFM). The nano-Newton tip-sample interaction forces of AFM were sufficient to damage soft biological tissues, and it was largely due to the need to reduce these forces for the study of biological specimens that a pico-Newton imaging system based on disturbances to a freely oscillating cantilever (called non-contact, or TappingMode™ by Digital Instruments) was developed. Simultaneously, another system based on AFM imaging in fluids, which reduce or eliminate Van der Waals and surface adhesive forces, was developed. Development of both techniques has taken place throughout the 1990's and continues to be an intensely active research area for SPM use in biology. In-vitro and even in-vivo [1] imaging has been achieved, although imaging artifacts and slow scan rates continue to be a challenge. The combination of TappingMode™ AFM in a specially designed liquid-holding enclosure, or fluid cell, is now the preferred method for biological imaging.

During the 1990's, the potential of scanning probe microscopy for nanomanipulation, or directed assembly, also emerged. Investigations to date have included three dimensional pick and place operations involving atoms in ultrahigh vacuum [2], two dimensional manipulations of micron through nanometer sized objects and molecules in air as well as ultrahigh vacuum

[3, 4, 5, 6, 7], and two-dimensional manipulations of carbon nanotubes [8, 9]. Some of these experiments have involved haptic control [7, 9]. These studies have also identified the important role of the sample-substrate interaction [3] in addition to the tip-sample interaction in nanomanipulation. Within the biological communities, the uses of AFM for force dissection [10], and for sub-cellular probing with functionalized tips [11, 12] have been reported. These experiments constitute proof-of-principle that SPM-based nano-positioning and nano-tasking are both possible.

## 1.2 Current State of the Art in SPM Control Architecture

Because of a lack of suitable sensors that can be used in a feedback scheme, motion control in micro/nano scale systems is primarily in an open loop. This is true in SPM systems; the architecture of a standard system used for imaging is closed loop in  $z$ , but open loop in  $x$  and  $y$ . In the experimental systems used for SPM-based manipulation, the standard  $(x, y)$  raster motion is interrupted and signals produced by human intervention (Refs. [2-9]) are fed directly into the SPM to control the  $x$  and  $y$  motion of the probe tip. The precision of the implementation relies on the collaboration of the piezoelectric actuators which are known to suffer from a variety of problems such as creep and hysteresis. In addition, the thermal drift of the piezoelectric actuator is very significant. Compensation for these effects for manipulation [5] and image post-processing for sensing [13] are both active research areas.

## 1.3 Research Strategy

Our recent investigations of biological specimens have focused on manipulations of DNA similar to those which have been achieved for carbon nanotubes. Future investigations will include manipulations of cells, and on cell surfaces. Our aim is to combine the high resolution imaging which has been achieved by SPM for biological specimens with the growing field of SPM-based nanomanipulation. To achieve efficient and reliable manipulation in a micro/nano environment, it is essential to possess capabilities of sensing, processing and actuation in dynamic interactions. We acquire the signals which would normally be projected as an SPM image and use them as the sensing component within a feedback control loop formulation to accurately steer the probe's tip along a prescribed trajectory. Example prescribed trajectories includes tracking the surface of a cell with accurate precision

required in operation of insertion or dissecting cell tissue. The methodology is automated by the feedback mechanism to allow for several desired key operations frequently used in biological and medical fields.

## 2 SPM Experimental Results

### 2.1 Samples and Preparation

A drop of *Larix Gmelini* (common larch tree) DNA suspended in water was deposited on an untreated mica substrate and allowed to dry. The first experiments were carried out within twenty four hours after deposition; the second experiments, within one week after deposition.

### 2.2 Instrumentation

Atomic force microscope imaging, manipulation and analysis were performed using a Digital Instruments Nanoscope IIIa operated in ambient air. Etched silicon tips with a nominal tip radius of 5-10 nm and a half cone angle of  $17^\circ$  were used for all experiments. The tip is fabricated on a single beam cantilever with a nominal spring constant of  $k = 20-100$  N/m (stiff cantilever). All images shown were acquired using the "E" scanner (13 mm x 13 mm).

### 2.3 Imaging and Manipulation Experiments

A DNA sample was deposited on an untreated mica substrate as described above and imaged using TappingMode™ AFM. Images are shown in Figure 1 of (a) the bare mica surface and (b) the same surface covered with DNA strands. The image remained stable over several scans, i.e., no displacement of the strands during imaging was observed. The large tangle of strands indicated by the arrow in (b) served as a landmark. This was positioned at the center of a 3 micron image area. The AFM was then switched to operation in Contact mode over a centered 1 micron area, still using the TappingMode™ cantilever and tip but without oscillation. No image was visible during the scan. The scan rate was 0.803 Hz. The AFM was then returned to operation in TappingMode™ with no tip change. The effect of the Contact mode operation, shown in Figure 1 (c), was to move all of the DNA material to the outside of the 1 micron scan area. Contact mode force measurements performed on the

stiff TappingMode<sup>TM</sup> cantilever indicate that the vertical force may have been on the order of 100's of nano-Newtons. Another effect, observed during several experiments, was that while the resonant frequency of the cantilever oscillation returned to its previous value for the subsequent imaging, the drive amplitude was consistently higher, which may indicate that sample material had adhered to the tip. We also note that although the landmark DNA strands were positioned in the center of the image, they are still outside of the 1 micron Contact mode scan area in Figure 1 (c), due to a combination of inaccurate centering when going from the 3 micron to the 1 micron scan size, and thermal drift, which was consistently observed to shift the images downward.

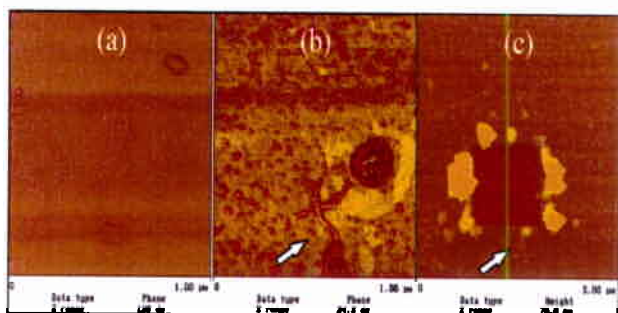


Figure 1: TappingMode<sup>TM</sup> phase images of (a) bare mica, (b) mica with DNA strands, and (c) TappingMode<sup>TM</sup> height image of the effect of the high force Contact mode over a one micron area. The feature in (b), indicated by the arrows, was not intercepted in (c).

The next experiments were performed in line mode, with the slow scan axis disabled. They were performed one week later and the DNA strands had dried and coalesced into mats. This indicates that the surrounding environment had changed, which could affect both tip-sample and sample-substrate interactions. The initial TappingMode<sup>TM</sup> images remained stable over several scans. The AFM was then switched to operation in Contact mode with the slow scan axis disabled. The line scan was performed for  $1/0.803 \text{ Hz} \times (512/2) = 320$  seconds. No image was visible during the scan. The initial setpoint value of 1.25 Volts (V) was the previous TappingMode<sup>TM</sup> value. The effect of lowering the setpoint (S.P.) to 1.00 V, thereby reducing the force (Contact mode), was investigated over a similar-looking section of material. The results of these scans are shown in Figures 2 and 3. In Figure 2, a TappingMode<sup>TM</sup> phase image with contrast enhancement shows the two line scans and the piled-

up material at the scan edges. The displacement of the two lines is due to thermal drift during the time which elapsed between the two scans. We note that the lighter force S.P. = 1.00 V line is wider than the S.P. = 1.25 V line. This effect was confirmed by Section analysis shown in Figure 3. Figure 3 (a) shows the line scan profiles along a line perpendicular to both. The width of the S.P. = 1.00 V line is about 312.5 nanometers (nm), and the width of the S.P. = 1.25 V line is about 175.8 nm. From the same figure, the depth of the S.P. = 1.00 V line is roughly 5 nm, and the depth of the S.P. = 1.25 V line is roughly 15 nm. The depth profile information was corroborated by lengthwise profile measurements taken down the center of each scan line. The lengthwise profiles are shown in Figure 3 (b) and (c). The dashed line indicates our estimate of where the true surface is. The depth of the lighter force S.P. = 1.00 V line from the dashed line is about 5 nm and the depth of the S.P. = 1.25 V line is about 15 nm. Both lines show very clean profiles along their lengths, indicating that material was easily moved aside at these forces. The piled-up material seemed to be greater along the scan direction (Figure 3 (b) and (c)) than at the sides (Figure 3 (a)).

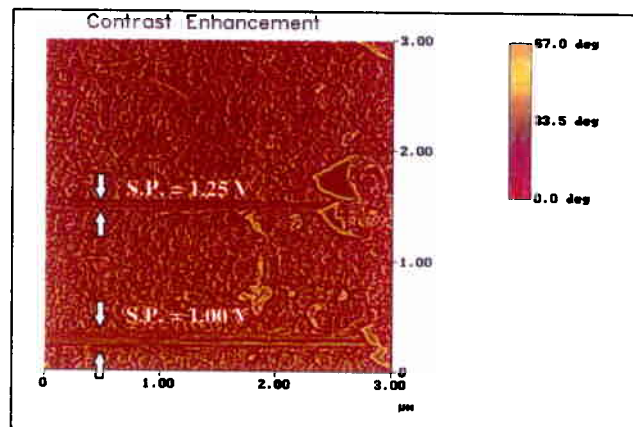


Figure 2: TappingMode<sup>TM</sup> phase image with contrast enhancement showing the effect of the high force Contact mode along a line (a) with a setpoint (S.P.) of 1.25 V and (b) with a setpoint (S.P.) of 1.0 V.

### 3 Adaptive Learning Designs

In the experimental systems used for SPM-based manipulation, other researchers (Refs. [2-9]) have demonstrated the feasibility of interrupting the standard (x, y) raster motion and introducing signals di-

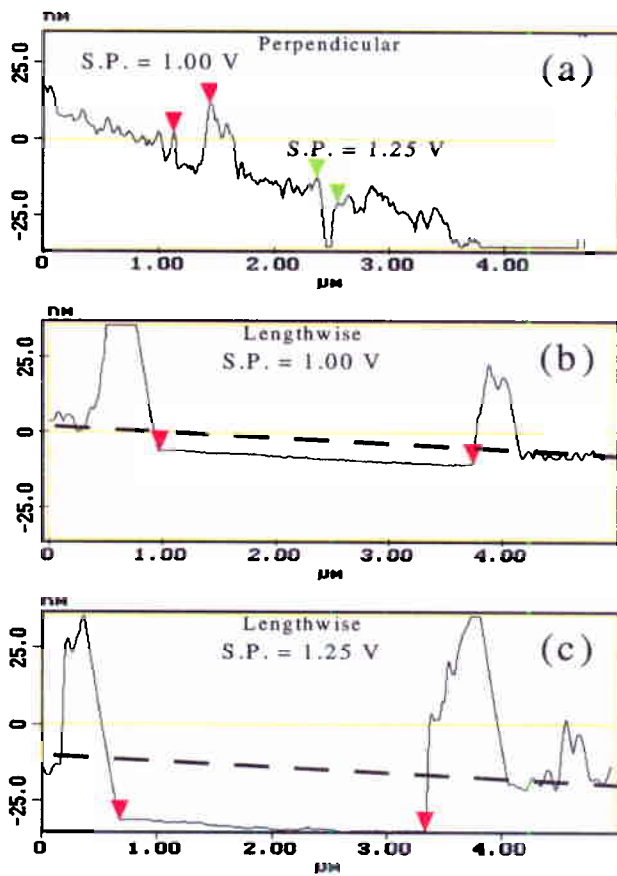


Figure 3: Section analysis of the effect of the high force Contact mode along a line measured (a) perpendicular to both lines, (b) lengthwise along the line with setpoint (S.P.) = 1.0 Volts, and (c) lengthwise along the line with setpoint (S.P.) = 1.25 Volts.

rectly into the SPM to control the  $x$  and  $y$  motion of the probe tip. We extend this approach with the introduction of an adaptive control step, integrated on a chip. Once such a chip is designed, it will provide an adaptive micro-processor that requires no programming. We will place this on-chip processing capability in a closed loop within the SPM robotic system so that instantaneous corrective actions are taken on the fly, resulting in controlled manipulation of the end-effector or probe. This approach differs significantly from the current state of the art which uses micro-controller or digital computers to access the measured signals, then process them via programs, then execute the actuation.

Our approach is to apply the paradigm learning process based on continuous input/output measurements as used in neural networks [14]. Both the network dynamics and the co-state dynamics are inte-

grated forward in time, while the parameters are being updated, at a slower rate. The slow rate update can approximate the integral operator in the parameter update. We are developing the adaptive on-chip learning process in the context of a modular neural network circuit architecture. The architecture of the chip is such that the tasks of adaptation and processing are dedicated to identical but separate networks. The adaptive network can be driven by the reference signal, the processing network can be driven by the feedback response while the error signal is used to adapt the common parameters of the two networks in order that the response asymptotically tracks the reference signal.

## 4 Summary and Conclusions

First manipulations of *Larix Gmelini* (common larch tree) DNA on untreated mica in ambient air have been investigated, using the approach of imaging in TappingMode™ and manipulation in Contact mode. For this biological specimen, the Contact mode forces were too high, resulting in uncontrolled pile-up of material especially in front of the advancing tip, rather than controlled manipulation. We have observed that operation in Contact mode with a lighter force resulted in removal of material along a path the was wider than with a stronger force; this was an unexpected result, which we will continue to investigate. In our next experiments, we will investigate line scans for setpoints of increasing force without changing from TappingMode™. We will also begin investigations in liquid environments.

Substantial thermal drift of the piezo may be observed in Figures 1 and 2. This resulted in the failure of the probe tip to intercept a centered landmark while implementing a micron-sized manipulation direction, as shown in Figure 1. We are developing an adaptive on-chip learning process in the context of a modular neural network circuit architecture, which may contain elements of landmark recognition by "feel". Such an adaptive chip integrated within the SPM robotic system will result in a continuously updated closed loop feedback control in  $(x, y)$ , in contrast to the current open loop  $(x, y)$  architecture.

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