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Paper Session I-C - Atomic Force Microscopy of DNA and Design Parameters for a Zero-G Operable Unit

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Atomic Force Microscopy of DNA and Design Parameters for a Zero-g Operable Unit

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Abstract

The International Space Station will finally provide the opportunity of a permanent zero-g laboratory facility where researchers could conceivably analyze the results of experiments in situ. There are numerous advantages to the rapid turn around of answers provided by this environment. A team of researchers and students in cooperation with the Florida Space Institute have imaged DNA and other biological specimens in an attempt to define the basic design parameters of a succesful AFM and STM unit for use in a zero-g environment such as ISS (Express Rack), Shuttle (Middeck Locker) and KC-135 (reduced gravity program). Broward Community College and Stephen F. Austin State University have utilized the experiences with student flights aboard the KC-135 as a starting point for future instrumentation and experiment design in the area of microscopy. We present images of DNA in contact mode as evidence of the feasibility of this work without vacuum systems. Vibrational isolation issues, including accoustical shielding have been addressed in preliminary designs. Student designs for the automation of microscopy operations demonstrate the success of Research Based Science Education. The design study team believes that AFM and STM microscopy will be a vital part of many space missions as we move toward the goal of further exploring Mars.

Introduction

As we set the stage for the future exploration of Mars over the next decade many questions in the search for life (past and present) begin to surface. Among the many questions, those pertaining to the robustness and survivability of the DNA molecule in potentially "hostile" environments appear to be of reasonable importance. What constitutes a "hostile" environment? Is the molecule mechanically fragile? If so, under what conditions? How sensitive is it to radiation in a "dried" state? These are a few of the questions that have been presented at meetings of the Association of Small Payload Researchers (ASPR). In the past two years ASPR members have entertained two main topics in this area of research. These are the radiation sensitivity, and the mechanical fragility of the DNA molecule. This interest was coupled with the desire to set the stage and address the feasibility of space borne microscopy equipment. Over the past year DNA samples have been imaged in "contact" mode with an atomic force microscope (AFM). The bulk of the work was done at the Microscopy and Imaging Lab of the Physics and Astronomy Department of Stephen F. Austin State University. Although the contact mode images yield less visual information than the preferred tapping mode images, they provide vital information about mechanical rigidity otherwise unavailable.

Methods

Genomic fish DNA was provided by the Texas A&M University Department of Wildlife and Fisheries Genotoxicology Lab. The standardized extraction techniques are discussed in the literature (Ritter et. al 1999). In order to image the molecule the original sample was diluted

several times using nano-pure de-ionized water in a sterile environment. The original sample provided a concentration of approximately 20 mg/ml. Successful imaging was attained at concentrations below 1 mg/ml. The samples were placed on several substrates. These were Mica, Graphite, and evaporated gold on quartz. The gold substrates were annealed to insure a 1,1,1 configuration. The gold samples yielded some of the first clear images in the Summer of 2000. Earlier images were obtained on the Mica substrate.

The preparation of each sample involved placing a small droplet of the diluted DNA solution on the 0.8 cm^2 substrate and allowing it to dry. A digital instruments Nanoscope II Atomic Force Microscope was used for imaging. The unit also has a Scanning Tunneling Microscope (STM) stage that can be used with conductive samples. Both the AFM and STM systems were used. The AFM mode dragging of the cantilever across the surface at a rate of 10-30 Hz provides a qualitative measure of the mechanical rigidity of "dried" DNA adhered to an atomically flat surface.

The Images

We present five images that are representative of most of the work. Figure 3 shows an evaporated gold on quartz surface with what are believed to be coiled DNA "globules". The image was scanned at the micrometer range. The "bare" gold samples were imaged and examined prior to the addition of the DNA solution and subsequent drying. Those calibration images showed none of the features of figure three.

Figures 1 and 2 are calibration images that are representative of the microscope's resolution at the time of imaging. Figure 1 shows Mica and figure 2 shows graphite.

Figures 4 and 5 show the DNA molecule on the surface of gold for the same sample of figure 3. This time the imaging was done at the nanometer range in the regions that showed the very fine filamentary structure between the larger globules of figure 3.

At the nanometer range it is important to find a relatively atomically flat region devoid of large "bumps" (such as coiled DNA).

The scale of the images indicates that the Z-axis height of 3nm matches the expected size. The width, however, appears larger as the result of imaging in contact mode.

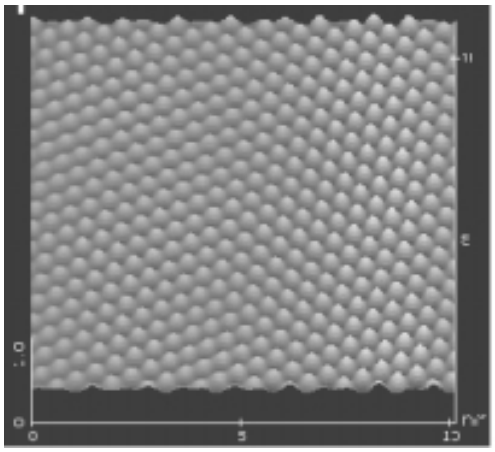

Figure1
(Mica , X & Y axes are 10nm)

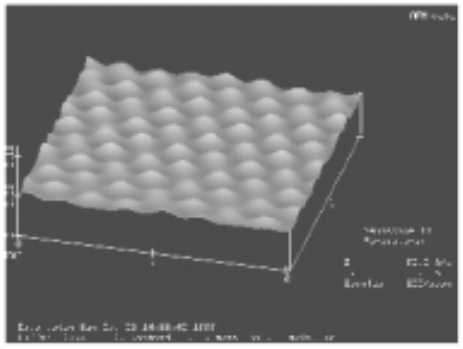

Figure2
(Graphite, X & Y axes are 2nm)

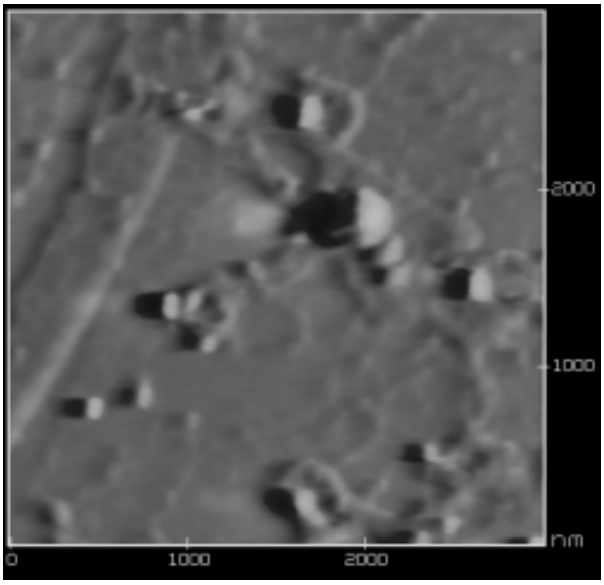

Figure 3
DNA on evaporated gold 3 micrometers on the X and Y axes.
The tallest Z axis features are 46 nm
Note the fine filamentary structures

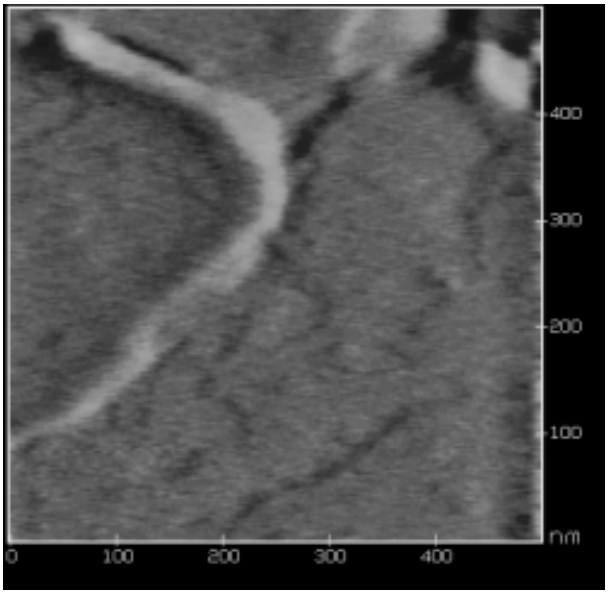

Figure 4
DNA “filament” at higher resolution. X and Y axes 500 nm
DNA image Z-axis ~ 3.8 nm

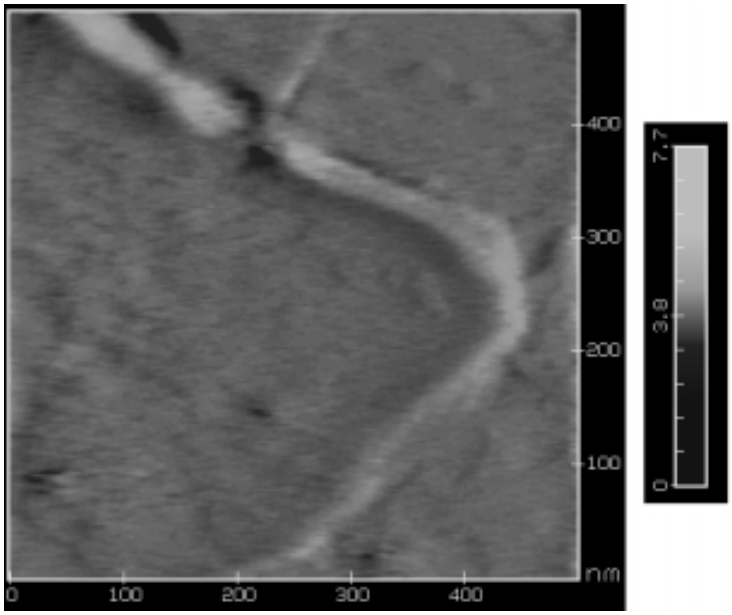

Figure 5
DNA on gold. Same region as above shifted to identify imaging artifacts.

Applicability for research aboard ISS

A significant part of the research aboard the International Space Station will involve micro-gravity materials science and the handling of biological samples. AFM and STM microscopy are powerful tools for both areas of research. The DNA images presented were obtained using a unit operating at one atmosphere. The unit can easily fit in an STS orbiter Mid-deck Locker or an ISS Express rack. Making it available aboard ISS would provide a high resolution microscopy capability that typically requires voluminous equipment. This would be the case with traditional TEM, SEM or other vacuum systems.

Design Parameters

The Association of Small Payload Researchers (ASPR) imaging group has identified the following concepts as desirable in establishing the design parameters of a Zero-g operable unit.

- Modification of the Tip
- Implementation of a revolving cartridge with spare tips
  (Both AFM and STM tips)
- Implementation of a revolving cartridge with multiple samples
  (After an initial flight, controllable or programmable from the ground.)
- Must fit in a Mid-deck Locker and EXPRESS Rack with an optional second locker for added control electronics and data storage.
- Vibrational Isolation (Can be easily attained with a double glass enclosure separated by a light vacuum.)

Student Involvement

Students have participated in the design phase through discussions and input of operational experiences in the Zero-g environment. Broward Community College's student team has flown aboard the KC-135 reduced gravity program twice. They have also designed appropriate mechanical interfaces and control electronics applicable to the microscopy project. The KC-135 experiments in fluid dynamics have provided insight on the effective handling of samples involving liquids. Several student teams have also worked on DNA radiation dosimetry experiments (Ritter 1999). The undergraduate student involvement is part of a BCC initiative to integrate different disciplines into the curriculum and provide students with the opportunity to participate in larger projects.

New stage for future work

The imaging of genomic DNA indicates that the mechanical rigidity of the molecule can be successfully studied with Atomic Force Microscopy in contact mode. The applications for Space Station work center around the capability of analyzing chemical samples and crystals grown in microgravity on site. An AFM, in addition to imaging, allows the probing of the mechanical properties of molecules. The Association of Small Payload Researchers (ASPR) expects to continue the design and test of a reduced gravity and Zero-g unit.

Acknowledgements

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