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Atomic Force Microscopy Based DNA Sensing and Manipulation

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Atomic Force Microscopy Based DNA Sensing and Manipulation

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by

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Abstract

Sequencing DNA provides a positive impact for the biomedical community by understanding a wide variety of applications such as human genetics, disease, and pathogens. The reason the Arkansas Micro & Nano Systems lab is involved with research in DNA sequencing is due to the current, leading industry method. Nanopore sequencing was developed by Oxford Nanopore Technology in which its sequencing method separates double stranded DNA to electrically characterize individual nucleotides traveling through a charged nanopore. Unfortunately, nanopore sequencing uses biological materials that require a shelf life and drives high cost. Therefore, the Arkansas Micro & Nano Systems lab has developed a DNA sequencing method using atomic force microscopy (AFM) to eliminate any shelf life of materials. One of the main functions of sequencing DNA using atomic force microscopy is using force spectroscopy to control the movement of DNA by creating an electrostatic force between a cantilever tip and strand of DNA. Two different force spectroscopy methods were developed by graduate students, Dr. Bo Ma and Lucas Bartmann, to control DNA.

The focus of this report is to test and evaluate both spectroscopy methods developed by previous graduate students from the Arkansas Micro & Nano Systems lab and determine which procedure is more effective. Because atomic force microscopy will be used to control and move DNA, this undergraduate thesis will also entail a top-down approach of how to prepare DNA on a mica surface and how to use a CoreAFM in liquid mode to scan images of DNA. Explaining the procedure to locate DNA using an AFM and determining which spectroscopy method is more effective in moving DNA will hopefully provide a more efficient process of sequencing DNA using atomic force microscopy.

Contents

1 Introduction

1.1 DNA Sequencing

Sequencing deoxyribonucleic acid (DNA) allows for the discovery of the order of nucleotides in a gene. The power behind DNA sequencing has a wide range of applications stretching from further knowledge of human evolution to better understanding pathogenic variants [1]. DNA is composed of four nucleotides: adenine (A), thymine (T), guanine (G), and cytosine (C). Each nucleotide consists of a sugar attached to a phosphate and a nitrogenous base. Due to hydrogen bonding between nitrogenous bases, DNA is double stranded with base pairs that are either adenine and thymine or guanine and cytosine [2]. By understanding an organism's genetic code, the bounds of how sequencing DNA can benefit the biomedical industry and human longevity seems practically limitless. Therefore, there are constant innovations that are occurring to improve DNA sequencing methods.

1.2 Current Industry Method for Sequencing DNA

Although there have been several methods of sequencing DNA throughout the past few decades, the most relevant method for this research is the current technique of nanopore DNA sequencing created by Oxford Nanopore Technology. The current method of identifying nucleotides in a gene is facilitated by forcing a DNA to travel through a nanopore by the help of a protein and enzyme. DNA is separated into two single strands by an enzyme as it is forced through one of the nanopores that lies within an electro-resistant membrane. As each nanopore is connected to an electrode and a sensor chip, the current of the pore within the membrane can be measured [3]. Disruption of current occurs when DNA travels through a nanopore and because each nucleotide has a distinct electrical property, base calling can occur by electrical

characterization. Therefore, DNA sequencing can occur by measuring the current flow inside a nanopore as a single strand of DNA is travelling through the membrane.

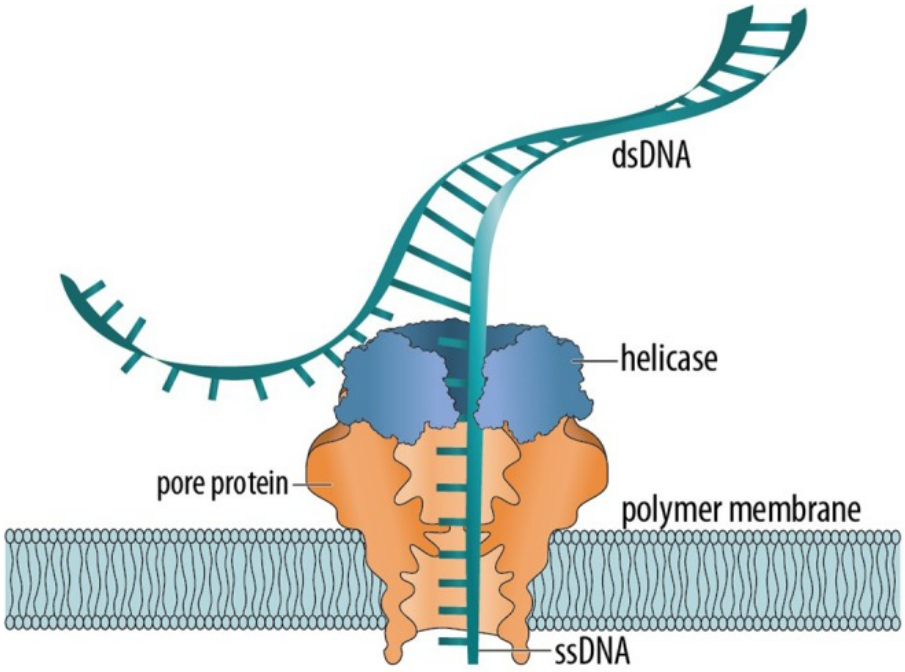

Figure 1: Schematic of DNA forced to travel through a nanopore and separate its double strand with the use of an enzyme to sequence its nucleotides [4].

Oxford Nanopore Technology has developed an efficient method to sequence DNA by electrically characterizing nucleotides that pass through a nanopore. In addition, a major strength to Oxford Nanopore Technology's sequencing method is that their manufactured devices that perform the sequencing procedure can be as small as a mobile phone with a relatively low cost of the device itself. However, there are still concerns about Oxford Nanopore's sequencing methodology that encourage further research and development of new DNA sequencing procedures. The focused concern about nanopore DNA sequencing is the shelf life of the biological materials used [5]. Due to using biomaterials to separate double helix DNA and force travel through a nanopore, a shelf life is mandatory when using proteins and enzymes. This leads

to customers who regularly sequence DNA to periodically purchase biological materials from Oxford Nanopore Technology to complete nanopore sequencing. Leading to increased costs for consumers and unproductive time while shipping of biomaterials occur.

1.3 Sequencing DNA using Atomic Force Microscopy

In order to maneuver away from using biological materials to sequence DNA, the Arkansas Micro & Nano Systems Lab has researched and developed a practice for sequencing DNA using atomic force microscopy. Atomic force microscopy (AFM) is a high-resolution imaging technique used to measure the topographical, electrical, optical, etc. properties of a sample surface [6]. AFM is an essential tool for imaging and cutting surfaces at the nanometer level. How a surface is measured at the nanometer level using atomic force microscopy is due to four main components: a cantilever tip, laser, photodiode, and feedback electronics.

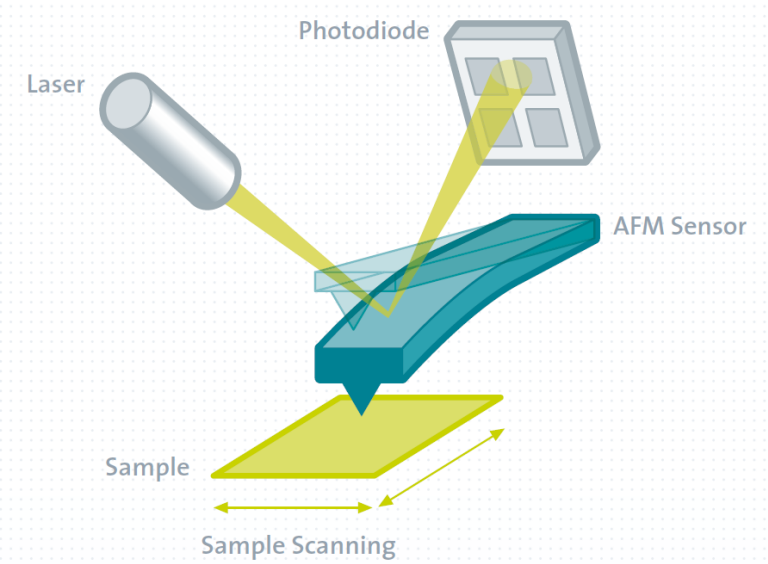

Figure 2: Illustration of how an AFM produces a topographic image using a cantilever tip.

As the cantilever tip constantly moves in the z-direction due to the roughness of a sample's surface, the deflection of the tip is tracked by a laser that is reflected from the back side of the cantilever to a photodiode. From the photodiode, information about the cantilever's coordinates

is stored and a feedback loop is enforced from the AFM's electronics so that a constant interaction can occur between the cantilever tip and the sample surface [6]. The culmination of each data point about the tip's coordinates leads to a topographic scan of the sample.

In regards of how the Arkansas Micro & Nano Systems Lab applied atomic force microscopy towards sequencing DNA, it was theorized that the cantilever tip from an AFM in contact with a sample surface could have an interaction with DNA that could lead to controlling the movement of DNA. Due to the adhesion force between a sample surface and a strand of DNA, an AFM instrument can image a surface and locate DNA using a cantilever tip without moving the strand. In addition, it was theorized that after locating DNA using AFM imaging techniques, using force spectroscopy in an AFM instrument can apply an electrostatic force between a cantilever tip and DNA strand that is greater than the adhesion force that is preventing the strand to not move. Discussing the electrostatic force between a cantilever tip and DNA strand has introduced the use of spectroscopy in an AFM instrument. AFM force spectroscopy is a single point measurement focused on a cantilever approaching, poking, and then withdrawing from a sample's surface [7]. The interaction between the cantilever and sample's surface produces a force versus tip sample separation graph by measuring the deflection of the tip versus movement from the piezo.

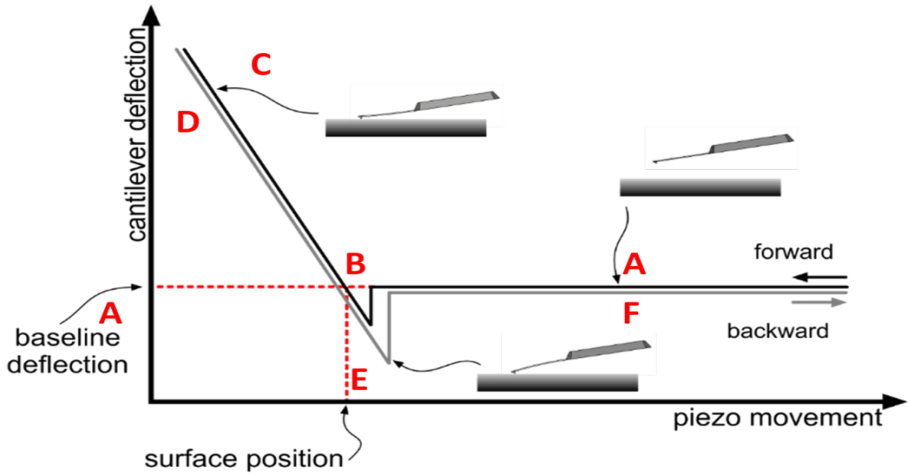

Figure 3: Example of what a force spectroscopy graph looks like and interaction between the tip and sample at each stage.

From steps A through F, it is shown in Figure 3 what a force versus height graph looks like as a cantilever tip approaches a sample, makes contact, and then withdraws from the surface. The advantage of using spectroscopy in an AFM instrument is that as the cantilever tip pokes a sample's surface containing DNA, the electrostatic force between the cantilever tip and DNA strand is greater than the adhesion force that is making the DNA stick to the surface. In turn, a bond or attachment occurs from the cantilever and DNA leading to movement of the DNA strand at the user's control. Verification that DNA has successfully attached to the cantilever is shown on a force versus height spectroscopy graph. Figures 4 and 5 are force spectroscopy graphs that were collected from Ph.D. student, Bo Ma, and M.S. student, Lucas Bartmann, who were both previously apart of the Arkansas Micro & Nano Systems Lab. Part D for Figures 4 and 5 show successful DNA attachment to a cantilever tip. The reason it was known by both graduate students, Bo Ma and Lucas Bartmann, that successful DNA attachment occurred can effectively be explained using parts C and D from Figure 5. The black line in the force spectroscopy graph shows the cantilever approaching the sample and then making contact whereas the grey line indicates the tip is pressed on the surface and then withdrawn to go back to its original position. As the tip withdraws from part C of Figure 5, a force is shown to make the height of the cantilever tip considerably oscillate. This considerable oscillation from the tip is due to the electrostatic interaction with DNA. Graph C indicates that the tip was not able to withdraw from the surface and move back to its initial position. Meaning that the DNA is too large for the tip to control. However, graph D from Figure 5 shows an interaction has occurred between the cantilever and strand of DNA as well as the tip was still able to withdraw to its original position. Thus, having complete control to move the attached DNA strand.

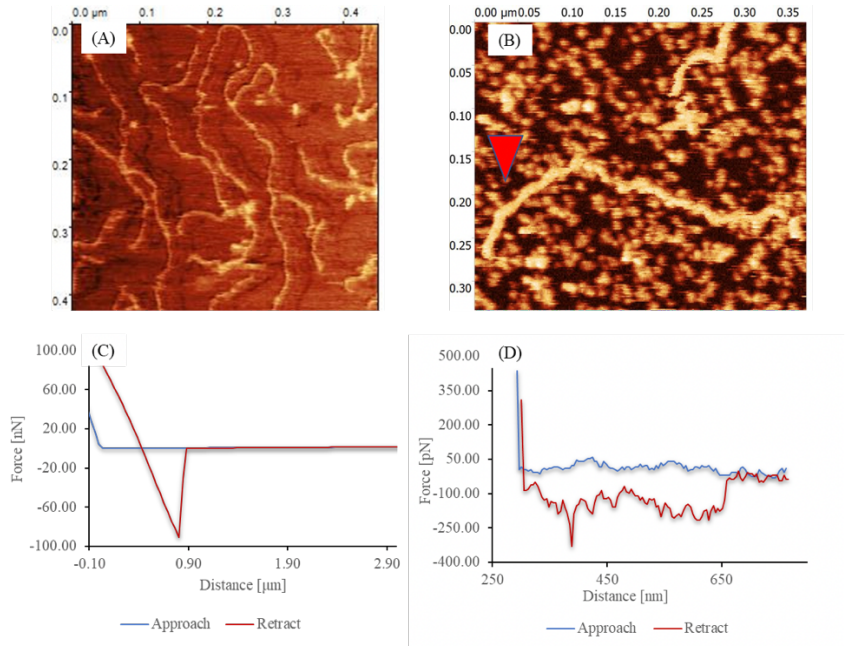

Figure 4: From Dr. Bo Ma's dissertation, (A) Shows a long, tangled DNA that was not suitable for DNA liftoff. (B) A DNA strand within 2.2 µm of length that is suitable for liftoff. (C) Force spectroscopy graph between a tip and mica surface. (D) Force spectroscopy graph of DNA lift off [8].

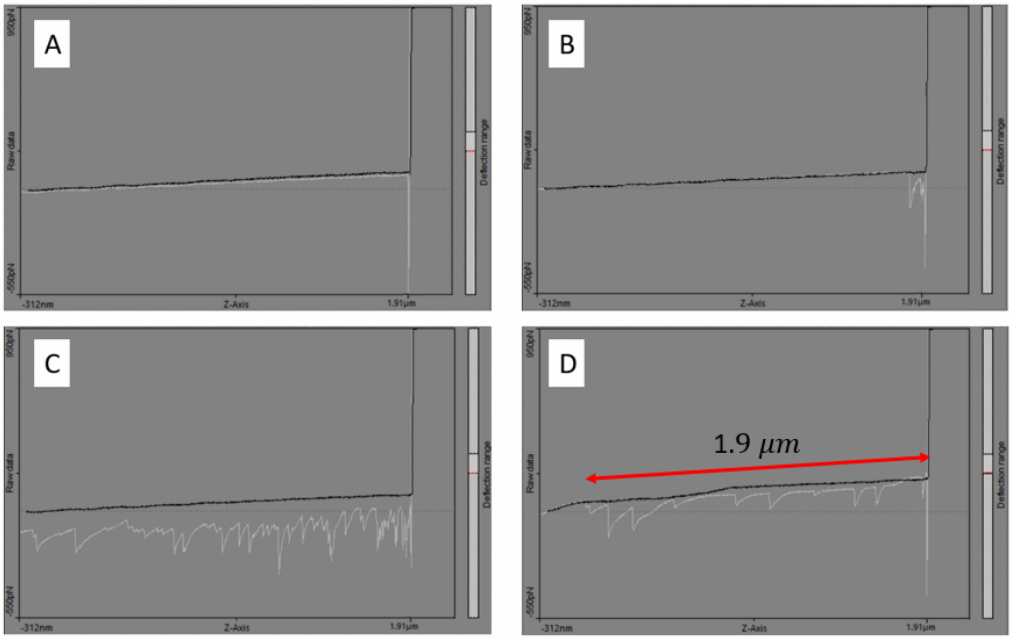

Figure 5: From Lucas Bartmann's thesis, (A) Force spectroscopy graph between a tip and mica surface (B) Spectroscopy graph of DNA on a surface but no attachment to the cantilever tip (C) Spectroscopy graph of DNA attachment to tip but too large of strand to successful lift-off. (D) Force spectroscopy graph of successful DNA lift off [9].

In order to complete DNA sequencing using atomic force microscopy, the DNA strand would then be moved over a sensor chip for electrical characterization. However, the scope of this thesis is to analyze the spectroscopy methods used by Dr. Bo Ma and Lucas Bartmann.

2 Research Objective

The scope of this report is to analyze and determine which spectroscopy method between Dr. Bo Ma and Lucas Bartmann was more effective in successfully lifting off DNA. After imaging and locating DNA, Dr. Bo Ma would align the cantilever tip as directly above the DNA as possible and then perform a single point measurement to lift off DNA. Dr. Bo Ma's method required the cantilever tip to be precisely above the DNA strand and a suitable speed from the tip as it withdraws from the surface so that DNA can attach to the cantilever and lift off. Nevertheless, Dr. Bo Ma was successful in moving DNA using single point force spectroscopy. On the other hand, Lucas Bartmann would locate DNA and then use the grid function found in Nanosurf AFM's to perform 64 spectroscopy measurements over a given square area. Lucas Bartmann's method led to much more trials of trying to lift off DNA leading to more chances of successful DNA lift off. However, it was acknowledged by Lucas that after using the grid function for a spectroscopy measurement, the cantilever tip was completely destroyed after each DNA lift off attempt. Therefore, the emphasis of this undergraduate thesis is to analyze the reliability and efficiency between Dr. Bo Ma and Lucas Bartmann's DNA liftoff methods.

3 Materials and Methods

This section explains what materials were used and how atomic force microscopy was utilized to make DNA images and force spectroscopy measurements.

3.1 Experimental Apparatus

DNA images were conducted on a Nanosurf CoreAFM in liquid mode scanning. As shown below, a mica surface containing DNA was placed in the center of a 3D modeled petri dish that had a fixed position for the mica. The petri dish, filled with liquid, was placed at the center of the piezo with prongs on both sides to keep the sample in one place. The cantilever tip was carefully placed above the mica and within the liquid.

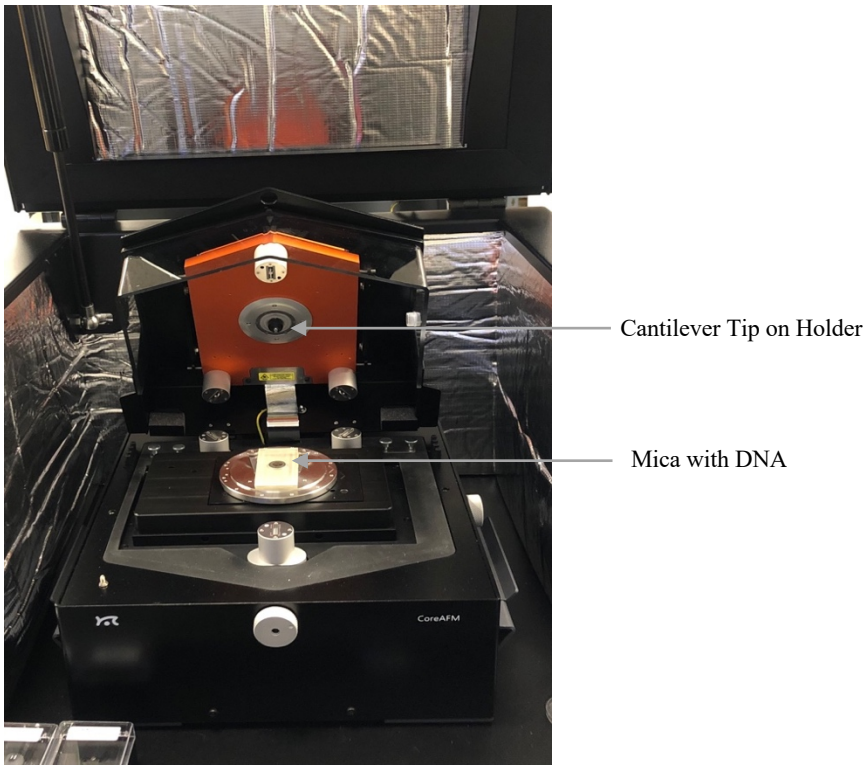

Figure 6: Experimental setup to scan DNA images using CoreAFM.

3.2 Preparation of Chemicals

It is imperative that as the CoreAFM conducts an image, DNA must not move during operation. The reason to make sure DNA is stationary during imaging mode is due to the fact that if there is not an adhesion force between the DNA strand and sample surface, the cantilever tip would continuously push DNA away. Resulting in a poor-quality image and increased difficulty to identify DNA strands. Fortunately, Dr. Patrick Heenan and Dr. Thomas Perkins

from the University of Colorado at Boulder published a paper in ACS Nano that provided a procedure to stick DNA on a mica surface for AFM liquid mode scanning. The procedure utilized three chemicals to create an adhesion force between DNA and a mica surface: $NiCl_2$, deposition buffer, and imaging buffer.

	Molar Units of Each Content	Weight of Each Content in Powder Form
$NiCl_2$	100 mM $NiCl_2$	0.4754 g $NiCl_2$
Deposition Buffer	10 mM $MgCl_2$ 25 mM KCl 10 mM $HEPES$ (pH 7.5)	0.1904 g $MgCl_2$ 0.3728 g KCl 0.4766 g $HEPES$ (pH 7.5)
Imaging Buffer	10 mM $NiCl_2$ 25 mM KCl 10 mM $HEPES$ (pH 7.5)	0.4754 g $NiCl_2$ 0.3728 g KCl 0.4766 g $HEPES$ (pH 7.5)

Table 1: Molar units of each content used to make $NiCl_2$, deposition buffer, and imaging buffer. In addition, weight of each content in powder form is shown for mixture with pure water.

The powder forms of each content that made up $NiCl_2$, deposition buffer, and imaging buffer were mixed with pure water. The powder form of $NiCl_2$ was mixed with 20 mL of pure water as the powder forms that made up deposition buffer and imaging buffer were each mixed with 200 mL of pure water. Having large solutions for each chemical led to multiple trials of DNA imaging in liquid mode without having to produce $NiCl_2$, deposition buffer, and imaging buffer before each DNA scan.

3.3 Deposition and Attachment of DNA to Mica Surface

The procedure below was published by Dr. Patrick Heenan and Dr. Thomas Perkins from the University of Colorado at Boulder of how to deposit and attach DNA to a mica surface.

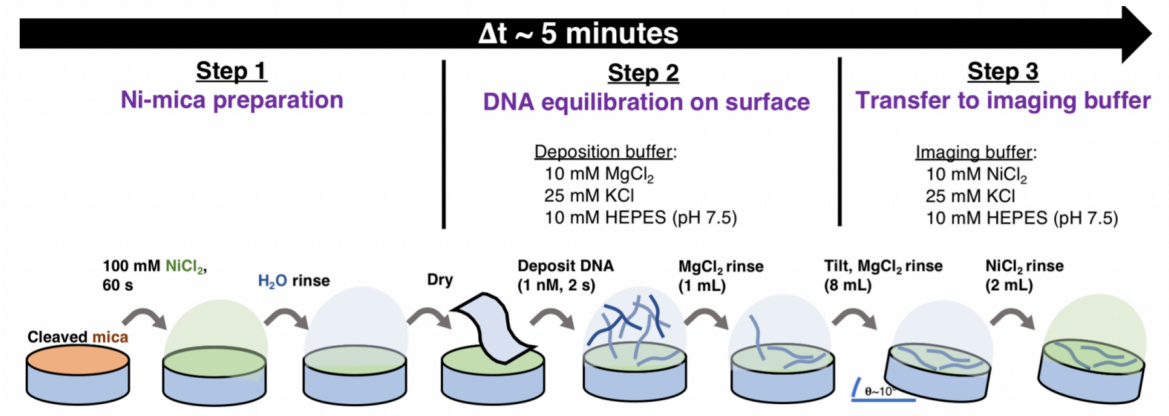

Figure 7: Procedure for depositing and attaching DNA to a mica surface [10].

1. Stick a clear mica to a thin (preferably circular) metal surface using double sided tape. With a razor blade, cut the edges of the mica so that each layer is separated from one another. With single sided tape, press on the top layer of the mica and then gently remove the tape. Applying and removing tape from the mica will eliminate any dust on the surface. A uniform, circular design should be stuck to the tape after removing the strip from the mica. It is imperative that dust is eliminated from the mica so continue to apply and remove tape until the uniform design is on the strip.
2. Drop 20 μL of 100 mM of $NiCl_2$ onto the mica surface using a pipette. Leave the liquid on the surface for 60 s.
3. Rinse 50 mL of pure water onto the mica surface to wash the $NiCl_2$ away.
4. Using filter paper, gently place the paper on the edge of the mica to dry the surface. Continue to place the filter paper around the edges of the mica so that the surface is dry. Blow N_2 on the mica to make sure that the surface is adequately dry.
5. Drop 20 μL of DNA, diluted with deposition buffer (100x dilution), onto the dry mica surface. Wait 2 s.
6. Rinse the surface with 1 mL of deposition using a pipette.

7. Apply a 10-15 degree tilt to the mica and then rinse the surface again with 8 mL of deposition buffer.
8. Move the mica surface back to level position and then apply 2 mL of imaging buffer. As the petri dish is filled with imaging buffer, gently place the mica into its dedicated position. The dedicated position to place the mica surface inside the petri dish is indicated by a circular indention similar to the shape of the mica.

3.4 Cantilever Tip and Parameters Used

The cantilever tip used for scanning in liquid mode was a qp-BioAC. Due to previous success from recent graduate student, Lucas Bartmann, of using a qp-BioAC cantilever tip on a Nanosurf FlexAFM, the decision was made to continue using the same type of cantilever. The qp-BioAC has 3 distinct tips that vary in size and resonant frequency.

Information for qp-BioAC			
Cantilever Tip	CB1	CB2	CB3
Thickness	$0.40 \pm 0.03\ \mu m$		
Length	$40 \pm 5\ \mu m$	$60 \pm 5\ \mu m$	$80 \pm 2\ \mu m$
Width	$20 \pm 2\ \mu m$	$25 \pm 2\ \mu m$	$30 \pm 2\ \mu m$
Res. Frequency	$65 - 115\ kHz$	$35 - 65 kHz$	$23 - 37\ kHz$
Force Constant	$0.15 - .55\ N/m$	$0.06 - .18\ N/m$	$0.03 - .09\ N/m$
Tip Height	$6 - 8\ \mu m$		

Table 2: Data about the qp-BioAC that had to be added into the CoreAFM software.

The shortest tip (CB1) was used to conduct DNA scans, however liquid scanning is not strictly limited to only using a qp-Bio tip. Different cantilevers tips that are suitable in liquid mode can also be used for liquid scanning.

After the cantilever was carefully lowered into the petri dish, the appropriate parameters were then selected. Liquid mode was always selected, forces selected were either dynamic or static force (further explanation is given in 3.5), and the appropriate cantilever was selected. If information about the cantilever is not included, an edit option is available to add data for a new tip. In terms of the force initially used for DNA scanning, make sure the AFM is in static force first.

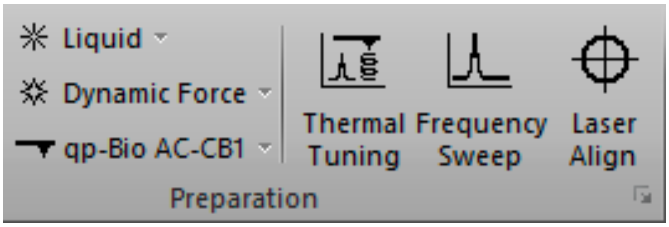

Figure 8: Parameters selected for DNA imaging.

3.5 Laser Alignment

Once the cantilever was carefully submerged in the petri dish and the appropriate parameters were selected, the laser was properly aligned. The laser had to be centered on the back end of the cantilever so that a strong signal communicating the cantilever's movement could be accurately sent to the photodiode. In addition, the reflection of the laser from the cantilever had to be directed towards the center of the photodiode. The laser alignment feature in addition to the video option on the CoreAFM made it possible to properly align the laser.

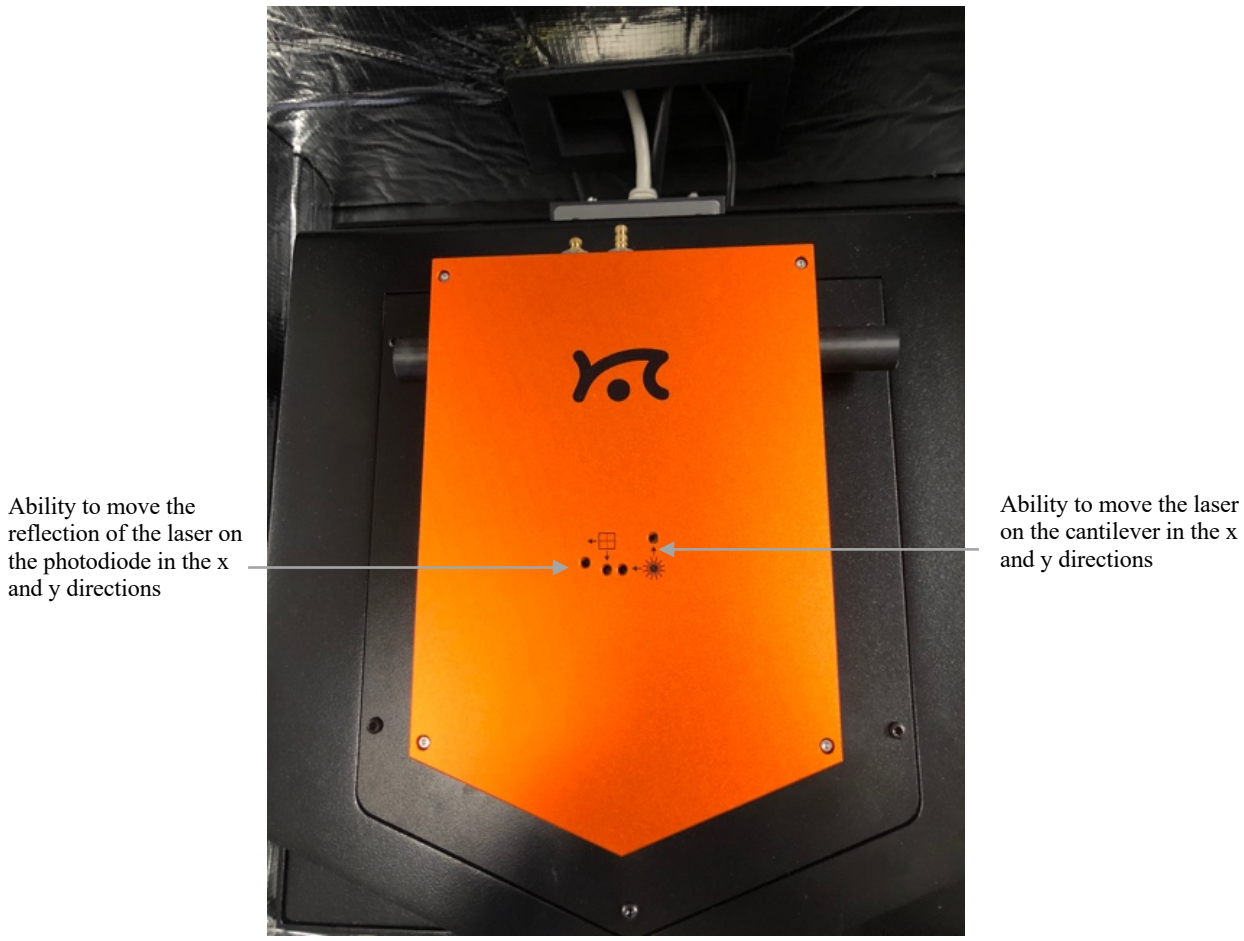

Figure 9: Screw holes that allowed the laser to move its direction on the cantilever and its reflection on the photodiode in the x and y directions.

The ability to move the laser's direction on the cantilever and photodiode in the x and y directions allowed for a strong signal to occur between the laser and photodiode. A relatively strong signal in liquid mode is around 50-60% with normal and lateral deflections below 5%.

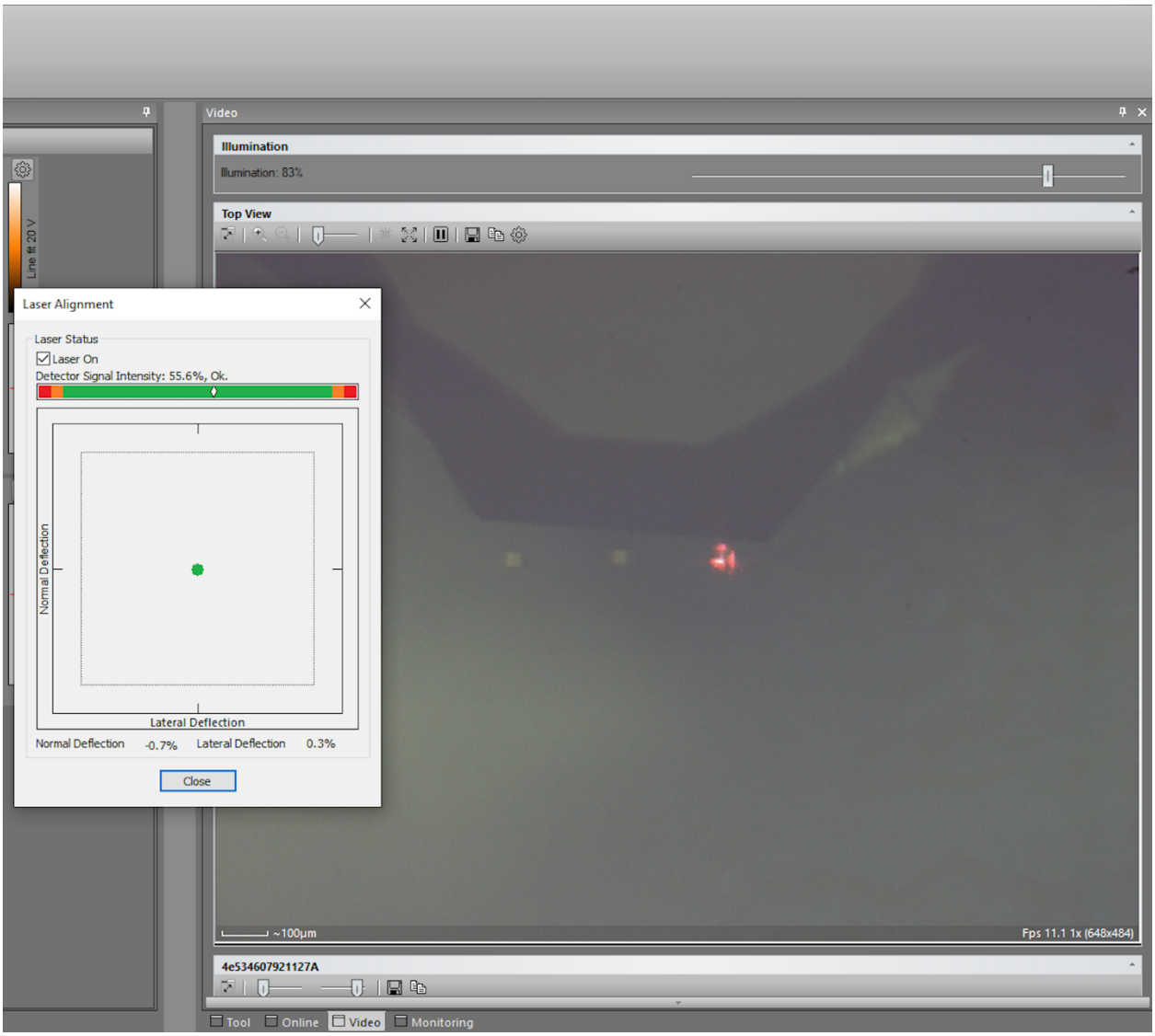

Figure 10: Example of a strong signal in liquid mode using the laser alignment and video features of the CoreAFM software.

3.6 Thermal Tuning and Frequency Sweep

Thermal tuning and frequency sweep are extremely important steps that measure the cantilever's resonant frequency and spring constant. Vibration effects occur from the cantilever as it makes contact with the sample's surface. If the cantilever's resonant frequency and spring constant are not accurately measured within the CoreAFM software, DNA scans will be extremely poor. With this in mind, once the laser was centered on the cantilever and photodiode

with a signal of around 50-60%, the next step towards scanning DNA was selecting thermal tuning. The reason it is important to use thermal tuning during AFM liquid operation is because it allows the user to get an estimate of what the resonant frequency and spring constant is for the cantilever being used. The user is able to create a range of what the resonant frequency should approximately be for the cantilever tip and selecting thermal tuning can then measure the vibration of the cantilever [11]. It is important to note that thermal tuning must be done in static mode.

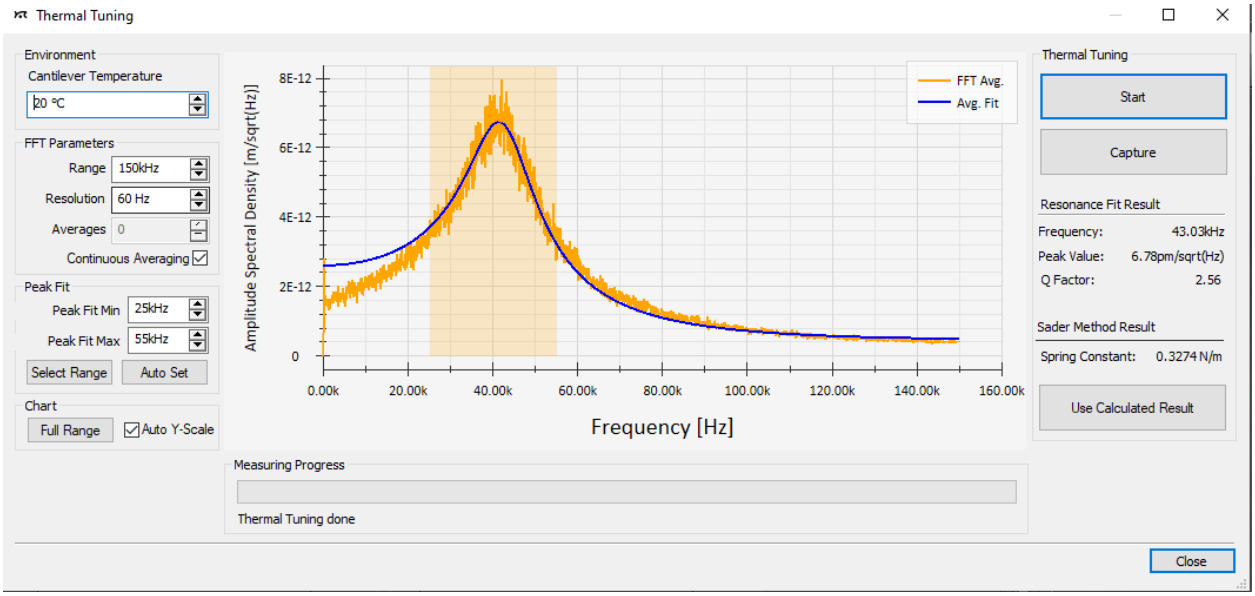

Figure 11: Thermal tuning example during liquid operation for a qp-BioAC 50 tip.

After an estimation of what the resonant frequency and spring constant should be for the cantilever being used through thermal tuning, a frequency sweep can then begin. Frequency sweep in liquid mode identifies multiple vibrational amplitudes that can be the resonant frequency of the cantilever. Sometimes, it may be difficult to decipher which peak represents the cantilever's resonant frequency and spring constant. Fortunately, through thermal tuning, an estimation of what the tip's resonant frequency and spring constant has already occurred. Therefore, the vibration frequency can then be moved to a peak that shares the same resonant

frequency as the thermal tuning results. It is important to note that frequency sweep only corresponds to a dynamic mode force. The tip applying a dynamic force, or tapping force, on the sample's surface during DNA scanning is utilized in order to improve imaging in a liquid environment and not damage DNA.

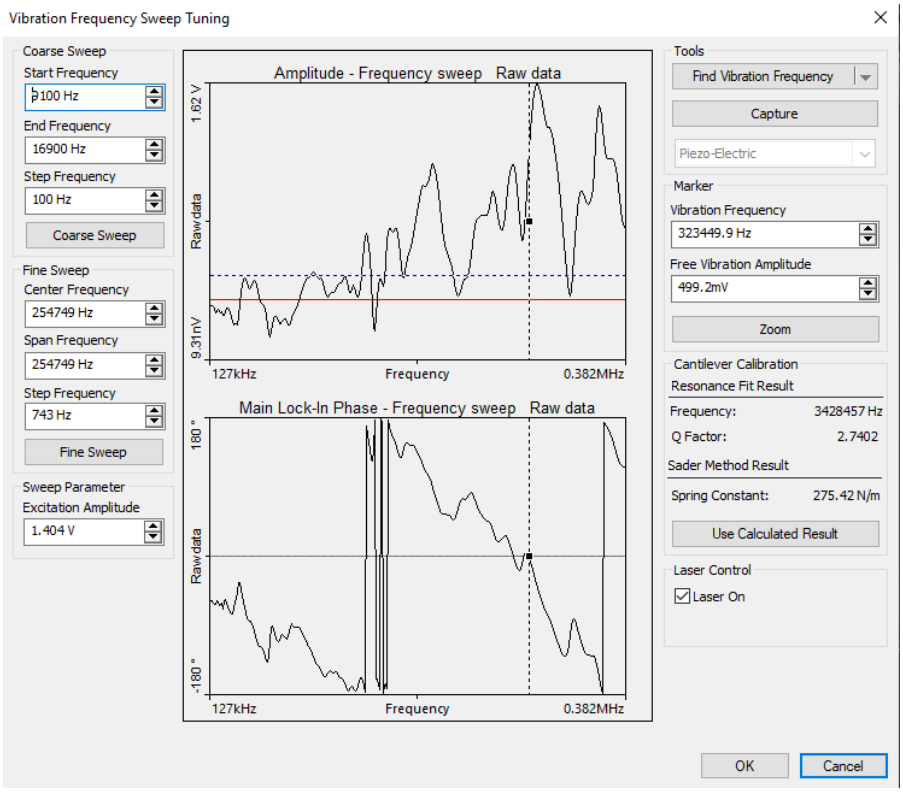

Figure 12: Frequency sweep example during liquid operation for a qp-BioAC tip.

3.7 Approaching the Sample and Scanning Surface

The cantilever tip is able to approach the sample, in dynamic mode force, once the resonant frequency and spring constant is selected. After the tip has appropriately approached the sample, the left tool bar can manipulate the parameters and z-controller of the scan. The parameters and z-controller should not have to be drastically changed if the procedures prior to the scan were adequately done. Meaning that sufficiently depositing the DNA onto the mica surface, having a laser signal of around 60%, and accurate measurements from thermal tuning and frequency

sweep can lead to locating DNA in AFM imaging operation. Although increasing time/line and points/line will lead to a longer duration for the DNA image to complete, it can give a better chance at locating DNA. For warning, AFM scanning in liquid mode takes a tremendous amount of experience and time. Make sure to work on troubleshooting skills in order to solve issues relatively quickly while scanning in a liquid environment.

4 Results and Discussion

4.1 DNA Imaging Using Atomic Force Microscopy

Several DNA images were obtained after performing the DNA deposition procedure and properly setting up the CoreAFM.

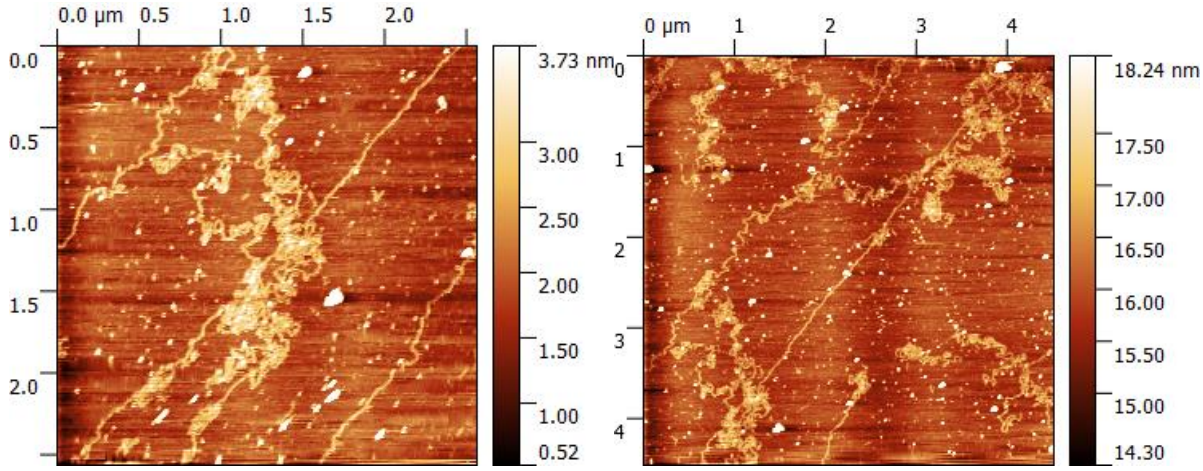

Figure 13: DNA images using a Nanosurf CoreAFM.

Gwydion software was used to process z-axis images from the Nanosurf CoreAFM. Utilizing the z-axis scan to identify DNA was beneficial to understand the height of DNA. Using figure 13 as an example, various heights can occur from scanning DNA. The image on the left of figure 12 is a 2.56 μm scan that contained DNA with a maximum height of 3.73 nm. Whereas the image on the right was a 4.51 μm scan with a maximum height of around 18.24 nm of DNA. What this implies is that various overlapping can occurring when depositing DNA for the reason that a double stranded DNA has a height of around 1-2 nm [12]. In

addition, bunching/clumping can occur to DNA. The image below shows DNA that is too bunched together for any type of liftoff to occur. It is preferred to move DNA that is linearized so less issues occur between the DNA strand and cantilever tip. Therefore, in order to have successful DNA liftoff, it is mandatory to stay away from any DNA that is either overlapping or bunched up.

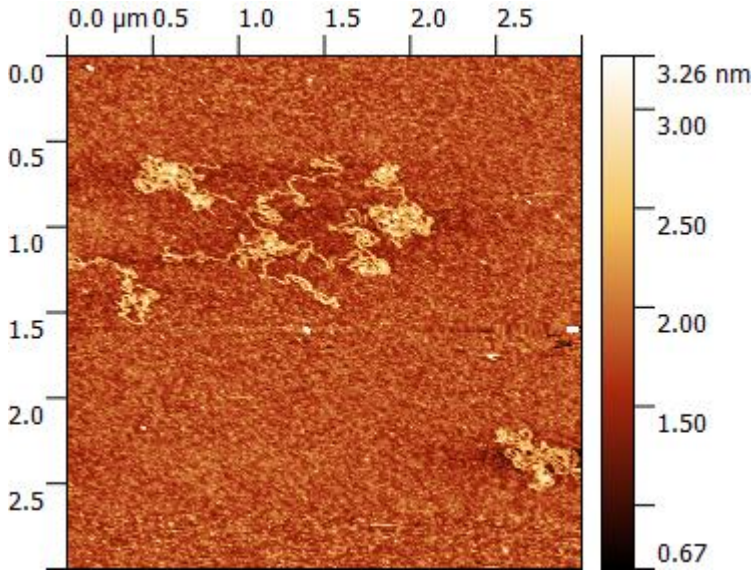

Figure 14: DNA that is too bunched up together to possibly move with force spectroscopy.

Below shows DNA strands that are more suitable for a cantilever to lift off (circled in black). Both images in figure 14 show DNA that are linearized and not overlapping. However, the length of the DNA can have a factor on the cantilevers ability to control the DNA's movement. It is strongly recommended that researchers who are trying to lift off DNA using atomic force microscopy use a Covaris g-tube to shorten DNA lengths with a centrifuge [13]. Previous students from the Arkansas Micro & Nano Systems lab have recommended using an rpm of 7200 for 60s to shorten DNA strands. However, using the recommended rpm and following the Covaris g-tube procedure led to DNA shards that were too small to identify under an atomic force microscope. Unfortunately, due to lack of time, a suitable centrifuge rpm was not

determined. But it is still recommended to shorten DNA using a Covaris g-tube for better chances of DNA lift off.

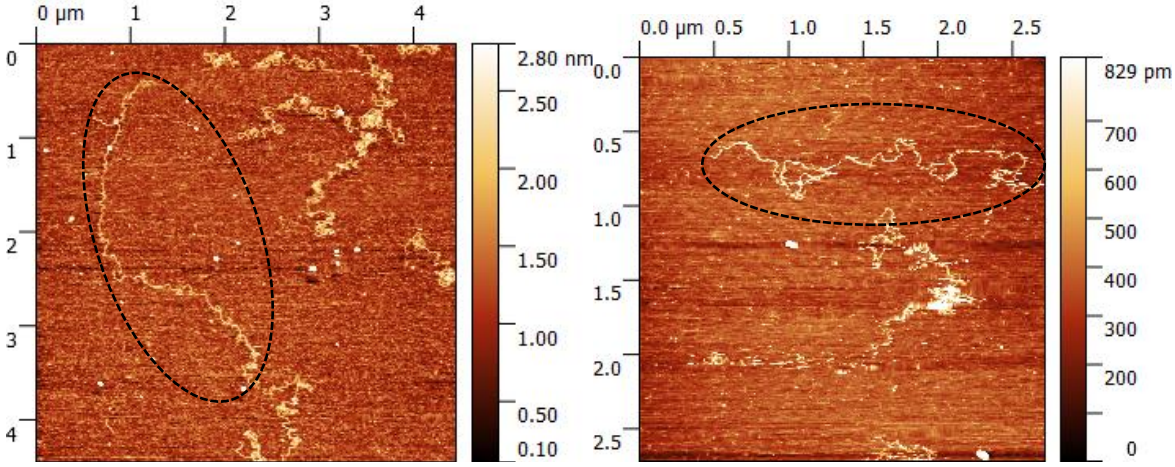

Figure 15: Images of DNA that are linearized and not overlapping.

4.2 DNA Nanomanipulation Using Atomic Force Microscopy

Unfortunately, there was no success at lifting DNA off the mica surface. Due to limited time, Lucas Bartmann's method was only evaluated using the grid function in force spectroscopy. A DNA strand was located and imaged at 1 micron. 64 force spectroscopy measurements were then conducted (8 spectroscopy point measurements for each of the 8 lines).

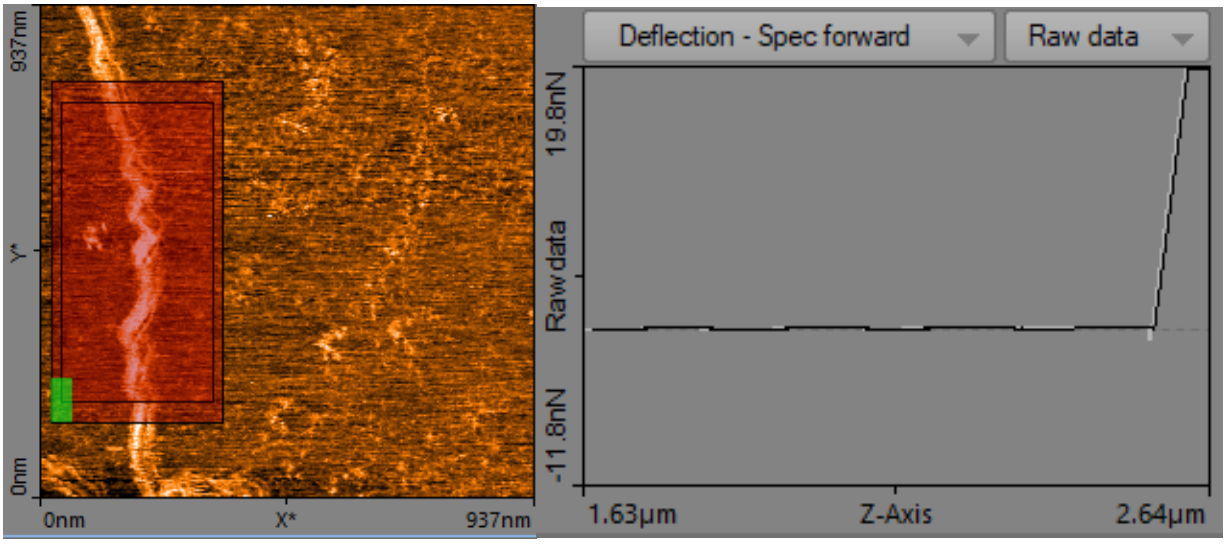

Figure 16: Grid function applied to a DNA scan and 1 of 64 force spectroscopy measurements.

The graph above indicates that the surface of the sample was barely poked for the reason that the deflection measurement (represented by the dip in the grey line plot) was relatively minimal. Therefore, a strong enough interaction between the DNA strand and cantilever tip could not occur with such a small force being applied by the cantilever. Due to limited time, no additional force spectroscopy measurements were conducted.

4.3 Discussion

It is evident that there wasn't enough progress to make an informed decision about if Dr. Bo Ma or Lucas Bartmann had a more effective spectroscopy method to lift off DNA. Due to lack of time before graduating, both approaches were not extensively evaluated enough. In addition, problems did arise during the semester that led to delays on research. First, nitrogen was not used for the first half of the semester to dry off the mica surface during the DNA deposition procedure. Only Kimwipes were used while nitrogen wasn't an option to dry off DNA. Because of this, it was noticed that DNA would not sufficiently stick to the mica surface and instead wash off. This was major factor for not locating DNA for the first half of the semester. Once nitrogen was obtained to dry off the mica surface, DNA sufficiently stuck to the surface and was located using AFM imaging. Also, it was noticed that the device that held the cantilever was broken for a few weeks during the 2022 spring semester. Malfunctions with the tip holder led to tremendous difficulty to approach the sample. The Arkansas Micro & Nano Systems lab decided to send back the tip holder to Nanosurf for repair and was given a different tip holder during the mean time. The tip holder given by Nanosurf during the latter half of the 2022 spring semester was effective to approach the sample and led to imaging DNA on a mica surface. Although both spectroscopy methods weren't evaluated enough, it is recommended to use the grid function to conduct several attempts at lifting DNA off. Using the grid function gives a higher probability of

lifting DNA off a mica surface rather than a single, point measurement. Lucas Bartmann did experience cantilever damage after using the spectroscopy grid function, but the amount of force distributed by the cantilever can be changed. For researchers who will attempt to lift DNA using force spectroscopy with an atomic force microscope, it's encouraged to evaluate the amount of nano newtons the cantilever tip provides so that a suitable force value can be used to liftoff DNA and prevent any damage to the cantilever. The methodology provided in this undergraduate thesis of how to stick DNA to a mica surface and scan an image using an atomic force microscope in liquid mode can be used for future evaluation of DNA liftoff. The deposition procedure and steps used within the Nanosurf software can be highly beneficial to control DNA movement and eventually sequence DNA. Therefore, although the ultimate goal to determine the best force spectroscopy method for DNA manipulation was not found, this undergraduate thesis can adequately prepare the next researcher to formulate the optimal DNA liftoff procedure.

5 Conclusion and Future Work

Overall, it is recommended to continue to determine which spectroscopy measurement should be prioritized when attempting to sense and manipulate DNA. During the process of identifying the best spectroscopy method, it was acknowledged that scanning in liquid mode with a CoreAFM and locating DNA takes a high level of experience and time. The reason it can be difficult to scan in liquid mode is because variables and issues can compound on one another to prevent a high-quality DNA scan from occurring. Meaning that multiple issues starting from attaching DNA on a mica surface to finding the correct resonant frequency of the cantilever can build on one another and lead to poor AFM images. Although there were not enough attempts using Dr. Bo Ma's method and Lucas Bartmann's procedure to confidently decipher which

spectroscopy approach is best, I am hopeful that my methodology from DNA sample deposition to liquid mode scanning can provide the next researcher to determine the answer.

It is strongly recommended when troubleshooting issues with the CoreAFM to isolate a variable. Meaning that every other step should be held constant so that the single variable can be evaluated on if it is negatively impacting the process to locate DNA in liquid mode scan. With this methodology written, it's very encouraged to back track and solve issues that might occur from scanning DNA. In my opinion, making sure a strong signal of around 60% from the laser alignment should be one of the first obstacles to overcome (as well as properly placing the cantilever tip in the holder). Once a laser alignment is resolved, determining the proper resonant frequency and spring constant of the cantilever tip should be the next obstacle to resolve. Becoming advanced with thermal tuning and frequency sweep measurements will facilitate in finding the proper data for the selected cantilever tip so proper feedback occurs to the photodiode. Lastly, making sure the deposition procedure actually attaches DNA to the mica surface should be the last problem to overcome. Even when an AFM image is complete, sometimes the scans will not contain DNA. Therefore, it is imperative that the DNA properly attaches to the mica surface so that scanning throughout the mica can result with finding DNA. Effective DNA attachment can occur by evaluating and being careful with each step of the deposition procedure.

In terms of future work, further analyzation of Dr. Bo Ma and Lucas Bartmann's spectroscopy methods should be continued to make an educated evaluation on which procedure is the most reliable and efficient. Future work should be focused on using the spectroscopy mode of a CoreAFM as controlling DNA with a cantilever tip is still novel. It is strongly recommended

to use this methodology and have enough experience and time with AFM liquid scanning to really dive into AFM force spectroscopy.

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