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Characterization and Manipulation of Double-Stranded DNA using Atomic Force Microscopy

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Characterization and Manipulation of Double-Stranded DNA using Atomic Force Microscopy

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ABSTRACT

An atomic force microscope (AFM) is used to scan high-resolution images on the nano scale. The lambda DNA used for this project are 48,502 base pairs in length and are double-stranded. This project utilizes the NanoSurf Core AFM in order to characterize and manipulate strands of lambda DNA which have been deposited on a mica surface. The deposition process of the DNA on the mica surface was developed by the National Institute of Standards and Technology and the University of Colorado - Boulder. The AFM is used in imaging mode to scan the mica surface to locate the DNA. When a linearized strand of DNA is identified, the AFM is then switched to spectroscopy mode which allows the user to attract the DNA strand to the cantilever tip. This electrostatic force between the DNA strand and cantilever tip enables the DNA to be moved to a location of the user's choice. In the case of this research, the DNA needs to be moved approximately 1 millimeter into a graphene nanoribbon (GNR) sensor which will record the electromagnetic force exuded by the base pairs as they pass through the sensor. Each base pair corresponds to a different signal and can therefore be identified and sequenced by examining this signal. Previously, the NanoSurf Core AFM has been used to scan and lift off the DNA from the mica surface, however it has not been used to manipulate the DNA into the GNR sensor. The identified, successful process of lifting off the DNA was inefficient and needs to be optimized. After successful sequencing of the double-stranded lambda DNA, the next step will be to use the AFM to sequence single-stranded DNA. The objective of this research is to successfully imagine double-stranded Lambda and prove that spectroscopy can be used to lift the DNA strand off the prepared surface.

Contents

1 Introduction

1.1 Sequencing of DNA

Deoxyribonucleic Acid (DNA) define the characteristics of every organism on earth, from plants to animals to humans. With today's innovative technologies, we have been able to sequence, alter, and even duplicate DNA sequences. DNA sequencing allows us to identify characteristics and irregularities in the base pairs adenine (A), cytosine (C), thymine (T), and guanine (G) [1] that can be associated with various diseases. Since the development of DNA sequencing techniques, technology has advanced to increase sequencing speed and decrease cost [2]. Ultimately, DNA sequencing can be used to prolong life by catching predispositions to diseases and illnesses as noted in genetics. This theory can be applied to human, animal, and plant life.

1.2 Proven Industry Standard of DNA Sequencing

DNA sequencing was developed by Fred Sanger in 1977. The Sanger method used large, high powered computers to sequence DNA using fluorophore excitation [3]. This method was extremely accurate but required a large processing time. The Sanger method was later improved by a company called Illumina, who developed a way to massively parallel the fluorophore excitation process to drastically shorten the sequencing time [4]. However, the use of large equipment made it so sequencing was still expensive and non-mobile. Currently, Oxford Nanopore Technologies produces a small, portable DNA sequencing device [5]. These drives contain small holes called nanopores embedded within a membrane which give off an electrical signal as the negatively charged DNA is pulled through the pore by the current applied across the membrane. A microchip in the membrane measures the electrical signal of the DNA strand as it

flows through the corresponding channel. The signal can then be analyzed to determine each of the base pairs and therefore detect any patterns or irregularities within the DNA sequence.

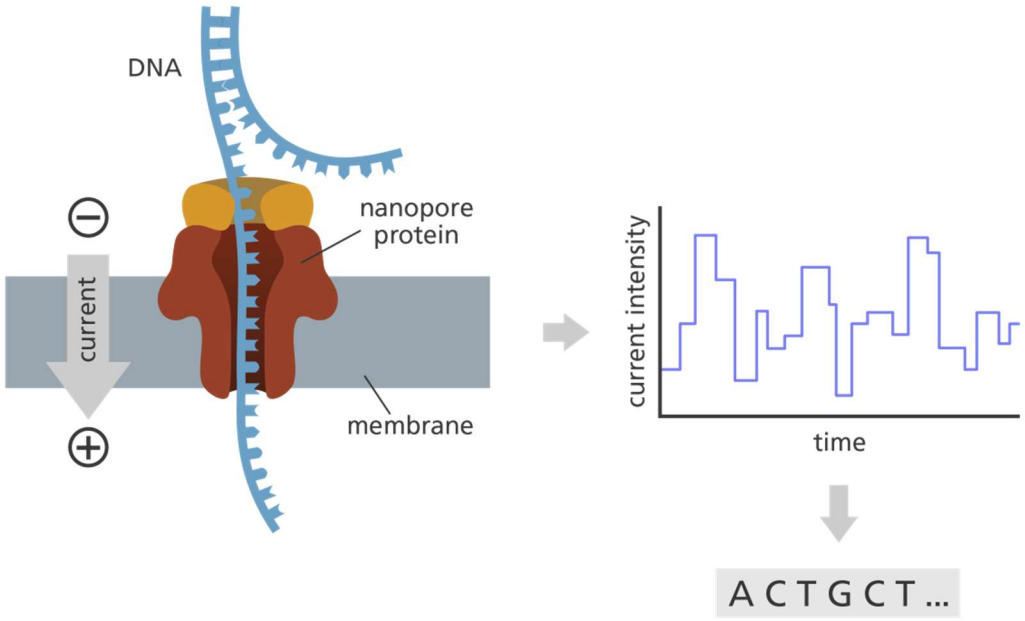


Figure 1: Double stranded DNA moving through a nanopore protein and eliciting an electrical current that can be analyzed to determine bases.

Oxford Nanopore has created the large-scale production of bespoke, proprietary pore-forming proteins to create pores in membranes. However, this use of proteins poses the issue of a limited shelf life due to the eventual degradation of the biological material used to sequence the DNA.

1.3 Sequencing DNA using an Atomic Force Microscope

Utilization of an atomic force microscope (AFM) allows for the characterization and manipulation of a single DNA strand so that it can be processed in a graphene nanoribbon (GNR) sensor. This sensor does not use biological proteins to sequence the DNA, instead relying on the

electromagnetic properties of the DNA base pairs and their interactions with the graphene to identify the base pairs.

In order to create a 3D image of a prepared sample, the AFM probe is vibrated above the surface. As the positioning of the laser deflection into the photodiode adjusts due to surface features, the change in positioning is analyzed to create an image of the surface.

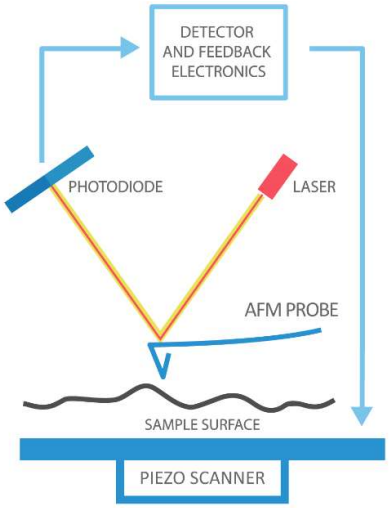


Figure 2: Interaction between AFM probe, surface, laser, and photodiode [6]

After an image is obtained of the sample surface, an ideal DNA strand can be identified and spectroscopy attempted. The spectroscopy mode approaches and presses into the surface, attaching the DNA to the AFM tip so it can then be dragged into the GNR sensor.

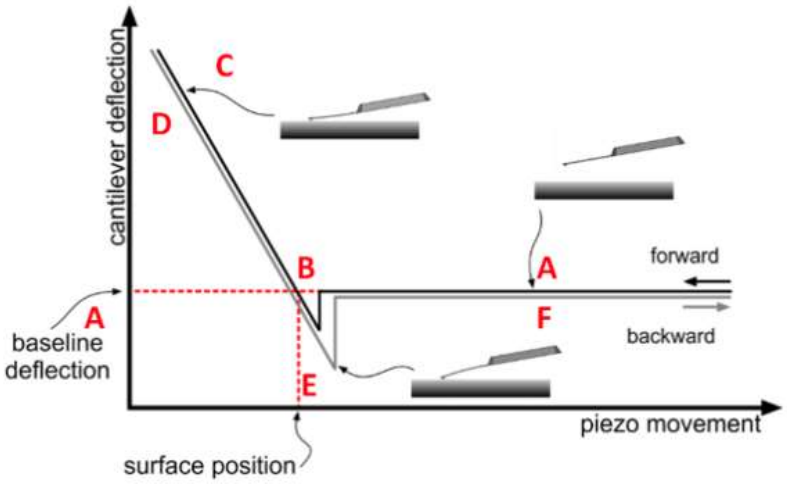


Figure 3: Core AFM Spectroscopy mode process (A) Cantilever approaches the surface. (B) Snap-in (C) Tip and sample are in contact and bends up upon further movement of the z-piezo. (D) Withdrawal (E) Pull-out: tip gets "stuck" in an adhesive dip (F) The cantilever has returned to its unperturbed state while the z-piezo further increases the tip sample distance.

Previously, the Core AFM has been proven able to successfully pick up a DNA strand. Characterization using the AFM allows for the location of specific DNA strands, while liftoff enables the DNA to be dragged into interaction with the GNR sensor.

2 Research Objective

In 2019, Lukas Bartman used the AFM Agilent with MAC-240 tips to scan and manipulate a single DNA strand [7]. This AFM utilized a magnetic field in order to vibrate the cantilever. However, after this achievement, the experimentation was moved to the Nanosurf Core AFM with qp-BioAC tips which uses resonant frequency to produce vibrations in the cantilever. Dr. Bartman was able to replicate his results on the Nanosurf Core AFM, however subsequent researchers have not been able to. Moving forward, the goal is to not only replicate Dr. Bartman's result but also improve on the method that was being used to pick up the DNA

and move it a distance. The NanoSurf Core AFM has been proven able to scan and pick up a DNA strand. However, only the scanning results have been replicated by other researchers and the liftoff technique used was inefficient. Also, for the DNA to be introduced to the GNR sensor it is necessary to drag the DNA strand a distance of 1 millimeter. This has previously not been accomplished using the NanoSurf Core AFM.

3 Materials and Methods

This section describes the necessary equipment and procedures required to create a DNA sample to be analyzed using the AFM.

3.1 Experimental Apparatus

The Core AFM is produced by NanoSurf and was the AFM model used throughout the following research. This equipment has a max imaging scan size of 100 µm x 100 µm x 20 µm [8]. The cantilever holder is inserted into the middle of the inside of the lid and lowered down above a sample. In this case, the sample can be seen on the stage of the AFM being held by a petri dish. The dish is full of imaging buffer and therefore before lowering the lid of the AFM, drops of imaging buffer must be applied to the cantilever holder until they envelop the tip in the holder. This is necessary when switching from air mode to liquid mode, as otherwise bubbles of air can form on the tip, interfering with the signal strength of the laser and resonance frequency and therefore decreasing image quality. A screw on the outside back of the stage isolation screen can be used to adjust image quality and screws in the front and right side of the AFM box can be used to adjust positioning of the cantilever over the sample.

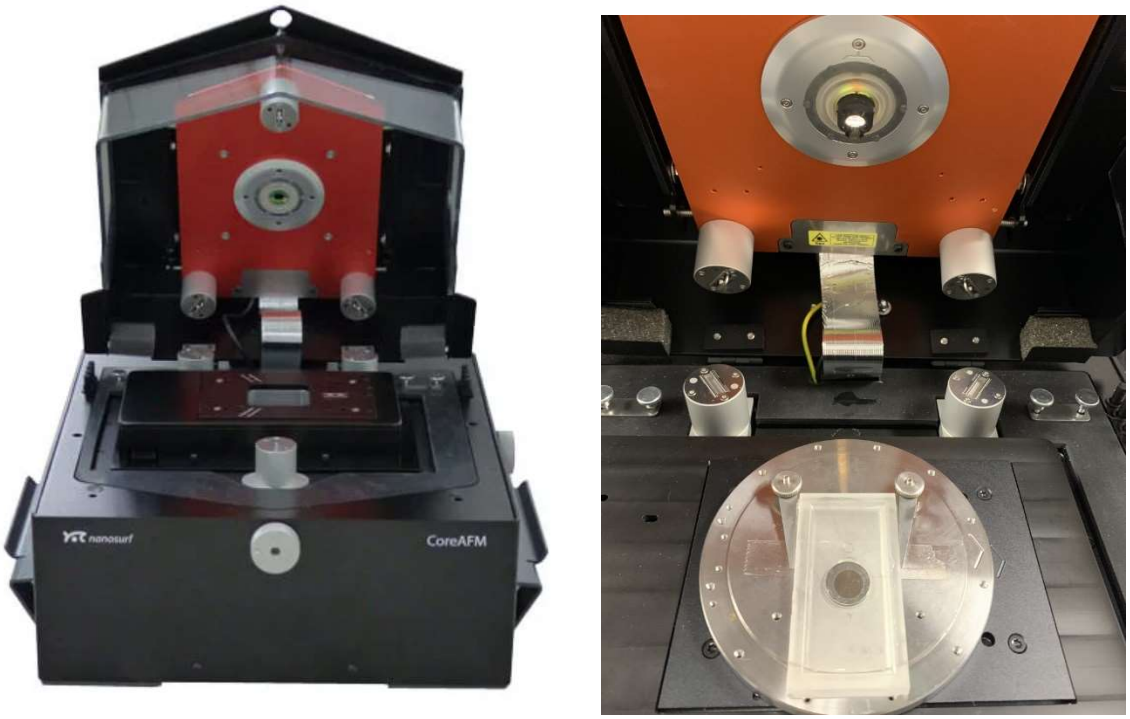


Figure 4: Open front view of Nanosurf CoreAFM

3.2 Preparation of Chemicals

To prepare the surface to be scanned in the AFM, it is necessary to follow a concise sequence of steps. The surface that is prepared using these steps and on which the DNA will be placed is mica. Mica is used due to its flat characteristic; its flatness and cleanliness is ensured each trial through cleaving. However, mica is negatively charged which requires binding agents to successfully attach the DNA to the surface [9]. The three chemical compounds that have been utilized to accomplish this are a nickel chloride solution, imaging buffer, and deposition buffer. Among these three compounds the chemicals used are $NiCl_2$, $MgCl_2$, KCl, and HEPES, each dissolved into distilled water to form solutions. The dilution ratio of 99 µL deposition buffer to 1 µL lambda DNA at a concentration of 500 µg/mL has been proven to produce more consistent results in the locating of DNA on the mica surface compared to a ratio of 119 µL deposition buffer to 1 µL lambda DNA.

The nickel chloride solution was 100 mM and was created by adding 0.4754 g of $NiCl_2$ to 20 mL of distilled water. To create the deposition buffer, 0.1904 g of $MgCl_2$, 0.3728 g KCl, and 0.4766 g of HEPES were mixed in 200 mL of distilled water. This created a solution with molecular weights of 10mM $MgCl_2$, 25 mM KCl, and 10 mM HEPES (7.5 pH). Similarly, the imaging buffer consisted of 0.4754 g of $NiCl_2$, 0.3728 g KCl, and 0.4766 g of HEPES dissolved in 200 mL of distilled water. The molecular weights of the imaging buffer were 10 mM $NiCl_2$, 25 mM KCl, and 10 mM HEPES (7.5 pH). Having 200 mL of each buffer allowed for multiple sample trials before creating more was necessary.

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 on how to deposit and attach DNA to a mica surface [9]. Step 5 has been modified from an original value of 20 µL of DNA in deposition buffer to 40 µL of DNA in buffer solution. This change allowed for faster and more consistent location of DNA on the mica surface when imaging using the AFM. However, this change also can result in DNA strands being stacked upon each other or coiled amongst themselves, not ideal for performing spectroscopy.

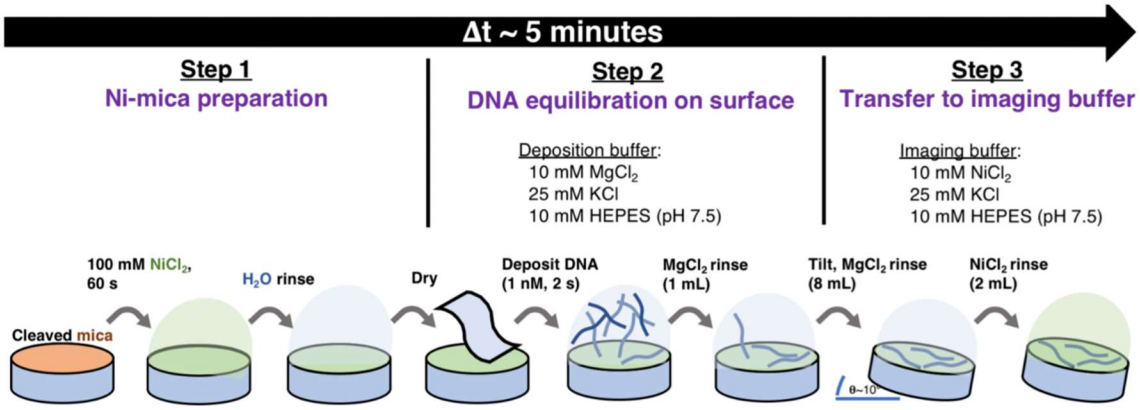


Figure 5: Procedure for depositing and attaching DNA to a mica surface

1. Apply a piece of double-sided tape to the center of a small metal disc.
2. Attach a clear piece of mica to the disc by pressing it into the double-sided tape.
3. With a razor blade, cut the exposed double-sided tape away from the metal disk.
4. 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 remain on 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.
5. Fill the petri dish with imaging buffer (approximately 10 mL).
6. Using tweezers to hold the metal disk midair, drop 20 μL of 100 mM of $NiCl_2$ onto the mica surface using a pipette. Allow the liquid to sit on the surface for 60 s.
7. Rinse the mica with 50 mL of distilled water to wash the $NiCl_2$ away.
8. Using filter paper, gently place the paper on the edge of the mica to absorb the water. Absorb as much liquid as possible from the surface of the mica by moving the filter paper around the edges, without touching the paper to the surface itself. When no more liquid can be removed using the filter paper, blow N_2 on the mica to fully dry any remaining water on the mica surface.
9. Drop 40 μL of DNA, diluted with deposition buffer (100x dilution), onto the dry mica surface. Wait 2 s.
10. Use a pipette of 1 mL deposition buffer to rinse the surface.
11. Apply a 10-15 degree tilt to the mica and then rinse the surface again with 8 mL of deposition buffer.
12. Move the mica surface back to level position and then apply 2 mL of imaging buffer.

13. Gently place the mica into the petri dish full of imaging buffer. The dedicated position to place the metal disk inside the petri dish is indicated by a circular indention similar to the shape of the mica.

The mica attached to the metal disk can be reused for many samples, until the mica becomes too thin to form a complete circle on the metal disk.

3.4 Cantilever Tip and Parameters Used

DNA characterization occurs in the imaging mode of the AFM. This process is completed using the qp-BioAC cantilever tip in dynamic mode. Several tip types have been used in conjunction with several tip holders in order to determine the optimal pairing of cantilever holder and tip. For example, the ARR-CONTR tip was used with the CantiClip Grooveless holder due to some previous success with the tip and imaging and spectroscopy were attempted. However, in the end the qp-BioAC was used in conjunction with the FlexAFM by Lukas Bartmann and produced successful imaging and spectroscopy results so it was carried over to the Core AFM and was used to produce the following results shown in this paper.

The qp-BioAC tip is utilized in this scenario due to its ability to function in both liquid and dynamic modes. Liquid mode is necessary as the DNA is suspended in a buffer solution and placed in a small tray filled with imaging buffer. During experimentation, the CantiClip Liquid/Air tip holder was used in conjunction with the qp-BioAC tip, due to the fact that this cantilever holder was compatible with both liquid and air modes. Dynamic mode allows the cantilever tip to create an image of the DNA on the mica surface without moving it, a possible effect of imaging in static mode, which would alter its location and make spectroscopy results

inconsistent. The qp-BioAC has three separate tips, with varying resonant frequencies and force constants.

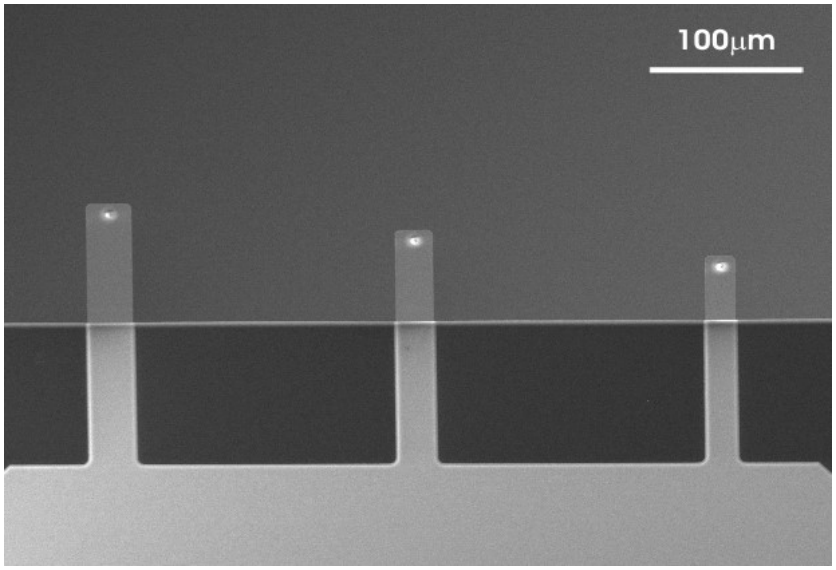


Figure 6: The three tips of the qp-BioAC, with tip number 3 on the left, tip 2 in the middle, and tip 1 on the right [10].

	CB1	CB2	CB3
Length	40 ± 5 µm	60 ± 5 µm	80 ± 5 µm
Width	20 ± 2 µm	25 ± 2 µm	30 ± 2 µm
Resonance Frequency	65 - 115 kHz	35 - 65 kHz	23 - 37 kHz
Force Constant	0.15 - 0.55 N/m	0.06 - 0.18 N/m	0.03 – 0.09 N/m
Thickness	0.40 ± 0.03 µm		
Tip Height	6 - 8 µm		
Resistivity	0.01 - 0.02 Ωcm		
Application	Contact mode / non-contact mode		

Figure 7: Manufacturer specifications of the qp-BioAC AFM probe tips [10]

Having multiple tips allows for one to be used for imaging and another to be used for spectroscopy. For this research, qp-BioAC tip CB1 was used for both imaging and spectroscopy. Tip 1 of the qp-Bio AC operates within a frequency of 32-39 kHz so in liquid mode so each trial required scanning to ensure the AFM was registering within such frequency range and using the

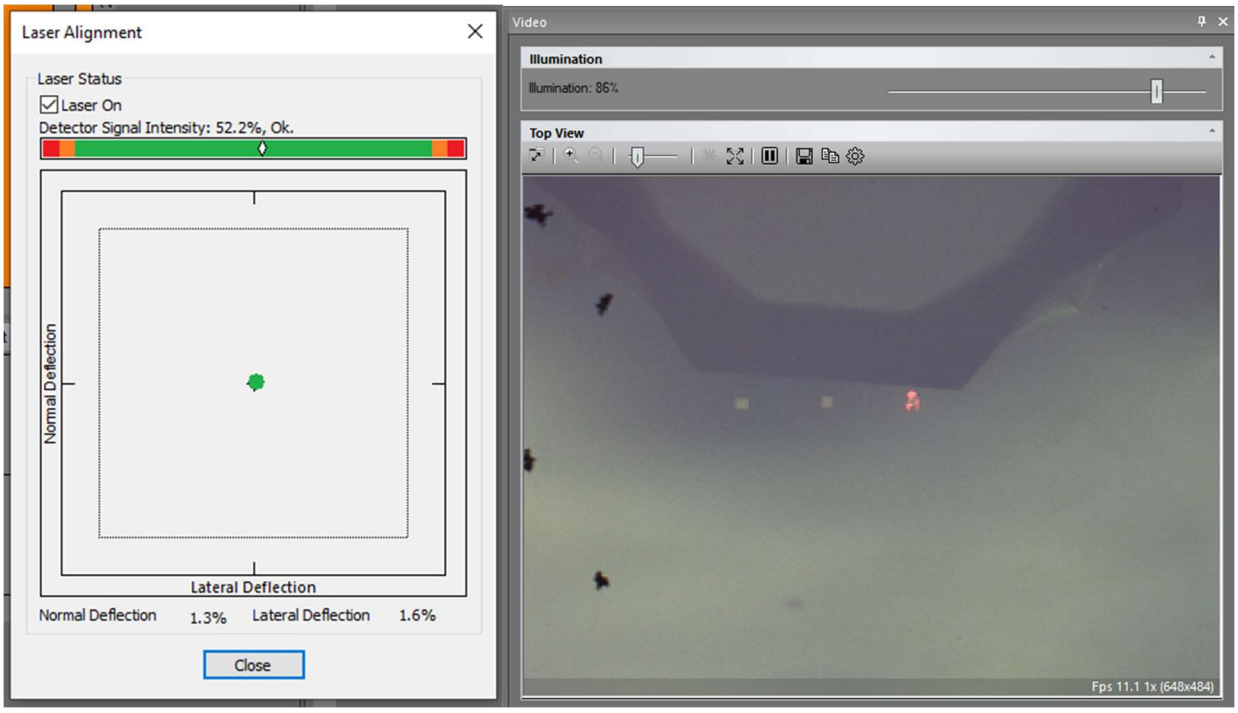
software to set the spring constant. From there, trials and experience were necessary to determine the correct AFM imaging settings to produce quality scans of DNA.

3.5 Laser Alignment

With the cantilever tip loaded in the cantilever holder, the holder is mounted magnetically to the AFM. Closing the lid and opening the Laser Align feature in the software allows for the detection of a strong signal. Using a small screwdriver, the laser and photodiode are adjusted using the holes in the top of the AFM until the laser can be seen on the desired cantilever tip (CB1) and the photodiode has a strong signal. This signal percent should be approximately 30% for the qp-BioAC tip CB1 in air mode. If there is difficulty locating the laser, the brightness can be adjusted lower which may make it easier to detect its location.

With the laser aligned successfully in air mode, the AFM software can be switched into liquid mode. The petri dish with the prepared mica sample can then be loaded onto the stage. At this point, to prevent air bubbles from forming and to lessen the impact of the liquid with the delicate cantilever tip, it is necessary to use a pipette to carefully place drops of the imaging buffer from the petri dish onto the cantilever tip. The tip is ready to be lowered into the petri dish when the tip is fully enveloped in liquid. Ensure the software is at Home before lowering the lid of the AFM closed. As you carefully and slowly lower the lid, make sure the cantilever holder is positioned above the mica by observing the positioning of the laser as it approaches the surface of the petri dish.

Due to the refraction of the laser in the liquid, the laser alignment will need to be readjusted. As it was already aligned, it will not be far off from the last positioning and is therefore easier to align to a good signal. Reopening the Laser Align feature, the screwdriver is used again to achieve a higher signal, generally approximately 60%.



Figures 8 & 9: Alignment of laser onto qp-BioAC tip 1 in liquid mode

3.6 Thermal Tuning and Frequency Sweep

Thermal tuning can only be performed in static mode. This software feature tests the cantilever tip's force constant and spring constant, averaging them over a range of frequencies. While the dry mode values for each tip of the qp-BioAC can be found on the box they came and are stored in, the values must be adjusted for liquid. To account for the dampening effects of liquid on the resonance frequency of the tip, the expected value range can be approximately divided by 3. For CB1, the air mode manufacturing values given are 65-115 kHz but in liquid mode the value of the resonance frequency registered by the software is generally 28-39 kHz (~1/3 air mode). After allowing the thermal tuning to run for approximately 10 seconds, stop it. You can then set the spring constant and make a note of the identified frequency.

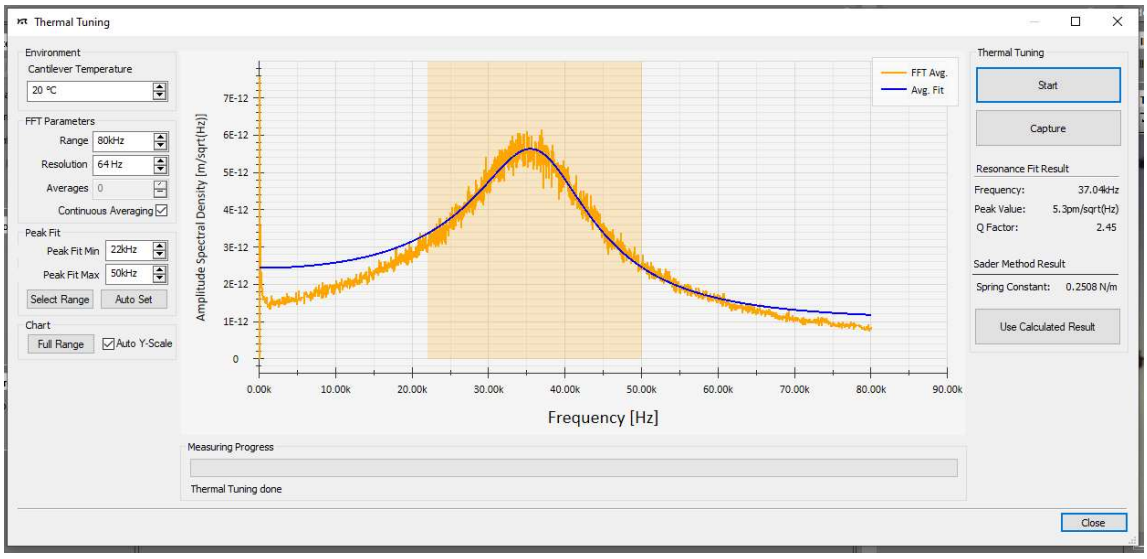


Figure 10: Thermal Tuning screen of qp-BioAC in liquid mode

A frequency sweep can be run using the Frequency Sweep feature in the software, but it also occurs automatically when the Approach button is activated. When the frequency sweep runs, the goal is to achieve a frequency close to that of the thermal tuning. If the initial frequency value is not close to the thermal tuning value, move the cure intersection to a peak in the curve closest to the thermal tuning frequency and run the frequency sweep again.

3.7 Sample Approach and Surface Imaging

When the cantilever holder is at home, it will be very far from the surface of the mica. To shorten the approach period, click and hold Advance until the surface features begin to appear in the video monitor. At this point, select Approach and the software will automatically begin a frequency sweep. Adjust the frequency sweep if needed as described in section 3.6.

Lambda DNA has a height in the z axis of approximately 2 nm so in each scan it was necessary to ensure that any feature registering that appeared to be DNA was in fact approximately 2 nm tall.

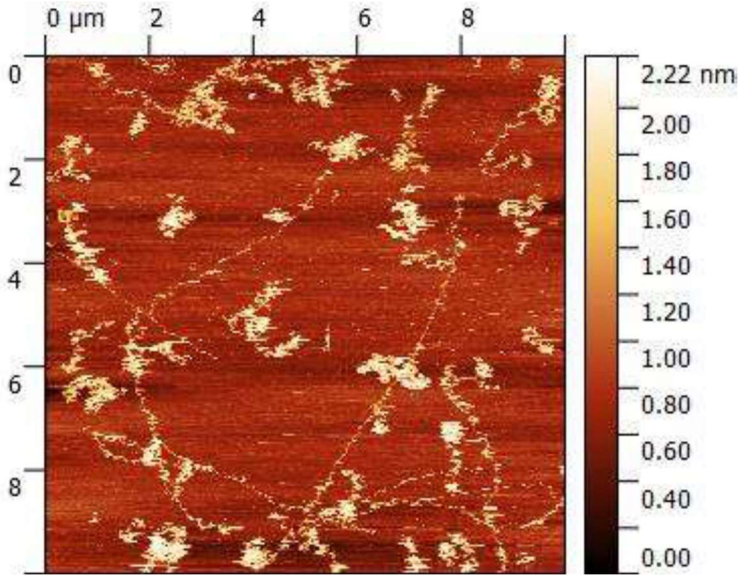


Figure 11: A 10 µm x 10 µm scan of lambda DNA with z-axis shown on side bar

As displayed in Figure 11, some DNA strands are more clumped while others are more linearized. For DNA to be processed through the GNR sensor the strand must be linearized so in deciding which strand to manipulate, the linearized ones are targeted. This image is not optimal quality for spectroscopy, as the image size is very large and the individual strands would be difficult to target.

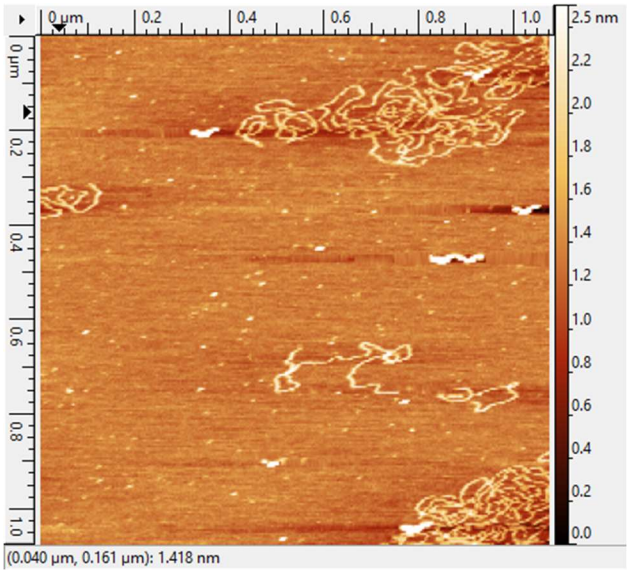


Figure 12: A 1.08 µm x 1.08 µm scan analyzed using Gwyddion

Figure 12 displays a better quality and closer image where individual DNA strands can be easily identified. However, this scan is still not optimal for spectroscopy due to the coiled nature of the DNA strands shown.

3.8 DNA Manipulation

In order to pick up and move the DNA, the AFM must be placed in static and spectroscopy mode. Spectroscopy mode allows for the cantilever tip to press down close enough to the end of the DNA strand for the electrostatic force to overcome the adhesion to the mica surface and stick to the cantilever tip. Once the DNA is stuck to the tip instead of the mica, it can then be dragged through the solution to the GNR sensor.

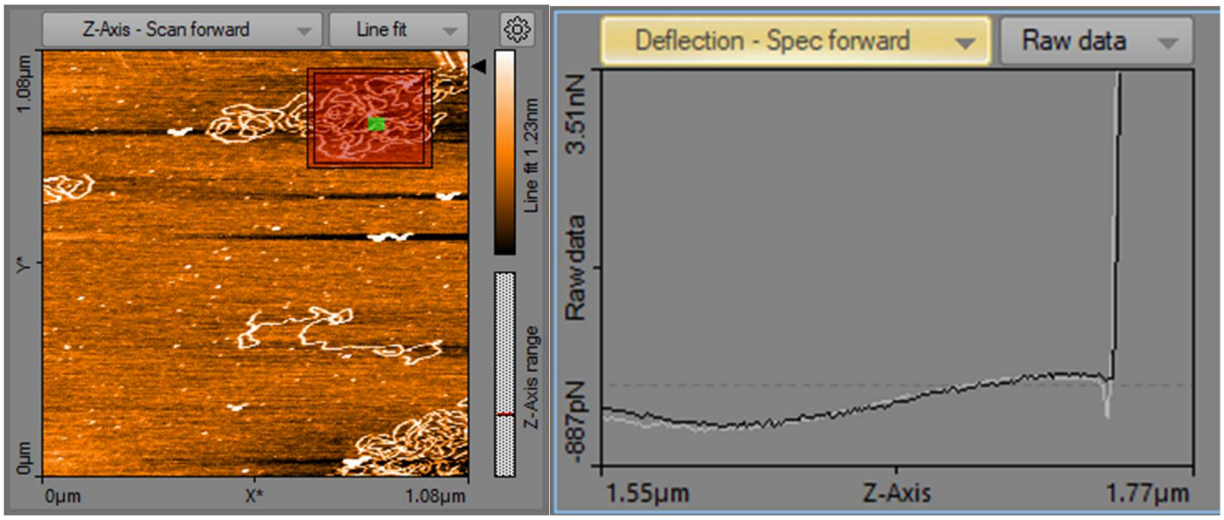


Figure 13: Spectroscopy measurement displaying contact with surface but no attachment of DNA to the AFM tip.

This can be attempted at the original length of the DNA, however, in order to overcome the adhesion of the entire DNA strand it is likely necessary to cut the DNA using a Covaris G-Tube. At this shorter length, there will be a lower adhesion force for the cantilever tip to

overcome and there will be less drag opposing the motion of the DNA which will help with any issues of detachment when moving. Cutting the DNA into shorter strands will also help to decrease the likelihood of strands being folded on top of themselves or coiled instead of the straight strands needed for manipulation. Lukas Bartman utilized the grid spectroscopy mode at 64 force points which required him to sit and wait until he observed a change in the force curve which indicates contact with the DNA strand. The goal is to optimize this process to make it faster and more efficient.

5 Conclusion and Future Works

This research has been ongoing for several years, however my personal involvement spans from Spring 2022 to Spring 2023. Past issues facing this research have been selecting the best tip as well as correct tip holder. Considering the use of the various chemical solutions required to attach the DNA to the mica surface, the AFM must be set in liquid mode for imaging and spectroscopy which requires liquid-compatible tips and tip holder. After attempting scanning with various tips, the selected qp-BioAC tip required time and experience in order to determine the optimal parameters, such as PID values, to be used in the AFM software. Considering that DNA imaging has been achieved, the next step is to successfully achieve DNA liftoff using the spectroscopy mode of the AFM.

Depending on the results, it will likely be necessary to shorten the DNA strands using a Covaris G-Tube. After shortening, the AFM will be able to completely lift off the DNA strands from the mica surface. With the adhesion overcome, the cantilever tip will drag the DNA across the mics surface. To access the GNR sensor, the DNA will need to be moved off the mica surface which poses an issue of surface height. Once the DNA strand is successfully moved from

its original adhesion point into the GNR sensor, the resulting electromagnetic signals will need to be examined and interpreted to identify the DNA base pairs. Upon successful manipulation of a DNA strand and fabrication of a graphene nanoribbon sensor, the two can be combined to sequence DNA without the use of biological proteins. Since the lambda DNA being used is double-stranded, the next goal moving forward in this research would be to sequence single-stranded DNA which is much smaller and therefore more difficult to scan and manipulate.

6 Acknowledgements

I would like to thank Dr. Tung for inviting me to join his research lab. No professor had ever reached out to me before with an opportunity and I am extremely grateful for that consideration that was shown. I would also like to thank Jeremy Meyer for his continued assistance in working through errors encountered throughout this research and Matthew Shubert for training me in the use of the AFM.

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