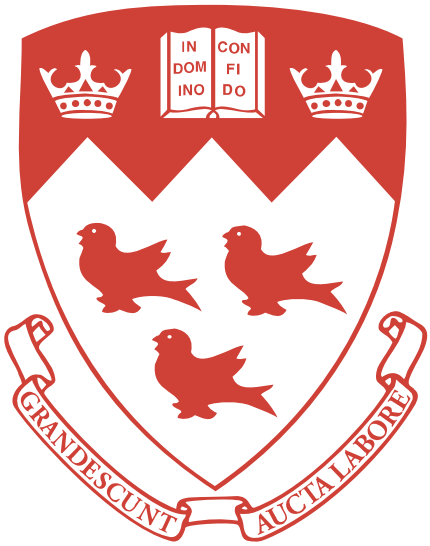
Mimicking the Piercing Mechanism of Mosquitoes Using Atomic Force Microscopy



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Abstract

This thesis focuses on the conception of a probe for atomic force microscopy that can replicate the mechanism used by mosquitoes to pierce the skin of their victims. First, a literature review to understand analytical mathematical models and how mosquitoes feed from their victims is necessary. An understanding on how they utilise their body to pierce the tissues of their victims as well as finding mechanical properties of such body parts that are required for the work. Then, a preliminary experiment is developed to practice with less fragile equipment and less scarce resources. This experiment needs to be partially transferable to the final experiment to conceive the probe. The preliminary experiment is then modified to have a protocol of the final experiment that focuses on conceiving the probe that can replicate the piecing mechanism of mosquitoes. The final experiment is complemented with analytical models that define how the probe can replicate the piercing mechanism of mosquitoes. The analytical models consist of defining the normal force applied to the probe's tip and a buckling analysis to ensure the structural integrity of the probe. It was found that the probe can be conceived. However, some imperfections were identified and must be corrected to use it in the future. The corrections are necessary in order to relate the analytical model to the probe that was conceived.

Abrégé

Cette thèse se concentre sur la conception d'une sonde pour la microscopie à force atomique qui peut reproduire le mécanisme utilisé par les moustiques pour percer la peau de leurs victimes. Tout d'abord, une revue de la littérature pour comprendre des modèles mathématiques analytiques et la façon dont les moustiques se nourrissent de leurs victimes est nécessaire. Il est essentiel de comprendre comment ils utilisent leur corps pour percer les tissus de leurs victimes ainsi que de trouver les propriétés mécaniques de ces parties du corps. Ensuite, une expérience préliminaire est développée pour s'entraîner à faire des manipulations avec de l'équipement moins fragile et avec des ressources moins rares. Cette expérience doit être partiellement transférable à l'expérience qui conçoit la sonde. L'expérience préliminaire est ensuite modifiée pour avoir un protocole utilisable pour l'expérience finale qui se concentre sur la conception de la sonde qui peut reproduire le mécanisme que les moustiques emploient pour percer la peau. L'expérience finale est complémentée par des modèles analytiques qui définissent comment cette sonde peut reproduire ce mécanisme des moustiques. Les modèles analytiques consistent à définir la force normale appliquée sur la pointe de la sonde et une analyse de flambage pour s'assurer de l'intégrité structurelle de la sonde. Il a été prouvé que la sonde peut être conçue. Cependant, certaines imperfections ont été identifiées et doivent être corrigées pour pouvoir l'utiliser. Les corrections sont nécessaires afin de relier le modèle analytique à la sonde conçue.

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- The research team from the McGill Nanofactory Lab who showed me how the various instruments work and who helped me with valuable insight throughout my research.

Nomenclature

Latin Symbols

D_i	Inner diameter of a cylinder
D_o	Outer diameter of a cylinder
E	Young’s Modulus
F_N	Normal force on the tip of an atomic force microscope cantilever beam
I	Second moment of inertia
k_N	Normal spring constant
l	Length of the cantilever beam
L	Euler’s buckling column’s length
L_e	Euler’s buckling effective length of buckling
P_cr	Euler’s buckling critical load
S	Sensitivity of the photodetector
t	Thickness of the cantilever beam
$V_\mathrm{a–b}$	Voltage applied to the cantilever
w	Width of the cantilever beam

Acronyms

AFM	Atomic force microscope
DOF	Degree of freedom
IPA	Isopropyl alcohol
SEM	Scanning electron microscope

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Chapter 1

Introduction

1.1 Problem Definition

It was found that recently, mosquitoes were responsible for the highest number of deaths surpassing all other animals in their lethal impact on humans. In fact, they almost caused twice as many deaths as those caused by humans to other humans — which come second on the list.

Table 1.1: Deaths caused by various animals in 2015 [1]

Animal	% of Total Deaths	Number of Deaths
Mosquito	54	830,000
Human	38	580,000
Snake	4	60,000
Sandfly	1.5	24,200

Mosquitoes not only kill people but also infect humans with various diseases like the malaria, the Zika virus and the yellow fever. Furthermore, even in places, like the province of Quebec, where most of the mosquitoes do not transmit any dangerous disease, people want to protect themselves against their bite because it is unpleasant even if it only means that the skin is irritated.

There are already means to protect people from mosquito bites, like using a mosquito repellents, but there is room for more methods being developed since mosquitoes are still the most deadly animals to humans.

Mimicking the mosquito piercing mechanism could help us to test, in a laboratory environment, fabrics for clothing or creams to check if they can get pierced by mosquitoes. Testing those materials could help in the development of physical barriers

that can be used to protect people against mosquito bites which could complement current methods of prevention, like mosquito repellents, or perhaps replace them entirely as better solutions.

1.2 Similar Research Studies

1.2.1 The Case for Painless Micro-Needles

Painless micro-needles are currently being developed. Mosquitoes are studied to understand how they manage to pierce a victim's skin and doing so while being undetected to replicate it in micro-needles that can be used, for example, to vaccinate people and causing less pain than conventional needles. The micro-needles use the principle of applying a dynamic load (caused by a vibratory motion) as opposed to a static load just like mosquitoes do. It is believed that it helps mosquitoes to pierce the tissues while being unnoticed. The micro-needles are serrated just like the mosquitoes' needles and there is a small tube that secretes a liquid that acts as a numbing effect which is a techniques mosquitoes use partially to act as a numbing effect thus making their piercing less painful for the victim. Figure1.1 shows a schematic of a micro-needle.

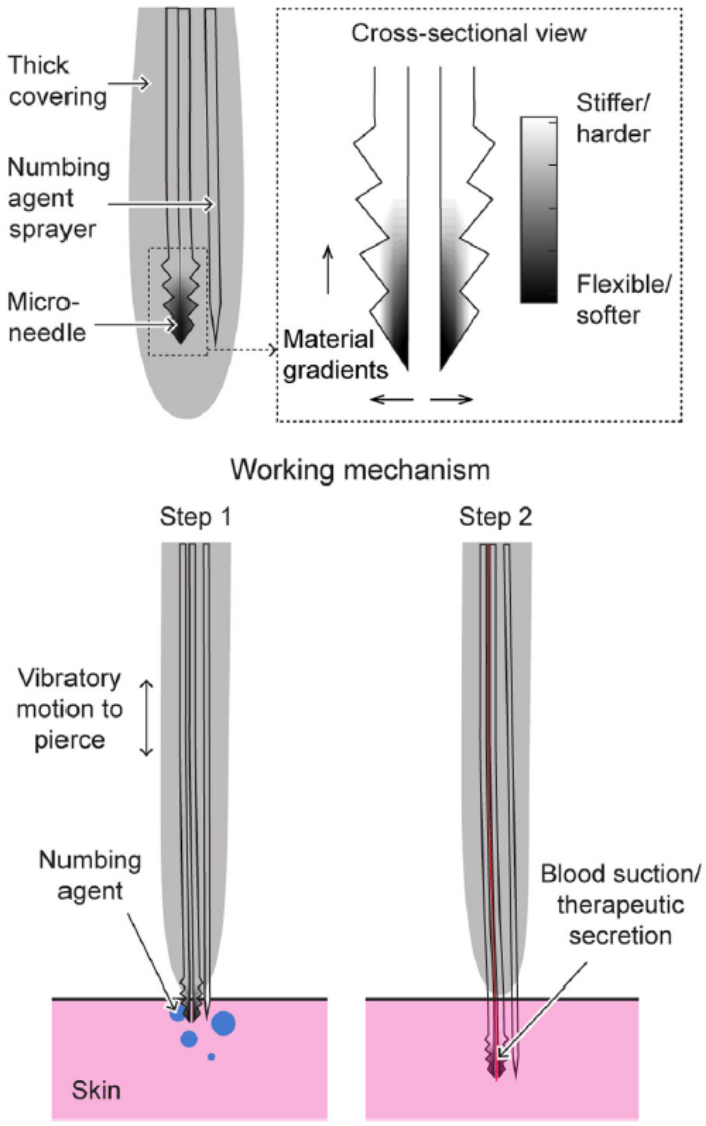

Figure 1.1: Schematic of a mosquito inspired micro-needle [1]

Different studies have found that by applying a dynamic load, the insertion force required to pierce the tissues is reduced. Thus confirming that it makes the piercing mechanism less painful and hence harder to detect. Figure 1.2 shows an example of that finding.

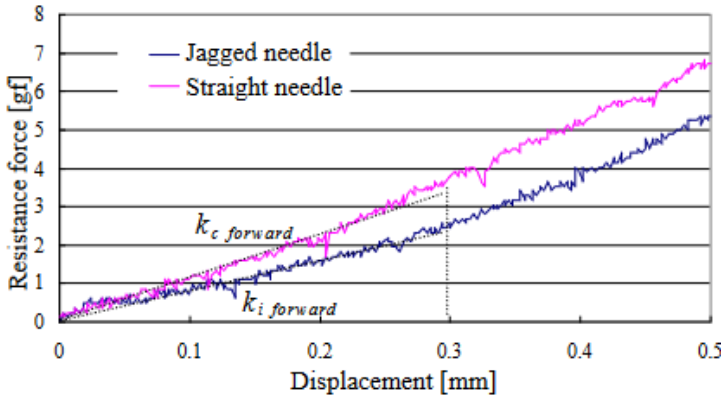

Figure 1.2: Chart of insertion force required for a conventional needle (pink) and a needle inspired by mosquitoes (dark blue) [2]

1.2.2 The Case for Mosquitoes' Needles Mechanical Properties Studies

It was found that the labrum of the mosquito, a needle that pierces tissues, is an anisotropic material since it exhibits different mechanical properties depending on where along the labrum measurements are taken. Depth-sensing nanoindentation techniques that are possible through atomic force microscopy [5] are used to indent the labrum and thus being able to test the labrum and find different mechanical properties at the indentations locations. For example, based on the storage modulus' variation along the labrum, the closer to the tip, the higher the stiffness of the labrum which can help to explain why the labrum's tip is used to pierce the victim's skin.

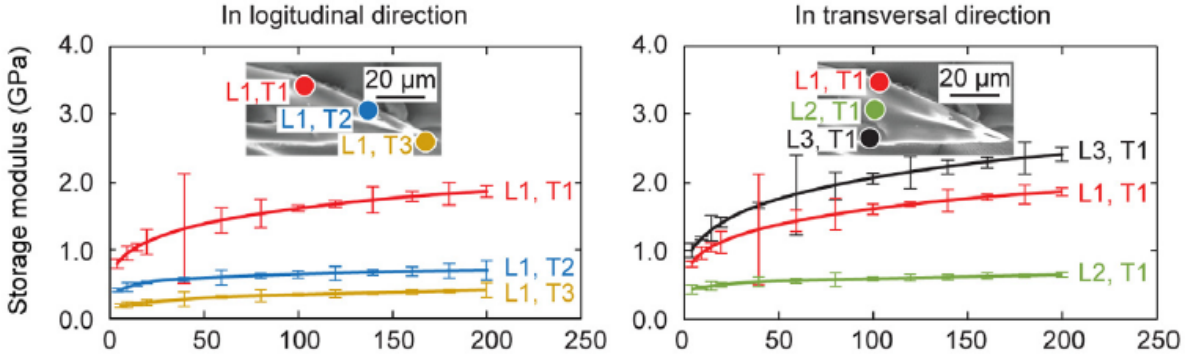

Figure 1.3: Storage Modulus variation on the longitudinal (left) and transverse (right) direction along the labrum [1]

1.3 Objectives and Applications

The overall objective of this thesis is to assess the feasibility of the conception of a tool that can replicate the piercing mechanism used by mosquitoes. This is to be achieved using atomic force microscopy by making a probe that utilises the sharp biological needle that mosquitoes use to pierce tissues to feed themselves from their victims.

The first objective is to develop a preliminary protocol using less fragile tools and instruments than those used in atomic force microscopy which are prone to breaking if the protocol is not rigorous enough and if there are mismanipulations made. The protocol must be made in such a way that most of the steps are transferable and easily adaptable to use them in atomic force microscopy and to use them with mosquitoes which are a scarce resource in the scope of this research.

The second objective is to adapt the preliminary protocol so that the biological probe can be conceived using atomic force microscopy and real mosquitoes. A theoretical model ought to be developed such that the biological probe is not only feasible but also useful for multiple applications.

The third and last objective is to conceive the biological probe using the adapted protocol and to compare it with the theoretical model.

The biological probe would be useful if it can apply, for example, the same insertion force that mosquitoes use when they pierce the victim's skin. This is what is meant by mimicking the piercing mechanism y mosquitoes. If that is achievable, fabrics and creams could be tested using the probe to evaluate if mosquitoes can pierce through them.

1.4 Outline

This thesis describes all the work that led to the conception of a biological probe that is designed to mimic the feeding mechanism of mosquitoes. Starting with the second chapter, a literature review of mathematical models and relevant research topics regarding mosquitoes are studied. Then, the third chapter describes a preliminary experiment with its protocol and results. Those two chapters are prerequisites to develop the final experiment on the fourth chapter. That experiment is where the probe

using the labrum’s tip of the mosquito is made and an analysis of the results is included. Chapter 5 gives the summary and the conclusion of the work.

Chapter 2

Literature Review

2.1 Normal Force Applied On an AFM Probe's Tip Theory

For the final experiment, an equation to relate the normal force experienced by the tip of the probe, F_N, is required. Such equation exists and depends on 3 parameters (courtesy of Dr. Cao):

$$F_{\mathrm{N}} = k_{\mathrm{N}} S V_{\mathrm{a-b}} \tag{1}$$

- k_N is the normal spring constant of the cantilever of the probe. It represents the stiffness of the cantilever perpendicular to the surface being probed (along the direction of the tip). When the tip exerts force on a surface, the cantilever bends and that bending is proportional to the normal spring constant. Therefore, k_N describes how much the cantilever bends for a given normal load. Its value will depend on the Young's Modulus of the beam and the dimensions of the cantilever.

$$k_N = \frac{Ewt^3}{4l^3}$$

  - E is the Young's Modulus of the cantilever which will solely depend on the material of the beam. It is the stiffness of the material. It describes by how much the material will deform under stress by comparing the ratio of stress over strain. Its units are Pa.
  - w, t, and l represent the width, thickness, and length, respectively, of the cantilever beam. The unit of those values is typically µm.

- The photodetector converts the reflected light signal from the cantilever into an electrical signal that can be measured (refer to Appendix A4). S is the sensitivity of the photodetector. It describes by how much the cantilever bends for a given voltage. Its units are nm/V.

- $V_{\mathrm{a-b}}$ describes the voltage that is applied by the shaker piezo to the cantilever. The higher the voltage, the higher the deflection and thus the higher the F_{N}. A typical voltage that can be applied in an atomic force microscope ranges from 0 to 10 V. Typically, F_{N} is expressed in µN.

2.2 Euler Buckling Theory

Different modes of buckling analysis exist and they are useful when determining the critical load that would cause a beam to buckle. Euler buckling occurs in long and slender columns that are under compressive loads. The loads must be along the length of the beam (in the longitudinal direction). Therefore, when Euler buckling occurs, the beam experiences its critical longitudinal compressive load P_{cr} that causes the column to buckle. Its value depends on three parameters [6]:

$$P_{\mathrm{cr}} = \frac{\pi^2 E I}{L_{\mathrm{e}}^2} \tag{2}$$

- E, the Young's Modulus of the material of the column.

- I, the moment of inertia of the column's cross section. The cross-section is in the radial direction (orthogonal to the direction of the critical load).

- L_{e}, the effective length for buckling of the column which determines the length of the beam that is prone to buckling if the critical load is reached. The lower the effective length, the greater the critical load is. therefore, shorter beams are more resistant to buckling than longer beams. its value depends on the total length L of the column and on the type of connection. Two connections of interest are:

  - *Fixed-fixed* columns which have two boundaries conditions. Both ends are fixed to surfaces and experience therefore no translation and no rotation. Whenever it is possible, it is preferable to design for *fixed-fixed* columns, because they provide the highest relative buckling strength when compared to the same column with a

different connection. It is the one that offers the smallest L_e, thus it can withstand a higher load before buckling. For a *fixed-fixed* column, $L_e = 0.5L$.

- *Fixed-pinned* columns which also have two boundary conditions. One end is fixed to a surface and the other end is pinned to a surface. Therefore, the fixed end has no translation and no rotation and the pinned end has no translation but can experience rotation. This potential rotation, if buckling occurs, causes the effective length to be longer than for the *fixed-fixed* column. In this case, $L_e = 0.7L$

2.3 Literature Review of the proboscis and the Feeding Mechanism of Mosquitoes

An understanding of the anatomy of the proboscis of mosquitoes is necessary to understand their piercing mechanism. The specie studied here in this thesis is the *Aedes aegypti*. The proboscis is a body part from the mouth of the mosquito which allows the mosquito to feed itself. It is composed of the labium and the fascicles. The fasciles are the two maxilla, the two mandibles, the hyphopharynx and the labrum. They are all shown on figure 2.1.

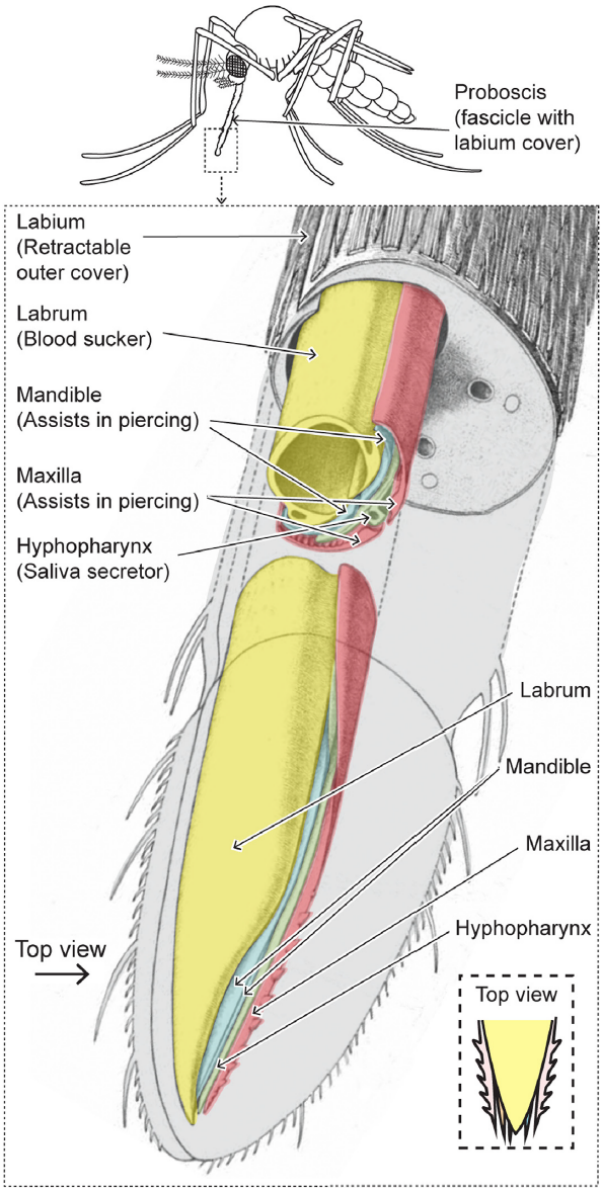

Figure 2.1: Schematic of a mosquito (top) and of the proboscis (bottom) [1]

The schematic on figure 2.2 can be used to explain the feeding mechanism of mosquitoes and to explain the role of the proboscis' components. The labium is what is seen by looking at a mosquito under a microscope or from the naked eye. It is a dark retractable body part which helps during the feeding process by shortening the effective length of the fascicle that can buckling thus requiring a greater load to make the fascicles buckle. On figure 2.2 *B*, the labium is the mouth part that is retracted (the bent part). Fascicle is a medical term that refers to a bundle of slender parts. Mosquitoes have 6 fascicles which are all covered by the labium and they are used during the feeding process. The two maxilla and two mandibles are sharp and serrated. Mosquitoes use them to anchor onto the victims' skin. On step *A*, the mosquito lands on the victim

and anchors its fascicles (which are hidden by the labium) on the skin of the victim. They allow the labrum to apply a vibratory motion which exerts a dynamic load to pierce the skin. It is believed that the dynamic load required to pierce skin is lower to its equivalent static load required to pierce human skin. Therefore, it makes the mosquito harder to detect by the victim. After successfully piercing the skin, shown on step *B*, the labrum continues penetrating the tissues until it pierces a blood vessel. This task is completed at step *C*, at which point, the hypopharynx secretes saliva acting as a numbing effect making the mosquito harder to be detected and it prevents the coagulation of blood within the blood vessels which facilitates the process of sucking blood. At this stage, the mosquito starts sucking blood from the pierced blood vessel through the labrum.

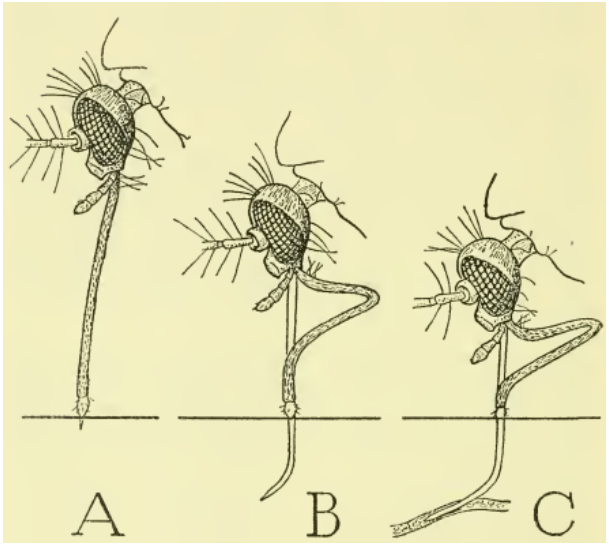

Figure 2.2: Schematic of the successive steps a mosquito follows to feed itself [3]

To prevent the mosquito from feeding of their victims and preventing the transmission of dangerous diseases, the tissues must be protected against the dynamic loads applied by the labrum because it is the member what pierces the skin.

2.4 Literature review of Mosquitoes' Piercing Mechanics and Properties

Piercing Insertion Force

An understanding of the insertion force that mosquitoes apply is important, because in order to develop a probe that mimics the piercing mechanism, it would be ideal to reproduce the same amount of force that mosquitoes generate when they pierce their

victims.

Studies have found, experimentally, the amount of force that mosquitoes use with their labrum to pierce through the skin. By having an inverted box with one mosquito and a lid made of screen cloth, the mosquito can land on their victim and pierce the skin to feed from them. By doing so, their legs are on the screen cloth, the labrum passes through it thus a force is read by the digital balance, because by piercing the skin, the mosquito is lifting itself up from the perspective of the digital balance. The digital balance used in this study had a precision of 0.1 µN. Different mosquitoes exerted different amounts of force which ranged from 5 to 40 µN and a majority of mosquitoes apply an insertion force ranging between 10 to 25 µN. The biological probe is to be designed for an insertion force range of 5 to 40 µN[4].

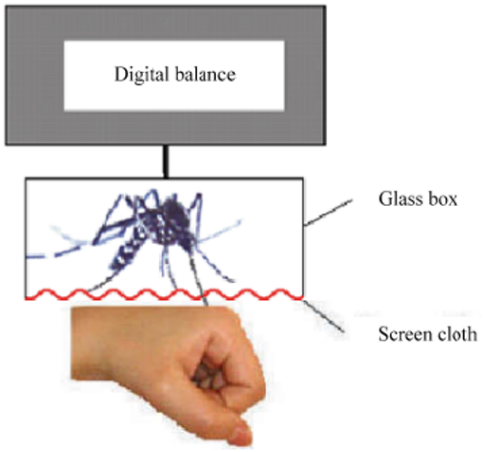

Figure 2.3: Schematic of the force measurement system [4]

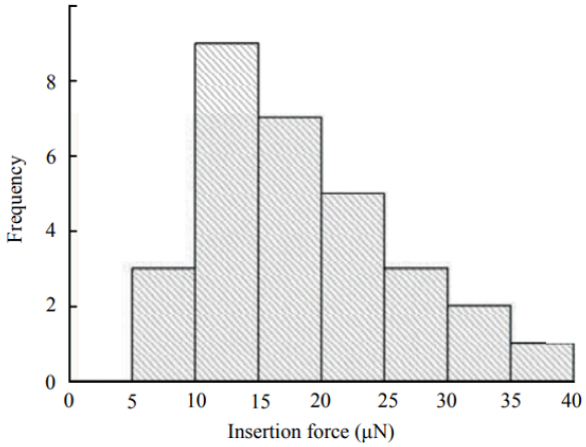

Figure 2.4: Chart of frequency versus insertion force [4]

Chapter 3

Preliminary Experiment

This chapter covers the development of the protocol to perform the preliminary experiment with a detailed explanation of the importance of each step. The discussion of results is also included. The experiment is about gluing singular micro-beads made of glass on small pieces of wafers. The goal is to have a protocol to successfully glue a single bead to the tip of a piece of wafer. The protocol requires an iterative process where the experiment is run multiple times with small variations to the protocol to constantly improve the method until a rigorous protocol that can be transferable to work with AFM cantilevers and mosquitoes is achieved.

The wafers are approximately 100 times thicker than the AFM cantilevers used in the final experiment. Therefore, they are stiff compared to AFM cantilevers, thus they are more forgiving in the case of doing mistakes during manipulations while developing the protocol. They are also cheap compared to AFM cantilevers which can cost hundreds of dollars each.

The micro-beads replace the mosquito's labrum from the final experiment. Just like the labrum, it is assumed that the beads are single use only, and they are abundant which allows for the development of the protocol through an iterative process because there will always be enough beads to run the experiment.

Many of the apparatus used in this experiment are also used for the final experiment. This is to maximize the similarities between this experiment and the one with mosquitoes.

3.1 Protocol

3.1.1 Step 1: Cleaning the Beads

Context

If a bead is used as a tip on an AFM cantilever beam, it can be used in *contact mode* to scan over a surface and get its topography (refer to Appendix A4). Their surface is blunt so they do not offer a resolution in the order of nanometers (as opposed to other tips) but they are inexpensive and experience very low wear so they can be used for a longer period of time. Furthermore, beads are glued to a cantilever so they can be removed and swapped. Sharp tips with greater resolution are permanent on the cantilever so once they lose the sharpness, the entire cantilever has to be changed which is expensive. in AFM studies, the beads need to have a clean bead so that the part of the bead in contact with the substrate is always on the same location on the bead. The beads are initially polluted by the presence of smaller beads that are stuck on the main bead that needs to be glued. If those smaller beads stay on the main bead, the part of the surface in contact with the substrate will always be different and hard to predict. Therefore, it is necessary to remove those smaller beads from the micro-bead of interest.

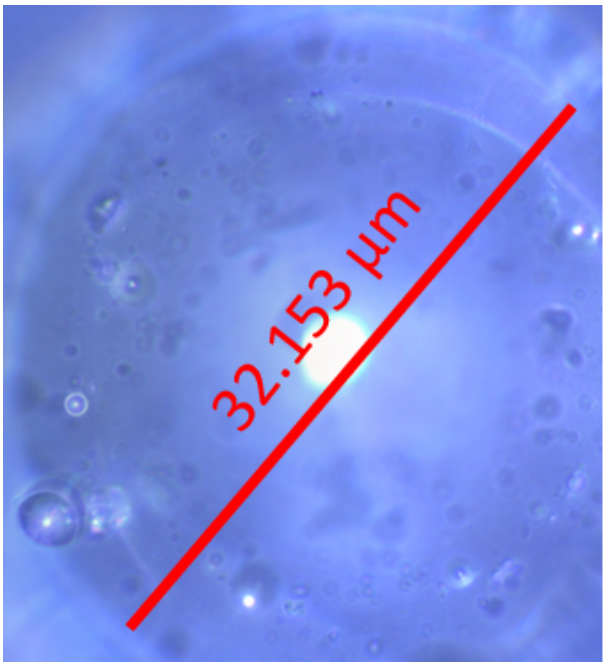

Figure 3.1: Polluted bead (50X magnification)

Manipulation 1: Cleaning the Beads and the Glass Slide

The method to clean the beads are to put them in the liquid pool of the ultrasonic cleaner (refer to Appendix A3). Everything that is to be in contact with the beads needs to be included in the liquid pool for cleaning so that the beads do not get contaminated in the next steps. The cleaning time used was 30 minutes. There are 2 items that need to be cleaned:

- A solution of isopropyl alcohol (IPA) in a sealed Petri dish. The beads are sealed inside the Petri dish so that they are easy to pick up later with the automatic dropper. They need to be in a solution so that the high frequency waves can reach the liquid containing the beads and generate the cavitation effect on the surface of the beads to clean them.
- The glass slide where the beads are to be dropped needs to be cleaned. On one side of the glass slide, a pen is used to draw orthogonal lines using a spacing of approximately 3 mm between each line. The reason is explained later.

The items are submerged in the pool of the ultrasonic cleaner to clean all their surface.

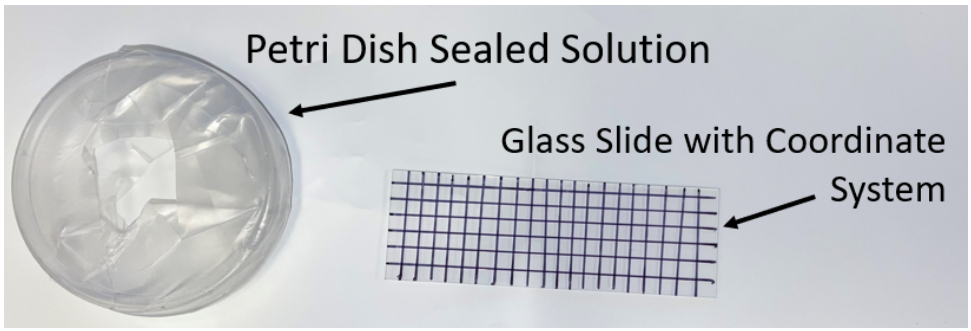

Figure 3.2: Items to clean in the ultrasonic cleaner

3.1.2 Step 2: Selecting Beads to Glue

Context

After cleaning the beads, the cleaned items are allowed to dry in ambient air. For this step, it is important to wear sterile nitrile gloves to avoid contaminating the cleaned material.

Manipulation 1: Dropping the IPA Solution Over the Glass Slide

A 2 mL sample from the IPA solution containing the cleaned beads is sucked with the automatic dropper which uses a sterile pipette. The isopropyl solution can erase the lines of the glass slide, thus the sample is dropped on the side of the glass that does not have the lines. Isopropyl alcohol quickly dries on the glass slide which is why it is the liquid used.

Manipulation 2: Selecting the Beads

When the IPA is dried, the glass slide is placed under the ZEISS microscope for examination. Initially, the magnifying lens of 5X is used to find a location along the glass slide where there is an agglomeration of beads.

Afterwards, a bead is selected for gluing. Using the 20X magnifying lens, the microscope takes pictures of the beads. Beads under 20 µm are hard to manipulate for gluing on a piece of wafer and beads of more than 40 µm are too heavy if they are glued to an AFM cantilever. Therefore, a scale is implemented in the picture of the beads to find one that has a diameter of 30 ± 5 µm. This is the first criterion to select a bead to glue. The second criterion is that the surface of the bead needs to be clean. Beads meeting the dimensional criterion are inspected with the magnifying lens of 50X. A conventional microscope cannot take clear pictures of an entire surface that is curved (refer to Appendices A1 and A2), therefore multiple pictures with different focuses are taken to ensure the beads are clean.

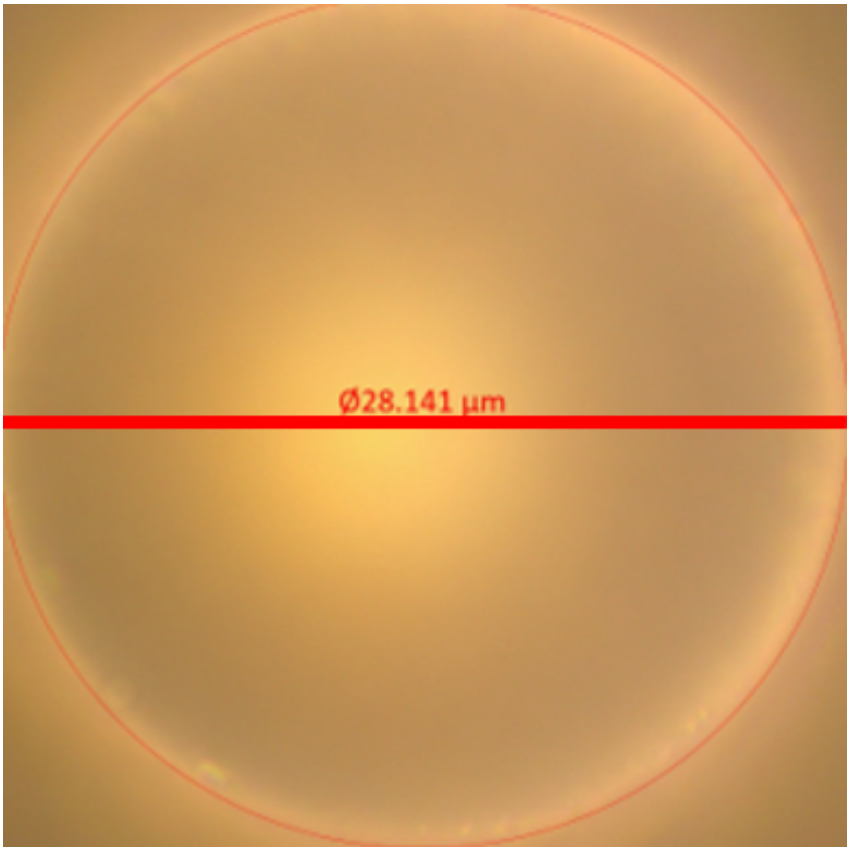

Figure 3.3: Bead properly cleaned by the ultrasonic cleaner (50X magnification)

Marking the Selected Beads

When a bead that meets the two criteria is found, a pen is used to mark where along the glass slide that bead is located so that it is easy to relocate the same bead under the Probe Station. The coordinate system can save 30 minutes to this experiment since it makes it very easy to relocate the bead among the hundreds of beads that are on the glass slide.

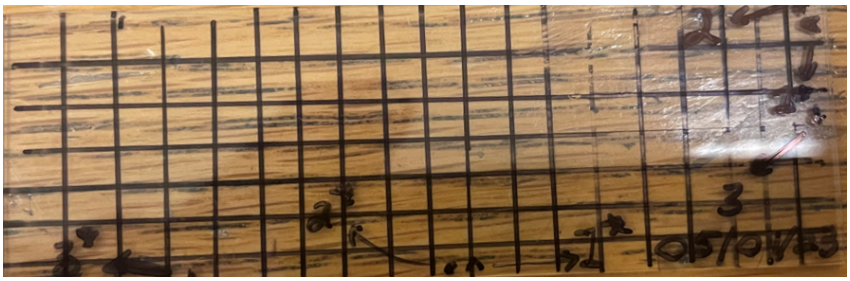

Figure 3.4: Marked glass slide to recognize the selected beads

3.1.3 Step 3: Cutting the Pieces of Wafers

Context

The beads need to be glued on the tip of a small piece of wafer. The wafers come in circular shapes in the order of a few centimeters. They need to be cut to be in the order of 1 cm or less. AFM cantilevers are small. The chip is usually 1 x 2 mm in dimensions and the cantilever is 200 µm long. Therefore, to make this experiment similar to gluing on an AFM cantilever, the wafer is cut into pieces of a few millimeters long.

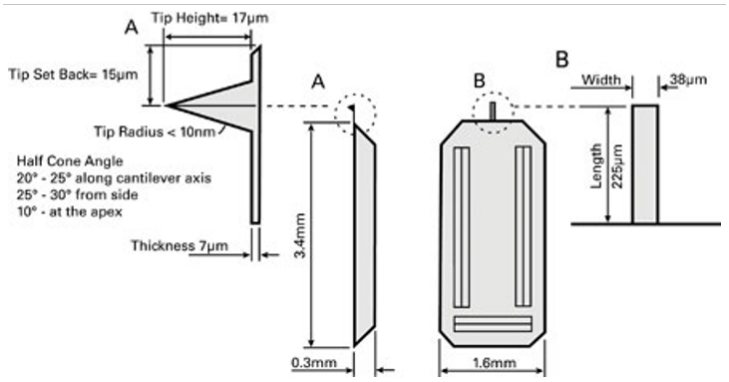

Figure 3.5: Schematic of a typical probe's dimensions[8]

Manipulation 1: Cutting the Pieces of Wafers

The wafer is cut using an X-ACTO blade by drawing straight lines using a glass slide over the wafer. Notice that the wafer was not cleaned in the ultrasonic cleaner because it will not be directly in contact with the bead. The epoxy on the wafer will be in contact with the bead instead.

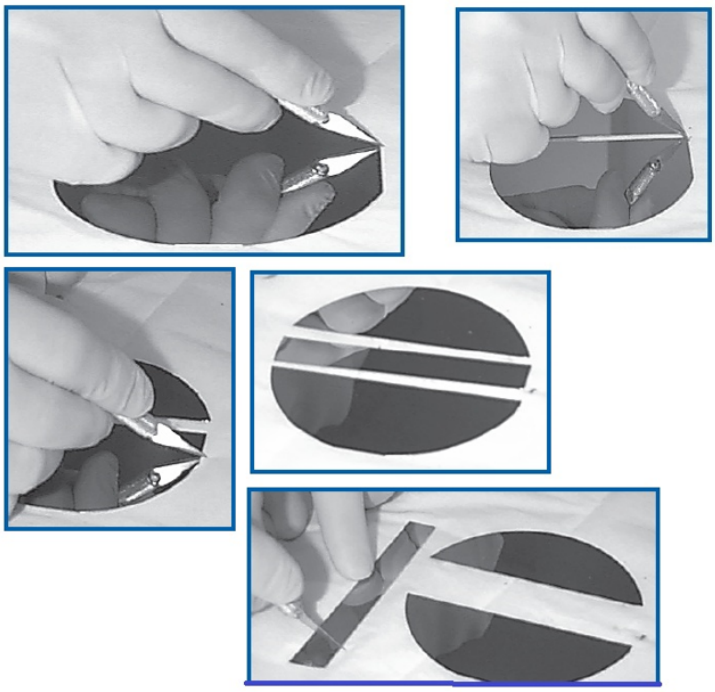

Figure 3.6: Cutting a wafer [9]

3.1.4 Step 4: Gluing the Selected Bead on the Wafer's Tip

Context

Less than 1 mg of 30-minutes epoxy [7] is necessary to glue a bead to a wafer. The epoxy can be handled for 30 minutes. Then, it takes 8 hours to be handled and 24 hours to be fully cured. The epoxy used is two-type with a 1:1 mixing ratio made of the hardener and the resin. If, for example there is an absolute error of 0.1 mg of difference in the mass of epoxy compared to the mass of hardener, the relative error of it for a total mix of, say, 0.4 mg is way larger than the relative error for a total mix of, say, 2 mg. Therefore, when weighing the epoxy parts, large quantities of 2 mg were used so that the relative error does not affect the bond strength of the epoxy.

Manipulation 1: mixing the epoxy

The epoxy is weighted on a scale and 1 mg of hardener was mixed with 1 mg of resin in a Petri dish. This epoxy is white when it is properly mixed. A different epoxy can be utilized if it allows enough time to perform the manipulations. Using epoxies with a handling time of less than 30 minutes is not ideal since it might not be enough time to run the experiment.

Manipulation 2: Applying Epoxy to the Wafers Pieces

A very small droplet of mixed epoxy is dropped on a glass slide. The epoxy is rubbed on the surface of the glass using a second glass slide. This allows to obtain a thin layer of epoxy on the glass slide. If the epoxy is too thick on the wafer, it can engulf the bead and hence compromise the experiment. Therefore, a a small layer of epoxy on the glass slide is necessary.

Using tweezers, the wafer piece is grabbed and the tip of one of the two surfaces is rubbed against the epoxy on the glass slide. The wafer is then inspected under a microscope to ensure it has enough epoxy.

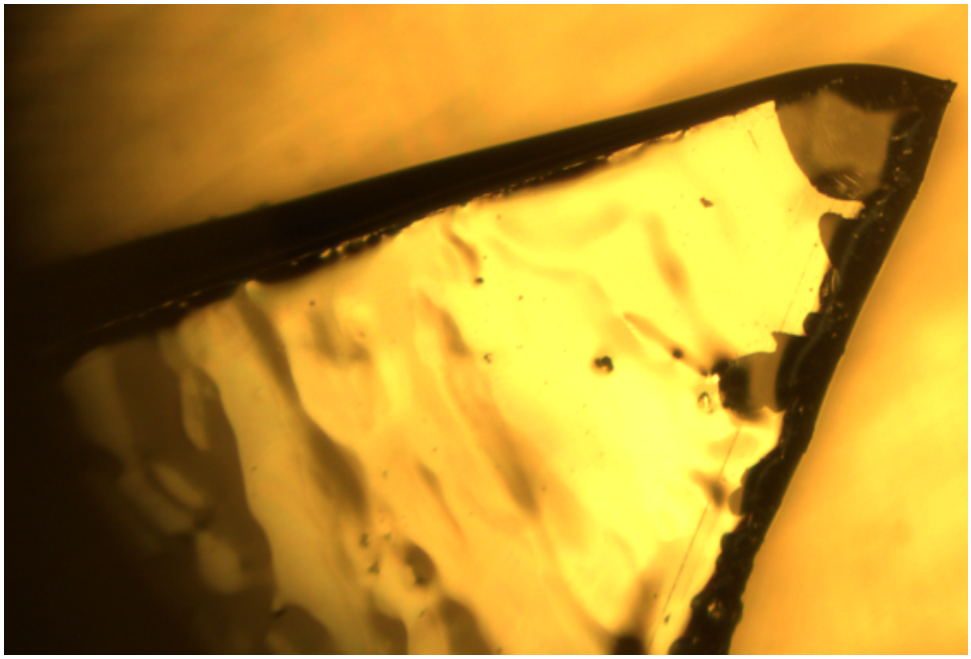

Figure 3.7: Wafer with glue (based from the presence of waves), 5X magnification

Manipulation 3: Mounting the Wafer to the Manipulator

The wafer is placed in the clamps (refer to Appendix A2). Only the tip of the wafer is not clamped and the face with epoxy faces downwards. Following Appendix A2, the clamp is bolted to the lever arm and the lever arm is bolted to the manipulator.

Manipulation 4: Relocating the Beads Under the Probe Station

The glass slide containing the labeled cleaned beads is place under the Probe Station. Using the labels, the selected beads need to be relocated under the probe station. Through pattern recognition with the beads in the vicinity of the chosen beads, the selected beads

are relocated.

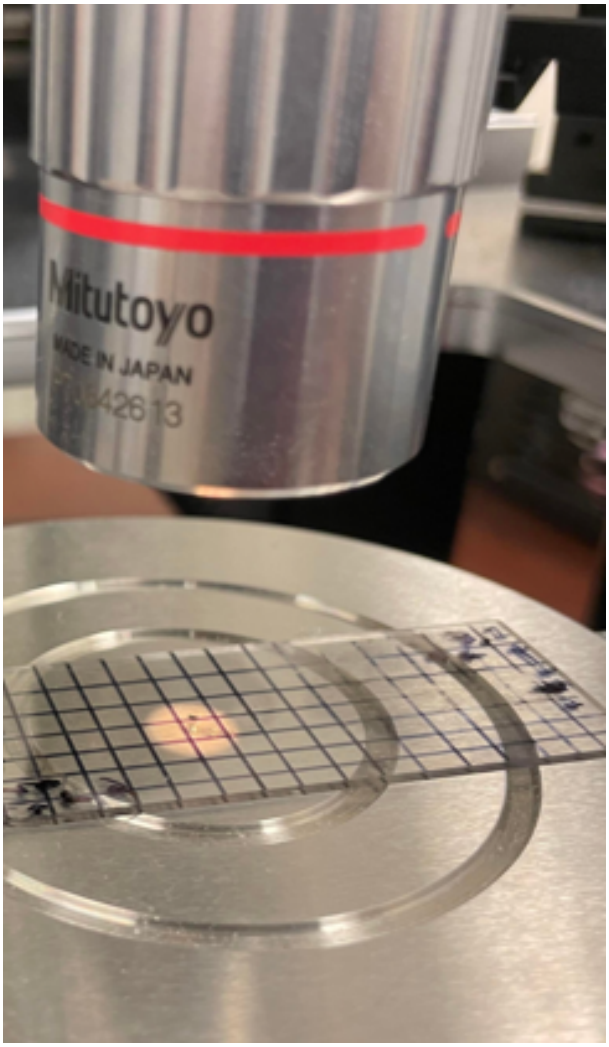

Figure 3.8: Glass slide ready for gluing

The square of the glass slide that is marked is placed under the probe station to locate the bead.

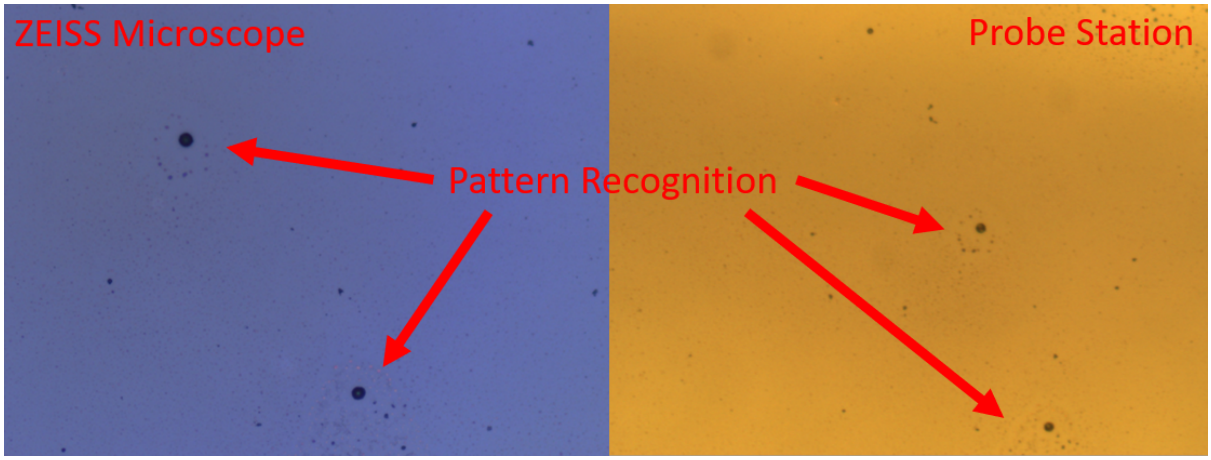

Figure 3.9: Beads pattern recognition from the ZEISS Microscope (left) to the Probe Station (right), 5X magnification

Manipulation 5: Gluing the Beads on the Wafer's tip

The wafer is initially placed approximately 2 mm away from the surface of the glass slide with the glued surface facing the glass slide.

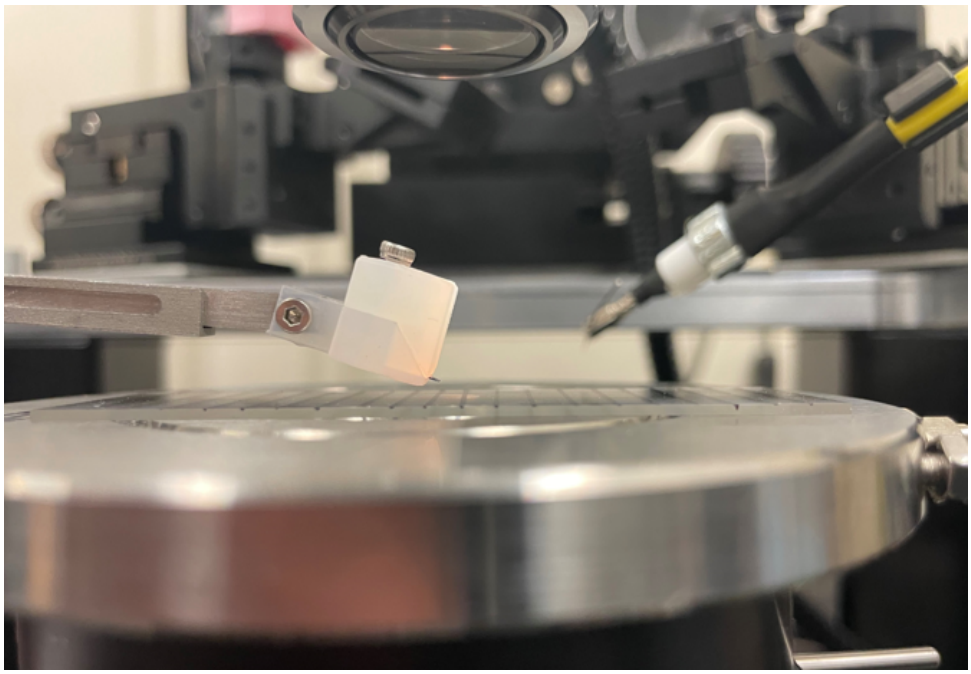

Figure 3.10: Wafer placed near the glass slide

The wafer is thus close enough to the glass slide to be able to see its shadow and by moving the manipulators (refer to Appendix A2), the shadow of the wafer's tip is placed on top of the selected bead. Using the manipulator, the wafer is slowly lowered on the glass slide. By looking at the voltage reading from the force sensor on the screen (refer to Appendix A2), when the wafer's tip touches the bead, there is a voltage reading. When

the voltage is read, the wafer is immediately lifted up using the manipulator. By moving the shadow laterally with respect to the glass slide, if the selected bead is not on the glass slide anymore, it is successfully glued on the wafer. Before inspecting the wafer, it is left still for 8 hours for it to cure.

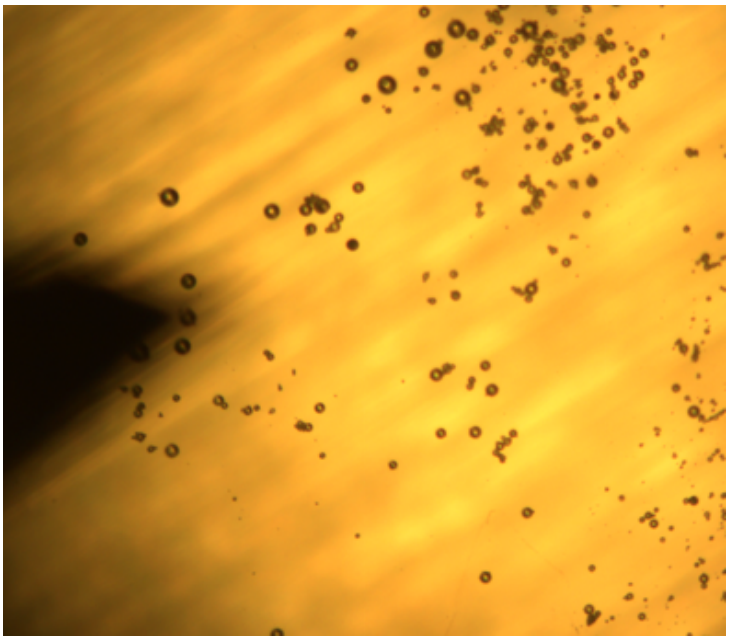

Figure 3.11: Wafer's shadow over the glass slide (5X magnification)

3.2 Results

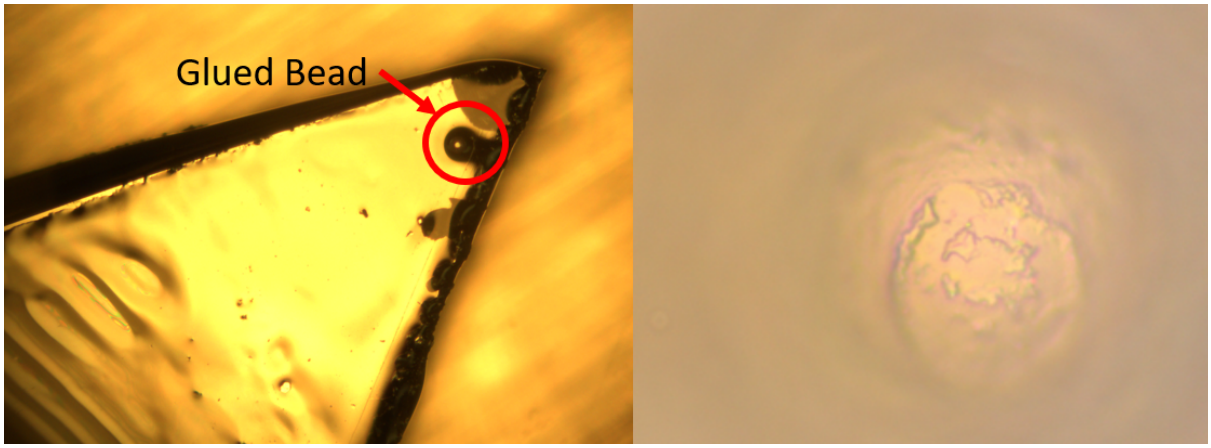

Figure 3.12: Position of the glued bead on the wafer (left), contaminated surface of the glued bead (right), 5X magnification

An example of a glued bead is shown on the figure above. The epoxy is not thick enough to engulf the bead, but the surface of the bead is polluted.

3.2.1 Results Analysis

Overview

A singular bead was successfully glued to a piece of wafer, therefore the goal of this experiment was met. The protocol can also be modified to glue a mosquito's labrum to an AFM cantilever beam. The experiment was reproducible since it was run multiple time with different wafers that have a singular bead glued to them. The protocol is still not perfect. There were problems with the results but they do not affect the work with mosquitoes. The glued beads were polluted and it was hard to glue them at the tip of the wafer. It seems like the bead's position shifts once it touches the wafer.

Difficulties Encountered

From the picture of the glued bead, the surface is not clean. When a clean bead was selected, it was assumed that the surface of the bead facing the glass slide, which is invisible to the ZEISS microscope before gluing, is clean like the surface that can be observed. Therefore, a hypothesis to explain the impurities on the surface is that the half of the bead that was invisible to the ZEISS microscope was polluted. This is unlikely because the pattern of the impurities is different from the small beads that are attached to the bigger beads before cleaning them in the ultrasonic cleaner (refer to figure 3.1). These pollutants look like circular lines.

The second hypothesis is that while gluing the bead, force was applied past the point of the bead being in contact with the piece of wafer. Therefore, the wafer starts sliding and the beads rolls on the epoxy. The rolling motion of the bead can explain why the impurities look like circular lines. This rolling can also explain why it is difficult to accurately glue the bead on a selected location on the wafer.

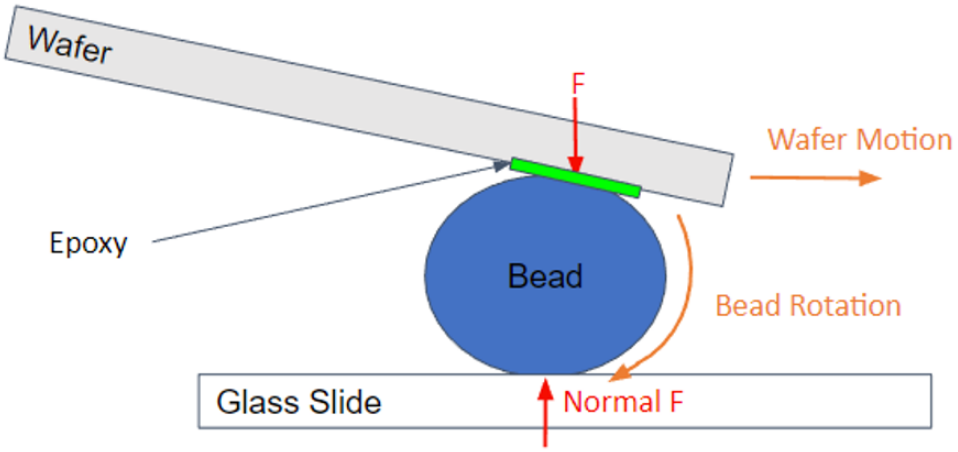

Figure 3.13: Schematic of the bead's rotation during the gluing process

If the bead is to be glued to an AFM cantilever beam, this problem needs to be solved. When gluing on AFM cantilever beams, the cantilever easily deflects as soon as a minimal amount of force is applied during the gluing process. Therefore, by visual inspection, it is possible to see that the bead is touching the epoxy thus if the force sensor is not sensitive enough to capture a force reading, it can be observed, through visual inspection, that the bead is touching the epoxy. In such case, the bead would not rotate so the surface remains clean and there can be a greater control on the gluing position along the cantilever.

These problems were not solved since the goal of the preliminary experiment is met.

Chapter 4

Final Experiment

This chapter first covers the calculations to determine where along the cantilever the labrum's tip needs to be glued. An Euler buckling analysis is also performed to ensure the labrum's tip will not bend from the compressive load exerted on it. The chapter also covers the protocol from the preliminary experiment that is adapted for gluing the labrum's tip of the mosquito to the atomic force microscopy cantilever. The experiment is performed and the results are analysed.

4.1 Labrum's Tip Gluing Position

Depending on where along the length of the cantilever beam the labrum's tip is glued, the normal force experienced by the tip, F_{N}, will vary as dictated by equation (1). l is the only parameter of the probe that can be varied to affect the F_{N}. The closer the labrum's tip is to the cantilever's base, the greater the normal spring constant (k_{N}) thus the greater the F_{N} experienced for a given voltage applied $V_{\mathrm{a-b}}$. The cantilever used for this experiment is the *TL-NCH* [10].

By gluing the labrum's tip at a distance of 110 µm, which is approximately half the length of the cantilever, the labrum's tip is theoretically capable of having a F_{N} ranging from 0 to 40 µN by varying $V_{\mathrm{a-b}}$ from 0 to 10 V, which are the limits of the AFM. This range of loads covers all the loads that the mosquitoes can apply on their victims based on the literature review. Following equation (1), the maximum value of F_{N} at a position $l = 0.110\,\mathrm{mm}$ for the maximum voltage is:

$$F_{\mathrm{N}} = k_{\mathrm{N}} S V_{\mathrm{a-b}}$$

$$F_{\mathrm{N}} = \frac{Ewt^3}{4l^3} S V_{\mathrm{a-b}}$$

$$F_{\mathrm{N}} = \frac{250000 \frac{\mathrm{N}}{\mathrm{mm}^2} \times 0.028\mathrm{mm} \times 0.003^3\mathrm{mm}^3}{4 \times 0.110^3\mathrm{mm}^3} \times 100 \frac{\mathrm{mm}}{\mathrm{V}} \times 10\mathrm{V}$$

$$F_{\mathrm{N}} = 4.08 \times 10^{-5}\mathrm{N}$$

- E is taken from the material of the AFM cantilever beam which is made of silicon.
- w and t come from the geometry of the AFM cantilever beam.
- S is taken as a generic value for these types of beam based on experimental studies (courtesy of Dr. Cao).
- V_{a-b} can have a maximal value of 10 V for the AFM of the Nanofactory Lab.

4.2 Euler Buckling Analysis for the Labrum's tip

An Euler buckling analysis is important for this probe, since it needs to withstand the normal compressive load, F_{N}, that ranges from 0 to 40 µN without failing by buckling. According to equation (2), F_{N} needs to be smaller than P_{cr} to ensure it will not experience buckling.

The probe containing the mosquito's labrum can be assumed to be *fixed-fixed*, since one end of the labrum is glued to the cantilever (thus fixed) and the labrum's tip can be considered to be fixed on the victim's skin. It could also be assumed to be *fixed-pinned*, because the labrum's tip can be assumed to be pinned on the victim's skin, because before it pierces the skin, it should be able to rotate around the labrum's tip in contact with the skin. The *fixed-pinned* case is a more conservative assumption, because it increases the effective length, L_{e}, of the labrum's tip. By increasing L_{e}, the critical load causing buckling decreases. If the labrum's tip will not buckle under a *fixed-pinned* boundary condition, it is safe to assume it will not buckle if it is considered to be *fixed-fixed instead*.

Following equation (2) for a *fixed-pinned* boundary condition:

$$P_{\mathrm{cr}} = \frac{\pi^2 EI}{L_{\mathrm{e}}^2}$$

$$P_{\text{cr}} = \frac{\pi^2 E}{L_{\text{e}}^2} \times (\frac{\pi}{64} \times (D_{\text{o}}^4 - D_{\text{i}}^4))$$

$$P_{\text{cr}} = \frac{\pi^2 \times 300 \frac{\text{N}}{\text{mm}^2}}{(0.7 \times 0.3)^2 \text{mm}^2} \times (\frac{\pi}{64} \times ((0.03\text{mm})^4 - (0.02\text{mm})^4))$$

$$P_{\text{cr}} = 2.14 \times 10^{-4}\text{N}$$

- E is the Young's Modulus of the labrum's tip. Its value is taken from the literature as defined in the previous section.
- I is the second moment of inertia of the cross-section of the labrum. It can be assumed to be a hollow cylinder with an outer diameter, D_{o}, of 30 µm and an inner diameter, D_{i}, of 20 µm. These values are based of the literature review and confirmed by measurements taken experimentally of the labrum.
- $L_{\text{e}} = 0.7 \times L$ based on the *fixed-pinned* boundary condition. The protocol explains the reason for picking $L = 300$ µm.

Therefore, the critical load of 214 µN is approximately 5 times greater than the maximal F_{N} the tip is designed for. The probe's tip has a safety factor of 5 for buckling and it is not a failure mode.

4.3 Protocol

The objective of this experiment is to glue the labrum's tip to the AFM tipless cantilever. Also, the labrum's tip needs to be orthogonal with respect to the cantilever's surface, since the analytical models assumed the labrum's tip is mounted normal to the cantilever's surface.

Each step of the protocol is explained and performed. The mosquitoes are initially dead and frozen, and they are stored in a freezer. Becoming proficient at this experiment takes a lot of practice. Due to the nature of the work, it requires patience and dexterity. Consuming large amounts of caffeine compromised the feasibility of the experiment, because it can cause a surgical tremor (shacking hands during surgeries) which makes most of the steps hard to perform. A calm mindset is important because surgical tremors are to be prevented.

A preliminary step to the protocol is to investigate the mosquitoes under a microscope to ensure they do not have a broken proboscis. 10 out of 300 mosquitoes had a full proboscis, they are a scare resource for this experiment.

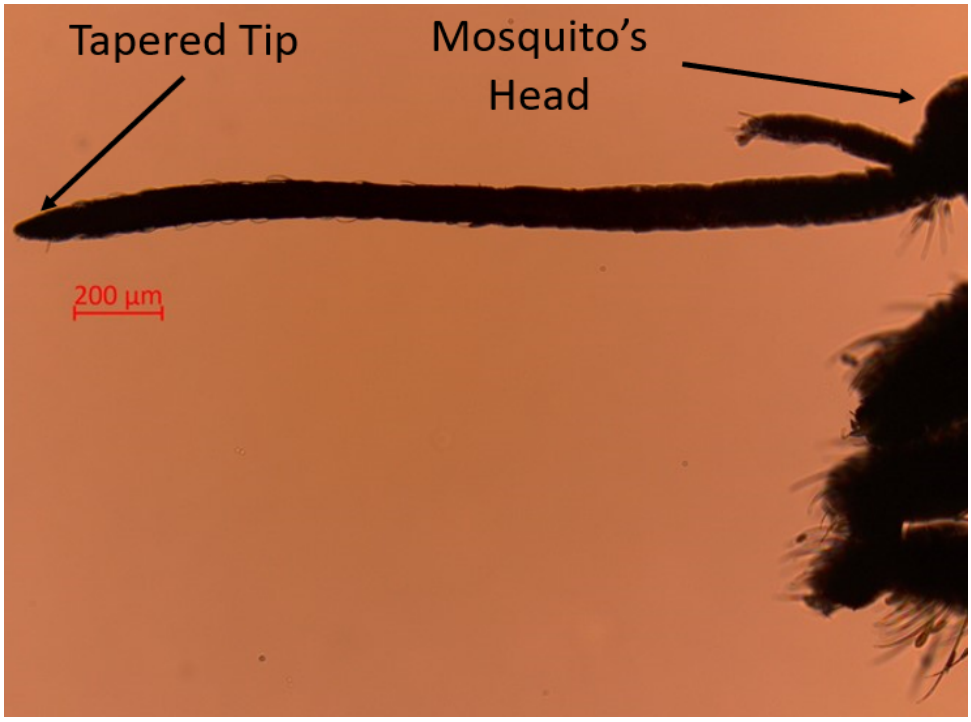

Figure 4.1: Mosquito (5X magnification)

A mosquito with a full proboscis can easily be identified since the proboscis has a tapered tip.

4.3.1 Step 1: Dissecting the labium from the cuticle

First, the labium covering the fascicles of interest needs to be dissected out of the proboscis. The labium is not to be glued to the cantilever beam as it is not what penetrates the victim's tissues. Nitrile gloves are greatly suggested for this step.

This step is done without the aid of any optical instrument over a glass slide. To dissect the labium, a scalpel was used to do an incision on the labium. This task is performed by gently pushing a scalpel on the proboscis until a small "toc" noise is heard. Next, the head of the mosquito is gently held back with tweezers. Ideally, no pressure is applied on the head of the mosquito because it can get ripped off and the experiment would be compromised. With another pair of slender tweezers, the labium is pinched and carefully pulled out of the proboscis. If the incision is properly made on the labium, it is easy to remove the labium thereafter.

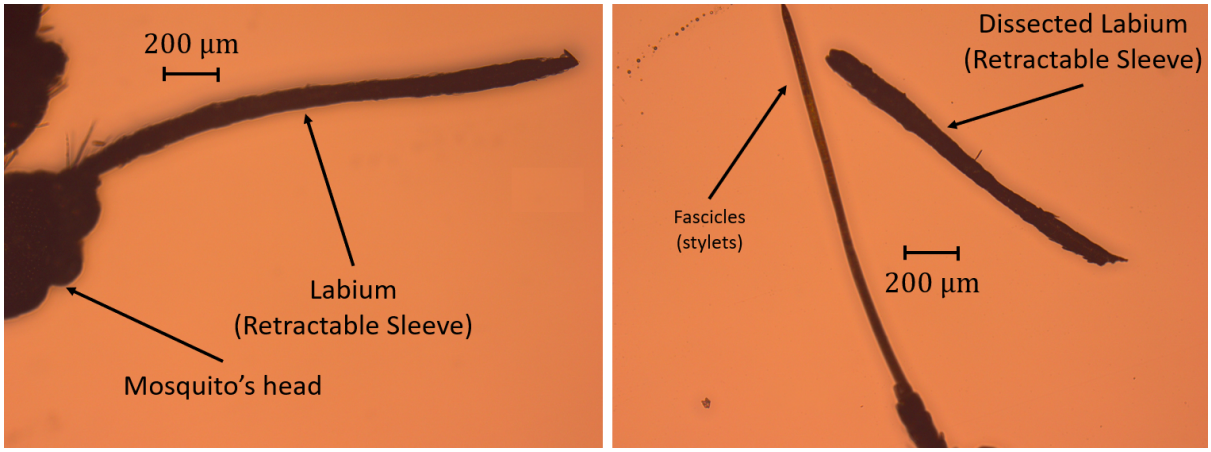

Figure 4.2: Dissected labium (5X magnification)

4.3.2 Step 2: Cutting the labrum's tip

This step is done without the aid of any optical instrument over a glass slide. The fascicles of the mosquito are now visible and, but since they are in a bundle together. From the Euler buckling analysis, it was determined that a labrum's tip of 300 µm will not buckle. Furthermore, after running this experiment multiple times, it was found that if the labrum's tip is smaller than approximately 200 µm long, it is hard to handle it afterwards. For these reasons, it is aimed to cut the tip of the labrum to a length of approximately 300 µm.

The tip of the labrum is gently cut with a scalpel. It takes practice to be able to repetitively cut the labrum's tip to a length of approximately 300 µm. Afterwards, sharp tweezers are used to push the labrum's tip over the glass slide because it will separate the mandibles and maxillae from the labrum. To know if this step is successful, after pushing the fascicles with a tweezer, the glass slide is investigated, if the fascicles are separated, this step was successful. Otherwise, the fascicles need to be pushed until they are separated. This step can take up to an hour as it is very challenging.

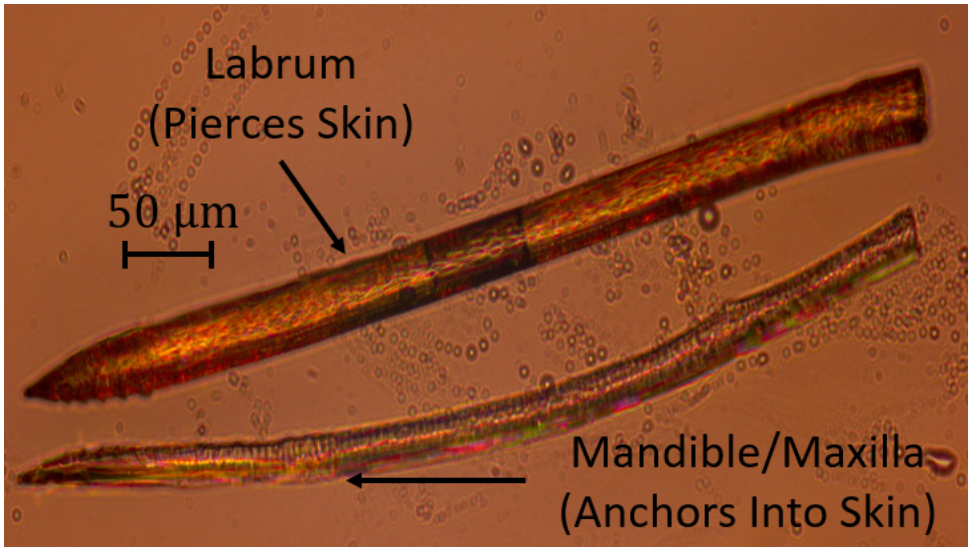

Figure 4.3: Labrum next to a fascicle (5X magnification)

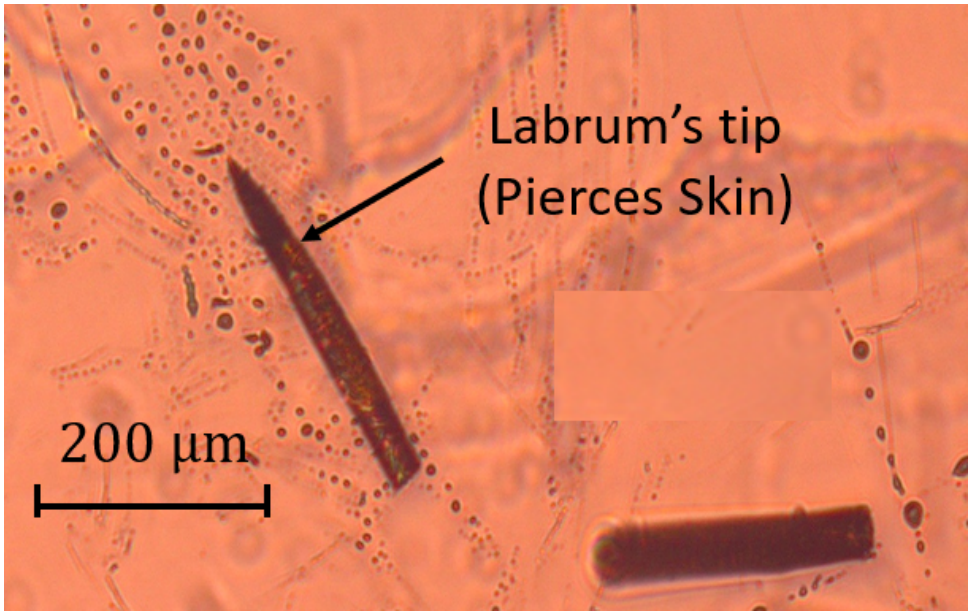

Figure 4.4: Labrum's tip cut to length (5X magnification)

The labrum is the fascicle that is not serrated and that is bigger in diameter than the others, thus once the fascicles are split apart, they are easy to distinguish.

4.3.3 Step 3: Gluing the Labrum's Tip to the Cantilever

This is the final step of the experiment to prove that it is feasible to make a probe for atomic force microscopy that used the labrum's tip of the mosquito. There are a lot of overlaps with the final step of the preliminary experiment where the bead is glued to the wafer.

Manipulation 1: Grabbing the Labrum's Tip with Tweezers

Sharp tweezers are used to gently tap the labrum's tip that is now cut to length. It is very complicated to touch the labrum piece with the tweezers and it can also take up to 1 hour based on the trials done. If this manipulation is successful, the labrum's tip will be on the tweezers instead of on the glass slide. This is to be confirmed by inspecting the tweezers under the microscope. This manipulation is iterative since tweezers tap the labrum and then they are inspected under the microscope and this is repeated until the labrum's tip is seen on the tweezers.

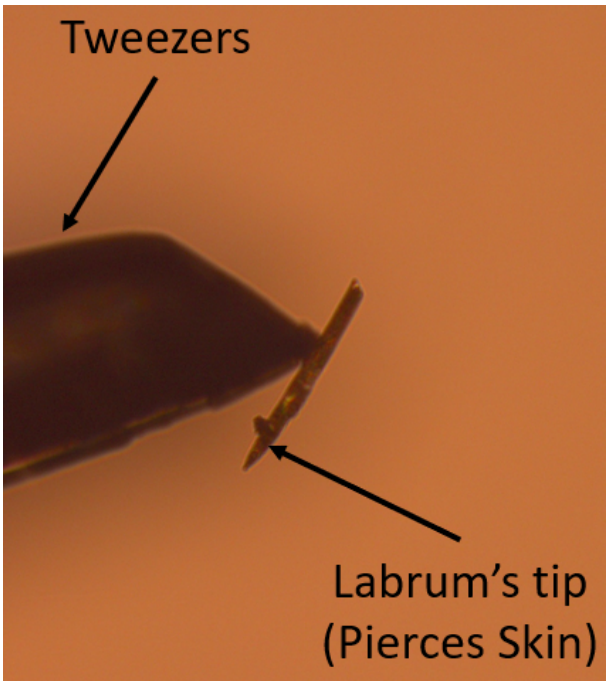

Figure 4.5: Labrum's tip on the tweezers

Manipulation 2: Applying Epoxy on the AFM Cantilever

Next, the same epoxy used for the preliminary experiment is used in the same way. A quantity of approximatelly 2 mL with a 1:1 mixing ratio is mixed. Only a small amount is dropped over a glass slide and spreaded near the edge of it. The glass slide is then placed under the Probe Station.

The AFM tipless cantilever is placed on the clamp using its housing. The cantilever is grabbed using tweezers, but if it is not grabbed properly it can easily be dropped. Keeping in mind that the thickness of the cantilever is 3 µm, it is very fragile, thus dropping it always breaks the cantilever if it hits any surface.

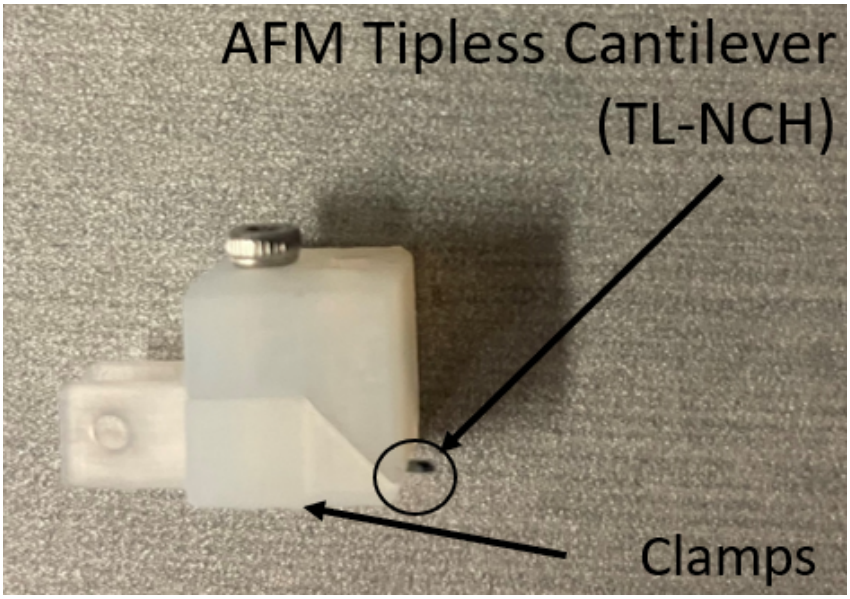

Figure 4.6: Cantilever clamped

The clamp containing the cantilever is mounted to the manipulator's assembly the same way the wafer was mounted (refer to Appendix A2). It is then placed on top of the edge of the glass slide that has epoxy. Using the manipulator, the cantilever is slowly lowered on the glass slide until it touches the epoxy. The force sensor is not sensitive enough to detect the contact of the cantilever with the epoxy but when they are touching, the cantilever will deflect, thus a visual inspection suffices to see that they are touching. Then, the cantilever is moved left and right and then taken away from the glass slide by using the manipulator.

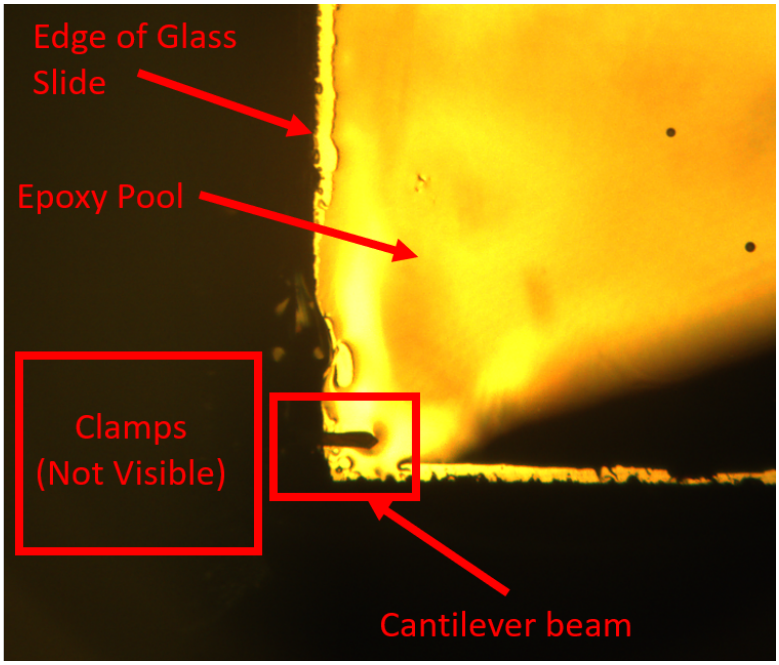

Figure 4.7: Epoxy applied on the AFM tipless cantilever

Manipulation 3: Gluing the Labrum's Tip to the cantilever

The tweezers with the labrum part is placed horizontally over the platform of the Probe Station (refer to figure 4.8). Having the labrum's tip over the tweezers, as opposed to underneath of it, is important. If the labrum's tip is under the tweezers, it can fall out during the gluing manipulation as it is poorly supported by the tweezers.

The manipulator is positioned sideways such that from the perspective of the microscope, the side view of the cantilever is observed (refer to figure 4.8). With this orientation, the bubble of epoxy over the cantilever is easily visible. It needs to be approximately at the center of the cantilever since it was determined the labrum's tip is to be glued at 110 µm along the beam. The The labrum's tip needs to be glued at the center of the epoxy bubble because otherwise, the labrum will shift towards the center of the bubble during the curing. If the labrum's tip is allowed to shift, it will not be orthogonal to the cantilever.

The cantilever and the labrum's tip need to be at the same height to be glued together. Only the height of the cantilever can be changed by using the manipulator (refer to Appendix A2). The height of the labrum's tip is fixed since the tweezers cannot move vertically. The Probe Station's microscope focuses on the labrum's end that need to be glued and the manipulator is used to change the height of the cantilever until it is also

focused meaning it is at the same height as the labrum's tip.

The cantilever is then very gently moved towards the labrum's tip using the manipulator. The labrum's tip is in contact with the cantilever once, through visual inspection, the cantilever pushes the labrum and the epoxy bubble starts to wrap around the labrum.

A picture is taken as soon as the cantilever is in contact with the labrum's tip to ensure that after the 8 hours curing period, the epoxy did not shrink and caused the labrum's tip to tilt.

A full cure takes 24 hours. Using different epoxies with different curing times would work as long as there is enough time to perform the manipulations before the epoxy cures.

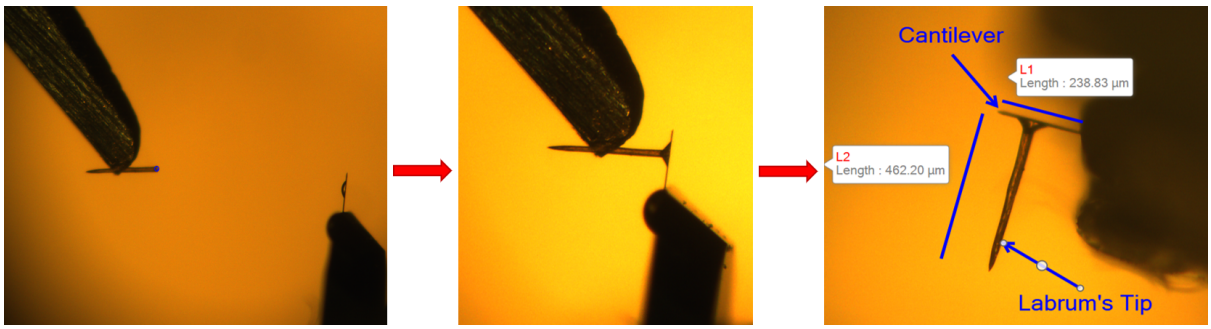

Figure 4.8: The cantilever and the labrum at the same height (left), the cantilever touching the labrum (middle), the cured probe (right)

4.4 Results

A better imaging of the probe can be done with SEM since the resolution is superior to the one offered by the probe station and the ZEISS microscope. The SEM also allows to obtain clear images of curved surfaces like this labrum's tip (refer to Appendices A1, A2 and A3).

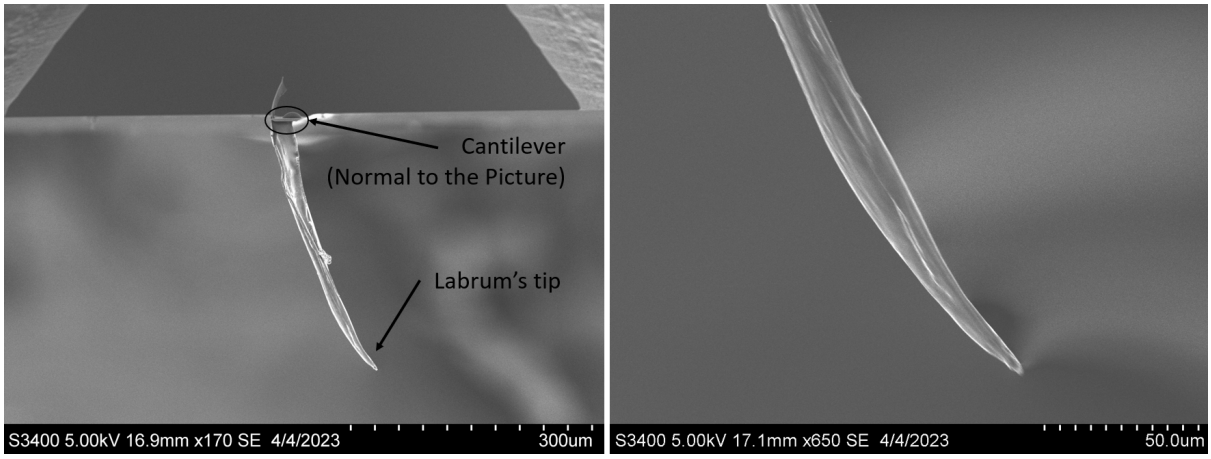

Figure 4.9: SEM imaging of the probe. Front view of the cantilever(left), close-up view on the tip (right)

The biological probe using a labrum's tip is successfully mounted to the tipless cantilever beam and the labrum's tip is tilted on the cantilever thus it is not orthogonal to the surface of the cantilever. It is clearly visible that the tip is not serrated, thus it the labrum as expected (since the other fascicles are serrated).

4.4.1 Results Analysis

Overview

The main objective of this research was met with this experiment. Making a biological probe using the mosquito's labrum's tip was proved to be feasible.

Difficulties

In order for the analytical models of the normal force applied on the tip, F_{N}, and of the Euler's buckling critical load, P_{cr}, to apply perfectly, the labrum's tip needed to be orthogonal to the cantilever. Therefore, the protocol needs to be improved to have a perfectly orthogonal tip mounted to the cantilever.

This probe can be used to test if it can pierce through different materials, but the analytical models needs to be reassessed to take in consideration the fact that the tip was tilted to obtain a good prediction of F_{N} based of the voltage applied by the piezo shaker and ensure the tip will not buckle.

Chapter 5

Conclusion

This thesis presented a literature review of topics of interests regarding mosquitoes to guide the experiments. A preliminary experiment was made to practice manipulations and to develop a protocol that is transferable to the main experiment which aims to conceive a probe for atomic force microscopy that uses the labrum's tip of the mosquito. The preliminary protocol was modified to perform the final experiment and mathematical models defined key parameters for the final experiment like the gluing position of the tip along the cantilever and the structural integrity of the probe. The feasibility of conceiving a probe for atomic force microscopy using the labrum's tip of a mosquito was shown to be possible. The protocol needs to be improved because the tip of the probe is not orthogonal to the cantilever, thus the mathematical models do not apply perfectly to the probe to predict the normal force experienced by the tip for a given voltage.

Bibliography

[1] D. Gurera, B. Bhushana, N. Kumarb, “Lessons from mosquitoes’ painless piercing,” *Journal of the Mechanical Behavior of Biomedical Materials*, Volume 84, August 2018, Pages 178-187. DOI:10.1016/j.jmbbm.2018.05.025.

[2] S. Aoyagi, Y. Takaoki, H. Takayanagi, C. Huang, T. Tanaka, M. Suzuki, T. Takahashi, T. Kanzaki, T. Matsumoto, "Equivalent Negative Stiffness Mechanism Using Three Bundled Needles Inspired by Mosquito for Achieving Easy Insertion,", October 2012.

[3] R. E. Snodgrass, "The Anatomical Life of the Mosquito," Volume 139, Number 8, November 1959, Page 58

[4] X. Q. Kong and C. W. Wu, “Measurement and Prediction of Insertion Force for the Mosquito Fascicle Penetrating into Human Skin,” *Journal of Bionic Engineering*, Volume 6, no. 2, pp. 143–152, Jun. 2009, DOI:0.1016/s1672-6529(08)60111-0.

[5] B. Bhushan, "Depth-Sensing Nanoindentation Measurement Techniques and Applications," March 2017 DOI:10.1007/s00542-017-3372-2

[6] C. H. Yoo and S. C. Lee, “Buckling of Columns,” *Stability of Structures*, pp. 1–73, 2011, DOI:10.1016/b978-0-12-385122-2.10001-6.

[7] 30 Minute Epoxy | Slow-Cure. bsi-inc.com. https://bsi-inc.com/hobby/slow_cure.html Accessed 28 October 2022

[8] BudgetSensors Atomic Force Microscopy, AFM Probes, https://www.tedpella.com/probes_html/budgetsensors-2.aspxAccessed 28 April 2023.

[9] how to cut SiO2 wafer. RBD TechSpot, (2013) https://www.rbdinstruments.com/blog/sputter-rate-standard/how-to-cut-sio2-wafer/ Accessed 22 September 2022

[10] TL-NCH AFM Probe. NanoAndMore. https://www.nanoandmore.com/AFM-Probe-TL-NCH Accessed 10 March 2023

[11] Ultrasonic Cleaning Principle. OPERATIONAL AND INSTRUCTION MANUAL. https://ultrasonicllc.com/images/uploads/general/1000M-2100M$_{Instruction_{M}anual.pdf}$ Accessed 8 October 2022

[12] Atomic Force Microscopy Working Principle — AFM Explained. Nanosurf https://www.nanosurf.com/en/support/afm-operating-principle Accessed 15 December 2022

[13] Scanning Electron Microscope - Advantages and Disadvantages in Imaging. MicroscopeMaster https://www.nanosurf.com/en/support/afm-operating-principle (2019) Accessed 14 January 2023

Appendix A1: ZEISS Microscope

The ZEISS microscopes is an instrument used to magnify microscopic objects to capture details that from the nature of the objects are impossible to see by the naked eye. The objects studied need to be placed over a glass slide that has the right dimensions to be properly mounted to the microscope. It can be used to take measurements or to evaluate the surface of an object to see imperfections or to see if the object is damaged. It uses different lenses that are mounted to the microscope that ranged from 5X magnification to 100X magnification for the ZEISS microscope from the *Nanofactory Lab*. The microscope can use bigger magnifications but those were not necessary for this research.

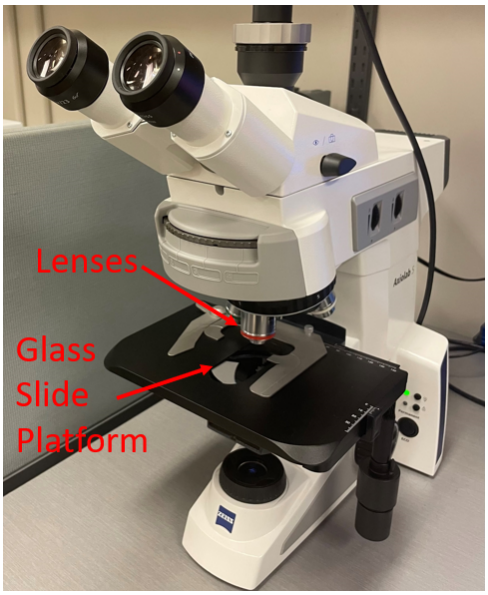

Figure 5.1: ZEISS Microscope

Limitations

The drawback of this type of microscope is it cannot capture a clear image of a curved surface. In which case multiple pictures need to be taken to visualise different regions along the surface. This was a difficulty encountered when dealing with beads from the preliminary experiment and when dealing with mosquitoes in the final experiment of this thesis. to look at those surfaces, multiple pictures needed to be taken by adjusting the focus in order to capture all the details of the surface studied.

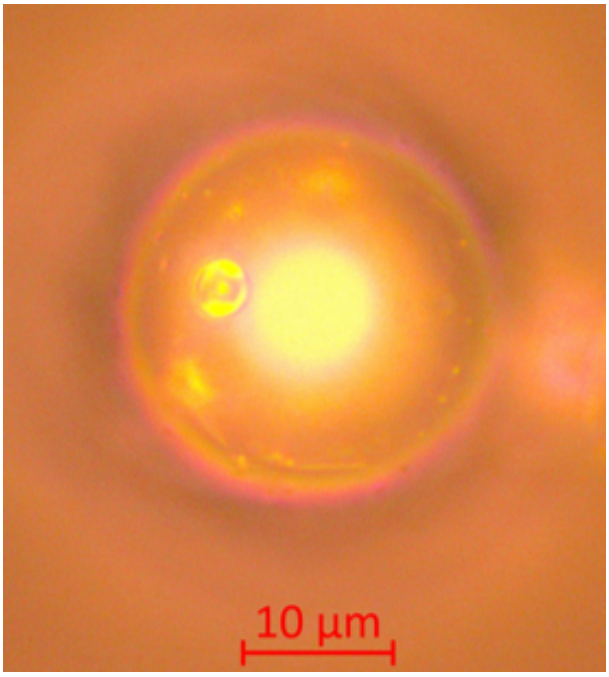

Figure 5.2: Blurry Bead

Figure 5.2 shows a bead where the surface is overall blurry but some details are clear. Multiple pictures are required to fully characterize curved surfaces like these.

Appendix A2: Probe Station

The probe station shares similarities with the ZEISS microscope and offers additional features which makes it indispensable for this research. It can be used to move microscopic parts using its manipulator and run different kinds of experiments.

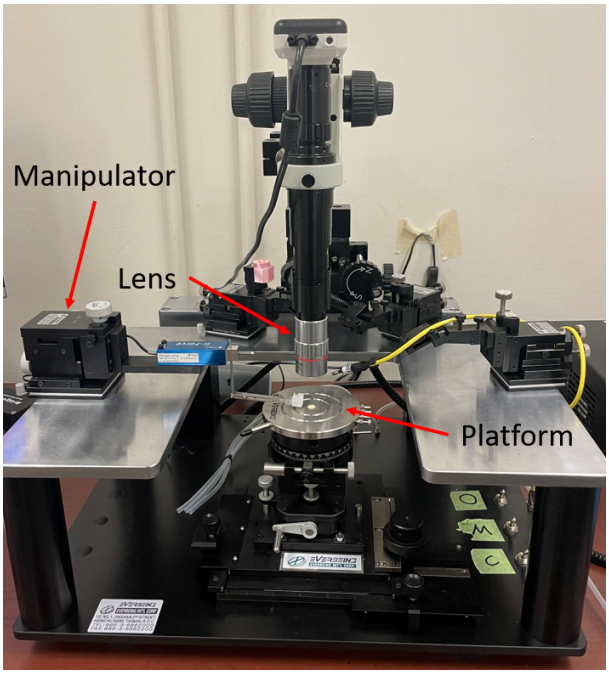

Figure 5.3: Probe Station

Similarities with the ZEISS Microscope

Both microscopes can be used with different magnifying lenses to study surfaces of objects. In the case of the Probe Station, it is equipped of a single lens that can be swapped but it also has built-in lenses that allows the user to vary the magnification. It can also be used to inspect objects on a glass slide but it does not need to be on a glass slide as the platform of the probe station is bigger and does not clamp the glass slide or any other surface onto which the object is mounted. The platform can also be removed if necessary as it was done when experimenting with mosquitoes.

Manipulators

The manipulator is what make this instrument unique and very valuable for this research. It allows to mount a lever arm on which clamps that were designed for this project can be used. Small pieces of wafers and AFM cantilevers can be mounted to the clamps for gluing beads or for conceiving the probe that uses the mosquito's needle in the final experiment. The clamps are held together with a bolt. They are self-tightening since the clamps' halves have tapped holes (M2 threads). To achieve this design, they were printed using the *FormLab 2* stereolithopgraphy (SLA) 3D printer using layer heights of 50 µm to capture the features of the tapped holes.

The manipulator uses three gears to control three degrees of freedom (DOF) that are in translation. Those can be used to position the clamps at a position of interest under the microscope.

The manipulator is also equipped with a force sensor. It senses vertical loads applied to it and can thus be used for experiments using very fragile equipment that is prone to breaking like AFM cantilever beams. It has a software that receives very sensitive voltage readings for very little loads. It can be used to ensure there is not an excessive force being applied to an AFM cantilever and hence ensuring it will not break.

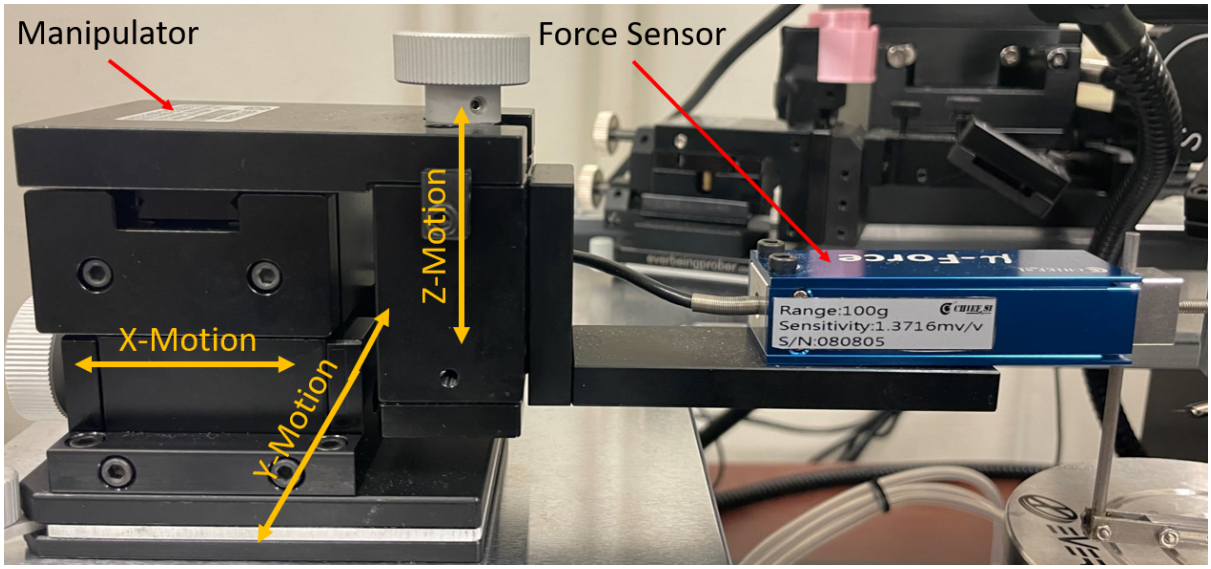

Figure 5.4: Manipulator of the Probe Station

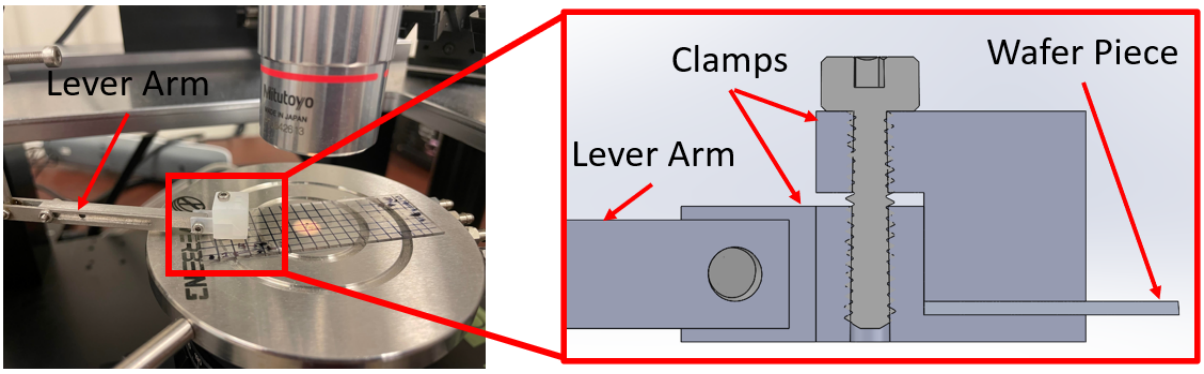

Figure 5.5: Clamps assembly

Limitations

The Probe Station cannot take a singular picture that captures with clarity a curved surface. It also offers a lower resolution than the ZEISS microscope so when the goal is to study the flat surface of an object, it is always better to use the ZEISS microscope. The image from the screen has very low frames per second,thus requiring an experienced user and makes experiments overall harder and take longer time to perform since every movement has to be made very slowly to avoid manipulation mistakes that are hard to capture on the screen due to this low amount of frames per second.

Appendix A3: Ultrasonic Cleaner

The ultrasonic cleaner allows to clean surfaces of objects on a microscopic level using the principle of cavitation. The cavitation is caused by transducers that generate ultrasonic waves that propagate in a liquid medium. The surfaces needs to be in contact with the liquid medium of the ultrasonic cleaner in order to get cleaned.

Figure 5.6: Ultrasonic cleaner

Cavitation Working Principle

When ultrasonic waves are generates within a liquid medium (typically water in the ultrasonic cleaner), the waves causes local high and low pressure regions within the liquid. These regions create bubbles within the liquid. Those bubbles are surrounded by high pressure because of the ultrasonic waves causing them to eventually implode by collapsing within themselves. This implosion generates a high burst of energy which is seen as a jet of the liquid originating from the center of the imploded vapor. Those liquid jets are shot in a random orientation within the liquid. This is know as cavitation [11].

When objects are submerged inside of the liquid experiencing cavitation, they get hit by those high energy jets of liquid which have dimensions in the order of microns. The jets hit any debris that is stuck on the surface of the submerged object and pushes it out of the surface to be cleaned. By allowing cavitation to occur over a period of time — which varies depending on the size of the surface to be cleaned and the nature of the debris — the surface in question can be entirely free of debris that can be otherwise hard to clean with chemicals.

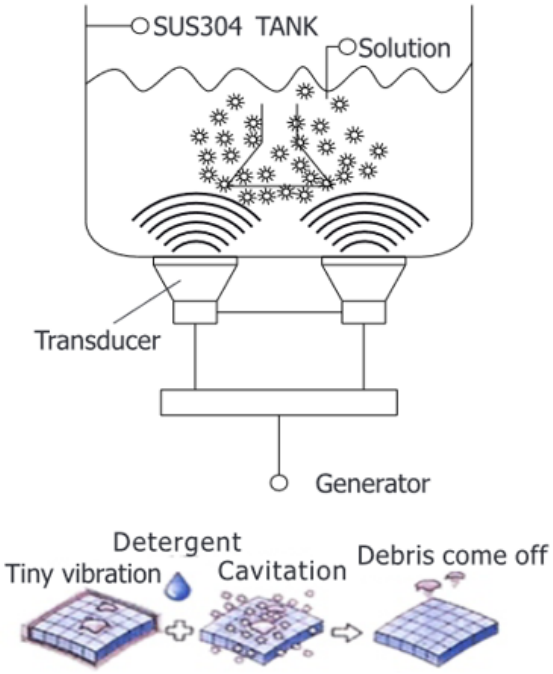

Figure 5.7: Schematic of the cavitation working principle [11]

Limitations

As the cavitation effect takes place, there is a temperature rise of the liquid medium over the entire operating time of the cleaner. If the water is allowed to get too hot it can exceed the recommended operating range by the manufacturer and hence damaging the instrument and reducing the efficiency of the cleaning.

The rapid expansion and collapsing event of the bubbles results in friction of the bubbles with the liquid medium which is the main contributor the the rise in the liquid's temperature.

Appendix A4: Atomic Force Microscopy

Atomic force microscopy (AFM) is a high resolution imaging technique allowing to perform a broad range of studies which all require the usage of a probe [12]. The probe of the AFM is composed of 3 parts:

- It has a chip which is attached to the shaker piezo of the AFM. The shaker piezo can be used to make the cantilever vibrate (it is inactive for topography studies but it mounts the chip to the rest of the AFM).
- It has a cantilever mounted to the chip. Depending on the nature of the study, it can be made of different materials and it can have different dimensions. For example, a silicon cantilever can have a length, thickness and width of 200, 3 and 30 microns respectively.
- It has a very sharp tip positioned on the cantilever at the center of its width and near the edge along the cantilever's length. It can be made of different materials and thus can vary greatly in cost because they can be made from silicon or diamond for example. The material selection can depend on how long the tip is to be used, the surface roughness of the surface in contact with the tip, the type of study to be made, etc.

Figure 5.8: Atomic force microscope

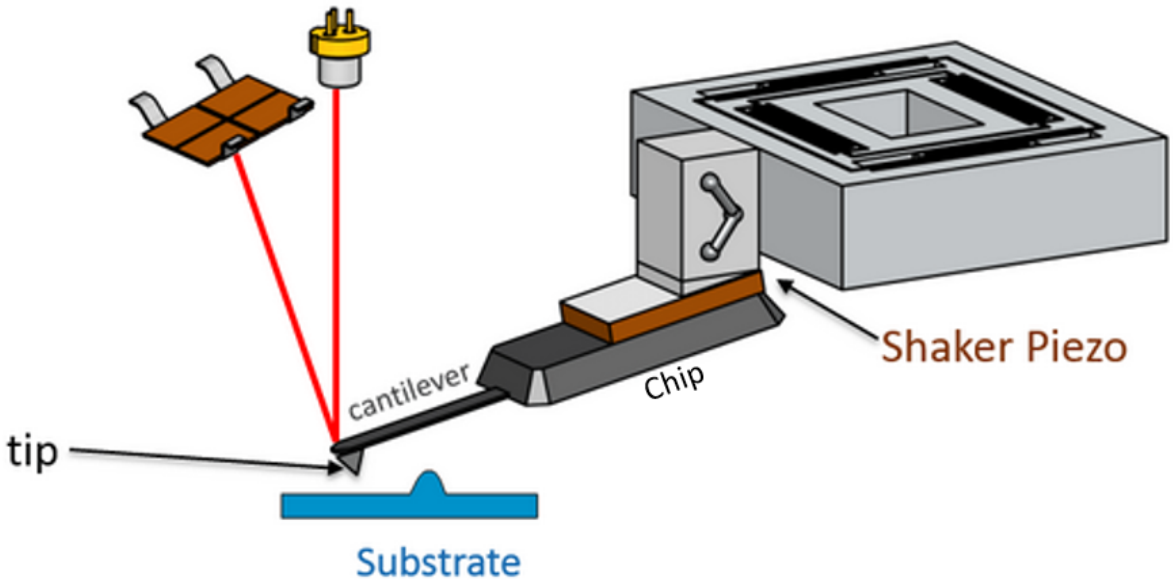

Figure 5.9: Schematic of the AFM probe [12]

For example, one can obtain the topography of a surface of interest, on a microscopic level, by scanning a probe over it. A cantilever that can easily bend for very small loads is preferred. In which case, the cantilever is generally made of silicon and will have a very low stiffness by having a width of approximately 3 microns. The tip of the cantilever is brought in contact with the surface of interest and it scans the surface. As it hits regions of higher or lower altitude, the cantilever deflects so that the tip is always at the top of the surface. There is a laser pointing on top of the tip's base and as the cantilever deflects, the laser is reflected to different angles depending on the bending of the cantilever (thus the height difference experienced by the tip). This laser is deflected on the position-sensitive-photo-detector which reads the angle as a vertical displacement of the probe's tip. By scanning over a surface using this method, the topography of the surface is obtained. The resolution for these studies can be in the order of a few nanometers and it is limited by the tip. A very slender and sharp tip is preferred for these studies as they can capture the surface's topography with a greater resolution. The described mode of operation is called *contact mode*.

Another study of interest that can be done using atomic force microscopy and which is of great interest for a probe that utilises mosquitoes is called *force spectroscopy mode*. It allows to apply different loads on the tip of the probe. If a probe uses the mosquito's labrum as the tip, operating the AFM in this mode would allow to test different insertion force that mosquito apply on their victims. This can be used to test different materials if such a probe is successfully made.

Limitations

Atomic force microscopy can be very expensive. The probes used can cost hundreds of dollars and they are very fragile so they are prone to breaking.

Appendix A5: Scanning Electron Microscope

A scanning electron microscope (SEM), uses electrons to get very high resolution images of a sample. It is very different from a traditional microscope (like the Probe Station or the ZEISS microscope) that use visible light to produce magnified images. In scanning electron microscopy, there is a focused beam of electrons that hit the object to scan the surface of it thus creating an image. As opposed to conventional microscopes, it can image, with high resolution, a curved surface. Electrons produce signals by interacting with the atoms on the surface of the object to get an image.The image resolution can vary from 1 to 20 nm (depending on factors like the type of surface studied), thus they are often used for nanotechnology and biological studies [13]. This thesis studies the biological needles of mosquitoes which are microscopic and generally not flat objects so the SEM is a very valuable apparatus for imaging.

Figure 5.10: Scanning electron microscope

Limitations

To obtain SEM images, the chamber of the microscope needs to be vacuumed so the nature of some materials can prevent them from being scanned with this microscope. Also, the material of some objects might charge as they receive the beam of electrons. This can emit secondary electrons causing a positive charge on the surface of the object thus distorting the image. To prevent the sample from charging, there are very thin layers of electrically conductive materials that can be applied on the surface to dissipate the charge.