Deposition of Avidin Protein on Silicon Oxide Substrate

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Abstract:

Chemomechanical functionalization of chemical surfaces is a field of chemistry and physics which has attracted a great deal of attention over the last decade. The atomic force microscope has played an instrumental role in the advancement of nanotechnology using such methods. In my research we investigated the effectiveness of depositing a stable Avidin protein monolayer on a silicon oxide surface. This was accomplished through a multi-step process involving surface chemistry. The atomic force microscope and ellipsometer were used in measuring the thickness and verifying the presence of each layer formed during this multi-step process.

Introduction/Background:

Nanotechnology, including the development of nanostructures and monolayers on the nanoscale, is at the forefront of science and research. Surface modification and surface chemistry have come to play a major role in this research. One of the first methods developed to manipulate and/or chemomechanically functionalize surfaces was microcontact printing. A well-known group making significant contributions to the field of surface chemistry and modification is the Whitesides Group. They have created self-assembled monolayers on organic surfaces from alkanethiols. The group accomplished this through the implementation of a PDMS stamp with Au or another noble metal. [1] Microcontact printing has proved to serve as a useful tool in surface modification. This method has been used in growing patterned polymers on silicon oxide surfaces. [2]

As technology improved nanostructures were able to be developed on surfaces. Among the methods used to accomplish this is dip-pen nanolithography (DPN.) Chad Mirken and his research group [4] are well known for their DPN methods. They have successfully created nanosize structures of specific shapes through "wet-chemical etching." DPN is used to create layers of Au, Ag, and Pd. Through a "direct-write method" the Mirken Group has been able to use various inks to create single and multi-component nanostructures. This is a significant improvement from previous surface modification technology.

Also found within the same realm of nanotechnology is nanografting. This method of surface modification is able to produce nanostructures of specific size and geometry. This method of nanofabrication demonstrates an improvement in the control of shape, size, and location of these structures on the surface. At the forefront of

nanografting is G. Liu of UC Davis and her research group, who have successfully produced nanostructures of single-stranded DNA through the use of nanografting. [3] This method requires the use of the atomic force microscope to scribe or shave off of a layer of surface molecules. This is conducted in the presence of a fluid which allows the chemicals to react and produce a new self-assembled monolayer where the scribing was performed. The nanostructures developed by this technique have been found to be stable and allow researchers to study the conductivity of these structures based on their size.

These nanografting experiments consist of imaging under a lower force to obtain an initial image of the surface. Following the initial imaging of the surface the tip is pushed down with a greater force on the surface in the presence of the reacting fluid or molecules, which is known as the scribing step of the process. The molecules in the reaction mixture almost simultaneously attach to the surface where the scanning tip has just scratched, thus creating the self-assembled monolayer.

Another effective method to manipulate surfaces on the nanoscale is chemomechanical surface patterning and functionalization, as demonstrated by Brent Wacaser. [5] His techniques use the atomic force microscope to scratch the surface of a hydrogen-terminated silicon wafer, which breaks the Si-H and Si-Si bonds. This technique enables the surface to react with various molecules such as alkenes, alkynes, alkyl halides, alcohols. Wacasser attaches an AFM tip to a fluid cell and immerses it in a liquid containing the reactive molecules. The fluid is placed on the silicon sample wafer and the tip is lowered down into the fluid and prepared to scribe. Smaller, more durable silicon-nitride coated tips are used in these experiments, making it easier to control the size of the modifications on the surface and reduce the damage to the surface. The same

tip is used throughout a single experiment to image, scribe, functionalize the surface, and then image again to see the results. Brent Wacasser's research represents several improvements in the ability to fabricate new surface molecules on the nanoscale with greater accuracy and less damage to surface.

Playing an essential and monumental role in the advancement of nanotechnology and development of nanostructures is the atomic force microscope (AFM.) The AFM is capable of producing images on the nanoscale, 10-9, as it is not an optical microscope. It functions by dragging a tip or probe across a surface. The part of the microscope to which the tip is attached is called the cantilever. As the cantilever and tip are being dragged along the surface a laser is deflected off the top of the cantilever into a photodiode detection system (See Figure 1). Through the use of advanced computer software the motion of the tip and cantilever are able to produce a three-dimensional image on the computer. This technology provides an avenue for vast improvements and developments in the nano-world. The two most commonly used modes on the AFM are contact and tapping. Contact mode is when the tip is dragged along the surface while always maintaining contact with the surface, while tapping mode is when the tip "taps" the surface at a certain frequency in order to obtain the desired image. On a normal scan using the atomic force microscope in contact mode, objects can be imaged or measured to widths as small as 5 nm across, or even smaller.

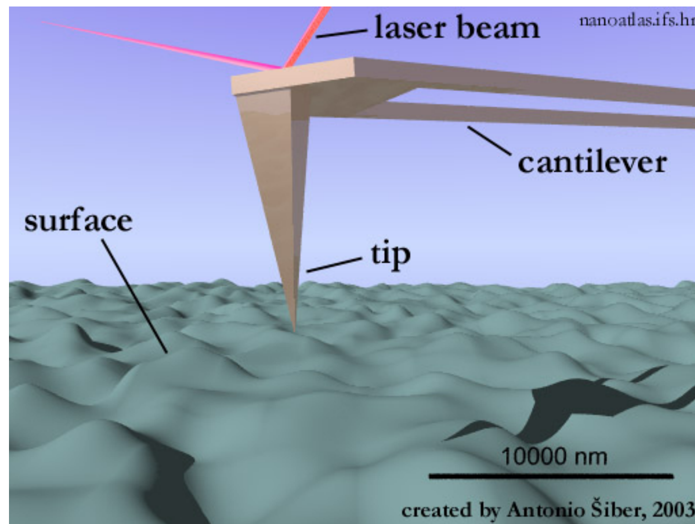


Figure 1

www.nanotech-now.com/.../antonio-siber.htm

Another key instrument used in nano-scale research is the ellipsometer. This instrument measures thicknesses of layers found on semi-transparent thin films. The ellipsometer contains a laser, a polarizer, and a quarter wave plate. The use changes the angle of polarization which in turn changes the state of polarization from linear to elliptical to circular. The laser is reflected off the layer we are trying to measure into a detector and then data is analyzed to obtain the desired results. The resulting reflection of the laser off the surface will vary depending on the polarization of the light, and the light which passes through the transparent substrate causes a phase-change in the incoming wave. This change in phase is dependent on the refractive index of the substrate. The ellipsometer is capable of measuring thicknesses of layers from 1 nanometer up to multiple micrometers in thickness. Further details of the function of the ellipsometer are beyond the scope of this paper, but this instrument has proved to be an excellent form of verification and analyzation of results in my research. Below is a schematic diagram of an ellipsometer.

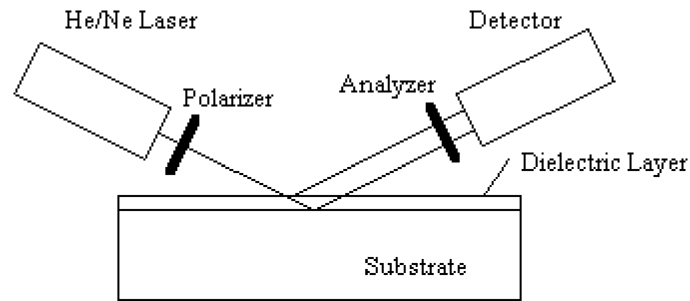


Figure 2

http://ece-www.colorado.edu/~bart/book/ellipsom.htm#principle

Similar to chemomechanical functionalization of silicon surfaces and nanografting, protein patterning is an alternative method to modify a surface. This method consists of putting down a stable monolayer and then scribing and patterning that layer with protein molecules. Avidin-biotin chemistry plays an essential role in this process. Avidin is a glycoprotein found in egg whites, and it is about 2-4 nm in height. Avidin is advantageous due to its tetrameric structure and its high resistance to denaturation in extreme conditions. This will allow it to form a very stable monolayer on our surfaces. Avidin is probably most widely known for its high binding affinity for biotin and the resulting avidin-biotin chemistry. As a result of the chemical nature of these two molecules they can be used to pattern protein on surfaces. First the avidin protein is deposited on the surface then a uniform layer of biotin is bound on top of the avidin. Finally the AFM tip is used to scrape through the biotin in the presence of an additional biotinylated protein molecule. The patterning of proteins in specific locations

on surfaces has many applications in the fields of biology and chemical engineering. Various molecules can be attached to protein molecules such as metals.

In my research I investigated the formation of an avidin protein monolayer on a silicon oxide substrate through a multi-step chemical process. My research consisted primarily of the formation of Avidin protein layers, which is a necessary step for protein patterning on a silicon surface. The objective of this project is to manipulate silicon surfaces through a three step chemical process involving a silane group, a glutaraldehyde cross-linker, and avidin protein. The Avidin protein monolayer needs to be uniform and stable. Avidin is used due to its high affinity for biotin, making it an effective method for patterning protein on surfaces. Producing an even and stable avidin monolayer is an essential step in this process. Ellipsometry is used to measure the different layers created and deposited in this process. The atomic force microscope is used to verify the formation of the monolayers and demonstrate the ability to remove these layers through scribing and nanoshaving techniques.

Procedure

The first step in avidin deposition on a silicon oxide surface is to clean the SiO_2 sample. The samples were cleaned with a heated pirana solution (concentrated H_2SO_4, 30% H_2O_2 in a 3:7 ratio.) This removes any excess carbon or unwanted molecules from the native oxide surface. We had relatively good success with the pirana solution, but a faster, more efficient method of cleaning the silicon wafers was introduced during the experimentation process. The plasma cleaner takes only 5-10 minutes and removes all contaminating molecules from the surface. You simply place the sample or samples in

the cleaning chamber, seal it, turn it on, and make sure there is the correct flow of oxygen for it to function properly. Once it is cleaned correctly let it run for the prescribed time and then remove the samples. This method generated clean of surfaces approximately five times faster than the previously used pirana cleaning method. As a result, from this point on we implemented the plasma cleaner as our method of cleaning the silicon oxide surfaces. After the surfaces were clean we measured the native oxide layer using the ellipsometer as explained in the introduction. Then I would deposit 3-aminopropyltriethoxysilane (APTES) on the SiO2 surface by pipeting it on the surface and letting it react at room temperature for 10 minutes. By doing this an amine group is attached to the oxide surface. This alone is not sufficient to attach the Avidin protein, but a cross-linker is necessary to deposit the protein on the surface. After the APTES had reacted, isopropanol was used to rinse off the surface. An alternative method involving APMS rather than APTES was also used with some success. The procedure for depositing this mono-silane molecule on the surface is the same as the APTES procedure with the addition of heating the sample in an oven rather than allowing it to react at room temperature. APTES proved to be the preferred method due to lack of stability with the APMS monolayer. Following the rinsing the thickness of the sample was again measured using the ellipsometer, which verifies the presence of an APTES monolayer on top of the native oxide. Following this step a glutaraldehyde cross-linker (Glutaric Acid) is pipeted on the surface and left to react at room temperature for approximately 2 hours. Once this reaction is complete the glutaraldehyde is carefully rinsed off with PBS buffer and then with Milli-Q water. Again ellipsometry was used to measure the new thickness of the

sample and verify the presence of the new glutaraldehyde layer. At this point the sample was ready to have Avidin protein deposited and allowed to react.

Before using the Avidin protein, I tested the samples with a different, less expensive type of protein known as Bovine Serum Albumim (BSA). I deposited this protein on the surface and let it react for varying times until a layer of protein was obtained. After the layer of BSA protein was successfully formed, I proceeded with the Avidin protein in a similar manner. Initially I would just pipet Avidin on the surface and let it react in air for a relatively short amount of time (2-3 hours.) I found this method did

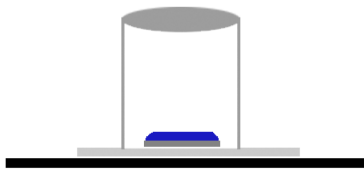


Figure 3

not give very promising results so I increased the reaction time significantly to times varying between 24 and 72 hours. I discovered that when I let the sample react for longer than 24 hours the samples would dry out leaving the resulting layers and data invalid and not useful. To combat this problem I created a humid environment in which the Avidin protein could be left to react for extended periods of time without drying out. (See figure 3) To accomplish this I would roughly measure out multiple layers of paper towels (approximately 20 layers) to form a roughly 6 x 6 inch square. Underneath these many layers I would place a single layer of parafilm down on the lab bench to reduce the possibility of contamination. I would then place the paper towels on the parafilm and

thoroughly soak them with Milli-Q water from a squirt bottle, at which point I would place the samples on top of them. I would then pipet the Avidin protein solution on the samples at varying concentrations and cover the entire system with a clean 500 mL glass beaker. To maintain the humid environment I used a transparent and durable tape combined with parafilm to seal the edges of the beaker to the lab bench. I would closely monitor the reaction until the time was complete. Once the prescribed time had passed I would carefully remove the samples from the paper towel surface and rinse them with PBS buffer and Milli-Q water. Lastly the new thickness of the samples was measured using the ellipsometer, which verified the deposition of the Avidin protein monolayer. Multiple samples were usually used during each step of the procedure to secure accuracy in the experimentation. The AFM was also used to image the APTES and glutaraldehyde surfaces as a second form of verification that they were depositing on the surface as indicated by ellipsometry.

Results/Discussion:

In my research there were two methods used to measure and verify the presence of monolayers in each step of the process. Ellipsometry was implemented to measure the thickness of each layer through a method involving the reflection of plain polarized light off the surface of the sample. The ellipsometry data is contained in Table 1 below. The table represents the layers of natural oxide on silicon following cleaning with pirana or plasma cleaner, APTES, glutaraldehyde, and BSA or Avidin protein. Multiple trials were performed first with BSA protein to be sure the methods would be successful prior to using the more expensive Avidin protein. Once stable layers of BSA were consistently

obtained I proceeded in forming Avidin protein layers using the same methods as described above. The following results were obtained.

Table 1

Sample	SiO_2 layer	APTES(15 min)	Glut. (1 hr 40 min)	Avidin (70 hr 45 min)
1	1.766 nm	.622 nm	.855 nm	7.712 nm
2	1.910 nm	.851 nm	.670 nm	7.999 nm

Sample	SiO_2 layer	APTES(10 min)	Glut.(2 hrs)	BSA (70 hrs)
1	1.969 nm	1.254 nm	1.338 nm	2.417 nm
2	1.979 nm	.635 nm	1.179 nm	2.326 nm
3	1.997 nm	.748 nm	1.563 nm	2.041 nm
4	1.979 mm	.995 nm	1.295 nm	2.778 nm
5	1.784 nm	1.306 nm	.860 nm	2.509 nm
6	1.804 nm	1.045 nm	.906 nm	2.219 nm
7	1.813 nm	1.064 nm	.944 nm	2.748 nm

Sample	SiO2 layer	APTES(10 min)	Glut.(1 hr 55 min)	BSA (17 hrs)
1	1.805 nm	.656 nm	1.618 nm	.179 nm
2	1.823 nm	.631 nm	1.275 nm	.153 nm
3	1.857 nm	.607 nm	1.346 nm	.190 nm

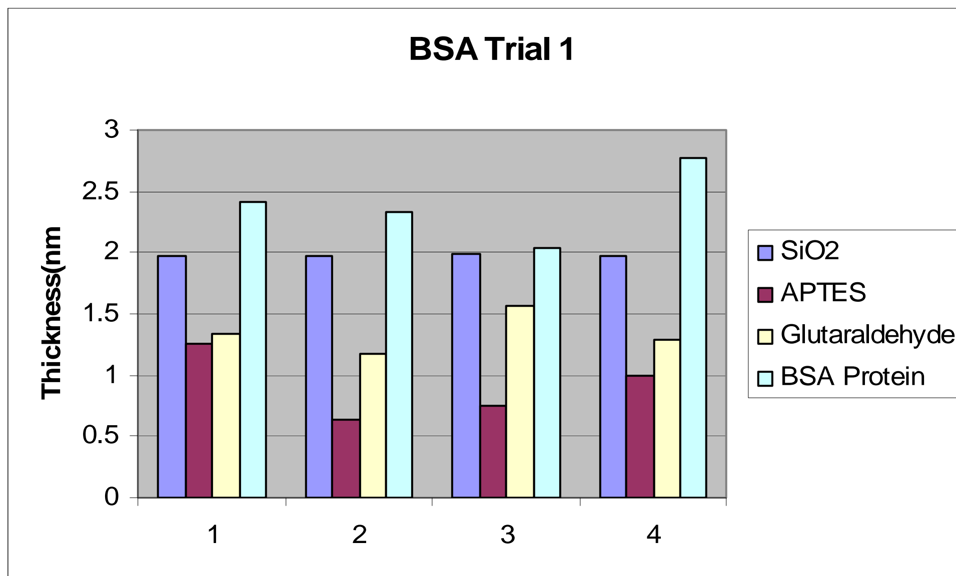
Sample	SiO2 layer	APTES(10 min)	Glut. (2 hrs)	Avidin (133 hrs)
1	1.837 nm	.657 nm	.692 nm	3.517 nm
2	1.825 nm	.689 nm	.835 nm	4.382 nm
3	1.853 nm	.682 nm	.896 nm	4.179 nm

Sample	SiO2 layer APTES(10 min)		Glut. (2 hrs)	Avidin (161 hrs)
1	2.680 nm	.801 nm	.877 nm	2.686 nm
2	2.752 nm	1.695 nm	.482 nm	3.030 nm
3	2.747 nm	1.346 nm	.596 nm	3.089 nm
4	2.670	.631 nm	1.257 nm	2.543 nm

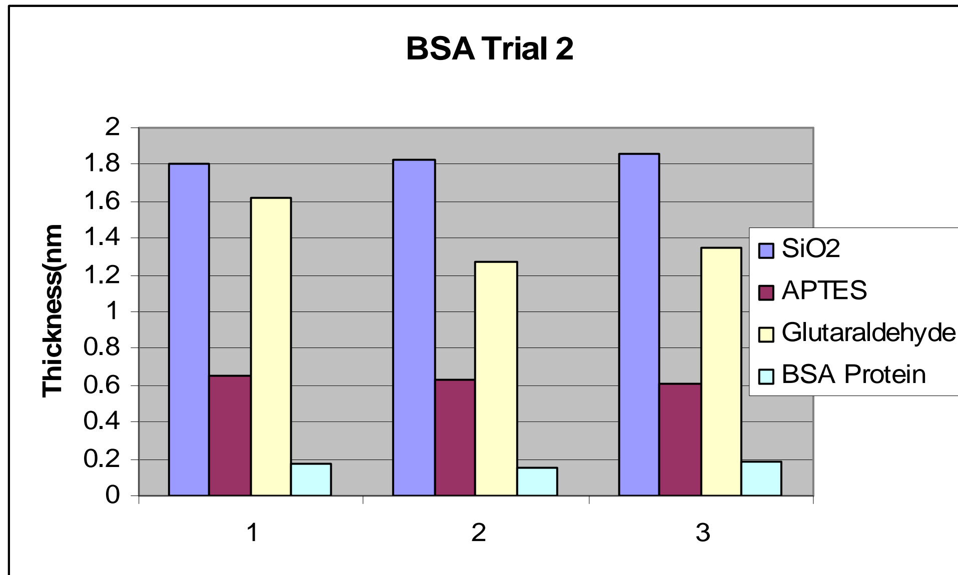
nm

The ellipsometry data from the table above has been organized into the following graphs depicting the varying thicknesses of each layer for both BSA and Avidin protein deposition. (See Graphs 1 - 5)

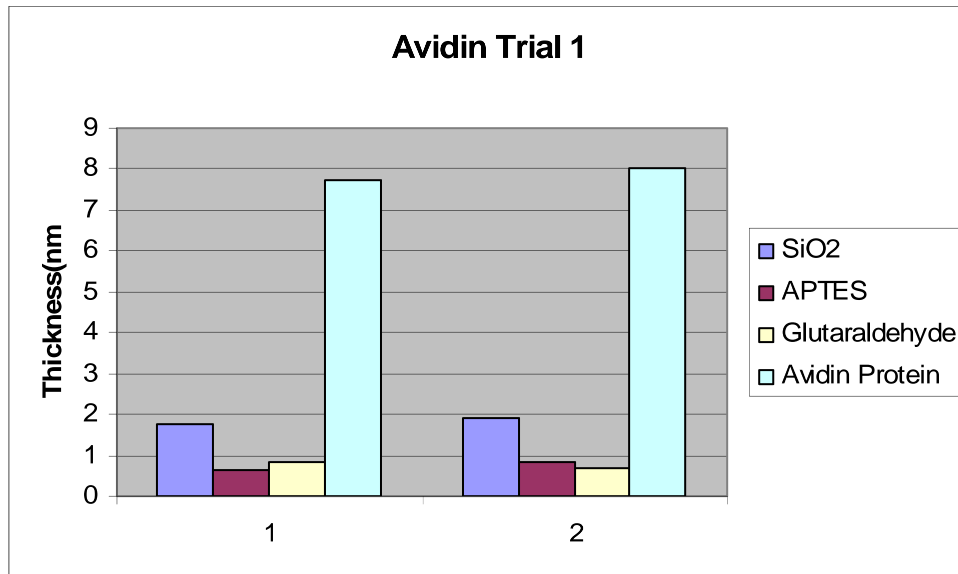
Graph 1



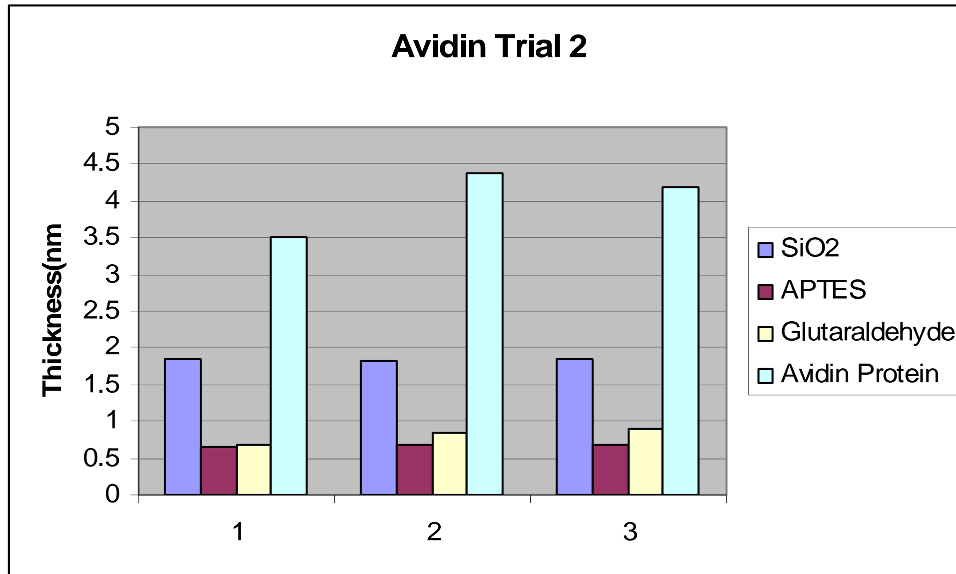
Graph 2



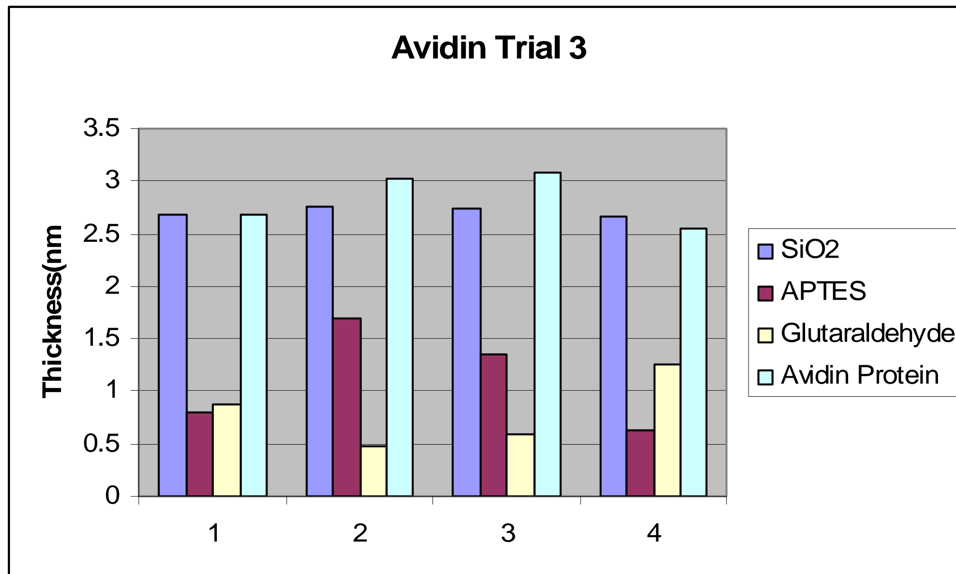
Graph 3



Graph 4

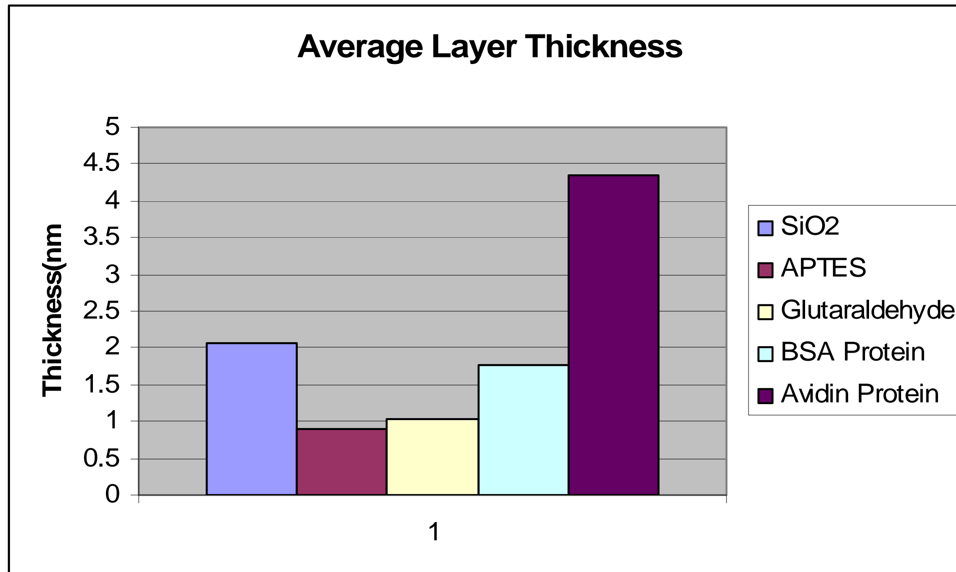


Graph 5



As displayed in graphs 1-5, the final thickness of BSA and Avidin protein can vary. Graph 6 below contains the averages of each layer throughout the various trials.

Graph 6



As apparent in the data found in the table and graphs above stable and consistent layers of native oxide, APTES, Glutaraldehyde, and protein were achieved along each step of the chemical process. The average thicknesses were 2.07 nm SiO_2, 0.89 nm APTES, 1.03 nm Glutaraldehyde, 1.76 nm BSA protein, and 4.35 nm Avidin protein. (1.56 nm APMS)

The average thickness of Avidin protein monolayer can be calculated in the following way:

Avidin Molecular Weight 66,000 g/mol

Average Protein Density 1.41 g/cm^3

(66000 g/mol)/(6.022x10^23 atoms/mol)/(1.41 g/cm^3)/(10^7 nm^3) = 77 nm^3

Assuming the Avidin protein molecule was a box each side would be approximately 4 nm in width. This would then give an estimated monolayer of Avidin protein with a thickness of 4 nm. With this calculation and estimation it is fairly obvious that the average thickness of Avidin protein of 4.35 nm obtained through ellipsometry methods verifies the formation of a protein monolayer.

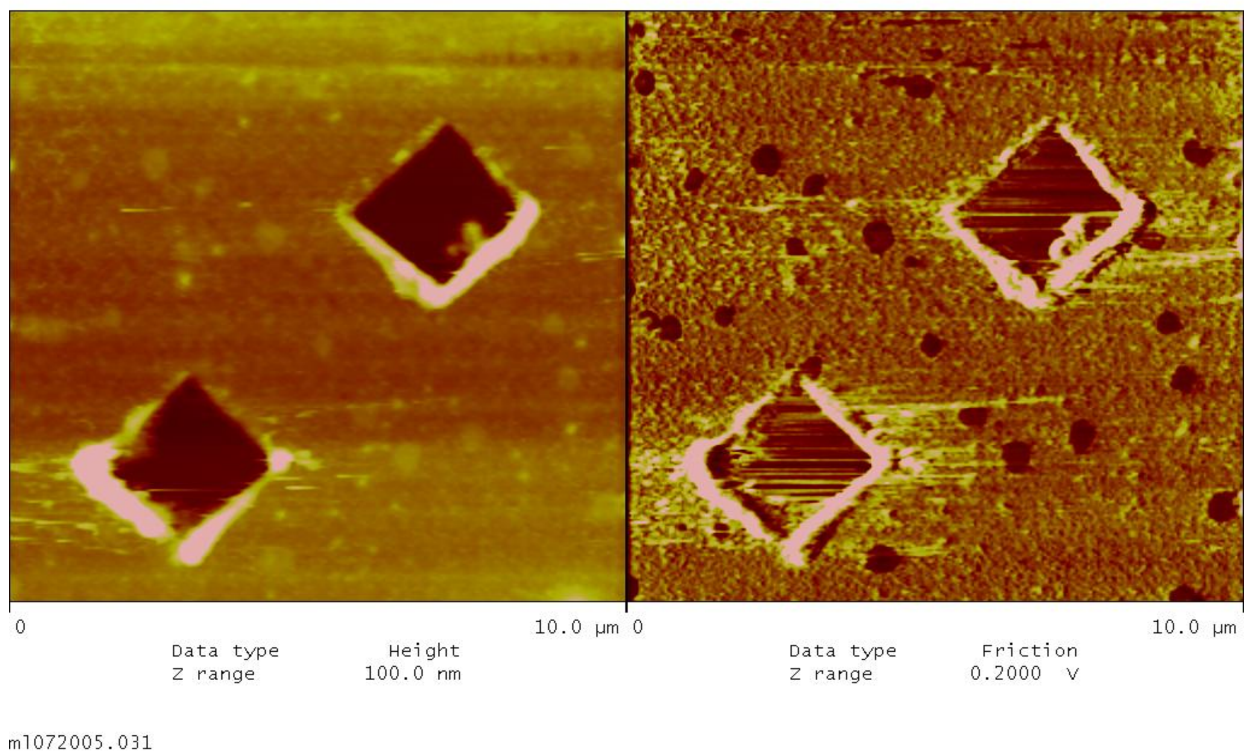


Figure 4

Another key method to verify the formation of each additional monolayer is the use of the atomic force microscope. During the second and third steps of the surface chemistry leading up to the protein deposition, I was able to image the APTES, APMS, and glutaraldehyde surfaces with the atomic force microscope. The AFM tip is dragged

along the surface in contact mode creating an image with height and friction data which provides valuable information concerning the nature of the surface. As displayed in the AFM image above (See Figure 4), the tip is used to scribe in the form of a box through the surface layers of APMS. This is also known as nanoshaving or removing the top layer on the surface. The above image is APMS on Silicon Oxide, and the squares are areas of the surface which have been scratched away demonstrating an apparent thickness.

Conclusion:

The data obtained from the AFM and ellipsometer measurements is conclusive in proving the presence of Avidin protein monolayers. These protein layers were obtained through the multi-step surface chemistry process described previously. The data displayed in the charts and graphs above demonstrates the presence of an average Avidin monolayer thickness of 4.35 nm.

One possible source of error in the experimentation was leaving the Avidin protein unrefrigerated over an extended period of time. Despite the evidence in favor of consistent, stable Avidin monolayer formation, the unrefrigerated protein could lead to contamination of the samples and incorrect data.

The formation of the avidin monolayer is the first of many key steps in the development of a protein patterning method. Avidin-biotin chemistry is to be used to attach biotinylated protein molecules to areas of the surface which have been previously scribed by the atomic force microscope tip. Also using this method, different groups can be attached to the biotin, making it possible to pattern other molecules on a silicon

surface. This in itself is a method of chemomechanical functionlization or surface modification which plays an essential role in the patterning of proteins on a silicon substrate as well as the future of this research project.

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