Electrical Properties of Chicken Herpes Virus Based on Impedance Analysis using Atomic Force Microscopy

Zhuxin Dong
Ph. D. Candidate, Mechanical Engineering
University of Arkansas

Brock Schulte
Masters Candidate, Mechanical Engineering
University of Arkansas

Abstract

Engineering research in health and wellness is well funded by medical professionals and government officials. The research is to create easier and cheaper ways to analyze the physical condition of a living organism. Much has been improved in the micro-electro-mechanical system (MEMS) and the total analysis system (TAS) line of business. This means alternate means to detect pathogens with electrical signals using mechanical structures are being developed swiftly. In this experimental research, electrical properties of a chicken herpes virus will be examined to better understand the means to detect their presence within an organism. In an attempt to classify the chicken herpes virus's electrical properties, a virus will be analyzed and the properties will be hypothesized. With the help of an impedance analysis system using atomic force microscopy (AFM)—of a polystyrene bead—the method of determining the dielectric properties of the virus will be resolved. By basing experimental data with existing data of a polystyrene bead, the researchers will gain optimal support of the hypothesized electrical properties of the chicken herpes virus. Therefore, the most favorable detection scheme of a chicken herpes virus will ultimately be utilized by an impedance analysis.

Background

In this experiment, the impedance (Z) of polystyrene beads will be analyzed and taken as a basis for virus detection schemes. The tip of an AFM as well as an Indium Tin Oxide surface of a glass wafer is to act as an impedance analysis system. Impedance is a measurement involving the opposition to a sinusoidal alternating current. It is composed of the resistance and reactance of a dielectric material and can be measured by the complex addition of the two. The impedance of a material can be determined with use of an electrode sending a current to another electrode while measuring the time delays and other differences between the two signals. In other words, the electric field around the interdigitated electrodes will be disturbed and the impedance is a measured value for this disturbance.

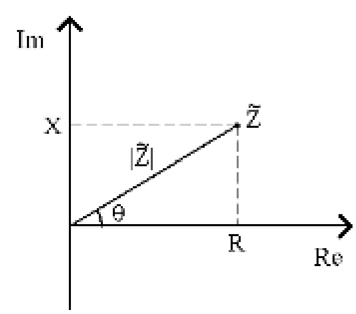


Figure 1: Impedance Measurement, Z=R+jX
R=Resistance
X=Reactance

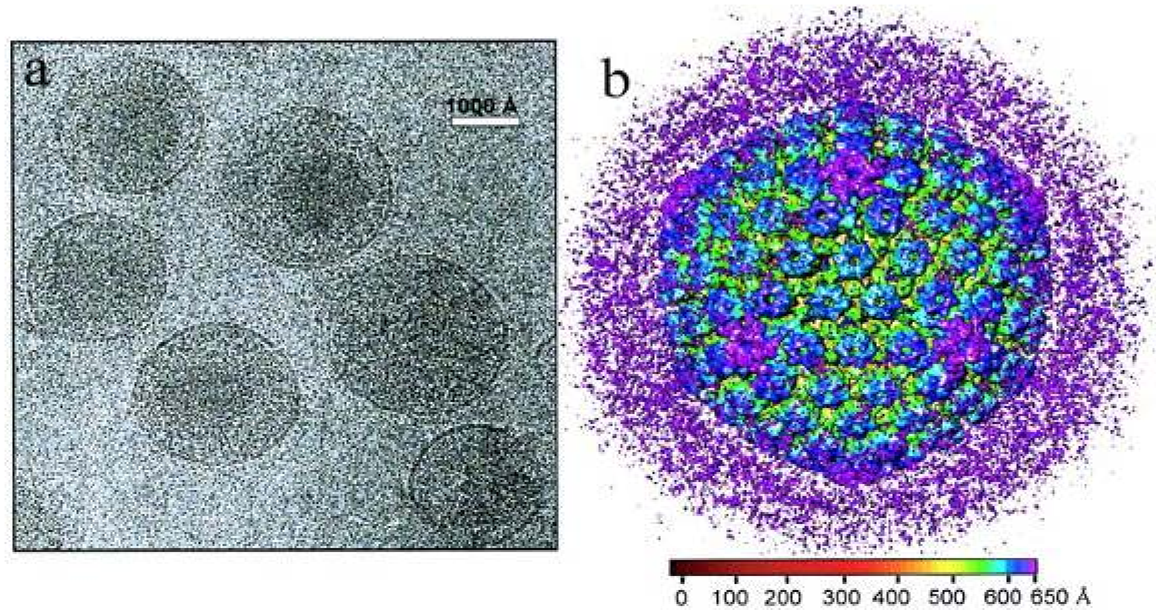


Figure 2: Chicken Herpes Virus Microscope View and Shape.

In figure 1 the θ represents the phase angle of the impedance. With a value for both impedance and phase angle one can calculate the resistive and reactive quantities. These values might be essential to determining a correlation between material properties and impedance.

$$R = Z\cos(\theta)$$

$$X = Z\sin(\theta)$$

Equation 1: Resistance and Reactance Back Calculated

The impedance analyzer is the HP 4192A. It was a used product when purchased, so the condition is assumed to be good. The main changes made to the analyzer was to add one meter long chords to the detection connectors as well as combine the two of the four inputs to one connector by soldering a junction between them. The other two connectors were treated the same creating only two electrical connectors. These two connectors were attached to the impedance chip electrodes. Some key specifications to the analyzer are:

1. 5 Hz to 13 MHz variable frequency
2. Swept measurement capability
3. Gain-phase measurements: amplitude, phase, group delay
4. Floating or grounded devices
5. Impedance measurement: |Z|, |Y|, Theta, R, X, G, B, L, C, D, Q, Delta, Delta%
6. GPIB

The frequency range used was in analysis was one kilohertz to one megahertz, at twenty kilohertz between reading.

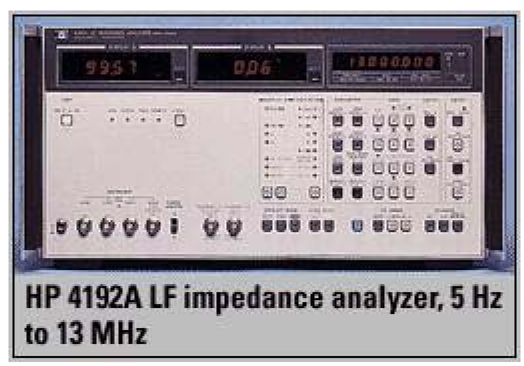


Figure 3: Picture of HP 4192a Impedance Analyzer.

National Instruments computer software, LabView, was used to decode the GPIB card. A downloadable program can be gotten from the LabView website, and can be used to read the GPIB card from the impedance analyzer. The two coinciding capture the values recorded by the analyzer into text files that can be saved to the hard drive of a computer.

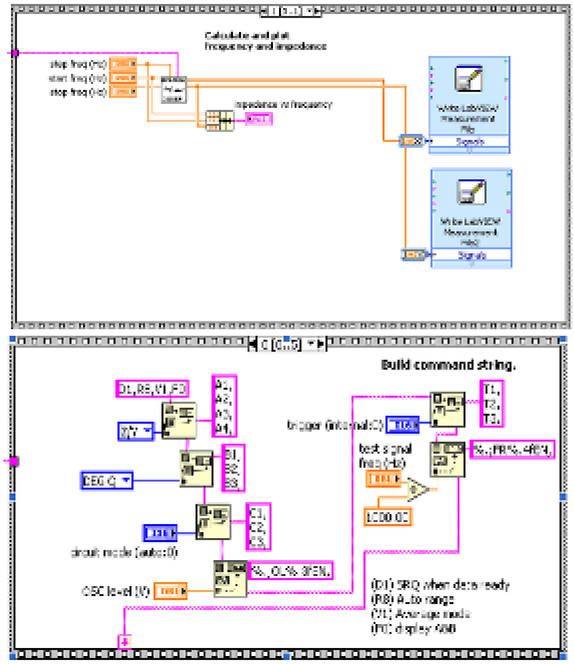


Figure 4: Lab View Reads and Writes The Impedance Analysis Data with this Configuration.

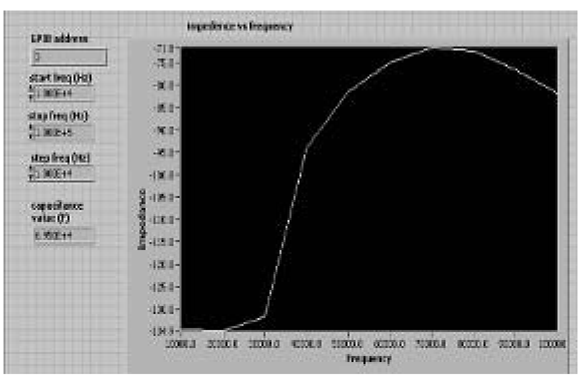


Figure 5: Lab View and the Impedance Analyzer Inputs and Outputs.

The signal obtained by each sample run will then be added to a control run of pure ITO surface to determine the impedance of the system. This process is to gather the curve fit of a circuit model as seen in figure #. The curve fit should correlate to the materials' electrical properties. The curve fit of the control sample of ITO will be able to calculate the components of the R_{inst} and CPE. With these known the capacitance of the bead and/or virus can then be calculated.

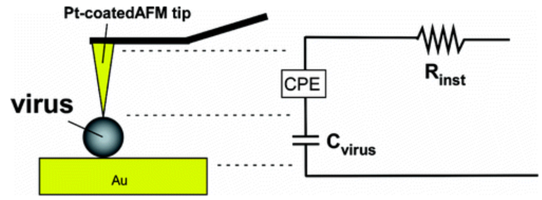


Figure 6: Circuit Model Used in ZView Software to Calculate the Bead and Virus Capacitance.

This calculation will be performed by a downloaded program called ZView. This program will be able to have inputs, of impedance curves, that will enable it to calculate the components of each electrical element. These components will then become constants that will be inserted into an equivalent circuit to calculate the other unknown, the bead capacitance.

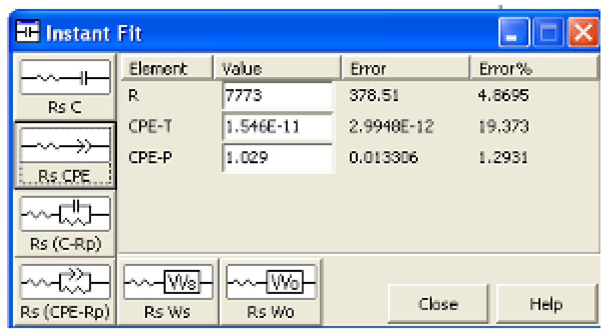


Figure 7: ZView Fit for R_{inst} and CPE.

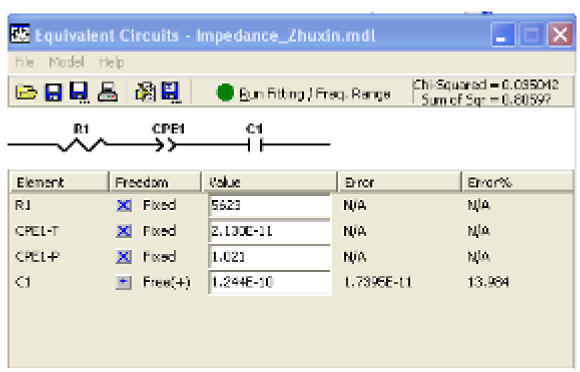


Figure 8: With Fixed R_{inst} and CPE ZView, to Curve Fit C_{virus}.

Also in this research the scan will be performed by an Agilent 5500 AFM. This apparatus has two scanners, a large scanner and a small. The large scanner has a scan size of one hundred micrometers square; whereas the small scanner has a scan size of ten micrometers square. The scanners can hold multiple types of nose cones that hold the tip (probe) with a spring. These nose cones consist of; current sensing modes, alternating current modes, and magnetic alternating current modes; just to name a few. The sole purpose of the scanner is to be able to take a three dimensional image of a micrometer and nanometer size objects.

For the case of impedance measurements the current sensing nose cone needs to be used in order to have an electrical connection between the tip and the sample. This will enable an electrical connection through the system into the bead and back to the analyzer.

Figure 9: Agilent 5500 AFM.

Figure 10: AFM Scanner

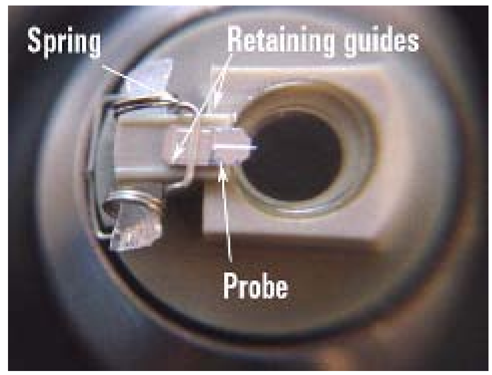


Figure 11: AFM Nose Cone with Probe Inserted

Finally a further understanding of the polystyrene beads may be essential to a conclusion of this research. Polystyrene is a material uses as a plastic in many applications. The beads used for this experiment are Polybeads© Microspheres made by Polysiences, Inc. A few parameters for the beads bought and used were mean diameter of the beads, weight per volume aqueous suspension of solids values, and the number of particles/ml. All of which are useful, but there is need for some dielectric properties of the polymer.

Experimental Procedures

Before measuring the impedance of virus, we test the impedance properties of polystyrene beads, of which the impedance characteristics have been found out. Then, the equivalent circuit built in ZView can be examined by comparing the measured beads impedance with the existing one. IF it is verified that the measured impedance matches what it is supposed to be, we can conclude we have the reliable values for the resistance and CPE part in the equivalent circuit. Hence, the experimental environment is able to be represented electrically, and eventually that makes a correct measurement possible for virus.

A. *Electrical Setup*

Agilent 5500 provides people with a Current Sensing AFM (CSAFM) function under contact mode. Our electrical connection for measurement is developed based on this operation mode. To begin with, a conductive surface is needed to sustain the beads. In this experiment, a glass slide is coated by an indium tin oxide (ITO) layer on the top, which is conductive. A droplet of beads solution is dropped on the surface and the glass wafer is dried by heating. Then the polystyrene beads sample is ready for scanning. In CSAFM function, an ultra-sharp AFM cantilever, coated with conductive film, probes the conductivity and topography of the sample surface simultaneously. CSAFM requires a special 10 nose cone containing a pre-amp. A bias voltage is able to be applied to the sample while the cantilever is kept as virtual ground. During scanning, the tip force is held constant and the current is used to construct the conductivity image of the surface. It has proven useful in joint I-V spectroscopy and contact force experiments as well as contact potential studies. Figure 12 shows the schematics how the measurement is done. The resonant frequency and force constant of the silicon AFM probes we use here are 13 KHz and 0.2 N/m respectively. And these probes are coated by Cr/Pt conductively on both sides. The special nose cone assembled has a sensitivity of 10 nA/V.

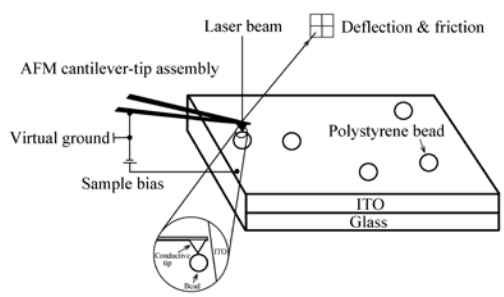


Figure 12: Schematics of CSAFM

However, for our purpose, some modifications are necessary. First of all, we set the sample bias at 0 V while keep the probe as a virtual ground during the entire course of scanning and measurement. Secondly, two wires are bonded with the metal spring on the nose cone and ITO surface respectively. Finally, they are connected to the clippers of the impedance analyzer. With this method, a closed circuit is available for impedance measurement whenever the probe tip is moved onto the top of a found bead. Figure 13 shows the electrical connection as described above.

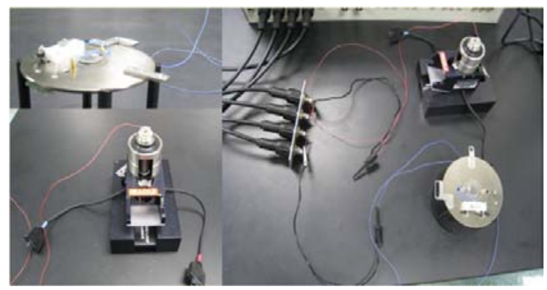


Figure 13: Electrical setup for AFM and Impedance Analyzer

B. *Impedance Measurement*

The impedance measurement of polystyrene beads can be divided into two main parts: one is the measurement of the beads and the other is the measurement of ITO surface without any beads. The measurement of ITO is used to measure the electrical characteristics of the entire experimental environment except the beads. That includes the characteristics of AFM internal structure, the scanner, the probe, the wires and the ITO surface itself, which work as the rest part of the closed electric circuit.

The other part is to measure the impedance of the entire closed circuit where a bead is brought in. Then, we can assume that, with certain method, the impedance of polystyrene beads can be computed as simple as we are dealing with a subtracting problem. And this method can be realized by setting up an equivalent circuit.

C. *Equivalent Circuit*

In ZView software, we can apply the data obtained from the impedance analyzer. When the analyzer is connected to ITO surface and the AFM probe, we can get a set of impedance data versus frequency from 100 KHz to 1MHz by 50K increment. An equivalent circuit has been set up to simulate the measuring environment as shown in Figure 6. It consists of a resistance, a CPE part and a capacitance, where the capacitance stands for the beads while the other two parts describe the rest of the components.

Thus, the measurement data of ITO is loaded into the software first and then an instant fit can be done to determine the values of the resistance and CPE part (see Figure 7). After these parameters are known, the equivalent circuit simulation is ready to go. Firstly, select the bead measurement data in active; secondly, open the equivalent circuit file; thirdly type the values for R1, CPE1-T and CPE1-P from previous instant fit and have them "fixed"; finally, set C1as "free" and run fitting to determine the value of the capacitor as shown in Figure 8.

D. Measurement of Virus

Why the impedance of the polystyrene beads is measured but not measure virus directly? That is because after the capacitance of the beads is obtained, it is able to be compared with previous result calculated and verified by other researchers. It our result matches the others', the impedance measurement of the virus, of which the electrical characteristics is unknown, can convince people with the same method.

<u>Experimental Data and Results</u>

A. Sample Preparation

A sample stock has plenty of polystyrene beads which have diameter ~200nm. Then, the stock is diluted into 3 dilutions: 50X, 100X and 500X. All the dilutions are sonicated for ~10mins to have beads suspend uniformly. Finally, a 2µl droplet from 50X is deposited on an ITO surface with glass substrate and dried for scanning.

B. AFM Scan of Beads on ITO

Figure 14 shows the operation status of the AFM software while scanning the beads. From the topography image, it is clear that there are many beads in the scan area and when we measure the cross section height of one bead, it is about 100.8nm high as shown in Figure 15. Therefore, we believe that is a bead and mover the tip onto the bead. In the topography image, the red cursor presents the location of the probe tip. Then the impedance analyzer is turned on to measure the impedance of that bead. After the measurement of beads is done, the probe tip is moved to the ITO surface to measure its impedance as shown in Figure 16. Hence, when the process above is repeated several times we can get enough data. Figure 17 shows the bead and ITO data curves.

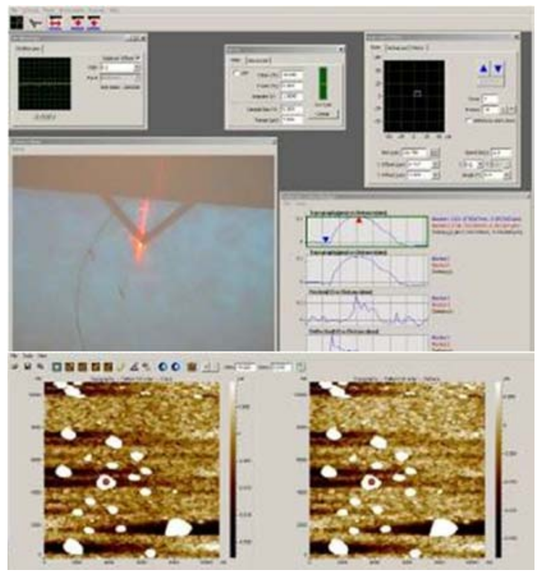


Figure 14: AFM operation menu, bead scan and measurement

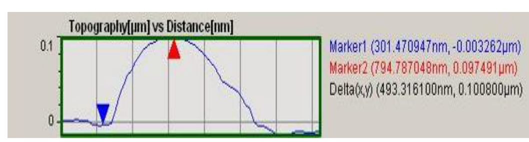


Figure 15: Cross section measurement for height

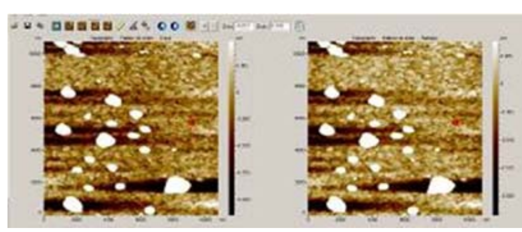


Figure 16: ITO surface scan and measurement

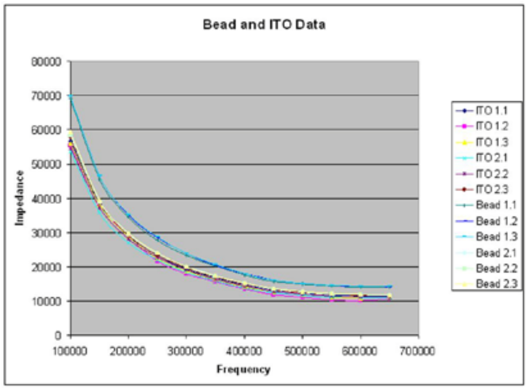
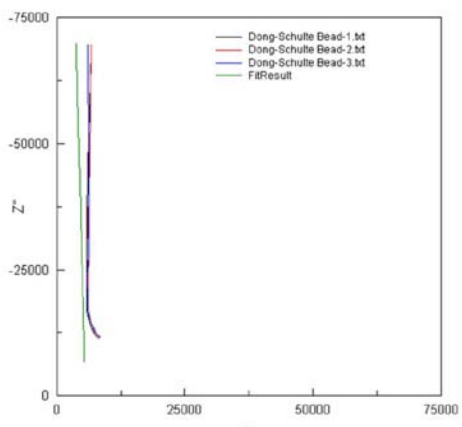


Figure 17: Bead and ITO data

C. Calculation of Bead Capacitance

The ITO data is input into ZView first to determine the values of the resistance and CPE part. Figure # illustrates how well is the instant is for determination of those values. Then 3 sets of bead data is loaded to determined the value of the capacitance. Figure 18 introduces the results for all the parameters and the value of the capacitor is 0.117nF. Since the error average is 13.86% of the collected data, we assume the bead's capacitance ranges from 0.096 to 0.139nF.

As the AFM tip is 200µm long and 40µm wide, we assume the contacting surface area is 8e-5cm^2. Therefore, the capacitance of beads can be represented in F/cm^2 from 1.2e-6 to 1.7e-5.



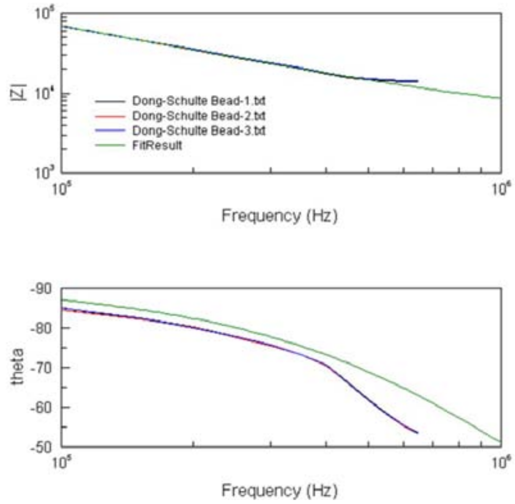


Figure 18: Instant fit and equivalent circuit simulation on Zview

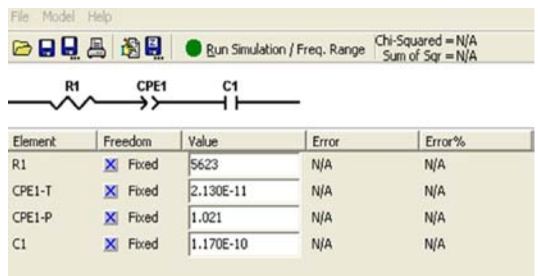


Figure 19: Results after equivalent circuit simulation

D. Scan of Virus

No data was taken for no distinct virus was found in any of the 4 scans we took (see Figure 20 & 21). We suppose that the buffer solution with the viruses crystallized to form a background that was too noisy to decipher what was what. From the scans the buffer by itself did not resemble the buffer with viruses, and no data was necessarily needed to conclude we could not find a virus vs. control surface on the scans.

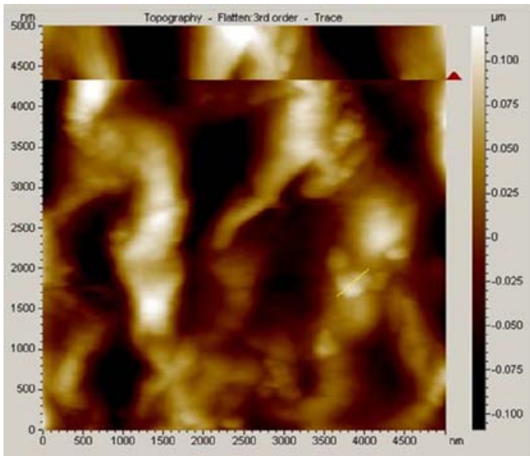


Figure 20: Topography scan for virus with buffer on ITO surface

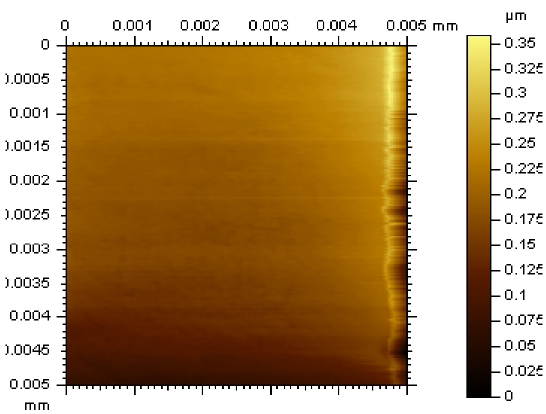


Figure 21: Topography scan for buffer surface only

Conclusions

Testing for capacitance values using impedance analysis can be achieved by the methods in the research. Bead capacitance values calculated seemed reasonable enough to conclude the method in this research will work for other substances. With better techniques of preparing virus samples, good scans may be achievable and electrical properties of the virus could be hypothesized. The techniques of converting the Bead capacitance values calculated to known electrical properties of Polystyrene need to be pursued to conclude this method will be able to determine electrical properties of other substances, like the Chicken Herpes Virus.

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