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Impedance Spectroscopy of Chicken Infectious Laryngotracheitis Virus Based on Atomic Force Microscopy

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Abstract — **The AC impedance spectroscopy of Chicken Infectious Laryngotracheitis Virus (ILTV, *gallid herpesvirus 1*) was measured using atomic force microscopy (AFM). The result indicates a 30% difference in the absolute impedance values between the control and virus samples within a 10-65 kHz frequency range. Using an equivalent circuit of the systems and an electrical curve fitting program, the data shows the average capacitance of a single virus particle is approximately 6 nF. Based on a similar analysis of the electrical properties of polystyrene beads, the overall dielectric constant of the ILTV was determined to be approximately 2.4.**

Keywords — ***AC impedance spectroscopy, ILTV, AFM, Equivelent cuircut, Dielectric constant, Polystyrene bead, Micro-electro-mechanical systems (MEMS), Total analysis system (TAS).***

I. INTRODUCTION

Over the passed few years, virus detection techniques are becoming increasingly important due to frequent occurrence of new pathogenic virus strains. At present, the means of virus detection and classification involve extensive serological procedures. These tests, such as ELISA and immune-fluorescent assay, include collecting a sample of bodily fluids to be analyzed [1]. The extensiveness of these techniques creates the need for a more generalized testing apparatus. The engineering research is to create easier and cheaper ways to analyze the physical condition of a living organism. Much has been improved in the MEMS and the TAS industry. The alternate means to detect pathogens with electrical signals using mechanical structures are being developed swiftly.

An alternative approach in detecting viruses can be through the material properties of a virus. As seen from previous work, shown in Figure 1, an AFM can be used to successfully scan and nanoindent viruses for the purpose of extracting the mechanical properties of individual virus particles [2]. In the present study the electrical properties of an ILTV particle was characterized by a similar AFM based technique. The electrical properties of the ILTV were examined to better understand the means to detect their presence within an organism. In an attempt to classify the ILTV's electrical properties, a virus will be analyzed through impedance spectroscopy and the properties will be determined. The ILTV is one of the most contagious viruses that can cause severe health problems in chicken flocks, which can lead to financial losses within the poultry industry. It is hypothesized that the viral electrical properties can be used to specifically identify ILTV through impedance spectroscopy. Confirmation of the hypothesis will be a breakthrough in the detection, classification, and isolation of ILTV.

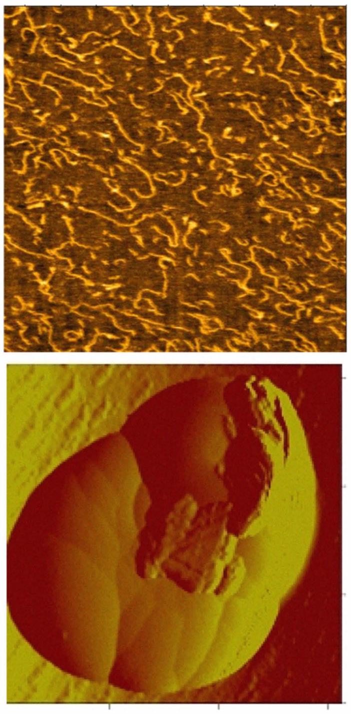

Figure 1: AFM based analysis of biomolecules. Topography of 3000 bp dsDNA ladders; and Nanoindented Avian Influenza Virus.

II. MATERIALS AND METHODS

A. Virus Sample Preperation

Infectious laryngotracheitis virus (ILTV; *gallid herpesvirus 1*) is a member of large *Herpesviridae* family. United States Department of Agriculture (USDA) reference strain of ILTV was purchased from the National Veterinary Services Laboratory (NVSL; Ames, IA). The stock ILTV titer, which is defined as plaque forming unit per milliliter (pfu/ml), was 10^5 pfu/ml. Generally, one pfu titer consists of 10^3-10^7 numbers of individual virus particles (termed as virion), thus total virion numbers for ILTV stock estimate approximately 10^8-10^{12} virions/ml. For both the inactivation and the fixation of ILTV,

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200 µl ILTV stock was mixed with paraformaldehyde to become a 4% concentration. To verify the virus inactivation, ILTV in 4% paraformaldehyde was infected to cultured cells and the plaque formation was monitored for the virus propagation. The inactivated viral samples were used in this study after being confirmed that no live ILTV propagation was observed.

10X dilutions of the inactivated stock virus samples were prepared using DI water. Each virus sample contained a 100 µl drop of solution on an indium tin oxide (ITO) surface. The sample was then allowed to dry in environmental condition in a biological fume hood.

B. Electrical Setup

In this experiment, an Agilent 5500 AFM was used. The AFM software provides a Current Sensing (CSAFM) function under contact mode. This mode allowed the electrical connection for the impedance analyzer. CSAFM requires a special 10° nose cone containing a pre-amp. A bias voltage is applied to the sample and set at zero to keep the cantilever as a virtual ground. A current sensing conductive AFM probe tip on a 10 µm scanner was put into the AFM. The Cr/Pt plated, silicon tip of R < 25 nm and kc of 0.2+50% N/m (Budget Sensors) with a resonant frequency of 13 kHz was used for the AC impedance measurements.

Since the impedance analyzer needs a closed circuit for the frequency sweep, wires were bonded to the nose cone's spring that holds the cantilever and to the sample's conductive surface. The sample surface has a 1500 Å conductive ITO layer that completes the closed circuit when the tip is in contact mode.

C. Impedance Measurement

Within the closed circuit an AC frequency was applied and the absolute impedance of the system can be recorded. 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. In figure 2 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 with use of (1). These values are essential to determining a correlation between material properties and impedance.

$$\begin{aligned} R &= Z\cos(\theta) \\ X &= Z\sin(\theta) \end{aligned} \tag{1}$$

Where: R=Resistance; X=Reactance.

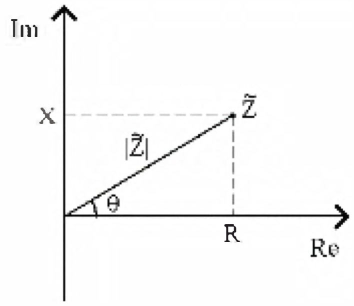

Figure 2: Impedance Measurement, Z=R+jX

To characterize the electrical properties the absolute impedance and phase angle values were obtained using an external impedance analyzer (HP 4192A) connected through the AFM tip. Data were recorded in the frequency range of 10 kHz to 1 MHz at increments of 10 kHz, all at 1VAC. Figure 3 shows the schematic of the virus-AFM-impedance analyzer experimental setup. This set-up was used to collect all the virus characterization data. National Instruments computer software, LabView, was used to decode a general purpose interface bus (GPIB). This card is used as the electrical interaction from the impedance analyzer to a computer. A downloadable program from LabView's website was used to read the GPIB card from the impedance analyzer.

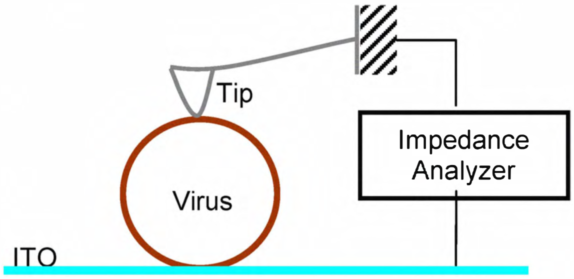

Figure 3: Schematic of the Experimental Setup.

D. Equivelent Cuircut and ZView

After the impedance spectrums were recorded the data were then converted into the resistance and reactance parts of impedance and plotted with a computer software program (ZView). The software creates a curve-fit of the collected data with a circuit model and calculates the model's components (Figure 5) to determine the virus capacitance values [3].

Figure 4: Equivalent Circuit Model without Virus.

R1: Instrument Resistance
CPE: Constant Phase Element of Instrument and Surrounding

Figure 5: Equivalent Circuit Model.

C1: Capacitance of Virus

As seen in MacCuspie's article, ZView is a strong program to calculate the components of an equivalent circuit. The program was downloaded from an online source and installed to be ready to use. The program has equivalent circuits pre programmed as well as an option to create a circuit. ZView also allows for impedance data to be inserted, as a text file in resistance and capacitance versus frequency form, into a plot. There are curve fitting options to allow an equivalent circuit to be fit to the plot. The circuit component can be made constant or variable; and the variable elements are then calculated.

E. Virus Dielectric Constant Measurement

First, the instrument resistance and constant phase elements had to be calculated. If the virus capacitor is said to be a short, the ZView program can calculate these two components. Data was recorded without the presence of a virus. Figure 3 then will be changed to have the tip touching the ITO substrate, closing the circuit. This then gives the electrical circuit shown in figure 4. The data was then used to calculate the two unknown components of the equivalent circuit. Second, the impedance data of the virus was plotted in ZView. Now the equivalent circuit of figure 5 is set up in the ZView program. The two now known components of the circuit are set as constants and the curve fit only considers the circuit change of the added capacitor, the virus. The capacitance of the virus was then known as well.

The impedance of the polystyrene beads was initially measure to find the capacitance. This was then compared with previous calculated and verified results. If the result is equivalent to other researchers, then the techniques used by the authors can be validated. Polystyrene nanobeads of similar size (200nm diameter) to the ILTV were used as the reference sample. The polystyrene bead is known to have a dielectric value approximately 2.6 [4]. Knowing this value as well as calculating the capacitance value of the bead, a reference capacitance can then be determined with (2) [5].

$$\varepsilon_r = \frac{C_x}{C_0} \qquad 2$$

Where: ε_r =Dielectric Constant; C_x =Known Capacitance; C_0 =Reference Capacitance.

III. EXPERIMENTAL RESULTS

Figure 6 is a topographical image of the nanobead and virus samples, as well as the substrate used as a control to compute the circuit resistor and CPE elements. Each image was within the ILTV size range of previous studies, 200 nm. Figure 7 represents the average absolute impedance spectrum based on multiple experimental runs. The control, ITO, is shown possessing lower impedance values throughout all frequency ranges. As expected the capacitance values of the bead, buffer, and virus cause an increase in absolute impedance of the system. It can also be observed that the absolute impedance values of the virus are different from that of the PS beads.

Taking data of the virus was then performed by the techniques used in finding the bead data. Once the absolute impedance was converted into the reactive and resistive parts, the data was inserted into ZView, plotted, and fit to the equivalent circuit shown in figure 5. Using the equivalent circuit model the capacitance values of the bead, buffer control, and virus are calculated and shown in Figure 8. It is evident that the bead capacitance and the virus capacitance vary. The capacitance values for the bead came out to be 6.5 nF, whereas the virus was 6 nF. The slight deviation can best be expressed as an electrical properties difference, and therefore is the most likely cause for the variance.

The result from an AC impedance spectroscopy shows a 27% difference between the ITO control and bead absolute impedance values. Likewise the difference between the ITO control and virus absolute impedance values is 30%. Using the experimental values of the reference capacitance, based on the bead data, the dielectric constant of the ILTV was calculated. The values from the experimental impedance spectra gave a dielectric constant of the ILTV to be ~2.4; whereas, that of the PS nanobead is ~2.6 [4].

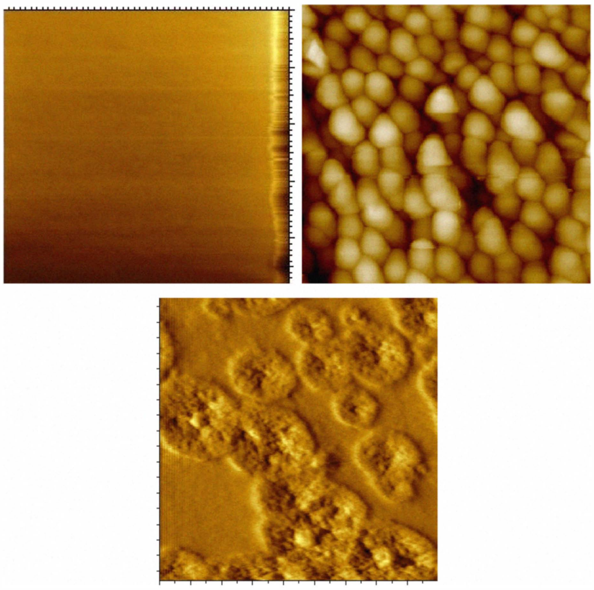

Figure 6: Topographical Images for Substrate (top left); Nanobeads (top right); and ILTV (bottom).

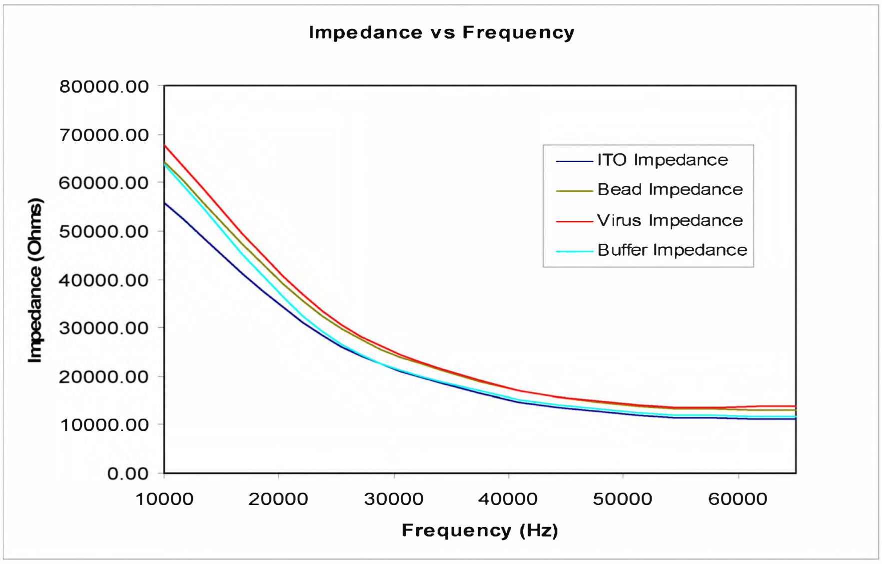

Figure 7: Bead-Virus-Substrates Impedance versus Frequency Spectra.

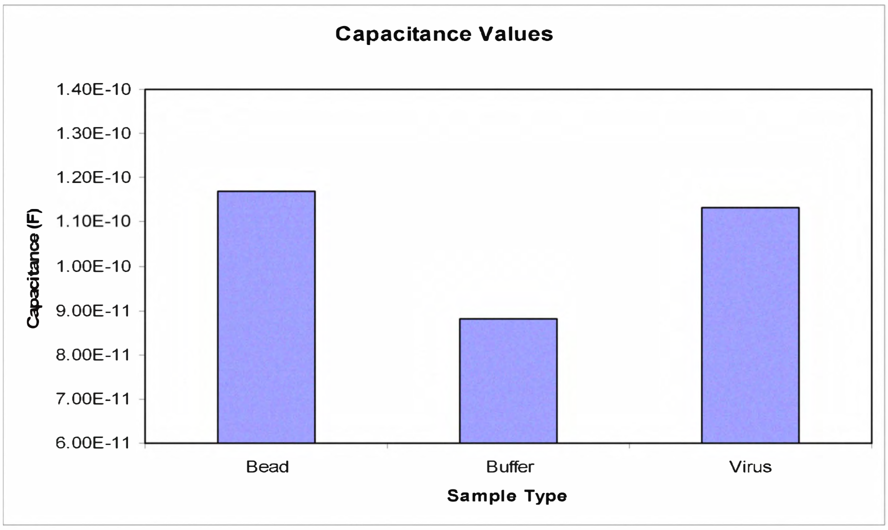

Figure 8: Capacitance versus Sample Type.

IV. CONCLUSIONS

With known differences between the ILTV and the control properties, an electrical detection of ILTV scheme can be based off an impedance spectrum of a chicken's bodily fluid. A TAS will be created with the impedance spectroscopy theory discussed in this paper. Further research on other detection schemes will ultimately be the deciding factor whether or not an impedance spectroscopy, based on the dielectric properties of the material, system will be utilized.

The ILTV will soon be able to be detected and isolated within a chicken flock before further contamination occurs. Finally, providing a quicker and easier detection technique will give overall financial gain to the poultry industry.

REFERENCES

[1] Nester, Eugene W., et al. *Microbiology, A human Perspective, 5th Edition.* McGraw Hill Companies Inc., New York, NY. 2007.

[2] Rokadia, Husein, et al. "Characterization of Avian Influenza Virus Subtypes using Atomic Force Microscopy." IEEE Nano 2009.

[3] MacCuspie, Robert I., et al. "Comparison of Electrical Properties of Viruses Studied by AC Capacitance Scanning Probe Microscopy." JACS Articles, 12/20/2007.

[4] Lee, Sang-Wook, et al. "Determination of Dielectric Constant of Dielectric Particles Using Negative Dielectrophoresis." 1996 IEEE Annual Report.

[5] Jackson, John David. *Classical Electrodynamics, 3rd edition.* 1998. New York: Wiley.