Towards Local Probe Vibrational Spectroscopy of Single Virus Particles

Christopher DuFort

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Professor Bogdan Dragnea

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Abstract

Recent success has been achieved in the synthesis of virus particles which encapsulate single gold nanoparticles. However, little information is known about how much resemblance there is with a true capsid. Through the integration of atomic force microscopy, surface enhanced Raman spectroscopy, and near-field scanning optical microscopy (SERS/AFM/NSOM) it will be possible to simultaneously characterize the optical, chemical, and morphological properties of single virus particles. Of particular significance is the ability to gain information on the symmetry of these particles. We have demonstrated the ability to image brome mosaic virus (BMV) in air and on a mica substrate with AFM. Current work is focusing on the construction of an atomic force microscope featuring a quartz tuning fork probe and a gold-coated etched glass tip. The advantage to traditional AFM probes is the relative non-invasive nature of the tip-sample interaction which is especially important when studying biological samples. Through the use of AFM/NSOM, following the identification of the virus-like particles it will be possible to observe their Raman spectra via SERS. Spatial manipulation of the particles will allow for comprehensive analysis, leading to a detailed mapping of their surface features.

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1. Introduction.

1.1 Atomic Force Microscopy (AFM). Since its invention in 1985 by Gerd Binnig and Christoph Gerberk, the Atomic Force Microscope (AFM) has been implemented in a variety of fields ranging from biology, chemistry, materials science, and electronics.[1] The ability to image surfaces in atomic resolution coupled with the ability to operate in multiple modes makes the AFM one of the most versatile scanned-proximity probe microscopes. Particular interest has been placed on the imaging of biological structures such as DNA, single proteins, living cells, and viruses.

The AFM generally consists of a laser, mirror, photodetector, amplifier, probe, and cantilever.[2] The basic principle is to maintain an atomically sharp tip at either a constant force or height above the sample surface. To accomplish this, the tip is attached to the underside of a reflective cantilever. An optical beam is bounced off the cantilever and deflected to a position-sensitive photodetector. The up and down motion of the tip scanning over the surface of the sample results in a deflection of the laser beam which is detected by the photodiode. The resulting signal is fed-back through the software control enabling the computer to keep the tip at either a constant height or force.[1]

Previous work operating in tapping mode AFM has shown considerable success in the imaging of viruses including tobacco mosaic virus, brome mosaic virus, turnip yellow mosaic virus, and cauliflower mosaic virus. Kuznetsov implemented the use of tapping mode AFM in the imaging of BMV and was able to resolve the individual

capsomeres that made up the capsid.[3] The measured diameter of the virus was approximated to be 28 nm. It is important to note that these measurements were done by height analysis; it is not accurate to take the lateral diameter because this will always be convoluted due to the shape of the AFM tip, sometimes as much as 2-5 times the correct value.[3]

Another method of force sensing involves replacing the cantilever based force probe with a tuning-fork probe.[4] This offers a number of advantages such as relatively non-invasive tip-sample interactions and it eliminates the need for a laser diode. Noise due to thermal mode hopping of the laser and drift from dissipation of the laser diode is also eliminated.[5] These advantages seem to suggest that the use of a nonoptical method of feedback would be ideal as is achieved with a tuning-fork probe. The tuning forks used in this application are the same as those found in common wristwatches as shown in Figure 1.

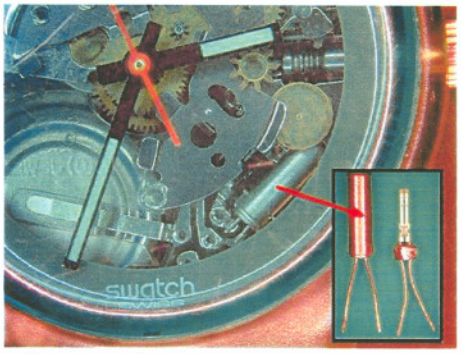

Figure 1. A typical tuning fork common to most commercial watches. These quartz crystals are also used as force sensors in atomic force microscopes.[6]

Another one of the principal advantages of using a quartz tuning fork as a force sensor lies in their very high oscillator quality factors, Q.[7,8] This property enables detection of very small resonant frequency changes and energy changes due to force interactions.[9]

Giessibl developed an atomic force microscope based on frequency modulation the schematic of which is shown in Figure 2.

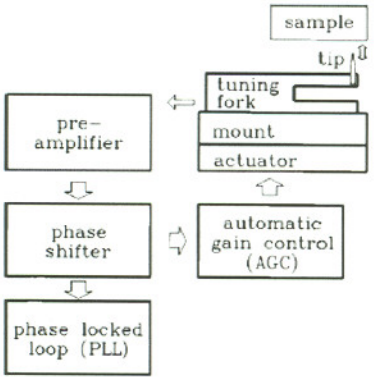

Figure 2. Schematic of the tuning-fork probe atomic force microscope developed by Giessibl. The basis is conversion of the oscillation frequency into a measurable dc signal.[8]

The automatic gain control circuit (AGC) was used to make adjustments ensuring constant vibration amplitude.[8] The output was also passed through a phase-locked-loop which functioned to produce a dc signal from the oscillation frequency which was proportional to the difference between an adjustable set-point and the aforementioned frequency. The tuning fork used by Giessibl had a frequency of 32.768 kHz, the prongs were each 3.0 mm in length, the width was 120 μm, and the calculated theoretical spring constant was 3143 N/m.[8]

1.2 Surface Enhanced Raman Spectroscopy (SERS). Raman spectroscopy is a powerful method used to investigate the vibrational frequencies of molecules yielding chemically specific information.[10] A significant weakness of this method arises from its inherent low signal strength. This can be overcome by placing small metal particles close to the molecule being examined; enhancements of up to 8 orders of magnitude have been achieved on Cu, Ag, and Au surfaces.[11] The principle of SERS was first demonstrated in

1974 by Fleischmann *et al.* in studies regarding the adsorption of pyridine at a silver electrode which had been electrochemically roughened.[10] More recently a gold coated AFM tip was used by Anderson to serve as a substrate for SERS.[12] A simple illustration of the power of SERS from these experiments is shown in Figure 3. In one experiment a gold coated AFM tip was used in conjunction with a Raman microprobe to induce enhancement of the Raman signal. Spectrum A shows the signal enhancement of the local surface Raman signal on a sulfur film as compared to spectrum B where there is no tip enhancement.[12]

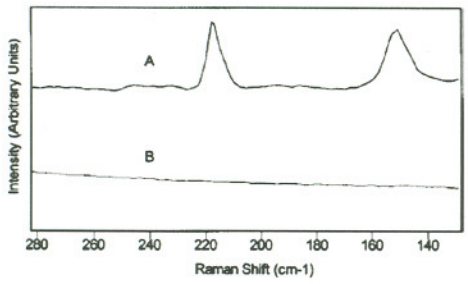

Figure 3. Plot A shows the Raman shift associated with a Raman signal enhanced by the presence of a gold coated atomic force microscope tip. Plot B shows the Raman shift in the absence of the gold coated tip.[12]

Results indicated that the coupling of AFM with Raman spectroscopy increased spectral resolution, selectivity, and sensitivity when compared to a conventional Raman microprobe.[12-13] The high degree of specificity displayed by implementing the use of an AFM tip offers the potential for single molecule targeted spectroscopy.[14]

1.3 Near-field Scanning Optical Microscopy (NSOM). Zenhausern, O'Boyle, and Wickramasinghe first demonstrated that it was not necessary to pass light through a small pinhole, but you could also reflect light from the tip of a scanning probe, such as an AFM tip.[15] The advantage of this method is the potential to achieve resolutions comparable to

the radius of the probe tip, which could be on the order of a few nanometers or less.[16] This makes possible the ability to do spectroscopy on individual molecules such as individual components of a virus capsid.[17-18]

2. Experimental.

2.1. Sample Preparation. All vials and beakers used to prepare AFM samples were first rinsed three times with pure water from a 0.22 μm syringe filter. The glass coverslips were first rinsed with filtered pure water, and then dried under a flow of nitrogen. The coverslips were then incubated in a glass beaker with a 3 mL solution of poly-L-lysine for 10 minutes. After this time the coverslips were rinsed once with pure water and again dried under a flow of nitrogen. Incubation was then done in a 1 mL solution of a brome mosaic virus (BMV) suspension for 30 minutes. The sample was then rinsed with pure water and dried under a flow of nitrogen. A vacuum jar was used to store the sample when not in use. The initial concentration of BMV used was 0.01 mg/mL, 10 μL of this solution was then diluted to 1 mL with filtered acetate buffer. When preparing samples on mica the sheet of mica was first cleaved with a razor blade and then subsequent treatment was the same as for the coverslip sample preparation. The mica was in the Muscovite form which produced a non-conducting, clean, and atomically flat surface.

2.2. Atomic Force Microscope (AFM) Settings. A VEECO Dimension 3100 AFM was used for all of the presented images. It was operated in tapping mode at a scan rate of 2 Hz. The scan sizes varied from 2-5 μm^2 with 262,144 data points taken per scan. The tips used were micromachined monolithic Si probes for use in tapping mode. They have a typical resonant frequency of 300 kHz, force constant of 40 N/m, width of 30 μm,

thickness 4 μm, and a tip radius of less than 10 nm. All samples were imaged within a few hours of preparation.

3. Results and Discussion.

3.1. Intact Brome Mosaic Virus. Initial experiments were carried with a commercially available atomic force microscope (VEECO Dimension 3100) to see if it would be possible to effectively image intact viruses. It was also desirable to determine if it would be possible to discern the difference between intact virus, empty capsid virus particles, and virus particles with gold cores. Brome mosaic virus (BMV) is approximately 28 nm in diameter and exhibits icosahedral symmetry[12] so it was expected to appear as uniform spheres during AFM imaging. An intact BMV sample was prepared on a glass substrate coated with poly-L-lysine and imaged with tapping mode AFM as shown in Figure 2. The poly-L-lysine was used because it coats the surface of the substrate with a dense layer of amine groups which function as a surface for ionic attachment. Figure 5 shows seven prominent uniform spherical viruses with diameters of approximately 28 nm which agrees well with theoretical results.[12]

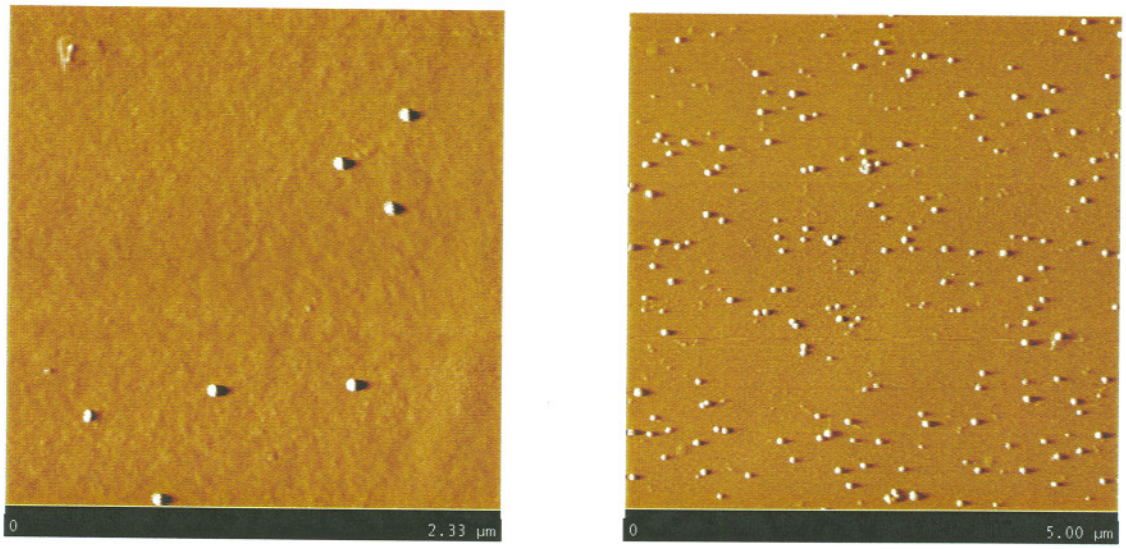

Figure 4. Atomic force image of intact brome mosaic virus (BMV) on glass surface (left) and mica surface (right) both were pre-treated with Poly-L-Lysine.

A mica substrate was also used (Figure 4) and results show a relatively large number of viruses adhered to the surface yielding uniform spheres with diameters of approximately 28 nm which also agreed well with theoretical results.

3.2. Empty Capsid and Gold Core Virus Particles. Imaging of empty virus capsids yielded somewhat ambiguous results as shown in Figure 5. It is difficult to pick out a series of uniform spheres of uniform height of approximately 28 nm that could be conclusively identified as BMV. The masses of material on the surface could come from a variety of sources. Residual protein could have been left in the sample prior to deposition onto the coverslip which aggregated to form the structures seen in Figure 5. Another possibility is that the empty capsid virus particles are significantly less stable in ambient conditions and upon contact with the AFM tip they collapse and take on irregular shapes. One option to overcome this could be imaging empty capsid virus particles in a buffer solution. Imaging was also done on virus particles synthesized with gold cores as

shown in Figure 5. The approximate heights of the uniform spheres were 27-33 nm. The spheres look distorted due to a convoluted tip so lateral measurements of diameter would be misleading. Height measurements would be consistent regardless of the shape of the tip.

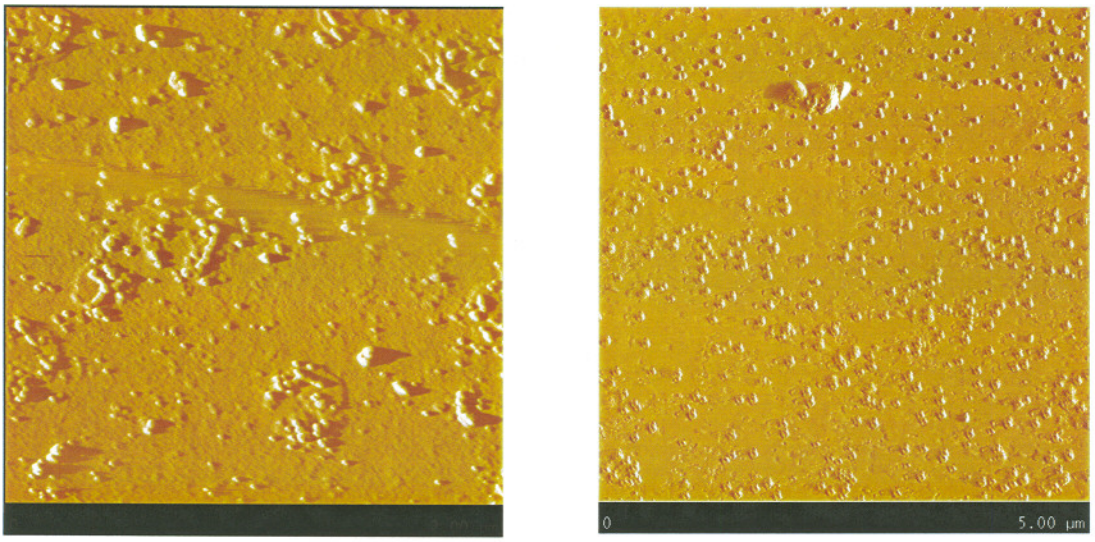

Figure 5. Atomic force image of empty capsid virus particles on a mica surface treated with poly-L-lysine (left) and virus particles with gold cores (right) on a glass surface treated with poly-L-lysine.

composition of the capsid to determine its similarity to intact BMV. The use of a gold-coated AFM tip and Raman spectroscopy will be used to investigate this. Upon detection of a virus with AFM the gold-coated probe will be held a few nanometers above the virus and serve as the substrate for surface enhanced Raman spectroscopy. The close proximity of the tip will allow for site-specific chemical characterization with a resolution of a few nanometers. Spatial manipulation accomplished with the tip allows for the entire capsid to be characterized with this method. Determination of whether or not a virus particle contains a gold core can be accomplished by following the general premise that only virus particles with gold cores will be capable of producing an enhanced Raman signal. Viruses owe much of their ability to function within their

natural systems to their highly specific structures. It must therefore be determined how much relation there is between synthesized virus particles and intact BMV.

3.3. Instrument Design. Other current work is focusing on construction of an instrument that will couple atomic force microscopy (AFM) with near-field scanning optical microscopy (NSOM). The bulk of the instrument will be made of stainless steel and will be attached to an existing optical microscope. The general design is shown in Figure 6.

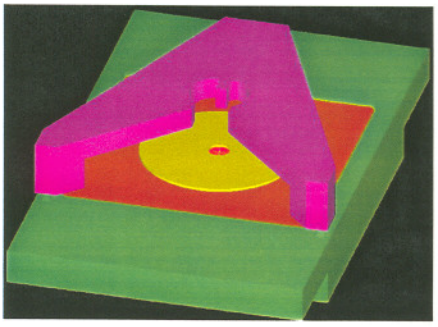

Figure 9. Basic design of the instrument to be built by our group. The tuning fork probe will be attached to the top piece, the disk will hold the sample, the red represents the piezo scanning stage, and the outer support is the mount which will be attached to an optical microscope.

Thus far the top piece has been completed. This will serve as the mount for the tuning fork probe and offers enough open space for the Raman laser to be used in close proximity to the sample. The main support stage is currently under construction and will serve as a mount to attach the apparatus to an existing optical microscope. The most intricate piece will consist of a tuning fork probe bonded to a piezo mount which will be attached to a Macor® substrate which is use due to its properties as an electrical insulator. The difficulties associated with this arise from working with materials on the order of a few millimeters, while necessitating highly precise positioning of parts. An amplifier will be embedded into the stage to minimize the distance the signal will have to travel. The amplified will then be sent to a feedback mechanism and a lock-in amplifier for signal processing. The geometry of the instrument design allows the placing of a

Raman laser in very close proximity to the AFM tip which will be the surface responsible for generating the SERS phenomena.

4. Conclusions and Future Work. Initial success imaging virus particles with a commercial AFM have proven encouraging. Height analysis and general structural observations seem to confirm that virus particles with gold cores have been synthesized. However, no chemical information can be gathered from this type of analysis. It is therefore desirable to develop a method of site-specific local probe analysis of single virus particles. This can be accomplished through the coupling of atomic force microscopy, surface enhanced Raman spectroscopy, and near-field scanning optical microscopy. Future work will consist of completing construction of such an instrument.

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