Atomic Force Microscopy with Touch: Educational Applications

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Abstract. Advances in microscopy are not only allowing scientists to investigate phenomena at ever-decreasing scale, but also permitting sensory data beyond the visual to be gathered. This chapter describes research on middle and high school students' use of remote microscopy with a new scientific tool called the nanoManipulator, which enabled them to reach out and *touch* live viruses inside an atomic force microscope. Using this technology, students were able to develop and explore their own line of scientific inquiry with adenoviruses and tobacco mosaic viruses. Research related to educational uses of the atomic force microscope is described as well as the development of the nanoManipulator and issues of remote access to the microscope over the Internet. We also discuss the microscopy instruction developed during our research and present student learning outcomes based on newspaper articles written by the students. Taking new microscopy techniques to middle and high school science classes and sharing the experience of doing science with them clearly had an impact on students. Students learned about various aspects of microscopy as well as virus morphology and function. They valued their own thinking processes and results, and weren't afraid to try new experiments to see what would happen. They learned that science can be exciting and involves the generation of new knowledge though novel explorations.

Introduction

My team is working on a nanoManipulator, which is a microscope that lets you manipulate a virus. You can squish, splat, mutilate, pull apart, or just about do anything humanly possible to the virus. It was the most interactive experience that I have ever had with a virus.

In the course of a week, I have learned so much. Coming into this experiment I knew so little about viruses, and now I can describe their size, some of their characteristics, and how viruses infect you and make you sick. The visiting scientists have inspired me and so many others to join a field in science… There are so many unanswered questions out there just looking for a solution. This experiment is just the beginning of a long line of discoveries.

(Written by Lundie, Age 15, for her school newspaper)

Recent advances in microscopy and connectivity of schools to microscopes via the Internet have opened up new worlds for precollege students to explore. Students can access expensive microscopy equipment through the Internet to perform experiments that previously were conducted only by university scientists.

For the last five years, educators and scientists from the university have worked together to provide middle and high school students access to atomic force microscopy though a specialized system our research team developed known as the nanoManipulator. The nanoManipulator consists of an atomic force microscope (AFM), advanced computer graphics, and a force-feedback joystick for instrument control. The system allows the user to not only control the motion of the microscope probe, but also provides feedback to the user about the haptic properties of the object being imaged. Haptics, by definition, involves both tactile and kinesthetic perception and the nanoManipulator system allows the user to “feel” properties of nanoscale objects such as hardness and shape, and allows the user to experiment with properties of stickiness and elasticity.

The science education project began as a pilot program to see if it was possible to remotely control an atomic force microscope (AFM) through the Internet from a local school. Two biology classes at a high school were connected via the Internet to the microscopy center located about 18 miles away at the University of North Carolina at Chapel Hill. The first year proved to be a success with students conducting atomic force microscopy experiments on adenoviruses. For the last five years, we have worked with high school biology classes and for the last two years have added middle school science classes to the outreach program. Nearly 600 public school students have had the opportunity to experiment with viruses using the nanoManipulator system. In this chapter we describe the microscope system, the connectivity issues related to linking public school classes to the microscope center, and the benefits of precollege students’ use of advanced microscopy.

Atomic Force Microscope

Since the 1980’s various scanning probe microscopes (SPMs) have been developed. These microscopes use a very small sharp tip to study a range of surface properties such as electrical, chemical, thermal, and mechanical properties at the atomic to micron scale. One such microscope is the atomic force microscope (AFM), also known as a scanning force microscope (SFM). In the AFM, the tip interacts physically with the surface of the sample under study to provide topographic images of the surface. In addition, the tip is a force sensor and can be used to study mechanical properties of nano-sized objects by using the tip to manipulate them. The AFM is particularly interesting to biologists because imaging and manipulations can be done in air and in liquid. This ability allows scientists to image and interact with single biological molecules in near physiological conditions. Since Binning, et al. [1] pioneered this microscope in 1986, AFM has been used to study structural and functional properties of biomolecules such as proteins[2], nucleic acids[3], and polysaccharides[4].

The AFM is composed of 1) a tip attached to the free end of a cantilever, 2) piezoelectric crystals, 3) a laser, 4) a quadrant photodiode detector and 5) a computer interface (Figure 1). The movements of the AFM are controlled by a dedicated desktop computer. The control software communicates directly with the AFM hardware; the software determines the tip motion and the size and location of the scan region over the sample inside the microscope. The tip is several microns long with a radius of curvature (R_c) between 10-50 nanometers. The cantilever length typically ranges from 100-200 micrometers. A piezoelectric crystal, is a material that changes in dimension with change in applied voltage. The crystal allows one to have atomic scale accuracy over the tip movement. Piezo crystals are used to move the tip in the three xyz-dimensions over the sample surface. The scan area typically ranges from ~10 nm^2 x 10 nm^2 to 100 μm^2 x 100 μm^2 with a z-range of ~15 µm. The cantilever bends up and down as the tip traces the ridges and grooves on the surface. The tip-surface interaction is monitored using the laser beam and the quadrant photodiode detector. The laser beam is bounced off the back of the cantilever on to the photodiode detector. The detector tracks the tip height via the position of the beam spot on the detector, which changes with the bending of the cantilever. In one imaging method, the computer controlled feedback loop between the photodiode and the piezo crystal maintains a constant tip height above the surface. The computer uses the tip adjustments made by the piezo to create the AFM images. Though the height data is accurate to an angstrom, the tip shape convolutes the lateral dimensions such that objects seem wider than they really are (Figure 2).

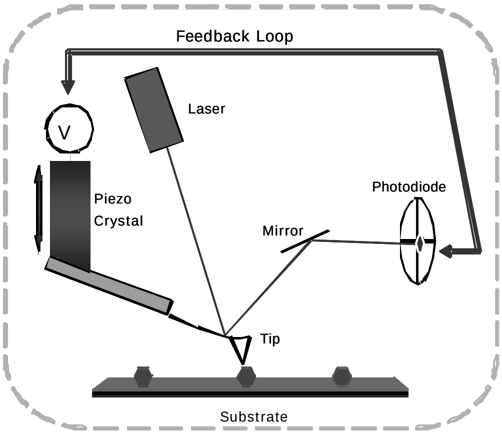

Figure 1: AFM setup

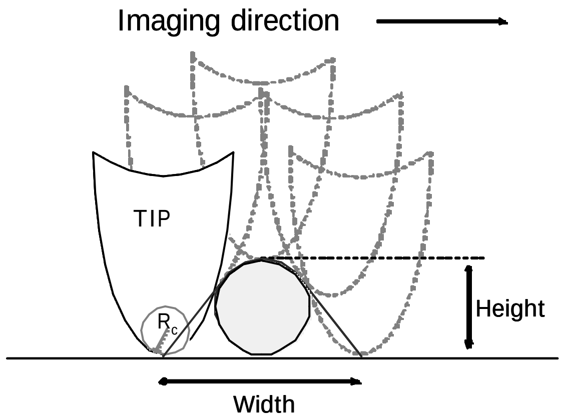

Figure 2: Tip Convolution

How are biomolecular interactions measured? First, biomolecules of interest are deposited on to a flat surface and/or on to the tip. Direct force measurements are made with the tip by pushing, poking, or pulling on these biological complexes. We can make force measurements using the AFM by detecting the degree of bending or twisting of the cantilever. The more force a tip applies; the greater the cantilever is displaced. By knowing the cantilever's spring constant and its displacement, Hooke's Law can be used to calculate the tip force. By varying the manipulation, one can measure friction, adhesion and elasticity (Figure 3). Viruses are of particular interest especially for their prevention and potential use in theraputics. Gene therapists are interesting in using recombinant virions as gene vectors because of their innate ability to infect cells and force the cells to express the viral proteins; recombinant viral particles would contain human genes. Through AFM imaging and manipulation of viruses, we can further understand viral stability and binding issues that occur during its infectious cycle.

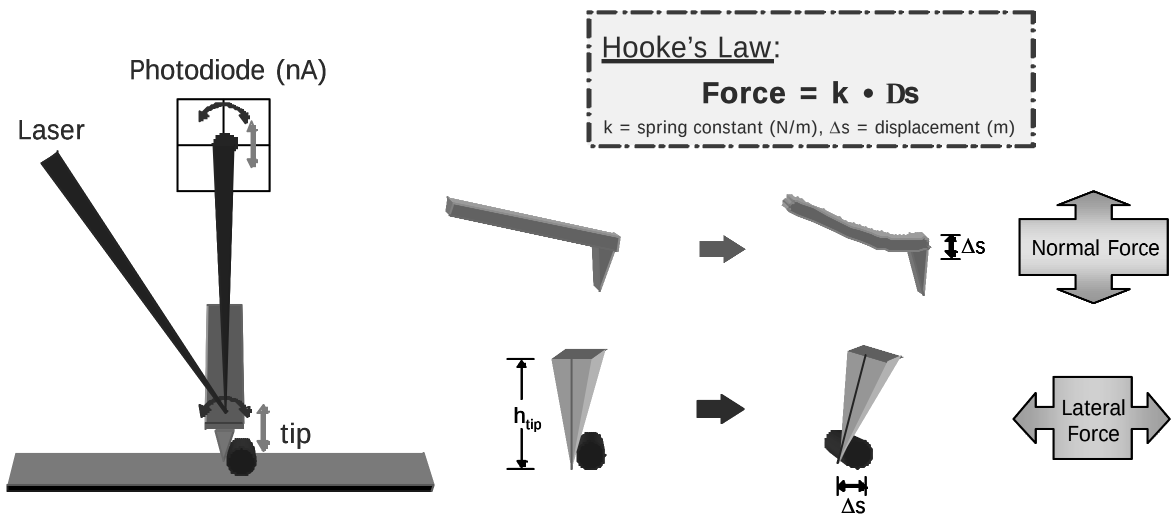

Figure 2: Force Measurements

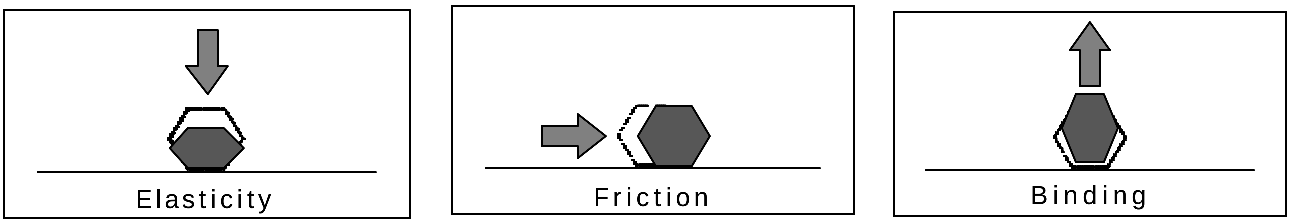

Figure 3: Virus Manipulations

The NanoManiulator System

The nanoManipulator [5] is an interactive, 3-dimensional interface to the AFM control system. The nanoManipulator communicates directly with the AFM control software and displays the scanned data in two ways. It displays the data visually, as a fully-interactive, 3-dimensional image, and it displays the data haptically, as a 3-dimensional surface that the user can touch and feel in real time, using a commercially available Phantom force-feedback joystick [6]. With the nanoManipulator interface, a scientist can see and *feel* the sample, whatever it may be, from viruses and DNA, to carbon nanotubes, or individual atoms.

The nanoManipulator system is much more than a display device, however. In addition to reading information from the microscope for display, the nanoManipulator sends control messages to the microscope that direct the motion of the AFM tip. In this mode, the Phantom force-feedback joystick becomes the active controller, and the scientist becomes the driver. By holding and moving the Phantom tip along the 3-dimensional surface created by the nanoManipulator, the scientist directly controls the motion of the AFM tip inside the microscope and can manipulate his or her sample. Now the scientist can push, poke, or cut viruses or measure the resistance of a carbon nanotube by applying an electrical current to an individual tube. Ultimately, by using the nanoManipulator, a researcher can build nano-sized gears and springs, machines over a million times smaller than exist now.

The nanoManipulator system and the AFM control software run on two different desktop computers. Figure 4 shows a diagram of the nanoManipulator system interface to the atomic force microscope. Both the nanoManipulator desktop computer and the AFM computer are connected to a network. This connection may be a direct link via an Ethernet connection, or through the Internet. To enable the nanoManipulator to communicate with the AFM control software, a message passing protocol was added to the AFM control software. This message passing protocol was designed using publicly available communication software, VRPN, developed at the University of North Carolina, Chapel Hill [7]. VRPN is a network-transparent interface between application programs, like the nanoManipulator, and hardware devices, like the AFM. This interface software uses TCP-IP standard internet message protocol [8], enabling remote communication between the nanoManipulator and the AFM across the Internet, or even the Internet2 [9].

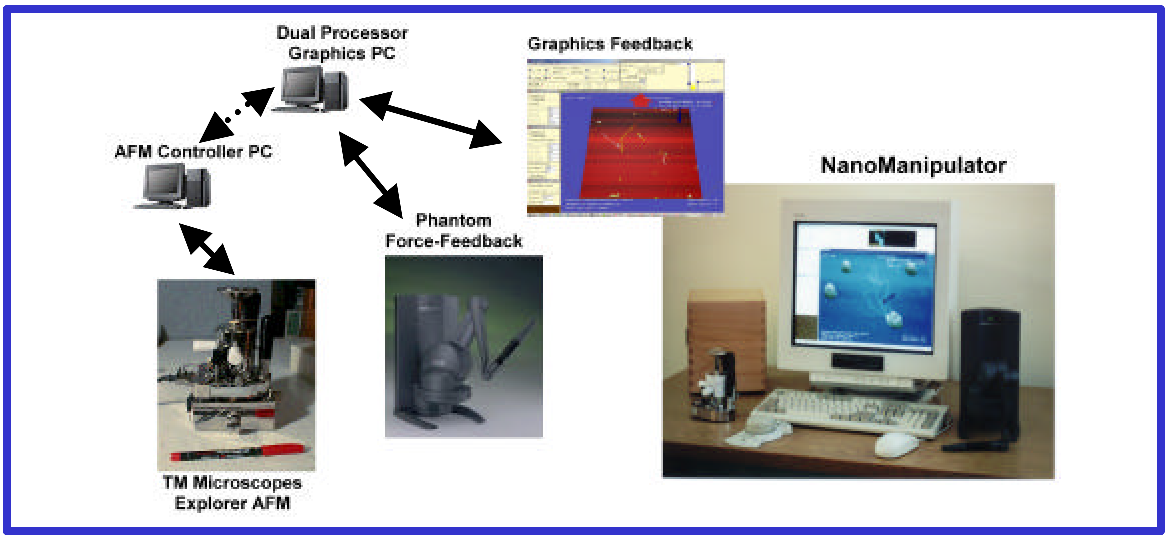

Figure 4. The NanoManipulator

The primary messages involved in the communication between the nanoManipulator and the AFM are the data stream from the microscope, which is sent line by line, as the microscope scans the sample, and control messages from the nanoManipulator, which enable manipulations by telling the microscope where to move the AFM tip in response to user-controlled Phantom motions. Because the nanoManipulator communicates to the AFM using standard TCP-IP Internet messages passing protocol, the nanoManipulator may be connected to the AFM from anywhere on the Internet. This means that the nanoManipulator does not have to be in the same lab as the AFM; it may be in the next room, across campus, or even across the country, or world.

The network system requirements for the nanoManipulator to communicate with the AFM are not extreme – it does not require a special dedicated line. Messages are streamlined, so that only surface data is sent from the microscope. Once the data arrives, the nanoManipulator reconstructs the information as a 3-dimensional image. This is much less expensive than sending live video images across the Internet, and therefore the amount of bandwidth necessary is not large. For example, a nanoManipulator system located at the Museum of Life and Sciences in Durham, North Carolina was used by students to manipulate viruses in the AFM located at the University of North Carolina, in Chapel Hill, NC, approximately 20 miles away. At the time, the museum had a partial T1 connection to the Internet, with a speed of 384 Kilobytes per second. Students were able to accurately touch, feel, and manipulate (pushing, cutting, and poking) live viruses in real time using this connection.

The critical component of the Internet connection for accurate manipulations is latency (the round-trip time for messages sent from one computer to another). In the case of the nanoManipulator, it is important that responses from the microscope return very quickly. This is most important while the Phantom force-feedback joystick is in use. In order for the scientist to accurately feel the virus and the path of his or her manipulation, two messages must be sent between the nanoManipulator and the AFM. First, the nanoManipulator must send a message to the AFM telling the microscope to move the tip in response to the Phantom, then the microscope must send a response to the nanoManipulator, which tells the nanoManipulator what the surface feels like at that new location. This communication represents one message round trip, as one message and response to and from the AFM is required. If the network system has large latency – if the time for the response from the AFM is long, then the scientist using the nanoManipulator will not be able to accurately feel the sample, and his or her manipulation will be off target. A latency of less than 50 milliseconds is necessary for accurate manipulations from remote locations on the Internet.

In our research we have taken the nanoManipulator to several schools in Orange County, North Carolina. One of the schools, Orange High school, is located about 20 miles from the University of North Carolina, Chapel Hill campus. The connection between the microscope and the high school was through the Internet via a T1 connection, with about 1.5 Megabits per second capability. In January 2002, the school upgraded to 6 T1 lines, which provided a connection speed of 10 Megabits per second. The latency for this connection was under the 50ms requirement. During the instructional activities at the nanoManipulator, students interacted with viruses in the AFM, located on the UNC campus. We also made available a Netmeeting [10] link between the high school and UNC, with both audio and video, so students could see and speak directly to the expert scientist monitoring the microscope, who in turn could see and speak to the students.

In the same year we took the nanoManipulator to Stanback Middle School, also in Orange County, North Carolina. At the time Stanback Middle connected to the Internet through a T1 link that initiated at Orange High School. We found at Stanback that the T1 connection did not provide enough bandwidth to allow communications between the AFM at UNC and the nanoManipulator, while other Internet activities were also ongoing. Our solution to this limited bandwidth situation was to limit all other Internet activities, including the use of Netmeeting, essentially providing a connection dedicated to the nanoManipulator. Once all other Internet activities ceased, we were able to communicate with the AFM at UNC with very low latency of 20ms, which enabled successful manipulations of the viruses inside the microscope.

Microscopy Instruction

The National Science Education Standards [11] calls for students beginning in 5th grade to work with microscopes as an introduction to microorganisms "establishing a foundation for developing understanding of molecular biology" (p. 155). Furthermore, the Standards argue for teaching students the "microstructure of matter" by exploring the "macroscopic and microscopic world of forces (and) motion… and the behavior of atoms and molecules" (p. 177). The early use of microscopes by students is also advocated by the American Association for the Advancement of Science as noted in the Benchmarks for Science Literacy [12]. The increased access of precollege schools to the Internet means that students can now remotely access electron [13,14] and atomic force microscopes [15].

In order for students to understand how the Atomic Force Microscope and nanoManipulator function during experimentation it is necessary to provide instruction on the basics of microscale, microscopy, the nanoManipulator system, and viruses. The entire instructional experience with the Atomic force Microscope and the nanoManipulator were designed to be completed in about 6 classes.

Viruses were selected for students to study because of their stability and availability. The study of viruses is included in the biology curriculum and is a topic of high interest to precollege students. Most middle and high school students have heard of viral diseases such as AIDS, Ebola, measles, and flu. For several years we provided adenoviruses for students to use in their investigations but found that they emerged from the experience with a limited understanding of viruses and have subsequently provided both adenoviruses (icosahedral shaped) and tobacco mosaic viruses (rod shaped) for student investigations.

The instruction begins with a presentation that includes an overview of the microscopy, descriptions of the atomic force microscope and the nanoManipulator system, an introduction to the morphology and general physiology of typical viruses, and a discussion of microscale. Most public school precollege classes have 20-35 students and a major issue is how to organize the instruction to allow each student time to work directly with the microscope. To accommodate the large numbers of students and a limited instructional time, we established a series of instructional stations for students to move through every 25 minutes [16]. The stations are described in Table 1.

Table 1. Instructional Stations

Station	Instructional Activities
Training Station	Students practice using the nanoManipulator with stored virus images. Students develop a research question to explore in subsequent investigations.
Powers of Ten	Students are introduced to micro and macroscale. Students watch the video “Powers of Ten.”
Relative Size	Using the hallway, students walk the length of the hall comparing relative sizes of model bacteria, viruses, and atoms.
Interview a Scientist	Visiting nanoscale scientists share their work with students.
Simulation of AFM	Students construct a a 3D paper model of a virus, and make an image of the virus using a mechanical simulator. By moving the tip over the virus, students also move an attached ink pen that records the tip movements on paper.
Virus Investigations	Working in groups of 4, students use the AFM and nanoManipulator to explore viruses and collect data to answer a question they have about virus morphology or function.
Newspaper Story	Upon completion of the stations, students write a newspaper article describing their experiences and what they learned.

Learning Outcomes

As a result of participation in this series of instructional activities, students developed a broader understanding of microscopy and a richer understanding of the atomic force microscope, viruses, and the processes of scientific inquiry.

Knowledge of microscopy. Prior to instruction, most students could only name the light microscope when asked to list as many different types of microscopes as they could. By the end of the experience, not only could most students name the atomic force microscope (AFM) as an alternative to the traditional light microscope, but also many of them were also able to describe in some detail how the AFM works. In their newspaper stories written at the end of the experience, many students clearly described the scanning and imaging process used by the AFM to create an image of objects that are too small to be imaged by light, such as viruses. Miki, one of the students in the project, wrote in her newspaper article, "The point of the microscope is used to feel the virus, like a blind person would use his finger to feel a penny." The concept of creating an image by scanning as opposed to using light was noted by Clint who wrote, "I also changed what I thought about microscopes. I always thought that they were something that you would take a picture with or look through some kind of lens, not use a little point to feel an object and look at it with computer generated pictures."

Another aspect of microscopy students were able to concisely describe following the experience was the remote control of the microscope over the Internet – as Sarah put it, "At [the university], the itsy, bitsy nano tip pokes and cuts at the virus as we move the [Phantom] over here [at the middle school]. A number of students seemed enthralled by being able to control a microscope in a university lab that was miles away from their school. How often does a middle school student express delight with microscopy as Carly did when she wrote, "What we were seeing on the monitor was what was under the microscope. It was hooked up to the Internet so we could see what was under the AFM on the computer screen. How cool is that?"

Students' knowledge of microscopy seemed to have been broadened beyond their previous conceptions and even caused some students to compare the advantages of using the AFM over traditional light microscopes. Amanda seemed to prefer the AFM when she wrote, "The microscopes we use in class are called light microscopes, they're difficult to see through and we can't <u>feel</u> what we examine and we cannot view our subject on the computer or save our experiment on the computer." Perhaps the most concise description of how students' thinking about microscopy changed because of this experience comes from Nick, "I never knew that a microscope could do that sort of thing."

Real Science. When reading students' stories about the experience, a theme that emerges is that the students valued the reality of the experience. They often emphasized how interesting the experience was because it was 'real,' using real viruses and exploring something that people were truly interested in knowing more about. Phrases such as that by Carl (his emphasis) "after the stations we got to use the nanomanipulator to investigate <u>real</u> viruses!" and Megan "when we used it [the nM], the information was live and we were actually messing with the real viruses" show up a number of times in student stories. Along with emphasizing that they were experimenting with real viruses, students also commented that they were surprised that they got to handle expensive equipment. Carl expressed this by writing, "I had no idea that a nanomanipulator was a machine that allowed you to touch and move viruses and was amazed that we seventh grade middle school students were given permission to use it."

Along with the reality of using actual viruses and using "real" science equipment, many students also focused on the idea that people were interested in their results. Many apparently felt empowered by this experience to value their own results and to believe that they could have something to contribute to scientific knowledge, unlike most textbooks assignments where the information is perceived to be already known and their job is to learn, not to contribute to knowledge. Comments ranged from new equipment development ideas such as Brett's, "I have numerous ideas but one of my best ones is to make a tip of the AFM to have a hole at the very end of it. Using this tip I would see what would happen to a virus when you put air into it and when you suck all of the air out of it" to satisfaction that their results may prove useful to someone, such as expressed by Cole, "I am pleased with my own work and glad that my work was quality enough for her [a scientist] to save and use in her paper later in her career." Some students displayed a sense of empowerment by writing comments similar to Brett, “you never know, a seventh grader could find a new virus or a cure” and Torrey, “so don’t feel like the scientist is the only one who could make a cure or be responsible for a cell or virus although it is important, because you can form anything and be rewarded for your work.” Conor even had the confidence to draw his own conclusions and support them with experimental evidence, “What I did was push a virus into dirt. This is my story and I am sticking to it.” The empowerment exhibited by students to value their work and value their thinking shows the significance of the microscopy investigations on students’ understandings of the processes of science.

“Outrageously fun experience.” This title written by Mariel, one of the students in the project summarizes the opinion of many students about this experience. Many students indicated that they thought of science as normally boring, but that this project really brought out some of the excitement and fun of doing science. For some students, microscopes and science do not sound like an interesting topic, yet after this experience many commented on how much fun it was. Typical comments included this from Brian, “At first I thought it was going to be boring and stupid. It actually turned out to be quite a learning experience for me” and this from Jordan, “I have gained a lot of respect for scientists and can actually see why they find this fun.” Even students who may normally not enjoy science had positive comments to make. “Well once I started thinking that I would be working with viruses during science, I thought it would be very boring. I’m not an expert in science like some others in my class so when I thought of viruses I thought of cells and how bad I did on that section of learning. …Learning about viruses, measurement and scientists didn’t seem fun to me at first but I was wrong, it was!” (Kathryn) “I think this experimentation was one of he coolest things I’ve ever done in science. Further, I [normally] hate science!” (Allison) Along with expressions of how much they enjoyed the experience, some students indicated that they are more seriously considering science as a future career. For example “I am really interested in being a scientist now” (Nick) and “Most of the students in our class were clueless about how cool it is to be a scientist.” (Katie) Comments such as these indicate that we need to better educate students about the fun and excitement of science so that they don’t leave school with primarily negative impressions, and getting students involved with leading edge science may be a way to do it. The uniqueness of the experience seemed to play a role in the positive attitudes developed by students, as exemplified by Christina, “I was thrilled to know that this type of experiment had not yet been completed successfully by one of my peers. Knowing this I was overly thrilled, and for the first time in my high school career I was ecstatic about something I had done in science.” Students seemed to particularly enjoy pushing the boundaries and exploring relatively unknown territory.

Microscopy with Touch. The impact of the haptic components of the experience is currently being studied as part of a series of research studies [16, 17]. Preliminary findings indicate that students who have haptic experiences with the nanoManipulator compared to students who have limited haptic experiences, tend to have better attitudes toward using microscopes and doing microscopic investigations, and are significantly more likely to have 3-dimensional understandings of viruses, and hold more accurate representations of virus shapes. Further research is needed to more fully document how the use of haptics in microscopy education alters students’ conceptions of objects that are not visible, even with light microscopes.

Summary

Taking new microscopy techniques to middle and high school science classes and sharing the experience of doing science with them clearly had an impact on students. Students learned about various aspects of microscopy as well as virus morphology and function. They valued their own thinking processes and results, and weren't afraid to try new experiments to see what would happen. They learned that science can be exciting and involves the generation of new knowledge though novel explorations. As one student recently said, "as a result of doing these investigations, I learned that science can be fun. Before, I just thought science was something you read about in textbooks."

The success of remote access by public school students to expensive microscopy equipment has the potential to take science out of the hands of only a few privileged scientists, and place it into the hands of students from wide ranges of abilities and backgrounds. The cost of the connection is modest, the cost of the equipment needed at the school is dramatically decreasing, and the primary expense is the cost of having a technician available in the microscopy center to place the samples in the microscope and monitor the imaging. We are currently in the process of developing a low cost haptic joystick that can replace the Phantom used in the nanoManipulator system. The inexpensive joystick and the addition of a reasonably inexpensive graphics card to a school's desktop computer can open access to these experiences to students in a wide range of schools. These developments have the potential to make *science for all* a reality.

Furthermore, the addition of haptics to atomic force microscopy adds a whole new dimension to microscopic studies for students. Being able to touch nanoscale objects that are being imaged gives the user additional data and alters the perceptual reality of the investigations. Being able to roll, turn, squish, or feel the texture of a virus sharpens the senses to the multidimensionality of virus morphology.

In summary, remote microscopy has been shown to be a viable avenue for students to experience the cutting edge of science. Students not only learn about microscopy, but they have the opportunity to experience science in unique ways that promote science as a process of inquiry.

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