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Summer Internship Summary Paper

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Summer Internship Summary Paper

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Section 1: ***Describe your internship project and discuss your specific role.***

At Lawrence Livermore National Laboratory I was assigned to work with Dr. Alexander J. Malkin, an expert in biological Atomic Force Microscopy (AFM). In addition to working with Dr. Malkin, I also worked with a post-doc in the lab Dr. Marco Plomp. I learned the techniques of AFM in my first week, and began imaging *Bacillus anthracis* spores for microbial forensics. The goal of this project was to understand the affects of formulation on the spore coat protein structure. In addition to imaging bacterial spores, I also developed procedures for imaging live *Bacillus anthracis* cells in liquid. This work shows promise as a method to understand how biological countermeasures work, and aid in the development of new countermeasures in the case of a biological attack. The experience was a highly positive one, as my supervisors gave me considerable amounts of freedom to work on my project independently. They were always around to discuss results and procedures, but never dictated what work was to be done. The lab is a small one, which was nice because it was extremely easy to get instrument time which allowed me to be very productive during the internship. I would recommend this lab as a great internship opportunity for future DHS scholars and fellows. A synopsis of the work completed is given in the remainder of this section.

Project Abstract:

The visualization of biological molecules and assemblies can provide enormous insight into protein structure-function relationships, as well as practical applications to fields such as microbial forensics. Atomic force microscopy (AFM) is a powerful tool for imaging soft biomaterials such as cells, spores, and proteins with nanometer resolution. The goal of this work is to understand pathogen architecture and its application to microbial forensics and medicine. Initial work has focused on imaging *Bacillus* species; both live cells and dormant spores. Experiments have shown AFM capable of visualizing the fine structures of cell wall peptidoglycan and spore coat proteins. Work completed thus far indicates that AFM will be able to resolve some persistent questions in microbiology concerning structure-function relationships at cell surfaces, as well as assist in understanding the formulation and processing of spores used for bio-terrorism. In addition to imaging results, we have developed a robust method for the attachment of cells to surfaces for imaging in liquid.

Introduction:

In tapping mode AFM (TM-AFM) (Figure 1), the cantilever is oscillated at its resonance frequency, such that the tip moves in and out of contact with the sample (tapping), as the piezoelectric scanner moves the sample in the X and Y directions. During imaging, a feedback loop maintains constant cantilever oscillation amplitude which is measured by the photodetector. Changes in amplitude trigger the feedback loop to adjust the height (Z) of the piezoelectric scanner as to maintain the constant amplitude. TM-AFM gives high resolution images of soft samples which are difficult to attach to a surface, without destroying their fine structures.

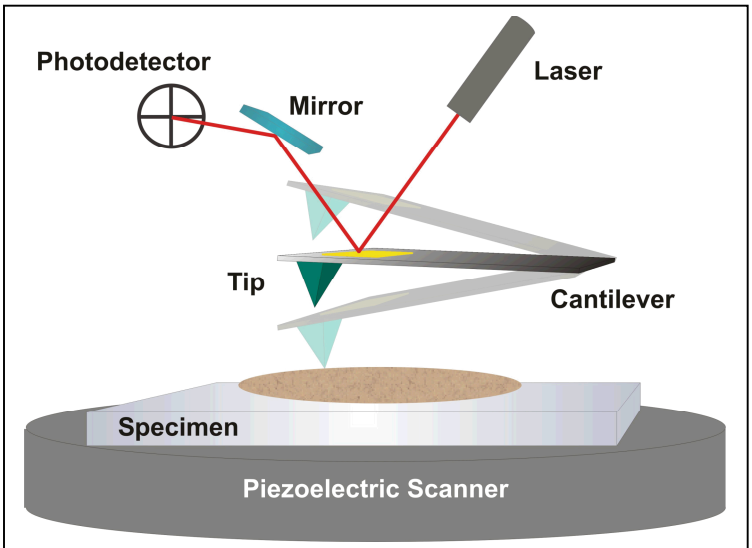

Figure 1 – Tapping Mode Atomic Force Microscopy

Tapping mode AFM is also capable of high resolution imaging in liquid, which allows for the investigation of cell surfaces under native conditions. This is vital because when cells are dried they begin to collapse and loose there rigidity, which is manifest as ridges on the surface. Though some structural features remain, it is difficult to say unequivocally that those features seen in the air are not an effect of the drying process. Additionally, the cells are no longer alive when dried, and changes in surface morphology due to antibiotics can not be seen. When imaging any sample in liquid, attachment of the sample to the substrate is extremely important. If the sample is only loosely held by the surface, the forces associated with scanning the AFM tip will cause the sample to become dislodged. When a cell detaches from the surface, it can no longer be imaged, and it floats away. Since this is the case, we have developed a robust method for chemically attaching cells to surfaces with the help of Dr. Jane Bearinger.

Methods Developed:

The covalent attachment method using carboxylic acid (COOH) groups on the surface of gram positive and gram negative cells, a chemical cross-linker, and an amine

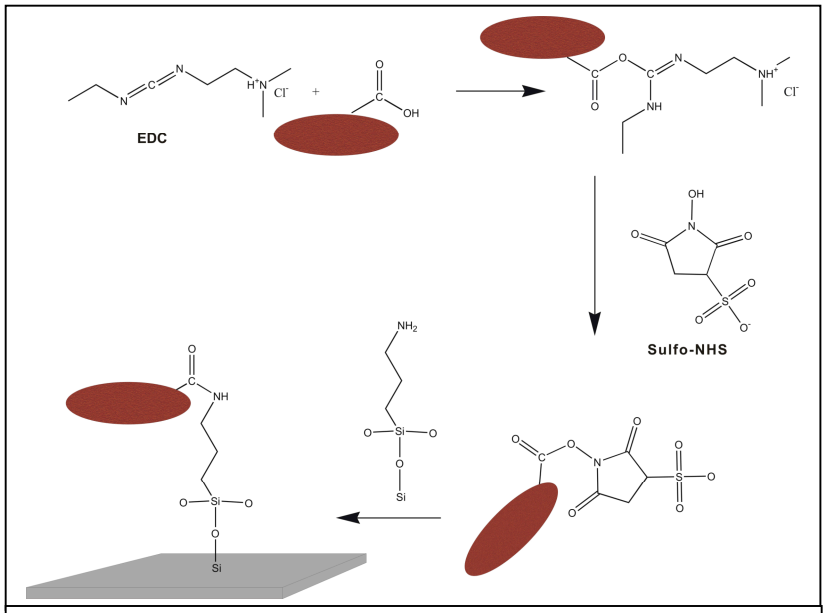

Figure 2 – Chemical Attachment of Cells to Surfaces

terminated silicon surface to give a robust cellular attachment for imaging in liquid. The chemical steps are shown in figure 2, and will only be discussed briefly. The cells are washed to remove residual media, and are allowed to react with the chemical cross-linker sulfo-NHS and the activating agent EDC. After the cells have been chemically modified, they are separated from the reaction side products and wastes. The clean cells are incubated with an amine terminated surface, allowing for the formation of a chemical bond between the carboxyl group of the cell and the amine of the surface. The surface is then washed to remove all unbound cells and imaged.

Data/Results:

I was able to obtain high resolution images of bacillus cells in liquid showing a structure filled with small holes. We believe that this indicates a peptidoglycan structure of vertical scaffolds, not horizontal sheets as other scientists have proposed. Additional investigations are ongoing to obtain additional AFM images of the peptidoglycan structure form different bacillus species. The structure of peptidoglycan can be seen under different resolutions in figure 3.

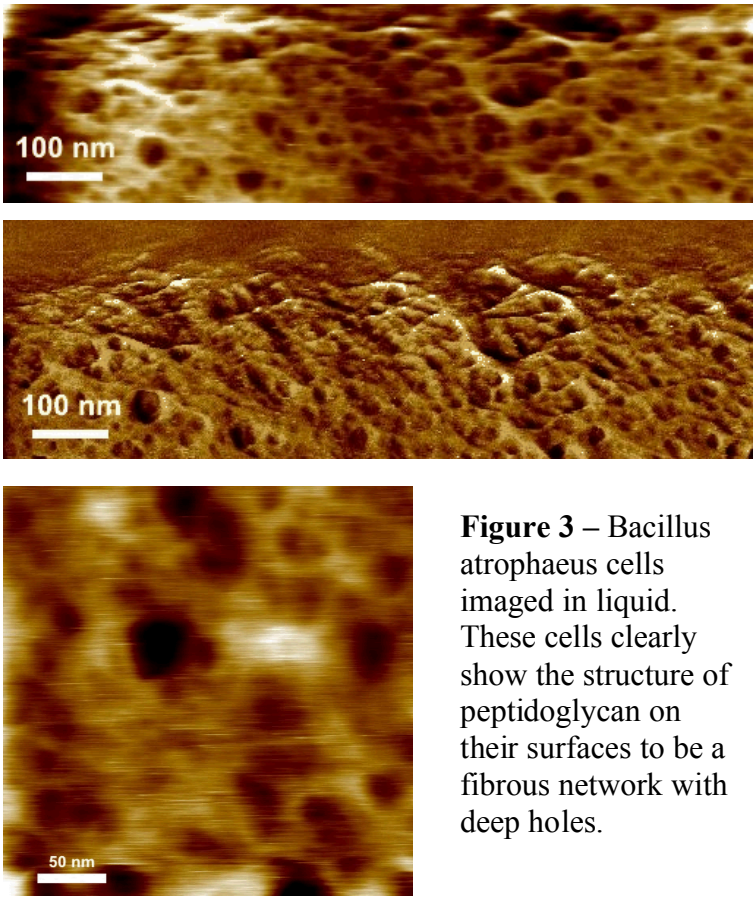

Figure 3 – Bacillus atrophaeus cells imaged in liquid. These cells clearly show the structure of peptidoglycan on their surfaces to be a fibrous network with deep holes.

In addition to imaging live bacterial cells, I have also imaged dried *E. coli* to test the chemical attachment technique. Currently, *Bacillus anthracis* has only been imaged dried and work is continuing to obtain high resolution structures of the surface of anthracis in liquid.

Besides cells, I spent some of my time working on the imaging *Bacillus anthracis* spores, to help determine formulation signatures for microbial forensics. AFM is able to determine the quality of the spore preparations quickly and easily. As preparation of bacterial spores is critical to the weaponization, AFM may prove an essential technique for assigning blame for an attack. We have seen that different preparations of spores yield different surface structures of anthracis spores. Work is currently on going to develop a formulation signature chart for the Department of Homeland Security and the Federal Bureau of Investigation. Images of *Bacillus anthracis* spores can be seen in figure 4. In addition to these spore studies, I did some work on another project that has been classified.

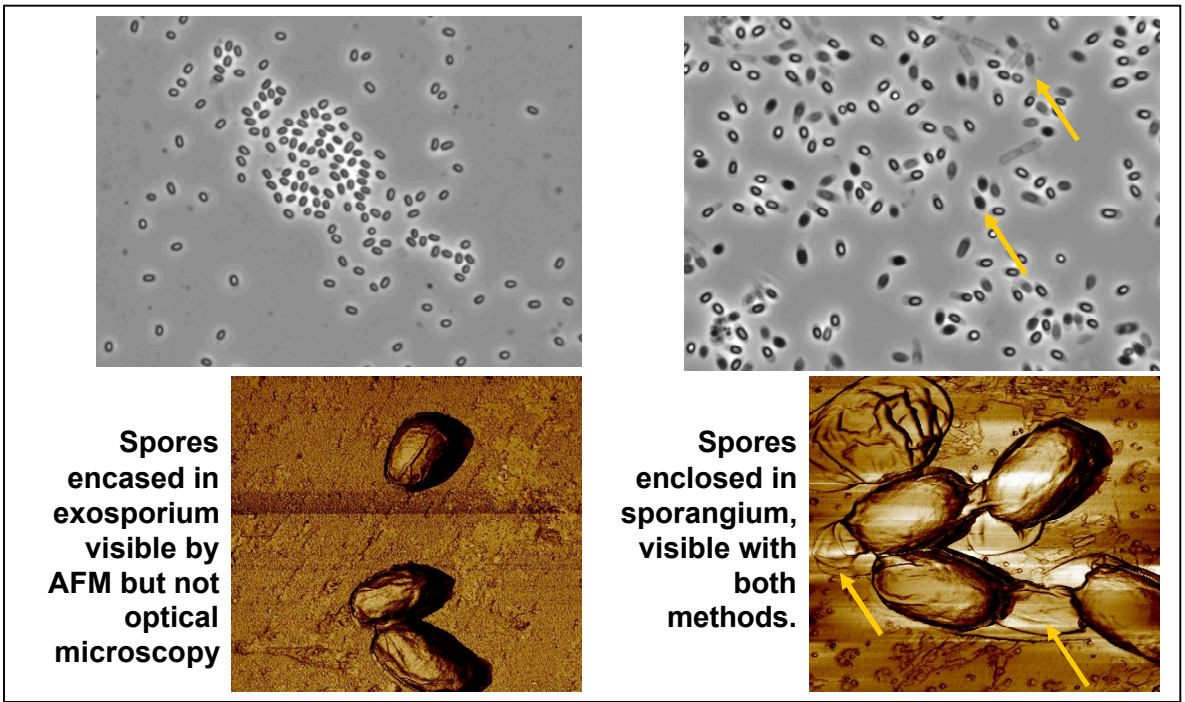

Figure 4 – *Bacillus anthracis* Spores. (Top: Phase Microscopy, Bottom: AFM)
The spores on the right show a tight exosporium indicating completion of sporulation. The spores on the left show a baggy sporangium indicating incomplete sporulation.

Section 2: ***Discuss your achievements during the internship including contributions to publications, presentations and accomplishment of project milestones.***

In the 10 week period, I was able to contribute significantly to two projects, both having to do with the imaging of live bacterial cells using AFM. One project was the covalent attachment of cells to silicon surfaces. I worked with Dr. Jane Bearinger to develop surface chemistries to couple live cells to patterned surfaces for many applications. The technique works for attaching cells to any amine terminated surface, and can be used for fluorescence microscopy as well as atomic force microscopy and other related microscopies. The work that I have done for the covalent attachment of bacillus species

and *E. coli* to surfaces will be published in an upcoming publication authored by Dr. Bearinger.

The second publication stems directly from my project to achieve high resolution images of living bacillus species in liquid, showing the three dimensional structure of peptidoglycan. I am currently finishing this project; however, since Lawrence Livermore National Laboratory requires me to submit my paper for review prior to my departure from the laboratory, I am not finished with the work at the time this paper had to be submitted to them. Currently, Dr. Malkin feels that the work will be submitted to the Biophysical Journal with in a couple of months.

Section 3: ***Discuss how the internship experience impacted your academic and/or career planning. Include a description of lectures/activities sponsored by the hosting facility that were of benefit to your personal and professional development.***

The time that I have spent at Lawrence Livermore National Laboratory this summer has exposed me to a world of science outside the realm of academia. There is a vast difference between research done at an academic instiution and that done at a national laboratory. The national laboratory works on projects in a high collaborative manner, with clear project goals and deadlines. During the 10 week stay at the national laboratory, I have learned that the research done by scientists at the national labs is generally focused on practical applications, such as detection, deterrence or prediction. Almost no fundamental research goes on at the national labs, which is completely different from the work done at universities. As I consider which path I would like to

pursue for a career, I have realized that both academia and national laboratory research is not what I am really interested in. The one thing that the lab did show me that I was no aware of was the ability for scientists to transition from the bench into the realm of science policy. I believe that there is a large disconnect between policy makers funding the sciences and scientists working in the trenches. My experience at Lawrence Livermore National Laboratory has lead me to investigate the possibility of entering the arena of politics/policy with the hope of closing the gap between society en mass and the scientific community.

Lawrence Livermore National Laboratory helped to foster this idea by holding weekly DHS brown bag lunches, where laboratory personnel came and spoke about their work, and what they believed the big challenges for science at the present and in the future are and would be. This was extremely interesting because almost everyone said that they believed more scientists needed to work at conveying the importance of the research done through government funding to average citizens as well as to policy makers.

Section 4: ***Discuss any ideas you may have of areas of research that should be considered to help the DHS accomplish its mission and goals.***

After attending a number of seminars concerning the development of radiological, nuclear, chemical and biological detection systems I think it would be useful for the DHS to establish an internship for students to spend their time at some of the military testing grounds for these new technologies. It is very easy for people in the lab to come up with a concept and to make it work in the laboratory setting, however making that platform

function in the real world is more difficult. I believe that if scientists and engineers had a practical understanding of what sort of conditions DHS needs these detectors to operate under, than the designs will be better which could bring technologies to the first-responders faster. It might also be enough to do a 1 week trip for students in the summer to one of these testing facilities, as a part of their 10 week commitment.

Another area of study that might also be useful to the DHS is in science policy. I know that there are a significant number of students who are interested in getting an understanding of how science policy is made. It might be a good addition to the summer program to offer the opportunity to do an internship with a congressman or governmental agency where students could see if this area is of interest to them. I know that there are lots of summer internships in Washington DC not associate with DHS now. This might be relatively simple thing to do because if a congressman/woman did not have to pay that student then they might be interested in having the help.

Addendum:

Thanks for a great opportunity. I have also attached a .pdf version of the poster that I gave at the Lawrence Livermore National Laboratory Poster Symposium ~ Haley Hill

Auspices Statements:

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