State Space Equations

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MC10
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I. SPACE EQUATIONS

$$F = k\Delta x \tag{1}$$

$$\omega_1 = \begin{bmatrix} 0 \\ 0 \\ \theta_1 \end{bmatrix}$$

II. BACKGROUND THEORY

AFM became a preferred method of imaging in many fields, including molecular and cell biology, solid state physics, chemistry, et al. AFM's advantage lies in that it's resolution is not limited by focusing of light through lenses. Therefore, diffraction limits do not limit the spatial resolution of the image. AFM images the surface of materials using a precisely aligned laser that reflects off an oscillating, tipped cantilever being moved across the sample. The distance between the sensing tip and the surface is controlled and adjusted by the TRAX software.

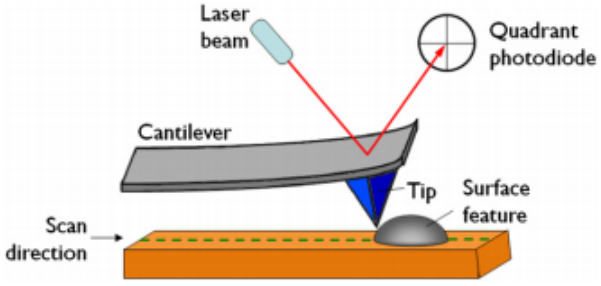

FIG. 1. AFM set up

The cantilever feels a force

$$F = k\Delta x \tag{2}$$

where F is the force felt by the cantilever, k is the stiffness of the cantilever, and Δx is the cantilever deflection. This small deflection is captured by the photodiode, which creates the topographical image.

The AFM used in this experiment has three primary modes: static force, dynamic force and phase contrast. Static force mode simply measures the deflection of the laser as the cantilever is dragged over the surface. In this imaging mode, the cantilever steadily contacts the direct surface. Both the cantilever tip and sample face degradation after significant use in static mode. Dynamic, or tapping, mode oscillates the cantilever at a resonant frequency. When the tip comes close to the surface, Van der Waals forces (attractive or repulsive forces between molecules) affect the deflection of the laser from the cantilever. Phase contrast mode expands on dynamic mode by also measuring the cantilever's vibration to a reference signal. The forces felt between the tip and the surface of the material alters the cantilever's resonance frequency. This measurement can give the image enhanced contrast when the tip and material have significant interaction.

III. MATERIALS AND METHODS

We used a traxAFM from Nanoscience Instruments. We selected the appropriate cantilever tip for dynamic mode scanning. We imaged two samples: a calibration grid and a sample of staphylococcus bacteria. The silicon oxide on silicon calibration grid contained elevated squares at approximately 25 nm with 10 μm periodicity. The staphylococcus was one of a variety of samples provided by Nanoscience Instruments.

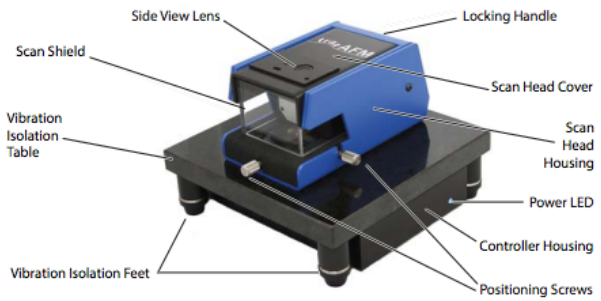

FIG. 2. AFM Instrument

Before imaging, we performed a frequency sweep which first coarsely and then finely tuned the resonant frequency of the cantilever. By eye, we advanced the tip as close to the surface without making contact. The program then instructed the tip to make the final approach for imaging. This process was repeated in between samples and between image collection.

We began image collection with the calibration grid. We collected images and compared their quality as we

altered the AFM parameters, including scan range, time per scan line, and offset. We were able to zoom in to one particular square on the calibration grid to receive maximum resolution. The calibration grid collections allowed us to make an educated guess for the input parameters for imaging staphylococcus. We analyzed both images in Gwyddion using the extract profile and measuring distance tools to identify characteristic measurements, such as width, length, height, and for staphylococcus, diameter.

IV. RESULTS

The normal scan of the calibration grid used the default parameters of 256 points/line at a scanning rate of 1s/line to produce a 47.18μm by 47.63μm image.

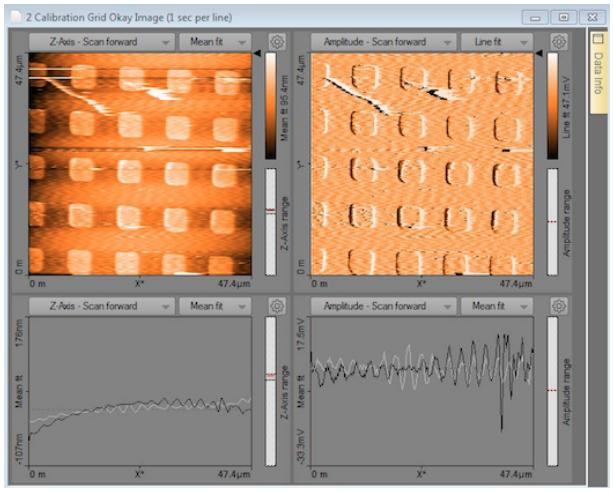

FIG. 3. Calibration Grid Scan

We were able to measure the height, width, and length of this image in figure 3 using the built in profiling tools provided by the Trax software. The sample red line in figure 4 displays where the profile shown in the graph was taken across the sample. First, we compared our measured periodicity to the known periodicity of the sample (10 μm). We made multiple measurements across the calibration grid and found a periodicity of 9.78 $\pm$ 0.03 μm (all uncertainties are standard error). We measured the width and length of the squares in the calibration grid to be 5.13 $\pm$ 0.05 μm. We measured the step height, Δz, to be 26.17 $\pm$ 1.49 nm, which is within agreement of the reported height of 25 nm.

From the image in 4, we selected a subsection of 18.9x18.9 μm to zoom in on and re-scan, see 5. As the image becomes more zoomed in, the background noise becomes more prominent. This AFM can resolve features to the nanometer resolution. The calibration grid provides a known pattern with set dimensions to test the AFM's resolution and signal to noise ratio. We confirmed this by re-measuring the step height of the grid features

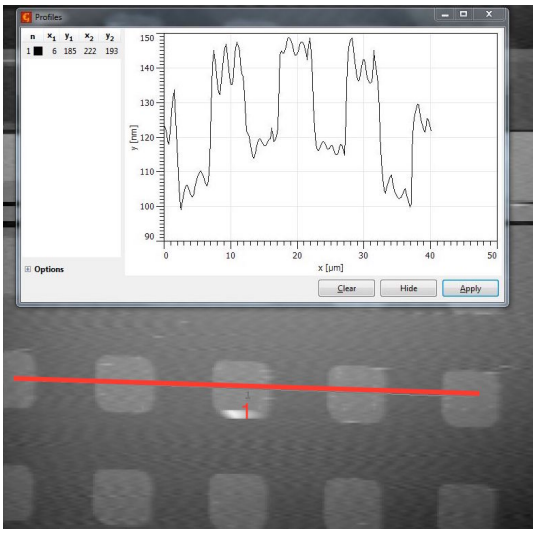

FIG. 4. Calibration Grid Profile

across multiple images.

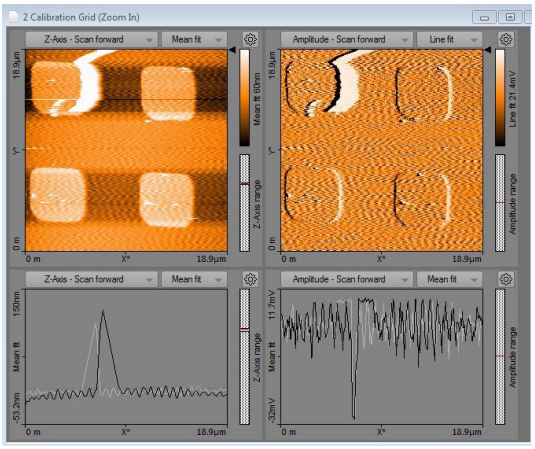

FIG. 5. Calibration Grid Zoomed in

The scan range parameter controls the dimensions the AFM images the sample. The parameters can restrict the area and height the cantilever oscillates. The maximum scan range for this AFM is 70x14μm (1x0.2 nm resolution). We took images using different scan ranges. The image in figure 6 has a limited range, giving a smaller image of 25.1x25.1 μm. By adjusting the z-axis scan range, the user can optimize the vertical sampling for a better image quality to get a more accurate reading on feature dimensions.

The offset parameter translates the cantilever, shifting the area over which the AFM captures the image. To demonstrate this, we altered the offset half way through an image capture (see figure 7). This does not have a direct effect on image quality in regards to noise or resolution. The offset tool allows the user to shift the imaged region without physically moving the sample in the AFM. This is useful as manually translating the sample to im-

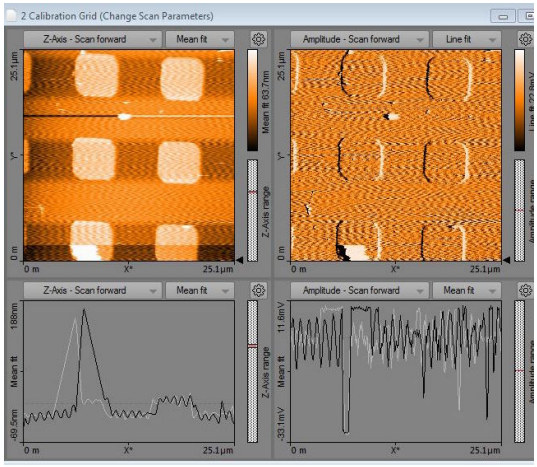

FIG. 6. Altered scan range parameters

age a desired feature on a micrometer scale would prove very difficult and prone to human error.

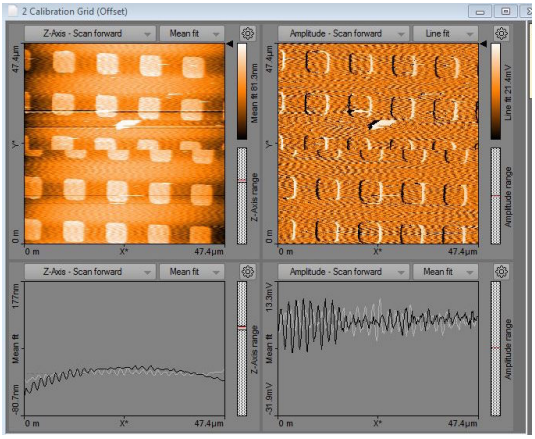

FIG. 7. Changed system offset half way through image

Time spent on scan per line directly effects the image quality of the image. The more time spent scanning, the better the image quality. When the AFM scans the material too quickly, the image becomes blurry and the photodiode cannot capture every slight deflection from the cantilever. When the cantilever scans over the sample more slowly, the detector captures more detail, resulting in better resolution. We scanned the same section at 0.5, 1, and 1.5 seconds scan time per line. Figure 8 displays these three images (in that order).

Finally, we imaged a sample of staphylococcus aureus (figure 9). Staphylococcus is a commonly found bacteria, known for its distinct spherical shape and 'grape-like' clustering. To enhance image quality, we imaged at a scan per line time of 3 seconds, a scan range for a 5x5 μm image, and increased the cantilever oscillation amplitude. The profile in figure 10 reflects the spherical topography of the bacteria. The bacteria are not evenly spaced on the sample; many grow on top of each other.

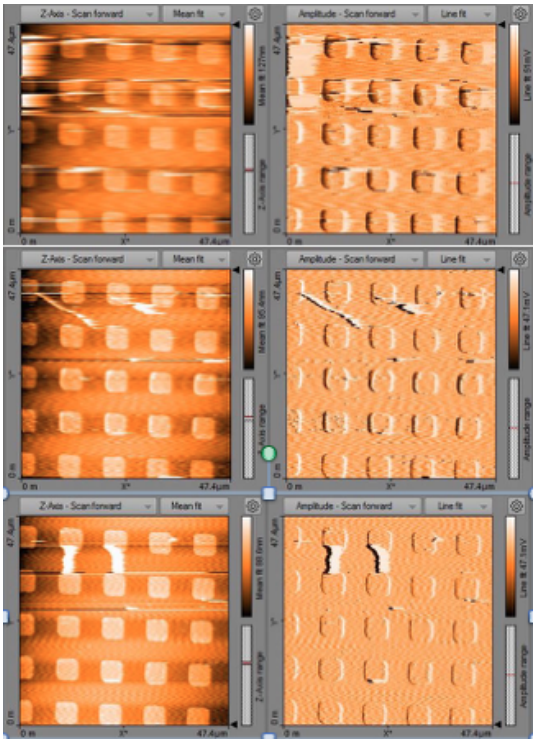

FIG. 8. Calibration Grid Scan/line

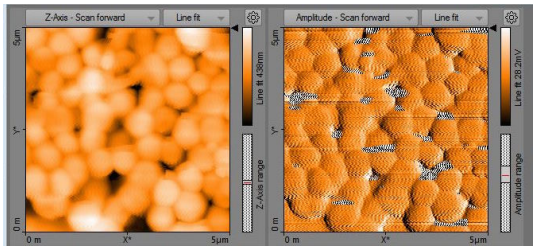

FIG. 9. Staphylococcus

The profile depicted in Gwiddyon shows a gradient in feature height, indicating that certain bacteria have grown on top of others. From this image, we measured an average diameter of 0.63 ± 0.01 μm (sample measurement in figure 10). This value is comparable to the diameter found in literature (0.6 μm). The uncertainty in our measurement may stem from the fact that not all bacterium grow to be the exact same size as variation exists in the population. Biologists may use this image to determine the sustainability of staphylococcus populations per unit area.

V. DISCUSSION

AFM imaging can collect topographical features from samples at the nanometer resolution. The technique is very different from traditional microscopes and does not suffer from the same spatial resolution issues. Using

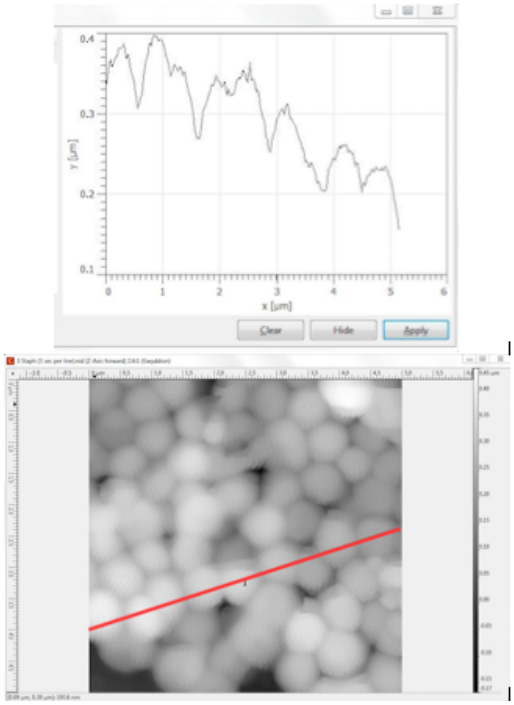

FIG. 10. Staphylococcus Profile

AFM, we took multiple images of a calibration grid with nanoscale features (i.e. step height). We altered parameters such as scan range, time per scan line, and offset, to see how these values affected image quality. These steps were important in learning how to optimize the AFM for image quality. These parameters change based on each unique sample. The calibration grid came with known information about its periodicity and step height. We verified the measurements made from our images by comparing to the given values. Both periodicity and step height matched within uncertainty to the known values at 9.78 ± 0.03 μm and 26.17 ± 1.49 nm respectively. We also measured the length and width of the calibration squares to be 5.13 ± 0.05 μm.

After testing how the AFM parameters affected image quality, we then attempted to image a provided staphylococcus sample. We upped the scan per line time to 3 seconds to achieve better resolution. We changed the scan range to focus on a particular subset of the sample and upped the cantilever amplitude for better z-range to visualize the 'grape like' clustering known to staphylococcus, shown in figure 9. Staphylococcus aureus is a spherical bacteria. We characterized our sample by measuring the average diameter of individual bacterium. We measured multiple bacterium and found an average diameter of 0.63 ± 0.01 μm. Staphylococcus is a commonly found and studied bacteria and we confirmed this diameter value in literature (0.6 μm).

The AFM is a very sensitive imaging technique. Some of the artifacts seen in our images (bright spots, lines, additional contrast) are caused when the AFM is disturbed during imaging. The AFM was placed on a lab bench, where multiple student groups were working. Any ambient vibrations, tapping, or hitting the bench affected image quality.

VI. CONCLUSION

We collected AFM images of a calibration grid and a staphylococcus sample with nanoscale resolution. We first optimized the oscillation frequency of the cantilever in dynamic mode by performing a coarse followed by a fine frequency sweep. We approached the tip to the sample as close as possible by eye and then allowed the software to make the final approach.

We began by taking multiple images of the calibration grid, altering the AFM imaging parameters each time. We observed the differences in image quality after changing each parameter. The time per scan line had the most direct effect on image quality. The more time the cantilever spent imaging per scan line, the better the image resolution. When scanned too quickly, the image becomes blurry. We compared the known values of periodicity and step height for the calibration grid squares to our measured values, finding them in agreement. These results are displayed in Table 1. We also measured the length and width of each individual square to be 5.13 ± 0.05 μm.

We then reconfigured the AFM parameters to image staphylococcus. This took some trial and error as we did not know prior to imaging surface information such as height of the structures. After adjusting the tuning, we captured a sample of staphylococcus and measured the diameter. Our measured diameter of 0.63 ± 0.01 μm is comparable to the value found in literature 0.6 μm (with some variation due to population variety).

Material	Feature	Value	±	Known Value
Cal. Grid	Step Height	26.17 nm	1.49 nm	25nm
Cal. Grid	Periodicity	9.78μm	0.03μm	10μm
Staphylococcus	Diameter	0.63μm	0.01μm	0.6 μm

VII. BIBLIOGRAPHY

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[3] Bacterial Cell Structure. Steane, Richard. "www.biotopics.co.uk/g11/bacterialcell.html"
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VIII. APPENDIX: FIGURES

VIII.1. AFM Measurements

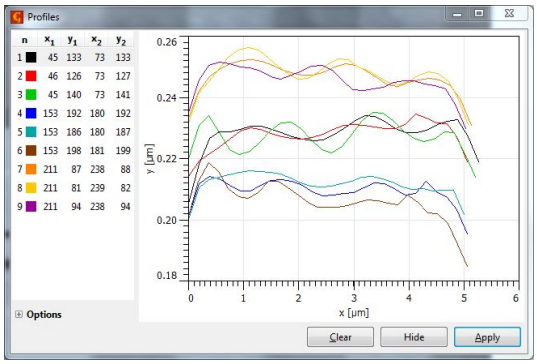

FIG. 11. Calibration Grid width/length measurements