Atomic Force Microscopy (AFM) Module labreport

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November 2017

Abstract

AFM was used to characterize DNA/PEI polyplexes. Three samples were imaged at two magnifications. All polyplexes seemed to be spherical in shape. Average particle size was calculated by the use of the software ImageJ and confirmed consistency in size, but with a high degree of uncertainty.

1 Introduction

The report, written by group B1, is part of the AFM module in the specialization course TFY4525. AFM is a microscopical technique widely used in modern education and industrial laboratories. To be able to use the tool a given procedure has to be followed. This is explained in the Methods and Material part.

In the labtext several questions were posed and these are answered in the Theory section. The layout continues to follow the pattern indicated in the labtext by answering the questions given on beforehand in the Discussion.

2 Theory

2.1 Answers to posed questions

1. What is the basic principle for AFM? Explain in your own words how AFM can be used to obtain atomic resolution of a surface.

The AFM has a cantilever with a small, sharp probing tip that is scanned very closely across the sample's surface. Due to the small distance between the tip and sample, atomic range forces act between them. The tip measures these forces. And the force acting on the tip is determined by detecting the deflection of the cantilever.

In order to achieve atomic resolution, two conditions have to be fulfilled. Those are ultra-clean and flat surfaces prepared in vacuum systems. For the AFM system, the measurement of the deflection from the last paragraph is used to control the tip-surface distance on an atomic scale. From a pure physical perspective, the forces acting on the tip will lead the tip to snap onto the sample. From this an effective nanometer-range flattening of the tip is induced.

2. In AFM there are many different modes of operation. The two most common

modes of operation for topographic imaging are tapping mode and contact mode.

a. What is the difference between contact mode and tapping mode AFM?

The difference between them is implied in their respective names. In contact mode the tip is raster-scanned across the surface and deflects as it moves over the surface. AFM's tip is in hard contact with the surface. In the tapping mode, a stiff cantilever is oscillated close to the sample. The tip intermittently touches/taps the surface.

Difference between them is therefore constant contact with the sample for the tip in the contact mode and intermittent contact with the sample in the tapping mode.

b. What are the advantages and disadvantages for these two modes?

Advantages of contact mode AFM are high scan speeds and that samples with extreme changes in vertical topography can occasionally be easier scanned. Disadvantages are that lateral forces can give a distortion of features in the images, high forces normal to the tip-sample interaction. The combination of these two can result in reduced spatial resolution and could lead to damaging of soft samples.

For the other mode in question, tapping mode, the advantages are higher lateral resolution on many samples, less damage to soft samples imaged in air due to lower forces and the interesting fact that lateral forces are virtually eliminated. A major disadvantage is that the scan speed compared to contact mode is slower.

c. If you have a biological material with a relatively soft surface, which of the two modes would you choose? Why would you choose this mode?

The preferred choice would be tapping mode. Reason for that would be due to the improved lateral resolution on soft samples in the tapping mode. When using the contact mode the combination of lateral forces and high normal forces could damage soft samples.

3. What is the convolution effect? Explain what it is and how you could avoid or at least partially compensate for this effect in an image.

The convolution effect is that the AFM tip can influence measurements. Any AFM image is a convolution of the shape of the probe and the shape of the sample. The effect makes protruding features appear wide and holes appear smaller.[1] The convolution effect increases as the radius of curvature of the tip increases and as the slopets of the facets increase.

An image can be partially corrected, and thereby partially negating the effect, if the shape of the tip is known.

2.2 DNA/PEI complexes formation

The DNA/DEI polyplex formation occurred in two ways: through a microfluidic channel and bulk mixing on a table. DNA is negatively charged and PEI is positively charged. When these two components get into contact with each other, they will instantly self-assemble into polyplexes. The

microfluidic channel is ideal to control a process like that due to its dimension at the microscale. Nanosight was used to characterize the polyplexes as the last step of the process.

The Nanosight results showed that the polyplexes were rather polydisperse. This indicated that the microchannel creation process did not go optimally.

2.3 AFM theory

AFM is an important tool for imaging, measuring and manipulating matter at the nanoscale. It has two modes: contact mode and tapping mode. The working principle of the AFM is that it measures the atomic range forces that arise due to the small distance between the tip at the end of the cantilever of the AFM and the surface.

The Nanosurf AFM, used in this module, refers to the two modes from the paragraph above with different names. Contact mode is static force and tapping mode is dynamic force. In the static force mode, "static" deflection of the cantilever is used as the error signal for the Z-controller. The term Z means the z-axis. It could visualize itself in fast motion in the z-axis and flatness in the x and y-axis. In the dynamic force, changes in the dynamic behaviour of the cantilever are detected by measuring changes in its vibtration amplitudes. At that point it is excited with a sinusoidal signal with a frequency close to the cantilevers free resonance frequency.

3 Methods and Material

The polyplexes formed in previous labsessions will be analyzed with the AFM. But before a characterization can take place, the sample has to be prepared.

A quartz substrate is cleaned with a ISO-5 (a cleanroom designation) wipe, wetted with a cleaning liquid. The quartz is rubbed on both sides. The liquid consists of ethanol first, followed by acetone. Visual control should be used to inspect the quartz. A final cleaning with ethanol must be done to remove acetone residues.

Three square regions on the substrate are sectioned out by the use of tape. A name should be given to each region. Micropipette is used to deposit 10 μL for each of the solutions. Deposition should be followed by drying with Nitrogen. Pressure should be held low for the Nitrogen. For two of the samples: bulk mix 10 μL and undiluted 10 μL the process in this paragraph should be done once. For the undiluted 30 μL, the process should be repeated three times.

Nanosurf AFM was used to acquire the images. The mode was dynamic force and the tip was an ACL-A tip. Speed in the measurements was:

- bulk 10 μm * 10 μm sample: 1 second/line

- bulk 4 μm * 4 μm sample: 0.6 second/line

- Undiluted 10 μL, 10 μm * 10 μm sample: 0.6 second/line

- Undiluted 10 μL, 4 μm * 4 μm sample: 0.6 second/line

- Undiluted 30 μL, 10 μm * 10 μm sample: 0.6 second/line

- Undiluted 30 μL, 4 μm * 4 μm sample: 0.6 second/line

Resolution is 256 points/line.

The DNA/PEI was prepared one day before the AFM characterization and stored in room temperature. The samples were covered and stored in room temperature for 30 minutes.

All the images were taken on the same day.

4 Results and discussion

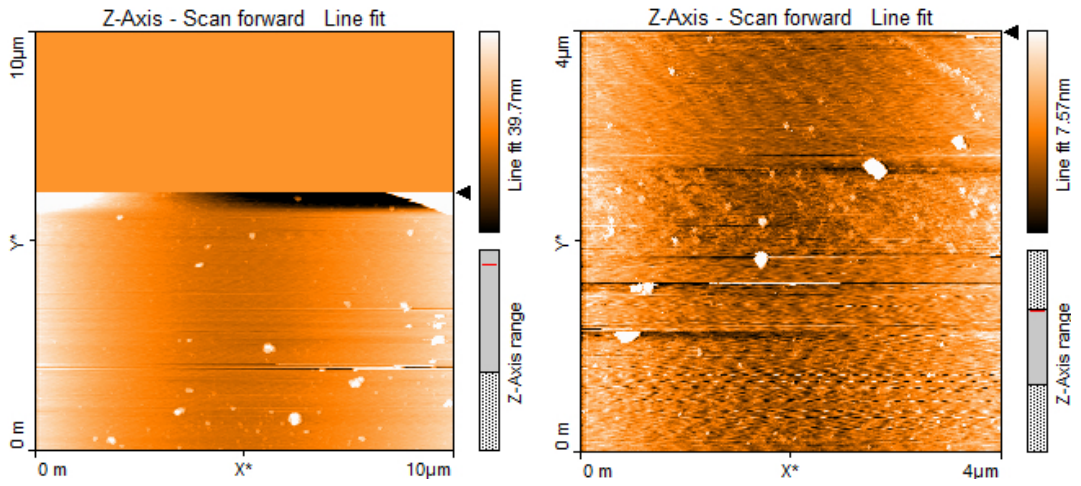
4.1 AFM micrographs

Figures 1-3 show the AFM micrographs taken of the three samples, and each sample is imaged at 10 µm × 10 µm and 4 µm × 4 µm magnification. For the bulk mix sample (figure 1), the 10 µm × 10 µm image is unfortunately amputated due to time limitation. In both magnifications, spherical polyplexes are visible. The concentration is low, and they are unevenly distributed across the surface.

For the microfluidics fabricated polyplexes in the 10 µl sample (figure 2), a high concentration of spherical polyplexes are evenly distributed on the surface. Especially for the 10 µm × 10 µm magnification, an artifact in the image makes the depth at vertical edges seem different from the central part, making it more difficult to visualize the polyplexes.

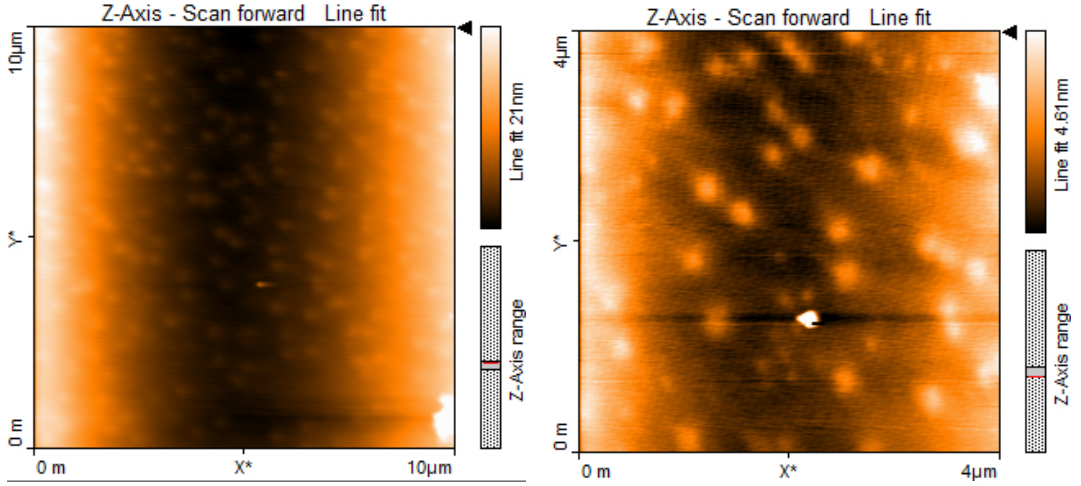
For the microfluidics fabricated polyplexes in the 30 µl sample (figure 3), polyplexes are not so easily detected. A large particle, possibly dust or a very large agglomerate, is clearly visible, with a few spherical polyplexes spread on the rest of the surface. Again, the same artifact as in image 2 (a) makes the detection more difficult.

For the microfabricated polyplexes of different sample volume in figure 2 and 3, the expected difference was to see a higher concentration in the latter sample, as the drop would have evaporated in a relatively smaller area than for the first sample. However, as figure 3 show one large particle and a few polyplexes, there might have been some problem when applying the solution to the sample stage. Effort was made to locate a better area to scan, but due to time limitation this was not succesful. Comparing the bulk mix (igure 1) with the microfluidics fabricated 30 µl sample (figure 3), the concentration is much higher for the microfluidics fabricated sample. The reason for this might be a difference in original concentration in the two samples, as these concentrations are unknown to the authors.



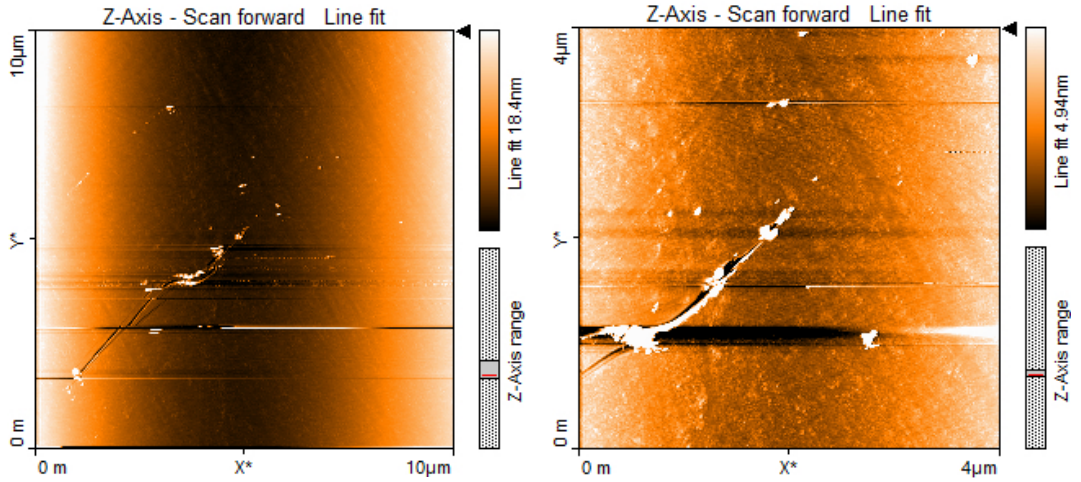
(a) (b)

Figure 1: AFM micrographs of the bulk mix sample of DNA/PEI polyplexes, 10 µm × 10 µm (a), and 4 µm × 4 µm (b). The figure in (a) is amputated due to time limitation.



(a) (b)

Figure 2: AFM micrographs of the 10 µl sample of microfluidics fabricated DNA/PEI polyplexes, 10 µm × 10 µm (a), and 4 µm × 4 µm (b).



(a) (b)

Figure 3: AFM micrographs of the 30 µl sample of microfluidics fabricated DNA/PEI polyplexes, 10 µm × 10 µm (a), and 4 µm × 4 µm (b).

4.2 Size distribution

The polyplexes imaged in subsection were quantified using the image analysis software ImageJ.[2] Detection of polyplexes was done using the 10 µm × 10 µm micrograph for the bulk mix sample, and the 4 µm × 4 µm for both microfluidics fabricated samples, due to differences in image quality. To obtain the best results, the micrographs were cropped before analysis to only include the part with visual polyplexes, avoiding the vertical edges as the analysis tool is sensitive to the difference in brightness. After analysis, very large particles were manually erased from the data sample before further calculations and plotting, as they were assumed to be agglomerates and not representative for the polyplexes. Very small particles were also erased. As these were several orders of magnitude smaller than the smallest polyplexes, they were assumed to be noise.

Table 1 lists the average particle radius in µm and standard deviation for the three samples. The average particle size does not differ much between the three, but contrary to expected results, the standard deviation is larger for the microfluidics fabricated DNA/PEI polyplexes compared to the bulk mix fabricated polyplexes. This might be due to smaller polyplexes and more agglomeration for the microfluidics fabricated polyplexes, but other explanations might also be possible. The distribution of particle sizes can be seen in figures 4-6. The data sample is too small to say anything certain about the distribution, but the figures visualize what table 1 states, that the polyplexes are fairly equal in size between the three samples, and distributed in the range 20 to 100 nm in radius.

Table 1: Size distribution of DNA/PEI polyplexes.

Sample	Average particle radius (nm)	Standard deviation
Bulk mix 10 µl	48.0	19.7
Microfluidics fabricated 10 µl	48.6	30.3
Microfluidics fabricated 30 µl	42.2	20.8

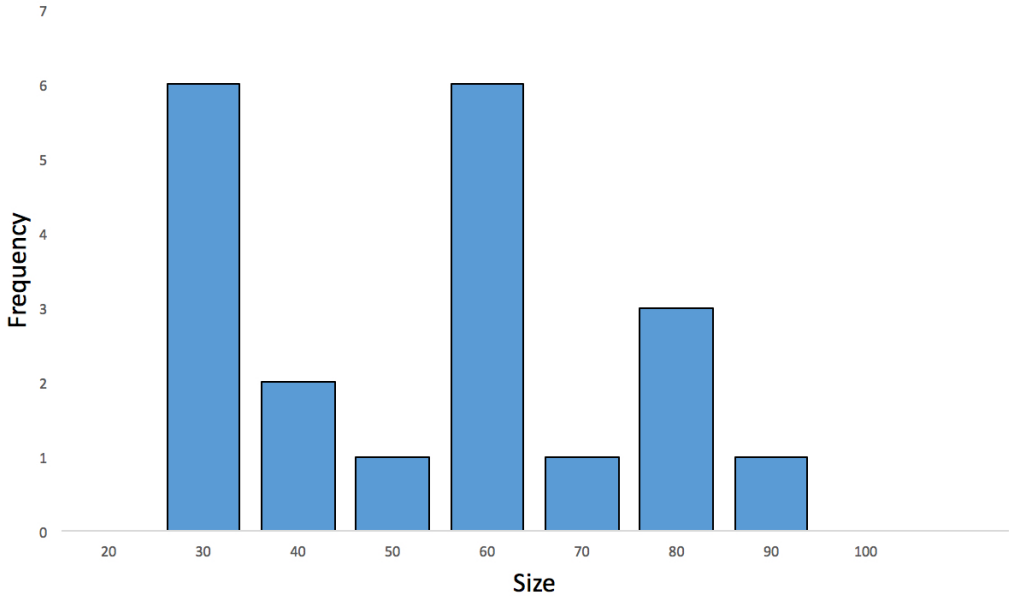


Figure 4: Histogram of polyplex size for the bulk mix DNA/PEI polyplexes.

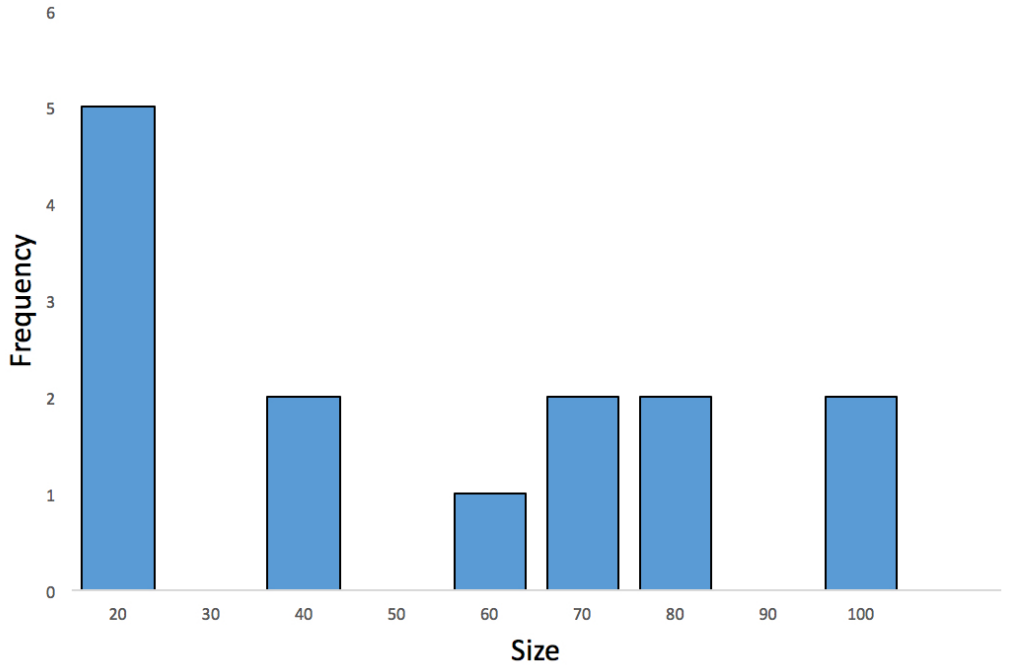


Figure 5: Histogram of polyplex size for the microfluidics fabricated DNA/PEI polyplexes, 10 µl sample.

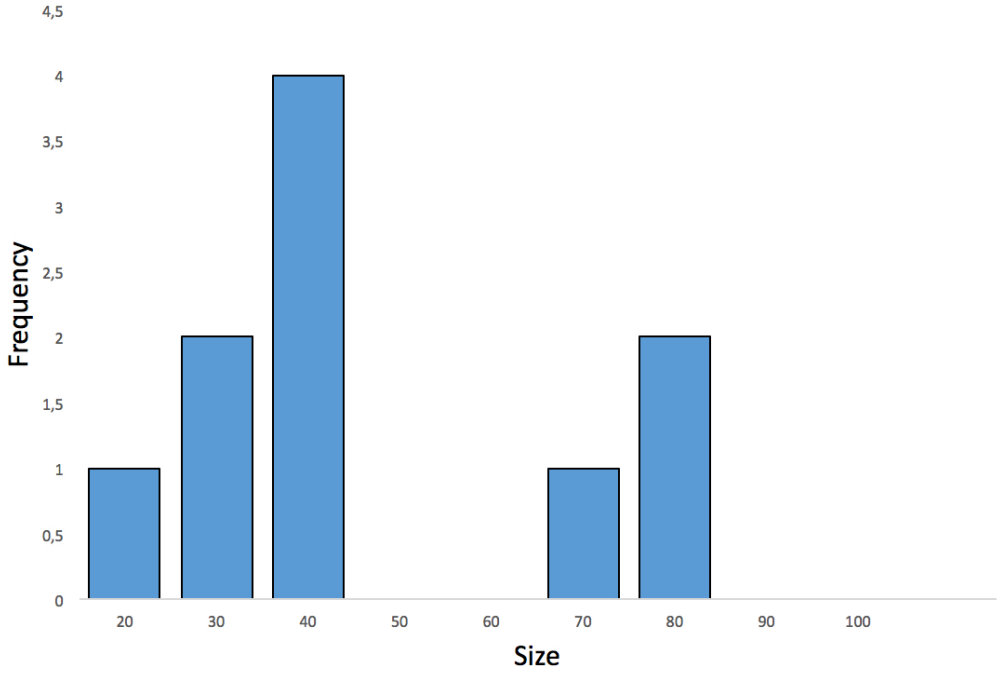


Figure 6: Histogram of polyplex size for the microfluidics fabricated DNA/PEI polyplexes, 30 µl sample.

Polyplexes seemed to be spherical in all three samples, and it was not possible to detect any rodshaped polyplexes. The resolution is high enough to detect any supercoiled or open circular DNA, but none were visible.

The polyplexes in this report have also been characterized using Nanosight, an instrument giving the size distribution by measuring the Brownian motions of the particles in a liquid solution. For the bulk mix sample, the size was measured to be about 50 nm in radius, and for the microfluidics fabricated polyplexes the size was measured to be about 80 nm in radius. These measurements unfortunately include a high degree of uncertainty. Nevertheless, they are in the same order of magnitude as the results elaborated on this this report. The measurements for both characterization techniques were performed on the same day, so the state of the polyplexes should have been very similar.

The polyplex samples were stored in room temperature overnight before imaging. If the samples were stored in room temperature for a longer period of time, there would be an increase in the risk of agglomeration or DNA degradation. Outside the natural environment of the living cell, nucleic acids are prone to degradation if buffer content, temperature and storage time is not monitored carefully.[2] Agglomeration occurs due to attractive forced between the particles in a liquid, and this is known to be a problem for different types of polyplexes.

5 Conclusion

AFM micrographs were obtained for three samples of DNA/PEI polyplexes. The images showed differences in concentration but spherical shape for all deteced polyplexes. The size distribution was found by an image analysis software, confirming consistency in size with differences in variance. The sizes obtained are in the same order of magnitude as measured for the same particles using Nanosight.

References

[1] *AFM.com Tip-Sample convolution*. http://afmhelp.com/index.php?option=com_content&view=article&id=65:convolution. Accessed: 2017-11-28.

[2] Debomoy K. Lahir and Bill Schnabel. "DNA Isolation by a Rapid Method from Human Blood Samples: Effects of MgCI2, EDTA, Storage Time, and Temperature on DNA Yield and Quality". In: *Biochemical Genetics* 31 (1993), pp. 321–328.