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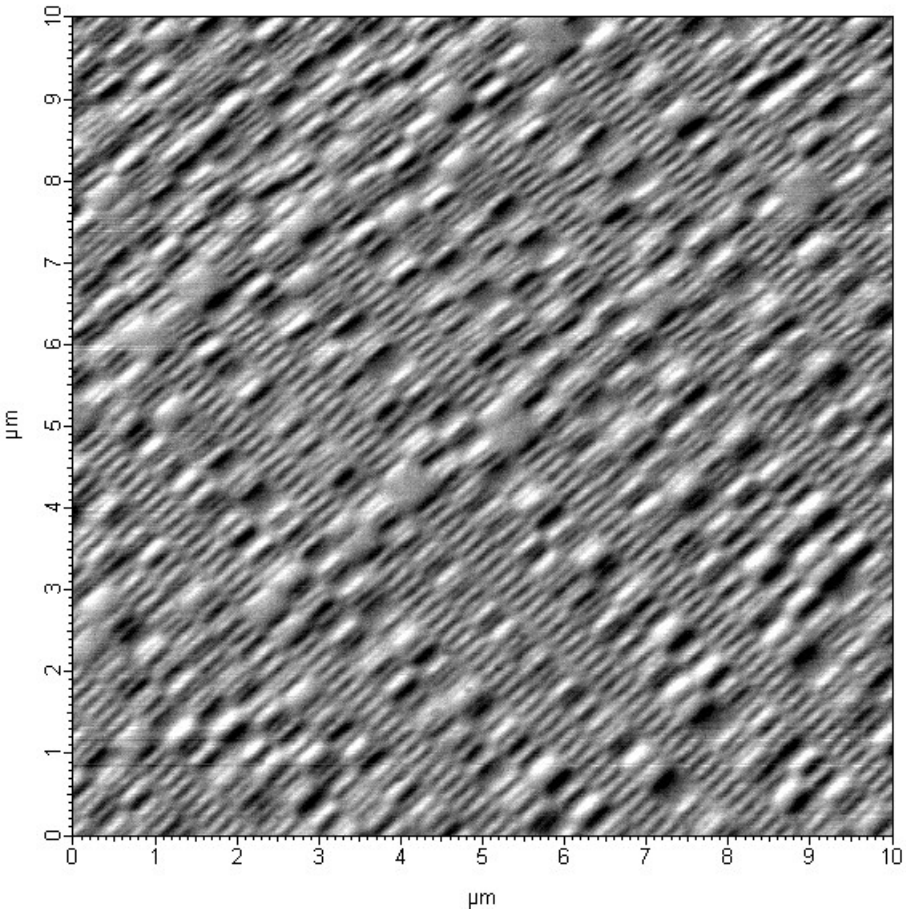
MFM and Nanomanipulation of magnetotactic bacteria



A Project Work by Alexander Lurf and Peter Oser

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MFM and Nanomanipulation of magnetotactic bacteria

Introduction

Our task was to perform MFM measurements on magnetotactic bacteria (MB).

Further we tried to manipulate our samples with an AFM tip.

Abstract

The preparation of the samples has been done in a microbiology laboratory. The bacteria have been in a formaldehyde solution. They have been centrifuged and then applied to a microscope slide. The MB could clearly be seen through a regular light microscope.

In order to learn how to operate the AFM we made some measurements on the bacteria with regular 70 kHz AFM tips.

Then we went on to make MFM measurements on magnetic samples with relatively large magnetic structure to get a feeling for the magnetic tips. Starting with an old music tape, over a 1.44 MB HD disk to a 40 GB hard disk we got to smaller magnetic structures on our samples. After that we tried to get pictures with magnetic structures of MB but we did not see anything.

So we turned our attention to manipulate the MB with a non magnetic 350 kHz tip. Again we started to use different samples to gain experience in using the scratch mode on the AFM. First we used Plexiglas samples and clear plastic binder samples but made no results until we used a gold sample.

Finally we managed to cut MB in two, using the scratch panel provided by the AFM software.

Sample preparation

We got the samples from Dr. Schüler in a solution of formaldehyde. We needed to have few bacteria in a single layer on a slide to be able to work with them on the AFM. We did so by centrifuging them at 800 rpm for 5 minutes. After that a dark spot of bacteria was gathered at the bottom of the container. They have then been applied to a slide using a spatula or a eppendorf pipette. Finally we dispensed them with distilled water.

Slides prepared this way have been used for AFM and MFM measurements and also scratching experiments.

For MFM experiments we used pieces of tapes, 1.44 MB HDD and a 40 GB Harddisk attached to a slide with plasticine. The HDD can be seen in figure 1

figure 1: 40 GB sample for MFM meassurements

For scratching experiment we attached plexiglas and clear plastic binder samples with double sided tape to slides. The gold foil has already been prepared to a slide.

AFM

We started making measurements on slides with MB in AFM mode to get to know the AFM micoscropy.

Figure 2 shows an amplitude trace image of a MB. The amplitude trace is the first derivate of the height information, but gives one a good impression of the outlook of an bacteria.

In firgure 3 you can see a single bacteria in a 3D image and colouration as height information.

The MB are approximately 3.5 µm in length and about 0.5 µm in width.

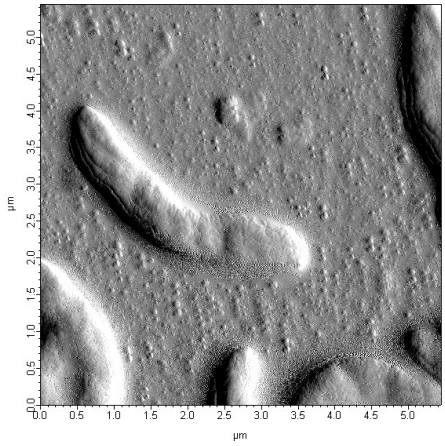

figure 2: AFM amplitude trace of a single bacteria

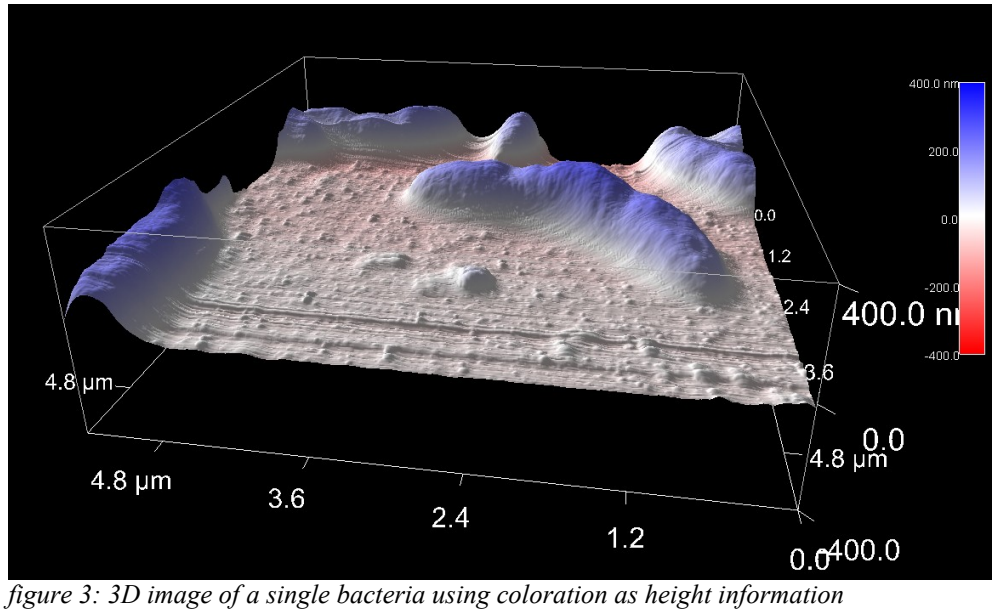

figure 3: 3D image of a single bacteria using coloration as height information

MFM

MFM is a variation of the AFM mode with metallic coated and magnetized cantilevers. Further there is a MFM mode called NAP panel and NAP channel panel in the MFP-3D software in the menu MFP controls.

There you can change the delta height and the scan ratio.

The measurements are done in two steps. The cantilever goes over each line in a regular tapping mode and then repeats the line in a special height called delta height to gain the magnetic structures. You can see the additional information in 3 extra windows called Nap height trace, NAP amplitude trace and NAP phase trace. Most clearly the magnetic structures could be seen in the NAP phase trace.

The magnetisation of the cantilever

The cantilever has been magnetised with one of the fixing magnets of the AFM. We first did this by adducting the magnet with one of his poles close to the cantilever and then pulled it away. We tried to keep the gap between the cantilever and the magnet smaller than 1 millimetre. After a few measurements we found out that the magnetization of the cantilever was not exactly repeatable.

So we looked for a reproducible method and found one using a precession lever (figure 4). We clamped the fixing magnet in the head of the lever and adjusted it in the way that the magnet was less than one millimetre away from the cantilever in extended position. In retracted position it was more than a centimetre away. We put the retracted lever next to the head of the AFM, extended and retracted it again. On this way it was possible to magnetise the cantilever always on the same way. It was just necessary that the lever is always standing in the same position in front of the AFM head.

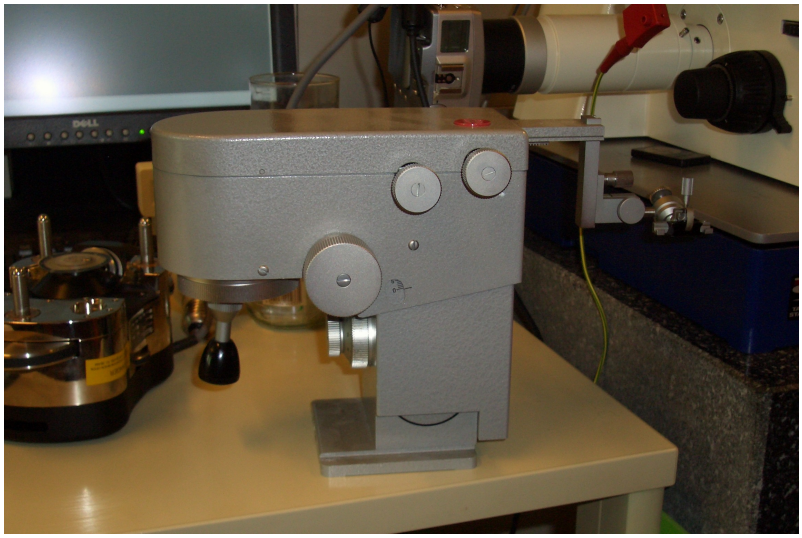

figure 4: precession lever for magnetizing the cantilever

The fixing magnets were necessary for the magnetisation of the cantilever, so we needed another way to fix the glass slides on the AFM. We used plasticine to fix the probes, because it does not affect the magnetisation of the probe, which is possible if they have a low magnetisation.

MFM measurements

In all the following measurements we used the MFM standard tip. First of all we tried to make a measurement of a sample of an old music tape, to learn how to use the MFM mode. On this first measurement it was easy to identify the magnetic structure on the tape. We made this gauging with a delta height of 20 nm. That means that the cantilever was in a distance of 20 nm over the probe.

After this first success with the magnetic tip we tried to measure smaller magnetic information. So we made a sample of a 1.44 MB HD disk and measured with a delta height of 5 nm. The magnetic structures with dimensions of about 2 µm could clearly be seen. (figure 5)

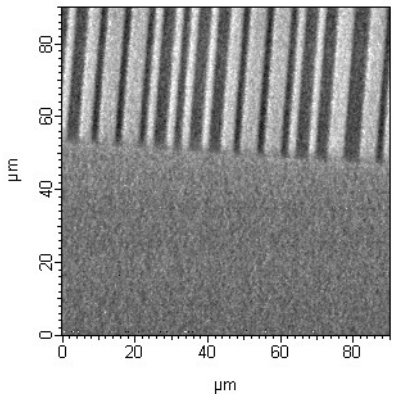

figure 5: 1.44 HDD

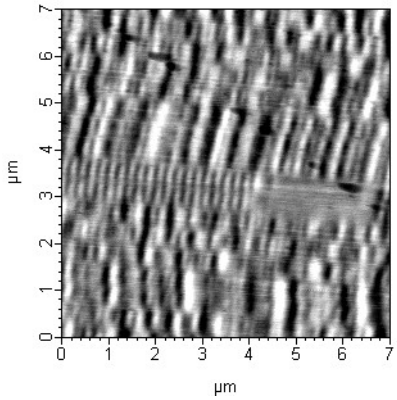

figure 6: 2 GB hard disk

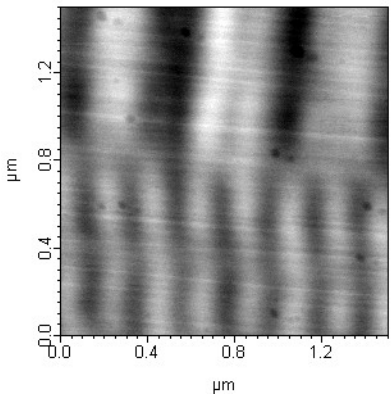

figure 7: 2 GB hard disk detail scan

Accordingly we tried to measure smaller magnetic structures than the information of the disk. So we took a sample of a 2 GB hard disk. We made scans on this hard disk with edge lengths of 20, 5 and 2 µm without finding continuous structures. So we made a big overview scan with an edge length about 90 µm, and there we thought to see some structures. Therefore we made another scan with an edge length of 30 µm, a scan rate of about 1 Hz and a delta height of 25 nm. At this scan a muster was observable. Therefore we made detail scans of this area with an edge length of 7 µm (figure 6) and 1.5 µm. At the last measurement we got figure 7, on which magnetic information with a dimension of about 0.05 µm could be seen. Typical settings for MFM measurements are listed in table 1.

scan	***scan rate***	***delta height***
music tape	1 Hz	20 nm
1.44 HDD	1 HZ	5 nm
2 GB HD	1 Hz	25nm
40 GB HD	0,4 - 1 Hz	35 - 40 nm

table 1: typical scan settings in MFM mode

After that we made a measurement of a 40 GB hard disk. This time we immediately found magnetic structures. The first scan was about an edge length of 20 µm and a delta height of 40 nm. Then we made a high-resolution scan with 512 points and lines, a delta height of 40 nm and an edge length of 10 µm (figure 8). Detailed scans with different delta heights of this area followed. One of these detail scans can be seen in figure 9, a scan with a delta height of 35 nm and an edge length of 2.5 µm. Adjacent an overview scan with an edge length of 90 µm and a delta height of 35 nm has been made, on which we found an interesting structure. A detail scan of this structure can be seen on figure 10, which was measured with an edge length of 19 µm and a delta height of 50 nm.

After these measurements we were secure with the exposure to the MFM and we knew that it is possible to measure magnetic structures which are smaller than 50 nm.

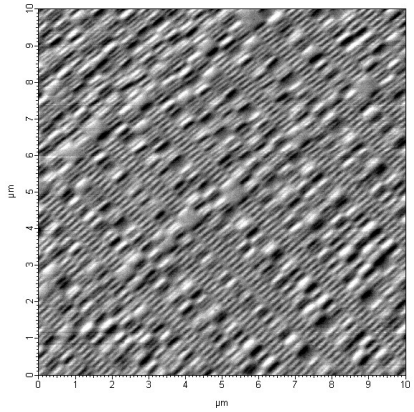

figure 8: 40 GB hard disk

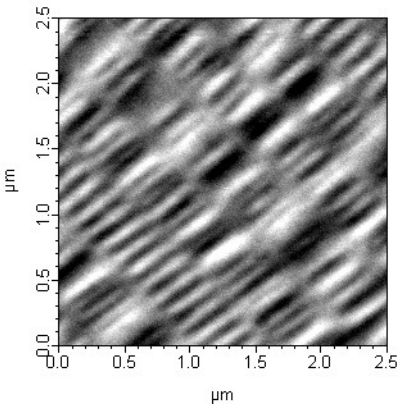

figure 9: 40 GB hard disk detail scan

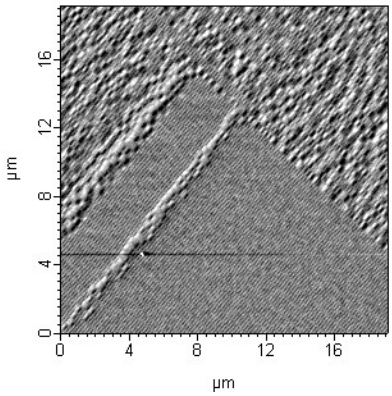

figure 10: unindentified structure on 40 GB hard disk

So we started to scan MB with the MFM mode. The procedure in scanning MB was to make a normal AFM scan first to find a good area with an adequate number of MB and do a scan over a single or over a small number of MB and then to start with MFM measurements. We scanned 5 different slides. At each slide many areas have been scanned and the delta height as well as the scan ratio have been varied. The results were always the same: We didn't find any difference between the normal AFM phase trace and the NAP phase trace. After these findings we tried measurements with another cantilever and exchanged the MFM standard tip with a MFM low moment tip. Still no magnetic structure was being found.

Scratch

The second major part of our research was to manipulate or scratch the MB using the AFM. For this purpose we used a 350 kHz tip which has a harder cantilever than the 70 kHz one. Scratching works only in AFM contact mode as you have to apply force continuously.

Scratching is done by using the scratch panel included in the MFP-3D software. It is located in the menu MFP controls.

This panel allows you to chose where and into which direction you want to scratch. First you chose the centre point of the scratch and then if the point is the start, end or the middle of the scratch (symmetrical) (figure 11). Further you can adjust the force and the way the force shall be applied (constant, increasing, decreasing) and the scratch angle whereas horizontal is 0°. You can see how the scratch is going to be done in the last active AFM window (Height Trace, Amplitude Trace or Phase Trace).

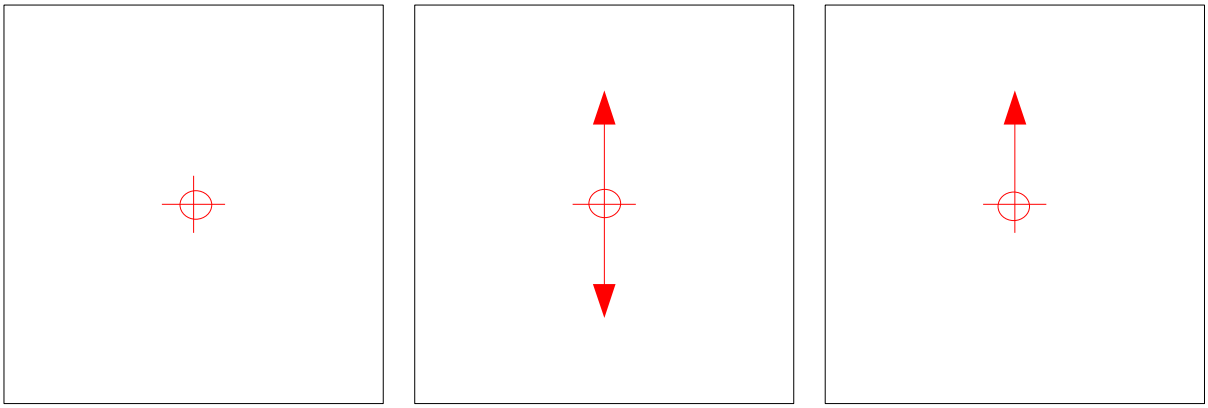

Figure 11: scratch mode

(a) Centre of scan *(b) Symmetrical* *(c) start*

We started trying to scratch a piece of Plexiglas, but after several tries without a positive result we tried a piece of clear plastic binder attached to a slide. But again without success. Both of the samples seem to be either too flexible or too hard for the cantilever.

Because of that we tried a gold sample next. This we hoped to be neither flexible nor too hard. Again we failed to scratch single lines into the gold material, but surprisingly when doing a scan over a large area we saw rectangle structures of a previous scan in contact mode with a high Set Point of 3V. We verified this result by doing another scan with a set point of 3.5 V. We then did a overview scan rotated by 10° to better see the scratched structures. The results are shown in figure 12 (marked with red arrows). We believe that a single scratch line is too fine to be detected by a following scan (at least on the surfaces named above). Nevertheless we have been able to show that it is possible to scratch into a gold surface.

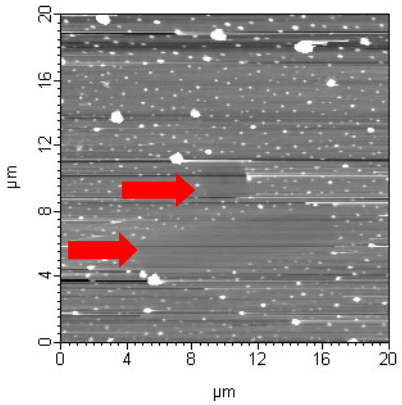

figure 12: scratched areas on gold surface

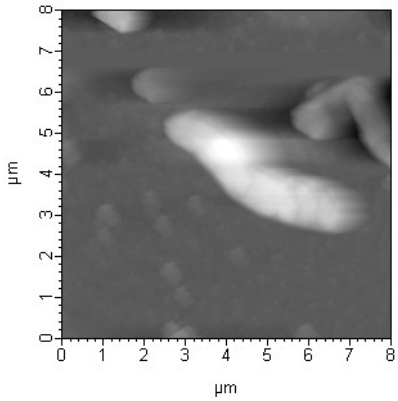

figure 13: bacteria before scratch

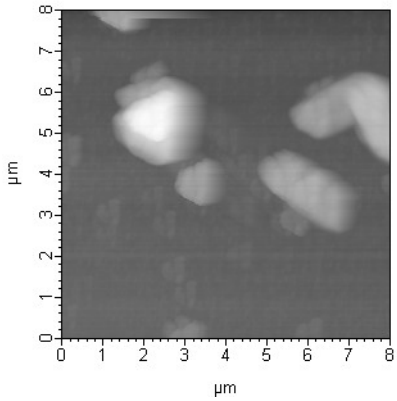

figure 14: bacteria after several scratches

Our first attempts of scratching bacteria were not successful as we used not enough force. Then we increased the force and we had success for the first time. We scratched several times using different forces before doing the next scan. The bacteria seemed to be cut in pieces and also a bit disarranged. This can clearly be seen in figure 13 and figure 14. Now we had to find out which force works best for the bacteria prepared on our slide. It took several attempts to find out the best force/speed combination for manipulating bacteria. Values of speed and force are shown in table 2. The first three lines are the first attempts where we cut the bacteria in pieces and moved it. The last two lines are values that worked fine for us.

force	*mode*	*speed*	*result shown in fig.*
4 V	constant	0,5 µm/s	14
2 V	constant	0,5 µm/s	14
3 V	constant	0,5 µm/s	14
3,5 V	constant	0,1 µm/s	16

table 2: scan settings for scratching

The result of the last value combination can be seen in figures 15 and 16. We managed to slice a magnetic bacteria at a defined location without moving it in a noteworthy way. This result has also been repeated to be sure the results are no accident.

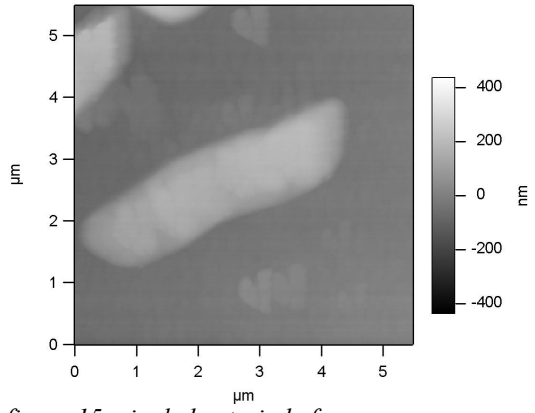

figure 15: single bacteria before scan

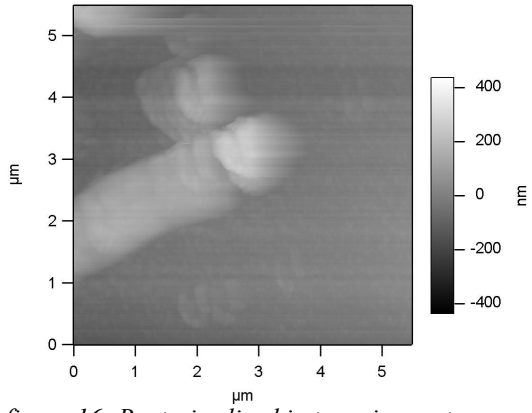

figure 16: Bacteria sliced in two pieces at a defined position

Acknowledgements

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