

Grade: 1,0

Protocol for the experiment
Scanning Ion Conductance Microscopy

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Table of content

1	Introduction	1
2	Technical background.....	1
2.1	Working principle of SICM.....	1
2.1.1	Setup of SICM.....	1
2.1.2	The dependency of the pipette-sample distance on the ion current	2
2.2	Different imaging modes of SICM.....	4
2.3	Axial and vertical resolution of SICM	4
3	Labwork - Part 1	5
3.1	Execution of the experiment.....	5
3.2	Results	7
3.3	Discussion.....	18
3.4	Conclusion.....	21
4	Labwork – Part 2.....	22
4.1	Execution of the experiment.....	22
4.2	Results	24
4.3	Discussion.....	31
4.4	Conclusion.....	32
5	References	34

1 Introduction

Scanning ion conductance microscopy (SICM) was developed in 1989 at the University of California by the team of Paul Hansma and has since offered the possibility of imaging the surface topography of soft and non-conductive materials on the nanometre scale [1] [2].

By means of an electrolyte-filled nanopipette, which is placed together with the sample in an electrolyte liquid, the sample is scanned line by line and the measured current is used to determine the height at each point of the sample. Through continuous further development, this method has been used to date not only to examine flat polymer films, but also living cells, proteins and DNA (deoxyribonucleic acid) [3].

In this experiment, which is divided into two parts, data was taken with the microscope SICM. In the first part, the properties of the SICM were investigated with special attention to the noise reduction and the achievable resolution of the images. For this purpose, graphs of the saturation current and its frequency spectrum, as well as IZ-curves and topography images were acquired under changes of settings such as bandwidth, trigger and pipette radius. In addition, topography images were taken in two different modes: hopping mode and constant current mode. Using the acquired data, quantitative noise components and resolution were determined with Excel and Gwyddion, and acquired images were qualitatively compared [4] [5].

In the second part, the focus was on the microscopy of different samples and the determination of their geometrical and physical properties. For this purpose, topography images of a decane droplet and two different cells in hopping mode were acquired. Also, timelapse series of these were performed. The data sets were analysed with Gwyddion, whereby shape parameters were determined quantitatively. In addition, dynamic processes of a cell were described [6].

2 Technical background

2.1 Working principle of SICM

2.1.1 Setup of SICM

SICM is a microscope in which an electrolyte solution-filled pipette with a nanometre-sized opening acts as a probe that scans sample surfaces line by line (see figure 1). For a measurement, the sample and pipette are placed in a vessel containing an electrolyte solution, with the sample

at the bottom of the vessel and the pipette attached above it by a holder. The holder enables movement of the pipette in the z-direction by means of an attached piezo, while movement in the xy-direction is accomplished with a piezo-controlled platform on which the assembly is placed. The piezos are addressed by the controller during the measurement.

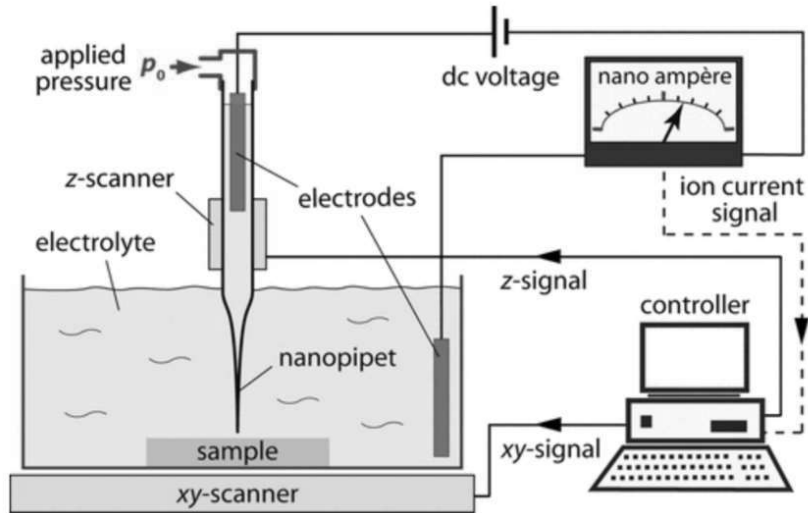


Figure 1: Setup of SICM [1]

The pipette and the free electrolyte solution each contain an electrode to which a dc (direct current) voltage is applied so that a current flows through the opening of the pipette, which is measured by an ammeter containing a low-noise current amplifier [1] [6].

If there is a large distance between the pipette and the sample, the constant saturation current flows. As the pipette approaches the sample, the current drops with decreasing distance between pipette and sample. The current value can be used to derive the distance between the pipette and the sample and thus the sample height [1].

2.1.2 The dependency of the pipette-sample distance on the ion current

The relationship between current and pipette-sample distance can be illustrated by the IZ- curve, which occurs when the pipette approaches the sample perpendicularly (see figure 2a)).

This current flow in the area of the pipette opening can be described using an electrical circuit diagram. It consists of two resistors connected in series through which the current flows due to the applied voltage (see figure 2b)).

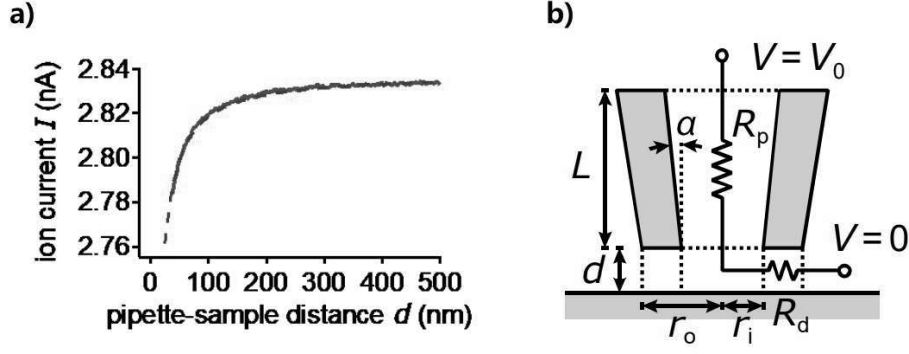


Figure 2: a) IZ-curve that occurs when the pipette approaches the sample perpendicularly, b) Geometry and circuit diagram of the pipette, where α is the opening angle, r_i the inner radius, r_o the outer radius of the pipette and d the pipette-sample distance.

R_p is the constant resistance and R_d , the resistance which depends on the pipette-sample distance [6].

The resistance R_p has a constant resistance value that is independent of the pipette-sample distance and only depends on the geometry of the pipette and the properties of the electrolyte solution (see formula 1):

$$R_p = \frac{1}{\pi \sigma r_i \tan(\alpha)} \quad (1)$$

Where σ is the conductivity of the electrolyte, α the opening angle and r_i the inner radius of the pipette.

The variable resistance R_d is added to the constant resistance as the pipette approaches the sample. This resistance behaves like a potentiometer and increases with decreasing distance from pipette to sample. Hence, the resulting current $I(d)$ can be defined as follows (see formula 2):

$$I(d) = \frac{V_0}{R_p + R_d(d)} \quad (2)$$

Here V_0 is the applied voltage and $R_d(d)$ is the distance-dependent resistance.

If the pipette is far away from the sample, the constant saturation current flows, since the value of $R_d(d \rightarrow \infty)$ approaches zero [1]. If formula 1 is inserted for R_p , the saturation current I_0 is given as (see formula 3):

$$I_0 = I(d \rightarrow \infty) \cong \frac{V_0}{R_p} = \pi \sigma r_i \tan(\alpha) V_0 \quad (3)$$

If the current is measured on the basis of the applied voltage, the nanometre-sized inner radius of the pipette can be calculated if the opening angle of the pipette is known [6].

2.2 Different imaging modes of SICM

Two commonly used modes of the SICM are the constant current mode and the hopping mode.

In the constant current mode, the height of the pipette is controlled by a feedback loop that addresses the z-piezo so that the current and the pipette-sample distance remain constant as the pipette moves across the sample. However, collisions between the pipette and the sample can occur if there are large height steps on the sample surface [2].

To prevent this, there is a hopping mode in which the pipette approaches the sample vertically until the current has dropped to a previously entered percentage of the saturation current. When this trigger value has been reached, the pipette is retracted to a defined retract distance and the process is repeated over the whole sample surface. Typical trigger values are in the range of 1 % of the current drop, which is approximately a pipette-sample distance of r_i .

It should be noted that the retract distance should not be too small, otherwise collisions with possible height steps may occur during the lateral movement of the pipette. However, if the retract distance is too high, the measurement takes a lot of time, since the pipette takes longer in z-direction to approach the sample until the trigger value is reached [6].

2.3 Axial and vertical resolution of SICM

The axial resolution is influenced by the temperature-related Johnson-Nyquist noise, which affects the resistance components of the microscope, in this case, the pipette resistance. The axial resolution is hence defined as follows (see formula 4):

$$\sigma_{axial} = \sqrt{\frac{4k_B T \Delta f}{R_p}} \quad (4)$$

Where k_B is the Boltzmann constant with a value of $1.4 \cdot 10^{-23} \text{ J / K}$, T is the temperature and Δf is the bandwidth of the measurement.

Since the IZ-curve takes a steeper course at smaller pipette-sample distance, the influence of the current noise on the final height value is the lowest in this range. For this reason, higher trigger values in hopping mode result in less noisy topography images [6].

The lateral resolution describes the minimum distance at which two objects or points can be distinguished as two different ones [7]. It depends on the pipette's inner radius, which determines the lateral sensitivity. As an estimate, the lateral resolution is approximately three times the inner radius of the pipette. In order to be able to make a quantitative statement about the lateral resolution, a height profile of a height step of the sample is recorded. From the height of the step the 0.12-fold and the 0.88-fold are calculated and the lateral distance is determined, which lies between the two height values [6].

3 Labwork - Part 1

3.1 Execution of the experiment

At the beginning of the first part of the laboratory work, a grid-shaped imprint was prepared from Sylgard 184 polydimethylsiloxane (PDMS), which was used as a sample in the SICM. For this, 1 g of PDMS was mixed with 0.1 g of curing agent and a drop of the mixture, which had a weight of approximately 0.1 g, was placed on the template grid. The mixture was placed in a Petri dish under a desiccator and was degassed for 20 minutes so that all air bubbles had disappeared. The sample was then baked for 40 minutes at 80° to become solid.

While the sample was being baked, the P39 and P37 pipettes were produced with the laser beam puller P-2000. First, the program was entered, which led to the production of either the P39 with a diameter of 500 nm or the P37 with a diameter of 100 nm. A capillary was clamped in the device, which was heated three times in the middle by the device and pulled apart so that it broke in the middle. The results of each run were two pipettes with nanometre-sized openings. Hereby direct contact with the tips of the pipettes was avoided to prevent breakage. The procedure was repeated for both types of pipettes, so that in the end there were at least four pipettes of each type.

After the baking process, the baked PDMS grid was removed from the template and placed in a petri dish with vacuum grease at the bottom so that the grid had good adhesion to it. The dish was filled with approximately 2 ml of a phosphate buffered sodium solution (PBS) and placed in the SICM.

Two identical runs of the experiment were performed using a P39 pipette in the first run and a P37 in the second. The corresponding pipette was filled with PBS using a syringe and attached to the holder of the SICM above the Petri dish so that it barely contacted the PBS solution. If necessary, the stage had to be moved in x- and y-direction with screws so that the pipette was optimally positioned above the sample. Subsequently, one silver electrode was inserted into the pipette and one into the Petri dish. Care was taken to ensure that the bath electrode did not touch the edge or

bottom of the Petri dish, but was free in the liquid so that the current measurement was not distorted. After pipettes, electrodes and the sample were placed, the camera was folded over the setup and the controller, light and power supply for the piezo actuators were switched on.

The offset current was calibrated before starting the measurements so that it amounted to approximately 0 nA with a voltage of 0 V applied to the electrodes. A bandwidth of 10 kHz was then set and a voltage of 100 mV was applied to the electrodes so that a current flowed.

First, the waveform of the saturation current was recorded over time and a frequency spectrum was created from it. To do this, the pipette just contacted the surface of the PBS liquid. Since it was found that the periodic interference signal strongly influenced the current signal, a Faraday cage was placed around the sample to shield it. The bandwidth was changed from 10 kHz to 1 kHz for comparison, and the current waveform of the saturation current, as well as the corresponding frequency spectrum, was recorded. In order to lose as little information about the signal as possible, the bandwidth for further measurements was set to 10 kHz, since more signal components were filtered out at 1 kHz.

For the approach of the pipette, the ramp mode was switched on with a trigger of 0.5 % on the computer, so that the pipette was continuously moved up and down a few micrometres by means of the z-piezo. At the same time, the rough adjustment was made by turning a screw so that the pipette was lowered a few millimetres and the IZ-curve became visible in the mapped current curve on the computer. The approach was controlled with a microscope located under the sample. For the recording of the IZ-curve, the hopping mode with a trigger of 1 % and a retract distance of 4 μm was used. With these settings, data from the IZ-curve was recorded once with a bandwidth of 10 kHz and once with one of 1 kHz.

Then, two-dimensional 40 μm x 40 μm topography images of the grating were taken in hopping mode. For this purpose, a pre scan was first made with a low resolution. Then three images were taken with a resolution of 80 pixels in x- and y-direction and with a retract distance of 2 μm , since the sample was flat. The trigger was changed after each measurement from 0.5 % to 1 % and 1.5 %. After scanning the topography of the specimen, an unwanted tilt of the sample that distorts the height values of the images was corrected on the computer. Each image was saved for later analysis.

To create a height profile, a single line of sample recording was performed with a high pixel count of 1000. For this, the pipette scan direction was rotated by 45° so that a straight step could be recorded, and a trigger of 1.5 % was selected. Again, the slope of the sample was calculated and the graph of the height profile was saved.

In the conclusion of the first part of the lab, a $40\text{ }\mu\text{m} \times 40\text{ }\mu\text{m}$ topography image was recorded in constant current mode. For this, the current setpoint was set to 99 % of the respective saturation current of the pipette, which corresponded to 4.16 nA for the P39 pipette and 3.66 nA for the P37 pipette. The resolution was set to 128 pixels in x- and y-direction. During the measurement, the controller parameter integral-Gain (I-Gain) was adjusted manually, which defines the responsiveness of the z-piezo to changes in current and altitude. A value for the I-Gain was required, at which a sharp image was generated, but the pipette did not start to oscillate. For the P39 pipette the I-Gain was set to 1.3, for the P37 to 2.5. With the determined I-gain the images were finally taken and saved [6].

3.2 Results

For the evaluation the text files recorded during the measurement were processed, calculations were performed and diagrams were generated using Excel and Gwyddion. In addition, recorded topography images and graphs of the SICM device were used for visualization.

With the use of the set voltage and the measured saturation current, which settled after the pipette was immersed for some time, the inner pipette radii were determined. The pipette radius r_i is defined as follows (see formula 5):

$$r_i = \frac{I_0}{V_0 \pi \sigma \cdot \tan(\alpha)} \quad (5)$$

Where I_0 and V_0 are the measured current and voltage. σ is the conductivity of the electrolyte, which has the constant value of 1.5 S/m at room temperature. The opening angle α of the pipette is also constant and has a value of 3° .

For the P39 pipette, a current of approximately 4.15 nA was measured after entering a voltage of 100 mV. By inserting the values into formula 5, the following results were obtained:

$$r_{i,P39} = \frac{4.15 \cdot 10^{-9}\text{ A}}{100 \cdot 10^{-3}\text{ V} \cdot \pi \cdot 1.5 \frac{\text{S}}{\text{m}} \cdot \tan(3^\circ)} = 168.04\text{ nm}$$

For the P37 pipette, the radius was calculated in the same way. With the voltage of 100 mV and a resulting current of 3.68 nA, this resulted in an inner radius of (see Formula 5):

$$r_{i,P37} = \frac{3.68 \cdot 10^{-9} A}{100 \cdot 10^{-3} V \cdot \pi \cdot 1.5 \frac{S}{m} \cdot \tan(3^\circ)} = 149,01 \text{ nm}$$

A noise spectrum was recorded with both pipettes at different bandwidths. During the recordings, they had a large distance to the sample surface, so that the current was constantly at the maximum. In addition, the sample was already surrounded by a Faraday cage, so that the current measurements were not influenced by coupled periodic disturbances such as the 50 Hz mains voltage.

Figure 3b) shows the noise signal of the P39 pipette at a fixed bandwidth of 10 kHz. Its frequency spectrum is shown in figure 3a), which depicts the different noise components of the noise signal plotted against their frequency. In this spectrum, a drop in the signal can be seen from about 11.7 kHz, so that the intensities of higher frequencies become minimal from about 44.5 kHz.

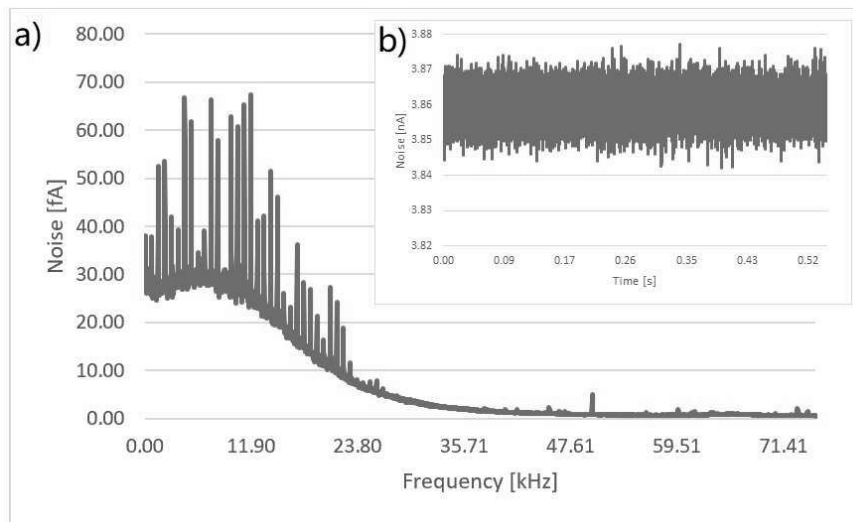


Figure 3: a) Frequency spectrum of the saturation current of the P39 pipette at a bandwidth of 10 kHz, b) Noise signal of the respective saturation current

At the bandwidth of 1 kHz, it can be seen in figure 4b), that the noise signal is less dense than this of 10 kHz. The signal of the frequency spectrum drops here already at about 0.7 kHz and from about 4.2 kHz the frequency components have the minimum constant intensity (see figure 4a)). The noise components which have a frequency smaller than 0.7 kHz are identical to those which occur at the same frequency in the 10 kHz spectrum.

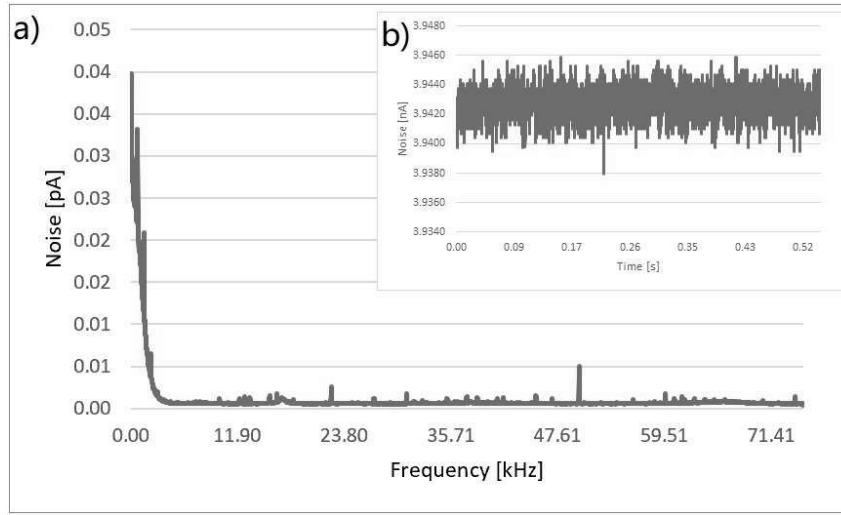


Figure 4: a) Frequency spectrum of the saturation current of the P39 pipette at a bandwidth of 1 kHz, b) Noise signal of the respective saturation current

Figures 5 and 6 show the noise signals and their frequency spectra recorded with the P37 pipette once at 10 kHz and at 1 kHz bandwidth. Again, it can be seen that at the 10 kHz bandwidth, the noise signal appears denser than that at 1 kHz (see figure 5b) and 6b)). Moreover, its noise density looks similar to that of the P37 pipette with a bandwidth of 10 kHz (see figure 3b)). In this frequency spectrum, a drop in the signal can be seen at about 12.1 kHz, with the constant intensity minimum being reached at about 46.8 kHz (see figure 5a)).

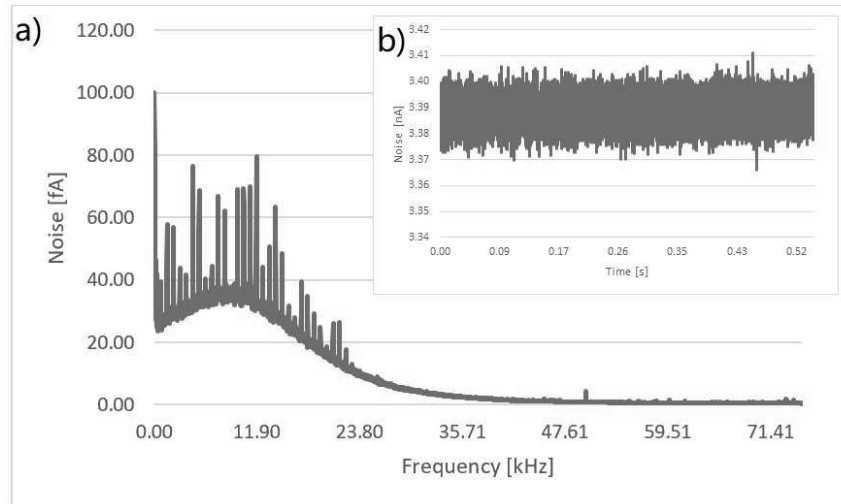


Figure 5: a) Frequency spectrum of the saturation current of the P37 pipette at a bandwidth of 10 kHz, b) Noise signal of the respective saturation current

A current drift can be seen in the noise signal of 1 kHz (see figure 6b)). The signal of the frequency spectrum drops at about 0.6 kHz and reaches the constant minimum at a frequency of 4.1 kHz (see

figure 6a)). Frequency components with a frequency lower than 0.6 kHz correspond approximately to those of the 10 kHz spectrum of the P37 pipette.

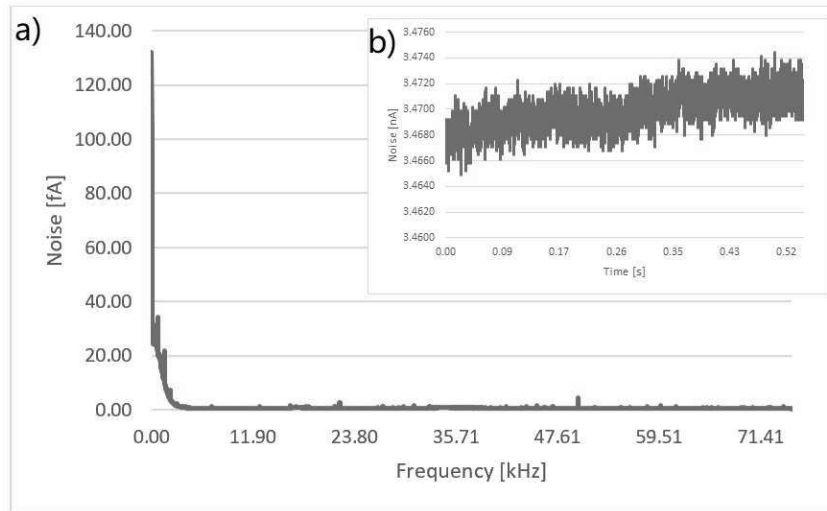


Figure 6: a) Frequency spectrum of the saturation current of the P37 pipette at a bandwidth of 1 kHz, b) Noise signal of the respective saturation current

The plots of the IZ-curve, where the pipettes were approximated to the sample with a threshold of 1 % and a retraction distance of 4 μm in hopping mode, can be seen in figures 7 to 9. The curves represent the current as a function of the deflection distance of the pipette.

The IZ-curve of the P39 pipette at a bandwidth of 10 kHz is shown in Figure 7. In the curve, a drop in current can be seen at approximately 11 μm deflection of the pipette.

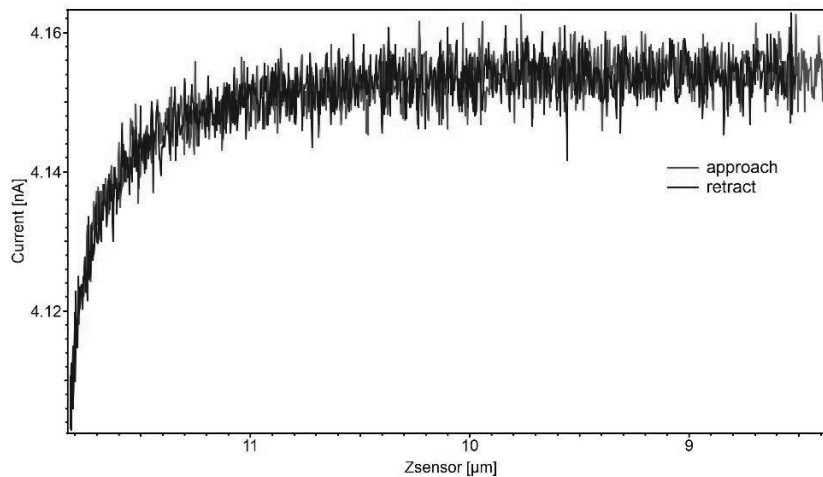


Figure 7: IZ-curve of the P39 pipette at a bandwidth of 10 kHz, the deflection distance of the pipette is plotted on the x-axis, the measured current on the y-axis.

For the constant area of the curve, where the pipette is far away from the sample and the current is constant and maximum, the ion current noise was calculated from the data. For this purpose, the standard deviation $\sigma_{\bar{x}}$ was determined from the measured values for a pipette deflection smaller than 10.05 μm using the following formula in Excel (see formula 6):

$$\sigma_{\bar{x}} = \sum_{i=1}^n \sqrt{\frac{1}{n-1} (x_i - \bar{x})^2} \quad (6)$$

Here n is the number of measurements, $\bar{x}$ the mean value of the measured data and x_i the current value. Thus, for the P39 pipette with a set bandwidth of 10 kHz, an ion current noise of 2.88 pA was obtained.

For comparison, the theoretical ion current noise limit was calculated with formula 4:

$$\sigma_{P39,10 \text{ kHz}} = \sqrt{\frac{4 \cdot 1.4 \cdot 10^{-23} \text{ J/K} \cdot 293 \text{ K} \cdot 10 \cdot 10^3 \text{ Hz} \cdot 4.15 \cdot 10^{-9} \text{ A}}{100 \cdot 10^{-3} \text{ V}}} = 2.61 \text{ pA}$$

The theoretically calculated ion current noise is thus a bit smaller than the standard deviation of the measured values.

A second IZ-curve with a bandwidth of 1 kHz was recorded with the P39 pipette (see figure 8). In this one, it can be seen that there is less noise in the curve than at 10 kHz and a current drop was observed at approximately 13 μm deflection.

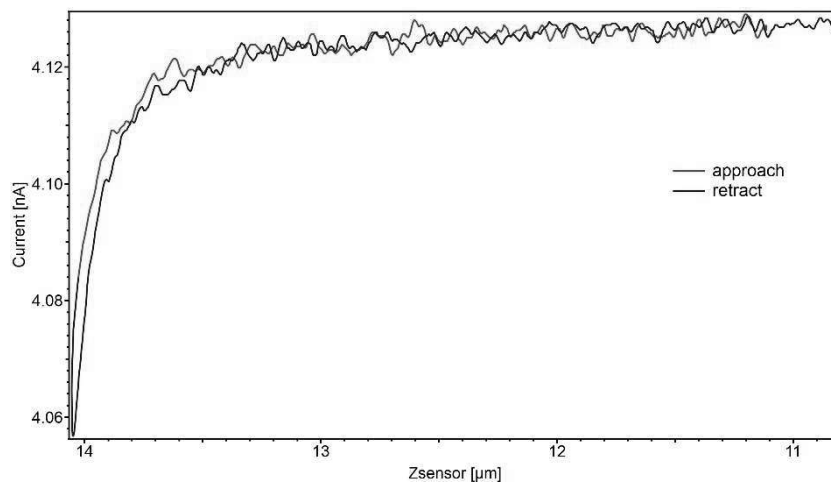


Figure 8: IZ-curve of the P39 pipette at a bandwidth of 1 kHz, the deflection distance of the pipette is plotted on the x-axis, the measured current on the y-axis.

As before, the ion current noise was calculated from the data in the constant range where the pipette was far away from the sample. For this, the standard deviation of all measured values, which occurred at a deflection smaller than 12.05 μm , was determined (see formula 6). It resulted in 986.24 fA, which was smaller than that at 10 kHz. The theoretical value was amounted to (see formula 4):

$$\sigma_{P3, 1 \text{ kHz}} = \sqrt{\frac{4 \cdot 1.4 \cdot 10^{-23} \text{ J/K} \cdot 293 \text{ K} \cdot 10^3 \text{ Hz} \cdot 4.13 \cdot 10^{-9} \text{ A}}{100 \cdot 10^{-3} \text{ V}}} = 823.2 \text{ fA}$$

Hence, also here the theoretically calculated value is a bit smaller than the determined value from the measured values.

The measurement was repeated with the P37 pipette with a bandwidth of 10 kHz, whose IZ-curve is shown in Figure 9. The noise of this pipette looks similar to that of the P39 pipette, but the curve has a drop at about 8 μm deflection of the pipette.

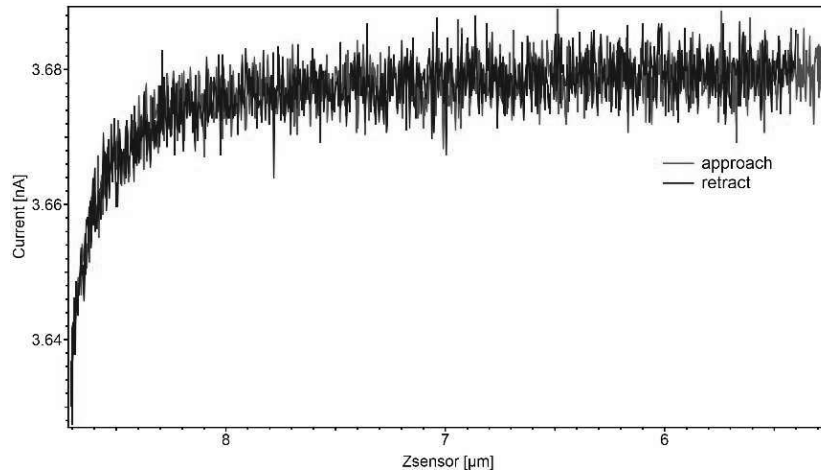


Figure 9: IZ-curve of the P37 pipette at a bandwidth of 10 kHz, the deflection distance of the pipette is plotted on the x-axis, the measured current on the y-axis

For the determination of the ion current noise, the values at a deflection smaller than 6.97 μm were calculated with formula 6. It amounts to 3.25 pA and is thus higher than the standard deviation of the P39 pipette. The theoretical ion current noise was calculated as follows (see formula 4):

$$\sigma_{P3, 10 \text{ kHz}} = \sqrt{\frac{4 \cdot 1.4 \cdot 10^{-23} \text{ J/K} \cdot 293 \text{ K} \cdot 10 \cdot 10^3 \text{ Hz} \cdot 3.68 \cdot 10^{-9} \text{ A}}{100 \cdot 10^{-3} \text{ V}}} = 2.46 \text{ pA}$$

Also in this measurement the theoretical value of the ion current noise is a bit smaller than the calculated standard deviation from data.

Using the P37 and P39 pipettes, two-dimensional topography images of the sample were acquired in hopping mode with current drop thresholds of 0.5 %, 1 % and 1.5 %, respectively (see figures 10 and 11).

Figure 10 shows the three base corrected images of the P39 pipette at a bandwidth of 10 kHz. Dark spots represent height values around 0 nm, bright spots are high areas with height values around 160 nm. The grating structure can be seen on all images, but in figure 10a) with a trigger of 0.5 % significantly more noise compared to figure 10b) and 10c) can be seen, so that the image appears pixelated. The other two images 10b) and 10c) look similar. However, it can be seen that 10c) has slightly less noise than 10b), which is why the image looks the sharpest.

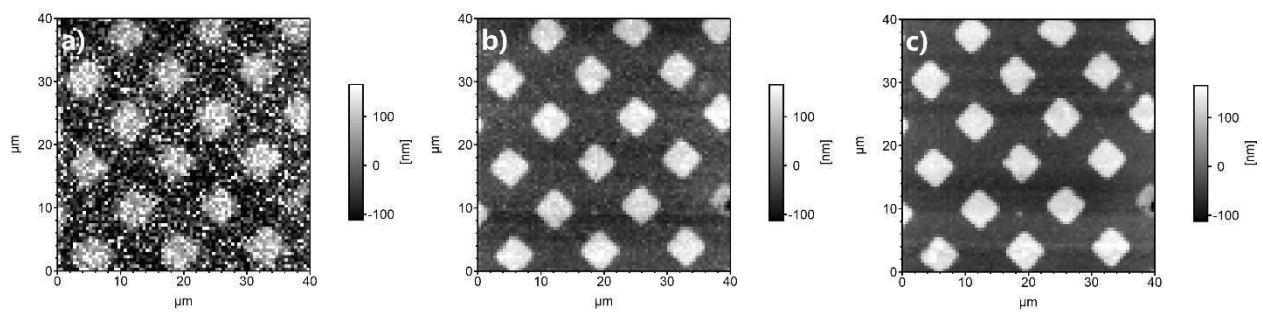


Figure 10: Topography images made with the P39 pipette in hopping mode, a) Current drop trigger of 0.5 %, b) Current drop trigger of 1 %, c) Current drop trigger of 1.5 %

The base corrected topography images from the measurement with the P37 pipette at a bandwidth of 10 kHz are shown in figure 11. It can be seen that in figure 11a) with a threshold of 0.5% the image is very noisy and no grid can be seen as in figure 10a) of the P39 pipette, but only single pixels. In Figure 11b), with the threshold of 1 % current drop, a relatively sharp image of the grid can be seen, which appears slightly more pixelated than the image 10b) of the P39 pipette. Figure 11c), which shows an image with a trigger of 1.5 %, is the sharpest of this series and looks similar to image 10c) of the P39 pipette.

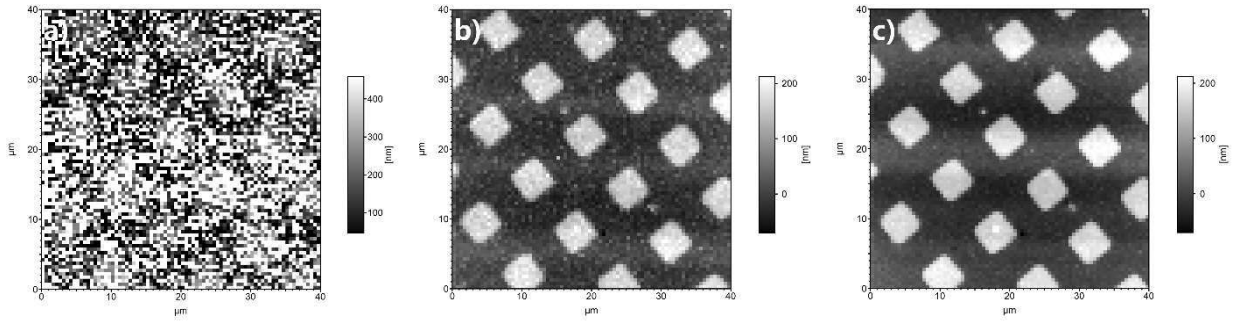


Figure 11: Topography images made with the P37 pipette in hopping mode, a) Current drop trigger of 0.5 %, b) Current drop trigger of 1 %, c) Current drop trigger of 1.5 %

Using Gwyddion, the height noise of the topography images was determined for both pipettes with the three different trigger values 0.5 %, 1 % and 1.5 %. For this purpose, the corresponding IBW file was opened in Gwyddion and the image was flattened with the Gwyddion functions "Plane Level" and "Fix Zero". A mask was placed over a height minimum in the image and the height noise was determined with the function "Statistical quantities" (see table 1). The height noise of the P37 pipette with a used trigger of 0.5 % is only an approximation, because in this image no structures were recognizable for evaluation.

Table 1: Height noise in the topography images of the P39 pipette and P37 pipette at different trigger values

Current drop trigger	Height noise of P39 Pipette	Height noise of P37 Pipette
0.5 %	88.76 nm	(399.8 nm)
1 %	14.91 nm	15.89 nm
1.5 %	6.24 nm	11.29 nm

The values in Table 1 show decreasing noise with increasing trigger, especially a decrease can be seen between the values of the 0.5 % trigger and the 1% trigger. In addition, noise levels are consistently lower for the P39 pipette compared to the P37 pipette, with the difference in noise levels between the two pipettes being highest at approximately 300 nm for a trigger of 0.5%. For the other triggers of 1 % and 1.5 %, the height noise results of the two pipettes have a smaller difference of approximately 1 and 5 nm, respectively.

In addition to the topography images, a height profile along a topography step was created for each of the two pipettes at a bandwidth of 10 kHz and a current drop trigger of 1.5 %, whereby a base correction was performed beforehand (see figure 12 and 13).

In the height profile of the P39 pipette three topography steps of the grid with a height of about 160 nm can be seen (see figure 12). Especially at the minima and maxima, which have a constant height, the graph looks noisy.

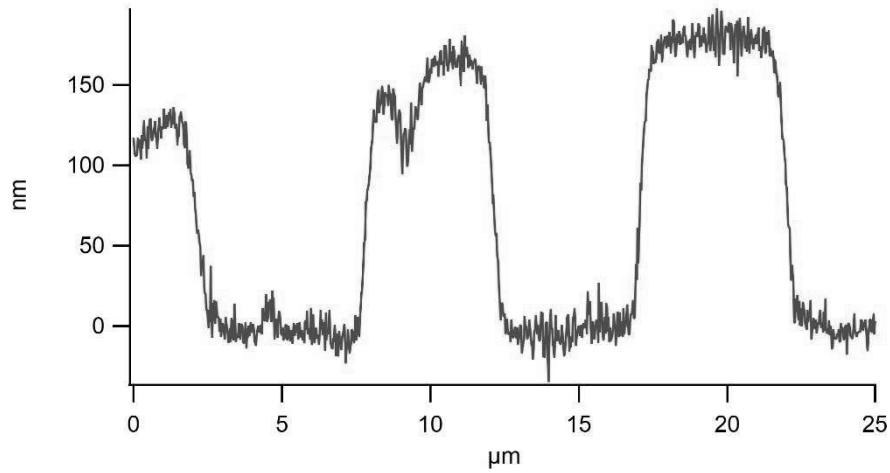


Figure 12: Height profile recorded with the P39 pipette at a threshold of 1.5 % and a bandwidth of 10 kHz

The lateral resolution was calculated based on the slope between the 2nd minimum and 3rd maximum using Excel. For this, first the difference of the mean values of the minimum and maximum was determined. From the difference, the 0.12-fold and 0.88-fold were calculated and the lateral distance was determined that lies between the two height values. This amounted to 475 nm. In addition, a theoretical value of the lateral resolution was calculated using the previously calculated inner pipette radius (formula 7):

$$\begin{aligned}\Delta x_{P39} &= 3r_i \\ &= 3 \cdot 157.51 \text{ nm} = 472.53 \text{ nm}\end{aligned}\tag{7}$$

Where r_i is the inner radius of the pipette previously calculated [6]. The experimental and theoretical resolution thus have a similar value of about 475 nm.

Likewise, a height profile of the P37 pipette was created (see figure 13). In this profile three topography steps with a height of about 180 nm can be seen, where maxima and minima appear noisy.

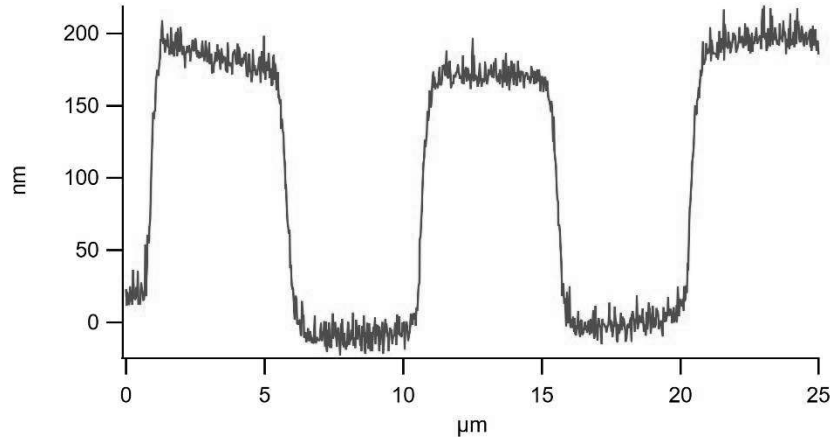


Figure 13: Height profile recorded with the P39 pipette at a threshold of 1.5 % and a bandwidth of 10 kHz

The lateral resolution was determined by the slope between the 2nd minimum and 3rd maximum. As with the P39 pipette, the mean values of the maximum and minimum were calculated, the difference was formed and the lateral distance was then determined between 0.12 and 0.88 times the difference. This leads to a lateral resolution of about 475 nm. The theoretical lateral resolution is calculated as follows (formula 7):

$$\Delta x_{p3} = 3 \cdot 142.93 \text{ nm} = 428.79 \text{ nm}$$

Thus, the theoretically calculated resolution is smaller than the resolution determined from the data.

Finally, topography images of the grating were acquired in constant current mode for both pipettes P39 and P37 with a bandwidth of 10 kHz and a current setpoint of 99 %. Figure 14 shows the image taken with the P39 pipette.

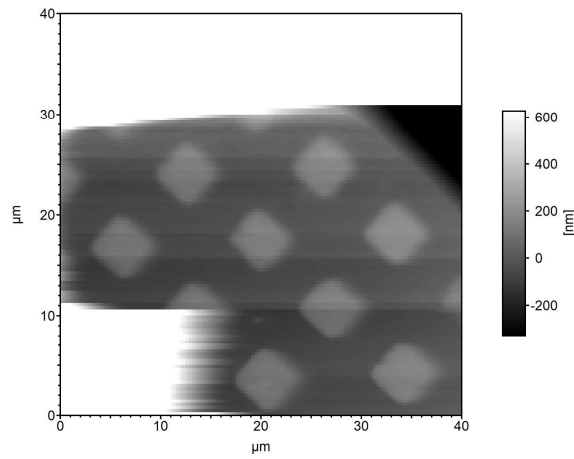


Figure 14: Topography image recorded in constant current mode with the P39 pipette, a current setpoint of 99 % and a bandwidth of 10 kHz

Compared to the topography images acquired in hopping mode, the image appears less pixelated, but the edges look blurred. In addition, the grid in this image was only displayed fragmentarily, since large areas were not mapped.

Figure 15 shows the topography image taken with the P37 pipette. Compared to the images acquired in hopping mode, the image looks similarly pixelated, but also a periodicity can be seen where lighter and darker pixels periodically alternate in a line.

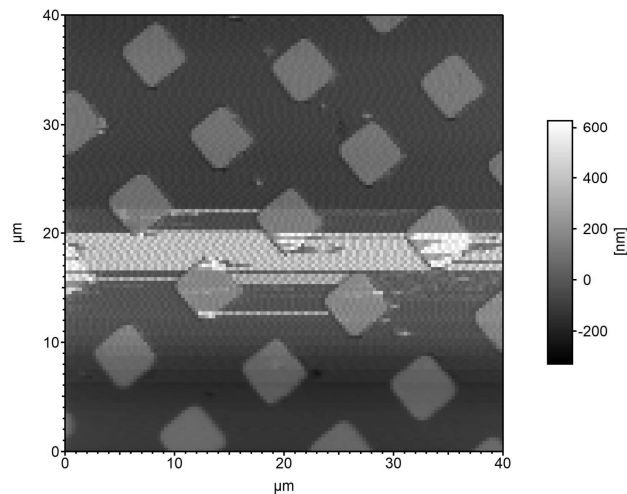


Figure 15: Topography image recorded in constant current mode with the P37 pipette, a current setpoint of 99 % and a bandwidth of 10 kHz

Furthermore, artifacts with strongly fluctuating values can be seen in some scan lines in the centre of the image, which occur especially after height steps.

3.3 Discussion

The results showed that the calculated inner pipette radii of the P39 and P37 had similar values of 168.04 and 149.01 nm, respectively. Optimally, the P39 pipette should have a radius of 250 nm after manufacturing, while the P37 pipette should have a radius of 50 nm [6]. The deviation of the calculated radii could be due to deviations of the actual radii caused by the manufacturing process with the laser beam puller P-2000. On the other hand, the discrepancies between calculated and theoretical radii could also have been influenced by reading errors of the current, since a wrong current value may have been read by mistake due to the fluctuating values. However, since the closely spaced saturation currents settled relatively constant even after a longer period of time, it can be assumed that in fact the manufactured pipettes did not have the desired radii.

The evaluation of the noise spectrum showed that for both, the P39 and P37 pipettes, at a bandpass of 10 kHz, there was a drop in this frequency range, namely once at about 11.7 kHz for the P39 pipette and once at about 12.1 kHz for the P37 pipette. Similarly, with a bandpass of 1 kHz, there was a drop in the frequency spectrum around this frequency for both pipettes. For the P39 pipette, the frequency components dropped at about 0.7 kHz, for the P37 pipette at about 0.6 kHz. Minimal deviations from the desired drop of 10 kHz and 1 kHz resulted, since lowpass filters do not work perfectly in experimentality. In addition, it can be assumed that reading errors of the frequency spectrum happened, since the values fluctuated strongly.

As expected, there was more and higher frequency noise in the noise signal at 10 kHz than at 1 kHz because a larger frequency range was suppressed at 1 kHz. Significant differences in the frequency spectrum and the noise signal of the P39 and P37 pipettes could not be detected except for a minimally different current intensity, since it can be assumed that they have a similar radius. It was also observed that when the same pipette was used, the unsuppressed signal components below the threshold of 10 kHz or 1 kHz remained identical with changes in the bandpass. The reason for this is that the same interference from external electric and magnetic fields is coupled into both measurements and, in addition, the same instrumental noise occurs depending mainly on the room temperature and the pipette.

The noise was also visible after approaching the pipettes in the saturation current. For the P39 pipette with a bandpass of 10 kHz, the determined ion current noise calculated from the data was 2.88 pA, which was a bit smaller than the theoretical value with 2.61 pA. The reason for this could be that for the theoretically calculated value of the ion current noise the formula of the instrumental noise was used, which depends on temperature, bandwidth and pipette radius (see formula 4). However, it can be assumed that in experimentality, despite the Faraday cage, remaining interferences such as magnetic and electric fields, whose strength vary depending on the environmental

conditions, were additionally coupled into the signal and they thus influenced the current signal. Furthermore, for the calculation of the theoretical value the temperature was estimated to be 293 K, but it was not measured during the experiment. Therefore, the instrumental noise could possibly have been greater due to a higher room temperature. Another reason for the deviation from the experimental to the theoretical value is that the low-pass filter did not filter out the frequency components exactly at 10 kHz.

When changing the bandpass to 1 kHz, the experimental ion current noise decreased to 986.24 fA. This was expected, since a larger part of the noise was suppressed in the saturation current. As at 10 kHz, the experimental ion current noise was about a bit larger than the theoretical with 823.2 fA, which, can also be attributed mainly to coupled interference signals, as well as the temperature conditions and the deviations of the lowpass filter.

For the P37 pipette with a bandwidth of 10 kHz, the signal in the saturation current region looked similar to that of the P39 at the same bandwidth. Also in the calculations, the experimental value of 3.25 pA and the theoretical one of 2.46 pA roughly corresponded to that of the P39 pipette at 10 kHz. The ion current noise of the P37 should have been somewhat smaller than that of the P39 pipette, since the current from the latter is smaller (see formula 4). However, as the pipette radii were estimated similarly, in this case only the bandwidth was the decisive factor of the strength of the ion current noise. The differences between the two pipettes may be due to statistical errors.

Also, in the measurement with the P37 pipette, the experimental value was bigger than the theoretical one. Here, as for the P39 pipette, environmental conditions such as interference fields and temperature are assumed to be the main reasons for that.

When recording the topography images with the P37 and P39 pipettes in hopping mode, the images became sharper and less noisy with increasing trigger values from 0.5 % to 1 % and 1.5 %. The calculated values of the height noise confirmed the observations. Especially between the trigger of 0.5 % and 1 %, the height noise decreased significantly for both pipettes from 88.76 nm to 14.91 nm for the P39 pipette and from 399.8 nm to 15.89 nm for the P37 pipette. The lowest height noise value was present for both pipettes at a trigger of 1.5 % and was 6.24 nm for the P39 pipette and 11.29 nm for the P37 pipette. This can be explained on the basis of the IZ-curve, since a steeper course of the current curve occurs with decreasing pipette-sample distance. Thus, at higher trigger values, where the pipette had a smaller distance to the sample, the ion current noise had less influence on the determined height values and hence led to a better resolution [6].

The resolution of topography images of the two different pipettes differed more strongly with decreasing trigger values, at which the images of the P37 pipette appeared increasingly more pixelated than those of the P39 pipette. This is especially visually noticeable at a trigger of 0.5 %.

Also, the determined height noise values of the P37 pipette were larger than those of the P39 pipette for all measurements. The values were particularly far apart at the trigger of 0.5 %, where the noise values had a difference of about 300 nm. At the trigger of 1%, the difference of the values was reduced to 1 nm difference. At the trigger of 1.5%, the difference between the noise values of the pipettes increased again and was approximately 5 nm. The large difference at a trigger of 0.5 % may have happened due to previously calculated slightly higher instrumental noise of the P37 pipette in the combination with the low trigger value. Since the ion current noise loses influence at a higher trigger, this led to similar results of the noise of the P39 and P37 pipette at 1% and 1.5 % trigger. It is assumed that the different height noise values of the pipettes at the higher triggers are of statistical origin and depended on which location of the grid was selected for the measurement. In the height profile the lateral resolution of 475 nm, was identical for both pipettes, which can be attributed to the similar pipette radii. However, it was originally assumed that the lateral resolution of the P37 pipette would be better, as smaller pipette radii have higher lateral sensitivities [6]. Depending on the choice of stage, changes in lateral resolution may occur. In addition, the maxima and minima of the profile showed strong scatter, so that the lateral resolution could only be determined approximately.

Since the theoretical lateral resolution was determined in each case using the previously calculated radii, these were also in a similar size range at 472.53 nm for the P39 pipette and 428.79 nm for the P37 pipette, but the value for the P37 pipette was minimally lower. That was expected, because the calculated radius of it was smaller and thus the lateral sensitivity should be greater. It can be seen that the theoretical resolution of the P39 pipette is approximately the same as that calculated from the data. For the P37 pipette, the theoretical value is smaller than the actual one. The deviations could be of statistical origin depending on the slope selected for the calculations of the experimental data.

In contrast to the images taken in hopping mode, the images taken in constant current mode had large misses and artifacts. In particular, the image taken in constant current mode with the P39 pipette has large imperfections and blurred edges, which could be due to a low I-gain of 1.3. This would make the responsiveness of the z-piezo too slow, so it could not react fast enough to large height changes [2]. The gap in the lower right could have occurred due to a slope in the sample, as the z-piezo could not respond quickly to the height change. In the case of the faulty upper part of the image, the pipette could have been defective due to a collision with the sample, so that proper scanning was no longer possible.

The I-gain of 2.5 for the measurement with the P37 pipette was significantly higher than that of the P39 pipette measurement. Due to this, instabilities of the control loop and oscillations of the

pipette could have occurred, which led to the periodic artifacts in the entire image. The strong artifacts in the centre of the image could have been triggered by a height step, which caused the pipette to oscillate even more.

3.4 Conclusion

Within the scope of the experiment, the properties of a SICM, as well as external interferences and methods to suppress them, could be investigated.

Due to the fact that the pipette radii were probably similar, often no significant differences between the data of the two pipettes could be detected during the measurements. Therefore, the influence of pipette radii on noise and lateral resolution could not be fully elucidated.

The measurements showed that strong ion current noise influenced the measurements, which in experimentality was higher than theoretically calculated. It is assumed that despite Faraday's cage external electric and magnetic fields were coupled into the measurement, and also that the temperature was higher as expected, so that it came to the higher experimental value of the ion current noise. Decreasing the bandpass to reduce the noise components in the ion current noise, as well as selecting a high trigger value in the hopping mode to reduce the influence of the ion current noise in the topography image, proved to be an effective means to enhance the image quality.

However, a middle ground must be chosen both when selecting the bandpass and when selecting the trigger value, since the former threatens to cause too much signal loss if the values are too high, and the latter increases the risk of collision with the sample if the values are too large. In this experiment, a bandpass of 10 kHz and a trigger value of 1.5 % led to the best results.

The images taken in constant current mode, showed artifacts and imperfections compared to the hopping mode and were thus significantly worse in terms of image quality than the hopping mode images. The cause for the image errors is assumed to be a non-optimally selected I-gain.

Since the pipette radii were not manufactured correctly in this experiment, the influence of the pipette radii with correctly manufactured pipettes should be carried out in renewed recordings in order to determine their influence on the image quality. In addition, further shielding against external electric and magnetic fields could be taken, and the room temperature could be lowered to reduce instrumental noise.

For the constant current noise recordings, further measurements would need to fine-tune the I-gain so that the image quality is improved. If this were achieved, a better comparison of the images in hopping mode and constant current mode could be made, so that the optimal mode could be identified for this measurement.

With the improvements mentioned above, in the future, the images taken with the SICM could be more reproducible and with a better resolution.

4 Labwork – Part 2

4.1 Execution of the experiment

In the second part of the laboratory work, a decane droplet was first used as a sample. To prepare it, a small amount of decane was placed on the bottom of a Petri dish and was flushed with 2 ml of phosphate buffered sodium solution (PBS). Subsequently, the prepared Petri dish was placed on the stage of the SICM and a P37 was filled with PBS and fixed in the holder above it. A silver electrode was inserted into the pipette and into the Petri dish, and devices such as the controller, the light and the power supply for the piezo actuators were switched on. The offset current was then calibrated so that the current had a value of approximately 0 nA at a voltage of 0 V.

For all measurements, a bandwidth of 10 kHz was set and a voltage of 200 mV was applied, since a good signal-to-noise ratio was expected with these settings. For the approach of the pipette, the ramp function on the computer was switched on, in which the pipette was continuously moved up and down by means of the piezo. In addition, for coarse adjustment, the screws of the SICM were manually turned so that the pipette was lowered closer to the sample. Whether the pipette was close enough to the sample was checked with the microscope under the sample and the IZ-curve displayed on the computer.

Afterwards, a two-dimensional 50 μm x 50 μm pre scan was recorded in hopping mode, using a low resolution of 50 pixels in the x- and y-direction, a trigger of 1 % and a retract distance of 5 μm . In this way, a suitable droplet was identified and the stage with the Petri dish was moved in the xy-direction using screws so that the desired droplet was located under the pipette. After that, a 10 μm x 10 μm high resolution topography image was taken of the drop, for which a retract distance of 2 μm was set, since the approximate height of the drop was known from the pre scan and less time was needed with the lower distance. The trigger remained unchanged at 1 %. The required number of pixels in x- and y- direction was calculated using the formula $\#Px = S / 3r_i$. Where S is the scan size in the corresponding direction, which was 50 μm . r_i is the inner pipette radius, which was approximately 50 nm for the P37 pipette. This resulted in 66 pixels in x- and y-direction for optimal resolution. With the above settings, a topography image was acquired and saved. In addition, a single line scan with a high resolution of 1000 pixels was performed, which was used for the creation of a height profile.

For the next measurement, surfactant sodium dodecyl sulfate (SDS) was added to the sample as the dissolution of the decane drop was to be induced. To ensure that 10 mmol of SDS was in the Petri dish, 2 ml of the 20 millimolar SDS solution was added to the PBS. The SICM settings were the same as those used for the previous measurement. Multiple frames of the dissolving droplet were recorded over time and the images were stored in chronological order.

Subsequently, in the third part of the lab, neuronal cells were investigated with the SICM. For this, the corresponding cells were washed in a Petri dish with sterile PBS by removing and adding 2 ml of PBS three times each, so that the cell ended up purified in 2 ml of sterile PBS.

The sample was placed in the SICM, which was prepared as for the measurement before. A $100\text{ }\mu\text{m} \times 100\text{ }\mu\text{m}$ pre scan was performed from the neurons. For this, a trigger of 1 %, a retract distance of $4\text{ }\mu\text{m}$ and a low resolution of 32 pixels in x- and y-direction were chosen. After identifying a growth cone and a cell body of a neuron, a high-resolution scan of each was performed. Since the cell body was relatively thick, a retract distance of $4\text{ }\mu\text{m}$ was chosen for the measurement. The trigger was 1 %, the scan size $60\text{ }\mu\text{m} \times 60\text{ }\mu\text{m}$ and the resolution 120 pixels in x- and y-direction. The number of pixels in the x- and y-directions was calculated as before on the basis of the scan size and the inner pipette radius, but then selected three times smaller, as the measurement would otherwise be too time-consuming.

For the growth cone, the scan size was increased to $70\text{ }\mu\text{m} \times 70\text{ }\mu\text{m}$, as this occupies a larger area, and the retract distance was reduced to $2\text{ }\mu\text{m}$, as the structures are flatter than the cell body. To have the same resolution as the image with the cell body, 140 pixels in x- and y-direction were chosen. In addition to the SICM images, optical images were taken of the sample using a camera. One was taken with phase contrast for comparison and one without.

In the last part of the laboratory, living mouse embryonic fibroblasts (MEF) were examined with a P39 pipette. For this purpose, they were washed in the same way as the neurons by removing and adding 2 ml of the sterile L15 medium of the sample in three steps. The Petri dish containing the MEFs in the sterile L15 fluid was placed in the SICM and the pipette was placed over it. The SICM was prepared as before and the pipette was approximated to the specimen. A pre scan of $100\text{ }\mu\text{m} \times 100\text{ }\mu\text{m}$ was performed of the cells with a trigger of 1 %, a resolution of 32 pixels in x- and y-directions, and a retract distance of $5\text{ }\mu\text{m}$. The high-resolution scan of the cell was performed with a scan size of $75\text{ }\mu\text{m} \times 75\text{ }\mu\text{m}$ and a trigger of 1 %. The retract distance was chosen to be large at $5\text{ }\mu\text{m}$ because the cell was relatively tall. The resolution, on the other hand, was set relatively low at 75 pixels in the x- and y-directions so that the acquisition time was not too long. With these settings, topography images of the cell were recorded over time and stored in chronological order [6].

4.2 Results

The recorded data were displayed and evaluated using Gwyddion. For this purpose, the respective IBW files were opened so that the corresponding image and its height values were displayed by means of colouring. Dark spots represent height values around 0 μm , bright spots are higher areas. Then each image was processed with the function "Flatten base", so that the lowest height values received the value 0 μm and the other values were adjusted in relation to it. The individual evaluations were carried out with the images processed in this way.

During the experiment, an image of a decane drop was taken (see figure 16 a)). To determine its volume, the drop was first marked with a mask in the already flatten-based image using the Gwyddion function "Mark by threshold". This mask was set by a height threshold, which was defined at about 0.3 μm , so that all pixels containing at least this height value were included in the mask (see figure 16 b)). The volume of this marked area was determined using the "Measure individual grains" function, resulting in 67.69 μm^3 .

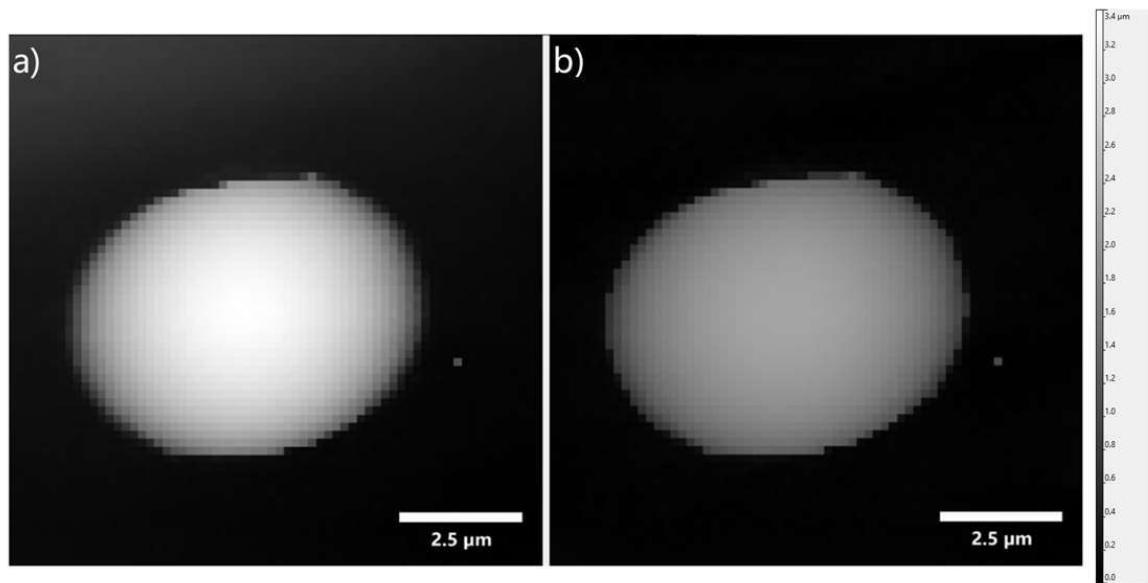


Figure 16: a) Decane drop shown in Gwyddion, b) Respective decane drop marked with a mask

In addition, the radius of curvature of the droplet, as well as its contact angle with the surface below it, was estimated. For this purpose, the high-resolution single-line image through the widest part of the drop was used. In Gwyddion, a line was drawn along the drop and the height profile was determined using the Gwyddion function "Extract Profiles along arbitrary lines" (see figure 17).

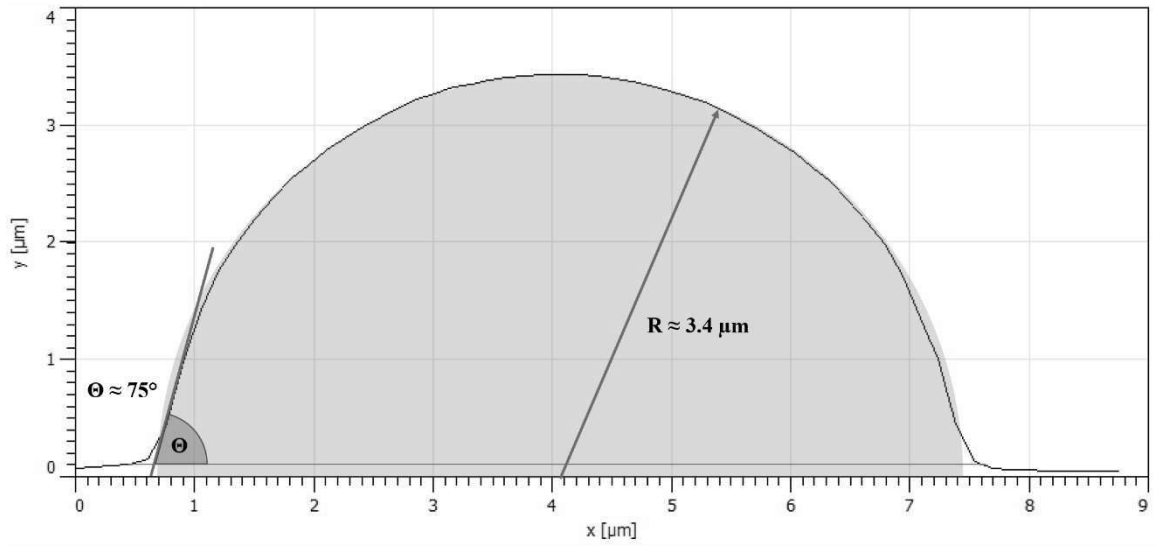


Figure 17: Height profile of the decane drop with an inset semicircle, radius R and contact angle θ .

The height profile represented the drop as a semicircle, into which a semicircle was fitted using PowerPoint and its approximate radius of $3.4 \mu\text{m}$ was measured manually [8]. In addition, the contact angle of approximately 75° was determined.

With these data, the pressure Δp was calculated using the Young-Laplace equation, which describes the relationship between radius, surface tension and pressure inside the drop (see formula 8):

$$\Delta p = \frac{2\gamma}{R} = \frac{2 \cdot 51.2 \cdot 10^{-3} \frac{\text{N}}{\text{m}}}{3.4 \cdot 10^{-6} \text{ m}} = 301,18 \text{ mbar} \quad (8)$$

Where γ is the surface tension with the value of $51.2 \cdot 10^{-3} \text{ N/m}$ for the decane to water interface and R the previously determined radius of the droplet [6].

After the addition of 10 mmol of SDS ($t = 0$), an image was taken every 3.3 min while the droplet was dissolving (figure 18). In the images 18a) to 18d), it can be seen that at the beginning initially sporadic decane detaches from the edge regions and disperses into the PBS liquid. From minute 13, the droplet begins to dissolve over a large area (18e) to 18h)) before completely dissolving at minute 26 in image 18i).

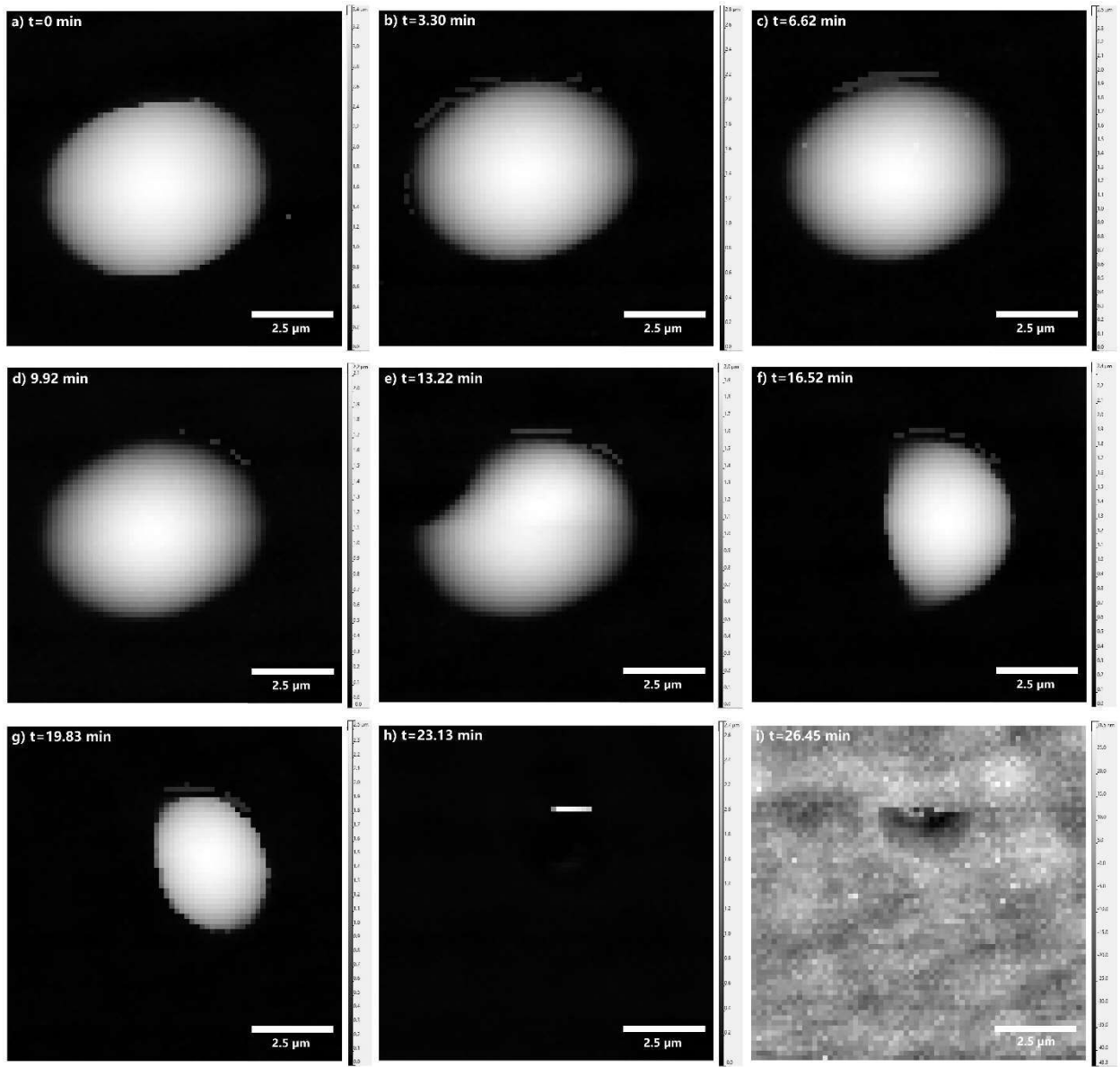


Figure 18: Time-lapse image series from the dissolution of the decane droplet

The volume of each flattened image was determined by marking the area of the drop with a mask with the Gwyddion functions "Mark by threshold" and determining the respective volumes with "Measure individual grains".

By means of the volumes the dissolution rate can be determined, which is defined as (see formula 9):

$$\alpha = \frac{V(t)}{(t_0 - t)^{3/2}} \quad (9)$$

Here $V(t)$ is the volume during the corresponding time t and t_0 is the time at which the droplet has completely vanished [6].

If the volume $V(t)$ is plotted as a function of $(t_0 - t)^{3/2}$, the slope corresponds to the average dissolution rate (see figure 19).

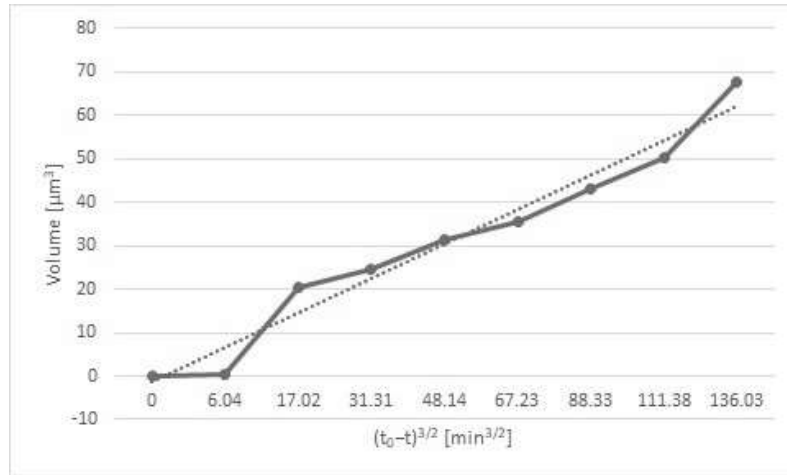


Figure 19: Graph of the volume as a function of $(t_0 - t)^{3/2}$ with an inserted straight line, whose slope corresponds to the average dissolution rate

In addition, the dissolution rate was calculated at each time point using formula 9 and the mean value was determined from these, which corresponds to the average dissolution rate. The calculated dissolution rates are shown in Table 2.

Table 2: Calculated dissolution rate at each time point

Time [min]	Volume [μm ³]	Threshold in Gwyddion [μm]	Dissolution rate [μm ³ /min ^{3/2}]
0.00	67.69	0.30	0.50
3.30	50.27	0.48	0.45
6.62	42.99	0.50	0.49
9.92	35.48	0.40	0.53
13.22	31.42	0.47	0.65
16.52	24.71	0.40	0.79
19.83	20.39	0.48	1.20
23.13	0.32	0.50	0.05
26.45	0	-	0.50

The mean value of the individual dissolution rates and the slope of the curve was approximately $0.58 \mu\text{m}^3/\text{min}^{3/2}$. The calculated value was inserted into the original volume dissolution function to calculate the volume with the new determined dissolution rate at each time point of an image recording (see formula 9). Figure 20 shows the original measured volumes and the curve calculated with the formula for comparison. Significant deviations in the curves can be seen, especially in the area where the droplet is about to dissolve completely and the original volume curve from the data has kinks.

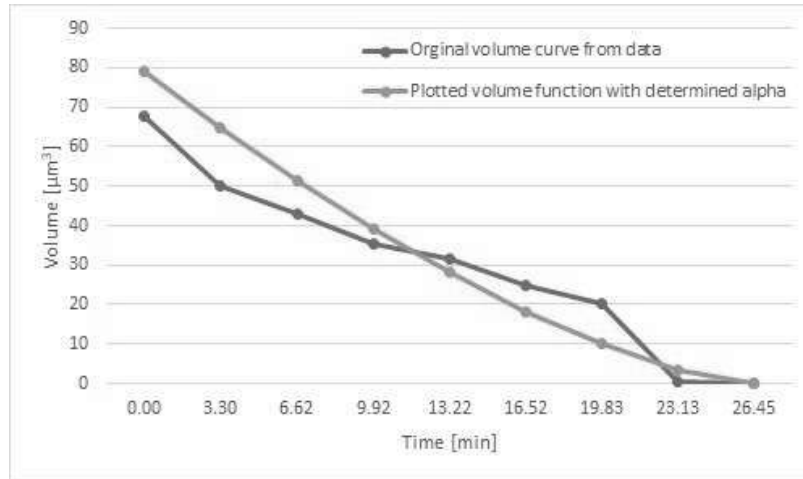


Figure 20: Graph showing the original measured volume values over time and the recalculated volume values using the determined dissolution rate

The subsequently acquired optical images of the mouse neurons, one being a conventional optical image and one an image with phase contrast, are shown in figure 21. It can be seen that in image 21(1) without phase contrast, only a few blurred structures can be seen and it is not possible to identify neuronal structures. In contrast, on the phase contrast image 21(2), fine elongated and larger roundish structures are visible, which can be assumed to be neuronal structures such as axons and cell bodies.

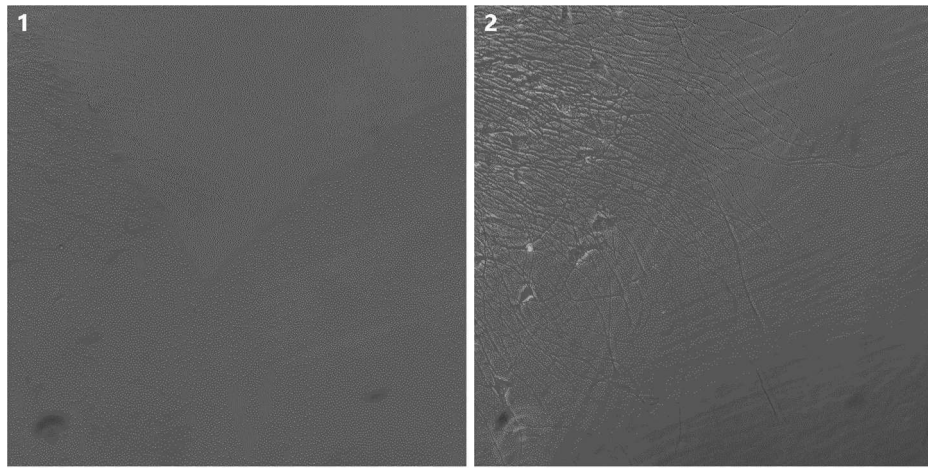


Figure 21: Optical images of neuronal cells recorded without phase contrast (1) and with phase contrast (2)

Two regions in the optical image 22(2) were additionally imaged with the SICM. One was a region at the end of axons, where growth cones were suspected, and the other was a round structure, which was assumed to be a cell body.

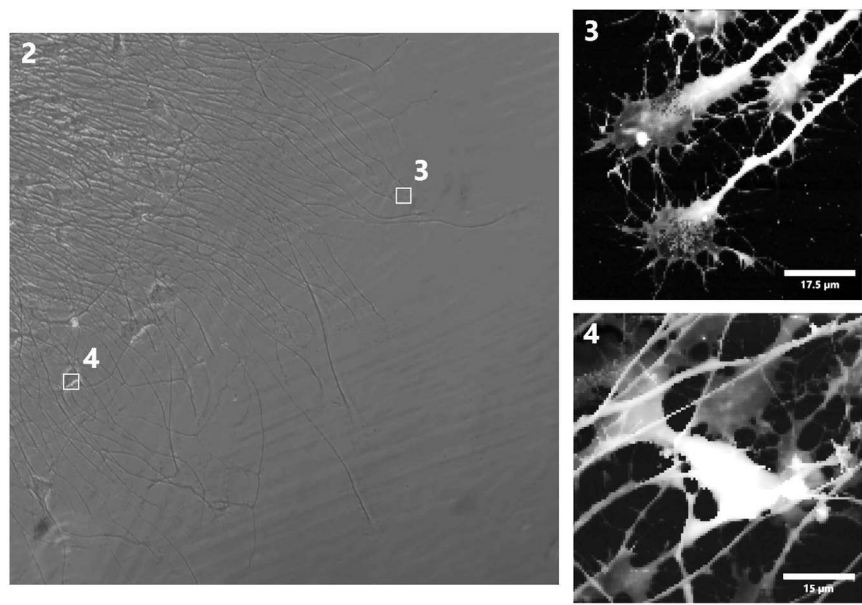


Figure 22: Optical phase contrast image (2) with marked areas of the SICM images of the growth cone (3) and the cell body (4)

The images taken with the SICM look more pixelated compared to the optical images (see figure 22(3) and figure 22(4)). Image 22(3) of the presumed growth cone shows elongated structures with branching at their ends. The other image 22(4) of the presumed cell body shows elongated structures and broad overlapping layers.

Finally, a 29-minute image series of a live mouse embryonic fibroblast (MEF) cell was acquired (see figure 23). An image of the cell was captured with the SICM every 3.97 minutes.

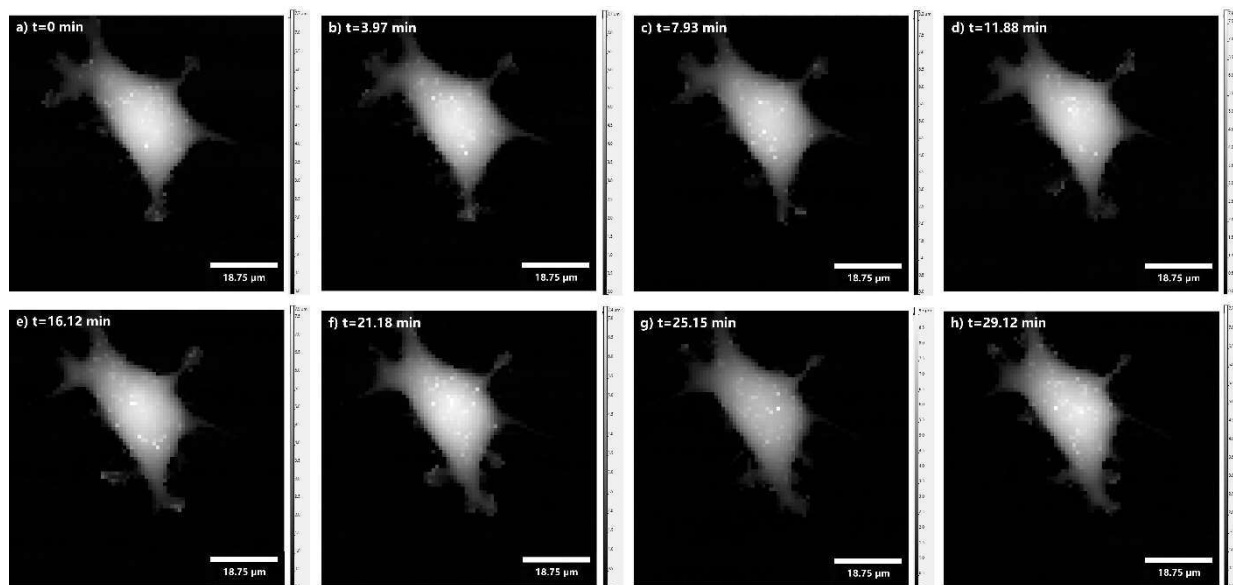


Figure 23: Time-lapse image series of a living MEF cell

In the image series, hardly any movement of the central cell body can be observed, only the shape seems to change over time, as the cell appears slightly more elongated in image 23h) than at the beginning in image 23a). In contrast, the protrusions of the cell were visibly in motion over the imaging period. Over time, it can be seen that the protrusions at the top of the cell shorten, whereas those at the bottom become wider, orient further to the right, and forming new structures. Particularly, a difference between figure 23f) and 23g) can be seen clearly, since structures in the upper left edge shorten considerably there.

From the first recorded cell (see figure 23a)), the shape parameters were also determined using the Gwyddion functions "Mark by threshold" and "Measure individual grains". These are listed in table 3.

Table 3: Cell shape parameters of the MEF cell

Cell shape parameters	Value
Maximum height	7.68 μm
Surface Area	1047 μm^2
Projected Area	840 μm^2
Volume	3010 μm^3
Boundary length	228.2 μm

4.3 Discussion

Using the images of the SICM and Gwyddion, the volume of the decane drop of $67.69\text{ }\mu\text{m}$ could be determined. However, deviations from the actual volume of the droplet may have occurred due to measurement and evaluation errors. For example, ion current noise overlapped the current measurement, which can lead to height noise in the final data [6]. During the evaluation with Gwyddion, a mask was placed on the drop by eye and the threshold was set manually. Since the edges of the drop had a low height and it was therefore challenging to see the boundaries of the drop, this could have led to deviations in the volume value.

Also, when determining the radius of the droplet and its contact angle using the height profile, a semicircle was manually placed inside the droplet so that it would approximately fit inside it. Again, the size of the semicircle, corresponded to the subjective observation of the evaluator, making the radius and the calculated internal pressure only an estimation. The contact angle was also only an approximation, since the edges of the drop are slightly rounded to the surface and the angle was measured by hand, resulting in deviations from the actual angle value.

With a calculated value of about 301.18 mbar , the internal pressure is below the pressures of everyday life. For example, the pressure in a bicycle tire is about 2 bar and in a soccer ball about 0.8 bar , which is 3-6 times higher [9] [10]. The difference in size between the ball, which has a diameter of 22 cm , and the droplet, which has a diameter of $6.8\text{ }\mu\text{m}$, is 100 times larger [11]. The pressure-size ratio is thus significantly higher in the droplet. The reason for this could be that in nanometre and micrometre scale the pressure to volume ratio changes and the pressure thus has a larger influence on the system.

When investigating the non-linear dissolution dynamics of the droplet, the dissolution rate α was found to be $0.58\text{ }\mu\text{m}^3/\text{min}^{3/2}$, which is a factor of 50 higher than the expected value of $0.01\text{ }\mu\text{m}^3/\text{min}^{3/2}$. In addition, the volume-time curve plotted from the data was found to have kinks and the shape differed with that of the plotted function with the calculated alpha. Reasons for the discrepancies could be that the formula assumes a uniformly decreasing hemispherical shape, however the droplet was not optimally hemispherical and deformed over time during the dissolution process. As a result, the surface area exposed to the SDS changed steadily in relation to its volume. Especially at the end starting at minutes 13.22, the droplet takes on a non-circular non-uniform shape and also the volume-time curve shows strong kinks from this point on.

Another reason for the deviation of the dissolution rate could have been the quality of the SDS solution. If therefore a higher SDS content would have been in the PBS liquid, this would lead to a faster dissolution of the droplet.

Furthermore, in the measurement of the dissolution dynamics, there was height noise in the recorded data and the volumes were determined using a mask set by eye, which is why there can be further deviations in the values.

In the last two parts of the experiment, it was shown that it is possible to take high resolution images of cells with the SICM in the micrometre scale to investigate smallest structures and movements. It was noticed that the SICM images appeared more pixelated compared to optical images. This was probably due to the fact that the SICM images represent much smaller image areas, which were more difficult to resolve, and that it was done in hopping mode, where each time the pipette was approached, a point was recorded that appeared as a pixel on the final image. When determining the cell shape parameters of the MEF cell, it was difficult to set a mask in Gwyddion because the cell had many bumps and protrusions. Therefore, it was challenging to see the boundaries of the cell by eye and to set the mask's threshold correctly, making the parameters error-prone.

4.4 Conclusion

The experiment showed that SICM is a suitable method for investigating the surface of micrometre-sized soft materials.

In hopping mode, topography images of a decane drop and two cells could be recorded. Based on the images it was possible to determine geometrical parameters of the sample, such as the volume, with Gwyddion and to create height profiles. When determining the parameters, marking the sample with a mask turned out to be a source of error, since its size can vary depending on the subjective assessment of the evaluator. In addition, errors could have occurred due to the influence of presumed ion current noise during the measurement, so that the height values may show slight deviations from the experimental ones.

In addition to the static images, it was possible to take time-lapse recordings with the SICM, which allowed the observation and investigation of dynamic processes of the droplet and the living MEF cell over a longer period of time. When recording the dissolution process of the decane droplet, the calculated dissolution rate deviated greatly from the expected value, which could be explained due to erroneous volume values, the non-uniform dissolution of the decane droplet and the quality of the SDS.

For future measurements with the SICM it would be useful if an algorithm would take over the placing of the mask in Gwyddion during the evaluation. Furthermore, further shielding of the SICM besides the Faraday cage would be useful to reduce the ion current noise and thus the height noise of the measurements.

In this way, the evaluation could be made more reproducible and more precise statements could be made about the parameters of a sample.

5 References

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