

Scanning Ion Conductance Microscopy (SICM)

Labwork Nanoanalytics and Interfaces
WS 2021/22

Group 4

Supervisors:

Jan Seifert
Hendrik von Eysmond

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1 Introduction

1.1 Motivation

Scanning ion conductance microscopy (SICM) is a scanning probe microscopy technique that enables high-resolution and noncontact imaging of a sample's topography [1]. Therefore, the technique is especially applied for the investigation of biological samples, biophysical materials, as well as for interfacial and electrochemical science [1]. As SICM is an invasion-free method, it is perfectly suited for the measurement of living cells as it is required for medicine and biology [2].

In the first part of this practical general knowledge about the technique of SICM was acquired. Therefore, the influence of the pipette diameter on the behavior of the measured current, as well as on the ion current noise was observed. Furthermore, topography images taken with different threshold values and pipette diameters were investigated, and the differences of images taken in constant current and hopping mode were studied. In the second part of the practical SICM was applied more practically by measuring the topography of a decane drop, and by imaging its dissolution. Moreover, the topography of biological samples, a fixed neuron cell, and a living mouse embryo fibroblast (MEF) cell, were investigated.

1.2 General Principle and Motivation

For measuring the topography of a sample by SICM a glass nanopipette filled with an electrolyte is brought in close proximity of the sample that is surrounded by an electrolyte. A constant bias voltage is generated between two electrodes, where one is located in the electrolyte in the pipette, and the other one outside in the electrolyte. As a result, an ion current in the order of nA's is induced in the pipette that is dependent on the pipette-sample distance in a sensitive manner. A piezo-scanner is used to raster-scan the pipette across the sample surface. Thus, depending on the pipette-sample distance an ion current can be measured precisely. This enables recording high-resolution topography images of the sample surface, without mechanical contact between the pipette tip and the sample. The complete setup of the SICM can be mounted above an optical microscope for detailed sample investigation [3]. The construction of a SICM can be seen in Fig. 1.

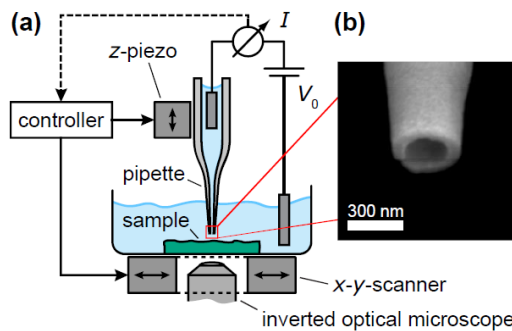


Figure 1: Setup of a SICM (left), nanopipette (right) [3]

1.3 Ion Current Measurement and Current vs. Distance Behaviour

Since the induced ion current is in the order of nA it only can be measured using a low-noise current amplifier that is usually implemented as a current-to-voltage converter. The output voltage V_{out} is proportional to the inverted input current I by a feedback resistance R_f [3]:

$$V_{out} = -I \cdot R_f \quad (1)$$

Thus, to measure currents in the order of nA, the feedback resistance has to be in the order of GΩ [3].

As mentioned in 1.1, the measured ion current is dependent on the pipette-sample distance [3]. This dependency can be investigated by lowering the pipette towards the sample surface [1]. The measured values of the ion current can be plotted against the pipette-sample distance in an *I*-*Z*-curve, as it can be seen in Fig. 2.

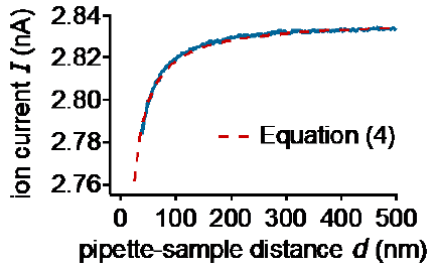


Figure 2: *I*-*Z*-curve [3]

The plotted *I*-*Z*-curve shows that the current behaves almost constant for a far pipette-sample distance above 200 nm [3]. If the distance decreases the ion current declines due to the sample impeding the flow of the ions [1]. It can be assumed that the ion current drops by 1 % from the constant value, if the pipette-sample distance *d* has the same size as the inner opening radius *r_i* of the pipette [3].

Usually, such a nanopipette has a conical shape with an inner and outer radius *r_i* and *r_o*, and an inner half-cone opening angle α . If the pipette is close to the sample, the ion current is determined by a constant resistance *R_p* and a distance-dependent resistance *R_d*, that together build an equivalent electrical circuit. The constant resistance, also called pipette resistance, can be calculated by [3]:

$$R_p \cong \frac{1}{\pi \sigma r_i \tan(\alpha)} \quad (2)$$

where σ describes the electrical conductivity of the electrolyte. The distance-dependent resistance is determined by the following equation [3]:

$$R_d(d) \cong \frac{1}{2\pi\sigma} \frac{\ln(r_o/r_i)}{d} \quad (3)$$

The ion current through the pipette can then be determined by [3]:

$$I(d) = \frac{V_0}{R_p + R_d} \cong \frac{I_0}{1 + \frac{\tan(\alpha) \ln(r_o/r_i)}{2 d/r_i}} \quad (4)$$

with *I₀* as the maximum ion current. Thus, when the tip of the pipette is far away from the sample the ion current can be approximated as [3]:

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

Obviously, the ion current would vanish if there is no distance between pipette and sample.

1.4 Imaging Modes

Several different imaging modes are existing for the SICM. The easiest one is the so-called constant current mode in which the pipette-sample distance is held at a constant distance by

a z-piezo over a feedback loop. The sample can then be laterally scanned. However, for uneven samples this imaging mode might not be suitable, since the pipette might crash with the sample when moving laterally [3].

A more advanced mode is the hopping mode. Here, the pipette is vertically approached to the sample, and is retracted, when an ion current drop threshold is reached. Both the retraction distance and the ion current drop threshold are predefined. The pipette is then moved laterally, and approached again. In this way, the surface of the sample is scanned line by line. However, it must be ensured that the retraction distance is predefined sufficiently large that the pipette will not crash with the sample when moving laterally. Furthermore, it must be considered, that when reaching the ion current threshold, the pipette further approaches to the sample, as the response rate of the z-piezo is finite. Therefore, when assuming the response rate as 1 ms and the threshold as $d \approx r_i$, the velocity for scanning the sample should not be chosen higher than $1r_i$ per 1 ms [3].

1.5 Resolution of the SICM

The vertical resolution in hopping mode is influenced by the instrumental noise of the ion current measurement. The resulting ion current noise σ_I can be calculated by the following equation [3]:

$$\sigma_I = \sqrt{\frac{4k_B T \Delta f}{R_p}} \quad (6)$$

It is dependent on the Boltzmann constant ($k_B = 1.38 \cdot 10^{-23} \frac{J}{K}$), the absolute temperature T , the measurement bandwidth Δf and the pipette resistance R_p . Since the ion current noise is dependent on the ion current vs. distance behavior, the influence of the ion current noise is non-linear. It becomes less for a higher pipette sample distance, thus, when choosing higher thresholds for retraction of the pipette [3].

The lateral resolution, also called physical resolution, is mainly determined by the opening size of the pipette. It is calculated numerically by measuring the topography of a sharp step. Due to the limited lateral sensitivity the step is never sharply imaged. Therefore, the lateral distance is taken as lateral resolution where the height of the step, that is imaged smeared, increases from 12 % to 88 % of the full step height. Usually, it can be assumed that the lateral resolution is limited to $3r_i$ [3].

The pixel resolution, thus, the number of pixels measured, should be matched with the lateral resolution as the pixel resolution can theoretically get increased very high, but the lateral resolution will limit the overall resolution.

2 Material and Methods I

2.1 Preparation of Grating and Pipette

First, a replica of the calibration grating TGX was prepared. As material polydimethylsiloxane (PDMS) was used. The grating had a step rate of 180 nm and a periodicity of 10 μm . The replica was fabricated by mixing 1.026 g of the base and 0.1 g of the curing agent, thus, in a ratio of approximately 10:1. A drop of this mixture was then given on the calibration grating, that was placed in a glass petri dish before, so that the grating was completely covered by the PDMS. After that, the petri dish was put in the desiccator for 20 minutes to degas the mixture. Next, it was put in the oven for 40 min at 80 °C for hardening. The last step was peeling the hardened PDMS off from the grating without destroying the replica.

While the PDMS was hardening in the oven, the pipettes were fabricated. In total, six pipettes of the type P37 (theoretical inner opening radius 100 nm) and six pipettes of the type P39 (theoretical inner opening radius 500 nm) were prepared using the laser beam puller P-2000. Therefore, a glass capillary was put in the device, the admired diameter of the capillary was chosen (P37 or P39), and finally, the device produced two pipettes out of one capillary.

2.2 Measurement of the Grating and Evaluation of the Pipette

For measurement of the grating replica, the replica was first mounted into a small glass petri dish by fixing it with some fat. Then, the petri dish was filled half with phosphate buffered saline (PBS). A P37 pipette, filled with PBS was mounted into the SICM. Next, the electrodes were placed. One electrode was put in the petri dish with the PBS solution, the other one was stuck into the pipette. Additionally, a Faraday cage was placed around the electrodes, the pipette and the sample, to reduce external noise during the measurement. After that, the pipette was lowered until it immersed the PBS, and the ion current was measured. The spectral noise density of the ion current measurement was recorded.

Next, the pipette was brought close to the sample surface, so that an *I*/*Z*-curve could be recorded. As threshold 1 % of the ion current drop was set.

Then, three topography images of the same location of the grating replica were recorded in hopping mode. For all three images, different current drop thresholds, of 1.5 %, 1.0 %, and 0.55 % were used.

Furthermore, an image of the grid was recorded in constant current mode by using a constant threshold of 1 %.

All these measurements done for the P37 pipette were also made for the P39 pipette afterwards.

3 Materials and Methods II

3.1 Decane Drop Preparation and Measurement

On the second day of the labwork, a sample of decane drops was prepared. This was done by dipping a capillary into decane and spreading a small amount of it on the bottom of a petri dish. The capillary was then flushed with 2 ml of phosphate buffered saline (PBS), so that the decane drops were completely covered.

For measurement of one decane drop, the petri dish was mounted in the SICM, and the Faraday cage was put over the setup to avoid external noise. Then, a P37 pipette was approached to the mixture in the petri dish. After that, one decane drop appropriate for measurement was chosen, and the stage was adjusted in a way, that the pipette was above that drop. In hopping mode, an image of the topography of this drop was recorded.

In the next step, a surfactant, here sodium dodecyl sulfate (SDS), at a concentration of 10 mM was added to the decane/ PBS mixture in the petri dish. The induced dissolution of the drop was tried to image with the SICM.

3.2 Topography Image of Fixed Neuron Cell and Growth Cone of Neuron Cell, Correlation with Optical Phase Contrast Microscopy

For this part of the experiment, a petri dish with fixed neuron cells was observed. The solution around the fixed cells was replaced by 2 ml of PBS, and the petri dish was mounted into the SICM, with a Faraday cage around the sample setup. Then, a growth cone was searched, and a P37 pipette was brought in its proximity to record a topography image. Next, a neuron cell was searched in the same area, and its topography was imaged.

Furthermore, one microscopy image was taken with phase contrast as an overview, where the complete cell and the growth cone were visible. Additionally, another photo of the same area was taken as conventional optical image without phase contrast for comparison.

3.3 Topography Image of a Living MEF Cell

In this part of the experiment, a petri dish with living MEF cells was observed. First, the cell culture was washed, and the solution around the cells was replaced by 2 ml of L15 medium. Then, the petri dish was placed in the SICM, with the Faraday cage around. After having chosen an appropriate cell, a P37 pipette was brought close to the sample, and a series of topography images was recorded to investigate dynamical processes of a MEF cell. In total, six images in an interval of 5 minutes each were taken.

4 Results and Discussion I

4.1 Pipette P37

4.1.1 Inner Opening Radius of Pipette

When lowering the pipette until it immersed the PBS, but was still sufficiently far away from the sample surface, the ion current was calculated as 2.88 nA, while the applied bias voltage was 100.77 mV. Assuming the inner half-cone opening angle of the pipette as 3° , and the electrical conductivity of PBS as $1.5 \frac{S}{m}$, the inner opening radius of the pipette can be calculated *via* equation 5:

$$r_i = \frac{I_0}{\pi \sigma \tan(\alpha) V_0} = \frac{2.88 \cdot 10^{-9} A}{\pi \cdot 1.5 \frac{S}{m} \cdot \tan(3^\circ) \cdot 0.10077 V} = 1.16 \cdot 10^{-7} m = 116 nm$$

The calculated inner opening radius of 116 nm is slightly higher than the theoretical radius of 100 nm. This might be due to inaccuracies of the machine producing the pipettes. Usually, the radius of the P37 pipettes becomes larger by a few nm when producing them with this machine. Another reason for a higher calculated radius might be that the value of the ion current of 2.88 nA used in this equation is determined out of the measured *I*-*Z*-curve. Therefore, this value is dependent on the measurement and statistical uncertainties. The exactness of the PBS's conductivity might additionally play a role.

4.1.2 Spectral Noise Density

The blue curve in Fig. 3 shows the measured current density for a P37 pipette, in dependence on the applied frequency. The orange curve represents the cumulative current.

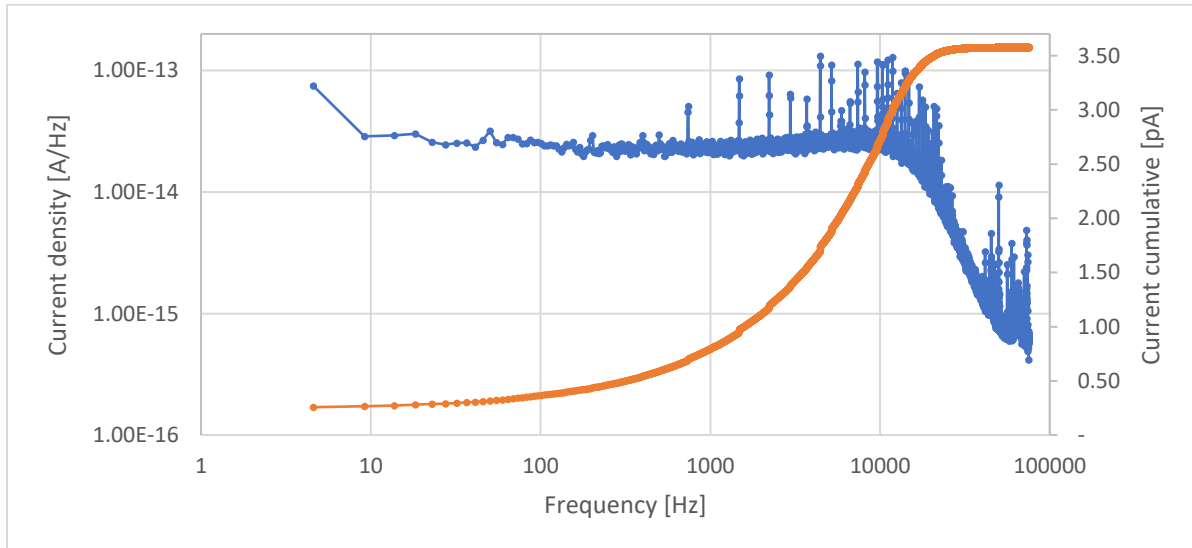


Figure 3: Spectral current noise density for P37 pipette

For lower frequencies up to 10 kHz the current density is at a constant level of about $2.5 \cdot 10^{-14} A/Hz$. For higher frequencies than 10 kHz the current density is decreasing. Moreover, the current noise density curve shows that for an increasing frequency, where the current density is still at the constant level, the noise increases. Generally, a higher frequency of e.g. 10 kHz enables a more detailed measurement than using a frequency of e.g. 1 kHz since fast changes would not be visible anymore. Therefore, it can be concluded that the bandwidth of 10 kHz, used in this practical, represents a suitable frequency for measurement with the P37 pipette.

4.1.3 IZ-Curve, Ion Current Noise

The IZ-curve recorded with the P37 pipette for a bandwidth of 10 kHz is depicted in Fig. 4. The curve shows the ion current in dependence of the deflection distance. A high deflection distance is defined as the z-piezo being fully extended downwards. Thus, when having a high deflection distance, the pipette-sample distance is low.

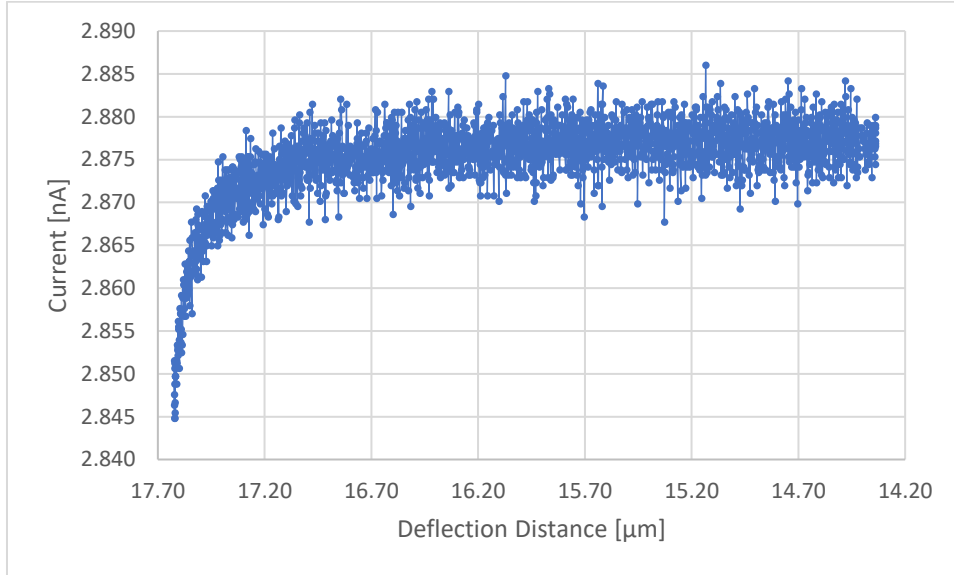


Figure 4: IZ-curve for P37 pipette and bandwidth of 10 kHz

The shape of the recorded IZ-curve looks as expected. For a low deflection distance, thus, for a high pipette-sample distance, the current is in a constant range at about 2.88 nA. For a higher deflection distance, thus, when approaching the pipette to the sample, the current drops exponentially to a value of about 99 % of the initial current.

The ion current was calculated as 2.88 nA for the pipette being far away from the sample, meaning the deflection distance being lower than 15.7 μm . The ion current noise σ_I from the ion current was determined by measuring the standard deviation of the IZ-curve for the pipette being far away from the sample. The noise was determined as $2.59 \cdot 10^{-12}$ A. The theoretical noise limit is calculated by equation 6:

$$\sigma_I = \sqrt{\frac{4k_B T \Delta f}{R_p}} = \sqrt{\frac{4 \cdot 1.38 \cdot 10^{-23} \frac{J}{K} \cdot 293 K \cdot 10000 Hz}{34906405 \Omega}} = 2.15 \cdot 10^{-12} A$$

With R_p calculated from equation 2:

$$R_p \cong \frac{1}{\pi \sigma r_i \tan(\alpha)} = \frac{1}{\pi \cdot 1.5 \frac{S}{m} \cdot 116 nm \cdot \tan(3^\circ)} = 34906.4 k\Omega$$

When comparing the theoretical and measured ion current noise, it can be observed that the two values are in the same range. The minimal deviation between the two values might be explained by statistical reasons when calculating the noise out of the IZ-curve. This means, when using the data up to another limiting value than 15.7 μm , the noise limit would probably change a bit. Another reason for the slight deviation can be inaccuracies in determining the resistance R_p of the pipette using r_i and the half-cone opening angle α .

4.1.4 Topography Images

In Fig. 5 three topography images that were recorded on the same location of the grating replica with a P37 pipette are depicted. All these images were recorded in hopping mode. However, the current drop thresholds during the measurements were different. The image on the left has a threshold of 0.55 %, the image in the middle 1 %, and the right one was taken using a threshold of 1.5 %. By setting a threshold, it is defined how much the current should drop when approaching the pipette to the sample until the pipette will be retracted.

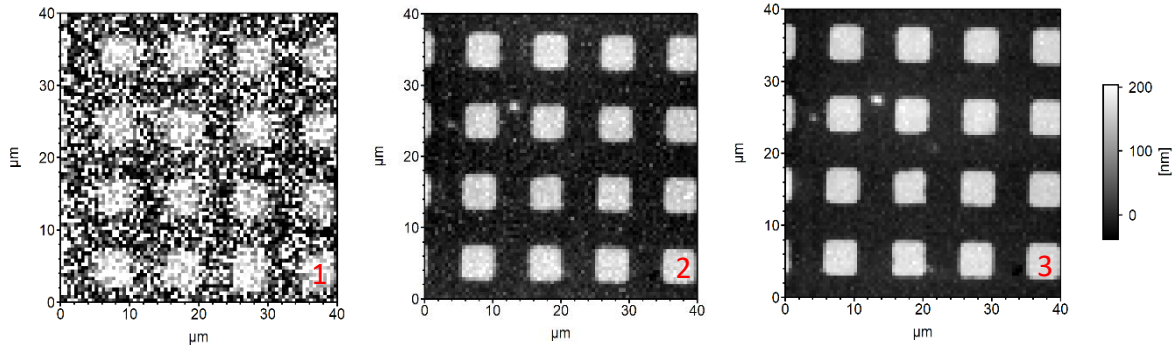


Figure 5: Topography images of grating replica with P37 pipette and thresholds of 0.55 %, 1 % and 1.5 %

When comparing the three images, it can be seen that the resolution of the images becomes more pixelated with a lower threshold value. In image 1, the grid can hardly be recognized. Image 2 depicts a quite sharp grid, yet, there are some bright pixels. In image 3, the topography of the grid is sharply visible and has a homogeneous structure. It can be concluded that a threshold of only 0.55 % leads to strong impact of electrical noise what makes it impossible to sharply depict structures. When increasing the threshold, the noise decreases. Although the image sharpness is dependent on the threshold set, the height of the grid seemed to be determined quite accurately. All pictures show bright areas that represent a height of 200 nm, and dark areas that stand for 0 nm in height.

The height noise, thus the range in which the current occurs noisy for a specific threshold value, for the images in Fig. 5 can be seen in Tab. 1. The values were determined with Gwyddion.

Table 1: Values measured for height noise with a P37 pipette

Threshold Value [%]	Height Noise [nm]
0.55	36.6
1	19.1
1.5	10.6

As it is listed in Tab. 1 the height noise decreases with an increasing threshold value. This also makes sense when looking at the images in Fig. 5 that become sharper with an increasing threshold value, as mentioned previously. A threshold of 0.55 % is such a low current difference compared to the stable current value, so that this difference is within the noise range (also see IZ-curve in Fig. 4). The current difference when using a threshold of 1 % and more towards the stable current value is clearly higher than the noise current. So, it can be concluded that higher threshold values deliver sharper images.

Fig. 6 depicts the height profile of the grating replica taken with a P37 pipette and a threshold value of 1 %.

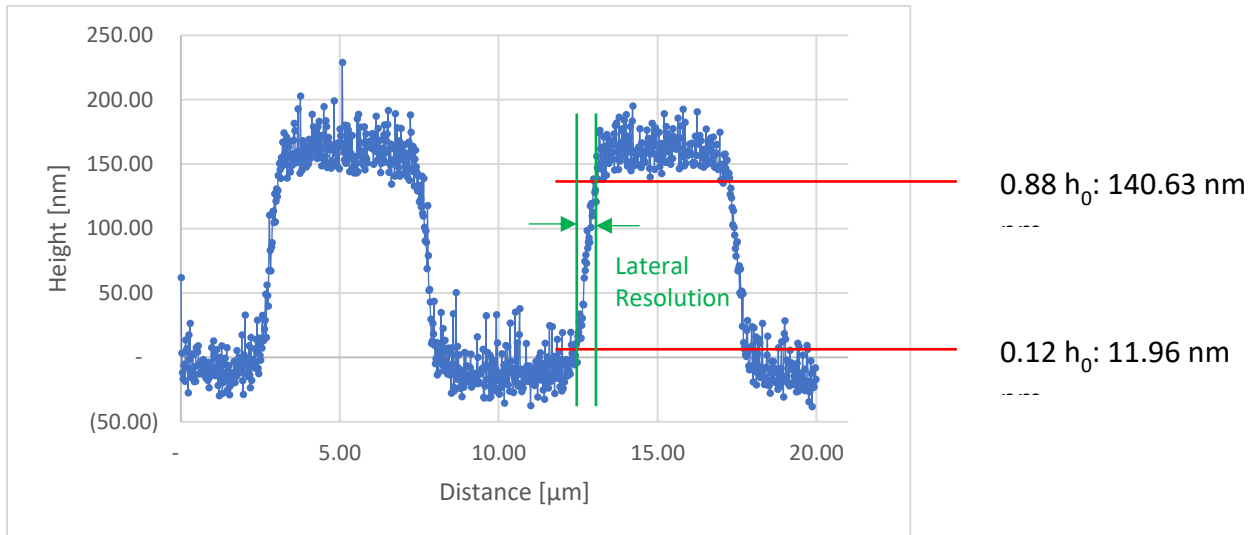


Figure 6: Height profile of grating replica measured with a P37 pipette and a threshold of 1 %

Out of the second minimum and the second maximum of the height profile the lateral resolution of the P37 pipette was determined according to section 1.4 as 500 nm. Thus, the determined lateral resolution is clearly higher than the theoretically calculated lateral resolution of:

$$3r_i = 3 \cdot 116 \text{ nm} = 348 \text{ nm}$$

The deviation between the calculated lateral resolution out of the diagram and the theoretically calculated value might be explained by the fact that there is a lot of height noise present in the diagram. Since the minimum and maximum plateau are noisy it is hard to determine the correct values on the distance-axis due to fluctuations of the height and multiple occurrences of the 0.12 and 0.88 values. By only going one measurement point forwards or backwards on the distance axis, the resolution changes by 20 nm. Another reason might be that the value of $3r_i$ to calculate the lateral resolution is a numerical consideration. Thus, it only gives the lateral resolution approximately.

4.1.5 Constant Current Mode

In Fig. 7, the grating replica is depicted when recording it in the constant current mode with a threshold value of 1 %. Overall, the image is very sharp. The edges between low and high points of the sample are clearly visible. Compared to the images taken in constant hopping mode, with 1 % and 1.5 % as threshold values (Fig. 5), the image recorded in constant current mode seems to be less pixilated. Generally, the constant current mode enables to take topography images faster, as the pipette has not to be moved up- and downwards. However, since the pipette is just moved laterally over the surface, it might crash with the sample. As PDMS is a soft sample this did not happen here.

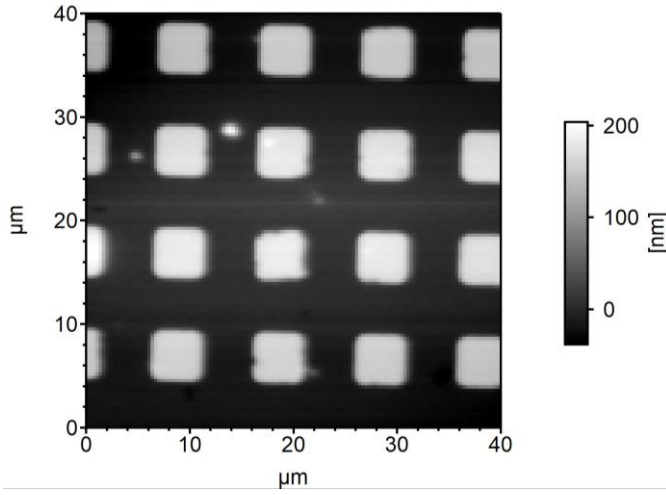


Figure 7: Grating replica imaged in constant current mode with a P37 pipette and a threshold of 1 %

4.2 Pipette P39

4.2.1 Ion Current Measurement, Inner Opening of Pipette

The P39 pipette was lowered in the same way as the P37 pipette until it immersed the PBS. For this pipette, the constant ion current was measured as 12.94 nA, while the applied bias voltage was 99.7 mV. The inner half-cone opening angle of the pipette was assumed as 3° , and the electrical conductivity of PBS as $1.5 \frac{S}{m}$. The inner opening radius of the pipette, calculated *via* equation 5, is then given by:

$$r_i = \frac{I_0}{\pi \sigma \tan(\alpha) V_0} = \frac{12.94 \cdot 10^{-9} A}{\pi \cdot 1.5 \frac{S}{m} \cdot \tan(3^\circ) \cdot 0.0997 V} = 5.26 \cdot 10^{-7} m = 526 nm$$

The calculated inner opening radius of 526 nm is slightly higher than the theoretical radius of 500 nm. This might be due to inaccuracies of the machine producing the pipettes. Furthermore, as mentioned for the P37 pipette, the value of the current used to detect the radius is determined out of the measured curve what additionally might lead to deviations.

Another aspect to be considered is that when not using the ion current determined from the *I*Z-curve, but read it directly from the device, where it is given as 12.2 nA, the inner opening radius of the pipette is calculated as 495 nm, what would be closer to the theoretical radius of 500 nm. This indicates that there are some measurement imprecisions, and probably inaccuracies in the pipette production.

Nevertheless, it can be stated for both the P37 and P39 pipette the radii are in a range that they were expected to be. Thus, in the following it makes sense to compare the results obtained for the P37 and P39 pipette because of their radii being sufficiently different.

4.2.2 Spectral Noise Density

The blue curve in Fig. 8 shows the measured current density for a P39 pipette, in dependence on the applied frequency. The orange curve represents the cumulative current in dependence on the frequency.

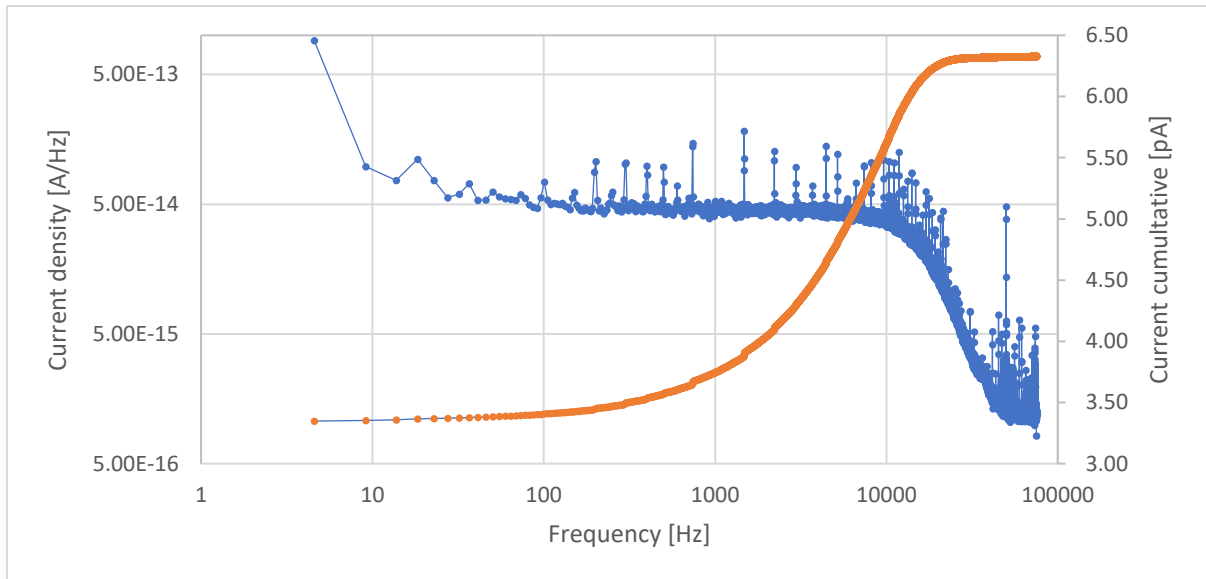


Figure 8: Spectral current noise density for P39 pipette

When observing the current noise density of the P39 pipette, it can be seen that the constant value of about $5 \cdot 10^{-14}$ A/Hz is almost twice as high as the value determined for the P37 pipette. The curve in general behaves as described previously for the P37 pipette. Since the inner opening radius of the P39 pipette is higher than of the P37 pipette, it makes sense that the values for the current density and for the cumulative current are higher, because more ions, and therefore, a higher current can flow through the pipette. It can be concluded that when measuring with the P39 pipette, a bandwidth of 10 kHz is appropriate.

4.2.3 IZ-Curve, Ion Current Noise

The IZ-curve recorded with the P39 pipette for a bandwidth of 10 kHz and a threshold of 1 % is depicted in Fig. 9.

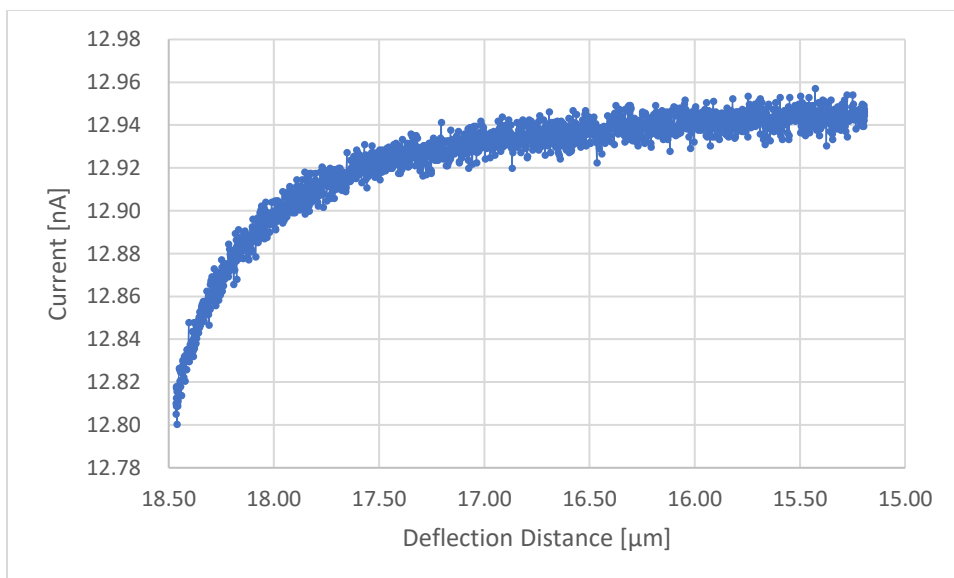


Figure 9: IZ-curve for P39 pipette and bandwidth of 10 kHz

The shape of the recorded IZ-curve looks as expected. As explained previously for the P37 pipette, the current drops to approximately 99 % of the initial current value, when approaching the pipette to the sample. However, for the P39 pipette it can be observed that

the I - Z -curve drops over the complete deflection distance. Thus, there is no plateau for the I - Z -curve as it was for the P37 pipette. Nevertheless, for a high pipette-sample distance, the curve is almost flat. The ion current of the pipette being far away from the sample (for a deflection distance lower than $16.5 \mu\text{m}$) was calculated as 12.94 nA . Thus, the current calculated for a high pipette-sample distance for the P39 pipette is much higher than the current measured under the same conditions for a P37 pipette. Since current is defined as the amount of charge per time it seems to be logical that more ions are able to pass the pipette having the larger radius. So, when measuring with the P39 pipette, the current is higher.

The ion current noise σ_I of the P39 pipette was determined as $4.85 \cdot 10^{-12} \text{ A}$. The theoretical noise limit is calculated by equation 6:

$$\sigma_I = \sqrt{\frac{4k_B T \Delta f}{R_p}} = \sqrt{\frac{4 \cdot 1.38 \cdot 10^{-23} \frac{\text{J}}{\text{K}} \cdot 293 \text{ K} \cdot 10000 \text{ Hz}}{7697990 \Omega}} = 4.58 \cdot 10^{-12} \text{ A}$$

With R_p calculated from equation 2:

$$R_p \cong \frac{1}{\pi \sigma r_i \tan(\alpha)} = \frac{1}{\pi \cdot 1.5 \frac{\text{S}}{\text{m}} \cdot 526 \text{ nm} \cdot \tan(3^\circ)} = 7698 \text{ k}\Omega$$

When comparing the theoretical and measured ion current noise, it can be observed that the values are similar. The slight deviation might be due to the same statistical reasons as mentioned for the P37 pipette.

Generally, the ion current noise determined for the P39 pipette is approximately twice as high as the value determined for the P37 pipette. Since the ion current is also higher for the P39 pipette this is logical.

4.2.4 Topography Images

In Fig. 10 the topography images of the grating replica recorded with a P39 pipette are depicted. The images were recorded in hopping mode, with current drop thresholds of 0.55 %, 1 %, and 1.5 %.

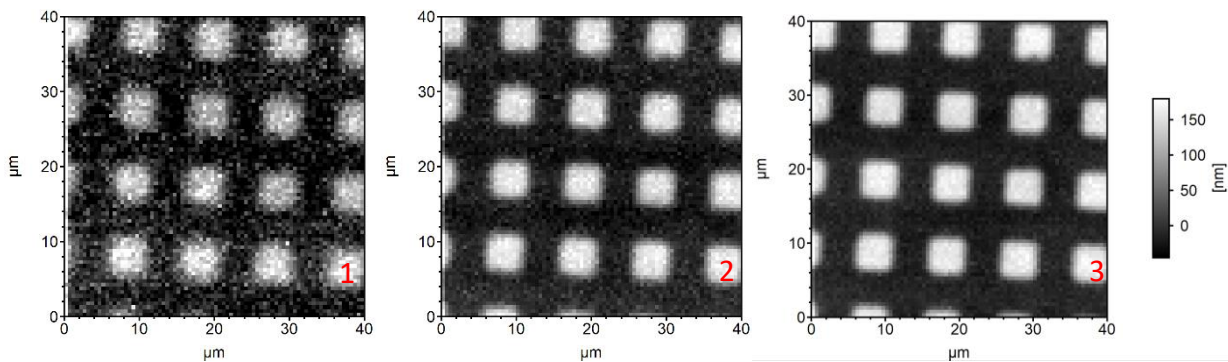


Figure 10: Topography images of grating replica with P39 pipette and thresholds of 0.55 %, 1 % and 1.5 %

As for the P37 pipette it can be observed that the topography images become more pixilated for a decreasing threshold value. However, for the image taken with a threshold of 0.55 % there is less noise visible than for the image taken with the P37 pipette. The images taken with thresholds of 1 % and 1.5 % look similar to the images taken with the P37 pipette. Nevertheless, for the images 2 and 3, the recorded structures seem to be slightly sharper when using the P 37 pipette. Especially the transitions between high and low areas seem to be

blurrier when using the P39 pipette. This is due to the better lateral resolution of the P37 pipette. The observation that the image recorded with the P39 pipette for a threshold value of 0.55 % looks less noisy than the image taken with the P37 pipette, might be explained by the fact that the ratio of the noise to the determined current value is higher for the P37 pipette. Thus, when only having a threshold value of 0.55 % this has less impact when measuring with a higher current since 0.55 % of the P39 pipette's current is more out of the noisy area than when using the P37 pipette. For such a low threshold value the better lateral resolution of the P37 pipette has no impact. This effect can also be seen in Tab. 2 as the determined height noise for a threshold value of 0.55 % is lower for the P39 pipette than for the P37 pipette with a threshold of 0.55 % (see Tab. 1). For the other threshold values of 1 % and 1.5 % the height noise is similar.

Table 2: Values measured for height noise with a P39 pipette

Threshold Value [%]	Height Noise [nm]
0.55	29.7
1	17.7
1.5	14.6

Fig. 11 shows the height profile of the grating replica measured with a P39 pipette and a threshold of 1 %.

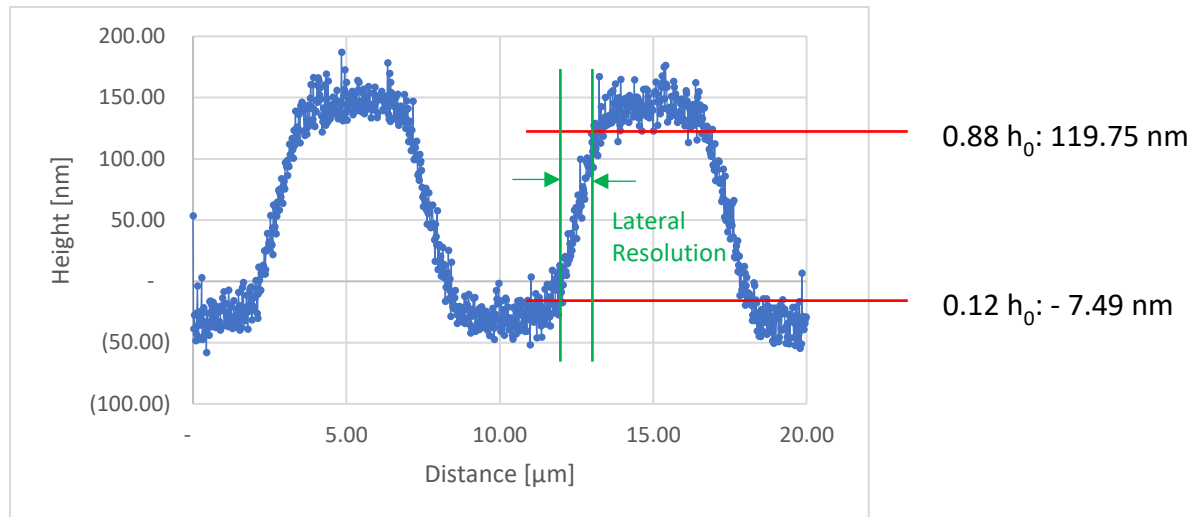


Figure 11: Height profile of grating replica measured with a P39 pipette and a threshold of 1 %

The calculated lateral resolution for the P39 pipette of 1120 nm is much lower than the theoretical lateral resolution of:

$$3r_i = 3 \cdot 526 \text{ nm} = 1578 \text{ nm}$$

The deviation between the calculated lateral resolution out of the height profile and the theoretical calculated one can be explained, as for the P37 pipette, by the fact that there is lots of height noise present in the height profile. This makes it difficult to estimate the correct values at the distance axis. Furthermore, as it was discussed in 4.2.1, when only using the current of 12.2 nA, the radius of the pipette would be smaller, leading to a better lateral resolution.

4.2.5 Constant Current Mode

The image recorded in constant current mode with the P39 pipette and a threshold of 1 % is depicted in Fig. 12.

Compared to the images taken in the hopping mode (Fig. 10) this image is less sharp. Also compared to the image recorded in constant current mode with the P37 pipette the edges between high and low areas are blurrier. This is due to the better lateral resolution of the P37 pipette. In general, it was not possible to take a good image in constant current mode with the P39 pipette. The areas at the bottom and at the top of the image are totally smeared by white stripes. It might be that the pipette crashed the sample, and therefore, falsified the image. It can also be seen that the height is not measured consistently since the grey and white areas should have the same height.

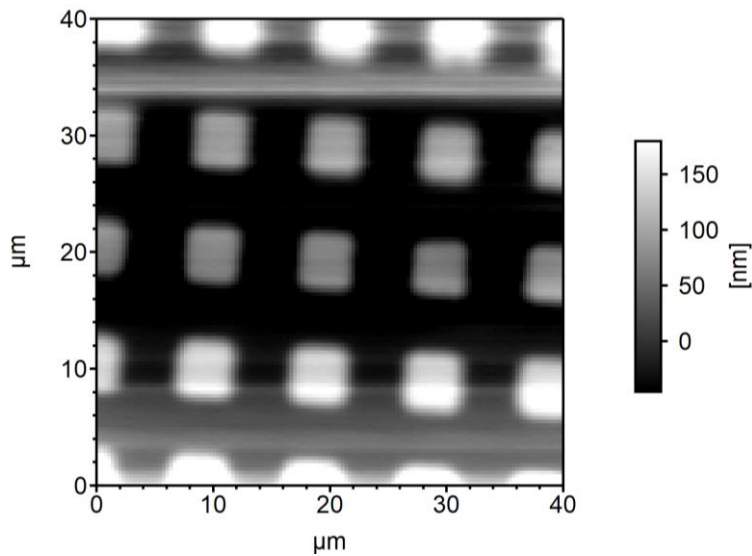


Figure 12: Grating replica imaged in constant current mode with a P39 pipette and a threshold of 1 %

5 Results and Discussion II

5.1 Measurement of Decane Drop

5.1.1 Volume of Decane Drop

The decane drop's topography measured in this experiment is depicted in Fig. 13. The determination of the drop's volume was made with the software Gwyddion. Therefore, a threshold of 50 nm was set on the image, so that only the area with z-components above this threshold was considered. In Fig. 13 the parts above 50 nm in height are marked in red. The volume of this red area, and thus the volume V_0 of the drop, was determined as $18.02 \mu\text{m}^3$.

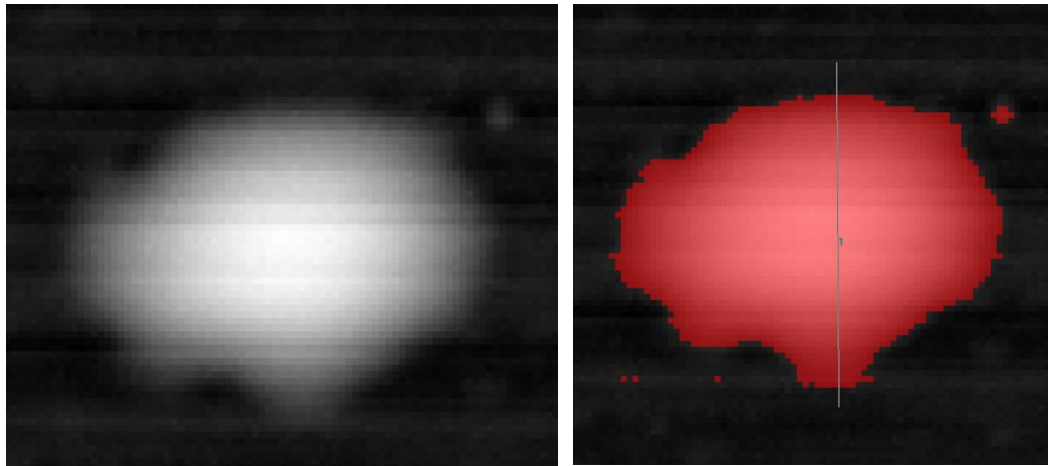


Figure 13: Topography of decane drop (left), with threshold set at 50 nm (right)

It can be assumed that the calculated volume of the decane drop of $18.02 \mu\text{m}^3$ is quite accurate. This is as the area marked red in Fig. 13, where the volume is determined from, approximately has the shape of the decane drop's topography on the left side. Nevertheless, the threshold will never be exactly depicting the drop. Therefore, the real volume might be slightly deviating from the determined value. The artifacts that are additionally marked in red are not taken into account when measuring the volume of the decane drop with Gwyddion.

5.1.2 Height Profile, Contact Angle and Internal Pressure of Decane Drop

The height profile of the investigated decane drop can be seen in Fig. 14. The profile was made along the grey line across the drop that can be seen in Fig. 14 on the right side.

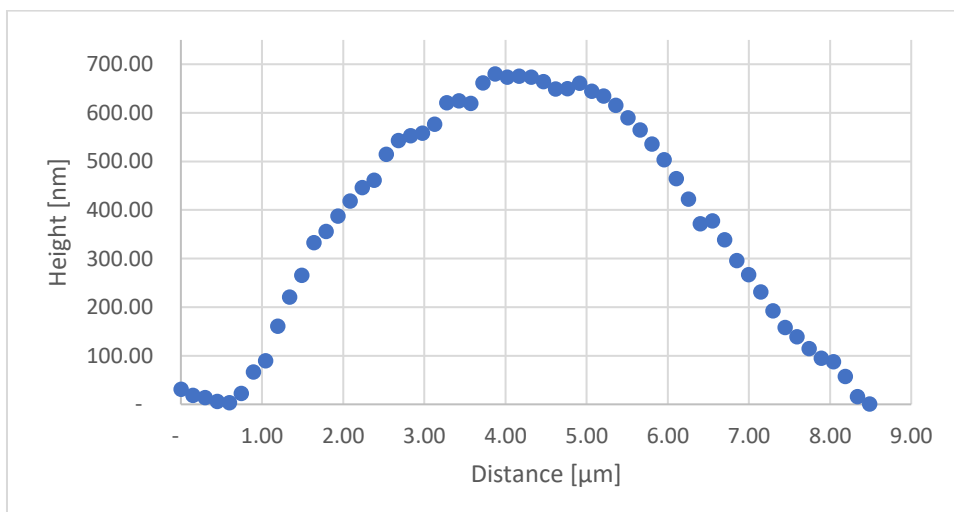


Figure 14: Height profile of decane drop

The height profile shows that the height of the drop is approximately symmetrically distributed. The maximum height of the drop can be read out of the profile. It is about 680 nm.

The contact angle of the drop Θ_c was calculated by determining the $\tan^{-1}$ of the slope of six points on the left and six points on the right side of the curve with Excel. The result showed two different contact angles for the left and the right side of the decane drop. On the left side, the contact angle was calculated as 18.56° , and on the right side as 8.42° . Theoretically, the contact angle should be the same on the left and right side of the drop. Different values for these angles indicate that the drop is not situated evenly. This might be due to inhomogeneous surface properties because of roughness or dirt if the drop is situated directly on a surface. If the drop is completely covered in PBS, it might be slightly deformed due to the PBS when adding it.

The radius of curvature was determined by attaching a circle on the height profile of the decane drop in Excel. The radius of this circle was calculated as $8.56 \mu\text{m}$. Fig. 15 shows the curve section of the arc over the height profile.

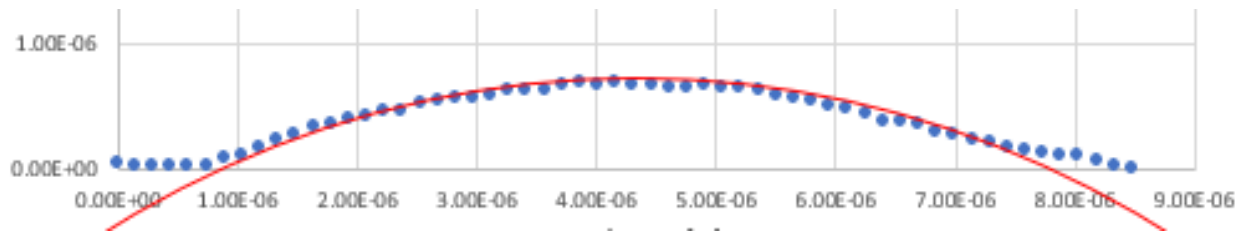


Figure 15: Curve section of arc over height profile for determination of the radius of curvature

By applying the Young-Laplace equation, the pressure difference of the drop towards the ambient was calculated as:

$$\Delta p = \frac{2\gamma}{R} = \frac{2 \cdot 51.2 \frac{\text{mN}}{\text{m}}}{8.56 \cdot 10^{-6} \text{ m}} = 11962.6 \text{ Pa} = 0.12 \text{ bar} \quad (7)$$

The surface tension γ of the water/decane interface was assumed as $51.2 \frac{\text{mN}}{\text{m}}$. Actually, the measurement took place in an aqueous PBS solution.

The calculated internal pressure of the decane drop of 0.12 bar is approximately 8 times lower than the ambient pressure on earth, that is about 1 bar. For comparison, the oxygen partial pressure in the human arteries of 75-100 mmHg (= 0.1-0.13 bar) is in the range of the inner pressure measured for the decane drop in this experiment [4]. The normal systolic blood pressure of a human of 120 mmHg (= 0.16 bar) is also similar.

5.1.3 Addition of Surfactant, Dissolution of Decane Drop

Fig. 16 shows the topography images of a decane drop that were taken after adding the surfactant SDS to the PBS. As it can be seen, the volume of the drop decreases with time, until no drop can be seen any more in photo 9. Furthermore, it can be investigated that in the beginning (photo 1 to 5), the drop dissolves more slowly than in the end (photo 6 to 8). This is due to the fact that the drop becomes smaller with time, and thus, having a larger volume-specific surface what leads to a higher material flow, and thus, to a faster dissolution.

The drop evaluated in this task (5.1.3) is not the same drop that was observed before, because the drop had not sufficient volume to observe its dissolution. Therefore, the photos of another group were taken.

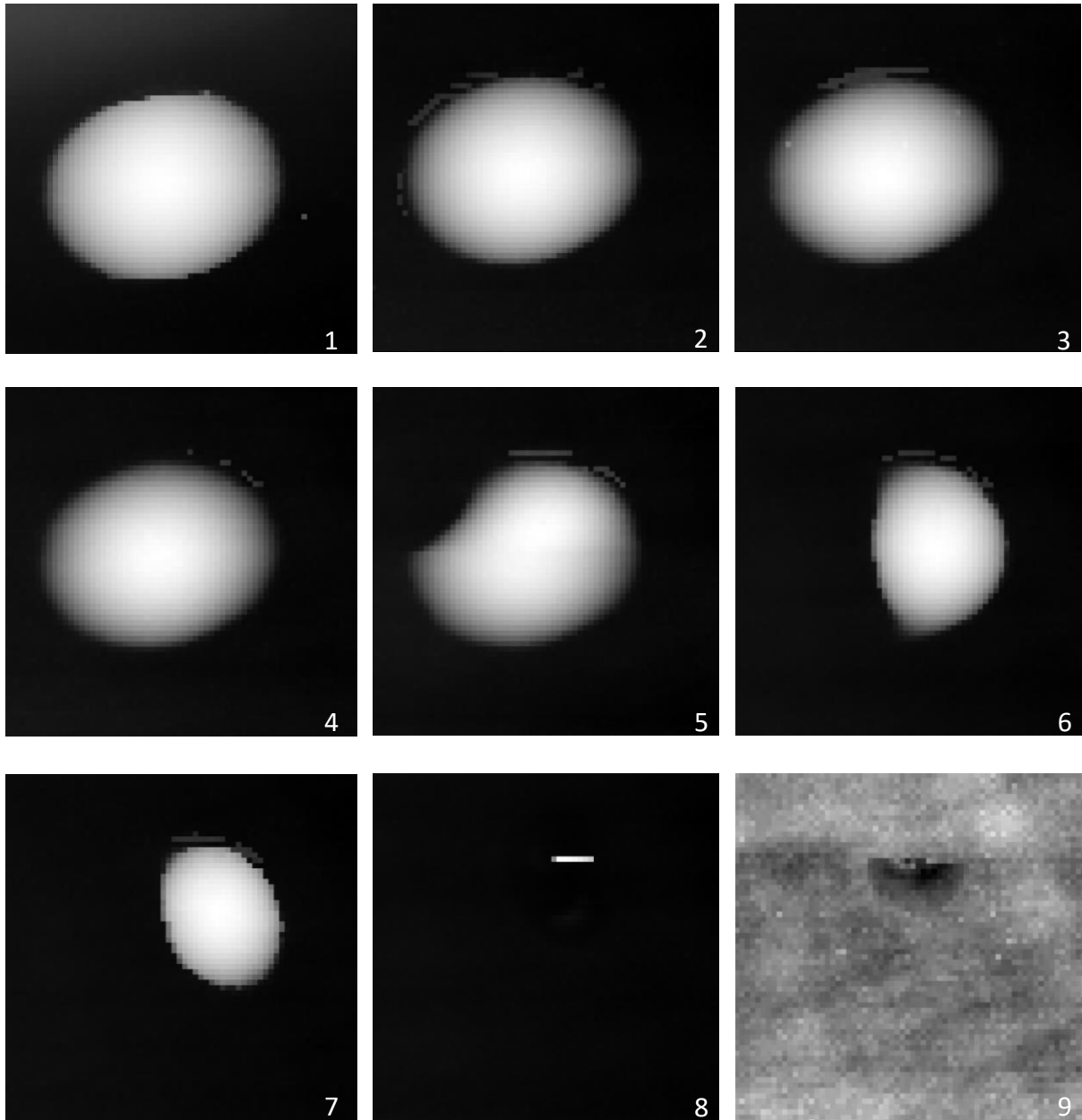


Figure 16: Dissolution of decane drop after adding SDS

In Tab. 3 the measured volume of the dissolving decane drop and the time are listed.

Table 3: Parameters for dissolution of the decane drop

Image	Volume Measured [μm^3]	Time [min]	Volume ^(2/3) [μm^3]
1	67.32	0.00	16.55
2	50.48	3.30	13.66
3	43.49	6.62	12.37
4	35.85	9.92	10.87
5	32.04	13.22	10.09
6	24.78	16.52	8.50
7	20.49	19.83	7.49
8	0.32	23.13	0.47
9	0.00	26.45	0.00

As already mentioned, the present diffusive dissolution is non-linear. Theoretically, the volume should have a time dependent behaviour that can be described by the following equation [3]:

$$V(t) = a(t_0 - t)^{3/2} \quad (8)$$

To calculate the diffusion rate a , a linearization was made. The values for the volume $^{2/3}$ can also be seen in Tab. 3. The parameter t_0 describes the time when the drop is completely vanished, thus, $t = 26.45$ min.

Fig. 17 depicts the linearized volume of the decane drop that is dissolved in dependence of the time.

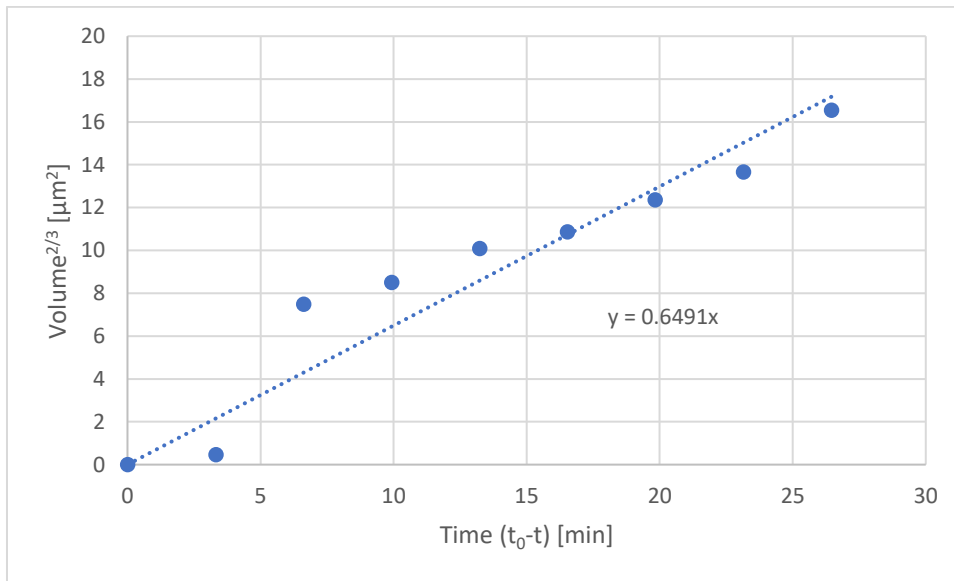


Figure 17: Dissolution of decane drop

For determination of the diffusion rate a least square fit was made with Excel, resulting in $a^{2/3} = 0.65 \mu\text{m}^2/\text{min}$. When now linearizing the value for a , the obtained dissolution rate for the decane drop dissolved in this experiment is calculated as $a = 0.52 \mu\text{m}^3/\text{min}^{3/2}$. In general, a typical value for the dissolution rate is $a = 0.01 \mu\text{m}^3/\text{min}^{3/2}$ [3]. Obviously, the theoretical and the measured value are deviating from each other. This might be due to the decane drop in this experiment being significantly smaller than the drop observed for the theoretical value in literature. For smaller drops there is a higher capillary pressure what is accelerating the dissolution compared to a bigger drop [5]. Other reasons might be a different ambient temperature, or an inaccurate determination of the drops' volume.

5.2 Topography of Fixed Neuron Cell

Fig. 18 shows an optical overview image of the investigated sample containing neuron cells. The neuron cell (pink) and the growth cone (blue) further observed are marked. The picture was taken with phase contrast. Therefore, the different structures in the sample are clearly visible.

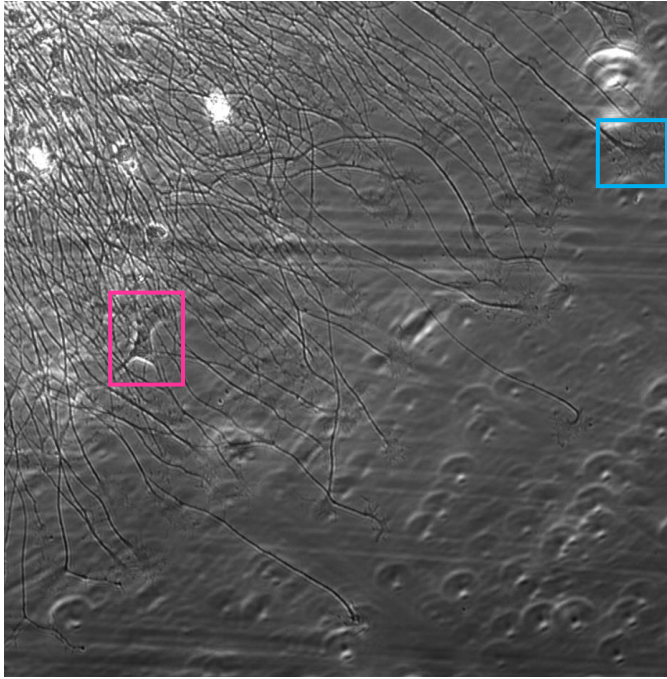


Figure 18: Phase contrast overview image, neuron cell left (pink frame) and growth cone right (blue frame)

In general, there are several axons visible in Fig. 18. When having a more detailed look, growth cones at the ends of the axons can be observed. This indicates that these axons are searching for new synaptic connections and are further growing. There can also be detected a few neuron cells in the sample.

A more detailed view of the marked neuron cell can be taken when looking at Fig. 19. The cell has an elongated shape with several dendrites. The thin structures above and around the cell are axons of other cells. For evaluation of the pink marked cell with Gwyddion, a threshold of $0.75\ \mu\text{m}$ was set. The determined parameters of the neuron cell can be seen in Tab. 4. It has to be considered that when setting a threshold for evaluation, all structures above this threshold, that are connected, are included in the measurement of the parameters by Gwyddion. When comparing the cell, and the marked areas in Fig. 18, it can be seen that the neuron cell is almost completely marked. However, some axons around and on top of the cell are additionally marked, what might falsify the measured parameters. Furthermore, it is complicated to determine at which point the cell ends. Regarding the complete area and the volume, the falsification due to the present axons should not have a huge influence, because the axons are quite narrow. Yet, the axons have a more relevant influence on the perimeter, because there are many of them.

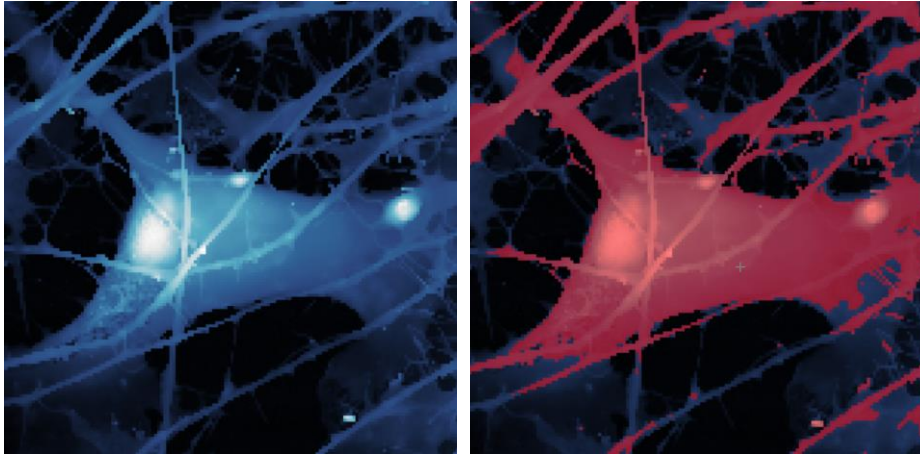


Figure 19: Topography image of neuron cell (left), threshold set at 0.75 μm for evaluation (right)

Table 4: Parameters measured with Gwyddion for neuron cell

Shape Parameters	Measured Values
Area [μm^2]	848.9
Maximum Height [μm]	3.3
Average Height [μm]	1.4
Volume [I]	$1.2 \cdot 10^{-12}$
Perimeter [μm]	658.1

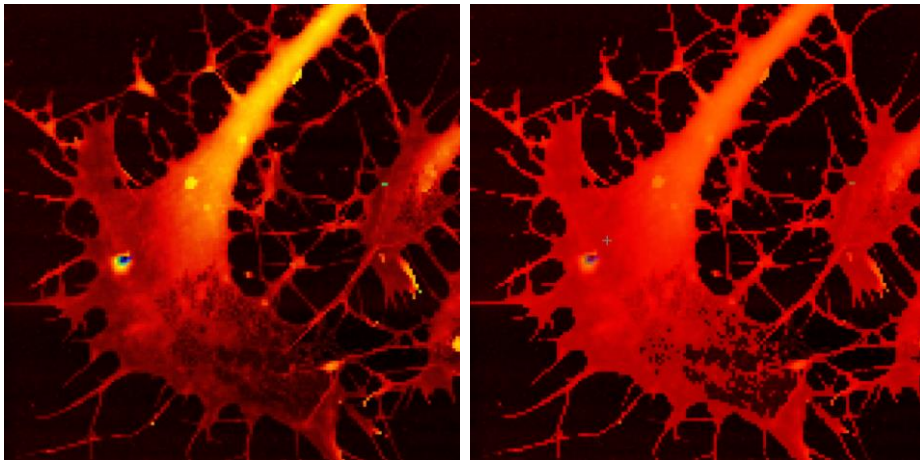


Figure 20: Topography image of growth cone (left), threshold set at 0.1 μm for evaluation (right)

The growth cone marked in Fig. 18 can be observed in Fig. 20 in a more detailed way. Tab. 5 shows the parameters measured for the growth cone. As for the evaluation of the neuron cell, it also must be taken into account, that by setting the threshold at, in this case, 0.1 μm the shape of the growth cone is not represented by 100 %. Furthermore, the image does not show the complete growth cone. As a result of the growth cone's low average height, its total volume is low, although, the area is much larger than e.g. the area of the neuron cell analyzed above. There is one height peak of 5.1 μm in the growth cone, that is also visible in Fig. 20 on the left. Due to the reason that the rest of the growth cone is quite flat this might be a detachment or a measurement error. It can be seen that the axon is brighter, and thus, higher than the growth cone itself.

Table 5: Parameters measured with Gwyddion for growth cone

Shape Parameters	Measured Values
Area [μm^2]	1256
Maximum Height [μm]	5.1
Average Height [μm]	0.4
Volume [l]	$0.5 \cdot 10^{-12}$
Perimeter [μm]	1120

Fig. 21 shows the identical sample section as Fig. 18. For comparison Fig. 18 is pasted next to Fig. 21. However, in this image it is much harder to recognize the different structures of the sample. A reason for this is that the image is a conventional optical image, taken without phase contrast. The obvious difference between an image taken with, and without phase contrast shows the necessity to record an image with phase contrast. Only then, it is possible to see tiny, but important structures. This especially plays an important role when investigating biological samples, but also when observing others. Phase contrast enables to detect structures of interest without further preparation of a sample.

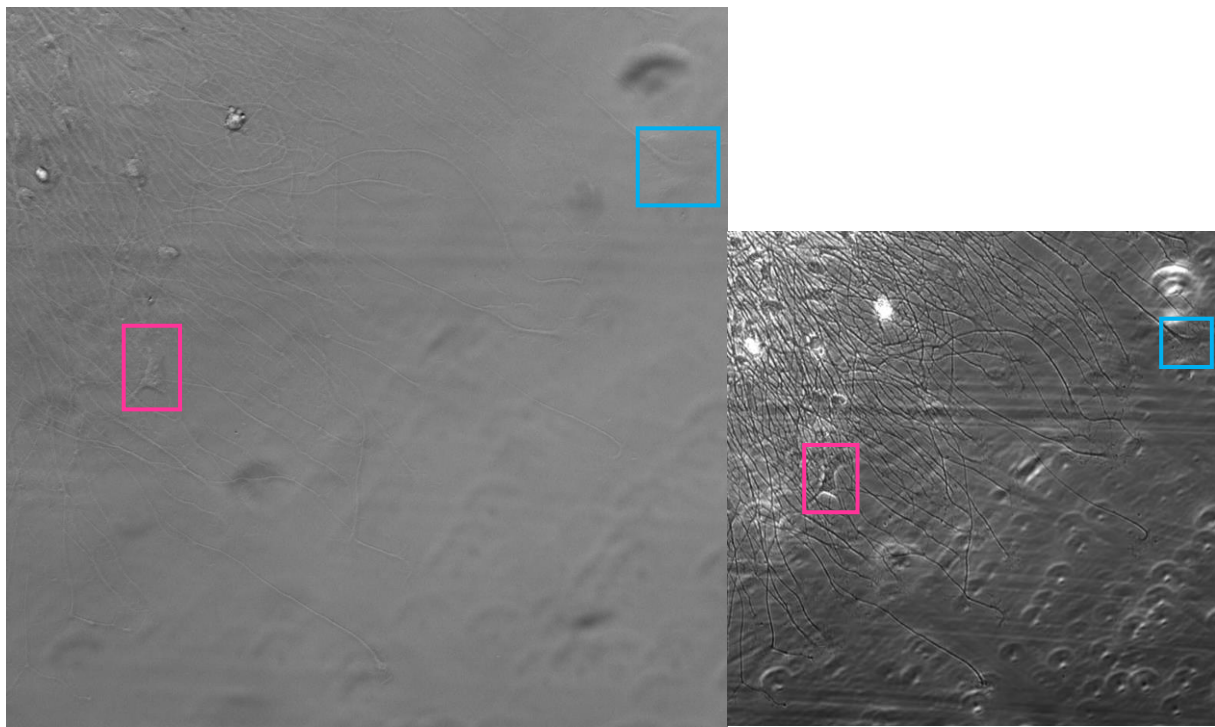


Figure 21: Microscopic overview image without phase contrast (left), neuron cell left (pink frame) and growth cone right (blue frame), microscopic overview image with phase contrast (right, same as Fig. 18)

5.3 Topography Image of Living MEF Cell

Fig. 22 shows the series of topography images that were recorded of a living MEF cell in time intervals of 5 minutes.

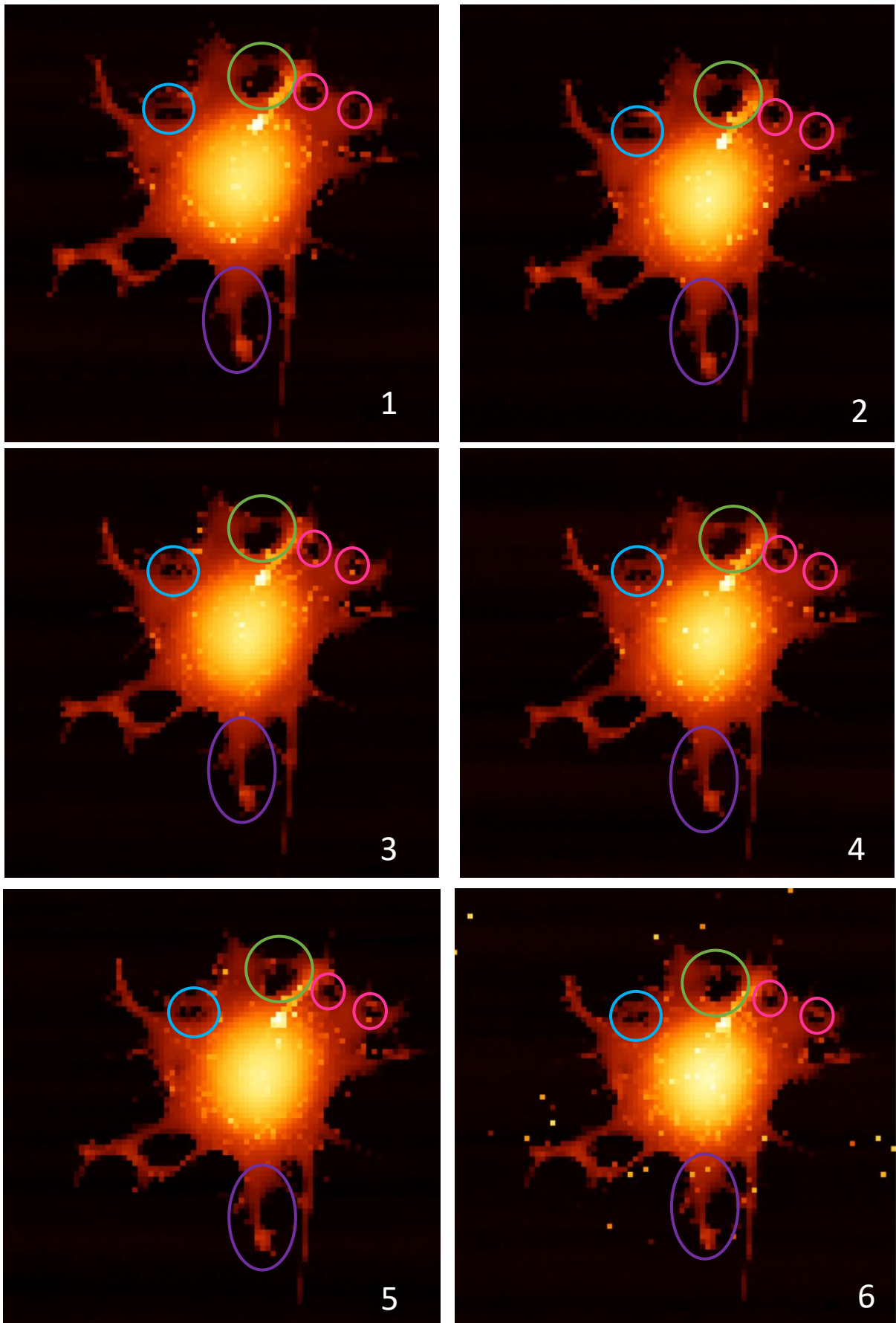


Figure 22: Series of topography images of living cells

For evaluation of the time dependent behaviour of the cell, shape parameters of the first and last image, that were taken in the series, were compared. To determine the shape parameters with Gwyddion, the area of interest, thus, the cell, was marked by a threshold of 0.1 μm . In Fig. 23 it can be seen that this threshold appropriately represents the cell.

Table 6: Comparison of shape parameters of living MEF cell at start and stop of image series

Parameters	Image 1	Image 6
Area [μm^2]	1736	1664
Maximum Height [μm]	5.3	5.0
Average Height [μm]	1.6	1.7
Volume [l]	$2.8 \cdot 10^{-12}$	$2.7 \cdot 10^{-12}$
Perimeter [μm]	501.4	475.1

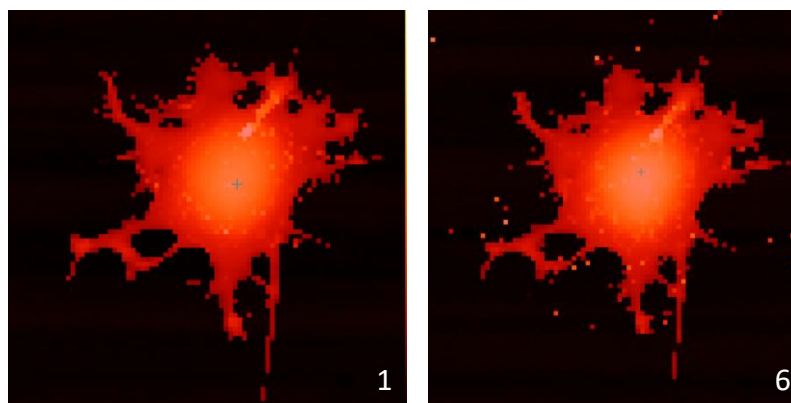


Figure 23: First and last image of MEF living cell marked by threshold of 0.1 μm

Tab. 6 shows that the area of the MEF cell between image 1 and 6 shrunk. It can also be seen that the maximum height decreased. However, the average height increased. Volume and perimeter of the cell decreased. Although these changes in the parameters compared are slight, they confirm that the cell observed is a living cell, and therefore, changes its structure with time.

These small changes can be seen by eye in Fig. 23. E.g. in the part marked in blue, it can be seen that the cell closes this part. In the green area the cell seems to move apart. In the purple area the structure becomes slimmer over time. The pink area also changes by enclosing the cell-free part. The tiny points outside of the cell that can be seen in Fig. 22, image 6 might be either artifacts, or small particles belonging to the cell, or false triggers due to electrical noise. It might be that some small changes happen below the resolution limit, and can therefore not be detected. Nevertheless, in total it can be concluded that SICM is a suitable method to detect even minimal changes of a living cell.

6 Conclusion

On the first day basic knowledge about SICM was acquired. The behavior of the ion current dropping by approximately 1 % was investigated when approaching the pipette to the sample. By using two different inner pipette radii (116 nm and 526 nm) it could be seen that the initial current for the larger radius was higher. While observing topography images of a grating replica, taken with different thresholds in hopping mode, it was obvious that the images became less pixilated when using higher threshold values, as there occurred less height noise. Moreover, due to a higher lateral resolution of the P37 pipette, these images were sharper than that taken with the P39 pipette. Last, images in constant current mode were recorded. This imaging mode enables to take fast and sharp topography images as the pipette has not to be retracted before moving laterally. However, it must be considered that the pipette should not crash (and damage) the sample. Thus, this mode can only be applied for flat (and maybe for very soft) samples.

The experiments conducted on the second day showed that SICM is a suitable method to observe dynamical processes. The process of non-linear dissolution of a decane drop could be investigated. Furthermore, the method enabled to observe a fixed neuron cell, but also the investigation of movement of a living MEF cell with time accurately. By measuring the topography of samples SICM enables the determination of shape parameters, e.g. volume or height, of these samples in further evaluation steps.

In general, SICM is an advanced method to record the topography of samples in a detailed and reliable way. Since the method enables to analyze living cells contactless it provides huge potential in the future of biology and medicine.

7 Literature

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