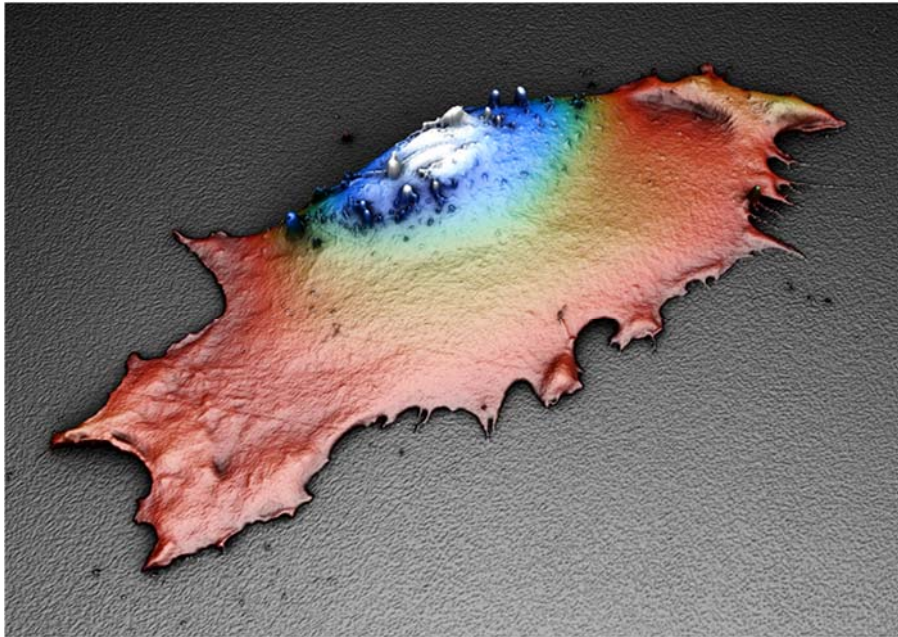




# Scanning Ion Conductance Microscopy

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## Instruction Manual

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Assistant room number: C9 A38/P34  
Experiment room number: C9 P34

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## I. Preparation

In order to conduct this experiment, knowledge of the following topics should be acquired by reading this instruction manual:

- o General principle and setup of the SICM
- o Ion current and distance behavior
- o Imaging modes (constant current mode, hopping mode)
- o Vertical and lateral resolution of the SICM

You should be able to answer the following questions before the experiment:

- How does an SICM work and what are its main components?
- How does the current measurement work?
- How does the ion current change as a function of the pipette-sample distance? What is an *I*-*Z*-curve and how does it look like?
- What is the hopping mode and how does it work? What limits the pixel rate there?
- What is the lateral resolution of the SICM and how could it be measured?
- What is the difference between physical resolution and pixel resolution?

## II. Background

### 1. Introduction to scanning ion conductance microscopy

The development of scanning probe microscopy was triggered by the invention of the scanning tunneling microscope (STM) in 1981 by Binnig, Rohrer, and coworkers.<sup>1</sup> One member of this microscope family is the scanning ion conductance microscope (SICM), which was developed in 1989 by the Hansma group.<sup>2</sup> The SICM uses an electrolyte-filled nanopipette, which is brought in vicinity of a sample in an electrolyte environment (Figure 1a). By applying a constant bias voltage between an electrode inside and another one outside the pipette, an ion current on the order of nA is driven through the pipette, which strongly depends on the pipette-sample distance and can therefore be used to detect the sample surface, before mechanical contact between pipette and sample occurs. With the use of piezo-scanners the pipette can be raster-scanned across the sample to record high-resolution topography images of the sample surface in a non-contact, invasion-free fashion, making the SICM highly-suited for the investigation of soft and fragile, especially biological samples.<sup>3</sup> Although some attempts have been made to use other types of probes such as microfabricated ones,<sup>4,5</sup> glass pipettes are used in most setups today, since they can be easily fabricated with a sub-micrometer opening (Figure 1b) and offer a large field of additional applications.<sup>6</sup>

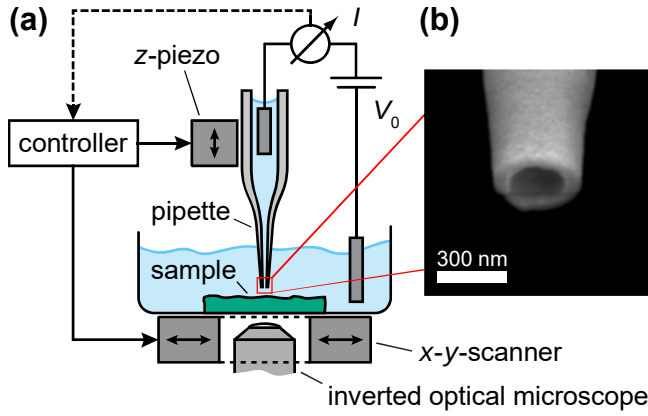


Figure 1. (a) Schematic of a scanning ion conductance microscope (SICM): A glass nanopipette is brought in vicinity of a sample in an electrolyte environment and can be moved with respect to the sample using a piezo-scanner. A constant bias voltage  $V_0$  is applied between an electrode inside and outside of the nanopipette, inducing an ion current  $I$  on the order of nanoampere. For sample inspection the SICM is usually mounted on a inverted optical microscope. (b) Scanning electron microscope image of the tip of a typical SICM nanopipette.

## 2. Ion current measurement

The ion current is usually measured using low-noise current amplifiers (often commercial patch clamp amplifier). In the simplest case, the current amplifier is implemented as a transimpedance amplifier or current-to-voltage converter (see Figure 2). For that the inverting input and the output of an operational amplifier is connected with a feedback resistance  $R_f$ . The output voltage  $V_{out}$  of the operational amplifier is then proportional to the current  $I$  with

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

Using feedback resistances on the order of  $G\Omega$  then allows measuring currents in the nA range.

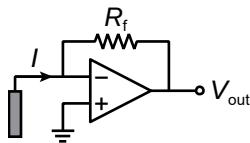


Figure 2. Simplified transimpedance amplifier as current-to-voltage converter for current measurement by using an operational amplifier with a high feedback resistor  $R_f$ . The output voltage is then proportional to the current,  $V_{out} = -I \cdot R_f$ .

## 3. Ion current vs. distance behavior

An important aspect of the operation principle of the SICM is the dependence of the ion current through the pipette on the pipette-sample distance. This dependency can be observed, when the ion current is measured while the pipette is vertically approached to the sample (often denoted as “ $I$ - $Z$ -curve”). Initially the ion current is constant, when the pipette is far away from the sample (Figure 3,  $d \gg 0$ ). When the pipette approaches the sample, the ion current decreases (Figure 3,  $d \rightarrow 0$ ), although the pipette does not contact the sample. As a rule of thumb, the ion current drops by approximately 1% when the pipette-sample distance is equal to the inner opening radius of the pipette ( $d \approx r_i$ ).

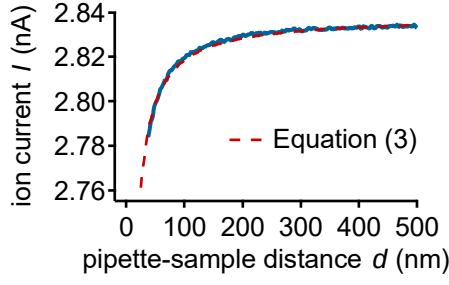


Figure 3. Ion current as a function of pipette-surface distance recorded using a nanopipette with inner opening radius  $r_i = 60$  nm. The distance-dependence of the ion current can be well described by the model using Equation (4).

To provide a quantitative description of the distance-dependence of the ion current, the geometry of the nanopipette tip has to be considered. SICM nanopipettes typically have a well-defined conical shape (Figure 4a), quantified by the inner and outer opening radii,  $r_i$  and  $r_o$ , respectively, and inner opening angle  $\alpha$ . When the pipette is in proximity to the sample, the ion current can be described by an equivalent circuit, which consists of a constant resistance  $R_p$  and a distance-dependent resistance  $R_d$ .<sup>7</sup>

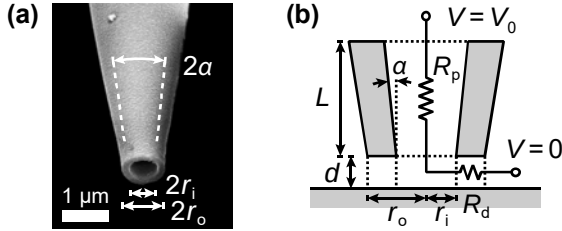


Figure 4. (a) Scanning electron microscope image and geometry of an SICM nanopipette tip with inner and outer opening radii,  $r_i$  and  $r_o$ , respectively, and inner opening angle  $\alpha$ . (b) Model of the tip geometry and equivalent circuit diagram, consisting of constant pipette resistance  $R_p$  and distance-dependent resistance of the tip-sample region  $R_d$ .

The constant resistance is mainly composed of the electrical resistance of the electrolyte in the pipette's inner space and is therefore denoted as "pipette resistance". For a conical, long ( $L \gg r_i$ ) pipette the pipette resistance follows<sup>8</sup>

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

where  $\sigma$  is the electric conductivity of the electrolyte, which mainly depends on the concentration and the type of the ions in the electrolyte. In this experiment, phosphate buffered saline (PBS) with a conductivity of about  $\sigma = 1.5$  S/m at room temperature is used. The distance-dependent resistance of the tip-sample region can be approximated by<sup>9</sup>

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

The total ion current through the pipette therefore is given by

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

where  $I_0 = I(d \rightarrow \infty)$  is the maximum ion current for the pipette far away from the sample

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

For  $d \rightarrow 0$  the ion current decreases to zero.

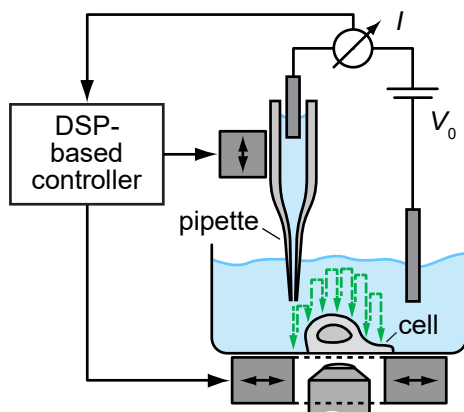
## 4. Imaging modes of the SICM

### 4.1. Constant current mode

As for other scanning probe microscopes, a variety of imaging modes exist for the SICM. The first setups were operated in the “constant current mode”, where the z-piezo is controlled by a feedback loop, which keeps the ion current and consequently the pipette-sample distance constant, while the pipette is laterally scanned over the sample.<sup>2,10</sup> Practically, this mode is limited to flat samples, because the pipette might get laterally into physical contact with the sample when scanning across steep slopes on the sample. Therefore, the most frequently used SICM imaging mode is the so-called “hopping mode”.<sup>18,19</sup>

### 4.2. Hopping mode

In the “hopping mode” the pipette is vertically approached to the sample until a predefined ion current drop threshold (“trigger” value) is reached, and the vertical position of the pipette there is stored. As a rule of thumb, for an relative ion current drop of about 1%, the pipette is in a distance of about  $d \approx 1r_i$  away from the sample surface.<sup>11</sup> Afterwards, the pipette is (quickly) retracted from the sample by a certain distance, moved laterally, and then approached again (Figure 5). By pixel-wise detecting the sample height, the topography of the sample is measured. Thereby, line by line a topography image of the sample is recorded.



**Figure 5. Schematic procedure of the SICM hopping mode, which enables one to image also samples with steep slopes, which frequently occur on biological samples such as cells. In hopping mode the pipette is pixel-wise vertically approached to the sample until a predefined ion current drop is reached. Then, the vertical pipette position is stored and the pipette retracted. Afterwards, the pipette is moved laterally and the next pixel is recorded.**

### 4.3. Imaging speed in hopping mode

In the experiment, contact between the nanopipette and the sample should generally be avoided. Therefore, the retract distance has to be chosen large enough so that the pipette does not contact the sample during lateral movement. On the other hand, the pixel rate is limited by performance of the z-piezo. After the ion current threshold is detected, the pipette continues to move downwards due to the finite response rate of the z-piezo, which is about 1 ms for the setup used here. Furthermore, the ion current threshold is detected at a distance of typically  $d \approx 1r_i$  away from the sample surface. Therefore, as a rule of thumb, the maximum approach velocity is approximately  $1r_i$  per 1 ms. For example, this gives  $100 \text{ nm/ms} = 100 \text{ } \mu\text{m/s}$  for a pipette with  $r_i = 100 \text{ nm}$ . If the pipette has to be retracted

and approached again by 10  $\mu\text{m}$  between the pixels, the maximum pixel rate would then be about 10 Hz.

## 5. Resolution of the SICM

For the resolution of the SICM, the vertical and the lateral direction have to be considered separately.

### 5.1. Vertical resolution

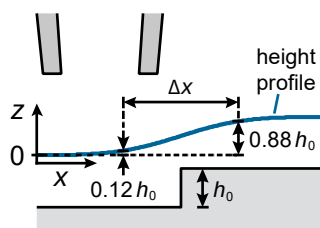
The vertical resolution (or, more precisely, the vertical sensitivity) depends on the imaging mode used. In the hopping mode, the detected sample height is directly affected by the instrumental noise of the ion current measurement. The ion current noise is limited by the Johnson-Nyquist noise in the resistive components, mainly the pipette resistance,<sup>12</sup> which is typically on the order of a few pA and can be estimated by

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

where  $k_B = 1.4 \cdot 10^{-23}$  J/K is the Boltzmann constant,  $T$  is the temperature, and  $\Delta f$  is the measurement bandwidth. The ion current noise is related to vertical resolution by the ion current vs. distance behavior. Thereby, choosing higher trigger values reduces the influence of the ion current noise on the detected sample height due to the non-linear distance behavior (see also Figure 8).

### 5.2. Lateral resolution

In contrast to the vertical resolution, the lateral resolution is mainly given by the pipette's opening size and caused by the pipette's limited lateral sensitivity when approaching objects sideways. Due to the complex geometry, the imaging process of the SICM has been investigated mainly numerically so far.<sup>13-15</sup> One possibility to quantify a microscope's lateral resolution is to measure the topography image of a sharp step. Due to the limited lateral resolution, the step will appear smeared out.<sup>13,14</sup> The lateral resolution of the SICM can then be determined by measuring the lateral distance, within which the height increases from 12% to 88% of the full step height (Figure 6).<sup>13</sup> From numerical considerations the fundamental limit of the lateral resolution is proposed to be about  $3r_i$ .<sup>13</sup>



**Figure 6. Schematic for the estimation of the lateral resolution of the SICM.** In the topography image of a sharp step of height  $h_0$ , the lateral distance  $\Delta x$ , where the height increases from  $0.12h_0$  to  $0.88h_0$ , can be used as a measure for the lateral resolution of the SICM.

### III. Experiment

#### 1. Laboratory day 1

The SICM used for the experiment is an expensive research microscope. The experimental work may therefore be started only after briefing by the instructor. If you are not sure, always ask the instructor first. In the experiment the following tasks should be performed:

1. Prepare replica of the calibration grating (TGX with 180 nm step height) using Sylgard 184 polydimethylsiloxane (PDMS). Please handle the expensive grating with care!
  - a. For that weight about 1 g of the base into a plastic petri dish and add curing agent in mass ratio of 10:1. Thoroughly mix base and curing agent with a spatula. Clean the spatula thoroughly with tissue paper and ethanol or isopropyl alcohol after usage.
  - b. Then place grating in a glass petri dish and carefully drop (about 0.1 g) of the mixture on the grating.
  - c. Degas the mixtures for 20 min in the desiccator to remove air bubbles. Cure the sample in the oven for 40 min at 80°C. Afterwards carefully peel off the PDMS from the grating.
2. Pipette fabrication (can be done while PDMS is in oven):
  - a. Fabricate about four pipettes each from program P38 and P36 (approximate opening diameter 100 nm and 500 nm, respectively) using the laser beam puller P-2000.
  - b. Use the stereo microscope to make photographs of the tip regions of one pipette from both programs and copy them for later analysis. At home determine the outer opening angle  $\alpha_o$  of the pipette (see Figure 7) and estimate the inner opening angle of the tip  $\alpha$  from the assumption that the ratio of inner to outer diameter of the capillary is conserved during pulling.<sup>16</sup>

$$\frac{\alpha}{\alpha_o} \cong \frac{r_i}{r_o} \cong \frac{R_i}{R_o} = 0.58 . \quad (7)$$

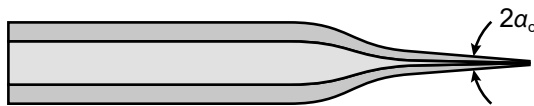


Figure 7. Determination of the outer pipette opening angle  $\alpha_o$  from the stereo microscope image.

3. Set up the SICM instrument and discuss the different components. Prepare the first measurement on the grating replica:
  - a. Mount the grating replica into a petri dish (if necessary, using vacuum grease) and fill the petri dish with about 2 ml of PBS.
  - b. Fill a P36 pipette with PBS and mount it into the SICM.
4. Lower the pipette until it immerses in the PBS and watch the ion current. Measure the ion current  $I_0$  and use Equation (5) in combination with your results for  $\alpha$  from 2.b to

estimate the pipette's inner opening radius  $r_i$  at home. Do not forget to note the applied bias voltage for that.

5. Record the spectral noise density of the ion current measurement. What can you read from the spectrum?
6. Approach the pipette to the sample surface and record an  $I$ - $Z$ -curve (down to about 1% ion current drop) and discuss the shape. Export the data for further analysis (for example as an Excel file). At home measure the measure the ion current noise  $\sigma_I$  (standard deviation) from the ion current for the pipette being far away from the sample surface (see Figure 8) and compare it with the theoretical noise limit from Equation (6) (you need the measurement bandwidth for that).

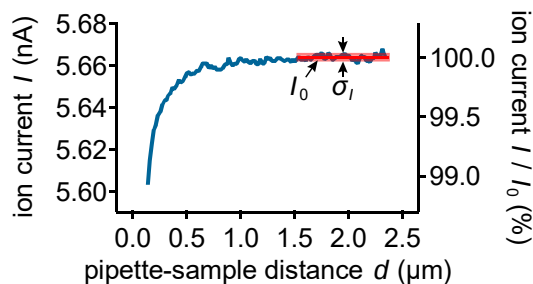


Figure 8. Exemplary  $I$ - $Z$ -curve for estimation of the ion current noise  $\sigma_I$  from the standard deviation of the ion current. Here the measured noise  $\sigma_I = 1.3$  pA is slightly above the expected noise limit of 1.1 pA from Equation (6) for a bandwidth of  $\Delta f = 2.5$  kHz and a pipette resistance of  $R_p = V_0/I_0 = 35$  M $\Omega$ .

7. Record three topography images on the same location of the grating replica in hopping mode using different current drop thresholds or “trigger” values (e.g. 1.5%, 1.0%, and 0.5%).
  - a. Discuss the visual differences between the images.
  - b. Choose one of the images and use the recording software to display a height profile along a topography step and export it for analysis at home (for example as an Excel file).
  - c. At home, use the height profile to measure the height noise  $\sigma_z$  (standard deviation of the height on a flat region).
  - d. Also record a height profiles to measure the lateral resolution (see section II.5.2) according to Figure 6. Compare the values for the different thresholds with the theoretical limit of  $3r_i$  (use your own result for  $r_i$  from above).
8. Repeat steps 4 to 7 with a smaller P38 pipette and compare the results with those from the P36 pipette measurement.
9. If the remaining time allows for it, record a SICM topography image of a fixed cell. A petri dish with fixed fibroblasts in PBS solution will be provided by the instructor.

## 2. Laboratory day 2 (for the block course only)

On the second experiment day set up the SICM and perform the following tasks:

1. Decane contact angle measurement:

- a. Prepare a sample of decane drops. Dip a capillary into the decane, spread a small amount on the bottom of a plastic petri dish, and flush it with about 2 ml PBS. Use gloves and avoid contact with the decane.
- b. Approach a P38 pipette. Then retract the piezo, adjust the stage that the pipette is above a smaller decane drop and record a hopping mode topography image.
- c. Use the recording software to generate a height profile centrally across the drop and export the profiles (for example as an Excel file).
- d. At home, use the Young-Laplace equation to calculate the internal pressure in the drop  $\Delta p = 2\gamma/R$ , where  $\gamma$  is the surface tension and  $R$  is the radius of curvature. Use  $\gamma = 51.2 \text{ mN/m}$  for the surface tension of the water/decane interface<sup>17</sup> and measure  $R$  from the height profile (see Figure 9). Compare the result with pressure values from everyday life.

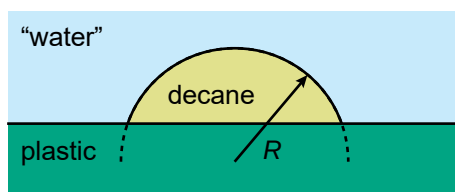


Figure 9. Determination of the radius of curvature  $R$  of the decane drop.

2. SICM topography image of a fixed cell and correlation with optical phase contrast microscopy:
  - a. A petri dish with fixed cells (e.g. fibroblasts) will be provided by the instructor. Replace the solution with about 2 ml PBS and mount the petri dish into the SICM.
  - b. Approach a P38 pipette and record topography images of a single cell. Record at least one overview image, where the whole cell is visible, one on the cell body, and one on a protrusion. For each of them record a low-resolution pre-scan to adjust the retract distance as small as possible.
  - c. Remove the petri dish from the SICM and transfer it to the optical microscope setup. Be careful not to rotate the sample (would make it complicate to find the cell again).
  - d. Use a low magnification objective to find the same cell again. Change to a higher magnification objective and record a conventional image and a phase contrast image for comparison.
  - e. At home, relate the optical and SICM images and mark the SICM scan areas in the optical image.
3. SICM topography image of live cells:
  - a. A petri dish with live cells (e.g. A6 epithelial cells) will be provided by the instructor. Replace the solution with about 2 ml of L15 medium and mount the petri dish into the SICM.

- b. Depending on the current cell density, single cells or a confluent monolayer will be present. Approach a P38 pipette and record a series of several topography images. Describe the dynamical processes observed?

### 3. Protocol

The protocol should give a brief description of the SICM. The major part of the protocol should be the presentation of the results obtained during the experiment and their detailed discussion and interpretation. The protocol must contain a signed declaration that the elaboration of the report was performed by you and your group (see below).

Name: .....

Matriculation number: .....

I hereby declare that my elaboration (or that of my group) was made independently and is not a copy (even partially) of an existing protocol or of the instruction manual. All external sources (also the instruction manual) have to be cited. I am aware that a violation of this rules is plagiarism and leads to the experiment being graded as failed (grade 5) and can result in an exclusion from the labwork.

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Date

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Signature

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