Construction and Usage of a Scanning Ion Conductance Microscope

Project thesis "**Interaction with surfaces**" (134.114) by
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Contents

Introduction

Scanning ion conductance microscopy [1] is a form of scanning probe microscopy. A physical probe is moved over the surface using a piezo actuator and a certain ion current is measured. In the case of the SICM the probe is generally a micropipette made from glass with a chlorinated silver wire inside. By decreasing the distance between the tip of the pipette and the surface the ion current which flows through the electrolyte becomes smaller. The main goal of this project thesis was to get the function which sets the ion current and the distance to the surface into relation. Since one can get all three variables of position through the piezo controller it is possible to reconstruct the texture of the surface.
The SICM's working principle and the theoretical model it is based on are described in detail in [2].
Even though the principle is simple, getting results is tricky. The gadgets, software and precautions which have to be taken, will be explained in the next few chapters.

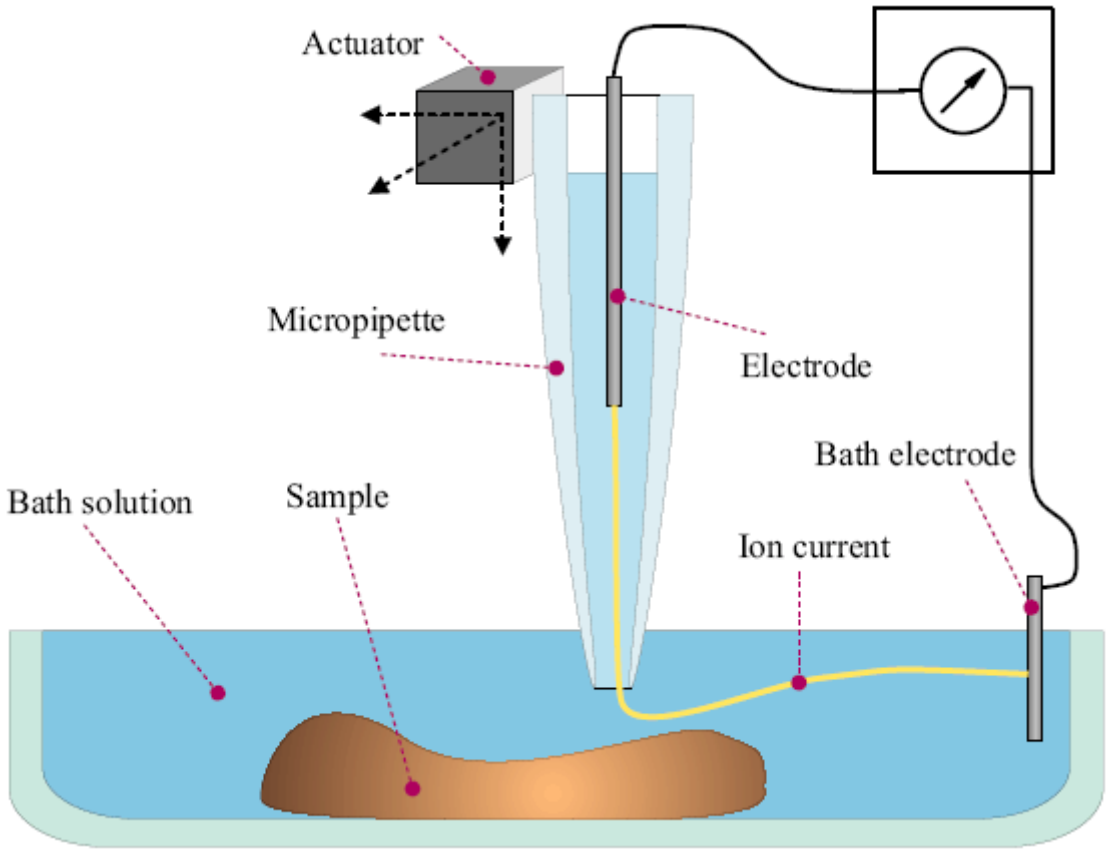

Figure 1a: Principle of the SICM

The Scanning Ion-Conductance Microscope

P. K. Hansma, B. Drake, O. Marti,* S. A. C. Gould, C. B. Prater

A scanning ion-conductance microscope (SICM) has been developed that can image the topography of nonconducting surfaces that are covered with electrolytes. The probe of the SICM is an electrolyte-filled micropipette. The flow of ions through the opening of the pipette is blocked at short distances between the probe and the surface, thus limiting the ion conductance. A feedback mechanism can be used to maintain a given conductance and in turn determine the distance to the surface. The SICM can also sample and image the local ion currents above the surfaces. To illustrate its potential for imaging ion currents through channels in membranes, a topographic image of a membrane filter with 0.80-micrometer pores and an image of the ion currents flowing through such pores are presented.

THE FAMILY OF SCANNING PROBE microscopes (*1–4*) is broadening the frontiers of surface imaging. These microscopes scan various sharp probes over samples to obtain surface contours—in some cases at the atomic scale (*2*). We report results from the SICM. It is designed specifically for biology and electrophysiology in that it can image soft nonconductors (such as membranes) that are covered with an electrolyte.

A schematic view of the SICM is shown in Fig. 1. A micropipette is filled with electrolyte and lowered through a reservoir of electrolyte toward an insulating sample surface while the conductance between an electrode inside the micropipette and an electrode in the reservoir is monitored. As the tip of the micropipette approaches the surface, the ion conductance decreases because the space through which ions can flow is decreased. The micropipette is then scanned laterally over the surface while a feedback system raises and lowers the micropipette to keep the conductance constant. The path of the tip follows the topography of the surface.

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Preliminary experiments were performed in our lab in 1986 by J. Saad and G. Tarleton (*5*). They were able to measure the topography of machined plastic pieces using an eyedropper as a probe. These experiments were not pursued further because the scan ranges then available with our microscopes (*6*) were not much larger than the openings in available micropipettes.

However, x,y,z piezoelectric translators with larger scan ranges are now available, and microscope design has evolved enough that experimentation with various scanning probes is relatively easy. The pipette of the SICM is brought near the surface with two fine screws that are adjusted by hand while the separation is monitored with an optical microscope (*7*). A third fine screw that is driven with a stepper motor (*7*) brings the pipette within range of a single-tube x,y,z piezoelectric translator (*8*). This translator has a 9.3-μm lateral range and a 3.0-μm vertical range (*9*).

The micropipettes for early experiments were made from 1.5-mm outer diameter (OD), 0.75-mm inner diameter (ID) Omega Dot (*10*) capillary tubing. Later versions were made with similar tubing (*11*) on a Brown-Flaming (*12*) puller. Although these pipettes are designed for measurement of intracellular potentials and patch clamping, similar micropipettes have been used in other scanning probe microscopes, namely the near-field scanning optical microscope and the micropipette molecule microscope (*3*). We estimated our micropipette tip diameters with a nondestructive bubble pressure method developed by Mittman *et al.* (*13*).

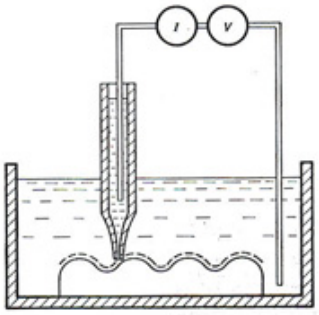

Fig. 1. The SICM scans a micropipette over the contours of a surface by keeping the electrical conductance through the tip of the micropipette constant by adjusting the vertical height of the probe.

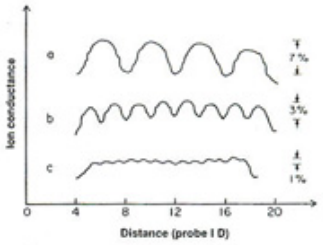

Fig. 2. Resolution test for the SICM. A pipette with an ID of 0.71 mm and an OD of 1.00 mm was scanned at constant height over three grooved plastic blocks with spacing of (a) four times, (b) two times, and (c) the same as the ID of the pipette. A 0.1*M* NaCl solution covered the blocks and filled the pipette. Note that even the grooves spaced by the ID of the pipette could be resolved.

Figure 1b: First paper that introduces the concept of the SICM

Motivation

The collaboration with Univ. Ass. Dipl.-Ing. Dr. techn. Ille Gebeshuber started in the lecture "Introduction to Nanotechnology and Nanoanalytics" in the winter term 2006/2007. In this lecture we heard that she was supervising a few biophysical diploma and project theses and since we also wanted to make a project thesis with a biophysical topic, we contacted her. So we had a meeting at the end of February with the conclusion that the job of our first project thesis was to rebuild the SICM which had previously been constructed by Stefan Schraml in his diploma thesis in 2003 ([2]).
The target of this project was to facilitate measurements with the SICM at the IAP (Institut für Allgemeine Physik). There are only a few SICMs worldwide and they are mainly used to measure the texture of the surface of biological samples, such as erythrocytes.
The main reason for choosing this topic for our first project thesis was that it dealt with information from a field of research which was totally new to all of us.
We especially hoped that through the technical developments and progresses made during the last few years we could improve the SICM of Stefan Schraml and make faster and more accurate measurements.

Components of the SICM

The working place of the SICM is divided in two parts. One part includes the measurement area which is situated on the table which is mechanically stabilized by a heavy stone. The other part includes the instruments used for performing the measurements and the PC. All the cables which connect both parts should be fixed on the stabilized table to prevent vibrations, which could disturb the measurements.
The figures below show the basic assembly of the SICM.
The manuals of almost all instruments are either saved on the PC as a pdf-file or a printed version is available.

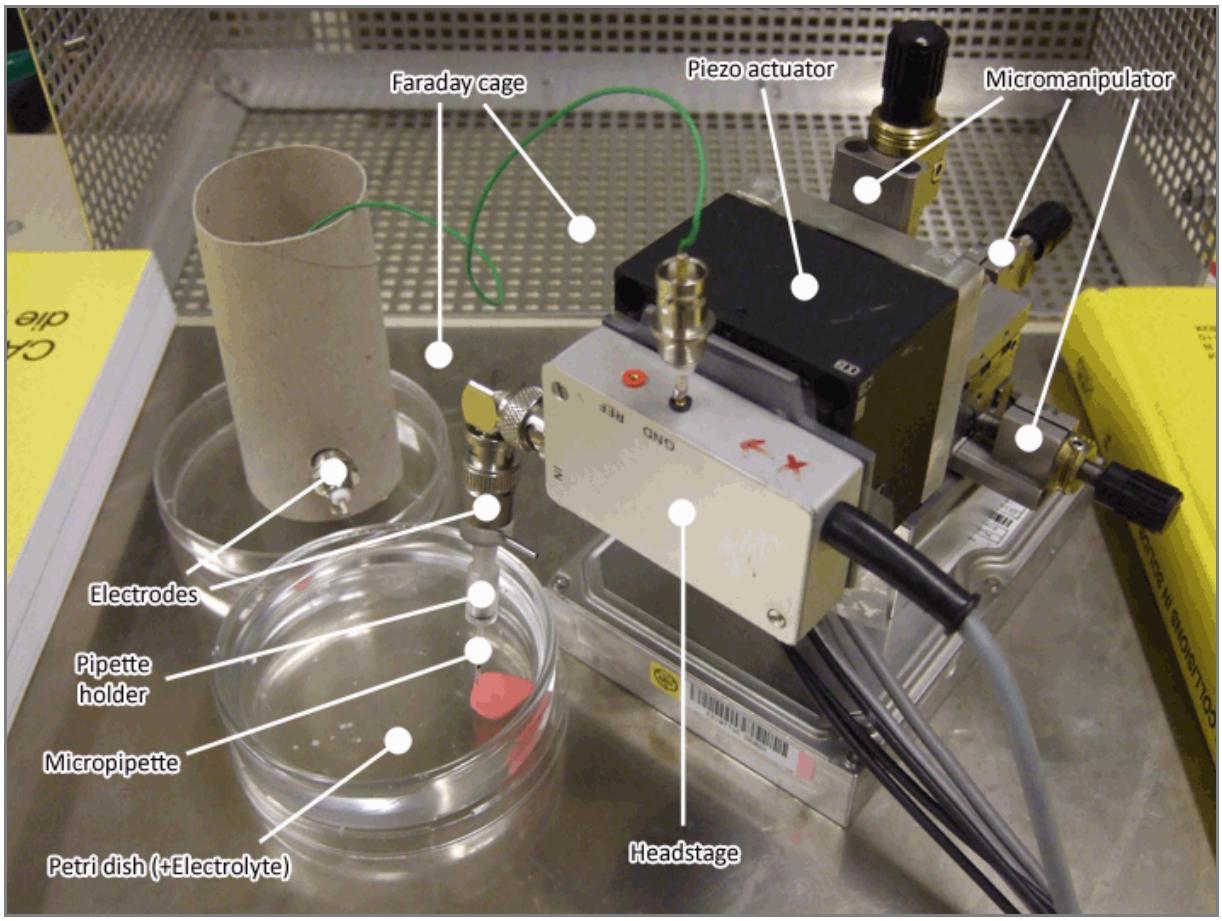

Figure 3a: Measurement area

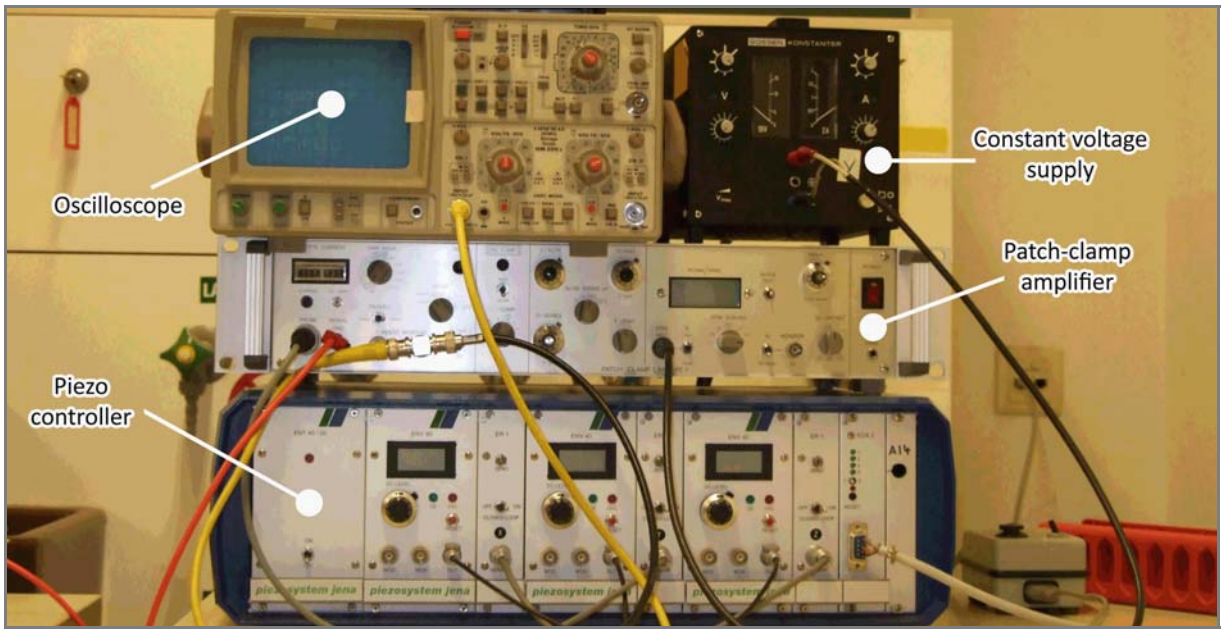

Figure 3b: Instruments

Micropipette

The micropipettes are produced out of glass capillaries (inner diameter: 0.75 mm, outer diameter: 1.5 mm, length: 75 mm) using a pipette puller. Their characteristic properties are the tip length, the tip opening diameter and the wall thickness. Another important feature is the glass type which influences the pulling procedure and the measurements. In our experiments we used borosilicate glass which is the best solution in our case. One can use also other glass types like soft or quartz glass but for example soft glass is toxic to cells.

We got our micropipettes from Dr. Jürg Graf from the AKH Wien ([8]) and there were still pipettes from Stefan Schraml available. Since we didn't have a pipette puller we couldn't get experiences in the pulling procedure but there is for example a description of it in [2]. Of course it's best to use newly pulled pipettes. Otherwise the storage of the pipettes should be dust-free in a dissicater and with a small amount of silica gel to prevent clogged tips.

For filling the pipettes with the electrolyte one can use a small plastic pipette with an outer diameter smaller than 0.75mm. The electrolyte we used was a saline solution with a NaCl-concentration of 150 mmol/l.

Pipette holder

The pipette holder consists of Teflon and polycarbonate with a small pipe to control the pressure inside, which is necessary for other measurements with the patch-clamp amplifier. It has an inner diameter of 1.5 mm and is fixed on a fitting piece which connects the front part with a BNC plug. In this part the metal stick with the soldered electrode has to be plugged into a metal tube with an inner diameter of about 1 mm. The holder is connected to the probe via the BNC plug and must be shielded from external electric fields with a Faraday cage.

Further information about the pipette holder is available in [4] and [7].

Headstage (Probe)

The headstage or probe is mounted on the piezo actuator which is fixed to the micromanipulator. Since the metal casing of the headstage carries the ground signal it must be insulated from the piezo actuator. Also the GND (=short for 'ground') pin jack carries a high quality ground signal and so it is used for the bath electrode. The assembly of the SICM is unusual compared to other scanning probe microscopes because the probe moves during the scan and not the sample but the advantage of this solution is that one can look at the sample during the scan with the optical microscope. It is very important to be insulated from the ground if one touches the headstage because otherwise it can be damaged by static electricity. The headstage is connected to the patch-clamp amplifier with one cable.

Electrodes

As electrodes we used silver wires with a diameter of 0.1 mm since they must be smaller as the inner diameter of the micropipette. One needs electrodes of second order, so they must be covered for example with a chlorine layer. Second order means that the potential of the electrodes is only indirectly dependent on the concentration of the surrounding electrolyte. An easy way to produce them is to put silver wires in Danchlor for about 12 hours. In the diploma thesis of Stefan Schraml there are also other ways mentioned to get useable electrodes, but the way we did it is probably the fastest and most comfortable one. For an exact measurement both electrodes should be produced using the same procedure because they should have the same properties and potential. The chlorinated silver wires have a dark-grey colour and should be renewed every month. The ground line electrode is soldered to a BNC plug and connected via a cable with the GND port of the headstage. The other one is soldered on a metal stick which is plugged into the pipette holder and thread inside the micropipette which is filled with the bath solution. The electrode should reach to the top of the micropipette and one has to handle the electrodes carefully because of the porosity caused by the chlorinating process.

Piezo actuator

It is mounted on the micromanipulator and controlled via the piezo controller. For horizontal moves one must use the x- and z-axis and for the height the y-axis. The maximum range in every direction is 100 µm and in the closed loop mode it's 80 µm. In our experiments we used the closed loop mode because in this mode the hysteresis and the drifting are controlled by the piezo controller.

Micromanipulator

The micromanipulator Newport M-461-XYZ is the only part which cannot be controlled via the PC. It's made of steel and used for the approximate positioning of the pipette near the sample area. A full turn with a screw leads to a displacement of 240 µm and the maximum range is 13 mm in every direction.

Piezo controller

The piezo controller is produced by Piezosystems Jena and the front panel is divided in five parts. The panel on the left side has the on/off switch. The right one (EDA2 = digital-analog- and analog-digital-converter) has ports which allow an external control of the whole instrument. We used the RS232 serial port for controlling the piezo system via the pc. Each of the panels in the middle (ENV = voltage

amplifier) is responsible for the movement into a specific direction, namely along the x-, y- or z-axis. Every panel has a display, which shows the current position, ports for the switching of the piezo actuator and a screw to turn the piezo actuator manually.
For further information consider [3] and [5].

Patch-clamp amplifier

The model EPC7 we used is supplied by HEKA and the front panel is divided in five parts. The whole device consists of the patch clamp amplifier and the pre-amplifier called probe or headstage, which was described above. Its function is to amplify the signal coming from the headstage and to output it as a voltage. Through several bandpass and other filters it also improves the signal's quality by decreasing its noise. The best way to understand the function of the patch-clamp amplifier is to read the user manual because it is impossible to describe it in a short and easy way.
For our measurements we used the Search-Mode with an input voltage of two volts. The Search- and Voltage-Clamp Mode measure the current with a constant voltage and so they are ideal for our purpose.
For further information take a look at [4] and [6].

Oscilloscope

It is necessary to monitor the output voltage of the patch-clamp amplifier before starting and during the measurement because a voltage overflow can damage the microcontroller.

Microcontroller

The microcontroller is an analogue/digital converter and it is used to transform the analogue signals from the patch-clamp amplifier to a digital signal for the data acquisition performed with the PC. The output of the patch-clamp amplifier is connected via a cable to the 0-pin of the micromanipulator and the PC via a USB cable.

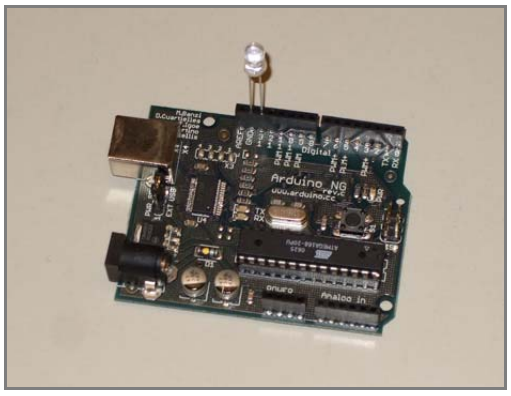

Figure 3c: Microcontroller

Constant voltage supply

The constant voltage supply is used for setting the input voltage of the patch-clamp amplifier. We used a constant voltage of two volts.

Faraday cage

The Faraday cage is used for reducing the influence of external electric fields on the measurement. Without it it is almost impossible to get usable results.

Inverted microscope

With this microscope and the micromanipulator one can approach the tip of the micropipette to the target area. It can also be used to check if the tip of the micropipette is useable or not.
In the future the microscope will be used as the base for all devices which are currently placed in the measurement area and for this it must be insulated and the working plate must be enlarged.

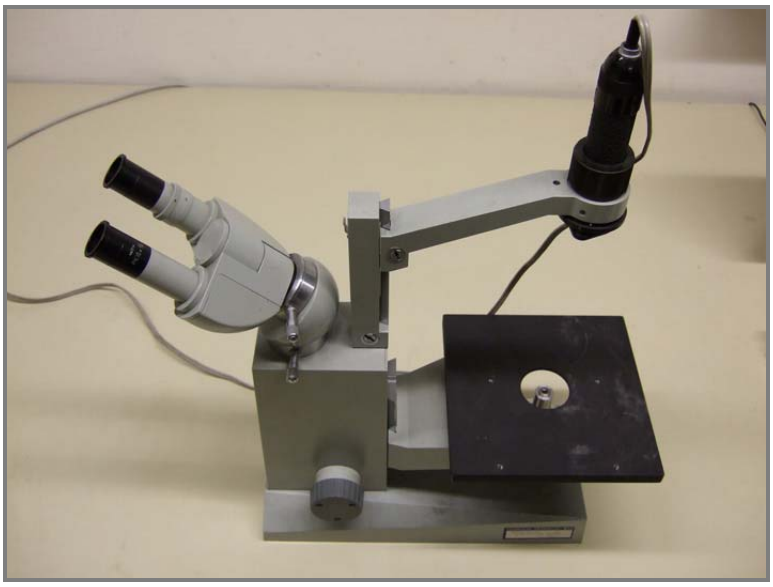

Figure 3d: Inverted microscope

Personal computer

Via the LabView-code the whole measurement is controlled with the PC and all the information used for the analysis is saved. So the PC has to be connected to the piezo controller via the serial port and to the microcontroller via USB.
The diagrams of our measurements were created with Microsoft Excel.

Heavy stone and its frame

The heavy stone is used for vibration damping which is essential for high precision measurements, since the tip of the pipette is only micrometers above the surface. A draft of the frame that carries the heavy stone is shown in Fig. (3e).
We used a gravestone, which we got from [9] and the frame was constructed at the laboratory of the TU Vienna.

Frame

Dimensions in mm
Scale of 1:10

Used Profiles:

- Profile : 50 x 50 x 5 mm
- Profile : 50 x 50 x 5 mm

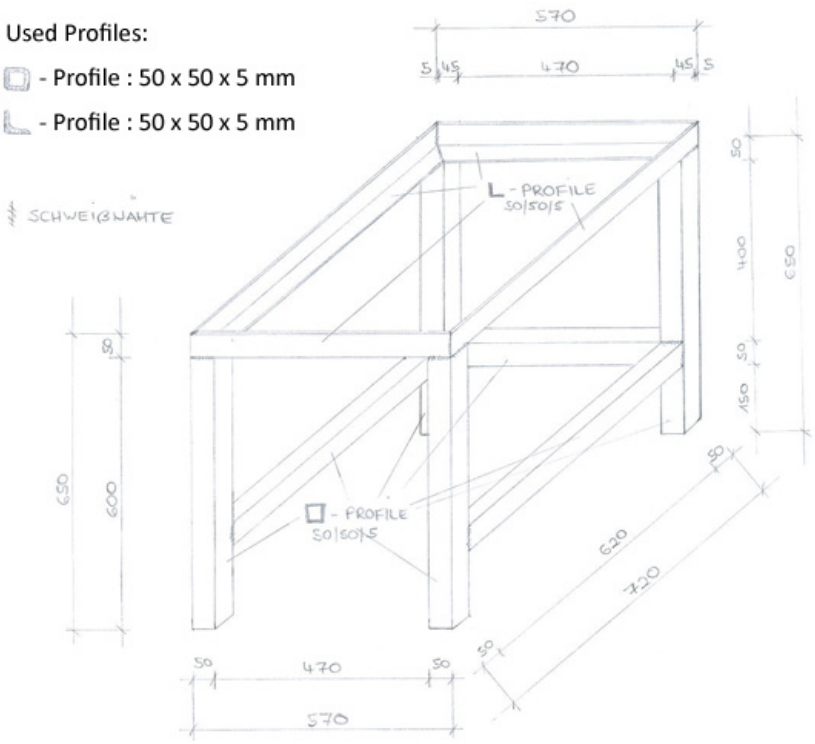

Figure 3e: Frame for heavy stone

Controlling the SICM with LabView

Almost the whole SICM, despite the micromanipulator, can be controlled via a PC, which has LabView installed.
For the input of the current that flows through the electrolyte we use a microcontroller which is connected to the PC over USB. Its job is to convert the analog signal obtained from the patch-clamp amplifier into a digital signal. With the LabView-code one can communicate with the microcontroller over the USB port like over a standard serial port. It is of course possible to directly input the analog signal for example through the PC's soundcard, but since also the piezo system is controlled over a serial port, the LabView-program becomes simple because one has to use only one type of I/O component. First we also tried to input the analog signal through the soundcard, but during the project thesis we started using the microcontroller because its resolution is in our case far better. That does not mean that by using the microcontroller we increased the signals resolution, but it hints to the fact that our PC's soundcard does not meet the requirements as far as the accuracy and the resolution of the signal are concerned.
Since the amplitude of the signal shows an exponential dependence of the distance between the patch-clamp-pipette and the sample one wants to probe, it is used for determining the texture of the surface. While the LabView-program moves the piezo actuator and thereby the pipette towards the surface, all necessary information, in this case amplitude, time and vertical piezo position, are written down in a textfile which is then saved to the computer's hard disk. If the signal amplitude decreases and becomes smaller than a certain minimal amplitude, which can be set in the LabView-interface, the program stops moving the piezo actuator and quits outputting the information to the textfile. The obtained textfile can be opened with Excel and by applying a macro we wrote one can directly view a diagram which shows the exponential decrease of the amplitude caused by the approach of the pipette to the surface.

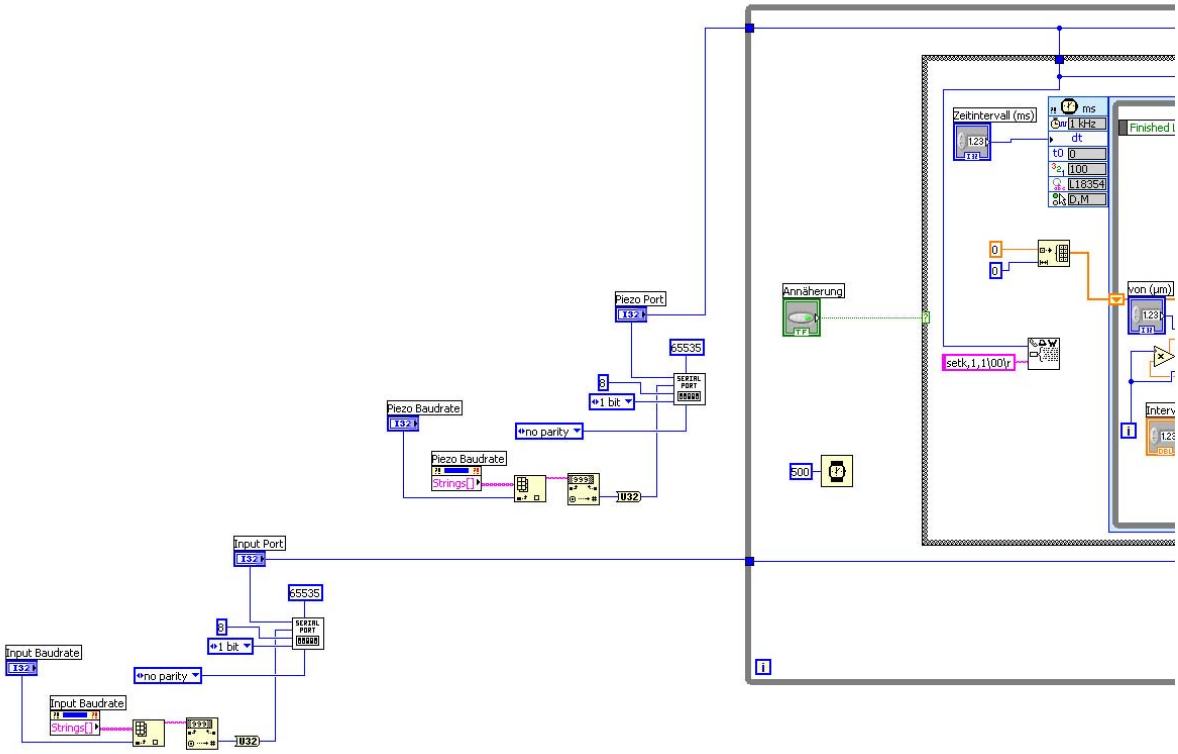

Figure 4a: Input and Output of signals in the LabView-code

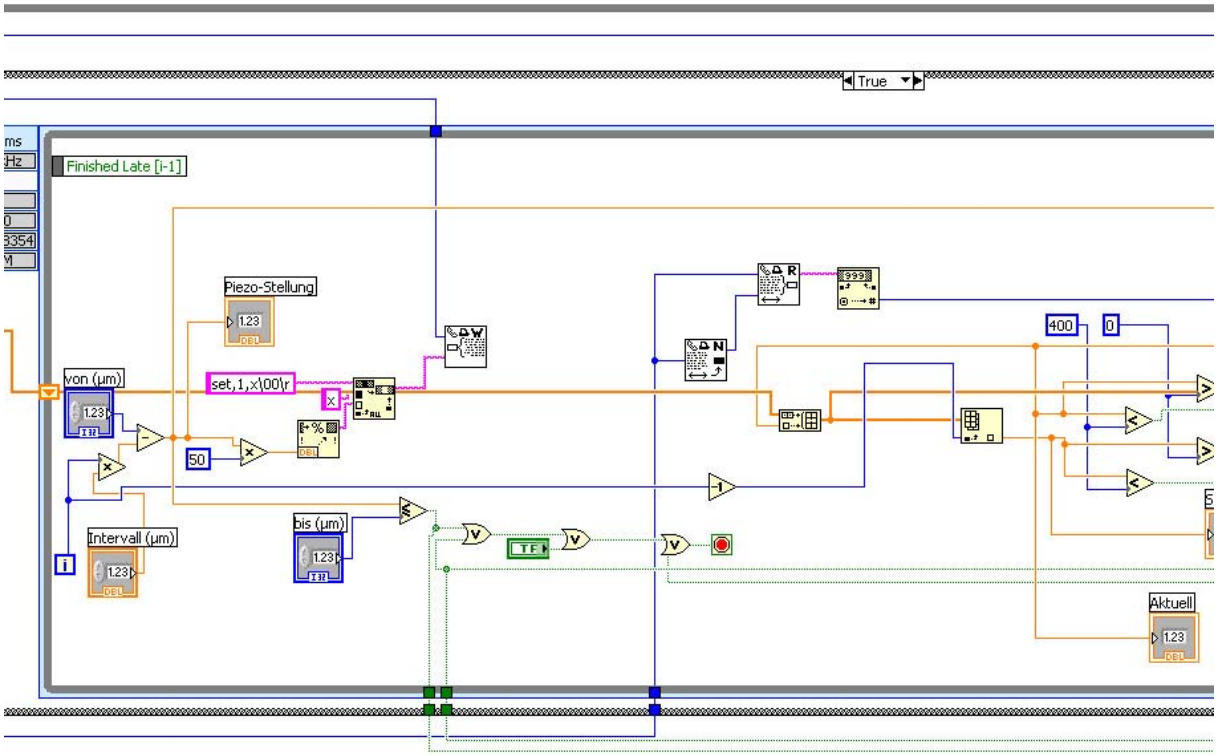

Figure 4b: Minimal-Amplitude-Check for the inputted signal

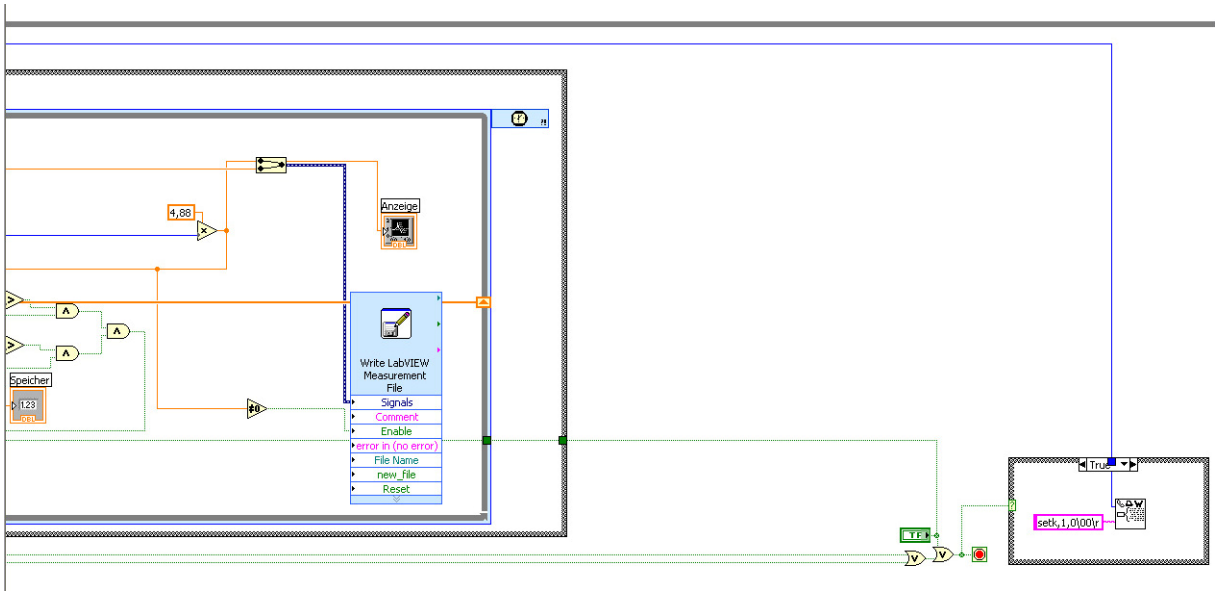

Figure 4c: Storage of obtained information and code for resetting the piezo to its starting position

The Interface

In Fig. (4d) a screenshot of the LabView-interface (LVI) is shown.

The default value of the ports (1,3) and baudrates (2,4) should not be changed. Especially the input baudrate of 115200 should not be decreased because that would lead to a lack of information.

The values of 'from (µm)' (5) respectively 'to (µm)' (6) specify the highest respectively lowest position of the piezo.

By changing 'Interval (µm)' (7) one can set the width of the vertical piezo steps in µm and the field 'Time Interval (ms)' (8) specifies the period of time that elapses between two piezo movements. The time interval should not be to small (<100ms) because this could lead to the problem that the piezo moves faster downwards than the signal from the patch-clamp amplifier arrives at the PC. That can cause a demolition of the pipette because when the LabView-program recognizes that the amplitude is below its minimal value, the piezo has already moved a few steps down and has probably penetrated into the sample one wanted to probe.

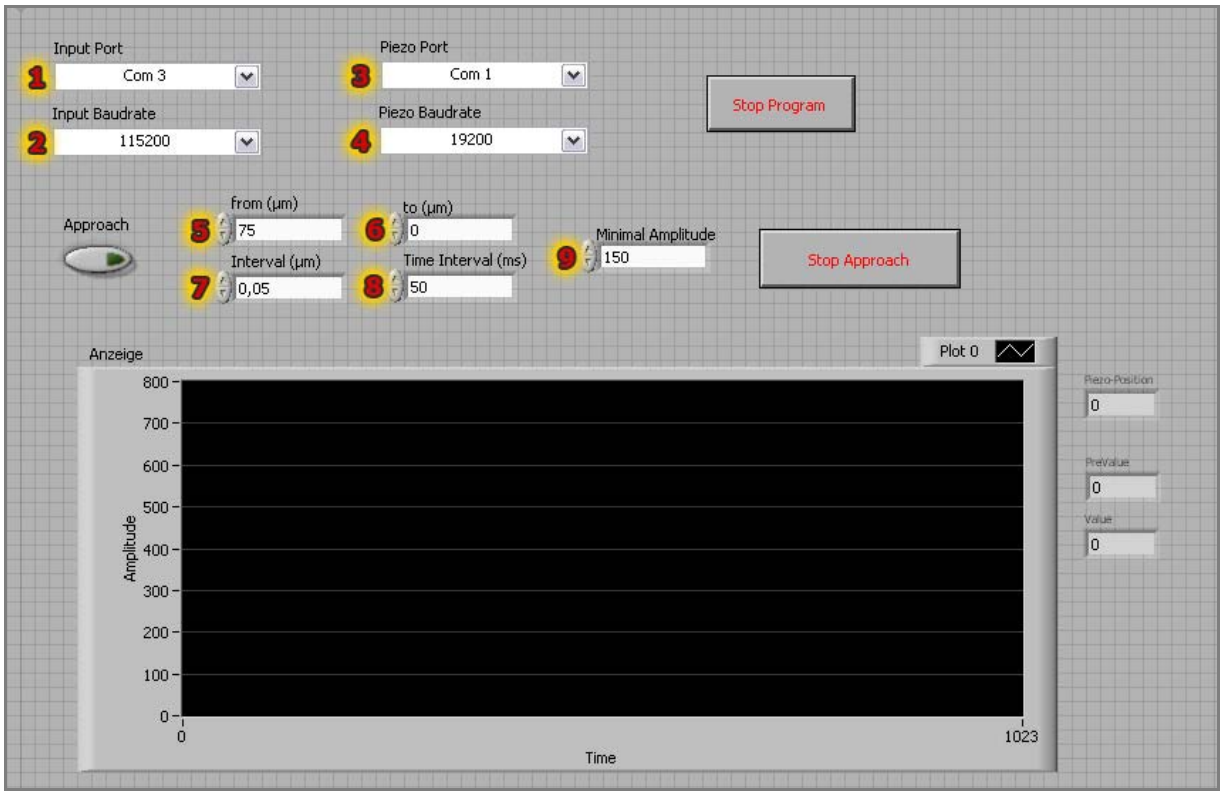

Figure 4d: Screenshot of the LabView-interface

The amplitude is given in arbitrary units because it is inputted in this way through the microcontroller. To calculate the amplitude in volts, one just has to multiply the signal with a certain factor, which need not necessarily be known since in our case only the relative decrease of the signal is important. The 'Minimal Amplitude' (9) determines the amplitude of the signal (in a.u.) below which the movement of the piezo has to be stopped.

Performing an experiment with the SICM

After all the instruments are turned on and connected to each other as described in the chapters before, one can start performing an experiment.
At first one has to take a glass pipette and look at it with the microscope. If the tip of the pipette is not damaged and its inside is not constipated through any impurity, it can be filled with electrolyte. Make sure that the pipette, especially the tip, is continuously filled with the electrolyte and that there are no air bubbles included because that would lead to incorrect results of the measurements. If everything is fine up to now, one can clamp the pipette at the pipette holder. The silver wire should reach as far to the tip as possible, of course without damaging or constipating it.
Before plugging the pipette holder to the headstage one has to be sure that the patch-clamp amplifier and the microcontroller are not connected to each other, because a too high signal from the amplifier can harm or in worst case destruct the microcontroller and the PC. If they are disconnected one can plug the pipette holder to the headstage and dip the pipette into the electrolyte which the sample containing vessel is filled with. Then one has to bring the second electrode into the electrolyte, which is already connected with the GND port of the headstage. The current flow that should occur now ensures that the pipette is in good condition. One can check the amplitude of the signal with the oscilloscope and by changing either VHOLD or the inputted stimulation signal the amplitude can be set to a specific starting level, which should in every case be below 5 volt if one doesn't want to harm the microcontroller as we mentioned before.
If one took care of all critical points discussed, the patch-clamp amplifier can be connected to the microcontroller, which should already be linked to the PC.
Now one can open the LabView-file 'Piezo-Steuerung.vi'. To start the measurement, run the program and turn the approach button on. Now the pipette will move towards the surface depending on how the configurations are set in the LVI. If the amplitude did not decrease below the minimal amplitude during the whole approach, the distance between the tip and the surface is obviously too large. That means that after the program has stopped, one has to lower the pipette with the micromanipulator. Since a rotation of 360° leads to vertical displacement of 240 µm, one has to be careful when handling the micromanipulator's rotary knob, because the pipette should not move down more than the difference between the starting and the final position of the piezo in µm since that could lead to the problem that it penetrates into the sample and gets damaged which would have the effect that one would have to redo all instructions discussed in this chapter. From our own personal experience we can say that when approaching to the surface with the micromanipulator, patience is worth a mint.

Results of the measurements

As the figures show, the measured decrease of the amplitude is the same as expected from theory, namely an exponential one. The steep descent of the current starts when the pipette is about 1 µm above the surface and that is what complicates fast measurements, since a too fast approach to the surface can lead to a demolition of the pipette caused by penetration.
The figures below show approaching curves with different pipettes (new and old ones) and different approaching speeds. In our measurement the results with the different pipettes were the same.

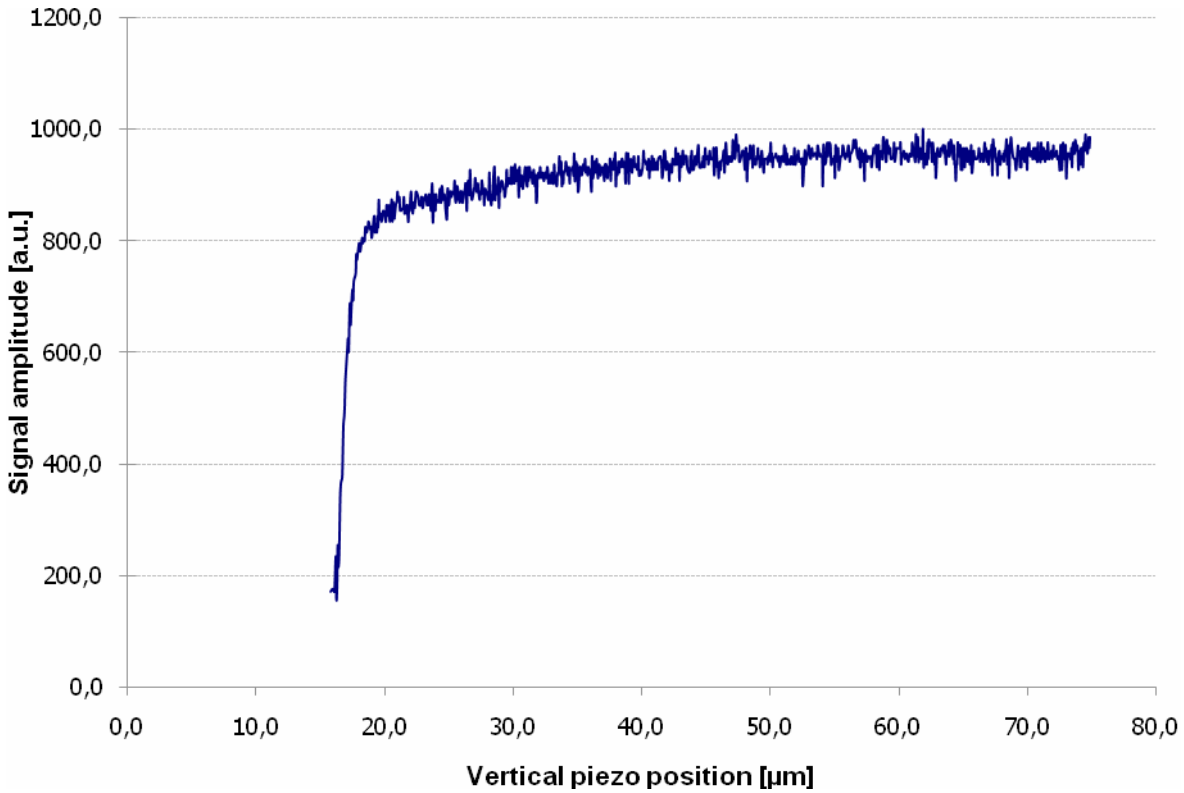

Figure 6a: Fast approach – Time interval: 100 ms – Interval: 0,05 µm

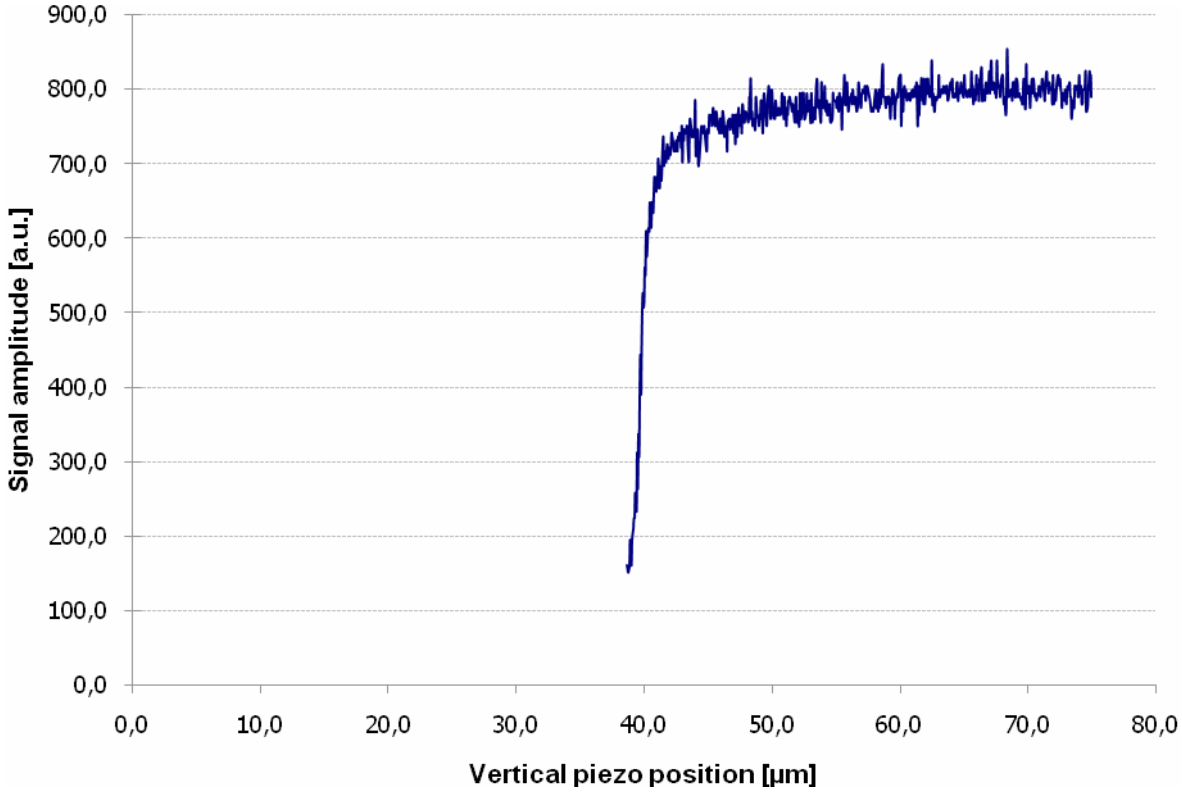

Figure 6b: Fast approach – Time interval: 100 ms – Interval: 0,05 µm

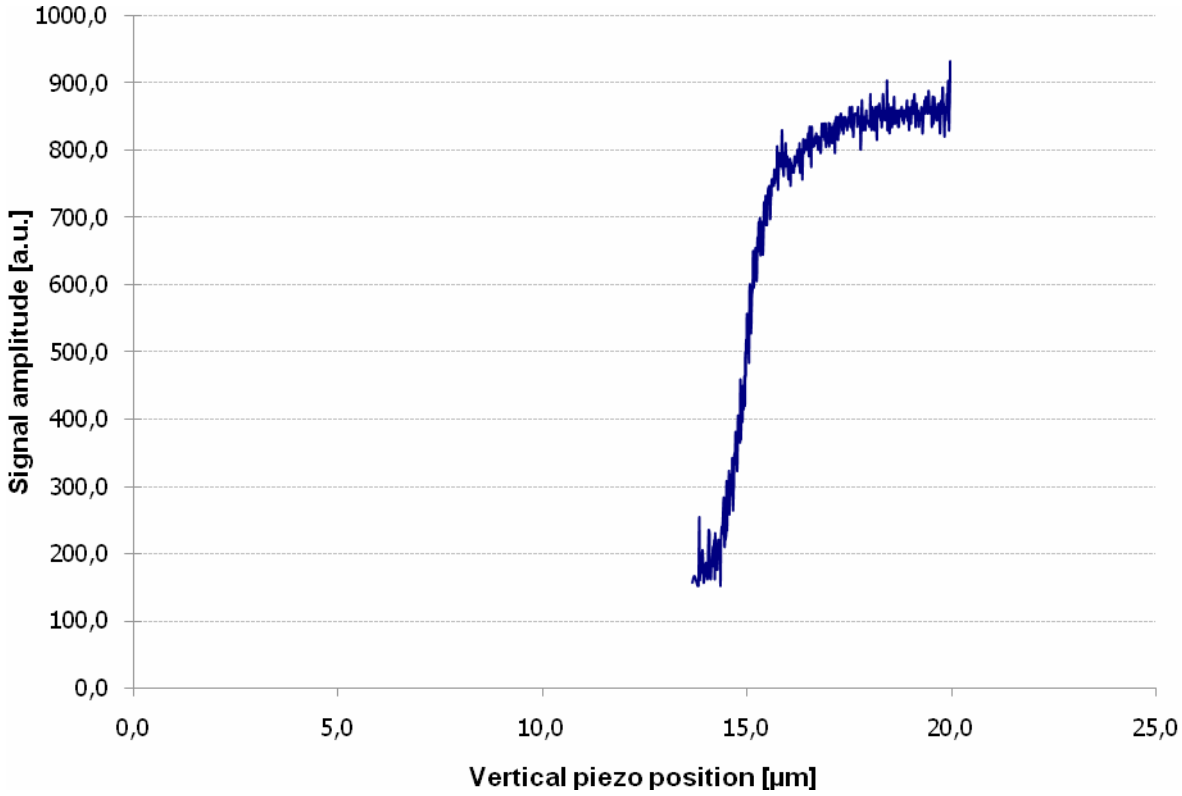

Figure 6c: Slow approach – Time interval: 200 ms – Interval: 0,05 µm

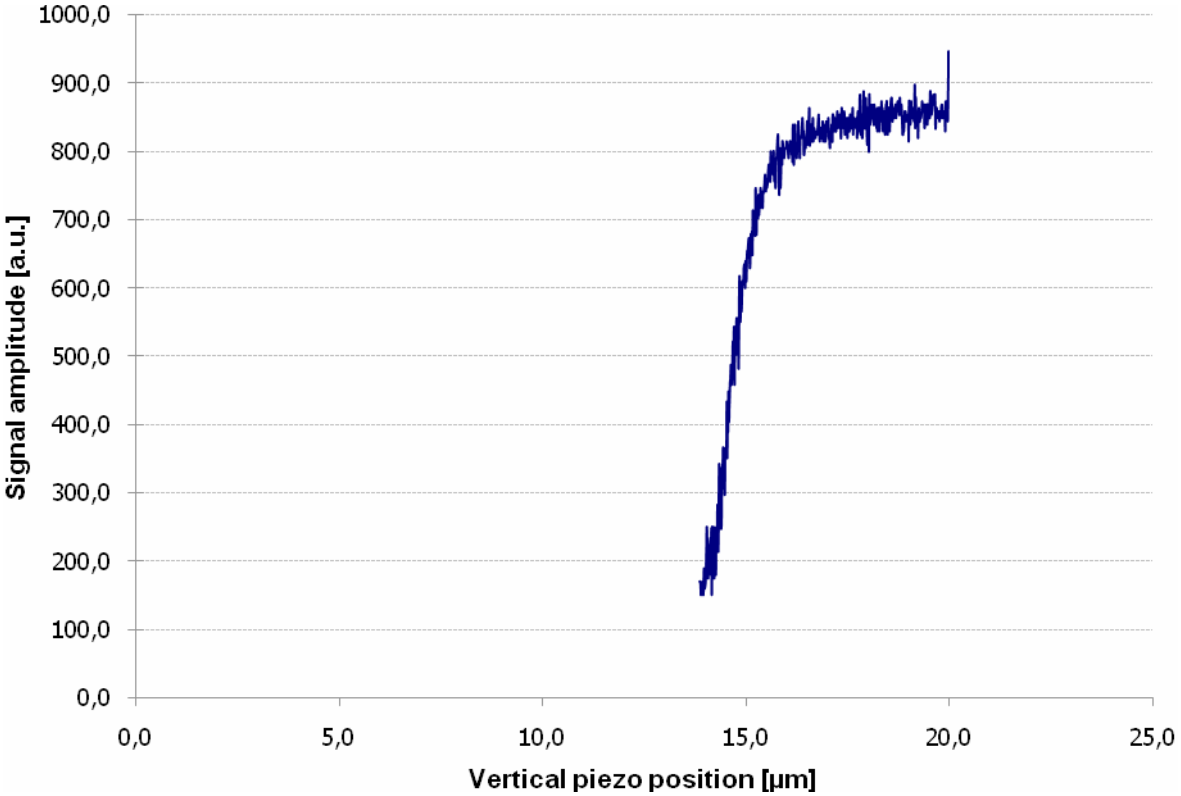

Figure 6d: Slow approach – Time interval: 200 ms – Interval: 0,05 µm

Proposals and Outlook

For further studies, especially for raster scans, which were not performed during this project thesis, speed and accuracy should be improved. Therefore better equipment is needed. Here a list of recommendations to improve the SICM.

Microscope: An inverted microscope large enough to mount all the devices from the measurement area on it should be obtained. This is maybe the most important equipment needed, because without it one is unable to place the micropipette near enough to the surface.

Pipettes: A puller to produce pipettes should be purchased.

Micromanipulator: For performing raster scans in the future it is absolutely necessary to replace the micromanipulator by a computer-controlled positioning device, like for example a piezo actuator with a higher range than the one currently used. The advantage of such a device is that also the rough approaching process can be controlled with the PC and in this way the demolition risk for the pipettes gets minimized since approaching them to the surface is pretty critical when using the micromanipulator.

LabView: Since we only measured approaching curves at certain positions, the LabView-program we wrote is just useable for this kind of scan, but not for performing raster scans. So everyone who goes on with this work has to write the needed subroutines.

References and contacts

[1] The Scanning Ion-Conductance Microscope – P.K. Hansma, B. Drake, O. Marti, S.A.C. Gould, C.B. Prater – Science Vol. 24, p. 641, February 1989

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[6] http://schraml.stikipad.com/attachment/asset/19212/m_epc8+7.pdf

[7] http://www.instrutech.com/lit%5Cepcholder_lit.pdf

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