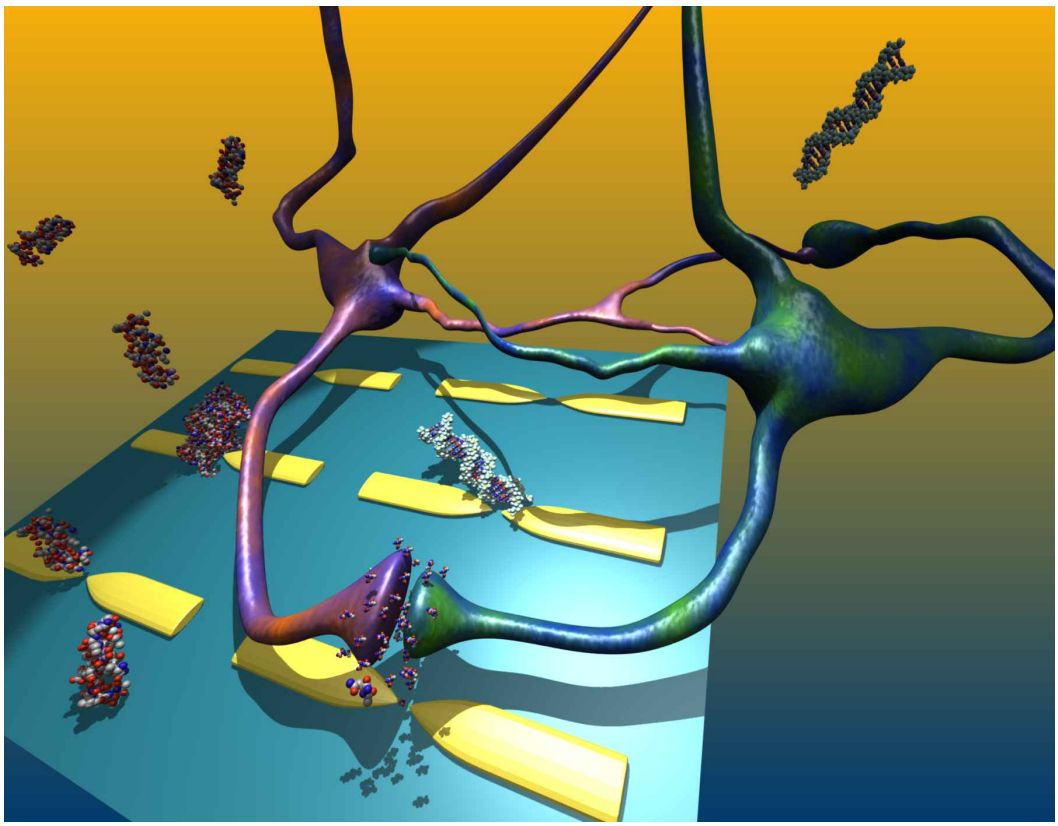
Ferienpraktikum
Nanoelektronik

Cell-chip coupling for bioelectronic devices

Lab manual



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1 Microelectrode array recordings from HL-1 Cells

1.1 Preparation of an Ag/AgCl reference electrode

Background:
A reference electrode is an electrode, which ideally maintains a constant potential in a solution. Stable reference electrodes are an important tool in all electrochemical measurements, where the potential of a working electrode has to be set versus a fixed reference potential. The potential is generated by the exchange of ions at the interface between electrode and solution. A high stability of electrode reference potentials can be achieved by employing a well-defined redox system with constant concentrations of the participants of the redox reaction. Each redox system is characterized by an individual value on the electrochemical potential scale. The basis of this scale (0.0 V) is formed by the standard hydrogen electrode. All other redox potentials are referenced versus this potential.

When performing electrical measurements with cells, the potential of the extracellular solution should be defined by a stable reference potential. The Ag/AgCl-electrode which is used in many electrochemical experiments is a good choice as it is biocompatible under measurement conditions. It is made by electrochemical oxidation of a thin silver wire in hydrochloric acid.

The basic reaction is given by: $Ag + HCl \rightarrow AgCl + ½\ H_2$

When an Ag/AgCl electrode is brought into contact with a 1 M potassium chloride solution at 25°C, it develops a potential of around +0.23 V versus the standard hydrogen electrode. Since the salt concentration in an extracellular solution is maintained roughly at a constant value, the silver chloride electrode exhibits a stable potential for electrical cell measurements.

Setup:

- Voltage source
- Silver electrode
- Glass beaker
- 1M HCL solution

Tasks:

1. Grind off electrode for cleaning and roughening.
2. Put the electrode into the socket.
3. Insert the setup upside down into the glass beaker such that the electrode is immersed into the HCl-solution.
4. Switch on power, turn to 1-2 V and wait until silver wire has become brown/black.
5. Get electrode out of the setup.
6. Rinse carefully with MilliQ water

1.2 Recordings of HL-1 cell signals

Background:
HL-1 cells belong to a cardiac muscle cell line that exhibits contractile activity and can be passaged in culture as well as recovered from frozen stocks. Since these cells are relatively easy to culture and show spontaneous electrical activity, they are an ideal candidate for cell-chip communication experiments. After a few days in culture, confluent layers of HL-1 cells exhibit synchronous beating and propagation of action potentials. In our experiments we want to monitor these action potentials using microelectrode arrays as extracellular recording devices. To this end, cells are plated three or four days prior to the experiments on microelectrode chips which are stored in the cell culture. For our electrical experiments, we use an automatic setup which is controlled by a personal computer.

Cell-Chip Coupling for Bioelectronic Devices

Setup:

- Microscope
- MEA-amplifier system with head stage, main stage, and controlling computer
- MEA-chip & cell culture
- Ag/AgCl reference electrode
- Norepinephrine stimulation solution

Tasks:

1. Put MEA-chip with the cells carefully into socket. **(Make sure that no electrolyte is spilled into headstage !!)**
2. Attach the reference electrode and put it into the solution.
3. Optical observation of cells
    - Maneuver stage with chip under objective
    - Focus microscope and confirm whether cells are growing on microelectrodes
    - Can you see beating cells?
4. Electrical recordings of spontaneous HL-1 cell acitivity
    - Start Med64 and observe cell signals for ~1 minute
    - Record and analyze 1 minute of data
        - Calculate spike frequency
        - Estimate propagation speed and direction
5. Stimulation of HL-1 cells with norepinephrine
    - Start recording for 90s
    - After 30s add 15 µl of norepinephrine solution to cell culture
    - Describe effects of chemical stimulation on cell activity for the remaining 60s.
6.

Note:

If time permits we will do a complementary experiment using calcium imaging to optically verify activity of HL-1 cells.

2 Whole cell patch clamp measurement

2.1 Preparation: Mixing intracellular (IC) patch solution

Background:
In intact tissue the extracellular fluid fills the space between the individual cells (also called interstitial fluid). It originates from the blood plasma leaking out of the blood vessels. The ionic composition of the extracellular fluid is essential for the vitality of the cells, because it influences, for example, cell adherence, cell volume and membrane potential.
In cell culture systems the interstitial fluid is substituted by the cell culture medium, which provides all nutrients (and growth factors) ensuring optimum cell growth. It should mimick as closely as possible the physiological conditions.
For electrophysiological experiments the cell culture medium is usually replaced by a simple salt solution containig the same salt concentations as in the cell culture medium or the interstitial fluid, respectively.

For the patch clamp experiments with HEK293 cells we use an extracellular patch solution the composition of which is given in table 1. For the preparation of the solution, we provide stock solutions of the salts in a concentration of 1M. The pH buffer HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) has to be weighed and added in powder form.

Component	Final concentration [mM]	Volume of 1 M stock solution for **100 ml** patch solution [ml]
NaCl	140	14
KCl	5	0.5
$CaCl_2$	1	0.1
$MgCl_2$	5	0.5
HEPES	10	Weight

Table 1: Composition of the extracellular patch solution for HEK 293 cells.

Tasks:
- Calculate the required amount of HEPES powder (MW = 238.31g/mol).
- Pipette the given volumes of the stock solution into a clean 100 ml beaker.
- Fill up to ~90 ml, add the weighed HEPES powder and adjust the pH to 7.4 with 0.1M NaOH (pH meter).
- Use a long-necked round bottomed flask to fill up to 100ml.
- Check the osmolarity (osmometer).

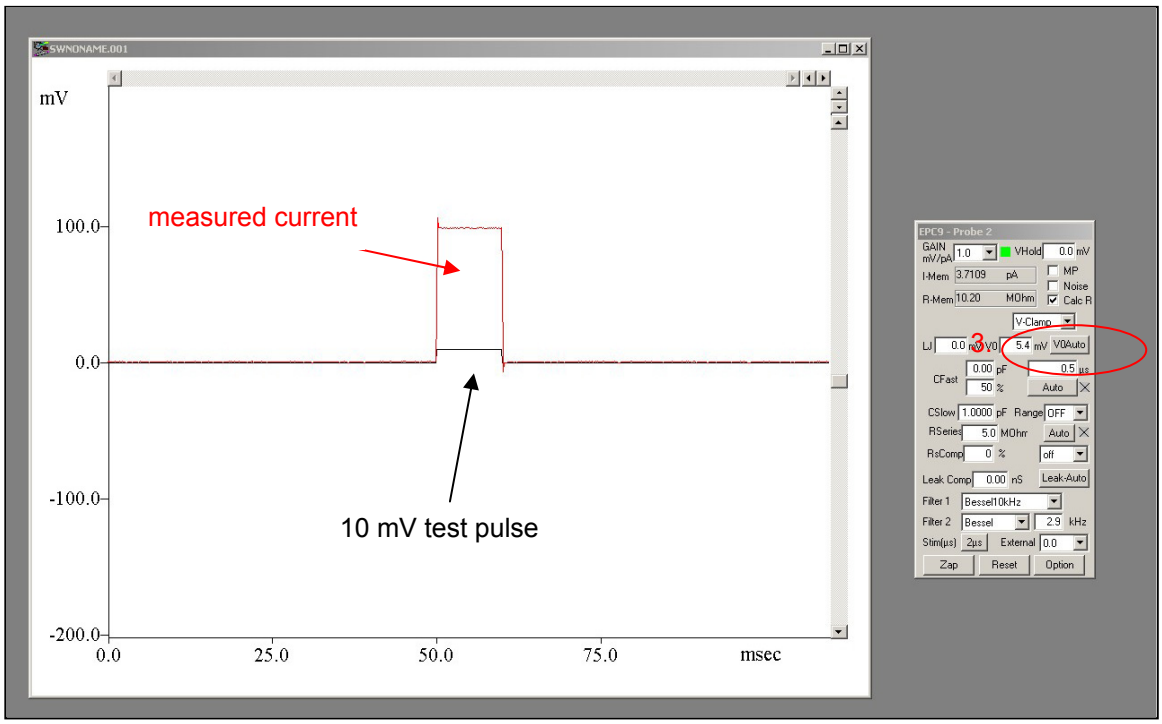
2.2 Experimental Setup

A typical patch-clamp setup is shown in picture 1. The most important components are:
- Microscope
- Micromanipulator(s)
- Pipette-holder(s) with Ag/AgCl-electrode and integrated pre-amplifier
- Main amplifier (also called patch-amplifier)
- Computer (for controlling stimulation and data acquisition)
- Grounded Faraday cage (in the background of the picture)

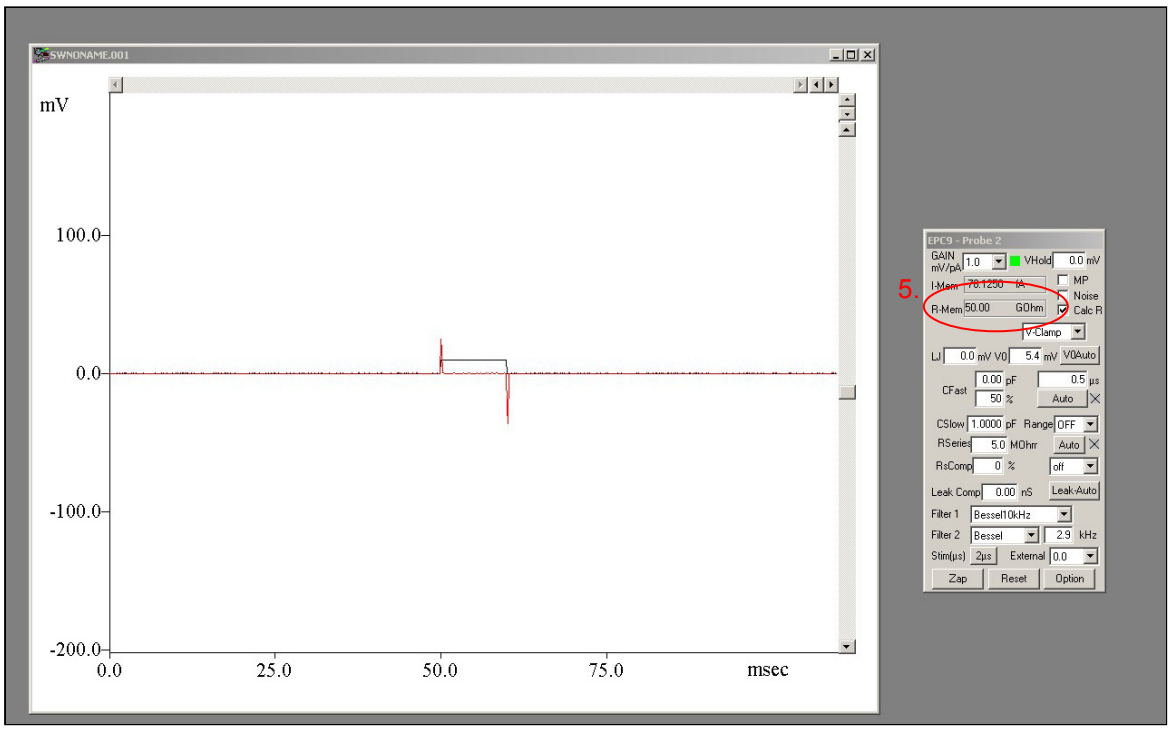
Picture 1: Patch-clamp-setup.

2.3 Step-by-step Procedure

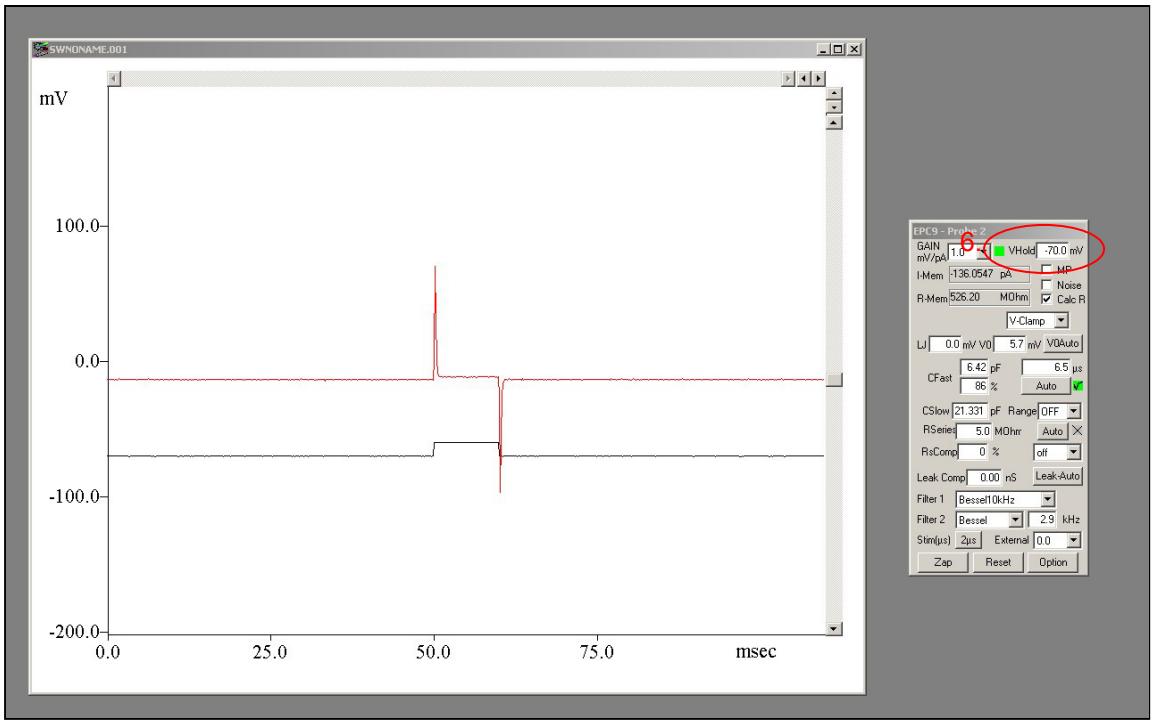
1. Apply overpressure to pipette and keep it by pinching of the tube.
2. Move the pipette into extracellular measurement solution.
3. Press “V0 Auto“ to compensate offset potentials caused by polarisation at the electrodes or junction potentials between bath medium and pipette.



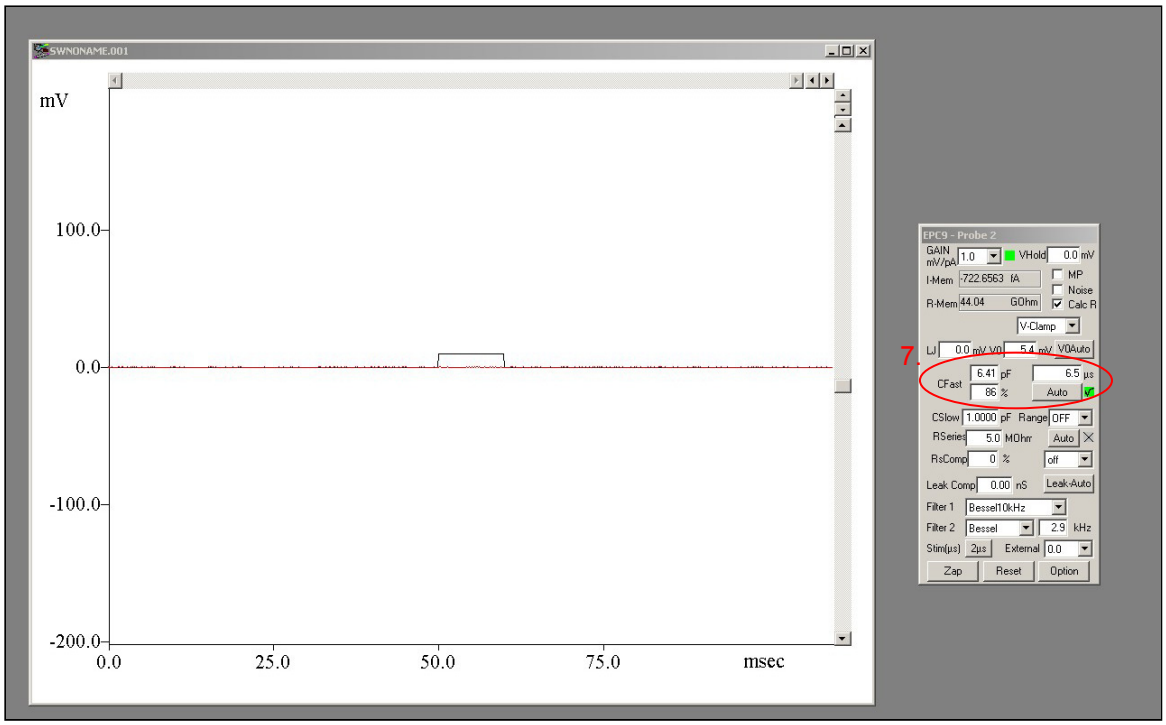
4. Move the pipette close to the cell. R_{Mem} should rise about 0.3 – 1 MΩ. Additionally, a small dent can be seen due to effusion of medium out of the pipette.



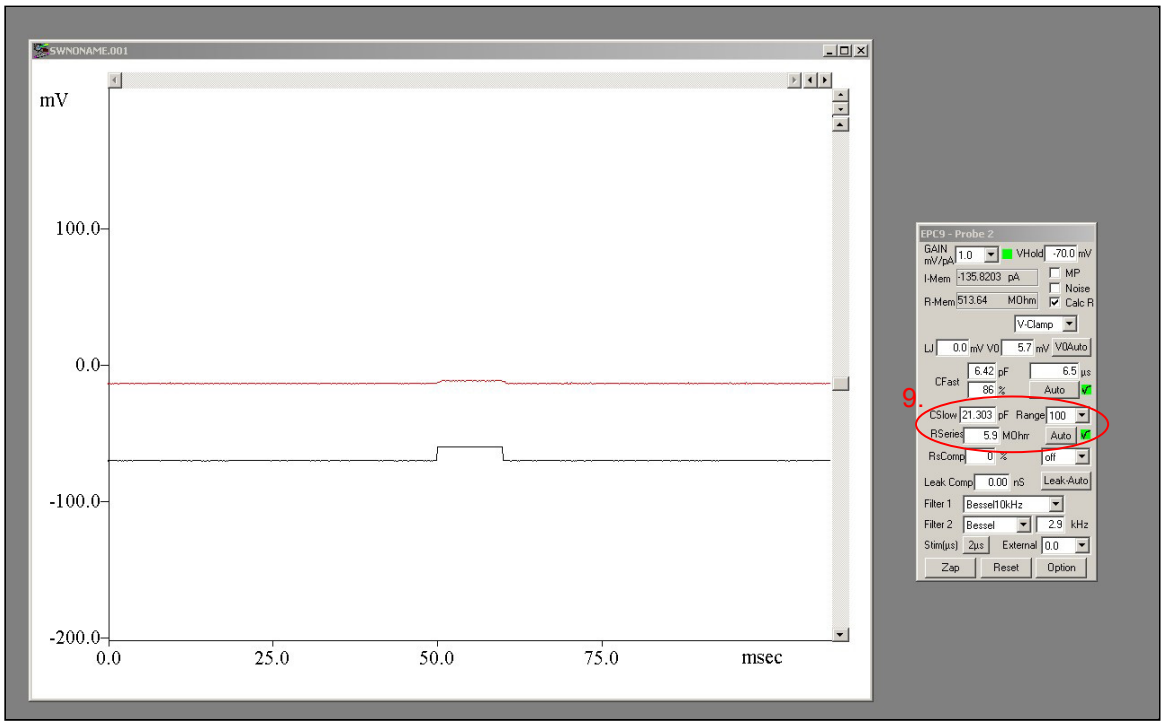
5. Remove the overpressure. Eventually this may lead to a “giga seal” (R_{Mem} > 1 GΩ), if not, gentle underpressure can be applied to facilitate sealing.
6. Set “VHold“ to –70mV.
7. After sealing press “CFast / Auto“. All fast capacitive currents, caused by charging of the pipette and the contacted membrane-patch, are compensated (CFast = 5-15 pF).



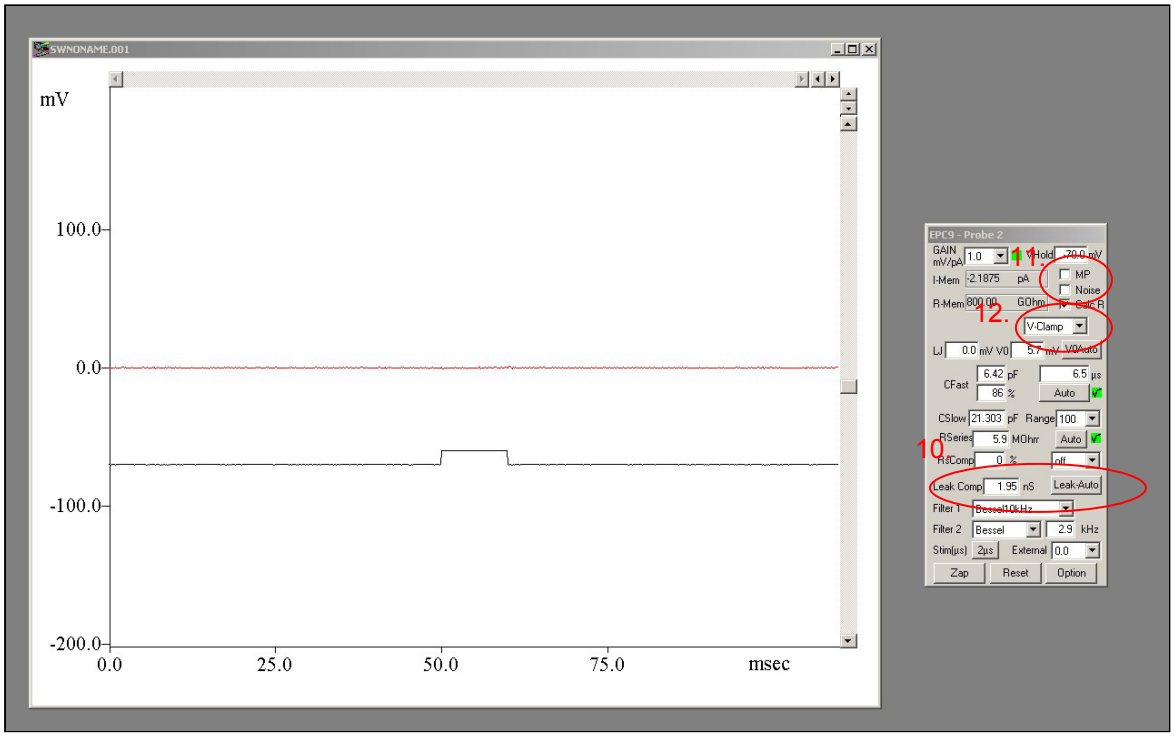
8. Open the cell by quick but gentle suction.



9. Press “CSlow / Auto” to compensate slow capacitive currents and the series resistance of the cell. CSlow = 10 – 100 pF (depending on the size of the cell), RSeries should be about 2-3 times the original pipette resistance.



10. Press “Leak-Auto” to compensate leak currents. Leak Comp should be < 5 nS.
11. The membrane potential can be checked by pressing “MP”. (< -30 mV).
12. Choose the “patch-mode”. (Voltage-Clamp or Current-Clamp)



13. Now you can perform your measurements by applying different kinds of pulse protocols.

Tasks:

- Follow above instructions and try to successfully patch a cell.
- Note your observations (and probably take a picture).
- Evaluate data for your protocol.