HB

multichannel *
systems

HiClamp

USER MANUAL

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IMPRINT

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GENERAL INFORMATION

Important Safety Advice

Warning: Make sure to read the following advice prior to installation or use of the device and the software. If you do not fulfill all requirements stated below, this may lead to malfunctions or breakage of connected hardware, or even fatal injuries.

Warning: Always obey the rules of local regulations and laws. Only qualified personnel should be allowed to perform laboratory work. Work according to good laboratory practice to obtain best results and to minimize risks.

Warning: The device and the software are not intended for medical uses and must not be used on humans. MCS assumes no responsibility in any case of contravention.

Warning: Pinch points exist. Keep fingers clear of moving parts.

The product has been built to the state of the art and in accordance with recognized safety engineering rules. The device may only

- be used for its intended purpose
- be used when in a perfect condition.

Improper use could lead to serious, even fatal injuries to the user or third parties and damage to the device itself or other material damage.

Malfunctions which could impair safety should be rectified immediately.

Grounding

This product is grounded through the grounding conductor on the power cord. To avoid electric shock, the grounding conductor must be connected to earth.

Orient the Equipment Properly

Do not orient the equipment so that it is difficult to manage the disconnection device.

High Voltage

Electrical cords must be properly laid and installed. The length and quality of the cords must be in accordance with local provisions.

Only qualified technicians may work on the electrical system. It is essential that the accident prevention regulations and those of the employers' liability associations are observed.

Each time before starting up, make sure that the power supply agrees with the specifications of the product. Check the power cord for damage each time the site is changed. Damaged power cords should be replaced immediately and may never be reused. Check the leads for damage.

Damaged leads should be replaced immediately and may never be reused. Do not try to insert anything sharp or metallic into the vents or the case. Liquids may cause short circuits or other damage. Always keep the device and the power cords dry. Do not handle it with wet hands.

Requirements for the Installation

Make sure that the device is not exposed to direct sunlight. Do not place anything on top of the device, and do not place it on top of another heat producing device, so that the air can circulate freely.

The HiClamp weighs more than 47 kg. Always grip it tightly and do not carry it alone, but with the aid of another person.

The movements of the well plate carrier can lead to vibrations of the workbench on which the HiClamp is set up. Therefore, the HiClamp must be set up on a rigid, vibration-free base. The base must also be sufficiently solid to carry the weight of the device.

The HiClamp should be operated only in an air conditioned room. A room temperature of 20 °C (or less) is recommended. Make sure that the device is not subject to direct sunlight. It may overheat.

If the air cannot circulate freely around the external power supply, the device may overheat. Do not shield the power supply by laying anything on top of the acryl glass cover.

The external power supply is only for use with the HiClamp. Do not connect it to other devices.

Handling of the motor driven axes

Do not move the axes by hand. Breakage may occur. Always use the software controls to move in vertical or horizontal level. The capillaries of the probe are sharp and may lead to injuries. Stay at a safe distance during operation and protect your eyes. Especially take care not to move your hands in the range of the vertical axis.

Do not try to plug anything other than 0.4 mm wire or the provided connectors into the sockets of the recording axis. Damage may occur.

Handling of the chloride coated silver wires

The silver chloride coated silver wires are sensitive to light. Always keep them in dark during storage. Make sure that all electrodes (including the reference electrodes) are still well-chlorided before you start a recording. They should look dark grey, not shiny. The Ag/AgCl layer deteriorates over time, leading to a DC offset and a voltage drift over time.

We recommend that you use the provided connectors to connect the electrodes to the vertical axis. If you want to plug the silver wire directly into the sockets of the vertical axis, use only 0.4 mm silver wire for the electrodes. A wire with a greater diameter will damage the connectors of the vertical axis irreversibly.

Handling of recording electrode needles

The needles are sharp and break easily. Always handle them with care. In rare cases, the needles may splinter when pressure is applied. Stay at a safe distance and protect your eyes.

Operation of the peristaltic pump

Empty the waste bottle at regular intervals, at least once a day if the HiClamp is in use. The peristaltic pump must not be operated if the waste bottle is full.

Regular backups

You (or the administrator) should perform backups of the HiClamp data files at regular intervals and to appropriate recording media for preventing data loss. Data loss may be caused by power failure, system and software errors.

INI file modifications

If you remove or edit text of an INI file, the software may cause severe problems. Some INI files relate to hardware functions. A modification of INI files may lead to malfunctions or even severe damage of the hardware. Always keep a copy of the original INI file. Only advanced users should modify program files. This warning message applies to all INI file modifications.

Explanation of the Symbols used

Caution / Warning   DC, direct current   Pinch points

Guarantee and Liability

The general conditions of sale and delivery of Multi Channel Systems MCS GmbH always apply. They can be found online at
https://www.multichannelsystems.com/sites/multichannelsystems.com/files/documents/Terms%20and%20Conditions.pdf

Multi Channel Systems MCS GmbH makes no guarantee as to the accuracy of any and all tests and data generated by the use of the device or the software. It is up to the user to use good laboratory practice to establish the validity of his / her findings.

Guarantee and liability claims in the event of injury or material damage are excluded when they are the result of one of the following:

- Improper use of the device.
- Improper installation, commissioning, operation or maintenance of the device.
- Operating the device when the safety and protective devices are defective and/or inoperable.
- Non-observance of the instructions in the manual with regard to transport, storage, installation, commissioning, operation or maintenance of the device.
- Unauthorized structural alterations to the device.
- Unauthorized modifications to the system settings.
- Inadequate monitoring of device components subject to wear.
- Improperly executed and unauthorized repairs.
- Unauthorized opening of the device or its components.
- Catastrophic events due to the effect of foreign bodies or acts of God.

Operator's Obligations

The operator is obliged to allow only persons to work on the device, who

- are familiar with the safety at work and accident prevention regulations and have been instructed how to use the device;
- are professionally qualified or have specialist knowledge and training and have received instruction in the use of the device;
- have read and understood the chapter on safety and the warning instructions in this manual and confirmed this with their signature.

It must be monitored at regular intervals that the operating personnel are working safely. Personnel still undergoing training may only work on the device under the supervision of an experienced person.

WELCOME TO THE HICLAMP
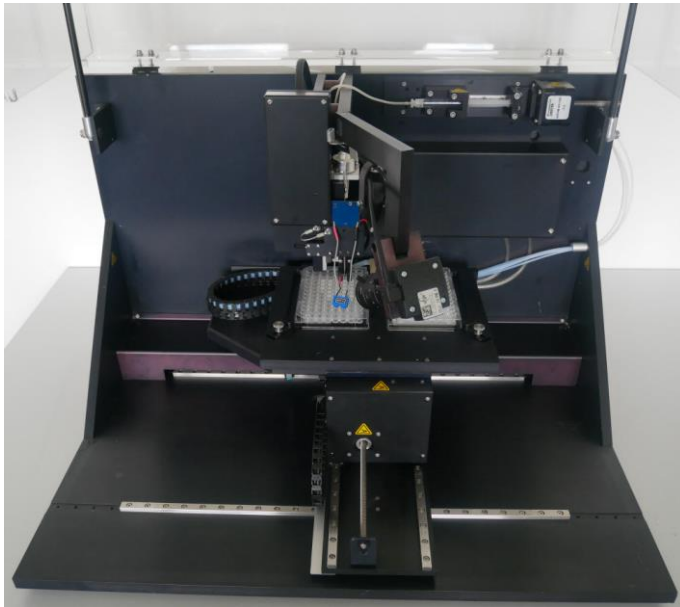
The HiClamp is a fully-automated all-in-one solution for high-throughput functional secondary screening of drug targets based on the standard *Xenopus* expression system. The HiClamp allows an automated recording using the TEVC method unattended and over night.

Main Features

- Compact and functional design works fully computer-controlled.
- High performance TEVC amplifier, records current of up to $\pm$ 105 µA.
- Export features for further data evaluation.
- Integrated washing station with a peristaltic pump based four channel perfusion system.
- Non-destructive usage of test compounds.
- Small sample volumes (~200 µl).

Operation Summary

The HiClamp functionality is based on the use of a novel system in which the oocyte is exposed to a test solution by moving it physically into the solution of interest, whereas in a standard system the solution is applied on the cell. The test solutions are disposed in a 96-microtiter plate which is fixed on a table (in the left part). The oocyte is fixed in the support held (oocyte basket) in the center of the automate and the table moves to immerge the cell in the desired solution. The table moves in X, Y and Z direction under software control of very precise motors.

The HiClamp software program is an "icon based" program and was designed to simplify the user interface while maximizing the possibilities for electrophysiological recordings. No special programming knowledge is required to use the HiClamp software. The program sequence is an ensemble of operations that are executed sequentially according to a set of icons that are stored in the program sequence. Then, each icon corresponds to a given procedure. For example, the oocyte icon will contain the type of message injected into the oocytes, the number of cells available etc. At difference, the icon corresponding to the amplifier will store the information controlling the amplifier gain, filter conditions, voltage clamp set up, voltage steps etc. To access the content of a given icon the user double clicks on the icon stored in the program sequence.

After defining the recording protocol, simply start the recording sequence by mouse click. The run will proceed automatically until finished or interrupted by the user.

SOFTWARE AND HARDWARE

The HiClamp Software

Operate the HiClamp, collect and evaluate the data by using the HiClamp software. The easy-to-use graphical user interface of the HiClamp software makes daily work with the HiClamp quick and easy. Recording is started by a single mouse click. The HiClamp controls the run for all 96 oocytes automatically. Thus, the recording can go on overnight, unsupervised.

You can define different experimental setups not only for different well plates, but also for specific selections of wells on the same plate. Several control features to save time and compounds are provided.

Automated analysis features are included in the HiClamp software, but you can export the data to your custom evaluation software as well. You can graph the data and generate reports with the HiClamp software. The large amount of data generated by the HiClamp can be managed with the included DataMerger software.

SOFTWARE AND HARDWARE
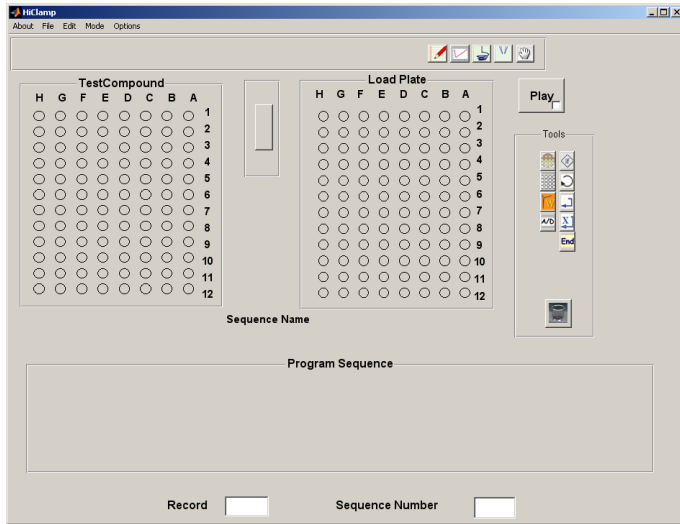
HARDWARE
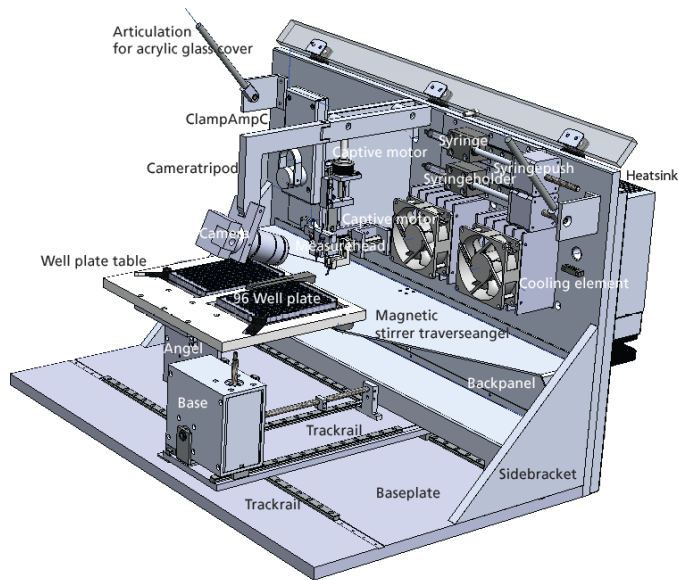
The HiClamp is compatible with standard lab equipment and can be easily integrated in your working environment. Software controls replace any knobs on the device. The HiClamp is straightforward and easy to operate; handling does not require special skills or special equipment.

Recording and cultivation of *Xenopus* oocytes is performed using disposable standard 96 well plates, which are commercially available from several providers. The oocytes are plated into the wells in a couple of minutes and can be kept for several days. The oocytes do not have to leave the plate anymore; you can easily transfer the oocytes from the incubator to the HiClamp and back again.

A quick alignment process under camera control guarantees that the glass microelectrodes in the measuring head impale the oocyte precisely centered before recording.

The ClampAmpC is a specifically designed digital TEVC amplifier and is completely automated.

The Syringe System arranges the aspiration of the cell when loading it into the basket via low pressure.

The Perfusion System with inlet and outlet tubes and external peristaltic pump is for managing the wash and test station placed between the 96 well plates on the table.

Setting Up the HiClamp

Provide a power supply in the immediate vicinity of the installation site.

Important: Make sure that you do not place any AC voltage sources, that is, any electrical devices or cables in the immediate vicinity of the HiClamp robot (especially not near the amplifier or near any parts belonging to the perfusion system, for example, the bath reservoir or the waste bottle), as they can introduce a 50 Hz noise or other electrical interferences to your recordings. Move electrical devices or cables a few inches away if you observe any problems.

Place the HiClamp on a rigid, stable, and vibration-free base in an air conditioned room. Place the computer and its accessories next to it. Make sure that you do not place the monitor or the computer too near to the HiClamp robot or the perfusion system.

Connecting the HiClamp

Connect all cables as described below.

⚠️ Warning: Carefully lay and secure the cords. Remember that someone could easily trip over a loose cable.

Note: All electrical connections are clearly marked, and the plug coding prevents confusion. The cords should be plugged in without the use of excessive force.

The following illustration shows the back panel of the HiClamp.

Connecting the video camera (Cam)

The USB cable of the video camera is fixed to the rear panel of the HiClamp. Please connect the cable of the video camera to an USB port of the computer.

Connecting the peristaltic pump stand (Pump)

Plug the female connector into the male socket of the peristaltic pump. Then plug the male valves connector into the female socket on the HiClamp's back panel.

On / Off Toggle Switch I / O (Power)

Toggle switch I / O for turning the HiClamp device on and off.

Connecting the HiClamp to the provided computer (USB)

Connect the HiClamp directly to the provided computer by the provided USB cable. The appropriate connector on the rear panel of the computer is tagged with a "HiClamp" label.

Note: Please do not use one of the USB 3.0 ports of your computer for connecting the HiClamp.

Power In for the HiClamp (24 VDC HiClamp)

Connect the HiClamp to the power supply unit here. This power supply powers the HiClamp main unit only.

Power In for the Cooler (24 VDC Cooler)

Connect the Cooler to the power supply unit here. This power supply powers the Cooler main unit only.

⚠️ Warning: If the air cannot circulate freely around the external power supply, the device may overheat.
Do not shield the power supply by laying anything on top of it. Make sure it is not exposed to direct sunlight.

Note: All electrical connections are clearly marked, and the plug coding prevents confusion. The cords should be plugged in with no excessive force.

The Peristaltic Pump Perfusion System with four Channels

For washing the oocyte between two measuring circles there is a washing station placed between the well plates. The washing station can also be used for applying test compounds to the cells.
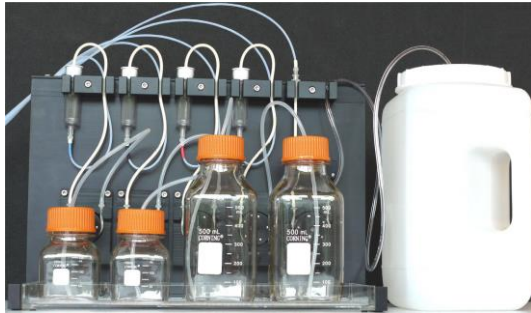
The test and washing station is connected to five tubes: Four inflow and one outflow channel. The fifth channel is connected to the waste bottle via peristaltic pump which is running backwards. Please read chapter "Mechanical Functionality of the HiClamp".

The operation of the perfusion system is fully software controlled.

Do not start the perfusion without double checking the connected bottles and the waste bottle.

Warning: Spilled liquid can damage or even destroy the electronics of the HiClamp. Please be careful when setting up the perfusion system and when starting the perfusion of the washing station. Take care that inlet and outlet tubing are properly connected so that flooding of the table and the mechanical components of the HiClamp is efficiently prevented.

Mechanical Functionality of the HiClamp

The specific characteristic of the mechanical components of the HiClamp device is their mobility in three dimensions. The movement of the table complex in dependency of the movement and adjustment of the measuring complex is the main idea of the HiClamp's functionality.
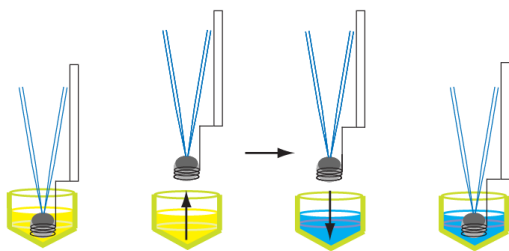
The table complex moves on track rails in X, Y and Z direction under software control of very precise motors.
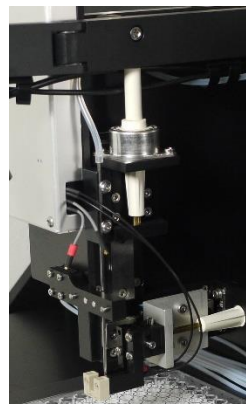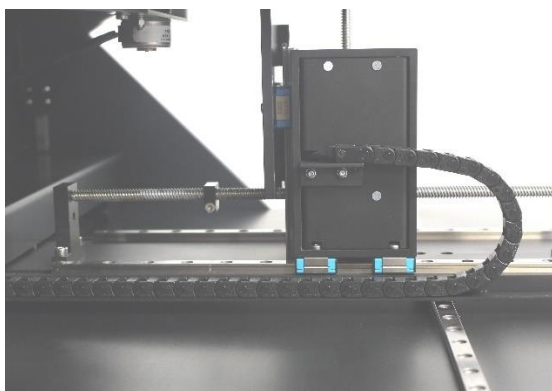
The basket with the oocyte inside moves in Y direction only, but is not moving during recording minimizing recording artifacts and oocyte damage during transport.

The HiClamp is based on a innovative system in which the cell (or preparation) is exposed to a test solution by moving it physically into the solution of interest, whereas in a standard system the solution is applied on the cell. The provided solutions are disposed in a 96 well plate which is fixed on the left

part of the table. The cell (or preparation) is carried in the basket in the center of the HiClamp. After adjustment of table complex and measuring head, the basket keeps stationary while the table moves to immerge the cell in the desired solution.

For washing the cell between two measuring cycles there is a washing station placed between the well plates.
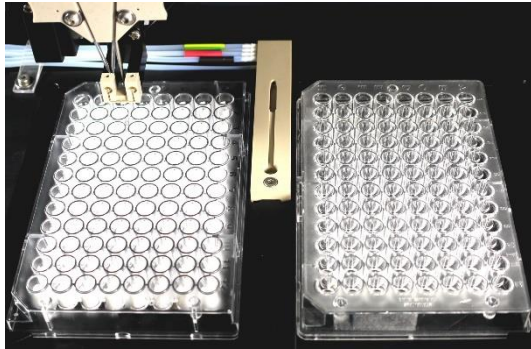
The well plate on the right hand side is called "Feeder Plate" and contains the oocytes to be tested.

The well plate on the left hand side is called "Compound Plate" and contains the solutions of interest. Each compound well should be filled with 230 µl. Additionally a micro stirrer is placed inside which is moved by magnetic forces.
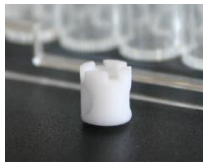

Immersion of the oocyte in the compound liquid is not sufficient to ensure a quick access of the drug to the cell membrane. Passive diffusion of the molecule of interest alone to the membrane would be too slow and mechanical stirring is therefore indispensable to ensure a fast access of the compound to the membrane protein. Miniature stirrers were specially designed and conceived with a proprietary structure to allow mixing of the solution in the 96 well format. Placing the stirrers in the microtiter wells offers the additional advantage to reduce the volume of the well and therefore to minimize the amount of solution required for the experiment.

Automated Loading of Oocytes from Compound Well Plate

The oocytes are stored in the 96 well plate called feeder plate on the right side of the table. They will be automatically transferred to the compound plate on the left side for measuring the cells. For transportation and measuring the oocytes are carried in the basket.
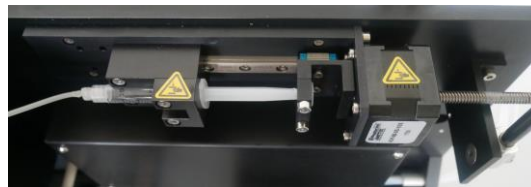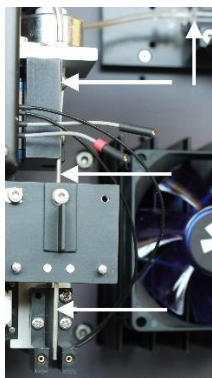
Cartoon of the automated oocyte transport from 96-well feeder plate to the oocyte basket:

Automated loading of oocytes from feeder plate
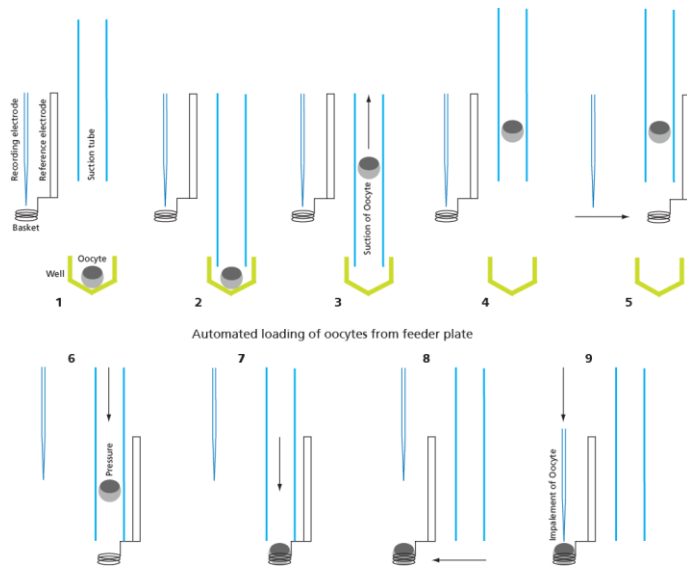
The oocyte is in the selected well of the feeder plate (1). The suction tube is lowered into the well (2). The oocyte is aspirated (3). The suction tube moves to the basket (4). The suction tube is lowered directly above the basket (5). The suction tube emits the oocyte (6). The oocyte is placed in the basket (7). The basket moves with the oocyte beneath the position of the TEVC electrodes (8). The electrodes are lowered, and the oocyte is impaled for recording (9).

Finally, the recording sequence starts when the oocyte is successfully impaled, and an appropriate resting potential is measured by both intracellular glass electrodes.

SOFTWARE AND HARDWARE

SOFTWARE

Program Concept

To maximize the system capacity while simplifying the use of the HiClamp device for data acquisition and analysis, HiClamp and the corresponding data analysis program are built around an object oriented concept. In such concepts the object, this is the icon, contains information to effectuate the appropriated and desired operation. In HiClamp, objects are represented as icons that symbolize the action to be executed.
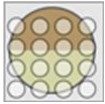
For example, the icon above symbolizes the loading plate that contains the oocytes to be measured.

For execution the information of this object, the HiClamp automatically loads the next available oocyte, measures the resting potential and puts the oocyte back in its original position if appropriate conditions that means the holding potential is not met.

This icon based system offers several advantages that become evident when considering the analysis. Data analysis is effectuated by a few clicks on the appropriate icons, which avoids the learning of a specific language while offering the maximal flexibility. Results obtained from different days of experiments are powerfully managed and merged by a module of the software and results can be presented either as graphics or excel compatible data report sheets.

Installing the HiClamp Software

System Requirements

Software: One of the following Microsoft Windows ® operating systems is required: Windows 10 or 8.1 (32 or 64 Bit) with the NT file system. English version supported; other language versions may lead to software errors.

Hardware: An USB 2.0 High Speed serial bus must be provided for the communication between the HiClamp and the computer. 1 GB RAM working memory is strongly recommended.

Recommended Operating System Settings

The following automatic services of the Windows operating system interfere with the data storage on the hard disk and can lead to severe performance limits. These routines were designed for use on office computers but are not very useful for a data acquisition computer.

- Turn off Windows System Restore.
- Turn off automatic Windows Update.
- Turn off Optimize hard disk when idle (automatic disk fragmentation).
- Deselect Windows Indexing Service for all local disks.
- It is also not recommended to run any applications in the background when using the HiClamp. Remove all applications from the Autostart folder.

Caution: You have acquired a high performance data acquisition and analysis computer. Do not modify the system, do not install new hard- or software, or another operating system without asking MCS or your local retailer for advice. MCS recommends not to install virus scanners or firewalls because these programs are known to interfere with the HiClamp to computer connection and with the data transfer. Virus scanners might cause a delay in the data transfer when they scan each data package and file, which can then result in an instability of the HiClamp program. If you need to install a virus scanner, please deactivate it before starting the recording protocol. MCS cannot guarantee that a system that was modified by the user is fully operational. Even data loss may occur.

Please check the system requirements before you install the software. MCS cannot guarantee that the software works properly if these requirements are not fulfilled.

Important: Please make sure that you have full control over your computer as an administrator. Otherwise, it is possible that the installed software does not work properly.

Installing the HiClamp Software with Computer Connected

The operating system and the HiClamp software are already installed on the provided computer. However, you may need to reinstall or update the HiClamp software on the same or another computer. Please check the system requirements if you are going to install the software on another than the provided computer. MCS cannot guarantee that the software works properly if these requirements are not fulfilled. Power up the connected computer and wait until it is ready.

• Double HiClamp Setup.exe on the installation volume. The installation assistant will open and guide you through the installation procedure.
• Follow the instructions of the installation assistant. The HiClamp software and all necessary drivers will be installed on your computer.

Starting the HiClamp

To start the HiClamp, just switch on the data acquisition computer first. Press the I / O Power switch on the rear panel of the HiClamp. The HiClamp is starting now.

Starting the HiClamp Software

You may use the computer and the software offline, that is, the HiClamp is switched off, to review and analyze your recorded data. However, if you start the software to operate the HiClamp, the easiest way is to start the HiClamp first and then start the software.

Important: Please make sure that you have full control over your computer as an administrator. Otherwise, it is possible that the HiClamp program does not work properly.

Double-click the HiClamp icon on the desktop.

- OR –

Select HiClamp software from the "Start" menu. The program starts.

Re-zero of the Axis

At that time the HiClamp table should move to the rightmost position. The re-zero sequence of all the axis of the HiClamp will be done sequentially. The HiClamp is ready to start only when this initialization phase ends, and the program window appears with the play button visible. This sequence takes approximately one minute.

TEVC RECORDING PROTOCOL

Preparations for Recording

Installing Solutions

Solutions applied to the washing station are pumped by the peristaltic pumps. Verify that the tubings are properly disposed and placed in the solution. Clicking the "Manual" icon activates the manual command dialog which allows the manual control of the pumps.
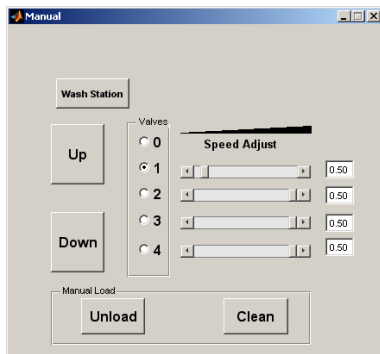
Filling of all used tubings is a precondition for proper function of the washing station during recording. Please verify that the liquids flow regularly without the appearance air bubbles in the perfusion chamber.

The Manual Window

The manual window is designed for the manual control of the solution, for placing the basket into the washing station, for unloading the oocyte and for the cleaning of the basket. A single click on the valve buttons switches on the desired pump. When all pumps are turned off, the radio button for the value 0 is highlighted in blue.

The sliders and values on the right indicate the rotation speed of the inlet peristaltic pumps. Note that the rotation speed of the outlet suction pump cannot be adjusted and keeps on running at optimum as long as the HiClamp is switched on.

Electrode Alignment

Mounting and Adjusting the Electrodes

First, mount the provided glass capillaries on the measuring head. Use a binocular and adjust the tip distance between 300 and 400 μm. Also be careful to align the tips to be at about the same height.

Then, fill the capillaries with either 3 M KCl or with a mixture from KCl and KAc which reduces crystallization (1 M and 1.5 M, respectively). Filling should not exceed about 70 % of the capillary length.

Try to insert the filling needle or micro loader as close as possible to the tip region. Try to avoid having any air bubbles in the capillary after filling. The tip region usually fills by itself, because the capillaries have a filament inside, but this takes sometimes a few minutes. Be patient and wait until the tip region fills spontaneously. Tipping against the capillary in order to speed up the process very often ends up in having many small air bubbles in the tip region making the capillary unusable.

After filling, insert the provided AgAgCl wires. A small drop of mineral oil can be finally added on the top of each electrode opening to prevent evaporation.

Please put the oil on top of each glass capillary after inserting the electrode wires! Otherwise, the AgAgCl coating will soak up the oil and destroy the electrical properties of the electrode.

Important: The recommended electrode resistance is in the range of 100 kilohms to 1 megaohm. The electrode resistance also limits the maximum gain that may be used. It is considered a normal behavior of electrodes that the resistance increases over time. It is recommended to use only high-quality oocytes (with high membrane potential and low leak current) to avoid blockade of the electrode tips. For an optimum clamp performance of the TEVC amplifier, the electrode impedance should not exceed the recommended range.

Warning: Use only a well-chlorided silver wire for all electrodes. Use a diameter of 0.4 mm if you plug the wire directly into the connectors of the vertical axis in order to avoid damaging the vertical axis. We recommend the use of the provided connectors for a better handling.

Once the measuring unit is installed on the HiClamp it is necessary to adjust the maximal depth at which the electrodes will be moved during oocyte impalement. Please use the camera for optical control of this alignment.

Press on the "**Electrodes**" icon to open the "**Electrodes**" window**.**
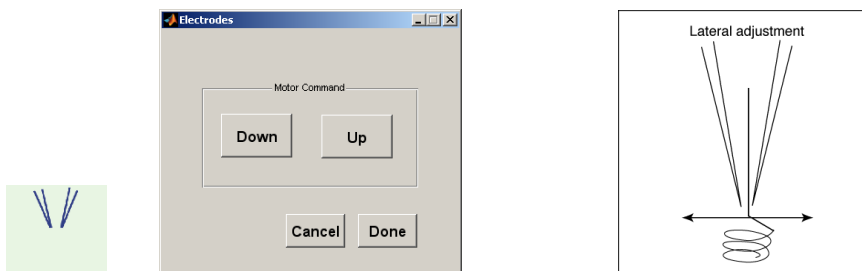
Press on the "Down" button for slowly moving the electrodes down towards the oocyte basket. Use the camera for optical control. Lower the electrodes progressively into the center of the basket, just a little bit deeper than necessary to penetrate the oocyte. Note that the camera is mounted on a rotating arm to facilitate the adjustments. If the electrodes are not centered use the allen key (small hexagonal head screw) to move the position of the electrode holder base plate.

If the electrodes are not correct aligned backward or forward use the icon „Adjust Basket".
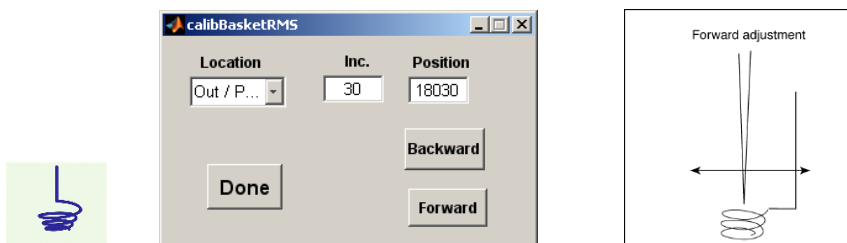
First move the basket in lateral and frontal direction by 30 units until the electrodes are in correct position.

The adjustment is finished when the electrodes are lowered in the center of the basket in maximal depth. This position of the electrodes is defined and saved when closing the window. Please use the "Cancel" button to move the electrodes up and to restart the alignment procedure. Use "Done" when you have found the right position. The position of the basket is stored in the file BasketDefault.mat that is saved on your disk.

Important: Do not remove or edit the BasketDefault.mat file as this would destroy the calibration parameters for the basket.

Test Model Cell

Testing the Amplifier with the Test Model Cell

Before you start to do real experiments with oocytes, we recommend getting familiar with the HiClamp using the **Test Model Cell** (TMC).

The TMC mimics the electrical (passive) properties of a real oocyte impaled with microelectrodes (electrode resistance ˜450 kOhm, membrane resistance 100 kOhm, membrane capacitance ~ 200 nF)). Before you start, you have to connect the TMC to the two electrode wire inputs (small connectors) and to the inputs where you would connect the oocyte basket (larger connectors). The red plugs have to be connected on the left side, respectively. When you now start the HiClamp and the HiClamp software, the amplifier window opens in the basic mode in which you can test the electrode impedance by clicking on the "**R_Test**" button.
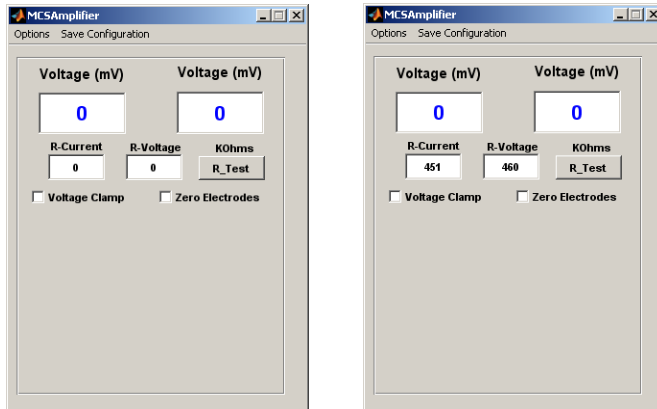
Resistance values similar as shown above should be displayed with your TMC connected.

If you know activate the "**Debug Mode**" via the "**Options**" menu, the display changes and you have access to all amplifier parameters. When you activate "**Voltage Clamp**" the TMC will be clamped to 0 mV with a holding current of about 0 µA.
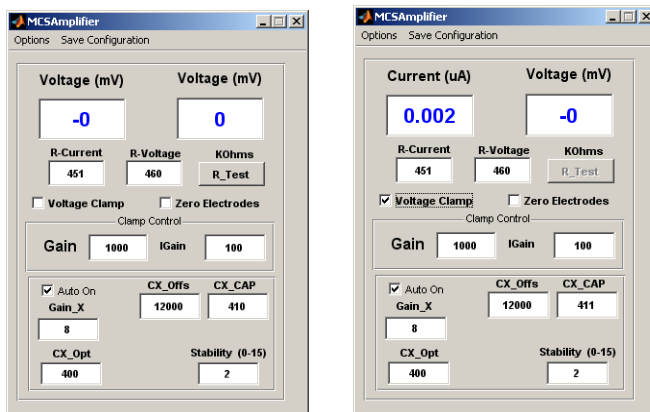

Tuning the Amplifier

The build in digital amplifier can perform an automatic capacitance compensation in real-time during the voltage-clamp recordings. This is an unique feature which is necessary to maintain stable clamping during the movements of the oocyte basket. This automatic capacitance compensation can be switched on and off with the "**Auto On**" check box.

Please note that "Auto On" should be checked during all recordings. Otherwise, amplifier oscillations could disturb your recordings or even damage the oocyte.

In order to get the highest stability, the function of the automatic capacitance compensation can be "tuned" although the default values should work under most circumstances.

For tuning switch off the "Auto On" first. You will notice that the displayed value in the CX_CAP window will change immediately. The exact value depends now on many factors like the amplifier, position of the TMC etc.

For tuning, change the "Cx_Offs" value until the "CX_CAP" window shows a value close to 400. Now you can reactivate the "Auto On" again and your amplifier is tuned.
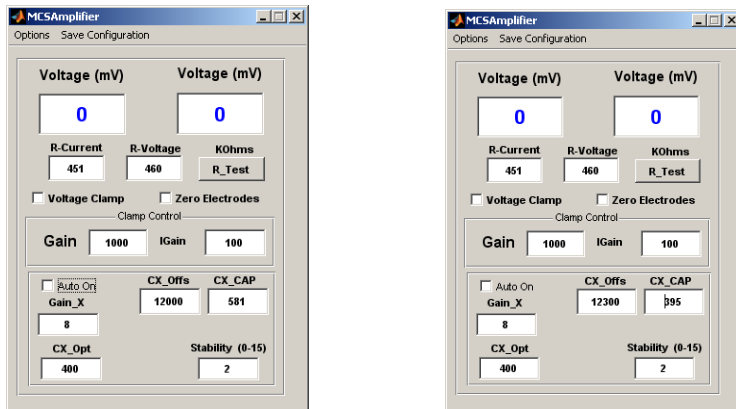

Please note that you right now performed tuning of the TMC. Repeat this tuning during your first recording with a real oocyte, which is placed in the basket. The basket should be in the washing station, impaled with electrodes, but clamp switched off. You do not have to repeat this tuning for every oocyte or recording.

Now, after tuning, you can test the performance of your system with a simple ""Voltage Step Protocol" (IV protocol) with, for example, 10 short voltage steps from -120 to +60 mV in 20 mV increments.
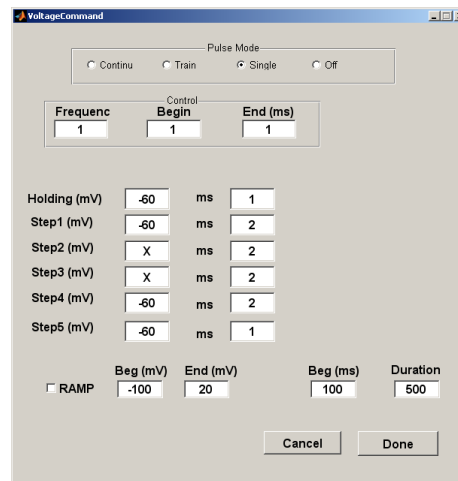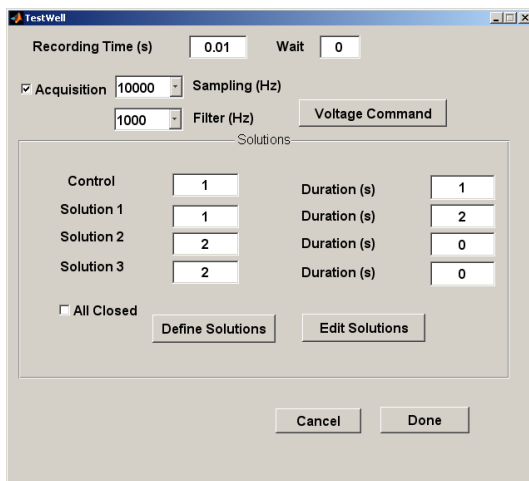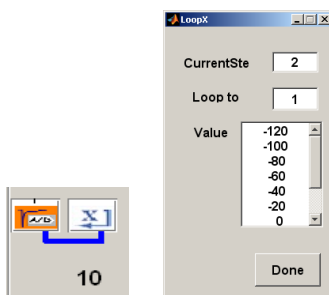
After executing and completing this voltage step protocol, the data display should show current traces looking like this. Please see the picture below.
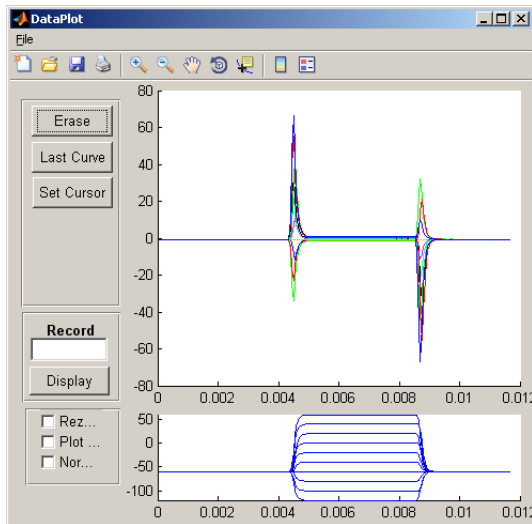
The traces shown above have been recorded with gain 1000 / 100 and an 1 kHz recording filter.

Selecting Oocytes for TEVC Recording

Selecting Oocytes

Please read chapter "Preparation of Oocytes" in Service and Maintenance.

Plating Oocytes

Please read chapter "Plating Oocytes" in Service and Maintenance.

Loading the Oocytes Plate

Place the 96-microtiter plate containing the oocytes in the right slot on the table. Tighten the fixation of the plate (lower right) and verify that the holding screw is properly secured. Poorly fixed oocyte plate can cause movement of the plate and result in a basket crash, or other dysfunction of HiClamp.

Starting a Recording Sequence

A program sequence is a chronology of operations that are executed sequentially according to a set of icons that are stored in the program sequence. Each icon symbolizes a fixed procedure, for example loading the oocyte or setting up the amplifier. Please see chapter "Icons and associated Effects" below. Each icon contains a given set of information to execute the desired actions. For example, the "Oocyte" icon symbolizes the type of message injected into the oocytes, the number of cells available etc. At difference, the icon corresponding to the amplifier will store the information controlling the amplifier gain, filter conditions, voltage clamp set up, voltage steps etc. To access the content of a given icon the user double click on the icon stored in the program sequence. The first and simplest example presented below illustrates such procedure.

A first and simple Example

The simplest example would be to test how oocytes are expressing membrane proteins and, in the case of ligand gated channels, would consist at measuring the amplitude of the current evoked by a brief pulse of agonist. To execute such operations, it is necessary to first load an oocyte into the recording basket, impale the electrodes, and apply the agonist while recording the data.
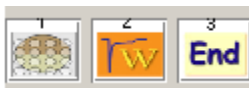

The icons sequence above presents such a sequence. To prepare this sequence one icon at a time is selected from the tools by a single click on the desired icon. Following the assembly of such sequence it is now necessary to store in this HiClamp sequence the properties including the type of message injected into the oocytes (mRNA or cDNA injected), the number and position of the oocytes in the feeder plate, the solution that is applied and for how long, the holding potential etc.

Selections are done by a double click on the icon in the program sequence. A double click on the first oocyte icon opens the "Load Plate" dialog. This window allows to enter parameters about the well plate and about the type of DNA injected into the oocytes as well as the position of this oocytes etc.
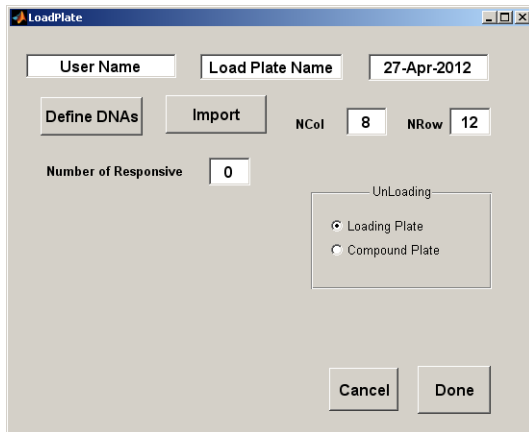

A double click on the second icon opens the "Test Well" dialog. This window allows to select the recording conditions, data acquisition, filter and sampling settings, drug application in the perfusion etc.
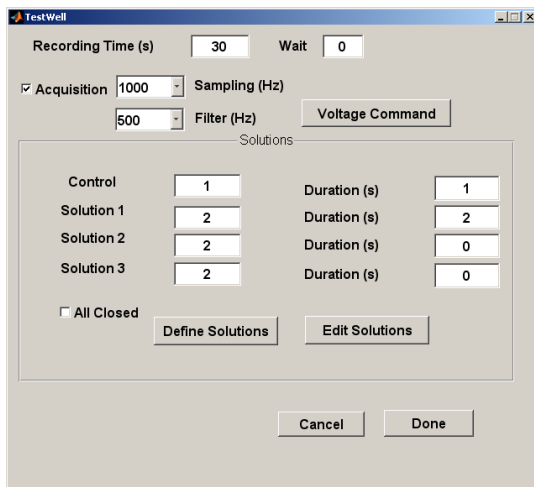
If you turn on the data acquisition by checking the box "Acquisition", the second icon will change it´s appearance and "A/D" will be displayed instead of "W".

If you click the button "Voltage Command" the respective dialog opens which allows you to define voltage protocols in step and ramp mode.
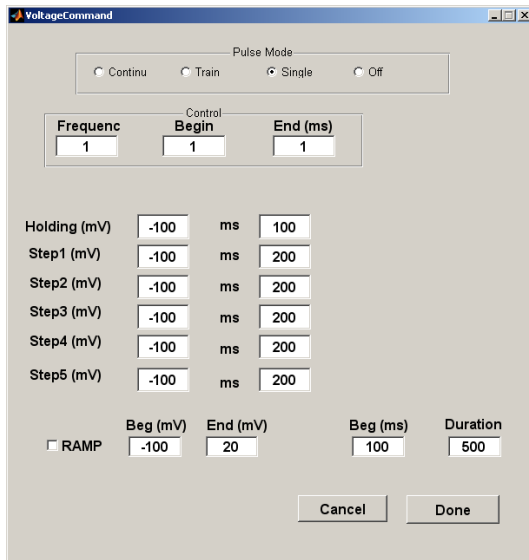
Pushing the button "Play" will start HiClamp. The button "Play" will be replaced by "Stop" as soon as the sequence is activated. Hitting the "Stop" button will stop the procedure. Importantly, however, time should be allowed to complete the entire stop procedure before interacting with the automate.

The window "DataPlot" will automatically pop out upon capture of your first data set. All data are automatically stored on the hard disk in defined files (see file organization).

Before we get into more sophisticated protocol sequences, you should learn about the meaning of all icons and the functions behind them.

**Icons and the associated Effects**
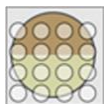
Oocyte Loading

This icon is used to select the oocytes that will be taken for the measurements. So, it is normally the first of a program sequence. Oocytes setting, such as the injected message, number of cells, date of injection etc. are stored in the corresponding data file. Access to these parameters is done by a double click on the "Oocyte Loading" icon and filling the appropriate parameters.

All parameters are automatically stored in the data sequence that is stored in the experiment folder. Multiple message types (cDNAs or mRNAs) can be defined in a single loading plate allowing determination of multiple protein functions.
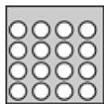

Compound Plate

This icon is used to define the assembly of compounds in the compound plate and the sequence of events that will be measured. Parameters such as incubation time, holding voltage, compound localization, concentrations etc. are defined by the user by a double click on the icon.
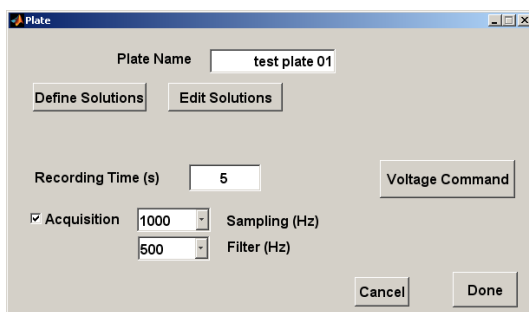
If you check the "Acquisition" box, options for setting sampling and filter frequency and the "Voltage Command" button appears. Double click on "Define Solutions" opens another window in which you can define and attribute solutions or compounds to the respective compound well (A1 to H12). If you want to define a sequence of concentration starting for example at A1, you just have to type the concentrations separated by a space.
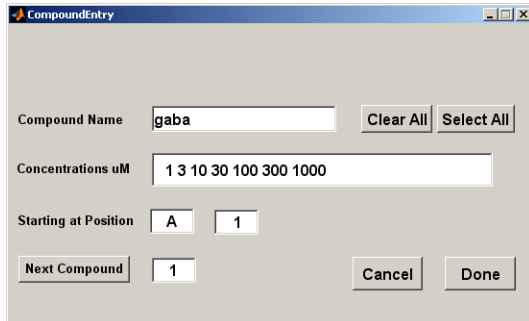
After clicking on "Done" the list window appears. Once defined you can change the active definition with the "Edit" function.
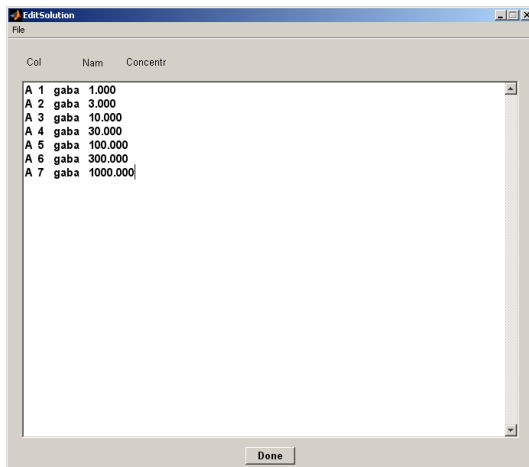

 Wash/Test

The "Wash/Test" icon is used to move the oocyte into the wash station where compounds can be washed, or a test solution can be applied to test the oocyte response. Parameters corresponding to this step can be accessed after a double click on the icon. These parameters include the amplifier settings, the voltage step control and drug application for the four available channels. The wash / test command is often used to determine the leak current of the cell, and its response to a standard solution. Note that measurements will be carried out only, if the data acquisition mode is turned on.

 The "If" Step

The "if" icon is designed to set up a detection mode that can be used for testing a given condition (holding current or response) of the cell. Typical example of the use of the "if" are shown in the concentration activation curve example. The "if" applies on data that have been captured in the last data acquisition epoch. For example, by the wash / test in the acquisition mode or A/D. Parameters governing the "if" action are set up in the associated window that is accessible by a double click on this icon in the program sequence. On the first double click, the cursor changes mode from the arrow to a circle to indicate the need for a user action. The user should then point to the icon where the program sequence should step back when the "if" conditions are not met.

Following a double click on the desired icon the condition window is displayed.
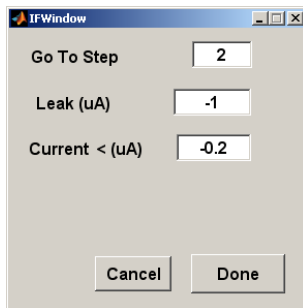
"Go To Step" indicates the icon to which the "if" is returning when the conditions are not met.

The "Leak" (expressed in μA) and current (expressed in μA) are used for the evaluation of the "if".

Unless the cursor is set up (see below) values are determined on the 10 first data points for the leak current and on the entire data epoch for the current. When the conditions are set a red line is represented in the upper part of the sequence program to indicate how the sequence executes. Equation is designed for future applications.

Loop with Increment

The loop with increment causes the program sequence to loop back to the pointed icon and to increment the position of the tested compound in the plate. For example, in the concentration activation curve the loop with increment allows to measure successively the responses evoked by a series of agonist concentrations. A double click on the loop icon changes the cursor from arrow to circle, indicating that the user can now point to the icon where the loop should go back. A double click on the desired icon turns on the corresponding window.
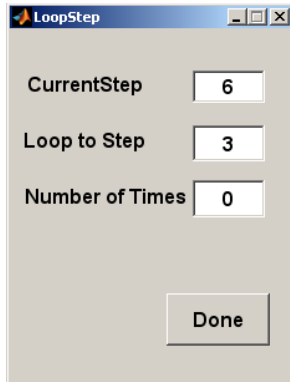
The current step indicates the icon position at which the loop was inserted. The loop to step indicates the icon at which the loop is pointing and where the program sequence will step back upon execution of the loop. The number of times indicates how many times the loop will be executed. It is of good practice to always verify that this number corresponds to the desired number of time of executions.

This value can be manually changed to reflect the desired number of time. When the button done is pushed this window automatically closes and a blue line is represented under the program sequence to indicate how the loop will take place. The number indicated in blue under the loop icon indicates the remaining number of passages. As error in setting up the loop are easily made it is important to always double check before starting a given sequence.

 Loop no Increment

The loop with no increment acts almost similarly to the loop. However, at difference with the loop with increment, this loop causes no modification of the compound to be measured. This loop can be used to measure a multiple number of time the same compound.
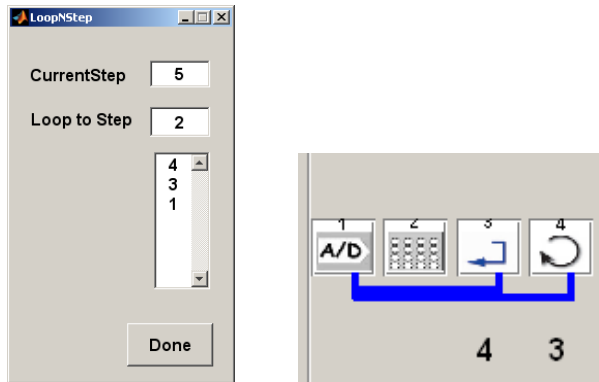

Another important difference between loop with increment and without increment is observed in the loop associated window. In the loop without increment the user can set up a list of numbers (lower editable panel) indicating the number of times the loop will be executed for each passage.

If the list contains less entry than the number of compounds the list will be repeated. In this example the first compound will be measured four times, the second three times and the third one time.

 Loop including a variable Component

The loop including a variable element can be used for defining voltage step protocols. Whenever you include the place holder "x" instead of a number, for example in the "Voltage Command" window and you loop back to this definition, you can define in the appearing "LoopX" window the values replacing the "x" incrementing with every loop count. In the shown example the potential steps go from -100 mV to +20 mV in 20 mV increments. The holding potential between the steps is -60 mV.
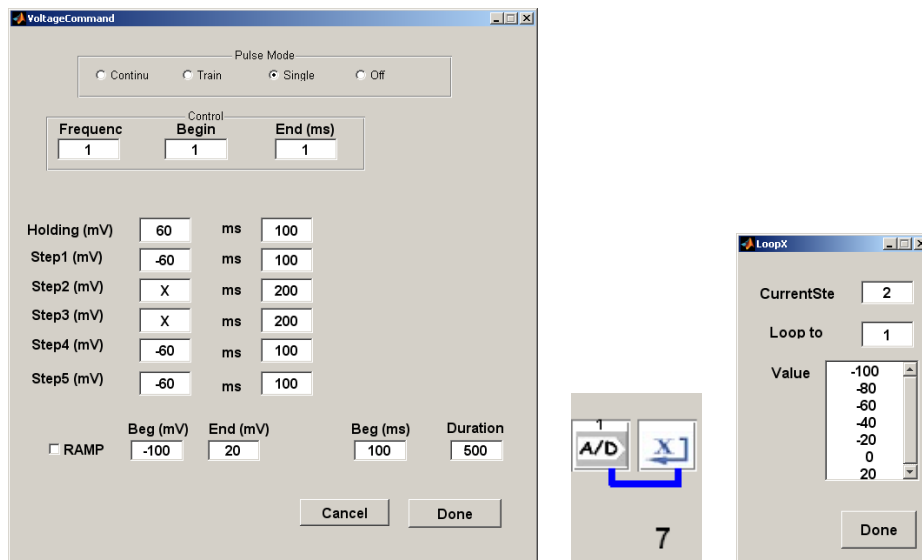
 Next DNA

Adding this icon as the last step of a program sequence allows measurements on multiple messages expressed in oocytes. A green line is automatically drawn indicating where the program will loop back upon execution of this step.

 End of the Sequence

This icon is normally placed at the end of the program sequence. Its execution causes the machine to stop, close the perfusion and remove the last cell that was measured.

Data Acquisition Icons

Execution of the icons described above allows the control of HiClamp movement and action. Additional icons are, however, necessary to perform data acquisition. Three icons control the data acquisition and can be used in the program sequence. At the end of each data acquisition the window DataPlot is turned on to display the last recorded data period.

Important: The timing of the data acquisition must be verified and the program should be set to loop back before data acquisition is completed.

 Starting Data Acquisition

This icon is typically used to start the data acquisition and to move the basket into the different compounds as shown in the concentration activation curve example. Execution of this icon initiates the data acquisition and sets up the voltage command and the amplifier conditions. The execution of the program sequence continues independently from data acquisition.

 Recording in the Washing Station

This icon indicates that the data acquisition mode has been set in the parameters of the wash / test icon. Setting up the data acquisition causes the recording of a data epoch, the parameters of which are set by the user. Execution of the program will resume only when the recording time is completed and the automate will remain in the wash chamber for the entire duration set in the corresponding parameters.

: **Recording in Plate**

Similarly to the wash / test icon, this icon is displayed only when the data acquisition mode is turned on in the corresponding plate parameters. Execution of this icon will turn on the data acquisition and maintain the program sequence at this step until completion of the recording time. This mode is particularly useful to determine voltage dependent characteristics in a given solution, for example in the determination of the properties of voltage dependent channels.
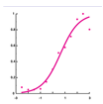
 Super Icons and loading stored Sequences

As particular program sequence may often be used in multiple experiments it is simpler to define them as Super Icons. Super Icons can be designed by the user or used from a standard icon pool. To set a Super Icon that will be represented in the upper part of HiClamp program it is sufficient to save the currently defined sequence. Upon saving the program sequence HiClamp issues a request window for setting up or not the Super Icon that can be chosen amongst *.jpeg files. To activate a Super Icon it is sufficient to click once on the Super Icon and the program automatically loads the program sequence.

Note that all the fields concerning compounds will automatically be emptied. An alternative way of loading a stored sequence consists in opening a sequence previously saved in a given experiment. Activation of the command "File -> Open" allows to browse through previously saved sequences and the user can chose any sequence generated by HiClamp. Upon loading a stored sequence, a dialog windows open requesting if the parameters of the sequence must be kept. Hitting the button "Yes" confirms that all the values, including the compound names and concentrations will be kept in the newly defined sequence. Alternatively, answering "No" will load the sequence but empty the compound names and concentrations previously defined. Note that names, values and concentrations must be filed in before using the sequence.


The Notebook

An electronic laboratory notebook is built in the HiClamp program. Access to this logbook is automatic when the first sequence is activated. The notebook window cannot be turned off during the experiment but can be minimized and recalled by a single click on the corresponding icon. The notebook window is divided in two parts. The upper section presents the notes that have been entered during the experiment. These notes are stored in the corresponding *.lab file in the data folder (see data file structure below). Notes are entered in the lower section of the window and can be modified as long as they are not entered or automatically stored by the program. The time for the beginning and ending of the experiment is automatically generated and stored in the logbook file. For security purposes the logbook is encoded and cannot be edited with a text processing program. Attempts to edit the logbook should therefore be avoided as they may destroy the file and your records.

Measuring multiple Oocytes

Playing the simple example presented before measures the properties of the first oocyte and stops.

To take advantage of the HiClamp it would be important to be able to measure an entire set of oocytes. This is readily possible by introducing a "loop" that will repeat the sequence as many time as desired. Following the addition of the "loop" icon the sequence is now the following.
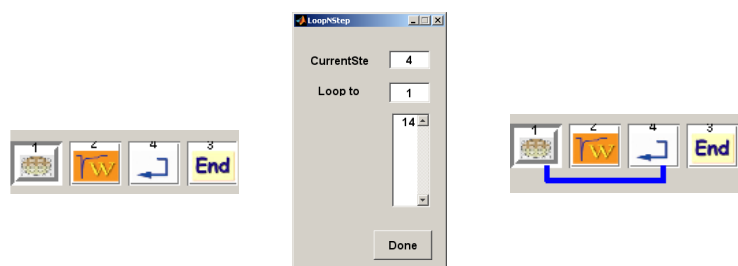

Note that the choice of the "Loop with No Increment" is indicated by the square arrow. Having introduced the loop it is now necessary to indicate at which step the program should loop back.

This is readily done by a double click first on the loop icon and the action is indicated by a change in the pointer from the arrow to a circle. A double click on the target step (in our case the load plate icon) opens a control window in which the number of times the loop should be executed is entered (for example 12 times to capture the response of an entire column of the microtiter plate).

Following the entry (done) the program sequence displays the steps that will be executed with a blue line in the lower part indicating how the loop will execute and at which steps it returns. The number of times the loop will be executed is indicated by the number displayed below the icon loop.

The Concentration Activation Curve

A first example of a program sequence is the determination of the concentration activation curve for a ligand gated channel. To establish such relationship the response of a cell exposed to a series of ligand concentration is measured and the concentration activation curve is obtained by plotting the peak of the evoked current (or, in some cases, the area under the curve) as a function of the logarithm of the agonist concentration. A sequence typically used for these measurements is illustrated in the next screenshot.
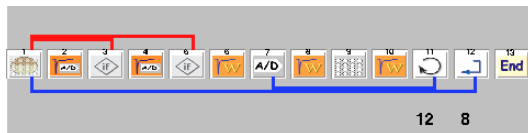
In step 1, the oocytes are automatically loaded from the loading plate (feeder). Oocytes displaying a membrane potential less than -15 mV are automatically rejected. Following a successful recording of the membrane potential the leak current is then determined in step 2 (10 s recording). Step 3 determines if the holding current satisfies the desired conditions (typically less than -1 μA). Step 4 and 5 are used to assess the cell response and cells displaying a response superior to 200 nA are retained. The concentration activation curve is then measured between steps 7 to 11. Note the red lines indicating the logical steps of the "if" whereas blue lines indicate the logical steps of the loop. Note also the difference between the two loops (with and without increment respectively). The sequence terminates once the desired number of cells is measured (here 8) or the number of available oocytes is reached.

The icon end indicates that the program stops, unload the remaining oocyte and closes the perfusion.

The program sequence is established either by inserting the icons in the proper sequence, by a single click on the icon tool bar, or by loading a predefined sequence. Loading of a predefined sequence can be done either using menu File "Open" to open a file sequence or by a single click on a "Super Icon" displayed in the upper part of the window.

**Recording Voltage-Gated Channel Activity**

Although analysis of ligand-gated and voltage-gated channels share significant similitude experimental paradigm significantly differ. While in the case of ligand-gated channels measurements generally start before moving the cell into the well containing the solution of interest recordings of voltage gated channel activity can be quite different as we shall illustrate in the next section.

The Voltage Protocols

Voltage protocols used for the activation, inactivation or characterization of voltage-gated channels include single steps, multiple steps or ramps. Voltage steps are programmed in the voltage command window. In the simplest case, lets consider that the sequence comprises the following elements:
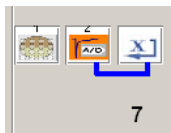
In the first step the oocyte is loaded, as previously described. The voltage protocol is defined in the voltage command window that can be seen after opening the control of the step 2.
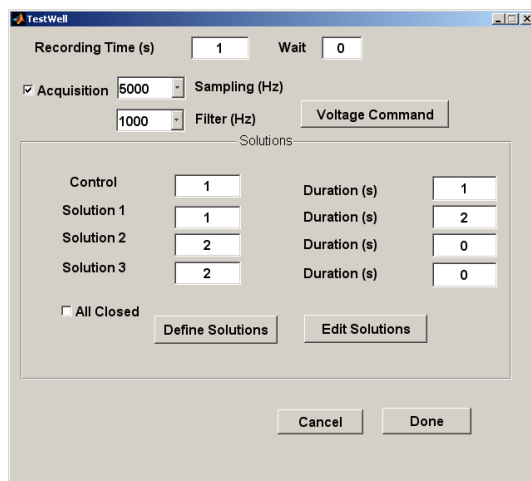
Opening the voltage command, by pressing the corresponding button, reveals the control variables accessible for the definition of single or multiple voltage steps.
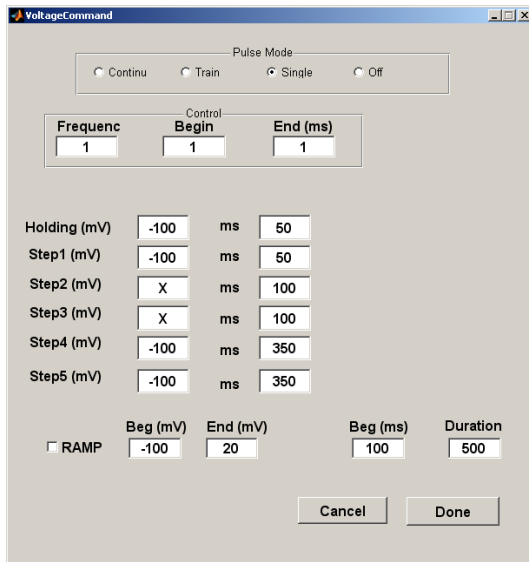
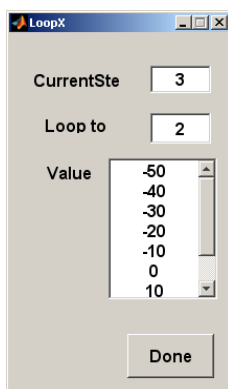A single recording data epoch (or sweep) is defined by its holding voltage -100 mV, and the number of steps the values of which are indicated in "LoopX" window.

The values above indicate which steps have to be sequentially performed. For example, to record the activity of a potassium channel it would be necessary to step the voltage between different values ranging from -50 to +10 mV starting from a holding potential of -100 mV. Activation of this voltage protocol would yield a sequence of seven voltage steps ranging from -50 mV to +10 mV by increment of 10 mV.
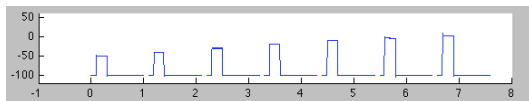
A typical example of currents recorded in a cell expressing a voltage gated potassium channel in response to a series of voltage steps is shown.
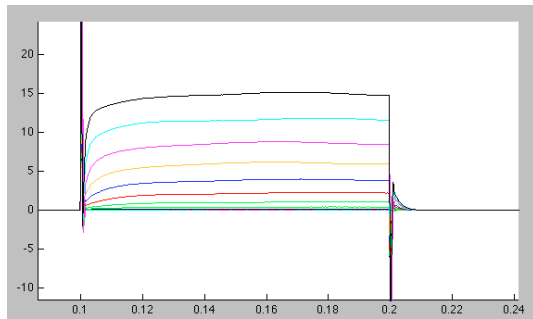
As for ligand-gated channels, data are readily analyzed in DataMining using the cursors, and can be expressed either as current in function of the voltage step or conductance. Results can be fitted with Boltzman equation to describe the parameters best representing the channel properties. Note the small tail current observed at the pulse offset. These data can be further analyzed using multiple step controls with, for example, a constant activation pulse and a second step of variable amplitude allowing the determination of the reversal potential. Such "tail current" protocols are easily implemented using the voltage command window.

Note that the voltage command window also offers the possibility to apply repetitive steps at regular time intervals.

DATA ANALYSIS

As the HiClamp is intended to perform experiments in an unattended manner this means that data captured by the robot should be self-containing and easily identifiable. Typical experiments will run for hours and often during day or night. Moreover, data concerning a particular set of experiments may be captured in a single day or on several days and the data analysis software must allow flexible analysis to be performed and regrouping of data coming from different experiments.

Data analysis is performed using two complementary program called **DataMining** and **DataMerger**. **DataMining** was designed for the analysis of a single experiment and to store results in a form that is compatible for multiple analysis. **DataMerger** was designed to combine experiments and prepare results for report presentation.

Before examining in more details **DataMining** and **DataMerger** programs it is, however, of value to examine the data files created by the **HiClamp**.

Data File Organization

```
1-17-Dec-2010-1.mat
1-17-Dec-2010-1.seq
1-17-Dec-2010-2.mat
1-17-Dec-2010-2.seq
1-17-Dec-2010-3.mat
1-17-Dec-2010-3.seq
1-17-Dec-2010.lab
1-17-Dec-2010.vic
```

A rapid look at the disk organization reveals that HiClamp creates folders that are organized by year, month and day of experiment. Each HiClamp sets its own folders according to its reference number set in the preference. HiClamp files include *.mat, *.seq, *.lab and *.vic.

Note: Do not erase any of these files otherwise data will be corrupted and reading will be disrupted.

A given folder might contain one or more *.mat and *.seq files, each of them corresponding to the activation of a play sequence. The *.mat contains the program sequence that was played whereas the *.seq contains all the sequence of events that have been captured during the particular experiment.

The *.lab stores the laboratory notebook. Note that for laboratory safety, this sequence is encoded and cannot be read into a text processor. The *.vic sequence stores all the data that have been captured.

Data are stored in a random access manner with each of the data epoch having independent record length. Critical parameters such as voltage steps, setting of the amplifier etc. are stored for each record and can be read during analysis.

DataMining
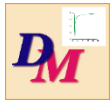
The **DataMining** was specially designed for the treatment of data captured with the HiClamp. The aim of this program is to provide the user with a convenient interface allowing efficient data retrieval and analysis. Upon activation of this program a window resembling the HiClamp environment is displayed.

The screenshot shows the main window of DataMining software.

DataMining window is divided in the tools for the upper panel, the program (middle panel) and the sequence (lower panel). Selection the desired file to analyze is made by activating the command **Open** in "File" menu**.** Selecting the desired sequence (*.seq) opens the sequence that is displayed in the program panel (middle panel) and the screen might look as in the following screenshot.
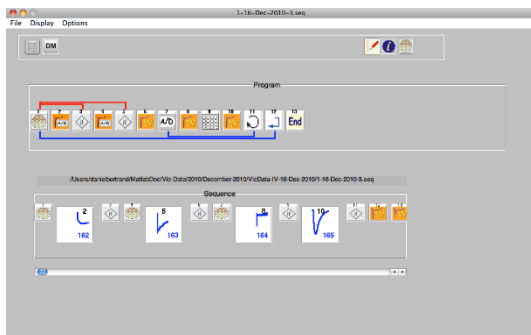

The program sequence that is displayed in the middle panel is identical to the program sequence used for the control of HiClamp. The lower panel illustrates the sequence of events that took place during the data acquisition in a "chart paper like" recording. In this example you can see that the HiClamp took three oocytes from the loading plate before finding one responsive cell as indicated by the three oocyte loading icons that are seen in the initial section of the sequence. Note that the sequence continues with the recordings (following the response in position 10, record 166, indicated in blue). To scroll through the sequence, please use the toolbar placed underneath the sequence.

DataMining takes full advantage of the icon-based concept allowing the user to efficiently analyze data with a single click. The concept is that selection of an icon representing a data acquisition module, such as steps 2, 4 or 7, in the example above, must trigger the analysis of data that have been captured during the experiment and that correspond to this particular step of the program sequence. Selection of the icon 4 should display all the oocytes that have been retained as displaying appropriate leak current.

A click on the icon 7 should display all the data that have been captured for a given oocyte. Each oocyte is represented versus its position in the loading plate. A typical display obtained by this action is illustrated in the screenshot below.

As the HiClamp system contains two plates with the oocyte loading plate and the compound plate, two modes of representation can be envisaged. A first mode, illustrated below, consists in representing data versus the position in the loading plate. Alternatively, data can be represented versus the compound position. Switching between these two representation modes is obtained by a click on the oocyte (or plate) symbol in the upper right panel. The mode of representation corresponds to the icon displayed at the instant of the data selection.
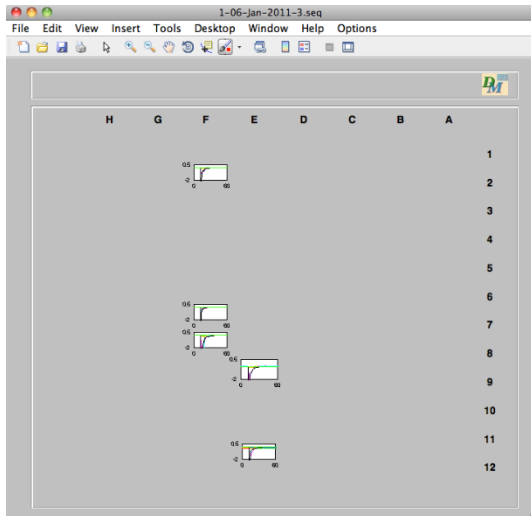
Recordings obtains in a series of oocytes are represented versus their respective position in the loading plate. The empty positions correspond to cells that did not meet the desired criteria (resting potential, leak currents and agonist evoked current).

A single click on the lower left corner of any of these small graphs displays the corresponding current traces as shown in the screenshot.
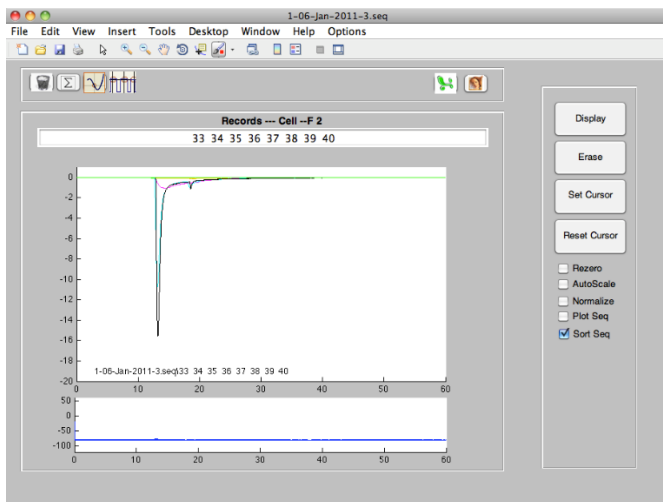
Another form of representation for the same data can be obtained by selecting the mode Plot Seq. When this mode is selected data are represented as on a chart paper (see screenshot).
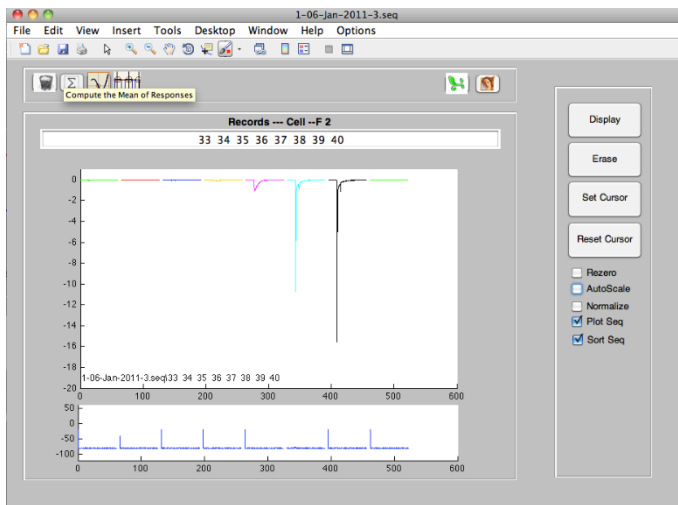
Measuring the Current Amplitudes

"Measurement of the peak ACh-evoked current" is carried out automatically within boundaries defined in the "Set Cursor" command. This command is activated by a single click on the button presented in the right panel. Activation of the "Set Cursor" changes the display as follows.

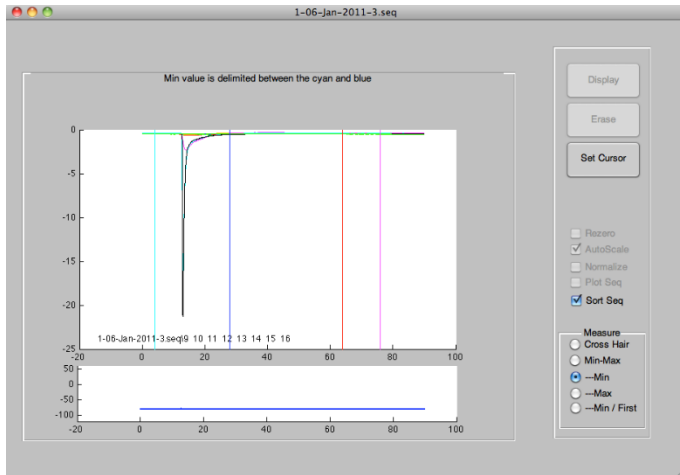Several cursor options are possible allowing measurements at the cyan and blue lines. Selection "Cross Hair" returns the delta current between the two lines with the blue line at the minimum. Min Max returns the delta current taking the minima and maxima between the two lines. –Min returns the delta current between the value measured at the cyan position and the minimal value measured up to the blue line.

–Max returns the maximal delta current measuring the minimal value at the cyan position and maximal between the two lines. –Min/First returns the minimal value measured between the cyan (taken on the first measured curve) and blue line at the current curve. Position of the minimal and maximal values determined for different curves can always be visualized using the button. Positions will be represented on the data curves by a blue and red circle.

Once cursors are set the ensemble of the data captured in a single run can be analyzed by choosing the calculator mode and by a single click on the A/D after activation of the calculator that is display in the right part of the commands.
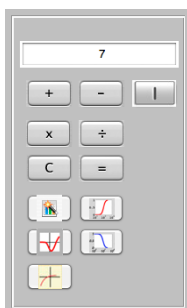
Following the selection of the A/D the calculator should display the number 7, which corresponds to the position of the A/D in the data acquisition program. Activation of the concentration activation curve is performed by a click on the corresponding symbol (red curve). The successive window allows the selection of the concentration of agonists that were tested in the experiment.

Following the selection of concentrations, the graphs corresponding to the concentration activation curves are automatically displayed.
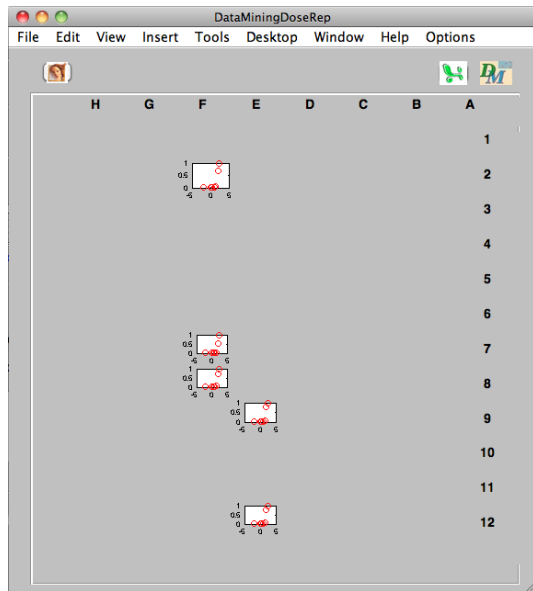
In this graph the curves measured for each of the cell tested are represented as function of the cell position in the loading plate. In this particular experiments five cells have been measured during the sequence No. 3. Data can either be analyzed further within DataMining or saved for later analysis with DataMerger. DataMerger is a companion program specially designed for the analysis of results obtained in different experiments. A single click on the upper right icon saves data for DataMerger. This will create a *.mat file that can later be open into DataMerger.

The e-Labbook of the HiClamp

The electronic laboratory book that opens when HiClamp is set to run is displayed when the sequence is read in DataMining. In this example the e-labbook is presented as follows:


Note that each sequence is indicated with the date, time of activation and end. A double click on a selected sequence automatically opens this sequence in DataMining.

DataMerger

DataMerger program was especially conceived for the analysis of data selected with DataMining and for combining experiments acquired in different session. Typically, experiments obtained on day one might need to be compared with experiments from day two or analyzed simultaneously.

DataMerger allows analysis of single point measurements, multiple measurements, concentration activation or inhibition curves, or voltage-dependent channel activity. Examples below are illustrating the power of DataMerger for the data analysis and preparation of table and / or data summary.

In the concentration activation curve illustrated in the DataMining example, curves were saved as ACh.mat. These data can be read into DataMerger using the command **Open** in "File" menu and choosing the file ACh.mat. The following window will appear.
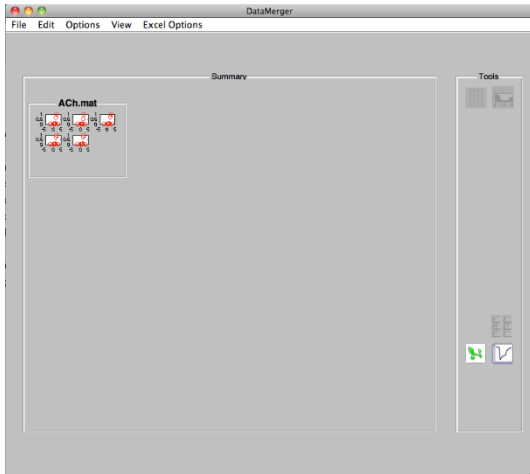
Note that each concentration activation curve is represented by a corresponding graph. Curves can then be traced either in isolation by a single click on the lower left corner of the graph or individual traces can be retrieved by CTRL (right click). Activation of this command browse the data file and retrieves the corresponding curve allowing constant visualization of the data quality. Concentration activation curves can be traced one by one, by selecting each graph, or all together by the option "Plot All" that will display the ensemble of curves.
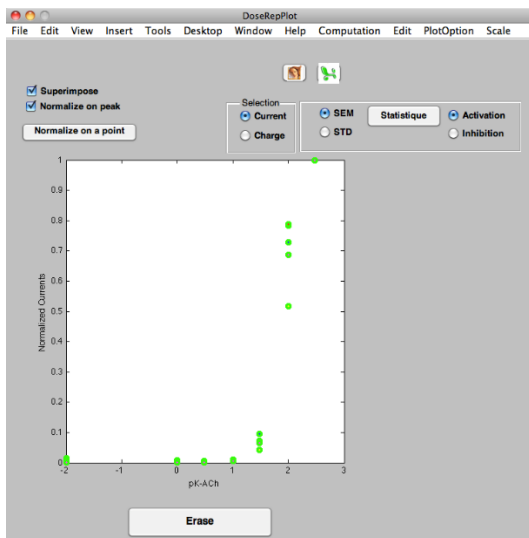
Statistic

Activation of the statistic button displays the average and SEM for the points that can be fitted by a single or dual Hill equation. Note that each curve can be fitted separately and the mean of curve can later be computed and analyzed. Values corresponding to the original data points and normalized values are stored in an excel compatible format by activation of the excel icon.

For the single Hill equation and a concentration activation curve the equation used for the curve fitting is in the form:

$$Y = 1 / 1 + (EC_{50} / x)^{\wedge} n_H$$

where: y = the fraction of evoked current, EC_{50} = concentration for 50 % activation, nH = the apparent cooperativity, x = agonist concentration.

Dual concentration-activation curves are fit using the equation:

$$Y = a / 1 + (EC_{50H} / x)^{\wedge} nHH + (1-a) / 1 + (EC_{50L} / x)^{\wedge} nHL$$

where: y = the fraction of evoked current, EC_{50H} = concentration for 50 % activation and nHH = the apparent cooperativity of the high affinity component, a the fraction of high affinity component, EC_{50L} = concentration for 50 % activation and nHL = the apparent cooperativity of the low affinity component , x = agonist concentration.

Concentration inhibition curves are fitted using comparable single or dual Hill equation with:

$$Y = 1 / 1 + (x / IC_{50})^{\wedge} n_H$$

where: y = the fraction of remaining current, IC_{50} = concentration for 50 % inhibition, nH= the apparent cooperativity, x = antagonist concentration.

Dual concentration-inhibition curves are fitted using the equation:

$$Y = a / 1 + (x / IC_{50H})^{\wedge} nHH + (1-a) / 1 + (x / IC_{50L})^{\wedge} nHL$$

where: y = the fraction of remaining current, IC_{50H} = concentration for 50 % inhibition and nHH = the apparent cooperativity of the high affinity component, a the fraction of high affinity component, IC_{50L} = concentration for 50 % inhibition and nHL = the apparent cooperativity of the low affinity component , x = antagonist concentration.

Example of a Curve Fitting

Examples of a curve fitting for the concentration activation using SIMPLEX.

Curve fitting of the data presented above can be effectuated in different mode. A first mode consists in determining the best fit for each of the curve. This is done by selecting any one of the curve (click on the desired data point).

When a curve is selected the corresponding data points are represented as stars and the curve fitting parameters are displayed in the right window.
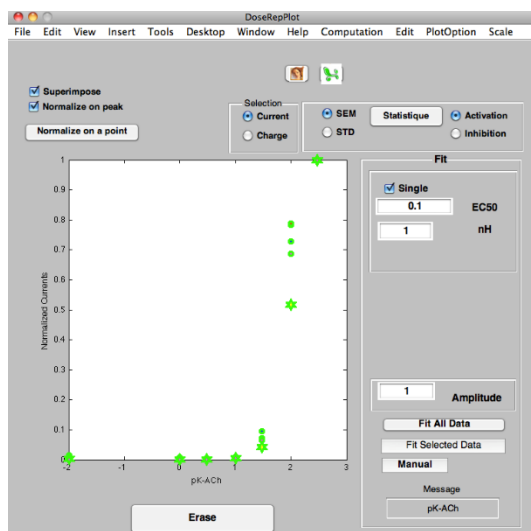
To optimize curve fitting it is often recommended to provide a first guess of the parameters. In this example we shall select 100 µM for the EC_{50}.
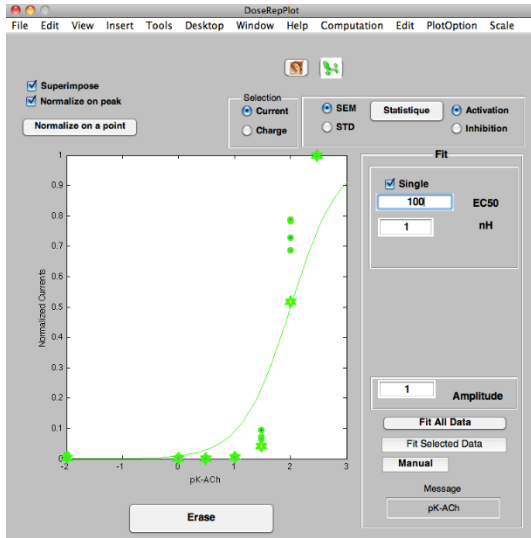
A continuous line corresponding to the guess parameters is displayed upon keying the desired value and the display should appear as shown.

Pressing "Fit Selected Data" initiates the curve fitting procedure and result with the best fit is displayed.
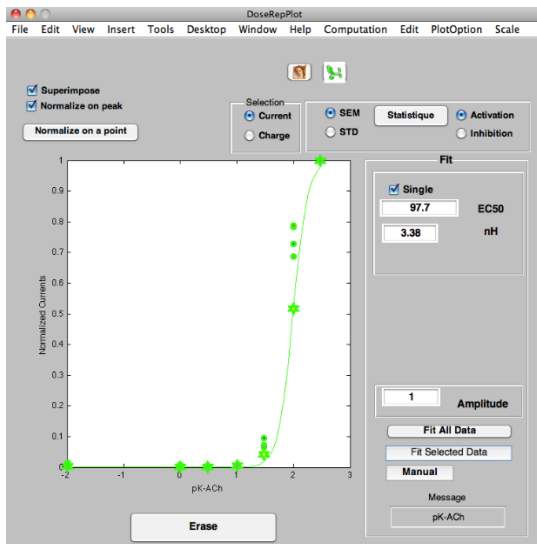
Values indicated in the windows correspond to the best fit obtained from the guess starting point. Pressing "Fit All Data" initiates the curve fitting procedure on each of the curve and results in:
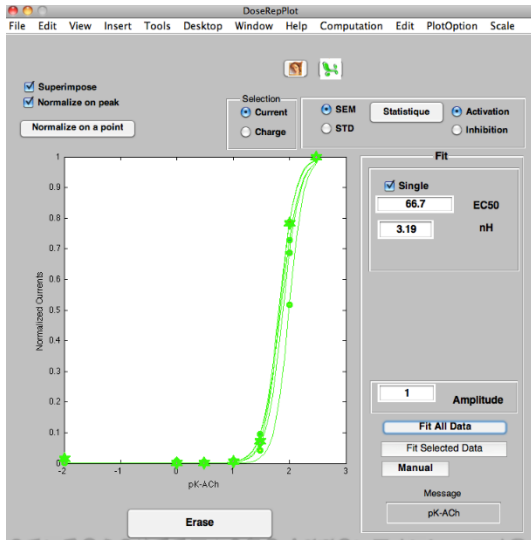
Pressing the "Statistics" button computes the standard error of mean and display the average points together with the SEM.
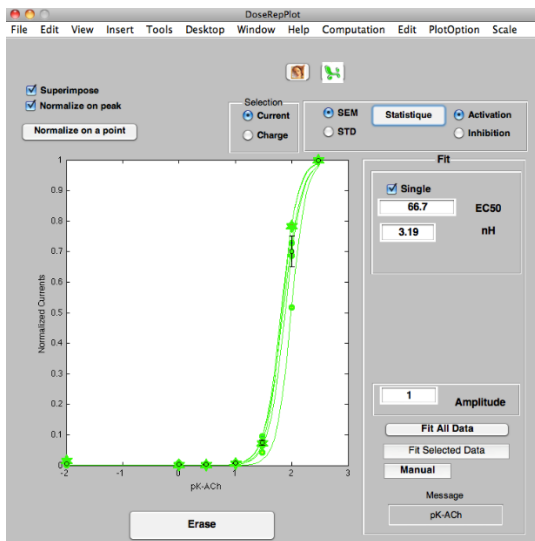
Selecting the average curve activates the display of an additional button and computation of the "Mean of Curves" can be effectuated.
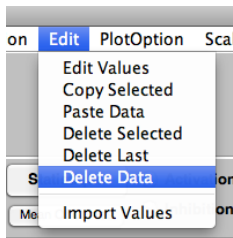
As in this example the mean of curves fits nicely the average curve no further adjustment is necessary. Data can now be removed by selecting "Delete Data" in the menu.

Leaving only the average curve in the display. Data can be further processed and saved as an excel data sheet for easy reporting by pressing the "Excel" button.

The corresponding Excel data sheet should be as following:

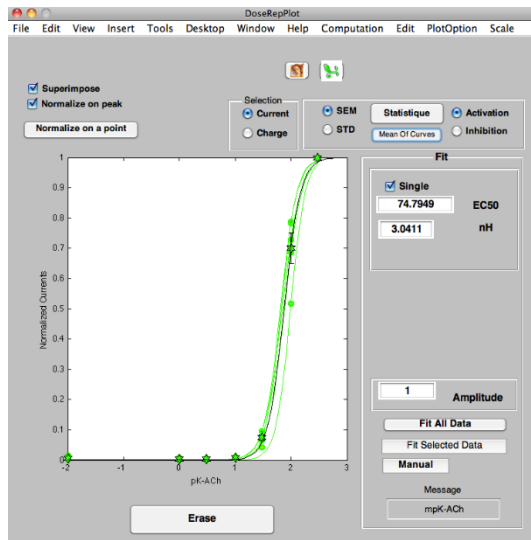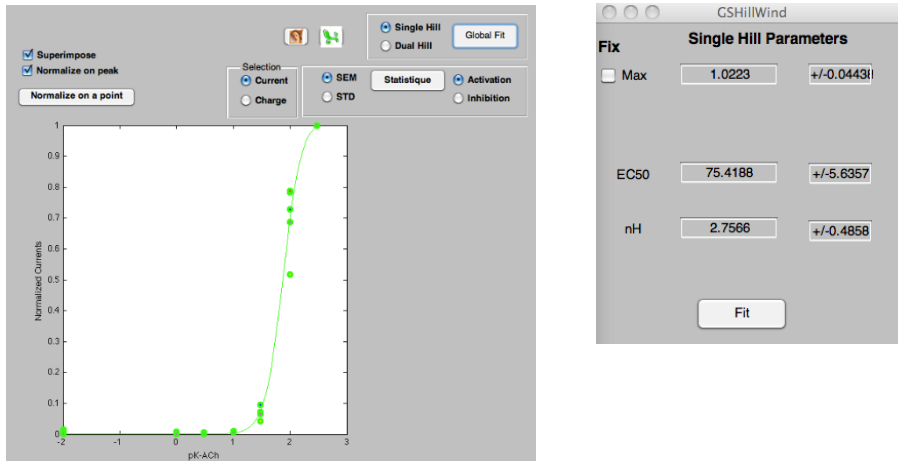| pK-Ach	File = /Users/danielbertrand/MatlabDoc/Vic-Data/2011/January-2011/VicData-X-06-Jan-2011/							
Activation								
Message		**Concentratiol**	0	1	3	10	30	100	300
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	F- 2	0.0305	0.0244	0.0275	0.107	1.02	10.7	15.5
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	F- 7	0.0305	0.0214	0.0275	0.0671	0.513	6.3	12.2
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	F- 8	0.0916	0.0366	0.0275	0.223	2.07	15.7	21.6
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	E- 9	0.15	0.15	0.107	0.201	1.97	16.4	20.9
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	E-12	0.397	0.0519	0.0336	0.162	1.97	21.1	26.9
		Average	0.14	0.0568	0.0446	0.152	1.51	14	19.4
		SEM	0.0679	0.0238	0.0156	0.029	0.314	2.54	2.55
Normalized								
Message		**Concentratiol**	0.01	1	3	10	30	100	300
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	F- 2	0.002	0.0016	0.0018	0.00688	0.0654	0.688	1
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	F- 7	0.0025	0.0018	0.0023	0.00551	0.042	0.516	1
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	F- 8	0.0042	0.0017	0.0013	0.0103	0.0959	0.727	1
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	E- 9	0.0072	0.0072	0.0051	0.00964	0.0944	0.788	1
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	E-12	0.0148	0.0019	0.0013	0.00602	0.0732	0.784	1
		Average	0.0061	0.0028	0.0023	0.00767	0.0742	0.701	1
		SEM	0.0023	0.0011	0.0007	0.00097	0.01	0.05	0

			EC50	nH	Scale			
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	F- 2	76.1	2.9	1			
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	F- 7	97.6	3.4	1			
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	F- 8	69.3	2.7	1			
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	E- 9	64.2	3	1			
alpha7	/VicData-X-06-Jan-2011/1-06-Jan-!	E-12	66.7	3.2	1			
		Average	74.8	3.04	1			
		SEM	6.04	0.113	0			
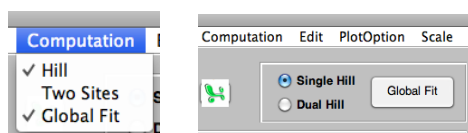
Example of curve fitting using "Global Fit"

An alternative to SIMPLEX curve fitting is to fit all the available data using the technique of "Global Fit". To activate the global fitting procedure, it is first necessary to select the option in the "Computation" menu bar.

Once the selection of "Global Fit" is made a new option set is displayed allowing the selection of single or dual "Hill" equation.
In our example data points are readily fitted by a single "Hill" equation and activation of the "Global Fit" yields the following results.

Result from the "Global Fit" are indicated in the new floating window and best fit obtained with this method is illustrated by the continuous line. Note the similitude of results obtained with the SIMPLEX curve fitting method. The check box (Max) allows to fix the maximal value to the desired constant and new fitting can be initiated by pressing the "Fit" button.

As before results are readily obtained in the form of a spreadsheet by pressing the Excel button. Results are displayed as follows:

| pK-ACh	File = /Users/danielbertrand/MatlabDoc/Vic-Data/2011/January-2011/VicData-X-06-Jan-2011/1-06-Jan-2011.vic							
		ACh	ACh	ACh	ACh	ACh	ACh	ACh
Activation								
Message	Concentratio	0	1	3	10	30	100	300
alpha7	/VicData-X- F- 2	0.0305	0.0244	0.0275	0.107	1.02	10.7	15.5
alpha7	/VicData-X- F- 7	0.0305	0.0214	0.0275	0.0671	0.513	6.3	12.2
alpha7	/VicData-X- F- 8	0.0916	0.0366	0.0275	0.223	2.07	15.7	21.6
alpha7	/VicData-X- E- 9	0.15	0.15	0.107	0.201	1.97	16.4	20.9
alpha7	/VicData-X- E-12	0.397	0.0519	0.0336	0.162	1.97	21.1	26.9
Message	Concentratio	0.01	1	3	10	30	100	300
alpha7	/VicData-X- F- 2	0.00197	0.00157	0.00177	0.00688	0.0654	0.688	1
alpha7	/VicData-X- F- 7	0.0025	0.00175	0.00225	0.00551	0.042	0.516	1
alpha7	/VicData-X- F- 8	0.00424	0.00169	0.00127	0.0103	0.0959	0.727	1
alpha7	/VicData-X- E- 9	0.00716	0.00716	0.00511	0.00964	0.0944	0.788	1
alpha7	/VicData-X- E-12	0.0148	0.00193	0.00125	0.00602	0.0732	0.784	1
		EC50	nH	Scale				
		Max	EC50	nH	pEC50			
		1.02E+00	7.54E+01	2.76E+00	-4.12E+00			
	CI 95%	4.44E-02	5.64E+00	4.86E-01	3.13E-02			

Merging Data from different Experiments

Experiments may have been conducted on the same or different days in identical or different conditions. DataMerger was designed to facilitate data analysis and to merge data sets. For example, if two data sets have been collected and analyzed separately, data are easily merged using the procedure:

Open DataMerger Program.

Open the different *.mat produced with DataMining. Data will appear in DataMerger and sorted according to the *.mat name.
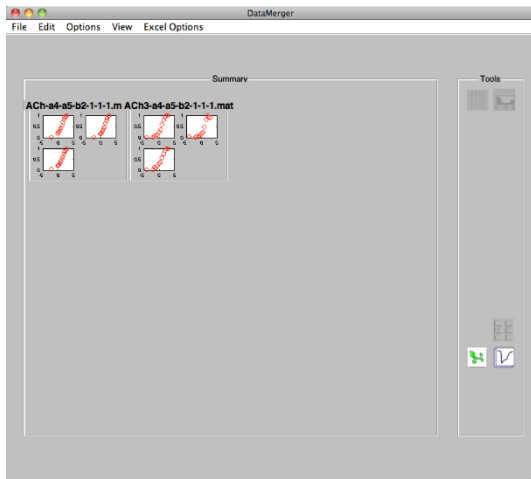
To merge data press "CTRL" and click on the first data set. The pointer will be changed from the arrow to a circle indicating that the merge is activated.

Maintaining the "CTRL" key, click on the panel you want to merge. The two panels should change and results should be merged as follows:
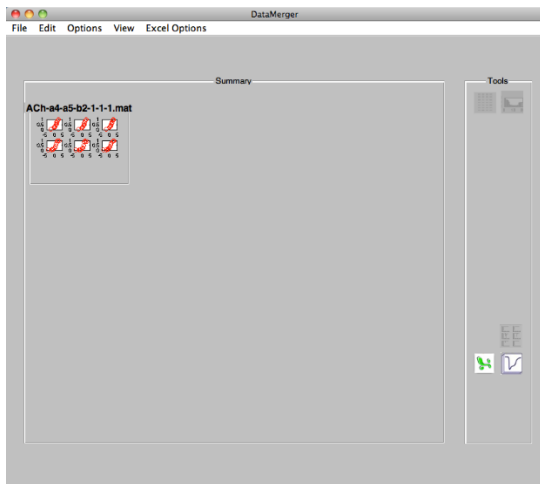
Using the Option "Plot all" displays all the curve at once.

Note: If the curves have not the same number of points or not the same concentration, statistics and curve fitting with Simplex will be restricted. Curve fitting can, however, be performed using the global fit that now analyzes all the curves as a single ensemble.

EXAMPLES

The Voltage Activation Curve of Kv1.4

Two alternatives can be used for the measurement of the voltage activation curve by measuring with the current activated during a voltage step and measuring of the relaxation current.

The program sequence for both examples was the same and voltage steps were defined by setting Step 1 to -50, 20 increments of 5 mV each. Setting Step 2 to -50 mV evoked a tail current of sufficient amplitude for the measurement of the relaxation.
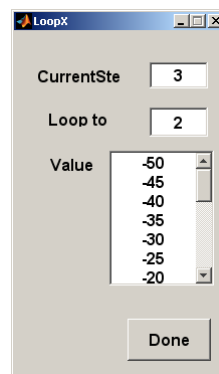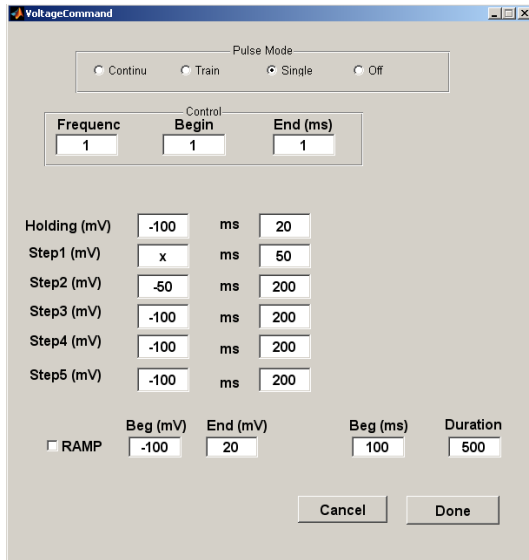
In the first case the current is measured during the voltage step and needs to be subtracted for the passive properties of the cells. Recording performed in oocytes expressing the channel KV1.4 illustrate how a progressive depolarization of the membrane activates the potassium channel.

The Screenshot shows the Activation of KV1.4
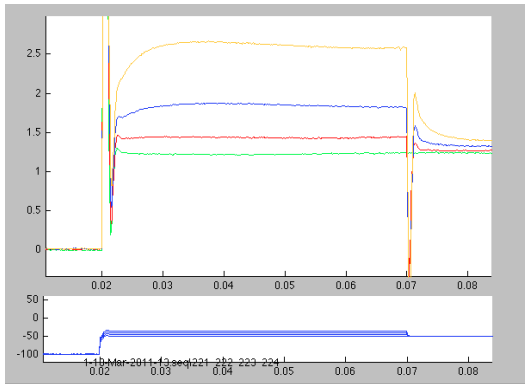
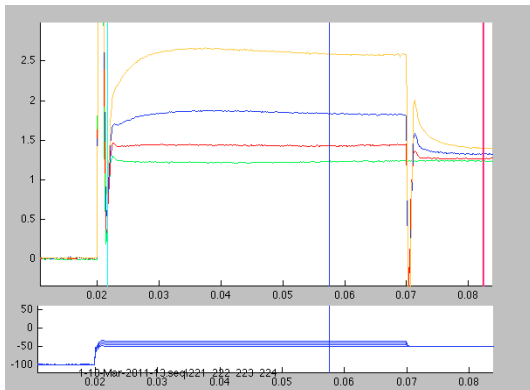Placing the crosshair (in the crosshair mode) immediately at the end of the capacitance discharge and at the end of the voltage step allows a first estimation of the current activated by the voltage step. Ideally, measurement of the current should be subtracted from the current passing through the passive properties of the membrane which is usually obtained by subtracting the current measured for the same voltage step in presence of a selective blocker.

Positioning the Crosshair for KV1.4 Activation

After setting the crosshair at the desired position the selection of the calculator is made and the appropriate sequence step is selected by a single click. The number corresponding to the step is displayed in the calculator window and results are readily obtained by selecting the potassium IV button.

The window displaying all the IV curves measured in different oocytes is displayed and the desired IV can be displayed separately by selecting the appropriate graph. The current-voltage window displaying the voltage activation curve is displayed.

Current-Voltage Activation Curve for KV1.4
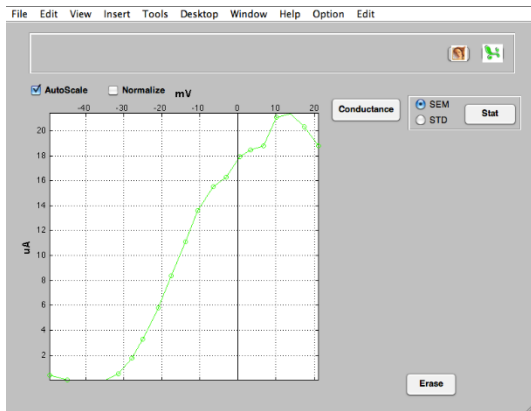
A plot of the corresponding conductance can be obtained by pressing the button "conductance" and setting the appropriate reversal potential for the considered ion (-80 mV in our example).

Data can be fitted using the typical procedure of selecting the desired curve, selecting the initial parameters, and activation of the curve fitting procedure. Data are fitted using a single **Boltzman** equation. Note that results of the curve fitting are applied to the IV curve allowing to compare the result of the fit with the data.

Plot of the Conductance for KV1.4 and Curve Fitting
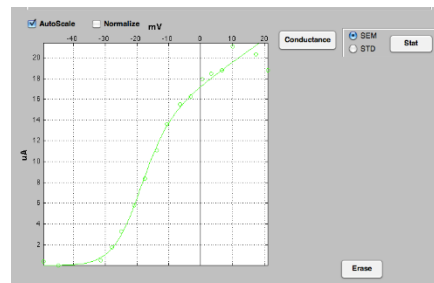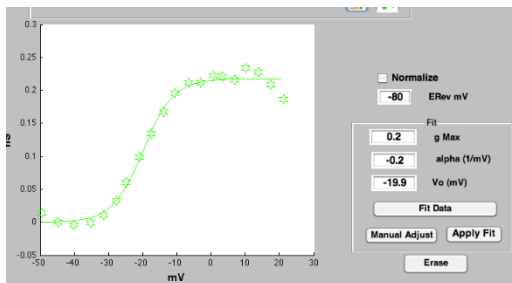
In the second case the current evoked by a voltage step is measured at the very beginning of the relaxation curve. This second method offers the advantage of a direct measurement of the current without requirement of the blockade by a selective inhibitor. Positioning of the crosshair is adjusted after a blow up of the section of interest using the magnifying tool.

Positioning of the Cursor on the Relaxation Tail
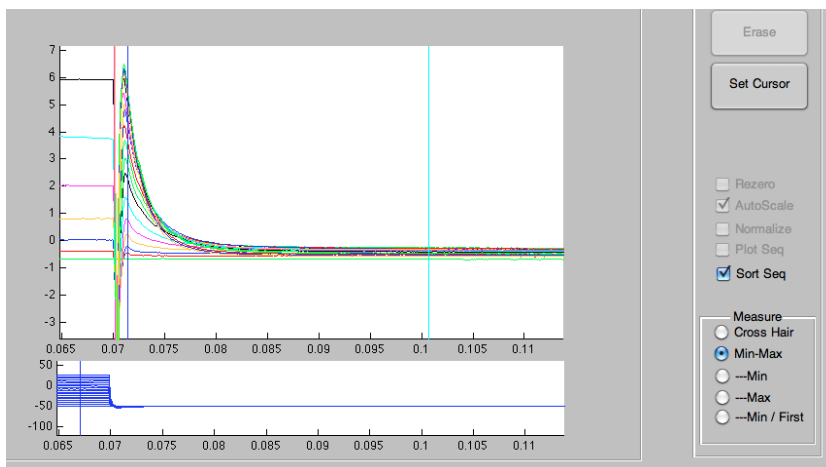
Note that the blue cursor is placed where the current is measured, the cyan (min value) towards the end of the recording and the dark blue in the voltage window at the position of the voltage step that activated the KV1.4 current and selection of "Min-Max".

Results are obtained as described above for the activation steps and yielded comparable activation profiles. The main advantage of this second procedure is to allow measurements at a fixed potential and thereby alleviate the needs for the subtraction of traces in presence of a blocker. This method is often used to characterize the voltage activation or blockade of K-channels such as HERG channels.

Measurement of the relaxation current at a fixed potential is also often used for the characterization of blocking agents (Windisch et al., 2011).

Voltage Activation measured on the Relaxation of Tail Current
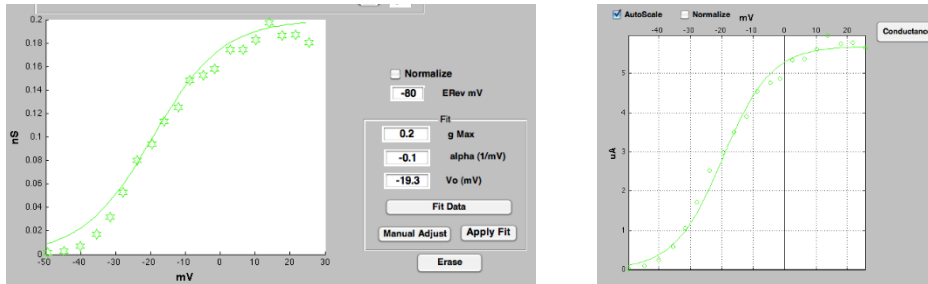
The Voltage Activation Curve of NaV 1.5

To determine the voltage activation relationship for NaV 1.5 a standard series of voltage steps are applied at periodic intervals. Voltage steps of regular increments can be set in the voltage command interface either from Wash/Test, A/D or Plate with acquisition. Currents recorded in response to a series of voltage steps in an oocyte expressing the NaV 1.5 are illustrated in the screenshot below. The voltage protocol is illustrated in the lower traces. Automatic detection of the peak inward current can be done using "Min-Max" setting with the cyan and blue crosshair positioned as on the figure.

Voltage steps evoke inward currents in NaV 1.5:
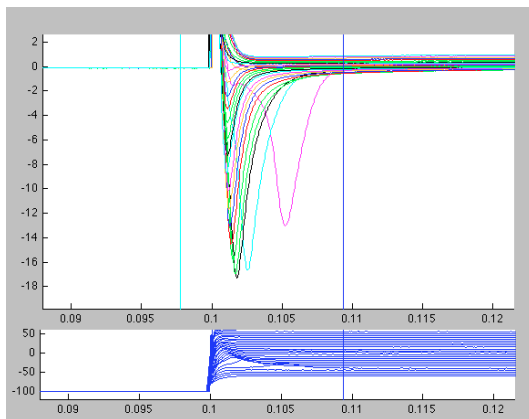
Inward currents are analyzed using the "calculator mode" and selecting the appropriate icon corresponding to the voltage step protocol. Display of the results is obtained by selecting the icon indicating the typical IV curve of a sodium channel.

The representation of the loading plate with oocytes recorded for the given experiment setup is represented and each single IV curve is shown as a "mini" graph as the following screenshot. In this experiment a single oocyte was probed and the desired IV can be drawn by selecting the "mini" graph represented on the left of the figure above.

Plot of IV curves for the NaV 1.5
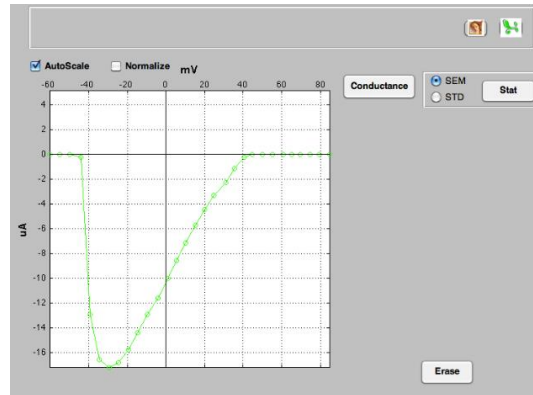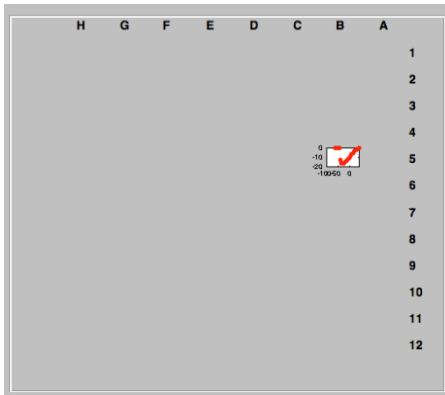
The plot of the conductance as a function the voltage step is readily obtained by activating the conductance button. Note that the reversal potential is automatically proposed in the Erev window, please see the next screenshots.

Conductance Plot and Reversal Potential
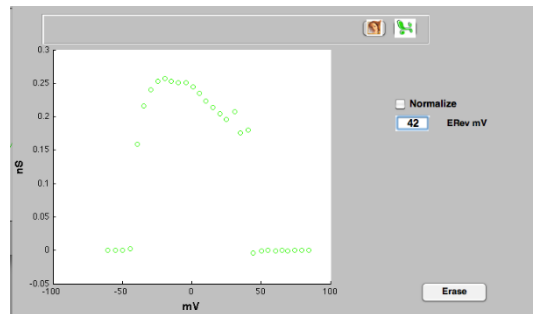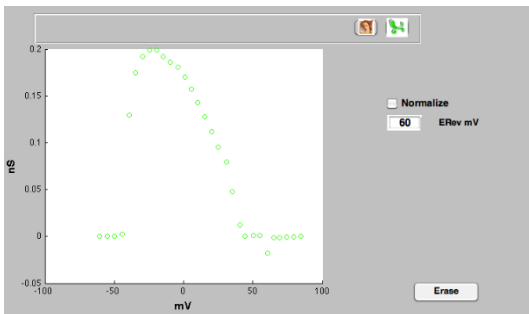
Adjustment of the reversal potential deduced from the left screenshot result the graph presented in the right screenshot. Importantly, conductance plot have large uncertainties for values close to the reversal potential and meaningless points should be removed.

Removal of the desired sets of points is done by selecting delete points in the option window. A dashed rectangle is drawn and adjusted with the mouse pointer encompassing the points that must be removed.

Deleting an ensemble of points:
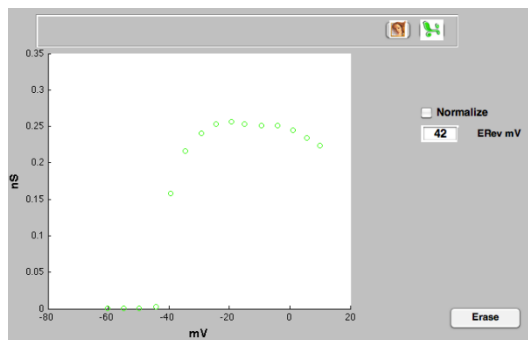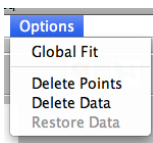
Curve fitting of the desired curve is done by selecting the curve of interest and with the Boltzman equation. Following selection of the curve, done by a single click on any one of the corresponding symbol, a window displaying the parameters of the Boltzman equation is displayed.

Curve fitting of the conductance with a Boltzman equation:
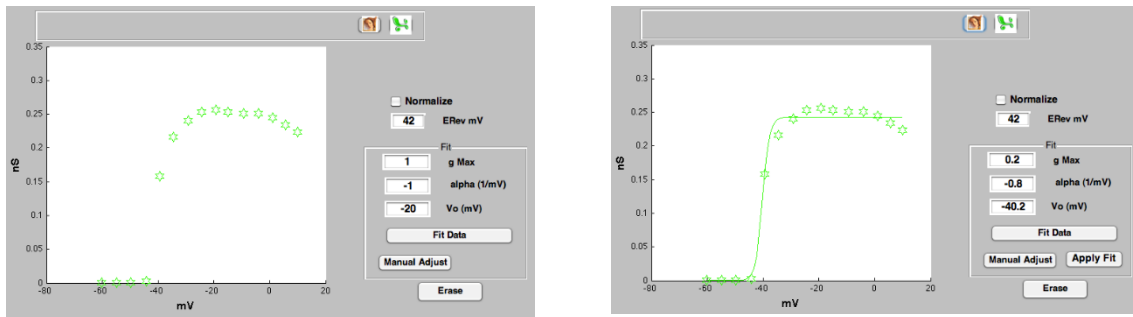
On the completion of the curve fitting results from the conductance fit are applied to the IV curve to assess the quality of the fit on the current voltage relationship and yielding the plot illustrated in the screenshot above. Note that multiple curves and statistic are treated equally using this process.

Curve fitting of the IV Curve for NaV 1.5
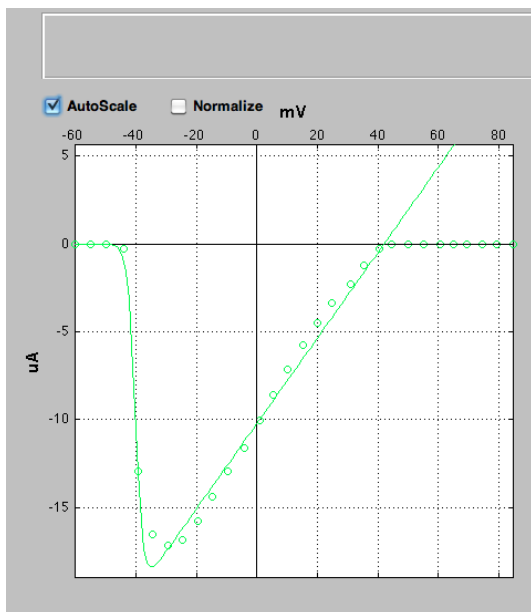
TROUBLESHOOTING

Most problems occur seldom and only under specific circumstances. In most cases, it is only a minor problem that can be easily avoided or solved.

If the problem persists, please contact your local retailer. The highly qualified staff will be glad to help you. Please inform your local retailer as well, if other problems that are not mentioned in this documentation occur, even if you have solved the problem on your own. This helps other users, and it helps MCS to optimize the instrument and the documentation.

Please pay attention to the safety and service information (chapter "Important Safety Advice". Multi Channel Systems has put all effort into making the product fully stable and reliable, but like all high-performance products, it has to be handled with care.

SERVICE AND MAINTENANCE

Please make sure that you read this chapter thoroughly. Proper handling and cleaning of the HiClamp will ensure reliable and long life time of the equipment. Most often, troubles are simply due to mishandling. See also chapter "Troubleshooting".

Cleaning of the System

Always unplug the HiClamp devices prior to cleaning. To clean the exterior surfaces, use a lint-free cloth to remove loose dust. For more efficient cleaning, use a soft cloth dampened (not soaked) with an aqueous solution of 75 % isopropyl alcohol, or a mild detergent.

Cleaning of the Basket

The basket is the most delicate part of HiClamp.

Note: Work on the basket only when absolutely necessary and with great care!

The following sequence is designed to allow cleaning of the basket with a maximum of security. Remove the electrodes as usual. Lower the table (button down on the manual command). Grab the basket holder on the side as shown on the picture.
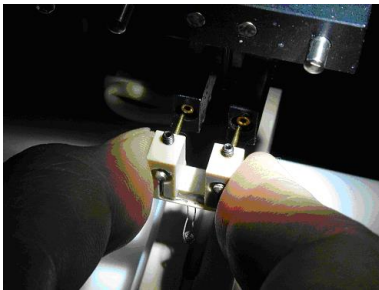
Gently flush the basket with water without touching it.

Replace the basket in position.

Warning: If you noticed that the basket is not aligned or anything special, you probably need to replace the basket.

Replacing or Realigning

If by accident the basket was damaged it is necessary to replace the basket. It is strongly recommended not to attempt to repair the basket because both the wire position and its shape are critical for the proper functioning of HiClamp.

As the basket is a critical part of HiClamp it was designed as an easily replaceable part using the following procedure.

1. Remove the electrodes as usual.
2. Lower the table (button down on the manual command).
3. Grab the basket holder on the side as shown on the picture above
4. Replace with the new basket
5. Align the basket if necessary.
6. Select the option "Adjust Basket" from the menu bar.
7. Select the basket position to "Load Basket". The basket will move into the loading position and the suction tube is lowered towards the basket. The suction tube will be used as the reference point to which the basket needs to be aligned.
8. Use the two small Allen keys screws to bring the basket in the closest juxtaposition with the suction tube but without contact.
9. Use the "Backward" or "Forward" button to align the basket to move the basket at the desired position.
10. Press "Done" to close the "CalibBasket" window.

HiClamp is now ready for your next measurements.

Preparation of Oocytes

Rough selection by filtration

1. Fill a 100 ml beaker with 80 ml of Barth's solution. Place the oocyte mesh sieve into the beaker for grading the oocytes by size. The mesh of the sieve should be completely immersed in the fluid.
2. Pipette an amount of oocytes onto the sieve. Approximately half the sieve should be covered with oocytes. Too many oocytes on the filter will lead to an inefficient filtration.
3. Gently move the filter about two centimeters up and down (in the fluid) to separate the oocytes by size.
4. Transfer the oocytes of appropriate size (that did not go through the sieve) into a 60 mm Petri dish filled with Barth's + gentamicin.
5. The filtered oocytes are incubated at 18 °C for 1 h.

Note: The incubation step is necessary for identifying damaged oocytes in the next step.

Fine selection

Use a stereo microscope and the golf-club shaped glass tool to check each single oocyte for the following criteria.

Outer form:

- No visible damage of the cell.
- Well separated colors (dark and light brown).
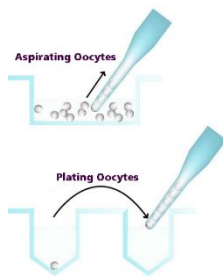- No residues of follicular tissue.

Size:

Uniform size of about 1.2 mm.

Note: Selecting oocytes is an important step. Perform it very carefully to obtain best results.

Plating Oocytes

You need a well plate filled with Barth's + gentamicin.

Important: Visually check all well plates before use. Use only unsterile and very flat plates. Sterilization may lead to warped plates. Warped plates will decrease the injection and expression efficiency.

1. Aspirate a number of oocytes from the dish with a transfer pipette by applying a low negative pressure, for example, with a rubber ball.
2. Move from well to well with the transfer pipette, carefully dropping one oocyte into each of the wells of the well plate. Grip only the glass pipette and do **not** squeeze the rubber ball, to avoid that too many oocytes will leave the pipette. The oocyte should drop into the fluid by its weight alone. If occasionally more than one cell drops into a well, this can be corrected after the plating will have been finished.
3. Due to the higher density of the vegetal pole, the oocytes will usually settle with the animal pole facing up if the (sticky) follicle cells were completely removed. Immediately check the position of each oocyte under a stereo microscope, and correct it carefully with the golf-club shaped glass tool, if the orientation is not correct. A manual correction should be necessary for less than five percent of the oocytes if the follicle cells were completely removed.
4. Cover the well plate with its lid, and seal the rim with Parafilm to reduce evaporation, and store it at 18 °C until injection.
   It is possible to inject the cells right after plating, but the oocytes should not be further disturbed, for example not be washed or otherwise treated until they have been allowed to adhere to the well bottoms (about 2 to 3 hours). The oocytes can also be injected on the next day, after being stored overnight at 18 °C. If injected on the next day, it is recommended to check the viability again under the microscope, and replace bad oocytes before the injection.

TEVC RECORDING BACKGROUND

TEVC Recording with the HiClamp

The small-sized digital TEVC amplifier is integrated within the HiClamp and fully computer-controlled.

It operates either in true current clamp or voltage clamp mode and uses a PI (Proportional Integral) based technique. The ClampAmpC records up to ± 105 µA of current, and the 16-bit A/D-conversion together with the over-sampling method results in an overall resolution of 1 nA. The sampling frequency of up to 20 kHz guarantees high-performance data acquisition of fast signals. The ClampAmpC features a short rise time of less than 500 µs, suitable even for recording from fast sodium channels.

The recording sequences can be flexibly designed exactly to your requirements. Raw data can be reviewed and evaluated offline with the HiClamp software, and also exported to custom analysis software.

Preparations for Recording

Recording with the HiClamp is easy, but nevertheless, we recommend that you make a few test recordings with non-injected oocytes and the provided sample recording protocol.

It is also a good idea run a test recording with the test model cell first.

Amplifier settings: Please make sure that the amplifier settings are appropriate for your ion channel type. For more details, please see section "TEVC Recording - Background". For a first test, the settings for slow ion channels are fine.

Ion channel kinetics Gain (P Coefficient) Integrator (I Coefficient)

slow 800 100   fast 2000 100

Glass micro electrodes: We recommend using a ready-made glass micro electrodes provided by Multi Channel Systems, especially for new HiClamp users. You can use custom-built electrodes as well, but you may observe problems if the design of electrodes does not follow the required specifications. Please see "Ordering Information" for details.

Electrolyte solution for filling the glass electrodes of the measuring head. Recommended solutions are 1.5 M KAc, 1 M KCl, pH 7.2 or 3 M KCl. The main disadvantage of a pure KCl solution is the fast formation of salt crystals that will shorten the lifetime of the electrodes. The KAc/KCl solution does not tend to crystallize, which is an advantage for storing the measuring head over night. You may want to vary the KCl concentration of the KAc/KCl solution for optimizing your experiments. We have used concentrations in a range from 0.1 M to 1 M KCl. Please keep in mind that the electrode impedance increases with a decreased overall ion concentration in the electrolyte.

Reusing a TEVC Probe

Multi Channel Systems MCS GmbH recommends replacing the recording electrodes each time when loading a new oocyte well plate onto the HiClamp, for achieving best recording results. But in many cases, a set of electrodes can be reused several times. It is also advisable to check the oocyte quality. It seems that bad oocytes tend to clog the recording electrodes more easily than oocytes of a very good quality.

To increase the lifetime of measuring heads, Multi Channel Systems recommends the following:

* Use 1.5 M KAc, 1 M KCl, pH 7.2 as electrolyte for filling the electrodes to avoid crystallization, which is a known issue with pure KCl solution.

* Use only high-quality oocytes to avoid that membrane residues from non-viable oocytes clog the electrode tips.

APPENDIX

Technical Support

Please read the "**Troubleshooting**" part of the manual or help first. Most problems are caused by minor handling errors. Contact your local retailer immediately if the cause of the trouble remains unclear. Please understand that information on your hardware and software configuration is necessary to analyze and finally solve the problem you encounter.

If you have any question or if any problem occurs that is not mentioned in this documentation, please contact your local retailer. The highly qualified stuff will be glad to help you.

Please keep information on the following at hand

- Description of the error (the error message text or any other useful information) and of the context in which the error occurred. The more information on the actual situation you can provide, the easier it is to track the problem.
- The serial number of the device. You will find it at the rear side of the device.
- The software and hardware version you are currently using. On the "Help" menu, click "About". The displayed dialog box shows the version numbers.
- The operating system and service pack number on the connected computer.
- The hardware configuration (microprocessor, frequency, main memory, hard disk) of the connected computer. This information is especially important if you have modified the computer or installed new hard- or software recently.

Preparation of Xenopus Oocytes

The materials and methods described in the following are kindly provided by the Bayer AG, Leverkusen. The described procedures have been optimized over several years for best performance of the oocytes and highest throughput. We recommend that you follow the instructions for obtaining best results.

Refer to standard protocols if you need more detailed information on the subject.

 Warning: Only qualified personnel should be allowed to perform laboratory work. Always make sure you fulfill the requirements of local regulations and laws. Work according to good laboratory practice to obtain best results and to minimize risks.

Materials

Biological Materials

Female frogs of *Xenopus laevis.*

Technical Equipment

- Shaker for the tubes (during defolliculation)
- Stereo microscope for quality control of oocytes
- 8- / 12-channel pipette or Tecan Columbus Microplate Washer for filling the well plates and washing the oocytes
- 96 well plates with conical bottom

It is very important that the well plates are produced carefully and have minimum variations.

Do not use coated plates, because oocytes will not adhere to the well bottom of coated plates! Check each single plate before use. The plate should be even and it should not be distorted in any way.

Note: If you use warped plates, you will encounter problems during injection. Check each plate carefully before use.

Oocyte sieve for grading oocytes by size: Remove the bottom of a 50 ml Falcon tube. Place a polyimide mesh with an 800 µm grid over the cut end and fix it with glue.

Oocyte transfer pipette: Cut and fire polished glass Pasteur pipette with an opening of ca.1.5 mm. The cut end should be straight, that is, with a fixed diameter of about 1.5 mm, for at least 10 mm so that a number of oocytes can line up inside the pipette as pearls on a string.

See also chapter "Plating Oocytes".

- Rubber ball for oocyte transfer pipette
- Glass tool for moving oocytes: Glass Pasteur pipette, sealed over a Bunsen burner, and melted into a golf-club like shape.
- Petri or cell culture dishes, 100 mm
- Petri dishes, 60 mm
- Beaker, 100 ml
- Razor blade
- Forceps
- Parafilm and standard laboratory equipment

Chemicals

Collagenase

For defolliculation:

- Fresh 1.52 mg/ml collagenase in Barth's solution without Calcium. The concentration has to
  be optimized according to the collagenase batch and experimental conditions, see also chapter "Defolliculation".
  Do not prepare solutions in advance as collagenase activity may decrease rapidly even if the solution is stored at -20 °C.
- Collagenase from Cl. histolyticum ca. 0.17 to 0.28 U/mg (according to Wünsch,1963) lyophilized.

Gentamicin

- Stock solution: 50 µg/ml gentamicin (free base) in Barth's solution. 1 ml aliquots with 50 mg/ml gentamicin (free base)
  are stored at -20 °C.
- Working solution: Dilute 1 ml of gentamicin stock in 1 l Barth's solution.
- Gentamicin sulfate salt, potency approx. 600 µg Gentamicin per mg.
- Alternatively, to gentamicin, a mixture of penicillin and streptomycin can also be used to suppress growth of microorganisms.

Barth's solution

- pH 7.4 (with NaOH)
- 88 mM NaCl
- 2.4 mM $NaHCO_3$
- 1 mM KCl
- 0.33 mM $Ca(NO_3)2 * 4 H_2O$
- 0.41 mM $CaCl_2 * 2 H_2O$
- 0.82 mM $MgSO_4 * 7 H_2O$
- 5 mM Tris/HCl

Barth's solution without Ca^{2+}

- pH 7.4 (with NaOH)
- 88 mM NaCl
- 2.4 mM $NaHCO_3$
- 1 mM KCl
- 0.82 mM $MgSO_4 * 7 H_2O$
- 5 mM Tris/HCl

Oocyte Removal

1. Remove the appropriate amount of ovarian tissue surgically from one side of the frog. Please refer to standard protocols on this subject (Stühmer and Parekh 1995).
2. Transfer the ovarian lobes into a new large Petri or cell culture dish (for example 100 mm Falcon) filled with Barth's without Ca^{2+}.

Collagenase Digestion and Defolliculation

Ovarian tissue contains immature and mature oocytes, as well as connective tissue from which the oocytes must be separated. Ovarian tissue should be removed completely by collagenase digestion. Oocytes are enveloped in a follicle cell layer which can cause trouble when plating oocytes into well plates. Remaining pieces of follicular tissue causes oocytes to stick to the walls. Oocytes will not move into correct positions in the middle of a well by themselves. Additionally, the follicular cell layer sometimes hampers the impalement by the injection needle causing damage to the oocytes or clogging of the tip. Therefore, it is important that isolated oocytes are completely free of the surrounding follicular cell layer.

The whole procedure should be completed after about 2 to 2.5 hours. Please adjust the collagenase concentration if this is not the case.

1. Divide the tissue with a razor blade and a forceps into smaller, approximately 0.5 cm^2 large pieces.
2. Transfer the clumps into 50 ml Falcon tubes containing 40 ml of 1.5 to 2.0 mg/ml collagenase in Barth's without Ca^{2+}. A volume of up to 7.5 ml of tissue can be put into a single tube. For more tissue, use additional tubes. Otherwise, the collagenase digestion would take too much time.
3. Place the tubes onto the shaker and let them shake gently at 20 °C. Check the progress after 90 min (and then every 15 min), and vigorously shake the tube briefly by hand to accelerate the process.
4. Typically, 120 minutes after beginning of the treatment, practically all oocytes should be isolated and the majority of them should have already lost their follicular cell layer. If not, continue the collagenase treatment for a maximum of 30 min.
5. Wash the oocytes extensively with Barth's solution (minimum of 5 times with 30 ml) to remove the collagenase completely.
6. Then fill up the tube (approx. to 45 ml) and put it back onto the shaker for 10 minutes.
7. Change the solution to Barth's without Ca^{2+} and put it onto the shaker again for another 10 minutes. Practically all oocytes should be defolliculated now. Shake the tube vigorously to remove the follicle cells completely, if necessary.
8. Wash the oocytes with Barth's solution until the supernatant is clear and free of follicular cell layer fragments.

Selecting Good Oocytes

Rough selection by filtration

1. Fill a 100 ml beaker with 80 ml of Barth's solution. Place the oocyte mesh sieve into the beaker for grading the oocytes by size. The mesh of the sieve should be completely immersed in the fluid.
2. Pipette an amount of oocytes onto the sieve. Approximately half the sieve should be covered with oocytes. Too many oocytes on the filter will lead to an inefficient filtration.
3. Gently move the filter about two centimeters up and down (in the fluid) to separate the oocytes by size.
4. Transfer the oocytes of appropriate size (that did not go through the sieve) into a 60 mm petri dish filled with Barth's + gentamicin.
5. The filtered oocytes are incubated at 18 °C for 1 h.

Note: The incubation step is necessary for identifying damaged oocytes in the next step.

Fine selection

Use a stereo microscope and the golf-club shaped glass tool to check each single oocyte for the following criteria.

Outer form:

- No visible damage of the cell.
- Well separated colors (dark and light brown).
- No residues of follicular tissue.

Size:

Uniform size of about 1.2 mm.

Note: Selecting oocytes is an important step. Perform it very carefully to obtain best results.

Plating Oocytes

You need a well plate filled with Barth's + gentamicin.

Important: Visually check all well plates before use. Use only unsterile and very flat plates. Sterilization may lead to warped plates. Warped plates will decrease the injection and expression efficiency.

1. Aspirate a number of oocytes with a transfer pipette by applying a low negative pressure, for example, with a rubber ball.
2. Move from well to well with the transfer pipette, carefully dropping one oocyte into each of the wells of the well plate. Grip only the glass pipette and do not squeeze the rubber ball, to avoid that too many oocytes will leave the pipette. The oocyte should drop into the fluid by its weight alone.
3. If occasionally more than one cell drops into a well, this can be corrected after the plating will have been finished.

TECHNICAL SPECIFICATIONS

General Characteristics	
Operating temperature	10 °C to 20 °C
Storage temperature	0 °C to 50 °C
Dimensions (W x D x H)	640 x 600 x 440 mm
Weight	47 kg
Power supply	230 VAC @ 50 Hz, 115 VAC @ 60 Hz
Amplifier	
Type	Integrated TEVC amplifier
Operation	Fully automatic and computer controlled
Reference electrode	Two independent active bath clamp
Max. sampling rate	20 kHz
Data resolution	16 bit
Current electrode output	
Current range	-105 µA to + 105 µA
Effective current resolution	1 nA
Voltage range	-100 V to + 100 V
Voltage electrode input	
Input range	- 500 mV to + 500 mV
Voltage resolution	0.015 mV
Clamp voltage setpoint range	- 500 mV to + 500 mV
Clamp voltage setpoint resolution	1 mV
Typical rise time in voltage clamp mode	< 1 ms
Software	
HiClamp software	DataMining and DataMerger software
Operating system	Microsoft Windows ® 10 or 8.1 (32 or 64 Bit) with NTFS, English version
Connection to the computer	USB 2.0 High Speed

CONTACT INFORMATION

Local retailer

Please see the list of official MCS distributors on the MCS web site.

Mailing list

If you have subscribed to the Newsletter, you will be automatically informed about new software releases, upcoming events, and other news on the product line. You can subscribe to the list on the contact form of the MCS web site.

multichannelsystems.com