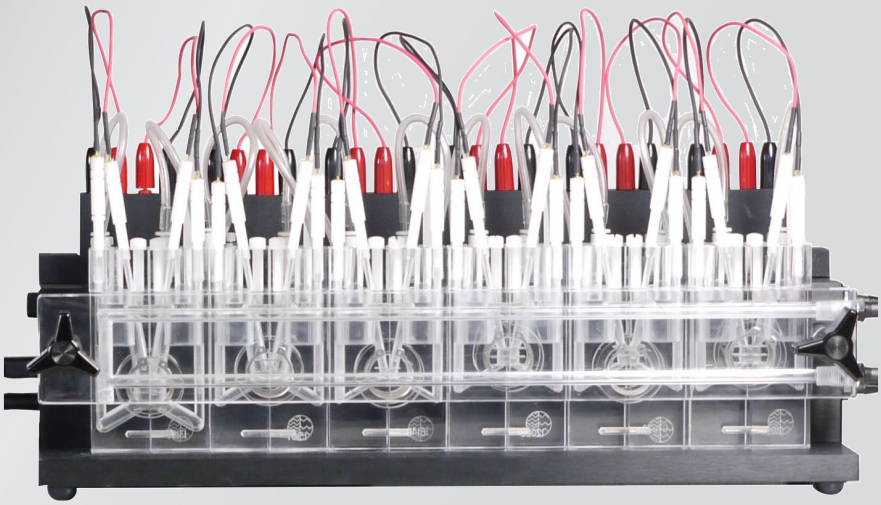


SmartUSSING-System

USER MANUAL

Version Number 1

TABLE OF CONTENTS

GENERAL INFORMATION

Safety

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 fulfil 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.

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 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.

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

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.

Explanation of the Symbol used

 Caution / Warning  DC, direct current

WARRANTY

The SmartUSSING-System is covered by our warranty to be free from defects in materials and workmanship for a period of two years from the date of shipment. If a failure occurs within this period, we will either repair or replace the faulty component(s). This warranty does not cover instrument failure or damage caused by physical abuse or electrical stress (inputs exceeding specified limits). In the event that instrument repairs are necessary, shipping charges to the factory are the customer's responsibility. Return charges will be paid by Warner Instruments, Inc.

Cleaning Instructions: Wipe exterior surfaces with a clean damp cloth.

High Voltage: Not for clinical use on humans. To avoid risk of electric shock, do not open an amplifier housing. There are no internal user-serviceable parts, please contact us for repair.

Installation Clearances: Instrument shall have 5 cm clearance on both sides when rack mounted and 12 cm side clearances otherwise. Instrument should not be stacked on top of heat producing equipment without a minimum spacing of 9 cm.

Note: If this equipment is used in a manner not specified by the manufacturer in this user manual, the protection provided by the equipment may be impaired.

Important for customers outside of the U.S.: Please be sure to contact us before return shipping any goods. We will provide instructions so that the shipment will not be delayed or subject to unnecessary expense in clearing U.S. Customs.

Compliance Statement

WEEE/RoHS Compliance Statement EU Directives WEEE and RoHS

To Our Valued Customers:

We are committed to being a good corporate citizen. As part of that commitment, we strive to maintain an environmentally conscious manufacturing operation. The European Union (EU) has enacted two Directives, the first on product recycling (Waste Electrical and Electronic Equipment, WEEE) and the second limiting the use of certain substances (Restriction on the use of Hazardous Substances, RoHS).
Over time, these Directives will be implemented in the national laws of each EU Member State.

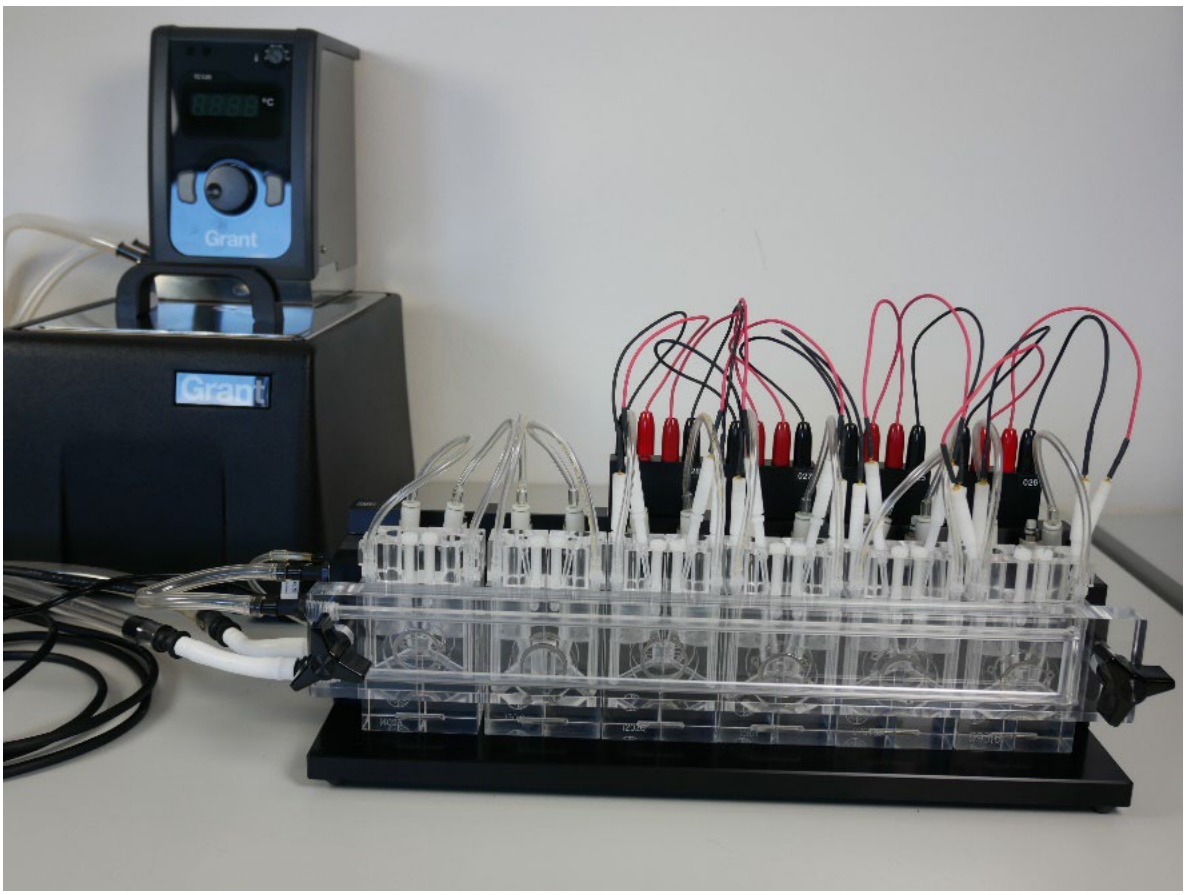
Once the final national regulations have been put into place, recycling will be offered for our products which are within the scope of the WEEE Directive. Products falling under the scope of the WEEE Directive available for sale after August 13, 2005 will be identified with a “wheelie bin” symbol.

Two Categories of products covered by the WEEE Directive are currently exempt from the RoHS Directive – Category 8, medical devices (with the exception of implanted or infected products) and Category 9, monitoring and control instruments.

Most of our products fall into either Category 8 or 9 and are currently from the RoHS Directive. We will continue to monitor the application of the RoHS Directive to its products and will comply with any changes as they apply.

Do Not Dispose Product with Municipal Waste

INTRODUCTION



A SmartUSSING System, used to measure transport across epithelial membranes, is generally comprised of a chamber and perfusion system, and, if needed, an amplifier and data acquisition system. The heart of the system lies in the chamber with the other components performing supporting roles. The 'Classic' chamber design, first introduced by the Danish physiologist Hans Ussing in the early 1950's, is still in wide use today. However, several newer designs are now available that optimize for convenience and for diffusion or electrophysiology based measurements.

Epithelia are polar structures possessing an apical (or mucosal) and basolateral (or serosal) side. It is the movement of electrolytes, non-electrolytes, and H_2O.

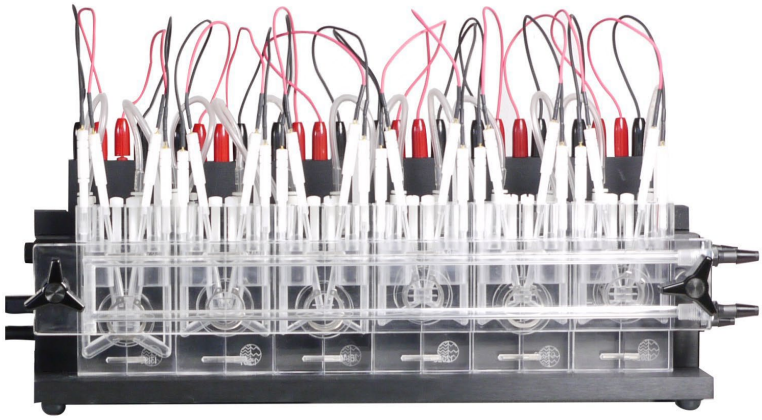
Ussing systems have been used to make measurements from native tissue including stomach, large and small intestine, gall and urinary bladder, skin, and trachea, as well as from tissue derived cell monolayers from various sources including renal tubes, pancreas, and salivary and sweat glands. A well designed Ussing chamber supports an epithelia membrane or cell monolayer in such a way that each side of the membrane is isolated and faces a separate chamber-half. This configuration allows the researcher to make unique chemical and electrical adjustments to either side of the membrane while maintaining complete control.

An electrophysiology based SmartUSSING-System focuses on measuring transepithelial electrical responses to experimental perturbations. These systems are used to quantify the operation of electrogenic pathways in the membrane (for example, ion pumps, channels, etc). As such, an electrophysiology based system carries the additional hardware requirement of a voltage and/or current clamp amplifier, a data acquisition system, and data acquisition and analysis software.

Basic measurement parameters in electrophysiology based systems include transmembrane voltage (Vt), epithelial membrane resistance (Rt), and short circuit current (ISC).

While a single SmartUSSING chamber block can hold from 1 to 6 chambers, multiple systems can be operated in parallel on a single computer giving you the flexibility to work with up 24 chambers in parallel. Each block is controlled with its own USSING Control software.

HARDWARE COMPONENTS



The SmartUSSING-System is complete with up to six included chambers and amplifiers, air/gas fitting kit, electrode set and support stand with option to connect thermocirculator for heating front and back plate. Ussing chambers are available in a variety of sizes and shapes, and the choice of chamber depends on the species and epithelia under study.

Chamber Types

Different types of USSING chambers are available. All Ussing chambers have the following features in common: a means to hold the membrane while minimizing tissue damage, a means for holding and exchanging solutions, a means to facilitate precise electrode placement, and a means to control solution temperature and gas load.

Example: Vertical Chamber

Tissue diffusion chambers are available with both circular and oblong openings, depending upon the tissue type under study. For example, for intestinal tissue studies, oblong openings can increase the effective surface area of the sample. A low volume tissue chamber that reduces the amount of compound required to conduct permeability studies is also available. Up to six chambers of either type can be placed in the heat block at one time, allowing convenient execution of six simultaneous transport studies under precise experimental control.

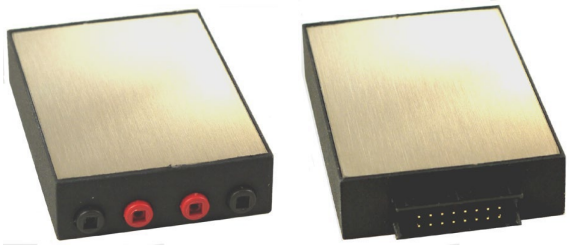
Micro-Reference Electrodes

The Warner “Ready to Use” electrodes fit both: “Vertical and Horizontal Chamber Systems” without modification. These electrodes allow measurement of the electrical parameters of tissues and cell monolayers. The electrodes have a shaft diameter of 2.5 mm and a shaft length of 5.0 cm.

The silver/silver chloride electrode uses a refillable glass barrel that can contain any suitable electrolyte solution. Usually, the filling solution is the buffer used for the external medium, or silver chloride (AgCl)-saturated 3 M potassium chloride (KCl). The glass barrel surrounds a chloride silver wire and terminates with a ceramic tip. The microporous ceramic tip limits the flux of ions into and out of the electrode, and in most applications, serves as a suitable replacement for bridge systems.

The electrode connects to a wire 100 millimeter in length, which terminates in a standard 1 mm pin plug. Electrodes caps for standard volume chambers provide exact position of the electrodes in the chamber. The “Low Volume” and “Horizontal Chambers” do not require these caps. Microelectrodes are not included in the systems and must be ordered separately.

Epithelial Voltage Clamp Amplifier



The small amplifier is specifically designed for studies of epithelial transport and the electrical properties of tissue. It features high common mode rejection, multiple clamp speed selection, membrane resistance measurement circuitry, and a watertight headstage that includes a model membrane.

Air Manifold

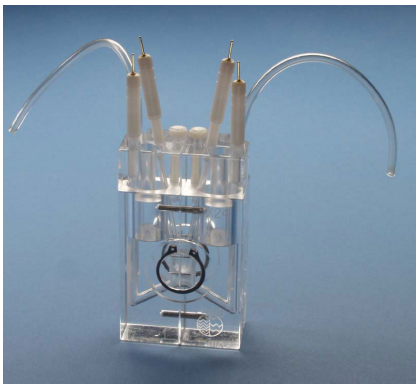
The air manifold for this diffusion chamber has been specially designed for use with mixed oxygen gases. Oxygen in the presence of hydrocarbons is combustible. Do not use this manifold to deliver gases which contain traces of hydrocarbons. Medical grade gases are recommended. Other than to replace air lines, do not open the air manifold by removing fittings as this may introduce contaminants into the oxygen cleaned environment.

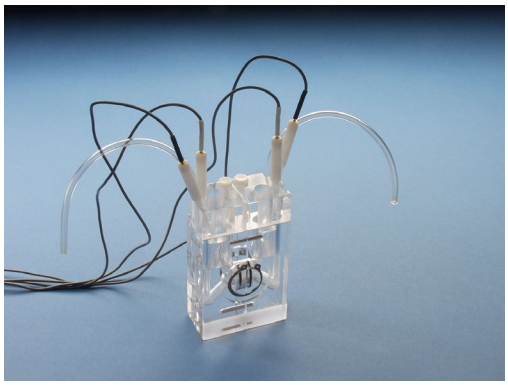
Thermocycler

For setting up the thermocycler, please read the “Grant” manual.

SETTING UP THE HARDWARE

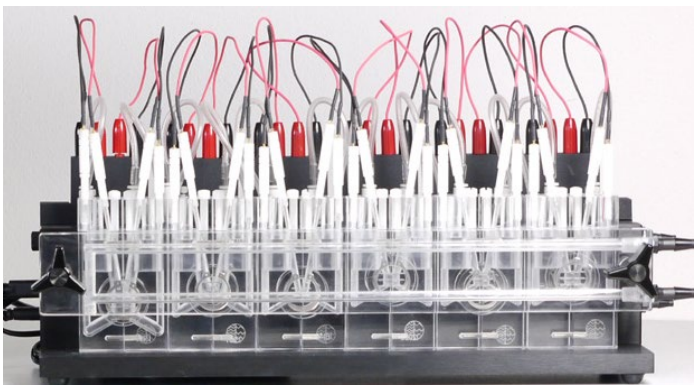
Setup of the individual USSING Chamber



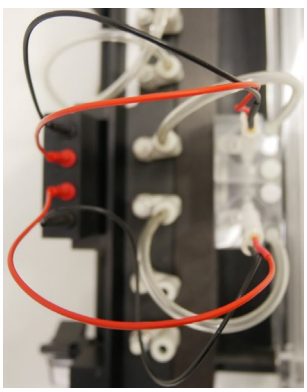


Set up your individual USSING chamber: Insert the tissue and the four microelectrodes. Connect the electrical cords and the gas tubes. Please read the respective datasheet.

Setting up the Rail and the Ussing Chamber



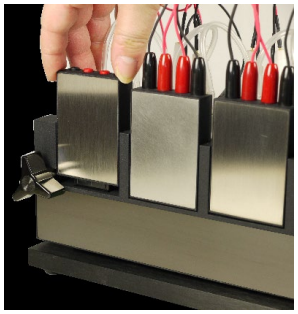
After setting up the USSING chamber, please insert the chambers into the rail. Connect the electrical cords from the microelectrodes to the respective amplifier. Connect the air manifold. Please see the photos.



Setting up the Rail and the Amplifier

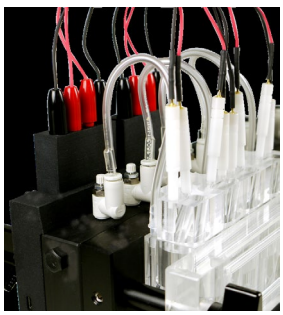
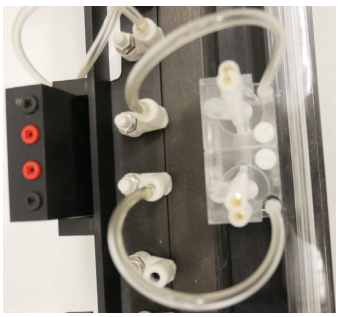
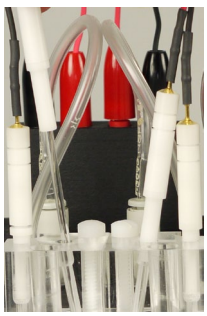
Please insert the amplifier in correct orientation into the rail. Join the connector of the amplifier to the connector of the rail by pressing the amplifier carefully. Connect the electrical cords from amplifier to the correct microelectrodes of the USSING chamber, please see the photos.





Attaching the Air Manifold to the USSING Chamber

The air manifold for this diffusion chamber has been specially designed for use with mixed oxygen gases. Oxygen in the presence of hydrocarbons is combustible. Do not use this manifold to deliver gases which contain traces of hydrocarbons. Medical grade gases are recommended. Other than to replace air lines, do not open the air manifold by removing fittings as this may introduce contaminants into the oxygen cleaned environment.


To attach the Air Manifold:

1. Attach air lines to the chambers using the holes on the outside edge at the top of each half-cell.
2. Place a bubble trap between the air supply tank and the air manifold; then bubble the gas through water in this trap. Low humidity gas directly from the gas supply tank will cause significant fluid loss from the chamber if it is not humidified prior to delivery to the air manifold.
3. The inlet pressure to the air manifold should be 20-30 psi. Adjust the flow to provide adequate mixing and oxygenation, generally to 15-20 cc/mm in each half-cell, but this may vary according to application or experiment.

Setting up the Rail and the Thermocycler

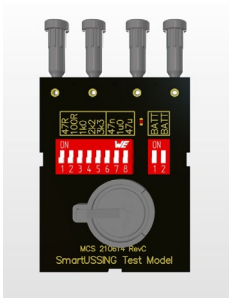
Unpack the tube set.

Connect the short white tubes to the heating in front of the USSING chamber and to the heating in the back of the chamber. Connect long transparent tubes via adapter to the white tubes. Connect these tubes from the front of the rail to the thermocycler and back from the thermocycler to the rail.

Connect a short tube from the front of the heating to the back of the heating for circulation of the warm water in the heating element.

Setting up the Amplifier and the Test Model Cell

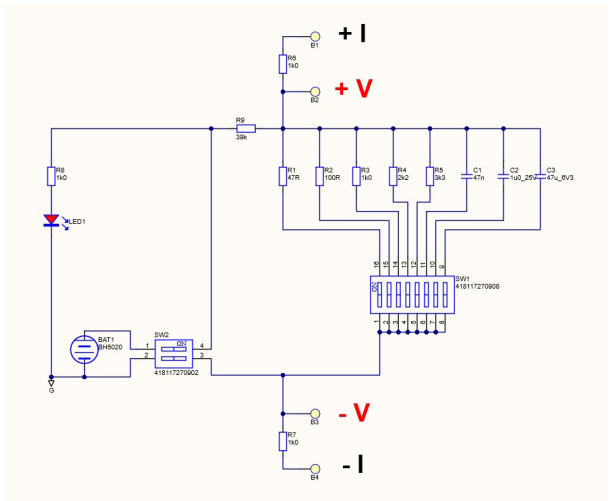
The test model probe imitates an USSING chamber and is perfect to test the SmartUSSING system and the USSING Control software.



Signal Generator: Circuit Diagram

Batterie “On” and “Off”.

47R
100R
1kO
2k2
3k3
47n
1uO
47u



SOFTWARE

Software Installation

System Requirements

Software: The following Microsoft Windows ® operating systems is required: 64-Bit Windows 10, Windows 8.1 (English and German versions supported) with the NT file system. Other language versions may lead to software errors.

Hardware: SmartUSSING System and a data acquisition computer.

Please contact Multi Channel Systems or your local retailer for more information on recommended computer hardware specification. Please note that there are sometimes hardware incompatibilities of the data acquisition system and computer components; or that an inappropriate computer power supply may lead to artefact signals. It is not recommended to run any applications in the background when using the Ussing Control software. Please remove all applications from the “Autostart” folder.

Important: You need to have installed the latest data acquisition driver to operate the device, which is automatically installed with Ussing Control software. The installation may be invalid if the data acquisition does not respond. Please contact Multi Channel Systems MCS GmbH or your local retailer in this case.

If a computer was acquired from MCS, the Ussing Control software will be preinstalled. Updates are available for free download on a regular basis from the MCS web site. It is recommended always to install the latest software version. To install the software, download and start the respective *.exe file and follow the instructions on the screen.

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 in the Ussing Control software. These routines were designed for use on office computers but are not very useful for a data acquisition computer.

- Turn off the screen saver.
- Turn off automatic Windows update.
- Power Options: Set power scheme to high performance. Never turn on system standby.
- Be careful when using a virus scanner.

It is also not recommended to run any applications in the background when using the data acquisition software.

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

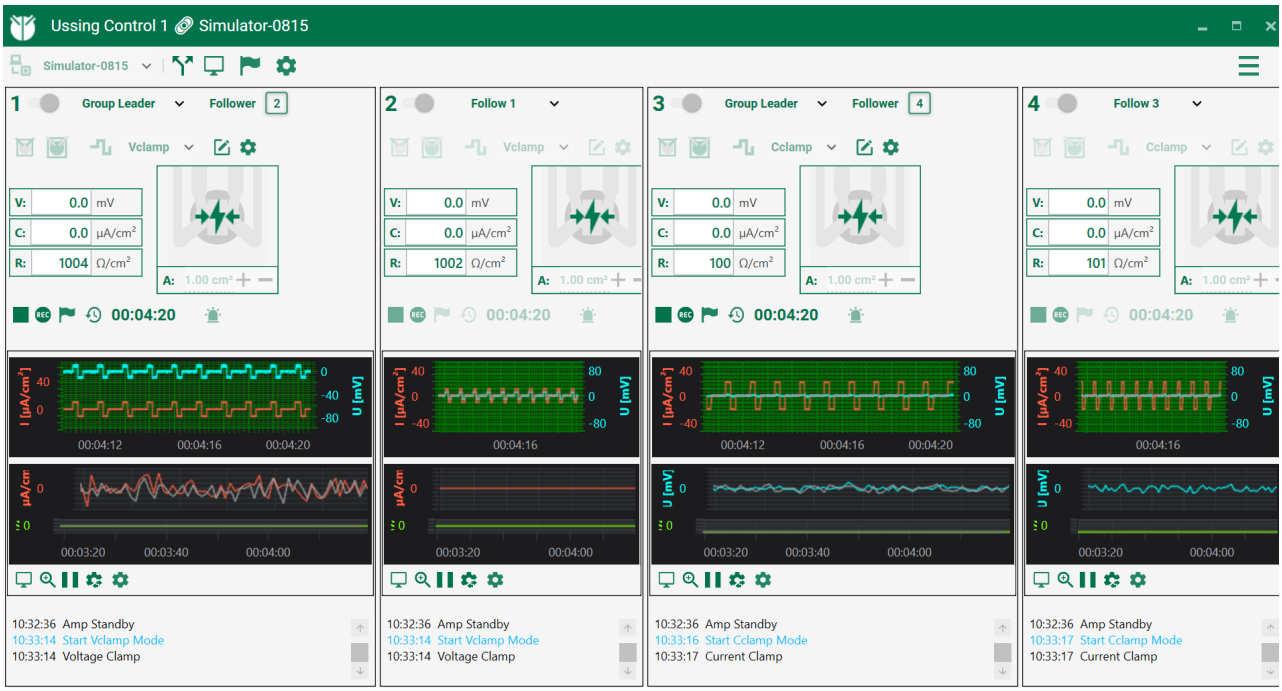
Important: Please make sure that you have full control over your computer as an administrator. Otherwise, it is possible that the installed software does not work properly.

USSING CONTROL SOFTWARE

The Ussing Control software is the heart of the system and allows full control of the amplifiers during data acquisition. Data is displayed in real time and is saved for later review or export. Exported data is compatible with a wide variety of data analysis and graphing programs. Raw data in hdf5 format exported to Excel are displayed with calculated voltage, current and resistance levels.

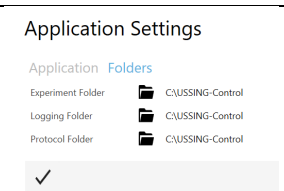
Main Menu

The chambers of the USSING System can be operated in parallel or independent from each other.



Global Toolbar

Table: Global Toolbar

	Connect Device	Connect or disconnect the USSING device.
Simulator-0815	**Select Device**	Please select the connected device. If no device is available, you can use the simulator.
	Bind / Unbind Chambers	Press button to bind or unbind all chambers.
	Toggle Follower Views	Merge or separate follower views with or from leader view.
	Set Marker Click the “Set Marker” icon to set a timestamp marker for all chambers.	
	Global Application Settings Edit global application settings. **Folders:** Set the data paths for the “Experimental Folder”, the “Logging Folder” and the “Protocol Folder” folders.	

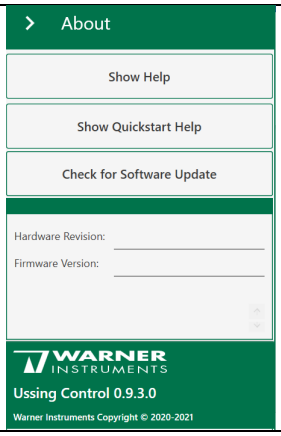
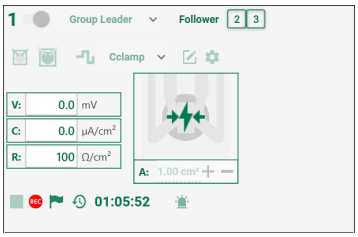
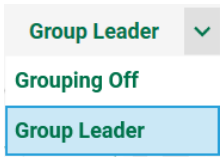
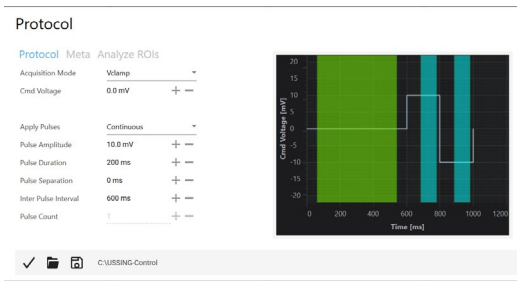
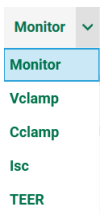
	About Show Help, show Quickstart Help, check Software for Updates. **Show Help** "Show Help" will open the latest version of the manual directly from the MCS web site. **Show Quickstart Help** "Show Quickstart Help" will open the latest version of the Quickstart manual directly from the MCS web site. **Software Update** It is recommended to use the "Check for Software Updates" function regularly. Free upgrades of the Ussing Control software are released on a regular basis.	

Table: Chamber and Display Control

Chamber Control		
	**Switch** SmartUSSING chamber "**On**" or "**Off**".	
	Set a chamber as "**Group Leader**" or "**Follower**" to bind chambers to a group. The followers are connected to the group leader and imitate the group leader's actions. Select "**Grouping Off**" for independent control of that chamber.	
	Toggle "**Follower UI**". Show or hide the users interface of the selected chamber.	
	**Electrode Offset** Compensate electrode offsets of this chamber and all followers.	
	**Fluid Resistance** Measure fluid resistance R of this chamber and all followers.	
	**Protocol** View, load, save and edit protocols.	
	**Monitor** Select a predefined acquisition mode of this chamber and all followers. **Data Acquisition Modes:** Voltage clamp, Current clamp, Isc, Teer	

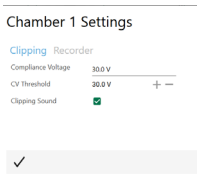
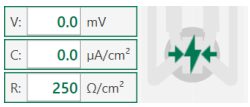
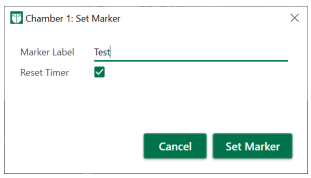
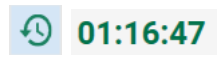
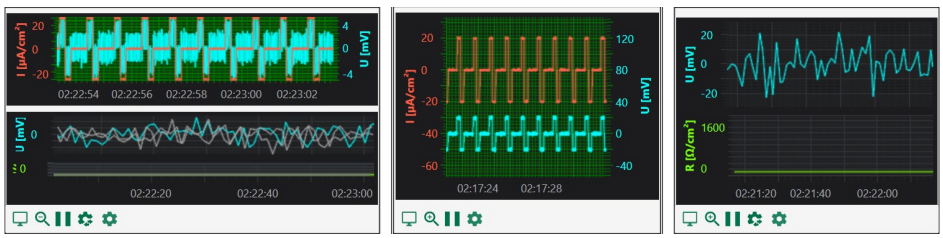
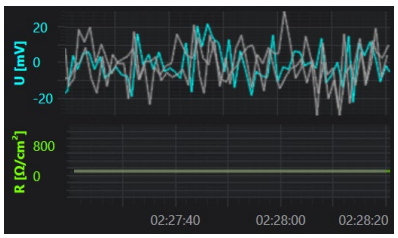
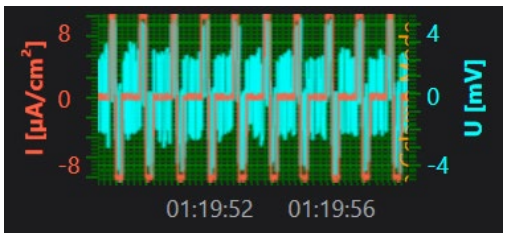
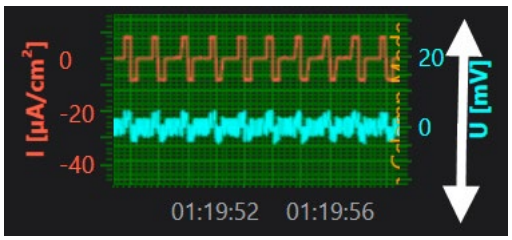
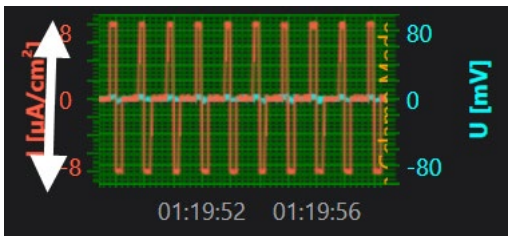
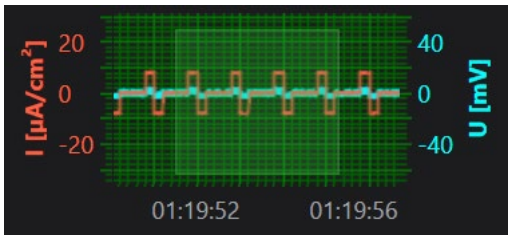
	**Lab Book** Open, view, edit and save individual lab book for this chamber.	
	**Chamber Settings** Edit chamber related settings.	
	**Display** V: Voltage in mV C: Current in µA/cm² R: Resistance in Ω/cm²	
	**Start and Stop** Start and stop acquisition mode of this chamber and all followers.	
	**Recording** Start and stop of this chamber and all followers.	
	**Marker** Set timestamped marker for this chamber and all followers.	
	**Reset Timer**	
	**Clipping**	Signals when the compliance voltage is clipped.
Display Control		
	**Toggle Data View**	Click to toggle between the different data view options. A selected view may be magnified.
	**Magnify /Reduce**	Press button to magnify the view. Press button again to reduce to normal size.
	**Pause and Start Plot Update**	
	**Transfer Axis Ranges**	Apply axis ranges to all group members.
	**View Settings**	Edit view related settings.

Table: Display Zoom Options

Inspect data while data view is running.	
	**Analysis** Merge of analyzed data streams of the “Group Leader” and all “Followers” color coded at a glance in the “Group Leader” plot display.
	**Doubleclick Display** Zoom data to maximum size in this display via doubleclick.
	**Pan Axis (X- and Y Axis)** Pan: Move the right mouse over the axis.
	**Zoom Axis (X- and Y-Axis)** Zoom: Move the mouse wheel.
	**Zoom Rectangle (Time Axis)** Stop the “Plot update”.  Draw a rectangle over the axis region of interest to zoom in.
	**Zoom whole Display** Click button “Magnify”  to enlarge the display of interest.

Protocol

Load, view, edit and save protocols.

“Close”: Click to close the dialog,
“Load”: Click to load protocols.
“Save”: Click to save protocols.

Tabbed Page: **Protocol**

Use the tab "Protocol" to define the parameter for the pulses to be used. Choose the "Data Acquisition Mode" from the drop down menu. Define the "Cmd Current" via up down box or overwrite the value.

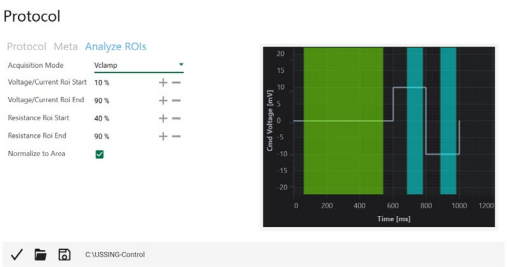
Define the way of pulse application from the drop down menu.: "Apply Pulse" “Continuous”, on “Count” or “Off”.

Define the pulse parameter in the up down boxes: "Pulse Amplitude", "Pulse Wait", "Pulse Duration", "Pulse Separation", and "Pulse inter pulse interval IPI".

Tabbed Page: **Meta**

Define a “Protocol Name”, register the “Author” or write an “Comment”. Date and time when the protocol was “Created” and the “Application” are displayed.

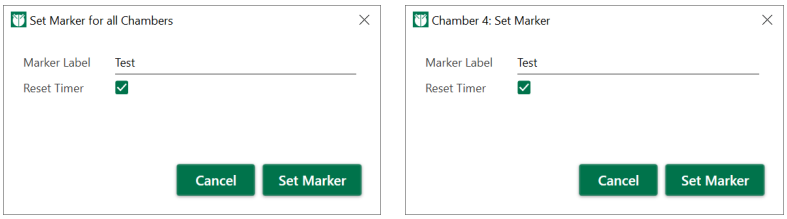
Tabbed Page: **Analyze ROI’S**



Choose the “Acquisition Mode" from the drop down menu.

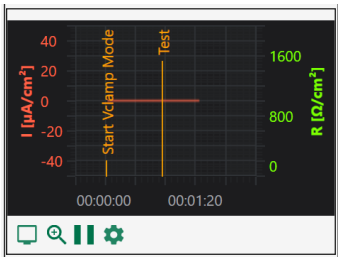
Define “Voltage/Current” and “Resistance as well for the “Start of the region of Interest” as for the “End of the ROI”. “Normalize to Area”, if necessary.

Marker



Please add a "Marker Label" and "Reset Time" by clicking the check box. The time will be zeroed when the marker is started with the "Set Marker" button.

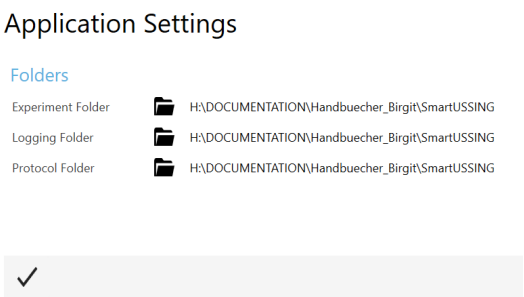
Set markers for all chambers in the upper global toolbar or set one marker in the chamber control window for one chamber.



Settings

Three Types of “Settings” are available: “Application Settings” in the global tool bar for common settings, “Chamber Settings” in the chamber control for individual settings and “View Settings” for the screen in the display control.

Application Settings in the Global Tool Bar



Folders

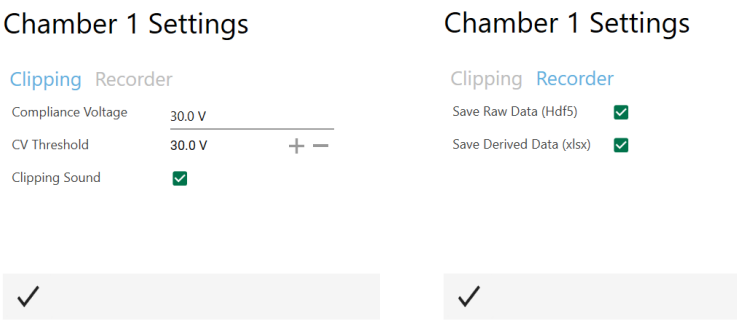
Please define the path to the respective folders: "Experiment Folder", "Logging Folder" and "Protocol Folder".

Data Types

The types of data occurring in the "Ussing Control" software:

Experiment data: Raw data per electrode channel "*Date-Time-Channel-Number.urd".
Logging data: Log file data "*.ulog".
Protocol data:

Chamber Settings in the Chamber Control



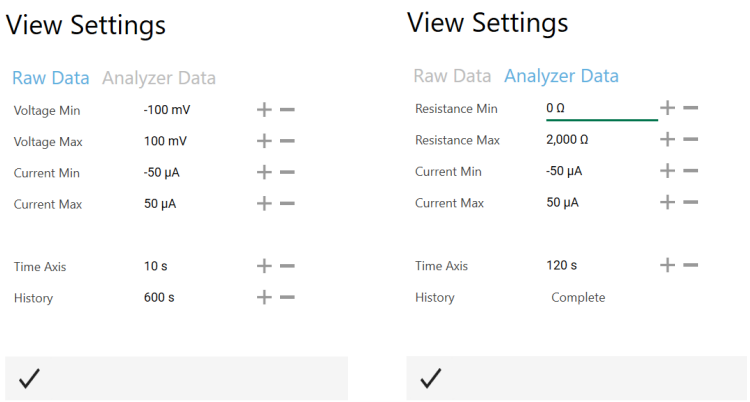
Tabbed Page: **Clipping**

See the “Compliance Voltage” and define the compliance voltage threshold “CV Threshold”. Click the check box “Clipping Sound” to be acoustical warned, when clipping happens.

Tabbed Page: **Recorder**

Click the respective check boxes to “Save Raw Data” in Hdf5 format or to “Save Derived Data” in Excel format *. xlsx.

View Settings in the Display Control



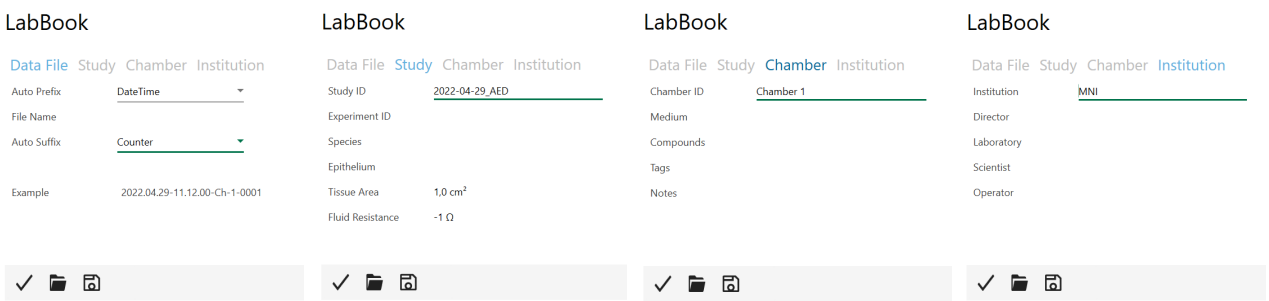
Tabbed Page: **Raw Data**

Define the parameter used for the raw data which can be observed on the data display: “Voltage Min/Max”, “Current Min/Max”, “Time Axis” and the time in which the “History” should be stored.

Tabbed Page: **Analyzer Data**

Define the analysis parameter: “Resistance Min/Max”, “Current Min/Max” and “Time Axis”. The “History” of data will be stored completely.

Lab Book



Technical Specifications

General Characteristic	
Operating temperature	4 °C to 40 °C , (40 °F to 104 °F)
Storage temperature	0 °C to 40 °C, (0 °F to 104 °F)
Shipping temperature	0 °C to 40 °C, (0 °F to 104 °F)
Operating humidity	20 % to 80 % RH, non-condensing
Storage humidity	20 % to 80 % RH, non-condensing
Shipping humidity	20 % to 80 % RH, non-condensing
Altitude	2000 m
For indoor use only!	
Classification	Class I
Installation	Category II
USSING System	
Dimensions of the USSING rail (W x D x H)	380 mm x 75 mm x 20 mm
Weight of the USSING rail	661 g
Power, maximal	60 W
AC input	100 V to 240 V ~ 2 A @ 50 - 60 Hz
DC output	24 V = 2.7 A
Compliance	± 50 V
Sample frequency	10 kHz
Current range	± 100 µA and ± 10 mA, switchable
Data resolution	24 Bit
Number of chamber(s) Modular:	1 to 6 chambers
Type of chambers Customized:	Vertical and horizontal
Connection for the USSING rail	USB-C
Software	
Operation System	Microsoft Windows ® 10, English version
Online data acquisition software	USSING Control
Offline data analyzing software	USSING Analyzer
Connection to the computer	USB 2.0 High Speed

CONTACT INFORMATION

Contact Information

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