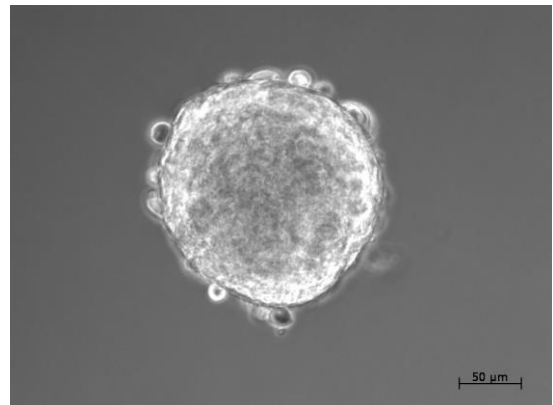




MEA Application Note: MEA Recordings from intact islets of Langerhans



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Contents

1 About this Application Note	4
2 Theoretical background	4
2.1 <i>Islets of Langerhans</i>	4
2.2 <i>Stimulus-secretion coupling (SSC)</i>	5
2.3 <i>Membrane potential oscillations of beta cells</i>	5
3 Material	6
3.1 <i>Chemical reagents</i>	6
3.1.1 <i>Islets of Langerhans preparation and cultivation medium</i>	6
3.1.2 <i>Extracellular recordings</i>	7
3.1.3 <i>Lab equipment and consumables</i>	7
3.2 <i>Isolation of islets of Langerhans</i>	9
3.2.1 <i>Isolation of murine islets</i>	9
3.2.2 <i>Isolation of human islets</i>	10
4 Experimental setup	11
4.1 <i>MEA2100-Beta-Screen-System Setup</i>	11
4.1.1 <i>MEA2100-Beta-Screen MEA Chip and Beta-Screen Chamber</i>	12
4.1.2 <i>Setup of the MEA2100-Beta-Screen-System hardware components</i>	13
4.1.3 <i>Setup of the Beta-Screen Chamber</i>	13
5 Recordings with the MEA2100-Beta-Screen-System	15
5.1 <i>Important preparations to start the recordings</i>	16
5.1.1 <i>Connection of the perfusion and negative pressure on the Beta-Screen headstage</i>	16
5.1.2 <i>Installation and connection of the PPS2 perfusion pump</i>	17
5.1.3 <i>Installation and connection of the Constant Vacuum Pump (CVP)</i>	17
5.1.4 <i>Hardware and software preparations to start an experiment</i>	19
5.1.5 <i>Positioning of the islets and start of an experiment</i>	22
5.1.6 <i>Example of electrical activity recorded with the Beta-Screen-System</i>	23
6 Contact Information	25

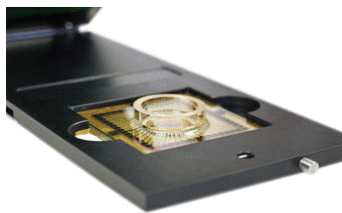
1 About this Application Note

The intention of the MEA Application Notes is to show users how to set up experiments with the MEA-Technology based on typical applications that are used worldwide. The documents have been written by or with the support of experienced MEA users who like to share their experience with new users.

This application note includes suggestions on how to prepare and record electrical activity from intact islets of Langerhans on top of MEA electrodes. There are two different systems available with different recording techniques for the recordings from intact islets.

- 1) The MEA2100-Mini-System is the ideal solution for continuous, undisturbed recordings of cultivated islets inside an incubator.
- 2) The Beta-Screen-System allows acute parallel recordings of beta cell activity for up to 40 intact islets of Langerhans with one system.

Chronic recordings with incubator-ready system **MEA2100-Mini-System**



Acute higher throughput recordings **MEA2100-Beta-Screen-System**



2 Theoretical background

2.1 Islets of Langerhans

The reason why islets of Langerhans obtain a remarkable amount of attention is due to their ability to produce and secrete insulin, the body's only hormone which can lower blood glucose concentration. The impairment of proper insulin secretion can result in several debilitating diseases, e.g. diabetes mellitus or congenital hyperinsulinism (CHI). Furthermore, Type-2 diabetes mellitus (T2DM), the most abundant endocrine disease, is an increasing health problem worldwide which has reached epidemic levels. The growing prevalence underlines the importance to further understand the physiology and pathophysiology of these fascinating islets.

Postprandial increase of blood glucose concentration leads to an increase of glucose metabolism in pancreatic beta cells which is a prerequisite of insulin secretion. Besides glucose, other monosaccharides like fructose and mannose as well as various types of amino acids and fatty acids can influence insulin secretion. The incretins, a group of intestinal hormones like GLP-1 (glucagon-like peptide-1) and GIP (glucose-dependent insulinotropic peptide) are additional stimuli in the presence of glucose.

2.2 Stimulus-secretion coupling (SSC)

Blood glucose concentration tightly correlates with the metabolic activity in beta cells. Increasing glucose concentration leads to a higher ATP/ADP ratio with increasing electrical activity which in turn controls insulin secretion. This process is called stimulus-secretion coupling (SSC).

A postprandial increase of blood glucose concentration leads to an augmented entry of glucose into the beta cells. In rodent beta cells glucose enters via low- K_m (Michaelis constant) Glut-2 transporters. In contrast Glut-1 and Glut-3 are the predominant glucose transporters in human beta cells. Glycolysis is the metabolic pathway that converts glucose into the intermediates pyruvate and NADH. Glucokinase (GCK), with its K_m in the range of the blood glucose concentration (~ 5 mM) is the first enzyme of the glycolysis. GCK is the glucose sensor and hence the rate-determining enzyme for glucose metabolism. The adaptation of the necessary amount of insulin for the organism is therefore firmly regulated by the activity of GCK. The glycolytic reduction equivalent NADH is shuttled into the mitochondria by the malate-aspartate and the glycerol-phosphate shuttle systems. The inhibition of these shuttle systems completely suppresses glucose-induced insulin secretion demonstrating that only ATP resulting from the glycolytic reduction equivalents can influence K_{ATP} channels.

The mitochondria are an essential link in the SSC of beta cells. Within the mitochondria NADH is directly used by respiratory chains to pump H^+ into the intra-membrane space of the mitochondria. This process leads to an electronegative environment within the mitochondria, a prerequisite for a proper function of F_1/F_0 -ATPase. The increase of the ATP/ADP ratio leads to a decrease of the open probability of K_{ATP} channels, which leads to a depolarisation of the beta cells via a yet unknown depolarising current. When the depolarisation of the beta cell exceeds the threshold for the opening of C_{av} channels, these channels open and Ca^{2+} ions enter the cell, which leads to an increase of the intracellular Ca^{2+} concentration. The influx of Ca^{2+} ions directly trigger insulin secretion via exocytosis of insulin-containing vesicles. In rodent beta cells APs are generated by L-Type Ca^{2+} channels, whereas in human beta cells, a combined interaction between specific Na^+ and Ca^{2+} channels is observed.

2.3 Membrane potential oscillations of beta cells

A remarkable and unique feature of pancreatic beta cells is the capability to generate membrane potential (V_m) oscillations in response to stimulatory glucose concentrations. This phenomenon leads to a pulsatile insulin secretion. It is still a matter of debate whether the oscillations of insulin are important for insulin sensitivity by avoiding down-regulation of insulin receptors. It is also unclear how approximately one million islets in the pancreas can achieve synchronized oscillatory insulin secretion. At subthreshold glucose concentrations the beta cell V_m is silent due to its hyperpolarization to a resting potential of about ~ 70 mV. An increasing glucose concentration leads to the generation of electrical activity arranged in AP bursts and electrical inactive interbursts. The oscillatory pattern of electrical activity in beta cells is also called slow waves. The synchronization of oscillations within an islet of Langerhans is due to a tight coupling of the cells via gap junctions. The burst and interburst length of an actively firing islet is glucose concentration dependent, i.e. with an increasing glucose concentration bursts are getting longer and interbursts shorter. The half maximal effective concentration (EC_{50}) for glucose in mouse islets is reached at ~ 11 mM whereas continuous spike activity takes place at ~ 20 mM. The fraction of plateau phase (FOPP), which shows the calculated percentage of burst time, can be used for the quantification of the V_m oscillations.

A special feature of pancreatic beta cells is that continuous stimulation of glucose concentration generates oscillations of electrical activity, intracellular Ca^{2+} concentration and insulin secretion.

3 Material

The chemicals and laboratory equipment used are divided according to their application fields and arranged according to their appearance during the workflow.

3.1 Chemical reagents

3.1.1 Islets of Langerhans preparation and cultivation medium

The collection of islets of Langerhans was carried out in working buffer, prepared with A+AB solution. The A+AB solution contained 135 mM NaCl, 5.6 mM KCl, 1.2 mM MgCl₂, 1.28 mM CaCl₂, 3 mM glucose, 10 mM HEPES, 1 g/L bovine serum albumin (BSA) and 10 % penicillin/streptomycin solution (+10.000 units/ml penicillin +10.000 µg/ml streptomycin). The pH 7.4 was achieved by adding 1 M NaOH until the desired pH was reached. The digestion solution was set up using A+AB solution and 2 mg/ml of Collagenase P were added. RPMI 1640 supplemented with 10% foetal bovine serum (FBS) and 1% penicillin/streptomycin (10.000 units/ml penicillin and 10.000 units/ml streptomycin) was used as culture medium.

Table 1: Chemicals and the corresponding distributors for the A+AB solution.

A+AB solution		
NaCl	135 mM	Roth, 3957.1
KCl	5.6 mM	Roth, 6781.1
MgCl ₂ (hexahydrate)	1.2 mM	Roth, HN03.2
CaCl ₂ (dihydrate)	1.28 mM	Roth, T885.2
D-(+)-glucose	3 mM	Sigma Aldrich, G8270
HEPES	10 mM	Roth, 9105.3
Albumine fraction V	1 g/L	Roth, 8076.2
PenStrep	1 %	PAA GmbH, P11-010
pH 7.4 was adjusted by adding 1M NaOH		

Table 2: Chemicals and the corresponding distributors for the culture medium.

Culture medium		
RPMI 1640		Gibco, 21875-091
FBS	10 %	Gibco, 2013-07, REF 10270-106
PenStrep	1 %	PAA GmbH, P11-010

Table 3: Chemicals and the corresponding distributors for the digestion solution.

Digestion solution		
A+AB solution		
Collagenase P	2 mg/ml	Roche Diagnostics GmbH, REF 11213 865 001, LOT 14556423

3.1.2 Extracellular recordings

MEA experiments were carried out in extracellular solution. The solution consisted of: 140 mM NaCl, 5 mM KCl, 1.2 mM MgCl₂, 2.5 mM CaCl₂, 10 mM HEPES. The pH was adjusted to 7.4 by adding 1 M NaOH. For pharmacological applications glucose and drugs of interest should be added to the extracellular solution.

Table 4: Chemicals and the corresponding distributors for the extracellular solution.

Extracellular solution		
NaCl	140 mM	Roth, 3957.1
KCl	5 mM	Roth, 6781.1
MgCl ₂ (hexahydrate)	1.2 mM	Roth, HN03.2
CaCl ₂ (dihydrate)	2.5 mM	Roth, T885.2
HEPES	10 mM	Roth, 9105.3
pH 7.4 was adjusted by adding 1M NaOH		

3.1.3 Lab equipment and consumables

Table 5: Equipment and the corresponding distributors for the islet of Langerhans culture.

Dissection equipment	
1x scissor	Miltex, 5-2 scissor
1x surgical scissor	4' martin 11-740-11
1x 55' forceps	FST
2x Semken forceps	FST 11008-13
3x Baby-Mixter haemostat-curved 14cm	FST 13003-10
1x Ultra-Asept 3R 5ml LL	Medicshop.de HSW 2405030124
2ml syringe Inject Luer solo	B Braun Melsungen AG, 4606027
27G needle, 0.4 x 20mm, Sterican	B Braun Melsungen AG

Table 6: General laboratory equipment and the corresponding distributors.

Laboratory Equipment & Consumables	
Centrifuge	Hettich Universal 16-A, 0034-K
Water bath	Julabo SW-20C
Plasma cleaner	Plasma Cleaner/Sterilizer PDC-32-G-2, Laseranalytik Starna GmbH
Incubator	

Table 7: Equipment for an up to date MEA recording setup (MEA2100 Beta-Screen- and / or Mini-system)

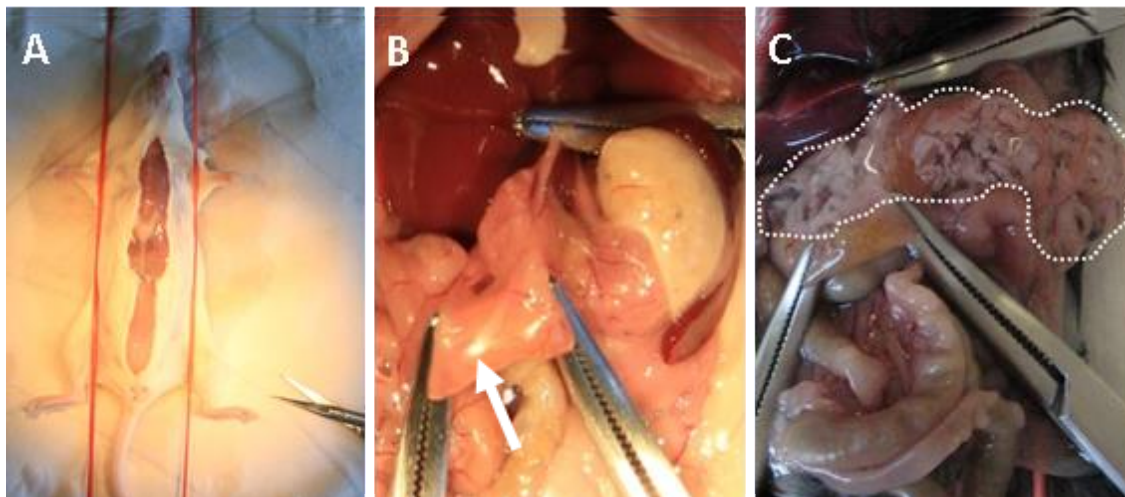
MEA setup equipment	
MEA2100-Beta-Screen-System	Multi Channel Systems, Reutlingen
MEA2100-Mini-System	Multi Channel Systems, Reutlingen
Temperature controller, TC02 (optional)	Multi Channel Systems, Reutlingen
Data acquisition computer (optional)	Multi Channel systems, Reutlingen

3.2 Isolation of islets of Langerhans

3.2.1 Isolation of murine islets

The experiments were carried out with islets of Langerhans isolated from fed 23 – 25 g NMRI or C57Bl/6 mice (Janvier, France). They were sacrificed by application of CO₂ and a subsequent cervical dislocation. The principles of laboratory animal care were followed (NIH publication No. 85-23, revised 1985) and experiments were done according to German laws.

The mouse was fixated with the ventral side facing upwards. A transversal incision was carried out inferior to the ribs. The pancreatic tissue was collected after inflation with digestion solution containing Collagenase P (Roche Diagnostics GmbH, Mannheim, Germany). Thereafter, haemostatic clamps were applied: The common bile duct and the anterior and inferior duodenum were closed to ensure proper inflation. Through the sphincter of Oddi the Collagenase P solution was injected into the pancreatic duct (see Figure 15 A). The inflated pancreas was then pooled in an iced 15 ml tube. The following digestion was carried out at 37° C under permanent shaking (30 to 40 rpm) in a water bath. Subsequently two cleaning and dissociation cycles were performed. After that the cell pellet was resuspended applying constant shear force with a record syringe and centrifuged at 500 G for 1 min. After discarding the supernatant containing fat, blood and pancreatic tissue, the pellet was transferred into fresh A+AB solution. The obtained islets of Langerhans were hand-picked under a binocular microscope. Darkened glass dishes were used to enhance the contrast of the brightly white islets and the surrounding tissue particles. The Langerhans islets were cultured up to 3 days in RPMI medium (10 % FBS, 1 % PenStrep) at 37°C and 5 % CO₂.



Preparation of mouse islets: Applying the haemostatic clamps correctly and inflating the pancreas with Collagenase P solution are the most critical part of the isolation process besides the digestion time. **A** The mouse was fixated with the ventral side facing upwards. A transversal incision was carried out inferior to the ribs. In **B** the sphincter of Oddi (white arrow) and the ductus hepaticus and pancreaticus are clamped. In order to apply the Collagenase P solution to the pancreatic tissue, three hemostatic clamps were used to close the anterior (right clamp) and inferior (left clamp) duodenum and the ductus hepaticus (upper clamp) connected to the liver. With the clamps in place, the applied Collagenase P solution readily entered the ductus pancreaticus. **C** Presents the result of a proper inflation. The colour of the pancreas changed to brightly white.

3.2.2 Isolation of human islets

Human islet recordings recorded with the MEA-technology were obtained from biopsies taken during pancreatic surgery according to the approvals of the ethics commissions of the Medical Faculty and the University Hospital of the University of Tübingen (No. 359/2013BO2) and the Universitätsmedizin Greifswald (BB 050/13) or from organ donors isolated according to the Edmonton Protocol. The islets were isolated by injecting collagenase (2–4 mg/ml, Serva, Heidelberg, Germany) into the biopsy material and by handpicking after digestion at 37°C. For measuring electrical activity of human islets in pancreas slices a biopsy was embedded in 4 % low-melting agarose. Slices of 300 µm thickness were cut with a Leica VT1000 S vibrating blade microtome (Leica, Wetzlar, Germany). Islets and pancreatic slices were cultured overnight in DMEM or CMRL1066 (human, 5 mM glucose). All media were supplemented with 10 % fetal calf serum, 100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin.

More information about recordings with human islets can be found in the following publication “Human Islets Exhibit Electrical Activity on Microelectrode Arrays (MEA)” (Schönecker et al., 2015).

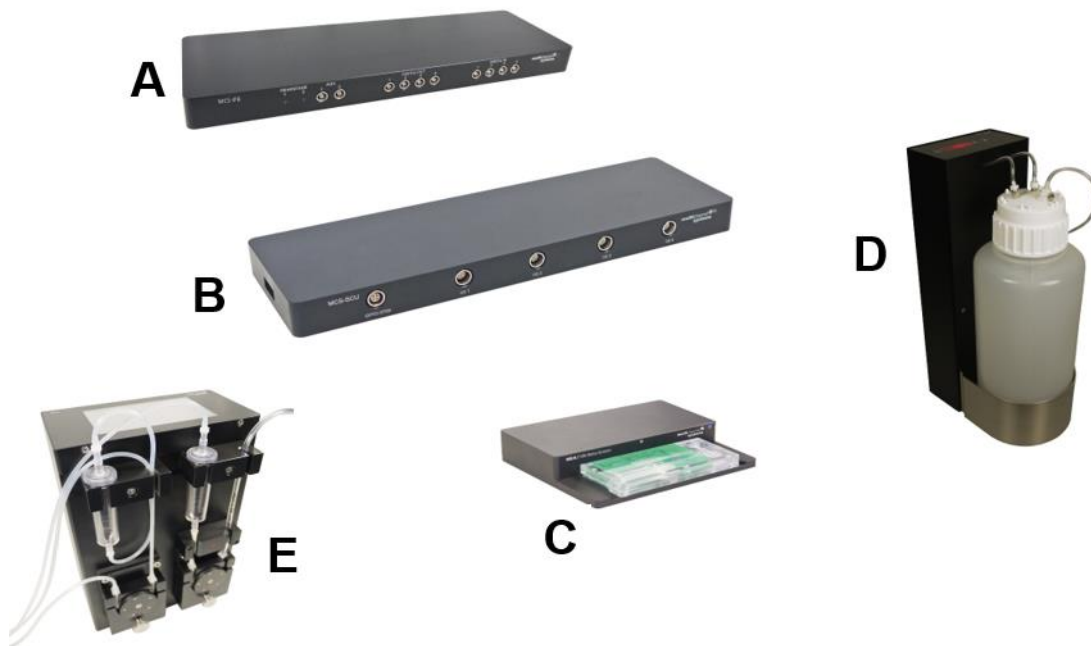
4 Experimental setup

This chapter describes how to prepare and to record islets of Langerhans with the MEA2100-Beta-Screen System.

4.1 MEA2100-Beta-Screen-System Setup

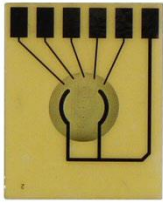
The Beta-Screen-System is specifically designed for electrophysiological recordings of multiple islets of Langerhans optimized for drug screening.

The first element of the MEA2100-Beta-Screen-System is the headstage. A specifically designed MEA with 5 electrodes can be used per headstage. The Beta-Screen chamber is equipped with a heating plate for a constant temperature in the close vicinity of the recording electrodes. The cable of the MEA2100-Beta-Screen headstage connects to the signal collector unit MCS-SCU. Up to four MEA2100-Beta-Screen headstages can be operated with one signal collector unit MCS-SCU and up to two signal collector units can be connected to one interface board MCS-IFB-*in-vitro*. Hence, in total a system can record from up to 40 electrodes (8 individual 5-channel headstages). Electrode raw data acquired from the probe is amplified and digitized by the analog / digital converter that is integrated in the headstage at sampling rates of up to 50 kHz per channel. Data is transferred via signal collector unit to the interface board with an eSATAp cable.



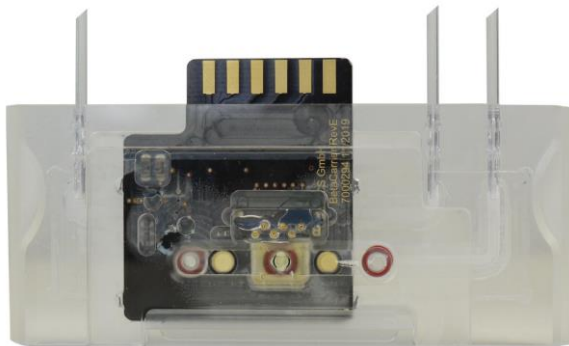
Main hardware components of the MEA2100-Beta-Screen-System **A** The interface board (MCS-IFB-in-vitro) collects data from one or two attached signal collector units (MCS-SCU). **B** The Signal Collector Unit (MCS-SCU) is the signal collector for up to four headstages. **C** The MEA2100-Beta-Screen Headstage is equipped with 5 electrodes and a heating plate for constant temperature in the MEA chamber. **D** The constant vacuum pump CVP is equipped with a precision differential pressure sensor to measure the target pressure in relation to the actual ambient pressure. **E** The peristaltic perfusion system PPS2 with one inlet and one outlet pump. Both pumps can be operated independently and the percentage difference is adjustable.

4.1.1 MEA2100-Beta-Screen MEA Chip and Beta-Screen Chamber



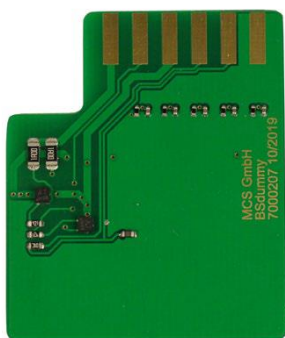
The Beta-Screen Chamber is made of Plexiglas and should be used as consumable which can be ordered separately. The MEA2100-Beta-Screen MEA chip contains 5 TiN (Titanium Nitride) electrodes with a diameter of $30\text{ }\mu\text{m}$ and two reference electrodes, which are connected to ground. The electrodes are surrounded by holes with a diameter of $10\text{ }\mu\text{m}$ for the positioning of the islet via negative pressure from the bottom side of the MEA.

Note: If necessary, please add an additional AgAgCl wire to ground the system.



The Beta-Screen Chamber can be constantly heated to a level of 37°C . The fluidics through each MEA chamber is realized via PPS2 pump for the inflow and the suction of the extracellular solution (recording solution) and a Constant Vacuum Pump CVP with pressure control for the low pressure from below. This helps to keep the islets in place on the recording electrodes.

Test Chip for MEA2100-Beta-Screen-System



The provided test model probe Test-5MEA simulates a MEA with a resistor of $220\text{ k}\Omega$ and a 1 nF capacitor between ground and electrode for each of the 5 electrodes. It can be used for testing the noise level of the electrodes of a MEA2100-Beta-Screen-System.

4.1.2 Setup of the MEA2100-Beta-Screen-System hardware components

Connecting Headstages, Signal Collector Unit (MCS-SCU), Interface Board (MCS-IFB) and Computer

1. Provide a power supply in the immediate vicinity of the installation site.
2. Place all devices on a stable and dry surface, where the air can circulate freely, and the devices are not exposed to direct sunlight.
3. Set up the computer with installed Beta-Screen program.
4. Install the software from the installation volume if it is not already installed. The MEA2100-Beta-Screen-System is a plug and play device. The driver is automatically installed together with the data acquisition program.
5. Set up the MEA2100-Beta-Screen headstage(s) on a solid table or workbench.
6. Setup the Peristaltic Perfusion System(s) PPS2 to control the fluidic.
7. Setup the Constant Vacuum Pump(s) CVP to apply low pressure to the MEA chamber below the electrodes.
8. Connect the headstage/s via the provided cable/s to the signal collector unit MCS-SCU. Connect the signal collector unit MCS-SCU-in vitro via iX-B cable to the MCS-IFB-in vitro interface board.

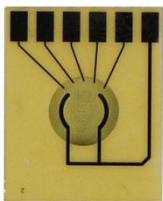


Warning: Do not stack the MCS-SCU device onto the MCS-IFB or any other device, because of heat development!

9. Connect the one or both USB 3.0 micro ports of the interface board to a free USB 3.0 port of the data proceeding acquisition computer. Do not use an USB hub and avoid USB ports on the front panel of the computer, as they are often connected to the mainboard via an internal hub.
10. Connect the interface board via power supply unit to a power outlet of the same electrical system (connected to the same ground / earth wire) as all other components of the setup, for example, the computer or shielding.
11. If necessary, connect the system to an external ground.
12. Turn the toggle switch at the rear panel of the interface board on.
13. Check the power LED. It should light up as soon as the power line is connected, and the toggle switch is switched on. If not, check the power source and cabling.
14. Start the data acquisition program and select the MEA2100-Beta-Screen-System as data source.

(For more details please see MEA2100-Beta-Screen Manual)

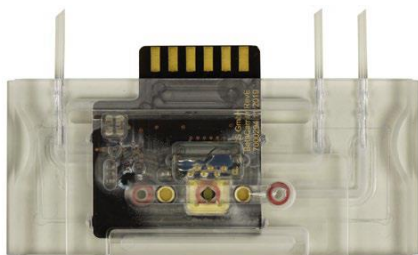
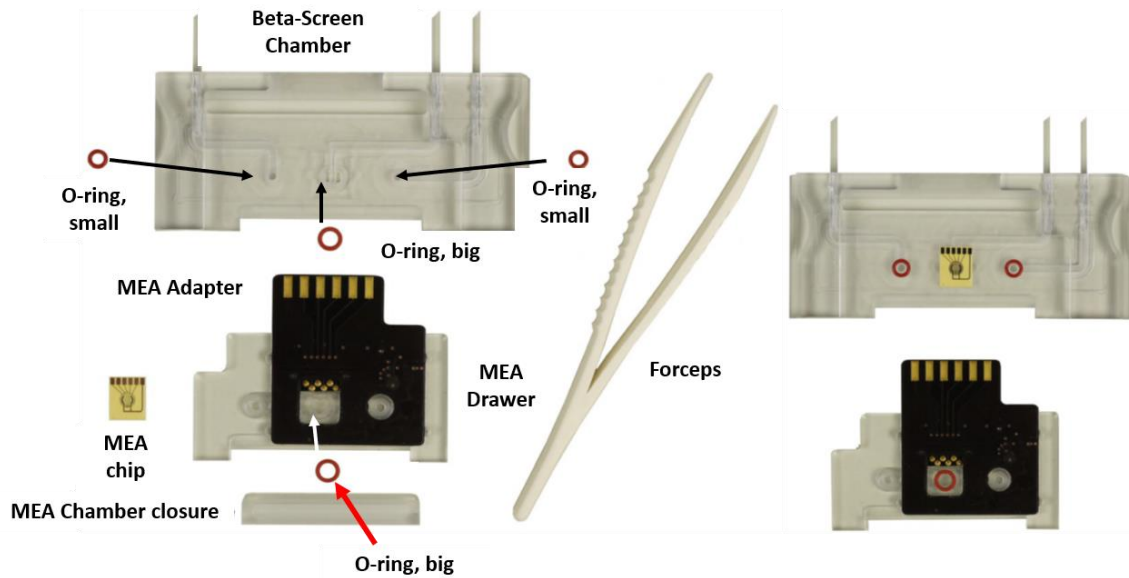
4.1.3 Setup of the Beta-Screen Chamber



Please follow the instructions with great care!

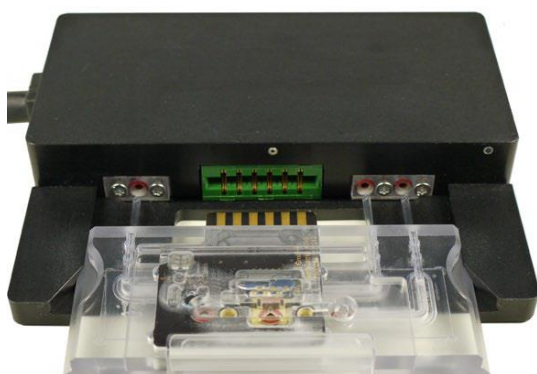
Configuration of the components:

- MEA chip
- Beta-Screen chamber
- MEA drawer
- MEA adapter
- MEA chamber closure
- O-rings, two smaller, two bigger rings
- Forceps



Place the components of the Beta-Screen MEA chamber on a flat table surface as shown on the picture. Insert the respective o-rings into the provided slots with the forceps. Do not mistake the different sizes of the o-rings! Use the forceps to insert the MEA in correct orientation over one of the bigger O-rings. Turn the drawer so that the other big O-ring is oriented to the side of the MEA and push the drawer with the MEA contact adapter carefully into the MEA chamber. Use the MEA Chamber closure to complete the setup of the Beta-Screen Chamber.

Shut the chamber with the MEA chamber closure of the drawer to complete the setup.



Connect the complete Beta-Screen Chamber to the MEA2100-Beta-Screen headstage. Insert the tubes carefully into the respective slots and connect the adapter to the provided plug in.

5 Recordings with the MEA2100-Beta-Screen-System

The Beta-Screen electrodes are specifically designed for the placement of islets via suction. The electrodes are placed on top of a polyimide foil and the electrode material is titanium nitride. Negative pressure can be applied from the bottom side of the MEA. The electrodes are surrounded with positioning holes to ensure a proper positioning of the islets on top of the electrodes. A recording chamber is equipped with five electrodes. Negative pressure can be applied with a vacuum pump (CVP) and recording solution can be perfused with a peristaltic perfusion system (PPS2) with one inlet and one outlet pump.

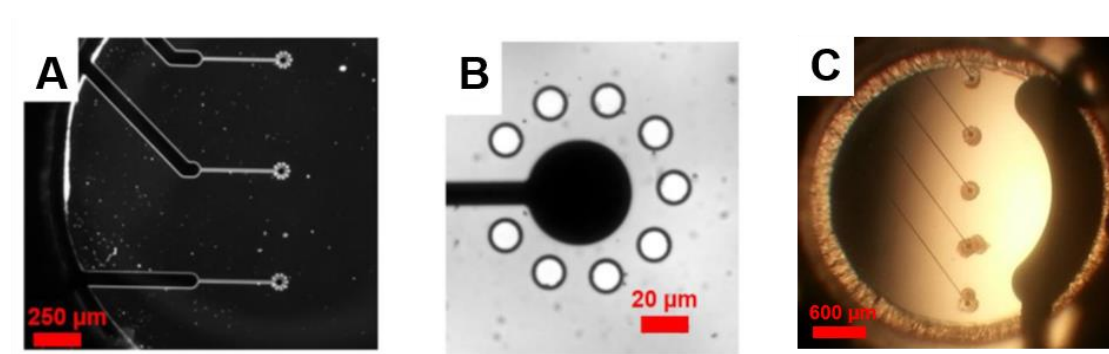
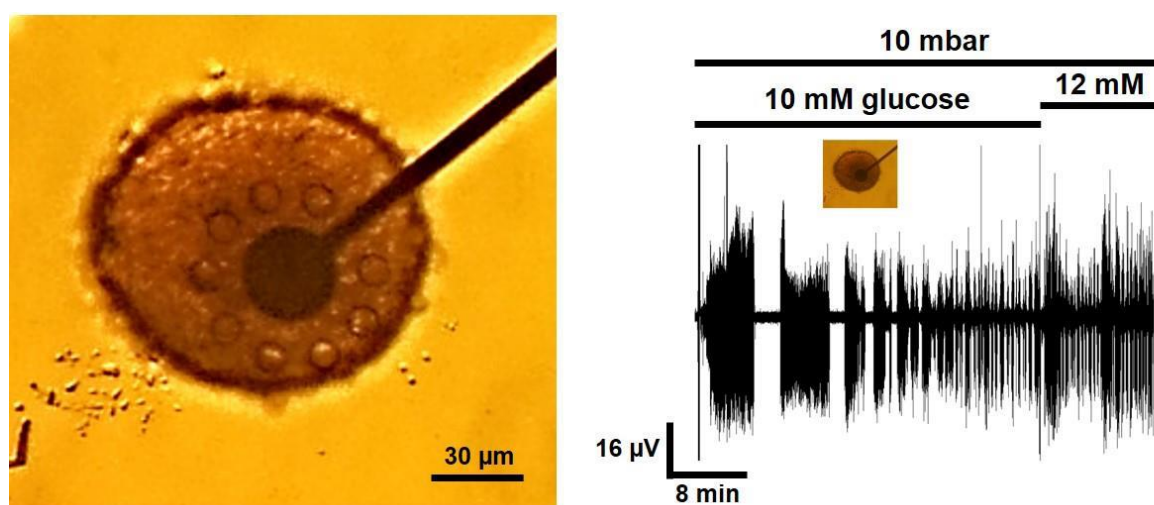


Figure 14: Microscopic image of the electrode design and mouse islets placed on top of the electrodes. **A** Three electrodes located in a recording chamber with electrode tracks coming from the left. **B** Beta-Screen electrode with surrounding positioning holes. **C** Mouse islets placed on five Beta-Screen electrodes.

One example recording of electrical activity of an intact islet with the new electrode design is shown in the figure below. After pipetting the islets into the recording chamber they positioned themselves on top of the electrodes. The use of 10 mbar negative pressure during the pipetting turned out to be ideal for the positioning process. Moreover, not only could the placement be facilitated, but even the signal to noise ratio is significantly improved in comparison to acute recordings with a standard MEA-System, due to a much better contact to the electrodes.



Proof of principle recording with a MEA2100-Beta-Screen-System electrode. **(left)** Microscopic photo of a mouse islet placed with 10 mbar negative pressure on top of a Beta-Screen electrode. **(right)** Trace with electrical activity of the islet shown on the left side in 10 and 12 mM glucose. Signal to noise ratio is improved in comparison to acute recordings.

5.1 Important preparations to start the recordings

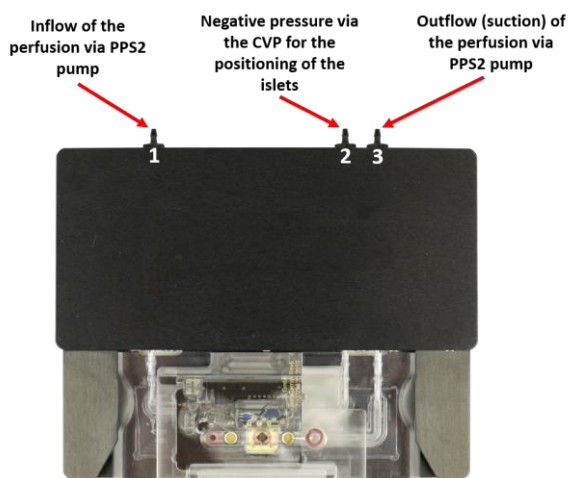
Connect all cables of the main hardware components as indicated in the manual and described in chapter 4.1.2. The fluidics through each MEA chamber is accomplished via PPS2 pump for the inflow of recording solution and the suction. The Constant Vacuum Pump (CVP) with pressure control generates the negative pressure for the positioning of the islets on the recording electrodes. For inserting the MEA chip into the Beta-Screen Chamber place the O-rings as described in chapter 4.1.3 and close the chamber with the MEA chamber closure.



MEA2100-Beta-Screen MEA setup. Example of a Beta-Screen setup with all components necessary for proper recordings.

As shown in the setup example above no specific anti-vibration table is needed for the recordings but could be used if available. The recordings are possible without a Faraday cage, but in case a cage is available it could be used to avoid noise sources.

5.1.1 Connection of the perfusion and negative pressure on the Beta-Screen headstage

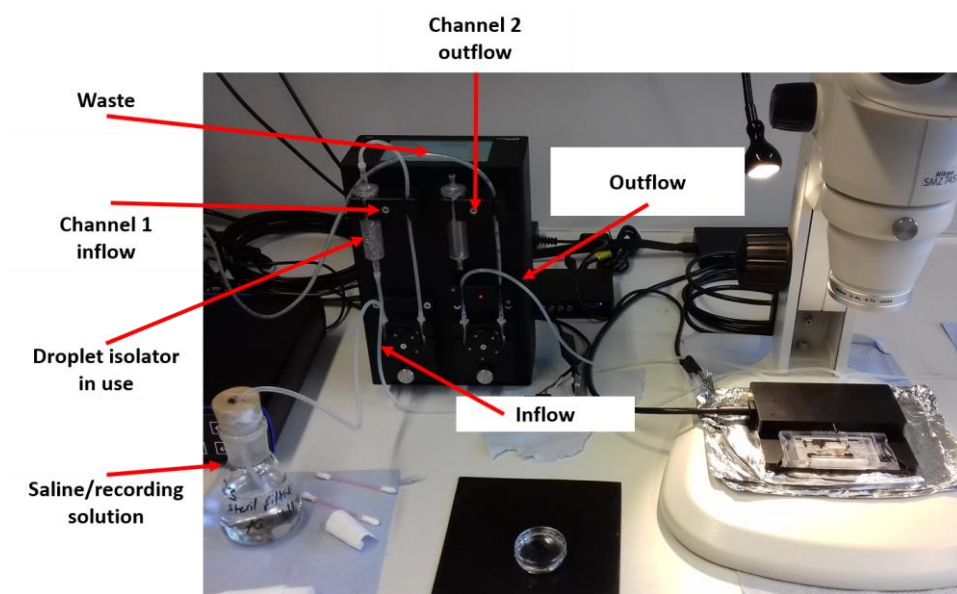


Connect the inflow and the outflow tubes of the PPS2 perfusion pump to the inflow (1) and outflow (3) connector of the headstage. Connect the CVP vacuum pump tube to the connector for the negative pressure (2).

5.1.2 Installation and connection of the PPS2 perfusion pump

1) Connect the reservoir with fresh extracellular solution (recording solution) to channel 1 of the PPS2 and insert a droplet isolator between the pump wheel and inlet tube of the Beta-Screen headstage. Leaving out the droplet isolator could result in line hum and additionally, to periodically appearing noise picked up from the pump electronics.

2) Connect the outlet tube from the head stage directly to channel 2 of the PPS2. Do not use a droplet isolator here! It would lead to slow build-up of negative pressure and sudden release by one strong slurp, causing a strong electric artifact on the measurement. Due to chamber design and the Silver/Silver Chloride electrode in the chamber, line hum imposed on the saline-filled tube will not reach the electrodes.



5.1.3 Installation and connection of the Constant Vacuum Pump (CVP)

The constant vacuum pump CVP with pressure control is a vacuum pump with a precision pressure sensor and a waste bottle. The selected pressure can be easily changed during the experiment. The CVP is especially suitable to work with the MEA2100-Beta-Screen-System or perforated MEAs in MEA-Systems.

The CVP consists of the pump housing with the control panel on top, and the waste bottle with a volume of 5 L. The cup of the waste bottle is equipped with three tube connectors. Two tubes come from the pump housing; they are connected to the pressure sensor and the pump, respectively. The third one is used to connect the waste bottle to your experimental setup. To remove the connectors, press the metal button and pull out the tube. Take care not to mix up the tubes.

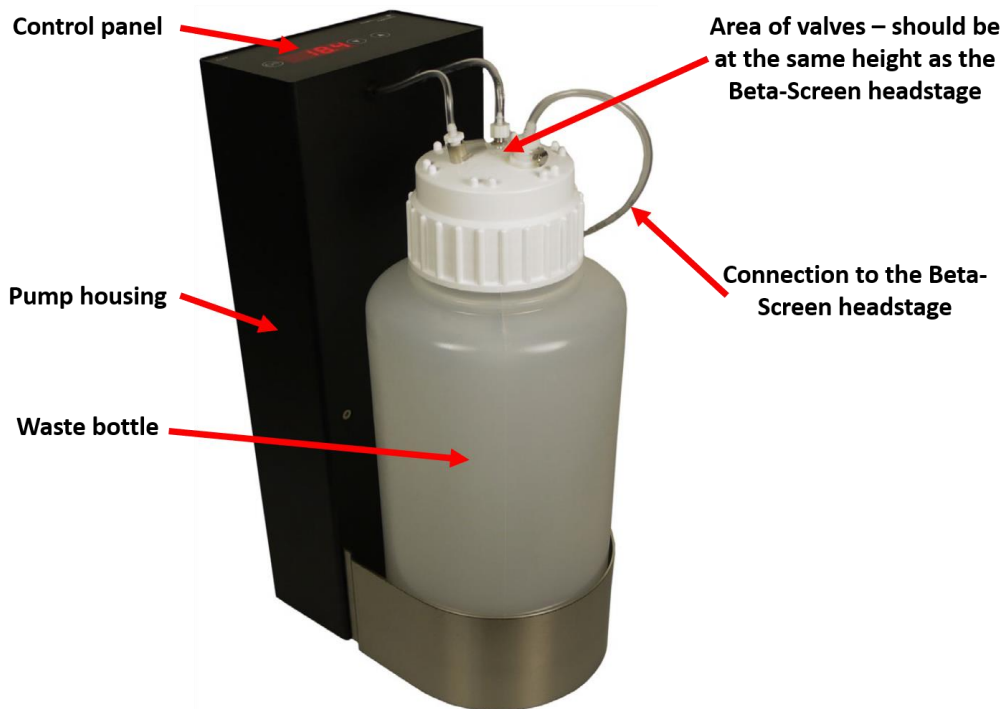
An air pump is switched on and off by the use of a solid-state relay (SSR) with an inherent triac. The air pressure in a bottle is measured with a precision sensor and compared with the selected pressure. According to the result, the SSR is fired in order to regulate the pressure in the bottle.

1) Connect the suction port tube from the head stage to the CVP on the other side. Please place a three-way valve in-between to be able to open and close the tube independent from the CVP. The use of the three-way valve also enable the tube to be filled with saline.

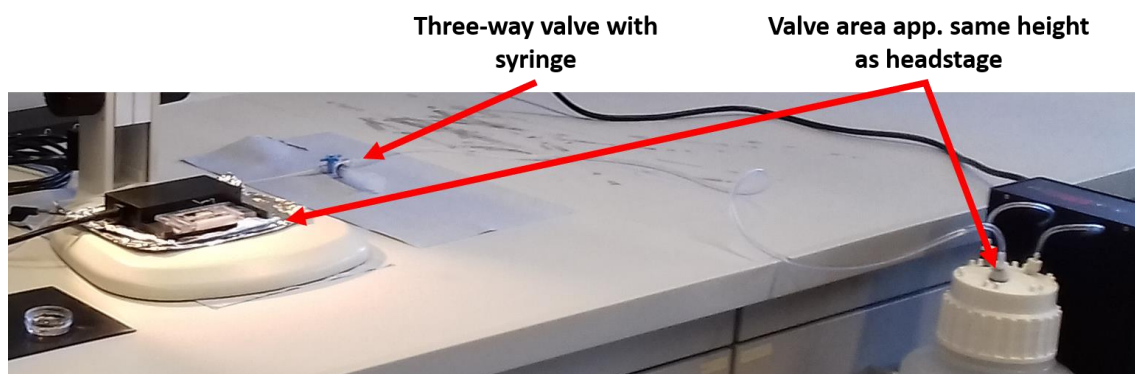
2) Fill the complete tubing and the fluidic block of the Beta-Screen headstage completely with saline. To fill the tubes please use a syringe, e.g. with 10 ml volume.

3) Please make sure that the area of the valves where the tubes reach the pump (see figure below) reaches approximately the same height as the Beta-Screen headstage! This is very important to improve the stability of the suction properties. Moreover, please try to keep the tubes as short as possible.

4) Usually the range of the mbar values are in-between 10 and ~ 40. The value can vary depending on the setup and tube length.



The valve area of the Constant Vacuum Pump (CVP) should be at the same height as the Beta-Screen headstage

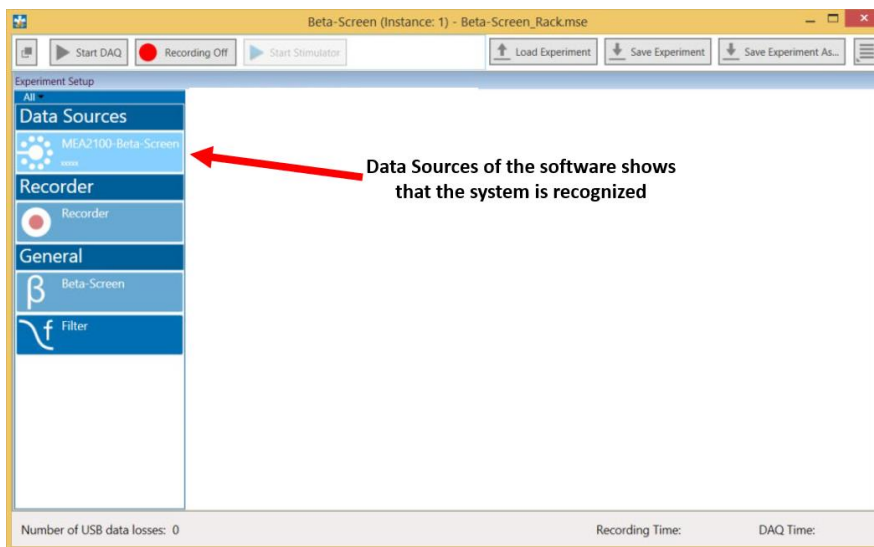


5.1.4 Hardware and software preparations to start an experiment

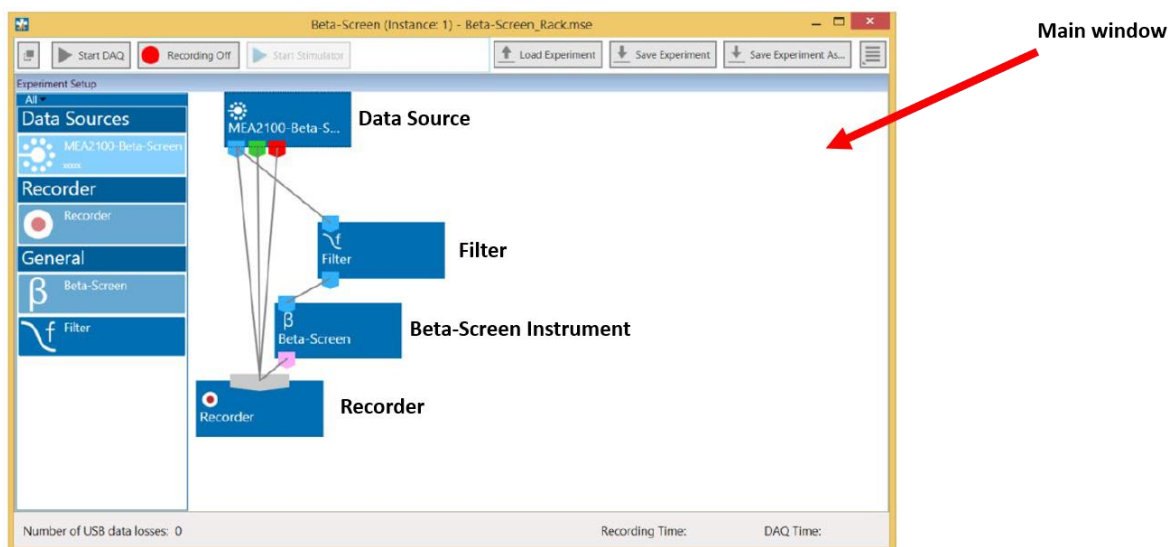
This chapter aims to explain how to prepare the system for the recordings and how to place the islets into the Beta-Screen chamber. More information about the software features are available in the Beta_Screen_Manual (<https://www.multichannelsystems.com/products/mea2100-beta-screen-system#docs>).

5.1.4.1 Software preparations to start an experiment

Please download the Beta-Screen Experimenter software from the Multi Channel Systems website via the following link <https://www.multichannelsystems.com/software/beta-screen-experimenter> . Install the software and make sure that the software recognizes the Beta-Screen system.



Once the hardware components are recognized, you can add the necessary instruments for the recordings, filtering and online data analysis. Each instrument can be dragged and dropped into the main window.



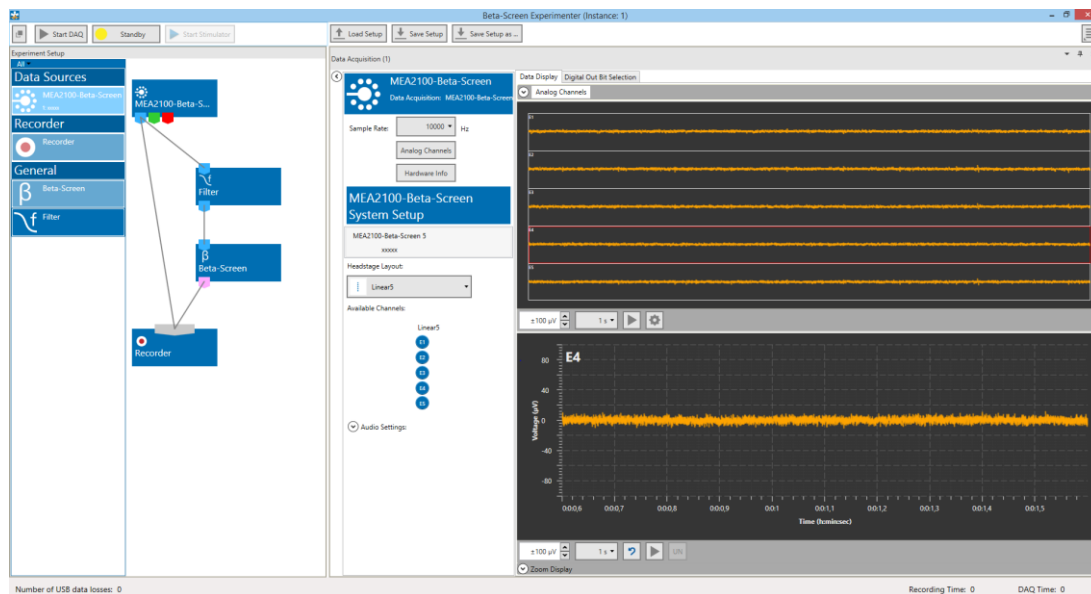
The “**Data Source**” instrument has three output ports: Raw Data (blue), Analog Data (green) and Digital Data (red). Please always connect the blue “Raw Data” port to the recorder so the raw and unfiltered data will be stored. In most cases the “**Filter**” tool is useful to improve the signal-to-noise ratio of the recordings. Especially for recordings with mouse islets, a low-pass filter is recommended. Please always compare the raw data trace with the filtered trace to have a direct comparison of how the filter effects the signal-to-noise ratio.

Recommended values for the low pass filter for mouse islet recordings are around 25 and 50 Hz. Please keep in mind that the signal shape and frequency can significantly vary between species. One important example is the comparison of the electrical activity from mouse and human islets recorded with the Beta-Screen. Electrical activity of mouse islets appeared much slower than the activity of human islets and a low pass filter could significantly cut the signals.

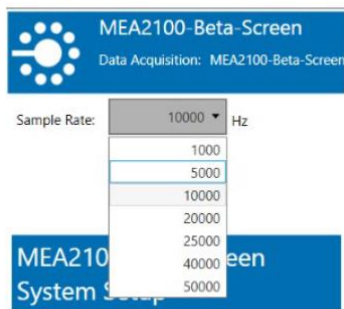
The “**Beta-Screen Instrument**” has specifically designed tools to visualize beta cell activity over a long period with implemented spike and burst (FOPP) analysis tools. The recordings can be user defined e.g. as single dose or as a dose-response experiment. Moreover, the integrated heating device can be directly controlled.

Step by step description to get the Beta-Screen Experimenter software ready for recordings

- 1) Drag and drop the **Data Source**, **Filter**, **Beta-Screen** and the **Recorder** into the main window.
- 2) Connect the Raw Data port (blue) directly to the Recorder!
 - Important to have the untouched raw data stored.
- 3) Also connect the Raw Data port to the Filter tool in case you want to filter your data. (If you do not use the Filter please connect the raw data to the Beta-Screen instrument and follow the description in step 5).
 - Use a low pass filter e.g. for mouse islet recordings (50 or 25 Hz).
- 4) Connect the Filter to the Beta-Screen instrument to be able to use the analyses tools.
- 5) Connect the Beta-Screen device to the recorder to directly store the analysis parameters.



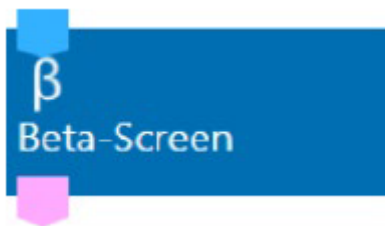
Sample Rate



Select the "Sample Rate" in "Hz" from the drop down window.

The MEA-Technology from Multi Channel Systems is not only developed for beta cell recordings and is therefore able to record data with the highest possible sample rate up to 50 kHz. For beta cell recordings it is enough to use a sampling rate of ~ 5 kHz. The settings for the sample rate can be found within the “**Data Source**” tool.

The Beta-Screen instrument



The Beta-Screen instrument is designed to

- visualize beta cell activity over a long period
- perform spike and burst (FOPP) analysis
- define and conduct dose-response experiments
- control the integrated heating device of a connected Beta-Screen headstage

Design your experiment with the 4 different experiment modes

The 4 experiment modes are “Predefined”, “Fixed Durations”, “Manually Timed” and “Basic”.

Predefined → run experiments with a predefined dilution series and predefined timing of the experiment phases

Fixed Durations → run experiments with a flexible number of cycles (definition on the fly) but a predefined timing of the experiment phases

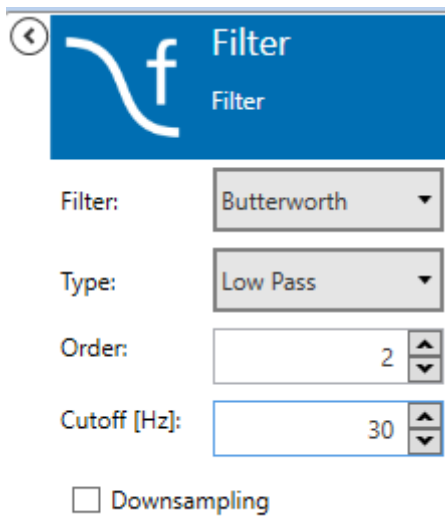
Manually timed → just like the Fixed Duration mode, except for the timing being completely manual

Basic → continuous measurement (no cycle phases) using defined markers only to indicate events

If you are not exactly sure about the experimental design and if you are doing the first recordings with the Beta-Screen we recommend using the **Basic Mode**. With the Basic Mode you are completely flexible, and you can change the conditions of your recording whenever you want. Please make sure to set the markers once you changed the drug or the concentration of a drug or glucose series.

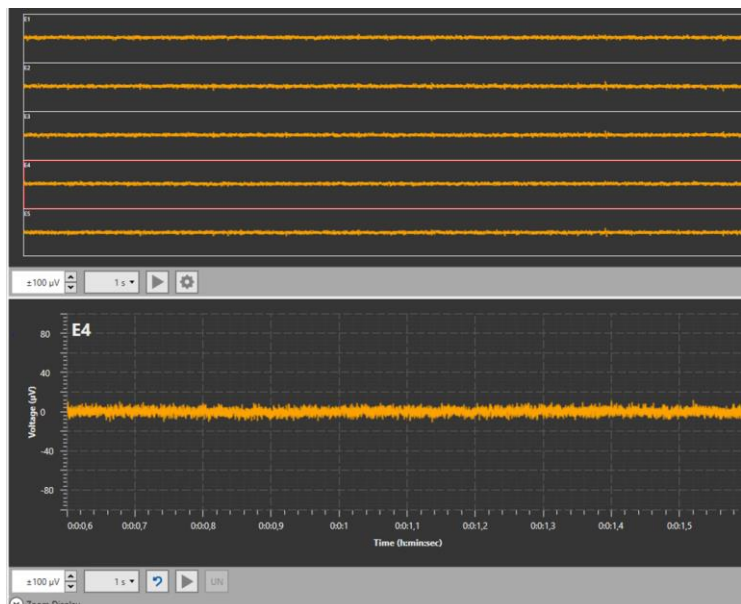
Low Pass filter for mouse recordings

In case you want to use a filter please choose the filter settings within the “Filter” tool, e.g. a Butterworth low pass filter of 30 Hz.



5.1.5 Positioning of the islets and start of an experiment

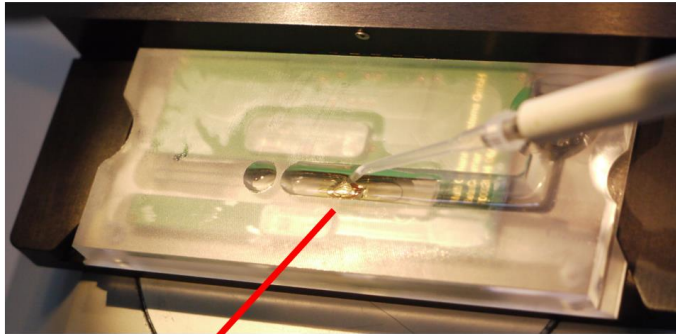
The first step is to start the PPS2 perfusion pump to fill the perfusion tubes and the recording chamber with recording solution. The outflow of the perfusion (suction) should be a stable slurping (alternate suction of air and liquid) and the electrical background noise (baseline) must be monitored via the software. The baseline should be stable and within an acceptable range of around ± 10 to $20 \mu\text{V}$.



The next step is to completely fill the tubes belonging to the Constant Vacuum Pump (CVP). Please use a three-way valve and a syringe to fill the tubes completely with saline. In addition, please make sure that the chamber under the Beta-Screen-MEA inside the Beta-Screen Chamber is also filled with saline. You can carefully fill the chamber via the tube from the three-way valve to the chamber. Try to avoid air bubbles inside the tubes! The air bubbles can interfere with the performance of the CVP which at the end can lead to problems with the positioning of the islets.

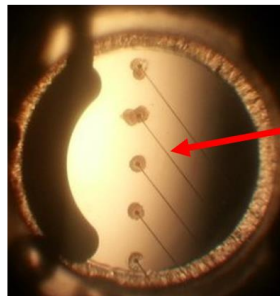
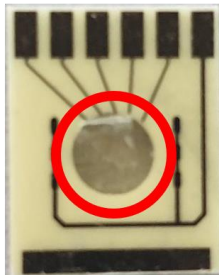
The last step is the placement of the islets into the Beta-Screen chamber. Please use a 10 μ l pipette (alternatively you can also use a 100 μ l pipette) and bend the tip roughly to an angle of 45 degrees (see figure below). Take 5 islets without any air bubbles into the pipette and turn the CVP pump on. Pipette the islets into the chamber and try to come as close as possible to the electrode area. Use a stereo microscope for optical control as shown above. Do not touch the electrode area! The electrodes are placed on a thin polyimide foil which can be destroyed with the pipette tip.

Pipette the islets into the Beta-Screen recording chamber



Bend the tip of a 10 or 100 μ l pipette and pipette 5 islet as close as possible to the electrode area.

Activate the CVP just before you pipette the islets into the chamber



Example of nicely positioned islets on top of the electrodes

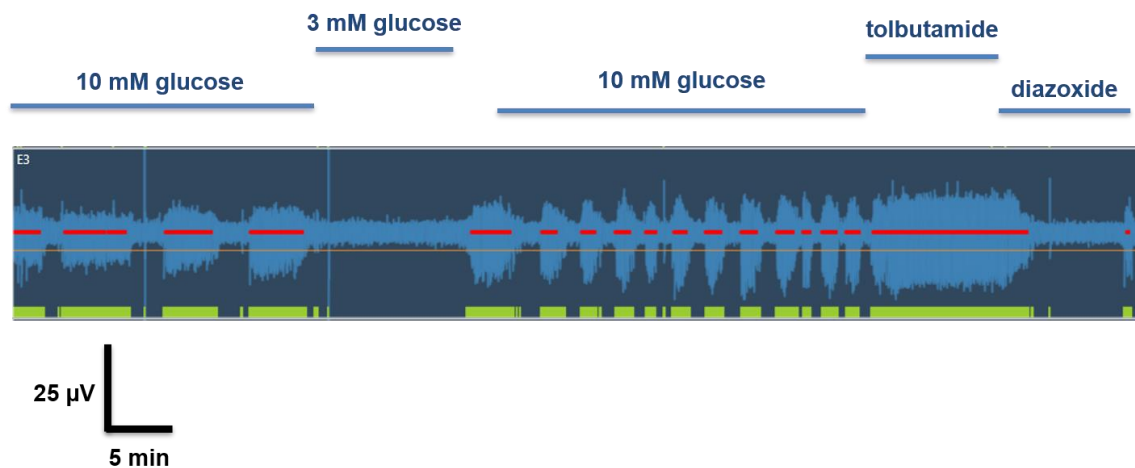
After the placement of the islets make sure that the perfusion volume is high enough to constantly cover the islets with recording solution. It could be that the CVP also sucks solution through the positioning holes. This strongly depends on how many islets are placed on top of the electrodes (ideally 5 islets) and how good they are positioned. Try to keep the perfusion velocity low to avoid artefacts.

If the islets are positioned wait a view minutes to monitor the performance on the individual electrodes and once you are fine with the activity you can start the recordings. Keep in mind that the electrical activity is relatively slow and a usual recording period under a specific condition (e.g. a defined glucose concentration) can vary between 5 to 25 minutes. The time frame of a recording also depends strongly on what exact parameters you are interested in.

5.1.6 Example of electrical activity recorded with the Beta-Screen-System

The electrical activity can vary between different species. To the best of our knowledge mouse and human islet activity has been already recorded with the MEA-technology.

Beta-Screen – Example trace of electrical activity from a mouse islet with

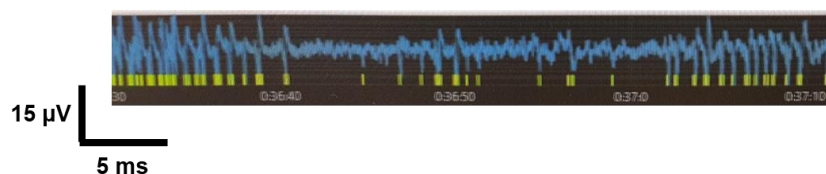


The upper example shows a trace of a mouse islet recording with different glucose concentrations and the K_{ATP} -channel modulators tolbutamide and diazoxide. The red line within the recorded trace represents the online analyses of the characteristic oscillatory burst activity which you can extract directly as fraction of plateau phase (FOPP). The analysis parameters can be directly exported as ASCII file as shown in the excel table below.

E3	E3 Burst Count	E3 FOPP [%]	E3 Mean Burst Duration [ms]	E3 Mean Inter Burst Interval [ms]	E3 Mean Spikes Per Burst
10 mM glucose	10	61	80424,87	52107,8	143,6
3 mM glucose	0	0			
10 mM glucose	10	43	74011,2	80953,8	174,3
tolbutamide	4	82	36147,4	30133,1	72,7
diazoxide	1	20	10648,5	440916	16

The table shows the analysis of electrode 3 from the example above. You can nicely see the different percentages of the fraction of plateau phase (FOPP).

Activity within an active burst



Electrical activity within an active burst and the detections of the "Spike Detector" (yellow areas).

At the end of each measurement, the following parameters are extracted, all based on spike time stamps and burst data:

- spike count
- burst count
- burst durations
- fraction of plateau phase (FOPP)
- inter burst intervals
- spikes per burst

After finishing an experiment, a log and all analysis results are automatically exported in an Excel compatible format.

6 Contact Information

Local retailer

Please see the list of official MCS distributors on the MCS web site
<https://www.multichannelsystems.com/sales>.

User forum

The Multi Channel Systems MCS GmbH user forum provides the opportunity for you to exchange your experience or thoughts with other users worldwide .
<https://multichannelsystems.forumieren.de/>

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 newsletter on the contact form of the MCS web site.
www.multichannelsystems.com