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Microelectrode Array (MEA) Manual

Imprint

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1 Introduction

1.1 About this Manual

The MEA manual comprises all important information about the microelectrode arrays (MEA) for use with MEA2100- and ME2100-Systems or (USB-) MEA- or ME-Systems from Multi Channel Systems. The MEA manual focuses on general information on the MEA design**,** use, and handling, and more specific information on different MEA types. It also includes recommendations on sterilization**,** coating, and cleaning procedures, from scientifical papers or from recommendations of other MEA users.

For more details on issues that refer to the amplifier, like grounding or mounting the MEA, please refer to the manual for the MEA amplifier you use. You will find more information about MEA(2100)- or ME(2100)-Systems and its components in general in the respective manual. For more details on the data acquisition and analysis program Multi Channel Suite or MC_Rack, please refer to the respective manuals.

It is assumed that you have already a basic understanding of technical terms. No special skills are required to read this manual.

The components and also the manual are part of an ongoing developmental process. Please understand that the provided documentation is not always up to date. Please check the MCS web site (www.multichannelsystems.com) from time to time for downloading up-to-date manuals.

Those parts in this manual that refer to the applications and not to the product itself, for example, coating of MEAs, are only a summary of published information from other sources (see references) and has the intention of helping users finding the appropriate information for setting up their experiments. Multi Channel Systems MCS GmbH has not tested or verified this information. Multi Channel Systems MCS GmbH does not guarantee that the information is correct. Multi Channel Systems MCS GmbH recommends to refer to the referenced literature for planning and executing any experiments.

2 Important Information and Instructions

2.1 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.

2.2 Guarantee and Liability

The General conditions of sale and delivery of Multi Channel Systems MCS GmbH always apply. The operator will receive these no later than on conclusion of the contract.

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 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.

2.3 Important Safety Advice

Warning: Make sure to read the following advice prior to install or to use 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: Obey always 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 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.

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

- Malfunctions which could impair safety should be rectified immediately.
- Regard the technical specifications of the various MEA types, especially the temperature range and the safe charge injection limits for stimulation.
- Do not autoclave or expose pMEAs to heat more than 50 °C.
- Do not touch the electrode field in any way.
- Do not use any liquids or cleaning solutions with a high pH (> 7) for a longer period of time on MEAs of a silicon nitride insulation type. Basic solutions will damage TiN electrodes.

3 Microelectrode Arrays (MEAs) — Overview

3.1 Extracellular Recording with Microelectrode Arrays

A microelectrode array (MEA) is an arrangement of typically 60 electrodes allowing the targeting of several sites in parallel for extracellular recording and stimulation.

The warranty of a MEA chip is six months from the date of delivery.

Cell lines or primary cell preparations are cultivated directly on the MEA. Freshly prepared slices can be used for acute recordings, or can be cultivated as organotypic cultures (OTC) on the MEA.

Recorded signals are amplified by a filter amplifier and sent to the data acquisition computer. All MEAs (except EcoFlex- or FlexMEAs) are only for use with MEA2100- or USB-MEA-Systems for extracellular recording from Multi Channel Systems MCS GmbH. FlexMEAs may be used with components of ME2100-System and USB-ME-Systems from Multi Channel Systems MCS GmbH. EcoFlex- and FlexMEAs are designed for use in *in vitro* or *in vivo* studies. Please see setup manuals "Setup (USB-) MEA-Systems and (USB-) ME-Systems" for more information.

Several MEA geometries are provided for a wide variety of applications. Almost all excitable or electrogenic cells and tissues can be used for extracellular recording *in vitro*, for example, central or peripheral neurons, cardiac myocytes, whole-heart preparations, or retina.

There are various applications for MEAs in the fields of neurobiology or cardiac electrophysiology.

Typical neurobiological applications are: Ion channel screening, drug testing, safety pharmacology studies, current source density analysis, paired-pulse facilitation (PPF), long term potentiation (LTP) and depression (LTD), I / O relationship of evoked responses, circadian rhythm, neuro-regeneration, developmental biology, microencephalograms (EEG), and microelectroretinograms (ERG).

Typical applications in the cardiac field are: Activation and excitation mapping, measuring of the conduction velocity, longterm characterizations of cell types (especially stem cells), culture pacing, drug testing, safety pharmacology studies, monitoring of QT-related prolongation and arrhythmias, cocultures and disease / implantation model.

For more information on published applications or procedures for biological preparations, please see the application notes on the MCS web site:

http://www.multichannelsystems.com/downloads/documentation

3.2 MEA Design and Production

A standard MEA biosensor has a square recording area of 700 µm to 5 mm length. In this area, 60 electrodes are aligned in an 8 x 8 grid with interelectrode distances of 100, 200, or 500 µm. Planar TiN (titanium nitride) electrodes are available in sizes of 10, 20, and 30 µm, and three-dimensional TiN electrodes have a diameter of > 12 µm at the base with a very fine tip. Standard MEAs are useful for a wide variety of applications. Different geometries match the anatomical properties of the preparation. Most MEAs are available with a substrate-integrated reference electrode replacing the silver pellet in the bath. All electrodes can either be used for recording or for stimulation.

In principle MEA electrodes are not arranged symmetrical, so the MEA chip has to be placed inside the amplifier in the recommended manner.

Several other MEA types and layouts that are dedicated to special applications are also available, please see chapter "MEA Types and Layouts" for more details.

The biological sample can be positioned directly on the recording area; the MEA serves as a culture and perfusion chamber. A temperature controller controls the temperature in the culture chamber. Various culture chambers are available, for example, with leak proof lid or with semipermeable seal. An incubator is not necessarily required, long-term recordings in the MEA culture chamber are possible over several weeks or even months.

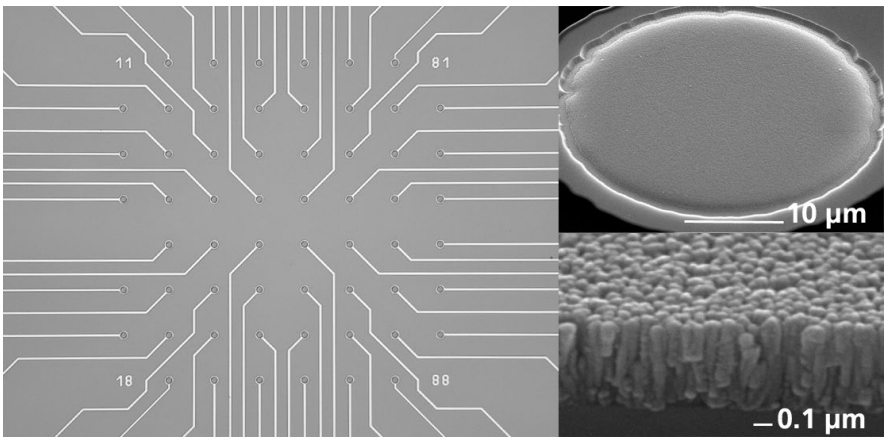
For cell or slice cultures, MEAs have to be coated with standard procedures before use to improve the cell attachment and growth, please read chapter "MEA Coating".

Spike activity can be detected at distances of up to 100 µm from a neuron in an acute brain slice. Typically, signal sources are within a radius of 30 µm around the electrode center. The smaller the distance, the higher are the extracellular signals. The higher the spatial resolution, the lower the numbers of units that are picked up by a single electrode, that is, the less effort has to be put into the spike sorting.

Multi Channel Systems provides MEAs with the highest spatial resolution in the market. HighDenseMEAs have electrodes with a diameter of only 10 µm arranged in a distance of only 30 µm (center to center). The challenge of manufacturing very small electrodes and at the same time keeping the impedance and the noise level down has been met by introducing the electrode material Titanium nitride (TiN).

The NMI TT in Reutlingen, Germany (www.nmi.de), produces MEAs from very pure fine quality and highly biocompatible materials. The NMI is a research institute, with which Multi Channel Systems has collaborated in many projects and over many years.

Quality controls and production processes have been improved over the last years so that MEAs are always of a fine consistent quality at very reasonable prices.



3.3 Electrodes, Tracks, and Insulation

Microfold structures result in a large surface area that allows the formation of electrodes with an excellent signal to noise ratio without compromising on the spatial resolution.

TiN (titanium nitride) is a very stable material that, for example, is also widely used for coating heavy equipment. All MEAs with TiN electrodes have a long life and can be reused several times if handled with care. If used for acute slices, MEAs can be used for approximately one year. Additionally available are EcoMEAs equipped with gold (Au) electrodes.

Long-time experiments with cell cultures and rigid cleaning methods shorten the MEA lifetime, but you can still reuse a MEA about 30 times, depending on the coating, cell culture, and cleaning procedure. All MEAs (except pMEAs) show excellent temperature compatibility and are stable from 0 °C to 125 °C, that is, they can be autoclaved.

The impedance of a flat, round titanium nitride (TiN) electrode is < 100 kΩ for 30 µm electrodes and approximately 250 to 400 kΩ for electrodes with smaller diameters. The smaller an electrode, the higher is the impedance. On one hand, lower impedance seems desirable, but on the other hand, a smaller electrode and interelectrode distance results in a higher spatial resolution.

Multi Channel Systems provides MEAs with TiN electrodes with sizes of 10, 20, or 30 µm and gold electrodes with 100 µm, which all show an excellent performance and low noise level. The average noise level of 30 µm and 10 µm electrodes is less than 10 µV and 15 µV peak to peak, respectively.

Gold electrodes (EcoMEAs) are only available with a low spatial resolution and are useful for medium throughput screening, where costs are a limiting factor.

All planar TiN electrodes are positioned on a round pad with a diameter of 40 µm. If you like to check the electrodes with a light microscope, you will need an upright microscope to see the MEA from above. With an inverse microscope, you are only able to see the (bigger) pad from below, not the electrode itself.

The electrodes are embedded in a carrier material, usually glass. Standard tracks made of titanium (Ti) or indium tin oxide (ITO) are electrically isolated with silicon nitride (SiN). Standard contact pads are made of titanium nitride (TiN) or indium tin oxide (ITO). ITO contact pads and tracks are transparent, for a perfect view of the specimen under the microscope.

4 MEA Types and Layouts

Various types of MEA biosensors are available for all kind of extracellular multi channel recordings.

Typical MEAs for *in vitro* applications have 60 microelectrodes arranged in an 8 x 8 layout grid embedded in a transparent glass substrate. You can cultivate the tissue or cell culture directly on the MEA. EcoFlex- and FlexMEAs are made for *in vivo* and *in vitro* applications.

MEA types differ in the materials used for the carrier and the recording area, and in the geometry, that is, electrode size and interelectrode distances. The electrode size and interelectrode distances are used for categorizing MEAs: The first number refers to the interelectrode distance, for example 200 µm, and the second number refers to the electrode size, for example 10 µm, which results in the standard MEA type 200/10, for example.

Standard versions are available with an internal reference electrode (abbreviated "iR") and with various culture chamber interface options. Culture chambers are available with and without lid.

Please ask for custom layouts, that is, MEA layouts according to your specifications.

In this chapter, each MEA type is briefly described and noted.

- Standard MEAs with flat round TiN electrodes in an 8 x 8 layout grid for all applications.
- MEAs with 6 x 10 layout grid and 500 µm inter electrode distances.
- HighDenseMEAs with the highest spatial resolution and a double recording field of 5 x 6 electrodes each.
- HexaMEAs featuring a hexagonal layout, perfect for recording from retina.
- ThinMEAs with a "thickness" of only 180 µm, ideally suited for high-resolution imaging.
- Transparent MEAs with transparent TiN electrodes for the combination of electrophysiological and optical methods.
- Three dimensional MEAs, 3D-MEAs with tip sharped electrodes to penetrate the dead cell layers of a slice. 60-3DMEAs and 120-3DMEAs are available.
- Very cost efficient and robust EcoMEAs on PCB (printed circuit board) base for applications with lower spatial resolution and higher throughput, especially for established cardiomyocyte cultures, large slices, or whole-heart preparations.
- Perforated MEAs allow perfusing the acute slice from up- and downside. For use with MEA2100 headstages equipped with perfusion element (MEA2100-PE) and MEA1060 amplifiers with perfusion ground plate (MEA-PGP) or with.
- Small perforated MEAs with 32 recording and 12 stimulation electrodes specified for use with MEA2100-32- and USB-MEA32-STIM4-System.
- 6-well MEAs feature a round MEA layout, separated in six segments of 3 x 3 electrodes, like a pie-chart. Using the 6-well MEA with macrolon triangle or round chamber ring, you have 6 separate culture chambers on one MEA, for example, for drug application in a screening experiment.
- 4 quadrant 1000 MEAs with electrode layout organized in four quadrants and a center line.
- Square MEAs with TiN (Titanium nitride) electrodes in 50 x 50 µm square size in a 8 x 8 layout grid.

- PEDOT-CNT MEAs with carbon nanotube – poly 3,4-ethylene-dioxythiophene electrodes and gold tracks and contact pads have very low impedance values of approximately 20 kΩ. They are ideal for stimulation and have excellent biocompatibility and cell adhesion.

- 256MEAs with 252 recording electrodes in a 16 x 16 layout grid for use with USB-MEA256-System.

- 6-well MEAs for use with USB-MEA256-System. 252 electrodes in 6 blocks of 6 x 7 electrodes in a round layout for use with 6-well macrolon triangle or round chamber rings. You have 6 separate culture chambers on one MEA, for example, for drug application in a screening experiment.

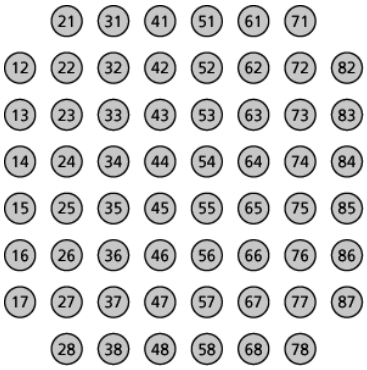
- 9-well MEAs for use with USB-MEA256-System. 256 electrodes in nine blocks of 26 recording, two stimulation and reference electrodes each. Using the 9-well MEA with macrolon quadrant, you have 9 separate culture chambers, for example, for drug application in a screening experiment.

- 120MEAs and a perforated 120pMEA with 12 x 12 layout grid for use with MEA2100-120-System only.

- FlexMEAs made of flexible polyimide 2611 foil, perfect for *in vivo* and specific *in vitro* applications, for example, whole-heart preparations. Available with 36 (FlexMEA36) or 72 (FlexMEA72) TiN (Titanium nitride) electrodes.

- EcoFlexMEAs made of flexible polyimide (Kapton) as well, but very cost efficient and more robust than FlexMEAs from polyimide foil. Available with 36 (EcoFlexMEA36) or 24 (EcoFlex24) gold electrodes.

4.1 Standard Electrode Numbering



The numbering of MEA electrodes in the 8 x 8 grid follows the standard numbering scheme for square grids: The first digit is the column number and the second digit is the row number. For example, electrode 23 is positioned in the third row of the second column.

These numbers are the same numbers that are used as channel numbers in the MCS data acquisition software, for example the Multi Channel Suite or MC_Rack program. Using MC_Rack please make sure that you have selected the two-dimensional MEA layout as the "Channel Layout" in "Data Source Setup". For more details, please refer to the MC_Rack manual or help.

Important: MEAs are not symmetrical! That is, why the **writing** (for example NMI, LEITER, MEA type) on the MEA chip should be on the right side viewed from the front, with the sockets of the amplifier in the back. MEAs with one big internal reference electrode should be placed with reference electrode to the left side in the amplifier. Otherwise, the MEA layout will not match with the pin layout of the channel map in the data acquisition software.

Other electrode grids are described in the next chapter, and in the Appendix.

4.2 Standard MEA

60MEA200/30iR-Ti, 60MEA200/30iR-ITO, 60MEA200/10iR-Ti, 60MEA100/10iR-TI, 60MEA500/30iR-Ti, 60MEA500/30iR-ITO, 60MEA500/10iR-Ti

Standard MEAs have 60 electrodes in an 8 x 8 layout grid with electrode diameters of 10 µm or 30 µm, and interelectrode distances of 100 µm, 200 µm. The MEAs with an interelectrode distance of 500 µm have a 6 x 10 layout grid.

Versions 200/10, 200/30, 100/10 are available with an internal reference electrode as indicated by the abbreviation iR. You connect the internal reference electrode directly to the amplifier's ground, so there is no need for silver pellets for grounding the bath. Please refer to the MEA manual delivered with your MEA amplifier for further information.

The flat, round electrodes are made of titanium nitride (TiN). MEAs with TiN electrodes are very stable. Therefore, the MEAs can be reused several times and are perfect for long-time experiments (up to several weeks and even months). The electrode impedance ranges between < 100 kΩ for 30 µm electrodes and 250 to 400 kΩ for 10 µm electrodes, depending on the electrode diameter. Generally, the smaller the electrode, the higher is the impedance.

Tracks are made of titanium (Ti) and contact pads are made of titanium nitride (TiN) or indium tin oxide (ITO); insulation material is silicon nitride. ITO contact pads and tracks are transparent, for a perfect view of the specimen under the microscope.

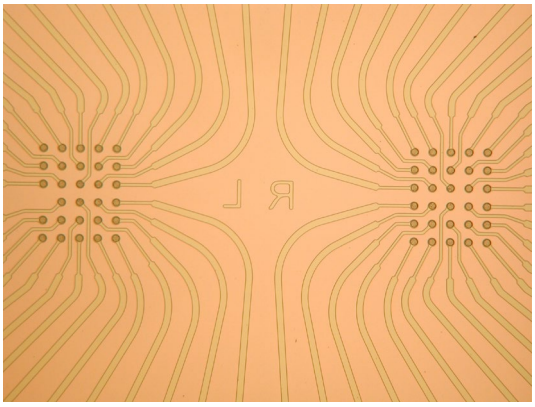
Important: MEAs are not symmetrical! That is, why the **writing** (for example NMI, LEITER, MEA type) on the MEA chip should be on the right side viewed from the front, with the sockets of the amplifier in the back. MEAs with one big internal reference electrode should be placed with reference electrode to the left side in the amplifier. Otherwise, the MEA layout will not match with the pin layout of the channel map in the data acquisition software.

Using standard MEAs

Standard MEAs can be used for a wide variety of applications. They are robust and heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures. Generally, they can be used for acute experiments as well as long-term cultures.

4.3 High Density MEA: 60HDMEA

60HDMEA30/10iR-ITO



10 µm electrodes are arranged in two recording fields with 5 x 6 electrodes each.
The interelectrode spacing is only 30 µm center to center.

The very high electrode density of the two recording fields on a 60HDMEA is only possible by the special TiN electrode material and production process. This MEA type is especially useful for applications, where a high spatial resolution is critical, for example, for multitrode analysis.

For example, the very high spatial resolution of the high density MEAs is very useful for recording from retina ganglia cells. The double recording field can also be used for coculturing two slices, each on one recording field. The flat, round electrodes are made of titanium nitride (TiN).Tracks and contact pads are made of transparent indium tin oxide (ITO); insulation material is silicon nitride.

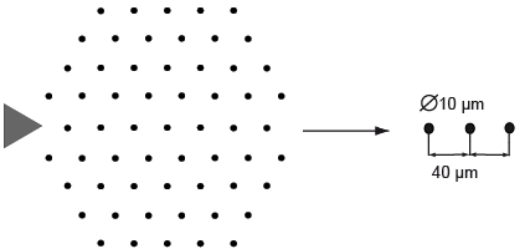
60HDMEA30/10iR-ITO MEAs are available with internal reference electrode.

Using 60HDMEAs

The same material is used for standard MEAs and high density MEAs. Therefore, they are equally robust and heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures.

4.4 Hexa MEA: 60HexaMEA40/10

60HexaMEA40/10iR-ITO



Electrode layout for 60HexaMEA40/10iR-ITO.

HexaMEAs feature a hexagonal layout, perfect for recording from retina.

The specific layout of the electrodes resembles ideally the regularity of the retina's architecture. The insulation material is silicon nitride.

Electrodes in the center have a diameter of 10 µm with an interelectrode distance of 40 µm. This HexaMEA provides an internal reference electrode.

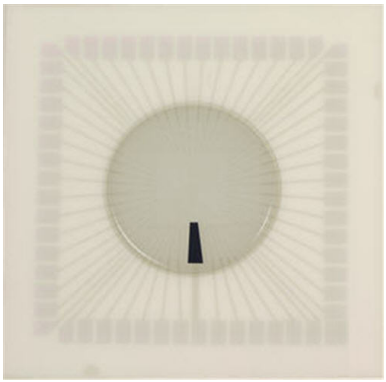
The electrodes of 60HexaMEA40/10iR-ITO are configured with invariable interelectrode distance of 40 µm, and with TiN electrodes of 10 µm diameter. They include a big internal reference electrode. The tracks and contact pads are made of ITO.

Using 60HexaMEAs

The same material is used for standard MEAs and HexaMEAs. Therefore, they are equally robust and heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures.

4.5 Thin MEA: 60ThinMEA

60ThinMEA200/30iR-ITO, 60ThinMEA30/10iR-ITO



60ThinMEAs are only 180 µm "thick", ideally suited for high-resolution imaging. 60ThinMEAs are like standard MEAs, but the electrodes are embedded in a very thin and delicate glass substrate on a robust ceramic carrier. The thin glass allows the use of oil immersion objectives with a high numerical aperture.

Like standard MEAs, 59 electrodes and one reference electrode are arranged in an 8 x 8 layout grid with electrode diameters of 30 µm and interelectrode distances of 200 µm.

60ThinMEAs are also available in a double 5 x 6 layout grid with 10 µm TiN electrodes and 30 µm interelectrode distance like the High Dense MEA (60ThinMEA30/10iR-ITO).

The flat, round electrodes are made of titanium nitride (TiN).

Tracks and contact pads are made of transparent ITO; insulation material is silicon nitride.

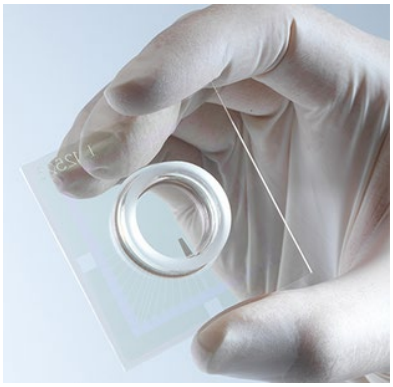
Using 60ThinMEAs

60ThinMEAs are heat-stabilized and can be autoclaved. They can also be coated with different procedures for cell and tissue cultures.

They should be handled with great care because of the thin and delicate recording area.

4.6 Transparent MEA

60tMEA200/30iR-ITO, 120tMEA100/30iR-ITO



Transparent MEAs are ideally suited for the combination of electrophysiological and optical methods, because of transparent leads with transparent electrodes. You have free sight towards the cells on top of the electrodes.

Transparent MEAs are available with 60 or 120 electrodes. The flat, round electrodes are made of very thin titanium nitride (TiN), which are transparent. Tracks and contact pads are made of transparent ITO; insulation material is silicon nitride (SiN).

60tMEA200/30iR-ITO

Like standard MEAs, 59 electrodes and one reference electrode are arranged in an 8 x 8 layout grid with electrode diameters of 30 µm and interelectrode distances of 200 µm.

120tMEA100/30iR-ITO

120tMEAs with 12 x 12 layout grid for use with MEA2100-120-System only. 120tMEAs have electrode diameters of 30 µm and interelectrode distances of 100 µm.

Using transparent MEAs

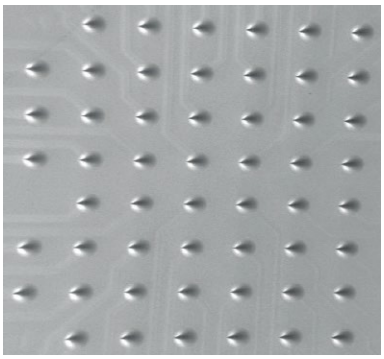
60tMEAs are heat-stabilized and can be autoclaved. They can also be coated with different procedures for cell and tissue cultures.

They should be handled with great care because of the thin electrodes in the recording area.

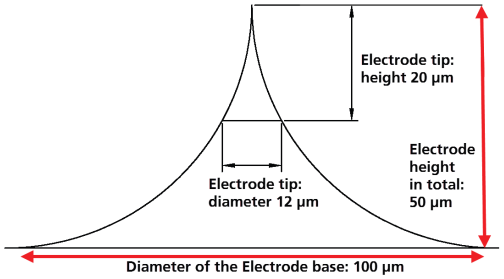
Important: Please do not treat a MEA with transparent electrodes in a Plasma Cleaner!
To make it hydrophilic use PBS overnight.

4.7 Three dimensional MEA: 60-3DMEA and 120-3DMEA

60-3DMEA200/12/40iR-Ti,
60-3DMEA200/12/50iR-Ti,
60-3DMEA200/12/80iR-Ti,
60-3DMEA250/12/100iR-Ti



120-3DMEA250/12/100iR-Ti



3DMEAs are the ideal solution for acute slices, because the three dimensionally shaped electrodes are intended to penetrate dead cell layers. Using conventional flat electrodes, the electrodes may interface with the damaged cell layer rather than with the healthy cells. The three dimensional electrode of a 3D MEA may be able to penetrate this cell layer and contact the healthy cells above better.

The tip-shaped electrode results in a larger surface area, etched from glass with a diameter of 100 µm.

60-3DMEAS: The spatial resolution is limited. 59 electrodes and one reference electrode are aligned in an 8 x 8 grid with interelectrode distances of 200 µm.

120-3DMEAs: The spatial resolution is limited. 120 electrodes and four reference electrode are aligned in a 12 x 12 grid with interelectrode distances of 250 µm.

The Titanium nitride (TiN) electrodes are 20 µm high and have a diameter of about 12 µm at the base, ending in a fine small tip.

Tracks and contact pads are made of Titanium (Ti); the insulation material is Silicon nitride (SiN).

Using 3DMEAs

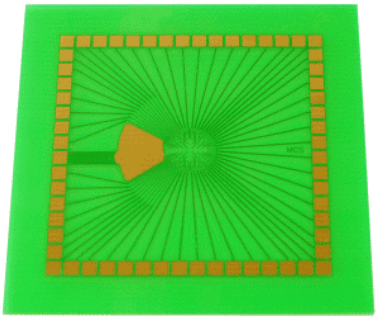
The same material is used for standard MEAs and 3DMEAs. Therefore, they are also heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures.

Due to the production process and the fine tip, there may be more variations in the electrode impedance, which is important for stimulation experiments, especially with current.

4.8 Eco MEA: 60EcoMEA

60EcoMEA

60EcoMEAs are available on opaque printed circuit board (PCB). They are low price variants for medium throughput applications like small screens where material costs play a bigger role than in more scientific MEA applications.



60EcoMEAs are opaque and are therefore useful only for applications where you do not need a visual control under a microscope, for example, for established cell cultures.

Due to the special production process on PCB, electrodes of 60EcoMEAs are available only with a diameter of 100 µm and an interelectrode distance of 700 µm. Thus, 60EcoMEA are useful for applications where a high spatial resolution is not important, but which emphasize on low price consumables. They have proven to be especially useful for recordings from established cardio-myocyte cultures. They are not useful for establishing a new cell culture, as the cell performance cannot be monitored. Multi Channel Systems recommends using standard 200/30 MEAs for establishing the cell culture first, then switch to 60EcoMEA.

60EcoMEAs are provided in the typical 8 x 8 layout with internal reference electrode. Custom layouts following your personal specifications are possible at very reasonable prices. Please ask your local retailer for details. Electrodes, tracks, and contact pads are made of pure gold. Due to the soft gold material of the contact pads, the contact to the amplifier pins is excellent.

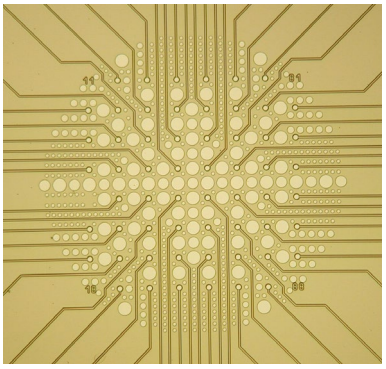
Using 60EcoMEAs

Like standard MEAs, 60EcoMEAs are very robust. They can be coated with different procedures for cell and tissue cultures. The gold electrodes are very robust, too, and are the only MEA electrodes that will endure more severe cleaning methods. EcoMEAs on PBC base can be autoclaved.

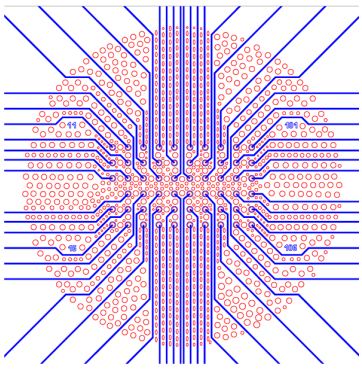
New 60EcoMEAs are hydrophobic. They should be coated with nitrocellulose or treated with a plasma cleaner before use.

4.9 Perforated MEA: 60pMEA

60pMEA200/30iR-Ti



60pMEA100/30iR-Ti



Acute slice recordings on common glass MEAs are done from the cells at the bottom of the slice, which are in contact with the MEA electrodes. These cells get less oxygen and nutrients from the perfusion medium, and therefore are likely to give smaller signals and might eventually die first. Perforated MEAs present a solution to this problem as they allow a perfusion of the tissue from both sides at the same time, thereby optimizing the oxygen supply of the acute slice.

Perforated MEAs are identical in size and function to the regular MEAs. The recording electrodes are arranged in 8 x 8 standard layout grid in 60pMEA200/30iR-Ti, and in 6 x 10 layout grid in 60pMEA100/30iR-Ti. The electrodes are integrated into a thin polyimide foil. This thin foil is fixed on a ceramic or glass waver for mechanical stability. In the middle of the waver, under the electrode field, there is a hole that makes it possible to access the electrode field from below. The area around the electrodes is perforated to allow a perfusion of the tissue from both sides. The total area of the holes averages 0.8 mm, the diameters of the holes varies between 20 µm and 90 µm.

These pMEAs are designed for use with MEA2100-System headstages with perfusion element (PE) and MEA1060 amplifier equipped with a perfusion ground plate (PGP). The PE or PGP replaces the standard ground plate of the headstage or MEA1060 amplifier. Please note that there are different types of the MEA-PGPs for different amplifier types (MEA1060-UP-PGP, MEA1060-UP-BC-PGP, and MEA1060-INV / INV-BC-PGP). Additional to the use of 60pMEAs together with the MEA2100-(2x) 60-System, you can use 120pMEAs with MEA2100-120-System equipped with a perfusion element (PE) integrated in the ground plate of the headstage.

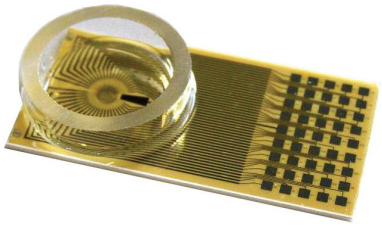
For an overview of suggested configurations to work with 60pMEAs, see the MEA Application Note “Acute Hippocampal Slices on pMEAs”.

Using 60pMEAs

Perforated MEAs have a robust ceramic carrier or they are mounted on glass as usual, but the electrodes are embedded in polyimide foil. Therefore, they are heat stable to 50 °C only, and cannot be autoclaved. Please do not use an ultrasonic bath for cleaning.

4.10 Perforated MEAs for Use with MEA2100-32-System and USB-MEA32-STIM4-System

pMEA-32S12-L1, pMEA-32S12-L2, pMEA-32S12-L3, pMEA-32S12-L4



pMEA-32S12-Lx

For the USB-MEA32-STIM4-System small perforated MEAs have been designed. Please see USB-MEA32-STIM4 manual for detailed information. Additionally, the MEA2100-32-System is adapted for these small type of MEA. The pMEAs are different in size, but identical in function to the regular pMEAs. Layout 1 (pMEA-32S12-L1) of the perforated MEAs designed for the MEA2100-32- and for the USB-MEA32-STIM4-System has been optimized for acute hippocampal slices.

The flat, round electrodes are made of titanium nitride (TiN) with a diameter of 30 µm for the recording electrodes, and 50 µm for the stimulation electrodes. The stimulation electrodes cannot be used for recording, and vice versa. The interelectrode distances vary from 150 to 200 µm. MEAs with titanium nitride (TiN) electrodes are very stable. Therefore, the pMEA can be reused several times and is perfect for long-time experiments (up to several weeks and even months). The electrode impedance is < 100 kΩ. Tracks and contact pads are made of titanium nitride (TiN), the insulation material is polyimide, respectively.

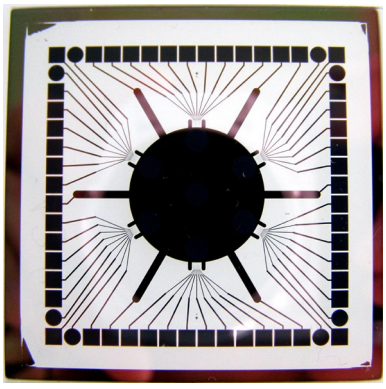
The electrodes are integrated into a thin polyimide foil. This thin foil is fixed on a ceramic waver for mechanical stability. In the middle of the waver, under the electrode field, there is a hole that makes it possible to access the electrode field from below. The area around the electrodes is perforated to apply suction to the slice from below. The total area of the holes averages 0.8 mm, the diameters of the holes varies from 20 to 90 µm. Please read chapter "Working with the USB-MEA32-STIM4 Amplifier" in USB-MEA32-STIM4 manual or “Setting up the MEA” in the MEA2100 manual.

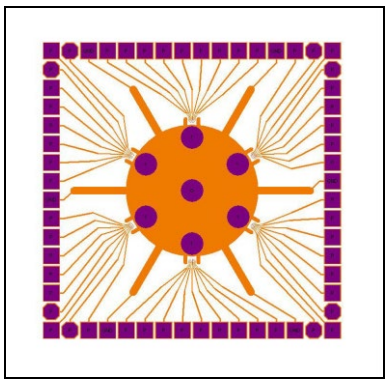
Using pMEAs

Perforated MEAs have a robust ceramic carrier, but the electrodes are embedded in polyimide foil. Therefore, they are heat stable to 50 °C only, and cannot be autoclaved. Please do not use an ultrasonic bath for cleaning.

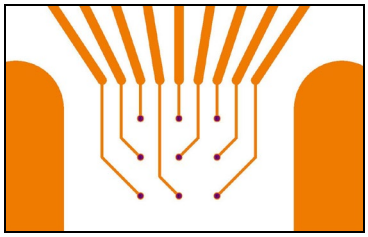
4.11 MEA with 6 Wells: 60-6wellMEA

60-6wellMEA200/30iR-Ti

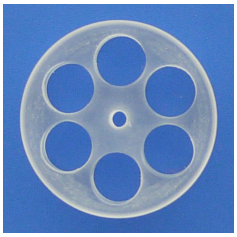


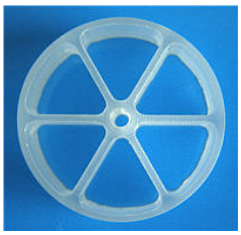


60-6wellMEAs are MEA chips with six independent culture chambers, separated by a macrolon ring. Inside each well, in between the marked two bars coming out of the circle in the middle of the MEA, there is a field of nine electrodes with an internal reference electrode. The electrode in the center of the MEA is for grounding.



60-6wellMEAs are developed, for example, for safety-pharmacological screenings of drug induced QT-prolongation. Multi Channel System MCS GmbH provides a software solution for these experimental intentions, the QT-Screen-Lite program. The 60-6wellMEA allows running six experiments with identical surrounding conditions at once. Two types of macrolon rings are available: Rings with six triangular chambers and rings with round chambers.





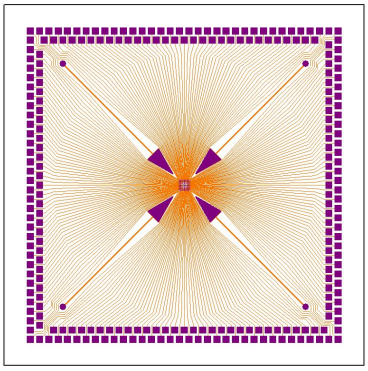
The electrodes of the 60-6wellMEA are from titanium nitride (TiN), the isolation is made up of Silicon nitride (SiN). Contact pads are from titanium nitride (TiN), and tracks are from titanium (Ti). The diameter of the electrodes is 30 µm, the distance from center to center is 200 µm.

Using 60-6wellMEAs

60-6wellMEAs can be used for a wide variety of applications. They are robust and heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures. Generally, they can be used for acute experiments as well as long-term cultures.

4.12 256MEA for Use with MEA2100-256- and USB-MEA256-Systems

256MEA30/8-ITO, 256MEA60/10iR-ITO, 256MEA100/30-ITO, 256MEA200/30-ITO, and 256ThinMEA



The 256MEAs have to be used with MEA2100-256- and USB-MEA256-Systems. Please refer to the respective manual for detailed information.

The 256MEA contains 252 recording, and four ground electrodes arranged in a 16 x 16 layout grid embedded in a transparent glass substrate. The contact to the amplifier is provided by a double ring of contact pads around the rim of the MEA. The standard material for MEAs is also used for 256MEAs: The electrodes are from titanium nitride (TiN) with a silicon nitride (SiN) isolator, and contact pads and tracks are made of transparent indium tin oxide (ITO).

The spacing of the electrodes in the 16 x16 grid averages 30, 60, 100 µm or 200 µm between the electrodes. The electrode diameter of 30 µm results in an impedance of <100 kΩ. The electrode diameter of 10 µm results in an impedance of approximately 250 to 400 kΩ. The dimension of the glass carrier is 49 x 49 x 1 mm as usual. 256MEAs are stable in a temperature range from 0 ° - 125 °C.

The 256MEA is only MEA type, which is rotationally symmetric.

256ThinMEAs are only 180 µm "thick", ideally suited for high-resolution imaging. 256ThinMEAs are like standard MEAs, but the electrodes are embedded in a very thin and delicate glass substrate on a robust ceramic carrier. The thin glass allows the use of oil immersion objectives with a high numerical aperture.

Like 256MEAs, 252 electrodes are arranged in a 16 x 16 layout grid with electrode diameter of 30 µm, and interelectrode distance of 200 µm (256ThinMEA200/30-ITO).

Using 256MEAs

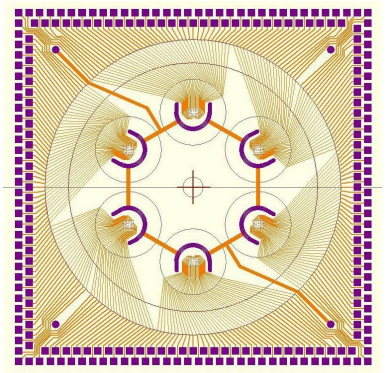
The same material is used for standard MEAs and 256MEAs. Therefore, they are equally robust and heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures.

Using 256ThinMEAs

256ThinMEAs are heat-stabilized and can be autoclaved. They can also be coated with different procedures for cell and tissue cultures. They should be handled with great care because of the thin and delicate recording area.

4.13 MEA with 6 Wells for Use with MEA2100-256- and USB-MEA256-Systems

256-6wellMEA200/30iR-Ti



The 256-6wellMEA200/30iR-ITO has 256 electrodes and has to be used with MEA2100-256- and USB-MEA256-Systems. Please refer to the respective manual for detailed information.

The dimension of the glass carrier is 49 x 49 x 1 mm as usual. The MEAs with 6 wells are stable in a temperature range from 0 ° - 125 °C.

The 256-6wellMEA contains 252 recording, 6 reference and four ground electrodes arranged in 6 electrode blocks with a 6 x 7 layout grid for the recording electrodes. The reference electrode is around each block. They are embedded in a transparent glass substrate. The contact to the amplifier is provided by a double ring of contact pads around the rim of the MEA. The standard material for MEAs is also used for 256-6wellMEA: The electrodes are from titanium nitride (TiN) with a silicon nitride (SiN) isolator, and contact pads and tracks are made of transparent indium tin oxide (ITO).

Using the 256-6wellMEA with macrolon ring, you have six separate culture chambers on one MEA, for example, for drug application in a screening experiment.

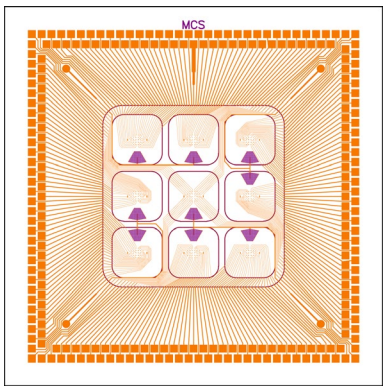
The spacing between the recording electrodes in the 6 x 7 grid averages 200 µm between the electrodes. The electrode diameter of 30 µm results in an impedance of < 100 kΩ.

Using 256-6wellMEAs

The same material is used for standard MEAs and 256-6wellMEAs. Therefore, they are equally robust and heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures.

4.14 MEA with 9 Wells for Use with MEA2100-256- and USB-MEA256-Systems

256-9wellMEA300/30iR-ITO



The 256-9wellMEA300/30iR-ITO has 256 electrodes and has to be used with MEA2100-256- and USB-MEA256-Systems. Please refer to the respective manual for detailed information.

The dimension of the glass carrier is 49 x 49 x 1 mm as usual. The MEAs with 9 wells are stable in a temperature range from 0 ° - 125 °C.

The 256-9wellMEA contains 234 recording, 18 stimulation or recording, and four ground electrodes arranged in nine electrode blocks with a 6 x 5 layout grid for the recording electrodes, two stimulation or recording electrodes, and one reference electrode per each block. They are embedded in a transparent glass substrate. The contact to the amplifier is provided by a double ring of contact pads around the rim of the MEA. The standard material for MEAs is also used for 256-9wellMEA: The electrodes are from titanium nitride (TiN) with a silicon nitride (SiN) isolator, and contact pads and tracks are made of transparent indium tin oxide (ITO).

Using the 256-9wellMEA with macrolon quadrate, you have nine separate culture chambers on one MEA, for example, for drug application in a screening experiment.

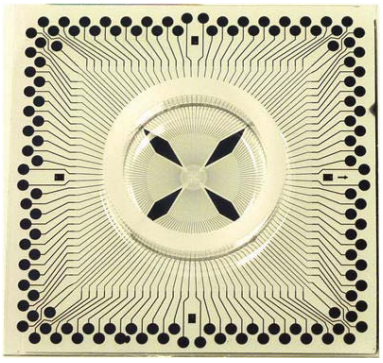
The spacing between the recording electrodes in the 6 x 5 grid averages 300 µm between the electrodes. The electrode diameter of 30 µm results in an impedance of < 100 kΩ. The dimension of the square stimulation electrode is 50 x 200 µm.

Using 256-9wellMEAs

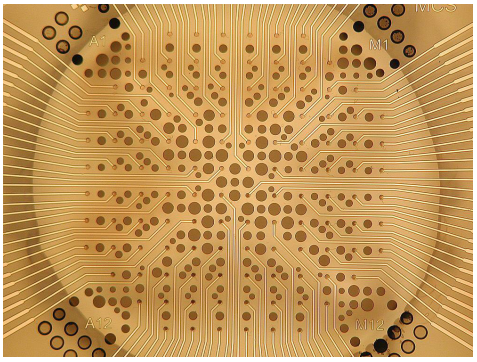
The same material is used for standard MEAs and 256-9wellMEAs. Therefore, they are equally robust and heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures.

4.15 120MEA for Use with MEA2100-120-System

120MEA200/30iR-Ti, 120MEA100/30iR-Ti, 120pMEA200/30iR-Ti



120MEA200/30iR-Ti



120pMEA200/30iR-Ti (electrode field with perforation)

The 120MEA200/30iR-Ti has 120 electrodes and can only be used with the MEA2100-System connected to a headstage HS120 with 120 electrodes. Please refer to the MEA2100-System manual for detailed information.

The dimension of the glass carrier is 49 x 49 x 1 mm as usual. The MEAs with 120 electrodes are stable in a temperature range from 0 ° - 125 °C.

The 120MEA200/30iR-Ti contains 120 recording, four reference and four ground electrodes arranged in a 12 x 12 layout grid. They are embedded in a transparent glass substrate. The contact to the amplifier is provided by a double ring of contact pads around the rim of the MEA. The standard material for MEAs is also used for 120MEA200/30iR-Ti: The electrodes are from titanium nitride (TiN) with a silicon nitride (SiN) isolator, and contact pads and tracks are made of titanium nitride (TiN).

The spacing between the recording electrodes in the 12 x 12 grid averages 100 or 200 µm between the electrodes. The electrode diameter of 30 µm results in an impedance of < 100 kΩ.

This MEA is also available as perforated 120pMEA200/30iR-Ti. The electrodes are from Titan Gold Titan, all other MEA parameters are the same. The inner diameter around the electrodes of 3 to 4 mm^2 is perforated and the total area of holes is 12 % of these area.

Using 120MEAs

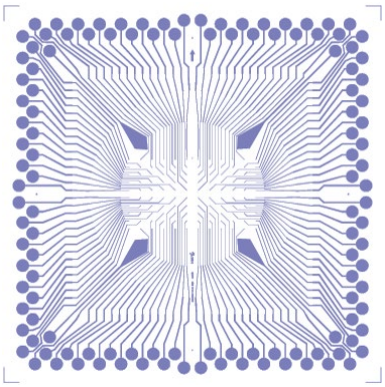
The same material is used for standard MEAs and 120MEA200/30iR-Ti and 120MEA100/30iR-Ti. Therefore, they are equally robust and heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures.

Using 120pMEA200/30iR-Ti

Perforated MEAs have a glass carrier, but the electrodes are embedded in polyimide foil. Therefore, they are heat stable to 50 °C only, and cannot be autoclaved. Please do not use an ultrasonic bath for cleaning.

4.16 120MEA1000-1500/30iR-Ti for Use with MEA2100-120-System

120MEA1000-1500/30iR-Ti



120MEA1000-1500/30iR-Ti

The 120MEA1000-1500/30iR-Ti has 120 electrodes and can only be used with the MEA2100-System connected to a headstage HS120 with 120 electrodes. Please refer to the MEA2100-System manual for detailed information.

The dimension of the glass carrier is 49 x 49 x 1 mm as usual. The MEAs with 120 electrodes are stable in a temperature range from 0 ° - 125 °C.

The 120MEA1000-1500/30iR-Ti contains 120 recording, four reference and four ground electrodes arranged in a 12 x 120 layout grid. They are embedded in a transparent glass substrate. The contact to the amplifier is provided by a double ring of contact pads around the rim of the MEA. The standard material for MEAs is also used for 120MEA200/30iR-Ti: The electrodes are from titanium nitride (TiN) with a silicon nitride (SiN) isolator, and contact pads and tracks are made of titanium nitride (TiN).

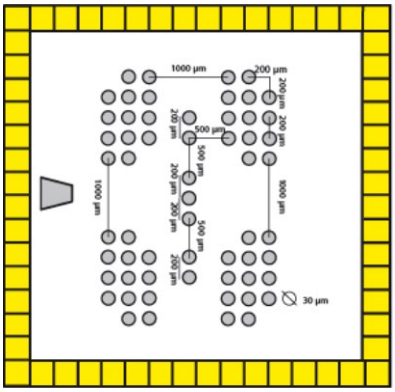
The wide spacing between the recording electrodes in the 12 x 10 grid is 1000 µm in vertical and 1500 µm in horizontal direction. The electrode diameter of 30 µm results in an impedance of < 100 kΩ.

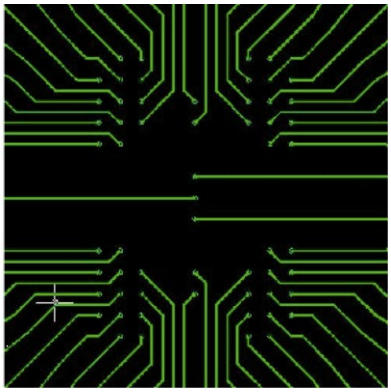
Using 120pMEA1000-1500/30iR-Ti

The same material is used for standard MEAs and 120MEA200/30iR-Ti and 120MEA1000-1500/30iR-Ti. Therefore, they are equally robust and heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures.

4.17 Quadrant MEA: 60-4QMEA1000

60-4QMEA1000iR-Ti





The 60-4QMEA1000 has 60 electrodes organized in four quadrants (13 electrodes each) with a center line (7 electrodes). The electrode diameter is 30 µm, and the interelectrode distance varies: Inside the quadrants the distance is 200 µm, from quadrant to quadrant the distance is 1000 µm, and to the center line it is 500 µm.

The 60-4QMEA1000 is available with an internal reference electrode.

The flat, round electrodes are made of titanium nitride (TiN). MEAs with TiN electrodes are very stable. Therefore, the MEAs can be reused several times and are perfect for long-time experiments (up to several weeks and even months). The electrode impedance is < 100 kΩ.

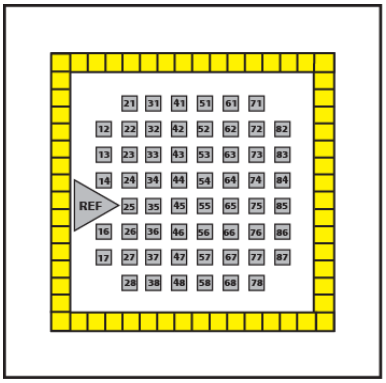
Tracks are made of titanium (Ti) and contact pads are made of titanium nitride (TiN); insulation material is silicon nitride (SiN).

Using 60-4QMEA1000

The 60-4QMEA1000 can be used for a wide variety of applications. They are robust and heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures. Generally, they can be used for acute experiments as well as long-term cultures.

4.18 Square MEA: 60SquareMEA

60SquareMEA200/50iR-Ti



60SquareMEAs have 60 electrodes in an 8 x 8 layout grid with square electrode of 50 x 50 µm size and interelectrode distances of 200 µm. They are available with an internal reference electrode. You can connect the internal reference electrode directly to the amplifier's ground and will not need silver pellets for grounding the bath anymore.

The flat, square electrodes are made of titanium nitride (TiN). The electrode size of 50 x 50 µm guarantees very low noise. MEAs with TiN electrodes are very stable. Therefore, the MEAs can be reused several times and are perfect for long-time experiments (up to several weeks and even months). The electrode impedance ranges about 100 kΩ.

Tracks are made of titanium (Ti) and contact pads are made of titanium nitride (TiN); insulation material is silicon nitride.

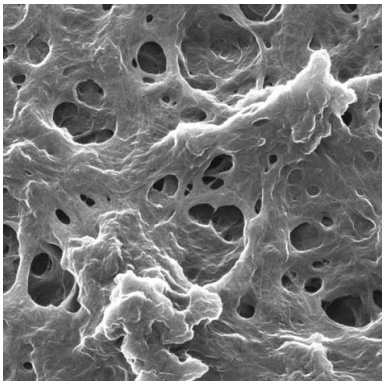
Important: MEAs are not symmetrical! That is, why the **writing** (for example NMI, LEITER, MEA type) on the MEA chip should be on the right side viewed from the front, with the sockets of the amplifier in the back. MEAs with one big internal reference electrode should be placed with reference electrode to the left side in the amplifier. Otherwise, the MEA layout will not match with the pin layout of the channel map in the data acquisition software.

Using 60SquareMEAs

MEAs with square electrodes can be used for a wide variety of applications. They are robust and heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures. Generally, they can be used for acute experiments as well as long-term cultures.

4.19 PEDOT-CNT MEAs: 60PedotMEA

60PedotMEA200/30iR-Au



Carbon nanotube structure of PEDOT electrodes.

60PedotMEAs have - like standard MEAs - 59 electrodes and one reference electrode that are arranged in an 8 x 8 layout grid with electrode diameters of 30 µm and interelectrode distances of 200 µm. The flat, round electrodes are made of PEDOT-CNT carbon nanotube – poly 3,4-ethylene-dioxythiophene.

Contact pads and track material is made of titanium nitride (TiN) covered by a layer of gold (Au). The insulation material is silicon nitride.

This type of MEA is characterized by very low impedance values of approximately 20 kΩ.They are ideal for stimulation and have excellent biocompatibility and cell adhesion.

Important: MEAs are not symmetrical! That is, why the **writing** (for example NMI, LEITER, MEA type) on the MEA chip should be on the right side viewed from the front, with the sockets of the amplifier in the back. MEAs with one big internal reference electrode should be placed with reference electrode to the left side in the amplifier. Otherwise, the MEA layout will not match with the pin layout of the channel map in the data acquisition software.

Using 60PedotMEAs

MEAs with PEDOT-CNT electrodes can be used for a wide variety of applications. They are robust and heat-stabilized. They can be autoclaved and coated with different procedures for cell and tissue cultures. Generally, they can be used for acute experiments as well as long-term cultures.

4.20 FlexMEA

FlexMEAs are made of flexible polyimide foil, perfect for *in vivo* and specific *in vitro* applications. Only 12 µm "thick" and weighing less than 1 g, the FlexMEA biosensor is very thin and light weight.

The FlexMEAs are available with 32 (64) recording electrodes plus two (four) indifferent reference electrodes and two (four) ground electrodes in a 6 x 6 (8 x 9) electrodes grid. More layouts can be provided on request. The flexible base is perforated for a better contact with the surrounding tissue.

The electrodes are from titanium nitride (TiN), contact pads and track material from pure gold.

For cleaning, rinse with distilled water, optional with ethanol 70 %. Please do not use an ultrasonic bath!

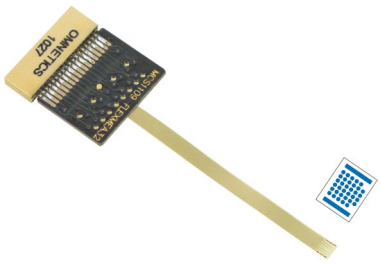
When using an autoclave at 121 °C, please make sure that the FlexMEA will not be exposed to the moisture. The FlexMEA itself must be dry and additionally sealed in a sterile package.

Using FlexMEAs

Warning: Do not use an ultrasonic bath for cleaning. The manufacturer recommends sterilization by rinsing with alcohol.

FlexMEAs are usually connected to a head stage preamplifier that is connected to a filter amplifier or programmable gain amplifier (see also the ME-System product line of Multi Channel Systems). Via provided adapters FlexMEAs can be connected to 32-channel miniature preamplifiers MPA32I from Multi Channel Systems for *in vivo* experiments. There is no need for an adapter if the FlexMEA should be connected to the 32-channel miniature preamplifier MPA32I-Flex.

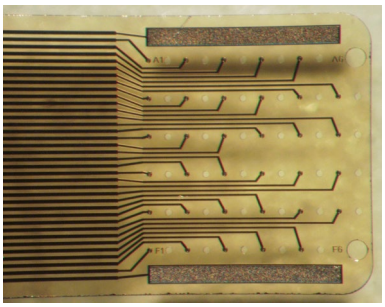
FlexMEA36-OM



The FlexMEA36-OM has 32 recording electrodes plus two internal reference electrodes and two ground electrodes in a 6 x 6 electrodes grid. The titanium nitride electrodes have a diameter of 30 µm, and the distance between the electrodes is 300 µm. The polyimide foil is perforated with holes of 50 µm diameter, ensuring optimal tissue contact.

When using the FlexMEA36-OM together with a ME2100-HS32 headstage or a standard 32-channel miniature preamplifier MPA32I, you do not need an adapter for the connection. Please read the data sheet FlexMEA36-OM, and the ME2100-System or MPA32I manual for more information.

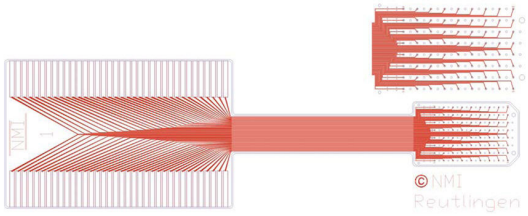
FlexMEA36



The FlexMEA36 has 32 recording electrodes plus two internal reference electrodes and two ground electrodes in a 6 x 6 electrodes grid. The titanium nitride electrodes have a diameter of 30 µm, and the distance between the electrodes is 300 µm. The polyimide foil is perforated with holes of 30 µm diameter, ensuring optimal tissue contact.

When using the FlexMEA36 together with a standard 32-channel miniature preamplifier MPA32I, you need the ADPT-FM-32 adapter to connect the FlexMEA36 to the standard MPA32I. There is no need for an adapter if you use the FlexMEA36 specified 32-channel miniature preamplifier MPA32I-Flex. Please read the data sheet FlexMEA36, and the MPA32I (-Flex) manual for more information.

FlexMEA72



The FlexMEA72 has 64 recording electrodes plus four internal reference electrodes and four ground electrodes in an 8 x 9 electrodes grid. The titanium nitride electrodes have a diameter of 100 µm, and the distance between the electrodes is either 625 µm or 750 µm. The polyimide foil is perforated with holes of 100 µm diameter, ensuring optimal tissue contact.

When using the FlexMEA72 together with two standard 32-channel miniature preamplifier MPA32I, you need the ADPT-FM-72 adapter to connect the FlexMEA72 to two standard MPA32Is. Please read the data sheet FlexMEA72 or ADPT-FM-72, and the MPA32I manual for more information.

4.21 EcoFlexMEA

EcoFlexMEAs are made of flexible polyimide (Kapton). They are less flexible as FlexMEAs, but therefore more robust in handling and sterilization. With a thickness of 50 µm and low weight the EcoFlexMEA is perfect for *in vivo* and specific *in vitro* applications, respectively.

The EcoFlexMEA is available with 24 or 36 electrodes, two internal reference electrodes, and two ground electrodes. Custom layouts can be provided on request.

The electrodes, contact pads and track material are made of pure gold. EcoFlexMEAs are stable at a temperature range from 0 °C to 125 °C and can be autoclaved.

The EcoFlexMEA can directly be connected to a standard 32-channel miniature preamplifier MPA32I, you do not need an adapter. An additional connector on the side of the EcoFlexMEA36 can be used for connecting a silver pellet or a silver wire for grounding the bath. Please read the data sheet EcoFlexMEA, and the MPA32I manual for more information.

Using EcoFlexMEAs

EcoFlexMEAs are made for use with ME2100-HS32 headstages of the ME2100-System or with 32-Channel Miniature Preamplifier MPA32I for *in vivo* or *in vitro* applications

EcoFlexMEAs can be directly connected to the ME2100-HS32, please read also the ME2100-System manual. When connected to a MPA32I it is connected to a filter amplifier or programmable gain amplifier (see also the ME-System product line of Multi Channel Systems).

EcoFlexMEA36

The EcoFlexMEA36 has 32 recording electrodes, two internal reference electrodes, and two ground electrodes in a 6 x 6 electrode grid. The recording electrodes have a diameter of 50 µm, the distance between the electrodes from center to center is 300 µm. The electrodes, contact pads and track material are made of pure gold. EcoFlexMEA36 is stable at a temperature range from 0 °C to 125 °C and can be autoclaved.

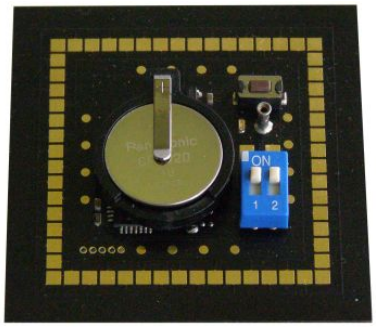
The EcoFlexMEA36 can directly be connected to a ME2100-HS32 headstage of the ME2100-System or to a standard 32-channel miniature preamplifier MPA32I. The connector on the right side of the MEA (see picture) can be used for connecting a silver pellet or a silver wire for grounding the bath. Please read the data sheet EcoFlexMEA36, and the ME2100-System manual and the MPA32I manual for more information.

EcoFlexMEA24

The EcoFlexMEA24 has 24 recording electrodes, two internal reference electrodes, and two ground electrodes in a 2 x 10 + 4 electrode grid. The recording electrodes have a diameter of 80 µm, the distance between the electrodes from center to center is 300 µm. The electrodes, contact pads and track material are made of pure gold. EcoFlexMEA36 is stable at a temperature range from 0 °C to 125 °C and can be autoclaved.

The EcoFlexMEA24 can directly be connected to a standard 32-channel miniature preamplifier MPA32I, you do not need an adapter. Please read the data sheet EcoFlexMEA24, and the MPA32I manual for more information.

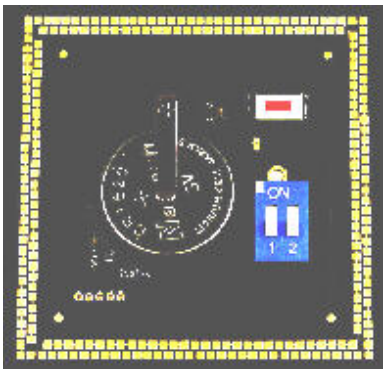
4.22 MEA Signal Generator: 60MEA-SG



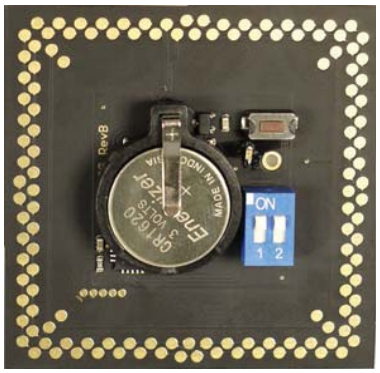
60MEA-SG

The 60MEA-Signal Generator is a convenient tool for MEA-Systems first time users. It can replace a MEA for learning and / or teaching purposes. The device has the same dimensions and contact pad layout as a 60-channel MEA chip and is compatible with all MEA1060 amplifier types and with the MEA2100-System with 60-channel headstage, MEA2100-HS60 or MEA2100-HS2x60.

The **MEA-SG** produces sine waves or replays a variety of biological signals. These signals are fed into the MEA amplifier as analog signals. With this artificial data, you are able to test the functionality of the hardware and software system, without the need for a biological sample on a real MEA. Please use the 256MEA-SG for the MEA2100-256-System and the USB-MEA256-System and the 120MEA-SG for MEA2100-120-System connected to a headstage with 120 channels, the MEA2100-HS120.



256MEA-SG



120MEA-SG

For FlexMEAs, connected to ME-Systems and for wireless headstages, connected to the Wireless-System you can use a specially adapted signal generator ME/W-SG.

ME/W-SG

5 MEA Handling

Warning: If possible, use only liquids or cleaning solutions with a neutral pH = 7 on MEAs. Do not expose MEAs with silicon nitride insulation or TiN electrodes to basic liquids (pH > 7) or aggressive detergents for a longer period of time. Basic or aggressive liquids may damage TiN electrodes irreversibly.

Warning: It is absolutely necessary to rinse the MEAs thoroughly with distilled water after treatment with detergent, particular when using Terg-A-Zym before heat sterilization (dry heat sterilization is not recommended). Otherwise, the potential rests of the detergent may burn into the glass carrier of the MEA and may destroy the electrodes.

Warning: Do not touch the electrode field in any way during the coating or cleaning procedure. Keep all instruments, tissues, pipette tips, and similar at a safe distance from the recording area. The electrodes are easily damaged (except EcoMEA electrodes).

5.1 Hydrophilic Surface Treatment

The surface of new MEAs is hydrophobic, and even hydrophilic MEAs tend to become hydrophobic again during storage. A hydrophobic surface prevents attachment and growth of the (hydrophilic) cells. The first step in preparing a MEA for use is therefore to ensure that the surface is hydrophilic enough for coating and cell adhesion.

To test this without contaminating the surface, place a small drop of water on the MEA surface outside the culture chamber. If the drop does not wet the surface, you likely need to perform one of the following steps, in particular when using new arrays.

Literature

Ulrich Egert, Thomas Meyer (2004); Heart on a Chip — Extracellular multielectrode recordings from cardiac myocytes *in vitro*, "Methods in Cardiovascular Research", S. Dhein and M. Delmar (eds.)

5.1.1 Pretreatment with PBS

An easy way to make dry MEAs hydrophilic is to fill the culture chamber on the MEA with PBS (Phosphate Buffered Saline) to soak the electrodes. Place the filled MEA on a heating plate at ±30 °C for at least five hours before using. Please refill the culture chamber with PBS during this time to prevent drying out. This pretreatment increases the hydrophilic behavior of the surface and the electrodes.

5.1.2 Plasma Cleaning

Laboratories with access to electron microscopy facilities are likely to have a sputter device or a plasma-cleaning chamber (for example Plasma Technology, Herrenberg, Germany or PDC-32G from Harrick Plasma, Ithaca, NY, United States). The MEA surface is exposed to a gas plasma discharge, which will make the surface polar and thus more hydrophilic. The treatment gives a very clean and sterile surface that can be coated readily with water-soluble molecules. Note that the effect wears off after a few days.

Plasma cleaner parameters: MEAs can be treated with low-vacuum plasma for 1 to 2 minutes at 0.2 mbar and 50 to 100 W. Additional supply of atmospheric air or O_2 is recommended.

5.1.3 Protein Coating

If protein coating is acceptable in the planned experiments, there is another quick and simple way to render the surface hydrophilic.

1. Sterilize the MEAs as described below.
2. Place approximately 1 ml of a concentrated, sterile protein solution (for example, albumin, fetal calf serum or similar) onto the culture region for about 30 min.
3. Wash the culture chamber thoroughly with sterile water afterwards. The MEA can then be directly used for cell culture.

5.1.4 Preculturing

Another pragmatic method is to coat the hydrophobic MEAs and to plate the cell cultures on the MEA, and let it grow for some days (up to weeks) until the cells have transformed the surface so that it is sufficiently hydrophilic. The "preculture" will generally show very bad growth and viability, and needs to be discarded before plating the culture that will be used for experiments.

Please note that the MEA and the electrode performance may suffer under cell culturing. Therefore, the above-mentioned methods are preferable.

5.2 Sterilization

Sterilization of MEAs is not necessary for acute slices.

Glass MEAs with TiN electrodes and EcoMEAs with gold electrodes can be sterilized with standard methods for cell culture materials using either rinsing with 70 % alcohol**,** UV-light (about half an hour depending on the intensity), vapor autoclavation, or dry-heat sterilization up to a temperature maximum of 125 °C.

Warning: Do not autoclave or sterilize perforated MEAs by heat**.** These MEA types are not thermoresistant and will be irreversibly damaged.

5.2.1 Sterilization with Ethanol and Ultraviolet Light

→ Rinse MEAs with 70 % ethanol. Do not immerse the MEA into the alcohol for a longer time otherwise the ring on the MEA will probably get off.

→ Let MEAs air-dry overnight on a sterile workbench (laminar flow hood) with UV light turned on.

5.2.2 Steam Sterilization (Autoclavation)

→ Autoclave MEAs at 125 °C for 15 min.

→ FlexMEAs: When using an autoclave at 121 °C, please make sure that the FlexMEA will not be exposed to the moisture. The FlexMEA itself must be dry and additionally sealed in a sterile package.

5.2.3 Dry-Heat Sterilization

Dry-heat sterilization is possible if a stream autoclave is not available, which the better choice is. Please clean the MEA thoroughly with distilled water before using the oven, otherwise potential rests of any material may burn into the glass carrier of the MEA and may destroy the electrodes.

→ Thermally sterilize **MEAs** in an oven at **121 °C** for 15 min.

→ Thermally sterilize **FlexMEAs** in an oven at **121 °C** for 15 min.

→ Thermally sterilize **EcoFlexMEAs** in an oven at **121 °C** for 15 min

→ Thermally sterilize **pMEAs** in an oven at **50 °C** for 2 hours.

5.2.4 Sterilization with Hot Water

→ Expose MEAs to hot water (90 °C) for 1 min.

5.3 MEA Storage

To maintain a hydrophilic surface after hydrophilization, it is recommended to store the MEAs filled with distilled water until use. Dry MEAs will get hydrophobic again after some time.

Store MEAs filled with sterile distilled water at 4 °C in the dark (that is, in the fridge, to prevent microbiological contaminations) to maintain a hydrophilic surface.

5.4 MEA Coating

Coating of MEAs with various materials is used for improving the attachment and growth of cell cultures or cultured slices. Coating is generally not required for recordings from acute slices.

Coating of MEAs has the same purpose than coating of other culture dishes. Therefore, you can generally use the same standard protocols that you have established for coating culture dishes for your cell cultures, provided that the involved chemicals are not aggressive and damage the electrodes (see recommendations for the various MEA types).

In the following, some standard coating procedures are shortly described. You should try out which coating procedure proves best for your application. The listed materials are only recommendations; you may use any equivalent equipment. Most coatings are stable for several uses of the MEA and do not have to be removed after use (except nitrocellulose).

Please note that the materials and procedures described in the following are only a summary of published information from other sources (see references) or from personal communications with MEA users, and has the intention of helping users finding the appropriate information for setting up their experiments. Multi Channel Systems MCS GmbH has not tested or verified this information, and therefore cannot guarantee that the information is correct. Please refer to the referenced literature for planning and executing any experiments.

5.4.1 Coating with Nitrocellulose

Coating with nitrocellulose is a fast procedure that works with several cell types and tissues and that is also successful with slightly hydrophobic MEAs. This method has the advantage that the cells stick well to the surface. Nitrocellulose does not form a uniform layer on the MEA. The coating leaves patches of nitrocellulose, which serve as a glue for the tissue, on the MEA surface. The tissue is not likely to get detached even under severe mechanical disturbance (by perfusion, for example). MEAs coated with nitrocellulose can be stored for a few days. Nitrocellulose coating has to be removed after use.

Main advantages of this method are that nitrocellulose is cheap, coating is fast and easy, and it is also easily removed after use.

Note: Nitrocellulose solutions cannot be stored for a longer period of time. The solution forms a visible gelatinous precipitate after extended storage of at least half a year and will not produce satisfactory adhesive coatings anymore. Prepare a fresh solution if there are visible precipitates.

Materials

- Protran or other standard nitrocellulose membrane (Whatman, PerkinElmer)
- 100 % Methanol (Carl Roth GmbH + Co. KG, UN-No. 1230)

Nitrocellulose solution

→ For preparing a stock solution, dissolve a piece of 1 cm^2 nitrocellulose membrane in 10 ml methanol. Stock solutions may be stored at room temperature in polystyrene tubes. For the working solution, dilute the stock solution 10 : 1 with methanol. You can adjust the concentration to meet your requirements.

Procedure

1. Directly before use, pipette 3 – 5 µl of the working solution onto the recording field. The recording field should be completely covered.
2. Remove the coating solution and let the MEA air-dry. It takes just a few seconds for the methanol to evaporate.

Literature

Ulrich Egert, Thomas Meyer (2004); Heart on a Chip — Extracellular multielectrode recordings from cardiac myocytes *in vitro*, "Methods in Cardiovascular Research", S. Dhein and M. Delmar (eds.)

5.4.2 Coating with Polyethyleneimine (PEI) plus Laminin

Polyethyleneimine (PEI) has been successfully employed for dissociated cell cultures and proven to enhance cell maturation in culture compared to polylysine coated plates. Polyethyleneimine is a positively charged polymer and thus changes the charge on the glass surface from negative to positive. The tissue sticks even better with this method than with the nitrocellulose method, but the polyethylenimine forms a uniform layer that can get more easily detached from the surface, for example, by the perfusion. This coating method can optionally be combined with laminin.

Materials

- Poly(ethyleneimine) solution (PEI) (Sigma-Aldrich, Inc., P3143)
- Boric acid, crystalline (Fisher Scientific, A73-500)
- Borax (sodium tetraborate) (Sigma-Aldrich, Inc., B0127)
- 1 N HCl
- Laminin, 1mg/ml (Sigma-Aldrich, Inc., L2020)

Borate buffer

- 3.10 g boric acid
- 4.75 g borax

→ Dissolve in 1l distilled water at 80 °C.

→ Adjust pH to 8.4 with 1 N HCl.

PEI stock solution

0.05 – 0.1 % PEI dissolved in borate buffer.

Laminin solution

20 µg/ml laminin in plating medium.

Procedure

Note: It is necessary to thoroughly rinse off unbound PEI from the plates before use, as dried PEI is toxic.

1. Pipette 500 µl PEI solution onto the MEA. The recording field should be completely covered.
2. Incubate at RT for 1 h, or at 4 °C over night.
3. Remove the PEI solution and thoroughly rinse 4 x with distilled water.
4. Air-dry the MEA.
5. Sterilize with UV light for at least 1 h after coating.
6. (Place a drop of sterile laminin solution onto the MEA and incubate for 30 min. Aspirate, do not rinse, and directly seed your cells. Alternatively, mix the cells with laminin solution before plating.)

Literature

Ulrich Egert, Thomas Meyer (2004); Heart on a Chip — Extracellular multielectrode recordings from cardiac myocytes *in vitro*, "Methods in Cardiovascular Research", S. Dhein and M. Delmar (eds.)

Lelong, IH, et al. (1992); J. Neurosci. Res. 32:562-568

5.4.3 Coating with Polyornithine (plus Laminin)

Poly-D-lysine can be used as an alternative for polyornithine.

Materials

- Polyornithine
- Laminin, 1mg/ml (Sigma-Aldrich, Inc., L2020)

Polyornithine solution

→ 500 µg/ml polyornithine in distilled water

Laminin solution

→ 5 µg/ml laminin in plating medium or PBS (phosphate buffered saline).

Procedure

1. Incubate the MEA with polyornithine solution at RT for 2 – 3 hours or overnight at 4 °C.
2. Aspirate the polyornithine solution and rinse the MEA 3 x with distilled water before direct use or before the following coating with laminin. MEAs coated with polyornithine can be stored at 4 °C for several weeks.
3. Incubate pre-coated MEA with laminin solution for at least 1 h.
4. Aspirate the laminin solution and directly plate cells.

Literature

Cellular Neurobiology, A practical approach, ed. By Chad and Wheal, IRL Press, Oxford

5.4.4 Coating with Poly-D-Lysine (plus Laminin)

Poly-D-lysine has been used by several groups. Results seem to be equivalent to a coating with polyornithine. Some users complained about **cell clumping** and resulting cell death when using poly-D-lysine and had better results when using polyethylenimine (PEI).

Materials

- Poly-D-lysine 5 mg / 10 mL (= 0.05 % w/v) stock solution (Sigma-Aldrich, Inc., P7280)
- Laminin solution 1 mg/ml (Sigma-Aldrich, Inc., L2020)

Laminin solution

→ 20 µg/ml laminin in plating medium or PBS (phosphate buffered saline).

Procedure

1. Incubate the MEA with poly-D-lysine solution and incubate at 4 °C over night.
2. Rinse MEA with sterile distilled water 3x to remove toxic unbound lysine and let the MEAs air dry under sterile conditions (laminar flow) before plating the cells, or before the following coating with laminin. MEAs can be stored at 4 °C for up to two weeks.
3. Incubate pre-coated MEA with laminin solution at 4 °C over night.
4. Aspirate the laminin solution and directly plate the cells.

Literature

Goslin et al. 1988, Nature 336, 672-674

Maeda et al. 1995, J.Neurosci. 15, 6834-6845

Gross et al. 1997, Biosensors & Bioelectronics 12, 373-393

5.4.5 Coating with Poly-D-Lysine (plus Fibronectin)

This coating method is used, for example, for culturing dissociated suprachiasmatic nucleus (SCN) neurons (on standard 60MEA200/30). It is very stable and therefore especially useful for long-term cultures.

Materials

- Poly-D-lysine 5 mg / 10 mL (= 0.05 % w/v) stock solution (Sigma-Aldrich, Inc., P7280)
- Fibronectin (BD BioCoat™ Fibronectin Cellware) (BD Biosciences)

Fibronectin solution

→ Prepare a stock solution of 25 µg/ml fibronectin in distilled water or PBS (phosphate buffered saline) and store it at 4 °C.

Poly-D-Lysine plus fibronectin solution

→ Prepare a 0.01 % (w/v) poly-D-lysine solution, and add fibronectin 1:1 (resulting in a final concentration of 12.5 µg/ml).

Procedure

1. Pipette 10 µl of the poly-D-lysine plus fibronectin solution onto the recording field. Pipette about 50 µl of sterile distilled water near the rim of the culture chamber.
2. Incubate for 1 h in an incubator set to 35 °C, 65 % relative humidity, 9 % O_2, 5 % CO_2; or 37 °C, 100 % humidity, 5 % CO_2. To avoid a dry out of the liquid, place the MEA in a big Petri dish with lid on.
3. Rinse 2 x with sterile distilled water.
4. Let MEAs air-dry overnight on a sterile workbench (laminar flow) with UV light turned on.

5.4.6 Coating with Fibronectin

Fibronectin is a more biological coating alternative, especially used for heart tissues. The adhesion tends to be very stable, which allows longer cultivation times.

Materials

- Fibronectin (BD BioCoat™ Fibronectin Cellware) (BD Biosciences)

Fibronectin solution

→ Prepare a stock solution of 1 mg/ml fibronectin in distilled water or PBS (phosphate buffered saline) and store it at 4 °C. The stock solution is diluted with water or PBS to a final concentration of 10 µg/ml before use.

Procedure

1. Cover the MEA surface with 300 µl fibronectin solution and incubate the MEA at 37 °C for at least 1 h.
2. Aspirate the solution and rinse the MEA 2 x with PBS (phosphate buffered saline).
3. Plate the cells onto the MEA immediately after coating.

Literature

Ulrich Egert, Thomas Meyer (2004); Heart on a Chip — Extracellular multielectrode recordings from cardiac myocytes *in vitro*, "Methods in Cardiovascular Research", S. Dhein and M. Delmar (eds.)

5.4.7 Coating with Collagen

Coating with collagen is useful for short-term cultures. It tends to detach from the surface if used for long-term cultures.

Materials

- DMEM Dulbecco's Modified Eagle Media (DMEM) / F12 (Gibco/Invitrogen, 21331-020)
- N Hydrochloric acid, pH 3.0
- Acid-soluble type I collagen solution (3 mg/ml, pH 3.0) Cellmatrix Type I-A (Nitta Gelatin Inc.)

Preparation buffer

→ 200 mM HEPES in 0.08 N NaOH

Collagen solution

→ Add 1 ml of 10 x DMEM/F-12 medium to 8 ml Cellmatrix Type I-A and stir gently.

→ Add 1 ml of preparation buffer and stir gently.

→ Incubate the mixture at 4 °C for 30 min to remove any air bubbles, if necessary.

→ Store at 4 °C until use.

Procedure

1. Sterilize the MEA before the coating with collagen and perform all following steps under sterile conditions.
2. Incubate the MEA at 4 °C for at least 1h.
3. Fill the MEA with collagen solution until the bottom of the culture chamber is completely covered. Immediately remove the collagen solution with a glass pipette. The solution can be reused.
4. Incubate the MEA in a CO_2 incubator for 30 min. Rinse the MEA with sterile distilled water. Fill the MEA with culture medium and keep it sterile in a CO_2 incubator until use (for up to one week). Check for contaminations before use.

5.5 Cleaning of used MEAs

5.5.1 General Recommendations for Cleaning MEAs

The cleaning procedure depends on the kind of coating and on the kind of biological preparation. In the following, a few general considerations are listed.

- If you have recorded from an acute slice without coating, you can simply rinse the MEA with distilled water and the MEA should be fine.
- If necessary, the MEA can then be cleaned with any cleaning agent, for example, a standard dish-washing detergent. When cleaning coated MEAs, parts of the coating may go off. You have to recoat a MEA when the coating is not sufficient anymore, that is, when you observe problems with cell attachment or recording.
- If more severe methods are needed, the MEA can also be cleaned in an ultrasonic bath for a short moment. But this method is a bit dangerous, because there are ultrasonic baths that are too strong and will destroy the MEA. The behavior should be tested with an older MEA first. Generally is using an ultrasonic bath not recommended.
- EcoMEAs are easier to clean, because the golden electrodes are not so easily damaged.

5.5.2 Cleaning Detergents

Terg-A-Zym

TergAZyme "Enzyme Active Powdered Detergent" is a concentrated, anionic detergent with protease enzyme for manual and ultrasonic cleaning. It is excellent for the removal of any type of proteinaceous soils, tissue, blood, and body fluids from glassware, metals, plastic, ceramic, porcelain, rubber and fiberglass with no interfering residues. Ideal as a cleaning agent in reverse osmosis and ultra-filtration systems. USDA authorized.

Rinse with distilled water first, then apply 1% Terg-A-Zyme solution (Sigma) for several hours. Rinse the MEAs again with distilled water and dry them directly before use.

5.5.3 Cleaning of pMEAs

Perforated MEAs have a robust ceramic or glass carrier, but the electrodes are embedded in polyimide foil. Therefore, they are heat stable to 50 °C only and cannot be autoclaved.

Please do not use ultrasonic bath for pMEAs!

→ Rinse with distilled water first, then apply 1% Terg-A-Zyme solution (Sigma) for several hours. Rinse the pMEA again with distilled water and dry them directly before use. Sterilization via rinsing with 70 % ethanol is possible. Do not immerse the pMEA into the alcohol for a longer time otherwise the ring may probably get off!

5.5.4 Cleaning of EcoMEAs

The gold electrodes of EcoMEAs are very robust and are the only MEA electrodes that will endure more severe cleaning methods. You can check the need for cleaning under a stereo microscope: The electrodes should be shiny and look golden. If they are gray, or if they show a film, you should clean them.

→ Carefully clean the electrodes with a swab and distilled water under microscopic control.

→ EcoMEAs made of PCB (printed circuit board) have a temperature range from 0 – 125 °C. They can be sterilized by autoclavation.

5.5.5 Cleaning of EcoFlexMEAs

EcoFlexMEAs made of polyimide (Kapton) have a temperature range from 0 – 125 °C. They can be sterilized by autoclavation.

→ If necessary, carefully clean the electrodes with a swab and distilled water under microscopic control.

5.5.6 Cleaning of FlexMEAs

→ Rinse with distilled water first, optional with ethanol 70 %.

FlexMEAs made of polyimide foil have a temperature range from 10 – 125 °C. They can be sterilized by autoclavation.

When using an autoclave at 121 °C, please make sure that the FlexMEA36 will not be exposed to the moisture. The FlexMEA36 itself must be dry and additionally sealed in a sterile package.

Please do not use an ultrasonic bath for FlexMEAs!

5.5.7 Removing Nitrocellulose Coating

Note: It is very important that you clean MEAs that have been coated with nitrocellulose and remove all biological material first before removing the coating. If you applied methanol on an uncleaned MEA, you would rather fix the cell debris on the MEA than actually remove the coating.

1. Directly after usage, biological material is rinsed off under running water and the MEA is cleaned with pH-neutral cleaning agents or enzymatically if necessary.
2. Rinse the MEA 2 x with methanol. If nitrocellulose is not sufficiently removed by rinsing, incubate the MEA filled with methanol for 15 to 30 min to dissolve the cellulose nitrate.
3. Rinse the MEA with distilled water.

5.5.8 MEA Cleaning with EDTA-Collagenase

Materials:

- Collagenase Type I (Sigma-Aldrich, Inc., C0130)
- 0.5 mM EDTA
- Phosphate buffered saline (PBS) (Gibco/Invitrogen, 14190-144)

Collagenase solution:

→ Dissolve collagenase type I in PBS at 20 U/ml.

Method:

1. Fill the MEA culture chamber with 0.5 mM EDTA and incubate for 30 min.
2. Rinse the chamber 3 times with PBS.
3. Fill the MEA with collagenase solution and incubate for at least 30 min at 37 °C.
4. Discard the collagenase solution and rinse the MEA with distilled water at least 3 times.
5. Air dry the MEA, preferably under a laminar flow hood.

5.5.9 MEA Cleaning with Terg-A-Zyme

Materials:

- Terg-A-Zyme (Sigma-Aldrich, Inc., Z273287)
- Distilled water

Terg-A-Zyme solution:

→ Prepare a 1 % solution of Terg-A-Zyme in distilled water.

Method:

1. Place the MEA in 1 % Terg-A-Zyme solution overnight at room temperature.
2. Apply gentle shaking or rocking, if possible.
3. After Terg-a-Zyme treatment, rinse the MEA thoroughly with distilled water. (Terg-A-Zyme solution can be stored at 4 °C and reused for about a week).
4. Dry the MEA and apply hydrophilic surface treatment, if necessary (Please see above).
5. If the MEA is going to be used for cell or tissue culture, autoclave the MEA at 121 °C for 30 min.
6. Do not fix cells or tissues on a MEA. Detergent treatment will not remove fixed tissues.

Important: **NEVER** wipe the electrode field or touch it otherwise!

Warning: It is absolutely necessary to rinse the MEAs thoroughly with distilled water after treatment with detergent, particularly when using Terg-A-Zyme before heat sterilization (dry-heat sterilization is not recommended). Otherwise the potential rests of the detergent may burn into the glass carrier of the MEA and may destroy the electrodes.

6 Culture Chamber and Ring Options

You have several options regarding culture chamber interface rings (without ring, glass ring, plastic ring without and with thread) and culture chambers, which are especially useful for long-term cultures or experiments. For more details or pricing information, please ask your local retailer.

6.1 MEA2100-CO2-C

The MEA2100-CO2-C is a climate chamber for MEA2100-Systems. Connect the chamber via magnetic forces on the lid of a MEA2100 headstage to create a 5 % CO_2 atmosphere (humid or non humid) around the biological probe. Connect a tube with an inner diameter of ± 2.3 mm to the tube connector.

6.2 Sealed MEA Culture Dish

MEA-MEM Chamber to cover Glass Rings on MEAs

In order to allow long-term cultivation and recording, Multi Channel Systems recommends the use of teflon membranes (fluorinated ethylene-propylene, 12.5 microns thick) developed by Potter and DeMarse (2001). The membrane is produced in license by ALA Scientific Instruments Inc. and distributed via the worldwide network of MCS distributors. The MEA chamber **MEA-MEM** is developed by Multi Channel Systems.

The sealed MEA culture chamber MEA-MEM with transparent semipermeable membrane is suitable for all MEAs with glass ring. A hydrophobic semipermeable membrane from Dupont that is selectively permeable to gases (O_2, CO_2), but not to fluid and H_2O vapor, keeps your culture clean and sterile, preventing contaminations by airborne pathogens. It also greatly reduces evaporation and thus prevents a dry-out of the culture.

Reference

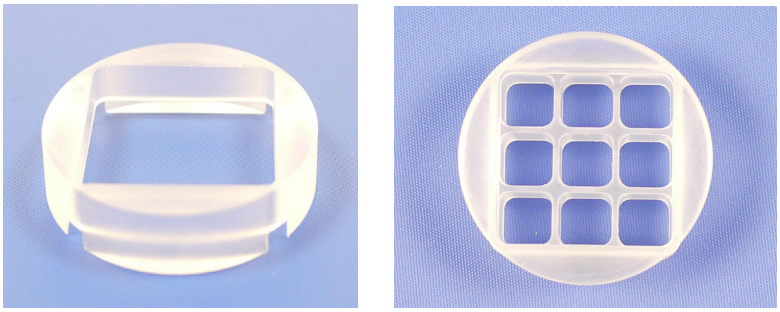
- Reference: Potter, S. M. and DeMarse, T. B. (2001). "A new approach to neural cell culture for long-term studies." J Neurosci Methods 110(1-2): 17-24.

6.3 MEA Culture Chamber with Lid

Another possibility is to use a MEA culture chamber with lid (available from Multi Channel Systems), which is suitable for all MEAs with plastic ring and thread. It can be adapted by inserting metal perfusion cannulas for setting up a continuous perfusion.

6.4 Culture Chamber for 9-Well MEAs

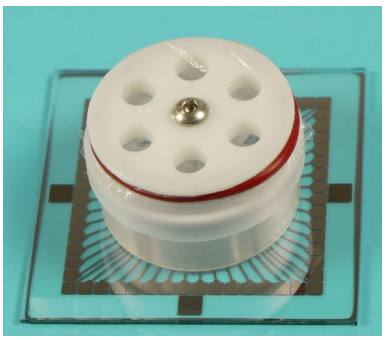
The culture chamber ring 9well-CC for 256-9wellMEAs is suitable for the 9well macrolon quadrat (available from Multi Channel Systems). To use the macrolon quadrat in combination with the 9well-CC as a culture chamber, please insert a foil between macrolon quadrat and 9well-CC ring.



9well-CC for 256-9wellMEA300/30iR-ITO

6.5 Culture Chamber for 6-Well MEAs

The 6well-CC culture chamber is suitable for 6well macrolon rings on 60-6wellMEAs (available from Multi Channel Systems). The removable membrane cover for 60-6wellMEAs is available for triangle and round chamber rings.



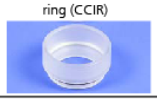
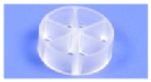
6well-CC

6.6 Ring Options

The following table shows all available ring options. Glass rings (-gr) are available in two heights of 6 or 12 mm. Plastic rings (-pr) are available in four heights and without or with thread (-pr-T).

The triangle (-tcr) and round (-rcr) chamber rings are suitable for the MEAs with 6 wells, the macrolon quadrates (-mq) for the 256MEAs with 9 wells.

MEA Ring Options

	Glass rings (-gr)	Plastic rings (-pr)	Plastic rings with thread (-pr-T)	6-well rings	9-well rings
Culture chambers/ lids to be placed on rings	MEA-MEM ID: 24.5 mm, OD: 31 mm 		Culture chamber lid (CCL)  Culture chamber (CC)	Culture chamber (6well-CC) + ALA MEA-MEM-SHEET 	Culture chamber (9well-CC) + ALA MEA-MEM-SHEET 
	ALA MEA-INSERT 		Culture chamber interface ring (CCIR) 	Set of 5 culture chambers (6well-CC-Set) 	
	ALA MEA-MEM-PL 				
Rings (standard)	Glass ring (-gr) 6 mm high ID: 19 mm, OD: 24 mm 	Plastic ring (-pr) 6 mm high ID: 26.5 mm, OD: 30 mm 	Plastic ring with thread (-pr-T) 6 mm high ID: 26 mm, OD: 30 mm 	Triangle chamber ring (-tcr), 10 mm high OD: 30 mm 	Plastic quadrate (-mq), 9 mm high OD: 24 mm 
				Round chamber ring (-rcr), 10 mm high OD: 30 mm 	
Rings (optional, on request)	Glass ring 12 mm high ID: 19 mm, OD: 24 mm 	Plastic ring 3 mm high ID: 26.5 mm, OD: 30 mm 	Plastic ring with thread 15 mm high ID: 26 mm, OD: 30 mm 		

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Please note: Not all rings can be placed on all MEAs. Please check exact order description.

7 Recording with MEAs

7.1 Mounting the MEA

7.1.1 Cleaning the Contact Pads

You should always clean the contact pads with alcohol before placing it into the MEA amplifier. Even if you do not see any contaminations, a very thin grease layer from touching the pads with bare fingers, for example may be present and results in a bad contact between the pads and the amplifier pins. A bad contact will result in an increased noise level on the affected channel. This is the most prominent handling error.

→ Carefully wipe the MEA contact pads with a clean and soft tissue moistened with pure alcohol.

7.1.2 Positioning the MEA

Important: MEAs are not symmetrical! That is, why the **writing** (for example NMI, LEITER, MEA type) on the MEA chip should be on the right side viewed from the front, with the sockets of the amplifier in the back. MEAs with one big internal reference electrode should be placed with reference electrode to the left side in the amplifier. Otherwise, the MEA layout will not match with the pin layout of the channel map in the data acquisition software.

When placing a special MEA into the amplifier, for example 120MEA, HighDense MEA, 4Q1000 MEA or HexaMEA, please make sure that the orientation of the MEA is correct, respectively.

7.1.3 Grounding the Bath

Make sure that the bath is connected to the amplifier's ground.

→ Attach the provided silver wire or Ag/AgCl pellet to the amplifier's ground and place it into the bath.

→ — OR — If you use a MEA with internal reference electrode, connect the ground to the reference electrode socket (pin 15) with the provided connector.

Please see the manual of the respective MEA amplifier for more information about mounting MEAs and grounding.

7.2 General Performance / Noise Level

You can test a MEA before use by filling it with a standard saline buffer, for example PBS (phosphate buffered saline), and recording the noise level of the MEA and the amplifier.

MEA amplifiers have a maximum noise level of ±8 µV. The noise level on the MEA depends on the electrode size and material. The smaller the electrode, the higher is the noise level. TiN electrodes have a larger surface area due to their microfold structures, and therefore they have generally a lower impedance and a lower noise level than electrodes of the same size that are made from other materials (for example, Pt or Au electrodes).

The total maximum noise level for a MEA and the amplifier should be about ±40 µV peak to peak for 10 µm TiN electrodes and ±10 µV for 30 µm TiN electrodes.

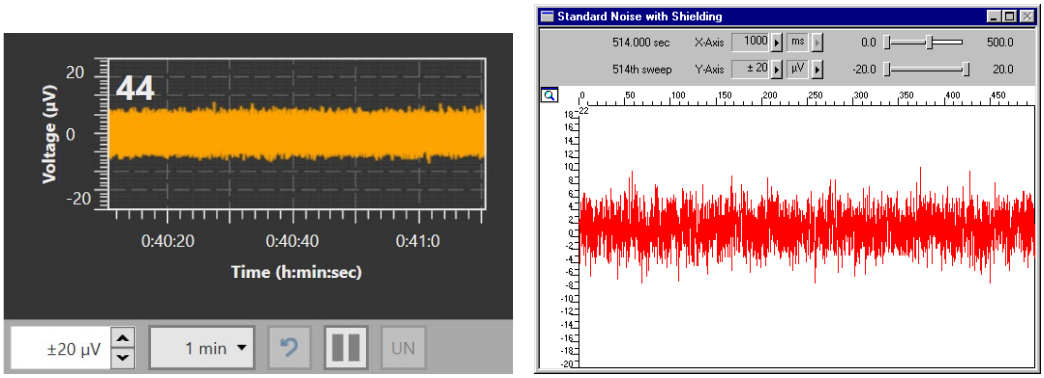
The initial noise level may be higher if the MEAs are hydrophobic. New MEAs should be made hydrophilic before use, please treat them in a plasma cleaner.

The following pictures show the behavior of MEAs, recorded with a MEA2100 or with a MEA1060-BC amplifier.

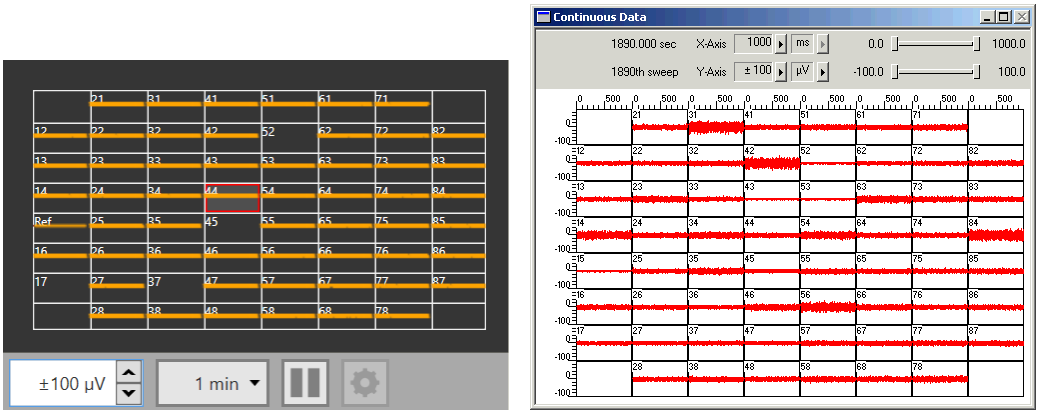
Both data acquisition software types from Multi Channel Systems are used: Multi Channel Experimenter on the left and MC_Rack on the right

Typical noise level of a used standard 60MEA200/30iR-Ti (round 30 µm Tin electrodes)

This picture shows the typical noise level of a standard 60MEA200/30iR-Ti on most electrodes, Some Electrodes show an increased noise level after a longer cycle of use. The bath was grounded with the internal reference electrode 15. Time axis: 1000 ms, voltage axis: ±50 µV. You should ground some of the electrodes if you want to use this MEA for recording again.

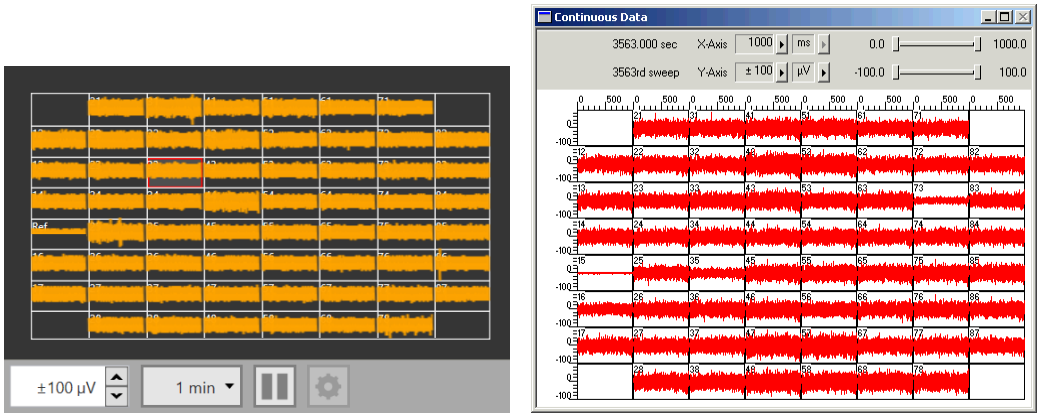


Same MEA, zoom to single channel #44 or #22. Time axis: 1 min, voltage axis: ±20 µV.



Same MEA after grounding defective electrodes. Time axis: 1000 ms, voltage axis: ±100 µV.

Typical noise level of a standard 60MEA200/10iR-Ti (round 10 µm Tin electrodes)



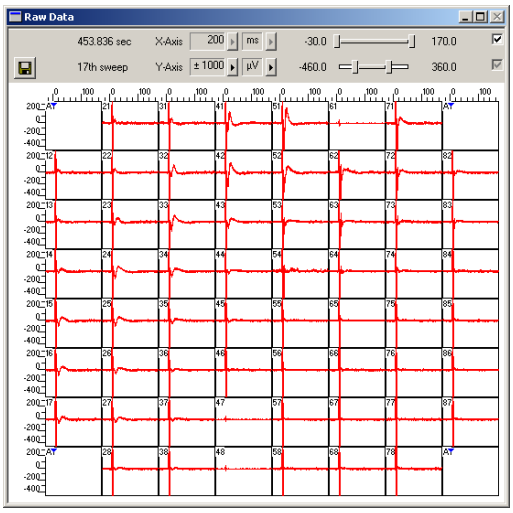
Noise level of a new standard 60MEA200/10iR-Ti. Bath grounded with the internal reference electrode 15. Time axis: 1000 ms, voltage axis: ±100 µV.

8 Stimulation

8.1 Using MEA Electrodes for Stimulation

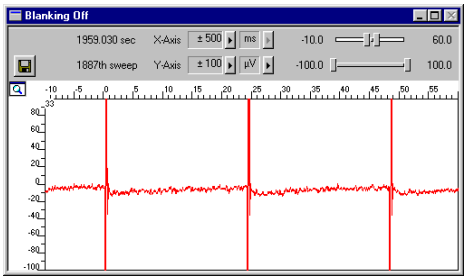
You can use **any MEA electrode(s)** for stimulation. Simply connect the stimulus generator outputs to the MEA amplifier. In the MEA2100-System the stimulator is integrated in the headstage. The stimulation electrodes are selected via software. Please see the manual for the respective MEA amplifier and stimulus generator for more details. This and the following chapters are intended for helping you to optimize the stimulation with MEA electrodes.

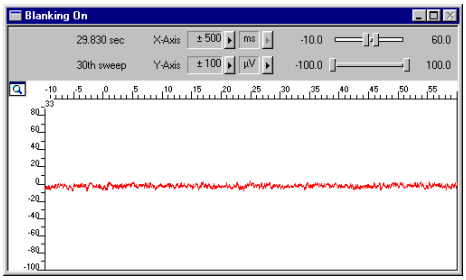
All electrodes suffer under electrical stimulation, especially under long-term stimulation. The wear depends on the stimulus and on the electrode type. When stimulating via MEA electrodes and with standard MEA amplifiers, you will see a stimulus artifact on all amplifier channels during stimulation due to the high charge that is injected into the circuit, and the following saturation of the filter amplifiers. The time constant of the stimulus artifact depends on the amplifier bandwidth; if the lower cutoff frequency is quite low, for example, 1 Hz, the stimulus artifact will be longer than with 10 Hz, for example. In most cases, it will not be possible to record true signals that are close to the stimulus pulse. This can be avoided by using a MEA amplifier with blanking circuit. The stimulating electrode can generally not be used for recording in parallel to stimulation, because the injected charge is so high, and the time constant for discharging so low.



The screen shot shows a prominent stimulus artifact on all channels, followed by a response. The stimulating electrode No. 61 has been grounded.

The next pictures demonstrate the blanking feature. On the left screen shot, you see the stimulus artifacts on a non-stimulating electrode without blanking. On the right, you see the same electrode and stimulation pattern, but with blanking. The stimulus artifacts have been completely avoided, making it possible to detect signals shortly after the stimulus.





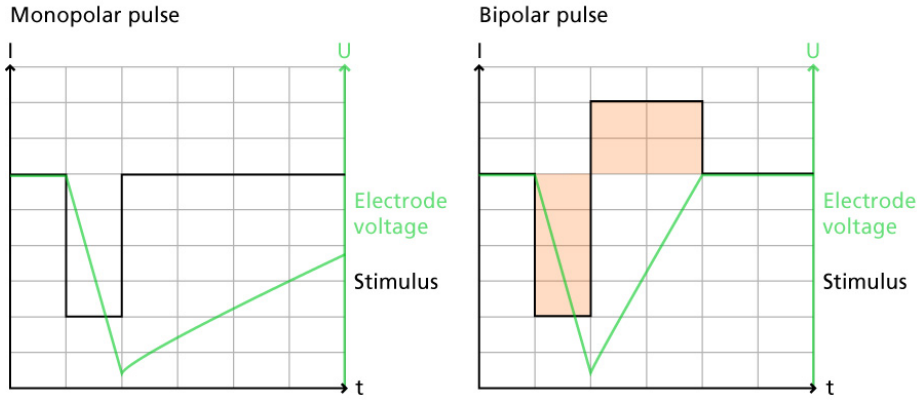
8.2 Capacitive Behavior of Stimulating Electrodes

Regarding the generally used stimulus pulses, stimulating electrodes behave as plate capacitors. The charge cannot flow back to the stimulus generator due to the high output resistance and thus is kept in the electrode. The electrode needs a quite long time to discharge itself after stimulation. As a result, stimulus artifacts interfere with the recording, and electrodes deteriorate over time due to electrolysis. You can avoid that by choosing an appropriate stimulus protocol that actively discharges the electrode after the pulse.

When using **voltage** driven stimulation, the electrodes are discharged when the voltage level is set to zero at the end of the (monophasic) pulse. Not so in **current** mode. When applying a negative current pulse, the electrode is charged and needs to be actively discharged by applying an inverted pulse with a matching product of current and time that is you need to stimulate with biphasic pulses for current driven stimulation to reduce both the stimulus artifact and to avoid an electrode damage. The easiest way is to use the same signal amplitude and the same duration with an inverse polarity. For voltage driven stimulation, monophasic pulses are fine.

The following illustration shows the effect of a biphasic current pulse on the discharge of the stimulation electrode. As you can see, the first monophasic pulse is followed immediately by a pulse of the opposite polarity and the same product of current and time.

Effect of a bipolar pulse on the electrode voltage



8.3 Aspects of Electrode Size and Material

Titanium nitride (TiN) electrodes are generally more robust than electrodes from other materials, for example platinum (Pt). In the Appendix, you find safe charge injection limit curves that document maximum current and stimulus durations for standard TiN electrodes. Please note that these curves document the limits. Stimulus pulses should be kept safely below these limits. The safe charge injection limit of platinum (0.4 mC/cm^2) is much smaller than for TiN (23 mC/cm^2). This fact results in a considerably lower charge that you can inject into the electrode before faradic reactions occur that will lead to electrolysis of the electrode.

Please note that, when using voltage driven stimulation, the current flow to the electrode depends on the electrode impedance. The **lower** the **impedance**, the **higher** is the **current**. Please make sure to obey the safe charge injection limits always. Generally, TiN electrodes have lower impedances than Pt electrodes, and larger electrodes also have lower impedances than smaller.

When using TiN electrodes, it is extremely important to not charge the electrodes positively, as this will lead to electrolysis. (This is not an issue for Pt electrodes.) Therefore, when using voltage driven stimulation, it is important to apply negative voltages only. Positive voltages will shortly charge the electrodes positively, even though the electrode is discharged at the end of the pulse. As a consequence, biphasic voltage driven stimulation is not recommended. When using current stimulation, it is required to use biphasic stimulation, and to apply the negative phase first, to avoid a positive net charge on the electrode.

8.4 Recommended Stimulus Amplitudes and Durations

The higher the amplitude and the longer the stimulus, the higher is the impact on the electrode performance. Therefore, the amplitude and duration should be as low as possible. It is advisable to start with a low amplitude and duration, and then increase it slowly until responses are evoked.

The allowed product of amplitude and duration is directly proportional to the electrode surface. The higher the amplitude, the shorter is the maximum duration of the pulse, and vice versa. Do not apply pulses with a higher amplitude or for a longer time than is recommended for the electrode type. TiN electrodes have a rough surface structure and therefore have a larger surface than electrodes of the same size but made of a different material. The safe-charge injection limits in the appendix describe the relationship between maximum pulse amplitude and time for TiN electrodes.

As a consequence of the points discussed above, Multi Channel Systems recommends using negative monophasic voltage pulses or biphasic voltage pulses to make sure that the voltage level of the stimulating electrode is zero, and thus the electrode is discharged, at the end of the pulse.

According to the experience of MEA users, voltage pulses should be < 1 V (–100 mV to –900 mV) for neuronal applications to avoid damage to electrode and cells. Generally, pulse durations between 100 to 500 µs are used. (See also Potter, S. M., Wagenaar, D. A. and DeMarse, T. B. (2005). "Closing the Loop: Stimulation Feedback Systems for Embodied MEA Cultures." Advances in Network Electrophysiology Using Multi-Electrode Arrays. M. Taketani and M. Baudry, Springer; Wagenaar, D. A., Madhavan, R., Pine, J. and Potter, S. M. (2005). "Controlling bursting in cortical cultures with closed-loop multi-electrode stimulation." J Neurosci 25(3): 680-8.)

For pacing cardiomyocytes, higher voltages and durations are generally required, for example, –2 V for 2 ms. As these pulses are not supported by standard MEA electrodes, the use of larger stimulating electrodes is recommended.

Warning: When using MEA electrodes of TiN material, use only negative voltages pulses or biphasic current pulses applying the negative phase first. Always regard the safe-charge injection limits as described in the appendix of this manual. Otherwise, electrodes can be irreversibly damaged by electrolysis.

9 Troubleshooting

9.1 About Troubleshooting

The following hints are provided to solve special problems that have been reported by users. Most problems occur seldom and only under specific circumstances. Please check the mentioned possible causes carefully when you have any trouble with the product. 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 Multi Channel Systems to optimize the instrument and the documentation.

Please pay attention to the safety and service information (chapter "Important Information"). 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.

9.2 Technical Support

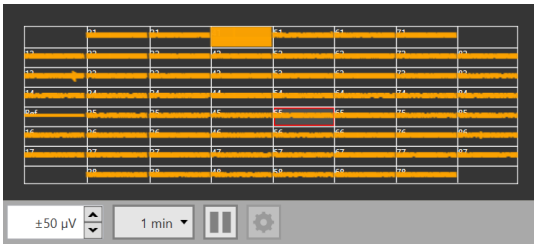
Please read the Troubleshooting part of the user manual first. Most problems are caused by minor handling errors. Contact your local retailer immediately if the cause of trouble remains unclear. Please understand that information on your hardware and software configuration is necessary to analyze and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

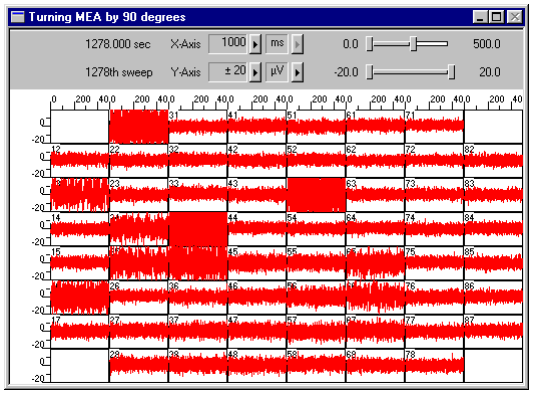
Please keep information on the following at hand

- Description of the error (the error message text or any other useful information) and of the context in which the error occurred. Try to remember all steps you had performed immediately before 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 MEA. You will find it on the MEA case.
- The amplifier type and serial number. You will find it on the device.
- 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.
- The version of the recording software. On the "Help" menu, click "About" to display the software version.

9.3 Noise on Single Electrodes

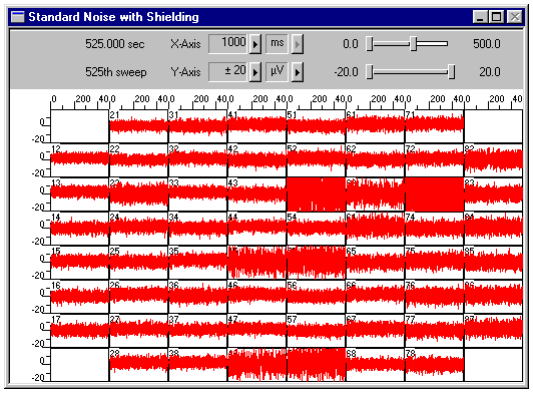
Please see the Multi Channel Experimenter software on the left picture, MC_Rack on the right. The noise level on single electrodes is significantly higher than expected or you see artifact signals. In the following example (60MEA200/30, filled with PBS (phosphate buffered saline), silver pellet as bath electrode, shielded). Electrode No. 41 (Multi Channel Experimenter), and electrodes No. 53, 63, 73, 45, 55, 48, 58 (MC_Rack) show a high noise level.





Possible causes:

? The electrode or the contact pin of the amplifier may be defective. To test this, do the following.



Open the amplifier and turn the MEA by 90 degrees.

Close the amplifier again and start the recording.

If the same electrode in the MEA layout is affected, the amplifier's contact is not ok. If another electrode is now affected and the previously affected electrode is ok now, the MEA electrode is not ok, but the amplifier is fine. The following screen shot shows the same MEA than above that has been turned clockwise by 90 degrees. You see that different channels are now affected, which indicates that the amplifier is fine but some electrodes on the MEA are defective.

— OR —

→ Use the test model probe to test the amplifier. If the noise level is fine without the MEA, bad MEA electrodes cannot be the cause.

MEA is defective

MEAs wear out after multiple uses or over a longer time of use, for example for long-term cultures. This is considered a normal behavior. MEAs are also easily damaged by mishandling, for example if wrong cleaning solutions or too severe cleaning methods are used or if the recording area is touched. If you observe a bad long-term performance of MEAs, consider a more careful handling.

Possible causes:

? The contact pads are contaminated.

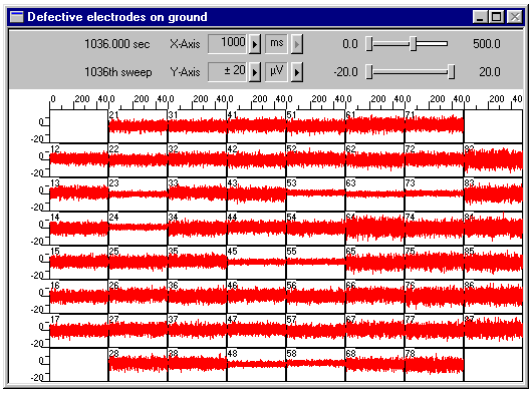
→ Clean the contact pads carefully with a swab or a soft tissue and pure (100 %) alcohol.

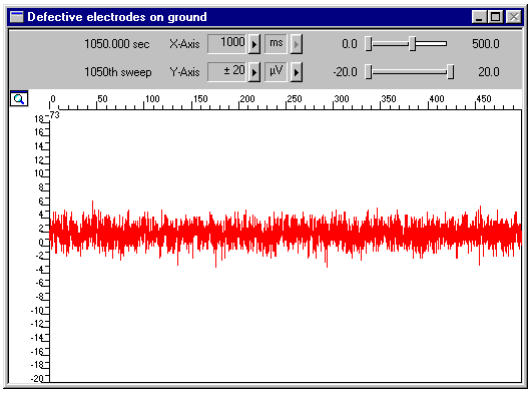
? The contact pads or the electrodes are irreversibly damaged. You could have a look at the electrodes under a microscope: If they appear shiny golden, the titanium nitride is gone and the electrode is irreversibly damaged. Electrodes may be damaged without changing their visual appearance, though.

→ Pick one of the bad channels after the other and ground it. See the MEA amplifier's manual for more information on grounding channels. In most cases, only one of the electrodes that appear bad is actually defective, and the other ones are only affected by the single defective electrode. Ground as many electrodes as you need for a good general performance.

In the following example, all defective electrodes have been grounded.

Grounded electrodes show a noise level that is lower than that of good electrodes (MC_Rack).





→ If too many electrodes are defective, use a new MEA.

Contact pin is defective

Please see the manual for the respective MEA amplifier.

9.4 Overall Noise / Unsteady Baseline

The baseline is unstable, signals are jumping or drifting.

Possible causes:

? Bath electrode is not connected to ground.

→ Connect the internal or external bath electrode to one of the ground inputs of the amplifier.

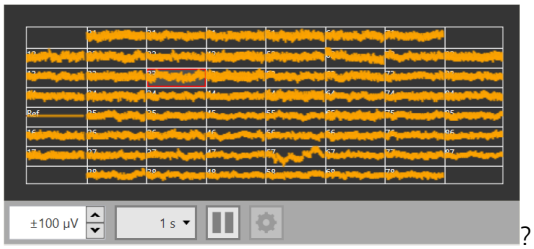
? AgCl bath electrode needs is not well-chlorided.

→ Rechloride the electrode or use a new one.

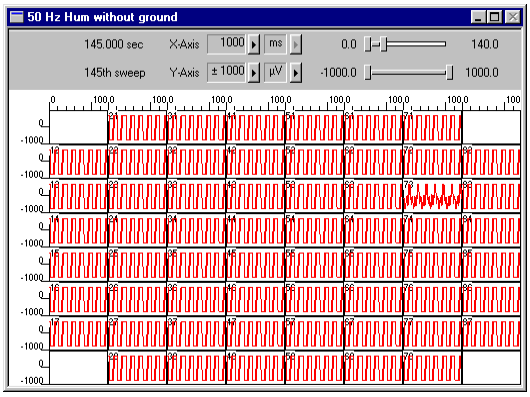
? 50 Hz hum: 50 Hz is the frequency of mains power in Europe. If the shielding and grounding of the setup is not sufficient, electrical signals are picked up from the environment.

→ Use a proper shielding. For example, you can place aluminum foil over the amplifier that is connected to any metal part of the MEA amplifier. You can also use special shielding equipment like a Faraday cage.

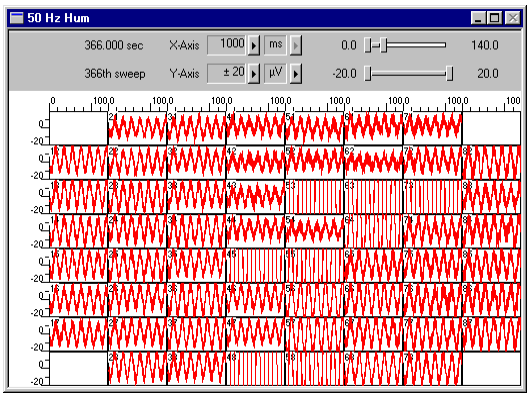
The following screen shot shows a recording of a 60MEA200/30 **without bath electrode** and **without shielding**. You see that the signals are so high that the amplifier gets saturated, and you see a very strong 50 Hz hum.

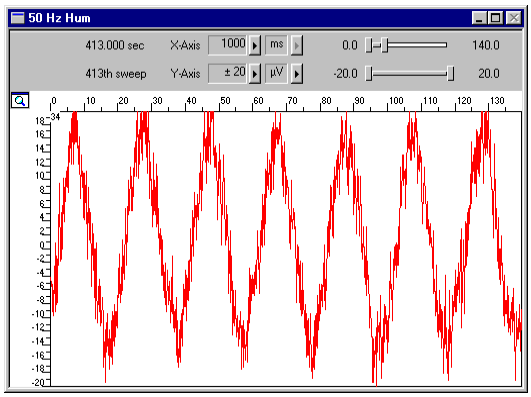


???

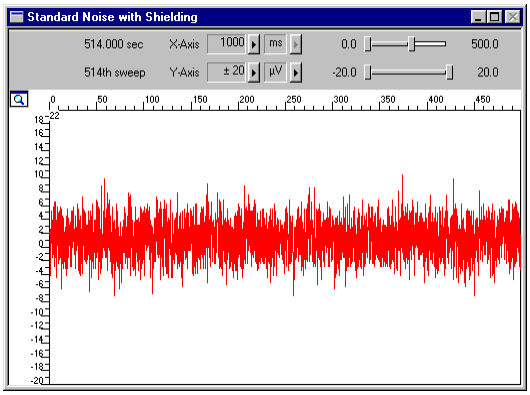


The next pictures show the same MEA with bath electrode (silver pellet), but without shielding. The baseline is very unsteady and oscillates with a frequency of 50 Hz.





The next screen shot shows the effect of shielding: The noise level is neglectable, and the baseline is steady. The shielding has been achieved with a metal plate connected to the metal part of the 68-pin MCS high grade cable connector and placed above the amplifier. You could also use aluminum foil or a Faraday cage for the same effect, for example.



9.5 Missing Spikes or Strange Signal Behavior

MEAs wear out after multiple uses or over a longer time of use, for example, for long-term cultures. The insulation layer gets thin over time. This is considered a normal behavior.

Possible causes:

? The insulation layer is too thin. As a result, the MEA gets the behavior of a low pass filter. This means, that the signal frequency may be shifted to a lower frequency, and spikes are missing.

→ Optically control the MEA with a microscope. If concentric colored rings (Newton rings) are visible (due to light interference), the insulation layer is too thin and you should use a fresh MEA.

? The insulation layer has been abraded and is missing in parts. This will result in a short circuit between the electrodes or tracks and the bath. You will still see signals, but as an unspecific smear over the complete array.

→ Use a fresh MEA.

10 Appendix

10.1 Contact Information

Local retailer

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

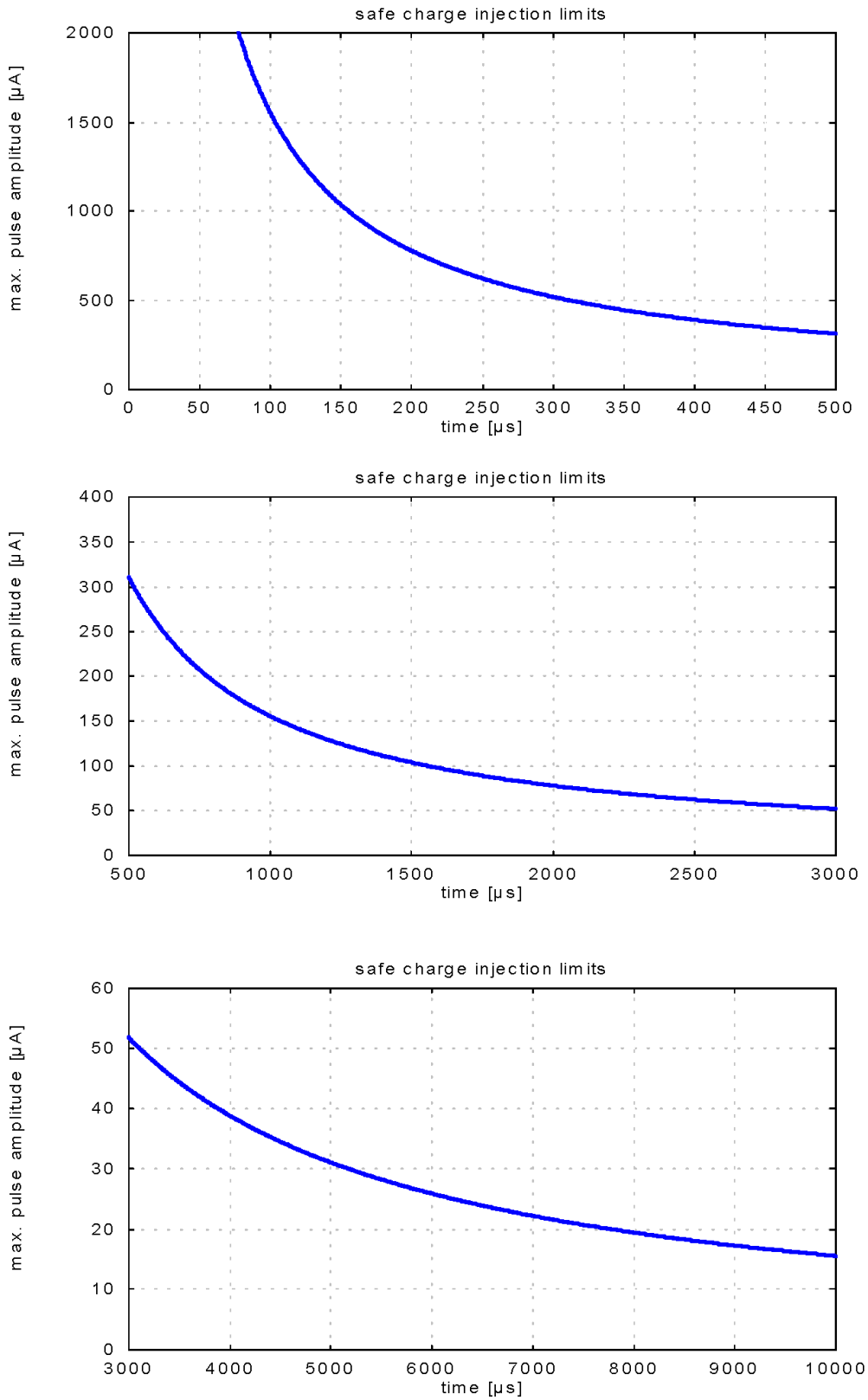
Mailing List

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 mailing list on the contact form of the MCS web site.

www.multichannelsystems.com

10.2 Safe Charge Injection Limits

Safe Charge Injection Limits of Micro Electrode Arrays with TiN Electrodes (diameter: 30 µm)



Safe Charge Injection Limits of Micro Electrode Arrays with TiN Electrodes (diameter: 10 µm)