

Microelectrode Array (MEA) User Manual



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Table of Contents

1	Introduction	5
1.1	About this Manual	5
2	Important Information and Instructions	5
2.1	Operator's Obligations	5
2.2	Guaranty and Liability	5
2.3	Important Safety Advice	7
3	Microelectrode Arrays (MEAs) — Overview	8
3.1	Extracellular Recording with Microelectrode Arrays	8
3.2	MEA Design and Production	9
3.3	Electrodes, Tracks, and Insulation	10
4	MEA Types and Layouts	11
4.1	Standard Electrode Numbering	11
4.2	Standard MEAs	12
4.3	HighDenseMEAs	12
4.4	HexaMEAs	13
4.5	ThinMEAs	13
4.6	3-D MEAs	14
4.7	EcoMEAs	15
4.8	FlexMEAs	16
5	MEA Handling	17
5.1	Hydrophilic Surface Treatment	17
5.1.1	Plasma Cleaning	17
5.1.2	Protein Coating	17
5.1.3	Preculturing	18
5.2	Sterilization	18
5.2.1	Sterilization with Ethanol and UV Light	18
5.2.2	Steam Sterilization (Autoclavation)	18
5.2.3	Dry Heat Sterilization	18
5.2.4	Sterilization with Hot Water	18
5.3	MEA Storage	19
5.4	MEA Coating	19
5.4.1	Coating with Nitrocellulose	19
5.4.2	Coating with Polyethyleneimine (PEI) (plus Laminin)	20
5.4.3	Coating with Polyornithine (plus Laminin)	21
5.4.4	Coating with Poly-D-Lysine (plus Laminin)	22
5.4.5	Coating with Poly-D-Lysine (plus Fibronectin)	22
5.4.6	Coating with Fibronectin	23
5.4.9	Coating with Collagen	24

5.5	Cleaning of Used MEAs	25
5.5.1	General Recommendations for Cleaning MEAs	25
5.5.2	Cleaning of 3-D MEAs	25
5.5.3	Cleaning of EcoMEAs	25
5.5.4	Removing Nitrocellulose Coating	25
5.5.5	MEA Cleaning with EDTA-Collagenase	26
6	Culture Chamber Options	17
6.1	Sealed MEA Culture Dish	26
6.2	MEA Culture Chamber with Lid	27
6.3	Removable Recording Chamber	27
7	Recording with MEAs	29
7.1	Mounting the MEA	29
7.1.1	Cleaning the Contact Pads	29
7.1.2	Positioning the MEA	29
7.1.3	Grounding the Bath	29
7.2	General Performance / Noise Level	30
8	Stimulation	33
8.1	Using MEA Electrodes for Stimulation	33
8.2	Capacitive Behavior of Stimulating Electrodes	34
8.3	Aspects of Electrode Size and Material	34
8.4	Recommended Stimulus Amplitudes and Durations	35
9	Troubleshooting	37
9.1	About Troubleshooting	37
9.2	Technical Support	37
9.3	Noise on Single Electrodes	38
9.4	Overall Noise / Unsteady Baseline	40
9.5	Missing Spikes or Strange Signal Behavior	41
10	Appendix	43
10.1	Contact Information	43
10.2	Ordering Information	44
10.2.1	MEA Systems	44
10.2.2	MEA Amplifiers	44
10.2.3	Accessories	45
10.3	MEA Data Sheet	46
10.4	MEA Layouts	47
10.5	Safe Charge Injection Limits	48

1 Introduction

1.1 About this Manual

The MEA User Manual comprises all important information about the microelectrode arrays (MEA) for use with the MEA System from Multi Channel Systems. The MEA User 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 scientific 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 user manual for the MEA amplifier you use. You will find more information about the MEA System and its components in general, especially the data acquisition card, in the MEA System User Manual. For more details on the data acquisition and analysis program MC_Rack, please refer to the MC_Rack User Manual.

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 user 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](#).

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 Guaranty 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 guaranty 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.

Guaranty 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

Those parts in this user manual that refers to the applications, and not to the product itself, for example, coating of MEAs, is 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.

2.3 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.

- 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 3-D MEAs or FlexMEAs to heat.
- Do not touch the electrode field in any way.
- Always put the provided **yellow plastic plate** beneath a 3-D MEA before placing it into the MEA amplifier. Avoid any mechanic pressure or stress when handling 3-D MEAs.
- 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 several (typically 60) electrodes allowing the targeting of several sites in parallel for extracellular recording and stimulation.

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 FlexMEAs) are only for use with the MEA System for extracellular recording from Multi Channel Systems MCS GmbH. FlexMEAs may be used with components of the ME System from Multi Channel Systems MCS GmbH. FlexMEAs are designed for use in *in vitro* or *in vivo* studies.

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 and the MEA System in the fields of **neurobiology** and **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, neuroregeneration, 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 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/support/applicationnotes.htm>

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 electrodes are available in sizes of 10, 20, and 30 μm ; and three-dimensional Pt electrodes have a diameter of 40 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.

Several other MEA types and layouts that are dedicated to special applications are also available, please see [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 see MEA Coating on page 19.

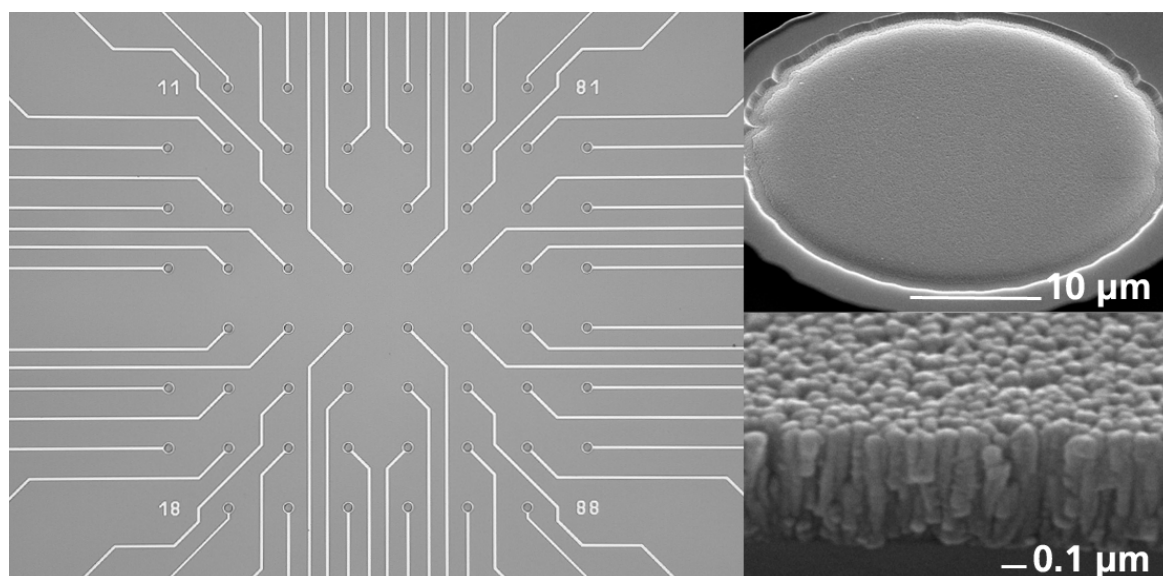
Spike activity can be detected at distances of up to 100 μm from a neuron in an acute brain slice. Typically, signal sources are within a radius of 30 μm around the electrode center. The smaller the distance, the higher are the extracellular signals. The higher the spatial resolution, the lower the numbers 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 a new electrode material: titanium nitride (TiN).

The NMI 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.

3-D MEAs (with platinum electrodes) are produced for Multi Channel Systems by Ayanda Biosystems in Lausanne, Switzerland (www.ayanda-biosys.com).

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 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. Long-time experiments with cell cultures and rigid cleaning methods shorten the MEA lifetime, but you can still reuse an MEA about 30 times, depending on the coating, cell culture, and cleaning procedure. All MEAs (except FlexMEAs and 3-D MEAs) 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 electrode ranges between 20 and 400 kilohms, depending on the diameter. 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 electrode sizes of 10, 20, or 30 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.

Pt electrodes (3-D MEAs) have also a fine noise level, but bigger electrodes and a lower spatial resolution. They are used in experiments that emphasize on the higher surface area of a 3-D MEA rather than a high spatial resolution. 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 . 3-D MEAs feature tip-shaped electrodes with a base 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 or indium tin oxide (ITO) are electrically isolated with silicon nitride (PEVCD). The contact pads are of the same material as the tracks are (except 3-D MEAs). 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 multichannel 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.

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, 100 μm) and the second number refers to the electrode size (for example 10 μm), which results in the standard MEA type 100/10, for example.

Standard versions are available with an internal reference electrode (abbreviated i. r.) 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,

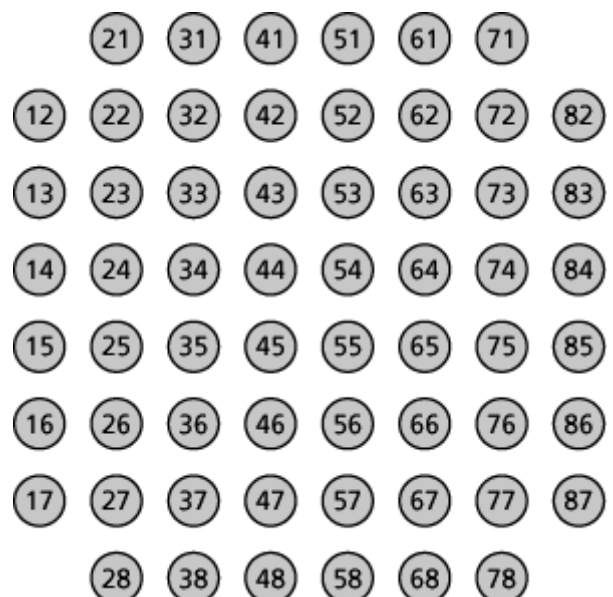
- [Standard MEAs](#) with flat round TiN electrodes in an 8 x 8 layout grid for all applications
- [HighDenseMEAs](#) with the highest spatial resolution and a double recording field of 5 x 6 electrodes each
- [HexaMEAs](#) featuring a hexagonal layout, perfect for recording from retina
- [ThinMEAs](#) with a "thickness" of only 180 μm , ideally suited for high-resolution imaging
- [3-D MEAs](#) — the ideal solution for acute slices, because the tip-shaped Pt electrodes are intended to penetrate dead cell layers, or for applications where a very high electrode surface area is required
- Very cost efficient and robust [EcoMEAs](#) for applications with lower spatial resolution and higher throughput, especially for established cardiomyocyte cultures, large slices, or whole-heart preparations
- [FlexMEAs](#) made of flexible polyimid material, perfect for *in vivo* and special *in vitro* applications, for example, whole-heart preparations

4.1 Standard Electrode Numbering

The numbering of MEA electrodes in the 8x8 grid (standard MEAs, ThinMEAs, 3-D MEAs, ecoMEAs) 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 MC_Rack program. Please make sure that you have selected the two-dimensional MEA layout as the **Channel Layout** in MC_Rack. For more details, please refer to the MC_Rack user manual or help.

Other electrode grids are described in the Appendix.



4.2 Standard MEAs

The following standard MEAs are available: 100/10, 200/10, 200/10 i. r., 200/30, 200/30 i. r., 500/10, 500/10 i. r..

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 , or 500 μm .

Versions 200/10, 200/30, 500/10, and 500/30 are available with an internal reference electrode as indicated by the abbreviation i. r.. You can connect the internal reference electrode directly to the amplifier's ground and will not need silver pellets for grounding the bath anymore. Please refer to the MEA1060 user manual delivered with your MEA amplifier for more 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 30 k Ω and 400 k Ω , depending on the electrode diameter. Generally, the smaller the electrode, the higher is the impedance.

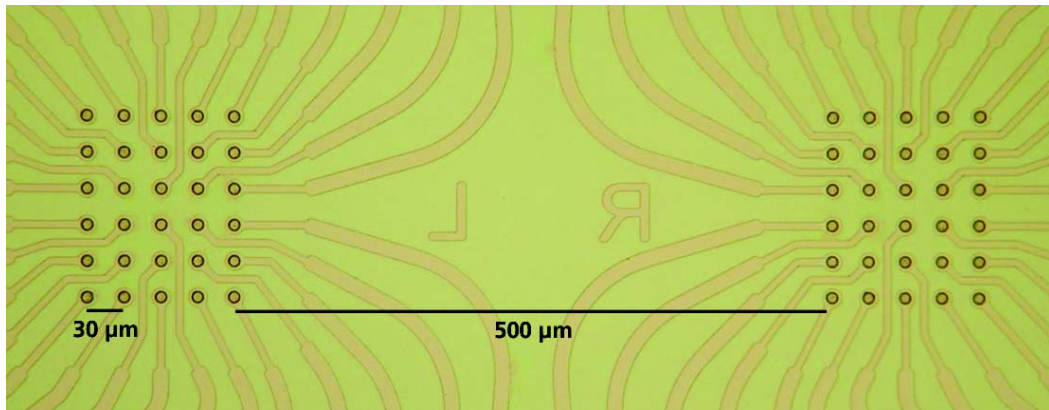
Tracks and contact pads are made of titanium or ITO; insulation material is silicon nitride. ITO contact pads and tracks are transparent, for a perfect view of the specimen under the microscope.

Using standard MEAs

Standard MEAs can be used for a wide variety of applications. They are robust and heat-stable. 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 HighDenseMEAs

10 μm electrodes are arranged in two recording field 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 HighDense MEA 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 HighDense 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 ITO; insulation material is silicon nitride.

Using HighDenseMEAs

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

4.4 HexaMEAs

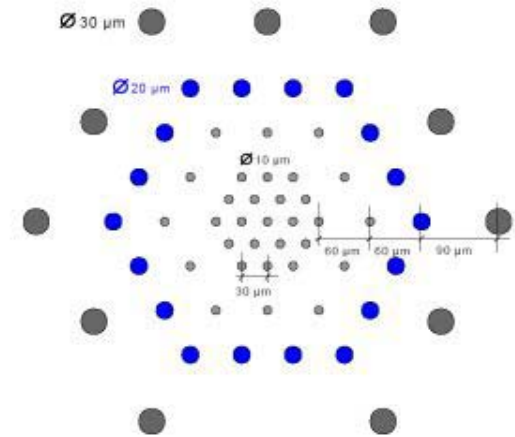
HexaMEAs feature a hexagonal layout, perfect for recording from retina.

60 electrodes are aligned in a special configuration with varying electrode diameters (10, 20, 30 μm) and interelectrode distances (see picture). The specific layout resembles ideally the regularity of the retina's architecture. The density of neurons is more important in the center than in the peripheral. This is matched by the density of electrodes on the MEA, which is also higher in the center than in the peripheral.

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

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

Electrodes in the center have a diameter of 10 μm with an interelectrode distance of 20 μm , where the peripheral electrodes have a diameter of 20 μm and 30 μm .



Using HexaMEAs

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

4.5 ThinMEAs

ThinMEAs are only 180 μm "thick", ideally suited for high-resolution imaging. ThinMEAs 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, 60 electrodes are arranged in an 8 x 8 layout grid with electrode diameters of 10 μm and 30 μm , and interelectrode distances of 100 μm or 200 μm .

The flat, round electrodes are made of titanium nitride.

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

Using ThinMEAs

ThinMEAs are heat-stable 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.

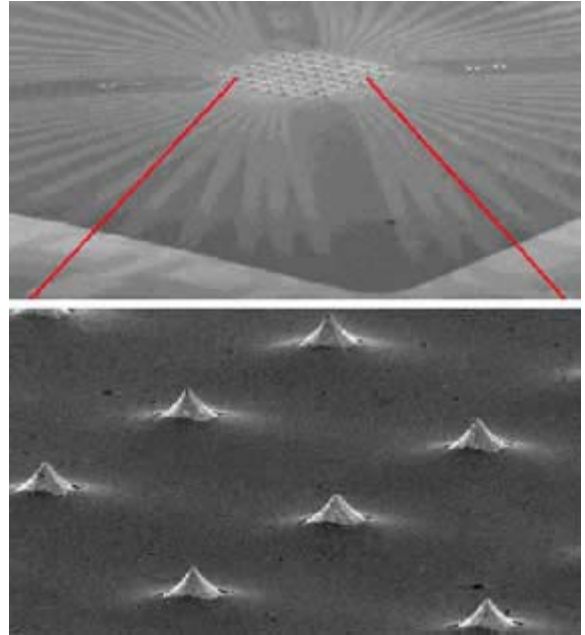
4.6 3-D MEAs

3-D MEAs 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 3-dimensional electrode of a 3-D 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.

The spatial resolution is limited. 60 electrodes are aligned in an 8 x 8 grid with interelectrode distances of 200 μm .

The platinum electrodes are 50 to 70 μm high and have a diameter of about 40 μm at the base, ending in a fine small tip.



Tracks and contact pads are made of platinum; insulation material is SU-8.

3-D MEAs are produced for Multi Channel Systems by Ayanda Biosystems in Lausanne, Switzerland (www.ayanda-biosys.com).

Using 3-D MEAs

Due to the production process, there may be **more variations in the electrode impedance** in comparison with TiN electrodes, which is important for stimulation experiments, especially with current.

3-D MEAs consist of several layers that are glued together. This leads to the fact that these MEAs are very sensitive to distortions and deflections.

A yellow plastic plate is provided to stabilize the form of 3-D MEAs. 3-D MEAs should always be used in combination with these plates.

These MEAs are only stable to temperatures of up to 80 °C; they are not suited for autoclavation because of the high temperature and pressure that is applied during the autoclavation procedure.



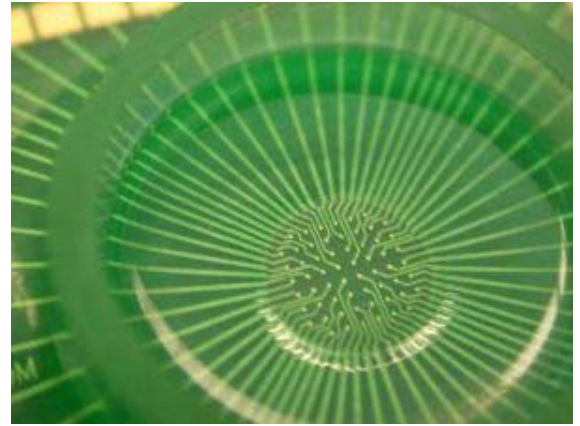
Warning: 3-D MEAs are sensitive to distortions and deflections. Always put the provided **yellow plastic plate** beneath a 3-D MEA before placing it into the MEA amplifier. Do **not** autoclave 3-D MEAs or sterilize 3-D MEAs by **heat**. Avoid rapid temperature changes even if they are within the recommended temperature range. Distortions of the MEA due to mechanic pressure or temperature will lead to bad contacts and irreversibe damage the MEA.

3-D MEAs are generally used for acute slices and therefore do not need to be coated.

When stimulating with 3-D MEA electrodes, please note that the safe charge injection limit of Pt electrodes is much lower than of TiN electrodes. See also [Recommended Stimulus Amplitudes and Durations](#).

4.7 EcoMEAs

EcoMEAs are a very cheap variant for medium throughput applications like small screens where material costs play a bigger role than in more scientific MEA applications. New production processes and the use of new materials made it possible to create this high-quality MEAs at very low prices.



EcoMEAs 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, electrodes of EcoMEAs are available only with a diameter of 100 μm and an interelectrode distance of 700 μm . Thus, ecoMEAs are useful for applications where a high spatial resolution is not important, but which emphasize on cheap consumables. They have proven to be especially useful for recordings from established cardiomyocyte cultures. They are not useful for establishing a new cell culture, as the cell performance cannot be monitored. Multi Channel Systems recommends to use standard 200/30 MEAs for establishing the cell culture first, then switch to EcoMEAs.

Standard EcoMEAs are provided in the typical 8 x 8 layout. 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 EcoMEAs

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

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

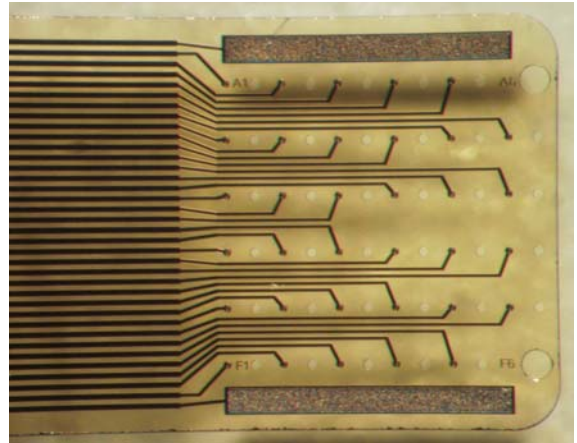
4.8 FlexMEAs

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

FlexMEAs have 32 electrodes plus two indifferential reference electrodes and two ground electrodes. More layouts can be provided on request. The flexible base is perforated for a better contact with the surrounding tissue.

The electrodes have a diameter of about 30 μm with an interelectrode distance of 300 μm .

Conducting material is pure gold.



Using FlexMEAs

FlexMEAs are usually connected to a headstage preamplifier that is connected to a filter amplifier or programmable gain amplifier (see also the ME System product line of Multi Channel Systems). FlexMEAs can be directly connected to a 32-channel miniature preamplifier from Multi Channel Systems for *in vivo* experiments. FlexMEAs are stable at a temperature range from 10 $^{\circ}\text{C}$ to 40 $^{\circ}\text{C}$.



Warning: Do **not** autoclave or sterilize **FlexMEAs** by **heat**. These MEA types are not heat-stable and will be irreversibly damaged if the temperature is too high.

5 MEA Handling



Warning: If possible, use **only** liquids or cleaning solutions with a **neutral pH = 7** on MEAs. Do **not** expose MEAs with a 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: Do **not** to **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 an 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; Heart on a Chip — Extracellular multielectrode recordings from cardiac myocytes *in vitro*, "Methods in Cardiovascular Research", S. Dhein and M. Delmar (eds.) (2004)

5.1.1 Plasma Cleaning

Laboratories with access to electron microscopy facilities are likely to have a sputter device or a plasma-cleaning chamber (for example PDC-32G from [Harrick Plasma](#), Ithaca, NY, United States). MEAs can be treated in these chambers with low-vacuum plasma for about two minutes. 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.

5.1.2 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.

5.1.3 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.

Silicon nitride MEAs with TiN electrodes can be sterilized with standard methods for cell culture materials using either **70 % alcohol**, **UV-light** (about half an hour depending on the intensity), **vapor autoclavation**, or **dry-heat sterilization**.



Warning: Do **not** autoclave or sterilize **3-D MEAs** or **FlexMEAs** by **heat**. These MEA types are not heat-stable and will be irreversibly damaged.

5.2.1 Sterilization with Ethanol and UV Light

1. Rinse MEAs with 70 % ethanol.
2. Let MEAs air-dry over night on a sterile workbench (laminar flow hood) with UV light turned on.

5.2.2 Steam Sterilization (Autoclavation)

→ Autoclave MEAs at 134 °C for 3 min.

5.2.3 Dry Heat Sterilization

- Thermally sterilize MEAs in an oven at 121 °C for 15 min.
- Thermally sterilize **3-D MEAs** in an oven at **56 °C** for 8 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 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

Nitrocellulose solution

- For preparing a stock solution, dissolve a piece of 1 cm² 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

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

Literature

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

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

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 3x 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

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 200/30 MEAs). 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 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 % O₂, 5 % CO₂; or 37 °C, 100 % humidity, 5 % CO₂. To avoid a dry out of the liquid, place the MEA in a big Petri dish with lid on.
3. Rinse 2x with sterile distilled water.
4. Let MEAs air-dry over night 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 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 2x with PBS
3. Plate the cells onto the MEA immediately after coating.

Literature

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

5.4.9 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)
- 0.01 N Hydrochloric acid, pH 3.0
- Acid-soluble type I collagen solution(3mg/ml, pH3.0) Cellmatrix Type I-A ([Nitta Gelatin Inc.](#))

Preparation buffer

- 200 mM HEPES in 0.08 N NaOH

Collagen solution

1. Add 1 ml of 10x DMEM/F-12 medium to 8 ml Cellmatrix Type I-A and stir gently.
2. Add 1 ml of preparation buffer and stir gently.
3. Incubate the mixture at 4 °C for 30 min to remove any air bubbles, if necessary.
4. 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 CO₂ incubator for 30 min.
5. Rinse the MEA with sterile distilled water.
6. Fill the MEA with culture medium and keep it sterile in a CO₂ incubator until use (for up to one week).
7. 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 **pH-neutral cleaning agent**, for example, a standard dish-washing detergent). When cleaning coated MEAs, parts of the coating may go off. You have to recoat an 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.
- EcoMEAs are easier to clean, because the golden electrodes are not so easily damaged.

5.5.2 Cleaning of 3-D MEAs

1. Rinse the culture chamber of the 3-D MEA thoroughly with distilled water.
2. Rinse the 3-D MEA with 70 % ethanol for a few minutes.
3. Rinse the 3-D MEA with distilled water for 1 minute to remove the ethanol.
4. Air-dry the MEA, preferably under a laminar flow hood.

5.5.3 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.

5.5.4 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 2x 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.

5.5.5 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 3x 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 3x.
5. Air-dry the MEA, preferably under a laminar flow hood.

6 Culture Chamber 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 Sealed MEA Culture Dish

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 ALA-MEA-MEM membrane is produced in license by ALA Scientific Instruments Inc., and distributed via the world-wide network of MCS distributors.



The sealed MEA culture chamber with **transparent semipermeable membrane** is suitable for all MEAs with **glass ring**. A hydrophobic semipermeable membrane from Dupont that is selectively permeable to gases (O₂, CO₂), but not to fluid and H₂O 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.

6.2 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.



6.3 Removable Recording Chamber

As an alternative to the fixed culture chambers, you can use silicone rings that adhere to a dry MEA surface and can be removed without leaving any residue. This is especially useful for **acute** experiments.

The removable recording chamber should be stored immersed in distilled water (simply put it into a bottle filled with distilled water) for best adhesion properties.

You dry the silicone chamber with a clean tissue, put it onto the dry MEA (with no rings), and fill the chamber with your recording buffer. Then, you can mount the slice onto the recording field and perform your experiments. After the experiment, you simply remove the chamber from the MEA, and rinse off the slice.



Sources of supply

Product	Product No.	Supplier
flexiPERM conA, Single-Well Removable & Reusable TC Chamber, Non-Toxic Silicon, area: 3.1 cm ² , diameter: 2 cm, volume: 4 ml	96077434	Greiner Bio-One

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



Warning: **3-D MEAs** are sensitive to **distortions** and **deflections**. Always put the provided **yellow plastic plate** first into the MEA amplifier, and then place the 3-D MEA on top of it. Otherwise, the pressure applied by the MEA amplifier will irreversibly damage the MEA.

When placing an MEA into the amplifier, please make sure that the orientation of the MEA is correct. The **writing (NMI)** should be on the **right** side (viewed from the front, with the sockets of the amplifier in the back). (For 3-D MEAs: The writing (**BOT ME60 V4**) should be on the **right bottom**.) Otherwise, the MEA layout will not match with the [pin layout](#).

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 an MEA with **internal reference electrode**, connect the ground to the **reference electrode socket (pin 15)** with the provided connector.

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

7.2 General Performance / Noise Level

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

MEA amplifiers have a maximum noise level of $\pm 8 \mu\text{V}$. 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 electrodes).

The total maximum noise level for an MEA and the amplifier should be about $\pm 40 \mu\text{V}$ peak to peak for $10 \mu\text{m}$ TiN electrodes and $\pm 10 \mu\text{V}$ for $30 \mu\text{m}$ TiN electrodes.

The larger Pt electrodes of the 3-D MEAs generally show a noise level comparable to the $30 \mu\text{m}$ TiN electrodes.

The initial noise level may be higher if the MEAs are hydrophobic. New MEAs should be made hydrophilic before use.

Typical noise level of a used standard 200/30 MEA

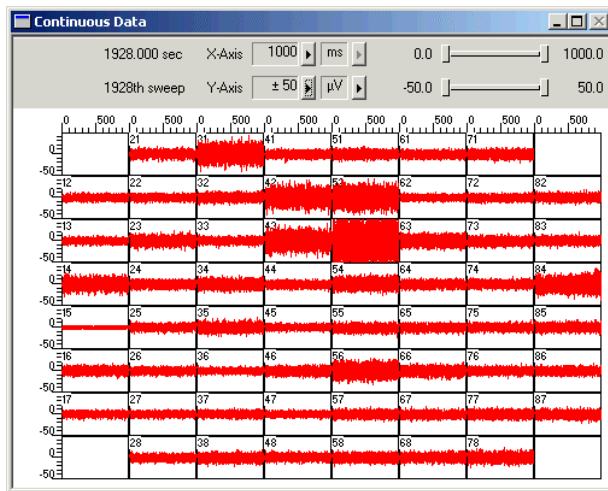


Figure 1 This picture shows the typical noise level of a standard 200/30 MEA on most electrodes, recorded with a MEA1060-BC amplifier. Electrodes 43, 52, 53, and 84 show an increased noise level after a longer cycle of use. The bath was grounded with the internal reference electrode 15. Time axis: 1000 ms, voltage axis: $\pm 50 \mu\text{V}$. You should ground some of the electrodes if you want to use this MEA for recording.

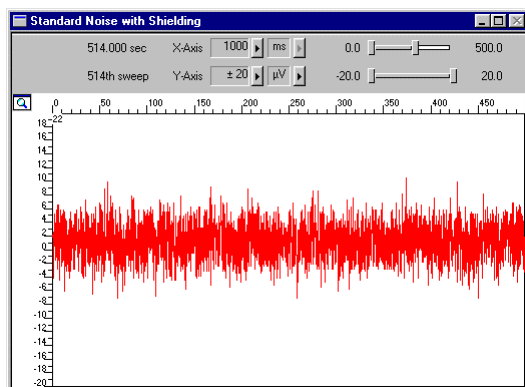
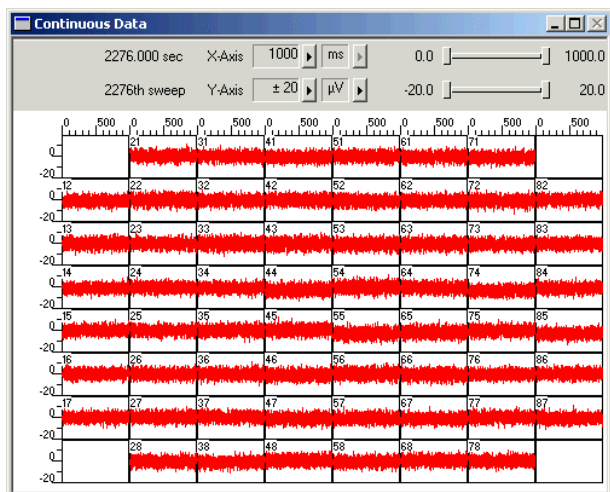


Figure 2 Same MEA, zoom to single channel # 22. Time axis: 500 ms, voltage axis: $\pm 20 \mu\text{V}$.

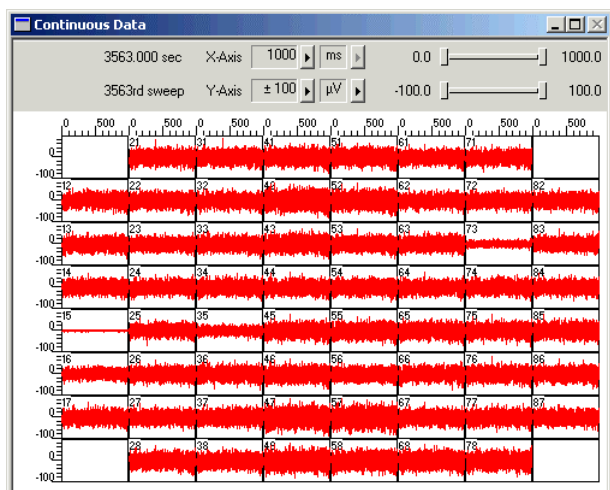
Typical noise level of a new 3-D MEA

Bath grounded with a silver pellet. Time axis: 1000 ms, voltage axis: $\pm 20 \mu\text{V}$.



Typical noise level of a new standard 100/10 MEA

Bath grounded with the internal reference electrode 15. Time axis: 1000 ms, voltage axis: $\pm 100 \mu\text{V}$.



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. Please see the user manual for the respective MEA amplifier and stimulus generator for more details. As an alternative, you can also use special MEAs with four pairs of **large (250x50 μm) stimulating electrodes** (MEA 200/30-stim) and a special stimulation adapter, or target cells with an **external electrode** for stimulation. 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 e. g. 10 Hz. 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 is so low.

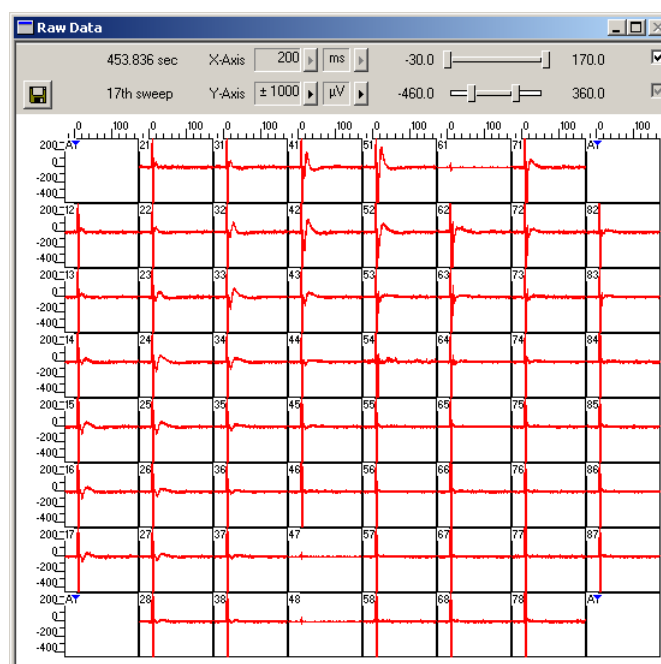
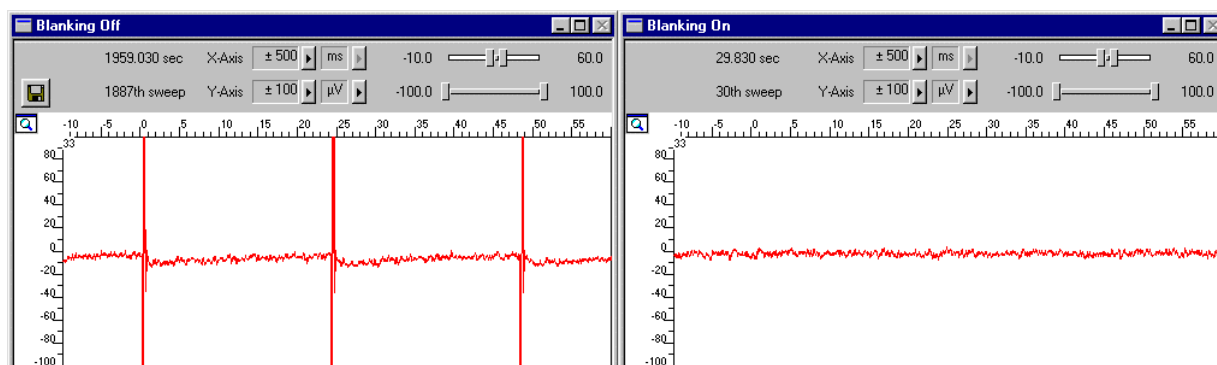


Figure 4 The screen shot shows a prominent stimulus artifact on all channels, followed by a response. The stimulating electrode No. 61 has been grounded.

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.



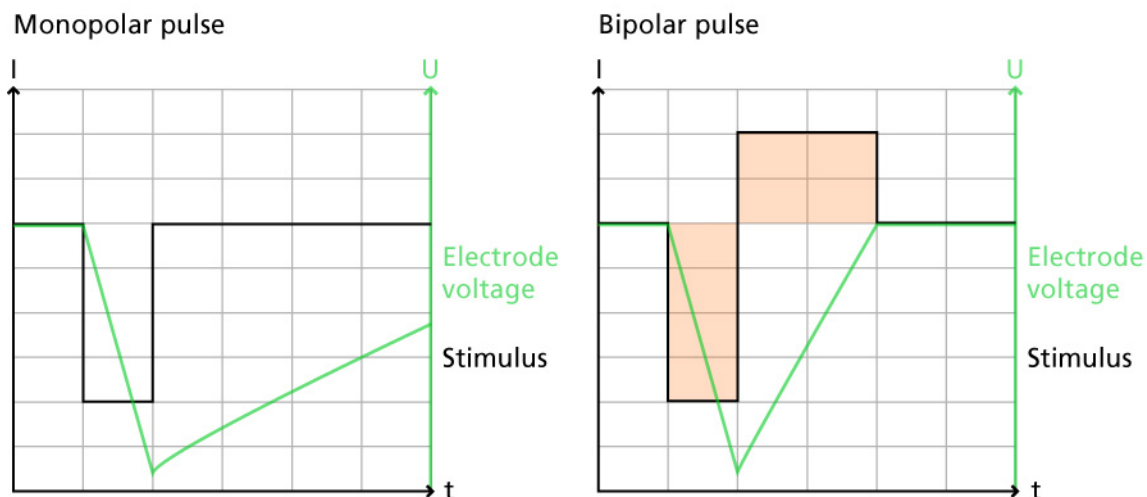
8.2 Capacitive Behavior of Stimulating Electrodes

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 stimulating 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 nitrite (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. For more information on safe charge injection limits of 3-D MEAs, please contact [Ayanda Biosystems](#).

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 **safe-charge injection limits** in the appendix describe the relationship between maximum pulse amplitude and time. The higher the amplitude, the shorter is the maximum duration of the pulse. Do not apply pulses with a higher amplitude or for a longer time than is recommended for the electrode type.

As a consequence of the points discussed above, Multi Channel Systems recommends using **negative monophasic 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 100–500 μ s 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. A special MEA with four pairs of large (250x50 μ m) stimulating electrodes (MEA 200/30-stim) and a special stimulation adapter is provided for such applications by Multi Channel Systems.



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 Safety Advice](#)" on page 7). 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

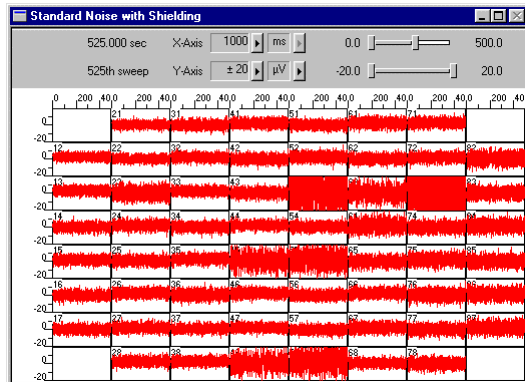
Please read the Troubleshooting part of the user manual first. Most problems are caused by minor handling errors. Contact your local retailer immediately if the cause of trouble remains unclear. Please understand that information on your hardware and software configuration is necessary to analyze and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

The noise level on single electrodes is significantly higher than expected or you see artifact signals. In the following example (200/30 MEA, filled with PBS, silver pellet as bath electrode, shielded), electrodes No. 53, 63, 73, 45, 55, 48, 58 show a high noise level.

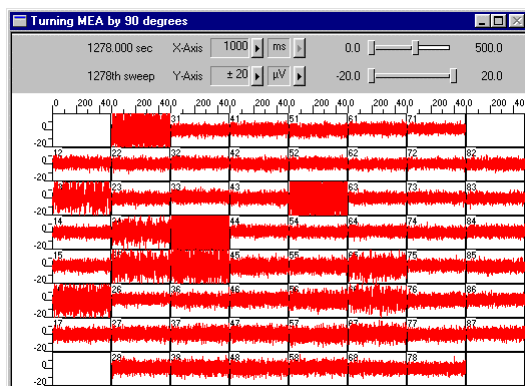


Possible causes:

- ? The electrode or the contact pin of the amplifier may be defective. To test this, do the following.
 1. Open the amplifier and turn the MEA by 90 degrees.
 2. 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°. 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 model test probe to test the amplifier. If the noise level is fine without the MEA, bad MEA electrodes cannot be the cause.

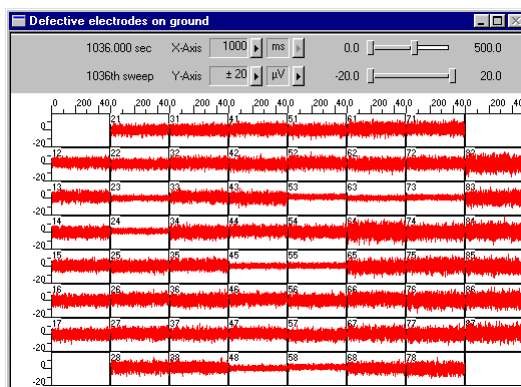
MEA is defective

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.

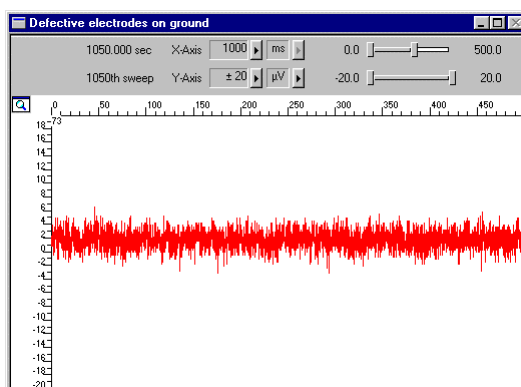
Possible causes:

- ? 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 TiN 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 user 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.



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

Contact pin is defective

Please see the user manual for the respective MEA amplifier.

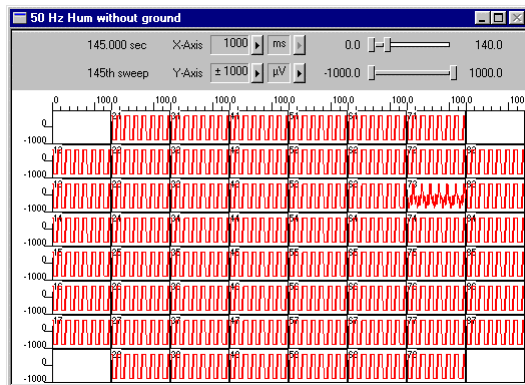
9.4 Overall Noise / Unsteady Baseline

The baseline is unstable, signals are jumping or drifting.

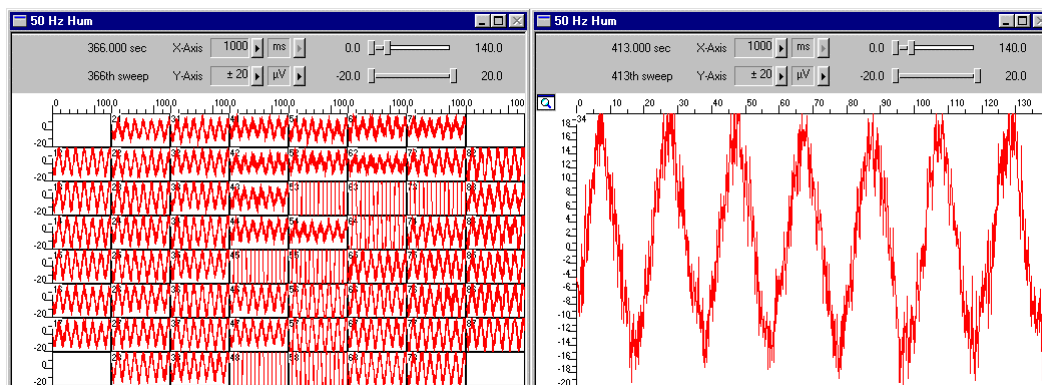
Possible causes:

- ? 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.

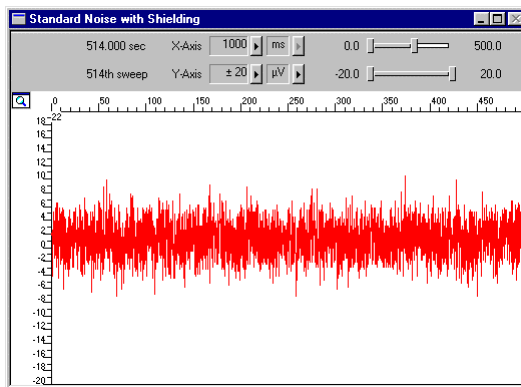
The following screen shot shows a recording of an MEA (200/30) **without bath electrode** and **without shielding**. You see that the signals are so high that the amplifier gets saturated, and you see a very strong 50 Hz hum.



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 neglectible, 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 electrode/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.

User forum

The **Multi Channel Systems User Forum** provides an excellent opportunity for you to exchange your experience or thoughts with other users worldwide.

Mailing list

If you have subscribed to the [NeuroElectronics Mailing List](#), you will be automatically informed about new software releases, upcoming events, and other news on the product line. You can subscribe to the list on the MCS web site.

www.multichannelsystems.com

10.2 Ordering Information

Please see the MEA data sheet for more information about available MEA types. Please contact your local retailer for pricing and ordering information.

10.2.1 MEA Systems

Product	Product Number	Description
MEA recording system for inverted microscopes, 60 electrode channels	MEA60-1System	Complete with 5 MEAs, data acquisition computer with MC_Card and IPS10W, MEA1060-1 amplifier, TC01, ALA MEA-PPORT2, and accessories
MEA recording system for upright microscopes, 60 electrode channels	MEA60-2System	Complete with 5 MEAs, data acquisition computer with MC_Card and IPS10W, MEA1060-2 amplifier, TC01, ALA MEA-PPORT2, and accessories
MEA recording system for inverted microscopes with advanced perfusion, 60 electrode channels	MEA60-1SystemE	Complete with 5 MEAs, data acquisition computer with MC_Card and IPS10W, MEA1060-1 amplifier, TC02, PH01, ALA MEA-PPORT2, and accessories
MEA recording system for upright microscopes with advanced perfusion, 60 electrode channels	MEA60-2SystemE	Complete with 5 MEAs, data acquisition computer with MC_Card and IPS10W, MEA1060-2 amplifier, TC02, PH01, ALA MEA-PPORT2, and accessories
MEA recording system for inverted microscopes, 120 electrode channels	MEA120-1System	Complete with 5 MEAs, data acquisition computer with MC_Card and IPS10W, 2 x MEA1060-1 amplifier, TC02, ALA MEA-PPORT2, and accessories
MEA recording system for upright microscopes, 120 electrode channels	MEA120-2System	Complete with 5 MEAs, data acquisition computer with MC_Card and IPS10W, 2 x MEA1060-2 amplifier, TC02, ALA MEA-PPORT2, and accessories

10.2.2 MEA Amplifiers

Product	Product Number	Description
MEA amplifier for inverted microscopes	MEA1060-1	Probe interface and 60 channel pre- and filter amplifier with custom gain and bandwidth
MEA amplifier for upright microscopes	MEA1060-2	
MEA amplifier with blanking circuit for inverted microscopes	MEA1060-1BC	Probe interface and 60 channel pre- and filter amplifier with custom gain and bandwidth. The blanking circuit prevents the amplifier from getting saturated and thus prevents stimulus artifacts.
MEA amplifier with blanking circuit for upright microscopes	MEA1060-2BC	

10.2.3 Accessories

Product	Product Number	Description
MEA culture chamber	CCIR	Suitable for all MEAs with plastic ring and thread. Simply screw the culture chamber onto the plastic holder on the MEA. An o-ring ensures that the chamber fits tightly and is leakproof. Autoclavable. Complete with lid.
MEA culture chamber lid	CCL	Additional or replacement lid for MEA culture chamber.
Sealed MEA culture dish	ALA MEA-MEM	MEA culture chamber with transparent semipermeable membrane suitable for all MEAs with glass ring. Simply slide the culture chamber over the glass ring. A hydrophobic semipermeable foil from Dupont that is selectively permeable to gases (O ₂ , CO ₂), but not to fluid, 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. Autoclavable. Comes complete with membranes.
	ALA MEA-SHEET	Set of 10 membranes for sealed MEA culture dishes.
	ALA MEA-MEM5	Set of 5 membranes and MEA-MEM-TOOL for sealed MEA culture dishes.
	ALA MEA-MEM-TOOL	Tool for smoothing and positioning the membrane on the ALA MEA-MEM culture chamber, so that the membrane is neat and flat on top of the culture chamber.
MEA perfusion insert	ALA MEA-INSERT	

MEA Microelectrode Array



MEA type	TiN electrodes, SiN isolator, and Ti contact pads 100/10-Ti, 200/10-Ti, 200/10iR-Ti, 200/30-Ti, 200/30iR-Ti, 200/30-Ti-stim with 8 stimulation electrodes, 500/10iR-Ti, 500/30iR-Ti, HexaMEA-Ti (10, 20, 30)	TiN electrodes, SiN isolator, and ITO contact pads 100/10-ITO, 200/30iR-ITO, HexaMEA-ITO (10, 20, 30)	ThinMEA ThinMEA (180 µm) 100/10-ITO, ThinMEA (180 µm) 200/30iR-ITO	3-D MEA-gr	EcoMEA	FlexMEA
Temperature compatibility	0 °C to 125 °C	0 °C to 125 °C	0 °C to 125 °C	0 °C to 80 °C	0 °C to 125 °C	10 °C to 40 °C
Dimensions (W x D x H)	49 mm x 49 mm x 1 mm	49 mm x 49 mm x 1 mm	49 mm x 49 mm x 180 µm (glass part) 8 g	49 mm x 49 mm x 1 mm	49 mm x 49 mm x 1 mm	12 µm height
Weight	8 g	8 g	8 g	8 g	8 g	< 1 g
Base material	Glass	Glass	Glass (on a ceramic carrier)	Glass	Gold	Polyimid 2611
Contact pad and track material	Titanium	Indium tin oxide	Titanium	Platinum	Gold	Gold
Electrode diameter	As specified; 10, 20, and 30 µm available	As specified; 10, 20, and 30 µm available	As specified; 10 and 30 µm available	40 µm (at base)	100 µm	30 µm
Interelectrode distance	As specified; 100, 200, and 500 µm available; not applicable to Hexa (10, 20, 30) MEA layout	As specified; 100 and 200 µm available; not applicable to Hexa (10, 20, 30) ITO MEA layout	As specified; 100 and 200 µm available	200 µm	700 µm	200 µm (16 electrode version), 300 µm (36 electrode version), 600 to 700 µm (72 electrode version)
Electrode height	Planar	Planar	Planar	Approx. 50 µm to 70 µm	Planar	Planar
Electrode type	Titanium nitride	Titanium nitride	Titanium nitride	Platinum	Gold	Titanium nitride
Isolation type	Silicon nitride 500 nm (PEVCD)	Silicon nitride 500 nm (PEVCD)	Silicon nitride 500 nm (PEVCD)	SU-8		
Electrode impedance	30 kΩ to 400 kΩ, depending on electrode type and diameter	30 kΩ to 400 kΩ, depending on electrode type and diameter	30 kΩ to 400 kΩ, depending on electrode type and diameter	approx. 400 kΩ (range from 250 to 500)	approx. 30 kΩ	approx. 50 kΩ
Electrode layout grid	8 x 8 (100/10, 200/10, 200/10, 200/30, 200/30 i. r.), 6 x 10 (500/10 i. r., 500/30 i. r.), hexagonal (Hexa (10, 20, 30))	8 x 8 (100/10, 200/30 i. r.), hexagonal (Hexa (10, 20, 30))	8 x 8	8 x 8	8 x 8	4 x 4 (14 electrode version), 6 x 6 (32 electrode version), 9 x 8 (72 electrode version)
Number of electrodes	60 i. r. = with internal reference electrode Optional: with 8 stimulating electrodes	60 i. r. = with internal reference electrode	60 i. r. = with internal reference electrode	60	60	Versions with 14, 32, and 72 electrodes available 14 and 32 electrode versions with 2 reference electrodes 32 electrode version with 2 ground electrodes

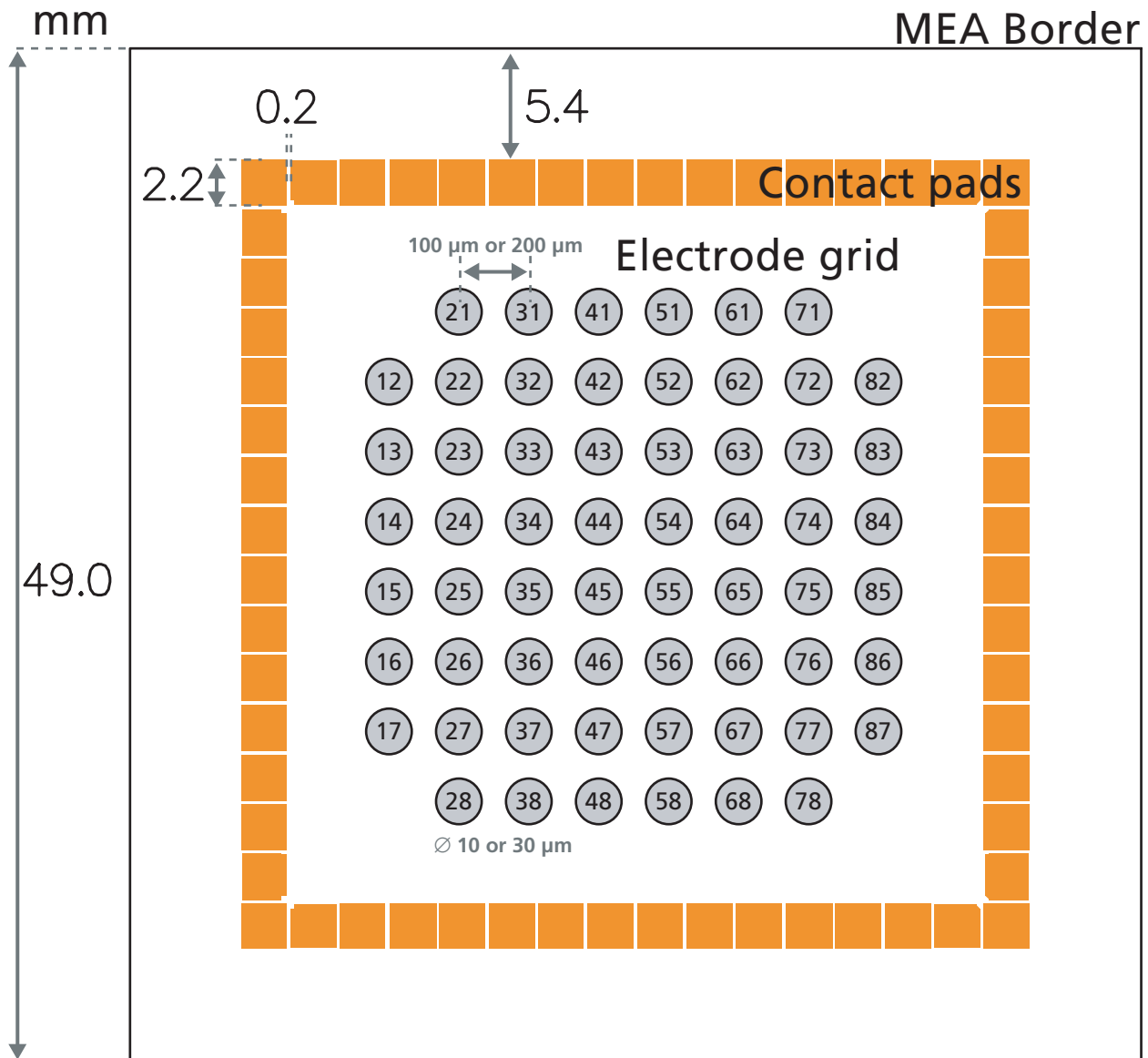
The product type specifies the interelectrode distance and the electrode diameter, that is, 100/10 means 100 µm interelectrode distance and 10 µm electrode diameter.

Culture chambers for MEAs

	Glass ring (-gr)	Plastic ring without thread for a lid (-pr)	Plastic ring with thread for a removable lid (-pr-T)	No ring for custom use (-w/o)
Inner diameter (ID)	20 mm	26 mm	26 mm	-
Outer diameter (OD)	24 mm	30 mm	30 mm	-
Height	6 mm	6 mm	6 mm	-
			with screw thread for lid	

Standard MEA Layout (8x8)

Standard MEAs (with TiN electrodes) in the 8x8 grid are available in versions **MEA 100/10**, **200/10 (i. r.)**, or **200/30 (i. r.)**; with **optional ITO tracks**. Dimensions refer to standard MEAs. (**ThinMEAs**, **3-D MEAs**, and **EcoMEAs** also follow the 8x8 grid. For other specifications like electrode material, diameter, and spacing, please refer to the MEA data sheet.)



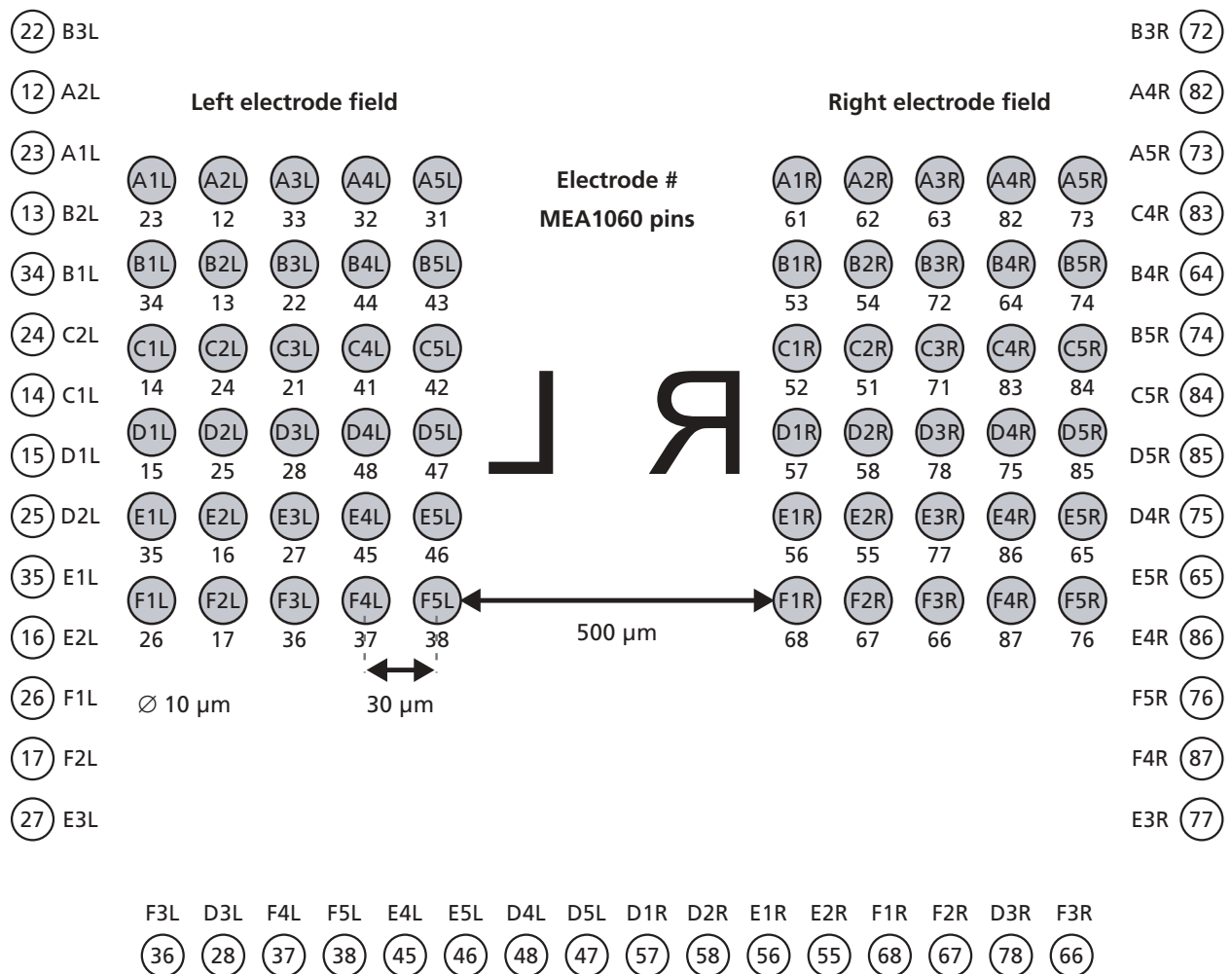
The numbering of MEA electrodes in the 8x8 grid follows the standard numbering scheme for square grids: The **first digit** is the **column number**, and the **second digit** is the **row number**. For example, electrode 23 is positioned in the third row of the second column.

These numbers are the same numbers that are used as channel numbers in the **MC_Rack** program. Please make sure that you have selected the **two-dimensional MEA layout** as the **Channel Layout** in **MC_Rack**. For more details, please refer to the **MC_Rack** user manual or help.

HighDenseMEA

Electrode layout

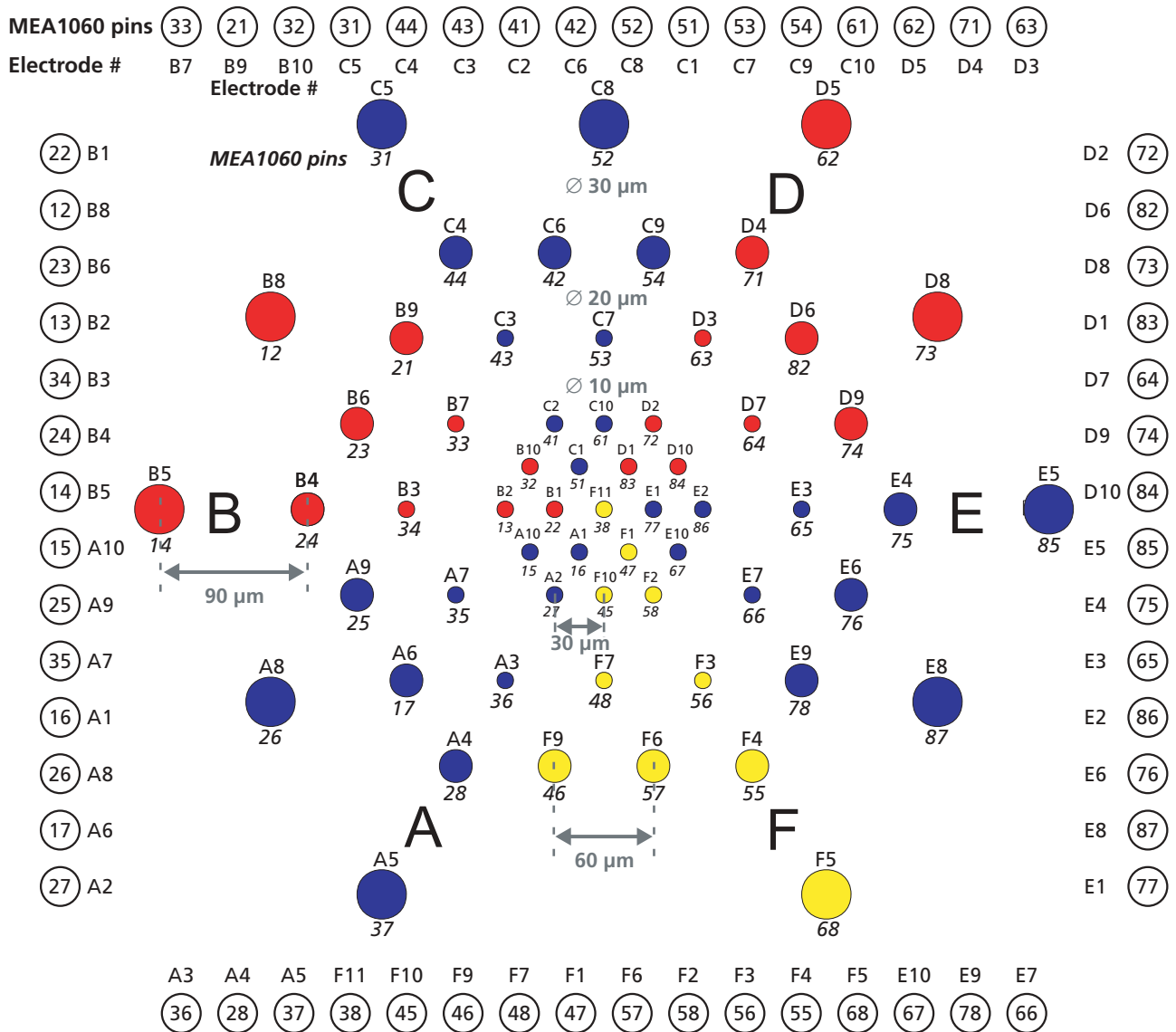
MEA1060 pins (33) (21) (32) (31) (44) (43) (41) (42) (52) (51) (53) (54) (61) (62) (71) (63)
Electrode # A3L C3L A4L A5L B4L B5L C4L C5L C1R C2R B1R B2R A1R A2R C3R A3R



The first letter of the electrode number code refers to the row number, the digit is the column number, and the second letter refers to the electrode field (left or right) of the HighDenseMEA. The specified MEA1060 pin numbers are the MEA System channel numbers that are used in the MC_Rack program.

HexaMEA

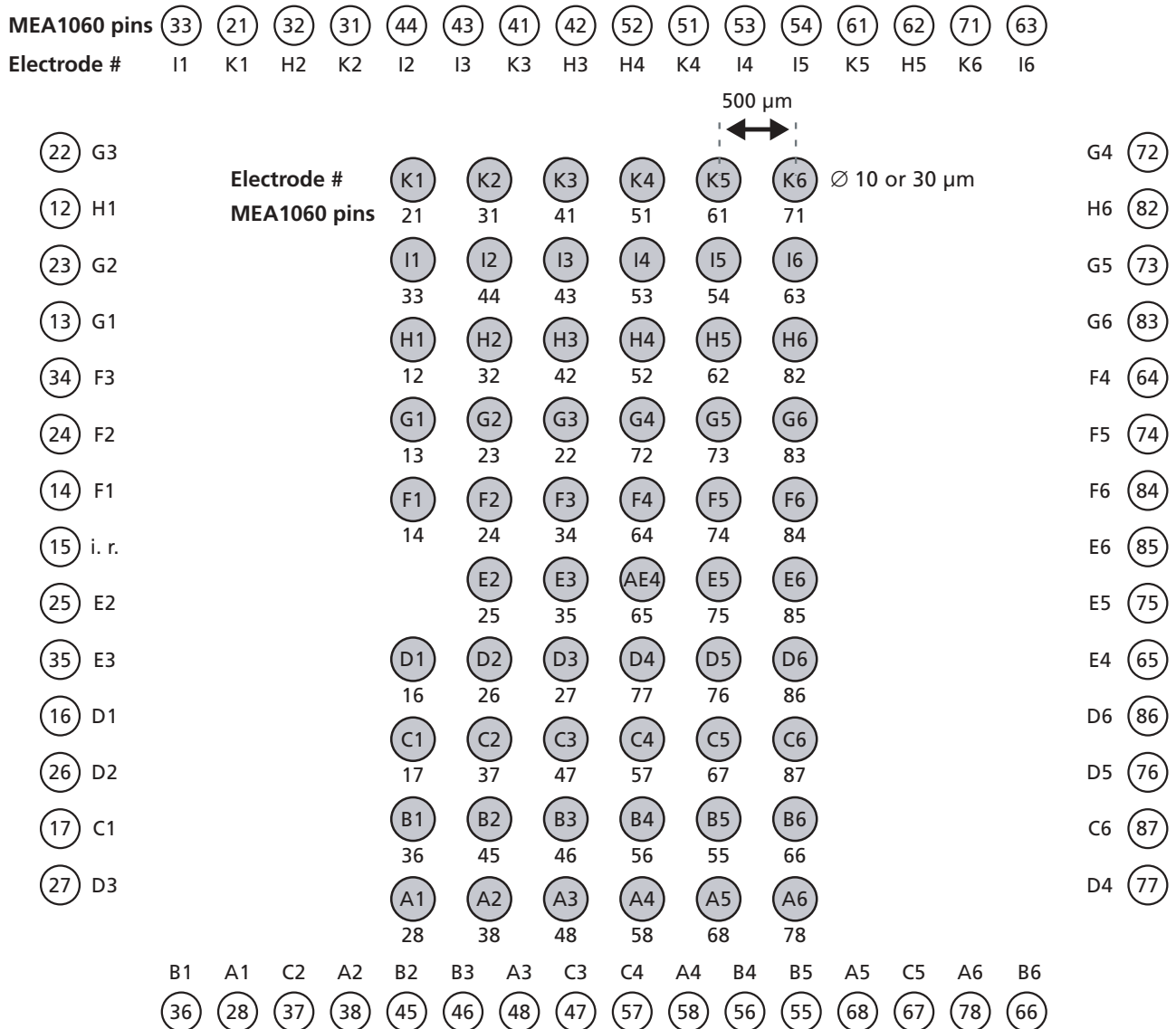
Electrode layout



The letter-digit code is the electrode identifier and refers to the position of the electrode in the hexa grid. The specified MEA1060 amplifier pin numbers are the MEA System channel numbers that are used in the MC_Rack program.

MEA 500/10 i. r., 500/30 i. r.

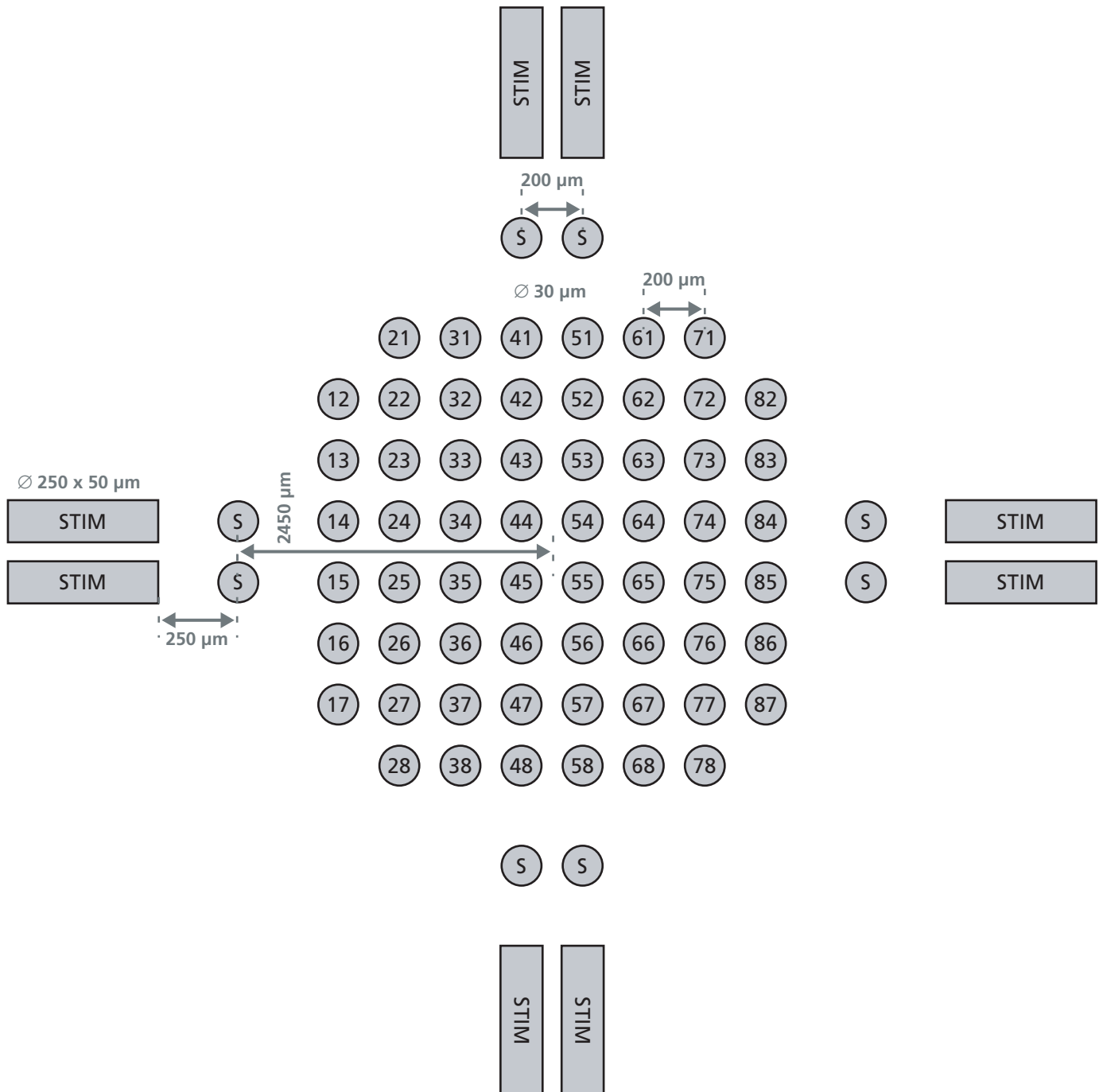
Electrode layout



The letter of the electrode number code refers to the row number, and the digit is the column number. The specified MEA1060 amplifier pin numbers are the MEA System channel numbers that are used in the MC_Rack program. The substrate-integrated reference electrode (i. r.) is connected to pin 15 of the MEA1060 amplifier.

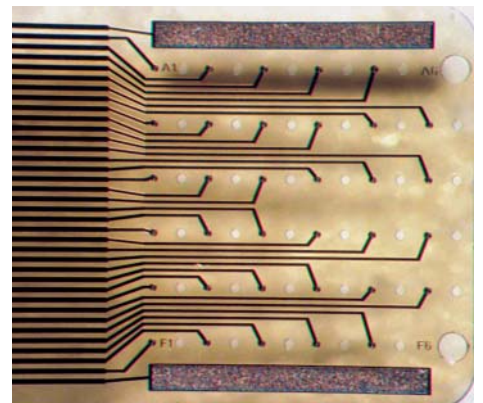
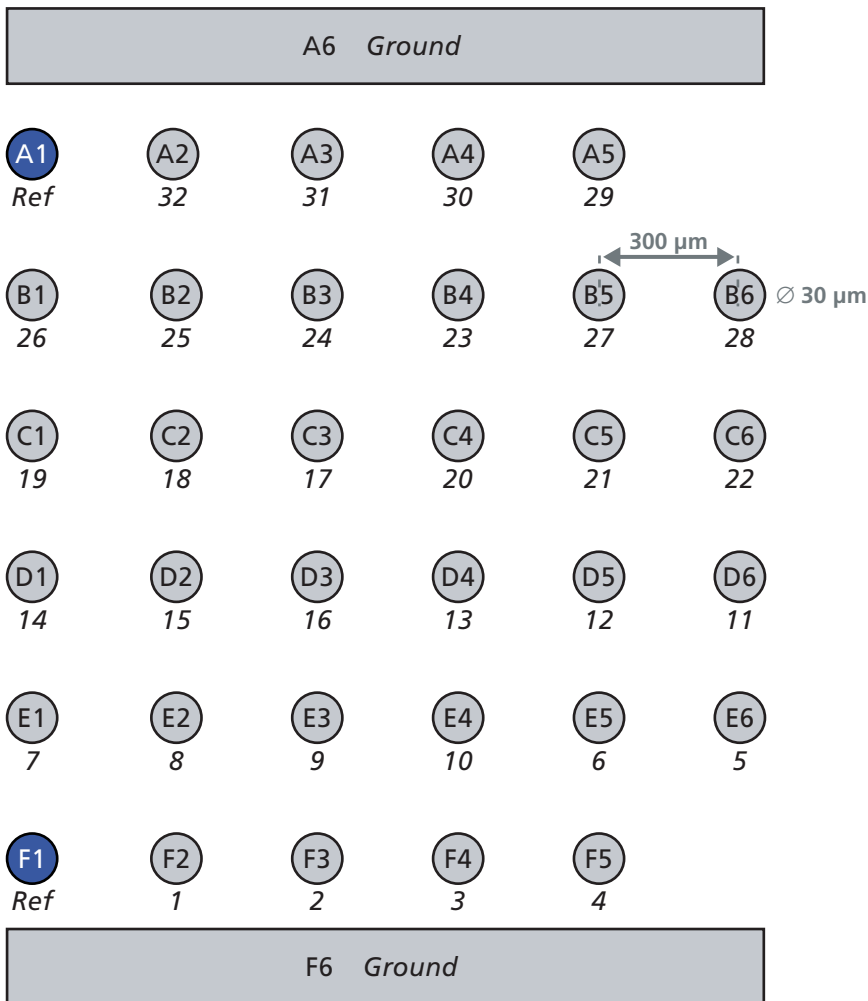
MEA-200/30-Stim

with 16 additional stimulating electrodes (8 à 30 µm, 8 à 250 x 50 µm)



FlexMEA

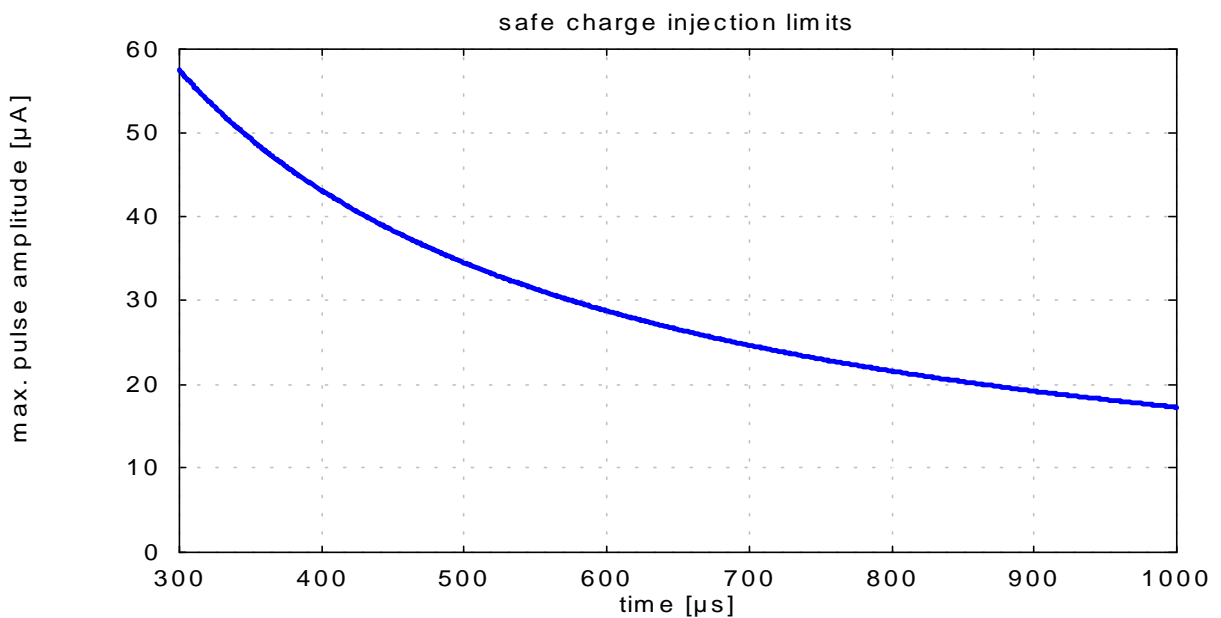
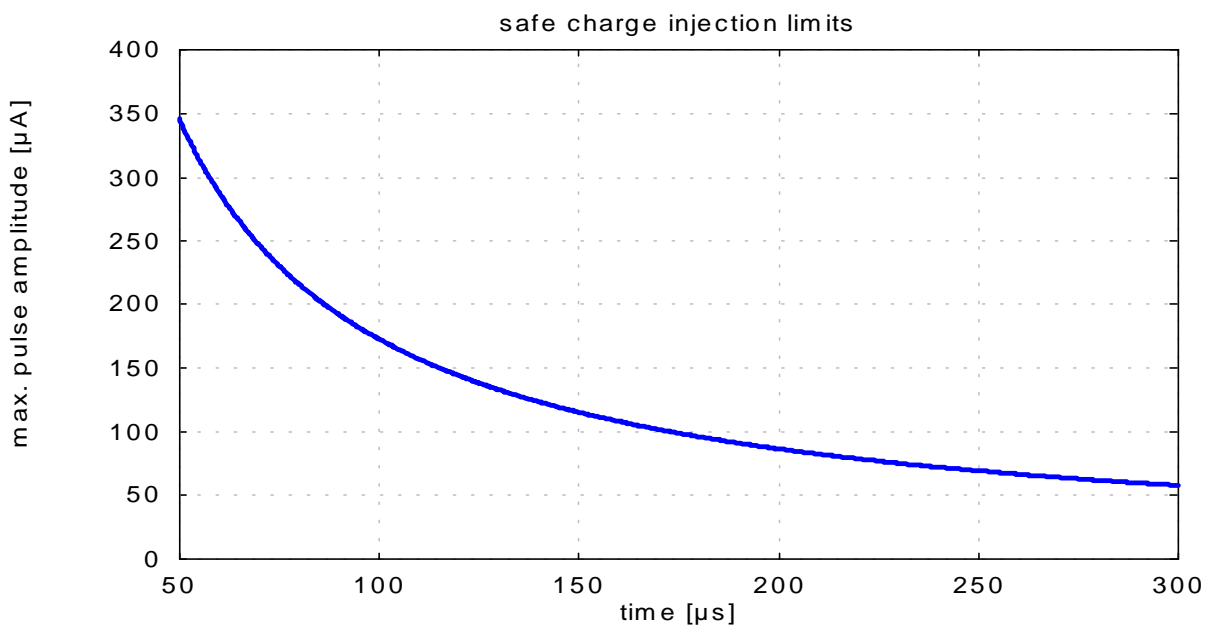
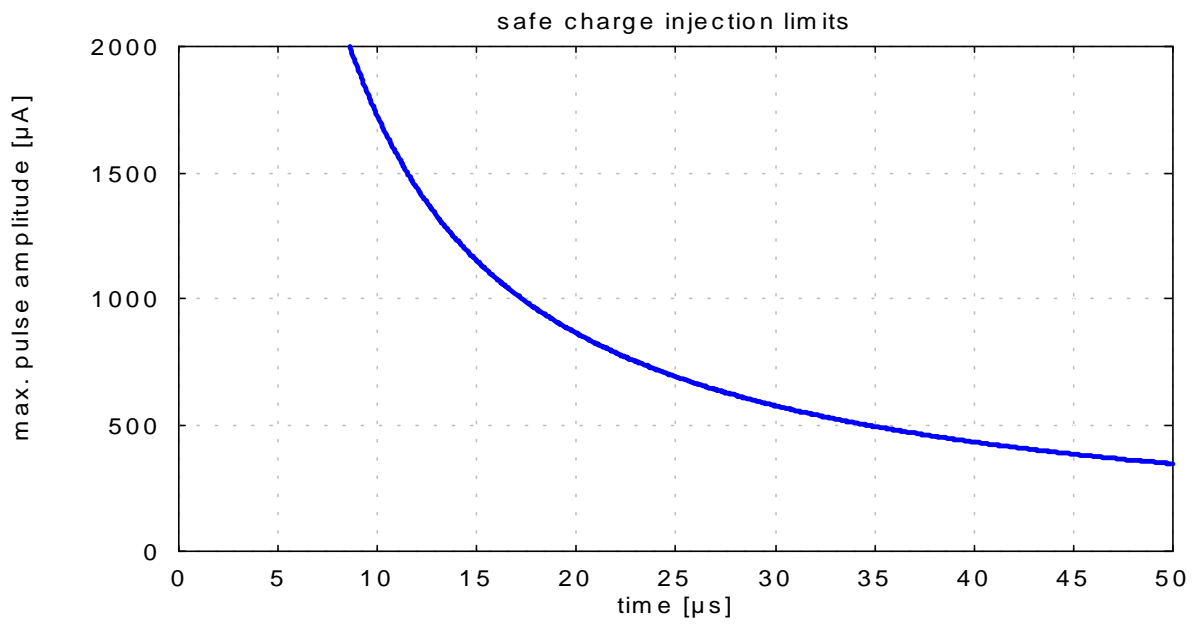
**36 Electrodes: 32 recording electrodes, 2 indifferent reference electrodes,
2 large ground electrodes; for use with miniature preamplifier MPA32I-FLEX**



The letter-digit code is the electrode identifier and refers to the position of the electrode in the grid. Below are the recording channel numbers (in italics) that refer to the channel numbers in the MC_Rack program. Please make sure that you have selected the linear channel layout in the MC_Rack program. See the MC_Rack user manual or help for details.

If you use more than one MPA32I-FLEX and a ME64 System, the signal collector SC2x32 leads the output channels of the second amplifier to channel number 33-64. Please see the SC2x32 data sheet for details.

Safe Charge Injection Limits of Multi Electrode Arrays with TiN Electrodes (diameter: 10 μ m)



Safe Charge Injection Limits of Multi Electrode Arrays with TiN Electrodes (diameter: 30 μ m)

