MicroElectrodeDevices

MEA60 Biochips

User Manual

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1 Before you start

1.1 Warning

The Micro-Electorde Array (MEA) biochips are only to be used as a specimen slide in combination with the MEA60, the MEA2100-System, and the MEA2100-Mini-System data acquisition system from Multi Channel Systems MCS GmbH, Reutlingen, Germany (http://www.multichannelsystems.com).

Proper use of the MEA60 Biochips includes the observance of all instructions presented in this user manual and adherence to the device, inspection and maintenance descriptions.

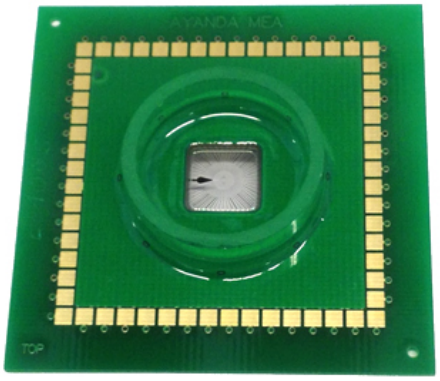
Any use outside of the scope of the instructions presented in this manual are considered to be improper. Any liability for any damages which may result from misuse or due to procedural faults will not be accepted by the product manufacturer.

1.2 Short description

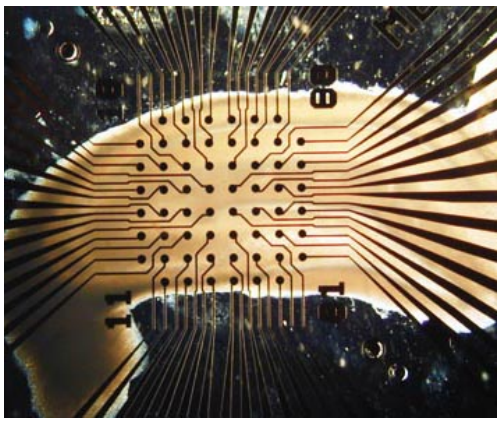
The MEA60 biochips are 60-channel specimen slides used for extraction of information from electrically active tissue slices and cell cultures, and have been adapted to the commercially available signal amplification and data acquisition systems from Multi Channel Systems MCS GmbH, Reutlingen, Germany.

It is made of a shielded printed circuit board including chip and wire connections. This device has to be placed into the MCS amplifier interface (see procedure in the next chapter).

Picture of a MEA60 biochip.
The global dimensions are 49mm x 49mm.



Picture of a typical hippocampus acute tissue slice preparation on the MEA60 biochip workspace.

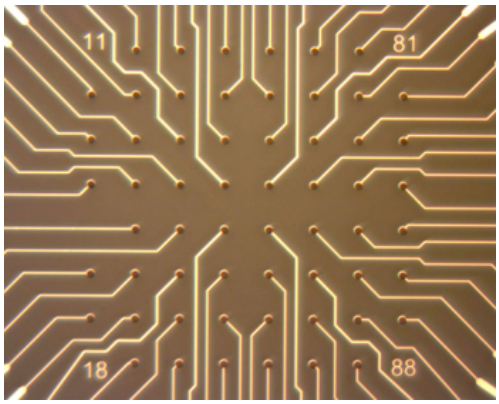


The MEA60 biochips are produced on a transparent substrate (dimension: 12,5mm x 12,5mm or 21mm x 21mm, thickness: 0.7mm) and mounted onto a printed circuit board, which fits into the MCS data acquisition systems. A glass ring that is sealed using a biocompatible epoxy is used to create a culture chamber.
The micro-electrode array chip is fabricated on a glass plate using standard micro-fabrication technologies. Thin-film platinum or transparent indium-tin oxide (ITO) electrodes are insulated using an SU-8 epoxy layer.
The standard electrode arrangement employed is an 8x8 matrix of ø10μm or ø30μm metallic electrodes with an interspacing of 100μm or 200μm (centre to centre), building a workspace about two square millimetres in the centre of the biochip. Different electrode geometries are available:

- **Planar MEA60 Biochips**

The planar MEA60 biochips, including platinum or indium-tin oxide electrodes, are mainly used for dissociated cell culture and organotypic tissue culture experimentation.

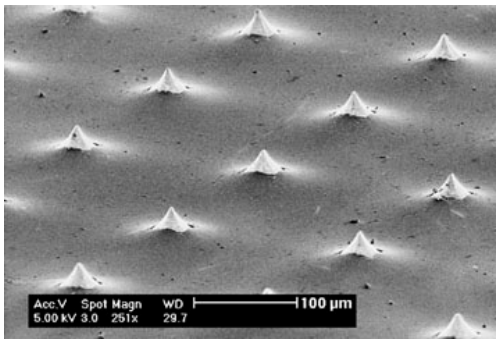
Picture of the workspace of a MEA60 biochip with a 8x8 electrode matrix, spacing 200μm, electrode diameter 30μm, electrode material made of platinum black.



- **3D MEA60 Biochips**

Planar electrodes present disadvantages for acute tissue slice experimentation. The outermost layers of cells of the slices are damaged during sample preparation. When acute tissue slices are placed onto planar electrodes, it is difficult to record its electrical activity due to the distance between the active cells within the tissue and the recording electrodes.
The best way to overcome this problem is the use of three-dimensional tip-shaped electrodes. By using 3D electrodes, it is possible to monitor the electrical activity of the tissue directly after placing it onto the electrodes. The geometry of the electrodes improves the stimulation and recording conditions by reducing the distance between the active cells and the electrodes (see Heuschkel, M et al., J. Neurosci. Meth., **114**:135-148, 2002 for more information).

SEM picture of several 3D tip-shaped electrodes. The tip-shaped electrodes have a height between 50μm and 70μm.



1.3 Scope of delivery

Before the MEA60 biochips can be properly installed at the customer's premises, the delivered parts must be checked against the order processing form for completeness.
MEA60 biochips
MEA specification sheet

- **MEA60 Biochip**
- **MEA spicification sheet**
- **MEA60 Biochip User Manual**

2 Proper MEA60 Biochip handling

2.1 How to avoid MEA60 biochip damage

Described below is a non-exhaustive list of possible sources for MEA60 biochip damage:

- **Common MEA60 biochip damage sources**

Do not touch the recording workspace of the MEA60 biochip with hard objects. This will avoid electrode or insulation layer damages, which will influence the global electrode characteristics.

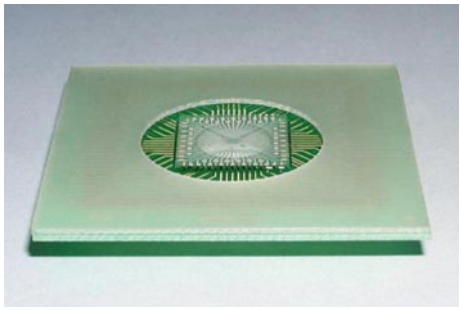
Do not use acetone of acids to clean the MEA60 biochip. The materials used to manufacture the MEA60 biochips may bel be damaged if exposed to acetone or acids.

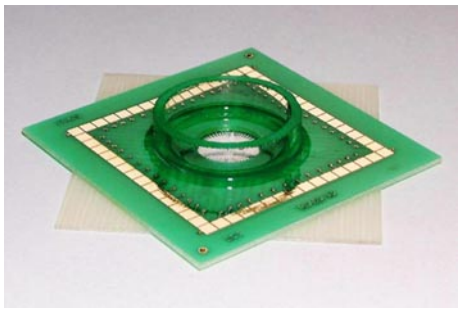
Do not heat the MEA60 biochip above 80°C and avoid any thermal chocks if heated. This will avoid stress between the different parts building the MEA60 biochip, which often induces damages to the electrical contacts between the glass chip and the printed circuit board.

Do not store the MEA60 biochips in fridge or refrigerator. This will result in delamination of the electrode insulation layer for the chip, damaging irreversibly the device. Best storage is obtained at room temperature.

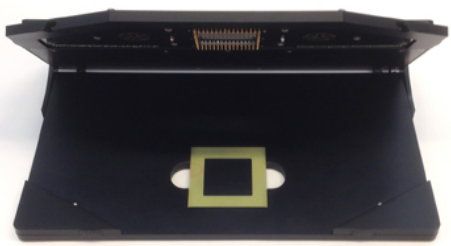
- **Avoid torsion of the MEA60 biochip**

Use a spacer in order to avoid damaging the MEA60 biochip during cell or tissue culture preparation and observation.
Due to the MEA60 biochip fabrication concept, the glass chip including the recording electrodes protrudes under the printed circuit board interface.
Do not generate any torsion of the MEA60 biochip. It may break the electrical contacts between the glass chip and the printed circuit board, damaging irreversibly the MEA60 biochip.
Usually, one or several spacers are provided with each MEA60 Biochip purchase order delivery. The spacer has to be placed under the MEA60 biochip as shown in the pictures on the right side. Note that the shape and material may differ from illustrated spacer.





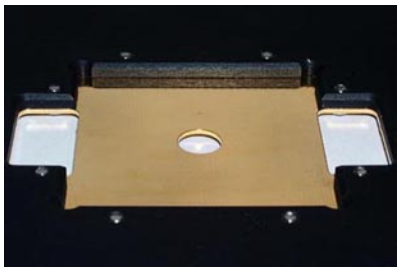
Use a spacer also to avoid damaging the MEA60 biochip during experimentation with the MEA60 system's interface from MCS.
Please, place the spacer into the amplifier interface before introducing the MEA60 biochip. Otherwise, the contact pins of the interface may break the electrical contacts between the glass chip and the printed circuit board, damaging irreversibly the MEA60 biochip.



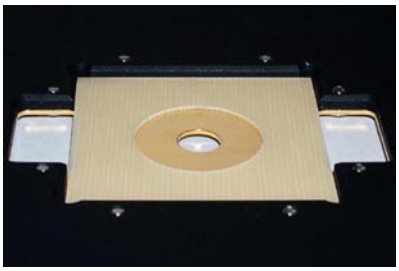
- **How to use MEA60 biochips in the MEA60 amplifier interface**

Find below the procedure to conduct an experiment using the MEA60 biochips:

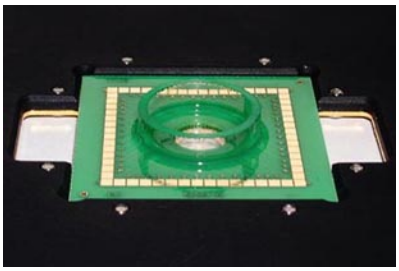
- First, clean the electrical pads of the MEA60 biochip with methanol or isopropanol in order to obtain a good electrical contact.
- A spacer is necessary to prevent irreversible damage of the MEA60 biochip while placed in the amplifier. Place the spacer at the bottom of the amplifier interface before placing the MEA biochip on top (see the pictures below).
- Place the MEA into the amplifier interface and verify that the text on the MEA chip “AYANDA MEA” is located at the top.



Amplifier interface



Place first a spacer



Place the MEA60 biochip on top

3 MEA60 Biochip Preparation

3.1 MEA60 biochip surface coatings

NOTE
The use of a coating on the MEA60 biochip surface may enhance cell or tissue adhesion. However, the use of a specific coating depends on the particular experiment to be carried out and will not always be necessary.

Several MEA surface coatings can be used with the MEA60 biochips:

- **Nitrocellulose**

Prepare a stock solution of a piece of 1cm² nitrate filter dissolved in 10ml methanol. Just prior to MEA60 biochip use, put 3-5μl of this solution onto the array and allow to dry (it takes a few seconds for the methanol to evaporate). This leaves patches of cellulose nitrate on the MEA-surface which serve as the glue for the tissue to be mounted.
After usage, biological material should be rinsed off under running tap water. The MEA60 biochip is then submersed in a hypochloride solution and cleaned for 15min in an ultrasonic bath. Afterwards, methanol is applied for 15 to 30min in order to dissolve the cellulose nitrate. The MEA60 biochip is then rinsed with distilled water. Best storage conditions before MEA60 biochip re-use should be in distilled water.

- **Polyethylenimine (SIGMA Cat N° P-3143)**

Polyethylenimine (PEI) has been successfully employed for dissociated cell cultures and proven to enhance cell maturation in culture compared to polylysine-coated plates (Lelong et al., J. Neurosci. Res., 32:562-568, 1992). PEI-coating has also been successfully employed with acute tissue slices (Heuschkel et al., J. Neurosci. Meth., 114:135-148, 2002).
Dissociated cell cultures: Prepare PEI stock solution (1g of SIGMA liquid PEI diluted in 10ml of de-ionised water). Dilute the stock PEI solution 1/10.000 times (1/100 and 1/100). Coat the MEA at room temperature for at least 2hours. Wash three times with de-ionised water and let it dry in the hood. Plate the neurons in their culture medium at the desired density (see also Bledi et al., Brain Research Protocols, 5:282-289, 2000).
Acute tissue slices: Prepare PEI stock solution (1g of SIGMA liquid PEI diluted in 10ml of de-ionised water). Dilute the stock PEI solution 1/100 times. Coat the MEA at room temperature for at least 2hours. Wash three times with de-ionised water and let it air dry.

- **Matrigel (Growth Factor Reduced BD Matrigel™ Matrix, Becton Dickinson, Cat N° 356230)**

Prepare a diluted 1:50 Matrigel solution in medium without serum, make aliquots and freeze at -20°C. Before use, warm up the aliquot (not higher than 4°C). Put about 250μl Matrigel solution on the MEA60 biochip and leave for 1hour at room temperature. Remove excess Matrigel solution and rinse once with medium and two times with distilled water. Finally, put the MEA 1-2 hours under a UV lamp. Treated MEA60 biochips could be kept for at least two days in incubator before cell plating. Plate the neurons in their culture medium at the desired density.

- **Polyornithine/laminine (polyornithine SIGMA Cat N° P-8638, mouse laminin Becton-Dickinson Cat N° 354232, Leibovitz L-15 medium GIBCO Cat N° 31415-029)**

Prepare polyornithine stock solution at 1mg/ml in H_2O and use laminin stock solution between 0.5 -1mg/ml provided by BD. Incubate the MEA with polyornithine (1μg/ml final; 1/1000 dilution in H_2O) between 2h-12h at 37°C in CO_2 incubator. Remove the polyornithine. Incubate the MEA with laminin (1μg/ml final; dilution in Leibovitz L-15 buffered with bicarbonates) between 2h-12h at 37°C in CO_2 incubator. Remove the laminine (don't let it dry!!!!) and plate the neurons in their culture medium at the desired density.

3.2 MEA60 biochip sterilisation

Sterilisation of the MEA biochip has to be done prior to cell or tissue culture preparation. For acute tissue slice experimentation, sterilisation can be omitted.

WARNING
Do not autoclave the MEA60 biochips. The MEA60 biochip does not resist temperatures above 80°C. Do not expose MEA biochips to prolonged UV light exposure.

Sterilisation should be realised using:

- **Rinse with methanol**
- **Thermal treatment in an oven: 8hours or over night at 60°C.**

3.3 MEA60 biochip cleaning procedures

WARNING
Do not use acetone to clean the MEA60 biochip.
Do not touch the workspace of the MEA60 biochip with hard material.
Do not use a mechanical cleaning method.
Do not expose MEA biochips to UV light.

Find below a cleaning method that is recommended for use with the MEA biochips:

- Rinse the inside chamber of the biochip thoroughly with distilled water.
- Rinse the MEA60 biochip with 70% ethanol for a few minutes.
- Rinse the MEA60 biochip with distilled water for 1min to remove the ethanol.
- Air-dry the MEA60 biochip, preferably under a laminar flow hood.

Another recommended cleaning method for MEA60 Biochips:

- Dissolve one drop of Tween80 (Sigma Ref. P8074) in 100ml distilled water.
- Fill MEA60 Biochip chamber with this Tween solution and leave for 2hours.
- Clean the electrode workspace using a soft brush (don't scratch the biochip surface !).
- Rinse the inside chamber of the biochip thoroughly with distilled water.
- Air-dry the MEA60 biochip, preferably under a laminar flow hood.

It is not possible to clean perfectly the MEA biochips. There is always some electrode degradation, which increases with the number of experiments done on the biochip.

After analysis of MEA60 biochips cleaned over 30 times with the Tween80 cleaning method, it has been found that the SU-8 epoxy insulation remains clean. However, cellular debris have been found on platinum electrodes, which generated a larger electrode noise level.