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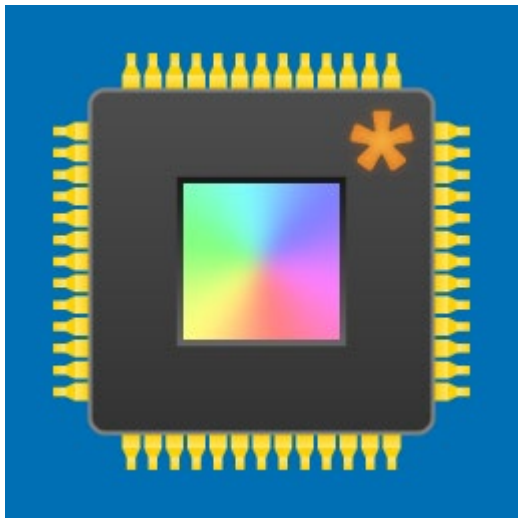
CMOS-MEA Application Note: Cultivation of primary cortical neurons on CMOS-MEAs



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1 Introduction and Acknowledgement

This application note provides a standard protocol for the cultivation of primary cortical neurons on CMOS arrays. For other cell types, adjustments in the procedure might be necessary. We thank **Dr. Günther Zeck** from the NMI Reutlingen, Germany, for providing the information to write this protocol.

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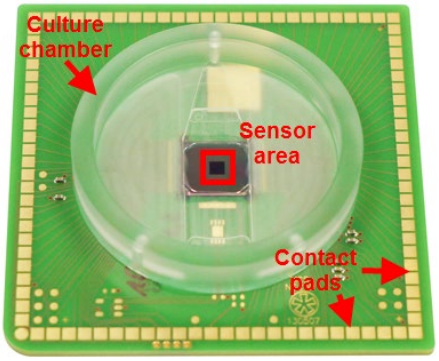
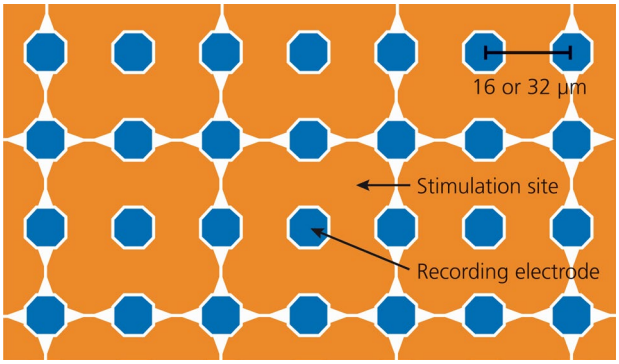
2 CMOS-MEAs

The CMOS-MEA arrays are based on complementary metal oxide semiconductor (CMOS) technology, facilitating fast, high resolution imaging of electrical activity. They are equipped with a culture- or slice-chamber to house the sample, while allowing the use of an upright microscope. The CMOS array has a **65 x 65 layout** and is available with 16 µm or 32 µm interelectrode distance (center to center). The electrode diameter is always 8 µm. Between the recording electrodes, there is a grid of **32 x 32 bigger stimulation sites**. The chip surface is coated with a planar oxide, similar to glass, enhancing the biocompatibility and biostability. CMOS arrays are **reusable** and should be handled, cleaned and stored appropriately to ensure an optimal lifetime.





2.1 Handling

Touch the CMOS arrays only at the culture chamber or at the rim. Avoid touching the contact pads and the central sensor area. Contact pads may be cleaned with a cotton swab and isopropanol or 70 % EtOH.

2.2 Preconditioning of new CMOS-MEAs

Only new CMOS arrays need to be treated like this. After first use, this is no longer necessary. Incubate new CMOS-MEAs for **two weeks** in the incubator with cell culture medium (NMEM-B27). Change all medium after one week. Apply the standard cleaning protocol afterwards.

2.3 Storage

For longer periods, store the CMOS arrays dry in the plastic boxes they came in. In between use, they can be stored in the fridge, filled with distilled water, to preserve the hydrophilicity of the surface. Do **not submerge** the complete array.

2.4 Cleaning

Different detergents are suitable to clean the chip. We recommend a 5 % solution of **Tickopur R60** diluted in distilled water. Remove cell debris with a water jet. Fill the culture chamber with this solution for at least two hours at 80° C. After Tickopur treatment, rinse thoroughly with distilled water. Dry the array and apply plasma treatment (see below), if available.

To remove persistent debris from the surface, it's possible to use a soft cotton swab with 70 % EtOH to carefully wipe the sensor area. Do not scratch the surface mechanically, otherwise the chip will be destroyed.

Likewise, the golden contact pads along the rim of the CMOS array can be cleaned with a cotton swab and alcohol.

2.5 Plasma Treatment

If available, plasma treatment in a plasma cleaner should be applied (for example PDC-32G from Harrick Plasma, Ithaca NY United States, or Plasma Flecto30 from Plasma Technology, Germany). The CMOS-MEA surface is exposed to an oxygen plasma, which makes the surface polar and thus more hydrophilic. This 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. CMOS-MEAs should be treated with low-power for up to **60 s at 0.2 mbar and 5 to 10 W**.

Warning: Extensive plasma treatment might damage the CMOS-MEAs. Keep treatment short and low power.

2.6 Sterilization

For sterilization, fill the culture chamber with 70 % **EtOH** and/or put them under **UV light** in a sterile hood for 30 minutes.

Warning: Autoclaving of CMOS arrays is possible, but not recommended as standard procedure.

3 Culture Protocol

The identical protocol can be applied to CMOS arrays with 16 or 32 µm sensor spacing.

3.1 Materials

- CMOS arrays with culture chamber (CMOS-MEA16-CC or CMOS-MEA32-CC)
- Chamber lids with semipermeable membrane (CMOS-CCL)
- Plasma Cleaner (optional, but recommended)
- Tickopur R60, 5 % solution
- Rat embryos, E17-18
- PEI solution, 0.0025 % in Borate buffer, pH 8.5 (Poly-ethylenimine, Sigma, Cat# 482595)
- Borate buffer, pH 8.5, sterile filter
  2.48 g H_3BO_3 in 200 ml H_2O, pH 5.5
  3.80 g $Na_2B_4O_7$ in 200 ml H_2O, pH 9.0
- NMEM-B27
  50 ml 10x MEM w/o Glutamine and $NaHCO_3$ (Thermofischer, Cat# 21430079)
  5 ml Sodium Pyruvate solution, 100 mM, (Thermofischer, Cat# 11360070)
  5 ml L-Glutamine solution, 200 mM, (Thermofischer, Cat# 25030081)
  20 ml $NaHCO_3$ solution, 5.5 %
  15 ml Glucose solution, 20 %
  10 ml B27 Supplement, (Thermofischer, Cat# 175004044)
  500 ml ddH_2O
  Sterile filter and store at 4° C; use within 10 days
- BrainPhys™ Neural Medium (Stemcell Technologies, Cat# 05790)

3.2 Preparation and Coating of CMOS Arrays (Day 0, Day 1)

Apply cleaning and sterilization as described. Apply **100 µl of PEI coating** solution to the sensor area of the CMOS array. Close the array culture chamber with the chamber lids with semipermeable membrane, to ensure the PEI drop does not evaporate, and place it in the incubator **overnight**. The next day, remove the PEI solution and carefully rinse the sensor area twice with distilled water. Add 200 µl NMEM-B27 and place the CMOS arrays in an incubator till needed.

3.3 Cell Dissociation (Day 1)

- Remove embryos at E17-E18 and dissociate cortical cells according to established protocols.
- Take 200 µl of cell suspension, add 10 µl trypan blue, mix, and take 10 µl to count **living** cells.
- Centrifuge the remaining cells, aspirate medium and re-suspend the cell pellet to the concentration of **2.1 x 10^5 living cells / ml** in NMEM-B27 + 5 % horse serum.

3.4 Cell Plating (Day 1, DIV 0)

Take coated CMOS arrays from the incubator and aspirate the 500 µl medium. Place a **100 µl drop of cell suspension** on the sensor area, nominally containing 21000 cells. The cell number of 21000 cells is relevant, the medium volume may vary.

Fill the CMOS chamber up to 500 µl with NMEM-B27 + horse serum. Keep the CMOS array in the incubator for 4 hours. Aspirate the medium and fill up the chamber carefully to 1 ml with NMEM-B27 culture medium **without** horse serum.

Keep the cultures in the incubator in a humidified atmosphere of 5 % CO_2 / 95 % air at 37 °C.

3.5 First Medium Change (Day 2, DIV 1)

Remove **al medium** one day after plating. Fill up with only **500 µl** of fresh NMEM-B27**.**

3.6 Cultivation (Day 4 / DIV 3 and up)

Gradually replace NMEM-B27 with **BrainPhys™ Neural Medium** as follows:
- DIV3: remove 250 µl of medium and replace with 250 µl of fresh Brain Phys medium
- DIV6: remove 250 µl of medium and replace with 250 µl of fresh Brain Phys medium
- DIV8: remove 250 µl of medium and replace with 250 µl of fresh Brain Phys medium

After that, change **half of the medium three times a week**. Cultures should become active after 7-10 days.

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