**Introduction**

In the last decades, micro-electrode arrays (MEAs) have become a widely used technology for recording extracellular signals arising from dissociated neuronal networks and acute brain slices. The commercially available systems provide 60-256 electrodes channels, which are typically spaced 20-200 μm apart. Compared to neural cell dimensions, this electrode distribution results in a spatially undersampling that does not allow, for example, to appreciate signal propagations at a global neuronal network level and at cellular compartment scales.

Recent advances in recording technology by using CMOS technology for MEA-based devices (1) allowed to overcome these limitations. In this work we present an innovative CMOS MEA chip that integrates 4096 electrodes at an inter-electrode distance of 21 μm, and that requires a signal at a minimal sampling rate of 64 kHz/channel. This remarkable technology paves now new perspectives for exploring signal propagations in dense cultures and in sparse neuronal networks [1–3], as well as field potential spreading in acute brain slices.

**Concept**

The CMOS-MEA chip, based on the Active Pixel Sensor concept commonly used for CCDs, acts as a sort of video camera. Square electrodes of 21 μm, integrating an amplifier underneath the electrode area, are arranged in a 64 by 64 layout with a separation of 21 μm. Signals are recorded at 64 kHz each electrode and are collected as frames, where each point represents the instantaneous extracellular voltage value for each electrode.

**Acquisition platform**

- pixel electrical charge (64-bit) encoded in the pixel color
- wide field of view (even under low density culture conditions)
- high sampling rate (40 kHz/channel)
- data pre-processing (high-pass, f<sub>C</sub>)
- device control and addressing
- analogue-to-digital conversion
- real-time pre-processing (high-pass, f<sub>C</sub>)
- spike detection and image/video based analysis
- off-line analysis (spike detection and image/video based)
- interface board (FPGA)
- bit stream re-ordering
- movie acquisition and visualization (average 62 MB/s)

**Basal recording**

Spontaneous electrophysiological activity is a main hippocampal neuronal culture (glutamate density of 600 nA/μm<sup>2</sup>, recording at 2100 V).

**Stimulation**

The first platform generation is a recording device that can be coupled with an external glass pipette for evoking neuronal activity. Indeed, by using a glass micropipette electrode placed in proximity of the neuron, it is possible to induce an electrical stimulus and to observe the propagation of the evoked response.

**Outlook and perspectives**

- high-resolution APS-MEAs offer:
  - reduced number (sampled 4096 electrodes, 2.6 x 2.6 mm<sup>2</sup>, 21 μm inter-electrode separation)
  - interfacing capability from network to cellular scales
  - detailed observation of activation sites and patterns propagations
  - activity measurements acquired from different networks (dissociated and brain slices)
  - additional possibility to evoke neuronal activity (external electrodes)
  - challenging opportunities for analyzing neural dynamics (image/video analysis, visualization tools, data mining)
  - identification of activation patterns and focal sites both for in vitro and in vivo conditions

**Dense disinherited hippocampal cultures**

Combination of high-resolution electrophysiological recording and immunofluorescence imaging enables to resolve activity of single neurons and interconnected microcircuits. (A) Hippocampal network (DIV14) coupled to an APS device and stained with neuN (neuronal cell nuclei – red) and MAP2 (neuronal processes and soma – green). (B) Electrophysiological activity (raw data) of three selected electrodes participating in a small micro-circuit. (C) Raster plot of all the active channels (active area) on a time scale of 2 min. Even under low density culture conditions, spontaneous activity involving the whole network are observed.

**Acute brain slice**

The APS-MEAs platform can also be used to study neuronal tissue in the example:

(A) recorded electrophysiological activities in the hippocampus of a neonatal mouse of 300 kHz (17 days old wild type mice); the same slice was preconditionally stimulating IC and AAP

**References**

2. www.3brain.com (for both burst onsets) are also presented to appreciate the recorded electrophysiological extracellular signals. (A) example of extracellular signal on two different electrodes obtained by applying a high-pass voltage stimulus of 2 V peak-to-peak.

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