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Active Pixel Sensor Micro Electrode Arrays (APS-MEA): perspectives and challenges using a high resolution neuroelectronic interface for functional electrophysiological imaging of in-vitro neuronal networks and acute brain slices

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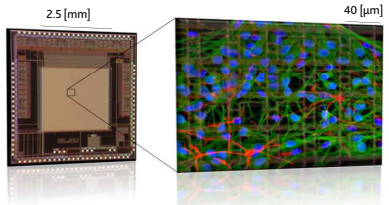
Active Pixel Sensor Micro Electrode Arrays (APS-MEA)

Introduction

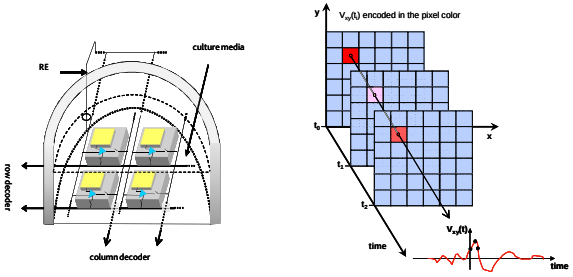
In the last decades micro electrode arrays (MEAs) have become a widely used technology for recording extracellular signals arising from dissociated neuronal networks and acute brain slices. The commercially available systems provide 60-256 electrode channels, whose inter-electrode distance is typically 100-200 µm. Compared to neuronal cell dimensions, this electrode distribution results in a spatial under sampling that does not allow, for example, to appreciate signal propagations at a global neuronal network level and at local sub-population scales.
The achievements of recent years 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 acquires signals at a minimal sampling rate of ~8 kHz/channel. This neuroelectronic interface provides new opportunities for exploring signal propagation in dense cultures and in sparse neuronal networks [2-3], as well as field potential spreading in acute brain slices.

- 4096 electrodes
- 21 x 21 µm in electrode dimensions
- 21 µm inter-electrode separation
- sampling rate of ~8 kHz/channel

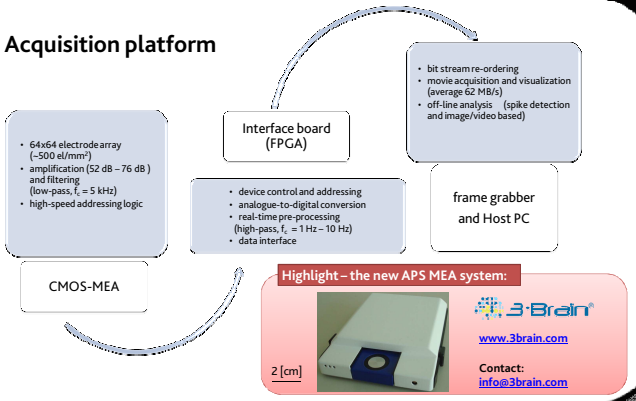


Concept

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 8 KHz for each pixel and are collected as frames, where each point represents the instant extracellular voltage value for each electrode.



Acquisition platform



Highlight – the new APS MEA system:

3Brain
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Dense dissociated hippocampal cultures

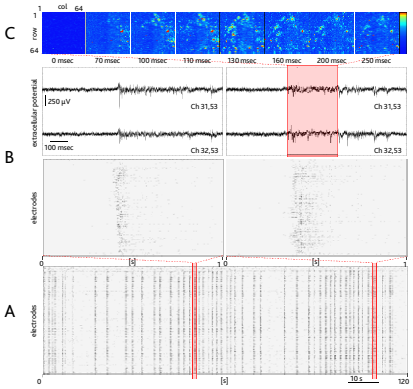
Basal recording

Spontaneous electrophysiological activity in a mature hippocampal neuronal culture (plating density of 600 cells/µl, recording at 27DIVs).

(A) raster plot of about 2000 active microelectrodes encompassing several network bursts over a period of 2 min.

(B) Two close-ups of the raster plot, corresponding to two burst onsets indicated with redlines in (C). Raw data of two exemplificative channels (for both burst onsets) are also presented to appreciate the recorded electrophysiological extracellular signals.

(C) Single burst involving the global network level, represented as an image sequence. The extracellular signals are shown in a false color map by computing the signal variance (bin size of 20 ms).



Burst classification

Spatial–temporal patterns of activity are acquired at high resolution from the whole active area, allowing thus a detailed observation of signal propagations. Different mathematical approaches can be adopted to recognize and classify burst patterns:

- wavelet decomposition: Center of Gravity (CoG) calculation of six burst activities recorded from an hippocampal culture at 27 DIV. CoG is computed on windowed maximum variance of the raw signal for each burst at 4 different resolution by using a 2d-wavelet filter bank. Three different pattern families can be easily identified [4].
- for a pattern classification based on CAT (Center of Activity Trajectory) computed both on spike trains and raw data

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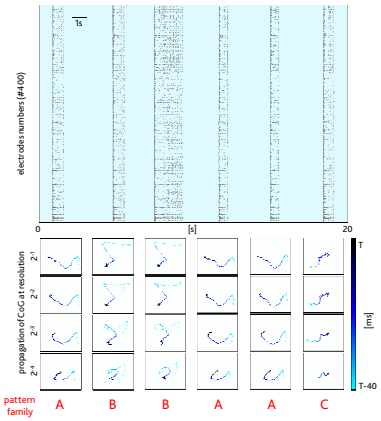


Stimulation

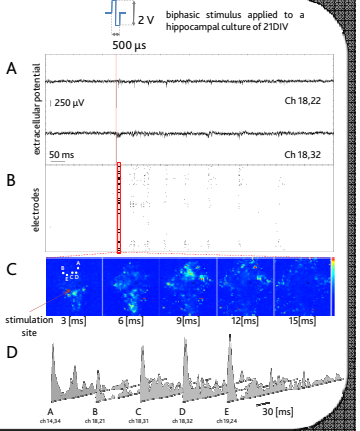
This first platform generation is a recording device that can be coupled with an external glass pipette to evoke neuronal activity. Indeed, by using a glass micropipette electrode placed in proximity of the neurons, it is possible to localize an electrical stimulus and to observe the propagation of the evoked responses.

(A) example of extracellular evoked signal on two different electrodes obtained by applying a biphasic voltage stimulus of 2 V peak to peak.

(B) raster plot of the evoked response on a time window of 1 sec after the stimulus

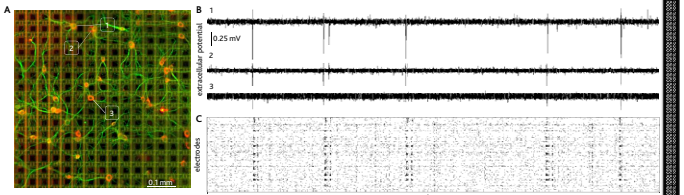
(C) false color map representation of the propagating response on the whole active area, during the first 15 ms after stimulation.

(D) Post Stimulus Time Histogram (PSTH) of 5 selected channels in a time window of 300 ms. The bin size is 4 ms as the blanking window after the stimulus. The PSTH is calculated on a series of 20 stimuli applyied in 60 sec.



Low density dissociated 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); image of a specific region of interest (16x16 electrodes). (B) Electrophysiological activity (raw data) of three selected electrodes participating in a small micro-circuit. (C) Raster plot of all the active channels (whole active area) on a time scale of 2 min. Even under low density culture conditions, quasi-synchronous activity involving the whole network are observed.



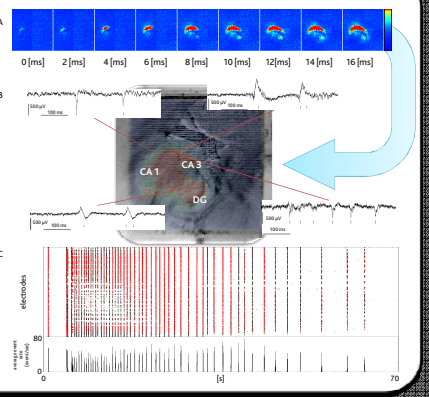
Acute brain slice

The APS-MEA platform can also be used to study neuronal tissue. In this example:

(A) recorded electrophysiological activation in the hippocampus of a horizontal slice of 300 µm (17 days-old wild-type mouse). The slice was preconditioned by adding BIC and 4AP.

(B) a great variety of waveforms are detected with respect to different areas of the tissue.

(C) raster plot of the inter-ictal events on a time window of 70 sec. In red the inter-ictal events participating to burst activity.
To detect the events we adopted a peak life time period of 10 msec and a refractory period of 15 msec [5]. Burst are detected if at least three events have a inter event interval less than 100 msec.



Outlooks and perspectives

- high-resolution APS-MEAs offer:
  - reduced network under-sampling (4096 microelectrodes, 2.6 x 2.6 mm², 21 µm inter-electrode separation)
  - zooming capability from network to cellular scales
  - detailed observation of activation sites and pattern propagations
- introduction of image-video concepts for spatial-temporal analysis and multi-resolution
- activity was acquired from dissociated networks (dense and sparse) and brain slices
- possibility to evoke neuronal activity (external electrodes)
- new challenging opportunities for analyzing neuronal dynamics (image/video analysis, visualization tools, data mining)
- identification of activation patterns and focal sites both for slices and dissociated neuronal networks

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