Engineered neuronal assemblies and functional connectivity analysis

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Outline

• CMOS based MEAs

• Neural signal analysis and connectivity

• Neural activity modulation by nanoparticles
Neural-interfaces: technological advances

“(…) Progress in **large-scale recording** of neuronal activity depends on the development of three critical components: the neuron-electrode interface, methods for spike sorting /identification and tools for the analysis and interpretation of parallel spike trains. In addition to increasing the numbers of recording sites on silicon probes, the development of on-chip interface circuitry is another priority. (…)”


- large under-sampling of the network activity (~10’000:100)
- limited number of microelectrodes (60-120)
- limited electrode pitch (~100 μm)

**need of new enabling technologies**

need of new analysis methods
CMOS based approach

Hierleman group ETH

- CMOS-based microelectrode array with 11'016 metal electrodes
- 128 addressable electrodes at a sampling rate of 20kH
- Now improved version

Herr et al., Biosensors and Bioelectronics, (2007), pp. 2546–2553

- 4225 Field Effect Transistor array with 1024 stimulating sites
- recording from all electrodes at a sampling rate of 25 kHz
- High-signal quality

maxwell biosystems

multichannel systems
CMOS based approach for in vivo

Nature (2017)
Fully Integrated Silicon Probes for High-Density Recording of Neural Activity

T. Harris et al., Janelia Research Campus
High-density CMOS based device

• IDEA: UE NEST project (2005-2008)
• Start-up (in Switzerland) (2011- www.3brain.com)


Samlab's
Sensors, Actuators and Microsystems Lab
A long-story: 15 years of experience in CMOS-MEAs

- 2012: BioCAM 4096
- 2015: BioCAM X
- 2019: BioCAM DupleX
- 2004: BioCAM Idea
High-density large scale CMOS-MEAs

**APOLLO**
Gen 0-1
- 4096 recording el.
- 42 µm pitch
- 2.7 x 2.7 mm² sensing area
- 3 x 3 mm² flat area

**ARTEMIS**
Gen 2
- 4096 recording el.
- 42 µm pitch
- 2.7 x 2.7 mm² sensing area
- 6 x 6 mm² flat area

**KHÌRON**
Gen 3
- 4096 recording el.
- 16 stim el.
- 81 µm pitch
- 5.1 x 5.1 mm² sensing area
- 6 x 6 mm² flat area

**TBD**
Gen 4
- Under development

**4096 recording el.**
**42 µm pitch**
**2.7 x 2.7 mm² sensing area**
**3 x 3 mm² flat area**
The Active Pixel Sensor (APS) technology

- redesigned in order to sense the electrophysiological signals
- each electrode/pixel integrates a pre-amplifier

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>No. of electrodes</td>
<td>4096</td>
</tr>
<tr>
<td>Electrode size</td>
<td>21 µm</td>
</tr>
<tr>
<td>Electrode separation</td>
<td>21 µm</td>
</tr>
<tr>
<td>Active area</td>
<td>~ 7 mm²</td>
</tr>
<tr>
<td>Spatial density</td>
<td>~ 580 el/mm²</td>
</tr>
<tr>
<td>Sampling rate</td>
<td>7.7 - 125 kHz</td>
</tr>
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</table>
| Data rate                  | ~ 0.5 Gbit/s           

The high-density APS-MEA platform

- oriented to image/video concepts

Host Computer
  - Frame grabber
  - Data storage and Visualization

Camera Link
  - Interface Board
    - FPGA (pre-processing)
    - Serializer

ADCs

Multiplexed Channels
2D networks on high-density APS

High-resolution:
~580 el/mm²

Covered area:
~ 7 mm²

Low-resolution:
~19 el/mm²

Covered area:
~2,7 mm²
2D networks on high-density APS

- **Full resolution** (~580 el/mm$^2$)
- **Low resolution** (~19 el/mm$^2$)
- **Medium-scale** (~2.7 mm$^2$)
- **Large-scale** (~7 mm$^2$)

High-resolution APS offers full resolution (~580 el/mm$^2$), while low-resolution APS provides a simplified view (~19 el/mm$^2$). The medium and large scales offer different resolutions and coverage areas.
Example: dissociated cultures – whole network synchronous activity

Post natal 14 DIVs mouse culture
30 msec synchronous event

Embryonic 22 DIVs rat culture
100 msec synchronous event
Example: structural and functional identification of sub-networks

<table>
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<tr>
<th></th>
<th>Basal</th>
<th>Bic 30 µM</th>
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<tbody>
<tr>
<td>MFR</td>
<td>0.63</td>
<td>0.88</td>
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<tr>
<td>Exc</td>
<td>1.14</td>
<td>1.36</td>
</tr>
<tr>
<td>Inh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTEST</td>
<td>**</td>
<td>*</td>
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</tbody>
</table>

Excitatory not GABAergic neuron
Inhibitory GABAergic neuron
Example: coupling electrophysiological and topological info

F. Ullo et al., Front. Neuro Anatomy 2014
Example: epileptic model for anticonvulsant compound testing

Ferrea et al. 2012, Front. Neural Circuits
Example: compound effect on purkinje activity in cerebellum slice

- CTRL
- CyPPA 5uM
- NS 309 5uM
- Apamin 0.2uM

80 min compound

baseline

Ca^{2+}-activated K^+ channels modulators alter PCs firing

In collaboration with evotec

A Ugolini et al. – Fens 2018
Engineered neuronal assemblies: data analysis

“(…) Progress in large-scale recording of neuronal activity depends on the development of three critical components: the neuron-electrode interface, methods for spike sorting /identification and tools for the analysis and interpretation of parallel spike trains. In addition to increasing the numbers of recording sites on silicon probes, the development of on-chip interface circuitry is another priority. (…)”


need of new enabling technologies

need of new analysis methods
Functional connectivity estimated by means of Cross-Correlation based techniques and information theory methods

Functional-effective connectivity methods: Transfer Entropy revisited

\[ TE_{y \rightarrow x} = \sum_{x_t x_{t-1} y_{t-1}} x_t x_{t-1} y_{t-1} \]

- for a reference spike train \( x \), and a target spike train \( y \)
- the couple \((k, l)\) defines the TE's order

Considering \( d \) varying from 1 to a fixed number, we can build a temporal function \( TE(d) \)

Simple cross-correlation revisited

Innovative Methodology

INTERACTION BETWEEN CORTICAL PRINCIPAL CELLS AND INTERNEURONS

FIG. 2. Short-latency, monosynaptic interactions between neuron pairs. A: excitatory drive by a putative pyramidal cell (red triangle). Note large, sharp peak at $\sim 2$ ms in the cross-correlogram. Reference event is the spike of the putative pyramidal neuron (time 0). Inset: higher temporal resolution of the histogram. Averaged waveforms of the units (filtered: 600 Hz to 5 kHz) are also shown. On the bas[es of spike duration, the target cell was classified as a putative interneuron (blue circle; see text). B: superimposed traces of the neuron pair from 2 recording sites with the largest amplitude for each spike. Arrow, monosynaptically driven spikes. C: inhibitory suppression. Reference event: spike of the putative interneuron (blue circle). Note strong and immediate suppression of target spikes. The 2 neurons were recorded from different shanks (200-μm lateral separation). Red line indicates exponential fit of suppression time course. D: reciprocal monosynaptic interactions of neurons recorded from the same shank. Reference event: spike of the putative interneuron (blue circle). Note excitation of the putative interneuron and strong suppression of the pyramidal cell (red triangle) spikes by the interneuron. Shading indicates the blank period of spike sampling (see METHODS).
Functional-effective connectivity methods: Cross-Correlation revisited

\[ C_{xy}(\tau) = \frac{1}{\sqrt{N_x N_y}} \sum_{s=1}^{N_x} \sum_{ti=(\tau-\sqrt{\tau}/2)}^{(\tau+\sqrt{\tau}/2)} x(t_s) y(t_s - ti) \]

CC is able to detect the inhibitory links

\[ CC_{xy\_peak\_value} = \max_{\tau = [-W/2, +W/2]} \left\{ C_{xy}(\tau) - \frac{1}{W} \sum_{\tau = -W/2}^{+W/2} C_{xy}(\tau) \right\} \]

Pastore et al., 2018, Plos Computational Biology
Filtered Normalized Cross-Correlation Histogram (FNCCH)

- It has been validated on in silico neural networks with 1000 neurons
- Identification of inhibitory links!
- Improvement of excitatory link detection
- Very good delay reconstruction
- Very good degree distribution reconstruction

Functional-effective connectivity
Connectivity & dynamics

Graph Theory can be used to:

- explore and compare structural and functional brain networks
- classify => topology

Graph theory and connectivity

Topology of the network

- **Clustering Coefficient (CC):** quantifies the number of connections that exist between the nearest neighbours of a node.

- **Mean Path Length (PL):** minimum number of edges that must be traversed to go from one node to another.

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Network models

Random (RND) networks

Each pair of nodes has an equal probability of being connected
Degree distribution follows a Gaussian distribution

Scale-Free (SF) networks

Few nodes connected to many others (hubs)
Degree distribution follows a power law

Small-World (SW) networks

Between totally regular and random
Highly clustered but short path length

B+C: It is hypothesized to reflect an optimal configuration associated with rapid synchronization and information transfer


Network topology: MEA-60 and MEA-4k

Small-world topological properties found in large-scale networks
Scale-free networks
Rich-club: privileged sub-networks
Modulation of network dynamics

Modulation by chemical compounds: specific for receptors but difficult for a spatially confined delivery

Modulation by direct electrical stimulation: unspecific but spatially confined (you need an electrode properly placed)

What about **remote non invasive** neuro modulation?

Optogenetics and optical stimulation could be a partial answer.
Pros: specificity
Cons: still invasive; it implies a genetic modification of the cells...
Engineered networks with piezo-electric nanoparticles

Barium titanate nanoparticles
BTNP

ultrasound induced stimulation

Camilo Rojas, PhD

Gianni Ciofani

**BaTiO$_3$ Nanoparticles**

- wrapped in Arabic gum
- hydrodynamic size: 479.0 ± 145.3 nm (by DLS)
- biocompatible

- commercially available

  - with tetragonal crystalline phase (perovskite-like) → **piezoelectric**
  - with cubic crystalline phase → **non-piezoelectric**

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![Diagram of basal activity, stimulus phase, and post-stimulus activity with ultrasound pulse train details](image)
Acoustic stimulation: excitation

Base

Stim

Post
Acoustic stimulation: excitation

US pulse train

1st stim

2nd stim

3rd stim

piezoNP

cubicNP

ctrl

MFR_{ON}/MFR_{OFF}

Baseline

Stim

Baseline

Stim
US stimulation mediated by piezoelectric nanoparticles induces an excitatory response in cultured neural networks


- mechanical deformation
- electro-elastic model of BTNP
- local change in electric potential
- increased open probability of voltage-gated channels
- action potential
Engineered networks with Gold Nano Rods

Gold Nano Rods
GNR

When gold particles are synthesized at the nanoscale they improve their surface plasmon resonance, acquiring very interesting plasmonic properties. Thanks to these properties, gold nanoparticles find numerous applications in different fields, such as:

- Cancer therapy
- Biomedical sensing and imaging
- Drug delivery
- Nanophotonics
- Neural activity modulation

Yoonkey Nam

Korean Advanced Institute of Science and Technology
Engineered networks with GNRs: photo thermal inhibition

- Inhibition of neural activity -
Engineered microsystems: brain-on-a-chip

- Ultrasound stimulation (exc)
- IR pulse stimulation (inh)
- Biopolymer based scaffold
- Modular interconnected iPSC neuronal networks
- Micro-nanotech device
- Microfluidic IN-OUT
- High-density 3D protruding CMOS chip
- Electrical Recording
- Metabolism monitoring
- Electrical Stimulation
- Neurotransmitter detection
- Microfluidics

- Dynamics and connectivity analysis
- Functional-structural 3D network modules
- Functional excitatory and inhibitory connections
- Remote neuromodulation Structural connectivity
Tools and technologies for analyzing engineered model systems: e.g., high-density large scale MEA devices

Reliable analysis methods to infer connectivity. Ground truth problem, in silico models, in vitro models. Connectivity methods are at the basis to infer topology. A large number of nodes is needed...

Further engineered neuronal systems with nanoparticles for neural activity modulation:
Piezo nano-particles for stimulation
Gold nano-rods for inhibition

In vitro 3D models for brain-on-a-chip applications, towards engineered brain organoids and patient specific medicine
Credits

Paolo Massobrio (computational aspects)
Laura Pastorino (biomaterials)
Pasqualina Farisello (cell biology)

Mariateresa Tedesco
Thiru Kanagasabapathi
Monica Frega
Virginia Pirino
Andrea Spanu
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Nicolò Colistra
Camilo Rojas Cifuentes

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Thanks for your attention!