Engineered neuronal assemblies and functional connectivity analysis

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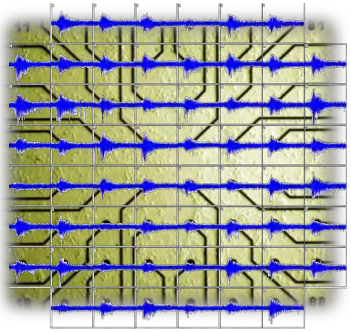
1st Brandyschool2019

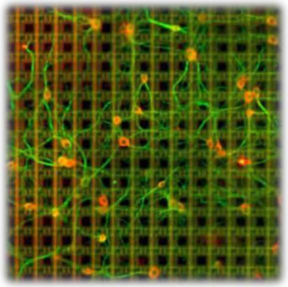
25 June 2019

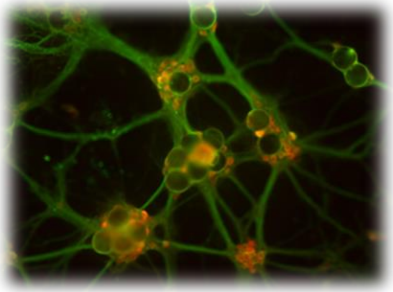
Outline

- CMOS based MEAs

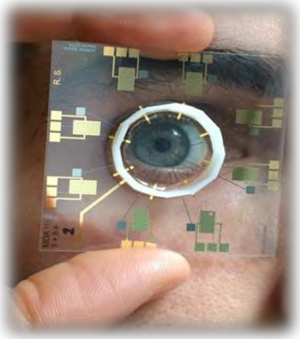


- Neural signal analysis and connectivity





- 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. (…)"

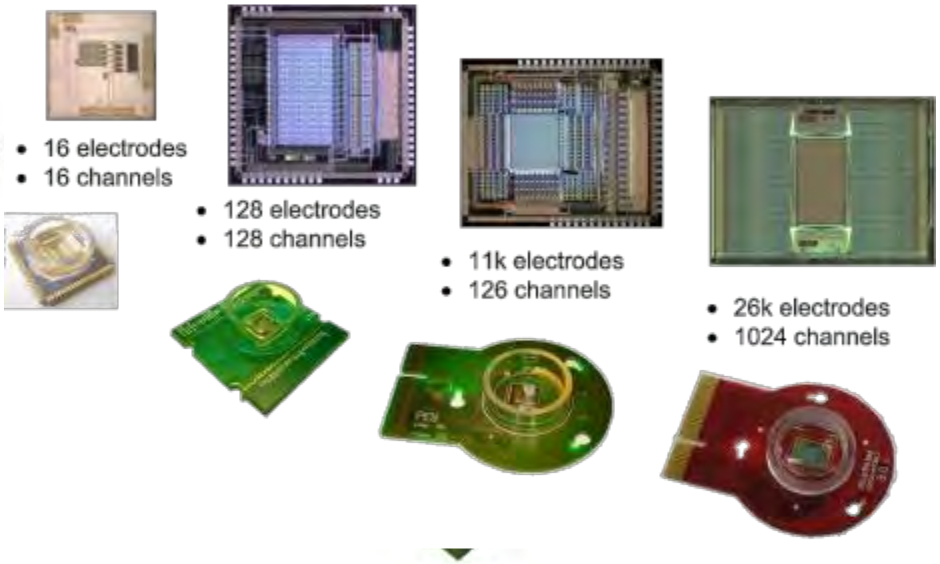
from G. Buzsáki, " Large-scale recording of neuronal ensembles", Nature Neuroscience, Vol. 7, No. 7, May 2004

- 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

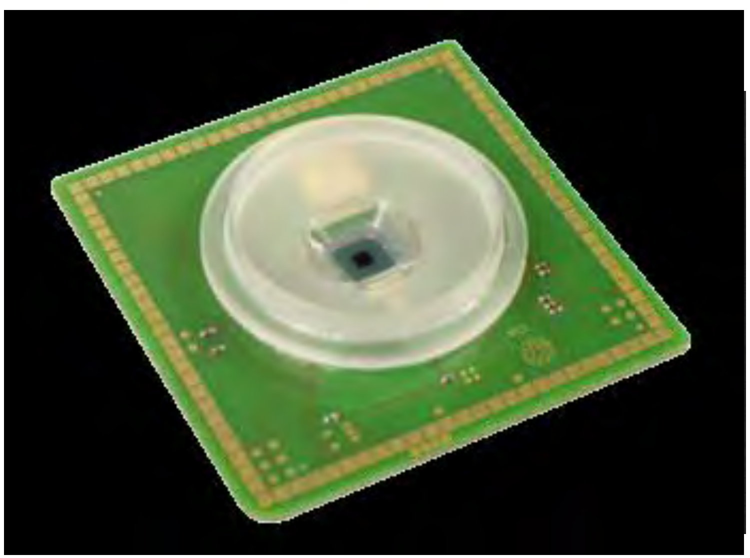


Hierleman gorup ETH

- 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

maxwell BIOSYSTEMS

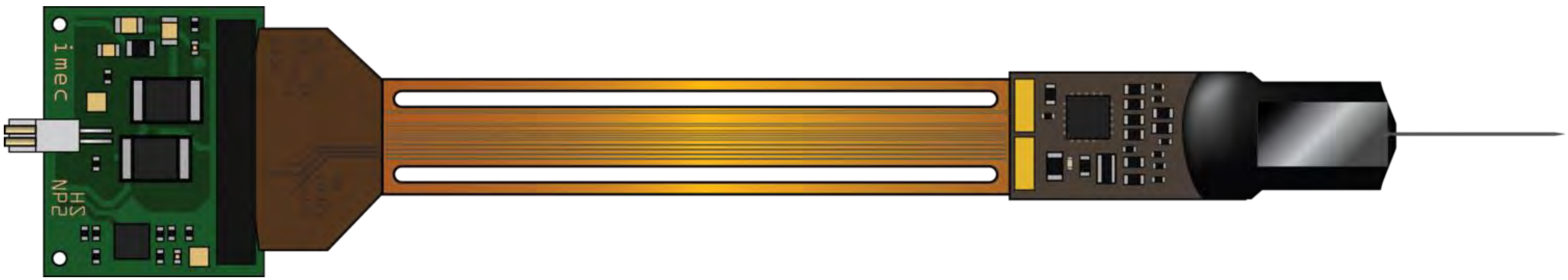


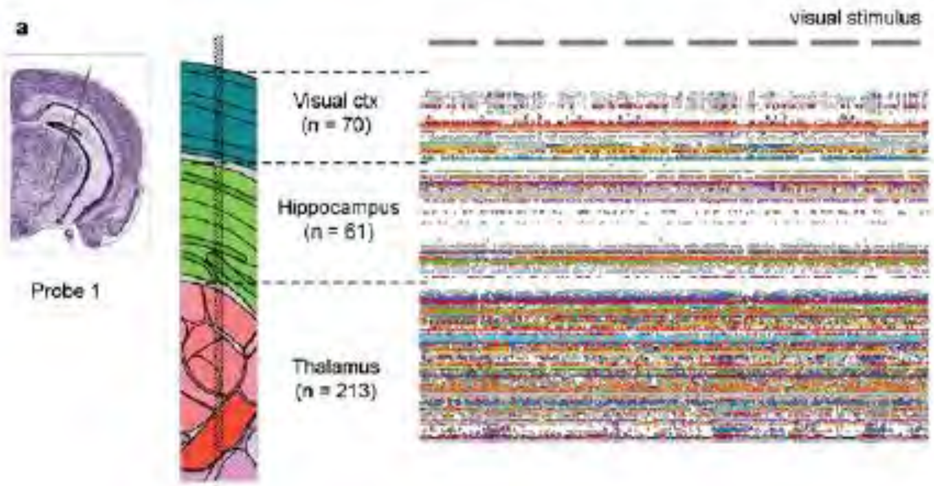
- 4225 Field Effect Transistor array with 1024 stimulating sites
- recording from all electrodes at a sampling rate of 25 kHz
- High-signal quality

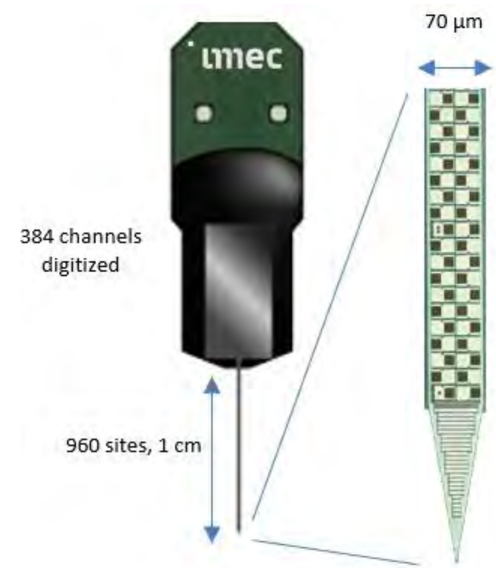
multichannel systems

CMOS based approach for in vivo

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

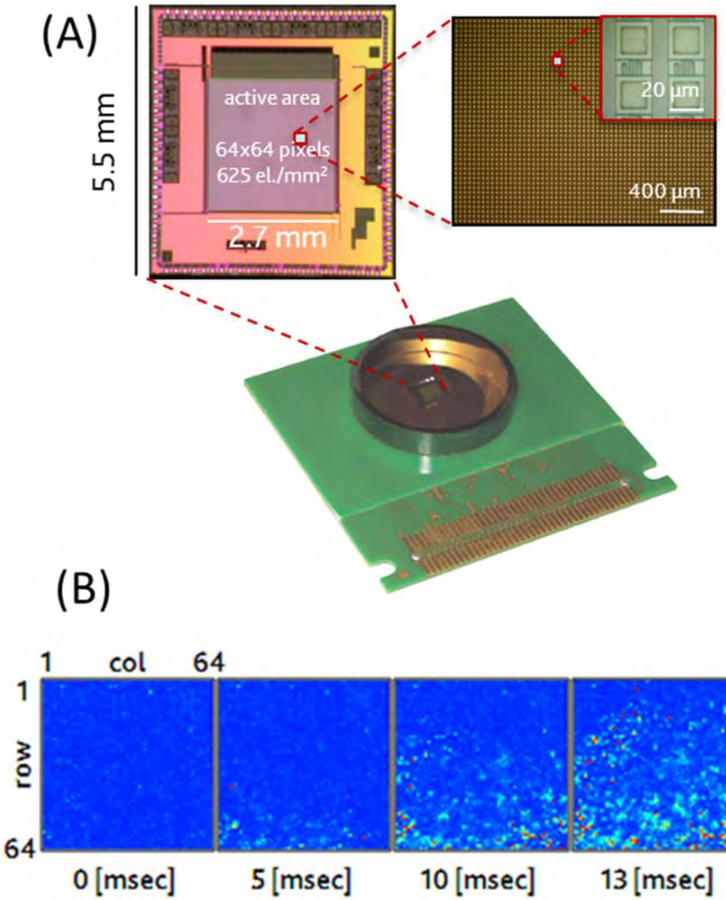






T. Harris et al., Janelia Research Campus

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

Berdondini et al. Lab on a Chip, 2009; Maccione et al., Front. In Neuroeng., 2010, Gandolfo et al., j. Neural Eng., 2010

A long-story: 15 years of experience in CMOS-MEAs

3·Brain

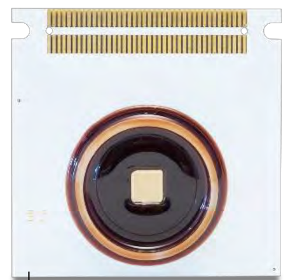
High-density large scale CMOS-MEAs

APOLLO
Gen 0-1

4096 recording el.
42 µm pitch
2.7 x 2.7 mm^2 sensing area
3 x 3 mm^2 flat area

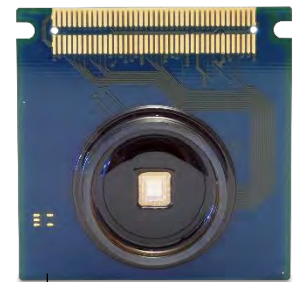
ARTEMIS
Gen 2

4096 recording el.
42 µm pitch
2.7 x 2.7 mm^2 sensing area
6 x 6 mm^2 flat area



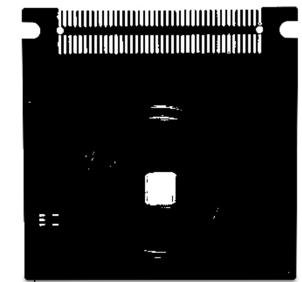
4096 recording el.
16 stim el.
81 µm pitch
5.1 x 5.1 mm^2 sensing area
6 x 6 mm^2 flat area

KHÌRON
Gen 3



4096 recording el.
4096 stim el.
60 µm pitch
3.8 x 3.8 mm^2 sensing area
6 x 6 mm^2 flat area

TBD
Gen 4



Under development

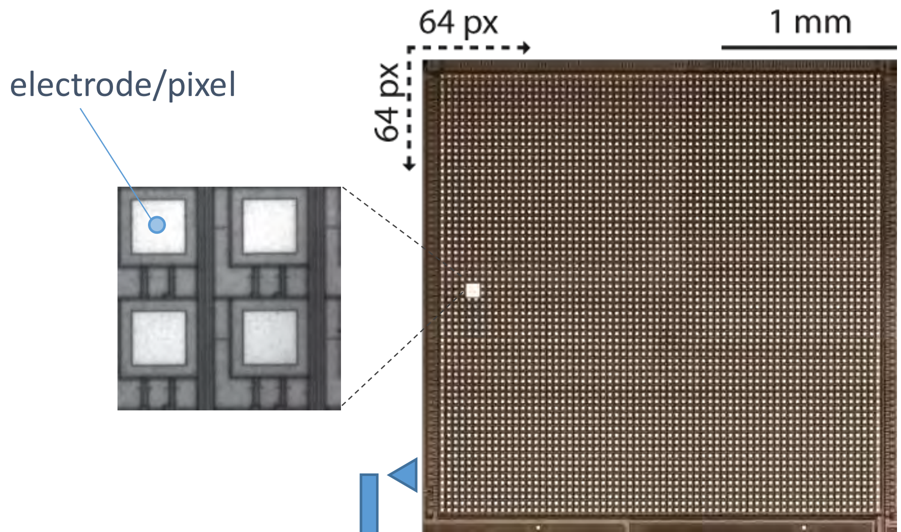
3·Brain

High-density CMOS based device

The Active Pixel Sensor (APS) technology

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

no. of electrodes	4096
electrode size	21 µm
electrode separation	21 µm
active area	~ 7 mm^2
spatial density	~ 580 el/mm^2
sampling rate	7.7 - 125 kHz
data rate	~ 0.5 Gbit/s



Multiplexed Channels

The high-density APS-MEA platform

- oriented to image/video concepts

Host Computer
Frame grabber
Data storage and Visualization

Camera Link

Interface Board
ADCs
FPGA (pre-processing)
Serializer

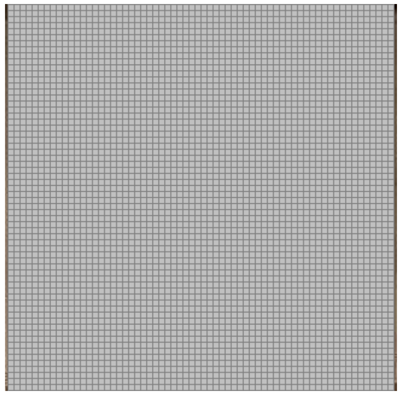
2D networks on high-density APS

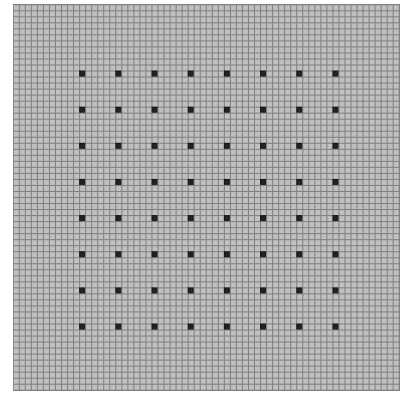
Full resolution

Low resolution

High-resolution:
~580 el/mm^2

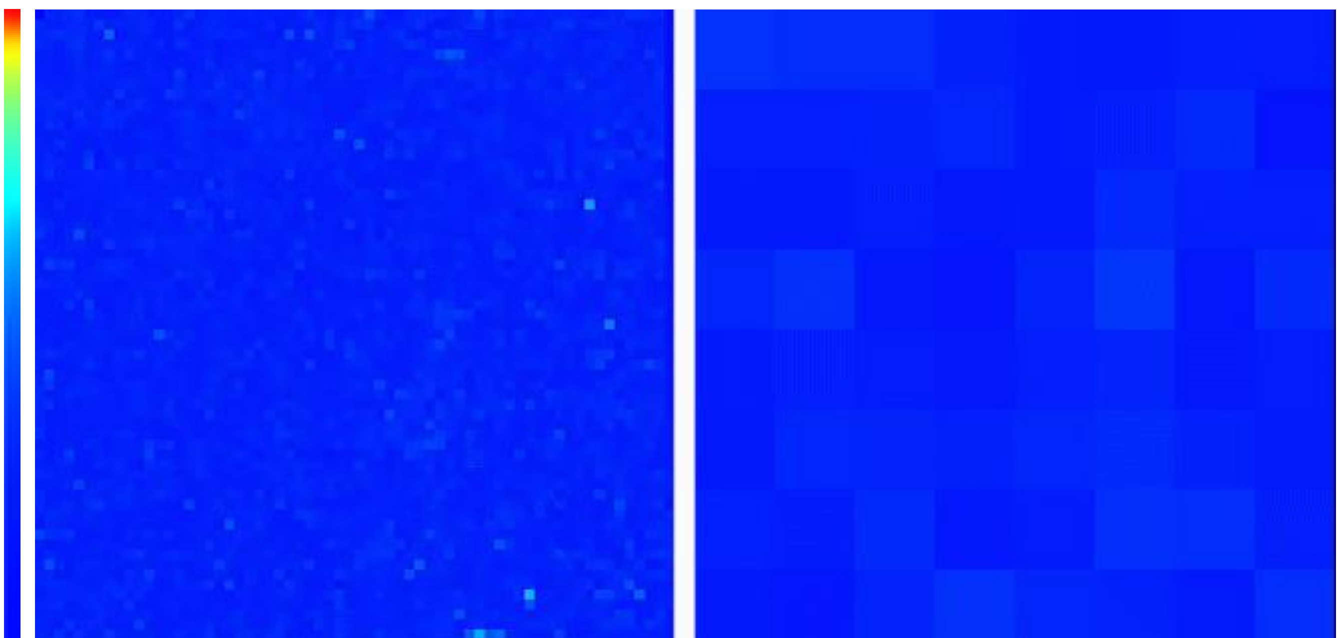
Covered area:
~ 7 mm^2





Low-resolution
~19 el/mm^2

Covered area:
~2,7 mm^2



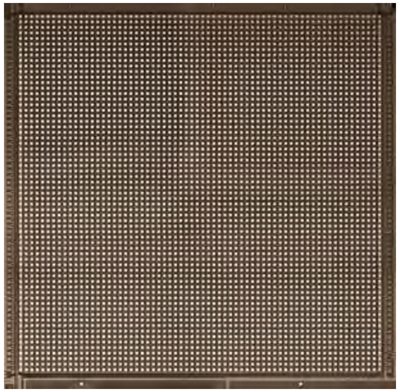
2D networks on high-density APS

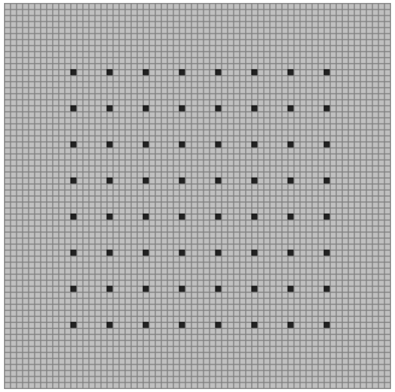
Full resolution

Low resolution

High-resolution
(~580 el/mm²)

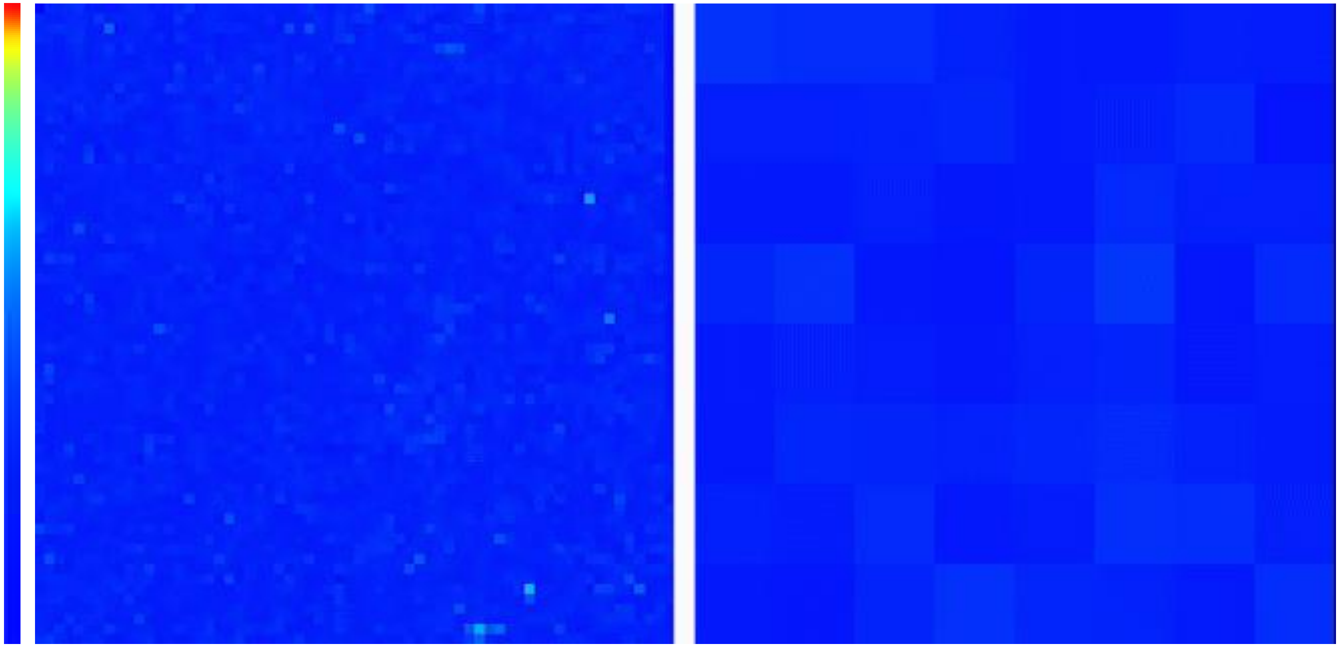
Large-scale
(~ 7 mm²)



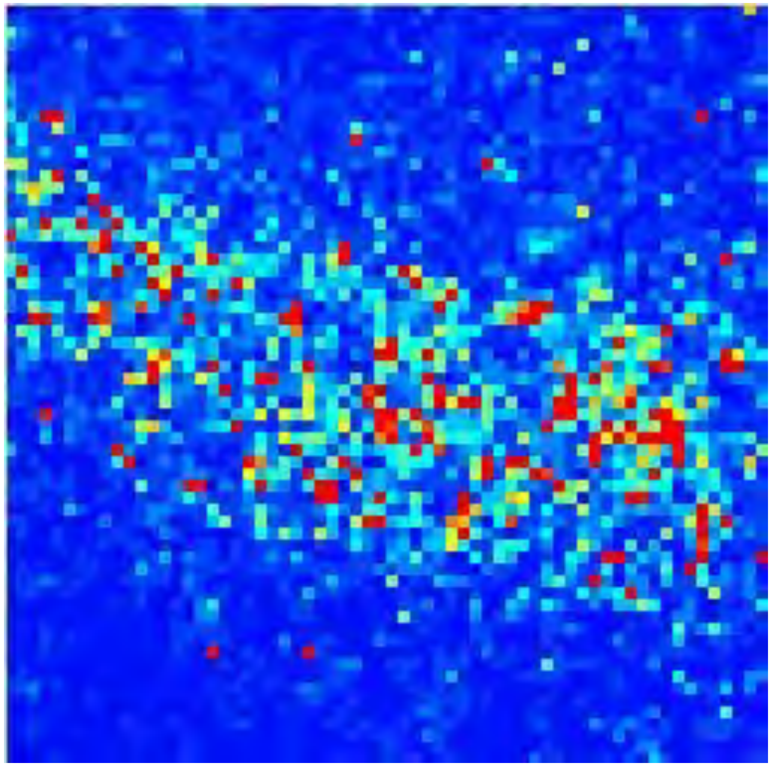


Low-resolution
(~19 el/mm²)

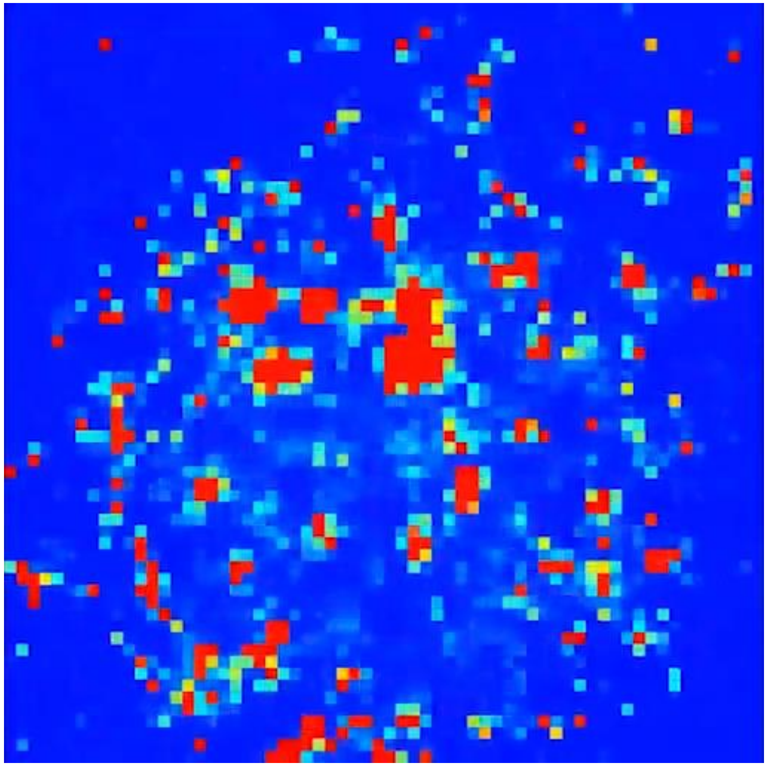
Medium-scale
(~2,7 mm²)



Example: dissociated cultures – whole network synchronous activity

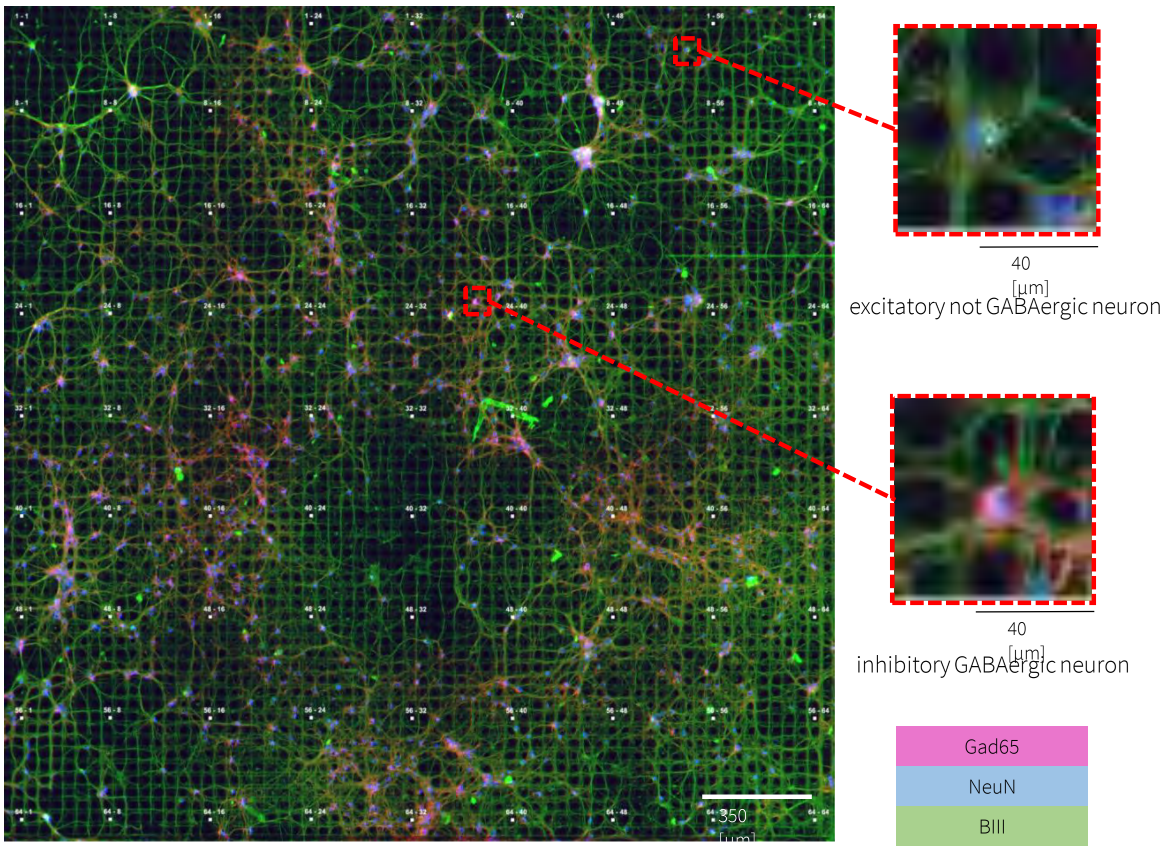


Post natal 14 DIVs mouse culture
30 msec synchronous event



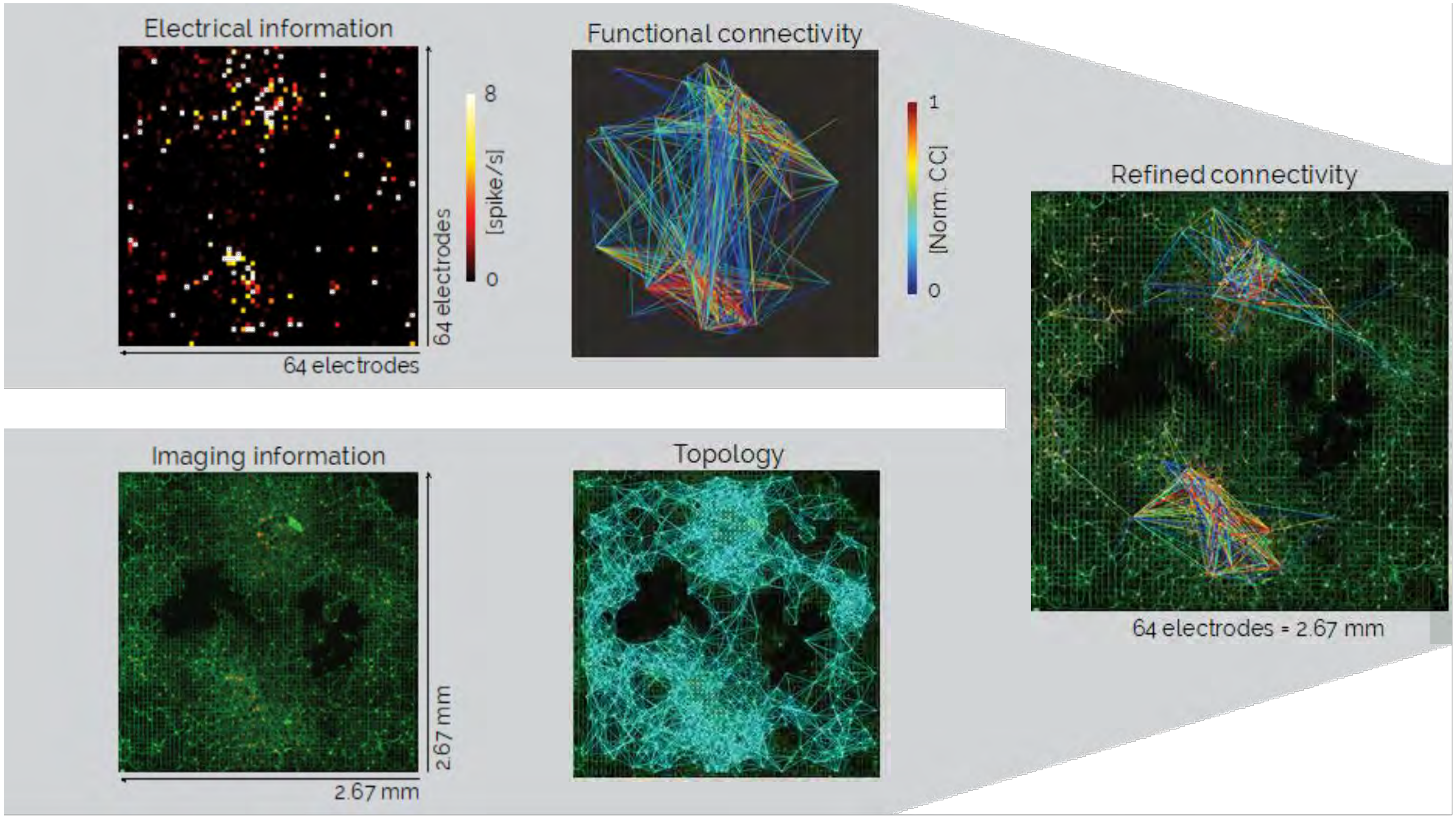
Embryonic 22 DIVs rat culture
100 msec synchronous event

Example: structural and functional identification of sub-networks



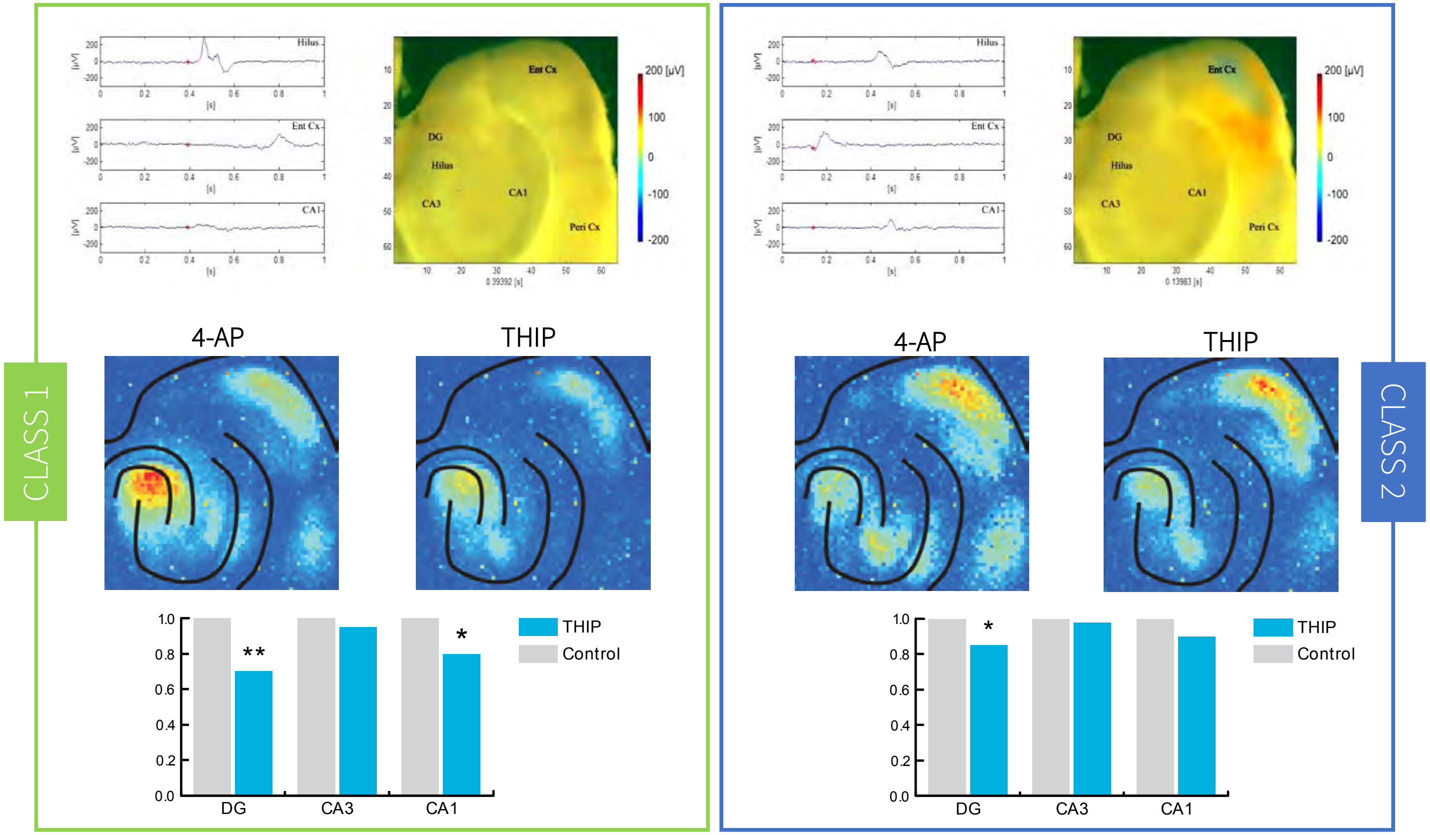
	Basal		Bic 30 µM		TTEST
MFR	mean	stdErr	mean	stdErr	
Exc	0.63	0.05	0.88	0.08	**
Inh	1.14	0.08	1.36	0.13	-
TTEST	**		*		

Example: coupling electrophysiological and topological info



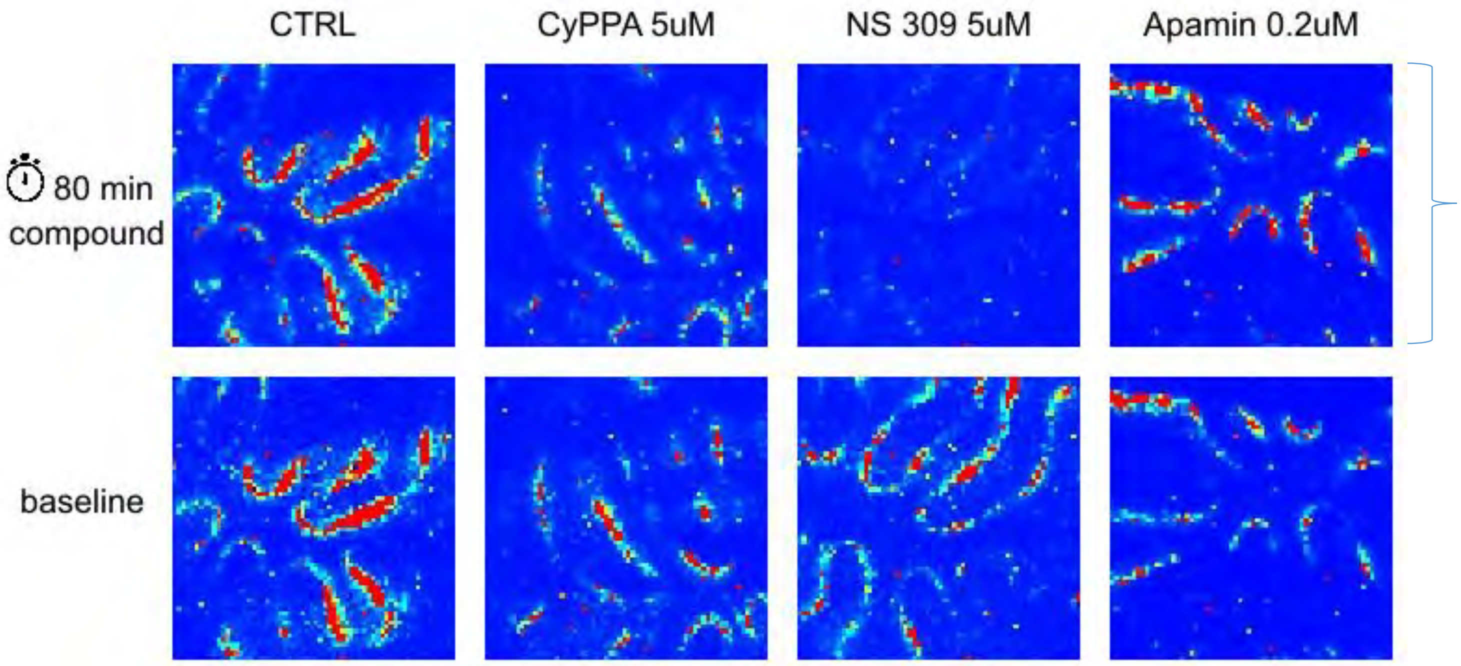
F. Ullo et al., Front. Neuro Anatomy 2014

Example: epileptic model for anticonvulsant compound testing



Ferrea et al. 2012, Front. Neural Circuits

Example: compound effect on purkinje activity in cerebellum slice



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

In collaboration with

evotec

A Ugolini et al. – Fens 2018

3·Brain

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. (…)"

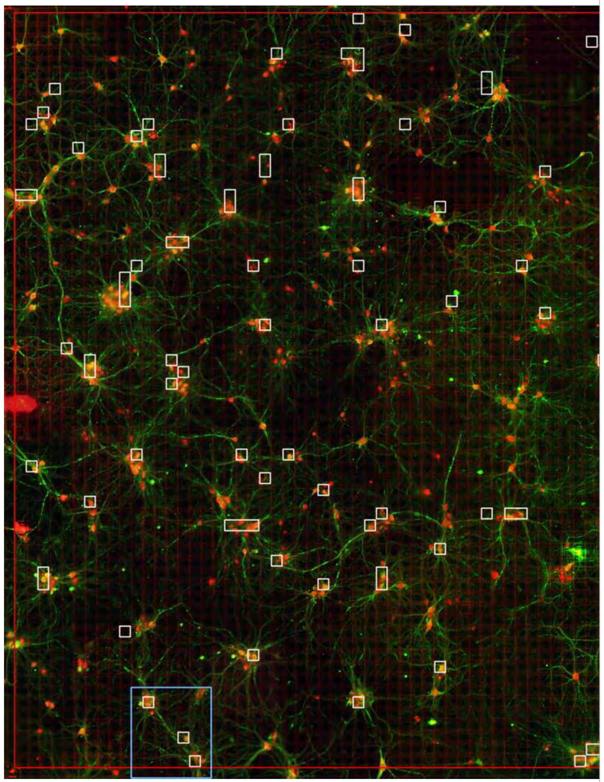
from G. Buzsáki, " Large-scale recording of neuronal ensembles", Nature Neuroscience, Vol. 7, No. 7, May 2004

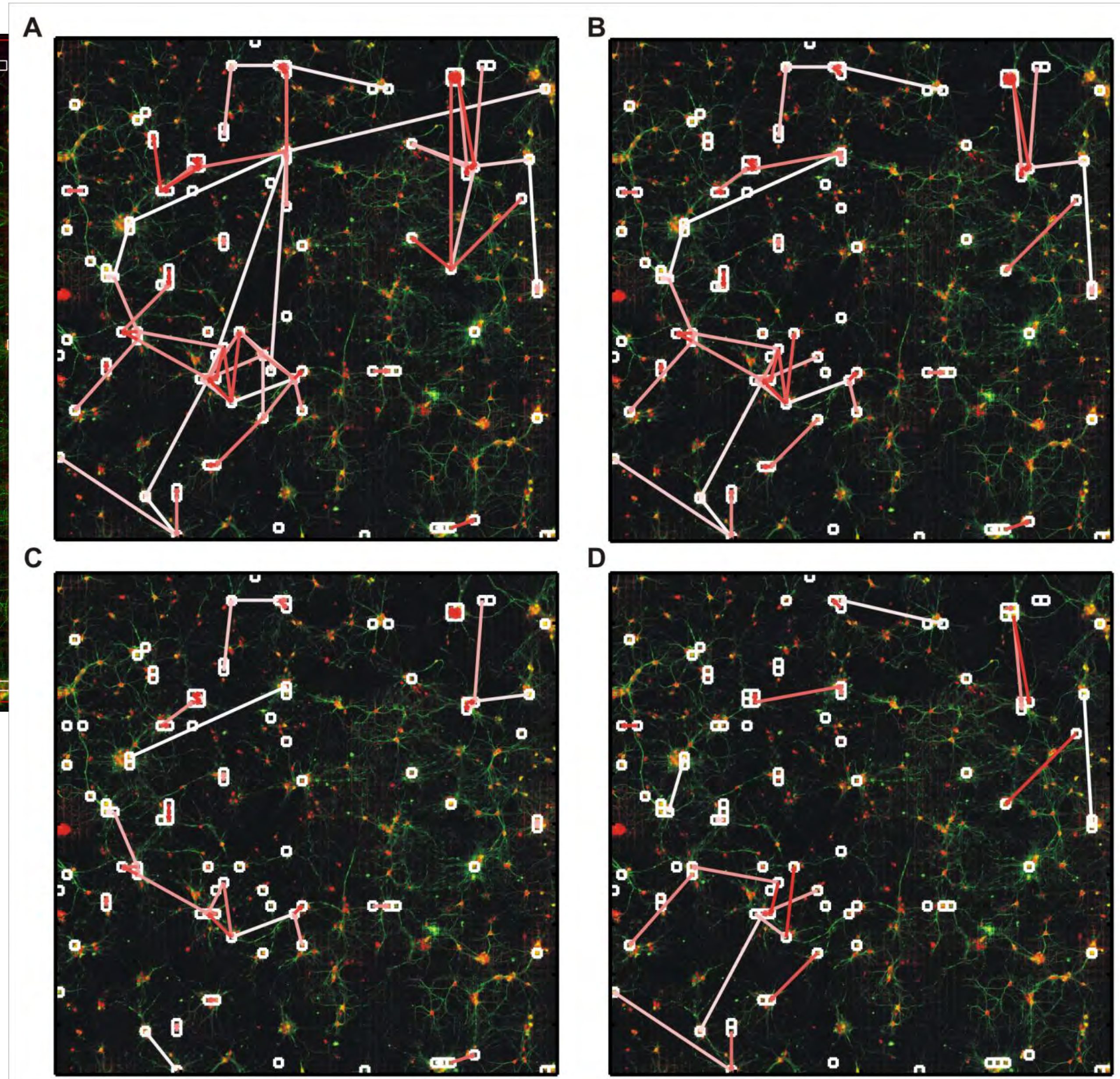
↓

need of new enabling technologies

need of new analysis methods

Structural vs functional connectivity





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

Garofalo et al. Plos One, (2009)

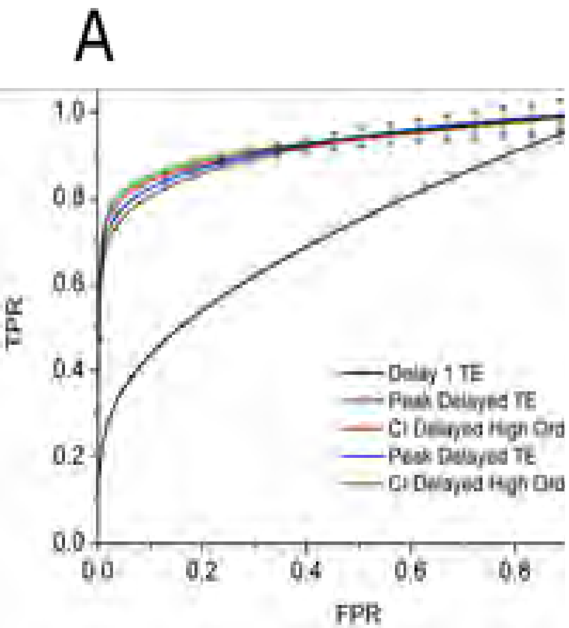
Maccione et al. J. Neurosci Methods (2012)

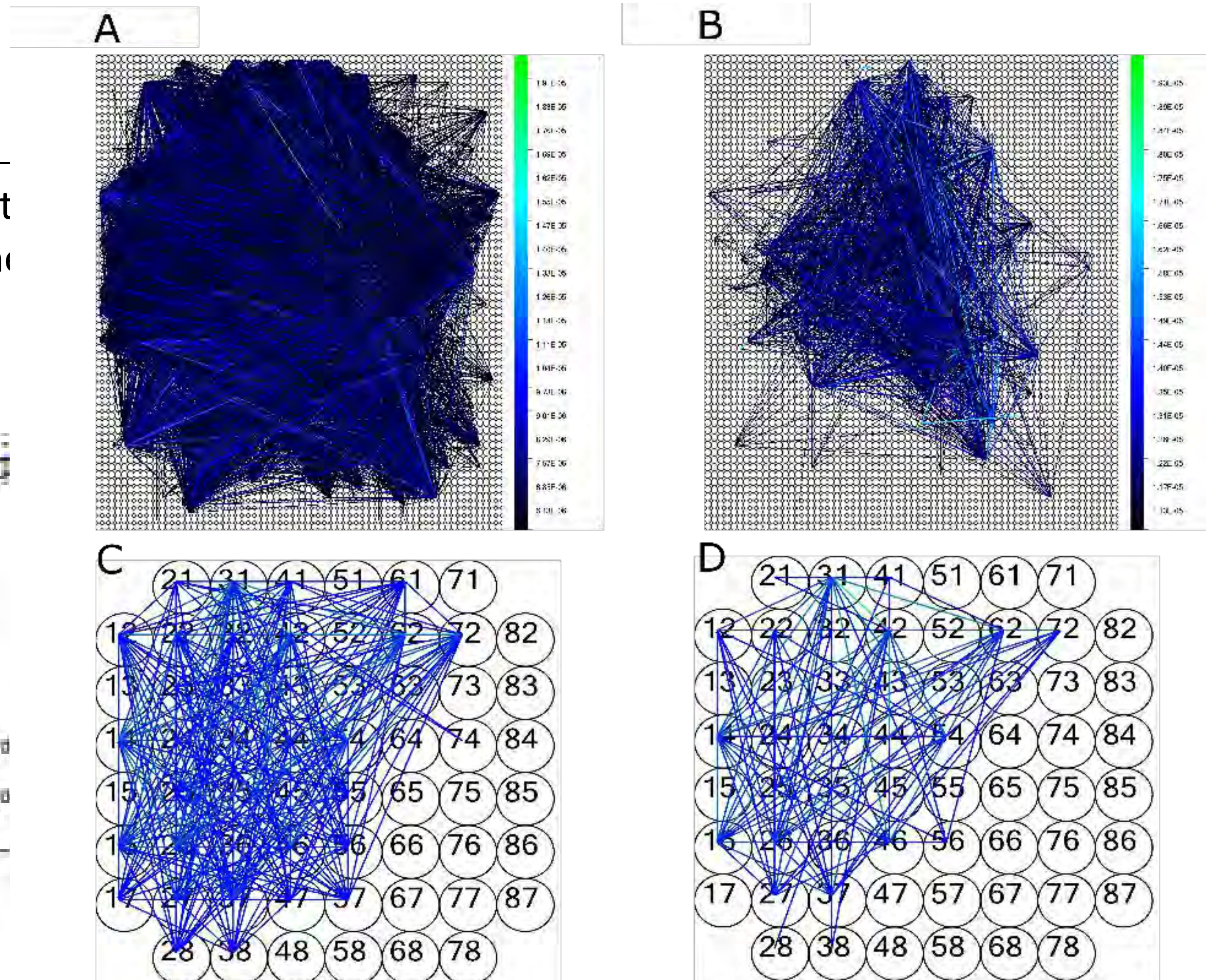
Functional-effective connectivity methods: Transfer Entropy revisited

Vito Paolo Pastore, PhD student

$$TE_{y->x} = \sum_{x_t x_{t-1} y_{t-}}$$

- for a reference spike t
- the couple (*k*, *l*) defin





Pastore et al. Frontiers in Neuroinformatics (2016)

Simple cross-correlation revisited

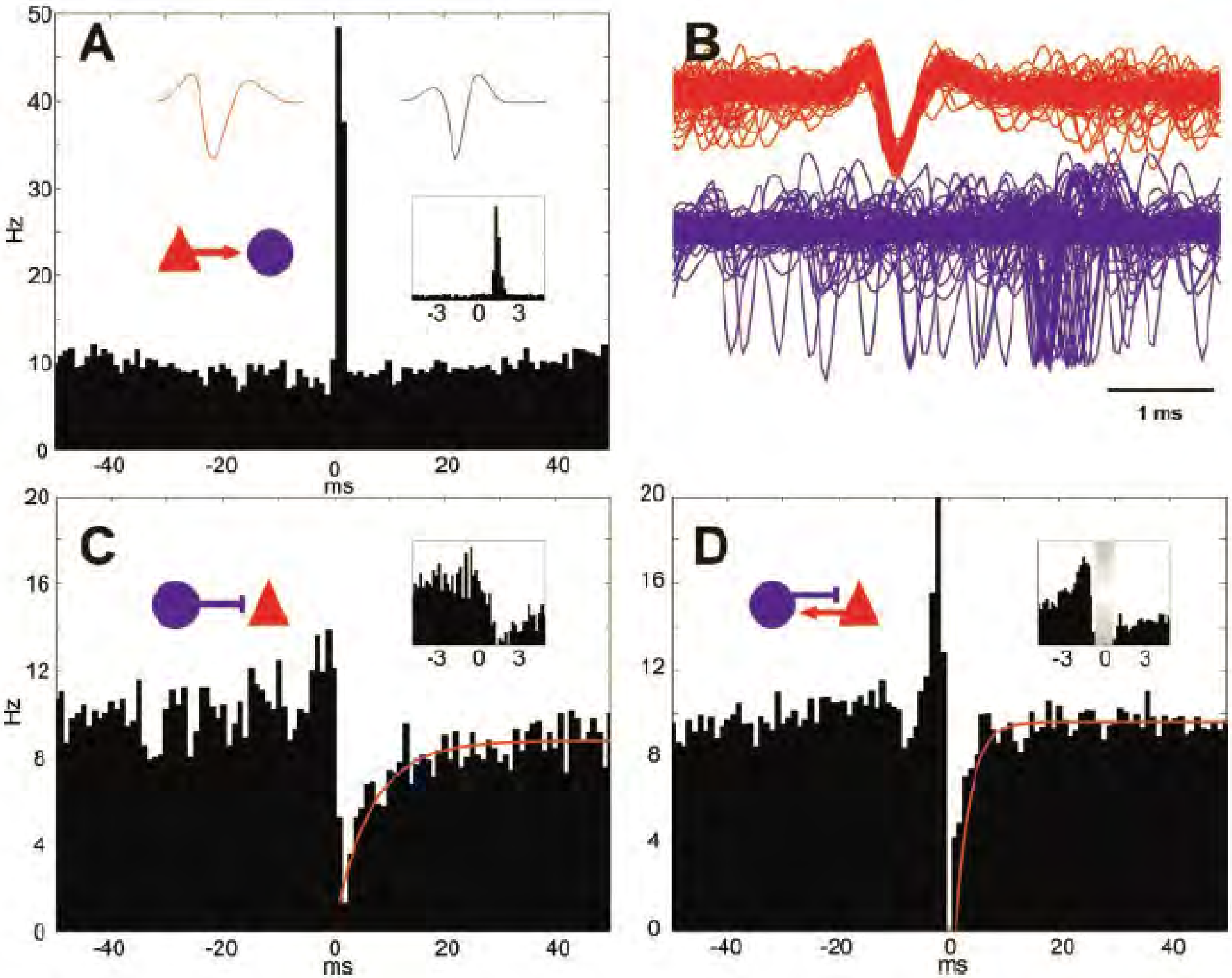
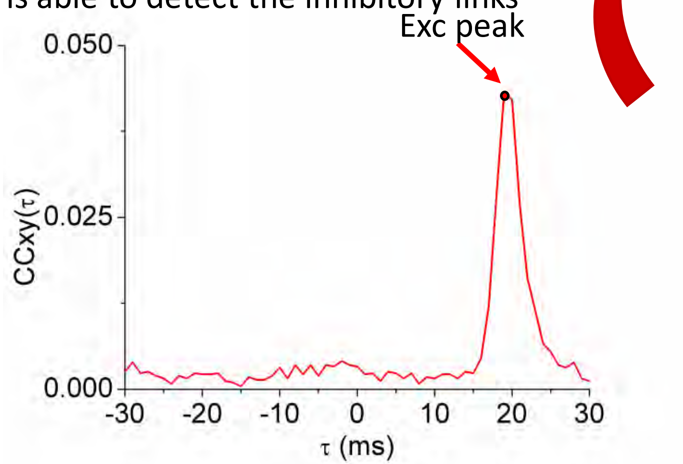


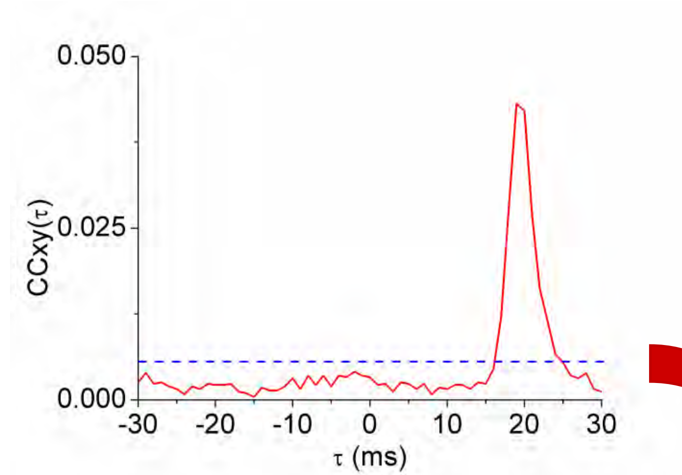
FIG. 2. Short-latency, monosynaptic interactions between neuron pairs. *A*: excitatory drive by a putative pyramidal cell (red triangle). Note large, sharp peak at ~2 ms in the cross-correlogram. Reference event is the spike of the putative pyramidal neuron (*time 0*). *Inset*: higher temporal resolution of the histogram. Averaged waveforms of the units (filtered: 600 Hz to 5 kHz) are also shown. On the bases 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).

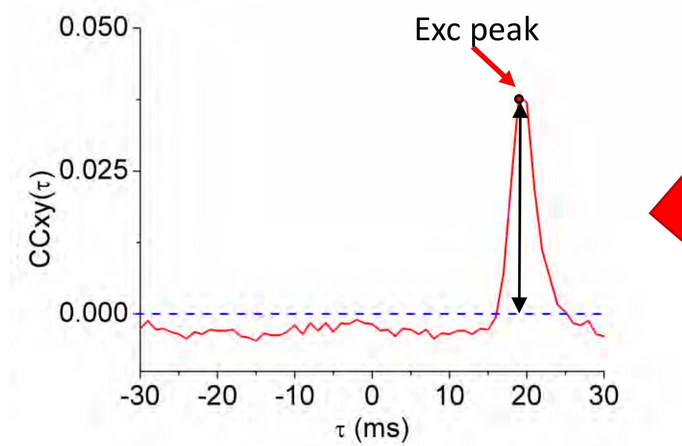
Functional-effective connectivity methods: Cross-Correlation revisited

$$C_{xy}(\tau) = \frac{1}{\sqrt{N_x N_y}} \sum_{s=1}^{Nx} \sum_{ti=(\tau-\nabla\tau/2)}^{(\tau+\nabla\tau/2)} x(t_s)\, y(t_s - t_i)$$

CC is able to detect the inhibitory links





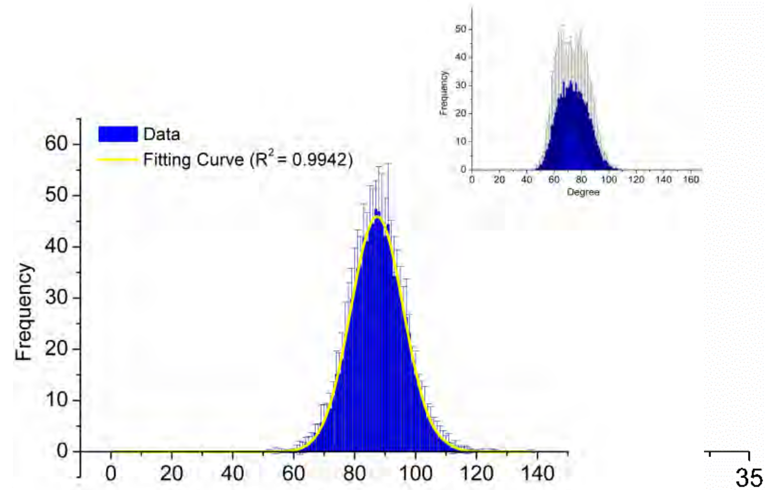


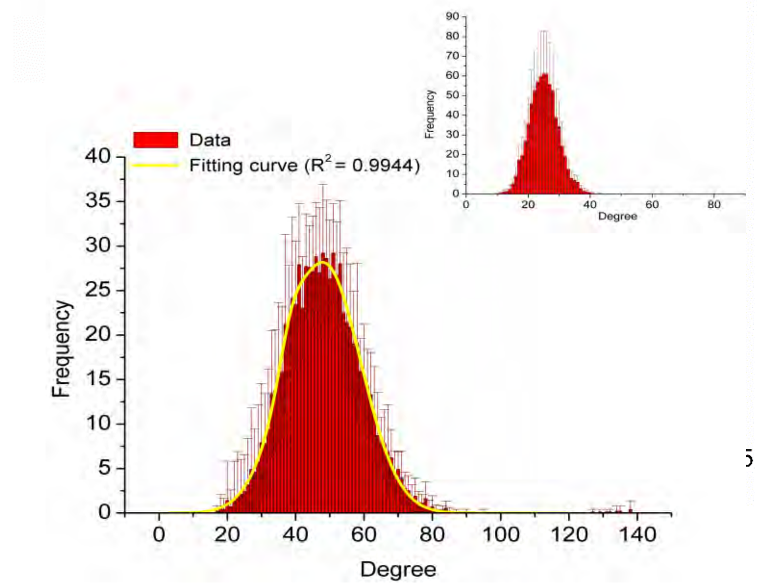
$$CC_{xy\_peak\_value} = \max_{\tau = [-W/2,\ +W/2]} \left\{ \left| C_{xy}(\tau) - \frac{1}{W} \sum_{\tau=-W/2}^{\tau=+W/2} C_{xy}(\tau) \right| \right\}$$

Pastore et al., 2018, Plos Computational Biology

Filtered Normalized Cross-Correlation Histogram (FNCCH)

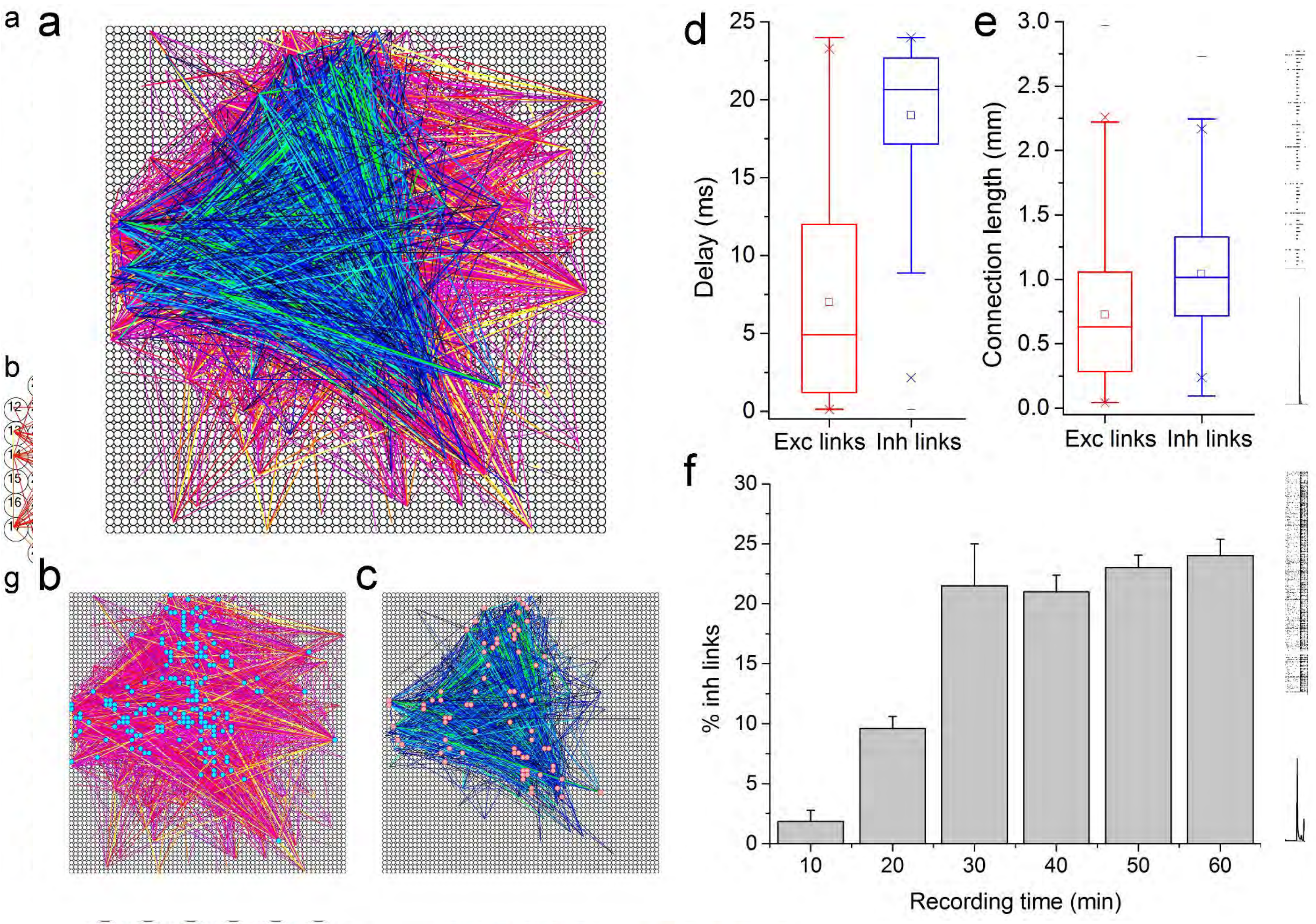
- It has been validated on in silico neural networks with **<u>1000</u>** neurons
- Identification of inhibithory links!
- Improvement of excitatory link detection
- Very good delay reconstruction
- Very good degree distribution reconstruction



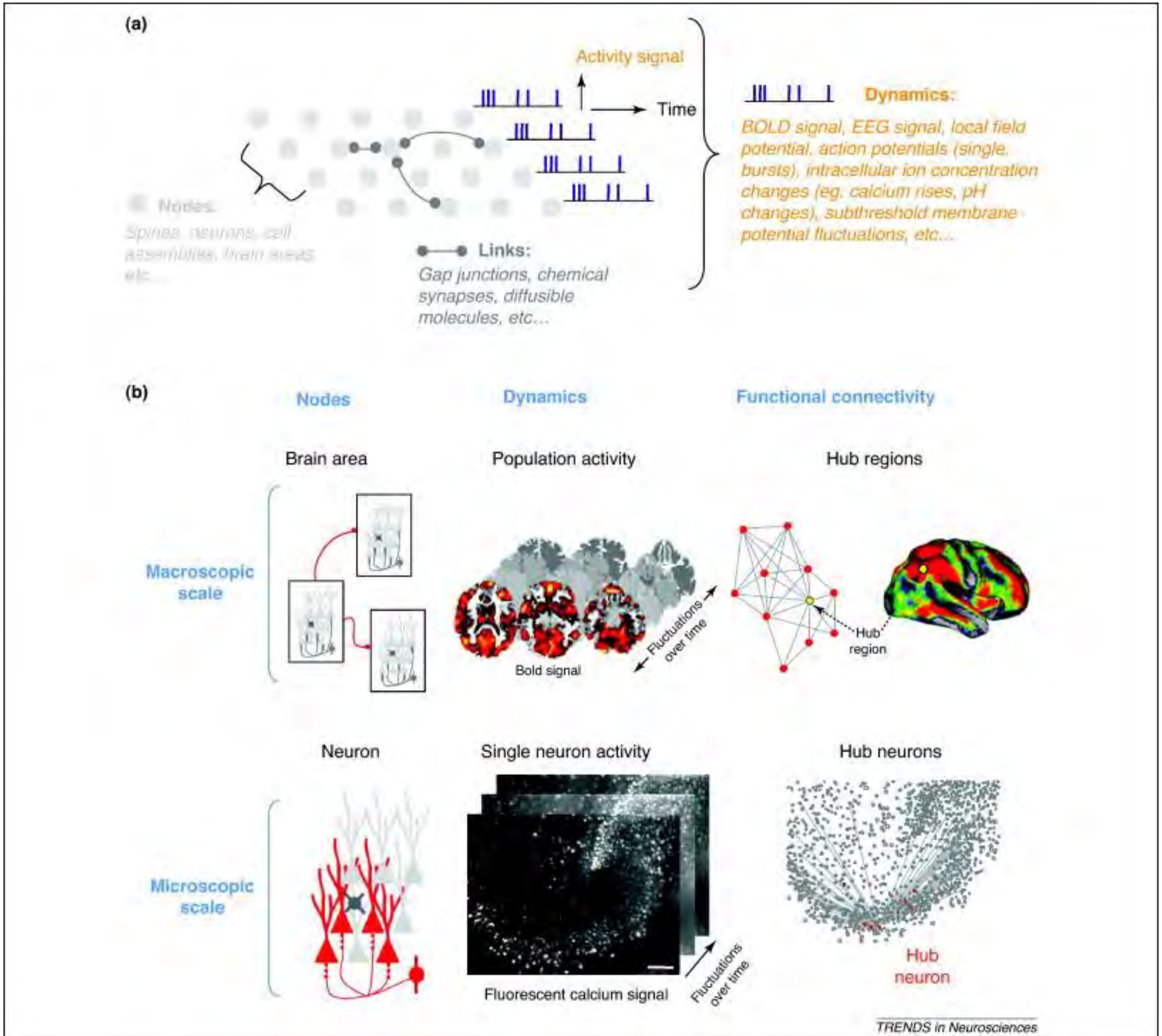


Pastore et al. Plos Comp. Biol. (2018)

Functional-effective connectivity



Connectivty & dynamics



Graph Theory can be used to:

explore and compare structural and functional brain networks

classify => topology

Feldst, S., Bonifazi P., Cossart R., "Dissecting functional connectivity of neuronal microcircuits: experimental and theoretical insight". *TINS, 34*, 225, (2011)

Graph theory and connectivty

Topology of the network

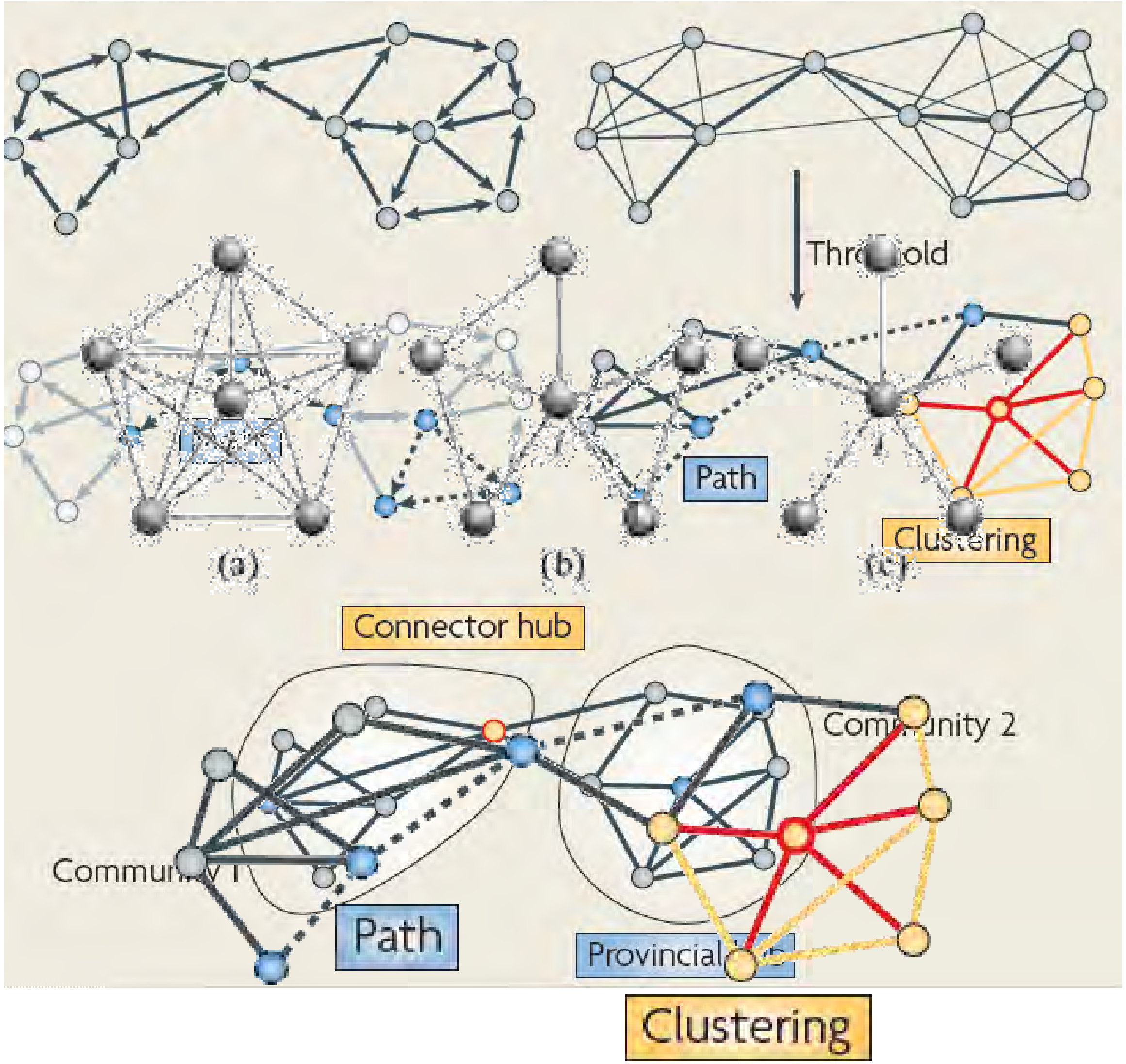
parameters

Clustering Coefficient (CC):

quantifies the number of connections that exist between the nearest neighbours of a node.

Mean Path Length (PL):

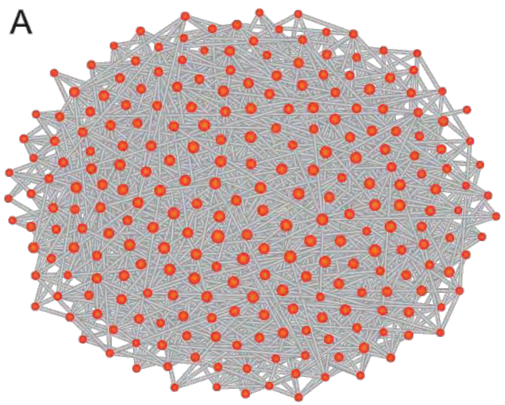
minimum number of edges that must be traversed to go from one node to another.



Bullmore, E., Sporns, O., (2009) "Complex brain networks: graph theoretical analysis of structural and functional systems". *Nature,* , 10, 186-201.

Network models

Random (RND) networks

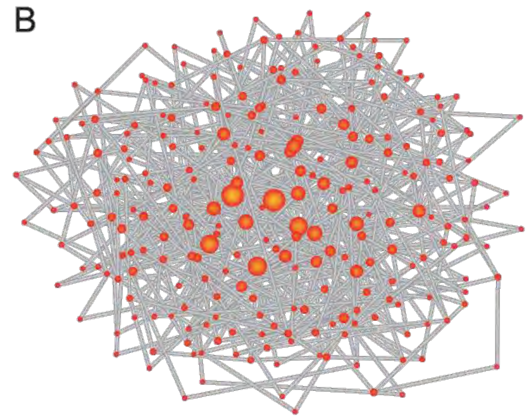


Each pair of nodes has an equal probability of being connected

Degree distribution follows a Gaussian distribution

Erdos P., Renyi A. Publicationes Mathematicae, Vol. 6, pp. 290-297, 1959

Scale-Free (SF) networks

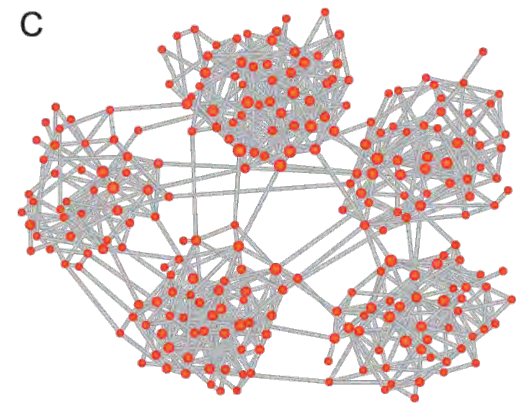


Few nodes connected to many others (hubs)

Degree distribution follows a power law

Barabási A-L., Albert R. Science, Vol. 286, pp.509-512, 1999.

Small-World (SW) networks



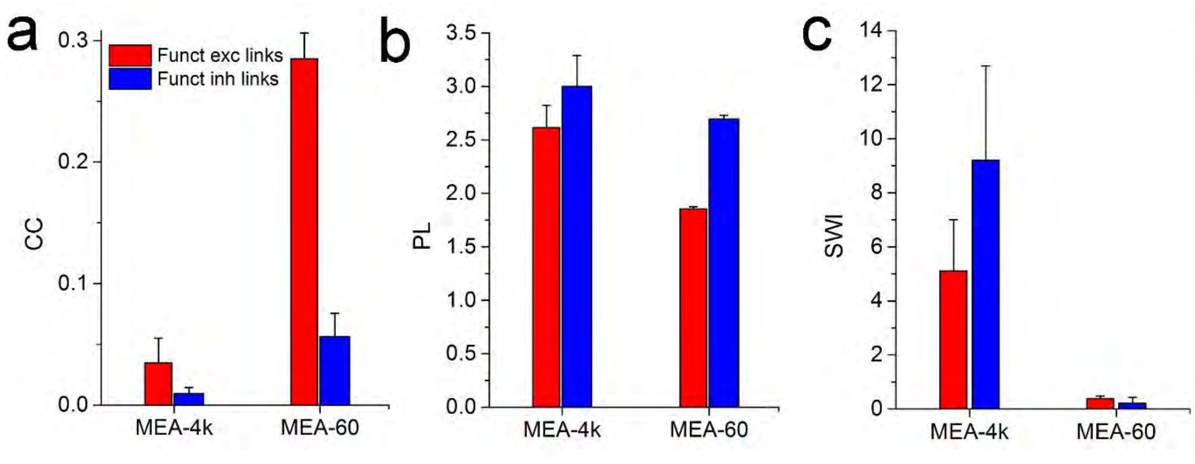
Between totally regular and random

Highly clustered but short path length

Watts D.J., Strogats S.H. Nature, Vol. 393, pp.440-442, 1998.

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 **<u>remote non invasive</u>** neuro modulation?

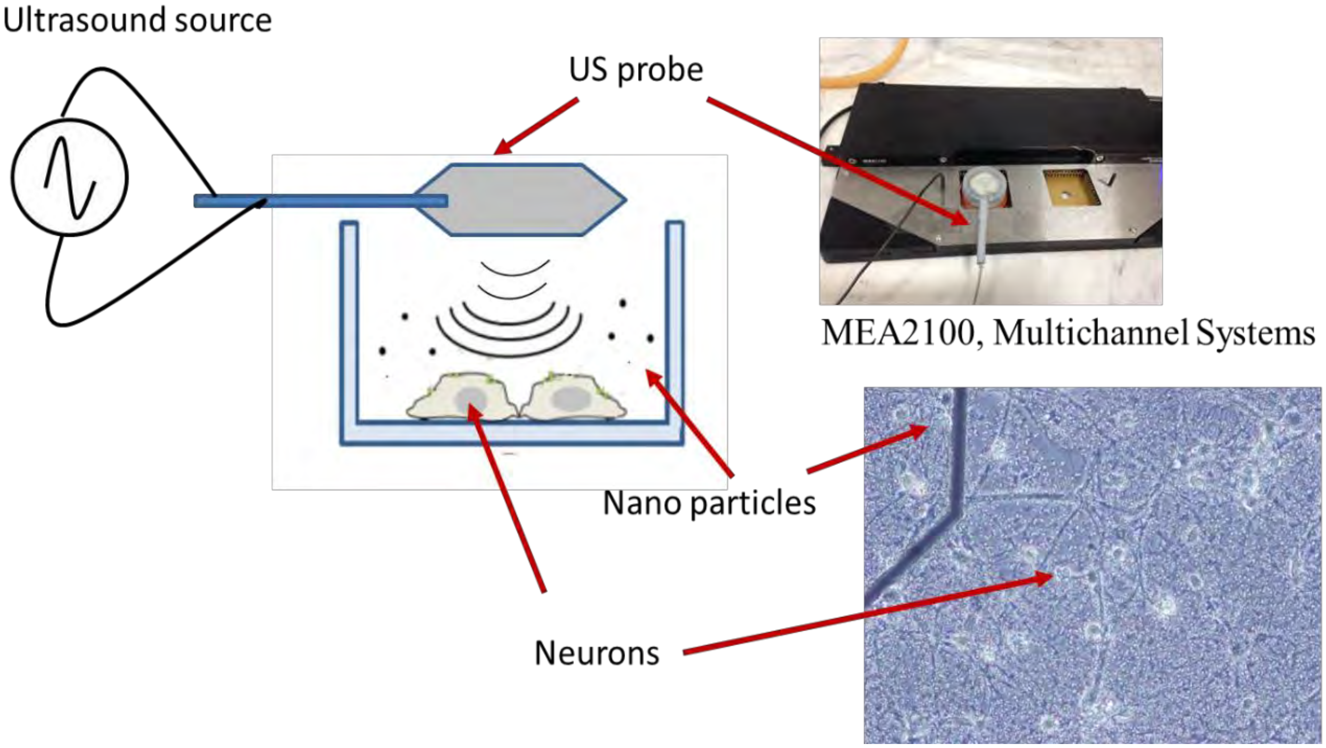
Optogenetics and optical stimulation could be a partial answer.
Pros: specificity
Cons: still invasive; it implies a genetic modification of the cells…

Engineered networks with piezo-electric nanoparticles

Camilo Rojas, PhD

Barium titanate nanoparticles BTNP

ultrasound induced stimulation

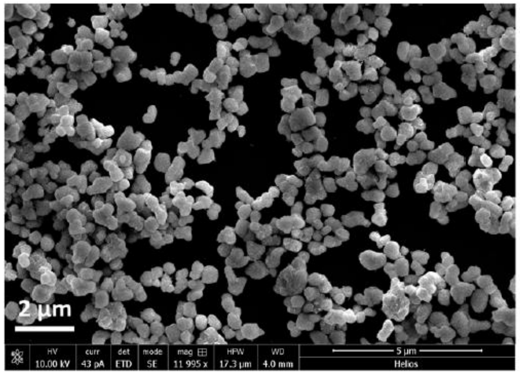


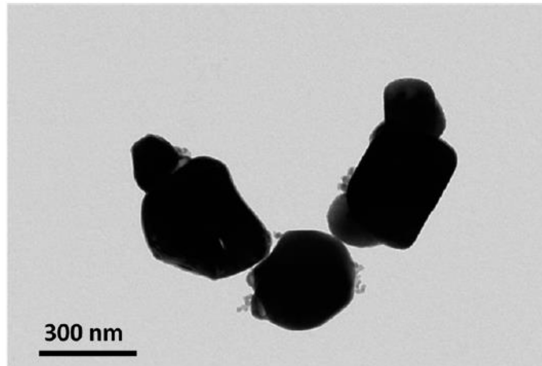
Gianni Ciofani

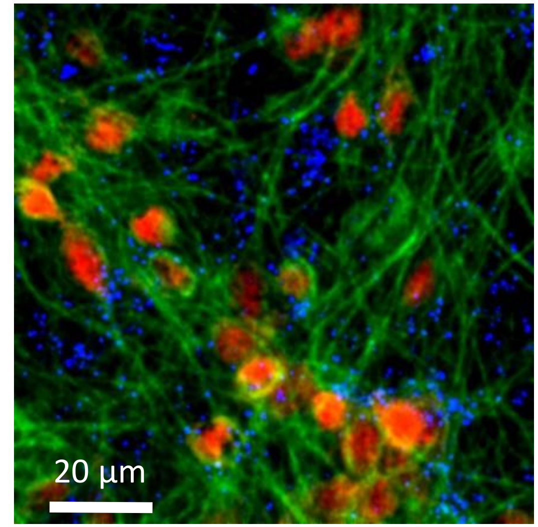
ISTITUTO ITALIANO DI TECNOLOGIA

Rojas et al. J. Neural Eng., (2018)

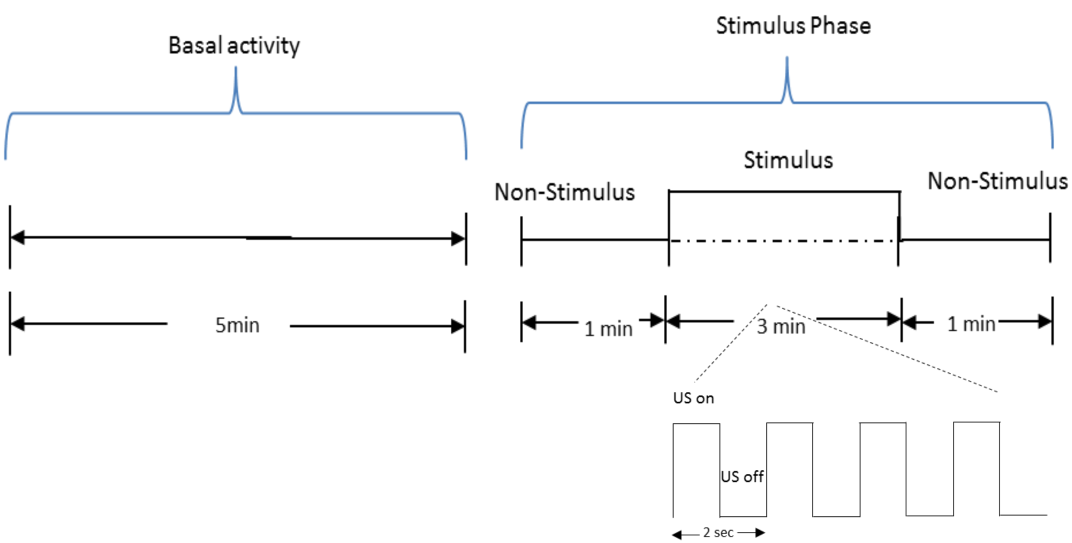
$BaTiO_3$ Nanoparticles







- 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**



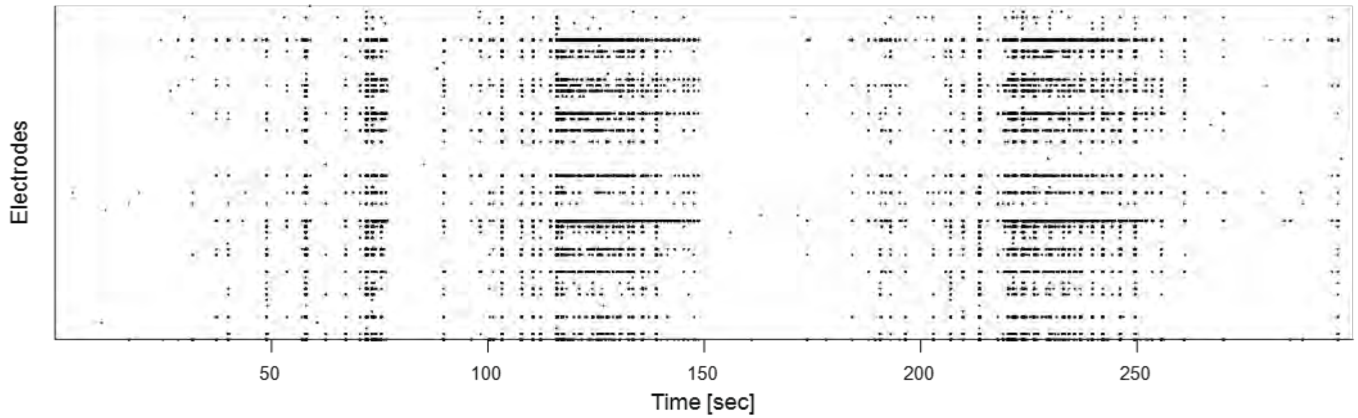
ultrasound pulse train
period: 2 sec
duty cycle: 50%

1MHz ~2kPa

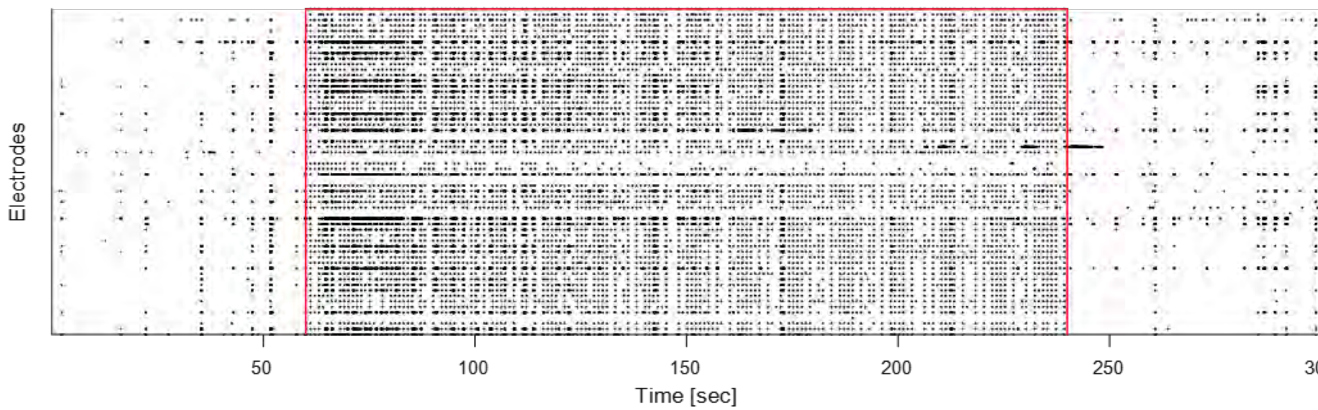
Acoustic stimulation: excitation

BTNP

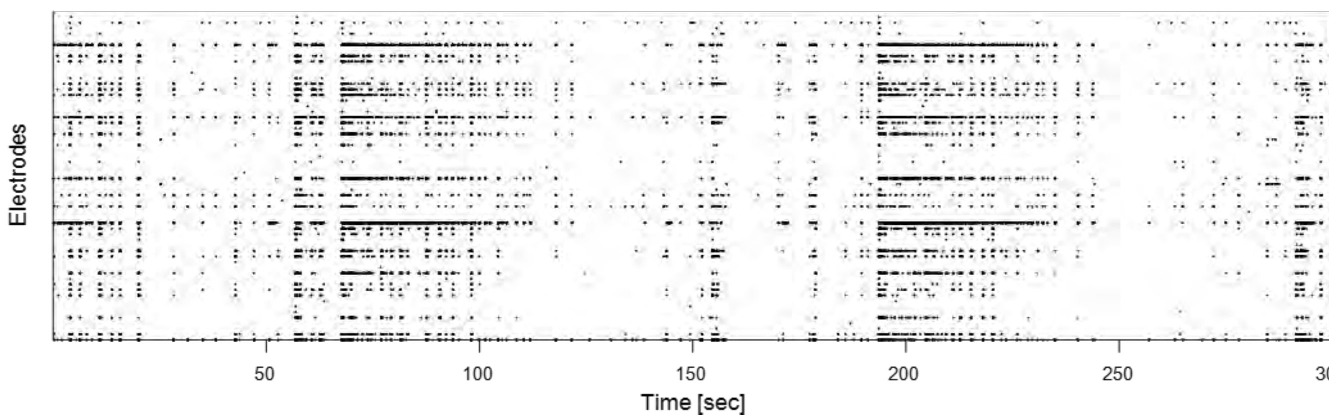
Base



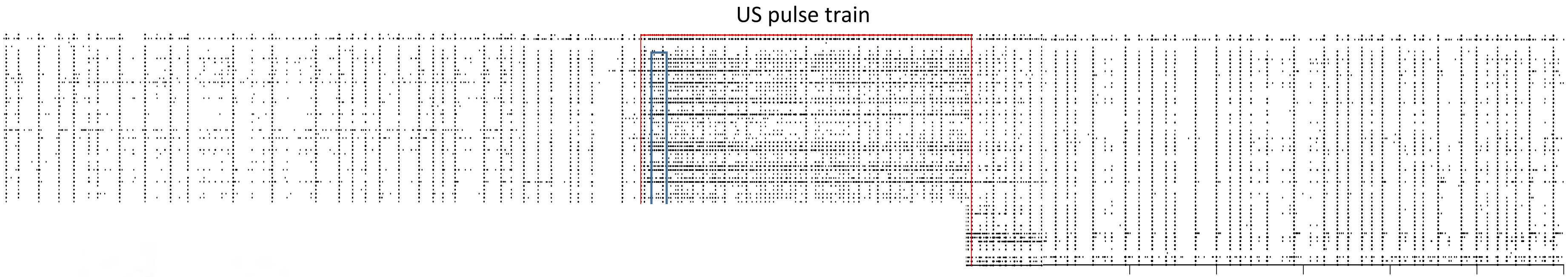
Stim

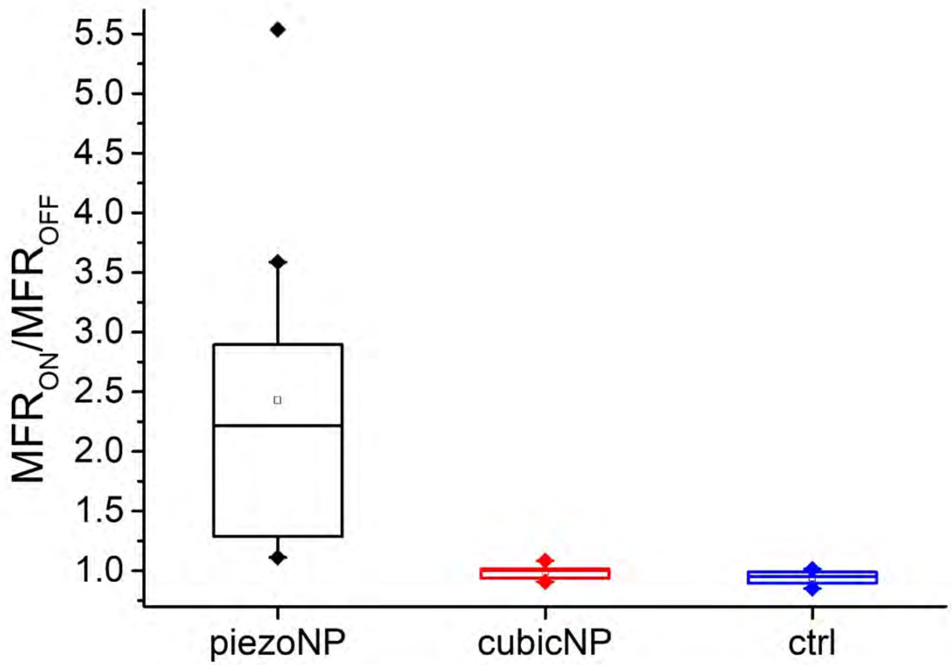


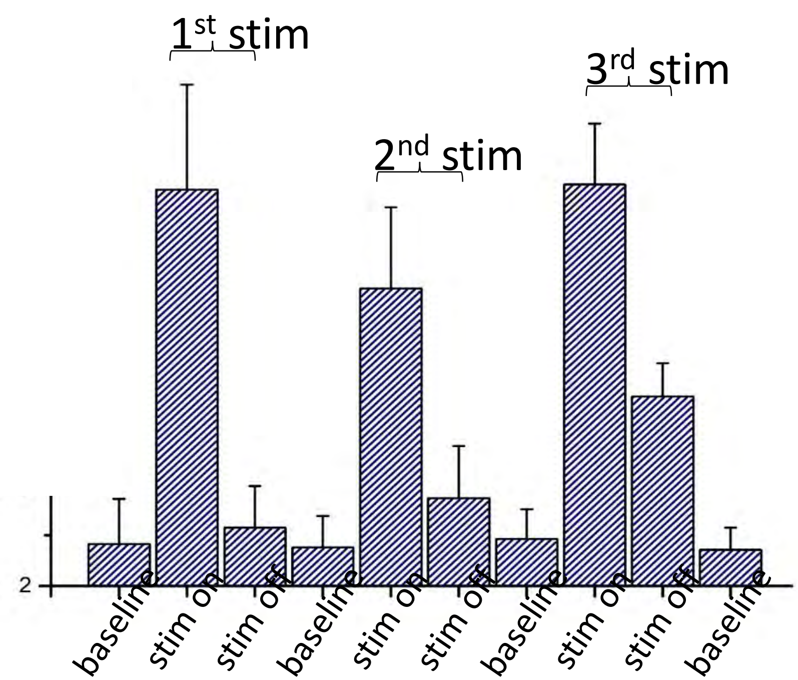
Post



Acoustic stimulation: excitation







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

Which mechanism?

A. Marino *et al. ACS Nano* Vol.9, 7678 (2015)

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

Andrea Andolfi, MS

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:

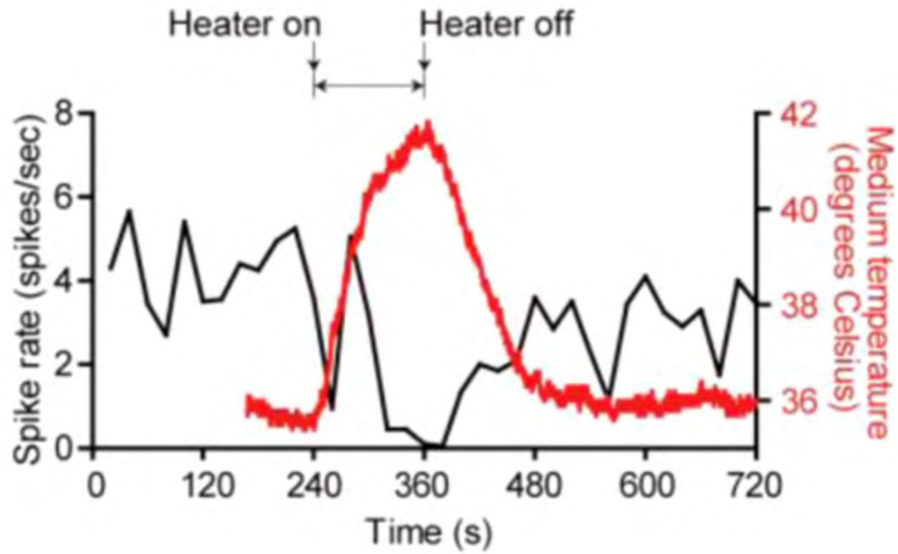
- Cancer therapy
- Biomedical sensing and imaging
- Drug delivery
- Nanophotonics
- **Neural activity modulation**

Yoonkey Nam

KAIST

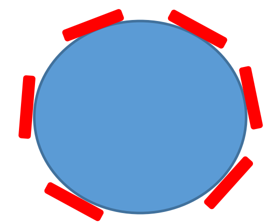
Korean Advanced Institute of Science and Technology

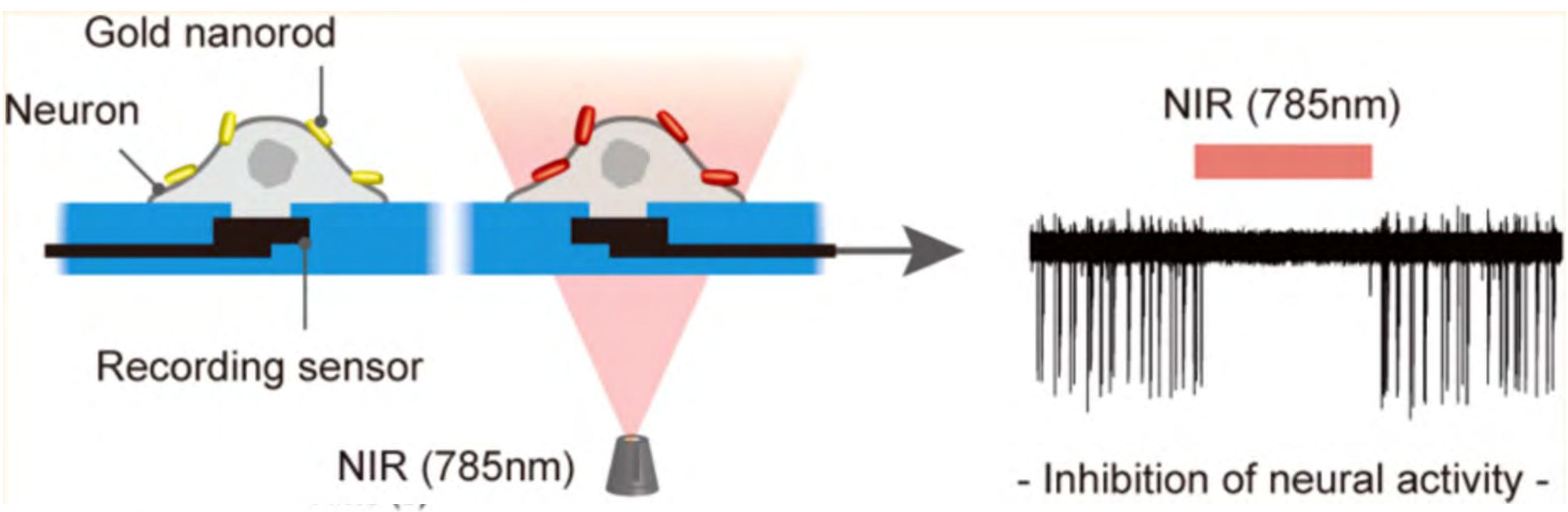
Engineered networks with GNRs: photo thermal inhibition



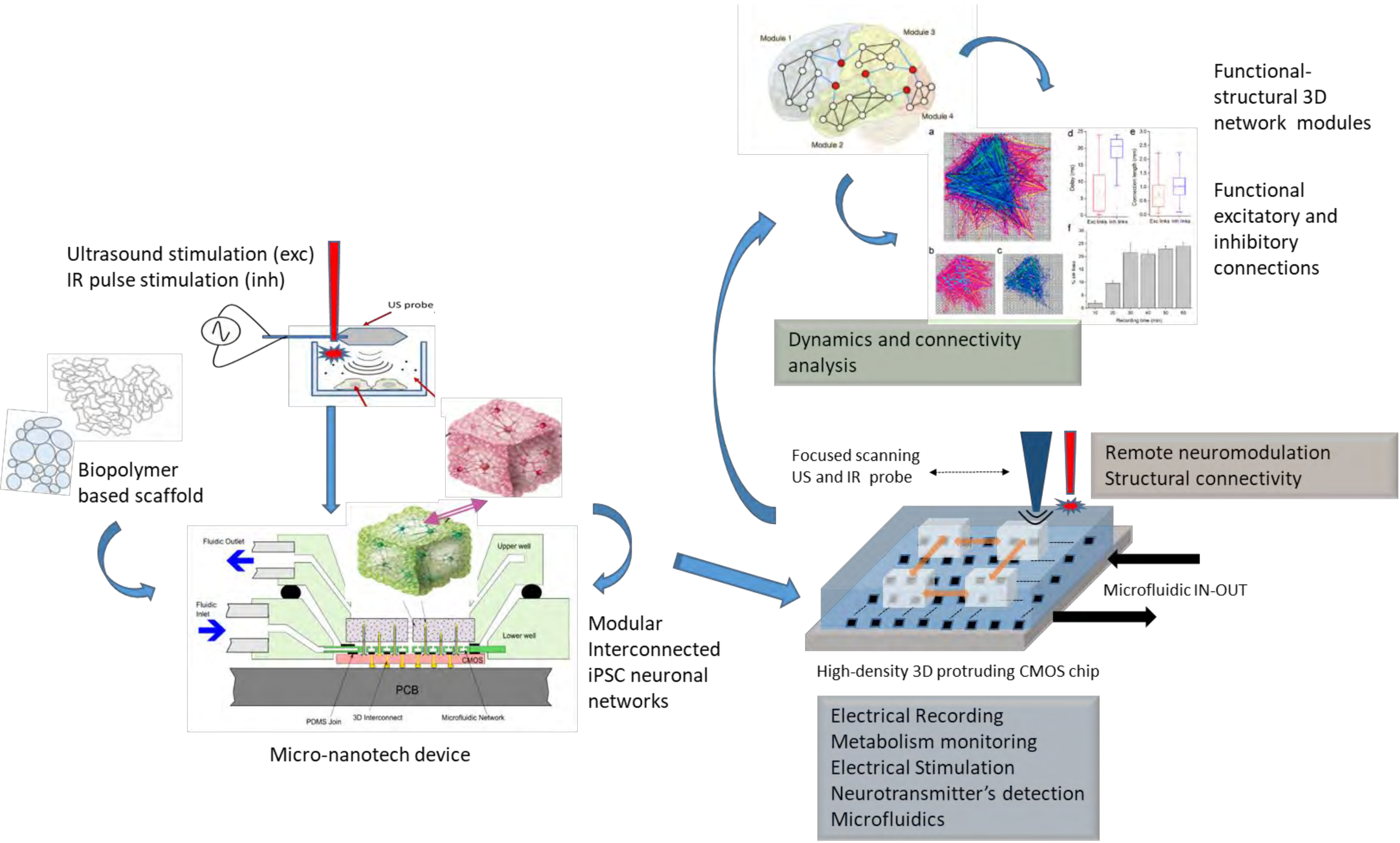
macroscale

Functionalized microbeads





Engineered microsystems: brain-on-a-chip



Summary and conclusions

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

Dibris

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Laura Pastorino (biomaterials)
Pasqualina Farisello (cell biology)

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Thiru Kanagasabapathi
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Michel Decré

www.neuroengineering.eu

Next Edition (8th) June 2020

Thanks for your attention!

School of NeuroEngineering "Massimo Grattarola"