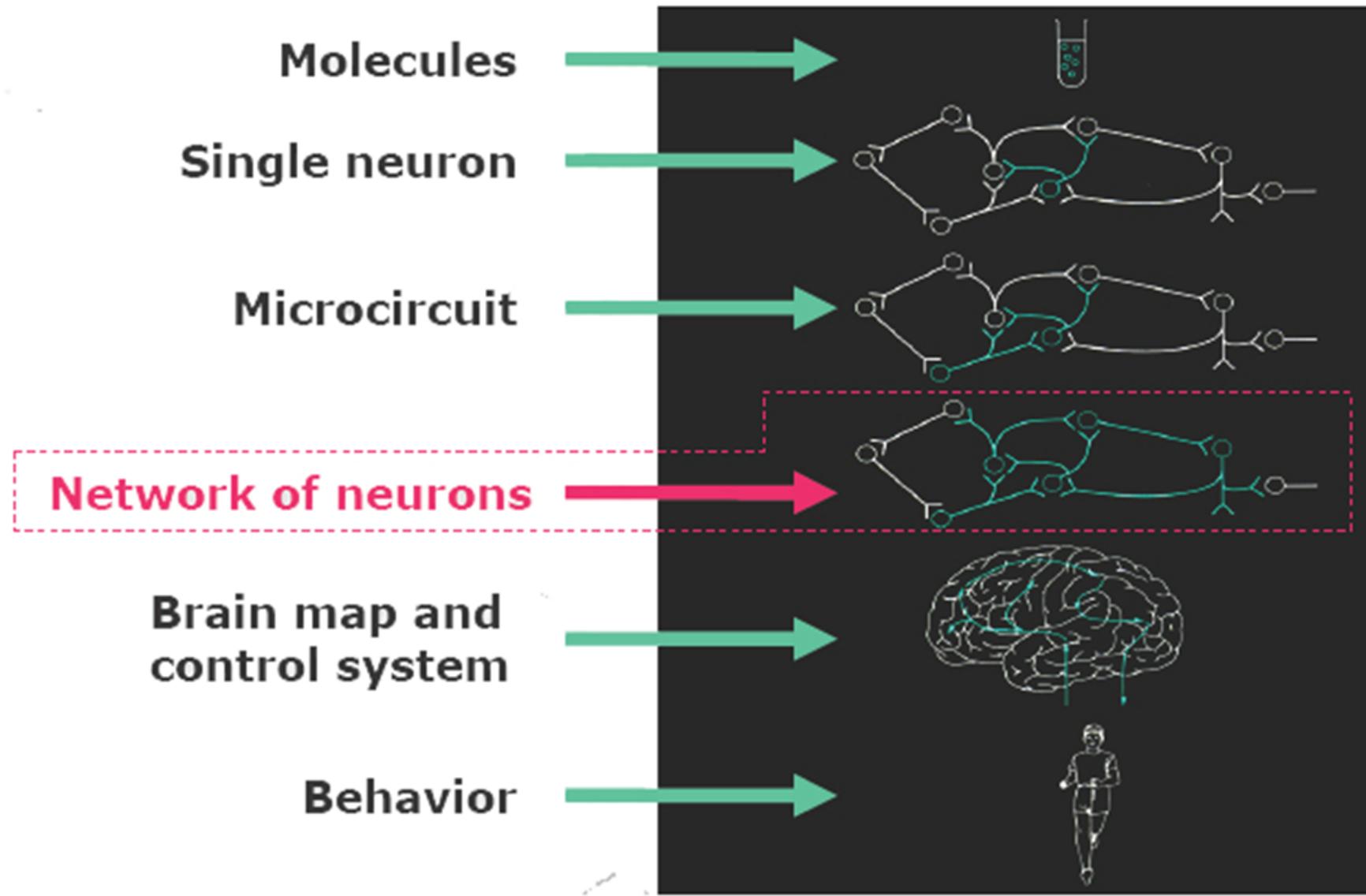




Neuroengineering 2021/22

NEUROENGINEERING FOR BIOLOGY 1-
Electronical tools to interface neuronal networks

Functional scale



GOAL

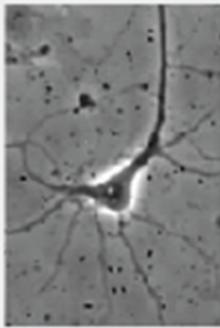
Both the single-neuron level and the CNS-level are well known but the dynamics of neural networks is still far from elucidated. For this reason, studies at this level of analysis are essential to deeply understanding neural pathways.

This question opens some very interesting technological challenges

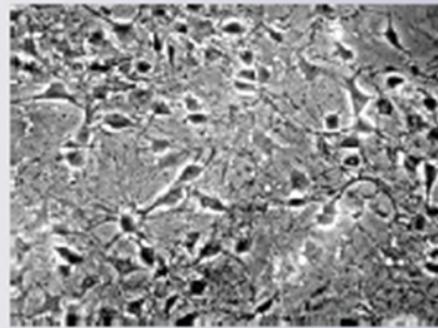
In vitro and In Vivo EXPERIMENTS

in-vitro

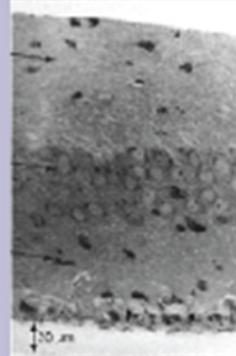
single-neuron
level



neuronal networks of
dissociated cells



brain
slices

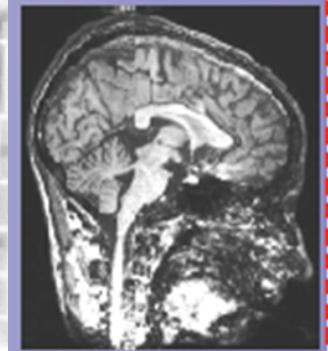


in-vivo

CNS, PNS
level



CNS, PNS
level



Functional connectivity
Modelling complexity
Reduced access

In VITRO EXPERIMENTS

- In-vitro experiments can be used to study the small functional cellular structures:
 - Slices: are functional naturally grown tissues extracted from the brain and then analyzed
 - Cultured neurons: are embryonic dissociated neuronal cells which are cultured in vitro and built the neuronal network directly in vitro. The system is then completely autonomous but it is only a model of natural functional networks.
 - Human patient specific IPS cells differentiated to neuron-like... great challenge for future research!
- **Multimodal approach is pursued to get the maximal information rate usually the different approaches are consecutive in time (depending on the goal)**

Technical specifications

The users' requirements:

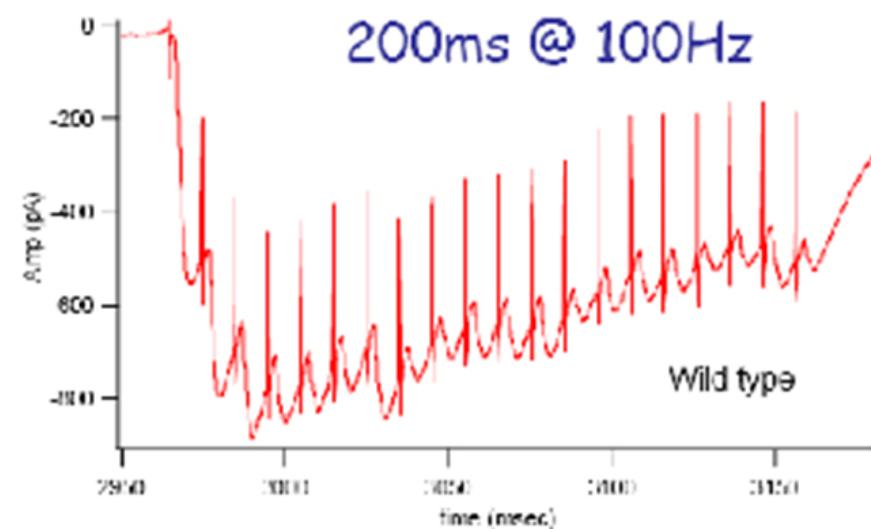
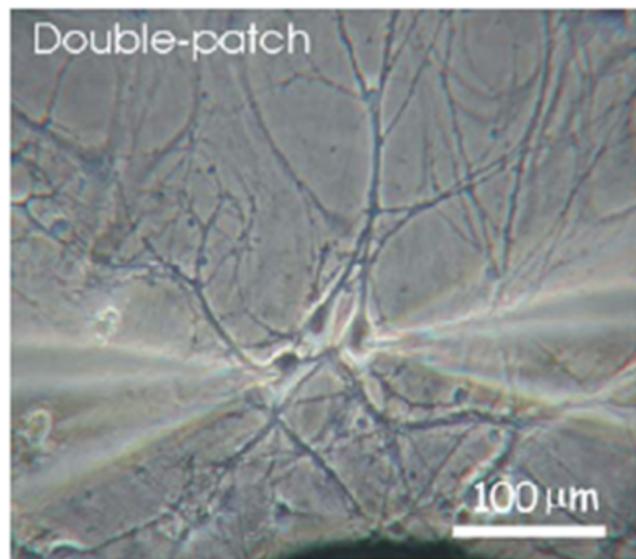
1. Simultaneous record + stimulate of **hundreds individual** neurons
2. Long acquisitions (days and months): maintain stable contact
3. Monitor transmembrane potentials (-80;+30mV)
4. SNR able to catch subthreshold transmembrane potentials ($\pm 0.5\text{--}10$ mV with a rise time of <1 ms and a slow decay time of 100–1,000 ms), and spike occurrence and spike oscillations (up to 50Hz)
5. record APs with amplitudes of ~100 mV and duration of 1–500 ms (long APs for recording from cardiomyocytes).

SPIRA AND HAI Multi-electrode array
technologies for neuroscience and
cardiology NATURE NANOTECHNOLOGY
VOL 8 FEBRUARY 2013

Intracellular electrophysiology

Traditionally...

The functional properties of neurons have been investigated using conventional electrodes , such as glass micropipettes, thus allowing neurophysiologists to disclose a detailed picture about the single cell properties, e.g. the receptor sensitivity and ion channel gating



Intracellular electrophysiology: patch clamp

Complete description of cause-effects links

Correspondence between morphology and function

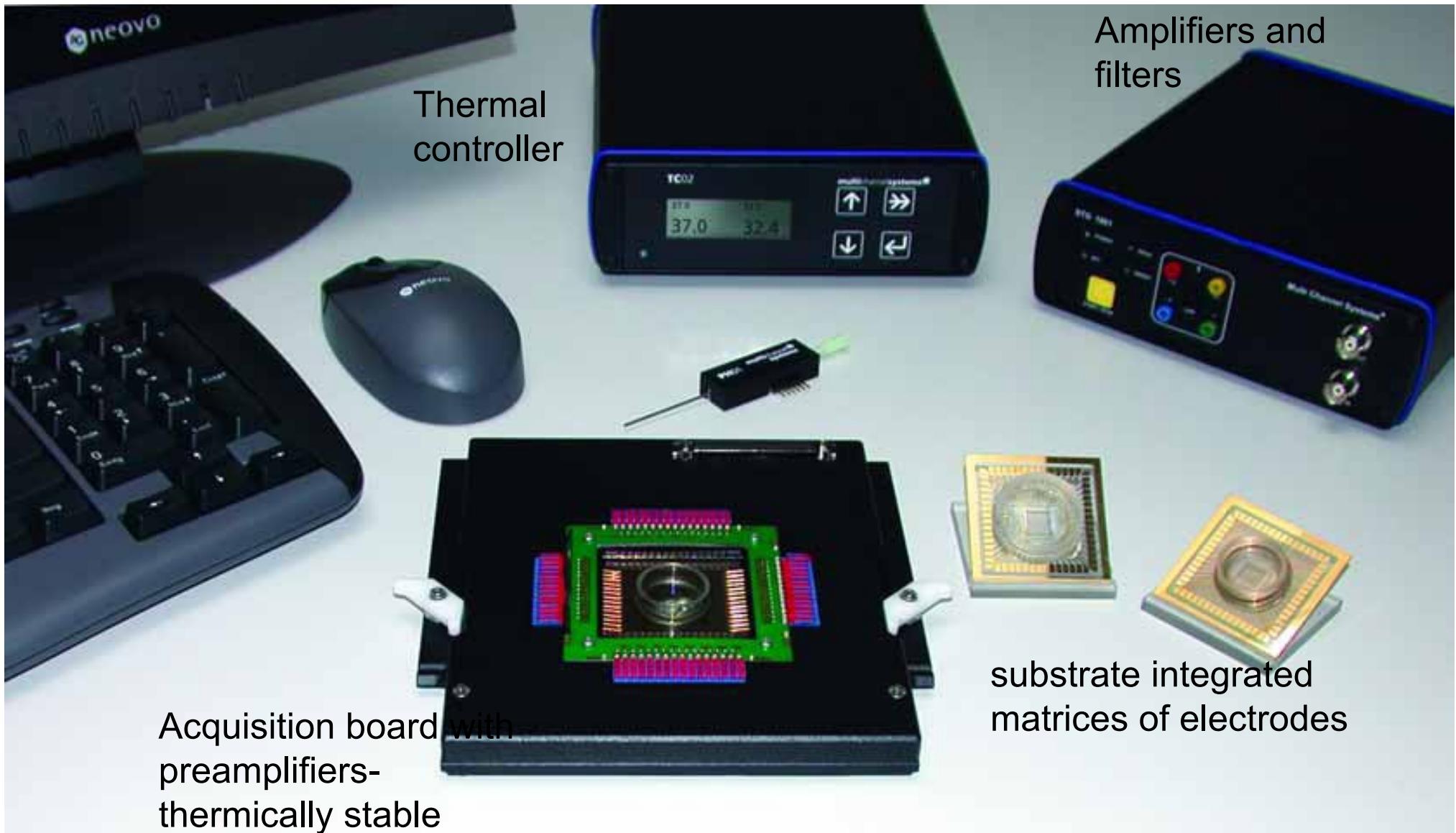
Accurate readout of the entire dynamic range of voltages without distortion

Invasivity -> short registration, non repeated

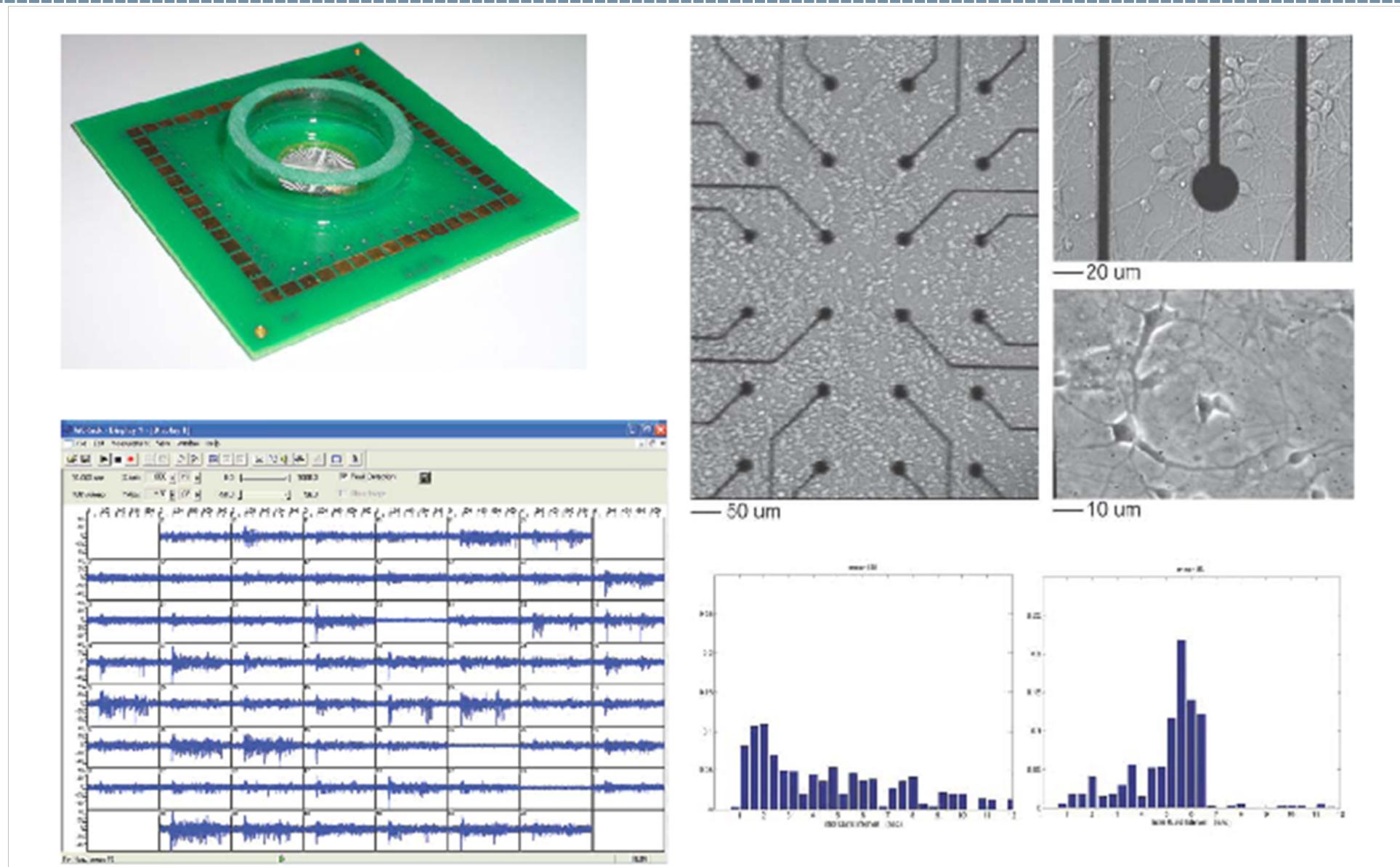
Encumbrance of manipulators -> contemporary registration of few neurons

Mechanical and biophysical instability -> cannot be used to monitor long-term electrophysiological correlates of plasticity

Multi electrodes arrays (MEA)



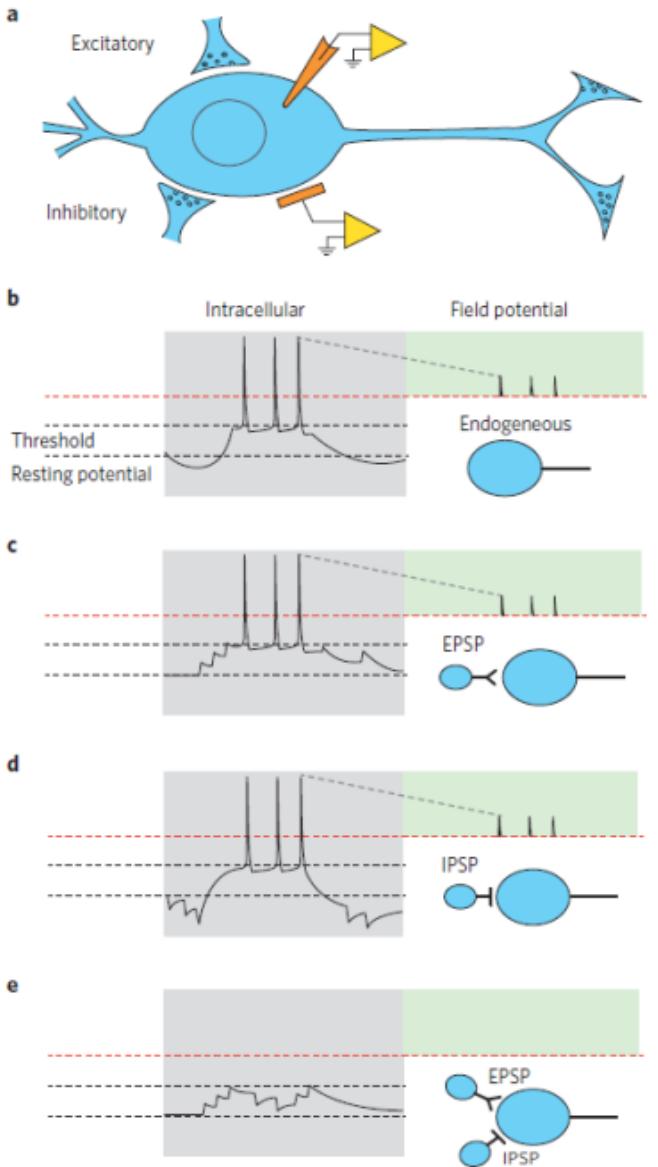
Non-implantable MEA recordings



The extracellular space is conductive as well, and although the resistance is very low, it is not zero.

According to Ohm's law ($V=R*I$), the extracellular current results in a small voltage that can be measured with extracellular electrodes. Extracellular signals are smaller than transmembrane potentials, depending on the distance of the signal source to the electrode.

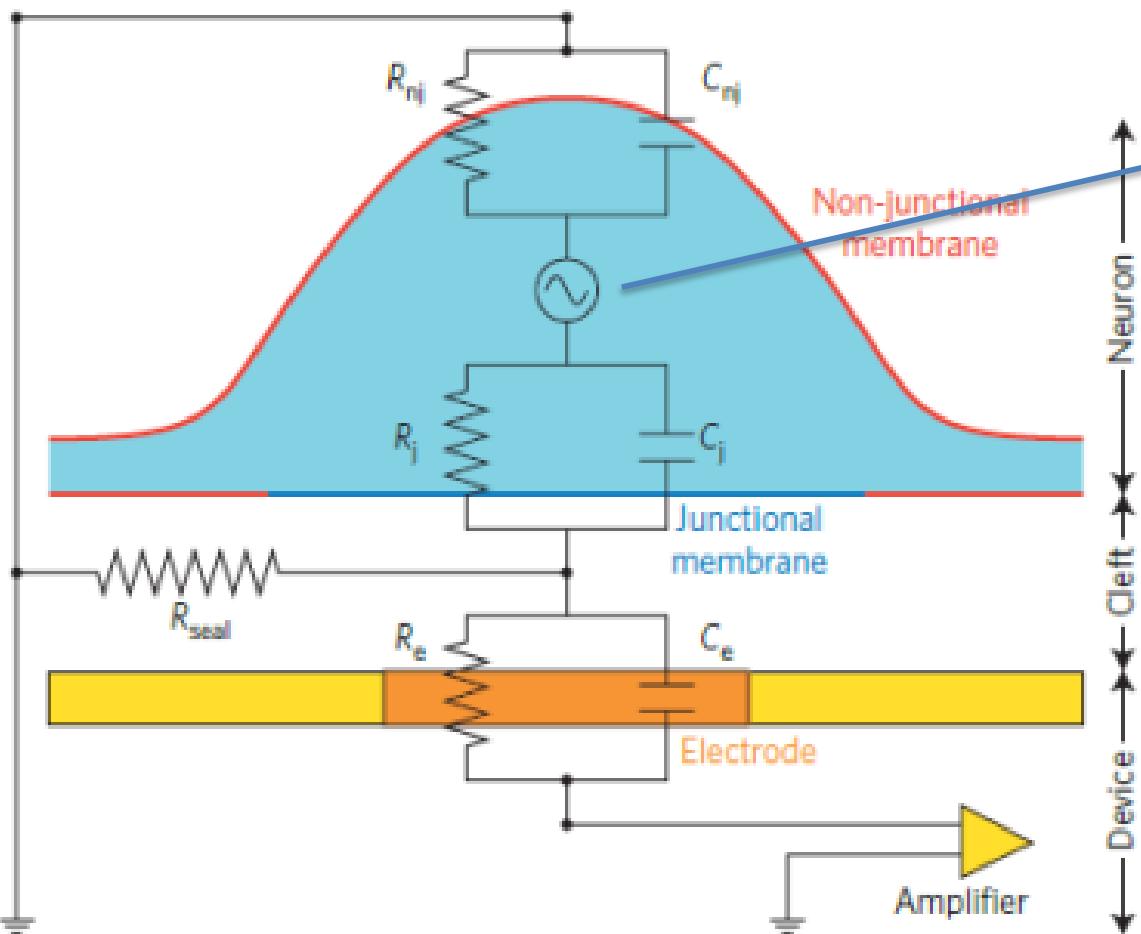
Extracellular recording: the problem of dark neurons



“Whatever sorting algorithm is applied, it remains the limit that MEA recordings could not provide information on as to whether a firing of an individual neuron is triggered by endogenous mechanisms, a barrage of incoming excitatory inputs or the cessation of inhibition... this information is typically available only to intracellular recordings across neuron membrane.”

Spira and Hai, 2013

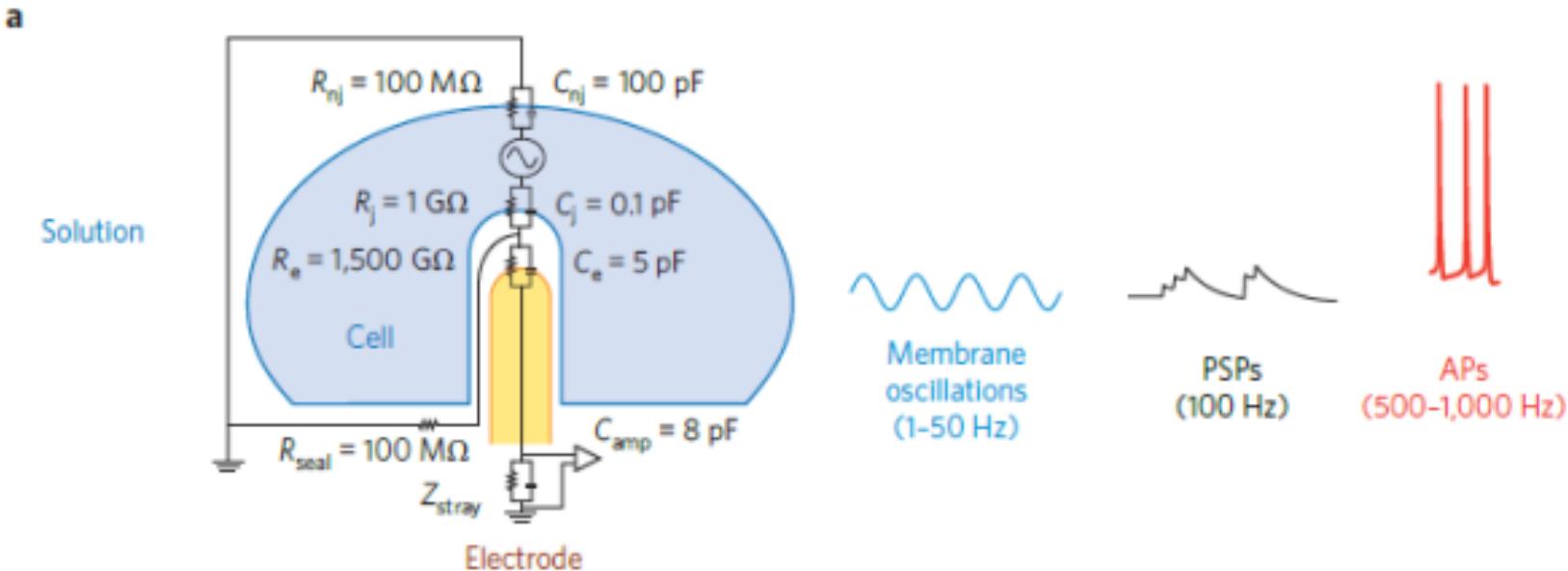
Electrical circuit analogue of neuron/electrode interface



Current generator
which simulates the
action potential

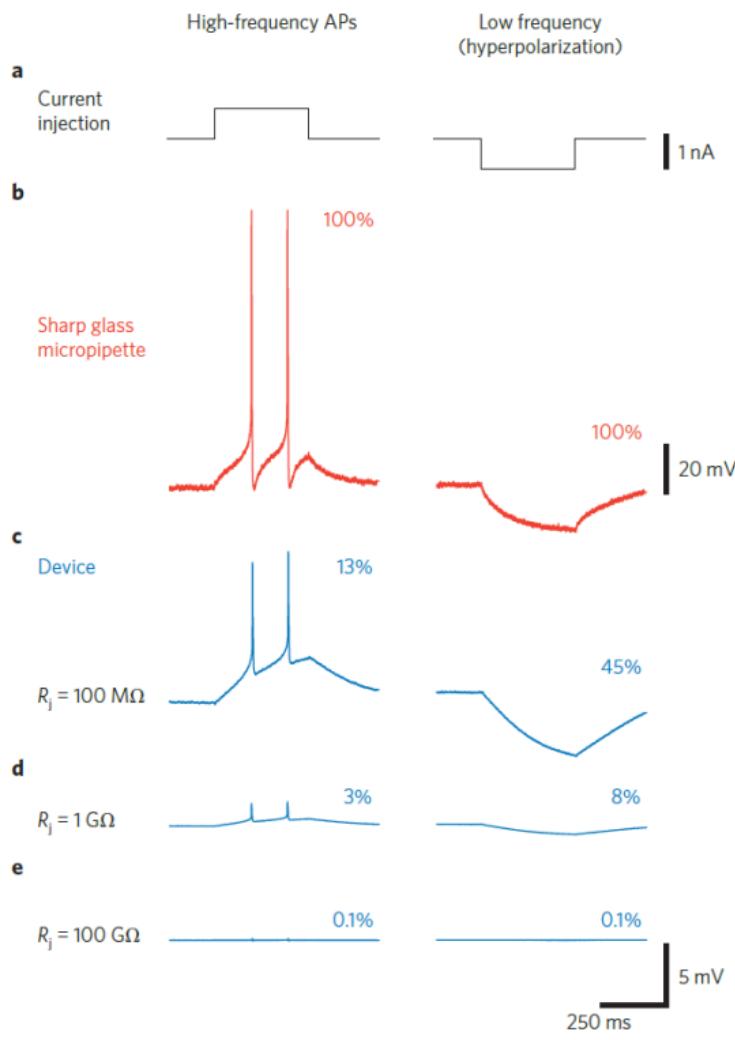
The adherence of the junctional
membrane on the electrode pads
improves the SNR

MEA: SNR and distortion



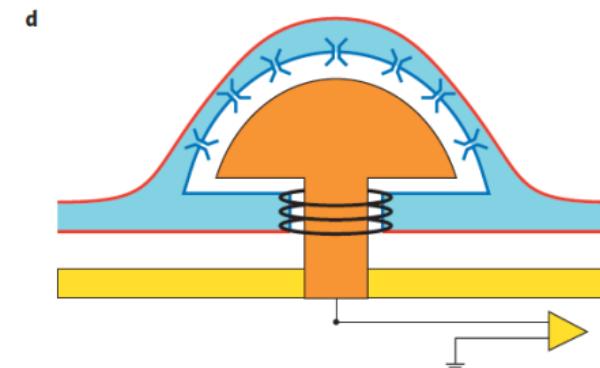
COUPLING: ratio between the maximal voltages recorded by the device in response to the maximal voltage generated by an excitable cell.

SNR and electrode impedance



↑ SNR
↓ Electrode Impedance
↑ Size of electrodes (size of the junctional membrane)
↓ spatial resolution

POSSIBLE IMPROVEMENT IN ELECTRODE:
gold Mushroom-shaped protruding microElectrode gM μ E-based

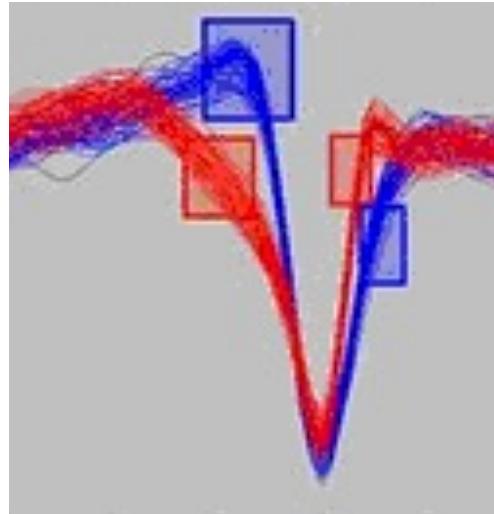


Spike sorting

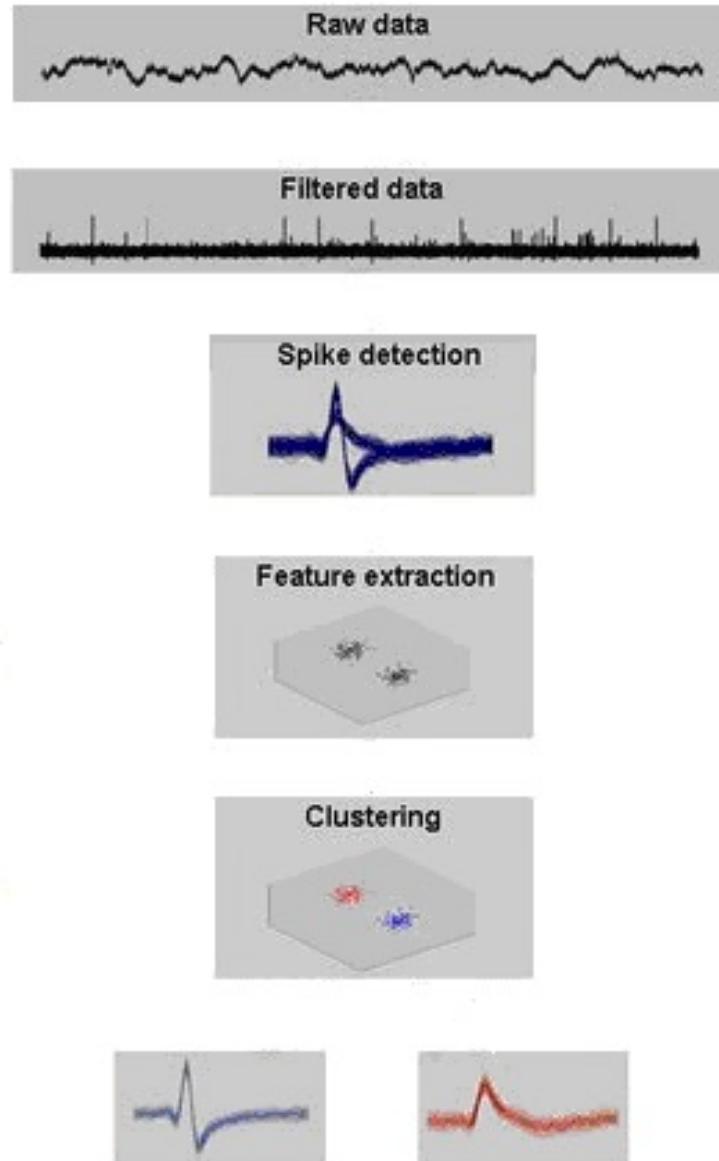
Two cells afferent to the same electrode will in general have a different covered area.

Even if they cover the electrode in the same way, their spike waveform will be different because in general they have a different nature and ionic channel density (V_j).

Assumption: the shape of the spike of each neuron is stationary



Spike sorting

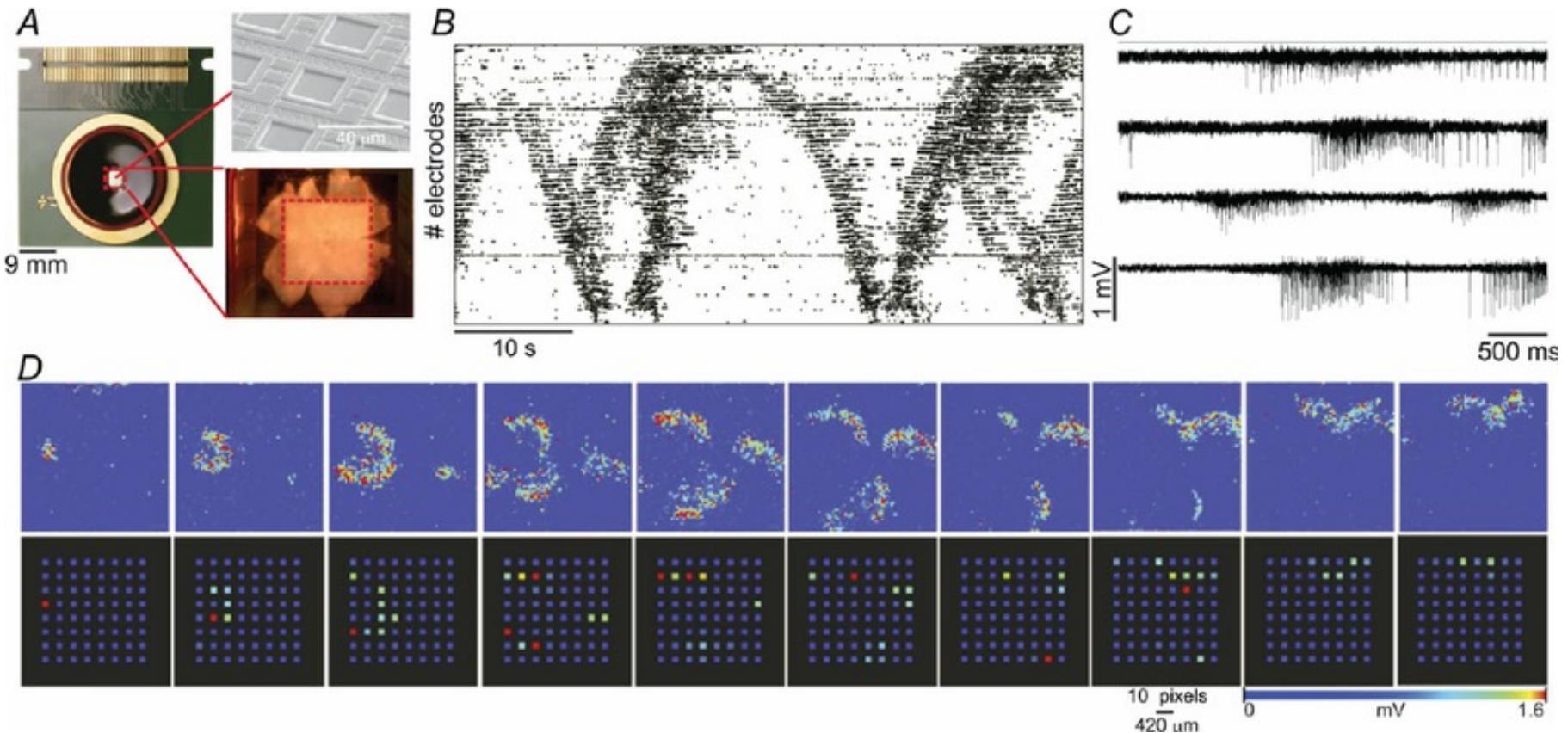


High density MEA (Active Pixel Sensor APS MEA)

GOAL: increase the spatial resolution

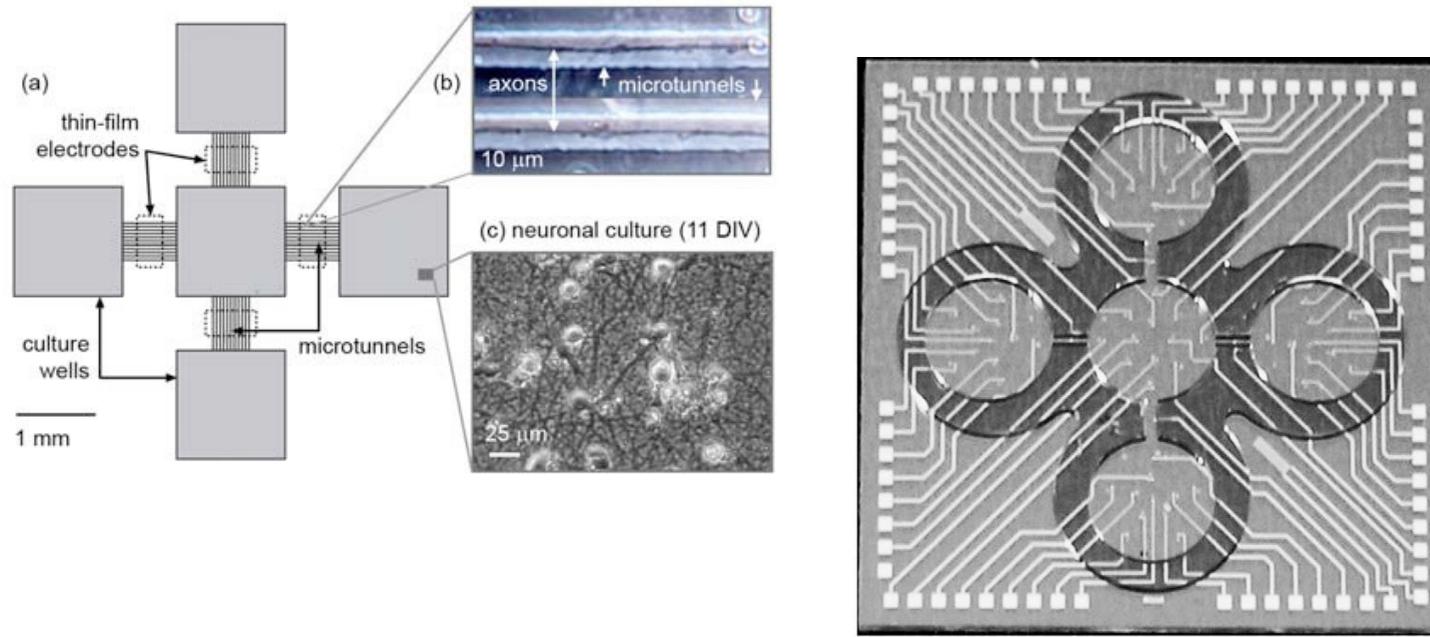
WEAKNESSES:

- worsen the signal-to-noise ratio
- Great deal of computational power to extract data and to sort them out



MEA stimulation

Low selectivity: medium is conductive
Clustering microfluidic solutions to confine stimulus



Pros and Cons

Spatio-temporal recordings of network activity

Large scale acquisitions (network level): modulation of local properties and impact at the network level

Long and repetitive time recordings (up to about one hour)

Low correspondence between morphology and function

High temporal resolution, low spatial resolution

Low selectivity in stimulation

No registration of subthresholds potentials (low SNR) -
>Dark neurons



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NEUROENGINEERING FOR BIOLOGY 2 –
Optical tools for studying neuronal networks

Optical stimulation

Science 1971: Fork Direct laser stimulation

Science 1983: Farber and Grinvald dye mediated stimulation

Neuron 2002: GENETIC METHODS FOR PHOTOSTIMULATION. Another approach to increase the sensitivity of neurons to light is to express a genetically engineered photoactivated sensor in them. (Zemelman et al. 2002)

Cage compounds are molecules that are rendered inactive by the addition of chemical groups, typically nitrobenzyl groups, which are broken up by the absorption of light.

In 2010, optogenetics was chosen as the "Method of the Year" across all fields of science and engineering by the interdisciplinary research journal [*Nature Methods*](#). At the same time, optogenetics was highlighted in the article on "Breakthroughs of the Decade" in the academic research journal [*Science*](#). These journals also referenced recent public-access general-interest video [Method of the year video](#) and textual [SciAm](#) summaries of optogenetics.

«But will optogenetics ever be used to treat disease? Several clinical trials are already underway, the results of which are anticipated eagerly. However, rather than optogenetics being used to treat patients directly, it is more likely that new treatments for brain disorders (and other disorders) will derive from the kinds of fundamental discovery research made possible by optogenetics.» ([Josselyn eLife 2018](#))



Neuroengineering 2021/22

NEUROENGINEERING FOR BIOLOGY 2 –

Optical tools for studying neuronal networks

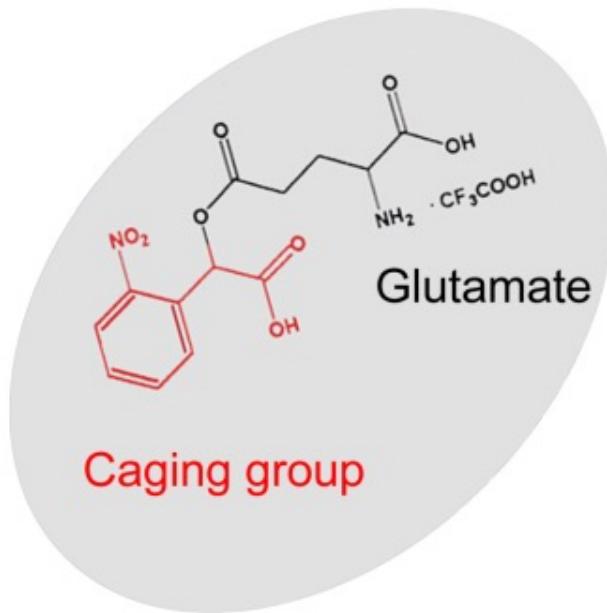
PART 1 –

Optical stimulation of in vitro neuronal cultures

ONE-PHOTON UNCAGING

The basic approach is to cage the compound (Black) with a blocking group (red). Thus, caged compound can be switched into the active form by short UV pulses.

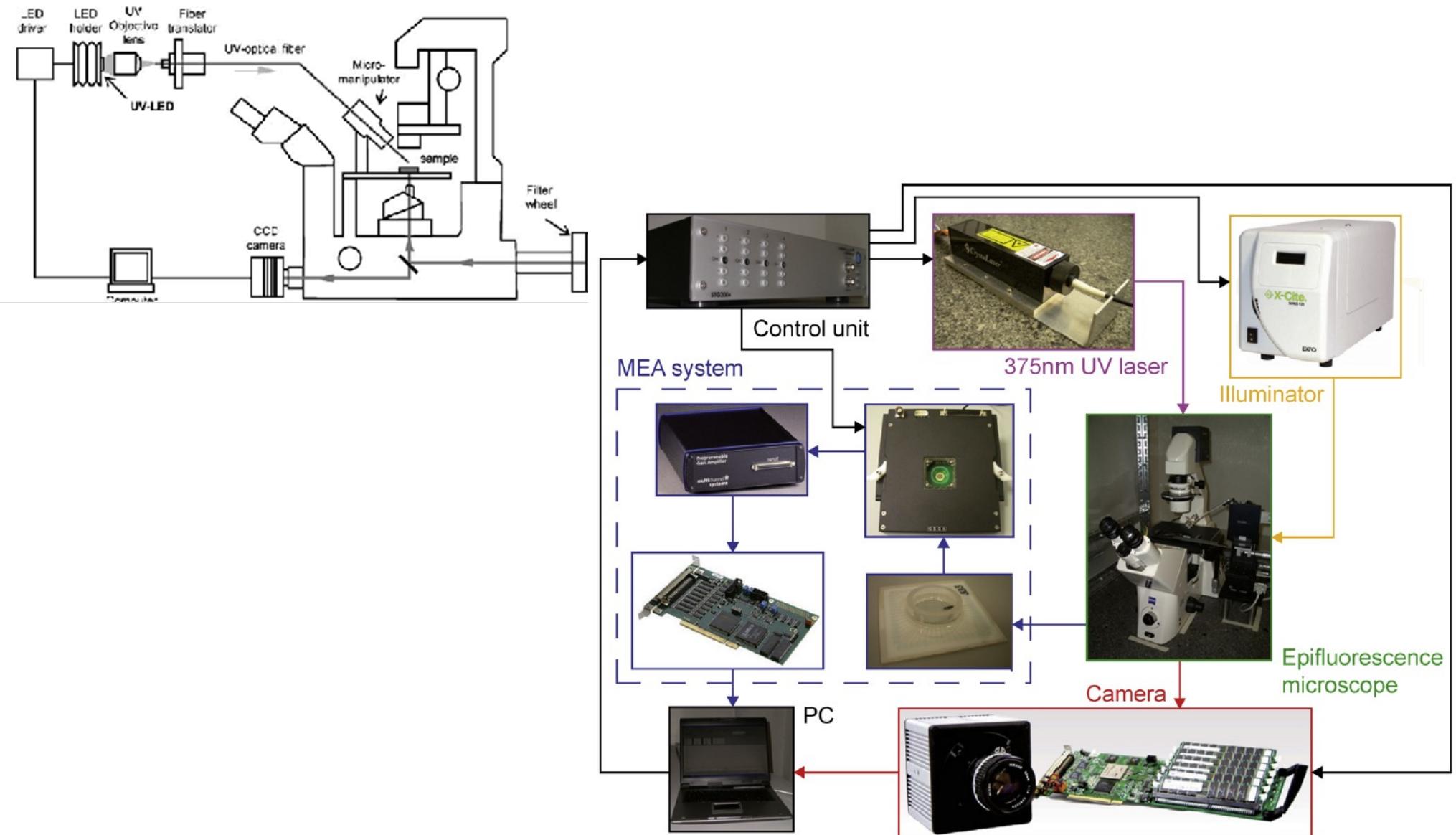
This allows us to obtain high spatial and temporal control during stimulation.



PROS

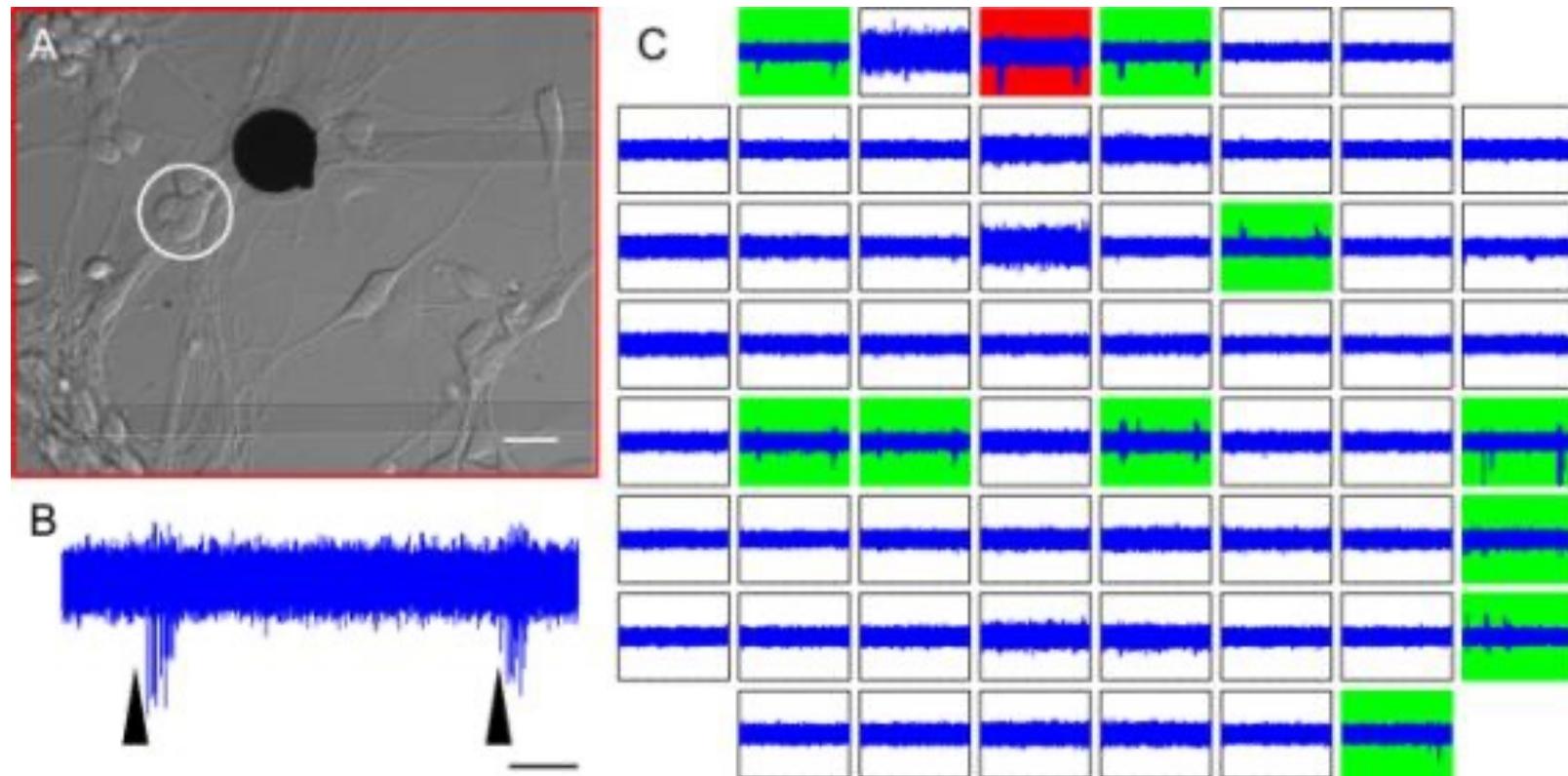
- Glutamate is one of the most common neurotransmitters in the CNS
 - Physiological stimulation
- The UV pulse can be highly focused
 - High selectivity

Setup: opt. Stim by optical fiber

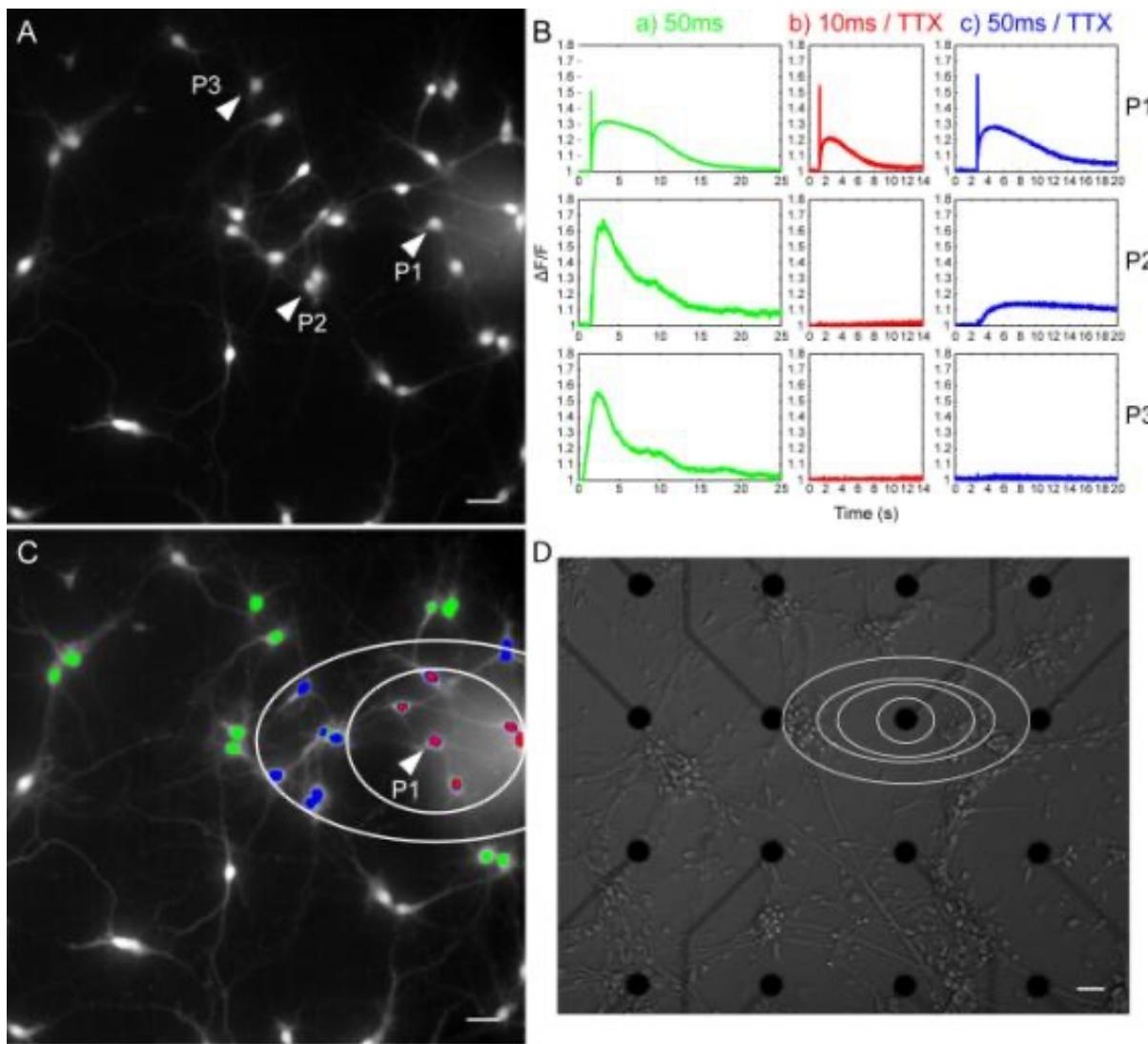


Optical stimulation

Stimulation:
by light caged compounds
Recording:
MEA



Optical stimulation + Ca optical recording



Stimulation:
by light caged
compounds
Recording:
Ca⁺⁺ optical
recording

Optical stimulation
can be confined in
an area of about a
couple of MEA
electrodes.



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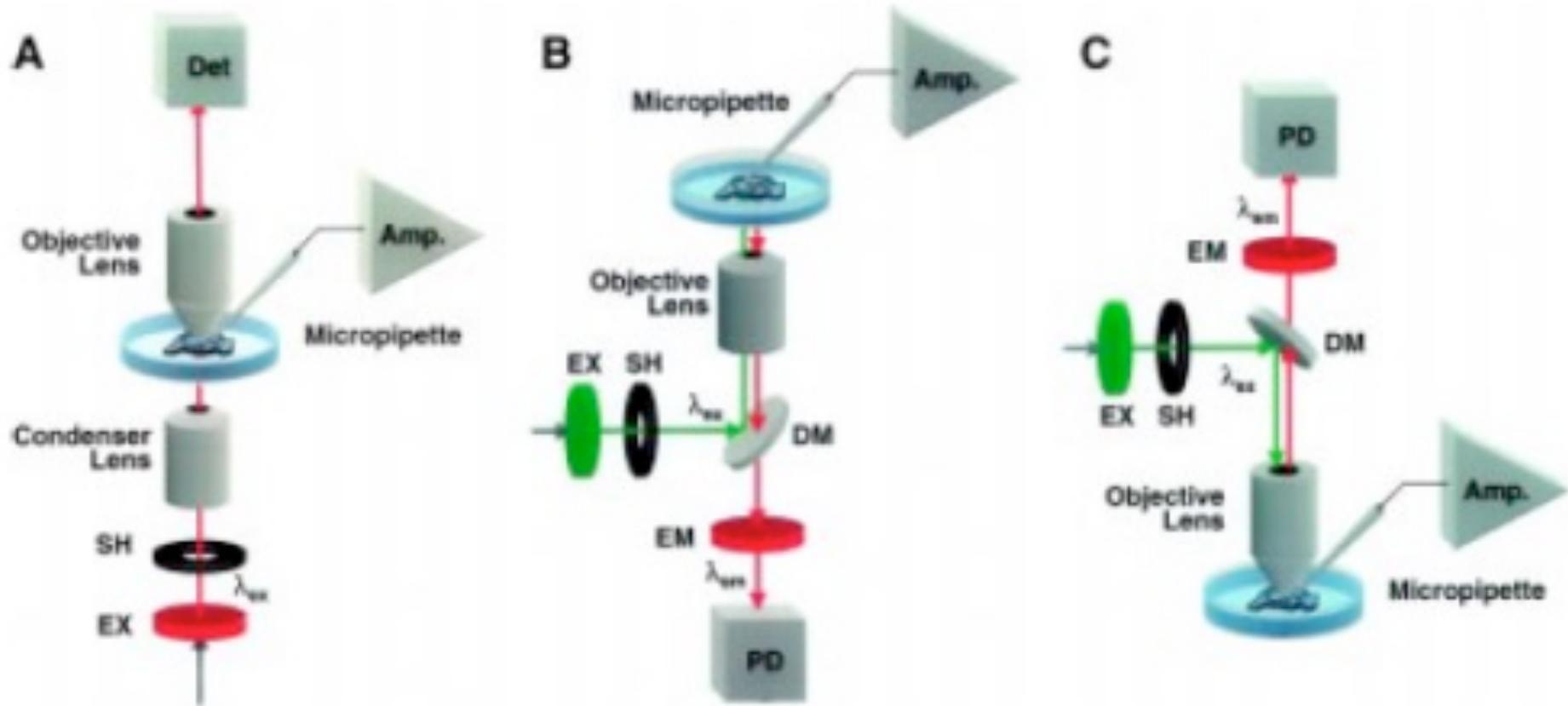
NEUROENGINEERING FOR BIOLOGY 2 –

Optical tools for studying neuronal networks

PART 2–

Optical recording of in vitro neuronal cultures activity

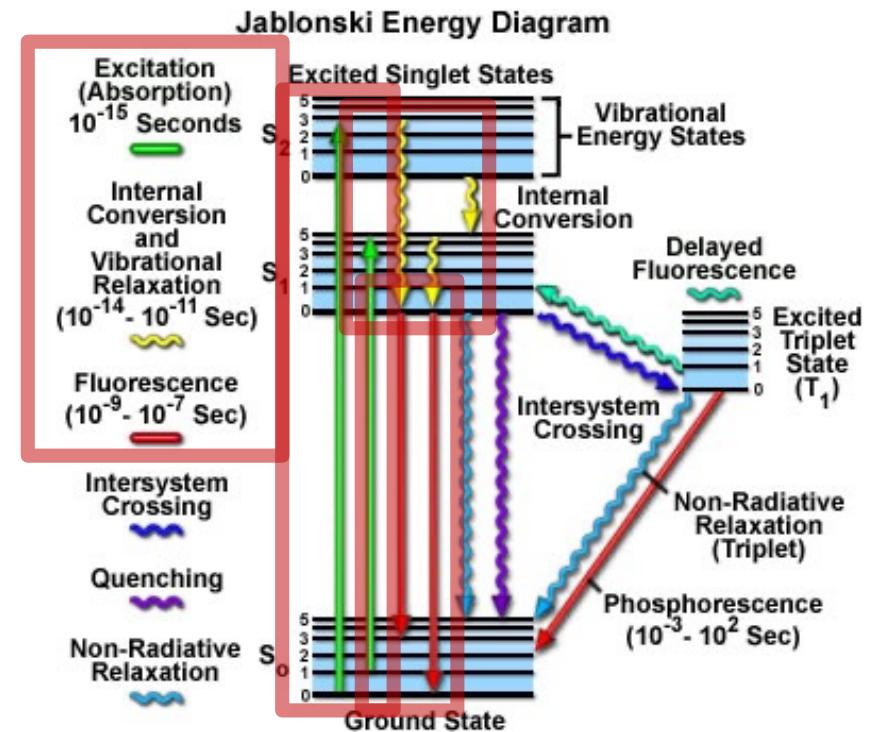
Type of microscopes



Fluorescence

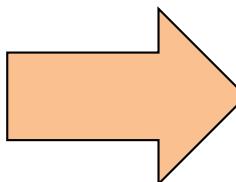
Fluorescence is governed by three events:

- Excitation (or absorption)
- Vibrational relaxation
- Emission



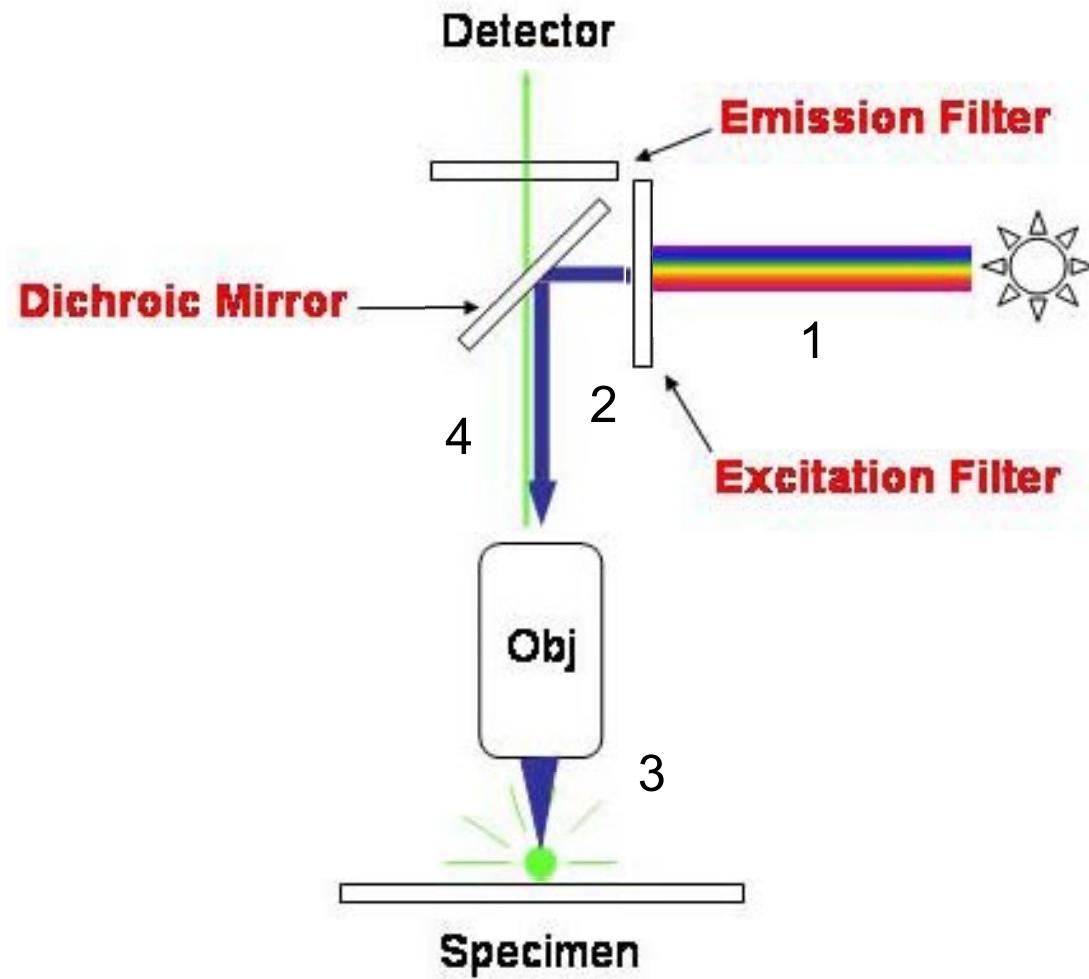
Fluorescence is a quick process: it is measured in billionths of seconds

Energy loss in vibrational relaxation
causes emitted photon to have less
energy than absorbed one



Emitted photon has a different
wavelength (“color”) than absorbed one

Fluorescence microscopy

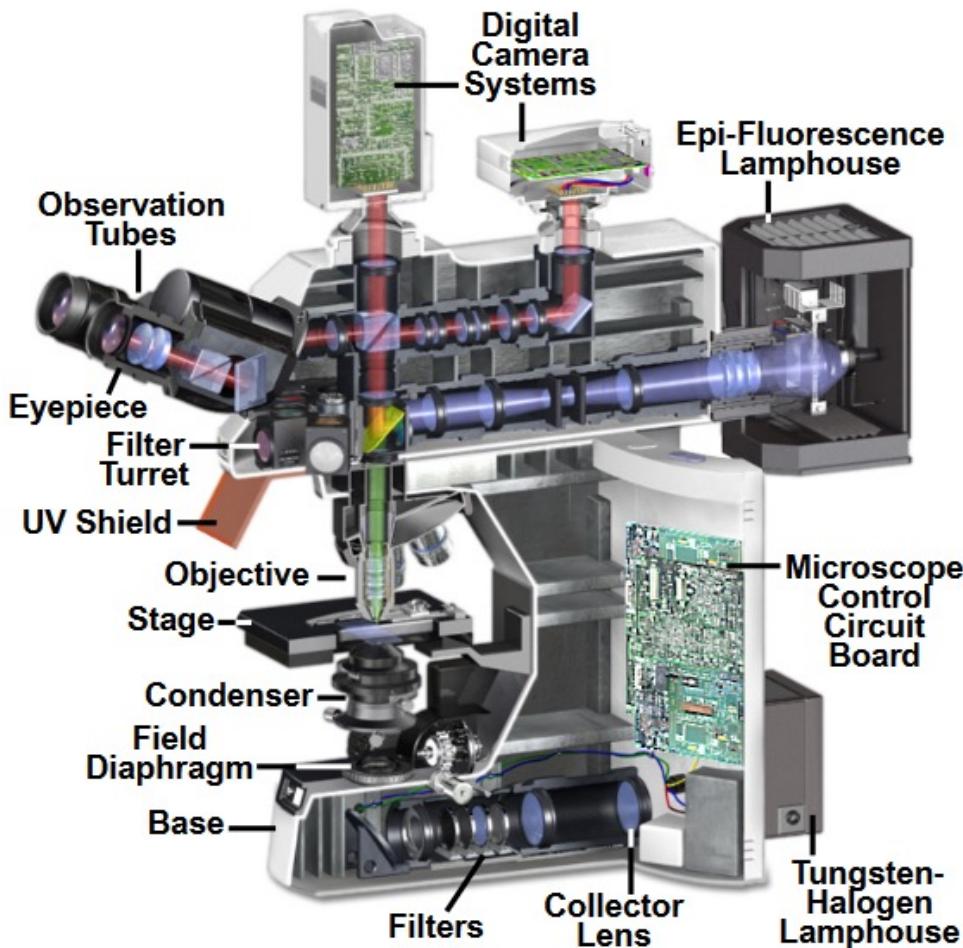


Fluorescence microscopy:

1. White beam from source to excitation filter
2. Monochromatic beam ($\text{wavelength} = \lambda_1$) from excitation filter to sample
3. Absorption and monochromatic beam emission ($\text{wl} = \lambda_2, \lambda_2 > \lambda_1$)
4. Monochromatic beam from sample to detector

Epifluorescence microscopy

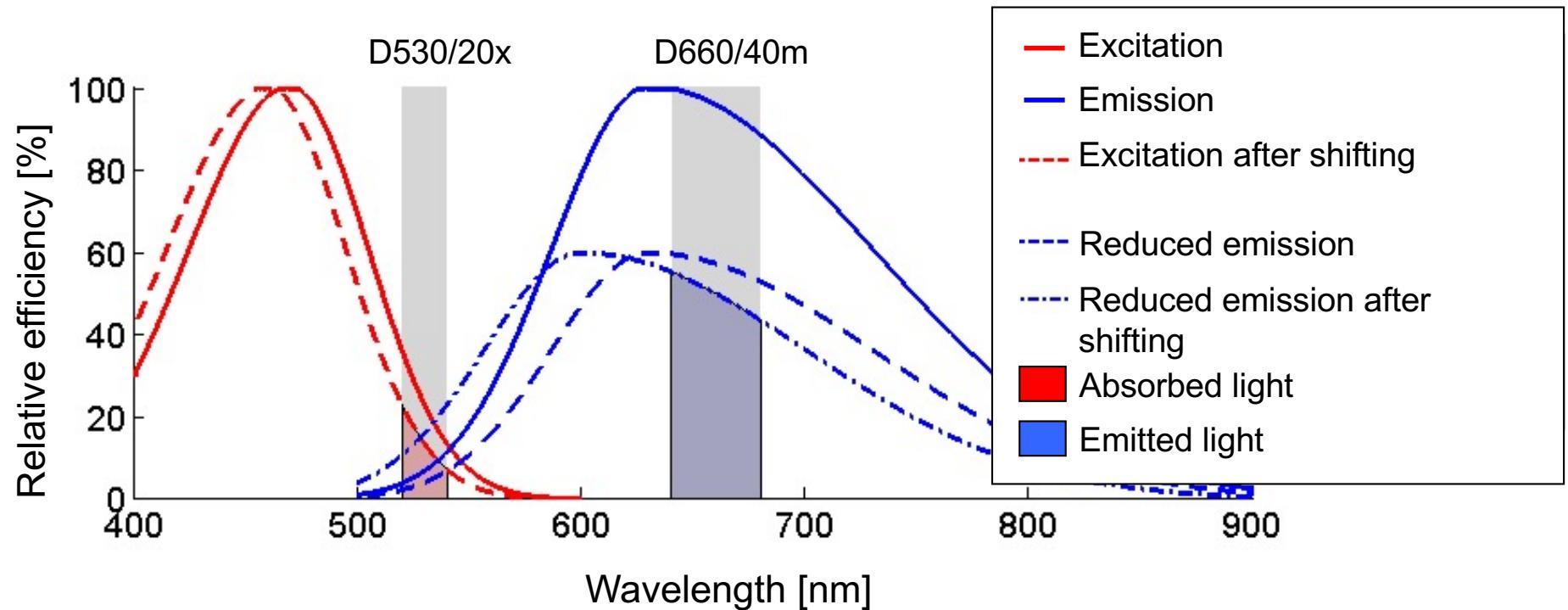
Figure 1 - Epi-Fluorescence Microscope



Epi-fluorescence microscope equipped for both transmitted and reflected fluorescence microscopy

VSDs: principle of working

VSDs allow OPTICAL detection of ELECTRICAL activity



VSDs efficiency is a function of local electrical voltage.

If excitation light is constant, output light intensity depends only on sample electrical properties.

Comparison of technologies for Neuronal culture reading

	Patch clamp	Mea	VSDs
Temporal resolution	+++	+++	Depends on camera, could limit the spatial resolution
Spatial resolution	Single neuron	Pool of neurons around the same electrode (partially improved by post processing)	Single neuron ...depends on the trade-off with the field of view (objective)
Field of view	Max few neurons	Full coverslip culture	Trade off with spatial resolution
SNR	Subthreshold potentials	Spikes (dark neuron)	Spikes (on neurons compartments)
Link activity and morphology	Perfect for the recorded neurons	NA	Good in the field of view
Difficulty	+++	++	+
....			