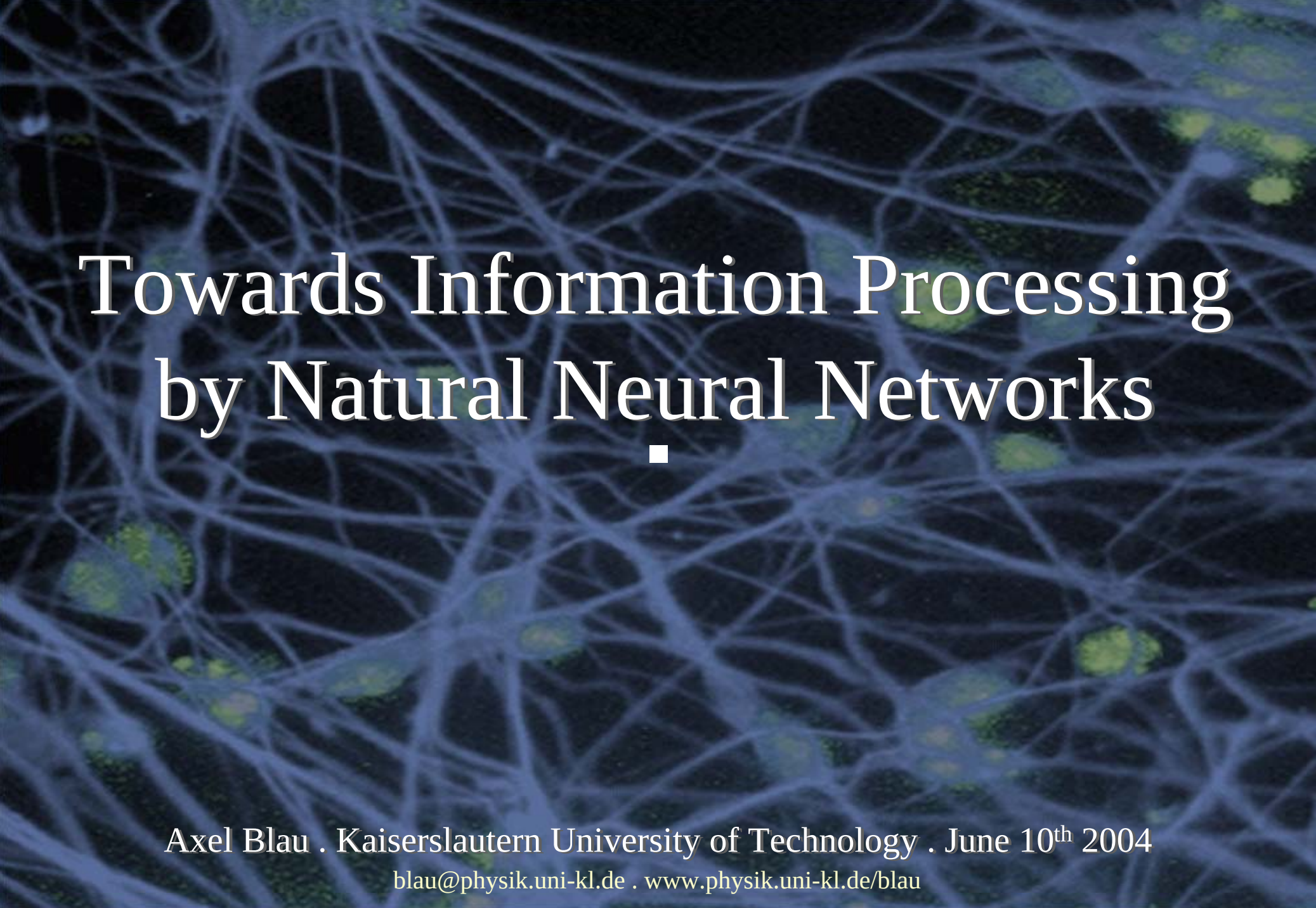




Towards Information Processing by Natural Neural Networks

■

Axel Blau . Kaiserslautern University of Technology . June 10th 2004

blau@physik.uni-kl.de . www.physik.uni-kl.de/blau

Towards Information Processing by Natural Neural Networks

- Why natural neural networks?
- Technological aspects of biohybrid information processing systems
- Pattern recognition by natural neural networks
- What's next?

Why Natural Neural Networks?

	number of processors	clockspeed/ processor	bps*	volume [m ³]
Intel Pentium IV (2001 A.D.)	1	1GHz	10^{11}	10^{-4}
IBM ,ASCI White' (2000 A.D.)	$\sim 10^4$	1GHz	10^{15}	1400
IBM ,Blue Gene' (2004 A.D.)	10^6	1GHz	10^{17}	330
Human Brain (~10 ⁵ B.C.)	10^{11}	200 Hz	$10^{16...27}$	10^{-3}



*bps: bits per second

What the brain is good at ...

Parallel processing of information (e.g. pattern recognition), abstraction and creative association.



How could we exploit these mental abilities?

Can we

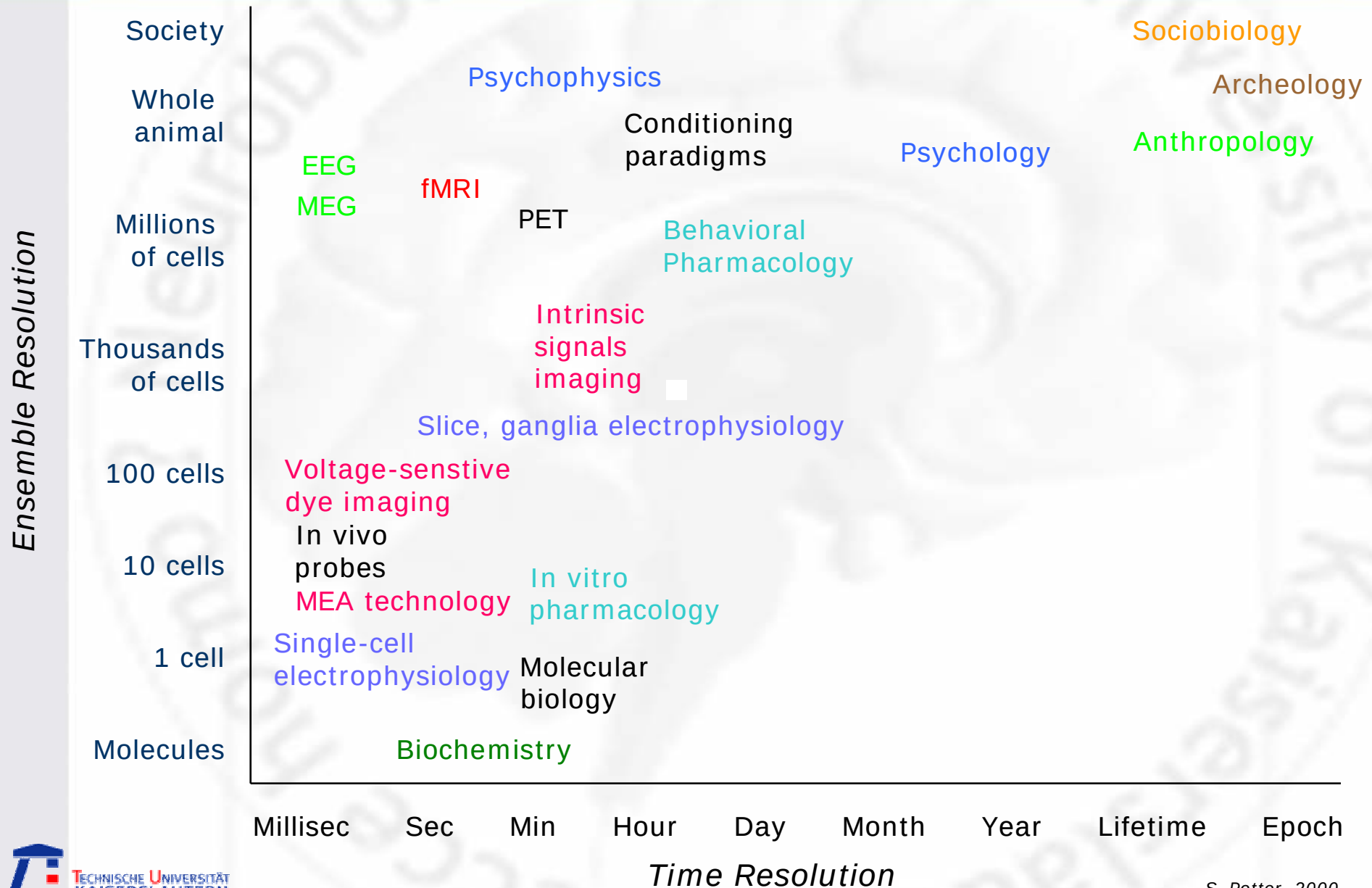
- combine natural neurons with standard Si-technology to build biohybrid computing entities
- that will allow us to explore novel methods of information processing
- because brains have more powerful parallel computing abilities than any contemporary Si-based computer.

?

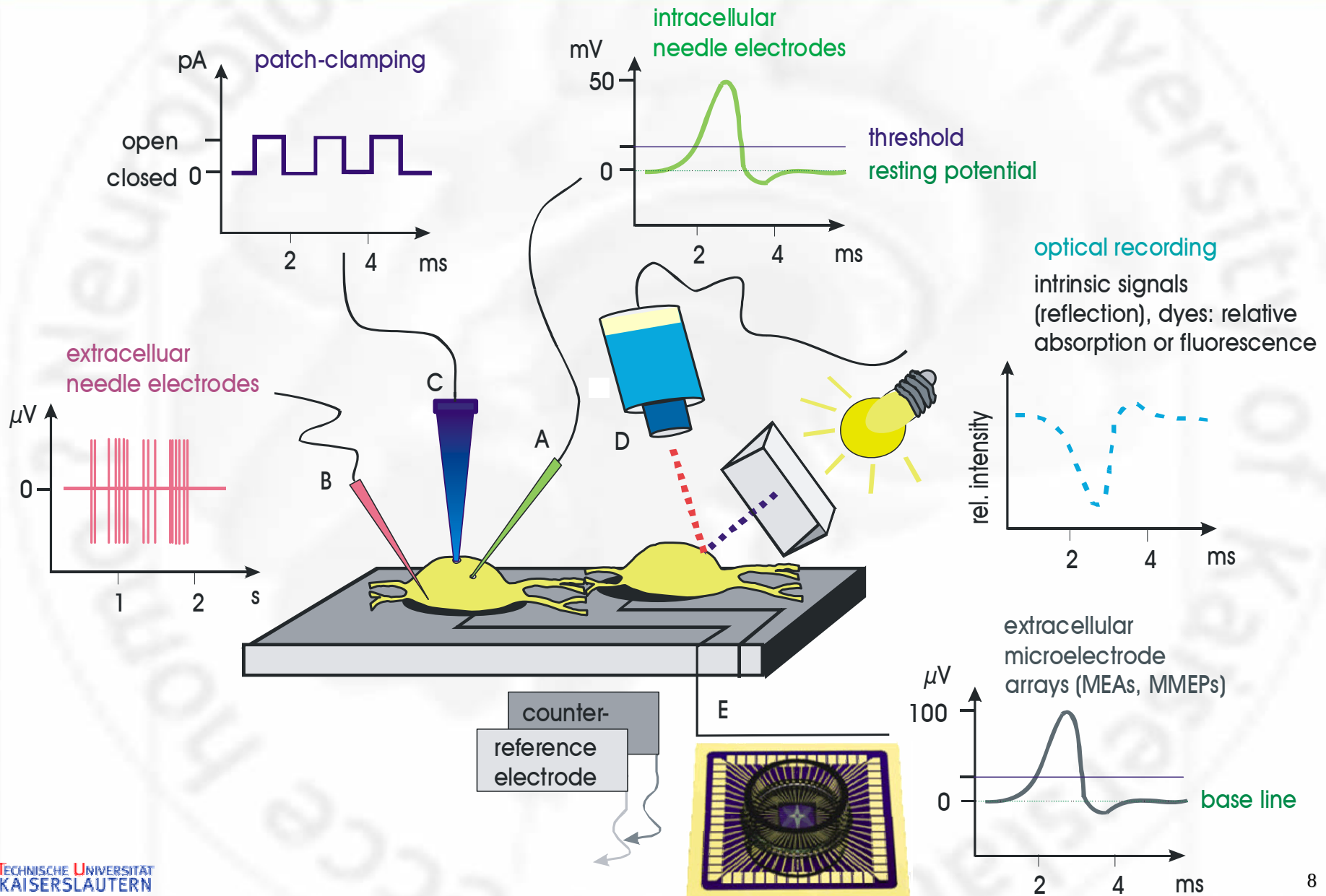
Towards Information Processing by Natural Neural Networks

- Why natural neural networks?
- **Technological aspects of biohybrid information processing systems**
- Pattern recognition by natural neural networks
- What's next?

Methods to study the brain



How can it be done? *In vitro* signal recording techniques ...



What else has to be considered?

Requirements for studying signal processing in neural networks:

Biocompatibility Biology-friendly materials, stable in physiological environments

Cell Culture Long-living neural networks

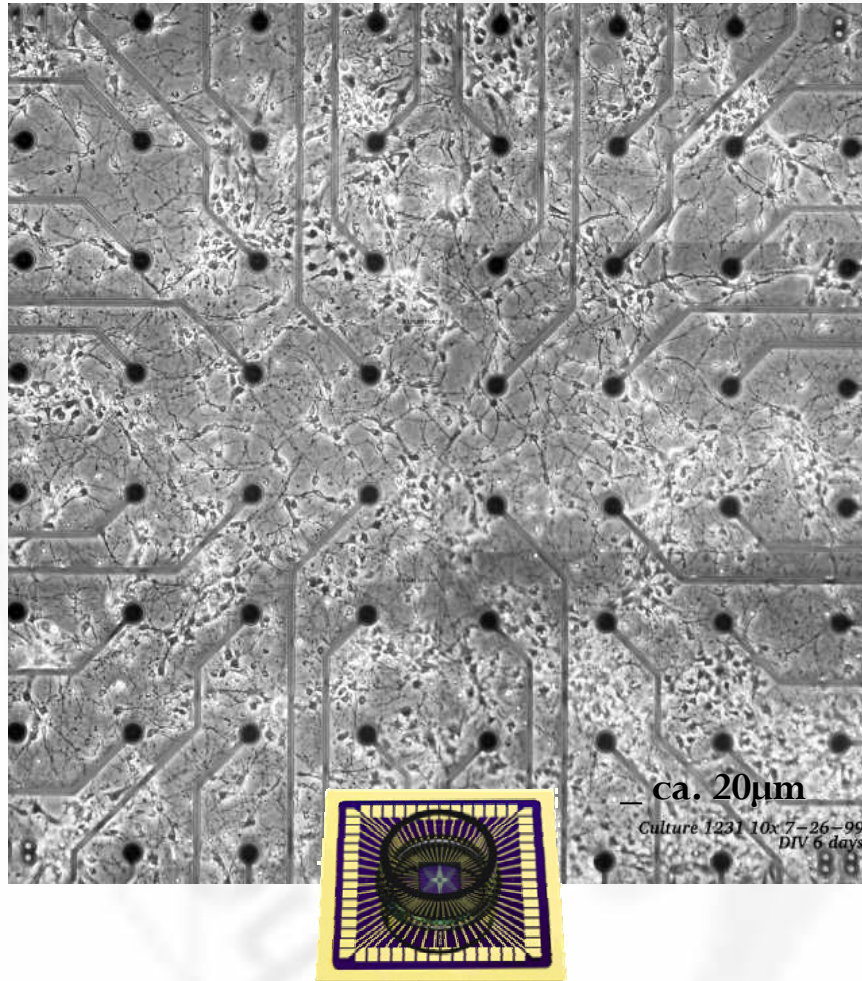
Cell Adhesion Biochemically adapted wetware-hardware interface

Fluidics Suitable life-maintaining (perfusion) chambers

Electronics Miniturized low-noise recording and stimulation electronics

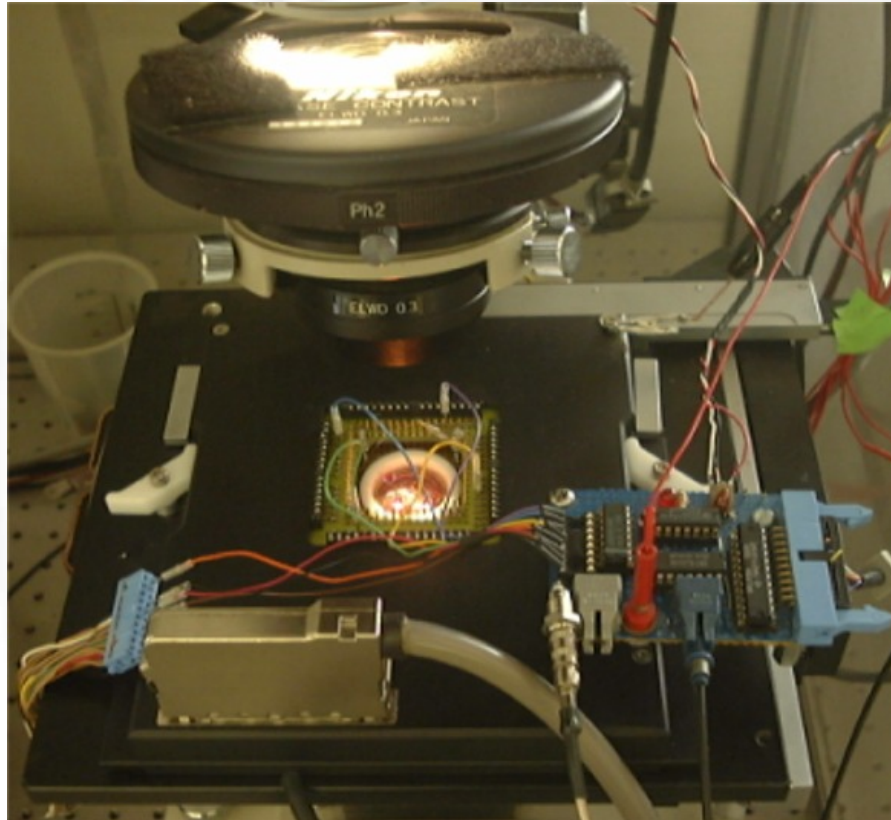
Data Analysis Data reduction and analysis algorithms

Current multi electrode array (MEA) technology ...



- 5*5 cm² array
- 1 mm² overall electrode area
- 60 gold electrodes (TiN-coated)
- Ø 10-30 μm
- 200 μm inter-electrode distance
- DMEM + FBS nutrient mix
- recording (30-400 μV)
- stimulation (biphasic 100-900 mV)
- 230 Euro per MEA

Current electronics technology ...

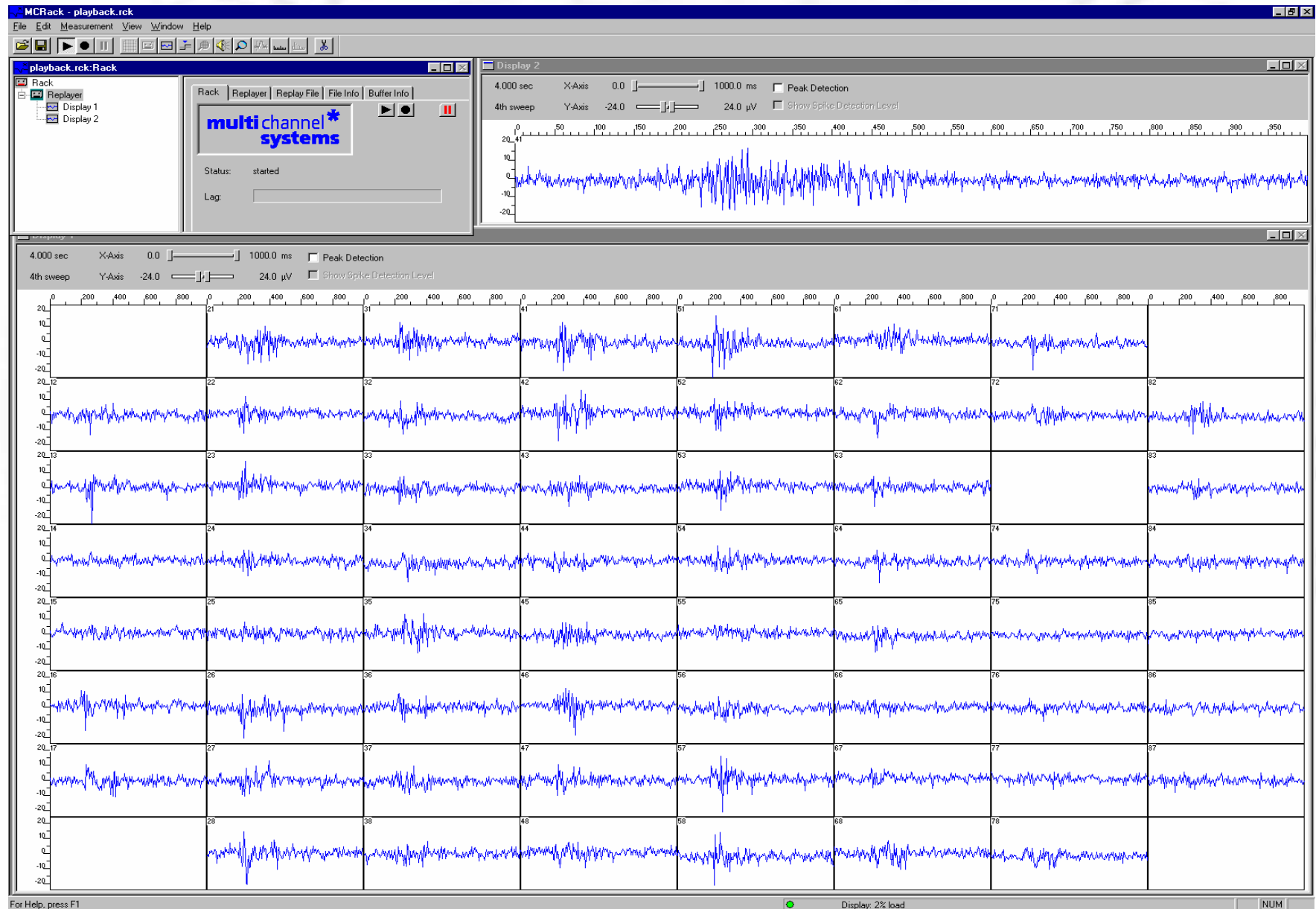


- Amplification 1000
- 25 kHz samp. rate/ch. @ 12 bit
- Temperature control
- Predetermined electrical stimulation
- 40 000 Euro with computer

Towards Information Processing by Natural Neural Networks

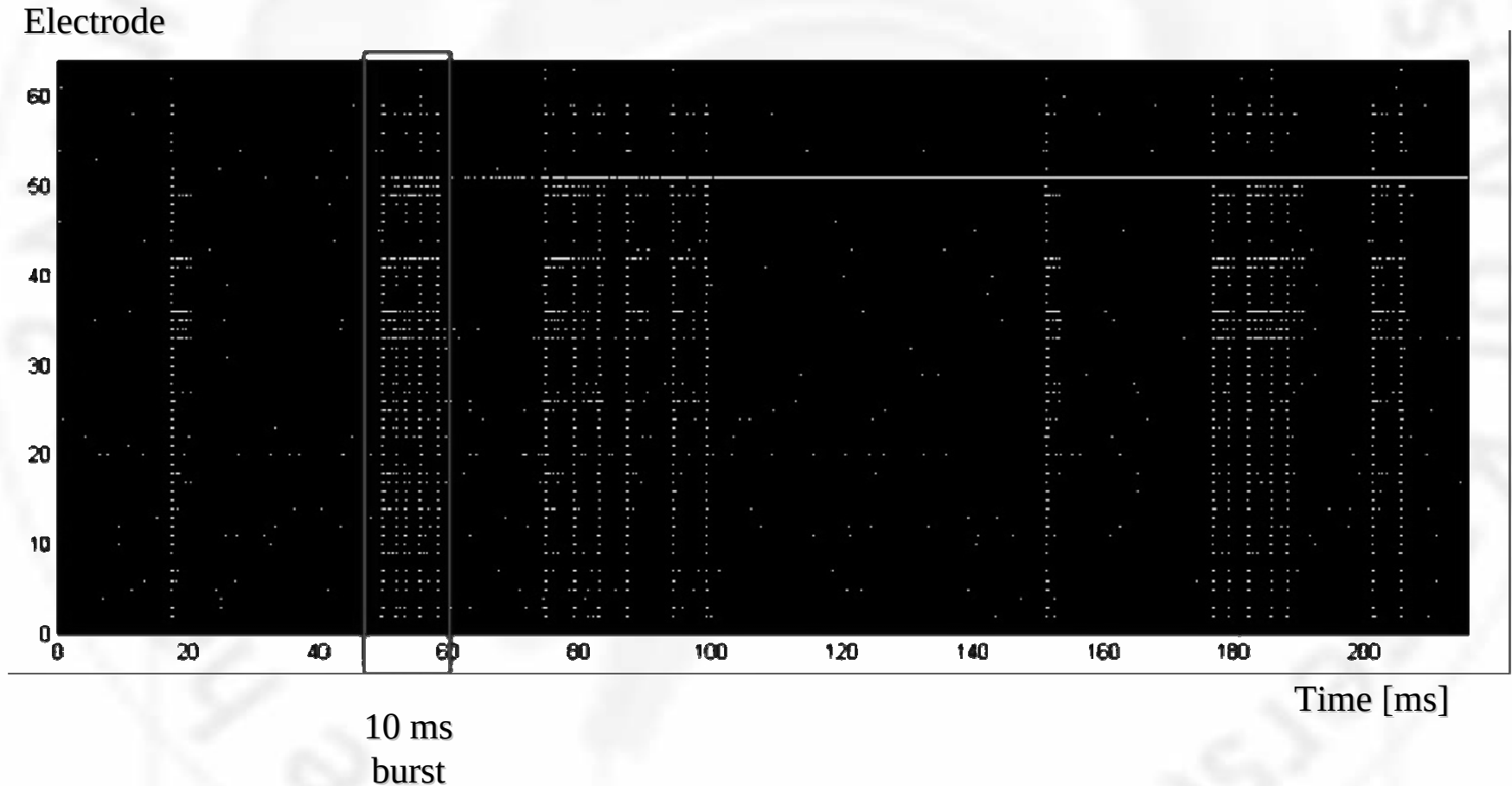
- Why natural neural networks?
- Technological aspects of biohybrid information processing systems
- **Pattern recognition by natural neural networks**
- What's next?

Digitized neural activity from a MEA recording



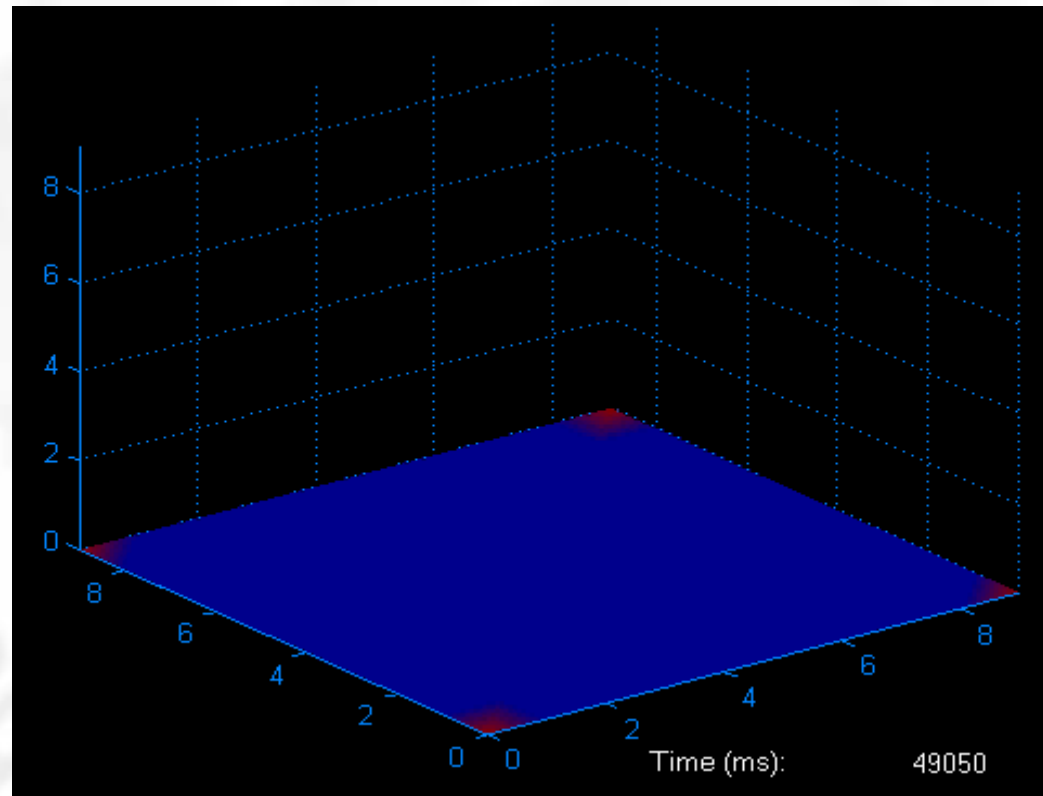
Digitized spontaneous activity

Spontaneous activity is dominated by bursts that are characteristic for stimuli-deprived neural networks (“Kaspar Hauser” effect).



5 Seconds of spontaneous neural activity

Location-Intensity-Time-map of an extracellular recording from an 8*8 MEA:



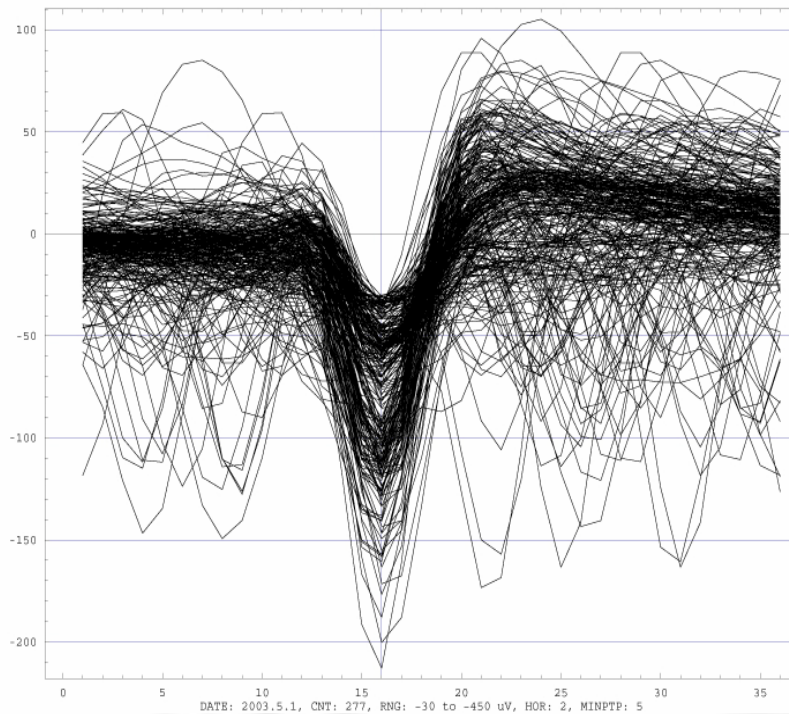
T. DeMarse, 2000

Identification of individual neurons

Spike discrimination by Independent Component Analysis (ICA) to separate and sort activity from different neurons contributing to the signal on one electrode.

Raw superimposed signals from 5 neurons on one electrode

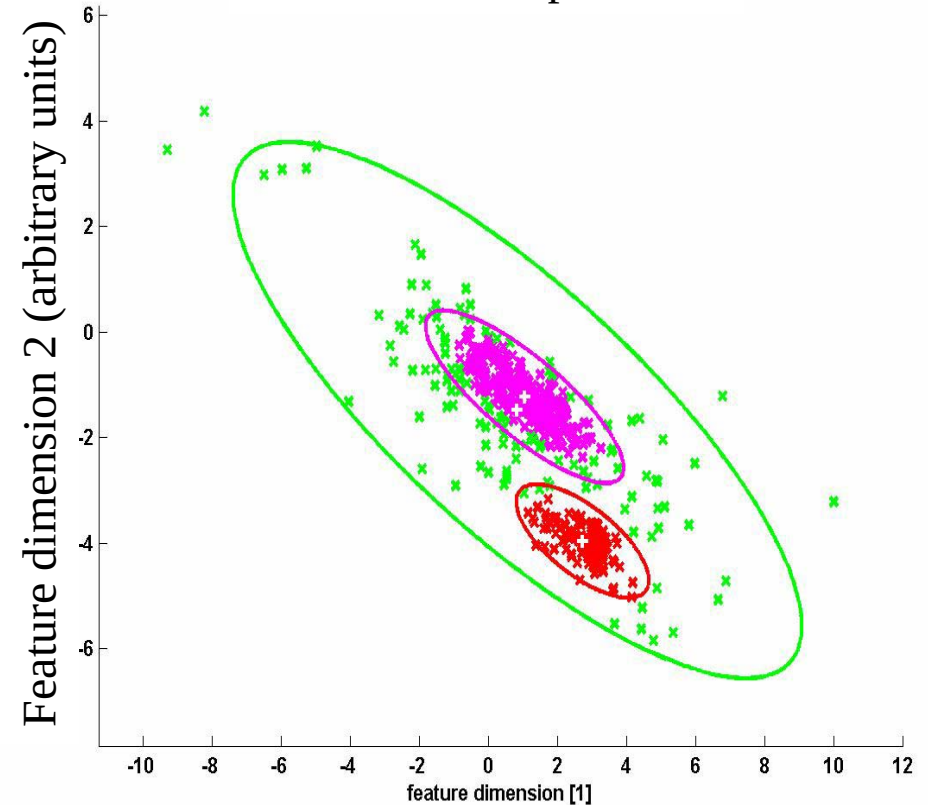
Signal amplitude (μV)



ICA
➔

Time [ms]

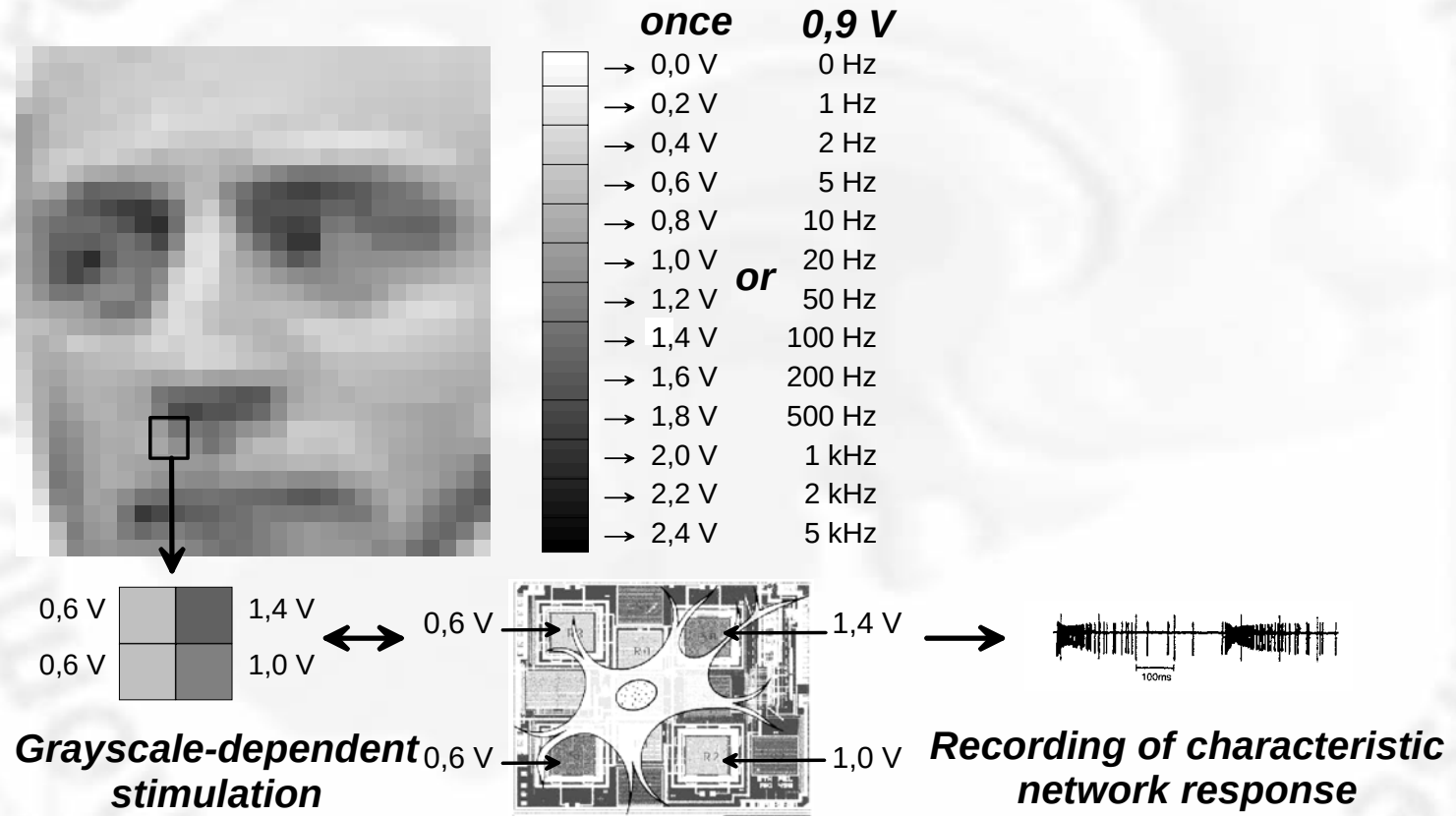
Classified spikes



Feature dimension 1 (arbitrary units)

Which **coding scheme** should we use to teach the neurons something?

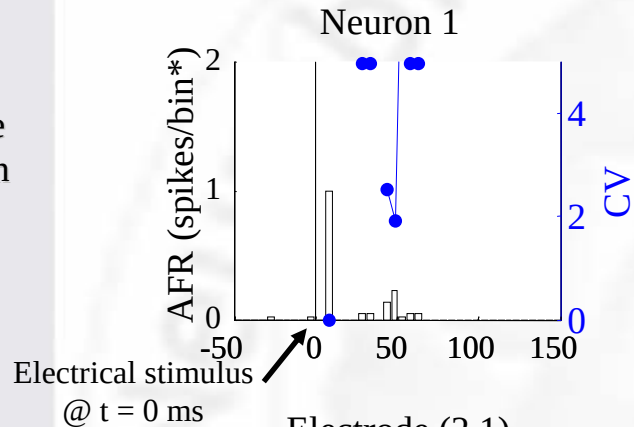
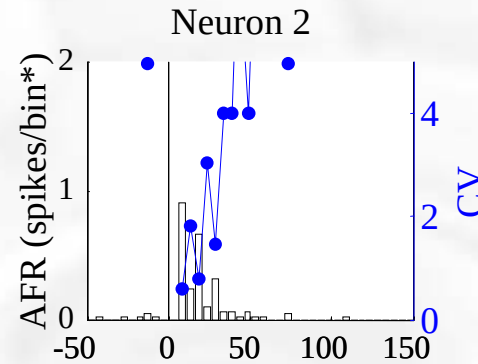
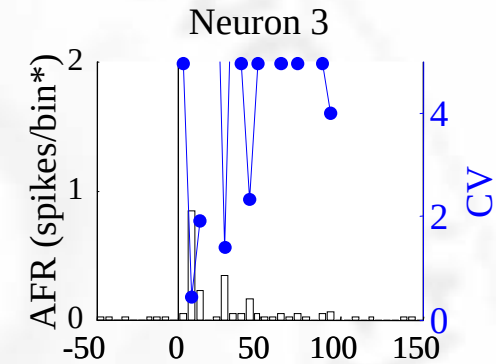
Voltage versus frequency



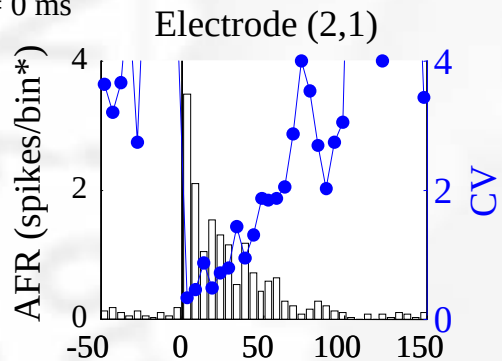
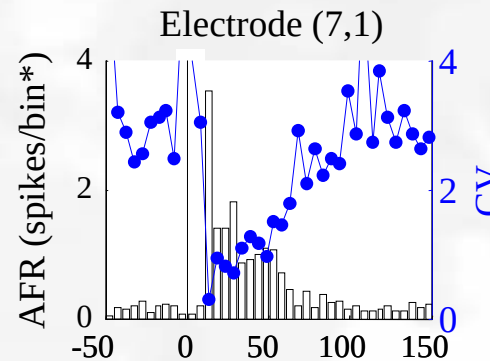
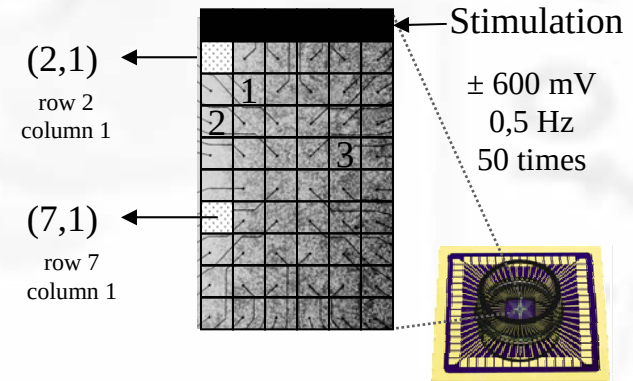
Exploring the reproducibility of evoked neural responses

Quality criterion: coefficient of variation (CV) = standard deviation divided by its mean

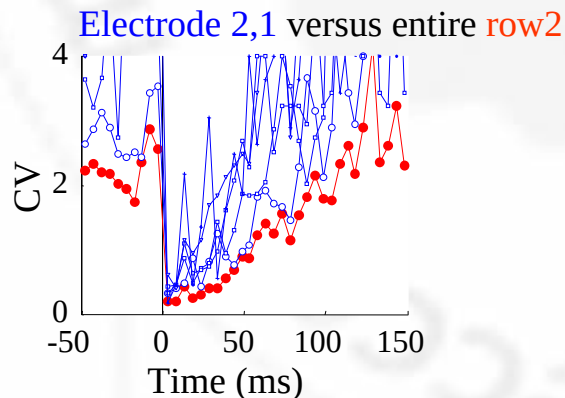
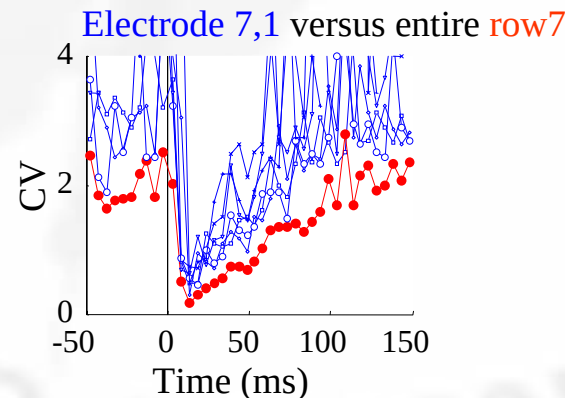
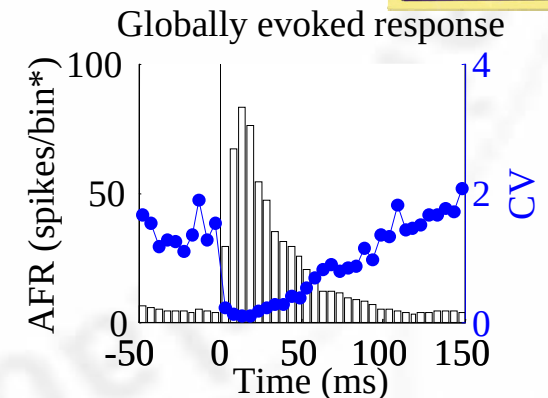
Single neuron



Single electrode
(3-5 neurons)



Electrode
versus row



Exploring the coding schemes ...

Finding: By **pooling the evoked activity** over larger populations of neurons it is possible to **increase** the **reproducibility** and **reliability** of the neuronal response over larger time windows **at the cost of spatial resolution**.

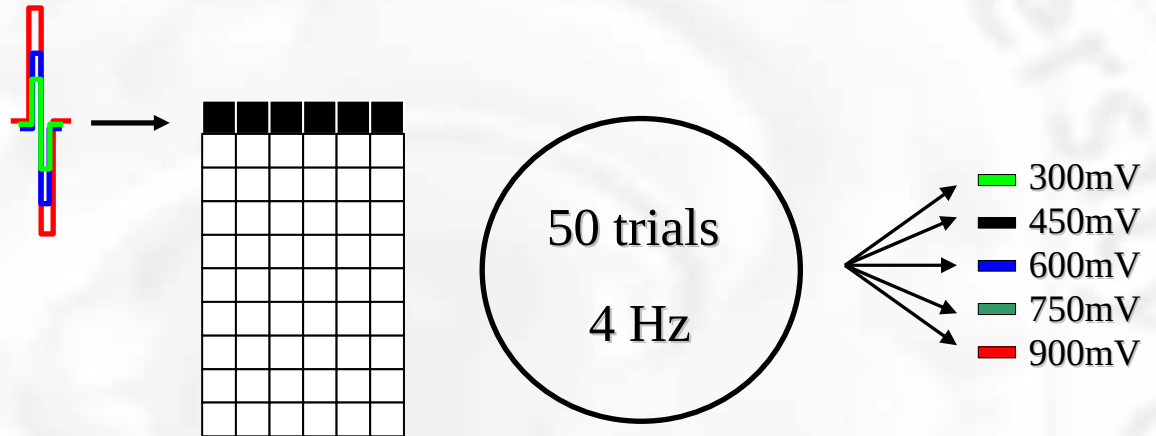
Question: What is the **time limit**?

Approach: Have a look at the brain: information processing of a chunk of information usually takes **less than one second** ...

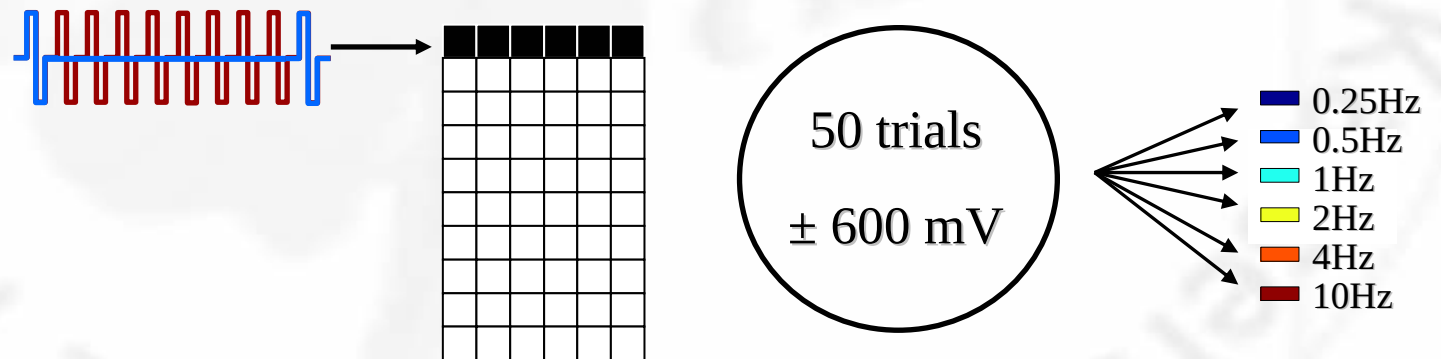
Hypothesis: $3 \text{ ms (1 spike)} < \text{coded information} < 1 \text{ s}$

Let's look at the effect of the two stimulation schemes ...

Amplitude-coded?



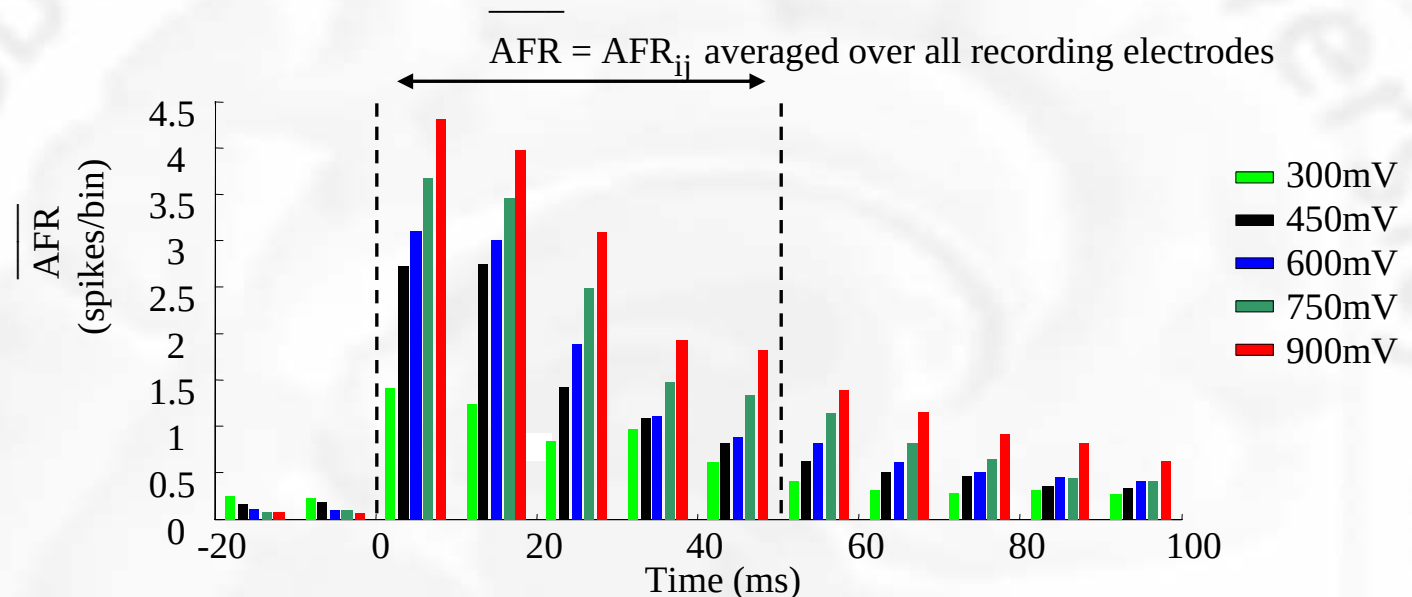
Frequency-coded?



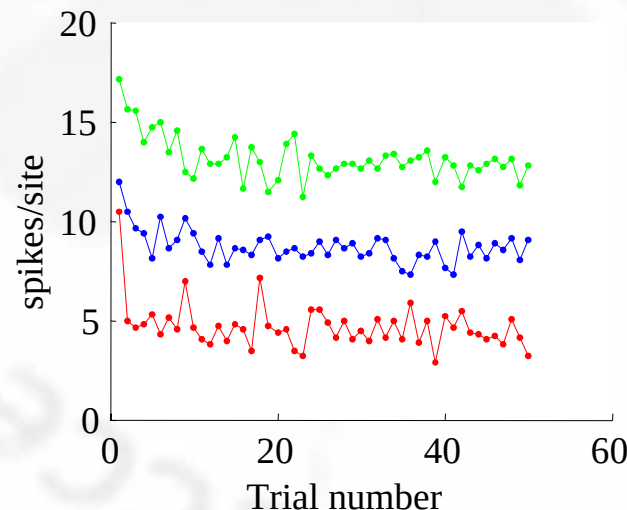
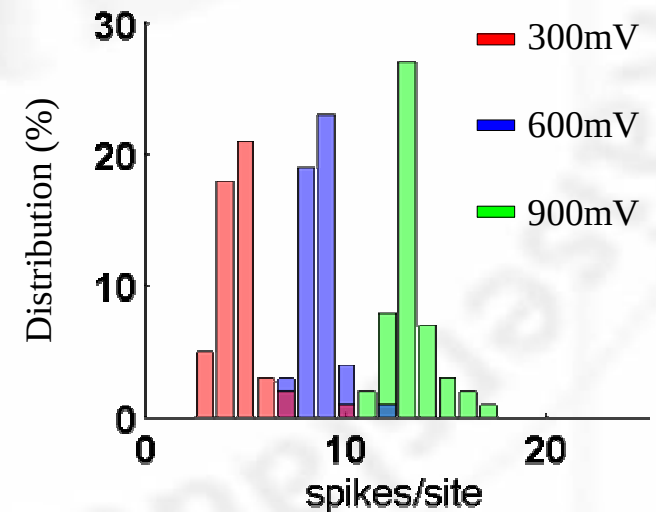
Neural response to voltage-coded stimuli

Good discrimination if the response is averaged over the entire 60 electrodes.

Average
global
response



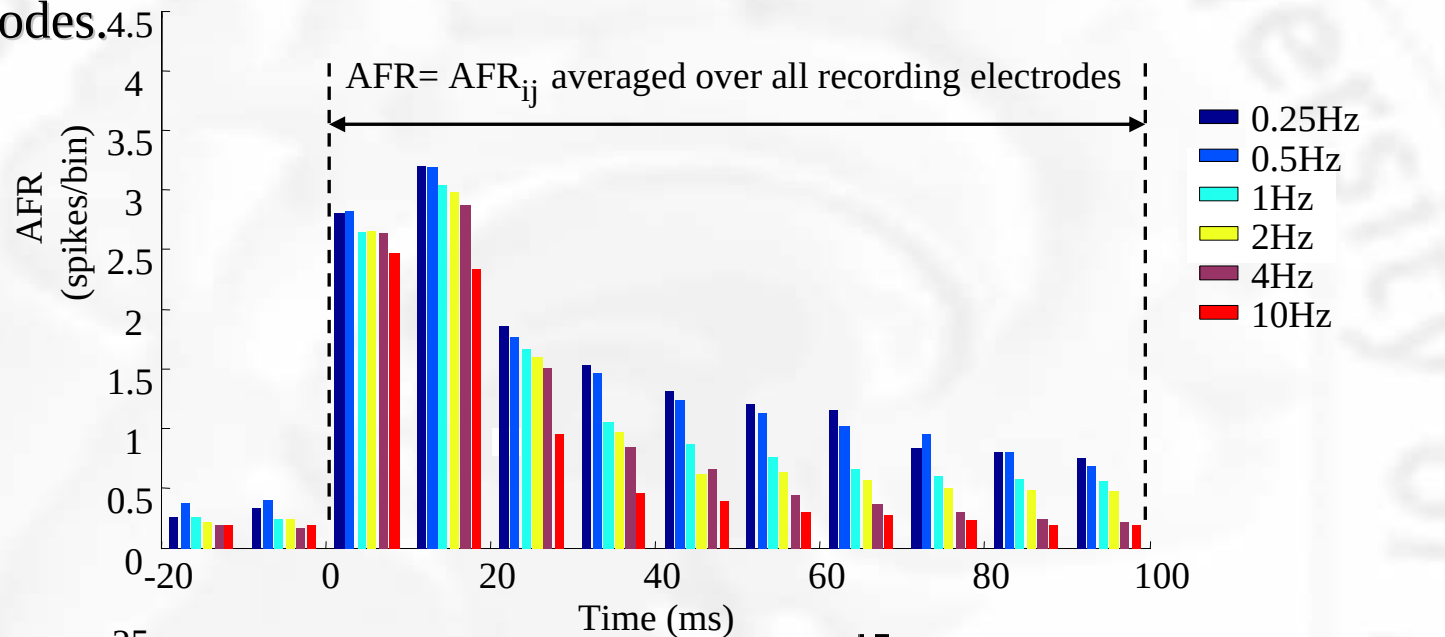
Single trial
global
response
(for a bin size
of 51 ms)



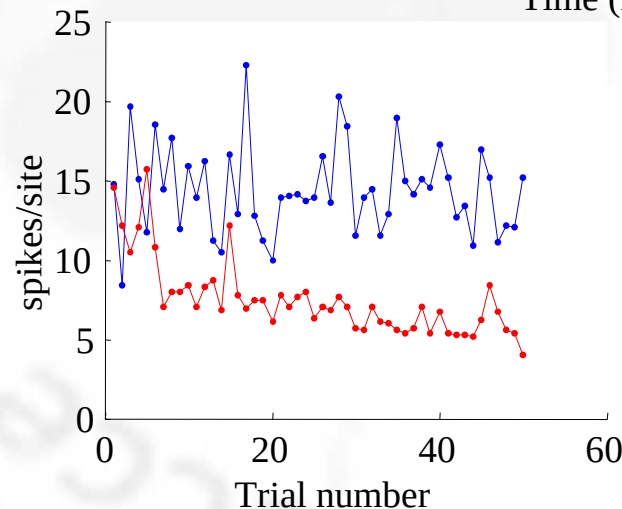
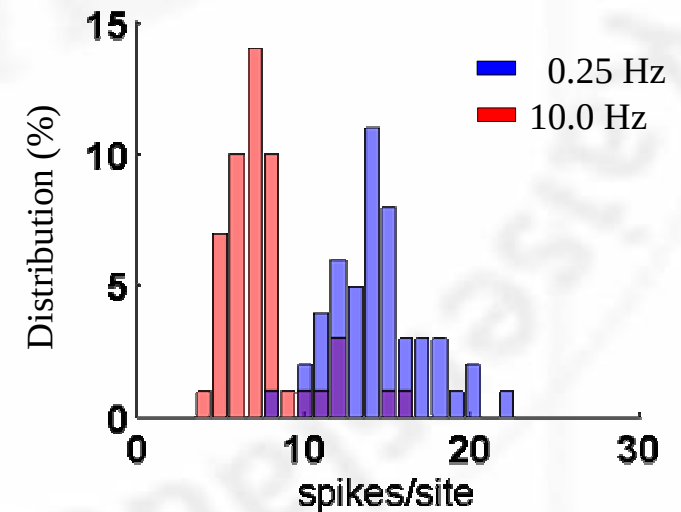
Neural response to frequency-coded stimuli

Only fair and inhomogeneous discrimination if the response is averaged over the entire 60 electrodes.

Average
global
response



Single trial
global
response
(for a bin size
of 101 ms)



What we observed so far ...

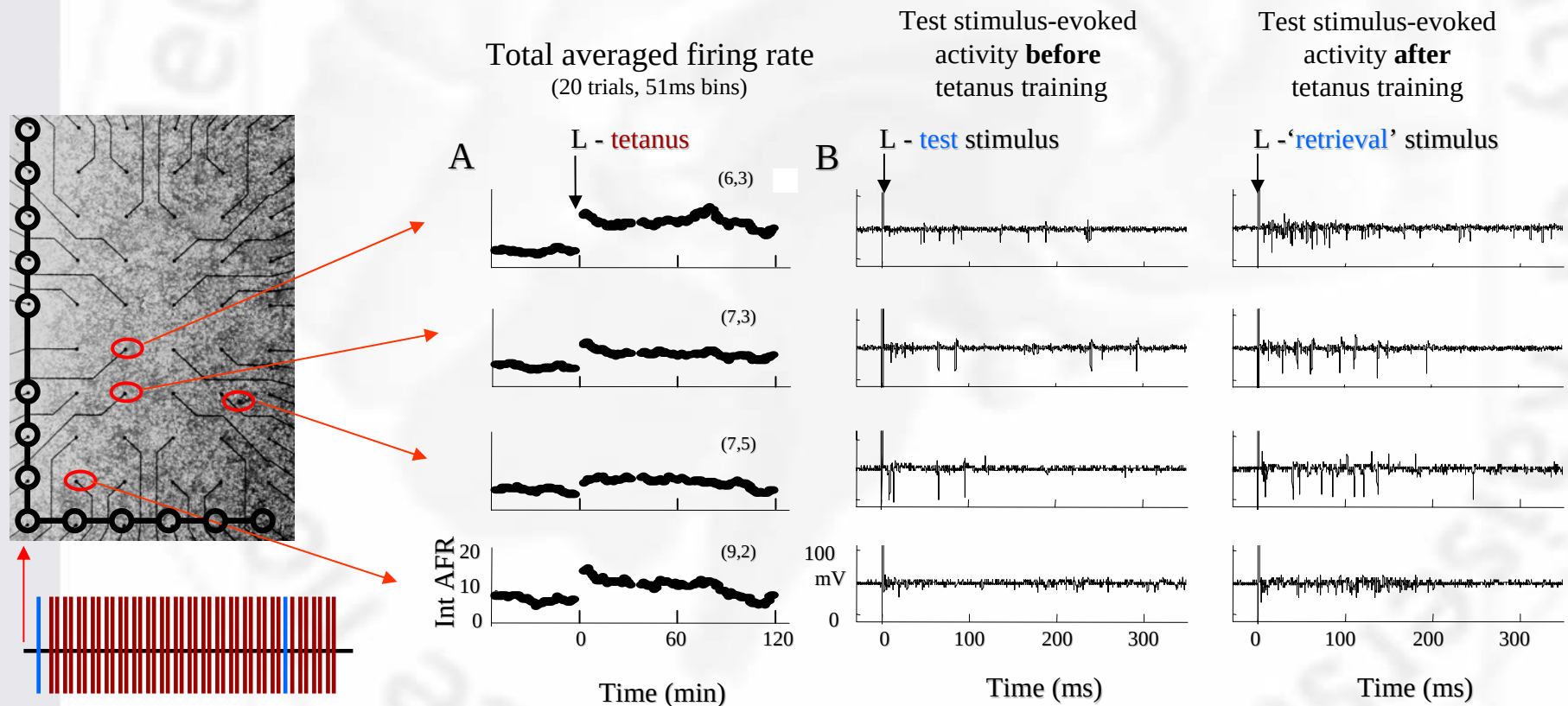
By pooling the activity evoked over a population of neurons of the order of 100-150 units it is possible to **distinguish reliably the response** of the neuronal culture **to stimuli varying in intensity or frequency**.

Question: Which of the two is the better strategy **to train a network** – e.g. for pattern recognition?

Frequency-coded training for pattern recognition and feature extraction

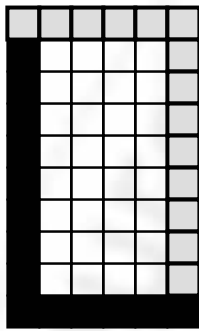
Protocol for 'teaching' and 'memory retrieval':

- **Test** = 'retrieval' stimulation (before and after training): different patterns of stimulation at a frequency of 0.5 Hz, $\pm 900\text{mV}$ (└-shaped, ┐-shaped, ┘-shaped, ┌-shaped, bar-shaped, ...)
- Training procedure: high frequency └-shaped **tetanic** stimulation (250 Hz, $\pm 900\text{mV}$)

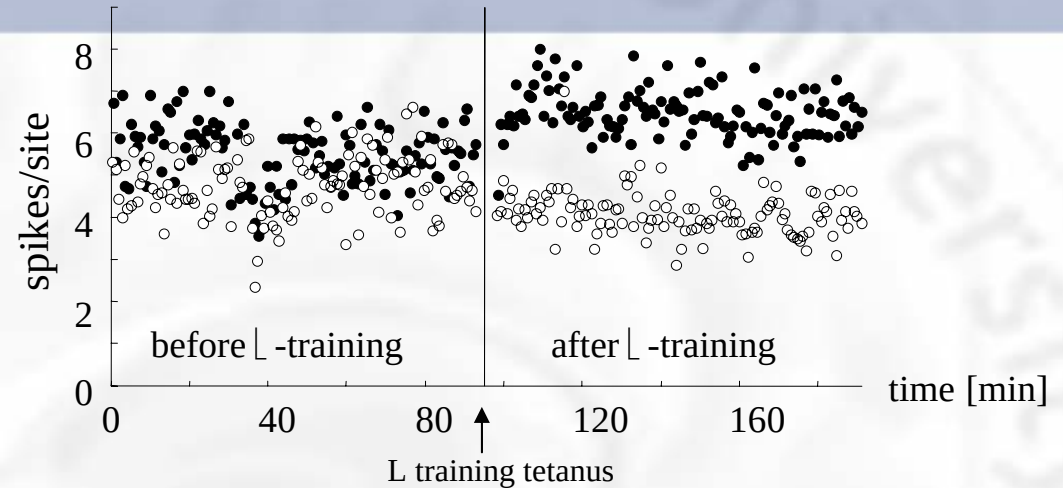


L-shaped training **tetanus** or **test** stimulus

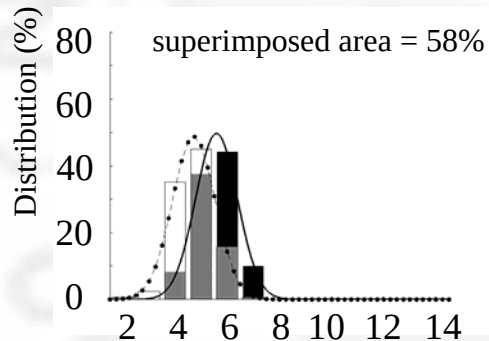
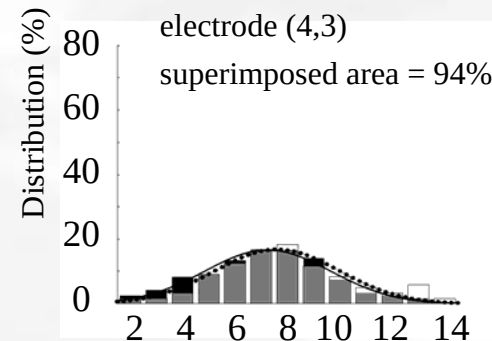
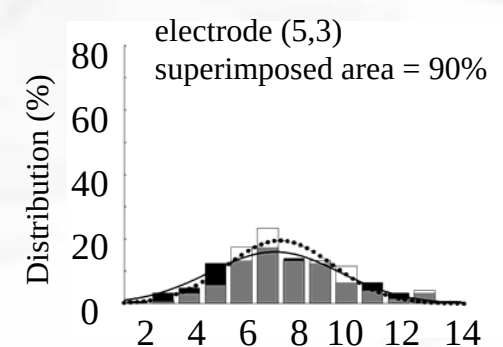
Can the neural network actually discriminate different stimuli?



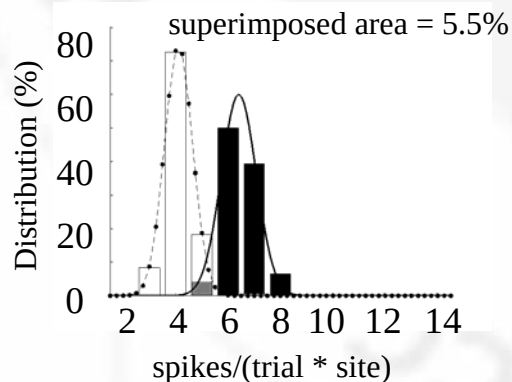
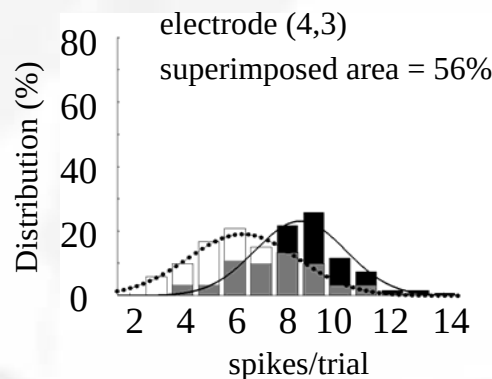
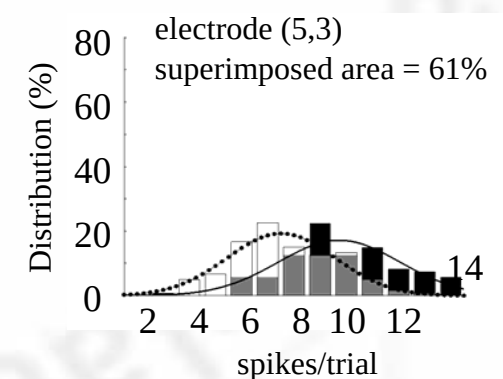
□ - Test stimulus
■ - Test stimulus



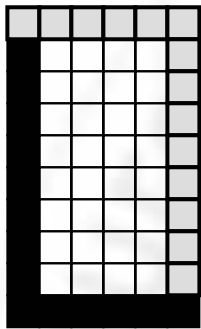
Before
L-training



After
L-training

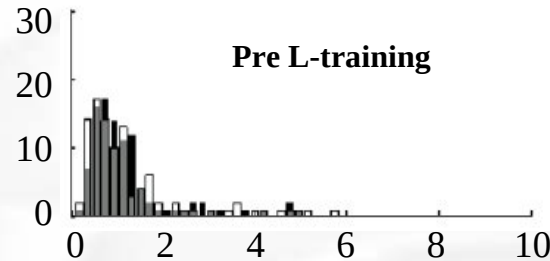
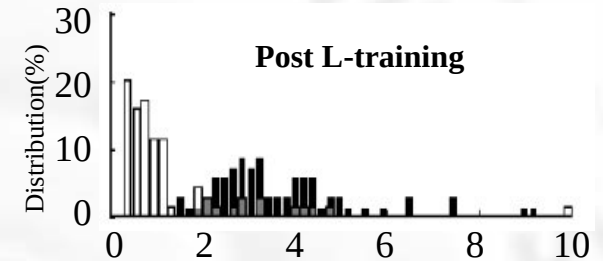


Were we just lucky with that one very smart neural network?

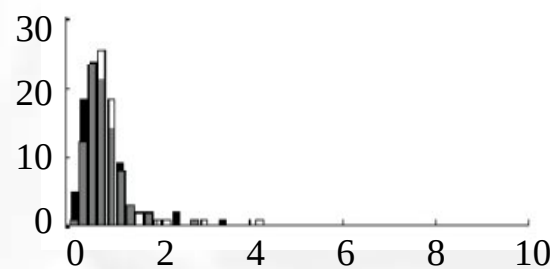
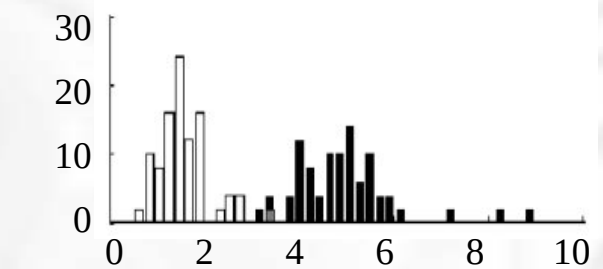


□ - Test stimulus
■ - Test stimulus

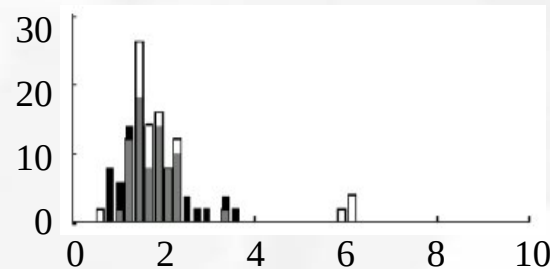
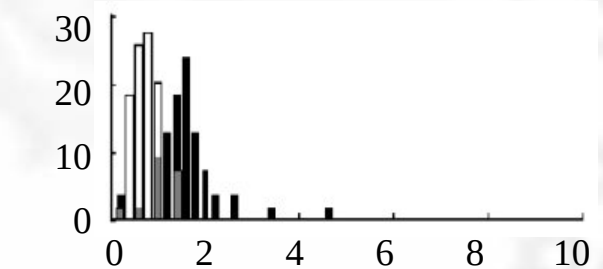
Network #1



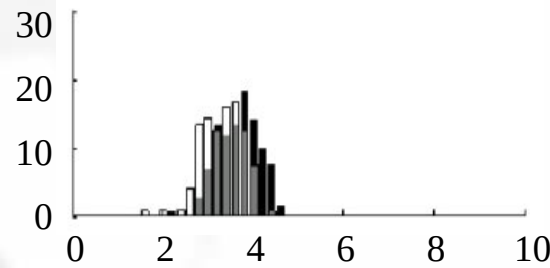
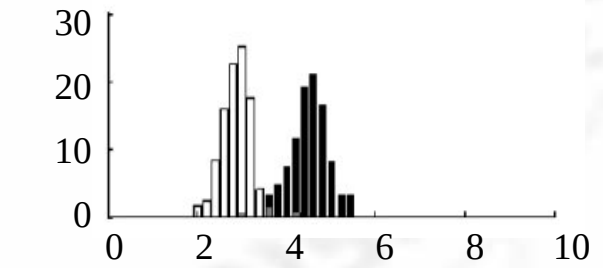
Network #2



Network #3



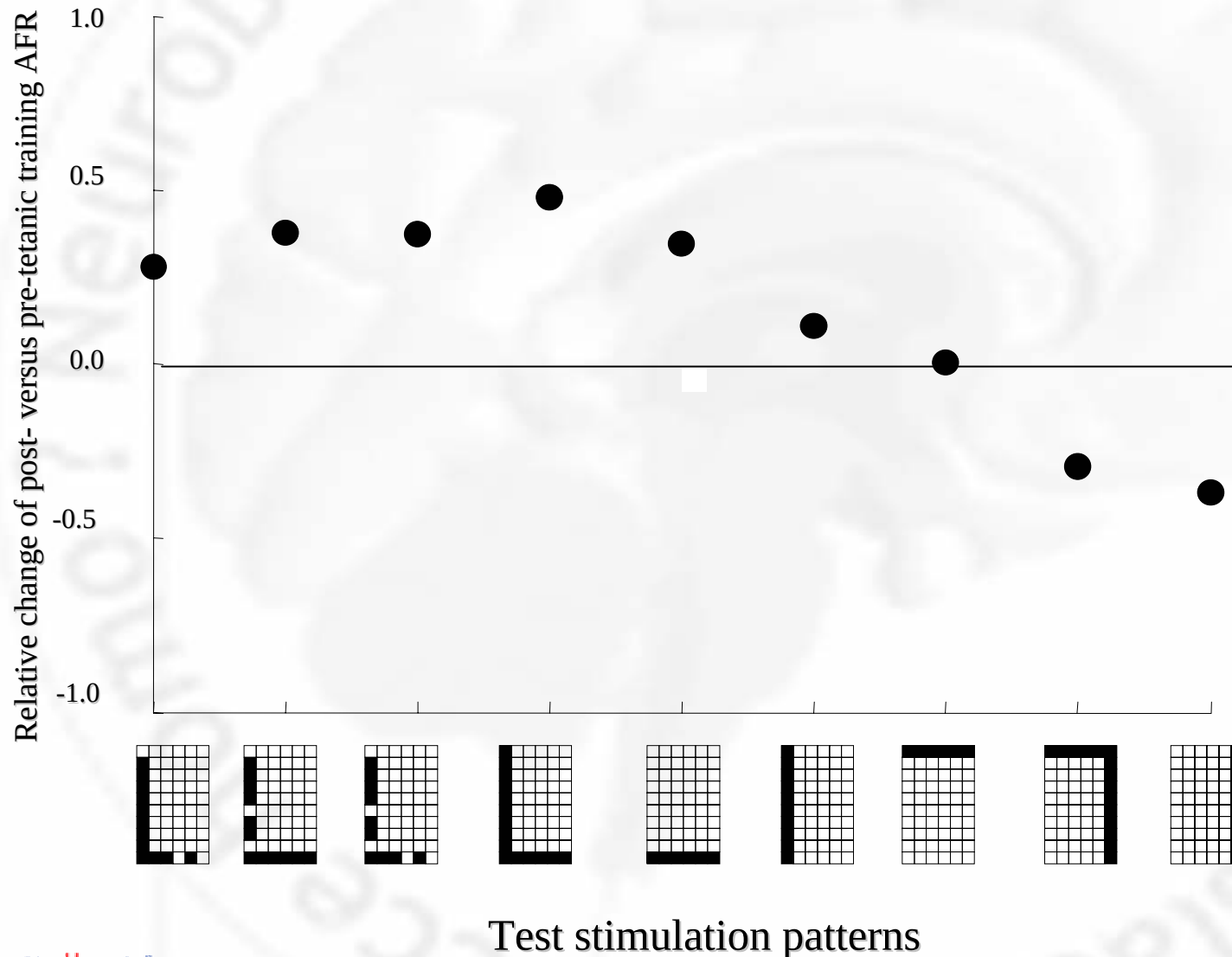
Network #4



spikes/(trial * site)

What part of the letter L does the network actually recognize?

Relative change of the mean response after L-training (total time window: 2*30 minutes)



Neural networks can filter out what they have learned ...

Neuronal filtering: L-feature extraction from rectangular test stimulus after L-tetanization

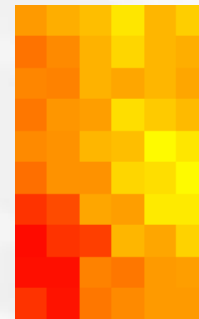
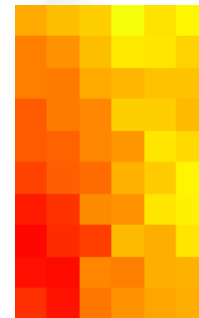
Original 8 bit
rectangle image
(= test stimulus)



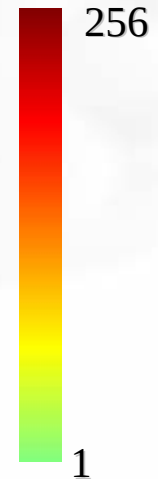
Pre L-training



Post L-training



Relative
AFR



Pattern recognition - Outlook

- Extension of experiments to **distinguish different patterns** at the same time
- Exploration of **voltage-coded** network **training**
- Exploration of **new spike and pattern classification algorithms**
- Repetition and **verification of experiments** with neural networks on high-density electrode arrays in CMOS technology (see following chapter)

Towards Information Processing by Natural Neural Networks

- Why natural neural networks?
- Technological aspects of biohybrid information processing systems
- Pattern recognition by natural neural networks
- **What's next?**

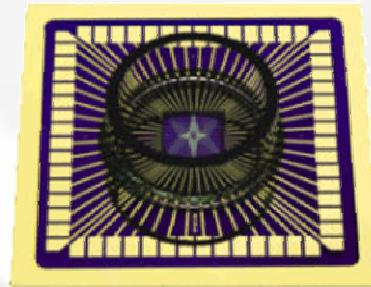
Limitations of current systems ...

- **Small number of electrodes** (60) – (low spatial resolution)
- **High price** (> 40 000 € + 230 € for each electrode array)
- **No real-time stimulation** hardware and software (fixed stimulation protocols)
- **No automated life-support** (manual feeding)
- **No portability** (costly laboratory infrastructure)

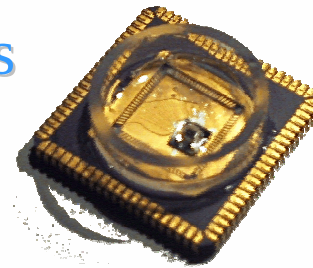
... may be overcome by developing **new technologies!**

New Electronics & Transducers

Current:
MEA-technology
25 cm², 60 electrodes
~ 230 Euro



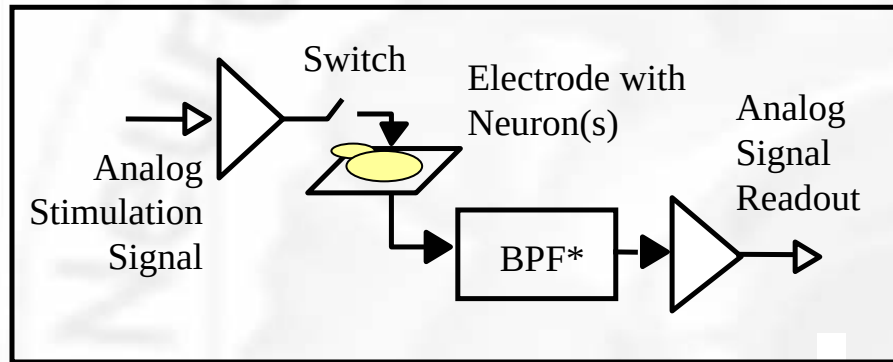
versus



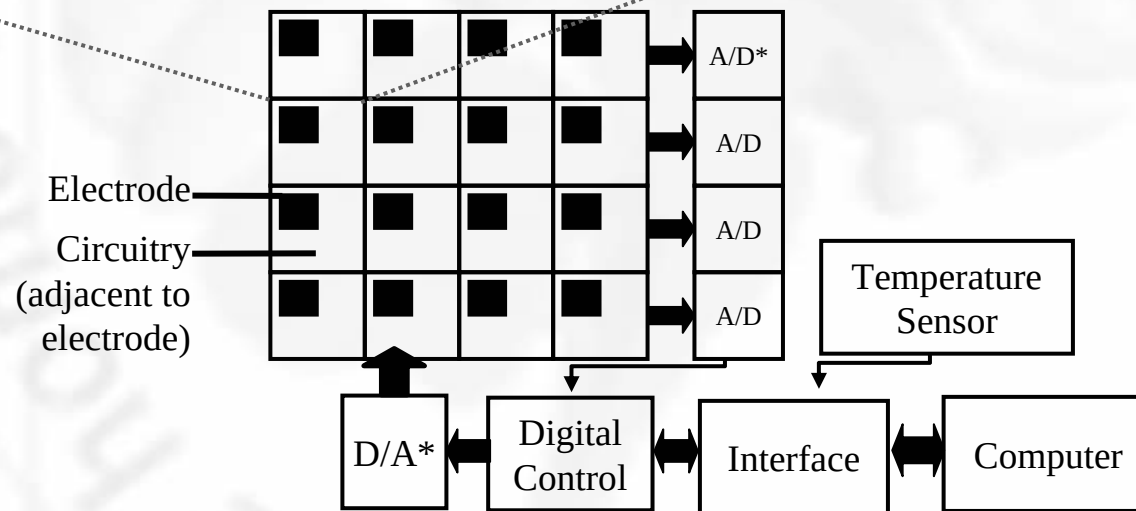
Near future:
CMOS-technology
1 cm², 1024 electrodes
< 10 Euro

CMOS chip and circuit design

High-density electrode array with on-chip amplifiers and stimulators

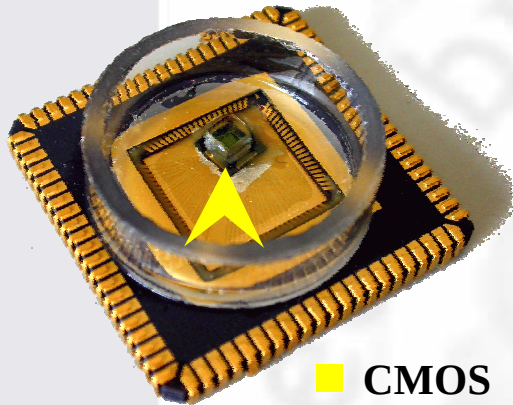


Inexpensive
Complementary Metal
Oxide Semiconductor
(CMOS) technology



* BPF: band pass filter; A/D: analog to digital converter; D/A: digital to analog converter

■ 4*4 Electrode array (1024 electrodes in the future)



■ CMOS

■ 1 D/A Converter

- sampling rate: 120 kHz
- resolution: 8 bit

■ Reference Electrode

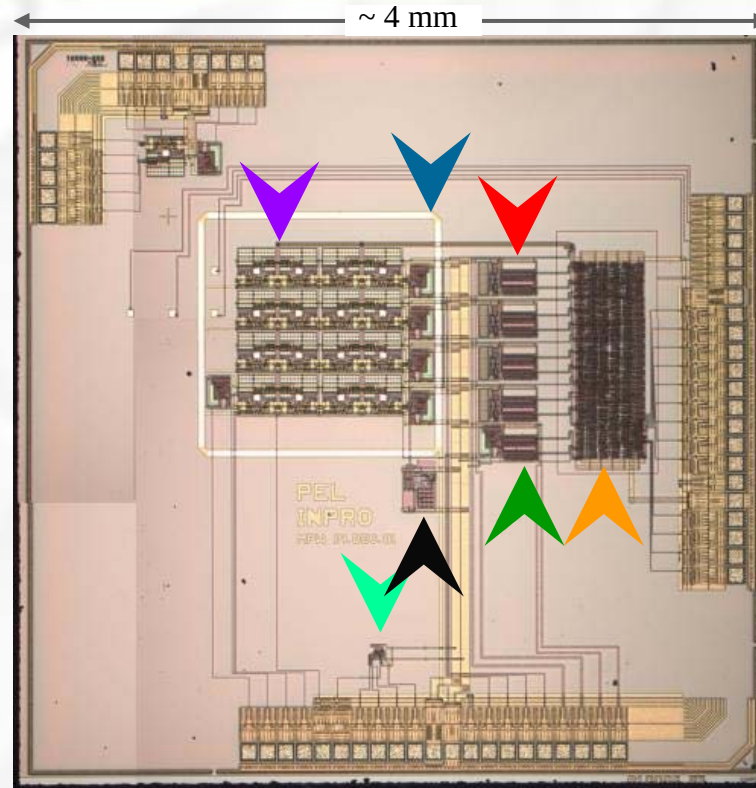
- electronic shielding
- reference for amplifiers and A/D

■ Temperature Sensor

- control of ambient temperature of cell culture

■ 4 A/D Converters

- sampling rate: 80 kHz each
- net rate: 20 kHz / electrode
- resolution: 8 bit



■ Digital Control

- communication to PC
- control of A/Ds, D/A, electrode selection

■ Biasing

for current-controlled voltage sources distributed in the array

Each unit is equipped with:

- electrode
- readout
 - amplifier with bandpass
 - buffer A/D decoupling
- stimulation
- flip-flop & switch to enable stimulation
- buffer for driving electrode

Stimulation

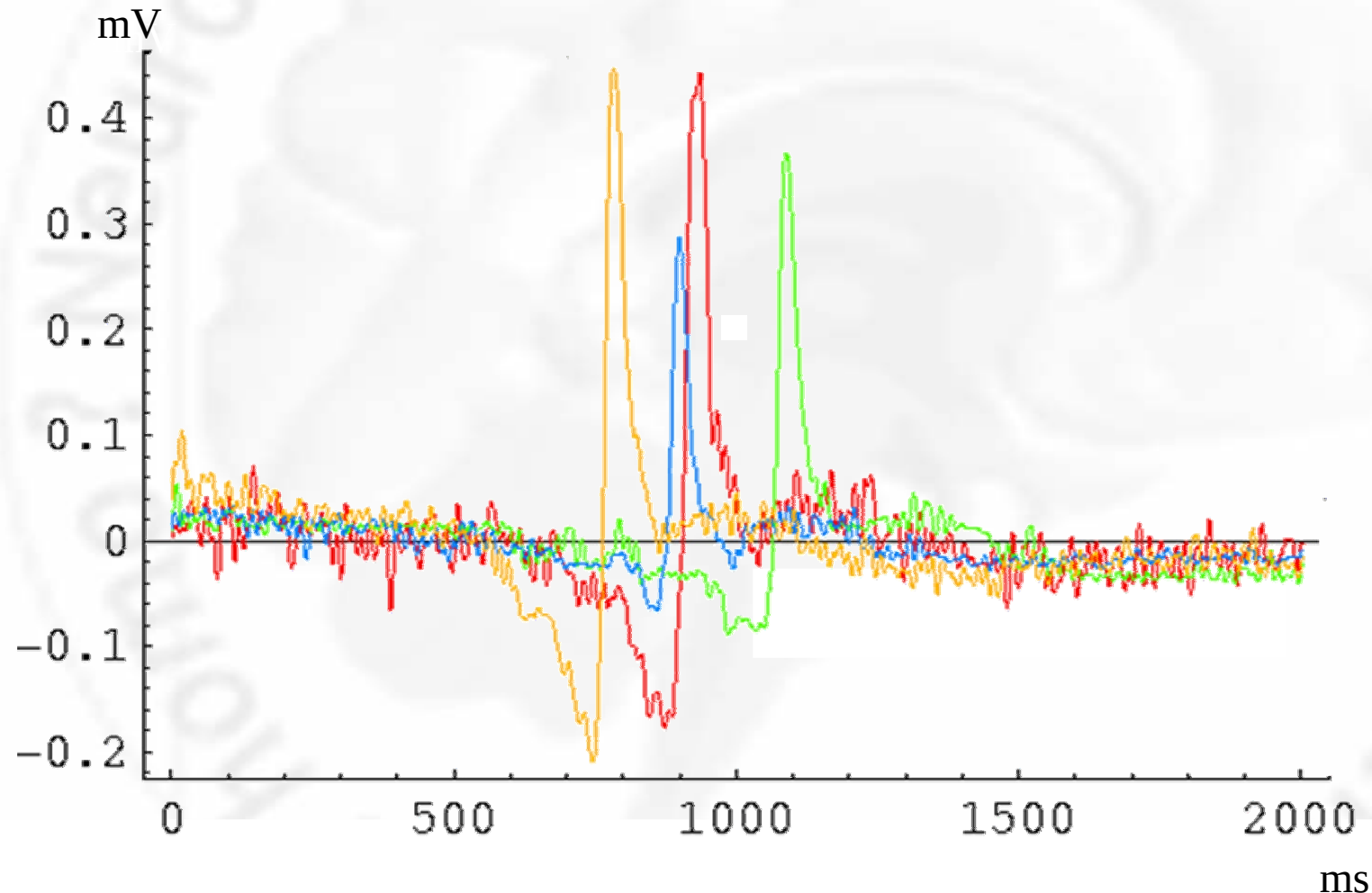
- select electrodes for stimulation
- provide 120-kHz, 8-bit signal form

Recording

- all electrodes at 20 kHz, 8 bit
- record also during stimulation

First bioelectrical measurements with such CMOS devices

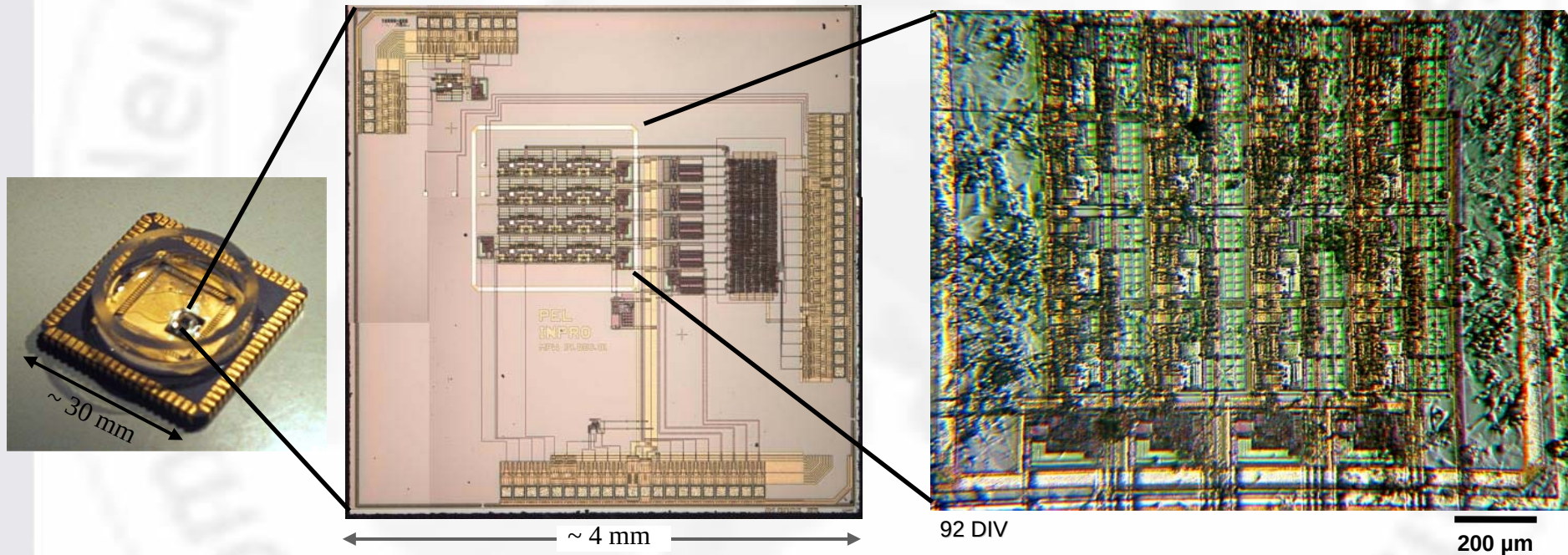
Spikes (extracellularly recorded action potentials) from cardiomyocytes from embryonic chicken (E7)*



* Signal amplitudes from cardiomyocytes are about 100x higher than those recorded from neurons.

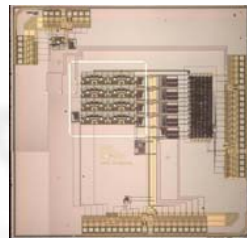
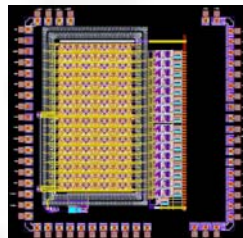
Biocompatibility of 2nd generation CMOS chip

Dissociated neural cultures can live on CMOS-chips for more than 240 days.



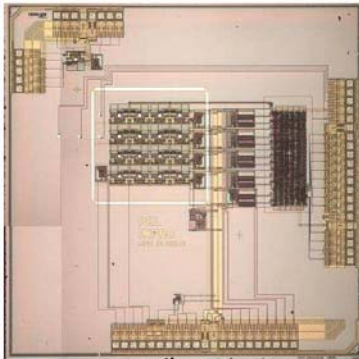
Feature comparison: 2nd versus 3rd CMOS generation

Feature	2nd	3rd	Comments
N° of electrodes	16	128	
Noise	< 9 μ V	< 5 μ V	Integrated over 10 Hz – 100 kHz
Signal processing	single-ended	fully differential	Protection against power supplies
Gain	1000	1000 or 3000	Selectable, different cells have different signal amplitudes
Bandpass filter	100Hz – 10kHz	adjustable	Flexible filter to record from different cell types or different phenomena (e.g. alpha-, beta-, gamma-oscillations, ...)
Sampling rate	20kHz	20kHz - 160 kHz	Reduction of random noise by oversampling → high resolution



The future of *in vitro* recording devices ...

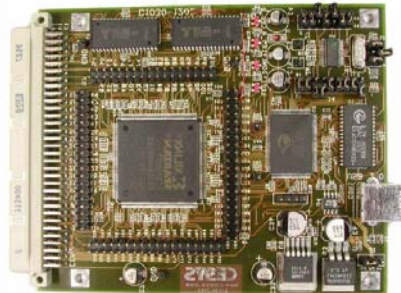
Hybrid Bio-CMOS Chip



16-Electrode Prototype

- Electrode Array
- Analog Design
- AD- & DA-converters
 - Digital Control
 - Digital Interface

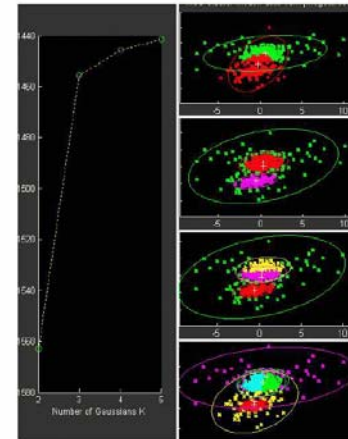
Reconfigurable Logic



Commercial fpga-board

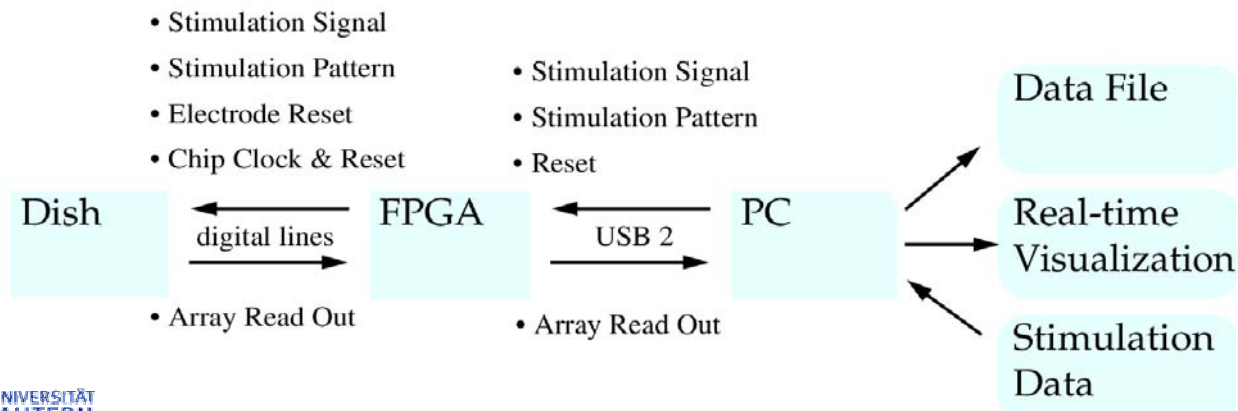
- Spike Detection
- Feature Extraction
- Buffering

Software



Discrimination of spikes recorded from a single electrode populated by two neurons.

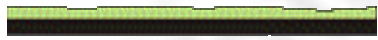
- Spike Discrimination
- Statistical Analysis
- Stimulus Generation





Enhanced Cell / Silicon Interface & Microfluidics

Adhesion Strategies ~ Overview



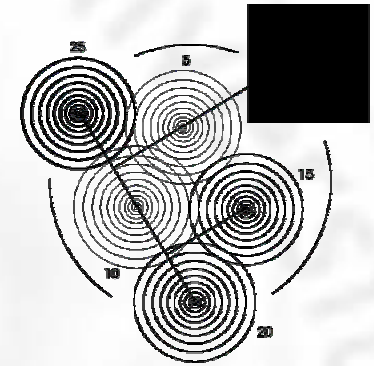
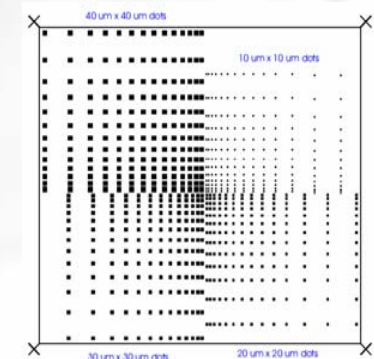
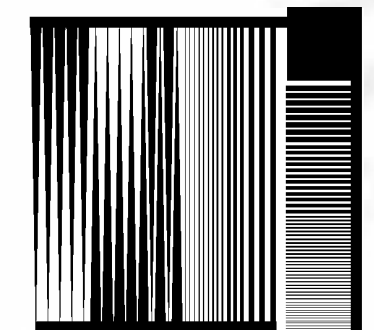
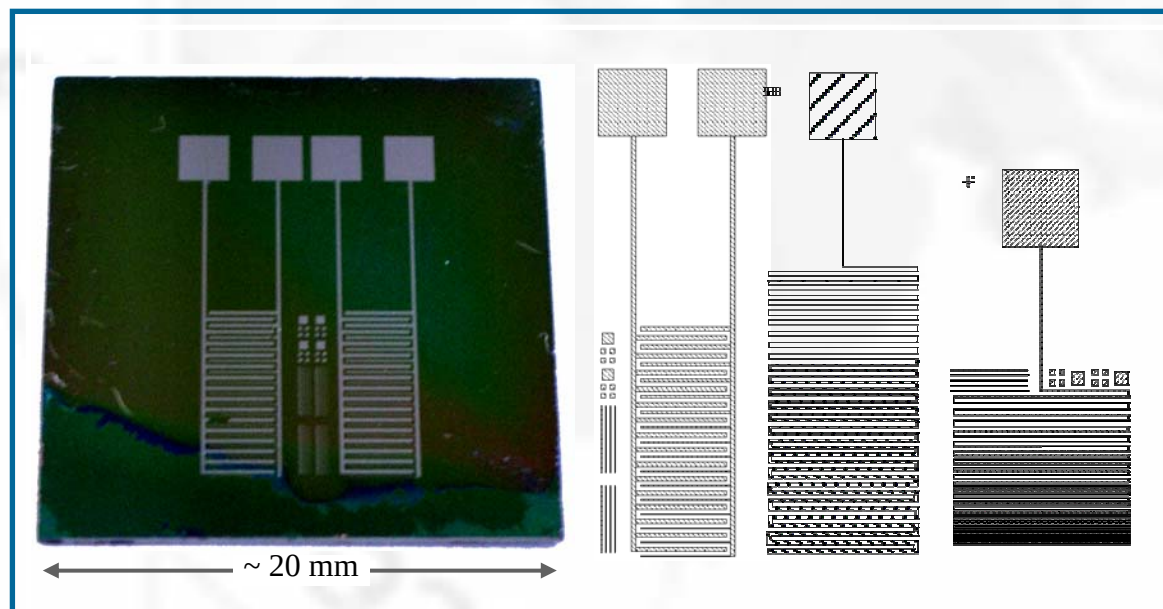
Global coating of any substrate (polylysine, polyethyleneimine, laminin, ...)



Electrochemical deposition on (Pt-) electrodes (biologically functionalized phenol derivatives, ...)

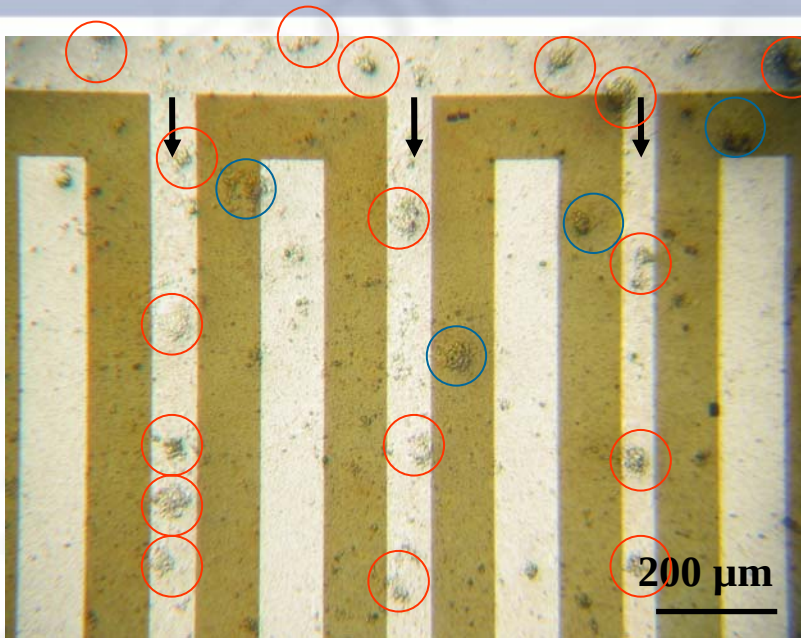
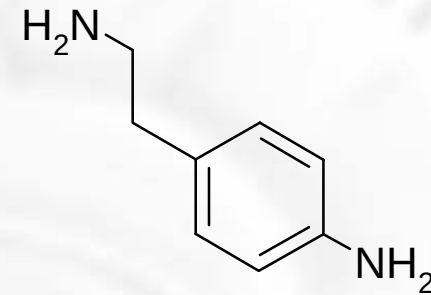


μ-Stamping on any substrate



Investigation of geometrical and chemical parameters on long-term cell adhesion strength.

Electrochemical deposition for localized cell adhesion

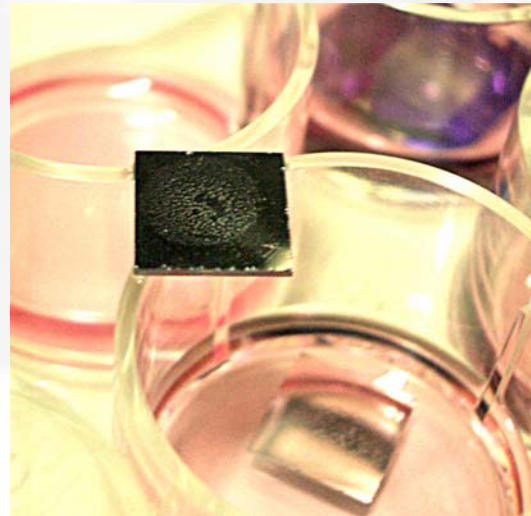
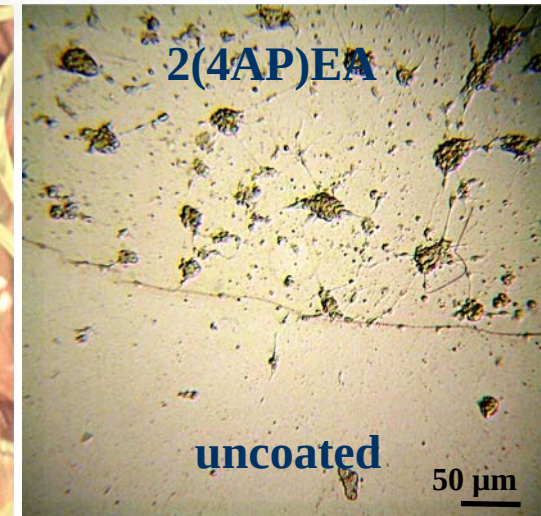


2-(4-Aminophenyl)-ethylamine
= 2(4AP)EA



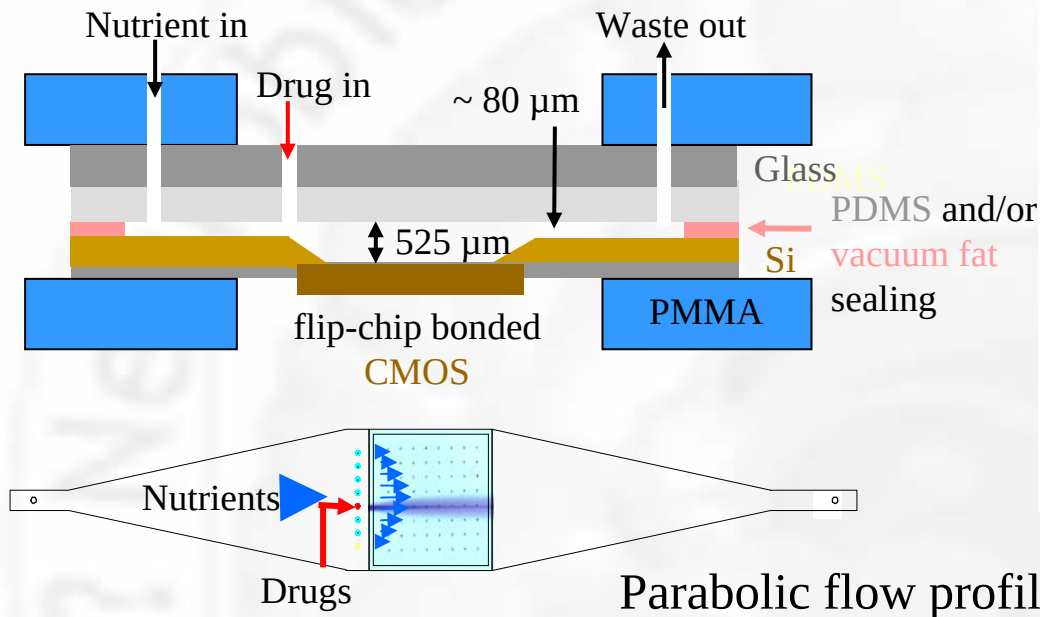
Interdigitated electrode (Pt on Si, ETHZ): Upper comb was modified by 2(4AP)EA

Pt electrode (ETHZ): A circular area was modified by 2(4AP)EA

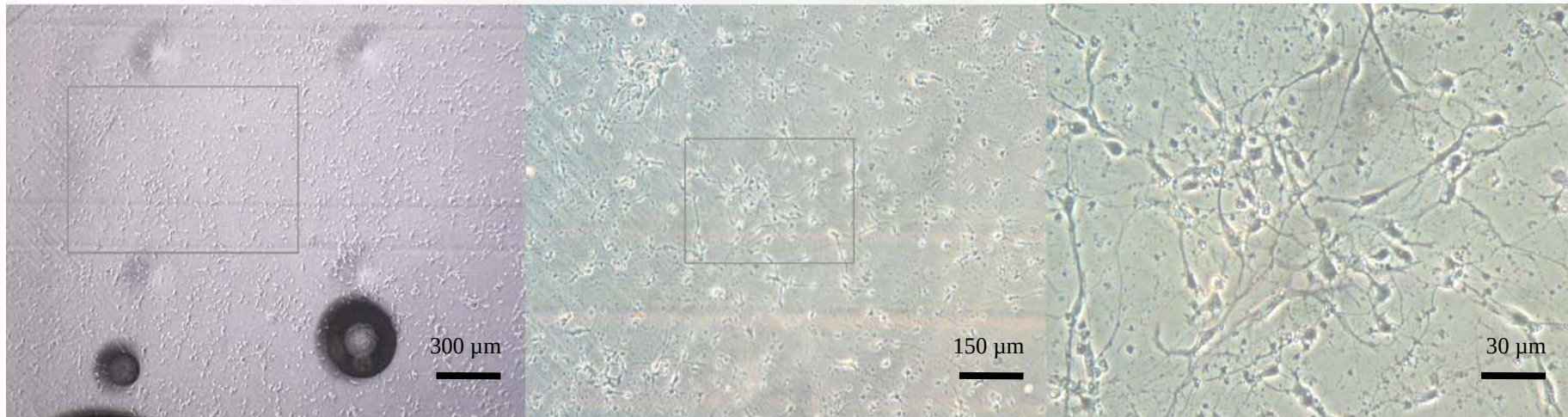


7 days in vitro (DIV)

Perfusion chambers for automated nutrient and drug delivery

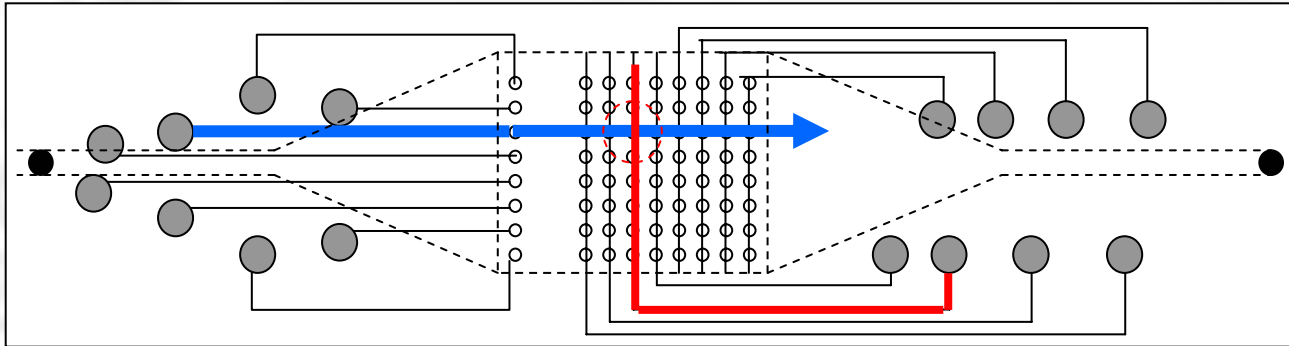


Neurons after 14 DIV

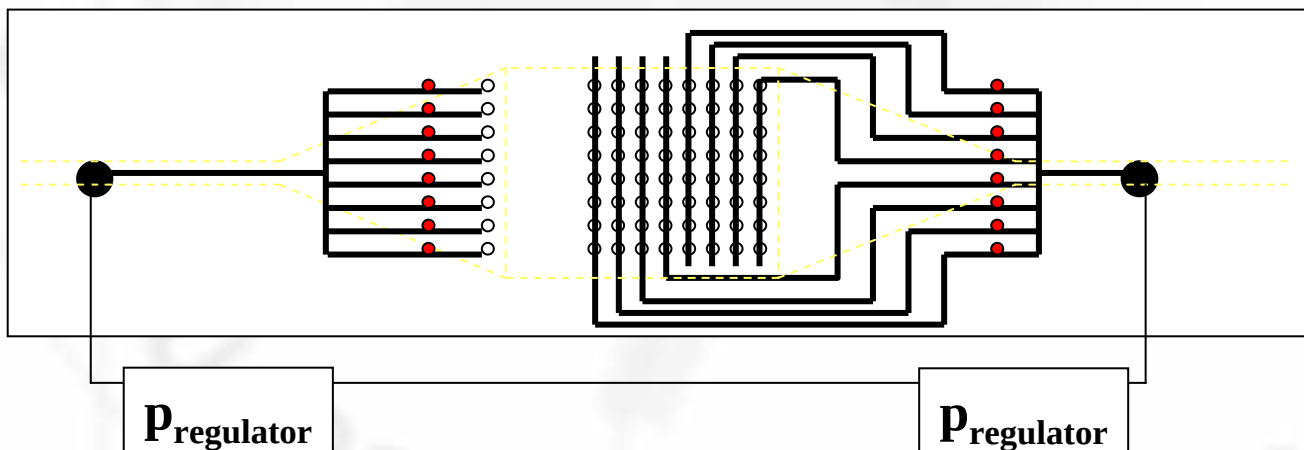


Outlook for next generation drug delivery design

Step 1: Addressing of individual 'pixels': laminar **drug flow** is pushed down by perpendicular **nutrient flow**



Step 2: Enhanced flow control by additional valves (●)



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