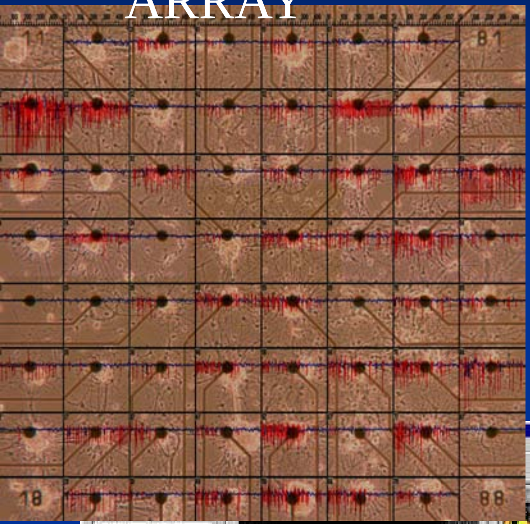
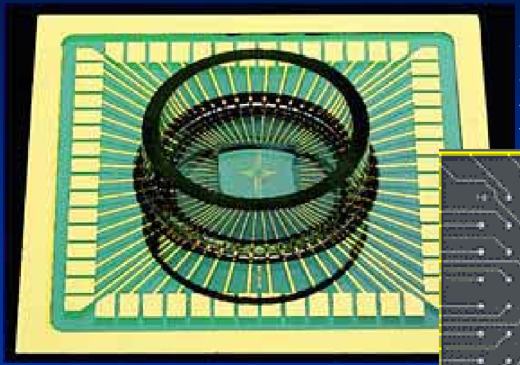
Brain on a Chip:
Can We Build One?

Bruce C. Wheeler
J. Crayton Pruitt Family
Department of BioMedical Engineering
University of Florida

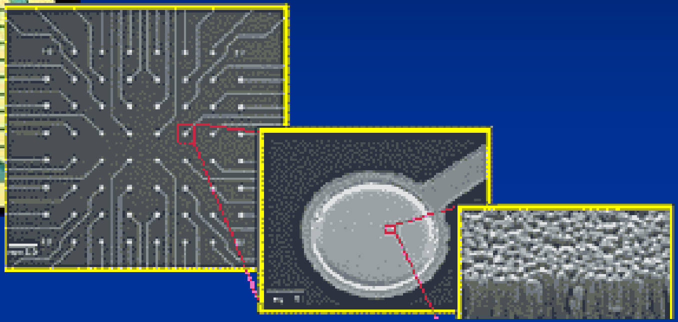
What is a Brain On A Chip

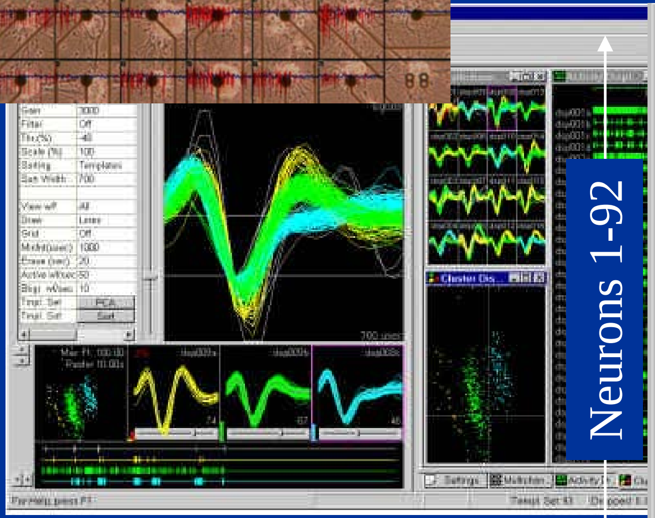
NEURONS GROWN ON AN ARRAY

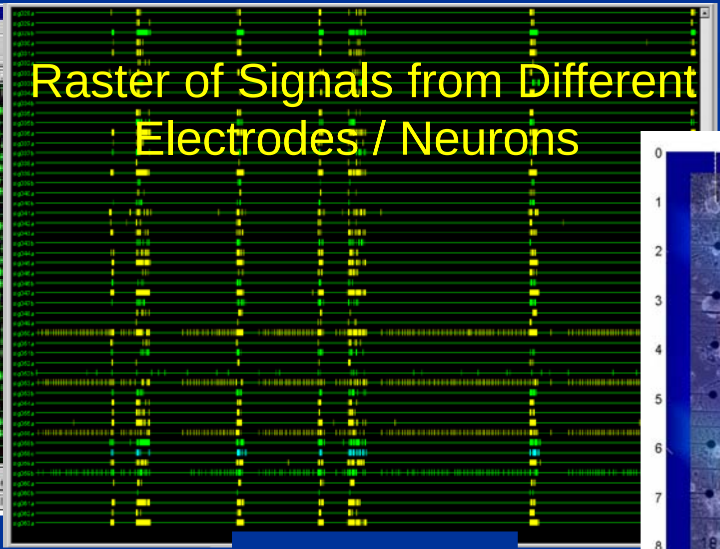


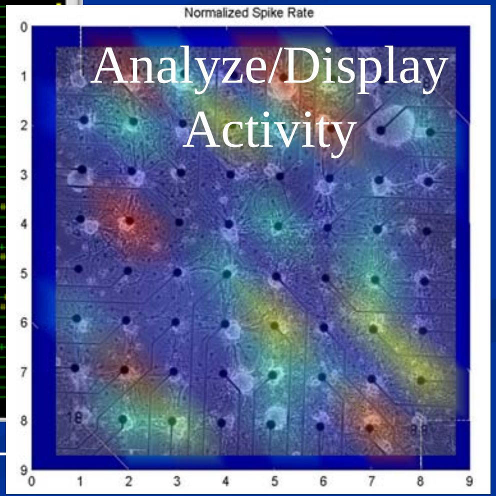


ELECTRODE ARRAY
MultiChannelSystems, Reutlingen Germany









Time 1.5 sec

RECORDED / STIMULATED ACIVITY
Plexon Inc. Dallas TX

Grand Challenge: Constructing Increasingly Realistic In Vitro Neural Circuitry

- Assist in Neuroscience Investigations
  - Easy chemical access, stimulation, recording
- Disease Models – Stroke, Epilepsy, Stem Cell / Regeneration
- Pharmaceutical Testing, Development
- Circuits: Cortex to Thalamus and Back
- Neural Network Models
- Learning and Memory
- Study the Question: Does Form Influence Function

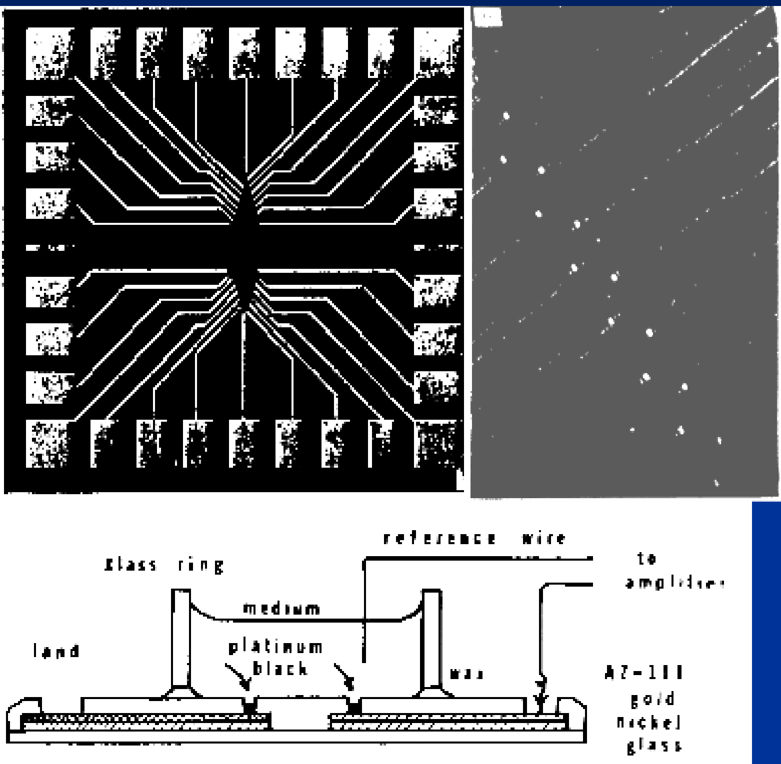
Can We Build a Brain on a Chip?
Elements of Design

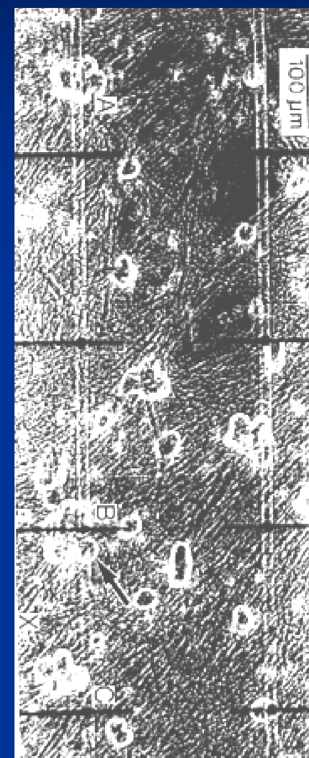
- The Chip
- Nature's Designs
- Lithography
- 3D Scaffolding & Lithography
- 3D Fluidics
- Analysis
- Design by Stimulation
- Media / Cell Type
- Optical Recording / Stim (not today)

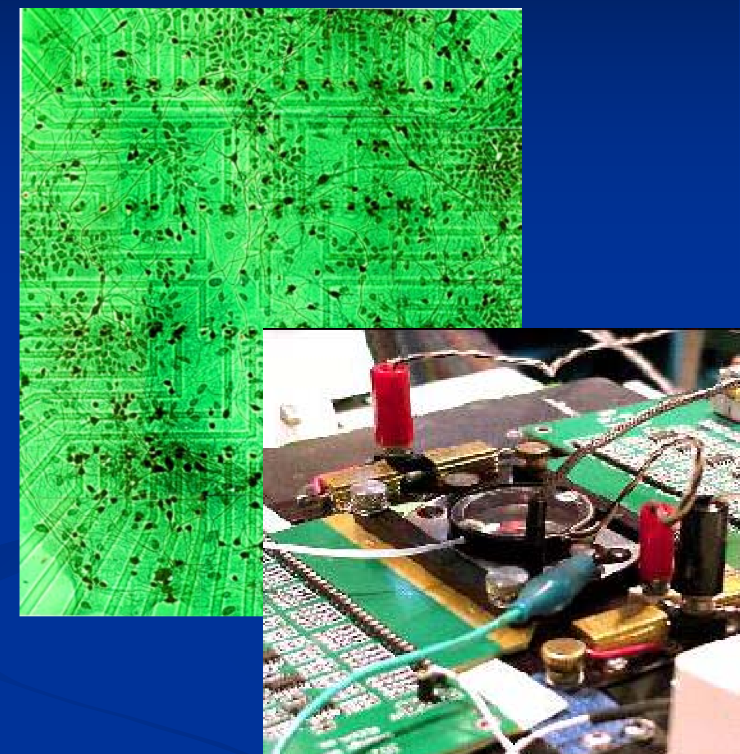
An Engineer's Approach: You Don't Understand it Until You Build It.

Chip Part of Brain on Chip Has a Long History

Earliest: Thomas 1972, Pine 1980, Gross 1979







First Array. Cardiac Cells.
Single etched line.

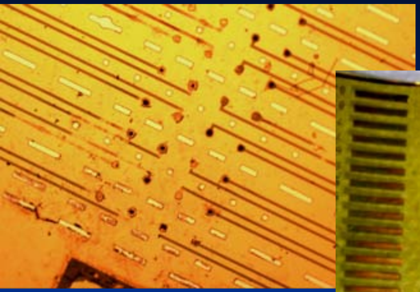
Thomas, Springer, Loeb, Berwald-Netter, Okun. A miniature microelectrreode array to monitor the activity of cultured cells. Exp. Cell Res. 74(`1)61-66, 1972

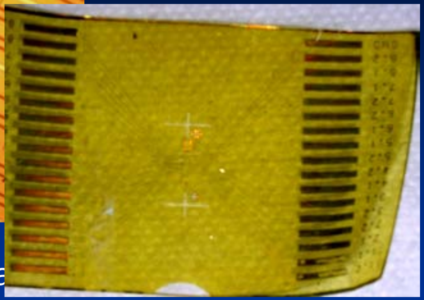
First Cultured Neurons

Pine, Recording action potentials from cultured neurons with extracellular microcircuit electrodes.

Gross: First Modern MicroElectrode Array
1979 Ganglia
1982 Culture

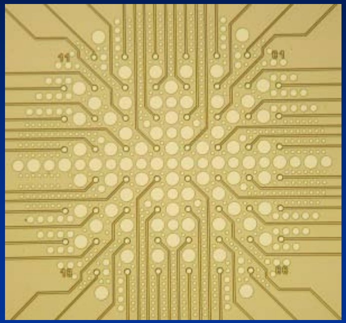
Toward Disposable Devices



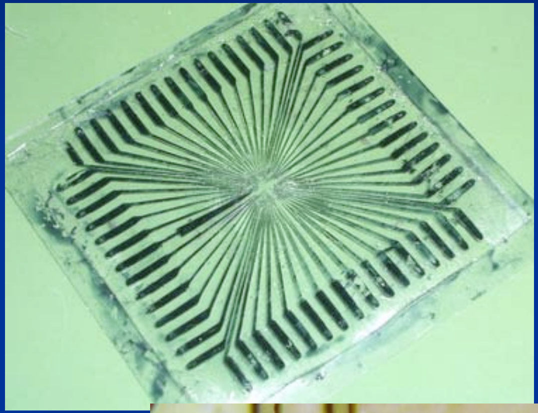


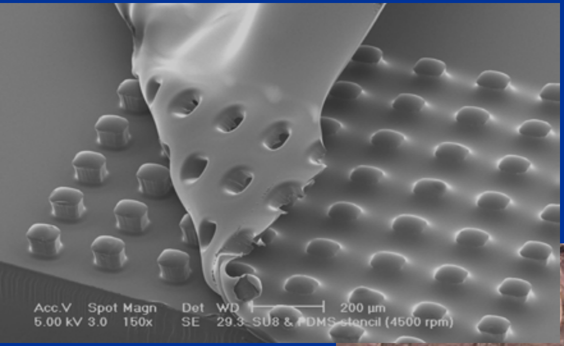
Boppart, Wheeler, Wa
IEEE Trans. Biomed. Eng.
39, 37-42, 1992.

Perforated/
flexible arrays

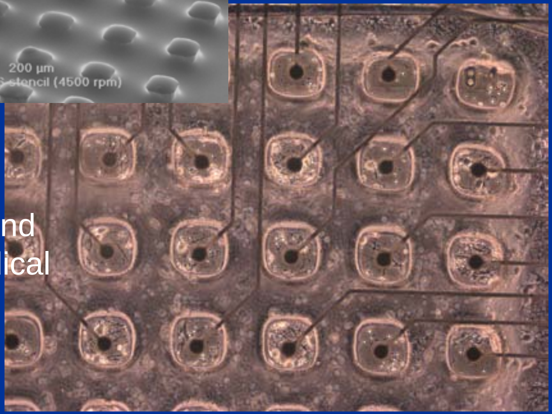


Multichannel
Systems, 2005



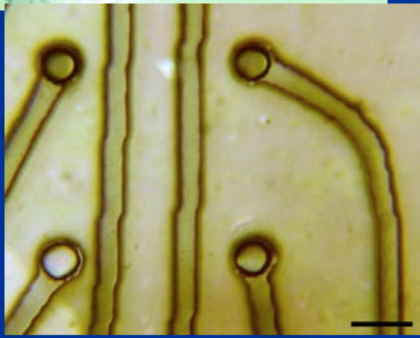


Disposable
PDMS
Insulator



Nam, Y., Musick, K., and
Wheeler, B.C. Biomedical
Microdevices., 2006

PDMS Array
with
Conductive
Polymer
Electrodes

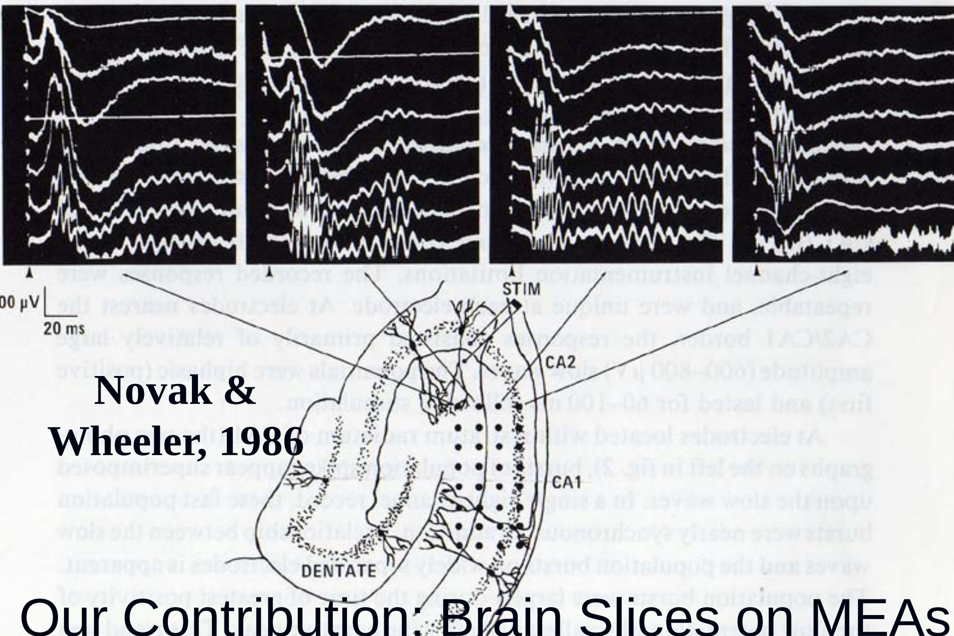


Murr, Ziegler, Benfenati, Blau,
Replica-molded polymer
microelectrode arrays
(polyMEAs), MEA2008

Designing Brains on Chips: Using Nature's Designs

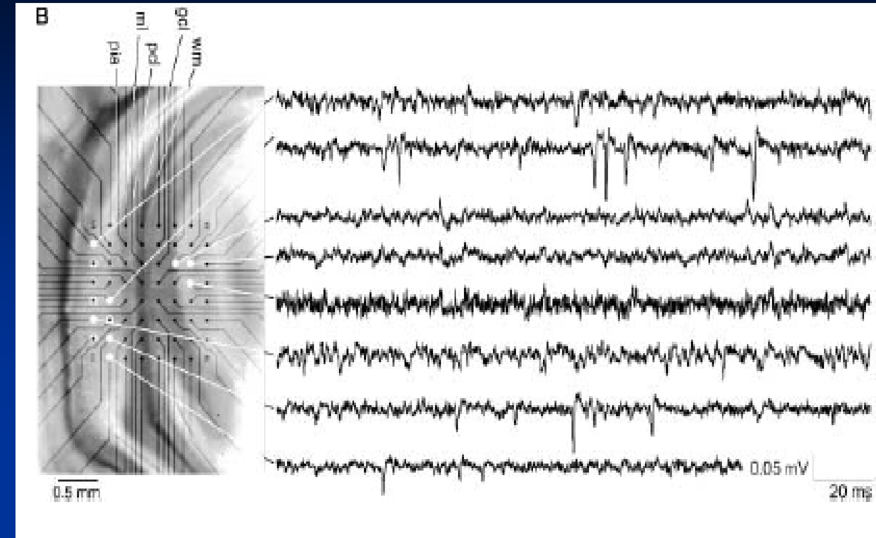
- Brain Slices
- Cultured Neurons
  - most often taken from embryonic or neonatal rats
  - hippocampal, cortical, dorsal root ganglion, spinal
  - weeks before electrically active -- hard experiments
- Isolated Retina
- Cardiac Myocytes

Brain Slice on Chip: Many Examples: here are three



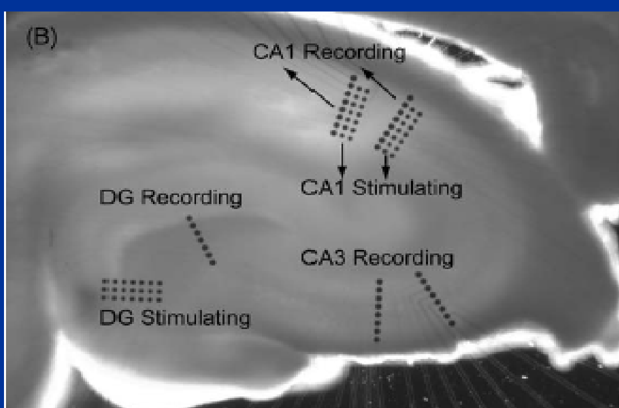
Novak & Wheeler, 1986

Our Contribution: Brain Slices on MEAs



Egert: mapping cerebellar activity

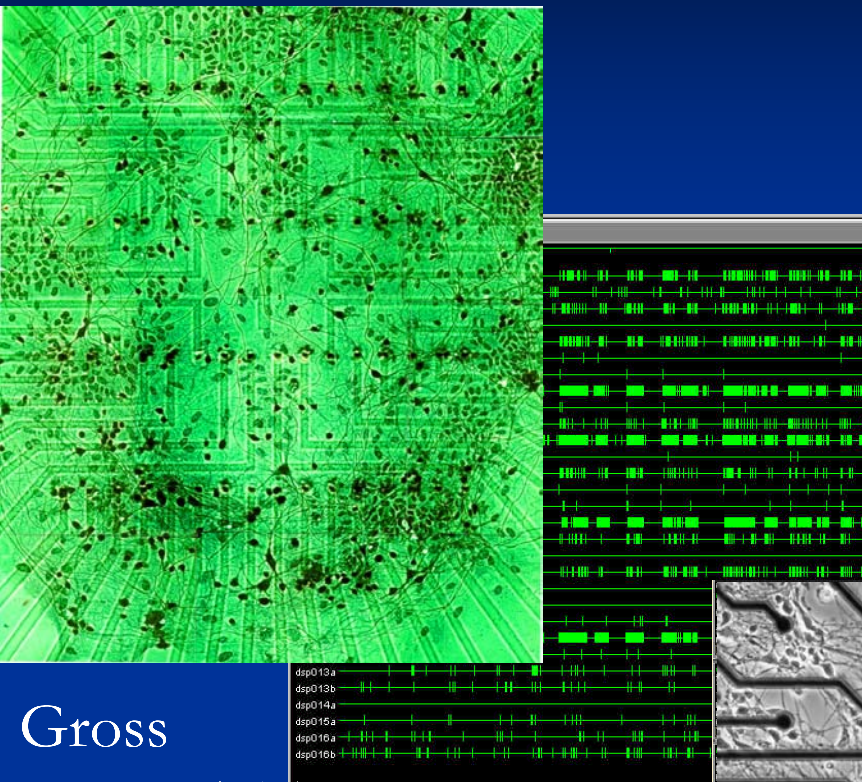
Egert, et al. Exp Brain Res (2002) 142:268–274



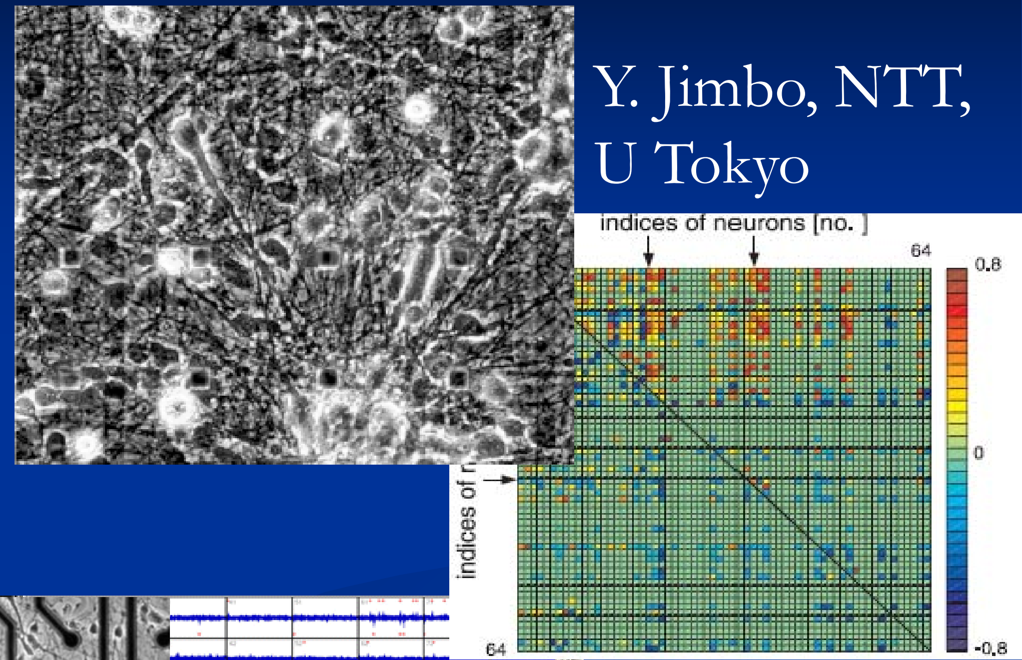
Gholmieh et al., Journal of Neuroscience Methods 152 (2006) 116–129

Berger Lab: Trisynaptic Pathway Modeling and Functional Replacement

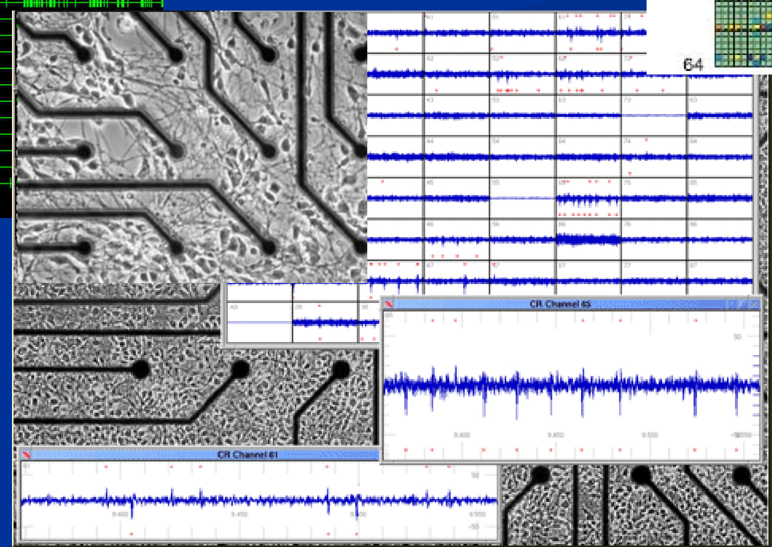
Cultured Neurons on a Chip



Gross
U. North Texas



Y. Jimbo, NTT,
U Tokyo



Steve Potter, Ga Tech

Designing Brains on Chips

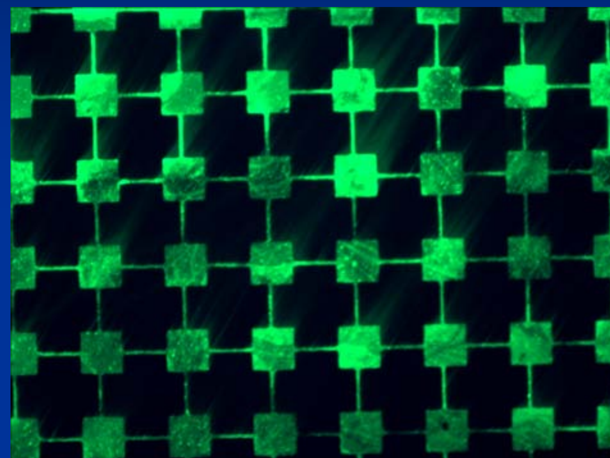
By Surface Lithography / Chemistry / Topography

Works Quite Well
Highly Structured Networks Can be Created
Ready to Be Exploited Further
Used for Many Cell Types
Assaying of Chemical Cues

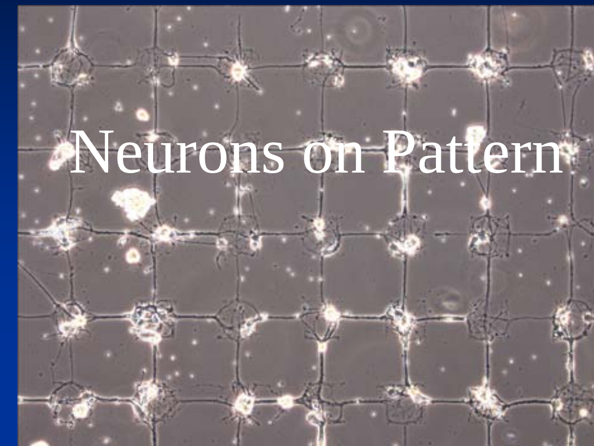
Design by Lithography Micro Contact Printing

PDMS Stamp

Length.: 80 µm
Node Diam.: 15 µm
Width.: 5 µm
Relief: 20 µm



Fluorescence Image of Stamped Protein



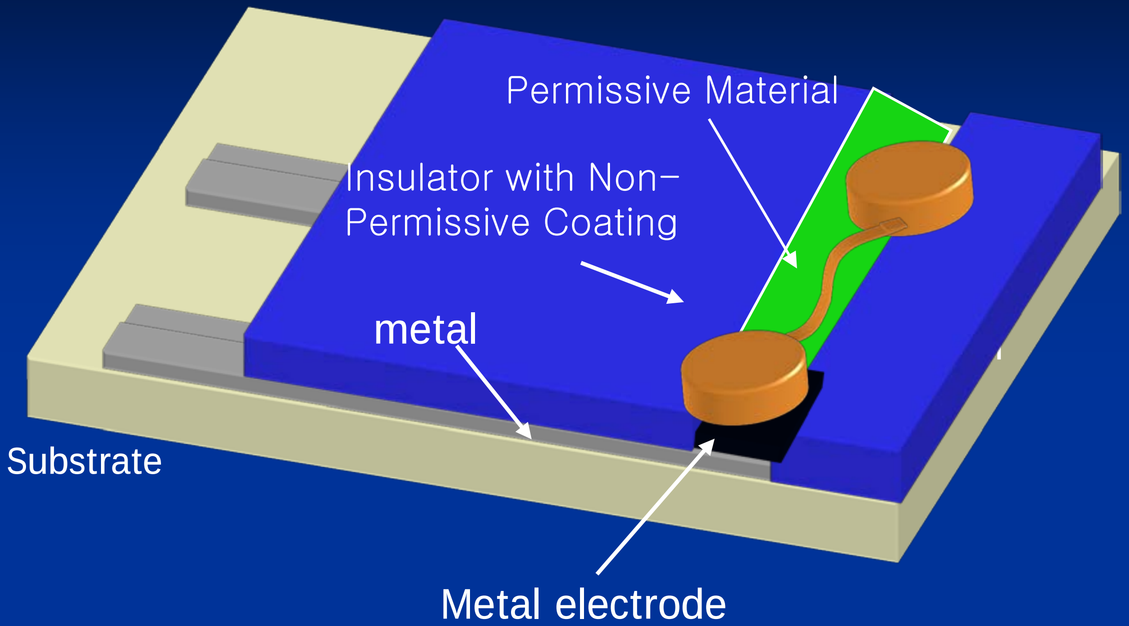
Neurons on Pattern

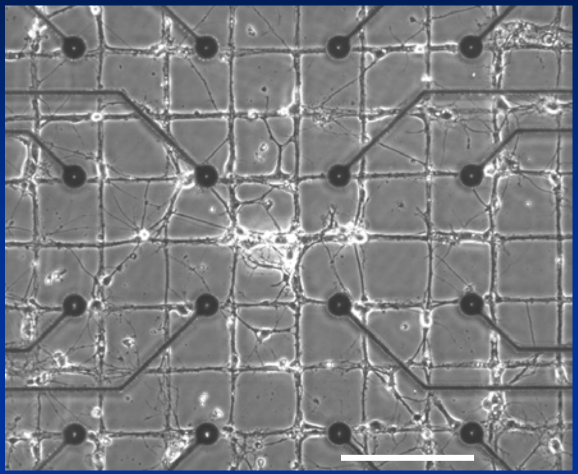
Aligned Double Stamping

Branch, D.W., Corey, J.M., Weyhenmeyer, J. A., Brewer, G. J., & Wheeler, B.C. (Jan 1998) “Micro-stamp patterns of biomolecules for high-resolution neuronal networks,” Med. & Biol. Comp. & Eng., 36, 135-141

Wheeler, B.C., Corey, J.M., Brewer, G.J., & Branch, D.W (1999), “Microcontact printing for precise control of nerve cell growth in culture,” J. Biomech. Eng., 121, 73-782863-2870

Design by Lithography

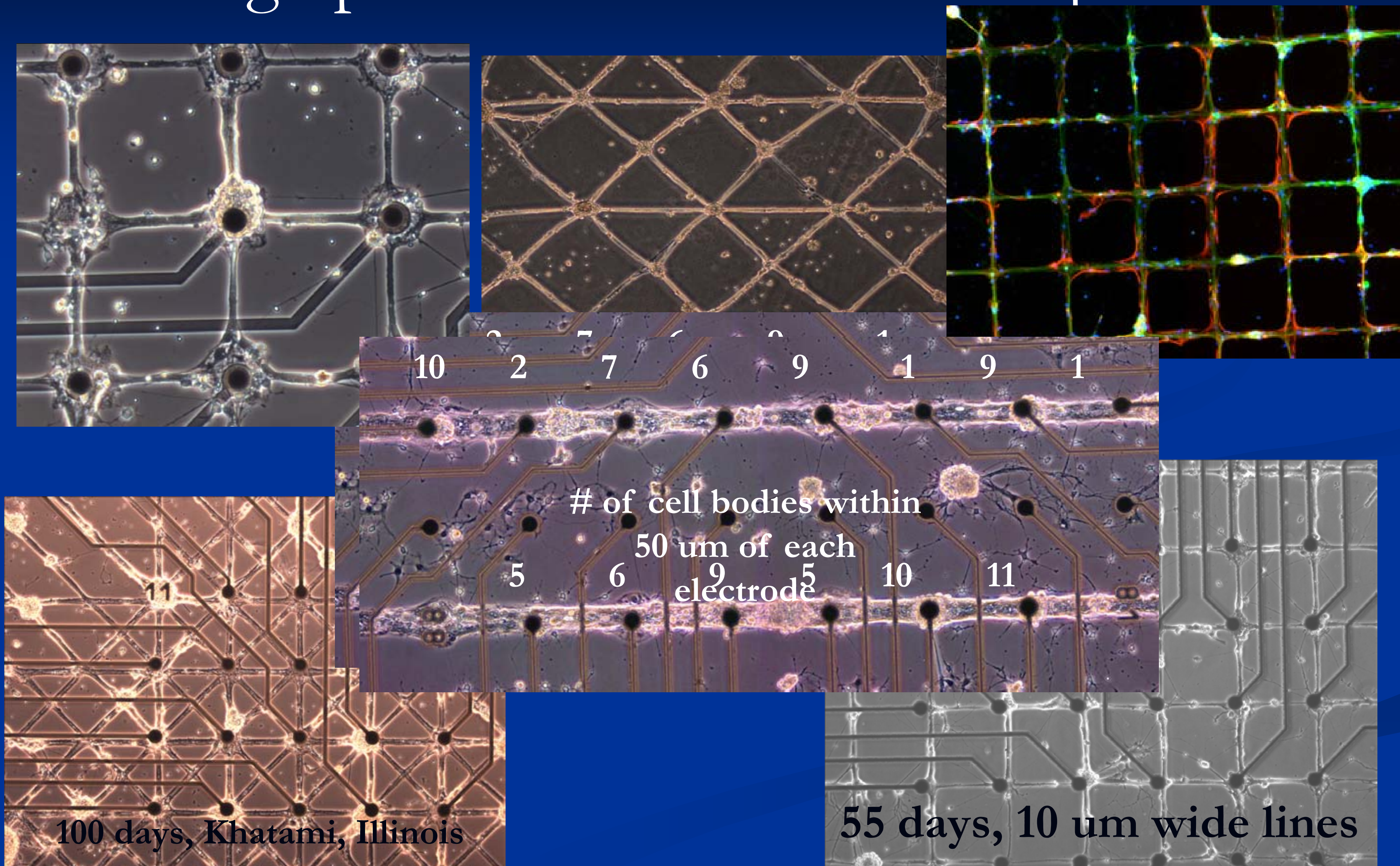




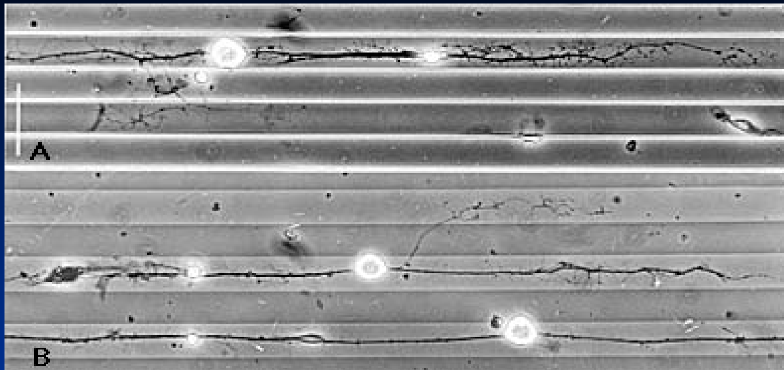
- **Microcontact Printing**
- **Photoresist Patterning**
- **Microfluidic Deposition**
- **Laser Ablation**
- **Microchannels**
- **Covalently linked or physisorb**

- **Metals:** platinum, indium tin oxide, titanium nitride, gold
- **Insulators:** silicon nitride, silicon dioxide, glass, polyimide, PDMS
- **Permissive:** polylysine, laminin, …
- **Nonpermissive:** PEG, chondroitin sulfate, …

Lithographic Results Can Be Exceptional



Design by Surface Topography or Physical Confinement

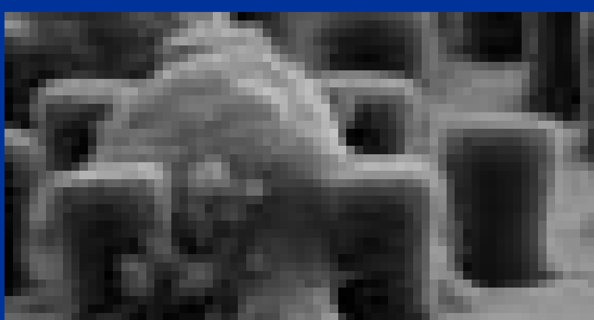


Guidance by Surface Channels

S. Britland, C. Perridge, M. Denyer, H. Morgan, A.S.G. Curtis, C.D.W. Wilkinson, Experimental Biology Online , 1:2 , ISSN 1430-3418, 1996.

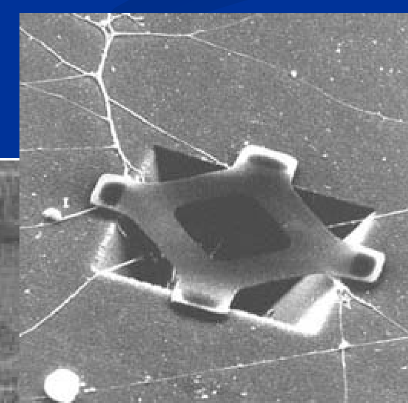
Neurons in PDMS microchannels

F. Morin et al. / *Biosensors and Bioelectronics 21* (2006) 1093-1100



Günther Zeck and Peter Fromherz PNAS 2001 98: 10457-10462.

Pine, CalTech



Circuit Design: Logic Gates

B

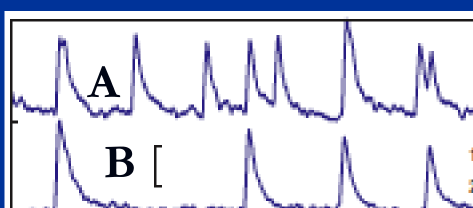
1

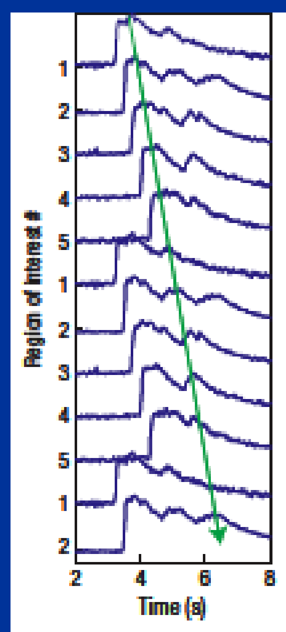
2

A

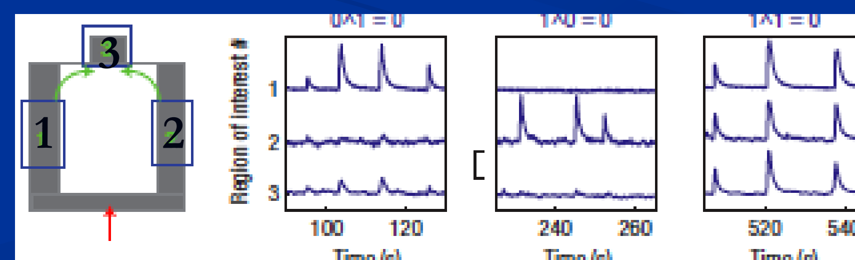
Delay Line

Diodes





AND Gate

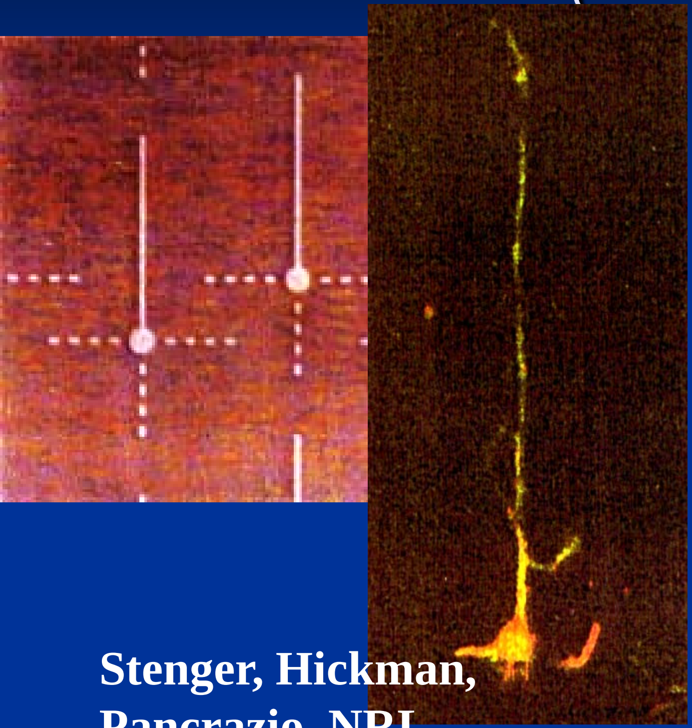


Feinerman, Rotem, Moses, Reliable Neuronal Logic Devices …
Nature Physics Dec 08

Design: Organizational Concepts

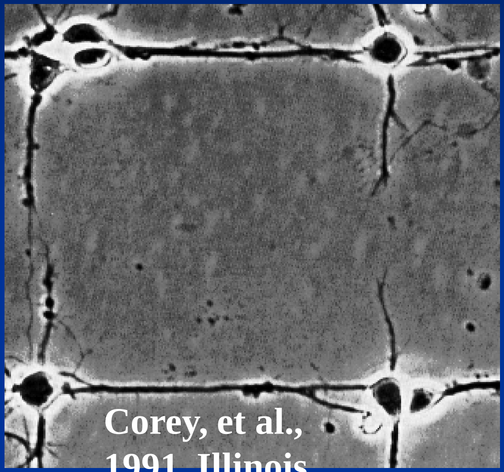
What is the unit of construction?

Design Choice:
Single Oriented Cell Bodies and Axons
(Circuit designer's approach?)

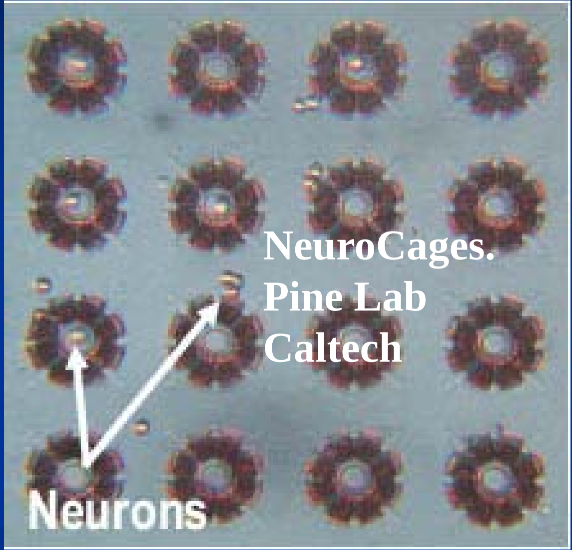


Stenger, Hickman, Pancrazio. NRL.

Stenger DA et al. *J Neurosci Methods* 1998 Aug 1;82(2):167-73



Corey, et al., 1991. Illinois

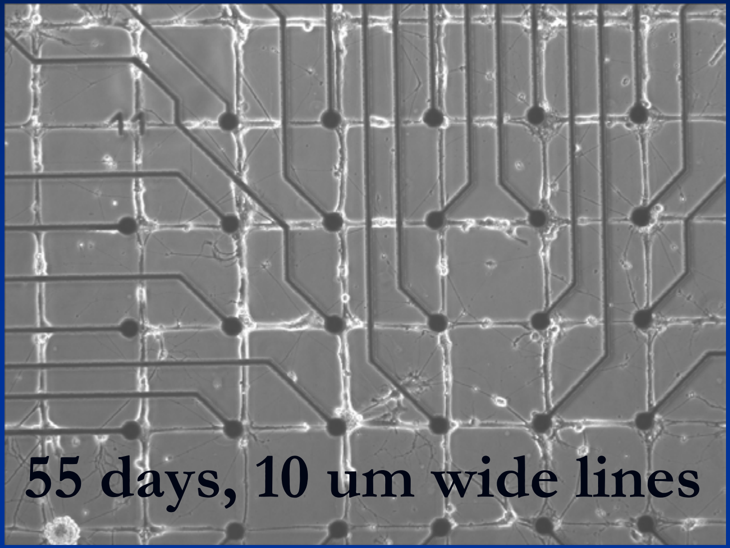


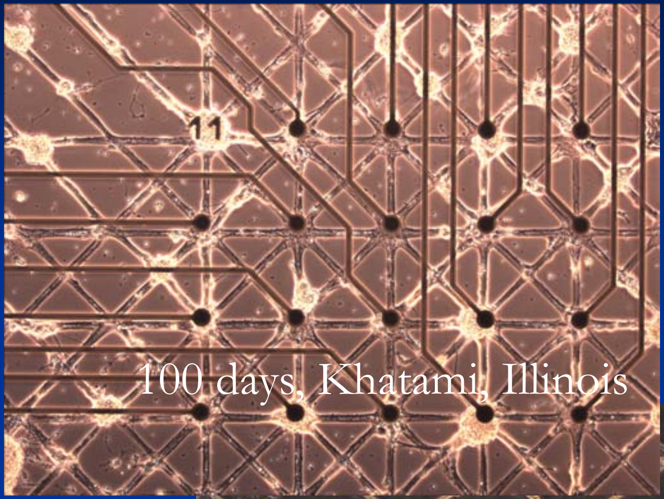
NeuroCages. Pine Lab Caltech

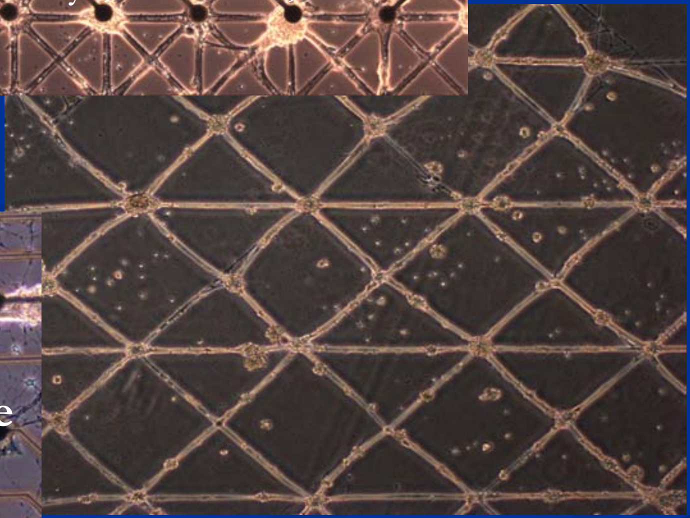
Nam. Illinois (now at KAIST)

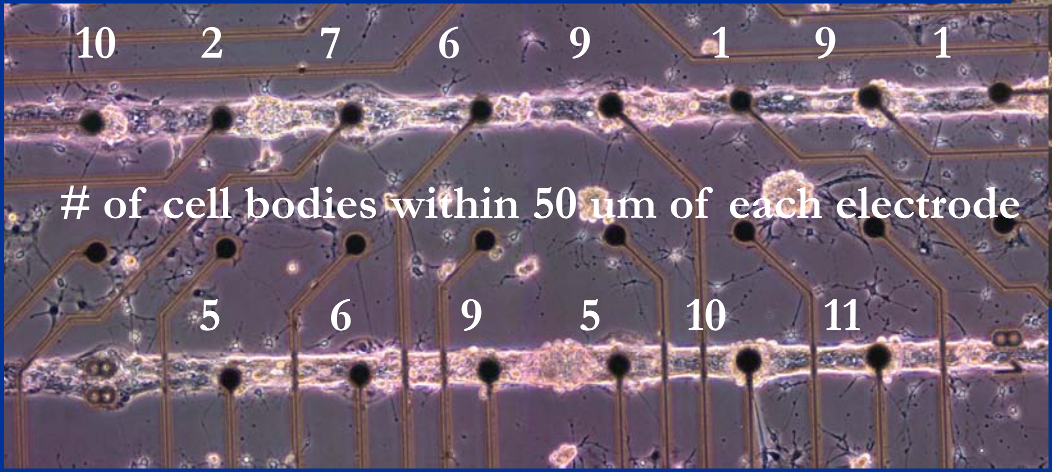
14 days, 3 um wide lines

Design Choice: Narrow or Wide Bundles?



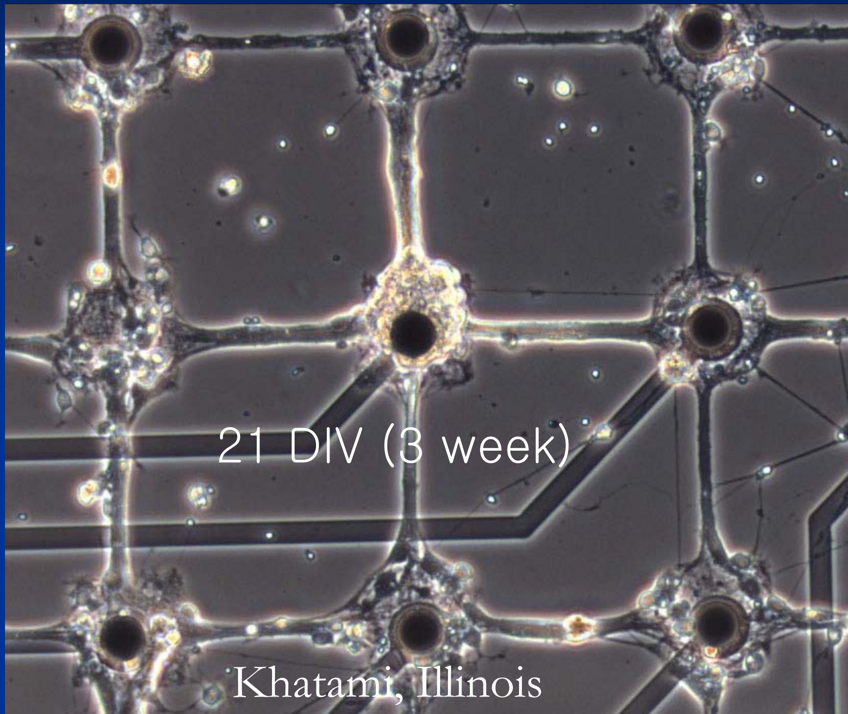


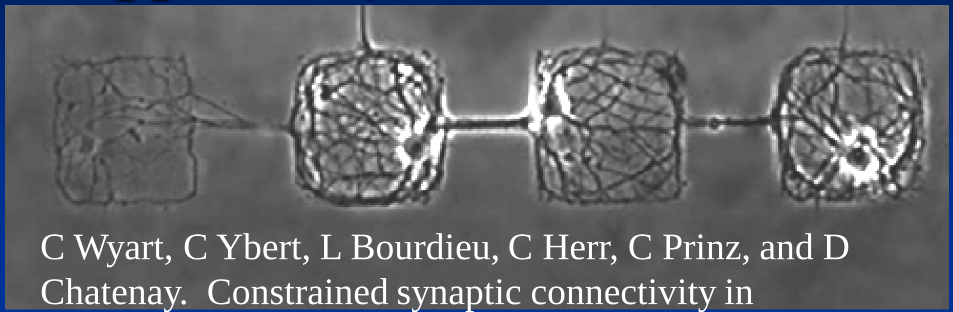




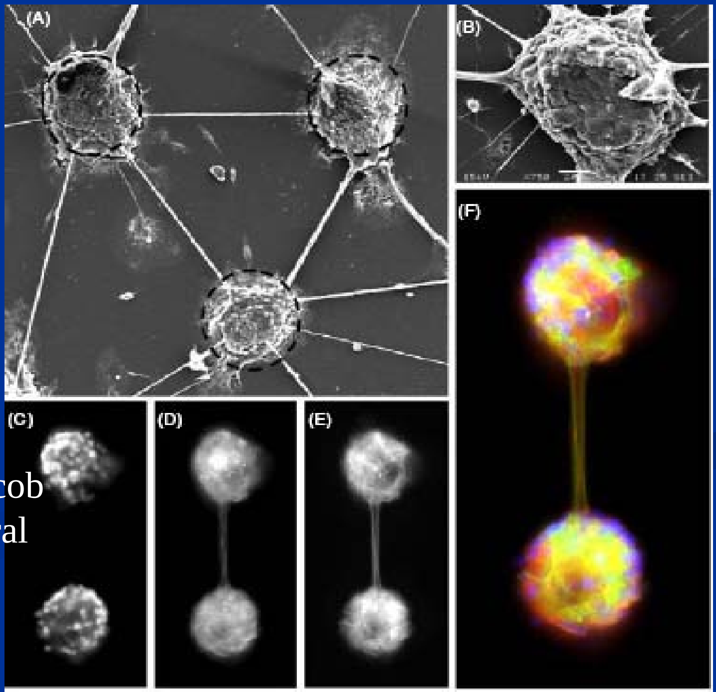
Design Choice: Clusters and Spokes

(connectionist approach?)





C Wyart, C Ybert, L Bourdieu, C Herr, C Prinz, and D Chatenay. Constrained synaptic connectivity in functional mammalian neuronal networks grown on patterned surfaces. Journal of Neuroscience Methods 117, 123-131(2002)

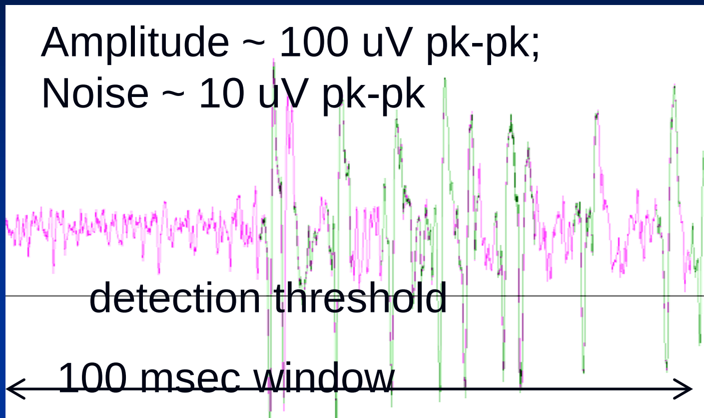


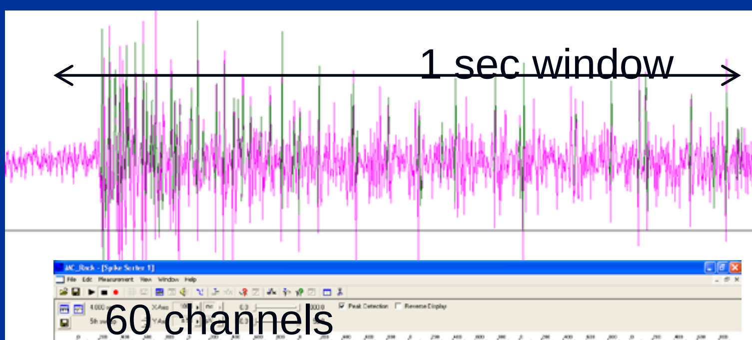
R Sorkin, T Gabay, P Blinder, D Baranes, E Ben-Jacob and Y Hanein, Compact self-wiring in cultured neural Networks, J. Neural Eng. 3 (2006) 95–101

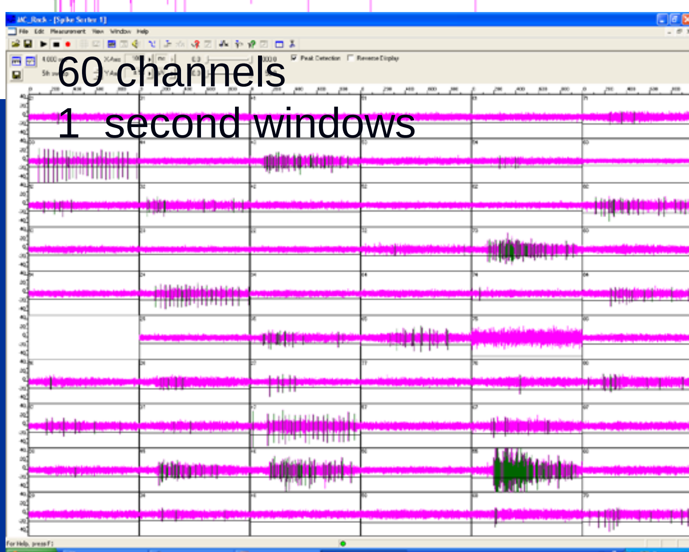
What Might A Brain on A Chip Say?
What Can We Learn?
What Can It Learn?

First … the complexity of the signals

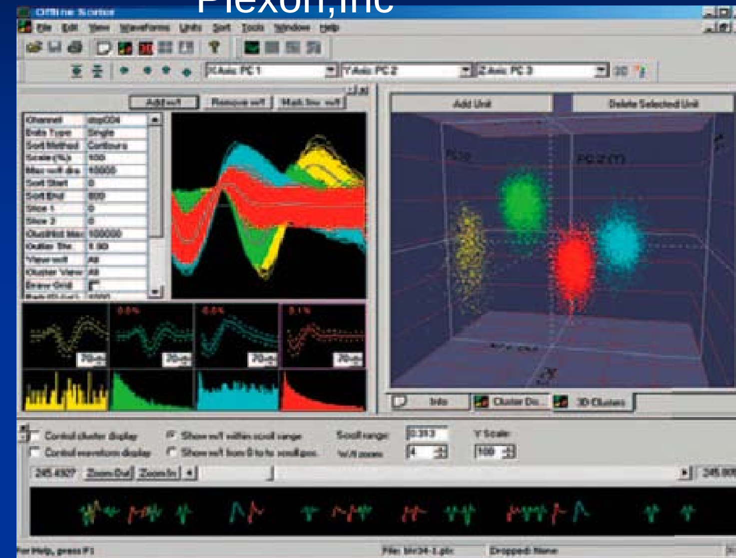
Single and Multichannel Activity

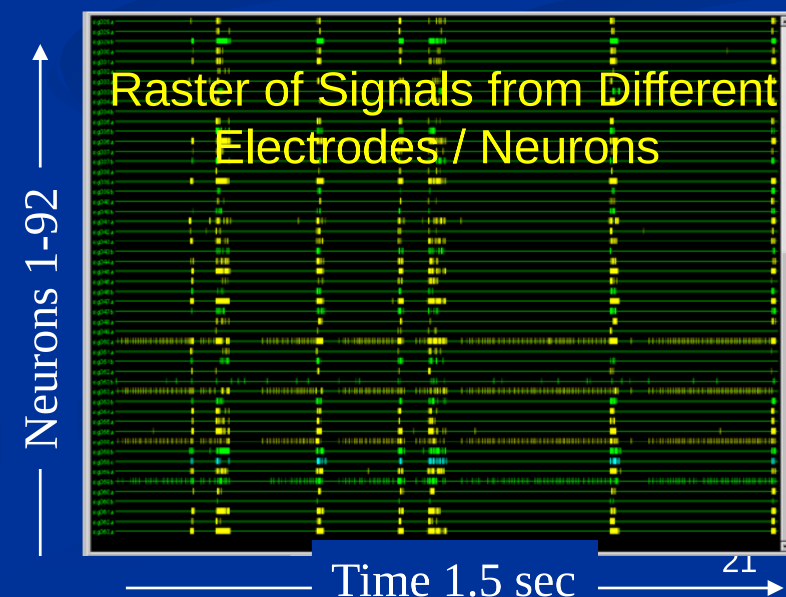




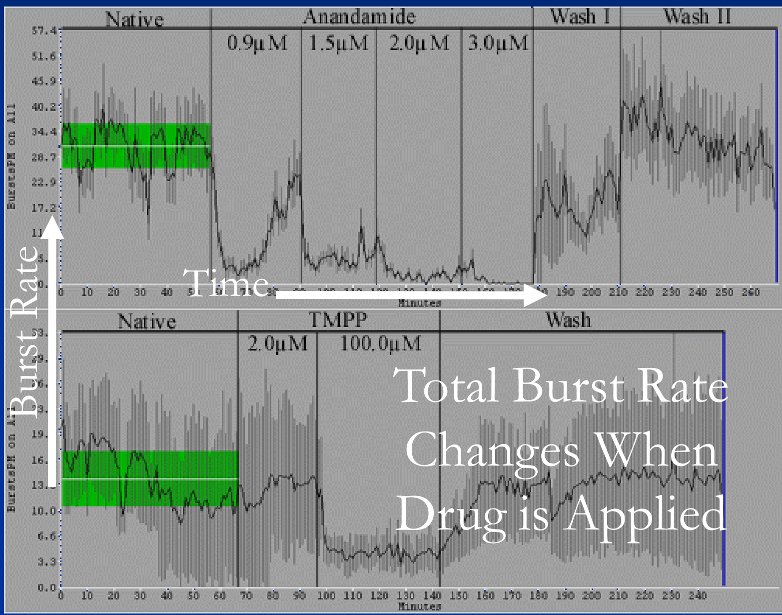


Spike Sorting System
Plexon,Inc

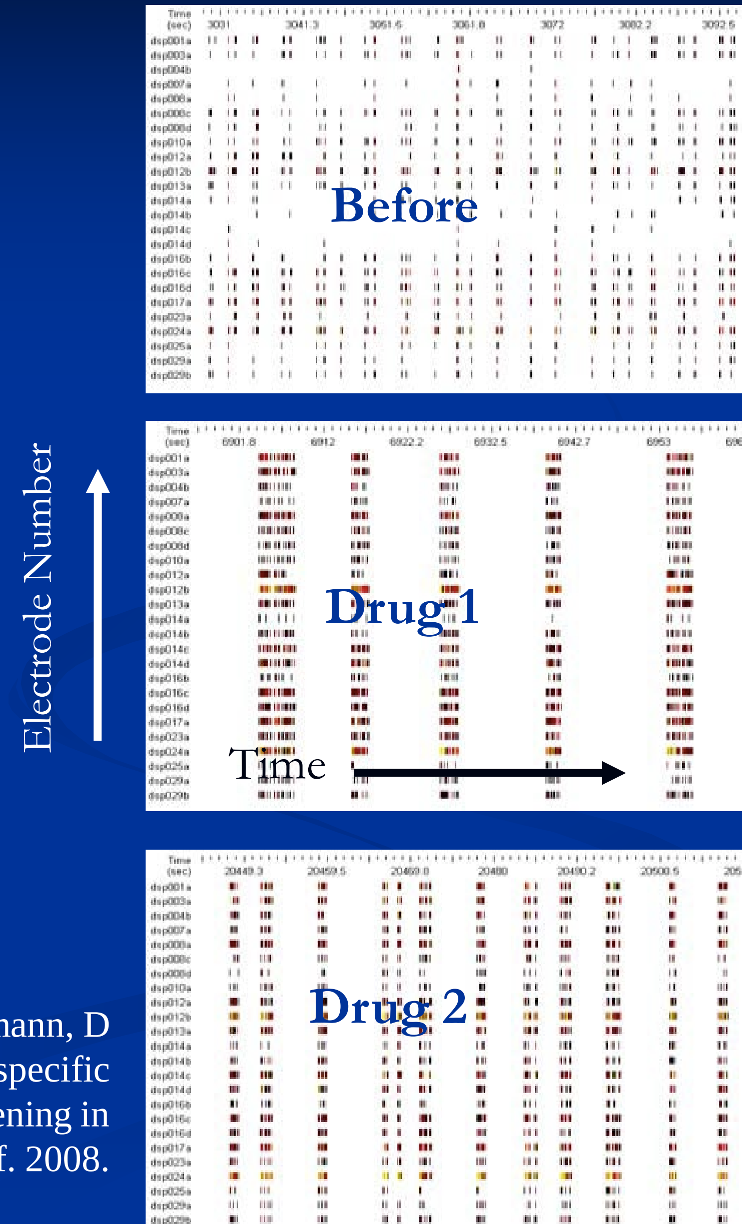




Brains on Chips Can Report Drug Exposure and Dose



Patterns Change with Drug



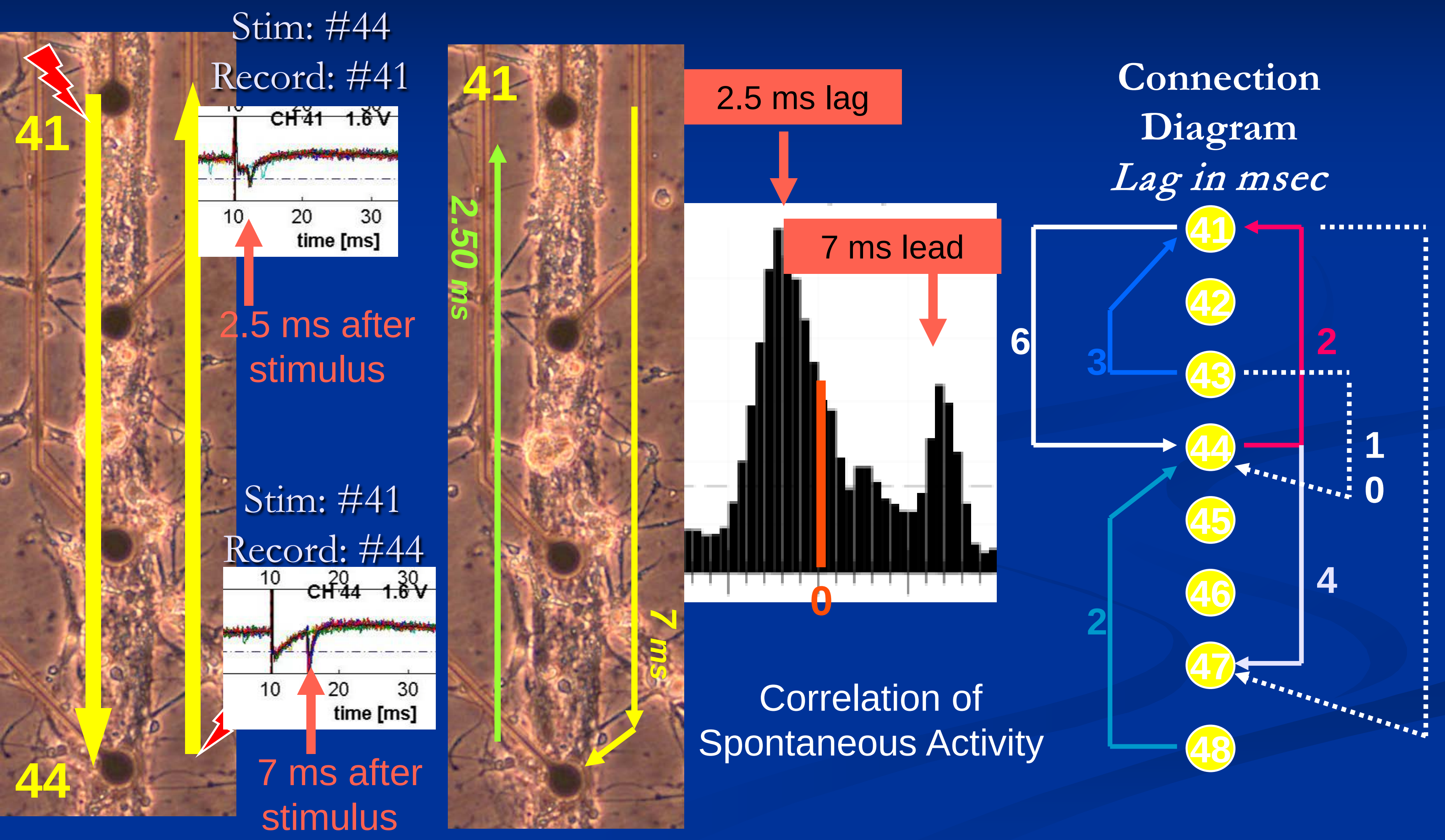
Guenter Gross

U. North Texas

http://www.cnns.org/

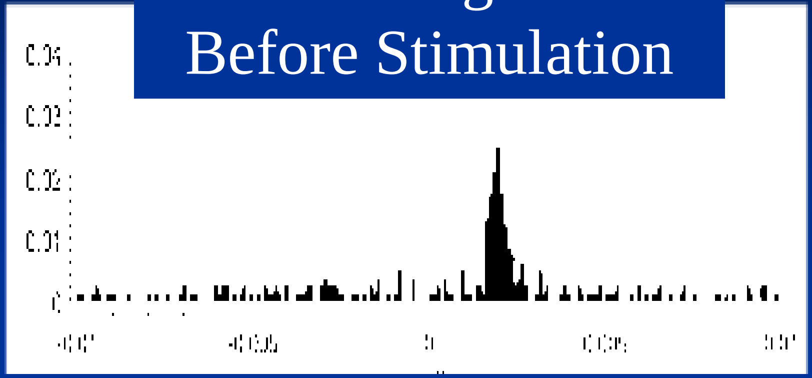
O. Schroeder, A. Gramowski, K Juegelt, C Teichmann, D Weiss, Spike train data analysis of substance-specific network activity: Application to functional screening in preclinical drug development, MEA Conf. 2008.

Stimulation / Correlation Reveals Connections

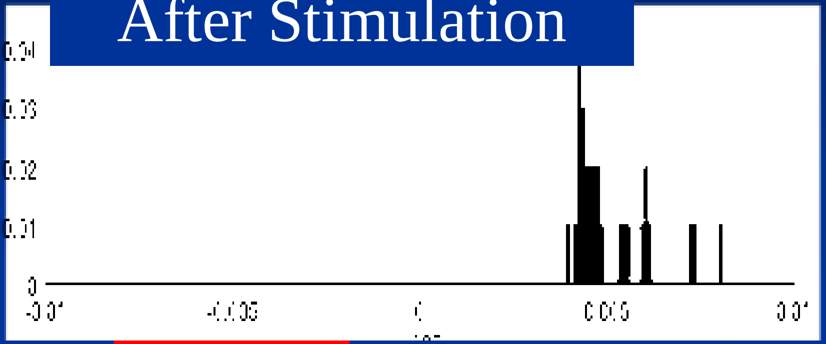


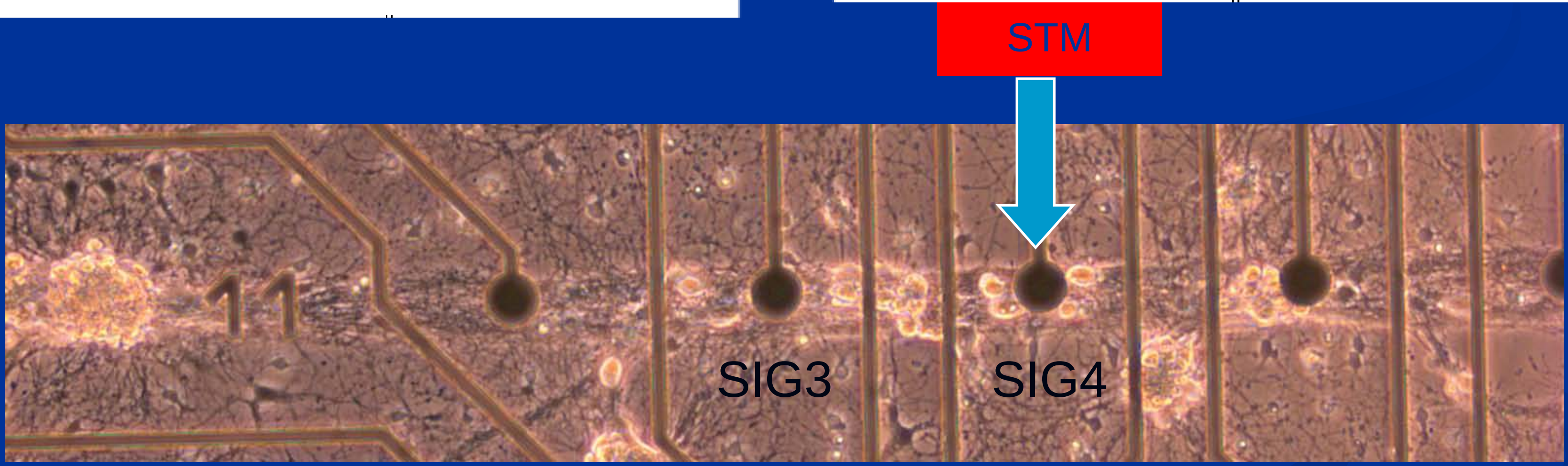
Cells Can Learn

Correlogram Before Stimulation

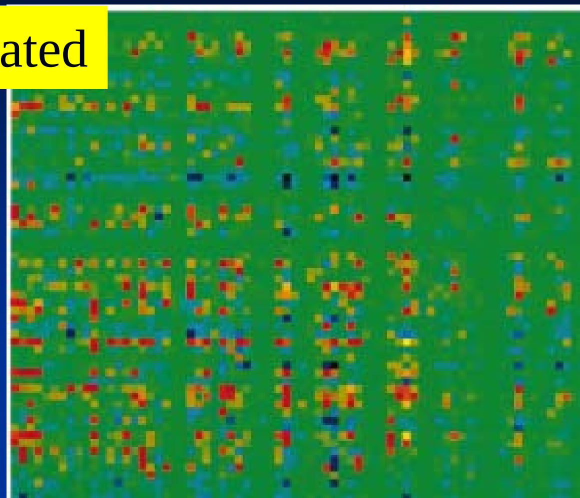


Correlogram After Stimulation

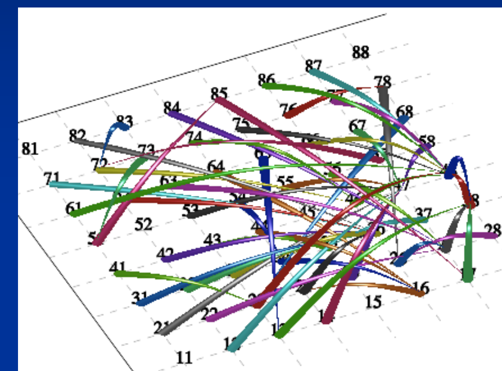




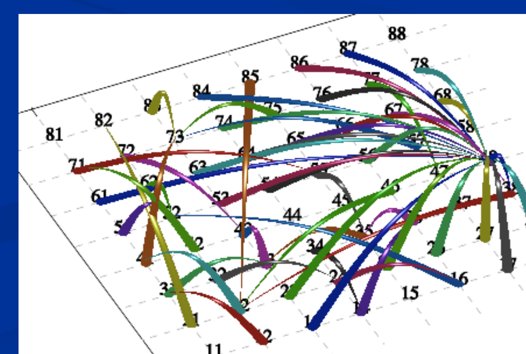
Relationships are Complex



Matrix of Changed Stimulus to Recording Functions

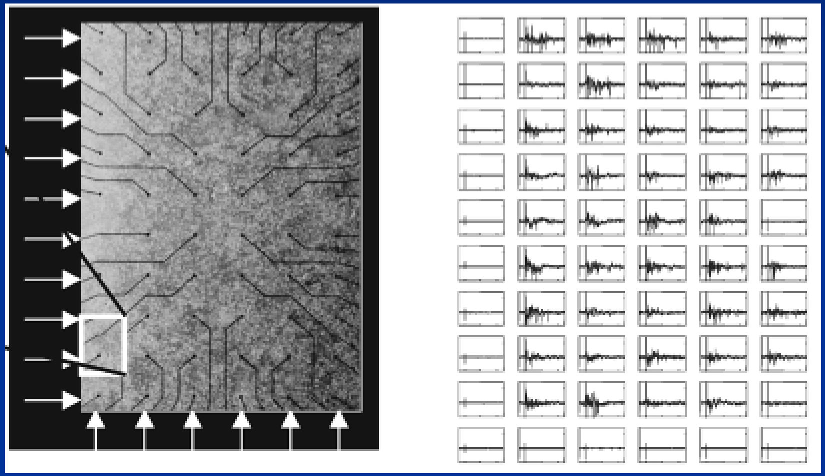


Change in Connectivity Pre to Post Learning Stimulus



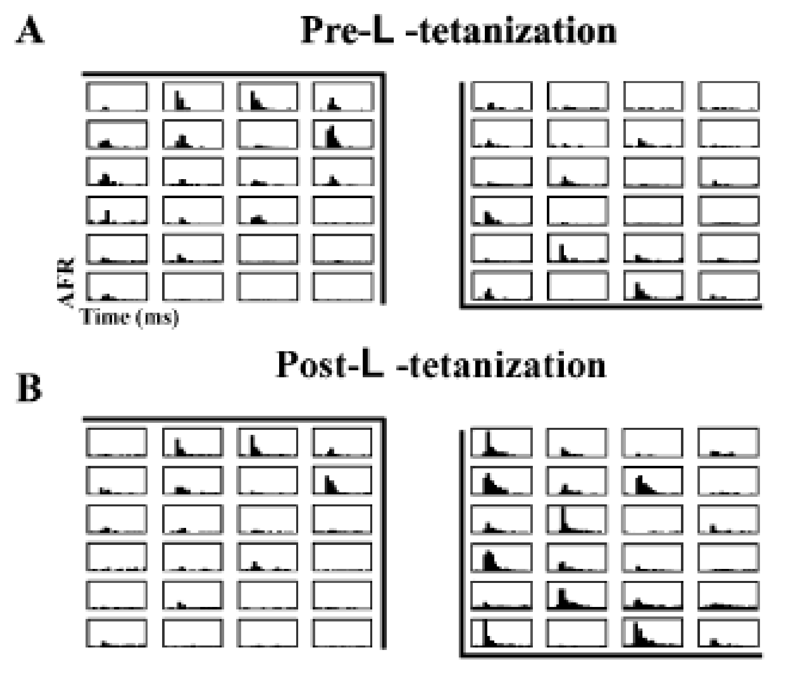
Jimbo, Tateno, Robinson,
Simultaneous induction of
pathway-specific potentiation …,
Biophys J. 76, 670, 1999.

Some Patterns Are Simpler



Dense Neuronal Culture on Electrode Array.
Arrows: Stimulated electrodes.

Right: electrical (action potential) responses



Neurons "Learn" to distinguish L from Ⅎ

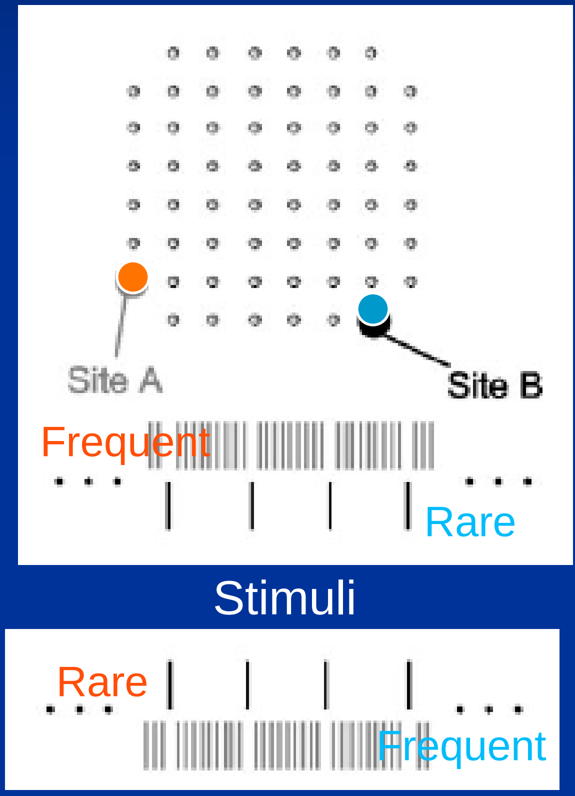
Ruaro, Bonifazi, Torre,
Toward the Neurocomputer ...
TBME, 52, 3, 371, Mar 2005

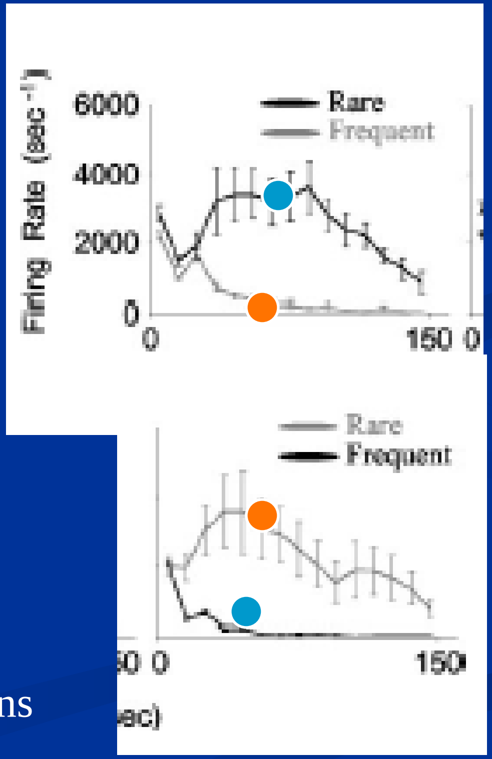
One Memory Paradigm

Remembers recent history

- if rare -- large output
- if frequent -- small output

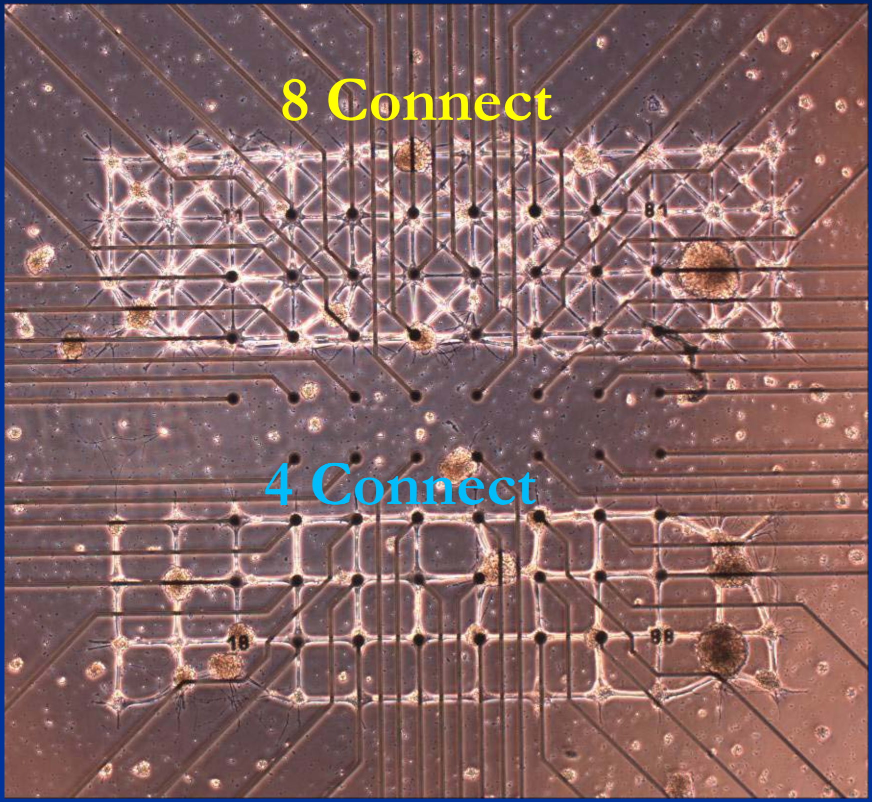
Switches state if frequency changes

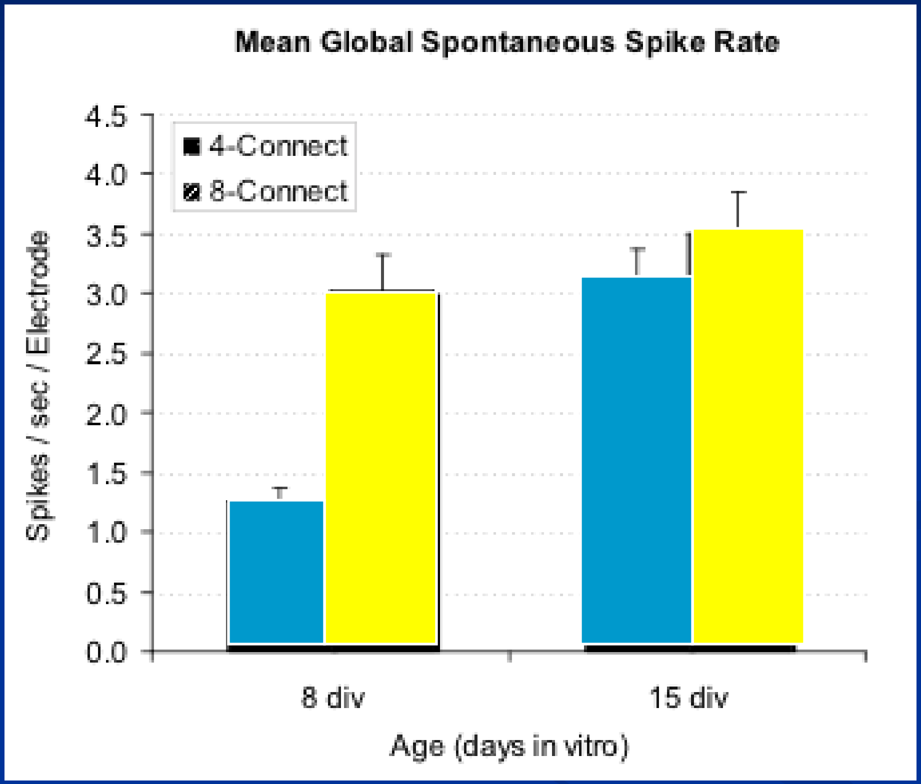




Eytan, Brenner, Marom, Selective daptation in networks of cortical neurons J. Nsci. 23(28) 9349, 2003.

Activity Can be Influenced by Connectivity



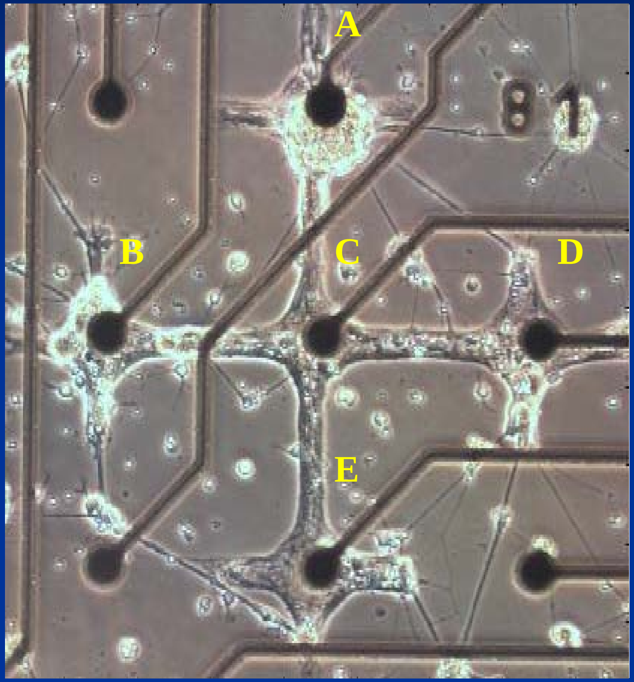


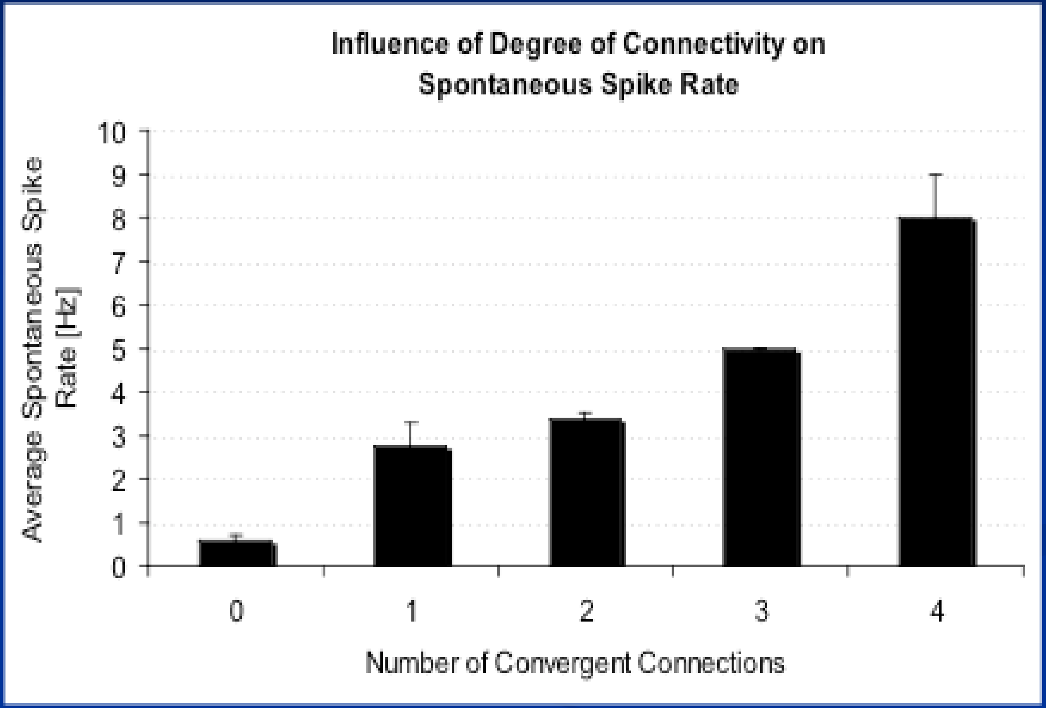
Spontaneous Activity was recorded and analyzed on a weekly basis

Earlier Development of activity on more connected network

Khatami, Thesis, Illinois

Even in Simple Patterns, Connectivity Determines Activity



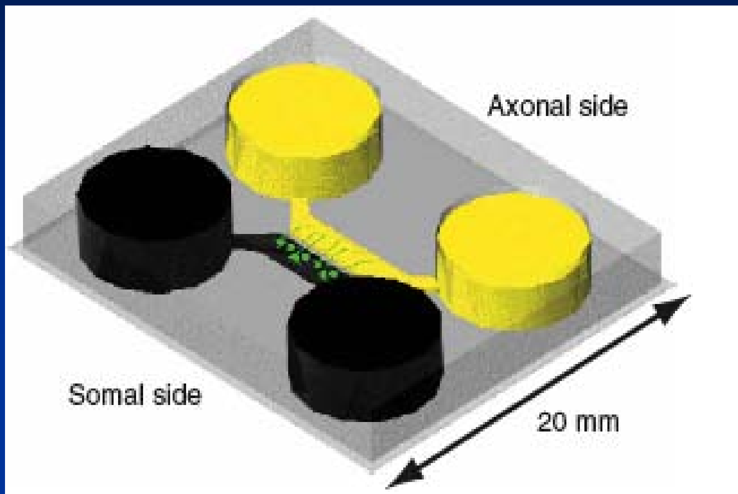


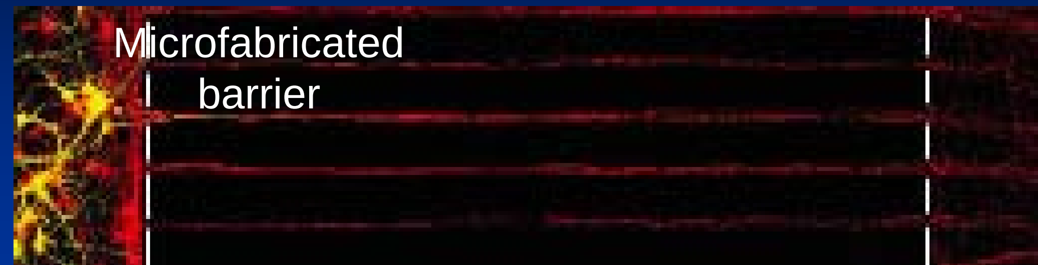
The more inputs, the more activity

Khatami, Thesis, Illinois

MicroTunnels Offer Unique Opportunities

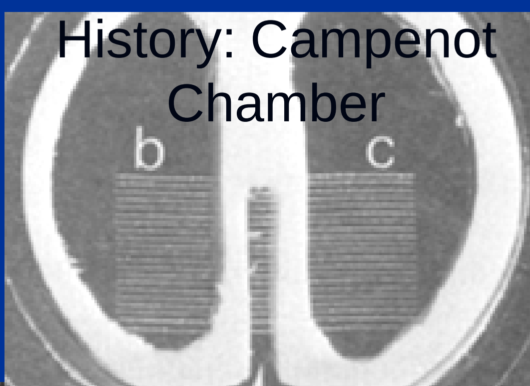
MicroTunnel for Axonal Separation



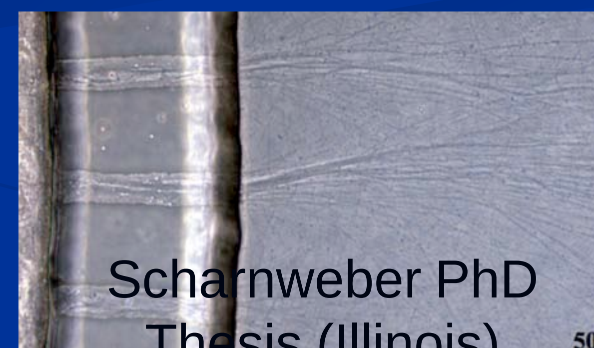


Jeon Lab (UC Irvine – now SNU)

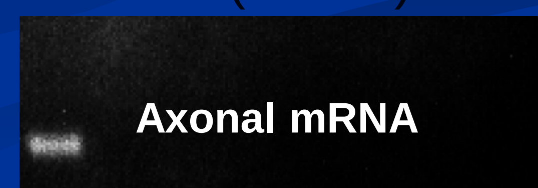
Taylor, AM et al. *A microfluidic culture platform for CNS axonal injury, regeneration and transport*. Nature Methods, 2005. **2**(8): p. 599-605.



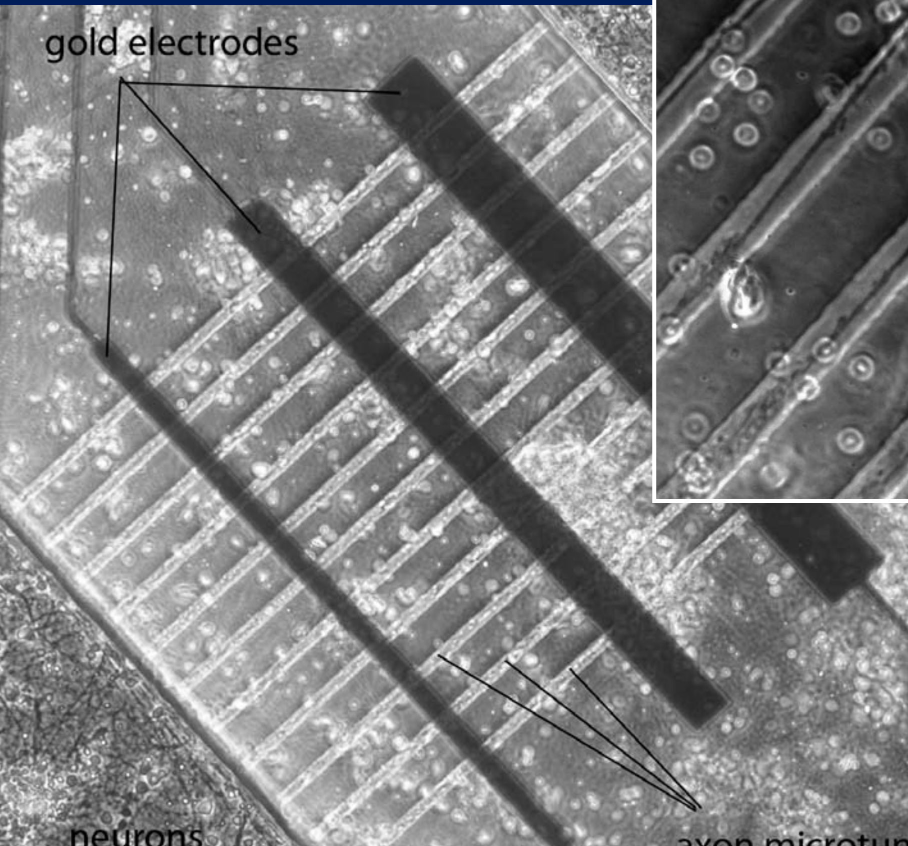
Campenot, PNAS 74, 4516-4519, 1977

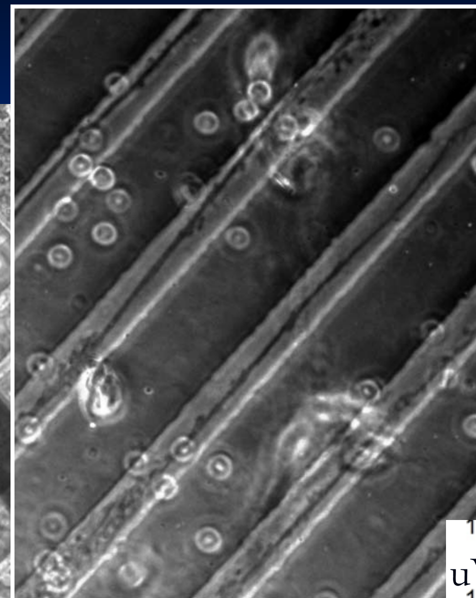


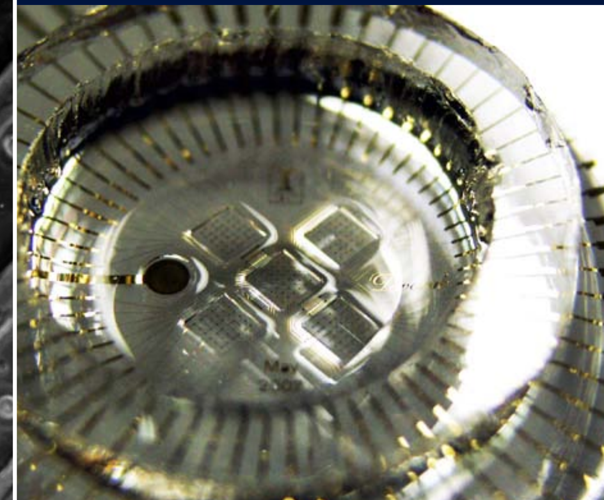
Scharnweber PhD Thesis (Illinois)

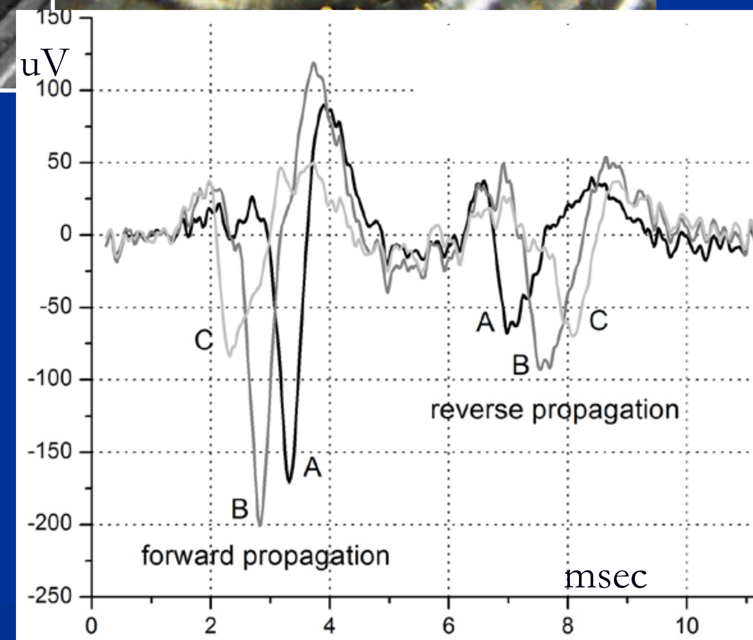


Electrodes inside Tunnels







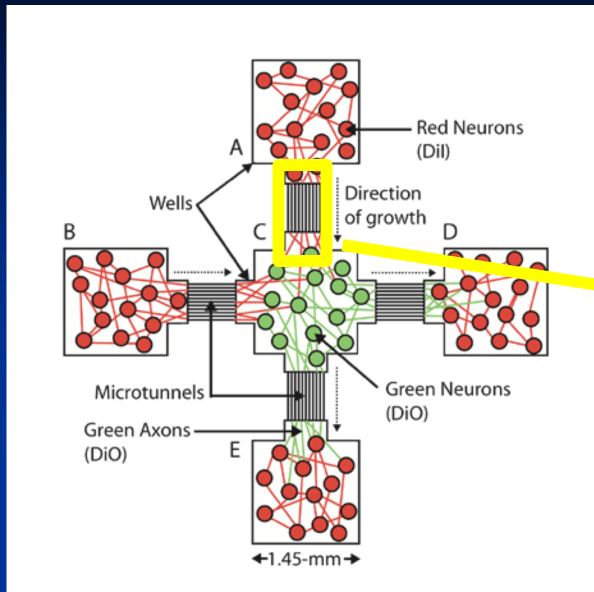


Tunnel Resistance: 16 Mohm

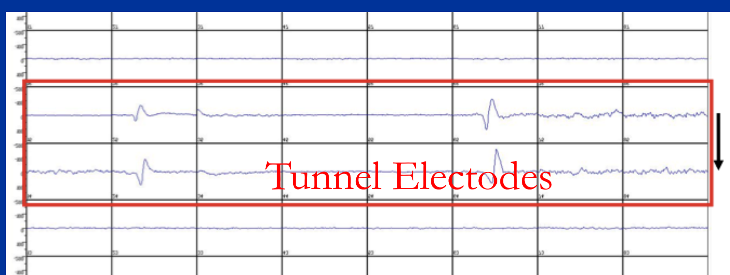
Large Amplitude Signals

Dworak, B. and Wheeler, B., *Lab on a Chip*, 2009

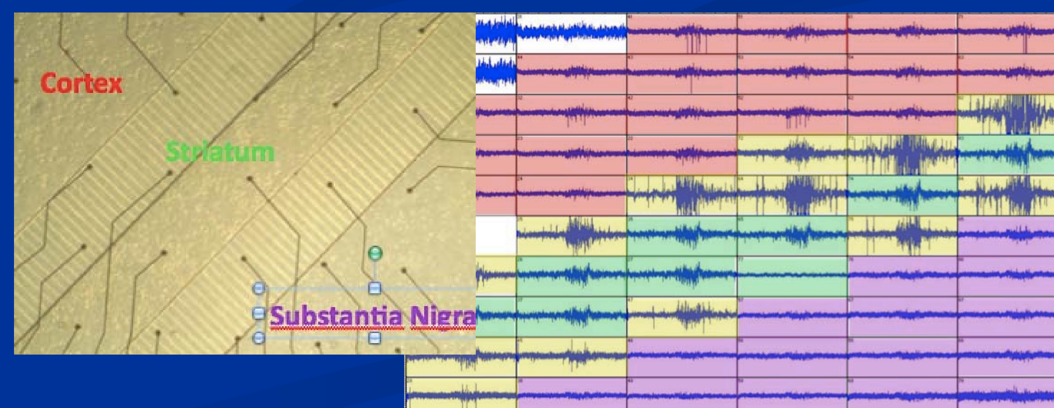
Unidirectional Connections by Sequential Plating



Unidirectional Growth



Unidirectional AP Propagation



MultiCompartment Bursting

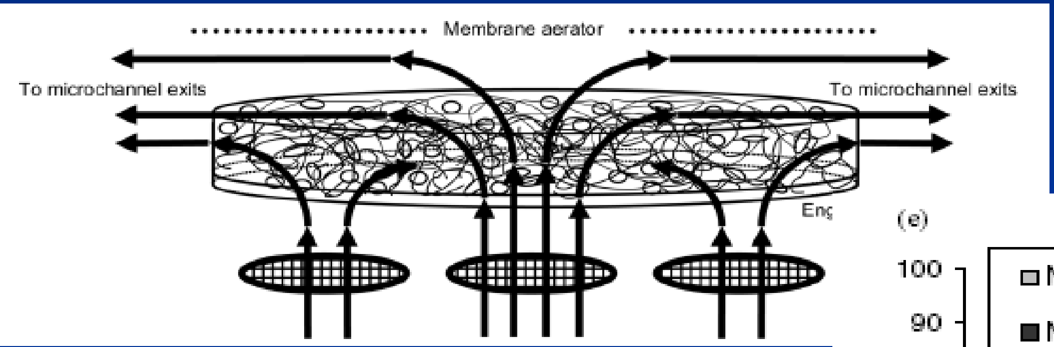
3-D Neural Networks in Culture

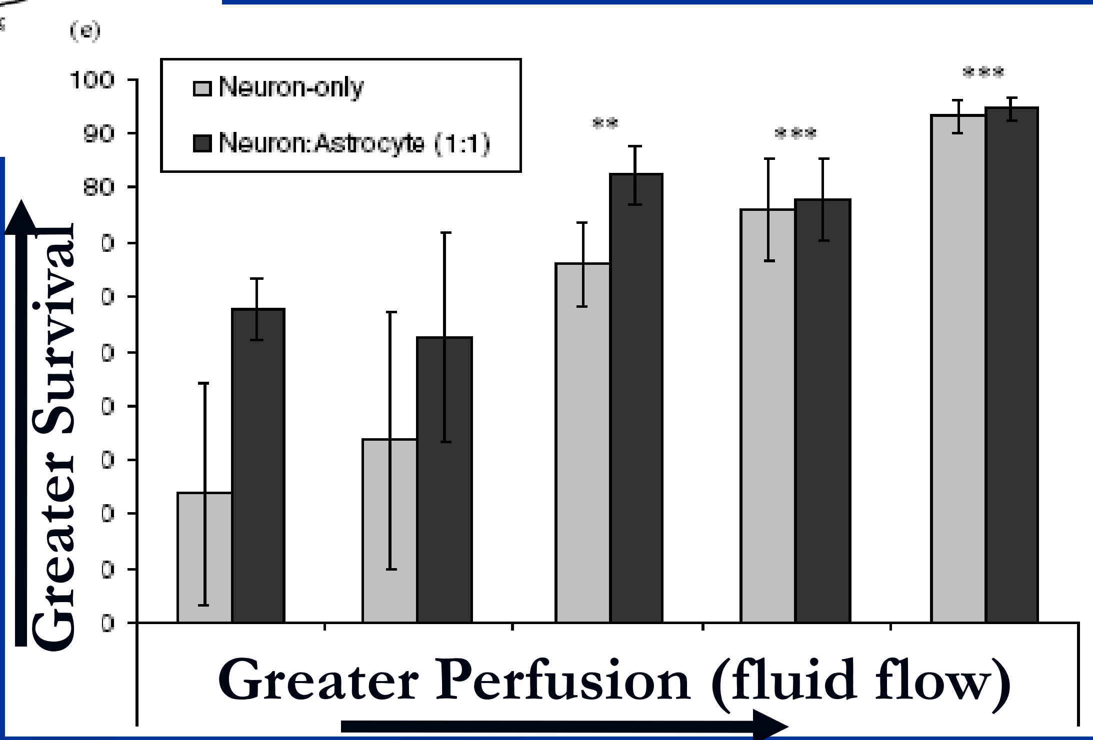
- More natural -- More surfaces for cells
- Model
  - Head injury – mechanical
  - Inserting electrodes
  - Spinal Cord Regrowth
- Very Difficult to:
  - Keep cells alive
  - Image the cells
  - Record and Stimulate
- Needs Vasculature
  - No cell in brain is more than 100 um from capillary

Design: Bio-Fluidics to Help Maintain 3-D Neural Cultures

Laplaca (GaTech) Perfusion of 3D Neural Cultures Enhances Survival

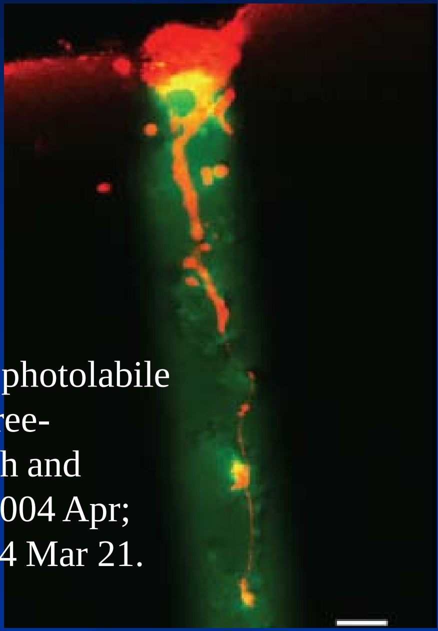
Cullen DK, Vukasinovic J, Glezer A, Laplaca MC Microfluidic engineered high cell density three-dimensional neural cultures J Neural Eng. 2007 Jun;4(2):159-72. Epub 2007 Apr 4.



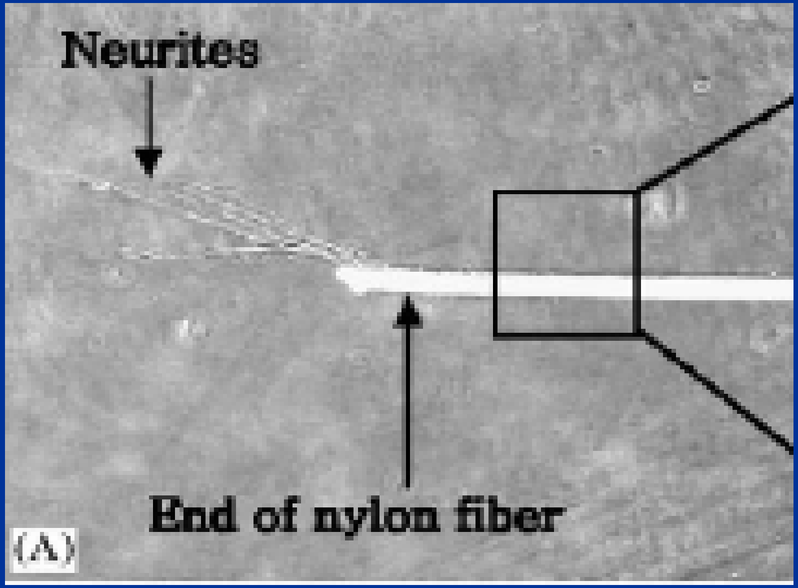


Creating Structure in 3D

Laser Modification for Neural Tracks within Gels



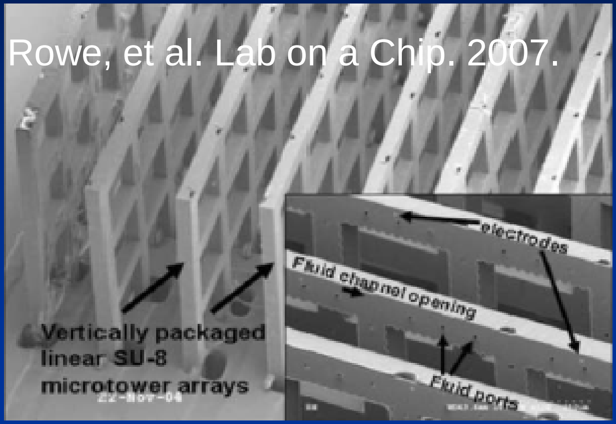
Luo Y, Shoichet MS A photolabile hydrogel for guided three-dimensional cell growth and migration Nat Mater. 2004 Apr; 3(4):249-53. Epub 2004 Mar 21.



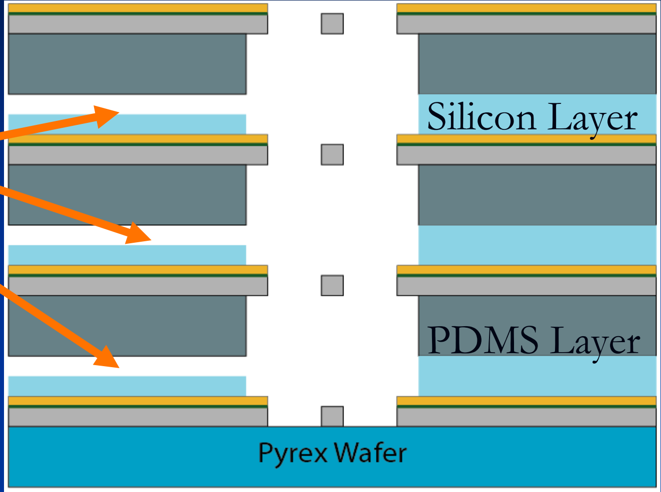
Neurons follow Fiber

Ravi V. Bellamkonda, Peripheral nerve regeneration: An opinion on channels. scaffolds and anisotropy Biomaterials 27 (2006) 3515–3518

3D Fluidics/Electrodes for 3D Culture

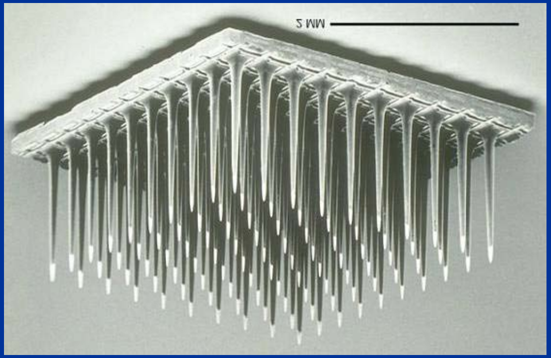


Fluid
Ports

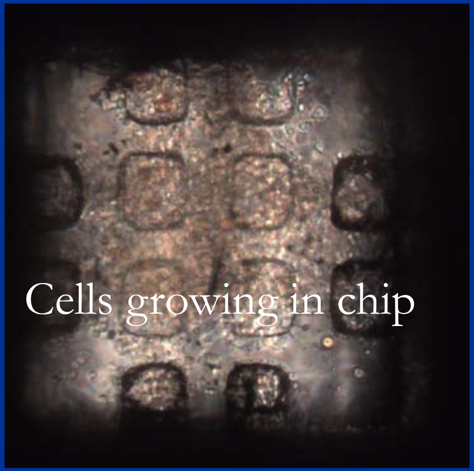


Electrode
Leads

Musick, et al. Lab on a Chip. 2009.



Could use – Utah or Michigan probes or microwires; drug delivery puffer would be nice



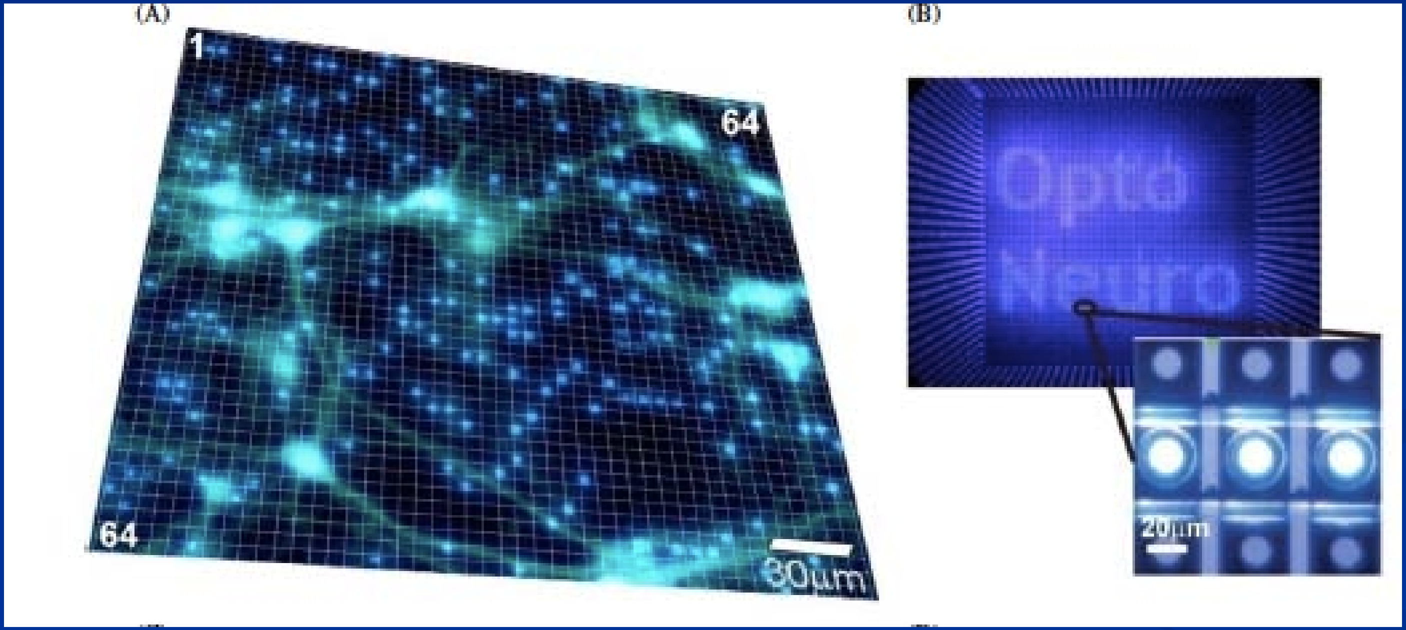
Signals from Electrodes on Top Layer

Middle
Layer

Bottom
Layer

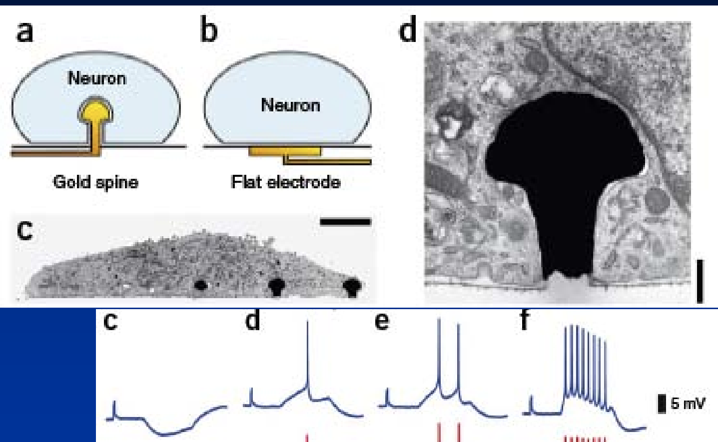
New Recording and Stimulating Technology

Optogenetics and Optical Stimulation



Multi-site optical excitation using ChR2 and micro-LED array, Nir Grossman, Vincent Poher, Matthew S Grubb, Gordon T Kennedy, Konstantin Nikolic, Brian McGovern, Rolando Berlinguer Palmini, Zheng Gong, Emmanuel M Drakakis, Mark A A Neil, Martin D Dawson, Juan Burrone and Patrick Degenaar. J. Neural Eng. 7 (2010) 016004, doi:10.1088/1741-2560/7/1/016004

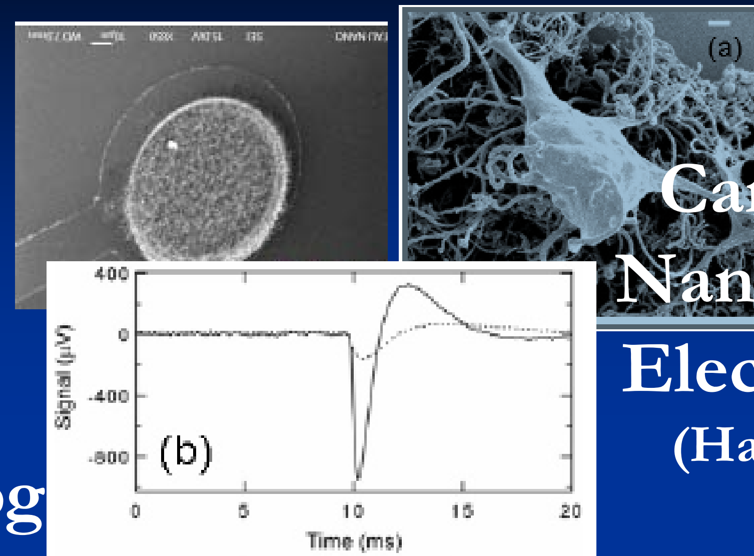
Improving Electrode Neuron Coupling



In Cell Recording Technology

(Spira Group)

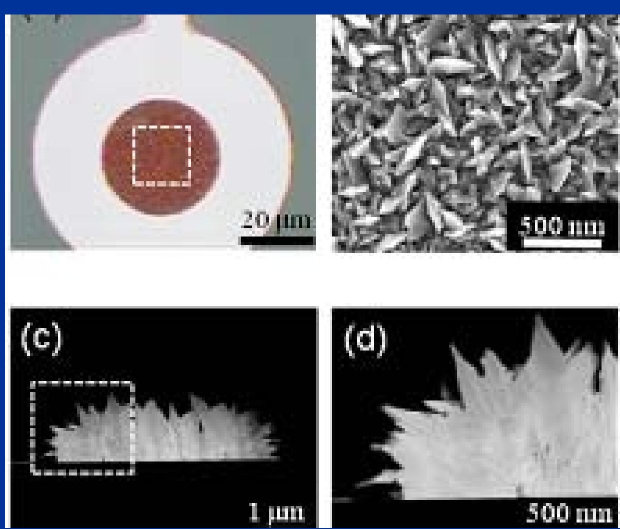
In-cell recordings by extracellular microelectrodes, AviadHai, Joseph Shappir&Micha E Spira, Nature Methods, 7/3, 2010, 200-203, doi:10.1038/nmeth.1420



Carbon Nanotube Electrode

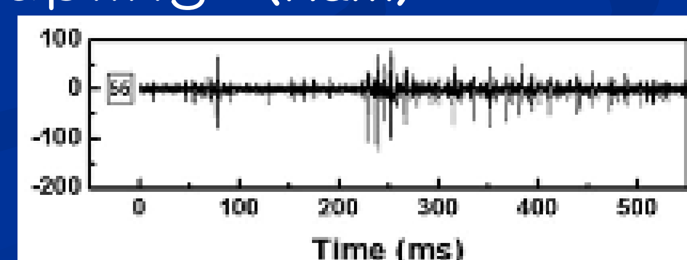
(Hanein)

Electro-chemical and biological properties of carbon nanotube based multi-electrode arrays TamirGabay, Moti Ben-David, ItshakKalifa, Raya Sorkin, Ze'ev R Abrams, Eshel Ben-Jacob and Yael Hanein Nanotechnology 18 (2007) 035201

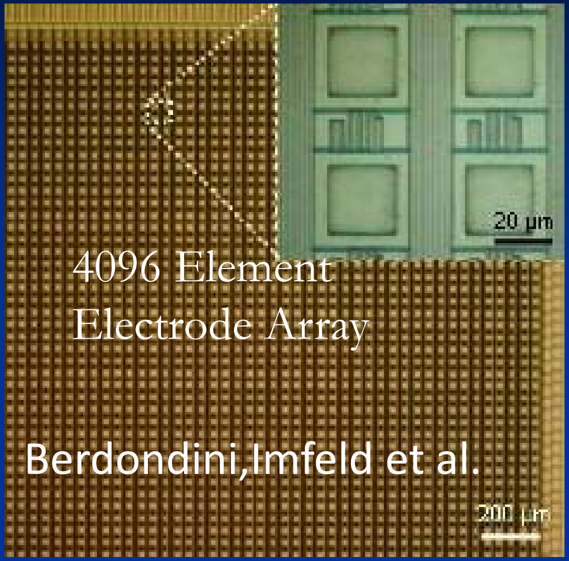


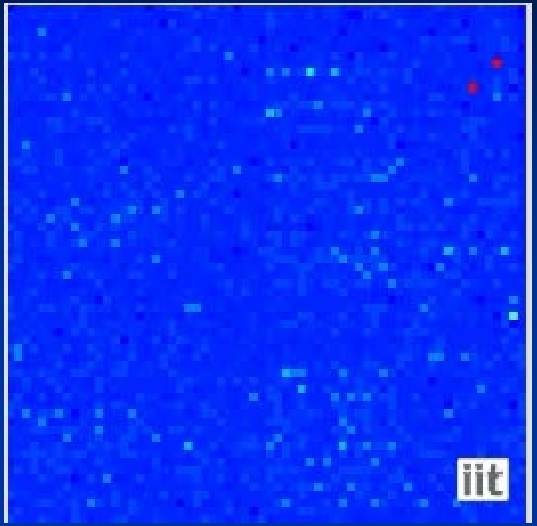
'Flake' nanostructure electrode for neuron coupling (Nam)

* J. Kim, G. Kang, Yoonkey. Nam, Yangkyu. Chio, Nanotechnology, 2010



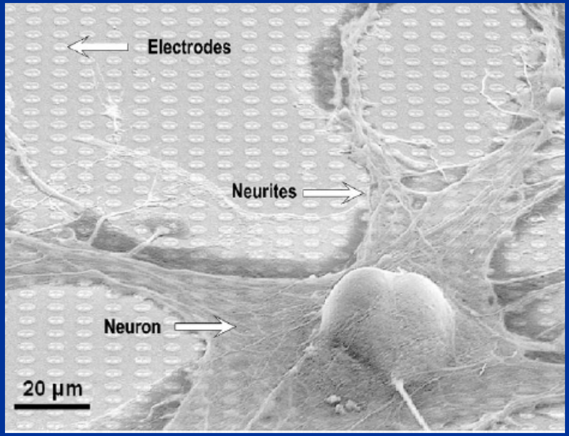
Massively Integrated FET Arrays

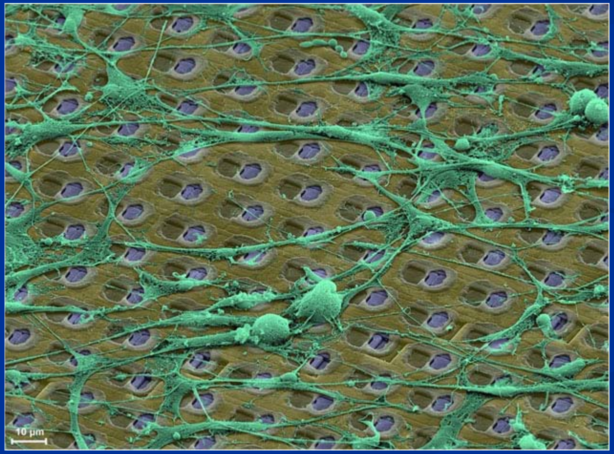




Remarkably Large
Amplitude Signals
Implies Excellent
Coupling to FETs

**More Movies than
Spike Trains**





A. Hierlemann, ETH
http://www.bsse.ethz.ch/bel/research/BioElectronics

11,016 electrodes
7 um diam, 18 um pitch
Stimulation & recording
Noise level 5 – 6 uVrms

Fromherz Group: A 128*128 CMOS Biosensor Array … IEEE J. Solid State Ckts. 38(12) 2003.

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SankarAlagapan, Eric Franca (grad students)
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