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Sandia National Laboratories

Engineered surfaces and microfluidic structures for controlling cell morphology, migration, and cell-cell interactions

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U.S. DEPARTMENT OF ENERGY | National Nuclear Security Administration | LDRD LABORATORY DIRECTED RESEARCH & DEVELOPMENT

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Outline

- **Biological computation**
- Neural engineering
  - Background
  - Neuron polarization
  - Microfluidic compartmentalization
  - Embedded microfluidic structures
  - Porous nanostructures
- Conclusions and Future Work

Biological neural networks have substantial computational capabilities

Cray XT5 Jaguar (ORNL), hex-core

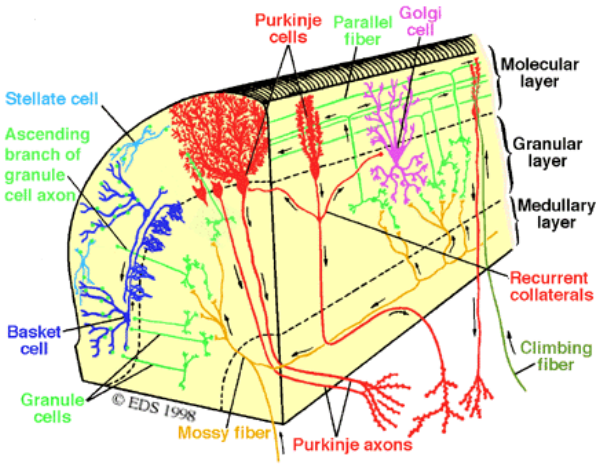
~2x10^{15} flops/s, **~10 MW, room-sized**

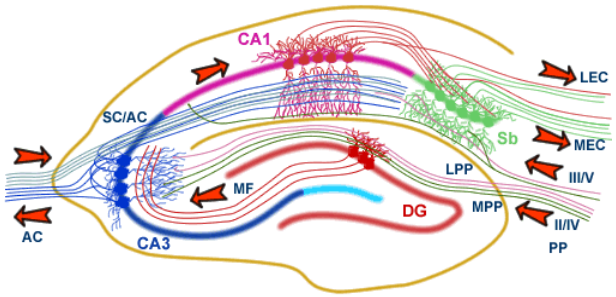
~100 Gbits/in^2

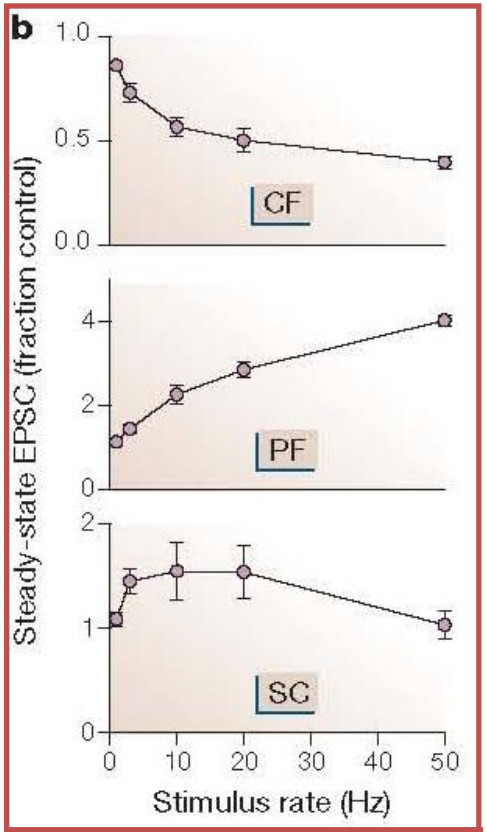
access-limited (processor vs. memory)

- Brain, 10^{11} neurons, 10^{15} glial cells
- >10^{16} flops/s, **20W, 1200 cm^3**
- **~large memory storage capacity**
- **fast memory access**

Influence of network architecture and stimulus frequency on network function

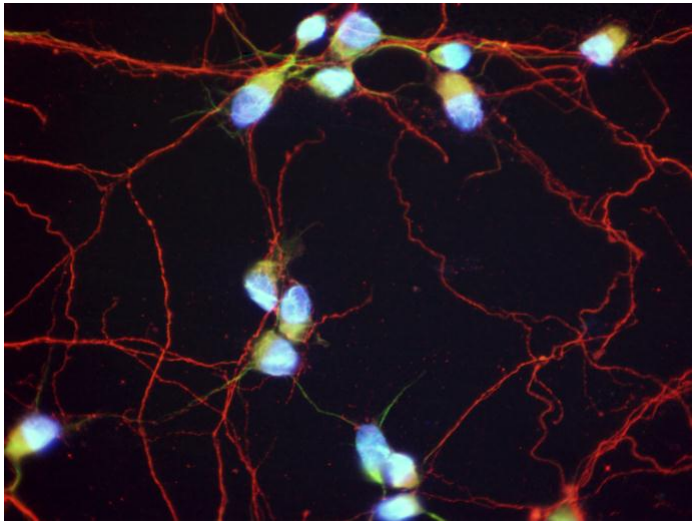




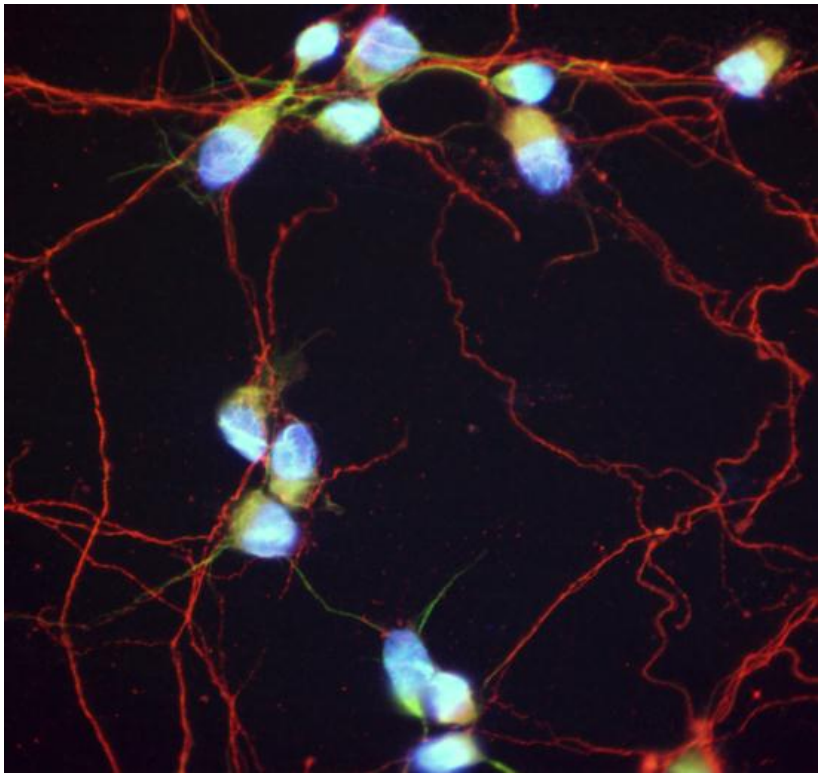


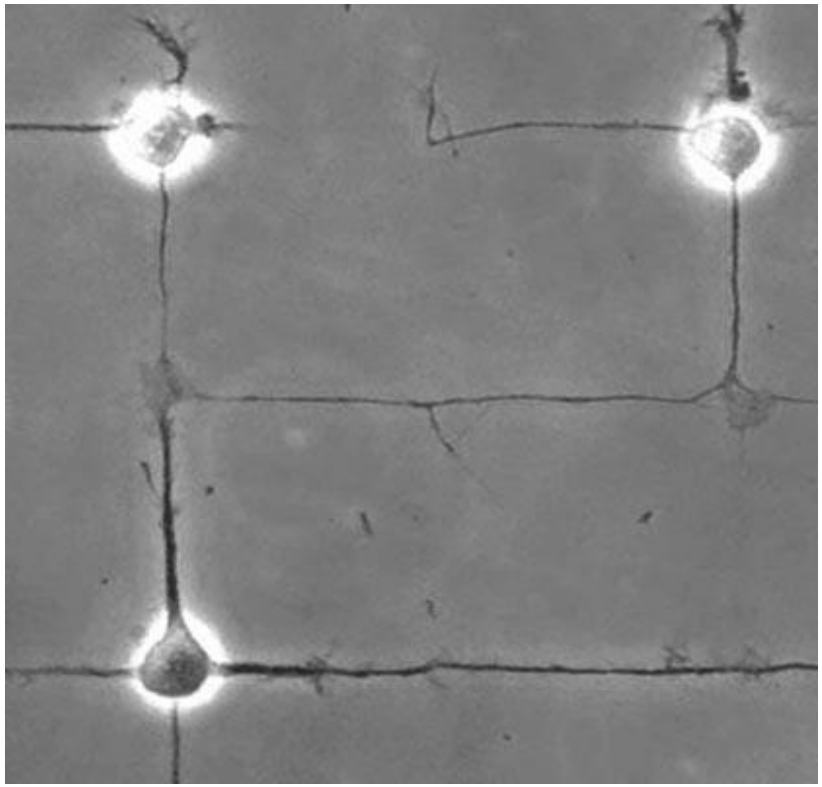
Abbot, Regehr 2004

Re-engineer dissociated neurons into networks to examine the effect of architecture on function



Utilize microfabrication techniques to engineer living neural networks





position cells, control polarization, and direct synaptic connections

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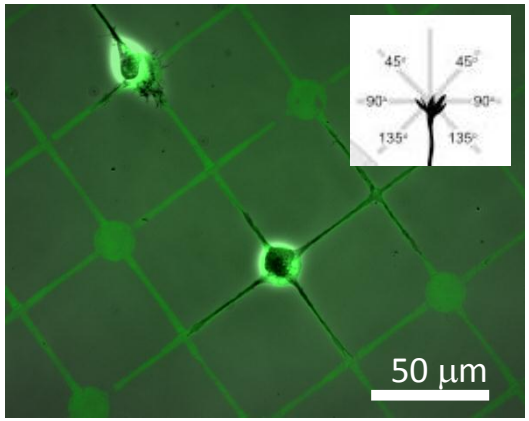
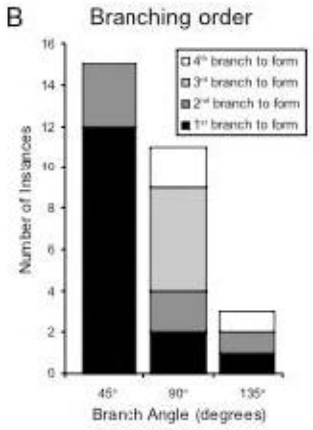
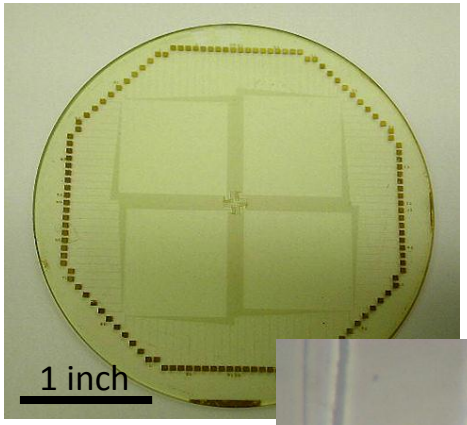
- Conclusions and Future Work

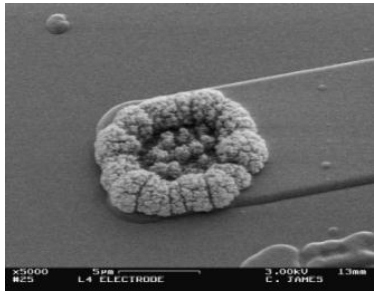
Engineered neural networks using microfabrication techniques

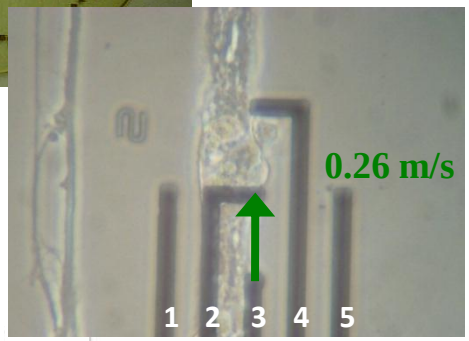


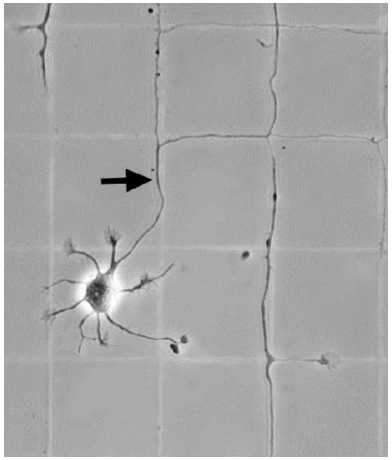


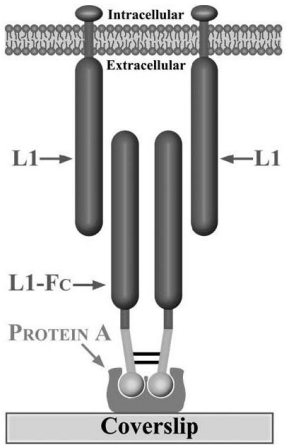
Withers et al., J Neurobio 2006, 66, 1183



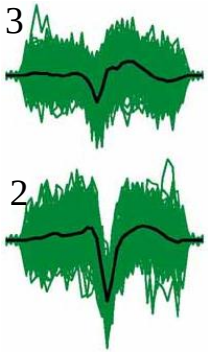


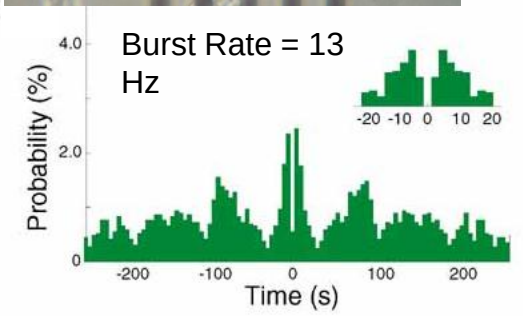






Oliva et al., Neurochem Res 2003, 28, 1639



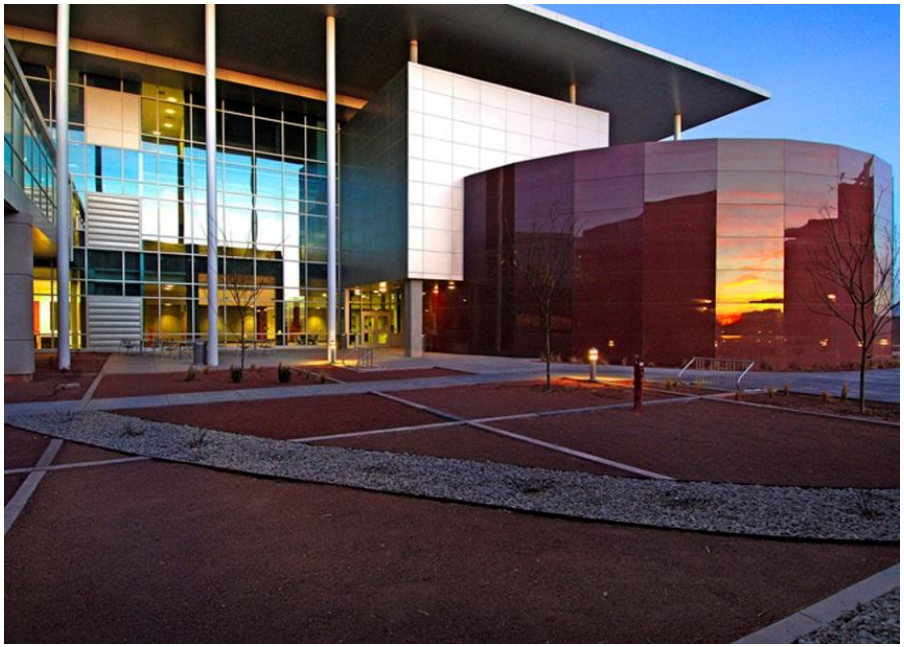


James et al., IEEE TBME 2000/2004, 47/51, 17/1640

Microsystems and Engineering Science Applications (MESA)

Highly collaborative research

- microsystems engineering (1700), biological science (8600), nanomaterials synthesis (1800), cognitive science (1400)



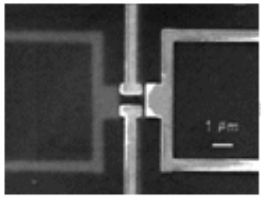
As an accredited Department of Defense Trusted Design Center, Sandia provides Trusted ASIC Design Services for both radiation-hardened and non-radiation-hardened foundries (350nm, 180nm, 130nm, 90nm), including in-house, IBM, National Semiconductor and other Trusted Foundries.

Center for Integrated Nanotechnologies (CINT)

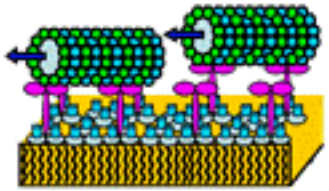
US Department of Energy Office of Science User Facility and Nanoscale Science Research Center

- Sandia National Laboratories (96K ft^2)
- Los Alamos National Laboratories (36K ft^2)

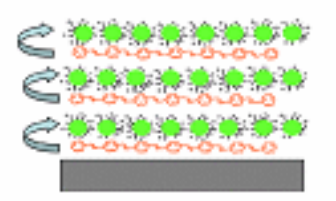
Nanoscale Electronics and Mechanics



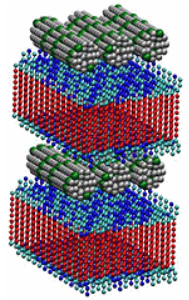
Soft, Biological and Composite Nanomaterials



Nanophotonics and Optical Nanomaterials



Theory and Simulation of Nanoscale Phenomena



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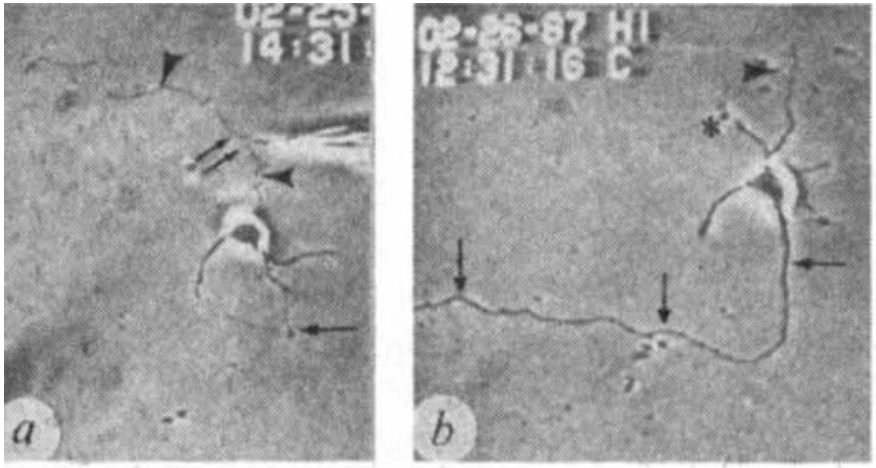
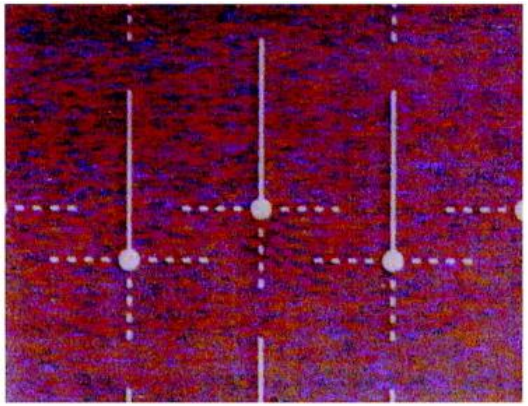
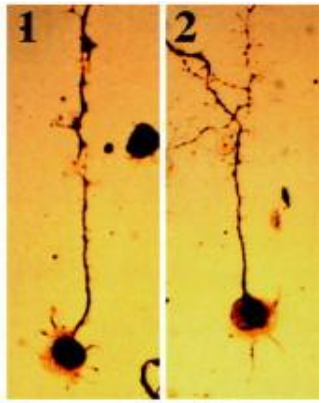
Control of neuron polarity using substrate-bound chemical cues

• Culture dissociated primary rodent neurons on artificial substrates

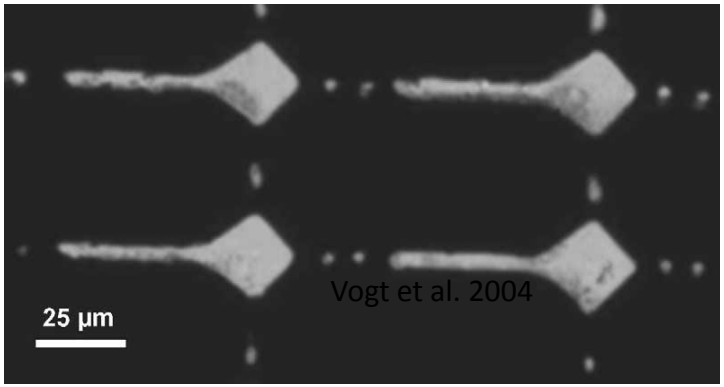


Dotti and Banker (1987) - the first neurite to grow the longest develops into an axon





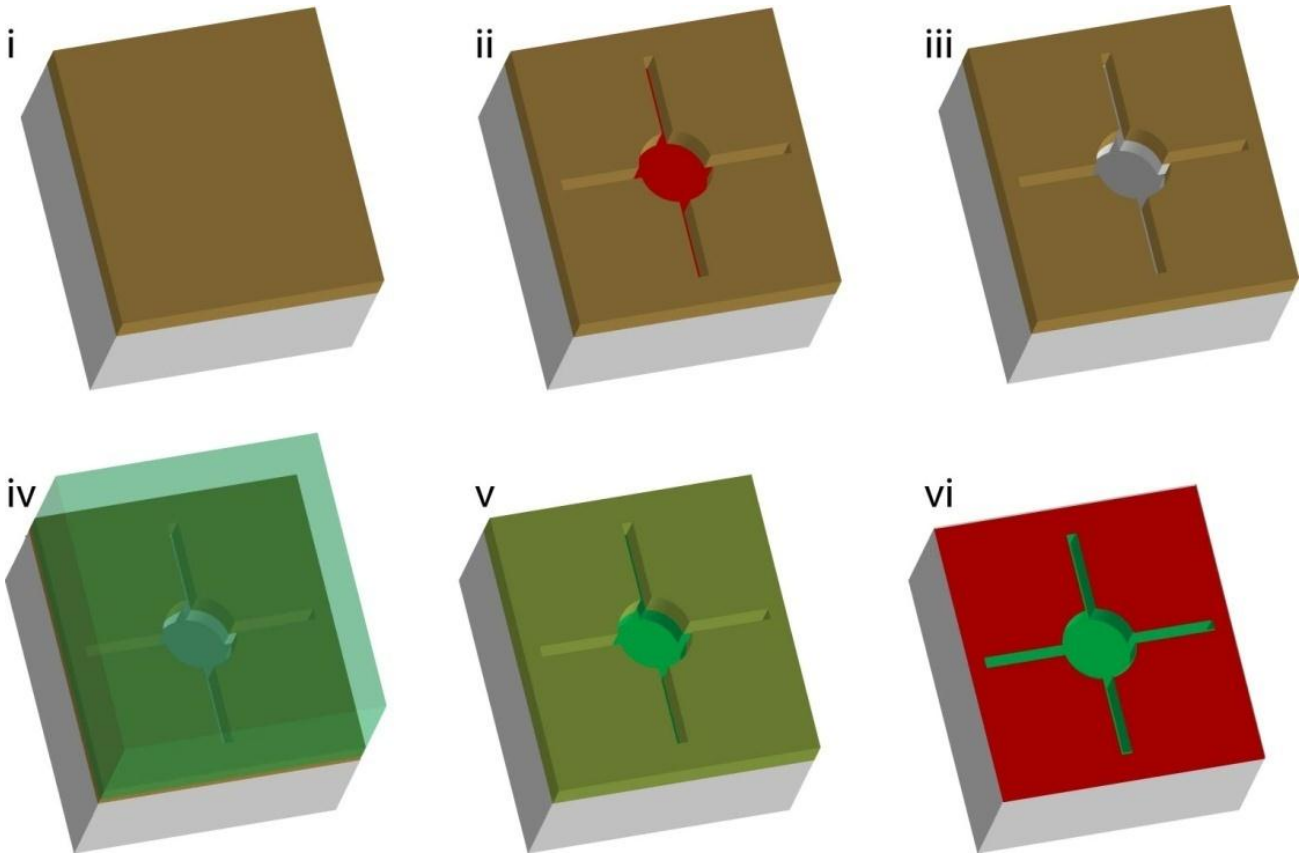
Stenger et al., (1998) – continuous and interrupted chemical cues



Vogt et al., (2004) – continuous and interrupted chemical cues; electrophysiology

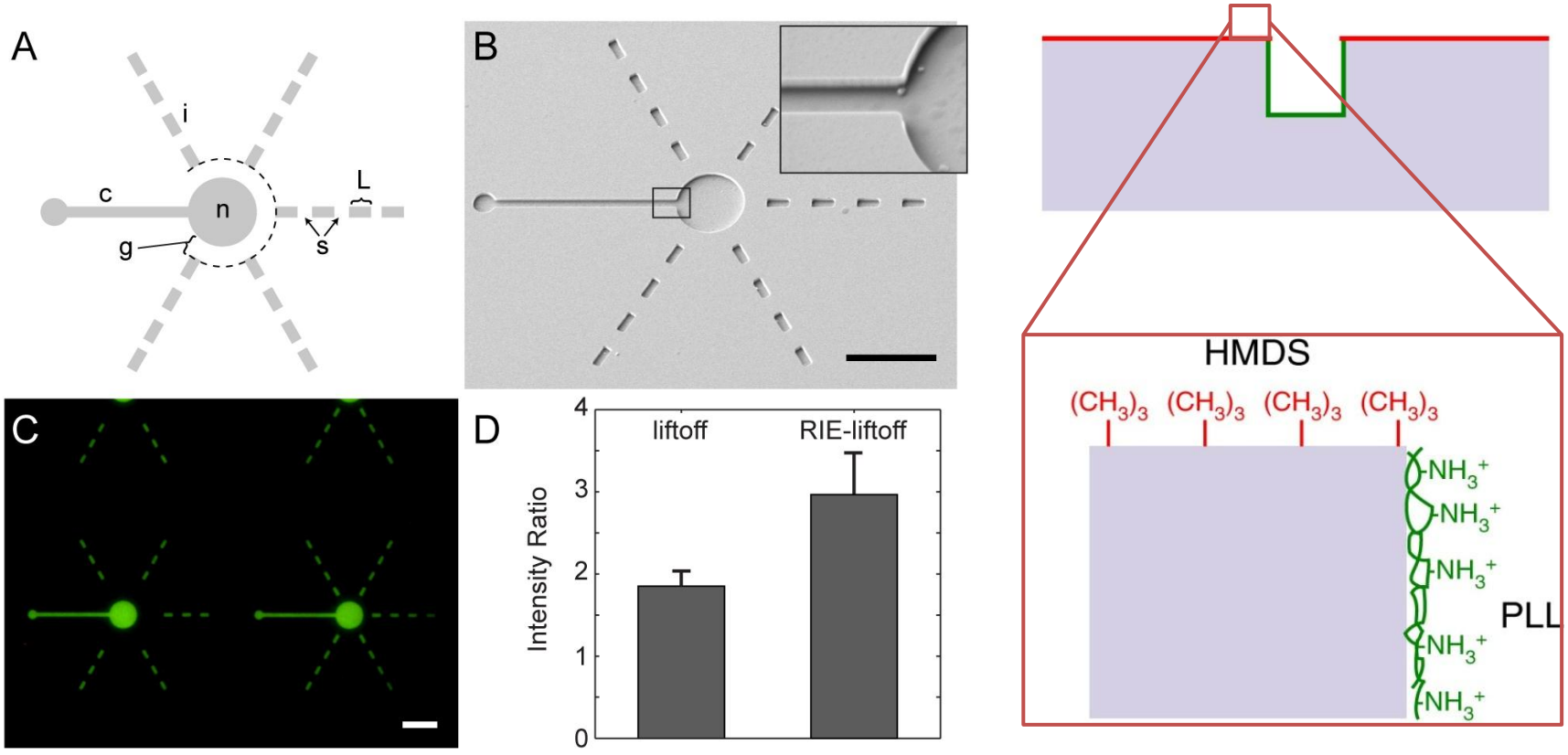
Topographical & chemical cues for network engineering

- Photolithographic techniques to create bi-functional surfaces



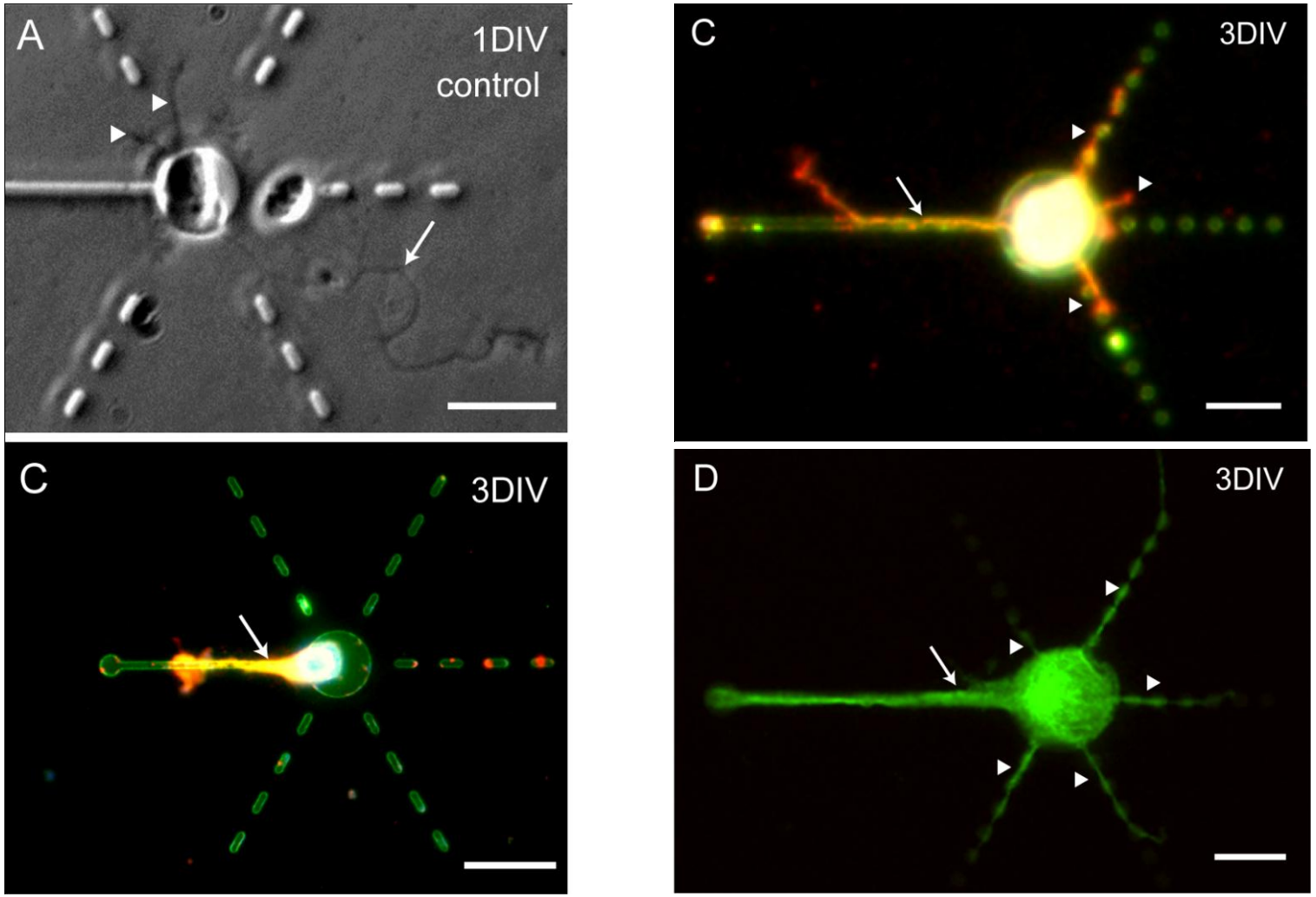
Bi-functional surfaces for promoting cell adherence and repulsion

• Individual neuron guidance cues consisting of adhesive (PLL) and repellent molecules (HMDS) embedded into a glass substrate



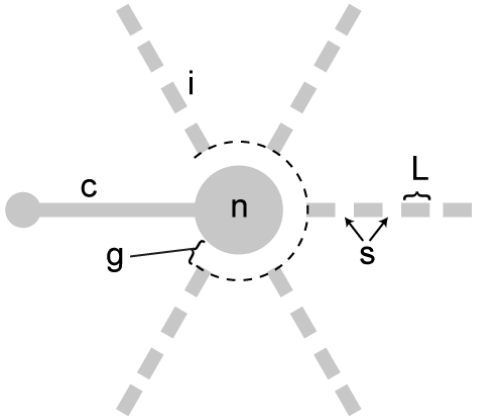
C.D. James et al., Biomaterials 2011, 32, 8860

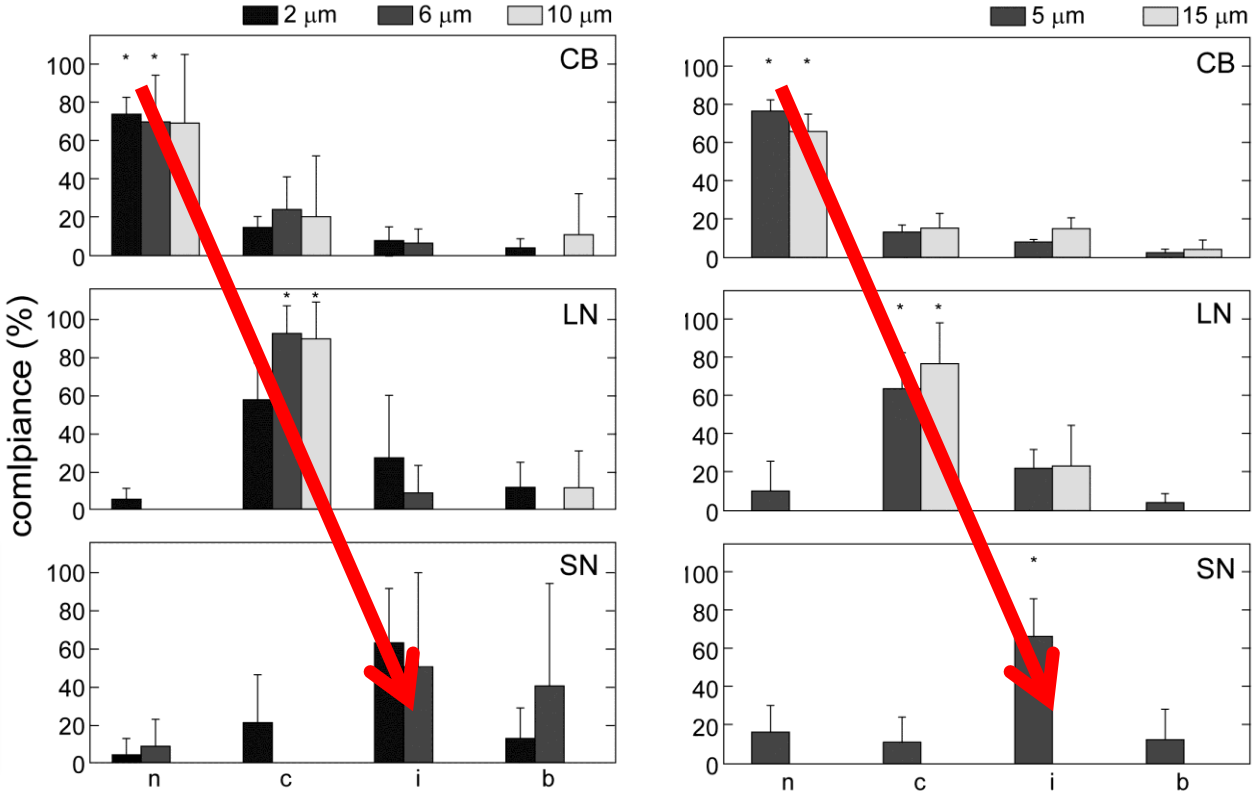
Guidance cue geometry & neuron polarization



Specific guidance of cell attachment and neurite outgrowth

Cell bodies attach to nodes (n), long neurites extend along continuous lines (c), short neurites extend along interrupted lines (i)



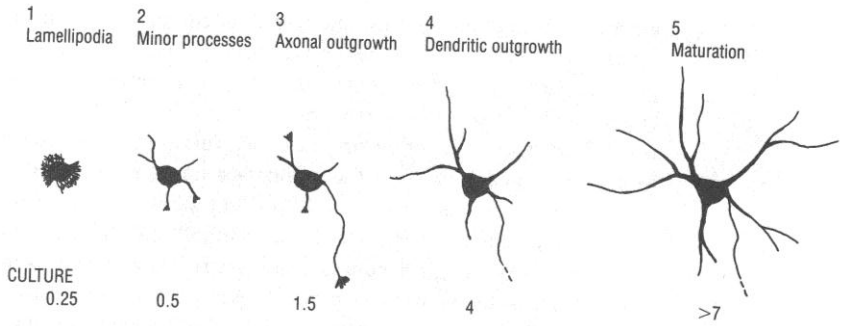


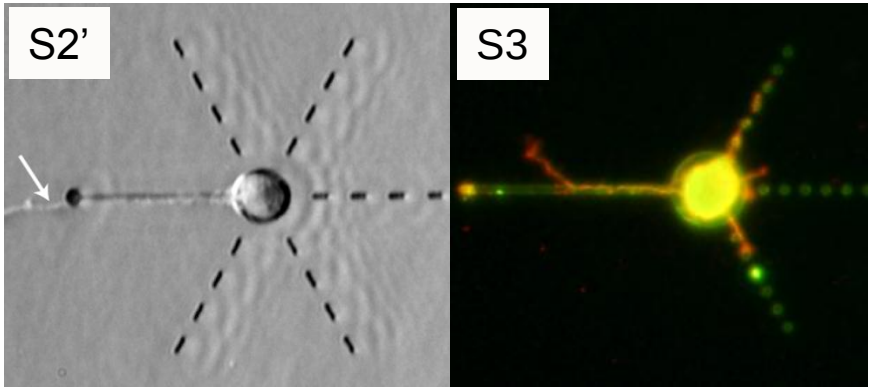
-small *g* provides no growth differential
- large *g* prevents dendrite formation

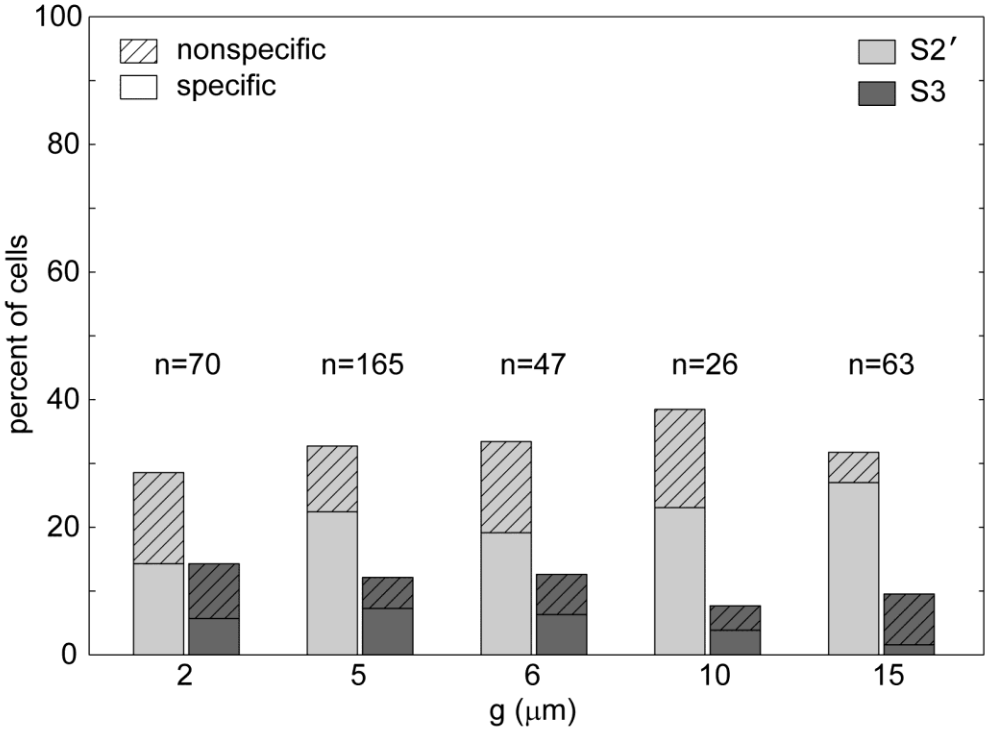
C.D. James et al., Biomaterials 2011, 32, 8860

Guidance of neurons towards cytoarchitectural maturity

Banker and Goslin (1998):







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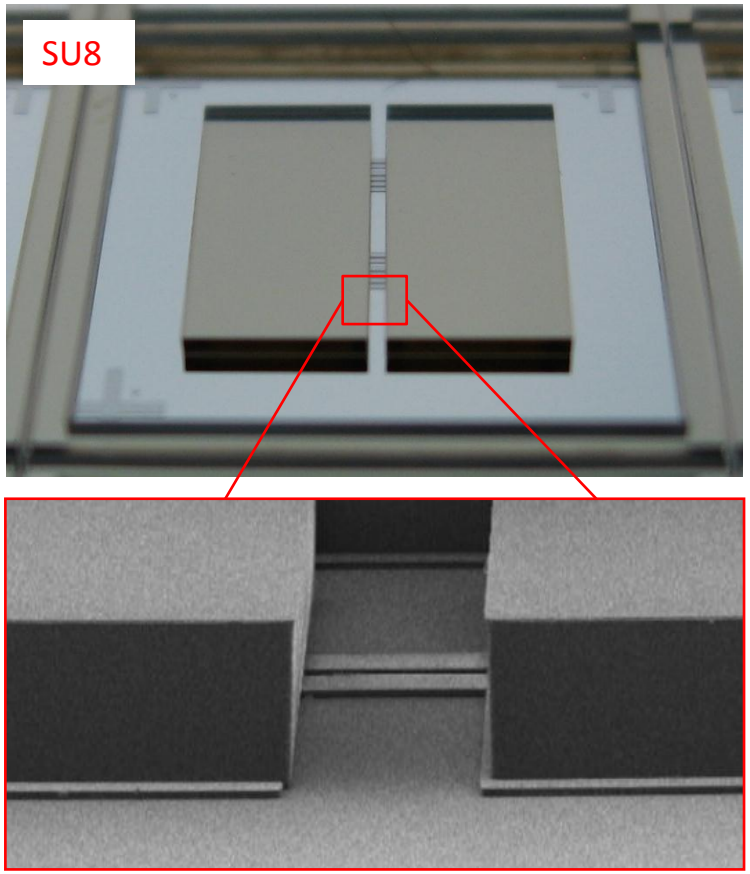
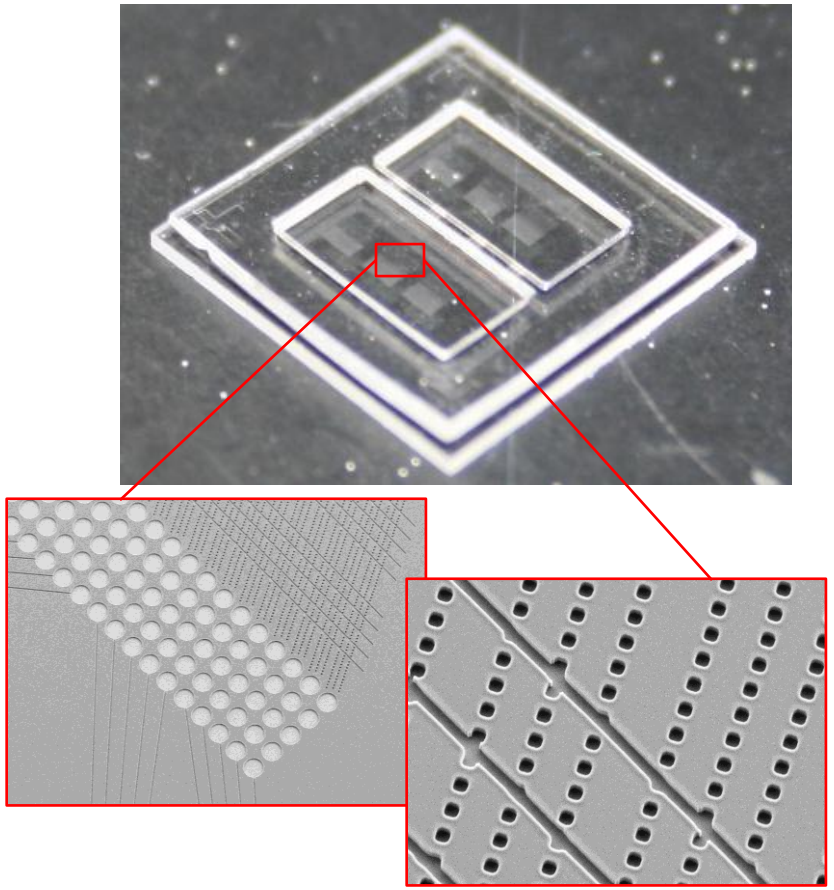
Engineered networks using microfluidic compartmentalization and surface guidance cues

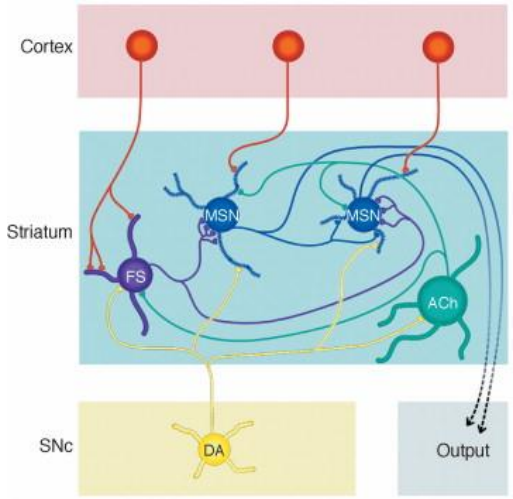


SU8 master for creating microfluidic compartments for engineered cell networks

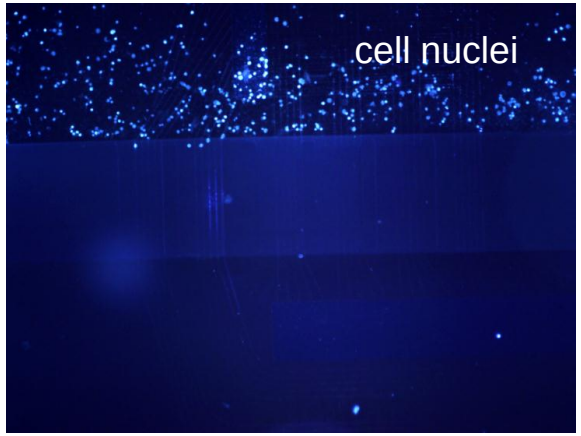


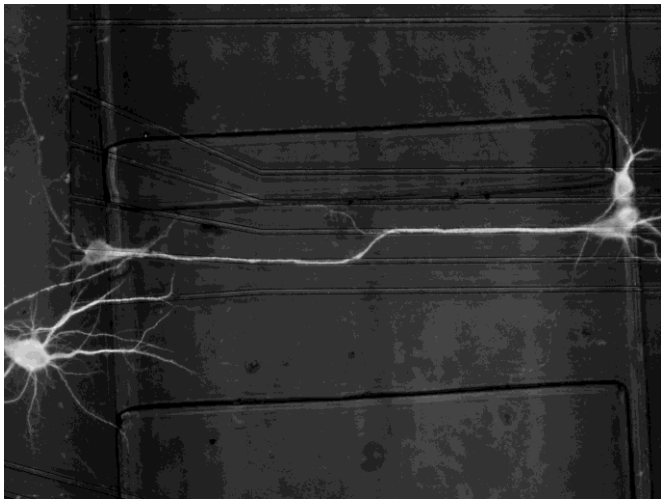
PDMS chambers bonded to glass substrates with guidance cues

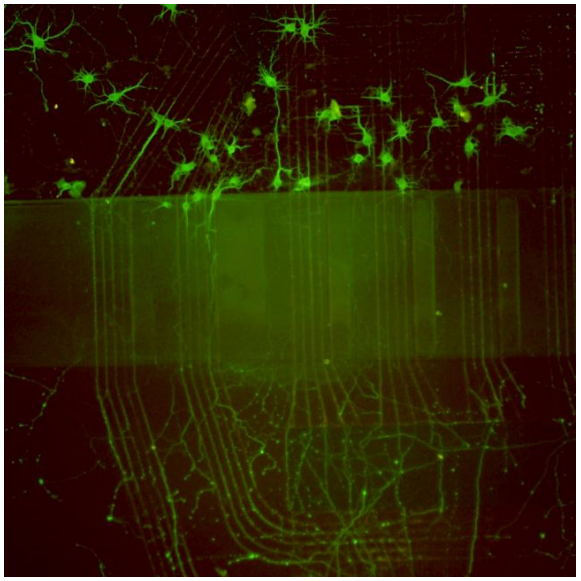
Compartmentalization of multiple neuronal cell types to construct heterogeneous networks

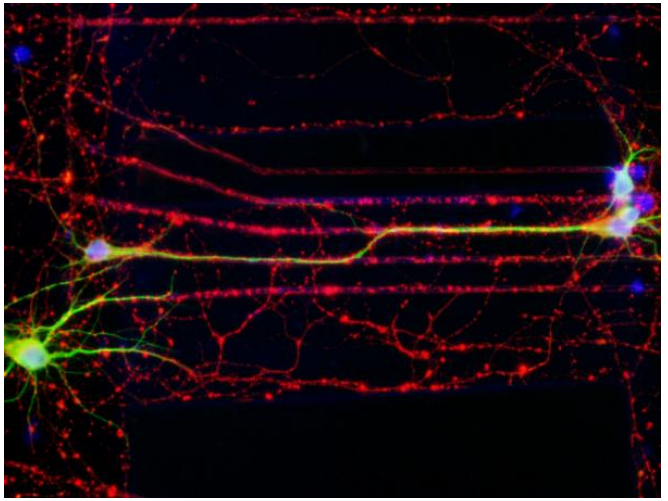


Tepper et al., *Curr Opin Neurobio,* 2004

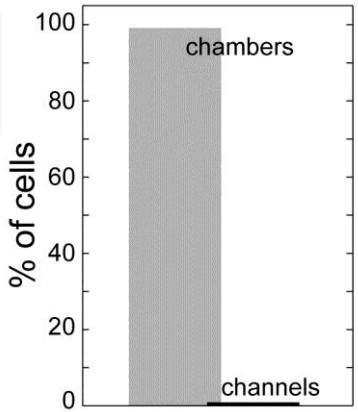




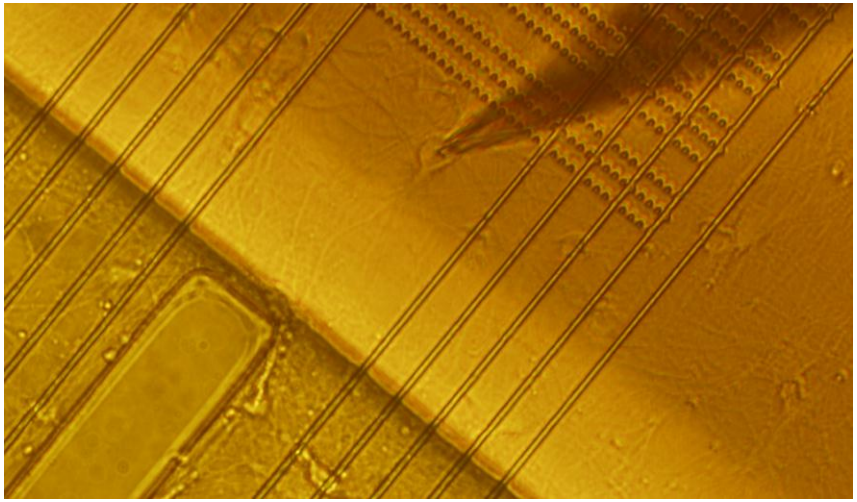


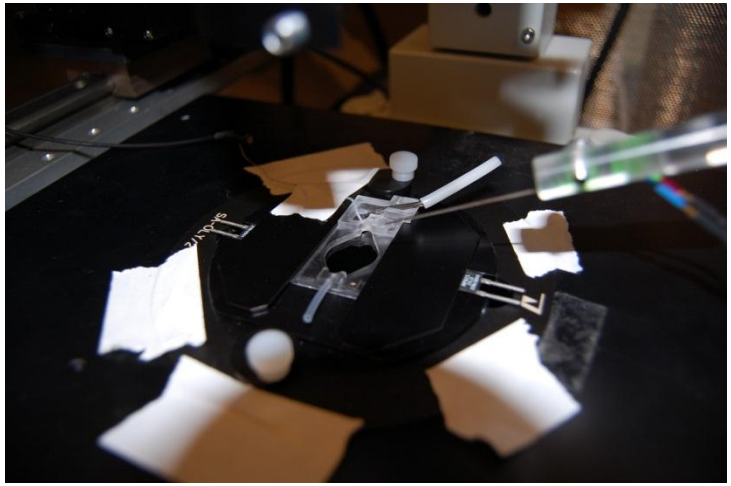


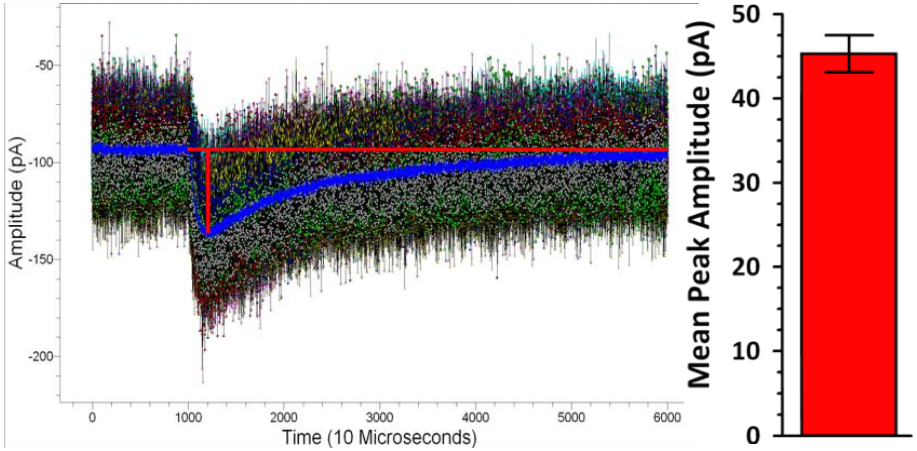
10 μm channels



Electrophysiological analysis of engineered corticostriatal networks

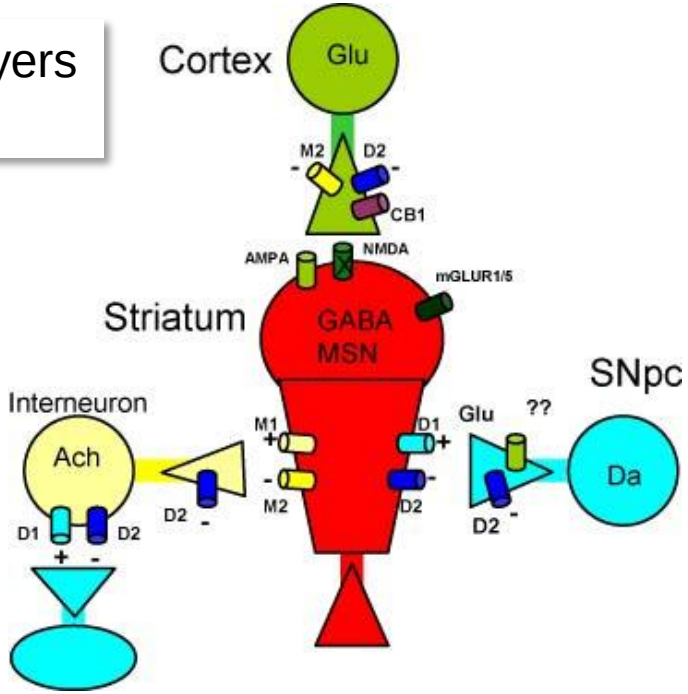


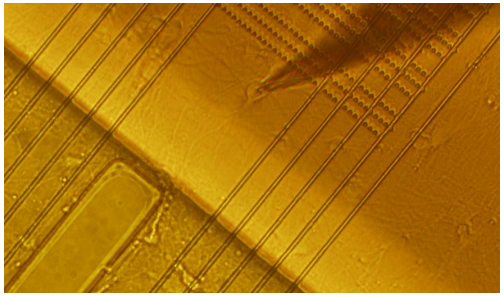


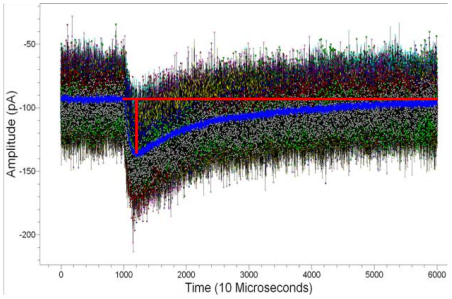


Engineered CSNs to identify important *in vivo* phenomena

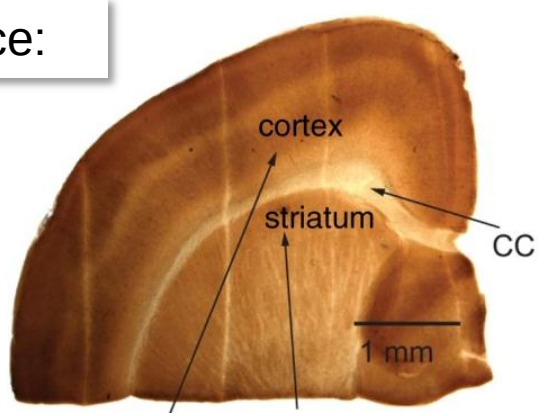
Major players in CSNs:

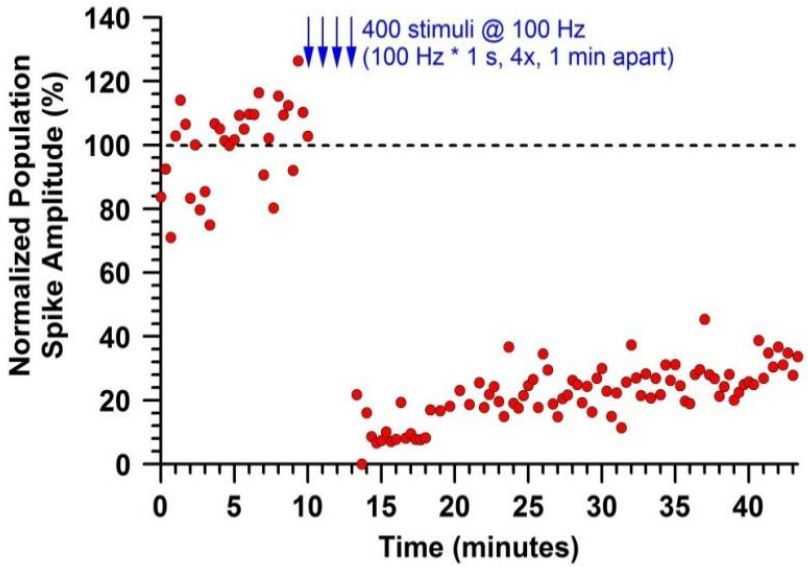




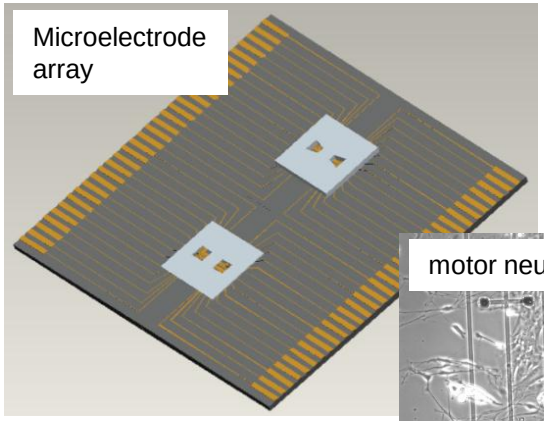


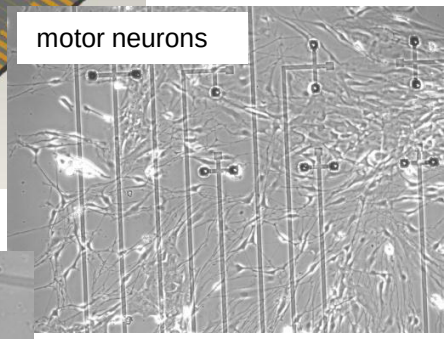
Brain slice:

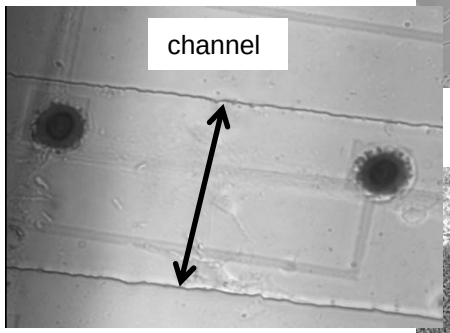


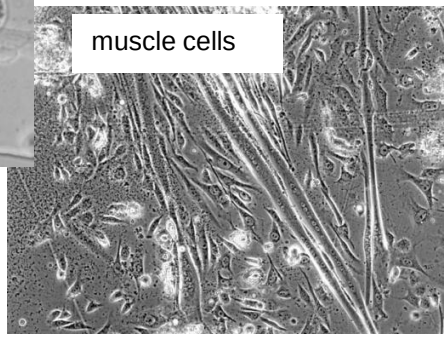


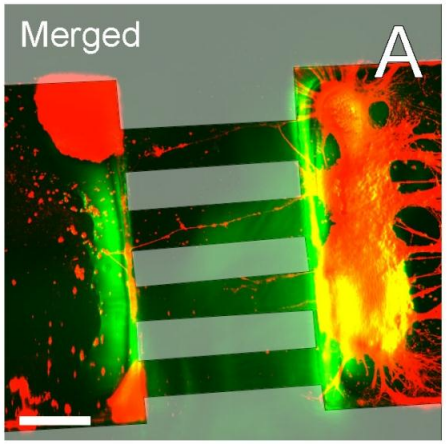
Engineered neuromuscular junction networks

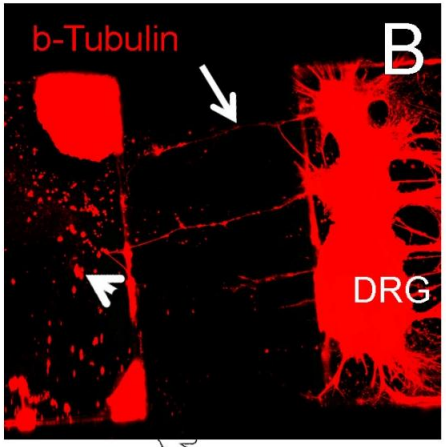


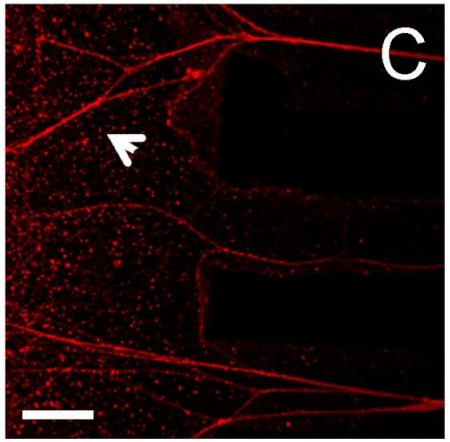


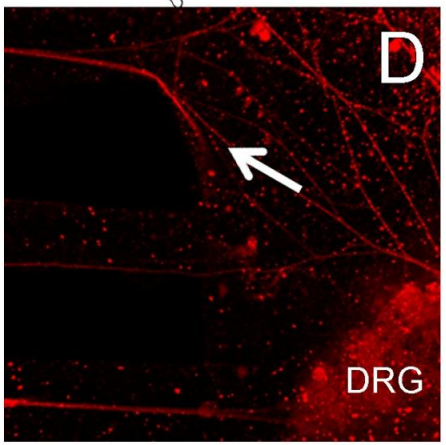






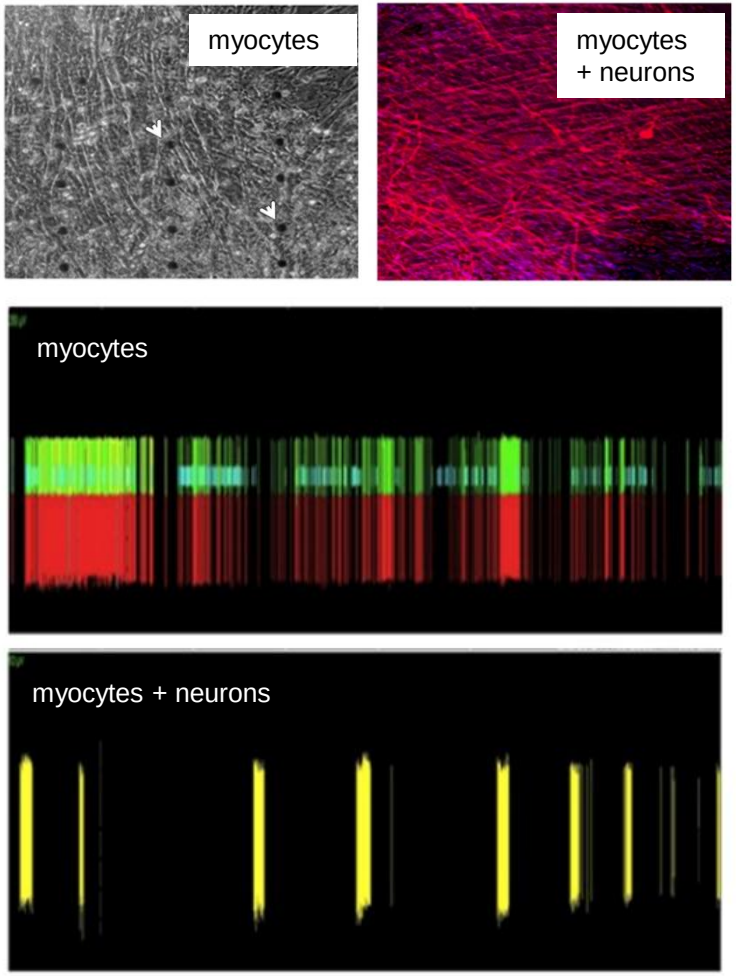


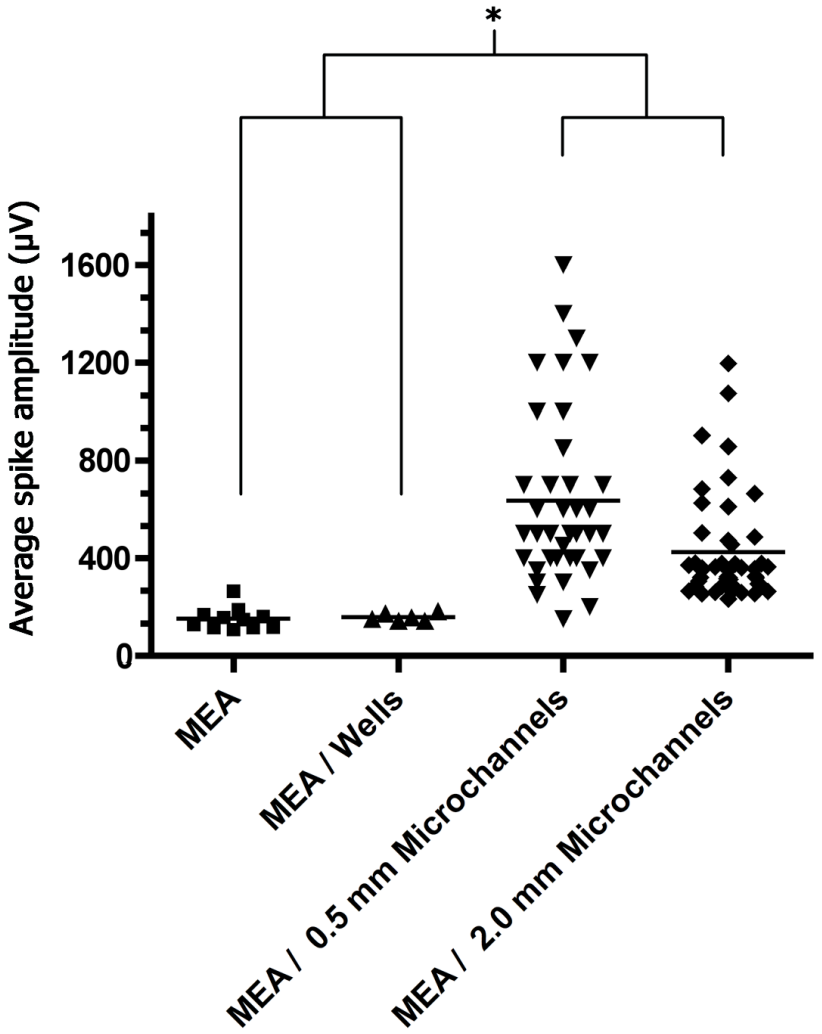




M. Romero-Ortega, UTA, in prep

Modulation of myocyte electrical activity due to co-culture with neurons





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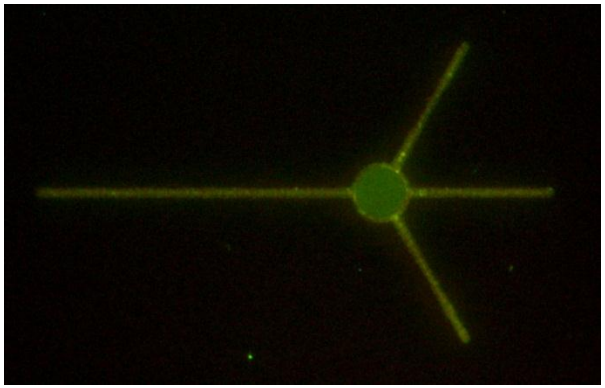
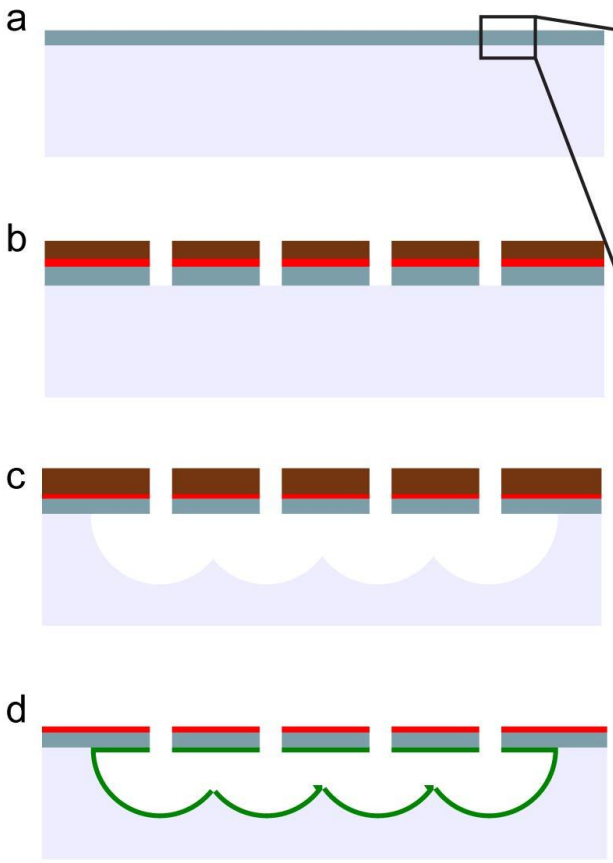
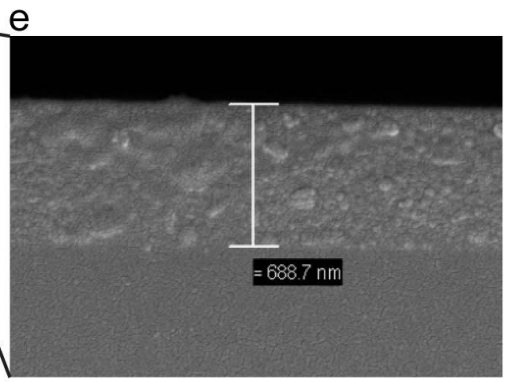
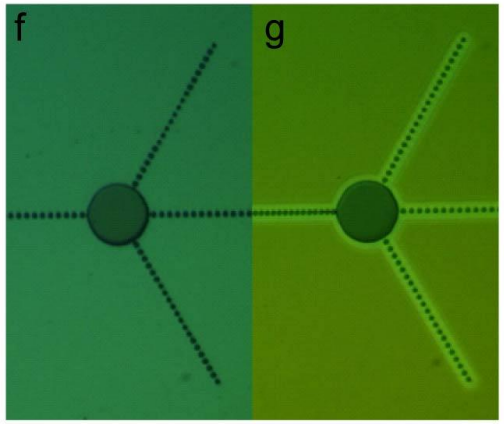
Embedded microfluidic channels for 3D guidance of neurons

1. Deposit LPCVD SiN on fused silica
2. Pattern irrigation vias
3. Etch glass in BHF
4. Poly-lysine coating
5. Liftoff

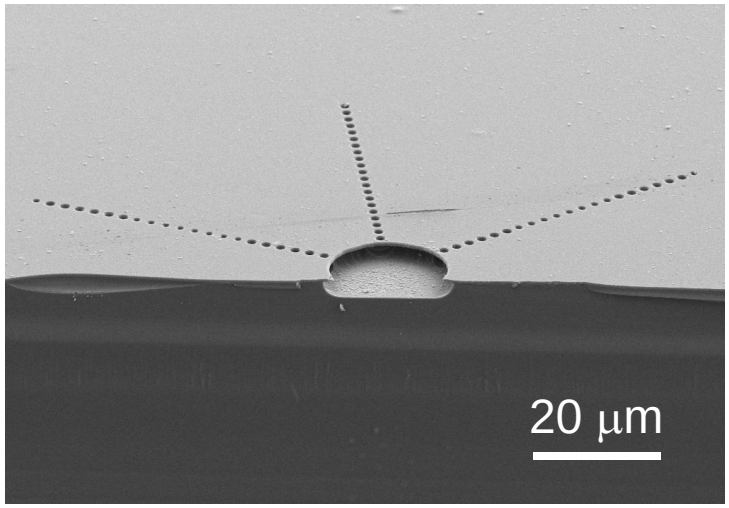


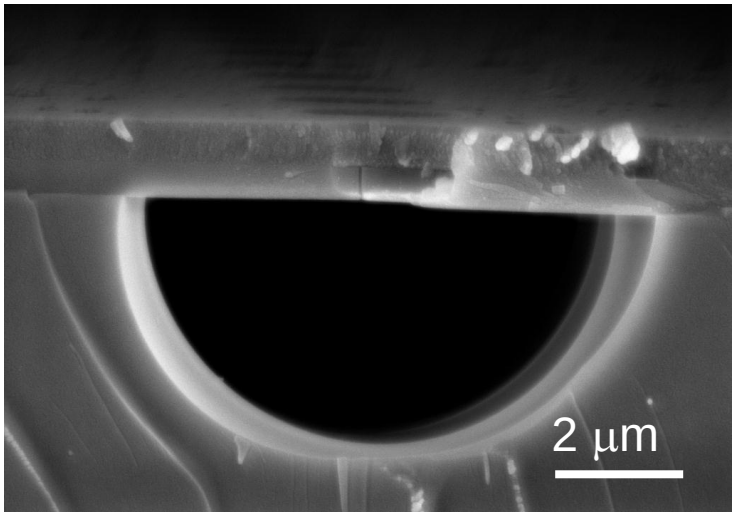


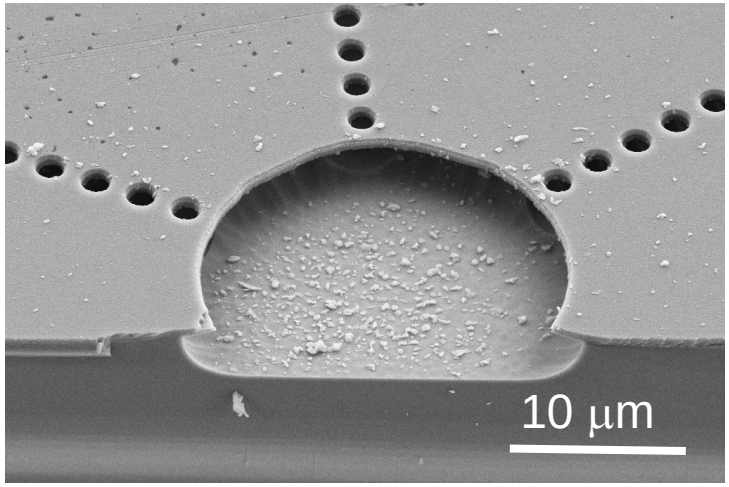


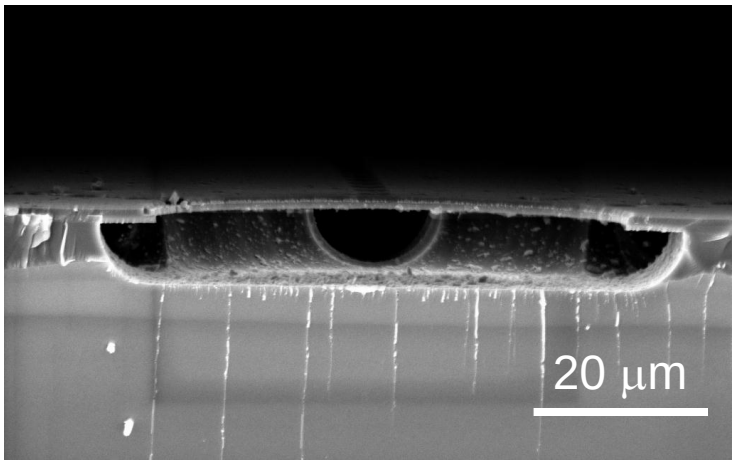


Microchannels provide structural support and maintainance of fluidic access

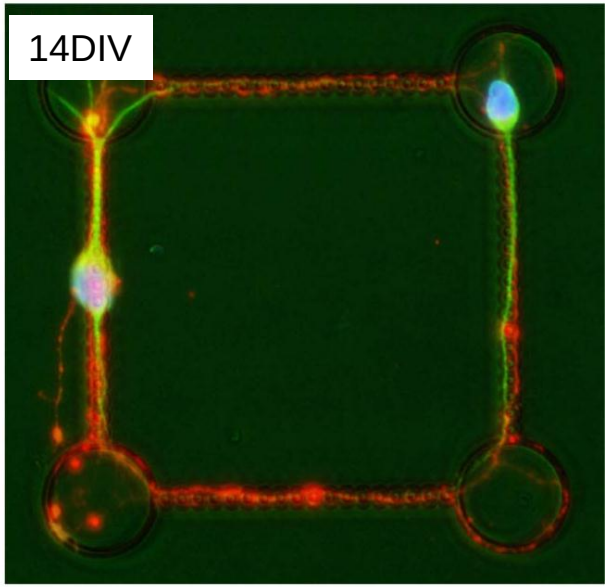


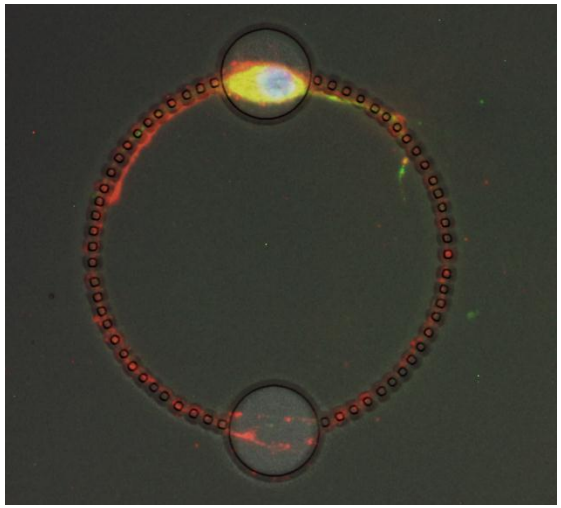


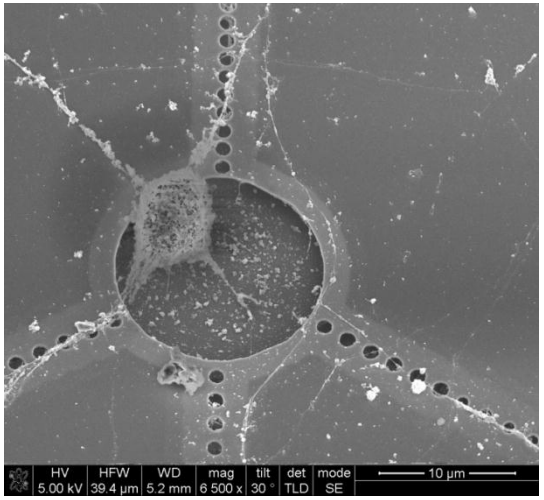


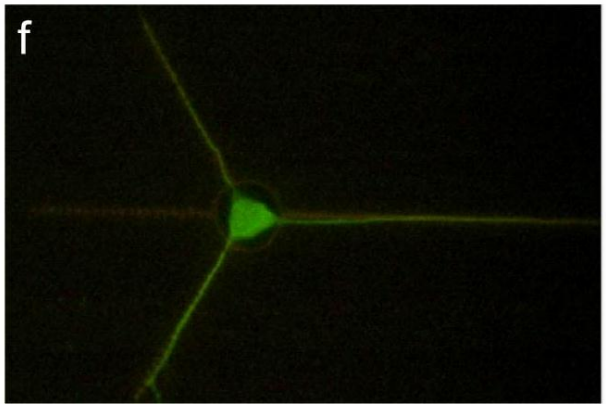


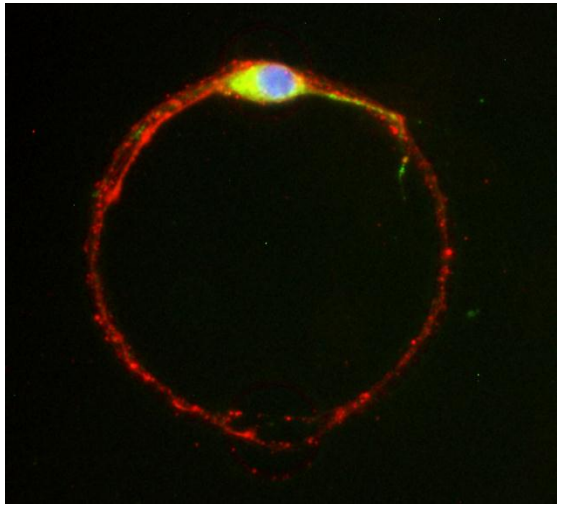
Long-term culture of neurons coupled to buried microchannels

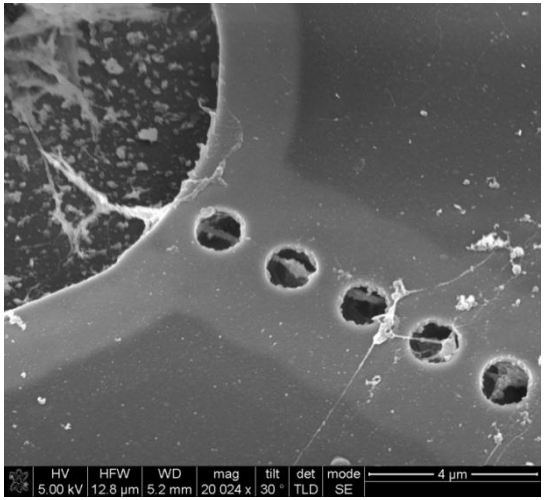












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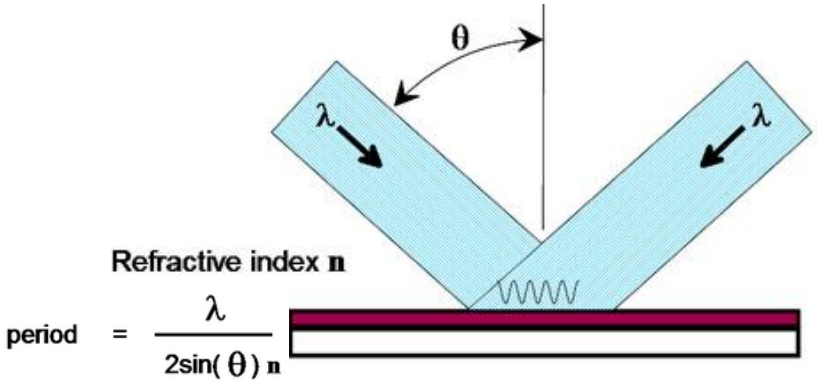
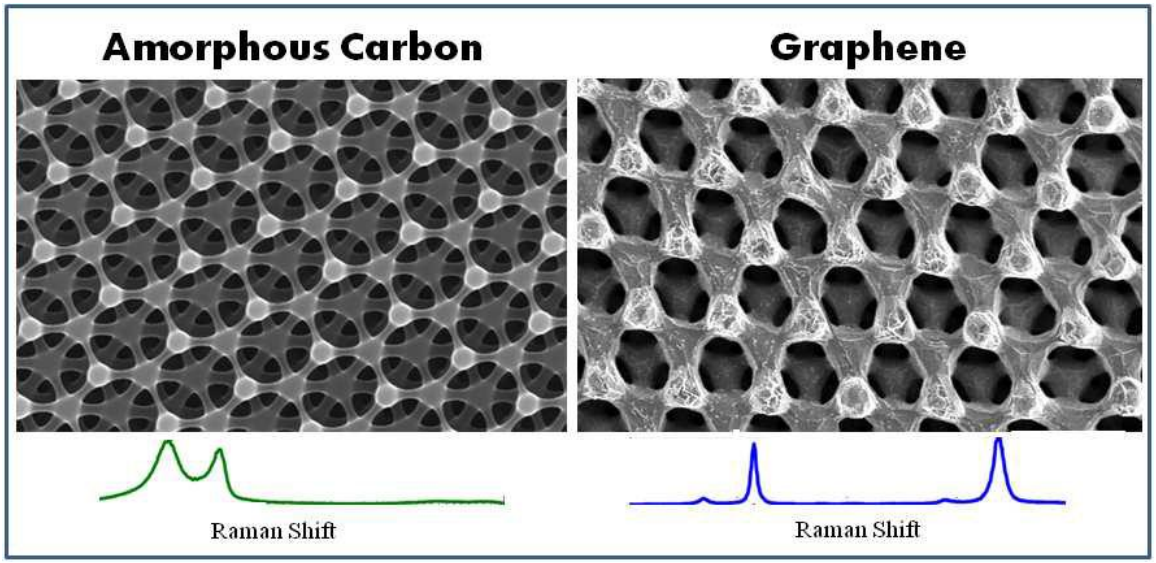
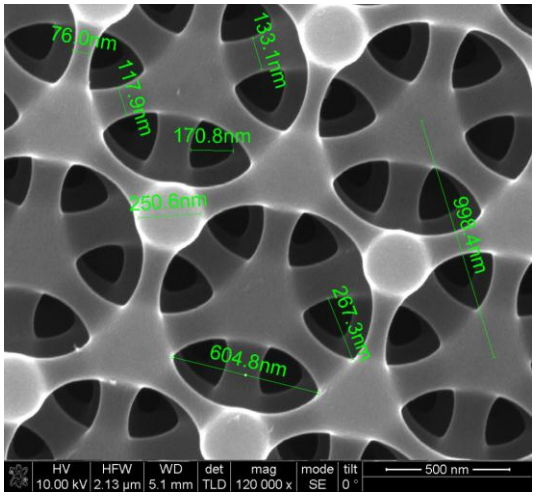
Porous carbon nanostructures fabricated by interferometric lithography

- Microscale geometrical structures: cell body attachment, neurite outgrowth, polarization
- Nanoscale structures: dendritic spine development



http://www.almaden.ibm.com/st/chemistry/lithography/interferometric/

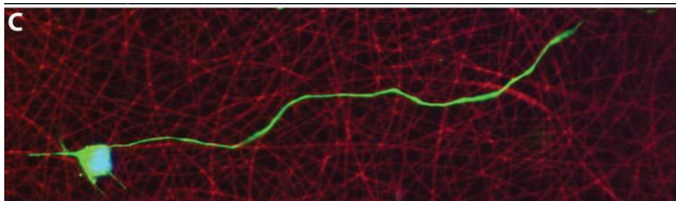




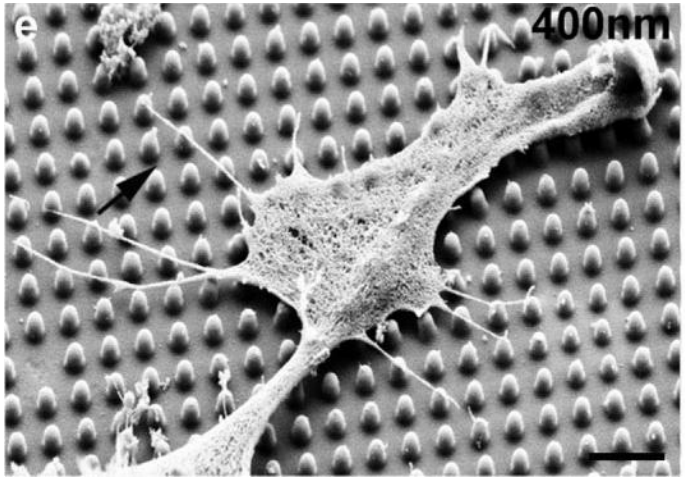
Burckel et al., *Small,* 2009, 24, 2792-2796

Dissociated neurons on nanoporous substrates

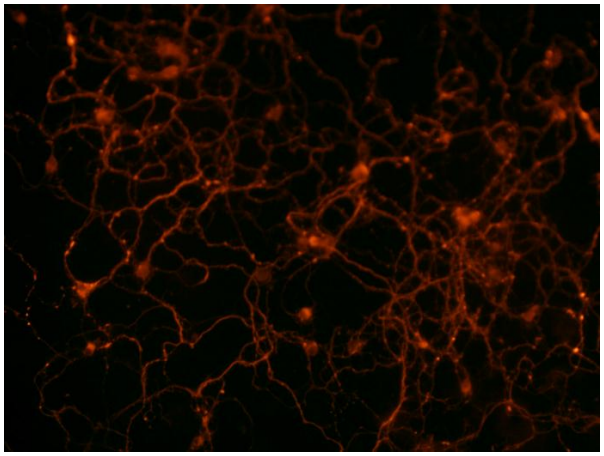
Nanostructured surfaces affect the maturation of dissociated cells in culture

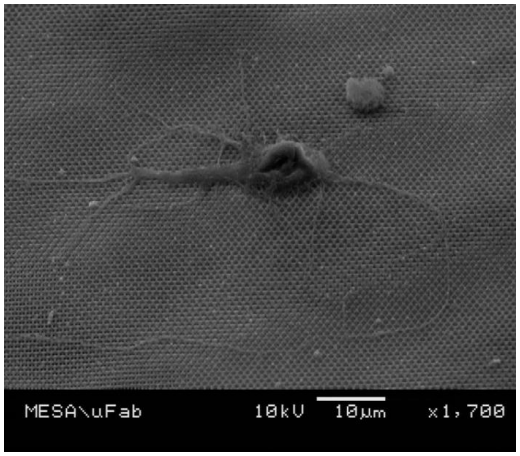


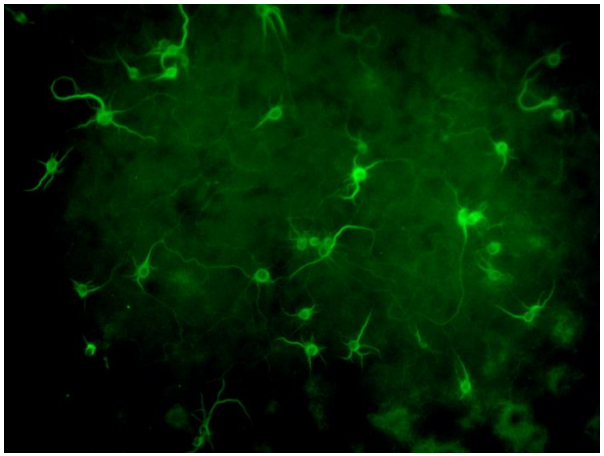
Gertz et al., *Dev Neurobio,* 2010

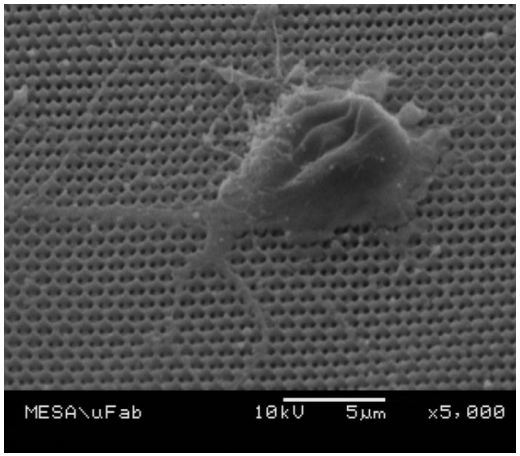


Migliorini et al., *Biotech Bioeng,* 2011

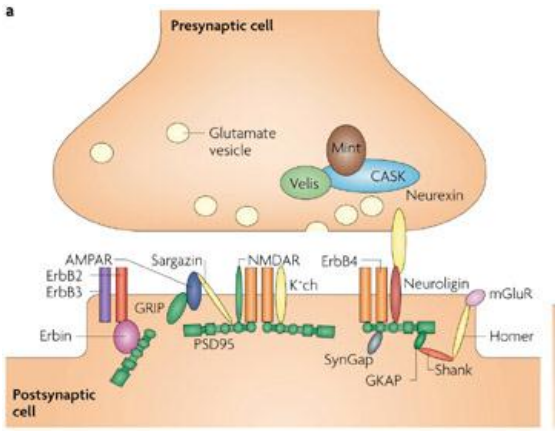




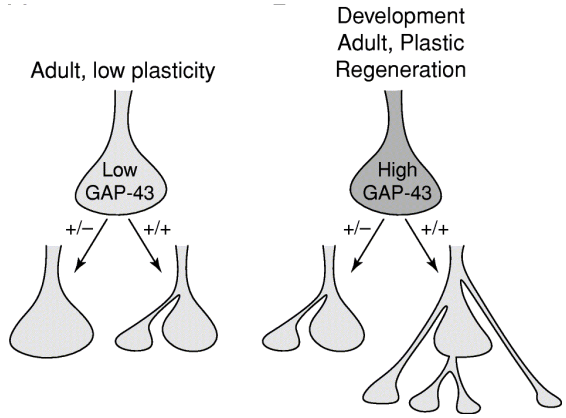




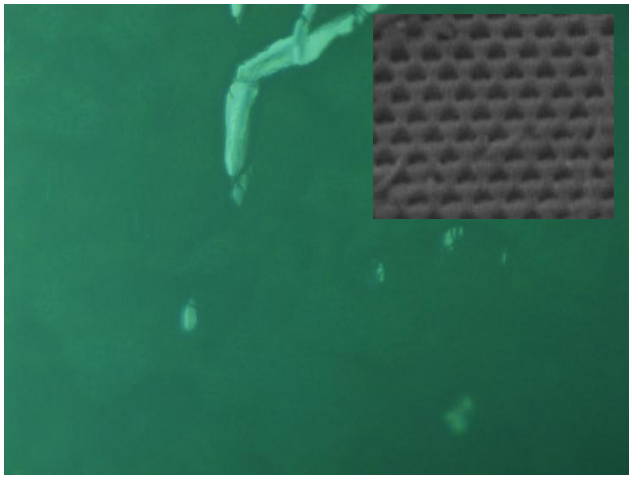
Synaptic staining of networks grown on nanostructured surfaces

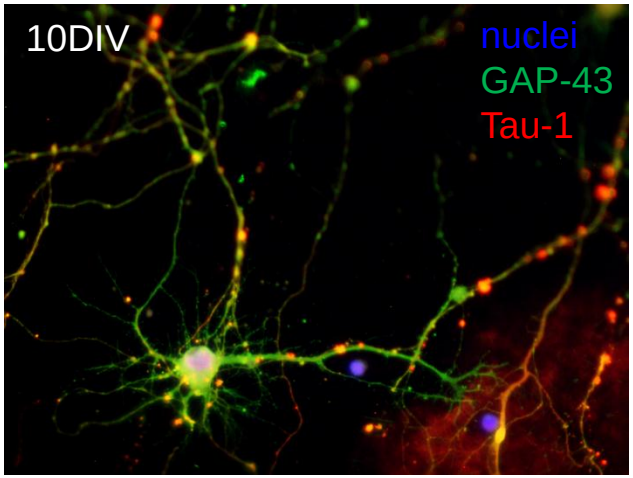


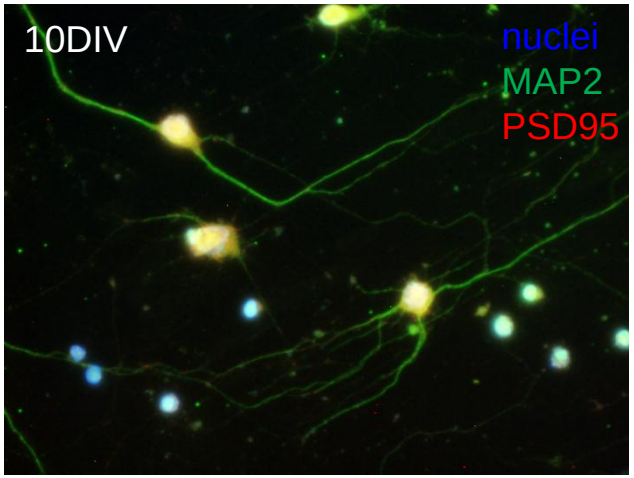
Sekino et al., *Neurochem Inter.* 2007



Benowtiz et al., *Trends Neuro.* 1997







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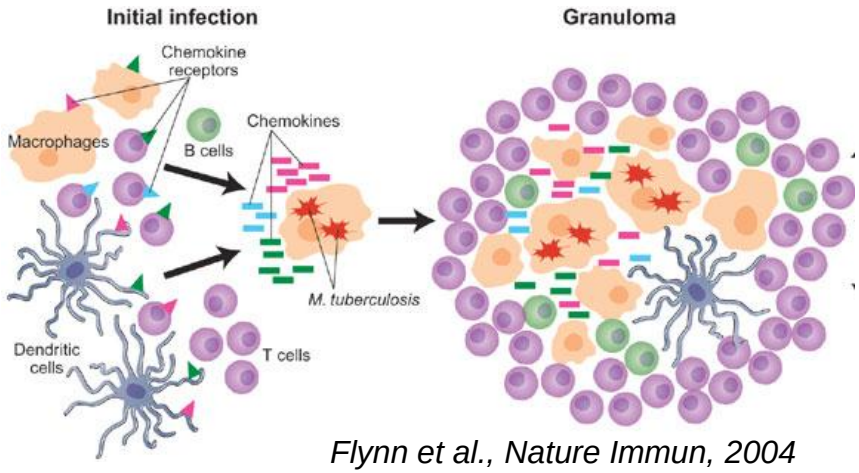
Conclusions and Future Work

- Microfluidic compartmentalization and embedded chemical and topographical cues can be used to engineer heterogeneous multicellular networks
- Nanostructured materials have the potential to influence sub-micro organization of cellular structures

- In vitro blood brain barrier model to examine pathogenesis in the central nervous system
- 3D microscaffolds with nanotexture for engineering cellular networks to study granuloma formation during immune response

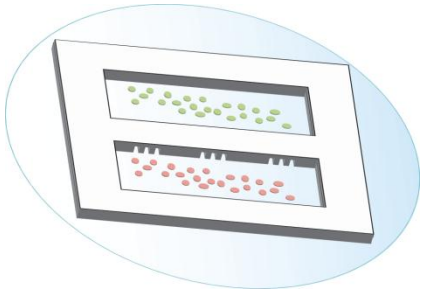
In vitro microfluidic model for interrogating pathogenic infections

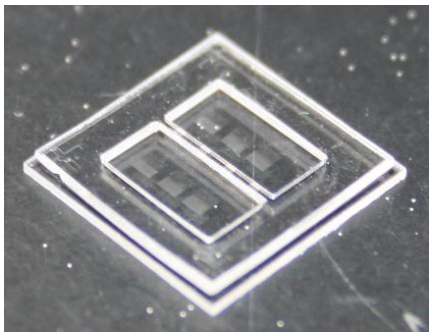
- Granulomas are a prototypical host response to infection that results in control of infection
- **Monitor pathogenesis in real-time with controlled environmental variables**
- Develop 3D gel scaffolds with compartmentalization; perform cell migration assays

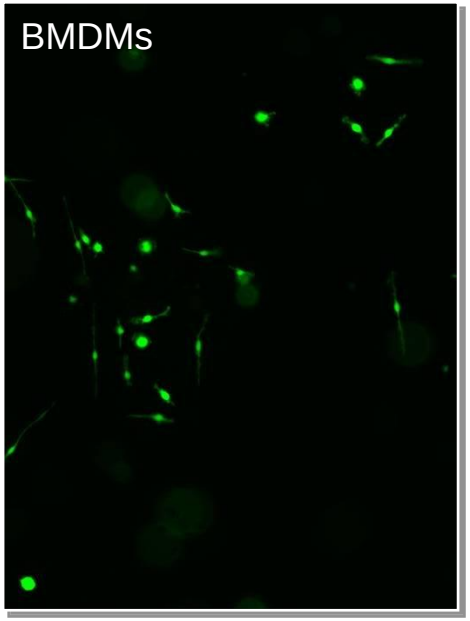


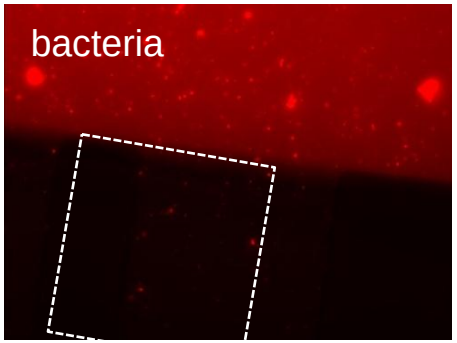
Flynn et al., Nature Immun, 2004

in vitro cell aggregates









Acknowledgments

- Conrad James, Patrick Mickel, Adrian Schiess, Erika Vreeland, Jamie Howell, Deanna Lopez, Ronen Polsky, Jason Harper, Susan Brozik – SNL, Microsystems Center: Biosensors and Nanomaterials
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