Microfluidic Platform for Culture and Live Cell Imaging of Cellular Microarrays

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Outline

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    - Microfluidics
    - Clonal Microarrays
- Problem Statement
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- Last Semester's Prototype
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- Testing - Gradient Comparison
- Integration
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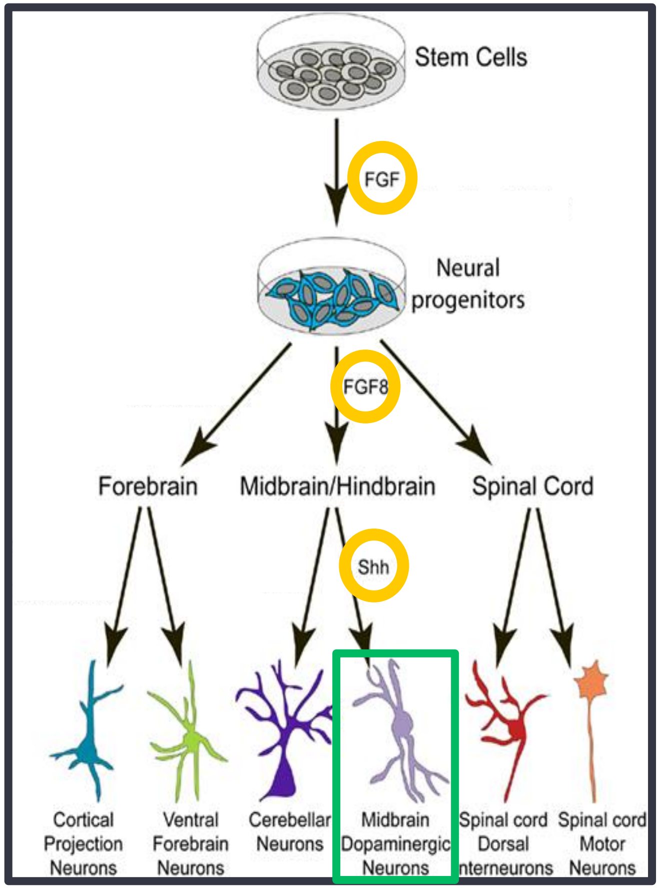
Motivation – Neurodegeneration

- Neurodegenerative diseases
  - Loss of neuron structure and function
  - Nervous cells have limited proliferation capacity[1]
  - Current therapies treat symptoms, not underlying causes[2]
- Neural stem cells can generate all neuron types[1]
  - Directed differentiation into specific neurons
  - Replace damaged or dead neurons



Stem Cell-based Regenerative Medicine.

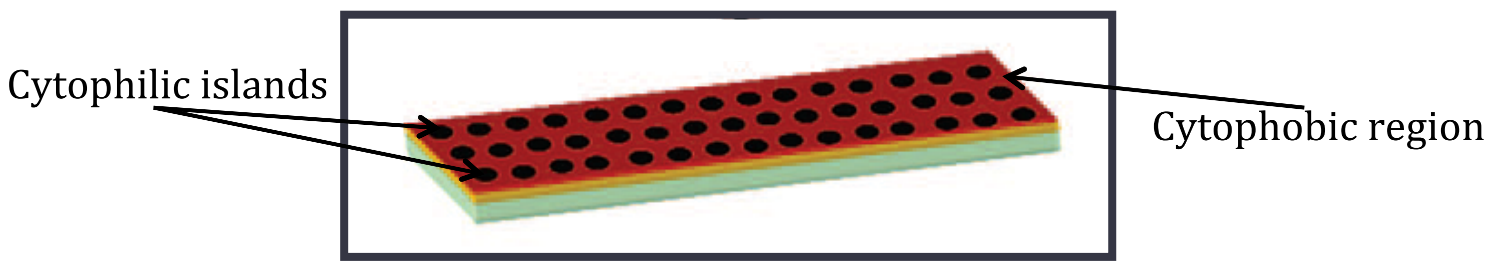
From "Pluripotent Stem Cells for the Study of CNS Development"

Motivation – Microfluidics

- Microfluidics can generate gradients *in vitro*[4]
  - Minimize reagent volumes
  - Allow for high resolution and sensitivity
  - Permit laminar flow and low shear stresses
  - Facilitate high-throughput analysis
- Temporal gradient investigation
  - Gradients form during embryonic development[5]
  - Varying concentrations direct stem cell fates

Motivation – Clonal Microarrays

- Gold-coated glass patterned with cytophilic islands[3]
  - Islands separated by cytophobic regions
  - Cells seeded on device at clonal densities
- Enable high-throughput screening of gene function
- Compatible with automated microscopy
- Need a high-throughput way to establish different colonies



Cellular Microarray.
From "High Throughput Screening of Gene Function in Stem Cells Using Clonal Microarrays"

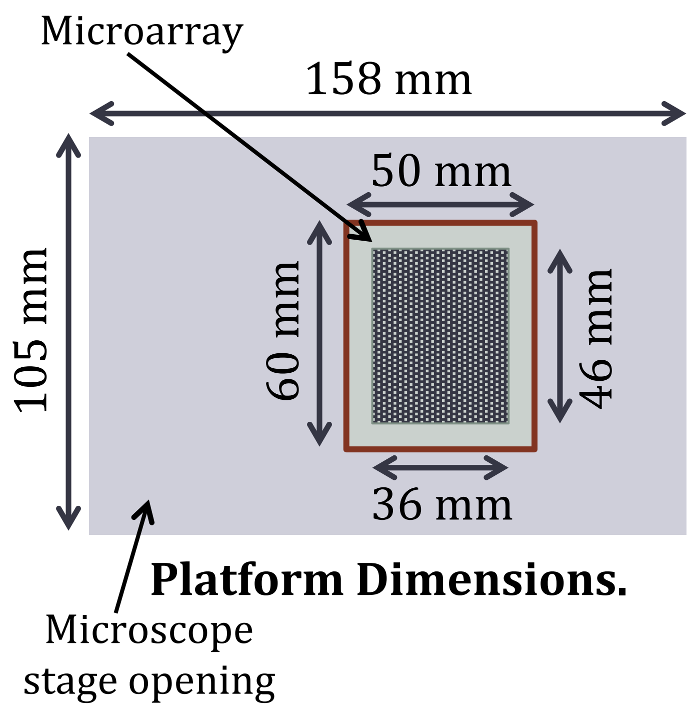
Problem Statement

Integrate cellular microarray with microfluidic platform that:

- Is compatible with a standard microscope stage
- Enables live-cell imaging and high-throughput analysis
- Generates a concentration gradient across a microarray
- Creates a water-tight seal with a microarray
- Is reusable for multiple microarrays

Design Specifications

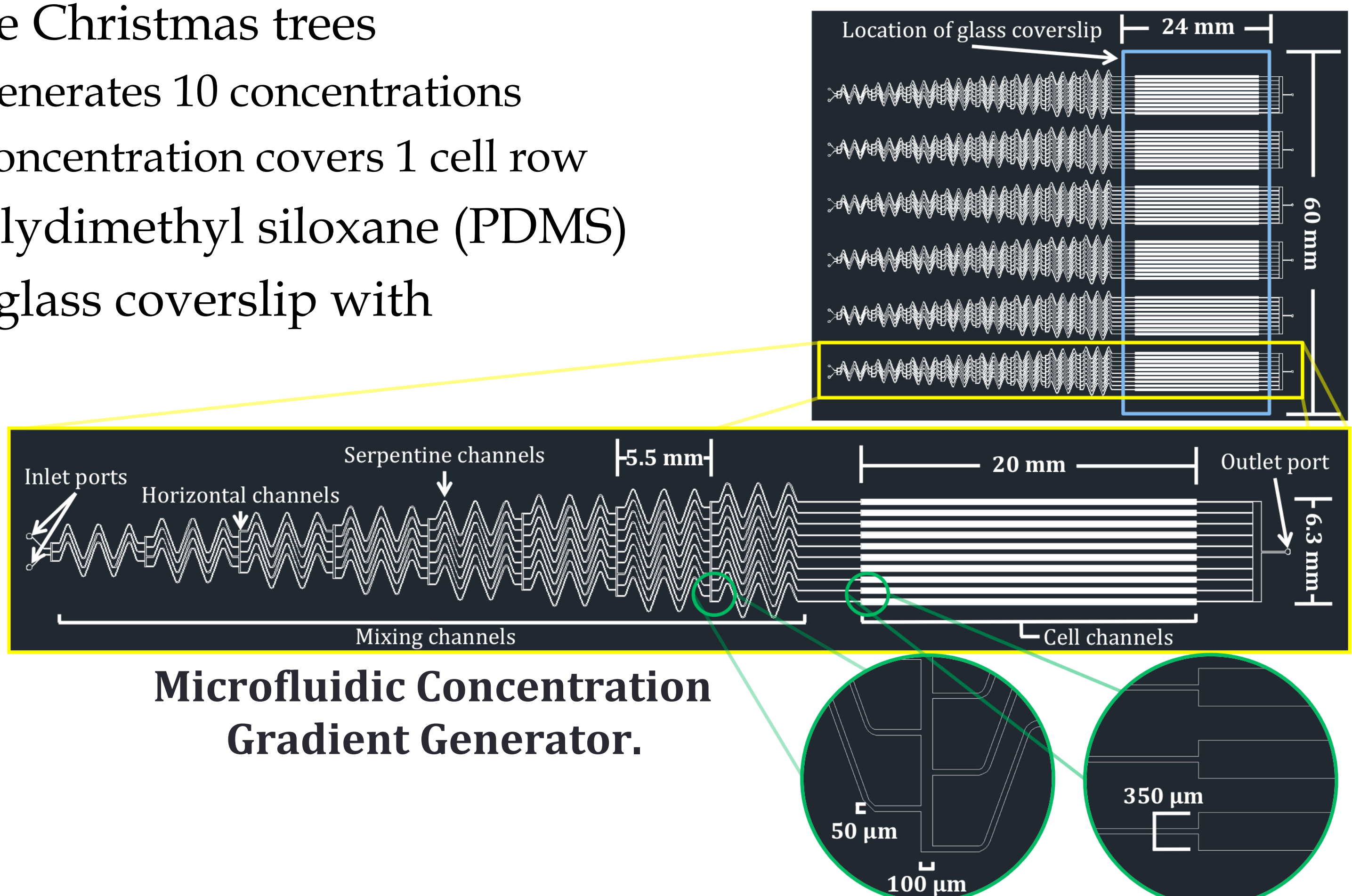
- Dimensions and weight
  - Platform: 158 x 105 mm
  - Microarray glass: 50 x 60 mm
  - Patterned microarray area: 36 x 46 mm
  - Maximum weight: 0.5 kg
- Ergonomics
  - Accurate fabrication and alignment
- Performance requirements
  - Maximize number of gradients
  - Maximize cellular pixels (r = 150 μm)
  - Long term experimentation (1–10 days)



Platform Dimensions.

Last Semester's Prototype

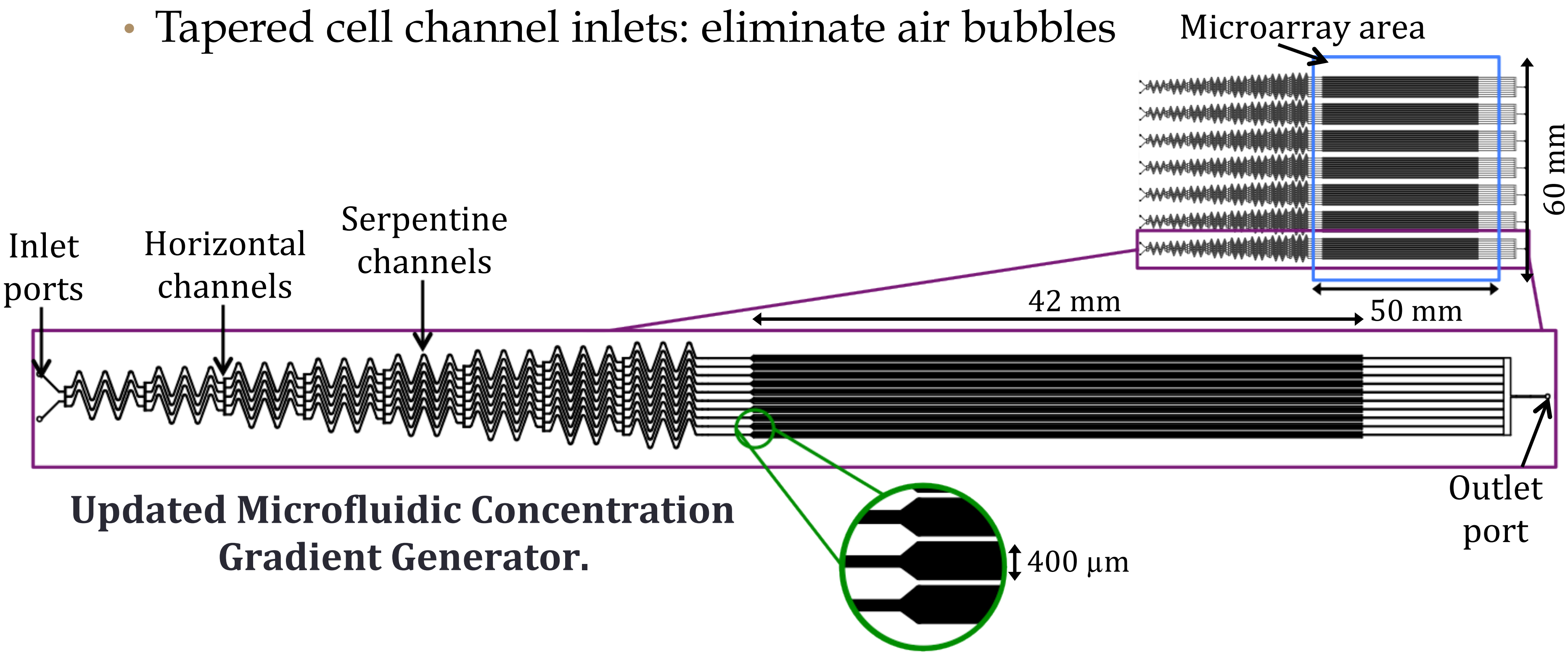
- Christmas tree design[6]
  - 6 separate Christmas trees
    - Each generates 10 concentrations
    - Each concentration covers 1 cell row
- Material: polydimethyl siloxane (PDMS)
- Removable glass coverslip with microarray



Microfluidic Concentration Gradient Generator.

This Semester's Updates

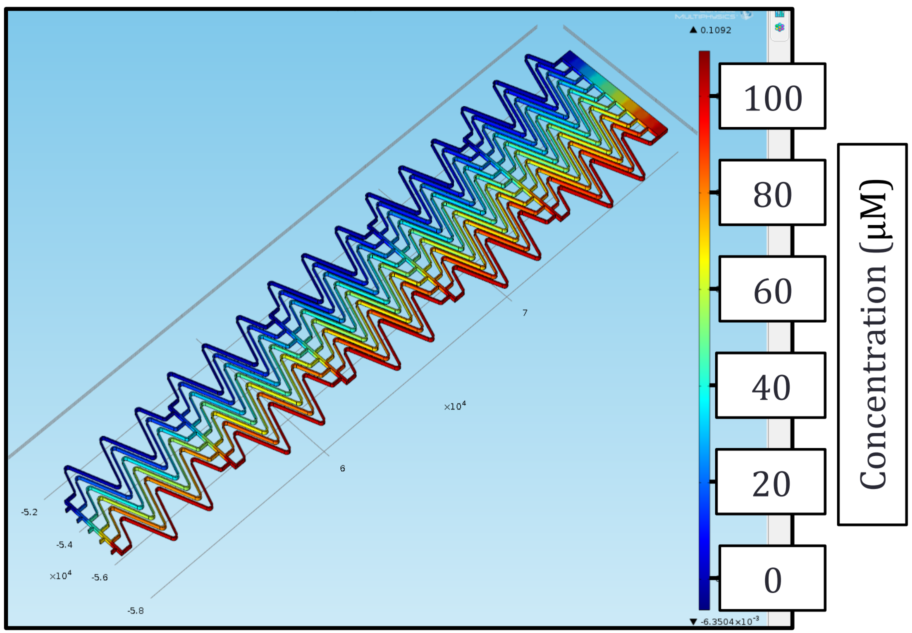
- Modified Christmas tree design[6]
  - 7 trees: maximize potential number of conditions
  - Longer cell channels: maximize pixels per condition
  - Increased cell channel widths: better alignment with microarray
  - Tapered cell channel inlets: eliminate air bubbles



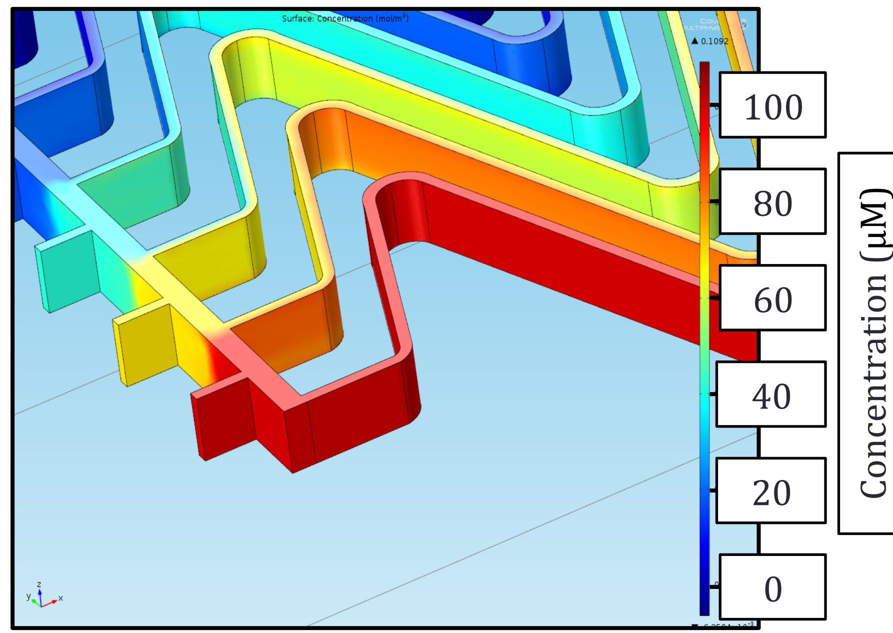
Updated Microfluidic Concentration Gradient Generator.

Testing – Gradient Comparison

- 3D simulation: COMSOL
  - Finite element analysis software
  - Simulated design functionality



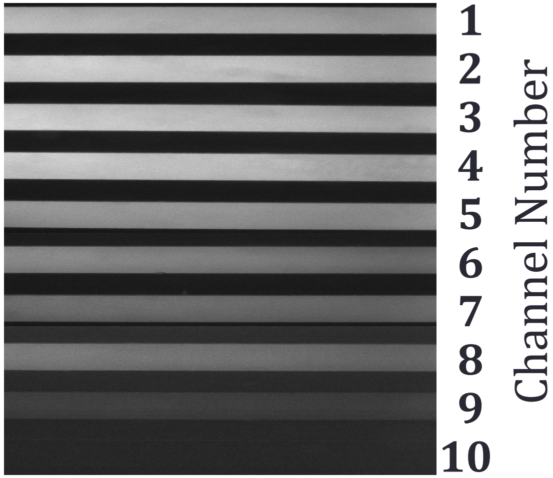
COMSOL Analysis in Mixing Channels.



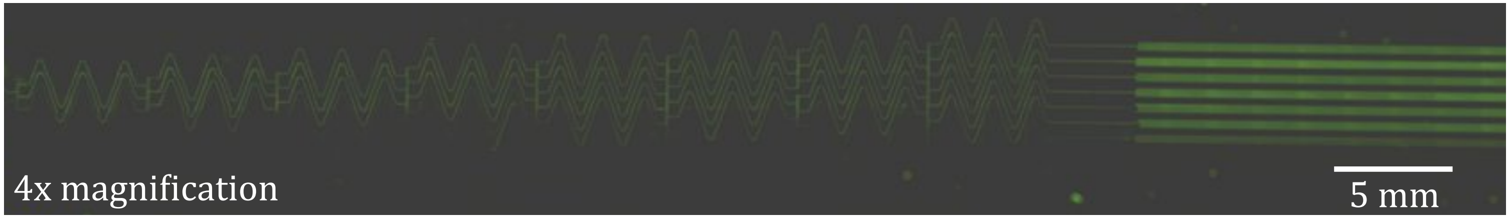
Demonstration of Fluid Mixing.

Testing – Gradient Comparison

- Experimental: gradient generated
  - 25 µM dextran-fluorescein isothiocyanate (FITC)
  - Imaged with fluorescence microscope

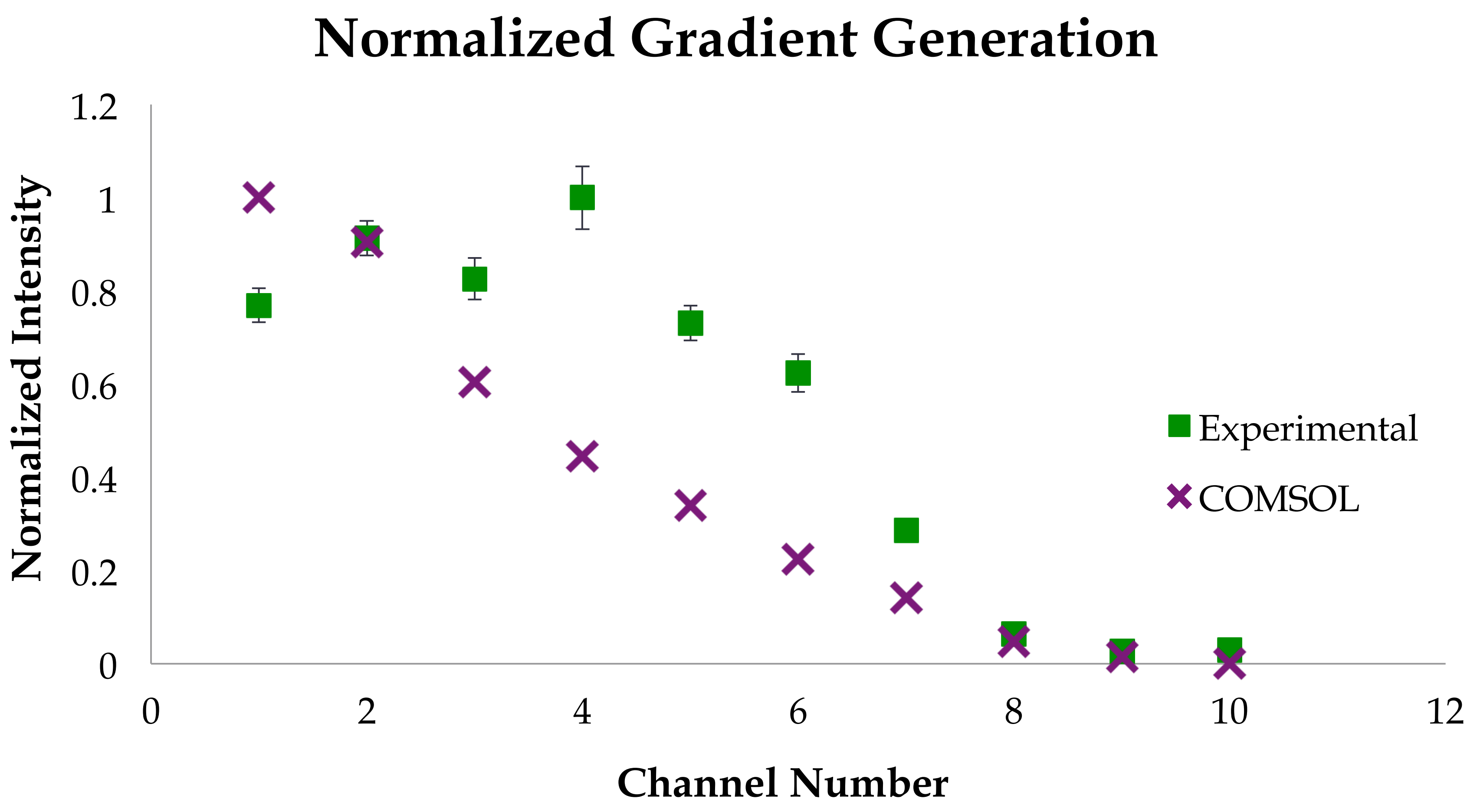


Channels in Experimental Device.



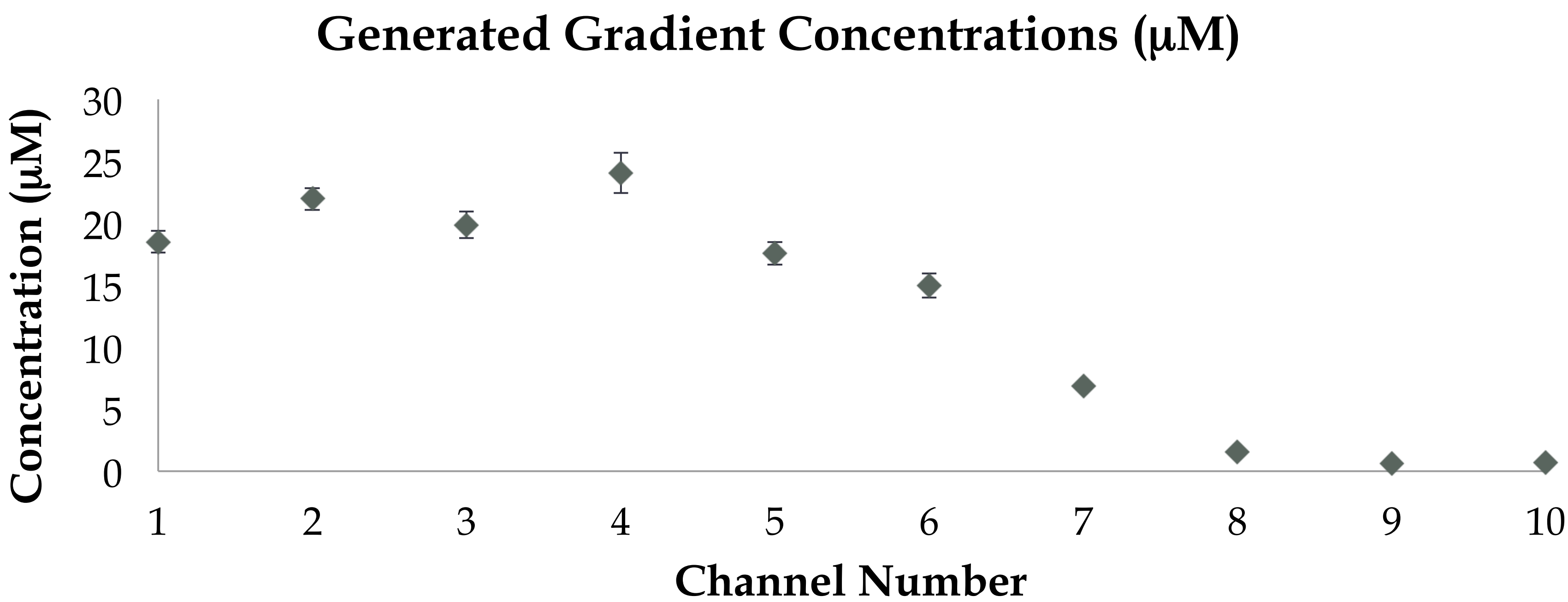
Fluorescent Gradient Generation in Experimental Device.

Testing – Gradient Comparison



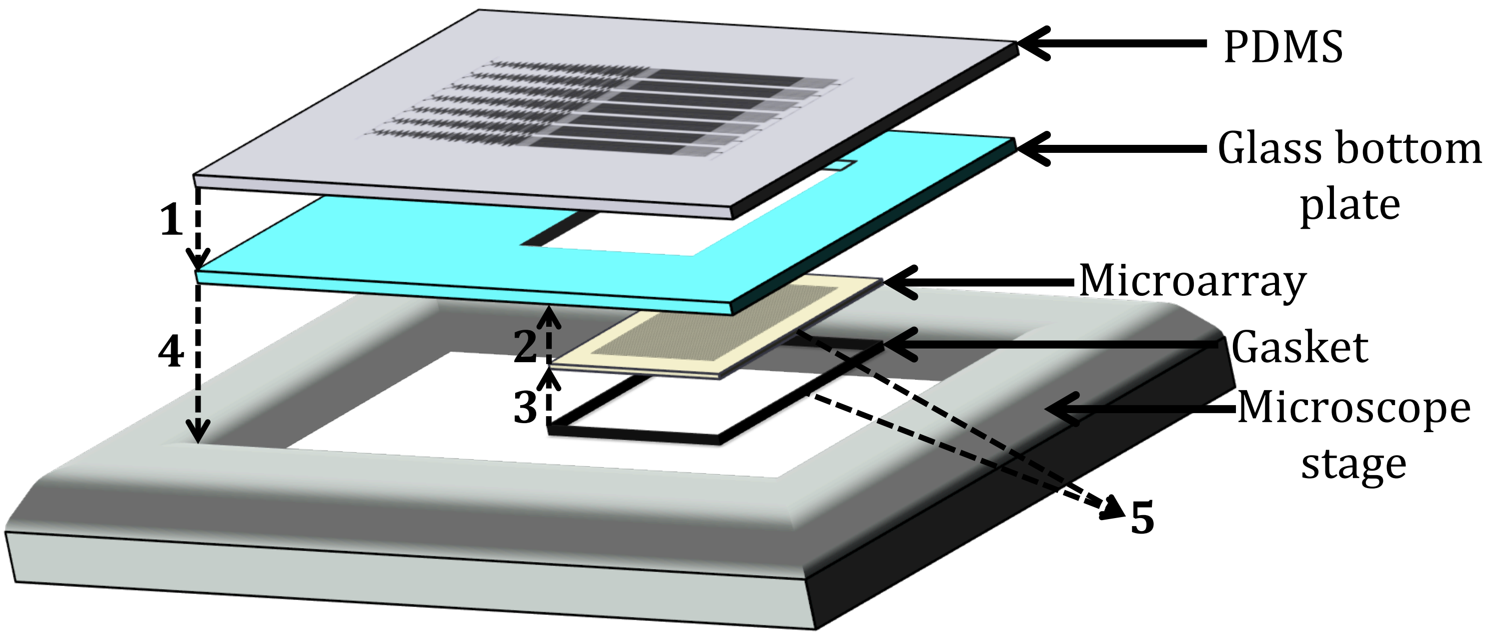
Testing – Standard Curve

- Standard normalized curve to relate intensity to concentration
  - Normalized intensity = 0.0416 × Concentration (μM)
  - 25 μM dextran-FITC at 4 μL/min



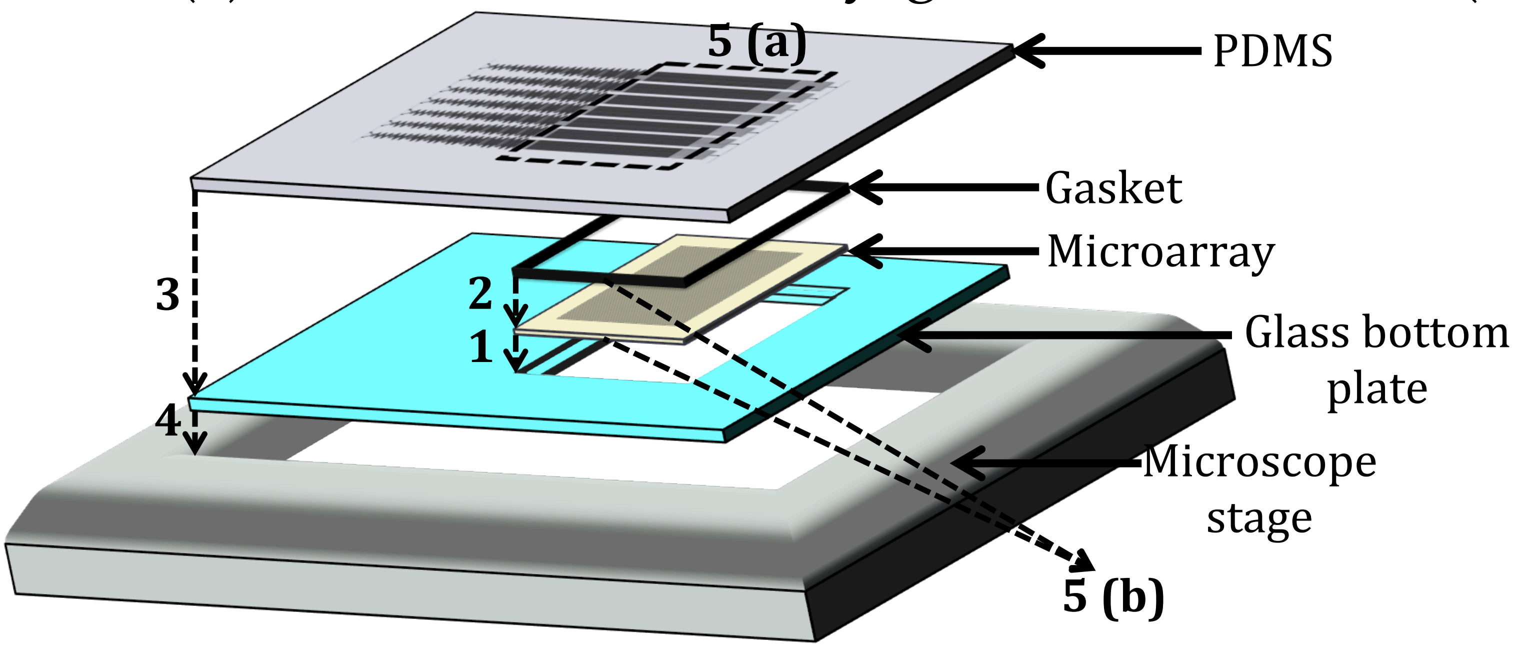
Integration – 1st Alternative

1. Plasma oxidize PDMS to glass bottom plate
2. Align microarray and insert underneath
3. Insert gasket and sealant
4. Insert into microscope stage and run experiments
5. Remove microarray, gasket, and sealant



Integration – 2nd Alternative

1. Insert microarray into etched glass bottom plate
2. Insert gasket and sealant
3. Align and plasma oxidize PDMS to glass bottom plate
4. Insert into microscope stage and run experiments
5. Cut PDMS (a) to remove microarray, gasket, and sealant (b)



Integration

	1st Alternative	2nd Alternative
Advantages	• Reusable components: PDMS, glass bottom plate, gasket • Compatible with electronic aligner	• Reusable component: gasket • Alignment from the top • One alignment step
Disadvantages	• Need to secure gasket • Potential for molecules to be absorbed into PDMS and released later	• More fabrication needed per experiment • Microarray must be covered during plasma oxidation

Future Work

- Microfluidic device
  - Make new silicon master with updated photomask
    - Investigate two-layer device if necessary
  - Determine concentrations generated through fluorescence testing
- Integration
  - Test fluid flow over glass interfaces
    - Find sealant that will also enable microarray removal
  - Verify ability to accurately align all components
- Cell incorporation
  - Ensure viability
  - Determine response to factors of varying concentrations

Acknowledgements and References

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1. NINDS. (2011, 8 December). National Institute of Neurological Disorders and Stroke – The Life and Death of a Neuron. Available: http://www.ninds.nih.gov/disorders/brain_basics/ninds_neuron.htm
2. University of California, San Francisco. (2011, 8 December). Biology of Neurodegeneration. Available: http://ind.ucsf.edu/causetocure/biology
3. R.S. Ashton, *et* al., "High Throughput Screening of Gene Function in Stem Cells Using Clonal Microarrays," *Stem Cells,* vol. 25, pp. 2928-2935, 2007.
4. G. M. Whitesides. "The origins and the future of microfluidics," *Nature*, vol. 442, no. 27, pp. 368-373, Jul. 2006.
5. G. Lupo, *et al.*, "Mechanisms of Ventral Patterning in the Vertebrate Nervous System," *Nat Rev Neurosci,* vol. 7, pp. 103-114, 2006.
6. N. L. Jeon, *et al.*, "Generation of solution and surface gradients using microfluidic systems," *Langmuir,* vol. 16, pp. 8311-8316, 2000.

Questions?