# Microfluidic Generation of Alginate Force Probes to Quantify Intra-tumoral Compression.

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Mentor: Dr. Joseph Chen, University of Louisville

NSF REU Award ID #2348869

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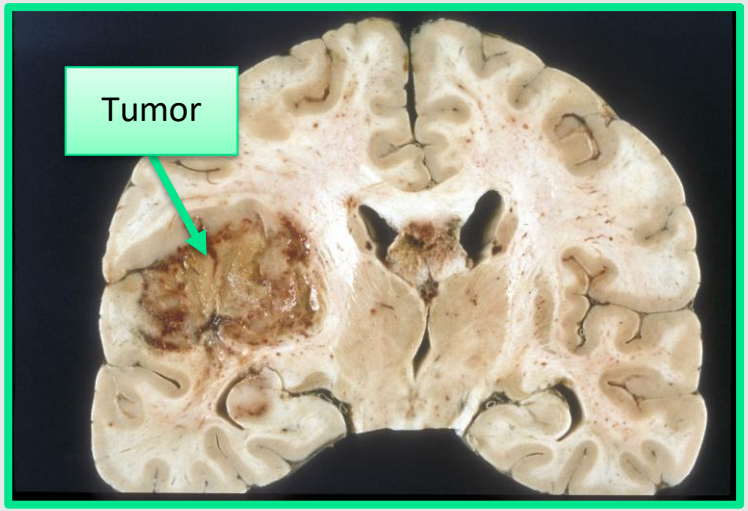
# Breaking Down the Project Title:

| | | |
|---|---|---|
| Microfluidics | →→→ | Fluid flow at a small scale for controlled, laminar flow. |
| Alginate Force Probes | →→→ | Biocompatible, deformable, measurable, cell-sized. |
| Intra-tumoral Compression | →→→ | The state of mechanical, compressive stress within a cancer cell. |

I am developing a material and method to quantify the state of stress within cancer cells.

# Glioblastoma

- A deadly type of brain cancer.
- It is the most common malignant tumor that affects the central nervous system.
- Its 5-year fatality rate is 93%.

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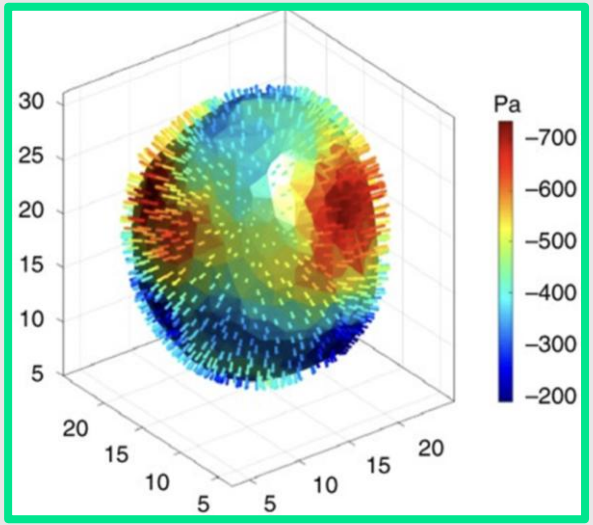
Quinn T. Ostrom, et al. 2019, CBTRUS Statistical Report.

# Intra-Tumoral Compression

- Under-developed research area compared to molecular and genetic structures of cancer.
- Evidence suggests a may cause a potential increase to tumor aggression.
- Difficulty: Unable to directly measure.




Compressive Stress

Nader, G. P. et al, 2021, Cell

# Objective

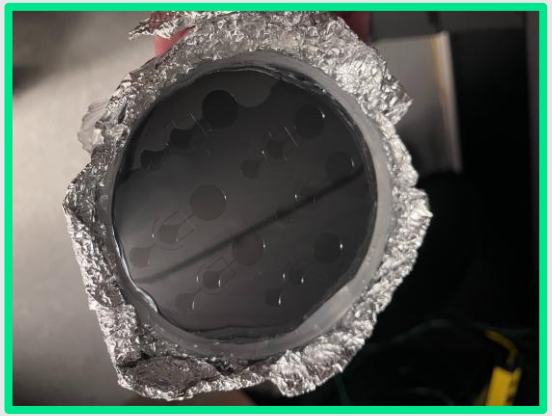
To quantify the compressive stress of Glioblastoma by placing cell-sized, measurable, deformable force probes inside of tumor spheres and then measuring their deformations over time.

# Experimental Approach

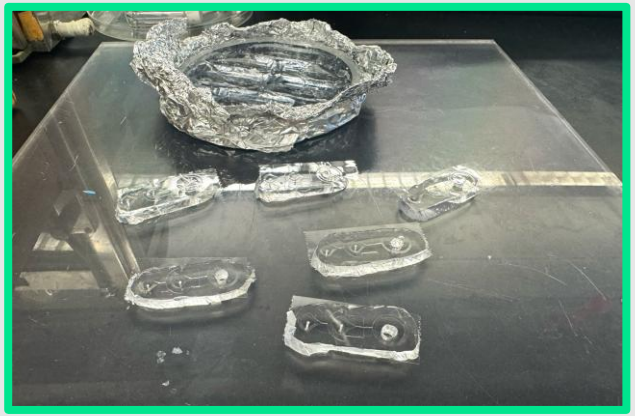
1. Make PDMS Microfluidic Devices
2. Make Alginate Force Probes (Beads)
3. Verify Bead Size and Fluorescence
4. Grow Bead-Embedded Tumor Spheres
5. Measure Bead Deformation

# Making PDMS Microfluidic Devices:

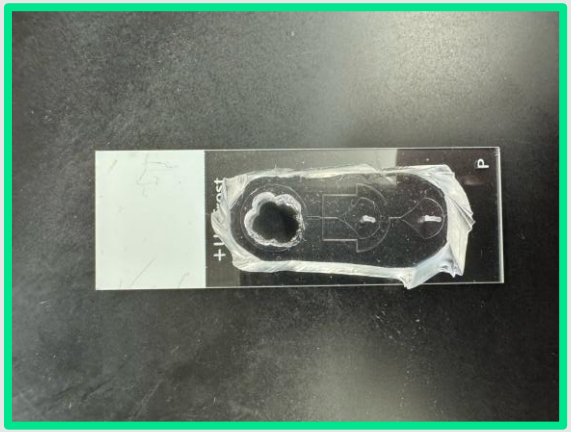
Pour PDMS on Silicon Wafer.

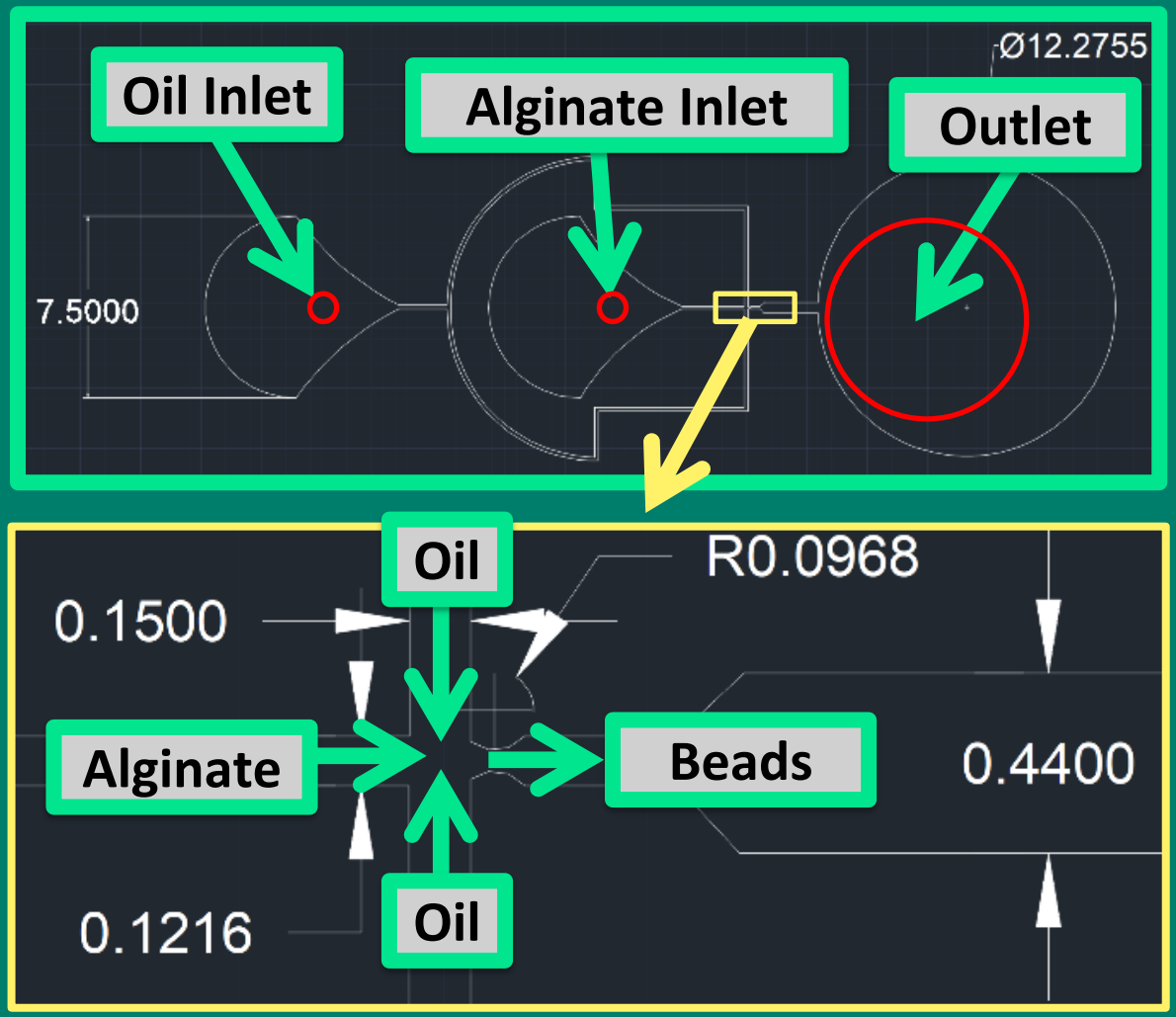


Cut Out Devices.



Bond Device to Glass Slide



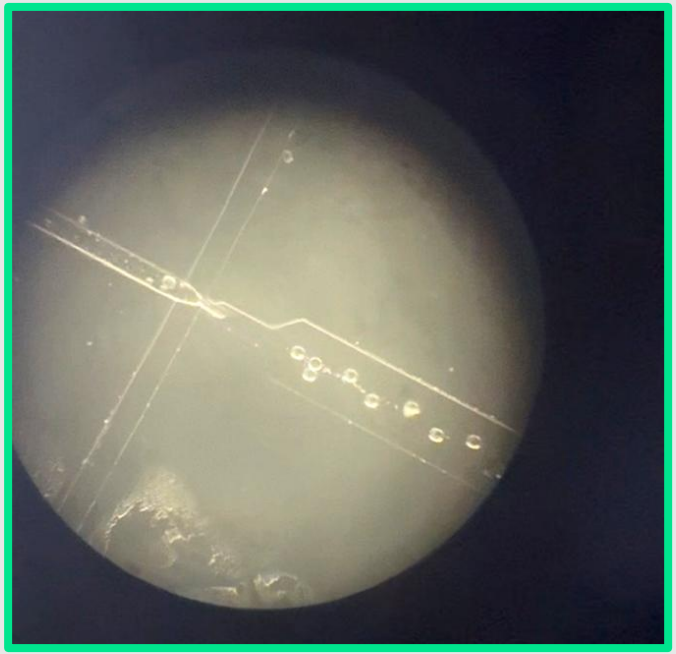

Ø12.2755
Oil Inlet
Alginate Inlet
Outlet
7.5000
Oil
R0.0968
0.1500
Alginate
Beads
0.4400
Oil
0.1216

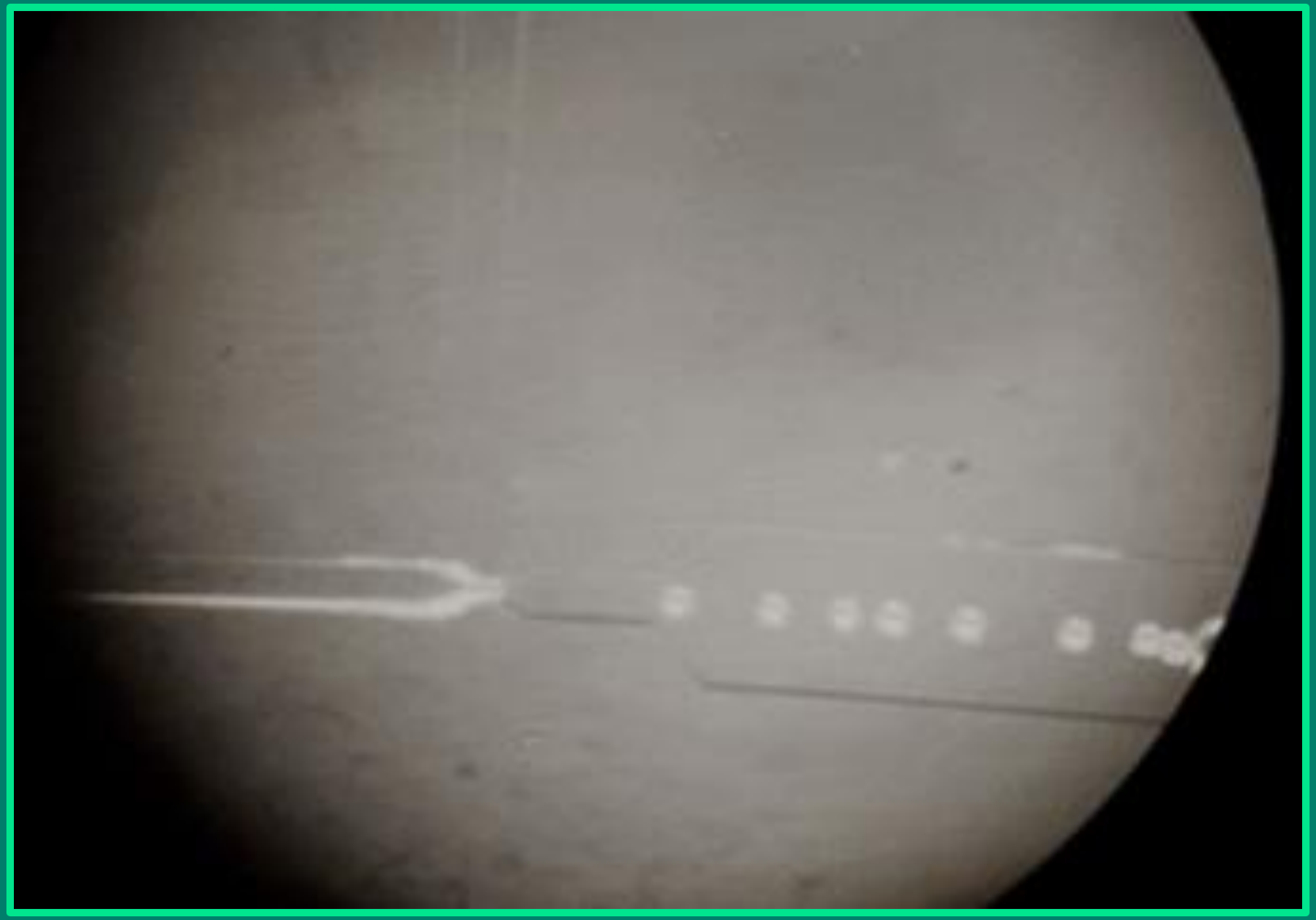
# Making Beads:

Connect Device to Pump

Set Device Under Microscope

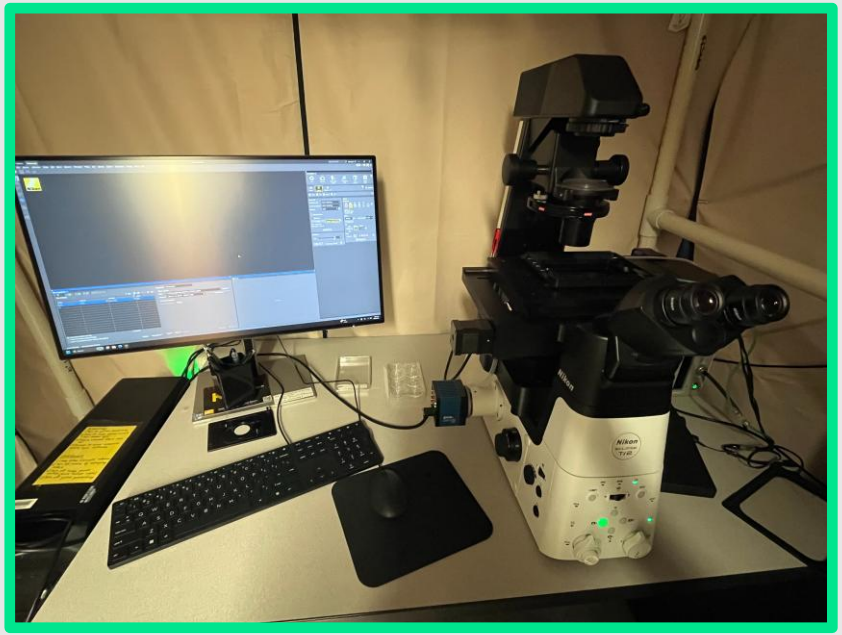

Pump Fluids and Begin Calibration



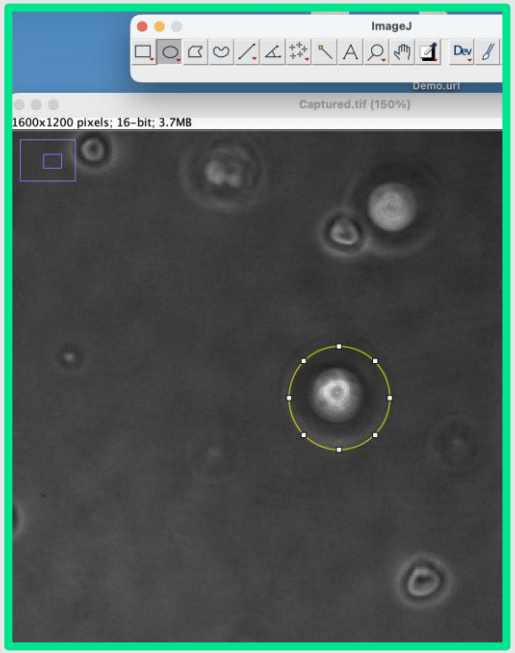


# Verify Bead Size and Fluorescence

Take Images with Nikon TI-2 and NIS Elements Software



Measure Diameter Using ImageJ Software



# Growing Bead-Embedded Tumor Spheres:

Tumor sphere: 3D spherical aggregation of cancer cells.
Homogeneous, lab-grown cancer cells, designed for biological testing.

The tumor spheres I will be growing are made with Glioblastoma cells.
Beads and cells will be placed together.

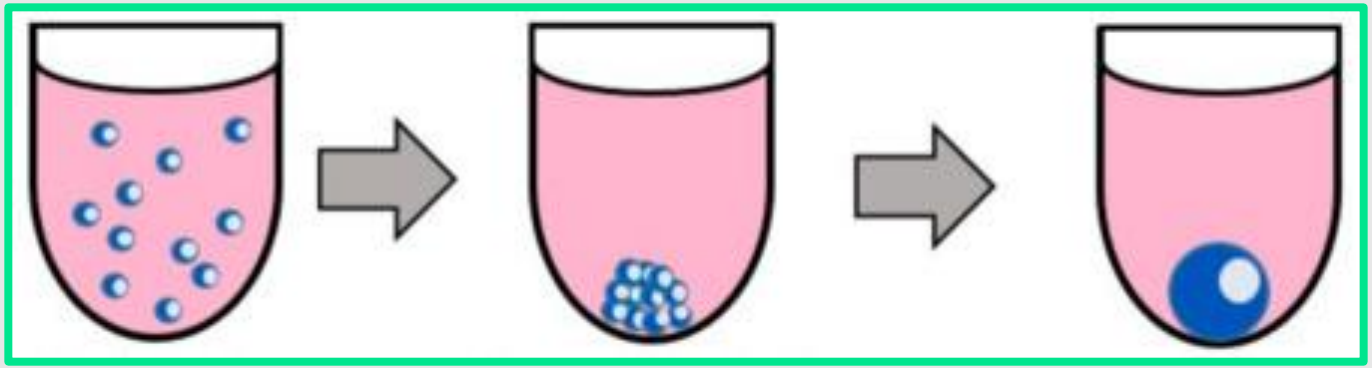


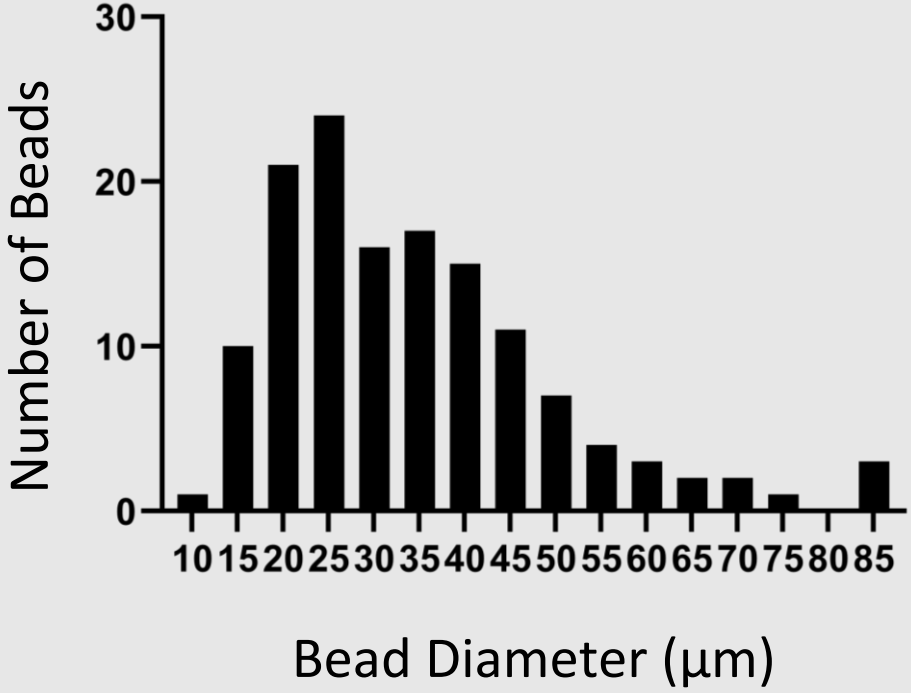
Diagram of cells collecting into a tumor sphere.

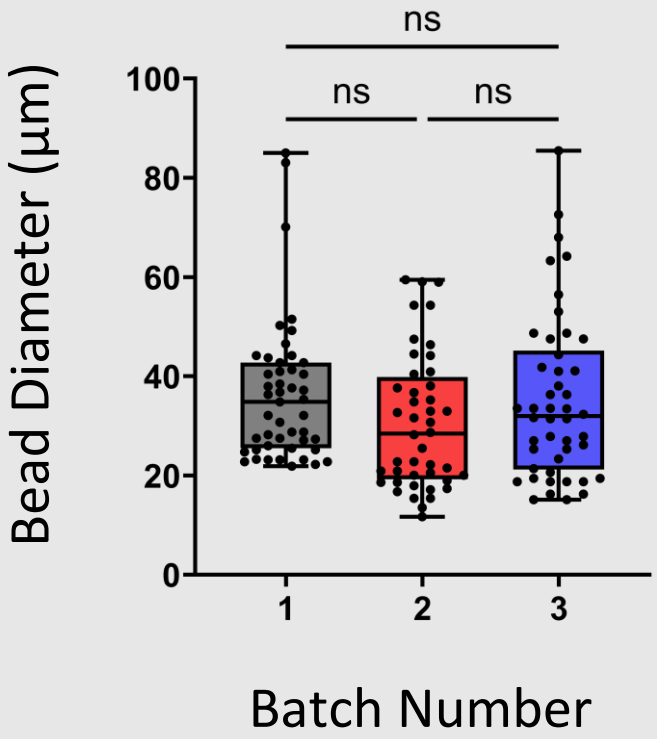
Bu, Nam-Ung, et al. 2020, MDPI.

# Results

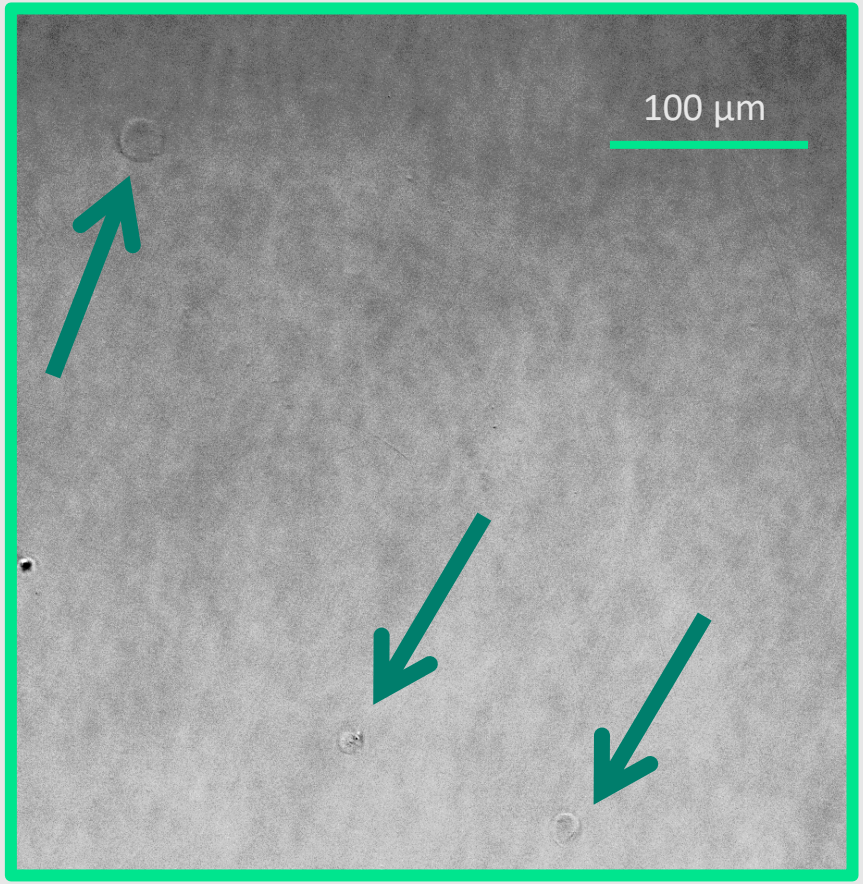
1. Validation of Cell-Sized Beads
2. Validation of Bead Fluorescence
3. Images of Bead-Embedded Tumor Spheres

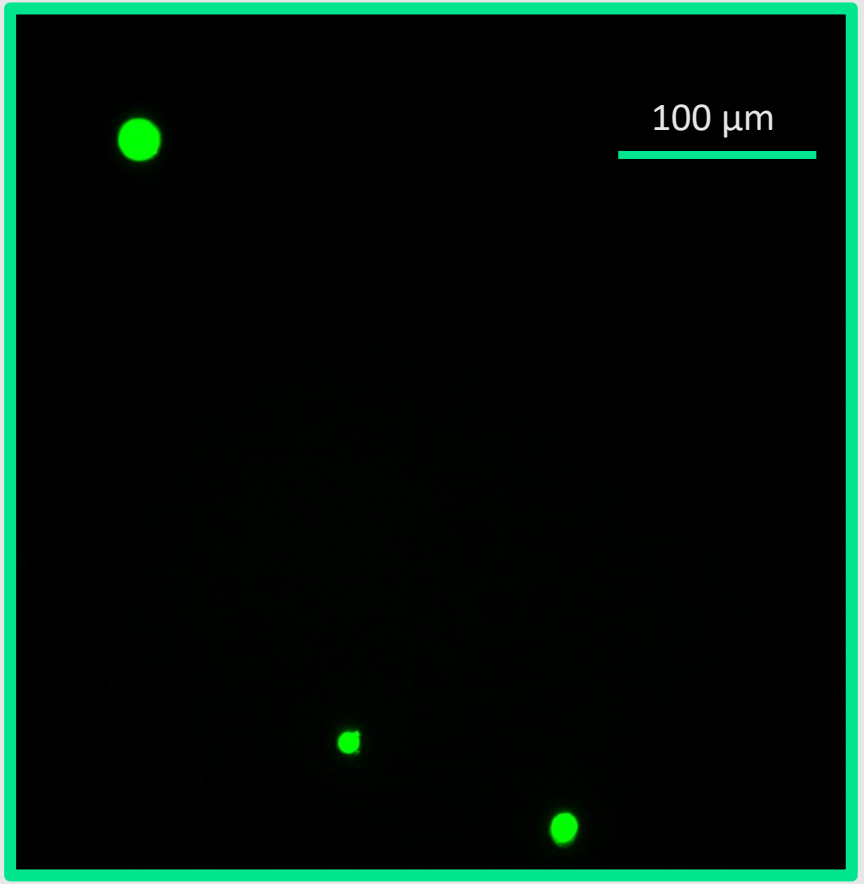
# Validation of Cell-Sized Beads



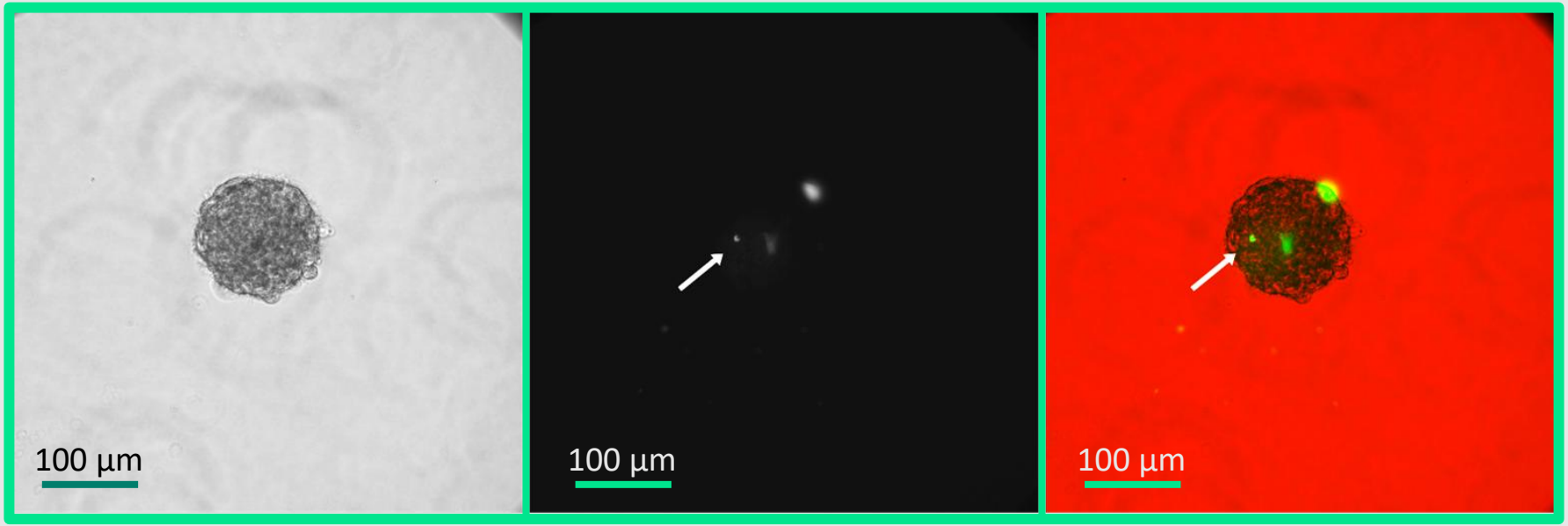



# Validation of Fluorescence



# Bead-Embedded Tumorspheres




Brightfield FITC Composite

# Conclusions and Future Work

- Successfully generated cell sized, deformable, measurable force probes.
- Successfully integrated force probes into glioblastoma tumor spheres.
- Sets up future work to calculate the compressive stress via finite element analysis of bead deformation.

# THANK YOU!

Presented by
Caleb Sutton

I gratefully acknowledge the support of my mentor, Dr. Joseph Chen; the previous leaders of this project, Zach Fowler and Bryce Thompson; and the rest of Dr. Chen's lab.

# ASK QUESTIONS!

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# Extra Slides!

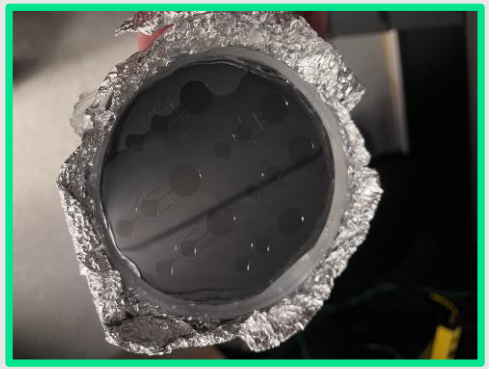
# Details on the Testing Fluids

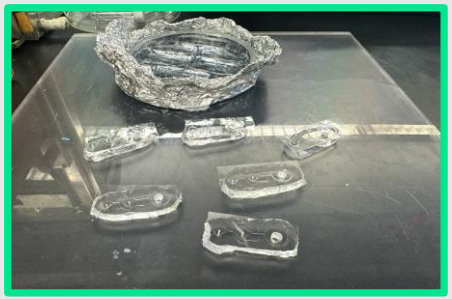
**5% Span-80 in Soybean Oil**
Soybean Oil: Common Cooking Ingredient
Why Soybean Oil? It's Hydrophobic
Span-80: Surfactant, Emulsifier
Why Span-80? Creates Barrier to Prevent Coalescence of Beads

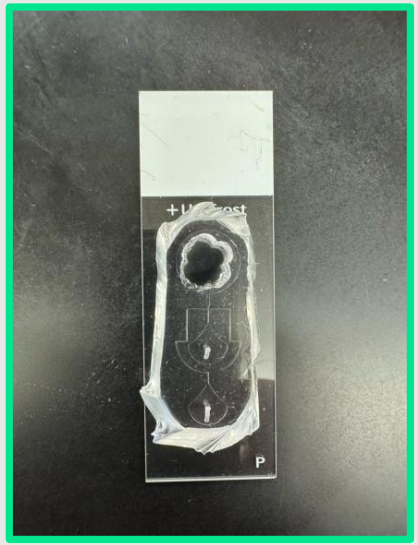
**1% Alginate in Water**
Sodium Alginate: Extracted from Brown Seaweed
Why Sodium Alginate? Highly Viscous (stable)
Water: Reverse Osmosis, Pure
Why Water? Reduce Viscosity. Allows Hydrophobic Interaction with Oil

**1% Calcium Chloride in Water**
Calcium Chloride: Ionic Salt
Why Calcium Chloride? Bonds with Sodium, Polymerizes Beads

# Making PDMS Microfluidic Devices: (Detailed Steps)







Pour 10:1 ratio SylGard 184 Base to Curing Agent, 45-50 grams total.

Wrap a photolithography-patterned silicon wafer (mask) in aluminum foil.

Pour PDMS onto the mask.

Vacuum all air bubbles out.

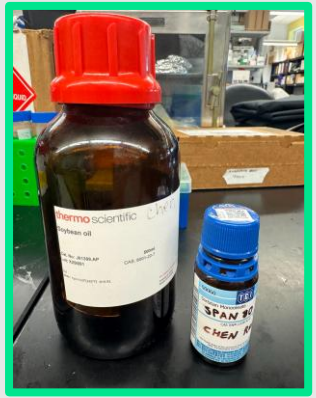
Bake at 70° C for 12+ hours.

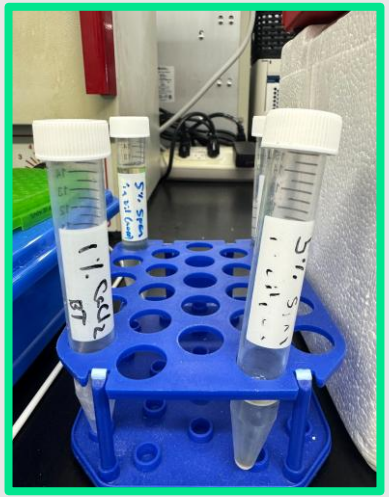
Cut out the devices and punch out the inlets and outlet.

Plasma treat devices to glass slides.

Bake at 70° C for 1+ hour.

# Making the Testing Fluids

3 Fluids:
Oil, Alginate, Calcium Chloride

Be Aware of Concentration and Total Amount:

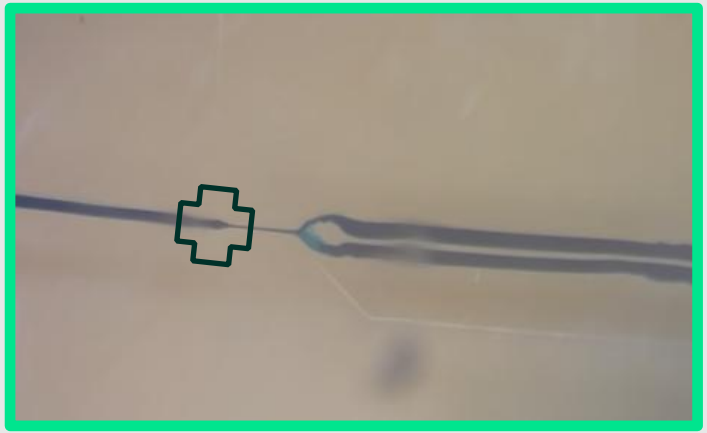
Use a Pipette to Measure Amounts (Small, Accurate Measurements)

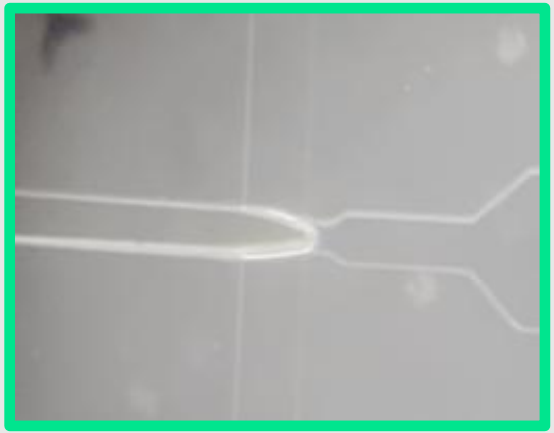
Place into 15 mL Conical and Mix Well

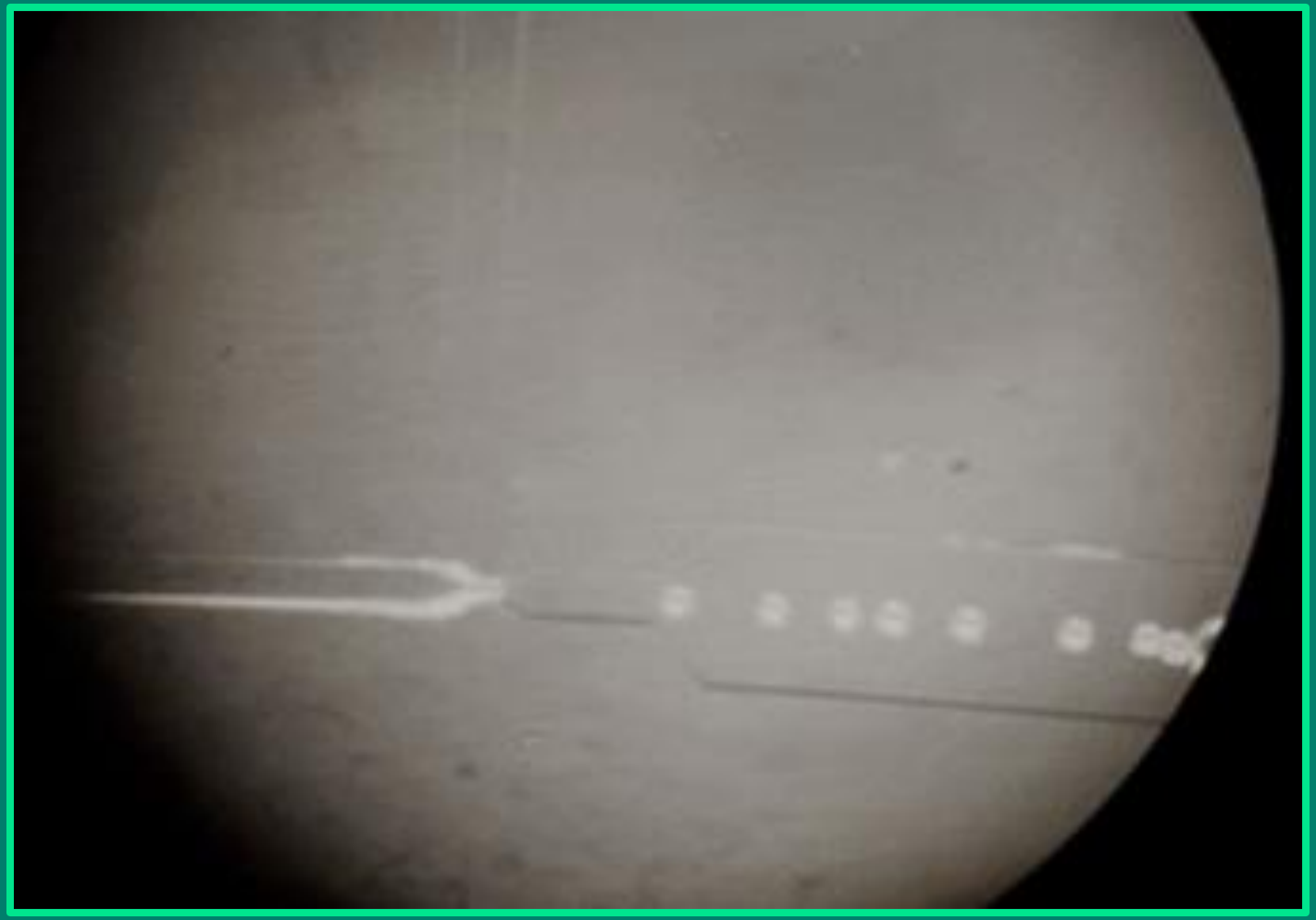
# Details on Bead Generation

2 Main Variables:  Alginate Flow Rate and Oil Flow Rate.
Too much or too little of either results in no beads.
The ratio of flow rates is **crucial** to bead generation.

Other variables include:  Device, device state, syringe, tubing, etc.







# Collecting Beads

Place CaCl Solution in Outlet

Collect Beads suspended in CaCl solution from Outlet

Collect 1 mL Sample per Device