



2018 SPRING BIOE 263L FINAL PRESENTATION

A single cell screening technique via a modified mancini assay to quantify monoclonal antibody production of hybridoma cells

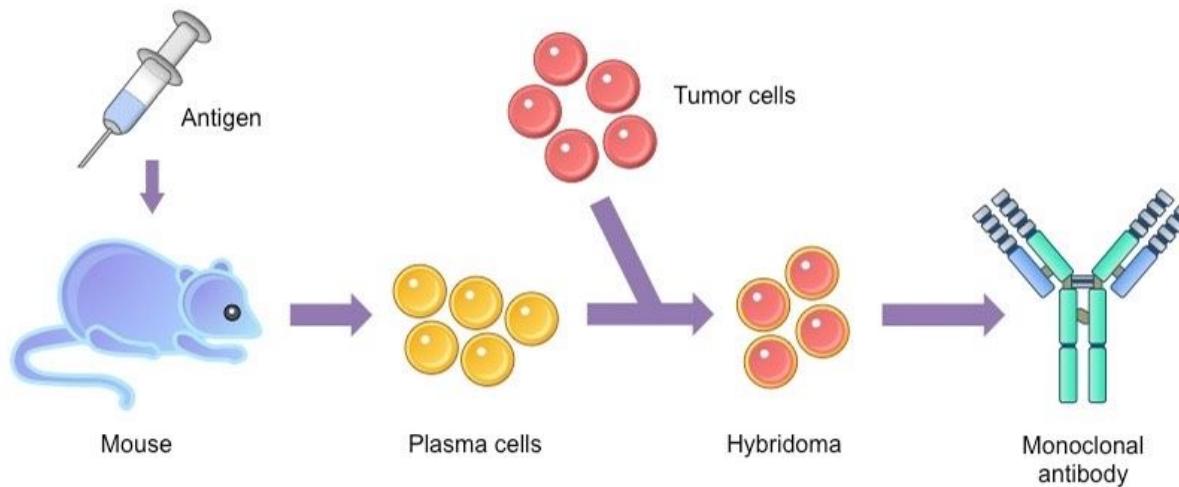
Kevin Qi, Ting-Wei Wu, Nicole Sugiono 2018.05.02

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1. Introduction
2. Experiment Design
3. Approach
4. Results
5. Conclusion and Future Work

Introduction

- Hybridoma cells are antibody producing factories



Introduction

- Population level screening
 - Optimizes the average
 - Costly
- Single cell level screening
 - Selects the best
 - Faster and cheaper

Introduction

- Why (or why not) mancini?
 - Cheap and accessible
 - High sensitivity and selectivity
 - Not infinitely scalable

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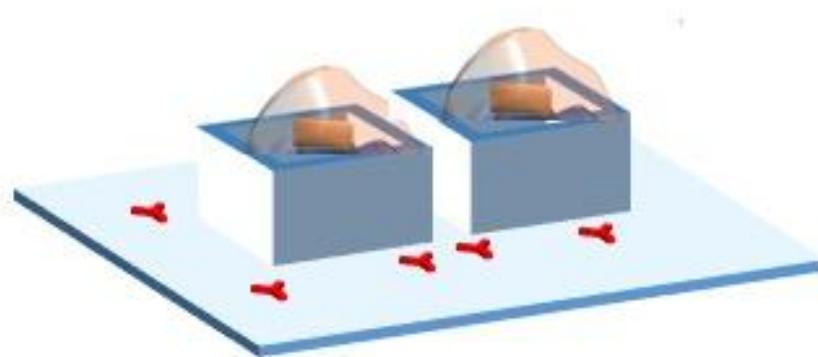
1. Introduction
2. **Experiment Design**
3. Approach
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Experiment Design

- **Objective:** To extend the mancini assay to the single cell level for quantifying antibody production in hybridoma cells.
- **Aims:**
 1. Single cell trapping and isolation
 2. Immunoprecipitate from a single hybridoma cell
 3. Quantification of antibody production

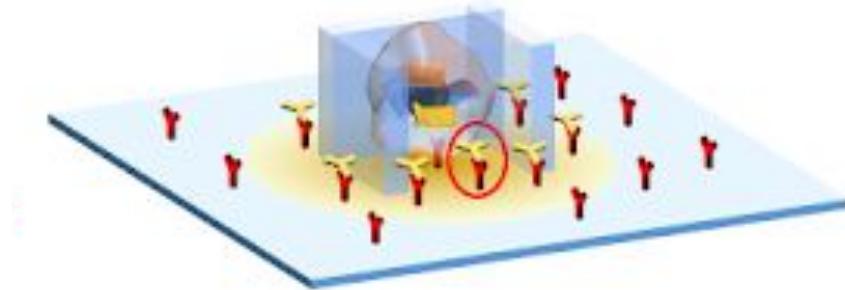
Aim 1: Single Cell Trapping and Isolation

- Trapping
 - Cells can't move around during immunodiffusion
- Isolation
 - Differentiate IP formations from different cells



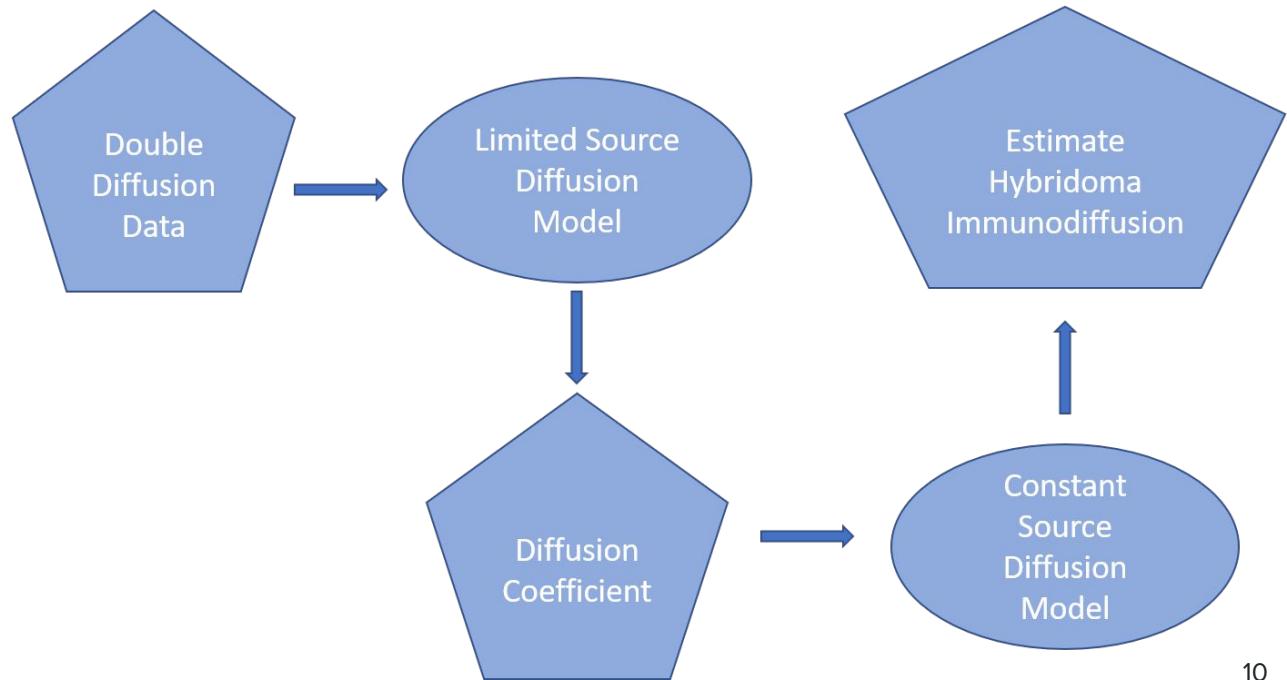
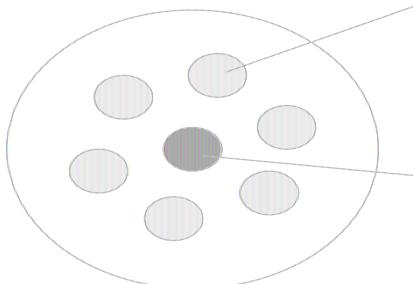
Aim 2: Immunoprecipitate from a single cell

2. Mancini assay using single isolated hybridoma cell in device
 - a. Use confocal microscopy to measure IP directly beneath each cell



Aim 3: Quantification

1. Develop Diffusion Model



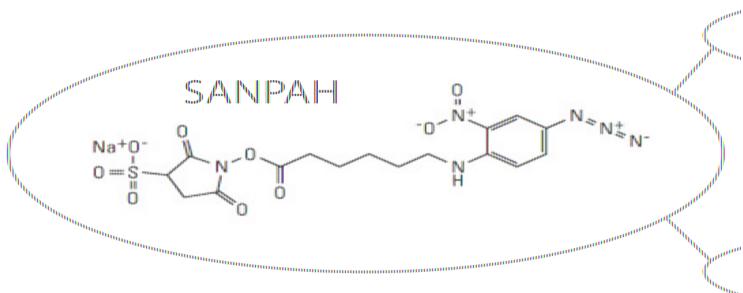
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Chemical

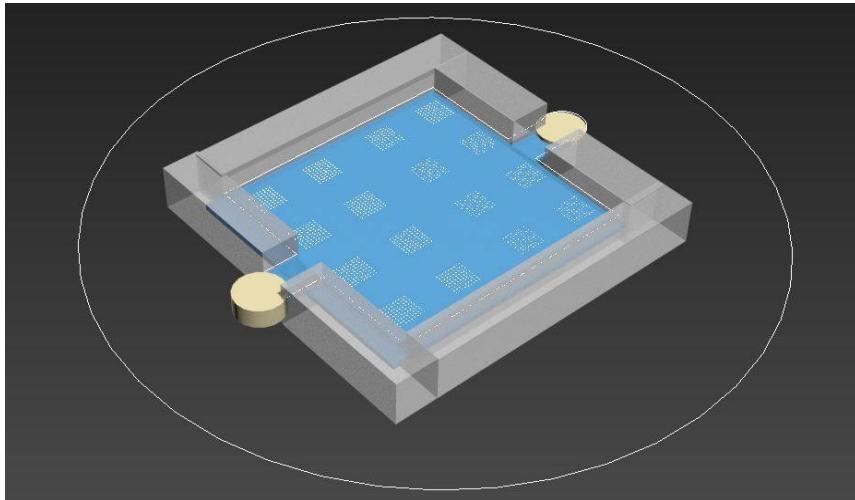
A single cell trapping modified mancini array

- Chemical approach using sulfo-SANPAH and Collagen
 - SANPAH: Optically activated crosslinker
 - Use photolithography to pattern SANPAH/collagen

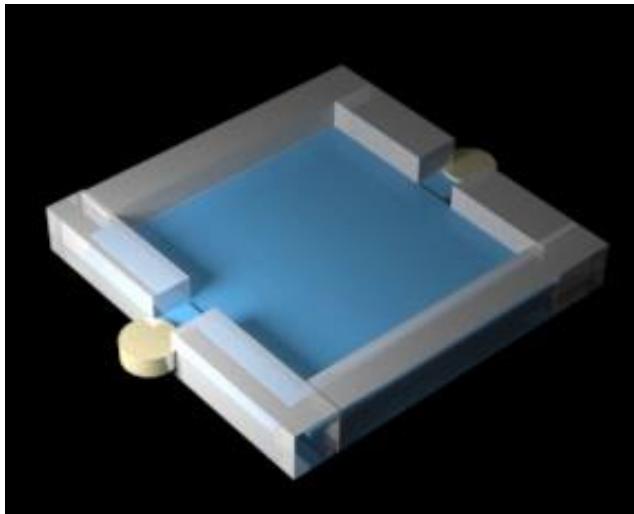


Mechanical

A single cell trapping modified mancini array



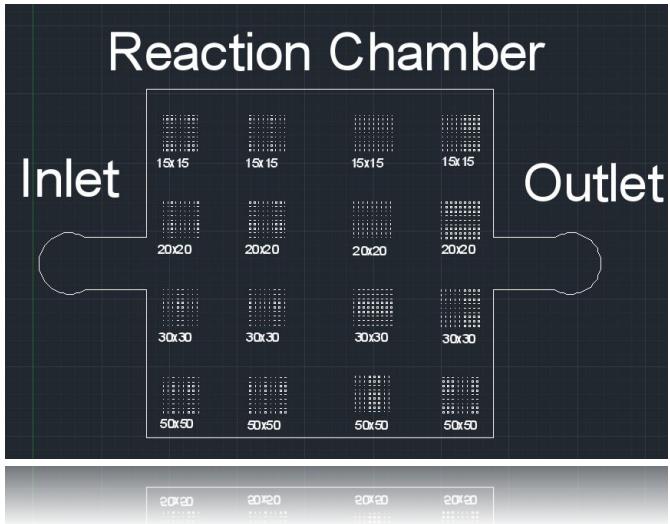
- The top view of designed device with illustrated microwells inside of reaction chamber.



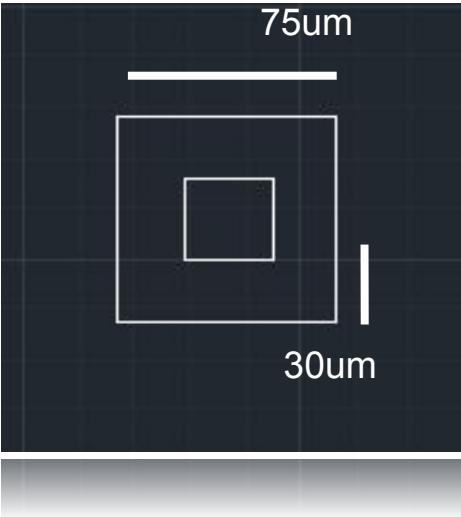
- The simulated device diagram with filled hybridoma cell line suspension.

Mechanical

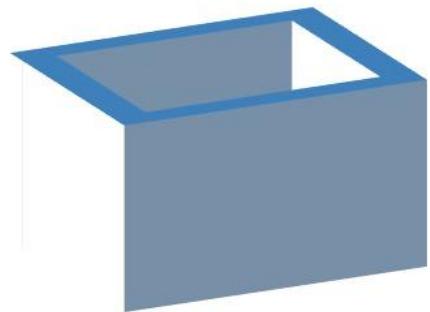
A single cell trapping modified mancini array



- Autocad schematic of device

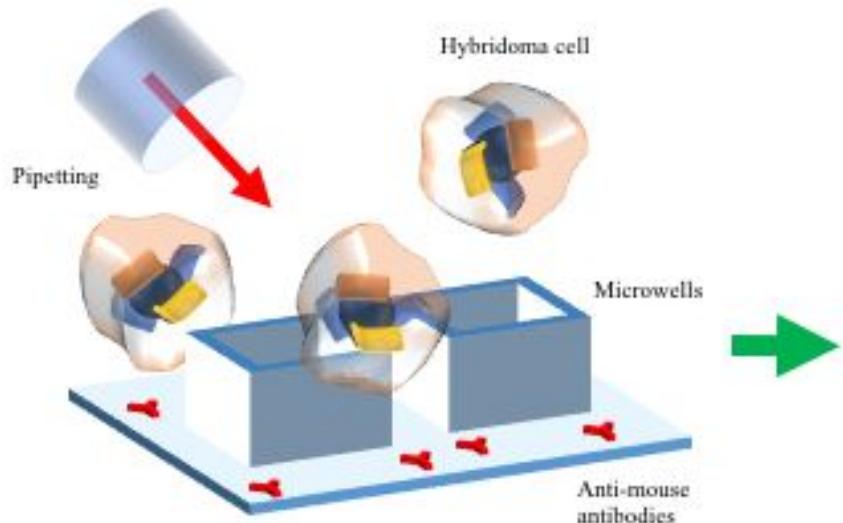


- Single cell trapping site

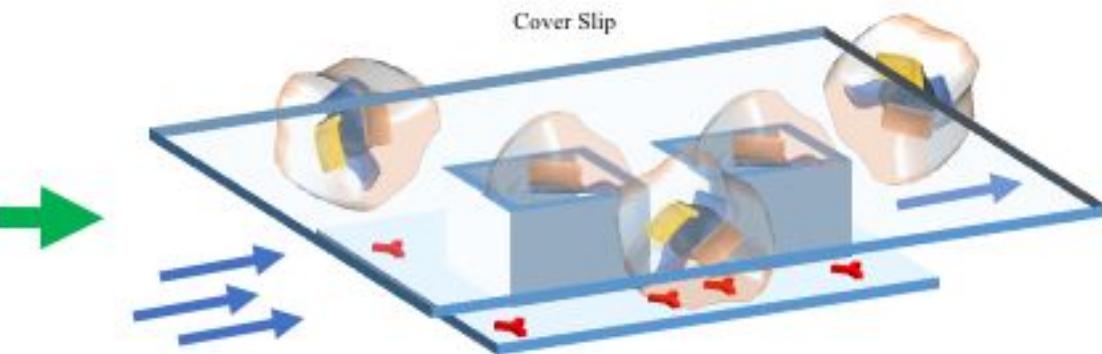


- Microwell

Working Mechanism

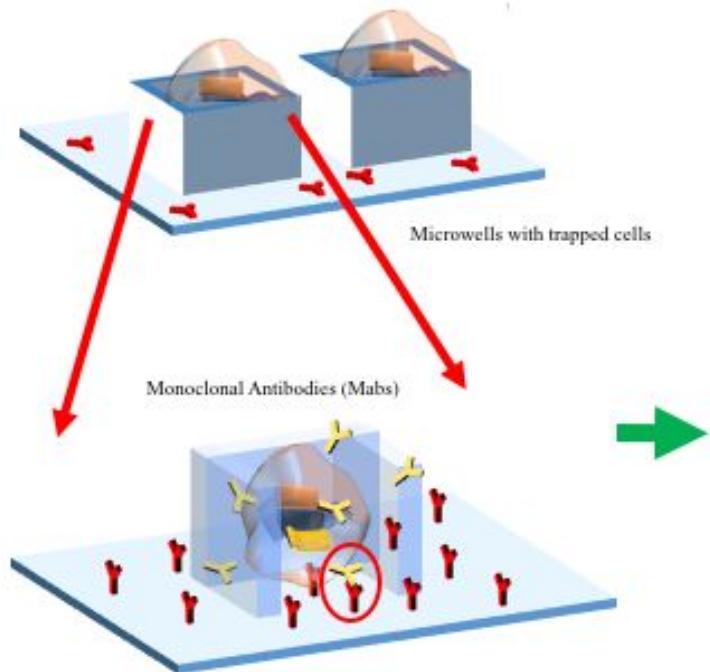


(a) Hybridoma cell line suspension would be pipetted on the patterned agarose pad.

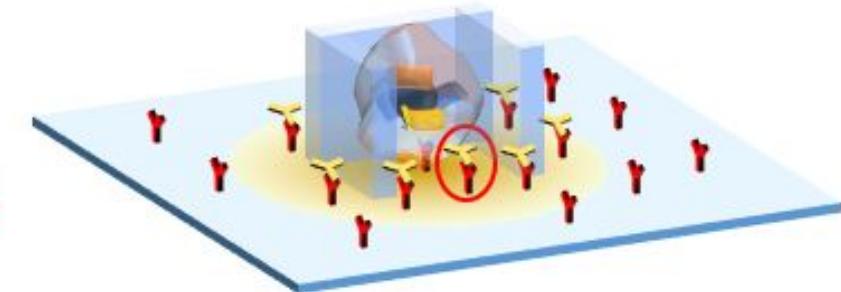


(b) A coverslip would be placed on the agarose pad for bonding on the surface of agarose wells, which traps each cell. Unisolated cells would be washed away then.

Working Mechanism



(c) For a single cell trapping site, MAbs produced by the hybridoma cell would form immunoprecipitate with anti-mouse antibodies.

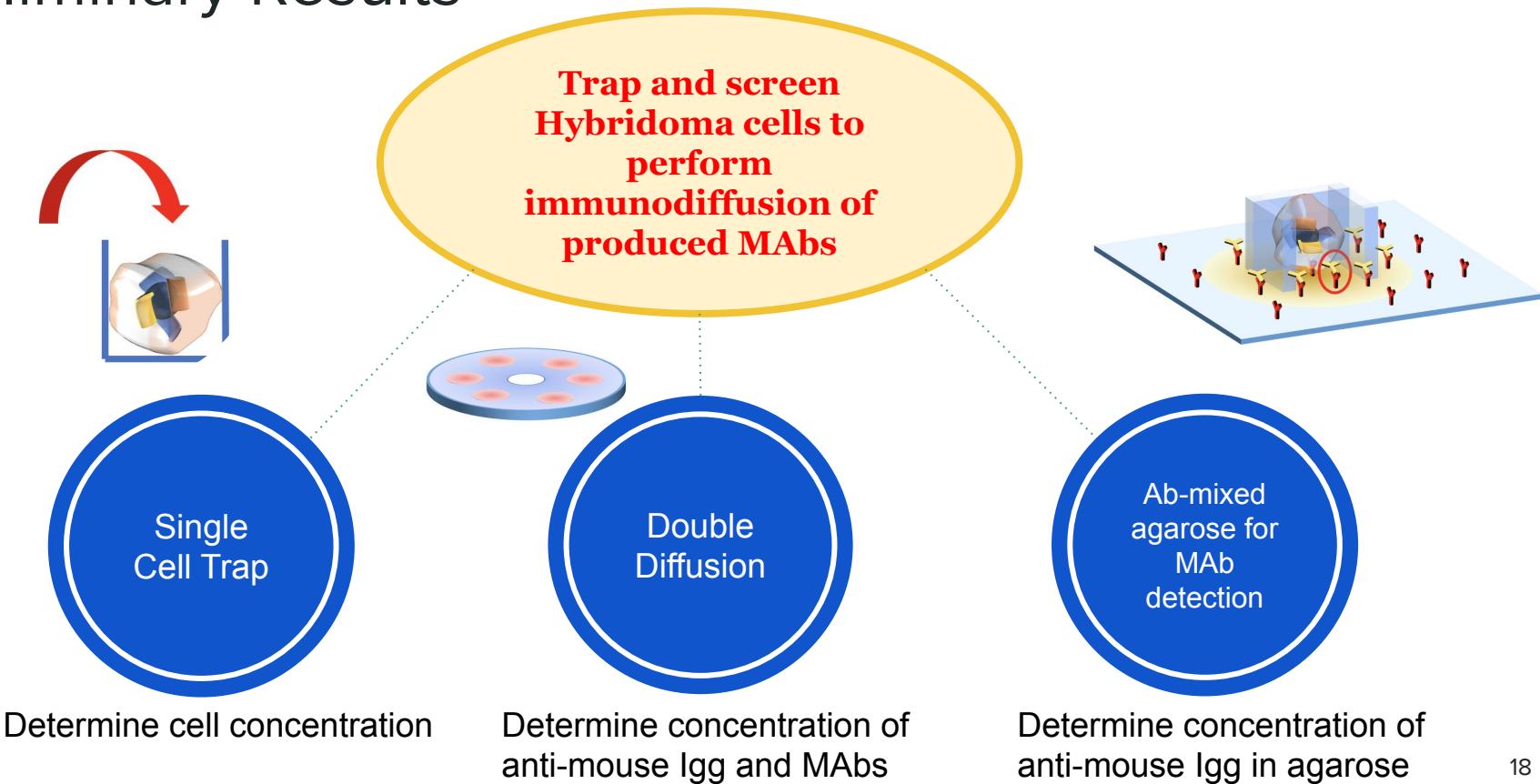


(d) A large crosslink network between MAbs and anti-mouse antibodies would form a zone of equivalence by immunodiffusion to the surrounding area.

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Preliminary Results



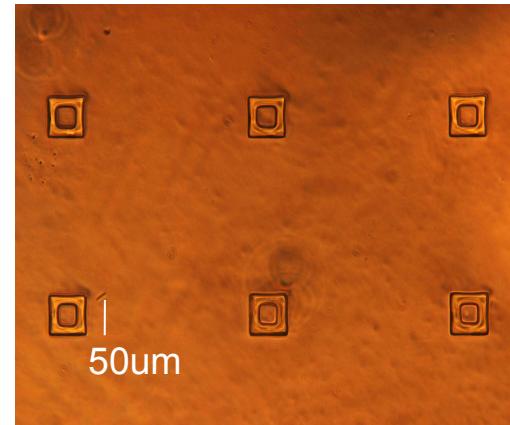
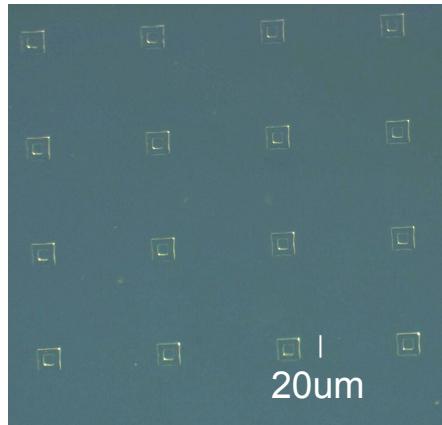
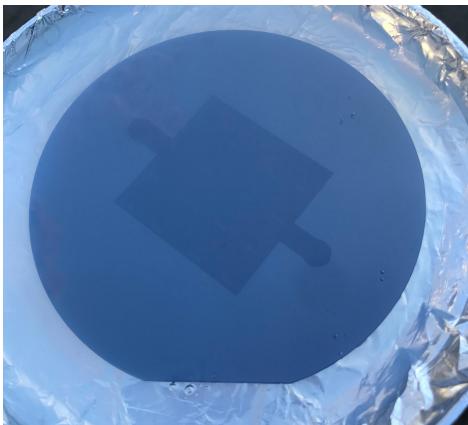
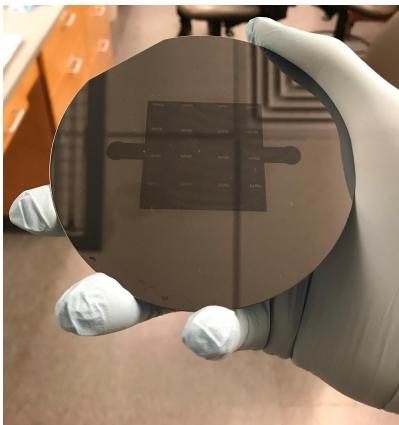
Determine cell concentration

Determine concentration of
anti-mouse IgG and MAbs

Determine concentration of
anti-mouse IgG in agarose

Preliminary Results 1

Real Fabricated Device Imaging



Fabricated Silicon Mold

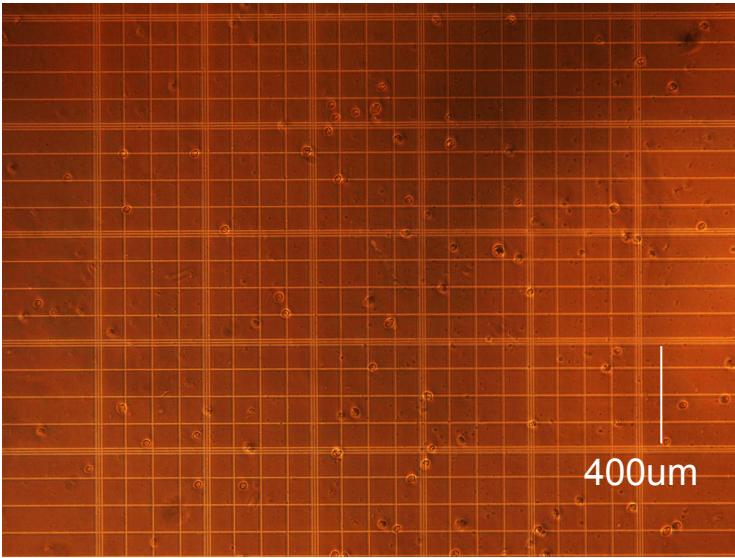
Patterned agarose pad

20x20 μm microwells

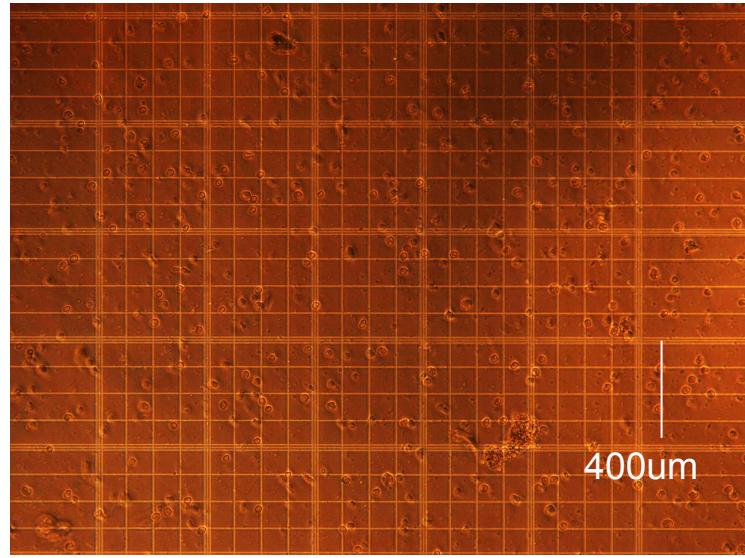
50x50 μm microwells

Preliminary Results 1

Hybridoma Cell Suspension Imaging



Hybridoma cell suspension with the concentration
 $\sim 85 \text{ cells}/400\text{mm}^2 = 0.2125 \text{ cell}/\mu\text{l}$

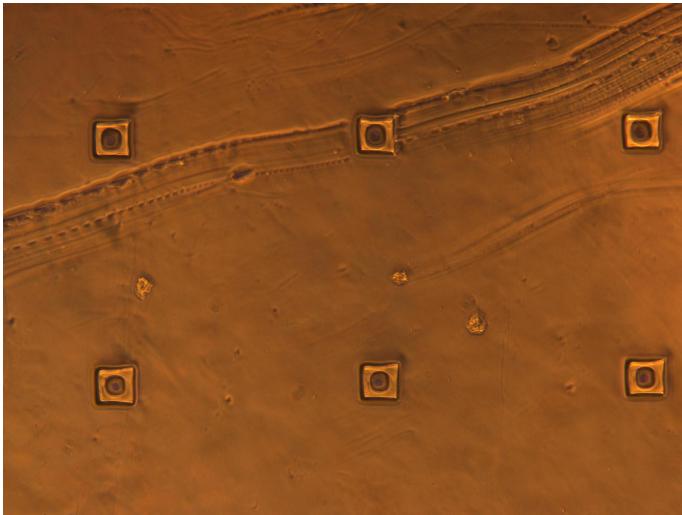


Hybridoma cell suspension with the concentration
 $\sim 330 \text{ cells}/400\text{mm}^2 = 0.825 \text{ cell}/\mu\text{l}$

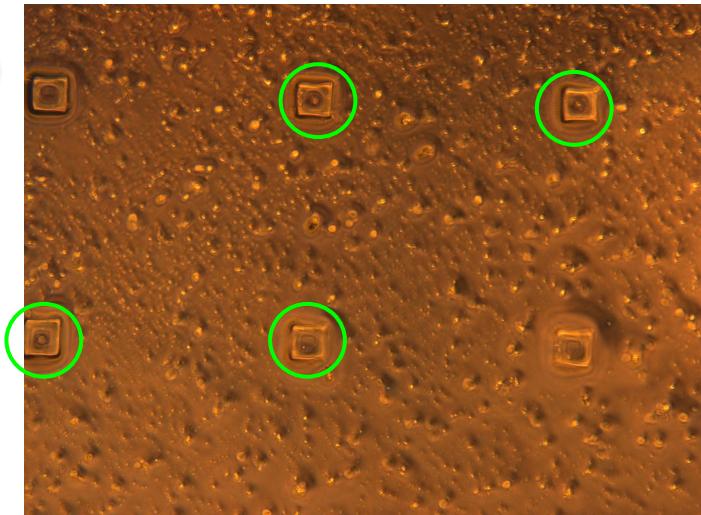
Preliminary Results 1

Single Cell Trapping Performance (0.825 cell/uL)

Before introducing cells



After introducing cells (4/6 trapping efficiency)



Fabricating agarose pad with defined trapping sites and pouring hybridoma cell suspension to discover trapping efficiency and wash out untrapped cells



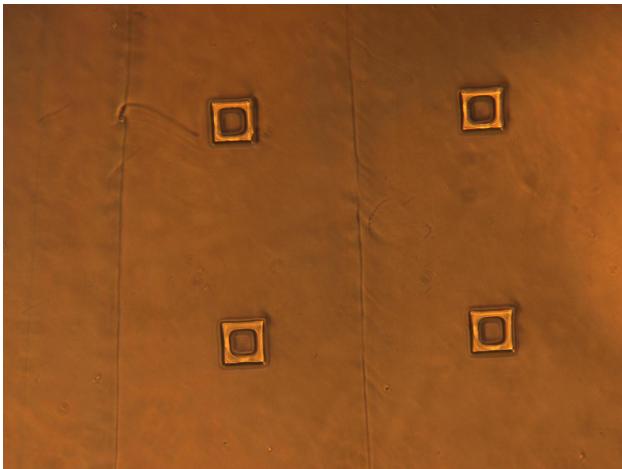
A microscopy image showing a dense population of cells on a slide. Several cells are highlighted with square selection boxes. One cell in the upper right quadrant is circled with a green line.

After wash

Preliminary Results 1

MDA-MB231 Breast Cancer Cell Trapping Performance (0.825 cell/ul)

Before introducing cells

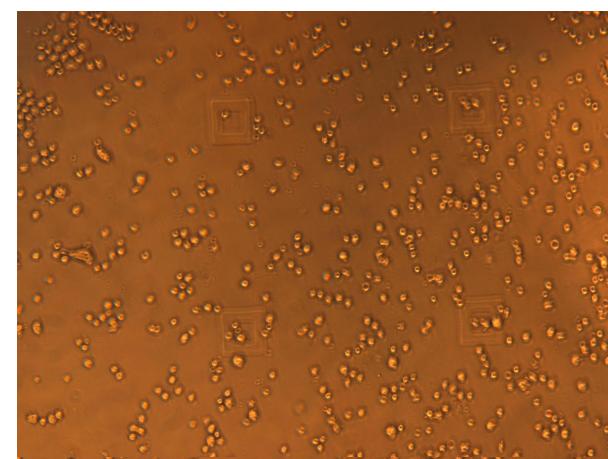


3

4



After introducing cells (3 cells per well)

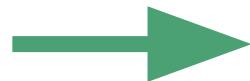
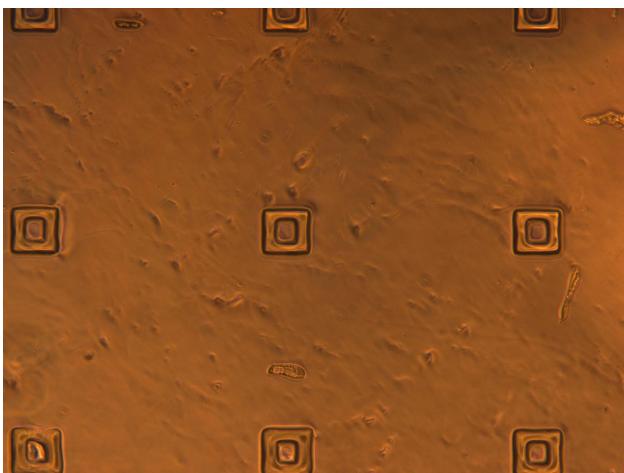


Fabricating agarose pad with defined trapping sites and pouring cancer cell suspension to discover trapping efficiency and wash out untrapped cells

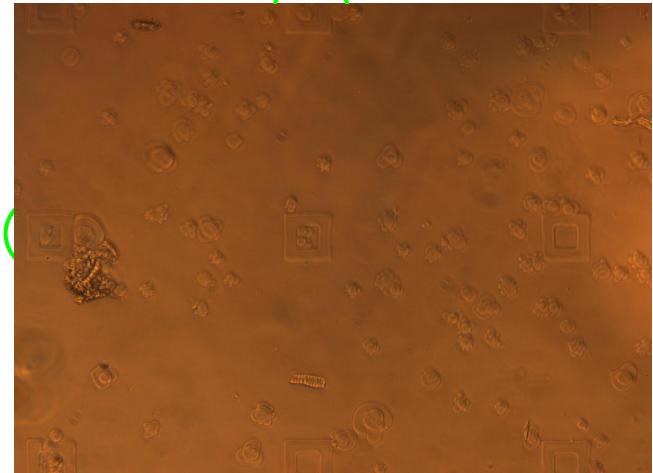
Preliminary Results 1

MDA-MB231 Breast Cancer Cell Trapping Performance (0.825 cell/ul)

Before putting coverslip



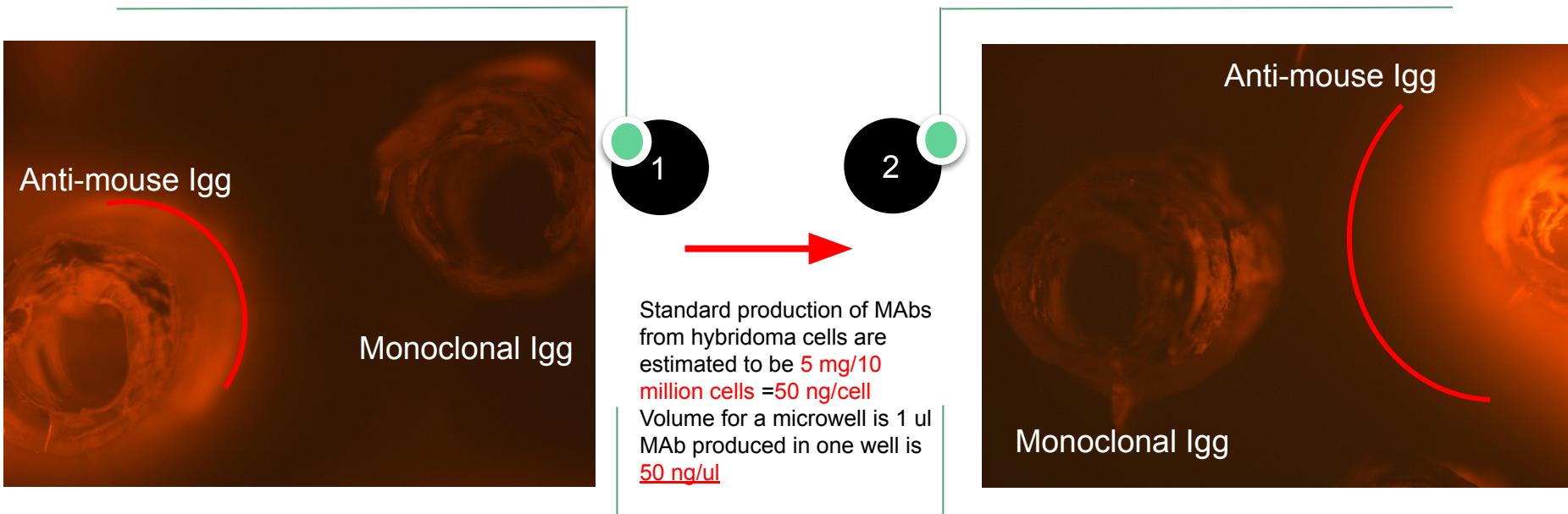
After putting coverslip



Fabricating agarose pad with defined trapping sites and pouring cancer cell suspension to discover trapping efficiency and wash out untrapped cells

Preliminary Results 2

Double Diffusion across Troponin I mouse monoclonal IgG and Rhodamine anti-mouse IgG



Anti-mouse IgG: 10-fold dilution of 2mg/ml: **200 ug/ml**
Monoclonal IgG: 10-fold dilution of 200ug/ml: **20 ug/ml**

Anti-mouse IgG: 10-fold dilution of 2mg/ml: **200 ug/ml**
Monoclonal IgG: 400-fold dilution of 200ug/ml:
0.5 ug/ml = 50 ng/ul

Preliminary Results 2

1. Higher concentration for anti-mouse Igg

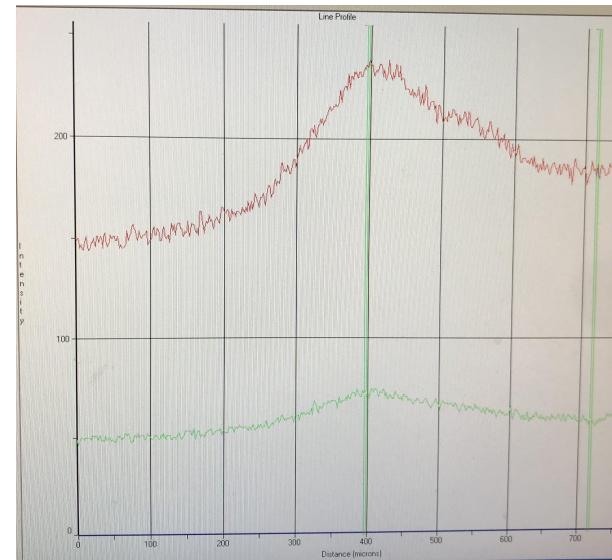
Anti-mouse Igg: 10-fold dilution of 2mg/ml: **200 ug/ml**

Monoclonal Igg: 400-fold dilution of 200ug/ml:

$$0.5 \text{ ug/ml} = \underline{50 \text{ ng/ul}}$$

Anti-mouse Igg

Monoclonal Igg



1. Distance for MAbs:
296.85 um

2. Distance for
Anti-mouse Igg:
320.26 um

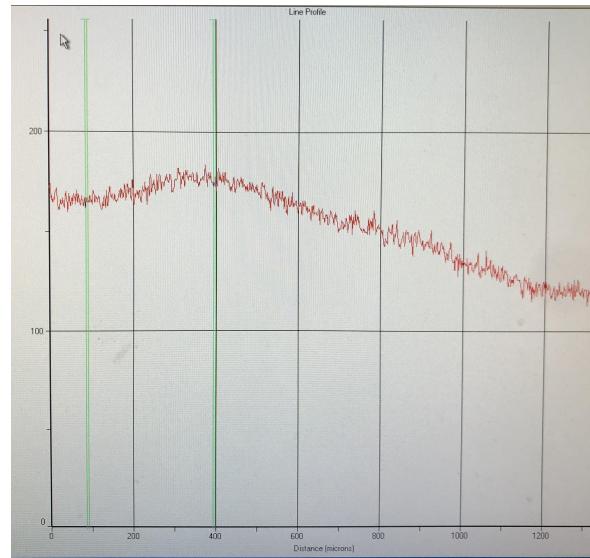
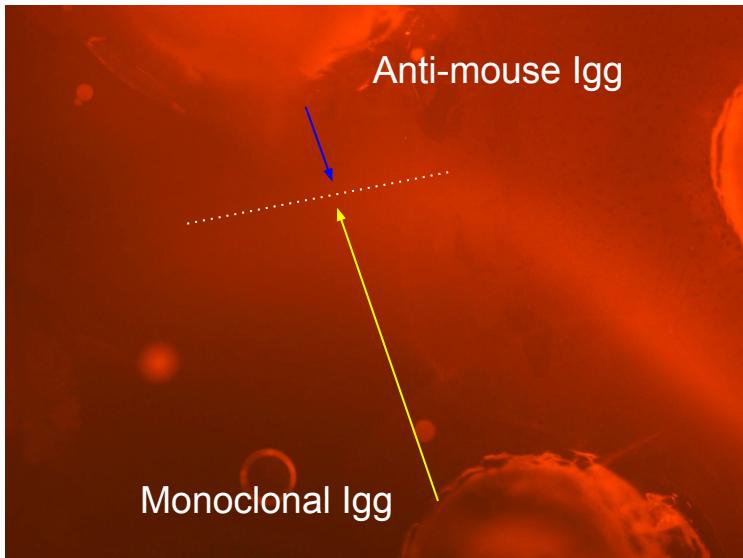
Preliminary Results 2

2. Lower concentration for anti-mouse Igg

Anti-mouse Igg: 10000-fold dilution of 2mg/ml: **0.2 ug/ml**

Monoclonal Igg: 400-fold dilution of 200ug/ml:

$$0.5 \text{ ug/ml} = \underline{50 \text{ ng/ul}}$$



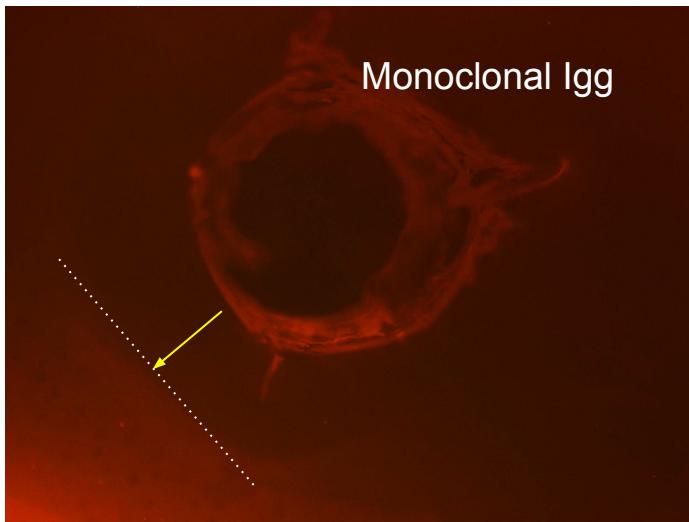
1. Distance for MAbs:
803.3 um

2. Distance for
Anti-mouse Igg:
258.18 um

Preliminary Results 3

IP formation of Monoclonal Igg (400-fold) on anti-mouse Igg-mixed agarose pad

Anti-mouse Igg: 400-fold dilution



Distance for MAbs: 336.56 um



Anti-mouse Igg: 10-fold dilution



Distance for MAbs: 0 um?

Results

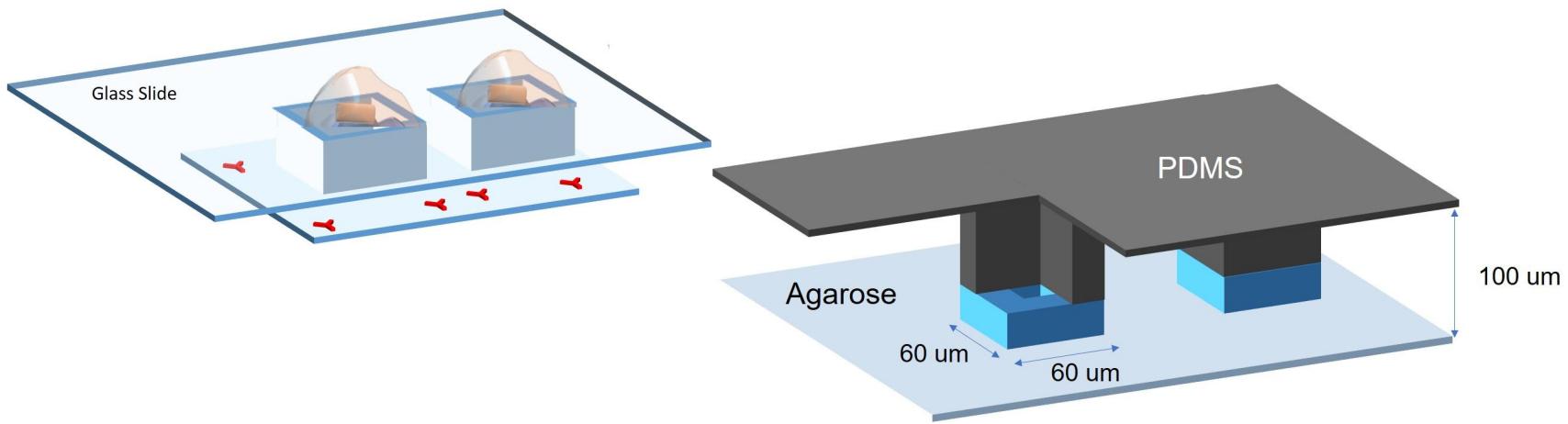
1. Cell concentration: 330 cells/400mm² with **66%** capture efficiency
2. Mouse MAbs produced by hybridoma cells: 5x(10⁻⁷) mg/ml, which is 400-fold for our ready-use Troponin I mouse MAbs (200 ug/ml).
3. Anti-mouse IgG: 10-fold dilution of 2mg/ml to expect zone of equivalence around 300 um away from each cell trapping location.
4. For final device, we should mix 400-fold dilution of 2mg/ml anti-mouse IgG to expect zone of equivalence around 340 um away from each cell trapping location.

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2. Aims and Significance
3. Approach
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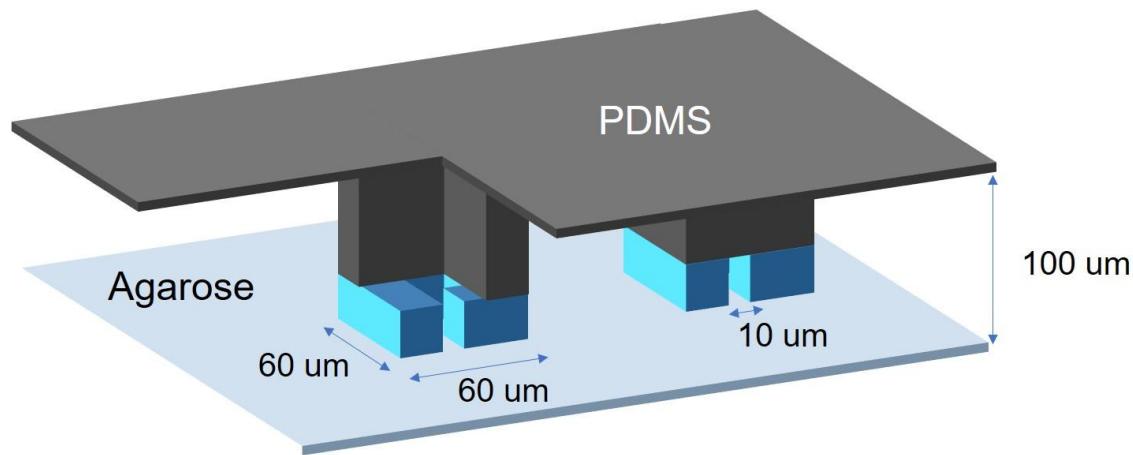
Future Work

1. Microfluidic channels with patterned PDMS



Future Work

2. Supply trapped cell with nutrients from fluid flow



Future Work

- Agarose surface treatment to prevent hybridoma adherence.
- Sterilization of device
- Immunodiffusion with live hybridoma cells
- Calibration curve between IP line distance and antibody production

References

- (1) Priest, D.G., Tanaka, N., Tanaka, Y., Taniguchi, Y. Micro-patterned agarose gel devices for single-cell high-throughput microscopy of *E. coli* cells. *Scientific Reports*. 7: 17750 (2017)
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- (3) C. Piggee, "Therapeutic antibodies coming through the pipeline," *Anal. Chem.*, 80: 2305-2310, 2008.
- (4) S. Lawrence, "Pipelines turn to biotech," *Nat. Biotechnol.*, 25: 1342, 2007.
- (5) M. Baker, "Upping the ante on antibodies," *Nat. Biotechnol.*, 23: 1065-1072, 2005.
- (6) S. K. Dessain, S. P. Adekar, J. B. Stevens, K. A. Carpenter, L. M. Skorski, B. L. Barnoski, R. A. Goldsby and R. A. Weinberg, "High efficiency creation of human monoclonal antibody-producing hybridomas," *J. Immunol. Meth.*, 291: 109-122, 2004.
- (7) Y. Tokimitsu, H. Kishi, S. Kondo, R. Honda, K. Tajiri, K. Motoki, T. Ozawa, S. Kadokami, T. Obata, S. Fujiki, C. Tateno, H. Takaishi, K. Chayama, K. Yoshizato, E. Tamiya, T. Sugiyama and A. Muraguchi, "Single lymphocyte analysis with a microwell array chip," *Cytometry A*, 71A: 1003-1010, 2007

Acknowledgments:

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