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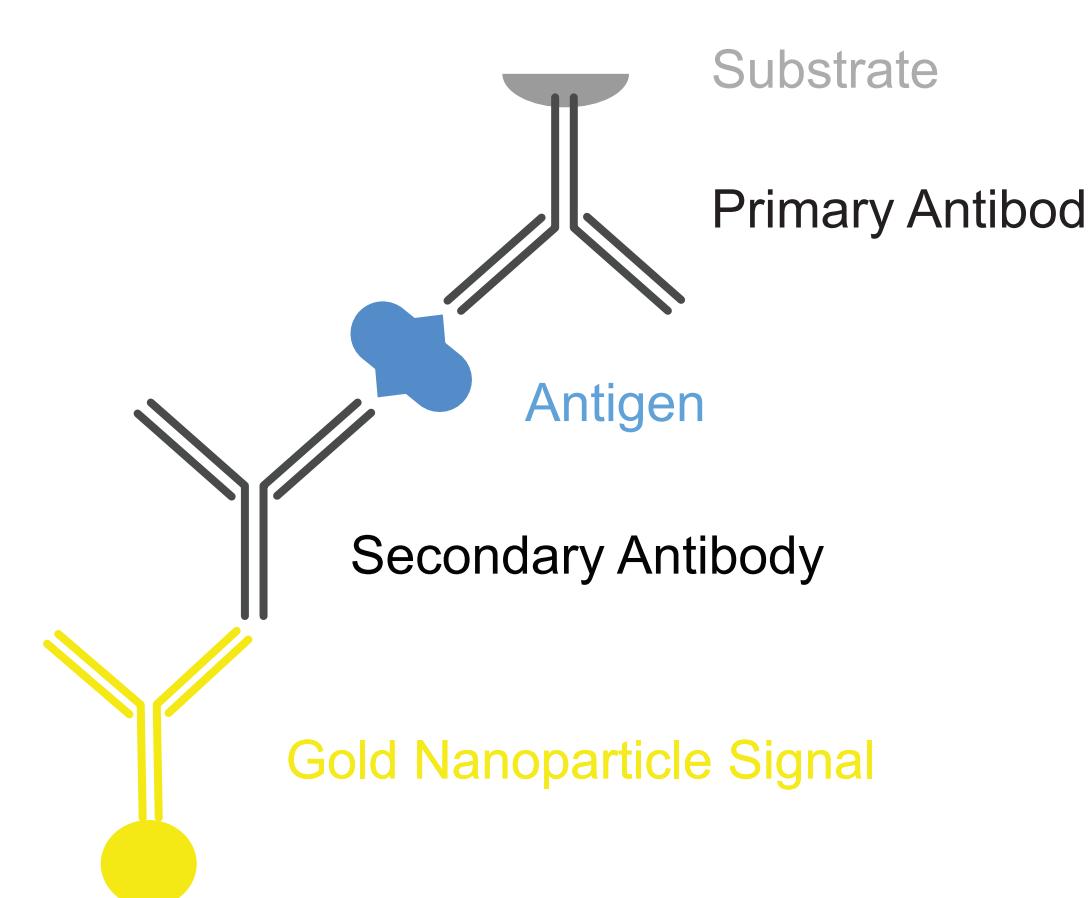
## 1. Introduction

### 1.1 Disease Outbreaks

- Diseases and pandemics cause an estimated 25% of deaths worldwide, resulting in irreparable social and economic damage<sup>1</sup>
- A major cause of mortality is the inability to treat patients due to a lack of affordable and timely diagnostic tests to prevent disease transmission
- Insufficient diagnostics compounded the detrimental effects of the Ebola virus outbreak in West Africa through the wrongful quarantine of healthy individuals<sup>2</sup>

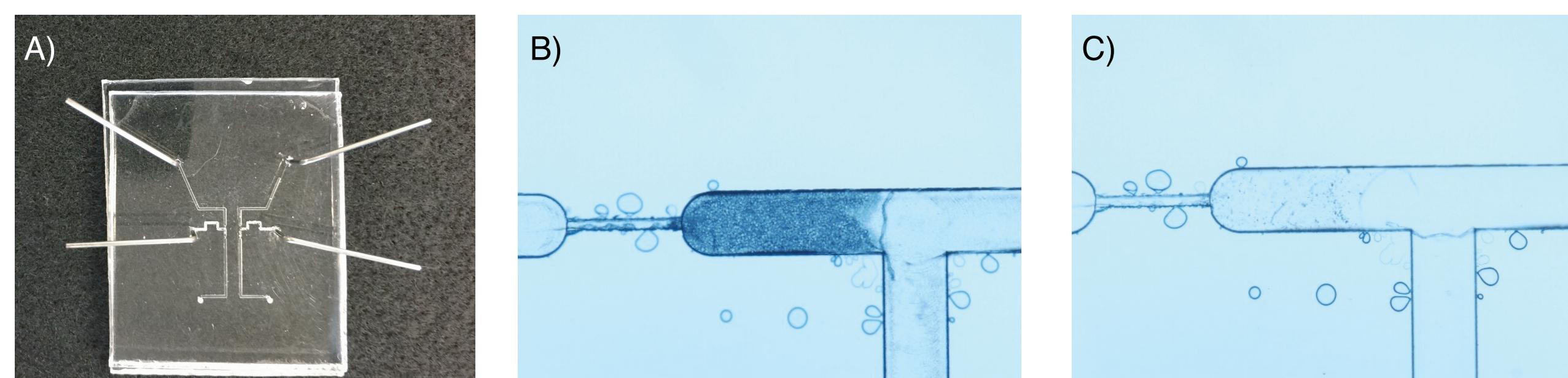
### 1.2 Sandwich Immunoassay

- The sandwich immunoassay is highly stable and incorporates easily interchangeable components to detect antigens such as viruses or bacteria
- With the successful linkage of all five components, shown to the right, a positive response is detected through the capture of gold nanoparticles causing a color change



### 1.3 Current Microfluidic Technology

- Current planar surface immunoassays could be improved by using volumetric capture substrates to increasing the surface area and improve detection performance
- However, 3D substrates are problematic for light-based assays due to light scattering and often require an additional highly viscous index-matching fluid, such as the one shown below:



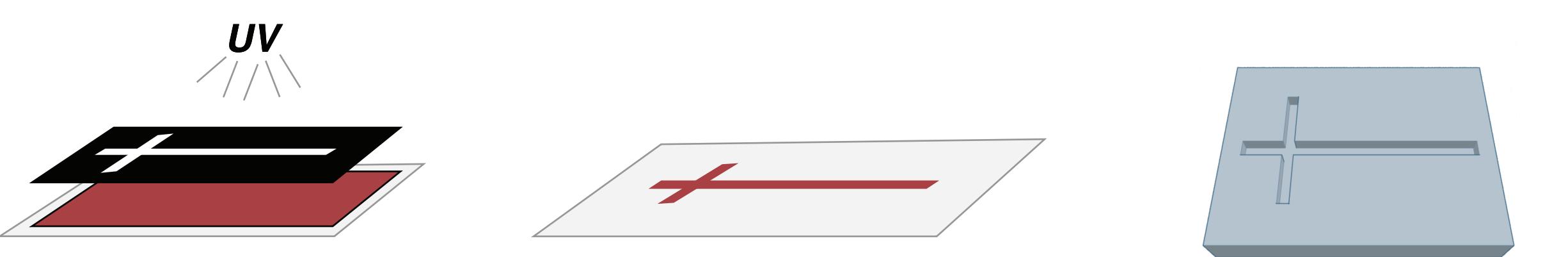
Glass beads on a microfluidic chip (A), before (B) and after (C) the addition of a sucrose-index matching fluid<sup>3</sup>

### 1.4 Innovation

- Proactively responding to disease outbreaks requires a better diagnostic platform that can provide real time data at point of care
- Building a marketable device to meet this goal requires a vast simplification from the one shown above
- By using low melting point agarose instead of the glass above, the agarose beads can be melted into a clear mass to create the same effect, but with a more reliable and cost-effective method

## 2. Methodology

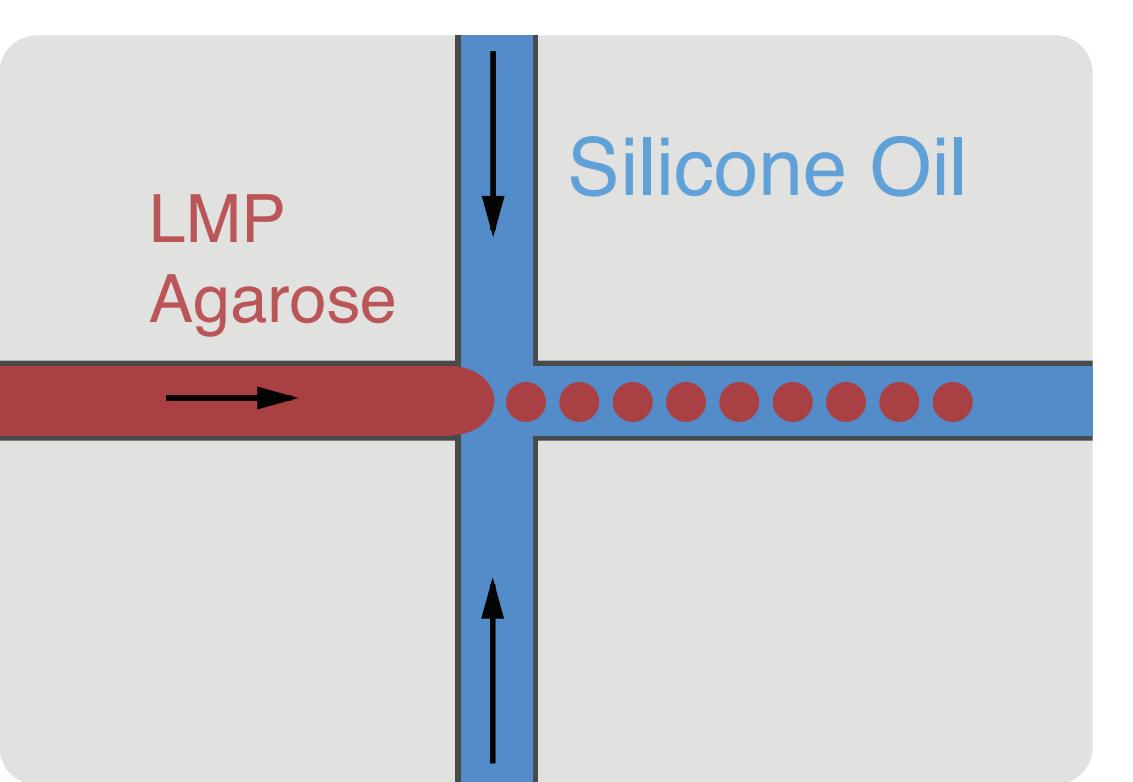
### 2.1.1 Dry Film Photoresist



- A patterned transparency is laid over several layers of laminated dry film
- The area exposed by the transparency hardens and adheres to the glass plate, while the rest of the material is washed off
- PDMS, a plastic polymer, is poured over the mold until the material hardens and forms a microfluidic chip

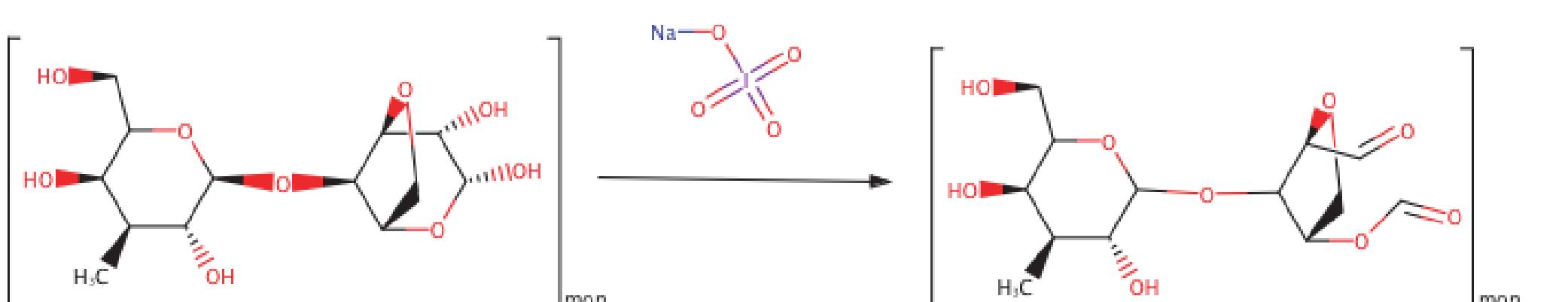
### 2.1.2 Droplet Generation

- Using a controlled fluid flow, agarose droplets are pinched off in a process called coflow to create a water in oil emulsion
- Using the hydrophobicity of silicone oil, the agarose beads will not mix and instead create distinct droplets

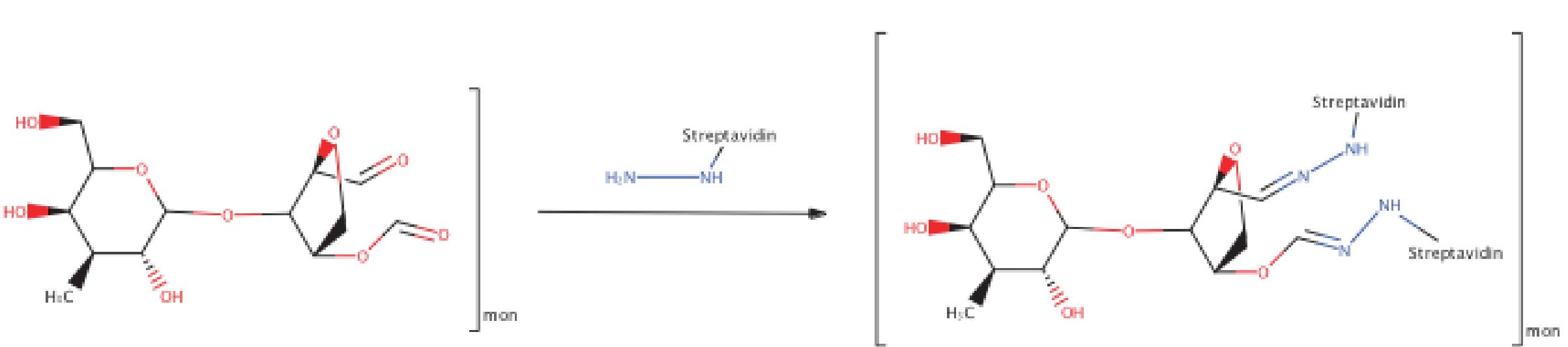


### 2.2 Functionalization

Each agarose molecule is exposed to sodium periodate<sup>4</sup>



The agarose is then exposed to a streptavidin-hydrazide complex, leaving two active streptavidin sites per monomer for biotin-avidin binding.



- Streptavidin has a very strong affinity for biotin and is the strongest non-covalent bond found in nature
- Using this binding site, a multitude of biological markers activated with a biotin tag can be anchored on the agarose substrate
- These markers include short sequences for PCR, antibodies, and a multitude of other options

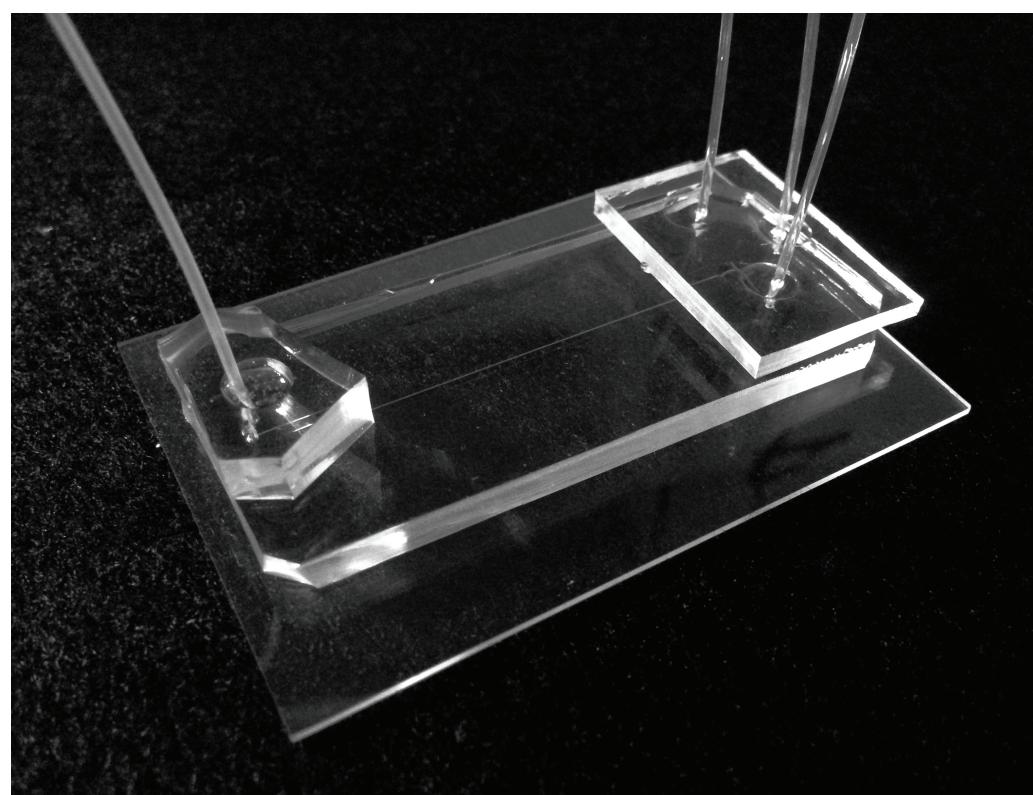
## 5. Next Steps

### 5.1 Quantification of Optical Improvement

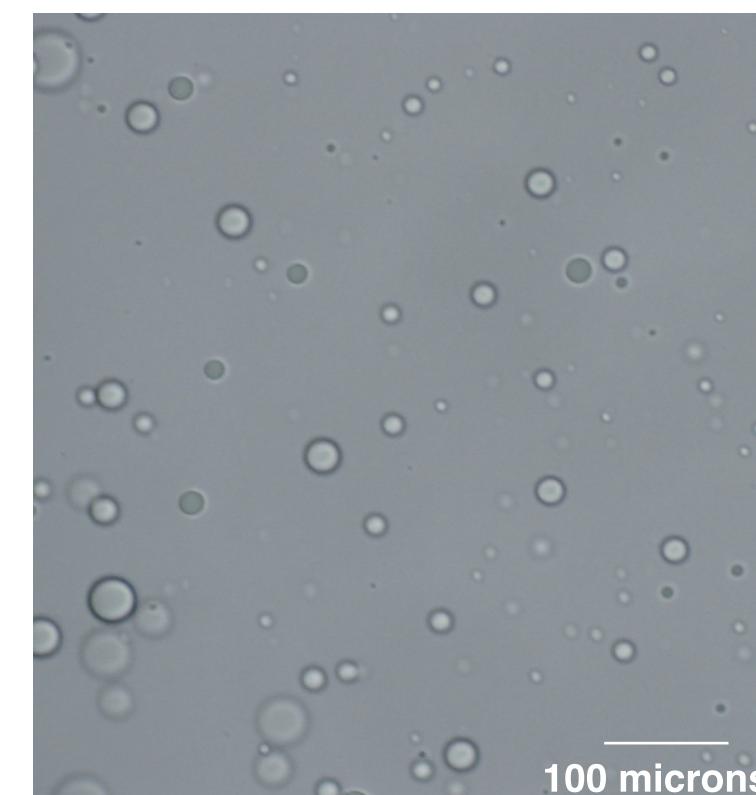
- The properly sized LMP agarose beads and the procedure for activation have been prototyped and tested, the next step is to implement the diagnostic assay on chip
- A baseline direct assay will be used to test optical improvement before and after melting and to confirm bead activation - see 3.2
- Using an immunoassay, human IgG will be measured at different concentrations to determine diagnostic specificity and sensitivity

## 3. Preliminary Results

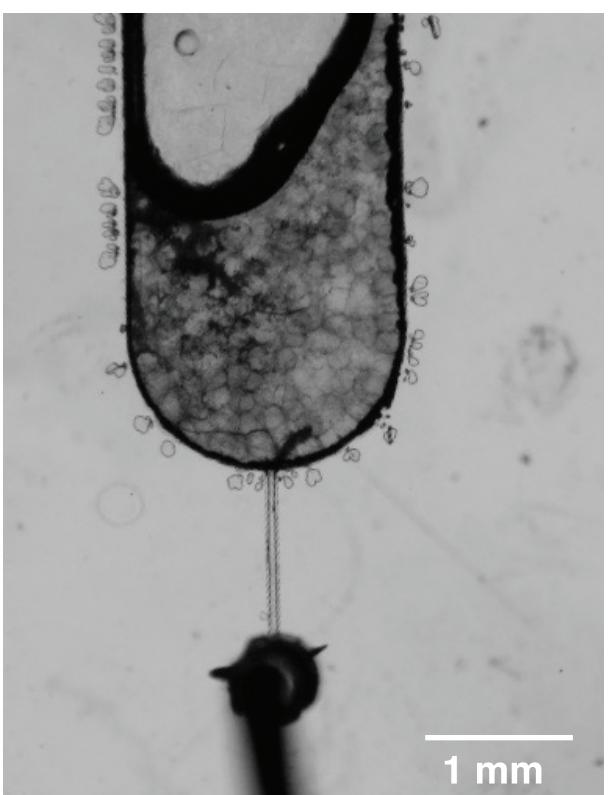
### 3.1 LMP Agarose Bead Production and Testing



Droplet generator microfluidic chip made with PDMS on a glass slide



10-20  $\mu\text{m}$  LMP agarose beads in silicone oil produced by chip pictured to the left



100 micron LMP agarose beads packed into microfluidic device

### 3.2 Macro-Scale Optical Improvement and Detection

Negative Control		Positive Control
Non-functionalized agarose + gold	Functionalized agarose w/o gold	Functionalized agarose + gold
Pre-melting		
After melting at 80°C		

## 4. Citations

- Yeh, Y. et al. (2014). Annals of Biomedical Engineering, 42(11), 2333–2343
- Oleribe et al. (2015). Ebola virus disease epidemic in West Africa: lessons learned and issues arising from West African countries.
- Wiederoder, M.S., et al. (2014). Paper presented at the 18th annual MicroTAS
- Spencer, L. A., et al. (2005). Methods in Molecular Biology, 302, 297–314.

### 5.2 Future Goals

- Ultimately, this device will obtain a 1 ng/ml detection limit to compete with comparable devices and hold potential for medical applications
- The device will additionally function as a platform for a variety of diagnostic tests using the interchangeable nature of the sandwich immunoassay