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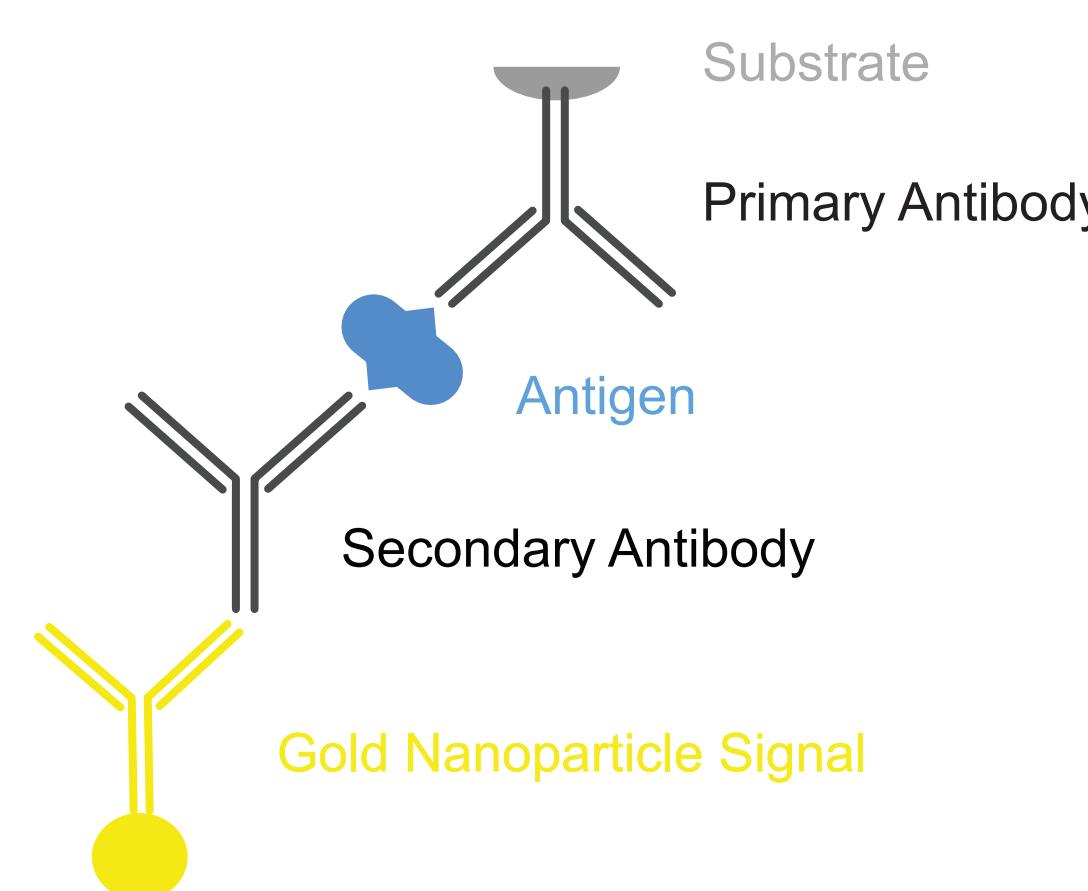
1. Introduction

1.1 Disease Outbreaks

- Diseases and pandemics cause an estimated 25% of deaths worldwide, resulting in irreparable social and economic damage¹
- 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 individuals²

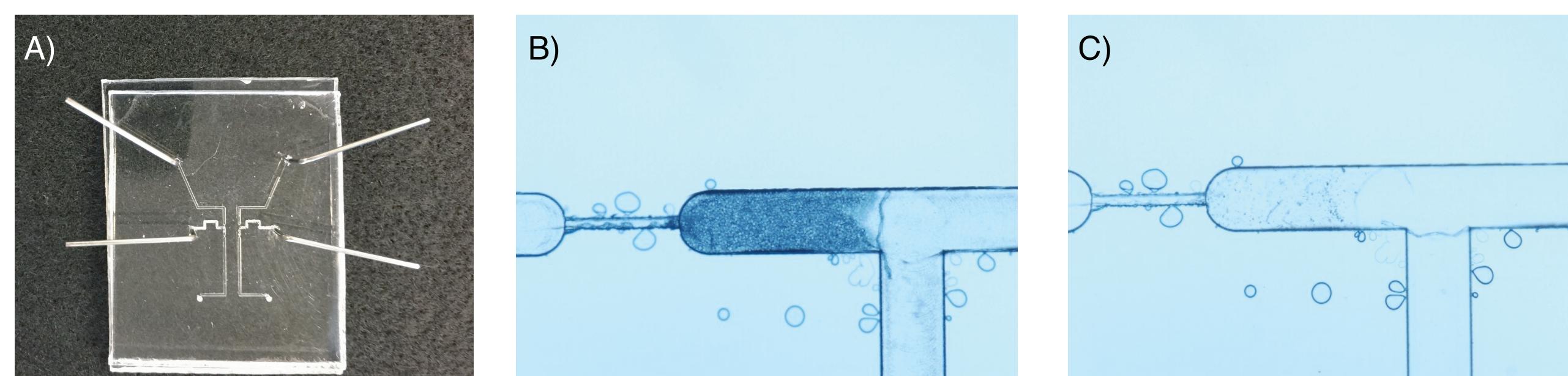
1.2 Sandwich Immunoassay

- 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:



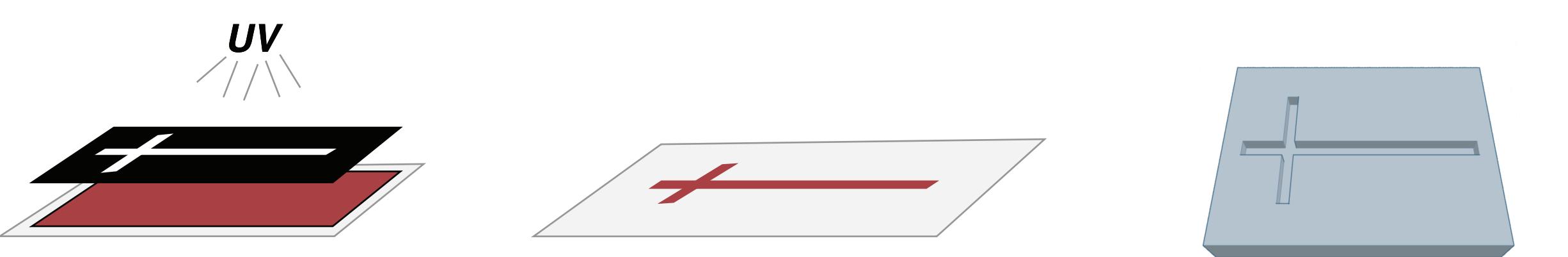
Glass beads on a microfluidic chip (A), before (B) and after (C) the addition of a sucrose-index matching fluid³

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

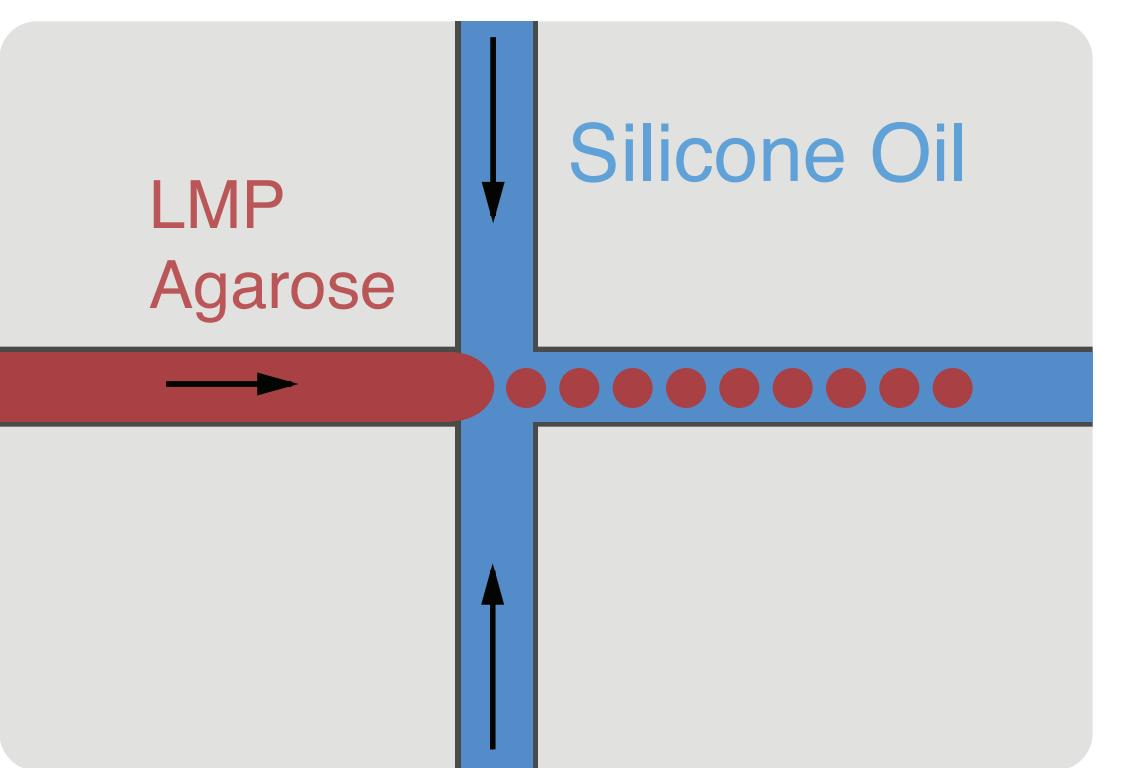
2.1.1 Dry Film Photoresist



- 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

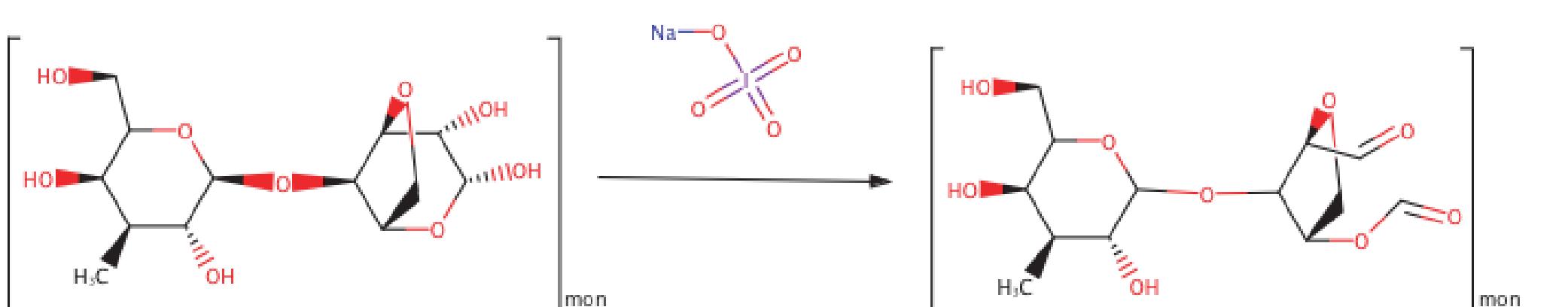
2.1.2 Droplet Generation

- 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

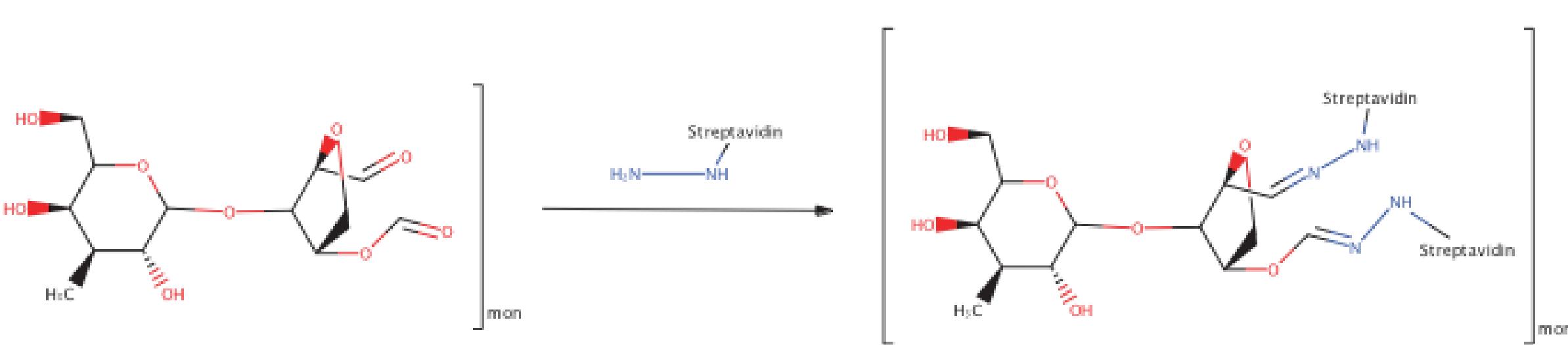


2.2 Functionalization

Each agarose molecule is exposed to sodium periodate⁴



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

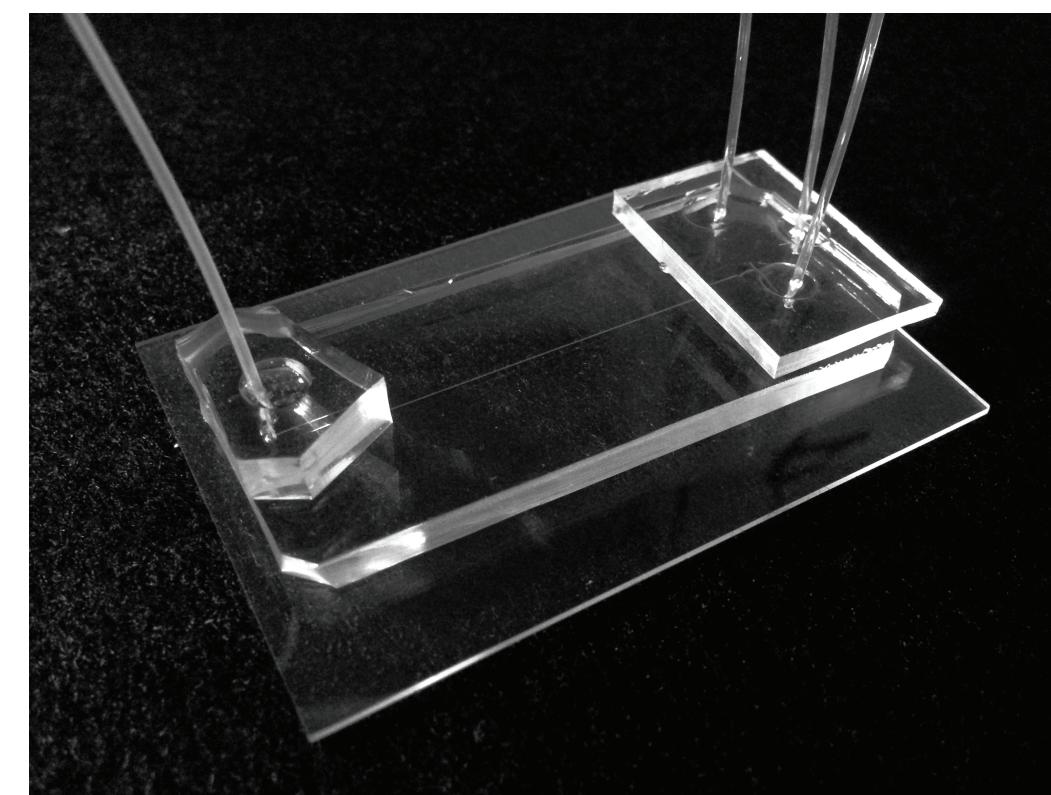
5. Next Steps

5.1 Quantification of Optical Improvement

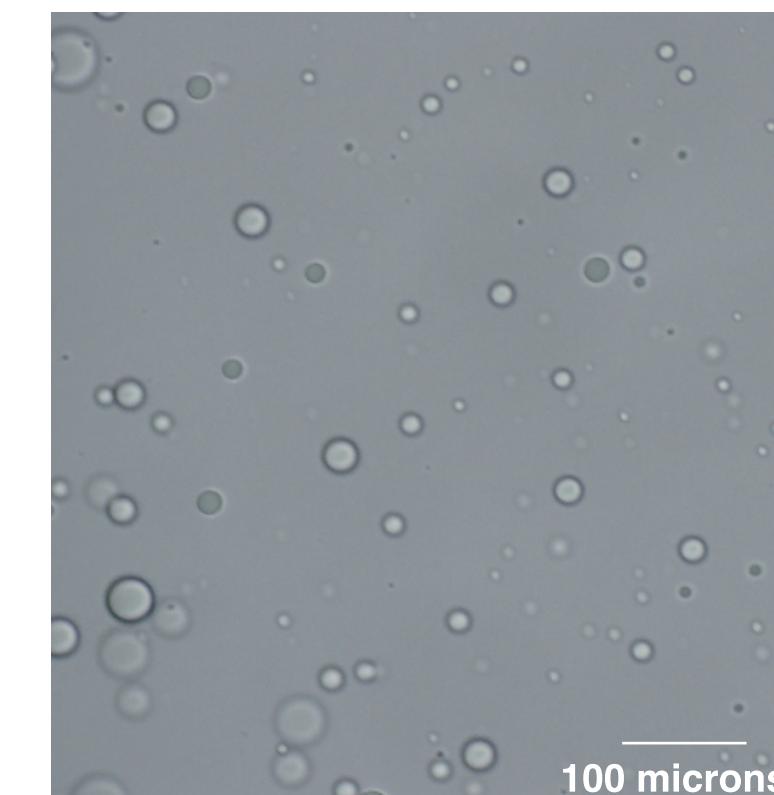
- The properly sized LMP agarose beads and the procedure for activation have been prototyped and tested, the next step is to implement the diagnostic assay on chip
- A baseline direct assay will be used to test optical improvement before and after melting and to confirm bead activation - see 3.2
- Using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

3. Preliminary Results

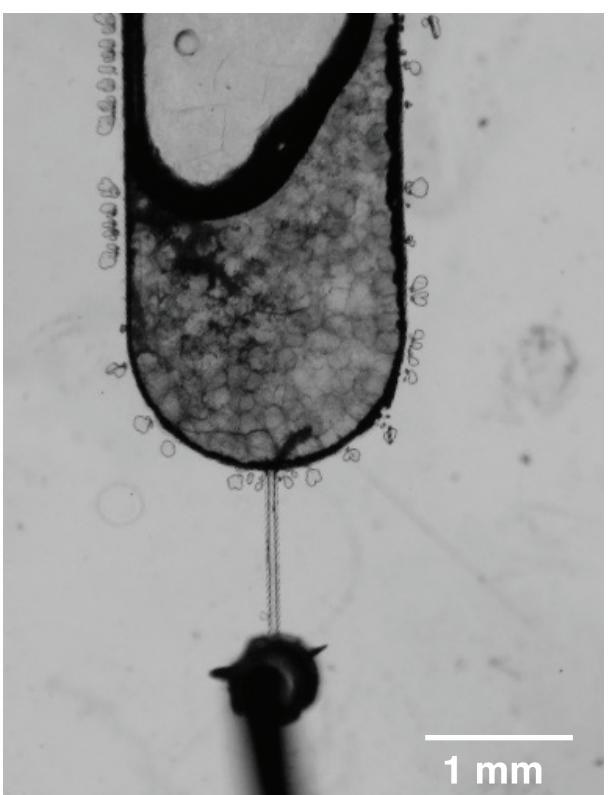
3.1 LMP Agarose Bead Production and Testing



Droplet generator microfluidic chip made with PDMS on a glass slide



10-20 μm LMP agarose beads in silicone oil produced by chip pictured to the left



100 μm 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