

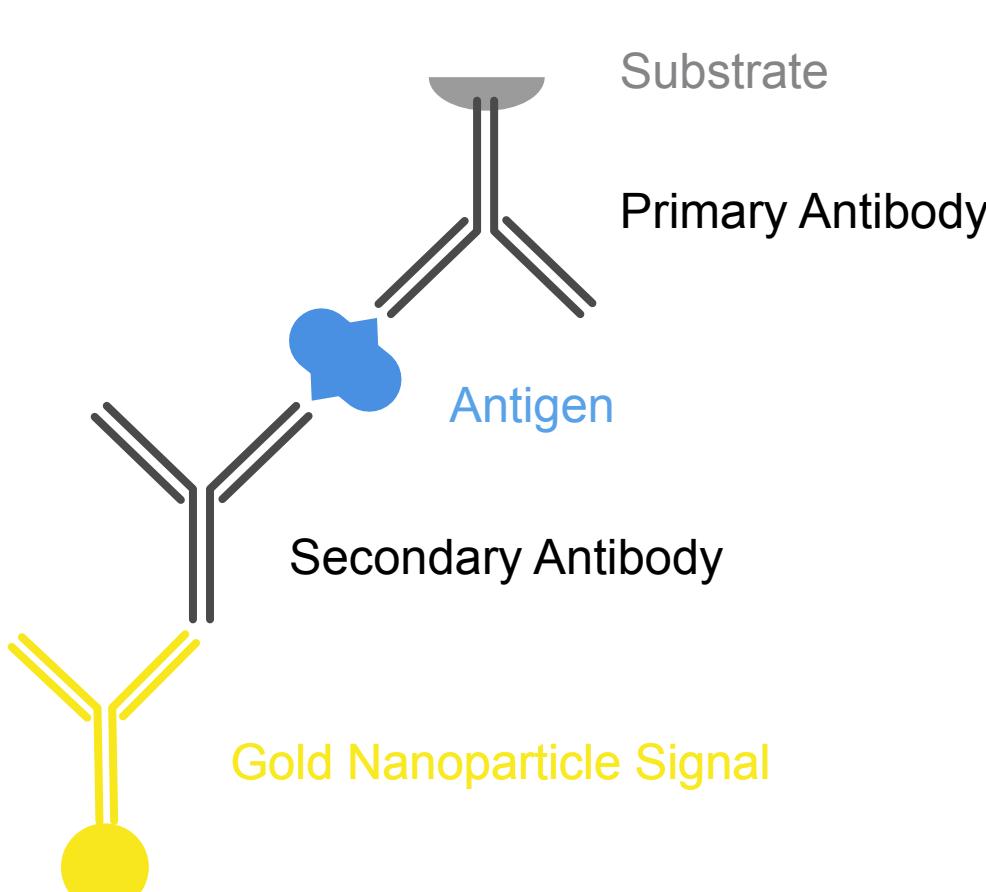
1. Introduction

1.1 Disease Outbreaks

- Diseases and pandemics cause an estimated 25% of deaths worldwide, resulting in irreparable social and economic damage¹
- Insufficient diagnostics compounded the detrimental effects of the Ebola virus outbreak in West Africa through the wrongful quarantine of healthy individuals²

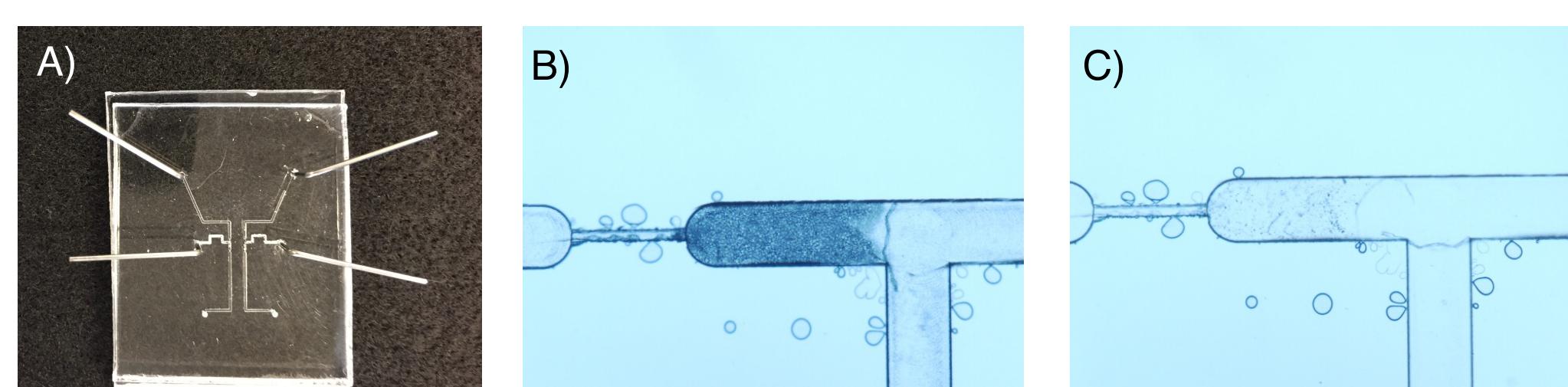
1.2 Sandwich Immunoassay

- The sandwich assay uses five interchangeable components to detect a small volume of antigens with high specificity
- If no antigen is present, the gold nanoparticle will flow through and won't collect. Inversely, a positive response will turn more red when the gold nanoparticle collects



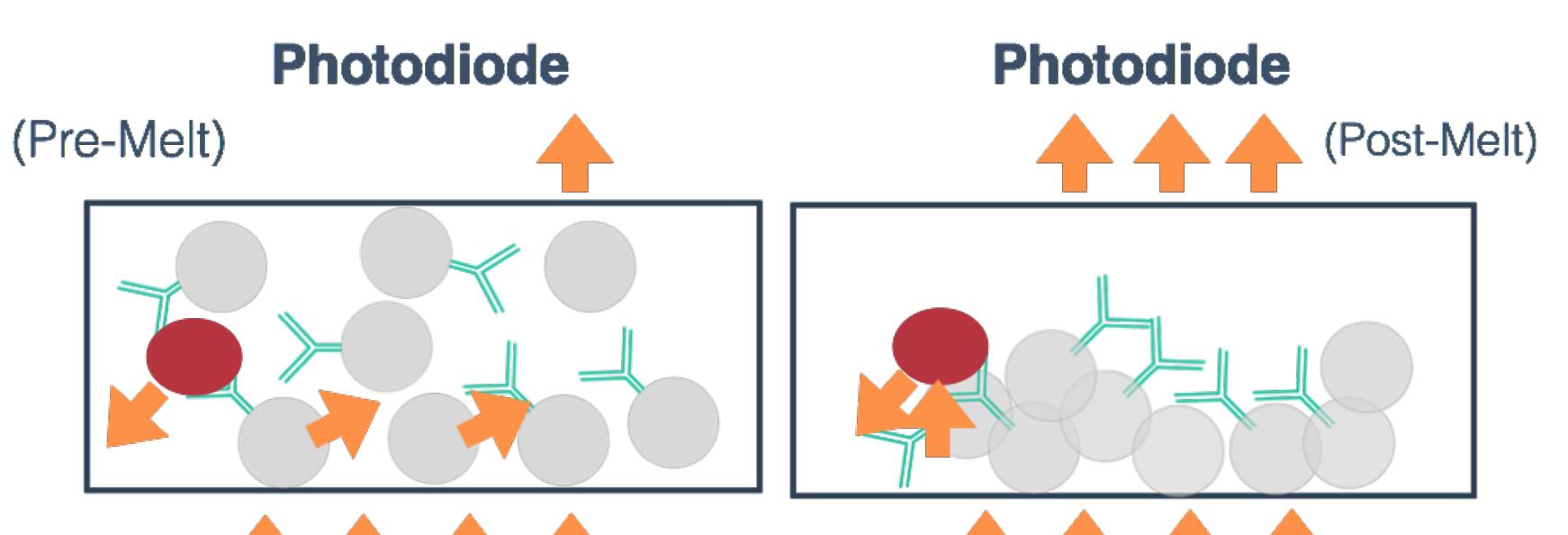
1.3 Current Microfluidic Technology

- Volumetric capture substrates reduce diffusion time by increasing activated surface area for antigen capture
- 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:



1.4 Innovation

- By melting agarose beads, light scattering can be reduced to greatly improve detection performance
- A cheap LED/photodiode quantification system can differentiate from a clear negative control and darker positive control



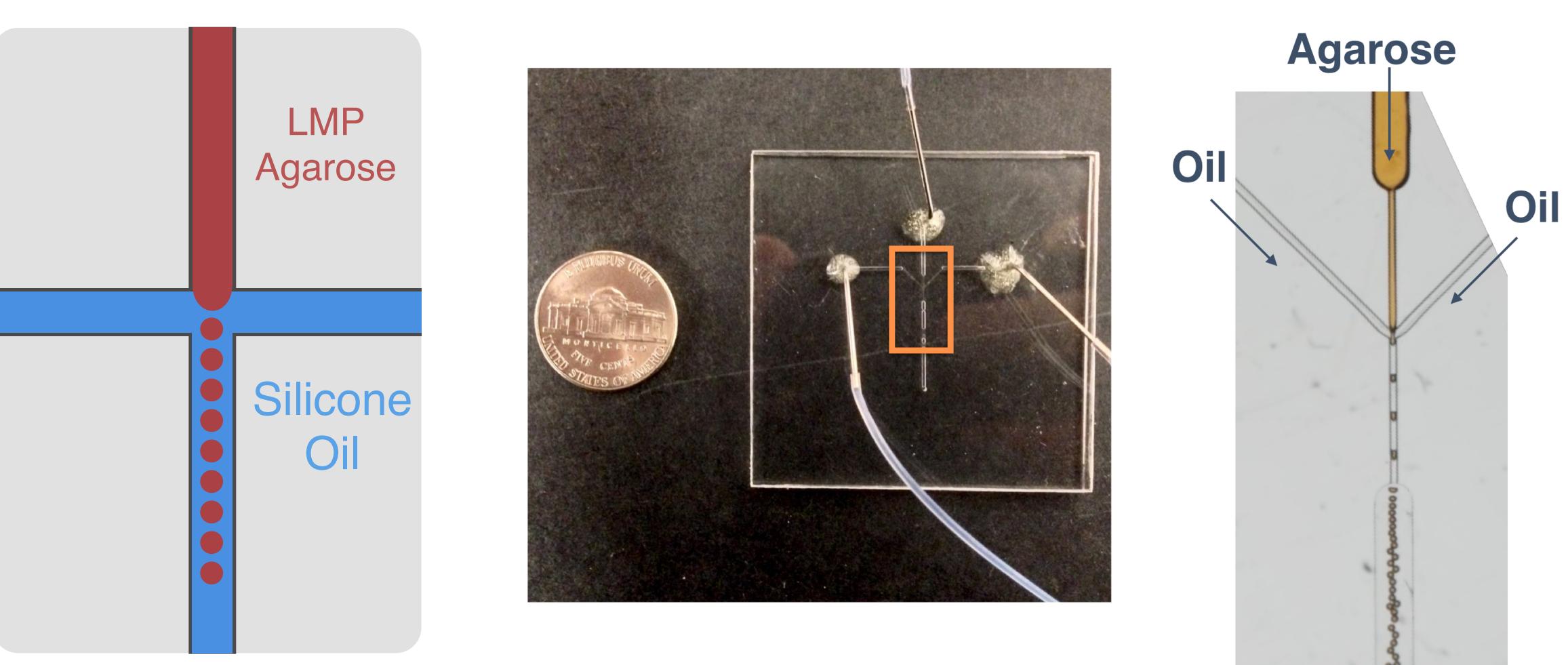
4. Citations

- 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.

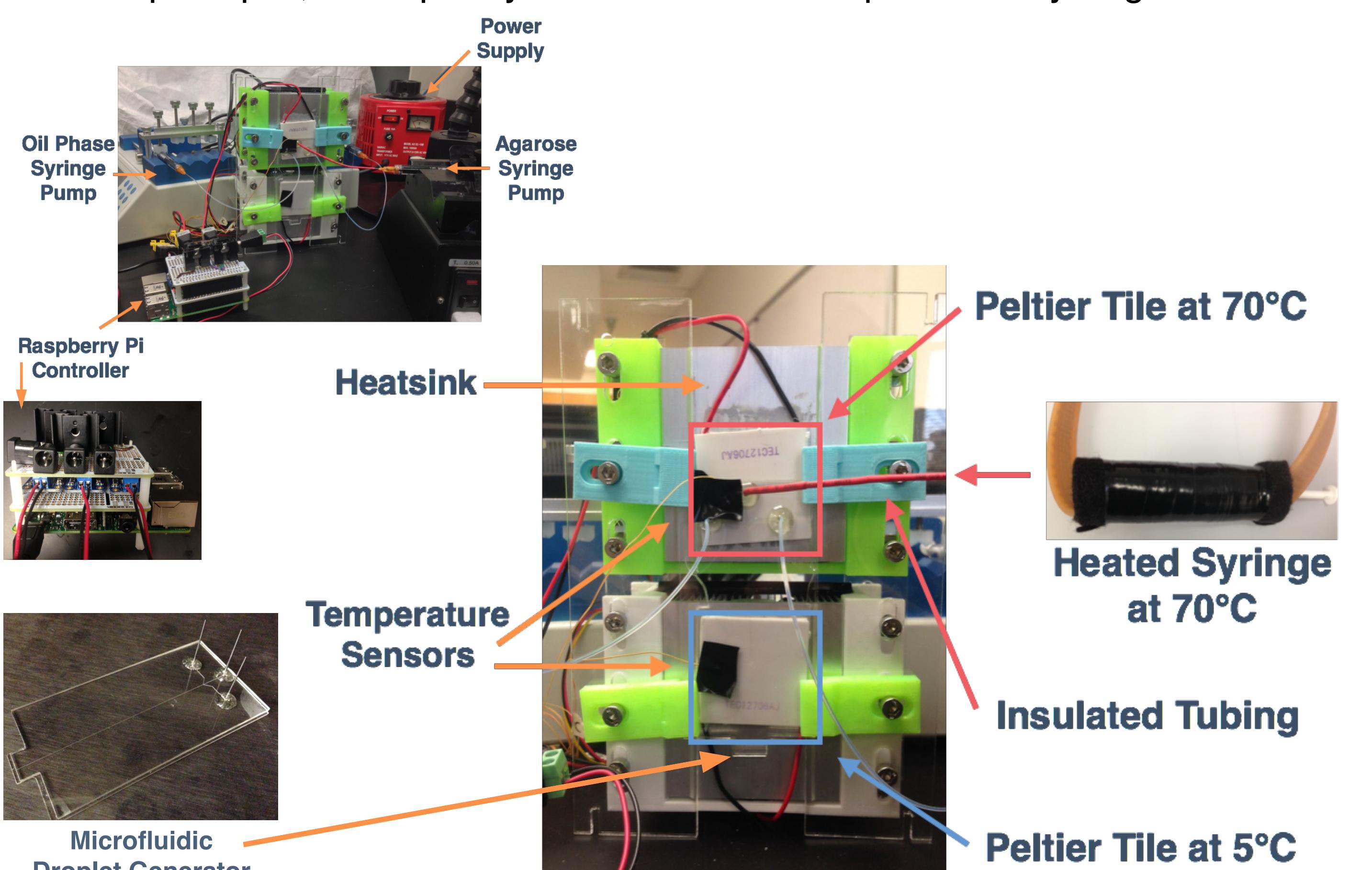
2. Methodology

2.1 Droplet Generation

- Using a controlled fluid flow, agarose droplets are sheared off in a process called coflow to create agarose in oil emulsions

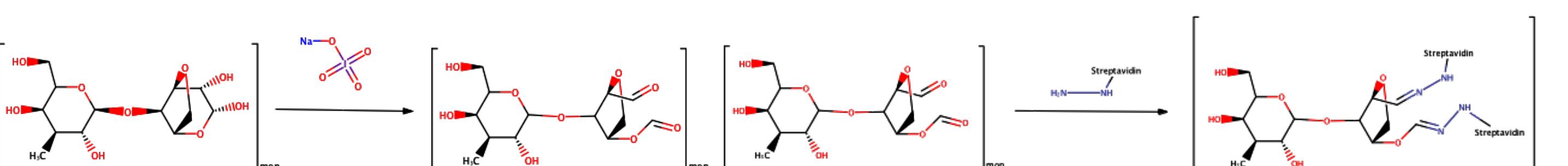


- However, when $\leq 25^{\circ}\text{C}$, LMP agarose gels and is too viscous for coflow
- A custom, two-temperature heat stage was developed to first heat the gel and keep it liquid, then quickly cool the distinct droplets into hydrogel solids



2.2 Functionalization

Each agarose molecule is incubated with sodium periodate, then a streptavidin-hydrazide complex, leaving two active streptavidin sites per monomer for biotin-avidin binding⁴

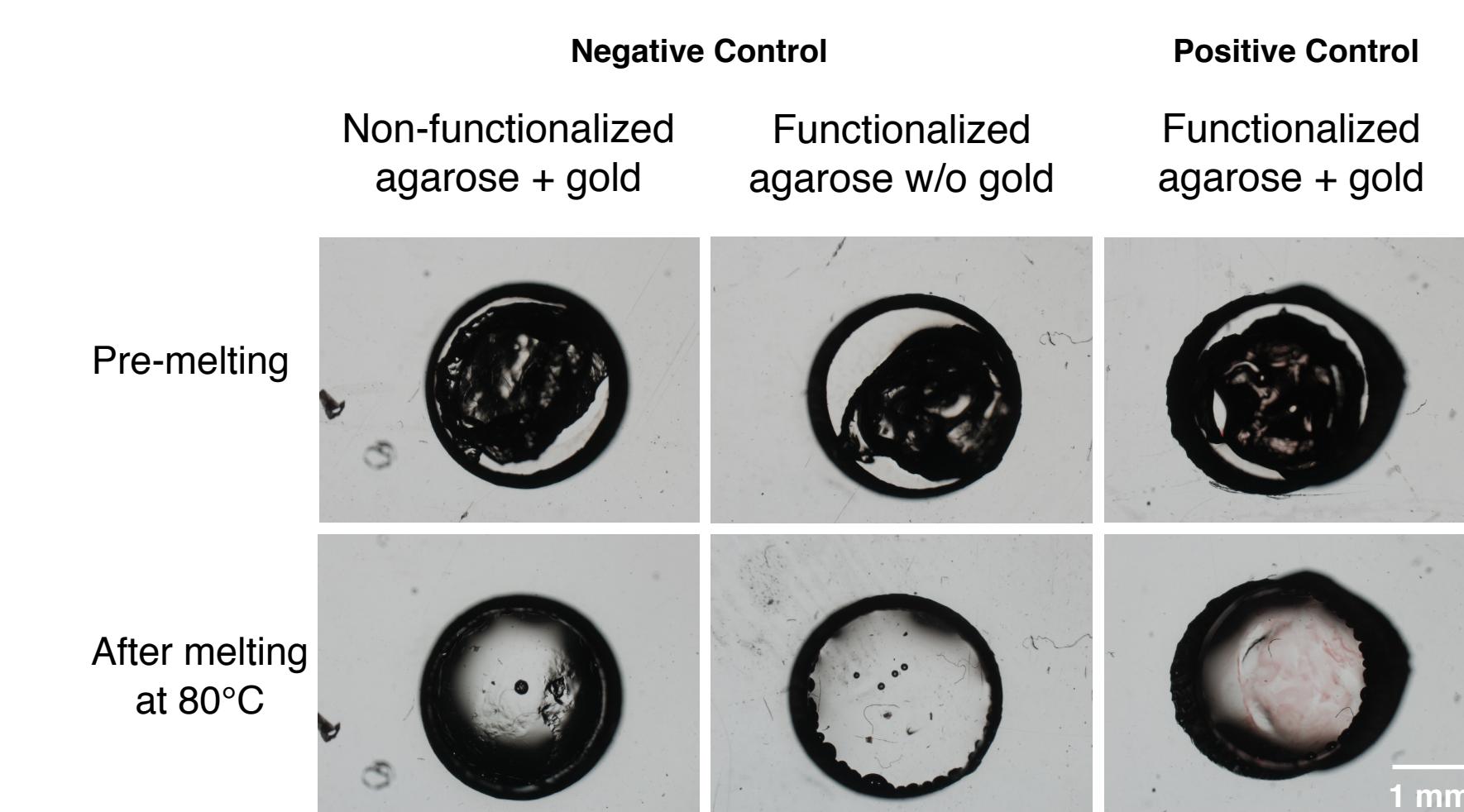


5. Next Steps

- 30 μm diameter LMP agarose beads can now be produced in large quantities through an automated device and the assay has been demonstrated in bulk
- The next step is to demonstrate the assay with the micro-scale agarose beads inside of a microfluidic chip and publish the final results
- Future work could demonstrate this device as a platform for recognizing different antigens, further lower the detection limit, or test the reagent stability for POC
- Further, this device could be developed into an integrated handheld detection device. The photodiode and LED system needs to be developed and for the transmission optical assay to work, the opaque Peltier tile needs to be replaced with a clear Fluorine doped Tin Oxide (FTO) Glass heater to melt the beads when needed

3. Preliminary Results

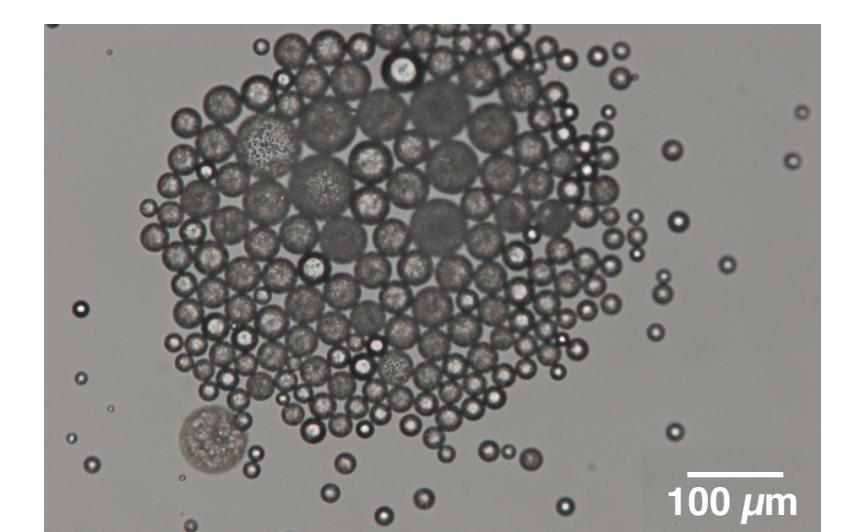
3.1 Macro-Scale Optical Improvement and Detection



- A bulk test was conducted to demonstrate both the optical improvement of melting agarose beads and the functionality of the assay
- The bottom right bead is red colored indicating the presence of gold nanoparticles captured in the agarose bead matrix, while both negative controls are completely clear

3.2 Microscopic LMP Agarose Beads

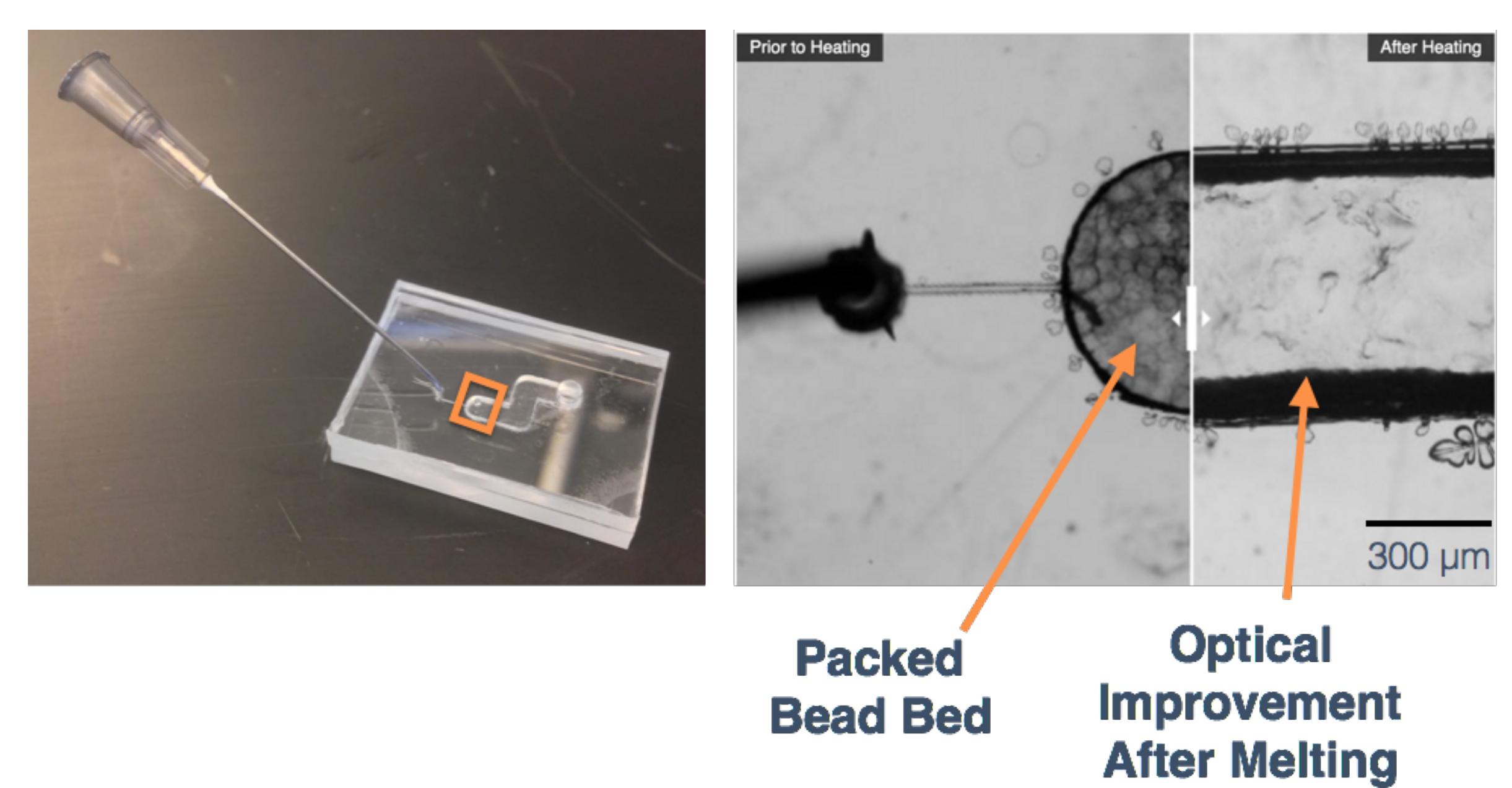
- Using the dual-temperature heat stage, LMP agarose beads can be produced in large quantities and through a largely automated process because of the 1 mL, heated dead volume



Agarose beads collected after generation

3.3 Bead Packing Demonstration

- A demonstration of packing agarose beads into a PMMA microfluidic device. The height and width restriction causes the beads to collide and collect
- With the beads constrained in one location, sample fluid and diagnostic fluids can be washed through to conduct the optical assay



Packed Bead Bed
Optical Improvement After Melting