# Validation of Ultrasound Molecular Imaging Agents for Diabetic Kidney Disease

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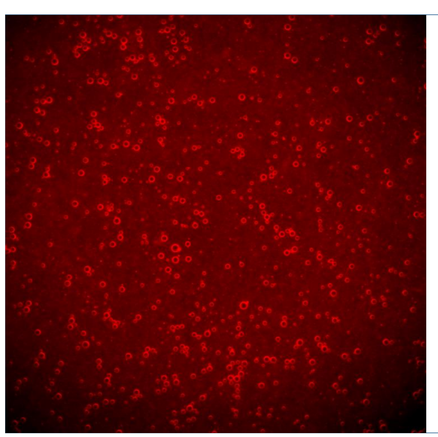
## Microbubble Preparations

Fluorescently-tagged microbubbles were generated and modified to fit necessary experimental parameters. The initial goal was to ensure microbubble compatibility with Di-I fluorescent dye, as well as with a microfluidic chip to mimic physiological blood flow conditions.

- Fluorescent Di-IC18 was reconstituted to a final concentration of 2.5 mg/mL.
- Lipid vials were obtained from refrigerator storage, and 2 µL of the dye was pipetted in.
- Gas exchanging the vials produced the gas that the microbubbles would be made of.
- The VialShaker system produced microbubbles in the lipid vials.
- The Accusizer was used to verify a stock concentration of 1 x $10^{10}$ microbubbles/mL.

The dyed microbubbles were imaged using the Widefield imaging system. A concentration of 1 x $10^{8-9}$ microbubbles/mL was verified for any microbubble/cell interaction assays.

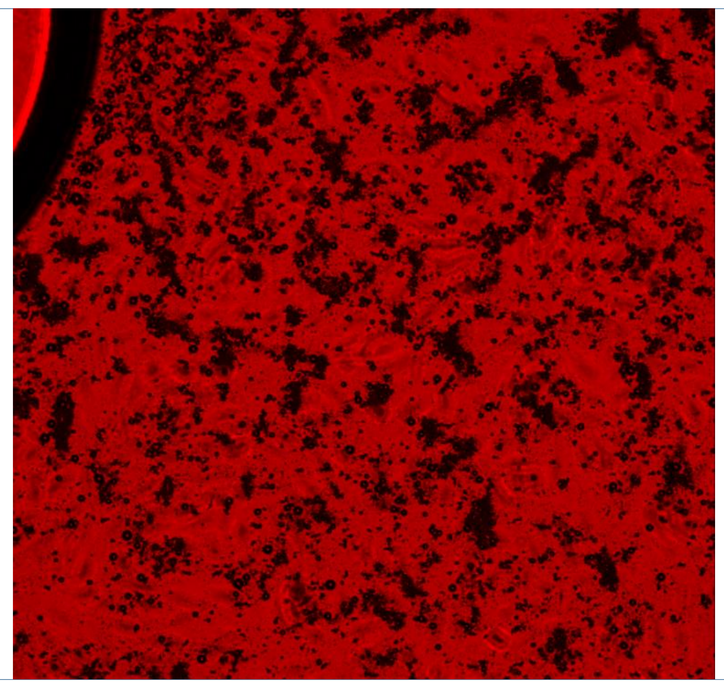


Results of Widefield imaging third trial of stagnant fluorescent microbubbles with a concentration of 1 x $10^{8}$ microbubbles/mL.

## Background

Diabetic kidney disease, as the name suggests, is kidney disease onset by existing diabetes. Damage to the kidneys due to this disease significantly inhibits their filtering ability, causing harmful toxins to accumulate in the bloodstream.

The use of medical-grade microbubbles as biomarkers has recently become popular due to their biocompatibility and general non-invasiveness. Targeting ligands can be attached to microbubbles to allow the bubble to bind to receptor proteins on the walls of blood vessels. Certain diseased cellular expressions could be targeted by these modified microbubbles, functioning as biomarkers to identify DKD proactively.



Microbubble and Cell Fluidic Interactions Imaged.

## Cells and Microfluidic Devices

HUVEC cells, or human umbilical vein endothelial cells, were used due to their applications in fluidic experiments. These cells were cultured, passaged, and centrifuged to ensure adequate confluency. An Ibidi flow chamber was utilized to create fluidic conditions for the cells. After the cells' passaging, they were seeded to take root in this microfluidic chip. Shear stress would be applied over the top of the cells so that cell alignment in response to flow could be examined.

The final steps performed as a part of this semester's portion of research involved flowing microbubbles through an Ibidi chamber seeded with HUVEC cells to confirm initial behavior between untagged microbubbles and unmodified cells. This would rule out any extraneous interactions that could simply be counted as normal when the ligands were employed. Visual results are displayed in the image below.

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