Validation of Ultrasound Molecular Imaging Agents for Diabetic Kidney Disease via Analysis of Bio-Molecular Interactions via MATLAB Software and Microfluidics

Emma Rosentreter; Rachel Walmer, PhD; Mitesh Rathod, PhD; William Polacheck, PhD; and Kennita Johnson, PhD

Joint Department of BIOMEDICAL ENGINEERING
UNC CHAPEL HILL | NC STATE UNIVERSITY

INTRODUCTION

- Ultrasound molecular imaging (USMI), using targeted microbubbles, is a rapid approach to characterizing disease progression through direct identification of diseased cell clusters. This may be applied to medical conditions such as diabetic kidney disease [1].
- The primary aim of this project is to establish an experimentally confirmed hypothesis that microbubbles targeted to vascular endothelial growth factor receptors (VEGFRs) will adhere specifically to tumor endothelial cells overexpressing VEGFR2.
- This will determine the relationship between microbubbles and cells in static and fluidic conditions to assist in the fabrication of more robust targeted ultrasound contrast agents.
- The focus specific to this semester was to establish a standard image processing protocol to identify microbubbles in a microfluidic chamber seeded with cells and to note their movement when no tagging exists, comparing their behavior with and without cells.

METHODS

Our experimental setup (*Figure 1*) was comprised of *in vitro* optical imaging in microtubes and *in vitro* imaging in microfluidic devices.

Experimental Parameters	Microtube	Microfluidic Device
Microscope	Inverted, Brightfield	Fluorescent, Widefield
Device Size (mm)	Diameter: 0.2	Volume: 50 x 5 x 0.2
Cells	None	HUVEC cells
Fluorescent Dye	None	2.5 mg/mL Di-I
Ligand Tag	None	None
Microbubble Concentration (#/mL)	1×10^8	1×10^8
Flow Rate (µL/min)	10	500
Images Acquired	.tiff image series	.ets files

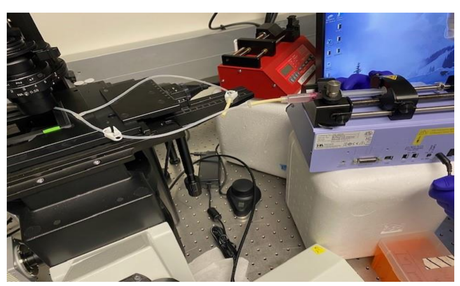

Figure 1: Experimental setup for microfluidic chip analysis. A T connector was used to connect a syringe pump with a dilution of fluorescently labeled microbubbles as well as cell media for flushing to the microfluidic chamber.

RESULTS

Image processing tools were generated in MATLAB® to determine essential parameters regarding microbubble movement in static and fluidic conditions. Three separate MATLAB® files were created to:

1. Apply image registration to correct for any dimension errors during imaging (all figures). Note: The correlation between image frames increased once registration was applied.
2. Generate a streak image that would track the movement of a single microbubble over time (*Figure 2*)
3. Locate all stationary microbubbles in the video using *imfindcircles* on the streak image (*Figures 3 and 4)*

Optical imaging of unlabeled, untagged microbubbles under static and flow conditions in microtubes

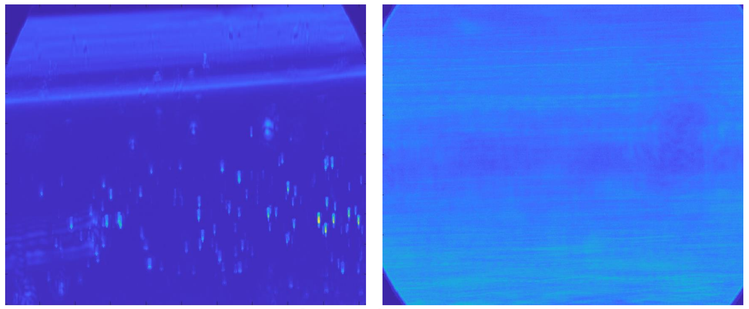

Figure 2: Comparative view of a streak image generated using our software with microbubbles under static (left) vs. flow (right) conditions. Microbubbles show up as bright blue to yellow spots in the static image, and faint microbubble traces can be seen in the flow image.

Fluorescent imaging of Di-I labeled, untagged microbubbles under static and flow conditions in microfluidic chips with cells

We imaged the fundamental biochemical interactions between fluorescently labeled, untagged microbubbles and endothelial cells. Video data were analyzed using MATLAB. Some limitations of our experiment were that the cells and microbubbles would not appear on the same plane under the microscope and that injection of the cell solution sometimes altered the field of view of the microscope due to bumping.

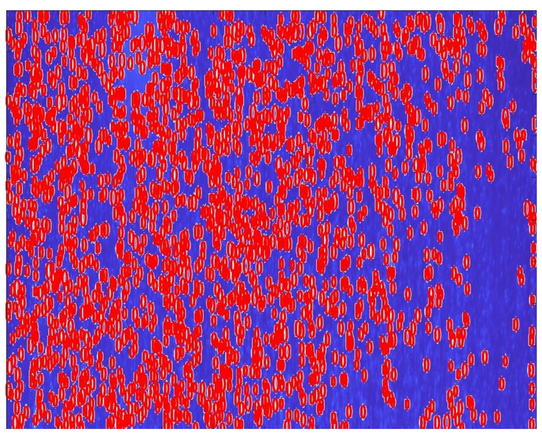

Figure 3: Microbubble detection under static conditions in the microfluidic device using the described image processing tools. Microbubbles appear as blue dots, overlaid with detection events (red circles).

CONCLUSIONS

- The video data collected during the experiments was successfully uploaded through our image processing pipeline, which quantified microbubble movement in static and fluidic conditions.
- This study attempted to visualize and characterize interactions between microbubbles and cultured cells in microfluidic devices. Understanding these interactions guides decisions around tagging microbubbles for targeted imaging.
- Furthermore, it provides a method by which to validate targeted microbubble adherence to diseased cells.
- The characterization of these interactions must be approached from multiple standpoints, including establishing control behavior for untagged microbubbles.

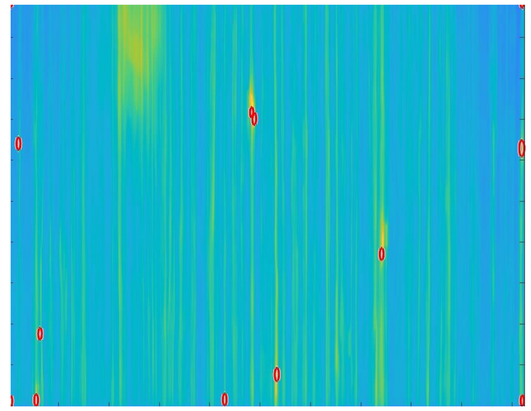

Figure 4: Microbubble tracking under flow in the microfluidic chip using image processing tools. Microbubbles appear as vertical yellow streaks in the image. Median filtering was used to denoise the image and reduce erroneous detection events (red circles).

FUTURE WORK

- Further directions for this project will include a study to determine the differences between labeled and unlabeled microbubbles through microscopic imaging.
- Tagging microbubbles is an important future experimental concept for this project to note how microbubbles may signal abnormally functioning cells.
- Finally, investigating the compatibility of different types of targeting ligands and biomarker receptors will allow us to verify the specificity of each interaction.
- This work will further the application of targeted microbubbles to identify diabetic kidney disease progression.

REFERENCES

1. "Diabetic Kidney Disease." *National Institute of Diabetes and Digestive and Kidney Diseases*, National Institutes of Health, Feb 2017, www.niddk.nih.gov/health-information/diabetes/overview/preventing-problems/diabetic-kidney-disease.
2. "Lipophilic Tracers – DiI, DiO, DiD, DiA, and DiR." *Molecular Probes, Inc.*, 2008, tools.thermofisher.com/content/sfs/manuals/mp00282.pdf.
3. "Ultrasonographic Characteristics of Diabetes Impacts in Kidneys' Morphology." *Open Journal of Radiology*, Scientific Research Publishing Inc., Oct 2014, http://dx.doi.org/10.4236/ojrad.2014.44039