Interferometric Reflectance-Based Nanoparticle Imaging with Patterned Illumination

Introduction: A significant issue in current medical standard-of-care is the accurate detection of infectious diseases. Viruses, bacteria, parasites, and other microorganisms causing these diseases are difficult to detect directly due to their micro- and nanometer length scales. Existing diagnostic techniques typically rely on indirect detection through monitoring bulk tissue changes in a patient, analyzing biological samples *in vitro*, or determining an infection based on the patient's symptoms and immune response. While these techniques are effective in certain cases, indirect detection methods increase the difficulty of achieving a proper diagnosis which can lead to harmful consequences for patients [1].

One such primary diagnostic tool experiencing this limitation is the optical microscope. New optical technology has improved microscopy's capabilities in imaging small-scale objects, but many modern systems have become diffraction limited. Diffraction limits occur when the particles of interest are smaller than the imaging wavelength of light. This sizing issue results in light scattering that prevents nanoparticles from being resolved with conventional microscopy techniques. This limit has been bypassed previously using methods such as fluorescence microscopy, where the particle of interest is indirectly detected by imaging a fluorescent dye that has been bound to the particle. Such techniques are successful, but they have significant drawbacks including the need for extensive sample preparation, augmentations to the sample prior to analysis, and expensive imaging hardware [2]. These factors create significant barriers of entry for these modalities from becoming common disease diagnosis platforms in developing and developed countries. **Thus, a substantial need exists for an affordable diagnostic platform capable of nonspecifically detecting nanoscale biological particles.**

Proposal: I propose a new microscope design combining the imaging modalities of Single-Particle Interferometric Reflectance Imaging Sensors (SP-IRIS) and Fourier Ptychography (FP) Microscopy for high resolution, high throughput imaging of biological nanoparticles.

SP-IRIS, developed in Dr. Selim Unlu's lab at Boston University, utilizes wide-field interferometric imaging techniques to acquire weak scattered light signals from nanoparticles over a large sample region. These signals provide information regarding nanoparticle geometry and have been used for label-free detection of viruses at attomolar concentrations (Figure 1). These factors make SP-IRIS a desirable option for both large sample virus diagnostics and biological nanoparticle characterization applications. However, drawbacks including the requirement of mechanical sample scanning and device limitations in detecting differences between floating and adhered nanoparticles limit the system's current abilities as a diagnostic tool [1].

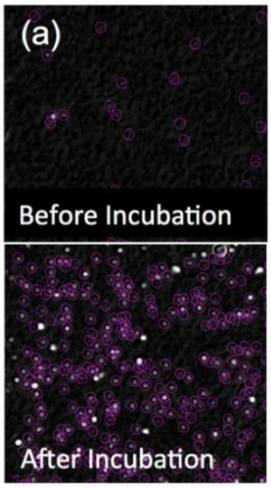

Figure 1: Label-Free Virus Particle Visualization with SP-IRIS Microscope [1]

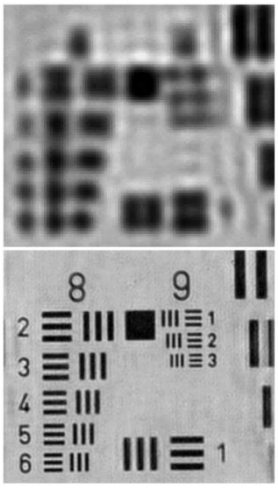

Figure 2: Standard Microscope (Top) and FP-Reconstructed (Bottom) Image [3]

Fourier Ptychography techniques could remove these existing issues in SP-IRIS technology. FP is a computational microscopy approach wherein different angled illumination patterns are projected on the sample via an LED array to obtain low-resolution image sets. These images can be recombined to create images with higher resolution and wider field-of-view than standard microscope techniques (Figure 2). These angled

illumination measurements also enable tomographic and 3-D reconstruction of the imaged sample. With the capabilities of FP in achieving near real-time imaging while providing high-resolution images, the synthesis of FP with SP-IRIS could create a highly sensitive and specific nanoparticle detection platform with volumetric information regarding each particle [3]. These additions would remove the need for depth sectioning in the SP-IRIS system and would allow the user to differentiate between floating and static particles as well as provide additional information for nanoparticle characterization.

Year 1: Proof-Of-Concept Prototype The first year will focus on proof-of-concept research illustrating the successful combination of SP-IRIS and FP. I have already constructed an SP-IRIS bench-top microscope and will be validating the instrument's operation prior to adding FP. This modification will require the addition of a programmable LED array for angled illumination, adapting FP algorithms for reflection microscope geometries, changing SP-IRIS forward modeling to use FP images, and determining whether volumetric FP results are viable with SP-IRIS imaging methods. This year's goals will be achieved when floating and static customized carbon nanotubes can be identified with the system and an improvement in particle visualization is achieved with the combined system over SP-IRIS alone.

Year 2: System Design and Speed Improvement: The primary work in this phase will focus on achieving real-time imaging using the combined software platforms from both modalities. Additional hardware and software modifications will likely be necessary to determine whether different illumination patterns, LED arrays, lens setups, or other aspects of the system can improve the imaging quality or speed. This year's success criteria will be satisfied once real-time imaging of floating carbon nanotubes in a microfluidic channel is achieved. This phase can be extended into Year 3 if additional time is required for real-time imaging with the system.

Year 3: System Validation in Biological Particles: The third year will investigate the device's applications in biological imaging. The system will be tested for its sensitivity to biological particle detection and characterization of different nanoparticles. The throughput and speed of this system will also be tested by analyzing samples with increasing particle counts under different fluid flow conditions. Should this device exhibit reliable results in identifying and characterizing biological samples, the use of this device in clinical trials at Boston University's medical hospital will be explored.

Intellectual Merit: Achieving high-resolution, high throughput imaging of nanoparticles would open opportunities for nanoparticle imaging in many other scientific fields including the semiconductor industry. This technology also uses relatively low-cost optical components allowing other research facilities to build their own systems. This research will be published in research journals and presented at conferences.

Broader Impacts: This technology would be viable as a low-cost, high sensitivity and specificity diagnostic platform for infectious diseases. The high throughput capabilities of this proposed device would be significant for detecting diseases with low concentrations of biological markers in the body. The results from this project will also be published in multiple journal articles and presented at optics-focused and biological research-related conferences.

[1] Avci, O., Ünlü, N. L., Özkumur, A. Y., & Ünlü, M. S. (2015). Interferometric Reflectance Imaging Sensor (IRIS)--A Platform Technology for Multiplexed Diagnostics and Digital Detection. Sensors (Basel, Switzerland), 15(7), 17649–65. http://doi.org/10.3390/s150717649

[2] Jesus, D. M.; Moussatche, N.; McFadden, B. B. D.; Nielsen, C. P.; D'Costa, S. M.; Condit, R. C. Vaccinia Virus Protein A3 Is Required for the Production of Normal Immature Virions and for the Encapsidation of the Nucleocapsid Protein L4. Virology 2015, 481, 1−12.

[3] Tian, L., Liu, Z., Yeh, L.-H., Chen, M., Zhong, J., & Waller, L. (2015). Computational illumination for high-speed in vitro Fourier ptychographic microscopy. *Optica*, *2*(10), 904. http://doi.org/10.1364/OPTICA.2.000904

I want to achieve my long term goal of improving the capabilities of medical imaging and the accessibility of medical diagnostics to the general public through the development of novel optics-based imaging tools.

I realized my passion for optical imaging design through my first undergraduate research project at the Diffuse Optical Spectroscopic Imaging (DOSI) laboratory at the University of California, Irvine. My project redesigned the existing non-invasive breast cancer monitoring optical system used in the lab's medical clinic into a portable, low-cost device using off-the-shelf components and 4G LTE telecommunications technology. I was the primary researcher for characterizing and validating the hardware, synchronizing the components together, and programming the device's firmware and user interface for automatic data acquisition. As a second year biomedical engineering undergraduate with only basic knowledge of programming and electronics, this project challenged and motivated me to seek additional training in Java, C++, and LabView programming languages as well as advanced circuit design courses through MIT opencourseware. Over the two years I spent developing and refining this benchtop prototype, I found myself loving the hours spent scouring over hardware component data sheets, developing the most efficient firmware program designs, and troubleshooting this diffuse optical device to work reliably for clinical use. When I finished a prototype providing equivalent measurements to the lab's clinical system with faster imaging speeds and lower cost components occupying one-fourth the existing system's size, I felt validated in my own abilities for building new technology and felt an urge to continue exploring optical device development in my future. My talks with clinicians in both my daily lab work and during my two presentations of this technology in undergraduate research symposiums showed me the need for small-scale non-invasive optical systems in medical facilities where device space is limited and maintaining patient safety is critical. These factors ignited a passion for optical device development and medical-related research for my future career.

I gained further insight on the potential applications of optical instruments in medicine through my undergraduate senior design team project. My team investigated the use of my prototype as an affordable *in vitro* diagnostics platform for detecting *Helicobacter pylori*, a bacteria causing stomach cancer and peptic ulcers in infected patients. This project's goal was to remove ratio mass spectrometry and carbon isotopes in standard *H. pylori* diagnostic methods through the detection of small ammonia concentration changes in a patient's breath from the bacteria using diffuse optics. The high cost and limited availability of these existing diagnostic techniques create significant barriers in developing countries where *H. pylori* infections are most prevalent. While the full scope of this proof-of-concept project could not be completed in the one-year project timeframe, my group showed promising results in detecting parts-per-million ammonia concentration changes using low-cost diffuse optical technology that allowed the project to be funded and continued within the lab by other researchers. As team leader, this project taught me how to perform group research and effectively delegate various research tasks, develop and adhere to timelines to maintain research progress, and maintain constant, effective communication between all group members to keep everyone updated regarding the project's status. In addition, researching on the significant need for low-cost medical diagnostic technologies in developing countries and how optics could fill this gap inspired me to pursue this realm of research in my graduate career. This field has a number of substantial unmet needs that I could make an impact on through pursuing an advanced science career.

The final factor in my decision to pursue a PhD was my post-graduation position, where I served as the project manager for a joint project between the DOSI lab and LG Electronics. This

project's goal was to design a commercially-viable DOSI system, test this new technology for evaluating chemotherapy treatment techniques in eradicating breast cancer through the noninvasive monitoring of tumor tissue composition, and prepare the device for FDA-regulated clinical trials. I was hired to both perform research for this system's development and act as the project manager for the lab's portion of the project. In my own research, I contributed to the physical construction of new systems, programming the next-generation system's firmware, and validating VCSEL-based light sources and new avalanche photodiode detectors for improving the next-generation system's sensitivity to minute changes in the breast tumor molecular composition. The project management part of my position improved upon my leadership abilities developed from my senior design project by requiring clear communication with non-native English speaking researchers in a different time zone and rigid enforcement of research deadlines to match the fast-paced requirements of industry. From performing both research and project management simultaneously, I realized that I could effectively handle the rigorous schedule and time management required in graduate school.

This project with LG solidified my decision to return to graduate school because I realized a gap existed in my abilities as an engineer and researcher. This position added to my existing expertise in optimizing developed optical technology and time management skills, but I did not feel I was gaining the experience to develop novel optical systems for medical applications from first principles and fundamental research. I realized I needed the extensive knowledge basis on optics and electronics and the research skills that only a PhD could provide.

The first step in achieving this goal through my graduate school career will be through my work with Dr. Lei Tian and Dr. Selim Unlu at Boston University. As explained in my research proposal, I believe the synthesis of Dr. Tian's research on computational microscopy and angled illumination techniques with Dr. Unlu's nanoparticle interferometric sensing technology can create a high-throughput, high sensitivity virus and disease diagnostic platform. I will utilize my knowledge from my work in electronics and optical hardware design as a baseline for this project upon which I can fully develop this system through the knowledge and experience gained in my PhD career. I will utilize this NSF fellowship to develop this combined platform, improve its design, and investigate the potential diagnostic applications of this system for the medical industry.

Intellectual Merit

One of the side projects I performed during my undergraduate career established a correlation between MRI and DOSI-based breast density measurements. Prior research showed a relationship between highly dense breast tissue and increased cancer risk, and this research attempted to establish an alternative quantitative optics-based density measurement tool to MRI to help in rapidly evaluating a patient's risk of cancer development. My contribution in calculating breast density from MRI images helped show a correlation between the techniques and received publication in Breast Cancer Research.

My work in testing VCSEL light sources for their amplitude modulation capabilities during the LG project contributed to a conference proceeding in the 2015 SPIE BiOS conference. This work illustrated that VCSELs could be amplitude-modulated at RF frequencies equivalent to standard laser diodes while consuming less power at a cheaper overall cost, thus allowing their use in new DOSI technology iterations. In addition, my post-bachelor's work with LG Electronics where I evaluated the existing clinical system for its short term multi-hour and long term multi-year stability in detecting tissue optical properties with high specificity is in the final editing stages prior to submission for publication.

The post-bachelor's research I performed continues aiding current scientific and medical research through the DOSI lab's multi-center breast cancer monitoring clinical trial. This trial is ongoing in eight different locations around the United States, and I organized the manufacturing, programming, troubleshooting, and validating for these diffuse optical systems prior to their shipment to each location. I am particularly proud of this achievement, as my work enables other scientists and researchers to continue ongoing research determining the effectiveness of diffuse optics in monitoring breast cancer hemodynamics during chemotherapy and utilize this technology for new clinical research applications around the country.

Broader Impacts

In addition to my direct research contributions to the scientific community, my work allowed me to mentor a biomedical senior design team during the 2015-2016 academic year. I advised the team on electronics hardware component selection, microcontroller programming, laser characterization techniques, and other aspects of their project. This team developed a prototype low-cost optics-based tissue hydration sensor and won $14,000 to continue developing the device and start a company through the BioEngine Fellowship. This experience was very rewarding because I had immediate validation that my contribution of knowledge on optical and electronic system design helped this group achieve their own success in their scientific careers and start a business that could bring the technology to the public.

The most significant contribution of my past research to society has been through my development of new optical systems for the DOSI lab's multi-center clinical trial and my work with LG Electronics. The systems I helped develop for the multi-center trial enables higher through-put on obtaining breast cancer patient measurements during chemotherapy. This faster accumulation of data provides greater support for the existing findings from the technology, allows new cancer types and chemotherapy treatments to be monitored at multiple clinic locations, and supplies additional evidence supporting this technology as a viable medical device for the eventual FDA approval process. My contribution aids in the system validation stage for the long-term goal of bringing this device into the doctor's office as a common cancer hemodynamics monitoring tool.

Future Goals

My next step after my PhD will be new optical device development in the medical industry. Should my proposed device be successful, I will investigate the possibility of starting a new company commercializing this design. My goal would be to develop an accurate, label-free, high-throughput diagnostic platform for infectious disease identification marketed initially to developed countries with available funding for new medical devices. Once established, the company would design low-cost iterations of the system for developing countries. These regions have higher disease infection rates, little to no medical technology for diagnostic tests, and sparse funding for medical supplies and tools. Given this platform's simple design, producing a low-cost device would be feasible and would enable the technology to reach the people most in need of diagnostic tests. After industry, I would like to return to academia as a professor after working in industry to pass my knowledge onto the next generation.

I believe the NSF fellowship would provide me with the freedom to research this topic and develop the best possible optical imaging system for improving medical diagnostic technology.