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].

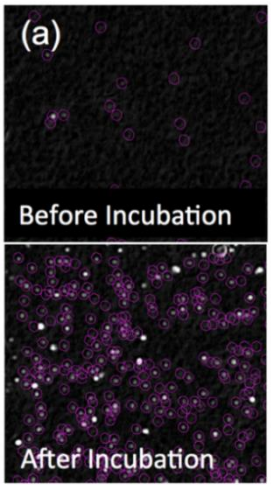

Figure 1: Label-Free Virus Particle Visualization with SP-IRIS Microscope [1]

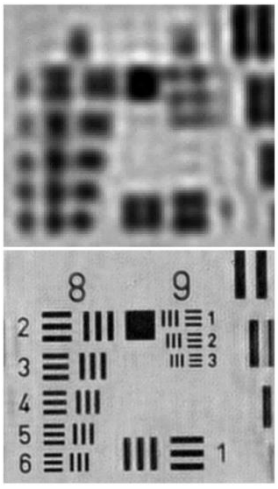

Figure 2: Standard Microscope (Top) and FP-Reconstructed (Bottom) Image [3]

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