

Personal Statement: Nathan C. Lindquist

Pursuing a career in science or engineering was never a difficult decision, but rather one of which field to study. All science did, and still does, interest me. Growing up, the television shows I liked were ones like *Newton's Apple*, *Nova*, or *Bill Nye the Science Guy*. And my favorite birthday present was any LEGO™ set or chemistry kit. But in high school, it was my physics teacher who really encouraged me to study the most basic of sciences, and I went to college to become a physics major. My undergraduate mentor, Dr. Richard W. Peterson, former president of the American Association of Physics Teachers, was the one who really inspired me to learn as much physics as I could, to apply myself to experiments, lab work and research, and to take seriously the role of a scientist as a teacher and leader. I remember that much of my physics work was “fun,” to the bewilderment of my classmates, who ended up voting for me to be the president of our local chapter of Sigma Pi Sigma, the physics honors society.

As an undergraduate, two experiences proved very important to the development of my scientific career. The first was when I was chosen to be part of a pilot NSF funded REU program at CERN, in Geneva, Switzerland through the University of Michigan in Ann Arbor during the summer of 2001. There I was able to experience large-scale physics collaboration first hand, learn the importance of international scientific cooperation, and begin to see myself as part of a “bigger picture,” and not only as an individual hovering over my own small projects. The worldwide scientific community is very important, something that was somewhat difficult to discern as an undergraduate at a small liberal arts college. Another important experience was during my senior year, when I chose to study the phenomenon of *sonoluminescence*, a peculiar effect that manifests itself as the conversion of sound into light, all taking place in the heart of a tiny air bubble floating in water. This year-long project was full of experimental challenges, but finally a whole lot of satisfaction the first time everything worked right, and the tiny bubble began to glow. Dr. Peterson told me that I had “seen the light,” and I realized that I needed to pursue advanced study, to keep learning, and to keep looking for those discovery moments.

In September 2003 I began a PhD program in the physics department at the University of Minnesota. There I completed my basic physics graduate coursework, passed my qualifying exams, and started my research working in a solid-state physics group studying liquid crystal compounds. However, in May 2005 after receiving my Master's degree, my wife needed an internship to finish her Master's degree. So we left to live and work in rural Mexico for one full year, as English teachers and science and math tutors. It was during that year, and during some previous summers working as an intern at *Medtronic, Inc.*, when I decided that my real interests were not in fundamental physics, but rather in a more applied and interdisciplinary field. So in coming back to the University of Minnesota, I began looking for a research group where I could learn something really new and exciting—something that would perhaps have a broader impact to society. I decided to focus on biotechnology.

Now, I'm studying for my PhD in the department of Electrical Engineering, in collaboration with the BioTechnology Institute and groups from Chemistry and Chemical Engineering. This is an exciting new area of study—microfluidic biotechnology and optical biosensing. I am eager to extend my fundamental physics knowledge to important biological and medical problems. I believe that modern biotechnology can be a key motivator for a better world. My long-term career goals, either in industry or academia, now very much involve understanding the problems of biotechnology, medicine, systems biology, and proteomics—and finding ways to solve them. A Doctoral Dissertation Fellowship would enable me to finish the research I've begun and to work with continued support. I thank you for your consideration.

Metallic Nano-Structures and Surface Plasmon Resonance for Optical Biosensing

Nathan C. Lindquist

Background

Thin metal films play important roles in countless applications, from electrical connections in computer chips, to highly reflective mirrors. One attribute that makes a metal film so versatile is its high concentration of electrons, which can be set into an oscillating motion by an external electromagnetic field. My research focuses on one class of electron oscillations in noble metals—gold or silver—known as Surface Plasmon (SP) waves, or Surface Plasmon Resonance (SPR). An SP wave is a sloshing motion of the electrons on the surface of the metal, like a water ripple on a pond. With proper nanostructuring, incident light—which is an electromagnetic field—is channeled to set these electrons in motion. The SP energy is concentrated within 100 nanometers of the metal surface, probing the local optical refractive index with high precision. In my research, we have engineered these metallic nanostructures to exhibit unusual optical properties not found in natural materials. Such novel “plasmonic” materials are broadly applicable and useful, in particular, for SPR biosensing [1-6].

The capability to analyze complex biomolecular interactions in a quantitative, high-throughput manner is the prerequisite to many important medical applications. For example, to test each of the tens of thousands of human proteins against a single new drug molecule in a reasonable amount of time requires a high capacity, high precision instrument. Exploiting SPR is an attractive path towards such a goal [7]. When molecules bind to the metal surface, the SP wave is perturbed. This can be monitored in real-time. Furthermore, **in contrast to prior efforts**, SPR measurements can be done without fluorescently labeling the molecules, which can modify their biological function. Conventional SPR instruments such as BIAcore™ (GE Healthcare), however, have **limited sensitivity** for detecting small biomolecules, **limited throughput and resolution**, and require **expensive**, bulky optics. Given this, a new class of SPR biosensors is emerging based on the recent discovery [8] of Extraordinary Optical Transmission (EOT) through a gold film perforated with periodic arrays of nanopores. SP waves oscillating in and around the nanopores modify the color of light that is transmitted through the nanopores. Therefore, the presence of molecules in and around the nanopores is easily detected (Fig. 1). In contrast to BIAcore™, the nanopore SPR sensor provides a number of clear advantages: (1) the transmission measurement setup **simplifies the optical design**, alignment and imaging, **reducing the cost** of the system; (2) each nanopore has an extremely small footprint, enabling **high-resolution and high-throughput** sensing; (3) the unique geometry of the nanopores enable even more **novel detection schemes**, such as suspending a cell membrane over each nanopore [6] to study transmembrane proteins, which are the **single most important class of drug targets**.

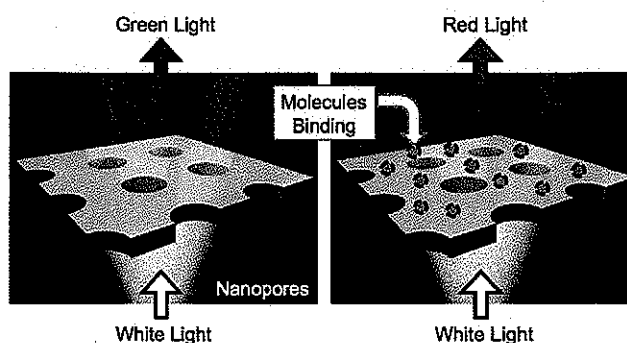


Figure 1. Nanopore array molecular sensing scheme.

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Goals and Objectives

In this work, new high-throughput SPR sensing technologies will be developed, in several stages. **First**, a low-cost, high-sensitivity system capable of real-time protein-protein binding measurements was developed [1], as these measurements are key to developing new drug molecules that bind strongly to target proteins. **Second**, since currently available technologies

can spot more than 3,000 proteins on a glass slide [9], we developed an SPR imaging platform, capable of measuring thousands of spots simultaneously [2-5]. **Third**, for studying the tens of thousands of human proteins, or where only a very small amount of molecules are available, a very small sensor area is needed. Due to the propagation of the SP waves on the gold surface, methods are required to control the SP waves [10], blocking sensor-to-sensor interference [11], and maintaining high-sensitivity [4]. **Fourth**, extensive computational modeling and simulations, in conjunction with further experimental characterization, will be used to push the limits of sensitivity and imaging resolution. **Fifth**, novel nanofabrication techniques will be employed, since currently used nanofabrication techniques are for proof-of-concept manufacturing only, and not appropriate for large-scale systems. **Finally**, nanopore SPR sensing substrates will be used to study transmembrane proteins [6], and other clinically relevant proteins or DNA.

Design and Methodology

As an SP wave propagates along the gold surface, its wavelength changes when it encounters any thin layer of molecules. By first anchoring capture molecules to the gold surface, it is possible to measure the binding strengths between the immobilized proteins and drug molecules flowing over the surface by monitoring the behavior of the SP waves. Our sensing mechanism is based on the extraordinary optical transmission (EOT) effect [8]. Each nanopore (150 nm diameter) milled through a thin gold film (200 nm thick) captures and scatters incident light, efficiently creating SP waves. When the nanopores are arranged in a periodic lattice, at certain wavelengths these SP waves constructively add together and intensify, “funneling” through the nanopores far more efficiently than the incident light. Molecules on the gold surface sharply modulate the propagation and funneling behavior of these SP waves. By measuring the color of the “funneled” light, molecular binding events can be monitored in real time and their binding strengths measured. A thin gold film patterned with thousands of nanopores (Fig. 2) offers the unprecedented capability of measuring the interactions between thousands of human proteins.

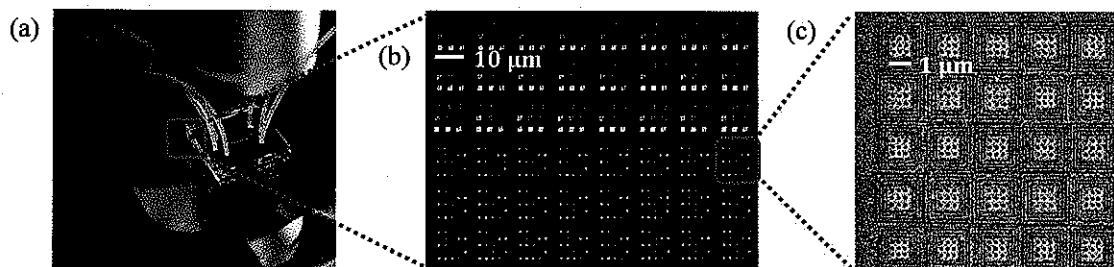


Figure 2. Nanopore array substrate: (a) Microscope and microfluidics setup. (b) Light transmission through nanopore arrays. (c) Zoomed electron microscope image showing the many nanopore arrays.

The brightness of each SPR “pixel” (Fig. 2b) changes in real time as proteins bind to the gold surface. Each small group of pixels, or sensing spots on the substrate, can be covered with unique capture proteins. As candidate drug molecules or other molecules flow over the surface, an image is taken every few seconds to simultaneously record the changing brightness from every sample spot. Such multiplex measurements provide a vast amount of information involving thousands of protein-protein interactions, their kinetic behavior and binding strengths.

Potential Significance of the Research

The ability to build a **low-cost, high-resolution, high-throughput** SPR system for protein-protein interactions or transmembrane protein research will have broad impact on biological researchers, many of whom may not easily afford commercial SPR instruments that can cost \$500k and don’t have high-throughput, high-resolution capability, or who cannot easily build and align the complicated optics themselves. Our research can also have an impact on the broader

areas of systems biology, proteomics (studying of the set of all proteins), and high-throughput characterization of antigen-antibody binding kinetics. If successful, our platform will give the **unprecedented capability** to study thousands of protein interactions and binding strengths with target molecules, key to **new drug discovery, disease research and clinical diagnostics**.

Progress to Date and Schedule for Completion

To date, we've made significant progress [1-6,10,11]. Our recent work on sub-micron resolution SPR imaging was featured as a cover article [4, *see below*], and was one of the top ten most accessed papers in January 2009 (<http://www.rsc.org/loc>). Fig. 3a shows sample real-time biomolecular binding data from several sensor spots on one of our samples. However, there is still work to be done, especially in three areas (outlined as objectives **four, five** and **six** above):

(1) More extensive computational characterizations are needed to push the limits of sensitivity and sensor design (Fig. 3b). (2) Large-area nanofabrication techniques need to be developed. We are currently exploring nanosphere (a)

lithography, but another option is nanoimprint lithography. (3) Beyond the basic sets of biomolecules that we have been using (biotin and streptavidin) and developing important proof-of-concept experiments [1,4,6], our research, in collaboration with other groups, will soon involve more

clinically relevant biomolecules, such as transmembrane proteins. This will broaden the impact of our work; and demonstrate the nanopore SPR sensing platform as a viable instrument for high-throughput, label-free (no fluorescence tags), real-time biomolecular assays. I've begun working towards these final three goals, and to graduate, will finish them by summer 2010.

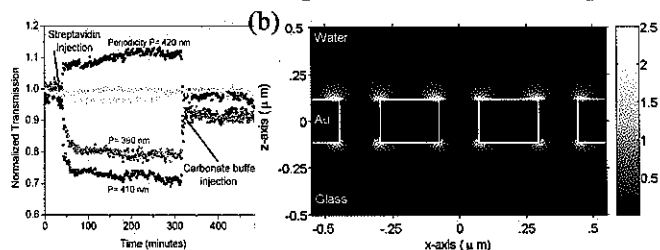


Figure 3. (a) Sample real-time data. (b) Computational modeling of the surface plasmon wave intensity in an array of nanopores.

Key References

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