Nanohole Arrays in Ultra-Low Concentration Biosensing

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***Abstract*— Millions of medical tests are performed annually in the United States. However, current medical testing infrastructure and methods are prone to delays and errors. Biosensing using plasmonic nanohole arrays (NHA) can meet the demand for accurate and accessible testing methods. NHAs are metallic nanoscale structures with voids that allow nano-sized particles to pass through while accumulating biomarkers on its surface. Using finite-difference time-domain (FDTD) simulations, a method to quantify electrodynamic interactions, researchers can observe the presence and concentration of accumulated biomarkers accurately and efficiently. This technique enables the detection of a variety of biomarkers, such as immunoglobulins and antigens, at ultralow concentrations. These detection results can be interpreted by medical professionals to diagnose diseases at early stages.**

I. Introduction

Medical diagnoses are often the first step in treating or preventing medical complications. Yet, up to 12 million (~5%) medical cases are estimated to be misdiagnosed annually, ~13.5% of them being infectious diseases [1]. Extant research suggests that biosensing, the detection of biological particles with an analytical device, is more rapid and accurate using plasmonic nanohole arrays (NHAs) compared to existing diagnostic technologies such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISA) [2,3].

The structure of NHAs [Fig. 1] consists of a silicon nitride substrate coated in a gold metal film with an evenly spaced array of cylindrical voids [Fig. 2][2,3]. Yanik et al. detected a variety of DNA and RNA viruses with minimal processing of samples using this structure, displaying the simplicity and versatility of biosensing with NHAs [2]. Their experiments showed that NHAs are capable of detecting a large range of particles at ultralow concentrations without complex purification processes [2]. More recent experiments have further optimized the silicon nitride layer by creating a silicon wafer patterned with UV light and coated in a light-sensitive material [3]. These new innovations in silicon technology suggest NHAs are a viable solution to medical testing [2,3].

Zhu et al. further observed the behavior of electromagnetic phenomena existing on the NHAs [3]. They affirmed that the conductive coating on the NHA interacts with electrons in biomolecules to create propagating high-energy electromagnetic waves called surface plasmon polaritons (SPP) [3]. The frequency of these waves varies based on the refractive index (RI) of the accumulated particles [3]. When photons interact with SPPs propagating on the underside of the NHA, the transmission of light through the NHA is enhanced, resulting in a phenomenon known as extraordinary optical transmission (EOT) [2-4]. The EOT effect allows researchers to observe and measure light transmission [2,3]. Unlike traditional photonic biosensors, these plasmonic devices can couple incident light directly, enabling researchers to observe EOT as the light exits the NHA [2].

Additionally, Zhu et al. utilized the finite-difference time-domain technique (FDTD) to observe the levels of light transmission that result from bioaccumulation of varying RI [3]. Novel FDTD simulations enabled the measurement of light transmission without fabrication and the use of an electron microscope for experiments [3]. In this method, variables are isolated and time is discretized, allowing for the direct measurement of light transmission without effect from other variables [3]. Furthermore, spectral shifts of light transmission at differing RIs were credited to an effect called red shifting [2,3,5]. As the RI of the bioaccumulation increases, the velocity of incident light decreases, causing the light to stretch and increase in wavelength [5]. Based on these shifts, they found that specific RIs could be matched to various bioparticles and further matched to the wavelength of transmitted light [2,3]. The higher the concentration of such particles, the more red shifting will occur [2,3].

There remains a limited understanding of these optical properties in experimental settings and possible limitations to wider applications of NHAs in biosensing since SPPs may only penetrate the depth of an accumulated particle [2,3]. In our research, we sought to apply novel FDTD simulation techniques to observe the transmission spectra and red shifts through an NHA in relation to different RIs. Our results affirming the optical behaviors of NHAs coupled with bioaccumulation of varying RIs support broader applications to medical testing and diagnostics.

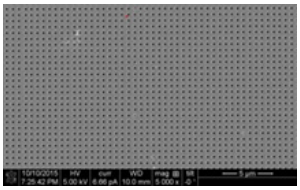

Figure 1: The surface of an NHA with a gold film and silicon nitride substrate is captured by a Scanning Electron Microscope (SEM).

II. METHODS

To predict the relationship between bioaccumulation, RIs, and light transmission in an experimental setting, we modeled and simulated an NHA in Lumerical Inc. FDTD Simulator. Our design [Fig. 2], based on designs of Zhu et al., consisted of a 125 nm thick conductive gold film placed above a 100nm thick substrate. Like previously completed simulations [3], the RI of the environment surrounding the NHA was set to 1.33, that of water. Water suspension reflects all escaped light internally, preventing any light from escaping the system [3].

A 3-by-3 nanohole array was evenly spaced across the plane and situated so its height would match that of the sensor. The light source was set along the negative z-axis to model the injection of light on a sensor when studied under an electron microscope. An FDTD calculator was applied normal to the y-axis to detect the light transmissions within our tests. Finally, an 8nm thick protein layer was added to the surface of the gold layer and nanoholes. This simulates the accumulation of particles taken from a biological sample. Light transmission was recorded from a series of simulations with protein layers of different RIs. FDTD analyses were utilized to compute light transmission within each simulation scenario.

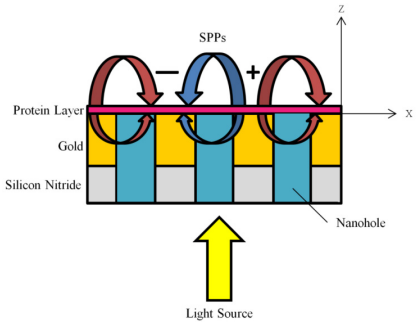

Figure 2: SPPs are illustrated at the metal-dielectric interface. A light source is injected from the negative z direction. Direction of light does not affect the behavior of SPPs or EOT, but it must be normal to the y-axis.

III. Results

After adjusting RIs in multiple trials, we observed differing transmission peaks, confirming that we can diagnose a biological irregularity using NHA. Figure 3 displays the wavelength results of our FDTD simulations, measured in nanometers (nm). Without protein accumulation (green), the maximum light transmission was observed at 685 nm. This means the wavelength of transmitted light without bioaccumulation measured 685 nm. When the refractive index of the simulated protein layer measured 1.53 (blue), 1.6 (red), and 1.7 (teal), the transmission peaks were observed at 692nm, 694nm, and 698nm, respectively. Thus, when the bioaccumulation refractive index rose, the transmission peak moved further to the right.

We reason that red shifting causes transmitted light to increase in wavelength as the RI increases. We further deduce that as the incident light slows and refracts at greater angles due to red shifting, the EOT will decrease. This effect can be observed by the decreasing peak levels of transmission (measured in a.u.) of each respective curve. This means researchers can identify the RI of any biomarker with the wavelength of light transmitted through it. For example, if an immunoglobulin of RI 1.6 accumulates on a biosensor, it can be detected based on the wavelength of light it transmits. By comparing different levels of light transmission, biosensing with NHAs can inform the presence and concentrations of specific molecules. These detection results can be further utilized by medical professionals to diagnose diseases.

IV. Conclusions

Using the FDTD technique, NHAs are capable of discerning differences in transmission peaks between molecules and thus allow researchers to detect the presence of biological irregularities using known RIs. Our findings suggest that using FDTD to measure the EOT that originates from bioaccumulation on an NHA effectively distinguishes between samples with or without proteins present. This is supported by our FDTD simulations, which show the usability of NHAs in accurately differentiating between biological particles by comparing transmission peaks. Therefore, this method will allow for the detection of biological differences at a broad spectrum of wavelengths and a wide variety of particles. Additionally, published research [2] shows binding sites or receptors can be mobilized on NHAs to detect a variety of biological particles under ultra-low concentrations and within the same sample. This enables the detection of a wide variety of proteins and particles, informing the diagnosis of diseases before they become more serious. Once further optimization is reached, plasmonic biosensing could provide an effective and efficient way for doctors to determine the biological composition of samples, diagnosing and thus preventing future diseases.

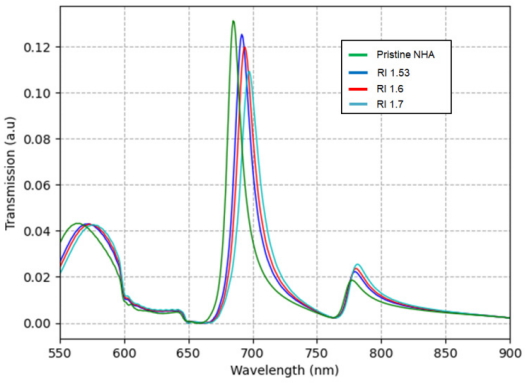

Figure 3: EOT peaks varied based on the RI of protein layers and wavelengths of light.

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