

Using Optical Fiber Tips as Biological Nanosensors

by

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Abstract

The capability of creating plasmonic nanostructures on the optical fiber tip with conventional nano-fabrication technologies would enable the transition of localized surface plasmon resonance (LSPR) based label-free biosensing technology from laboratory environment to field and space applications. The use of optical fiber platform for LSPR biosensing enables highly integrated and portable solutions for point-of-care applications in immunoassays and DNA hybridization assays. Multiple detections can be integrated into a single fiber bundle for parallel analyses. Moreover, this fiber probe is very suitable for remote sensing in space applications where savings in reagents are very valuable. In this report we present an approach to create arrays of metallic nanoantennas on the end facets of optical fiber utilizing planar substrate nanofabrication technologies such as electron beam lithography and lift-off processes. Compared to commercial surface plasmon resonance (SPR) sensing systems (Biacore AB), our proposed device offers several advantages, including (1) it can be inserted into the fluids and specimens for in situ chemical and biological detection, (2) the fiber device can be used as a SERS probe, a function not found in conventional SPR detection systems, and (3) it allows remote sensing. The fabrication methods allow rapid and inexpensive prototypes of nanostructures on optical fiber tip. Highly specific and sensitive detection of short strand DNA (ssDNA) using this fiber-optic label-free biosensor will be presented.

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Department Chair/ Advisor (signature) Date

Honors College Director (signature) Date

Introduction

A biosensor is a device capable of sensing biochemical reactions within a solution it comes in direct contact with.

The components of a biosensor should include the analyte studied, the sample deliverance, the detection, and the signal. For this experiment, the biosensor in study was used to detect the binding of strands of corresponding DNA. The delivery of the sample was done by varying the concentrations of target DNA used. The detection of the sample was done by the input of light reflected into the spectrometer and displayed using the computer program, Ocean Optics SpectraSuite, as a wavelength curve. The DNA under study was comprised of two sets of corresponding DNA strands, NEG and ARC. Each DNA oligonucleotide was 20 base pairs long with the probe DNA strands containing a thiol group attached to the 5' end. This thiol group has a large affinity for the gold nanodots on the sensor tip and would form the strong sulfur bonds used to attach the probe to the tip. Once this bond is made, a shift in the wavelength of light detected should occur and ideally, the shift should be around 10nm. Once the probe is bound to the gold, the tip can be submerged into the corresponding target DNA. If the probe DNA binds to the matching target DNA, a shift in the light wavelength should be detected. In other words, the NEG probe should only bind to NEG target and ARC probe should only bind to ARC target.

In order to establish proof that the probe DNA correctly adheres with the correct target DNA, control experiments would be performed. The probe DNA would be submerged into junk DNA obtained from HudsonAlpha. This junk DNA, Herring Sperm DNA, would possibly have nonspecific site binding to the probe DNA but should result in little to no significant wavelength shift. As another control experiment, the probe DNA would be submerged into the opposite target DNA. The NEG probe would be introduced to the ARC target and the ARC probe introduced to the NEG target. Similarly, this would also create

nonspecific site binding to the probe DNA but should also not result in significant wavelength shifts.

Materials and Methods

Materials

All reagents used in experimentation were of chemical grade. The two sets of DNA used, NEG and ARC, were obtained from Sigma Aldrich. The NEG probe DNA had a base pair sequence of 5'-/5ThiolMC6 -D/TGT AGA AAA ATA ACC GGT TG- and the ARC probe DNA had a base pair sequence of 5'-/5ThiolMC6-D/GTG CTC CCC CGC CAA TTC CT-. Each probe DNA had its own corresponding target DNA so that the NEG probe binds with NEG target and ARC probe binds with ARC target. The buffer used for the solution of the target DNA, SSPE buffer, was obtained from HudsonAlpha. For the control experiments, Herring Sperm DNA at 10 mg/mL was given from HudsonAlpha from Trevigen, Inc. The sperm DNA was diluted to 0.1 mg/mL with PbS buffer. This buffer, PbS, was obtained from Sigma Aldrich. The chemical grade solvents used for cleaning the tip consisted of ethanol, acetone, methanol, isopropyl alcohol, and deionized water. These were obtained from the University of Alabama in Huntsville.

Methods

Experimental Setup

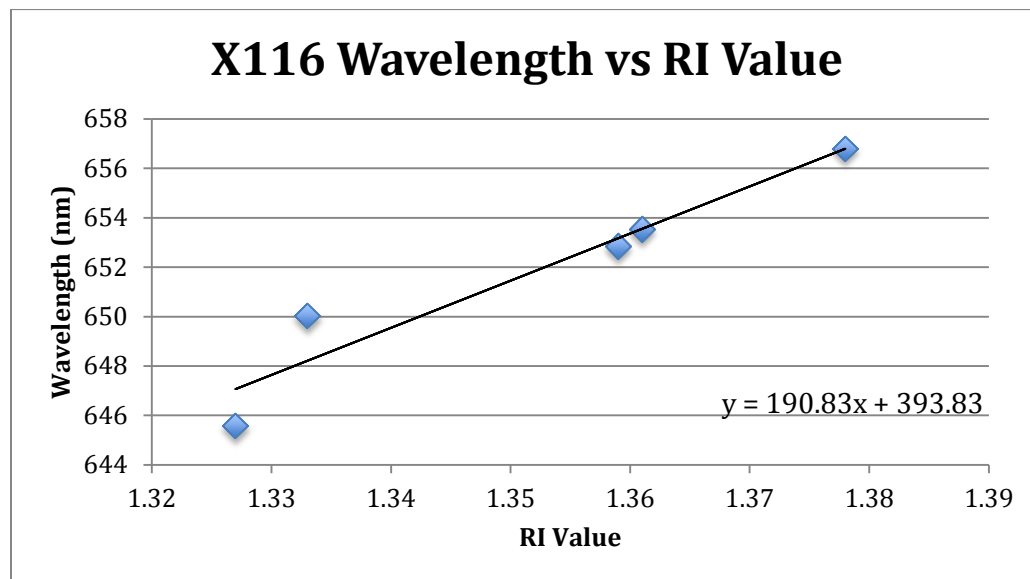
The experimental setup consisted of four major parts including the coupler, the spectrometer, the sensor tip stand, and the light stand. The coupler was connected to three items: the halogen light source, the spectrometer, and the sensor tip. The spectrometer was connected to a Dell PC computer and used the program, Ocean Optics SpectraSuite, to display the spectrum. The halogen light source was put on a metal stand and the sensor tip had a separate stand to suspend the tip above a clamp.

Obtaining a baseline

Before starting the recorded experiment, the sensor tip must be cleaned of any impurities or other contaminants. The sensor tip is rinsed in ethanol to clean the tip. A

baseline wavelength is achieved if the sensor tip returns to this wavelength three times after being washed in deionized water. If the tip does not return to this line, acetone and isopropyl alcohol may be used to further clean the tip of lingering impurities.

Sensor Tip Sensitivity



Graph 1: Sensitivity Graph of tip X116

The sensitivity of the sensor tip was determined by creating a graph of the wavelength of different materials plotted against their known refractive index number. The chemicals used in determining the sensitivity of the tip were acetone, methanol, ethanol, isopropyl alcohol, and water. The sensitivity would be used to determine if the sensor tip in experimentation would be perceptive enough to determine small wavelength shifts accurately.

Probing the tip

Both sets of probe DNA, NEG and ARC, were diluted to 100 microMolar concentration with deionized water. These diluted samples were stored in a freezer at -4°C while not being used. Seven microliters of probe were added to a microcentrifuge tube and clamped into place underneath the sensor tip. Rubber tubing was used seal the connection of the tip to the microcentrifuge tube in order to avoid evaporation of the DNA. Once the sensor tip was placed in the DNA solution, the setup was allowed to sit for 24 hours. After

this waiting period, the probed sensor tip was rinsed gently three times with deionized water. From the displayed spectrum, a MatLab program, Fit2Max, was used to determine the exact peak position of the transmittance spectrum.

Binding the Target

The original target DNA was diluted to 100 microMolar concentration with deionized water. These stock solutions were then diluted eleven times with SSPE buffer, giving twelve samples of each target ranging from concentrations of 100 microMolar to 1 femtoMolar. The probed tip used the corresponding target DNA for testing. First, the probed tip was placed in the smallest concentration of target for ten minutes and then rinsed gently three times with deionized water. The transmittance spectrum peak was recorded and, using the Fit2Max MatLab program, was directly pinpointed. The same tip, without any probe being being washed off, was placed directly into the second lowest concentration for ten minutes. This was then rinsed three times with deionized water and the transmittance spectrum peak was recorded. This procedure was used through all twelve dilutions of the target DNA. Once each peak was obtained and recorded, these could be compared to the probe peak to determine the total wavelength shift.

Testing Controls

For the opposite target DNA controls, a sample of each target DNA was diluted from 100microMolar to 10microMolar. The high concentration allows for an accurate control test, because at this concentration there should be a highly significant wavelength shift if binding occurs. A sensor tip probed with ARC solution was submerged in 10microMolar NEG target for 10 minutes, and a sensor tip probed with NEG solution was submerged in 10microMolar ARC target for 10 minutes. After 10 minutes elapsed, both tips were gently rinsed with water three times, and the transmittance spectrum peaks were recorded. The NEG target peak was compared to the ARCprobe peak, and the ARC probe peak was compared to the NEG probe peak, to determine if any binding occurred.

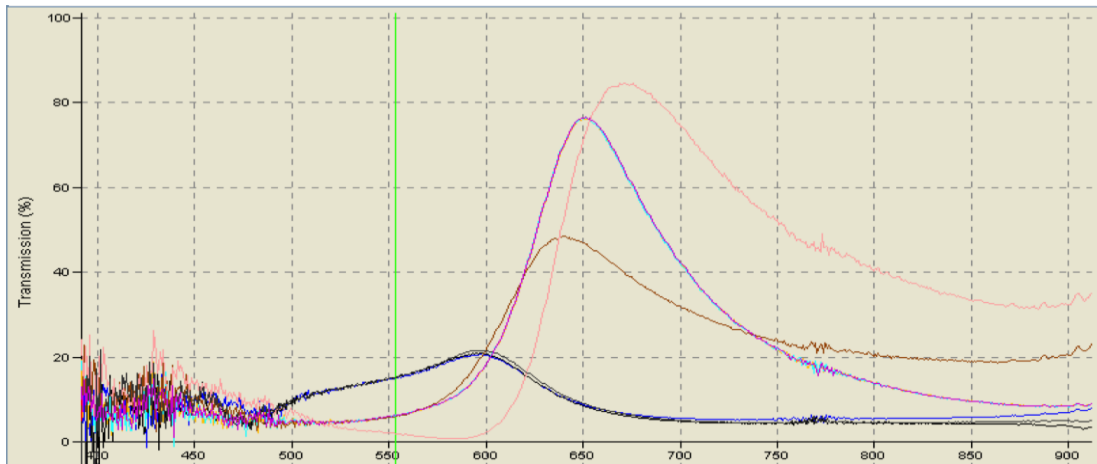
For the junk DNA control experiments, Herring Sperm DNA was used on both the ARC and NEG probe sequences. The original Herring Sperm DNA was diluted from 10mg/mL to 0.1mg/mL, using PbS buffer. A sensor tip probed with ARC probe was submerged in the Herring Sperm solution for 10 minutes. It was then rinsed gently three times with water, and the transmission spectrum peak was recorded and compared with the probe's peak. This process was repeated using the NEG probe.

Results and Discussion

Results

Numerical data was obtained from the transmission graph produced using the SpectraSuite program. The wavelength of reflected light at its peak position is calculated using the Fit2Max program in MatLab. This peak wavelength is dependent on the solution in which the sensor is immersed. As can be seen in the spectrum graph, the peak of the transmission curve for the sensor in water is approximately 50nm greater than the peak of the curve when the sensor is in air. This 50nm shift is expected to remain nearly constant and independent of the sensor tip being used. Other transmission curves seen in the spectrum graph are those of the sensor in a probe solution, in a control target solution, and in several dilutions of the corresponding target. Ideally, the control solution should not result in any significant change in peak wavelength and the probe solution should produce a peak wavelength approximately 10nm greater than that of the baseline. In the spectrum graph, it can be observed that the probe and control solutions behaved as expected, as the control overlay is overlapping that of the baseline, and the probe overlay is above and to the right of the baseline. With each target concentration used, the wavelength can be seen to

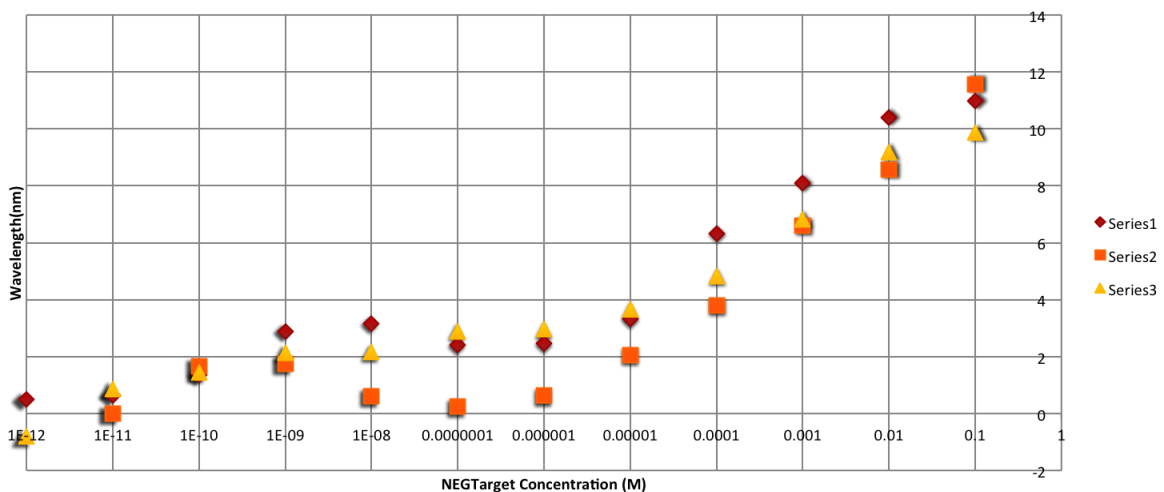
gradually shift to the right.



Graph 2: Sample Spectrum Graph

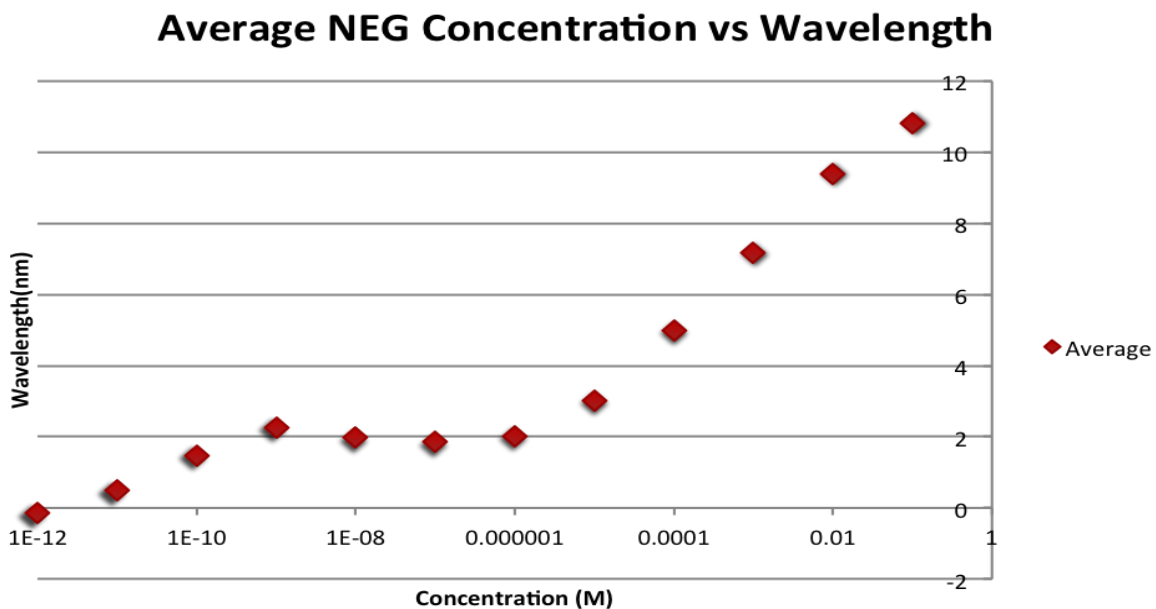
To determine binding of the NEG target to the NEG probe, the sensor was placed in NEG probe solution overnight, and then placed in a series of NEG dilutions ranging from concentrations of 1femtoM to 100microM. The NEG probe had an average wavelength shift of 4.882nm from the baseline for the three data sets. After all the target dilutions had been tested, a graph was plotted using the target concentration as the independent variable and peak wavelength shift being the dependent variable. The peak wavelength shift was calculated by finding the difference of the wavelength of the probed sensor tip from the wavelength of the probed sensor placed in the target solution tested. This graph allows for observations of the quantity of target to probe binding. The quantity of binding can best be observed by viewing the graph of average target binding for each dilution, as seen below.

NEG Concentration vs Wavelength Shift



Graph 3: Three experimental runs using the NEG Probe

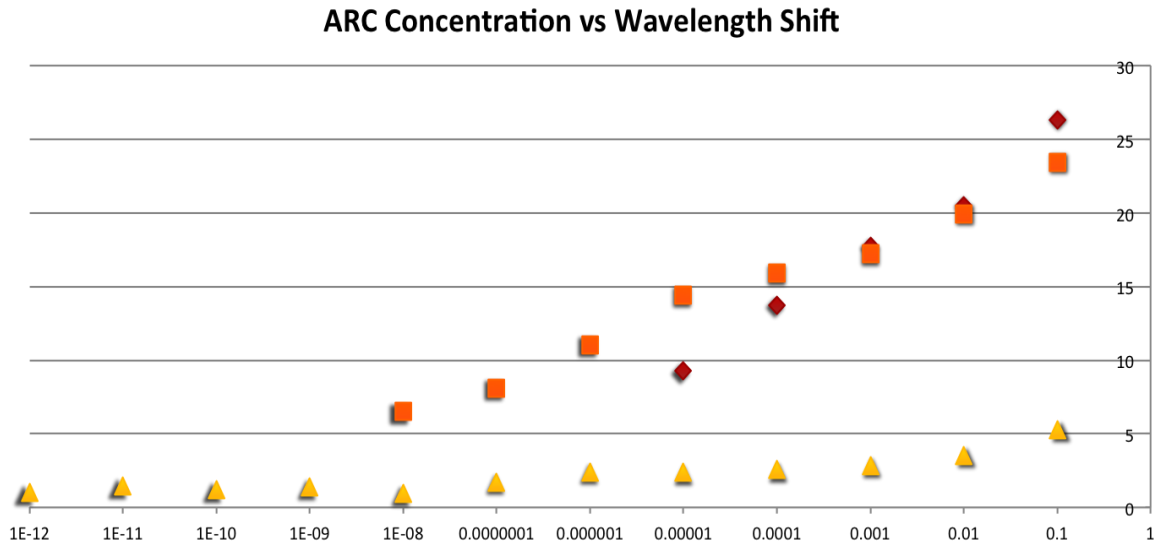
An average wavelength shift for each dilution was obtained by taking the averages from each of the three trials performed. The averages were also plotted against concentration. For the first four dilutions, 1femtoM to 1picoM, the data produced a constant slope suggesting binding was highly proportional to the concentration of target used. However, from 1picoM to 1nM, the slope decreased suggesting that less target was binding to the sensor with each concentration used. After the 1nM concentration, the slope increased and became as expected again. At the final concentration of 100microM, the curve appears to begin a slope resembling a plateau. This indicates that there has been the maximum amount of probe to target binding within the sensor.



Graph 4: Average of NEG Probe experiment

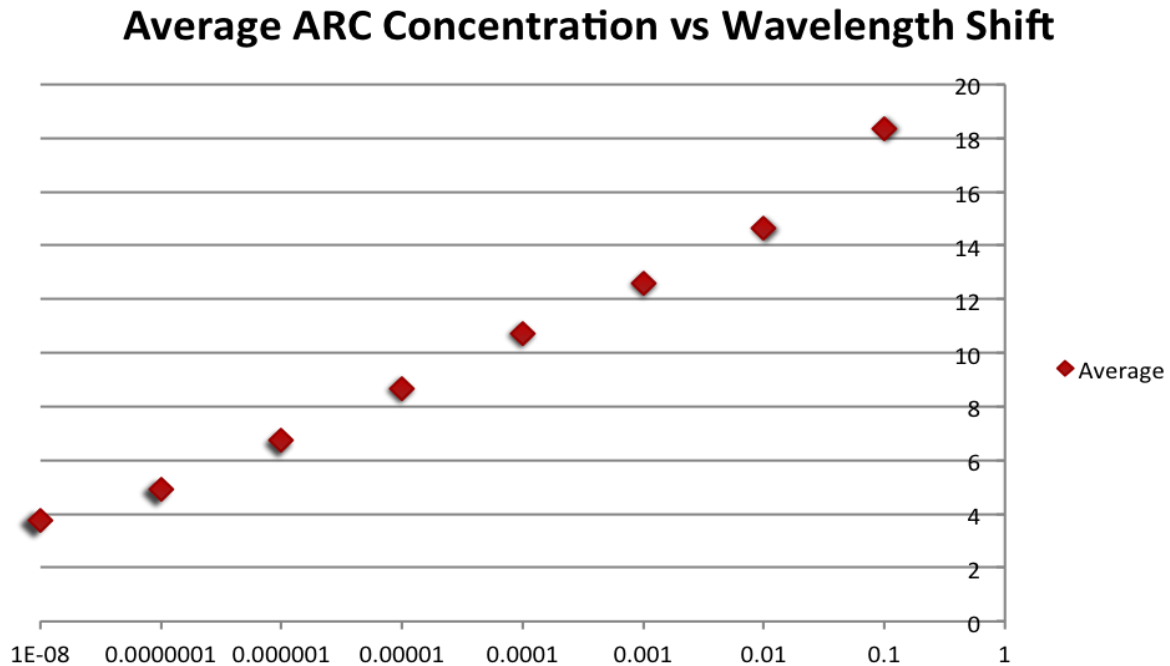
To determine binding of the ARC target to the ARC probe, the sensor was placed in ARC probe solution overnight, and then placed in a series of ARC dilutions ranging from concentrations of 1femtoM to 100microM. The ARC probe had an average wavelength shift of 5.485nm from the baseline for the three data sets. After the corresponding target dilutions were tested, a similar graph was made as with the NEG experiments, with

concentration plotted as the independent variable and peak wavelength shift as the dependent variable, as seen below.



Graph 5: Three experimental runs using ARC Probe

An average wavelength shift for each dilution was obtained by taking the averages from each of the three trials performed. The averages were also plotted against concentration.



Graph 6: Average of ARC Probe experiment

To establish proof that specific binding is occurring between each probe and its corresponding target, control experiments were performed. In the first control experiment, each probe sequence was submerged in a 10microMolar solution of the opposite target DNA. When the NEG probe was placed in ARC target, there was a shift of 0.315nm to the left of the probe's transmission spectrum peak. This number is insignificant, proving that the ARC target does not bind to the NEG probe. Similarly, when the ARC probe was placed in NEG target, there was a shift of 0.217nm to the right. This result is also insignificant, and therefore proves that the NEG target does not bind to the ARC probe. Since there is no significant shift in wavelength when the probes are placed in their opposite targets, but there is a significant shift when probes are placed in their corresponding targets, it can be concluded that there is binding only between corresponding DNA sequence sets.

In the second control experiment, both probe sequences were submerged in "junk" DNA. Herring Sperm DNA was used as junk DNA in this experiment. Submersion of the ARC probe in 0.1mg/mL Herring Sperm DNA solution resulted in a wavelength shift of 1.695nm to the right. This number is insignificant compared to the large amount of probe binding. Likewise, submersion of the NEG probe in the Herring Sperm DNA solution resulted in a wavelength shift of 0.117nm to the left. This number is insignificant, proving that there is little to no nonspecific binding of probe to DNA.

Conclusion

From the experiment, consistent binding was found in the NEG probe to NEG target sequence. Also noted was as the concentration of target increased, the shift in the light wavelength detected also increased. Control experiments were used successfully to prove that each probe only binds to its corresponding target. There is little to no nonspecific binding between probes and their opposite target, as well as between probes and other "junk" DNA sequences.