A Fiber-Based Optical Transducer for Wash-Free Analysis of Biomarkers

FINAL REPORT

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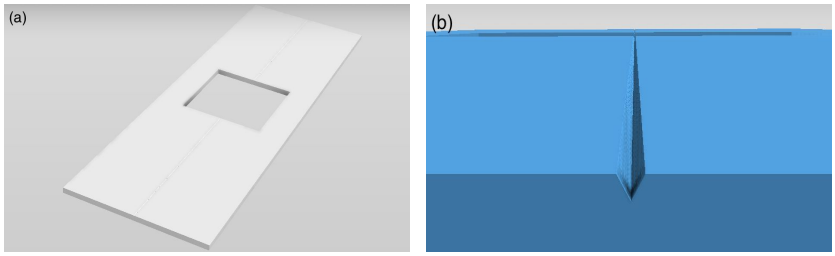

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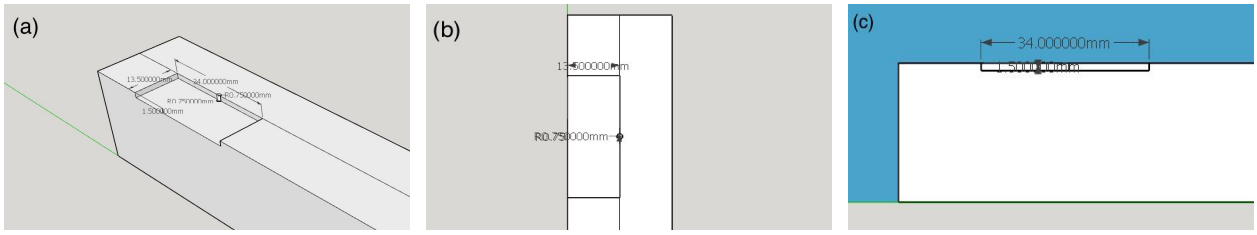

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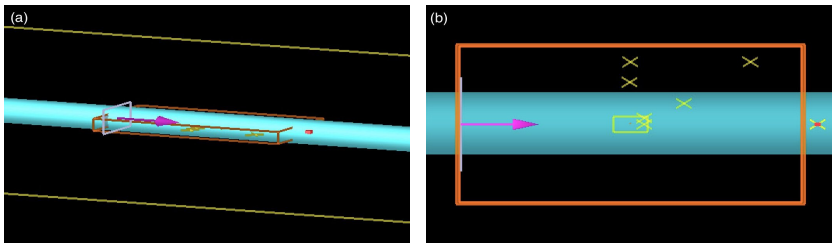
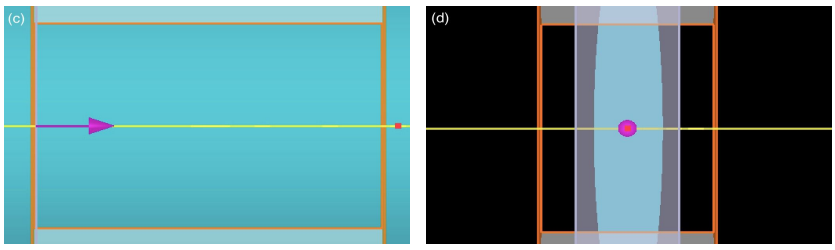


(a)3D version (b)Front view



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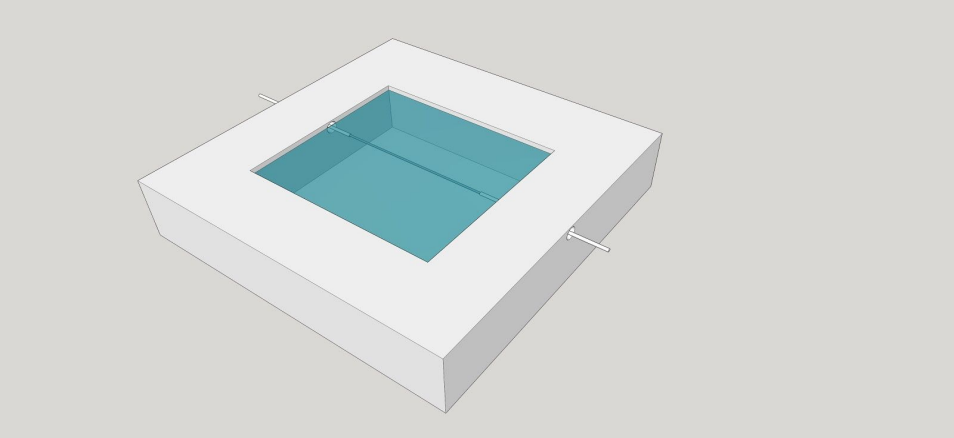

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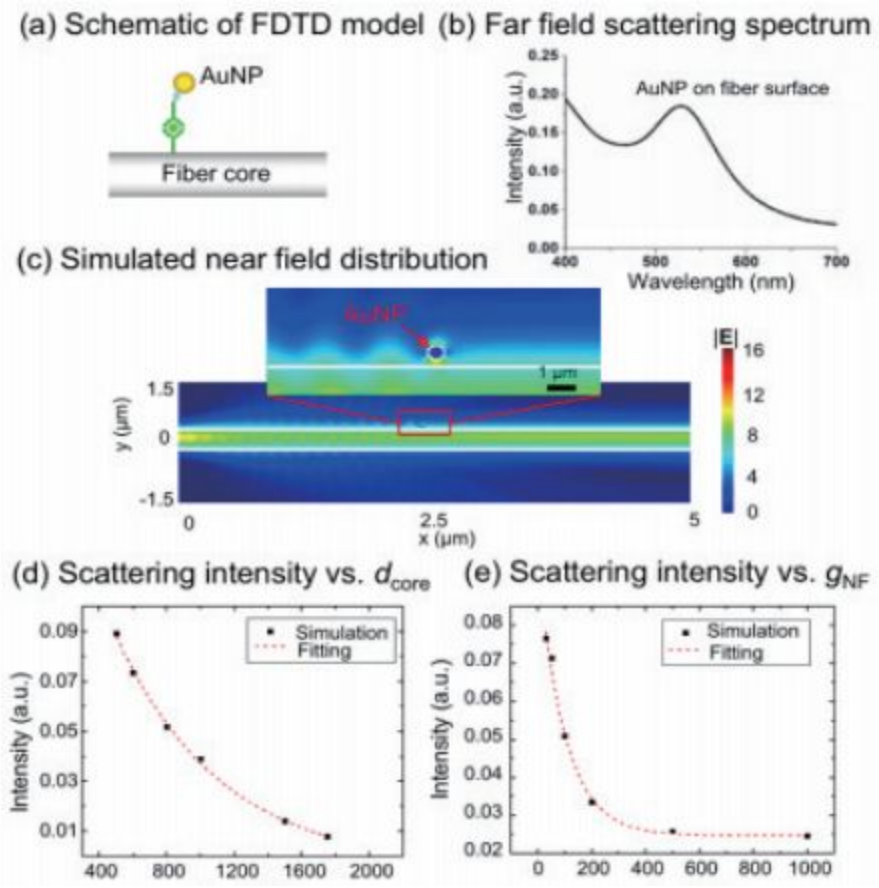

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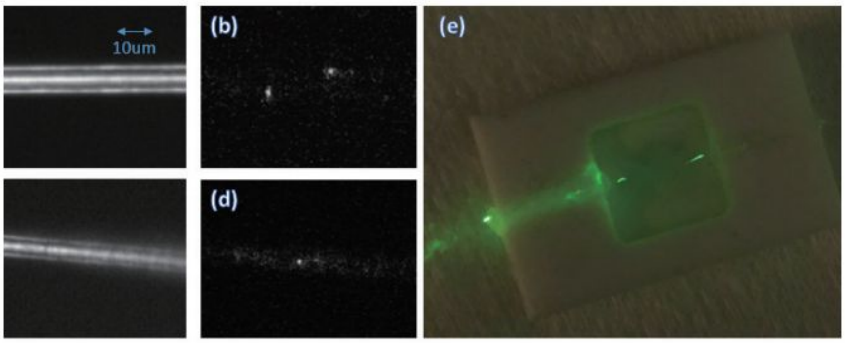
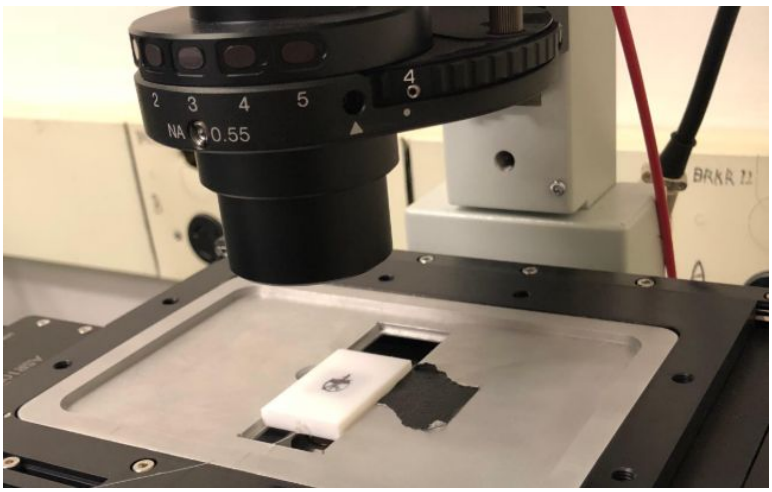


Figure 7. Image of the holder on the microscope we used for observing the light scattering by AuNP.



List of Definitions

Mechanotransduction: the process of that cells sense physical forces and translate them into biochemical and biological responses.

Optical fiber component (Thorlab single mode):

1. **Core**: the most inner silica with relatively high reflection index and radius of 8 micrometers.
2. **Cladding**: outer doped silica with relatively low reflection index and radius of 10 micrometers.
3. **Coating**: the most outer plastic cover.

Total internal reflection: the complete reflection of a light ray at the boundary of two media, when the ray is in the medium with greater refractive index.

Evanescent field: an oscillating field that does not propagate as an electromagnetic wave but whose energy is spatially concentrated in the vicinity of the source.

1 Introductory Material

1.1 Acknowledgement

This work was funded by the ECpE department at Iowa State University. All the chemicals used in this project were provided by the LIOS Research Group in ECpE department.

1.2 problem Statement

In this century, more and more people are diagnosed with cancers, which is a group of abnormal cell growthing and spreading to other parts of the body to cause the body cannot work functionally. In 2016, an estimated 1,685,210 new cases of cancer were diagnosed in the United States and 595,690 people died from the disease [1]. In the early stage of cancer, the patient can live longer if they could be diagnosed earlier, since the number of cancer cells is still relatively small in cancer's early development. But it brings a problem: it is challenging to detect the cancer biomarkers in the very early stage.

In this project, we aim to apply optical principles to measure the light scattering of the nanoparticles immobilized on the fiber surface owing to analyte-ligand interactions, and collect preliminary results of a fiber-based optical force transducer that can probe forces as low as nanonewtons. The optical force transducer consists of a bare fiber (without coating and a majority of cladding) with nanostring, which is used to attach cells, and a laser source. For visualizing the forces, we utilize the evanescent field, which occurs at the interface between core and cladding of the fiber during the total internal reflection. The evanescent field can react with the cells to emit light, whose intensity can be used to determine the force.

1.3 Operating Environment

In a study of the coupling of fiber and nanoparticles, we use the exposed fiber functionalized with -CHO group to attach gold nanoparticles. The exposed fiber would be

observed under the microscope while the room should be completely dark. In the final testing, we use the exposed fiber with nanostring to attach cells. The observation condition is the same as the previous testing. The fiber must be kept at room temperature in dry container.

1.4 Intended Users and Intended uses

The product of our project can speed up the detection of biomarkers which can be used in the fields of biomedical science, clinical research, environmental monitoring, and food safety. Furthermore, the product can perform biomarker analysis for clinical diagnostics, improving the sensitivity of biosensors for detecting the low-abundance biomarkers.

The researchers who interested in the field of mechanotransduction would be the primary users of our product. By observing the induced light intensity, researches can visualize cell mechanotransduction.

1.5 Assumptions and Limitations

The potential users of our product are the researchers in the field of mechanotransduction. Since our product is beneficial for them to visualize cell mechanotransduction.

During the testing period, the laser safety goggles must be worn for protecting the eyes, as the background needs to be dark enough to observe the laser through the fiber. The length of the optical fiber should be about 1 meter long. The cost to produce the product shall not exceed one thousand dollars.

In case of the limitations, it’s impossible to remove all the cladding from the optical fiber. Because both core and cladding are made of glass, we have to use Hydrogen Fluoride (HF) to etch the cladding part. But the size of the optical fiber is in micrometer scale.

1.7 Previous Work and Literature

In the research paper “Blood-based biomarkers of aggressive prostate cancer”[4] and “Biomarkers in cancer staging, prognosis and treatment selection”[5]. We observe the rapid and accurate diagnostic is fundamental to quality care. Despite significant advances in diagnostic technologies, new tests are needed to precisely identify a specific biological signature with high accuracy and also provide results in a short amount of time. Disease biomarkers circulating in human body fluids are the promising diagnostic targets. Cancer cells at an early-stage tumor site can express protein biomarkers which subsequently circulate in blood. The paper guide us to take advantages of biomarkers for in-vitro diagnostics, the biomarkers in body fluids need to be characterized quantitatively.

The Nobel Prize in 1960 for the first immunoassay measuring insulin levels and the enzyme-linked immunosorbent assay (ELISA) in the early 1970s to the modern development of highly sensitive and specific multiplex microarrays, immunoassays enable quantitative analysis of proteins in a myriad of biological experiments. [6]The research paper “Enzyme immunoassay techniques” described the techniques of immunoassay, it is fundamental to the immunoassay test we have developed. [7]

2 Proposed Approach and Statement of Work

2.1 Functional requirements

To solve the problem, we need to create a numerical model of the optical force transducer to optimize the parameters, and fabricate the optical fiber to characterize light scattering by the attached nanoparticles. On the theory and numerical modeling, we used simulation software to build a numerical model for nanofiber with gold nanoparticles; in addition, we used the model to optimize the sensor parameters. In the fabrication and process development, we have processed the optical fiber by stripping the outside layers, adding FC connector and attaching gold nanoparticles. At the end of the phase, we tested the gold nanoparticles immobilization process.

Functional requirement:

- The coating layer and cladding layer should be completely removed
- The gold nanoparticles should be evenly attached onto the optical fiber
- The fiber holder should be made to fit the size of the single mode optical fiber
- The parameter of the numerical model should optimize the efficiency of optical force transducer (nanoparticle size, operation wavelength)
- The laser experiment must be performed in the laser hood room

Non-functional requirement:

- The optical fiber should be cleaned thoroughly after each experiment to minimize the contamination
- The design of the fiber holder/stabler should be simple enough to save more time and resources

2.2 Constraints considerations

Standards:

- IEEE Standard for Sensor Performance Parameter Definitions," in IEEE Std 2700-2014 , vol., no., pp.1-69, Aug. 12 2014
  doi: 10.1109/IEEESTD.2014.6880296
    - - The standard is related to our senior design project, which defines the standard of sensor performance parameters, specifically, the terminology, units, conditions, and limitation of each sensor. By providing the specific condition and measurement of each sensor, the document precisely lay out the range of measurement, noise, delay and sensitivity level. The sensors covered in this standard are accelerometer, magnetometer, gyrometer/gyroscope, barometer/pressure sensor, hygrometer/humidity sensor, temperature sensor, ambient light sensor, and proximity sensor.
- IEEE Guide for Installation Methods for Fiber-Optic Cables in Electric Power Generating Stations and in Industrial Facilities," in IEEE Std 1428-2004 , vol., no., pp.0_1-23, 2005
  doi: 10.1109/IEEESTD.2005.96276
    - The document is intended for guiding the operation of fiber-optic cable which includes application and installation of optical cable. It covers the design considerations of optical cable, introduces the characteristics of fiber and relevant measurement needed, at the end, it described the installation of the designed cable and specify the testing result. This document is related to our senior design which is about optical fiber, it has work through the process of installation of optical cable to show the professional method of handling optical fiber.

- IEEE Recommended Practice for Validation of Computational Electromagnetics Computer Modeling and Simulations," in IEEE Std 1597.2-2010 , vol., no., pp.1-124, Feb. 25 2011
  doi: 10.1109/IEEESTD.2011.5721917
    - This standard provides examples of simulation and computer modeling techniques which can be applied in electromagnetic field, it also compares data to help serving complement measurement and electromagnetic design tasks of complex modeling problem. The process of simulation is introduced,furthermore, the model validation is also included in each entry.

2.3 Technology considerations

Strengths:

- Robust and strong signal: the scattering of gold nanoparticles is immune to photobleaching and can be measured without using emission and excitation filters
- Inexpensive and miniaturized sensor system: the fiber sensors can be prepared and functionalized easily
- Analysis is easily multiplexed: the incident light will pass through the fiber during a test, and the scattered images from all the detection zones will be acquired

Weakness:

- During the simulation period, the time takes to run is long

2.4 Safety considerations

Cable installation:

- When working with cleaners, wipes and adhesives glue inside the cables, it is required to wear gloves and act carefully

- Working area should be free of combustible gases
- Caution should be exercised when working with fiber-optic cables to avoid lightning induced surges

Laser safety:

- When working with fiber coupling, never look directly at the laser source which locate at the tip of connector
- The laser beam should never face to the installer
- To avoid eye damage, special safety glasses must be worn to filter the infrared light

Termination:

- When cutting, cleaving the optical fiber or the cables, caution should be exercised to avoid fiber fragments cut
- After handling the bare fiber, the table must be cleaned completely, and fiber scraps should be collected and disposed carefully
- Eating and drinking should be prohibited on the fiber working table to prevent ingestion of optical fiber

Surface Chemistry:

- Most chemicals used in the experiment are toxic, all the chemical solutions should be used under the hood
- Gloves must be worn during the surface chemistry experiment
- The chemical solutions should be placed in the refrigerator
- The mixed chemical solutions should be placed in a closed container

2.5 Objective of the task

There are two objectives of the task which are 1) the integration of optical fiber and plasmonic nanoparticles, 2) Demonstrate fiber-based proximity assays for cardiac biomarker. For objective 1, we need to perform simulations and experiments to understand the light coupling between waveguide modes and plasmonic nanoparticles which will experimentally characterize the light scatterings of AuNPs attached on the fiber surface and suspended in solution. For objective 2, we aim to obtain an optimized wash-free immunoassay protocol, which means the limit of detection of the fiber based assay will be quantitatively studied and compared to the ELISA result.

Numerical model and component of optical force transducer:

- Single mode optical fiber holder (for microscope use)
- Cubic model of optical fiber stabler for surface chemistry
- Complete model of optical force transducer with gold nanoparticles
    - Optimize the sensor parameters: fiber diameter, nanoparticle size, and operation wavelength
    - Characterize the figure of merits (force sensitivity) and noise sources
- Comsol (simulation)
- SolidWork (Design the research components)
- Scanning Electron Microscope (observing gold nanoparticles)

Fabrication:

- Optical fiber preparation
    - Remove coating layer and cladding layers
- Surface chemistry
    - Capture -NH2 coated AuNPs
    - Capture -COOH coated AuNPs
    - Immobilize gold nanoparticles
    - Examine fiber under optical microscope and SEM

- Laser experiment:
    - Installing connectors (FC/PC), polish the fiber tip
    - Testing the laser beam
- Demonstrate the application of the optical force transducer in cell mechanotransduction
- Characterize the light scattering by the gold nanoparticles attached to the optical fiber
- Functionalize the fiber core and test the gold nanoparticles immobilization process

3. Testing and Implementation

3.1 Interface Specifications

- Lumerical (simulation)
- SolidWorks (Design the research components)
- Scanning Electron Microscope (observing gold nanoparticles)
- Chemicals used in surface chemistry (PVA, GA, Acetone, DI water, and AuNPs solutions)

For the software and simulation part, we use the solid work to design fiber holders for observing purposes under the microscope. We used solid work to design the shape of the fiber in precise measurement, and then ask ETG to print out the real holder which is shown in figure 2. The fiber holder is used as a tool to stabilize the optical fiber under the microscope. Also, we used the FDTD to do the simulation. The basic model of the optical fiber was built in FDTD, and gold nanoparticles were also added.

For observing the gold nanoparticles, we send the samples to SEM to observe whether the gold nanoparticles have successfully attached.

3.2 Hardware and software

- Lumerical (simulation)

Lumerical is a software that could simulate the 2-D and 3-D model by designing the exterior structure and applying the physics equations. In our project, we use FDTD to design a 2-D model of optical force transducer made by the optical fiber and gold

nanoparticles, and then optimize the efficiency by adjusting the parameters of the nanoparticle size and operation wavelength.

- SolidWorks (Design the research components)

SolidWorks is a computer program that can build models in 2D sketch. We use SolidWorks to design the components of our project like the optical fiber holder.

- Scanning Electron Microscope (observing gold nanoparticles)

The Scanning Electron Microscope is used for observing the attachment of the gold nanoparticles on the optical fiber, it uses electrons to form an image with high resolution, so it can closely observe the particles at high magnification which cannot be replaced by the fluorescence microscope [2].

- Chemicals used in surface chemistry(PVA, GA, Acetone, DI water, and AuNPs solutions)

The chemicals are used for attaching the gold nanoparticles on the optical fiber.

3.3 Process

Before assembling the optical force transducer, we need to finish creating the numerical model with FDTD. We need to find out the size of appropriate single mode optical fiber and the wavelength of the incident laser. After the raw model of the transducer has been created, we can start taking care of the optical fiber that will be used in the sensor. The first thing for us is to remove the cladding part off the sensing part of the optical fiber. We need to conduct some experiments to determine a appreciate etching rate first. After the etching process, we can move to work on the attachment of the gold nanoparticles on

the fiber core using the surface chemistry plan. In the final stage of our senior design project, once we can make sure the last three major processes are working, we can start to use a single mode optical fiber with a FC connector to perform the etching and surface chemistry processes. Then, we can try some AuNP with different concentrations to see the performance of the device under a microscope and coupled with a laser.

3.4 Functional/Non-functional Testing

The initial functional testing includes measuring the light scattering of the gold nanoparticles immobilized on the fiber core and compare the light scattering intensity on the fiber core to the remaining gold nanoparticles solution. The scattering intensity is measured at two polarizations. The detected intensity represent good signal, and the undetected intensity represent uneliminated noise in the background or non-adsorbed gold nanoparticles. By performing the initial functional testing, we can determine the noise level and observe the percent of noise being effectively removed by controlling the concentration of gold nanoparticles.

The fiber will be etched by hydrofluoric acid to expose the fiber core, and the fiber sensors were prepared by coating the sensor with protein. A Conjugated AuNPs (Nanopartz Inc.) at five different concentrations from 1 x 108 NPs/mL to 6.75 x 1010 NPs/mL will be attached to single mode optical fiber. The fiber was coupled to a laser source with wavelength at λ =532 nm and power of P = 100 mW (CL532-20-S, CrystaLaser).The images of the AuNPs scattering were captured using a microscope.

The fibers will be mounted on a customized fixture with v-groove holder and characterized using a laboratory microscope equipped with a CMOS sensor. Two sets of

experiments will be performed: 1) AuNPs adsorbed on the surface of fiber core with different size of gNF: the gap size will be adjusted using a chemical linker, polyethylene glycol (PEG), with different molecular weights; 2) Non-adsorbed AuNPs suspended in buffer solution: the fiber surface will be passivated to prevent the absorption of AuNPs. During the measurements, the microscope image will be focused on the side surface of the fibers to collect the scattered light from the AuNPs. The light scattering will be acquired using different integration times (0.5 sec, 1 sec, and 2 sec) set on the CMOS sensor.

In case of immobilized AuNPs, we expect to identify AuNPs down to a single particle level. Single-to-noise ratio of measured AuNP scattering will be demonstrated as a function of laser power. We will also measure the signal as a function of PEG chain lengths. We will learn how the scattering signal decays when the AuNPs are moved away from the fiber surface. The tests using immobilized AuNPs will allow us to predict the range of signal intensity that is useful in the following tasks to develop the biomarker detection assay. On the other hand, we expect to minimize the signal from the non-absorbed AuNPs. Owing to the fast rotation of suspended AuNPs, light scattering from these AuNPs is depolarized. The images taken for two crossed polarizations will be compared, and the difference will be calculated. The results will show how the background signal will be estimated to improve the sensitivity of this wash-free biosensor.

3.5 Model and Simulation

In the model and simulation, we build a numerical model to study the coupling of fiber and nanoparticles. First of all, we created a 2D waveguide with gold nanoparticles using the finite-difference time-domain simulation. The 2D model has a fiber which is an infinitely wide dielectric slab, and the gold nanoparticles will be placed on the fiber; moreover, the numerical model is capable of calculating light propagation in optical fiber, predicting the LSPR-enhanced scattering of a waveguide mode. Secondly, we converted the 2D model in 3D. After the 3D model has been built, we can perform detailed analysis of the parameters. The simulation effort can be broken down into three steps, 1) Determine the resonance of AuNPs and the propagation mode of optical fiber, 2) Calculate the AuNP scattering intensity in the far field for a given waveguide mode, and 3) Study the LSPR-enhanced light scattering by selecting the AuNPs for a given wavelength.

3.6 Issues and Challenges

The challenges would be developing an assay protocols to validate the performance advantages of the new biosensor. The objective is to obtain an optimized wash-free immunoassay protocol, but the detection of fiber-based assay is limited.

To establish a robust and repeatable surface chemistry for the functionalization of the fiber surface, we need to expose the fiber core first. In this case, we have difficulties to remove the cladding layer of the fiber. We developed a method to etch the cladding layer, applying Hydrofluoric acid can remove the cladding layer, therefore we can possibly

decrease the diameter of the fiber to 10 micrometers. For better performance, we need to figure out the etching rate of hydrofluoric acid on the fiber.

3.7 Results

As a result, from the simulation result from **Fig.5** , we can see the optimized wavelength of the incident laser is 532 nm. For the experiment results as seen in **Fig.6**, it showed that the optical transducer we developed can detect a very low concentration of AuNP. From the results, we believe that the optical wash-free can be used for cancer biomarker detection. However, there is still a problem for this device. Due to the current holder design, the fiber etching process is not uniform. From the end to the center, the diameter of the fiber ranges from 1 um to 10 um. That causes the etched part is curved. In this situation, it is difficult to perform the microscope observation. Because each point of the fiber surface needs different levels of focus.

4 Estimated Resources and Project Timeline

4.1 Personnel effort requirements

Sub-group	Theory and numerical modeling	Fabrication and process development
Members	Jiameng Li and Qinming Zhang	Yalun Tang and Quan Wang
Tasks	1. Study fundamental principles of optical fiber, plasmonics, and nanoparticles 2. Learn how to use COMSOL Multiphysics, including the Wave Optics Module and Structural Mechanics Module. 3. Build a numerical model for the nanofiber with metal nanoparticles. 4. Optimize the sensor parameters: fiber diameter, nanoparticle size, DNA/PEG, and operation wavelength. 5. Characterize the Figure of Merits (force sensitivity) and noise sources	1. Study the skills to handle optical fibers and the fluorescence microscope 2. Prepare a bare optical fiber (stripping the jacket, buffer, and cladding layers), add a FC/PC connector to fiber, and polish the fiber tip 3. Immobilize gold nanoparticles (40 nm, 60 nm, 100 nm) on the exposed fiber, exam fiber under optical microscope and SEM 4. Characterize light scattering by the nanoparticles 5. Functionalize the fiber core (silica) using PEG or DNA. Test the gold nanoparticles immobilization process.

4.2 OTHER RESOURCE REQUIREMENTS

In this project, we need to use the chemicals from the LIOS group for surface chemistry on the bare optical fiber. And we also use the laser devices, optical fibers, and microscopes from LIOS group. MRC offers us 49% HF for wet etching.

4.2 FINANCIAL REQUIREMENTS

ECpE department at Iowa State University offers our senior design group 1000 dollars for developing the optical fiber based biosensor.

4.3 PROJECT TIMELINE

Task/Month	9	10	11	12	1	2	3	4
Prepare bare fiber								
Surface chemistry(for fiber core)								
Coupling of fiber and nanoparticles								
Characterize light scattering								
Detection of nanoparticles								
Design modeling using simulation tool								

Design holder for the optical fiber								
Optimize sensor parameters								
Prepare optical fiber for immunoassay								
Enhance biosensor performance								

5 Closure Materials

5.1 CONCLUSION

In our senior design project, we try to develop an optical fiber based biosensor, which uses evanescent field to interact with some nano-sized targets, connected with the sensing component by nanosprings, such as nano-particles, cells, and biomarkers. The sensing component is the decladded part of the optical fiber. By achieving this goal, our team is divided into two groups: Theory and numerical modeling group and Fabrication and process development group. Theory and numerical modeling group works on the modeling and simulating in an EM simulation tool called FDTD to find out the optimized parameters of the optical biosensor. Fabrication and process development group aims to try fabricate the sensor device. As a result, our fabricated optical transducer is able to detect a low concentration of AuNP (smaller than 0.25 ug/ml). In the future, the project will be continued to work on reducing the diameter of the fiber below 1 um and trying to make the etching more uniform by using a new etching platform. After that, we will move to demonstrate fiber-based proximity assays for cardiac biomarker by using our optical transducer.

5.2 References

[1] Paluch, E. K., Nelson, C. M., Biais, N., Fabry, B., Moeller, J., Pruitt, B. L., … Federle, W. (2015). Mechanotransduction: use the force(s). BMC Biology, 13, 47. http://doi.org/10.1186/s12915-015-0150-4

[2] Xiong, B., Huang, Z., Zou, H., Qiao, C., He, Y., & Yeung, E. S. (2017). Single Plasmonic Nanosprings for Visualizing Reactive-Oxygen-Species-Activated Localized Mechanical Force Transduction in Live Cells. ACS Nano, 11(1), 541-548. doi:10.1021/acsnano.6b06591

[3] Huang, Q., Lee, J., Arce, F. T., Yoon, I., Angsantikul, P., Liu, J., . . . Sirbuly, D. J. (2017). Nanofibre optic force transducers with sub-piconewton resolution via near-field plasmon–dielectric interactions. Nature Photonics, 11(6), 352-355. doi:10.1038/nphoton.2017.74

[4] M. L. Liong, C. R. Lim, H. Yang, S. Chao, C. W. Bong, W. S. Leong, P. K. Das, C. S. Loh, B. E. Lau, C. G. Yu, E. J. Ooi, R. K. Nam, P. D. Allen, G. S. Steele, K. Wassmann, J. P. Richie, and C. C. Liew, "Blood-based biomarkers of aggressive prostate cancer," PLoS One, vol. 7, p. e45802, 2012.

[5] J. A. Ludwig and J. N. Weinstein, "Biomarkers in cancer staging, prognosis and treatment selection," Nature Reviews Cancer, vol. 5, pp. 845-856, 2005.

[6] R. M. Lequin, "Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA)," Clin Chem, vol. 51, pp. 2415-2418, 2005.

[7] S. D. Gan and K. R. Patel, "Enzyme immunoassay and enzyme-linked immunosorbent assay," Journal of investigative dermatology, vol. 133, p. e12, 2013.

[8] T. Porstmann and S. T. Kiessig, "Enzyme immunoassay techniques. An overview," Journal of Immunological Methods, vol. 150, pp. 5-21, 1992.

Appendix I - Operation Manual

As seen in **Fig.7**, the demo of the optical wash-free transducer need to be under a fluorescence microscope. In this section, we introduce how to perform the light scattering observation under a microscope.

1. At the beginning, coupling the device with the laser is required.
2. Turn on the power of the microscope and the light source.
3. Put the device on the microscope like **Fig. 7**.
4. Choose 20X objective lenses.
5. Due to the light source, you can see the light transmitted from the objective lenses and through the back of the fiber holder. Therefore, you can see what position the microscope is observing now. Move the objective so that the light shines on the edge of the hole (where the fiber core is) of the fiber holder.
6. Adjust the focus so that you can see the edge from the microscope. Then move the objective along the edge son that the fiber coating can be seen.
7. Move the objective along the fiber. During this process, you will move from the coating to the decladded part of the fiber. Therefore, you need to adjust the focus (decrease the distance between the holder and the objective) once you cannot see anything.
8. Turn off the light source of the microscope. Then you will see some light scattering on the fiber core.

Appendix II: Other versions

Our single mode optical fiber consists of three parts which are coating layer, cladding layer, and core. A main step of our experiment is to remove the cladding layer to enhance the sensitivity of the fiber. We considered using Butane Gas Blow Torch to remove the cladding layer which can apply heat to melt the cladding layer of the fiber, but the result shows that temperature of heat is tough to control, thus the melting rate of fiber is unpredictable. The overheat of the fiber could damage the core layer of the fiber, and we need a better solution to remove the cladding layer evenly.

To achieve a better solution which meets client's specification, we changed to etching method in order to remove the cladding layer efficiently without damaging the functionality of the fiber core.