Optical Wash-free Transducer for Biomarker Analysis

PROJECT PLAN

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Revised: 12/07/17 v3

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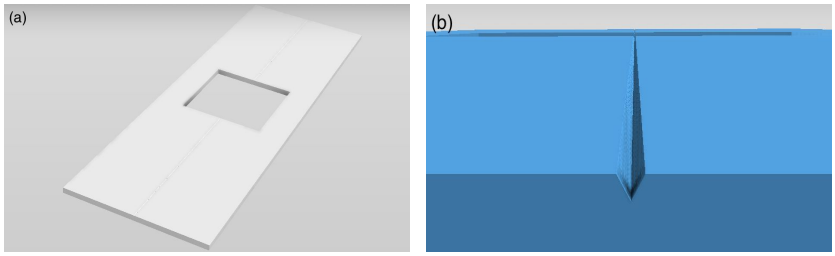
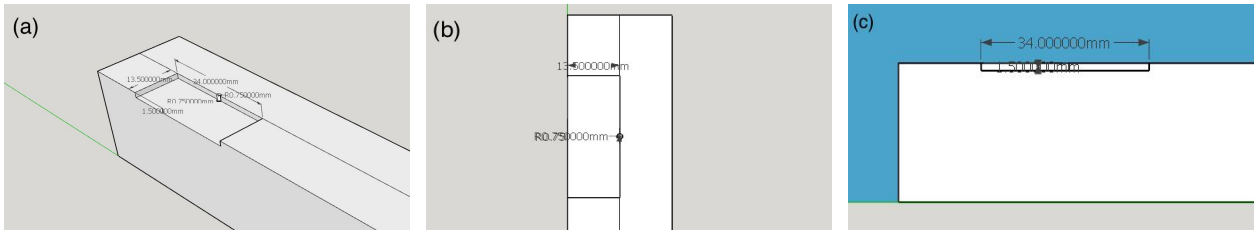


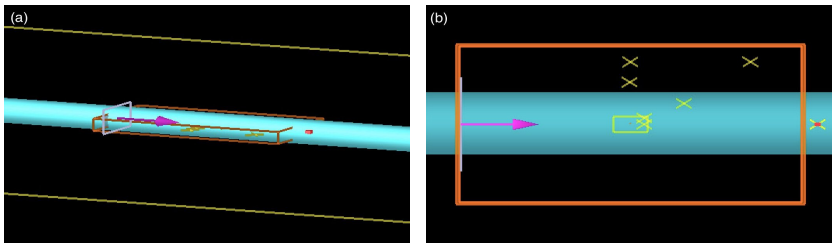
(a) 3D version (b) side view V-groove

Figure 2. Etching platform

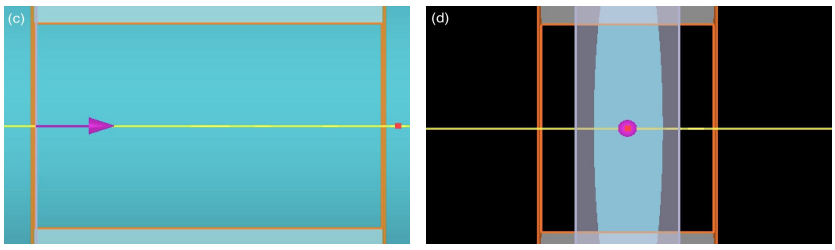


(a) 3D version (b) Vertical view (c)Front view

Figure 3.Simulation model.



(a)3D version (b)Front view



(c)Vertical view (d)Side view

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

1.2 problem Statement

Mechanotransduction refers to how cells react physical forces (mechanical stimuli) and translate the forces into biochemical outputs. In mammalian cells, their cytoskeletons allow them to exert forces in nano newton; and physical forces play an important role on biological choices of cells such as stem cell differentiation and tumor formation [1]. By applying our resources, and collaborations, we are committed to study these challenging topics using a photonic approach.

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 nN. The optical force transducer is consist of a bare fiber (without coating and majority of cladding) with nanostring, which is used to attach cells, and a laser source. For visualizing the forces, we utilize 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 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 in room temperature in dry container.

1.4 Intended Users and Intended uses

The product of our project will speed up the detection of biomarkers which can be use 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 will be needed for protecting the eyes, as the background need 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 optical fiber is in micrometer scale.

1.6 Expected End Product and other Deliverables

Our expected end product is an optical force transducer used to visualize the mechanotransduction under the microscope. To achieve a high sensitivity, we use simulation tool to optimize the parameters of the optical fiber, which is the major part of

our optical force transducer. We expect we can get the optimized parameters by the end of Fall 2017, and finalize our product design by the end of Spring 2018.

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 will develop.[7]

2 Proposed Approach and Statement of Work

2.1 Functional requirements

To solve the problem, we should create numerical model of the optical force transducer to optimize the parameters, and fabricate the optical fiber to characterize light scattering by the attached nanoparticles. In the theory and numerical modeling, we will use simulation software to build a numerical model for nanofiber with gold nanoparticles; in addition, we will use the model to optimize the sensor parameters. In the fabrication and

process development, we will process the optical fiber by stripping the outside layers, adding FC connector and attaching gold nanoparticles. At the end of the phase, we will be able to test 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

2.2 Constraints considerations

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

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 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, also it compares data to help serving complement measurement and electromagnetic design tasks of complex modeling problem. The process of simulation is introduces, the model validation is 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 necessary 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 Possible Risks and risk management

The possible risks includes the handling of optical fiber core, it is impossible to remove the cladding layer completely, therefore, we need to try multiple methods to find the best way to solve the problem. Another risk we found is the running time of the simulation, it takes about 48 hours to run the numerical optical model. Instead of waiting for the simulation, we would need to move on to the experiment part in case of saving more time.

More challenges in protein biomarker analysis also include the long assay time, low testing throughputs, high cost, and large footprint of the detection instrument. For the diseases that develop quickly, such as some cardiovascular diseases, new technologies with a short assay time are needed to fulfill the requirement of rapid diagnosis. In particular, some biomarkers present at high physiological concentrations (1 ng/mL – 1 μg/mL) and the change of their concentrations could be as large as 1000-fold in case of

infection or injury. For these biomarkers, the assay time is the top priority, and the sensitivity becomes the second.

2.6 Project Proposed Milestones and evaluation criteria

The aims of the project are designed to apply optical principles to reduce the time of detection and user intervention when maintaining the constant sensitivity for quantification of disease biomarkers. The key milestones in our proposed project are the development and fabrication of the fiber nanoparticle sensor, design and construction of a compact detection instrument and validation of the resulting capability using diseases biomarkers.

To investigate the fabrication of the fiber nanoparticle sensor which includes coupling between the core of optical fiber and gold nanoparticles, we will create an electromagnetic simulation model to gain insight regards to the waveguide modes of fiber and LSPR modes of gold nanoparticles. To evaluate the simulation, we need to determine the accuracy of operation wavelength; following the simulation, we would be able to evaluate the signal and noise level of the biosensor based on the light scattering of nanoparticles on the fiber surface.

To design a compact detection instrument, we need to establish the wash-free proximity assay platform which will choose a cardiac biomarker to be the demo assay. In the experiment stage, we need to apply the surface functionalization to the fiber core, immobilization of ligands and use of a blocking step to eliminate nonspecific binding. To evaluate the result, we would compare the assay to observe whether it can achieve high sensitivity, low nonspecific binding and low background. The final result will be compared with ELISA measurement.

2.7 Project tracking procedures

We will follow the team schedule to track our progress, and we will set goals for current week and next week to accomplish tasks on time. Each of the team member will be

assigned to different work, and we will discuss our contribution and progress in the member's weekly meeting which happens twice a week.

2.8 Objective of the task

There are two objectives of 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 aims to obtain an optimized wash-free immunoassay protocol which means the limitation 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:

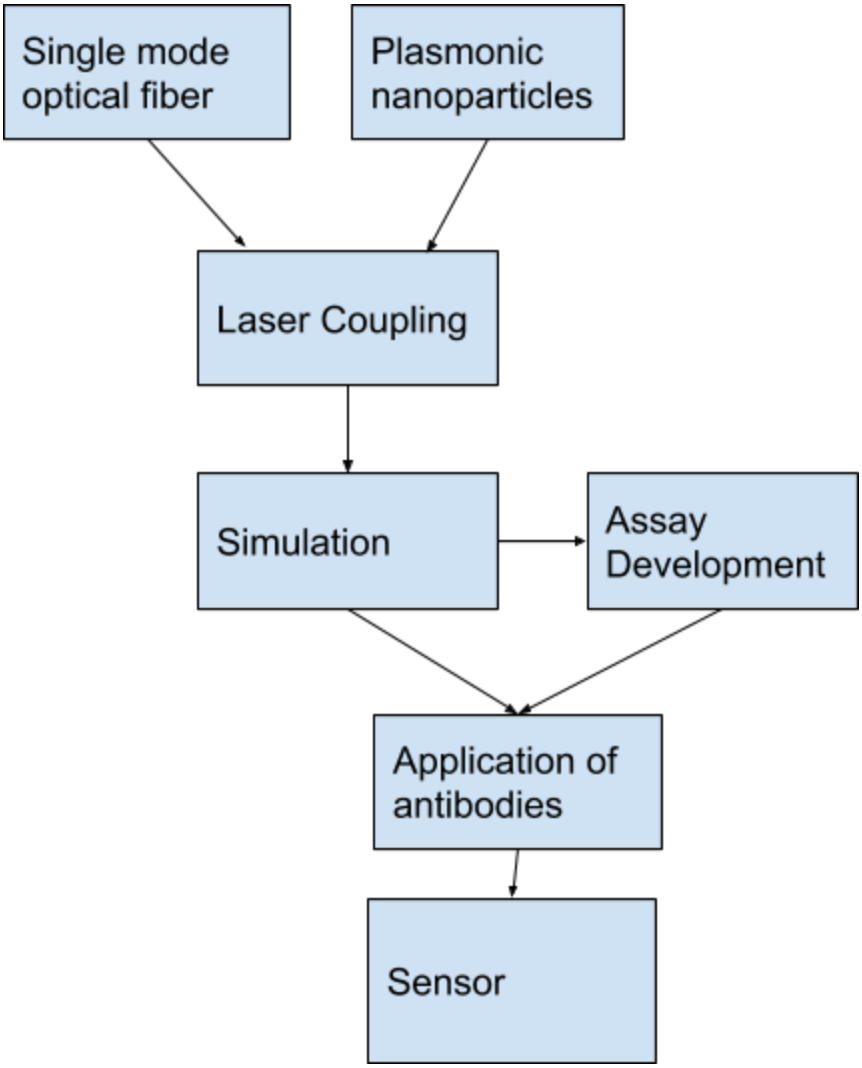
- Single mode optical fiber holder (for microscope use)
- cubic model of optical fiber stabler for surface chemistry
- complete model of optical force transducer with gold nanoparticles
    - optimize the sensor parameters: fiber diameter, nanoparticle size, and operation wavelength
    - characterize 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, cladding layers
- Surface chemistry

    - capture -NH2 coated AuNPs
    - capture -COOH coated AuNPs
    - immobilize gold nanoparticles
    - exam fiber under optical microscope and SEM
- Laser experiment:
    - making 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 on the optical fiber
- Functionalize the fiber core and test the gold nanoparticles immobilization process

2.9 Task approach



For the integration of optical fiber and plasmonic nanoparticles, we need to use single mode optical fiber and plasmonic nanoparticles to study the coupling of fiber and nanoparticles. We will strip the buffer and cladding layers of single mode fibers to expose the fiber core, at the same time, the fiber will be connected to a laser source with wavelength of 532 nm. The core is made of doped silica, it will be etched to the designed diameter by dipping the exposed fiber core in 49% hydrofluoric acid, at the meanwhile, the fiber will be inspected under an optical microscope to measure the diameter. The fiber will be mounted on a customized fixture with v-groove holder and characterized using a

microscope equipped with a CMOS sensor. The microscope image will be focused on the surface of fiber to collect the scattered light from gold nanoparticles.

Following the fiber experiment, we we build 2D and 3D models to study the waveguide with gold nanoparticles. The simulation will be measuring the resonance of gold nanoparticles and the propagation mode of optical fiber, and calculate the gold nanoparticles scattering intensity.

After the parameters have been optimized by studying the numerical model, we will prepare optical fiber for immunoassay. A series of surface chemistry experiment will be performed for functionalization of the fiber surface.

Fiber surface chemistry protocol I:

I. PVA-GA Surface chemistry for Amine-terminated materials

**Prepare containers (centrifuge tubes) for the following processes, including the cleaning process. The fiber should be soaked into the container and gently shaked.*

1. Clean the fiber using Acetone, DI and dried in air
2. Prepare PVA (Polyvinylamine) solution (1% - 2% dissolved in DI water). PVA is stored in the fridge (Sweeney 3152)
3. Soak the fiber in PVA for 12 -24 hr (overnight) at room temperature
4. Clean the fiber by soaking in DI water 3x
5. Prepare GA (Glutaraldehyde) 20% solution in DI. GA is stored under the fume hood in Sweeney lab.
6. Soak the fiber in GA solution for 5 hours
7. Clean the fiber by soaking in DI water
8. At this point the fiber tip is functionalized with –CHO group. It is ready to capture amine (-NH2) samples.
9. If the following experiment can't be carried out immediate after the pervious step, fiber should be stored in DI water. DON'T let it dry in air.

II. Capture -NH2 coated AuNPs (gold nanoparticles)

1. Dip the fiber in DI water (2 mins) for quick cleaning.
2. Soak the GA-coated fiber in the solution of AuNPs (with NH2) for 1-4 hrs. Gently shake the fiber during the conjugation process.
3. Pull the fiber out of AuNP solution and dip it in the DI water for a quick cleaning (2 mins)
4. The fiber is ready for imaging. Fiber can be dried at this point.

We will investigate the wash-free detection of a disease biomarker after the surface functionalization process. The LOD of the proposed platform will be evaluated by performing the dose-response characterization. We will begin with the ligand (CRP antibody) coated fiber described in the previous subtask. The initial tests will measure a dilution series of CRP antigen dissolved in PBS. The green laser will be coupled to the fiber sensor to excite the desired waveguide mode, and we will need to observe and record the increase of scattering intensity in the image.

2.10 Expected Results and Validation

By performing the 3-D simulation, we expect to determine the operation wavelength, fiber diameter, diameter of gold nanoparticles, scattering efficiency for a single nanoparticle. Importantly, we will determine the scattering efficiency as a function of the relative distance between the gold nanoparticles and fiber core. In case of immobilized gold nanoparticles, we expect to identify gold nanoparticles down to a single particle level. Single-to-noise ratio of measured gold nanoparticles 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.The proposed method measures the resonant light scattering by AuNPs, whose stability is excellent in the buffer solution. Thus, we anticipate that the fiber-nanoparticle approach will be successfully validated as a sensitive and highly stable detection assay.

The final product of our senior design project is an biosensor, which uses the bare part as its sensing component attaching nanoparticles by nano-spring. By coupling the optical fiber with a laser, we should be able to see some emitted light, whose intensity is determined by the distance between the sensing surface and the sensing targets, from the nanoparticles under a microscope.

3 Estimated Resources and Project Timeline

3.1 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.

3.2 OTHER RESOURCE REQUIREMENTS

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

3.2 FINANCIAL REQUIREMENTS

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

3.3 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								
Characterize the figure of Merits								
Prepare optical fiber for immunoassay								
Construct compact assay apparatus								
Enhance biosensor performance								

4 Closure Materials

4.1 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 using a single mode optical fiber from Thorlab first. After the experimental fabrication successes and getting the optimized parameters, Fabrication and process development group can start to fabricate the goal product and use it to do some experiments with cells.

4.2 References

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