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Final Project Report: Glucose Monitoring Group

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Abstract:
Retroreflectors can potentially be used in the design of a minimally invasive glucose-monitoring device. The primary objective of this senior design project is to design, build, and test a system to show that retroreflectors can be detected through a semi-opaque medium similar to human tissue. The secondary objective is to determine if the system can detect the retroreflectors through blood with and without gold nanoparticles. The design constraints of the project are described as well as the design of the apparatus, the test setup and procedure protocols for the project, the results of these tests, and a conceptual design. Data from the test procedure is collected by using a HeNe laser that shines through one converging lenses, an iris, a beam splitter, a semi-opaque medium to emulate human tissue, and gold nanoparticles that mimic glucose molecules in blood. The light hits a retroreflector that sends the light back through the beam splitter to a photodiode that is hooked up to a digital multimeter to measure the detected signal. A full factorial two factor Design of Experiments(DOE) with three levels is used to test the apparatus. Nanoparticle concentration and angle of incidence on the retroreflector are the dependent variables. The DOE is run two times for the water medium, but the testing of semi-opaque media are unable to be performed due to scattering of the beam. In deionized water, minimal interaction effects between nanoparticle concentration and angle are observed, and light scattering appears to increase as nanoparticle concentration increases. In blood, significant interaction effects are observed, and light scattering appears to decrease as nanoparticle concentration increases. This unexpected trend is likely due to unforeseen interactions between the nanoparticles and the solutes in blood. It is concluded that the apparatus is sufficient to provide results for the water medium, and insufficient for more opaque media due to scattering. Altered nanoparticles are needed for testing in blood.

Executive Summary
Research indicates that it may be possible to build a minimally invasive blood glucose monitor using retroreflectors. The primary objective of this project is to design, build, and characterize a system that measures retroreflected light through a semi-opaque medium similar to human tissue. The second objective is to determine if the system can detect the retroreflectors through blood with and without gold nanoparticles. The design solution is not harmful to human tissue and takes into account social, economic, and political impacts.

The test setup contains many components: a 632.8 nm Helium-Neon laser, a 27cm lens, a 2.54cm lens, an iris, a makeshift screen, mounting equipment, a beamsplitter, gold nanoparticles, semi-opaque media, retroreflectors, a photodiode, a protoboard, an oscilloscope, a digital multimeter (DMM), and sheep's blood. The test procedure developed is a full factorial design of experiments (DOE) with two variables and three levels. The two variables are nanoparticle concentrations and the angle that light is incident on the retroflectors. The nanoparticles are to be tested at a percentage of the original 2.82 pM concentration: 0, 50%, and 100%. The angle of incidence is varied between 30^o, 45^o, and 60^o. The DOE is run on each of the semi-opaque media being used to replicate the effects of human tissue in the following order: water, milk, pig skin. The same DOE is used for the testing of the blood, except with nanoparticle concentrations of 0, 25%, and 50%.

In order to set up the apparatus for testing, the laser beam is leveled with the optical bench. The 27cm lens, retroreflectors, beamsplitter, 2.54cm lens, cuvet holder, makeshift screen, and photodiode are all carefully positioned. The photodiode is then connected to the protoboard which is hooked up to the DMM in order to get an initial maximum signal off of the retroreflectors. The tests are then run with careful precision in the order indicated by the DOE.

The testing phase presents several unforeseen challenges. The DOE is unable to be run on all media except the water due to the large amount of light that is scattered in the medias. The originally planned blood test is also unsuccessful, but the DOE is performed on diluted blood.

Trends are noticed in the water data, and it is noticed that there are interaction effects between the nanoparticle concentration and the retroreflectors. As nanoparticle concentration goes up, the signal back from the retroreflectors decreases. This result is expected because nanoparticles scatter light. Interaction effects are also noticed in the blood tests. However, the tests performed with whole blood have the opposite trends of the water data. As the concentration increases, the signal increases. This result is likely due to charge interactions between the blood and the nanoparticles that are not present when the nanoparticles are diluted using deionized water. When placed in whole blood, the charged may nanoparticles aggregate with the charged particles in the blood, and the entire complex would fall out of solution. The loss of suspended solutes would cause the blood to scatter less light, resulting in a higher diode response. A higher concentration of nanoparticles would nucleate more complexes, pulling more solutes out of the blood, and causing a higher diode response.

It is recommended for further research that the surface chemistry of the nanoparticle be altered so that charge interactions would not occur. Also, a different laser should be used with a higher wavelength, or a translucent dermal implant be researched and tested. Different retroreflectors with alternative geometry and more surface area may possibly make the testing easier and more sensitive, but also the resolution would be larger, and more light coming from the retroreflector could be detected.

The conceptual design for eventual implantantion would involve coating two sides of the four-sided retroreflector with a substrate that binds both glucose and gold nanoparticles, leaving the other two sides clear for use as reference sides. The retroreflectors spin around a central ball bearing so that a laser held still would transiently measure the signal reflected from all four sides, thus being able to compare the reference sides to the glucose-measuring sides. The retroreflectors are placed inside a cylindrical tube with filters on either end that allow plasma to enter, while filtering out components of the immune system that would envelope the gold nanoparticles and expunge them from the body. This cylindrical device can be injected into vascularized tissue. The external device contains a laser, a beam splitter, a photodiode, a voltmeter and a memory card. The device outputs a differential voltage and can tell the users if their glucose level is high. The memory card keeps a record of past readings that can be taken to a doctor for analysis.

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1. Introduction

Research has indicated that there is a possibility of designing a minimally invasive glucose monitoring device in which a light source shines through the skin to an implanted retroreflector. The reflected signal can then be used to find the level of glucose in the blood. Retroreflectors have shown strong potential as a detection tool. For example, the University of Houston has shown that retroreflectors in conjunction with gold nanoparticles are able to detect viruses that attack human tissues [1]. This technology, although relatively new, has the potential to detect anything in blood, given the proper research. As a result, the study of retroreflectors could be extremely helpful in medical applications.

The primary objective of this senior design project is to design, build, and test a system to show that retroreflectors can be detected through a semi-opaque medium similar to human tissue. The device should be able to detect only retro-reflected signals beneath the tissue and disregard ambient noise in the signal. The second objective is to determine if the system can detect the retroreflectors through blood with and without gold nanoparticles.

2. Design Constraints

The social, economic, and political impacts of this senior design project should be taken into account in order to fulfill the obligations and responsibilities of engineers. On a long term basis, this research may one day provide affordable materials for glucose monitoring, reduce suffering and improve quality of life for diabetics. The device is to be safe to use, and the light used should be able to pass through tissue safely. Medical costs should be reduced, but the reaction of Medicare and pharmaceutical companies should also be considered. Noninvasive testing reduces

waste of testing materials, and therefore positively effects the environment. Manufacture of the device should not harm the environment.

On a short term basis, the experimental apparatus is to be safe to use with no sharp edges and using no dangerous light or chemicals in testing without taking the proper precautions. Waste should be disposed of properly. The data taken during experimentation should be truthfully and accurately reported. Credit to the University of Houston and Trinity University is given where appropriate. By keeping these obligations and responsibilities, the project is ethical and more likely to succeed.

3. Specification of Working Criteria

The design criteria of the experimental setup include five items listed in order of importance:

1) Not harmful to human tissue
2) Retroreflector detectable through semi-opaque medium
3) Within the budget of the project (~$1000 to spend)
4) Low noise in signal

Each criterion is given a weighted percentage for importance. “Not harmful to human tissue” has a 35% weight, and is the most important criterion because this research desires to have a procedure that is less painful than current invasive procedures for glucose monitoring. The second criterion has a 30% weight. The retroreflector has to be detectable through the medium, a key element of the apparatus. The third criterion has a 20% weight due to the limited budget of one thousand dollars. The fourth criterion has a 15% weight due to the importance of low noise and stability in the test setup. Stable readings of detection from the retroreflector are desired.

4. Design of the Model

For this project, the only dependent variable that needs to be found is the intensity of the signal from the laser through various tissues. A schematic of the apparatus can be seen in Fig. 1.

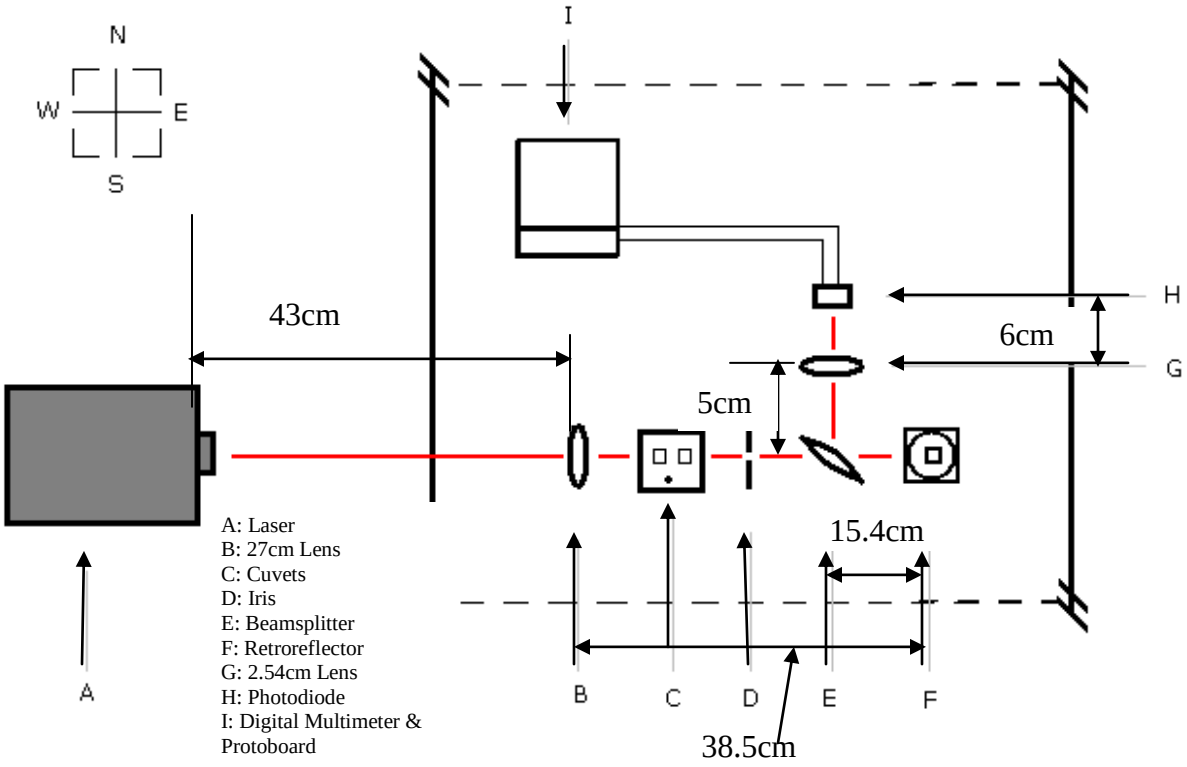


Figure 1. Test Apparatus.

4.1. System Components

The components of the setup include a steel tabletop and optical workbench, laser, laser stand, retroreflectors, a Plexiglas holder, a rotational stage, a block for the retroreflectors, the focusing lenses, two magnetic stands, a beam splitter and holder, a photodiode, semi-opaque medium, nanoparticles and solution, two cuvets, and translational stages. For the blood testing, sheep blood and a thinner cuvet are used. Figure 1 shows a top view of the experimental apparatus.

4.1.1. Steel Tabletop & Optical Bench

The steel tabletop and optical bench secure the magnetic stands. Together, these serve as the base of the experimental apparatus setup. The steel tabletop comes from the side of an old machine from the Trinity University machine shop. The optical bench is provided by Dr. Pursell from Trinity's chemistry department.

4.1.2. Laser & Mount

The laser is a 632.8nm Standard JDS Uniphase Helium Neon Laser manufactured by Edmund Optics. The laser is on loan from the University of Houston. The placement of the laser can be seen as component A in Fig. 1. The laser mount is fabricated by Manuel Garza from the Trinity Machine shop and is shown in Fig. 2. The laser is placed on top of the mount, and the mount is screwed into the steel tabletop.

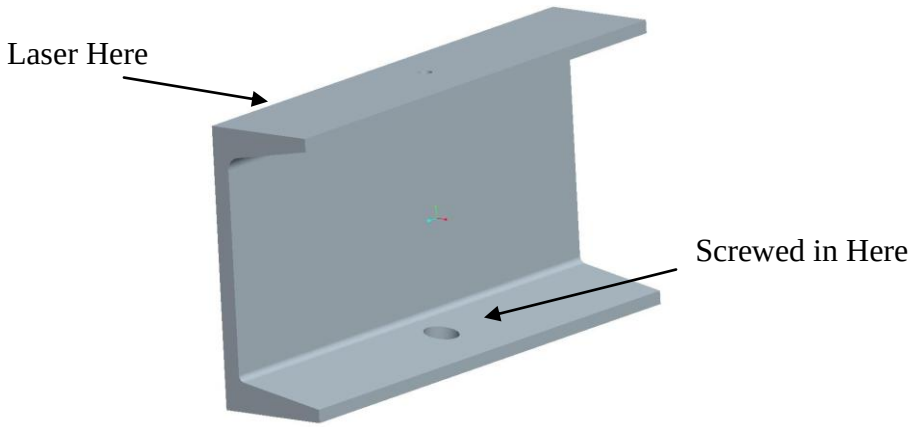


Figure 2. Laser Mount.

4.1.3. Retroreflectors

The retroreflectors used in the test system are provided by the University of Houston Research Experience for Undergraduates program. Figure 3 shows the linear retroreflectors with their aligned planes. Ideally, incident light that hits the retrorefletive structure at within a range of angles is reflected back to the source.

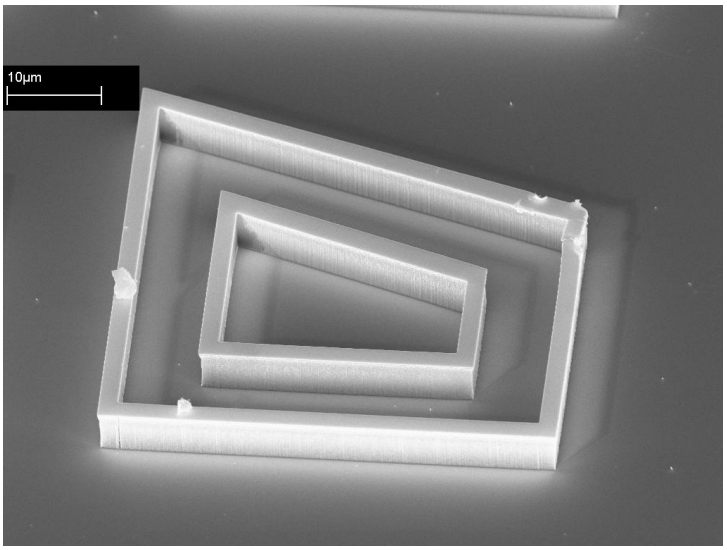


Figure 3. Retroreflective array unit.

The area occupied by this retroreflective unit (an outer wall with an embedded wall of smaller size inside) is roughly 50 microns x 50 microns. The retroreflective surface is a fraction of this 50 micron x 50 micron area, as it is the walls of this structure that function as the linear retroreflectors. These units are arranged in 4x4 grids on the silicon surface of the retroreflector chip, all tightly packed. The total area of this grid of retroreflectors is approximately 300 microns x 300 microns. Component F of Fig. 1 shows the placement of the retroreflectors in the setup.

4.1.4. Plexiglas Holder

The Plexiglas structure constructed by Manuel Garza, shown in Fig. 4, holds the opaque medium cuvet and the nanoparticles in solution cuvet. The placement of the holder can be seen in component C of Fig. 1. The Plexiglas holder is held up by a magnetic stand as shown in Fig. 5.

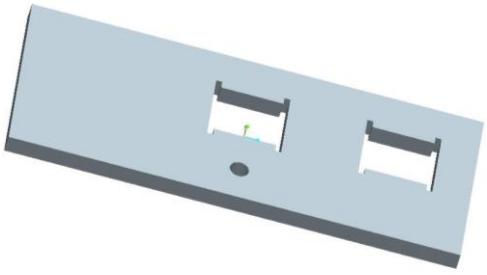


Figure 4. Plexiglas Holder.

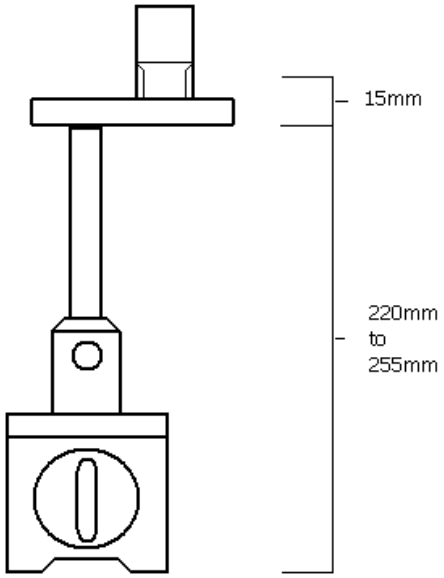


Figure 5. Plexiglas Holder Mount.

4.1.5. Rotational Mount & Block

A rotation mount is used to vary the angle of the retroreflector with respect to the laser beam. This mount is purchased from Thorlabs. The mount controls the angle with a precision of 2 degrees. On top of the mount is a Plexiglas mount as shown in Fig. 6, also constructed by Manuel Garza. It holds the reflector normal to the laser when the rotational mount is at angle zero. The retroreflector is attached by double sided tape is carefully aligned so that the retroreflectors are directly over the center of rotation.

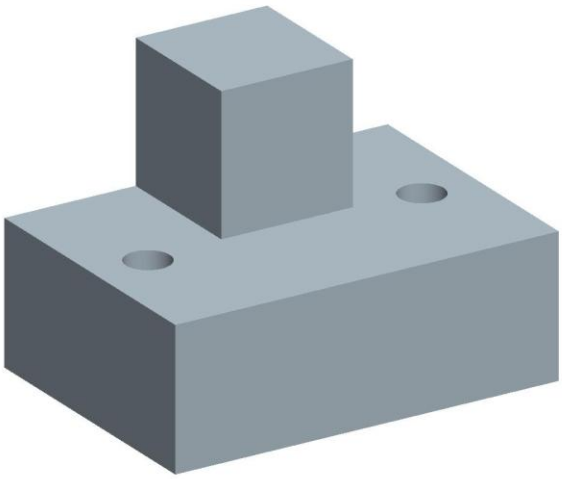


Figure 6. Plexiglas Retroreflector Mount.

4.1.6. Focusing Lenses

One converging lens is used in the test setup in order to narrow the laser beam to a smaller diameter. Ideally, another lens is used in order to collimate the beam, but the light does not collimate in practice. A larger response from the retroreflectors is observed when only one lens is used. The converging lens, provided by Dr. Pursell, has a 27cm focal point and a diameter of 4cm. Another lens with a 2.54cm focal length is placed between the photodiode and the beam splitter in order to collect all of the light that is reflected back onto the beam splitter off of the

retroreflectors. The lens converges the reflected light into a small dot that the photodiode can then detect. The lenses are attached to optical mounts, provided by Dr. Pursell. The 27cm focal length lens can be seen in Fig. 1, component B. The placement of the 2.54cm focal length lens in the apparatus can be seen in component G of Fig. 1.

4.1.7. Beamsplitter & Holder

The beamsplitter is a 50% beamsplitter (50% reflectance, 50% transmittance) made by Edmund Optics. The beamsplitter is circular and is positioned in such that incident light hits at a 45^{o} angle. The mount for the beamsplitter, shown in Fig. 7, is a Thorlabs adjustable lens mount made for holding circular lenses. It has a metric screw hole on the bottom (M4), and can fit a lens with a diameter ranging from 5mm-46mm. The placement of the beam splitter and holder in the experimental setup can be seen in component E of Fig. 1.

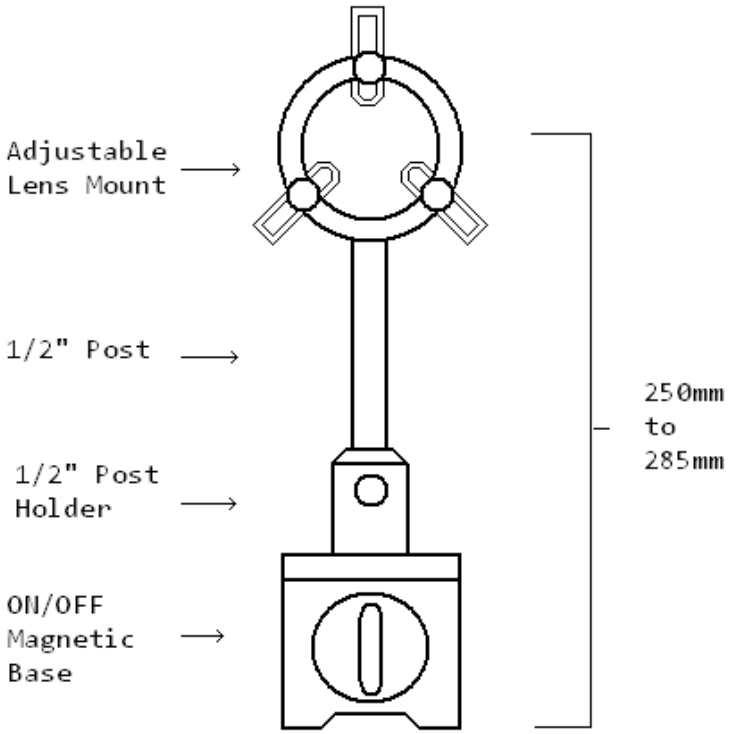


Figure 7. Beamsplitter Mount.

4.1.8. Photodiode & Digital Multimeter (DMM)

The photodiode is the light-detecting component of the system. An unbiased, blue response Edmund Optics Silicon Detector detects the light reflected from the retroreflectors and transforms light energy to an electrical current via the photovoltaic effect. This photodiode is chosen based on the wavelength of the laser beam and the low signal detection environment. The photodiode comes secured by an outside casing and is held by a magnetic base as seen in Fig. 8.

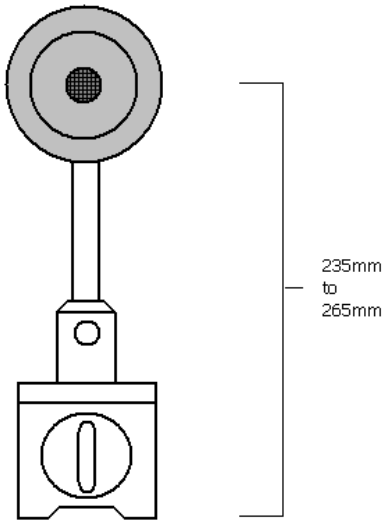


Figure 8. Photodiode Mount.

A DMM reads the current produced by the photodiode and the voltage across a 223 kΩ resistor. The photodiode position in the apparatus setup can be seen in component H of Fig. 1.

4.1.9. Magnetic Stands

The beamsplitter, Plexiglas holder, and the photodiode are mounted on top of magnetic base stands and adjustable posts as seen in Figs. 6, 7, and 8. The adjustable posts allow for height

variability in order to manipulate the laser into a position where it can reach the reflector. The magnetic base stands have an On/Off feature that allows for adjustments. Once the component has been situated properly, the magnets are turned on, and the components do not move, allowing for more accurate and reproducible testing.

4.1.10. Iris

An iris is added before the beamsplitter in order to eliminate excess scattering from the incident beam. It is manufactured by Thorlabs, and its placement is shown as component D of Fig. 1.

4.1.11. Makeshift Screen

A cardboard shield is added north of the iris in order to block the light scattering and reflecting off of the cuvets. This screen is cut from the side of a box. Without this shield, the photodiode detects the light that is scattered by the cuvets and the nanoparticles.

4.1.12. Nanoparticles & Solution

Gold nanoparticles in solution are used to test the amount of light scattered by the presence of gold nanoparticles. The gold particles are provided by Ted Pella, Inc. Literature indicates that a nanoparticle of diameter 140nm is optimal for scattering for a 633 nm wavelength light [2]. The gold nanoparticle solution ordered from Ted Pella, Inc. contains 20 mL of 150 ± 2 nm particles at a concentration of 2.42 pM [1]. Their positioning in the apparatus setup can be seen in component C of Fig. 1. The particles are held in solution inside of a cuvet on the Plexiglas Holder in Fig. 4. The concentrations used for testing are 100%, 50%, and 0% of the stock concentration, or 2.42 pM, 4.21 pM, and 0 pM, respectively. The dilutions are carried out using deionized water as a diluent. One mL of stock solution is placed in the 100% nanoparticle cuvet, 500 μL of deionized water and 500 μL of stock solution are placed in the 50% nanoparticle cuvet, and straight deionized water is placed in the 0% cuvet.

4.1.13. Semi-Opaque Medium

Optical transmission through increasingly opaque media is tested. Tests are to be conducted using water and milk before moving on to the pig skin. If the transmitted light intensity is satisfactory for the preliminary tests, then the system is ready for pig skin testing. The media are chosen based on their composition and how closely they resemble the composition of skin. Water, milk, and pig skin, respectively, are increasingly more similar to human tissue. The positioning of the media in the optical system can be seen on component C of Fig. 1.

4.1.14. Cuvets

Fisher Scientific plastic cuvets are used as a container for the gold nanoparticles and the liquid semi-opaque media. The cuvets are the same as the ones used for photometric analysis, so they have a clear window with about 80% transmittance and a known pathlength of 1 cm. They are held on the Plexiglas Holder as shown in Fig. 4.

4.1.15. Translational Stages

Two translational stages purchased from Thorlabs are used underneath the retroreflector block. One stage moves in the North-South direction (see Fig. 1 for orientation) and the other stage moves up and down. These are needed in order to make the testing easier and more precise. The stages allow for minute changes in the placement of the retroreflector. The placement of the stages can be seen in component F of Fig. 1.

4.1.16. Sheep Blood and Cuvets

For the testing with blood, whole sheep blood, purchased from Hemostat, is placed in a cuvet with a shorter pathlength (1mm) than the cuvets from the skin testing (10mm) in section 4.1.14. Whole blood is chosen because it contains both plasma and red blood cells. The plasma fraction contains particles such as glucose, hormones, and proteins, and sheep red blood cells are similar to human red blood cells. All of these components would be present *in vivo*.

5. Methods: Testing the Apparatus

The testing phase of the design process is to determine the unknown characteristics of the measurement apparatus. For this project, the only experimental variable that needs to be found is the intensity of the signal through the various tissues or the blood with and without nanoparticles. A schematic of the prototype can be seen in Fig. 1.

5.1. Test Setup Protocol

This procedure is used to set up the apparatus so that it can operate with the most precision possible. Leveling and precise locations of the components are needed for accuracy and consistency between tests. The apparatus is expected to be as level as possible after it is set up. Note: See Fig. 1 for the North, South, East, West orientation.

1) Level the laser beam with the optical bench. It is important to never lean on the bench at any time.
2) Place the 27cm focal length lens 43cm east from the laser end.
3) Make sure the beam is passing through the center of the lens.
4) Place retroreflector 38.5cm east of the 27cm lens. Make sure the beam is hitting the retroreflector. For alignment of the retroreflector, turn the rotational stage until the silicon chip is oriented at 45°. Locate the retroreflective portion of the chip. The retroreflector is a dull grey color and on the order of a third of a millimeter in diameter. Position the beam so that it impacts this retroreflective portion.
5) Place the beamsplitter about 15.4cm to the west of the retroreflector. For alignment of the beamsplitter, adjust the height of the post until the laser is as close to the middle of the lens as possible. Put the beamsplitter close to a 45° position.

6) Place the 2.54cm focal length lens north of the beamsplitter about 5cm. For alignment of the collection lens, adjust the height of the lens until the reflected beam is impacting it in as close to the middle as possible.
7) Place cuvet holder between 27cm lens and beamsplitter. Do not place any cuvets in holder yet.
8) Place iris between beamsplitter and cuvet holder, and ensure that beam is passing through center. For alignment of the iris, start with the diaphragm in the fully open position. Adjust the height of the iris until no reflection off the front surface is noticeable. Close the diaphragm slightly, and further adjust the height of the iris. Repeat this process until the diaphragm is in its closed position and little to no reflection can be seen off the front side.
9) Place the photodiode about 6cm north of the 2.54cm lens. Ensure that the beam is hitting the center of the photodiode. For alignment of the photodiode, trace the laser light beyond the collection lens with a thin object. The diode is placed at the point at which this beam is the sharpest. Avoid moving the diode away from the focal point of the collection lens if at all possible.
10) Connect photodiode to circuit.
11) Connect digital multimeter to circuit.
12) Turn on laser and take reading in order to establish the maximum signal.

5.2. Test Procedure Protocol for Medium Testing

A detailed set of directions on how tests are conducted using the apparatus for testing the media is listed below. As pictured in the test apparatus in Fig. 1, the light path goes from the laser, through the medium and nanoparticles, to the beamsplitter and to the retroreflector, back to the

beamsplitter and into the detector. This test measures the amount of light reflected off of the retroreflector through the medium.

1) Check DOE assignment to see the tests that need to be performed next.
2) Remove protective layers from test system.
3) Put on safety equipment, including gloves to reduce skin oil contact and laser safety glasses.
4) Gather appropriate cuvets, based on DOE.
5) Place cuvets in holder. Be careful to put the nanoparticle cuvet in the position closest to the beam splitter.
6) Turn on laser. Be careful to ONLY use the switch on the power surge and not the switch on the back of the laser. Pressing this switch can throw off the alignment of the beam.
7) Verify that the beam is passing through the cuvets properly and impacting the middle of the plastic pane.
8) Turn off the lights in the laboratory. Keep a flashlight close by to avoid bumping into things and to assist in system reorientation.
9) Once cuvets and media are in place, the beam is deflected slightly. Begin fine alignment of test system. DO NOT lean on the optical bench at any stage during this process.
    a. Realign iris.
    b. Realign beamsplitter.
    c. Realign retroreflector.
    d. Realign 2.54cm lens.
    e. Realign photodiode.

f. Begin voltage maximization process. Observe the voltage displayed on the DMM. The objective is to adjust the mechanical components of the system until this voltage is as high as possible. The retroreflector alignment has the most impact.

g. Put slight pressure on the rotational stage at different axes, noting the behavior of the voltage on the DMM. If pressure is applied in a direction and the voltage increases, then some slight adjustment may be necessary in this direction. Be careful to only move the rotational stage in small amounts at a time, as the patterned area is very small and can easily be lost.

h. Put slight pressure on the photodiode in various directions and adjust if any change is noticeable.

10) Orient retroreflectors for DOE testing.

11) Wait approximately 30 seconds for the current from the photodiode to stabilize.

12) Record the voltage seen on the DMM. Use as many significant figures as possible.

13) Turn the lights in the room back on.

14) Check which portion of testing is to be completed next, and repeat procedure starting with step 4.

5.3. Test Procedure Protocol for Blood Testing

The test procedure protocol for blood testing is the same as the medium testing protocol in Section 5.2 with a few differences.

1) The cuvets used are the blood cuvets as described in section 3.1.16.

2) There is no medium used.

3) The nanoparticle concentrations in this DOE are 0%, 25%, and 50%. The 0% is made with 1mL:1mL blood to water, forming a diluted blood solution. The 50% concentration is made with 500µL:500µL nanoparticles to diluted blood solution. The 25% is made with 250µL:250µL:500µL water to nanoparticles to diluted blood solution.

5.4. Design of Experiments

Minitab is used in order to obtain a full factorial Design of Experiments (DOE) for the test procedure. The DOE is a full factorial design with two variables and three levels, as seen in Table 1. This DOE is used for testing with the mediums.

Table 1. Full factorial DOE with two variables and three levels (Medium Testing).

Run	NP Conc. [pM]	Angle of Incidence
1	2.82	45
2	0	60
3	2.82	60
4	2.82	30
5	0	45
6	0	30
7	1.41	60
8	1.41	30
9	1.41	45

Table 1 shows one replicate out of the ten replicates that are performed. The angle of incidence on the retroreflector and the nanoparticle concentration are varied in order to have nine tests to perform. Because the DOE is replicated ten times, there are ninety data points total. This DOE is performed separately with each media as explained in section 3.6. The dependent variables are discussed in the following sections. The full DOE can be seen in Appendix A.

Table 2 shows the similar DOE used for the blood testing.

Table 2. Full factorial DOE with two variables and three levels (Blood Testing).

Run	NP Conc. [pM]	Angle of Incidence
1	1.41	45
2	0	60
3	1.41	60
4	1.41	30
5	0	45
6	0	30
7	0.705	60
8	0.705	30
9	0.705	45

5.4.1. Variable Angle of Incidence

The retroreflectors are only active at certain angles due to the geometry of the microstructures on the surface. In practice, this is a problem because the retroreflectors are not always guaranteed to be at the exact angle required. To account for this, multiple measurements are to be taken at various angles (high and low of 45^o and 30^o respectively) by rotating the retroreflector on the rotational mount.

5.4.2. Variable Nanoparticle Concentration for Medium Testing and Blood Testing

The concentration of nanoparticles in solution for medium testing is varied between 0 nM and 2.82 pM in order to determine what effect varying concentration has on reflected light intensity. Serial dilutions are performed in order to increase the accuracy of the nanoparticle concentration because it allows for a smaller initial quantity to be used. These dilutions are made by first taking a sample of the original 2.82 pM particles and placing it in a cuvet. This sample is the most concentrated. The rest of the solution is then diluted to 50% concentration, and a small sample is removed and placed in a cuvet. This sample is the second most concentrated solution. This process is repeated to make the 25% solution. Serial dilutions are more accurate than simple dilutions because smaller quantities are measured during each dilution than in a simple dilution.

The dilutions follow a geometric pattern, allowing smaller concentrations to be reached while using more accurate measuring devices. For example, 9 mL can be measured more accurately than 900 mL.

The dilutions for the blood testing vary concentration between 0 pM and 1.41 pM. These dilutions are also carried out serially.

5.4.3. Variable Media

The different media are to be used in an order of least opaque to most opaque, with a pass/fail assessment of reflected light intensity assigned to each medium as they are tested. The media testing flow chart is shown below in Fig. 9.

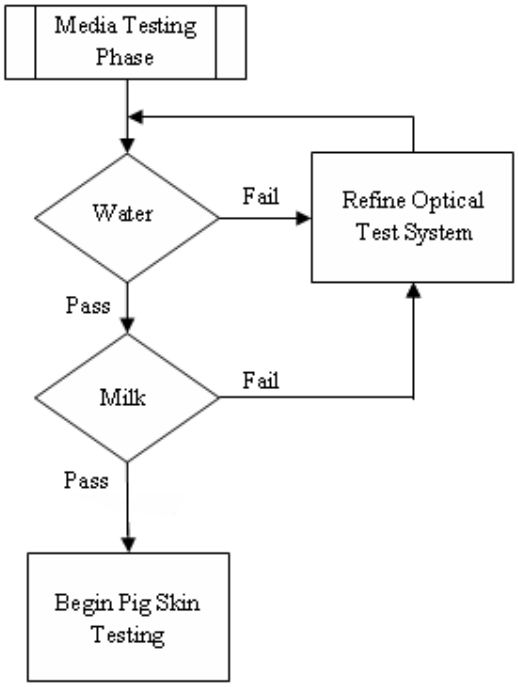


Figure 9. Process Flowchart for Media Testing Phase.

Water serves as a baseline, with maximum optical penetration expected. Milk is slightly more difficult to penetrate, as it less opaque than water. Milk contains many lipid groups as well as saccharide groups, more closely representing the human skin composition. Once these tests pass, then testing of pig skin begins. Variable thicknesses of the skin are to be tested in order to create a good mix of data points for our quantitative analyses. The DOE is to be performed separately for each of these media.

6. Results of Tests

Results are found for the water tests and the blood test. However, other data are unable to be taken.

6.1. Water Tests

The first experiment of the retroreflector behavior involves the use of water as a skin medium. This experiment is intended to obtain preliminary data and to debug any potential test setup and protocol issues. The diode response is the average reading from the ten replicates. The full data sheets for Test I and Test II are shown in Appendix B.

6.1.1. Water Test I

Figure 10 shows the boxplot results for the first water test. This test is performed without the translational stages described in Section 4.1.15.

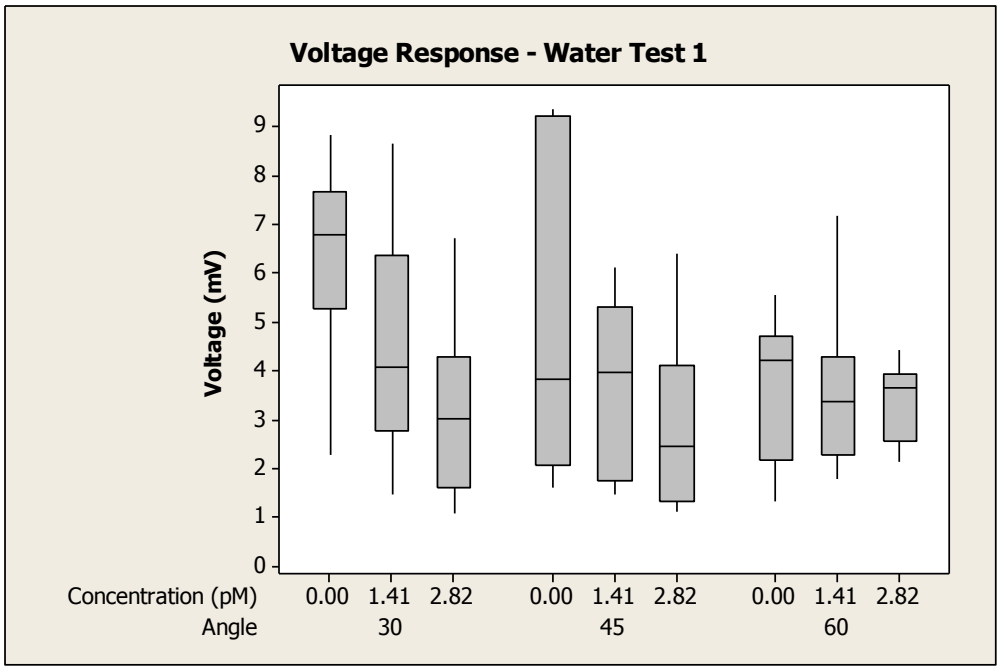


Figure 10. Boxplot of data from Water Test I. (The boxes represent the range of voltage values detected during testing for each concentration at each angle. The horizontal line in the box represents the mean voltage value within this range.)

6.1.2. Water Test II

Figure 11 shows the boxplot results for the second water test. This test is performed with an improved setup with the translational stages described in Section 4.1.15.

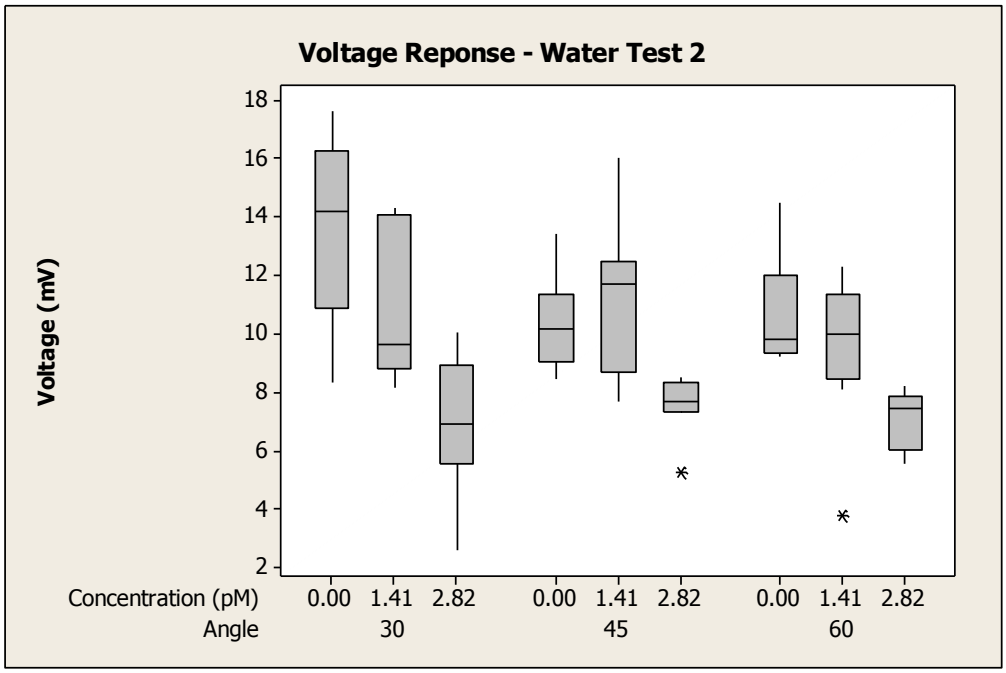


Figure 11. Water test II boxplot. (The boxes represent the range of voltage values detected during testing for each concentration at each angle. The horizontal line in the box represents the mean voltage value within this range. The star (*) represents an outlier.)

Table 3 shows the mean and standard deviation for each combination of concentration and angle.

Table 3. Water test II mean and standard deviation.

Angle	Concentration (pM)	Average (V)	Standard Deviation (V)
30	0	13.566	3.112188226
	1.41	10.861	2.564741486
	2.82	6.942	2.29156327
45	0	10.301	1.555345264
	1.41	11.231	2.481435293
	2.82	7.583	0.927949352
60	0	10.748	2.041098833
	1.41	9.502	2.462937722
	2.82	7.024	0.959840264

6.2. Medium Testing Results

As shown in the flowchart from Fig. 10, the next step after the water medium is to test milk and then pig skin. Chicken skin is also tested because of failure of the pig skin.

6.2.1. Milk

No milk data is obtained. Milk is too thick to allow light to pass through as a beam, so attempts are made to dilute the milk using water. After diluting 30 parts water to 1 part milk and obtaining no results, it is determined most time efficient to move onto skin since the milk test is not essential to the project.

6.2.2. Pig Skin

No pig skin data are obtained. The pig skin is too thick and does not transmit a useable beam of light.

6.2.3. Chicken Skin

No chicken skin data are obtained. The chicken skin scatters light such that the beam is not useable.

6.2.4. Blood Testing Results

A boxplot of the results obtained in the blood test can be found in Fig. 12.

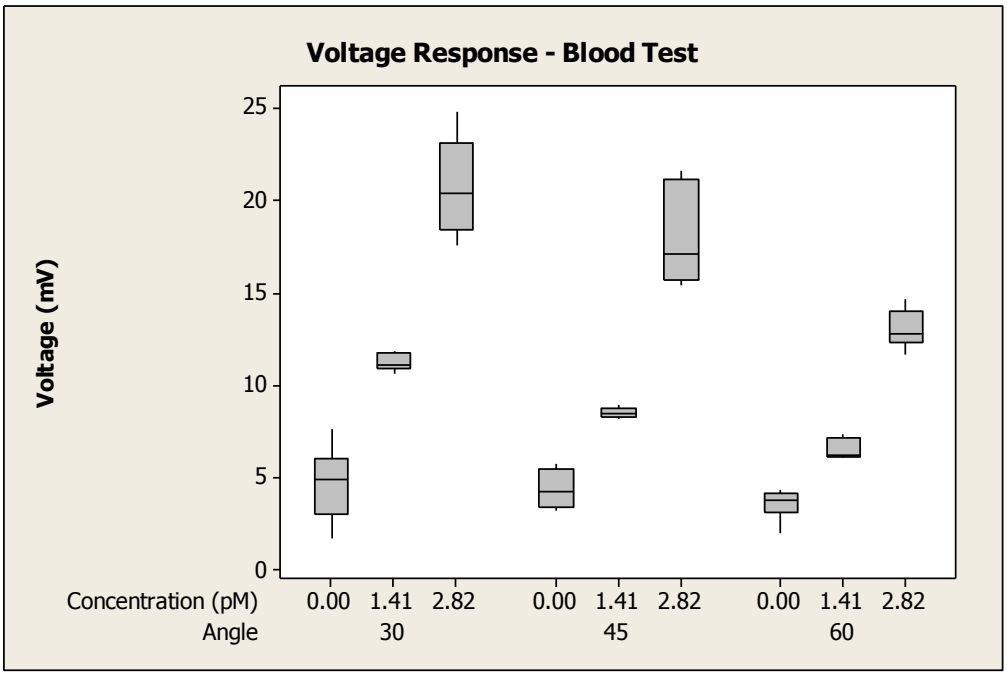


Figure 12. Boxplot of blood test.

Table 4 shows the means and standard deviations of the diode response to nanoparticle concentration and angle.

Table 4. Blood test means and standard deviations.

Angle	Concentration(pM)	Mean(V)	Standard Deviation(V)
30	0	4.654	1.910928803
	1.41	11.251	0.446926541
	2.82	20.825	2.615518857
45	0	4.323	0.983588103
	1.41	8.507	0.238795589
	2.82	18.184	2.754621168
60	0	3.539	0.744766183
	1.41	6.504	0.5009369
	2.82	13.023	0.985236012

7. Discussion

The trends of the tests from Section 6 are discussed.

7.1. Water Test I

The data set shows a very large standard deviation. One reason for the large standard deviation is that the testing procedure is still in the process of debugging and improvement. During the testing, the iris has been repositioned three times and each time the change alters the amount of light striking the retroreflector and changes the diode response. Another possible cause for the error is that the small beam hits the retroreflector area differently each time when the angle is changed. There is a significant amount of human error involved in the current test protocol because the retroreflectors have to be moved vertically and horizontally by hand. This problem is rectified in the second water test by the addition of translational stages that increase the precision of movement. Trends in the voltage are seen when plotted against the concentrations. This relationship is confirmed by an Analysis of Variance (ANOVA). Because the ANOVA shows there is a trend, the large standard deviations seen can likely be explained by noise. As a result, further testing should help lower the scatter. Conversely, there is not yet a confirmed trend in the voltage response with respect to the angle from these preliminary data.

7.2. Water Test II

As stated in section 6.1, the second water test is performed with translational stages to eliminate some operator error. This set of data shows a large range of values (see Fig. 12), although the range is smaller than in Water Test I (Fig. 11), indicating that test procedures improved. The trend in the boxplot for Water Test II (Fig.12) also shows that at 30^{o} and 60^{o}, the diode response acts as expected. As the concentration of nanoparticles increases, the diode response correspondingly decreases due to increased scattering by the nanoparticles. At 45^{o}, the trend

shows a peak at 1.41 pM of nanoparticles and a sharp decrease at 2.82 pM. This effect is unexpected and is most likely due to interaction effects between the angle and the concentration. The data from this test indicate that angle of the incident light on the retroreflector is not completely independent of concentration of nanoparticles, and that the angle can affect the measured reflected light off of the retroreflector.

7.3. Medium Testing

The milk, pig skin, and chicken skin tests yield no results. The skin scatters the beam too much to be useable, even when the skin appears translucent to the eye. These tests indicate that a different laser needs to be tested because the laser used in this test setup is not strong enough to penetrate through semi-opaque media.

7.4. Blood Testing

The blood testing results indicate that, contrary to expectations, the diode response increases at higher concentrations (see Fig. 13). This result is likely due to charge interactions between the blood and the nanoparticles that are not as problematic when the nanoparticles are diluted with deionized water. Whole blood consists of two fractions: plasma and red blood cells. The plasma fraction contains many particles such as glucose, hormones, ions, and proteins, and many of these particles are polar or charged. The gold nanoparticles are suspended in a citrate buffer to prevent charge interactions between particles. When placed in whole blood, the charged may nanoparticles aggregate with the charged particles in the blood, causing the entire complex to fall out of solution. The loss of suspended solutes would cause the blood to scatter less light. Thus, higher concentrations of nanoparticles appear to pull more solutes out of the blood, causing a higher diode response. The trend is consistent at all angles with very little overlap between the data points. The standard deviation is small, indicating that testing error is not a problem unless it is a systematic error. The responses at all angles and 0 pM nanoparticle concentration is very close, indicating that the retroreflectors are reasonably consistent. The

response values begin to differ between angles when nanoparticles are added. This suggests that interaction effects are likely present, similar to the results seen in Water Test II.

8. Budget & Timeline

A budget and timeline is used during the project in order to keep the group within budget and on schedule.

8.1. Budget

The project falls within the budget of $1000, with a total amount spent of $987. Appendix C shows a complete list of our expenditures. Appendix D shows a timeline of how the budget was spent. Figure 13 shows a categorical breakdown of where the money was spent.

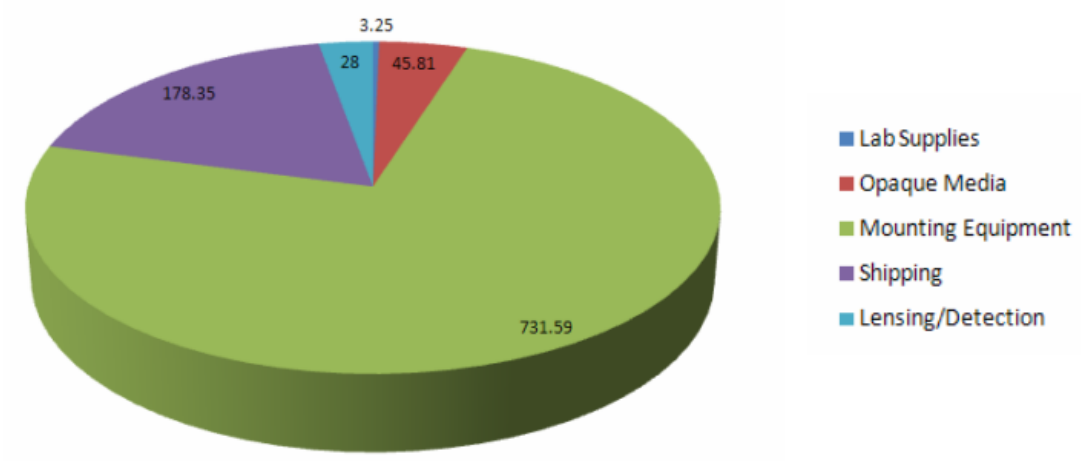


Figure 13. Budget Breakdown

The majority of the budget was spent on mounting equipment for the test setup. Shipping costs also occupy a large portion of the budget mostly because of the quantity of items shipped as well as the high cost of those that had to be shipped overnight.

8.2. Timeline

Appendix E.1 and E.2 show a breakdown of the schedules for the Fall ‘07 and Spring ‘08 semesters. The group worked a total of 830 hours out of 1190 allotted hours. This discrepancy in the number of hours worked versus the total number of hours allotted is explained by the unexpected results from the milk/chicken/pig testing. More hours would have been spent testing if detection had been successful through these semi-opaque media. A breakdown of how time was spent on the project follows: approximately 1.5 months spent on background and research, 4 months spent on test system setup, 2 months spent on testing and optimization of the system, and 1 month spent on data interpretation.

Many months were spent on the test system setup due to the iterative nature of the design. The test system set up did take longer than expected because of unexpected problems such as leveling the laser and placing the lenses. However, the data interpretation took less time than expected because the data from the tests for the semi-opaque media did not need interpreting because they failed.

9. Conclusions & Recommendations

Based on the results of the experiments, an evaluation of the test apparatus, conclusions, and recommendations for further work are made in this section.

9.1. Evaluation of Design

The designed test apparatus fulfills some of the working criteria, but other criteria require reworking of some system components to be fulfilled. The test system is theoretically safe to utilize in humans due to the use of a normal, visible wavelength that does not cause harm to the tissue. Also, the power output of the laser is low enough for the intended purpose so that no harm is done to the tissue. Detection through the translucent medium water is successful; however,

detection through a semi-opaque medium is not successful. The test system is within the budget, but if additional funding is provided, purchases of more sophisticated equipment, such as a more suitable laser, may allow fulfillment of all the working criteria. There is significant noise in the test apparatus, evidenced by the rapid shifting of diode response within a single test.

9.2. Water Tests

Based on these tests, as expected, it is concluded that as the nanoparticle concentration increases, the signal from the retroreflector decreases. The nanoparticles scatter the light causing less light to reach the retroreflector. For these tests, the test apparatus is sufficient for determining the relationship between the nanoparticle concentration and the detected signal.

9.3. Medium Testing

The test system is not sufficient to determine if a retroreflector can be detected through a semi-opaque medium because the laser used cannot provide a visible beam past the media. Because these tests failed, no further conclusions can be made.

9.4. Blood Testing

Based on the blood tests, the test setup is not sufficient to go through 100% blood. When the blood is diluted by 50%, a signal can be detected from the retroreflector. However, the testing shows that the surface of the nanoparticles must be altered in order to be functional in blood, which contains charged particles, such as potassium ions, sodium ions, and proteins, which aggregate with the current particles.

9.5. Recommendations for Future Testing

For future testing, the iris should be set once and never altered during the course of the experiment. It is recommended that a different laser with a higher wavelength is used in a similar setup. This laser may be able to pass through a semi-opaque medium and provide a

usable beam that can hit the retroreflector. If a different laser does not work, a translucent dermal implant that eliminates the complications of a semi-opaque medium should be researched and tested. Retroreflectors with a different geometry and more surface area can be used in order to make the signal easier to obtain. These retroreflectors provide higher sensitivity and more distinguishable readings. Increasing the surface area would increase the resolution, and decrease the diffraction. Future testing would also involve the use of nanoparticles that are altered such that they no longer aggregate with the components of blood.

10.The Conceptual Design

The concept for the future design is portable, easy to use, and convenient for most people. The conceptual design of a system for eventual implantantion would involve coating two sides of the four-sided retroreflector with a substrate that binds both glucose and gold nanoparticles (the measuring sides), leaving the other two sides clear for use as reference sides. The retroreflectors would spin around a central ball bearing so that a stationary laser would transiently measure the signal reflected from all four sides, thus being able to compare the reference sides to the glucose-measuring sides. The retroreflectors would be placed inside a cylindrical tube with filters on either end that would allow plasma to enter, while filtering out components of the immune system that would envelope the gold nanoparticles and expunge them from the body. The filter may be based on size exclusion since glucose molecules are on the order of 0.5 nm while components of the immune system are on the order of around 10μm [4],[5]. Uncharged nanoparticles are still required, as filtration will not exclude small ions, such as potassium. This cylindrical device could be injected under the skin into vascularized tissue. The external device would contain a laser, a beam splitter, a photodiode, a voltmeter and a memory card. The device gives the user a reading which is the delta between the measured and reference readings and tells

the user if his or her glucose levels are high, low, or normal. The memory card stores readings throughout the day and can then export those readings to a computer in order to visualize trends. This characteristic is especially helpful to doctors who can analyze the glucose level trends of the patients.

References

[1] Kemper, Steven M., Tim Sherlock, Kaajal D. Shah, Paul Ruchhoeft, Robert L. Atmar, and Richard C. Willson. "A Highly Sensitive, Low-labor Pathogen Detector Based on Retroreflector-linked Immunosorbant Assay." University of Houston. June 2007.

[2] Ted Pella, Inc. <http://www.tedpella.com/gold_html/NanoParts.htm> (Accessed: October 4, 2007).

[3] Ruchheoft, Paul. Interview on 16 April 2008. University of Houston.

[4] Marjorie, L.L., Waring, A.J., Hammer, D.A. "Interaction of the Influenza Hemagglutinin Fusion Peptide with Lipid Bilayers: Area Expansion and Permeation." Biophysical Journal Vol. 73 (September 1997): 1430-1439.

[5] Wadsworth Center, NYS Department of Health. 2008. New York State Department of Health. <http://lib.trinity.edu/research/citing/electronic/multipageinternet.shtml>.

Appendix A. Design of Experiments.

Angle	Concentration(%)	Value		Angle	Concentration (%)	Value
60	0			45	0	
60	0			60	100	
30	0			45	50	
60	50			45	50	
30	100			30	50	
60	0			30	0	
45	50			30	50	
30	0			30	0	
45	100			45	0	
30	100			60	50	
30	50			30	0	
60	0			30	50	
30	100			60	50	
45	100			30	100	
30	0			30	0	
30	50			45	100	
30	50			30	50	
60	0			45	50	
45	100			60	0	
30	50			45	50	
30	0			60	0	
60	0			30	0	
60	50			60	100	
60	100			30	100	
45	100			45	100	
60	50			30	50	
60	100			45	100	
60	50			60	100	
45	50			45	50	
45	0			30	100	
45	0			45	0	
60	50			45	100	
45	100			60	100	
60	0			45	50	
30	50			45	50	
60	100			30	100	
45	0			45	0	
60	100			30	0	
45	50			60	50	
60	50			60	100	
60	50			60	100	
45	100			30	100	
30	100			45	0	
30	100			45	0	
60	0					
45	0					

Appendix B. Water Test Results

Water

0.004815 = 50%
0.00963 = 100%
0% = H_2O

Angle	Concentration	Voltage mV
60	0	4.68
60	0	4.74
30	0	7.63
60	0.004815	3.16
30	0.00963	1.07
60	0	4.02
45	0.004815	3.55
30	0	4.35
45	0.00963	1.10
30	0.00963	1.91
30	0.004815	4.71
60	0	3.21
30	0.00963	2.83
45	0.00963	1.96
30	0	5.58
30	0.004815	3.12
30	0.004815	3.14
60	0	1.31
45	0.00963	~~1.89~~ 1.36
30	0.004815	3.44
30	0	6.32
60	0	4.63
60	0.004815	3.85
60	0.00963	2.14
45	0.00963 B1	2.08 1700 nM
60	0.004815	3.56
60	0.00963	3.44
60	0.004815	2.41
45	0.004815	2.56
45	0	1.59
45	0	1.61
60	0.004815	~~2.11~~ 2.43
45	0.00963	2.79
60	0	2.12
30	0.004815	1.63
60	0.00963	2.54
45	0	2.19
60	0.00963	2.69
45	0.004815	1.84
60	0.004815	1.76
60	0.004815	1.80
45	0.00963	1.18
30	0.00963	1.61
30	0.00963	1.61
60	0	2.18
45	0	2.21

somewhere I changed a ris

resistor value: 223.5 kΩ

Ambient:

100% : 0.04mV
50% : 0.04mV
0% : 0.04mV

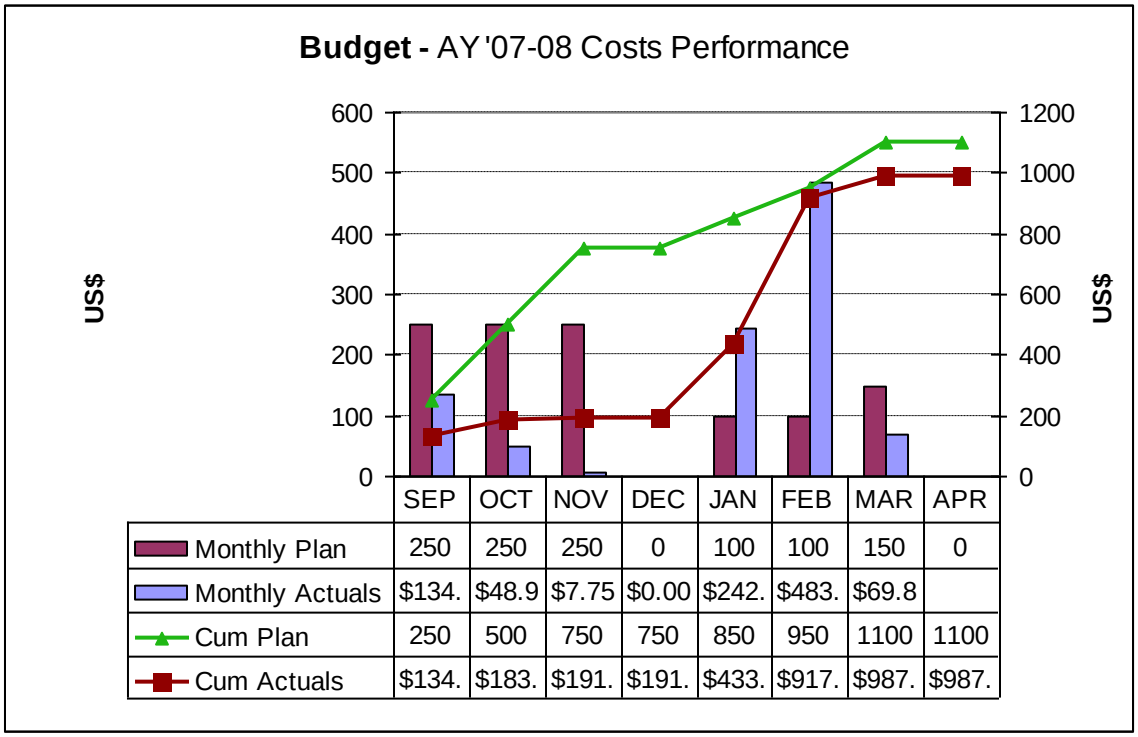
45	0	2.24
60	0.00963	2.54
45	0.004815	1.50
45	0.004815	1.47
30	0.004815	1.47
30	0	2.25
30	0.004815	4.72
30	0	6.60
45	0	5.38
60	0.004815	3.98
30	0	7.50
30	0.004815	6.33
60	0.004815	5.18
30	0.00963	3.40
30	0	~~8.77~~ 8.82
45	0.00963	~~3.33~~
30	0.004815	6.42
45	0.004815	5.99
60	0	4.40
45	0.004815	4.40
60	0	5.56
30	0	7.72
60	0.00963	4.43
30	0.00963	5.90
45	0.00963	6.39 (!)
30	0.004815	8.65
45	0.00963	5.57
60	0.00963	3.99
45	0.004815	6.12
30	0.00963	3.72
45	0	5.83
45	0.00963	3.60
60	0.00963	3.82
45	0.004815	5.08
45	0.004815	5.06
30	0.00963	3.15
45	0	9.16
30	0	10.99
60	0.004815	7.17
60	0.00963	3.89
60	0.00963	3.88
30	0.00963	6.72
45	0	9.35
45	0	9.33

Appendix C. Expenditures

Itemized Budget

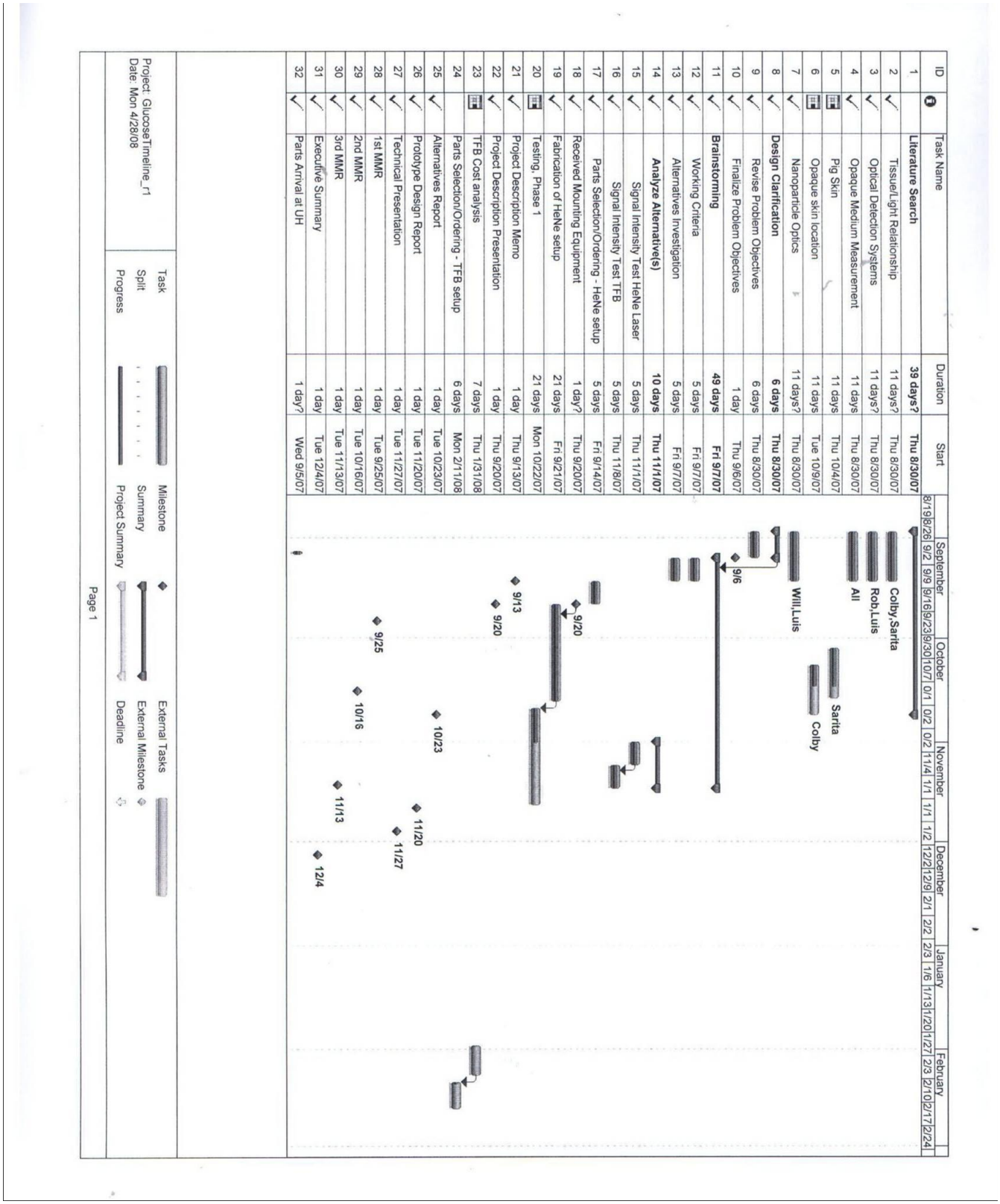
Date	Item(s)	Company	Total
9/26/2007	Plate Mount & thread adapter	Edmund Optics	$56.00
9/26/2007	Post Holders & mounts	Thor Labs	$78.59
10/12/2007	Lens Mount	Thor Labs	$48.93
11/14/2007	Tweezers	Tedpella, Inc.	$7.75
1/18/2008	Rotational mount & mounts	Thor Labs	$155.66
1/23/2008	Nanoparticles	Tedpella, Inc.	$87.00
2/1/2008	photodiode	Edmund Optics	$168.50
2/1/2008	Iris & mount	Thor Labs	$51.06
2/13/2008	Translational stages	Thor Labs	$319.06
2/14/2008	Credit for returning Iris	Thor Labs	-55.1
3/3/2008	Pig Skin	HEB & Culebra Meat Market	$9.81
		Total Spent:	$927.26

Appendix D. Budget Timeline.



	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR
Monthly Plan	250	250	250	0	100	100	150	0
Monthly Actuals	$134.	$48.9	$7.75	$0.00	$242.	$483.	$69.8	
Cum Plan	250	500	750	750	850	950	1100	1100
Cum Actuals	$134.	$183.	$191.	$191.	$433.	$917.	$987.	$987.

Appendix E.1. Fall ’07 Project Timeline



Appendix E.2. Spring ’08 Timeline