Confocal Synthetic Optical Holography Compatible Live-cell Imaging Module

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ECE 398 PSC Design Review #2

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1. Introduction

1.1. Motivation

Confocal Laser Scanning Microscopy (CLSM) is an optical imaging technique for obtaining high resolution image mostly used in biological science. A confocal microscope uses a pinhole to prevent any out of phase light from reaching the detector, allowing for high accuracy for the single pixel. Using scanning mirrors controlled by galvos, this image is taken across a square region and used to reconstruct the image in the focal plane.

Holography is when an illuminated object is superposed with a reference wave. The resulting interference pattern, a hologram, encodes the complex optical field scattered from the object within a single image. Holography has been implemented with wide-field techniques, but has only recently been used with scanning microscopy techniques such as CLSM.

Challenges using traditional holography with scanning optical microscopy are solved by synthetic optical holography (SOH), an approach that allows for millisecond-scale phase-resolved imaging. SOH operates simultaneously at visible and infrared frequencies using synthetic reference waves from a single reference arm. Other high resolution techniques for imaging cells, like SEM, destroy cells. Many other types of imaging, like imaging of fluorescently-labeled antibodies, also kill cells by requiring them to be fixed.

While SOH has been used with scattering-type scanning near-field optical microscopy (SNOM), it has not been fully implemented with CLSM for live cell imaging (LCI). Doing so would allow for higher resolution label-free phase imaging, keep the imaged cells unharmed during LCI, and provide valuable information about cell density, volume, and dry mass. It would allow for SOH to provide valuable information for cellular processes that occur over the course of hours, which is the case for many biologically and disease relevant processes. This design aims to incorporate these components to create a valuable tool for using holography with LCI with CLSM, a widely used technique that has several advantages over traditional widefield microscopy. Cells and organoids that are difficult to differentiate with traditional LCI under CLSM can be more easily differentiated using holography.

I selected this project due to my experience in optics and cell culture. I chose this because I was interested in learning more about and working with holography and live cell imaging.

1.2. Objectives

Goals and Benefits:
Add SOH capabilities to the confocal microscope
Allow for high resolution, label-free phase-resolved imaging
Keep cells alive during imaging process
Low cost and ease of implementation will allow SOH to be adopted widely, due to the existing prevalence of scanning microscopy systems and beneficial features of holography.
Allow for easier differentiation of cells and organoids under CLSM during biological processes
Features and Functions:
Low Production Cost, Low Market Cost (<$1000)
Easy to assemble
Easily replaceable components in case a part fails
“Plug-and-Play” compatibility with any generic confocal microscope (snug lock-on attachment of mirror to dish, does not block other parts of the confocal microscope, compatible with fluorescence imaging under CLSM)
Allows for phase-based calculation and encoding of scattering signal
Keeps cells alive for at least 1 hour to allow for imaging on a biologically relevant timescale
Optically measure dry mass, cell density, and cell volume

2. Design

2.1. Block Diagram

![Block Diagram Illustrating high level overview of the system. Specific interactions between blocks are detailed in the block descriptions.](image)

2.2. Block Descriptions

Base Confocal System: Components of the system that vary depending on the application. Since the components for SOH are intended for sale, the modules should be general enough to be adapted for different laser systems, microscope systems, etc. This allows for a wider variety of potential future applications, especially if optical contrast agents like
quantum dots or other fluorescent techniques are used in conjunction with the CLSM-SOH-LCI system. These are largely under the purview of other subgroups, although I have made contributions to the other parts, notably the confocal imaging program.

Confocal Imaging Program: A computer interface in LabView for interacting with the confocal microscope system. This will allow a computer to directly plug into an outlet and output a signal to the microcontroller to change the height of the piezo motors, components of the ECE 445 stage. It also communicates with the Okolab Uno Controller to record culture conditions. The latency is not of major importance, as most of the image analysis and computation is done in post-processing. Images are acquired as rapidly as on a standard confocal system, which can take images on the order of seconds.

Laser System: A 633 nm HeNe laser source, which has an associated controller to modulate laser power output. The laser system outputs light, which goes through a series of optical component (lenses, mirrors, beam splitters, half wave plates) to reach the confocal microscope system and tune the beam’s wavelength, size, and power. It takes power from the wall outlet (technically, from a power strip connected to the wall outlet).

Confocal Microscope System: The existing microscope system that the LCI and SOH apparatus are added to. We specifically are using an inverted Leica SP8 microscope. Light travels from the laser system through this to the ECE 445 stage, which sits atop the microscope stage. The system includes components such as the photodetectors (PMTs) that have an associated controller and communicate with the confocal imaging program, based on a light signal that travels back from the mirror, through the glass bottom dish to the PMTs. The program interfaces with it to read measurements like beam power, size, stage height, and current galvo position. It takes power from the wall outlet (technically, from the aforementioned power strip) to power components such as the galvo motors that control the scanning mirrors. The galvos’ position and scanning are controlled by the confocal imaging program. Our objective is non-immersion based with an NA of .75, but the design is compatible with any non-immersion objective used for CLSM.

ECE 445 Team Stage: It takes power from the wall outlet (technically, from the aforementioned power strip) and puts it through an AC/DC converter. Includes a PCB, an AC/DC converter, a controller, and piezo motors. The controller reads the current height of the system and sends that to the imaging program. The imaging program can send heights to the controller to modulate the individual height of each piezo. The piezos oscillations create the reference wave used for SOH. Light that passes through the confocal system goes past the hole in the stage to reach the cell viability module, specifically the glass bottom plate which sits atop it.

Cell Viability Module: Components of the system specifically related to keeping the cells alive. Absolutely necessary for long-term imaging. The Okolab Uno Controller and Condition Control System can be ignored for imaging that takes place on the scale of minutes.

Outlet: No batteries are used in the system, so an outlet is used as a power source instead. This will provide power for all components of the system and takes no inputs.

Okolab Uno Controller: A commercial touchscreen device that interfaces with the Okolab Condition Control System. Controls gas, heat and humidity release condition for the Okolab
Condition Control System, which directly delivers these to the dish containing cells. Connects to computer containing confocal imaging program through a USB, but powered by the wall outlet.

Figure 2: Okolab Uno Condition Control System Assembly (Left). Okolab Uno Gas and Condition Control System Interface (Right).

Okolab Condition Control System: The condition control system is powered by the wall outlet and interfaces with the Okolab Uno Controller. It modulates humidity ($H_2O$), gas flow ($CO_2$), and temperature for the live cell imaging module based on inputs from the controller. The control system includes the gas canisters and thermal source used for LCI, as seen in Figure 2. It takes feedback from the culture conditions in the dish to determine the true values of these conditions. These values are used to adjust how much $H_2O$, $CO_2$, and heat should be delivered. This information is fed back to the controller, which can send it to the confocal imaging system.

Transflection Module: Optical and biological components of the system

Mirror: This is a circular aluminum mirror with average reflectance in excess of 90% in the 450 nm to 2 µm range, specifically the ME05-G01 (Ø1/2" Round Protected Aluminum Mirror, 3.2 mm thick). A protective SiO$_2$ overcoat is layered over bare aluminum, creating an oxidation-resistant surface which has a smaller chance of tarnishing than protected silver in a high humidity environment. Aluminum is used due to the lowest cost, which is useful for prototyping. The surface is treated with poly-lysine to allow cells to adhere to it, although this may be unneeded due to SiO$_2$ having relatively high biocompatibility. Light passes through the cells attached to it, which are surrounded by a phosphate buffered saline (PBS) solution. The light then hits the mirror, and bounces back. The mirror is attached to the glass bottom dish with spacers, which are 3D printed pegs that hook onto the top outer rim of the dish and hold the mirror in place. This assembly can be seen in the Figure 4 schematic. The pegs are coated in a biocompatible polymer (PLGA, although PEG may be used for prototyping purposes due to its ready availability) to prevent interaction with the surrounding fluid and minimize resulting cell death. The mirror must be less than 200 microns from the glass bottom dish to ensure, but must be more than 50 microns away to prevent the cells from being squished and dying due to disruption of the membrane. Muscle fiber cells (merge together to form syncytia where multiple...
nuclei reside in one cell) and megakaryocytes (bone marrow cells responsible for the production of blood platelets) are among the largest cell types reach up to 100 microns in diameter, but adherent cells in culture spread out onto a surface to take up less space than their diameter. HeLa stem cells, the oldest immortalized stem cell line and a commonly used cell type, collapse to a height of 40 microns once adhered, and further collapse to a height of 20 microns once confluent, which justifies the choice in height restrictions.

Adherent Cells: Adherent C2C12 mus musculus myoblasts (immortalized house mouse muscle cells) will be used for the purpose of testing the CLSM-SOH-LCI system, using indicators such as cell viability and cell density. Cells are kept in PBS (an isotonic buffer solution), to preserve cell pH, osmotic pressure, and ion concentrations. Media cannot be used due to its scattering properties, even though it would allow the cells to survive for a longer period of time. The phase-resolved imaging is used to collect information about these cells, which can be used for. The cells are adhered to the mirror, which has been treated with poly-lysine to promote adhesion. Cells will be cultured in DMEM.

Glass Bottom Dish: Petri dish with a hole cut in the base, which is replaced with clear glass to improve optical image fidelity, as seen in the Figure 5 schematic. A 35 mm diameter dish is used, with the lens having a diameter of 12 mm, roughly the same size as the mirror. Gas, heat, and humidity are delivered from the Okolab system to the dish to preserve the conditions of the cells, allowing them to survive for a longer period of time outside media. The dish can be assembled for prototyping purposes to save money by cutting the bottom and attaching the treated glass manually. The dish itself improves the quality of high resolution cell culture images and reduces surrounding evaporation and contamination.
2.3. Schematics

Figure 3. (a) Wide-field image-plane holography. (b) Synthetic optical holography (SOH) implemented in a scattering-type scanning near-field optical microscope (s-SNOM). AFM, atomic force microscope; BS, beam splitter; L1, L2, lenses; PZM, piezo-actuated mirror.

Figure 4: Live Cell Imaging Module. The new design concept has the black pegs attach to the sides of the petri dish, instead of the cover as seen above.
Figure 5: Glass Bottom Dish diagram. The optical glass improves the optical quality of images taken. The cells form a monolayer on above the optical glass.

2.4. Software Diagram

User Interface:

Figure 6. Software flow chart that shows how a user interfaces with the SOH stage, specifically through the sub-block of the confocal imaging program that handles it. Labview compatible.
The above schematic shows how the confocal imaging program interfaces with the ECE 445 Senior Design Stage. The stage checks its height and adjusts it based on input from the user. A voltage is applied to each piezo motor to adjust the stage. If there is no need to keep checking stability (for SOH), the adjustments end, but if there is, the piezo motor heights cycle, adjusting by either incrementing or decrementing the voltage. When the voltage returns to zero, the piezos relax.

3. Requirements and Verifications

3.1. Requirements and Verification Table

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Verification</th>
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<tbody>
<tr>
<td><strong>Adherent Cells</strong></td>
<td><strong>Cell Viability</strong></td>
</tr>
<tr>
<td>1. 90% +/- 10% of cells are still alive after 1 hour (any lower and the cells, but a loss of 20% is acceptable just from minor issues with culturing the cells. On the scale of a few minutes, there is little to no cell death in PBS, but the lack of delivered gases, heat, and humidity starts to rapidly kill cells within an hour. High proportions of viable cells means the “live cell” component was successful)</td>
<td>1. Verification Process for Item 1: (a) Trypsinize cells (3 minutes) from mirror after 1 hour in live cell imaging module (b) Resuspend cells in media and transfer to microcentrifuge tube (c) Add Trypan blue, then measure in haemocytometer to count how many cells are present total and how many are dead (d) Calculate cell viability and density using the haemocytometer cell counts</td>
</tr>
<tr>
<td><strong>Mirror</strong></td>
<td><strong>Mirror</strong></td>
</tr>
<tr>
<td>1. Reflect 99.9% +/- .1% of incident light (any higher error allows out of phase light to reach the confocal pinhole, which is removed resulting in no image)</td>
<td>1. Verification Process for Item 1: (a) Set up multiple detectors at a variety (4) different angles in relation to the incident light (below microscope, in beam return path) (b) Place dish with bottom cut out atop slide, do not add PBS, attach mirror apparatus (c) Image cells using 633 HeNe Laser (d) Analyze images to determine light scattering due to glass (multi-angle laser light scattering, or MALLS)</td>
</tr>
<tr>
<td>2. Space of 125 +/- 75 microns between mirror and bottom of glass bottom dish (any higher and SOH can’t be performed, any lower and cells start to squish and die)</td>
<td>2. Verification Process for Item 2: (a) Attach mirror apparatus to dish (b) Align interferometer to determine the distance to the mirror (c) Align interferometer to determine distance to the dish (d) Calculate distance between dish and mirror</td>
</tr>
</tbody>
</table>

Glass Bottom Dish

Glass Bottom Dish
1. Light scattering through glass is less than .1% deflection (higher will compound with other scattering effects and result in a blank image, as no light will be able to go through the pinhole)

1. Verification Process for Item 1:
   (a) Set up multiple detectors at a variety (4) different angles in relation to the incident light (above microscope, in beam path)
   (b) Place glass bottom dish atop slide, do not add PBS
   (c) Image cells using 633 HeNe Laser
   (d) Analyze images to determine light scattering due to glass (multi-angle laser light scattering, or MALLS)

<table>
<thead>
<tr>
<th>Okolab Controller System  (Environment)</th>
<th>Okolab Controller System</th>
</tr>
</thead>
</table>
| 1. CO\(_2\) is being delivered at the specified flow rate (15 mL/min +/- 1 mL/min, which is what the cells need to stay healthy) at room temperature | 1. Verification Process for Item 1:
(a) Turn on Okolab, set value for CO\(_2\) delivery 
(b) Attach thermal mass flow meter 
(c) Wait 5 minutes 
(d) Read resulting mass and calculate equivalent volume for culture conditions |
| 2. Humidity (H\(_2\)O) is being delivered at the specified flow rate (15 mL/min +/- 1 mL/min, which is what the cells need to stay healthy) at room temperature | 2. Verification Process for Item 2:
(a) Turn on Okolab, set value for H\(_2\)O delivery 
(b) Attach thermal mass flow meters 
(c) Wait 5 minutes 
(d) Read resulting mass and calculate equivalent volume for culture conditions |
| 3. A temperature of 37\(^\circ\) C (+/- .1\(^\circ\) C) is being maintained | 3. Verification Process for Item 3:
(a) Turn on Okolab, set value for temperature 
(b) Setup glass bottom dish on stage 
(c) Insert thermometer into dish 
(d) Wait ten minutes 
(e) Read resulting temperature |

<table>
<thead>
<tr>
<th>Confocal Microscope System</th>
<th>Confocal Microscope System</th>
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</table>
| 1. Galvo scanning mirrors scan beam in 5 mm (+/- .05 mm) by 5 mm (+/- .05 mm) square (greater error means the scan is clipping and will not accurately encapsulate all areas of interest for the cell. If the error is any greater, cell density, volume, and dry mass calculations from the scan will be inaccurate) | 1. Verification Process for Item 1:
(a) Setup tissue phantom with known, sized fluorescent beads on stage 
(b) Start CLSM galvo scanning 
(c) Use ImageJ to create a scale bar 
(d) Compare set image and field of view to measured values to calculate error |

<table>
<thead>
<tr>
<th>ECE 445 Team Stage System</th>
<th>ECE 445 Team Stage System</th>
</tr>
</thead>
</table>
| 1. Voltage is supplied to the piezos by the microcontroller (which controls the height) at 12.1 V +/- .1 V | 1. Verification Process for Item 1:
(a) Attach 20 Ohm Resistor as load 
(b) Attach oscilloscope across load |
(c) Plug linear rectifier unit into wall
(d) Ensure output voltage remains within 12.0 V and 12.2 V

<table>
<thead>
<tr>
<th>Confocal Imaging Program</th>
<th>Confocal Imaging Program</th>
</tr>
</thead>
</table>
| 1. Latency should be less than 5 seconds (so that images can be taken rapidly to track biologically relevant processes) | 1. Verification Process for Item 1:
   (a) Turn on program timing function in LabView
   (b) Image single layer under SOH
   (c) Ensure time for image to be obtained is under 5 seconds. |

3.2. Calculations

The forces acting on the mirror during an experiment are the buoyant force from the media, the tension force from the pegs, and the force of gravity. However, as the mirror should be able to stay suspended even when no media is in solution, the following calculation is done without taking buoyant force into account. Assume there is an inscribed square in the circular mirror. There are 4 pegs attached to four corners of an inscribed square in the circular mirror. Treat this system as if all four of them being attached to the center of mass for the mirror. According to Thorlabs, the mirrors are 0.05 lbs each, or 22.67962 g each.

The amount of gas, specifically CO$_2$, to be delivered to the dish for LCI conditions is cited as 10% of total culture volume in literature. As total dish volume for a 35 mm dish is 2.5 mL, 10% comes out to be .25 mL. This means that .25 mL of gas should be delivered per second for optimal cell culture conditions, or 15 mL of gas per minute. The same value for gas delivery rate is acceptable for both H$_2$O and CO$_2$.

Pixel quality is determined by using a CCD camera beam profiler to measure the potential resolution of the beam before and after passing through the given objective. This values can then be used to

The confocal imaging program size came out to be a 437 line long, 15 KB Matlab (.m) file. This incorporates a 105 line, 2.86 KB .m file that creates the hologram, prepares the image, constructs the reference field, does a fast fourier transform, filters, shifts back to the space domain, reconstructs the image, and plots and displays the original and reconstructed images against amplitude and phase. Under the initial, standardized protocol, each image came out to be a 2.3-2.6 MB PNG file, depending on the reference wave.

3.3. Simulations

Using a computational model in Matlab, four different variables (humidity levels, CO$_2$ levels, temperature, and time) and their individual effects on cell viability were simulated. A wide range of values were swept through to see how each factor affected cell viability while the other three were held constant at the values described in the Requirements and Verifications
table above. The results were plotted in Excel. The most notable of these was temperature, as shown below.

![Cell Viability vs. Temperature](image)

Figure 7. Simulated cell viability counts after 1 hour at the given temperature. Humidity and gas flow were held at values conducive to keeping cells alive, as described in the Requirements and Verifications Table.

A simulation was then run to compare cell viability with and without the LCI components to modulate environmental conditions. For the values within our tolerances, these results indicate that minimal deleterious effects on viability would be observed. This shows that the LCI system should successfully drastically increase the number of living cells that can be imaged.

![Cell Viability vs Time Comparison](image)

Figure 8. Simulated comparison of viability with and without LCI module. Temperature was set by comparing room temperature (25°C) to ideal cell culture conditions (37°C). Similarly, gas flows were set to zero for standard conditions and to the values given in the Requirements and Verifications Table for the live cell imaging module.
A simple finite element analysis (FEA) simulation was carried out in Solidworks on the proposed design for the pegs to test whether they would be able to support the forces exerted on them by the mirror, how much stress the dish would be under, how the surroundings could be affected. The FEA simulation indicated that the mirror would be effectively supported by the pegs.

4. Tolerance Analysis

The most important component specific to the LCI module is the viability. If cells cannot stay alive, this method of holography has far more limited uses compared to existing widefield holography techniques. We can analyze this using any of a host of viability assays. One simple way is to trypsinize the cells for 3 minutes, resuspend the cells in 1 mL of media, and transfer 10 microliters of the suspension to a small microcentrifuge tube. Adding trypan blue to it 1:1 stains all cells with broken cell membranes. Propidium iodide is an alternative to Trypan blue that serves the same function. The mix can be added to a haemocytometer, where the total number of cells and number of living cells can be counted to determine cell viability. Cell density can also be an indication of healthy conditions for the cell. This is especially likely if less total cells are present after seeding at the same concentrations. When cell viability drops below 80% for the LCI module, we lose biologically relevant interactions between cells and should no longer consider the “live cell” component successful. We then calculate cell density (number of cells in a certain area), volume, and dry mass to compare it to the values we get from the phase-resolved imaging. We can also isolate the cause of cell death by finding the above values for cells attached to the mirror in media vs the values for cells left in TBS for 1 hour.

It is also a good idea to do an LDH assay, a variant of MTT/MTS used to calculate cell death. The LDH assay is a colorimetric assay to quantitatively measure lactate dehydrogenase (LDH) released into the media from damaged cells as a biomarker for cellular cytotoxicity and cytolysis. Adhered cells can be left in TBS for different time spans (15 minutes, 30 minutes, 45 minutes, 1 hour, 1.25 hours, 1.5 hours, and 2 hours). Carrying out the LDH assay will show how many cells are still alive for a specific seeding density, such as 5000 cells/mL, as a standard logarithmic curve relating absorbance to cell count can be obtained. This allows for determination of the cause of cell death.

Normal body temperature for humans is 37°C, and most mammals have normal body temperature within a few degrees of that, which dictates the ideal temperature for cell growth. CO₂ is part of the most common buffering system used in tissue culture media, and the amount that should be delivered is 10 to 20% of the total dish volume to preserve culture conditions. This is determined due to bicarbonate ions being balanced by dissolved carbon dioxide, which forms carbonic acid in solution. Carbonic acid can then convert to CO₂ and bubble out of the media, changing culture pH. Keeping CO₂ levels high in the dish prevents bicarbonate from converting to carbonic acid, thereby preventing the loss of CO₂ from the media and helping pH stay constant.
5. Cost and Schedule

5.1. Cost Analysis

<table>
<thead>
<tr>
<th>Student</th>
<th>Hourly Rate</th>
<th>Hours</th>
<th>Labor Cost</th>
<th>Total Labor Cost (with overhead)</th>
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<tr>
<td>Sreyesh Satpathy</td>
<td>$30/hour</td>
<td>80</td>
<td>$3200</td>
<td>$8000</td>
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### Parts

<table>
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<tr>
<th>Cost</th>
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<tbody>
<tr>
<td>C2C12 Mouse muscle cells</td>
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<td>Provided</td>
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<tr>
<td>Cell Culture Media and PBS</td>
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<td>Provided</td>
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<tr>
<td>Okolab Uno</td>
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<tr>
<td>Provided</td>
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<tr>
<td>CO₂ Gas Containers</td>
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<tr>
<td>Poly-lysine</td>
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<td>$50</td>
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<td>$103</td>
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<td>PLA 3d printed spacer (20)</td>
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<tr>
<td>Total Cost</td>
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All parts have already been ordered.

### Schedule

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<tr>
<th>Week</th>
<th>Task</th>
<th>Responsibility</th>
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</thead>
<tbody>
<tr>
<td>Date</td>
<td>Task Description</td>
<td>Author</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>10/30/16</td>
<td>Prepare RFA</td>
<td>Sreyesh Satpathy</td>
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<tr>
<td>(Week 1)</td>
<td>Create LCI Module Design</td>
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<tr>
<td>11/6/2016</td>
<td>Research/Compare Part costs, Address biocompatibility</td>
<td>Sreyesh Satpathy</td>
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<tr>
<td>(Week 2)</td>
<td>issues</td>
<td></td>
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<tr>
<td>11/13/2016</td>
<td>Order Parts, Assemble live cell imaging module, Take</td>
<td>Sreyesh Satpathy</td>
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<tr>
<td>(Week 3)</td>
<td>confocal cell images</td>
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<td>11/20/2016</td>
<td>Run tests and analysis for cell viability, confocal LSM,</td>
<td>Sreyesh Satpathy</td>
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<tr>
<td>(Week 4)</td>
<td>and fix LCI module issues</td>
<td></td>
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<tr>
<td>11/27/2016</td>
<td>Prepare Design Review, Re-seed cells</td>
<td>Sreyesh Satpathy</td>
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<td>(Week 5)</td>
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<tr>
<td>12/4/2016</td>
<td>Image cells using LCI module with confocal SOH</td>
<td>Sreyesh Satpathy</td>
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<tr>
<td>(Week 6)</td>
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<tr>
<td>12/11/2016</td>
<td>Analyze images</td>
<td>Sreyesh Satpathy</td>
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<tr>
<td>(Week 7)</td>
<td>Run comparison of obtained info to traditional methods</td>
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<td></td>
<td>for cell density</td>
<td></td>
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<tr>
<td>12/18/2016</td>
<td>Prepare Elevator Pitch</td>
<td>Sreyesh Satpathy</td>
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<tr>
<td>(Week 8)</td>
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6. Safety and Ethics

6.1. Safety

Electrical Concerns: In the stage design, the first step converts the signal from the power outlet of a 120V AC signal to 12V DC signal. This process is powered by the outlet through a power cord which is protected by a plastic around the wire inside. Whenever the power cord is torn or uncovered, users should immediately stop all processes and turn off the power. Afterwards, the users should purchase another power cord, or protect the uncovered cord by a friction tape. Be aware that any of the circuits should not be placed in extreme environments. Temperatures above 70°C are hazardous to the modules and the power system. Other components of the system, such as the Okolab and the computer containing the Confocal Imaging Program, have their own commercial adapters, but plug directly into a wall socket.

Optical Concerns: Although we are indirectly using lasers for confocal microscope, users...
should avoid staring lasers directly. In order to image live cells, the power of the laser should be 0.2% of the 10mW source. When exposed for long periods of time, the eyes are likely to be harmed.

Biological Concerns: C2C12 lines are mouse derived, but are still proliferating mammalian cells and can be dangerous for those untrained in using them. Only people who’ve taken all the requisite DRS safety training and are officially on the protocol can handle these cells. The CLSM-SOH-LCI system can be used with human cell lines as well, which can be more dangerous and require greater ethical considerations, but problems are minimized and mitigated by following established protocols, procedures and DRS guidelines. Proper sterile technique should be observed, and wells should be bleached or autoclaved following the completion of experiments.

6.2. Ethics

Our ethics guidelines for this project are based on IEEE Code of Ethics.

1. To accept responsibility in making decisions consistent with the safety, health, and welfare of the public, and to disclose promptly factors that might endanger the public or the environment;

In order to implement a safe performance, we must use the confocal microscope only in the allowed lab after training and approval. As mentioned ‘Safety’, users will not be handling any device or circuits in extreme environments.

2. To avoid real or perceived conflicts of interest whenever possible, and to disclose them to affected parties when they do exist;

All intellectual property rights and patents are under the jurisdiction of Professor Scott Carney and Dr. Martin Schnell.

3. To be honest and realistic in stating claims or estimates based on available data;

All data we report will be based on real experiments. We will be honest with our claims and estimates to the best of our abilities.

4. To reject bribery in all its forms;

We will not accept bribes.

5. To improve the understanding of technology; its appropriate application, and potential consequences;
Conventionally, Synthetic Optical Holography (SOH) is used for quantitative phase mapping of confocal microscopy by adding a linearly moving reference mirror. Using this method, the reference mirror needs long travel range for creating a linear-phase synthetic reference wave. Consequently, in order to improve this drawback, our design uses the method of changing the height and tilts the glass slide by using four piezo-electric controllers, which reduces the travel range compared to the conventional method.

6. To maintain and improve our technical competence and to undertake technological tasks for others only if qualified by training or experience, or after full disclosure of pertinent limitations;

In order to improve our technical competence, we will ask helps from professors, TAs, our clients. With help from their knowledge, we can get advice and consult about limitations and restrictions of the project. It may be necessary to consult with potential customers due to their insight into conventional approaches to live cell imaging.

7. To seek, accept, and offer honest criticism of technical work, to acknowledge and correct errors, and to credit properly the contributions of others;

In order to complete our project with precise measurements, we need to read many related papers for calculations. When any of their intellectual properties are used, we need to properly credit the contributions.

8. To treat fairly all persons and to not engage in acts of discrimination based on race, religion, gender, disability, age, national origin, sexual orientation, gender identity, or gender expression;

We will treat all fairly.

9. To avoid injuring others, their property, reputation, or employment by false or malicious action;

We will avoid injuring others.

10. To assist colleagues and co-workers in their professional development and to support them in following this code of ethics.

We will work with and support others.

7. References


Available: https://www.atcc.org/Products/All/CRL-1772.aspx