Phase Contrast Microscope condenser for observation of multi-well cell culture plates

DEPARTMENT OF
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Abstract

Phase Contrast Microscopy is a technique used for viewing transparent specimens, without harming the specimen, by passing through phase shifts of light. This is particularly useful for live cells. Our client, BrainXell, uses phase microscopy to examine purity of specific neuron types. Pre-existing designs include using multiple sources of refracted light to create multiple focal points and using semi-circular objective lens and multiple annulus for refractions. A universal issue experienced with this type of image is to have high resolution but only over a small area, typically smaller than the standardized well-plates. Our team seeks to expand the area of contrast to the entire well-plate, while maintaining the quality of resolution. To achieve this, the team plans on designing two prototypes outlined in the design matrix: Extra Lens and Mechanical Condenser Focusing. The Extra Lens design will increase the magnification and intensity of the light. The Mechanical Condenser Focusing design will focus on adjusting the microscope in order to increase the area without jeopardizing the resolution. After fabrication, the team will test their designs and evaluate the efficiency of their product.

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I. Introduction

Motivation

The purpose of this design project is to increase the effective area seen when using phase-contrast microscopy. In many labs across the world scientists studying cell cultures may run into the very problem we are working to fix. The dark area seen at the middle of the visual field of a phase-contrast microscope is the best area for viewing cells. This spot lets the user view cells in the most detail including internal structures and viewing of long axon tails of neurons. Outside of this area is a bright ring where the user of the microscope loses the details of the cell and the cells becomes merely an outline. This problem causes issues for consistency in labs and is a main contributor to laboratory errors while using a microscope. [Appendix B]

Existing Devices and Current Methods

The Enhancing Polarized Light Microscopy and Confocal Scanning Microscope are two of the major competing designs on the market. The Enhancing Polarized Light microscopy uses semi-circular objective lenses and multiple annuluses for refractions to improve quality of image by using this contrast-enhancing technique[5]. This technique has a high degree of sensitivity for both qualitative and quantitative analysis[5]. The Confocal Scanning Microscope uses multiple sources of refracted light and multiple focal points to allow for imaging of thick specimens through a technique called optical sectioning[4]. A drawback of this method is live specimens are sensitive to fluorophores, so photobleaching cannot be prevented[4]. These microscopy techniques are similar to phase contrast, and both have pros and cons in comparison.

Three main patents exist within the realm of this project. The first, Patent US 9041788B2, considers an apparatus that includes a light illumination, illumination optical system and a calculation device to calculate the plurality of the first electronic image [1]. This device has a similar illumination optical system and calculation to phase contrast microscopy. The next patent is US 8576483B2, this invention contains; an illumination optic system, a first and second image creation optic system, and an illumination focused diagram system [2]. Although this device has a more complex illumination optic system, it may still be similar to our design in sections. The final patent in consideration is US 6924892B2. This device includes a source of polarized light, an intensity of light detector, a condenser for providing light to the specimen, and support mounting [3]. This patent has a variation retarder with multiple sections -- each addressable by a control single, which is similar to our light diffraction mechanism in Design 4.

Problem Statement

When using phase microscopes on different sized well plates, the area of high resolution seen on each well is smaller than the entire area seen by the microscope[Appendix B]. Our team seeks to expand the area of high resolution phase contrast to the entire well by creating a customized condenser that keeps the same level of resolution as the Nikon Eclipse Ts2 Microscope.

II. Background

Relevant Physiology and Biology

The condenser alteration will not directly interact with the cells inside of the 96 well plates, so we don't need to worry about biohazards interfering with the cells. When changing the condenser, we may need to adjust the illumination; the illumination needs to be strong enough to see the cells without killing them. This is also a precaution when adjusting the focal point. These considerations are important to ensure that we don't harm the cells used by labs if they implement our design.

The process of phase contrast microscopy is characterized by the diffraction of incoming light on the specimen. This causes the light going through the denser specimen to become "out of phase" with the surrounding light. This diffracted light goes through an aligned phase ring on the objective lens that brings it back into phase with the surrounding light. When the background light and previously diffracted light combine at the image plane, negative interference of the waves creates the appearance of brightly contrasted edges of cells. This allows the viewer to view features of living, clear specimens that would be otherwise invisible in typical brightfield microscopy.

Reflection

theta=theta'

$$\frac{sin(\theta 1)}{sin(\theta 2)} = \frac{v1}{v2}$$

note $v1 > v2$ if $\theta 1 > \theta 2$

Refraction

n is the index of refraction (can find on standards table)

$$\lambda 1 * n1 = \lambda 2 * n2$$

$$n1 * sin(\theta 1) = n2 * sin(\theta 2)$$

Figure 1:
Equations relating to phase contrast optics

As seen in Figure 1, the index of refraction is related to the angle of the incoming light, which is determined by the condensing lens. The objective lens must coordinate with the condenser in phase contrast, since the image distance of the condenser and object distance of the objective lens must lie in the same location.

Development and Process flow

The development process of our design followed key stages of the design process. First general research was done to provide background about phase microscopy, Inverted microscope, purpose of microscope condensers. Then we found optics equations that gave us a background of limitations for prototypes and allowed us to understand microscopy from an optical perspective. Important equations about thin lens, reflection, and refraction, are provided in figure 2. Researching current patents was crucial in order to see how others approach our problems and to not copy any previous inventions. These existing designs also served as inspiration for our designs, as we are mostly seeking to optimize existing phase microscopy equipment. By combining our designs, existing lenses, and the researched optics equations, we can mathematically optimize our designs.

Thin Lens Equations

$$\frac{1}{f} = (n-1)\left(\frac{1}{R1} - \frac{1}{R2}\right)$$

f>0 converging

f<0 diverging

$$\frac{1}{do} + \frac{1}{di} = \frac{1}{f} \qquad di = \frac{f*do}{do-f} \qquad M = \frac{hi}{ho} = -\frac{di}{do} = \frac{f}{f-do}$$

M= magnification f = focal length

di = image distance do = object distance

hi = image height ho=object height

R = radius of curvature

Figure 2: Thin lens equations

About the Client

BrainXell, is a company who manufactures neurons from differentiated stem cells for research and development, specifically in the area of central nervous system research. BrainXell was founded in 2015 as part of the Discovery to Product program here at the University of Wisconsin Madison. The team at BrainXell uses phase-contrast microscopy to check the quality of their neurons; hence, the need for high quality resolution from the microscope. [6]

Design Specifications

There are several key design specifications for our project. The design must be able to work with pre-existing equipment such as: Nikon Eclipse Ts2, 96 well plates with opaque walls, and other interchangeable standard condensers. The condenser must maintain a minimum resolution of 1.22µm and 0.959µm with different magnification objective lenses. The condenser must allow the user to increase the area of effective phase contrast viewing in standard 96-well plates with opaque walls. We must stay within the specified budget of $1500 for the prototype. Besides these key factors, we have freedom in our design process. [Appendix B]

III. Preliminary Designs

Design 1 - Oil to Increase the Numerical Aperture (NA)

For this design we will be using oil in place of air as the immersion medium in order to increase Numerical Aperture. A higher numerical aperture is achieved by increasing the refractive index of the immersion medium. This is seen in the equation NA = (refractive index)*sin(angular aperture) [7]. Many microscopes use air with a refractive index of 1.33 as their immersion medium, but we will be changing this to oilw, which has a refractive index of 1.51 [7]. This will allow us to drastically increase the numerical aperture and hence increase the resolution.

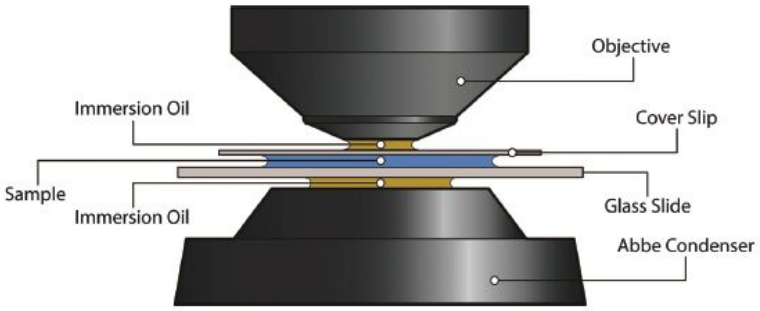


Figure 3: Design 1, using immersion oil (shown in figure) to replace what is normally air

Design #2 - Microscope Condenser Attachments

We will not change anything about the phase microscope body or anatomy. This design will make sure we don't lose any of the resolution because we are not changing anything about the existing lenses. These extensions will connect to the objective lens part of the microscope, and will contain a light, annulus, and condenser that will be angled to the side of the focal point, to it and further extending it.

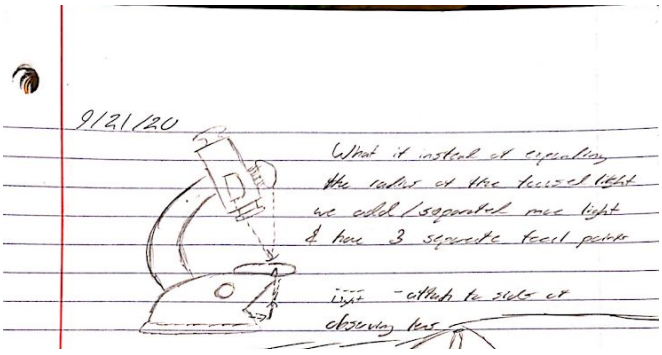


Figure 4: Design 2. The lines in the image represent the change of angles of light

Design #3 - Condenser Annulus Adjustment

For this design we will not change anything about the microscope body or condenser, just the condenser annulus, which lies directly below the condenser. We would like to make the opening wider to allow for more light to pass through and into the condenser. This will allow us to maintain the resolution because the only thing we are altering is the amount of light that passes through the condenser annulus. Making this change will cause more light to travel throughout the microscope, hopefully allowing it to cover a wider area of the specimen to increase the area that is easily visible and contrasted. This could possibly also be paired with a change in the condenser lens, if we need to alter the angle of the light as well.

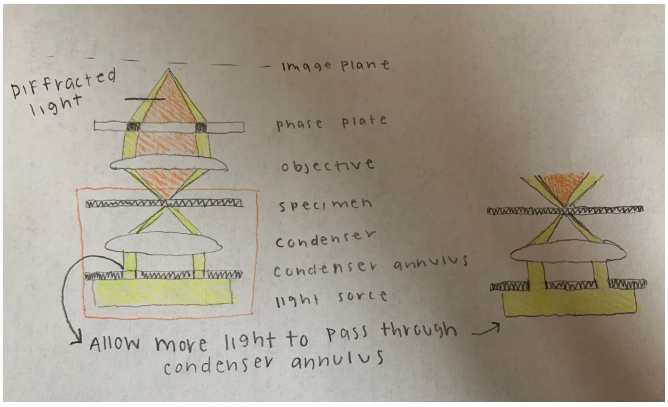


Figure 5: Design 3. This models the typical form a condenser annulus, with specified changes to increase area of phase contrast

Design #4: Additional lenses to narrow cone of light

As seen in the second photo, the problem we see with decreased effective area of phase contrast results from "clipping" of the cone of light coming off the specimen. The decreased amount of this non-diffracted "background light" causes an issue when combining the diffracted and un-diffracted light at the viewer's eye. My solution was to add an additional set of lenses between the condenser and objective lenses that essentially narrow the incoming cone of light. The decreased angle of the cone would pass through the well plate with less clipping at the edges, and should - in theory - result in a more effective area.

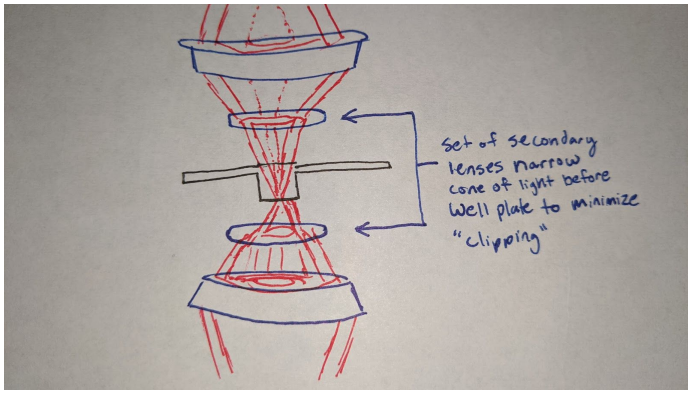


Figure 6: Design 4. Extra lenses between typical condenser structure

Some possible issues with this design are that it could result in an unacceptable change of resolution. Additionally, narrowing the cone of light and then expanding it again could cause some unforeseen distortion in the final image. This would be unacceptable, as the shape should be precise for the viewer to properly analyze the specimen. Finally, since this is dealing with lenses, it would have to be specific to the condenser/objective set. You wouldn't be able to use a standard set of secondary lenses for all magnification levels.

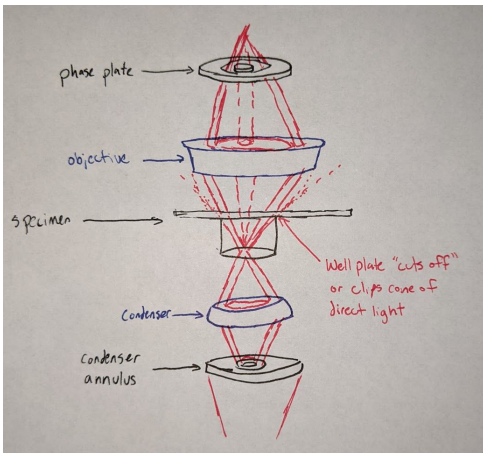
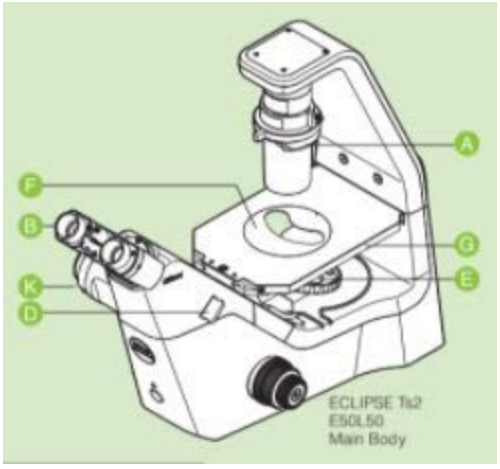


Figure 7: Whole picture showing "clipping" of light cone with dotted lines

Some positives with this design are that it is strictly mechanical. This could be added to any existing microscope. Additionally, the design is fairly simple to build, as it would just go around the objective lens and below the condenser. Finally, if the math is done correctly, this design could realistically be done with store-bought lenses, as all we need the lenses to do is focus a beam of light and expand it.

Design #5 - Mechanical Solution Consideration

This design does not change the condenser, but rather changes the mechanics of where it is placed and how to align it. A possible problem with why there are rings in the images is that the illumination and objective are out of line with each other. The Nikon microscopes say they are suited for well plates, but without an option to change the alignment of the condenser, the extra depth of the well plates is not accounted for, and thus our image has a low area. This design keeps the current condenser, the same objective set up, but we will make an attachment to move the condenser to match the tip of cone of illumination to the correct spot (which will have to be tested to find exactly) and get the optimized phase imaging. Below is a picture of the model used by BrianXell, to show the section that we are focusing on.



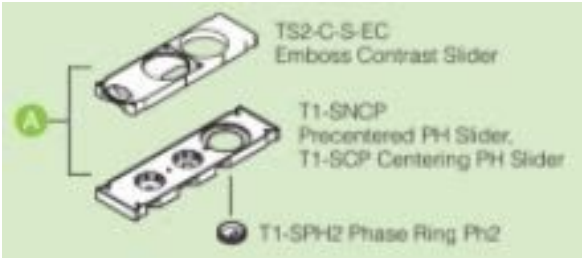


Figure 8: Mechanical Approach Design. Images of Nikon Eclipse Ts2 found from it's manual [8].
Above part A, we will add threads or an extension to change the vertical height of the condenser in reference to the middle plate, F

IV. Preliminary Design Evaluation

Design Matrix

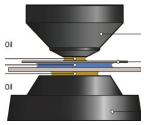
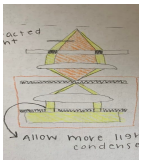
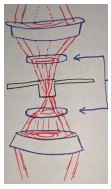
			Using Oil instead of Air to increase refraction index 		Objective Lens Attachment 		Condenser Annulus Adjustment 		Extra Lenses 		Mechanical Condenser Focusing	
Rank	Criteria	Weight	Score out of 10	Weighted Score	Score out of 10	Weighted Score	Score out of 10	Weighted Score	Score out of 10	Weighted Score	Score out of 10	Weighted Score
1	Effective Area seen by phase contrast	25	9/10	22.5	8/10	20	7/10	17.5	7/10	17.5	6/10	15
2	Resolution	25	3/10	7.5	8/10	20	8/10	20	6/10	15	7/10	17.5
3	Adaptability	20	5/10	10	9/10	18	7/10	14	6/10	12	7/10	14
4	Cost efficiency	15	6/10	9	3/10	4.5	7/10	10.5	4/10	6	6/10	9
5	Simplicity	10	7/10	7	6/10	6	8/10	8	5/10	5	6/10	6
6	Safety	5	8/10	4	10/10	5	10/10	5	10/10	5	10/10	5
	Sum	100	**Sum**	60	**Sum**	73.5	**Sum**	75	**Sum**	60.5	**Sum**	66.5

Figure 9: Design Matrix

Summary of Design Matrix

Effective area seen by phase contrast

These criteria were chosen to be the same weight because both are an integral part of inspecting a sample effectively. Our main focuses in this project are to expand the area of high resolution phase contrast and the effective area seen by phase contrast. High resolution is difficult to obtain but equally as important in assessing cell cultures. The oil design won the area seen category since it would, in theory, change only the numerical aperture of the condenser and would increase the area most efficiently, but would lose some resolution. The resolution category was a tie between the extra lens design and the condenser annulus adjustment. The extra cone design will not change where the cone is focused, and therefore keep the original resolution intact. The condenser annulus adjustment would fix the focal point by physically moving the focal point by changing the position of the condenser.

Adaptability

In labs globally there already exists high functioning, expensive equipment such as; microscopes, condensers, and mass produced and standardized well-plates. For this reason, we do not want to come up with a completely new design for all these components. Or even a design that requires a change in more than one of these components. It is important that our product is made to adapt to or replace one piece of already existing equipment. It is also important that the piece work for light and dark well plates, as well as a variety of sized well-plates. So that the technology can be used diversely. The extra lens design also won this category because of its ability to be attached to any existing condenser/objective combination. Additionally, it should be able to focus the light through any well plates, not just the specific ones we are designing for.

Cost Efficiency

Cost efficiency is also important, since this is made as an addition to already existing equipment, it needs to not add a financial burden on labs. The more effective the cost per product is, the more accessible it is to scientists and lab technicians everywhere. Cost is not the most important criteria because marketability is not the top priority for us or the client. The extra lens design proved to be most cost effective since it only requires use to buy lenses. If designed the way we intend, and if the math checks out, these lenses could be standard focusing lenses purchased from the internet, which wouldn't be very expensive.

Simplicity

Simplicity is rated at a 10 for importance because all the designs are complex and labor intensive. The more complex a design, the smaller the room for error becomes and the harder the design is to execute. However, it is rated as second lowest because executing the design

requirements are more important than the labor required to build a prototype. The higher rated a design is, the less complex it is. The oil design and annulus adjustment designs tied to win this category. Both of these designs only involve adjusting one part of the existing microscope, compared to the others that either require us to build an entirely new device or work with complex optics.

Safety

Safety is the least important criteria because we are following FDA approved products as our inspiration. However, we chose the extra lens design to win this category, since it has no moving parts, requires very little manipulation once installed, and doesn't pose any threat to lab safety or cleanliness.

Proposed Final Design(s)

The final design was a group decision by taking the combination of our design matrix winner (the condenser annulus adjustment) and our client's recommended design (the lens addition). To do this we will slightly adjust the condenser annulus opening to allow more light in and add an extra lens to properly direct the lens towards the specimen. The mix of allowing more light in and adding a lens to direct it will allow for more light to pass through a wider area of the specimen, pushing more light out of phase, and correctly directing it to the viewing area. Adjusting the lenses may result in a loss of resolution, but hopefully this will be accounted for by finding the correct angle to allow for maximum viewability of the specimen.

V. Fabrication/Development

Materials

We will be using FDM printing, so the material for the annulus's housing will be PLA (Polylactic Acid), which is a type of plastic that is relatively cheap, and prints quickly. FDM printing is not extremely precise, however we do not need precision in the fractions of millimeters for the annulus to hold the lenses properly. For the lens we will purchase condensers, deconstruct the condenser, and then use the pre-existing glass lens for our prototypes.

Methods

Since the prototype will require attachments to both the condensing and objective lenses, a great amount of custom fabrication will be required. In addition to the non-standard fabrication methods required, the project will likely need to be changed and updated several times, since we don't have consistent access to a microscope for precise measurements. Both of these issues are

addressed well by the rapid prototyping capabilities of 3D-printing, and that is why we will use the method of FDM (First Deposition Modeling) 3D printing for our project.

The reasons we will use FDM printing over other more precise methods of 3D printing are cost, speed, and size of the prototype. The printed components are housings to hold lenses at calculated distances from the existing objective and condensing lenses. This will not require a degree of internal complexity or structural integrity. However, the fittings may have to be re-designed several times, so several iterations will have to be printed. To minimize cost, FDM printing is the cheapest method, as well as the fastest. Its main drawback is its lack of precision, which is not a significant negative for this project.

The prototype will consist of two lens housings that will be printed as two halves each. The lenses will be placed in the halves at the correct places, and the halves will be joined together around each lens using epoxy bonding. This creates a sealed surface where the lenses cannot move within the housings. The housings will then be attached to the microscope using a press-fit seal over the objective and condensing lenses.

Testing

Preferably, after fabrication we will conduct tests on a Nikon Eclipse Ts2 inverted microscope, but we can use any inverted microscope with a removable condenser to test our condenser. The testing we will perform is to use our condenser and take pictures of the well plates we are observing. Then we will compare this to pictures of the same well plates under the Nikon eclipse TS2 microscope, or in the case of a different model we will test compare our condenser to the original condenser on that model. We will measure and compare the effective phase contrast (dark circles) found by using our condenser versus the original condenser. We will also make qualitative observations on the resolution of both. We will repeat this process for different mediums, different magnifications of the objective, and different light sources to make sure the design is adaptable to different environments.

By measuring and analyzing the area and resolution of our new design compared to the existing design, we will be evaluating the most important aspects of the clients requirements. Once we assess our results we will either redesign parts of the condenser that we found problems with, or move forward. The next step, if resolution and area are up to par, is to design an easy fabrication and application plan so that the clients next desire (marketability) can come to fruition.

VI. Results

Since the device has not been created yet, there is no data to analyze. The future data will be analyzed using Matlab, to understand the mean, median, mode, standard deviation, and variance of the data. Through the analysis of the data, it can be better understood what type of distribution is occurring and how the data is spread. The team will strive to obtain data that is spread consistently and align with expected numbers. A graph will be made to illustrate how the data is being spread and how close the standard deviation is to the predicted data.

VII. Discussion

If successful, the application of this device will allow for more specific selection of differentiated stem cells, which will aid in research of central nervous system disorders and ultimately help people who suffer from central nervous system disorders. There is a broader application to any study of live cultures involving phase microscopy. Under better magnification, researchers can be more selective and narrow with their studies under the microscope.

Once the device is made and tested we have to see if it is logical to use our design over other existing phase microscope designs. Because there are other, optimized, microscopes that can be used on well-plates it may be a better financial option for a user to simply purchase another microscope that already exists. However, if financially viable, our condenser may replace condensers in labs with Nikon Eclipse microscopes wishing to use well-plates

VIII. Conclusion

In order to expand the area of high resolution phase contrast, our team researched phase contrast microscopy, microscope physics and competing designs. This research showed us how to follow our design requirements: to increase viewable area, maintain resolution, and fit our design to the Nikon Ts2 microscope. After weeks of trying to grasp the complexities of phase contrast microscopy and pondering how to best solve our design problem we decided on our final design: a combination of the condenser annulus adjustment and extra lens addition. Testing the prototype will allow us to determine if the new design increases area and maintains resolution - our two main project constraints. Looking back at the semester the main thing we struggled with was grasping the complications behind altering the microscope. We have not been able to identify the source of error in the microscope because of the current COVID-19 situation and not being able to use lab facilities to test the microscope used by the client. In the future, we will continue to meet with our client, Mike Hendrickson to obtain a better understanding of the problem. Future work will be obtaining access to a microscope in order to test our fabricated designs to see if they will improve imaging of live neuron cells.

IX. References

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X. Appendix

Appendix A- PDS

Function:

BrainXell, represented by Mr. Michael Henrickson faces a common problem experienced by many lab technicians globally. When using phase microscopes on different sized well plates, the area of high resolution seen on each well is smaller than the entire area seen by the microscope. Our team seeks to expand the area of high resolution phase contrast to the entire well by creating a customized condenser. The condenser will keep the same level of resolution as the current condenser used by the client, and function with a variety of different well plates.

Client requirements:

- Expand the area of contrast
- Be used for black and white well plates
- Condenser should fit already existing equipment
- A new condenser is easier to distribute than new well-plates or a new microscope
    - This leaves open the option of trying different lighting techniques

Design requirements:

1. Physical and Operational Characteristics
    a. *Performance requirements:*
        i. The condenser must allow the user to increase the area of effective phase contrast viewing in standard 96-well plates with opaque walls from <25% to >75%.
        ii. The condenser must maintain a resolution of 1.22µm with 10X magnification objective lenses and 0.959µm with 20X magnification objective lenses with a tolerance of ±25%.
        iii. The condenser must be compatible with a Nikon ECLIPSE Ts2 microscope.
        iv. The condenser must be removable and interchangeable with other standard condensers.
        v. The condenser must be compatible with standard 96-well plates with opaque walls.

    b. *Safety:*
        i. The condenser does not interact with the electrical components of the microscope, so no additional marking is required.
        ii. The condenser cannot produce any residue that may damage the specimen or harm the user.
        iii. The condenser must not perforate any safety equipment or damage the body of the user during interaction.

iv. The condenser surface cannot exceed 44℃ for risk of thermal burning. [1]

c. *Accuracy and Reliability:*
   i. The goal for this project is to maintain the accuracy and reliability of current phase contrast microscopy -- BrainXell uses the Nikon ECLIPSE Ts2.
      1. The Nikon ECLIPSE Ts2 uses the ELWD (extremely long working distance) condenser has a numerical aperture of 0.3, working distance of 75 mm and magnification of 10x, 15x, and 20x. [2]
      2. These specifications should be maintained or improved to allow for consistent accuracy and reliability.
   ii. The condenser should also be reliable enough to give consistent results each time images are taken.

d. *Life in Service:* Establish service requirements, including how short, how long, and against what criteria? (i.e. hours, days of operation, distance traveled, no.of revolutions, no. of cycles, etc.)
   i. The condenser must withstand up to 24 hours of continuous laboratory use.
   ii. The condenser must withstand 10+ years of daily laboratory use
   iii. The condenser must remain usable after 10,000 cycles of removal.

e. *Shelf Life:*
   i. The condenser for a phase microscope has no expiration date if stored properly and used in the correct operating environment.

f. *Operating Environment:*
   i. The ideal operating environment for a phase microscope is between 32º-104ºF and the maximum humidity should be no more than 85%.
   ii. Storing conditions for the condenser must be dry, with low humidity, and not in direct sunlight.
   iii. A dust cover must be used when the microscope is not in use for an extended period of time. [3]

g. *Ergonomics:*
   i. There are many ergonomic issues with the condenser. The useability of the condenser is important to reach Köhler Illumination. The placement of the condenser must fit the size of the light source and the focus to achieve accurate results. Without proper fitting of the condenser, quantitative results are less accurate and harder to calculate. The condenser is adjusted with the aperture and will give the highest quality image. The aperture must be in an easily located position on the microscope and will have the correct height in relation to the objective. [4]

h. *Size:*
   i. The condenser must fit in the space of current standard condensers:
   ii. The current condenser used in the Nikon ECLIPSE Ts2 is the ELWD Condenser which must not exceed 75 mm in length.[2]

i. *Weight:*
   i. The condenser must weigh between 90-200 grams.[5]

j. *Materials:*

i. Condenser materials should be taken into consideration with the watts of lamp used. Common materials for condensers include plastic or metal.
ii. The field lens should be constructed out of glass.[6]

k. *Aesthetics, Appearance, and Finish:*
i. The form and texture of the finish of the condenser must be standardized with the Nikon ECLIPSE Ts2 microscope.
ii. The color and shape should be consistent with current condensers (black and cylindrical) [2]

2. Production Characteristics

a. *Quantity:*
i. Only one unit is needed for testing in this course. If the results are satisfactory with the users, more can be made for use by other users experiencing phase resolution issues with their current condensers.

b. *Target Product Cost:*
i. The specified budget for the prototype is $1500.
ii. The product cost should be at or below the cost of the Nikon Phase Contrast ELWD 0.3NA Condenser at $1,150. [7]

3. Miscellaneous

a. *Standards and Specifications:*
i. FDA approval is not required for the fabrication of a Class I microscope condenser, as well as, all GMP regulations. This is true as long as the condenser is not labeled or otherwise represented as sterile. [8]

b. *Customer:*
i. The customer needs the condenser to be able to be adaptable to different kinds of plates, with focus on the Grainer-96 plates
ii. The client prefers resolution of the edges of the visible lens to not be opaque and to be equally as transparent as the center of the visible lens.
iii. The customer would also like the resolution and contrast to be our highest priorities, with limiting tradeoff between lower resolution for more area.

c. *Patient-related concerns:*
i. Microscopes should generally be sanitized and cleaned after 200 hours of service, or more frequently depending on daily usage.
ii. Microscope condensers are extremely fragile pieces and must be cleaned thoroughly and carefully. [9]
iii. There is no specified shelf life for this device

d. *Competition:*
i. *Patent US 9041788B2* - This apparatus includes a light illumination, an illumination optical system, a calculation device used to calculate the plurality of the first electronic image. [10]
1. This device has a similar illumination optical system and calculation.

ii. *Patent US 8576483B2* - This invention contains an illumination optic system, first and second image creation optic system, an illumination- focused diaphragm section . [11]
    1. This device has a more complex illumination optic system, however, may be similarities in sections.

iii. *Patent US 6924892B2* - This device includes a source of polarized light, an intensity of light detector, a condenser for providing light to the specimen, and support mounting. [12]
    1. Has a variation retarder with multiple sections, each addressable by a control signal. Similar to our light diffraction mechanism.

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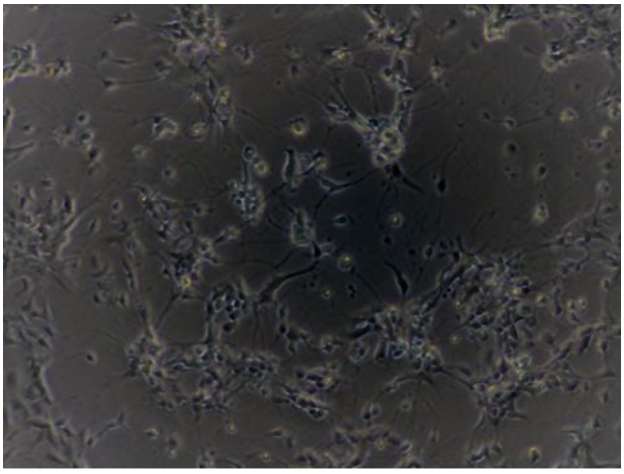
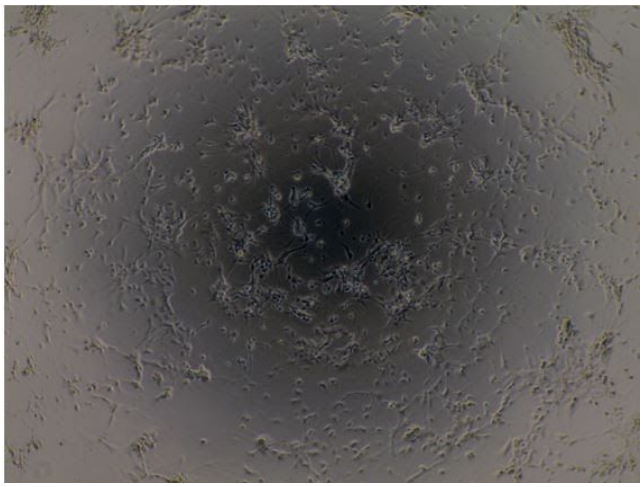
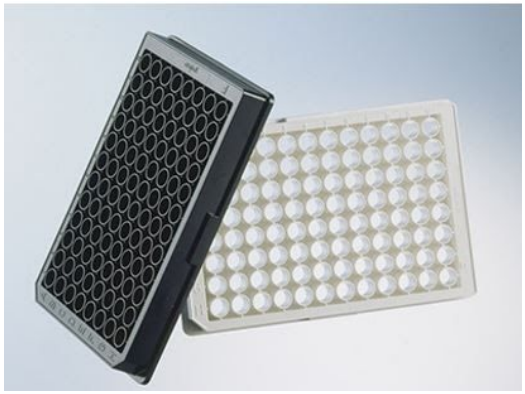
Appendix B- Additional Figures





Picture Provided by Mr. Michael Hendrickson, The top two show areas of high resolutions, but decreasing area (rings seen best on the right) of phase contrast. The bottom right image is a picture of standard 96-well plates, both dark and opaque



Calculations for Resolution

The equation we were given for resolution is as follows: d = 1.22(wavelength)/(NA condenser + NA objective)

The condenser they use is a Nikon ELWD (Extremely Long Working Distance) condenser with NA of 0.3.

The objective lenses they use are:

- 10X Magnification: NA 0.25
- 20X Magnification: NA 0.4

The wavelength of light we are using is 550nm

Therefore the resolutions of the different magnifications are:

- 10X: 1.22 micrometers
- 20X: 0.959 micrometers