DEPARTMENT OF
Biomedical Engineering
UNIVERSITY OF WISCONSIN–MADISON

Phase Contrast Microscope condenser for observation of multiwell cell culture plates

BME 200/300 - Final Report, December 9th, 2020

Client: Mr. Michael Hendrickson
BrainXell

Advisor: Dr. Kris Saha
University of Wisconsin-Madison - Department of Biomedical Engineering

Team Members:

Kylie Gaspar	*Team Leader*
Ben Hildebrandt	*Communicator*
Katherine Budde	*Co-BWIG*
Samuel Herzog	*Co-BWIG*
Carson Evenstad	*BSAC*
Lauren Hicks	*BPAG*

Abstract

Phase contrast microscopy is a technique used for viewing transparent specimens by passing light through the specimen and creating an image from the shifts in light waves. Phase contrast is particularly useful for our client, BrainXell, who uses phase contrast microscopy to examine the purity of specific neuron types. BrainXell commonly experiences inconsistent phase contrast imaging across a single well of a well plate. The edges of the image show a significant decrease in cell visibility and phase contrast quality. The goal of the project was to increase the effective area of phase contrast to the entire image by creating an extension for an inverted phase microscope that maintains the current resolution or quality of phase contrast.

To optimize the image, our team had several preliminary designs that would redirect the path of the light from the existing condenser through the specimen, to the image plane. The team selected two different designs for fabrication. Specifications and testing of these designs were done using a Fisher Scientific Micromaster Inverted Microscope. The designs were 3D printed with a black resin to avoid light interference, additional standard lenses were ordered and assembled in the corresponding print. Though BrainXell specializes in neuronal cells, testing of the designs were evaluated by imaging HEK cells. Images from using the Fisher Microscope alone were compared to the images from using our two designs attached to the Fisher Microscope. By using additional lenses to concentrate the path of light, we found a significant change in the area of the color on the edge of the image.

I. Introduction

Motivation

Phase microscopy has been utilized since 1934, and has been innovated and refined to the current models we have today [1]. These models allow the user to capture high-contrast images of live, transparent specimens without distorting their health by adding any dyes or solutions as is used for other microscopy techniques. The downside of phase microscopy is that the resolution can fluctuate greatly in a single image. We believe the reason for the fluctuation is when various sample holders, that the microscope is not calibrated for, are used to hold the specimens. The absence of calibration causes clipping of light by the sample holders or inaccurate measurements of in and out of phase light. The change in light path causes the area of high contrast resolution to greatly narrow. The difference of resolution causes observation of an entire cell plate to be much more strenuous and time-consuming than what an optimized design would be. And when researchers have to observe many cell plates every day, the wasted time truly adds up. To combat the stress of inaccurate phase microscopy reading, we are looking to a low cost, easily adjustable and will expand the central maxima of the light. We hope our design is able to help researchers across the world in their efforts to utilize phase microscopy.

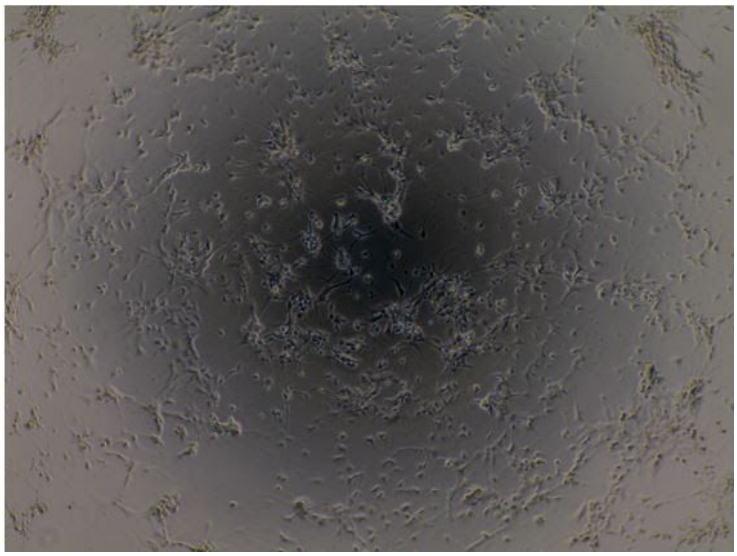


Fig. 1: Picture provided to us by Mr. Michael Hendrickson of BrianXell. This is an entire image seen by a user viewing neurons using a Ts2 Nikon Eclipse Inverted Microscope. The center, dark area, of the image has high resolution phase contrast but a decreased resolution is seen by moving outwards from the center of the image, towards the edges of the viewable area.

Existing Devices: ***Current Methods of Phase Microscopy***

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 objective lenses and multiple annuli to determine the optical properties of the specimen. The contrast-enhancing technique has a high degree of sensitivity for both qualitative and quantitative analysis. The illumination optics system is related to our design from the concentration of their polarized light being comparable to the illumination of optic systems that would enable us to be able to avoid clipping of well plates[2].

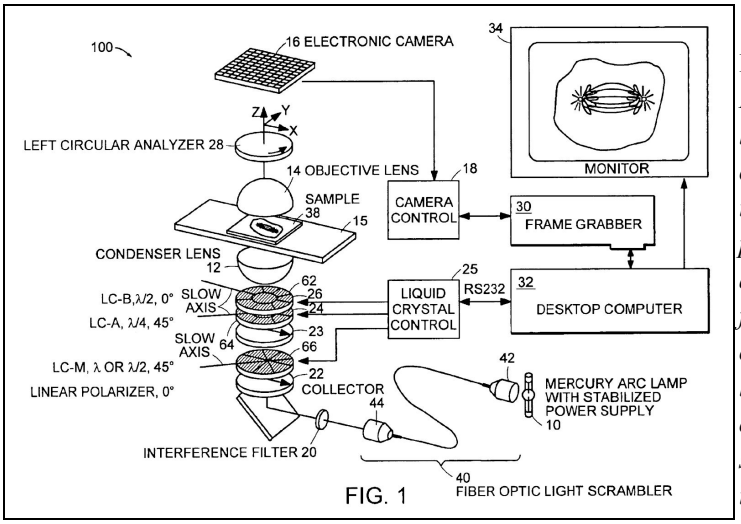


FIG. 1

Fig. 2: *Schematic diagram of "Enhancing Polarized Light" design that captures the effect light area through use of multiple circular objective lenses which resolutes a complex illuminate optics system that is able focus polarized light (polarize light it used for more accurate data measurement from the electric field of the light being concentrated on a certain plane) and measure the intensity of light. Thes intensity value is needed for calculating the optical properties of the specimen, such as reflectance and refractive index.*

The Confocal Scanning Microscope utilizes 'pinhole' to eliminate out of focus light and is suitable for both live and fixed cells and tissues. The advantage of a confocal microscope compared to wide-field microscopes is that discrete optical sections can be collected while eliminating the out of focus light around the current plane of focus. Because of the high precision, only high intensity beams of laser light are used. The Confocal Scanning Microscope is related to our project from the elimination of out of phase light in the microscope, out of phase light is causes destructive interference with the in phase light going through our specimen, destructive interference creates the fluctuations of areas of high and low resolutions in our imaging [3].

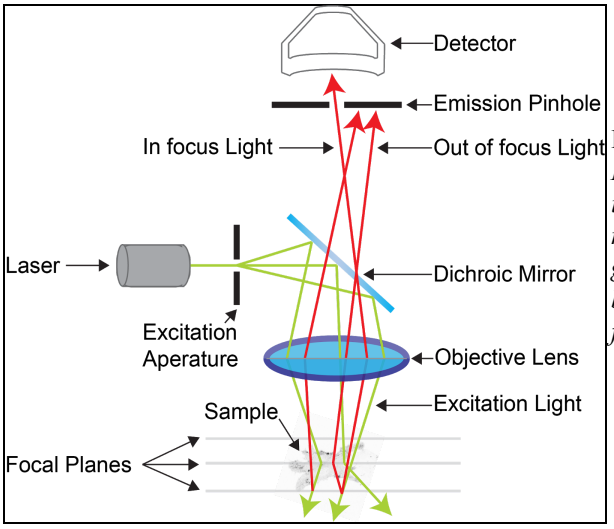


Fig. 3: Shows a *schematic diagram of a Confocal Scanning Microscope apparatus that uses arrows to indicate high intensity beams of light that are separated by differences in refraction of phase and out of phase light. The process guarantees that only in phase light will be used to observe by the detector. The Dichroic Mirror is able to source and focus all in phase light to properly observe the sample.*

Problem Statement

When using phase contrast microscopes on different well plates, the area of effective phase contrast is limited to the center of the plate w1ell. Our team seeks to design a solution to expand the area of high resolution phase contrast to the entire well. The design solution should maintain the quality of resolution and be compatible with a variety of well plates and microscopes.

II. Background

Relevant Biology and Physiology

One design specification requirement we had to abide by is not to alter the method of holding live cells or moving live cells. So biohazards are of little concern. For our design we did change the illumination, so it must be sure that we do not injure the cells, yet we still must allow proper light intensity to go through the specimen to allow for proper viewing. These considerations are important to ensure that we do not harm the cells used by labs that implement our design.

The process of phase contrast microscopy is characterized by the diffraction of incoming light on the specimen. Diffraction of light causes the light going through the denser specimen to become "out of phase" with the surrounding light. The 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. The contrast allows the viewer to view features of living, clear specimens that would be otherwise invisible in typical brightfield microscopy.

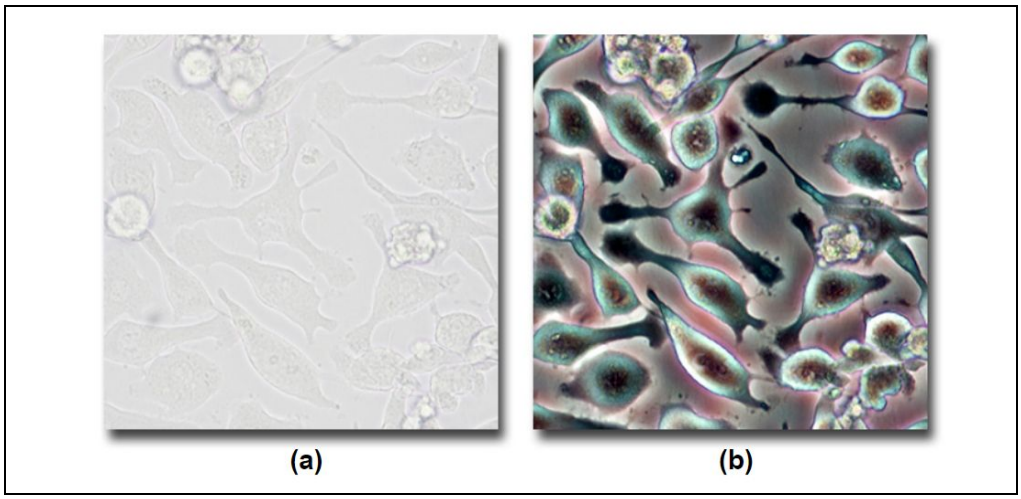


Fig. 4: *Comparison of cells viewed under (a) typical brightfield microscopy and (b) phase contrast microscopy from MicroscopyU [4]. The "halo" effect around the edges of the cells under phase contrast allows for the viewing of thin or transparent cells in a much more detailed manner. The problem the client is seeing decreases the effectiveness of phase contrast, and the image becomes more like (a) towards the edges of the wells.*

Required Physics

Understanding optics and being able to apply certain principles of physics was required to create all designs considered. The optic equations below are principal equations for refraction, and for light passing through lenses. Refraction occurs when light waves pass through a medium, the light changes trajectory based on the medium it passed through. The physical characteristics of various mediums are represented by n , which is the index of refraction, found from the standard reflective index. The angle of the path of light is represented by θ , which is typically measured from the vertical axis. λ is the wavelength of light.

Refraction

$$n_1 * sin(\theta_1) = n_2 * sin(\theta_2)$$

$$\lambda_1 * n_1 = \lambda_2 * n_2$$

These two equations can be applied to solver for either θ_1 or θ_2 , which, in our case, determines the angle that the light leaves the design and goes into the specimen.

The equipment used for microscopy uses various lenses, and the radius of curvature, R, of those lenses also affects the resulting image. So does the image distance, d_i , and object distance, d_o .

Thin Lens Equations

$$1/f = (n-1)(1/R_1 - 1/R_2)$$

$$\text{Numerical Aperture NA} = nsin(\theta)$$

$$1/d_o + 1/d_i = 1/f$$

When considering converging, or concave, lenses, f>0. When considering diverging, or convex, lenses f<0. These equations allowed the team to select appropriate lenses for our final design and allowed for knowledge of limitations of prototypes by giving an applicable understanding of microscopy from a mathematical perspective. By combining our designs, existing lenses, and the researched optics equations, we mathematically optimized our designs [5].

Client Information

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 at the University of Wisconsin-Madison. BrainXell uses phase-contrast microscopy to check the quality of their neurons; hence, the need for high quality resolution from the microscope. We defined high quality resolution as the ability to see different parts of the neurons. For example, in high quality resolution one can see the axon, dendrites, and cell nucleus [12].

Design Specifications

All design specifications given by BrainXell were important and considered during the duration of the project. First, the condenser must allow the user to increase the area of effective phase contrast viewing in standard 96-well plates with opaque walls. Second, the condenser must maintain a minimum resolution of 1.22μm and 0.959μm with magnification at 10x and 20x original image (Fig. 5). Third, the design must be compatible with pre-existing equipment, most importantly the equipment used by BrainXell, such as: Nikon Eclipse Ts2, 96 well plates with opaque walls, and other interchangeable standard condensers. We must stay within the specified budget of $1500 for the prototype.

$$d = (\lambda \div 2)(NA_{Condenser} + NA_{Objective})$$

$$d = resolution$$

$$\lambda = wavelength = 550nm$$

$$NA = Numerical\ Aperture$$

$$d_{10x} = (275nm)(0.3 + 0.25) = 1.22\mu m$$

$$d_{20x} = (275nm)(0.3 + 0.4) = 0.959\mu m$$

Fig. 5: *The calculations used to find the resolution of the existing condenser for two different magnifications. The formula used is d=λ/2(NA), with NA as Numerical Aperture [M. Wilson]. The calculated resolutions of 1.22 micrometers (10x magnification) and 0.959 micrometers (20x magnification) will serve as the control values if resolution is changed in the designs.*

We defined more design specifications during the researching and prototyping phase. The design must elevate or even terminate any amount of clipping of light done by the 96-well plates. Therefore, the design must be able to change the illumination optics system in order to decrease the area of the focal point, *f,* which will minimize clipping and aid in the expansion of effective contrast area. The design must control the amount of light passed through the objective lens. If there is too much brightness there will be poor image resolution, discussed in the Results section, demonstrated effectively by our design that increased light area by 35%.

III. Preliminary Designs

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

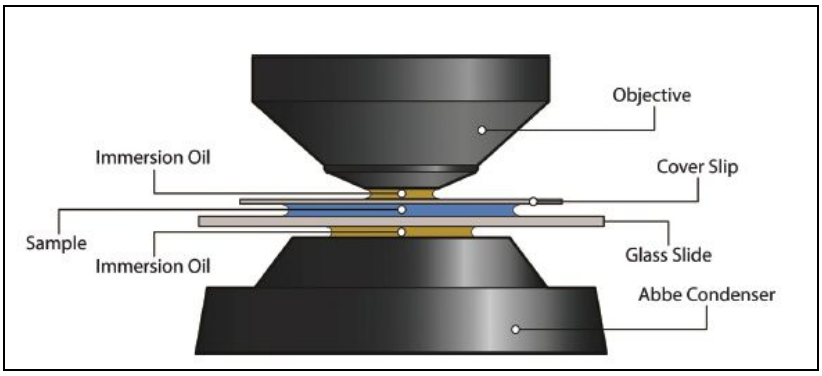


Fig. 6: *Design 1, using immersion oil (shown in figure) above and below the microscope slide. Oil immersion decreases the Numerical Aperture (NA) of the condenser. Decreasing the Numerical Aperture changes the angle of the cone of light coming and would affect the resolution, since it is included in the equation d=λ/2(NA) [6].*

For the oil design design we would have been 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. Seen in the equation NA = (refractive index)*sin(angular aperture) [6]. Many microscopes use air with a refractive index of 1.33 as their immersion medium, but we would be changing the immersion medium to oil, which has a refractive index of 1.51 [6]. Changing the immersion medium would allow us to drastically increase the numerical aperture to a maximum of 1.51, and increase the resolution.

Design 2 - Objective Lens Attachment

The objective lens attachment design does not change anything about the phase microscope body or anatomy. The objective lens attachment design ensures we do not lose any of the resolution because we are not changing anything about the existing lenses. These extensions connect to the objective lens part of the microscope, and contain a light, annulus, and condenser that is angled to the side of the focal point.

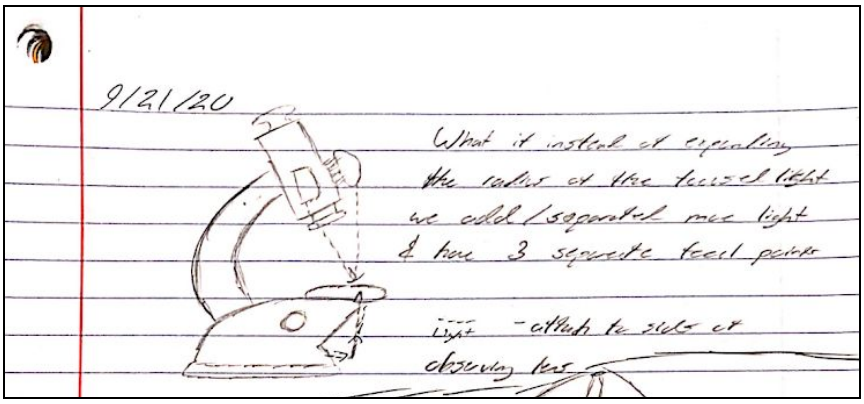


Fig. 7: *Rough drawing of Design 2 - Objective Lens Attachments. The additional light sources on the sides of the microscope help to further illuminate the areas where phase contrast is decreased - along the edges of the well. The angles of these sources of light would be calculated to minimize the potential of light clipping in the wells.*

Design 3 - Annulus Adjustment: Final Design

For the Annulus Adjustment design we did not change anything about the microscope body or condenser, just the condenser annulus, which lies directly below the condenser. We increased the open area of the annulus by 25%, and 35% to allow for more light to pass through and into the condenser. We believed the increased light would 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 the adjustment 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 design does not address the clipping of the light we believe to happen on the 96-well plate walls. The modified annulus could possibly be paired with a change in the condenser lens, or if we need to alter the angle of the light as well.

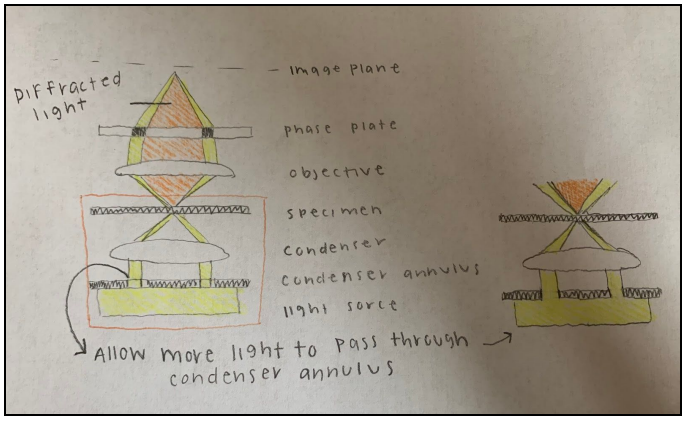


Fig. 8: *Drawing of Design 3, modeling the typical form of the condenser annulus, showing the specified changes to increase area of phase contrast. The design increases the annulus ring that creates the cone of light by 25%, and another annulus that increases the light by 35%. The increase allows 25% , and 35% more light into the system, increasing the area of the cone of light, and saturating in the edges of the wells where phase is diminished.*

Design 4 - Extra Lenses Design: Final Design

As seen in figure 10, the problem of decreased effective area of phase contrast is hypothesized to result from "clipping" of the cone of light coming off the specimen. The decreased amount of non-diffracted "background light" causes an issue when combining the diffracted and un-diffracted light at the viewer's eye. The solution was to add an additional set of lenses, one concave lense and another convex lens, between the condenser and objective lenses that narrow the cone of light. The decreased angle of light passes through the well plate with less clipping at the edges, and results in a different area of phase contrast than the original design.

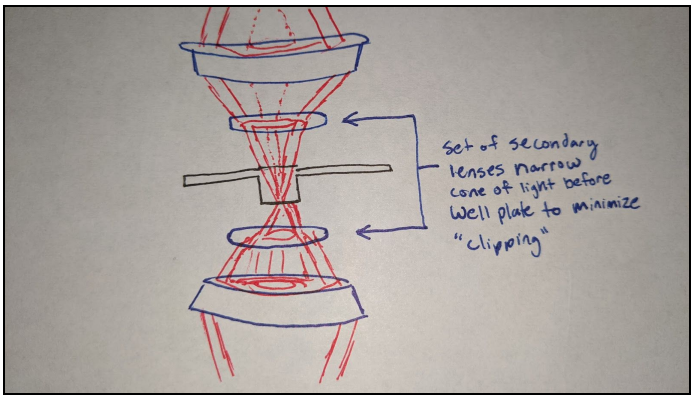


Fig. 9: *Drawing of Design 4, modeling the placement of extra lenses. Narrowing the cone of light may also affect the Numerical Aperture at the specimen, so resolution may be changed. If the angle of light is changed before entering the well, clipping may be able to be reduced. For details on light cone clipping, see Fig. 10 below.*

Some possible issues with the extra lens design are that it could result in a decrease of resolution, lower than the allowed 0.959µm resolution specification. Additionally, narrowing the cone of light and then expanding it again could cause some unforeseen distortion in the final image. Distortion of the cell image would be unacceptable, as the shape should be precise for the viewer to properly analyze the specimen, there are no testing criteria to assess if the cells become distorted. Finally, since the design is dealing with lenses, it would have to be specific to the condenser/objective set. There cannot be a standard set of secondary lenses for all microscope models.

The benefits of the design is strictly mechanical. The lens attachment design could be added to any existing microscope provided measurements are given. Additionally, the design is fairly simple to build, as it requires a single 3D print and then the lenses can be placed in the print with no additional equipment. The lenses are also able to be bought, not fabricated. By using the physics equation from the Background session, a distance of 3mm and diameter of 20mm was known, and we solved for the proper curvature of the lenses, R. Two lenses were found with these curvatures, R, to achieve a light angle of 24.66° upon entering the well-plate.

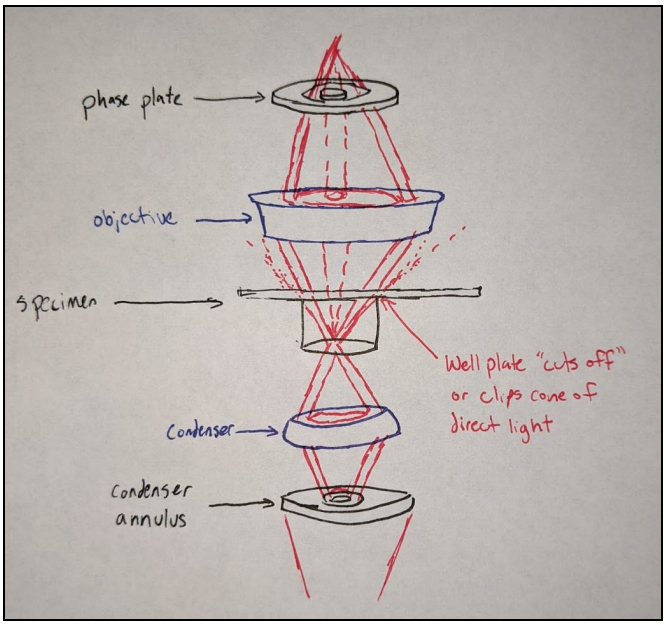
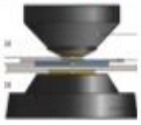


Fig. 10: *Drawing of the light cone pathway, modeling the clipping of the cone of light. The diameter of the cone of light is greater than the diameter of the well at the height of the top of the well plate. The cone results in the reflection of light at the edges of each well and the attenuation of out-of-phase light reaching the specimen at the bottom of the well. Clipped light is our leading theory for the source of decreased phase contrast at the edges of the wells.*

IV. Preliminary Design Evaluation

Design Matrix

Table. 1: *The table contains four designs that we considered before selecting two for fabrication. Each idea was given a score from 1-10 in each of the six categories. The highest-scoring idea in each category is highlighted in yellow (muted yellow for ties). Since certain categories were more important than others, the categories were weighted. Each idea received a weighted average of their scores in the six categories and the Extra Lenses idea received the highest score of 81.5/100. The Annulus Adjustment received the second highest at 74/100, so the group elected to proceed by fabricating and testing both.*

			Using Oil instead of Air to increase refraction index 		Objective Lens Attachment 		Condenser Annulus Adjustment 		Extra Lenses 	
Rank	Criteria	Weight	Score	Weighted Score	Score	Weighted Score	Score	Weighted Score	Score	Weighted Score
1	Effective Area seen by phase contrast	25	9/10	22.5	7/10	17.5	7/10	17.5	8/10	20
2	Resolution	25	5/10	12.5	6/10	15	6/10	15	8/10	20
3	Adaptability	20	5/10	10	8/10	16	9/10	18	10/10	20
4	Cost efficiency	15	6/10	9	3/10	4.5	8/10	12	9/10	13.5
5	Complexity	10	7/10	7	4/10	4	7/10	7	3/10	3
6	Safety	5	8/10	4	9/10	4.5	9/10	4.5	10/10	5
	Sum	100	**Sum**	64.5	**Sum**	61.5	**Sum**	74	**Sum**	81.5

Summary of Design Matrix

The six main criteria we considered while constructing our design matrix, in order of importance, include effective area seen by phase-contrast and resolution, adaptability, cost efficiency, complexity, and safety. The main focus of our project is to increase the in-phase area significantly compared to the current method, without compromising the resolution. Adaptability served as an important factor as well since there are already standards for lab equipment, so we wanted to make sure our design was able to accommodate those standards. With adaptability, we also want our design to be easily accessible, no matter the existing equipment in a user's lab. Cost efficiency is necessary to consider because we wanted to create a product that would be affordable to all laboratories and cost-effective to mass-produce. In terms of complexity, all of the designs are complex and labor-intensive; however, it is rated as the second-lowest because executing the design requirements are more important than the labor required to build a prototype. Lastly for the criteria we are considering is safety, since we are following ISO-14001 certified devices [7], such as the Nikon Eclipse Ts2 Microscope, as our inspiration we do not anticipate an issue for safety.

Although the Oil design, which would use a different medium to increase the refraction index, scored highly on effective area, however, it scored lowly on resolution and adaptability. After discussing the design with our client we decided that a different medium would not be a suitable option.

The Objective Lens Attachment design ranked evenly with the Extra Lenses design except for in the cost efficiency and complexity categories which allowed us to rule out an objective lens attachment.

The Annulus Adjustment design, ranked evenly with the Additional Objective Lens Attachment design but excelled in the cost efficiency and complexity categories which led us to continue to research an Adjustment of the Condenser Annulus.

The Extra Lenses design scored highest in four out of the six categories and received our client's recommendation which led us to continue researching the Extra Lenses design as well as the Annulus Adjustment.

Proposed Final Designs

After evaluating the design matrix and discussing with the client, the final decision was to fabricate both the Annulus Adjustment (placed second in the matrix) and the Extra Lenses (client's recommendation). Both designs are able to be fabricated with reflective ease and time efficiency. Having two designs to test will give the team better insight into which design works better. Selecting both designs allows for a better chance of solving the design problem while staying within semester time constraints. The Annulus Adjustment allows for 25% and 35% more light to pass through the specimen and will impact the viewable area. In order to fabricate the annulus, the team sketched CAD drawings of the design to be 3-D printed at the makerspace. The Extra Lenses will adjust the angle the light hits the plate to 24.66°, and will reduce any light that is clipped off by the edges of the wells, the decrease in clipped light will not be quantified, but secondarily observed by the results of the images. Fabrication of the Extra Lenses design involved using optics equations to find the best lens that can be ordered from market, 3-D printing a lens holder to attach to the microscope, and buying the correct types of lenses.

V. Fabrication/Development Process

A. Annulus

Materials

The most important aspect of the annulus is to only allow light through the ring, and block out additional light. The performance requirement demanded we use a sufficiently dense material that would block out almost 100% of incoming light despite the thin design. The control annulus from the microscope solves the problem by using sheet metal, presumably aluminum, with a matte black finish to prevent reflection. However, using such material would require the use of a laser or waterjet cutter, neither of which were easily accessible during the pandemic. Instead, stereolithography (SLA) 3D-printing was chosen as the preferred method of fabrication due to the high precision and greater range of materials available, and FormLabs standard "Black" resin was chosen for the annulus material.

STANDARD RESINS

CLEAR FLGPCL04 | WHITE FLGPWH04 | GREY FLGPGR04 | BLACK FLGPBK04 | COLOR BASE FLGPCB01

	METRIC[1]		IMPERIAL[1]		METHOD
	Green[2]	Post-Cured[3]	Green[2]	Post-Cured[3]	
Tensile Properties					
Ultimate Tensile Strength	38 MPa	65 MPa	5510 psi	9380 psi	ASTM D 638-10
Tensile Modulus	1.6 GPa	2.8 GPa	234 ksi	402 ksi	ASTM D 638-10
Elongation at Break	12 %	6 %	12 %	6 %	ASTM D 638-10
Flexural Properties					
Flexural Modulus	1.3 GPa	2.2 GPa	181 ksi	0.5 ksi	ASTM C 790-10
Impact Properties					
Notched IZOD	16 J/m	25 J/m	0.3 ft-lbf/in	0.46 ft-lbf/in	ASTM D 256-10
Thermal Properties					
Heat Deflection Temp. @ 1.8 MPa	42.7 °C	58.4 °C	108.9 °F	137.1 °F	ASTM D 648-07
Heat Deflection Temp. @ 0.45 MPa	49.7 °C	73.1 °C	121.5 °F	163.6 °F	ASTM D 648-07

[1] Material properties can vary with part geometry, print orientation, print settings, and temperature.

[2] Data was obtained from green parts, printed using Form 2, 100 µm, Clear settings, washed and air dried without post cure.

[3] Data was obtained from parts printed using Form 2, 100 µm, Clear settings, and post-cured with 1.25 mW/cm² of 405 nm LED light for 60 minutes at 60 °C.

Fig. 11: *Table from FormLabs (manufacturer of the utilized SLA printers) regarding the Black standard resin and its material properties. Properties taken into consideration were the flexural modulus (post-cured) and the ultimate tensile strength (post-cured). Curing is always done at the Makerspace, and is not allowed to be avoided.*

The two main factors we took into consideration for materials for the SLA printing were color and reflectivity. Structural stability was not seriously considered for the part, as it would not be placed under any significant forces. From the materials that were available, the best fit was the standard "Black" resin. It is the darkest available resin which will therefore absorb the most light [8]. It also has a relatively low surface reflectivity due to the method of printing in very thin layers. The flexural strength (quantified flexibility) of the material was relatively low at 2.2 GPa [9], but the flexibility of the material was not a critical factor in our consideration, as it was not to be subjected to loading or stretching.

Methods

The fabrication of the annulus was done using Stereolithography (SLA) 3D-printing at the makerspace. A FormLabs Form 2 printer was used for the SLA process. In order to maximize the matte finish on the surface of the material and to avoid support residue on the final surface, the part was printed in a diagonal orientation.

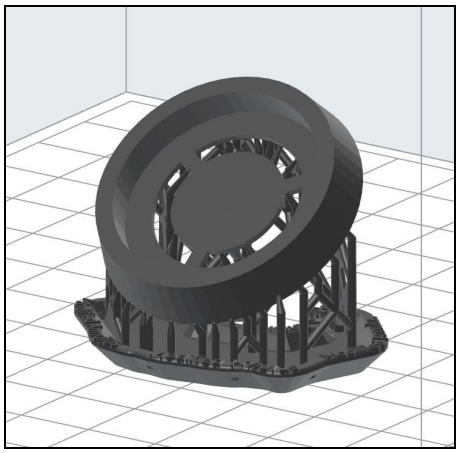


Fig. 12: *The annulus is shown after automatically generating supports with the PreForm pre-printing software. The diagonal orientation allows supports to only be placed on the bottom face of the part. The top face will be free of support residue. The diagonal orientation also allows for diagonal layering of printed material, which will limit the reflectivity of the flat surfaces.*

Final Prototype

The final annulus prototypes were printed accurately to the specifications. The measurements of the printed parts and the CAD model were compared, and all major dimensions (Inside Ring Radius, Outside Ring Radius, Outside Diameter, and Part Thickness) fell within a standard 5% tolerance for engineering accuracy.

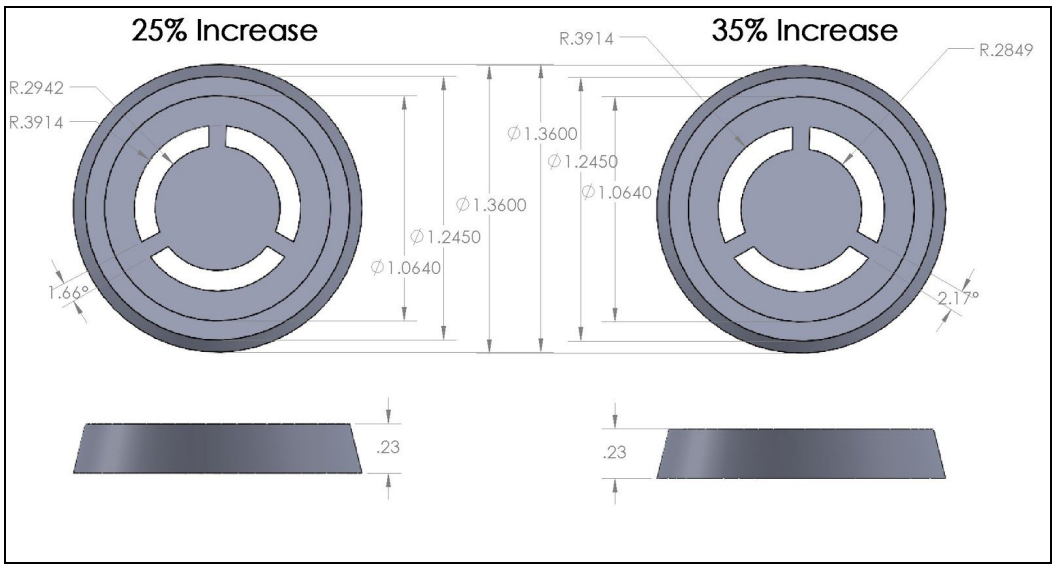


Fig. 13: *The annulus measurements of the CAD Model including Inside Ring Radius (0.294 in and 0.285 in), Outside Ring Radius (0.391 in), Outside Diameter (1.36 in), and Part Thickness (0.23 in). These dimensions were compared to the printed parts and were within the preset 5% tolerance.*

When placed in the annulus slide on the testing microscope, the printed prototype fit in the designated spot in the same way the annulus of the Fisher Scientific standard annulus fit. It was able to produce a light ring similar to the original when the microscope's adjustable aperture was set to its lowest setting. The printed prototype was robust enough to withstand the minimal pressure of the spring that kept it in place in the annulus slide.

Fig. 14: *The original (control) annulus (right), 25% increased annulus (middle) and 35% increase annulus (bottom). The annulus slide (top) has the far-right spot where the annuli are held in place by the thin aluminum strip that acts as a spring. Below the image is a scale bar in centimeters.*

B. Extra Lenses

Materials

For the Extra Lenses design, there were two materials to consider: the material of the lenses themselves, and the material for the superstructure. For the lenses, our key factor was cost. Since lenses can be quite expensive, it was imperative for us to budget them into our total. We set a maximum budget for the lenses at $50. Most commercial-quality glass lenses were outside of our price range, especially for the accurate focal length and numerical aperture that we needed. However, experimental-grade lenses are still cut to size and are free of chips and superficial stains [10]. Experimental-grade lenses were purchased from Edmund Optics and after shipping cost $40 - well underneath the budget.

In order to finalize the measurements, calculations were done by hand to find the "ideal angle changes" of each lens to achieve a final cone of light that would not be clipped by the top of the well plate. On the website Edmund Optics (our selected source of our lenses), available sizes of lenses were compared to the ideal angle changes to find the ideal lenses. This was done by selecting an appropriately-sized plano-convex lens and finding the plano-concave lens that would compliment it the best. The angle changes of each lens were calculated using

trigonometry on the lens' diameter and focal points. The ideal angle change for the convex stage was 24.84° and the 18mm diameter/-19.6mm focal length lens yielded a 24.66° angle change and was therefore selected as the lens to use.

The material for the superstructure had two key factors: printability and adjustability. We needed a material that would be able to be accurately printed within the specifications of the CAD model and could press-fit onto the condenser in case tolerances were too tight. To meet the specifications, the SLA method of 3D printing was chosen, since the First Deposition Modeling (FDM) method of printing can yield unreliable tolerances. Additionally, the part needed to be slightly flexible to press fit onto the condenser, and several flexible resins are available for the Makerspace's SLA printers. The aptly-named "Flexible" material was chosen due to the tensile strength of the material (7.7-8.5 MPa) [9]. The only other flexible option was "Elastic" material (tensile strength 1.38 MPa). Since the "Elastic" material was up to 16% of the tensile strength of "Flexible," the choice to use "Flexible" was obvious when we wanted to minimize the chance of rupture when press-fitting the cone to the condenser.

Fig. 15: *Datatable from FormLabs describing the material properties of "Flexible" resin. The Ultimate Tensile Strength was the property that was focused on, since the material needed to press-fit onto a variety of condensers without shearing. The Post-Cured tensile strength of the material is listed as 7.7-8.5MPa, which is significantly higher than the other flexible "Elastic" material (1.38MPa). If the tensile strength is too low, there is risk of rupture when the device is applied to the microscope.*

FLEXIBLE RESIN

FLFLGR02

	METRIC[1]		IMPERIAL[1]		METHOD
	Green	Post-Cured[2]	Green	Post-Cured[2]	
Mechanical Properties					
Ultimate Tensile Strength[3]	3.3 - 3.4 MPa	7.7 - 8.5 MPa	483 - 494 psi	1100 - 1230 psi	ASTM D 412-06 (A)
Elongation at Break[3]	60 %	75 - 85 %	60 %	75 - 85 %	ASTM D 412-06 (A)
Compression Set[4]	0.40 %	0.40 %	0.40 %	0.40 %	ASTM D 395-03 (B)
Tear Strength[5]	9.5 - 9.6 kN/m	13.3 - 14.1 kN/m	54 - 55 lbf/in	76 - 80 lbf/in	ASTM D 624-00
Shore Hardness	70 - 75 A	80 - 85 A	70 - 75 A	80 - 85 A	ASTM 2240
Thermal Properties					
Vicat Softening Point[6]	231 °C	230 °C	448 °F	446 °F	ASTM D 1525-09

[1] Material properties can vary with part geometry, print orientation, print settings and temperature.

[2] Data was obtained from parts printed using Form 2, 100 µm, Flexible settings, and post-cured with 80.5 mW/cm2 of 365 nm fluorescent light for 60 minutes.

[3] Tensile testing was performed after 3+ hours at 23 °C, using a Die C dumbbell and 20 in/min cross head speed.

[4] Compression testing was performed at 23 °C after aging at 23 °C for 22 hours.

[5] Tear testing was performed after 3+ hours at 23 °C, using a Die C tear specimen and a 20 in/min cross head speed.

[6] Thermal testing was performed after 40+ hours with a 10 N loading at 50 °C/hour. Cracks formed in samples during testing.

Flexibility was considered to be a necessary factor, since the ability to press-fit the design onto a variety of condensers would greatly improve the marketability of the design. If the design can be directly applied to any condenser with a Numerical Aperture of 0.3, fewer model-specific designs would have to be made, and instead several standard parts could be created. Similar to a drill bit, our design can be made in different sizes to fit the corresponding Numerical Aperture size and connect at the condenser analogous to our design.

Methods

Identically to the annulus designs, the fabrication of the superstructure was done using Stereolithography (SLA) 3D-printing at the makerspace. A FormLabs Form 2 printer was used for the 3D-printing process. In order to minimize residue and markings on the inside surface of the cone, the part was printed in a diagonal orientation. Flexible material requires support touchpoints of at least 0.1 cm compared to the 0.025 cm touchpoints of standard materials, so support residue is greatly increased on parts printed with Flexible material.

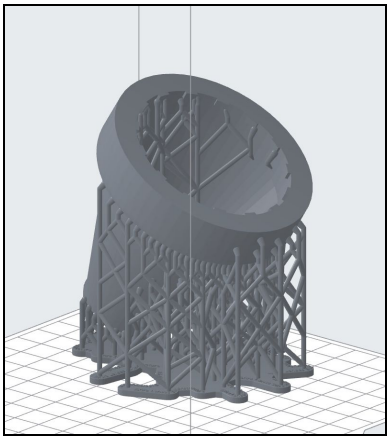


Fig. 16: *The Extra Lenses Superstructure oriented in the pre-print software Pre-Form. The automatically-generated support structures are clustered on one side of the external surface, and minimized on the inside surface to minimize the residue on the inside surface, as residue may interfere with the cone of light. Flexible material requires denser support structures with larger touchpoints, as the material is more prone to warping than standard materials during the printing process.*

The assembly process was designed to be simple. To make use of the flexible material, the lenses were press-fit into the bottom of the cone from the top. The press-fit assembly allows for them to still be removed if necessary, but keeps them in place when inverted or subjected to small forces when adding or removing the superstructure to a condenser.

Final Prototype

The final prototype was assembled and met nearly all tolerances. All major measurements (Outside Diameter, Outside Angle, Inside Angle, Bottom Lens Diameter, Top Lens Diameter, and Cone Height) were accurate to the 5% tolerance. The superstructure press-fit as intended onto the test microscope's condenser. The lenses did not perfectly press-fit into their respective slots, as the tolerances on the lens diameters were 6.6% and 7.5% smaller than listed on the parts' specifications on Edmund Optics' website for the convex and concave lens respectively. The inaccurate lens diameters resulted in the lenses falling out of the superstructure whenever inverted. The loose fit did not detriment the testing process, however; the product needs to be fixed for ease of use by consumers.

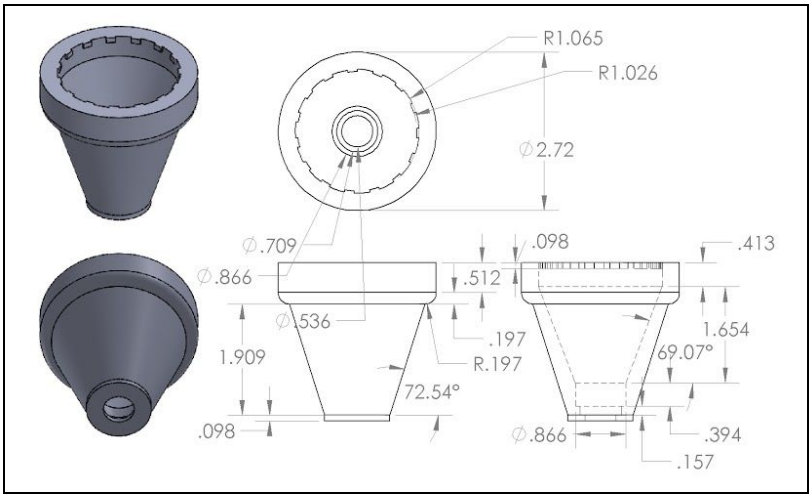


Fig. 17: *Engineering Drawing of the Extra Lens Superstructure parts showing all critical dimensions. Those measured and compared to tolerances include Outside Diameter (2.72 in), Outside Angle (72.54°), Inside Angle (69.07°), Bottom Lens Diameter (0.709 in), Top Lens Diameter (0.866 in), and Cone Height (1.909 in). All critical dimensions fell within the 5% tolerance limit.*

The outside surface was completely smooth on one half and very rough on the other half that had supports. For superficial reasons, the outside surface would have to be sanded down in the future, but the residue did not detract from the performance of the part. The internal surface was relatively free of any support residue, and none of the internal residue measured longer than 0.25 cm, which was the distance required to interfere with the cone of light.

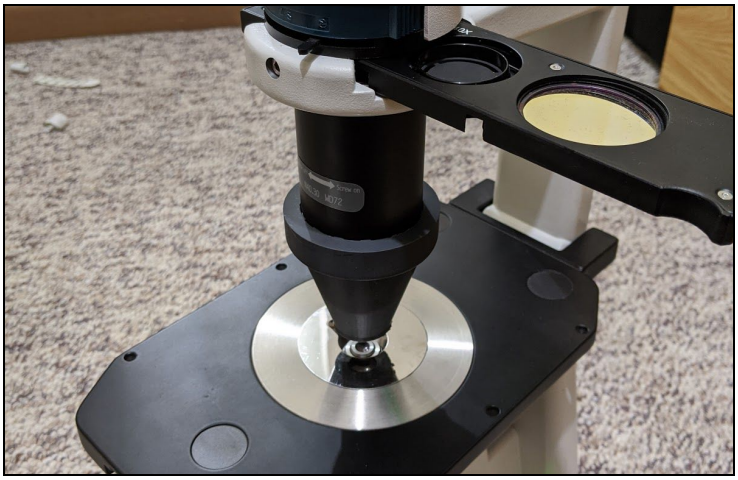


Fig. 18: *Printed prototype (dark grey inverted cone) press-fitted onto the condenser (black cylinder above cone). The flexible material created a tight grip on the end of the condenser, and flanges designed into the top of the cone fit into an inset ring 0.8 cm above the bottom of the condenser to further solidify the attachment.*

The press-fit of the top of the cone performed as designed. The flanges interlocked directly with the indent on the condenser and held the superstructure on with enough force that it took significant, but not excessive effort to remove, which would allow a consumer to successfully use the device when needed, rather than donating a separate microscope to our device.

Testing

The testing protocol for the Annulus Adjustment and Extra Lenses designs was as follows:

1. Adjust the aperture on the microscope until the background light is minimized and phase contrast is maximized. Maintain positioning for the remainder of testing.
2. Set the magnification objective lens to 10x.
3. Identify a spot in a well where many cells are clearly visible using the control annulus.
4. Take a picture of the view using a cellphone camera.
5. Without moving the plate, remove the annulus slide and replace the control annulus with the 25% Increase annulus.
6. Take a picture of the view using a cellphone camera.
7. Repeat steps 5-6 with the 35% Increase annulus.
8. Repeat steps 2-7 for two additional wells in the same plate.
9. Repeat steps 2-8 using the 20x objective instead.
10. Repeat steps 2-7 for the second well plate. There should be a total of 36 images taken.
11. Repeat steps 2-10, but replace only use the control annulus. Instead of changing the annulus, add the extra lens superstructure. There should be an additional 24 images taken.
12. Analyze the photos using ImageJ software by converting the images to grayscale, and scaling the contrast up.
13. Use ImageJ to count the number of cells in each photo, comparing the modified annuli to their respective controls.
14. Use ImageJ to measure the "dark area" of each photo by calculating the pixel number below a specified darkness threshold for each set of controls and modified images.

The tests were performed in a well-lit, non-laboratory setting using two well plates of laboratory-grown human embryonic kidney (HEK) cells that were subsequently fixed and stored in a refrigerator at approximately 36°F ±5°F. HEK cells are not used by BrainXell, but they are relatively simple to culture and have high yield quality, which is helpful for our analysis, discussed later [16]. Plate 1 had cell growth that was largely isolated, and small clusters were very visually individualized. Plate 2 had clustered cell growth along the edges of the plates, creating a less-distinguishable mass of cells. These two environments allowed the test to compare the phase contrast on densely-populated wells and sparsely-populated wells.

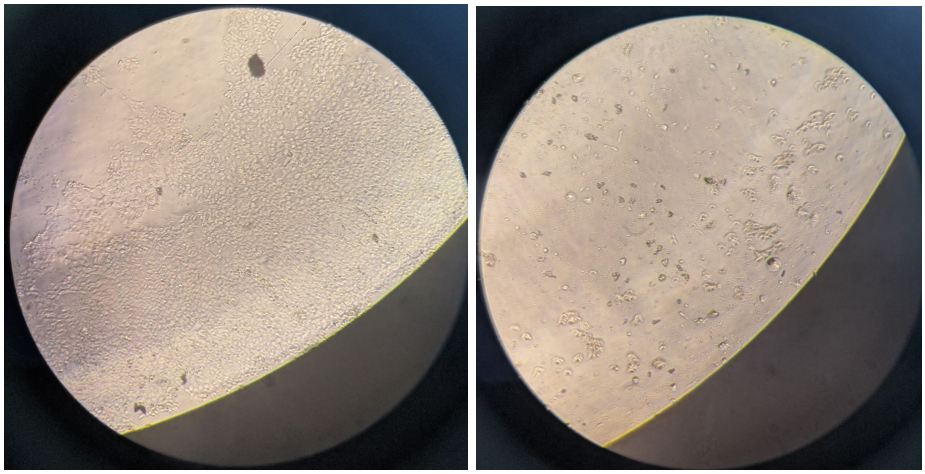


Fig. 19: *Comparison of HEK cell growth between Well 1 (right) and Well 2 (left). Well 1 has small clusters of dispersed cell growth which allows for greater contrast along the edges of the clusters, but less cells overall. Well 2 has much denser cell growth, but the contrast between individual cells is diminished.*

Some possible complications with the testing are how the contrast in each image can be quantified. When using neuronal cells, an easy way to quantify the effectiveness of the phase contrast is to count the axon tails that are visible. Axon tails are clear and nearly impossible to see without phase contrast microscopy. However, HEK cells have no such appendages, so the quantification of phase was done by calculating the pixel area of "dark spots" in the background illumination in ImageJ software. The areas with darker background illumination yielded much better phase contrast along the edges of cells, so it was safe to assume that dark areas were areas of effective phase contrast.

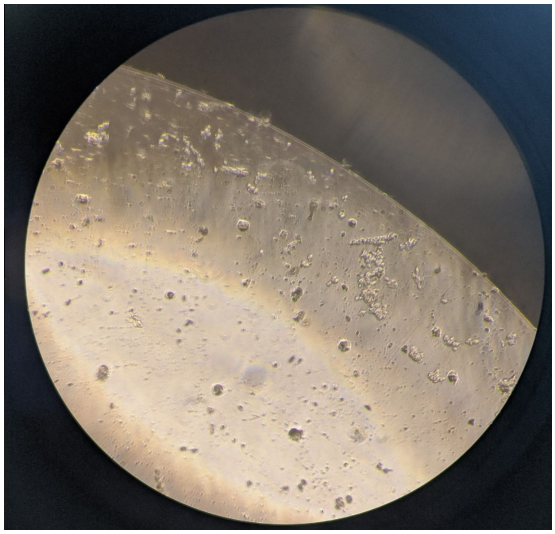


Fig. 20: *Example image showing increased phase contrast on "dark areas" in the background illumination. The outer edge of the well has increased contrast on the edges of the cells identified by the white outlines along the cells. The contrast is diminished to the bottom-left when the background illumination increases.*

VI. Results

To assess the quality of our design, we compared measurements of images taken with our designs and compared them to images taken without condenser modification. We measured the effective dark area of the viewable well, counted the number of visible cells, and did statistical analysis to determine if there was a significant change in dark area measurements when using our attachments. Dark area was measured in ImageJ by using the protocol below.

1. Import desired image into ImageJ
2. Convert the image to grayscale by selecting image, type, 8-bit.
3. Adjust brightness/contrast by selecting adjust, brightness/contrast
    a. Drag minimum up until all background disappears and contrast is prevalent
4. Measure the area of the dark space by selecting the wand tool in the bar
    a. Circle the dark area
    b. Select analyze, measure
5. Record number for area
6. Repeat steps 1-5 for each image

The Extra Lenses design showed a significant decrease in dark area of the image when analyzing the results of the test in ImageJ. These results are shown in the decrease in average dark area per image for both 10x and 20x magnification (Figures 21 and 22 below). In 10x magnification a decrease of 8.2% in dark area was observed when comparing the control and modified designs. In 20x magnification a decrease of 9.4% in dark area was noted.

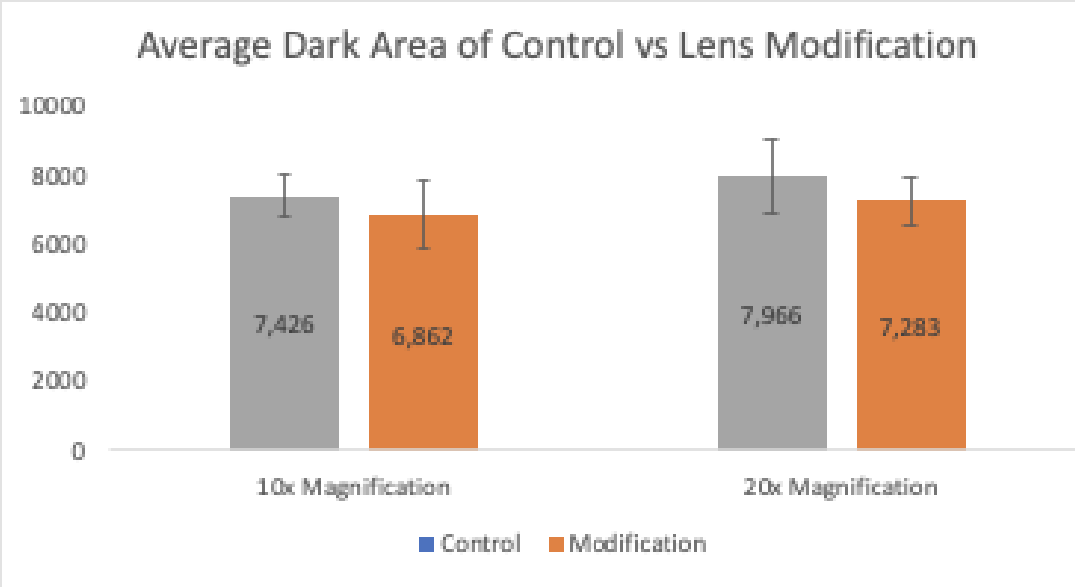


Fig. 21: *Lens modification average comparison of dark area. In 10x magnification, a significant decrease of 8.2% was observed when comparing the control and lens modification. In 20x magnification, a similar decrease of 9.4% was observed.*

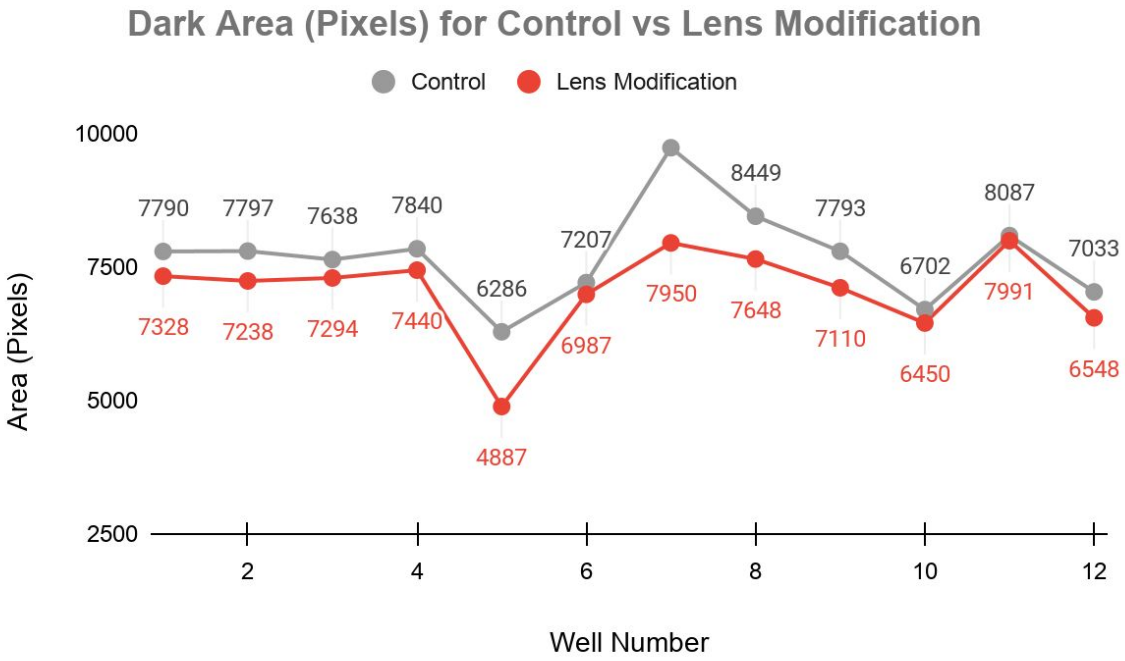


Fig. 22: *Lens modification data point comparison of dark area. The graph above displays the dark areas for the control and modified designs. The control design was found to have a higher number of dark area pixels for each of the 12 tests.*

Using the 25% and 35% increased annuli we came to a conclusion that the designs were ineffective at changing the dark area. At 10x magnification both annuli saw minimal changes in dark area, with a decrease of 4.1% dark area in the 25% annulus and a decrease of 11.2% in the 35% annulus design. At 20x magnification a similar decrease in dark area was found, with a decrease of 4.1% dark area in the 25% annulus and a decrease of 11.0% in the 35% annulus design, compared to the dark area of the control. This change in area was due to the "washing" out of cells by the high amount of light let into the well. Results of the average data point dark area per image are seen in graphs Fig. 23 & Fig. 24 below.

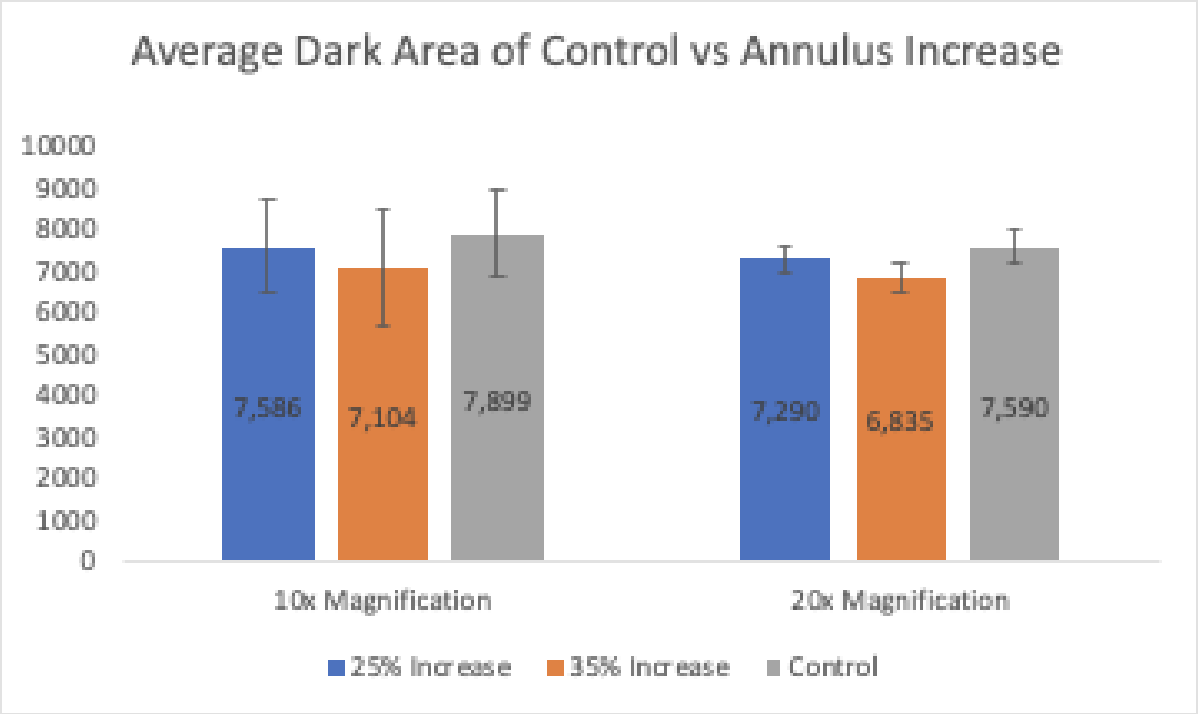


Fig. 23: *Annulus Adjustment average comparison of dark area. In 10x magnification a decrease in dark area was observed for both the 25% (4.1% decrease) and the 35% (11.2% decrease) designs. In 20x magnification there was also a decrease in dark area -- 4.1% for the 25% increase and 11.0% for the 35% increase.*

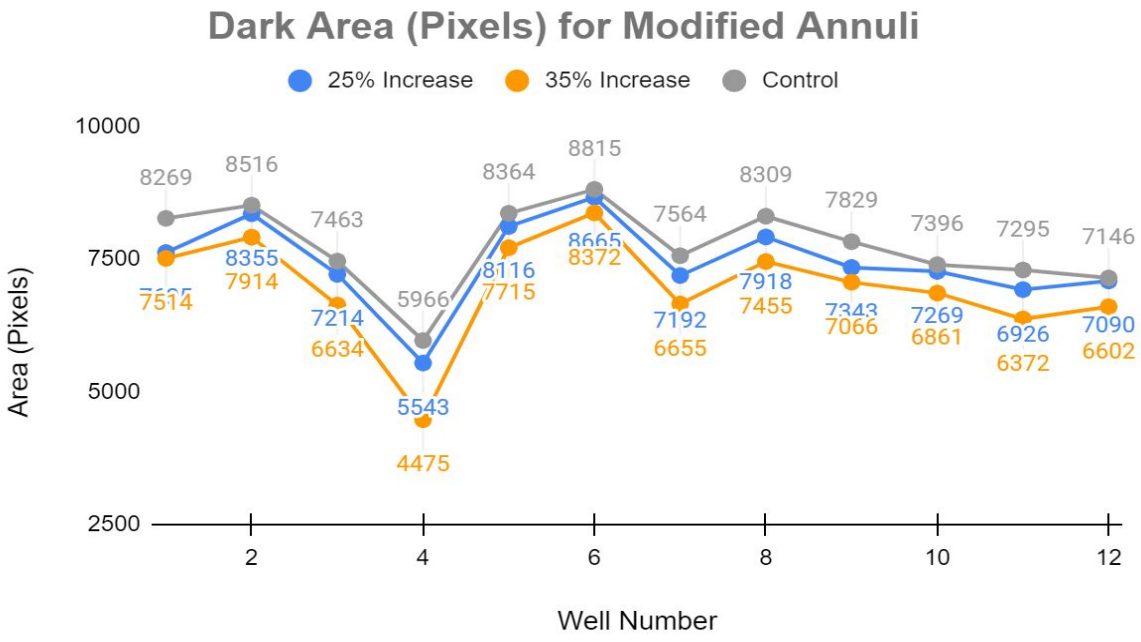


Fig. 24: *Annulus Adjustment Data Point Comparison of Dark Area. The graph above displays the dark areas for each image taken by the control and modified designs. The data followed a trend for almost every data point of being highest for control and lowest for 35% increase.*

A summary of the results can be seen by the statistical analysis performed on the data using matlab to show whether each design caused a statistically significant change in the dark area. Using a single variable t-test, with a standard 0.05 significance value, the mean dark area of the Extra Lenses design was compared to the mean of the control group. Three t-tests were run, one with only objective magnifications of 10x, another with 20x, and one t-test with all the combined data. Since the control group matches the respective Lens Holder image, the magnification difference was not a changing variable in the t-test. As shown in Table 2 below, all Extra Lens means of dark area were significantly different than the mean of the dark area of the control. A single variable ANOVA test was ran to compare the mean of the dark area when using no microscope adjustments, the 25% increase in light area and the 35% increase in light area Anuli. Again, three ANOVA tests were run with three different data sets, one with only the wells and images taken using 10x objective magnification, another with only the images from 20x magnification, and another with the combined data. Using a 10x magnification, the anuli were not statistically significant in their mean dark area compared to each other and the control. However, the 20x magnification was shown to have statistically significant different means. The reason for this was because the dark area was decreased compared to the control, but at the expense of the resolution of the image.

Table 2: *Statistical analysis done in MATLAB produced the numbers below. The code can be found in the Appendix. A significance level of 0.05 was used which accounts for a 95% confidence interval, suitable for our purposes as a standard engineering tolerance [17]. A single variable t-test rejected the null that the mean of dark area was equal for the Extra Lenses and the control. An Anova test revealed overall that the Annuli dark areas were not statistically significant from each other and the control, however with just using the data from a 20x magnification, the means were different from each other, this is because of how much light the 35% annulus let in, greatly decreasing the dark area.*

Significant change in dark areas from p value compared to control (α=0.05)		
Magnification	Extra Lenses	Annuli (25% and 35%)
10x	Yes, p=0.0022	No, p=0.5301
20x	Yes, p=0.0382	Yes, p=0.0131
Both 10x, 20x data	Yes, p=0.0024	No, p = 0.1033

The cell count analysis proved that our designs made little to no impact on cell visibility in the well plates. The protocol below shows the methods used to gain the information [18].

1. Download cellcounter.jar to ImageJ plugins folder, restart ImageJ
2. Import desired image into ImageJ
3. Activate cell counter plugin by selecting Plugins, Cell Counter
4. Initialize counter, select desired counter type
5. Place counter on each visible cell
6. Record counter type number
7. Repeat steps 2-6 for each image

The protocol was used to gather cell count data for each well plate (P1, P2), magnification (10x, 20x), and design (annulus adjustment and lens attachment). Fig. 25 below shows how similar the cell count remained for each different well plate, magnification, and design.

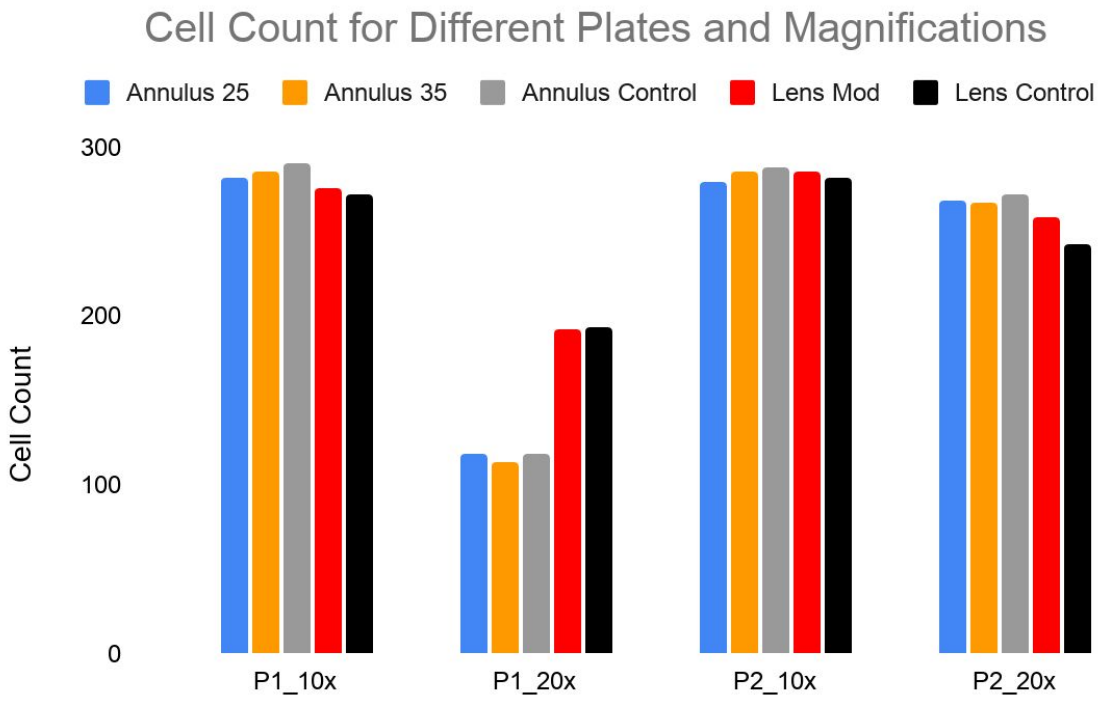


Fig. 25: *Cell counts for different well plates and magnifications. The results above show the average cell counts for each design, well plate, and magnification. For each testing situation, both the annulus and lens designs had similar results when comparing between them.*

VII. Discussion

We found that one of our designs increased the effective area of phase contrast. The extra lens design actually narrowed the cone of light to a point where the area of effective phase contrast expanded. The Annulus Adjustment failed to expand the area of phase contrast, though at 20x magnification there was a change in area, it was due to over lighting of the image. We saw the phase contrast for the Annulus Adjustment design decrease in overall qualitative image appearance with an increase in brightness. The images become brighter in succession from the control to the 35% expanded annulus as seen in Fig. 24. The cell count for the images was close to the cell count of their corresponding control image, but when using smaller references, such as dendrites, we believe the count would be significantly different, showing a change in quantitative resolution of the images.

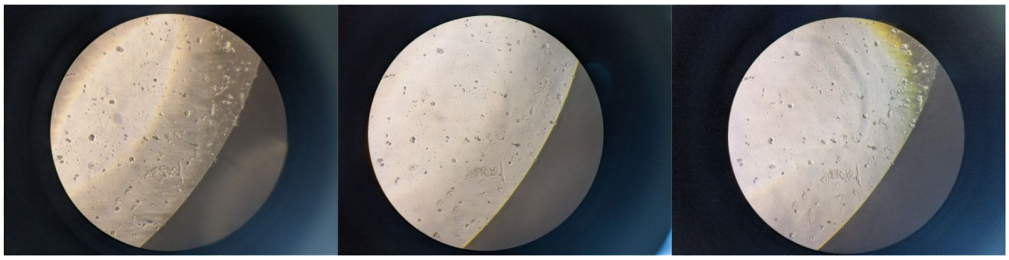


Fig. 26: *(left to right) Control, 25% expanded annulus, 35% expanded annulus. All of these images are pictures of the same well plate with the camera in the same position, but the annulus was changed for each of the images. The Images show a gradual increase in brightness. Similarly, the dark area decreases in area meaning the phase contrast becomes less clear.*

The current semester has led to unexpected changes and our testing was not as precise as we had hoped. For example, our ability to assess the final designs for the clients needs was difficult because we could not use neurons, which would allow for more specific analysis of resolution. To assess the quality of resolution, we would want to count the number of cellular components visible, which would be the axon, dendrites, and nucleus for neurons. Since we were not able to acquire neurons provided by the client, we used HEK cells which are significantly less detailed for analysis of cellular components.

Additionally, we experienced an inverse image compared to BrianXell, where the image was light in the center of the picture, and dark on the outside. Brighter areas have worse phase contrast than darker, more shaded regions of the image. However, for our client we were seeking to expand the quality of the center of the image. From this, our analysis of dark area results tell two things. First, by decreasing the dark area of the image, successful in the Extra Lens design, we expanded the quality of the center of the image, giving a more consistent image throughout the viewable area, which is beneficial. However, with a decrease in dark area is a decrease in

phase contrast for the Fisher Scientific microscope, this is bad for this model of microscope. If given access to the Nikon Eclipse microscope used by the client, we predict the Extra Lens design would be successful. Our design is easily interchangeable with other microscopes, however measurement of the design will vary and have to be recalculated for each different model.

A source of error in testing was our camera. The phone camera that we were using automatically adjusts the exposure of light of the image, or how much light it would let in to create an image. Auto-adjusting the exposure meant that all of the images were toned down in brightness and would actually appear brighter and more washed out on the actual microscope. Although the auto-exposure setting helped us to analyze the pictures better in ImageJ, it did not provide accurate information as to how our final designs performed in-person.

For future research, our team can look into three different approaches. These approaches include the complete removal of the annuli, increasing the number of light sources, and using an apodized phase plate. Observing the effects of removing the annuli completely could lead to a higher resolution since the annuli limit aperture which can decrease resolution. Experimenting with multiple sources of light to observe if the increased amount of light would decrease the halo effect. Implementing an apodized phase plate which would add neutral density material that will reduce the intensity of the light diffracted off of the specimen.

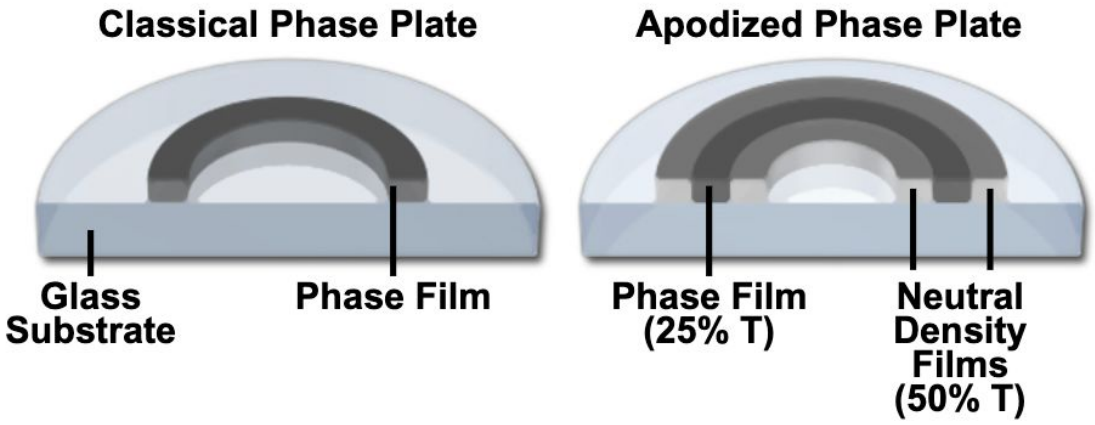


Fig. 27: *Classical Phase Plate (left) does not contain the neutral density film which allows for the intensity of the light to be diffracted. Apodized Phase Plate (right) would include a neutral density film placed along the phase film to reduce the intensity of the light diffracted.*

VIII. Conclusion

Phase contrast microscopy enables researchers and scientists to better understand cells in their natural, live state, however, there have been issues with the resolution of the entire image created by phase microscopes. Images created with phase contrast microscopy are composed of high-resolution dark areas that are surrounded by bright low-contrast areas. The irregular change in phase light makes phase microscopy difficult to form accurate data and it is a modern-day problem for researchers around the world. To resolve these issues with phase microscopes, our team set out to innovate an apparatus that is able to diminish the inconsistency of high and low resolution areas.

After our research and design phase, two separate designs were selected, Extra Lenses and Annulus Adjustment design. The Extra Lenses design was a cone that can be attached to the end of the microscope, the cone contains two object lenses that would narrow the illumination optics system in an effort to eliminate the possibility of clipping. The Annulus Adjustment design was meant to allow more light into the microscope that would light up and illuminate our image, we thought the extra illumination would increase the amount of effective area and maintain the same resolution. Both designs decreased the dark area on the outside of the image. After testing, it was certain that the Annulus design, both increasing light by 25% and 25%, diminished the resolution of the original image and made it more difficult to obtain accurate data. The reason the Annulus Adjustment failed was that the increased area of light allowed into the phase microscope (25% & 35%) saturated the image with light. The image created was overly illuminated and diminished all dark high-resolution areas. Next, the Extra Lenses design created an image which decreased the dark area of the image due to the changed angle of light entering the specimen without clipping the walls of the well plate, thus making the image consistent with what is seen in the center of the control images.

Going forward with the project, the Extra Lenses design would have the most likelihood to aid in the problem of increasing effective areas of phase contrast. The design is already able to control light let into the microscope, has the same viewing area as the original condenser (same cell count), reduces the chance of clipping by the cell plates, and allows us to decide the position of the focal point (via optics equations). What we would do differently, would be to buy more objective lenses so we could try different combinations, which would enable us to be able to change the location of the focal point and through testing would be able to determine which location would have the most increased effective light area. We also would ideally have access to the same equipment as our client; a Nikon Eclipse Ts2 microscope, and neurons.

IX. References

[1]Murphy, D. (2020). Introduction to Phase Contrast Microscopy. Retrieved December 09, 2020, from https://www.microscopyu.com/techniques/phase-contrast/introduction-to-phase-contrast-microscopy

[2] Oldenburg, R. (2020). US6924893B2 - Enhancing polarized light microscopy. Retrieved December 09, 2020, from https://patents.google.com/patent/US6924893B2/en?q=Enhancing+Polarized+Light+Microscopy

[3]Fox, W. (2019, August 22). Confocal Microscopes. Retrieved December 09, 2020, from https://imb.uq.edu.au/facilities/microscopy/hardware-software/confocal-microscopes

[4]"Introduction to Phase Contrast Microscopy," *Nikon's MicroscopyU*. [Online]. Available: ' https://www.microscopyu.com/techniques/phase-contrast/introduction-to-phase-contrast-microscopy. [Accessed: 09-Dec-2020].

[5] Pedrotti, Leno. Basic Geometrical Optics. 2020, pp. Module 1.3, Accessed 9 Oct 2020.

[6] M Wilson, "Microscope Resolution: Concepts, Factors and Calculation," Learn & Share | Leica Microsystems, 02-Dec-2016. [Online]. Available: https://www.leica-microsystems.com/science-lab/microscope-resolution-concepts-factors-and-calculation/. [Accessed: 09-Dec-2020].

[7] "Inverted Routine Microscope Inverted Ts2." Nikon, 2003 Available: https://www.edmundoptics.com/f/1-249mm-experimental-quality-plano-convex-pcx-lenses/14470/. [Accessed: 08-Dec-2020].

[8]"Absorption, reflection and transmission of visible light - What happens when light and sound meet different materials? - OCR 21C - GCSE Physics (Single Science) Revision - OCR 21st Century - BBC Bitesize," BBC News. [Online]. Available: https://www.bbc.co.uk/bitesize/guides/zg7jng8/revision/3. [Accessed: 07-Dec-2020].

[9] Materials Data Sheet Photopolymer Resin for Form 1 and Form 2." FormLabs, 22-Jan-2019.

[10] "1 - 24.9mm Experimental Quality Plano-Convex (PCX) Lenses," Edmund Optics Worldwide. [Online]

[11] García-Fruitós, Elena. *Insoluble Proteins*. Humana Press, 2015, pp. 209-211.

[12] BrainXell. 2020. *About Brainxell — Brainxell*. [online] Available at: <https://brainxell.com/about-us> [Accessed 9 December 2020].

[13] "Nikon Phase Contrast ELWD 0.3NA Condenser", *spectraservices.com*, 2020. [Online]. Available: https://spectraservices.com/product/NIKONPCELWDCOND.html. [Accessed: 17- Sep- 2020].

[14]"A1 HD25 / A1R HD25 | Specifications". *Nikon Instruments Inc.*, 2020, https://www.microscope.healthcare.nikon.com/products/confocal-microscopes/a1hd25-a1rhd25/specifications. Accessed 9 Oct 2020.

[15] Roberts, W. (2020, January 09). What the HEK? A Beginner's Guide to HEK293 Cells . Retrieved December 09, 2020, from https://bitesizebio.com/45489/what-the-hek-a-beginners-guide-to-hek293-cells/

[16] Davidson, M., 2020. Molecular Expressions: Science, Optics And You - Intel Play QX3 Computer Microscope - Advanced Photo Gallery - Abbe Condenser Design Number Six. [online] Micro.magnet.fsu.edu. Available at:

[17] M. Cowles and C. Davis, "On the Origins of the .05 Level of Statistical Significance," *American Psychologist*, vol. 37, no. 5, pp. 553–558, May 1982.

[18] K. De Vos, "Cell Counter", *Imagej.nih.gov*, 2020. [Online]. Available: https://imagej.nih.gov/ij/plugins/cell-counter.html. [Accessed: 08- Dec- 2020].

[19] Penna., and MARTON. *CRAC Channel*. Springer New York, 2018.

X. Appendix

A.***Product Design Specification***

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.

References

[1]Greenhalgh, D., Lawless, M., Chew, B., Crone, W., E Fein, M. and Palmieri, T., 2003. Temperature Threshold for Burn Injury: An Oximeter Study. *Journal of Burn Care & Rehabilitation*, [online] 24, p.S169. Available at: <https://pubmed.ncbi.nlm.nih.gov/15353932/#affiliation-1> [Accessed 16 September 2020].

[2]"Nikon | Healthcare Products & Solutions (Microscope Solutions) | ECLIPSE Ts2 - Specifications/Dimensions", *Nikon.com*, 2020. [Online]. Available: https://www.nikon.com/products/microscope-solutions/lineup/inverted/ts2/spec.htm. [Accessed: 17- Sep- 2020].

[3]Microscope.com. 2020. *Safety & Maintenance*. [online] Available at: <https://www.microscope.com/safety-and-maintenance/> [Accessed 16 September 2020].

[4]Rottenfusser, R., Wilson, E. and Davidson, M., 2020. *ZEISS Microscopy Online Campus | Microscopy Basics | Microscope Optical Systems*. [online] Zeiss-campus.magnet.fsu.edu. Available at: <http://zeiss-campus.magnet.fsu.edu/articles/basics/opticalsystems.html> [Accessed 17 September 2020].

[5]Nikon, 2020. Nikon | Healthcare Products & Solutions (Microscope Solutions) | Condenser. [online] Nikon.com. Available at: <https://www.nikon.com/products/microscope-solutions/lineup/accessory/condenser/> [Accessed 17 September 2020].

[6]Davidson, M., 2020. *Molecular Expressions: Science, Optics And You - Intel Play QX3 Computer Microscope - Advanced Photo Gallery - Abbe Condenser Design Number Six*. [online] Micro.magnet.fsu.edu. Available at: <https://micro.magnet.fsu.edu/optics/intelplay/abbedesign6.html> [Accessed 17 September 2020].

[7]"Nikon Phase Contrast ELWD 0.3NA Condenser", *spectraservices.com*, 2020. [Online]. Available: https://spectraservices.com/product/NIKONPCELWDCOND.html. [Accessed: 17- Sep- 2020].

[8]Product Classification. (n.d.). Retrieved September 17, 2020, from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpcd/classification.cfm?id=5110

[9]Fellers, T. (n.d.). Cleaning, Care, and Maintenance of Microscopes. Retrieved September 17, 2020, from https://micro.magnet.fsu.edu/primer/anatomy/cleaning.html

[10]Ishiwata, H., 2020. *Method And Apparatus For Visualizing Phase Object*. [online] Scienceon.kisti.re.kr. Available at: <https://scienceon.kisti.re.kr/srch/selectPORSrchPatent.do?cn=USP2015059041788> [Accessed 16 September 2020].

[11]Takashi, Y. (n.d.). FOCUSING DEVICE, FOCUSING METHOD, FOCUSING PROGRAM AND MICROSCOPE - SONY CORP. Retrieved September 16, 2020, from http://www.sumobrain.com/patents/jp/Focusing-device-method-program-microscope/JP2011209573A.html

[12]Oldenbourg, R. (n.d.). Laboratory of Rudolf Oldenbourg. Retrieved September 16, 2020, from https://www.mbl.edu/bell/current-faculty/oldenbourg-lab/

B. Expenses

Item	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total
Category 1 - Lens (Online Order)							
	Plano Convex Lens	Edmund Optics	(#73-871)	11/12/2020	2	$6.30	$12.60
	Double-Concave Lens	Edmund Optics	(#75-691)	11/12/2020	2	$6.30	$12.60
	Shipping Costs/Taxes				1	$10.87	$10.87
Category 2 - Makerspace Protoype							
	Annulus Prints	Makerspace		11/6/2020	1	$2.76	$2.76
	Extra Lenses Superstructure	Makerspace		11/12/2020	1	$29.66	$29.66
	Extra Lenses Superstructure +35% Annulus	Makerspace		11/30/2020	$1.0	$20.44	$20.44
						TOTAL:	**$88.93**

C. Matlab Code: Statistical analysis of results

```
%%Lens Modification

%10x magnification

Control1= [7790,7797,7638,7840,7207]; %5th well exlcuded

LensMod1= [7328,7238,7294,7440,6987];

[h,p]=ttest(Control1,LensMod1)  %reject null, they are stat different strong
evident against null
```

```
%20x magnification

Control2 = [9732,8449,7793,6702,8087,7033];

LensMod2=  [7950,7648,7110,6450,7991,6548];

[h,p] = ttest(Control2,LensMod2)  %reject null, they are stat different week
evidence against null

%Lens mod together

Control = [7790,7797,7638,7840,7207,9732,8449,7793,6702,8087,7033];
%control1 + control2 together

LensMod = [7328,7238,7294,7440,6987,7950,7648,7110,6450,7991,6548]; %lens1
and lens2 together

[h,p] = ttest(Control,LensMod)  %still stat significant, strong evidence
against null

%%Annulus Modification

%10x magnification

Con1= [8269,8516,7463,5966,8364,8815];

twentymod1 = [7625,8355,7214,5543,8116,8665];

Thirtymod1 =[7514,7914,6634,4475,7715,8372];

Anovaprep1 = [Con1', twentymod1', Thirtymod1']  %control is first column,
etc

[p,tbl] = anova1(Anovaprep1)  %p = .5301 = not stat different

Con2 = [7564,8309,7829,7396,7295,7146];

twentymod2 = [7192,7918,7343,7269,6926,7090];

Thirtymod2 = [6655,7455,7066,6861,6372,6602];

Anovaprep2 = [Con2',twentymod2',Thirtymod2']

[p,tbl] = anova1(Anovaprep2) %p = .0131 something is stat different

Con = [8269,8516,7463,5966,8364,8815,7564,8309,7829,7396,7295,7146];

twenty = [7625,8355,7214,5543,8116,8665,7192,7918,7343,7269,6926,7090];

thirty = [7514,7914,6634,4475,7715,8372,6655,7455,7066,6861,6372,6602];

Anovaprep = [Con',twenty',thirty']

[p,tbl] = anova1(Anovaprep) %p = .1033
```