BME Design-Fall 2020 - KYLIE GASPAR
Complete Notebook

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KYLIE GASPAR

on

Dec 08, 2020 @08:54 PM CST

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Team contact Information

Ben Hildebrandt - Sep 11, 2020, 12:39 PM CDT

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Herzog	Sam				

Project description

KYLIE GASPAR - Oct 01, 2020, 4:36 PM CDT

Course Number: BME 200 / 300

Project Name: BrainXell: Phase contrast microscope condenser for observation of multi-well cell culture plates

Short Name: BrainXell

Project description/problem statement:

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.

About the client:

From BrainXell's "About Us" Webpage:

BrainXell was founded in 2015 by Prof. Su-Chun Zhang in conjunction with the Discovery to Product (D2P) program of University of Wisconsin-Madison and operates out of a new facility in University Research Park. The company is based on the proprietary technology of directed differentiation of human stem cells (including induced pluripotent stem cells) to highly enriched subclasses of neurons. BrainXell provides a range of high-purity, iPSC-derived human neurons for research and development with a focus on drug discovery.

KYLIE GASPAR - Dec 08, 2020, 12:37 PM CST

Impact and Motivation

Live cells of various types, including those used by the client, are transparent and need to be seen for analysis. Imaging live cells requires accuracy, which is why we want to keep the microscope's resolution and expand that area, so further quantitative and qualitative analysis can be done by professionals on whatever they wish to study. However, imaging equipment, phase microscopes, inverted microscopes, are expensive. It is not reasonable to make a new design of the entire microscope because not many labs would have access to buy a new microscope when they already have one on hand. This is why we are looking to create a simple attachment to existing microscopes, so that a low cost, effective, method can be widely implemented. If successful, this design will benefit the daily life of scientists who use phase contrast.

9/14 - Initial Client Meeting

LAUREN HICKS - Sep 14, 2020, 5:01 PM CDT

Title: Initial Meeting with Client

Date: 14 September 2020

Content by: Lauren Hicks

Present: Carson, Ben, Kylie, Katherine, and Sam

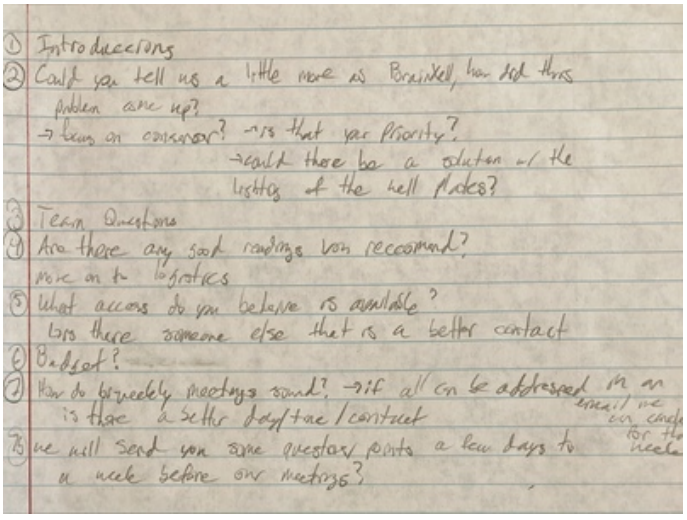
Goals: To get a better understanding of what the client wants for the project and how things will pan out due to Covid-19 restrictions.

Content:

- We went over introductions and our team roles.
- BrainXell - boutique biotech company that produces cells for CNS disorders research for big pharmacies. Our client, Michael Hendrickson, is a project manager and has a background in microscopy.
- If we are able to generate a solution for this company it would be helpful to alleviate annoyances from labs.
    - Phase allows for the contrast to enhance the quality of the viewing.
    - Standard Tissue Microscopes - condenser on the top (inverted microscope), objectives are on the bottom
    - The plates might be where the problem has arisen, well-plates. The cone of light coming in gets clipped by the wells.
        - One solution route - build a new condenser
        - Another solution route - changing design of objective
        - Another solution route (comes w constraints) - clear well-plates
- Constraints:
    - Well-plates are standardized, black plates are used for fluorescent
        - Grinder 96 well-plate opaque
    - Resolution - dropping resolution solves the problem (10% down)
- Using optic calculations for resolution, using breadboard systems
- Biweekly updates with the client - email is preferred (from Ben), and he will come to deliverables.
- Budget from BrainXell - they would buy use materials if we have a design.
- Microscopes, scrap inside of check and get buckle cells, make sure there is liquid in well-plates, 96 well plate, and replicate the issue. Would need a microscope and a breadboard system.

Conclusions/action items: Going forward we are going to be continuing our research now with the specified topics our client discussed with us. We will also be looking at gaining access to a microscope/well-plates so we are able to see the targeted problem with resolution.

KYLIE GASPAR - Sep 14, 2020, 12:14 PM CDT



IMG_0822.jpg(1.8 MB) - download Meeting Itinerary

2020/10/27-Testing Meeting

KYLIE GASPAR - Oct 31, 2020, 10:40 PM CDT

Title: Testing Meeting-Identification of problem

Date: 10/27/2020

Content by: Kylie Gaspar

Present: Ben, Kylie, Mike Hendrickson, Katie, Carson

Goals: Discuss how the problem is not seen by team member, Ben, on our loaned microscope. These are notes taken during the meeting with the client.

Content:

- Magnification = eye piece + objective settings
- Samples (of client) at bottom
    - because they grow on adhesive to plate
    - check swabs are floating-this is an issue
- Samples grown in wells
- Saline solution used-anything close to the pH of blood
- Prepare teaching samples
    - research what is suggested
- thin, **translucent**, samples

Ideas about problem

- change NA of condenser
    - angle light goes in, make it straighter
    - **NA directly affects resolution**
- Relay lens
    - between sample and condenser
        - this is our Extra Lens design
    - create a steeper angle
- Math-NA affects resolution
    - See if we can go from high NA to a steep, small, NA
- Alternate Phase Contrast Mechanism
    - whole new condenser
    - adjust for clipping

Conclusions/action items:

Look into practice samples to make at home, like phase microscopy 101

Look to speak with professors on Photolithography and/or Optical design

2010/09/11-First Advisor Meeting

KYLIE GASPAR - Sep 11, 2020, 2:14 PM CDT

Title: First Advisor Meeting

Date: 09/11/2020

Content by: Kylie Gaspar

Present: Kylie Gaspar, Ben Hildebrandt, Katie Budde, Lauren, Carson

Goals: Ask some questions regarding COVID policies and access to materials. Troubleshoot communication issues as well as team conflicts. Meet everyone.

Content:

- Get to know everyone, background
- Katie works with Skala in lab
- PROGRESS REPORTS due Thursdays
- CC client and Saha on emails
- Maybe they'll send us some parts, idk COVID is terrible (be safe)
- Finding new meeting times and getting in touch with Sam
    - As long as Ben and Kylie are both present for meetings, we can split them up.
    - Mentoring

why do the clients want, what do they get from it?

would it be helpful to add extra channels or only care about phase?

Conclusions/action items:

- Find meeting times
- Get in touch with Client
- Start PDS
- get a regular schedule down

2020/09/18-Second Advisor Meeting

KYLIE GASPAR - Sep 18, 2020, 1:04 PM CDT

Title: Advisor Meeting Post Preliminary PDS

Date: 09/18/2020

Content by: Kylie Gaspar

Present: All

Goals: Go over PDS and update Saha on our plans for next week (create design ideas and Design Matrix)

Content:

- Good of PDS
    - Team says constraints and client responsiveness
- Share how PDS works w client, ask for comments
- GMP- good manufacturing practice
    - Living material
    - FDA has strong requirements for how everything needs to be traced back to GMP
    - LETS DOCUMENT THIS SOON
    - Facilities at Hosman(?) center and hospital
- Is BrainXell more research grade or human grade? human grade needs GMP

Design Matrix time

- Top criteria
    - Resolution stays the same
    - Effective area seen by phase contrast
    - Sam says illumination=effective area by focusing light of condenser
    - Katie says cost effective so labs can afford it
- Big pharma
    - they have high end equipment
- Testing
    - design in client hands to test, potentially give to mick customer (grad student in Saha's lab)
    - get sense of images
    - how would we test ours?
        - compare our images to images of what they already have
- comment on PDS, more broad on competitors
    - has anyone identified this as a problem
    - if they have its buried in literature
    - look at patents, supplement with academic articles
- microscopy of life Course, Paul camponola and Skala, ask for relevant materials
    - reach out to skala and them, maybe ask for a zoom lesson
    - use people in BME
- Ask Hendrickson for images of the phase contrast
- codes and standards are friendly to our project

Conclusions/action items:

Work on Design matrix,

look at more literature for codes and stuff

talk to Skala and Paul for optics info

Email Hendrickson for photos

2020/10/09-Advisor Meeting Post Midsemester Deliverables

KYLIE GASPAR - Oct 09, 2020, 12:36 PM CDT

Title: Post Midsemester Deliverables Meeting

Date: 10/09/2020

Content by: Kylie Gaspar

Present: All

Goals: Get feed back on our presentation and report. Discuss adequate designs to move forward with. Talk about experimentation plan for playing w microscope.

Content:

- suggestions for presentations
- need slide numbers, screenshare would help
- peer evals would team groups up

Design

What to use in teaching lab

- animal cells
- maybe BrainXell cells
- ask John P or Kris or Skala
- include request for cells in testing plan
- keep multiwell plate from client in buffer saline 4 degrees
- ask BrianXell for multiwell plates

Conclusions/action items:

Next week, make fabrication plan, get microscope, test microscope. Meet again Monday 10/19

Designs for Design Matrix

LAUREN HICKS - Sep 28, 2020, 6:38 PM CDT

Title: Drafting Designs for Design Matrix

Date: 23 September 2020

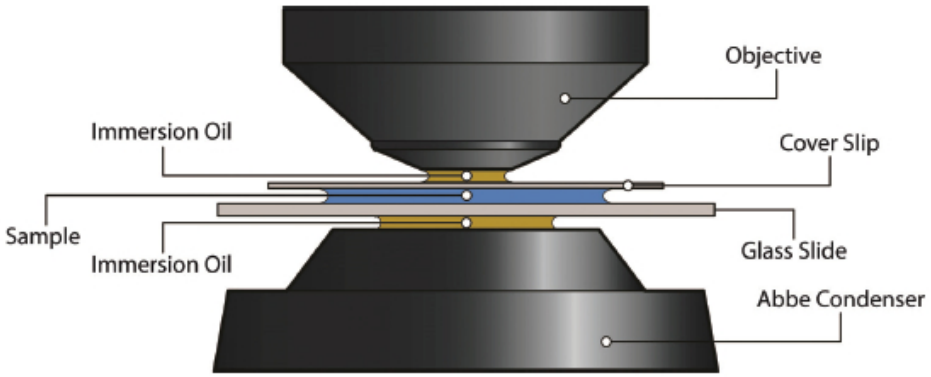
Content by: Team

Present: All

Goals: To create designs for the Design Matrix

Content:

- Lauren's Design #1 - Increasing the size of the condenser annulus by 25%, which would allow for more light to pass through with a greater surface area.
    - Sketch attached below.
- Lauren's Design #2 - Increasing the Numerical Aperture (NA) by using oil instead of air because oil has a higher refraction index.



- Carson's Designs -
    - Condensor attachments
        - We will not change anything about the phase microscope body or anatomy, this will make sure we will not lose any of the resolutions because we are not changing anything about it, these extensions will connect the objective lens part of the microscope, and will continue a light, annulus, and condenser that will be angled to the side of the focal point, connection to it and further extending it.
    - Changing the Phase Microscope Illumination Systems -
        - This idea will split the light into 2 separate rays of light, then will deflection to be parallel to each other. Depending on the limits, size, and diffraction, we can have to separate annulus to filter the incoming light and a convex condenser that will be able to filter both rays of light onto the specimen.

- Katie's designs:
    - Change the curvature of one of the condenser lenses so the focal point is a little above the specimen. this might be able to make it so the light is able to pass through a larger portion of the well plate - making more of it visible under phase contrast microscopy. I think that this might help it shift a wider range of the light out of phase to make them show up better.
    - Put more light beams through the condenser to allow it to pass a wider wave of light through the well, hopefully causing it to light up a wider area to push the specimen out of phase to help them show up better. I think that this could be done by altering the annular ring -- possibly widening the translucent area to allow for more light to pass through it -- could make the radius smaller or larger to achieve this.
    - Increase the intensity of the light. I'm not as sure about this idea, but maybe this could cause more of it to interfere and be knocked out of phase by the specimen to see if this could make a wider range of it visible.
- Ben's Designs:

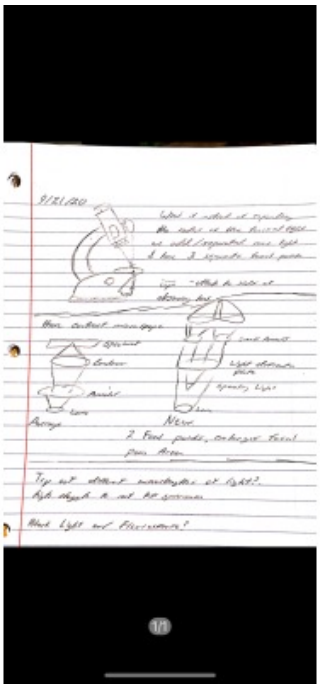
- Adjust the location of the specimen within the "cone of light" so that the light coming out of the condenser is focused roughly halfway up the well. Perhaps even lower than this. Although this will likely produce an unfocused image, it may decrease the amount of light entering along the edges or reduce the amount of "clipping" that the well plates do to the cone of light.
- Combine aspects of darkfield and phase contrast to possibly remove the outer ring of light. Darkfield microscopy focuses on only picking up the diffracted light of the specimen while refracting the rest of the light away. This could in theory get rid of the halo by creating solely dark background, but combination of techniques would prove to be difficult and finicky.
- Create some sort of refractive or absorbing apparatus to attach below the specimen plate in line with the condenser. This could either cut off the edges of the cone of light before it enters the well plate, which may make the cone of light thinner and therefore wouldn't result in clipping (however, this may also worsen the problem, as we are purposefully clipping the cone). This could be combined with increasing the width of the annular ring, to widen the cone. This combination of a wider cone and controlled clipping could result in getting a cone of light specifically designed for viewing well plates.
- Work with some sort of in-between lens that focuses the angle of the cone to be thinner before going into the well plate. The lens would have to go between the condenser and the bottom of the well plate, and the image it creates would have to be at the same location as the original. This would require a matching lens placed before the objective lens (but after the well plate) to expand the condensed cone of light back into its original form. The idea behind this solution would be to "increase the magnification" for the time that the cone of light travels through the specimen and then immediately decrease that "magnification" back to the intended value for the objective and condenser lens. The drawback from this design would be that the resolution may be decreased, and the lenses would have to be able to move to pre-determined positions for different pairs of objective/condenser lenses.

Conclusions/action items:

LAUREN HICKS - Sep 23, 2020, 7:27 PM CDT

IMG_1622.HEIC.pdf(2.9 MB) - download

CARSON EVENSTAD - Sep 24, 2020, 10:27 AM CDT



Microscope_Ideas.pdf(2 MB) - download These are drawing of my ideas, first is the microscope attachment, second in the new Illumination Optics system

2020/10/01-Original Design Matrix

KYLIE GASPAR - Oct 01, 2020, 4:40 PM CDT

Title: Original Design Matrix

Date: 01/01/2020

Content by: Kylie Gaspar

Present: Kylie, Sam, Ben, Carson

Goals: Put together a design matrix

Content:

			Using Oil instead of Air to increase refraction index		Hexagonal Light Structure		Reflect light to area of well		Change Condenser Curvature lens	
Rank	Criteria	Weight	Score (10 max)	Weighted Score	Score (10 max)	Weighted Score	Score (10 max)	Weighted Score	Score (10 max)	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

Effective area seen by phase contrast and resolution

These criteria were chosen to be the same weight because both are an integral part of inspecting a sample effectively. While our main goal in this project is to expand the area of high resolution phase contrast, it is equally important that we don't sacrifice too much resolution. High resolution is difficult to obtain but equally as important in assessing cell cultures.

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.

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.

Complexity

Complexity 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.

Safety

Safety is the least important criteria because we are following FDA approved products as our inspiration.

Conclusions/action items:

This Design Matrix was changed, we need to update it to more relevant designs we have drawings for. The criteria will stay the same.

2020/10/06-Design Matrix version 2

KYLIE GASPAR - Oct 06, 2020, 8:49 PM CDT

Title: Design Matrix Version 2

Date: 10/06/2020

Content by: Kylie Gaspar

Present: All

Goals: Update Design matrix to our current designs, as well as add in the mechanical approach (inspired by Professor Rogers opinion on the problem)

Content:

			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

Conclusions/action items:

Discuss with advisor and client to find which they think the best solution is. We need to find the source of the problem to know which the best approach is, for this reason we really need to get our hands on a Nikon Eclipse. More feedback will lead to more designs will lead to the best solution.

2020/10/26 - Adjusted Annulus Design

Ben Hildebrandt - Dec 08, 2020, 9:18 AM CST

Title: Adjusted Annulus Design

Date: 10/26/2020

Content by: Ben Hildebrandt

Present: N/A

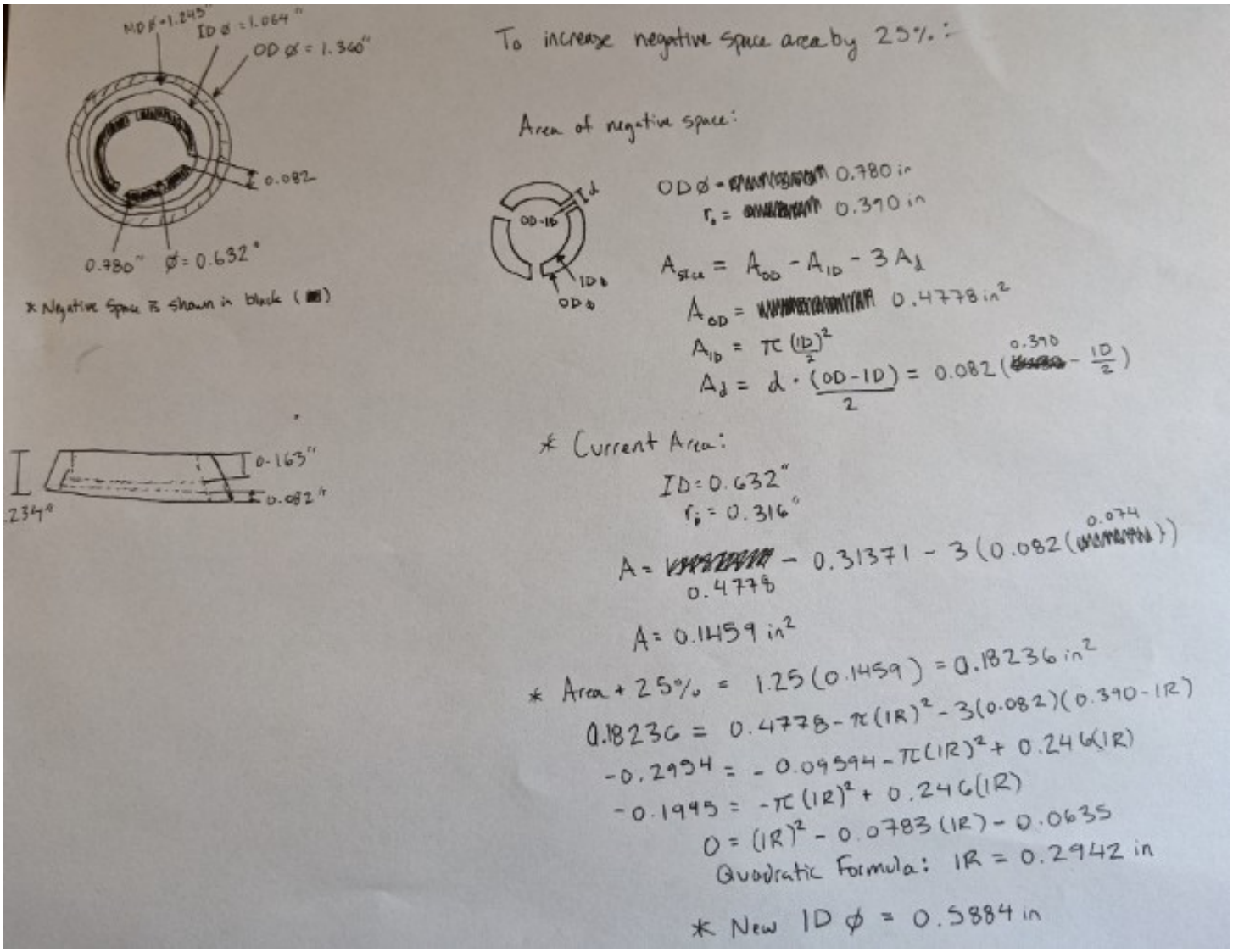
Goals: To calculate and CAD an annulus ring that allows 25% more light through

Content:

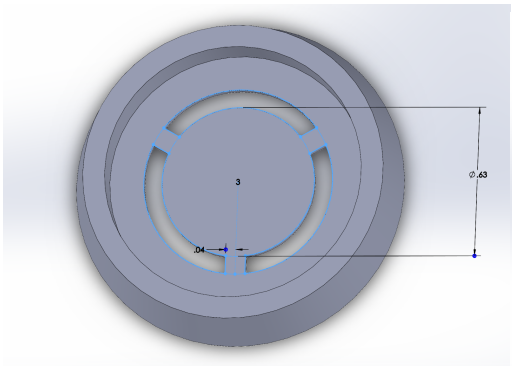
We received several condenser annuli with the microscope we are renting. One of these fits for both 10X and 20X magnification, which is our target. Therefore, it is logical to base our adjusted annulus off of this one (shown below).\

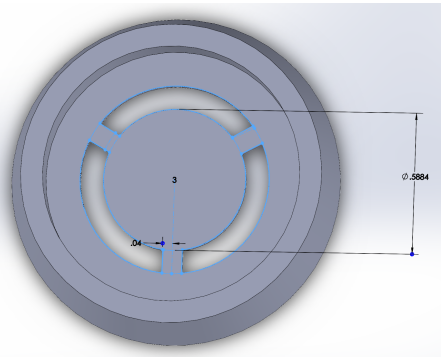
In order to replicate then subsequently modify the annulus in CAD, we took measurements of the current annulus using a digital caliper. These measurements can be seen in the image below, which also contains the calculations done to modify the inner diameter of the "negative space ring" to allow 25% more light through.

We wanted to maintain the shape and outside width of the cone, so the outside diameter (shown as OD in the image below) was not modified. The below calculations were made to calculate the new inside diameter to increase the area of the three negative space arcs by 25%. This area was calculated by subtracting the A_{inside} from the $A_{outside}$ and then subtracting 3 rectangles with areas of $(0.082*(r_o-r_i)$ to account for the small strips of metal that keep the middle circle in place. The width of these rectangles is also not to be changed.

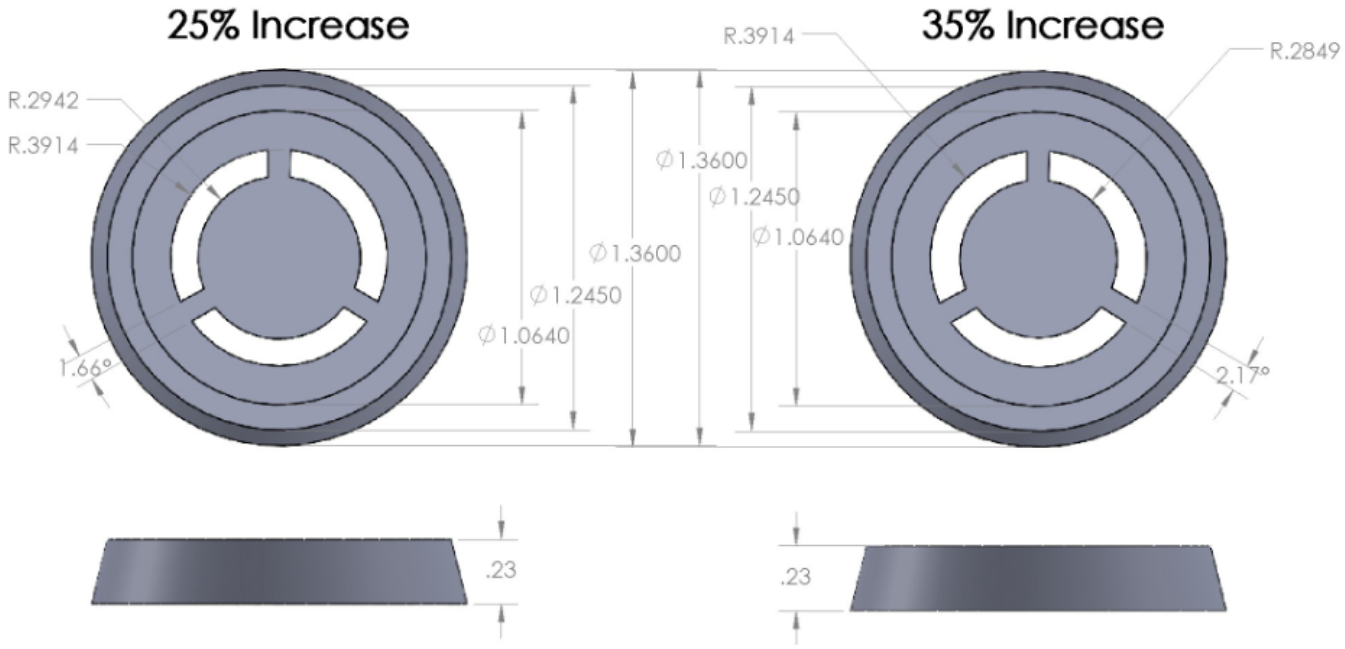


The resulting annulus was then modeled in SolidWorks to produce the following file (shown on the right, next to the original for comparison):





Following the design of the 25% increased annulus, the identical mathematical process was done to create an annulus with a 35% increased area as well. The engineering drawings for both of these parts can be seen below:



Conclusions/action items:

These annuli, once printed, could be easily swapped out with the existing annulus on our rented microscope. The ease of use will immediately allow us to test its effectiveness. It will need to be printed in a black material that does not allow any light to pass through. If this is not possible, we may have to purchase some metal and laser-cut the annulus instead.

2020/10/29 - Extra Lenses Design

Ben Hildebrandt - Oct 29, 2020, 11:18 AM CDT

Title: Extra Lenses Design

Date: 10/29/2020

Content by: Ben Hildebrandt

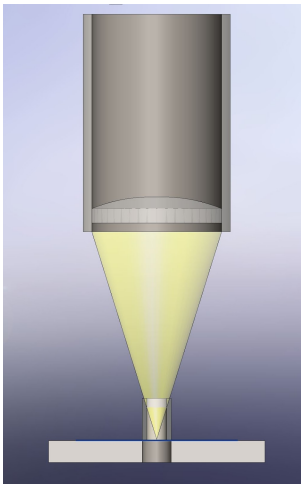
Present: N/A

Goals: To fully model and calculate the measurements for the extra lenses design

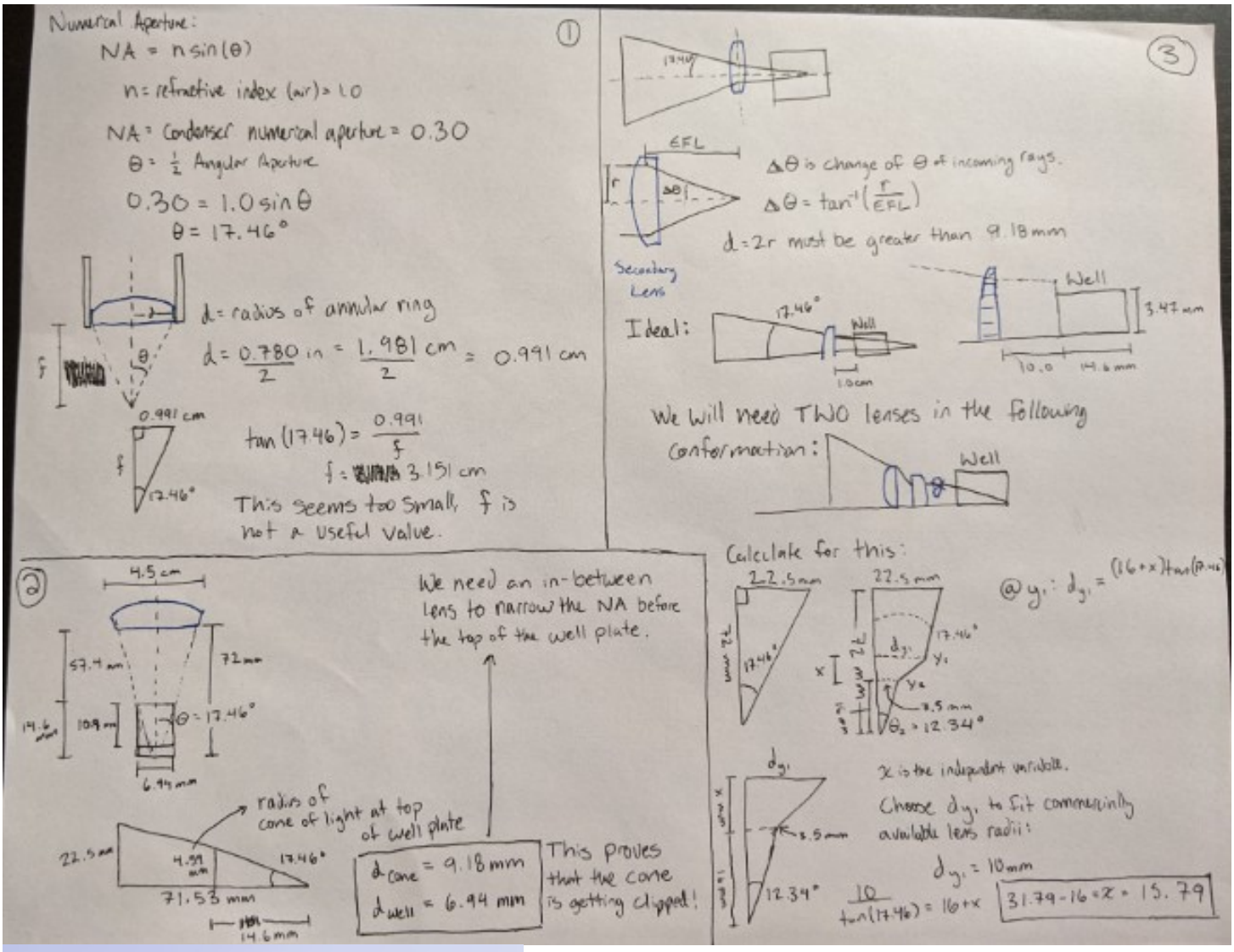
Content:

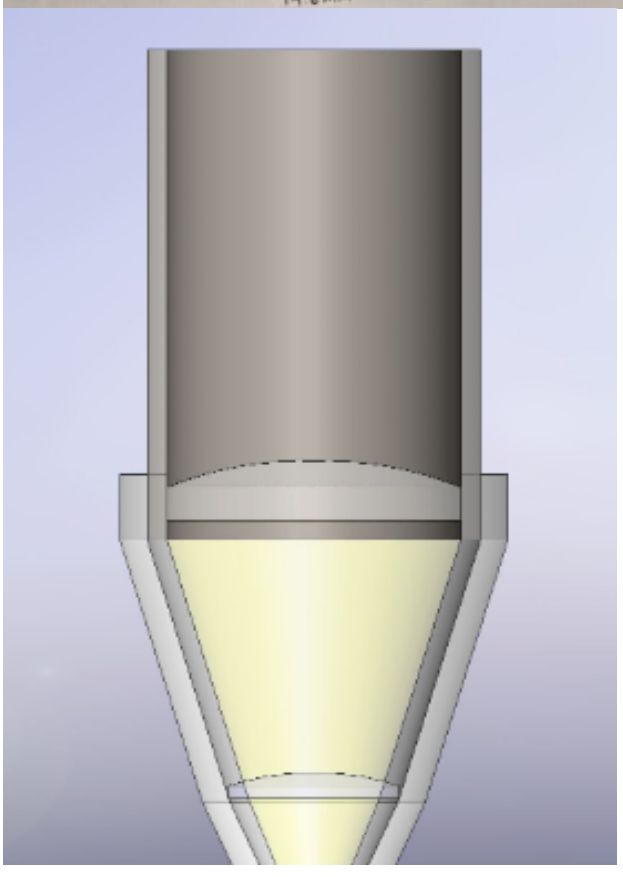
This design has a lot of open variables, so it would be necessary to do some preliminary calculations to get a handle on the problem. Included below is an image of these calculations that I did, divided into three distinct sections.

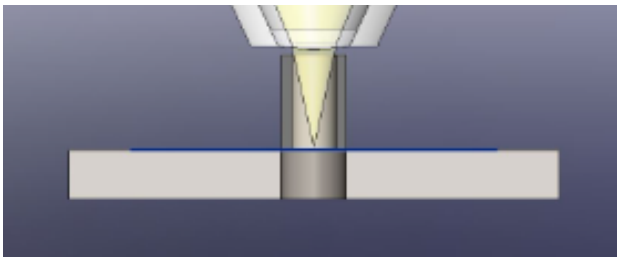
1. Section 1 was calculating the focal length of the cone of light assuming the cone of light's initial diameter was equal to that of the annular ring, at 0.991 cm. However, as the calculations in section 1 showed, this value was too small and did not result in a useful value.
2. Section 2 was much more useful. Assuming the cone of light's initial diameter was equal to that of the condenser lens at 2.25 cm, the focal length was calculated to be 72 mm, which is exactly the distance from the condenser to the specimen surface. Using similar triangles and the known numerical aperture of the condenser lens, I was able to mathematically prove that the light cone's diameter at the height of the well plate was greater than that of the well plate itself, proving that clipping is going on. This can also be seen in the image I included below:



3. Section 3 was calculating the distances between the lenses that would be added below the condenser to eliminate clipping. Many of the variables in this setup are still being determined, since it largely depends on what lenses are available for purchase. However, some assumptions were made: I assumed the final lens would be 3 mm above the top of the well plate, that the first lens would have a diameter of 20mm (which seems to be fairly common for commercially available lenses), and the diameter of the second lens would be 7 mm (which is also fairly common). The calculations yielded the vertical location of the first lens relative to the second (which is written as "x") and that allowed for the model shown below.


Numerical Aperture:
NA = n sin(θ)
n = refractive index (air) = 1.0
NA = Condenser numerical aperture = 0.30
θ = ½ Angular Aperture
0.30 = 1.0 sin θ
θ = 17.46°
d = radius of annular ring
d = 0.780 in / 2 = 1.981 cm / 2 = 0.991 cm
tan(17.46) = 0.991 / f
f = 3.151 cm
This seems too small, f is not a useful value.
①
③
EFL
Δθ is change of θ of incoming rays.
Δθ = tan⁻¹(r / EFL)
d = 2r must be greater than 9.18 mm
Secondary Lens
Ideal:
Well
3.47 mm
10.0
14.6 mm
1.0 cm
We will need TWO lenses in the following conformation:
Well
②
4.5 cm
57.4 mm
72 mm
14.6 mm
10.9 mm
θ = 17.46°
6.94 mm
We need an in-between lens to narrow the NA before the top of the well plate.
radius of cone of light at top of well plate
22.5 mm
4.99 mm
17.46°
71.53 mm
14.6 mm
d_cone = 9.18 mm
d_well = 6.94 mm
This proves that the cone is getting clipped!
Calculate for this:
22.5 mm
17.46°
22.5 mm
@ y₁: d_y1 = (16 + x) tan(17.46)
x
3 mm
3 mm
3 mm
y₁
y₂
8.5 mm
θ₂ = 12.34°
d_y1
x is the independent variable.
Choose d_y1 to fit commercially available lens radii:
d_y1 = 10 mm
8.5 mm
12.34°
10 / tan(17.46) = 16 + x
31.79 - 16 = x = 15.79





Conclusions/action items:

This design would be easily fabricated, since it would be a 3D-printed superstructure (shown in white on the model) and lenses that would fit snugly into it. However, the difficult part of this design lies with the math required to make it work. Since we do have an ability to test this with our microscope we have at home, it would be easy to set up a test plan. Unfortunately, most of the variables involved with this design are reliant on the lenses themselves, and there's no guarantee that a pair of commercially available lenses exist to make this design work the way it's intended to.

Further comparative calculations will have to be done before any lenses are purchased, which may delay the fabrication time significantly.

2020/11/13-Extra Lenses Final Design

Ben Hildebrandt - Dec 08, 2020, 10:23 AM CST

Title: Extra Lenses Final Design

Date: 11/13/2020

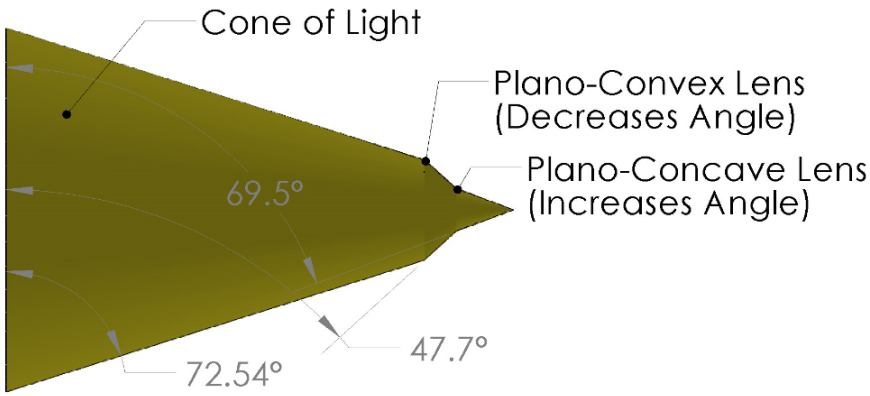
Content by: Ben Hildebrandt

Present: N/A

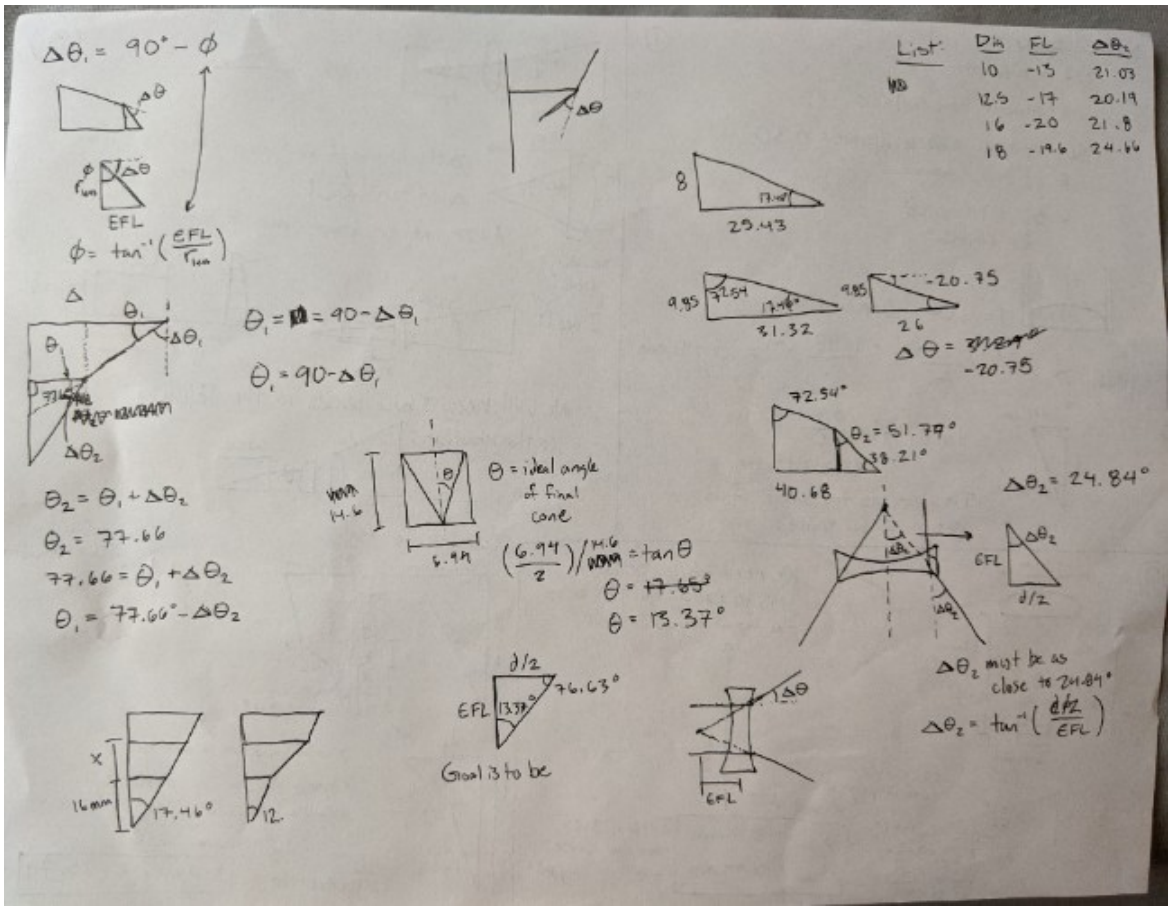
Goals: To finalize all measurements for the superstructure and to finalize what lenses to purchase.

Content:

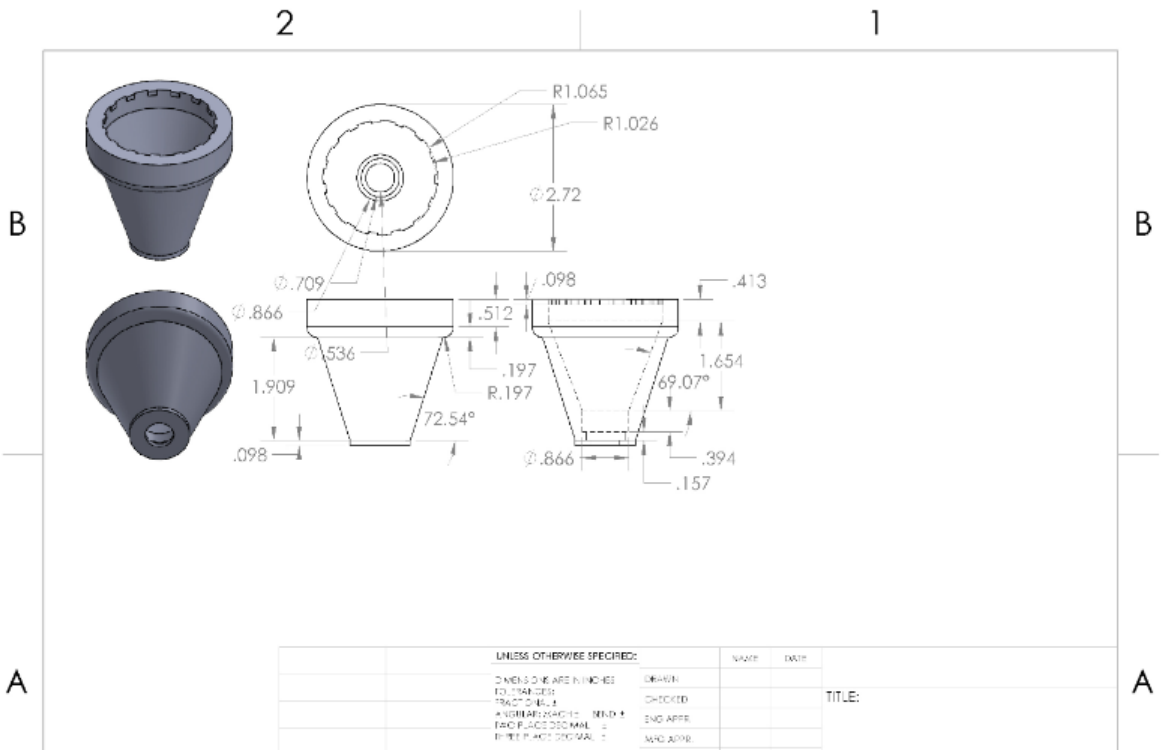
Originally when calculating the concept of the design, I assumed that using a combination of a normal plano-convex lens and the same lens inverted would effectively narrow and subsequently expand the cone of light. However, through more research, it seems that a plano-concave lens will be required to expand the cone of light after the plano-convex lens expands it. This can be explained in the below model of the cone of light:



In order to finalize the measurements, calculations were done by hand (image shown below) 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), I compared available sizes of lenses 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 lenses compared can be seen in the "List" section of the math image below. The ideal angle change for that 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.



Following the calculations, a superstructure was designed to house the lenses and contain the cone of light. This superstructure was designed with slots that allow the lenses to be press-fit into their locations in the cone of light through the top of the part. It also allows enough room between the bottom of the part and the microscope surface to slide standard well plates underneath. The superstructure needed to press-fit onto the condenser for our testing microscope, so the outside diameter of the condenser was used as the inside diameter for the top of the cone-shaped part. Finally, flanges were added to the inside surface of the top to further improve the press-fit on the condenser. These flanges aligned with an indented ring on the condenser cylinder approximately 0.8 cm from the bottom. The flanges were designed to lock into this indented ring and hold the superstructure tightly in place. An engineering drawing of the part is include below:



Conclusions/action items:

The angle change optimization was the most challenging mathematical part of the project so far. In hindsight, it would've worked much more efficiently to utilize a drafting software such as Solidworks for this math as well. Drawings can be fixed and dimensions can be changed very easily to visually optimize the design. If I had used CAD to calculate the angle here, it would've made my calculations much more accurate.

Regardless of the doubt about the methods, I feel fairly confident that the lenses I chose are ideal for this application. All the trigonometric approximations of their angle changes should create a cone of light that is sufficiently narrowed to create a non-clipped cone of light.

BPAG Expense Spreadsheet

LAUREN HICKS - Nov 19, 2020, 11:47 AM CST

https://docs.google.com/spreadsheets/d/14cm2kiGu0Gp1HLBKboS2kag_UgBkPYN6v0A6tmmoElE/edit?usp=sharing

KYLIE GASPAR - Dec 07, 2020, 11:25 PM CST

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**

In total we have spent $88.93 on this project for the fall 2020 semester

2020/11/07 - Fabrication of Modified Annuli

Ben Hildebrandt - Dec 08, 2020, 11:49 AM CST

Title: Fabrication of Modified Annuli

Date: Ben Hildebrandt

Content by: Ben Hildebrandt

Present: N/A

Goals: To decide on a material and print the modified annuli

Content:

The fabrication of the modified annuli should be fairly simple, as they can be 3D-printed as one part. 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 [1]. 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 [2], 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. The material properties table for the standard black table can be seen below and also in the references [2].

STANDARD RESINS

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

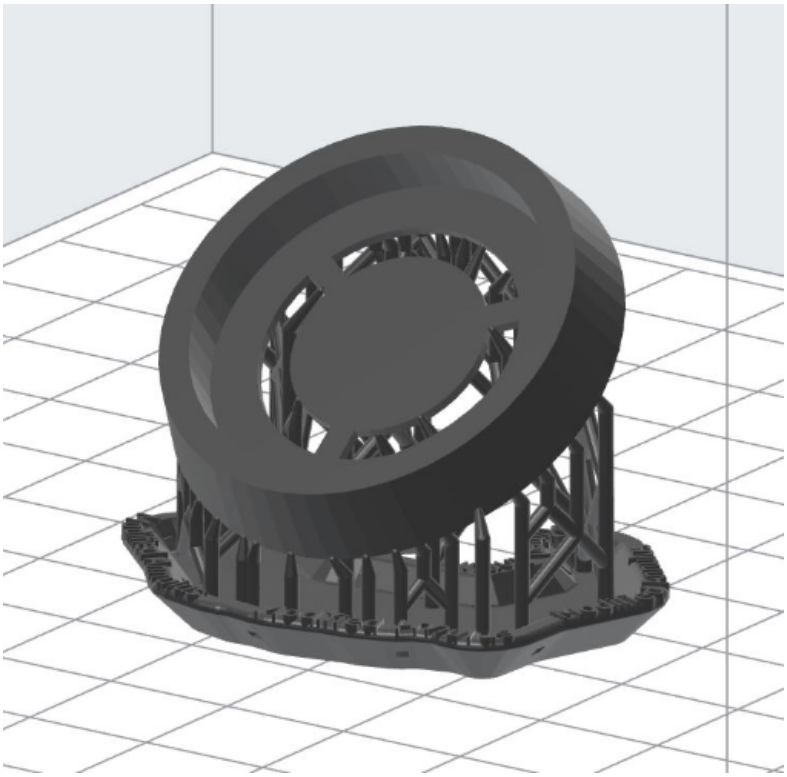
	METRIC[1]		IMPERIAL[1]		METHOD
	Green[2]	Post-Cured[3]	Green[2]	Post-Cured[3]	
Tensile Properties					
Ultimate Tensile Strength	38 MPa	65 MPa	5510 psi	9380 psi	ASTM D 638-10
Tensile Modulus	1.6 GPa	2.8 GPa	234 ksi	402 ksi	ASTM D 638-10
Elongation at Break	12 %	6 %	12 %	6 %	ASTM D 638-10
Flexural Properties					
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.

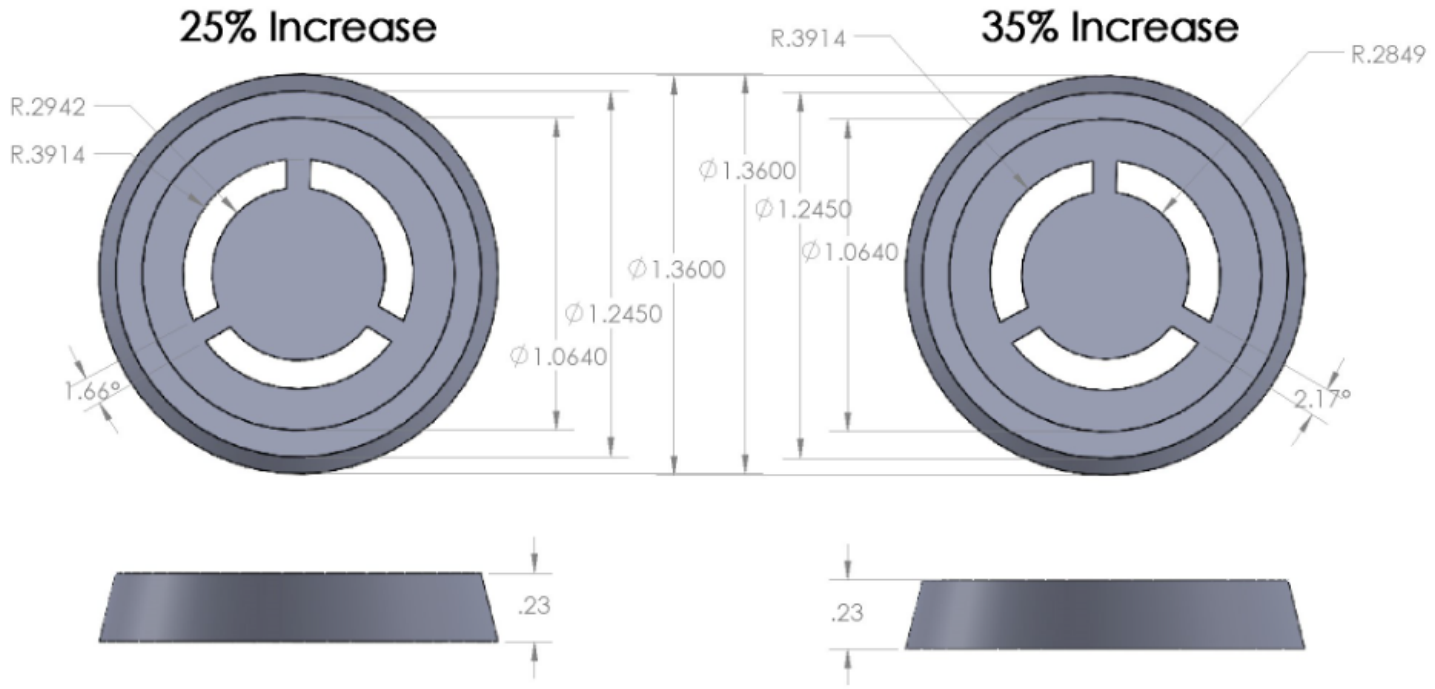
[2] Data was obtained from green parts, printed using Form 2, 100 µm, Clear settings, washed and air dried without post cure.

[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.

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.



In the above images, the final prototypes are shown. The diagonal orientation of the printed annulus limits the automatically-generated support structures to touch only one side of the annulus, leaving the front free of any markings. Additionally, the diagonal orientation of the part decreased reflectivity on the surface, since there were multiple layers along the surface, creating a slightly textured external surface. The image on the left shows the two final prototypes (25% Increase in middle and 35% Increase on Right) next to the original annulus (Left). Above the annuli is the annulus slide that holds the annulus when put into the microscope. All three annuli fit into the slide identically.



The printed parts were measured for tolerance on the following critical dimensions: 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.

References:

[1] “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].

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

Conclusions/action items:

In conclusion, the printed parts came out nearly perfectly. The supports, when removed, left little to no residue and the parts worked effectively as annuli - blocking out light everywhere except in the "negative space" of the ring. There is reason to believe that these modified designs will indeed allow 25% and 35% more light in respectively.

2020/11/24 - Fabrication and Assembly of Extra Lenses

Ben Hildebrandt - Dec 08, 2020, 9:56 AM CST

Title: Fabrication and Assembly of Extra Lenses Design

Date: 11/24/2020

Content by: Ben Hildebrandt

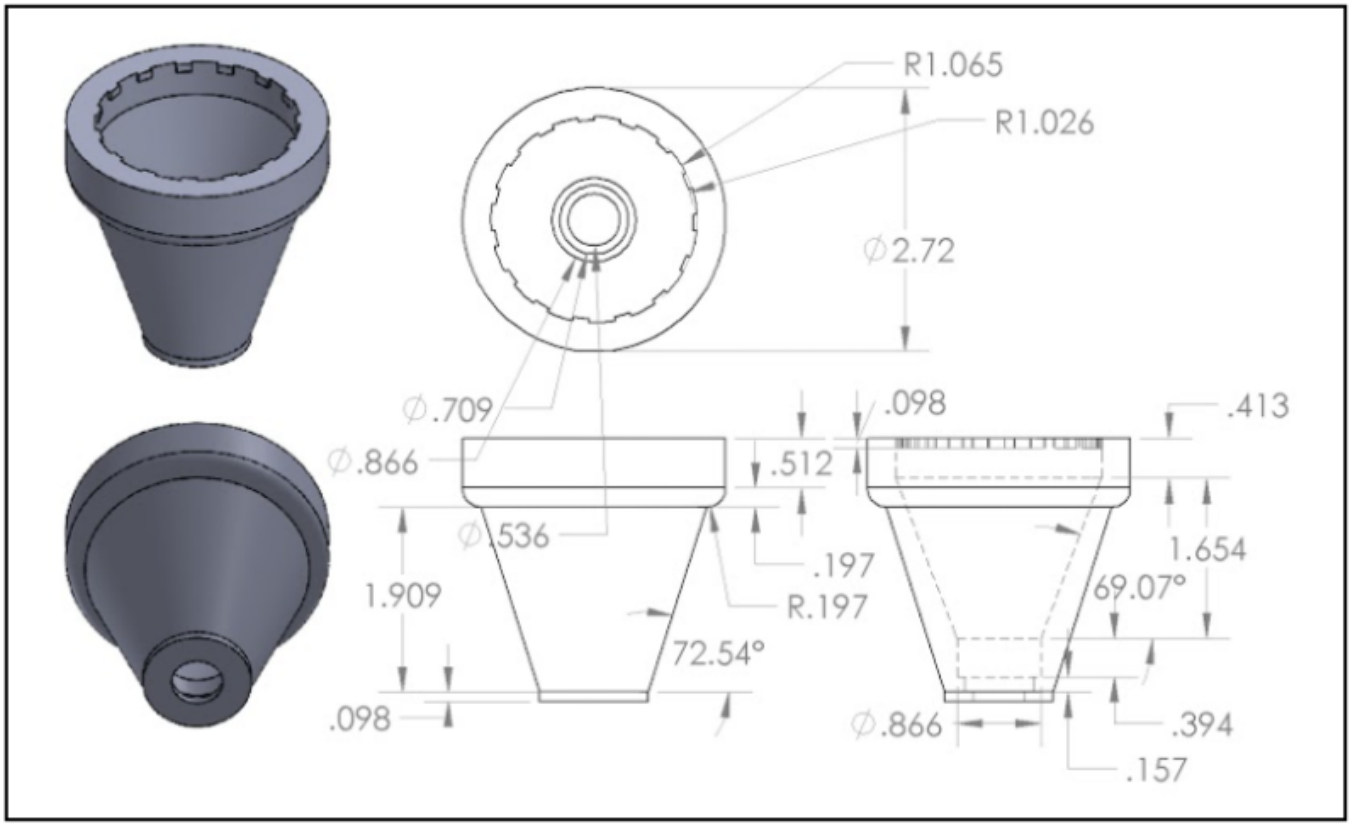
Present: N/A

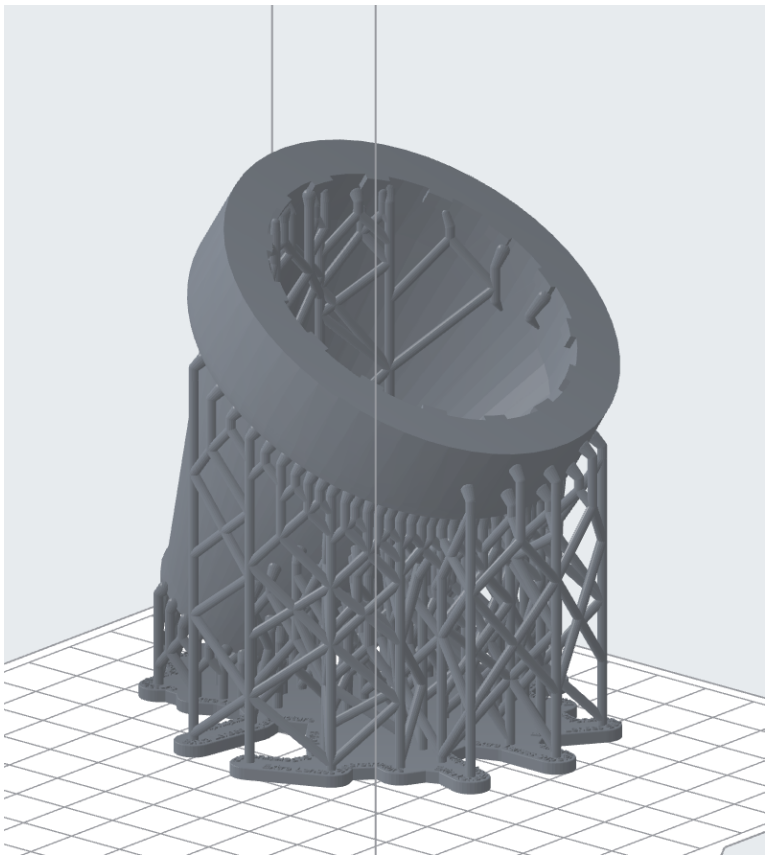
Goals: To print the necessary components for the Extra Lenses Design and to assemble the final prototype for testing.

Content:

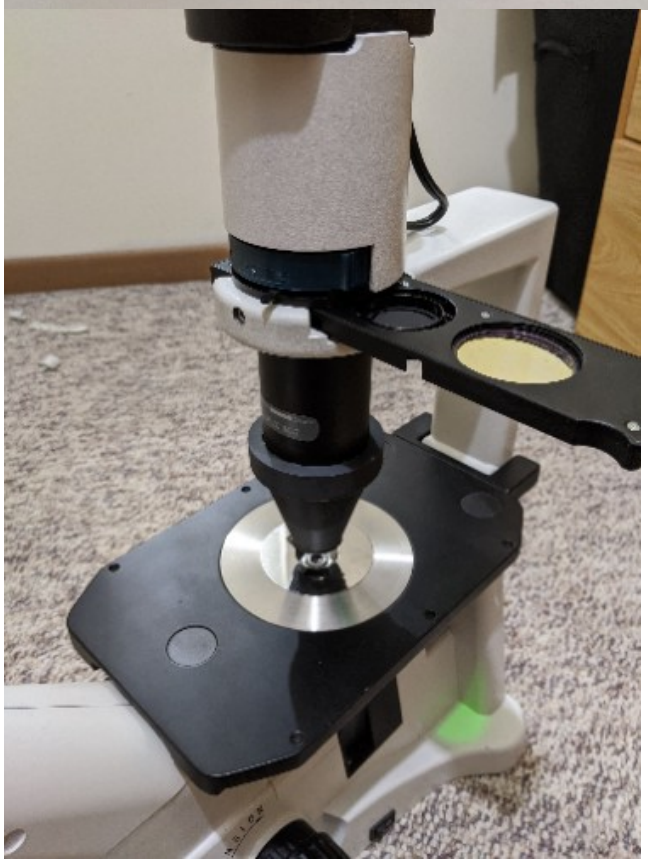
The extra lenses design would be comprised of primarily two parts: the outside superstructure and the lenses themselves. The lenses were ordered from Edmund Optics and consisted of a larger Plano-convex lens and a smaller Plano-concave lens. Both were made with Experimental-grade glass, which ensures the lenses are cut to the correct shape and size and are free of surface-level cracks or markings. The tolerances for these lenses are considerably lower, at ±10% on diameter and focal length [1]. This was not ideal, but it was the only grade of optical lens that fit our budget. Two of each lens were purchased as a failsafe, in case one of the lenses was cracked or unusable for any reason.

The material chosen for the superstructure (design shown below) needed to be slightly flexible, but sturdy enough to keep the lenses in place. It also needed to have a strong enough tensile strength to not break when press-fit onto the condenser as it was designed to do. For these reasons, "Flexible" material was chosen using the Stereolithography (SLA) method of printing at the Makerspace. The process of SLA printing uses a laser to solidify liquid resin layer-by-layer and is best used for materials that have flexible properties. The material properties table for "Flexible" resin can be found below and also in the sources [2].





The process of SLA printing also requires automatically-generated supports, which can be seen above on the right. The part requires a significant amount of support, as the "Flexible" material is more prone to warping during the printing process. The part was printed at a diagonal orientation to limit the touchpoints of the support structures to only half of the design. We also wanted to minimize the amount of touchpoints on the inside surface, as residue from the removed supports may interfere with the cone of light passing through the part. Following the printing process, the part was accurate to the specifications within a 5% tolerance on the following critical measurements: 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).



When the individual components seen above on the left were assembled, the intention was to press-fit the lenses into the slots on the bottom of the cone. However, the diameters on the lenses themselves were smaller than expected and were not able to press-fit into the material. Instead, the lenses were carefully placed in the bottom of the cone and held in place by gravity during testing. The lenses would fall out in the event of inversion. We didn't want to use adhesive, as this may have created spots on the lenses and chemically reacted with the resin of the cone. In the future, a better solution for holding the lenses in place must be engineered.

The press-fit on the condenser worked exactly as intended. The flanges interacted with the indented ring in the condenser and the flexible material stretched just enough that there was a seal on the condenser that could withstand small forces applied. Additionally, no residue or damage was done to the condenser when the device was removed.

References:

[1] “1 - 24.9mm Experimental Quality Plano-Convex (PCX) Lenses,” Edmund Optics Worldwide. [Online]. Available: https://www.edmundoptics.com/f/1-249mm-experimental-quality-plano-convex-pcx-lenses/14470/. [Accessed: 08-Dec-2020].

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

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.

Conclusions/action items:

In conclusion, the lenses that were ordered had tolerances that were a bit too high and we were unable to engineer a solution to fitting them in the superstructure without falling out. The 3D-printed part was fabricated within our specified 5% tolerance on all critical dimensions and its fit on the condenser worked as intended. The fabrication and assembly of this design is overall very simple and could be mass-produced relatively easily. With a part the press-fits onto standard condensers like the one we are testing with, the opportunity to have standard-sized devices is opened up. Since our part is flexible, it could stretch to fit any condenser that has a numerical aperture of 0.3 and a working distance of 75mm, both measurements being fairly standard.

Next steps with this design are to test it using a standard protocol that is written up.

2020/11/23-Physical Testing Protocol

KATHERINE BUDDE - Dec 02, 2020, 10:19 PM CST

Title: Physical Testing Protocol

Date: 11/23/2020

Content by: Kylie Gaspar/Carson Evenstad/Katie Budde

Present: NA

Goals: Create an detailed, repeatable, testing protocol.

Content:

This list will be done for the 1)original microscope/condenser set up, the 2)annulus designs with various negative areas, and the 3) lens holder design

1. *Select eye piece magnification and 10x objective magnification*
2. *Place well plate 1 (plated 11/16) in view, with light adjustment as needed*
3. *Take picture through eyepiece with phone camera (digital if on hand)*
4. *Move plate slightly and take another picture*
5. *Repeat until a total of three pictures have been taken for plate 1 with x magnification*
6. *Upload pictures to a computer for analysis on image J*
7. *Repeat steps 1-5 with 20x magnification*
8. *Repeat steps 1-6 for well plate 2 (plated 11/20)*
9. *Repeat entire process for remaining two set up environments*

-a total of 36 images will be analyzed

Image J analysis

*remember that we are looking to increase the area of viewable image, so the measurements do not need to be standardized in imageJ across the 36 pictures. A number of pixels of total area of the image can be compared to number of pixels of dark area of a single picture

1. *Import desired image into imageJ*
2. *Convert image to grey scale (Image -> Type -> 8-bit)*
3. Adjust brightness/contrasty (Adjust -> Brightness/Contrast)
    1. Drag minimum up until all background disappears and contrast is prevalent (usually around 100, but varies)
4. *Measure the area of the dark space by selecting the wand tool in the bar*
    1. Circle the dark area
    2. *Next select analyze, then measure, and record the number for Area*

*there are a variety of different tools to use for area measurement selection, wand tool is preferred, after that are the "area selection" tools and the "Analyze particles" tool. these can all be done on step 2

Resolution test from Carson on google doc?

Conclusions/action items:

2020/11/24 - Physical Testing Setup

Ben Hildebrandt - Dec 08, 2020, 12:06 PM CST

Title: Physical Testing Setup

Date: 11/24/2020

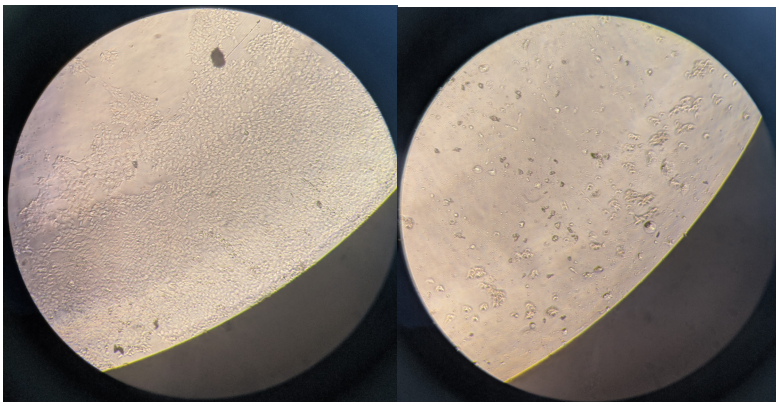
Content by: Ben Hildebrandt

Present: Ben Hildebrandt

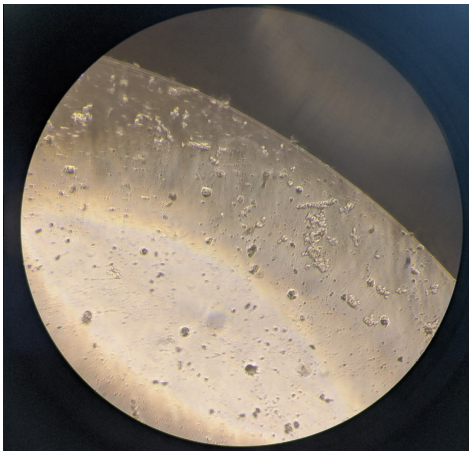
Goals: To perform the testing protocol

Content:

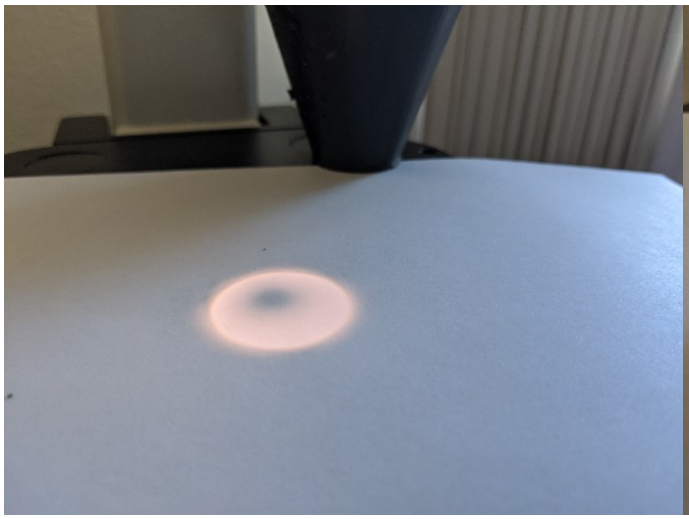
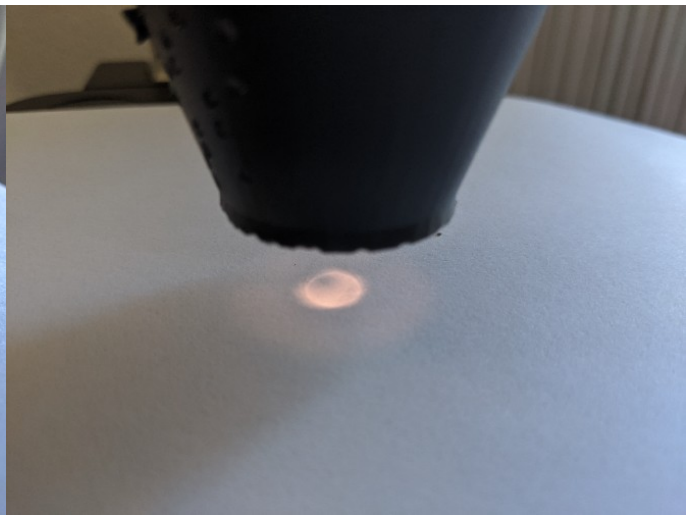
The tests were performed in a well-lit, non-laboratory setting using two well plates of laboratory-grown human embryonic kidney (HEK) cells that were subsequently fixed and stored in a refrigerator at approximately 36°F ±5°F. Plate 1 had cell growth that was largely isolated, and small clusters were 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.



The two wells shown above are from the different plates. Plate 1 is on the right and has more dispersed growth. Plate 2 is on the left and has very dense cell growth, which therefore has less contrast along the edges of individual cells. Some possible complications with this test 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. Shown below is an example of the dark spots yielding good contrast (the white outlines of cells) while the brighter spots diminish this effect.



The setup of the actual microscope was simple. The modified annuli were able to be exchanged without moving the microscope. The Extra Lens superstructure was also able to be removed with minimal interference with the well plates. As a proof of concept, the below images were taken showing the condensing of cone of light (shown by the light ring). Without the extra lenses device, the cone of light measured approximately 1.5" in diameter. When the device was added, the cone of light measured approximately 0.5" in diameter, showing evidence of a narrower cone of light.

Conclusions/action items:

The testing went relatively smoothly, but the results were fairly indeterminate. Before any image analysis, there was no discernible difference in the quality of images taken with any of the devices added on. The only discernible difference was a "washing out" effect with the modified annuli. They would allow more light in, and make the images brighter and the contrast worse.

The ImageJ analysis and subsequent results will be calculated by the team soon, and can be found in their respective sections in the notebook.

2020/11/23 - Statistical Analysis of Results

KYLIE GASPAR - Dec 08, 2020, 1:20 PM CST

Title: Statistical Analysis of Results-PUT IMAGES IN BOTTOM OF THIS DOC-one from each is ok

Date: 11/23/2020

Content by: Kylie Gaspar

Present: NA

Goals: Compare the relevant significance of the areas of contrast for the annulus design to the original phase microscope and the lens holder design to the original microscope.

Content:

Based on the mean of each sample if .5<s1/s2<2 is false we will do a standard Welches T-test to determine p value if the listed inequality is true, we will do an equal variance T-Test to determine p value. If the p value shows a statistical significance (.001<p<.05) we can determine that our designs improve the area of phase contrast.

After using the designated set up, 3 pictures will be taken of each environment and then analyzed using image J to measure the area of each picture. This data will be put into the table below, then used for the selected T-Test

The area in each cell below was found using imageJ, the dark area of the cell was measured in pixels with a free handtool and recorded below.

Chart of data from Lens Magnification testing, wells 1-6 had a 10x magnification and 7-12 had 20x

Well Number	Dark Area of Control (No Modification) in pixels	Dark Area with Lens Modification
1	7790	7328
2	7797	7238
3	7638	7294
4	7840	7440
5	**NA**	**NA**
6	7207	6987
7	9732	7950
8	8449	7648
9	7793	7110
10	6702	6450
11	8087	7991
12	7033	6548

Well 5 had poor images for both so the data was not included.

A standard, single variable t-test was ran (can be seen in MATLAB entry code) with a significance values of 0.05. Will all 12 wells put together it was found that there was a significant difference in dark area from the control to the lens magnification. With a p value of 0.0024, there was strong evidence against the null that the two mean dark areas were equal. This tells us, that for better or worse, the lens holder changes the lighting of the well plate.

Chart of data from Annulus Testing with two Annuli, wells 1-6 had a 10x magnification and 7-12 had 20x

Well Number	Dark Area of Control (No Modification) in pixels	Dark Area with 25% Annulus	Dark Area with 35% Annulus
1	8269	7625	7514
2	8516	8355	7914

3	7463	7214	6634
4	5966	5543	4475
5	8364	8116	7715
6	8815	8665	8372
7	7564	7192	6655
8	8309	7918	7455
9	7829	7343	7066
10	7396	7269	6861
11	7295	6926	6372
12	7146	7090	6602

Anova Testing was done on the three columns to compare means of dark areas, there was a significant change in mean, which again tells us that the Annuli affected the light reaching the image. However, there was much worse quality with the change in area. This would be able to be further quantified if a more detailed cell type was used, our team counted cells viewable in each picture, and they were all equal to their various control groups. If a cell had things like nuclei, and dendrites, we could count those instead and maybe have a difference in number of viewable objects, quantifying our "resolution". We did not have access to that, so our "resolution" is simply qualitative.

Conclusions/action items:

In the clients problem statement, we wanted to increase the dark area so that the entire image would be like the center of the the image. In our case, with a Fischer Scientific microscope, the center of the image is light and the outside is dark. So decreasing the dark area expanded the center image. It is still unclear if the center is better phase contrast than the outside, but I believe the fact that the image became more standard across the well plate is a good thing. The lens modification should be looked at further, maybe on a different microscope that experiences the same problem as the client.

The Annulus changes should not be looked at, as it simply saturated the sample with light, and negatively affect resolution, which is an important aspect of our project. If more evidence is needed to support this, access to a more detailed image should be used, and points counted and compared to control. If the count is less than control, our conclusion is correct and the Annuli negatively affect resolution. If more items are seen than the control, then we are wrong and the annuli does improve the resolution of the image, but I strongly doubt that being the case.

2020/12/07-MATLAB Analysis

KYLIE GASPAR - Dec 07, 2020, 11:31 PM CST

Title: MATLAB Analysis of Testing Results

Date: 12/07/2020

Content by: Kylie Gaspar

Present: NA

Goals: Show code used for analysis, it can be copied and used again for other data sets

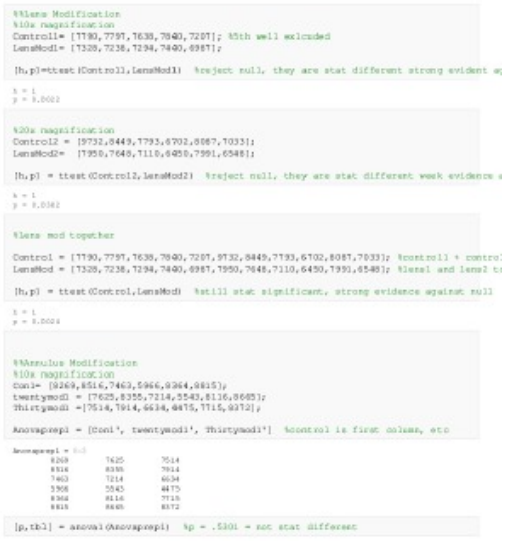
Content:

```
%%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 for more data points
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
```

Conclusions/action items:

Include and Explain these in the report and presentation. I will include the pdf below so that values and tables can be seen as well.

KYLIE GASPAR - Dec 07, 2020, 11:31 PM CST



```
%%Lens Modification
%10x magnification
Control1= [7790,7797,7638,7840,7207]; %5th well excluded
LensMod1= [7328,7238,7294,7400,6987];

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

h = 1
p = 0.0002

%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 a

h = 1
p = 0.0382

%lens mod together

Control = [7790,7797,7638,7840,7207,9732,8449,7793,6702,8087,7033]; %control1 + control
LensMod = [7328,7238,7294,7400,6987,7950,7648,7110,6450,7991,6548]; %lens1 and lens2 t

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

h = 1
p = 0.0004


%%Annulus Modification
%10x magnification
Con1= [8269,8516,7463,8966,8364,8815];
twentymod1 = [7625,8355,7214,8543,8116,8665];
Thirtymod1 =[7514,7914,6634,8475,7715,8372];

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

Anovaprep1 = 6×3
        8269        7625        7514
        8516        8355        7914
        7463        7214        6634
        8966        8543        8475
        8364        8116        7715
        8815        8665        8372

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

1

BME300stats.pdf(44.9 KB) - download

2020/09/14-Phase Microscope Imaging

KYLIE GASPAR - Sep 14, 2020, 3:37 PM CDT

Title: Initial Research on Phase Microscopy

Date: 09/14/2020

Content by: Kylie Gaspar

Present: NA

Goals: Understand the workings and purpose of phase microscopy

Content:

- Microscopy is based in the understanding that microscopes display images of objects. These objects interfere with the typical wave length, diffracting light. All light comes back together to make a coherent image of the object.
- "phase" object is when the microscopic object changes only the phase of the wave transmitted through it. (Versus amplitude of wave)
- Live cells are colorless and transparent, to stain them involved killing the cells (NOT good for BrainXell as they are making live neurons) it also can introduce changes in structure caused by processing the cells.
    - To combat this we can look at light waves

Phase explanation

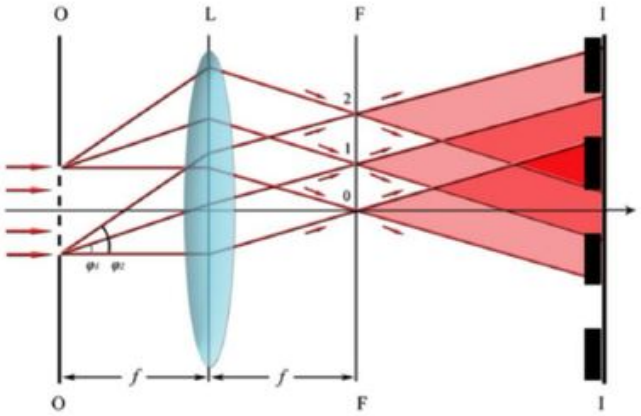
- Two waves from the same source are coherent
    - aka same constant relative phase
- Interference is when phase differences convert to intensity changes

Conclusions/action items:

https://ebookcentral.proquest.com/lib/wisc/reader.action?docID=840613

[1]T. Dmitry, T. Vladimir and T. Tishko, *Holographic Microscopy Of Phase Microscopic Objects: Theory And Practice*, 1st ed. World Scientific Publishing Company, 2011, pp. 5-17.

KYLIE GASPAR - Sep 14, 2020, 3:06 PM CDT



BME_300.JPG(33.5 KB) - download Optics and lenses. O=Object L=Objective F=Focal Plane I-Image Plane f=focal distance of objective

2020/09/16-Old Notes on Optics

KYLIE GASPAR - Sep 16, 2020, 6:34 PM CDT

Title: Notes on Optics

Date: 09/16/2020

Content by: Kylie Gaspar

Present: NA

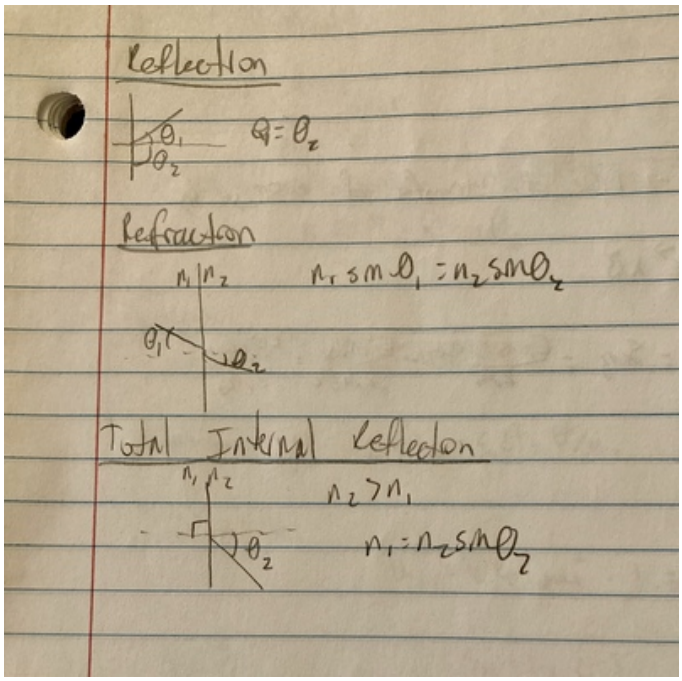
Goals: Have a reference on optics from personal notes in Physics 202. This is a start so that my team and I can build our knowledge up from this as a starting point.

Content: below

Conclusions/action items:

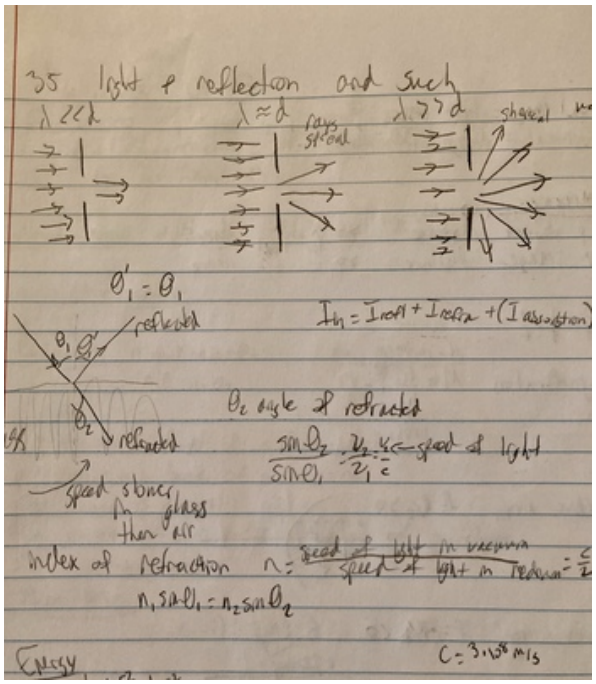
I'm making a crash course lesson from these notes. They will be summarized and used at a later date.

KYLIE GASPAR - Sep 16, 2020, 6:39 PM CDT



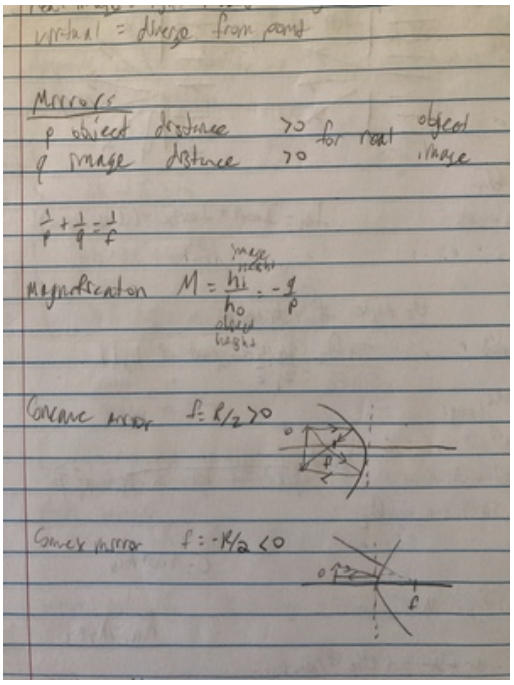
IMG_0823.jpg(2.9 MB) - download All notes from Kylie Gaspar fall 2019. Taken from course content in Physics 202 from UW-Madison, taught by Professor Matthew Herdon and Professor Gary Shiu

KYLIE GASPAR - Sep 16, 2020, 6:39 PM CDT



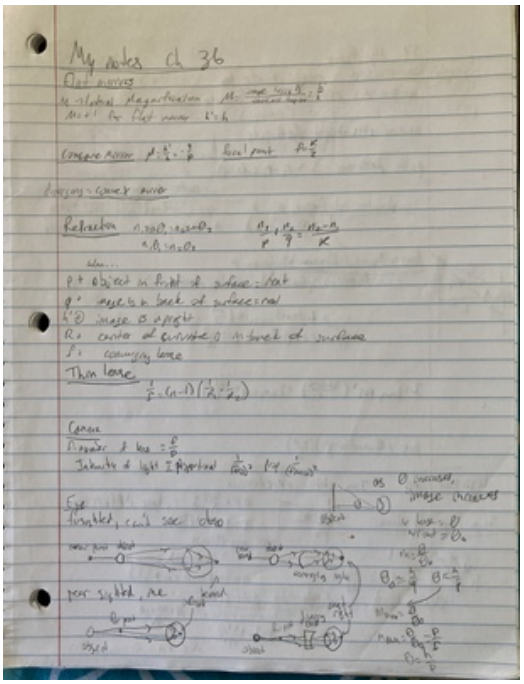
IMG_0824.jpg(3.1 MB) - download All notes from Kylie Gaspar fall 2019. Taken from course content in Physics 202 from UW-Madison, taught by Professor Matthew Herdon and Professor Gary Shiu

KYLIE GASPAR - Sep 16, 2020, 6:39 PM CDT



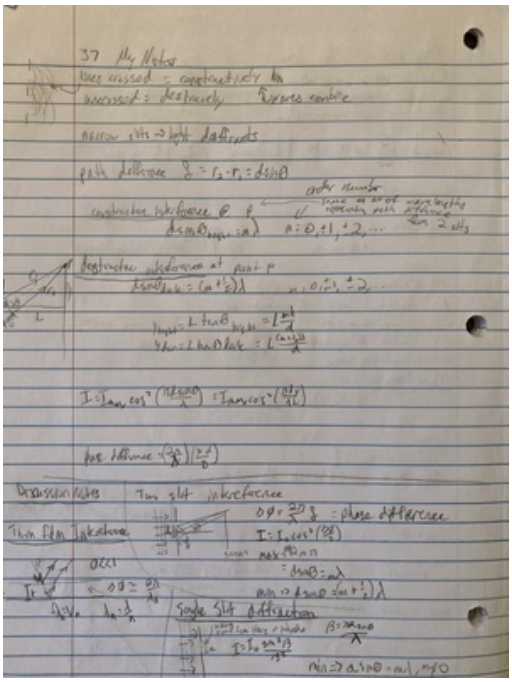
IMG_0825.jpg(4.3 MB) - download All notes from Kylie Gaspar fall 2019. Taken from course content in Physics 202 from UW-Madison, taught by Professor Matthew Herdon and Professor Gary Shiu

KYLIE GASPAR - Sep 16, 2020, 6:39 PM CDT



IMG_0826.jpg(3.3 MB) - download All notes from Kylie Gaspar fall 2019. Taken from course content in Physics 202 from UW-Madison, taught by Professor Matthew Herdon and Professor Gary Shiu

KYLIE GASPAR - Sep 16, 2020, 6:40 PM CDT



IMG_0827.jpg(3.4 MB) - download All notes from Kylie Gaspar fall 2019. Taken from course content in Physics 202 from UW-Madison, taught by Professor Matthew Herdon and Professor Gary Shiu

2020/09/21-Physics 202 notes and Summary

KYLIE GASPAR - Sep 21, 2020, 9:20 PM CDT

Title: Notes and equations on Optics from Physics 202

Date: 09/21/2020

Content by: Kylie Gaspar

Present: NA

Goals: Summarize all previous learned lessons on optics, lenses, and light from Physics 202 (taught in Fall 2019). Also make easy to access and apply equations for the whole team to reference

Content:

Intensity

Iin=Irefracted + Ireflected + Iabsorbed

Frequency remains unchanged

Reflection
theta=theta'

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

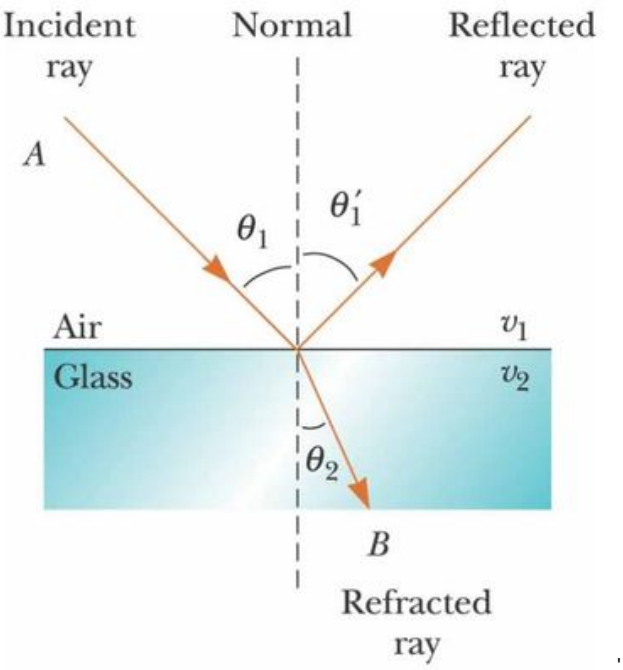
note $v1 > v2$ if $\theta 1 > \theta 2$

Refraction

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

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

KYLIE GASPAR - Sep 21, 2020, 8:33 PM CDT



3001.JPG(37.3 KB) - download

KYLIE GASPAR - Sep 21, 2020, 8:38 PM CDT

Real vs Virtual Images

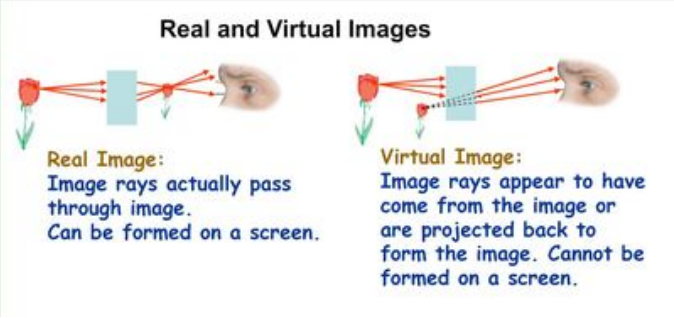
Real

- Rays pass through image
- Can be formed on a screen

Virtual

- Cannot be formed on a screen
- Rays appear to have come from image (but don't)

KYLIE GASPAR - Sep 21, 2020, 8:38 PM CDT



phsyics_202.JPG(96.9 KB) - download optics 101

KYLIE GASPAR - Sep 21, 2020, 9:11 PM CDT

Thin Lens Equations

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

f>0 converging

f<0 diverging

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

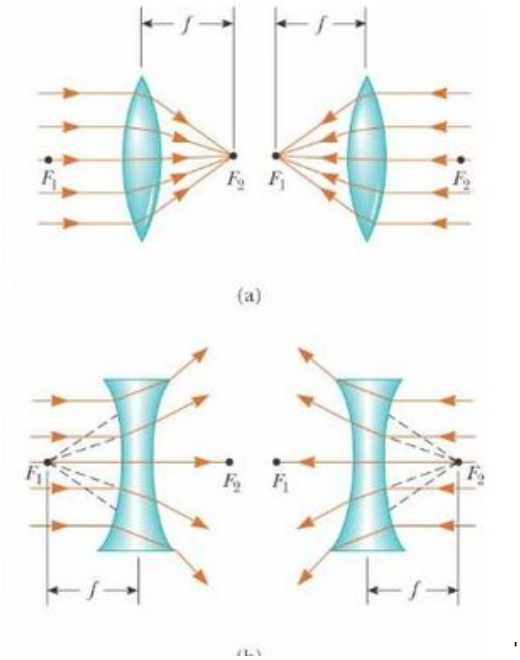
M= magnification f = focal length

di = image distance do = object distance

hi = image height ho=object height

R = radius of curvature

KYLIE GASPAR - Sep 21, 2020, 9:08 PM CDT

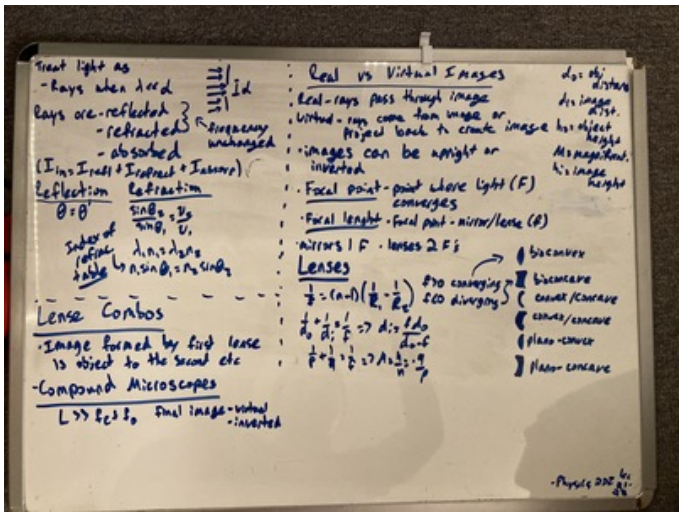


3002.JPG(25.2 KB) - download Top two converge Bottom two diverge

KYLIE GASPAR - Sep 21, 2020, 9:08 PM CDT

	>0 converging lens	<0 diverging lens
f	concave mirror	convex mirror
R	Center at image side	center on other side
do	object side	
di	real	virtual
M= -di/do	upright	inverted

KYLIE GASPAR - Sep 21, 2020, 9:07 PM CDT



IMG_0856.jpg(3 MB) - download Personal Notes summarizing the important aspects of optics in what pertains exclusively to understanding and solving our problem for phase microscopy.

KYLIE GASPAR - Sep 21, 2020, 9:19 PM CDT

Light and Interference

- Superpostion-when two waves meet the resulting wave is the sume of those two waves

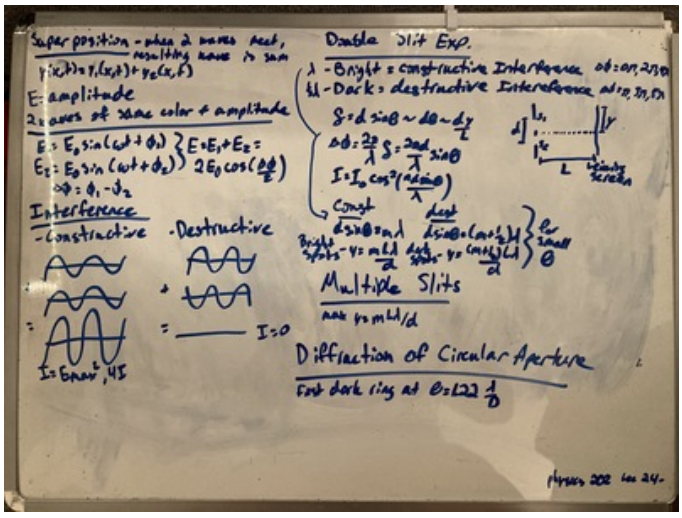
$$y(x,t) = y1(x,t) + y2(x,t)$$

- **Constructive** interference results in light spots
- **Destructive** interference results in dark spots

Diffraction from a Circular Aperture

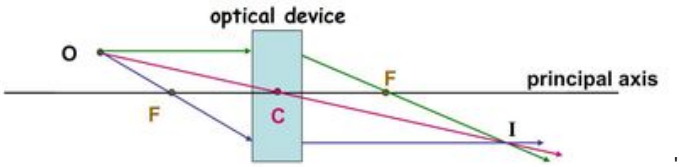
- The first dark ring at $\theta = 1.22 * \frac{\lambda}{D}$

KYLIE GASPAR - Sep 21, 2020, 9:09 PM CDT



IMG_0857.jpg(3.1 MB) - download Personal Notes summarizing the important aspects of optics in what pertains exclusively to understanding and solving our problem for phase microscopy.

KYLIE GASPAR - Sep 21, 2020, 9:20 PM CDT



physics_202_a.JPG(37.8 KB) - download optics 101

KYLIE GASPAR - Sep 21, 2020, 9:27 PM CDT

Conclusion/Action Items:

The lessons on constructive and destructive interference most likely does not specifically pertain to this project, but the concept of overlapping light is important in understanding how phase microscopy works.

When working with microscopy and/or multiple lenses, the image from the first lens (which can be found with equations) turns into the object of the following lens (which again can be applied to equations)

Next is to understand how this applies to condensers because the team has a relatively good understanding of the physics of the actual microscope. We need to apply how light interacts with the cells in the multi-well plates in order to effectively strategize design solutions.

2020/09/30-Professor Rogers lesson

KYLIE GASPAR - Sep 30, 2020, 4:01 PM CDT

Title: Notes from lesson with Professor Rogers

Date: 09/30/2020

Content by: Kylie Gaspar

Present: NA

Goals: Learn more and ask questions on optics

Content:

Questions prior to lesson

- what is the true purpose of the condenser?
- what does he think would help in our design? in his exp.?

Lesson

- Is everything aligned?
- illuminations should be focused axially
- look at Kohler Illuminations
    - Microscopy U
- Does the well-plate mess up the specs of microscope?
- Objective focus and illumination focus should meet
    - In pic of example of problem, the cells (objective) is focused but the illumination is not hence the ring

Conclusions/action items:

- **Questions**
    - why this model?
    - have you tired others?
    - can you focus the condenser?
        - if so, are you sure it's aligned with the objective?
    - Do the objective and illumination meet at the same plane?
- **To-Do**
    - Look up specifications/manual for microscope
- **Question for Saha**
    - **Can we focus on the mechanical aspect?**
    - **would it make sense to suggest a different microscope?**

2020/10/31-Phase Contrast 101-at home samples

KYLIE GASPAR - Oct 31, 2020, 11:02 PM CDT

Title: Phase Contrast 101- At home samples to mimic BrainXell's live neuronal cells

Date: 10/31/2020

Content by: Kylie Gaspar

Present: NA

Goals: See what can replace our current testing slides of cheek swabs in saline solution to more accurately mimic neuronal cells rooted on the bottom of the well plate.

Content:

The problem began with Ben not being able to mimic the poor phase contrast seen by our client, and we realized it was because he seeing almost no phase contrast at all (upon speaking to Mike Henrickson, client). it is becuase ceek swabs float in water, which makes them be in different planes. We need something to test that is clear, and at the bottom of the well. - the top of th ewell might be ok too as a last resort as long as the cells are all in the same plane of the well- plate.

Conclusions/action items:

KYLIE GASPAR - Dec 07, 2020, 9:48 PM CST

Much research for examples of a make home sample led to no findings. This is most likely because the concept of phase contrast imaging is both advanced and expensive. The advanced aspect leads to minimal "simple" tricks like a quick at home fix. And the expensive nature of the equipment means that it doesn't quite make sense to have a microscope of thousands of dollars and no access to samples. Luckily, our team has moved to live HEK cells for testing.

2020/11/12-Notes on HEK liver cells

KYLIE GASPAR - Dec 08, 2020, 2:40 PM CST

Title: Notes on HEK Liver Cells

Date: 11/12/2020

Content by: Kylie Gaspar

Present: NA

Goals: To take notes on HEK cells. Our team could not acquire live neuronal cells used by our client, so a student of our advisor was nice enough to plate some of the cells she is studying on our 96-well plate. The cells the student, Madeline Smerchansky, is studying are live HEK cells. My goal on this page is to take notes on the necessary safety and upkeep of these cells. As well as list the distinguishable differences and similarities to live neuronal cells.

Content:

"Of these, HEK 293 cells have become the mammalian cell line of choice for lab-scale protein production due to their ease of culture and high transfection effi ciency"

"Again, transient expression in HEK 293 cells offers a way of rapidly assessing the protein yield and quality. " - (Garcia-Fruitos)

Conclusions/action items:

Basically from reading what I found that HEK cells are easy to manufacture, keep and test on, this is definitely better than if we had to keep neuronal cells. They are dense however when they grow on a plate, this may be different from neuronal cells. We will have to see in our final testing results.

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

USED FOR REFERENCE- was mostly methods Penna., and MARTON. *CRAC Channel*. Springer New York, 2018.

2020/11/12-Old/Other Phase Contrast Designs

KYLIE GASPAR - Dec 08, 2020, 2:42 PM CST

Title: Other Phase Contrast mechanisms

Date: 11/12/2020

Content by: Kylie Gaspar

Present: NA

Goals: List other phase contrast designs, their pros and cons, mechanisms, and state yes/no for if the design could replace the condenser on the NIKON ECLIPSE

Content:

Shade-off is another very common optical artifact in phase contrast microscopy, and is often most easily observed in large, extended phase specimens. It would normally be expected that the image of a large phase specimen having a constant optical path length across the diameter would appear uniformly dark or light in the microscope. Unfortunately, the intensity of images produced by a phase contrast microscope does not always bear a simple linear relationship to the optical path difference produced by the specimen. Other factors, such as absorption at the phase plate and the amount of phase retardation or advancement, as well as the relative overlap size of the phase plate and condenser annulus also play a critical role. The intensity profile of a large, uniformly thick positive phase contrast specimen often gradually increases from the edges to the center, where the light intensity in the central region can approach that of the surrounding medium (the reverse is true for negative phase specimens). This effect is termed shade-off, and is frequently observed when examining extended planar specimens, such as material slabs (glass or mica), replicas, flattened tissue culture cells, and large organelles. - https://www.microscopyu.com/techniques/phase-contrast/introduction-to-phase-contrast-microscopy

Conclusions/action items:

2020/10/01-Misalignment solution

KYLIE GASPAR - Oct 01, 2020, 4:34 PM CDT

Title: Misalignment Solution

Date: 10/01/2020

Content by: Kylie Gaspar

Present: NA

Goals: Draw out a "simple" solution based on what Professor Rogers believes could be causing the problem of low resolution area in well plates using phase contrast microscopy.

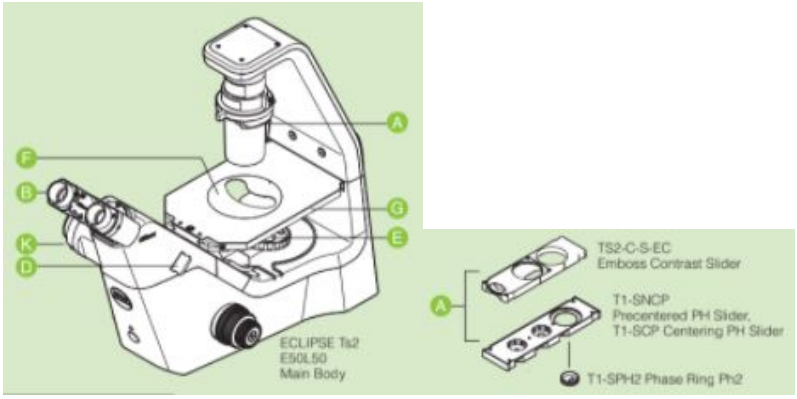
Content:

After speaking with Professor Rogers, he noted that microscopes are *theoretically* optimized by their manufacturers. I would like a second opinion to confirm this, but what I gathered from the meeting was that our client's problem with phase microscopy is not experienced by everyone if different equipment is used for phase contrast. This different equipment definitely includes a different microscope model and cell culture chambers. Rogers explained that typically just like an objective ifs focused, so are the condenser/illumination art when phase contrast is involved. There appears to be no focus knob from what we can tell in the product specification (there is no way to confirm this until Ben asks our client). This means that even if the product is marketed for a range of observation plates/well/etc, it cannot be optimized for all of them unless a knob exists to change the illumination to match the needs of each plate. (Again needing a second/third/fourth opinion on this) Rogers alluded to the fact that if the objective AND illumination are aligned properly, phase contrast will not have the problem our client is experiencing.

If this is the case there are ways to fix this. The goal is that the cone of illumination be "in focus" by meeting at the same spot the objective is focused on. Rogers told us that the cells are in view which means our objective was aligned to the well plate, but the illumination (black and while rings) is NOT aligned. To fix this we can simply keep the current condenser and find a way to move the objective as needed.

Conclusions/action items: MECHANICAL Design Solution

Do not change the condenser, but change 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.



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

Source

[1]"Eclipse Ts2", *Nikon Instruments Inc.*, 2020. [Online]. Available: https://www.microscope.healthcare.nikon.com/products/inverted-microscopes/eclipse-ts2. [Accessed: 01- Oct- 2020].

2020/10/06-Redesign approach

KYLIE GASPAR - Dec 07, 2020, 9:52 PM CST

Title: Redesign Approach

Date: 10/06/2020

Content by: Kylie Gaspar

Present: NA

Goals: Upload an idea I've had since the beginning of the project but for some reason haven't recorded

Content:

Design Idea

Using the lens equations from the Mechanics folder, reverse engineer the already existing lenses in the already existing condenser. We are far enough in this project now that I know that remaking a lens is too expensive and un-probable but if money and time didn't exist this is what I would do....

Use lens equations to figure out the curvature, thickness, and type of lens that gives an image area larger than what the client currently has. We would start by finding the area of a single well in a 96-well plate and then solve for the needed radius of curvature (R) of the lens. Then we would fabricate this lens and put in the condenser in place of the existing lens.

Conclusions/action items:

This design is above our level, budget, and resources. It also does not meet the marketability of the product that the client is looking for. We would need to outsource the creation of the lens to a company with adequate equipment to make a lens of the exact same quality as the existing lenses, otherwise the resolution of the image may go down.

2020/10/06-Midsemester Report

KYLIE GASPAR - Oct 06, 2020, 9:08 PM CDT

Title: Midsemester Report

Date: 10/06/2020

Content by: Kylie Gaspar

Present: NA

Goals: Type the Testing and Discussion section to be included in our Midsemester Report

Content:

Testing

- 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. We will measure and compare both areas of effective phase contrast (dark circles). We can 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 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.
- 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.

Discussion

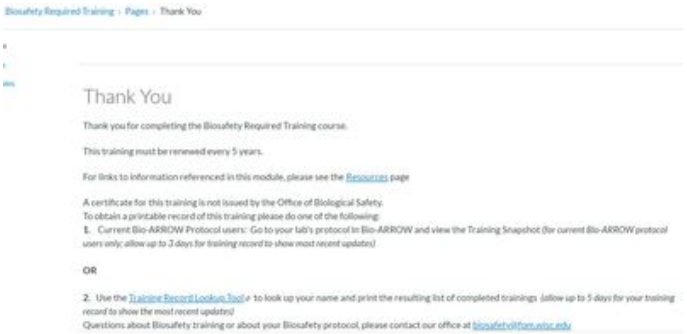
- Once the device is made and tested we have to see if it is logical to use our design over other existing phase microscope designs.
    - Another option for people with this problem may be to buy a better/different microscope that already exists
- We could also give what we found to Nikon to make suggestions on their design to maximize it.
    - With the fact we are working on a Nikon device, we have to be sympathetic and respectful to the company that did the tough work in making the entire inverted phase microscope.
- This issue has a broad application as live cultures and phase microscopy could become more selective if there is a better image under magnification.
- The use of this device will allow for more specific selection of differentiated stem cells, which will aide in research of central nervous system disorders and ultimately help people who suffer from central nervous system disorders.

Conclusions/action items:

Put this information into the midsemester report in a more formatted and descriptive manner, then let the team edit and make a few comments (just to get extra points of view and feedback).

Biosafety Training

KYLIE GASPAR - Sep 27, 2020, 5:29 PM CDT



Biosafety Required Training › Pages › Thank You

Thank You

Thank you for completing the Biosafety Required Training course.

This training must be renewed every 5 years.

For links to information referenced in this module, please see the Resources page

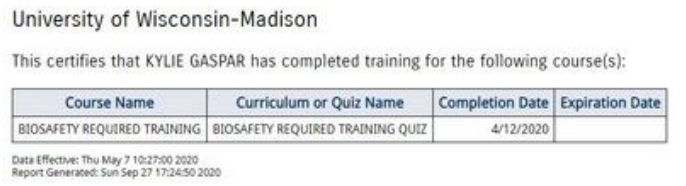
A certificate for this training is not issued by the Office of Biological Safety.
To obtain a printable record of this training please do one of the following:
1. Current Bio-ARROW Protocol users: Go to your lab's protocol in Bio-ARROW and view the Training Snapshot (for current Bio-ARROW protocol users only; allow up to 3 days for training record to show most recent updates)

OR

2. Use the Training Record Lookup Tool to look up your name and print the resulting list of completed trainings (allow up to 5 days for your training record to show the most recent updates)
Questions about Biosafety training or about your Biosafety protocol, please contact our office at biosafety@bse.wisc.edu

biosafety_training.JPG(134.6 KB) - download

KYLIE GASPAR - Sep 27, 2020, 5:30 PM CDT



University of Wisconsin-Madison

This certifies that KYLIE GASPAR has completed training for the following course(s):

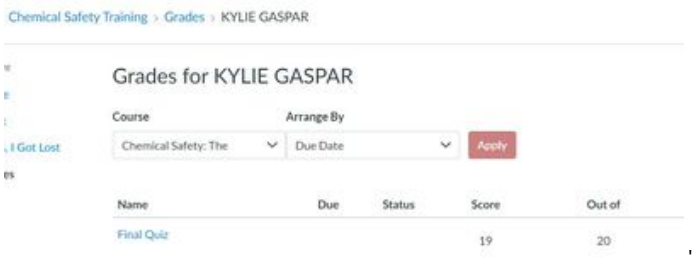
Course Name	Curriculum or Quiz Name	Completion Date	Expiration Date
BIOSAFETY REQUIRED TRAINING	BIOSAFETY REQUIRED TRAINING QUIZ	4/12/2020	

Data Effective: Thu May 7 10:27:00 2020
Report Generated: Sun Sep 27 17:24:50 2020

biosafety_training_2.JPG(46.7 KB) - download

Chemical Safety Training

KYLIE GASPAR - Oct 07, 2020, 10:27 PM CDT



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2020/10/29-First steps of Fabircation-3D printing

KYLIE GASPAR - Oct 30, 2020, 12:05 PM CDT

Title: Compiling Check List for Fabrication

Date: 10/29/2020

Content by: Kylie Gaspar

Present: NA

Goals: Combine all pieces of check list (below) to have a detailed fabrication plan. And be ready to order materials and 3D print from the Makerspace.

Content:

- Ultimaker PVA and breakaway are support if we need them for the Extra Lens holder
- Ultimaker Tough PLA- comes in black, stiff, tough, SDS downloaded on personal computer.
    - 8 cents/G
    - **$1.30 for annulus**
    - **$2.72 for lenses super structure**
- Ultimaker PC is non-transparent
    - 12cents/G
- Formlabs Black
    - Opaque and Black
    - 24cents/mL

Conclusions/action items:

Next steps, send in these orders to the Makerspace for the 3D printed structures. Continue research on where to buy the lenses for the superstructure.

KYLIE GASPAR - Oct 29, 2020, 4:28 PM CDT

Fabrication

1. Measurements of Nikon ts2
2. Places to buy from
    1. List of products needed

Part	Purpose	Where to Obtain	Cost	Links
Adjusted Annulus	Allowing more light into the microscope and pairing with the extra lens to extend the focused might	Makerspace	Varies https://making.engr.wisc.edu/3d-printers/3dprint-cost/	https://making.engr.wisc.edu
Extra set of objectives lens	Focus light that is supplied by the adjusted annulus	Unitron		https://microscopes.unitronusa.com/lx-microscopes-by-unitron/parts-accessories/objectives.html
Opaque 96 wells plates	Displays specimen	Pippet.com	~$35	https://www.pipette.com/SSI-Bio-96-Well-PCR-Plate
Adjusting tool	Shifting objectives lens to focus lens to different well plates Screw and Knob Adjustments	Thor Labs		https://www.youtube.com/watch?v=CCO9Z7-ufbg

3. Type of plastic for holder
    1. The standard for condenser annulus is an opaque material
    2. Using the Formlabs 3D printer we could use Black which is sturdy, black, and a melting point
        1. Pricing is $0.24 per gram
    3. Using the Ultimaker 3D printer we could use Tough PLA which is sturdy, high melting point, black material

2. Pricing is $0.08 per gram

4. <u>Process of completing it</u>

    1. Obtain Measurements of model Ts2 Microscope
    2. Use measurements to calculate the optimal lens placement
    3. Fabricate Annulus Adjustment Piece
    4. Prototype both objective lens/annulus adjustment and adjusting knob
    5. Test both designs by taking images and use image analysis to obtain qualitative and quantitative results

Content by Team- The check list was made as four things (underlined) we needed to know in order to fabricate our prototypes. Notes were taken underneath.

2020/12/07-2nd Round of 3D Printing

KYLIE GASPAR - Dec 07, 2020, 11:08 PM CST

Title: 3D Printing

Date: 12/07/2020

Content by: Kylie Gaspar

Present: NA

Goals: Put the information from the print into this document. It is of important note that the first round of printing was done by Ben H.

Content:

I met virtually with the makerspace on 11/30 at 9am to print a new lens holder. We could not find the original that Ben printed, so I inquired about it during my appointment and the worker did find it in a bin by searching for Ben's name and by looking at the file I was ready to print. I decided to print a second holder with the same STL file Ben used, but a different print orientation to minimize filament/support on the inside near where the lenses sit and the light goes through. This way we could have two option just incase. I also printed a new annulus with a 35% light area increase for more testing to see if this was better or worse than the 25%. I decided to print both designs on the same plate and with a Form2 Black print. The same as the 25% annulus print. ***The total came out to $20.44***, I paid by Wiscard after adding $30 to my account to hopefully cover the amount of the print. The print was ready Tuesday (12/1) around 9am, so less than a 24 hour turn around time. Then the prints were picked up by me and all three (35% annulus and 2 lens holders) were left at Ben's house for testing.

Conclusions/action items:

Now we have *four 3D prints*, and two lenses for our project.

- *25% Annulus* and *Lens Holder* (gone missing, found, and used for testing)- Printed by Ben
- Concave and Convex lenses-paid for by client, mailed to Ben's house
- *35% Annulus* and *backup Lens Holder* (used with Black Form2 material, was not tested on)-Printed by Kylie, outlined in this document.

KYLIE GASPAR - Dec 07, 2020, 11:00 PM CST

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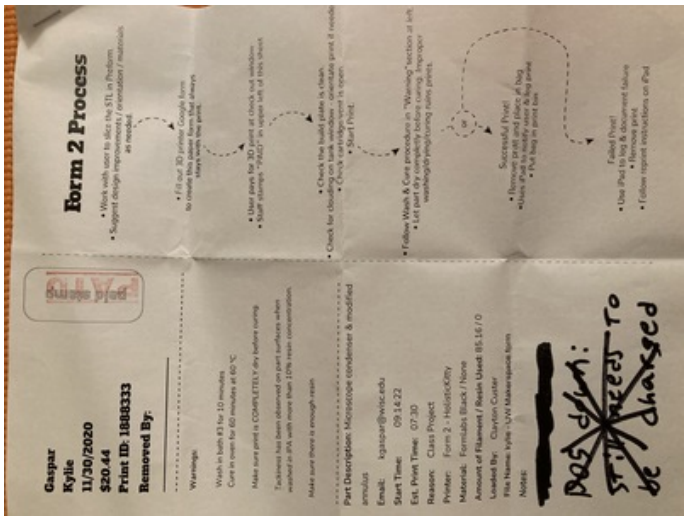
KYLIE GASPAR - Dec 07, 2020, 11:00 PM CST



IMG_1293b.jpg(2.7 MB) - download

Week of 09/27/2020

KYLIE GASPAR - Oct 01, 2020, 4:41 PM CDT

Title: Weekly Contribution Report

Date: 09/27/2020-

Content by: Kylie Gaspar

Present: NA

Goals: Illustrate summary of project accomplishments for this week.

Content:

We needed to expand on our design matrix so I explained all the design criteria:

Effective area seen by phase contrast and resolution

These criteria were chosen to be the same weight because both are an integral part of inspecting a sample effectively. While our main goal in this project is to expand the area of high resolution phase contrast, it is equally important that we don't sacrifice too much resolution. High resolution is difficult to obtain but equally as important in assessing cell cultures.

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.

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.

Complexity

Complexity 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.

Safety

Safety is the least important criteria because we are following FDA approved products as our inspiration.

Conclusions/action items:

This is all on a shared google document for the team to see, and eventually be turned into a deliverable. All the paragraphs need to be concluded with what design won the specific criteria.

KYLIE GASPAR - Oct 01, 2020, 4:49 PM CDT

Contribution to Presentation

I can see that my team has trouble summarizing in a concise manner, so in addition to the slide summaries I will put below, I also helped all of them make their slides less wordy.

Overview Slide

Who is BrainXell?

- Founded in 2015 by Prof. Su-Chun Zhang
- Boutique Biotech company
- High purity neuron types
- Phase Contrast Microscopy

The Problem with Phase Contrast Microscopy

High resolution: Low area

- Universal problem with well plates
- Must expand area of contrast
- Must keep high resolution
- Need an easy-to-change component
- Adaptable to existing equipment

Design Matrix Slide

Future works

- Confirm the cause of the problem
- Fabricate prototypes
- Test attachments
- Client Feedback
- Revise

Acknowledgements Slide

Week of 10/04/2020

KYLIE GASPAR - Oct 06, 2020, 7:02 PM CDT

Title: Weekly Contribution Report (half a week)

Date: 10/04/2020-10/06/2020

Content by: Kylie Gaspar

Present: NA

Goals: Display contribution to project prototype

Content:

This week I have been working on the Chemical Safety Training. After that is complete I will sign up for a time slot to use the teaching lab to play around with the inverted microscope they have. Due to other deadlines, I will finish the last two Canvas modules for this training tomorrow, (October 7th).

In relation to our report, I assigned the team parts to fill out based on evenly distributing the work load. I met individually with Katie, who missed our team meeting, to discuss logistics and answer any of her questions. I wrote two sections of the report which you can find under Design Ideas>2020/10/06-Midsemester Report. I also edited and formatted the entire document for submission.

Conclusions/action items:

My next steps are

- to take notes on the microscope in the teaching lab
    - report those to the team
- Make a detailed fabrication plan with numbers to prove the area of phase contrast WILL expand with our designs
- Discuss with the client and our advisor (Saha) to find a solution that they like better (because Mr. Hendrickson didn't seem to approve 2/4 of our ideas).
    - Basically just want confirmation we're on an OK path

Week of 10/18/2020

KYLIE GASPAR - Dec 07, 2020, 10:07 PM CST

Title: Weekly Contribution report

Date: 10/24/2020

Content by: Kylie Gaspar

Present: NA

Goals: Weekly contribution summary

Content:

This week I arranged a pick up of an inverted phase microscope from one of our client's friends, Joe Beck of Blackstar Assets. He loaned us the microscope as he is waiting for a buyer to buy it on Ebay, in the mean time our team will adjust our measurements to be in accordance with this Fischer microscope. After I picked up the microscope and thanked Mr. Beck, I dropped off the equipment at Ben Hildebrandt's house for use by him.

Conclusions/action items:

Contact info:

joesph.d.beck@gmail.com

1(608)320-9143

Week of 10/25/2020

KYLIE GASPAR - Dec 07, 2020, 10:20 PM CST

Title: Weekly Contribution Report

Date: 10/31/2020

Content by: Kylie Gaspar

Present: NA

Goals: Summary of weekly work

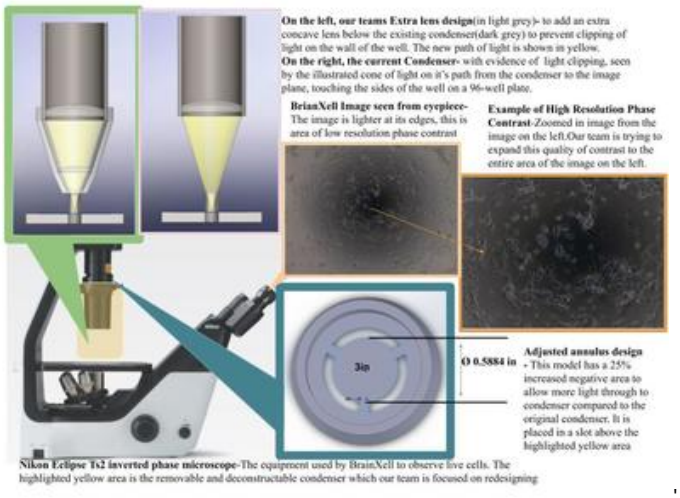
Content:

This week on Tuesday (10/27), we had a meeting with Mr. Hendrickson because Ben, who has the microscope, could not recreate the same images of dark circles (phase contrast) in the center and light rings on the outside. I took notes of the meeting, which I documented in the Team Folder under Client Meetings. After this meeting, I looked into better samples for at home testing than simple cheek swabs. I also helped finish the final design so that we can move forward with fabrication plans. I also made a list of different costs of materials, which can be seen in my Fabrication folder. To prepare for the Piazza post, I drafted a summery and made a visual graphic (seen below).

Conclusions/action items:

Figure out specific fabrication costs, dates, methods. As well as what to do about samples to put in the well-plates

KYLIE GASPAR - Dec 07, 2020, 10:20 PM CST



Elevator_Pitch_Graphic_3_.jpg(113.5 KB) - download

2020/11/01-Tuesday Team Meeting Plan

KYLIE GASPAR - Nov 01, 2020, 7:27 PM CST

Title: Team Meeting Plan for Tuesday (11/3)

Date: 11/01/2020

Content by: Kylie Gaspar

Present: NA

Goals: Organize meeting and work to be done for this week-next week.

Content:

To do (independently)

- Literature Research
    - Two options for at home slides that mimic live neuronal cells, must be rooted at bottom of well
    - Everyone else should find 2 articles (hopefully semi-different perspectives) on ***literature*** exploring
        - This problem
        - Other, non-conventional, phase contrast techniques
        - Imaging in general
- We need a plan on how we can fund 3D printing
- We need to find a SPECIFIC lens and WHERE to order it from
    - client may order directly
- Kylie + Ben + any volunteers to do math?
- After Friday-everyone read comments on our Piazza post and take notes on what they said, and ideas you have that came from reading everything
- We need to start getting quantitative numbers on the old design and our testing-areas
    - stats will be done on this

Meeting order

1. Printing
2. Lens
    1. Math
    2. Where can we get it
3. Quantitative numbers-testing areas
4. Literature research-can spend time doing some of this together
5. Piazza

Conclusions/action items:

Have this meeting with the team on Tuesday

Week of 11/01/2020

KYLIE GASPAR - Dec 07, 2020, 10:28 PM CST

Title: Weekly Contribution Report

Date: 11/06/2020

Content by: Kylie Gaspar

Present: NA

Goals: Describe weekly contributions

Content:

There is a lot to be done in different areas, so I made a meeting plan, seen in the entry above this one. Since we now have access to live cells, thanks to Madeline Smerchansky, I did research on what we need to know about HEK cells, the cells Madeline is plating for us.

Conclusions/action items:

Wait for cells to be plated and parts to be picked up from the Makerspace

Week ok 11/08/2020

KYLIE GASPAR - Dec 07, 2020, 10:34 PM CST

Title: Weekly Contribution Report

Date: 11/13/2020

Content by: Kylie Gaspar

Present: NA

Goals: Describe weekly contribution

Content:

I did research on other methods of phase contrast imaging to look for alternate design ideas, they all seem counterintuitve or uncomplete-able at this stage in the course. I finished my notes on HEK cells. I also was supposed to pick up the cells from Madeline, today, then go test on them with Ben, but they weren't left to adhere long enough, so we will hope they are ready for pick up on Monday. I also watched the Tong Lecture on the class Zoom.

Conclusions/action items:

smerchansky@wisc.edu

Week of 11/15/2020

KYLIE GASPAR - Dec 07, 2020, 10:49 PM CST

Title: Weekly Contribution Report

Date: 11/20/2020

Content by: Kylie Gaspar

Present: NA

Goals: TEST THIS WEEK

Content:

I planned a space and time for our team to test on Friday, in Engineering hall, however our lens holder design has yet to be printed so we decided to wait until Saturday morning (11/21). In the meantime, I picked up more cells from Madeline from the WID building on Friday (11/20), these cells were better plated according to her.

Conclusions/action items:

TEST

Week of 11/22/2020-Thanksgiving week

KYLIE GASPAR - Dec 07, 2020, 10:48 PM CST

Title: Weekly Contribution Report

Date: 11/04/2020

Content by: Kylie Gaspar

Present: NA

Goals: Cover content from 11/22-11/4

Content:

I had various email exchanges trying to located the lens holder print from Ben. I made a detailed testing plan, I met with a makerspace employee and printed the 35% annulus and a new lens holder. I picked those designs, as well as Ben's first lens holder print up on Tuesday 12/1. I dropped these off at ben's house. I also made an outline for the final poster, and final report. I assigned sections to write for the Report and did MATLAB analysis of the data Ben collected.

Conclusions/action items:

Wrap it all up in the report and presentation, all that's left is to fill out evaluations.

*Every week previous, and this week included, I created the weekly progress report. Filled out all the team sections for the Progress Report. I set up team meeting times, usually on Monday or Tuesday, reminded the team of Advisor and client meeting. I also updated the team every 2-4 days on the project's progress, what they needed to do, and what they could expect from the upcoming week, this was all done in GroupMe.

2020/09/27-LECTURE_Oral Presentations

KYLIE GASPAR - Sep 27, 2020, 5:45 PM CDT

Title: Oral Presentations Lecture

Date: 09/27/2020

Content by: Kylie Gaspar

Present: NA

Goals: Take notes on "Oral Presentations" by Professor Brenda Ogle in order to apply the information to the teams preliminary presentation due on 10/02/2020

Content:

- Be Specific and informative for outline
    - with key points
        - these key points become slide titles
- Write in point format, at most 6 points and 6 words/point
- No smaller than 18 point font
- Bigger fonts for titles/topics
- use color
- Proof read slides
- Practice
- Convey enough detail to show mastery but in an easily understandable way

Conclusions/action items:

Use this information to create a preliminary presentation outline for the team

Source: https://bmedesign.engr.wisc.edu/course/topics/communication/oral_presentations

2020/09/12 Phase Microscopy

Ben Hildebrandt - Sep 14, 2020, 12:56 PM CDT

Title: Microscope Parts

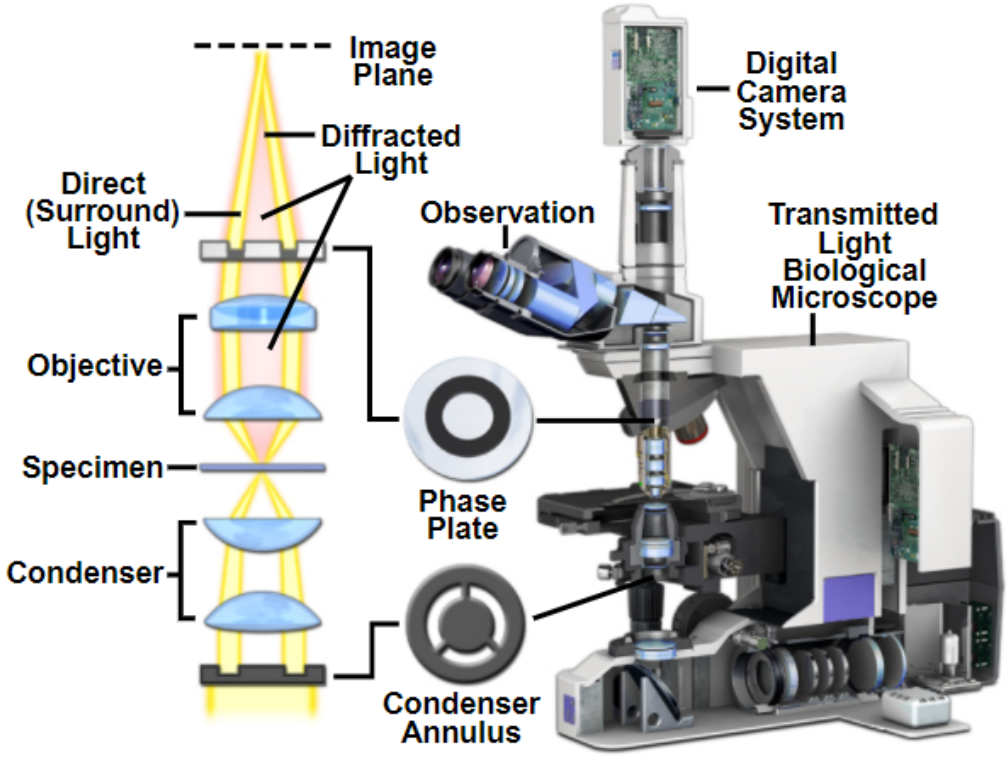
Date: 9/12/2020

Content by: Ben Hildebrandt

Present: N/A

Goals: Become better acquainted with the components of a phase contrast microscope

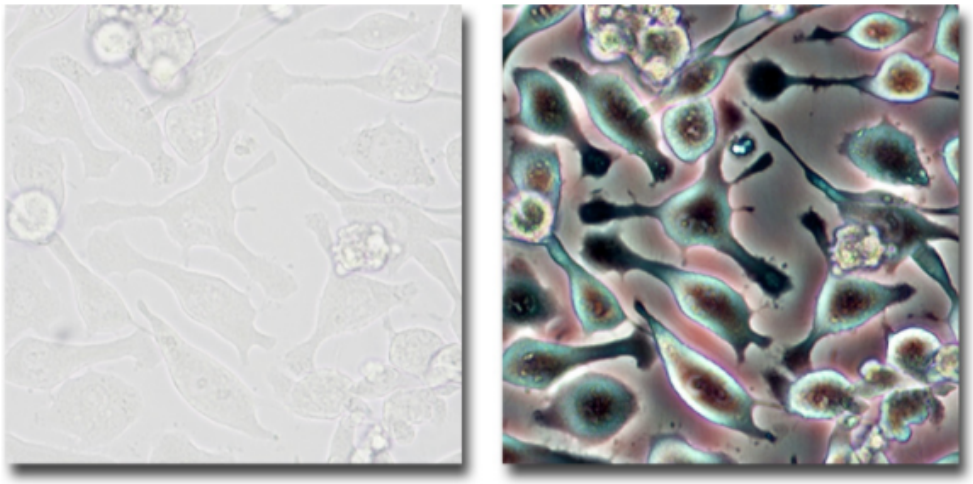
Content:



Shown above is a typical phase contrast microscope, with the different components shown. The part of the microscope that we will be working with is the condenser. We want to modify that part to allow the viewing of multiple specimens at once.

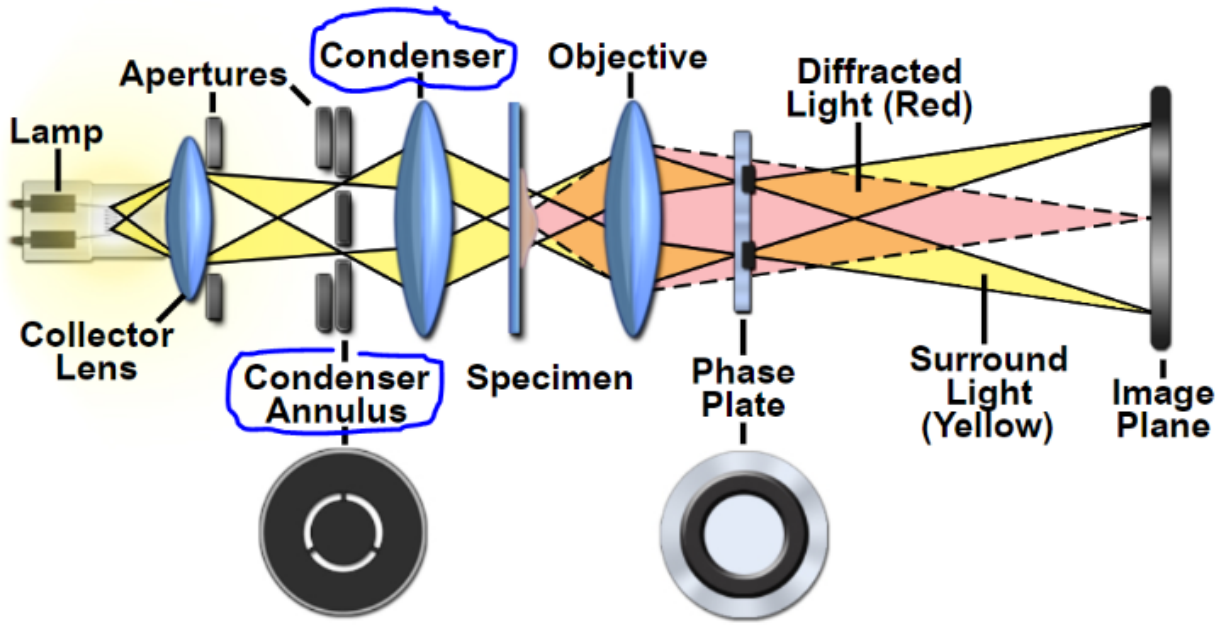
The main advantage of the phase contrast microscope is that it produces high-contrast images of transparent specimens, such as cells. Typically it would require dyes or death of the cell to view, but the phase contrast microscope allows the user to view living cells in a high contrast image.

Figure 2 - Living Cells in Brightfield and Phase Contrast



Above is an example of transparent cells in a light-field microscope (a) compared to a phase contrast microscope (b).

Figure 4 - Phase Contrast Microscope Optical Train



Above is the optical train of a phase contrast microscope. The condenser and condenser annulus are located directly below the specimen and server the function of redirecting the light into the specimen to be phase-shifted.

The purpose of the condenser annulus is as follows (from MicroscopyU, an Introduction to Phase Contrast Microscopy)

"The **condenser annulus** (illustrated in Figure 1 and Figure 4) is typically constructed as an opaque flat-black (light absorbing) plate with a transparent annular ring, which is positioned in the front focal plane (aperture) of the condenser so the specimen can be illuminated by defocused, parallel light wavefronts emanating from the ring. The microscope **condenser** images the annular diaphragm at infinity, while the objective produces an image at the rear focal plane."

Conclusions/action items:

2020/09/17 Calculating Resolution

Ben Hildebrandt - Sep 17, 2020, 10:25 AM CDT

Title: Calculating Resolution

Date: 9/17/2020

Content by: Ben Hildebrandt

Present: N/A

Goals: For the PDS, the resolution should remain the same, and needs to be quantified.

Content:

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

Conclusions/action items:

We want to maintain these resolutions within 25%.

2020/09/16 Safety Standards

Ben Hildebrandt - Sep 16, 2020, 9:13 PM CDT

Title: Safety Standards

Date: 06/16/2020

Content by: Ben Hildebrandt

Present: N/A

Goals: Research standard safety specifications for microscopes condensers and enter then into the PDS.

Content:

According to ISO 10934:2020 standards on Microscopes, the phase contrast condenser is listed as:

3.1.28.6

phase-contrast condenser

condenser (3.1.28) designed for **phase contrast (3.1.32.4)** microscopy which forms on the **phase plate (3.1.112)** in the **back focal plane (3.1.62.1)** of the **objective (3.1.106)** a suitably sized **image (3.1.75)** of a **diaphragm (3.1.38)** (generally annular) positioned in the **front focal plane (3.1.62.2)** of the *condenser*

This designates the phase-contrast condenser as term 3.1.28.6. I believe this may be able to be searched for in ISO standards to find standards pertaining to phase-contrast condensers.

Conclusions/action items:

This search led to nowhere, as the ISO 10934 standards do not specify anything for the condenser itself, but rather defines it. Consecutive searches yielded no results for "phase contrast condenser" in any other ISO documentation.

2020/09/16 Thermal Safety

Ben Hildebrandt - Sep 16, 2020, 9:16 PM CDT

Title: Thermal Safety

Date: 16/06/2020

Content by: Ben Hildebrandt

Present: N/A

Goals: Find information on acceptable temperature thresholds to prevent burning.

Content:

Tried to find reliable source. Many articles (including wikipedia) listed 44 degrees Celsius as the lower threshold of burning temperatures, but I couldn't confirm it with a scholarly article/journal.

Discovered the following article:

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].

This article tests and proves 44 degrees celsius as the lower threshold for burning human skin.

Conclusions/action items:

Content for PDS: "The condenser surface cannot exceed 44℃ for risk of thermal burning."

2020/09/17 Performance Requirements

Ben Hildebrandt - Sep 17, 2020, 9:51 AM CDT

Title: Performance Requirements

Date: 17/09/2020

Content by: Ben Hildebrandt

Present: N/A

Goals: To quantify the requirements of this project and establish numeric constraints.

Content:

The client, Mr Mike Hendrickson, was contacted regarding six questions to further quantify the project:

1. What specific microscope will we be designing this product for, and what specification of condenser is it set to replace?
2. What made/model of condensers do you currently use?
3. Is there a measurable resolution or field size that we are aiming for?
4. Are there any international standards pertaining to phase-contrast microscopy that you're aware of, or that you may have access to?
5. Does your lab have to abide by any international safety standards that our product needs to meet?
6. What is the formal budget of this project?

The response was as follows:

1. We have a Nikon ECLIPSE Ts2 (https://www.nikon.com/products/microscope-solutions/lineup/inverted/ts2/index.htm). You can download the brochure from that site, which has some technical specifications and drawings at the back. However, if you have access to a similar microscope, you should consider designing for it because that will just be easier, especially with COVID. In general, these instruments are very similar across manufacturers.
2. I think we have the one that comes with it: ELWD Condenser (NA 0.3, W.D. 75 mm)
    1. ELWD = extremely long working distance
    2. NA = numerical aperture
    3. WD = working distance, which is the distance from the front-most optical element to the focal plane
3. The field of view should match the entire optical system such that the eyepieces (or camera port) are filled with an image. The lateral resolution (X and Y) is given by this equation: d = 1.22λ /(NA-Objective + NA-Condenser)
    1. 1.22 is a factor derived from Rayleigh criterion concerning when two closely spaced objects can be resolved as not being a single object
        i. You have no control over this
    2. λ Is the wavelength of light (or the average wavelength) used for illumination
        i. You have no control over this (in this scenario)
    3. NA-Objective is the numerical of the objective
        i. You can look at available phase objectives (https://www.nikon.com/products/microscope-solutions/lineup/objectives/)
        ii. You could build your own, but this would not be trivial, and I'm not sure it would help here
        iii. I would suggest working with whatever is available
    4. NA-Condenser
        i. We're trying to keep this as high as possible
4. There are objective standards now, but I'm not sure about condensers. I would look at similar microscopes and their condensers from Olympus, Zeiss, and Leica.
5. No issue here.
6. I can spend $1,500 with additional approval, but potential more if needed.

I think your main constraint would related to the area of the bottom of a well (in a 96-well plate with opaque well walls) that can be properly view with phase microscopy. My guess is that it is currently <25% (maybe even <10%.) A good goal might be >75%.

Important information gathered from this response:

- We now know what condenser they currently use and how resolution is measured.
- We now know the goal of the project is to increase the area of the 96 well plate that can be properly viewed with phase microscopy (currently <25%, >75% is the goal).
- Budget is defined at $1500

Still confused about several things:

- There is no discernible documentation for the condenser they currently use (dimensions, weight, etc)
- The actual solution to the problem seems outside our realm of experience, since it will likely require detailed optics theory.
- There doesn't seem to be a standard Objective lens they use. This could be asked in a follow-up question.

Conclusions/action items:

Filled in respective sections in the PDS that needed quantifiable data.

2020/09/23 Extra Lenses Design

Ben Hildebrandt - Oct 05, 2020, 7:27 PM CDT

Title: Extra Lenses Design

Date: 9/23/2020

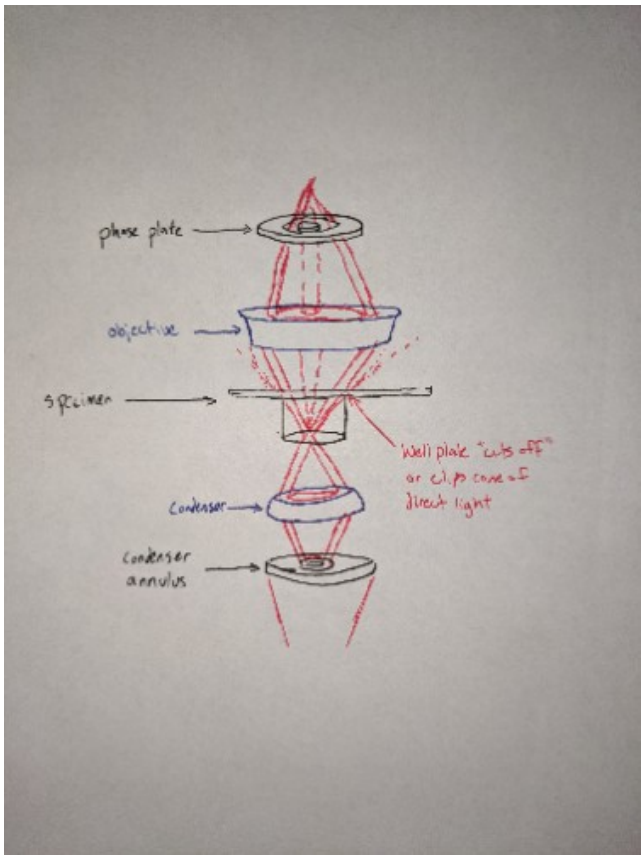
Content by: Ben Hildebrandt

Present: N/A

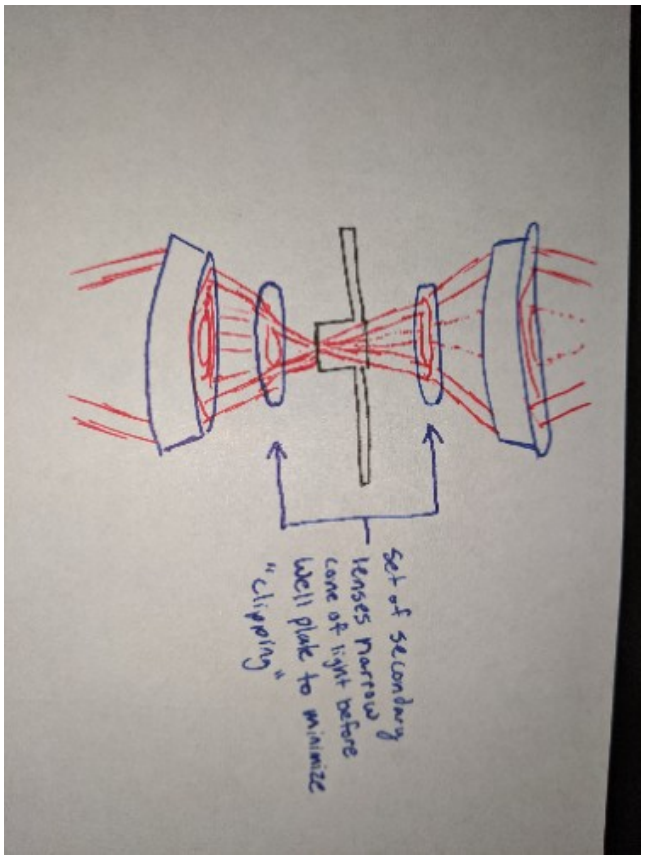
Goals: Describe the solution of using an extra set of lenses to narrow the cone of light.

Content:

As described by several microscopy forums and Prof. Rogers who we have collaborated with on microscopy education, the main issue we are dealing with is the "clipping" of the cone of light. In the below image, the "clipped" portions of the cone of light are shown as dotted lines. This decreases the amount of "background light" on the image, which theoretically could be the culprit of the decreased contrast in the outer edges of the image.



The "extra lenses" design addresses this issue by introducing a second lens in front of both the objective and condensing lens. As seen in the image below, the theory behind this design would be to narrow the cone of light BEFORE it enters the well plate to eliminate any "clipping." However, this would require some complex optical physics to be calculated to find the perfect lenses for this application. Ideally, we'd like to use standard lenses (as these are cheaper), which may or may not be possible with the specific orientation of the specimen.



Conclusions/action items:

While the theory behind this design checks out, it may seem more difficult to manufacture in practice. It would likely require somewhat custom lenses and very precise measurements in the light train, or else these lenses could massively disrupt or distort the final image.

2020/10/02 Weekly Contributions

Ben Hildebrandt - Sep 30, 2020, 11:19 AM CDT

Title: Weekly Contributions

Date: 9/30/2020

Content by: Ben Hildebrandt

Present: N/A

Goals: Succinctly describe what I did for the group this week.

Content:

This week was largely busy for me, so I wasn't able to donate as much time as I would've liked to the group. I participated in all the team meetings and finished slides in the presentation, design matrix paragraphs, and did my part for the presentation. However, as for individual research, I didn't do much as a result of having two exams this week to prepare for.

This week as communicator, I fulfilled my responsibilities of keeping in contact with the client and reaching out to possible sources for a lesson in optics. The person I reached out to was Prof. Rogers (who I currently have for statistics). He mentioned in class that he really enjoys microscopy and specifically mentioned phase contrast. He agreed to meet with us for a quick "lesson" and Q&A session about phase contrast.

As for gaining access to microscopes, it looks as if the labs our team members work for are mostly full. This is problematic, as our only hope is to be able to get into the teaching lab in ECB. This could prove to be difficult, since it is in high demand and COVID restrictions make that much more difficult.

Conclusions/action items:

Some goals for next week include fleshing out my design idea of additional lenses a bit more. I want to be able to design a superstructure to house them. However, this would require some math on what lenses we need to buy and some measurements of the Nikon ECLIPSE microscope. Both of these goals should be possible, as I have a significantly less busy week next week.

2020/10/05 Weekly Contributions

Ben Hildebrandt - Oct 05, 2020, 7:47 PM CDT

Title: Weekly Contributions

Date: 10/5/2020

Content by: Ben Hildebrandt

Present: N/A

Goals: Succinctly describe what I did for the group this week.

Content:

This week I was focused largely on further researching the "mechanical focus" idea that we formulated with Prof. Rogers last week. I discovered that there is no discernible focus knob for the condenser on an Eclipse Ts2, so there could be an issue with properly focusing the condenser on the well plate. This opens up a strictly mechanical solution opportunity that wouldn't necessarily require as much optical knowledge. We want to minimize the areas where we don't have expertise, but we all have experience designing a mechanical device for vertical movement.

Conclusions/action items:

Some goals for next week include CAD-ing up some potential design ideas for the new mechanical condenser focus. This idea has a lot of potential, so I believe this is important to get a fully-realized three dimensional design established.

2020/10/24 - Weekly Contributions

Ben Hildebrandt - Dec 08, 2020, 12:22 PM CST

Title: Weekly Contribution report

Date: 10/24/2020

Content by: Ben Hildebrandt

Present: NA

Goals: Weekly contribution summary

Content:

I picked up the microscope that Kylie got us access to and set it up in my room. I researched how to use the microscope that we have access to to try and recreate the issue that they are seeing. I also experimented with adjusting the condenser and objective lenses for kohler illumination. I was unsuccessful in recreating the issue they were seeing by using the default annulus and condenser.

Conclusions/action items:

My goal for next week is to show the team the knowledge I've gained from experimenting with the microscope. I hope to have workable images that prove that I recreated the problem successfully, but this may seem difficult with the experimenting I've been doing so far.

2020/10/30 - Weekly Contributions

Ben Hildebrandt - Dec 08, 2020, 12:28 PM CST

Title: Weekly Contributions

Date: 10/30/2020

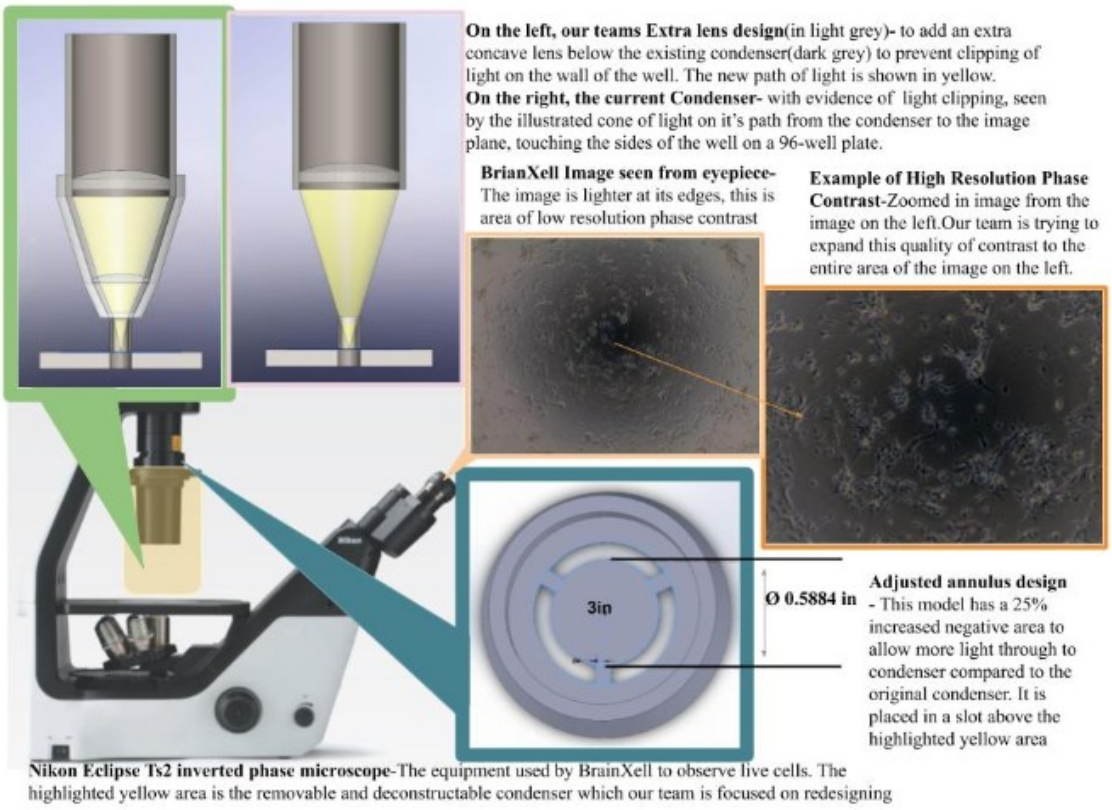
Content by: Ben Hildebrandt

Present: NA

Goals: Summary of weekly work

Content:

This week I helped to set up and attended a meeting with the client to ask questions, discuss our design ideas, and ask for help on setting up our loaned microscope. I also created two CAD models and did the necessary calculations to optimize each one to best simulate what our extra lenses design would do to the cone of light. The models were for our discussion post that we posted on Piazza this week, shown below:



Conclusions/action items:

An individual goal I have for this upcoming week will be to finalize the measurements for the extra lenses design based on commercially available lenses we can purchase. Our discussion post isn't getting very much attention. This is probably resultant of the confusing nature of the problem. We probably need to learn how to explain the project in general.

2020/11/06 - Weekly Contributions

Ben Hildebrandt - Dec 08, 2020, 12:33 PM CST

Title: Weekly Contributions

Date: 11/06/2020

Content by: Ben Hildebrandt

Present: N/A

Goals: Record weekly contributions

Content:

This week, I prepared the condenser annulus for printing and looked into some standard lenses that would be used for the extra lenses design. I also communicated with the client to finalize the payment methods for parts and printing. Essentially, the plan will be to use his credit card for the online purchases, and he will pay me back for the Makerspace, since they only take Wiscard payment.

Conclusions/action items:

My goal for the end of this week already is to have tangible printed parts in several different materials. This is done over the internet at the Makerspace. I believe it will also be possible to purchase lenses from Edmund Optics soon, I just need to do the math to figure out which sizes will work best. Once those measurements are finalized, I can design the finalized superstructure for the extra lenses design.

2020/11/12 - Weekly Contributions

Ben Hildebrandt - Dec 08, 2020, 12:36 PM CST

Title: Weekly Contribution Report

Date: 11/12/2020

Content by: Ben Hildebrandt

Present: N/A

Goals: Record weekly contributions

Content:

This week I met with a lab clinician named Madeline Smerchansky (smerchansky@wisc.edu) to coordinate the growth and fixation of Human Embryonic Kidney (HEK) cells in well plates for testing purposes. I also met with the group to discuss lens purchases. I performed calculations to find the correct lenses and coordinated with the client to purchase them. I also watched the Tong Lecture on the class Zoom.

Conclusions/action items:

Next week I want to CAD the final design to house the lenses and begin testing on the printed annulus prototypes. I don’t predict much success with these designs, but having actual cultures to view will be incredibly helpful. As for the extra lenses design, I plan to CAD the superstructure soon, now that I have the lenses we will be using.

2020/11/20 - Weekly Contributions

Ben Hildebrandt - Dec 08, 2020, 12:50 PM CST

Title: Weekly Contribution Report

Date: 11/20/2020

Content by: Ben Hildebrandt

Present: N/A

Goals: Record weekly contributions

Content:

I design the final superstructure for the extra lenses design, and met with the makerspace to print it out of Flexible resin. They said it would take about 26 hours to print, so it should be ready for the testing Kylie set time aside for this Saturday. Unfortunately, we had to postpone testing from Friday afternoon, since the part wasn't ready yet.

Conclusions/action items:

The highest priority is to assemble and test the final prototype. This will be done by this coming weekend and testing/documentation will be taking place up until thanksgiving break.

2020/11/25 - Weekly Contributions (Thanksgiving week)

Ben Hildebrandt - Dec 08, 2020, 12:58 PM CST

Title: Weekly Contributions (Thanksgiving Week)

Date: 11/25/2020

Content by: Ben Hildebrandt

Present: NA

Goals: Record weekly contributions.

Content:

This week, I continued to prepare our testing setup by taking test images, looking at all the wellplates with cells to find the best places to take pictures, and doing some preliminary testing to ensure the quality of the testing protocol. This week was quite stressful, since I had a few midterms to study for and our house went on lockdown due to a positive COVID-19 test of a roommate. This forced our testing to be done by only me, so the team agreed to a balance of workload that I perform the physical testing and the rest of the team splits up the ImageJ analysis work. Kylie will be performing the t-Test for the extra lenses design and an ANOVA test for the annuli.

Conclusions/action items:

Next week I want to have the testing results completely done, and a 3D-printed prototype in my hands. This is critical now that we are quickly running out of time. I also will update my LabArchives more outside of these weekly contribution reports, since this is in dire need of documentation. The Makerspace has not responded to any of my questions regarding the superstructure's printing, so Kylie and I reached out to Prof. Puccinelli to see if he could help us get into contact with someone at the Makerspace.

2020/12/05 - Weekly Contributions

Ben Hildebrandt - Dec 08, 2020, 1:05 PM CST

Title: Weekly Contributions

Date: 12/05/2020

Content by: Ben Hildebrandt

Present: N/A

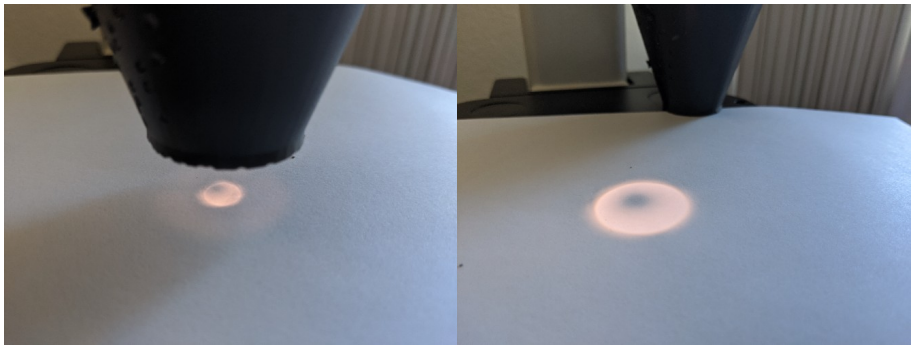
Goals: Record weekly contributions.

Content:

This week I performed all of the physical testing by preparing the different setups and taking photos with my camera. I also organized these photos and helped with the poster and report. The testing went smoothly, and the images were of varying quality. Most were clear enough to see many cell clusters, and these were able to be counted easily by the ImageJ software. Unfortunately, our testing yielded results that showed our designs either had no effect or were actively detrimental to the phase contrast quality in some cases. There is more information on this in our results section.

Conclusions/action items:

I believe there is some potential in the extra lenses. Although we did not see any noticeable improvement in the quality of the phase contrast when using the design, there was evidence of the cone of light being narrowed (see images below). The clear issue with the project as a whole was that we were never able to recreate the problem the client saw, with phase being effective in the middle, and then substantially decreasing towards the edges of the wells.



From now until they are due on 12/9, I will be working on my lab notebook as well as the final report. I will be focusing on the design portions of these deliverables, as I was the one who did all the calculations and CAD work, as well as fabrication and physical testing. I will also be preparing the microscope to be returned.

09/29/20 Existing Designs

KATHERINE BUDDE - Oct 05, 2020, 8:48 PM CDT

Title: Existing Designs

Date: 09/29/20

Content by: Katherine Budde

Present: N/A

Goals: Better understand the existing designs in our presentation

Content:

Enhancing Polarized Light Microscopy

- Inventor: Rudolf Oldenbourg
- Use semi-circular objective lens
- multiple annulus for refractions
- contrast-enhancing technique
    - improves quality of image
- High degree of sensitivity
    - used for both quantitative and qualitative studies
    - target at wide range of anisotropic specimens
- Microscope must be equipped with:
    - polarizer (placed in light path before specimen)
    - analyzer (placed in optical pathway between aperture and observation tubes)
- When electric field vectors restricted to single plane by filtration light is said to be polarized

Confocal scanning microscope

- Inventor: William J. Fox
- Used multiple sources of refracted light
- multiple focal points
- employ pair of pinhole apertures to limit specimen focal plane to confined volume
- images thick specimens by acquiring series of sections along optical axis
- optical sectioning of thick specimens
- Living specimens are sensitive to fluorophones and photobleaching cannot be prevented

Conclusions/action items: Finalize the preliminary presentation

Resources:

J. Murray, "Laser Scanning Confocal Microscopy", *Nikon's MicroscopyU*, 2020. [Online]. Available: https://www.microscopyu.com/tutorials/laser-scanning-confocal-microscopy. [Accessed: 06- Oct- 2020].

P. Robinson, "Polarized Light Microscopy", *Nikon's MicroscopyU*, 2020. [Online]. Available: https://www.microscopyu.com/techniques/polarized-light#:~:text=Polarized%20Light%20Microscopy&text=Polarized%20light%20is%20a%20contrast,Hoffman%20modulation%20contrast%2C%20and%20fluorescence. [Accessed: 06- Oct- 2020].

09/13/20 Well Plates

KATHERINE BUDDE - Sep 17, 2020, 2:58 PM CDT

Title: Well Plates

Date: 9/13/2020

Content by: Katherine Budde

Present: N/A

Goals: become acquainted with BrainXell's well plates and their overall uses

Content:

Intro to BrainXell's Technology:

-Design neural cells for drug screening and neuro development

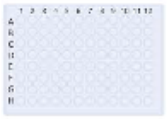
-Use iPSC (stem cells)

- adult cells -> stem cells -> neuroepithelial progenitors -> neurons
- need a homogenous pop with fast maturation – what BrainXell does
- allows them to make specific varieties of neurons
    - highly pure and rapid maturation
    - very large scale

Well Plates Research:

-Flat plate with multiple wells that are used as small test tubes

- 2:3 rectangular mix, most common is 96 (pictured below) because it can be used manually by lab tech or researcher and is compatible with automated equipment



- Standard tool in analytical research and clinical diagnostics
- Wells typically hold 10s of nanoliters to several milliliters
- Commonly made of polystyrene

- Every batch of BrainXell's neurons can fill a thousand 96-well plates

- Also culture and image cells while inside well plates

-Description

- Flat bottoms
- 25 to 340 µL total volume
    - Recommended working volume: 75 to 200 µL
- Sterilized by gamma radiation
- Alphanumeric code for well identification
- Greiner 96 well plates have 34 mm^2 culture area
- chimmeny well design
- can be black or white opaque

- Can't change plates -- specific ones have specific usages

Conclusions/Action Items: This is a good overview of important basic information to know about well plates. The website didn't say the exact well-plates they use, just that they are 96-well plates and are the Axion Biosystems MEA plates. I will need to ask the client what exact type of MEA plate they need to work with our microscope design so we know what to base it of off. I also think it would be helpful to know how fill they try to fill each of the wells so we know how this will effect the imaging process.

Resources:

"Consumables | Axion Biosystems", *Axionbiosystems.com*, 2020. [Online]. Available: https://www.axionbiosystems.com/products/consumables. [Accessed: 17- Sep- 2020].

"Intro to BrainXell's Technology | ALZFORUM", *Alzforum.org*, 2020. [Online]. Available: https://www.alzforum.org/file/intro-brainxells-technology. [Accessed: 17- Sep- 2020].

"Spinal Motor Neurons — BrainXell", *BrainXell*, 2020. [Online]. Available: https://brainxell.com/spinal-motor-neurons. [Accessed: 17- Sep- 2020].

09/15/20 Microscope

KATHERINE BUDDE - Sep 15, 2020, 2:04 PM CDT

Title: Well Plates

Date: 9/15/2020

Content by: Katherine Budde

Present: N/A

Goals: Become familiar with Phase Contrast Microscope and the Condensor

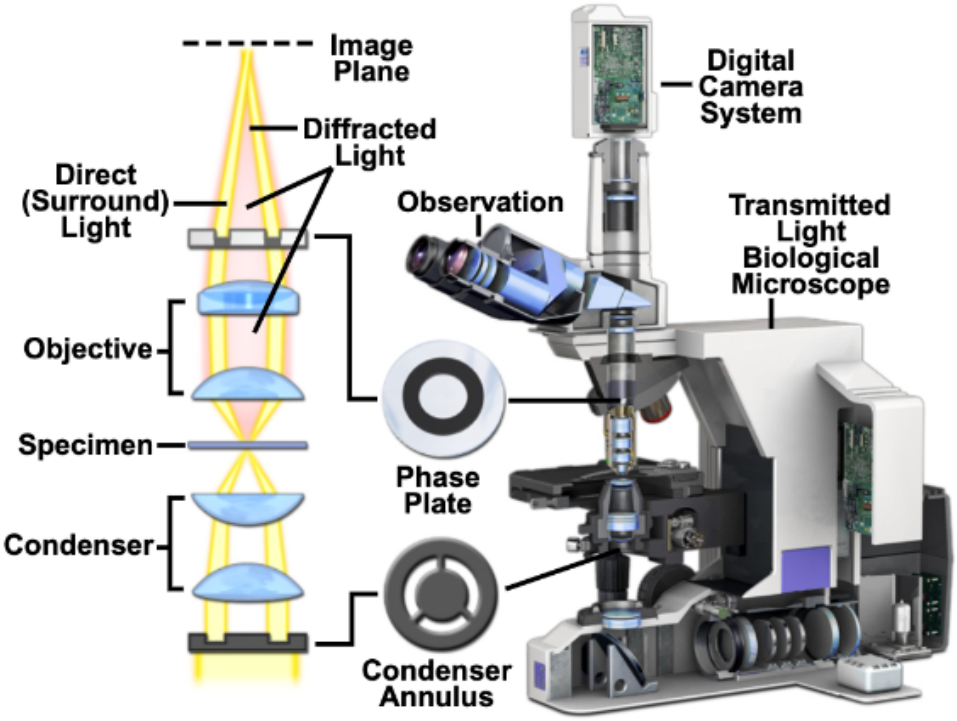
Content:

- Contrast-enhancing optical technique to produce high-contrast images (mainly of living cells)

- translate variations in phase into changes in amplitude -- results in image contrast

- allows living cells to be visualized without being killed, fixed, or stained

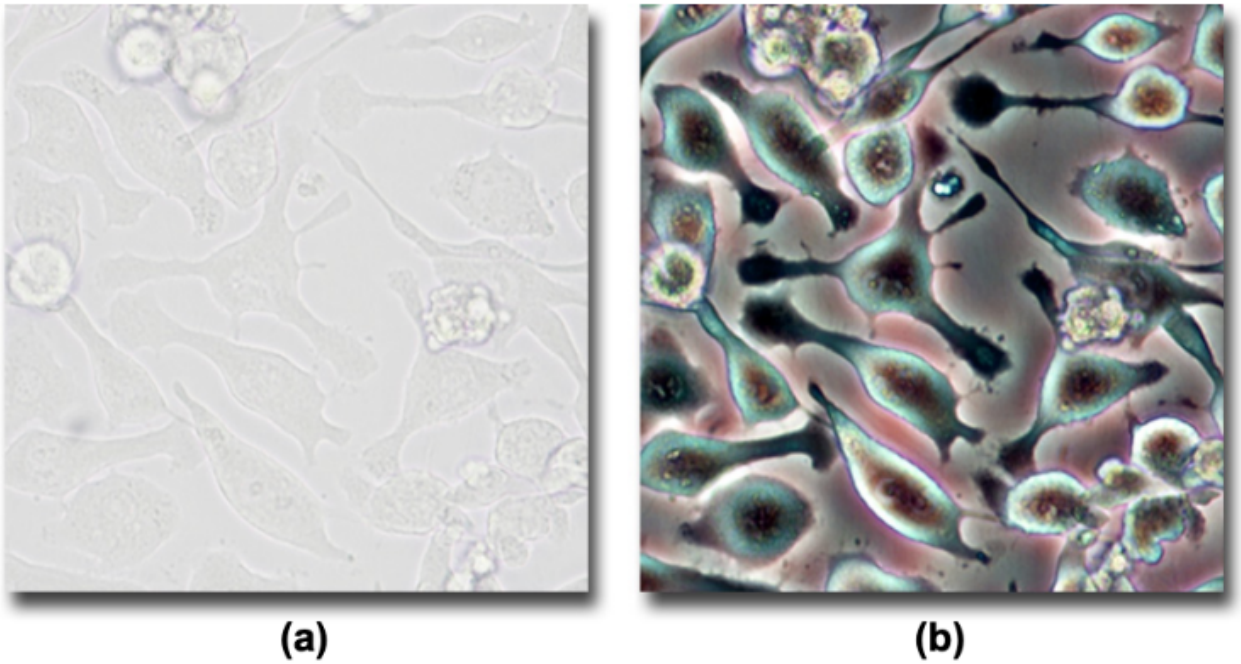
Figure 1 - Phase Contrast Microscope Configuration



- Figure 1 shows modern phase contrast microscope

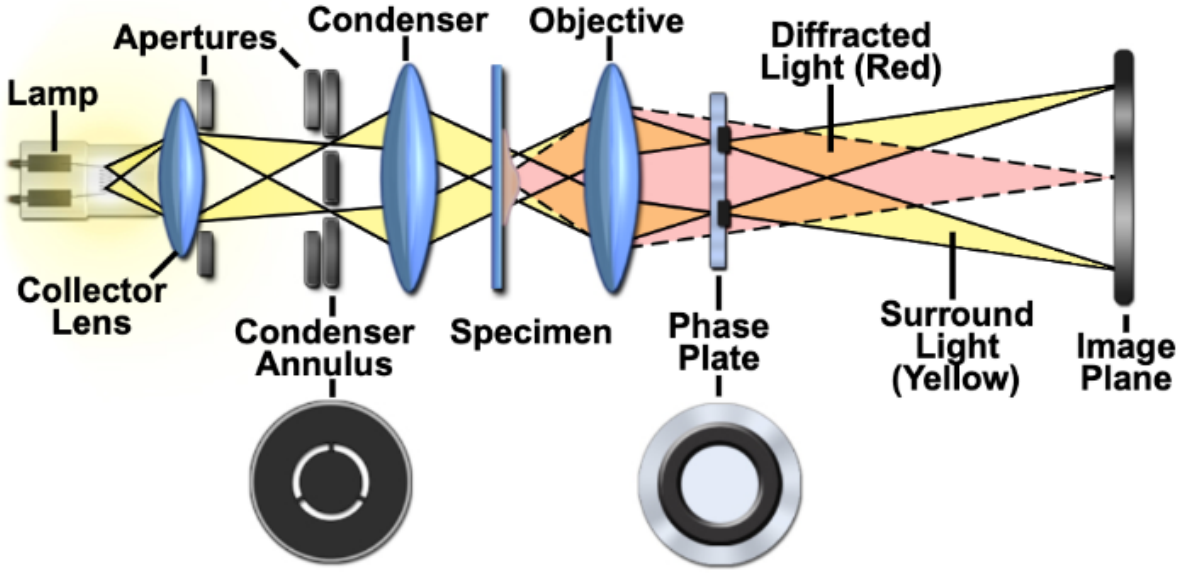
- so refined that extremely small molecules can be imaged

***Figure 2** - Living Cells in Brightfield and Phase Contrast*



- figure two shows the huge difference phase contrast allows in imaging and showing detail easily

***Figure 4** - Phase Contrast Microscope Optical Train*



- The condenser Annulus, shown above is positioned in front focal plane of condenser so specimen can be illuminated by "defocused, parallel light wavefronts emanating from the ring.

Conclusions/Action Items: This is an overview about the phase contrast microscope. I know that I am going to have to go much more in depth on the details, particularly on the condenser and condenser annulus, but for now this helps me understand the basics. Once I know more about the

scope of the project I will be able to research more in depth on what really matters.

Resources:

"Introduction to Phase Contrast Microscopy", *Nikon's MicroscopyU*, 2020. [Online]. Available: https://www.microscopyu.com/techniques/phase-contrast/introduction-to-phase-contrast-microscopy. [Accessed: 17- Sep- 2020].

09/17/20 PDS Research

KATHERINE BUDDE - Sep 17, 2020, 2:59 PM CDT

Title: Project Design Specifications

Date: 9/17/2020

Content by: Katherine Budde

Present: N/A

Goals: To determine accuracy/reliability, price, and quantity specifications of our project

Content:

Accuracy/Reliability

- Goal: maintain accuracy and reliability of current phase contrast microscopy - BrainXell uses Nikon ECLIPSE Ts2 with the ELWD condenser

- ELWD condenser has numerical aperature of 0.3, working distance of 75 mm, and magnification of 10x, 15x, 20x (these should be maintained or improved)

-should be reliable enough to give consistent results each time images are taken.

Quantity

- one one is needed for testing in this course

- if results are satisfactory, more can be made for others

Price

- Specified budget for prototype is $1500.

- Product cost should be at or below the cost of the Nikon phase contrast ELWD condenser at $1,150.

Conclusion/Action Items: This shows many aspects of our project design specifications and will help me finish my part of it. Next we will need to start designing solutions to our problem.

Resources

"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]

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

09/22/20 Phase Contrast Video

KATHERINE BUDDE - Sep 22, 2020, 11:52 PM CDT

Title: Phase Contrast Videos

Date: 9/22/2020

Content by: Katherine Budde

Present: N/A

Goals: To better understand it to come up with designs

Content:

"What PHASE CONTRAST microscopy is - all you ever wanted to know" - Microbehunter

- Rotate condenser for different types of contrast -- different touch stops for each objective

- Phase contrast objectives have phase rings when you look through them

- Condenser goes in under the plate

- phase contrast allows you to see more details (organelles), get halos around things to help set them apart from surrounding structures

- - optics convert changes in refractive index (we can't see, changes in color) into brightness changes

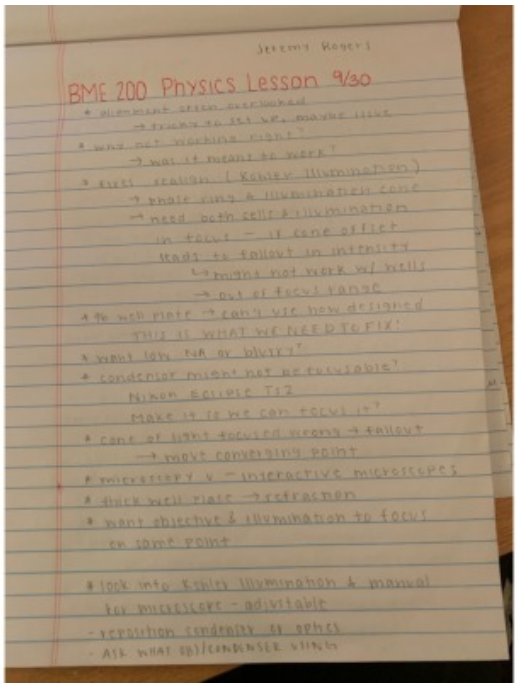
Because speciman has different refractive index than surrounding medium, it compresses the wave and shifts it over (phase n. shift) cant see this with our own eyes. At the end optics allow the light that went through specimen to interfere with light that didnt and those waves cancel each other out leading to wave reduced in amplitude

- shifts change in phase into change in amplitude

- allows you to see specimen without staining them

09/30/20 Physics Lesson

KATHERINE BUDDE - Oct 05, 2020, 7:45 PM CDT



PhysicsLesson.pdf(11.2 MB) - download

10/02/20 Kohler Illumination

KATHERINE BUDDE - Oct 05, 2020, 7:59 PM CDT

Title: Kohler Illumination

Date: 10/02/20

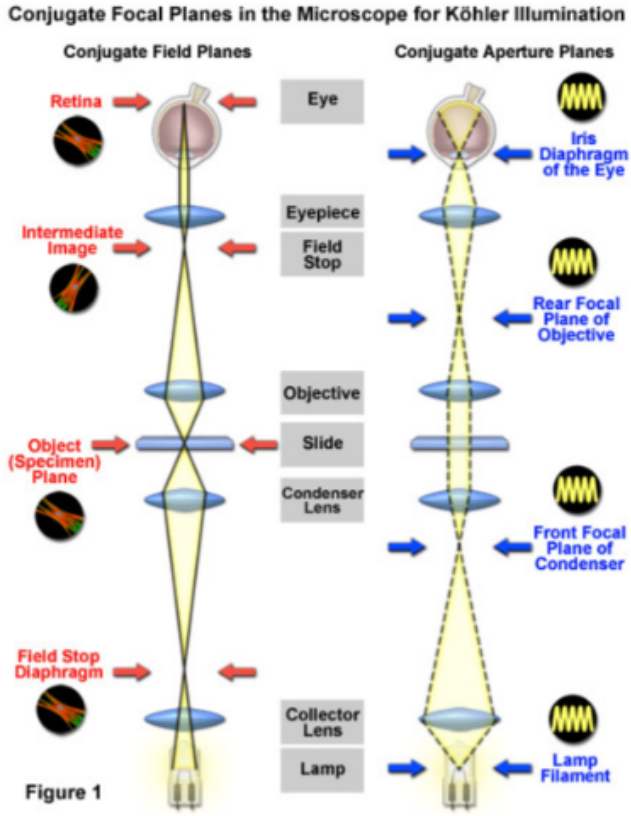
Content by: Kohler Illumination

Present: N/A

Goals: Learn about Kohler Illumination as discussed in our lesson with Professor Rogers

Content:

- First introduced in 1893 by August Kohler as a method of providing optimum specimen
- closing or opening condenser controls angle of light rays
- light at specimen not focused -- essentially grainless and extended.
- setting of condensers aperture, along with aperture of objective, determines the realized numerical aperture of microscope system
- As condenser opend, working NA increases, leading to greater resolving power and light transmittance



- Image of the conjugate and aperture field planes critical for establishing proper illumination in microscope
- The illumination system of the microscope, when adjusted for proper illumination, must be:
    - at least as large as field of view
    - light must be of uniform intensity
    - NA must vary from a maximum to a minimum that depend on optimal characteristics of specimen.

Conclusions/action items:

Discuss with the group to see how this can be applied to our designs.

Resources:

M. Davidson, "ZEISS Microscopy Online Campus | Microscopy Basics | Kohler Illumination", *Zeiss-campus.magnet.fsu.edu*, 2020. [Online]. Available: http://zeiss-campus.magnet.fsu.edu/articles/basics/kohler.html. [Accessed: 06- Oct- 2020].

11/10/20 multi-Kohler Illumination

KATHERINE BUDDE - Nov 10, 2020, 3:38 PM CST

Title: Enhanced field of view using multi-Kohler Illumination

Date: 11/10/20

Content by: Katherine Budde

Present: N/A

Goals: Better understand Kohler Illumination

Content:

- An inappropriate illumination affects contrast, reduces resolution, and destroys image
- Kohler illumination the image of the source is formed at infinity
- Optimum method of illumination in microcopy
- Their multi-Kohler Illumination concept provides a homogenous illumination source for the images
- shows improved performance
- can be used in a variety of integral imaging based 3d display systems
- Is a good idea, but I don't see how it can be applied to our project, because it kinda goes over our problem but we're already in too deep.

Conclusions/action items: After learning more about this I feel I better understand why it won't fully apply to our project.

Citation:

A. Tolosa et al., "Enhanced Field-of-View Integral Imaging Display using Multi-Kohler Illumination", *Optics Express*, vol. 22, no. 26, pp. 31853-31863, 2014. Available: https://www-osapublishing-org.ezproxy.library.wisc.edu/oe/fulltext.cfm?uri=oe-22-26-31853&id=306891. [Accessed 10 November 2020].

11/10/20 Condenser Free Phase Contrast

KATHERINE BUDDE - Nov 10, 2020, 3:05 PM CST

KATHERINE BUDDE - Nov 10, 2020, 3:25 PM CST

Title: Condenser Free Phase Contrast Microscopy

Date: 11/10/2020

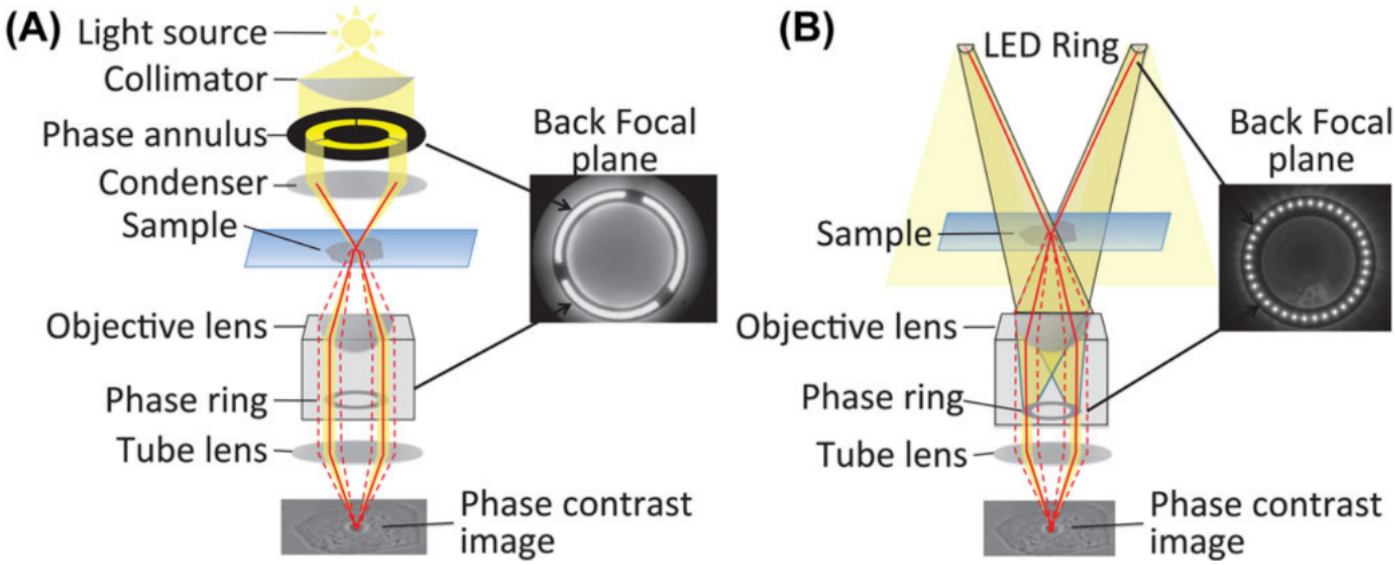
Content by: Katherine Budde

Present: N/A

Goals: Understand how condenser free microscopy works and hopefully better understand how our condenser works.

Content:

- Phase Contrast allows study of detail rich transparent specimens by addint contrast
- Without a condenser, a ring of light emitting diodes (LEDs) is positioned within the light field
    - this makes the illuminating ring in conjuction with the phase ring
    - it has been demonstrated that Zernike phase contrast is obtained
- Works across a range of magnifications, numerical apertures, and phase positions
- This can be useful in the future for X-rays and infrared rays



- Shows the difference between the Zernike phase contrast and the condenser free phase contrast (Above)
- Tested it on Nikon Ti eclipse -- what we were trying to test on for this class -- contains how it works in the article!

Conclusions/action items: Can do a lot more research into this if our current design works. Could be a good alternative because it is so much more adaptable to a range of phase positions and numerical apertures/objectives

Citation:

K. Webb, "Condenser-free Contrast Methods for Transmitted-Light Microscopy", *Journal of Microscopy*, vol. 257, no. 1, pp. 8-22, 2015. Available: https://onlinelibrary-wiley-com.ezproxy.library.wisc.edu/doi/pdfdirect/10.1111/jmi.12181. [Accessed 10 November 2020].

11/15/20: HEK Cells

KATHERINE BUDDE - Dec 08, 2020, 9:47 AM CST

Title: HEK Cell Reserach

Date: 11/15/20

Content by: Katherine Budde

Present: N/A

Goals: Understand more about HEK cells, which we will be using for our project

Content:

- HEK = Human Embryonic Kidney Cells
- very efficient about producing high amounts of recombinant proteins
- hardy, adherant, low-maintenance
- divide rapidly (double every 36 hours)
- second most widely used cell line after HeLa
- Health degrades if cultured for a long time
- risks of contamination

Conclusions/action items: Use this information in our final paper to describe HEK cells

Resources:

R. Roberts, "What the HEK? A Beginner’s Guide to HEK293 Cells", *Bitesize Bio*, 2020. [Online]. Available: https://bitesizebio.com/45489/what-the-hek-a-beginners-guide-to-hek293-cells/#:~:text=HEK293%20cells%20are%20Human%20Embryonic,in%20Van%20der%20Eb's%20lab. [Accessed: 08- Dec- 2020].

12/1/20: ImageJ Protocol Cell Counting

KATHERINE BUDDE - Dec 08, 2020, 9:32 AM CST

Title: Image J Protocol Cell Counting

Date: 12/1/20

Content by: Katherine Budde

Present: N/A

Goals: Understand how to effectively count cells in ImageJ

Content:

- Download cell counter.jar to plugins folder, restart
- Initialize: initializes current image or stack
- add: add counter type
- remove: removes counter type
- delete: delete last marker placed
- reset: reset counters to 0
- Results: shows numbers of counters

Conclusions/action items: Use this information to analyze our images in imageJ

Resources:

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

09/23/20 Microscope Design Ideas

KATHERINE BUDDE - Sep 23, 2020, 10:52 AM CDT

Title: Microscope Design Ideas

Date: 09/23/20

Content By: Katherine Budde

Present: Katherine Budde

Goals: Design microscope condensers to allow for expansion of viewable range while maintaining current resolution and magnification of the microscope.

Content:

1. Change the curvature of one of the condenser lenses so the focal point is a little above the specimen. this might be able to make it so the light is able to pass through a larger portion of the well plate - making more of it visible under phase contrast microscopy. I think that this might help it shift a wider range of the light out of phase to make them show up better.

2. But more light beams through the condenser to allow it to pass a wider wave of light through the well, hopefully causing it to light up a wider area to push the specimen out of phase to help them show up better. I think that this could be done by altering the annular ring -- possibly widening the translucent area to allow for more light to pass through it -- could make the radius smaller or larger to achieve this.

3. Increase the intensity of the light. I'm not as sure about this idea, but maybe this could cause more of it to interfere and be knocked out of phase by the specimen to see if this could make a wider range of it visible.

Conclusion/Action items: Collaborate with teammates to determine which of our ideas will work the best, create design matrix, continue researching anatomy of condensers to better understand how to apply these ideas. I should also continue to research how/if intensity plays a role in phase contrast microscopy to see if this is a plausible way to solve our design problems.

09/27/20 Condenser Annulus Adjustment

KATHERINE BUDDE - Oct 05, 2020, 8:07 PM CDT

Title: Condenser Annulus Adjustment Design Idea

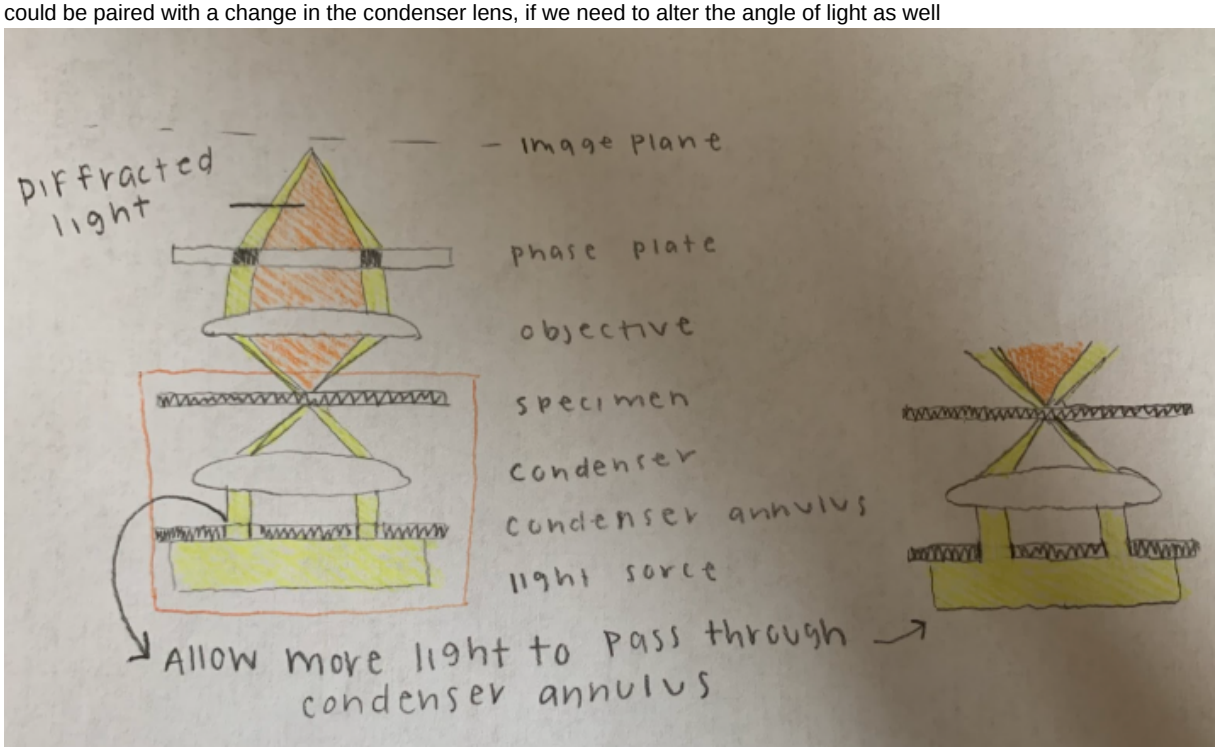
Date: 09/27/20

Content by: Katherine Budde

Present: N/A

Goals: Further research my condenser annulus design idea to understand how it works for our presentation.

Content:

- Doesn't change anything about microscope body/condenser (just condenser annulus)
- condenser annulus lies below condenser
- maintain resolution -- only changing amount of light passing through
- cause more light to travel through microscope
- hopefully will cover a wider area of specimen to increase the visible area
- could be paired with a change in the condenser lens, if we need to alter the angle of light as well
- 

Conclusions/action items: compare this design with others to see which is the best one to pursue for the remainder of the semester.

09/28/20 Oil Numerical Aperture

KATHERINE BUDDE - Oct 05, 2020, 8:15 PM CDT

Title: Oil to Increase NA idea (Lauren's idea, research for presentation)

Date: 09/28/20

Content by: Katherine Budde

Present: N/A

Goals: Better understand Lauren's design idea so I can best present it

Content:

- Use oil in place of air because it has a higher refractive index
    - Air refractive index = 1.000293
    - Oil refractive index = 1.51
    - oil would replace the empty space in the condenser
- Numerical Aperture = n*sin(angular aperture)
    - n = refractive index
- Numerical aperture measures ability to gather light and resolve fine specimen detail at a fixed object distance
- As numerical aperture increases it causes a greater light-gathering ability
    - this causes an increase in resolution
- an increase in refractive index causes a higher numerical aperture
- changing to oil increases the NA and hence the resolution

Conclusions/action items: Present this in our preliminary presentation on Friday 10/2

Resources:

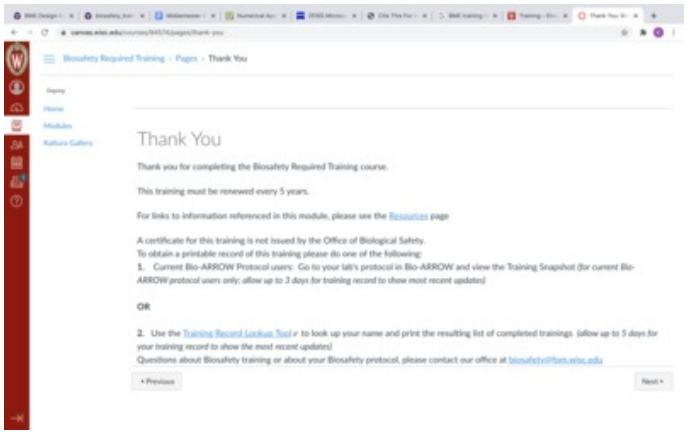
M. Davidson, "Numerical Aperture", *Nikon's MicroscopyU*, 2020. [Online]. Available: https://www.microscopyu.com/microscopy-basics/numerical-aperture. [Accessed: 06- Oct- 2020].

Biosafety and Chemical Training

KATHERINE BUDDE - Oct 05, 2020, 8:32 PM CDT

ChemicalSafety.pdf(15.7 MB) - download

KATHERINE BUDDE - Oct 05, 2020, 8:32 PM CDT



Screen_Shot_2020-10-05_at_8.26.32_PM.png(534.6 KB) - download

09/08/20: Literature Searching

KATHERINE BUDDE - Oct 05, 2020, 8:18 PM CDT

Title: Literature Searching

Date: 9/8/2020

Content by: Katherine Budde

Present: N/A

Goals: Learn about literature searching by watching the videos on the design website

Content:

- Most information is not online, not free, paper only
- Interlibrary loan can get you info from another library
- Use google books and engineering ebooks to find books
    - ENGnetBASE
    - Knovel
    - ASM Handbooks Online
- Deep Web
    - Use fee based databases
    - highly structured
    - Engineering Index (Compendex)
- Patents: google patents
- Get help from professors, librarians, fee-based info services
- Strategies to retrieve a manageable number of records
    - if too many, be more specific
    - if too few, be more specific
    - look for and use synonyms
    - be careful with terms like: new, development, effect
    - truncation, word endings
    - use phrase searching -- can improve results
- Types of information:
    - books
    - scholary articles
    - trade magazine articles
    - newspapers
    - conferences
    - gov documents
    - technical reports
    - patents
    - standards
    - product literature
    - blogs

Conclusions/action items: Begin to research my own literature about phase contrast microscopy using these skills.

Resources: https://bmedesign.engr.wisc.edu/course/topics/literature/literature_searches

09/10/20: Progress Reports

KATHERINE BUDDE - Oct 05, 2020, 8:19 PM CDT

Title: Writing Progress Reports

Date: 9/10/2020

Content by: Katherine Budde

Present: N/A

Goals: Better understand how to write an effective progress report for this class

Content:

Purpose of progress reports:

- Team snapshot
    - clarifies team position
        - communication within team
        - clarifies current achievements and hurdles
- Maximizes usefulness of limited class time
- Real life experience

Content

- Title, team names, date, problem statement
- Restatement of team goals
- specific accomplishments
- specific goals
- current difficulties
- project schedule
- activities
- expenses

Conclusions/action items: Fill out this weeks progress report now that I better understand the sections

Resources: https://bmedesign.engr.wisc.edu/course/topics/communication/progress_reports

09/12/20: Design, PDS

KATHERINE BUDDE - Oct 05, 2020, 8:21 PM CDT

Title: Design Process and PDS

Date: 9/12/2020

Content by: Katherine Budde

Present: N/A

Goals: Learn about the design process and how to write the PDS.

Content:

Steps in the design process

1. Problem definition: understand the problem as much as possible:
    1. goals of the project
    2. Necessary background information
        1. Medical Issues: understanding of constraints due to medical use of devise
        2. biological and physiological issues: basic knowledge of medical specialty
        3. technical issues: understanding of engineering aspects and competing tech
    3. Do this by:
        1. meeting with client
        2. meeting with advisors
        3. literature searches
        4. development of PDS
2. Preliminary Design
    1. brainstorm alternative solutions -- think of as many as possible
    2. sketches are very important
3. Choose single design to pursue
    1. compare designs using design matrix
    2. may combine positive aspects of several designs
4. Design Detailing
    1. continually evaluate design choice
    2. consider:
        1. dimensions
        2. materials, fasteners, etc.
        3. analysis (loads, flow rates, etc.)
        4. more sketches and drawings
    3. create 3D graphic rendering
5. Evaluate design
    1. look over design in entirety before building
6. Prototype
    1. finalize drawings and build a fully operational prototype
7. Evaluation of prototype
    1. test device in the field

How to write a PDS

- PDS includes:
    - a list of requirements and constraints
    - states the requirements a product must fulfill
    - helps designer completely understand the problem
    - based on input from customer, market analysis, research
- How to write:
    - fully define problem

    - list all specifications
    - list numerical values and tolerances
    - split specifications into smaller categories
    - assign importance to different specifications
- Writing good specifications
    - be specific and quantitative

Conclusions/action items: Now that I better understand the design process and PDS I will be able to work with my team to generate a better PDS

Resources: https://bmedesign.engr.wisc.edu/course/resources/design_process

https://bmedesign.engr.wisc.edu/files/course/topics/process/Turner_design_spec.pdf

09/17/20: Codes and Standards, Decision Matrix

KATHERINE BUDDE - Oct 05, 2020, 8:22 PM CDT

Title: Codes and Standards

Date: 9/17/2020

Content by: Katherine Budde

Present: N/A

Goals: Learn about codes and standards and the decision matrix

Content:

Codes and Standards

- Why use standards
    - legal necessity
    - consensus of design community
    - simplify drawings and callouts
    - more control over design and purchasing
- What is a standard
    - written description of criteria that is agreed to by formal processes
- types of standards
    - consensus standards: agreed to by formal consensus
    - defacto (adhoc) standards: developed outside formal procedures, by the marketplace
    - government regulations; adopted by government and written into law
- who generates standards
    - IEE Standards board
    - cost accounting standards board
    - ASTM international
- US process for standards development
    - accredited developer submits statement of need
    - if project approved by ANSI, draft test is written
    - draft is reviewed by many, rewritten, reviewed
    - developer submits request for final review
    - ANSI approves and publishes it
    - Standard is reviewed every 5 years for possible updates or removal
- International Standards
    - ensure quality, safety, compatibility
    - facilitate trade
    - climate change, energy
- International Organizations
    - ISO: international organization for standardization
    - IEC: international electrotechnical commission
- Finding standards - Diligence
    - expensive
    - not a single organizing agency
    - very difficult to find full text online
    - must work hard to find the standards you need
- Where to find standards
    - assist military database
    - NSSN data base from ANSI
    - library and reference librarian
    - research libraries and commercial libraries
    - specific organizations web sites

Decision Matrix Guidelines

- 3 distinct design alternatives
- criteria (6-10) reflect most important specifications (including cost and safety)
- Evaluate 1-5 for each design, calculate weighted total (adds up to 100 and ranks)
- List criteria by order of importance

- choose short titles for each design (image if possible)
- differentiate winning categories with shading/color
- Justify scoring criteria in the caption

Conclusions/action items: Start filling out the decision matrix

Resources: https://bmedesign.engr.wisc.edu/course/topics/considerations/codes_and_standards

https://bmedesign.engr.wisc.edu/files/course/matrix/DesignMatrixRequirements.pdf

09/23/20: Preliminary Presentation & Report

KATHERINE BUDDE - Oct 05, 2020, 8:23 PM CDT

Title: Preliminary Presentation and Report

Date: 9/23/2020

Content by: Katherine Budde

Present: N/A

Goals: Learn about the preliminary presentation and report

Content:

Preliminary Presentation Guidelines

- Understand the audience
- Prepare thoroughly for the presentation
    - title slide
    - overview
    - problem statement
    - background material
    - PDS summary
    - design alternatives
    - design matrix
    - future work
    - references and acknowledgements
- Practice the presentation
- bring supporting materials appropriate to the presentations

Basics of Oral Presentations

- Informative outline: follow order of main points
- Good slide structure
    - 1-2 slides/minute
    - point form
    - 6 points per slide/6 words per point
    - key words and phrases only
- Good fonts
    - at least 18 point
    - different size for main and secondary
    - use standard font
- Good color
    - contrast sharply with background
    - color to reinforce logic of structure
    - color to emphasize a point (only occasionally)
- Good background
    - attractive, light, simple
    - keep it consistent
- good graphs
    - graphs and pictures instead of chards and words
    - title
- Spelling and Grammer
    - proof read for spelling mistakes, repeated words, grammatical errors
- Practice
    - stimulate actual environment
    - don't read from slides
    - talk to audience
    - be professional
    - think of how to handle questions
- conclusion
    - 2-3 take home messages
    - deliver using tools discussed

- BME guidelines
    - understand audience
    - prepare throughly
    - bring supporting materials
        - additional slides
        - simple prototypes
        - copy of slides for advisor and client

Written Reports

Report Guidelines

- General recommendations
    - follow outline
    - every method have a result that is discussed
    - take time to edit and format
    - use titles and subtitles
    - cite sources throughout paper
    - meaningful figures with captions
    - number all pages
    - use SI units
    - add PDS in appendix
- Construction
    - Cover page
        - title
        - course name
        - date
        - client name and affiliation
        - advisor name and affiliation
        - team member names and roless
    - Abstract
        - write this last, summarize entire paper
    - table of contents
        - after complete report, Word will do this
    - Body of Report
        - Intro
            - motivation/impact
            - existing devices/current methods
            - problem statement
        - Background
            - research (physiology and biology)
            - research required to design prototype
            - client information
            - design specifications
        - Preliminary designs
            - include all considered
            - neat sketches, labeled
            - written summary of each
        - Preliminary design eval
            - design matrix
            - written summary of it
            - proposed final design
        - Fabrication/development process
            - materials
            - methods used
            - final prototype
            - testing
        - Results
            - analyze data and use statistical methods
            - include only relevant data
            - identification of and observation on salient features
        - Discussion
            - reveal implications of results
            - ethical considerations
            - changes needed

        - identify and describe sources of error
    - Conclussions
        - restate problem and final design
        - briefly summarize your findings
        - what worked and what didn't, what would you change
        - future work
    - References
        - IEE
    - Appendix
        - PDS
        - table with materials
        - extra data, computer code
        - complicated protocols followed

Conclusions/Action items: Now that I have done this research I have the means to work on our preliminary design presentation and report.

Resources: https://bmedesign.engr.wisc.edu/files/course/preliminary_presentations/preliminary_presentation_guidelines.pdf

https://bmedesign.engr.wisc.edu/course/topics/communication/oral_presentations

https://bmedesign.engr.wisc.edu/files/course/report/BMEDesign-Report_Guidelines.pdf

10/21/20: Intellectual Property

KATHERINE BUDDE - Dec 08, 2020, 9:23 AM CST

Title: Intellectual Property for Design, IP Agreements

Date: 10/21/20

Content by: Katherine Budde

Present: N/A

Goals: Learn more about intellectual property and patents

Content:

Intellectual Property

- If you tell someone your idea can you still patent it -- depends
    - public disclosure more than one year before application eliminates rights to patent
    - Public disclosure:
        - printed publications
        - website post
        - poster presentations
        - class presentations
        - public use
        - non-confidential discussions
        - offer for sale
    - most countries do not have a one year grace period
- don't have to build invention to apply
    - must describe in words and figures how to make and use invention
    - must display inventer in possession of invention
- who owns inventions
    - inventors: patent right granted to inventors
    - if no pre agreements, co-inventors have equal rights to invention
    - ownership by assignment -- company, usually signed document
    - UW ownership
        - if invention part of class project inventors allowed to own patent

IP agreements

- types of IP
    - Patents, biomaterials, copyright, trademarks, trade secrets
- why important
    - give owners/creators exclusive rights to creation
- license agreements
    - contract between patent owner and licensee giving licensee ability to make, use, or sell
- benefits of licensing a technology
    - reduces R&D costs
    - improved time to market
    - opportunity to enter new markets and expand quickly
- license agreement terms
    - exclusive or non exclusive
    - field of use
    - royalties
    - patent reimbursement
- Research License
    - commercial research licenses allow company to evaluate tech, not dev product
    - in case of material, can use in research not not sell to others.

Conclusions/action items: I hope to be able to use this information in future design projects.

Resources:

https://bmedesign.engr.wisc.edu/course/topics/literature/ip_agreements

https://bmedesign.engr.wisc.edu/course/topics/literature/ip_in_design

11/13/20: Tong Lecture

KATHERINE BUDDE - Dec 08, 2020, 9:26 AM CST

Title: Tong Distinguished Entrepreneurship Lecture

Date: 11/13/20

Content by: Katherine Budde

Present: N/A

Goals: Learn about entrepreneurship

Content:

-Widely Successful = Unicorn, don't come across them often

-Go through both failures and successes

-Biomedical is a very global field – share with other countries

-Successful Entrepreneurs: build companies become angel investor

- Angel investors get management/advisory roles in companies

-Take Financial Risks and Opportunity Risks

- It's a lifestyle decision

-Not just monetary profit, but also personal growth and helping people

-Most entrepreneurs pay others more than they pay themselves

-Entrepreneurship for people who think strategically and out of the box and problem solve

-Even if you fail, you'll probably want to keep trying – hard to change career after doing it

-Look into what it takes to set up a company before you start – lots of responsibility

-MBA helps with management of company

-Lots of risk that comes with managing a company

-Need to know risk tolerance limits – how far do you want to go

-Need to be very perseverant lots of setbacks

-Know when to quit; most difficult part in his opinion: very painful

-Biomed is a very complex field

-Need lots of multidiscipline experience

-Before getting into biomedical entrepreneurship

- Know your regulatory
    - How hard will it be to get approved?
- Know your funding options (government role?)
    - Clinical testing gets very expensive (especially if more invasive)
    - Takes a lot of years to get to revenue, investors like revenue asap
- Hardware?
    - Need a place to build it, supply chain (hard to set up), need samples
    - Makes things more complicated
- Know your market
    - Is there a market?
- Go global (foreign governments)
    - Helps everyone in the development of new devices

-Lessons learned

- By the best advice you can afford

        - Corporate set up (governance, share structure, type of company, tax implications)
            - Get a corporate lawyer – VERY expensive
        - IP
            - Get an IP lawyer
            - Protects your idea
        - Regulatory
        - Selling
            - Know the value of your company
    - Know the strings that come with funding
    - Know your partners
        - Know them well – wrong partners can derail the whole project
        - 1 person companies do well

-Know what you like

- R&D; Management; PR; OPS; Clinical; Sales & Marketing

-Not for everyone, but very rewarding

-Look beyond commercial success

-Lifestyle choice, lifelong journey

-Total commitment

Conclusions/action items: Use this information in my future career.

Week 1: 9/04-9/11

KATHERINE BUDDE - Dec 08, 2020, 9:56 AM CST

Title: Week 1 Contributions

Date: 9/11

Content by: Katherine Budde

Present: N/A

Goals: Recap everything I accomplished this week.

Content:

- got in contact with the rest of the group
- updated team website with member roles and team pictures
- began research on phase microscopes, well plates, and BrainXell
- Met with Dr. Saha

Conclusions/action items: Next week I am going to further research well plates to see how their size and color affect clarity under phase contrast microscopy.

Week 2: 9/11-9/17

KATHERINE BUDDE - Dec 08, 2020, 9:59 AM CST

Title: Week 2 Report

Date: 9/17

Content by: Katherine Budde

Present: N/A

Goals: Discuss what I accomplished this week

Content:

- Further research on phase contrast microscopes and condensers
- looked into accuracy and reliability of current microscopes
- researched well plates
- worked on PDS and progress report

Conclusions/action items: Next week I hope to gain a better understanding of the physics of the microscope and condenser. I also plan to come up with ideas on how to alter the condenser to provide a better field of view.

Week 3: 9/18-9/24

KATHERINE BUDDE - Dec 08, 2020, 10:01 AM CST

Title: Week 3 Report

Date: 9/24

Content by: Katherine Budde

Present: N/A

Goals: Go over my weekly accomplishments

Content:

- watched videos to get a better understanding of optics of phase contrast microscopy
- more research
- optics lesson with team
- came up with ideas for the design matrix

Conclusions/action items: Next week I plan to do more math on condensers to help the team decide which design will be the best solution.

Week 4: 9/25-10/01

KATHERINE BUDDE - Dec 08, 2020, 10:05 AM CST

Title: Week 4 Report

Date: 10/01

Content by: Katherine Budde

Present: N/A

Goals: Recap my weekly accomplishments

Content:

- Worked on design ideas
- sketched condenser annulus design and worked on preliminary presentation
- scripted, finalized, and recorded preliminary presentation
- upload progress report and video/slides to website
- met with Professor Rogers to better understand the physics behind microscopes

Conclusions/action items: Next week I plan to look into further detail about several concepts Professor Rodgers brought up. I also plan to go into more detail about the designs so we can start prototyping soon.

Week 5: 10/02-10/08

KATHERINE BUDDE - Dec 08, 2020, 10:08 AM CST

Title: Week 5 Report

Date: 10/08

Content by: Katherine Budde

Present: N/A

Goals: Weekly Recap

Content:

- Attended preliminary presentation and watched other groups presentations
- worked on preliminary report: problem statement, final design, conclusions
- report proofreading and finalizing

Conclusions/action items: The plan for next week is to work with the team to improve our final designs.

Week 6: 10/09-10/15

KATHERINE BUDDE - Dec 08, 2020, 10:10 AM CST

Title: Week 6 Recap

Date: 10/15

Content by: Katherine Budde

Present: N/A

Goals: Weekly Recap

Content:

- Looked into Kohler Illumination
- Researched ways to best prototype our design ideas

Conclusions/action items: Hopefully we will be able to gain access to a microscope soon to see it in person and come up with the best ways to prototype it.

Week 7: 10/16-10/22

KATHERINE BUDDE - Dec 08, 2020, 10:12 AM CST

Title: Week 7 Recap

Date: 10/22

Content by: Katherine Budde

Present: N/A

Goals: Weekly recap of accomplishments

Content:

- Attended team meeting
- researched fabrication plan
- created fabrication plan

Conclusions/action items: Next week I will work with the team to begin fabrication and testing.

Week 8: 10/23-10/29

KATHERINE BUDDE - Dec 08, 2020, 10:14 AM CST

Title: Week 8 Recap

Date: 10/29

Content by: Katherine Budde

Present: N/A

Goals: Weekly summary

Content:

- Attended meeting with client -- helped me better understand microscope issues
- did more design research

Conclusions/action items: Next week I hope to begin design prototyping.

Week 9: 10/30-11/05

KATHERINE BUDDE - Dec 08, 2020, 10:16 AM CST

Title: Week 9 Recap

Date: 11/05

Content by: Katherine Budde

Present: N/A

Goals: Weekly recap

Content:

- helped finalize elevator pitch
- attended Tuesday team meeting
- research to find cell options for microscope (lack of options)

Conclusions/action items: Keep doing research to find ways to test our designs and find literature articles to add to our reports.

Week 10: 11/06-11/12

KATHERINE BUDDE - Dec 08, 2020, 10:18 AM CST

Title: Week 10 Recap

Date: 11/12

Content by: Katherine Budde

Present: N/A

Goals: Weekly summary of accomplishments

Content:

- literature research to find more scholarly articles
- image analysis to find percent of effective area

Conclusions/action items: Next week I hope to analyze testing results. I also wish to find more articles to add in our report.

Week 11: 11/13-11/19

KATHERINE BUDDE - Dec 08, 2020, 10:20 AM CST

Title: Week 11 Recap

Date: 11/19

Content by: Katherine Budde

Present: N/A

Goals: Weekly summary of accomplishments

Content:

- attended guest lecture
- website update
- HEK cell research

Conclusions/action items: Next week I will help with testing the prototype and analyzing the results.

Week 12: 11/20-11/27

KATHERINE BUDDE - Dec 08, 2020, 10:22 AM CST

Title: Week 12 Recap

Date: 11/27

Content by: Katherine Budde

Present: N/A

Goals: Weekly Recap

Content:

- Image J analysis
- Protocol to objectively compare image results

Conclusions/action items: Next week I hope to finish as much image analysis as possible and put together our final presentation/report.

Week 13: 11/28-12/03

KATHERINE BUDDE - Dec 08, 2020, 10:24 AM CST

Title: Week 13 Recap

Date: 12/03

Content by: Katherine Budde

Present: N/A

Goals: Weekly summary of accomplishments

Content:

- lots of Image J Analysis
    - Counted cells in each image
    - Dark area measurments
- Graph creations from data
- helped with final poster creation and recording
- uploaded everything to website for final presentation

Conclusions/action items: Next week will be spent finalizing the report and lab archives. I also hope to ensure all of our data analysis was completed properly.

10/1/20 - Patent Research

CARSON EVENSTAD - Dec 07, 2020, 11:05 AM CST

Title: Patent Research

Date: 10/1/20

Content by: Carson Evenstad

Present: Carson Evenstad

Goals: To find Patents that are related to designs on our design matrix

Content

3. Miscellaneous

1. *Standards and Specifications:*
    1. 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]
2. *Customer:*
    1. The customer needs the condenser to be able to be adaptable to different kinds of plates, with focus on the Grainer-96 plates
    2. 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.
    3. The customer would also like the resolution and contrast to be our highest priorities, with limiting tradeoff between lower resolution for more area.

1. 3. *Patient-related concerns:*
    1. Microscopes should generally be sanitized and cleaned after 200 hours of service, or more frequently depending on daily usage.
    2. Microscope condensers are extremely fragile pieces and must be cleaned thoroughly and carefully. [9]
    3. There is no specified shelf life for this device
4. *Competition:*
    1. 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.
    2. *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.
    3. 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.

Conclusions/action items: Develope presentation slides

10/6/20 Preliminary Presentation slides

CARSON EVENSTAD - Oct 06, 2020, 8:11 PM CDT

Title: Prememinlary Presentation Slides

Date: 10/6/20

Content by: Carson Evenstad

Present: Carson Evenstad

Goals: Develop slides for the preliminary presentation regarding Existing designs and client constraints

Content: Pictures Provided

Conclusions/action items:

CARSON EVENSTAD - Oct 06, 2020, 8:11 PM CDT

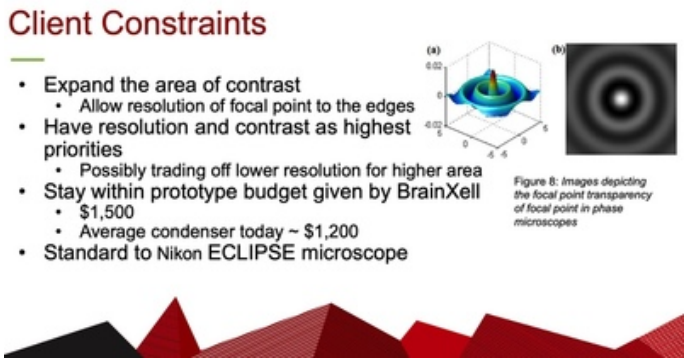


Image_10-6-20_at_8.10_PM.jpg(164.1 KB) - download

CARSON EVENSTAD - Oct 06, 2020, 8:12 PM CDT

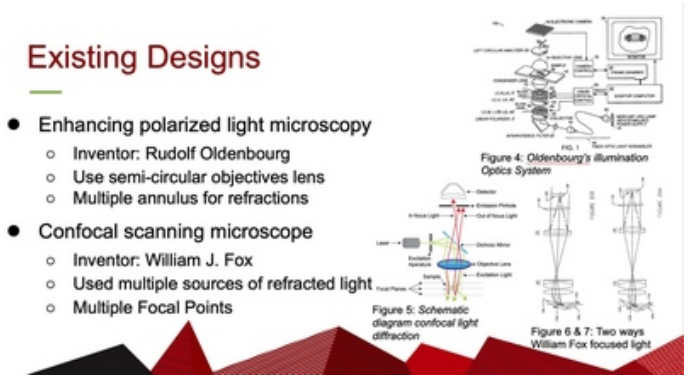


Image_10-6-20_at_8.10_PM_1_.jpg(181.8 KB) - download

10/6/20 Design Flow

CARSON EVENSTAD - Oct 06, 2020, 8:16 PM CDT

Title: Design Flow

Date: 10/6/20

Content by: Carson Evenstad

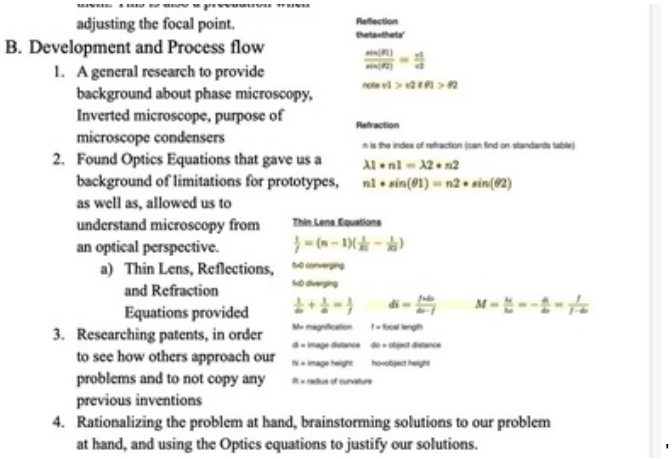
Present: Carson Evenstad

Goals: Produce a design Flow that shows all the research that we have to do up to this point and what we found.

Content: Pictures Provided

Conclusions/action items: Continue to the design phase

CARSON EVENSTAD - Oct 06, 2020, 8:16 PM CDT



adjusting the focal point.

B. Development and Process flow

1. A general research to provide background about phase microscopy, Inverted microscope, purpose of microscope condensers
2. Found Optics Equations that gave us a background of limitations for prototypes, as well as, allowed us to understand microscopy from an optical perspective.
   a) Thin Lens, Reflections, and Refraction Equations provided
3. Researching patents, in order to see how others approach our problems and to not copy any previous inventions
4. Rationalizing the problem at hand, brainstorming solutions to our problem at hand, and using the Optics equations to justify our solutions.

Reflection
thetaxtheta'

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

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

Refraction

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

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

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

Thin Lens Equations

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

f>0 converging

f<0 diverging

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

M= magnification f = focal length

di = image distance do = object distance

hi = image height ho=object height

R = radius of curvature

Image_10-6-20_at_8.14_PM.jpg(97.2 KB) - download

10/19/20 Materials List

CARSON EVENSTAD - Oct 19, 2020, 12:34 PM CDT

Title: Materials List

Date: 10/19/20

Content by: Carson Evenstad

Present: Carson Evenstad

Goals: Find all the parts we need for fabrication of our final design, explore the internet looking for materials, and documenting finding in spreadsheet

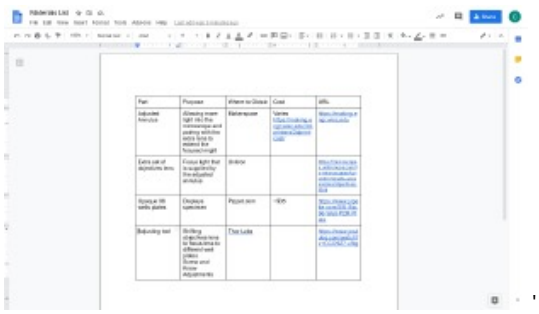
Content:

See attachments

Conclusions/action items:

We need to get a microscope in order to fine precious measurement for our objectives lens and annulus. We can't make either until we have a microscope to measure and work on.

CARSON EVENSTAD - Oct 19, 2020, 12:35 PM CDT



Materials_List_-_Google_Docs.pdf(213 KB) - download

11/5/20 Database articles for Phase Miccrosopy

CARSON EVENSTAD - Nov 05, 2020, 5:12 PM CST

Title: Database articles for Phase Microscopy

Date: 11//520

Content by: Carson Evenstad

Present: Carson Evenstad

Goals: Find article portraying to fixing resolution of phase microscopy with new and innovative ways. Learn mroe about the funamental principal of phase contrast microscope and trying to apply the optics equations.

Content:

Development of an in-vacuum x-ray microscope with cryogenic sample cooling for beamline p11 at PETRA II :

This is farly elaborate microscope with one component that I think could really help us in your project. This uses piezo motors in order to shift their scanner for the X ray portion of the microscope, I thing with could use a similar apparatus underneath the well plates. The piezo motors are able to shift an object vertically and horizontally deoending on the changing magnetic field insdie of the coil. This can adjust our well plates of the desired place in order to maximize resolution. These piezo motors are cost effective, as well as, extremely precise in their movements.

link to how a piezo motor operates: https://www.youtube.com/watch?v=Rh-JvbTKvXAL

Link to article: https://www-scopus-com.ezproxy.library.wisc.edu/record/display.uri?eid=2-s2.0-0016169171&origin=resultslist&sort=plf-f&src=s&st1=microscope+condensor+phase+contrast&nlo=&nlr=&nls=&sid=6cffd37297cbbafb0beb996731ed5aae&sot=b&sdt=b&sl=50&s=TITLE-ABS-KEY%28microscope+condensor+phase+contrast%29&relpos=3&citeCnt=2&searchTerm=

The Nomarski interference contrast technique modified for the use of apochromatic objectives and other supplementary parts

This microscope simply uses a new set of lens, apochromatic lens, they are used in photography and allow better correct of the lens and a more sperical aberration.

Link to the article: https://www-scopus-com.ezproxy.library.wisc.edu/record/display.uri?eid=2-s2.0-0016169171&origin=resultslist&sort=plf-f&src=s&st1=microscope+condensor+phase+contrast&st2=&sid=6cffd37297cbbafb0beb996731ed5aae&sot=b&sdt=b&sl=50&s=TITLE-ABS-KEY%28microscope+condensor+phase+contrast%29&relpos=3&citeCnt=2&searchTerm=

Conclusions/action items:

Start developing prototypes of our other designs and see if either of these ideas would applu to our problem

11/14/20 HEK cells research

CARSON EVENSTAD - Dec 07, 2020, 10:26 AM CST

Title: HEK cells back round research

Date: 11/14/20

Content by: Carson Evenstad

Present: Carson Evenstad

Goals: Learn about HEK cells, which are the cells we will be using in our testing protocol, this includes all needed background information to work with them, maintenance, and how to keep them happy.

Content:

HEK Cells Background Research

- What Is HEK?
    - HEK293, Human Embryonic Kidney cells, is the most commonly used in the laboratory due to it's adherence to injected DNA (a non-enveloped virus with a nucleoid containing double-stranded DNA).
    - DNA makes a cell into a STEM cell that is capable of growing and functioning as a producer of various proteins and cells.
    - Used from low maintenance and rapid division.

- Real-Life Uses
    - Cancer Research, Vaccine development, protein productions, and drug testing…. And now phase microscope testing.

- How to Maintain
    - 37 degrees Celsius / 5% CO_2
    - High Glucose Media DMEM / FBS
    - Split cell cultures every 2 or so days, and throw out sample after 13 splits
    - Easy Tranfect Kit can be bought this is how to hand them
    - Watch for Mycoplasma infections

HEK Cells Protocol for Phase Microscopy testing

Figure 3.1: Phase contrast images of healthy suspension-adapted HEK293 cells in stationary culture. The cells were plated at a seeding density of 5 × 10^4 viable cells/cm^2 in 293 SFM II medium and grown as an unattached culture in a 37°C incubator with a humidified atmosphere of 5% CO_2 in air. The images were obtained using 10X and 20X objectives (panels A and B, respectively) 4 days after plating.

Image of the HEK cells

Conclusions/action items:

Start uses these HEK cells to test our microscope apparatuses.

11/14/20 Resolution Testing

CARSON EVENSTAD - Dec 07, 2020, 10:33 AM CST

Title: Resolution Testing

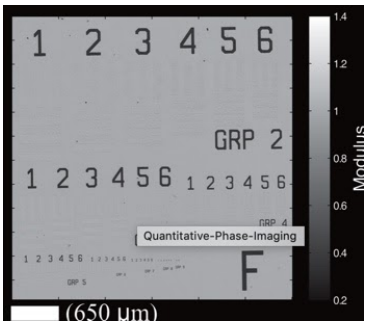
Date: 11/14/20

Content by: Carson Evenstad

Present: Carson Evenstad

Goals: Find a definite protocol to follow in order to test the resolution of our microscope parts, we already have a protocol for the area testing, but with this resolution test we will be able to test if our apparatus is able to save our problem statement (increase resolution)?

Content:



Protocol

- Use of **Resolution Target**
- This target as a series of different letters varying in shape and depths, this is to obtain different spatial frequencies, so measure the phase shifts.

- They use the sharpness of the edges to determine the amount of diffraction in the object lens, and by knowing the depths of the carved letters, a repeatable and accurate image can be used to find quantitative phase values.

Magnification	Lateral Resolution of images (µm)	Sensitivity to thickness (phase sample)	Sensitivity to thickness (rad)	Transfer of spatial frequencies
10x (NA = 0.25)	1.30	11	2π/126	Unity
20x (NA = 0.40)	0.81	11	2π/126	Unity
40x (NA = 0.65)	0.50	11	2π/126	Unity

This table is what they used to quantify their arguments, we can use this in our testing tables.

This is a great resource for the testing of phase microscopy

Useful website: https://www.phasefocus.com/resources/tech-notes/quantitative-phase-imaging-phasefocus-phase-calibration-target

Conclusions/action items: Start the testing phase of our project.

9/22/20 - Competing Ideas

CARSON EVENSTAD - Sep 22, 2020, 9:20 PM CDT

Title: Microscope Condensor Ideas

Date: 9/22/20

Content by: Carson Evenstad

Present: Carson Evenstad

Goals: Create Innovative ways to change the phase microscope in a way that will be cost-efficient, will expand the focal point centered on the specimen, and will not inhibit the resolution of the microscope

Content:

Microscope Condensor Extentions-

We will not change anything about the phase microscope body or anatomy, this will make sure we wil not lose any of the resolution because we are not changing anything about it, these extensions will connect the objective lens part of the microscope, and will continue a light, annulus, and condenser that will be angled to the side of the focal point, connection to it and further extending it.

Changing the Phase Microscope Illumination Systems -

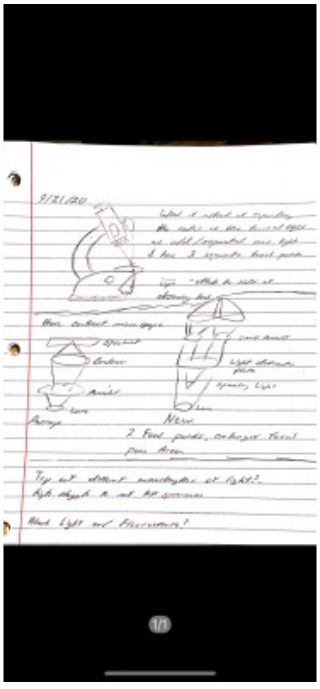
This idea will split the light into 2 separate rays of light, then will deflection to be parallel to each other. Depending on the limits, size, and diffraction, we can have to separate annulus to filter the incoming light and a convex condenser that will be able to filter both rays of light onto the specimen.

The other ideas are testing light at different intensities, without killing the specimen, and increasing the focal point. And the last idea was injecting the speicemen with fluorescent pigment and look into black lights.

Conclusions/action items:

Research the anatomy of the microscope to further understand its limits and abilities.

CARSON EVENSTAD - Sep 22, 2020, 9:22 PM CDT



Microscope_Ideas.pdf(2 MB) - download Some picture images of my ideas for the design matrix.

10/7/20 Design Ideas

CARSON EVENSTAD - Dec 07, 2020, 10:19 AM CST

Title: Desing Ideas

Date: 10/7/20

Content by: Carson Evenstad

Present: Carson Evenstad

Goals: Find previous, innovative ways the optics resolution problems are been faced in prior time that have been documented and try to adjust them to our current problem.

Content:

The first idea uses the use of nomarsk interference contrast in our microscope, this approach is used in the apochromatic lens (photographic lenses). This lens would be able to save our resolution problem but would have as much magnification as our current lenses, which might use a few cells in the cell count.

The second idea was the use of pice motors in order adjust the observation distance in order to change the location of the focal point and make the microscope self-focusing. These pico motors would be mounted to the sides of the microscope and by decision of out pf phase light, they would adjust the height of the microscope of the focus the light.

Conclusions/action items:

Keep building on these ideas and hopefully, one of them will well rounded enough to be incorporated.

09/13/20 Brainxell Microscope Research

CARSON EVENSTAD - Sep 16, 2020, 5:04 PM CDT

Title: BrainXell Research

Date: 9/13/20

Content by: Carson Evenstad

Present: Carson Evenstad

Goals: Obtaining knowledge about BrainXell and understanding the fundamental part of the microscope and how the part that we are fabrication functions, as well as, overall usage and conceptions.

Content:

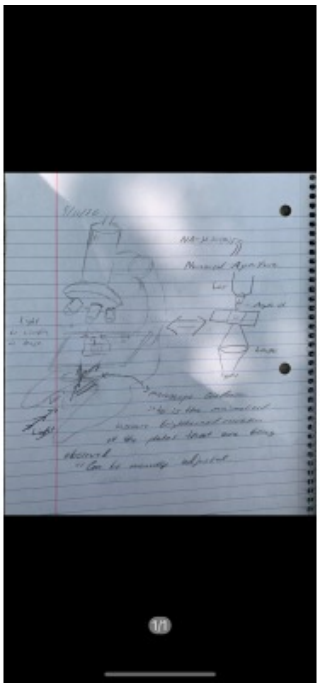
BrainXell has a company that sells neurons that can be injected into the body are being used to counteract/fight against neurological disorders. Their neuron has been advertised to be purely homogenous, with fast maturing stages, and can be made in high volumes. BrainXell reached out the University seeking a condenser for the microscope that will allow full visual of the plates. as well as, keeping the abiltity of advancing the contrast, brightness, resolution, the depth perception.

Attached is an image of the anatomy of a microscope with a condenser

Conclusions/action items:

Find patients and more research on micrscopes

CARSON EVENSTAD - Sep 16, 2020, 5:04 PM CDT



Microscope_image.pdf(4.1 MB) - download

9/16/20 Microscope Condensor Patents

CARSON EVENSTAD - Sep 16, 2020, 5:09 PM CDT

Title: Microscope Condensor Patents

Date: 9/16/20

Content by: Carson Evenstad

Present: Carson Evenstad

Goals: Researching already patents in microscope condensers and determine what we are still able to change and innovation to our condensers with violating copyright patents. Also more research on the regulation of fabrication (FDA approval) and anatomy of the microscope.

Content:

1. *Standards and Specifications:* 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.
2. *Customer:*
    1. The customer would like the condenser to be able to be adaptable to different kinds of plates, especially the Grainer-96 plates
    2. The client would prefer resolution of the edges of the visible lens to not be opaque and as transparent as the center of the visible lens.
    3. The customer would also like the resolution and contrast to be our highest priorities, with limiting tradeoff between them.

1. 3. *Patient-related concerns:*
    1. Microscopes should generally be sanitized and cleaned after 200 hours of service, or more frequently depending on daily usage.
    2. Microscope condensers are extremely fragile pieces and must be cleaned thoroughly and carefully.
    3. There is no specified shelf life for this device
4. *Competition:*
    1. 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. (1)
        1. This device has a similar illumination optical system and calculation.
    2. *Patent US 8576483B2* - This invention contains an illumination optic system, first and second image creation optic system, an illumination- focused diaphragm section
        1. This device has a more complex illumination optic system, however, may be similarities in sections.
    3. 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.

1. Has a variation retarder with multiple sections, each addressable by a control signal. Similar to our light diffraction mechanism.

Conclusions/action items:

Continue research

12/7/20 Poster Project

CARSON EVENSTAD - Dec 07, 2020, 10:58 AM CST

Title: Poster

Date: 12/7/20

Content by: Carson Evenstad, Kylie Gasper, Lauren Hicks, Ben Hildebrant, Katie Budde, and Sam Herzog**Present:** Carson Evensta**Goals:** Complete the poster as a team and go over my roles in creating the poster

Content: My main parts of the poster was the modification/ implication of pictures and figures, the design specification sections

Conclusions/action items: The next item, the final item on the do-to list, and the final report that will contain everything we have done the entire year by summarizing all of the important parts.

CARSON EVENSTAD - Dec 07, 2020, 11:00 AM CST

Image_12-7-20_at_10.49_AM.jpg(287.8 KB) - download The first figure is our completed poster that we did as a team and the design specification sections were one of the parts that I did.

CARSON EVENSTAD - Dec 07, 2020, 11:00 AM CST

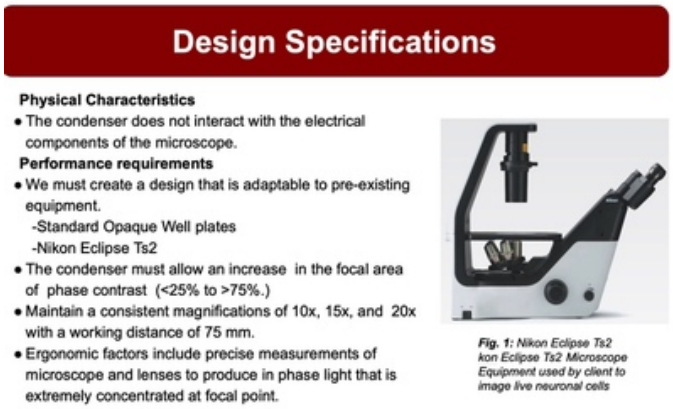


Image_12-7-20_at_10.48_AM.jpg(131.1 KB) - download The first figure is our completed poster that we did as a team and the design specification sections were one of the parts that I did.

Research week 1

SAMUEL HERZOG - Sep 16, 2020, 6:33 PM CDT

Title: Week 1 Research

Date: 9-14-2020

Content by: Sam Herzog

Present: N/A

Goals: To come to a better understanding of how the components of a phase-contrast microscope compile to make the image seen in the specimen field.

Content:

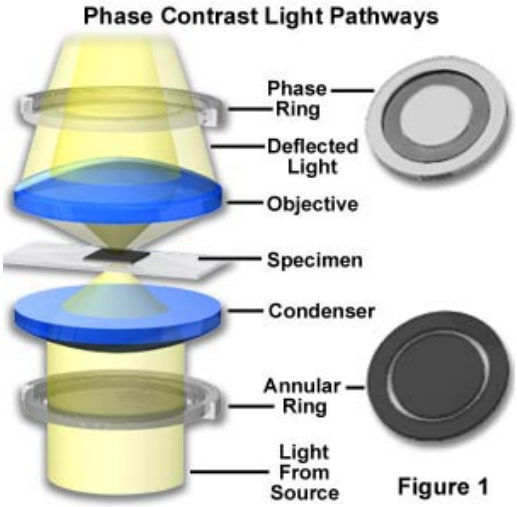


Figure 1

Phase-contrast microscopy is a method of microscopy that allows observation of objects that are hard to see with a brightfield microscope. The way they work is by making the light "out-of-phase". This means that the waves are out of sync by 1/2 wavelength. By making the waves out of sync by 1/2 of a wavelength, the light is amplified because of constructive interference. The higher amplitude allows for previously "invisible" objects to become visible. Phase-contrast microscopes are typically used for living things like cells.

Conclusions/action items:

ibidi, M., Techniques, M., Contrast, -., Well, µ., Luer, µ., Bottom, µ. and µ-Dish 35 mm, h., 2020. *Phase Contrast Microscopy | Principle And Applications | Ibidi*. [online] Ibidi.com. Available at: <https://ibidi.com/content/213-phase-contrast> [Accessed 16 September 2020].

Prof. Rogers notes

SAMUEL HERZOG - Oct 06, 2020, 12:50 PM CDT

Title: Meeting with Professor Rogers

Date: 9-30-2020

Content by: Sam Herzog

Present: n/a

Goals: Take notes on the basics of phase-contrast microscopy, and introduce our problem and get feedback.

Content:

- This could be a mechanical problem or it could be an optics problem.
    - The
- Why is the microscope not focusing?
    - Köhler Illumination
    - The Cone of light (illumination) needs to be focused. (I.e. optical solution)
    - The illumination cone is too high or too low and not focusing on the cells. (I.e. mechanical solution)
- Inverted microscopes change how the cone of light behaves around the well plate as well as the medium that the cells are in.
    - It could be that the solution that the cells are in is obscuring the light for best viewing.
- Make sure the objective point and focal point are in the same location. (KEY)
- Make sure to go through Nikon Eclipse Ts2 user manual and specifications.

Conclusions/action items: Successful session with Prof. Rogers. We learned a lot about different potential solutions to the problem as well as some testing methods to make sure we are doing accurate work.

Research PDS

SAMUEL HERZOG - Oct 06, 2020, 1:06 PM CDT

Title: Research for the PDS

Date: 9-17-2020

Content by: Sam Herzog

Present: n/a

Goals: To find sufficient information on the ergonomics, shelf life, and operating environment.

Content:

- Operating environment
    - Must be between 32^{o}-104^{o}F with humidity no higher than 85%.
    - The less humid the environment the better phase-contrast works.
    - Store out of the sunlight, in low humidity, and keep the microscope dry for ideal usage and shelf life.
    - Use a dust cover when not using the microscope.
- Shelf life
    - They don't really have a shelf life; however, there are ways to keep the microscope in a better condition.
- Ergonomics
    - The piece that we are dealing with on the microscope does not come into contact with any humans.
    - 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.

Conclusions/action items:

Nikon Esclipse Ts2 specifications/dimensions

SAMUEL HERZOG - Oct 21, 2020, 9:08 PM CDT

Title: Nikon Eclipse Ts2 specifications and dimensions

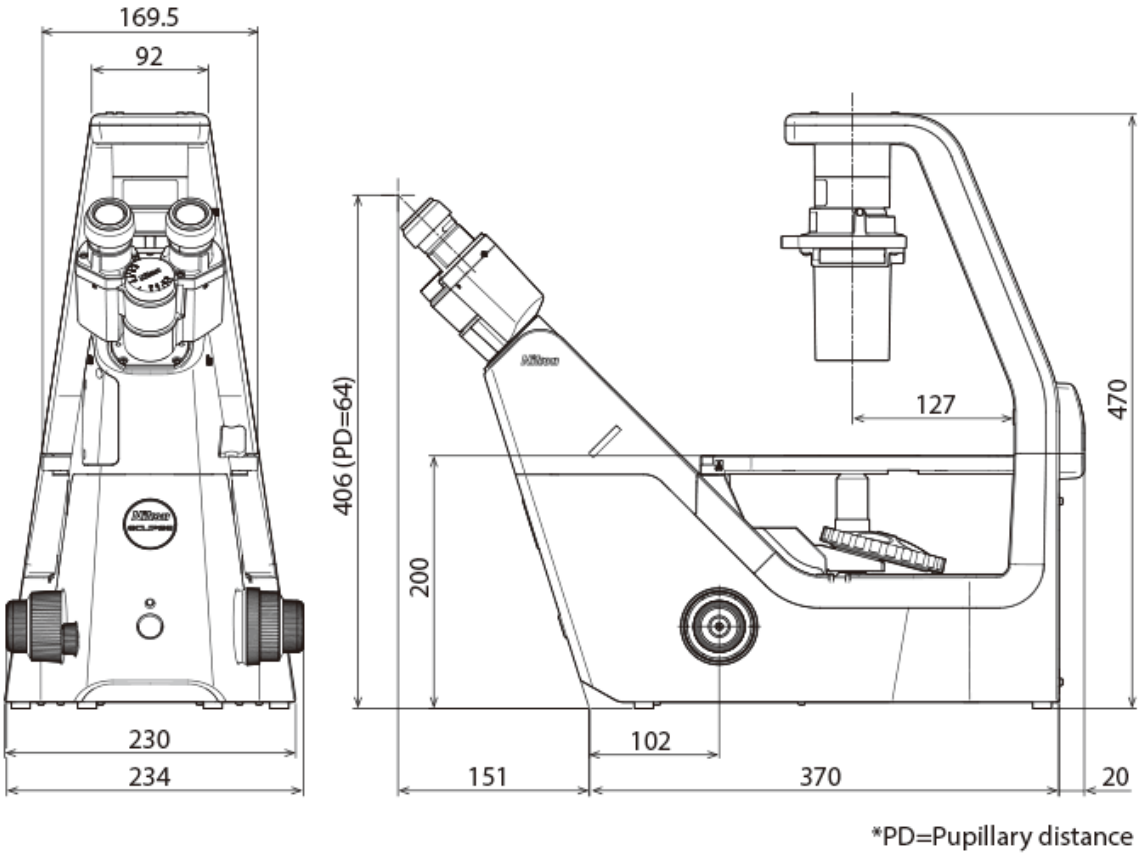
Date: 10-21-2020

Content by: Sam Herzog

Present: N/a

Goals: Outline the measurements of the condenser, and evaluate the space available to add another lens.

Content:



Condenser: ELWD Condenser (NA 0.3、W.D. 75 mm)

Conclusions/action items: https://www.nikon.com/products/microscope-solutions/lineup/inverted/ts2/spec.htm

Literature for Similar Microscopy Experiments

SAMUEL HERZOG - Nov 12, 2020, 2:19 PM CST

Title: Literature for Similar Microscopy Experiments

Date: 11-12-2020

Content by: Sam Herzog

Present: N/A

Goals: Find other experiments that are researching similar concepts as our team is. For example, the team may be testing to see the effects of changing the aperture or condenser of a phase microscopy system to improve efficiency.

Content: A team of scientists from Nanjing University of Science and Technology in China has looked into the idea of changing the aperture of the phase microscopy system to increase the effective area seen when using high-quality imaging techniques. This Is very similar to our project because we have the same goal in the long run. In their experiment, they decided to change the circular illumination aperture with an annular aperture. This increased the phase contrast at low frequencies and helped the team to reach the incoherent diffraction limit, which is useful in the imaging of cellular structures. One important difference is that Zuo's team used fixed cells instead of living cells in a well plate. With our hypothesis that the cone of light is being clipped by the well plate, the results of their experiment would not help us. It is worth looking into this issue as a potential solution to our problem provided that our original hypothesis does not hold.

Conclusions/action items:

Zuo, C., Sun, J., Li, J. *et al.* High-resolution transport-of-intensity quantitative phase microscopy with annular illumination. *Sci Rep* 7, 7654 (2017). https://doi.org/10.1038/s41598-017-06837-1

Design ideas for Condenser

SAMUEL HERZOG - Dec 07, 2020, 5:33 PM CST

Title: Design Ideas for Condenser on a Phase Microscope

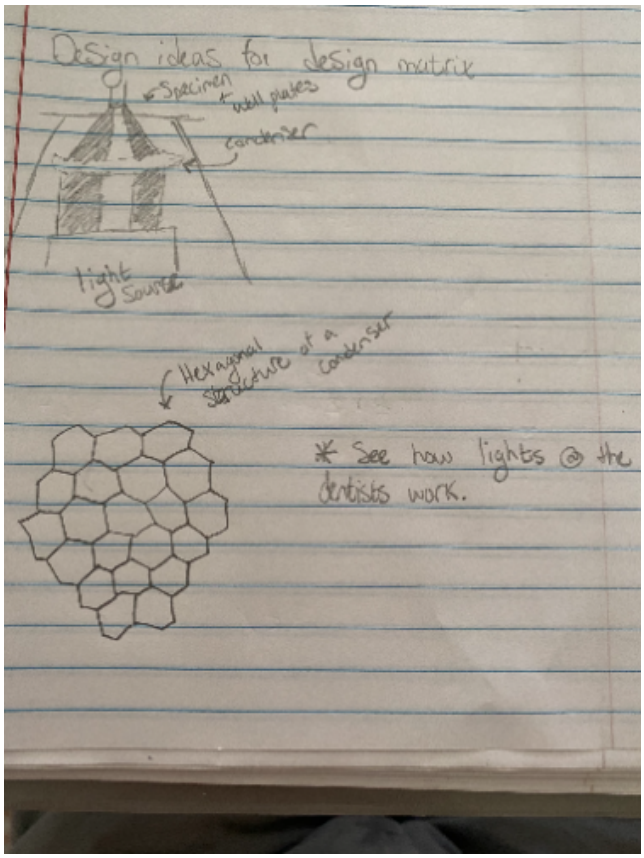
Date: 9-24-2020

Content by: Samuel Herzog

Present: N/A

Goals: To come up with possible solutions to the problem at hand.

Content:



In the top image, I have a slightly thinner condenser than one seen on the microscopes currently. In the bottom image, I have a hexagonal structure that might be better at illuminating the specimen. When I had my wisdom teeth removed, the light source had this background structure and seemed to concentrate on my mouth nicely and did not bother my eyes when I looked at it.

Conclusions/action items:

Final Design Ideas

SAMUEL HERZOG - Dec 07, 2020, 5:37 PM CST

Title: Final Design Ideas for Phase Contrast Microscope

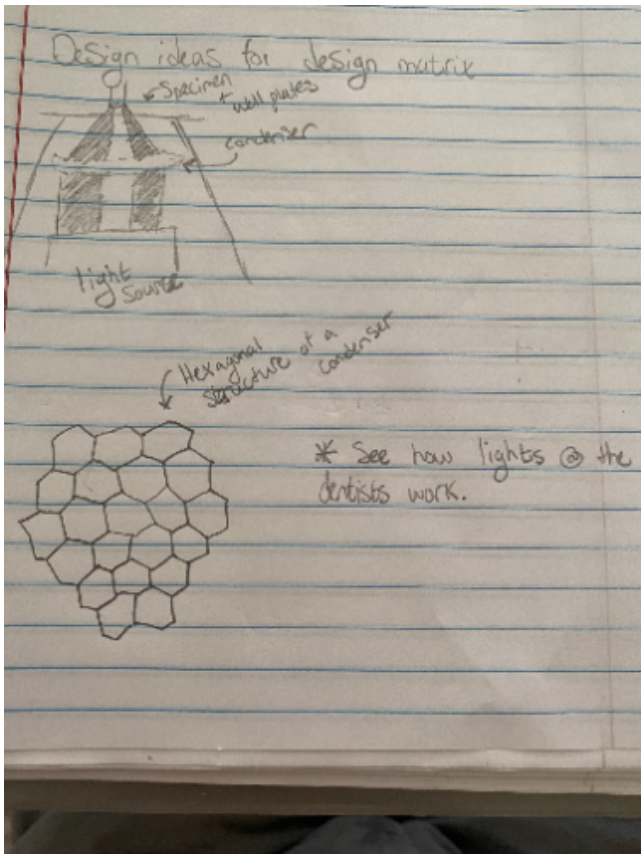
Date: 12-7-2020

Content by: Samuel Herzog

Present: N/A

Goals: Come up with a final design idea to increase the effective area of phase contrast.

Content:



In the top image, I have a slightly thinner condenser than one seen on the microscopes currently. In the bottom image, I have a hexagonal structure that might be better at illuminating the specimen. The light source is supposed to concentrate the light cone better to a more confined area. This will could solve the solution to our hypothesis: the light is being clipped by the edge of the well plate.

2020/10/20 - Makerspace 3D-Printing Materials

LAUREN HICKS - Nov 05, 2020, 4:45 PM CST

Title: Makerspace 3D-Printing Materials

Date: 10/20/2020

Content by: Lauren Hicks

Present: NA

Goals: To research feasible materials at the Makerspace for our prototype

Content:

- The standard for condenser annulus is an opaque material
    - Using the Ultimaker 3D printer we could use Tough PLA which is sturdy, high melting point, black material
        - Pricing is $0.08 per gram
    - Using the Formlabs 3D printer we could use Black which is sturdy, black, and a melting point
        - Pricing is $0.24 per gram

References:

Mini-Mart, U., 2020. *UW Makerspace Mini-Mart*. [online] UW Makerspace. Available at: <https://making.engr.wisc.edu/mini-mart/> [Accessed 20 October 2020].

Conclusions: I was able to find materials that are available for purchase at the Makerspace.

Action items: We can now use this information on our PDS.

2020/09/13 - Phase-Contrast Microscopes

LAUREN HICKS - Sep 16, 2020, 7:19 PM CDT

Title: Background Information on Phase-Contrast Microscopes

Date: 13 September 2020

Content by: Lauren Hicks

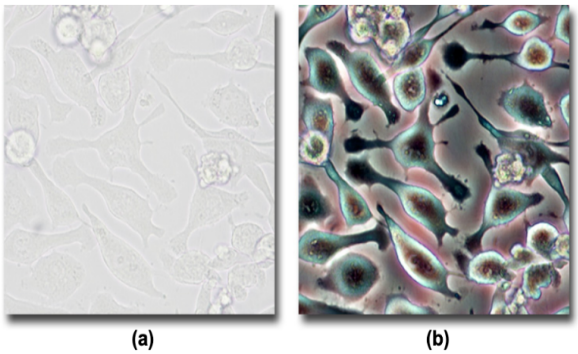
Present: N/A

Goals: To gain an understanding of what a phase-contrast microscope is, what is it used for, and what are some limitations.

Content:

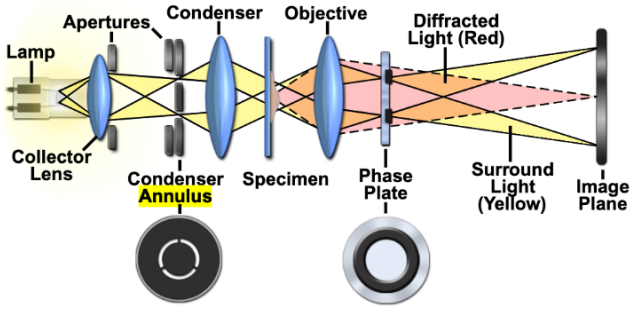
- Create high-contrast images of a transparent specimen, allows for living cells to observed (without staining, which can harm live cells)
- Phase object - light waves that are diffracted and shifted in phase by the specimen
- Below this figure shows how significant of a different phase contrast makes

Figure 2 - Living Cells in Brightfield and Phase Contrast



- Below this figure shows the order of a phase-contrast microscope

Figure 4 - Phase Contrast Microscope Optical Train



-
- Condenser Annulus - limits the angle of penetrating light waves
- Condenser - reduces the amount of light and increases the contrast of the image

Conclusions/action items: I have gained a basic understanding of what Phase-Contrast Microscopes are.

Resources:

Carr, K. and Davidson, M., 2020. *Basic Microscope Ergonomics*. [online] Nikon's MicroscopyU. Available at: <https://www.microscopyu.com/microscopy-basics/basic-microscope-ergonomics> [Accessed 16 September 2020].

Ockenga, W., 2020. *Phase Contrast*. [online] Leica-microsystems.com. Available at: <https://www.leica-microsystems.com/science-lab/phase-contrast/> [Accessed 17 September 2020].

2020/09/16 - Quantitative Standards

LAUREN HICKS - Sep 16, 2020, 7:16 PM CDT

Title: Quantitative Standards for Phase-Contrast Microscopes

Date: 16 September 2020

Content by: Lauren Hicks

Present: N/A

Goals: To identify the standards in size, weight, materials, and aesthetics of the objectives and condensers of phase-contrast microscopes.

Content:

- **condenser** - is an optical lens that renders a divergent beam from a point source into a parallel or converging beam to illuminate an object
- The below image shows how NA is related to the cone size and shape of the condenser:

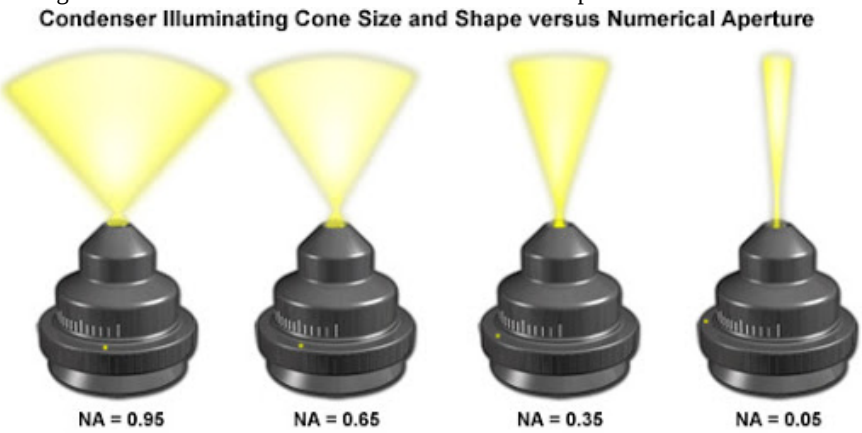


Figure 1

- The average size for a Nikon condenser (common manufacturer of microscopes/microscope accessories) ranges from 1.80-2.20 mm in diameter
- The average weight for a Nikon condenser ranges from 90-200 grams
- Condenser materials should be taken into consideration with the watts of lamp used. Common materials for condensers include plastic or metal. The field lens should be constructed out of glass.
- The form and texture of the finish of the condenser are standardized with the given microscope. The color and shape are two variables we are going to be researching further to see if they could have the desired effect.

Conclusions/action items: With the research I have conducted, I am able to complete a portion of the Preliminary PDS.

Resources:

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].

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].

2020/09/17 - PDS Research

LAUREN HICKS - Sep 30, 2020, 8:24 PM CDT

Title: Background Information on Phase-Contrast Microscopes

Date: 17 September 2020

Content by: Lauren Hicks

Present: N/A

Goals: To gain an understanding of what Numerical Aperture is, what is it used for, and what are some limitations.

Content:

- *Size:*
    1. The condenser must fit in the space of current standard condensers:
    2. The current condenser used in the Nikon ECLIPSE Ts2 is the ELWD Condenser which must not exceed 75 mm in length. [2]
- *Weight:*
    1. The condenser must weigh between 90-200 grams.[5]
- *Materials:*
    1. Condenser materials should be taken into consideration with the watts of lamp used. Common materials for condensers include plastic or metal.
    2. The field lens should be constructed out of glass.[6]
- *Aesthetics, Appearance, and Finish:*
    1. The form and texture of the finish of the condenser must be standardized with the Nikon ECLIPSE Ts2 microscope.
    2. The color and shape should be consistent with current condensers (black and cylindrical) [2]

Conclusions/action items: I have gained an understanding of what Numerical Aperture is.

Resources:

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].

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].

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].

2020/09/26 - Numerical Aperture

LAUREN HICKS - Sep

Title: Background Information on Phase-Contrast Microscopes

Date: 26 September 2020

Content by: Lauren Hicks

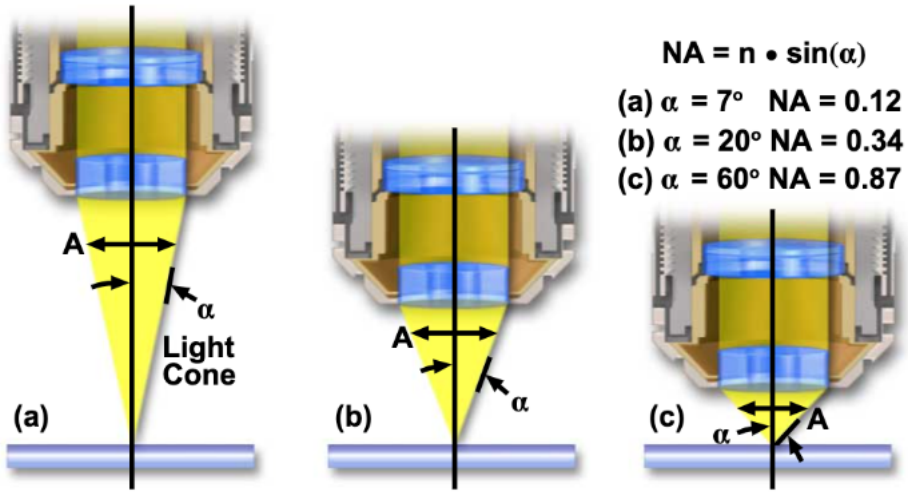
Present: N/A

Goals: To gain an understanding of what Numerical Aperture is, what is it used for, and what are some limitations.

Content:

- Numerical Aperture (NA) - measures the angular acceptance for incoming light
- With a medium with a higher refraction index (n) it will increase the NA
    - As the equation is NA = (n)(sin(α)
- Air refraction index = 1.00
- Water refraction index = 1.33
- Glycerin refraction index = 1.47
- Immersion oil refraction index = 1.51

Figure 1 - Numerical Aperture



Conclusions/action item

understanding of what Numerical Aperture is.

Resources:

Nikon's MicroscopyU. 2020. *Numerical Aperture*. [online] Available at: <https://www.microscopyu.com/microscopy-basics/numerical-aperture#:~:text=Numerical%20aperture%20is%20a%20measure,rays%20captured%20by%20the%20objective.&text=Higher%20numerical%20apertures%20can%20be,and%20the%20objec
[Accessed 26 September 2020].

2020/11/05 - Adaptive Illumination Techniques

LAUREN HICKS - Nov 10, 2020, 8:06 PM CST

Title: Adaptive Illumination Techniques

Date: 11/05/2020

Content by: Lauren Hicks

Present: NA

Goals: To research adaptive illumination techniques for phase contrast microscopy

Content:

- The reason that resolution goes down is that when the imaging comes near the well plate, the light cone is refracted differently and misaligns the phase ring.
- Due to the small diameter of the well plates, surface tension can create meniscuses that would refract the illumination cone.
- Two approaches
    - Tilting the entire illumination cone, a ring shift
        - fails when meniscuses are created
    - Mulitple discrete illumination angles to increase angular range
        - spatial resolution improved
        - halo-reduction
            - using a split plate to refract light

Conclusions/action items: Our prototype might have luck with testing since the research this article has conducted aligns with what we plan on fabricating for our prototype.

Research:

Hofmeister, A., Thalhammer, G., Ritsch-Marte, M. and Jesacher, A., 2020. Adaptive illumination for optimal image quality in phase contrast microscopy. *Optics Communications*, 459, p.124972.

2020/11/05 - Without Condenser

LAUREN HICKS - Nov 05, 2020, 9:51 PM CST

Title: Phase Contrast without Condenser

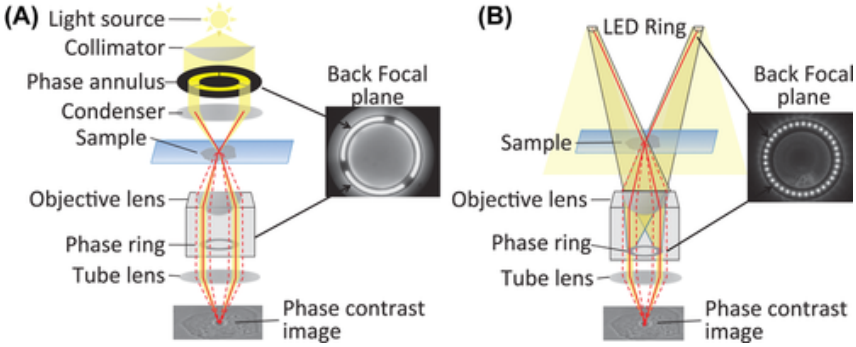
Date: 11/05/2020

Content by: Lauren Hicks

Present: NA

Goals: To research the effects of the elimination of the condenser from phase-contrast microscopy

Content:



- Allows for the object's back focal plane to light up the image (b)

Conclusions/action items: This could be a good direction for our group to consider if we are unsuccessful with our first prototype.

Research:

WEBB, K., 2014. Condenser-free contrast methods for transmitted-light microscopy. *Journal of Microscopy*, 257(1), pp.8-22.

2020/12/3 - Removal of Annuli

LAUREN HICKS - Dec 07, 2020, 9:49 PM CST

Title: Removal of Annuli

Date: 12/03/2020

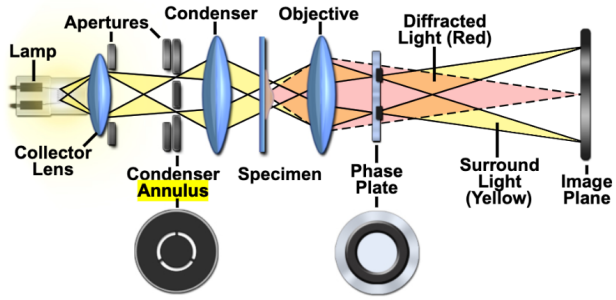
Content by: Lauren Hicks

Present: NA

Goals: To research future works for our design project

Content:

- Observing the effects of removing the annuli completely.
    - The annuli limits aperture which can decrease resolution.



    - This figure shows how the annulus is oriented in a phase-contrast microscope.

Conclusions/action items: The removal of the annulus might serve to be a pathway to look into for future work.

Research:

Nikon's MicroscopyU. 2020. *Introduction To Phase Contrast Microscopy*. [online] Available at: <https://www.microscopyu.com/techniques/phase-contrast/introduction-to-phase-contrast-microscopy> [Accessed 3 December 2020].

2020/12/3 - Increasing Light Sources

LAUREN HICKS - Dec 07, 2020, 9:49 PM CST

Title: Increasing the Number of Light Sources

Date: 12/03/2020

Content by: Lauren Hicks

Present: NA

Goals: To research future works for our design project

Content:

- Experimenting with multiple sources of light to observe if the increased amount of light would decrease the halo effect.

Conclusions/action items: The addition of light sources might serve to be a pathway to look into for future work.

Research:

Nikon's MicroscopyU. 2020. *Introduction To Phase Contrast Microscopy*. [online] Available at: <https://www.microscopyu.com/techniques/phase-contrast/introduction-to-phase-contrast-microscopy> [Accessed 3 December 2020].

2020/12/3 - Apodized Phase Plate

LAUREN HICKS - Dec 07, 2020, 9:48 PM CST

Title: Apodized Phase Plate

Date: 12/03/2020

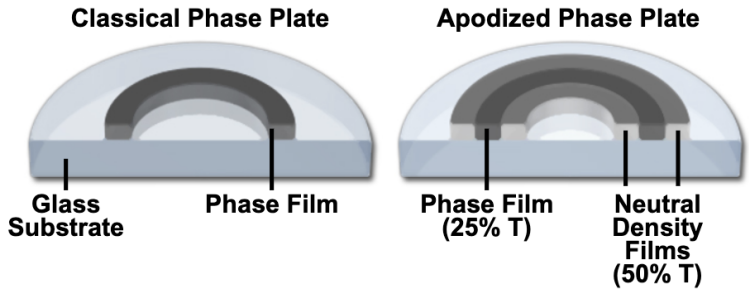
Content by: Lauren Hicks

Present: NA

Goals: To research future works for our design project

Content:

- Implementing an apodized phase plate
    - Means adding a neutral density material that will reduce the intensity of the light diffracted off of the specimen.



- This figure shows a neutral density film placed along the phase film to reduce the intensity of the light diffracted

Conclusions/action items: The usage of an apodized phase plate might serve to be a pathway to look into for future work.

Research:

Nikon's MicroscopyU. 2020. *Apodized Phase Contrast*. [online] Available at: <https://www.microscopyu.com/techniques/phase-contrast/apodized-phase-contrast> [Accessed 3 December 2020].

2020/11/03 - Design Ideas

LAUREN HICKS - Oct 05, 2020, 9:05 PM CDT

Title: Design Matrix Ideas

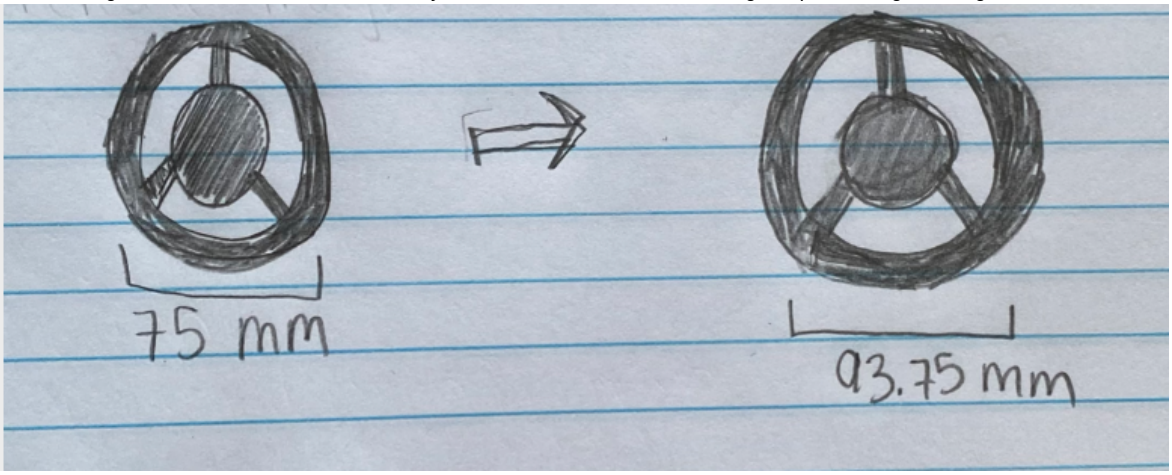
Date: 9/23/2020

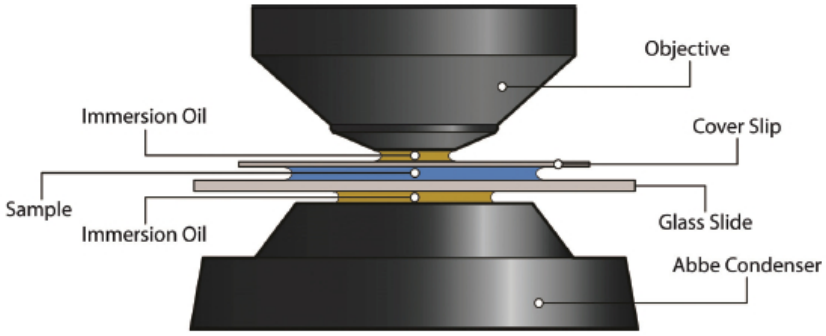
Content by: Lauren Hicks

Present: NA

Goals: To create two designs for the design matrix

Content:

- Design #1 - Increasing the size of the condenser annulus by 25%, which would allow for more light to pass through with a greater surface area.
  - 

- Design #2 - Increasing the Numerical Aperture (NA) by using oil instead of air because oil has a higher refraction index.
  - 

Conclusions/action items: I will present these designs to our group to see which ideas we should include in the design matrix.

2020/11/22 - Materials

LAUREN HICKS - Oct 21, 2020, 10:09 PM CDT

Title: Materials Available at Makerspace for Holder

Date: October 21, 2020

Content by: Lauren Hicks

Present: NA

Goals: To research materials available at the Makerspace for the production of a lens holder

Content:

- The standard for condenser annulus is an opaque material
    - Using the Ultimaker 3D printer we could use Tough PLA which is sturdy, high melting point, black material
        - Pricing is $0.08 per gram
    - Using the Formlabs 3D printer we could use Black which is sturdy, black, and a melting point
        - Pricing is $0.24 per gram

References:

Formlabs. 2020. *Formlabs 3D Printing Resin Materials Library*. [online] Available at: <https://formlabs.com/materials/> [Accessed 22 October 2020].

Mini-Mart, U., 2020. *UW Makerspace Mini-Mart*. [online] UW Makerspace. Available at: <https://making.engr.wisc.edu/mini-mart/> [Accessed 22 October 2020].

Nikon's MicroscopyU. 2020. *Optical Pathways In The Phase Contrast Microscope*. [online] Available at: <https://www.microscopyu.com/tutorials/optical-pathways-in-the-phase-contrast-microscope#:~:text=The%20condenser%20annulus%20(illustrated%20in,light%20wavefronts%20emanating%20from%20the> [Accessed 22 October 2020].

ultimaker.com. 2020. *Ultimaker Tough PLA Material: Create Durable Prototypes And Tooling*. [online] Available at: <https://ultimaker.com/materials/tough-pla> [Accessed 22 October 2020].

2020/09/18 - Week of 9/18 - 9/24

LAUREN HICKS - Dec 07, 2020, 9:53 PM CST

Title: Weekly Contribution Report

Date: 09/18/2020-9/24/2020

Content by: Lauren Hicks

Present: NA

Goals: Create a summary of project accomplishments for this week.

Content:

- Learned about optics and how they relate to phase microscopes through previous lectures in Physics 202 as well as Youtube videos.
- I did more research to create ideas for the design matrix.
    - See Research Notes => Initial Research => 2020/9/16 - Quantitative Standards
- I did research for the PDS
    - See Research Notes => Initial Research => 2020/9/17 - PDS Research

Conclusion: My goal is to strengthen my understanding of optics by reviewing more videos as well as practicing optic equation problems.

2020/09/25 - Week of 9/25 - 10/01

LAUREN HICKS - Dec 07, 2020, 9:54 PM CST

Title: Weekly Contribution Report

Date: 09/25/2020-10/1/2020

Content by: Lauren Hicks

Present: NA

Goals: Create a summary of project accomplishments for this week.

Content:

- Researched Numerical Aperture and its relationship to phase-contrast microscopy as well as different medium's refractive index.
    - See Research Notes => Initial Research => 2020/9/26 - Numerical Aperture
- Finalized designs for the Design Matrix
    - See Team Activities => Design Process => 2020/10/1 - Originial Design Matrix
- Worked on presentation.

Conclusion: Since I was unable to attend the meeting with Professor Rodgers, I will be watching the recording to learn more about optics and gain a better understanding of how we can work on our project solutions.

2020/10/02 - Week of 10/02 - 10/09

LAUREN HICKS - Dec 07, 2020, 9:54 PM CST

Title: Weekly Contribution Report

Date: 10/2/2020-10/9/2020

Content by: Lauren Hicks

Present: NA

Goals: Create a summary of project accomplishments for this week.

Content:

- I attended the Preliminary Oral Presentation section and actively asked questions to other groups.
- I worked on the Preliminary Report, specifically in the materials category as well as the abstract section.
    - See Research Notes => Initial Research => 2020/9/16 - Quantitative Standards

Conclusion: My goals this week are to collaborate with the team on finalizing our designs.

2020/10/10 - Week of 10/10 - 10/17

LAUREN HICKS - Dec 07, 2020, 9:54 PM CST

Title: Weekly Contribution Report

Date: 10/10/2020-10/17/2020

Content by: Lauren Hicks

Present: NA

Goals: Create a summary of project accomplishments for this week.

Content:

- Research Kohler Illumination and how it is applicable to our prototype.

Conclusion: Beginning a fabrication plan for our prototypes.

2020/10/18 - Week of 10/18 - 10/25

LAUREN HICKS - Dec 07, 2020, 9:54 PM CST

Title: Weekly Contribution Report

Date: 10/18/2020-10/25/2020

Content by: Lauren Hicks

Present: NA

Goals: Create a summary of project accomplishments for this week.

Content:

- I attended the team meeting where I was assigned the task of researching materials to use for our project.
- I then researched materials we can use through the Makerspace
    - See Research Notes => Materials => 2020/10/20 - Makerspace 3D-Printing Materials

Conclusion: I plan on working together with the team to establish a plan to create our prototype and to test it.

2020/10/26 - Week of 10/26 - 11/3

LAUREN HICKS - Dec 07, 2020, 9:54 PM CST

Title: Weekly Contribution Report

Date: 10/26/2020-11/3/2020

Content by: Lauren Hicks

Present: NA

Goals: Create a summary of project accomplishments for this week.

Content:

- Helped finalize our elevator pitch for Friday
- Went to our team meeting where we discussed what we need to achieve this week with fabrication.
- I began research on literature documents to find solutions to our problem.
    - See Research Notes => Literature Research
- I posted a question on pizza.

Conclusion: I plan on continuing research on different phase-contrast techniques.

2020/11/04 - Week of 11/04 -11/11

LAUREN HICKS - Dec 07, 2020, 9:54 PM CST

Title: Weekly Contribution Report

Date: 11/4/2020-11/11/2020

Content by: Lauren Hicks

Present: NA

Goals: Create a summary of project accomplishments for this week.

Content:

- Researched two literature articles, updated LabArchives
    - Research Notes => Literature Research
- Team meeting for the objective lens

Conclusion: My goal is to consult with the team on my research and see how that plays into our prototype.

2020/11/12 - Week of 11/12 -11/19

LAUREN HICKS - Dec 07, 2020, 9:54 PM CST

Title: Weekly Contribution Report

Date: 11/12/2020-11/19/2020

Content by: Lauren Hicks

Present: NA

Goals: Create a summary of project accomplishments for this week.

Content:

- Attended BME Guest Speaker Lecture
- Updated the BPAG Expense Spreadsheet
    - Team Activities => Materials and Expenses => BPAG Expense Spreadsheet

Conclusion: I plan on helping the team with testing our prototype and analyzing the results.

2020/11/20 - Week of 11/20 -11/27

LAUREN HICKS - Dec 07, 2020, 9:54 PM CST

Title: Weekly Contribution Report

Date: 11/20/2020-11/27/2020

Content by: Lauren Hicks

Present: NA

Goals: Create a summary of project accomplishments for this week.

Content:

- Reviewed the testing protocol
- Looked for ways to analyze our team's results after testing

Conclusion: Next week I hope to finish the testing and analysis of our results, as well as film the team’s final presentation.

2020/11/28 - Week of 11/28 -12/05

LAUREN HICKS - Dec 07, 2020, 9:57 PM CST

Title: Weekly Contribution Report

Date: 11/28/2020-12/05/2020

Content by: Lauren Hicks

Present: NA

Goals: Create a summary of project accomplishments for this week.

Content:

- Worked on our poster and final presentation.
- Attended the meeting with our advisor.
- Helped finalize the poster.
- Recorded the presentation.
- Watched BME Lecture 12/4 of Poster Presentations and peer-reviewed assigned projects.

Conclusion: Next week I hope to finish the final lab report and update lab archives.

2020/12/06 - Week of 12/06 -12/13

LAUREN HICKS - Dec 07, 2020, 10:01 PM CST

Title: Weekly Contribution Report

Date: 12/06/2020-12/13/2020

Content by: Lauren Hicks

Present: NA

Goals: Create a summary of project accomplishments for this week.

Content:

- Finalized the Final Report with group

Conclusion: Completed the project requirements.

2014/11/03-Entry guidelines

John Puccinelli - Sep 05, 2016, 1:18 PM CDT

Use this as a guide for every entry

- Every text entry of your notebook should have the **bold titles** below.
- Every page/entry should be **named starting with the date** of the entry's first creation/activity, subsequent material from future dates can be added later.

You can create a copy of the blank template by first opening the desired folder, clicking on "New", selecting "Copy Existing Page...", and then select "2014/11/03-Template")

Title: Descriptive title (i.e. Client Meeting)

Date: 9/5/2016

Content by: The one person who wrote the content

Present: Names of those present if more than just you (not necessary for individual work)

Goals: Establish clear goals for all text entries (meetings, individual work, etc.).

Content:

Contains clear and organized notes (also includes any references used)

Conclusions/action items:

Recap only the most significant findings and/or action items resulting from the entry.

2020/11/03-Template

John Puccinelli - Nov 03, 2014, 3:20 PM CST

Title:

Date:

Content by:

Present:

Goals:

Content:

Conclusions/action items: