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

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Abstract

Scientists who study live cells use phase microscopy to view their cells globally. Phase contrast requires distinct color contrast across the entire area

We looked for a universal, low cost way to adjust existing microscopes phase microscopy methods by designing a condenser modification to sit onto existing microscopes.

Our method was to make a modification specific to the side of a 96 well plates that adjusts the light in the well.

Our results shows that the lens holder did change the area of light and dark area from the original microscope set up, however at the cost of a lower resolution of cells. Additionally, we found that the more light let in by the annulus, the lower the area of phase contrast.

Problem Definition

Motivation

- BrainXell sees a small area of high contrast during phase contrast imaging of live neuronal cells.
- We looked to expand the area of effective phase contrast, while maintaining the high-quality contrast.

Background-Phase Contrast

1. Light comes from condenser
2. Goes through specimen
3. Creates final image from out of phase light due to cells it interfered with.

More lenses and refraction result in low resolution images

Design Specifications

- We must create a design that is adaptable to pre-existing equipment.
  - -Standard Opaque Well plates
  - -Nikon Eclipse Ts2 Microscope

Figure 1: Nikon Eclipse Ts2 Microscope Equipment used by client to image live neuronal cells

- Must not interact with the electrical components of the microscope
- Must increase in the focal area of phase contrast (<25% to >75%.)
- Maintain a consistent magnifications of 10x, 15x, and 20x with a working distance of 75 mm.

Extra Lenses Design

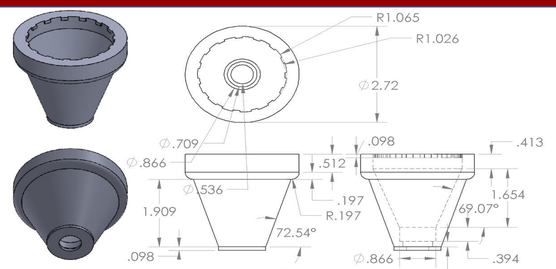

Fig. 2: Engineering Drawing in CAD of the 3D-printed lens holder for a concave lense at the tip of the cone, anda convex lens just above it

- Goal was to set additional lenses in the correct spot to narrow the cone of light
- Printed on a Form 2 printer using flexible material in order to mold to the condenser's shape with lenses purchased online.
- First lens condensed cone of light
- Second lens re-expanded cone of light to angle that wouldn't "clip" on the top of the well

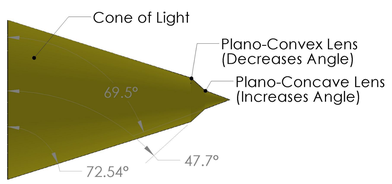

Fig. 3: Labeled Diagram of how the extra lenses condense and expand the cone of light.

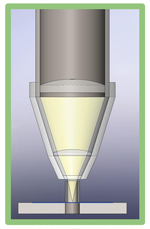

Fig 4. See through view of the illumination optics system used to condense the cone of light to above "clipping" on the well plate underneath

Future Works

Removal of Annuli

- Observing the effects of removing the annuli completely, since the annuli limit aperture which can decrease resolution.

Increased Light Sources

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

Apodized Phase Plate

Implementing an apodized phase plate which would add neutral density material that will reduce the intensity of the light diffracted.

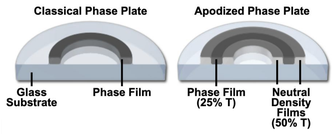

Fig 12; *A neutral density film placed along the phase film to reduce the intensity of the light diffracted*

Annulus

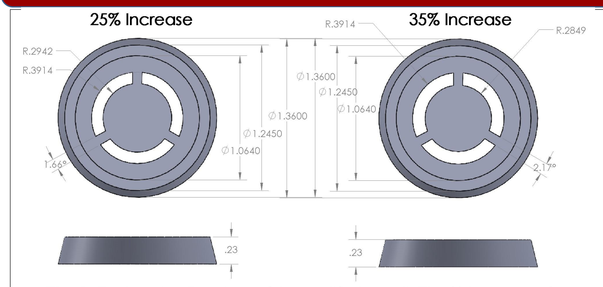

Fig. 5: Engineering Drawings of the modified annuli. The % increase refers to the negative (white) area seen on the frontal view of the design

- Goal was to increase "negative area" by 25 and 35 percent to allow more light in.
- Calculations were done to calculate inner diameter of ring (outside diameter was identical)
- Outside structure was designed to be identical to original annulus so they could be interchangeable

Fig. 6: Annulus Holder (Top), original (L), 25% (Mid), and 35% (R)

Discussion

A solution to this problems could be marketed and used easily because the design is set as an addition to existing devices, so there is no need for fabrication of expensive equipment. The results show that there is significance difference in area of contrast when adjustments are made with lenses used. We also learned that increasing the light through the annulus results in a lower quality image and should not be used. In the testing phase, we did not have access to neurons which made it harder to use the same quantification method as our client. The camera we used adjusted it's exposure automatically. Images washed out to the human eye appeared darker after taking the picture.

Acknowledgements

- BrainXell, Mr. Michael Henrickson, Dr. Kris Saha, Professor Jeremy Rogers, Madeline Smerchansky, and Joe Beck.

Testing

- Used a client provided microscope, fit our designs to Fisher Scientific Micromaster Inverted Microscope.
- We tested the lens holder design separately from the our designed annuli.
- Control pictures of each well were taken with the original microscope components.
- Our modified images are taken with either the lens holder attached or the modified annulus.
- To analyze our result, we used ImageJ. This program can measure the area of brighter phase contrast.
- We compared control images to images taken with final designs.
- The unit for area is in pixels.

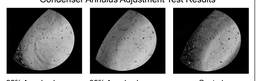

Fig. 7: *Testing Images from Condenser Annulus Attachment*

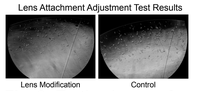

Fig. 8: *Testing Images from Lens Attachment*

Fig. 9: *The extra lens holder design is circled in green. The Design attaches to the extisting microscope condenser.*

Results

Significant change in dark areas from p value compared to control(α = .05)

Magnification	*Extra Lenses*	*Annuli (both)*
10x	Yes, p =.0022	No, p = .5301
20x	Yes, p=.0382	Yes, p =.0131

Extra Lenses- A significant decrease in dark area around the edge of the image was found when the extra lens design was used in conjunction with the existing condenser,

Annulus-When using the respective annuluses, no significant changes were seen with a 10x magnification, however when using 20x magnification, a significant decrease in dark area was found. There was a loss of phase contrast due to the "washing" out by the high amount of light let in by these designs.

Cell Count Test- We performed a cell count test for each set of well images, but the test yielded near-identical counts, showing that the number of visible cells was not affected.

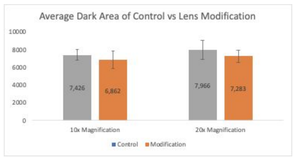

Fig. 10*: Lens Modifications Comparison Graph*

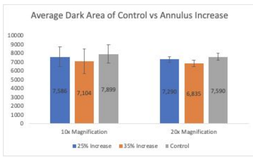

Fig. 11: Annulus Adjustment Comparison Graph

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