BrainXell: Phase Contrast Microscope Condenser for Observation of Multiwell Cell Culture Plates

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Overview

Background

- Who is BrainXell?
- Phase Microscopy
- The problem with phase contrast microscopy
- Existing Designs

Project

- Client Constraints
- Quantitative Constraints
- Designs
- Design Matrix
- Decision
- Future Works

Who is BrainXell?

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

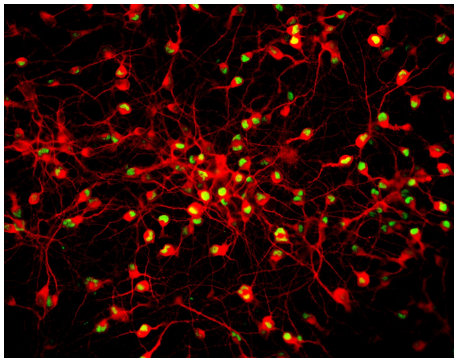


Figure 1: Layer V Glutamatergic Neurons grown by BrainXell

What is Phase Contrast Microscopy?

- For live cells
  - Developed by Frits Zernike in 1934
  - Passes a “cone of light” through specimen
  - Denser material diffracts light
  - Diffracted light is “out of phase”
- View “out of phase” light
  - Dark edges
  - Bright cells
  - Neurons, with long axon “tails”

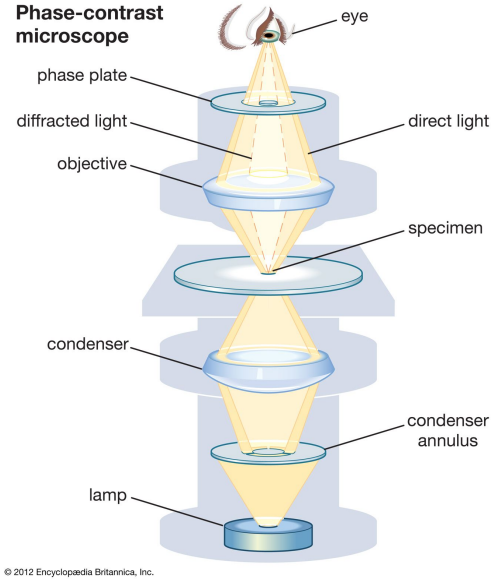


Figure 3: Light path of Phase-Contrast (source: Encyclopaedia Britannica)

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

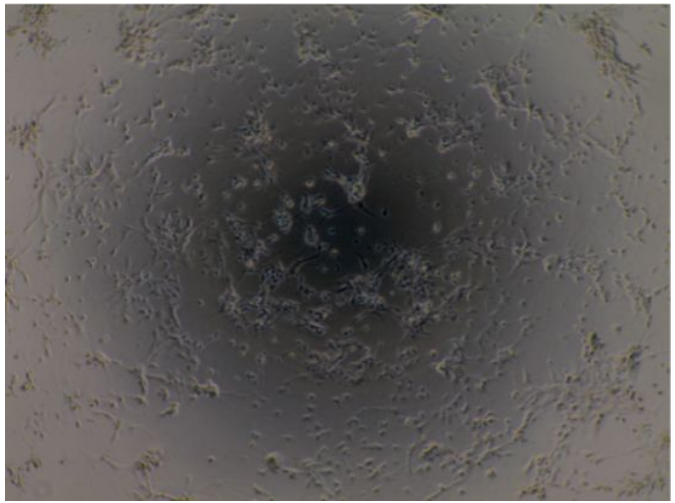


Figure 2: Example of ineffective area of phase contrast from BrainXell

Existing Designs

- Enhancing polarized light microscopy
  - Inventor: Rudolf Oldenbourg
  - Use semi-circular objectives lens
  - Multiple annulus for refractions
- Confocal scanning microscope
  - Inventor: William J. Fox
  - Used multiple sources of refracted light
  - Multiple Focal Points

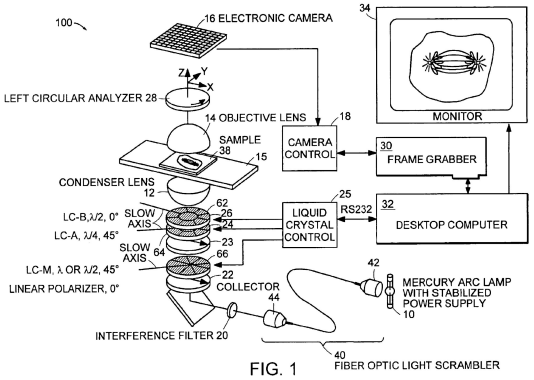


FIG. 1

Figure 4: *Oldenbourg's illumination Optics System*

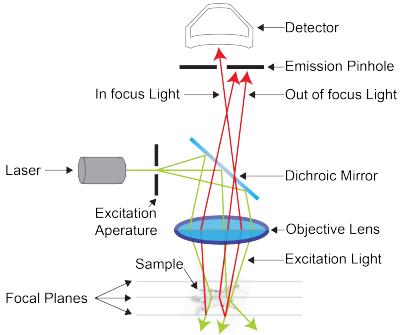


Figure 5: *Schematic diagram confocal light diffraction*

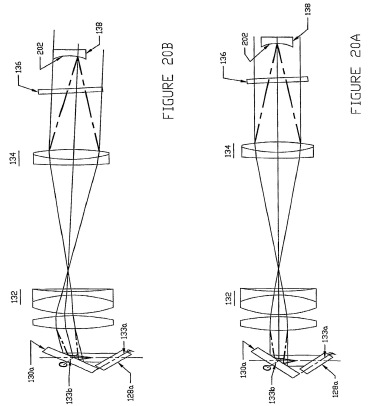


Figure 6 & 7: Two ways William Fox focused light

Client Constraints

- Expand the area of contrast
  - Allow resolution of focal point to the edges
- Have resolution and contrast as highest priorities
  - Possibly trading off lower resolution for higher area
- Stay within prototype budget given by BrainXell
  - $1,500
  - Average condenser today ~ $1,200
- Standard to Nikon ECLIPSE microscope

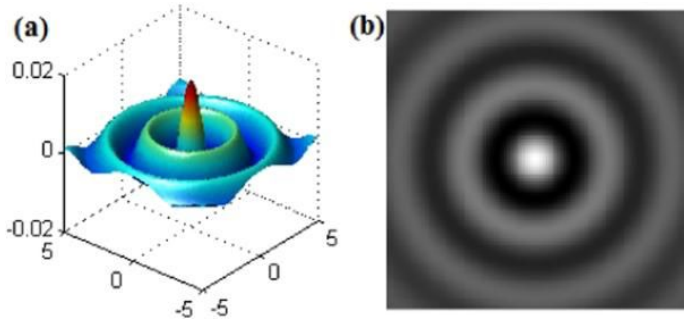


Figure 8: *Images depicting the focal point transparency of focal point in phase microscopes*

Quantified Constraints

- Increase effective area
  - ~25% to ~75%
- Maintain original resolution
  - 10X Magnification: 1.22µm
  - 20X Magnification: 0.959µm
  - Tolerance: ±25%
- Compatible with standard equipment:
  - Nikon ECLIPSE Ts2 microscope
  - 96-well plate (opaque)

Figure 9: Nikon ECLIPSE Ts2 microscope and standard 96-well plate (opaque)

Condenser Annulus Adjustment

- Allows for more light to pass through condenser annulus
- Doesn’t impact resolution
- Will increase amount of light going out of phase

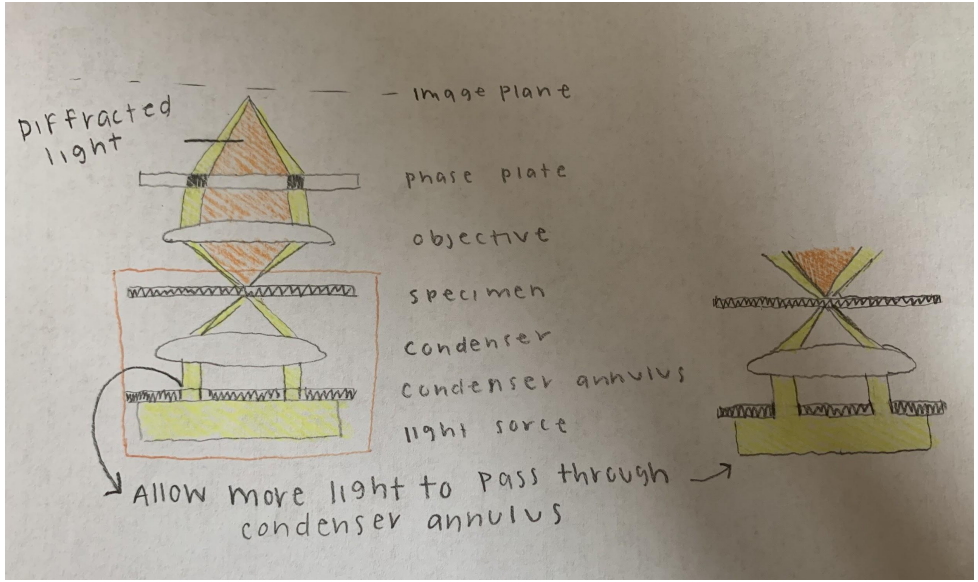


Figure 10: Sketch of annulus allowing more light from lamp into condenser

Using Oil to increase Numerical Aperture

- Oil has a higher refractive index
  - Air = 1.33, Oil = 1.51
- NA = nsin(theta)
  - NA = Numerical Aperture
  - n= refractive index
- Increase in NA causes increase in resolution

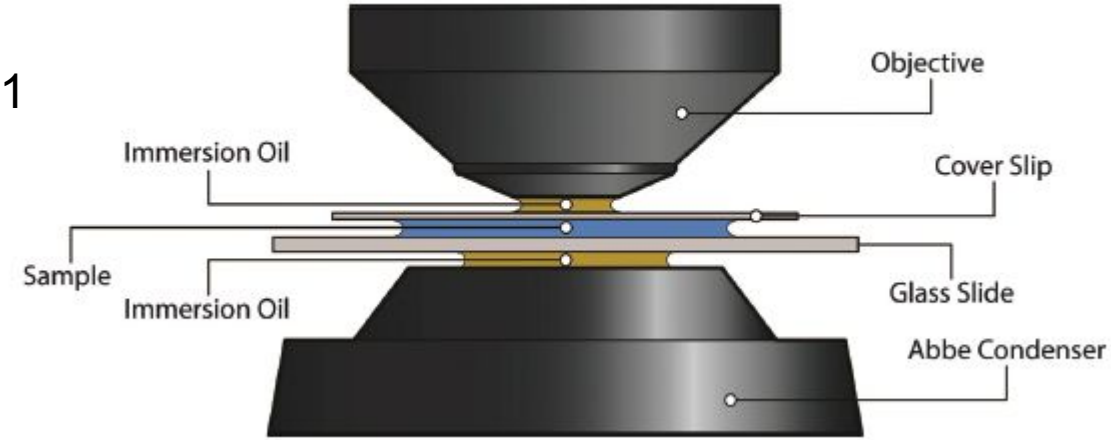


Figure 11: Diagram of an oil-immersed specimen slide

Objective Lens Attachments

- Doesn’t change the light path
  - Maintains original resolution
  - Guarantees visibility
- Fills in “clipped” portions of light
- Attaches to objective lens
  - 1-3 additional beams
  - Could be combined with other solutions

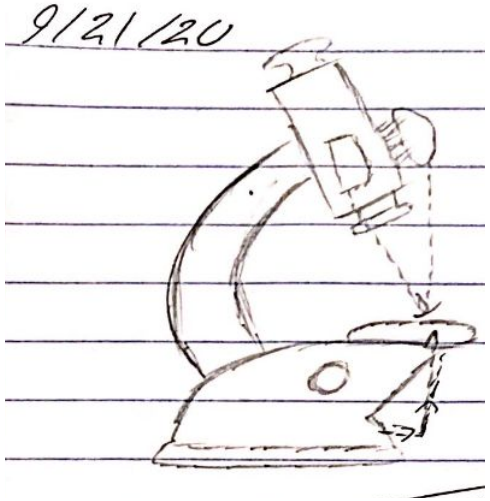


Figure 12: Initial sketch of attachments directed at specimen

Extra Lenses to Narrow Cone of Light

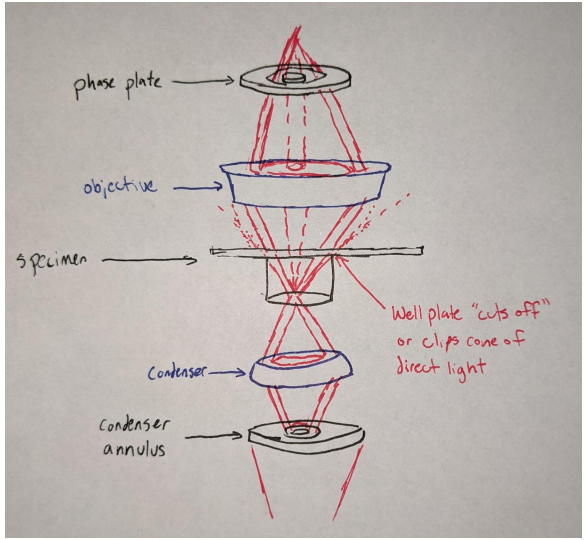


Figure 13: Sketch showing "clipping" of light (red dotted lines)

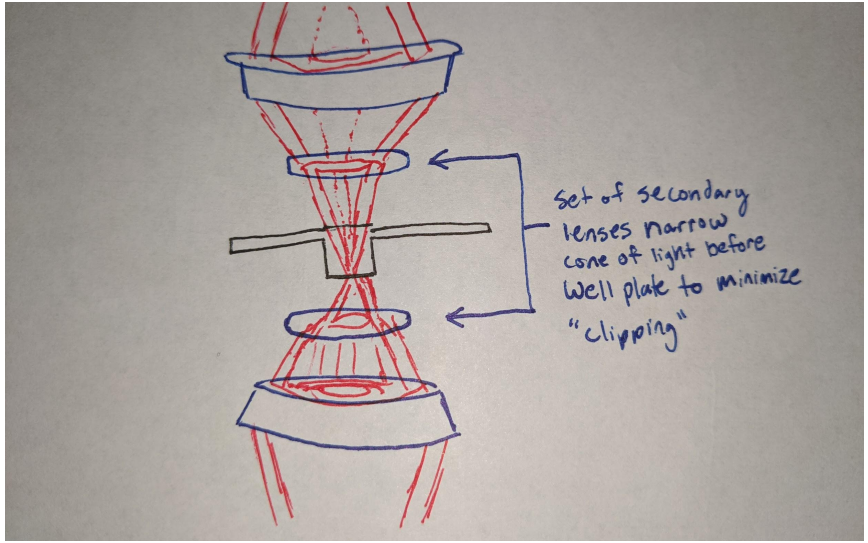


Figure 14: Sketch showing extra lenses narrowing the cone before and after the well plate

Design Matrix

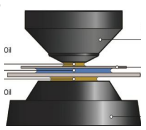
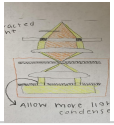
			Using Oil 		Objective Lens Attachments 		Condenser Annulus Adjustment 		Extra Lenses 	
Rank	Criteria	Weight	Score (10 max)	Weighted Score	Score (10 max)	Weighted Score	Score (10 max)	Weighted Score	Score (10 max)	Weighted Score
1	Effective Area seen by phase contrast	25	9/10	22.5	7/10	17.5	7/10	17.5	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

Figure 15: *Design Matrix*

Decision

- Hybrid of the annulus adjustment and extra lenses
- Incorporate mechanical condenser focusing knob
- Allows more light in
- Controls where light cone is focused

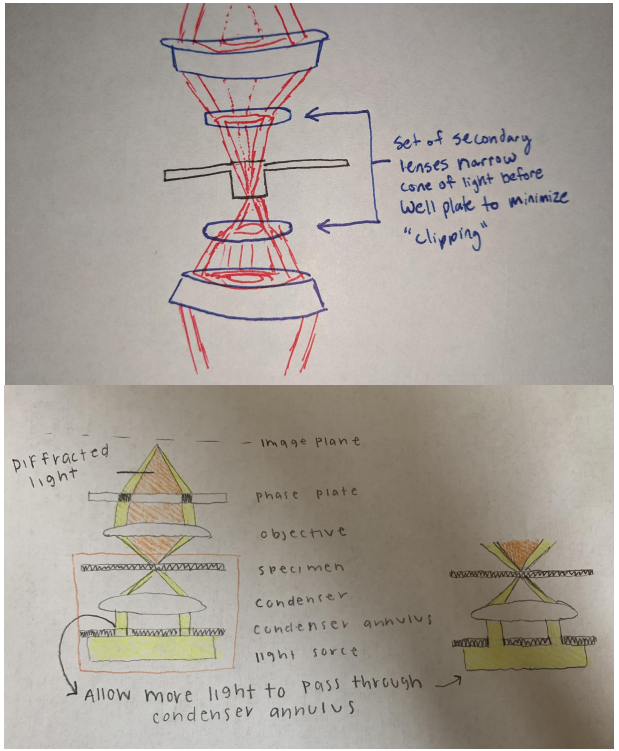


Figure 16: Sketches of extra lenses (top) and annulus adjustment (bottom) designs

Future works

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

Figure 17: Nikon Eclipse TS2, the microscope used by the client

Acknowledgements

Thanks to

- BrainXell
- Mr. Michael Henrickson
- Dr. Kris Saha
- Professor Jeremy Rogers

References

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