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Pre-Screening Individual Cells with Digital Holography

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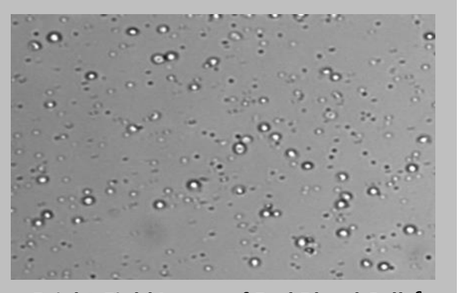
Abstract

Cell populations were thought to be homogenous, but recent research has uncovered that there is heterogeneity in protein and gene expression, and size within even small populations. Study of the causes of these differences will lead to advancement in many medical fields, namely cancer research and immunology. By combining the frontier of modern imaging, digital holography microscopy (DHM), with biological study, scientists can determine not only location and classification of cell types within a sample instantly, but also we can collect more data in single cell analysis than traditional microscopy. In this project, bright-field microscopy and confocal fluorescence microscopy was compared to both in-line and scattered DHM for visualizing fixed to live, multi-cell type samples in multiple dimensions. By surveying basic imaging scenarios and assessing the success of identifying cells, this project provides preliminary data towards advancing the utility of DHM in biomedical applications of single-cell analysis. As we explore this research further, it is possible that we can significantly speed up the pre-screening process using DHM which directly and positively impacts modern diagnostics.



Bright-Field Image of Red Blood Cells[6]

DHM Image of Red Blood Cells[8]

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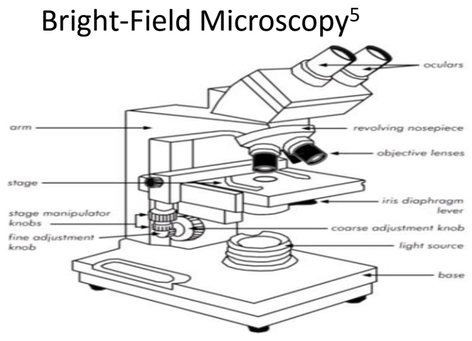
Red Blood Cell Interoferogram for DHM[3]

3D Reconstruction of Red Blood Cell[4]

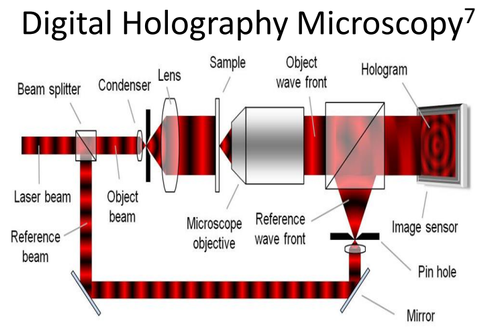
Background

Bright-Field Microscopy[5]

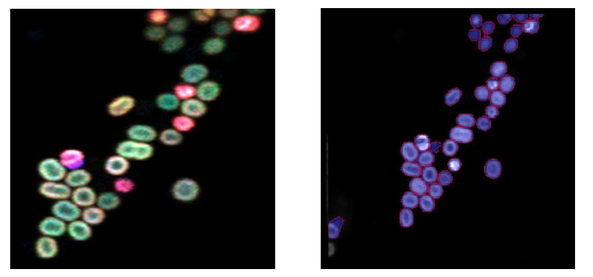


Vs.

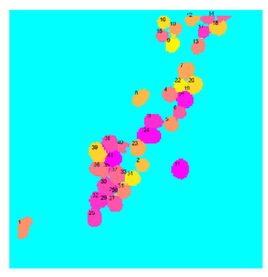
Digital Holography Microscopy[7]



Heterogeneity Among Cells



Cell Segmentation



Methods

Cell Culture

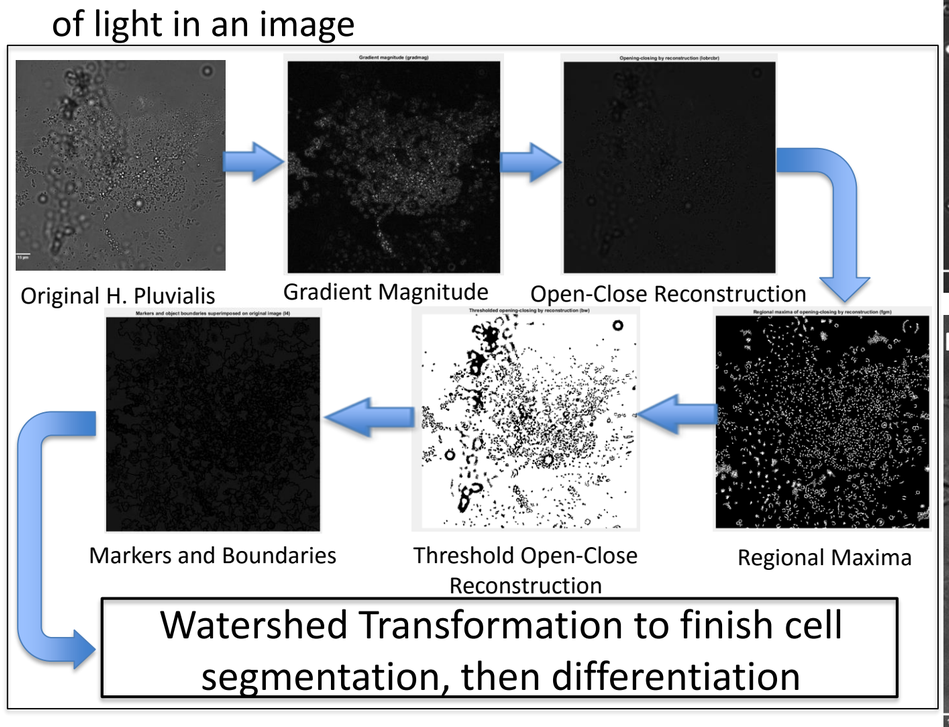
- Cultured Haematococcus pluvialis, Chlorella NC64A, Raw 2647, and HeLa cell types
- Adjusted cell to media ratio for best growth

Data Collection

- Mounted cell types on slides in various conditions
  - (Fixed) With and without mounting media (Prolong versus other sealant method)
  - (Live) With just PBS versus rinsed of PBS
  - (Live) With rinsed PBS versus with media
- Researched 3D cell culture for imaging
- Looked into various dyes
  - Fluorescent dye (CMO) that had a wavelength close to the absorbance necessary to emit light
  - Visual stains (Trypan Blue and Neutral Red)
- Analyzed single to multiple cell types
- Observing AR coated slides and coverslips effect
- Scattering with DHM for 3D cell imaging

Single Cell Analysis

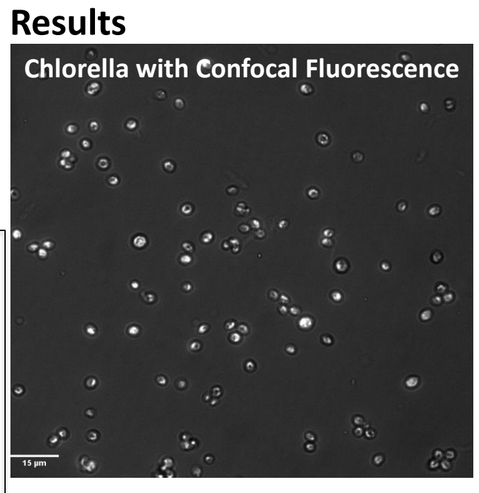
- Deciding which cell segmentation algorithm would be best for the input of the CCD camera
- Manipulating MATLab program to attempt to segment multiple cell types with an image input
- Using ImageJ to manipulate images for MATLab and exploring possibilities for better image processing
- Manipulating MATLab program to be able to differentiate cells based on size, shape, and intensity of light in an image

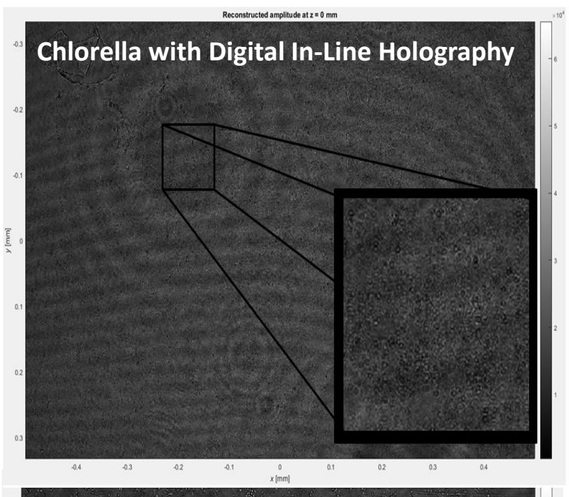


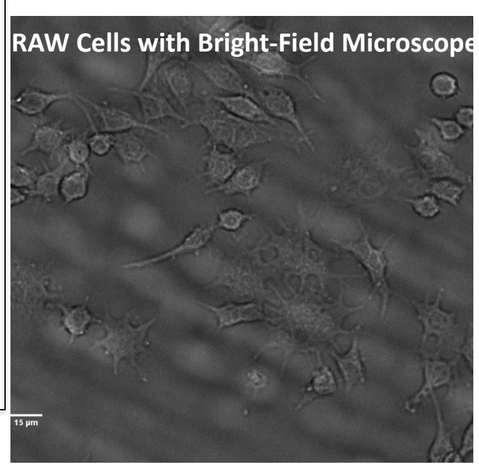
Conclusions

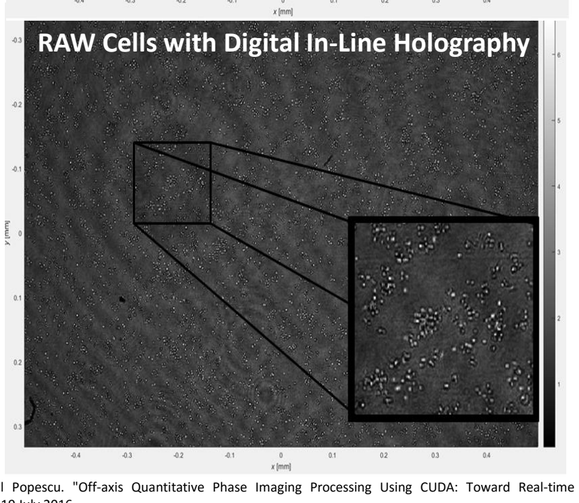
- Digital holography can be used to image biological samples, both fixed and live
- Digital holography yields higher resolution images than traditional microscopy and gives a better field of view
- Glass slides used for traditional imaging reflects and makes imaging a bit more difficult. Anti-Reflective coating on the slides may be useful
- Scattering with DHM is limited with basic laboratory slides
- Staining makes transparent specimen somewhat more detectable, but is not necessary to obtain a clear image and locate the specimen
  - Fluorescent dyes are not very helpful– given monochromatic nature of laser light source and filtering, light emission from the sample is not detectable
- Mounting mediums used for traditional biological imaging adds significant noise to the image, and removal of this noise *limits* the amount of data that can be collected from the image
  - *Limitation*: without mounting medium, the imaging "lifespan" of the biological images is significantly shortened, which means that there is not much time to manipulate the sample.
  - Agar coating on slides adds noise but is predictable enough of a pattern to subtract out of the final image
  - Mounting mediums add to preparation time

Results









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[3]Pham, Hoa, Huafeng Ding, Nahil Sobh, Minh Do, Sanjay Patel, and Gabriel Popescu. "Off-axis Quantitative Phase Imaging Processing Using CUDA: Toward Real-time Applications."*Biomedical Optics Express* 2.7 (n.d.): 1781-793. *OSA Publishing*. Web. 19 July 2016.
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[5]"What Are the Parts of the Brightfield Microscope? PreLab 3.8." *BIOL 1406, PreLab 3.8*. Austin Community College, n.d. Web. 19 July 2016.
[6]Willson, Amy. "Innovation and Expertise in the Science of Cell Counting." *Nexcelom Blog*. N.p., 16 Aug. 2012. Web. 19 July 2016.
[7]https://en.wikipedia.org/wiki/Digital_holographic_microscopy
[8]https://commons.wikimedia.org/wiki/File:DHM_image_of_human_red_blood_cells.jpg

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