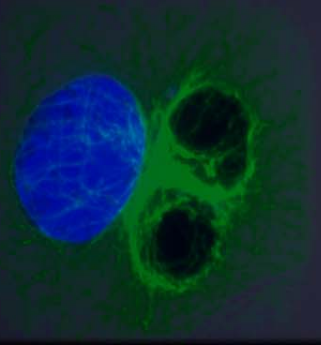
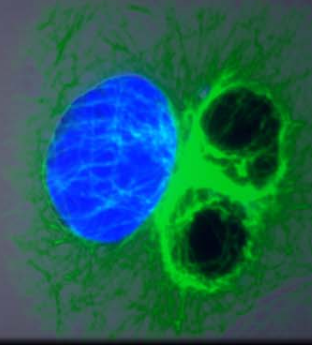


Analyzing COS1 Cells

for Studying Alzheimer's Disease



Vision Research Lab

Center for Bio-image Informatics

Ivan Villalba

Electrical Engineering

Cal Poly SLO

Oxnard College (2005)

Mentors

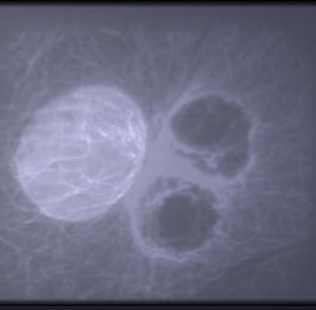
Jiyun Byun

DeeAnn Hartung

Faculty Advisor

Dr. B.S. Manjunath

Overview



- Introduction
  - Alzheimer's
- Image Analysis
  - Properties
  - Procedure
- Experimental Results
  - Cell Segmentation
  - Cell Density
- Future Work


Introduction

Alzheimer's

Characterized by two pathological hallmarks in the brain,

1. Extracellular Amyloid Plagues
   - Amyloid-Beta (Aβ) protein
2. Intracellular Neurofibrillary Tangles (NFTs)
   - Tau protein

Aβ Dysfunction —?→ Tau Dysfunction —Cell Death→ Alzheimer's

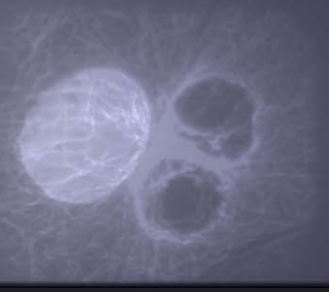
- Big Picture: Aβ and Tau
  - Examining molecular mechanism of cell death.

Why COS1 Cells?

- Model System
  - Monkey Kidney Cells
  - Cancer cell line
  - COS1 Cells transfected with Tau

- Determine cell survival/death under various treatments and time points for Alzheimer's disease.

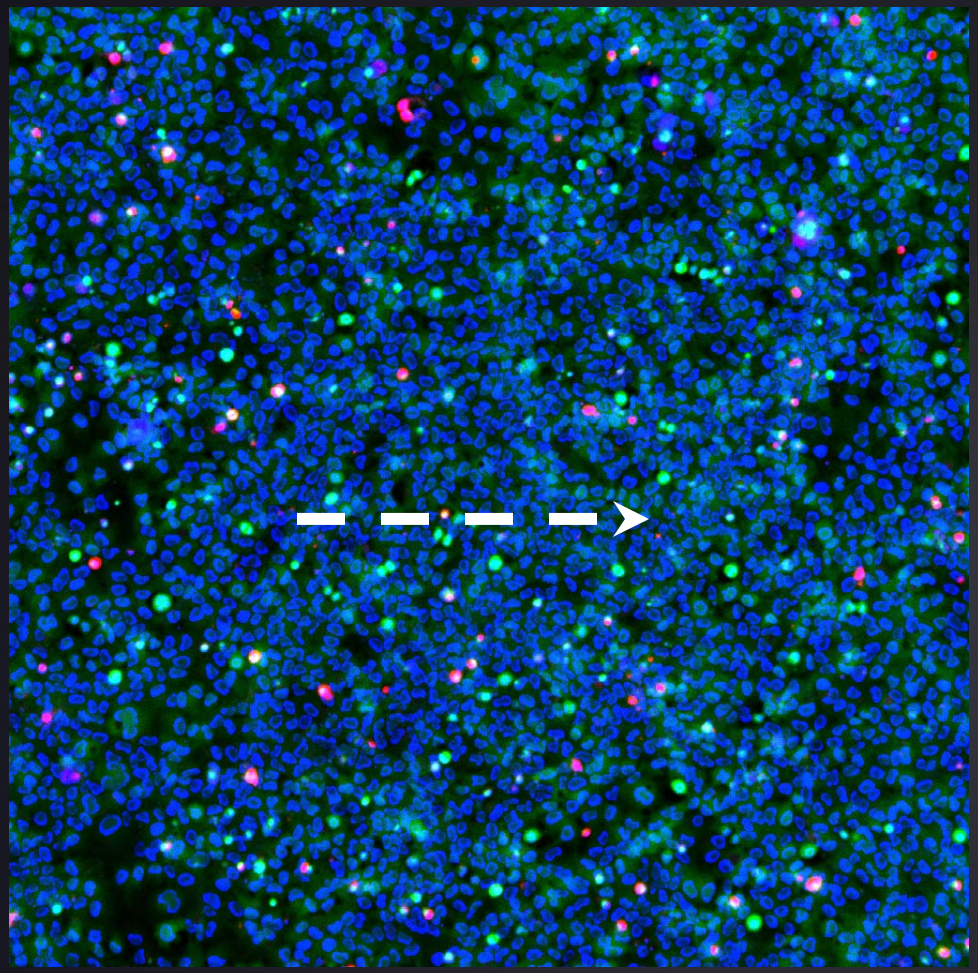
My Goals



- Cell survival/death ratio
  - Cell Count by segmentation
    - Live cells = total cells – dead cells
  - Cell shape: extract further information

    (e.g. Cell division ratio)

Motivation

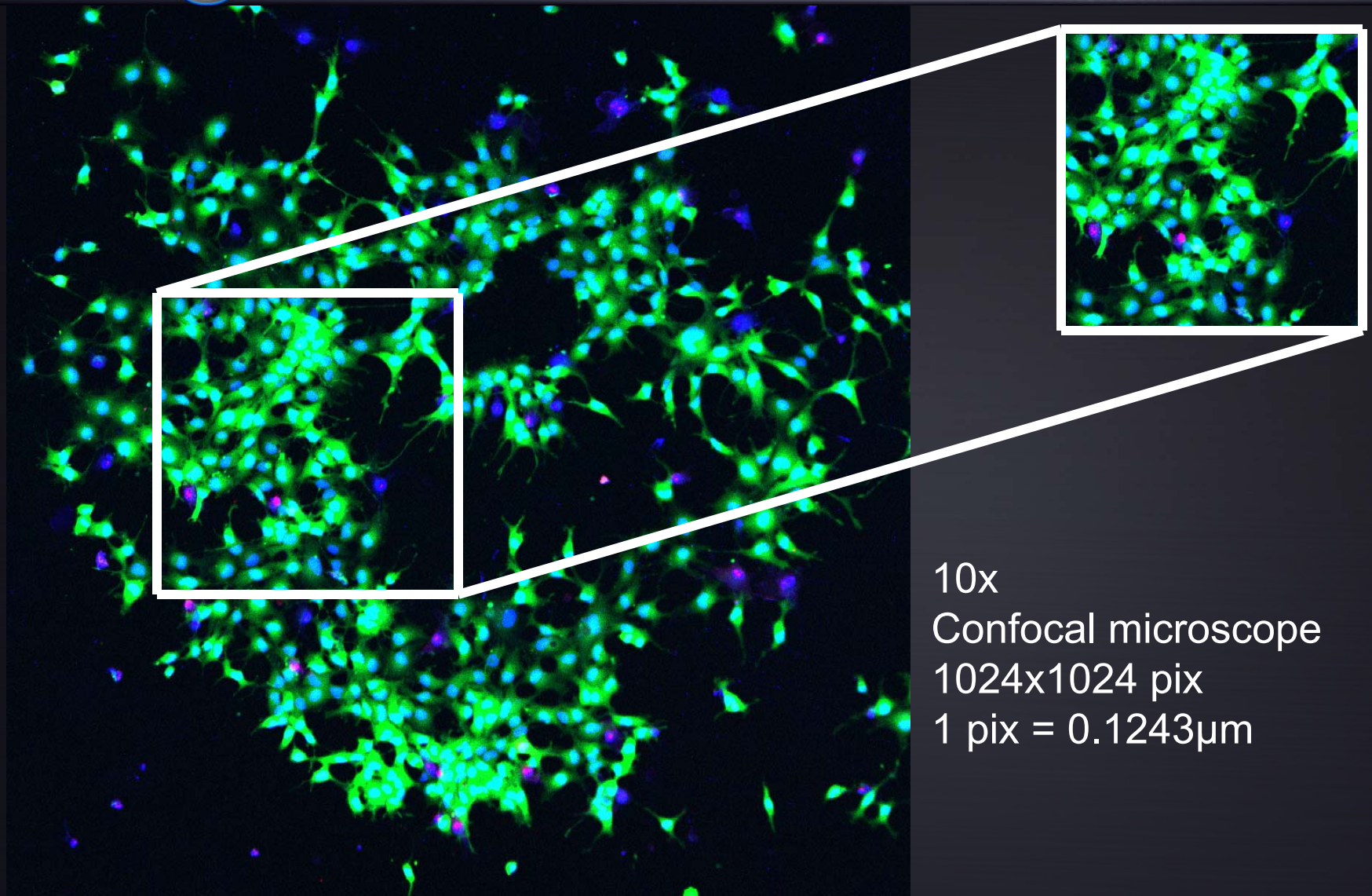


120 Hours

Various Treatments


Image Analysis

Image



10x
Confocal microscope
1024x1024 pix
1 pix = 0.1243µm

Image

Hoechst

– Total Cells

Propidium Iodide

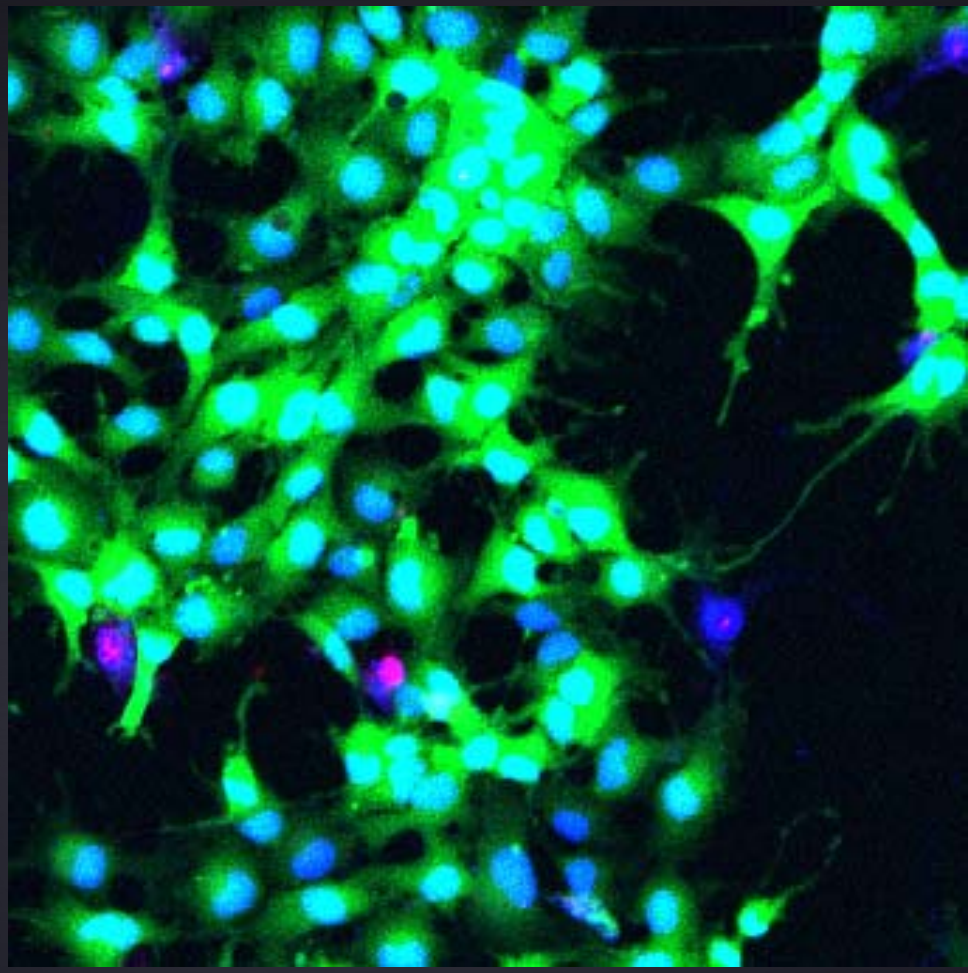
– Dead Cells

(Enters leaky membranes)

Calcein AM

– Live Cells

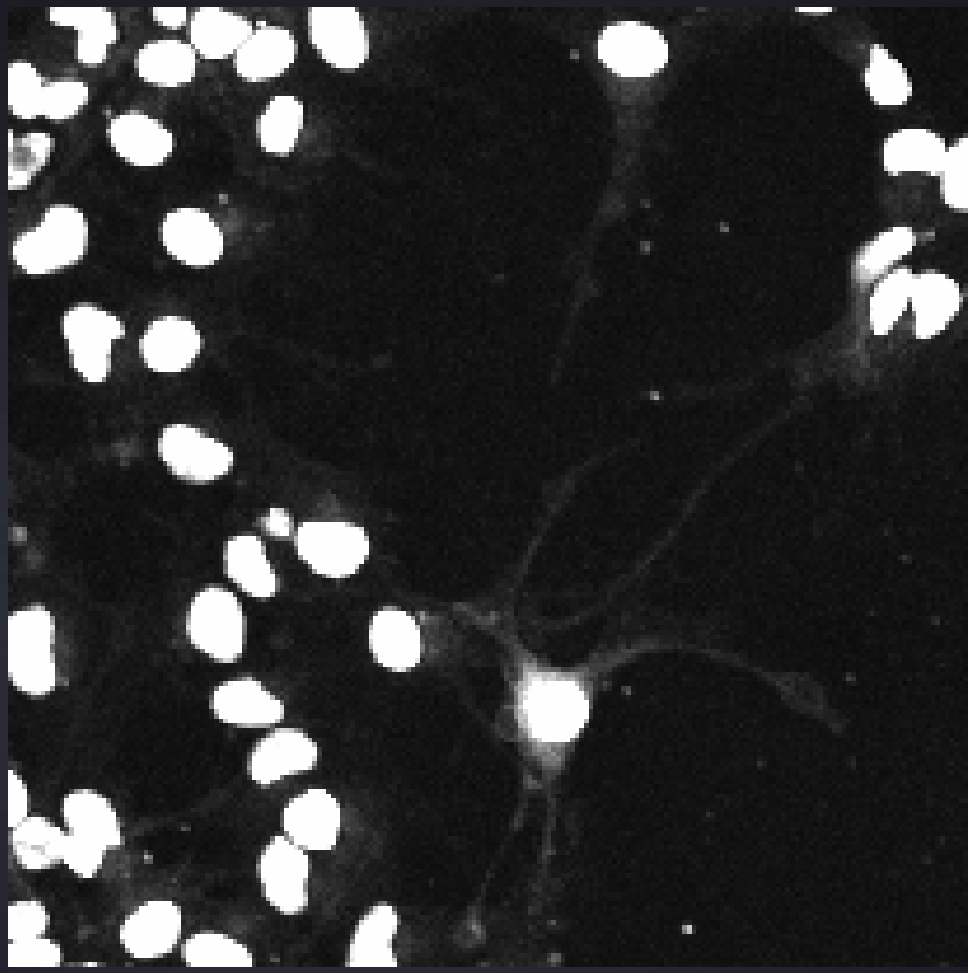
(Hydrolyzed by intracellular enzymes)



Hoechst Channel

Procedure

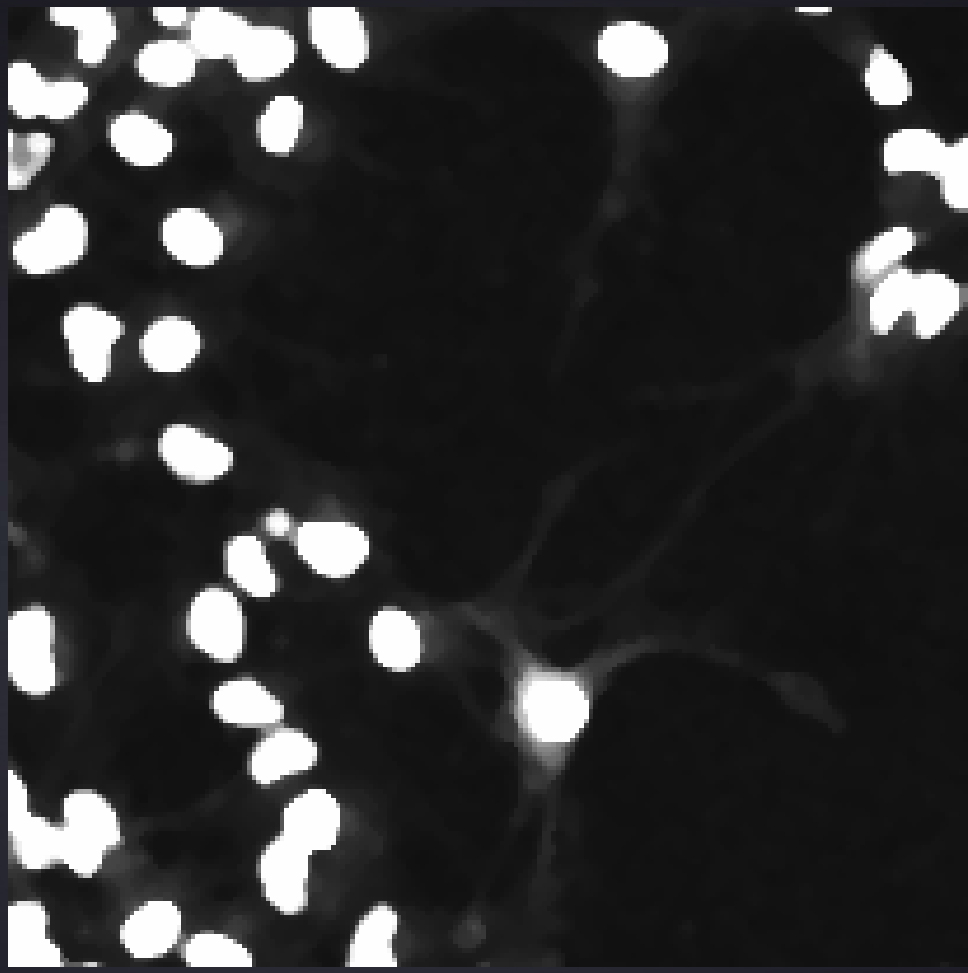
- **Isolate Channels**



Hoechst Channel

Procedure

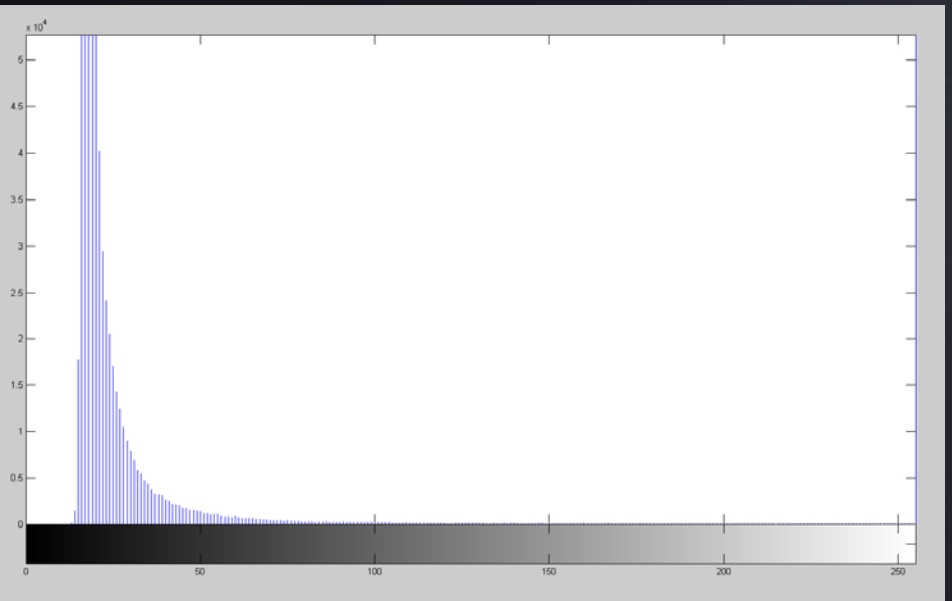
- Isolate Channels
- **Median Filtering**
  - **Noise Reduction**

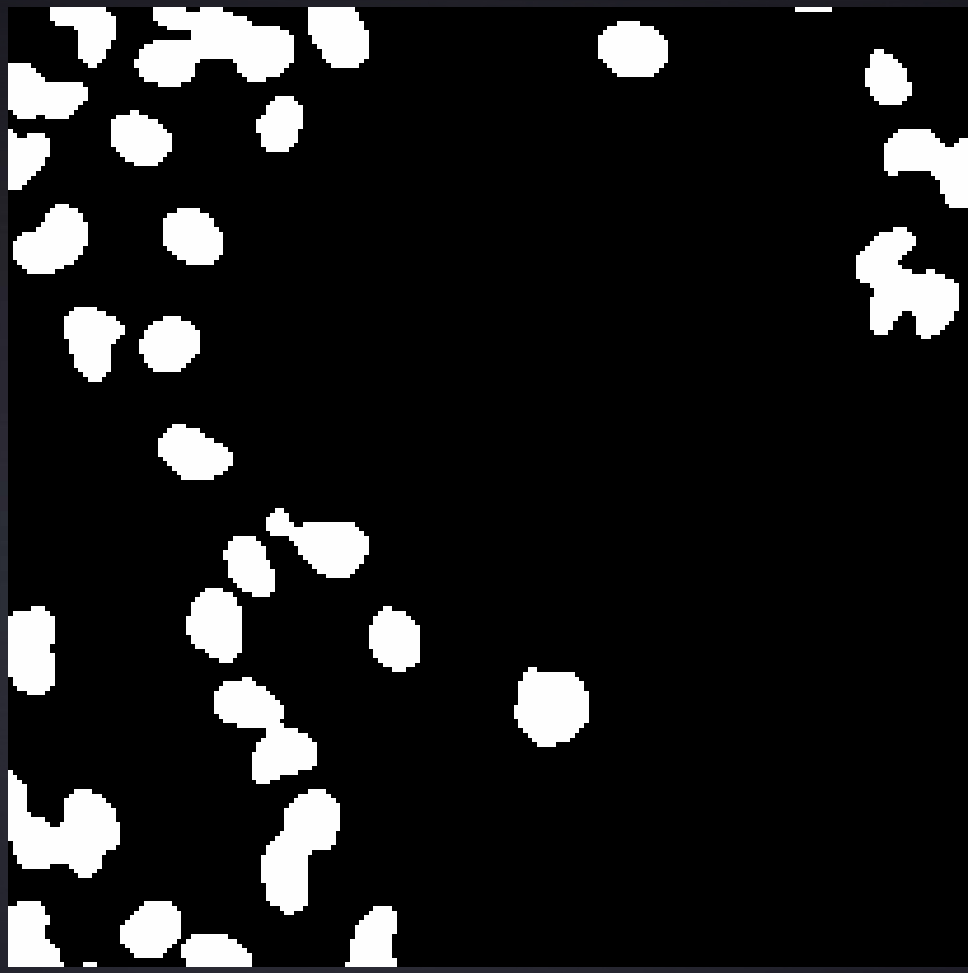


Hoechst Channel

Procedure

- Isolate Channels
- Median Filtering
- **Binary Image**
  - **Threshold**

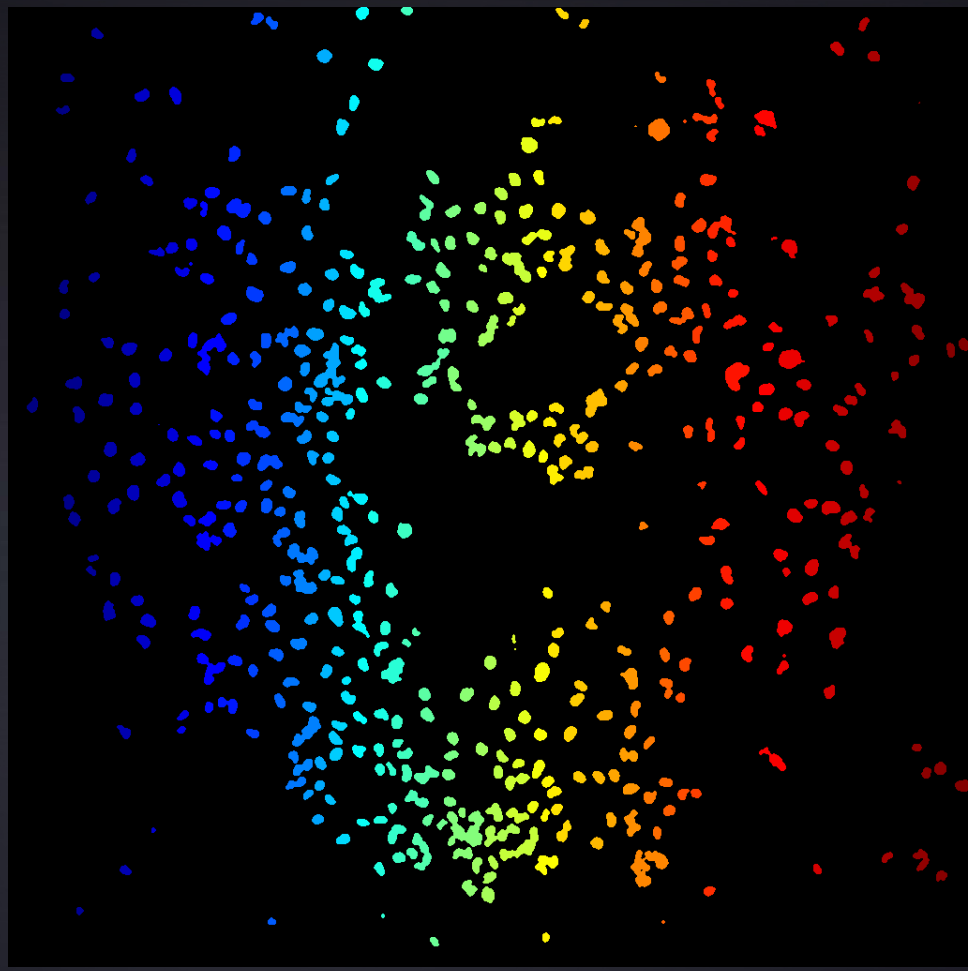




Hoechst Channel

Procedure

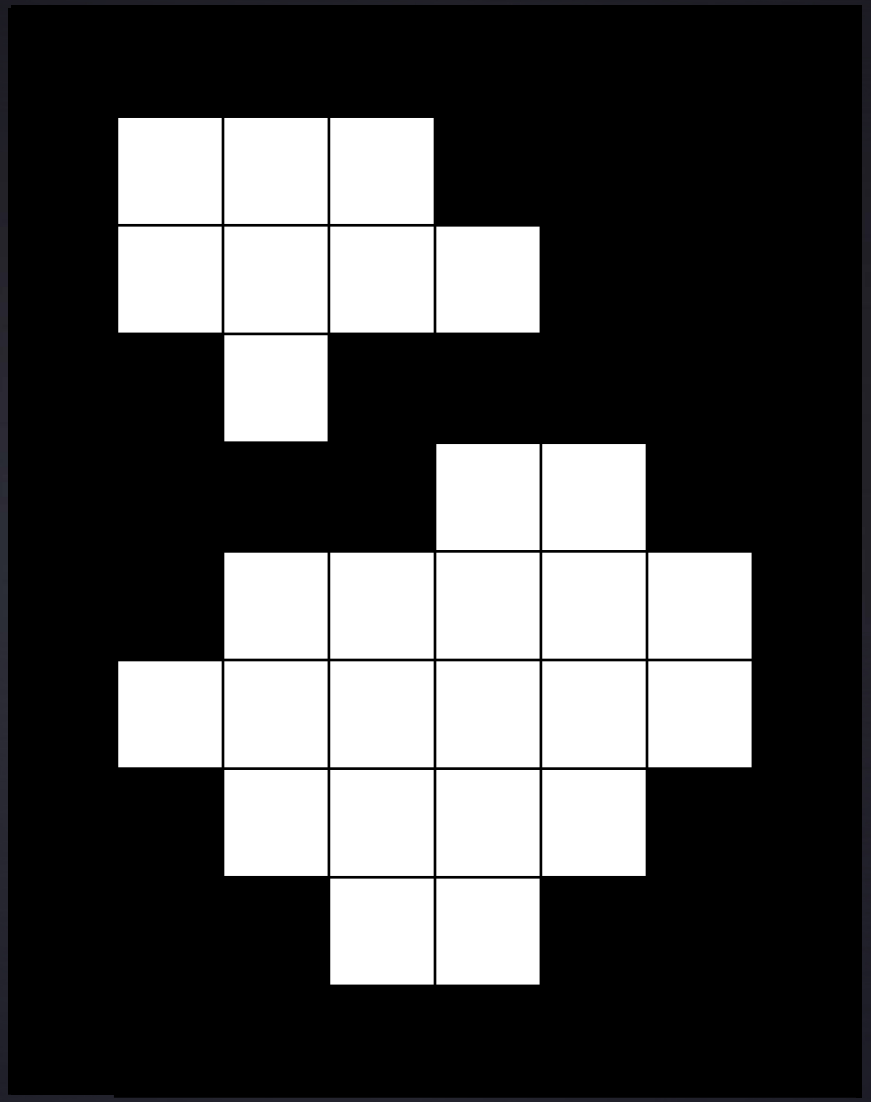
- Isolate Channels
- Median Filtering
- Binary Image
- **Label Image**



Hoechst Channel

Procedure

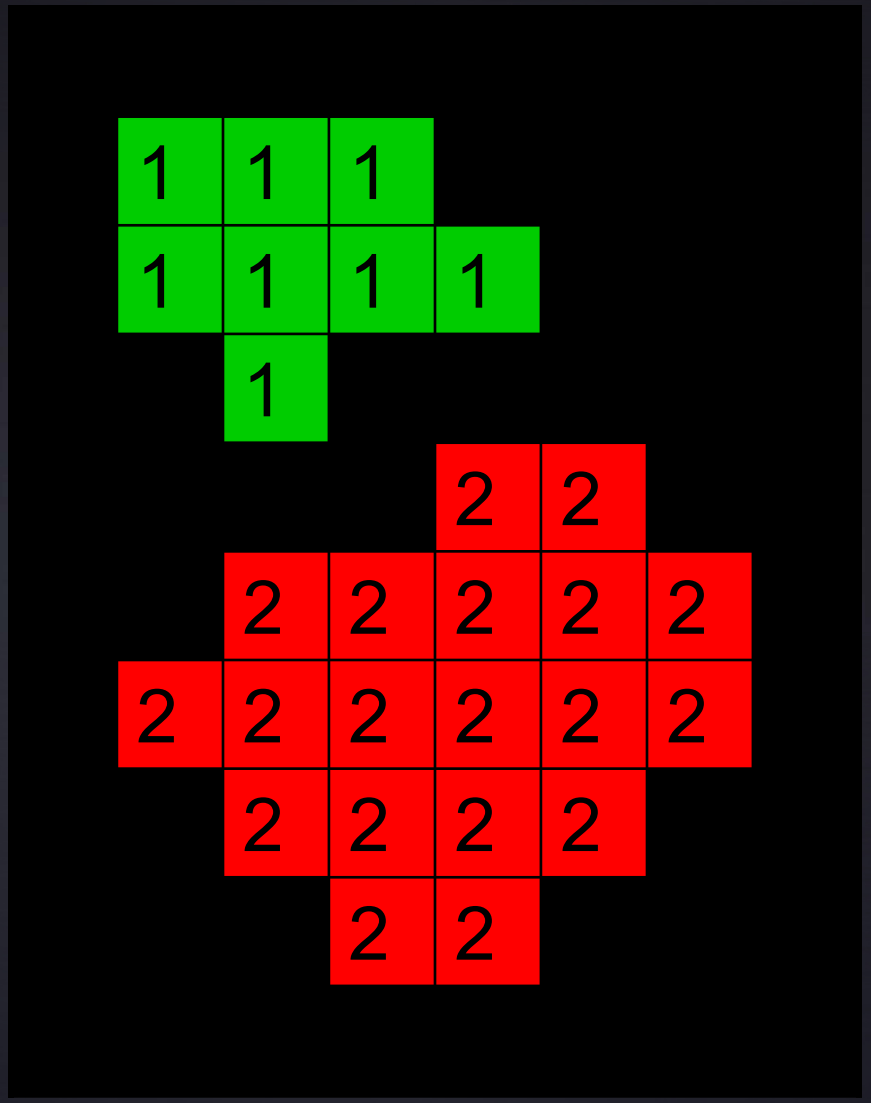
- Isolate Channels
- Median Filtering
- Binary Image
- **Label Image**



Example:
10x8 pixel
Binary image

Procedure

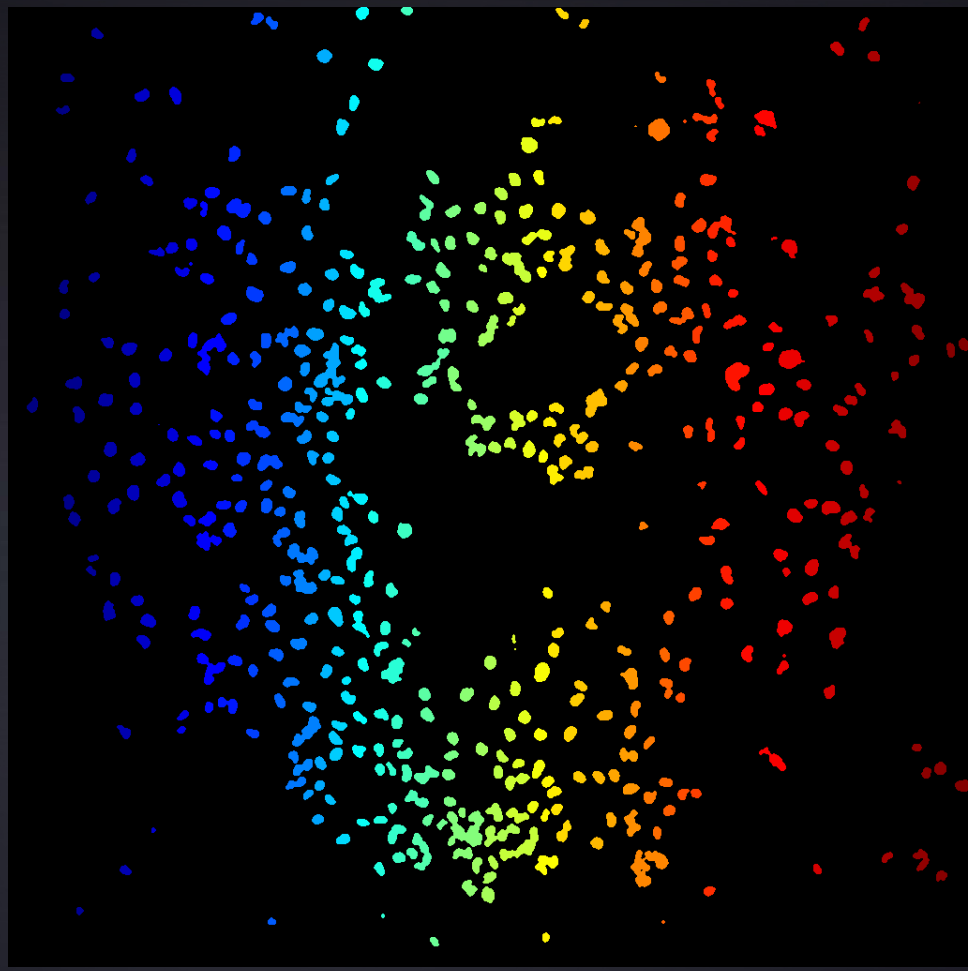
- Isolate Channels
- Median Filtering
- Binary Image
- **Label Image**



Example:
10x8 pixel
Label Image

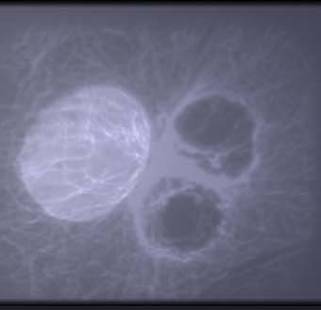
Procedure

- Isolate Channels
- Median Filtering
- Binary Image
- **Label Image**



Hoechst Channel

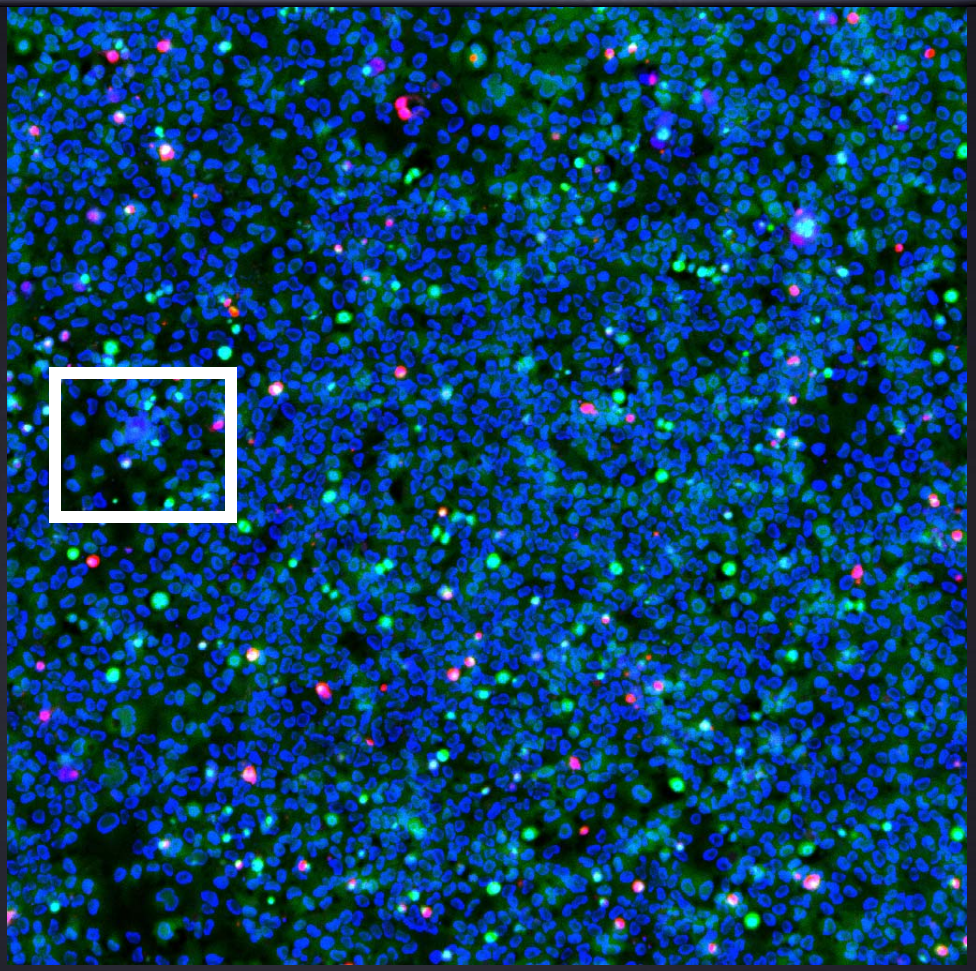
Procedure



- Isolate Channels
- Median Filtering
- Binary Image
- Label Image
- **Object Analysis**

Procedure

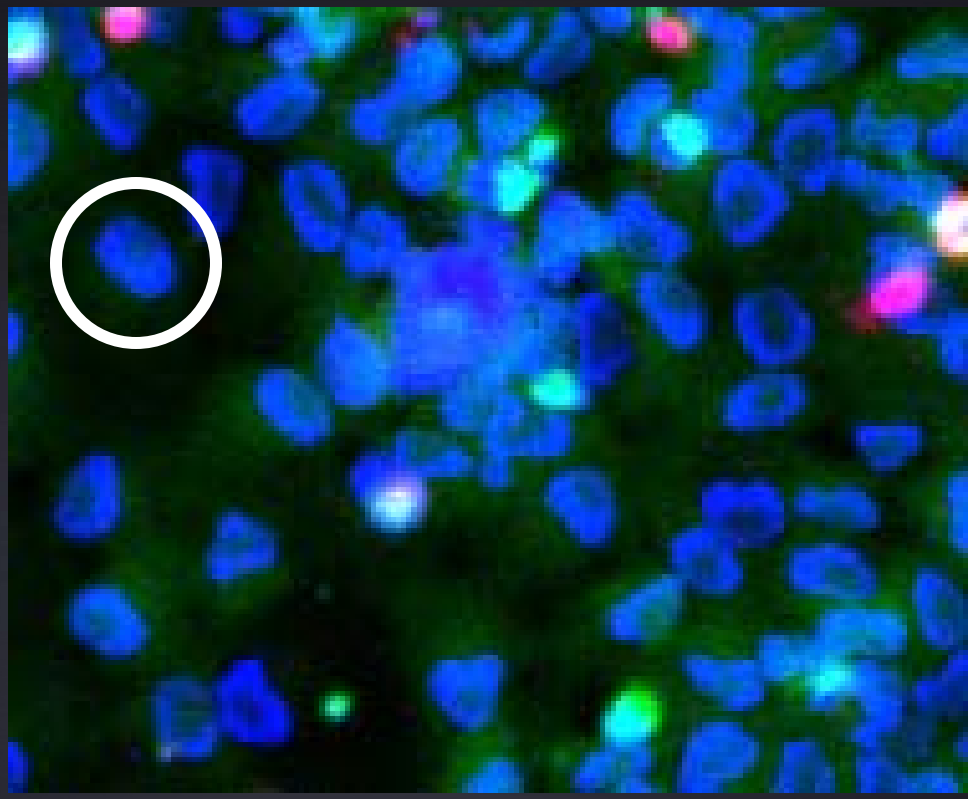
- Isolate Channels
- Median Filtering
- Binary Image
- Label Image
- **Object Analysis**



120 Hours Untreated

Procedure

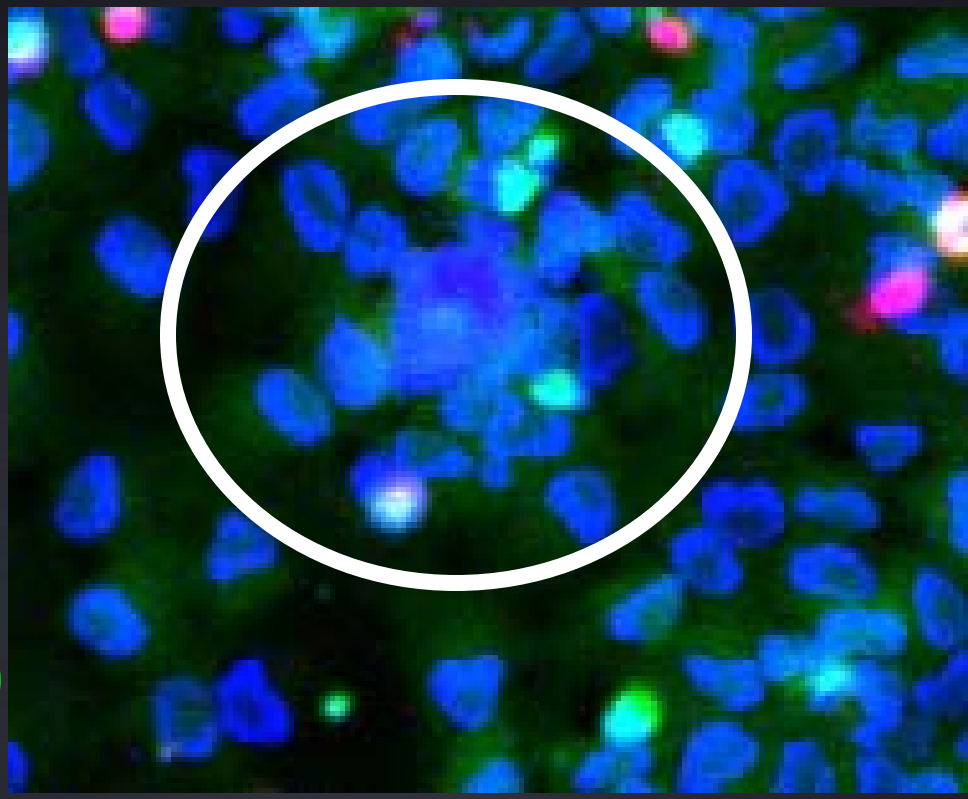
- Isolate Channels
- Median Filtering
- Binary Image
- Label Image
- **Object Analysis**
  - **Normal (1 cell)**



120 Hours Untreated

Procedure

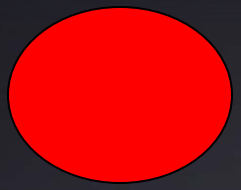
- Isolate Channels
- Median Filtering
- Binary Image
- Label Image
- **Object Analysis**
  - **Cluster (>= 3 cells)**



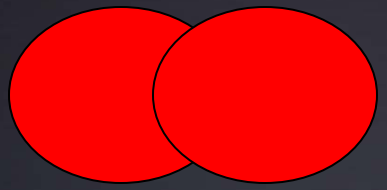
120 Hours Untreated

Procedure

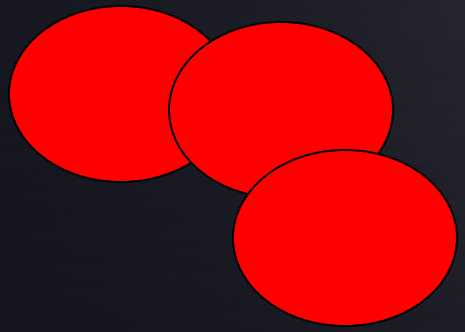
- Object Analysis
  - Normal



  - Peanut
    (Dividing cell)

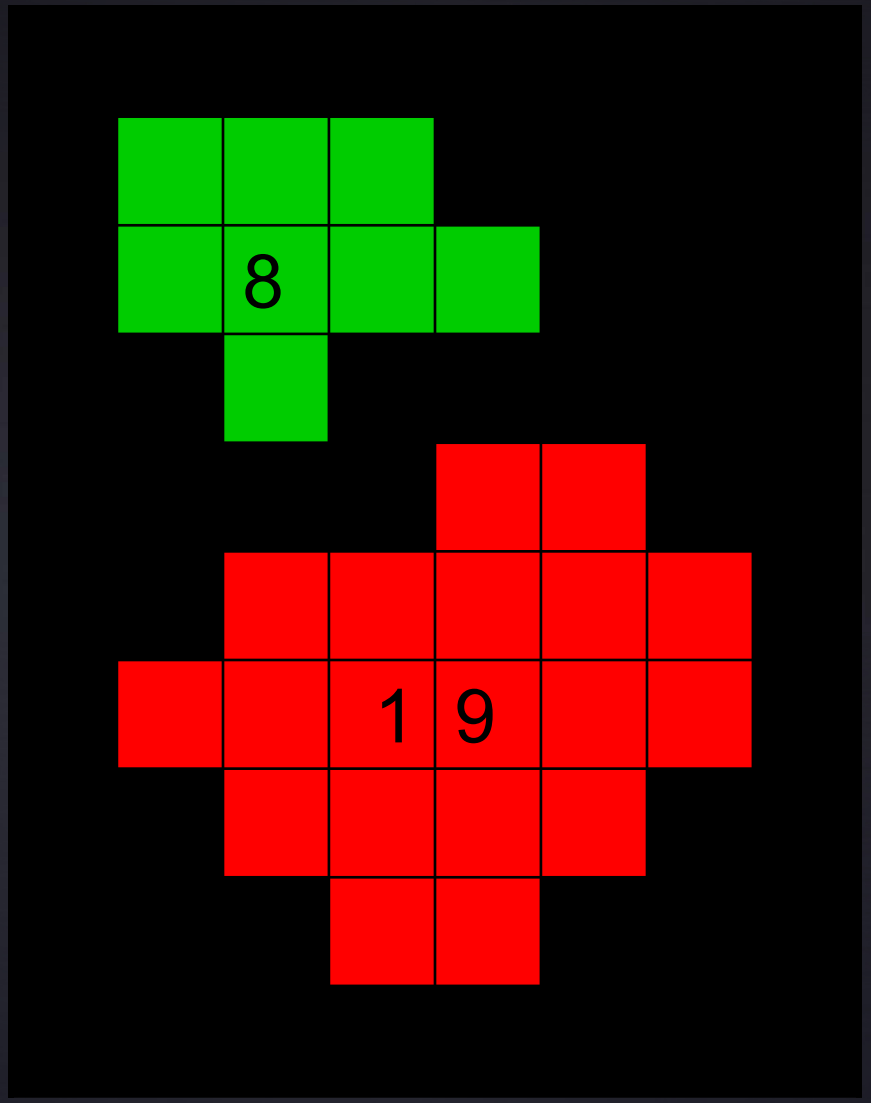


  - Cluster (>= 3 cells)



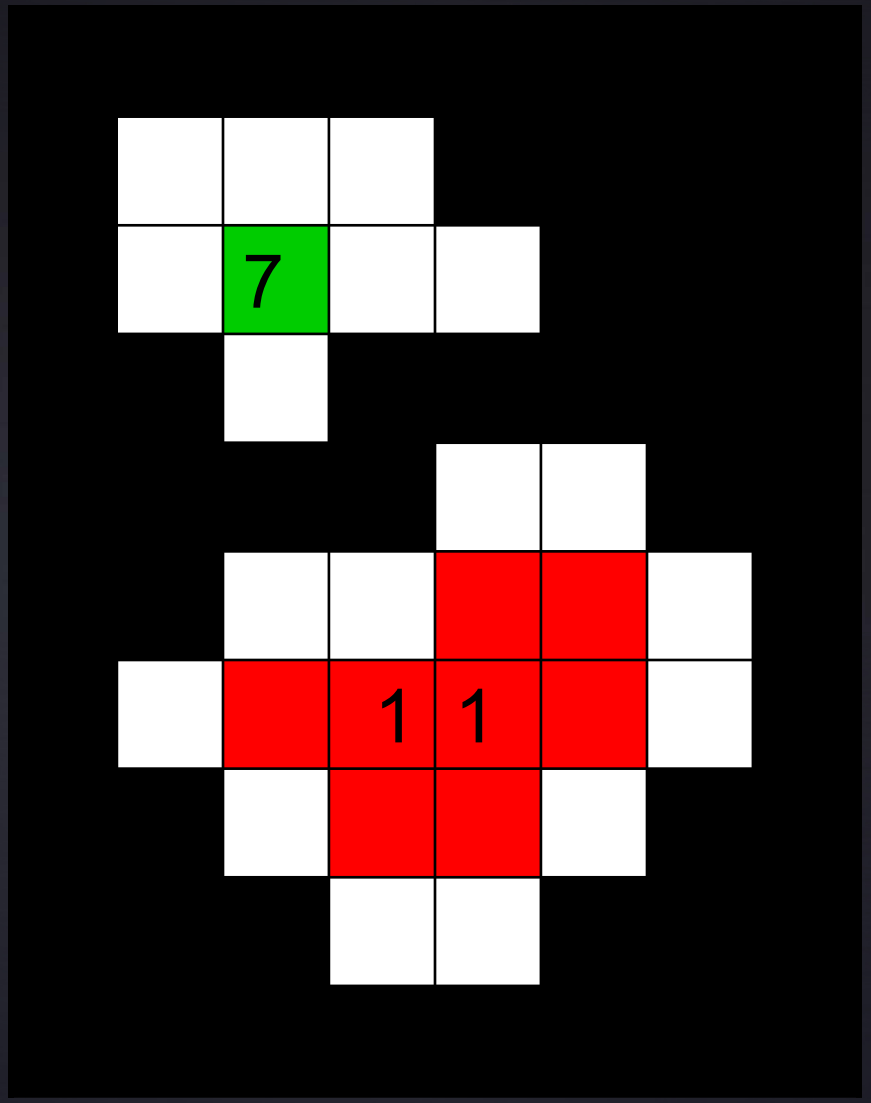
Statistics

- Area



Statistics

- Area
- **Perimeter**

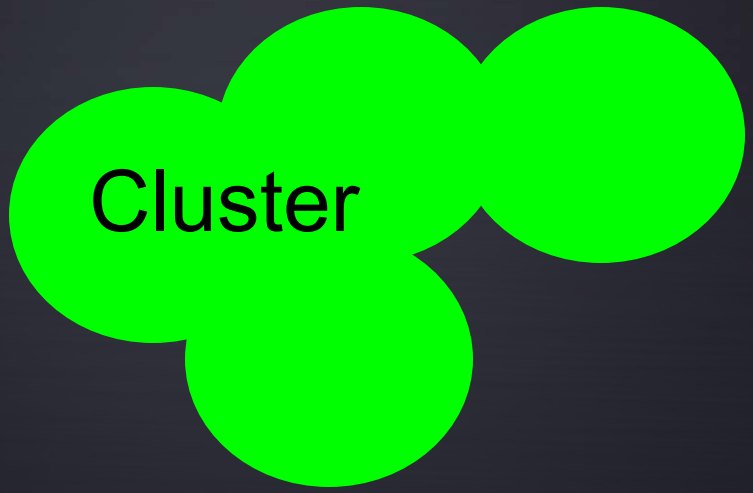


Statistics

- Area
- Perimeter
- **Compactness**

$$\text{Compactness} = \frac{4 \times \text{pi} \times \text{Area}}{\text{Perimeter}^2}$$

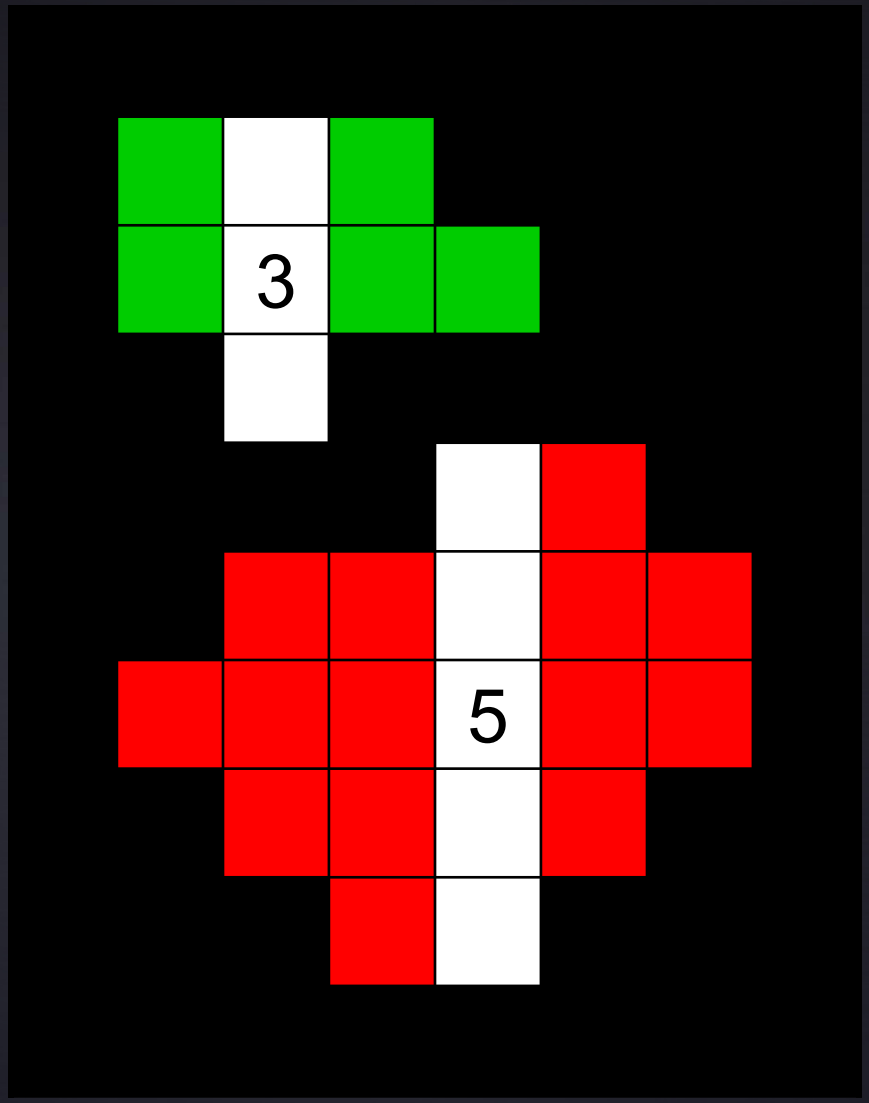
Compactness = 1



Low Compactness

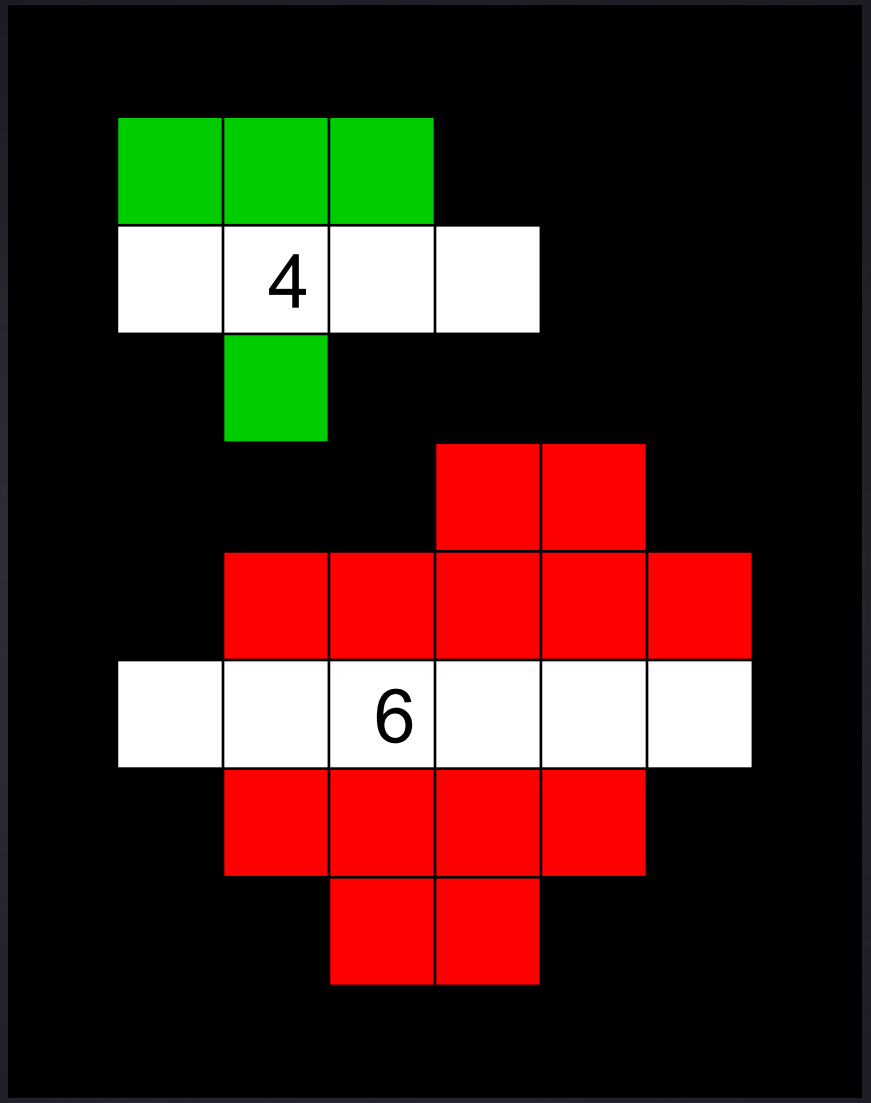
Statistics

- Area
- Perimeter
- Compactness
- **Minor Axis**



Statistics

- Area
- Perimeter
- Compactness
- Minor Axis
- **Major Axis**

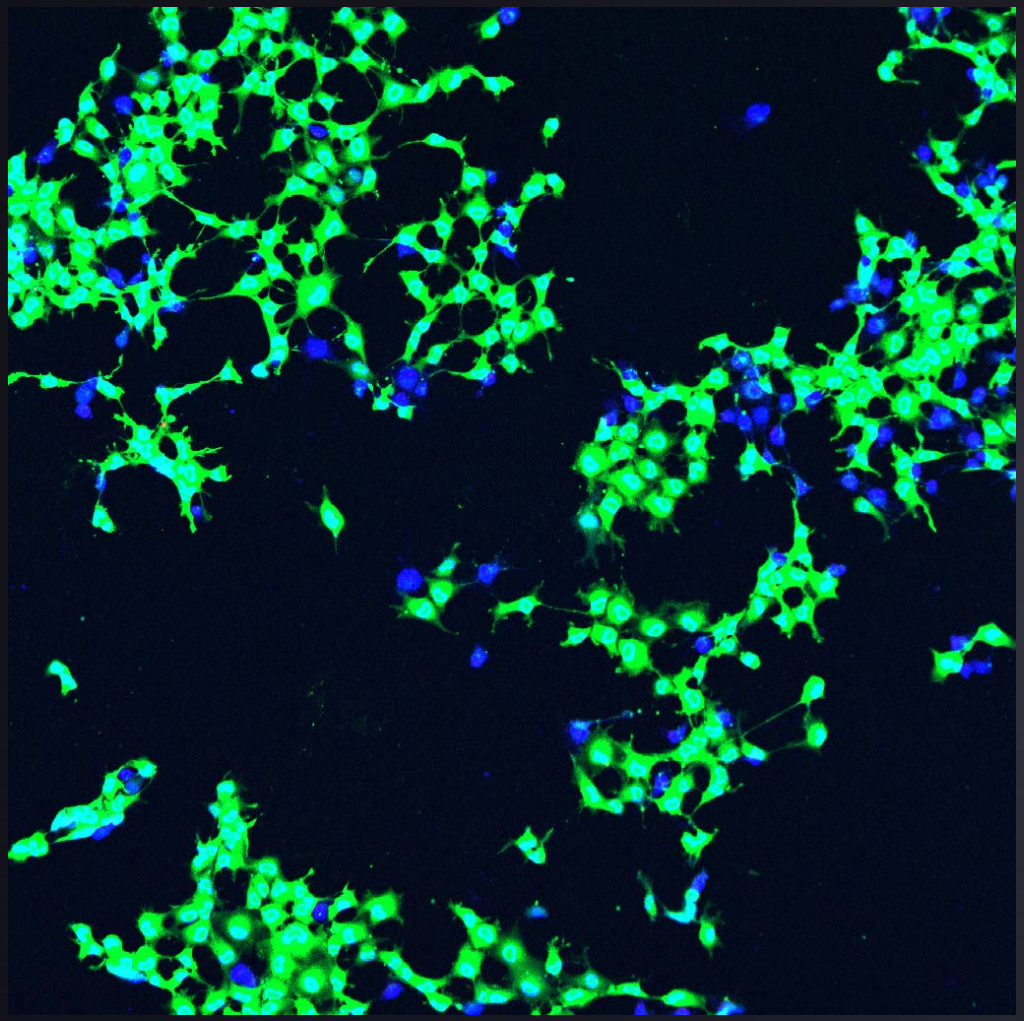


Statistics

- Area
- Perimeter
- Compactness
- Minor Axis
- Major Axis
- **Axis Ratio**

$$\text{Axis Ratio} = \frac{\text{Minor Axis}}{\text{Major Axis}}$$

Procedure



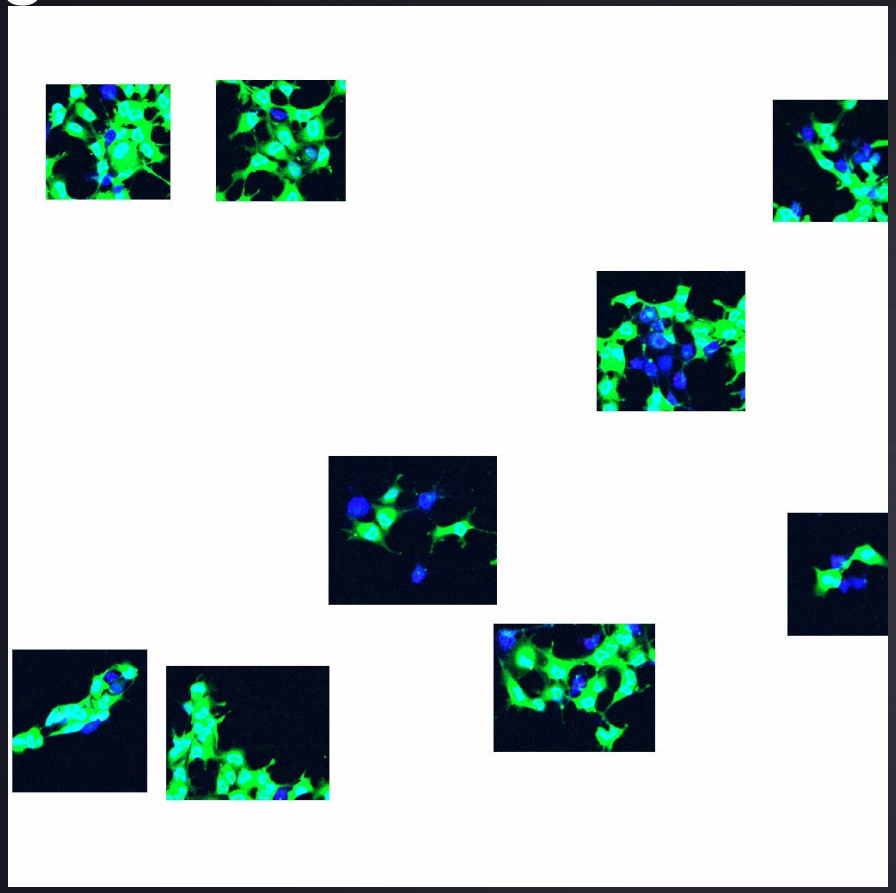
2hrs Untreated

Class Type:

1. Normal + Peanut
2. Cluster

Procedure

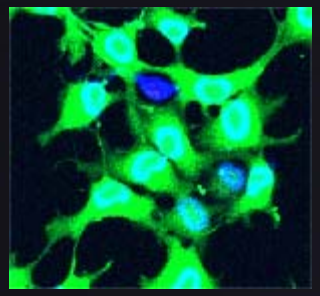
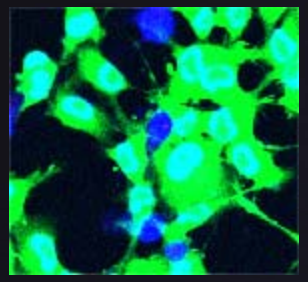
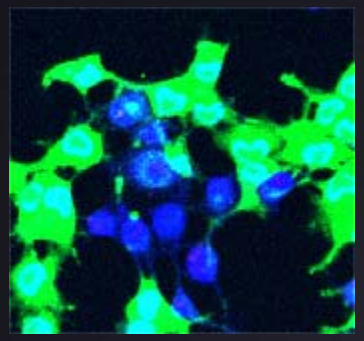
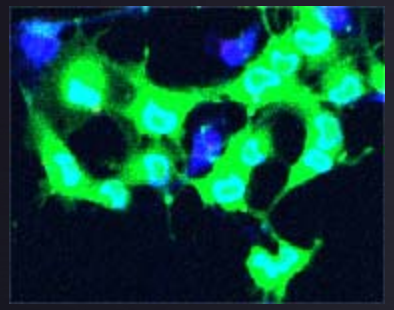
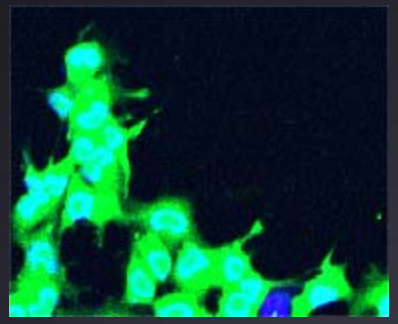
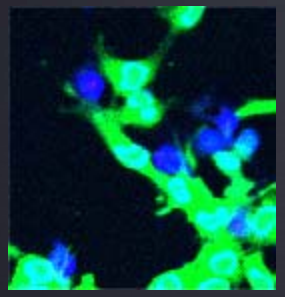
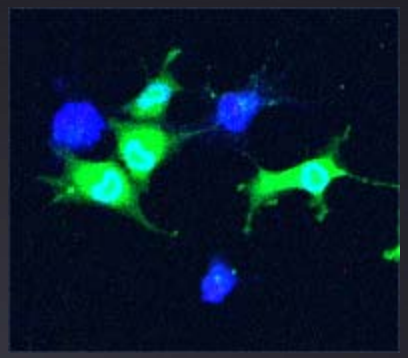
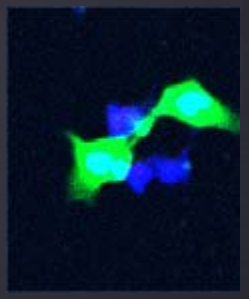
- Crop Regions



2hrs Untreated

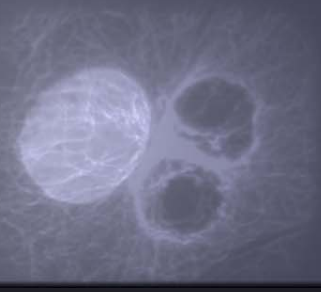
Procedure

- Crop Images

2hrs Untreated

Statistics

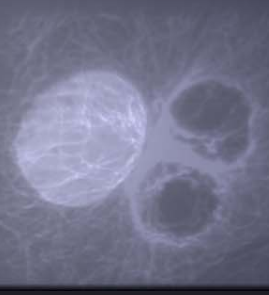


- Area
- Perimeter
- Compactness
- Minor Axis
- Major Axis
- Axis Ratio

- Clusters can be discriminated.
- Normal & Peanut are difficult to discriminate.

Experimental Results

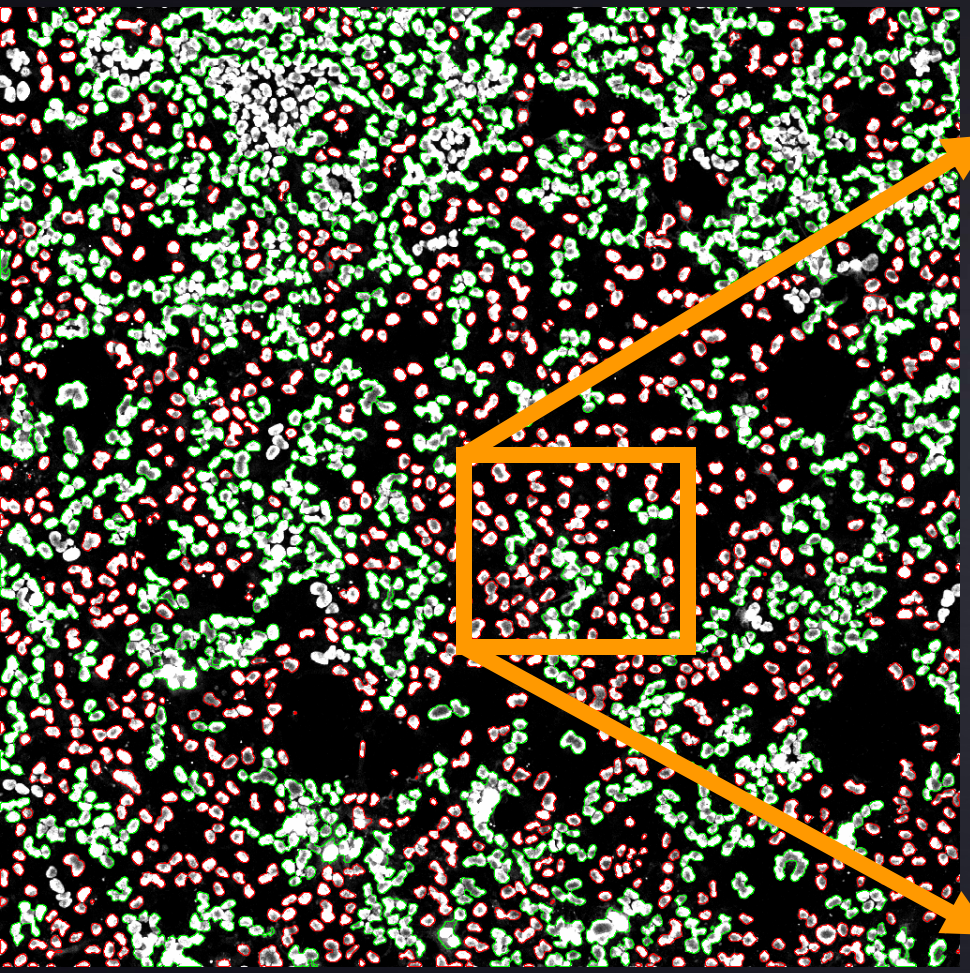
Dataset
Analyzed with MATLAB



- 42 “Untreated” Images from 7 different time points
  - 2 hrs (7)
  - 6 hrs (5)
  - 12 hrs (6)
  - 24 hrs (5)
  - 48 hrs (8)
  - 72 hrs (6)
  - 120 hrs (5)

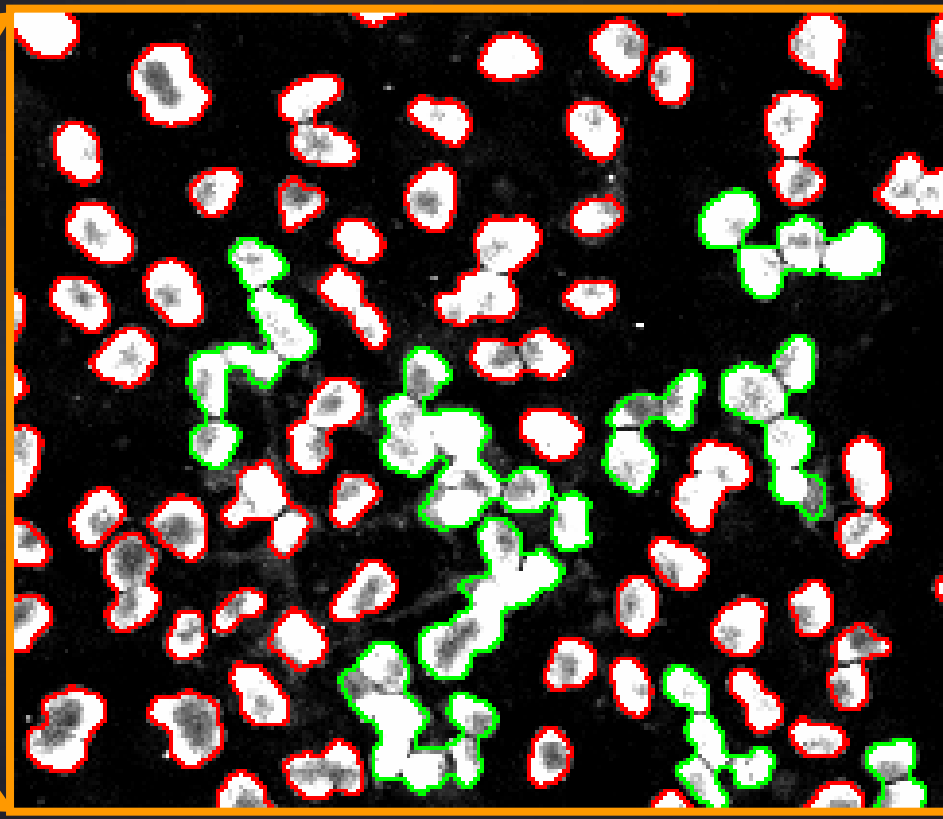
Experimental Results

120 Hours



Red = Normal + Peanut

Green = Clusters

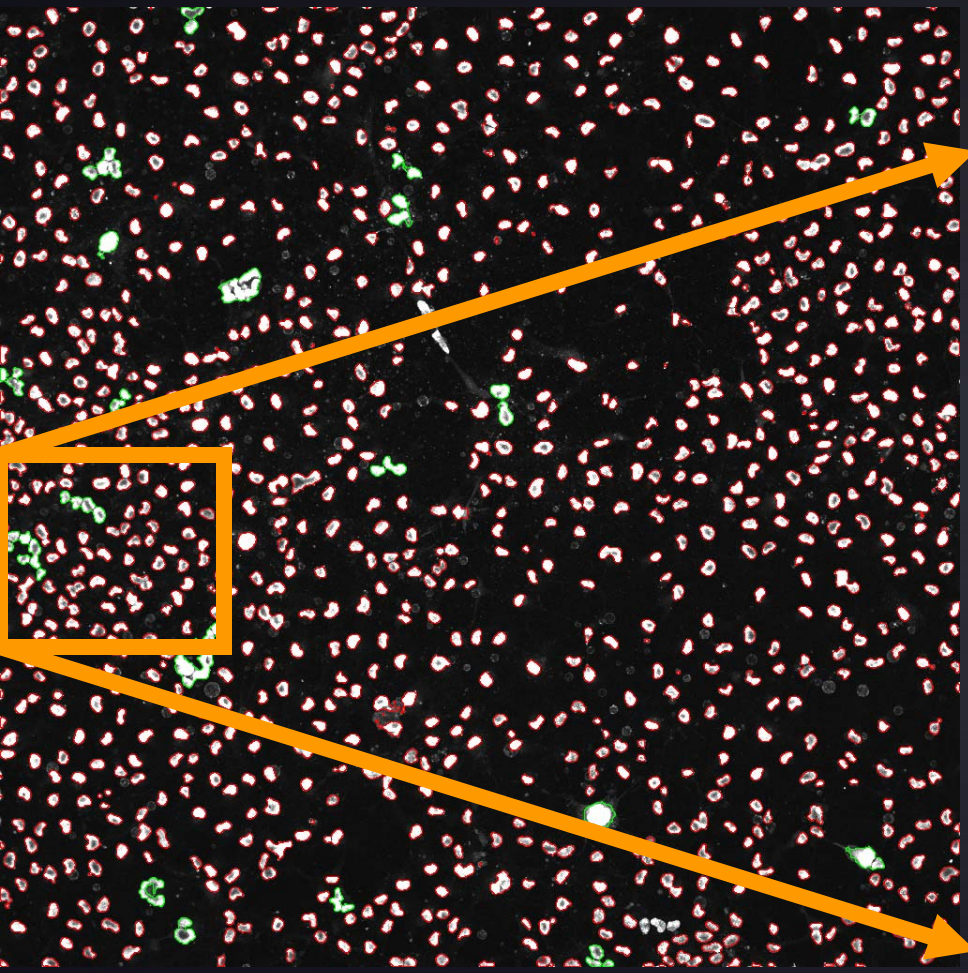


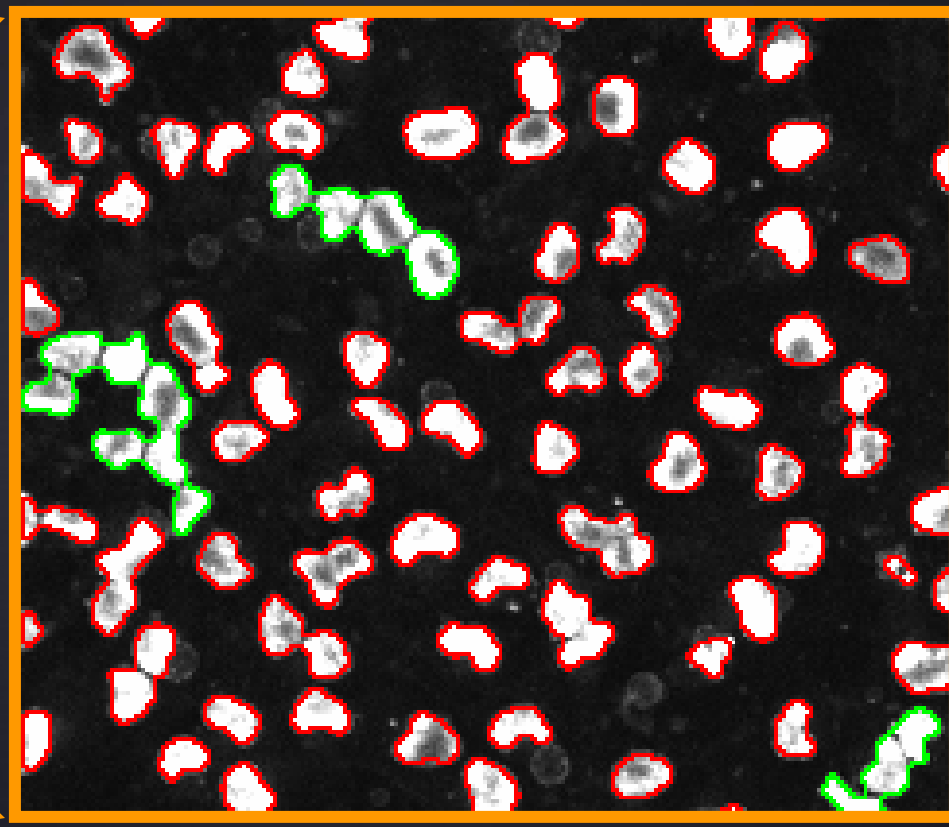
Experimental Results

24 Hours

Red = Normal + Peanut

Green = Clusters





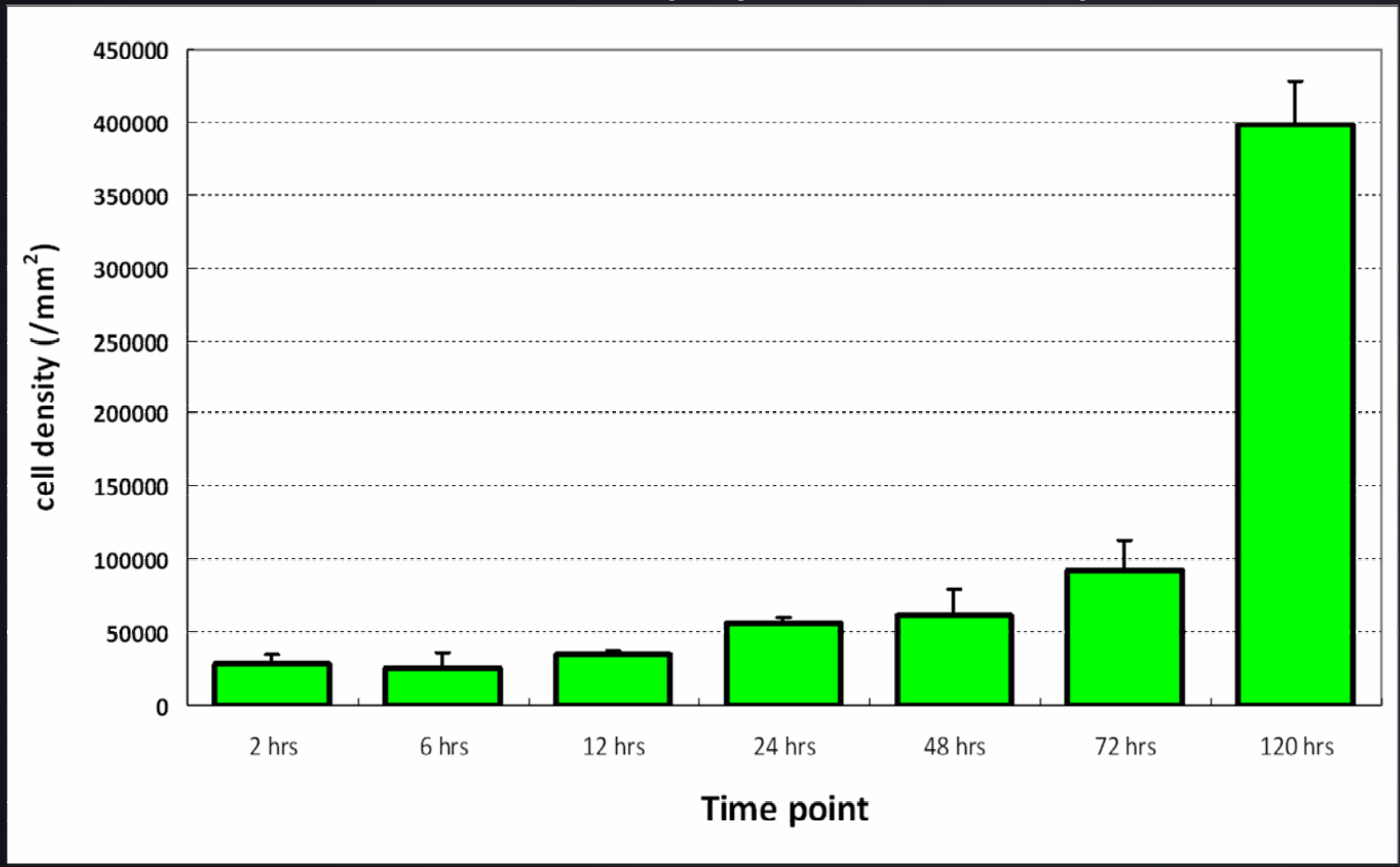
Cell Density

- Cell Density:
  - Cells = Number of “Normal & Peanut”
  - Area = Image Area – Cluster Area

$$\text{Cell Density} = \frac{\text{Cells}}{\text{Area}}$$

Experimental Results

Cell Density (Untreated)




Future Work

Future Work

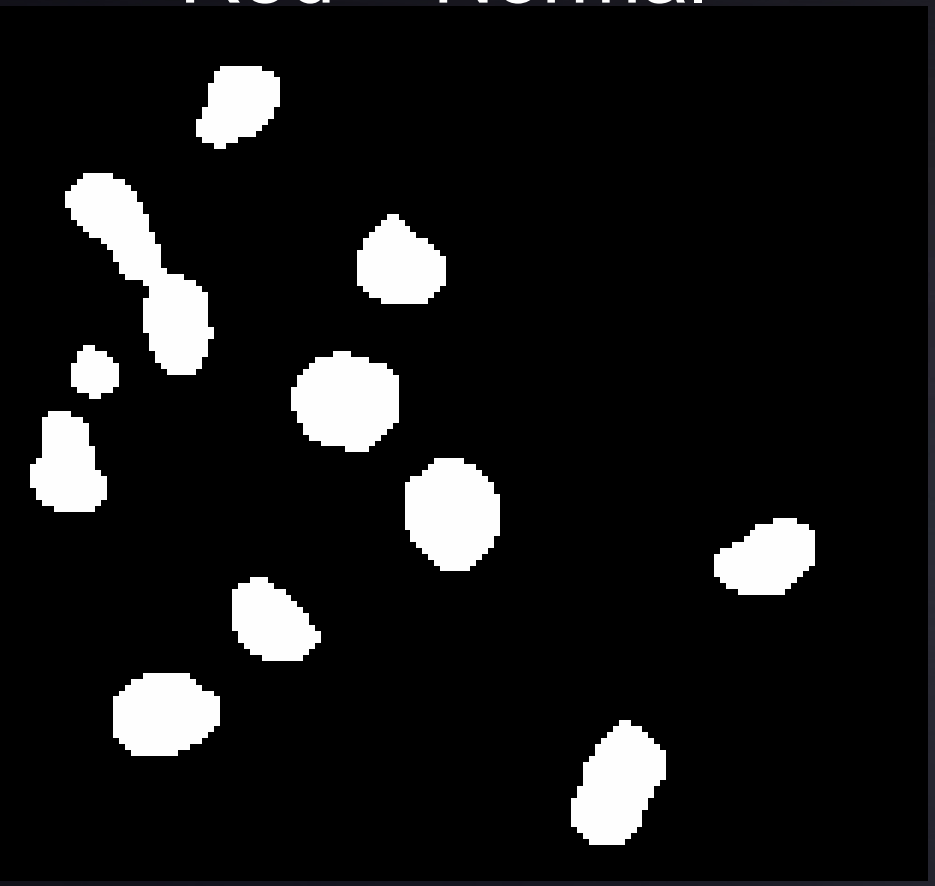
- Apply to various treatment
  - Untreated
  - 5 uM Staurosporine (stauro)
  - 10 uM Amyloid-Beta ()
  - 100 nM Taxol (Taxol)
  - 100 nM Taxol + 10 uM Amyloid-Beta (Tx-)
- Cell survival/death ratio.

Future Work

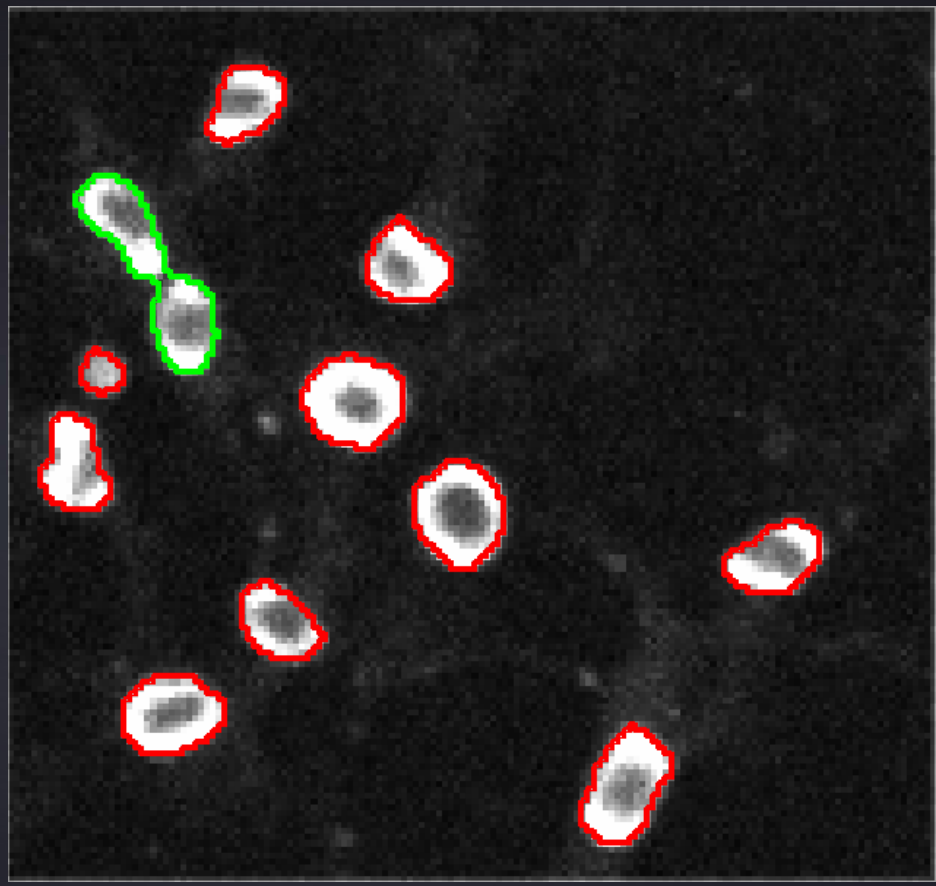
- Better Threshold
  - Grayscale Image → Binary Image
- Improve Segmentation

Future Work

Red = Normal

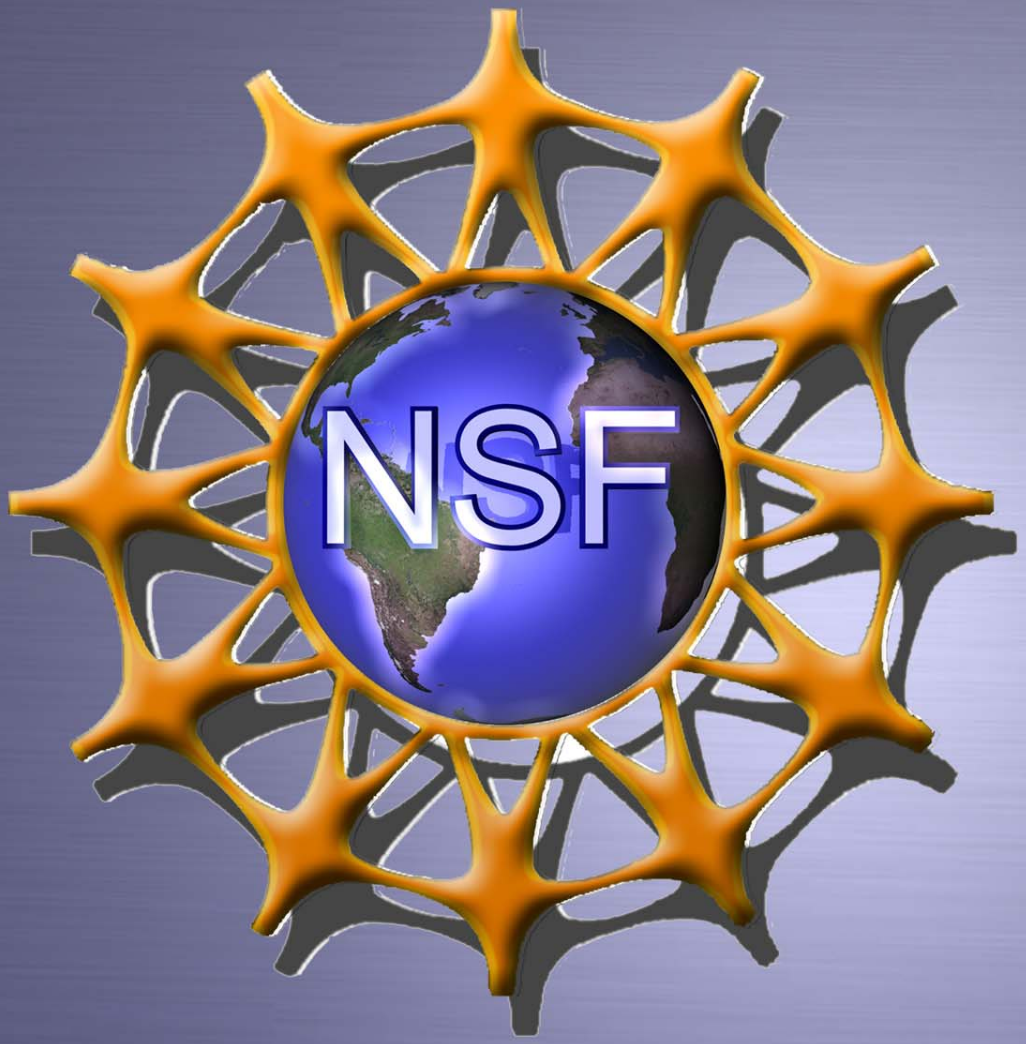


Green = Peanut



2hrs Hoechst Channel

Acknowledge



Acknowledge

Acknowledge

Acknowledge

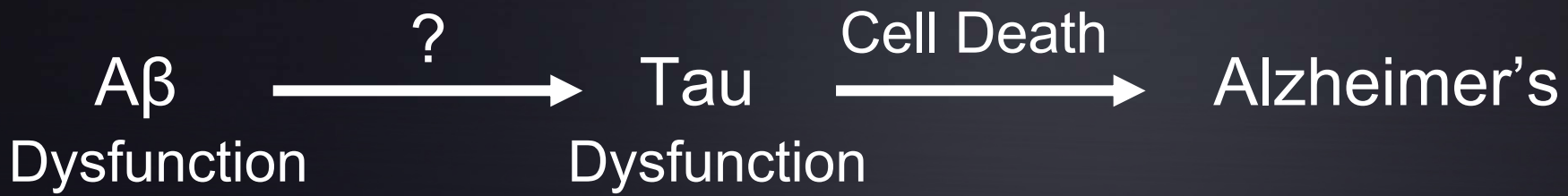
- Faculty Advisor
  - Dr. B.S. Manjunath
- Mentors:
  - Jiyun Byun
  - DeeAnn Hartung
- Center for Bio-Image Informatics


Questions?

Alzheimer's

Characterized by two pathological hallmarks in the brain,

1. Extracellular Amyloid Plagues
   - Amyloid-Beta (Aβ) protein
2. Intracellular Neurofibrillary Tangles (NFTs)
   - Tau protein



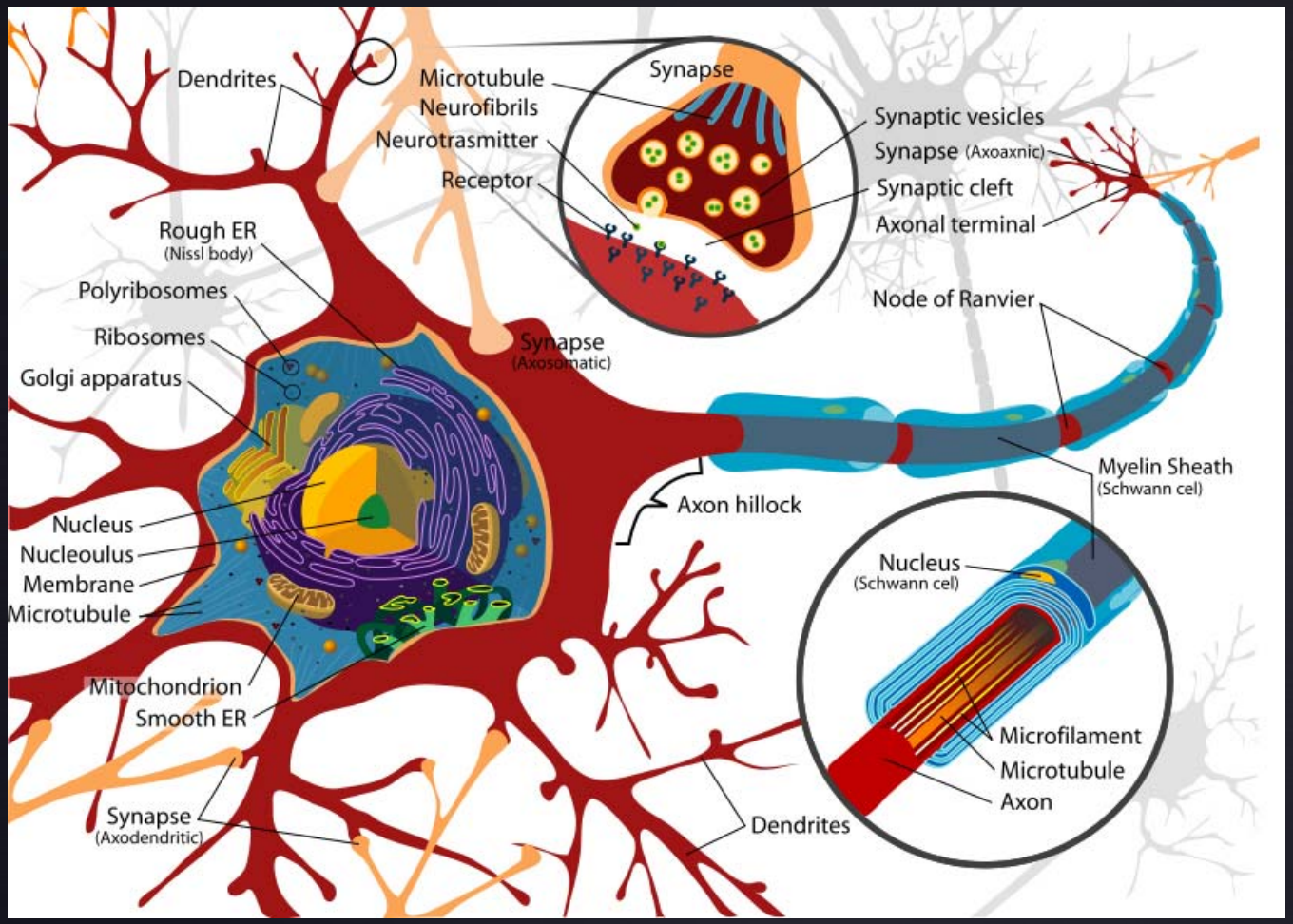
Alzheimer's

Amyloid-Beta (Aβ)

1. Amyloid Percursor Protein (APP)
   a. Function: Undetermined
2. Can't cause disease without Tau

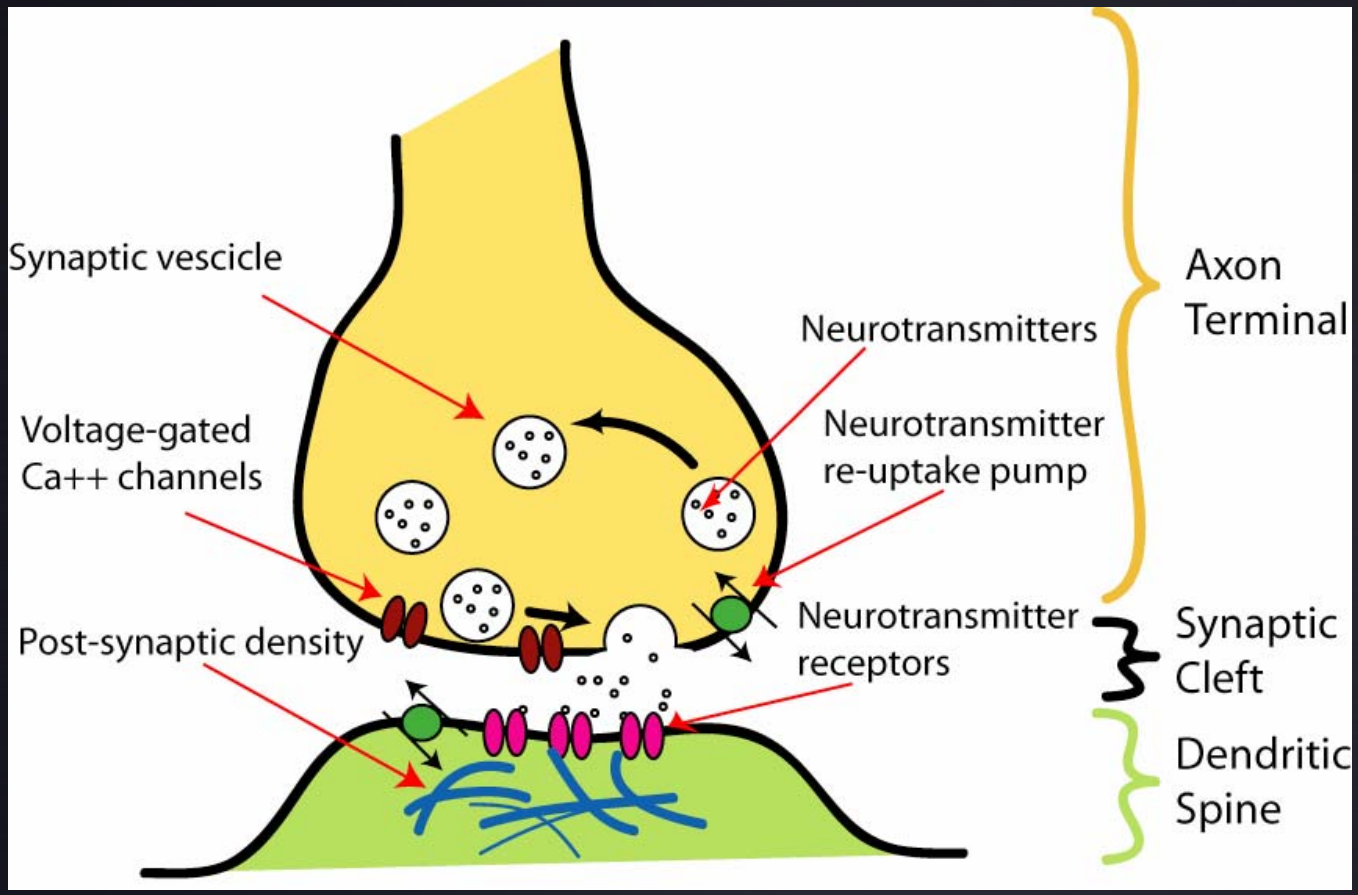
Alzheimer's

Amyloid-Beta (Aβ)



Alzheimer's

Amyloid-Beta (Aβ)

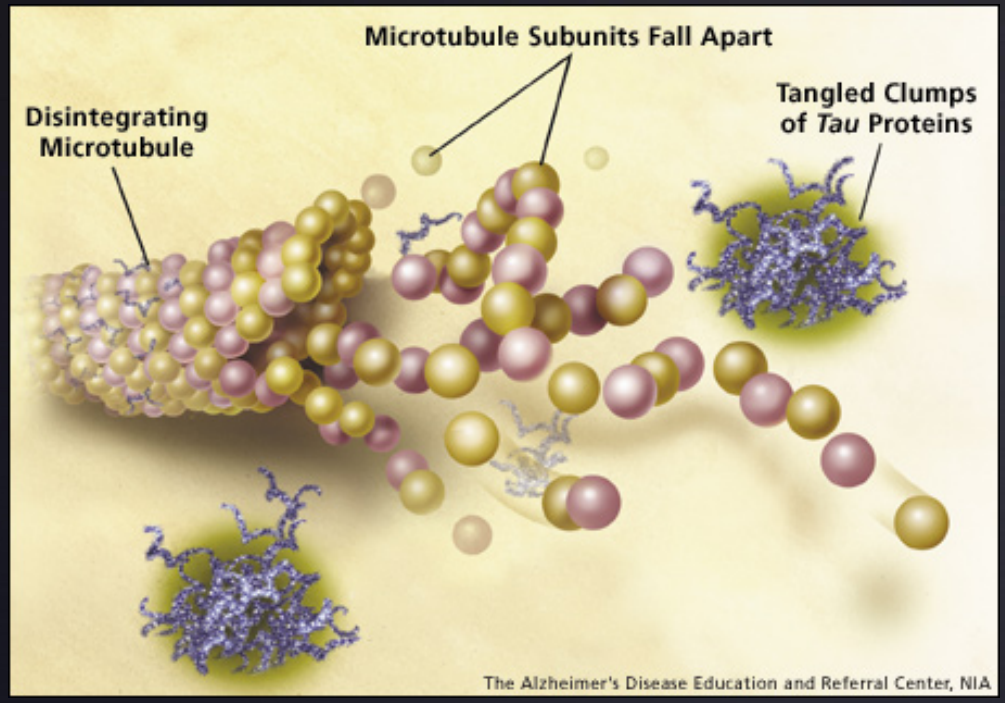


Alzheimer's

Tau Protein

1. Function:

Regulates neuronal microtubule dynamics



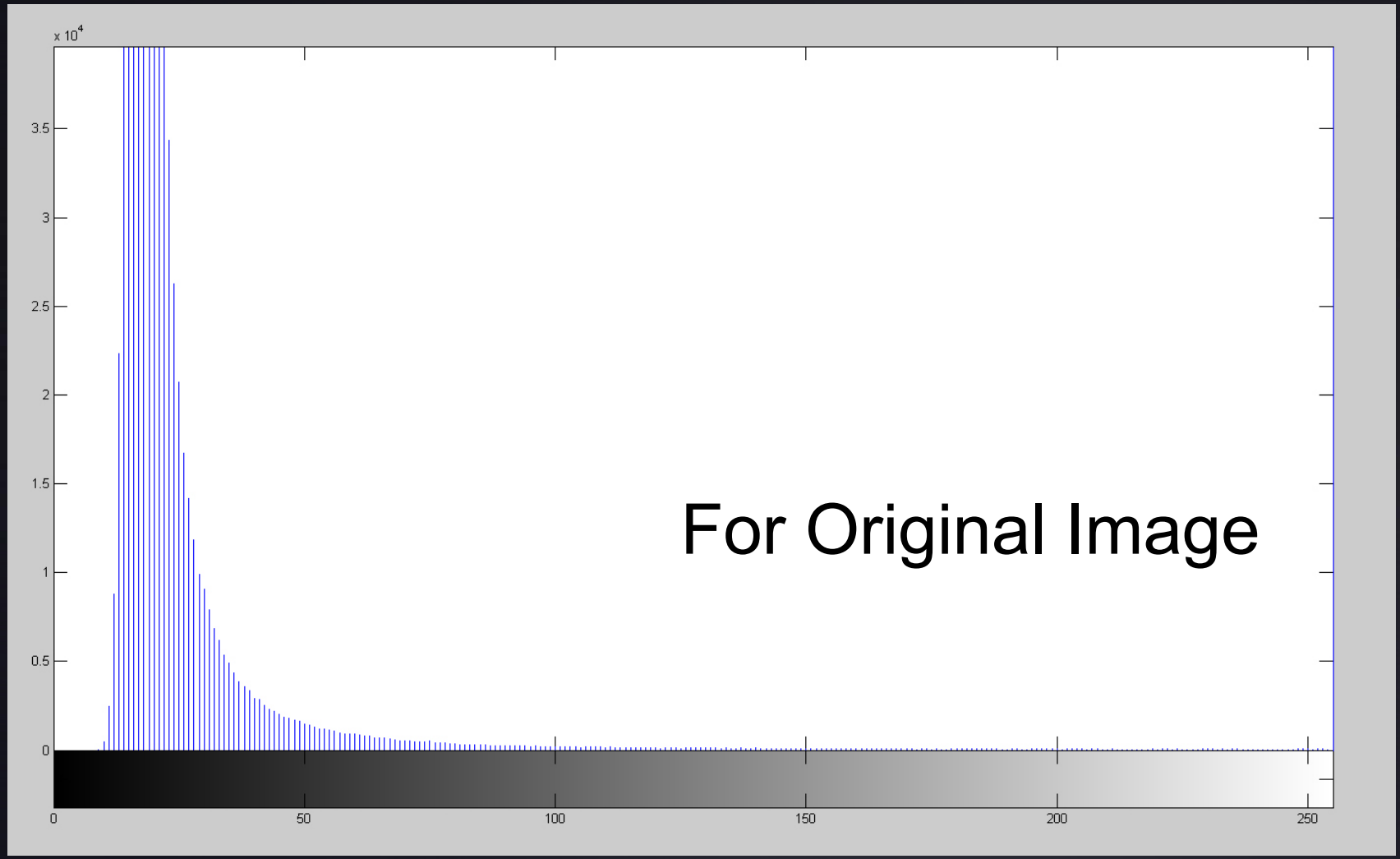
Alzheimer's

Experimental Setup

- Treatments
  - Untreated
  - 5 uM Staurosporine
  - 10 uM Aβ
  - 100 nM Taxol
  - 100nM Taxol + 10 uM Aβ
- Timepoints
  - 2 hours
  - 6 hours
  - 12 hours
  - 24 hours
  - 48 hours
  - 72 hours
  - 120 hours

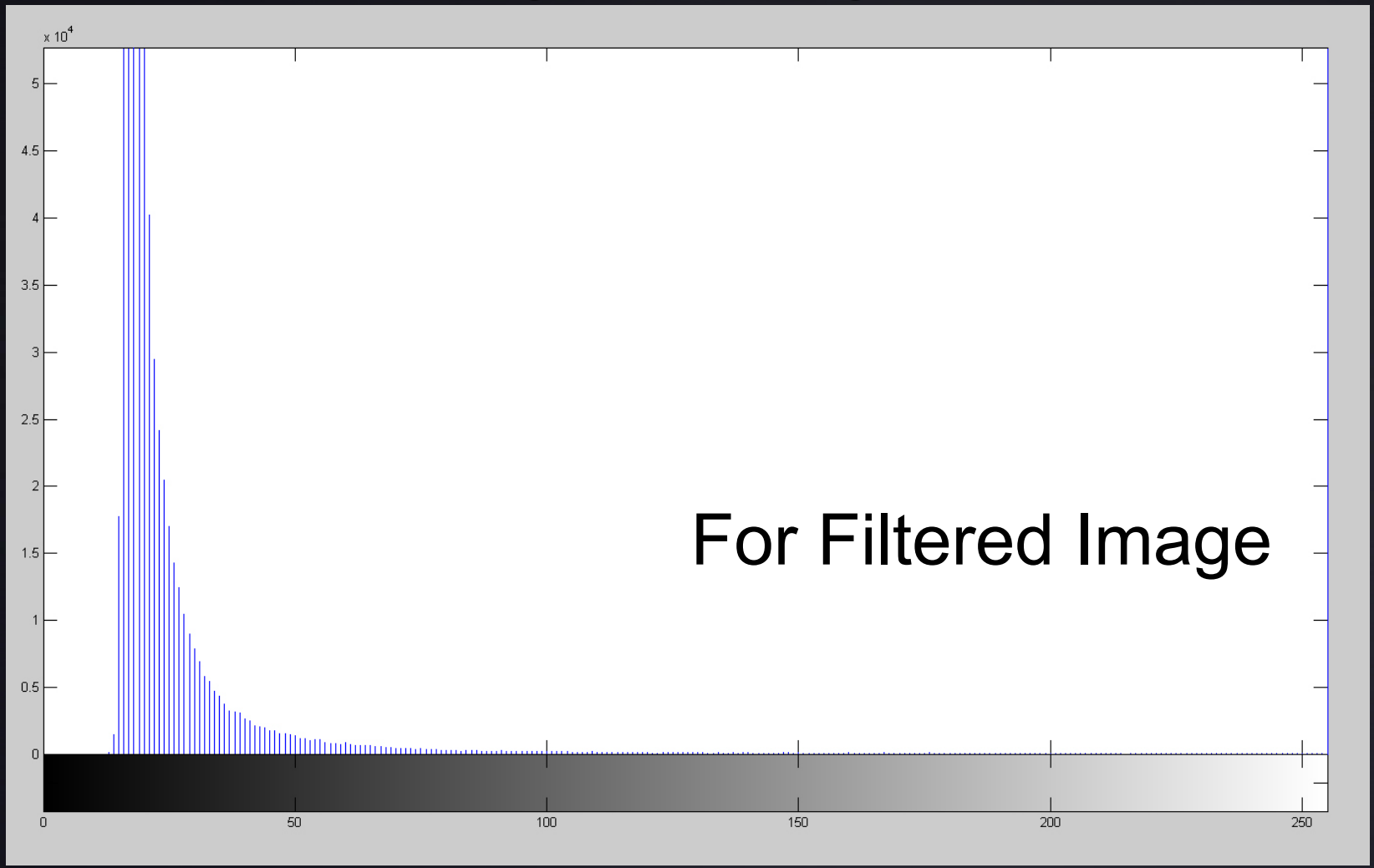
Threshold

Logical Image



Threshold

Logical Image



Threshold

Logical Image