AUTOMATIC CELL SEGMENTATION TO ANALYSE SINGLE CELL HETROGENEITY BASED ON MORPHOLOGICAL FEATURES

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OBJECTIVE

- To perform automatic segmentation and extract multiple morphological features from cells using MATlab.
- To elucidate the single cell heterogeneity among the cells based on shape and morphological features.
- To compare between single cell and overlapped cells in a image and infer the changes.

BACKGROUND

Problem Identification

ECM stiffness may cause heterogeneity

0.03 **0.3**

INTRODUCTION

INTRODUCTION

- The ensemble behaviors of cell population may not represent the behaviors of any individual cell.
- Behavior of each and every cell in a population is important to understand the accurate behavior of cells.
- The diverse collection of cells harboring distinct molecular signatures will have differential levels of sensitivity to treatment.
- So, it is essential to identify the differences that are important to differentiate between the cells.

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DATASET DESCRIPTION

Dataset

- Our dataset consists of images of two types of cells, Actin and Tubulin, nucleus at two different stress levels, 0.03 and 0.3.
- The stress levels are the mechanical stress experienced by the cells.
- The cells in 0.03 corresponds to healthy cells and at 0.3 are the diseased cells.
- Both the healthy and diseased state cells were monitored at three different times, 3 hours, 9 hours, and 24 hours

Data Framework

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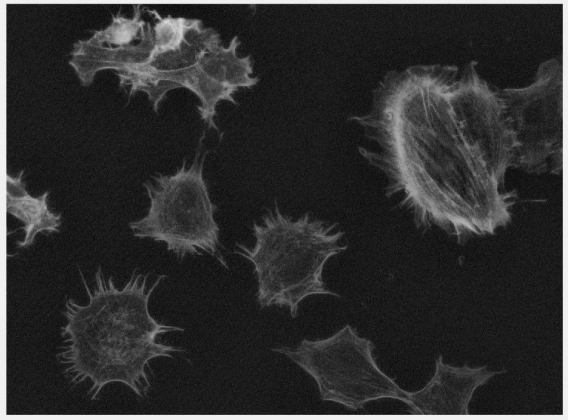
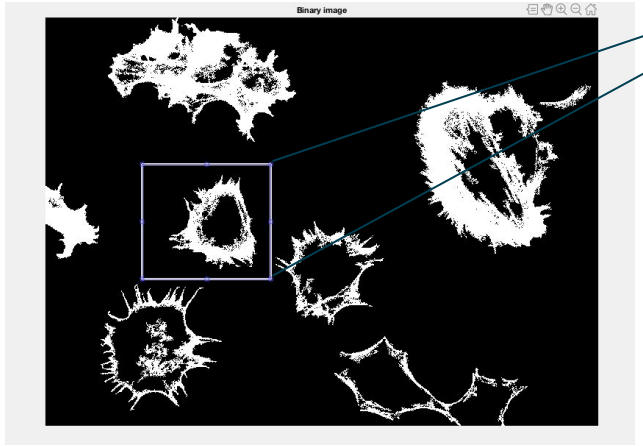
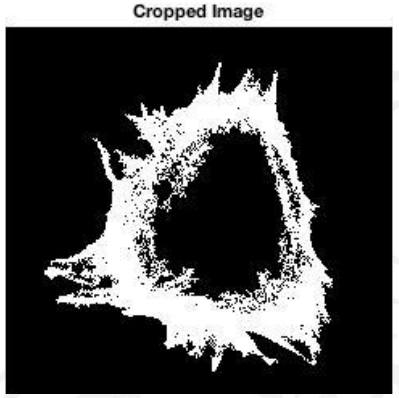
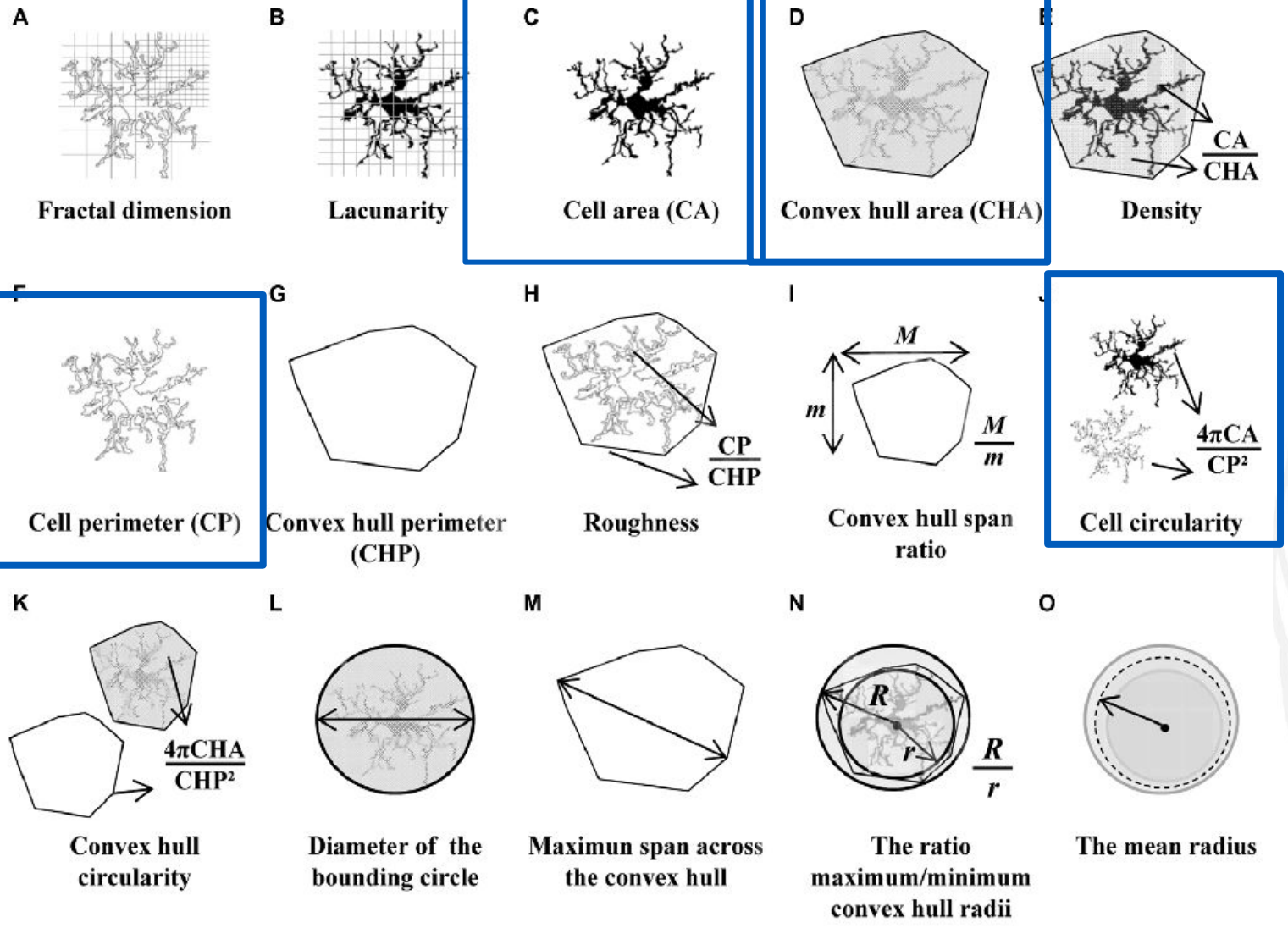
WORK FLOW

WORKFLOW









- **Area:** The area of the particle is calculated as the sum of the areas of each individual pixel within the borders of the cell.
- **Perimeter:** The perimeter of the particle, P, is defined as the total length of the object boundary.
- **Solidity:** Solidity, S, is the measurement of the overall concavity of a particle. It is defined as the image area, A, divided by the convex hull area, Ac.
- **Eccentricity:** The eccentricity is the ratio of the distance between the foci of the ellipse and its major axis length. The value is between 0 and 1.

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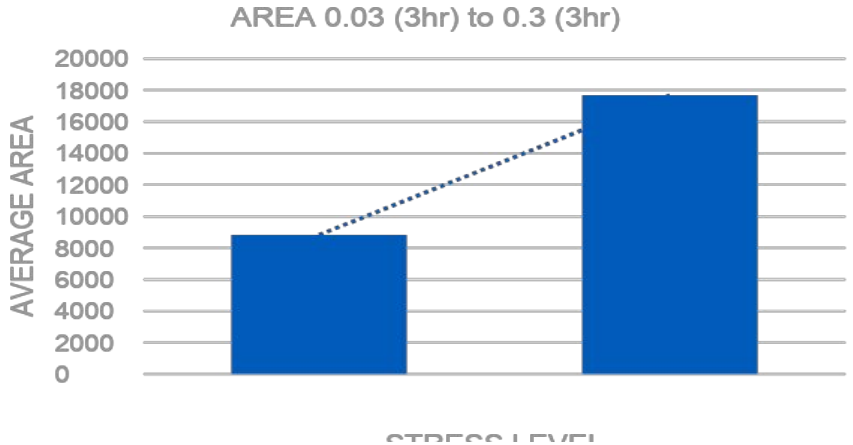
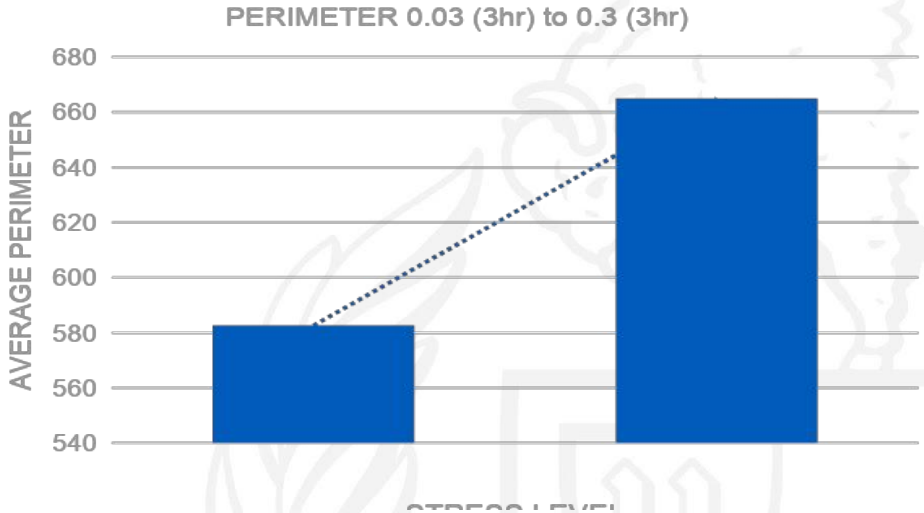
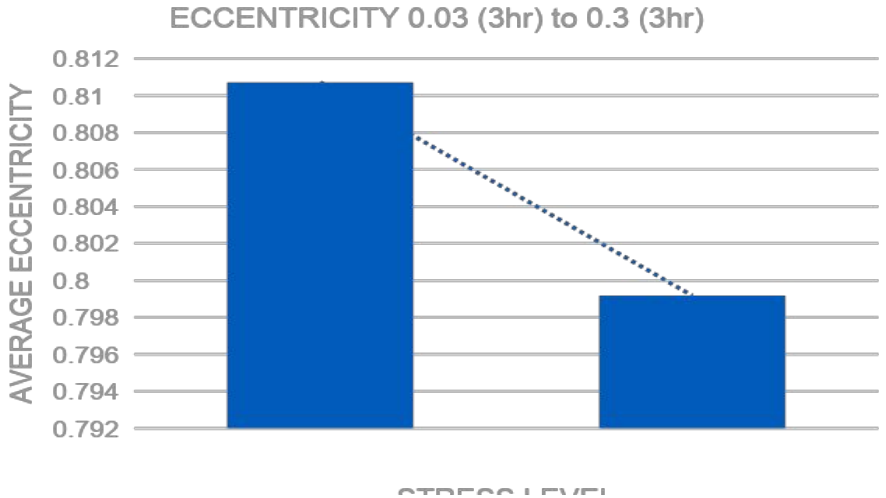
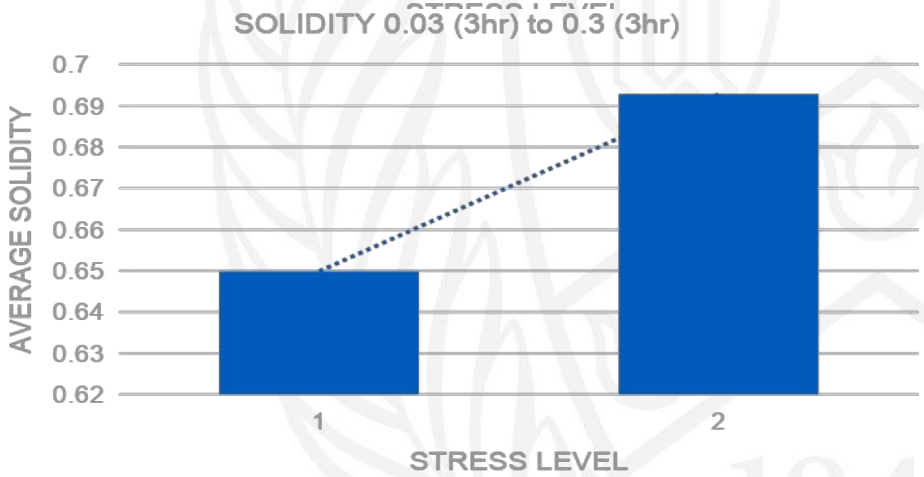
ANALYSIS

AVERAGE VALUE COMPARISON [ACTIN @ 3HR]

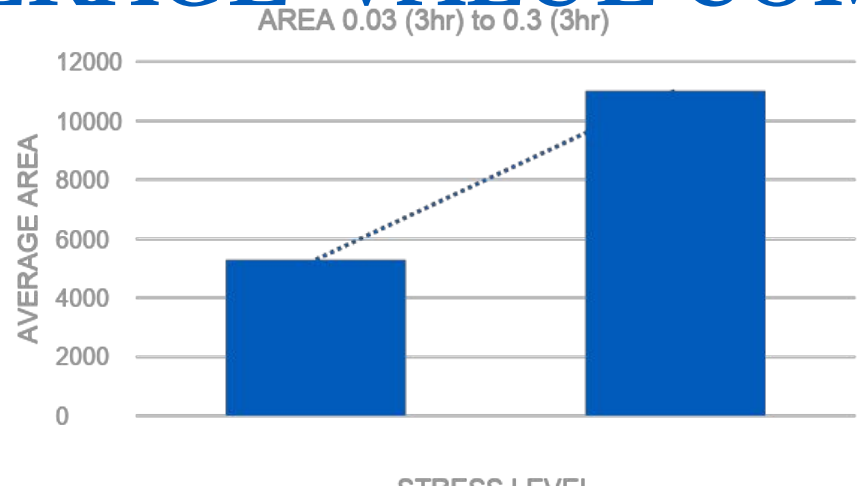


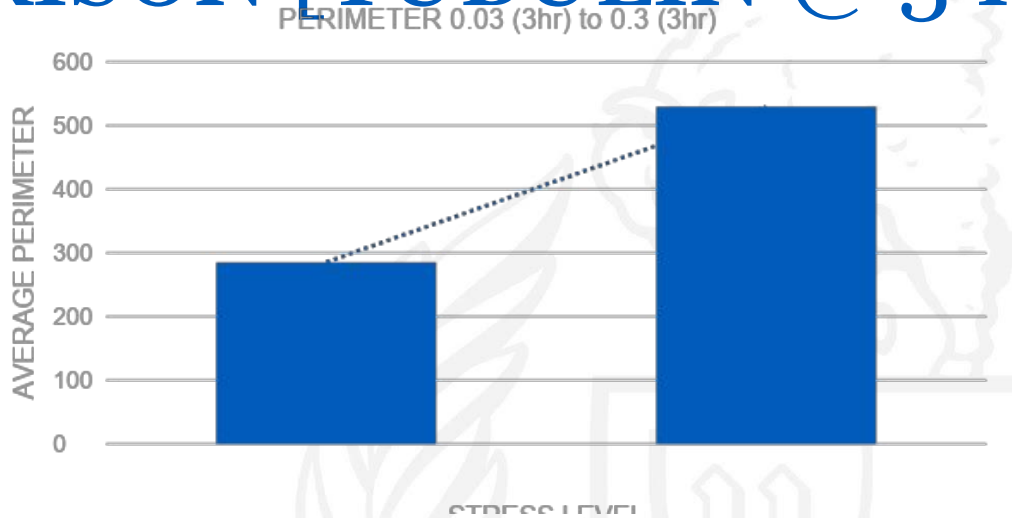


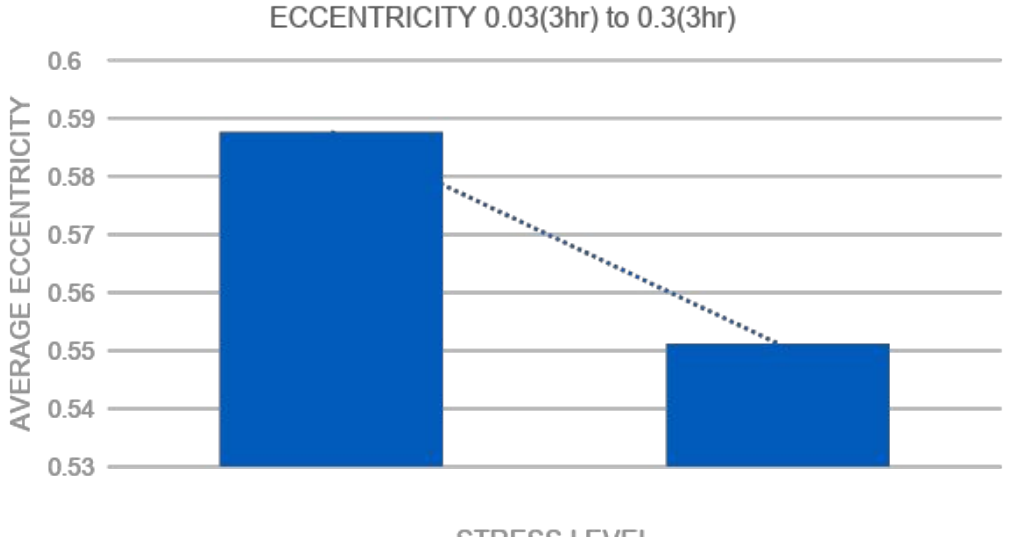


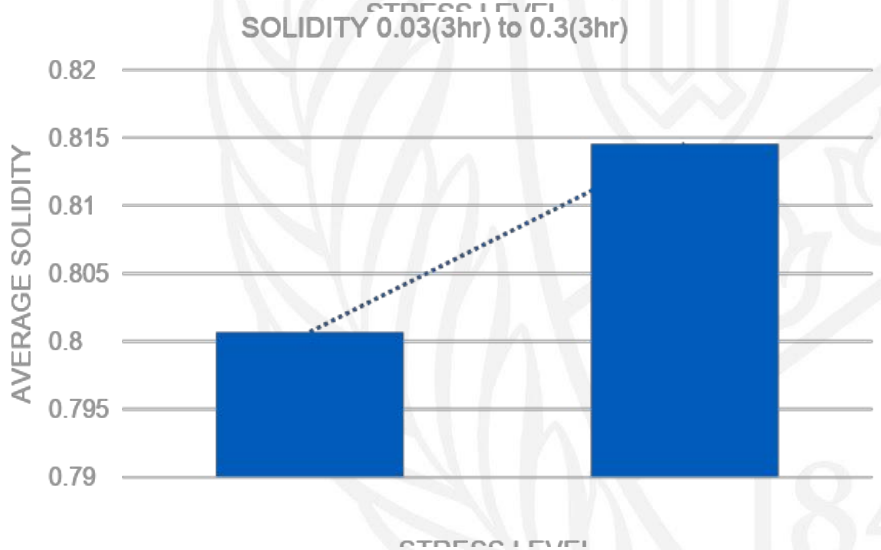


AVERAGE VALUE COMPARISON [TUBULIN @ 3 HR]

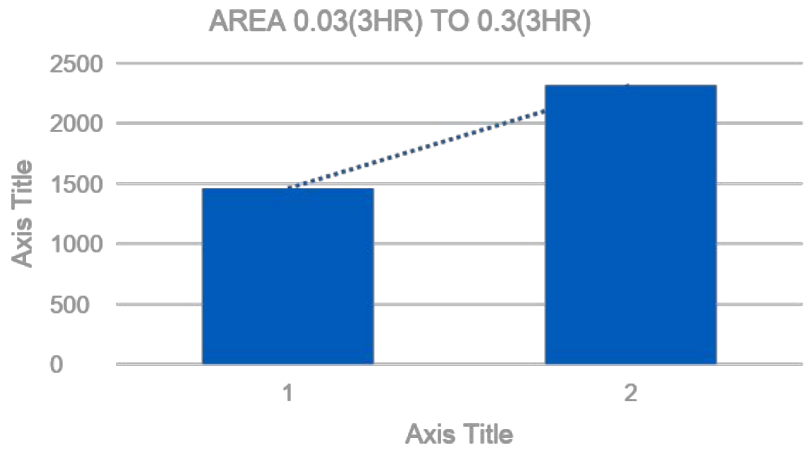


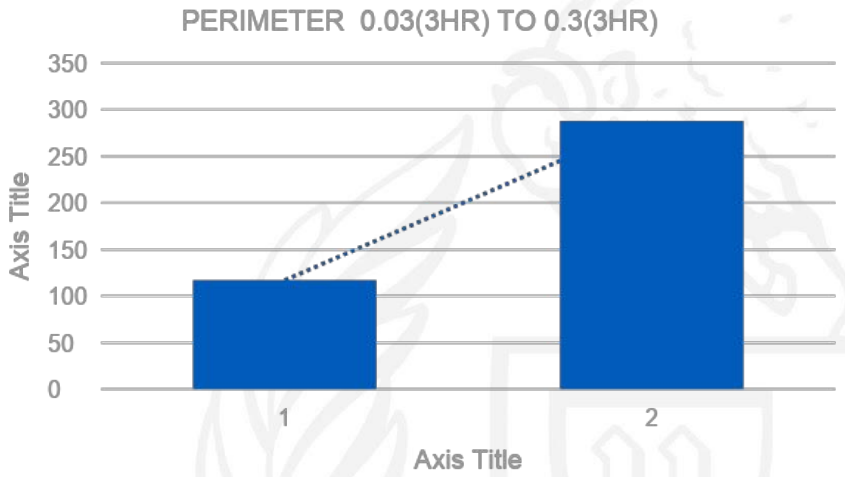


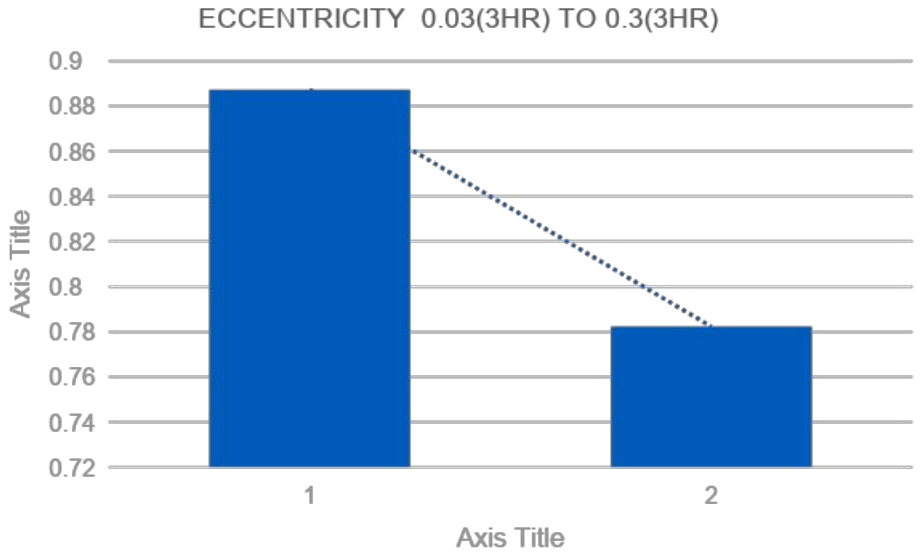


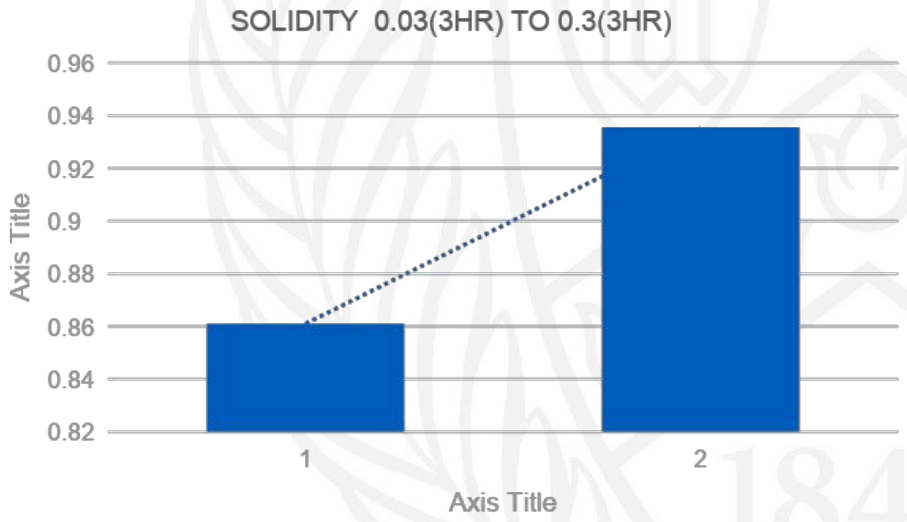


AVERAGE VALUE COMPARISON [NUCLEUS @ 3 HR]

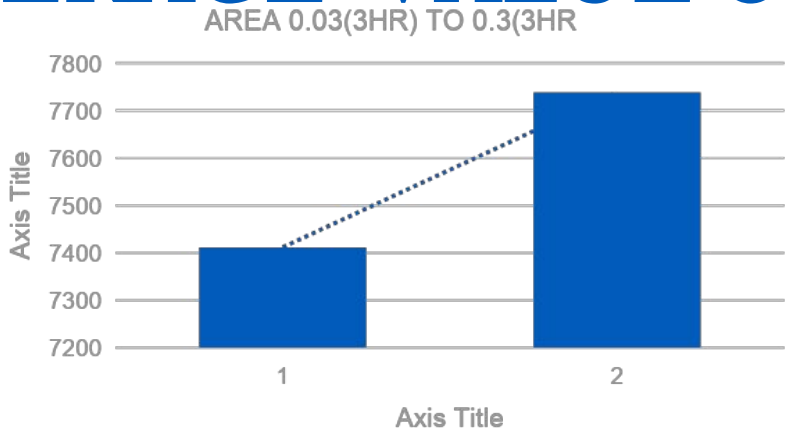


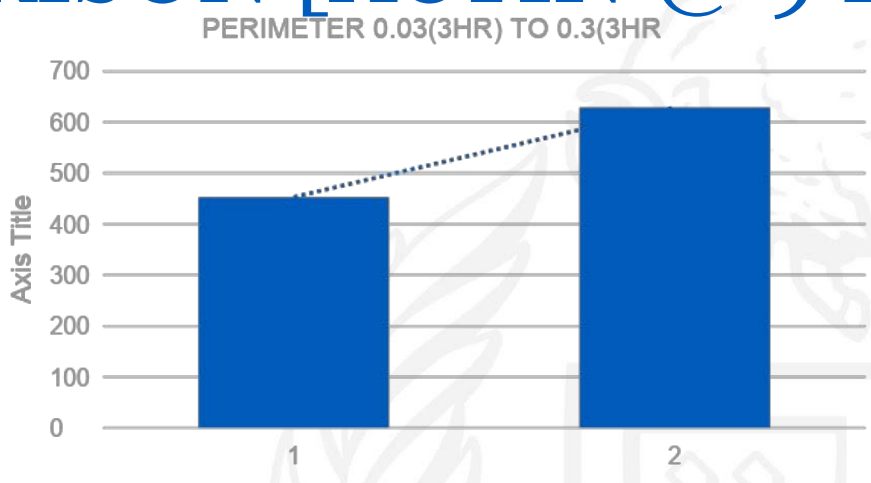


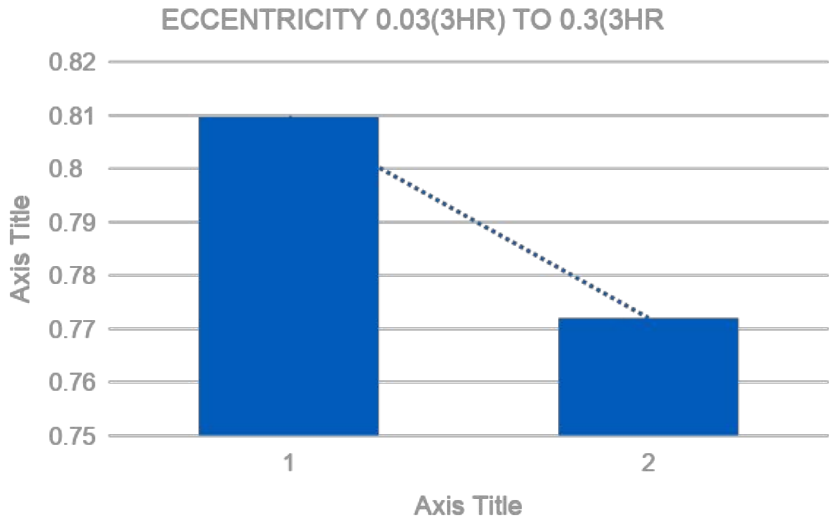


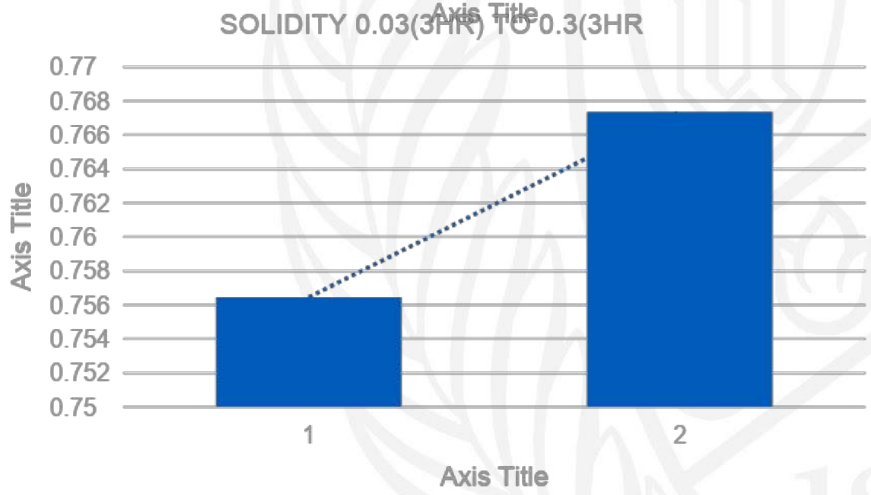


AVERAGE VALUE COMPARISON [ACTIN @ 9 HR]

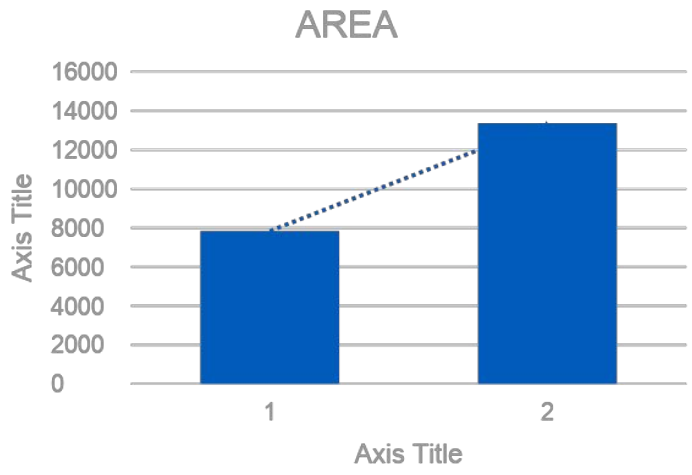


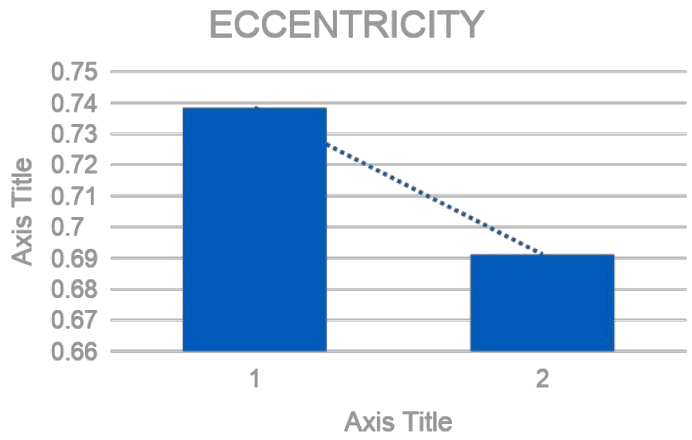


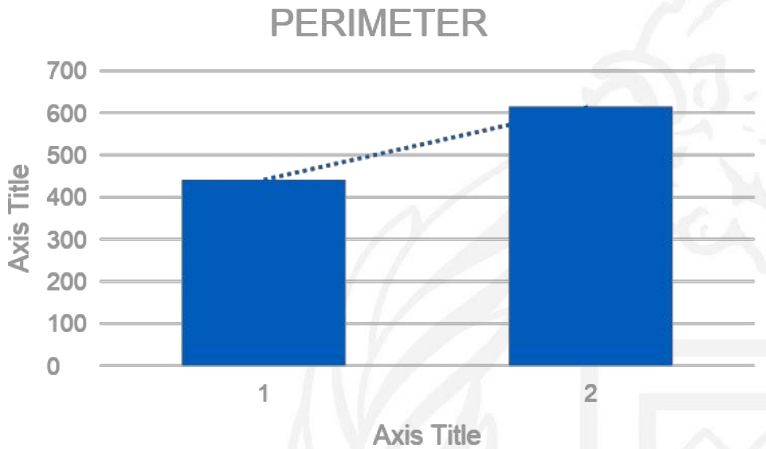


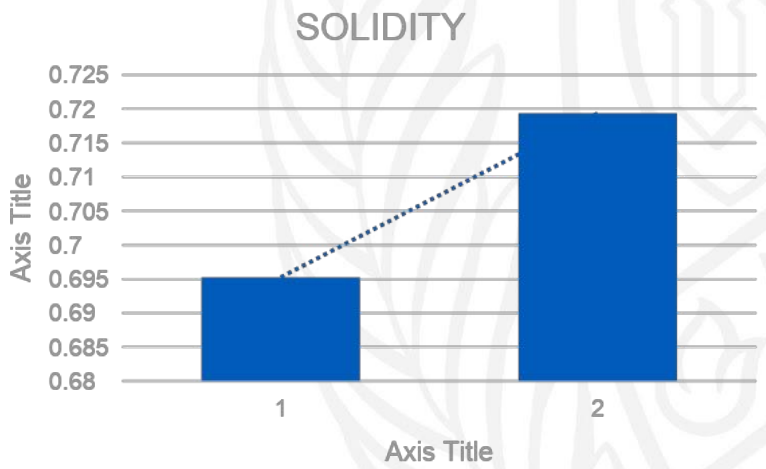


AVERAGE VALUE COMPARISON [TUBULIN @ 9 HR]

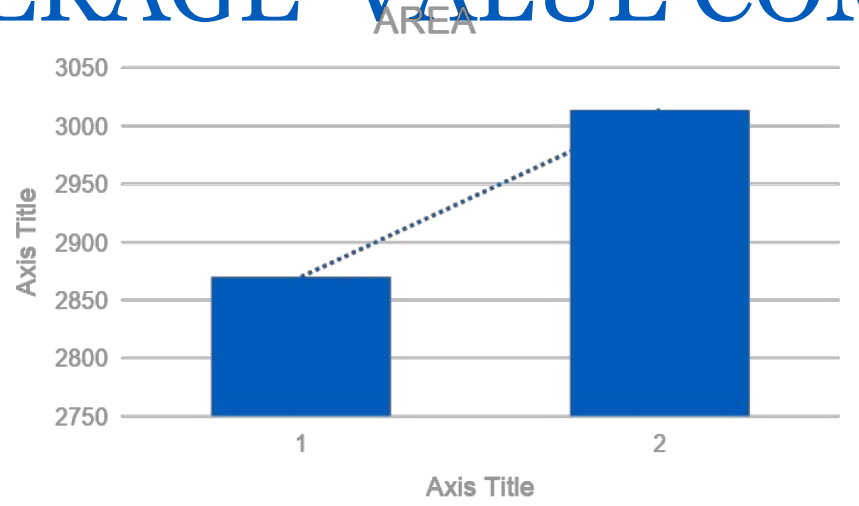


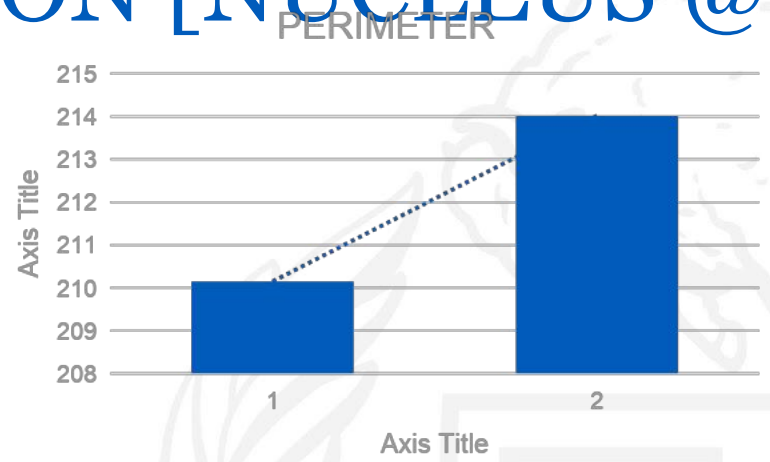


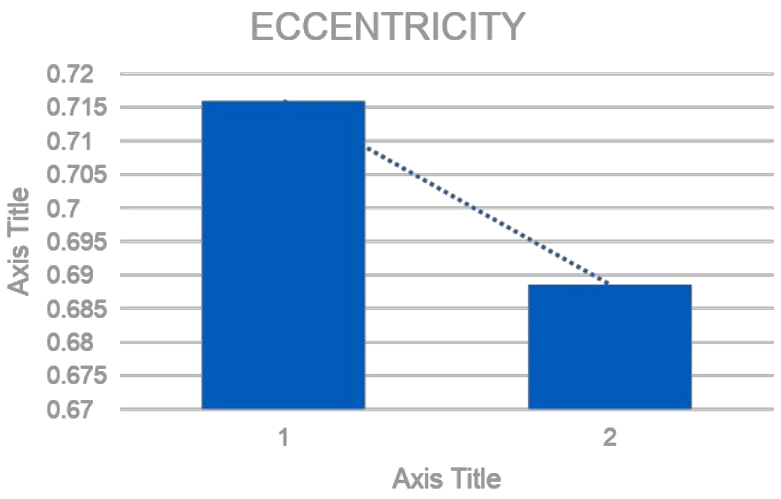


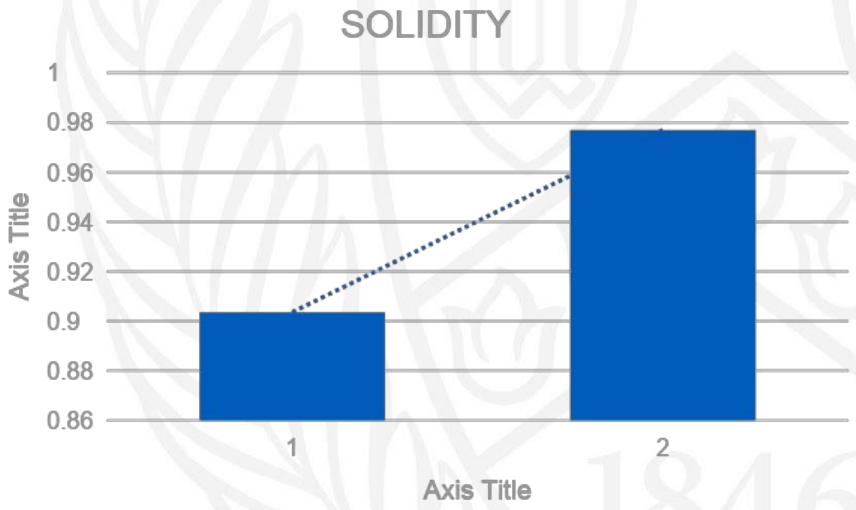


AVERAGE VALUE COMPARISON [NUCLEUS @ 9 HR]

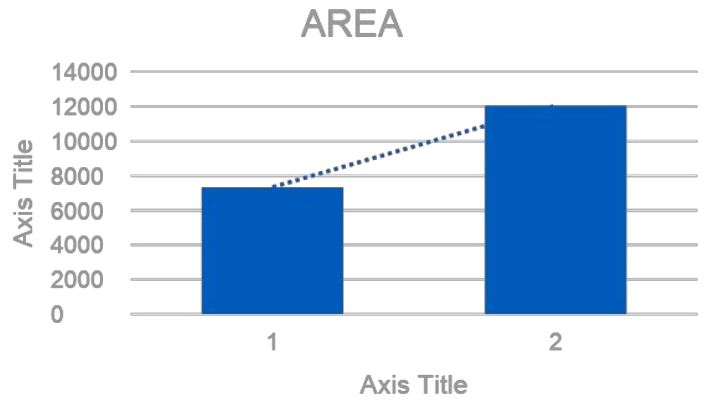


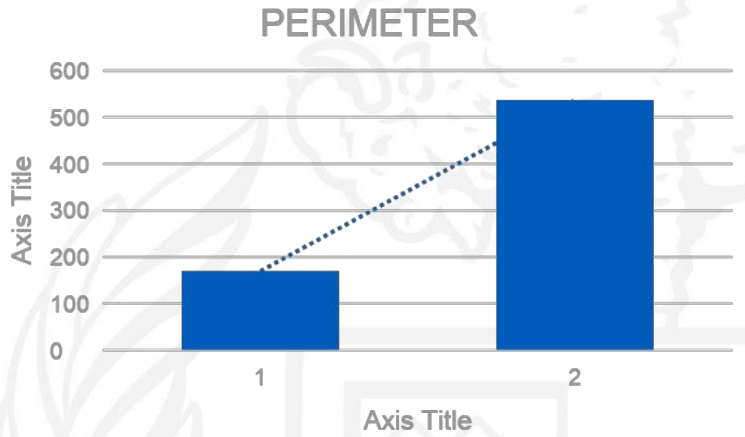


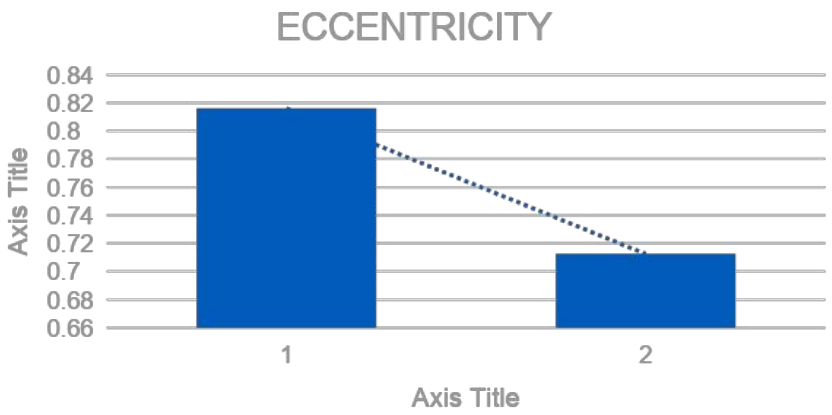


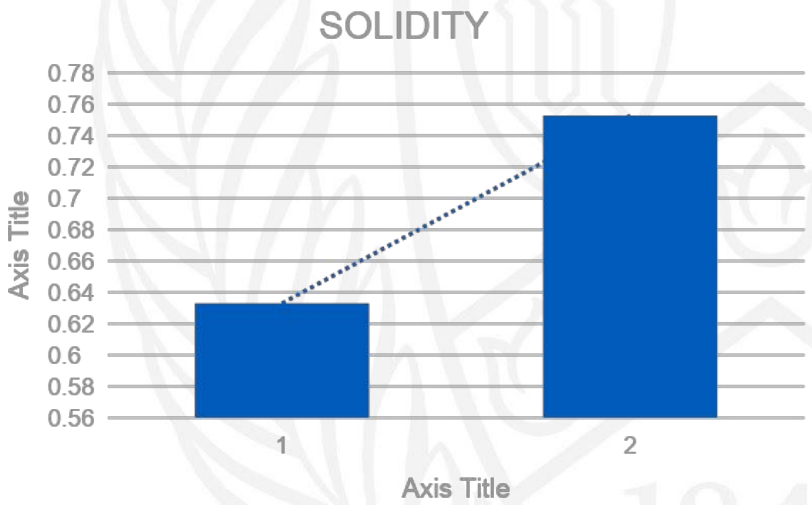


AVERAGE VALUE COMPARISON [ACTIN @ 24 HR]

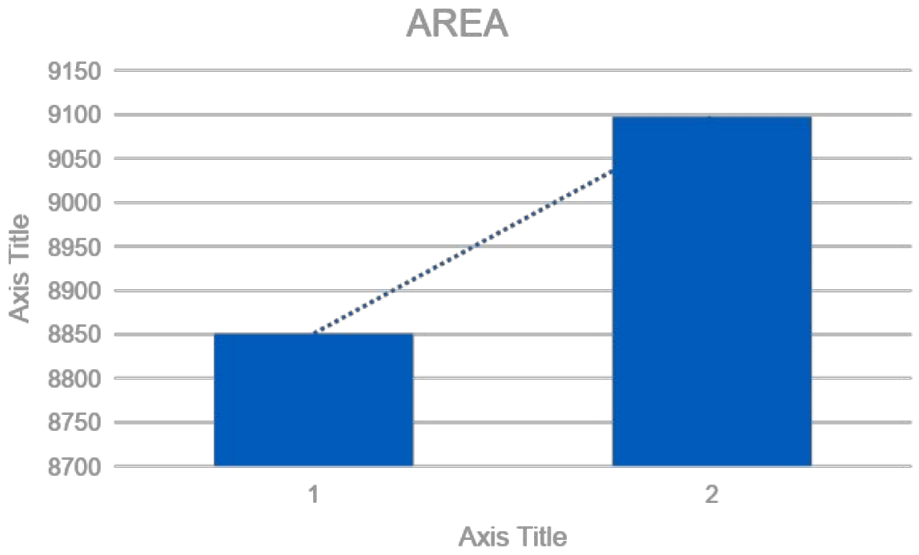


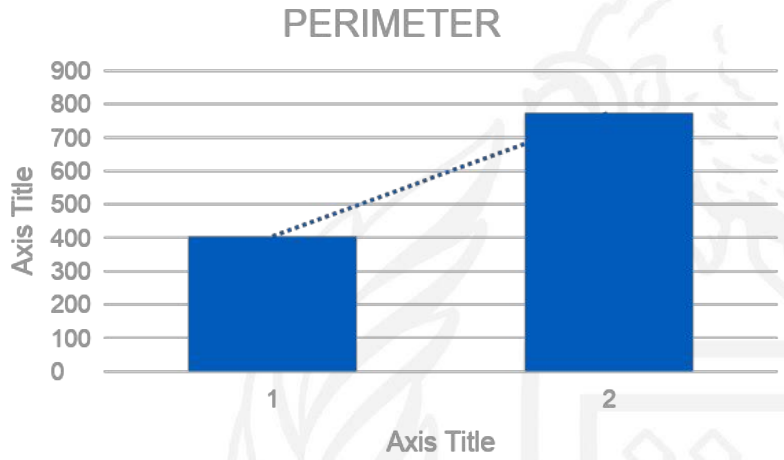


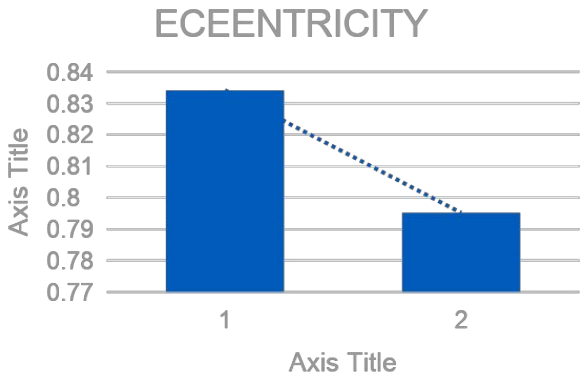


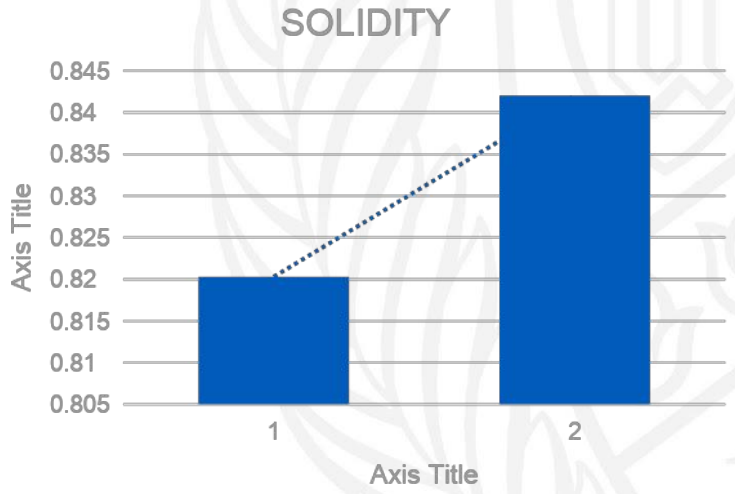


AVERAGE VALUE COMPARISON [TUBULIN @ 24 HR]









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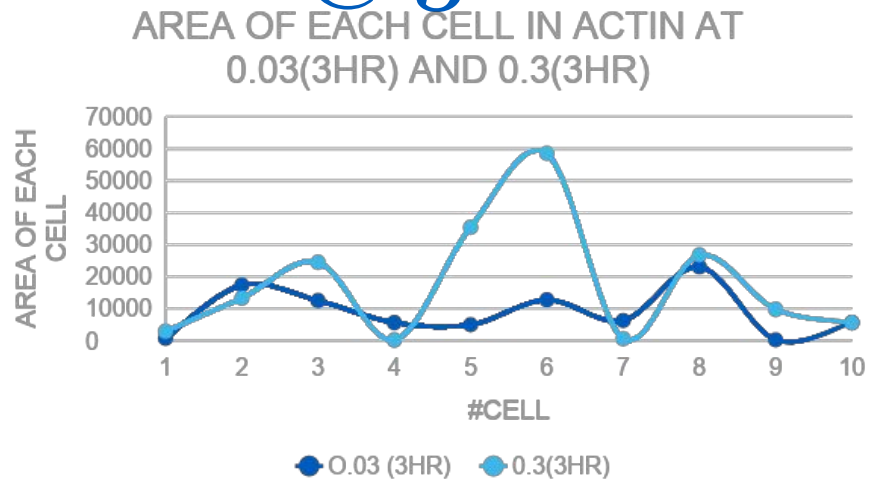
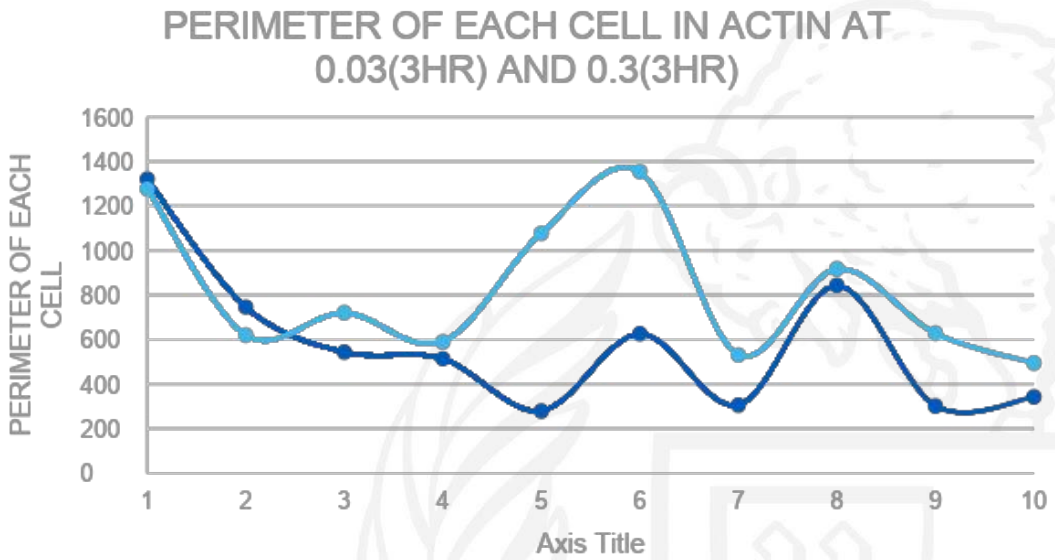
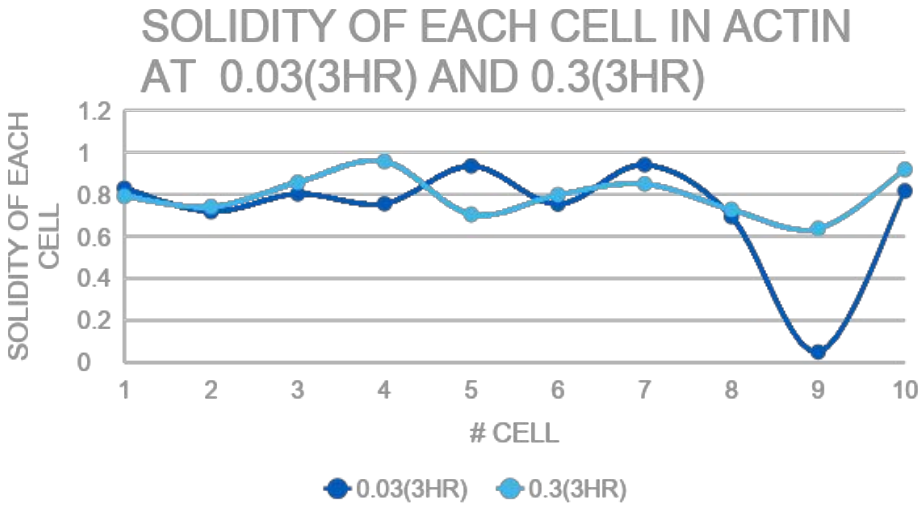
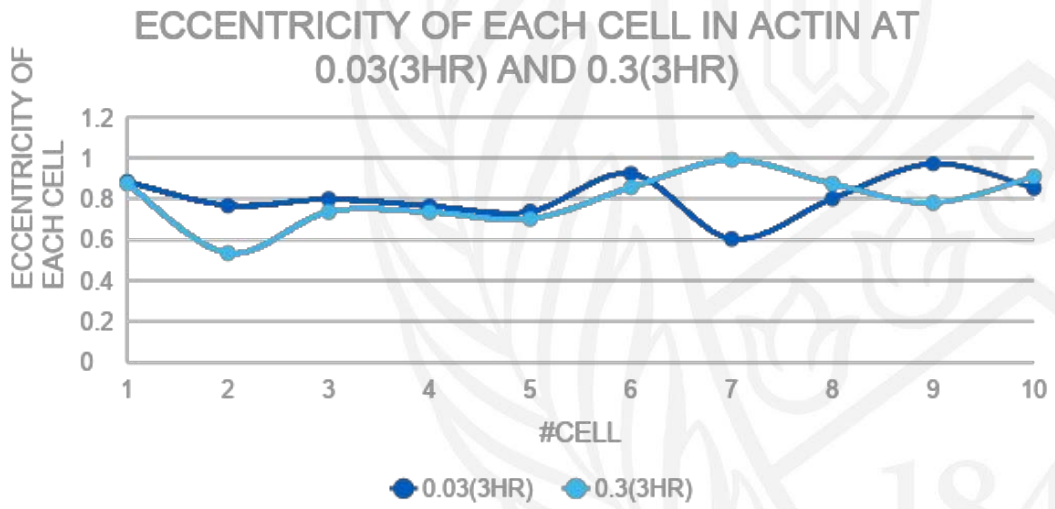
SINGLE CELL VALUE IN ONE IMAGE COMPARISON

ACTIN @ 3HR

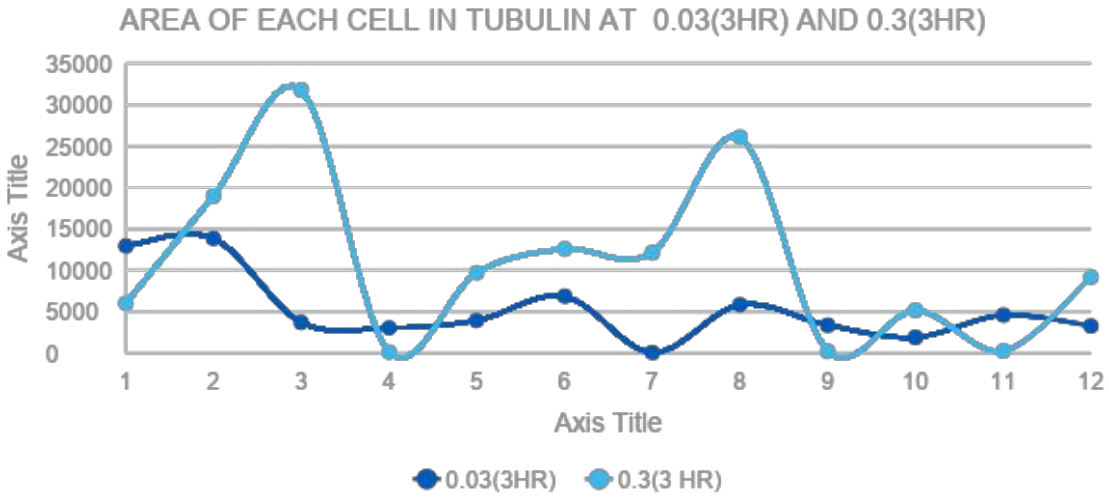

AREA OF EACH CELL IN ACTIN AT 0.03(3HR) AND 0.3(3HR)
AREA OF EACH CELL
70000
60000
50000
40000
30000
20000
10000
0
1 2 3 4 5 6 7 8 9 10
#CELL
O.03 (3HR)
0.3(3HR)

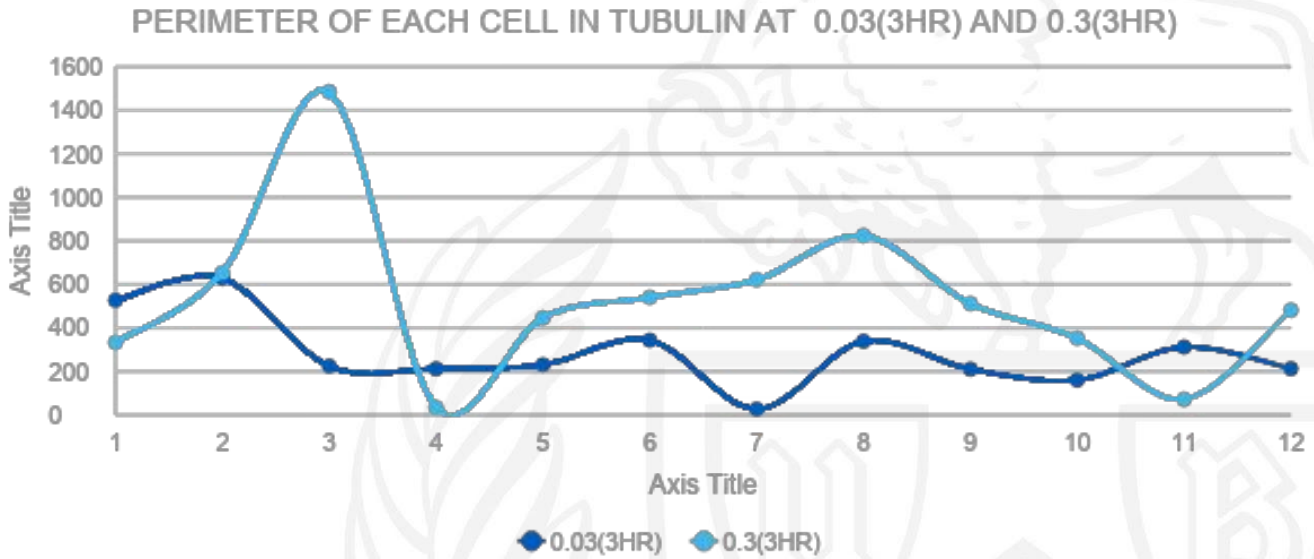

PERIMETER OF EACH CELL IN ACTIN AT 0.03(3HR) AND 0.3(3HR)
PERIMETER OF EACH CELL
1600
1400
1200
1000
800
600
400
200
0
1 2 3 4 5 6 7 8 9 10
Axis Title

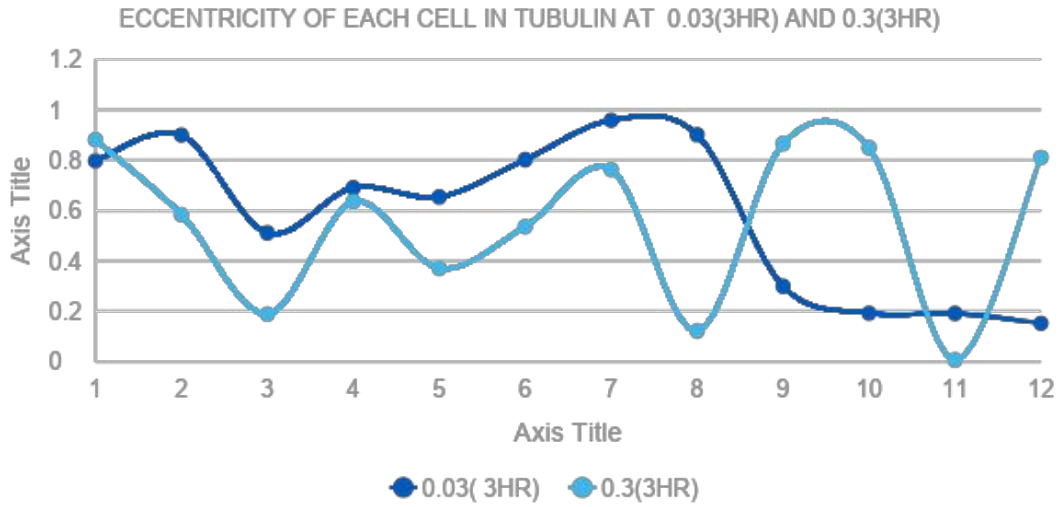

SOLIDITY OF EACH CELL IN ACTIN AT 0.03(3HR) AND 0.3(3HR)
SOLIDITY OF EACH CELL
1.2
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10
# CELL
0.03(3HR)
0.3(3HR)

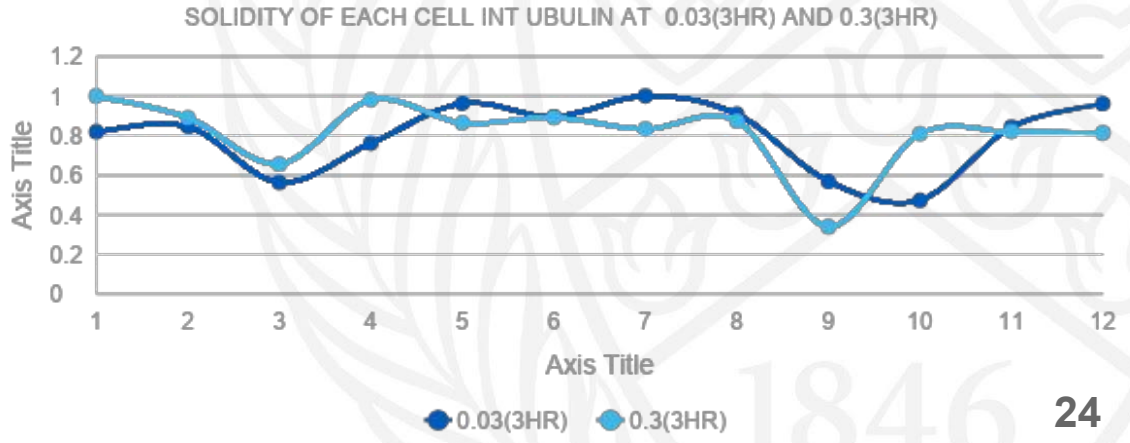

ECCENTRICITY OF EACH CELL IN ACTIN AT 0.03(3HR) AND 0.3(3HR)
ECCENTRICITY OF EACH CELL
1.2
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10
#CELL
0.03(3HR)
0.3(3HR)

TUBULIN @ 3 HR

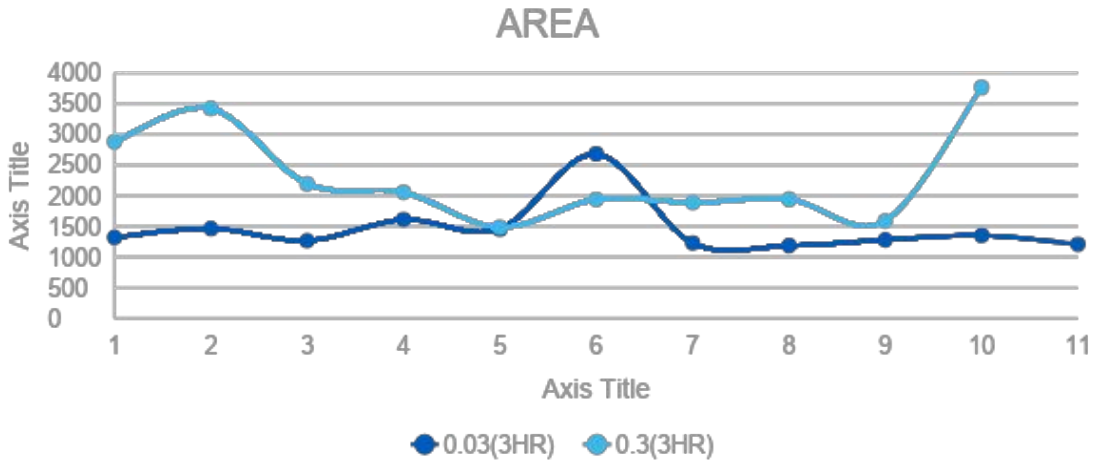

AREA OF EACH CELL IN TUBULIN AT 0.03(3HR) AND 0.3(3HR)
35000
30000
25000
20000
15000
10000
5000
0
Axis Title
1 2 3 4 5 6 7 8 9 10 11 12
Axis Title
0.03(3HR)
0.3(3 HR)

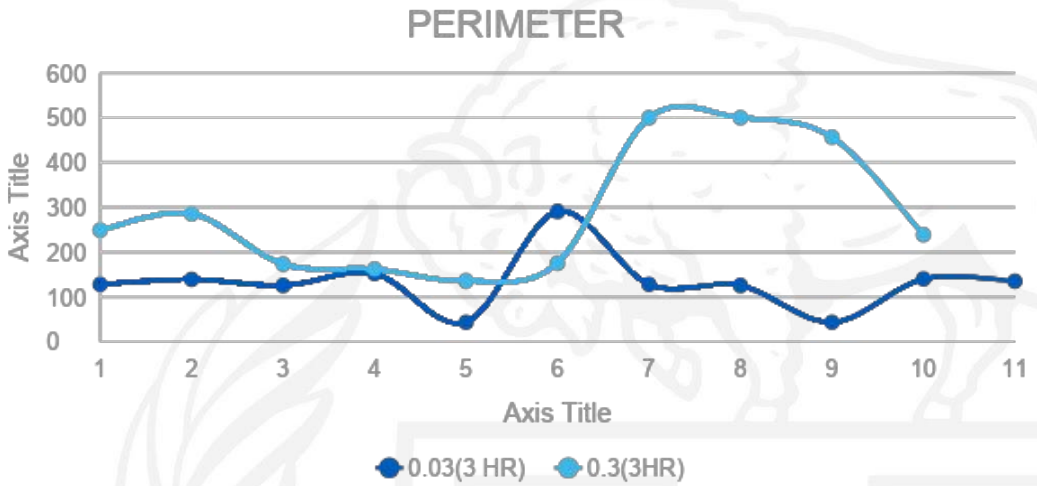

PERIMETER OF EACH CELL IN TUBULIN AT 0.03(3HR) AND 0.3(3HR)
1600
1400
1200
1000
800
600
400
200
0
Axis Title
1 2 3 4 5 6 7 8 9 10 11 12
Axis Title
0.03(3HR)
0.3(3HR)

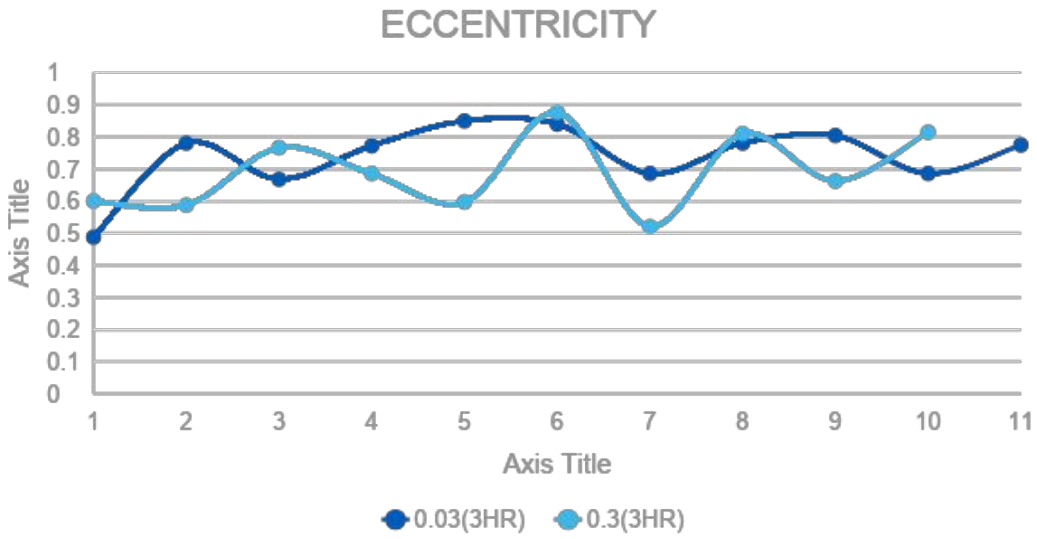

ECCENTRICITY OF EACH CELL IN TUBULIN AT 0.03(3HR) AND 0.3(3HR)
1.2
1
0.8
0.6
0.4
0.2
0
Axis Title
1 2 3 4 5 6 7 8 9 10 11 12
Axis Title
0.03(3HR)
0.3(3HR)

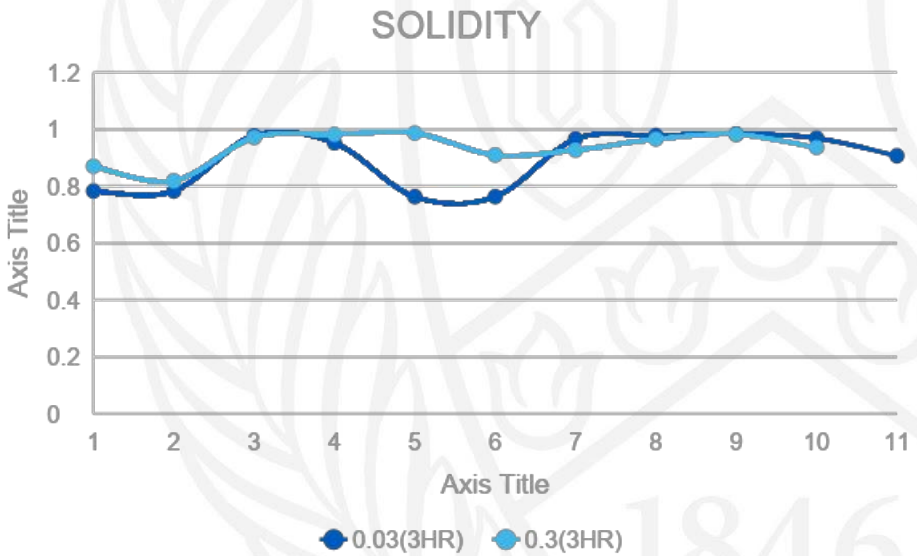

SOLIDITY OF EACH CELL INT UBULIN AT 0.03(3HR) AND 0.3(3HR)
1.2
1
0.8
0.6
0.4
0.2
0
Axis Title
1 2 3 4 5 6 7 8 9 10 11 12
Axis Title
0.03(3HR)
0.3(3HR)

NUCLEUS @ 3 HR


AREA
4000
3500
3000
2500
2000
1500
1000
500
0
Axis Title
1 2 3 4 5 6 7 8 9 10 11
Axis Title
0.03(3HR)
0.3(3HR)


PERIMETER
600
500
400
300
200
100
0
Axis Title
1 2 3 4 5 6 7 8 9 10 11
Axis Title
0.03(3 HR)
0.3(3HR)


ECCENTRICITY
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Axis Title
1 2 3 4 5 6 7 8 9 10 11
Axis Title
0.03(3HR)
0.3(3HR)


SOLIDITY
1.2
1
0.8
0.6
0.4
0.2
0
Axis Title
1 2 3 4 5 6 7 8 9 10 11
Axis Title
0.03(3HR)
0.3(3HR)

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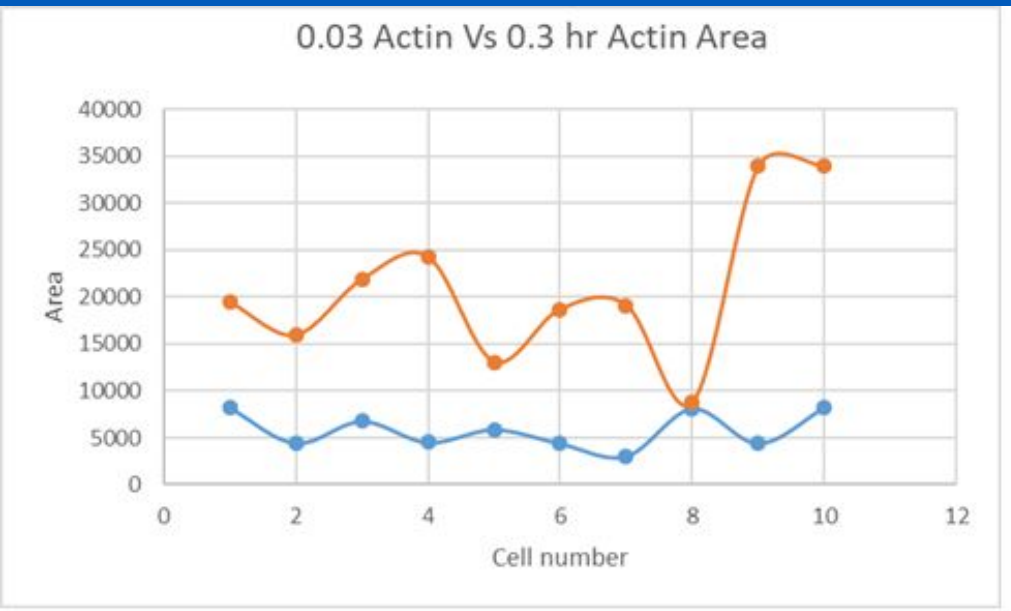
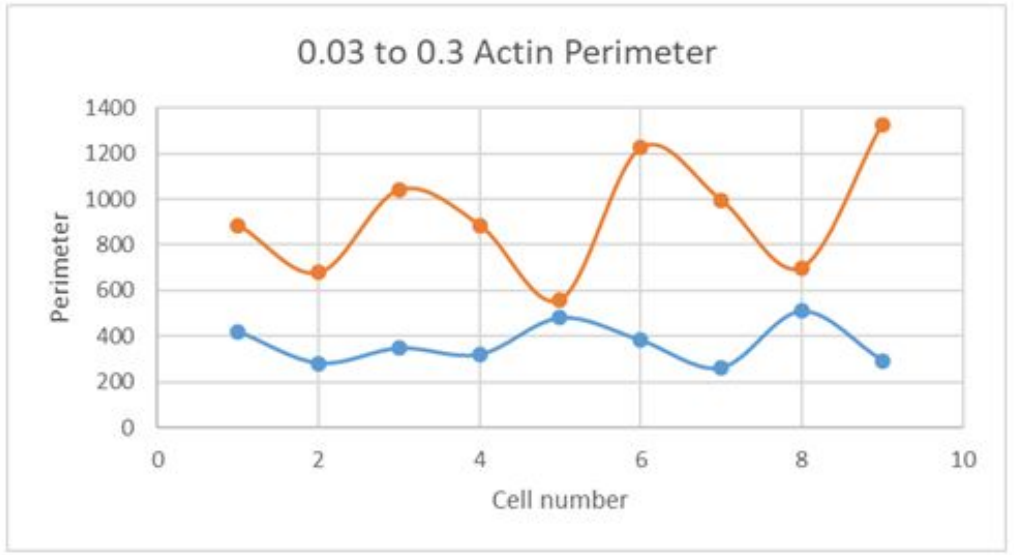
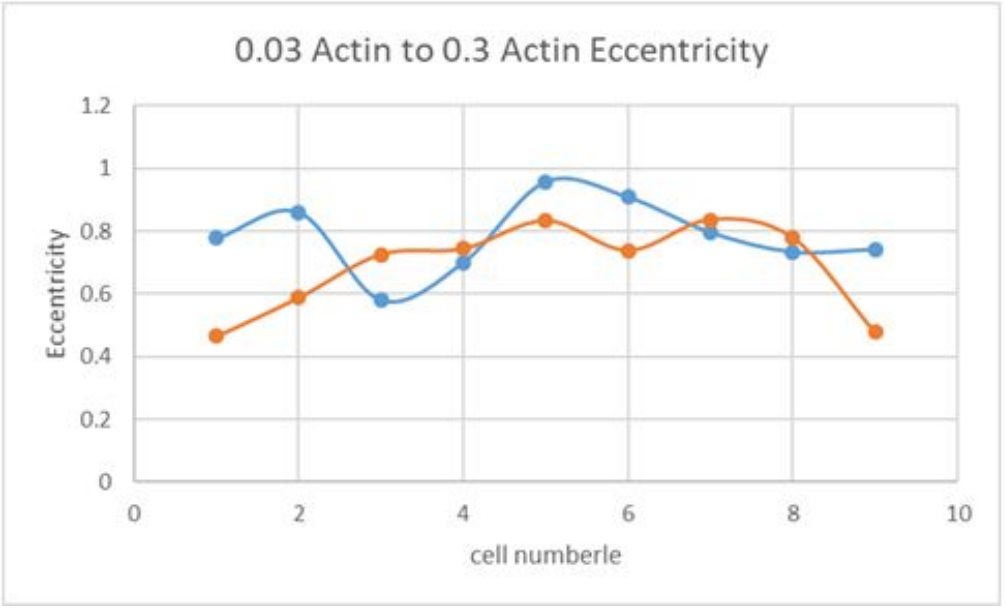
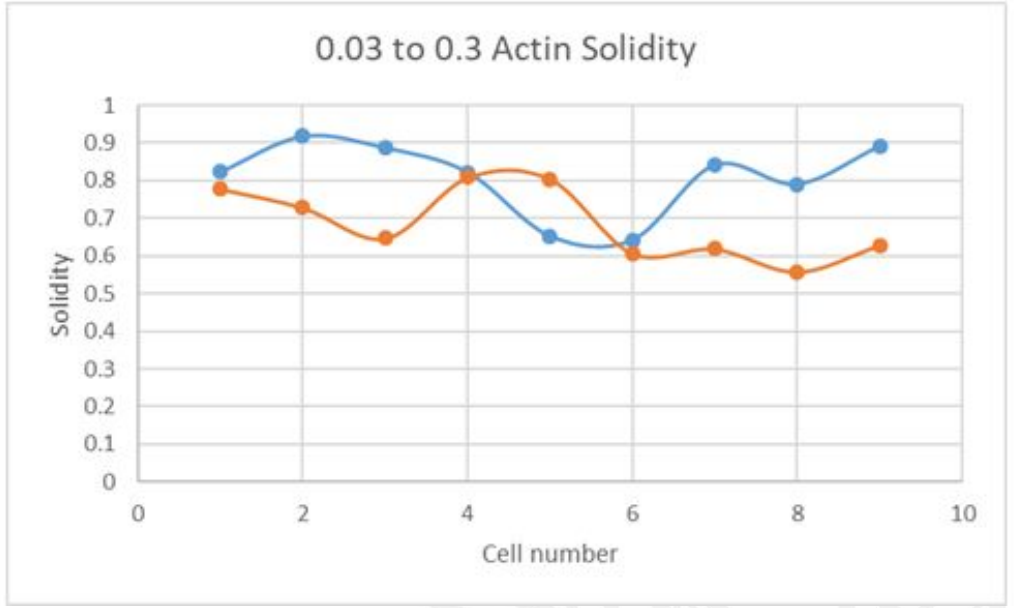
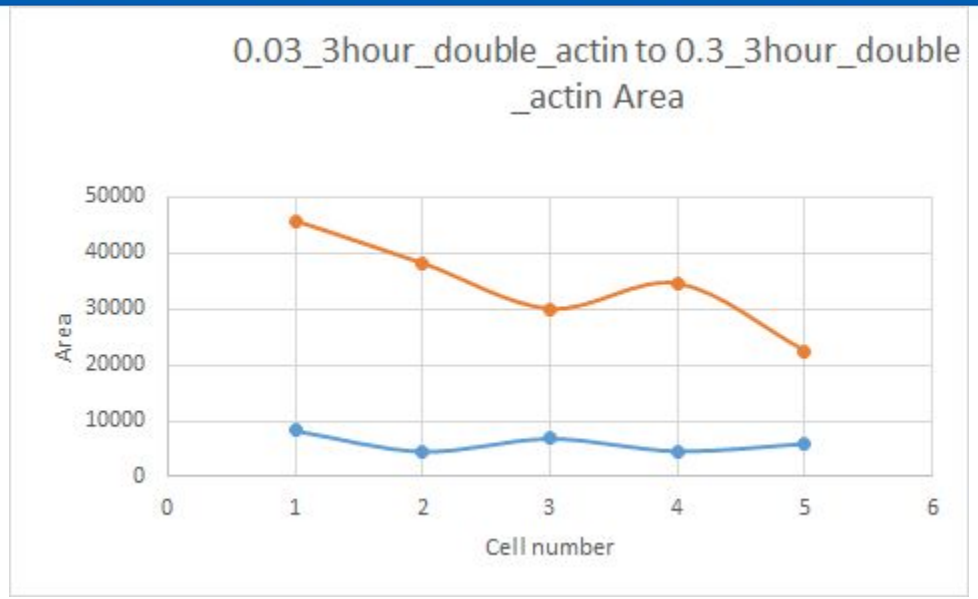
SINGLE CELL ANALYSIS FROM A GROUP OF IMAGES

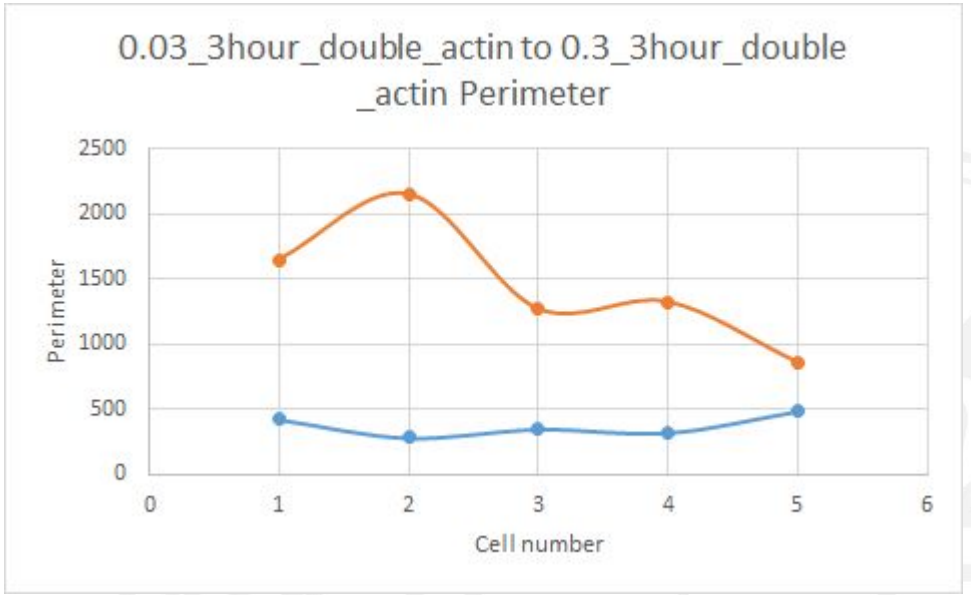

0.03 Actin Vs 0.3 hr Actin Area
Area
Cell number
0.03
0.3

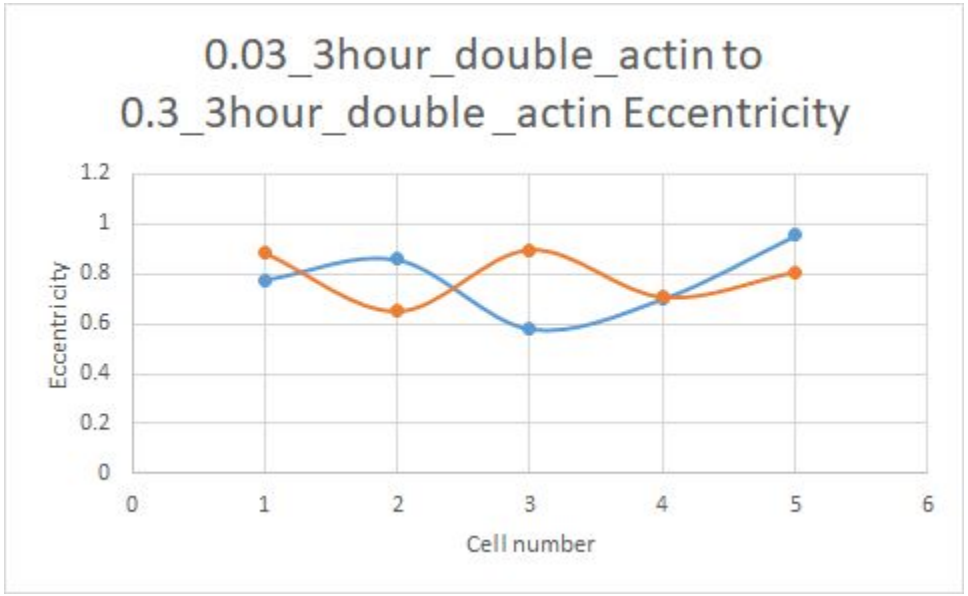

0.03 to 0.3 Actin Perimeter
Perimeter
Cell number
0.03
0.3

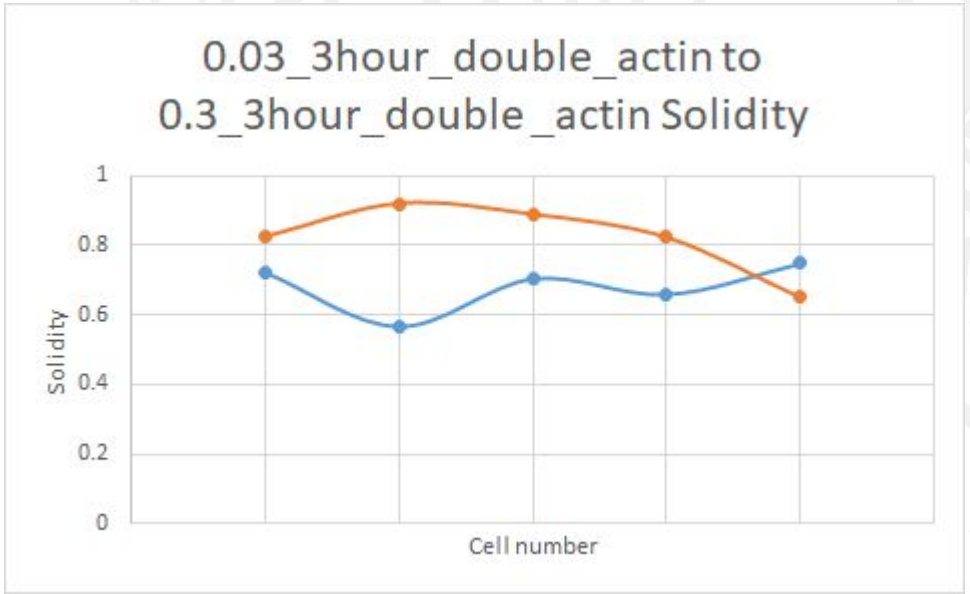

0.03 Actin to 0.3 Actin Eccentricity
Eccentricity
cell numberle
0.03
0.3


0.03 to 0.3 Actin Solidity
Solidity
Cell number
0.03
0.3


0.03_3hour_double_actin to 0.3_3hour_double _actin Area
Area
50000
40000
30000
20000
10000
0
0
1
2
3
4
5
6
Cell number


0.03_3hour_double_actin to 0.3_3hour_double _actin Perimeter
Perimeter
2500
2000
1500
1000
500
0
0
1
2
3
4
5
6
Cell number


0.03_3hour_double_actin to 0.3_3hour_double _actin Eccentricity
Eccentricity
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
Cell number


0.03_3hour_double_actin to 0.3_3hour_double _actin Solidity
Solidity
1
0.8
0.6
0.4
0.2
0
Cell number

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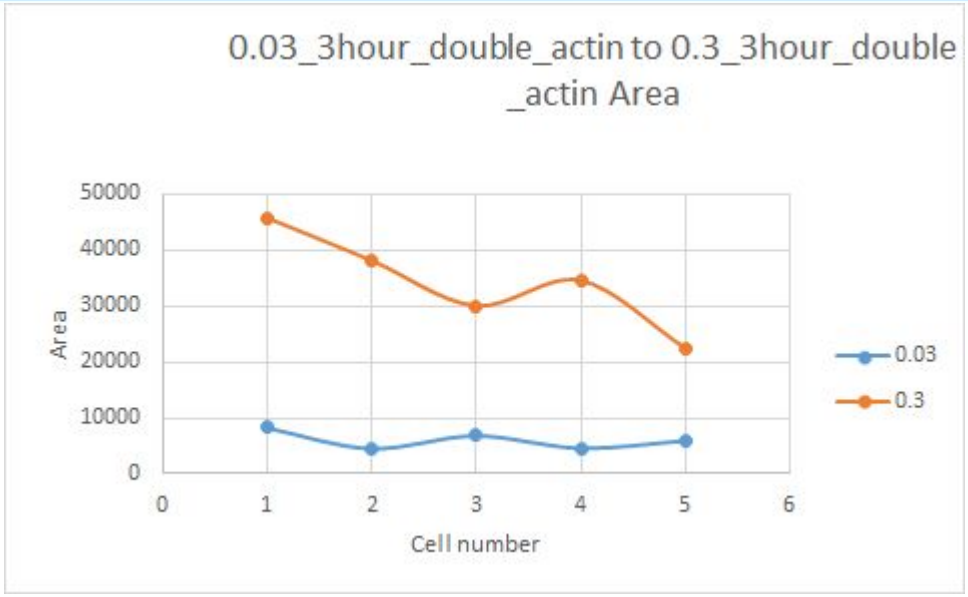
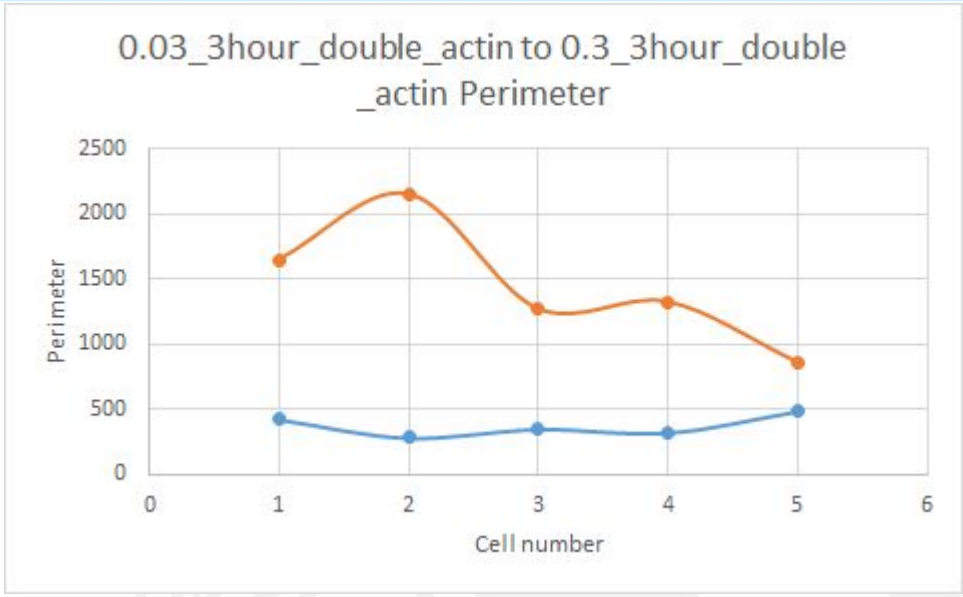
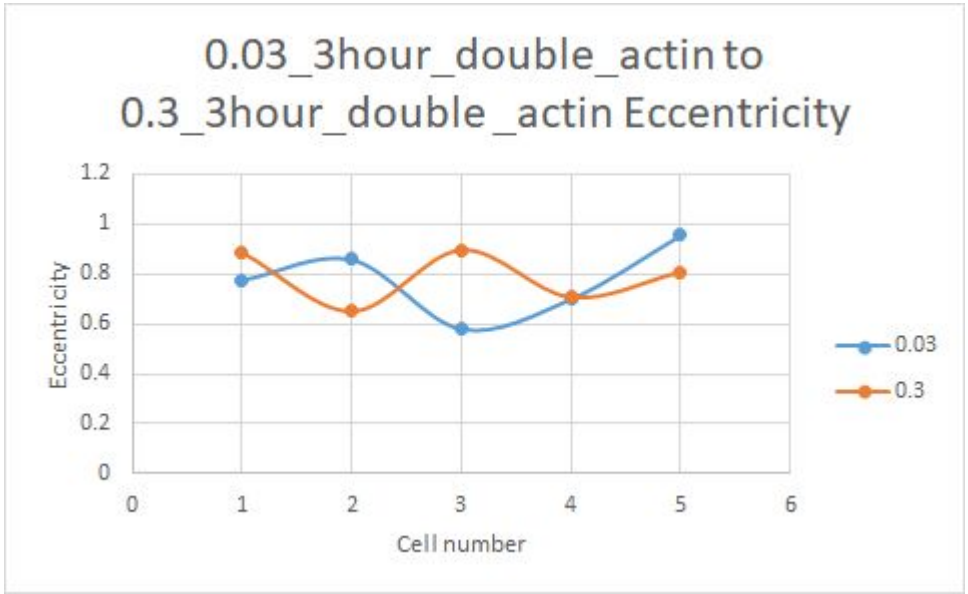
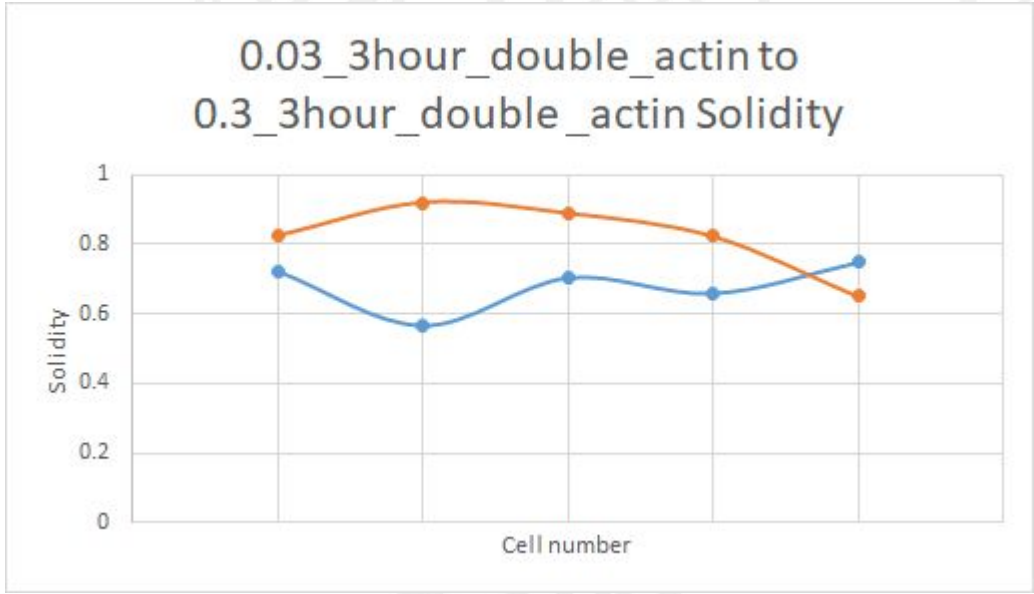
OVERLAPPED CELL ANALYSIS FROM A GROUP OF IMAGES


0.03_3hour_double_actin to 0.3_3hour_double _actin Area
Area
50000
40000
30000
20000
10000
0
0
1
2
3
4
5
6
Cell number
0.03
0.3


0.03_3hour_double_actin to 0.3_3hour_double _actin Perimeter
Perimeter
2500
2000
1500
1000
500
0
0
1
2
3
4
5
6
Cell number


0.03_3hour_double_actin to 0.3_3hour_double _actin Eccentricity
Eccentricity
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
Cell number
0.03
0.3


0.03_3hour_double_actin to 0.3_3hour_double _actin Solidity
Solidity
1
0.8
0.6
0.4
0.2
0
Cell number

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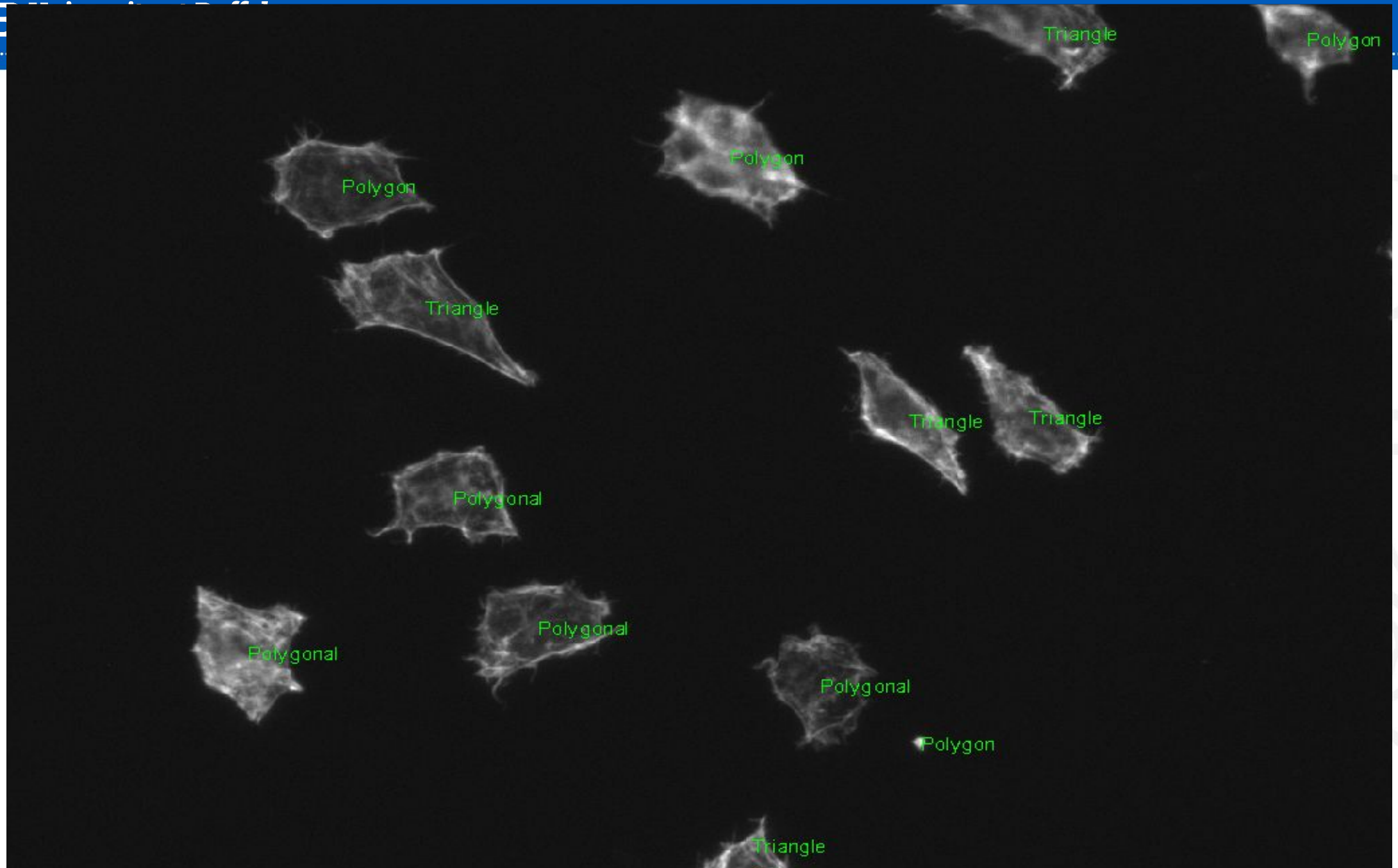
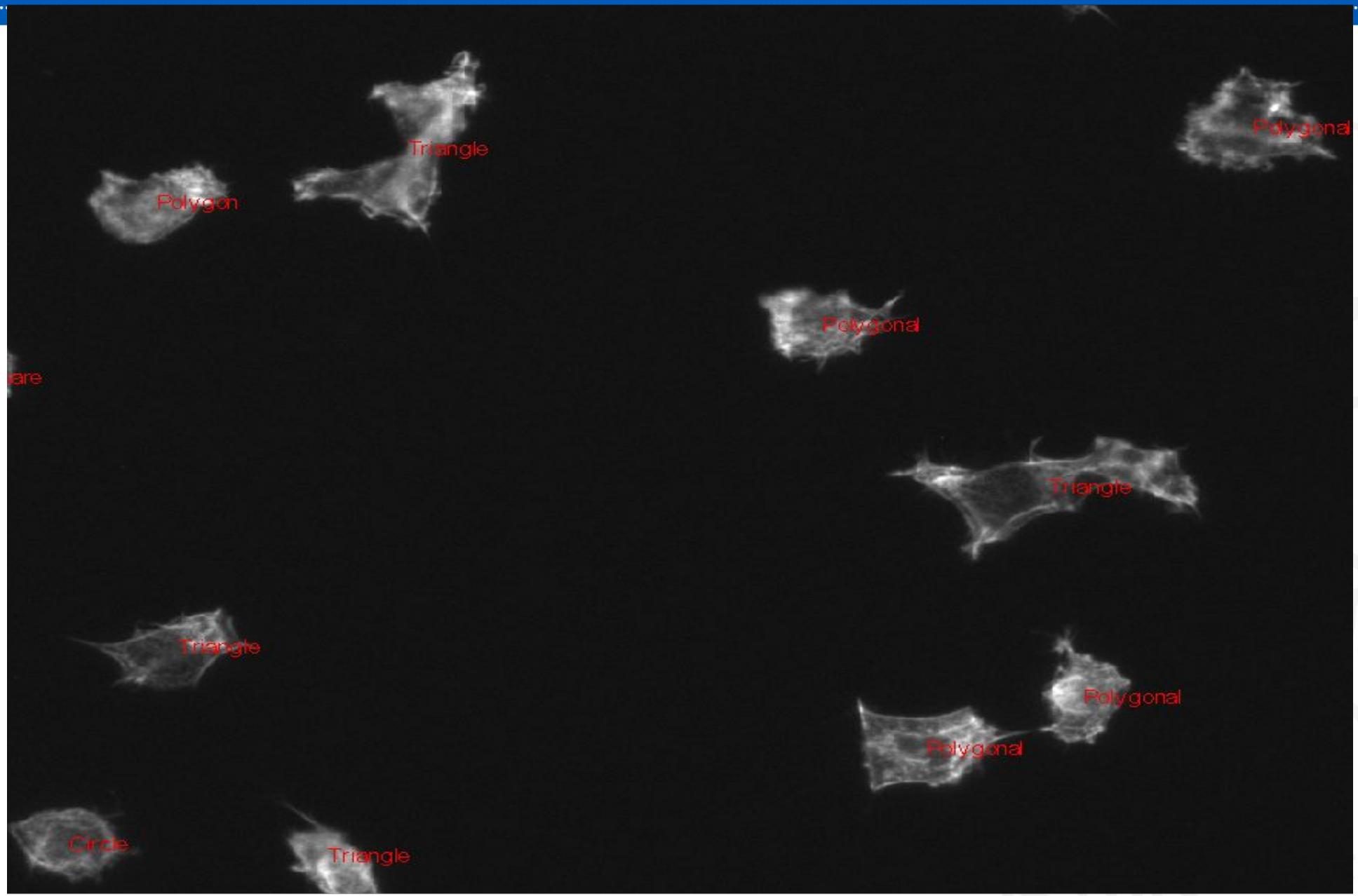
DETERMINING THE SHAPE EACH CELL

Circularity

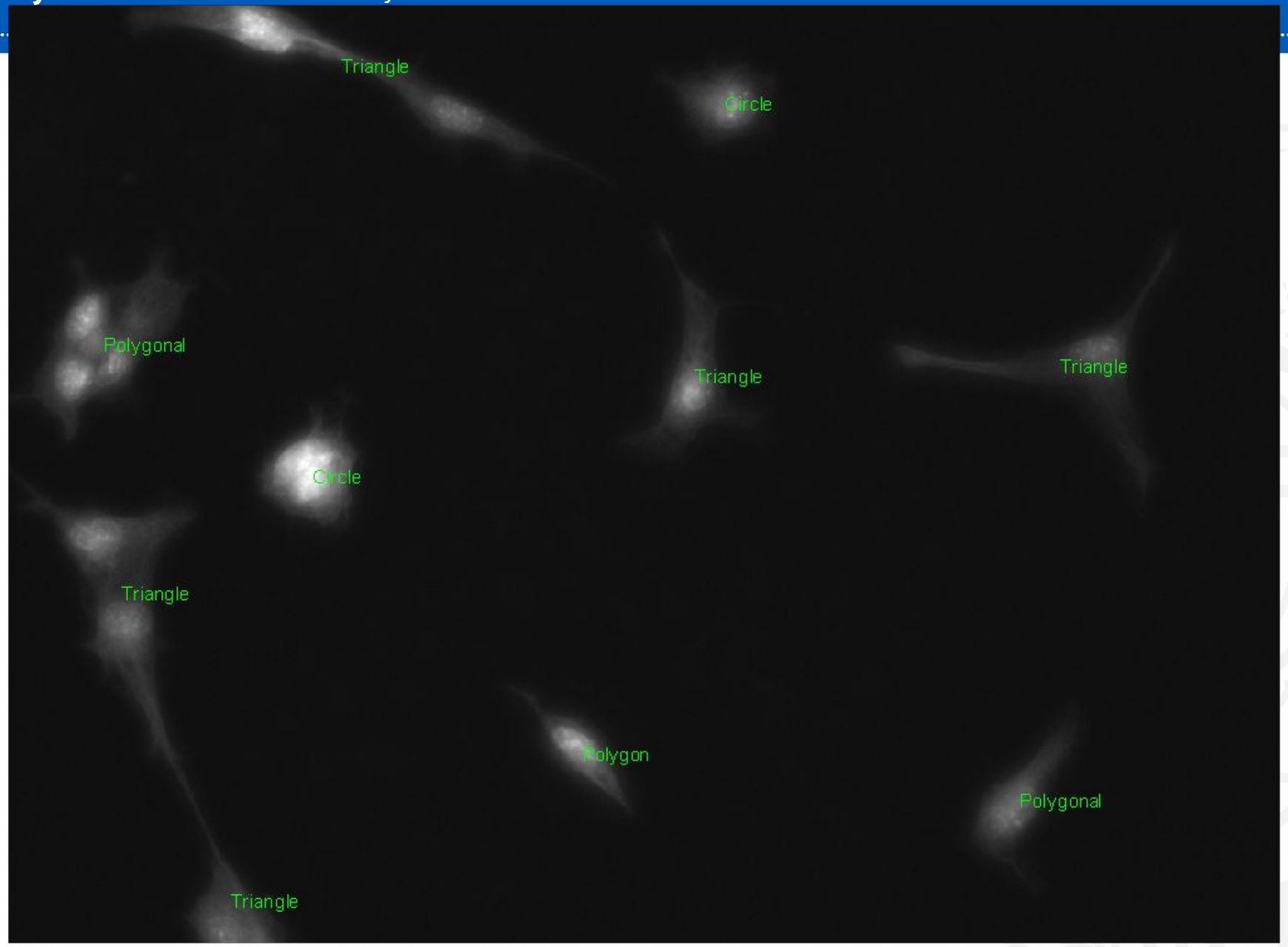
- Circularity is defined as the degree to which the particle is similar to a circle, taking into consideration the smoothness of the perimeter.
- Circularity is a measurement of both the particle form and roughness.
- Smooth circle a particle, the lower the circularity value.
- Circularity is a dimensionless value.


Triangle
Polygon
Polygon
Polygon
Triangle
Triangle
Triangle
Polygonal
Polygonal
Polygonal
Polygonal
Polygon
Triangle

Results


Triangle
Polygon
Polygonal
Polygonal
Triangle
Triangle
Polygonal
Polygonal
Circle
Triangle

Results


Triangle
Circle
Polygonal
Triangle
Triangle
Circle
Triangle
Polygon
Polygonal
Triangle

INFERENCE

CONCLUSIONS

INFERENCE 1:

- **Average morphological feature values of all the cells in a single image has a higher variability at 0.3 stress level as compared to 0.03, irrespective of the time point and the type of cytoskeleton.**
- **Gradual increase in the morphological feature value can be explained as the time of each observation increases.**

INFERENCE 2:

- **Single cell in the same group shows varied morphological features, i.e. cells represent single cell heterogeneity .**
- **Each cell should be studied individually to elucidate the changes in the morphological features at various stress levels.**

INFERENCE 3:

- Varied morphological features of single cell obtained from the image data set show single cell heterogeneity.
- This concludes that each cell in an image should be treated individually

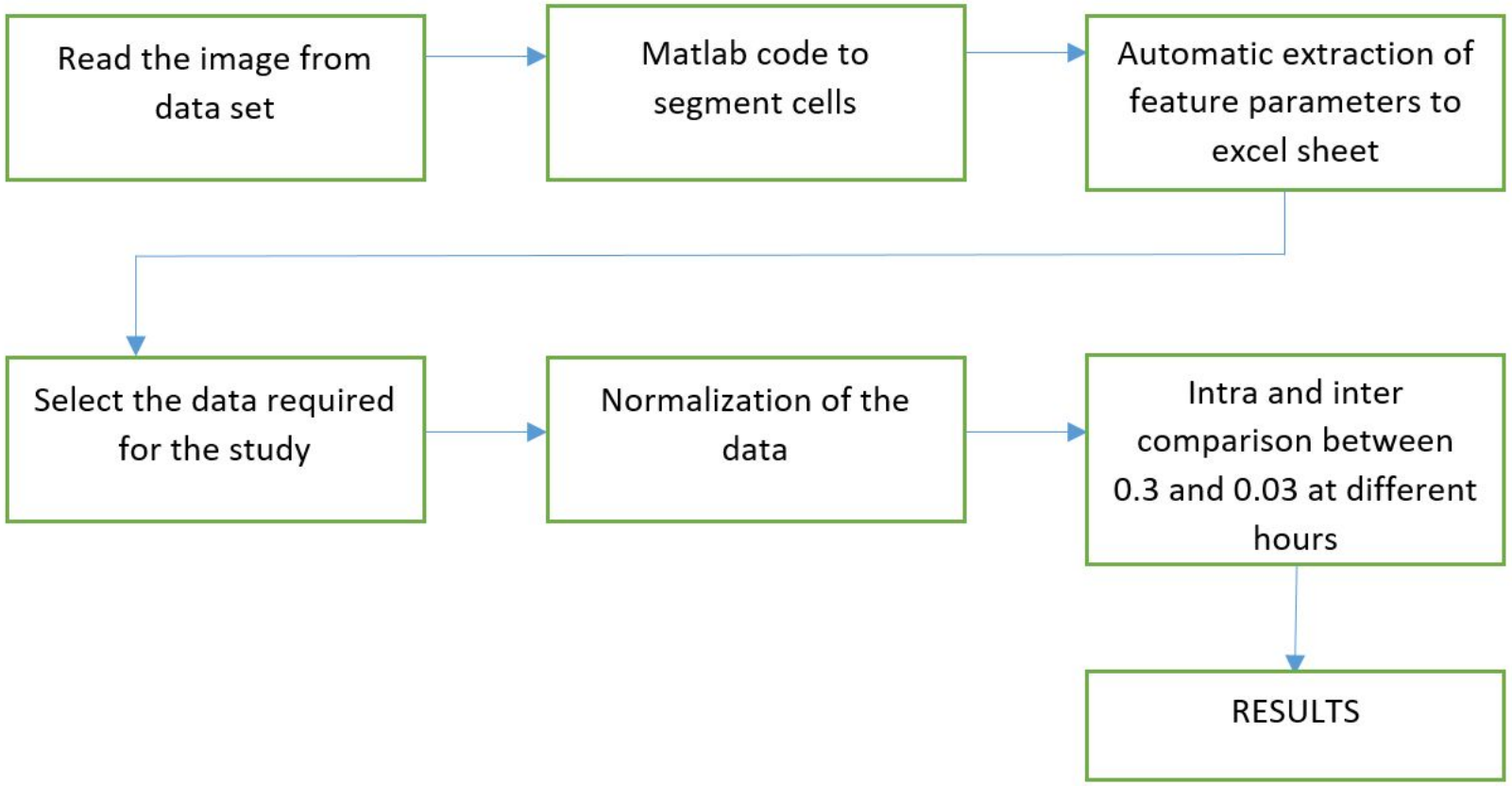
INFERENCE 4:

- All the cells in actin, nucleus and tubulin images show different shapes such as circle, polygon, hexagonal and triangle.
- This strengthens the existence of single cell heterogeneity.

INFERENCE 5:

- The single cell and overlapped cell show the same behavior when subjected to different stress at different time points.

PROCEDURE:


Read the image from data set
Matlab code to segment cells
Automatic extraction of feature parameters to excel sheet
Select the data required for the study
Normalization of the data
Intra and inter comparison between 0.3 and 0.03 at different hours
RESULTS

FUTURE SCOPES

- Implementing machine learning algorithm to automatically segment the cells and classify the features.

- To study the effect of cell textures on single cell homogeneity.

REFERENCES

ISO, ISO 13322-1, “Particle size analysis – Image analysis methods – Part 1: Static image analysis methods,” 2004. 10.

ISO, ISO 13322-2, “Particle size analysis – Image analysis methods – Part 2: Dynamic image analysis methods,” 2016.

Malvern Instruments Ltd, Morphologi G3 User Manual, 2010.

. Faria, Pons, Feyo De Azevedo, Rocha, & Vivier, “Quantification of the Morphology of Sucrose Crystals by Image Analysis”, Powder Technology, 133, 2015.

Schmid, Dvorak, Müller, & Müssig, “Characterizing Flock Fibers using Quantitative Image Analysis”, Flock, 30, 2009.