COMP2032 Introduction to Image Processing

“Extracting & Analyzing Cell Nuclei”

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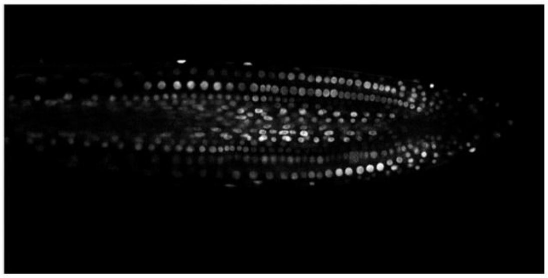
Process

The steps taken to detect and count nuclei cells in the images provided can be divided into three sections. Each section builds up on the next to produce the end result.

1. Image Enhancement

Step 1: Colour Space Conversion

- Green channel extraction



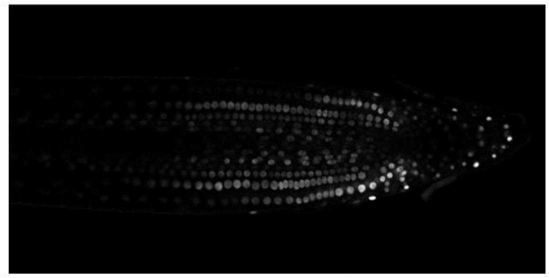


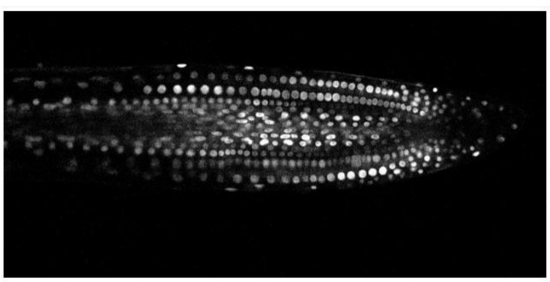
Image 1 **Image 2**

Image 3

Figure 1: Images after colour space conversion

Step 2: Image Brightening

- Brightness factor: 0.3



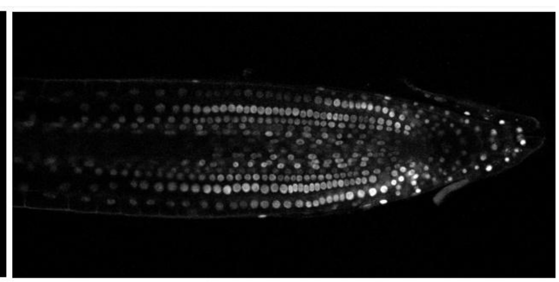


Image 1 **Image 2**

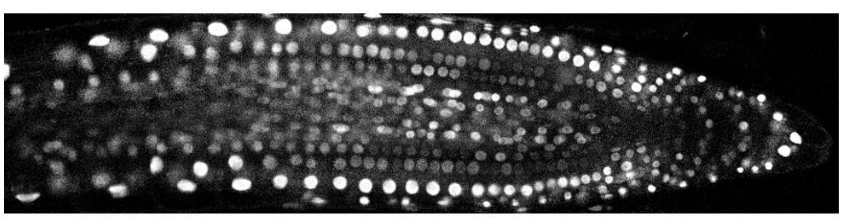


Image 3

Figure 2: Images after brightening by a factor of 0.3

Step 3: Clearing Border

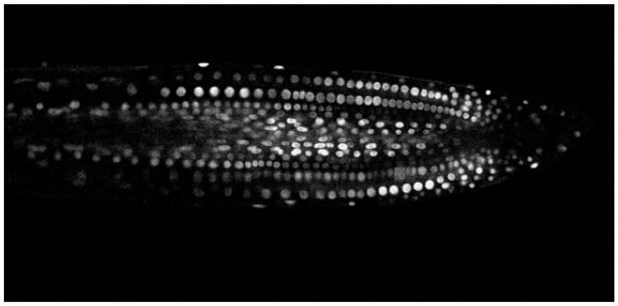


Image 1

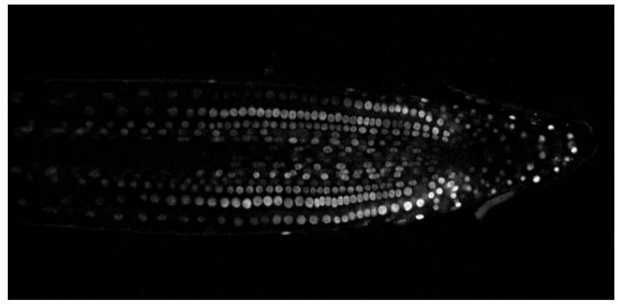


Image 2

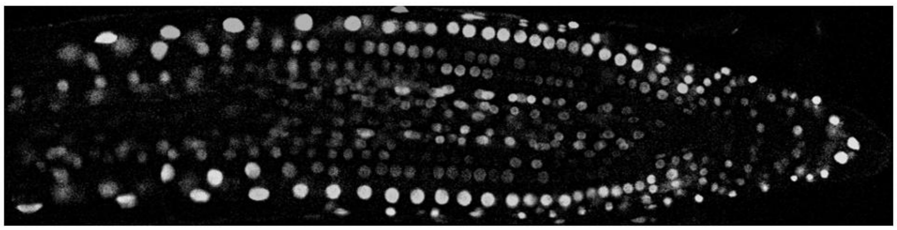


Image 3

Figure 3: Images after clearing borders

Step 4: Reducing Noise

- Median filter
- 5x5 mask

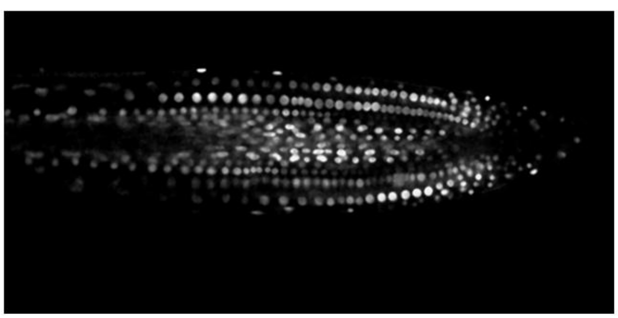


Image 1

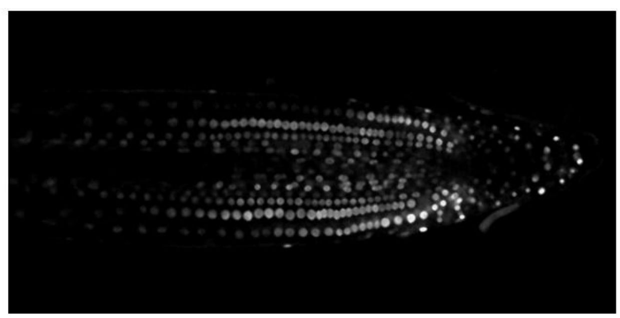


Image 2

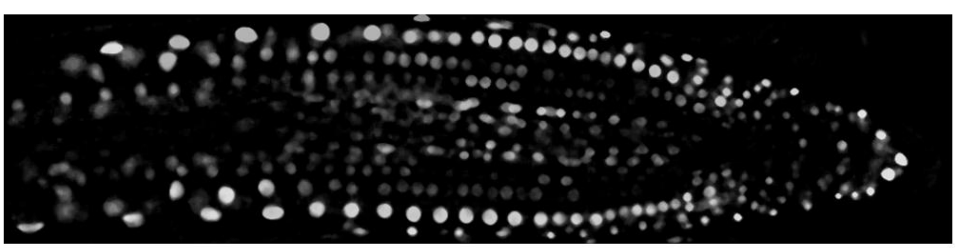


Image 3

Figure 4: Images after applying the 5x5 Median filter

Step 5: Sharpening

- Extracting edges using the Laplacian filter
- Subtracting edges from image for sharpening

Image 1

Image 2

Image 3

Figure 5: Images' edges extracted using the Laplacian filter

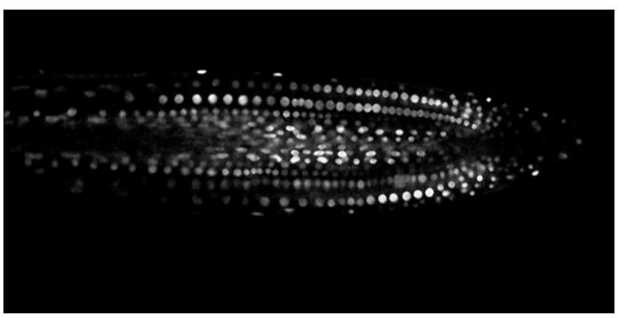


Image 1

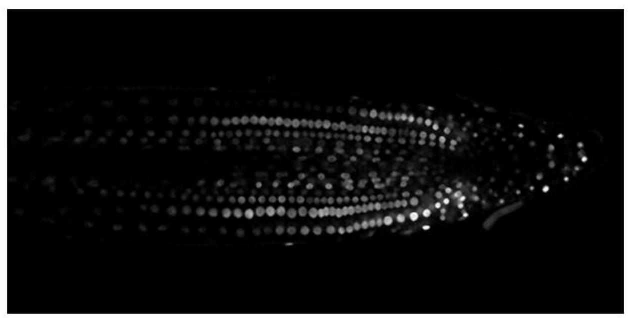


Image 2

Image 3

Figure 6: Images after sharpening by subtracting the edges

Step 6: Reducing Noise After Sharpening

- Median filter
- 5x5 mask

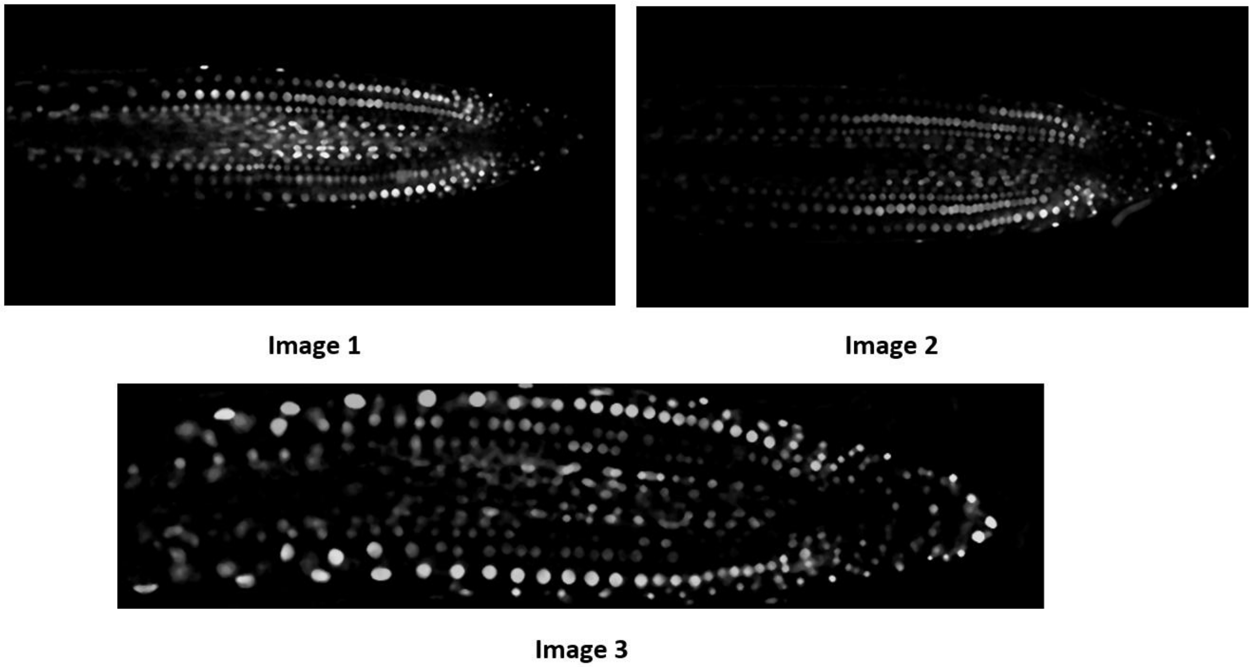


Figure 7: Images after applying the 5x5 Median filter again after sharpening

2. Segmentation of Cell Nuclei

Step 1: Canny Edge Detection

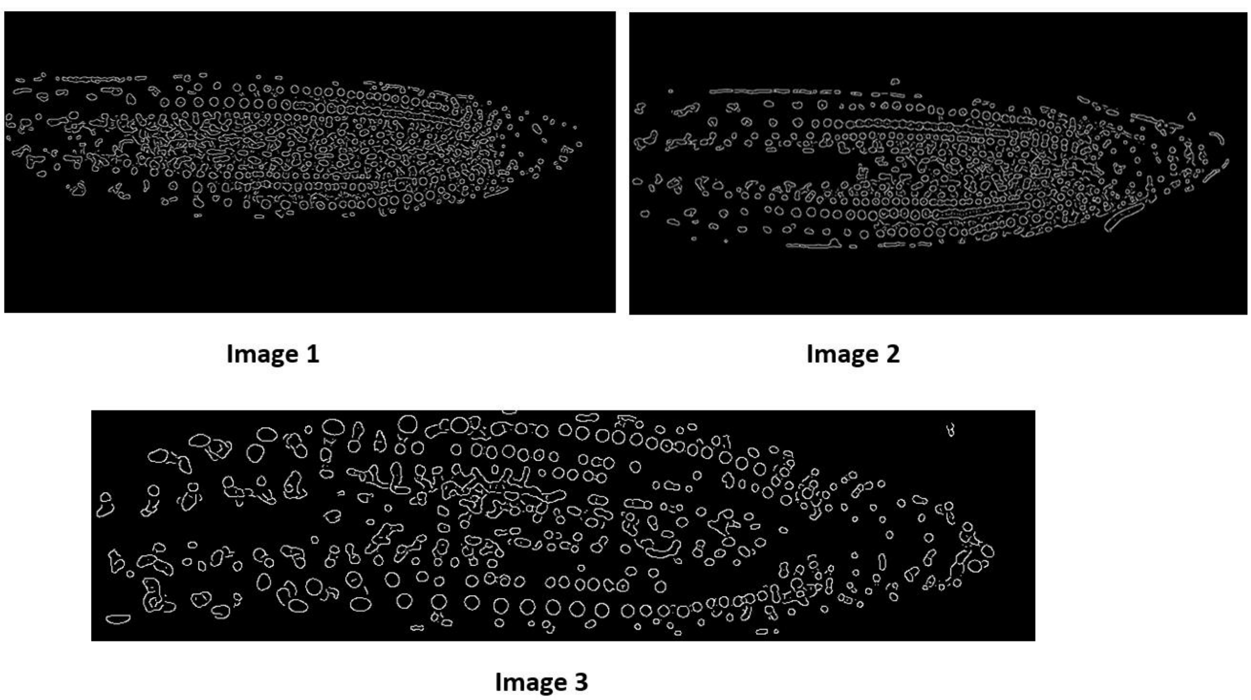


Figure 8: Images after canny edge detection

Step 2: Dilation

- Disk-shaped Structuring Element (SE)
- Radius: 1

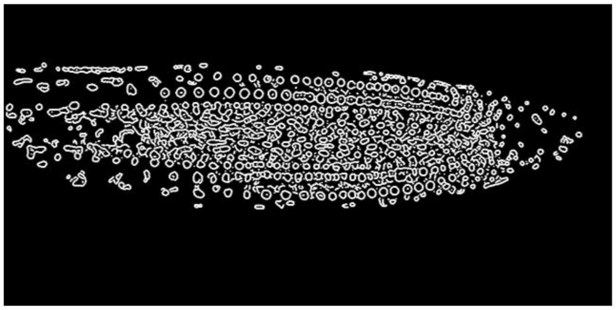
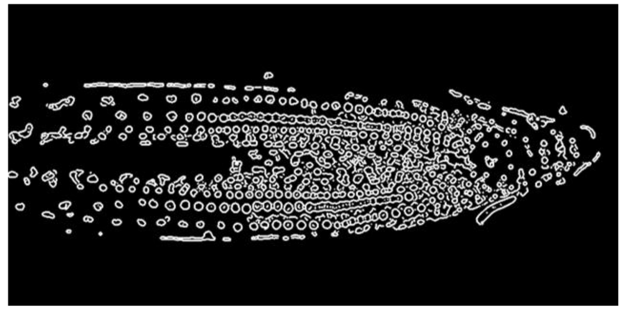



Image 1 **Image 2**

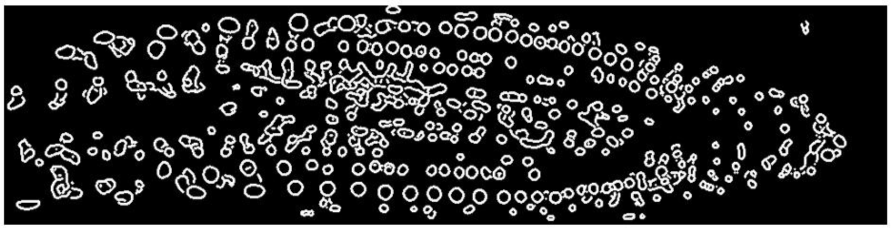


Image 3

Figure 9: Images after dilation is applied

Step 3: Filling Holes

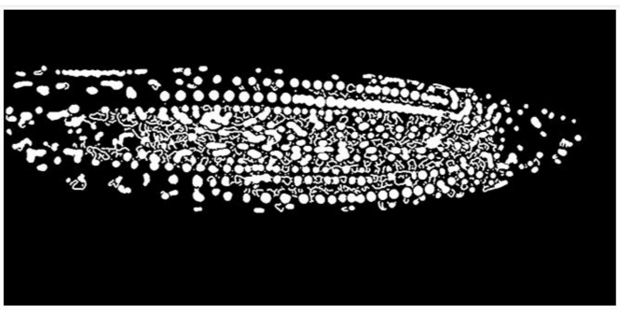
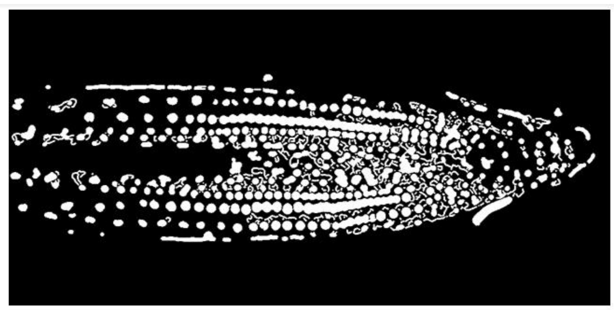



Image 1 **Image 2**

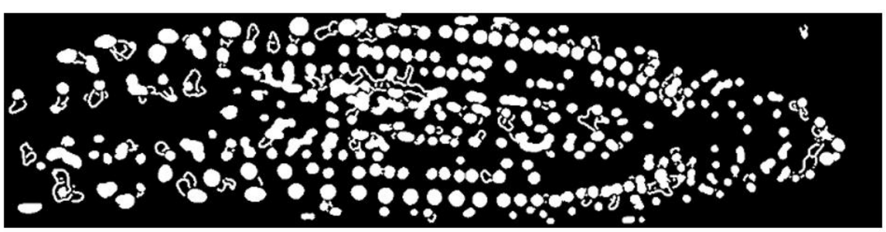


Image 3

Figure 10: Images after hole filling

Step 4: Opening Image

- Disk-shaped SE
- Radius: 2

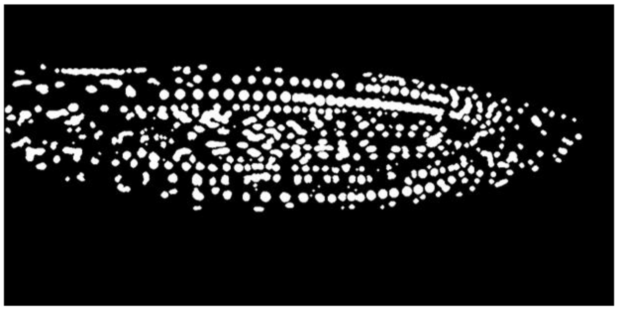


Image 1

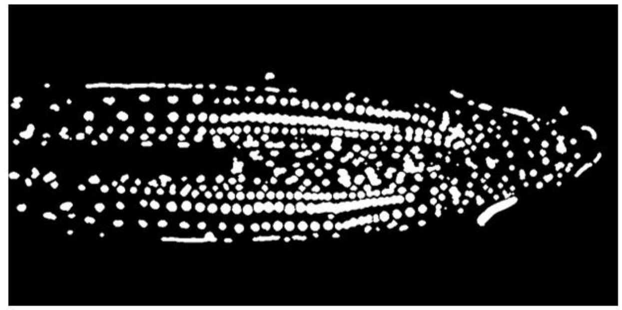


Image 2

Image 3

Figure 11: Images after morphological opening

Step 5: Erosion

- Disk shaped SE
- Radius: 1

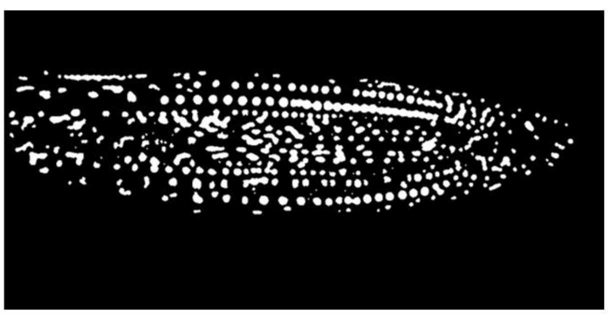


Image 1

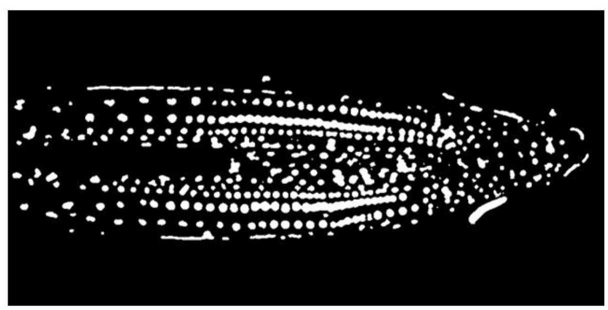


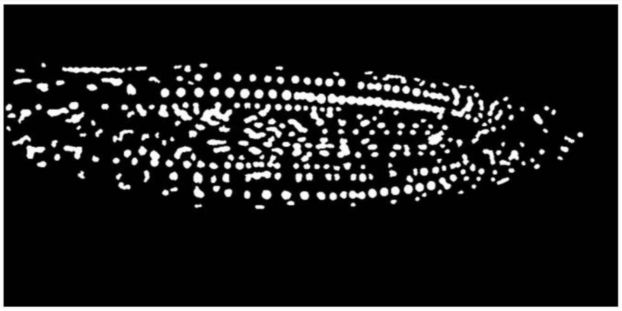
Image 2

Image 3

Figure 12: Images after erosion is applied

Step 6: Removing Small Particles

- < 35 pixels objects removed



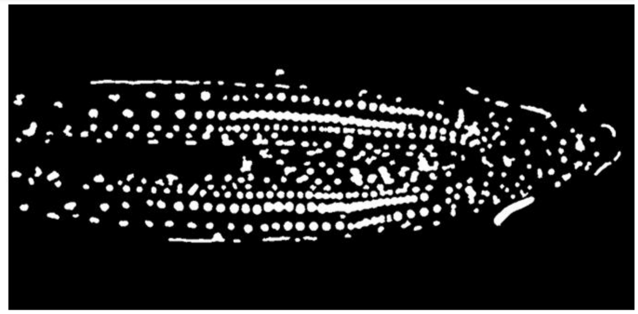


Image 1 **Image 2**

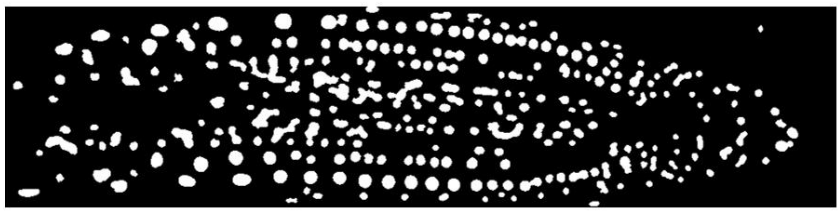
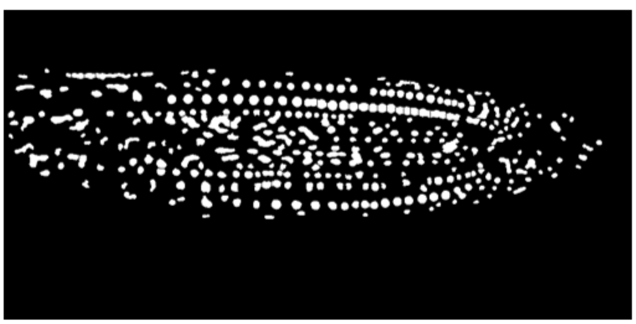


Image 3

Figure 13: Images after removing particles with less than 35 pixels

Step 7: Watershed Transform



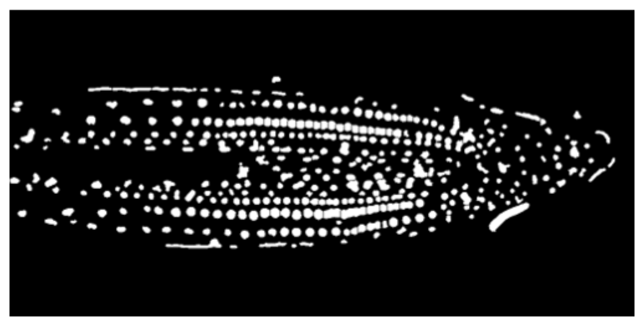


Image 1 **Image 2**

Image 3

Figure 14: Images after applying the watershed transform

3. Counting Nuclei

- Using bwconncomp

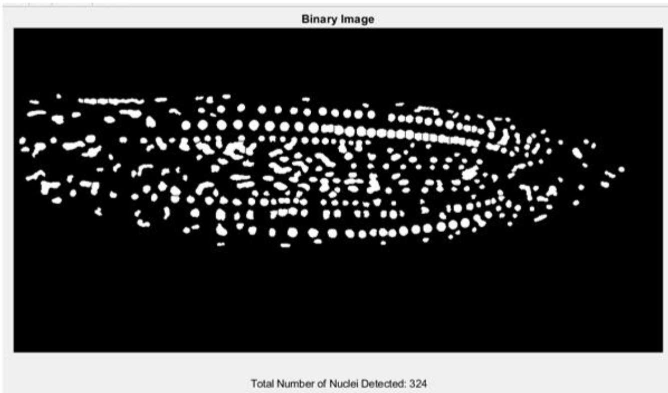


Image 1

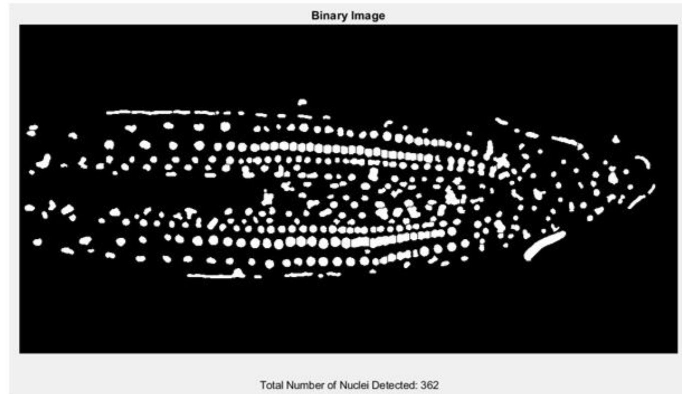


Image 2

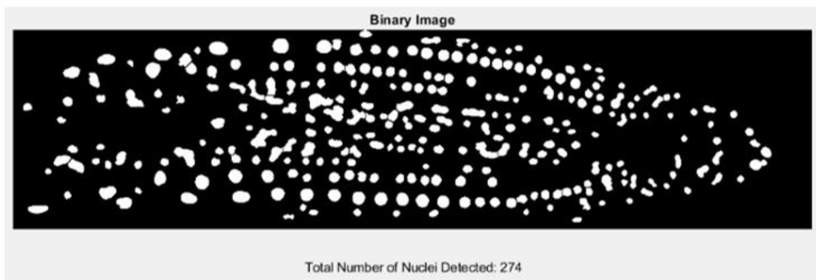


Image 3

Figure 15: Output from the nucleiCount function showing the number of nuclei detected.

Explanation

1. Image Enhancement

Colour Space Conversion

It was observed from the images that most nuclei are green in colour and as the main focus is on the cell nuclei, the green colour needed to be isolated. Therefore, colour space conversion to extract the green channel needed to be done so we can work easier in the low-dimension green colour.

The extraction of the green channel results in grayscale images which are easier to work with. This is because RGB images have 3 layers while grayscale images only have one. Anything that would be applied to a grayscale image needs to be applied once instead of 3 times, one for each layer, in the RGB image.

Image Brightening

After extracting the green channel, it was observed that some areas of the images were too dark making it difficult to observe the nuclei in those areas. Therefore, the image was brightened by a factor of 0.3.

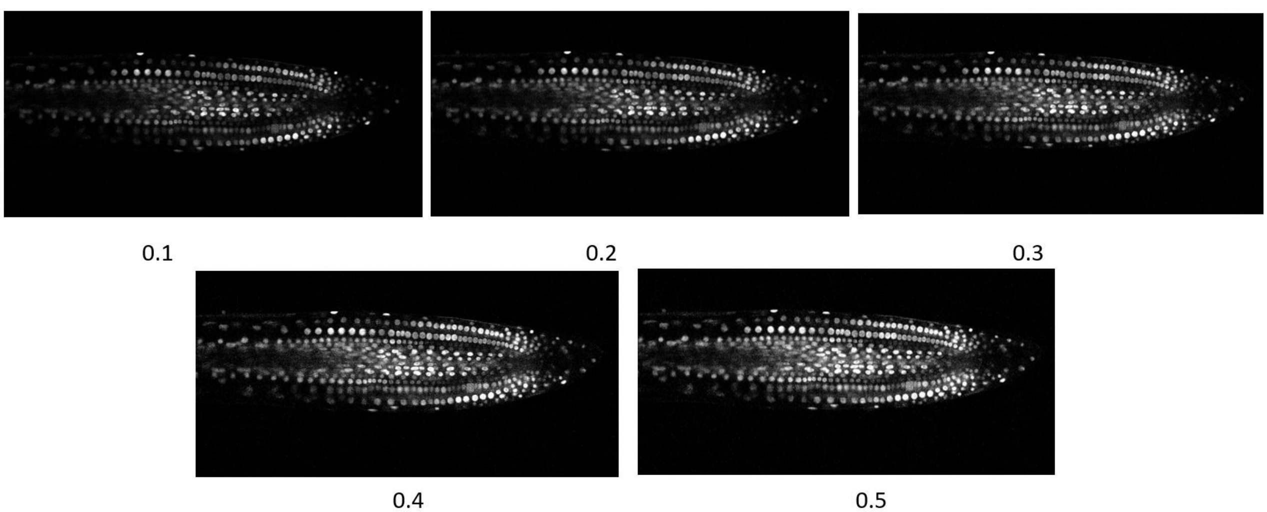
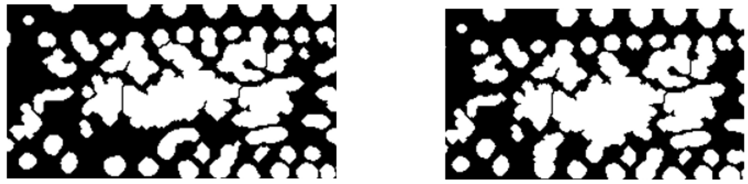


Figure 16: Results of different brightness factors

The image was tested with brightening factors from 0.1 to 0.5 before 0.3 was chosen as the most ideal. The brightness factor is important as it affects how the algorithm detects nucleus and counts them. Factors 0.1 and 0.2 did not increase the brightness enough for the nuclei to be detected while factors 0.4 and 0.5 resulted in large blobs of the image, that did not necessarily correspond to nuclei, to be counted.



0.4 0.5

Figure 17: Large blobs being counted as nuclei due to high brightness factors.

Clear Border

Objects touching the border can be incomplete in shape which makes us unable to obtain complete information about them. To avoid disrupting the analysis of shape, and size, those objects had to be removed so they could be excluded from the detection process.

Reducing Noise

Brightening the image brought to attention the noise present. Having noise in the image disrupts the results as noise particles would be inaccurately counted as nuclei if not removed. Several filters were tested to find out which one reduced noise most while still maintaining the shape of the nuclei.

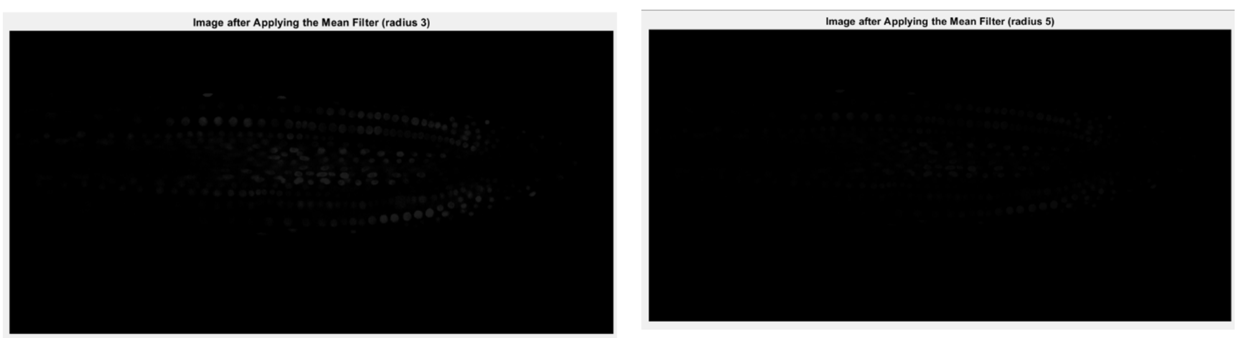
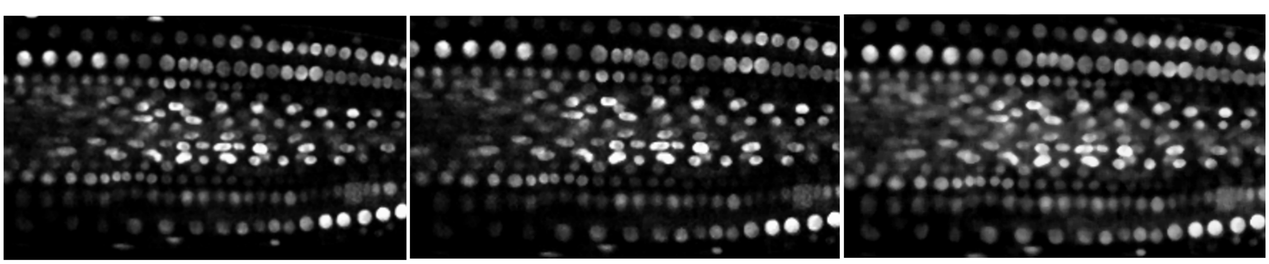


Figure 18: Results of applying the Mean filter. The images are too dark, so it was excluded.



Gaussian Median (3x3) Median (5x5)

Figure 19: Results of applying the Gaussian and Median filters.

After several tests, it was observed that the median filter with a 5x5 mask provided the best results. It reduced the noise most but still maintained the shape of the nuclei as shown in figure 19 compared to other filters. It also maintained the brightness of the image.

Sharpening

Although the median filter was the best choice amongst others, it was not perfect, and led to blurring of the image. The blurring caused some of the nuclei shapes to be lost which would make it difficult for the algorithm to detect them. Therefore, the image needed to be sharpened. The Laplacian filter was used as it uses the second derivative which is more sensitive to changes and would provide a better sharpening result.

Sharpening led to the sharpening of the remaining noise in the image as well. This is why the median filter was applied again with a 5x5 mask. The median filter was tested against the Gaussian filter. Although the Gaussian filter led to less blurring, the median filter was better suited for segmentation as it provided better results as shown in figure 20.

Gaussian Filter

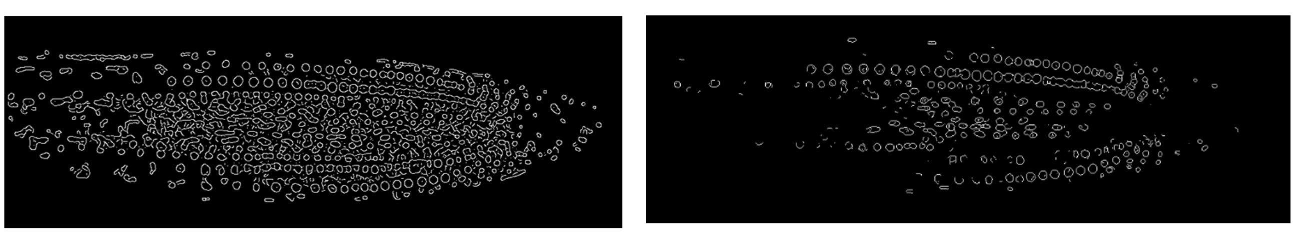
Median Filter (5x5)

Figure 20: Effects of the Gaussian filter vs the Median filter on segmentation.

2. Segmentation

Canny Edge Detection

A method was needed to detect the edge of the nuclei so they can be identified in the image. A comparison was made between the Sobel and Canny edge detection methods.



Canny

Sobel

Figure 21: Difference between the Sobel and Canny edge detection methods.

The Sobel edge detection method did not detect a majority of the edges in the image as compared to Canny which was more accurate and detected almost every edge.

Dilation

Some of the edges in the nuclei were unconnected after edge detection. Therefore, the image was dilated so the edges can connect and form the outline of the nuclei to be filled.

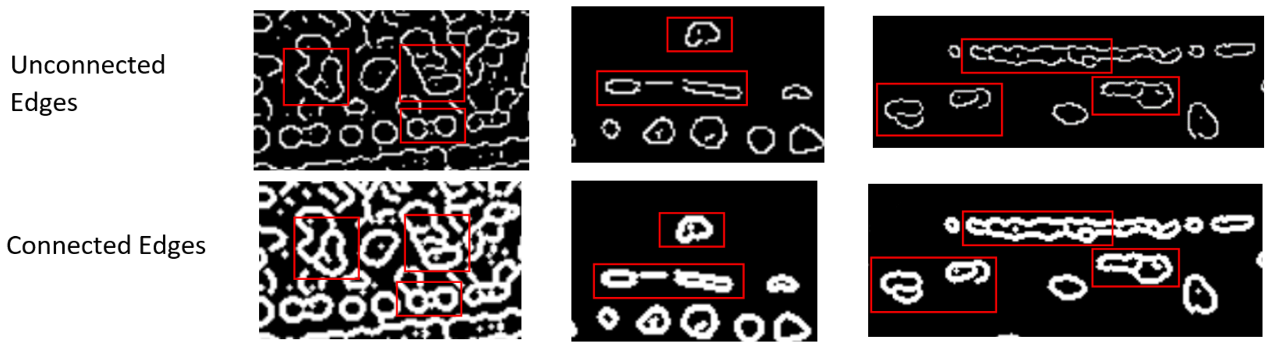


Figure 22: Examples of edges being connected after dilation.

Filling Holes

After detecting the edges and connecting them, the holes in the image needed to be filled. This was done to obtain a filled object which can cover the nuclei so the algorithm can identify them easier.

Opening Image

The Canny edge detection method detected several edges in the image, some of which are unneeded and do not belong to nuclei. In order to get rid of those edges, the image was opened. Opening was used as it first erodes and then dilates the image. Erosion gets rid of small holes present in the image and further breaks apart remaining weak edges while dilation ensures that the strong edges outlining the nuclei are still present.

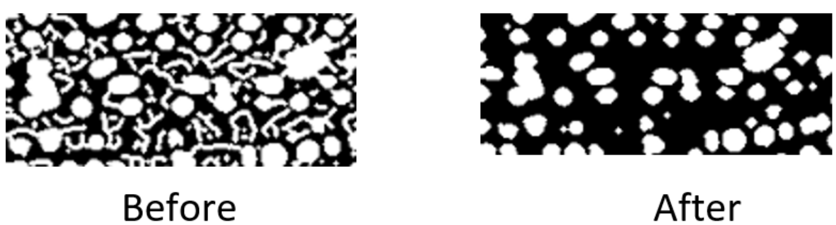


Figure 23: Example of an area before and after morphological opening.

Erosion

After opening, there were still some weak connections between nuclei in the image. To get rid of those connections, so that counting of nuclei can be more accurate, erosion was applied. Erosion breaks the connections by shrinking the nuclei. It also puts a distance between nuclei and makes small particles smaller so they can be easily removed.

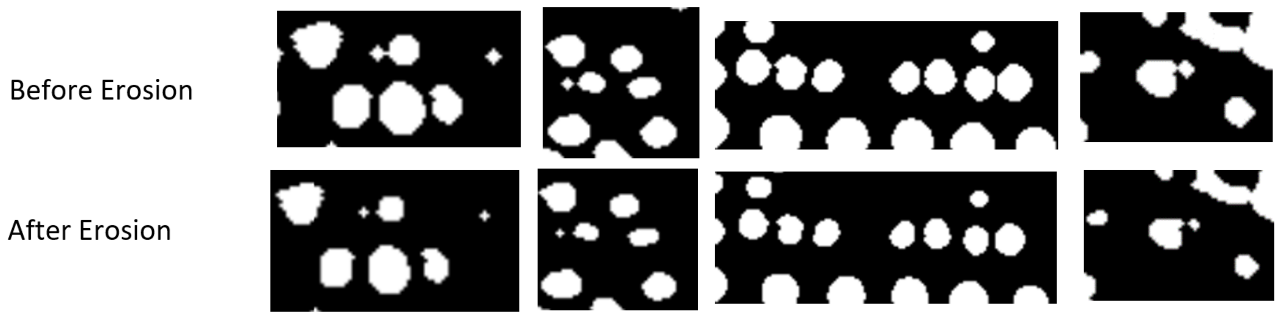


Figure 24: Examples of nuclei before and after erosion.

Removing Small Particles

Despite applying erosion, there were small particles still observed in the image. Therefore, to remove them, the bwareaopen function was used. This function opens areas of the image and removes all connected components that have less than 35 pixels. This function was tested with several values of pixels and 35 proved to be the most effective.

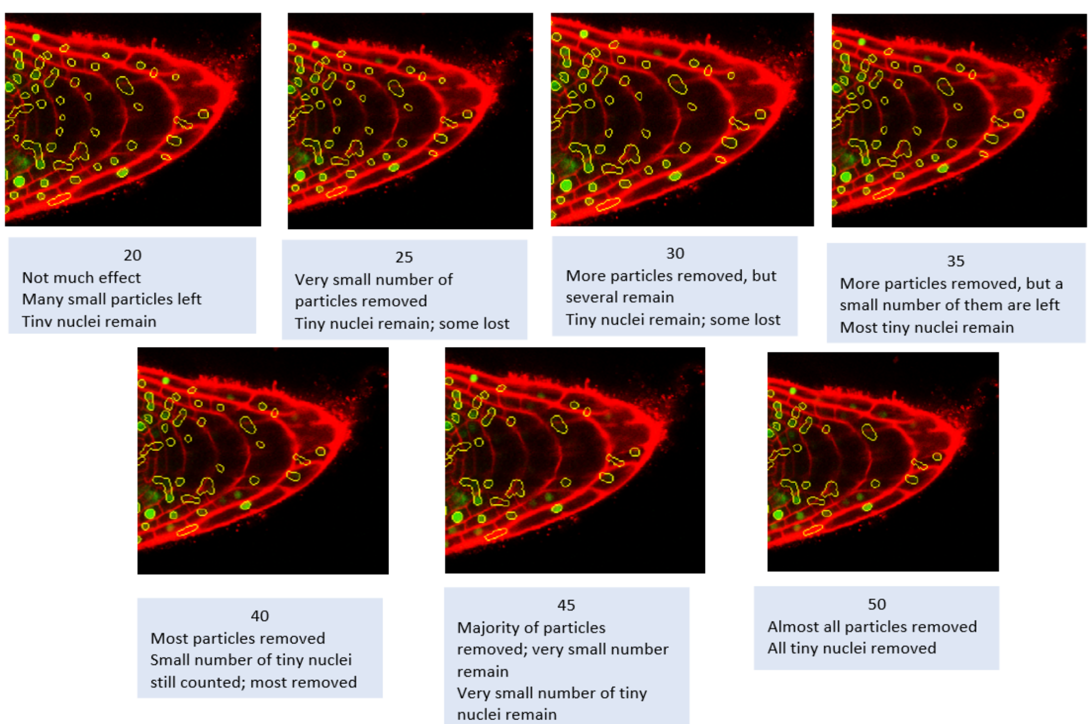


Figure 25: Comparison between different pixel values for removal of small particles.

Despite this not completely getting rid of all insignificant particles, it takes into account the tiny nuclei that would otherwise be undetected if the pixel value was higher.

Watershed Transform

To further separate the nuclei, the watershed transform was applied. It was chosen because it proves to be the most useful when segmenting touching objects. Several of the blobs detected by the segmentation algorithm were large due to the nuclei connecting and so, the watershed transform was applied to break those areas, and other nuclei connected to each other, apart.

However, after applying it, it was observed that the image was over segmented. Some of the small blobs were broken down into even smaller ones (figure 26B) and large blobs were broken down into a large number of small blobs (figure 26A). This led to an inaccurately high nuclei count.

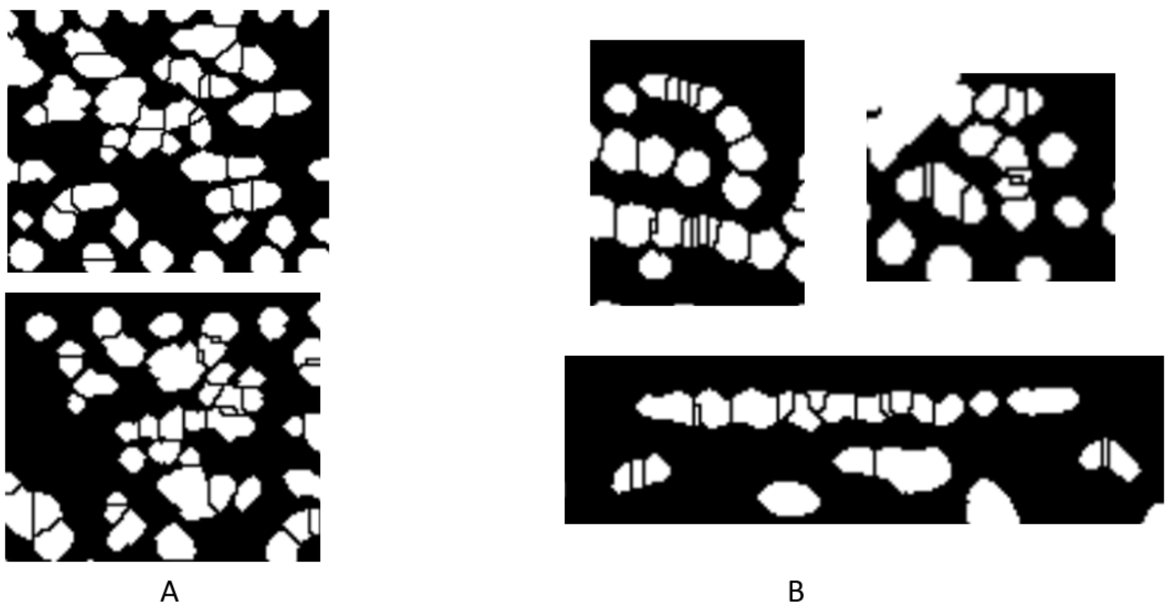


Figure 26: Examples of over segmentation. A represents large blobs being broken into may smaller blobs while B represents small bobs being broken into many smaller blobs.

In order to fix this, the imextendedmin and imposemin functions were used. The imextendedmin function filters out small local minima then, the imimposemin function modifies the distance transform to ensure none of the minima occur at the filtered-out places [1]. The imextendedmin function is used as a mask for the imimposemin function.

Strengths and Weaknesses of Each Process

The following section states the strengths, weaknesses and a fix for the weaknesses of the steps taken in the nuclei extraction process.

1. Image Enhancement

Colour Space Conversion

Strength: Extracts the green channel in which most nuclei are present and produces a grayscale image.
Weakness: Image is darker after green channel extraction and noise is more obvious.
Fix: Enhancement of the image.

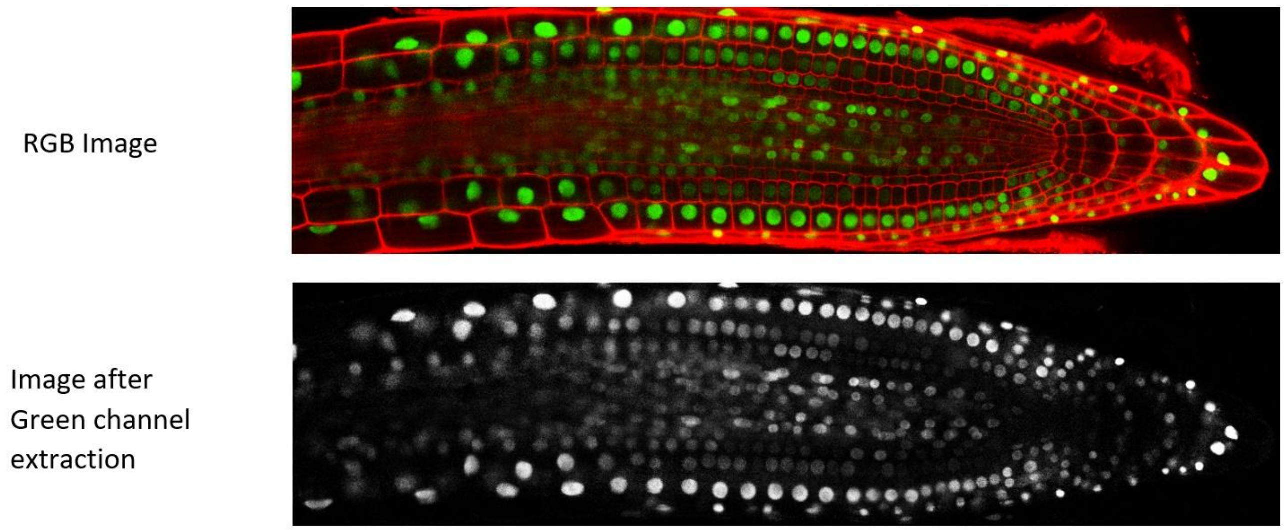


Figure 27: Comparison between the RGB image and the green channel image. The green channel image highlights the nuclei but is darker and noisier.

Image Brightening

Strength: Nuclei became clearer in the image.
Weakness: More noise can be observed.
Fix: Noise reduction using filters.

Clearing the Border

Strength: Cleans the border and removes incomplete objects and noise close to the border.
Weakness: The incomplete nuclei removed become undetected in the count.

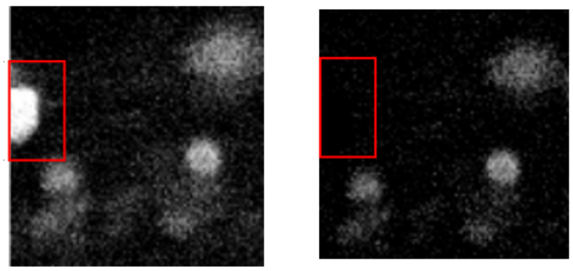


Figure 26: An example of a nuclei being removed due to its incomplete shape.

Removing Noise

Strength: Median filter smooths the image and preserves the nuclei edges. It is clean, sharp and does not heavily blur the image.
Weakness: Blurring still occurs causing some of the nuclei shapes to become unclear.
Fix: Sharpening using the Laplacian filter.

Before applying the Median filter

After applying the Median filter

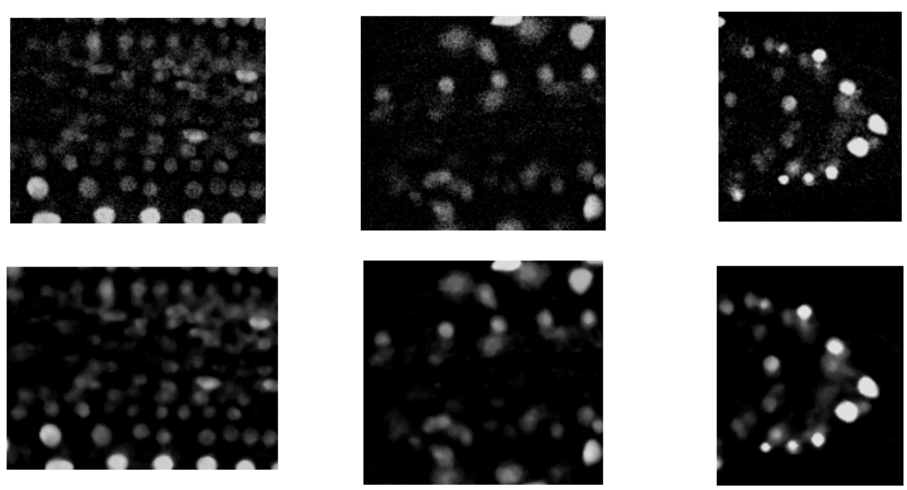


Figure 27: Examples of noise reduction in areas of the image after applying the Median filter.

Sharpening

Strength: The nuclei's outlines became more defined so their shape became clearer.
Weakness: Remaining noise in the image was also sharpened.
Fix: Applying the Median filter with a 5x5 mask again.

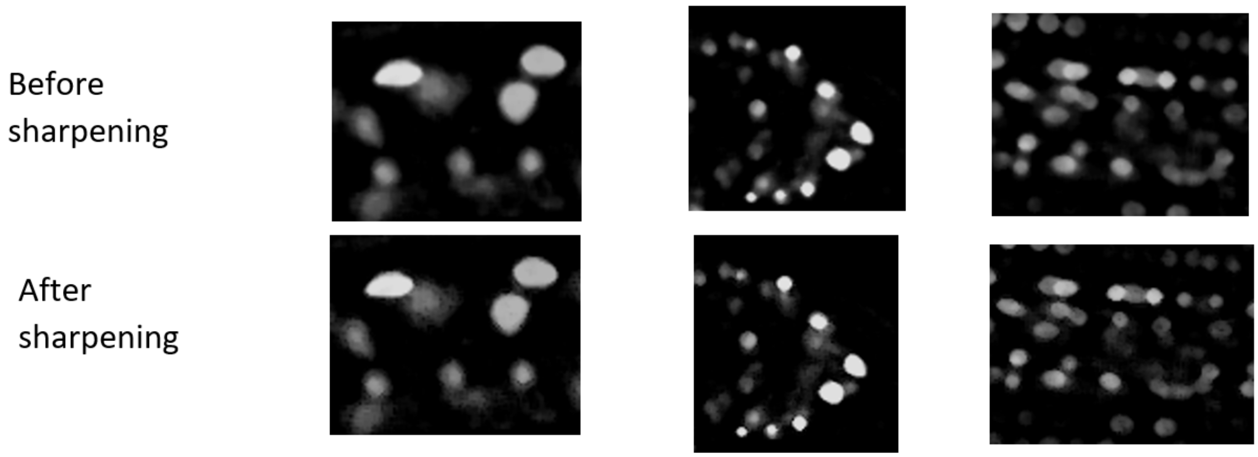
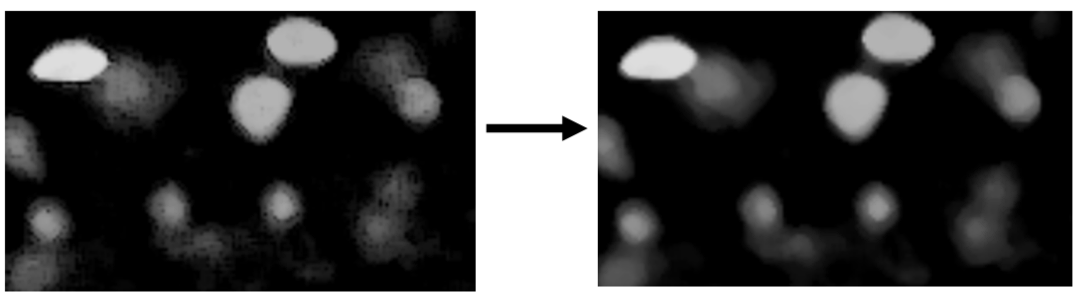


Figure 28: Examples of nuclei shapes being defined after sharpening.



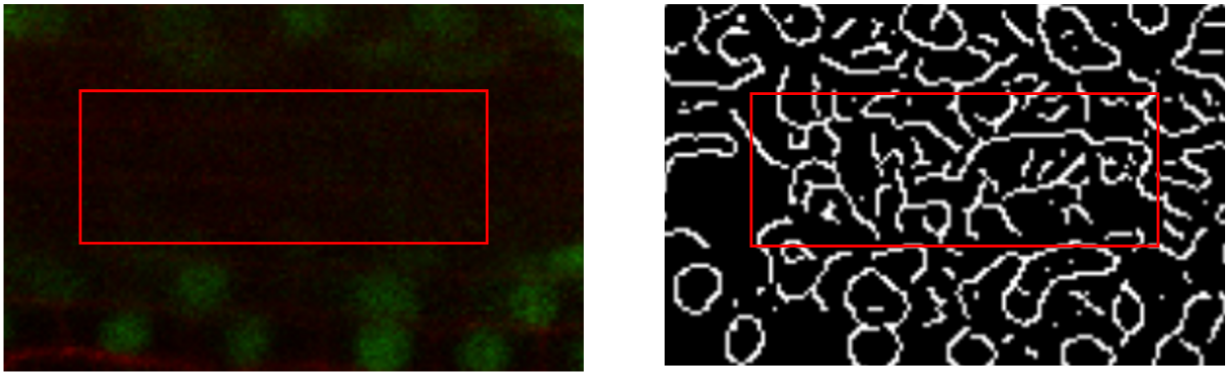
After sharpening | After applying the Median filter

Figure 29: Example of noise sharpened after sharpening and its reduction after applying the Median filter.

2. Segmentation

Canny Edge Detection

Strength: Detects both weak and strong edges.
Weakness: Detects every edge in the image; detects several edges even when they are not a part of the nuclei.
Fix: Applying morphological operations.



Area in original image | Area after edge detection

Figure 30: An example of an area where too many edges were detected despite them not corresponding to nuclei.

Dilation

Strength: Strengthens weak edges and connects them which helps in identifying whole nuclei.
Weakness: Dilates unneeded parts of the image like small particles and the non-nuclei edges. It also does not connect all edges.
Fix: Applying other morphological functions such as image opening and erosion.

Figure 31: Examples of unconnected edges even after dilation.

Filling Holes

Strength: Most of the nuclei were filled.
Weakness: Does not fill all holes; some nuclei had unconnected edges and so, were not filled.
Fix: Morphologically opening the image.

Opening Image

Strength: Gets rid of unnecessary edges, unfilled holes and small particles.
Weakness: Some small particles still remain.
Fix: Applying erosion.

Erosion

Strength: Gets rid of weak connections.
Weakness: Small particles were not rid of, they just became smaller.
Fix: Applying bwareaopen; which applies morphological opening on areas of the image.

Removing Small Particles

Strength: Gets rid of most small particles that are not nuclei while still keeping nuclei that are small in size.
Weakness: Does not completely get rid of all insignificant particles.

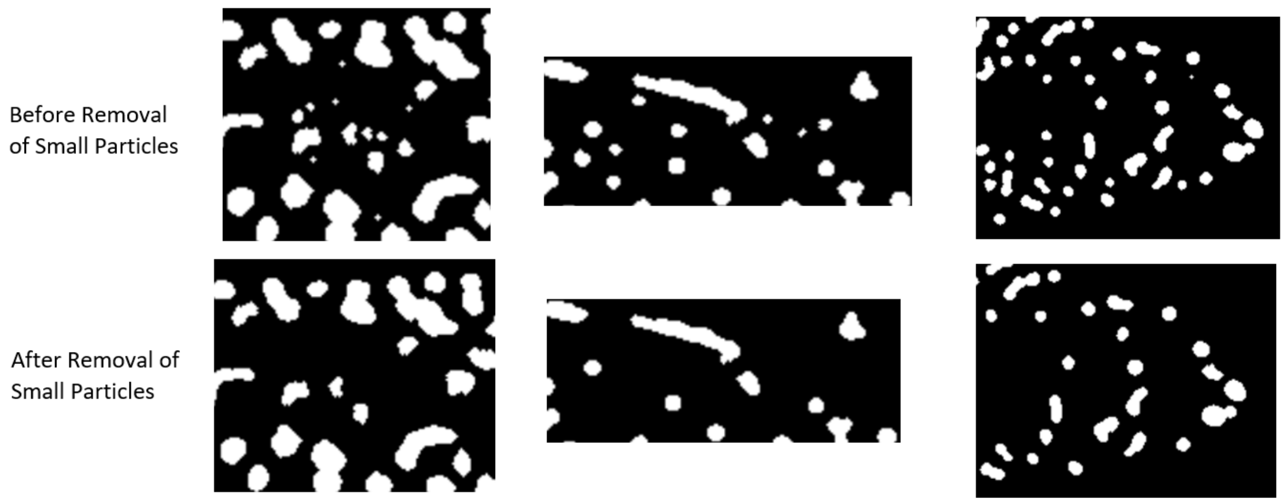


Figure 32: Examples of areas in the images before and after removal of small particles.

Watershed Transform

Strength: Breaks down large blobs into separate nuclei and separates touching nuclei.
Weakness: Oversegmentation; small nuclei were broken down into even smaller ones.
Fix: Imposing a minima (imimposemin) with a mask (imextendedmin).

Analysis of Nuclei

After segmentation and nuclei counting, further steps were made to analyse the detected nuclei. To extract quantitative data, the regionprops function and its different properties were used. The results of each of these distributions were plotted on a histogram and their mean and standard values calculated. The values calculated are all in terms of pixels.

From the images, it can be observed that most of the detected nuclei are round and so, with this observation in mind, the size and shape distributions were calculated.

1. Size Distribution

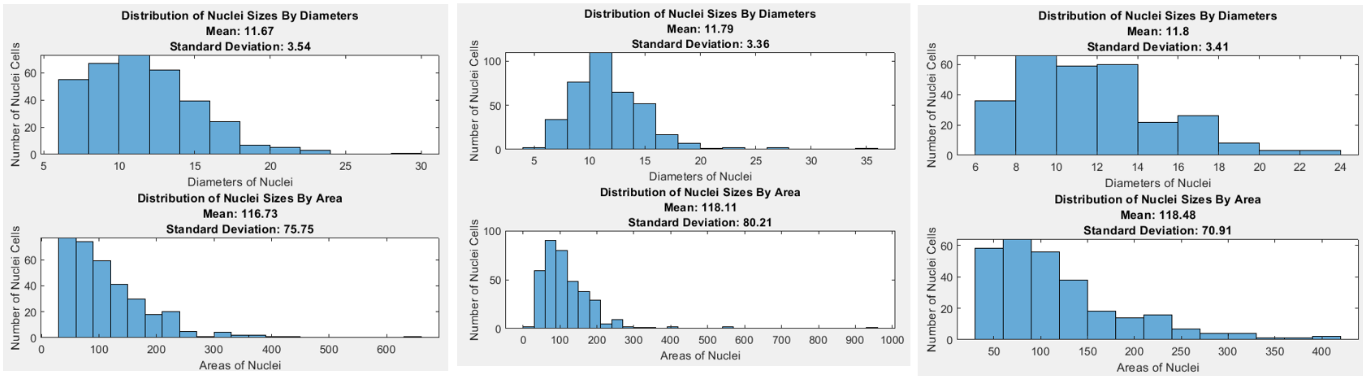


Image 1 Image 2 Image 3

Figure 33: Size distribution by area and diameter for each image

The nuclei size distribution can be found out using two methods, area and diameter. The ‘EquivDiameter’ property was used to obtain the diameter values while the ‘Area’ property was used to calculate the area.

2. Shape Distribution By Roundness

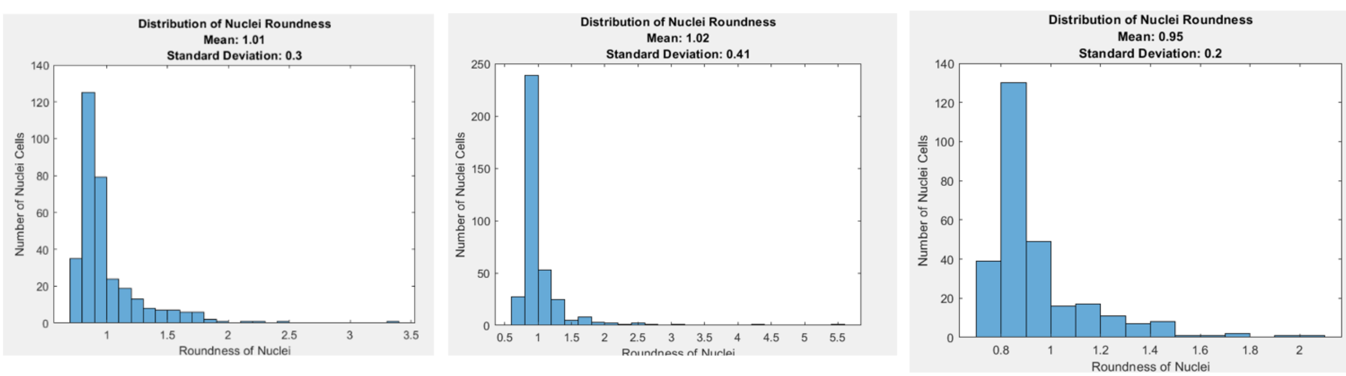


Image 1 Image 2 Image 3

Figure 34: Shape distribution of Nuclei by roundness for each image.

For the distribution of roundness of the nuclei, the roundness metric was used. The roundness metric can help us find out how round the nuclei are. It is calculated by the formula **(perimeter^2)/(4*pi*area)**. A perfect circle has a ratio of exactly 1 while a circle has a ratio less than 1.20. If the ratio is between 1.20 and 1.55, then the nuclei is rectangular. Otherwise, any shape with a ratio greater than 1.55 is irregular.

As the nuclei in the image are small, it is difficult to calculate exactly what their circularity is using the perimeter and area calculated in terms of pixels. Therefore, to allow for a margin of error, rather than taking circles to have a ratio of only less than 1, 1.20 is taken.

The shape of each nuclei is also marked at its center; perfect circles with a *, circles with an O, rectangles with a square and irregular shapes with an X.

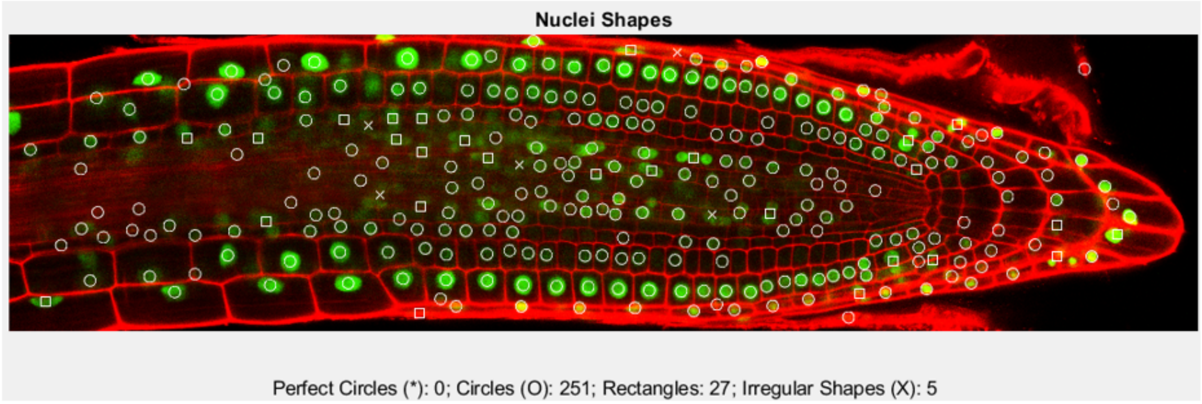
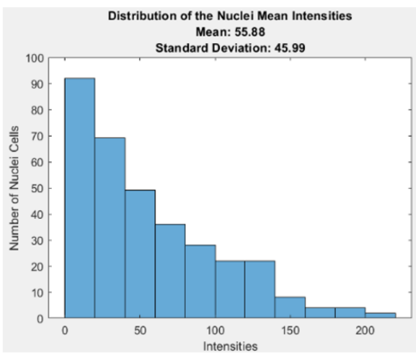
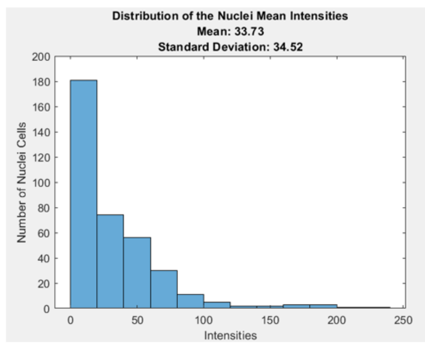


Figure 35: An example of the shapes of nuclei marked on image 3.

3. Average Brightness Distribution





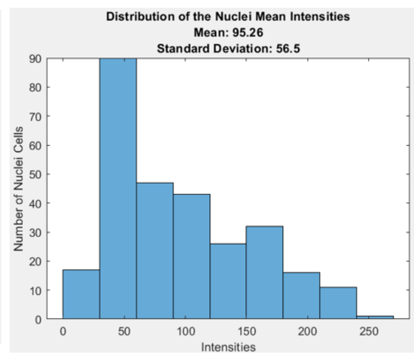


Image 1 Image 2 Image 3

Figure 36: Distribution of Nuclei mean intensities for each image.

For this distribution, the 'MeanIntensity' property was used. It calculates the mean intensity of different regions of an image. The nuclei detected in the binary image are compared with their corresponding areas in the grayscale image. This gives us the mean intensity of regions in the grayscale image using the white pixels of the binary image.

Evaluation

The distribution of the histograms is log-normal, showing that the nuclei sizes, shapes and brightness have a normal distribution. However, in each histogram it can be observed that there are outliers which increase the values of the mean. The higher standard deviations of the area and average intensity show how far the values are from the mean.

For the area, this is caused by large blobs of the image being detected and counted as one nuclei, as shown in figure 36, as well as tiny nuclei for image 2. The situation is similar for the shape distribution as the large blobs have a larger area and perimeter leading to a higher ratio of roundness than the other nuclei detected.

Figure 37: Examples of large blobs in the images causing an increase in the values of the mean and standard deviation.

While for the mean intensity, this is due to a difference in contrast between the grayscale and binary images. For example, as in figure 38, there are some nuclei in the grayscale image that are much darker than their corresponding nuclei in the binary image leading to a high contrast. This leads to some outliers that increase the mean and standard deviation.

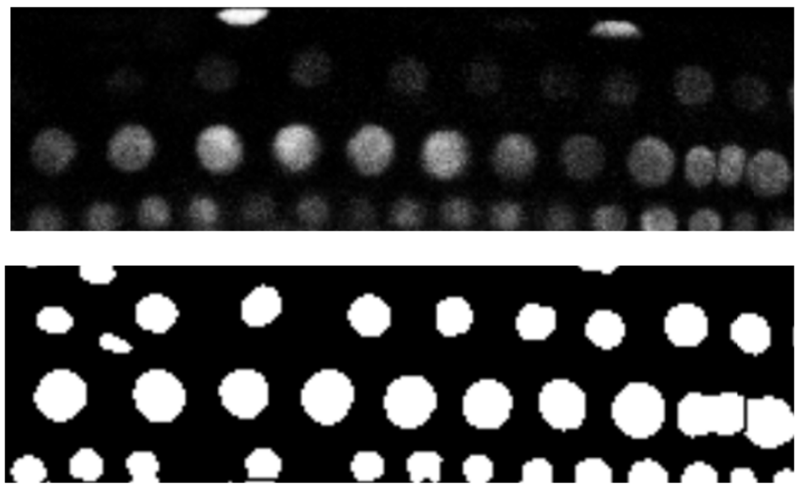


Figure 38: An example to show the high contrast between nuclei in the grayscale image and their corresponding nuclei in the binary image.

Conclusion

It is difficult to arrive at a solution which perfectly fits all three images as each one has different properties. The steps in the nucleiAnalysis function and their order are a result of several trials and errors to reach the best possible solution. Although it manages to count most of the nuclei in the image, it can still be further improved by finding a solution that manages to completely get rid of objects that aren't nuclei and only counts nuclei. However, although not perfect, this solution still provides a satisfactory result.

References

[1] Eddins, S. (2013, November 18). *Watershed transform question from tech support.* MathWorks Blogs. https://blogs.mathworks.com/steve/2013/11/19/watershed-transform-question-from-tech-support/

Appendix B: Final Output of All Images

Image 1 (StackNinja1.bmp)

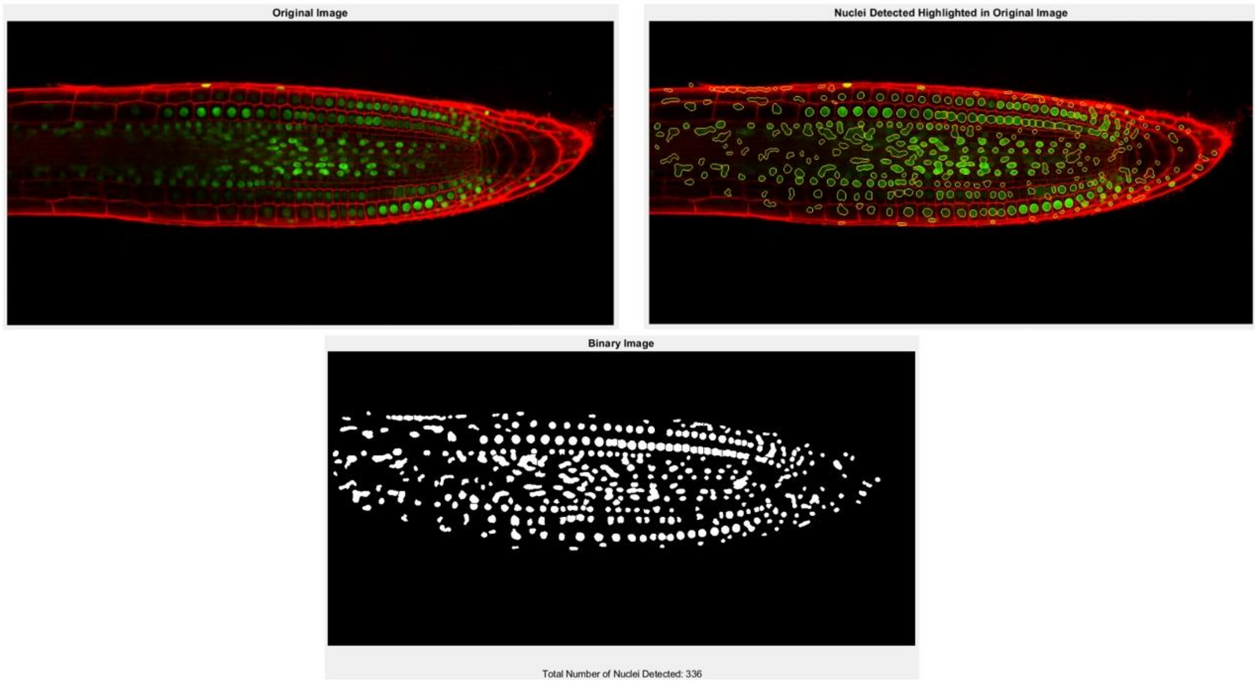


Figure 1: Output of Image 1 showing the original image, the overlay of nuclei detected, and the number of nuclei counted with the binary image.

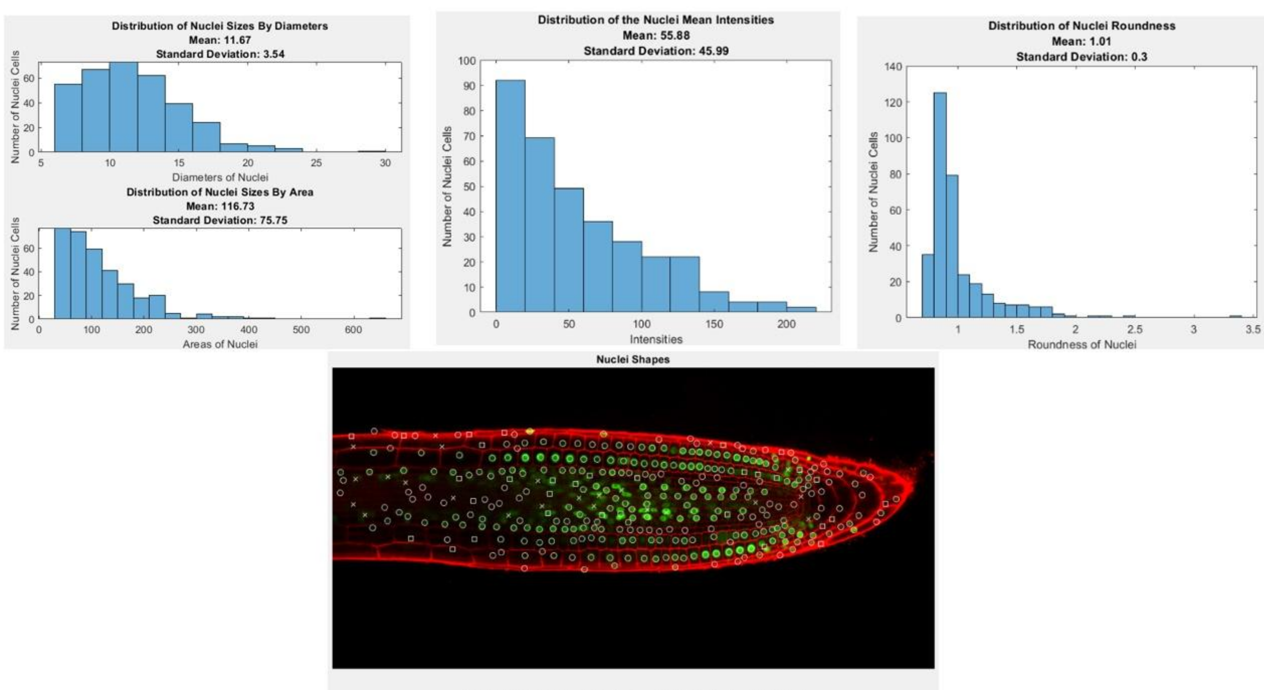


Figure 2: Output of Image 1 showing the histograms of the size, mean intensities, and roundness distributions of nuclei as well as the marking and count of nuclei shapes.

Image 2 (StackNinja2.bmp)

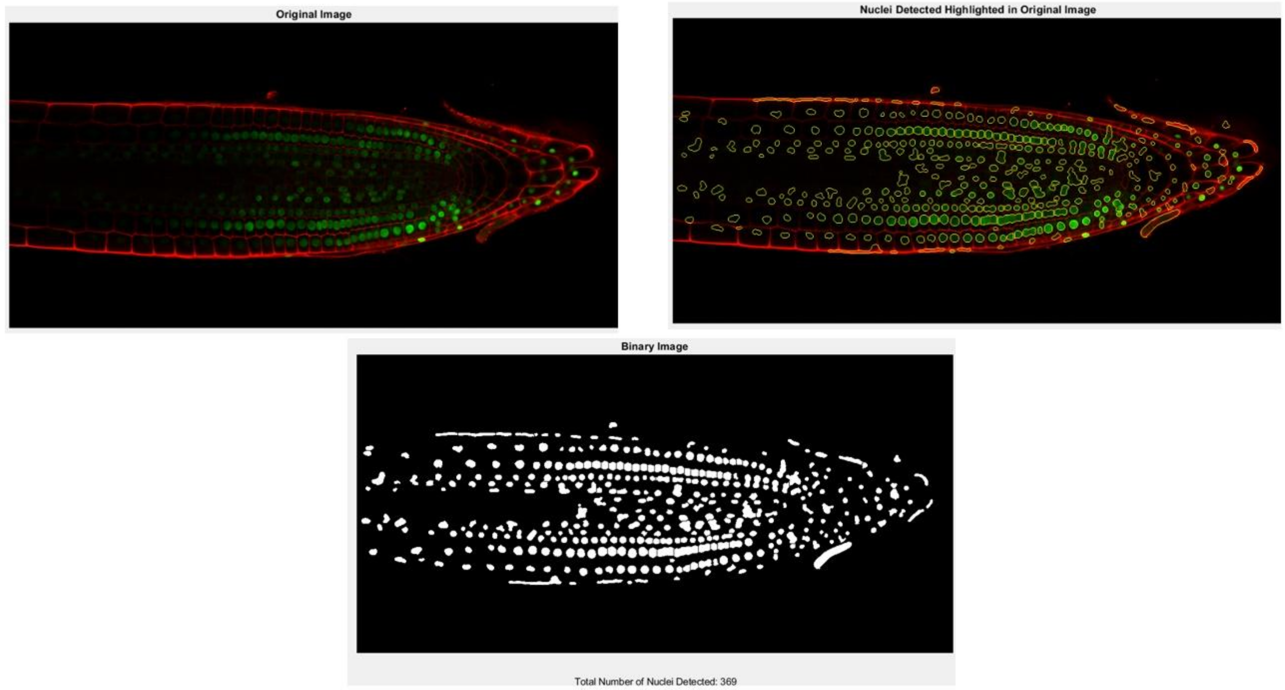


Figure 3: Output of Image 2 showing the original image, the overlay of nuclei detected, and the number of nuclei counted with the binary image.

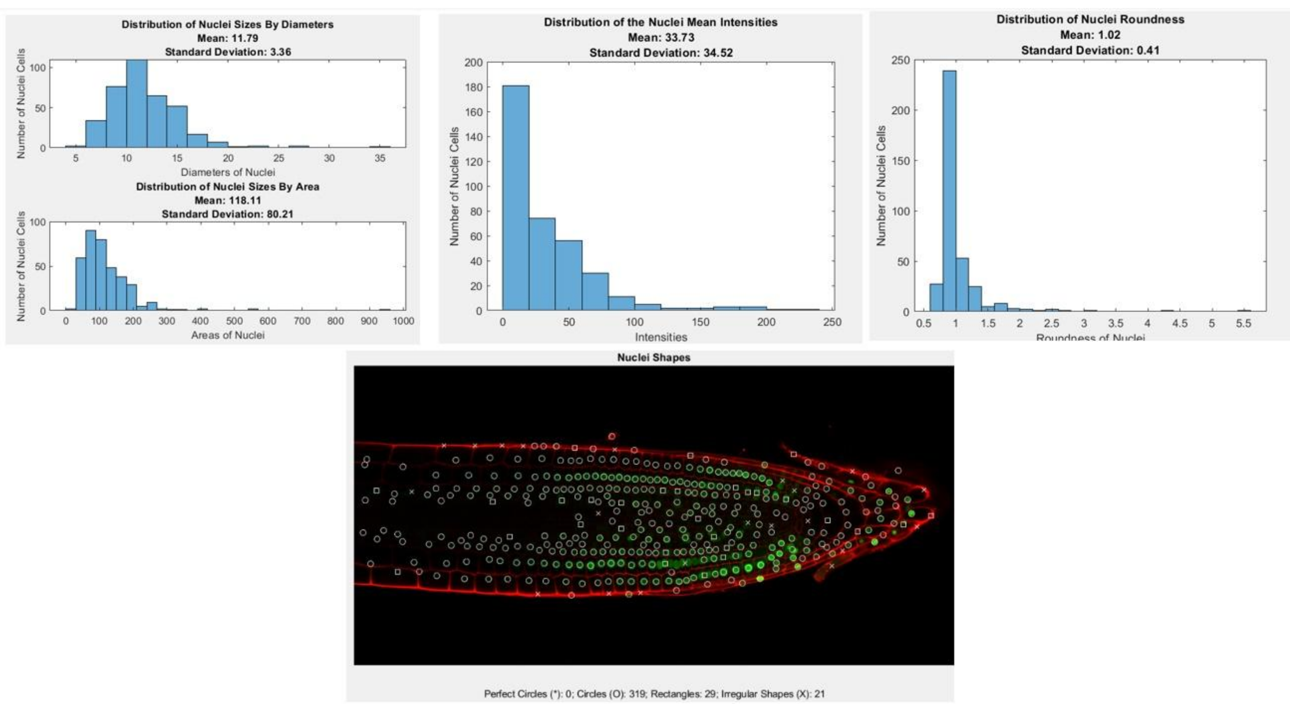


Figure 4: Output of Image 2 showing the histograms of the size, mean intensities, and roundness distributions of nuclei as well as the marking and count of nuclei shapes.

Image 3 (StackNinja3.bmp)

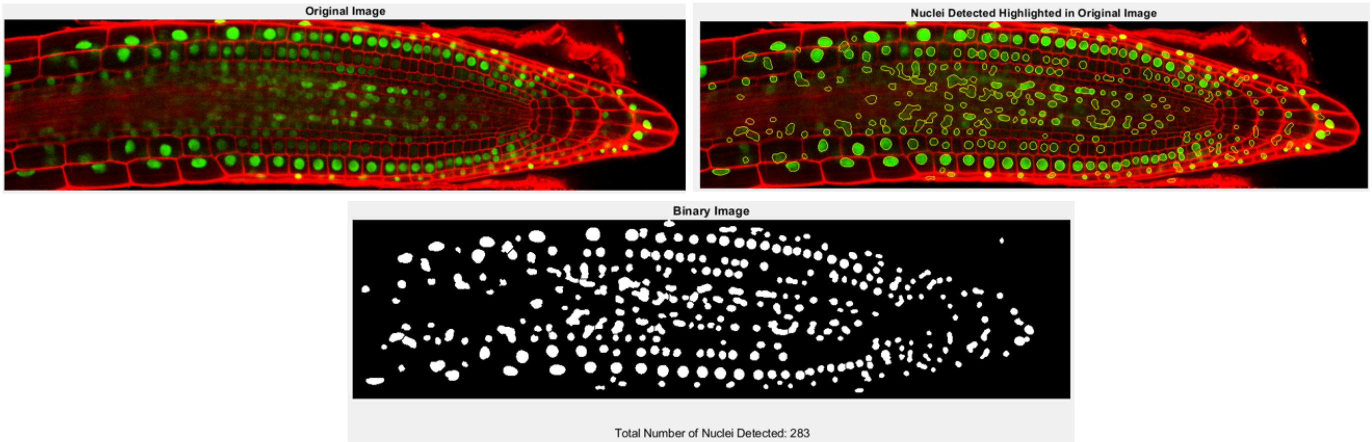


Figure 5: Output of Image 3 showing the original image, the overlay of nuclei detected, and the number of nuclei counted with the binary image.

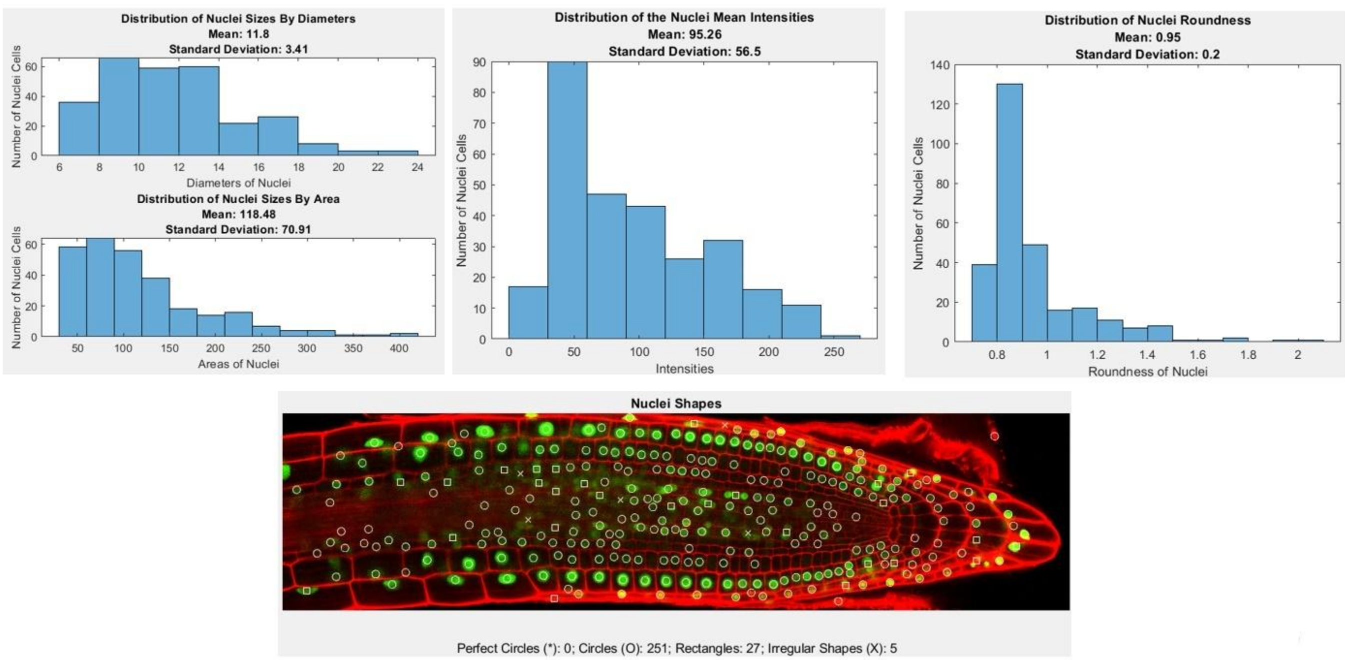


Figure 6: Output of Image 3 showing the histograms of the size, mean intensities, and roundness distributions of nuclei as well as the marking and count of nuclei shapes.