Counting Cell Nuclei

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Implementation Features:

In this part, key features of the implementation are outlined with brief descriptions. Explanation towards the obtained results will be provided in the second part and approach evaluations are covered in the last part of this report.

The main features of this image processing pipeline could be summarized into four aspects:

1. **Cell nuclei preservation**

   Achieved by applying local Otsu threshold method for thresholding (Stage 3) and disk shape structuring element for morphological image process (Stage 5).

   Throughout the pipeline, cell nuclei are preserved as much as possible in order to enhance the accuracy of quantitative data extracted from source image.

2. **Cell nuclei separation**

   The watershed transforms for segmentation (Stage 4) and opening operator for morphological image process (Stage 5) realized cell nuclei separation.

   The drawbacks caused by cell nuclei preservation have been minimized by segmentation process. Connecting cell nuclei will be segregated according to their disparities in shapes and colors.

3. **Noise reduction and interference elimination**

   Green channel extraction for color space conservation (Stage 1), median filter applied in noise reduction (Stage 2) and opening operator for morphological image process (Stage 5) are responsible for noise reduction and interference elimination.

   The noise and pixel fragmentations have been significantly eliminated by this pipeline which is essential for the accuracy of the export quantitative data.

4. **Quantitative data export**

   A labeling function is applied in the quantitative data export stage (Stage 6).

   In order to achieve high automatic, this program will automatically calculate the number of cell nuclei within the output image.

Image Processing Pipeline Overview:

In the second part of this report, each stage within this image processing pipeline will be demonstrated.

0. Environment configuration

Before executing the image processing pipeline, a configure file is loaded to set all related parameters to a target value. The access path towards processing images is loaded simultaneously.

```
%% Load parameters and source image path
configure;
```

This file is developed to simplify the process of changing parameters and file path towards source images.

1. Color space conversion

- Green channel extraction

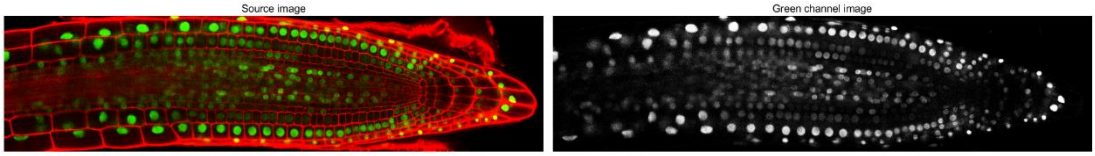

Figure 1. Preserve green channel layer

2. Noise reduction

- Median filter
- Filter size 3 × 3

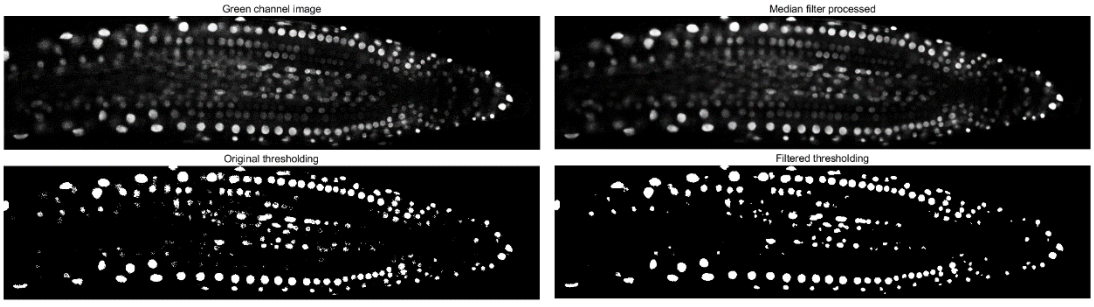

Figure 2. Median filer [3×3] noise reduction and result comparison

3. Thresholding

- Local thresholding
- Segmentation size 16 × 128

- Otsu threshold method

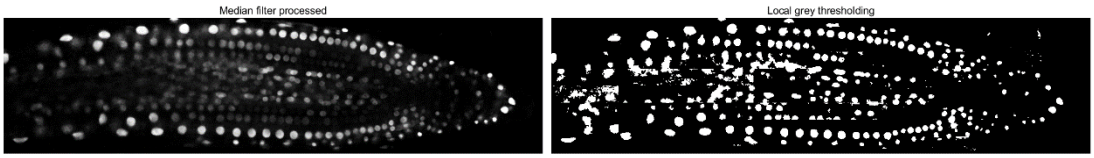

Figure 3. Local Otsu thresholding

4. Segmentation

- Watershed transform
- Minima deviation 0.4

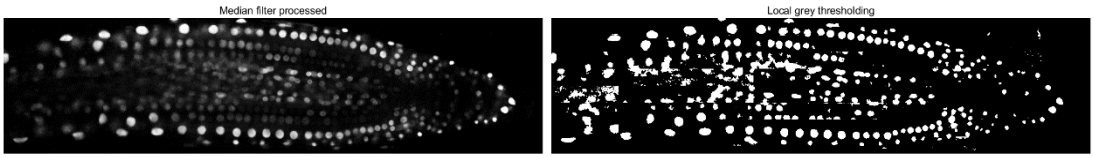

Figure 4. Watershed transform

5. Morphological image process

- Morphological operator Opening
- Erosion times 3
- Dilation times 3
- Structuring element shape Disk
- Structuring element radius 1

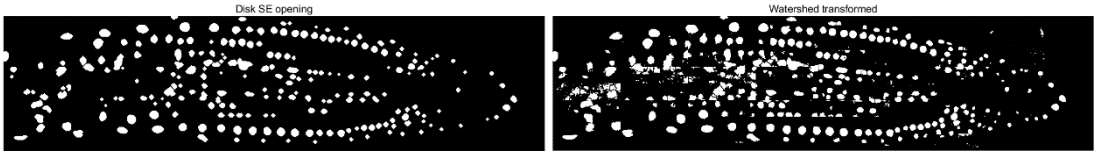

Figure 5. Disk shape structuring element opening

6. Quantitative data export

Figure 6. Separated partitions counting

Approach Evaluation:

In this part of this report, evaluations and comparisons of the applied method and most of their alternatives for each stage are provided.

1. Color space conversion

Figure 7. Comparison amongst 3 channel layers

For color space conversion, a lower-dimensional space could save a great amount of workload, so it is necessary to convert the source RGB image into a grey level image.

By applying layer separation to source image, 3 channel layers could be generated. Cell wall appears in red channel layer, cell nuclei appear in green channel layer and there is nothing could be observed from blue channel layer.

The green channel layer is extracted from source image for color space conversion. Since preserving cell nuclei figure is the main objective and most of the cell nuclei are colored in green. Green channel layer should contain most of the nuclei. Therefore, it is the most effective and easiest implementation.

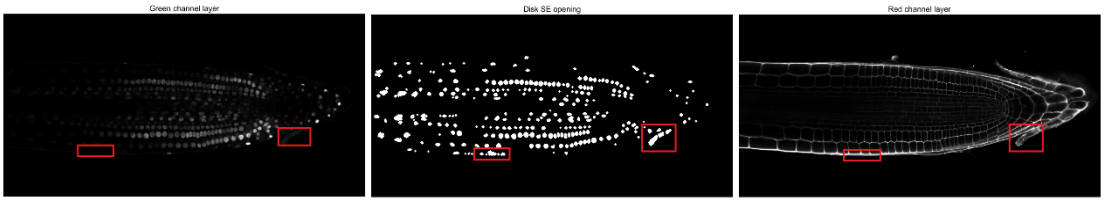

Figure 8. Preserving interferences demonstration

But this strategy will introduce extra noise into the resulting image. According to Figure 8, some interferences, part of the cell wall has been preserved simultaneously. Since these interferences are generated from cell wall, greenness focusing (G = (R + B) / 2) could be applied to eliminate these interferences.

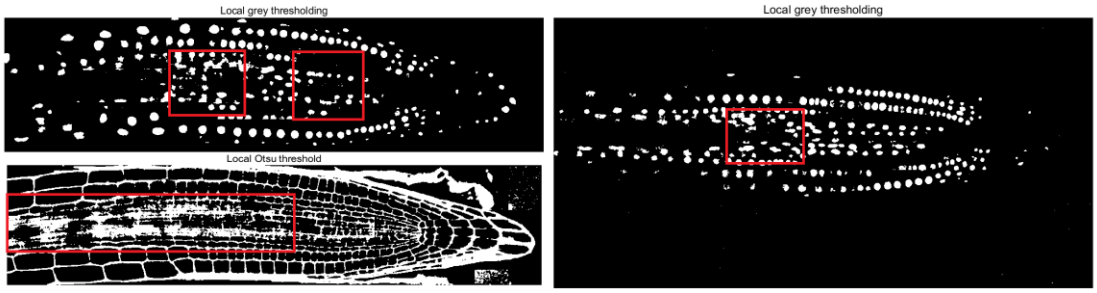

Figure 9. Interferences generated from red channel layer

However, this method will introduce more noise into the image because of the middle part of the root is blurry. So green channel extraction is comparing more acceptable.

2. Noise reduction

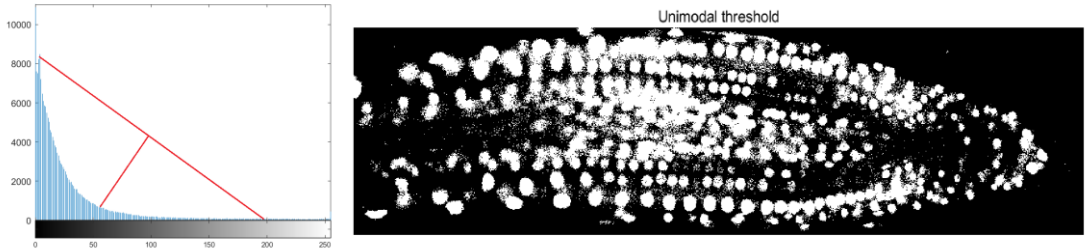

Figure 10. Unimodal threshold processed image

According to histogram of the image, unimodal thresholding is the most suitable threshold method. However, after directly applied the unimodal thresholding, great amounts of noise appeared in the processed image.

Consequently, a 3×3 median filter is applied for noise reduction.

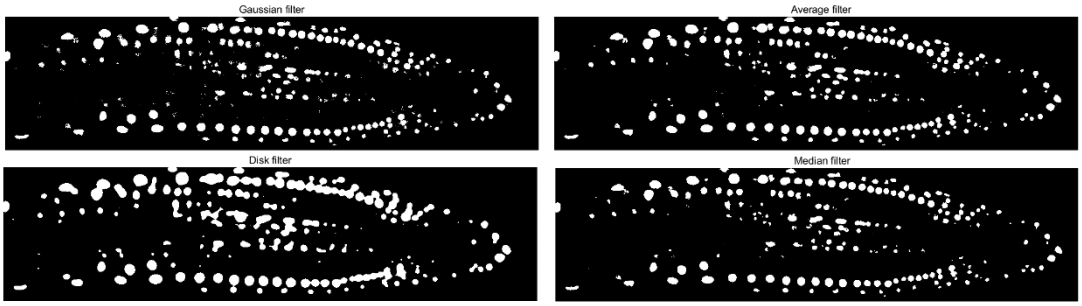

Figure 11. Comparison amongst different filters

According to the comparison, median filter could maintain the separability between nuclei while eliminating noise.

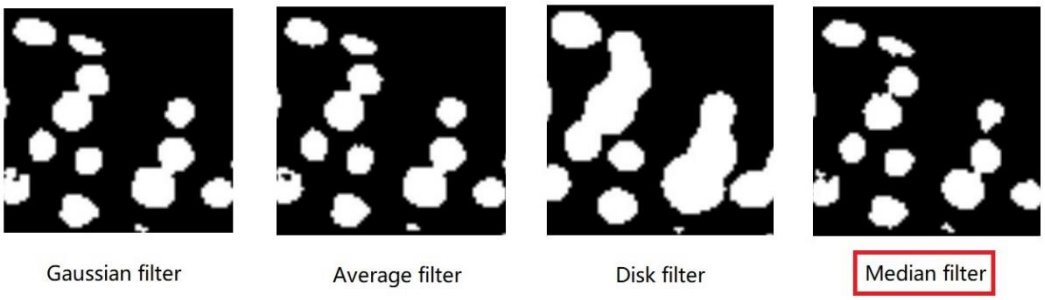

Figure 12. Partial comparison amongst different filters

By observing the partial comparison, disparities in separability of different filters' results are

obvious. Other filters will automatically smooth and enhance the sticking part between nuclei which jeopardized the image quality.

Figure 13. Median filter fragmentation result

According to figure 13, median filter generates fragmentations around nuclei with irregular shapes. In other words, it generates noise around some nuclei and enhances their irregularities. This results from the implementation of the filter which takes the median value only, from the filter window.

Since these noises and fragmentations could be reduced by applying morphological operation, this method's drawback is acceptable.

3. Thresholding

The choice of thresholding method mainly consists of two parts. First, apply local thresholding or global thresholding. Second, choice of function generates threshold value.

1) Local thresholding vs Global thresholding

Local thresholding is applied as a thresholding strategy and the source image is divided into image pieces of size 32 × 128.

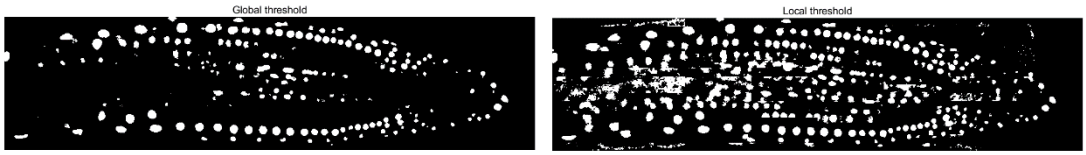

Figure 14. Global thresholding vs Local thresholding

Because of the unique structure of plant root, cell nuclei's colors within the center of root are comparingly darker than ones around the edge. Therefore, global thresholding might filter out most of the center cell nuclei and local thresholding could preserve more data.

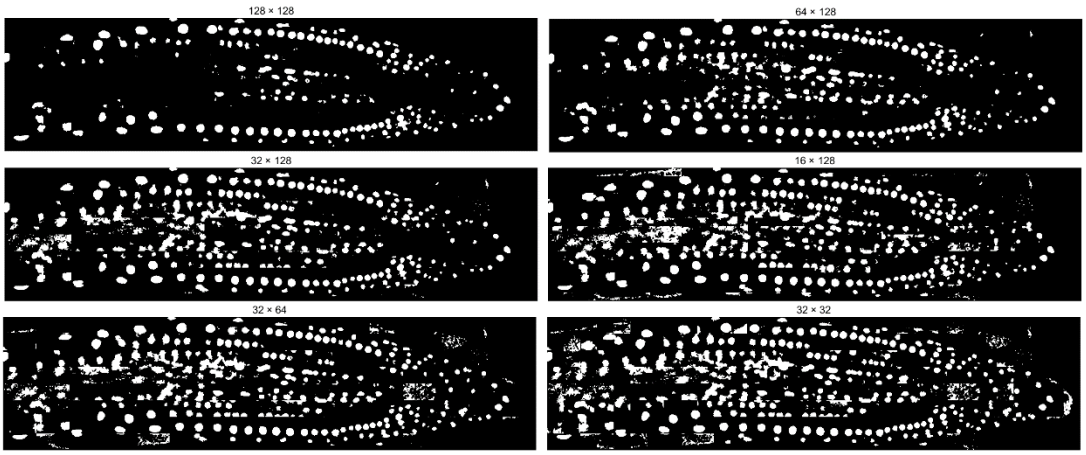

Figure 15. Comparison amongst different image pieces sizes

By comparing the result of thresholding with various image piece sizes (Figure 14). There appears a tradeoff between data preservation and noise elimination. This could be ascribed to short sight of local threshold method which takes fewer pixels for analysis. A lower threshold will be generated from image pieces with large blank space, which takes in some noise.

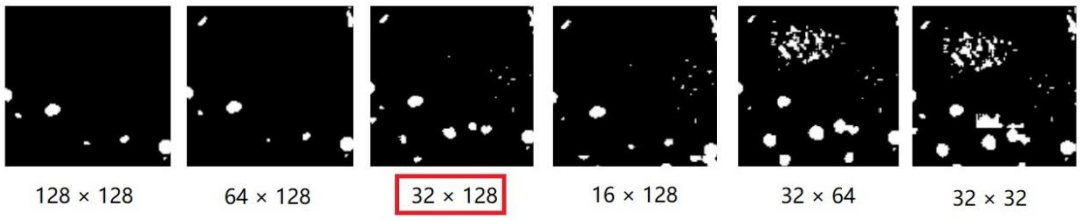

Figure 16. Partial comparison amongst different image pieces sizes

According to Figure 15, "32×128" is observed to be a balancing choice. Most of the nuclei have been preserving while only little noise has been introduced. This size might benefit from cells arrangement structure because it contains more cells in a horizontal direction.

However, local thresholding protrudes more noise from cell wall interference than global thresholding. And the process of splitting and merging images significantly increases program's time complexity. And applying target image piece size, a small amount of noise still exists, and some data loss could still be observed.

2) Choice of threshold methods

Different threshold methods have been applied for experiment. Including a self-implement unimodal thresholding method and adaptive thresholding method.

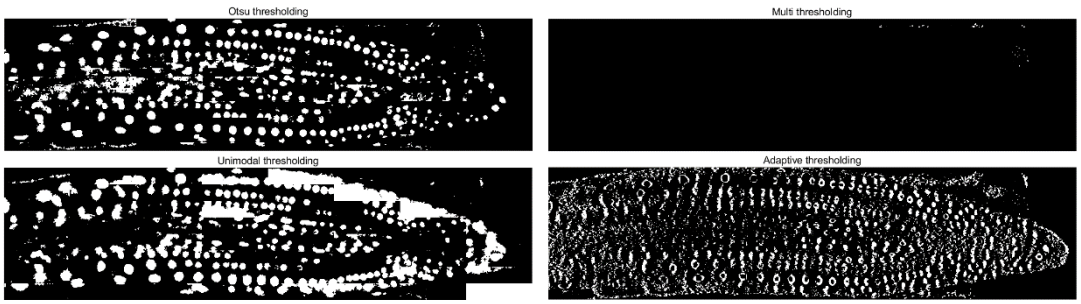

Figure 17. Comparison amongst different threshold methods

By observing above result, Otsu thresholding not only preserves most of the cell nuclei but also enhanced the separation between different cell nuclei. This could be result from the bimodal distribution of the grey level histogram. But part of the noise is failed to be eliminated and cell wall interference could be observed on boundaries of image pieces.

The image processed by multi thresholding is almost black, this might be result from the fragmentation of source image. The size of image pieces is too small for multi thresholding to generate a threshold. This causes a problem to unimodal thresholding in the same way. Huge noise blocks have been generated under this circumstance because the local threshold value is too low.

Although Adaptive thresholding preserves most of the nuclei, great amount of noise has been produced simultaneously which could jeopardize later process.

Consequently, Otsu thresholding is applied as the local threshold generating method.

4. Segmentation

Figure 18. Connecting parts of cell nuclei

According to above examples, after the local thresholding process, some cell nuclei are still connecting to each other. Connecting cell nuclei could be transformed into one single nucleus by morphological operators, which will significantly jeopardize the result. Consequently, an image mask is required to mask out the boundaries between cell nuclei. Two methods have been developed to generate target image mask.

1) Apply red channel layer as an image mask

A cell wall image mask could be generated from red channel layer of source image which

should be able to separate the connecting nuclei.

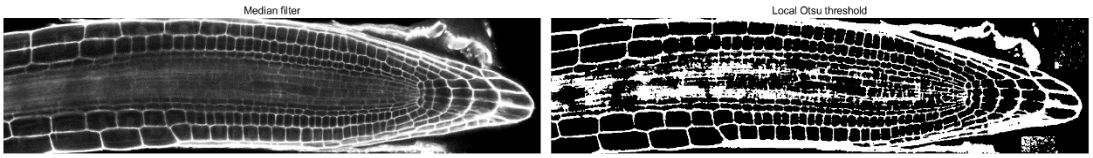

Figure 19. Cell wall image mask

Plant cells are surrounded by call wall, a thin cell wall layer exists within each one of the cell blocks. And as a result, cell blocks, especially for blocks in the middle of plant root, are extremely blurry.

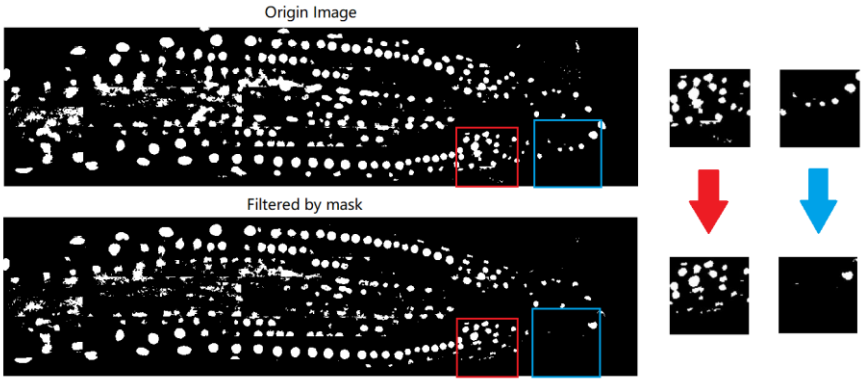

Figure 20. Comparison between origin image and masked image

According to the result, the image mask has enhanced the boundaries between part of the connecting cell nuclei (red comparison in above image). However, because red channel layer contains noise, blurry blocks in practical, some cell nuclei could be masked out in the meantime (blue comparison in above image).

2) Apply watershed transform to generate an image mask

This method is generated form an article published by Steve Eddins. Within this watershed transform, source image has been processed in four stages.[1]

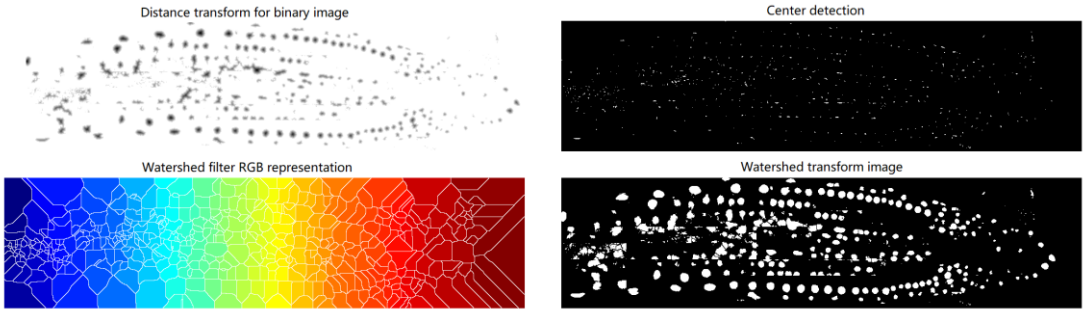

Figure 21. Process of watershed transform

[1] Steve Eddins. (2013) 'Watershed transform question from tech support', Blogs, MathWorks. Available at: https://blogs.mathworks.com/steve/2013/11/19/watershed-transform-question-from-tech-support/ (Accessed: 08 March 2020)

In the first stage, the Euclidean distance transform of the source image is computed by a distance transform function. For each pixel in source image, the distance transform assigns a number which is the distance between that pixel and the nearest nonzero pixel of source image.[2]

In the second stage, the center of each individual cell has been emphasized and selected out from the source image provided by distance transform. This process ensured the accuracy of watershed filter generating.

In the third stage, a watershed filter has been generated and demonstrated in RGB spread mode. This filter segregates source image into different blocks according to the result form center detection.

In the final stage, the watershed filtered image is functioned onto the original image as a mask.

Figure 22. Boundaries enhanced by watershed transform

By observing figure 22, watershed transform has successfully separated most of the connecting cell nuclei without eliminating existing cell nuclei. Especially for connecting nuclei located around the edges of root.

However, a great amount of fragmentation and noise has been generated after the mask process. That could be ascribed to the watershed process which generates small fragments between blocks. Also, some single nuclei have been separated into two or more individuals while some sticking nuclei remain connecting.

Comparing to red channel layer, watershed transform is more acceptable in terms of nuclei separating capacity and noise introducing capacity.

5. Morphological image process

The morphological operator applied in morphological image process could be discussed in two

[2] MathWorks. (2018) 'bwdist', Help Center, MathWorks. Available at: https://www.mathworks.com/help/images/ref/bwdist.html (Accessed: 08 March 2020).

parts. First, apply opening or closing as the basic building blocks. Second, choice of structuring element.

1) Opening vs Closing

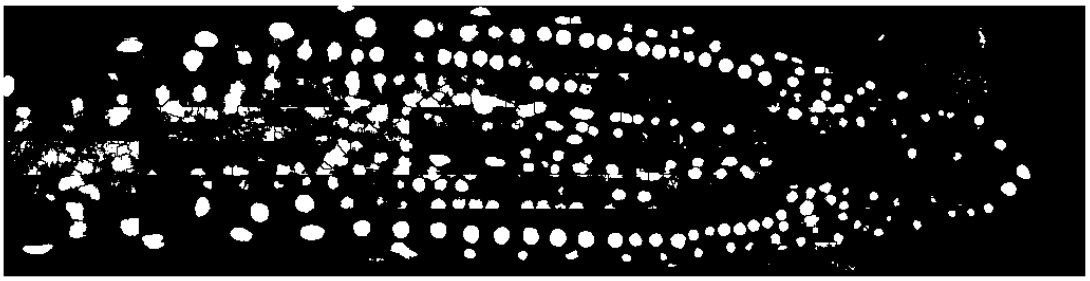

Figure 23. Present status of the image

By observing figure 23, present image contains a great amount of noise and fragmentations around cell nuclei which should be eliminated. Moreover, narrow gates and gaps between nuclei should be enhanced.

According to the requirements, opening operator should be the idea basic building block for this pipeline.

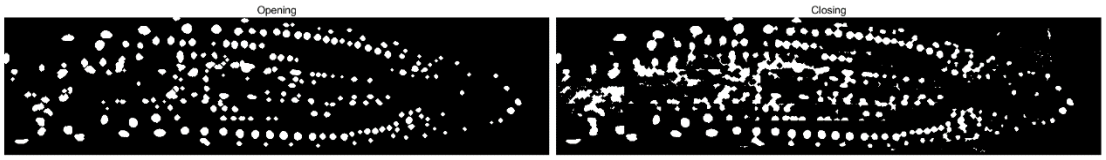

Figure 24. Opening vs Closing

Figure 24 has illustrated above assumption. Most of the noise and fragmentations within the image are been erased while boundaries between nuclei are successfully preserved. Since erosion process is executed before dilation process, fragmentations are flushed out of the image. Closing, opposingly, performs badly because it takes all fragmentations and noise into analysis.

However, some nuclei with smaller sizes have been eliminated simultaneously. But opening performs significantly better than closing.

2) Choice of structuring element

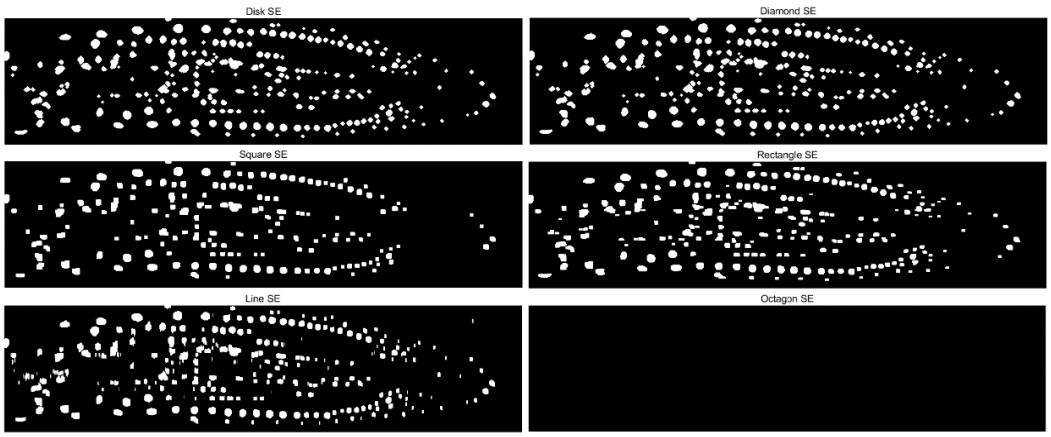

Figure 24. Comparison amongst different structuring elements

Figure 25 demonstrates the comparison amongst different structuring elements, disk SE, diamond SE and square SE performs better than the others.

Because the most common shape of cell nuclei is disk, structuring element with disk shape should have advantages over the others. Moreover, disk shape is sensitive to gaps between entities in all directions for human eyes.

However, disk shape structuring element could hardly separate mostly overlapped nuclei. According to the comparison, line shape one could enhance nuclei separation, but this only functions in single direction. Therefore, a disk shape structuring element is applied in morphological image process.

6. Quantitative data export

This stage is the final stage of the entire image processing pipeline. A function is applied to count the number of nuclei by labeling the connecting pixels with the same index.

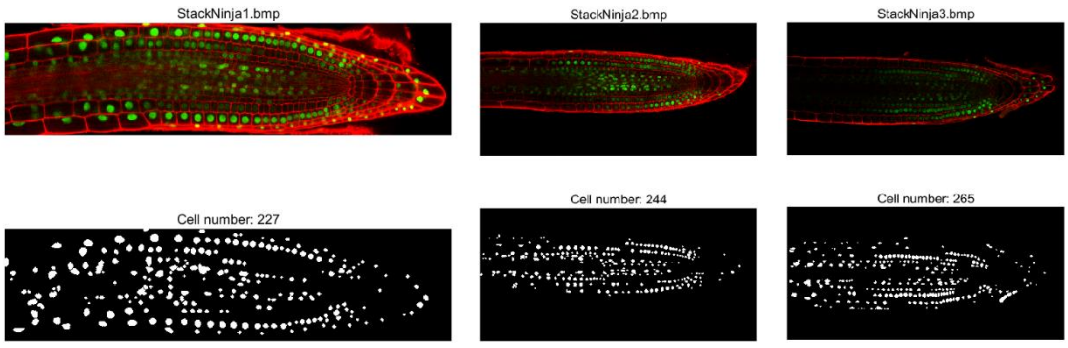

Figure 25. Outputs of source image set

This method significantly reduced users' workload and enhanced accuracy of the output quantitative data.

However, its accuracy based on the quality of output image. Because all separate noise pixels are counted as nuclei and connecting nuclei are counted as one single nucleus.

Conclusion

According to the analysis, this image process pipeline performs well in processing plant root image with clear separate nuclei figure. Within the practice, it maintains its robustness confronting blurry images containing sticking nuclei with various shades.

However, results from the limitation of knowledge, this pipeline performs poorly in generality and layer separation. For the former aspect, the pipeline is designed to detect cell nuclei with green color only and requires high purity in green channel layer of source image. And for the latter one, this pipeline detects all nuclei within the image, even if some of the nuclei belong to other layers.