An easy-to-use and effective tool for counting cell nuclei in confocal laser microscopy images

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Nowadays, **Confocal laser scanning microscopy** has been heavily used in a variety of filed, with respect to its effective intersect and obvious fluorescence. Images of this approach are often utilized for extracting numerical features by image processing and analysis methods. In this report, an effective pipeline is designed for **marking binary region of nuclei** given a set of confocal laser microscope images of plant roots. Basic image processing methods, such as color space conversion, noise reduction, thresholding and binary processing are included. As a result, it produces outputs of **great accuracy** and is also easy to implement.

Keywords: Confocal laser microscopy images, HSV color space, Noise Reduction, Gaussian Filter, Morphology, Binary image processing

Pre-processing

Before tackling the problem, several tools, such as "improfile()", "imtool()" and "imhist()", were used to investigate the basics of images, such as distribution of intensity.

Method

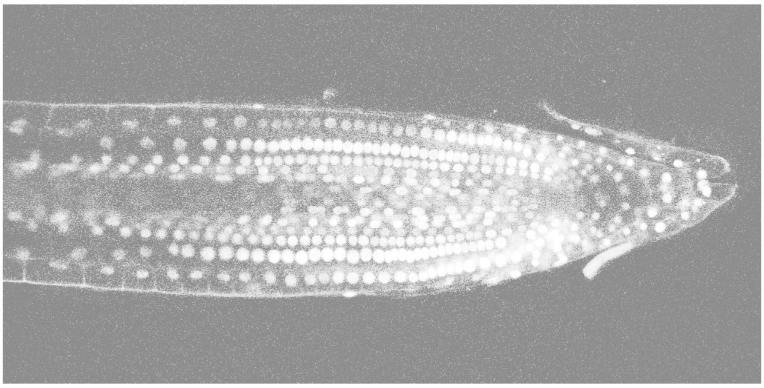
Color Space Conversion

Selecting suitable color space could benefit specific image processing task with both accuracy and efficiency. For counting nuclei in cells, color spaces are supposed to be constructive to nuclei segmentation beyond the plant root. According to Wesolkowski et al, more clear and continue edge is depicted using images of HSV color space, compared to those of other spaces when using same algorithms. Moreover, variation in Hue, Saturation and Intensity values could be captured by visual perception(Sural et al, 2002). This implied that HSV color space could be a considerable choice for this task. However, from the original picture, area of nuclei seemed to have obvious

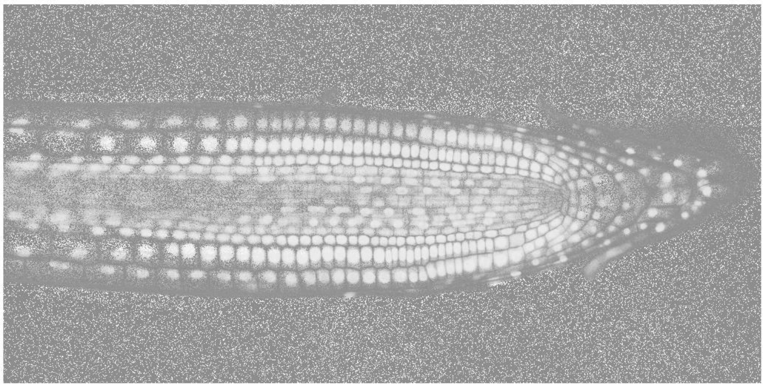
color difference with cytoderm, which indicates that RGB maybe a simple but suitable choice as well.
Thus, RGB and HSV color space were compared and analyzed with respect to the feature they preserved. Finally, HSV color space was supposed to be more suitable and the original image was then converted from RGB to HSV using "rgb2hsv()" method.
One of the givens pictures, for example, "StackNinja3.bmp", was first read and a copy in HSV color space was created using the method "rgb2hsv()". After that, "Green" channel and "Hue" channel were extracted respectively, since the area of nuclei was green while the cytoderm was red. Consequently, obvious difference could be expectedly detected in both "Hue" and "Green" channel.
As the Figure1(a) shows, the image of green channel held both nuclei and cytoderm information. Although there was not so much noise in the background compared to figure2, the edge between nuclei were obscure which could increase the difficulty of later segmentation. Moreover, redundant cytoderm may hamper upcoming process as well. However, nearly all cytoderm were removed and clear edges among nuclei can be found in Figure1(b). At the same time, the intensity of nuclei are much more balancing. Though there was some noise outside the plant, a pellucid edge formed by dismissing cytoderm divided the image into two parts. Thus, HSV color space was chose.

Noise Reduction

Since there was a lot of noise on the background, intuitively it could be conducted noise reduction based on previous image. It is important to select suitable filters for specific problems. A two steps noise reduction using median filter and gaussian filter was achieved.
As most of noise in the image were speckle noise, linear filter may not be able to achieve good performance and even spread these speckle noise and edges. Thus, median filter was chose first using the method "medfilt2()" as it was suitable for reducing noise of such speckle kind. After that, most speckle noise were removed.
However, some were remained. For those additive noise left "lonely", it could be suitable to use gaussian filter again. To achieve this, Predifined filter using "fspecial()" was combined with "filter2()". By conducting noise reduction again, the numerical value of those remained noise were sharply decreased, but those of nuclei were nearly not influenced.



(a)



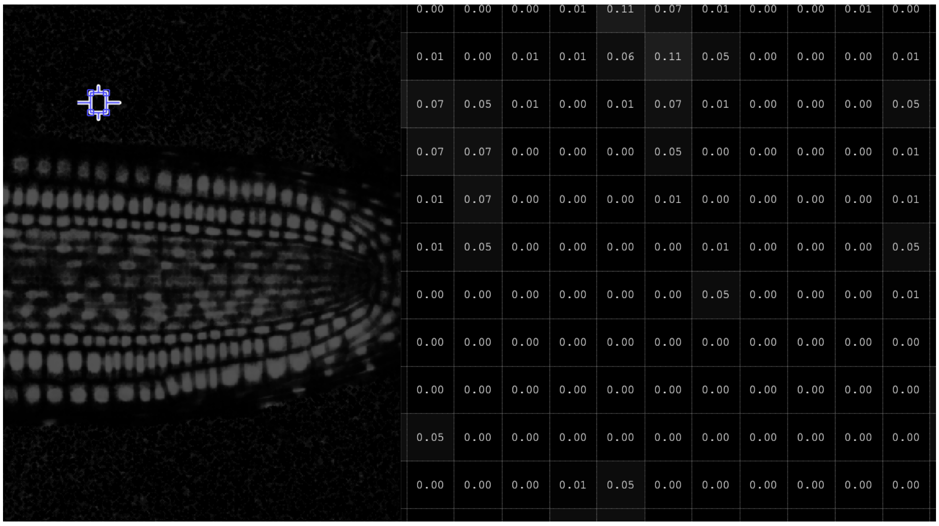
(b)

Figure 1: Color Space Conversion (a) Green channel (b) Hue Channel

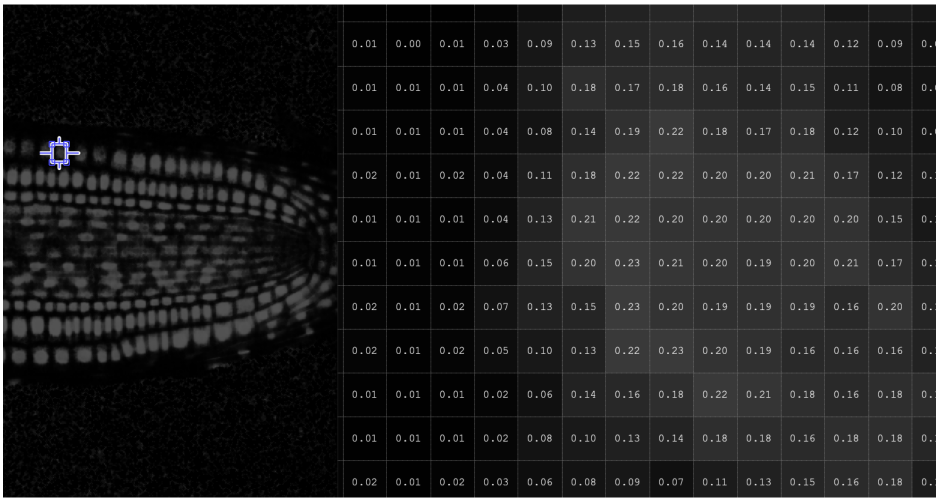
Thresholding

As there still existed some noise and area of nuclei was almost cropped, thresholding was conducted aimed at segmentation and noise reduction.
It was impossible to set a suitable value for thresholding as it determined the performance of final segmentation result. This value should keep nuclei information as much as possible and eliminate useless noise. Since noise in confocal laser microscopy images often come from low illumination, the value of noise was lower than those of plant.(Ben Hadj et al, 2013) Thus, value of noise and nuclei should be analyzed first and a threshold between them could

be selected. In order to get this threshold, "impixelregion" was used with pictures, which allowed to visualize corresponding area with numerical value when moving the mouse. As Figure2 shows, the value of noise was mostly between 0 and 0.15. The value of nuclei(including its edge) was usually more than 0.17. Thus, the threshold was set to 0.17 intuitively.



(a)



(b)

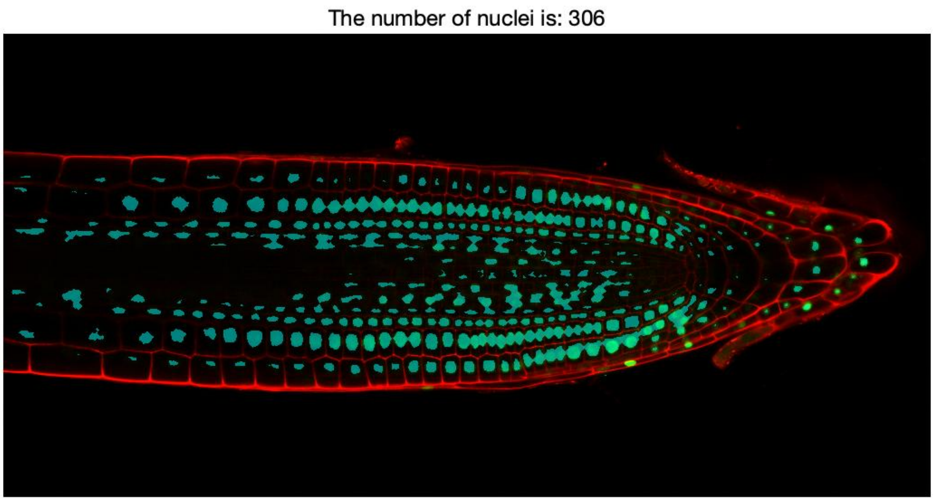
Figure 2: Thresholding Selection (a) Values of background noise which are usually between 0.0-0.11 (b) Values of nuclei edge which are usually between 0.17-0.25

Closing

As thresholding cannot makes the segmentation perfect, it was important to use other methods to help improve the result.
Mathematical morphology provided a rigorous approach and dilation was conducted first. Two linear structures were defined using "strel()" and the image was then dilated vertically and horizontally at the same time using "imdilate()". This step was designed for eliminate negative effect caused by thresholding, since it removed the edges to some extent. A corresponding step for erosion was designed for eliminate negative influence caused by previous dilation, such as making nuclei connected to each other. A circular structure was defined and erosion was conducted twice by "imerode()".

Counting

A method called "bwlabel()" was used help count the number of nuclei. It could help detect labels for the 8-connected objects found in binary image. The following figure shows the number of nuclei in the title of the figure. Especially, 8-connected was used in this pipeline, which means that two adjoining pixels are part of the same object if they are both on and are connected along the horizontal, vertical, or diagonal direction. The Figure3 shows a result with the count of nuclei as its title.

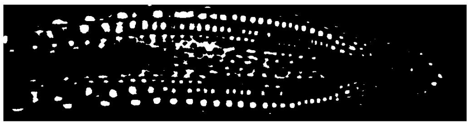


(a)

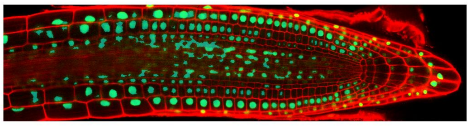
Figure 3: Counting the number of nuclei.

Result

The figure below show results produced by this pipeline using all of three pictures.



(a)



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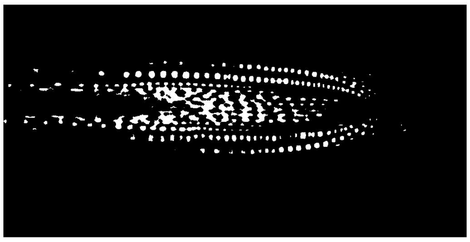
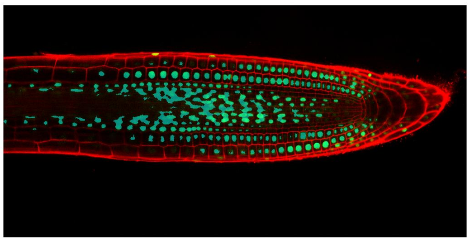
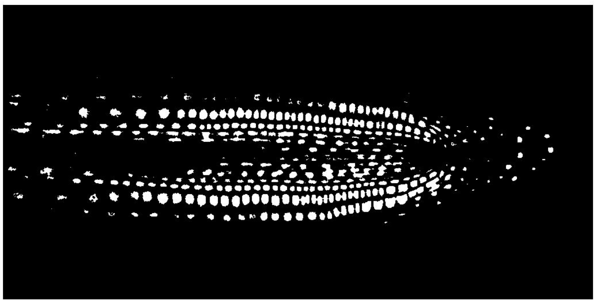


(c)

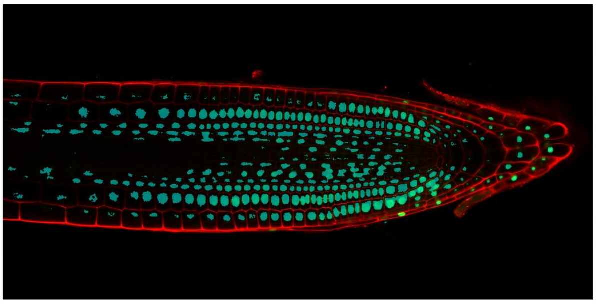


(d)

Figure 4: Result of picture 1 and 2 (a)(c) The binary segmentation result of nuclei area (b)(d) The mask overlay on original image



(a)

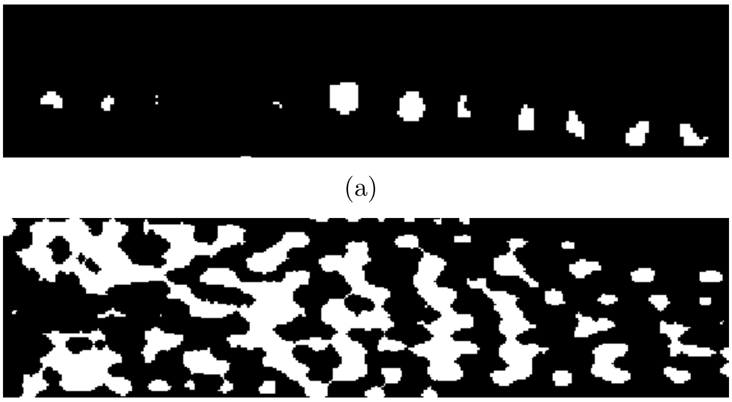


(b)

Figure 5: Result of picture3 (a) The binary segmentation result of nuclei area (b) The mask overlay on original image

The Figure4(a), Figure4(c) and Figure5(a) were the binary image marking regions corresponding to nuclei. As we can see from all of figures, both nuclei on the top(right) and bottom(left) of plant were segmented. Most of nuclei was separated by a pellucid edge. The shape of most of nuclei was disk-alike. In the middle part of the plant, there were a lot of obscure nuclei successfully segmented as well. There was nearly no noise on the background. The Figure4(b), Figure(d) and Figure5(b) were the masks overlay on the original images. As we can see from the figures, most of nuclei area was successfully segmented. The dice of segmentation result could be considerable if provided with a ground truth mask.
However, some nuclei here were segmented without a desired result. As the Figure6(a) shows, they were shaped different from their ground truth. It seems that they were influenced by thresholding and later erosion. Moreover, the area of those nuclei was much more "adhesive" in the middle of the root,

which could be seen in Figure6(b). It seemed to be a common problem among those results and these kind of adhesive nuclei could be found from all three pictures.



(a)

(b)

Figure 6: Shortage (a)Area which is not well shaped in result 3 (b)Nuclei were adhesive in result 2

Discussion and Evaluation

Color Space Conversion

Strengths

- Effectiveness: According to Wesolkowski et al, more clear and continue edge is depicted using images of HSV color space, compared to those of other spaces when using same algorithms. For this task, accuracy segmentation result could highly influenced by pellucid edges.

- Efficiency: Since a lot of important features were contained in hue channel for this task, it is easy and direct to extract the channel for later analysis and processing. It could save a lot of time without sophisticated calculations.

- Interpretability: With scientific definition and visual inspection, HSV color space could help with more interpretability compared with those generated by complex equations.

Weaknesses

- Since only hue channel was chose in this pipeline, the accuracy could be decreased to some extent, since it ignored some features maintained in saturation channel. Improvements could be made by linear combination of saturation and hue channel with suitable weights.

Noise Reduction

Strengths

- Sharpness: One of most obvious disadvantages for using linear filters is they degrade images and the edges of image (Research Gate, 2020). This is especially evident after using the average filter. As the figure shows, after conducting noise reduction with average filter for different times, image could become more and more obscure. However, median filter used in this pipeline does not have this issue. As Figure7 showed, most of speckle noise were removed first, without causing nuclei area as obscure as linear filter.

- Effectiveness: The Figure8 consisted of several image got by "impixelregion", showing the change of value after noise reduction in a same area, which coordinator of the center point is (511,100). As seen in the figure, most noise were first removed. Later, guassian filtering decreased the value of remained noise, which improve the contrast between noise and nuclei. This helped upcoming thresholding and made it much easier to find a suitable threshold as there was obvious difference between noise and nuclei.

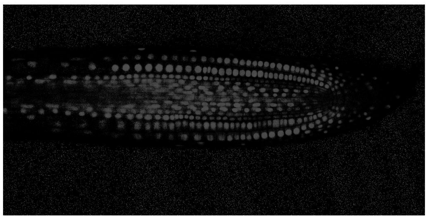
Weaknesses

- Inflexibility: Gaussian filtering needs more pre-defined parameters, which increase the inflexibility for tackling different samples as different setting may be required. This complexity also makes the process less automatic, compared using simple linear filters.

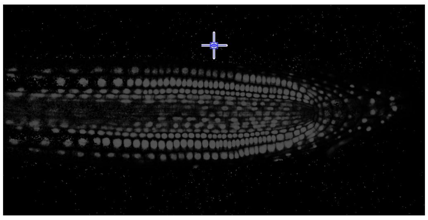
- Efficiency: More time need to took since the mathematical representation of gaussian is much more complex and consequently, more calculation. Especially compared to simple filters like mean filter, it is much more time-consuming.

Thresholding

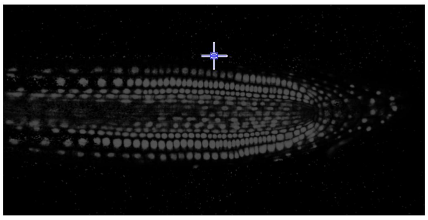
Strengths



(a)



(b)



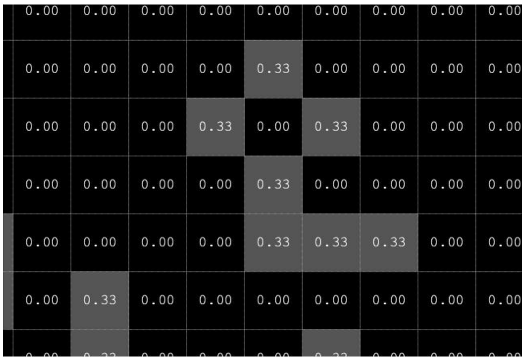
(c)

Figure 7: Noise Reduction: images before/after noise reduction (a) Original Hue (b) After conducting median filtering (c) After conducting gaussian filtering

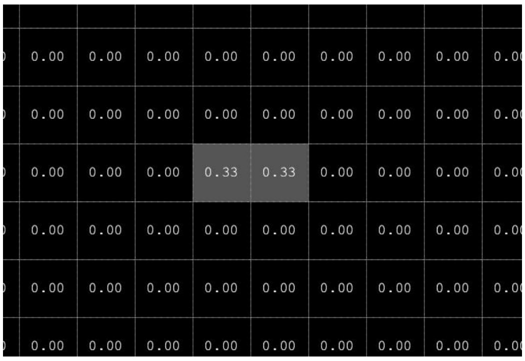
- Availability: Thresholding is one of the most frequently used methods in image processing.
- Efficiency: It is easy to use and without abundant of calculation, which help to improve the efficiency of processing.

Weaknesses

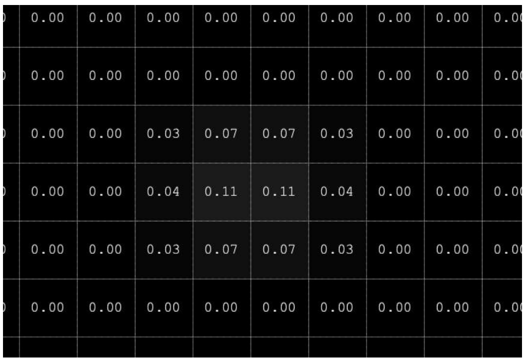
- Tradeoff between accuracy and noise: As stated before, thresholding can eliminated the noise but also potentially important features, which shows that there is a tradeoff between segmentation accuracy and noise.



(a)



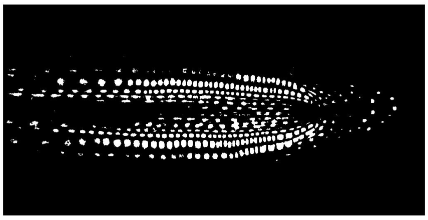
(b)



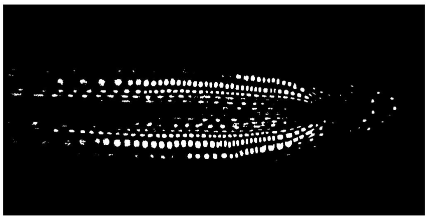
(c)

Figure 8: Noise Reduction: An example area of image before/after noise reduction (a) Original area (b) After conducting median filtering (c) After conducting gaussian filtering

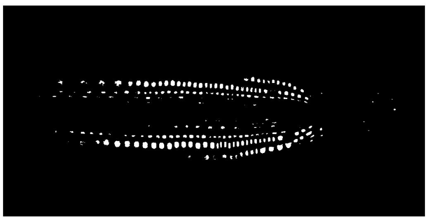
A high threshold may be able to help remove most of noise in confocal laser microscopy images, however, also some parts of ROI. For example, as Figure9 shows, more and more area of nuclei were removed as the value of threshold increased, though noise were reduced as well. Since 0.17 was selected as the value of threshold and the value edge of nuclei is also 0.17, it may erode the edge and make the area of nuclei much smaller.



(a)



(b)



(c)

Figure 9: Thresholding: Images with different threshold (a) Original Segmentation result (b) Result with threshold value increased to 0.25 (c) Result with threshold value increased to 0.30

Closing

Strengths

- Edge Smooth: Since it was possible that some edges has same value as threshold, they could be removed during the thresholding step, dilation was conducted first, which could cover the shortage made by previous thresholding.

- Noise Reduction: Following erosion could then help eliminate the negative influence caused by dilation and even remove some remaining noise.

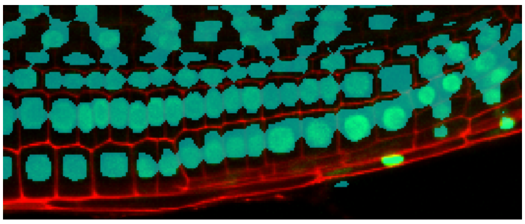
- Efficiency: Dilation and erosion are achieved by morphology processing, which reduces the serial computer processing time for binary operations. (JI et al, 1988) Moreover, they are especially useful since these operations could be regarded as a set of simple calculations.

- Availability: The computation approaches could be unaffected by what the shape of structuring elements are. Also, compared to other complex algorithms, the availability of dilation and erosion permits circular erosion in counting nuclei tasks.

Weaknesses

- "Adhesive" nuclei: The parameter of dilation should be determined carefully. If the parameter of "strel()" is set too big, the nuclei will be connected to each other since dilation was abused. As Figure10, nuclei seems to be adhesive to each other and count of nuclei was decreased. This was because that the area of nuclei were over dilated, which destroyed gap between them.

- Difficulty of understanding: Sometimes, it could be not easy to use morphology to achieve something, since it needs a lot of mathematical background knowledge.



(a)

Figure 10: "Adhesive" nuclei caused by unsuitable parameters of dilation

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