Adaptive Cervical Nuclei Segmentation Using Morphological Operation

Authors

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Abstract

In this project, to achieve a compatible result of cervical cell nuclei recognition in a clinic grand-challenge website, a new adaptive segmentation method was studied and implemented. By reviewing many previous works on cervical cell nuclei recognition, this new adaptive segmentation method was originated from another solid segmentation method [1], the main idea of the new approach is using classic morphological operation with new added statistical based connected object removal resort to properly remove as much garbage as possible. Then by running an experiment, the result show the new segmentation is reliable and robust to cope the given test case. Finally, compared with submitted result on this challenge website, the result yielded by the new approach appeared a compatible performance.

1. Introduction

1.1 Background

Nowadays, Pap Smear Screening is pretty crucial to diagnose probable cervical cancer. Practically, the Pap test is always not coming with a definite result of cancer detected, many factors may influence the final conclusion given, apart from all other non-technical factors, a reliable way to detect the given pap smear can at least try best reducing diagnostic error which may occurred by this process. In this case, giving a resort of detecting cervical cell nuclei as accurately as possible has been a significant step before recognizing the pathological cervical cell.

Narrow down to our Computer Vision study field, a reliable segmentation of the epithelial cells of a pap smear image is an important step in order to have correct morphometric measures [1]. Luckily, there were exhaustive research study in this issue, besides there is an up-to-date website: grand-challenge.org[4] where released many fancy medical image analysis challenges and exactly a cervical cell related project: Overlapping Cervical Cytology Image Segmentation Challenge.

In this challenge, we noticed that, the biggest issue of cervical image segmentation is that all given dataset come from real world Pap Smear are not so ideal. Specifically, due to the cytology feature of cervical cell, the cytoplasm overlapping, and uneven stained of these smear, detecting expected dark stained nuclei is definitely a non-trivial work. All the previous works related to cell detection inside and outside of this challenge is focusing on perusing more and more advanced automated segmentation analysis of cervical cytology specimens, however, none of them can give a generally adaptive and robust approach for clinical practice. But some of them indeed can give us hint to dig in.

1.2 Previous Work

As mentioned in last section, several previous study contributed to cervical nuclei detection were reviewed, some of them introduced cervical nuclei detection as a mid-step in their whole research and some are working on cervical nuclei only.

Izzati Muhimmah et al 2012[1] proposed a fully automated method for the segmentation of cell nuclei based on the morphological operation and watershed transformation in Pap smear test. The authors in this paper have developed a way to get the marked-boundaries image from back ground subtraction. Then they proposed the use of the morphological gradient as an alternative way for the estimation of the nuclei borders. The key point for their research is that they introduced watershed algorithm to solve the adjacent nuclei to improve the result.

Sahar Zafari et al 2015 [2] showed a segmentation of overlapping elliptical objects. They assumed the overlapping objects' approximated shaped have been known. Then they proposed a method with three steps: namely, seedpoint extraction, contour evidence extraction, and contour estimation. Seedpoint extraction is performed by a compound model consisting of morphological erosion and the Fast Radial Symmetry transform.

Daniela M. Ushizima et al 2015 [3] submitted their approach to this grand-challenge, they proposed a new unsupervised method implemented using Fiji that fuses key methods such as superpixel representation with Voronoi diagrams to detect cells and split cellular mass into individual nuclei and cytoplasm. We can use our design's test result to compare with theirs because we joined in the same challenge and used exact same dataset to our own design.

1.2 Overview

The following contents mainly covered our whole project framework from algorithm to experiment, then claimed an analysis to the result from new segmentation method, furthermore, A conclusion was drown which including further consideration about this design's future possible improvement points.

1.3 Proof of concept example

The algorithm used for this proof of concept part is the based-on contrast enhancement and background subtraction, which will also be used as the starting point of the final developed algorithm.

The object of this project is designing and implement automatic algorithm perform a cervical nuclei segmentation:

Give an input image as Figure 1(a), the output of the algorithm is expected to be Figure 2(b).
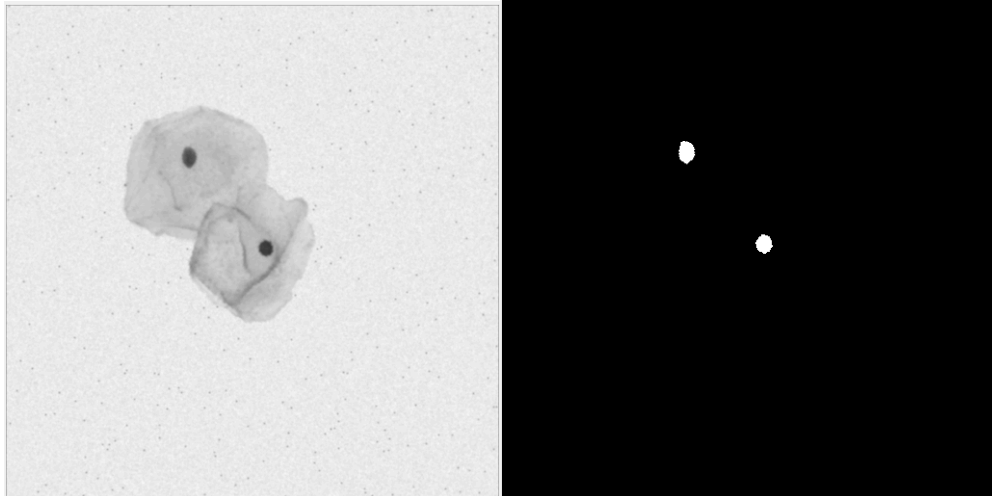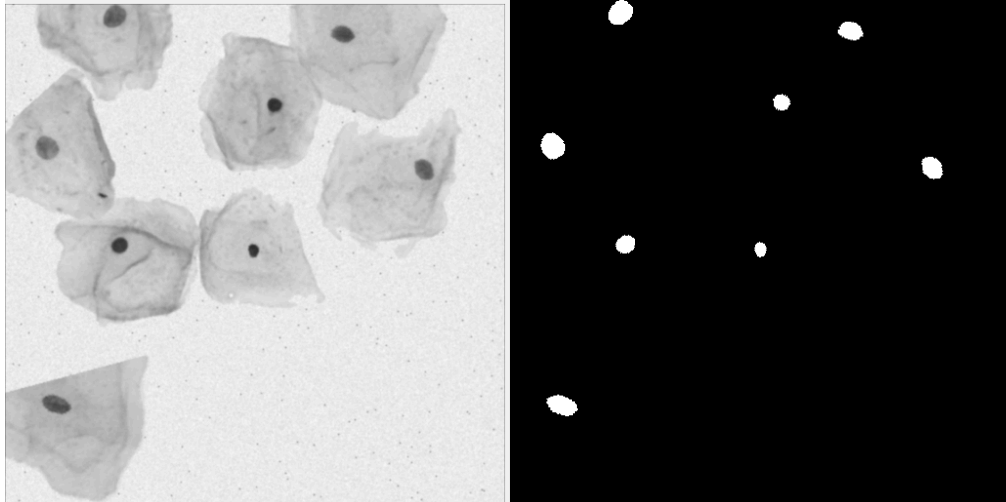
Figure 1 (a)Input image (b) Expected output(gound truth)

The output generated by the baseline algorithm is Figure 2. There are 2 objects in the ground truth and both were segmented out.
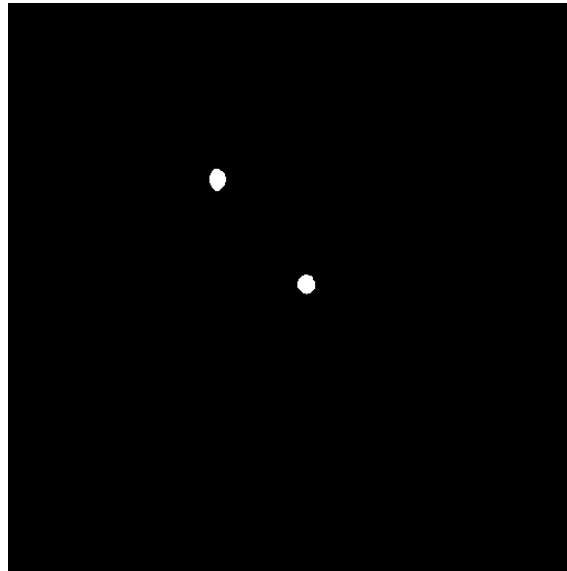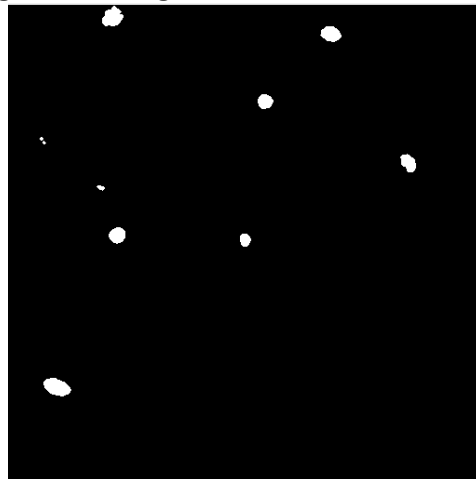

Figure 2 Output of the baseline algorithm

However, if apply this algorithm on a more complicate image, for example, Figure 3,

Figure 3 A complex image with the expeceted ground truth

The output of the baseline algorithm is Figure 4

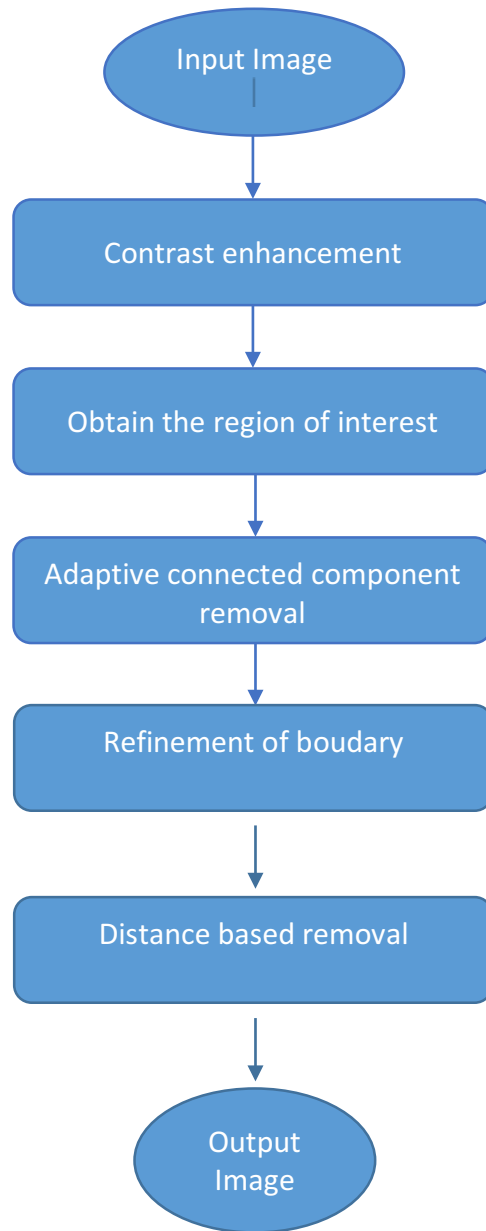*Figure 4 Output of the baseline algorithm applied to a complex image*

It can be observed that the part of the cytoplasm is segmented as the nuclei and the size of the correctly segmented nuclei are generally smaller than the ground truth.

2. Methods

2.1 Algorithm

2.1.1 Preprocessing

The procedure of preprocessing can be summarized as a pipeline below:

```
                    Input Image


                Contrast enhancement


              Obtain the region of interest


            Adaptive connected component
                       removal


               Refinement of boudary


                Distance based removal


                    Output
                    Image
```
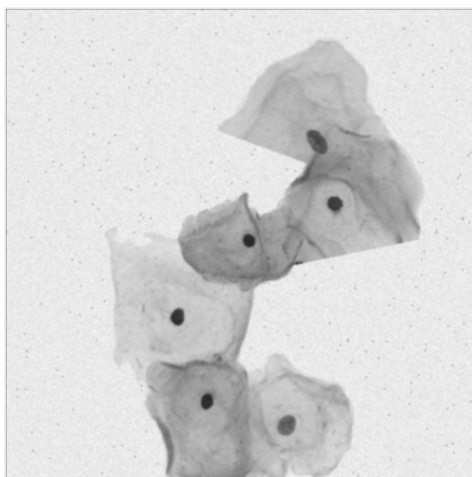
Figure 5 Original image

(a)

(b)
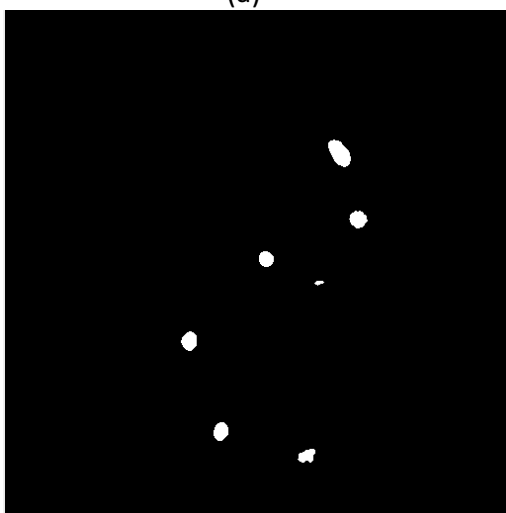
(c)
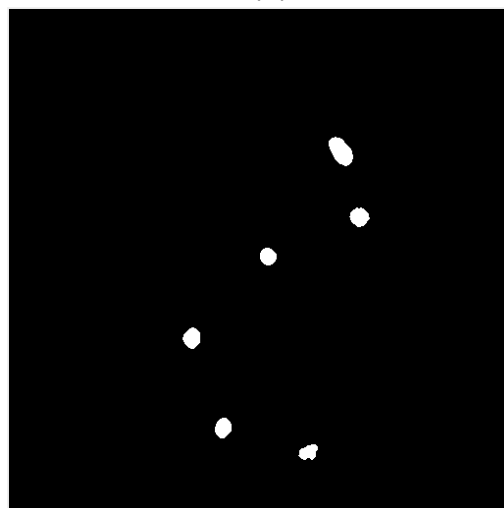
(d)

1. Contrast enhancement with background subtraction

The background subtraction yield from h-minima transform[1], this process can give a nuclei stand out image. H-minima transform will be used twice to remain the range of nuclei pixel value and subtract lower pixel value objects which were deeply stained cytoplasm and higher pixel value objects which were regularly stained cytoplasm and largely weighted in most of training set. Noticed that, the "lower" pixel value and "higher" pixel value, both are relative to expected remained nuclei pixel value. In this case, this process have to be tested more times so that can get a general applicable threshold value to guarantee the most of nuclei be saved.

2. Obtain the region of interest

To get the region of interest from the original image, I, a morphological opening is applied to it. Then the

Then this image is added to the contrast enhanced image, I_{CE} as:
$$C_n = I_{CE} + H$$
The generated image is Figure 5(a)
Then C_n is dilated with a disk(X) of radius 1 to smooth the edges.
$$M_d = C_n \oplus X$$
M_d is then threshold to give the binary mask of the region of interest.
The generated image is Figure 5(b)

3. Adaptive connected component removal

The binary image obtained from last step may have small connected component and they are likely to be impurities. In the algorithm proposed by Izzati's group, the fixed value size threshold, 500, was set. The connected component with size smaller than this value will be removed with morphological opening. However, this value if defined in pixel area, which is sensitive to scale of the input image. For example, for two images with exact same components with difference resolutions, one is 512 by 512 and the other one is 1024 by 1024. The same region of interests may have different sizes, so as the impurities. To reduce the sensitivity to image scaling, we propose finding the threshold based on the statistical distribution. Instead of using the fixed size threshold, the threshold is determined based on distribution of the sizes of all the objects in the image. The sizes of the connected components in the image are obtained and sorted.

Small outlier   Nuclei   Large outlier

0   max

Small threshold   Large threshold
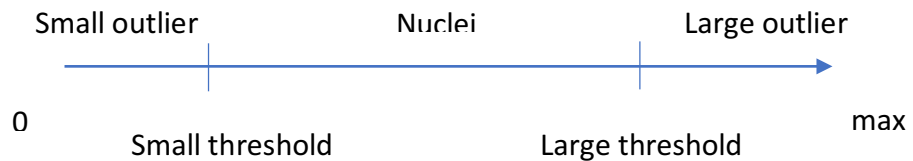
This method can also be used to remove the large impurities, e.g. the mistakenly segmented cytoplasm. The small threshold and large threshold will be given as input, for example, 20% and 10%. Areas with size distributed in first 20% and 10% will be removed.

The image before small object removal is shown as Figure 5(c) and the result of the preprocessing is shown as Figure 5(d).

2.1.2 Refinement of boundary

The result of the preprocessing is a coarse region of the nuclei. The boundaries will be refined in this stage.

1. Enhance the contrast of C_n from previous stages
2. Dilate the enhance C_n with a disk of radius 1. The result of this stage is denoted as M_d

$$M_d = C_n \oplus X$$

This stage different from the previous one as the Cn used here has been applied contrast enhancement.

3. Enhance the contrast of C_n from previous stages
4. Find the difference of the contrast enhanced image Ice and M_d. Enhance the contrast of the result image.

$$M_g = |I_{ce} - M_d|$$

The result of this step is show as Figure 4(a)

5. Threshold M_g to get the outline of the nuclei, denote as BW_2.
6. Distance based object removal

In the original pipeline proposed by Izzati's group, the outline of the nuclei will be combined with the output from the pre-process with OR operation. However, this may bring in some impurities to the result.

During the implementation, it was found out that:

1. The comparing the output of the pre-processing with the ground truth, the precision is generally over 90% while the recall is around 80%. This implies that the preprocessing procedure dose not generate much False Positive segmentation. Also, the object based precision is good. In another word, to improve the overall performance, the pixels generated by morphological edge detection, BW_2, can be selected regarding the result of preprocessing, M_d.
2. The basic idea of the selection is:
   - For any True points in BW_2, search around the same location in M_d. If there is a Ture pixel around this position, this pixel in BW_2 will be kept. Otherwise it will be cleaned.
   - The distance threshold is set to 5 pixels in x and y direction.

The result of this step is show as Figure 6(c)

7. Combine the cleaned BW_2 and preprocessing result with OR operation.
8. Apply adaptive connected component removal.

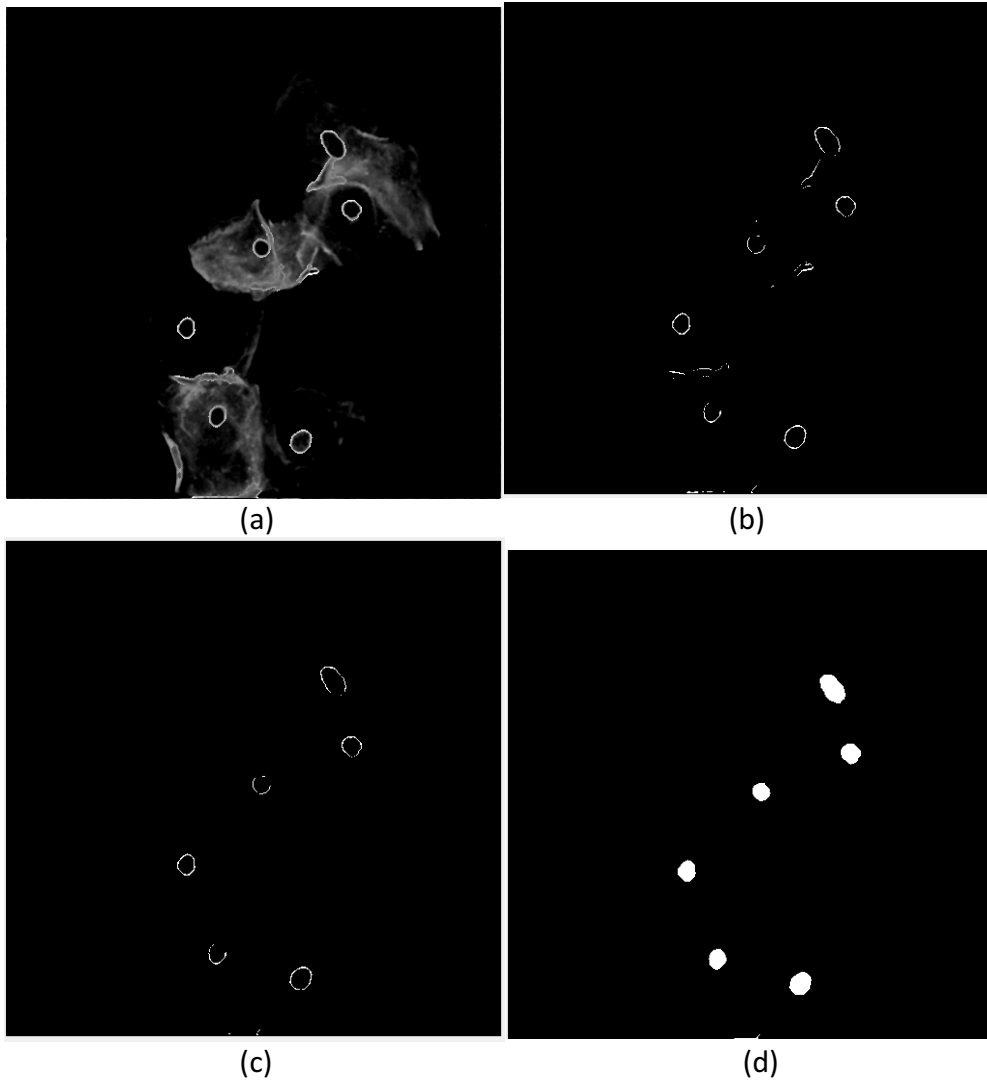The final output of this algorithm is show as Figure 6(d)

Figure 6 (a) Result of contrast enhanced morphological detection (b) Threshold edges (c) Result of distance based object removal. (d) Final output

2.2 Experiment

2.2.1 Hypothesis and Evaluation functions

The object of the experiment is:

1. Evaluated the effectiveness of the distance based object removal
2. Adjust the lower and high size threshold of adaptive connected component removal to improve the performance of the algorithm.

This project will adopt the same evaluation metrics for nuclei segmentation of the "Overlapping Cervical Cytology Image Segmentation Challenge - ISBI 2014", the criteria developed by Aksoy et al. [5]. An object can be considered as corrected segmented if and only if

$$\frac{A \cap B}{A} > 0.6$$

and

$$\frac{A \cap B}{B} > 0.6$$

where A is the segmented area generated by the algorithm and B is the pixel area provided by the ground truth.

The object based precision is calculated as:

$$Precision_{object} = \frac{TP_{object}}{TP_{object} + FP_{object}}$$

$$Recall_{object} = \frac{TP_{object}}{TP_{object} + FN_{object}}$$

$$Dice_{object} = \frac{2 * TP_{object}}{OUT_{object} + GT_{object}}$$

The pixel based performance will be evaluated with the following metrics:

$$Precision_{pixel} = \frac{TP_{pixel}}{TP_{pixel} + FP_{pixel}}$$

$$Recall_{pixel} = \frac{TP_{pixel}}{TP_{pixel} + FN_{pixel}}$$

$$Dice_{object} = \frac{2 * TP_{object}}{OUT_{pixel} + GT_{pixel}}$$

Only the correct segmented object will be used to calculate the pixel based performance.

2.2.2 Documented Data Set

The data set used for this project is the data set from the "Overlapping Cervical Cytology Image Segmentation Challenge - ISBI 2014". 45 training synthetic images, 90 testing synthetic images were provided by the challenge organizer. Both training and testing set includes ground truth images.
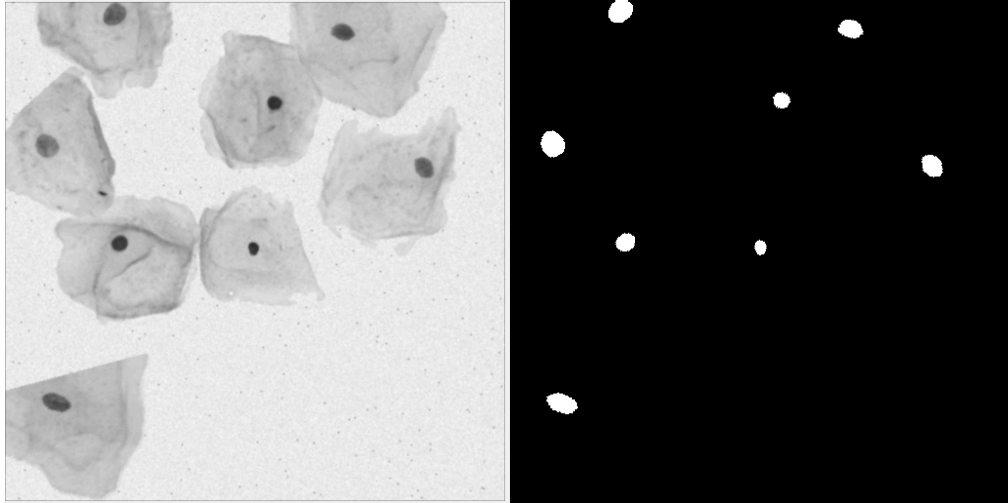
Figure 7 A example of the data set. Synthesis image(left) and ground truth(right)

2.2.3 Experiment procedures

The parameters need to be tuned is listed as follow:

threshold_low: the lower bound of the distribution where the areas with size distributed lower than this area will be removed.

threshold_high: the higher bound of the distribution where the areas with size distributed higher than this area will be removed.

distance: The distance threshold of the distance based object removal. For an edge pixel in BW2, search around the same location in Md (the binary image generated by the preprocessing). If there is a Ture pixel in Md around this position, this pixel in BW2 will be kept. Otherwise it will be cleaned. 'distance' is the parameter to define the size of the neighborhood.

The experiment will be carried out in the following procedure:

1. Hold the *distance* and *threshold_low* to 5 and 0.3. Test the algorithm on train set with *threshold_high* of 0, 0.1, 0.2, 0.3, 0.4. These values were select based on some general tests during the implementation.

2. Hold *threshold_high* to the best values found from the previous steps. Hold distance to 5. Test the algorithm on train set with *threshold_low* of 0, 0.1, 0.2, 0.3, 0.4. These values were select based on some general tests during the implementation.

3. Hold *threshold_low* and *threshold_high* to the best values found from the previous steps. Test the algorithm on train set with *distance* of 0, 2, 4, 6. These values were select based on some general tests during the implementation.

4. Apply the algorithm to the train set twice, once without distance based object removal and once with distance based object removal. Compare the result. For this step,

threshold_low, threshold_high and distance will be hold to best parameter values found from the previous stages.

5. After all the best parameter values are found from the previous stages, apply the algorithm on the train set. Also, apply the baseline algorithm to the test set.

6. Apply the best model on test set

3. Results

	hi = 0	hi = 0.1	hi = 0.2	hi = 0.3	hi = 0.4
obj_mean_dice	0.5095	0.4305	0.2729	0.1860	0.1147
pxl_mean_dice	0.4006	0.3270	0.1920	0.1215	0.0677
obj_precision	0.0000	0.0000	0.0000	0.0000	0.0000
pxl_precision	0.0000	0.0000	0.0000	0.0000	0.0000
obj_recall	0.4342	0.3476	0.2023	0.1292	0.0768
pxl_recall	0.0000	0.0000	0.0000	0.0000	0.0000

Table.1 evaluation result of procedure 1

According to Table 1, removing any large size object during the adaptive connected component removal will degrade the performance of the algorithm seriously. As a result, during the connected component removal procedure, only small objects will be removed and *threshold_high* is an invalid parameter for later stages.

	lo = 0.001	lo = 0.1	lo=0.2	lo = 0.25_a	lo = 0.25 _b	lo = 0.3	lo = 0.4
obj_mean_dice	0.8018	0.8065	0.8317	0.8411	0.7325	0.8432	0.8332
pxl_mean_dice	0.7734	0.7749	0.7810	0.7823	0.6773	0.7795	0.7746
obj_precision	0.7336	0.7424	0.7910	0.8208	0.6634	0.8477	0.8898
pxl_precision	0.8834	0.8832	0.8827	0.8834	0.8862	0.8846	0.8866
obj_recall	0.9041	0.9018	0.8947	0.8794	0.8625	0.8529	0.7995
pxl_recall	0.9494	0.9499	0.9519	0.9541	0.9544	0.9547	0.9614

Table.2 evaluation result of procedure2 & 4*
(lo = 0.25_a is the result with distance based object removal)
(lo = 0.25_b is the result without distance based object removal)

The result of Step 2 in recorded in Table 2. During the experiment, it was found that low_threshold = 0.3 has the best object based Dice coefficient while low_threshold = 0.2 has the best pixel based Dice coefficient. low_threshold = 0.25 was added to the experiment and it has the best performance in terms of pixel and object based Dice coefficient.

	baseline	d = 0	d = 2	d = 4	d = 6
obj_mean_dice	0.7925	0.8390	0.8567	0.8442	0.8392
pxl_mean_dice	0.7641	0.7969	0.8028	0.7902	0.7777
obj_precision	0.7286	0.8151	0.8325	0.8225	0.8190
pxl_precision	0.9928	0.9397	0.8847	0.8842	0.8828
obj_recall	0.8913	0.8829	0.9011	0.8849	0.8769
pxl_recall	0.8071	0.9162	0.9553	0.9553	0.9551

Table.3 evaluation result of procedure 3

low_threshold was fixed to 0.25 for procedure 3. d=2 has the best performance among all the parameters.

The final parameter for this model is:

threshold_low = 0.25
threshold_high N/A
distance = 2

Apply this model with obtained parameter on test set, the result is:

Dice_object = 0.8433
Dice_pixel = 0.7736
Precision_object = 0.8817
Precision_pixel = 0.8331
Recall_object = 0.8215
Recall_pixel = 0.9506

4. Discussion

The result of the first step indicated that, for this dataset, it is not appropriate to remove the outlier objects in terms of size. Though the final object based precision is 0.8325, some impurities was segmented as the nucleus. If their size is distributed at right tail of the distribution, they should be removed by the large connected component removal procedure. However, the result turned out that their size is close to the nucleus.

The result of the second step of the experiment indicates that a large portion of the False positive objects are small impurities. As increasing the threshold of the small connected component removal, the object based precision is increasing. The trade of increasing this threshold is the model failed to recognized some of the nucleus because object recall is negative correlated to this parameter. To leverage these two metrics, the major metric for the following experiments will be Dice coefficient.

Comparing the result using and not using distance based object removal in Table 2, a conclusion is that, distance based object removal can bring significant improvement to the model. Both object and pixel based Dice coefficient has an improvement over 10%. The best parameter for this procedure is 2 pixels, which indicates that most of the edge pixels generated by morphological edge detection is about 2 pixels away to the area generated in the preprocessing

stage. This size may have some relationship with size of the dilation kernel used to generate the edge pixels. The size of the kernel is 2.

The result of the baseline algorithm is
        Dice_object = 0.7925
        Dice_pixel = 0.7641
Both less than the algorithms developed in this project.
The testing result on test set is close to the test set. This indicates that the model is not overfitting with the test set.

	Precision (Object)	Recall (Object)	Precision (Pixel)	Recall (Pixel)	ZSI (Pixel)
Dani	0.959	0.895	0.968±0.055	0.871±0.069	0.914±0.039
Masoud	0.903	0.893	0.901±0.097	0.916±0.093	0.900±0.053
Lu	0.977	0.883	0.942±0.078	0.912±0.081	0.921±0.049

Table 4 Result of Challenge [4]

Table 4 is the Top 3 results of the challenge [4]. This result can only be used as a reference as this was generated with a test set without ground truth given in public. The object base precision of our algorithm is 0.8817 and the recall is 0.8215, less the Top 3 performance. The pixel precision of our algorithm is 0.8331, about 10% less than the top results, which indicates that our algorithm tend to over segmenting. Our pixel recall is 0.9506, outperformed the top 3 results.

Conclusion

In this project, we have implemented an adaptive morphological segmentation method which was originated from a morphological segmentation to join in a cervical nuclei segmentation challenge on-line, after evaluating this approach, the segmented nuclei via using this algorithm showed an expected result which is compatible with on-line submitted good result. For the future work, find a better and more general approach to remove large detected garbage can significantly improve dice result and object_precision.

References

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Program Documentation

Program Code