

# SEGMENTATION SYSTEM TUTORIAL

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## INTRODUCTION

### SYSTEM STRUCTURE

The system aims to segment microscopy images based on a supervised statistical modeling method. The general idea is to train a statistical template in terms of the shape and texture of target object structures by utilizing input sample images. This template will be used in the segmentation of a given test image by maximizing the normalized cross-correlation between the template and the test image. The ultimate goal of this system is to achieve the broad application in segmentations in multiple types of microscopy images. The entire system can be divided into two main parts: the training part and the test stage.

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#### TRAINING: ESTIMATING A STATISTICAL MODEL

In the training stage, we manually choose several typical textures from a set of sample images; by learning the shape variations as well as the mean texture, we create a mean template. The normalized cross-correlation (NCC) method is used to learn the mean model by maximizing the correlation confidence among the chosen templates. Finally, a principle component analysis (PCA) is conducted to create multiple shape variations.

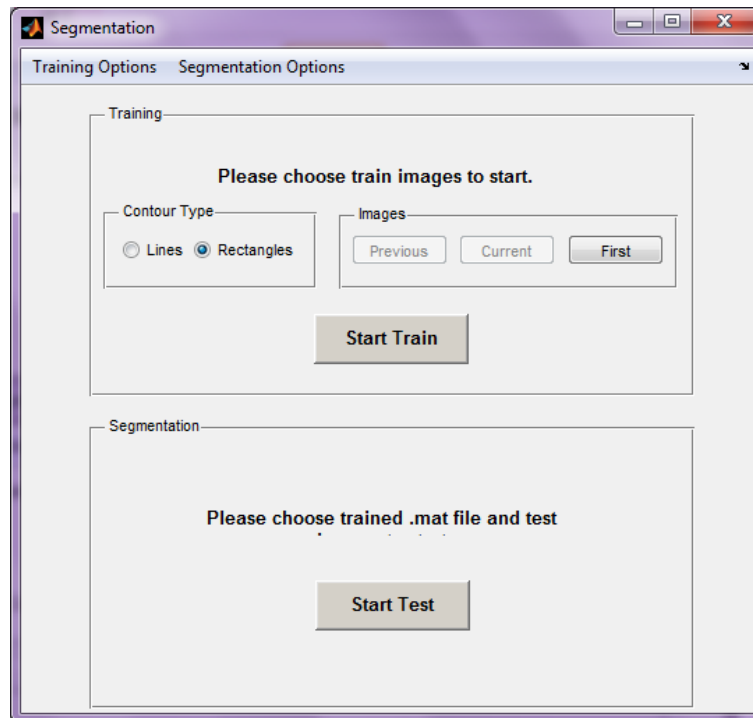
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#### TESTING: DETECTION AND SEGMENTATION

There are two primary parts involved in the test section: (a) detecting the structures of interest and potential segmentation for a given test image and (b) accurately performing the counter-detection to perfectly match the edges of the objects by maximizing the NCC between the rough detection area and the real targets.

### USER GUIDELINES

The GUI of this system integrates the training and testing stages to provide a comfortable user experience. The training stage is located in the training panel at the top of the GUI; the testing stage is located at the bottom (called “Segmentation”). Detailed instructions about how to use this system will be discussed in the following tutorial.



## TRAINING

### STEP 1: CHOOSING TRAINING IMAGES

There are two ways to import training images: by folder or by the file select dialog box, which enables multiple selections at the same time.

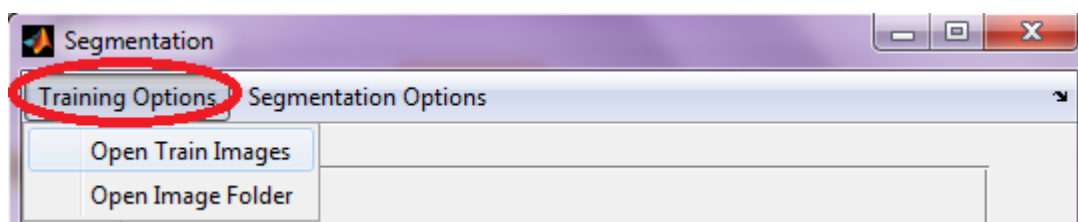
#### Open “Training Options” to load training images

- Click “*Open Train Images*”

This option enables you to choose to load the training images via the file dialog window. It also supports multiple selections.

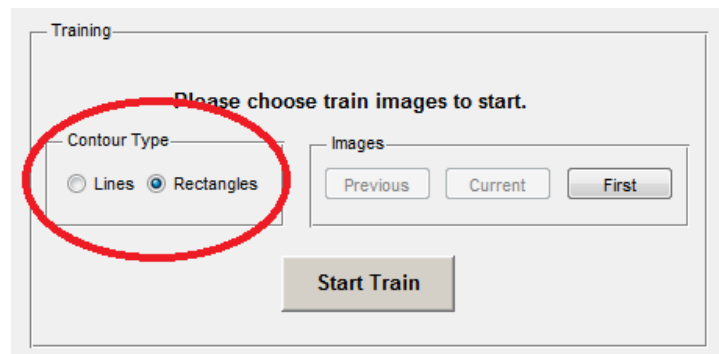
- Click “*Open Image Folder*”

This option enables you to import training images by folder, which makes the operation much easier when the training dataset is large.



### STEP 2: SET UP CONTOUR TYPES

You should be able to see the “Contour Type” configurations in the training panel (shown below).

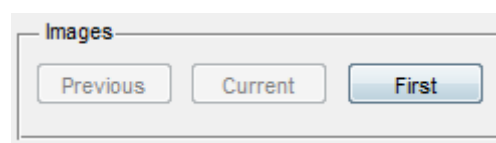


Selecting the “Lines” option enables you to draw line contours of objects you want to extract; the “Rectangles” options provides rectangle contours for all objects. If you do not have very strict requirements in terms of the accuracy of the contours, the rectangle contour option is recommended as it facilitates the cropping patches process much more effectively than lines. For more detailed information about how to crop patches from the training images, please refer to the following instructions.

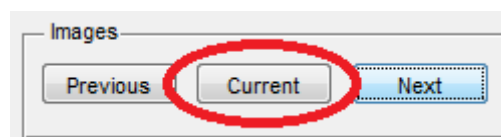
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### STEP 3: NAVIGATE TRAINING IMAGES

After choosing the contour type, you are ready to launch the training images to crop patches. You can navigate the images by the clicking the control buttons. When you initially load the training images into the system, you are only able to select the first image in the queue.



Once you begin reading images one by one, the other buttons (i.e., “Previous” and “Next”) are enabled. You can always to view the current image again by clicking the “Current” button.



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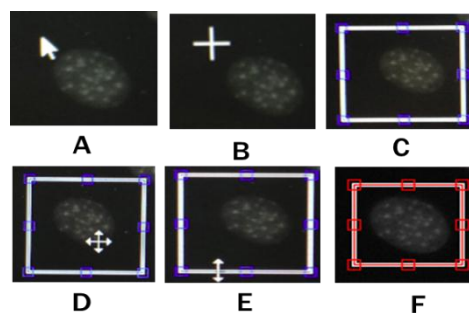
### STEP 4: CROP PATCHES FROM TRAINING IMAGES

After launching a selected training image, the cursor is originally presented in arrow form. Left-click any figure area to change the cursor to a cross symbol, which means you are currently in the working model and can crop the patch. Please note that the

centers of previously cropped patches are marked with a “red cross symbol” to avoid over-cropping the same patch.

## Rectangle Contours

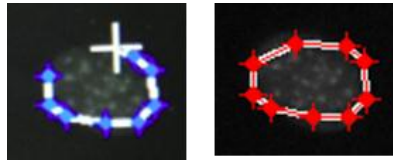
If you are using the “Rectangles” option to draw contours, left-click the area you want to extract, then stretch the rectangle to cover the object. If you want to move the rectangle, place the mouse on the rectangle area to move it. You are also able to resize the rectangle by stretching the edges when the mouse turns into the double-direction arrows. Double left-click to confirm your drawings. The rectangle becomes red instead of white, and you can no longer make any updates. Now the cursor becomes an arrow (i.e., back to the original state). If you want to crop another patch, you need to repeat the procedures accordingly. To stop the cropping, press the “Enter” button. Please note that you should not use the “Close” icon in the upper right corner of the figure to exit the cropping model as it will not store your training results. However, if you make a mistake or want to discard the current cropping, then you can use the “Close” icon to exit the current model. There probably will be an error message showing in the Matlab history panel. Just ignore it since it won’t influence the use. Then please click the “Current” button in the image navigation panel to re-start the cropping.



(A) Initial model. (B) Left-click to activate the working model (C) Draw a rectangle (D) Move the rectangle (E) Resize the rectangle (F) Double left-click to confirm

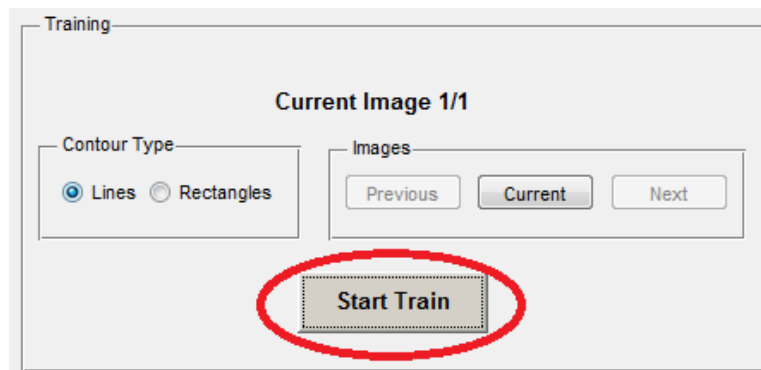
## Line Contours

Drawing contours with lines follows almost the same procedures as rectangles. The only difference is the drawing. When cropping the patches using lines, you need to place each point to line them up with an enclosed shape. You do not need to connect the starting and end points as the system will automatically close the shape once you double left-click to confirm the drawing. Press the “Enter” button to stop the cropping. Similarly as before, if you make a mistake or want to discard the current cropping, you can use the “Close” icon to exit, and then click the “Current” button to re-start the cropping procedure. Please ignore the error message when doing this since it won’t influence the segmentation result.

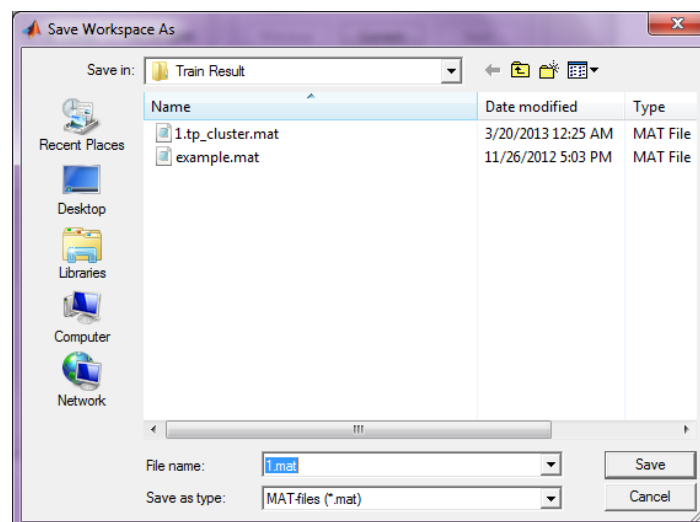


## STEP 5: START THE TRAINING

After completing the entire patch-cropping process, we are ready to start the training by clicking the “Start Training” button in the Training Panel.



A dialog box will open, asking you to name your training result file and indicate where to store it. The training result is stored in “.mat” format. Please be patient as the training period generally takes a while depending on how many patches you cropped.



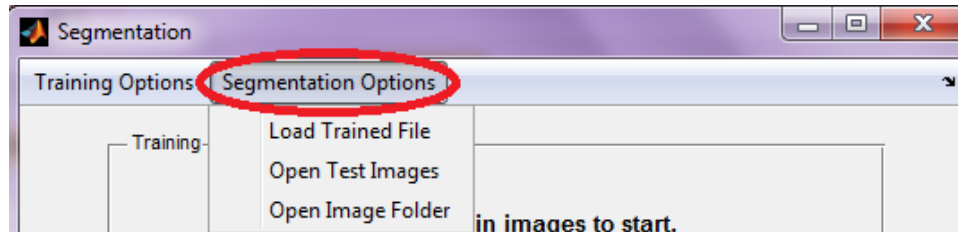
## SEGMENTATION

The segmentation stage is also known as the testing stage, as previously mentioned. In this period, you will use the training file to segment your datasets.

## STEP 1: CHOOSE A TRAINING RESULT

- Open “Segmentation Options”
- Click “Load Training File” to load the training result

The training file will be in the “.mat” format only, as obtained during the training stage.



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## STEP 2: IMPORT DATASET

There are two ways to import datasets: choose the images by the folder or use the file select dialog box, which enables multiple selections at the same time.

### Open “Segmentation Options” to load test images

- Click “Open Test Images”

This option enables you to choose to load test images via the file dialog window. It also supports multiple selections.

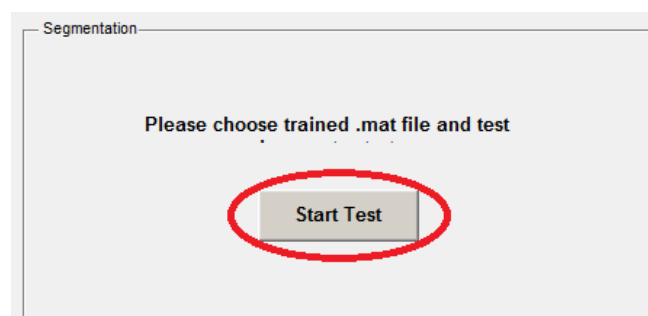
- Click “Open Image Folder”

This option enables you to import test images by folder, which makes the operation much easier when the dataset is large.

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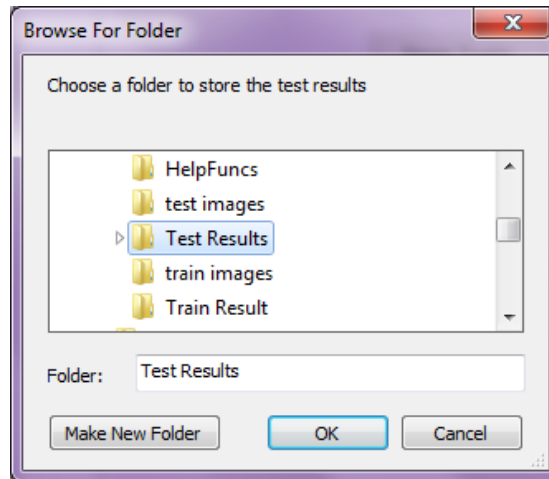
## STEP 3: START SEGMENTATION

Now press the “Start Test” button in the Segmentation Panel to start the segmentation.



The system will ask you to select a folder in which to store the segmentation results.





It will take some time for the system to process the segmentation. If possible, you should stop other applications to speed up the testing period.

## RESOURCES

You can find many other useful materials in the following information.

### *Article*

C. Cheng, W. Wang, J. A. Ozolek, G. K. Rohde, A template matching approach to segmenting cells and nuclei from microscopy images, to appear, Cytometry A, 2013.

### *Official Website*

<http://www.andrew.cmu.edu/user/gustavor>