PART 1 – QUICK START

I. Locate Your Images:

The first thing to do in Yeast-Analysis after you acquire your images is to specify the directory where the images are located.

I/O tab → Inputs → Browse → Choose folder → Open. (Figure 1)

You need to make sure the images are either lsm or tif images and they follow the naming convention used by Yeast-Analysis (check section: Naming Conventions).

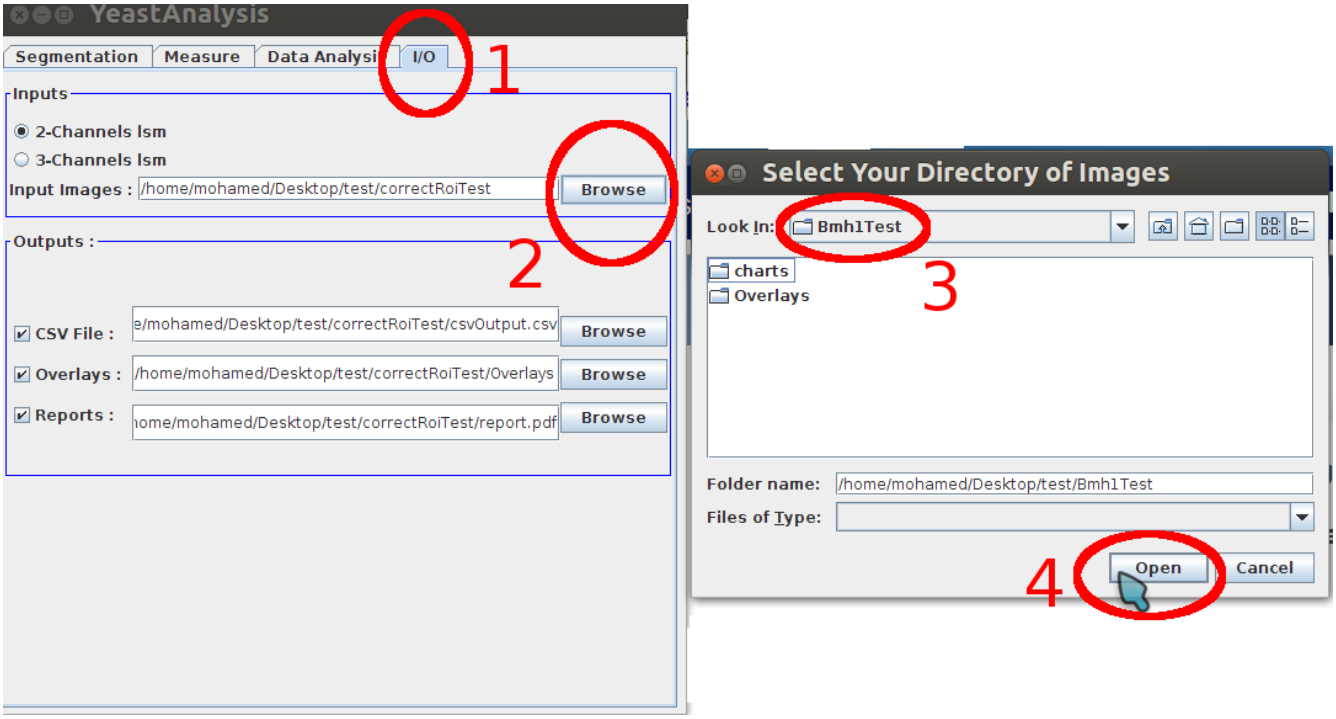


Figure 1 – Selecting Your directory of images.

II. Segment the Images:

Now you need to perform the segmentation step (locate the individual cells in images to be measured), Click the "Segmentation Tab" (Figure 2 -1), Choose the parameters for the segmentation process by clicking on "Settings" (Figure 2 – 2) or use the default values (Figure 2 – 3) and then click the "Segment" button (Figure 2 - 4).

During the segmentation process, you can wait the process to be done, or View the already segmented images, by clicking on "View Segmented Images" button.

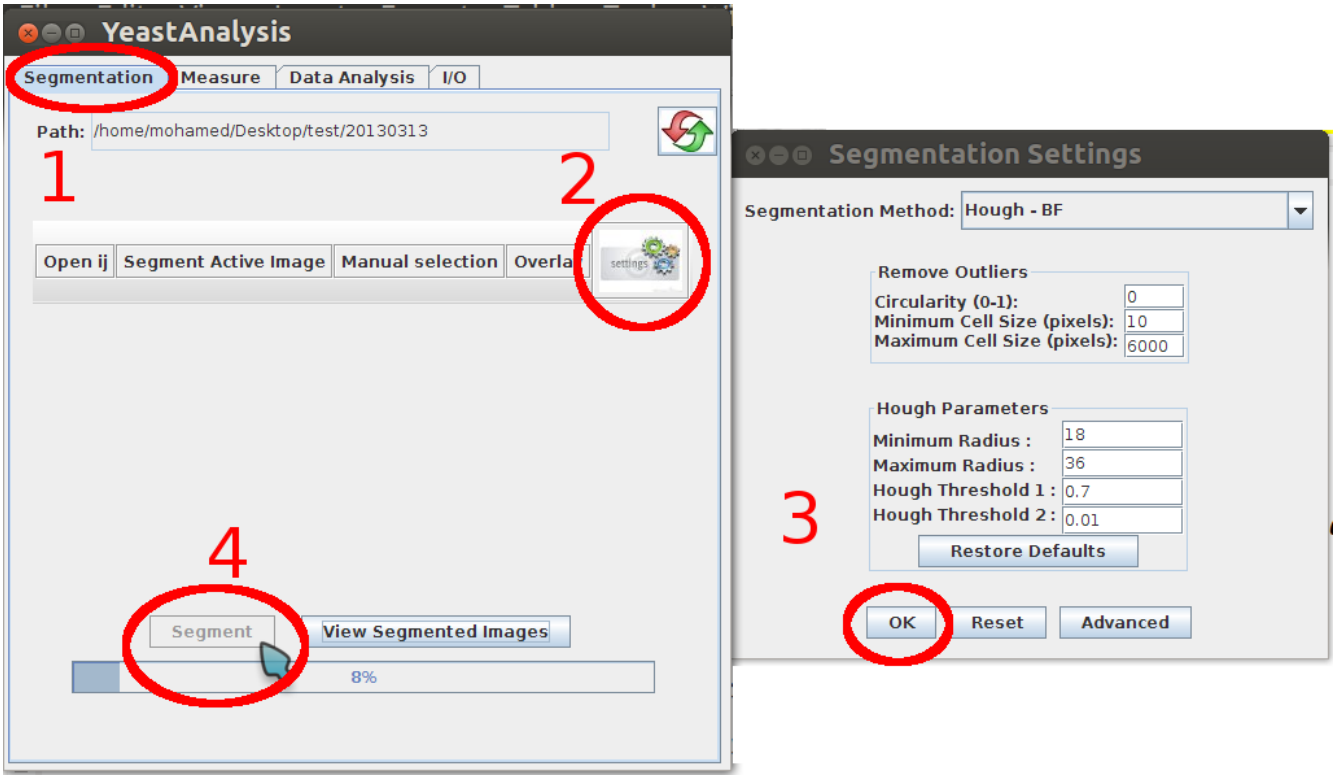
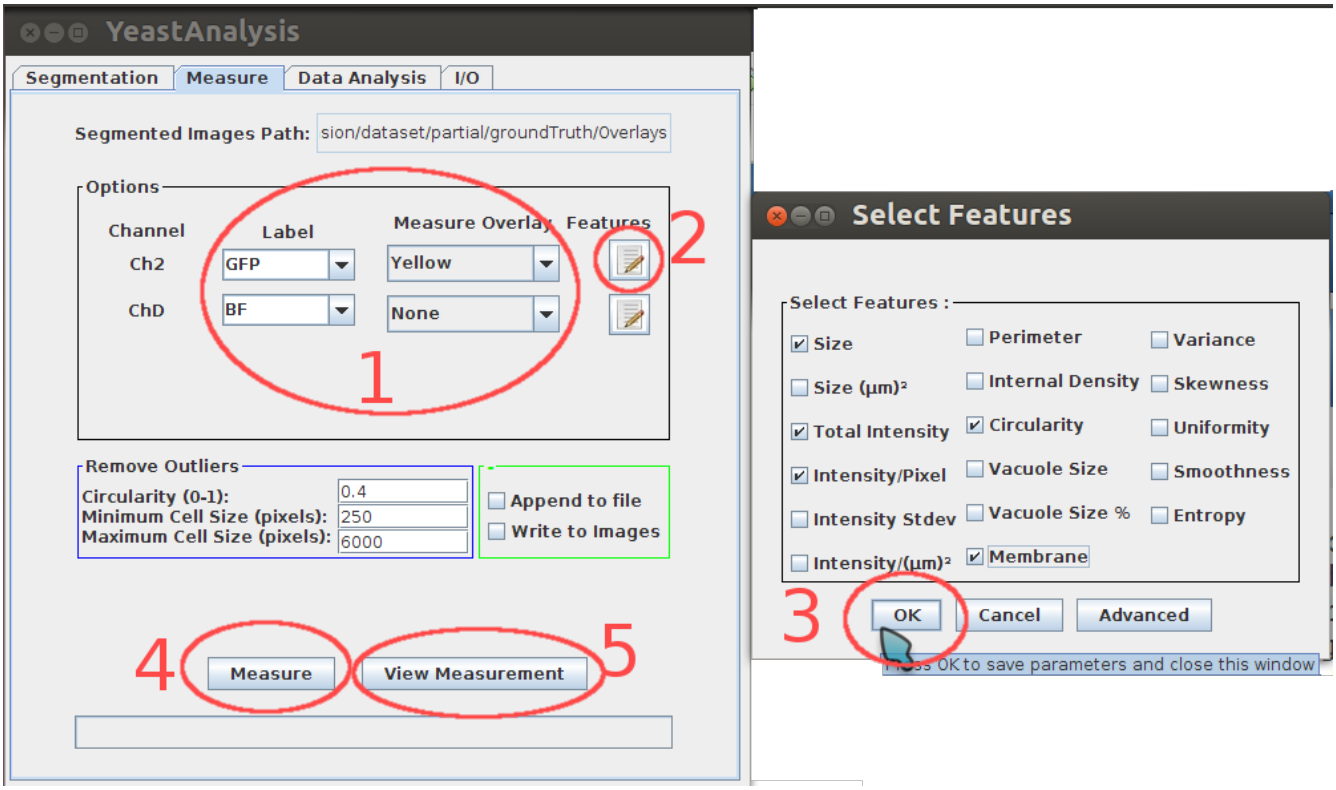


Figure 2 – The Segmentation Process.

III. Measure the Cells:

When the Segmentation is completed, you want to measure the individual cells for several features. Here you need to access the "Measure Tab" (Figure 3) and Choose the Channels you want to measure (Figure 3 -1) and the features you want to compute (Figure 3 – 2,3). And click on the "Measure" button (Figure 3 - 4).

After the Measurement is done, you can view them in Excel by clicking the "View Measurement" button (Figure 3 -5).



IV. Analysis

Now you might want to perform an automatic analysis for the measurement file. For this, click the Data Analysis Tab, and Choose the Keywords of the Cell groups you would like to compare. Choose also the type of charts you would like to generate for what features, then Click the "Analyze" Button. When the analysis process is done, click the "View Report" button to view your statistics in a pdf file.

PART 2 – User's Guide

I. Before You Start: Preparing Your Images:

A. Naming Convention

Yeast-Analysis follows a special naming convention of the images to realize the different groups that we would like to compare. This naming convention is as follows:

Unique-group-name_ImageNumber.

For example, if we have two group of cells one representing cells with Nha1 protein attached to GFP in a control wildtype medium, and another in NaCl medium. The Image names would be similar to this:

Nha1-GFP-control_01	*Nha1-GFP-NaCl_01*
Nha1-GFP-control_02	*Nha1-GFP-NaCl_02*
…...	*…...*
Nha1-GFP-control_XX	*Nha1-GFP-NaCl_XX*

it is important to notice that Yeast-Analysis uses the Underscore symbol to recognize the group names.

B. Folder Structure

All the Images should be placed under one directory. The Segmentation results typically goes under Overlays folder under the parent directory, the csv file and pdf report typicall goes under the parent directory along with the images.

II. Configure I/O directories:

A. Choosing the Input directory:

The aim of this application is to measure a set of images automatically; hence the first step is to choose a directory that contains the images to be measured. To choose your input directory go to the I/O tab, and for the Input Images textField, browse for your folder.

1. *I/O tab → Inputs → Input Images → Browse*

Under the same I/O tab.

B. Choosing the Output directories:

In the I/O tab; three output directories can be chosen, since the outputs of the application are a measurement file in CSV format, Outlines of the cells, and Overlays on the original images showing the location of the cells and there nucleus if presented. Note that Choosing an input Directory will automatically change the default output directories, and hence you have to change the output directories after choosing the input directory first (Currently changing the output directories is not functioning, and the CheckBox-es also are not working (i.e. Output is done automatically without user prompt)).

III. Segmentation:

A. Choose the Segmentation Algorithm

Since images are acquired under different conditions, different segmentation methods might perform better that the others; Under the segmentation tab, there are currently five different segmentation methods available:

1. *FS images*
2. *BF images*
3. *Sobel BF images*
4. *Hough-BF images*

5. *Manual segmentation*

B. Change Segmentation Settings

Under the Segmentation Tab, You can find the Settings button which allows setting a number of parameters related to the segmentation process. The first of these is the threshold parameter typically setting the value to 2.75 performed well on most of the images we tested.

Another setting is the outlier values; you can specify the minimum circularity value of the detected objects. And the minimum and maximum size in pixels for those objects.

C. Detecting Individual Cells

In case you have a cell that is not detected during the automatic process. You can choose "Single Detection" button. Note that this function works well only under bright field images. The image must be open using the embedded imageJ instance (Open imageJ) on the Segmentation Tab.

D. Segmenting An Open (Active) Image.

In case you would like to segment an individual image that is open on the screen, you can try the "Segment Active Image" button. The image must be open using the embedded imageJ instance (Open imageJ) on the Segmentation Tab.

E. Use the Interactive Segmentation

In case you would like to interactively segment the images and view the results directly, you can try the "Interactive Segmentation" method under Segmentation Method combo box in the Settings Dialog box.

Segmentation Tab → Settings → Segmentation Method → Interactive Segmentation → Segmentation Tab → Segment.

IV. Measurement

A. Configure Measurement Options

B. Selecting the measurement Features:

Now that the images are already segmented, we can start working with the measurement part under "Measure" tab. First we have the "Measure Options" Dialog; here, we can choose the channel that we would like to measure, In case we choose more than one channel, it would be better to choose a label prefix for each channel, to distinguish between the measured attributes in the measurement file. Setting this to "None" will label the features without any prefix. The Overlay Color option allows choosing different overlay colors to be measured. For example, if the cell segmentation were marked with a yellow overlay, and the nucleus with a cyan overlay. Having fluorescent signal of the cell cytoplasm under channel 1 and the nucleus signal under channel two. Then we can choose to measure Ch.1 and overlay color : yellow , and channel two with overlay color: cyan. Next comes the selection of features to be measured for each channel. By clicking the Edit button, you get a dialog box with the features available to be measured.

Moreover, under the Measure Tab you can choose the Outliers to be removed: you can specify the circularity threshold in which all the detected objects that has a circularity value below this threshold will not be included into measurements. Moreover, you can input the minimum and maximum cell size (area) in pixels. (Note that this step can also be performed during the segmentation process).

C. Measure Images and View Results

After setting all the parameters for the measurement process. You can click the "Measure" button under the "Measure" Tab. When this process is done , you can view the results by clicking "View Measurement". View Measurment automatically transform the csv file into an xls file so that the user can easily work with, in a SpreadSheet application. Saving the xls file will automatically save into the csv file too.

VI. Data Analysis & Visualization:

A. Fetching Keywords.

When you click on the data analysis tab, the keywords are automatically fetched from the csv file. These keywords represented the different cell groups that were detected. You can click the edit button and choose what cell group needed to be compare to what cell group. For example comparing Nha1-GFP-control vs Nha1-GFP-NaCl and Bmh1-GFP-50mM vs. Bmh1-GFP-200mM. In case you only have two cell groups, you don't have to change the keywords, otherwise, you can click the edit button and choose the different groups to be compared.

B. Size vs. Intensity analysis

if you choose this option for analysis, this will generate a scatter plot with the intensity values on the y-axis and the size on the x-axis. For this option, you need to make sure the features "size-micron" and "total intensity" were measured.

C. Select Features to analyze.

To measure an individual feature, you can select what feature you want to analyze and choose the type of chart you would like to visualize your results in. For example choosing the Feature Total Intensity to be measured and the chart type : Box plot, will generate a box plot diagram visualize the different group of cells.

D. Report Generation & Viewing.

When you are done with parameter setting, you can click analyze, and a pdf report file will be automatically generated, you can click view report to open the pdf file in your default pdf reader. Make sure to save your report to another location before your next analysis.

VII. Resetting the Application

Under the segmentation Tab, there is a Reset button, this will simply restart the application.

VIII. The properties File

User choices and several property values related to the application are automatically saved into a properties file "ya_prop.txt" which is created under the same location of the executable jar file of Yeast-Analysis.