Yeast Analysis Manual

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1. SPECIFYING DIRECTORY LOCATION

The first step in using this software is to specify the path for the directory of the images to be measured. From the IO tab, Browse for path of your images(cf. Fig. 1)

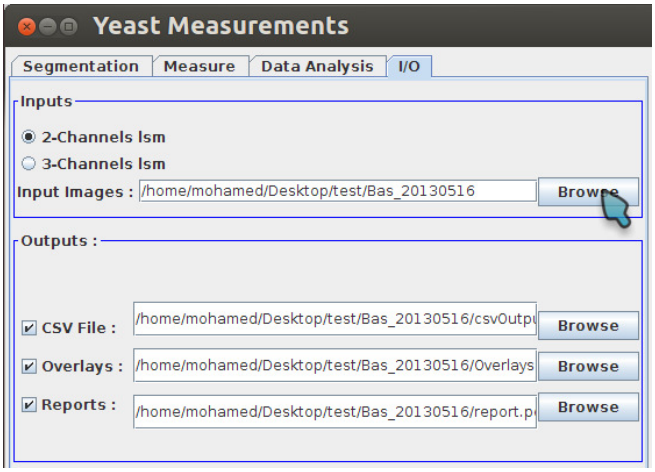

Figure 1. Choosing the path of images directory

2. SEGMENT IMAGES

The next step is to choose a segmentation method, and perform the segmentation. This process is done by accessing the Segmentation Tab, choose the appropriate segmentation method and press on "Segment" (cf. Fig. **??**). During the Segmentation, you can click on View Segmented Images. You can open an instance of imageJ from the application by pressing the "Open ij" button to view the segmented images.

2.1 detecting single cells

If some cells are not detected, you can press the "Single Detection" (test?) (an image must be open). and then press inside the cell to detect its boundaries (works on bright-field images of yeast cells)

2.2 Working with Image Overlay

In order to add the detected contour to the image Overlay, you can use the imageJ functionality (Ctrl+B). Or simply use the "Overlay" Button in the segmentation tab which automatically adds a cell to the overlay upon clicking inside a cell. To delete an ROI (cell contour), the easiest way is to use imageJ functionality by pressing the middle mouse button over the ROI and clicking "Backspace".

2.3 Saving Image

After adding and removing cell contours to the image, make sure to save it in the same folder where automatic segmentation images were created (by default foler name is Overlays)

3. MEASUREMENT

After segmentation, click on the measure tab, choose the attributes to be measured. Make sure to measure the appropriate features that you would like to visualize later. For example, choose the features : label, area, intDensity to generate area vs. intensity chart later.

3.1 Image naming convention

This system assumes that the images belonging to the same group has a unique name separated from a number by underscore (the first underscore is considered, so it is better to use only one underscore in image names). For examples to compare wildtype images of Bmh1-GFP against mutants. A working image nameing convention is $BY-Bmh1-GFP_xxandbmh1-GFP\_xx$ where xx is the image number. In this case, the software is able to distinguish between two groups BY-Bmh1-GFP vs. bmh1-GFP.

3.2 Viewing measurement

you can press "View Measurement" to view your measured data in SpreadSheet appliation (.xls file). you can also modify your data and save back in the same path. The software itself deals with the measurement file in CSV format. that is, It originally creates CSV file and creates a XLS copy for the user to work with. Later, when the software needs to analyze the measurement, it copies the XLS file back into CSV file.

4. REPORT GENERATION

After doing the measurement, Go to "Analyze" Tab to generate a report visualizing your measurement. The analysis compares groups in keyword1 field against those in keyword2 field. The keywords are automatically generated from the measurement file. However a user can manipulate them by writing Regex expressions.

4.1 Manipulating Keywords

You can compare groups against each other by specifying the image names as regex expression according to convention mentioned previously. to compare more than one pair, you can separate the expressions by semicolon.

For example the following values:

$Keyword1 = GFP-NHA1-0.5MNaCl\_\d.*; bmh1pUG34GFP-NHA1\_\d.*; Keyword2 = BY4741GFP-NHA1-0.5MNaCl\_\d.*; BY4741pUG34GFP-NHA1\_\d.*;$

would compare GFP-NHA1-0.5MNaCl against BY4741 GFP-NHA1-0.5MNaCl and bmh1pUG34 GFP-NHA1 against BY4741pUG34 GFP-NHA1.

4.2 Choosing Charts

You can choose the chart to be created into your report, currently you can create size vs intensity scatter plot showing the cell areas on the x-axis and intensity values on the y-axis. You can also choose a chart for any single attribute. The available attributes are automatically fetched from the measurement file.

4.3 generating the report

You can simply click on the "Analyze" button, and then after the analysis is done you can view the pdf report by clicking "View Report".