YeastAnalysis: An image analysis platform to quantify expression levels of GFP-tagged proteins in *Saccharomyces cerevisiae* cells.

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Abstract

Fluorescent protein-tags like GFP are nowadays widely used in yeast research. In order to use an image-based readout to study the levels and localization of individual proteins, methodology is needed allowing routine quantification of observations in images. This requires a certain workflow - a protocol of actions - and we have been developing image analysis software typically geared for yeast research (YeastAnalysis). The YeastAnalysis software follows a standard workflow starting with a segmentation of the yeast cells from the images. Next, each individual cell is characterized by a panel of features such as surface area, fluorescence intensity and texture. From the features a report is automatically generated including chart visualizations. With such visualizations two different strains can be compared: i.e., mutant vs. wildtype, or low salt concentration vs high concentration. The reporting also includes basic statistical analysis.

Image Acquisition

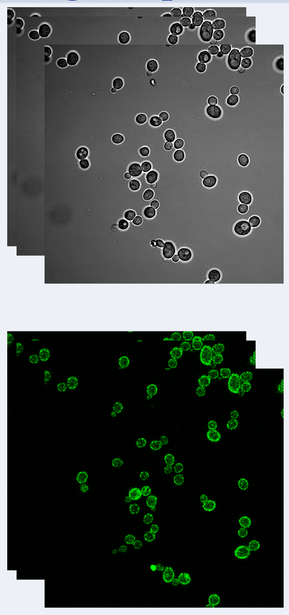
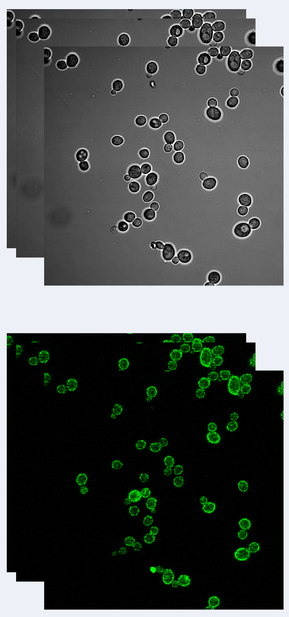
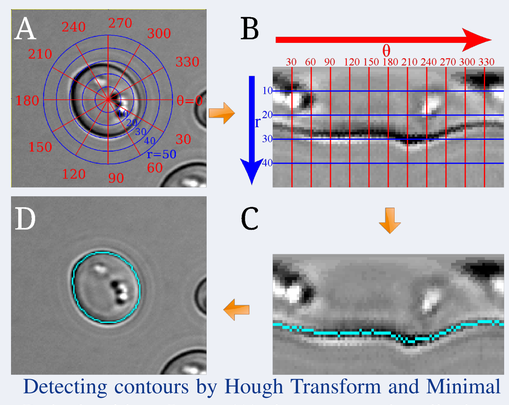


Image Segmentation



- Detecting Individual Cells in Images:
  - In general:
    - An Algorithm based on Hough Transform and minimal Path algorithm.
  - If contours are well highlighted:
    - Method based on Sobel Edge Detection Algorithm.
  - If Protein well distributed throughout the Cell:
    - Method based on Sigma Filter and Triangle auto-threshold.



Detecting contours by Hough Transform and Minimal Path algorithm. Image (A) shows a sample cell where the center point is detected by Hough Transform. In (B) a parametric plane is generated. The columns corresponds to the pixels along the radius of the largest possible circle (red lines). The rows corresponds to the circles surrounding the center point (blue lines, blue circles in image A). Image (C) shows the detected path from the first column to the last column in the parametric plane, after the application of dynamic programming. Image (D) shows the detected path as the actual contour of the cell.

Measurement

- Individual Cells can be measured for several features:
  - Size, Fluorescent Intensity, Perimeter, Textures (Histogram Variance, Histogram Skewness, Smoothness, Uniformity, Entropy)
- Saved to CSV and XLS files.

Label	Area (µm²)	Mean Fluorescence	Fluorescence Total Intensity	Membrane Area	Membrane Total Intensity
bmh1 Nha1-GFP_04.tif:Cell 1	11.25	8.39	10,868	5.04	6,049
bmh1 Nha1-GFP_04.tif:Cell 2	12.91	10.49	15,604	5.40	8,520
bmh1 Nha1-GFP_04.tif:Cell 3	16.03	11.12	20,528	6.25	10,756
bmh1 Nha1-GFP_04.tif:Cell 4	11.37	9.24	12,091	5.19	6,766
bmh1 Nha1-GFP_04.tif:Cell 5	12.10	9.51	13,247	5.32	7,040
bmh1 Nha1-GFP_04.tif:Cell 6	14.67	11.92	20,140	5.79	9,585
bmh1 Nha1-GFP_04.tif:Cell 7	12.22	9.55	13,438	5.31	6,737
bmh1 Nha1-GFP_04.tif:Cell 8	9.84	8.52	9,653	4.72	5,165
bmh1 Nha1-GFP_04.tif:Cell 9	9.57	8.64	9,526	4.64	5,305
bmh1 Nha1-GFP_04.tif:Cell 10	10.24	7.83	9,232	4.68	5,284
bmh1 Nha1-GFP_04.tif:Cell 11	11.39	7.89	10,349	5.13	5,249
bmh1 Nha1-GFP_04.tif:Cell 12	9.94	9.58	10,961	4.72	6,356
bmh1 Nha1-GFP_04.tif:Cell 13	9.22	11.20	11,891	4.56	6,358
bmh1 Nha1-GFP_04.tif:Cell 14	11.71	9.43	12,714	5.26	6,781
bmh1 Nha1-GFP_04.tif:Cell 15	9.31	9.07	9,721	4.52	5,476
...	...	...	...	...	...

Data Analysis

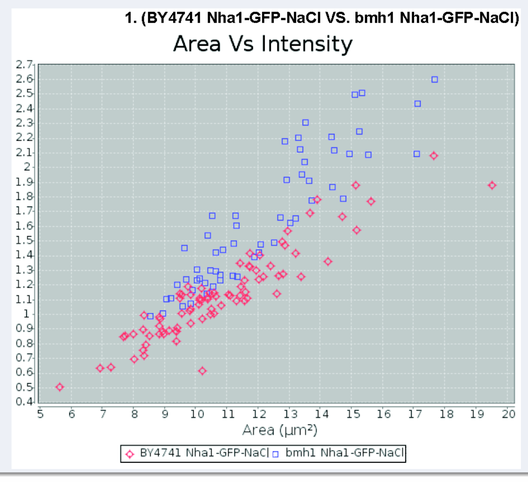
- Basic Statistical Analysis
  - Count, Mean, Median, Standard Deviation.
- Student t-test Analysis.
- Remove Outliers (Size Range, Circularity Constraints).

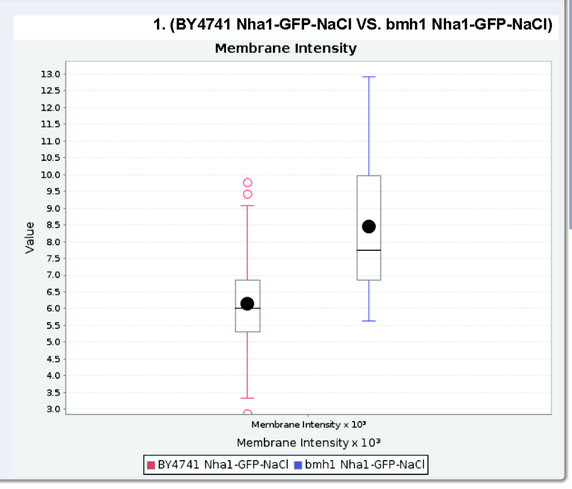
Basic Statistics		
	bmh1 Nha1-GFP-NaCl	BY4741 Nha1-GFP-NaCl
Count	57	84
Average Area	12.12	10.81
Area SD (σ)	2.27	2.34
Average Intensity	1.63×10^4	1.13×10^4
Intensity SD (σ)	0.45×10^4	0.30×10^4

Unpaired Student t-test		
	Area	Intensity
t-value	-3.327	-7.282
P-value (Assuming Null Hypothesis)	0.001	< 0.001

Visualization

- Various Visualization Charts in pdf report.
  - BarCharts, ScatterPlots, Box-and-Whiskers Plots, Pareto Charts.





Conclusion

YeastAnalysis has been successfully used in our laboratories and it offers us a quantification tool that supports in understanding many molecular processes. Further developments will direct in using the imaging in a systems biology setting testing for a larger number of experimental conditions and resulting in larger volumes of data. To have access to YeastAnalysis, please contact one of the authors by email To have access to YeastAnalysis, please visit the following URL: