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

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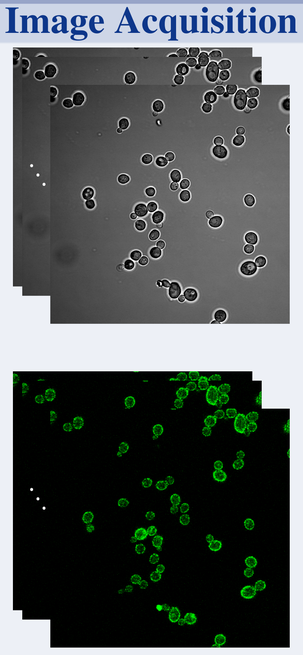
Background

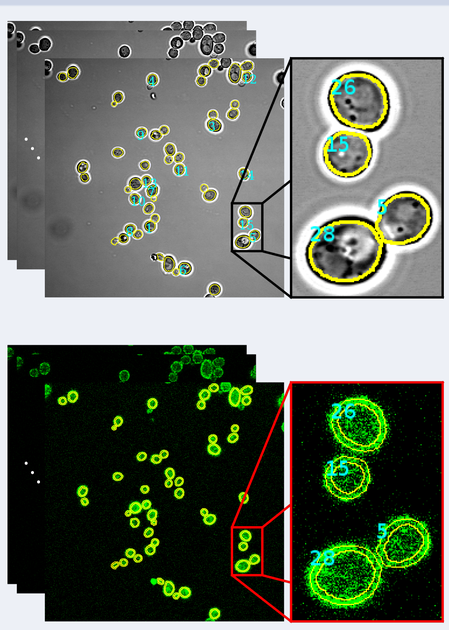
Fluorescent protein-tags like GFP are nowadays commonly used in yeast research. In order to use an image-based readout to study the levels and localization of individual proteins, a methodology is needed allowing routine quantification of observations in images. This requires a certain workflow - a protocol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

Image Acquisition

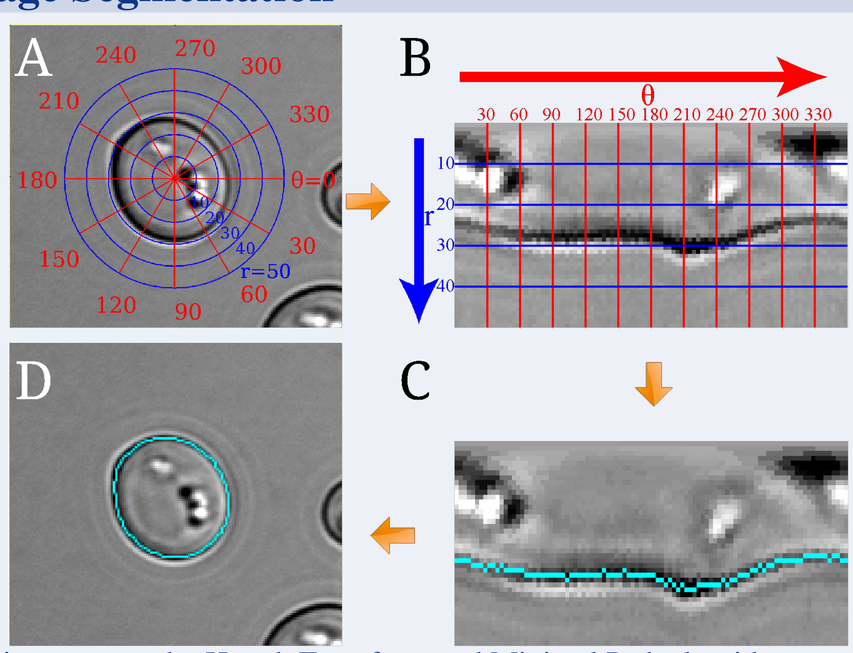


Images acquired as multiple channels by a confocal microscope. In this experiment, we acquired images of two strains (**Δ**bmh1 Nha1-GFP and BY4741 Nha1-GFP) cultured in two different mediums (0mM and 500mM NaCl).

Image Segmentation



Overlays on images showing the detected cells (in bright-field channel) and membranes (in fluorescence channel) for a sample image of **Δ**bmh1 Nha1-GFP strain.



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 correspond to the pixels along the radius of the largest possible circle (red lines). The rows correspond 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

- With the obtained masks, individual cells can be measured for several features. Key features are:
  - Size
  - Fluorescent Intensity
  - Textures
    - Histogram Variance
    - Histogram Skewness
    - Smoothness
    - Uniformity
    - Entropy
- Measurements are saved into 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
...	...	...	...	...	...

A sample from the measurement file showing some cells from the sample image of **Δ**bmh1 Nha1-GFP strain in 0mM NaCl medium.

Data Analysis

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

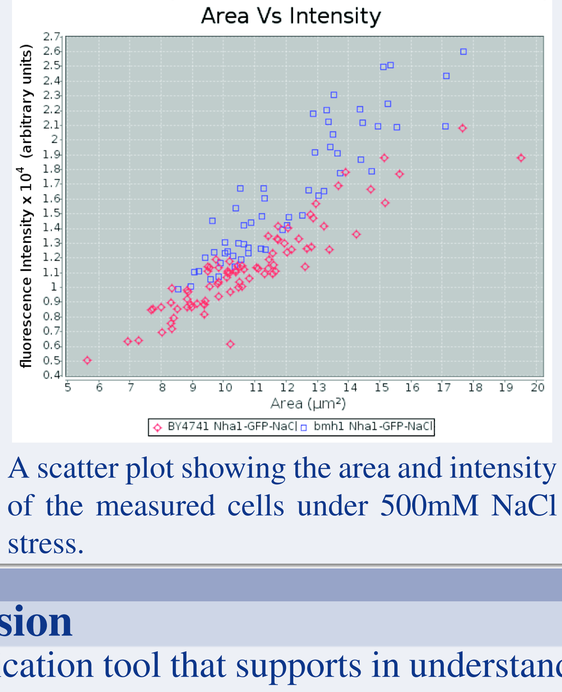
The measurement file is analyzed, and basic statistics about the cells are automatically generated in a (pdf) report.

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

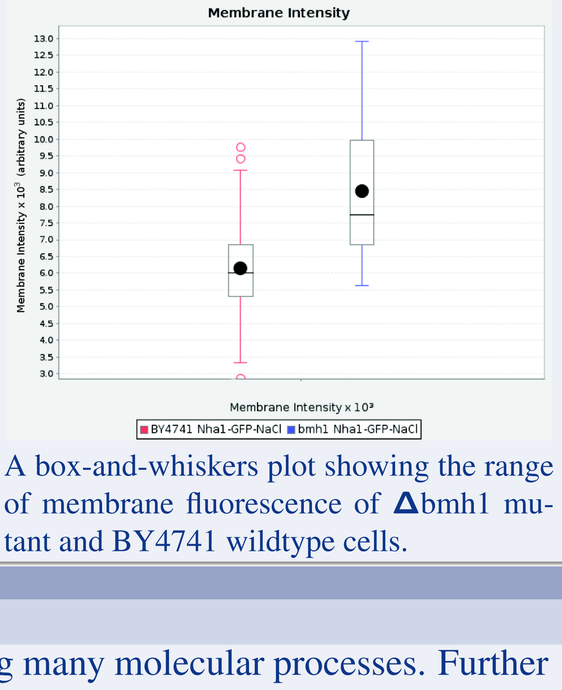
An unpaired Student's t-test is also automatically generated in the report.

Visualization

- Various visualization charts can be generated in the pdf report.
  - Bar charts
  - Scatter plots
  - Box-and-Whiskers plots
  - Pareto Charts



A scatter plot showing the area and intensity of the measured cells under 500mM NaCl stress.



A box-and-whiskers plot showing the range of membrane fluorescence of **Δ**bmh1 mutant and BY4741 wildtype cells.

Results

The statistics show that the average size and Nha1-GFP fluorescence intensity in the **Δ**bmh1 mutant cells is higher than that of BY4741 wildtype under 500mM NaCl stress. The t-test shows the significance of these findings. The intensity and size differences between the two strains are visualized in the scatter plot, and the range of membrane fluorescence in the box-and-whiskers plot. The Nha1-GFP fluorescence in the membrane is also higher in **Δ**bmh1 mutant cells.

Conclusion

YeastAnalysis has been successfully used in our laboratories and it offers 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. If you have interest in YeastAnalysis, please contact us by email at : m.tleis@liacs.leidenuniv.nl.

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