Analysis of the Quantitative Traits of Single Yeast Cells Imaged Through Microscopy

Primary Citation

Ginovart, Marta, Rosa Carbó, Mónica Blanco, and Xavier Portell. "Digital Image Analysis of Yeast Single Cells Growing in Two Different Oxygen Concentrations to Analyze the Population Growth and to Assist Individual-Based Modeling". Frontiers in Microbiology, January 4, 2018, 2628. https://doi.org/10.3389/fmicb.2017.02628

Additional citations

Lev, Sophia, Julianne Teresa Djordjevic. "Why is a Functional PHO Pathway Required by Fungal Pathogens to Disseminate Within a Phosphate Rich Host: A Paradox Explained by Alkaline pH Simulated Nutrient Deprivation and Expanded PHO Pathway Function". PLOS Pathogens, June 21, 2018, 1007021. https://doi.org/10.1371/journal.ppat.1007021

Sethiya, Pooja, Maruti Nandan Rai, Rikky Rai, Chirag Parsania, Kaeling Tan, Koon Ho Wong. "Transcriptomic Analysis Reveals Global and Temporal Transcription Changes During Candida glabrata Adaptation to an Oxidative Environment". British Mycological Society, December 28, 2019, 1878—6146. https://doi.org/10.1016/j.funbio.2019.12.005

Introduction

Biomass and cell wall components from the yeast species *Saccharomyces cerevisiae* are used for several different industrial purposes, including in the manufacturing of alcoholic beverages, as a microorganism used in probiotic supplements with health benefits, and as a source of functional ingredients for the food industry. Researchers wanted to improve methods for better understanding the growth process of *Saccharomyces cerevisiae* cells due to the importance of being able to control the growth of these cells for industrial applications. Specifically, they wanted to develop a method able to tell which of the four growth phases in the life cycle of yeast cells (lag phase, log phase, stationary phase, and death phase) cells in a given population were in at a given time point as a means of studying how the population was adapting to a given environment, as this information can vary based on the carrying capacity and growth rate of a population. The authors of this study aimed to find a method that could combine data about yeast cells from two different levels: the population level and the level of individual cells. The integration of these two sources of information was selected as a goal for the study because of its ability to connect interactions between individual microbes and interactions between microbes and the surrounding environment with the overall behavior of the population, which creates a model that accounts for biological heterogeneity in the population. The method obtained allowed the researchers to characterize the growth phase and budding state of the yeast cells

analyzed in order to determine at what time point in the growth process the cells transitioned between growth phases, thereby allowing the researchers to better understand the growth process of these cells and how it can be controlled to result in a greater number of cells or larger cells for industrial applications. The authors took images of yeast cells growing in two different conditions: aerobic (containing a normal amount of oxygen) and microaerophillic (containing decreased amounts of oxygen) and established a protocol for analyzing the images. Oxygen concentration was chosen as a variable because of its impact on the number and size of cells produced in a growth culture. The researchers then used the data obtained from these image analyses to determine a number of parameters describing each cell, including area and perimeter. This data was then used to determine what point the cells were at in their life cycle by finding the growth state and budding phase of the cells. These results were then compared to results obtained using the INDISIM (INDividual DIScrete SIMulations) model, a method for simulating the growth and behavior of microbial colonies. I intend on applying the method the authors of this paper obtained to a set of original data gathered as part of an effort to create an automated method for microscopy image analysis that would measure the same parameters as those measured by the authors of this study and then use the data to assess the budding state and growth phase of the cells imaged and at what time point the population entered that growth phase. The goal of this project would also be to compare cells grown in different conditions but would involve applying the method to pathogenic yeast species related to *Saccharomyces cerevisiae* grown under conditions of stress often imposed on these cells by the host. Examples of specific stress conditions include alkaline pH stress, for which the loss of the stress response often results in decreased virulence in pathogenic cells (Lev and Djordjevic 2018), and oxidative stress, which is often imposed on pathogen cells by host phagocytes (Sethiya et al 2019). This effort is focused on the response to such conditions of stress in the *Candida* clade pathogenic relatives of *Saccharomyces cerevisiae* as a method of analyzing the stress responses of pathogenic yeast species and how they compare to non-pathogenic species. The aim would be to obtain the same information the authors of this study obtained for their cells-at what time point cells transitioned between growth phases, the number of cells, and the size of cells-for cells exhibiting immune related stress responses.

Figure to reproduce

Figure 3 in this paper shows the results obtained from the protocol for measuring individual cells for two different replicates grown in two different conditions, where figure 3A represents the results for a replicate grown in aerobic conditions and figure 3B represents the results for a replicate grown in microaerophillic conditions. The data for this figure came from a series of images similar to the image shown in figure 1. More specifically, the researchers used the image analysis software image J to obtain four direct measurements-area, perimeter, major diameter, and minor diameter-using a series of modules in ImageJ and then used the resulting measurements to calculate the circularity of the cells. I intend

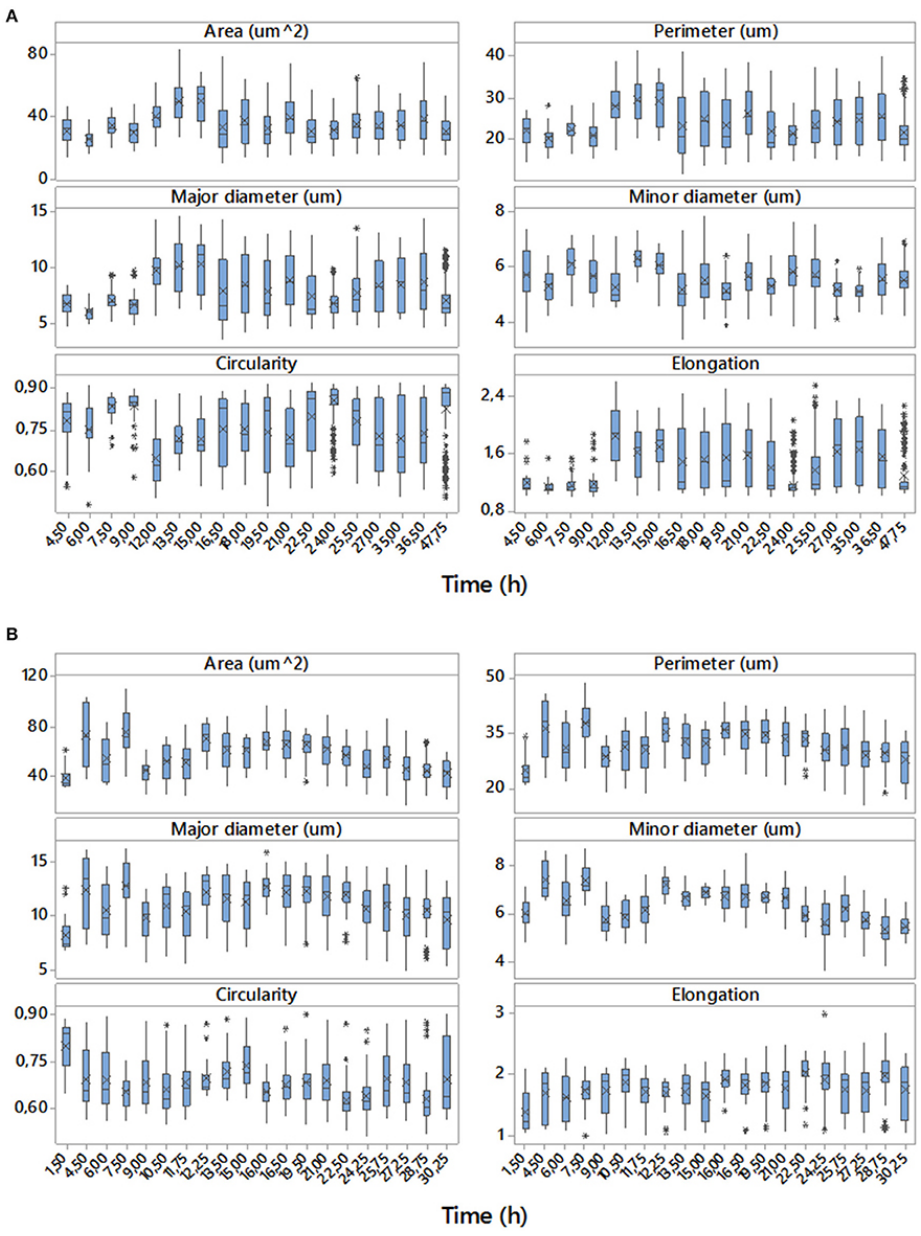

Figure 1: Figure 3

to apply the analysis protocol developed by the authors of this study to a series of similar but original photos of yeast cells of the same species, *Saccharomyces cerevisiae*, in order to generate the data necessary to recreate this figure with original data. The result will be values for the average area, perimeter, major diameter, minor diameter, circularity, and elongation of cells from different time points that can be graphed to create a figure similar to the one above. This information will have implications for the growth phase of the cells, such as how a decreased average circularity value over time can indicate an increased number of cells with buds and how an increase in area followed by a decrease can indicate cell division over time.

Materials and methods

Data

- On February 5th, 2025, I performed an eight-hour time course experiment in which microscopy images of *Saccharomyces cerevisiae* cells grown in standard YPD media were photographed at the time points of 0 hours, 2 hours, 4 hours, 6 hours, and 8 hours. 20 images were taken at each time point, for a total of 100 images across 5 time points.
- The data used in this project can now be found under the "data" folder in this repository. The images are divided into 5 subfolders corresponding with the 5 experimental time points.
- To recreate this analysis, the "data" folder can be downloaded along with the .ijm files found in the "script" folder in order to re-process the images and re-measure the cells. There is one ijm file for each of the parameters measured.
- Once the measurments have been obtained from the cells, the R scripts also provided in the "script" folder can be downloaded and used to re-create the final figure.

Methods

Software The image processing software ImageJ was used to process raw images and obtain the relevant measurements, including area, perimeter, major diameter, minor diameter, circularity, and aspect ratio. The resulting data points were saved in .csv format. One csv file was generated for the 20 images collectec at each of the 5 time points. Further data processing, which involved the calculation of range and mean for each parameter measured at each time point, was performed using the programming language R in the software R studio. Data visualization was also performed in R.

Image Processing The processing of raw images prior to the measurement and analysis process because by creating blurred copies of each of the images and then subtracting these copies from the original images in order to reduce noise. This was done by using the "Gaussian blur" tool found under the "Filters"

section of the "Process" section in the ImageJ user interface. The macros code needed to use this module can be seen in the ijm file located in the "script" section of this repository. After the Gaussian blur tool was applied with a radius of 2.00, the resulting image was saved as a copy, also in tif format. From there, the "Image Calculator..." tool, which can also be found under the "Process" tab on the ImageJ user interface or written into a macros program, was used to subtract the blurred copy from the original copy. The resulting image was converted into an 8 B greyscale format and then saved, also in tif format, for further processing. From there, the "Enhance contrast" tool, which can also be found in the "Process" section, was applied with a pixel saturation value of 0.35 and with the "Normalize" option selected in order to make the borders of the cells more easily detectable. The images were then segmented using the "Threshold" tool, which can be found by going to the "Image" tab and then the "Adjust" section, resulting in the images being converted to 1 B binary image files. The tool "Fill Holes" (found under the "Binary" section of the"Process" tab) was then applied.

Cell Measurement The first of the parameters, area, was measured using the tool "Analyze particles", which can be found in the "Analyze" section of the ImageJ user interface. A minimum area requirement of 1 um and circularity requirement of 0.1 were entered into the settings for this tool in order to ensure that non-cellular bits of visual noise were not counted. Circularity was measured the same way. The "Analyze Particles" tool automatically finds the area and perimeter of the cell being measured and returns the circularity value, which it calculates using the formula C = 4 x pi x A / P^2, where A is area and P is perimeter. However, as of ImageJ version 1.54g, perimeter was not returned by the function as an independent value. Elongation, another term for aspect ratio, was also measured using this tool. "Analyze Particles" measures the major diameter and minor diameter of the cell being analyzed and returns the elongation value it calculates by dividing major diameter by minor diameter. Similar to with perimeter, the version of ImageJ utilized in this project does not return the intermediate values obtained, in this case the values for major and minor diameter, to the user. I am currently looking into methods by which perimeter, major diameter, and minor diameter can be measured using a computational function that can be written into a program, as opposed to being measured manually. The researchers in the study cited and the beginning of this document measured these values manually. For all values, the output of either automated ImageJ programs measuring the parameter in question or the output of manual measurements can be found in the "analysis" folder.

Data Analysis and Visualization An R script was used to calculate the average and range for each value at each time point. This data was then visualized through the generation of a boxplot.