

Research



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A Matlab-based application for quantification of yeast cell growth on solid media

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Quantitative assessment of growth and survival is a suitable technique in studying biochemical, genetic and physiological processes in the cells. The budding yeast *Saccharomyces cerevisiae* is one of the most widely used eukaryotic model organisms for studying cellular mechanisms and processes in evolutionarily distant species, including humans. Yeast growth can be evaluated on both liquid and solid media by measuring cell suspension turbidity and colony forming units, respectively. Several software tools utilizing different parameters have been proposed to quantify yeast growth on solid media. Here, we developed a Matlab-based application which provides a rapid and robust quantitative yeast growth analysis from spot plating assay. Spot plating assay is a typical procedure to evaluate yeast growth in low-throughput laboratory settings, including growth on different nutrient sources or treatment with specific stressors. The app has a one-step installation process, a self-explanatory interface and shorter analysis steps compared with previous established methods, providing a useful tool for both expert and non-expert yeast researchers.

1. Introduction

Assessment of growth and survival are important parameters used in cell biology studies. In unicellular organisms, this can be pursued by the quantification of growth on both liquid and solid media by measuring cell suspension turbidity and counting the formation of colonies on agar plates, respectively. In particular, the growth of microbial cells, such as bacteria and yeast, on solid media mimics the conditions of real-world environment, where they are exposed to challenging environmental situations [1]. Thus, colony counting and size assessment are considered as accurate measurements reflecting real environmental conditions and suitable for both low- and high-throughput applications to cell fitness analysis scenarios [2–5]. Several software tools have been developed to quantify colony growth in yeast, such as ImageJ [6], Spotsizer [7], CellProfiler (a multi-purpose image analysis tool) [8], YeastXtract [9], HT Colony Grid Analyzer [10], Colonyzer [11], ScreenMill (an ImageJ macro) [12], Balony [13], SGAtools (web-based) [14] and gitter (R package) [15]. These platforms use different parameters such as definition of manual selection of radius of area to be quantified, generation of growth time relationship curve using size of colony, colony area/colony size versus volume (i.e. integration of intensity obtained from each pixel), or use of manually prepared replica-plated arrays having bigger colonies versus robot arrays with small colonies [6–15]. Importantly, for some of these tools, there is no provision for user-defined grid support and colony size [7,9–15]. Some platforms work with specific format of image file and resolution, need dedicated equipment for image acquisition, and their performance is affected by suboptimal image quality which includes distortions introduced by imager/camera [6,9–11,13–15]. Furthermore, the set-up of these tools is technically difficult and/or uses specific commands without a graphical user interface (GUI) that can make their use easier for wet-laboratory researchers [6,11,15].

Here we present a dedicated application (app) based on the Matlab platform for quantification of yeast growth from spotting assay analysis. Spot plating assay is a typical procedure to evaluate yeast growth in low-throughput laboratory settings, including growth on different nutrient sources or treatment with specific stressors. Our tool was inspired by the Image J-based method presented in Petropavlovskiy *et al.* [6], representing an advancement to their protocol. The Matlab application has the advantages of a one-step installation process, self-explanatory GUI with built-in image processing, fixed size of the spot, shorter analysis steps, displays the selected spots and saves the data as .jpg and .text file. The app has been tested with experimental data obtained in our laboratory from spotting assay experiments of *Saccharomyces cerevisiae* yeast cells exposed to osmotic stress, but it can be easily applied to any stress condition. Further validation has been performed by analysing the same images with ImageJ software and comparing the results obtained from both tools.

2. Material and methods

2.1. Yeast strains and media

Two strains of the budding yeast *Saccharomyces cerevisiae* were used in this study: W303-1B wild type (WT) (MAT α ade2 leu2 his3 trp1 ura3) and its derivative Δ rtg2 (rtg2 Δ ::LEU2). Cells were grown at 30°C with shaking (180 r.p.m.) in YPD medium (1% yeast extract, 2% bactopectone (Gibco, Life Technologies, Waltham, MA, USA)) and 2% glucose (Sigma-Aldrich, St. Louis, MO, USA). For spotting assay, cell growth was performed on YPD medium with 2% agar (Invitrogen, Life Technologies Waltham, MA, USA) in the absence or presence of 0.4 and 0.8 M sodium chloride (NaCl). Cell growth in liquid medium was monitored by measuring the optical density at 600 nm.

2.2. Spotting assay

Spotting assay was performed as described by Guaragnella *et al.* [16]. Briefly, the optical density (OD₆₀₀) of overnight yeast cultures (30°C, YPD medium, 180 r.p.m.) was adjusted to OD₆₀₀ = 1.0. Under sterile conditions, 5 μ l of 1 : 10 serial dilutions were carefully spotted by using micropipette on YPD $\pm$ NaCl agar plates placed on a grid. The spotted plates were incubated at 30°C for 2 days and plate images were taken every 24 h using ChemiDoc Touch Imaging System.

2.2.1. Note

For spotting assay, ensure the spots are round and it is recommended to dry the plates in the laminar flow hood for about 30 min with the lid slightly off. This will help to obtain the best results from spotting. It is important to ensure pipetting equal volume for all biological and technical replicates of different strains since subsequent analyses will be normalized relative to the control group. In order to ensure the spots are of same size and reproducible, a spotter or fogger can be used. Troubleshooting: Problems 1, 2, 3 and 4.

2.3. Laboratory and software equipment

The following materials, instruments/equipment were used for the experiments and analyses: disposable cuvettes; non-contaminated yeast culture and Eppendorf tubes; sterile petri dishes; spectrophotometer; ChemiDoc Touch Imaging System; Matlab software: Matlab installed in a Personal Computer (PC).

2.3.1. Note

In situations where gel imager is not available, images acquired with a digital camera of good resolution can be analysed using this application. If the digital camera is used, all images should be taken with the same camera having same resolution using same environment.

2.4. Quantification of cell growth by Matlab application

2.4.1. Timing: 10 min/installation and quantification

The application has been developed using Matlab platform and the 'Spotting Assay Quantification' (GUI), which is available for download under BSD license (Berkeley Source Distribution), that belong to Open-Source Software family of licenses with low restrictions from the MathWorks website [17]: Spotting Assay Quantification (GUI) for Windows, MacOS and Linux (requiring Matlab installed on computer).

Here follows the steps to perform quantification of spotting assay images by using Matlab app. Description also available at: <https://youtu.be/7rCEfoEHrdw>.

- (1) Open Matlab, go to 'APPS' tab and click on 'Get more apps'. A new window will open with the title 'Add-On Explorer'. In the search bar write 'Spotting Assay Quantification (GUI)'. The app will appear in the drop-down menu, click on the app. A new window will open displaying the app home page. Go to top right corner and click on 'Add', accept the license and the app will be automatically added in the apps store of your Matlab.
- (2) Navigate the current directory of Matlab to the folder in which you have placed the image for analysis.
- (3) Go to app store of Matlab and click on Spotting Assay Quantification application. A GUI will open as shown in figure 1.
- (4) Enter the radius size of the spot, number of rows and number of columns in the boxes in front of each section.
- (5) Click upload image and select the image to be analysed from the folder. The image will appear in the box in front of the button.
- (6) Click the start quantification button. A selection window appears showing input spotting assay image and a pointer. Drag the pointer to the centre of the first spot of first row and left click. Similarly, click in the centre of all the spots one after the other in all rows from left to right.

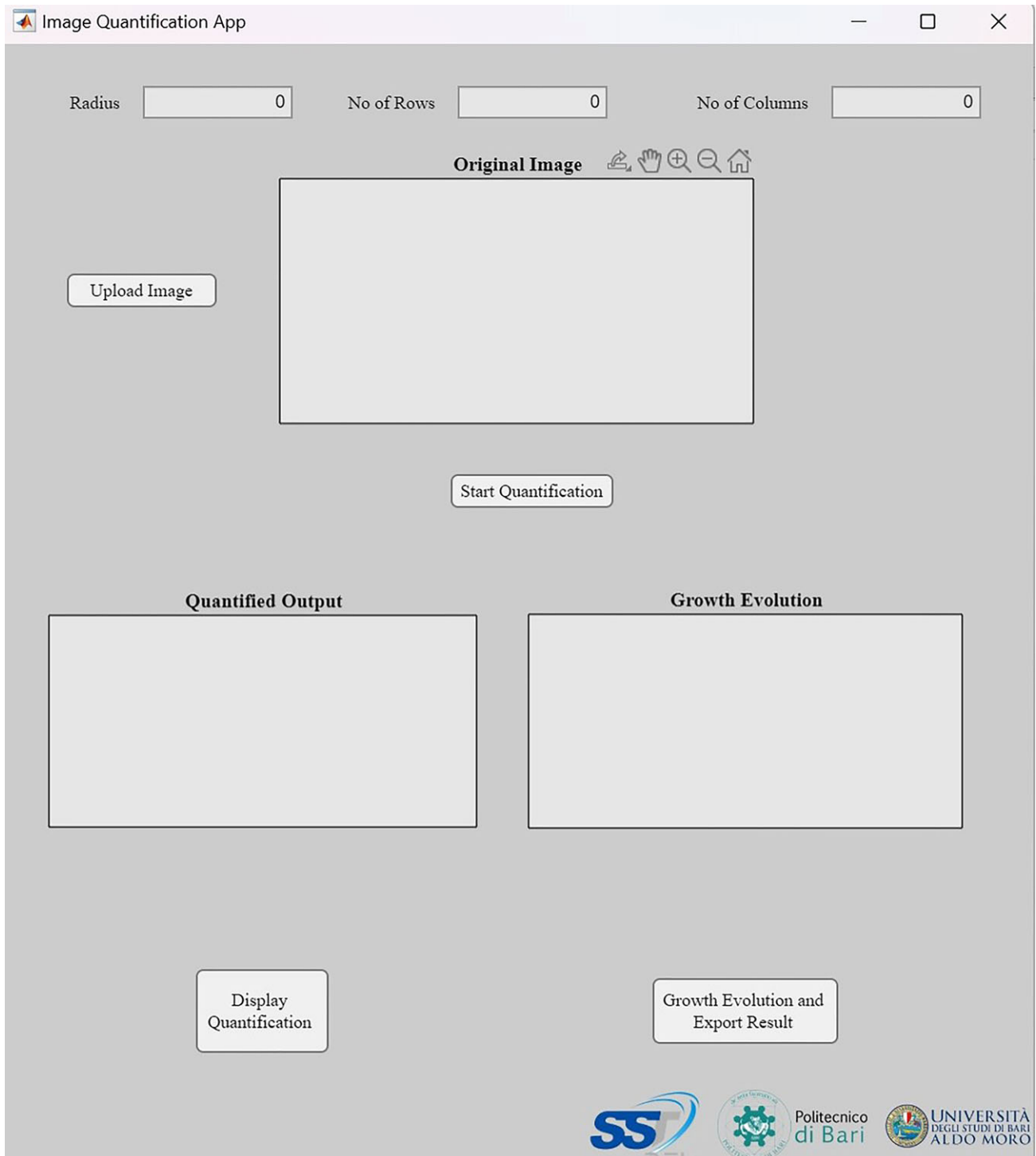


Figure 1. Display of Matlab-app interface for quantification of spotting assay.

- (7) When all spots are analysed, three windows will appear: the first with the original image; the second with quantified values of each spot and the third with growth evolution graph. Troubleshooting: Problems 5 and 6.
- (8) Click on display quantification to visualize quantified spots and values, in app display above display button.
- (9) Click growth evolution and export results button for in app display of growth evolution graph and to save images in .jpeg format and quantification values in the form of a .text file in parent folder.
- (10) Repeat steps 4 to 9 for the images of all other plates (that is, biological replicates).

The same radius should be used to perform all the analyses in one experiment. This is because changing the radius can affect the readings. Choose the radius of circle such that the size of masked area is equal to the size of largest spot on the plate. This can be achieved by selecting an arbitrary radius before the quantification by selecting one row and one column. Select the largest spot on the image in the selection window and check if the masked area covers the whole spot. If not, change the radius accordingly.

2.4.2. Application overview

Spotting Assay Quantification (GUI) has a Matlab source code. The app contains already-developed method for analysis of spotting assay able to accurately identify crowded cells, and its versatile design allows for analysis of different conditions and phenotypes. A user-

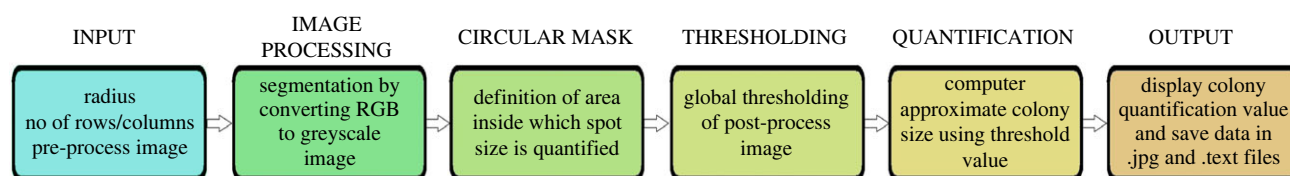


Figure 2. Flowchart diagram of Matlab-based algorithm for yeast growth quantification.

Table 1. Comparison of available software tools for yeast growth quantification on agar plate.

tool name	user defined grid	file format (JPEG/PNG)	user-defined colony size	all operating systems	GUI	flexible image acquisition	ref
Spotting Assay Quantification	✓	✓	✓	✓	✓	✓	current study
ImageJ	✓	]	✓	✓	]	✓	[6]
Spotsizer	]	✓	]	✓	✓	]	[7]
Balony	✓	]	]	✓	✓	]	[13]
CellProfiler	✓	✓	✓	✓	✓	]	[8]
Colonyzer	]	]	]	✓	]	]	[11]
gitter	]	]	]	✓	]	]	[15]
HT Colony Grid Analyzer	]	]	]	✓	✓	]	[10]
ScreenMill	]	✓	]	✓	✓	]	[12]
SGAtools	]	]	]	✓	✓	]	[14]
YeastXtract	✓	]	]	✓	✓	]	[9]

friendly graphical interface has the advantage of shorter analysis steps typically missing in most image analysis software (figure 1). Thus, spot size can be quantified following few steps. The algorithm is designed to display quantification value and image of selected spots. Comparison of available software tools, including this application, for yeast growth quantification on agar plate are reported in table 1. Different parameters, such as the file format, the defined grid and colony size, and the procedure for image acquisition have been considered. The absence of one or more of these parameters represents a bottleneck in quantification of colony size for laboratory workers. Flexibility in image acquisition and file format are among the parameters which can reduce the need for dedicated instruments.

User-defined grid and colony size: users can opt to select customized grids and sample size according to their need apart from the conventional configurations making the experimental set-up simpler. Operating systems and GUI: availability of application for multiple operating system and provision of GUI allows pliability to laboratory workers with no or little expertise in software installation and handling. The presented application incorporates all the above-mentioned features, keeping the quantification results comparable to previously developed methods.

2.4.3. The workflow of the algorithm

The workflow of the algorithm has six steps: input, image processing, circular mask, thresholding, quantification of spot size and output (figure 2).

2.4.3.1. Input

Analysis on this application starts with the input of three parameters including image of desired plate, number of rows/columns and radius. The algorithm is designed to allow user to vary area of circular mask (see section below) by manual input of radius. The user can input number of rows/columns (grid) of spots on the plate at the start of every analysis. This provision is made as the app is designed to work with arbitrarily placed but equal volume of spots. Once the radius is selected the area of mask is fixed for the whole analysis. In the next step, user is allowed to select the image and provide the location of cultures spot on the plate by clicking in the centre of each spot under consideration. This helps correct identification of all colonies in image as visual inspection and manual adjustment is available.

2.4.3.2. Image acquisition/processing

The images in the present report were obtained using the gel imager ChemiDoc usually in .jpg format by scanning the plates at 24-hour interval of incubation. The app is also able to analyse images obtained by snapping plates with digital camera with good resolution. Correction of variations in illumination is important in image analysis. Algorithm used in this application contains standard methods to address illumination variation. Plate images are segmented using white/black thresholding by converting the true colour image RGB to the greyscale image into colony area/background. Matlab built-in *rgb2grey* function is used for conversion of standard images to greyscale by retaining the luminance and removing saturation and hue information. For further improvement, *double* function to add double

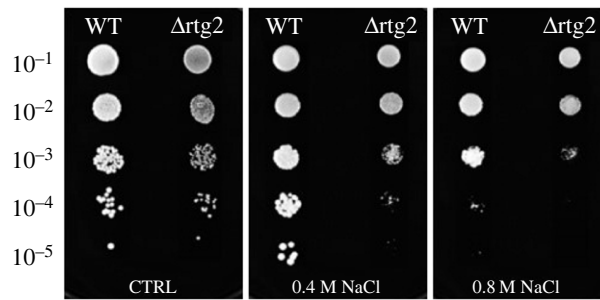


Figure 3. Effect of sodium chloride on growth of wild type and $\Delta rtg2$ cells. Wild type (WT) and $RTG2$ -lacking cells ($\Delta rtg2$) were grown overnight in YPD medium and diluted to 1.0 OD₆₀₀. Tenfold serial dilutions were spotted on YPD agar plates without (CTRL) or with 0.4 and 0.8 M sodium chloride (NaCl). Cell growth was analysed after 2 days of incubation at 30°C. Images were acquired by ChemiDoc Touch Imaging System.

precision to the image. This method was used to correct the luminance of processed images as raw image intensity measurements can preclude accurate quantification.

2.4.3.3. Circular mask

Circular mask is defined as the area within which spot size is quantified depending on the threshold value. The definition of mask is one of the most crucial steps in quantification of spot size. The size of circular mask area depends on the radius that is selected in the start of quantification. This concept is introduced as this approach helps quantify the spots of your choice depending on their size and discard extraneous spots that are due to contamination. The size of masked area cannot be changed during one analysis, it is advised to select the size of masked area equal to or a little bigger than the biggest spot on the plate. It is important to state here that the masked area (radius of area to be quantified) is set before every analysis and can be changed based on the experimental conditions.

2.4.3.4. Thresholding

In this step, each pixel of the image is compared with a predefined grey value, and pixels are changed to black/white for separation of background from the yeast colonies on agar plate; this procedure is known as 'thresholding'. The images are saved at an 8 bits per pixel colour depth, having a value between 0 and 255. An image from three-dimensional or two-dimensional greyscale image is generated by changing all values above a threshold to 1 and converting other values to 0. We implemented Otsu's method using Matlab built-in command *imbinarize*. During this step, a 'criterion function' is generated that ensures a clear separation between different regions. Otsu's method instinctively formulates restricting of a greyscale image to a binary or image clustering-based image thresholding. The algorithm considers backing bimodal histogram (background pixels and foreground pixels) ensuring two classes of pixels; it then generates the optimum threshold that not only disconnects the classes but also keeps the overall spread to a minimum value.

2.4.3.5. Quantification of spot size

The colony growth on plate is quantified as a function of spot sizes by analysing all pixels within the masked area. When the quantification is started, the user is allowed to select the spot that is under consideration. When a spot is selected by clicking, the algorithm generates a masked area around the point of selection and compares the grey value of every pixel within the masked area with the threshold value. This app assumes that the intensity of the pixels of the area covered by each colony on each spot has higher values than the surrounding area. Every pixel above the threshold value is graded 1 and the other values are graded 0. The percentage of number of pixels having 1 compared with 0 in the total masked area is displayed as the size of spot in terms of numerical values.

2.4.3.6. Output

After all the spots are quantified, the app provides a graph, displaying the trend of growth treated, the numerical data saved in the form of simple table in a text file and cumulative image of spots analysed in .jpg format.

2.5. Data analyses

All data are presented as mean $\pm$ standard deviation. All the experiments were performed with a minimum of three technical and biological replicates. Average absolute values of growth were determined via Matlab application or ImageJ protocol as indicated. Growth inhibition (GI) was calculated by subtracting from 100 the relative growth of stressed cells (exposed to 0.4 or 0.8 M NaCl) with respect to control. The data were analysed using one-way ANOVA followed by Tukey's *post hoc* test to determine differences between and within groups. The data were analysed using Microsoft Excel software, values of $p \leq 0.05$ (ANOVA-alpha) were considered significant. Troubleshooting: Problem 7.

2.5.1. Note

Since all the spots are quantified by the app, it is necessary to select a single dilution across the strains for data analysis. This should be the lowest dilution that shows distinct colonies. In the present study, the third dilution satisfied this condition (figure 3). Researchers should choose appropriate dilution based on the experimental conditions. The data obtained from the quantification was normalized by dividing the value of each spot from the test group (stressed cells) by the corresponding control at the same dilution.

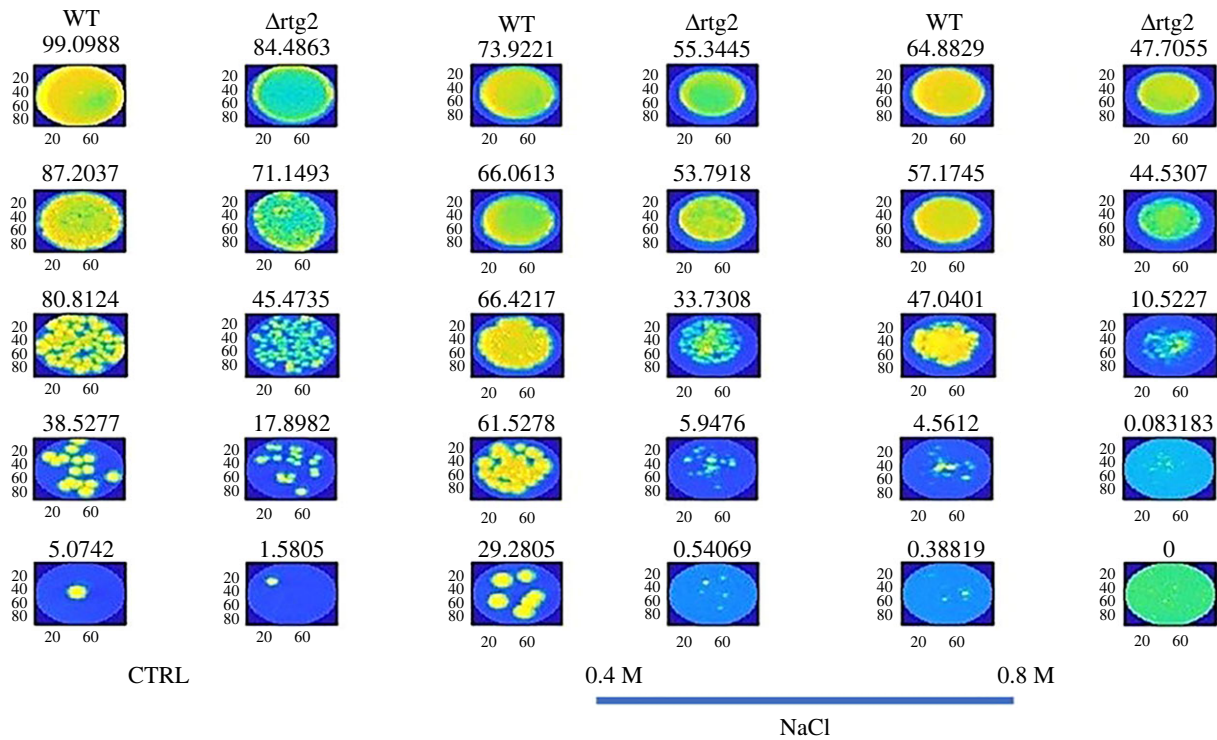


Figure 4. Spotting assay quantification. Masked area and numerical results from quantification process on wild type (WT) and *RTG2*-lacking strain (Δ rtg2) with and without NaCl stress at the indicated concentrations using Spotting Assay Quantification (GUI).

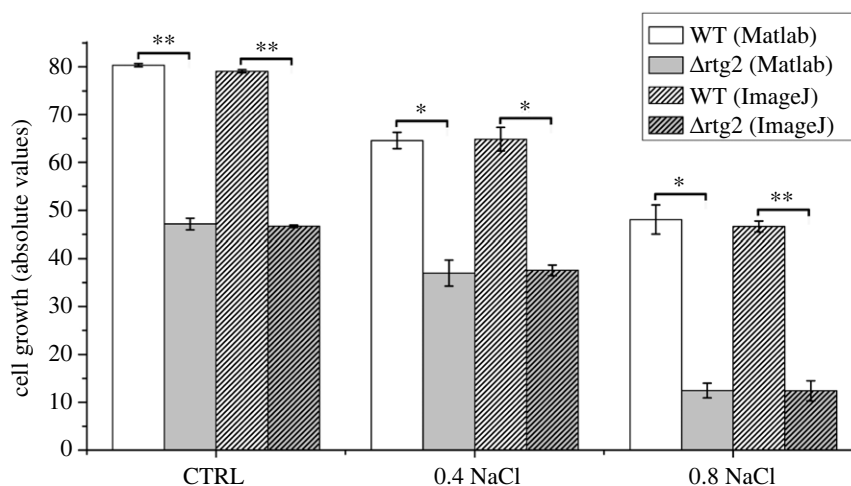


Figure 5. Cell growth of wild-type (WT) and *RTG2*-lacking strain (Δ rtg2) using Matlab application and ImageJ protocol. The third dilution on solid media was selected for calculation of cell growth. Average absolute values of three independent experiments are reported. Unpaired Student's *t*-test: statistically significant differences with **p* < 0.0001 and ***p* < 0.00001.

3. Results and discussion

In *Saccharomyces cerevisiae*, mitochondrial RTG-dependent retrograde signalling plays an imperative role in yeast stress response and cellular adaptation under various environmental conditions [18–21]. In particular, it has been shown that deletion of *RTG2*, the upstream positive regulator of RTG pathway, sensitized yeast cells to osmostress downstream of the mitogen-activated protein kinase Hog1 [16,22]. To validate our Matlab application, we spotted replicates of wild-type and mutant yeast strain lacking the *RTG2* gene on distinct agar plates with and without presence of mild (0.4 M NaCl) and high (0.8 M NaCl) salt stress. Results reported from distinct plates in figure 3 show an expected inverse correlation between cell growth and NaCl concentrations for both WT and Δ rtg2 cells.

However, a higher sensitivity with respect to WT was observed in the mutant when comparing the same concentration of stressor. This agreed with previously reported data [16,22]. Images and values from spotting assay quantification are reported on top of each spot in figure 4. The values present on the left and bottom of each spot display the percentage area of the spot up to that point.

The quantification values decreased proportionally in the serial dilutions, with lowest growth value obtained for the highest dilution and vice versa. Average of absolute growth values calculated from the third dilution via Matlab application are reported in figure 5. It was evident that increasing sodium chloride concentration produced a proportional growth inhibition in both WT

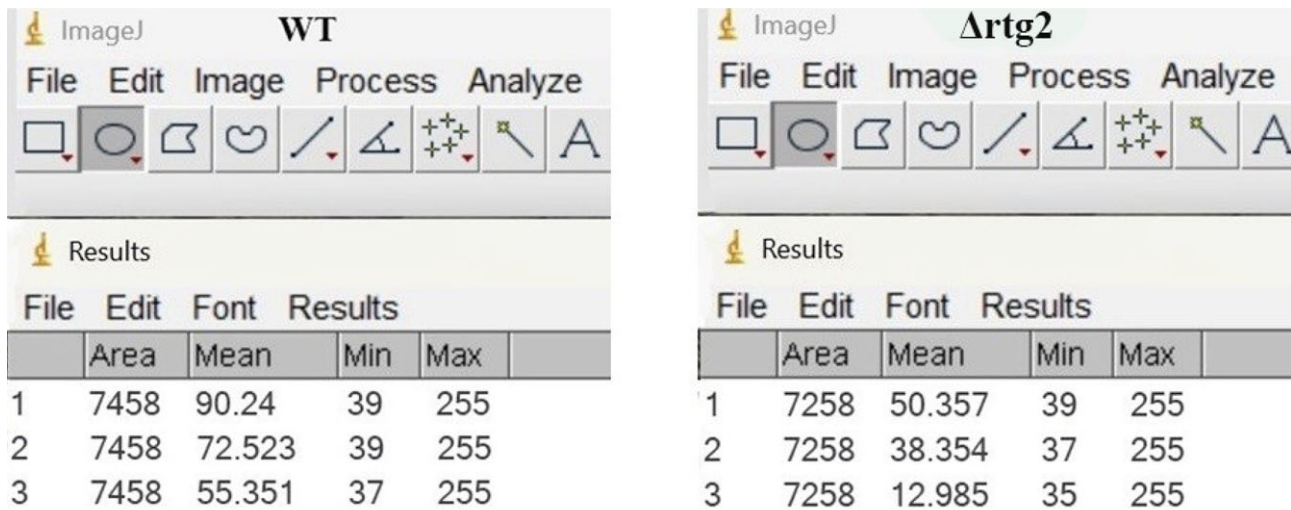


Figure 6. Numerical results from wild type (WT) and *RTG2*-lacking strain (Δ *rtg2*) without and with NaCl stress using ImageJ software. In both panels (WT and Δ *rtg2*), 1, 2 and 3 referred to control cells, 0.4 and 0.8 M NaCl treated cells, respectively. The mean of each sample corresponded to the numerical results calculated using ImageJ protocol [6].

Table 2. Cell growth absolute values calculated via Matlab application or ImageJ protocol.

sample	CTRL	0.4 NaCl	0.8 NaCl	GI (%) 0.4 NaCl	GI (%) 0.8 NaCl
WT (Matlab-app)	80.4 ± 0.32	64.6 ± 1.71	48.1 ± 3.02	19.7	40.2
Δ rtg2 (Matlab-app)	47.1 ± 1.19	37 ± 2.71	12.5 ± 1.54	21.5	73.6
WT (ImageJ)	79.1 ± 0.31	64.9 ± 2.45	46.6 ± 1.13	18.0	41.1
Δ rtg2 (ImageJ)	46.7 ± 0.24	37.6 ± 1.11	12.4 ± 2.10	19.5	73.4

and Δ *rtg2* strains (table 2). Quantification values obtained from distinct plates in independent experiments showed a relative growth inhibition of almost 20% and 40% in the presence of 0.4 M and 0.8 M NaCl respectively in WT cells. Conversely, 0.4 and 0.8 M NaCl inhibited the growth of Δ *rtg2* strain by almost 22 and 74%, respectively (table 2, electronic supplementary material, tables S16 and S17). By comparing the two strains, Δ *rtg2* showed higher sensitivity with respect to WT cells upon mild and high NaCl stress. This result revalidates the contribution of RTG pathway to osmoadaptation, as previously reported in both solid and liquid media [16–22]. In addition, the growth quantification revealed a significant increased sensitivity of Δ *rtg2* compared with WT also in the absence of stress. This result might be related to the role of RTG pathway in the diauxic shift, that is the physiological transition from a fermentative to a respiratory metabolism [23]. It must be noted here that the developed method quantifies average growth present in a selected area unlike existing methods [7,8]. Abnormal growth of colonies can perplex the results. So, after obtaining the results, researchers using our method should double check that the obtained results correspond to images. Moreover, the method only provides insight about survival and growth, but it does not provide information about the mechanism of toxicity or genetic alteration in certain growth environments. For such studies, cell death staining and individual colony counting techniques can be considered [24,25].

Our Matlab application was validated by comparing growth quantification data with the ones obtained by the analysis of the same spotting images using ImageJ protocol (figures 5 and 6) [6]. In the WT strain, a relative growth inhibition of 18% and 41% were obtained in the presence of 0.4 M and 0.8 M NaCl, respectively. Conversely, 0.4 and 0.8 M NaCl inhibited the growth of Δ *rtg2* strain by 20% and 73%, respectively (table 2, electronic supplementary material, tables S16 and S17). The average quantification values obtained with Matlab application and ImageJ-based protocol were comparable in terms of relative growth for both WT and mutant. However, by comparing the two methodologies, the Matlab application presents several advantages, such as an interactive GUI, lower variability due to fixed size of the spot and shorter analysis steps.

The similarity in results with previous experimental data obtained for liquid cultures and similar established methods proves the effectiveness of our method [6,16].

3.1. Limitations

The method presented in this work does not quantify the growth of individual colonies; instead it measures on average growth in a spot, as in [6]. For this reason, the results can be affected by the presence of abnormally large colonies and bubbles, cracks and hairs. That is, fewer large colonies may produce higher values than a lot of small colonies. After obtaining the values, researchers using our method should double check the results comparing with what they can see on the actual image of the plate.

It must be noted that the yeast colonies do not always grow linearly on agar plate and each colony reaches stationary phase depending on number of cells [23]. This implies that the growth time affects the quantification. Dilution and time at which plate image is taken are two crucial parameters that can alter the differences in growth between experimental groups. Thus, this method should not be used to compare growth differences at different times.

Another shortcoming of this method is lack of insight into genetic manipulation and type of toxicity because of specific conditions, as in other well-developed methods like colony-forming unit counting or cell death staining [6,23,24]. However, this method helps make spotting assay quantifiable.

4. Conclusions

In this work we described a novel and user-friendly Matlab application developed for the quantitative assessment of yeast cell growth and survival on solid media. This application is easy to install and has shorter analysis steps compared with other analytical methods. The method is based on the quantitative analysis of yeast colonies obtained from spot plating assay images and it is suitable for low-throughput applications. The method has been validated by quantifying the growth of wild-type and mutant cells lacking *RTG2* gene in the absence and presence of mild and high salt-induced osmotic stress and subtle differences could be revealed between the strains and stress conditions. Similar results were obtained when the same images were analysed following the protocols for ImageJ. Given the advantages of one-step installation process, shorter analysis steps and a self-explanatory graphical user interface, this tool will make analysis of growth on solid media from spotting assay images easier for both expert and non-expert yeast researchers.

4.1. Troubleshooting

4.1.1. Problem 1

Yeast spots are too large, or they look oval.

4.1.1.1. Solution

Yeast cells were not properly absorbed because the plates were wet before and/or after the experiment and/or moved during the experiment. Repeat spotting assay and dry the plate properly before and after the spotting. Be careful to avoid moving the plate after the spotting.

4.1.2. Problem 2

Spots sizes are abnormally too small or too large.

4.1.2.1. Solution

There is variation of culture volume in each prong of the spotter. Repeat spotting assay with equal volume.

4.1.3. Problem 3

Yeast spots are abnormally small sized.

4.1.3.1. Solution

Culture volume is too small. Repeat culture volume with higher culture volume for each dilution. If multiple plates are being spotted, make sure there is sufficient volume in each prong.

4.1.4. Problem 4

Spots are not round.

4.1.4.1. Solution

Care was not taken while lifting the spotter after placing the spot. Repeat assay and while backing up lift the spotter vertically.

4.1.5. Problem 5

Large colonies can be seen on the plate and quantification values obtained from spots do not relate to yeast growth.

4.1.5.1. Solution

Yeast colonies did not grow linearly, and large colonies were formed due to high volume. Repeat spotting by lowering the culture volume.

4.1.6. Problem 6

Large colonies are not seen on the plates, but quantification values obtained from spots do not relate to yeast growth.

4.1.6.1. Solution

Diameter of circular area selected before quantification for spot might be too large. Repeat the quantification and make sure to keep the diameter of circular selection equal to largest spot.

4.1.7. Problem 7

Abnormally large differences in standard deviation are observed in one or multiple experimental groups while there are no visually significant differences.

4.1.7.1. Solution

This may happen due to lack of consistency during quantification. Quantify the entire replicate that shows the highest deviation, including all samples.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material [26].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. E.W.: data curation, formal analysis, methodology, validation, writing—original draft; O.B.O.: data curation, formal analysis, validation, writing—original draft; N.G.: conceptualization, funding acquisition, supervision, writing—review and editing; C.G.: conceptualization, funding acquisition, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. Authors have no conflict of interest to declare.

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References

- Palková Z. 2004 Multicellular microorganisms: laboratory versus nature. *EMBO Rep.* **5**, 470–476. (doi:10.1038/sj.embor.7400145)
- Kamrad S, Rodríguez-López M, Cotobal C, Correia-Melo C, Ralser M, Bähler J. 2020 *Pyphe*, a python toolbox for assessing microbial growth and cell viability in high-throughput colony screens. *Elife* **9**, e55160. (doi:10.7554/eLife.55160)
- Miller JH, Fasanello VJ, Liu P, Longan ER, Botero CA, Fay JC. 2022 Using colony size to measure fitness in *Saccharomyces cerevisiae*. *PLoS ONE* **17**, e0271709.
- Zackrisson M *et al.* 2016 Scan-o-matic: high-resolution microbial phenomics at a massive scale. *G3: Genes Genom. Genet.* **6**, 3003–3014. (doi:10.1534/g3.116.032342)
- Bean GJ, Jaeger PA, Bahr S, Ideker T. 2014 Development of ultra-high-density screening tools for microbial 'omics'. *PLoS ONE* **9**, e85177. (doi:10.1371/journal.pone.0085177)
- Petropavlovskiy AA, Tauro MG, Lajoie P, Duennwald ML. 2020 A quantitative imaging-based protocol for yeast growth and survival on agar plates. *STAR protocols* **1**, 100182. (doi:10.1016/j.xpro.2020.100182)
- Bischof L, Převorovský M, Rallis C, Jeffares DC, Arzhaeva Y, Bähler J. 2016 Spotsizer: high-throughput quantitative analysis of microbial growth. *Biotechniques* **61**, 191–201. (doi:10.2144/000114459)
- Carpenter AE *et al.* 2006 CellProfiler: image analysis software for identifying and quantifying cell phenotypes. *Genome Biol.* **7**, R100. (doi:10.1186/gb-2006-7-10-r100)
- Shah NA, Laws RJ, Wardman B, Zhao LP, Hartman JL. 2007 Accurate, precise modeling of cell proliferation kinetics from time-lapse imaging and automated image analysis of agar yeast culture arrays. *BMC Syst. Biol.* **1**, 3. (doi:10.1186/1752-0509-1-3)
- Collins SR, Schuldiner M, Krogan NJ, Weissman JS. 2006 A strategy for extracting and analyzing large-scale quantitative epistatic interaction data. *Genome Biol.* **7**, R63. (doi:10.1186/gb-2006-7-7-r63)
- Lawless C, Wilkinson DJ, Young A, Addinall SG, Lydall DA. 2010 Colonyzer: automated quantification of micro-organism growth characteristics on solid agar. *BMC Bioinf.* **11**, 287. (doi:10.1186/1471-2105-11-287)
- Dittmar JC, Reid RJ, Rothstein R. 2010 ScreenMill: a freely available software suite for growth measurement, analysis and visualization of high-throughput screen data. *BMC Bioinf.* **11**, 353. (doi:10.1186/1471-2105-11-353)
- Young BP, Loewen CJR. 2013 Balony: a software package for analysis of data generated by synthetic genetic array experiments. *BMC Bioinf.* **14**, 354. (doi:10.1186/1471-2105-14-354)
- Wagih O *et al.* 2013 SGAtools: one-stop analysis and visualization of array-based genetic interaction screens. *Nucleic Acids Res.* **41**, W591–W596. (doi:10.1093/nar/gkt400)
- Wagih O, Parts L. 2014 gitter: A robust and accurate method for quantification of colony sizes from plate images. *G3 (Bethesda)* **4**, 547–552. (doi:10.1534/g3.113.009431)
- Guaragnella N, Agrimi G, Scarcia P, Suriano C, Pisano I, Bobba A, Mazzoni C, Palmieri L, Giannattasio S. 2021 RTG signaling sustains mitochondrial respiratory capacity in HOG1-dependent osmoadaptation. *Microorganisms* **9**, 1894. (doi:10.3390/microorganisms9091894)
- Ehtisham Wahid. 2023 *Spotting Assay Quantification (GUI)*. MATLAB Central File Exchange. See <https://www.mathworks.com/matlabcentral/fileexchange/124095-spotting-assay-quantification-gui> (accessed 12 November 2023).
- Torelli NQ, Ferreira-Junior JR, Kowaltowski AJ, da Cunha FM. 2015 RTG1- and RTG2-dependent retrograde signaling controls mitochondrial activity and stress resistance in *Saccharomyces cerevisiae*. *Free Radical Biol. Med.* **81**, 30–37. (doi:10.1016/j.freeradbiomed.2014.12.025)
- Guaragnella N, Stirpe M, Marzulli D, Mazzoni C, Giannattasio S. 2019 Acid stress triggers resistance to acetic acid-induced regulated cell death through Hog1 activation which requires RTG2 in yeast. *Oxid. Med. Cell. Longev.* **2019**, 4651062. (doi:10.1155/2019/4651062)
- Hijazi I, Knupp J, Chang A. 2020 Retrograde signaling mediates an adaptive survival response to endoplasmic reticulum stress in *Saccharomyces cerevisiae*. *J. Cell Sci.* **133**, jcs241539. (doi:10.1242/jcs.241539)
- Ruiz-Roig C, Noriega N, Duch A, Posas F, de Nadal E. 2012 The Hog1 SAPK controls the Rtg1/Rtg3 transcriptional complex activity by multiple regulatory mechanisms. *Mol. Biol. Cell* **23**, 4286–4296. (doi:10.1091/mbc.e12-04-0289)
- Di Noia MA *et al.* 2023 Inactivation of HAP4 accelerates RTG-dependent osmoadaptation in *Saccharomyces cerevisiae*. *Int. J. Mol. Sci.* **24**, 5320. (doi:10.3390/ijms24065320)

23. De Risi JL, Iyer VR, Brown PO. 1997 Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278**, 680–686. (doi:10.1126/science.278.5338.680)
24. Chadwick SR, Pananos AD, Di Gregorio SE, Park AE, Etedali-Zadeh P, Duennwald ML, Lajoie P. 2016 A toolbox for rapid quantitative assessment of chronological lifespan and survival in *Saccharomyces cerevisiae*. *Traffic* **17**, 689–703. (doi:10.1111/tra.12391)
25. Wloch-Salamon DM, Bem AE. 2013 Types of cell death and methods of their detection in yeast *Saccharomyces cerevisiae*. *J. Appl. Microbiol.* **114**, 287–298. (doi:10.1111/jam.12024)
26. Wahid E, Ocheja OB, Guaragnella N, Guaragnella C. 2024 A Matlab-based application for quantification of yeast cell growth on solid media. Figshare. (doi:10.6084/m9.figshare.c.7098771)