Growth Pattern of Yeast Cells Studied under Line Optical Tweezers

S. Charrunchon, J. Limtrakul, and N. Chattham

***Abstract*—Cell growth and division has been of scientists' interest for over generations. Several mathematical models have been reported derived from conventional method of cell culture. Here we applied optical tweezers to guide cell division directionally. The patterns of Saccharonmyces Bayanus yeast growth was studied under 1064 nm line optical tweezers generated by time-shared multiple optical traps. Yeast growth was found following the path of the generated laser patterns in linear shape as a result of localized heating effect due to absorption at the focal point.**

The area of grown yeast cells as a function of time was measure through image processing. Mathematical model for the growth rate under line optical trap has been determined and discussed.

***Index Terms*—Optical tweezers, saccharomyces bayanus, yeast, growth.**

I. Introduction

Optical tweezers was first introduced by Arthur Ashkin in 1986 as a powerful tool for manipulating microscopic objects without any direct contact using single laser focus beam [1]. A microscopic particle can be trapped at the focal point by tightly focused laser beam, typically through a high numerical aperture microscope objective [2]. Translation and positioning of a particle are possible as a result of the electric field intensity gradient of a laser generated from the transfer of momentum from light to a particle under the law of momentum conservation [3]. Thus far, Optical tweezers have proven to be an ideal tool for studying biological [4] and macromolecular systems [5] because they can trap objects as small as 5 nm and can exert forces exceeding 100 pN [6].

Multi-functional optical traps have been demonstrated in wide varieties of experiments, especially in biological systems, for example, DNA stretching [7] and organelle removal [8]. Transformation of a single laser beam at a focal point by various techniques generates multiple beams, namely, holographic optical tweezers (HOTs), phase-only diffractive optical element (DOE), computer-addressed spatial light modulator (SLM), the generalized phase contrast (GPC) and a mechanical scanning method using galvanometer mirrors.

Neuronal cell growth guided with multiple optical traps has been of particular interest recently [9] leading to advancement in medical technology. We used the similar technique producing multiple beams by a galvanometer mirror to guide the growth of yeast cells for the study of their growth pattern and growth rate in controlled environment

II. Experimental Details

A. Saccharomyces Bayanus

As a starting point for studying growth pattern of yeast cells, *Saccharomyces bayanus*, the second most important yeast for basic and applied studied in scientific and breeding project [10], was selected for this research. *S. bayanus* multiplied by budding was grown by common cultivation in potato dextrose broth (PDB). Its biochemical and biotechnological characteristics related to *S. cerevisiae*, a good model of higher eukaryotes. Moreover, this winemaking and cider fermentation yeast is in the kingdom of fungi including many species that are important in food production. The picture of *S. bayanus* in laboratory recorded from CCD camera in laboratory was shown in Fig. 1.

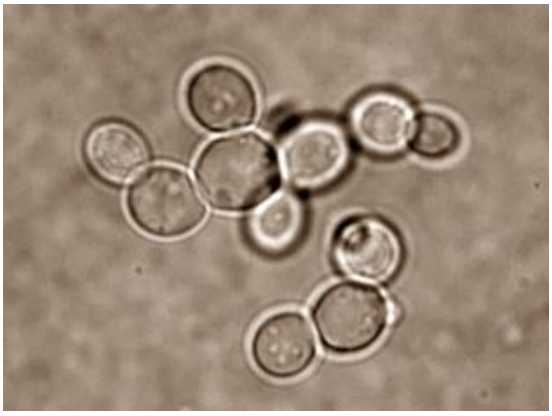

Fig. 1. Saccharomyces bayanus.

B. Optical Tweezers Setup

A time-shared multiple optical traps was set using 1064 nm fiber laser directing to a galvanometer mirror to obtain a desired pattern of multiple trapping beams under a Nikon Ti-U inverted microscope. The laser beam transmitted directly to CCD camera in linear shape pattern. The sample was placed in optical tweezers for study growth pattern of yeast cells. The diagram of optical tweezers was shown in Fig. 2.

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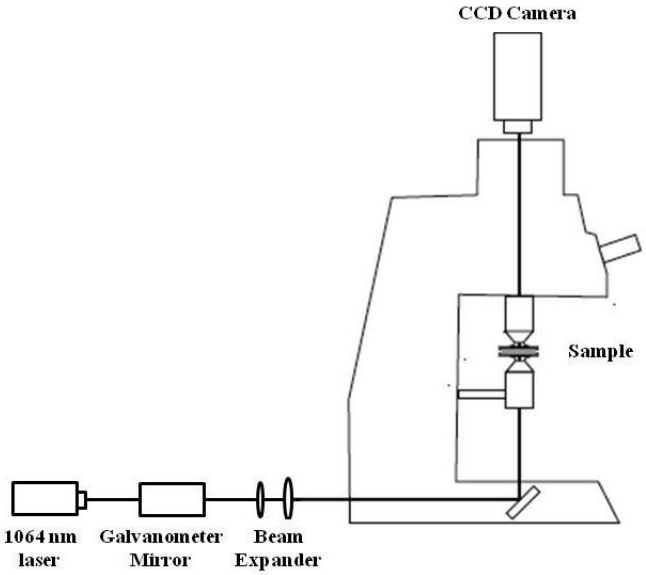


Fig. 2. 1064 nm line optical tweezers setup.

III. Growth Models

A. Cell Area Index (CAI)

We sought to measure the size of cell growth as a function of time. The Number of Pixels (NoP) [11] is the area occupied by yeast cell extracted from a frame every 5 minutes from the video recording as seen in Fig. 3.

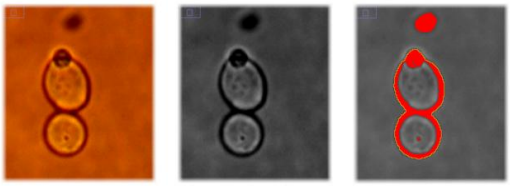


Fig. 3. Image processing to improve quality of frame capture from video. From left to right images, the boundary of yeast area was enhanced.

The NoP for every data point was normalized by dividing with the area of the cluster at start time (t=0) due to the variations in size of the yeast cells at the start of the experiment. This quantity is the Cell Area Index (CAI) given by equation (1).

$$CAI(t) = \frac{NoP(t)}{NoP(t_0)} \tag{1}$$

B. Modified Growth Model

The commonly used function for yeast growth model are the logistic, Gompertz and Richards function as shown in equation (2) – (4) respectively. All of the model use the logarithm of relative cell size, log(CAI), where CAI is the size of yeast cell at time t divided by the size of yeast cell at t=0. Since most of the functions do not contain biological parameters, Zwietering *et al.* [12] re-parameterized these growth curve functions, where A is the maximum value of the growth reached, μ is the maximum growth rate at the lag time and λ is the lag time. The time axis intercepts the tangent at the inflection point.

$$\log(CAI) = \frac{a}{1+\exp(b-ct)} = \frac{A}{1+\exp\{(4\mu/A)(\lambda-t)+2\}} \tag{2}$$

$$\log(CAI) = \frac{a}{\exp\{\exp(b-ct)\}} = \frac{A}{\exp\{\exp[(\mu\cdot e/A)\cdot(\lambda-t)+1]\}} \tag{3}$$

$$\log(CAI) = \frac{a}{(1+\nu\cdot\exp\{k(\tau-t)\})^{\frac{1}{\nu}}} = \frac{A}{\{1+\nu\cdot\exp[(\mu/A)\cdot(1+\nu)^{(1+\frac{1}{\nu})}\cdot(\lambda-t)+(1+\nu)]\}^{\frac{1}{\nu}}} \tag{4}$$

IV. Results and Discussion

A. Yeast Motion

S. bayanus sample was filled in between microscope glass cover slips sealed with silicone and placed under microscope in the absence of trapping laser beam. Living yeast cells moved freely due to currents in PDB solution caused by random molecular motion and temperature gradient. The random motion of yeast observed for 25 minutes recorded by CCD camera was shown in Fig. 4.

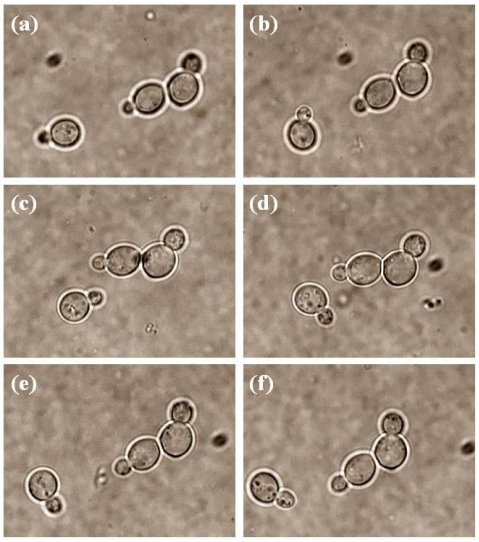


Fig. 4. Images from one representative yeast movement were recorded at (a) 0 min; (b) 5 min; (c) 10 min; (d) 15 min; (e) 20 min; and (f) 25 min.

B. Yeast Trapped under Line Optical Tweezers

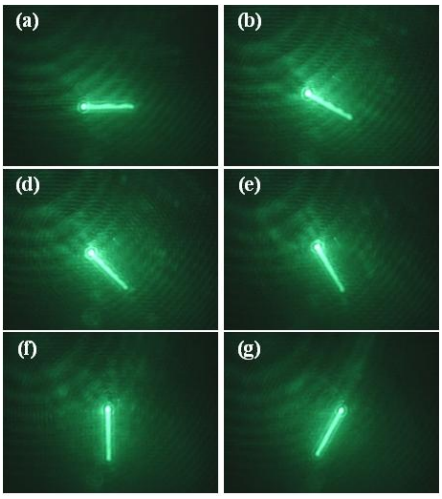


Fig. 5. The linear shape laser pattern was generated in various orientations with angles ranging as (a) 0o; (b) 22.5o; (c) 45o; (d) 67.5o; (e) 90o; and (f) 112.5o.

A time-shared multiple optical traps setup was set under a Nikon Ti-U inverted microscope using 1064 nm fiber laser directing to a galvanometer mirror to obtain a desired laser pattern. Linear shaped laser pattern generated in different kinds of angles was shown in Fig. 5.

In order to control yeast motion, we decided to trap them under optical tweezers using linear shape pattern of different angles as shown in Fig. 6. Yeasts were trapped successfully in these oriented lines.

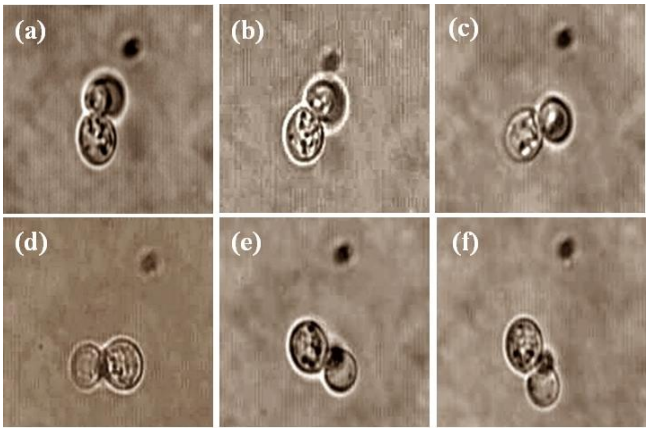


Fig. 6. Yeast was trapped by linear shaped laser pattern in different kinds of angles (a) 0o; (b) 30; (c) 60o; (d) 90o; (e) 120o; and (f) 150o.

C. Growth Pattern under Line Optical Tweezers

Optical tweezers setup was constructed to obtain a line pattern of trapping beams using 1064 nm fiber laser directing to a galvanometer mirror under a Nikon Ti-U inverted microscope. The generated laser pattern in linear shape recorded by CCD camera is shown in Fig. 7.

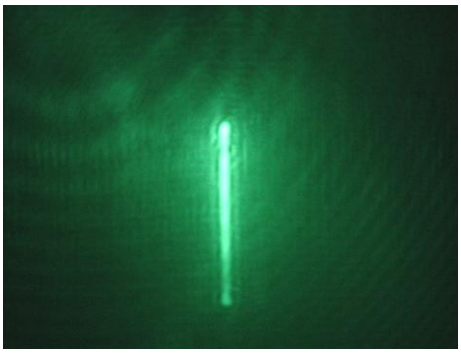


Fig. 7. Linear shaped laser pattern for trapping yeast cells.

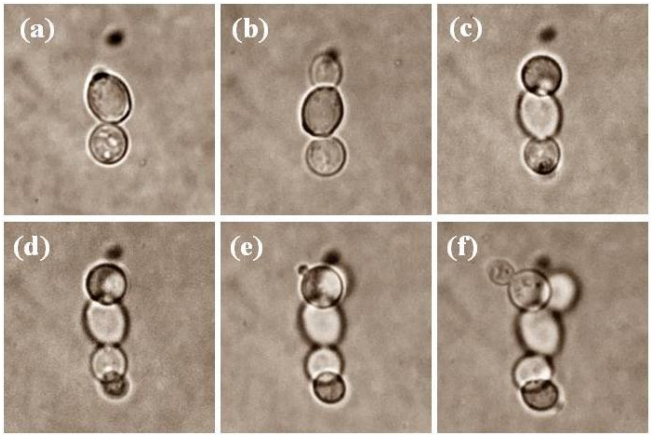


Fig. 8. Yeasts were trapped by linear shaped optical tweezers were recorded at (a) 20 min; (b) 110 min; (c) 205 min; (d) 245 min; (e) 345 min; and (f) 480 min.

After yeasts were trapped by linear shaped optical tweezers, we found that yeast growth followed this line shape. Images from one representative yeast growth recorded for 8 hours was shown in Fig. 8. This behavior can be interpreted as a result of localized heating effect due to absorption at focal point, thus, causing the induced yeast growth direction.

D. Growth Function

A single oval shape of yeast cell in controlled environment was trapped by linear shape optical tweezers in order to study fitted growth function. The budding happened along the laser line. We fitted a nonlinear regression model of yeast cell using logistic, Gompertz and Richards function respectively.

TABLE I: YEAST GROWTH CURVE FITTED BY LOGISTIC FUNCTION AND THE CORRESPONDING RESIDUAL PLOT

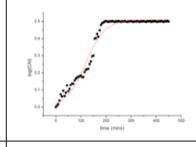
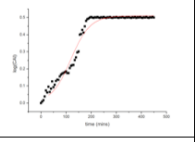
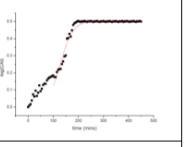
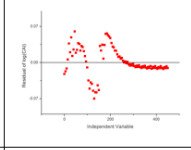
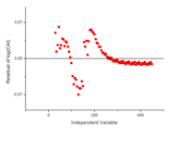
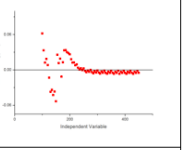
time	t > 0 mins	t > 30mins	t > 100 mins
log(CAI)			
Residual			
RSS	0.06822	0.06551	0.02181

TABLE II: YEAST GROWTH CURVE FITTED BY GOMPERTZ FUNCTION AND THE CORRESPONDING RESIDUAL PLOT

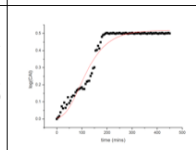
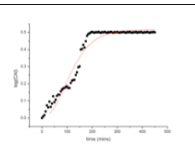
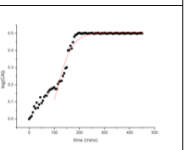
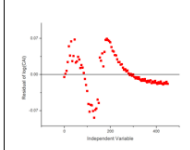
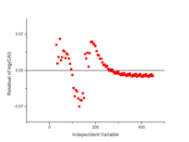
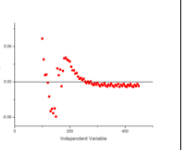
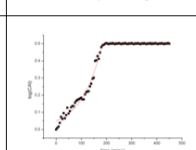
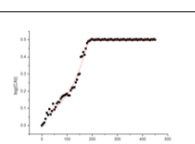
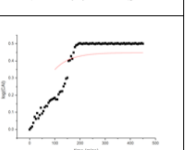
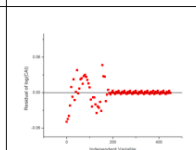
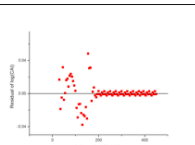
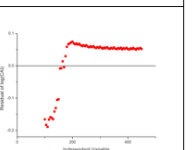
time	t > 0 mins	t > 30mins	t > 100 mins
log(CAI)			
Residual			
RSS	0.10859	0.10358	0.03258

TABLE III: YEAST GROWTH CURVE FITTED BY RICHARDS FUNCTION AND THE CORRESPONDING RESIDUAL PLOT

time	t > 0 mins	t > 30mins	t > 100 mins
log(CAI)			
Residual			
RSS	0.02219	0.01476	0.44873

The graphs were fitted by the functions at t>0 min, t>30 min and t> 100 min as shown in Table I-III. Residual sum of square (RSS) was calculated to find out the perfect fitted

function of yeast growth. A small value of RSS means the model fits the data well. According to table I and II, we found the smallest RSS in each graph at t>100 min implying that logistic and Gompertz functions are the best functions for yeast growth after 100 minutes respectively. However, the growth curve after 30 minutes fitted well with Richards function as shown in Table III.

Nevertheless, the best function for study yeast growth throughout the experimental data (t>0) is Richards function due to the smallest amount of RSS as shown in Fig. 9. The equation of this growth curve is given by

$$\log(CAI) = 0.50033(1+(21.01482)e^{-0.26646(x-171.0468)})^{-\frac{1}{21.01482}} \quad (5)$$

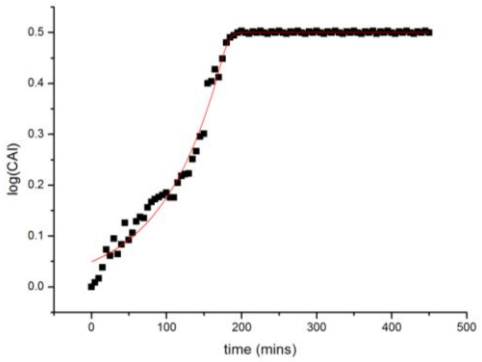


Fig. 9. The growth curve of yeast cell fitted by Richards function.

From re-parameterized Richards function in equation 4, we can find biological parameters from growth curve, A is the maximum value that the growth reached = 0.50033, μ is the maximum growth rate at the lag time = 0.00523 and λ is the lag time = 88.42719 min. The residual of growth curve of yeast cell was shown in Fig. 10.

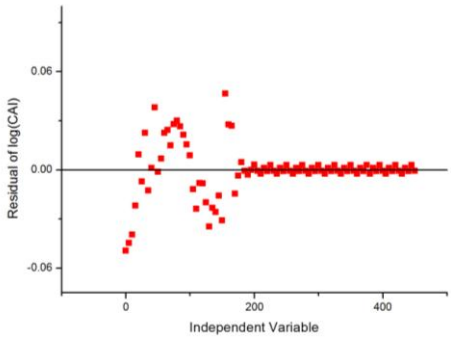


Fig. 10. The residual of growth curve of yeast cell at t >0 min fitted by Richards function.

E. Food Rate and Yeast Growth

Food is one of the important factor for living cell growth. The yeast cells in this experiment were grown by common cultivation in potato dextrose broth (PDB) with two different feeding rates, namely every 24 hours and every 48 hours. After that, they were trapped by 1064 nm linear shape optical tweezers.

The cell area indexes of the samples with two different feeding rates were plotted as a function of time shown in Fig. 11. The slope of this graph fitted by linear function is 0.00523 and 0.00263 for feeding rate every 24 and 48 hours respectively implying that food rate plays an important role for growth rate. The more we feed, the higher rate of yeast growth

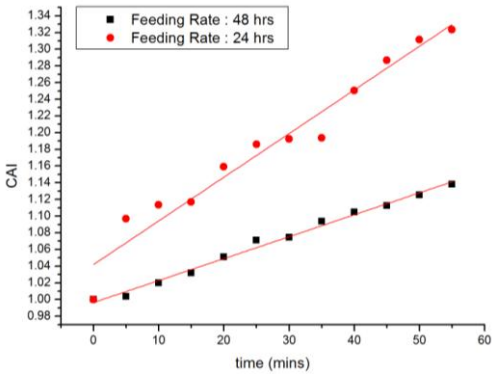


Fig. 11. The growth curve of yeast cell with feeding rate every 24 and 48 hours.

V. Conclusion

We were able to guide the growth pattern of yeast cells using line optical tweezers. Linear guiding growth pattern has been demonstrated successfully. The area of grown yeast cells was measured and plotted as a function of time. The best mathematical model for the growth rate under linear shape optical tweezers is Richards function. In addition, food rate plays an important role for growth rate of yeast cells.

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Dr. Chattham is a member and a referee of American Physical Society. Her Publication was highly commended for 2009 Luckhurst Samulski Prize from Taylor&Francis Publishing Group.