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MASTER THESIS WORK

USING OPTICAL TWEEZERS TO MEASURE ELECTROPHORETICAL MOBILITY OF YEAST CELLS UNDER DIFFERENT CONDITIONS

Pau Mestres Junqué

Supervised by Prof. Dmitri Petrov, (ICFO)

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Using Optical Tweezers to Measure the Electrophoretical Mobility of yeast cells under different conditions

Pau Mestres[1]

1 Institut de Ciències Fotòniques (ICFO), Mediterranean Technology Park, Castelldefels (Barcelona) 088660, Spain

E-mail: pau.mestres@icfo.es

Abstract. Living cells contain different charged groups such as membranes, nucleus, histones, etc... The interaction of these groups with electric fields will affect the basic properties and behavior of cells by determining how they communicate, grow or diffuse. In this work we measure the electrophoretic mobility (EPM) of single yeast cells during normal growth and at different growth stages under hyperosmotic stress using a combination of optical tweezers and a position detector. In the first case we find that EPM shows a non-monotonic behavior during the cell cycle; in the second case we find that in spite of the few morphological changes observed, the EPM presents important variations depending on the time spent under hyperosmotic stress conditions.

Keywords: Optical tweezers, yeast cells, stress response, electrophoretical mobility

1. Introduction

All living beings are made of one or more cells. Living cells are very complex systems that always stay in non-equilibrium states, failing to leads to death. In order to stay out of equilibrium, continuous consumption of energy and several regulating mechanisms are required. Some of these regulating mechanisms consist of ion-gates that maintain specific ion concentrations inside the cell, molecular motors driving cellular motion thanks to the flow of H^+ through membranes [1], and specifically charged proteins that keep cellular components such as DNA densely packaged [2]. In all these processes the charge distribution is vital for the proper functioning of these molecular groups.

The EPM of a particle is "the magnitude of the velocity divided by the magnitude of the electric field strength" when this particle is immersed in a liquid under the influence of an external electric field. Therefore, EPM is a parameter that relates the charge and the geometry of an object by seeing how it flows when a force via electric field is applied [3]. For example, EPM has been extensively used to separate DNA fragments and proteins of different charge and length [4]. The aim of this work is to monitor the

EPM of a single living yeast cell using a non-invasive optical trapping technique such as photonic force microscope (PFM) (i.e. the combination of optical tweezers and a high resolution position detector [5]).

The *Saccharomyces Cerevisiae* yeast cell was chosen as an object of study since it is an excellent model organism for cellular and molecular biology. As general biochemical mechanisms are highly conserved among eukaryotes, most of the current understanding of the processes occurring during the cell cycle came from experiments performed on yeast [6]. Besides being used in baking industry, yeasts are also relevant for the production of industrial chemicals and fuels [7]. Yeast cell sizes are around 5 μm, and being non-infectious, they can be easily manipulated thus not requiring very specific equipment.

2. Experimental

2.1. Setup

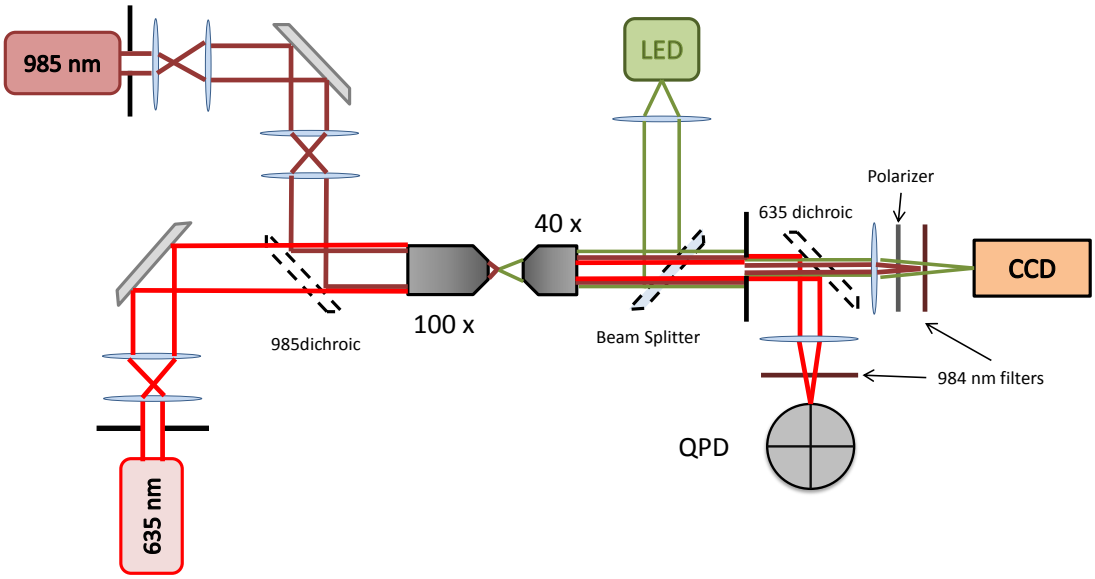

Figure 1. Photonic force microscope (PFM) setup

The optical setup consists of a Photonic Force Microscope (Figure 1). A 985 nm optical beam is focused by a 100X, NA=1.3 oil immersion objective (Nikon CFL PL FL

100x) was used for trapping using an inverted microscope configuration. A green LED in Kohler configuration was focused using a 40X objective for illumination. Finally, the detection system consisted of a 635 nm beam arriving coaxially with the trapping beam at the sample. Then, the forward-scattered light of the detection beam was collected by the 40X objective and sent to a quadrant photo diode (QPD) that was used as position detector. Acquisition was done using Labview at a rate of 2kHz. An extra optical channel was added for a CCD camera to monitor the trapping in the flow cell.

Two different flow cells were designed for this experiment (Figure 2 and Figure 4). The first flow cell was designed for measurements under normal growth conditions. Few modifications were needed in order to be able to shift from normal to hyperosmotic conditions while monitoring the same single living cell. In both cases the flow cells were self made by placing copper electrodes between two masks of parafilm. Later, they were sandwitched between 2 glass coverslips and heated for waterproof sealing. At last, the electrodes were connected to a wave generator that allowed us to apply different AC and DC fields.

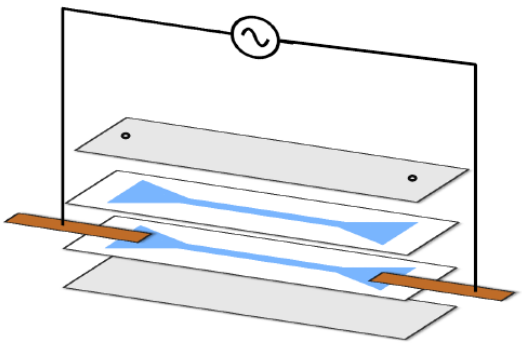

Figure 2. Scheme of different elements assembled to build a flow cell. During the experiment the AC current is applied at the electrodes as showed. Flow cell height was about 200 μm and the coverslip glass had a thickness around 170 μm. Entry holes were drilled to allow injection and output of the samples using a syringe.

2.2. Sample Preparation

The cell cycle of an individual yeast cell can be divided into four phases: mitosis, G1 (gap), synthesis and G2 (gap). Nuclear division occurs during mitosis. The period after this but before the initiation of nuclear DNA replication, during which the cell monitors its environment and its own size, is known as G1 phase. DNA synthesis takes place during the synthesis phase. The period between the completion of nuclear DNA replication and mitosis is termed the G2 phase. The cell cycle is primarily regulated at a point in the late G1 phase when bud forms and continues growing until it separates

from the mother cell after mitosis. Morphological changes undergone by the cell as it goes throw the cell cycle can be detected under the microscope (Figure 3).

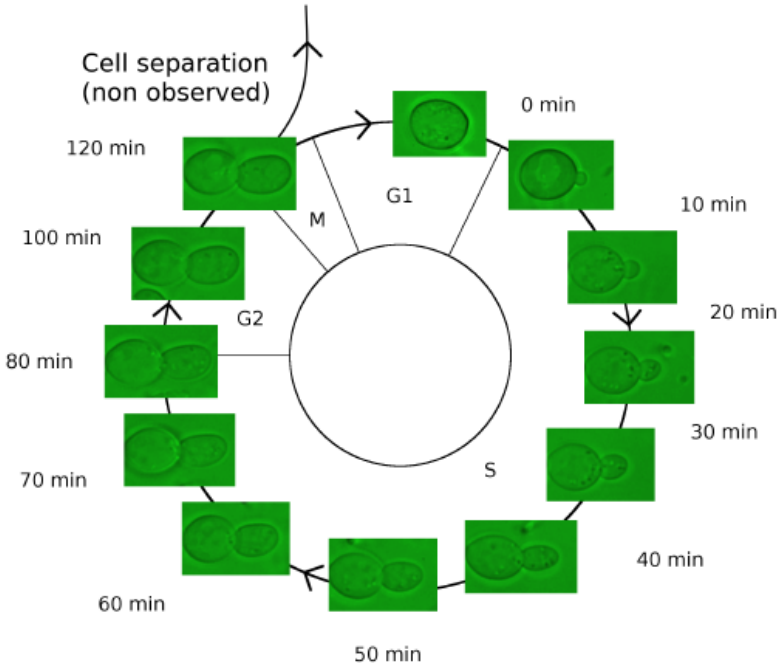

Figure 3. Cell cycle of a yeast cell trapped with 3mW of power (Figure adapted with permission of Mario Tonin)

Yeasts cells were extracted directly from commercially available yeast for bakery. After extraction, they were diluted and grown in yeast extract-peptone-glucose (YPD, Broth, Sygma Aldrich) medium with complete supplement under standard conditions. The pH of the medium was kept around 6.5 to ensure cell viability over the growing process. Finally, cells were added to the flow cell and monitored continuously without trapping until measurements started.

2.3. Measurement Protocol

We carried a preliminary study on the survivability of yeasts cells under trapping conditions and found that below 1mW of laser power cells easily escaped the trap because of brownian motion. Furthermore, if more than 5mW where applied, cell growth was usually affected and alignment of the mother cell and the bud along the optical axis occurred. For this reason, we decided to use 3mW trapping power, where cell remained trapped most of the time and no difficulties in its growth were appreciated.

2.3.1. Protocol for measurements during normal growth If an AC field ranging from 10V (minimum voltage to appreciate oscillations) to 21V (maximum source voltage) was applied at the electrodes during the whole experiment, the cell growth stopped -these correspond to field values of 750 to 1500 V/m in the trapping spot according to our chamber geometry-. For this reason the following measurement protocol was designed: we monitored several yeast cells in the flow cell. When one of them started to grow, we trapped and separated it from the rest of the cells to avoid interference from extra hydrodinamic effects (apart from those coming from flow cell geometry). Then, we applied an AC field and measured the EPM for 25 seconds. After this measurement, we turned the AC field off and let the cell continue growing while trapped. Finally, the AC field was turned on every ten minutes and new measurements were performed until the end of the experiment.

2.3.2. Protocol for measurements under hyperosmotic stress While monitoring the EPM under hyperosmotic stress we discovered that cell growth arrested as soon as the hyperosmotic medium (culture medium with 100g/L of glucose) substituted the standard culture medium. For this reason, a second protocol was designed to perform measurements of EPM at different stages of the cell growth: once a growing cell reached the desired phase we trapped it and measured its EPM as described before. Then, by moving the microscope stage using computer controllable screws, the cell was taken to point 6 (Figure 4), where flow was lower during the flow cell medium change . Finally, the cell was moved back to the same measurement spot 4 (Figure 4). At that moment, measurements of EPM under hyperosmotic stress were performed again every ten minutes as described before.

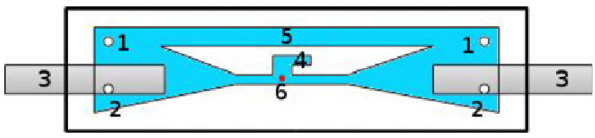

Figure 4. Flow cell to measure under hyperosmotic stress conditions. Channels for liquid are shown in blue. The holes labeled 1 were used as input and output for injection of growth medium with cells. The holes labeled 2 were used as input and output for injection of glucose solution. Electrodes are labeled 3. A channel (labeled 5) was added to divide the input Xux and reduce it at the trapping point 6. We added a narrow channel 4 without output where we kept the optically trapped cell while injecting the glucose solution.

2.4. Data processing

Collected data about position was smoothed using a 4th order Butterworth filter to cut frequencies one order of magnitude above and below the input signal used. Then, the

amplitude of oscillations was calculated using the formula:

$$Amplitude = 2\sqrt{\sigma(x)} \tag{1}$$

where x was the position along the axis the electric field was applied and σ the variance.

3. Results and Discussion

Using a DC electric voltage of known polarity we found out that yeasts cells move following the direction of the electric field, thus giving a positive sign to the EPM according to previous observations [8].

3.1. Measurements during normal growth

Figure 5 shows the amplitude of the oscillations of the monitored cell while applying a AC voltage of 13V at 1 Hz at different stages of the cell growth. It can be seen that the oscillations show a periodical behavior with small fluctuations that we attribute mainly to brownian motion.

The amplitude of these periodical oscillations were retrieved and plotted in Figure 5 (right) for 2 frequencies (10Hz and 1Hz). We see that EPM experiences an important growth -up to three times- in the first 20 minutes of the cell cycle . Then it decays back to at the same values as the beginning of the cycle. Morphologically the cell does not experience many changes during the EPM increase, just a slight growth of the bud. However, as the EPM decreases and reaches the normal values, we notice a significant growth of the bud until itssize is comparable to the mother cell. From these observations we give the following phenomenological explanation:
When the experiment starts (t=0), the mother cell has a certain charge distribution coming from the normal distribution of charged groups (Figure 5 (a)). This charge distribution provides the background oscillation amplitude for the optically trapped cell. When the cell enters the mitotic cycle, the chromosomes in the cell nucleus separate into identical sets; then, the remaining organelles, cell membrane an cytoplasm divide into two cells. Previous characterization of a yeast cell growth by Raman microspectroscopy combined with optical tweezers showed that, during the first 30 min of cell growth, the Raman peaks corresponding to proteins and lipids increase considerably [9]. Now, we observe that the generation of new cell constituents leads also to an increase in the EPM. After 50 min, when the bud formation is completed, the EPM decreases. Finally, when the cell reaches the late G2 phase (t=120 min) the charge distribution goes back to normal inside of each cell, showing the complete division and re-organization of both cells. Since the cell acquires a strong EPM only during a relatively short period of the mitotic phase, we conclude that it is during this stage that the cell carries an important redistribution of charged cellular components, even though the volume of the cell

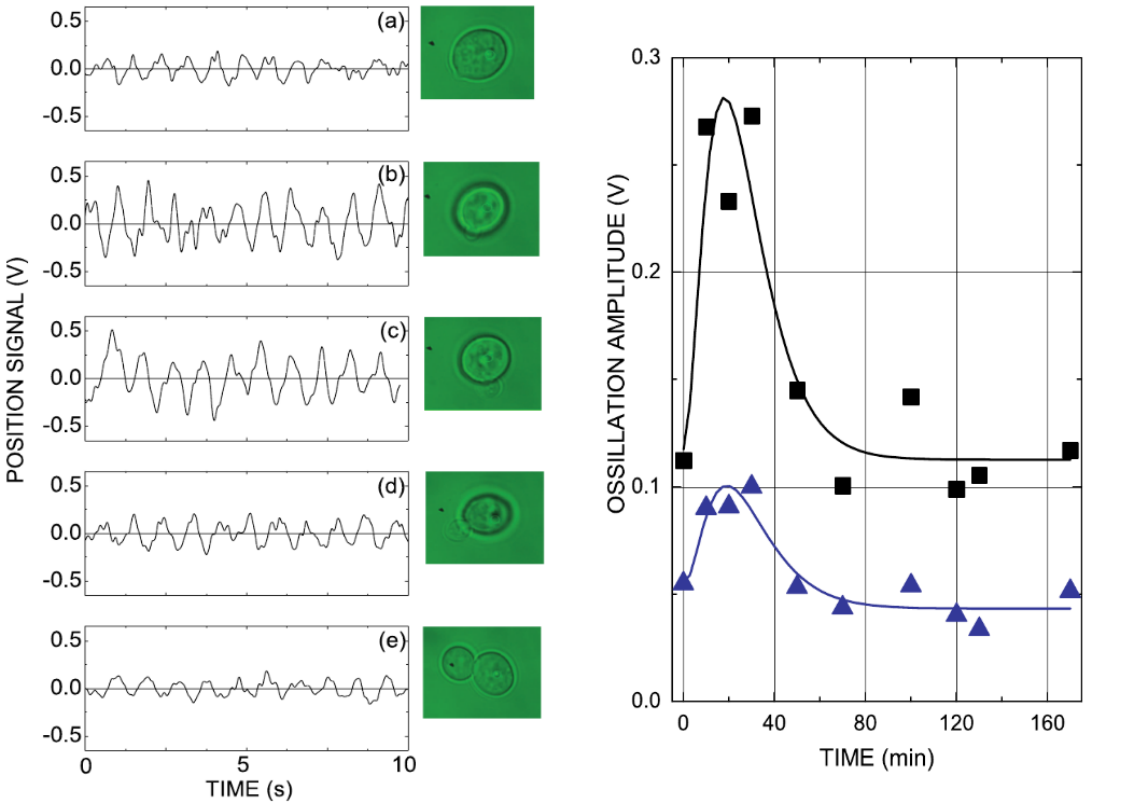

Figure 5. Oscillations of yeast cells when applying an electric field at different growth stages:(a) start of the experiment, (b) 10 min, (c) 20 min, (d) 50 min, (e) 120 min (left). Relative amplitudes of oscillations which are proportional to the EPM of yeast cells for 1Hz (squares) and 10Hz (triangles) (right). Solid lines were obtained by fitting the experimental data using a combination of polynomial and exponential functions and they are only used to emphasize the EPM behavior.

doesn't increase significantly. After this period, the organization of of charged cellular components returns slowly to normality as the daughter cell grows.

With a frequency of 10 Hz we observe smaller amplitudes of the cell oscillation due to the viscosity of the cytoskeleton. However, the general behavior was consistent with the results at 1 Hz.

3.2. Measurements under hyperosmotic stress

We studied the EPM stress response of yeast cells at three different growth stages: late G2, early budding and advanced budding phase. Since cell growth arrested as soon as the hyperosmotic pressure was induced, we can simply evaluate how EPM changes for a given phase of the growth at different times of hyperosmotic stress.

While monitoring the yeast cells under the microscope the following morphological changes were observed: (1) Cell growth stopped regardless of the stage at which the

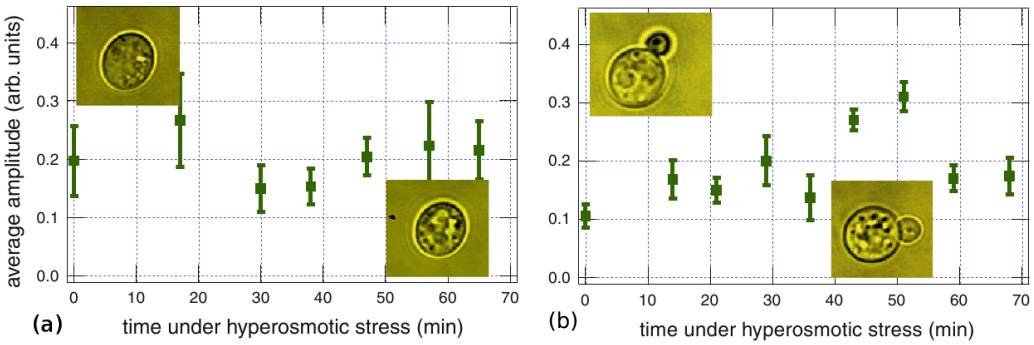

Figure 6. Oscillation amplitude of yeast cells under hyperosmotic stress during late G2 phase (a) and during early budding phase (b). Left insets show an image of the cell before applying hyperosmotic stress and right insets show the same cell under stress conditions. Small shrinkage and better contrast in the inner organelles can be appreciated.

hyperosmotic stress begins. (2) After adding glucose, shrinkage of the cell was observed along with morphological changes that caused an increase of the contrast of organelles (see insets in the following figures). (3) At the end of the measurements, after changing back the hyperosmotic medium for the normal culture medium, the cell growth resumed normally.

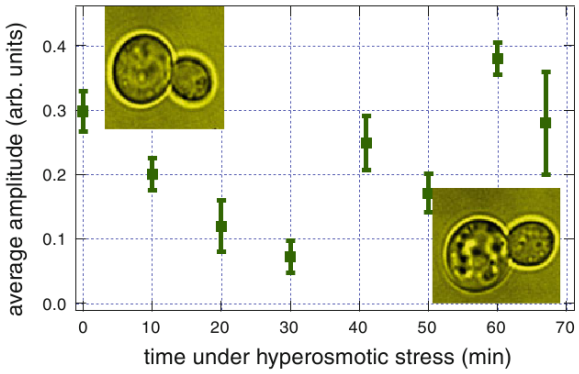

Figure 7. Oscillation amplitude of yeast cells under hyperosmotic stress during advanced budding phase. Left insets show an image of the cell before applying hyperosmotic stress and right insets show the same cell under stress conditions. Small shrinkage and better contrast in the inner organelles can be appreciated.

Measurements of the EPM time dependence under stress conditions at the late G2 phase or early budding phase (Figure 6) showed that EPM remains more or less constant. Nevertheless, when the cells were in an advanced budding phase an increase of the EPM was observed after 30-40 minutes under stress (Figure 7). Our interpretation is as follows:

When the cell membrane is subjected to hyperosmotic stress, an stress response mechanism is triggered. This evokes intracellular changes such as temporal arrest of the cell cycle, membrane depolarization [10] and a contribution of water from the vacuoles to the cytoplasm to compensate the leakage through the membrane due to the difference in osmotic pressure. This loss of water explains why the organelles could be observed with greater contrast after few minutes in the hyperosmotic medium.

One of the most important mechanisms known to occur in yeast cells to counter hyperosmotic stress is the production of glycerol, which acts as a protector against osmosis by re-equilibrating the osmotic pressure [11]. It was reported that an increase of the intracellular glycerol concentration occurs in approximately 40 min after the stress begins [12]. Based on this observation, we may relate the EPM increase for cells subjected to stress at advanced budding phase to this glycerol production mechanism. However, although the cell seems to stabilize and undergoes through several internal changes in order to resume the cell cycle, the osmotic pressure is still too high to allow the cell continue its growth. This is proved by the fact that, after removing the hyperosmotic medium, the cell growth resumes.

4. Conclusions

Changes in EPM may provide additional information by characterization of the biochemical processes in single living cells. For example, the measured EPM differences might be useful for separation of budding yeast cells without causing critical damage.

These results also prove that the study of electrical properties of single living cells under different conditions can be performed by means of PFM and a suitable flow cell, thus giving alternative measurements to standard biophysical assays.

In both measurements, low frequency modulation of the signal appears. We suggest that these modulations are caused because optical trapping occurs on the cellular organelles with highest refractive index. Nevertheless, these organelles do not necessarily coincide with the local charged groups. This spatial difference can give rise to small torques that slightly rotate the molecule giving rise to the low modulation frequencies observed.

Finally, we emphasize that many details of the EPM stress response remain unclear. Further studies under different stress conditions such as changes in pH or high temperatures would provide valuable information.

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