In this experiment budding yeast cells which were synchronized with α factor were imaged. In addition, FACS analysis was performed for some of the samples.

### A.1. Alpha Factor-Based Synchronization

Yeast *Saccharomyces cerevisiae* cells may grow and multiply as haploids or diploids. Haploid yeast cells are either MATα or MATα cells, and haploid cells of different types may fuse together to form a diploid cell. When cells grow as haploids, they convert their type every time they divide, i.e., MATα cells become MATα cells and MATα cells become MATα cells. An important part of this process called mating-type conversion is a site-specific cleavage in the MAT-locus that is performed by HO endonuclease. MATα cells of HO mutated yeast strain cannot convert to MATα cells, and if there are no MATα cells in the population, the cells cannot fuse to form diploid cells.

Mating (fusion) of α and a cells is induced by pheromones. MATα haploid cells secrete a pheromone called a factor and MATα haploid cells secrete a pheromone called α factor. When the receptors of a MATα cell recognize the presence of α factor, the cell growth stops in the G1 phase of the cell cycle and the MATα cell starts waiting for fusion with a MATα cell. Thus, α factor synchronization is based on an artificial introduction of α pheromone to a cell population of MATα cells which are HO mutated. After the cells are released from the resulting α factor arrest, they grow again as haploids but now in synchrony.

The genotype of the yeast strain that was used in the experiment was MATα (BY4741; MATα; his3D1; leu2D0; met15D0; ura3D0; YIL015w::kanMX4). The overnight grown culture was refreshed to an OD600 of 0.6 and then grown to an OD600 of 0.8 in YEP 20 g/l glucose in 2 hours and 15 minutes. The growth took place at +30°C in ten 250 ml bottles, each containing 150 ml medium. During growth the bottles were shaken at a rate of 200 rpm. At OD 0.8, the pH was adjusted to 4 with 1 M HCl. After the adjustment, α factor (Nova Biochem, Switzerland, prod. no. 05-23-5300, batch no. A18684) was added to a concentration of 1 µM to each bottle and the shaking was continued. After 60 minutes, the medium and α factor were removed by centrifuging the cells at 5000 rpm for 10 minutes at the working temperature +4°C (Beckman Coulter Allegra 25R centrifuge). The cells were resuspended in approximately 10 ml fresh and cold YEP glucose and then inoculated to 1.5 liters YEP glucose in order to let them grow without α factor arrest. The initial OD600 was 0.8.

During the subsequent sampling the cells grew in a fermentor (Braun Biostat CT-DCU 3). The temperature was kept at +30°C, pH at 5.5, and the medium was agitated at a rate of 1000 rpm. A 1 ml sample was taken from the fermentor every 2 minutes for 280 minutes in total.

### A.2. Microscope Imaging

Microscope imaging was performed for all the 140 samples that were taken from the fermentor. 15 µl of each sample was pipetted on a 1 mm microscope slide and each sample was covered with a #1.5 cover glass. Three different fragments were then imaged for each sample.

Imaging was performed with an upright bright field light microscope (Olympus BX51). An achromatic condenser with the numerical aperture adjusted to 0.4 and a 100x Universal Plan Fluorite oil immersion objective with 1.3 numerical aperture were used (Olympus UPLFL 100x OP). The light source was a 100 W Tungsten Halogen bulb. The immersion oil had a refractive index of 1.515−1.517 and was pipetted onto each sample slide at the microscope.

The magnification of the camera adapter was 0.5 leading to the overall 50 times magnification (Olympus U-TV 0.5x). The camera had a 1.5 million pixels CCD and a color filter array (Olympus DP-50). The used exposure time was 1/25 s. Exact information on the size of each pixel was not available, but it can be estimated to be around 5 × 5 µm. The images were saved in an uncompressed tiff-format with the image size 2776 × 2074 pixels. In the images the diameter of a full-grown yeast cell is approximately 100 pixels.

The software that was used for imaging included Pixera Corporation Viewfinder 1.0 and Pixera Corporation Studiolite 1.0 running under Microsoft Windows 2000 (SP3) on an AMD Thunderbird 1.3 GHz PC with 1280 Mb of memory. These software performed the color interpolation required in using a CCD camera with a color filter array.
A.3. FACS Analysis

The FACS analysis was performed for every third sample that was taken from the fermentor (starting from the second sample), i.e., every 6 minutes. The samples were chilled on ice immediately after harvesting. Cell doublets were separated by sonicating the samples 3 times for 30 seconds with output power 40 W (Branson Sonifier 450). In the last 11 samples the sonication times were increased while the output power remained the same. After sonicating, the cells were removed from the medium by centrifuging them at 13000 rpm for 2 minutes at the working temperature +4°C (Eppendorf Centrifuge 5415R). The supernatant was then discarded, and cells were fixed by gently shaking them in 1 ml of 70% ethanol for one hour.

After five days of preservation at +4°C, the fixed cells were centrifuged at 13000 rpm for 5 minutes at room temperature. They were washed with 35% ethanol, centrifuged again, washed with water, and centrifuged again. Then, the cells were suspended to 0.5 ml of 2 mg/ml ribonuclease A solution (50 mM Tris-HCl, pH 7.5). They were incubated by gently shaking the solution for 1.5 hours at room temperature.

Next, the cells were spun down with the centrifuge and suspended to 0.5 mg/ml of pepsine media (55 mM HCl). They were then incubated for 1.5 hours at room temperature in the same way as was done for the ribonuclease A solution. Then, the cells were centrifuged down and suspended to 0.5 ml of propidium iodide media (180 mM NaCl, 70 mM MgCl2, 100 mM tris, 75 µM propidium iodide). They were incubated by slowly shaking the solution at room temperature for 1 hour to stain the DNA. Finally, the DNA contents of the cells were estimated with a FACS analysis (Becton-Dickinson FACS Calibur).

A.4. Summary of Obtained Data

The data were obtained from three different sources. Firstly, 140 readings of oxygen and carbon dioxide concentrations along with the respective pH values were obtained from the fermentor at 2 minute intervals. Secondly, three times 140 images of yeast cells were obtained from the microscope (also at 2 minute intervals). Some of the images are not focused perfectly, and as a result, generally 1–2 images are successful for each sample. Each image contains approximately 5–15 cells, although some variation exists. Finally, the FACS analysis produced 5 results with 15 minute intervals during the α factor arrest and 47 results with six minute intervals after the α factor arrest. Each FACS result gives the DNA content of 20000 cells.