Y04C APPLICATION NOTE

Dynamic Live Cell Imaging of Yeast

INTRODUCTION

Yeast cells serve as an important class of model organisms for the study of eukaryotic cell biology. Applications of yeast cells include use in bioreactors, food preparation, synthetic biology, and infectious disease research. Key advantages are their ease of cell culture, amenability to genetic manipulations, and high degree of homology with human cells. Current methods for yeast research address cells on a population level, with measurements averaged over millions of individual cells. The recent trend towards single cell analysis reflects the demand for more detailed information such as intercellular variation, dynamic response profiles, intergenerational relationships, spatial information, and morphological features.[1,2] Advances in high resolution microscopy enable quantification of individual yeast cells,[3,4] but traditional culture chambers do not provide optimal conditions for tracking cells over time. The small size (~4 µm) and non-adherent nature of most yeast make live cell imaging experiments difficult with conventional methods.

CellASIC has developed the Y04C microfluidic plate for spatially localizing yeast to a single monolayer for long term, high quality live cell microscopy. The microfluidic chamber traps cells using an elastic ceiling (3.5-4.5 µm height) without preventing perfusion flow or hindering cell growth. Six upstream fluidic channels allow controlled exposure of the cells to different solutions during live imaging. The cells are in contact with a #1.5 thickness (170 µm) optical glass surface, enabling high quality imaging using an inverted microscope.

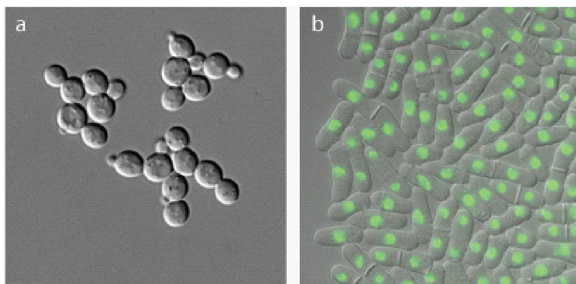

Figure 1. (a) Budding yeast and (b) fission yeast imaged in the Y04C microfluidic plate. The microfluidic chamber maintains the cells fixed in space in a single focal plane for time-lapsed perfusion live cell imaging. Images courtesy of Jan Wisniewski, NIH, and Hironori Niki, NIG, Japan.

The operation of the Y04C microfluidic plate was demonstrated using *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *S. cerevisiae*, or budding yeast, is a widely used model in cell biology. The cells are spherical, with a 4-10 µm diameter (strain dependent), and can survive as haploid or diploid cells. S. pombe, also called fission yeast, is a model organism commonly used to study cell division. They are rod shaped, with a 3-4 µm diameter and 7-14 µm in length. Both species can be cultured with synthetic liquid media, and have typical doubling times between 90 minutes to a few hours. The trap heights of the Y04C microfluidic plate (3.5-4.5 µm) were tuned for haploid cerevisiae and pombe cells, however other cell types with a similar size profile will also be amenable to the Y04C. For larger trap sizes, see the Y04D plate (5-7 µm).

PLATE DESIGN

The Y04C microfluidic plate is built on the ONIX platform developed by CellASIC (www.cellasic.com/ONIX). The plate has a SBS standard footprint compatible with typical microscope stage holders. The custom well layout was designed to maximize live cell imaging capabilities. The Y04C has 4 independent units (A-D), with each unit containing 8 wells (6 inlets, waste, and cell inlet). The four cell culture chambers are centralized under a single large imaging window (see figure 2). The chamber to chamber distance is 4.0 mm, reducing objective travel time and focus drift. The bottom surface of the plate is a #1.5 thickness (170 µm) optical glass slide to maximize quality of high resolution, high numerical aperture imaging. The plate houses all experiment solutions allowing control with an external pneumatic manifold (see figure 3). The manifold lets the user direct flow rates and select exposure solutions without perturbing the microscope stage. Additionally, a gas line allows control of the environment within the microchambers through a network of gas permeable air diffusion channels.

CELL TRAPPING

Each culture chamber has 3 cell trap regions. The traps are 1.0x1.0 mm in area, with heights of 3.5, 4.0, and 4.5 µm. Cells are loaded by pressure driven flow from well 8

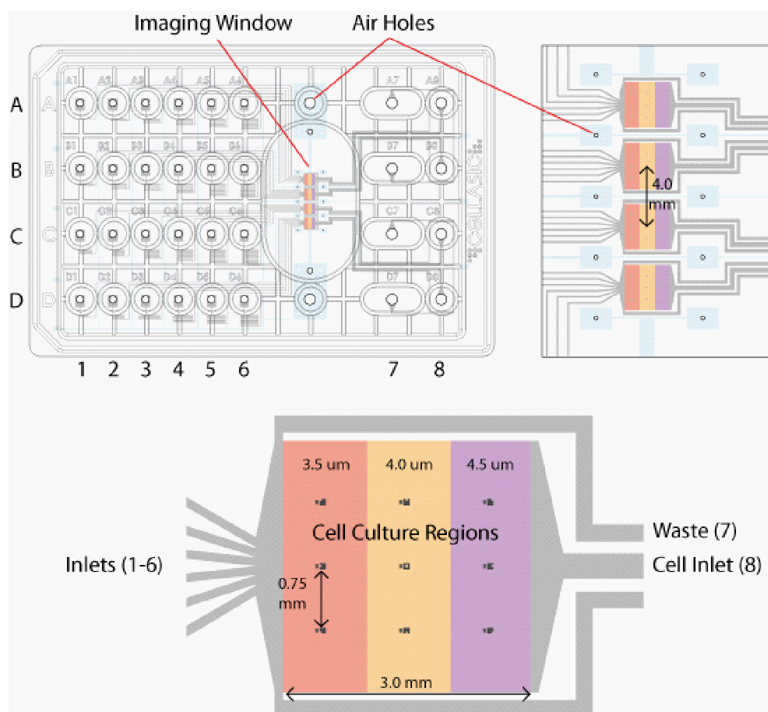

Figure 2. The Y04C plate contains 4 independent flow units (A-D), each with 6 upstream solution inlets. Additional wells are provided for cell in and waste. The culture chamber contains 3 rectangular trap regions with ceiling heights of 3.5, 4.0, and 4.5 µm. Cells are loaded from the right channel and pressure loaded into the appropriate trap site. Continuous flow of solutions from the inlets creates a dynamic exposure profile during live cell imaging. Nine position markers (black squares) are embedded within the trap channels.

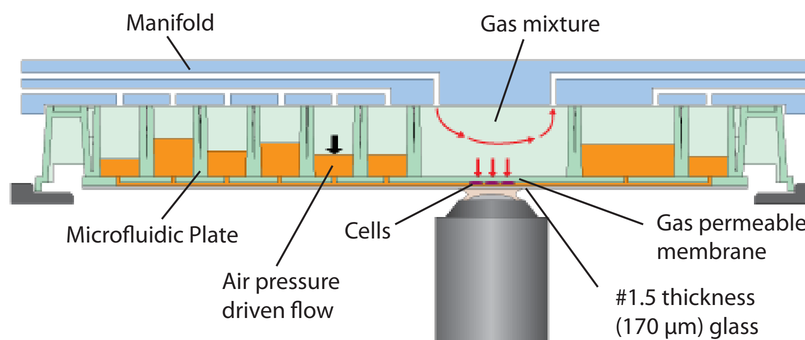

Figure 3. Side view schematic of the microfluidic plate on a microscope stage. The bottom surface of the microfluidic plate is a thin glass sheet, allowing high quality cell imaging. The plate is sealed to a pneumatic manifold, allowing user control of the flow profile during imaging. Additional air channels allow control of the gas environment.

into the back side of the traps. This pressure expands the elastic ceiling to allow cell entry. Depending on their size, cells will become trapped at varying distributions in the 3 trap locations. Excess cells are washed out through the two "arms" to well 7. For *S. cerevisiae*, it is typical to see cells localized in the 3.5 µm region. Trapped cells are held against the glass floor by the elastic ceiling when it returns to its rest position (see figure 4 a-d). Because the ceiling never touches the floor, there is adequate space for solution to flow past the cells. Adjusting the pressure and duration of the pressure loading step can modify the loading profile. Loading cells at different initial densities is the best way to alter the number of cells loaded (see figure 4e).

SOLUTION SWITCHING

Exposure solutions are introduced from the 6 inlet wells and flow through the chambers (to well 8) as well as out through the waste (well 7). The flow rate and exchange times are given in figures 5a and 5b, respectively. When the input solution is changed, a sharp fluid interface is created that moves across the culture area from left to right due to the highly laminar flow profile. The velocity of this front is given in figure 5a. The time it takes for the entire front to reach the end of the culture chambers is plotted in figure 5b. The actual local exchange time (the transition from solution 1->2 around the cells) happens much more quickly, typically in a few seconds. The small volume of the culture chamber enables fast solution exchange at flow rates from 2-30 µl/hour. This means that a typical experiment (with 300 µl per inlet well) can run for over 24 hours without perturbing imaging sequence.

A key feature of the Y04C plate design is that solutions can be changed during live cell imaging without perturbing the plate or microscope. This enables tracking of cell responses to changing solution environments. The Y04C allows 6 different solutions to be switched during the course

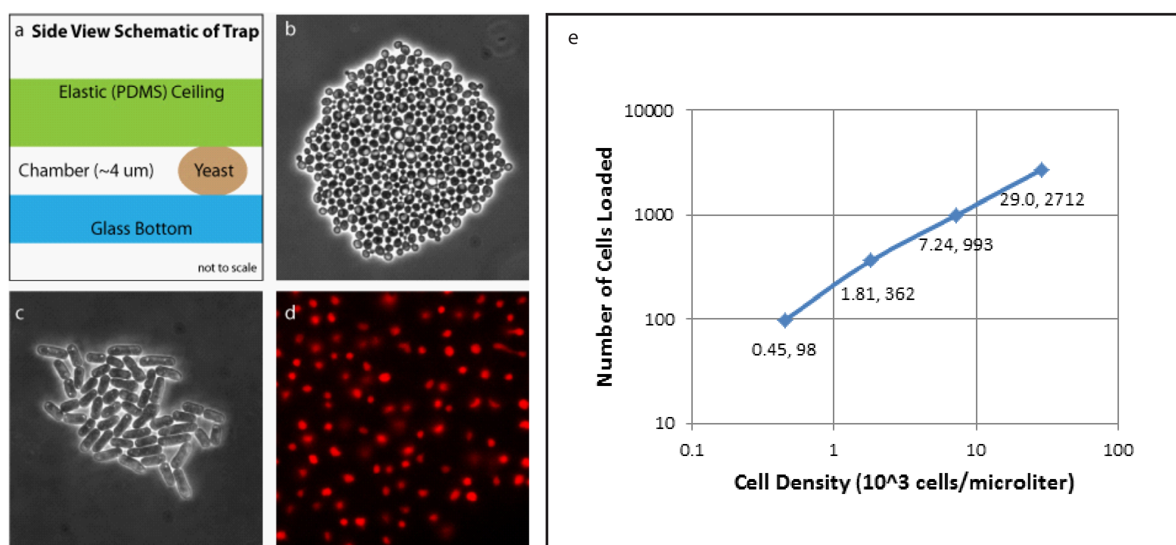

Figure 4. Imaging of yeast in the microfluidic trap. (a) Side view schematic of the cell trap chamber shows the glass bottom floor, a trapped yeast cell, and the elastic PDMS ceiling. (b) Phase contrast image (40X objective) of a single colony of S. cerevisiae in the 3.5 μm trap region after 12 hours perfusion culture. (c) Phase contrast image (40X objective) of a single colony of S. pombe in the 4.5 μm trap region after 12 hours of perfusion culture. (d) Fluorescence image of a colony of S. cerevisiae expressing RFP in the nucleus (100X objective). S. cerevisiae courtesy of Jan Skotheim, Stanford. S. pombe courtesy of Susan Forsburg, USC. (e) Initial cell loading profile as a function of cell suspension density. S. cerevisiae at 4 densities were loaded at 8psi for 5 seconds and counted in the 3.5 μm trap region.

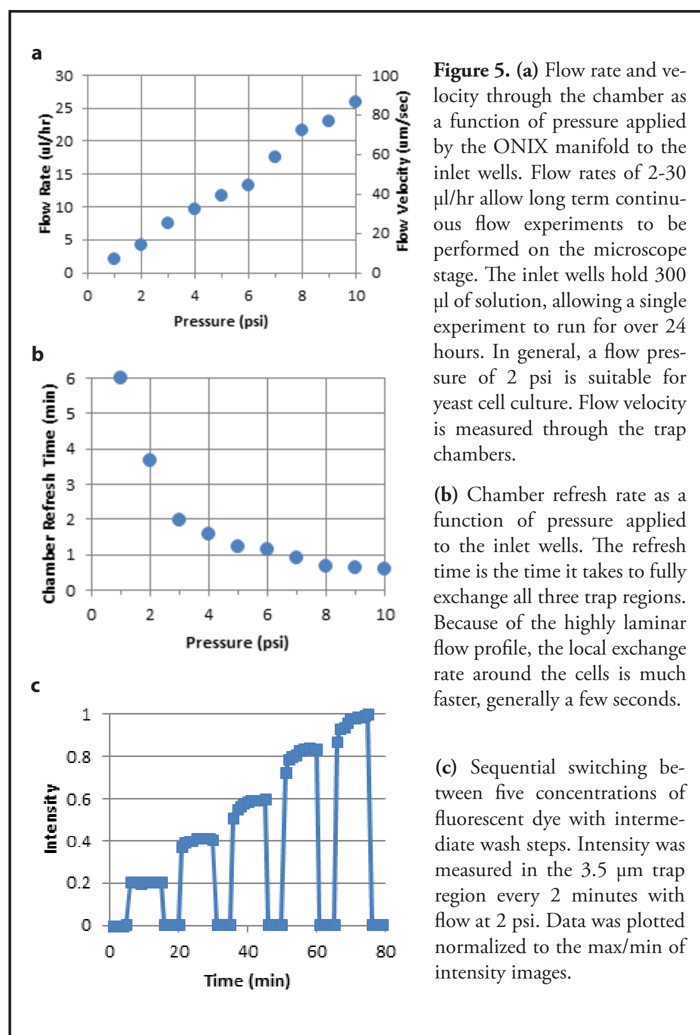

Figure 5. **(a)** Flow rate and velocity through the chamber as a function of pressure applied by the ONIX manifold to the inlet wells. Flow rates of 2-30 μl/hr allow long term continuous flow experiments to be performed on the microscope stage. The inlet wells hold 300 μl of solution, allowing a single experiment to run for over 24 hours. In general, a flow pressure of 2 psi is suitable for yeast cell culture. Flow velocity is measured through the trap chambers.

(b) Chamber refresh rate as a function of pressure applied to the inlet wells. The refresh time is the time it takes to fully exchange all three trap regions. Because of the highly laminar flow profile, the local exchange rate around the cells is much faster, generally a few seconds.

(c) Sequential switching between five concentrations of fluorescent dye with intermediate wash steps. Intensity was measured in the 3.5 μm trap region every 2 minutes with flow at 2 psi. Data was plotted normalized to the max/min of intensity images.

of the experiment. As one example, 5 concentrations of a fluorescent dye (20%, 40%, 60%, 80%, and 100% of dextran conjugated fluorescein, 3kDa, Invitrogen) were switched at 10 minute intervals with 5 minute wash steps in between (see figure 5c). Fluorescence intensity was measured in the 3.5 μm trap region with a 20X objective. Note the rapid and complete response of the solution, creating a clean "step function" in the culture region. Since all six channels converge near the culture chamber, the Y04C plate minimizes the dead volume during switching to a few nanoliters. Even with this small dead volume, there will be a brief interval (generally a few seconds) immediately after a switch where the old is washed out before the selected solution flows in. For sensitive kinetic experiments, it is recommended that a tracer dye be used to accurately follow solution flow profiles.

TIME LAPSE IMAGING

The favorable cell culture environment in the Y04C chamber allows long term maintenance of yeast under precisely controlled conditions. Since the cells are trapped in x,y,z space, it is possible to track cell responses over time on the same group of cells. This allows collection of kinetic response data on live cells not possible with other approaches. Moreover, as the cells are prevented from moving, intracellular dynamics can also be monitored with high resolution. Cell growth in the trap chamber was equivalent to expected values, indicating there is no detrimental effect of

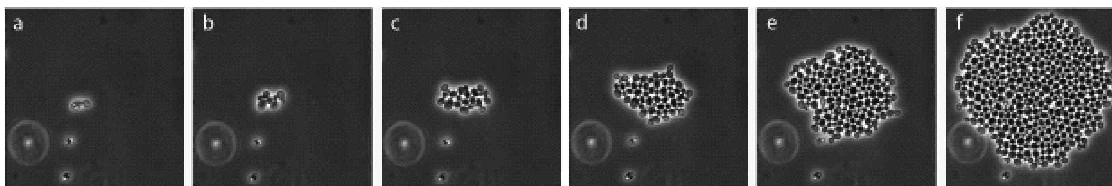

Figure 6 Growth of S. cerevisiae in the 3.5 µm trap at (a) 0, (b) 2, (c) 4, (d) 6, (e) 8, and (f) 10 hours with perfusion at 2 psi. Images taken with a 40X phase contrast objective.

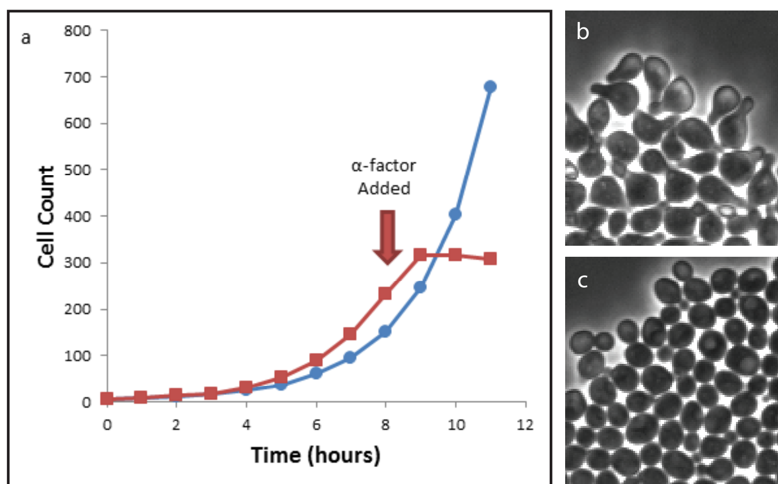

Figure 7. Cell growth response to addition of α-factor. (a) Cell growth curves of S. cerevisiae cultured in the Y04C plate with perfusion at 2 psi over 11 hours with (red squares) and without (blue circles) addition of 100 µM α-factor. Phase contrast images at 11 hours of (b) α-factor treated and (c) control cells.

the microfluidic culture configuration (see figure 6). As cells begin to overgrow the chamber, excess cells will flow out to the waste well (well 7). Generally at this point, the chamber is completely confluent, and flow transport will be reduced due to the large cell mass.

As a demonstration of monitoring cell growth response over time, *S. cerevisiae* was cultured in SC complete medium for 8 hours, then switched to medium containing α-factor (100 µM). These cells should respond to α-factor by arresting the cell cycle and growing schmoos. Two chambers were imaged simultaneously with a 40X phase contrast objective and monitored for growth rate. As expected, after exposure to α-factor, the yeast cells stopped growth and changed morphology (see figure 7). Similar experiments include adding an inducer/inhibitor of expression, changing concentrations of a compound over time, or performing time-varying exposures.

SUMMARY

The ability to track individual yeast cells with time lapse imaging is beneficial to a variety of applications. Current approaches are limited in the ability to maintain non-adherent yeast cells in a single focal position for time-lapse imaging and long-term monitoring of response to solution changes. CellASIC has developed the innovative Y04C microfluidic perfusion chamber specifically designed to trap yeast cells in a single focal plane without limiting solution exchange or cell growth properties. This design has been demonstrated for monitoring *S. cerevisiae* and *S. pombe* for long term culture, fluorescence quantification, solution exchange response, and time-varying inputs. Further, the ease-of-use, flexibility, and accessibility of this advanced technology platform should prove beneficial to a wide range of yeast cell biology applications.

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