

HIGH-RESOLUTION LIVE CELL IMAGING OF THE YEAST LIFE CYCLE

Olivier Frey¹, Fabian Rudolf², and Andreas Hierlemann¹

¹ETH Zurich, Dept. of Biosystems Science and Engineering, Bio Engineering Laboratory, Basel, Switzerland

²ETH Zurich, Dept. of Biosystems Science and Engineering, Comp. Systems Biology Group, Basel, Switzerland

ABSTRACT

Layout, operation, and results of a microfluidic device, which allows for anaerobic and aerobic cultivation of yeast cells over extended time (>3 days) are presented. The use of this device in combination with automated image analysis software and multiple fluorescence reporters (>4 fluorescence protein per strain) allows for studying timing and coherence of morphological features, the cell-cycle timing, stress-pathway activation, and the occurrence of protein aggregates during the whole cell life cycle. Moreover, the device enables to investigate the inheritance of such features over several cell generations under different metabolic conditions.

KEYWORDS

Live cell imaging, 2D cell culture, yeast, perfusion

INTRODUCTION

Elucidation of cell-to-cell variability and associated hereditary transmission are at the forefront of current research in single-cell analysis. In order to investigate the reasons for cell-to-cell variability – including, for example, stochastic noise in gene expression, different cell age or different microenvironments – experimental setups are required that enable to monitor cells and their progeny at single-cell resolution over their whole life span.

Most of the aging-involved processes are guided by molecular events. These can be observed by advanced light and fluorescence microscopy, which recently have been combined with cell cultivation in microfluidic chips [1-3]. Advances in both fields already allowed the generation of consistent single-cell data sets under realistic conditions at subcellular resolution [1,2,4].

Assessment of cell heterogeneity and its inheritance needs further developments and an improved synergy between microfluidic cell culturing and microscopy setups. This includes devices enabling simple and reliable culturing of different cell populations in parallel under precise and dynamic control of the microenvironment, as well as non-restricted optical access for high-resolution live-cell imaging and tracking in long-duration experiments.

In this contribution, we present a microfluidic chip featuring extremely simple but robust operation and cell loading, which has been used for live-cell imaging of several yeast strains in parallel. Population growth has been constrained in a horizontal plane and, therefore, enables high-resolution time-lapse imaging using automated microscope control and image acquisition.

EXPERIMENT

The microfluidic layout is shown in Figure 1a. It consists of three identical compartments, which allows simultaneous cross-talk-free experiments with three different yeast strains in parallel, and a surrounding channel structure for perfusion purposes. Microfluidic structures have been fabricated in PDMS, casted from a two-layer SU-8 mold, fabricated by standard photolithographic processes. The heights of the two layers can be adapted with regard to optimal flow-conditions and cell clamping force. Loading and operation of the microfluidic chip is extremely simple without compromising imaging resolution (see Figure 1). Cells (10^8 /ml) are loaded into dedicated areas of the O_2 -plasma-activated PDMS using a conventional pipette. The droplet is kept in place by a surrounding trench, which acts as capillary stop and prevents the liquid to be spread over the whole hydrophilic PDMS surface (1). A glass slide (150 μ m thick) is placed on top to seal the microfluidic network and clamp the cells between the soft PDMS and the glass (1-4 μ m gap, adjustable with respect to target cells). In this way, cells are immobilized, and population growth is constrained to the same focal plane. During the sealing step, excess medium is guided sideways into the overflow compartments (b). This prevents a spreading over the whole PDMS-glass interface, which would render a tight, irreversible bond impossible. Previous to the loading process, the PDMS layer is extensively degassed in a vacuum chamber and allows the uptake of air enclosures in the microfluidic network during the loading process. 20-30 minutes after loading, the microfluidic chip is completely filled without any remaining bubbles that could eventually upset fluid-flow conditions.

A larger microfluidic ring structure surrounds the three culturing sites and ensures continuous perfusion and nutrient supply. It has been designed so as to minimize the delivery time of oxygenated media into the chip (<3 min), while keeping flow-speeds in the compartments low (<50 μ m/s) in order to reduce shear-stress on the cells and to avoid cell displacement. We used a Wheatstone-bridge-analogous arrangement and introduced the flow resistor, R_F , which generates a pressure difference between the lower and upper channel (Fig. 1a). Different R_F values have been realized in the parallel fabrication process to have variability in internal flow-rates in different experiments. An electric circuit model, based on calculated flow-resistances, was required to optimize the inlets of each single cell-culturing compartment, R_{Celli} , in order to generate equal flow-rates in all of them.

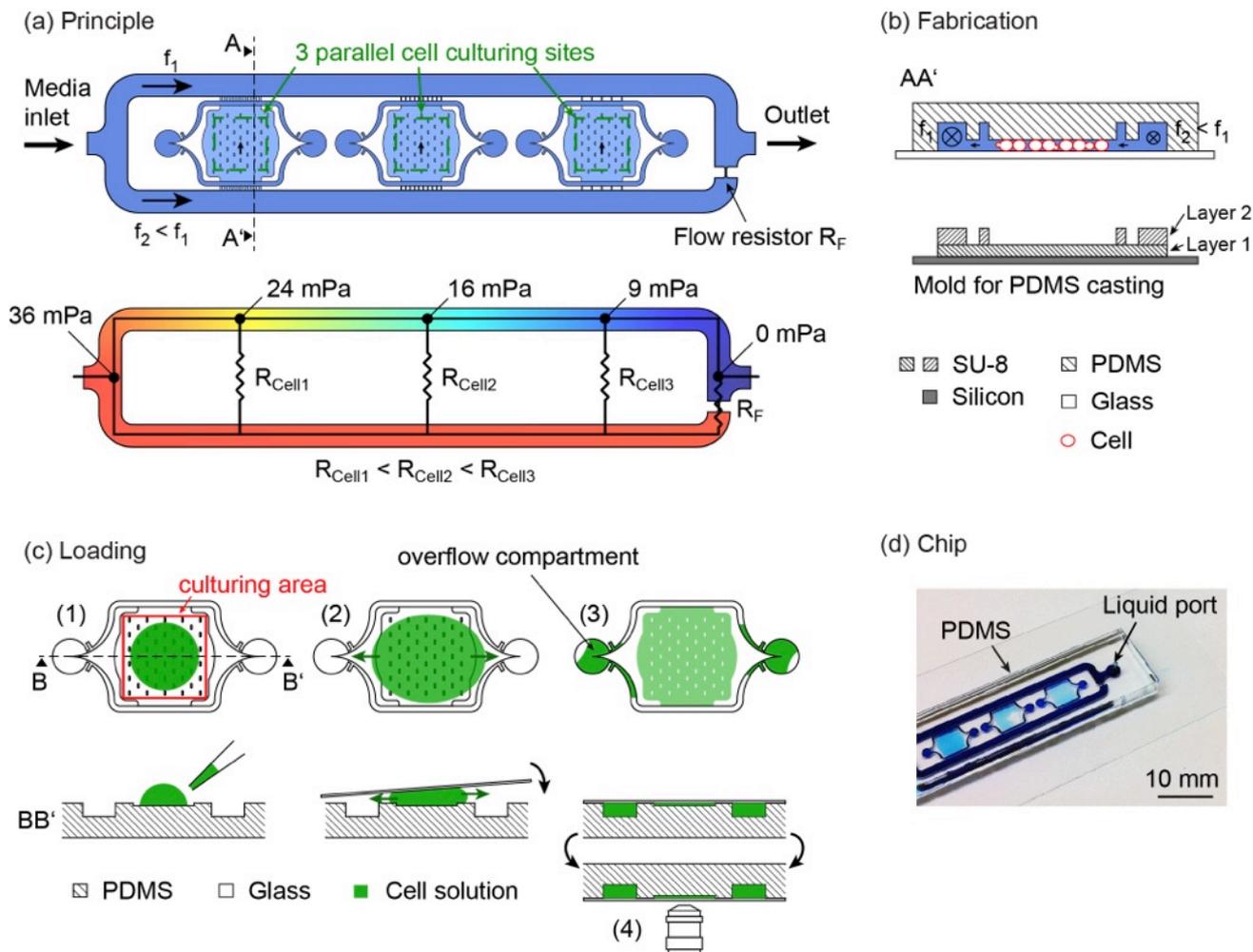


Figure 1: (a) Layout and pressure conditions in the microfluidic network (top view). The flow resistances of the three parallel cell-culturing compartments ($R_{Cell1-3}$) are adjusted to generate equal flow-rates. The pressure differences are resulting from the channel constriction, R_F (Wheatstone bridge analogy). (b) Side view of the PDMS chip and the two-layer SU-8 mold. (c) For loading, cell solutions are pipetted (1) on the activated PDMS surface (channels open to top), and the chip is then sealed with a glass slide pushing excess liquid sideways (2) into the overflow compartments and clamping the cells between PDMS and glass (3). For experiments, the chip is flipped, and liquid tubings are connected (4). (d) Fabricated chip filled with food color for visualization.

After loading with cells, the inlet of the chip (Fig. 1d) is connected to an oxygenated media tank, and the outlet to a standard syringe pump in withdrawal mode (5-10 $\mu\text{l}/\text{min}$). Standard tubings are plugged into previously punched in- and outlet holes in the PDMS layer. The device is placed on an inverted fluorescence microscope (Nikon Ti-Eclipse) with automated stage, a hardware autofocus device, and custom-made acquisition software (www.youscope.org). Time-lapse imaging (bright-field and fluorescence) is performed at up to 30 selected sites in parallel (5 to 10-min interval).

To characterize the performance of the chip, we used different *S. cerevisiae* yeast strains featuring different fluorescence markers. Figure 2, for example, shows frames of a typical time lapse movie of yeast cells cultured under continuous perfusion over 24 hours. The *S. cerevisiae* cells express a fluorescence marker for budneck formation (Myo1) appearing each time at the end of a growth cycle. This can be observed over the whole experiment. For the strain shown in Figure 3, an additional marker for “Start” inhibition (Whi5) has been introduced. The live-cell-imaging shows continuous and natural growth times throughout the “cell-sheet” even though the carbon source (glycerol) requires high oxygen concentrations ($>20 \text{ mM L}^{-1} \text{ h}^{-1}$) during the experiment. The presented perfusion approach is therefore suited for the requirement of aerobic conditions in the microfluidic chip.

Further, it is shown that yeast cells are not stressed under these conditions by growing a strain featuring a fluorescently tagged general stress response transcription factor (Msn2) in one of the parallel chambers. Stress can be induced during loading, as well as during the whole experiment as a consequence of the mechanical pressure on the cells. The Msn2 transcription factor disperses throughout the cytoplasm in unstressed conditions and relocates into the nucleus upon environmental stress, such as the absence of oxygen. Figure 4 shows a healthy yeast cell population during the first 8 hours in culture. After an experimenter-induced stop of perfusion and related oxygen supply, bright fluorescent spots appear due to the relocation of the transcription factor.

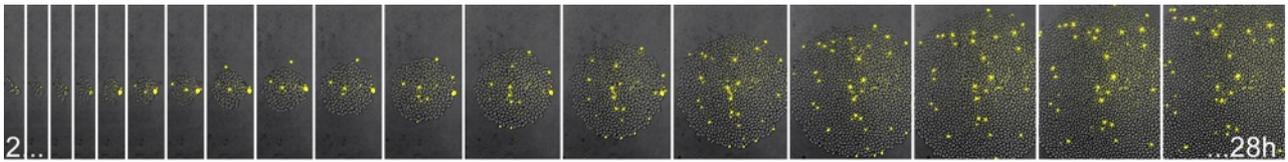


Figure 2: Frames (28 hours, regular capture) of a dual channel (bright-field and fluorescence) time-lapse movie of yeast cells tagged with Myo1-Citrine. Myo1 localizes at the bud neck from S phase to the end of mitosis. The bright yellow cells are dead cells. Cells are grown in glucose (anaerobic conditions).

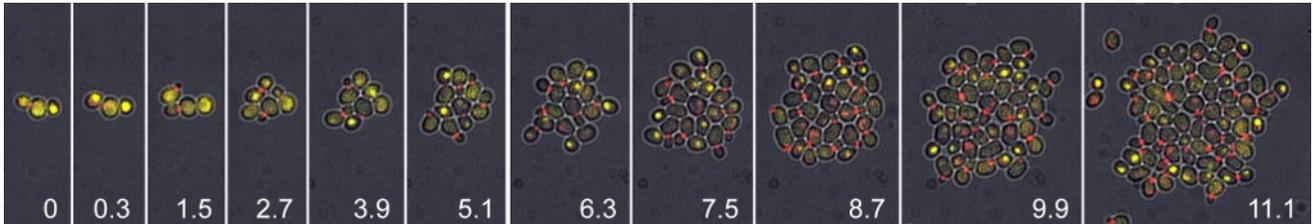


Figure 3: Frames of a 3-channel (1 bright-field and 2 fluorescence) time-lapse movie from yeast cells tagged with Myo1 (red) and Whi5 (yellow). Cells are grown in glycerol (obligatory aerobic conditions).

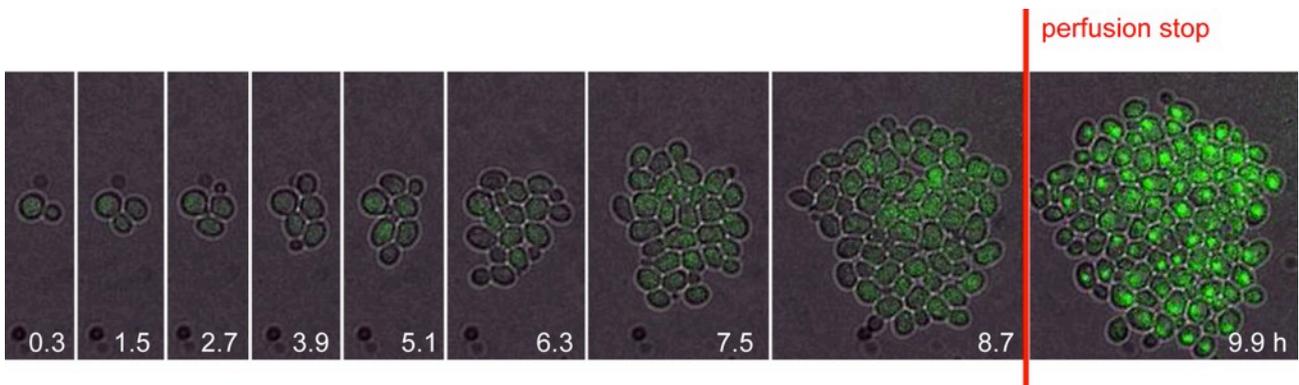


Figure 4: Initial frames of a yeast colony featuring Msn2 (green). The red line indicates the stopping of the perfusion and reduced oxygen availability. Cells are grown in glycerol (obligatory aerobic conditions).

With this system, we are currently studying the genealogy and cellular heterogeneity of cell populations under different metabolic conditions and try to quantify the molecular and subcellular changes associated with aging of yeast cells.

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CONTACT

Olivier Frey 41-61-387-3344 or olivier.frey@bsse.ethz.ch