Supporting Information

Pump-free multi-well-based microfluidic system for high-throughput analysis of size-control relative genes in budding yeast

Xianjie Kang[1, 2], Lingli Jiang[2], Xi Chen[2], Haiyu Yuan[1], Chunxiong Luo[1, 2],* Qi Ouyang[1, 2,3]*

[1]The State Key Laboratory for Artificial Microstructures and Mesoscopic Physics, School of Physics, Peking University, China, [2]Center for Quantitative Biology, Academy for Advanced Interdisciplinary Studies, Peking University, China. [3]Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, 100871, China.

*To whom correspondence should be addressed: ChunxiongLuo, Email: pkuluocx@pku.edu.cn, Tel: +86-10-62754743; Qi Ouyang, Email: qi@pku.edu.cn, Tel: +86-10-62756943.

1. Microfluidic chip fabrication

The chip was fabricated with PDMS (polydimethylsiloxane, RTV615, USA) using the standard soft lithographic technique. The mold of chip was constructed by patterning photoresist (SU-8, Clariant Corp.) on silicon wafer in a classical overlay lithography procedure. The photoresist relief mold consists of three parts (**Figure 1**a): the first growth chamber has a height of about 7.1μm and a cross-sectional area of 180μm × 180μm (red); the second growth chamber has a height of about 4.2μm and a cross-sectional area of 180μm ×180μm (purple); the last enrichment chamber has a height of about 40μm and a cross-sectional area of 250μm ×500μm (green). The second growth chamber is connected to the first growth chamber through three fine necks which are 30μm wide and 4.2μm high (purple); and the last enrichment chamber is connected to the second growth chamber through three fine necks which are 30μm wide and about 2.0μm high (blue) (see **Figure 1**a).

We first manufactured the lowest part of the chip mold. To manufacture the three fine necks of 2.0μm high, a bare 100 mm diameter, 500 μm thickness silicon wafer was spin coated with SU-8 3002 photocurable epoxy using a spin coating machine (MYCRO WS-400BZ-6NPP-LITE, USA) to a thickness of about 2.0 μm, followed with prebaking on a hot plate of 65℃ for 1 min and 95℃ for 5 min subsequently. After exposure to 365 nm UV light of intensity of 3.2 mW/cm^2 for 50 s with the corresponding mask, the coated wafer was postbaked at 65℃ for 1 min and 95℃ for 5 min subsequently. After development procedures a 2.0 μm thick mold was obtained

on the wafer.

We then manufactured the 4.2μm high part of the chip mold. The previous silicon wafer with 2.0 μm thick mold on was spin coated with SU-8 3005 photocurable epoxy using the spin coating machine (MYCRO WS-400BZ-6NPP-LITE, USA) to a thickness of about 4.2 μm, followed with the similar procedure as before. After development procedures a 4.2 μm thick mold was obtained on the wafer.

Similarly, we then manufactured the 7.1μm and 40μm high part of the chip mold on the same silicon wafer in succession. We finally obtained the complete mold of chip as shown in the bottom right of **Figure 1**a.

To fabricate the chip body, PDMS monomer and curing agent were mixed in a ratio of 8:1 by weight, poured over the silicon wafer mold, and degassed. After being cured at 75℃ for 30 min, the cured PDMS of thickness of about 0.5 cm was carefully peeled off from the mold and cut into individual module. To help punch the loading wells more accurately and conveniently, an inverted camera and corresponding software were used to define the position precisely. After punched all the loading wells, the PDMS component was then bonded to a 25 mm × 75 mm glass slide using air plasma. The resulting device had 32 separated loading wells and isolated growth environment for different budding yeast strains.

2. Verification that nutrient is abundant enough to sustain cell to grow exponentially.

Figure 3c shows measure example of mother cell doubling time (MCDT) and the corresponding daughter cell doubling time (DCDT). From the timing diagrams of cell division, we can obtain that $MCTP_1$ = 50 min, $MCTP_2$ = 110 min; $DCTP_1$ = 50 min, $DCTP_2$ = 130 min. Therefore, the conclusion is that MCDT = 60 min and DCDT = 80 min. Meanwhile, we had tracked cells of the example strain used in **Figure 3**c to test whether mother cell doubling time/daughter cell doubling time was consistently the same or not with continuous cell division. Some of the repeatedly measured data of MCDT/DCDT for the example strain were shown in **Table S-1**, from which we can found that MCDT/DCDT of the example strain didn't change significantly between two consecutive cell divisions. That is to say, the nutrient is abundant enough to sustain cell to grow exponentially. In fact, about ten measurements were performed to get each parameter (MCDT, DCDT, MCS and DCS) for each strain in the experiment, and the average results were calculated. During the about ten measurements, we had

confirmed that MCDT/DCDT was basically the same with continuous cell division for all of the strains.

Besides, whether the nutrient is abundant enough or not for cells to grow exponentially depends on the OD of the cell suspension, not the absolute volume of the medium. In the experiments, 2μL medium containing different strains with OD about 0.1 was dropped into each well of the chip respectively, followed with 20μL fresh medium. The OD of the cell suspension during the limited imaging time was low enough to sustain cell growth at the exponential phase, which was verified by our experimental observations and data in **Table S-1**. In fact, cells could grow exponentially more than 10 hours in our system. However, for our purpose of measurement, we didn't need such a long time.

<table>
<tr><th></th><th>From the 1st to the 2nd cell division</th><th>From the 2nd to the 3rd cell division</th><th>From the 3rd to the 4th cell division</th></tr>
<tr><td rowspan="8">MCDT /DCDT (min)</td><td rowspan="4">MCDT=65</td><td rowspan="2">MCDT=60</td><td>MCDT=60</td></tr>
<tr><td>DCDT=80</td></tr>
<tr><td rowspan="2">DCDT=80</td><td>MCDT=65</td></tr>
<tr><td>DCDT=75</td></tr>
<tr><td rowspan="4">DCDT=80</td><td rowspan="2">MCDT=65</td><td>MCDT=65</td></tr>
<tr><td>DCDT=85</td></tr>
<tr><td rowspan="2">DCDT=85</td><td>MCDT=60</td></tr>
<tr><td>DCDT=75</td></tr>
</table>

Table S-1. Measurements of MCDT/DCDT in the process of consecutive cell division.