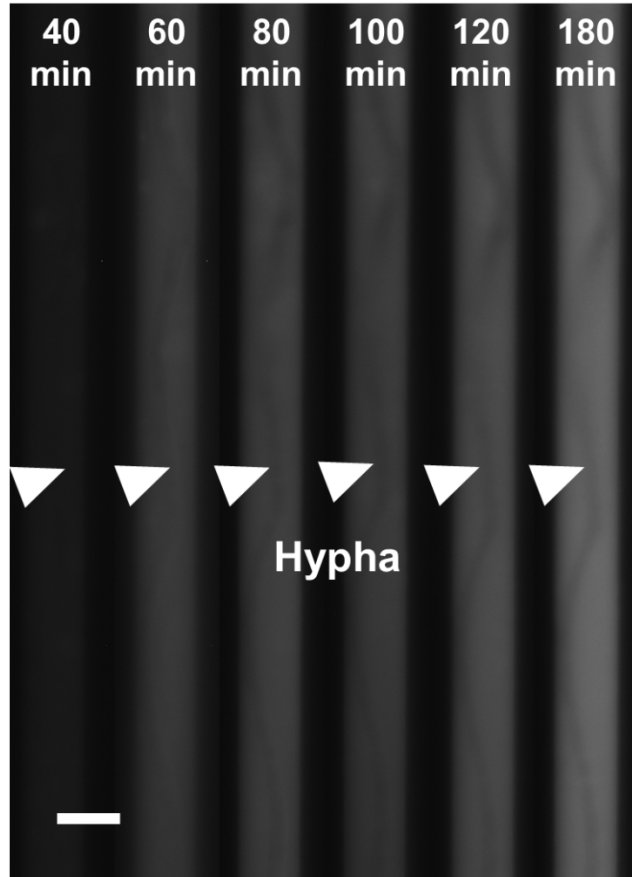


## **Supplementary information**

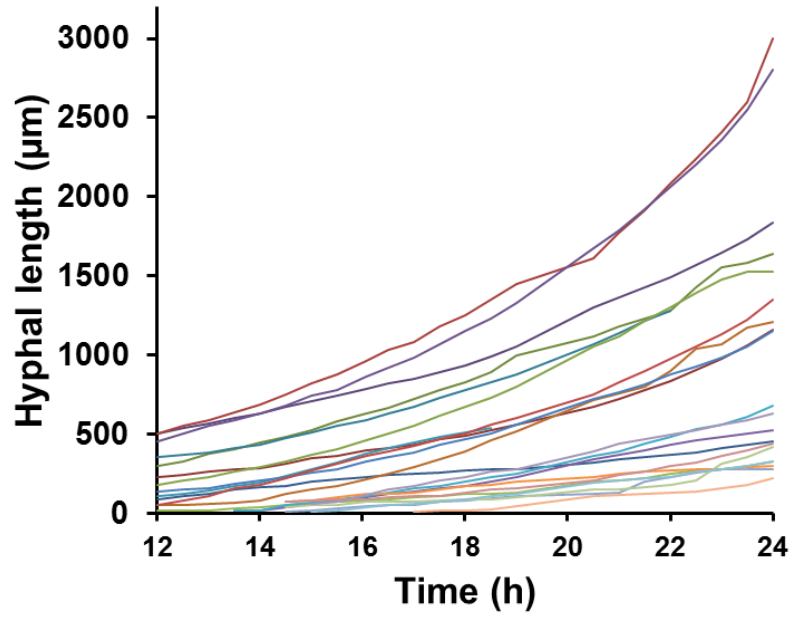
### **Compartmentalized microchannel array for high-throughput analysis of single cell polarized growth and dynamics**

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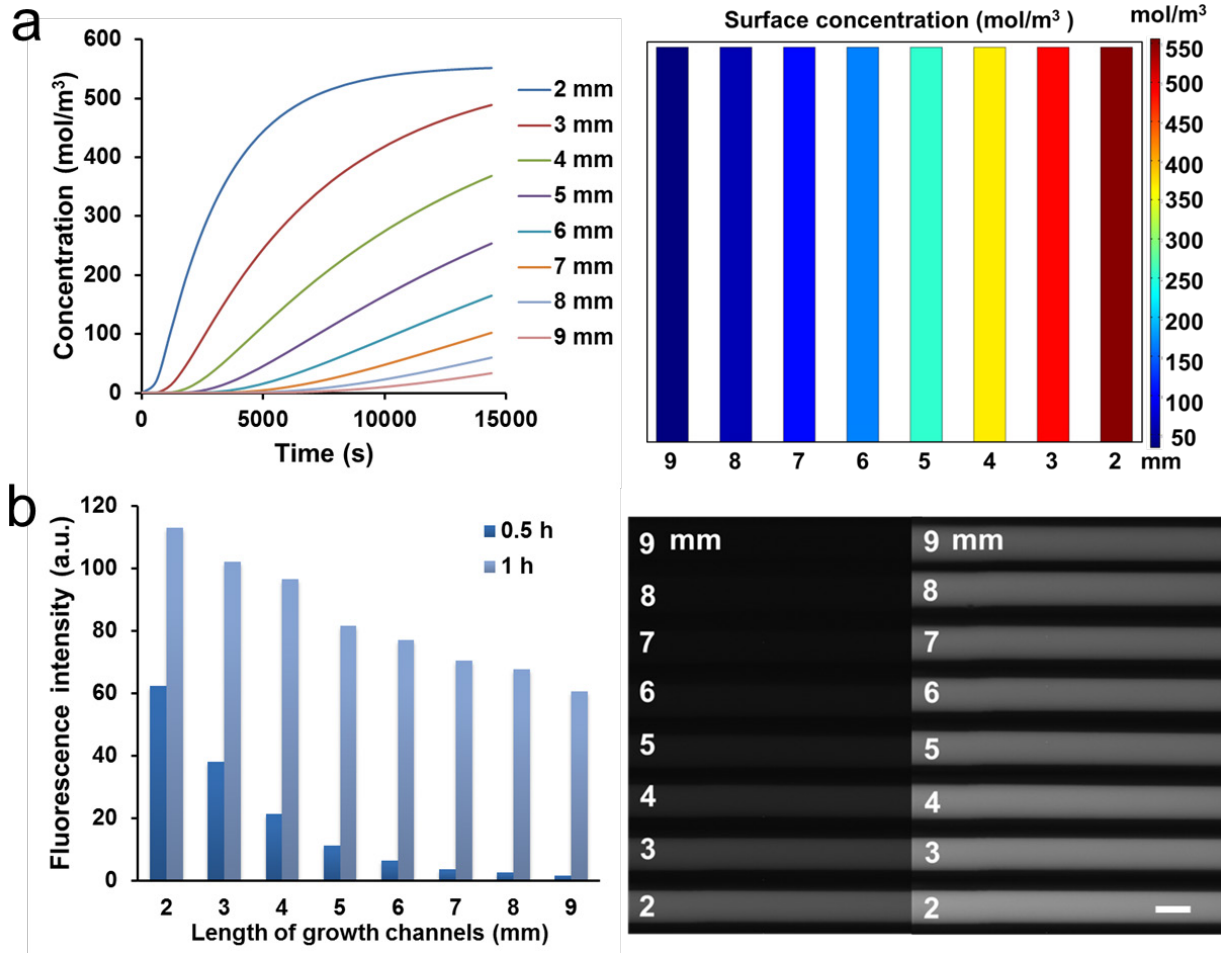
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**Supplementary Figure 1.** Diffusion of 2-NBDG into a channel filled with a growing hypha. The fluorescence intensity increases over time, indicating medium exchange is not blocked in the hyphal growth channels ( $2.5 \text{ mm} \times 10 \text{ }\mu\text{m} \times 10 \text{ }\mu\text{m}$ ) by the hyphal extension. Scale bar,  $10 \text{ }\mu\text{m}$ .



**Supplementary Figure 2.** Growth curves of 27 individual hyphae growing in the channels ( $2.5 \text{ mm} \times 10 \text{ }\mu\text{m} \times 10 \text{ }\mu\text{m}$ ) on a single microchip. In the experiment, wild-type *N. Crassa* is grown under the constant flow of 10% glucose in  $1\times$  Vogels' salts solution at  $0.3 \text{ }\mu\text{L}/\text{min}$ .



**Supplementary Figure 3.** Chemical concentration gradient on the microfluidic chip with different lengths of channels. **(a)** Numerical simulation of time-dependent diffusion of glucose in the hyphal growth channels with lengths of 2, 3, 4, 5, 6, 7, 8 and 9 mm using COMSOL Multiphysics software. The initial concentration of the glucose is 555 mol/m<sup>3</sup> (10% w/v). Right panel shows the glucose concentration in the 120 μm of regions from the trapping sites after 4 h of diffusion. **(b)** Visualization and quantification of the gradient profile after flowing 100 μM of 2-NBDG solution for 0.5 h and 1 h. Scale bar, 10 μm.

**Supplementary Video 1.** Hyphal extension of *N. crassa* histone H1-RFP strain NMF617 in a  $2.5 \text{ mm} \times 10 \text{ }\mu\text{m} \times 10 \text{ }\mu\text{m}$  growth channel. The video is taken for 5 min at intervals of 1 s.

**Supplementary Video 2.** Nuclear dynamics in an apical compartment of *N. crassa* histone H1-RFP strain NMF617 in a  $2.5 \text{ mm} \times 10 \text{ }\mu\text{m} \times 10 \text{ }\mu\text{m}$  growth channel. All nuclei unidirectionally move towards the growing tip. The video is taken for 5 min at intervals of 1 s.

**Supplementary Video 3.** Nuclear dynamics in parts of two adjacent subapical compartments of *N. crassa* histone H1-RFP strain NMF617 in a  $2.5 \text{ mm} \times 10 \text{ }\mu\text{m} \times 10 \text{ }\mu\text{m}$  growth channel. The nuclei move irregularly including oscillation, bypassing and passing through septa. The video is taken for 5 min at intervals of 1 s.