

Supplementary information on 'The microfluidic lighthouse: an omnidirectional gradient generator'

A. Nakajima^a, M. Ishida^b, T. Fujimori^b, Y. Wakamoto^{a,b} and S. Sawai^{a,b,c}

^{a.} *Research Center for Complex Systems Biology, Graduate School of Arts and Sciences, The University of Tokyo. Komaba, Meguro-ku, Tokyo 153-8902, Japan. E-mail: cssawai@mail.ecc.u-tokyo.ac.jp*

^{b.} *Department of Basic Science, Graduate School of Arts and Sciences, The University of Tokyo, Japan*

^{c.} *PRESTO, Japan Science and Technology Agency, Kawaguchi-shi, Saitama 332-0012, Japan*

Supplementary Figures

Figure S1

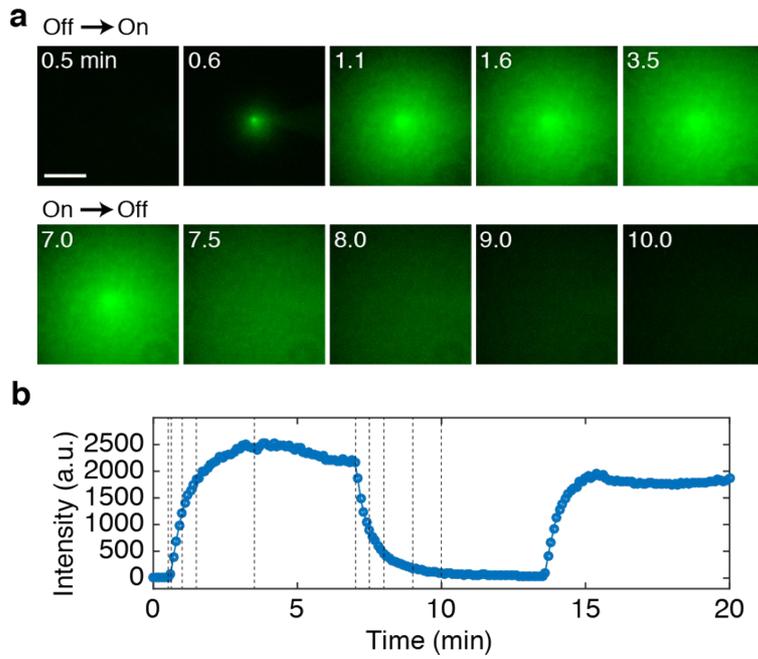


Fig. S1. The conventional gradient generation and removal using a glass needle occur at minutes-timescale. a) Confocal images of fluorescence intensity profile during gradient development (upper panels) and attenuation (lower panels). A glass needle (Femtotip, Eppendorf) loaded with 30 μ M fluorescein PB solution was positioned at the centre of the images. The needle was pressurized using an injector (Femtojet, Eppendorf) at $t = 0.5$ min (upper leftmost panel) and stopped at $t = 7.0$ min (lower leftmost panel). The images are in logarithmic scale. Scale bar, 100 μ m. b) Time course of the fluorescent intensity averaged over the observation area. Time slices in a) are indicated by vertical dashed lines. The glass needle was pressurized again at $t = 13.5$ min.

Figure S2

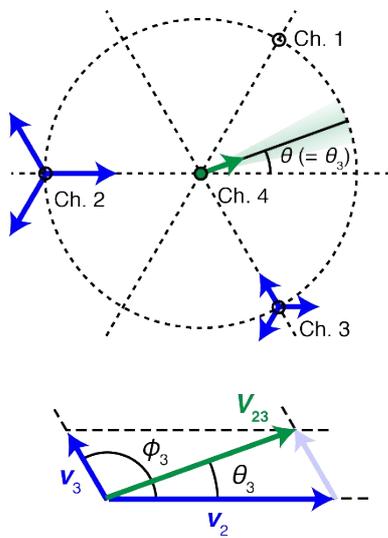


Figure S2. Schematic illustration of flow control. The stimulus stream from port 4 (Ch. 4; green) is oriented by the control stream from two ports, i and j . In this illustration, ports $i = 2$ and $j = 3$ are used (Ch. 2 and 3; blue arrows). The direction of the stimulus stream is determined by the velocity of the control stream at the centre immediately beneath port 4 (Ch. 4) (lower panel, \mathbf{V}_{23}). The flow velocity \mathbf{V}_{23} is a superposition of the flow velocity of the control stream from Ch. 2 (\mathbf{v}_2) and Ch. 3 (\mathbf{v}_3) at the position of Ch. 4.

Figure S3

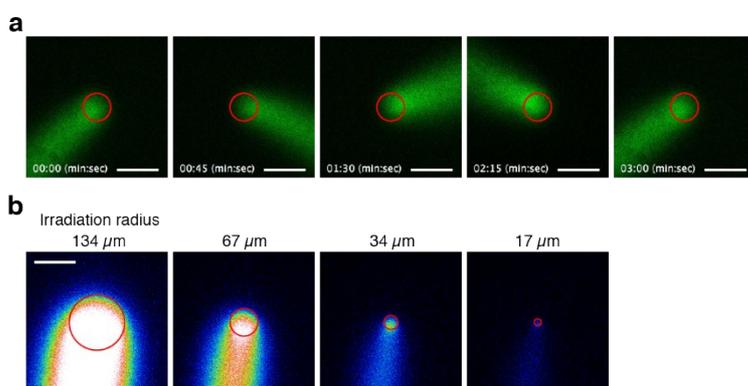


Figure S3. Spatial and temporal control of the stimulus stream formed in the basic lighthouse device in combination with flow photolysis of a caged compound. a) Generation of a rotating wave. CMNB-caged fluorescein was included in the solution. Fluorescence from liberated fluorescein (green). The stimulus stream was rotated every 3 min by changing p linearly over time and holding the total flow constant; $Q_{\text{sum}} = 26 \mu\text{L min}^{-1}$. b) Fluorescent intensity of liberated fluorescein (pseudo-colour) obtained at various illumination area sizes. UV light was irradiated in a circular domain at the centre of the observation area (red circle). The diameters of the circles were (panels from left to right): 134 μm , 67, 34, and 17. $Q_{\text{sum}} = 10 \mu\text{L min}^{-1}$. Scale bars, 100 μm .

Figure S4

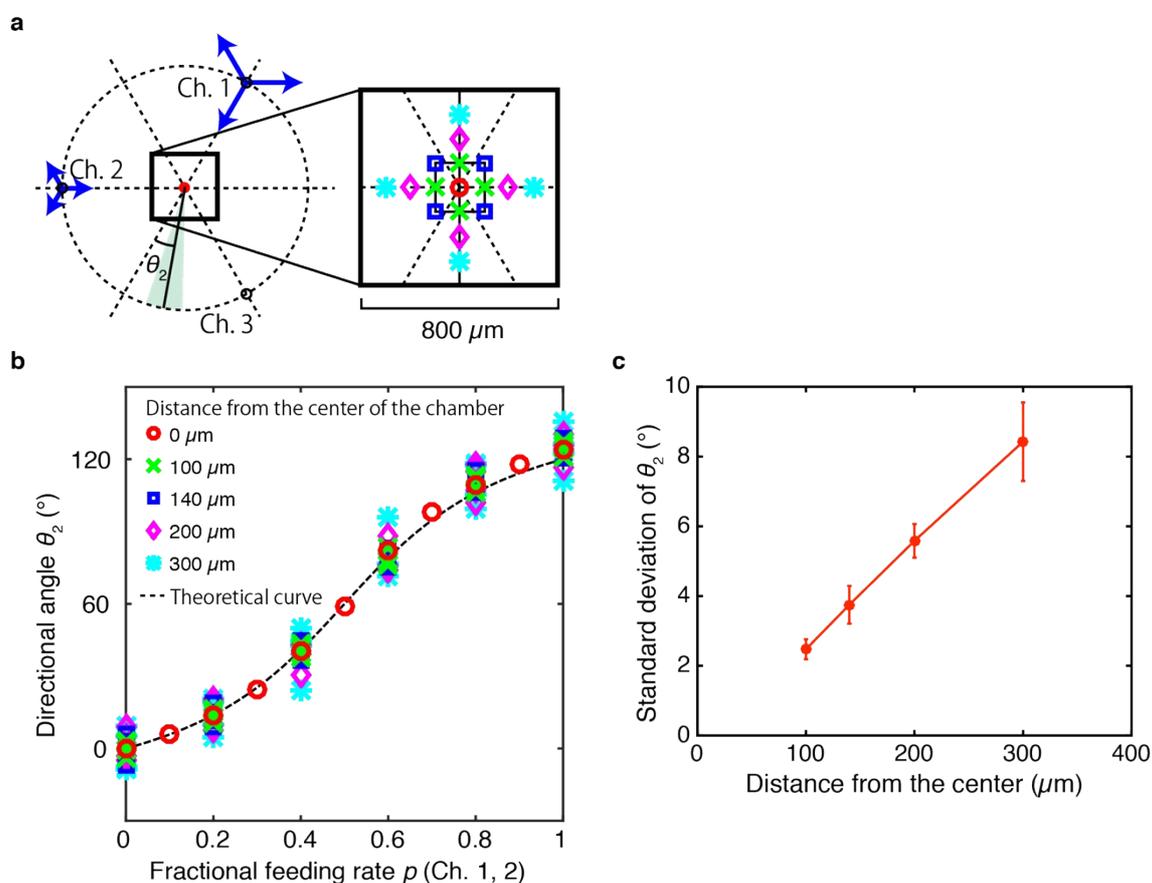


Figure S4. The directional angle of the stimulus stream is relatively conserved for off-centred UV-uncaging. a) Schematic illustration of the flow control and the positions of UV illumination (upper panel); 0 μm (red circle), 100 (green cross mark), 140 (blue square), 200 (magenta diamond), and 300 (cyan asterisk) from the centre of the chamber (upper panel). Fluid was fed from port Ch. 1 and 2; $Q_{\text{sum}} = 5 \mu\text{L min}^{-1}$. b) Measured flow direction plotted as a function of p (lower panel; the symbols and colours correspond to those in the upper panel). The theoretical curve for 0 μm (black broken line; Equation (1)). c) Deviation of the stimulus angle in b) plotted against the offset of the uncaging position. Error bars indicate the standard deviation over p .

Figure S5

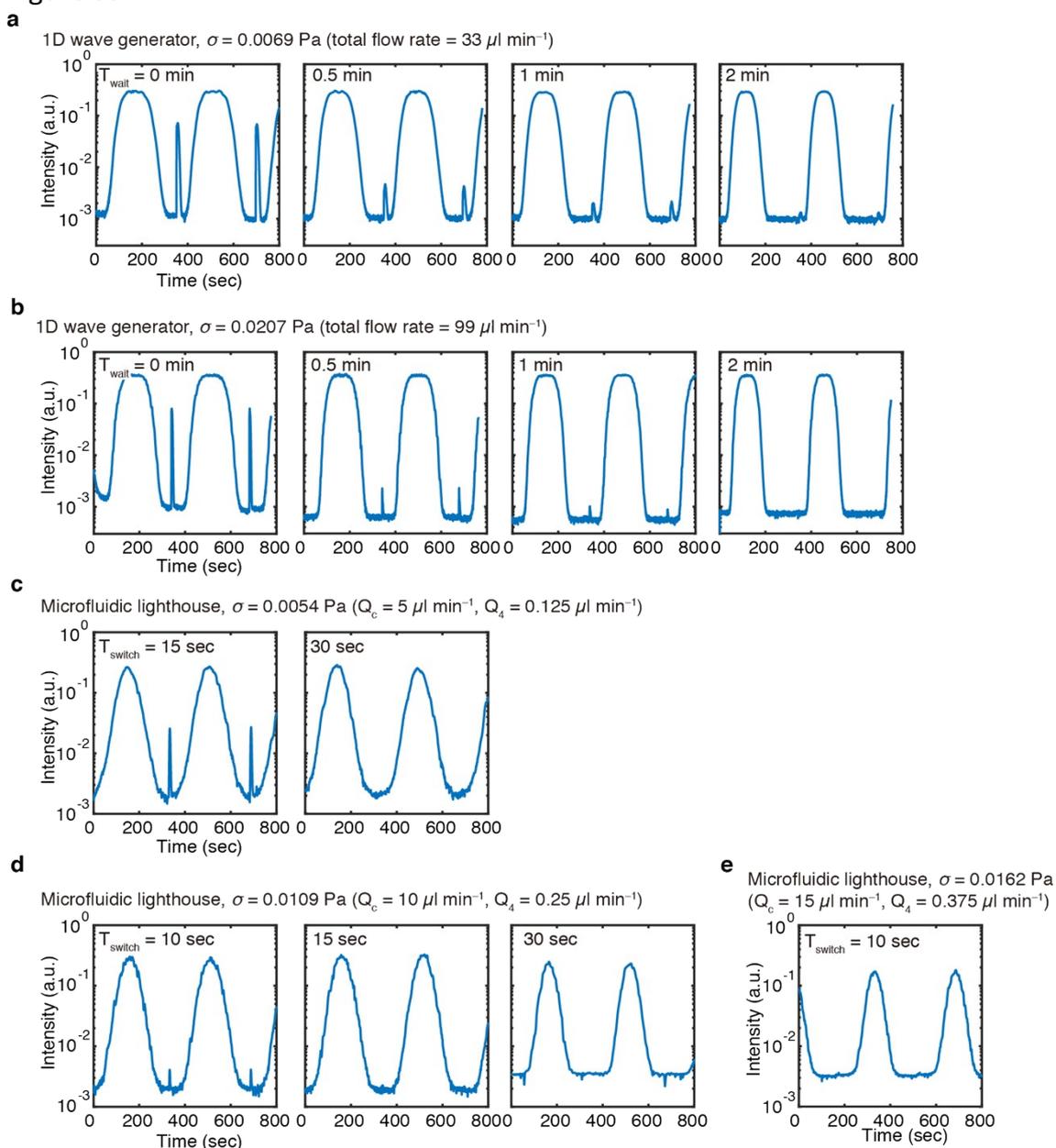


Figure S5. Temporal profiles of repetitive traveling wave stimuli. a,b) 1-D wave generator (3-inlet linear chamber) ¹¹ at the documented flow rate $33 \mu\text{L min}^{-1}$ a) and at a 3-fold higher flow rate of $99 \mu\text{L min}^{-1}$ b). The mean fluorescence intensity from a 512×512 pixel central region 3 mm downstream of the junction point. A bell-shaped gradient was moved by changing relative flow rates of two side flows. To repeat the wave, the stimulus flow was stopped for a time interval T_{wait} between each sweep. At time intervals T_{wait} shorter than 1 min, residual fluorescein was detectable when resetting (back-sweeping) the laminar flow position. c–e) Traveling wave stimuli in a microfluidics lighthouse at $Q_c = 5 \mu\text{L min}^{-1}$ (c), $10 \mu\text{L min}^{-1}$ (d), and $15 \mu\text{L min}^{-1}$ (e). The average intensity from a 512×512 pixel region at $x = 0.4$ mm, $y = 0.7$ mm from the centre of the chamber. T_{switch} is the fast-forward time of the laminar flow rotation outside the region of interest (from $\theta = 120^\circ$ to

360°; see Fig. 6a). σ is the estimated shear stress. Due to unidirectional rotation of the laminar flow, there is no profile disruption due to backsweep (e). The tail became detectable at time intervals at slow flow rates < 15 sec (c) and <~10 sec (d). Fluorescence intensity was background-corrected as described in ref. 11 (a-e).

Supplementary Movie Legends

Movie S1. Stimulus waves rotating in an anti-clockwise direction over a period of 3 min. The movie is from data shown in Fig. 2h. Scale bar, 200 μm .

Movie S2. Formation of a stimulus stream by uncaging. CMNB-caged fluorescein solution was introduced from Ch. 1, 2, and 3. UV light was irradiated at the centre (red circle). $Q_{\text{sum}} = 22 \mu\text{L min}^{-1}$. Scale bar, 100 μm .

Movie S3. Generation of alternating gradients with flow photolysis. The movie corresponds to data shown in Fig. 4b and c. Confocal images (left panel) of the uncaged stimulus stream (green) and their magnified images (right panel) in the yellow square region in the left panel. The red circle indicates the area of UV light application. Scale bar, 50 μm .

Movie S4. Generation of alternating gradients by the duplexed device. The movie is from data shown in Fig. 5b. Scale bar, 200 μm .