Supplementary Information:
Self-mixing in microtubule-kinesin active fluid from nonuniform to uniform distributions of activities

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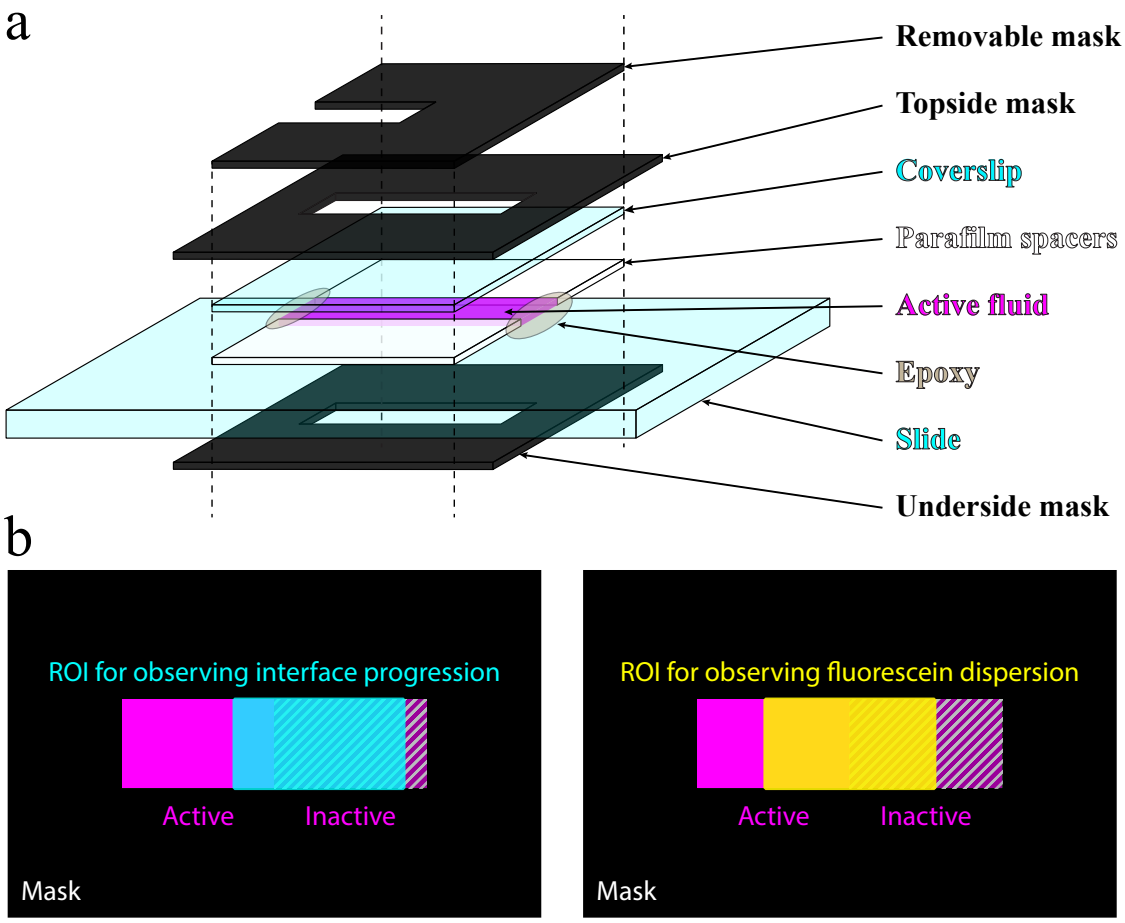
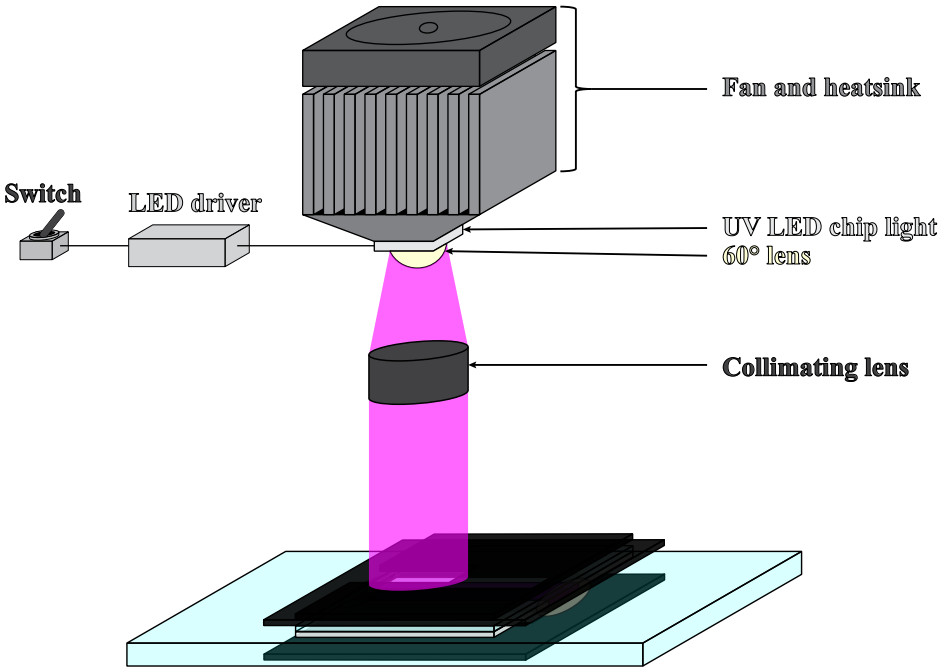
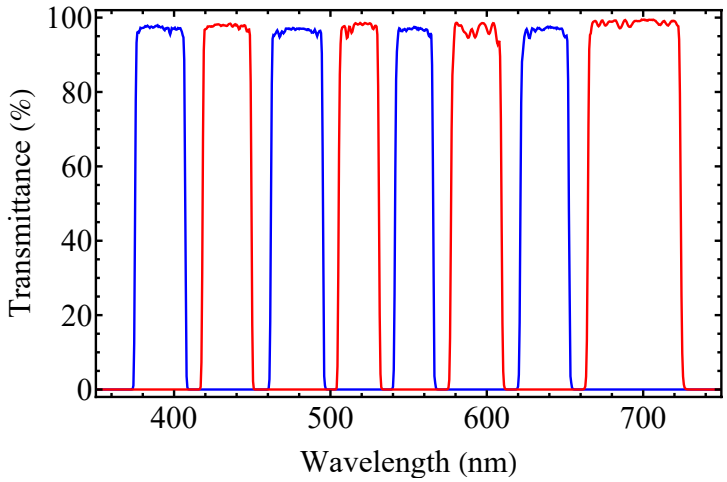
Table of Contents



Supplementary Fig. 1: We used a removable mask to activate one side of the sample. (a) Active fluid was loaded into a glass flow cell consisting of a polyacrylamide-coated glass slide and coverslip with parafilm as a spacer and sealed with epoxy. To prepare the active-inactive fluid system, we blocked one side of the sample (right half) with a removable mask. To prevent UV light from being scattered to the masked region by the epoxy and parafilm, which could cause unwanted fluid activation in the masked region, we further blocked the rest of the sample, including epoxy and parafilm, with 2 masks (one topside and one underside). After the UV exposure, we removed the mask to image the sample with fluorescent microscopy. (b) The sample is 20 mm long, which is wider than the field of view in our microscope even using a 4× objective, so we imaged 3 to 4 adjacent frames along the flow cell and stitched these frames into one large image. For the experiments monitoring active-inactive fluid interface progression (left), we selected the region of interest (ROI) as one quarter of active area and most of the inactive area (cyan rectangle) to observe the progression of the interface (Figs. 1–4). For the experiments monitoring the dispersion of fluorescein (right), we selected the ROI as half of the active and half of the inactive regions (yellow rectangle) to observe how one-sided dyes dispersed to the rest of the sample (Fig. 5).



Supplementary Fig. 2: Setup to apply UV light to the masked sample (Supplementary Fig. 1a).[1] UV light was emitted with a UV LED chip (Amazon, B01DBZK2LM) powered by an LED driver (McMaster, 4305N124) and cooled with a fan-powered heatsink (Amazon, B01D1LD68C). To ensure that the light exposure was consistent across the sample, we parallelized the emitted UV light beams with a 60° lens (part of the heatsink) and a collimating lens (part of the microscope, Nikon, MEA54000).



Supplementary Fig. 3: Excitation (blue) and emission (red) spectrums of our multiband pass filter cube (Multi LED set, Chroma, 89402–ET). The filter cube is compatible with the fluorescent spectrums of fluorescein (excitation: 490 nm; emission: 525 nm), Alexa 488 (excitation: 499 nm; emission: 520 nm), Alexa 647 (excitation: 650 nm; emission: 671 nm), and Flash Red (excitation: 660 nm; emission: 690 nm). Data source: www.chroma.com.

Supplementary Video 1: Mixing of activated and inactivated fluids. The fluid contained caged ATP, which could not fuel the kinesin motors until it was uncaged by exposure to ultraviolet light. After one side of the sample was exposed to ultraviolet light, the ATP molecules on that side of the sample were released and could fuel the kinesin motors to drive microtubules and create flows. The activated fluid blended with the inactive fluid until two fluids became one activity-uniform fluid. Cyan fibers are microtubules and red dots are tracers. The time stamp indicates hour:minute:second.

Supplementary Video 2: A one-dimensional, Fick's law-based model described how ATP distribution evolved from one side of a container to being uniformly distributed (top). The ATP was confined in a segment from $x = 0$ to $x = 20$ mm. The ATP distribution was converted to distribution of active fluid mean speed via Michaelis-Menten kinetics (Fig. 3b), which shows that initially only one side of the system was activated followed by evolution toward an activity-uniform state (bottom). Active fluid with a higher initial concentration of ATP (8 mM; red curve) evolved toward an activity-uniform state faster than active fluid with a lower initial concentration (1 mM; black curve). The time stamp indicates hour:minute:second.

Supplementary Video 3: UV-activated fluorescent dyes were suspended in inactive (top) and active (bottom) fluids. In the inactive fluid system, the dyes were dispersed only by molecular diffusion, whereas in the active fluid system, the dyes were further transported by active fluid flows and thus dispersed faster through the sample. Time stamp indicates hour:minute:second.

Supplementary Video 4: Simulated maps of ATP concentrations and flow speeds of active fluid for various pairs of α_0^* and D^*. In the no activity system ($\alpha_0^* = 0$; top), dispersion of ATP was driven only by molecular diffusion ($D^* = 16$). When the fluid was activated ($\alpha_0^* = 5$; middle), dispersion of ATP was mainly driven by active transport with the negligible contribution from molecular diffusion. However, when the ATP diffused significantly faster ($D^* = 64$, bottom), dispersion of ATP was driven mainly by ATP molecular diffusion with negligible contribution from active transport.

Supplementary Video 5: Checkerboard-patterned UV light (grid size $a = 1$ mm) was applied to an inactivated fluid system (00:00:12–00:01:06), which released caged ATP to activate kinesin motors and power the active fluid (middle). The motion of microtubules stirred the surrounding solvent, which carried and mixed the fluorescent dyes (left) to a homogeneous state in 10 minutes (00:10:30). The right panel represents merged microscopic images of fluorescent dyes and microtubules. Time stamp indicates hour:minute:second.

Supplementary References

1 Berezney, J., Goode, B. L., Fraden, S. & Dogic, Z. Extensile to contractile transition in active microtubule-actin composites generates layered asters with programmable lifetimes. *Proceedings of the National Academy of Sciences* **119**, e2115895119 (2022).