

Supplementary Information

Fluorescence-based Sorting of *Caenorhabditis elegans* via Acoustofluidics

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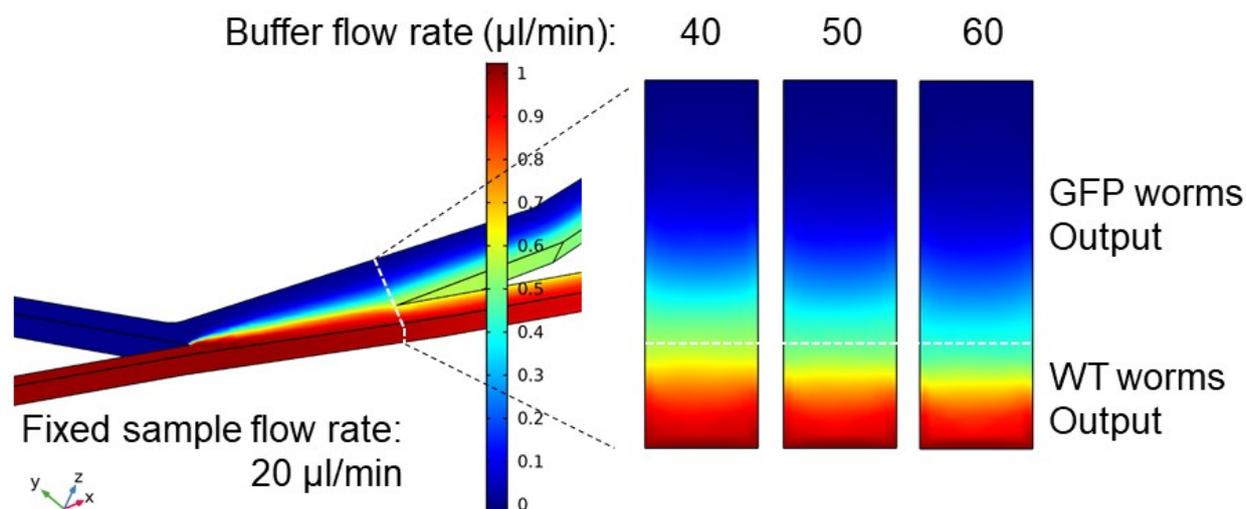


Figure S1. Simulation results of the worm flow concentration distribution with different flow rate ratios. The right parts are the cross-section concentration distribution of the channel's bifurcation part. The rate of worm flow was fixed as 20 $\mu\text{l}/\text{min}$, and the buffer flow was tuned from 40 $\mu\text{l}/\text{min}$ to 60 $\mu\text{l}/\text{min}$.

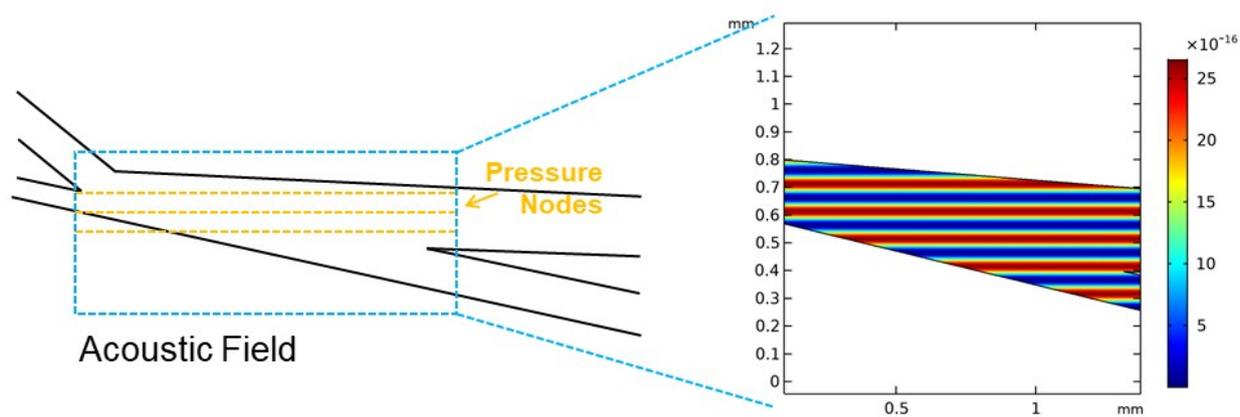


Figure S2. Schematic and simulation results of the standing surface acoustic field distribution at the acoustic sorting area.

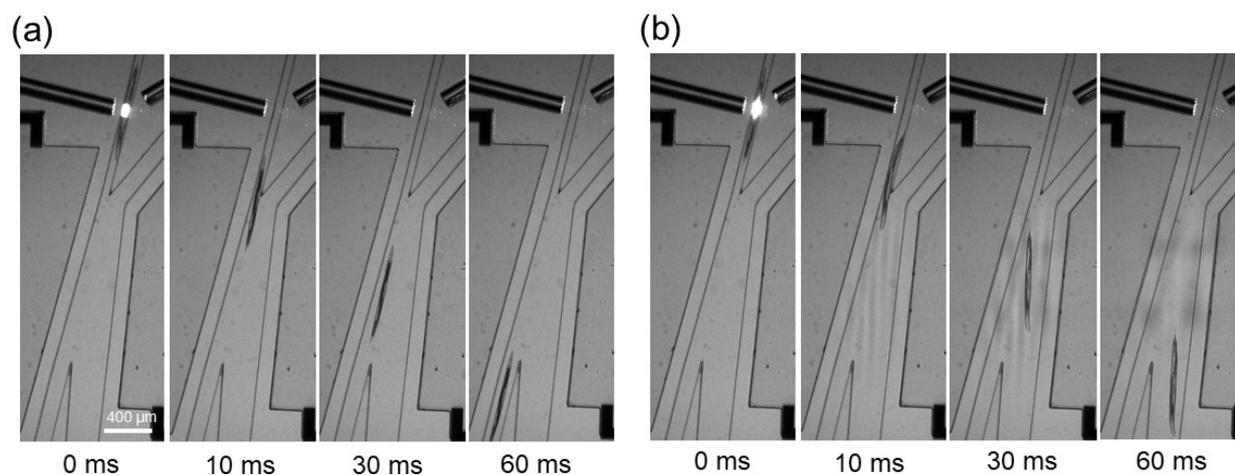


Figure S3. Acoustic sorting process of L4 GFP-expressing worm and wild-type worm. (a) Image sequence of a wild-type L4 worm detected and then sorted by acoustic field. (b) Image sequence displaying the detection and sorting process of a L4 GFP-expressing worm.

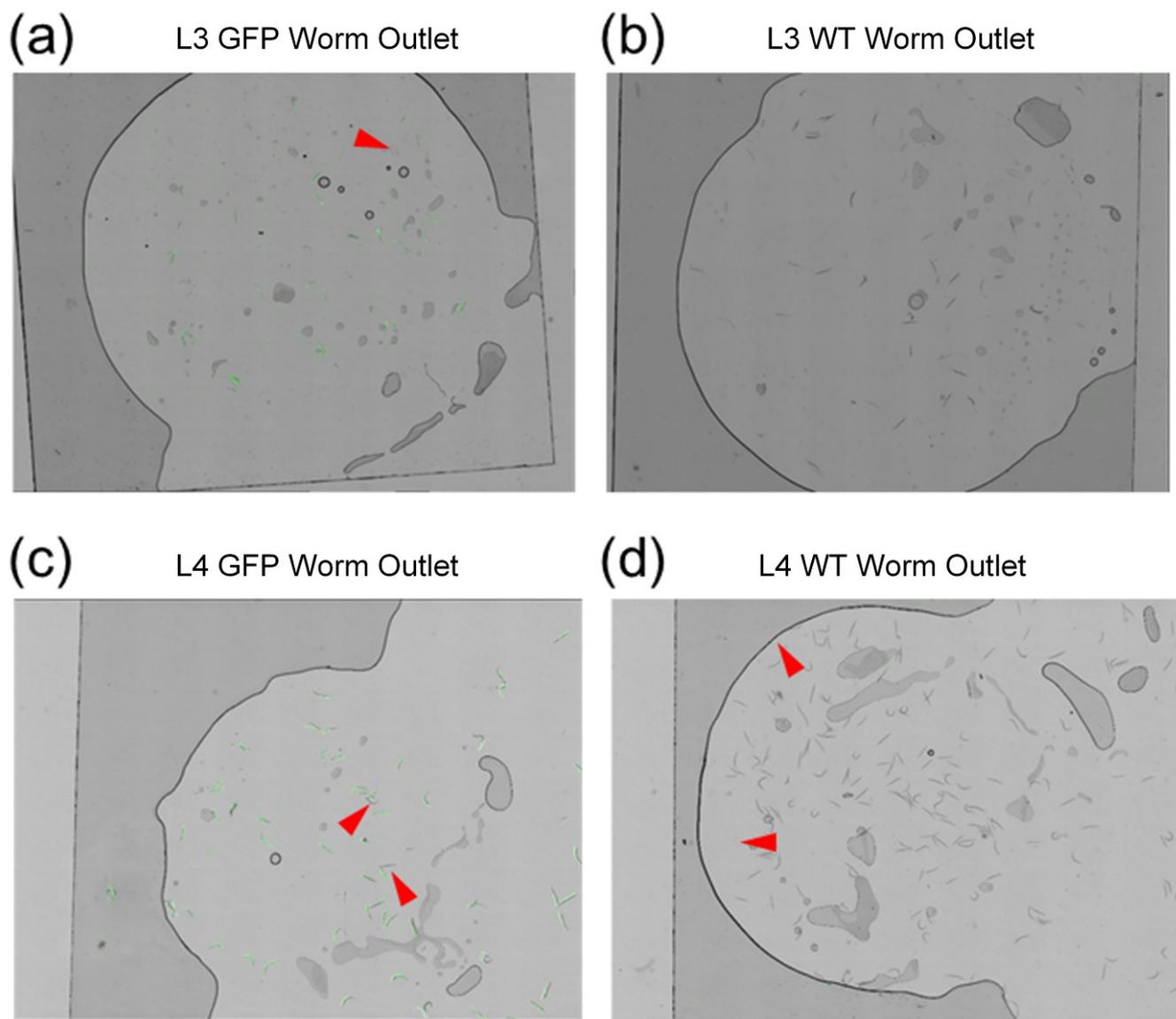


Figure S4. Original figures of Fig. 5a-d. Incorrectly sorted worms were pointed out by the red arrows.

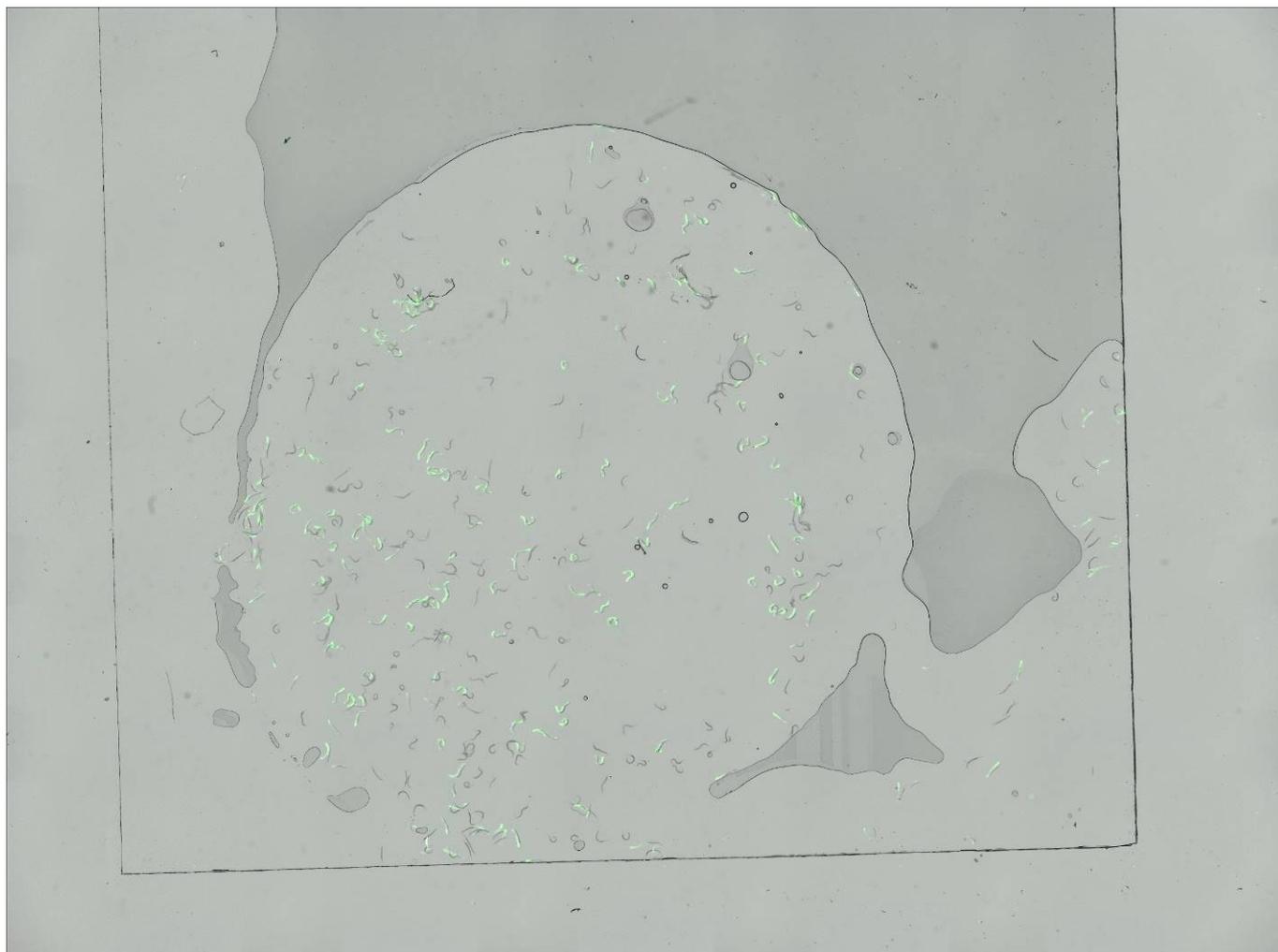


Figure S5. GFP-field and brightfield overlapped image of the unsorted L3 worm sample. After mixed the GFP worms and WT worms, we imaged a small sample of the mixture to estimate the worm concentration and the ratio of GFP worms to WT worms. Here, the amount of GFP worms is 166 and the amount of WT worms is 217, so the ratio is around 0.433 to 0.567.

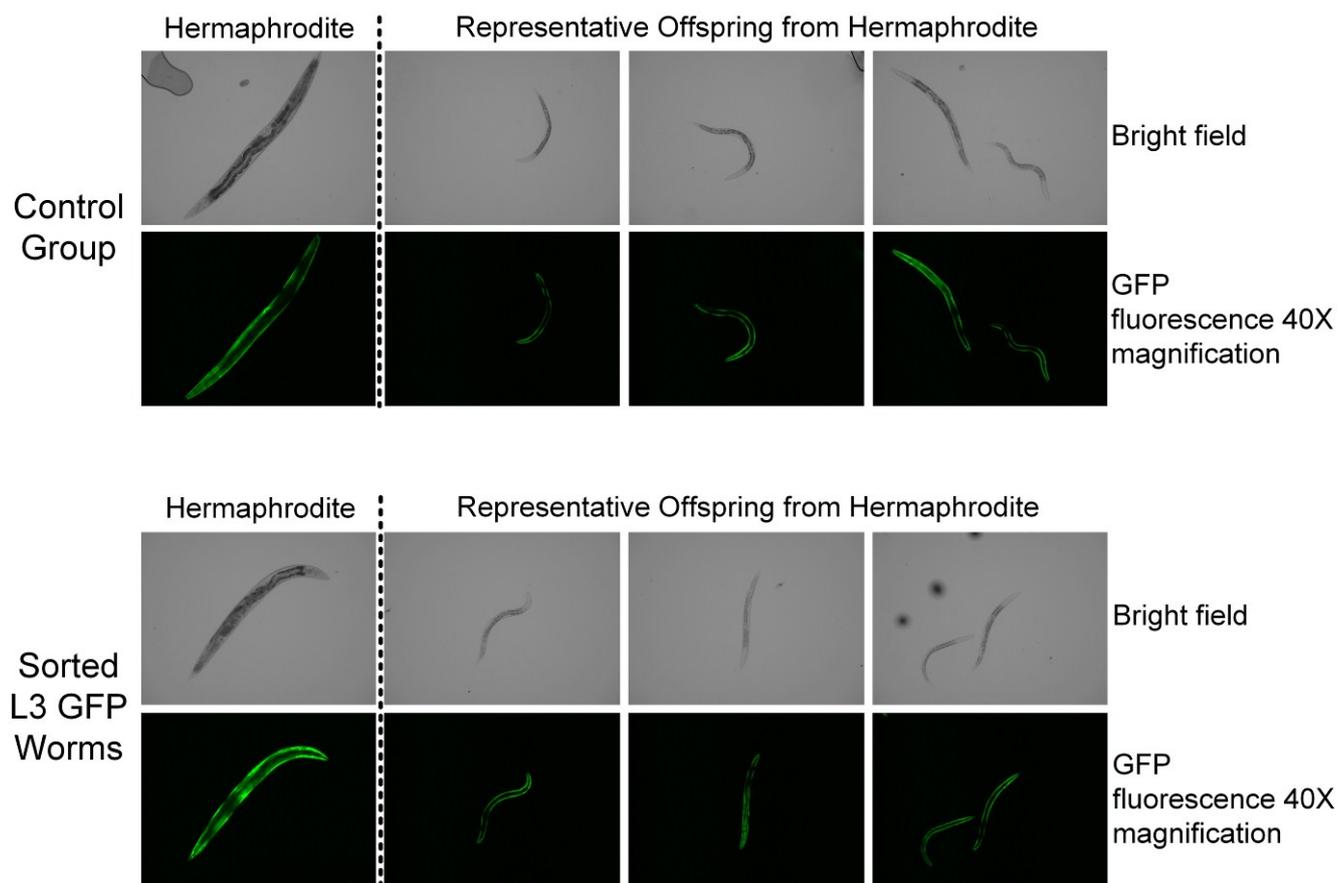
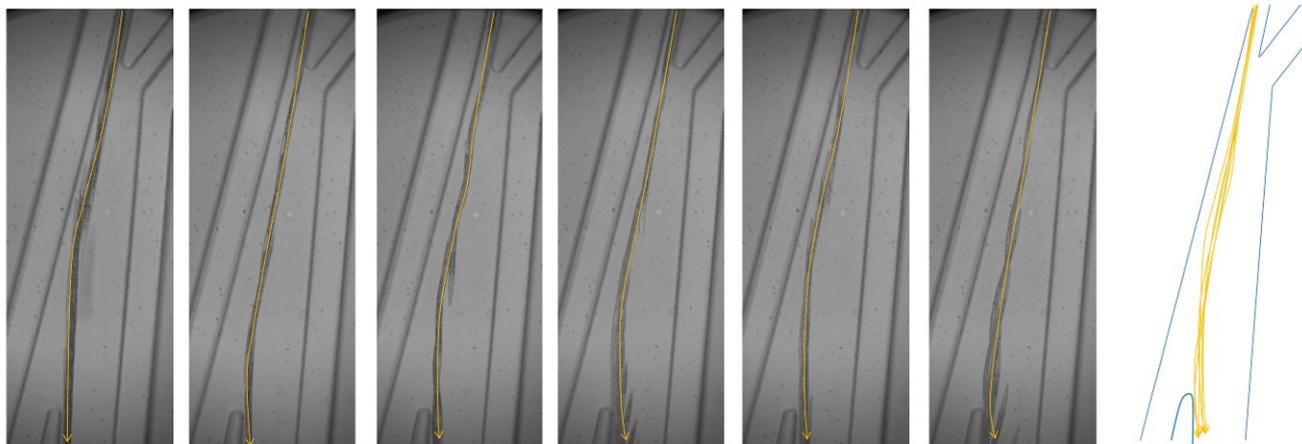
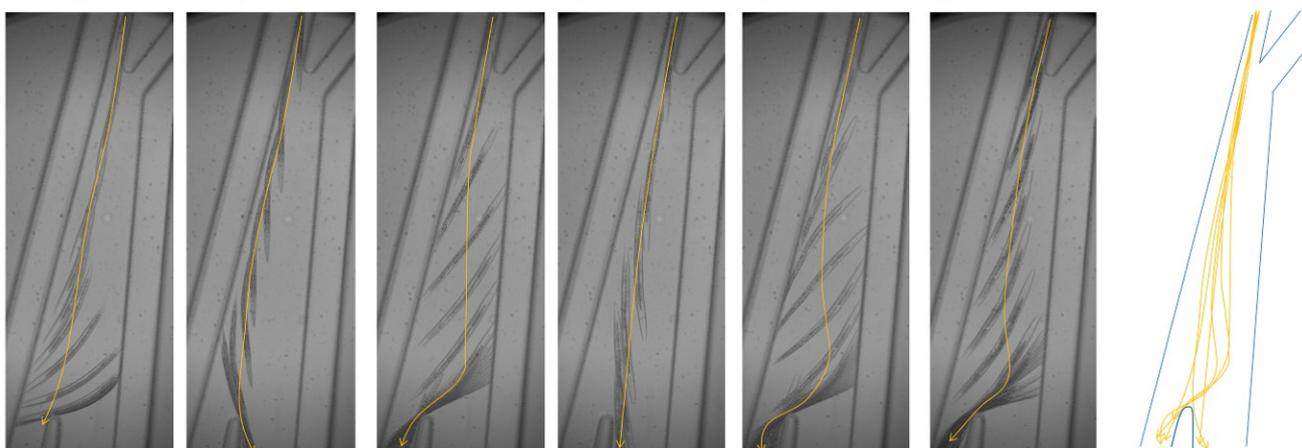


Figure S6. Representative images for a hermaphrodite from a control group and sorted L3 GFP-expressing worms and its offspring. The offspring of sorted GFP worms exhibit the expected fluorescence as compared to the control group.

Group 1. Acoustic power: $18.2 V_{pp}$. Worm sample flow rate: $20 \mu\text{l}/\text{min}$. Throughput: ~ 70 worms/min



Group 2. Acoustic power: $18.2 V_{pp}$. Worm sample flow rate: $40 \mu\text{l}/\text{min}$. Throughput: $120\sim 150$ worms/min



Group 3. Acoustic power: $18.2 V_{pp}$. Worm sample flow rate: $60 \mu\text{l}/\text{min}$. Throughput: >200 worms/min

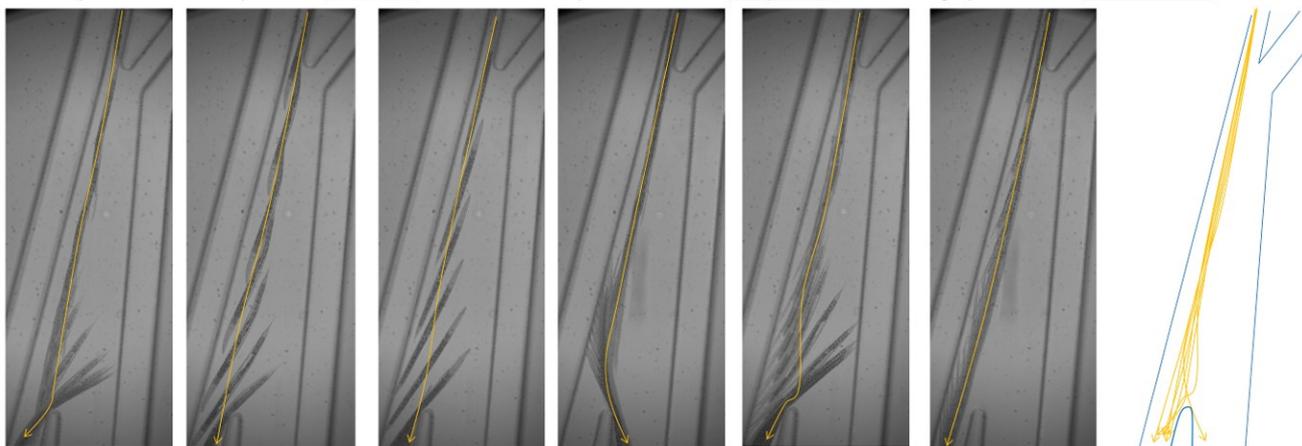
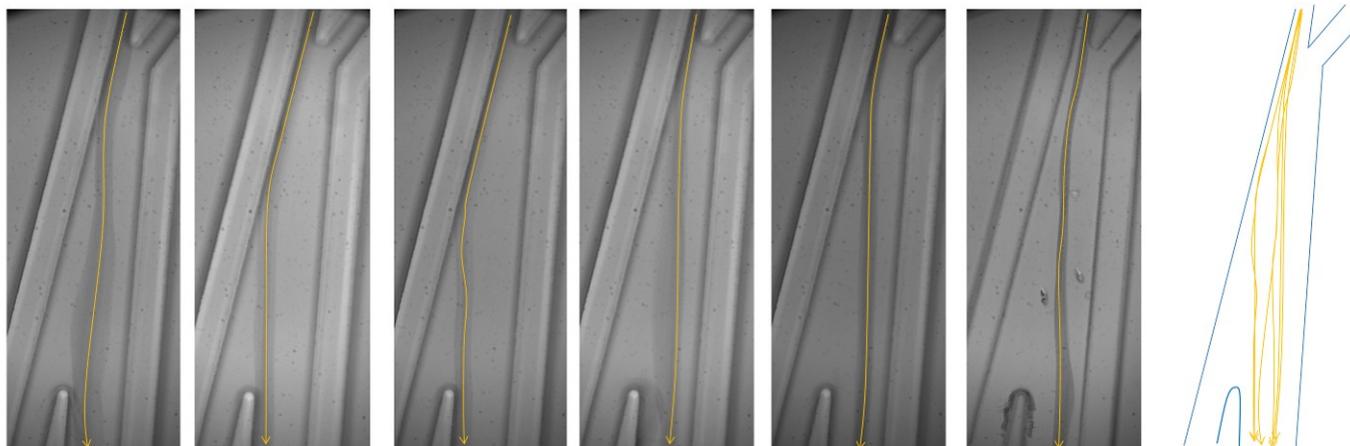
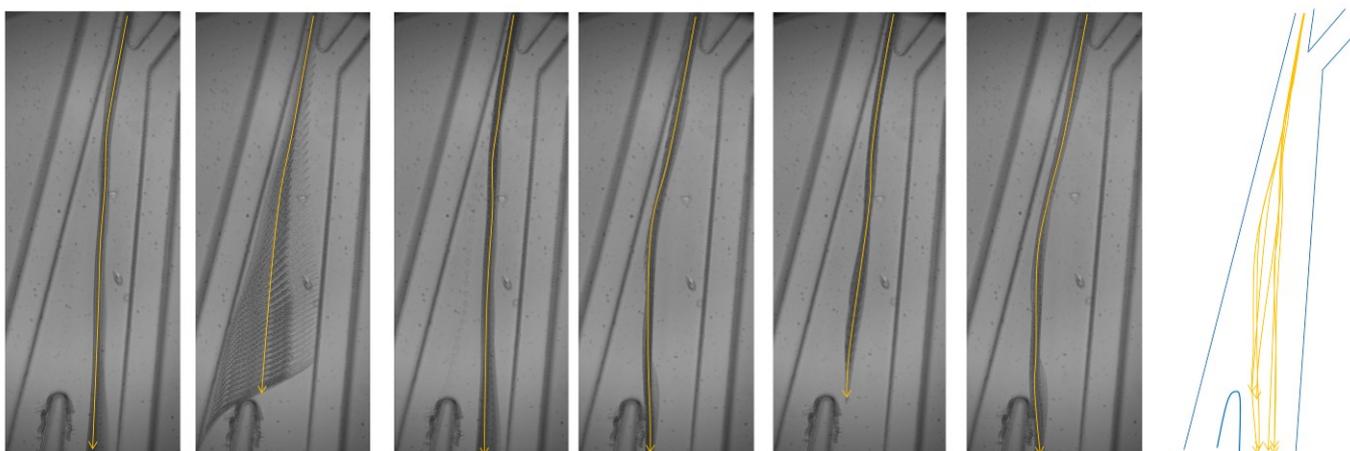


Figure S7. Trajectories of the L4 worms passing through the sorting channel in different flow rates when $18.2 V_{pp}$ acoustic power was applied. Each image was a stack of images that a L4 worms passed through the sorting channel. The trajectory was labelled by the yellow line. For each group, all the trajectories were summarized in the right schematic.

Group 4. Acoustic power: $25.6 V_{pp}$. Worm sample flow rate: $20 \mu\text{l}/\text{min}$. Throughput: ~ 70 worms/min



Group 5. Acoustic power: $25.6 V_{pp}$. Worm sample flow rate: $40 \mu\text{l}/\text{min}$. Throughput: 120~150 worms/min



Group 6. Acoustic power: $25.6 V_{pp}$. Worm sample flow rate: $60 \mu\text{l}/\text{min}$. Throughput: >200 worms/min

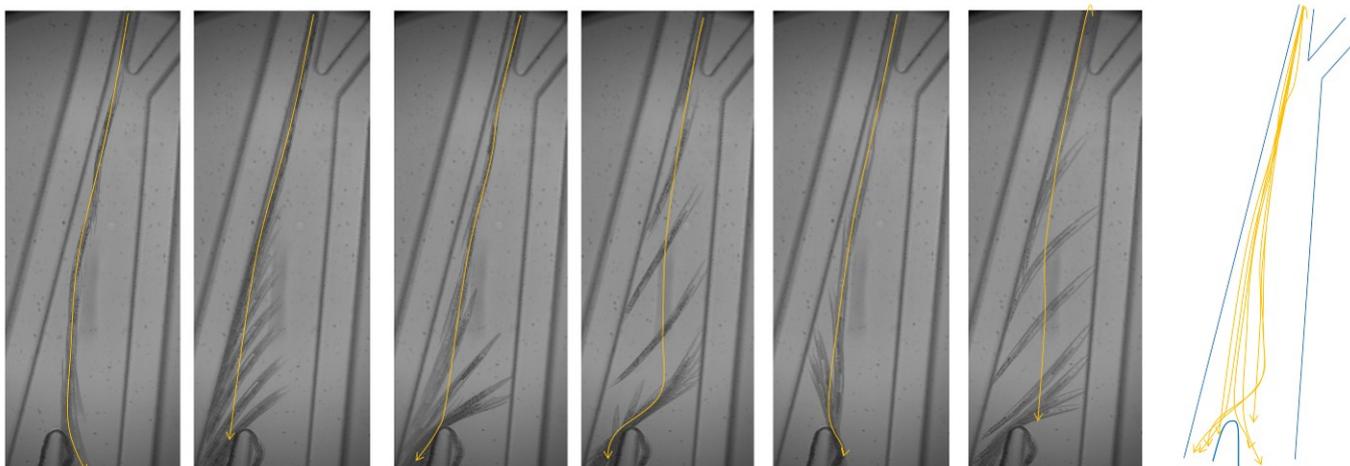
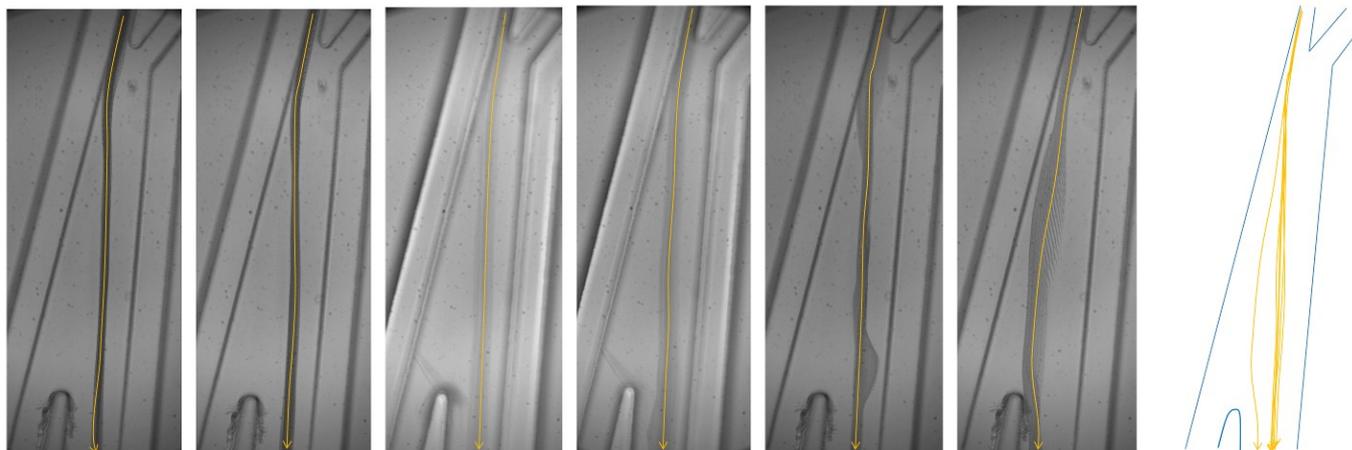
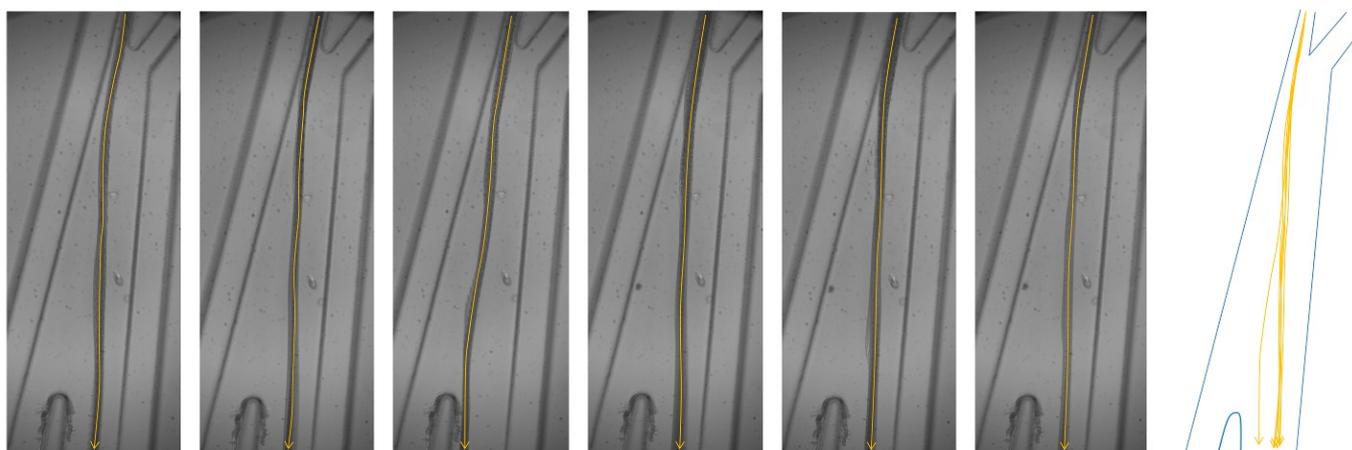


Figure S8. Trajectories of the L4 worms passing through the sorting channel in different flow rates when $25.6 V_{pp}$ acoustic power was applied.

Group 7. Acoustic power: $31.2 V_{pp}$. Worm sample flow rate: $20 \mu\text{l}/\text{min}$. Throughput: ~ 70 worms/min



Group 8. Acoustic power: $31.2 V_{pp}$. Worm sample flow rate: $40 \mu\text{l}/\text{min}$. Throughput: 120~150 worms/min



Group 9. Acoustic power: $31.2 V_{pp}$. Worm sample flow rate: $60 \mu\text{l}/\text{min}$. Throughput: >200 worms/min

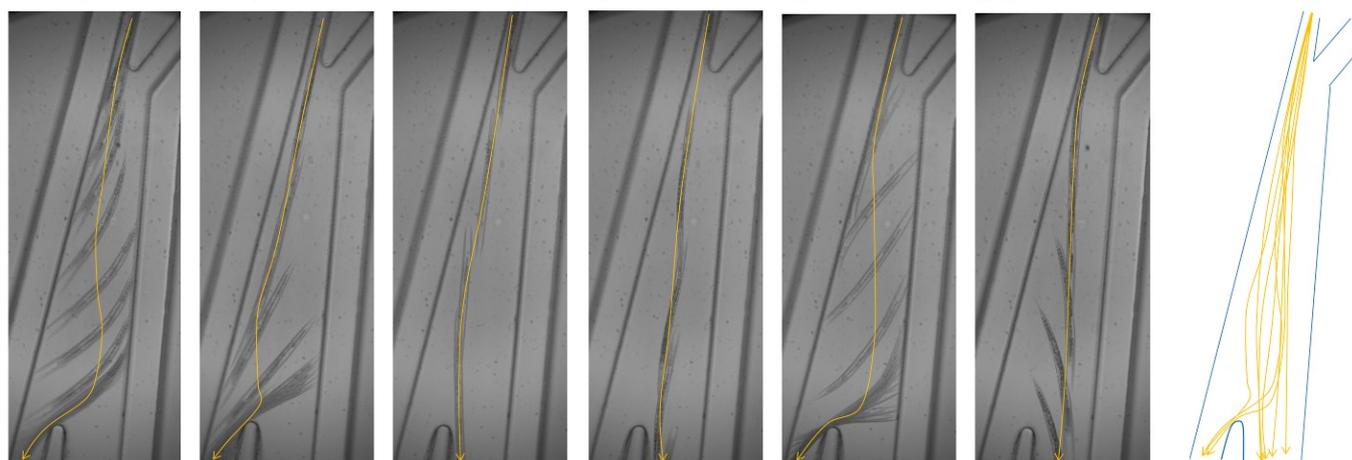


Figure S9. Trajectories of the L4 worms passing through the sorting channel in different flow rates when $31.2 V_{pp}$ acoustic power was applied.

Group 10. Acoustic power: $40.8 V_{pp}$. Worm sample flow rate: $60 \mu\text{l}/\text{min}$. Throughput: >200 worms/min

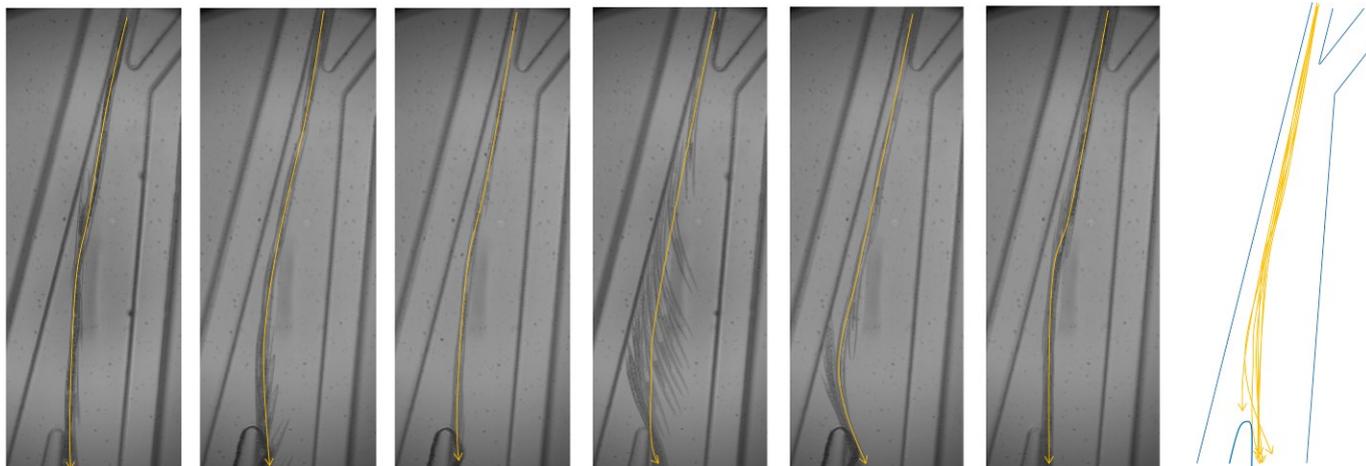


Figure S10. Trajectories of the L4 worms passing through the sorting channel in different flow rates when $40.8 V_{pp}$ acoustic power was applied.

Video S1: L3 worms sorting at a throughput around 70 worms/min.

Video S2: L3 worms sorting at a throughput around 120 worms/min.

Video S3: L3 worms sorting at a throughput around 210 worms/min.

Video S4: L4 worms sorting at a throughput around 220 worms/min.