

## Supplementary Methods and Materials

### 1. Microfluidic Device Fabrication

The microfluidic device was fabricated using multi-layer soft lithography.<sup>1, 2</sup> Two different molds were fabricated by photolithographic processes to create worm loading layer and the control layer as follows: a 30- $\mu\text{m}$ -thick negative photoresist (SU8-2025, Microchem) was spin-coated onto a silicon wafer for the worm loading chamber and the detection channel. The master for the control layer was made of a 15- $\mu\text{m}$  layer of negative photoresist (SU8-2010, Microchem) on a silicon wafer. The two molds and a blank wafer were treated with tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane vapor (United Chemical Technologies) in a vacuum desiccator to prevent adhesion of PDMS during the molding process.

For fabricating the flow layer, polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning A and B in 10:1 ratio) was dispensed onto the flow-layer master to obtain a 5 mm-thick layer. A mixture of 10:1 was then spin-coated onto the control layer to create a 50- $\mu\text{m}$ -thick membrane. The control layer was then allowed to relax at room temperature for 30 min. The flow layer was partially cured at 70 °C for 20 min, and the control layer at 65 °C for 9 min. The thick flow layer was then peeled off from the master, cut into small rectangles and individually aligned and bonded to the thin PDMS membrane on the control layer. This assembled device was fully cured at 70 °C for 2 hours. Once cured, the devices were removed from the wafer, and holes were punched to provide access to the various layers. Devices were then treated with oxygen plasma and irreversibly bonded to glass slides.<sup>3</sup>

### 2. Microfluidic device operation

This phenotyping and sorting process is gentle, and in our experiments, 100% of animals were viable, crawling on agar and thrashing in buffer normally immediately after the processing. To load an animal into the imaging chamber, the outlet channel for wild-type animals is left open, while the positioning control valve and mutant channel valves are closed. This allows fluid flow to continue and carry a worm into the field of view of the camera, until its head pushes against the positioning control valve. The presence of the worm significantly increases the fluid resistance of the channel, so the flow is dramatically reduced, preventing another worm from entering. If the worm is clearly wild-type or mutant, the user selects the “Wild-Type” or “Mutant” buttons on the control interface. If wild-type, the positioning valve opens and allows the worm to be released. If mutant, the wild-type outlet is closed and then the mutant channel and position valve are opened. The mutant channel and positioning valve are then reset to the closed position for the next animal.

### 3. *C. elegans* culture, sample preparation and screening

Animals were cultured according to established methods<sup>4</sup>. Mutagenesis was performed on age-synchronized L4 animals using EMS according to standard protocols.<sup>5</sup> F2 eggs were obtained by bleaching F1 adults using a solution containing about 1% NaOCl and 0.1 M NaOH, washed in M9 buffer, and cultured on Nematode Growth Medium (NGM) plates seeded with *E. coli* OP50 until L4 stage. Animals were washed and suspended in M9 solution containing 0.02 wt% Bovine Serum Albumin (BSA) for each experiment. Animals were screened under a compound microscope at 20X based on differences in the reporter expression pattern or intensity; potential animals of interest were sorted into the mutant outlet and were collected directly from tubing

connected to the mutant outlet with M9 solution containing 0.02 wt% BSA. Animals were subsequently transferred to individual plates for culture and further examination.

#### 4. Software Interface

The software interface written in Matlab® allows users to control various camera settings such as EM sensitivity and gain, and to control the exit time of animals. By selecting the appropriate buttons, an animal is sorted as either mutant or wild-type. If the image in the streaming video window is unclear to the user, selecting “stack” will acquire images at multiple focal planes (number of and spacing of images as specified by the user). Images can be flattened and processed according to the user selection. The following toolboxes are required for use: data acquisition, image acquisition and image processing.

The various options for flattening the z-stack are:

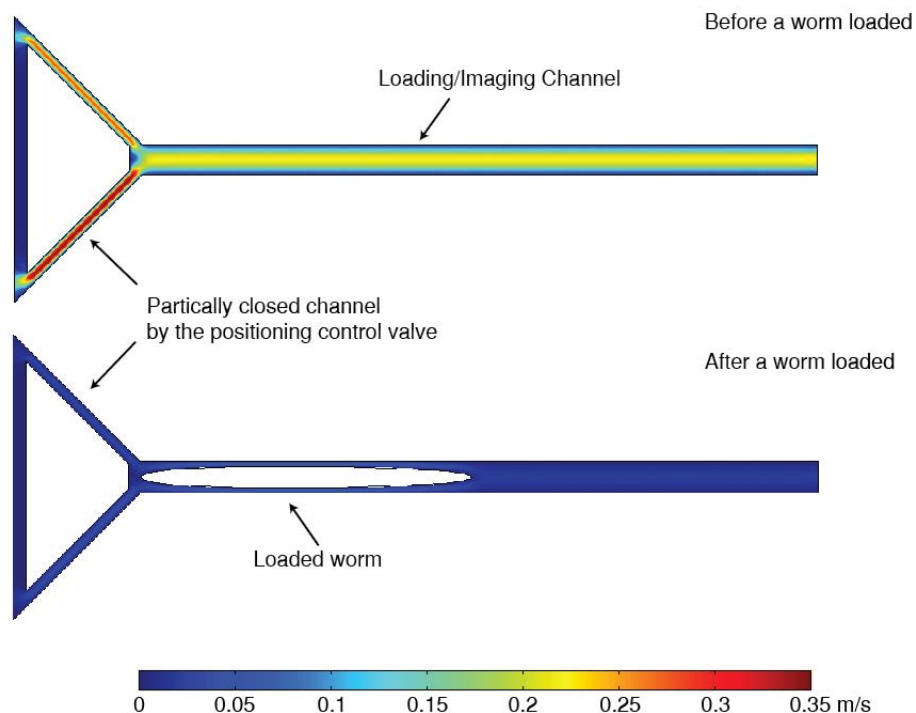
- **Summation:** this flattens the stack by making each x-y point equal to the summation of the values at that point over the z-direction
- **Maximum:** this flattens the stack by making each x-y point equal to the maximum of the values at that point over the z-direction.
- **Standard Deviation:** this flattens the stack by making each x-y point equal to the standard deviation of the values at that point over the z-direction.
- **In-focus:** this assumes that the slice with the highest standard deviation is the most in-focus and uses it for the subsequent image processing steps.<sup>6</sup>

The various options for image processing the flattened image are:

- **Gaussian:** applies a rotationally symmetric Gaussian low-pass filter to the flattened image.
- **Laplacian:** applies a filter approximating the Laplacian operator to the flattened image.
- **Laplacian of Gaussian:** applies a rotationally symmetric Laplacian of Gaussian filter to the flattened image.
- **Prewitt H:** applies the prewitt filter for emphasizing horizontal edges to the flattened image.
- **Prewitt V:** applies the prewitt filter for emphasizing vertical edges to the flattened image.
- **Sobel H:** applies the sobel filter for emphasizing horizontal edges to the flattened image.
- **Sobel V:** applies the sobel filter for emphasizing vertical edges to the flattened image.
- **Unsharp:** applies an unsharp filter for contrast enhancement created by the negative of a Laplacian filter and applies it the flattened image.
- **Range:** filters the image provided by the flattening step using the local range of the image.
- **Entropy:** filters the image provided by the flattening step using the local entropy of the image.
- **Standard deviation:** filters the image provided by the flattening step using the local standard deviation

## 5. Fluid Flow with and without a worm

A COMSOL fluid dynamics model was generated to show the effect of a worm on the flow and pressure profiles within the device. As can be seen from the figure below, the flow is decreased by an order of magnitude when a worm is present.



Supplementary Figure 1. Numerical models showing flow rate distribution along the worm loading channel before and after a worm is loaded. To simplify the numerical simulations, partially closed channel by the positioning control valve is assumed as channels with a small width (10 ~ 12  $\mu\text{m}$ ) along the edge of the original channel geometry. The presence of a worm causes at least 10-fold decrease in average flow rate: 0.153 m/s before a worm loaded, 0.0137 m/s after a worm loaded.

## 6. Supplemental Movie

We have included a movie to show the device operation. Sorting of three worms sequentially is shown twice, once in real time and a second time slowed down by 50%. Notice that the “wildtype” sorting valve is open most of the time unless a “mutant” is detected and the “wildtype” valve closes momentarily and the “mutant” valve “opens” in synchrony.

## References

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