Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

PDMS FILTER STRUCTURES FOR SIZE-DEPENDENT LARVAL SORTING AND ON-CHIP EGG EXTRACTION OF *C. ELEGANS*

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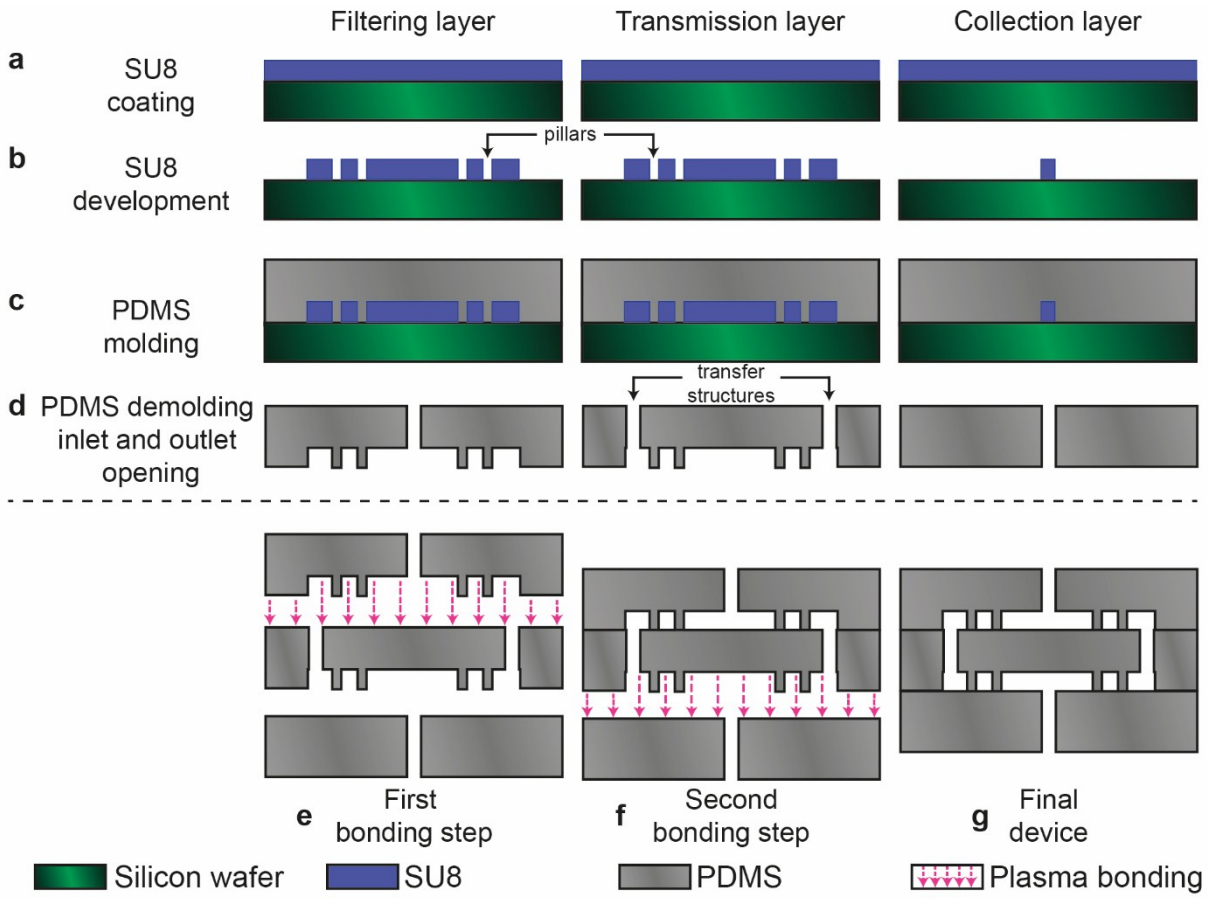

Fig. S1. Details of the fabrication process of the microfluidic chip. Starting from clean Si wafers, **(a)** a 80 µm thick SU8 layer was spin-coated, exposed and **(b)** developed to realize three different designs on three different wafers. Following a trimethylchlorosilane (TMCS) silanization of the three molds, **(c)** a 10:1 PDMS polymer to curing agent mixture was poured on the wafers, degassed and cured for 2 hours at 80 °C. **(d)** After peeling off the PDMS molds, inlets and outlets were punched with a biopsy punch, and in a first step **(e)** the patterned side of the filtering layer and the backside of the transmission layer were plasma-activated and bonded. **(f)** The fabrication was finalized by bonding the patterned side of the previously bonded part to the punched collection layer.

Supplementary Figures

Fig. S2. Typical diameter values of a few worms before creating a mixed population (left) and after the sorting experiments (right) for L1 and L2 populations. (a-f) Experimental diameter results, displayed for six different experiments. The horizontal black and grey dashed lines indicate the nominal onset of L1 and L2 larval stages with diameter values of 11.7 and 17 µm, respectively.

Fig. S3. Typical diameter values of a few worms before creating a mixed population (left) and after the sorting experiments (right) for L2 and L3 populations. (a-f) Experimental diameter results, displayed for six different experiments. The horizontal black and grey dashed lines indicate the nominal onset of L2 and L3 larval stages with diameter values of 17 and 22.1 µm, respectively.

Fig. S4. Typical diameter values of a few worms before creating a mixed population (left) and after the sorting experiments (right) for L3 and L4 populations. (a-f) Experimental diameter results, displayed for six different experiments. The horizontal black and grey dashed lines indicate the nominal onset of L3 and L4 larval stages with diameter values of 22.1 and 29.5 µm, respectively.

Fig. S5. Typical diameter values of a few worms before creating a mixed population (left) and after the sorting experiments (right) for L4 and young adult (YA) populations. (a-f) Experimental diameter results, displayed for six different experiments. The horizontal black and grey dashed lines indicate the nominal onset of L4 larval and YA stages with diameter values of 29.5 and 47.9 µm, respectively.

Fig. S6. Typical diameter values of a few worms before creating a mixed population (left) and after the sorting experiments (right) for L1 and young adult (YA) populations. (a-f) Experimental diameter results, displayed for six different experiments. The horizontal black and grey dashed lines indicate the nominal onset of L1 larval and YA stages with diameter values of 11.7 and 47.9 µm, respectively.

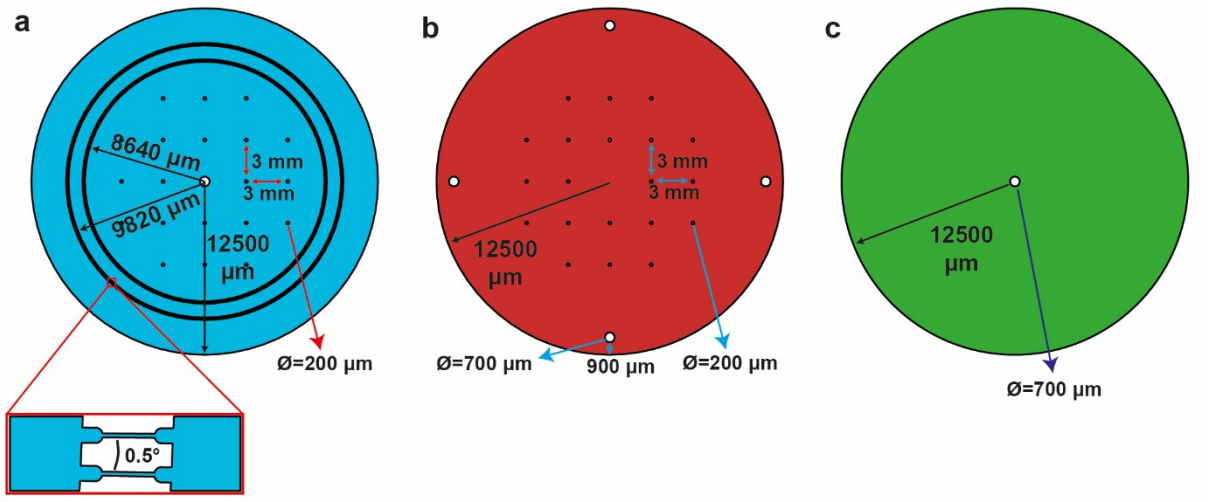

Fig. S7. Additional feature sizes of the three microfluidic layers. The critical features of **(a)** filtering, **(b)** transmission and **(c)** collection layers are noted. The symbol 'Ø' denotes the diameter value.

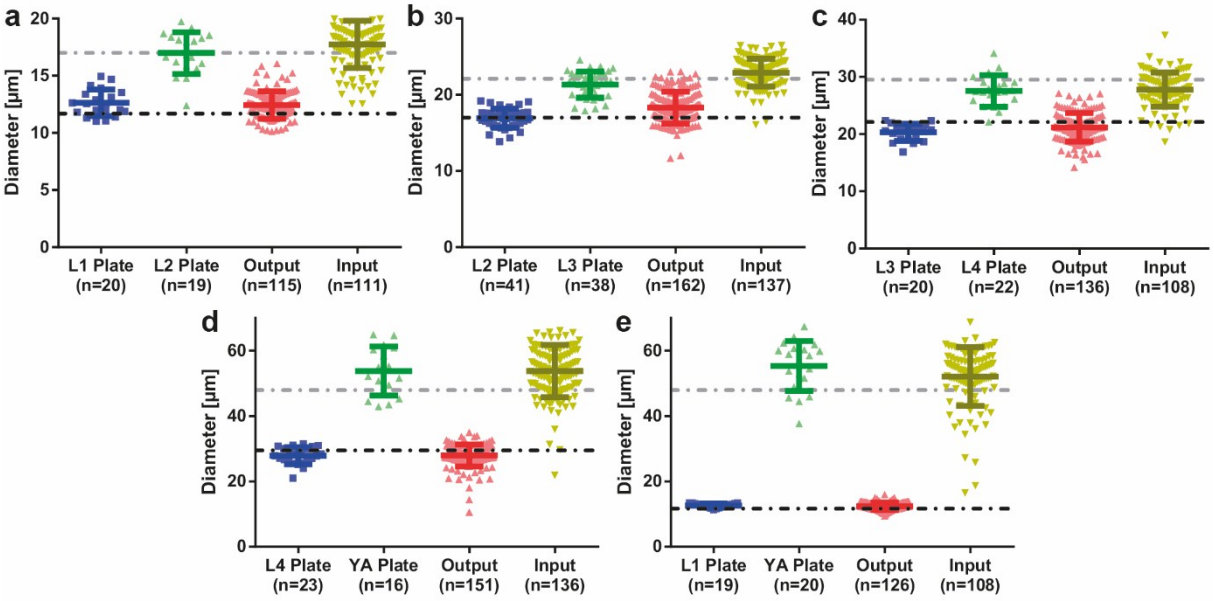

Fig. S8. Measured diameter values of worms before and after sorting from different mixed populations. The first two clusters of data in each panel are the diameter values of two selected worm populations obtained from plates (before the sorting experiment). These populations are mixed, sorted on the chip and, after the sorting procedure, the diameters of worms of the two sorted worm populations present in the Input and Output of the filter chip, respectively, are measured. The mixed populations are: **(a)** L1 and L2, **(b)** L2 and L3, **(c)** L3 and L4, **(d)** L4 and young adult (YA), and **(e)** L1 and YA, respectively. The black and gray dashed lines in each panel display the onset diameter values of the younger and older larval stages, respectively. Data is expressed as mean ± SD. All measurements are based on 6 experiments for each sorting experiment. The number "n" is the total number of worms measured for a particular condition.

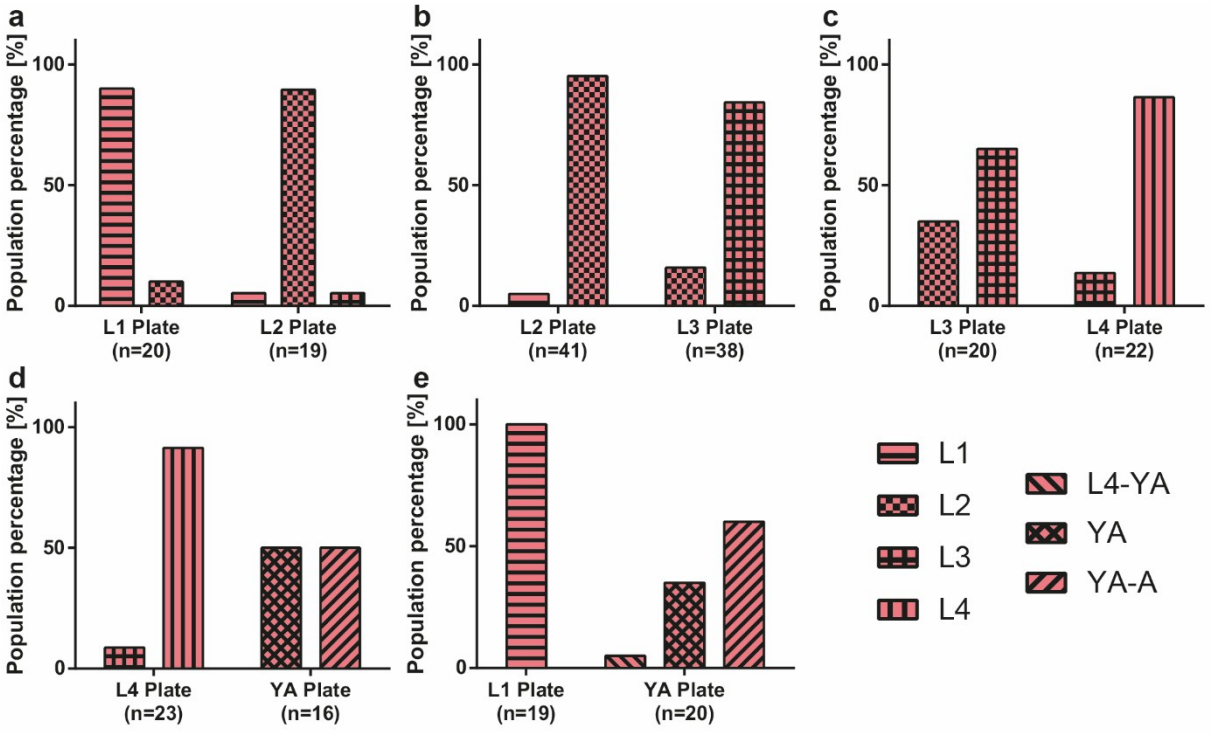

Fig. S9. Characterization of worm populations on NGM agar plates prior to sorting experiments. Larvae populations present on 'synchronized' NGM agar plates, as determined from the larval diameter, prior to mixing them for subsequent sorting experiments of (a) L1 and L2, (b) L2 and L3, (c) L3 and L4, (d) L4-YA, and (e) L1-YA. The total number of worms measured for characterizing a NGM agar plate, the number "n", is noted below the bar plots.

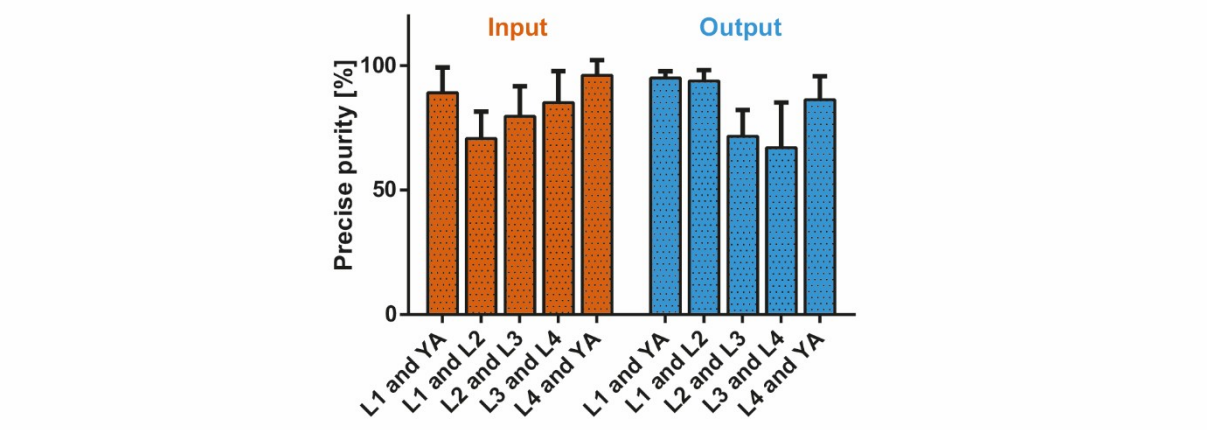

Fig. S10. Purity precision of the sorting experiments. Purity precision of the sorting of the initially mixed populations (L1 and YA, L1 and L2, L2 and L3, L3 and L4, and L4 and YA) at the input and the output of the microfluidic chip. We obtained a purity precision of 89, 71, 80, 85 and 96% at the input and 95, 94, 72, 67 and 86% at the output of the device for L1-YA, L1-L2, L2-L3, L3-L4 and L4-YA sorting experiments, respectively. Data is expressed as mean ± SD. Measurements are based on 6 experiments for each sorting experiment.

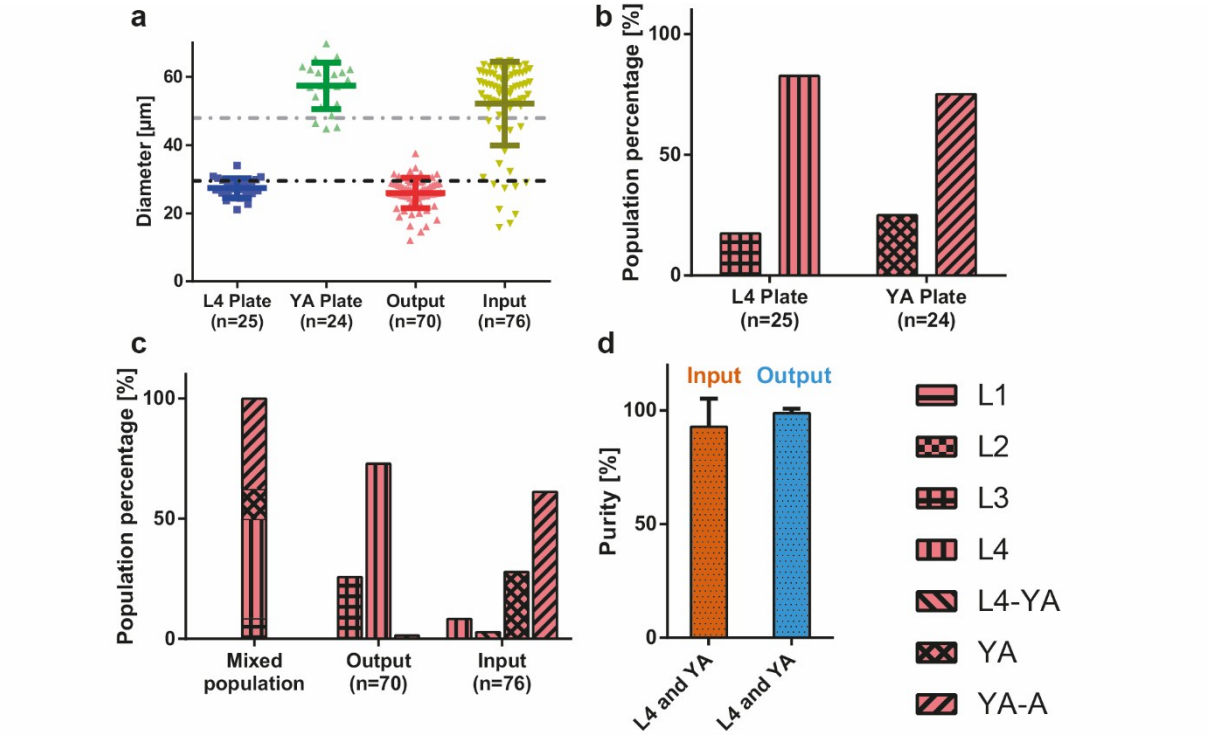

Fig. S11. Results of L4-YA sorting experiments performed using 800 worms from each L4 and YA worm population. (a) Measured diameter values of worms before and after sorting from L4 and YA worm populations. **(b)** Larvae populations present on 'synchronized' NGM agar plates, as determined from the larval diameter, prior to mixing them for subsequent sorting experiments of L4-YA. **(c)** Distribution of worm populations, as determined from the worm diameter, before sorting ("Mixed population" results) and after sorting ("Output and Input" results) for mixed worm populations of L4 and YA. **(d)** Sorting purity of the initially mixed L4 and YA worm populations at the input (only YA at Input would give 100% purity) and the output (only L4 at Output would give 100% purity) of the microfluidic sorting chip. Data is expressed as mean ± SD. All measurements are based on 3 experiments for each sorting experiment. The number "n" is the total number of worms measured for a particular condition.

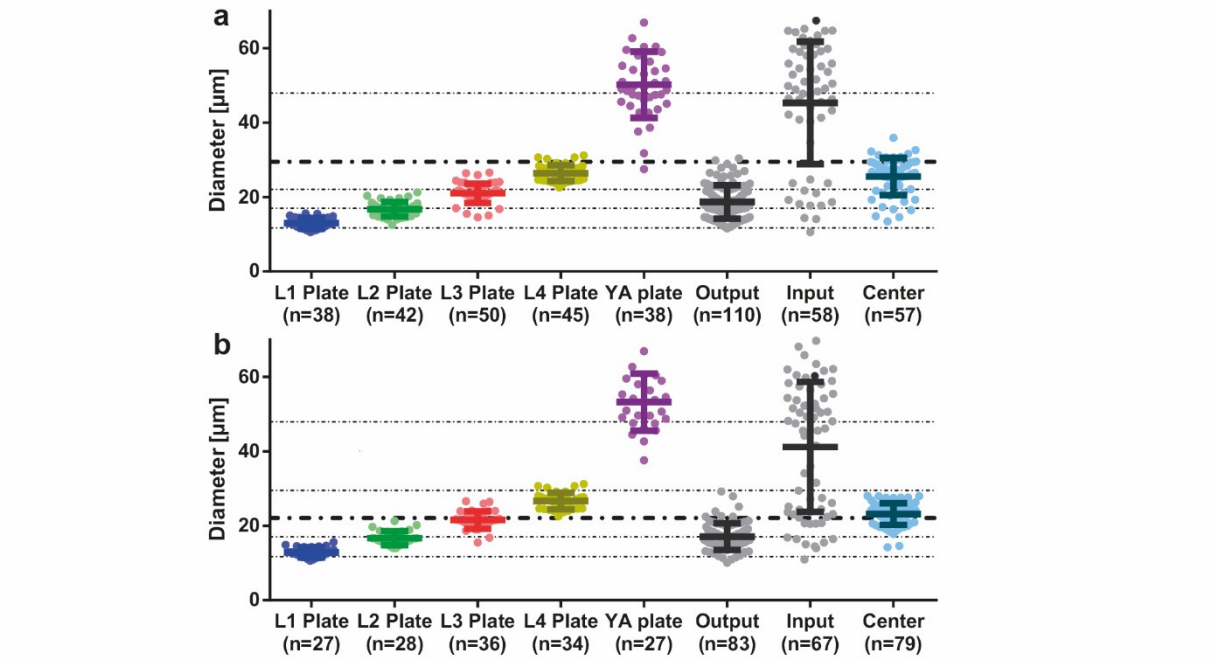

Fig. S12. Measured diameter values of worms obtained from dedicated plates (L1, L2, L3, L4, YA), which were used to prepare a worm mix that was sorted subsequently by our chip, after which we measured the diameter values of the worms in the Output, Input, and Center chamber of a sorting chip. The latter was configured with (a) 26 and 20 µm and (b) 20 and 15 µm filter structures, respectively. The light and thick dot-dashed lines in each panel display the onset diameter value of the untargeted and targeted worm populations of each chip, respectively. Data is expressed as mean ± SD. All measurements are based on 4 experiments for each sorting experiment. The number "n" is the total number of worms measured for a particular condition. The final output is a population of L3-L4 worms obtained from the Center chamber of the second device (b) in the cascaded configuration.

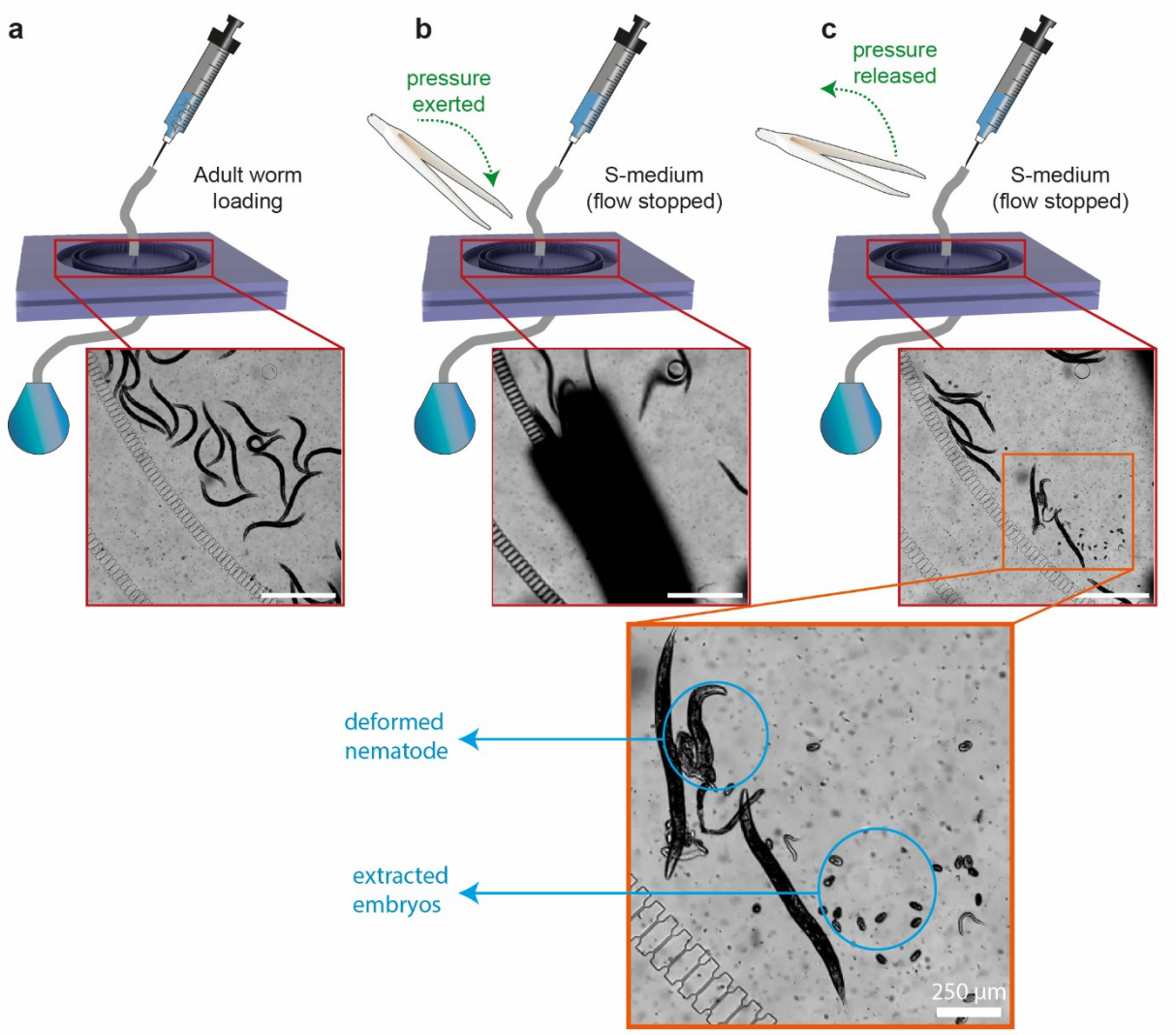

Fig. S13. Schematic illustration and real-time images of the experimental protocol for on-chip egg extraction. (a) After the chip filling and degassing steps, an adult worm population is loaded in the microfluidic device. **(b)** A tweezer is used to exert pressure locally on the PDMS chip and hence the adult nematodes are mechanically compressed. **(c)** After the release of the tweezer, some worms have deformed characteristics and embryos are extracted from these worms. Scale bars: 1000 µm (unless otherwise stated).

Supplementary Movies

Movie S1. A real-time video of sorting of L1 population from a combination of L1 and L2 populations

Movie S2. A real-time video of sorting of L2 population from a combination of L2 and L3 populations

Movie S3. A real-time video of sorting of L3 population from a combination of L3 and L4 populations

Movie S4. A real-time video of sorting of L4 population from a combination of L4 and young adult populations

Movie S5. A real-time video of sorting of L1 population from a combination of L1 and young adult populations

Movie S6. A real-time video of extraction of L3 larvae from a mixed worm population with cascaded microfluidic chips that were mounted on a motorized stage

Movie S7. A real-time video of on-chip egg extraction using a standard bleaching solution

Movie S8. A real-time video of collection of hatched L1 worms 12 hours after on-chip egg extraction using a standard bleaching solution

Movie S9. A real-time video of on-chip egg extraction using a tweezer for local and mechanical compression of adult nematodes