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

High-Throughput, Motility-Based Sorter for Microswimmers such as *C. elegans*

Supplement

Jinzhou Yuan[1], Jessie Zhou[2], David M. Raizen[2], and Haim H. Bau[1,3]

[1]Department of Mechanical Engineering and Applied Mechanics, University of Pennsylvania, Philadelphia, PA 19104

[2]Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

[3]Corresponding author: bau@seas.upenn.edu

Video: The video records nematode motion through the bifurcation region of the Y sorter. Animals occasionally enter the separation conduit. Only the ones with a sufficient propulsive power to overcome the adverse flow progress upstream through the separation conduit. Once the animal enters the collection conduit, it is carried with the flow into the collection chamber. Scale bar: 1mm. Play speed: real time.

S1. Gravity-Based Sorting

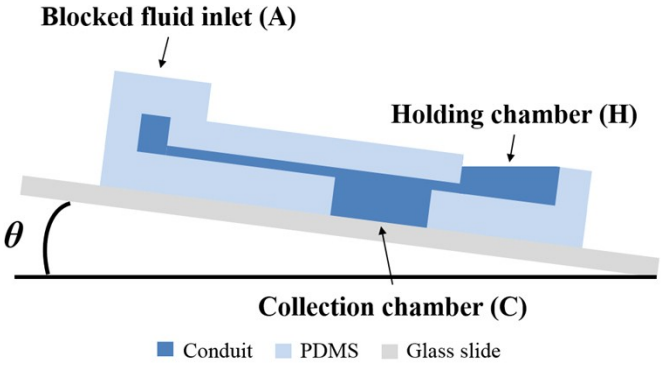

Fig. S1: A schematic depiction of the gravity-based sorter.

In this sorter, we use gravity as an alternative to adverse flow. The L sorter is tilted with an angle θ with respect to the horizontal plane (Fig. S1). Since the animals' density is greater than

that of the suspending liquid, animals must overcome gravitational forces to swim uphill. The greater the angle θ, the greater is the propulsive power needed to overcome gravity. Only animals with sufficient propulsive power will depart from the holding chamber and reach the collection chamber. The threshold propulsive power needed to depart the holding chamber is a function of the tilt angle θ.

To demonstrate the feasibility of this method, we filled a L sorter with M9 buffer and loaded its holding chamber with EMS mutagenized, wild-type young adult animals. The holding chamber was then tilted to an angle θ, and the sorting operation was carried out for two hours. The animals left in the holding chamber were extracted, suspended in M9 buffer, and confined between two glass slides, spaced 130 μm to 160 μm apart. The motion of these animals was tracked with a video camera and their velocities were deduced with the wrMTrck ImageJ plug-in. Similar recordings and velocity measurements were also carried out with the pre-sorted, mutagenized animals. The experiments were repeated twice with different tilting angles (n=1600 animals in each experiment). The average swimming speeds of the animals that were left in the holding chamber were 208μm ± 186μm and 273μm ± 206 μm when θ = 5°C and 15°C, respectively. The average swimming speed of the pre-sorted population was 314μm ± 198 μm. Our preliminary data suggest that the gravity-based method can select animals based on motility and that the threshold motility can be controlled with the tilting angle. Since no syringe pumps are needed in the gravity-based sorter, the method provides a reduced cost, simpler design, and greater convenience.

Table S1: Genetic complementation test results: Assessment of male behavior

Father^	Mother^	Fraction of male progeny feeding	Fraction of male progeny moving	N
+	+	0.13	0.20	46
qn44	*qn44*	0.56	0.88	25
+	+	0.05	0.19	21
qn52	*qn52*	0.53	0.68	19
+	+	0.13	0.20	46
qn44	*qn40*	0.32	1.0	41
+	+	0.05	0.19	21
qn44	*qn52*	0.80	0.83	40
+	+	0.00	0.05	22
qn44	*qn45*	0.39	0.44	18
+	+	0.05	0.19	21
qn52	*qn44*	0.90	0.67	21
+	+	0.05	0.19	21
qn52	*qn51*	0.74	0.79	19
+	+	0.10	0.19	21
qn52	*qn53*	0.87	0.74	38
+	+	0	0.05	22
qn44	*qn49*	0.67	0.84	43
+	+	0.20	0.40	25
qn44	*qn46*	0.42	0.63	19
+	+	0.00	0.05	22
qn44	*qn54*	0.00	0.09	22

N denotes number of animals tested.

^Each animal contained the transgene *qnIs303[Phsp-16.2:flp-13; Phsp-16.2:gfp; Prab-3:mCherry]*.

The behavior of the animals on an agar surface was assessed by an observer blinded to the genotype 2-3 hours after a 33^{o}C heat shock. If the pharyngeal pumping rate was >5 per 20 seconds, the animal was considered feeding. If the frequency of anterior body bends was greater than 5 per 20 second, the animal was considered moving.

For each complementation test, the behavior of a cohort of control animals of the *qnIs303[Phsp-16.2:flp-13; Phsp-16.2:gfp; Prab-3:mCherry]* parental strain was simultaneously assessed. Statistical comparisons between mutants and same-day control animals were performed using the two-tailed Fisher's Exact Test. Shaded rows were those in which there was significant suppression of quiescence of both feeding and locomotion ($p<0.05$).

Table S2: Genetic complementation test results: Assessment of hermaphrodite behavior

Father^	Mother^	Fraction of hermaphrodite progeny feeding	Fraction of hermaphrodite progeny moving	N
+	+	0.09	0.23	47
qn51	*qn51*	0.94	0.76	17
+	+	0.04	0.27	26
qn52	*qn51*	0.92	0.68	25
+	+	0.14	0.19	21
qn52	*qn53*	0.89	0.58	19
+	+	0.14	0.19	21
qn44	*qn52*	0.89	0.50	18
+	+	0.14	0.04	50
qn44	*qn40*	0.72	0.91	22

N denotes number of animals tested.

^Each animal contained the transgene *qnIs303[Phsp-16.2:flp-13; Phsp-16.2:gfp; Prab-3:mCherry]*.

The behavior of the animals on an agar surface was assessed by an observer blinded to the genotype 2-3 hours after a 33^{o}C heat shock. If the pharyngeal pumping rate was equal to or greater than 5 pumps per 20 seconds, the animal was considered to have been feeding. If the frequency of anterior body bends was equal to or greater than 5 per 20 second, the animal was considered to have been moving.

For each complementation test, the behavior of a cohort of control animals of the *qnIs303[Phsp-16.2:flp-13; Phsp-16.2:gfp; Prab-3:mCherry]* parental strain was simultaneously assessed. Statistical comparisons between mutants and same-day control animals were performed using the two-tailed Fisher's Exact Test. Shaded rows were those in which there was significant suppression of quiescence of both feeding and locomotion ($p<0.05$).