High-Throughput Sorting of Eggs for Synchronization of C. *elegans* in a Microfluidic Spiral Chip

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1. Device Geometry

Spiral type	Flat
First diameter	10 mm
Second diameter	32.4 mm
Turns	8
Final turns	6 (Inner two turns were removed)
Spacing between spiral turns	400 µm
Length of the spiral loops	464.7 mm
Inner depth	220 µm
Outer depth	160 µm
Inner outlet length	6 mm
Inner outlet width	750 µm
Outer outlet length	3.8 mm
Outer outlet width	1000 µm
Angle between the inner and outer outlets	58.4 °

Table S1. Geometry information regarding the spiral microfluidic channel. The 3D modeling tool (Rhinoceros Version 5.3.2, McNeel North America, USA) was used to model the chip.

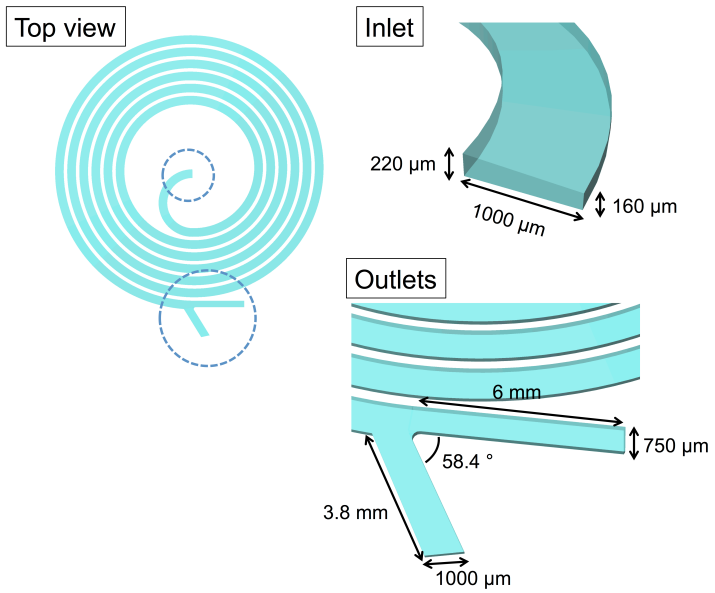

Figure S1. Geometry information regarding the spiral microfluidic channel.

2. Figures:

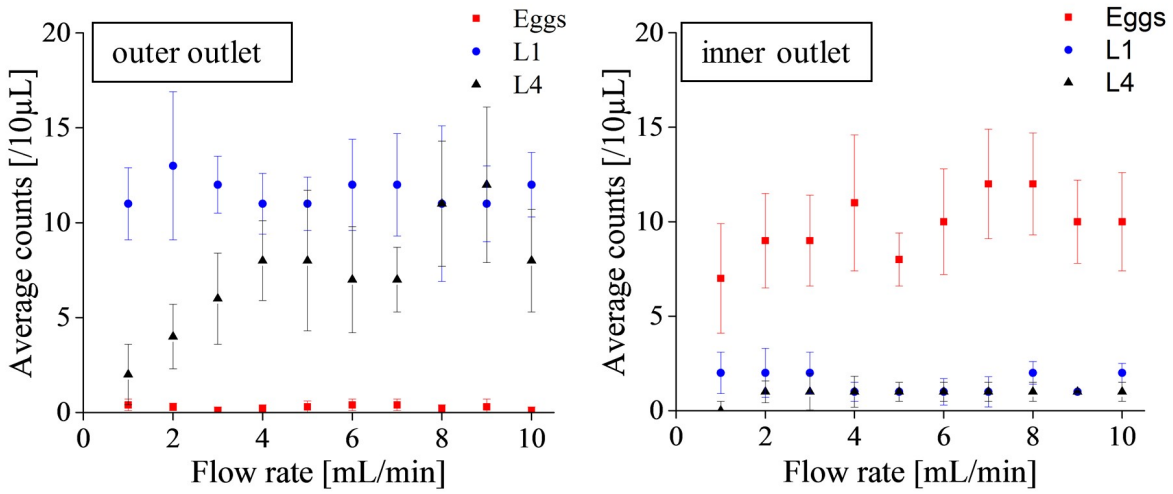

Figure S2. Average counts of organisms per 10 µL collected from the inner outlet and outer outlet after sorting of single populations of eggs, L1 and L4.

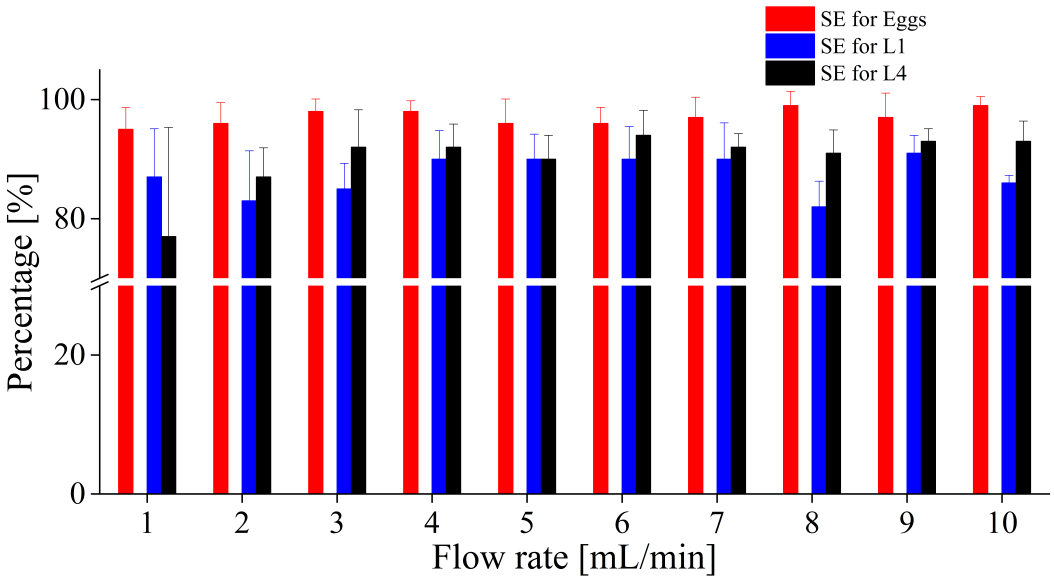

Figure S3. Average sorting efficiency (SE) of organisms per 10 µL collected from the inner outlet and outer outlet after sorting of single populations of eggs, L1 and L4.

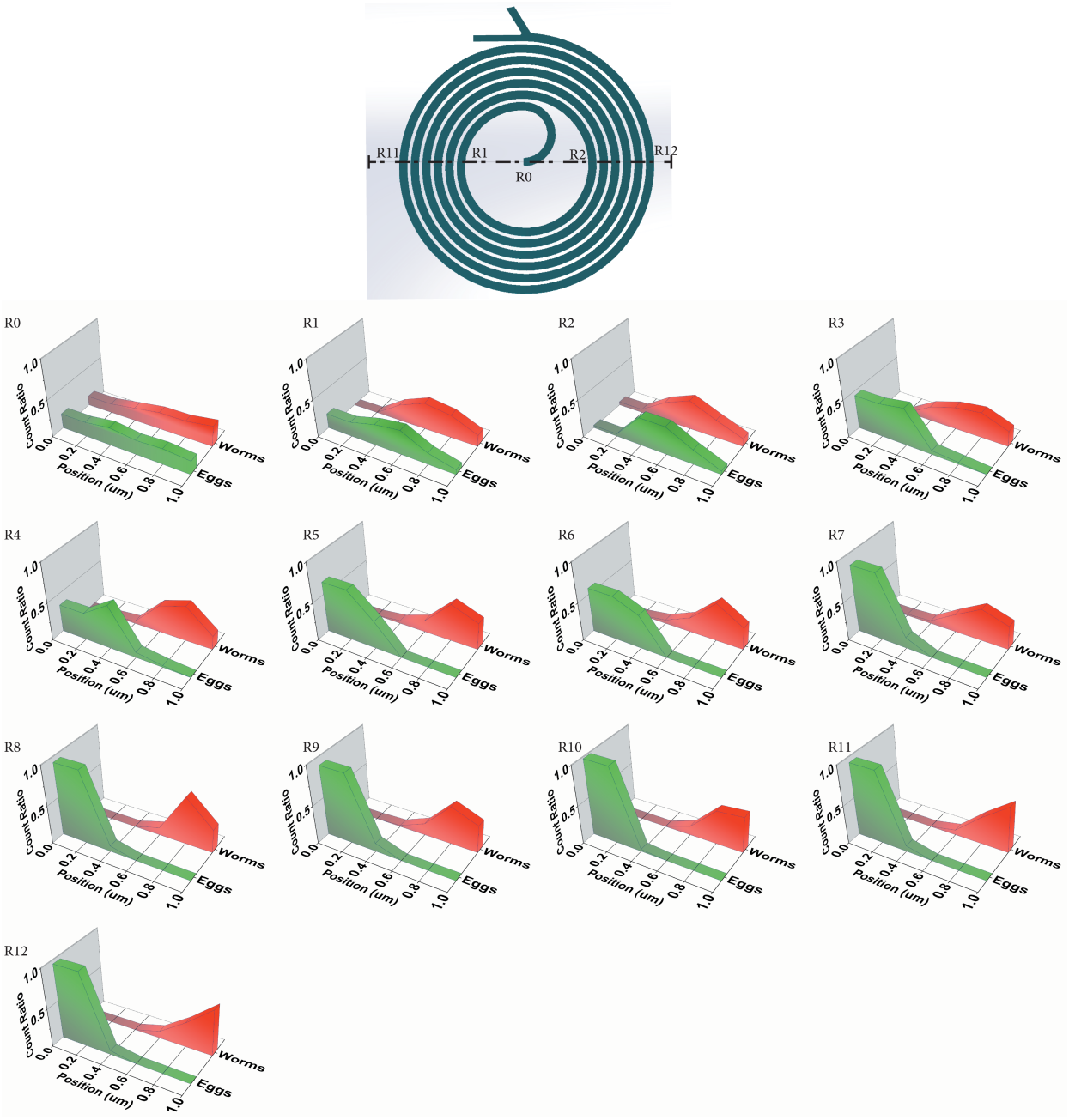

Figure S4. Spatial distribution of organisms (eggs and L4) within the channel during the sorting of eggs from eggs+L4 mixture. The loops are labeled R0, R2, R3…..R12 for every half loop from the inlet to the outermost loop.

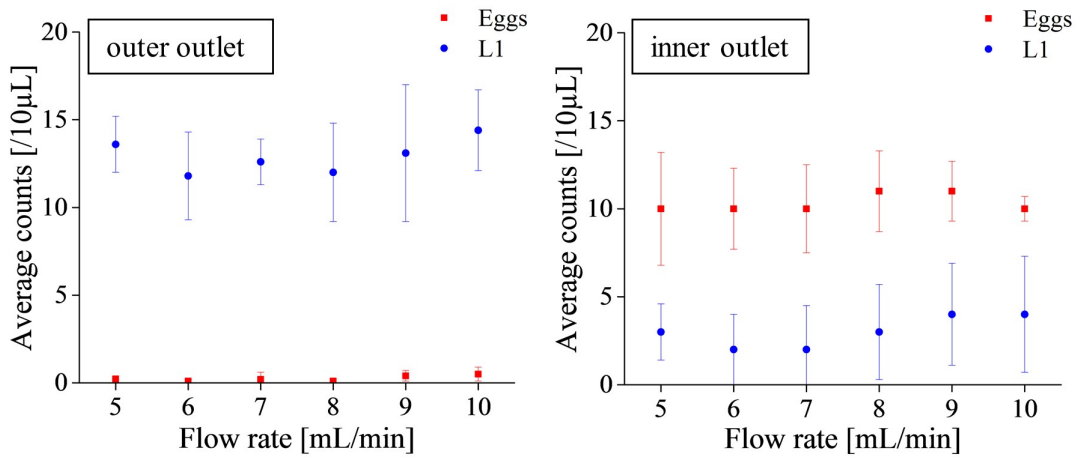

Figure S5. Average counts of organisms per 10 µL collected from the inner outlet and outer outlet after sorting of heterogeneous population of eggs + L1

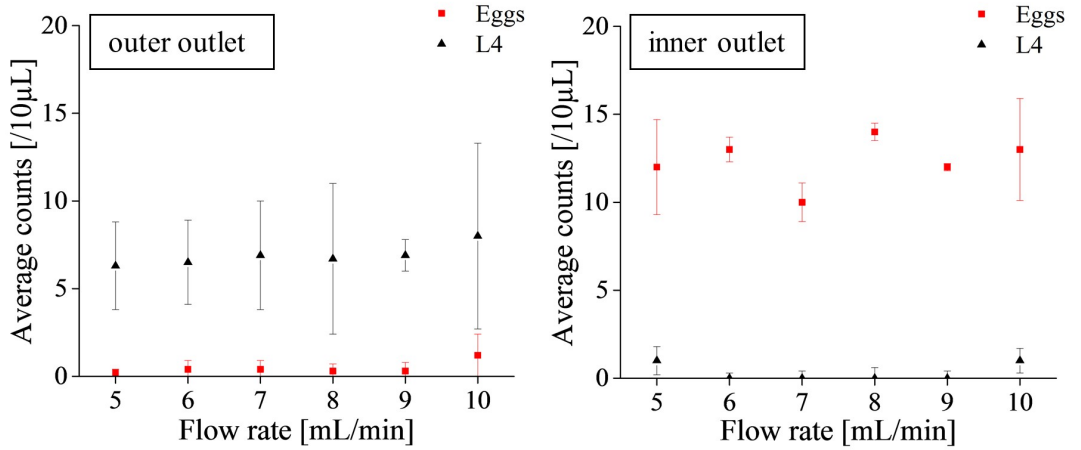

Figure S6. Average counts of organisms per 10 µL collected from the inner outlet and outer outlet after sorting of heterogeneous population of eggs + L4

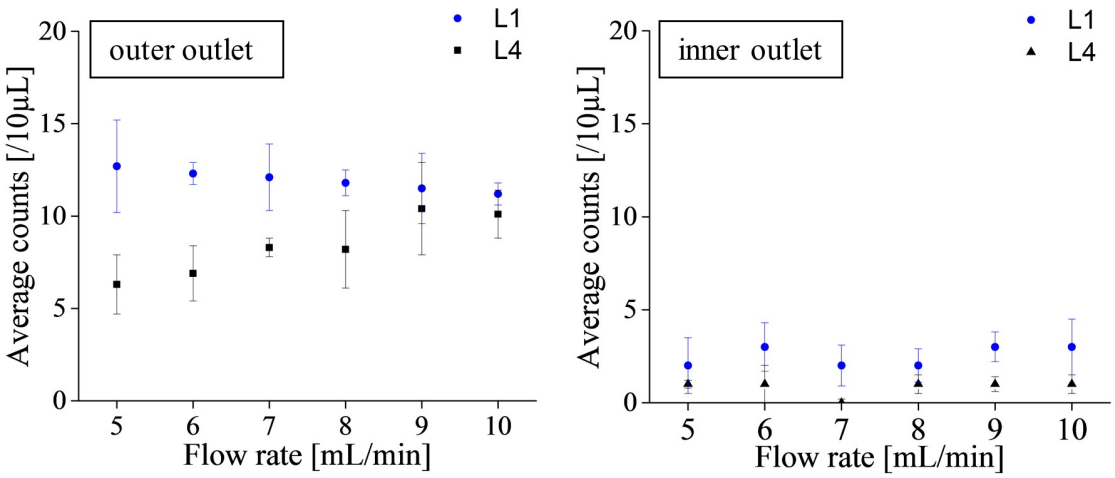

Figure S7. Average counts of organisms per 10 µL collected from the inner outlet and outer outlet after sorting of heterogeneous population of L1 + L4

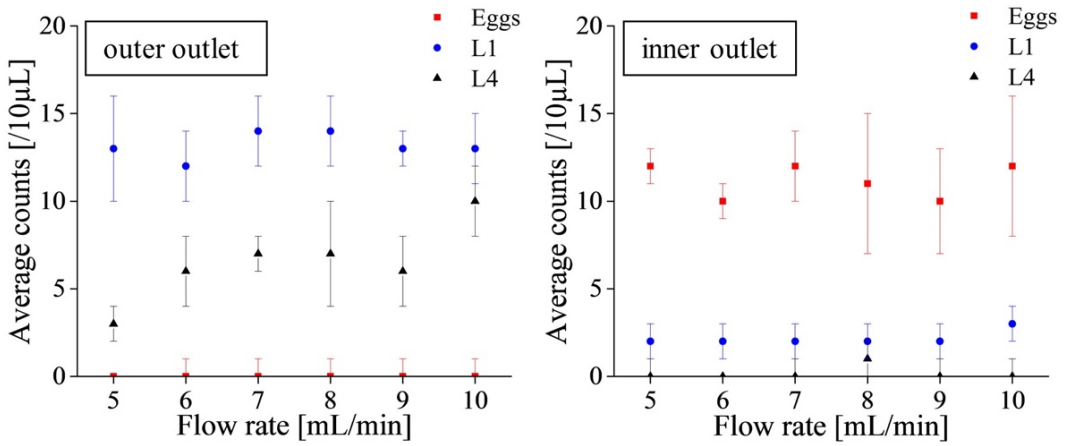

Figure S8. Average counts of organisms per 10 µL collected from the inner outlet and outer outlet after sorting of heterogeneous population of eggs + L1 + L4 (~7 organisms/10 uL)

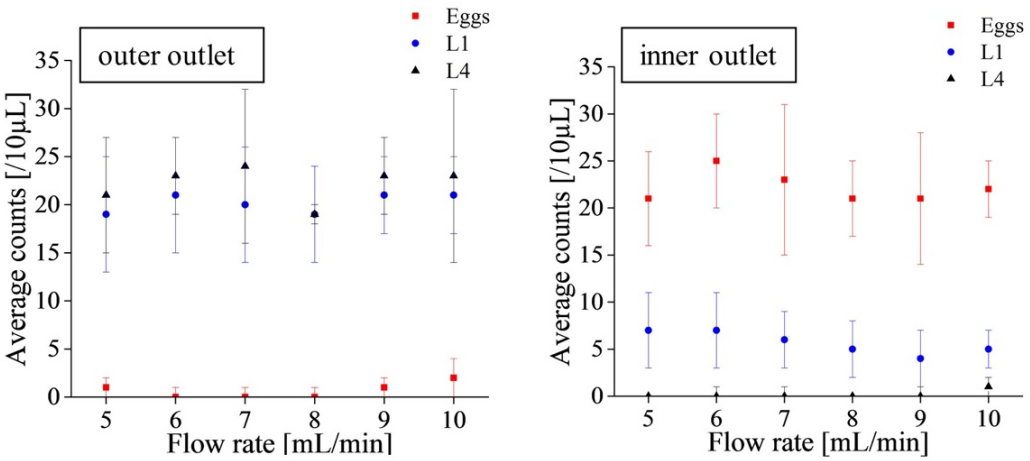

Figure S9. Average counts of organisms per 10 µL collected from the inner outlet and outer outlet after sorting of heterogeneous population of eggs + L1 + L4 at the doubled concentration (~14 organisms/10 µL)

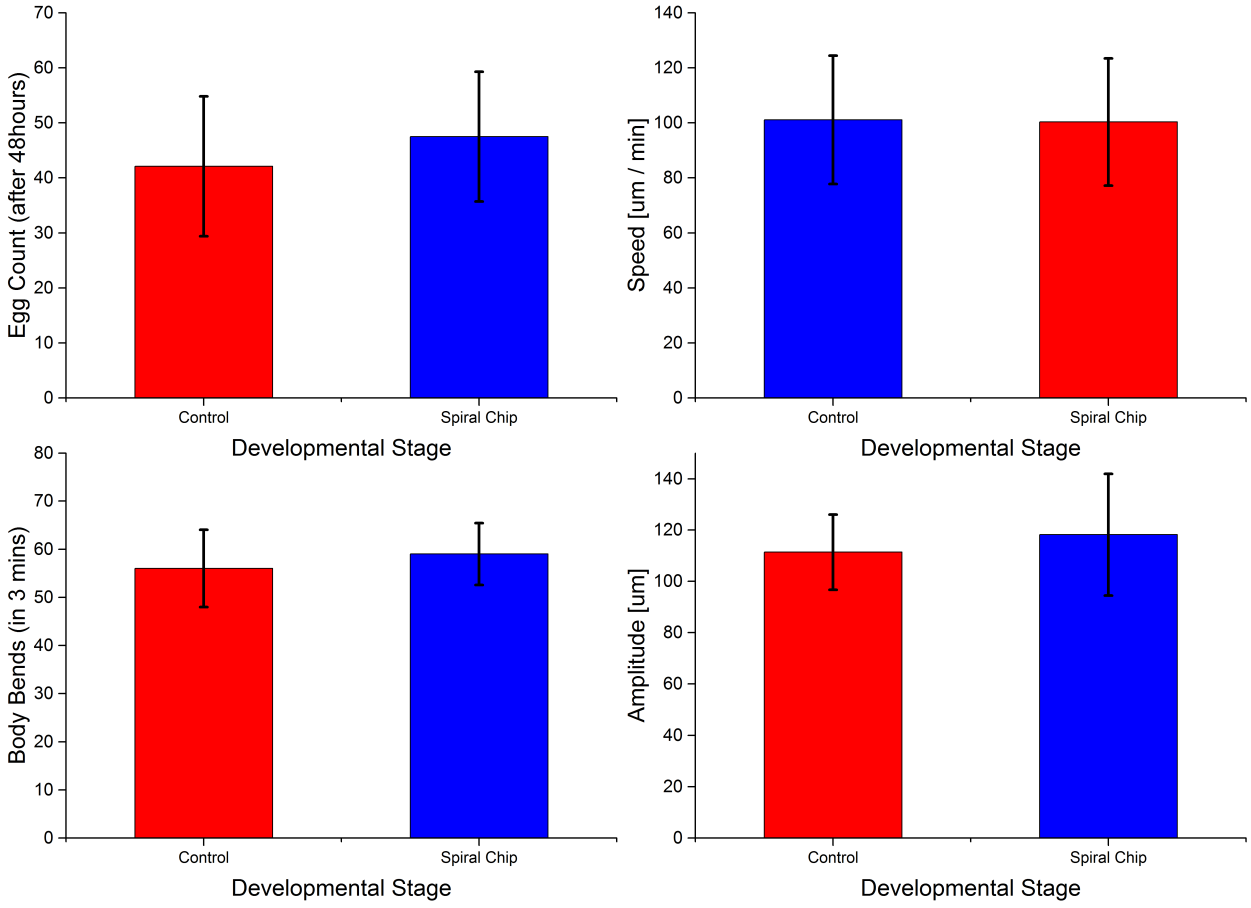

Figure S10. Viability Assay: Reproductive fitness (egg count) and behavioral (locomotion: velocity, body bends and amplitude) comparison between L4 worms collected from the spiral chip

at 10 mL/min and L4 worms (control) that did not undergo sorting in the spiral chip. Student t-test showed that there is no significant difference in these behaviors of both categories. $p > 0.05$ in all cases.

3. Equations for SE, SP, and percentage of L1 & L4 in the inner outlet:

(a) Sorting homogenous population:

- Sorting efficiency for Eggs= $\frac{\#\ of\ eggs\ in\ the\ inner\ outlet}{\#\ of\ eggs\ in\ the\ inner\ and\ outer\ outlet}$
- Sorting efficiency for L1= $\frac{\#\ of\ L1\ in\ the\ outer\ outlet}{\#\ of\ L1\ in\ the\ inner\ and\ outer\ outlet}$
- Sorting efficiency for L4= $\frac{\#\ of\ L4\ \square n\ the\ outer\ outlet}{\#\ of\ L4\ in\ the\ inner\ and\ outer\ outlet}$

(b) Sorting heterogeneous population containing two developmental stages:

- Eggs+L1:
- Sorting efficiency for Eggs+L1= $\frac{\#\ of\ eggs\ in\ the\ inner\ outlet}{\#\ of\ eggs\ in\ the\ inner\ and\ outer\ outlet}$
- Sample purity for Eggs+L1= $\frac{\#\ of\ eggs\ in\ the\ inner\ outlet}{\#\ of\ eggs\ in\ the\ inner\ outlet+\#\ of\ \square 1\ in\ the\ inner\ outlet}$
- Eggs+L4:
- Sorting efficiency for Eggs+L4= $\frac{\#\ of\ eggs\ in\ the\ inner\ outlet}{\#\ of\ eggs\ in\ the\ inner\ and\ outer\ outlet}$
- Sample purity for Eggs+L4= $\frac{\#\ of\ eggs\ in\ the\ inner\ outlet}{\#\ of\ eggs\ in\ the\ inner\ outlet+\#\ of\ L4\ in\ the\ inner\ outlet}$

- L1+L4:
- Sorting efficiency for L1+L4= $\frac{\#\ of\ L1+L4\ in\ the\ inner\ outlet}{\#\ of\ L1+L4\ in\ the\ inner\ and\ outer\ outlet}$
- % of L1 in the inner outlet = $\frac{\#\ of\ L1\ in\ the\ inner\ outlet}{\#\ of\ L1\ and\ L1\ in\ the\ outer\ outlet}$
- % of L4 in the inner outlet = $\frac{\#\ of\ L4\ in\ the\ inner\ outlet}{\#\ of\ L4\ in\ the\ inner\ and\ L4\ in\ the\ outer\ outlet}$

(c) Sorting heterogeneous containing three developmental stages, and sorting heterogeneous population containing doubled concentration of three developmental stages:

- Sorting efficiency for Eggs+L1+L4 = $\frac{\#\ of\ eggs\ in\ the\ inner\ outlet}{\#\ of\ eggs\ in\ the\ inner\ and\ outer\ outlet}$
- Sample purity for Eggs+L1+L4= $\frac{\#\ of\ eggs\ in\ the\ inner\ outlet}{\#\ of\ eggs+L1+L4\ in\ the\ inner\ outler}$
- % of L1 in the inner outlet = $\frac{\#\ of\ L1\ in\ the\ inner\ outlet}{\#\ of\ L1+L4+eggs\ in\ the\ inner}$
- % of L4 in the inner outlet = $\frac{\#\ of\ L4\ in\ the\ inner\ outlet}{\#\ of\ L1+L4+eggs\ in\ the\ inner}$

4. Estimated lift force and Dean's forces:

The Reynold's number of the channel, Re, is given by:

$$Re = \frac{\rho U D_{h\_channel}}{\mu}$$

Where $\rho, U, D_{h\_channel}$ and μ are density, maximum velocity, channel hydraulic diameter and dynamic viscosity, respectively.

The net inertial lift force, F_L, and Dean drag force, F_D, are estimated as[1]:

$$F_L = \frac{\rho U^2 a^4}{D_{h_{channel}}{}^2} f_L(Re, z_c)$$

$$F_D = 3\pi\mu a U_D$$

where a is the organism hydraulic diameter and $f_L(Re, z_c)$ is the coefficient of the net lift force. Also, U_D is the magnitude of the secondary flow and is estimated as:

$$U_D = 1.8\times10^{-4}\ De^{1.63}$$

where *De* is Dean number $De = Re\sqrt{\frac{D_h}{2R}}$ and *R* is the radius of curvature of the channel.

The organism centrifugal force can be estimated by[2]:

$$F_{cent} = \rho\pi a^3 v_{ot}^2/6R$$

where v_{ot} are the organism tangential velocity.

References:

1 M. Rafeie, J. Zhang, M. Asadnia, W. Li and M. E. Warkiani, *Lab Chip*, 2016, **16**, 2791–2802.

5. Estimated g-force in Spiral Chip

Estimated egg acceleration, $a = \frac{v^2}{R}$

Where v is the egg velocity in the spiral channel and R is the radius of curvature.
The egg velocity is given by:

$$v = \frac{Q}{A}$$

Where Q, Volumetric flowrate. For $Q = 5\ \frac{ml}{min} = 1.667 * 10^{-7}\ \frac{m^3}{s}$

Area, $A = 0.5 * (220 + 160) * 1000 * 10^{-12} m^2 = 190 * 10^{-9} m^2$

$$v = 0.877 \frac{m}{s}$$

$$a_{max} = \frac{0.438^2}{10 * 10^{-3}}\ for R = 10mm(first\ loop)$$

$a_{max} = 76.95\ \frac{m}{s^2} \approx 8$ g

6. Supplemental videos

Video S1. High-speed video shows separation of eggs from L1+eggs into the inner outlet at 5 mL/min. Eggs flow towards the upper part of the channel, entering the inner outlet. L1 flows towards the lower part of the channel, entering in the outer channel.

Video S2. High-speed video shows separation of eggs from L4+eggs into the inner outlet at 5 mL/min. It is noticeable that eggs flow towards the upper part of the channel, entering in the inner outlet. L4 flows towards the lower part of the channel, entering in the outer channel. In the video, the worms exhibit random shapes and orientations (straight or coiled) which make the separation challenging.

Video S3. High-speed video shows separation of eggs from L1+L4+eggs (~7 organisms/10 uL) into the inner outlet at 10 mL/min. L1 and L4 flows into the outer outlet. In the video, a group of eggs flow into the inner channel. The worms exhibit random shapes and orientations (straight or coiled) which make the separation challenging.

Video S4. High-speed video shows separation of eggs from L1+L4+eggs into the inner outlet at 10 mL/min after doubling the concentration to ~14 organisms/10 uL. L1 and L4 flow into the outer outlet. A group of eggs flow in the inner channel. Plus, its noticeable that some eggs hit the wall.