Supplementary Material (ESI) for Chemical Communications

A High-Throughput Microfluidic Worm-Sorter

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Electronic Supplementary Information

0.

Figure A shows ROC curves representative of several possible outcomes for a 6 cut-off points sorting scheme (or, in our case, 6 sorting channels).

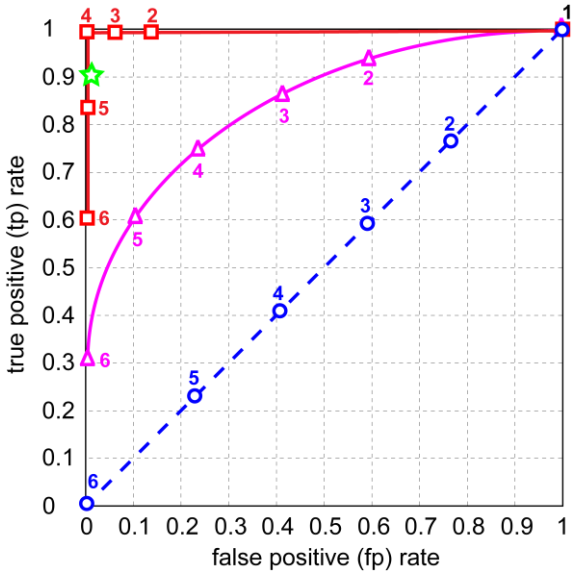
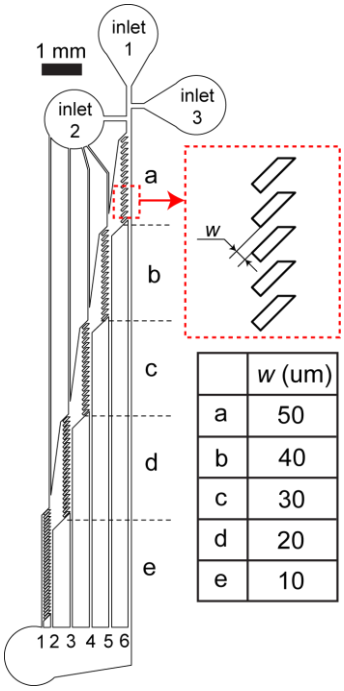


Figure A The dashed line (open circles) represents a random (without sorting) process, where as many adults can be found in all sorting channels as larvae. The solid line (triangles) represents a process with partial sorting achieved in channel 6, but recovering only 30% of adult worms. The solid line (square markers) represents a perfect separation with 100% adult recovery that is also "robust", i.e. no larvae are found from channel 4 onwards and all adults are collected from channels 4, 5 and 6. The star represents our specification of an efficient sorter.

1.

The device shown in Figure B was designed to filter the worms according to their size, since adults are both longer and wider than larvae. Adults typically measure between 50 and 70 µm in width, while worms in the largest larval stage (L4) average 25 µm. Based on this size differential, microfluidic devices with the dimensions illustrated in Figure B were fabricated and tested.



	w (um)
a	50
b	40
c	30
d	20
e	10

Figure B Schematic and associated dimensions of microfluidic filter devices for a separation strategy based on the size differential between adults and larvae.

Devices incorporate three inlets. Inlet 1 is used to load the worms into the device. Inlets 2 and 3 are used to add attractants or simply to "squeeze" the initial flow of worms towards the size of interest of the device (either to the right or to the left). In this case the numbering of the sorting channels is reversed with respect to the layout presented in Figure 1 of the main text, since larvae are expected to progress towards the left-hand side sorting channel and adults towards the right hand side sorting channel.

Despite the intuitive design, the locomotive behaviour of the worms renders the structure inefficient at separating adult worms from larvae. Specifically, adult worms are consistently observed attempting to squeeze between gaps much smaller than their geometric body size. Indeed, many are successful in these attempts due to the flexibility and compressibility of their own bodies and of the PDMS substrate (Movie 1 exemplifies this effect). In order to take advantage of the behaviour (or non-passive nature) of adult worms a new set of microfluidic devices was designed. These are presented in Figure C.

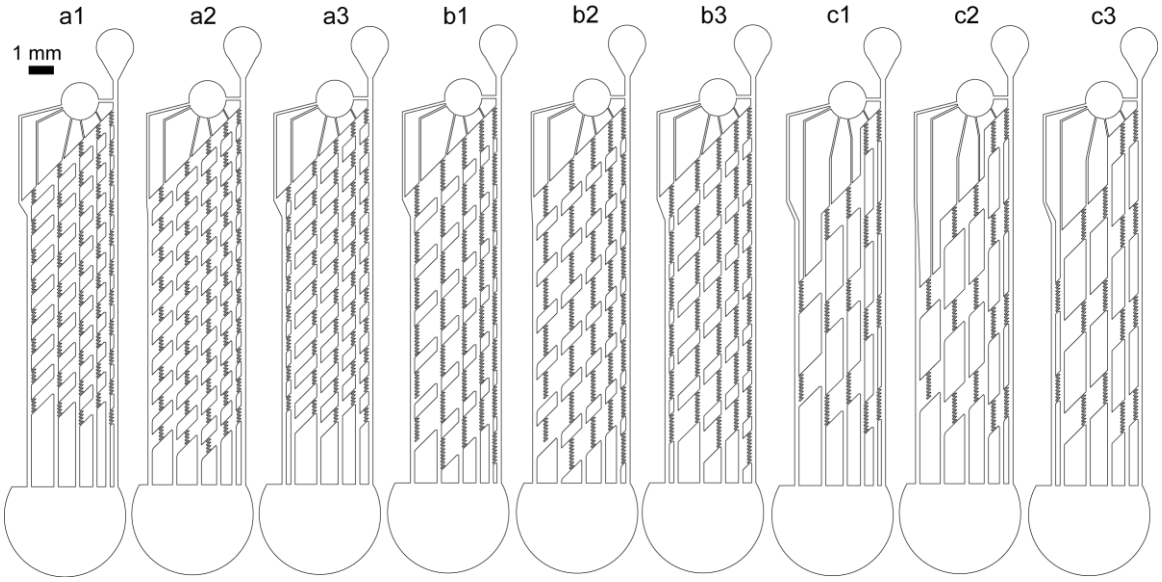


Figure C Schematics of microfluidic devices for the sorting of adult worms and larvae based on the observation that adult worms squeeze between gaps much smaller than their geometric body size. The distance between pillars in the rows of all designs was 50 µm. Designs ***ai*** to ***ci*** differ in the increasing length of the pillar rows and associated chambers. Designs ***x1***, ***x2***, ***x3*** differ in the localization of the pillar rows in the associated chambers (let-hand side, middle and right-hand side, respectively).

The devices in Figure C aimed to "encourage" adult worms to "squeeze" through rows of pillars and therefore progress towards the left-hand side of the device. Squeezing the fluid-flow of worms from the additional inlet should in principle confine larvae to sorting channels on the right-hand side of the device. In each device, the chamber width (and the distance between neighbouring rows of pillars) increased sequentially as one moved from right to left. This variation made it more difficult for larvae to reach the next row of pillars and interact with them, while the longer adult worms were able to progress towards sorting channel 6.

Good separation of adults and larvae was observed using the above strategy, especially with the addition of attractants (based on NaCl and E. Coli gradients). Unfortunately, sorting throughput was low since adults tended to delay their progress through the device through interaction with the pillar structures. This led to overcrowding unless low flow-rates and low densities of the worm suspension were used. A representative ROC curve analysis of a separation is presented in Figure D in addition to a video (Movie 2).

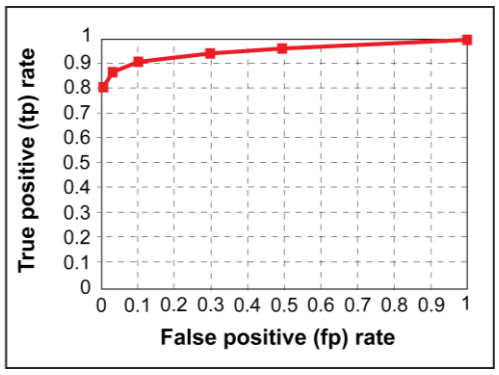


Figure D Representative ROC curve for the separation of adult worms and larvae using device **a1** (in Figure C) and E. coli as an attracting agent. Sample was introduced through inlet 1 at a volumetric flow rate of 5 µL/min, and a 10% E. coli suspension was introduced through inlet 2 at 10 µL/min. Data describe a system with a true positive rate of 86% and false positive rate of 3% at sorting channel number 5. A throughput of 25 worms/minute (1500 worms/hour) was achieved.

2.

The "pool-based" devices shown in Figure E were designed to sort on the basis of swimming performance.

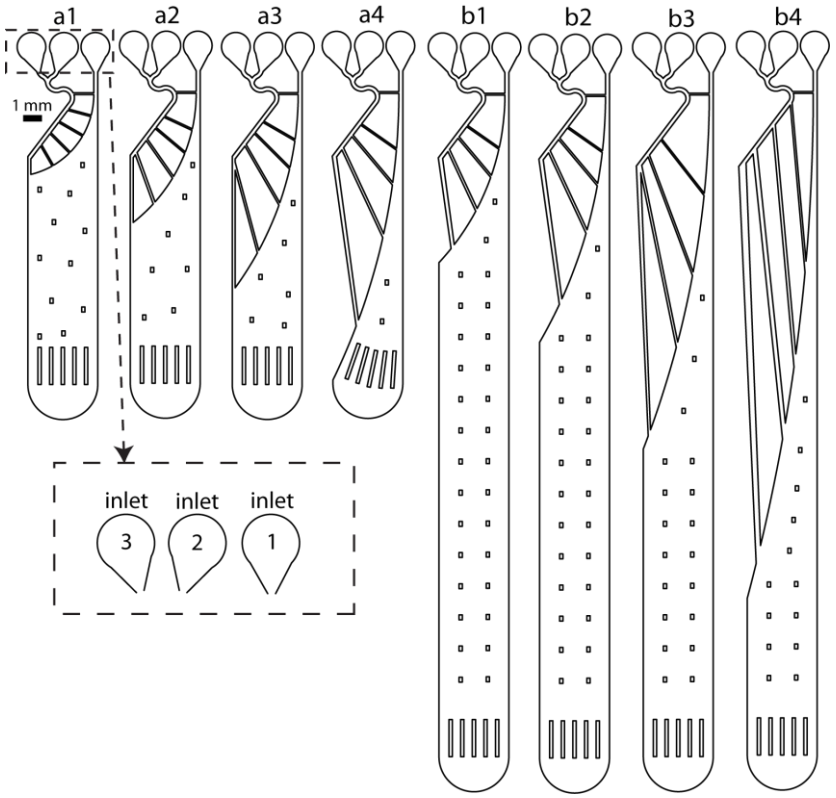


Figure E Pool-based microfluidic devices for sorting worms based on swimming performance. Pillars were included to avoid the ceiling of the pools from collapsing during fabrications and use. Devices $\mathbf{b_i}$ were fabricated with longer bodies than devices $\mathbf{a_i}$. Devices ***x1*** reach their full width at a shorter length than devices **x4**. Both sets of dimensional variation were targeted at allowing a wide diversity of attractant gradients and worm residence times.

During experiments with the devices shown in Figure E it was observed that the presence of attractant gradients has a direct effect on the swimming direction of worms (i.e. many swam to the left of the device, towards the attractant). Optimization of sorting using these devices was difficult for a number of reasons. First, fast flow fields reduced exposure of worms to the attractant gradient. Furthermore, the presence of a flow field seemed to act as a taxis stimulant in itself: adult worms had a strong tendency to orient and swim against the flow-field (upwards). This behaviour interfered with the left-orientation that was sought-after with the use of attractants.

Additionally, if the flow field was reduced enough to allow worms to sense the attractant gradients and orient towards them, two issues arose. First, large amounts of larvae were allowed to progress with the adult worms (increasing the false positive rate). Second, sorting throughput was significantly reduced. All the above contributed to the unsatisfactory separation results observed. A representative ROC curve analysis of a separation is presented in Figure F in addition to a video (Movie 3).

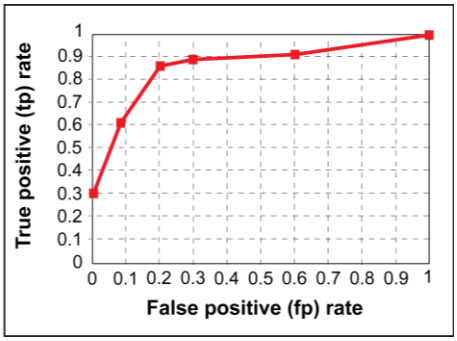


Figure F Representative ROC curve for the separation of adult worms and larvae using device **b1** in Figure E and NaCl as an attracting agent. Sample was introduced through inlet 1 at a volumetric flow rate of 5 μL/min. 1x and 2x Phosphate Buffered Saline was introduced at a flow rate of 10 μL/min through inlets 2 and 3 respectively. Data describe a system with a true positive rate of 32% and false positive rate of 1% at sorting channel number 6. A throughput of 20 worms/minute (1200 worms/hour) was achieved.

3.

The devices shown in Figure G were designed to filter the worms using pillar arrays.

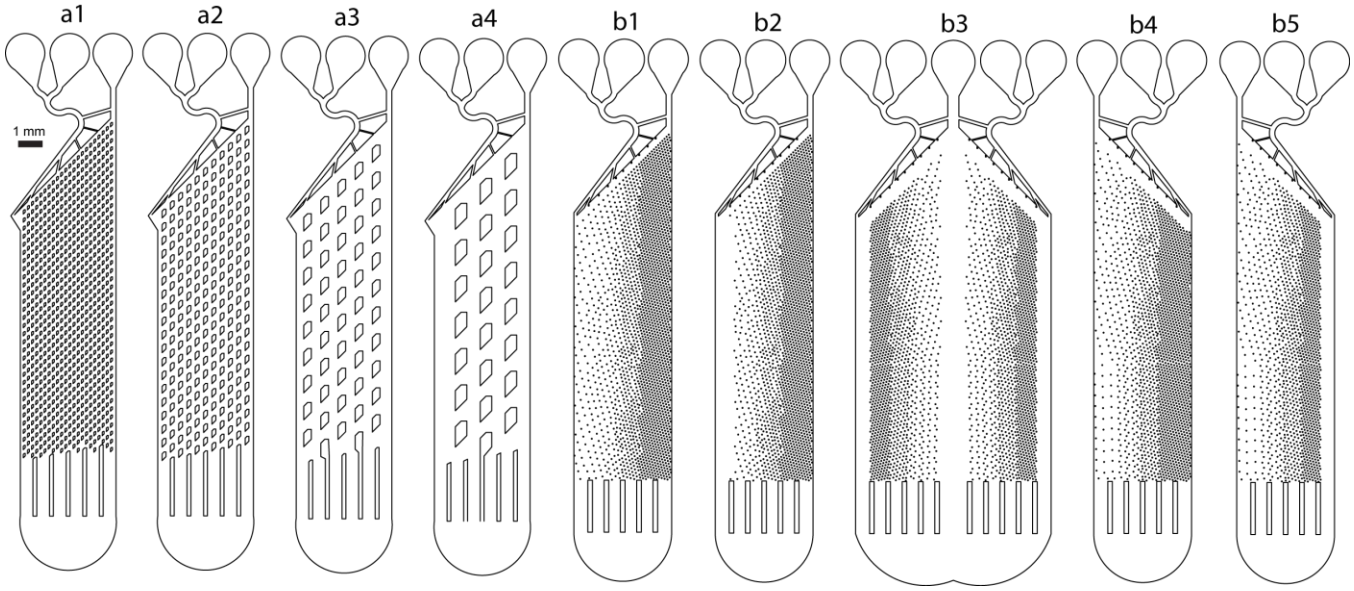


Figure G Schematics of pillar-based microfluidic devices for sorting adult worms and larvae. The distance between pillars in devices **a1**, **a2**, **a3**, **a4** was 100, 200, 400 and 600 µm respectively. The distance between pillars in devices **b1**, **b2**, **b3**, **b4** was variable within each device and ranged from 50 to 400 µm.

Devices **a1** to **a4** were used to test separation in terms of differential locomotion between *crawling* adults and *swimming* larvae. Additionally, attractants (based on NaCl and E. Coli gradients) were used to direct worms towards the side of the device of interest. Devices **b1** to **b5** were designed to improve separation by segmenting the body of the chip into sections containing different pillar separations. Devices **b1** and **b4** were prepared as "smart" filters: the full body of the device was filled with pillars, allowing adult worms to crawl and accumulate in regions of higher pillar density, while larvae were flushed through the device. This approach was non-continuous in operation, since after removal of larvae, adult worms needed to be flushed from the device, which significantly lowered throughput. Movie 4 presents part of an experiment carried out with device **b1**. Devices **b2**, **b3** and **b5** were designed to avoid "cramming" of adults by completely removing the pillars from regions where adults were expected to crawl (left side on device **b2**, both extremes of the body of the device **b3** and right side on device **b5**). Once out of the pillar maze, adults could only swim and the drag force of the fluid-flow expelled them from the device (in a continuous and more high-throughput manner). Additionally device **b3** contained a central pool to allow only strongly swimming adult worms to reach the pillar sections and therefore increase efficiency of larvae separation. Unfortunately, although the concept was successful in separating adult worms and larvae, a low true positive rate limited the recovery of adults. This was primarily due to the fact that worms could easily reorient in the pillar arrays and since crawling occurred at high speeds, adult worms sped through the device in whatever direction they were moving (regardless of the presence of attractants) and exited from any sorting channel.

A representative ROC curve analysis of a separation using device **b5** is presented in Figure H. Movie 5 exemplifies the "orientation problem" encountered when using these devices.

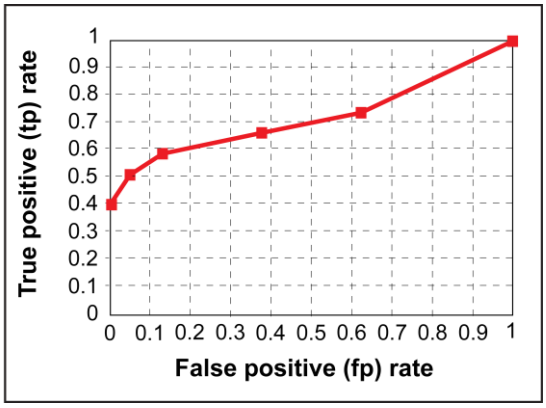


Figure H Representative ROC curve for the separation of adult worms and larvae using device **b5** in Figure G and E. Coli as an attracting agent. Sample was introduced through inlet 1 at a volumetric flow rate of 20 μL/min. 10% and 25% E. coli suspensions were introduced at a flow rate of 20 μL/min through inlets 2 and 3, respectively. Data describe a system with a true positive rate of 39% and false positive rate of 1% at sorting channel number 6. A throughput of 50 worms/minute (3000 worms/hour) was achieved.

4.

Movie 6 illustrates a portion of the experiment presented in Figure 2 of the main text. Sample was introduced through inlet 1 at 20 µL/min, with buffer being introduced through inlets 2 and 3 at 30 µL/min. As described in the main text analytical throughput was in excess of 200 worms/minute (12,000 worms/hour). Figure I shows representative ROC curves for this device under different operating conditions.

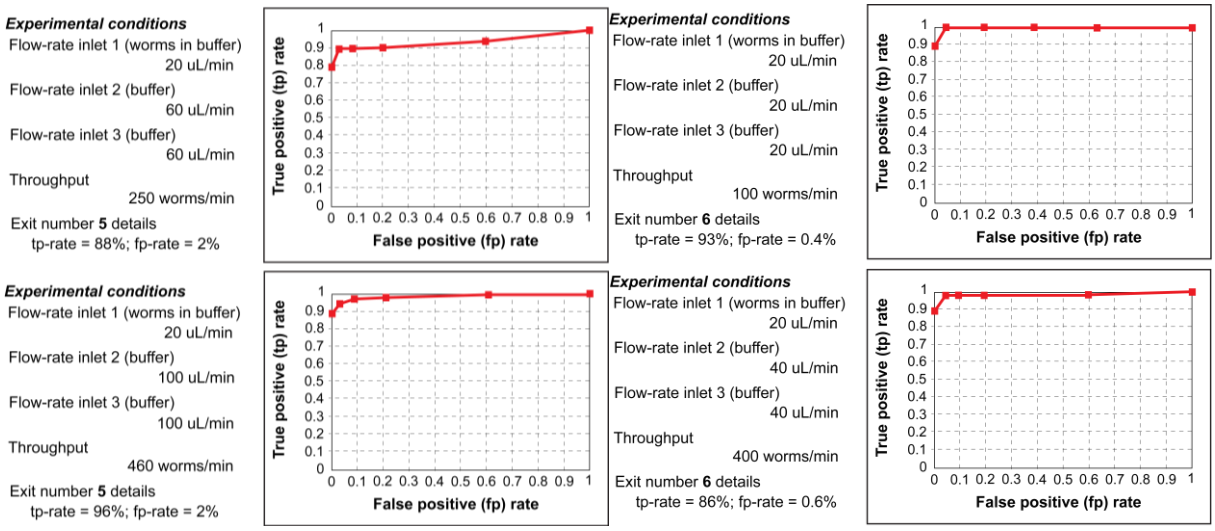


Figure I Representative ROC curves for the device described in Figure 2 of the main text under different operating conditions.

Figure J illustrates additional "maze" devices tested for this strategy. All devices in Figure J contain channels 150 µm in height, except for devices **g** and **h** that contain channels 600 µm in height.

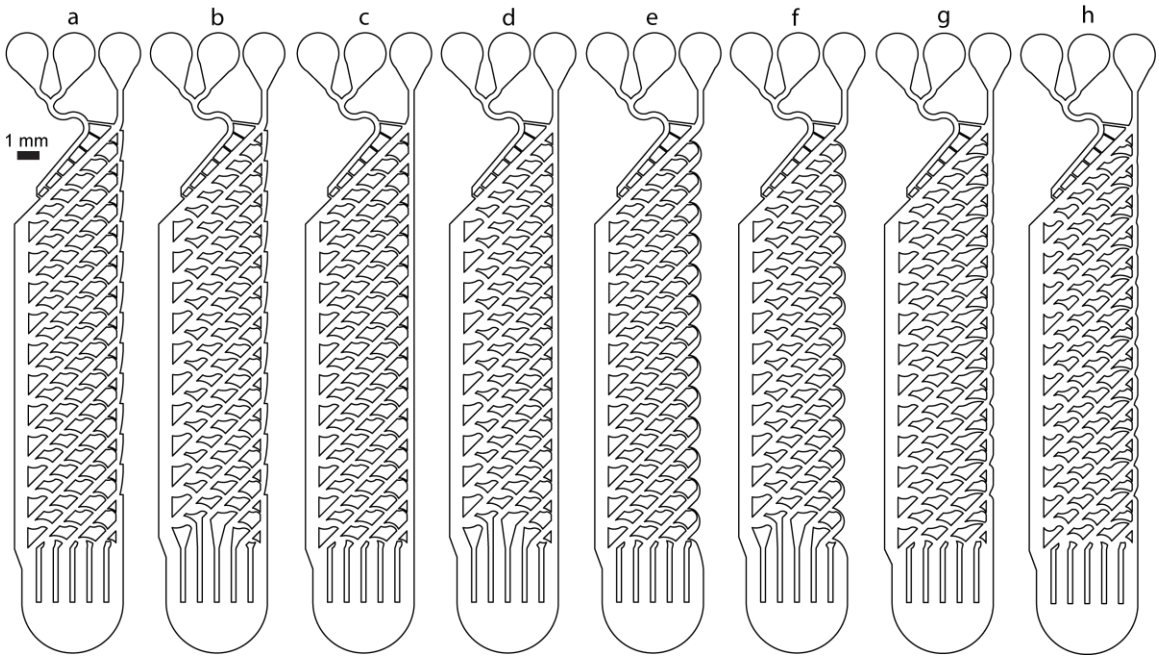


Figure J Microfluidic devices that incorporated the "smart" maze strategy. Device **a** is described in detail in Figure 2 of the main text. All devices were fabricated with channels 150 μm high, except **g** and **h** that contained channels 600 μm high.

Device efficiency was dependent on the relative flow rates (between inlet 1 and inlets 2 and 3) used in the experiments and, conveniently, independent of attractant gradients generated within the device. The separation mechanism was strictly based on the squeezing of the original flow from inlet 1, the orientation of the *main channels* (45° tilt downwards) and the size distribution of the interconnecting channels (as well as their curvature). Further devices that introduced variations in terms of *main channel* length and inclination (either fully horizontal or 45° tilt upwards) and on the number of *interconnecting channels* (from only two to four) were inefficient in separation and are not presented here. Analytical throughput was primarily controlled through variation of the concentration of the worm suspension and the flow rate through inlet 1. It was usually observed that at high concentrations worms plugged the device and a typical limit achieved with "thin" devices was 500 worms/min. Therefore devices **g** and **h** were fabricated with wider and deeper channels in an attempt to minimize plug formation at high concentrations. Figure K shows a representative ROC curve analysis of a separation using device **g** in Figure J. This experiment, which is partially reproduced in Movie 7, exhibited a throughput of 1200 worms/minute (72,000 worms/hour). At sorting channel 5, a true positive rate of 91% and false positive rate of 5% was achieved. At sorting channel 6 a true positive rate of 70% and a false positive rate of 2% was obtained.

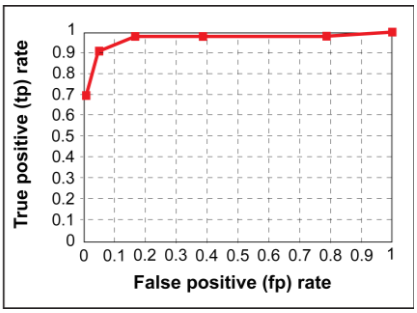


Figure K Representative ROC curve for the separation of adult worms and larvae using device **g** in Figure J. Sample was introduced through inlet 1 at a volumetric flow rate of 30 μL/min. Buffer solutions were introduced at a flow rate of 50 μL/min through inlets 2 and 3. Data describe a system with a true positive rate of 91% and false positive rate of 5% at sorting channel 5. A throughput of 72,000 worms/hour was achieved.