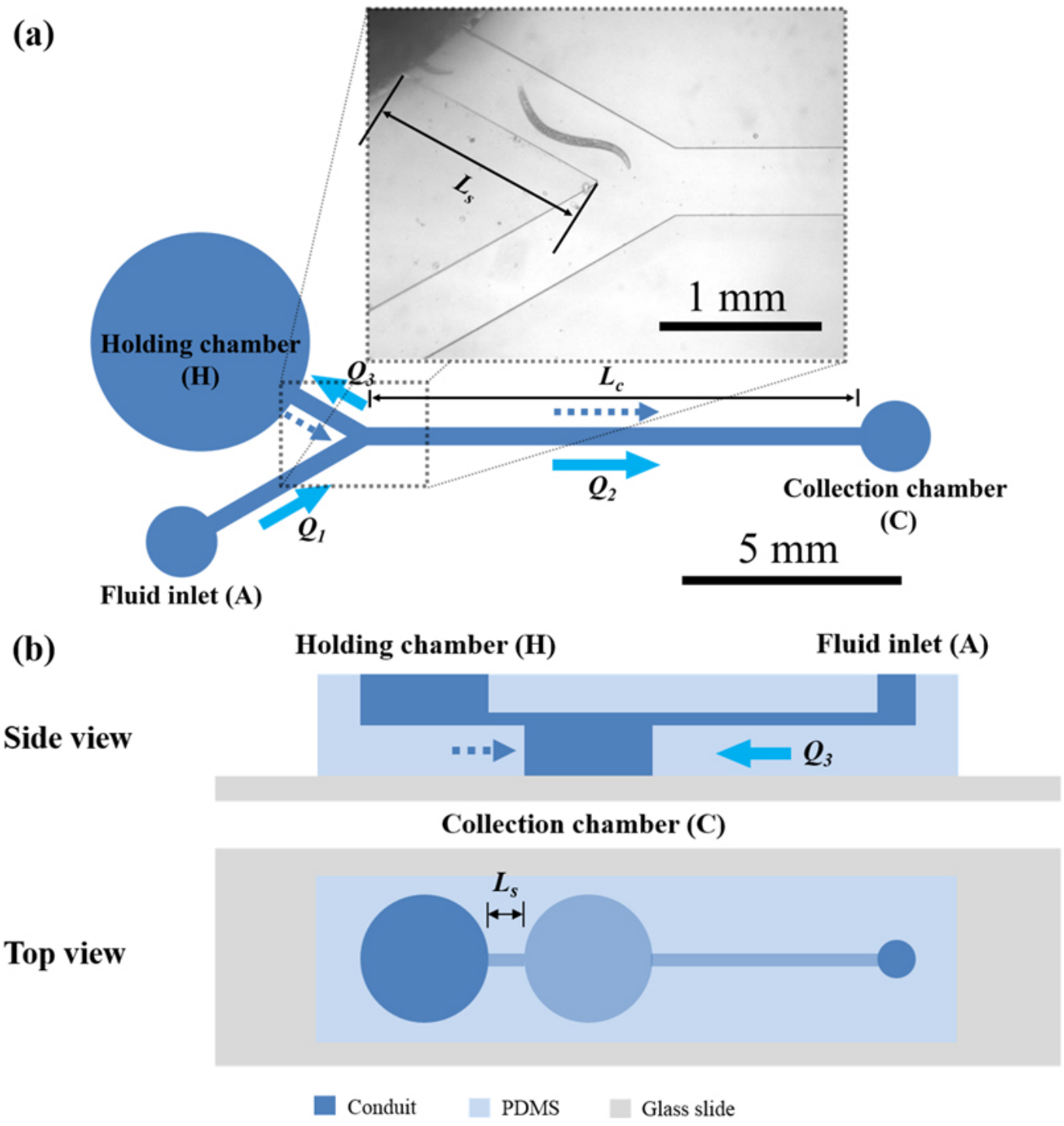
High-throughput, motility-based sorting of microswimmers for gene discovery

Organisms' motility varies with genotype, disease progression, drug treatment, aging, and environmental conditions and can serve as a useful metric to access drug effectuality, to identify drug resistant strains, and, more generally, various genotypes. In 1974, Sidney Brenner proposed using the 1 mm long, round nematode *Caenorhabditis elegans* (*C. elegans*) as a model organism to study nervous system function. *C. elegans* was selected because it is simple and easy to cultivate and mutate. In a typical genetic screen, animals are observed under the microscope and the investigator selects the ones that move qualitatively differently than the norm. Although this strategy has proven powerful in identifying over a hundred genes that impact animal locomotion, it is qualitative, lacks objectivity and sensitivity, is tedious, and is limited by the investigator's vigilance, competence, and time.

Ideally, one desires a quantitative, high throughput method capable of identifying even subtle, gene-induced variations in locomotion. In our paper, we describe a simple, microfluidic strategy that enables sorting many thousands of animals to isolate just a few whose propulsive thrust exceeds a preset, adjustable threshold. See the Figure. Our device consists of a holding chamber into which thousands of animals can be inserted. The holding chamber connects to an exit conduit that directs liquid flow into the holding chamber. Only animals with propulsive thrust that exceeds the drag induced by the flow in the conduit escape and are directed into a collection chamber. Animals with propulsive thrust below the threshold remain in the holding chamber. The desired threshold can be readily adjusted by controlling the flow rate in the inlet conduit. Many sorting modules can be accommodated on a single substrate to operate concurrently and a cascade of sorters can be used in series to either refine the sorting process or to isolate animals with various motilities. We qualified our device by successfully isolating mutants of *C. elegans,* known to have differing motilities, from an assorted population.



A schematic depiction of the sorting devices. (A) A Y sorter – a photograph and top view. The fluid inlet is connected to a syringe pump, which controls the flow rate Q1. The collection chamber is connected to a second syringe pump that operates in suction mode and controls the flow rate Q2. Q3 = Q1-Q2. Solid arrows and dashed arrows denote, respectively, flow direction and able animal direction of movement. (B) The L sorter: Side cross-section (top) and top view (bottom). The fluid

inlet was connected to a syringe pump, which controlled the flow rate Q3 in the separation conduit (Ls). Animals that moved with sufficient velocity to escape the length Ls of the sorting conduit sank to the bottom of the collection chamber and were thus isolated.

Once we have proved the utility of our device, we turned to examine an important biological question related to sleep. Sleep plays an important role in human health and productivity. Ongoing sleep deficiency is linked to serious diseases. Yet, in spite of its critical importance to human health, sleep remains poorly understood. It is recognized that sleep is genetically-regulated and researchers study the molecular mechanisms of sleep in model animals to identify the genetic underpinning of sleep disorders. *C. elegans* exhibits a quiescent behavior that shares many characteristics with sleep. Therefore, lessons learned about the genetic mechanisms that interfere with quiescence in *C. elegans* may be applicable to sleep.

The gene *flp-13*, which encodes neuropeptides and is expressed in the sleep-promoting ALA neuron, has been shown to regulate sleep-like, quiescent behavior in *C. elegans*. When *flp-13* is over-expressed in an artificial manner, by placing it under the control of an inducible promoter, the animals cease feeding and moving. To identify genes that interfere with the quiescence-inducing *flp-13*, we induced random mutations by soaking these animals in a chemical that modifies their DNA. Since the mutations are generated randomly in the DNA, only very rare mutations will disrupt the function of genes required for the quiescent behavior of interest. We thus made use of our sorting device to find these rare mutants. We placed a quarter million mutated animals over-expressing *flp-13* in our device and selected for animals able to swim out of the holding chamber. Four days later, we repeated the process with the progenies of animals isolated in the first round of sorting to finally isolate 13 mutants capable of suppressing the somnogenic effects of the *flp-13* gene. Subsequent genomic sequencing of seven of these mutants led to the identification of a *flp-13*-suppressor gene, previously unknown in *C. elegans*.

In conclusion, we have demonstrated a simple, high throughput sorter capable of efficiently separating microswimmers based on their propulsive thrust. Our sorter isolated rare mutants from a very large population and proved to be a useful tool for gene discovery and to investigate fundamental biological processes.

Publication

High-throughput, motility-based sorter for microswimmers such as C. elegans.
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