High-throughput, on-chip, whole-animal screening at subcellular resolution

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Innovations in microfluidic technologies enable rapid in vivo studies employing two-photon microscopy and femtosecond laser nanosurgery. Small animal models have become increasingly important for biological discovery. Notably, studies involving the nematode *Caenorhabditis elegans* have contributed significantly to three Nobel Prizes awarded in medicine or chemistry over the last six years. *C. elegans* has emerged as a powerful model organism due to the availability of a wide array of species-specific genetic techniques, along with its short development time and ability to grow in minute volumes. Additionally, *C. elegans* is transparent, which allows the use of powerful optical techniques that enable precise cellular and subcellular visualization within the living animal.

Conventionally, researchers manipulate *C. elegans* manually using small glass and metal picks and anesthetize the animals prior to high-resolution imaging. However, such manual manipulation is too slow and error-prone for high-throughput genetic and drug studies. Moreover, the anesthesia is slow to take effect and can have unexpected side effects. Anesthesia is also unsuitable for inquiries requiring physiologically active animals, such as studies examining neurophysiology, germ-line proliferation, or development. Thus, a method for precisely manipulating and immobilizing physiologically active animals, with high throughput and minimal physiological effects, is of great importance.

We recently developed the first high-throughput, on-chip, small-animal manipulation technologies for whole-animal studies at subcellular resolutions, as well as techniques that can immobilize physiologically active animals with the highest stability reported to date without using either chemical anesthetics or cooling. The devices that allowed these manipulations were fabricated using polydimethylsiloxane soft lithography and employed microfluidic valves to direct fluid flow.

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**Figure 1.** (a) Microfluidic worm-sorter layout and operation. (b) Illustration of the improved immobilization device using a flexible membrane to achieve three-dimensional imaging at subcellular resolutions (see Figure 2). (c, i–iv) Capture, isolation, and immobilization of an individual animal. (c, v) A close-up image of an immobilized animal, showing green fluorescent protein (GFP)-labeled posterior lateral mechanosensory neurons (scale bars i–iv: 250µm, v: 20µm).

**Figure 2.** Microfluidic immobilization enables key techniques, including three-dimensional two-photon imaging and femtosecond laser nanosurgery, to be applied to living and unanesthetized animals.
Figure 3. Microchamber chips for large-scale screening. (a) Chips consist of chambers connected to flow lines in which the flow path is controlled via multiplexed control lines and valves (scale bar: 500 µm). (b) Each chamber can be addressed independently, and the flow lines can be flushed with wash buffer through a dedicated line to prevent cross-contamination (scale bar: 500 µm). (c) The same flow lines can also be used to deliver worms. A special chamber geometry that consists of circularly arranged microposts is used to immobilize the animals quickly in a well-defined geometry by applying a flow pressure without using anesthetic (scale bar: 100 µm). (d) High-resolution images can be taken through the glass substrate of the chip. The GFP-labeled neuron was imaged using a white-light background to show a micropost (scale bar: 25 µm).

The microfluidic small-animal sorters we developed can rapidly isolate and immobilize individual animals (see Figure 1). The sorters consist of control channels and valves (gray) that direct the flow of worms in the flow channels. A worm is captured in the chamber by aspiration via the top channel, while the lower aspiration channels are inactive. The chamber is then washed to flush any other worms in the chamber (blue line) toward the waste or back to the circulating input. The chamber is isolated from all of the channels, and the worm is released from the top aspiration channel to be restrained by the lower aspiration channels (red line). This aspiration partially immobilizes animals to a degree where wide-field images can be taken. In order to further immobilize the animals for high-resolution imaging, we create a seal around them to restrict their motion completely. This is done using a flexible membrane (15–25 µm thick) that separates a top channel from the worm channel below: see Figure 1(b). The top channel can be rapidly pressurized to expand the membrane downwards, wrapping around the animal and forming a tight seal that completely constrains its motion in a linear orientation. Image acquisition and processing are then performed, and the worm is either collected or directed to the waste, depending on its phenotype (orange line). Quantitative analysis of the immobilization showed that its stability is comparable to chemical anesthetics, and there is no change in the lifespan or brood size of the immobilized animals. Additionally, visual observation of the animals and their neurons showed no signs of hypoxia or other distress. The high stability of this approach enables the use of a variety of powerful optical techniques, including femtosecond laser nanosurgery and three-dimensional multiphoton microscopy of whole-animals at subcellular resolutions (see Figure 2).

To perform precisely timed chemical and time-lapse screens, we have developed an array of multiplexed chambers (see Figure 3) for simultaneous incubation, immobilization, subcellular imaging, and independent screening of many animals on a single chip. Each individually addressable incubation chamber contains posts arranged in an arc. To image animals, fluid flow is used to push the animals toward the posts. This flow restrains the animals against the posts for immobilization and subsequent imaging and also allows the medium in the chambers to be exchanged through the microfluidic channels without losing the animals. To simplify the delivery of existing large-scale RNA interference and drug libraries, we have also developed a microfluidic interface device (see Figure 4) that connects these microfluidic chambers to large-scale multiwell-format libraries. Minute amounts of individual compounds from standard multwell plates can thus be routed to the incubation chambers, and the connection lines can be automatically washed between samplings. This device also functions as a multiplexed animal dispenser.

In various configurations, these technologies have the potential to significantly accelerate current genetic and drug screens and also enable completely new types of whole-animal assays. For example, we have employed femtosecond laser micro/nanosurgery to perform the first axonal regeneration study in C. elegans. These investigations have opened up new possibilities for genetic/drug discoveries in neural regeneration. To further explore this phenomenon, we are currently employing these microfluidic technologies to rapidly uncover the genetic and chemical factors involved in neural regeneration in living animals.

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Mehmet Fatih Yanik is an assistant professor at MIT. His on-chip photon storage invention was selected among the top 10 research advances of the year by Technology Research News magazine and awarded first place in the Innovator’s Challenge of Silicon Valley. He was selected as Outstanding Young Person by the Junior Chamber and was named one of the world’s top 35 innovators under age 35 by Technology Review magazine. He is a recipient of the NIH Director’s Innovator Award, the Alfred Sloan Award in Neuroscience, the Packard Award in Engineering, and the National Science Foundation Career Award.

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References