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On-chip whole-animal manipulation for high-throughput subcellular-resolution in-vivo drug/genetic screening

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Abstract—Techniques for rapid and automated small-animal manipulation and immobilization are necessary for high-throughput in vivo genetic/drug screens using cellular and subcellular features in multicellular organisms. We present a suite of technologies for complex high-throughput whole-animal genetic and drug screens. We demonstrate a high-speed microfluidic sorter that can isolate and immobilize Caenorhabditis elegans in a well-defined geometry, an integrated chip containing individually addressable screening chambers for incubation and exposure of individual animals to biochemical compounds, and a device for delivery of compound libraries in standard multiwell plates to microfluidic devices. The immobilization stability obtained by these devices is comparable to that of chemical anesthesia and the immobilization process does not affect lifespan, progeny production, or other aspects of animal health. The high-stability enables the use of a variety of key optical techniques. We use this to demonstrate femtosecond-laser nanosurgery and three-dimensional multiphoton microscopy to study neural regeneration at sub-cellular resolution in vivo.

I. INTRODUCTION

Small animal models have become increasingly important for biological discovery. Notably, studies involving the nematode Caenorhabditis elegans (C. 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 optically transparent, which allows the use of powerful optical techniques that enable precise cellular and subcellular visualization within the living animal.

Conventional research 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, and 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 previously demonstrated the first high-throughput, on-chip small-animal screening technologies at cellular resolution [1]. We have since developed techniques that can immobilize physiologically active animals with the highest stability reported to date without using either chemical anesthetics or cooling [2].

Our devices consist of multiple thin layers of poly(dimethyl siloxane) (PDMS) fabricated by soft lithography [3]. Two or three different layers are used to construct these devices: flow, control, and, when applicable, immobilization. The flow layers contain microchannels for manipulating C. elegans, for immobilizing them for imaging, and for delivering media and reagents. The flow layers also contain microchambers for incubating the animals. The control layers typically lie below the flow channels and consist of microchannels that when pressurized, flex a membrane into the flow channels, blocking or redirecting the flow [4]. Animals in the flow lines can be imaged through a transparent glass substrate using high-resolution microscopy. The immobilization layer is above the flow layer, and contains channels that are pressurized in the same manner as the control layer.

II. HIGH-THROUGHPUT SCREENING DEVICES

A. On-Chip High-Throughput Sorting

The microfluidic small-animal sorters we developed can rapidly isolate and immobilize individual animals (see Fig. 1). The sorters consist of control channels and valves (gray) that direct worms in the flow channels. Animals enter through inlet valve A. Single suction port C isolates an individual animal from a group (red line), and then any animals remaining in the chamber are washed away by closing valve A and opening valves B and E (blue line). Once the animal is isolated, all valves are closed and the animal is transferred to the multiple aspiration ports on the lower side of the chamber by opening valve D. Here the animal is immobilized in a linear configuration, and optical methods for imaging and manipulation can be used. Following this, the animal can be

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sent to another device/off chip by opening valve F, or to waste by opening valve E (orange line). The immobilization can be improved using the method illustrated in Fig. 1b. When the animal is held against the multiple aspiration ports, a channel above the chamber is pressurized, and the membrane separating the two channels expands downwards. The membrane wraps around the animal, completely constraining it against the multiple aspiration ports.

We have quantified the stability of this immobilization through automated tracking of fluorescently-labeled cell bodies. Movies were captured at 50x magnification of pmec4::gfp animals immobilized either by the anesthetic NaN3 at concentrations 10 μM (the highest concentration for which we could recover animals) or by our microfluidic device. Frames from the movies were first thresholded and the large connected regions were identified to determine the cell bodies. We recorded the centroids of these regions and calculated the instantaneous velocities. Analysis of these data showed that the movement of the immobilized animals was comparable to their motion when deeply anesthetized. We also quantified the effect of our immobilization technique on animal health. A population of animals immobilized using our microfluidic technique was compared to a population that was not run through the device. There was no statistically significant difference between the lifespans of the two populations, and both populations produced normal brood sizes and were free of axonal blebbing.

B. Large-Scale Time-Lapse Assays with Subcellular Resolution

To perform high-throughput time-lapse studies on small animals we have designed the microfluidic-chamber device shown in Fig. 2 for worm incubation and for continuous imaging at subcellular resolution. Individual chambers can be addressed using microfluidic valves. The medium in the chambers can be exchanged through the microfluidic channels for complex screening strategies, and precisely timed exposures to biochemicals (e.g., drugs/RNAi) can be performed. The use of microfluidic technology also reduces the cost of whole-animal assays by reducing the required volumes of compounds. An array of posts arranged in a semi-circular configuration is used to permit exchange of media keeping animals in the chamber. To image animals, a flow is used to push the animals toward the posts arranged in

Fig. 1. Microfluidic device for small animal sorting, immobilization and imaging. (a) Principle of operation of small animal sorter (see text). (b) Illustration of improved immobilization process. (c) Isolation, immobilization and imaging of an individual animal. (c.v) shows a close-up combination bright-field and fluorescent image of an immobilized animal illustrating gfp-labeled mechanosensory axons (scale bar i-iv: 250 μm, v: 20 μm).

Fig. 2. Multiplexed microfluidic incubation. (a) Device layout illustrating flow and control lines. (b) Illustration of individual chamber addressing. (c) Semi-circular post arrangement allows animal immobilization and imaging. (d) High-resolution image taken through glass substrate (scale bars a-b: 500 μm, c: 100 μm, d: 25 μm).
an arc inside the chambers (Fig. 2(b) and 2(c)). This flow restrains animals for subcellular imaging and manipulation.

Microchamber chips based on this design can be readily scaled for large-scale screening applications because the number of control lines required to independently address n incubation chambers scales only with log(n) [5]. The millimeter scale of the microchambers can allow hundreds of microchambers to be integrated on a single chip.

C. Microfluidic Multiwell-Plate Interface Chip for Compound-Library Delivery and Multiplexed Animal Dispensing

To simplify the delivery of existing large-scale RNAi and drug libraries, we have also developed a microfluidic interface device (see Figure 3) that connects these microfluidic chambers to large-scale multi-well-format libraries. Minute amounts of individual compounds from standard multi-well plates can thus be routed to the incubation chambers, and the connection lines can be automatically washed between samplings. The device consists of an array of aspiration tips that can be lowered into the wells of microwell plates. The chip is designed to allow minute amounts of library compounds to be collected from the wells by suction, routed through multiplexed flow lines one at a time, and delivered to the single output of the device. The output of the interface chip can then be connected to our microfluidic chamber device for sequential delivery of compounds to each microchamber. This device, when run in reverse, also functions as a multiplexed animal dispenser.

III. APPLICATIONS

To demonstrate the utility of the microfluidic immobilization method we developed, we have chosen to demonstrate two techniques that take advantage of the high-stability achieved: multi-photon microscopy and femtosecond-laser microsurgery (Fig. 4). Multiphoton microscopy enables optical sectioning with little out-of-plane absorption [6]. This allows high-precision three-dimensional subcellular imaging within minimal photobleaching and phototoxicity [7]. Another nonlinear optical technique, femtosecond-laser micro/nanosurgery enables precision ablation of sub-cellular processes with minimal collateral damage [8] and we have previously employed this technique to perform the first axonal regeneration study in C. elegans [9][10]. Our immobilization technique enables rapid and repeatable immobilization which permits femtosecond-laser microsurgery with sub-cellular precision. This provides a powerful tool for the discovery of potential drugs and genetic factors affecting neural degeneration and regeneration.

IV. CONCLUSIONS

We have reported on key integrated high-throughput technologies that enable high-throughput sub-cellular small animal studies. 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. The microfluidic immobilization method present in our small animal sorter is a rapid and highly repeatable technique for immobilizing small animals for imaging and manipulation of sub-cellular processes without anesthesia. We have used this to demonstrate multiphoton microscopy and femtosecond-laser microsurgery on physiologically active animals. We are currently using these devices to investigate the genetic and chemical mechanisms involved in neural regeneration.
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