An automated microfluidic system for screening *Caenorhabditis elegans* behaviors using electrotaxis

Dingsheng Liu,^{1,a)} Bhagwati Gupta,^{2,b)} and
Ponnambalam Ravi Selvaganapathy^{1,c)}

¹Department of Mechanical Engineering, McMaster University, Hamilton, Ontario L8S 4K1, Canada

²Department of Biology, McMaster University, Hamilton, Ontario L8S 4K1, Canada

(Received 11 December 2015; accepted 28 January 2016; published online 11 February 2016)

Caenorhabditis elegans (*C. elegans*) is a widely used animal model to study mechanisms of biological processes and human diseases. To facilitate manipulations of *C. elegans* in the laboratory, researchers have developed various tools that permit careful monitoring of behavior and changes in cellular processes. Earlier, we had reported a novel microfluidic assay device to study the neuronal basis of movement and to investigate the effects of cellular and environmental factors that can induce degeneration in certain neurons leading to movement disorder. The system involved the use of an electric field to perform electrotaxis assays, which allows detailed examination of movement responses of animals. One of the potential uses of this system is to perform genetic and chemical screenings for neuroprotective factors; however, it could not be done due to manual operations and low throughput. In this paper, we present an integrated microfluidic system that automates screening of *C. elegans* behavioral response using electrotaxis. The core component of system is a multilayer poly dimethyl siloxane (PDMS) device, which enables *C. elegans* loading, capture, flush, release, electrotaxis, and clean sequentially with the help of other components. The system is capable of screening *C. elegans*, at a throughput of more than 20 worms per hour, automatically and continually without human intervention. To demonstrate the effectiveness of the system, *C. elegans* neuronal mutants were screened, and the phenotype data were extracted and analyzed. We envision that the automatic screening potential of the system will accelerate the study of neuroscience, drug discovery, and genetic screens in *C. elegans*. © 2016 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4941709>]

INTRODUCTION

Caenorhabditis elegans (*C. elegans*) is a widely used multicellular model animal, mainly because of its short life cycle, semi-transparent body, easy maintenance, and complete cell line map. The studies based on *C. elegans* have led to insights in neurobiology, ageing, development, genomics, and drug discovery.^{1–3} Due to its simpler nervous system consisting of only 302 neurons, *C. elegans* is an ideal model system to understand neuronal signaling and function. One way to do this is through the analysis of movement based behavioral response to an external stimulus.⁴ Various neurodegenerative diseases such as Parkinson's disease⁵ and Alzheimer's disease⁶ can also be modelled in *C. elegans*, and the disease characteristics can be identified by distinctively different movement behavior from wild type animals. By studying the locomotion of *C. elegans* mutants, and co-relating it with the transformation of the neuronal system in these mutants, the mechanisms of these diseases could be understood. These studies have implications in humans and for developing suitable drugs for these diseases.^{3,7,8}

^{a)}dsliu401@yahoo.com

^{b)}guptab@mcmaster.ca

^{c)}Author to whom correspondence should be addressed. Electronic mail: selvaga@mcmaster.ca

The locomotion behavior of *C. elegans* has been elicited by a variety of external stimuli such as chemical agents,⁸ light,⁹ heat,¹⁰ and electric field.¹¹ In the recent past, microfluidic techniques have been used to provide precise spatiotemporal environments to deliver various stimuli to *C. elegans*, which make the behavioral studies of *C. elegans* more reproducible and adaptable.^{8,9,12–17} Moreover, microfluidic devices can be automated to handle single or a population of *C. elegans* in a high-throughput fashion, which enables large-scale assays such as drug and genetic screens.^{15–22} In previous work from our group, we designed a simple microfluidic device to examine the swimming behaviors of *C. elegans* under electric fields, termed electro-taxis, and demonstrated that changes in movement are reliable indicators of neurodegeneration and harmful effects of toxic chemicals on *C. elegans* health.^{23–25} However, the inherent manual operation of the devices makes the screening process labor intensive and time consuming, which limits largely the practical application of the method.^{23–25} In order to enable widespread use of the electrotactic assay, we have addressed the current limitations and developed an entirely new automated microfluidic system, which can perform electrotaxis assay for a batch of worms, without human intervention, one worm at a time while substantially increasing the throughput of analysis. This system can load, isolate, perform electrotaxis assay, and flush *C. elegans* in a computer-controlled sequence. The system consists of integrated microvalves to perform the fluid operation and electrodes to apply electric field in a coordinated sequence that is controlled by software that also captures the movement behavior. The results show the potential of our new microfluidic platform in rapid screening of *C. elegans* movement behavior under electric fields as well as future screenings of mutants and chemicals that affect neuronal health.

METHODS

Device design and experimental setup

The microfluidic system, as shown in Fig. 1(a), is mainly made up of a multilayer PDMS device, a set of pressure control and solenoid valves unit, a custom-built LabVIEW controller, a microscopy-camera-computer vision setup, and some accessories. Fig. 1(b) shows the detail of the multilayer PDMS device, which is a core component of the microfluidic system and is composed of three individual PDMS layers: a top layer for control channels, a middle layer for deformable thin membranes, and a bottom layer for flow channels. The worm solution flows into the device from the inlet channel (Fig. 1(b)) and flows out through the outlet channel. The electrotaxis channel at the center is used to isolate a single worm and perform electrotaxis experiments. The flushing channel is used to inject buffer that can clean the electrotaxis channel

FIG. 1. (a) Automated microfluidic system for screening *C. elegans*. (b) Configuration of a multilayer PDMS device in the system. (c) Operational procedure of the microfluidic system. (d) Schematic of *C. elegans* electrotaxis in a microchannel.

after an experiment and flush any worms that are in it so that it is ready for the next assay. A suction channel, which joins the middle of the electrotaxis channel and connects to a tiny vacuum pump, is used to capture a single worm flowing through the electrotaxis channel and isolate it for further experiments. Based on the configuration of the three PDMS layers, five microvalves²⁶ (valves A to E in Fig. 1(b)) are built into the device to direct liquid flow in flow channels and are controlled by five external solenoid valves. When a solenoid valve is on, a high-pressure air from a gas tank will be introduced to a relevant control channel prefilled with water. This high pressure exerted on the water deforms a thin PDMS membrane against the walls of a flow channel to close it; when the solenoid valve is off, the external high-pressure air will be disconnected from the control channel, and so the thin PDMS membrane will retract to open the flow channel. A pair of platinum wire electrodes is assembled on the opposite ends of the electrotaxis channel to apply electric field and induce *C. elegans* locomotion in the electrotactic channel. Using this system, behavioral assays can be conducted on a number of worms in a batch, continually, automatically, and under the control of a LabVIEW program which can be used to set the parameters of the measurement. Fig. 1(c) shows the operational procedure that is programmed into and controlled by the LabVIEW program. The letters A–E represent the relevant valves, as shown in Fig. 1(b). As noted in Fig. 1(c), “1” represents closing PDMS valves, and “0” represents opening PDMS valves. Fig. 1(d) shows sequence of fluidic operations in a graphical form, as indicated in Fig. 1(c) (operations 1–4). At the beginning (operation 1 in Fig. 1(c)), valve B is closed, and all the other valves are opened. *C. elegans* worms in M9 solution are driven by a high inlet pressure through the electrotaxis channel, see Figs. 1(b) and 1(d-1). The suction channel is held at a negative pressure produced by the vacuum pump. The size of the suction channel is optimized to ensure that one and only one *C. elegans* will be captured by the suction channel. Then (operation 2 in Fig. 1(c)), valve A is closed and valves C–E are kept open. Valve B is then opened to flush the electrotaxis channel of the chip with the M9 solution in order to remove any uncaptured worms, while still retaining the single worm captured by the suction channel (Fig. 1(d-2)). After flushing, all the valves are closed, and valve C is cycled through open and closed states a number of times to generate pressure pulses that ensures reliable release of the captured worm (operation 3 in Figs. 1(c) and 1(d-3)). Once released, the electric field is applied in the electrotaxis channel through the platinum wire electrodes that are embedded at the opposite ends of the channel (operation 4 in Figs. 1(c) and 1(d-3)). The motion of the worm in response to the electric field is captured by recording a video of its movement in the channel within the LabVIEW program (Figs. 1(d-3)–1(d-6)). Once the worm arrives at the spatial position predefined in the LabVIEW program, the polarity of the DC electric field is automatically switched (Fig. 1(d-4)). The *wild type* worm senses the change of the polarity, makes a turn, and swims back toward the opposite direction, i.e., the direction to the new cathode, which is also recorded (Fig. 1(d-5)). The LabVIEW program has control pre-set positions for switching the electric field as well as the number of times that the field should be switched. These controls can be set by the user at the beginning of the assay for the program to automatically control. After, the pre-set number of cycles is complete, the valves B and E are opened, and the rest of the valves are closed. The M9 solution is flushed through valve B to flow through the electrotaxis channel and clean it for the next run where a single worm is again captured for electrotaxis (operation 5 in Fig. 1(c-5)). Then, operations 1 to 5 in Fig. 1(c) are repeated to screen the next worm. The screening runs automatically without any intervention of a human as long as the feed solution containing worms are present. An inverted microscope (Leica) with 10× objective lens was used to observe worms within an 11 mm long region in the electrotaxis channel. A digital camera mounted on the microscope recorded in real time the electrotaxis processes and transferred the videos to a computer.

Device fabrication

The PDMS microfluidic device here was fabricated using standard soft lithography method.²⁷ The device was made up of three layers, a control layer (top), a thin PDMS membrane (middle), and a flow layer (bottom), which were fabricated individually, and then aligned

and bonded together after oxygen plasma treatment. A negative photoresist SU8 2025 (MicroChem, USA) was used to make a $100\text{ }\mu\text{m}$ high mold on a silicon wafer for transferring channel patterns to PDMS elastomer as a control layer. A PDMS pre-polymer (base to curing agent of 10:1) was poured on a silicon wafer and was spun and crosslinked to make a PDMS membrane with $100\text{ }\mu\text{m}$ thickness which was used as a middle layer to close and redirect liquid flow in flow channels. A positive photoresist AZ 40XT (MicroChemicals, Germany) was used to make a mold on a silicon wafer for a flow layer. The positive photoresist patterns developed were $60\text{ }\mu\text{m}$ in height, and then were reflowed by heating at 150°C form semi-circular cross sections in the flow layers. The device design is shown in Fig. 1(b). The electrotaxis channel is 4 cm in length and $300\text{ }\mu\text{m}$ in width, while the suction channel is designed very narrow, $30\text{ }\mu\text{m}$ in width, to capture the worms. The channels in the regions where the valves are integrated are very wide, $900\text{ }\mu\text{m}$ in width, to ensure that the valves can be closed easily and used robustly. The heights of the rounded channels vary throughout the device and depended on the channel width at that location. They were measured to be $80\text{ }\mu\text{m}$, $85\text{ }\mu\text{m}$, and $70\text{ }\mu\text{m}$ at the electrotaxis channel, suction channel, and valves regions, respectively. The round shapes ensure that the valves are completely closed when actuated. Two accessory channels with $300\text{ }\mu\text{m}$ in width were fabricated at two ends of the main flow channel. Two platinum wires with $100\text{ }\mu\text{m}$ in diameter were inserted through the accessory channels to the ends of electrotaxis channel, and used as electrodes. The accessory channels were sealed by placing a droplet of uncured PDMS at its end, allowing it to wick in slowly while quickly heating it to form a leak proof seal. While performing the assay, 16 V DC potential (4 V/cm electric field) was applied along the electrotaxis channel to direct worms swimming.

Device operation and characterization

The application of electric potential higher than 1.2 V to a channel filled with aqueous fluid is bound to initiate electrochemical reactions that evolve gases, which could nucleate into a bubble. Bubble nucleation can be prevented if the current is low such that the generated gases do not reach their solubility limit to form a bubble. Alternatively, flow of the liquid could also maintain the concentration of dissolved gases at a level lower than the critical level for nucleation. Since bubbles can affect the electric fields applied in the channels and hence the outcomes of the assays, we characterized the conditions for bubble generation on our devices. The regions at the ends of the electrotaxis channel where the electrodes are inserted are shown in Fig. 2. Our previous results²³ showed that the electric fields necessary for initiating electrotaxis of *C. elegans* in a microchannel are between 2 and 12 V/cm . Lower electric fields are generally preferred as higher one lead to reduction in viability of the exposed worms. Typical electrotactic experiments are $\sim 2\text{ min}$ in duration. Therefore, we applied potentials between 8 and 60 V , which corresponded electric field $2\text{--}15\text{ V/cm}$ in order to identify whether these conditions led to bubble generation. Figs. 2(a) and 2(b) show an example of this experiment where the generated bubble (shown in the dotted box) grew in size from 0 to 7 nl in volume within $t=90\text{ s}$ at 40 V potential. Fig. 2(c) shows the growth of bubbles with respect to time at various applied potential. We found that application of potentials 30 V and lower does not lead to bubble generation within 2 min . Higher potentials will lead to generation of bubbles, which will change the electric field distribution in the channel and hence affect the quality of the assay. Therefore, in further experiments, we chose 16 V (4 V/cm), which is high enough to consistently initiate electrotactic response in worms but low enough to avoid generating bubbles in the channel.

Worm capture is a key step in the assay, and determination of the suction pressure is an important criterion in determining the operating conditions of the assay. The capture of a worm at the suction capillary is shown in Fig. 3. Fig. 3(a) shows two worms being transported in the channel due to flow. Figs. 3(b)–3(e) show the capture of the first worm which blocks the suction channel and allows the remaining worms in the channel to be pushed to the outlet clearing the channel for electrotaxis. Fig. 3(f) shows the release of the worm by the suction channel. There are two ways that the worms are captured at the suction capillary, as shown in Figs. 4(a) and 4(b). More than 90% of the worms were held at their middle sections, while a minority of

FIG. 2. Bubble generation on the positive electrode. (a) Junction where the channel interfaces with the electrode indicating it is clear of any bubbles before application of a voltage. Scale bar: 1 mm. (b) Same junction area after application of 40 V over 90 s indicating formation and growth of a bubble (within the dashed-lined box). (c) Characterization of the bubble formation and growth over time at various voltages.

the worms ($<10\%$) were captured at their ends (head or tail). The capture of the worms at their ends at high suction pressures leads to insertion of a significant section of their bodies into the suction capillary that it could not be released when the suction pressure was removed. This led to clogging of the suction capillary and eventual failure of the device. In order to avoid it, a new mechanism of two series valves (shown in Fig. 1(b)) was designed. In order to release the

FIG. 3. Series of images showing the flushing and releasing operation. One worm was captured and remained at the end of the suction channel. Clear M9 solution flushed away the rest worms inside the channel from the left to the right. Yellow arrows indicate worms that are freely flowing and not captured. In panel (a), the freely flowing worm is moving very fast and hardly visible, but they are much clearer in panels (b)–(d). (a) and (b) One worm captured within 0.025 s. (c) and (d) A second free worm passes by the fixed worm within 0.025 s. (e) and (f) The captured worm was released to the electrotaxis channel, and swam to the new position within 0.05 s. Scale bar: 1 mm.

FIG. 4. Capture of *C. elegans* by the suction channel under vacuum. (a) Middle capture, the suction channel captured a worm at its body. Scale bar: 1 mm. (b) End capture, the tail or head of one worm was captured by the suction channel. (c) Capture efficiency of worms by the suction channel over vacuum at different flow rates. The flow rates indicated are set by the positive displacement pump at the inlet of the device.

worms, valve D was closed to shut off the suction pressure, and valve C was cycled between open and closed states three times. This created a cyclical pressure in the enclosed chamber between the tip of the suction capillary and the valves and led to release of 100% of the worms. We also characterized the effectiveness of capture of the worms at different suction pressures and inlet flow rates that are shown in Fig. 4(c). Capture efficiency was defined as $1/N$, where N represents the average number of worms passing by the suction channel before the channel captured one worm. It can be seen from Fig. 4(c) that the capture efficiency increases with decreasing the pressure (increasing the vacuum) and decreasing the flow rates, as expected. Higher vacuum produces stronger pull on the worms and ensures their capture quickly. Similarly, lower flow rate allows more residence time of the worm near the suction capillary, which also leads to higher probability of its capture. The capture efficiency was found to be over 95% at or below 650 mbar at 2000 $\mu\text{L/h}$.

RESULTS AND DISCUSSION

In order to validate the microfluidic system, we performed assays on wild-type N2 *C. elegans* and one dopamine (DA) neurotransmitter pathway mutant *dat-1*. DA is an important neuromodulator that regulates movements and psychological functions in both vertebrate and invertebrates. Various neurological disorders and behaviors diseases result from changes in DA levels. *dat-1* mutant worms lack the dopamine reuptake transporter, swim slowly because their internal dopamine levels increase without reuptake during moving.^{25,28}

FIG. 5. Series of images showing the electotaxis of *C. elegans* in a microfluidic channel. Electric field applied: 4 V/cm. Scale bar: 1 mm. Red + and – represent anode and cathode direction, respectively. (a) and (b) Worm moving to the right towards the cathode. (c) Worm stopped and started to turn when the electric field changed the polarity. (d)–(f) Worm moving to the left towards the new cathode. (g) Worm stopped and started to turn when the electric field changed again the polarity. (h) Worm moving again toward the new cathode direction.

All the animals were 68 h old (young adults), and assays were conducted on 20 worms of each type. Fig. 5 shows the operation of the microfluidic system to measure speed, turning time, and amplitude of motion of the worms for a single worm. The worms after capture are released into the electotaxis channel. Upon application of an electric field (Fig. 5(a)), the worm moves towards the cathode and continues moving in that direction as long as the electric field is applied (Fig. 5(b)). The speed of the worm is measured by recording the video of its motion and analyzing the video frames using image analysis software to assess its location over time. Similarly, the body bending frequency of the worm was calculated as the ratio of the number of the body bending to the time spent. Then, the electric field's direction is reversed (Fig. 5(c)) which causes the worm to recognize the switch in direction of the field and to turn. By correlating the switch in the electrical signal and the time it takes for the worm to recognize it and turn around, the turning response was characterized. The worm then continues to move towards the new cathode location (Figs. 5(d)–5(f)), where its speed can be measured again. Once it reaches the end of the viewing window, the electric field's direction is switched again (Fig. 5(g)) which forces the worm to turn and move in the opposite direction (Fig. 5(h)) where its speed is measured again. Next, channel was flushed to remove the worm, and the entire process was repeated for the next worm. The videos captured from the two types of worms were analyzed, and three phenotypic parameters involving swimming speed, body bending frequency, and turning time were extracted from every video for characterizing the locomotion behaviors of *C. elegans*. The swimming speed and body bending frequency were all calculated from sections of the video where the worms kept forward moving at steady state. The turning time is calculated as the time it takes for the worm to re-orient its body direction after the change in polarity of electric fields.

FIG. 6. Electrotaxis phenotypes of Wild-type N2 and mutant *dat-1*. Electric field applied: 4 V/cm. $**p < 0.01$. (a) Speed, (b) bending frequency, and (c) turn time responses.

Figs. 6(a), 6(b), and 6(c) show the worm swimming speed, body bending frequency, and turning time, respectively. From Fig. 6(a), *dat-1* swam more slowly than wild type as expected.^{25,28} Similarly, in Fig. 6(b), the bending frequency of *dat-1* is lower than wild type. The results show a strong and positive correlation between the swimming speed and the bending frequency for the two types of worms screened. In Fig. 6(c), *dat-1* has a longer turning time than wild type which has been reported earlier.²⁵ From Figs. 6(a)–6(c), it can be seen that *dat-1* is more muted in response to electric fields than wild type.

Throughout the experiment, the system operated smoothly and was able to perform all the steps automatically. User intervention was only required to change the batches of worms corresponding to the different mutant types that were tested. The screening time was 2 min per worm which is long enough for worms to finish four complete electrotactic runs along with associated turning in the 11 mm long observing region in the electrotaxis channel. Including the time spent in the other operation steps, the total screening time one worm took is less than 3 min producing a throughput >20 worms per hour. The electrotaxis time can also be flexibly adjusted for different mutants and experimental purposes. Further increase in throughput is possible by adding more flow channels in parallel in the PDMS device that will allow screening of a larger number of worms simultaneously.

CONCLUSIONS

In this work, we have presented an integrated microfluidic system for screening electrotactic behaviors of *C. elegans*. The system can enable *C. elegans* loading, capture, flush, release, electrotaxis, and clean sequentially. The performance of the system was verified on screening two types of *C. elegans*. The system runs smoothly, robustly, and continually without an intervention of a human, which enables electrotactic screening easier, quicker, and wider. The screening time of one worm can be adjusted flexibly based on the specific objectives of the assay. The device is also scalable to add more electrotaxis channels in parallel to get higher throughput. Thus, movement behaviour initiated on demand through electrotaxis in both wild type as well as genetically modified disease models can be measured in a large population of worms in an automated way and at high throughput. These studies are crucial in understanding the mechanisms of these diseases, effect of drug candidates on these mechanisms, as well as toxicity of the drug candidates or other chemicals on the organisms. Therefore, this system has potential to accelerate the study of neuroscience, drug discovery, and genetic screen carried on *C. elegans*.

ACKNOWLEDGMENTS

The authors acknowledge financial support for this project from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Institutes of Health Research (CIHR) through their joint Collaborative Health Research Program. P.R.S. also wishes to acknowledge the Ontario Ministry of Research and Innovation for the Early Research Award and the Canada Research Chair Award for support. The authors declare no competing financial interest.

¹S. Brenner, *Genetics* **77**, 71 (1974), available at PubMed 4366476.

²A. K. Corsi, *Anal. Biochem.* **359**, 1 (2006).

³T. Kaletta and M. O. Hengartner, *Nat. Rev. Drug Discovery* **5**, 387 (2006).

⁴“Behavior,” in *WormBook*, edited by A. C. Hart (The *C. elegans* Research Community, 2006), see <http://www.wormbook.org>.

⁵T. M. Dawson, H. S. Ko, and V. L. Dawson, *Neuron* **66**, 646 (2010).

⁶D. L. Price, R. E. Tanzi, D. R. Borchelt, and S. S. Sisodia, *Annu. Rev. Genet.* **32**, 461 (1998).

⁷C. I. Bargmann, *Science* **282**, 2028 (1998).

⁸D. R. Albrecht and C. I. Bargmann, *Nat. Methods* **8**, 599 (2011).

⁹J. N. Stirman, M. M. Crane, S. J. Husson, S. Wabnig, C. Schultheis, A. Gottschalk, and H. Lu, *Nat. Methods* **8**, 153 (2011).

¹⁰A. Mohammadi, J. B. Rodgers, I. Kotera, and W. S. Ryu, *BMC Neurosci.* **14**, 66 (2013).

¹¹C. V. Gabel, H. Gabel, D. Pavlichin, A. Kao, D. A. Clark, and A. D. T. Samuel, *J. Neurosci.* **27**, 7586 (2007).

¹²J. Qin and A. R. Wheeler, *Lab Chip* **7**, 186 (2007).

¹³W. Shi, J. Qin, N. Ye, and B. Lin, *Lab Chip* **8**, 1432 (2008).

- ¹⁴N. Chronis, M. Zimmer, and C. I. Bargmann, [Nat. Methods](#) **4**, 727 (2007).
- ¹⁵K. Chung, M. M. Crane, and H. Lu, [Nat. Methods](#) **5**, 637 (2008).
- ¹⁶A. Parashar, R. Lycke, J. A. Carr, and S. Pandey, [Biomicrofluidics](#) **5**, 024112 (2011).
- ¹⁷G. Aubry and H. Lu, [Biomicrofluidics](#) **8**, 011301 (2014).
- ¹⁸M. M. Crane, J. N. Stirman, C.-Y. Ou, P. T. Kurshan, J. M. Pehg, K. Shen, and H. Lu, [Nat. Methods](#) **9**, 977 (2012).
- ¹⁹C. Samara, C. B. Rohde, C. L. Gilleland, S. Norton, S. J. Haggarty, and M. F. Yanik, [PNAS](#) **107**, 18342 (2010).
- ²⁰C. B. Rohde, F. Zeng, R. Gonzalez-Rubio, M. Angel, and M. F. Yanik, [PNAS](#) **104**, 13891 (2007).
- ²¹S. K. Gokce, S. X. Guo, N. Ghorashian, W. N. Everett, T. Jarrell, A. Kottek, A. C. Bovik, and A. Ben-Yakar, [PLoS One](#) **9**, e113917 (2014).
- ²²J. Larsch, D. Ventimiglia, C. I. Bargmann, and D. R. Albrecht, [PNAS](#) **110**, E4266 (2013).
- ²³P. Rezai, A. Siddiqui, P. R. Selvaganapathy, and B. P. Gupta, [Lab Chip](#) **10**, 220 (2010).
- ²⁴P. Rezai, S. Salam, P. R. Selvaganapathy, and B. P. Gupta, [Lab Chip](#) **12**, 1831 (2012).
- ²⁵S. Salam, A. Ansari, S. Amon, P. Rezai, P. R. Selvaganapathy, R. K. Mishra, and B. P. Gupta, [Worm](#) **2**, e24558 (2013).
- ²⁶T. Thorsen, S. J. Maerkl, and S. R. Quake, [Science](#) **298**, 580 (2002).
- ²⁷J. C. McDonald, D. C. Duffy, J. R. Anderson, D. T. Chiu, H. K. Wu, O. J. A. Schueller, and G. M. Whitesides, [Electrophoresis](#) **21**, 27 (2000).
- ²⁸A. Vidal-Gadea, S. Topper, L. Young, A. Crisp, L. Kressin, E. Elbel, T. Maples, M. Brauner, K. Erbguth, A. Axelrod, A. Gottschalk, D. Siegel, and J. T. Pierce-shimomura, [PNAS](#) **108**, 17504 (2011).