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High-throughput screening in the *C. elegans* nervous system

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Abstract

The nematode *Caenorhabditis elegans* is widely used as a model organism in the field of neurobiology. The wiring of the *C. elegans* nervous system has been entirely mapped, and the animal's optical transparency allows for in vivo observation of neuronal activity. The nematode is also small in size, self-fertilizing, and inexpensive to cultivate and maintain, greatly lending to its utility as a whole-animal model for high-throughput screening (HTS) in the nervous system. However, the use of this organism in large-scale screens presents unique technical challenges, including reversible immobilization of the animal, parallel single-animal culture and containment, automation of laser surgery, and high-throughput image acquisition and phenotyping. These obstacles require significant modification of existing techniques and the creation of new *C. elegans*-based HTS platforms. In this review, we outline these challenges in detail and survey the novel technologies and methods that have been developed to address them.

Introduction

The roundworm *Caenorhabditis elegans* has a rich history as a model organism, particularly in the field of neurobiology. The connections between the animal's 302 neurons have been mapped [White et al., 1986], and its transparent body is conducive to calcium imaging [Kerr et al., 2000], optogenetics [Nagel et al., 2005], and other fluorescence-based techniques, allowing for observation of both single neurons and the entire nervous system. Many genes encoding ion channels, neurotransmitter receptors, and vesicle release machinery are well conserved between *C. elegans* and more complex vertebrates [Bargmann, 1998], leading to the development of nematode models for neurodegenerative diseases like Parkinson's disease [Braungart et al., 2004], amyotrophic lateral sclerosis [Oeda et al., 2001], and Alzheimer's disease [Levitan and Greenwald, 1995].

C. elegans offers the utility of a whole-animal model with the simplicity and convenience of single cells: the animal has well-defined tissues, distinct organ systems, and exhibits a variety of complex behaviors, yet its small size, short reproductive cycle, and ability to self-fertilize make it simple and inexpensive to maintain on both solid and liquid media [Brenner,

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1974; Sulston and Brenner, 1974]. These characteristics also make *C. elegans* an attractive candidate for high-throughput screening (HTS), and in particular for screens for modulators of nervous system function and development, axon regeneration, and neuropathology. However, there exist several challenges in using *C. elegans* for large-scale screens in neuroscience, including: 1) immobilizing the animal for imaging and manipulation of neurons, 2) housing individuals in parallel for longitudinal study, and 3) adapting low-throughput laser surgery techniques to high-throughput screening. This review will discuss some of the available tools and methodologies that have been developed to address each of these challenges, followed by a short survey of recently developed technologies for high-throughput image acquisition and phenotypic scoring in the nervous system.

Methods for reversible immobilization

Nematodes are mobile animals, and changes in movement provide a useful readout for screening in the nervous system. This characteristic also presents a significant challenge in using *C. elegans* for applications that require near-complete immobilization of the subject organism, like high-magnification imaging and microsurgery. Immobilization is most commonly achieved by placing the animal on an agar pad with glue [Lockery and Goodman, 1998] or polystyrene beads [Fang-Yen et al., 2012], or using paralytic drugs like sodium azide [Sulston and Hodgkin, 1988] and levamisole [Lewis et al., 1980]. None of these methods are practical for most HTS workflows. Gluing individual animals is time consuming and irreversible, preventing subsequent study of the animal; the use of polystyrene beads, while allowing for animal recovery, still requires manual transfer of individual animals. Paralytics can be applied to many individuals at once, and immobilization is reversed upon sufficient dilution of the drug. However, these compounds unavoidably affect the physiology of the animal, and removal/washout of the drug is difficult to achieve in multiwell plates without specialized equipment. Promising new immobilization methods have been reported that offer high-throughput reversibility, however. While the utility of most of these methods has only been demonstrated in microfluidic devices thus far, potential exists for application to other HTS platforms.

A variety of mechanical methods have been used to automatically and reversibly immobilize animals in microfluidic devices. Arrays of tapered microchannels or ‘clamps’ have been described that immobilize and subsequently release over a hundred animals in parallel [Hulme et al. 2007]. Similar designs to clamp animals in place for olfactory stimulation provided sufficient immobilization for calcium imaging in individual neurons [Chalasan et al., 2007; Chokshi et al., 2010; Chronis et al. 2007; Leinwand et al., 2015]. Another mechanical method uses pressure to deflect a flexible membrane made of polydimethylsiloxane (PDMS), an optically clear, gas-permeable polymer widely used in microfluidic devices, as a means for immobilization. Under pressure, the membrane forms a tight seal around the animal, ‘pinning’ it to a glass surface. This type of design has been used to immobilize animals for fluorescence imaging and nanosurgery of axons [Chokshi et al., 2009; Guo et al., 2008; Zeng et al. 2008].

Pluronic F127 (PF127), a copolymer that undergoes reversible liquid-to-gel transitions at physiological temperatures (around 22° C), is another promising alternative to traditional

immobilization agents. Krajniak et al. demonstrated the utility of PF127 in several microfluidic devices, using water baths or warming to room temperature to control the sol–gel transition of the PF127 solution [2010, 2013]. Upon gelation, animals were sufficiently immobilized for high-resolution imaging of neurons and individual synapses; image quality was comparable to that obtained by immobilization with sodium azide. The same group has also developed a simpler and more versatile PF127 immobilization technique that does not require microfluidics. Instead, animals in PF127 solution are placed on a photo-absorbing layer, and triggering gelation is achieved by tuning the intensity of a halogen lamp directed at the sample [Hwang et al., 2014]. PF127 has also been successfully incorporated into a droplet-based microfluidic device that generates up to 250 microdroplets of PF127 containing individual L1 larvae [Aubry et al., 2015]. Upon sol–gel transition, motion is sufficiently reduced for imaging of individual neurons under high-magnification objectives.

Carbon dioxide has been shown to immobilize worms in the context of a microfluidic device, although the mechanism of action is unclear [Chokshi et al., 2009]. Increasing the local concentration of CO₂ to around 75% within the device provided over two hours of immobilization with no long-term effects on animal behavior. CO₂ immobilization also reduces the photobleaching of fluorophores, which is known to be exacerbated by a high-oxygen environment. Thus, this method is suitable for long-term, time-lapse fluorescence imaging and could feasibly be adapted to non-microfluidic platforms.

Cooling *C. elegans* as a means of immobilization has been employed with some success in both microfluidic and multiwell plate formats. Thermal immobilization is advantageous because it is reversible, does not cause any physical deformation of the animal, and is one of the few methods that can eliminate pharyngeal motion, which is important for applications like laser microsurgery [Chung and Lu, 2009]. Microfluidic devices that briefly cool animals to 3–4°C have been used to immobilize animals for neuronal and even synaptic imaging [Chung and Lu, 2009; Crane et al., 2012]. Combined with automated image analysis and sorting, this method allowed for high-throughput screening of 20,000 mutants for abnormal synaptic phenotypes. In order to apply thermal immobilization to 96-well plates, Rohde and Yanik built an array of cooling pins that can be inserted directly into individual wells [2011]. Each element of the array is controlled independently so that animals are only exposed to cold temperatures immediately before or during image acquisition. Cooling with this system was used to successfully immobilize animals for axon microsurgery and subsequent time-lapse imaging of axon regeneration. This device is theoretically compatible with robotic handling and could increase the throughput of screens that require imaging the same individuals at multiple time points.

Parallel single-animal culture and containment technologies

C. elegans are typically cultivated on plates or in bulk liquid [Lewis and Fleming, 1995], where a large population of animals can be easily maintained and studied for days to weeks. This approach is high-throughput and adequate for when phenotypes can be pooled to discern changes at the population level. However, single-animal observation is more suitable for measurements of dynamic events such as axon regrowth, neural wiring during development, or neurodegeneration. Longitudinal single-animal culture and observation can

be performed by depositing individuals onto separate plates [Raizen et al., 2008], but this method is low-throughput and allows the animal to leave the field of view. Moreover, for high-resolution microscopy, animals need to be transferred (potentially repeatedly) from the culture medium to a microscope slide, which is both tedious and potentially injurious to the animal. In order for single-animal culture to be feasible for HTS, methods are needed to combine individual containment with the ability to cultivate large numbers of animals in parallel in a manner compatible with microscopy. A variety of novel nematode housing methods have been developed to address these needs.

Microfluidic devices, consisting of features patterned into PDMS, have proven suitable residences for individual *C. elegans*, and a number of microfluidic platforms capable of short [Chung et al., 2011; Ma et al., 2009] and long-term nematode confinement are now available [Hulme et al., 2010; Krajniak and Lu, 2010; Wen et al., 2012; Xian et al., 2013]. These devices generally consist of an array of liquid-filled chambers that confine individuals for the duration of the experiment. Since the size of the animal changes drastically during its life cycle, chambers are designed to accommodate individuals within a set range of developmental stages, from early [Krajniak and Lu, 2010] to late [Hulme et al., 2010; Wen et al., 2012] larval stage animals and adults [Xian et al., 2013]. More recently, a device that can successfully house embryos through the entirety of development and adulthood has been described [Uppaluri and Branwynne, 2015]. The chambers in such devices are typically connected to flow channels that can be used to deliver chemical compounds for screening or exchange waste and fresh media if long-term culture is required. Throughput is largely dependent on the number of chambers, and as many as 48 have been patterned onto a single device [Chung et al. 2011].

Detailed HTS-compatible protocols for liquid culture of *C. elegans* in 96- and 384-well microtiter plates have also been made available [Conery et al., 2014; Leung et al., 2011; Rangaraju et al., 2015; Solis and Petrascheck, 2011], and ultra high-throughput screens have even been performed in 1536-well plates [Leung et al., 2013]. Each well can act as a compartment for individual animals; provided that the bottom of the plate is transparent, phenotypes are easily observed with an inverted microscope. Multiwell plate-based methods have been used successfully to screen compound libraries for modulators of lifespan [Petrascheck et al. 2007; Ye et al., 2014], induction of gene expression [Leung et al., 2013], pathogenicity [Moy et al., 2009; Rajamuthiah et al., 2014], and protein aggregation relevant to neurodegenerative diseases [Gosai et al., 2010].

Other unique culture systems for isolating individual *C. elegans* have been developed from a variety of materials. A 45×45 array of microcompartments molded from agarose hydrogel and sealed with a glass coverslip was constructed to culture early larval stage animals (L1–L2) for several days [Bringmann, 2011]. The size and depth of the wells were designed to restrict movement and confine animals to the same focal plane, allowing for detailed confocal imaging of neuron rewiring during development. This system has since been used to observe neuronal and muscle calcium activity as well as animal behavior during lethargus [Schwarz and Bringmann, 2013; Schwarz et al., 2012]. A protocol on how to adapt the microcompartments to different larval stages, including dauers, was recently published [Turek et al., 2015]. A similar device molded from polyacrylamide, which is less prone to

tearing than agarose, has also been used to house and observe early larval stage animals [Nghe et al., 2013].

Using novel polymer chemistry, Pincus et al. developed a culture system consisting of polyethylene glycol gel pads cross-linked to glass slides [2011]. Eggs are picked onto drops of bacterial slurry deposited on the pad, which is then sealed with a layer of PDMS to trap the animals within the confines of the drop. Provided that sterile strains are used, this system allows for high resolution imaging of individual animals over the course of their entire lifespans.

Microdroplets of buffer or media surrounded by oil have been used to encapsulate individual nematodes [Belfer et al., 2013; Luo et al., 2008]. The small size of these droplets allows for large-scale parallel containment, minimal consumption of reagents, and rapid compound exchange. Devices capable of automatically generating large arrays of animal-containing droplets have been described for assessing neurotoxin effects in high throughput [Shi et al., 2010, 2008]. A version of these devices has been outfitted for substance exchange between the droplets and inflow of media, allowing for parallel long-term culture of 160 newly hatched animals to adulthood [Wen et al., 2015]. The *Caenorhabditis*-in-Drop method (CID) is a microdroplet-based system developed for studying quiescence in larval animals but capable of culturing animals for up to five days. NGM microdroplets containing FUDR to prevent reproduction and concentrated bacteria as a food source are deposited onto a PDMS chip and covered with mineral oil, and individual animals are manually placed into the droplets [Belfer et al., 2013].

Automation of laser microsurgery and nanosurgery

Laser microsurgery for ablating neuronal cell bodies has been crucial in delineating the function of individual neurons and their contribution to specific physiological functions and behaviors in *C. elegans* [Avery and Horvitz, 1989; Bargmann and Horvitz, 1991; Chalfie et al., 1985]. Nanosurgery, or severing individual axons, has been made feasible by the application of femtosecond lasers [Yanik et al., 2004] and is useful in studying both normal function of nerve fibers as well as their regrowth after injury. While laser surgery has proved invaluable in studies of the *C. elegans* nervous system, the technique is laborious and time-consuming, which limits its inclusion in HTS workflows. Typical protocols require picking individual animals onto an agar pad, immobilizing and orienting them, focusing the laser onto the cell body or axon of interest, and recovering the animals after surgery [Fang-Yen et al., 2012]. Recently developed techniques have been able to automate some or all of these steps, greatly increasing the throughput of laser microsurgery and nanosurgery and its potential application in HTS.

The first microfluidic device built for performing laser axotomies in *C. elegans*, dubbed the 'nanoaxotomy chip', was developed in 2008 [Guo et al.]. The device design includes an immobilization domain, where individual animals are immobilized by pressurized membrane deflection. The membrane flattens the worm against a glass surface, ensuring that the majority of the axon is in focus and improving the ease with which surgery can be performed as well as the image quality. An additional recovery domain allows worms to be

cultured after surgery and returned to the immobilization domain for imaging at later time points if desired. While the actual surgery must still be performed manually, the positioning, immobilization, and recovery of the animal are greatly expedited. This device was able to reduce typical nanosurgery throughput from ten minutes per animal [Yanik et al., 2004] to just one. A microfluidic device using similar immobilization methods but incorporating a software interface for performing laser surgery was later developed [Samara et al., 2010]. In order to perform the surgery, the software requires that the user select the neuronal cell body and the position on the axon where the laser surgery will be performed. This reduced processing time to only 20 seconds per animal, enabling the platform's use in screening a small-molecule chemical library for modulators of axon regeneration.

A fully automated microfluidic device for performing laser axotomies has recently been reported [Gokce et al., 2014]. A population of up to 250 animals is deposited into a loading chamber, from which individual worms are automatically loaded into a trapping area. The trapping area contains a series of flow outlets arranged in a sieve structure; the pressure drop across this structure linearizes the worm before an additional trapping membrane is activated. This immobilization method ensures consistent location of the neuron of interest within a constrained field of view, simplifying the image processing. In order to identify the axon of interest, low magnification brightfield images are used to find the centroid of the animal, where the field of view is then centered. The software then switches to a higher objective to collect a z-stack of fluorescence images, from which the circular soma is identified and brought into fine focus. Finally, the associated axon is identified, optimally focused, and moved to the laser spot for the axotomy. Using this fully automated workflow, ALM neuron axotomies were performed successfully on 236 out of 350 animals at a rate of only 17 seconds per worm. These rates facilitate large-scale, possibly genome-wide screens for modulators of axon regeneration.

A microfluidic device has also been used as a platform for high-throughput laser ablation of fluorescently tagged neurons [Chung and Lu, 2009]. In this device, animals are automatically loaded and positioned on the surgery platform and immobilized by a cooling channel. A z-stack of images is then acquired at 100 $\times$ magnification, which is fed to an image-processing pipeline that identifies individual neurons based on local fluorescence maxima. Cell bodies are distinguished from autofluorescent objects based on empirical knowledge of neuroanatomy, such as expected size and position of respective neurons. The laser is then automatically centered on the neuron's coordinates and subsequently fired to perform the ablation. This workflow was used for laser ablation of the AWB neuron pair in individual animals, demonstrating a throughput of 33 seconds per worm and an 89% success rate for ablation of both cells. Provided that the neurons of interest are tagged with cell-specific fluorescent markers, this platform could be utilized for large-scale studies of behavior, neuronal function, and development.

Novel tools for high-throughput image acquisition and phenotype scoring

There are many approaches to assessing neurological function and associated behaviors in *C. elegans*, including locomotion, electrophysiological activity, and neuronal response to stimuli. Coupling such measures with high-throughput screens requires robust, automated

scoring of the phenotype of interest. Here we will briefly survey a variety of novel technologies and image-analysis tools capable of automated phenotype scoring and that are currently compatible with or easily adapted to *C. elegans*-based HTS.

A fully automated, high-throughput method for assessing worm motility has been developed through a novel application of the cell monitoring system xCelligence [Smout et al., 2010]. This technology was originally designed to measure cell confluence via changes in electrical impedance, as measured across microelectrodes embedded in the base of specially designed 96-well plates. This readout can be readily converted to a motility index, as swimming nematodes repeatedly come into contact with the electrodes and cause changes in conductivity. It should be noted that this system has only been tested on parasitic nematode species thus far, but could feasibly be utilized with *C. elegans*. Another commercially available system for tracking *C. elegans* movement in microtiter plates, the WMicrotracker, uses infrared microbeams to quantify motion in real-time [Simonetta and Golombek, 2007]. Microbeams passing through the bottom of each well are scattered by moving animals. This allows changes in movement over time to be detected and measured in conventional multiwell plates.

Microfluidic arenas containing grids of pillars which *C. elegans* can use to crawl as if on solid surfaces ('artificial dirt') have been used as highly controlled environments for locomotory and behavioral measurements, and are compatible with introduction and removal of different compounds for screening. [Lockery et al., 2008] Such designs may also be adaptable to long-term individual or group culture, though no such applications have yet been published.

Using a very different technical approach, the *C. elegans* 'Lifespan Machine' was developed to measure lifespan of large *C. elegans* populations in high throughput [Stroustrup et al., 2013]. The platform is based on widely available and inexpensive flatbed scanners, which can obtain resolutions of 8 μm for imaging animals on agar plates. Scanners, each holding 16 plates with ~35 animals each, are kept in temperature-controlled incubators and are controlled by automated software tools. This system can easily acquire a large number of time-lapse images of thousands of animals and is suitable for large-scale screening. The automated image analysis pipeline associated with this platform is designed to quantify lifespan; however, because movement or lack thereof between serial images is used to determine lifespan, this method could potentially be adapted to directly measure movement phenotypes.

Electrophysiological measurements of the *C. elegans* pharynx provide high-resolution information about neurological function, but application to HTS has been limited by the low-throughput of available recording techniques. A microfluidic device designed for collecting electropharyngeograms (EPGs) from up to eight individuals in parallel has been reported, improving throughput and decreasing the manual labor required [Lockery et al., 2012]. Worms are loaded into separate 'recording modules', where the head or tail of the animal is secured in a tapered channel with a port for an electrode to be inserted. The device was able to reliably measure EPGs at a rate of 75%, with waveform characteristics similar to those obtained with canonical methods. Because the recordings are done on a microfluidic

platform, rapid compound delivery for small-molecule screening is also possible. Increasing the number of parallel recording modules or operating multiple devices in parallel could provide sufficient throughput for HTS applications. Similarly, the ‘Neurochip’ is a microfluidic device that is designed for collecting EPGs from individual adult animals [Hu et al., 2013]. This design includes microfabricated electrodes directly integrated into the device, simplifying the recording setup and producing EPGs with better signal-to-noise ratios. The device was also modified successfully for use with early larval stage (L2) animals, which are particularly difficult to record from due to their small size and high mobility. Individual recordings can be obtained from up to 12 animals per hour, a threefold increase in throughput compared to manual recording techniques.

A number of image analysis tools have also been made available for scoring complex *C. elegans* phenotypes from images acquired on different HTS platforms. Worm Toolbox is a software package designed to analyze image outputs from high-content, liquid-based screens in multiwell plate format [Wählby et al., 2012]. Bright field images are processed to outline wells and delineate single worms from clusters of overlapping animals. The software is then able to obtain measurements of texture, fluorescence intensity, opacity, and curvature for individual animals and can use these measurements to reliably distinguish living from dead worms. A program capable of assessing multiple phenotypes from images or video of animals on solid plates has also been developed [Jung et al., 2014]. This system includes image acquisition software as well as programs for measuring lifespan, crawling speed, body size, and egg laying. The Multi-Worm Tracker (MWT), a computer vision application that tracks individual *C. elegans* in real-time, is a useful tool for automating analysis of locomotor and behavioral outputs [Swierczek et al., 2011]. To identify individual worms and their positions in each frame of the video feed, the MWT segments objects that are darker than the background by a predetermined threshold. A worm whose location in the current frame resides within a 10 pixel region of a worm’s position in the previous frame is tracked as the same individual. A complementary offline program, Choreography, can extract information about crawling speeds as well as the frequency of reversals, turns, and bending motions. With the ability to track up to 120 individuals per plate, the MWT is suitable for analyzing chemotaxis, habituation and other behavioral readouts in high throughput.

Current limitations and emerging technologies

The tools and methods described above have significantly advanced the development of *C. elegans*-based HTS and its utility in the field of neurobiology; however, there remain several limitations to be considered when using *C. elegans* for certain HTS applications. The animal’s impermeable cuticle and arsenal of xenobiotic genes reduce the uptake of many small molecules, presenting additional complexities and cost considerations for high-throughput drug screening [Collins et al., 2006; Lindblom and Dodd, 2006]. A screen by Burns et al. of more than a 1000 small molecules found that for the vast majority, the internal concentration accumulates to less than half of that applied externally. The results of the screen were used to build a ‘small-molecule structure-based accumulation model’ (SAM) that can predict which compounds are likely to accumulate and show bioactivity in *C. elegans* [2010]. The use of this model may compensate for any deficiencies in drug uptake by prioritizing small molecule leads and improving screening efficiency.

In addition, the requirement for bacterial co-culture means that many compounds may be metabolized by bacteria in ways that alter their efficacy or otherwise challenge interpretation of results. A recent study by Zheng et al. showed that small molecule absorption in *C. elegans* is dependent on the method of delivery and whether the animals are cultured with live or heat-killed *E. coli*, suggesting that the efficiency of drug uptake may be improved by optimizing screening conditions [2013]. A related example is that the effect of metformin application on *C. elegans* lifespan was shown to be mediated via bacterial metabolism [Cabreiro et al., 2013; Onken and Driscoll, 2010]

Another ongoing limitation is the lack of available tools for transiently inducing gene expression in *C. elegans*, which may be desirable for some HTS workflows. While viral vector transfection is widely used for this purpose in cell-based systems and is readily amenable to HTS, no similar tools currently exist in the nematode. Some success in cell-specific induction of transgene expression has been achieved through the use of *hsf-1* mutant strains, which lack the protein responsible for mounting the heat shock response. Animals are transformed with constructs containing *hsf-1* coupled to a cell-specific promoter as well as the transgene of interest under the control of a heat-shock-responsive promoter; shifting the animals to higher temperatures induces transgene expression with both temporal and spatial control [Bacaj and Shaham, 2007]. To circumvent the need for cell-specific promoters, infrared lasers have been used to provoke heat shock and resultant transgene expression in specific cell types and tissues, including neuronal cells [Kamei et al., 2009]. Spatial resolution has been improved through the application of pulsed infrared lasers, allowing for induction of gene expression in individual neurons [Churgin et al., 2013; Suzuki et al., 2014]. However, this method requires generation of a different transgenic strain for each gene of interest, which remains low-throughput and labor-intensive. The emergence of the computer-assisted microinjection (CAMI) system, a new platform that automates transgenesis in *C. elegans*, may prove promising in alleviating this bottleneck [Gilleland et al., 2015].

Conclusion

Unique among all other animal models for neuroscience research, *C. elegans* is compatible with both microfluidic devices and multiwell plates, providing the basis for truly high-throughput screens using these animals. With the recent technical advances in *C. elegans* handling, culture, and phenotyping reviewed above and summarized in Table 1, it is now increasingly possible to conduct mass screens in whole, intact organisms for mutations or chemical compounds that alter complex neuronal phenotypes.

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Highlights

- *C. elegans* is a model for high-throughput screening (HTS) in the nervous system
- Traditional techniques for manipulating *C. elegans* are laborious and lowthroughput
- High-throughput immobilization, parallel culture, and phenotyping are key challenges
- We review the technologies and platforms developed to address these challenges

Table 1

A list of the tools and techniques that have been developed to address the major challenges associated with *C. elegans* high-throughput screening and a selection of associated references.

Challenges associated with <i>C. elegans</i> high-throughput screening (HTS)	HTS-compatible tools and techniques	Selected reference(s)
Reversible immobilization	Tapered microchannels and clamps	Hulme et al., 2007
	PDMS membrane deflection	Guo et al., 2008; Zeng et al., 2008;
	Pluronic F127	Krajniak et al., 2010
	Carbon dioxide	Chokshi et al., 2009
	Thermal cooling	Chung and Lu, 2009; Rohde and Yanik, 2011
Parallel single-animal culture and containment	Microfluidic chamber arrays	Hulme et al., 2010
	96-, 384-, and 1536-well microtiter plates	Leung et al., 2013; Solis and Petrascheck, 2011
	Agarose microcompartments	Bringmann, 2011
	Polyethylene glycol pads cross-linked to glass slides	Pincus et al., 2011
	Microdroplets	Belfer et al., 2013; Wen et al., 2015
Automation of laser surgery	Microfluidic devices for automated laser axotomy	Gokce et al., 2014; Samara et al., 2010
	Microfluidic device for automated cell ablation	Chung and Lu, 2009
High-throughput image acquisition and phenotyping	xCelligence cell monitoring system	Smout et al., 2010
	WMicrotracker	Simonetta and Golombek, 2007
	Microfluidic pillar grids ('artificial dirt')	Lockery et al., 2008
	Lifespan Machine	Stroustrup et al., 2013
	Microfluidic devices for electropharyngeogram (EPG) recording	Hu et al., 2013; Lockery et al., 2012
	WormToolbox	Wählby et al., 2012
	Multi-Worm Tracker	Swierczek et al., 2011