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Recent Advances and Trends in Microfluidic Platforms for *C. elegans* Biological Assays

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Abstract

Microfluidics has proven to be a key tool in quantitative biological research. The *C. elegans* research community in particular has developed a variety of microfluidic platforms to investigate sensory systems, development, aging, and physiology of the nematode. Critical for the growth of this field, however, has been the implementation of concurrent advanced microscopy, hardware, and software technologies that enable the discovery of novel biology. In this review, we highlight recent innovations in microfluidic platforms used for assaying *C. elegans* and discuss the novel technological approaches and analytic strategies required for these systems. We conclude that platforms that provide analytical frameworks for assaying specific biological mechanisms and those that take full advantage of integrated technologies to extract high-value quantitative information from worm assays are most likely to move the field forward.

INTRODUCTION

The advent of microfluidics has revolutionized biological studies. Microfluidic technologies, driven by a need for miniaturization of analytical techniques, allow for precise control and manipulation of liquids, reagents, cells, and small organisms (1). These devices or chips are typically made using an inert transparent polymer, polydimethylsiloxane (PDMS), and are patterned using soft lithography fabrication techniques (2–4). The relative ease of fabrication and the incredible utility of these devices have led to widespread adoption of microfluidics in biological studies (5, 6).

Caenorhabditis elegans research in particular has benefitted from the use of microfluidic platforms (7). This is in part because of the length scale of these microscopic nematodes, their short life cycle, and their transparent cuticles (8). These worms can be readily cultured on nematode growth media (NGM) agar plates in large numbers (8–11). However, handling and processing such small animals by conventional methods for quantitative or large-scale studies can be laborious and time consuming. Microfluidics-based platforms overcome these hurdles by allowing for robust and rapid manipulation of worms on-chip and have advanced the field by enabling conventionally impractical studies. For example, microfluidic devices have been developed for rapid worm immobilization, imaging, and sorting (12); controlled stimulus and drug delivery (13); and physiology and behavior tracking (14).

In this review, we discuss recent and notable applications of integrated microfluidic technologies for *C. elegans* research. There have already been a number of exemplary reviews that provide a general overview of this topic (7, 15–17) and still others that highlight microfluidics-based worm assays in the context of specific research areas (18–25). In the past two years, three publications have summarized the current progress in this field (26–28). In the most recent review, Cornaglia et al. (28) thoroughly highlighted recently published works that had clear applications in high-throughput and high-content screens. In contrast, the focus of this review is not to provide a comprehensive list and description of each major recent publication in this domain. Instead, this review aims to highlight particular publications that demonstrate the maturation of integrated microfluidic technologies for *C. elegans* biological assays. We also seek to draw a focus on techniques that provide a new analytical framework for *C. elegans* studies. Finally, we aim to provide insights into the future directions and standing challenges in the field.

We generally refer to microfluidic devices that rely on advanced external techniques, such as custom microscopy, automated image processing, or specialized control systems, as integrated microfluidic technologies. These systems are aptly named, as they require advanced integration of devices with hardware and software components beyond the basic flow-control and microscopy tools needed for microfluidics. There have been separate reviews on some of these topics (29–35), and this review does not generally discuss these technologies. Instead, we systematically indicate how specific integrated microfluidics tools enable studies that could not have been performed with either conventional or stand-alone techniques.

SENSORY ASSAYS

C. elegans sensory assays aim to probe the neuronal and behavioral response of the animal in response to stimuli. This model organism has a compact nervous system composed of 302 neurons, and the identity and structural connections of these neurons have been comprehensively mapped (36, 37). Additionally, in vivo neuronal activity of these animals can be readily observed using fluorescent microscopy and genetic labeling techniques, such as the GCaMP calcium reporter (38–40). For these reasons, sensory assays in *C. elegans* can provide unique insights on the neural basis of perception and behavior. Two sensory modalities often studied in *C. elegans* are chemosensation and mechanosensation. Chemosensation is analogous to the human senses of taste and smell,

and in worms, it refers to their ability to respond to chemical cues that influence their physiology, behavior, and fitness (41). Similarly, the nematode mechanosensory circuit responds to mechanical stimuli on the worm body and has parallels in the human senses of touch and hearing (42). Recent integrated approaches have enabled deeper understanding of these sensory circuits and helped in the quantification and standardization of assay techniques for future studies.

Integrated Approaches to Chemosensory Assays Reveal Novel Circuit and Whole-Brain Dynamics

A number of microfluidic devices have been proposed to test *C. elegans* response to chemical stimuli at individual or population levels. One notable early design by Chronis et al. (13), called the olfactory device (**Figure 1*a***), has proven useful for a variety of chemosensory studies (43–46). Their design allowed for precise on-off control of chemical stimuli to immobilized animals while recording their functional neuronal activity. Recently, Kato et al. (45) characterized the temporal dynamics of chemosensory neurons by presenting rapid stimulus fluctuations to animals and recording the resulting neuronal activity using this device. In another application discussed in more detail later, Bazopoulou et al. (46) characterized the age-dependent functional deterioration of chemosensory neurons. Whereas the olfactory chip enables neuronal recordings of individual animals to a precisely delivered chemical stimulus, another system proposed by Albrecht & Bargmann (47) enables behavioral recordings from a population of worms in the presence of spatiotemporally controlled chemical stimuli. This design consists of a micropillar arena with multiple stimulus reservoirs that can be externally controlled to send temporal pulses, spatial steps, or spatial gradients of chemical stimuli to the animals. Albrecht and Bargmann enhanced subsequent analysis by developing an automated behavioral tracking software that quantified assay results.

In a follow-up study by Larsch et al. (48), the major advantages of the two designs by Chronis et al. (13) and Albrecht & Bargmann (47) were incorporated into a single device. This study demonstrated the ability to simultaneously record both the neuronal activity and the behavior of freely moving worms in the presence of a spatiotemporally controlled chemical stimulus. Behavioral video captures of freely moving animals require long-term low-magnification microscopy, whereas fluorescent imaging of GCaMP-labeled neurons is susceptible to motion artifacts, can cause photobleaching and phototoxicity after extended imaging, and must have a high signal-to-noise ratio. The authors solved these seemingly conflicting requirements by integrating the micropillar arena design with a custom-adapted microscopy system (**Figure 1*b***). This system included a number of hardware and software components that enabled the simultaneous measurements. For example, a high-numerical aperture objective in conjunction with a low-noise camera was required to maximize the signal-to-noise ratio of the fluorescence signals recorded at low magnification. Moreover, a fast-switching pulse timer circuit allowed for illumination pulses that were synchronized with image capture, minimizing motion blur, photobleaching, and phototoxicity. Custom neuron tracking and worm segmentation algorithms were used later to quantify neuronal and behavioral response. After developing this platform, Larsch et al. (49) utilized it to advance the understanding of how *C. elegans* olfactory circuitry mediates gradient climbing. They showed that the AWA sensory neurons and AIA interneurons work in conjunction to detect small increases in odor concentration over time. Furthermore, their experiments indicated that AWA desensitization to chemical stimuli depends on concentration and odor history.

To assay response of animals to different oxygen and carbon dioxide cues, Zimmer et al. (50) previously designed a microfluidic platform that could rapidly deliver gaseous stimuli to worms while recording their functional neuronal activity via a genetically encoded calcium reporter (**Figure 1*c***). Their system flows gases through a two-layer device, with the gas stimuli chamber on

top of a worm immobilization and imaging channel. Rapid gas exchange occurs between the layers due to a thin gas-permeable PDMS membrane connecting the two layers. In a recent follow-up study, Schrödel et al. (51) integrated this system with a rapid two-photon microscopy platform, which they used together with a pan-neuronal calcium reporter, to track the global brain response

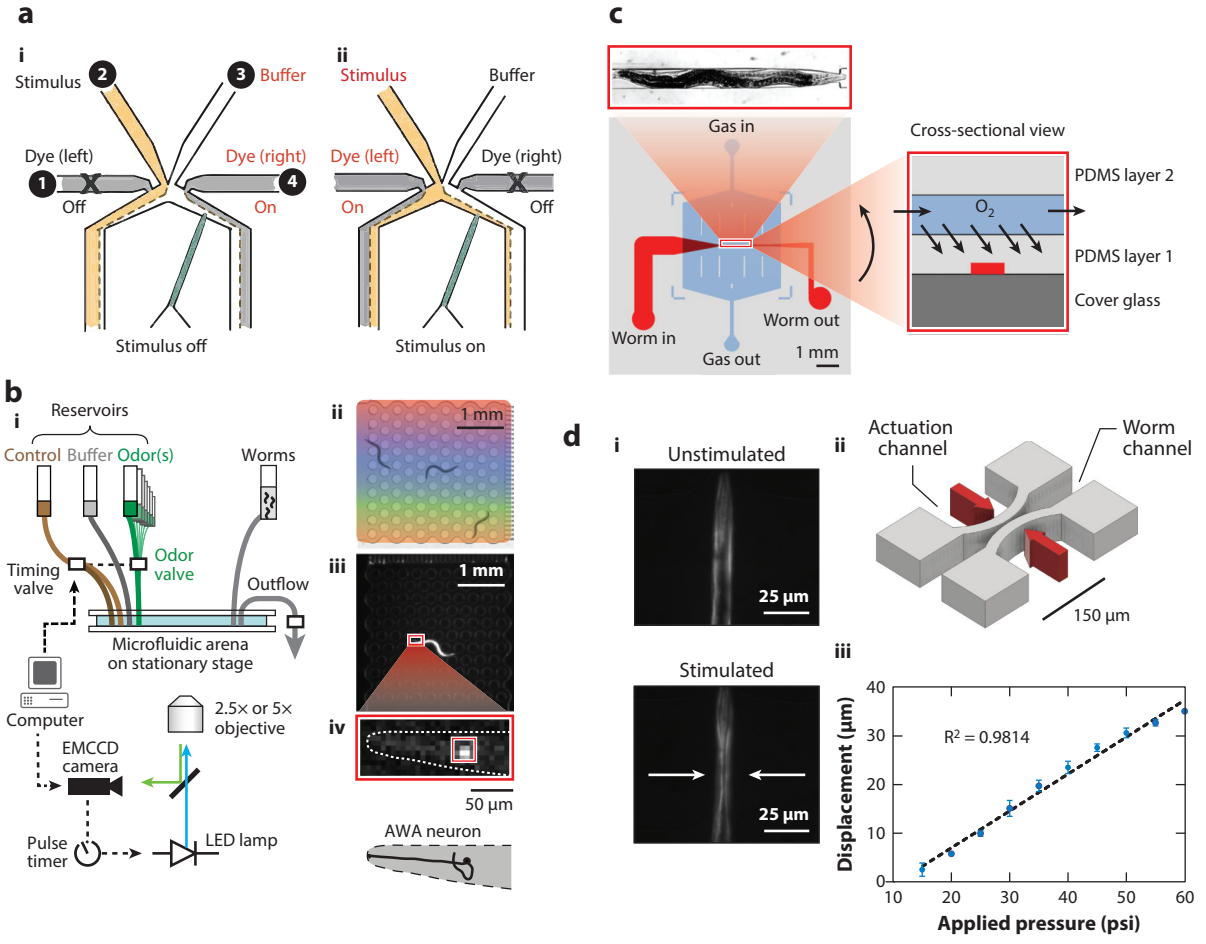


Figure 1

Microfluidic systems for studying *Caenorhabditis elegans* chemosensation and mechanosensation. (*a*) Chemical stimulus device developed by Chronis et al. (13) that can rapidly turn the stimulus on (*i*) and off (*ii*) as desired. Panel adapted with permission from Reference 13. Copyright 2007, Nature Publishing Group. (*b*, *i*) Overview of system developed by Larsch et al. (48) for simultaneously measuring behavior and neuronal response of *C. elegans* in response to spatiotemporally controlled chemical cues. (*b*, *ii*) The microfluidic arena device used in the system to produce the spatiotemporal chemical gradient, the spatial component of which is represented by the color-gradient overlay. (*b*, *iii*) Representative behavior image and (*iv*) neuronal signal of GCaMP-labeled AWA neuron captured on the device. Panel *b* adapted from Reference 48. (*c*) Gaseous stimuli delivery device developed by Zimmer et al. (50) wherein gas flow in the second layer above the worm rapidly diffuses to the imaging channel through thin PDMS membrane. Panel adapted with permission from Reference 51. Copyright 2013, Springer Nature. (*d*, *i*) Mechanical stimulation of a *C. elegans* animal inside a microfluidic system developed by Cho et al. (64), with the worm outline expressing brightfield fluorescence. (*d*, *ii*) Schematic showing mechanical stimulation channel geometry; red arrows indicate pressure applied. (*d*, *iii*) Characterization of applied valve pressure and resulting displacement of the worm body. Panel image components provided courtesy of Y. Cho. Abbreviations: EMCCD, electron-multiplying charge-coupled device; LED, light-emitting diode; PDMS, polydimethylsiloxane.

of worms to varying gaseous environments. Such a powerful integrated platform then enabled these researchers and their colleagues to uncover systems-level behaviors of the worm brain. They found that motor commands are embedded in global neural activity patterns in *C. elegans* (52) and that sleep-like behavior in these animals leads to a down-regulation of neural activity to a global quiescent state (53).

The studies discussed above demonstrate the power of integrated microfluidic platforms that combine relatively simple device designs with advanced microscopy and data analysis techniques. The results of these assays have elucidated the dynamics of individual neurons, olfactory circuit behavior, and global brain states. We surmise that the discovery of these neural properties hinged on the technological advancement of these platforms and thus could not have been ascertained by conventional or stand-alone chemosensory assays. It is likely that future studies will further characterize the mechanisms by which connected individual neurons give rise to complex dynamic behavior.

Quantitative On-Chip Mechanical Stimulation Reproduces and Standardizes Conventional Mechanosensory Assays

Similar to chemical signals, mechanical cues are critical for *C. elegans* survival (42). Two avoidance behaviors that have provided significant insights into sensory signal transduction are the responses of animals to gentle and harsh touch stimuli (54–58). The two stimuli are conventionally defined by the probing method used, for example, by stroking animals with an eyebrow hair, tapping the culture plate for gentle touch stimulation, or prodding the animals with platinum wire for harsh touch stimulation (59, 60). These assays were initially scored based on the resulting behavior, characterizing the worm movement after stimulation. More recently, there has been a push to develop both quantitative stimulus delivery techniques, such as force or body-wall displacement–based mechanical stimuli, and standardized response metrics, such as measuring electrophysiology or calcium activity of mechanosensory neurons (42, 60). However, these techniques for quantitative mechanical stimulation, such as force cantilever devices, generally suffer from being low throughput due to long manual loading of animals, or they are unsuited for worm recovery due to the need to glue animals in place during stimulation (61–63). These factors limit the use of these techniques for high-throughput mechanosensory assays.

To address these concerns, Cho et al. (64) and Nekimken et al. (65) reported on microfluidic devices for on-chip quantitative mechanical stimulation and functional neuronal imaging of *C. elegans*. Both of these groups used a single-layer device design, in which a worm is loaded into an imaging channel and subsequently allowed to acclimate to the loading process. Then, pneumatically actuated valves press the PDMS side walls onto the main channel, mechanically stimulating the desired sections of the worm body in a measured and precisely controlled fashion (**Figure 1*d***). Both groups used strains that expressed the GCaMP fluorescent calcium reporter in mechanosensory neurons to quantify responses to the stimuli. Additionally, each group characterized the deflection of the worm body in response to the applied pressure of the stimulation valve. This important characterization, which is done using both empirical imaging and finite-element simulation, guarantees the standardization of stimuli to each worm assayed.

Nekimken et al. (65) demonstrated that their device could elicit a response from the individual gentle touch neurons (ALM, AVM, and PVM) when using a high-frequency pulsing or buzz stimulus. They noted that these touch-receptive neurons could also be activated by using a blue light stimulus in their device. Importantly, they verified that their on-chip mechanical stimulation reproduced results from classical mechanosensory assays. They showed that mutants deficient in the MEC-4 channel, which transduces mechanical signals on touch-receptive neurons, do

not respond to mechanical stimulation on-chip. They recapitulated the receptive fields of the individual gentle touch neurons using their device. They also confirmed that repeated mechanical stimulation led to habituation of the calcium response in these neurons, which is analogous to the behavioral habituation to repeated mechanical stimuli shown in off-chip assays.

Although Cho et al. (64) showed similar validation steps in their mechanical stimulation device that recapitulated results from conventional mechanosensory assays, there are several notable distinctions between the two designs. First, the authors demonstrated that their system could be used to probe not only the gentle touch circuitry but also the harsh touch-sensing PVD neurons. This was enabled by the robust design of the microfluidic device that allowed for a larger range of operating pressure, and thus, tissue deformation. Second, they showed that they could elicit a tunable response from both gentle and harsh touch neurons depending on the pressure used during a single nonpulsed press. Third, Cho and coworkers demonstrated that their device is robust for long-term operation and thus could be automated, allowing them to perform a high-throughput drug screen for small molecules that act on the mechanosensory pathway. They characterized the effects of molecules from an orphan ligand library on the calcium dynamics of AVM neurons and found a few such compounds that significantly altered the response metrics (64).

Both of the microfluidic devices discussed in this section show promise for quantitatively and rapidly probing the mechanosensory circuit of *C. elegans*. It is important to note that for these studies, automated neuron tracking and fluorescent image processing play a central role in quantifying the neuronal response to stimulation. This further indicates that advances must rely on robust quantitative analysis to fully leverage the power of microfluidic devices. In summary, these approaches to on-chip mechanical stimulation using PDMS devices have demonstrated a new analytical framework for assaying mechanosensation. We expect that these designs could be adapted to explore the neuronal response to mechanical stimulation at different developmental time points, providing insights into how this circuitry adapts and changes with age. Finally, the methods presented in these studies could also be integrated with additional sensory stimulation designs to probe multimodal sensory integration.

DEVELOPMENTAL, LIFE SPAN, AND AGING ASSAYS

The short life cycle and life span of *C. elegans* make it an ideal system for studying development and aging in microfluidic devices (21, 24). *C. elegans* embryonic development begins with initiation of embryogenesis upon fertilization of the oocyte and continues outside the parent once the egg is laid. After the embryo hatches, postembryonic development continues through four distinct larval stages (L1, L2, L3, and L4) to adulthood. Individuals reach adulthood roughly 72 h after being laid and can survive as adults for up to three weeks at 20°C (8, 66). Recent device innovations have allowed for dynamic control of environmental conditions during life span assays and extended the ability to track developmental and age-dependent physiological changes.

Physiological Tracking in Long-Term Developmental Assays Is Enhanced by Microfluidics-Enabled Rapid Microscopy and Periodic Immobilization

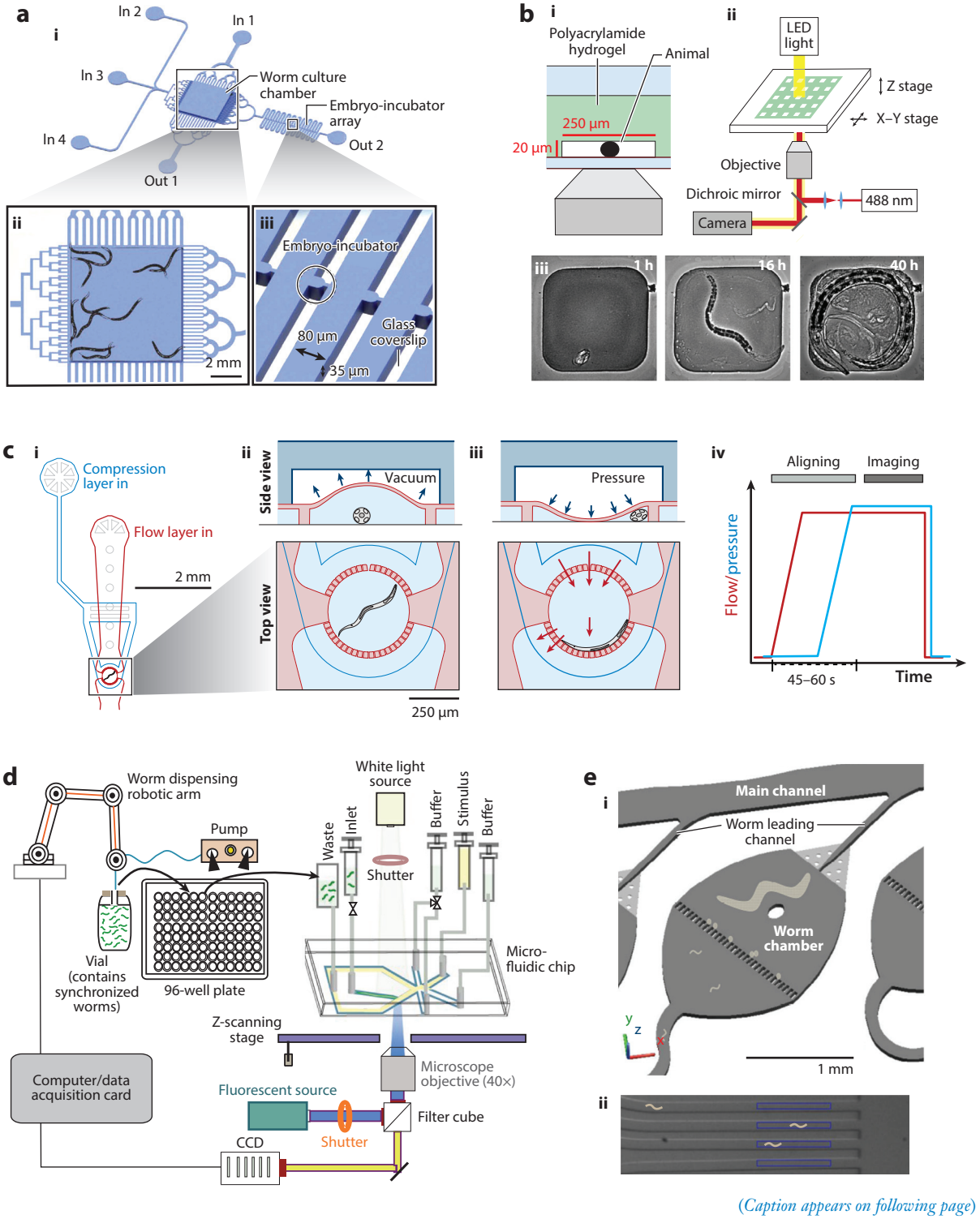
The use of microfluidics to study development has been previously explored (21, 24, 67, 68), but significant advances have been made in recent years. For example, Chung et al. (68) developed a microfluidic device for high-throughput parallel trapping and imaging of *Drosophila melanogaster* embryos. The embryos could be hydrodynamically loaded and oriented into the device via a single serpentine flow channel, allowing for simple loading. Recently, a similar principle was demonstrated for high-throughput capture and analysis of *C. elegans* embryos. Cornaglia et al.

(69) designed a microfluidic device that automatically trapped and immobilized newly laid worm embryos through the entirety of embryogenesis. Their design, highlighted in **Figure 2*a***, is divided into two areas. In one area, gravid adults are size synchronized and cultured to lay eggs. The eggs are then hydrodynamically transferred into an adjoining embryo trap array for immobilization. Here, high-resolution time-lapse microscopy can be used to assay embryonic morphogenesis for multiple embryos in parallel. The integration of multiple device design ideas allowed the authors to create a complete analytical platform for embryonic studies. Using this platform, the group demonstrated that there is a burst of mitochondrial biogenesis during late embryonic development and that perturbing mitochondrial function during embryonic development can trigger the unfolded protein response pathway.

To assay long-term *C. elegans* postembryonic development, microfluidic devices must be able to accommodate the vast changes in size of the animal as it molts through the four larval stages. A common technique is to use microchamber arrays, which are analogous to microwell plates, in which individual worms can be allowed to freely grow in a controlled environment, with liquid culture media continuously perfused through the chambers (70–73). Uppaluri & Brangwynne (70) recently demonstrated the use of such a design to calculate and track the changes in body length of developing worms. This analysis led the authors to uncover distinct growth rates for each larval stage. Additionally, by changing the food conditions on-chip, they demonstrated a size threshold–based model for molting. In an earlier microchamber array device used by Krajniak & Lu (67), worms were periodically immobilized for imaging by replacing the culture media in the chambers with a thermoreversible hydrogel. Thermally activated by an external temperature controller, this material transitioned from a liquid to gel state, immobilizing the animals for high-resolution imaging. This technique did not significantly affect the developmental progression of the worms. Cornaglia et al. (71) later adapted this design to a platform that could be used to track protein aggregation in worm models for both amyotrophic lateral sclerosis (ALS) and Huntington's disease. An important result from this study was that doxycycline, a commonly used antibiotic, significantly suppressed the size expansion of aggregates and slowed the loss of motility in the *C. elegans* ALS model strain.

Gritti et al. (72) and Keil et al. (73) also demonstrated the use of two notable integrated systems for long-term tracking of developing *C. elegans*. Gritti and coworkers fabricated microchamber arrays in which larval development could be tracked without the need for worm immobilization (**Figure 2*b***). Using light-emitting diode (LED) and laser illumination coupled to a motorized stage, this group could capture ultrafast Z-stack images (1–10-ms exposure times) of freely moving animals in the arrays. They used this system to track seam cell divisions, distal tip cell migration, and molting cycle gene expression oscillations over a period of 30–40 h through the different larval stages. This rapid microscopy system presents a clear advantage over other long-term development tracking techniques, as it does not require immobilizing or perturbing the developing larvae for image acquisition.

Keil et al. (73) designed a microfluidic chamber device that could repeatedly immobilize the worms in a specific orientation for high-resolution imaging (**Figure 2*c***). Once L1-stage animals were loaded into the chambers, they were allowed to move freely and develop normally. When imaging at a particular time point was desired, a membrane on the second layer of the device was pneumatically pressed against the worm while the flow layer pushed the animal toward the edges of the culture chamber. This enabled complete immobilization of the worm in a stereotyped fashion at any larval stage. This group demonstrated the advantages of their technique by tracking vulval precursor cell divisions with differential interference contrast microcopy and by tracking vulval fluorescing gene expression markers. Impressively, they also tracked PVD neurite outgrowth over a 24-h period with high-resolution, time-lapse, fluorescent Z-stacks, in which



(Caption appears on following page)

Figure 2 (*Figure appears on preceding page*)

Systems and devices for development and aging studies in *C. elegans*. (*a*) Device developed by Cornaglia et al. (69) for studying embryos. (*a*, *i*) Gravid adults are cultured on-chip in the first section (*a*, *ii*), and their eggs are hydrodynamically transferred to a second section (*a*, *iii*) that is used to immobilize and image the embryos. Panel adapted with permission from Reference 69 under the terms of the Creative Commons Attribution 4.0 International License, **https://creativecommons.org/licenses/by/4.0**. (*b*, *i*) Chamber structure and (*ii*) imaging platform used by Gritti et al. (72) for long-term imaging of postembryonic development. (*b*, *ii*) A motorized stage can be moved in an XY direction to image different chambers, while a piezo Z stage allows for focusing samples. (*b*, *iii*) Representative micrographs of an animal at different time points. Panel adapted with permission from Reference 72 under the terms of the Creative Commons Attribution 4.0 International License, **https://creativecommons.org/licenses/by/4.0**. (*c*, *i*) Device used by Keil et al. (73) for long-term culturing and high-resolution imaging of developing animals. (*c*, *ii*) Animals are allowed to move freely in the culture chamber until they are (*iii*) immobilized by a top layer that pushes down on them as the flow layer pushes them against the circular walls of the chamber. (*c*, *iv*) Time-controlled culture-channel flow and immobilization-chamber pressure allow for quick alignment followed by imaging. Panel image components provided courtesy of W. Keil. (*d*) Automated platform developed by Bazopoulou et al. (46) for tracking the age-dependent deterioration of the ASH neuron. Panel adapted with permission from Reference 46 under the terms of the Creative Commons Attribution 4.0 International License, **https://creativecommons.org/licenses/by/4.0**. (*e*) Device developed by Li et al. (92) to measure reproductive life span, where (*i*) animals are cultured in microchambers as they produce progeny which (*ii*) are passed through a counting region where they are automatically counted. Panel adapted with permission from Reference 92. Copyright 2015, Royal Society of Chemistry. Abbreviations: CCD, charge-coupled device; LED, light-emitting diode.

dendritic self-avoidance, retraction, and regrowth could be readily observed. This would have been difficult otherwise, as other techniques do not necessarily allow for periodic immobilization with stereotyped orientation each time the worm is restrained. An important step in this PVD neurite tracking analysis was the deconvolution of the three-dimensional (3D) image stack and subsequent straightening of circularly oriented features, which are done with machine learning estimation and classification algorithms. The integration of these algorithmic techniques with a stereotyped immobilization design allowed the authors to capture 3D time series of PVD neurite formation with a level of detail unmatched by other microfluidic platforms.

As seen in these reports, long-term physiological tracking of *C. elegans* development requires engineered solutions in both experimental design and data analysis. It should be noted that none of the systems discussed here altered development of the animal in any significant way, thus validating the use of these systems to obtain the results presented. A potential future direction to pursue using these systems would be to explore the behavioral and physiological consequences of intentionally perturbed development. Another general challenge in the field is the validation of a technique that could quantitatively study individual worms completely from initial embryonic development, through the larval stages, and across the adult life span. Arguably, the platforms presented here could perhaps be adapted for such a task, but no technique has thus far demonstrated the ability to monitor the worm at every stage with high temporal precision. Such a system would provide incredible insights into the developmental predictors for life span and aging.

Microfluidic Devices Can Enable Studies of Individual Animals During Life Span Assays but Require Standardization

Life span assays are crucial for studying the dynamics of aging. As mentioned previously, these assays are particularly useful in *C. elegans* because of its relatively short life span (66). Recent standardized protocols report that adult wild-type worms maintained on solid or liquid media live for approximately 21 days at 20°C (74, 75). (Note: The L4 stage is used as the day 0 reference point for the life span measurements reported in this section.) However, genetic and environmental conditions, minute differences in experimental protocol used, and trial-to-trial variability can significantly affect worm life span (74–78). These issues do not necessarily vanish for microfluidics-based life span assays. For example, Hulme et al. (79) were the first to report a device that could

culture individual adult animals for multiple days for life span assays. Their design utilized a combination of a microchamber array for culturing the animals and an adjoining immobilization channel for scoring their body features. However, the average life span of animals in their device was only 10 days at 24°C, and control experiments of similarly conditioned off-chip life span assays showed a mean life span of 12 days. The authors hinted that unquantifiable aspects of the worm environment might be responsible for these differences and showed that important aging metrics could nevertheless be extracted from on-chip life span assays.

More recently, alternative designs for microfluidics-based longevity studies have been reported and have demonstrated the advantages that such designs can provide over conventional methods. Dong et al. (80) reported a dual-chamber device that could be used to simultaneously culture two separate populations of worms from the L1 larval stage to adulthood and across the adult life span. In their design, worm feeding was done manually by periodically adding liquid bacterial cultures through pipette-connected inlets on each chamber. They could culture wild-type animals for a mean life span of 13 days post-L4 at 20°C on-chip. However, the main advantage of their platform was the ability to selectively exchange media between the two culture chambers. The authors leveraged this ability to recapitulate the effects of male-secreted compounds on wild-type hermaphrodites, previously detailed by Maures et al. (81). Dong et al. (80) separately cultured males and hermaphrodites on-chip and periodically exposed the hermaphrodites to media from the male culture chamber. The authors confirmed that hermaphrodites conditioned to this media had shorter life spans, showed signs of accelerated aging, and had altered body morphologies.

In a separate study, Wen et al. (82) describe a circular multilayer device featuring radial arrays for long-term culturing and imaging of individual worms in microchambers. In this design, a control layer underneath the culture chambers can be pneumatically activated, pushing a deformable PDMS membrane onto the animals and pressing them against the top of each microchamber for immobilization during fluorescent imaging. The most notable aspect of this platform was the demonstration of temporally controlled delivery of toxic copper ions to the animals, which has been shown to induce oxidative stress and shorten the life span of *C. elegans* (83). The authors used this stimulus to explore the protective effects of polydatin, a compound with known antioxidative properties. In this study and a follow-up study, the researchers probed the antiaging effects of polydatin and found that it could increase the life span of worms in both oxidative stress and normal conditions (82, 84). They showed that polydatin could extend the mean life span of animals in their device from 14 days to 19 days at 20°C, with a maximum life span of 35 days in the polydatin-treated group (84).

In a separate study, Xian et al. (85) developed WormFarm, a scalable microfluidics platform for assaying life span and healthspan metrics of *C. elegans* at the population level. Their system contained parallel microfluidic arenas and could separately control the food or genetic conditions in each device. They validated that their on-chip food and genetic conditions that altered life span mirrored results from NGM plate controls. Moreover, they used automated image processing of both brightfield and fluorescent movie captures to quantify additional life span and healthspan traits on-chip, such as worm width, motility, and fluorescent intensity. Overall, they demonstrated the mean life span in both their on- and off-chip assays control animals for roughly 10 days at 20°C and 16 days for their gene knockdown experiments.

Although these microfluidic devices have recapitulated results of earlier studies and have demonstrated their applicability for use in life span–based drug screens, high-throughput life span assay techniques have been developed using electrical and chemical engineering methods. The most notable are the Lifespan Machine by Stroustrup et al. (86), the high-density individual culture device proposed by Zhang et al. (87), and the WorMotel by Churgin et al. (88). The Lifespan Machine describes an adapted flatbed scanner setup on which conventional worm plates are

placed and periodically imaged (86). Although this system does allow for automated tracking of many simultaneous plate-based life span assays, it is significantly larger than other technologies and only acquires low-resolution images. Nevertheless, a follow-up study using this platform revealed that, in the presence of environmental and genetic perturbations, aging effects are temporally scaled to shortened and extended life spans (89).

Zhang et al. (87) used quantitative healthspan metrics to suggest a different perspective on aging. However, to conduct life span assays that allowed for high-content analysis of these metrics, the authors had to develop a new hydrogel-based parallel culturing device. This hydrogel device consisted of an array of roughly 100 individualized bacterial lawns in which worms were cultured long term. The platform was irreversibly sealed after wild-type worms were deposited into the lawns. The compact footprint of the device allowed the authors to capture videos of short- and long-lived wild-type animals. They found that the long-lived animals do not necessarily have better healthspan traits throughout their life span, indicating an extended period of poor health, or twilight, for older worms. A more recent study by Churgin et al. (88) also sought to develop a microfabricated device that can be similarly used for large-scale parallel life span assays, but it was not limited in ability to interact with worms. The authors developed the WorMotel, a specially patterned microwell array that can simultaneously culture and track up to 240 animals in parallel and is not irreversibly sealed, providing greater throughput and worm accessibility than other devices. One important result was that extended twilight can be also be observed in long life span mutants, providing further evidence against a temporal scaling model of aging.

It is clear that microfluidics-based long-term life span assays in *C. elegans* have room for improvement and have not yet matched the capabilities of the existing platforms described above. However, one advantage that microfluidic technologies can provide over other engineered solutions is the ability to actively and dynamically manipulate the worm microenvironment. For example, the three aforementioned passive technologies could not temporally adjust food or chemical availability over the lifetime of the animal. Another issue for device-based life span techniques is the challenge of standardization and repeatability. An important validation step for new microfluidic-based life span devices should be controls that show life span curves comparable to the standardized protocols (74, 75). Furthermore, if there are still differences in life span between techniques, then new devices should seek to precisely identify aspects of the microenvironment or device protocol that may cause these differences. Finally, new microfluidic devices should demonstrate robustness by characterizing trial-to-trial variability.

The significance of these issues for even simple plate-based life span studies has recently been highlighted by Lucanic et al. (78). These authors conducted a study to trace the sources of variability in life span studies and found that minute, seemingly inconsequential protocol details can significantly affect assay outcomes (77). Extensive effort was required by the three separate labs involved in this study to standardize the conventional life span protocols in *C. elegans*. However, even after standardization procedures, the authors found significant variability in the outcomes of the same life span assay, conducted in subsequent identical conditions, just months apart. Therefore, future microfluidics-based life span assay technologies must take special care to ensure that protocols can be standardized and that results can be reproduced in the presence of experimenter and batch variability.

On-Chip Animal Manipulation Provides Novel Techniques for Quantifying Age-Dependent Healthspan Metrics

An alternative approach to long-term life span studies on-chip are techniques that culture worms using conventional methods but characterize healthspan metrics using microfluidic devices. For

example, Bazopoulou et al. (46) described a fully automated system for characterizing the age-dependent deterioration of ASH neuronal activity in *C. elegans*. They developed a system in which worms were synchronized and grown in liquid cultures and subsequently moved to conventional multiwell plates. When imaging at a particular age was desired, a liquid-handling robotic system, capable of pipetting and pneumatically driving liquids, would move groups of animals from the wells into an adapted olfactory device, as mentioned previously, which was used to serially study each individual animal. In this study, the device was used to stimulate GCaMP-labeled ASH neurons and characterize the subsequent calcium response. This automated system allowed the authors to track the deterioration of ASH activity as the population of animals aged. Furthermore, they discovered that mutations and compounds that increased life span do not necessarily delay the onset of this deterioration. Finally, the researchers used their system to perform a large-scale drug screen of a library of US Food and Drug Administration (FDA)-approved compounds and found that tiagabine, an antiepileptic drug, enhanced ASH response in older animals. This study is an example of how integrating robust device design with deliberately engineered platforms can provide a new analytical tool to characterize aging.

Another important healthspan attribute studied in *C. elegans* is aging of the reproductive system. Wild-type animals typically lay self-fertilized eggs from the onset of adulthood for 3–8 days afterward, until available sperm are exhausted or oocyte quality declines (90, 91). Li et al. (92) recently designed a device to automatically assay age-related reproductive decline in *C. elegans*. Their device can culture up to 16 gravid adult worms in microchambers as they lay eggs throughout their reproductive life span. As the embryos hatch, the resulting L1s are automatically filtered out of each culture chamber and pass by a counting region. In this region, brightfield microscopy and image processing are used to count the number of progeny from each parent. The software does so by correlating the change in standard deviation of the intensity in images of the counting channels with the presence of a new animal. Thus, this system automatically quantifies the number and frequency of progeny laid by multiple animals in parallel. This platform is notable because it provides a new analytical tool for tracking reproductive life span and progeny profiles of *C. elegans* and significantly increases the efficiency of these conventionally time-consuming assays.

These publications further illustrate the unique role that integrated microfluidic tools can play in studying age-related physiological changes. Not only can they be used to quantify physiology when conventional methods prove impractical or labor intensive, but they can also provide new analytical techniques with which to study worm healthspan. Combining these tools with off-chip life span assays has shown that they can provide important insights into the dynamics of aging. Similarly, we expect that such techniques could be integrated with on-chip life span assays to increase the content value of these studies. Follow-up studies with the progeny-counting device could explore how drug treatments and chemical stimuli increase the reproductive window of *C. elegans*, as this could have potential implications for delaying the symptoms of reproductive aging in humans.

ANATOMICAL AND PHYSIOLOGICAL ASSAYS

As we have demonstrated throughout this review, the stereotypical anatomical and physiological features of *C. elegans* are often used as screening criteria for sensory, developmental, and aging assays. In this section, we aim to highlight specific studies that demonstrated the extraction of highly quantitative information from anatomical assays and studied the genetic components involved therein. We also discuss a few notable microfluidics tools that further extend the capabilities of on-chip physiological investigations of *C. elegans*. Finally, we discuss how these techniques

enable the discovery of both novel worm biology and potential therapeutic targets for human diseases.

High-Content Phenotyping Enabled by Microfluidics Predicts Relationships Between Genetic Components in Anatomical Assays

A fundamental challenge in biology is understanding how genotype influences phenotype. Quantitative phenotyping can help elucidate this relationship. Recently, San-Miguel et al. (93) and Hwang et al. (95) demonstrated two integrated microfluidic techniques to quantitatively phenotype neuronal and muscular substructures in *C. elegans*, respectively. San-Miguel et al. demonstrated a deep phenotyping approach to assaying subtle differences in the synaptic morphology of *C. elegans*. First, the researchers developed an image processing pipeline to extract a number of heuristic and statistical features from fluorescent images of the synaptic domain of the DA9 motor neuron. Next, they implemented a stepwise logistic regression classifier that allowed them to use these features to discern minute differences between the synaptic domains of wild-type and mutant animals. The authors then integrated this classifier with a microfluidics-based automated screening platform, previously developed by Chung et al. (12) for rapid phenotyping and screening (**Figure 3*a***). This allowed them to identify new mutants with altered synaptic domain morphology. These results were validated by a behavioral assay on a particular mutant that had visually imperceptible synaptic domain changes yet showed a significant time-dependent reduction in swimming ability. Additionally, hierarchical clustering of the extracted features corroborated genetic relationships among previously identified mutations and predicted new relationships for mutants isolated from the synaptic morphology-based genetic screen.

Stirman et al. (94) described a technique for trapping and arraying worms in a microfluidic device while light is used to optogenetically stimulate their body wall muscles. Following stimulation and imaging, image processing algorithms segment the worm body to extract quantitative features that describe the kinetics of muscle contraction and relaxation. In a recent follow-up study by Hwang et al. (95), researchers assayed wild-type and mutant animals with known sarcomere defects to characterize the effects of genetic changes on muscle dynamics (**Figure 3*b***). These authors demonstrated that they could extract high-content features from the on-chip optogenetic assay. Additionally, hierarchical clustering of parameters obtained from the study produced groups of genes and proteins that were known to alter sarcomere structure or function in similar ways. Furthermore, they demonstrated that conventional crawling and swimming assays could not reveal these patterns, potentially because of the animals' ability to compensate for their mutations by altering their locomotion patterns. These results validated that on-chip quantitative phenotyping combined with hierarchical clustering can reveal the relationships of different genetic components influencing anatomy and physiology, even when conventional techniques fail to do so.

These studies demonstrate that high-content phenotyping can greatly enhance microfluidics-based *C. elegans* studies. As such, researchers in the field should take full advantage of statistical learning techniques, such as hierarchical clustering and logistic regression, to discover relationships among genetic components of physiological and anatomical structures. It should be noted that these techniques do not necessarily need complicated microfluidic designs. In both of the studies mentioned here, for example, the devices used were progressive iterations of earlier robust designs (12, 94). Therefore, these studies suggest that the advancing quantitative phenotyping techniques can greatly increase the capabilities of older microfluidics designs. Additionally, the success of these results, and of those mentioned in previous sections, demonstrates the utility of high-throughput genetic screens based on quantitative functional and fluorescent markers.

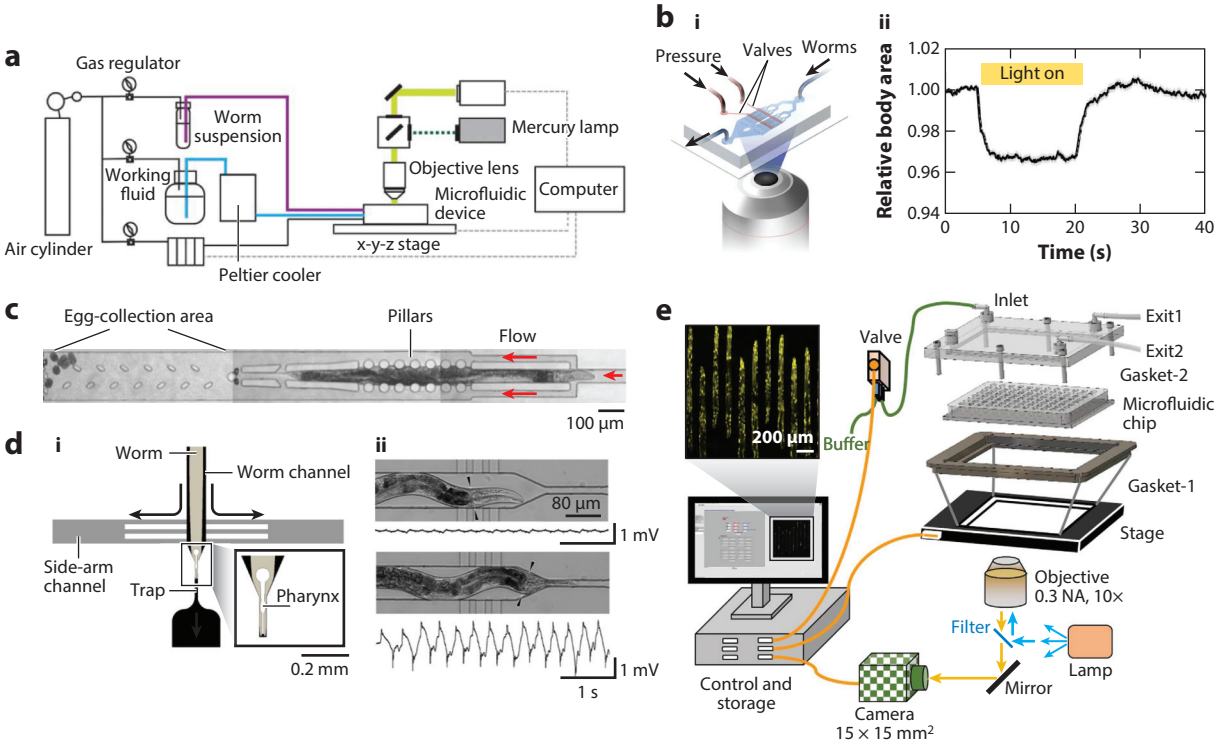


Figure 3

System and device designs for anatomical and physiological assays. (*a*) An automated sorting platform for anatomical screening first developed by Chung et al. (12) and adapted by San-Miguel et al. (93) for deep phenotyping. Panel components adapted with permission from K. Chung and H. Lu. (*b*, *i*) A microfluidic-based platform for optogenetically stimulating worm body wall muscle, first described by Stirman et al. (94) and later adapted by Hwang et al. (95). (*b*, *ii*) Quantitative tracking of animal size after stimulation can reveal dynamics of muscle contraction and relaxation. Panel components adapted with permission from J. Stirman, H. Hwang, and H. Lu. (*c*) Micrograph of a worm in the WormSpa device, which allows for long-term high-resolution imaging of the animal without significant stress or changes in physiology. Panel adapted with permission from Reference 96. Copyright 2014, Royal Society of Chemistry. (*d*, *i*) A device developed by Lockery et al. (100) for measuring the electrophysiology of the worm pharyngeal neuromuscular network, called the electropharyngeogram (EPG). Side arms also allow for delivery of chemicals during EPG recordings. (*d*, *ii*) Recordings are captured as worms are pushed against a head-trapping channel that is connected to an external electrode. Panel adapted with permission from Reference 100. Copyright 2012, Royal Society of Chemistry. (*e*) Overview of the platform used by Mondal et al. (103) for large-scale study of protein aggregation in *C. elegans*. The image outset from the computer shows a representative fluorescent image of protein aggregation in diseased worms immobilized in parallel channels. Panel adapted with permission from Reference 103 under the terms of the Creative Commons Attribution 4.0 International License, **https://creativecommons.org/licenses/by/4.0**.

Advanced Physiological Assays Provide Insights into Worm Biology and Human Diseases

Many of the devices discussed earlier have the capacity to track physiological changes in *C. elegans*. However, recent analytical devices have advanced the capabilities of on-chip physiological investigations. In one such study, Kopito et al. (96) developed the WormSpa, a device that allows the presentation of different environmental cues to individual worms without applying significant stress to the animal (**Figure 3*c***). The major novelty of this design is its ability to immobilize individual worms for extended periods of time (more than 24 h) for high-resolution imaging in a way that has been shown to not alter the physiology or life span of the animals.

This allows for probing of stress responses of the animal without the background stress conditions that other immobilization techniques can induce. The applicability of this modular design for integrated microfluidics platforms was demonstrated in a follow-up study by Lee et al. (97), in which they used the WormSpa to perform long-term time-lapse imaging of pharyngeal pumping. They developed an automated image processing algorithm that tracked individual animals' grinder movements frame-by-frame and quantified their dynamics. They used this process to assay pharyngeal pumping in response to varying food conditions. From the subsequent analysis, they found that pumping dynamics depend on the availability of bacteria in the environment and can have distinct modes, such as feeding bursts. Moreover, experiments using strains with mutations in serotonin receptors and pathways showed that this neurotransmitter plays an essential role in the induction of these feeding bursts.

Nearly all of the aforementioned assay techniques rely on the optical inspection of physiological features. However, electrophysiological techniques have also been used for in vivo characterization of *C. elegans* neuromuscular activity. For example, the electropharyngeogram (EPG) is an extracellular recording of the electrical activity of the *C. elegans* pharyngeal neural network that has been used to characterize its electrophysiology (98). However, obtaining this measurement conventionally is very tedious and does not lend itself well to large-scale assaying techniques (99). However, recently developed microfluidic devices enable the high-throughput and rapid measurements of EPG signals (100, 101). Lockery et al. (100) demonstrated a design (**Figure 3*d***) that could record EPG signals on-chip by trapping the worm head in an electrode-connected recording channel. In the device design, the worm is first injected into an inlet port with saline solution, which is required for EPG recordings. Hydrodynamic flow pushes the worm into the recording channel until its head is pushed into a constriction. An off-chip electrode is then used to record the EPG of the constricted channel. The authors demonstrated that they could assay up to eight animals in parallel and that this design could be used for simultaneous delivery of chemical stimuli that affect EPG activity. A robust implementation of this idea was explored by Hu et al. (101), who unveiled the NeuroChip. This device allowed for selective recovery of the animals after measuring the EPG recordings, enabling its use for genetic screens in addition to novel drug screens. Additionally, the authors showed that their design allowed them to capture higher-resolution EPG signals and could be adapted for use with both adult and larval animals. The development of these devices provides a unique on-chip analytical tool for electrophysiological studies.

In addition to providing insights into worm biology, *C. elegans* physiological assays have also proven to be powerful tools for understanding the biological underpinnings of human diseases (102). Microfluidics-based physiological assays can significantly enhance and enable such studies. As discussed previously, for example, Cornaglia et al. (71) designed a platform to longitudinally study protein aggregation in ALS model worms. This study led to the discovery of the potential neuroprotective effects of the commonly used antibiotic doxycycline. In another study, Mondal et al. (103) developed a system (**Figure 3*e***) that allowed them to probe *C. elegans* polyglutamine (PolyQ) aggregation, a model relevant to Huntington's disease, with remarkably high throughput. Their design consisted of a 96-well plate–compatible microchip with 40 straight traps per well to immobilize and image young adult animals. Hulme et al. (104) previously demonstrated the utility of such traps, and Lee et al. (105) recently demonstrated resistance arrays for long-term monitoring of cellular and subcellular features in *C. elegans*. Nevertheless, the unique integration of these traps with a novel 96-well device architecture allowed Cornaglia and coworkers to rapidly screen thousands of animals in parallel per run, for a total of nearly 100,000 animals screened over the course of their experiments. Additionally, they utilized an external stage and custom software to automate the capture and analysis of the thousands of high-resolution fluorescent Z-stacks per device. After validating their platform by testing the effects of previously studied compounds on

the PolyQ pathway, they performed a drug screen using an FDA-approved library to identify new potential therapeutics for protein aggregation. The study validated that four compounds in the library significantly reduced PolyQ aggregation in *C. elegans* and showed that new physiological assay tools in this field can provide novel insights into both worm biology and human diseases.

In summary, recent microfluidic devices have enabled the study of both nuanced physiological features and neuromuscular electrophysiology. Furthermore, these studies demonstrated that microfluidics-enabled studies in *C. elegans* need not aim only at uncovering novel worm biology, as it has long been understood that these results can have strong implications for human genetics and medicine (102). In future studies, we envision the additional integration of different microfluidic device designs to both increase throughput and provide new analytical tools. For example, coupling EPG recordings with functional calcium brain imaging might reveal additional insights about the pharyngeal neural network.

CONCLUSIONS

The field of microfluidics-based worm assays has matured significantly since its conception. Major signs of this progress include the integration of advanced quantitative techniques with microfluidic devices and designs, as well as the discovery of novel biology in *C. elegans*. It is thus likely that the best method for developing integrated microfluidic technologies going forward is to design these systems with the goal of solving conventionally challenging and lasting biological questions.

The maturation of this field should not be confused with stagnation, however. There is still room for a significant amount of growth in integrated microfluidic technologies that probe *C. elegans* biology. For example, for sensory system assays, there is a need for technologies that can study the polymodal nature of sensory integration. Additionally, no such platform exists for tracking sensory response and integration for each developmental stage and into adulthood for individual animals. Furthermore, there remains a standing challenge to develop a complete microfluidic system that can reliably track and probe animals from the initial embryonic stage, through larval development, and throughout the adult life span.

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LITERATURE CITED

1. Whitesides GM. 2006. The origins and the future of microfluidics. *Nature* 442:368–73
2. Duffy DC, McDonald JC, Schueller OJA, Whitesides GM. 1998. Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anal. Chem.* 70:4974–84
3. McDonald JC, Duffy DC, Anderson JR, Chiu DT, Wu HK, et al. 2000. Fabrication of microfluidic systems in poly(dimethylsiloxane). *Electrophoresis* 21:27–40

4. Qin D, Xia Y, Whitesides GM. 2010. Soft lithography for micro- and nanoscale patterning. *Nat. Protoc.* 5:491–502
5. Beebe DJ, Mensing GA, Walker GM. 2002. Physics and applications of microfluidics in biology. *Annu. Rev. Biomed. Eng.* 4:261–86
6. Sackmann EK, Fulton AL, Beebe DJ. 2014. The present and future role of microfluidics in biomedical research. *Nature* 507:181–89
7. San-Miguel A, Lu H. 2013. Microfluidics as a tool for *C. elegans* research. In *WormBook*, ed. The *C. elegans* Research Community. WormBook. **http://www.wormbook.org**
8. Corsi AK, Wightman B, Chalfie M. 2015. A transparent window into biology: a primer on *Caenorhabditis elegans*. In *WormBook*, ed. The *C. elegans* Research Community. WormBook. **http://www.wormbook.org**
9. Brenner S. 1974. The genetics of *Caenorhabditis elegans. Genetics* 77:71–94
10. Riddle DL, Blumenthal T, Meyer BJ, Priess JR. 1997. Introduction to *C. elegans*. In C. elegans *II*, ed. DL Riddle, T Blumenthal, BJ Meyer, pp. 1–23. Cold Spring Harbor, NY: JR Priess
11. Stiernagle T. 2006. Maintenance of *C. elegans*. In *WormBook*, ed. The *C. elegans* Research Community. WormBook. **http://www.wormbook.org**
12. Chung K, Crane MM, Lu H. 2008. Automated on-chip rapid microscopy, phenotyping and sorting of *C. elegans. Nat. Methods* 5:637–43
13. Chronis N, Zimmer M, Bargmann CI. 2007. Microfluidics for *in vivo* imaging of neuronal and behavioral activity in *Caenorhabditis elegans. Nat. Methods* 4:727–31
14. Zhang Y, Lu H, Bargmann CI. 2005. Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans. Nature* 438:179–84
15. Hulme SE, Shevkoplyas SS, Samuel A. 2008. Microfluidics: streamlining discovery in worm biology. *Nat. Methods* 5:589–90
16. Chronis N. 2010. Worm chips: microtools for *C. elegans* biology. *Lab Chip* 10:432–37
17. Bakhtina NA, Korvink JG. 2014. Microfluidic laboratories for *C. elegans* enhance fundamental studies in biology. *RSC Adv*. 4:4691–709
18. Lockery SR, Lawton KJ, Doll JC, Faumont S, Coulthard SM, et al. 2008. Artificial dirt: microfluidic substrates for nematode neurobiology and behavior. *J. Neurophysiol.* 99:3136–43
19. Crane MM, Chung K, Stirman J, Lu H. 2010. Microfluidics-enabled phenotyping, imaging, and screening of multicellular organisms. *Lab Chip* 10:1509–17
20. McCormick KE, Gaertner BE, Sottile M, Phillips PC, Lockery SR. 2011. Microfluidic devices for analysis of spatial orientation behaviors in semi-restrained *Caenorhabditis elegans. PLOS ONE* 6:e25710
21. Hwang H, Lu H. 2013. Microfluidic tools for developmental studies of small model organisms—nematodes, fruit flies, and zebrafish. *Biotechnol. J.* 8:192–205
22. Aubry G, Lu H. 2014. A perspective on optical developments in microfluidic platforms for *Caenorhabditis elegans* research. *Biomicrofluidics* 8:011301
23. O'Reilly LP, Luke CJ, Perlmutter DH, Silverman GA, Pak SC. 2014. *C. elegans* in high-throughput drug discovery. *Adv. Drug Deliv. Rev.* 69–70:247–53
24. Levario TJ, Lim B, Shvartsman SY, Lu H. 2016. Microfluidics for high-throughput quantitative studies of early development. *Annu. Rev. Biomed. Eng.* 18:285–309
25. Porto DA, Rouse TM, San-Miguel A, Lu H. 2016. *Microfluidic Platforms for Quantitative Biology Studies in Model Organisms*, ed. C Lu, SS Verbridge, pp. 1–18. Cham, Switz.: Springer Intl.
26. Gupta B, Rezai P. 2016. Microfluidic approaches for manipulating, imaging, and screening *C. elegans. Micromachines* 7:123–23
27. Shanmugam MM, Santra TS. 2016. Microfluidic devices in advanced *Caenorhabditis elegans* research. *Molecules* 21:1006
28. Cornaglia M, Lehnert T, Gijs MAM. 2017. Microfluidic systems for high-throughput and high-content screening using the nematode *Caenorhabditis elegans. Lab Chip* 22:3736–59
29. Erickson D, Li DQ. 2004. Integrated microfluidic devices. *Anal. Chim. Acta* 507:11–26
30. Kuswandi B, Nuriman, Huskens J, Verboom W. 2007. Optical sensing systems for microfluidic devices: a review. *Anal. Chim. Acta* 601:141–55

31. Megason SG, Fraser SE. 2007. Imaging in systems biology. *Cell* 130:784–95
32. Tarca AL, Carey VJ, Chen XW, Romero R, Draghici S. 2007. Machine learning and its applications to biology. *PLOS Comput. Biol.* 3:e116
33. Danuser G. 2011. Computer vision in cell biology. *Cell* 147:973–78
34. Wu J, Zheng G, Lee LM. 2012. Optical imaging techniques in microfluidics and their applications. *Lab Chip* 12:3566–75
35. Choi JR, Song H, Sung JH, Kim D, Kim K. 2016. Microfluidic assay-based optical measurement techniques for cell analysis: a review of recent progress. *Biosens. Bioelectron.* 77:227–36
36. White JG, Southgate E, Thomson JN, Brenner S. 1986. The structure of the nervous system of the nematode *Caenorhabditis elegans. Philos. Trans. R. Soc. B* 314:1–340
37. Emmons SW. 2015. The beginning of connectomics: a commentary on White et al. 1986 'The structure of the nervous system of the nematode *Caenorhabditis elegans*'. *Philos. Trans. R. Soc. B* 370:20140309
38. Kerr R, Lev-Ram V, Baird G, Vincent P, Tsien RY, Schafer WR. 2000. Optical imaging of calcium transients in neurons and pharyngeal muscle of *C. elegans. Neuron* 26:583–94
39. Tian L, Hires SA, Mao T, Huber D, Chiappe ME, et al. 2009. Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. *Nat. Methods* 6:875–81
40. Akerboom J, Chen TW, Wardill TJ, Tian L, Marvin JS, et al. 2012. Optimization of a GCaMP calcium indicator for neural activity imaging. *J. Neurosci.* 32:13819–40
41. Bargmann CI. 2006. Chemosensation in *C. elegans*. In *WormBook*, ed. The *C. elegans* Research Community. WormBook. **http://www.wormbook.org**
42. Goodman MB. 2006. Mechanosensation. In *WormBook*, ed. The *C. elegans* Research Community. WormBook. **http://www.wormbook.org**
43. Chalasani SH, Chronis N, Tsunozaki M, Gray JM, Ramot D, et al. 2007. Dissecting a circuit for olfactory behaviour in *Caenorhabditis elegans. Nature* 450:63–70
44. Chokshi TV, Bazopoulou D, Chronis N. 2010. An automated microfluidic platform for calcium imaging of chemosensory neurons in *Caenorhabditis elegans. Lab Chip* 10:2758–63
45. Kato S, Xu Y, Cho CE, Abbott LF, Bargmann CI. 2014. Temporal responses of *C. elegans* chemosensory neurons are preserved in behavioral dynamics. *Neuron* 81:616–28
46. Bazopoulou D, Chaudhury AR, Pantazis A, Chronis N. 2017. An automated compound screening for anti-aging effects on the function of *C. elegans* sensory neurons. *Sci. Rep.* 7:9403
47. Albrecht DR, Bargmann CI. 2011. High-content behavioral analysis of *Caenorhabditis elegans* in precise spatiotemporal chemical environments. *Nat. Methods* 8:599–605
48. Larsch J, Ventimiglia D, Bargmann CI, Albrecht DR. 2013. High-throughput imaging of neuronal activity in *Caenorhabditis elegans. PNAS* 110:E4266–73
49. Larsch J, Flavell SW, Liu Q, Gordus A, Albrecht DR, Bargmann CI. 2015. A circuit for gradient climbing in *C. elegans* chemotaxis. *Cell Rep.* 12:1748–60
50. Zimmer M, Gray JM, Pokala N, Chang AJ, Karow DS, et al. 2009. Neurons detect increases and decreases in oxygen levels using distinct guanylate cyclases. *Neuron* 61:865–79
51. Schrödel T, Prevedel R, Aumayr K, Zimmer M, Vaziri A. 2013. Brain-wide 3D imaging of neuronal activity in *Caenorhabditis elegans* with sculpted light. *Nat. Methods* 10:1013–20
52. Kato S, Kaplan HS, Schrödel T, Skora S, Lindsay TH, et al. 2015. Global brain dynamics embed the motor command sequence of *Caenorhabditis elegans. Cell* 163:656–69
53. Nichols ALA, Eichler T, Latham R, Zimmer M. 2017. A global brain state underlies *C. elegans* sleep behavior. *Science* 356:eeam6851
54. Sulston J, Dew M, Brenner S. 1975. Dopaminergic neurons in the nematode *Caenorhabditis elegans. J. Comp. Neurol.* 163:215–26
55. Chalfie M, Sulston J. 1981. Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans. Dev. Biol.* 82:358–70
56. Chalfie M, Sulston JE, White JG, Southgate E, Thomson JN, Brenner S. 1985. The neural circuit for touch sensitivity in *Caenorhabditis elegans. J. Neurosci.* 5:956–64
57. Way JC, Chalfie M. 1989. The *mec-3* gene of *Caenorhabditis elegans* requires its own product for maintained expression and is expressed in three neuronal cell types. *Genes Dev.* 3:1823–33

58. Li W, Kang L, Piggott BJ, Feng Z, Xu XZ. 2011. The neural circuits and sensory channels mediating harsh touch sensation in *Caenorhabditis elegans*. *Nat. Commun*. 2:315
59. Chalfie M, Sulston J. 1981. Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. *Dev. Biol*. 82:358–70
60. Chalfie M, Hart AC, Rankin CH, Goodman MB. 2014. Assaying mechanosensation. In *WormBook*, ed. The *C. elegans* Research Community. WormBook. **http://www.wormbook.org**
61. Park SJ, Goodman MB, Pruitt BL. 2007. Analysis of nematode mechanics by piezoresistive displacement clamp. *PNAS* 104:17376–81
62. Park SJ, Petzold BC, Goodman MB, Pruitt BL. 2011. Piezoresistive cantilever force-clamp system. *Rev. Sci. Instrum.* 82:043703
63. Petzold BC, Park SJ, Mazzochette EA, Goodman MB, Pruitt BL. 2013. MEMS-based force-clamp analysis of the role of body stiffness in *C. elegans* touch sensation. *Integr. Biol.* 5:853–64
64. Cho Y, Porto DA, Hwang H, Grundy LJ, Schafer WR, Lu H. 2017. Automated and controlled mechanical stimulation and functional imaging *in vivo* in *C. elegans*. *Lab Chip* 17:2609–18
65. Nekimken AL, Fehlauer H, Kim AA, Manosalvas-Kjono SN, Ladpli P, et al. 2017. Pneumatic stimulation of *C. elegans* mechanoreceptor neurons in a microfluidic trap. *Lab Chip* 17:1116–27
66. Collins JJ, Huang C, Hughes S, Kornfeld K. 2008. The measurement and analysis of age-related changes in *Caenorhabditis elegans*. In *WormBook*, ed. The *C. elegans* Research Community. WormBook. **http://www.wormbook.org**
67. Krajniak J, Lu H. 2010. Long-term high-resolution and culture of *C. elegans* in chip-gel hybrid microfluidic device for developmental studies. *Lab Chip* 10:1862–68
68. Chung K, Kim Y, Kanodia JS, Gong E, Shvartsman SY, Lu H. 2011. A microfluidic array for large-scale ordering and orientation of embryos. *Nat. Methods* 8:171–76
69. Cornaglia M, Mouchiroud L, Marette A, Narasimhan S, Lehnert T, et al. 2015. An automated microfluidic platform for *C. elegans* embryo arraying, phenotyping, and long-term live imaging. *Sci. Rep.* 5:10192
70. Uppaluri S, Brangwynne CP. 2015. A size threshold governs *Caenorhabditis elegans* developmental progression. *Proc. R. Soc. B* 282:20151283
71. Cornaglia M, Krishnamani G, Mouchiroud L, Sorrentino V, Lehnert T, et al. 2016. Automated longitudinal monitoring of *in vivo* protein aggregation in neurodegenerative disease *C. elegans* models. *Mol. Neurodegener.* 11:17
72. Gritti N, Kienle S, Filina O, van Zon JS. 2016. Long-term time-lapse microscopy of *C. elegans* postembryonic development. *Nat. Commun.* 7:12500
73. Keil W, Kutscher LM, Shaham S, Siggia ED, Cooper RC, et al. 2017. Long-term high-resolution imaging of developing *C. elegans* larvae with microfluidics. *Dev. Cell* 40:202–14
74. Sutphin GL, Kaeberlein M. 2009. Measuring *Caenorhabditis elegans* life span on solid media. *J. Vis. Exp.* 27:1152
75. Solis GM, Petrascheck M. 2011. Measuring *Caenorhabditis elegans* life span in 96 well microtiter plates. *J. Vis. Exp.* 49:2496
76. Vanfleteren JR, Braeckman BP. 1999. Mechanisms of life span determination in *Caenorhabditis elegans*. *Neurobiol. Aging* 20:487–502
77. Lithgow GJ, Driscoll M, Phillips P. 2017. A long journey to reproducible results. *Nature* 548:387–88
78. Lucanic M, Plummer WT, Chen E, Harke J, Foulger AC, et al. 2017. Impact of genetic background and experimental reproducibility on identifying chemical compounds with robust longevity effects. *Nat. Commun.* 8:14256
79. Hulme SE, Shevkoplyas SS, McGuigan AP, Apfeld J, Fontana W, Whitesides GM. 2010. Life span-on-a-chip: microfluidic chambers for performing lifelong observation of *C. elegans*. *Lab Chip* 10:589–97
80. Dong L, Cornaglia M, Lehnert T, Gijs MAM. 2016. On-chip microfluidic biocommunication assay for studying male-induced demise in *C. elegans* hermaphrodites. *Lab Chip* 16:4534–45
81. Maures TJ, Booth LN, Benayoun BA, Izrayelit Y, Schroeder FC, Brunet A. 2014. Males shorten the life span of *C. elegans* hermaphrodites via secreted compounds. *Science* 343:541–44
82. Wen H, Shi W, Qin J. 2012. Multiparameter evaluation of the longevity in *C. elegans* under stress using an integrated microfluidic device. *Biomed. Microdevices* 14:721–28

83. Harada H, Kurauchi M, Hayashi R, Eki T. 2007. Shortened life span of nematode *Caenorhabditis elegans* after prolonged exposure to heavy metals and detergents. *Ecotoxicol. Environ. Safety* 66:378–83
84. Wen H, Gao X, Qin J. 2014. Probing the anti-aging role of polydatin in *Caenorhabditis elegans* on a chip. *Integr. Biol.* 6:35–43
85. Xian B, Shen J, Chen W, Sun N, Qiao N, et al. 2013. WormFarm: a quantitative control and measurement device toward automated *Caenorhabditis elegans* aging analysis. *Aging Cell* 12:398–409
86. Stroustrup N, Ulmschneider BE, Nash ZM, Lopez-Moyado IF, Apfeld J, Fontana W. 2013. The *Caenorhabditis elegans* lifespan machine. *Nat. Methods* 10:665–70
87. Zhang WB, Sinha DB, Pittman WE, Hvatum E, Stroustrup N, Pincus Z. 2016. Extended twilight among isogenic *C. elegans* causes a disproportionate scaling between life span and health. *Cell Syst*. 3:333–45.e4
88. Churgin MA, Jung SK, Yu CC, Chen X, Raizen DM, Fang-Yen C. 2017. Longitudinal imaging of *Caenorhabditis elegans* in a microfabricated device reveals variation in behavioral decline during aging. *eLife* 6:e26652
89. Stroustrup N, Anthony WE, Nash ZM, Gowda V, Gomez A, et al. 2016. The temporal scaling of *Caenorhabditis elegans* ageing. *Nature* 530:103–7
90. Hughes SE, Evason K, Xiong C, Kornfeld K. 2007. Genetic and pharmacological factors that influence reproductive aging in nematodes. *PLOS Genet*. 3:e25
91. Luo S, Murphy CT. 2011. *Caenorhabditis elegans* reproductive aging: regulation and underlying mechanisms. *Genesis* 49:53–65
92. Li S, Stone HA, Murphy CT. 2015. A microfluidic device and automatic counting system for the study of *C. elegans* reproductive aging. *Lab Chip* 15:524–31
93. San-Miguel A, Kurshan PT, Crane MM, Zhao Y, McGrath PT, et al. 2016. Deep phenotyping unveils hidden traits and genetic relations in subtle mutants. *Nat. Commun.* 7:12990
94. Stirman JN, Brauner M, Gottschalk A, Lu H. 2010. High-throughput study of synaptic transmission at the neuromuscular junction enabled by optogenetics and microfluidics. *J. Neurosci. Methods* 191:90–93
95. Hwang H, Barnes DE, Matsunaga Y, Benian GM, Ono S, Lu H. 2016. Muscle contraction phenotypic analysis enabled by optogenetics reveals functional relationships of sarcomere components in *Caenorhabditis elegans*. *Sci. Rep*. 6:19900
96. Kopito RB, Levine E. 2014. Durable spatiotemporal surveillance of *Caenorhabditis elegans* response to environmental cues. *Lab Chip* 14:764–70
97. Lee KS, Iwanir S, Kopito RB, Scholz M, Calarco JA, et al. 2017. Serotonin-dependent kinetics of feeding bursts underlie a graded response to food availability in *C. elegans*. *Nat. Commun*. 8:14221
98. Raizen DM, Avery L. 1994. Electrical-activity and behavior in the pharynx of *Caenorhabditis elegans*. *Neuron* 12:483–95
99. Avery L, Raizen D, Lockery S. 1995. Electrophysiological methods. *Methods Cell Biol.* 48:251–69
100. Lockery SR, Hulme SE, Roberts WM, Robinson KJ, Laromaine A, et al. 2012. A microfluidic device for whole-animal drug screening using electrophysiological measures in the nematode *C. elegans*. *Lab Chip* 12:2211–20
101. Hu C, Dillon J, Kearn J, Murray C, O'Connor V, et al. 2013. NeuroChip: a microfluidic electrophysiological device for genetic and chemical biology screening of *Caenorhabditis elegans* adult and larvae. *PLOS ONE* 8:e64297
102. Markaki M, Tavernarakis N. 2010. Modeling human diseases in *Caenorhabditis elegans*. *Biotechnol. J.* 5:1261–76
103. Mondal S, Hegarty E, Martin C, Gökçe SK, Ghorashian N, Ben-Yakar A. 2016. Large-scale microfluidics providing high-resolution and high-throughput screening of *Caenorhabditis elegans* poly-glutamine aggregation model. *Nat. Commun.* 7:13023
104. Hulme SE, Shevkoplyas SS, Apfeld J, Fontana W, Whitesides GM. 2007. A microfabricated array of clamps for immobilizing and imaging *C. elegans*. *Lab Chip* 7:1515–23
105. Lee H, Kim SA, Coakley S, Mugno P, Hammarlund M, et al. 2014. A multi-channel device for high-density target-selective stimulation and long-term monitoring of cells and subcellular features in *C. elegans*. *Lab Chip* 14:4513–22

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Errata

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