Chapter 16

Microfluidic Devices for Behavioral Analysis, Microscopy, and Neuronal Imaging in *Caenorhabditis elegans*

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Abstract

Microfluidic devices offer several advantages for *C. elegans* research, particularly for presenting precise physical and chemical environments, immobilizing animals during imaging, quantifying behavior, and automating screens. However, challenges to their widespread adoption in the field include increased complexity over conventional methods, operational problems (such as clogging, leaks, and bubbles), difficulty in obtaining or fabricating devices, and the need to characterize biological results obtained from new assay formats. Here we describe the preparation and operation of simple, reusable microfluidic devices for quantifying behavioral responses to chemical patterns, and single-use devices to arrange animals for time-lapse microscopy and to measure neuronal activity. We focus on details that eliminate or reduce the frustrations commonly experienced by new users of microfluidic devices.

Key words Microfluidics, *Caenorhabditis elegans*, Quantitative behavior, Locomotion, Time-lapse microscopy, Neuronal imaging, PDMS, Chemical stimulation

1 Introduction

Microfluidics refers to plumbing at a small, submillimeter scale. Microfluidic-based technologies have found numerous applications in biology over the past decade, from miniaturization of molecular biology reactions to high-throughput analysis of cellular functions. Microfluidic devices offer the advantage of increased experimental productivity by collecting more data (conditions or repeats) in less time with fewer resources and lower costs. Further, predictable fluid physics at this length scale enables dynamic and reproducible control over the fluidic environment surrounding cells or small organisms, often increasing experimental reliability.

In *C. elegans* research, microfluidic devices have been particularly useful for presenting precise physical and chemical environments, immobilizing animals during imaging, quantifying

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behavior, and automating phenotypic screens. A wide variety of specific *C. elegans* microfluidic devices have been surveyed in several recent reviews (1–8). Microfluidic devices are generally described as "easy, fast, and inexpensive," especially when fabricated from elastomeric materials such as polydimethylsiloxane (PDMS). This description is technically true, but only if the user has prior experience with microfluidic devices, already has the equipment necessary for fabrication, and uses microfluidics often enough to reduce per-device costs by purchasing raw materials in bulk.

Microfluidic designs can be as simple as a single channel with one inlet and one outlet, or as complicated as a multilayered device containing dozens of fluidic ports and integrated, computer-controlled pneumatic valves. A simple design can be operated with a syringe reservoir and thin tubing, with fluid flow driven by hydrostatic pressure. A complicated system may require precisely balanced fluid and pneumatic line pressures and computer-actuated valves or syringe pumps. Simple and elegant systems that focus on ease of use are preferred, not just to reduce user frustration, but to improve reliability and reproducibility of an assay.

Here we describe the preparation and operation of several simple microfluidic *C. elegans* devices that are currently in use by many research labs. The first two devices are microfluidic arenas for observing locomotory behavior in response to precise and stable spatial patterns of chemical stimuli, either sharp or shallow chemical gradients. These are similar to plate-based chemotaxis assays, but with greater reproducibility and data content (9) (Fig. 1). These devices can be cleaned and reused dozens of times. The third and fourth devices are microfluidic worm traps for microscopy (10) and for high-resolution imaging of neural activity (11) using a fluorescent, genetically encoded calcium sensor (12) (Fig. 2). These devices are permanently bonded to a glass substrate, and most microfluidic systems are of this "single use" variety. All of these are fabricated from microfluidic molds prepared by photolithography in a cleanroom. Alternatively, lower-resolution devices can be created by cutting adhesive tape (xurography) with a consumer-grade craft cutter (13, 14), or even by hand (15), which can be useful for prototyping and educational applications. Two examples are included for monitoring individual animals in channels or in microwell arrays (Fig. 3). The following protocols are aimed at the user unfamiliar with microfluidic systems as well as the user who has experienced some difficulty in practice. While many details for preparing and operating microfluidic systems differ among published reports, here we highlight our preferred materials and methods and note alternatives when appropriate.

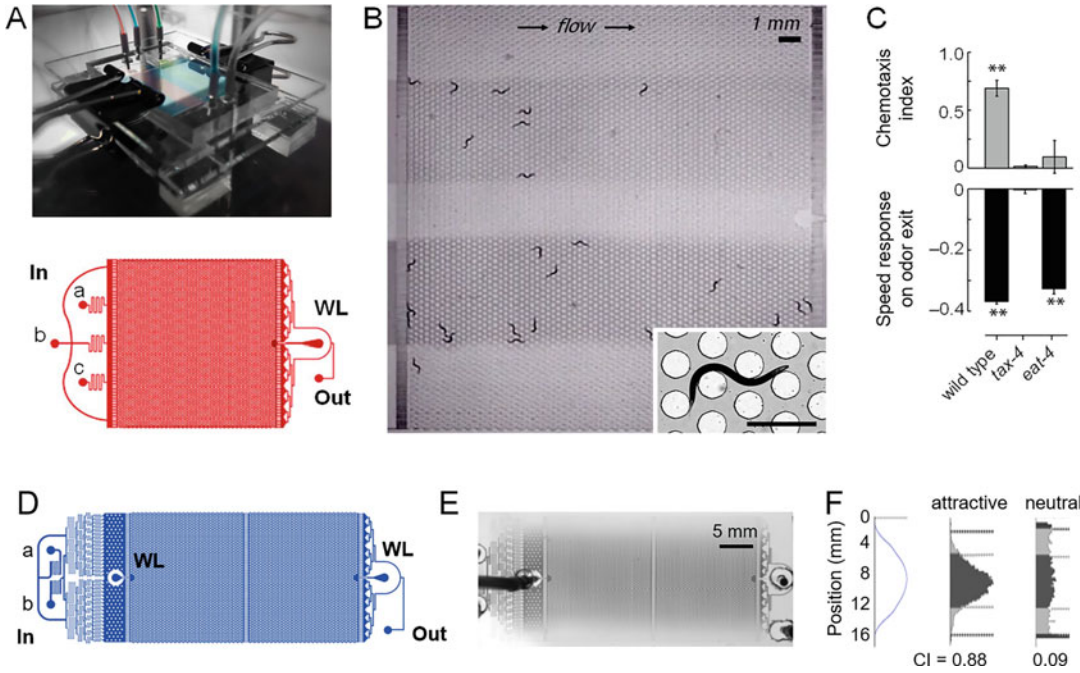

Fig. 1 Microfluidic devices for quantifying behavioral responses to spatial stimulus patterns. (**a**) The "stripe" device presents two stable chemical stripes that flow from three inlet (In) ports to an outflow port (Out). Animals are loaded through a worm loading (WL) port. A completed device is shown clamped and mounted to the video capture stage (above). (**b**) A video frame showing 25 wild-type *C. elegans* animals responding to two horizontal stripes of the attractant isoamyl alcohol (IAA, 0.92 μM top, 1.84 μM bottom) ***(9)***. Inset shows a young adult animal moving around microposts. Scale bar, 500 μm. (**c**) Quantitative behavioral data for chemotaxis index (the relative time spent inside versus outside the odor stripe, a measure of attraction to the stimulus) and for the change in speed upon exiting the odor stripe. Wild-type animals are attracted to the IAA odor and slow down upon exiting. Sensory mutant *tax-4(ks28)* shows no attraction or speed response to the odor, whereas glutamate-deficient *eat-4(ky5)* animals slow normally but do not reside in the odor due to the inability to turn appropriately ***(9)***. (**d**) The "gradient" device presents a symmetrical linear gradient from the midline to the outside edges from the inlet (In) ports to the outflow port (Out). This device contains two isolated arenas, separated by a worm barrier, to assess two animal populations at once. Animals are loaded through one of two worm loading (WL) ports. (**e**) A video frame showing a linear gradient across the device shown in **d**. Scale bar, 5 mm. (**f**) Chemical concentration across the vertical axis and corresponding histogram of animal position in response to attractive and neutral stimuli. Chemotaxis index (CI) calculated as the relative time spent inside the middle 50% band at higher concentration (dark gray) versus the outer regions at lower concentration (light gray)

2 Materials

Microfluidic devices and accessories should be handled with gloves and pre-cleaned by washing in water, then 95% ethanol, then water again, and dried in an air or N_2 stream (*see* **Note 1**). All solutions are prepared in sterile worm buffer at room temperature, unless noted, using aseptic technique.

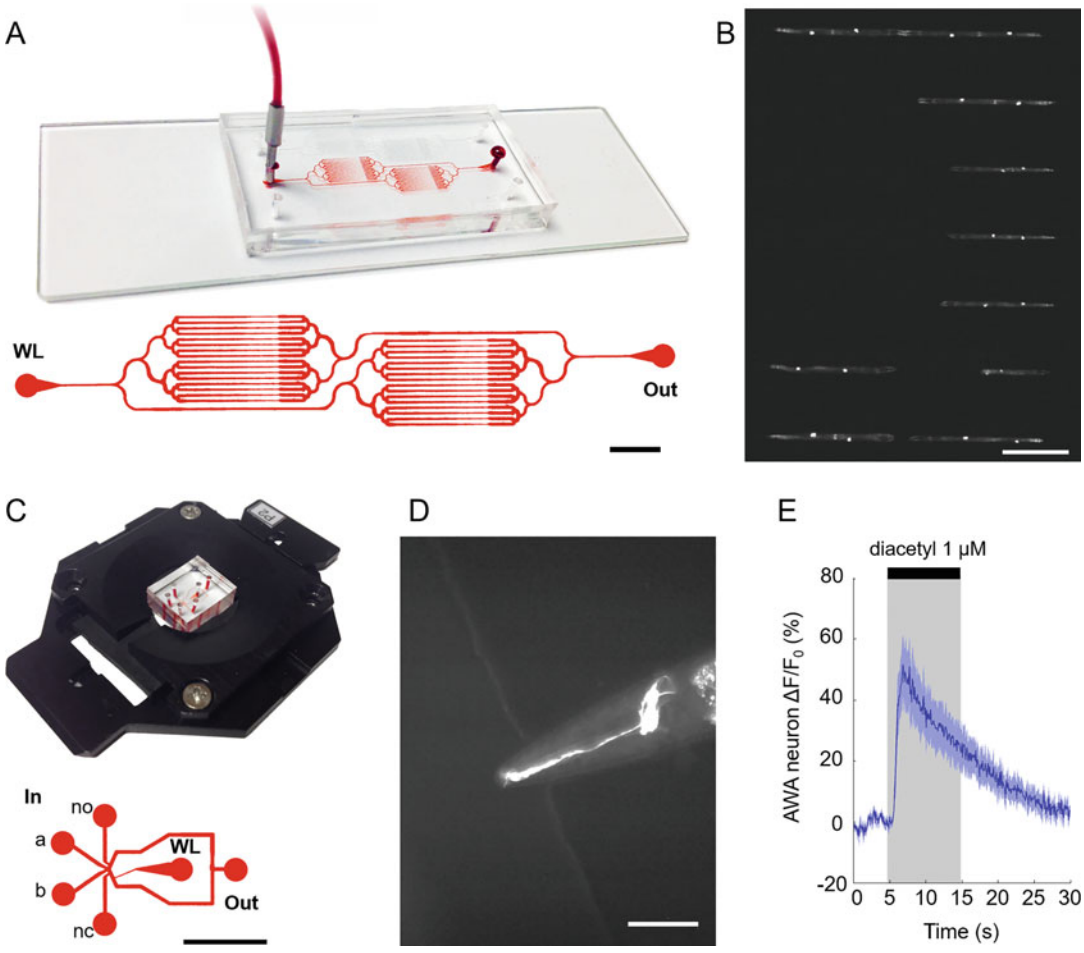

Fig. 2 Microfluidic devices for animal trapping and alignment, time-lapse microscopy, and neural imaging. (**a**) A parallel array of 32 tapering animal traps ***(***10***)*** is loaded via a single worm loading (WL) port. Scale bar, 2 mm. (**b**) Animals are aligned and remain in a fixed position during time-lapse imaging. Two distal tip cells are shown labeled with GFP (strain JK2868). Scale bar, 0.25 mm. (**c**) A neural imaging device ***(***11***)*** mounted in a microscope slide holder. Fluid streams from inlet (a) or (b) pass over the animal's nose depending on which control channel (no) or (nc) is open. Scale bar, 2 mm. (**d**) AWA chemosensory neurons are shown expressing the calcium sensor GCaMP2.2b (strain CX14887), Scale bar, 50 μm. (**e**) AWA neurons respond to addition of the odorant diacetyl (1 μM) with increased relative fluorescence intensity ($\Delta F/F_0$), indicating an "on" neural response ***(***16***)***

2.1 Equipment

1. Vacuum desiccator.
2. Weigh balance (150 g range).
3. Magnetic stir plate.
4. Air or N_2 gun.
5. Micropipettes (10 or 20 μL, 200 μL, and 1000 μL).
6. Serological pipettes (25 mL).
7. Lab oven (65 °C) with level shelves (check with bubble level).

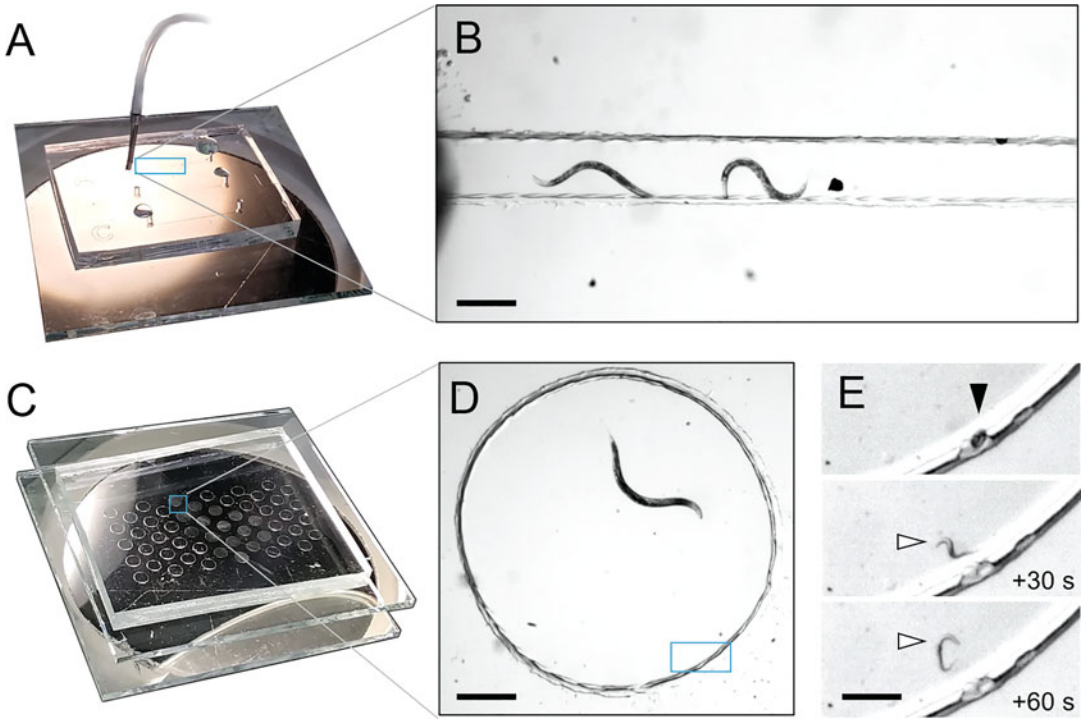

Fig. 3 Example microfluidic devices fabricated from stencil-cut adhesive tape on glass. (**a**) Three parallel 500 μm wide channels are loaded with animals by tubing or by surface droplet. (**b**) Young adult animals exhibit swimming locomotion in 100 μm tall channels shown, whereas <50 μm tall channels enable crawling locomotion (not shown). Scale bar, 0.5 mm. (**c**) An array of 2 mm diameter chambers filled with a pipette and sealed with a hydrophobic glass slide. (**d**) Individual young adult animals can be imaged over time and analyzed for behavior, fluorescent markers, developmental events, or other responses. Scale bar, 0.3 mm. (**e**) At hour 2 of a 15 h time-lapse, an egg (filled arrowhead) hatched releasing the L1 animal (open arrowheads) in three images taken 30 s apart. Scale bar, 0.1 mm

8. Plasma bonding system; optional, if permanently bonding the microfluidic device to a glass substrate (*see* **Note 2**).
9. For rapid prototyping of adhesive tape mold masters, *see* Subheading 2.7 for additional equipment.

2.2 Microfluidic Device Casting and Punching

1. Microfluidic master mold fabricated from plastic or a silicon wafer (*see* Subheading 3 for sourcing) or adhesive tape (*see* Subheading 3.8).
2. PDMS: polydimethylsiloxane kit; e.g., Sylgard 184.
3. Nitrile or powder-free latex gloves (powder may inhibit PDMS curing).
4. 150 mm Petri Dish (*see* **Note 3**).
5. Large weigh boat.
6. Plastic transfer pipettes.

7. Stainless steel scalpel holder and No. 10 blade.
8. Single-edge heavy-duty razor blade.
9. Scotch Magic Tape (3 M cat. 810).
10. Dermal punch, 1 mm.
11. Dark fine-tip marker.
12. Self-healing cutting mat.

2.3 Microfluidic Device Accessories

1. Glass slides, 1–1.5 mm thick, cut to desired dimensions if necessary (*see* **Note 4**) using a diamond-tipped scribe. Use cover slips (#2 or #1.5 thickness) for high-resolution microscopy applications.
2. TFOCS: (Tridecafluoro-1,1,2,2-tetrahydrooctyl) trichlorosilane for preparation of hydrophobic glass. *CAUTION*: *TFOCS is corrosive and toxic and must be used in a fume hood.*
3. Dremel rotary tool with workstation stand and 5/64″ diamond taper wheel point.
4. Metal clamp assembly, either: (a) Small 5/16″ capacity binder clips, (b) Warner Instruments platform P-2 for small devices less than 20 × 20 mm^2, or (c) a custom-fabricated device holder (*see* **Note 5**).

2.4 Solutions

1. Worm buffer (*see* **Notes 6** and **7**): Dissolve 5.85 g NaCl, 1.0 g K_2HPO_4, and 6.0 g KH_2PO_4 in 1 L deionized water. Check that pH is about 6.0 and adjust if necessary. Sterilize by autoclave.
2. Dye solution (100×): 10 mg/mL xylene cyanol in worm buffer (*see* **Note 8**). Dissolve 1.0 g xylene cyanol in 100 mL worm buffer and sterilize by autoclave or syringe-tip filter.
3. Loading buffer: 5% w/v Pluronic F127 with 0.5× dye solution. Prepare 1 mL aliquots and store at 4 °C.
4. Stimulus buffer(s): For example, a 1:10^7 dilution of isoamyl alcohol (IAA, 3-methylbutanol >99.8% purity) produces a robust attraction response (*see* **Note 9**).

2.5 Experimental Setup

1. Tubing set (5 total): Insert the metal end of a blunt 23 gauge Luer stub adapter into one end of a 50 cm length of 0.020″ inner diameter (ID) Tygon tubing (*see* **Note 10**). Insert a 1/2″ (13 mm) long 19 gauge stainless steel tube about one-third of the way (4–5 mm) into the other end of the Tygon tubing as in Fig. 4a (*see* **Note 11**).
2. Solution reservoirs (3 total): Connect a 30 mL syringe and 3 mL syringe to a 3-way Luer stopcock valve as in Fig. 4a (*see* **Note 12**). Remove the plunger from the 30 mL syringe. Connect a tubing set (from **item 1**) to the valve.

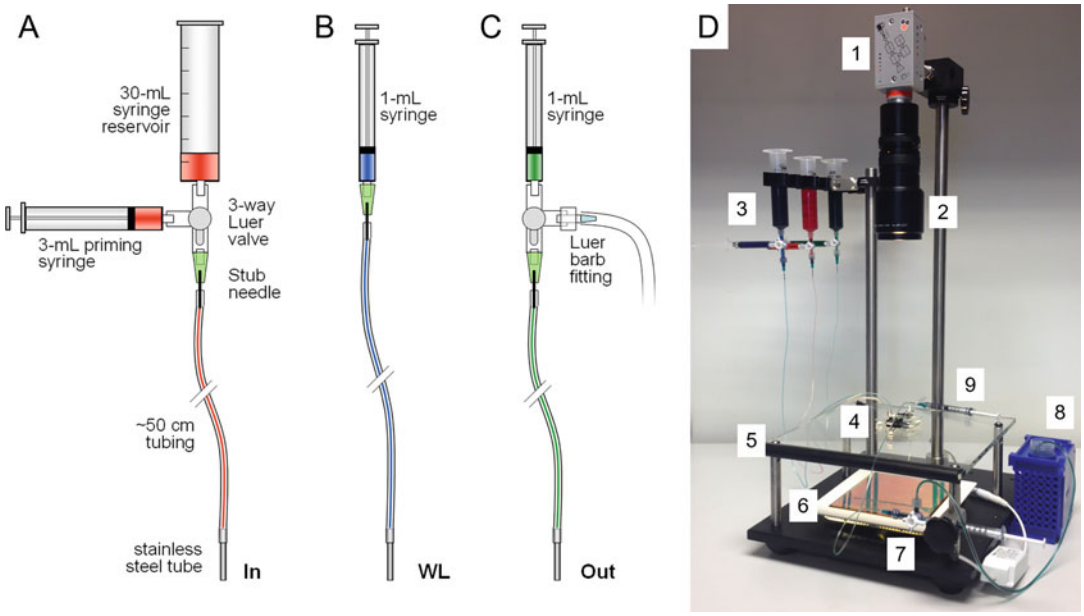

Fig. 4 Experimental setup for microfluidic flow via hydrostatic pressure. Tubing, syringes, and valves are shown for solution reservoirs (**a**), worm loading syringe (**b**), and outflow tubing (**c**). The complete video capture system (**d**) contains the (1) video camera, (2) zoom lens, (3) stimulus reservoirs, (4) microfluidic device, (5) glass stage, (6) LED backlight, (7) outflow syringe, (8) waste reservoir, and (9) worm loading syringe

3. Worm loading syringe: Connect a 1 mL syringe to one tubing set as in Fig. 4b.
4. Outflow tubing: Connect a 1 mL syringe, a tubing set, and a male Luer barbed fitting with ~50 cm of Tygon tubing (1/16″ ID, 1/8″ OD) as in Fig. 4c.
5. Reservoir rack and stand.
6. Solid 1/2″ long 19 gauge stainless steel blocking pins (*see* **Note 13**).
7. Video capture system as in Fig. 4d (*see* **Note 14**).

2.6 Alternative Materials

1. *Tubing*: Tygon and other lab tubing can leach chemicals such as plasticizers that elicit a weakly attractive behavioral response in *C. elegans*. We have found this effect to be negligible when testing robust stimuli or when all inlet fluids flow at an equal rate. Alternatively, high-purity Teflon PFA tubing (e.g., Upchurch IDEX, 0.020” ID, 1/16” OD) does not elicit any behavioral response in our experience, although it is substantially more expensive. This tubing is rigid and can be inserted directly, without a metal tube, into a larger PDMS inlet port: in Subheading 3.1 **step 10**, use a 1.5 mm dermal punch. This tubing also requires specific fittings (IDEX LuerTight, P-835) to connect with syringe reservoirs.

2. *Automated valves*: Automated valves are useful for precise temporal delivery of stimuli, such as a sudden application or removal of a stimulus. We prefer electrically actuated, computer-controlled microvalves with low swept volume and an inert wetted path.

2.7 Materials for Rapid Prototyping of Adhesive Tape Molds

1. Electronic cutting tool (such as Silhouette Cameo) with computer and cutting software installed.
2. Cutting tool accessories: Premium ratchet blade (such as SILH-BLADE-PREM) and 12″ × 12″ adhesive cutting mat.
3. Glass slides, 1–1.5 mm thick (e.g., 25 × 75 mm, 50 × 75 mm, or 50 × 50 mm).
4. Adhesive tape of desired thickness (e.g., Scotch 311+, 50 μm thick).
5. Tweezers.
6. Metal ruler or other straight edge tool.

3 Methods

This protocol assumes that the user has a microfluidic mold master (begin at Subheading 3.1) or pre-cast microfluidic devices (begin at Subheading 3.2). To obtain a photolithographic mold master, we recommend contacting the corresponding author of a publication presenting a microfluidic device of interest to request either a PDMS casting, a mold master, or the computer-aided design (CAD) file from which the master can be microfabricated. We encourage microfluidics-oriented labs to provide these resources openly or at moderate cost. There are a few publicly available resources for outsourcing the microfabrication of mold masters, including university-based and commercial vendors, although they tend to have fluctuating capabilities (for a list, see *albrechtlab.github.io*). Most universities with engineering departments house a microfabrication facility, and many will either produce a mold master for nominal cost or provide training to users interested in learning fabrication methods. In-house photolithography may be feasible for some labs, requiring approximately $25,000 in hardware and a cleanroom or hood. Fabrication steps are relatively quick (about 2–3 h) for simple device masters and are described in detail elsewhere [14–16].

Alternatively, microfluidic mold masters can be created by cutting adhesive tape on glass with a consumer-grade craft cutter (*see* Subheading 3.8). While these molds have lower geometric precision and stability than photolithographic molds, their rapid production (minutes versus hours) and far lower cost are useful for prototyping and educational applications.

3.1 Device Fabrication: PDMS Casting from a Mold Master

1. Weigh a 10:1 (w/w) ratio of Sylgard 184 PDMS base to curing agent into a large weigh boat (*see* **Note 15**).
2. Mix PDMS base and curing agent thoroughly (*see* **Note 16**).
3. Degas the mixture in the vacuum desiccator for 30 min to 1 h to eliminate bubbles (*see* **Note 17**).
4. Clean the mold master, Fig. 5a, in an air stream if dust is visible (*see* **Note 18**).
5. Pour the PDMS mixture onto the mold master in a large (150 mm diameter) Petri dish (*see* **Note 3**), and fill to the desired device thickness, approximately 4–5 mm (*see* **Note 19**).
6. Inspect the poured PDMS and remove bubbles or dust (*see* **Note 20**).
7. Bake at 65 °C on a level shelf for between 3 h to overnight.
8. Remove the PDMS casting: Cut along the mold master perimeter with a metal-handled scalpel (*see* **Note 21**), gently peel up, and remove as in Fig. 5b–d.
9. Cut individual devices from the PDMS casting with a single-edge razor blade as in Fig. 5e (*see* **Note 22**).
10. Punch inlet and outlet holes (*see* **Note 23**) using a 1 mm diameter dermal punch as in Fig. 5f–h (*see* **Note 24**). Clean any debris remaining in the holes (*see* **Note 1**).

3.2 Device Fabrication: Glass Substrates

Microfluidic channels are formed upon sealing the PDMS casting to a flat substrate, usually a glass slide or cover slip. A reversible leak-resistant seal can be made by compressing the PDMS casting between a hydrophobic glass substrate and a second glass slide with holes drilled for tubing connections (Fig. 6a). This configuration is considered "reusable" as it can be opened after an experiment for cleaning and recovery of animals. However, it may leak under excessive fluidic pressure; thus, flow is best driven by hydrostatic pressure.

An irreversible leak-proof seal is prepared by plasma bonding a glass slide or cover slip to the PDMS casting as in Fig. 6b. Flow in bonded devices may be driven by pressurized reservoirs or syringe pumps without risk of leakage, and they are preferred for long-duration experiments and for high-resolution microscopy requiring a thin cover slip as the substrate. However, bonded devices are usually considered to be single use, as cleaning and animal recovery are difficult.

3.2.1 Glass Plates for Reusable Devices

Perform these steps only for reusable, reversibly sealed microfluidic devices as in Fig. 6a, then proceed to Subheading 3.3.

1. Prepare a hydrophobic glass slide slightly larger than the PDMS device (*see* **Note 4**) by TFOCS vapor deposition (*see* **Note 25**).

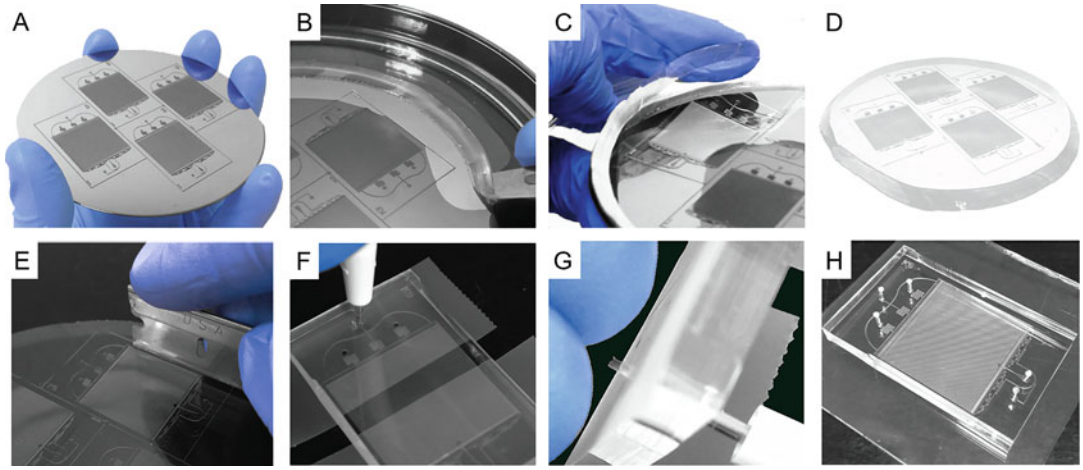

Fig. 5 Device fabrication steps. PDMS polymer is cast over a mold master such as a micropatterned silicon wafer (**a**). The cast PDMS is cut out with a scalpel (**b**), peeled from the mold master (**c**, **d**), and trimmed into individual microfluidic devices with a razor blade (**e**). Inlet and outlet ports are punched (**f**), removing excess punched material (**g**). The microfluidic device is cleaned and ready for assembly or for permanent bonding to a glass substrate (**h**)

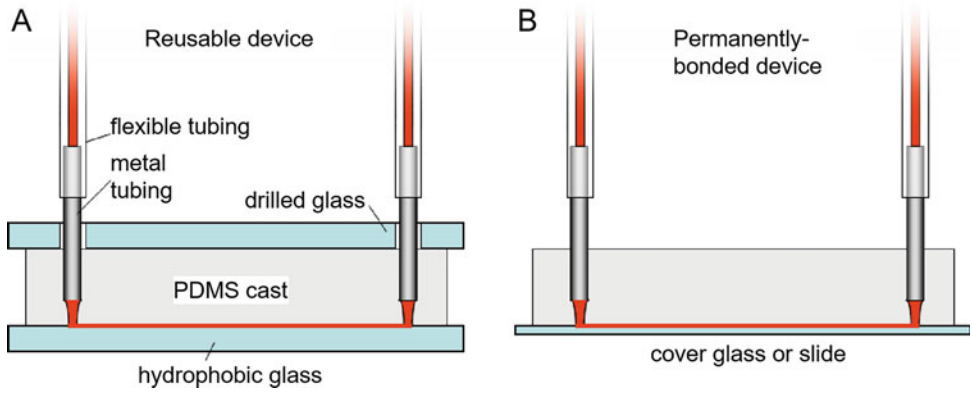

Fig. 6 Cross-sectional view of a reusable microfluidic device (**a**) and a permanently bonded microfluidic device (**b**)

2. Drill inlet holes into a second glass slide of equal dimensions: Align the PDMS device on the glass and mark hole locations (*see* **Note 26**). Drill holes under constant water lubrication (*see* **Note 27**), and clean thoroughly (*see* **Note 1**).

3.2.2 *Plasma Bonding*

Perform these steps only for permanently bonded microfluidic devices (Fig. 6b).

1. Seal a clean, dry, dust-free PDMS device (*see* **Note 1**) onto a 2″ × 3″ glass carrier slide such that the micropatterned features face up.

2. Clean the glass substrate (cover slip or slide) with ethanol while rubbing with a lint-free wipe (e.g., Kimwipe). Dry with the air/N_2 gun and remove dust with tape. Transfer to the glass carrier slide and insert into the plasma vacuum chamber (*see* **Note 2**).
3. Expose to air plasma for 60 s (*see* **Note 28**).
4. Invert the slide or cover glass onto the PDMS device (*see* **Note 29**) and briefly apply gentle pressure (*see* **Note 30**).

3.3 Device Preparation and Assembly

1. Before use, microfluidic devices should be cleaned by soaking in ethanol for several hours, rinsed with water (*see* **Note 1**), and baked for at least 1 h to evaporate residual ethanol (*see* **Note 31**).

   For permanently bonded devices, skip to **step 5**.
2. Seal a clean, dust-free microfluidic device against a clean, dust-free drilled top glass slide (*see* **Note 1**), aligning the drilled holes with the punched inlets. Remove any remaining dust with tape.
3. Seal a clean, dust-free hydrophobic glass slide against the micropatterned side of the device.
4. Clamp the device with binder clips as in Fig. 7 (or in a clamp apparatus, *see* **Note 5**). Holding the glass-PDMS-glass assembly firmly in the center, apply a binder clip to one side. Without releasing pressure, clip the opposite side. Test for proper compression force (*see* **Note 32**).
5. Block the worm loading port by inserting a solid blocking pin until it reaches ~2/3 (about 3 mm) through the thickness of the PDMS (*see* **Note 33**).
6. Degas the assembled microfluidic device in the vacuum desiccator for 10–30 min (*see* **Note 34**).

3.4 Device Filling

1. Prepare fluid reservoirs and tubing (as noted in Subheading 2.5). Position each reservoir on the rack and fill with stimulus or buffer solutions. Remove air bubbles (*see* **Note 35**) and fill the tubing (*see* **Note 36**).
2. Prepare outflow tubing. Fill with buffer, then draw 1 mL loading buffer into outflow syringe.
3. Remove the device from vacuum and connect the outflow syringe to the outflow port (*see* **Note 34**).
4. Gently inject loading buffer until it has completely filled the arena and begins to emerge as a droplet at an inlet.
5. Connect the buffer tubing to the inlet hole using a "drop-to-drop" connection as in Fig. 8 and aspirate any excess liquid (*see* **Note 37**).

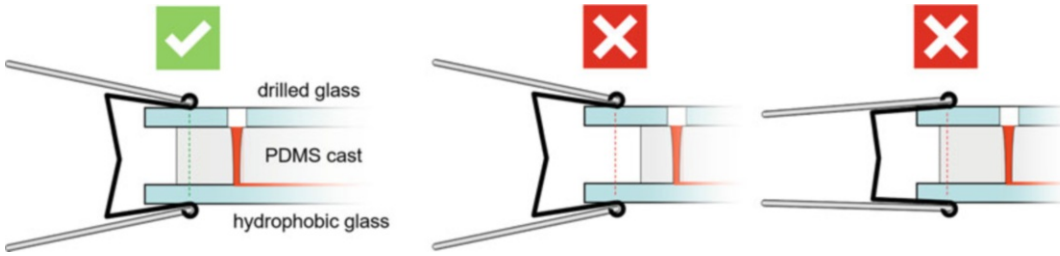

Fig. 7 Proper positioning of binder clip clamps (left). Avoid clamping where the glass slides are unsupported (center) or with a binder clip that is too small (right), as excess stress may crack the glass slide

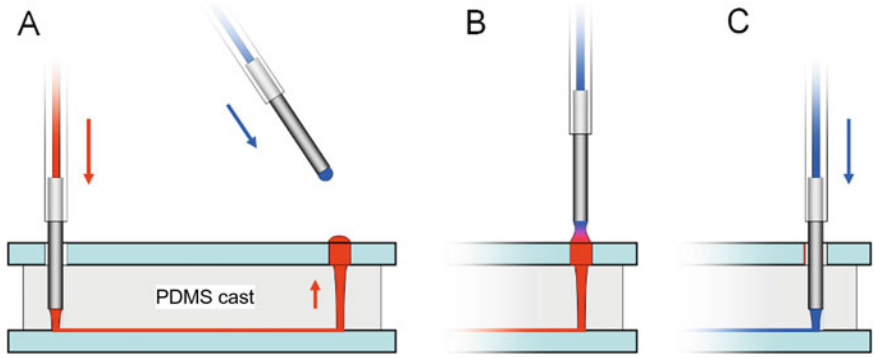

Fig. 8 Bubble-free, "drop-to-drop" insertion of tubing into a port on microfluidic device. Allow liquid to flow in the device (red) until a drop appears at the port, and inject liquid in the tubing to be inserted (blue) until a droplet hangs from the tubing pin (**a**). Bring the droplets into contact (**b**) and insert pin about 2/3 into the port (**c**)

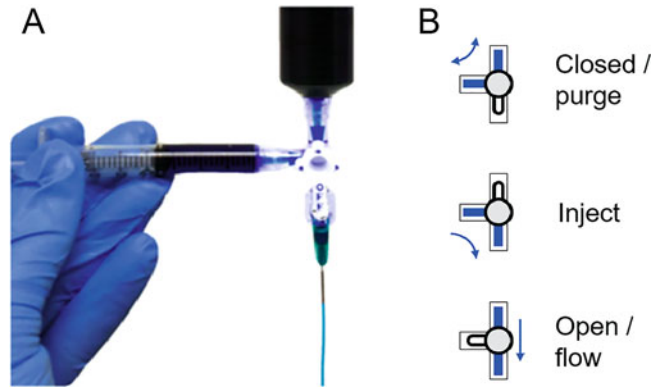

Fig. 9 (**a**) Solution reservoir filled with dye and valve in the Closed/Purge position. (**b**) Valve positions used to control fluid flow during operation of the microfluidic device. Arrows indicate fluid flow direction

6. Open the buffer valve briefly (turn to "Flow" position, Fig. 9) until another inlet hole fills, then insert the next stimulus tubing with a "drop-to-drop" connection (*see* **Note 38**). Repeat for all inlet reservoirs.

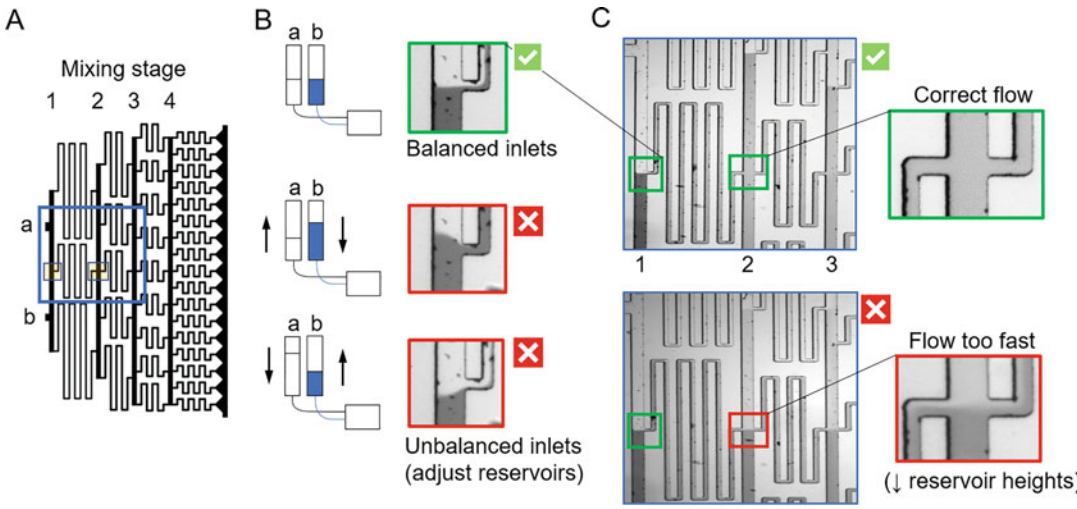

Fig. 10 Flow adjustments to achieve a linear chemical gradient with the "gradient" microfluidic device (Fig. 1d). (**a**) Subsection of upstream channels showing four numbered mixing stages. Fluids entering at inlets "a" and "b" converge at the center of stage 1 (left box) and should fully mix by the end of the serpentine channel (right box). (**b**) Balance inlet flows at the stage 1 center channel (left box in **a**). Lower the reservoir of inlet fluid that dominates in an unbalanced flow condition (red; middle and bottom) to achieve balanced flow (green, top). (**c**) Ensure complete mixing in the serpentine channel by checking at the center of stage 2 (right boxes in **a** and **c**). No gradient should be visible when flow is sufficiently slow (green box, top). Lower both reservoir heights if a gradient is visible due to fast flow and insufficient mixing (red box, bottom)

7. Once all ports are connected to tubing (or blocked), open a buffer valve and the outflow valve to flush the arena with worm buffer and displace the loading buffer (*see* **Note 39**).
8. Position the microfluidic device on the observation stage (*see* **Note 40**).
9. Adjust the relative heights of the stimulus reservoirs to establish the desired spatial pattern. Fluid flowrate is proportional to the height difference between fluid levels of the inlet reservoirs and outlet waste reservoir (*see* **Note 41**). For a stripe pattern device (Fig. 1a–c), raising a reservoir increases the width of the corresponding fluidic stripe. For a gradient pattern device (Fig. 1d–f), a balance of reservoir heights is necessary to maintain correct mixing for a linear spatial gradient (Fig. 10). Establish proper fluid flow prior to animal loading to avoid premature stimulus exposure.

3.5 Worm Loading and Device Operation

1. Pick 1–50 young adult *C. elegans* (*see* **Note 42**) onto an unseeded 60 mm agar plate (*see* **Note 43**).
2. Pour ~5 mL worm buffer onto the plate.
3. Fill the 1 mL worm loading syringe with worm buffer, purge any bubbles, and draw animals into the tubing (*see* **Note 44**) using a minimal volume of buffer (*see* **Note 45**).

4. Close the outflow valve, remove the blocking pin from the worm loading port, and briefly open the buffer valve to fill it with a buffer drop. Insert the worm loading tubing with a "drop-to-drop" connection (*see* Fig. 8).
5. Open the buffer and outflow valves (Fig. 9, "Flow" position) and gently inject animals into the arena (*see* **Note 46**).
6. Once all animals have entered the arena, pinch the worm loading tubing and clamp it with a mini binder clip to prevent flow into this channel.
7. Allow animals to disperse and adapt to the micropost environment for several minutes before initiating a recording and opening the stimulus valves (*see* **Note 47**).
8. Record animal behavior for 30–120 min (*see* **Note 48**) and analyze as desired, for example using "ArenaWormTracker" software (*see* **Note 49**) *(9)*.

3.5.1 Troubleshooting Device Operation, Common Problems, and Recommended Solutions

1. *Bubbles in a microfluidic channel or arena*. If the device was recently degassed, small bubbles will absorb into the PDMS (*see* **Note 34**). Wait 5–10 min and see whether the bubble has gotten smaller. If not, open the device, clean it, and set it up again as in Subheadings 3.3 and 3.4.
2. *Bubbles in inlet tubing*. Halt flow immediately by closing the outflow valve and pull out the tubing containing bubbles. Purge the bubbles by forcing them out with the syringe (valve position "Inject," Fig. 9), then reinsert using drop-to-drop contact (*see* Fig. 8).
3. *Bubbles or air in outflow tubing*. Small bubbles in the outflow tubing are ok and useful to estimate fluid flowrate (*see* **Note 41**), but large air-filled regions may slow gravity-driven flow. Be sure to fill the tubing completely to the waste beaker using the outflow syringe.
4. *Leakage at the fluorinated glass slide*. A poor channel seal may be caused by insufficient clamp pressure (*see* **Note 32**), insufficient flatness of the PDMS device due to ethanol swelling (*see* **Note 31**), or a fabrication defect. Increase the compression force, evaporate longer in the oven, or use a new, flat device.
5. **Leakage at an inlet**. A poor seal at an inlet port may be due to insufficient tube insertion depth (ideal is ~2/3 of the port depth as in Fig. 6) or to poor punch quality. The inlet port should be punched straight with smooth walls. Damaged punches may cut a notch along the hole causing a slow fluid leak (*see* **Note 24**).
6. *Dust or particles in the microfluidic channels*. Clean devices thoroughly (*see* **Note 1**) and assemble quickly or in a clean

hood. Wash inlet holes after punching. Filter solutions if necessary.

7. *Warped stimulus pattern*. Ensure no debris or bubbles disturb the flow path. If binder clips clamp too strongly and reduce channel depth at the edges, horizontal fluid stripes will bow toward the center.
8. *Fluid flow is too fast or too slow.* Adjust the height difference between reservoirs and waste beaker. For a height difference of 50 cm, flowrate in the behavioral arena is typically ~0.8 μL/s. Flowrate can be estimated as described in **Note 41**.

3.6 Device Cleanup and Storage

1. Shut off all valves (Fig. 9, "Closed" position) and remove tubing from all ports.
2. Rinse all syringe reservoirs and tubing with water and blow dry with an air or N_2 gun (*see* **Note 50**). Eject remaining liquid in worm loading tubing, rinse with water, and dry.
3. For reusable devices, open the microfluidic assembly, separate the PDMS from glass slides, and optionally recover animals to a seeded agar plate by rinsing the PDMS and glass with worm buffer.
4. Rinse the PDMS with water (*see* **Note 1**) and soak it overnight in ethanol to remove any adsorbed stimulus (*see* **Note 51**), then clean (*see* **Note 1**) and bake for at least 1 h to evaporate residual ethanol (*see* **Note 31**).
5. Devices may be stored at room temperature or in an oven at <65 °C, and reused dozens of times.

3.7 Microfluidic Devices for Microscopy and Neural Imaging

The methods described above for fabricating, cleaning, assembly, filling, loading, and operating microfluidic devices are general and can be used with most designs. Below, we describe two simple devices for arraying many animals in parallel for time-lapse microscopy *(*10*)* (Fig. 2a, b) and for high-resolution imaging of neural activity *(*11*)* (Fig. 2c–e).

3.7.1 Parallel Trap Array Device

This device aligns many animals within tapering channels for ease of imaging by microscopy. It is conveniently loaded through a single worm loading port and is often used for time-lapse microscopy in which individual animals can be observed over many hours.

1. Prepare the trap array device (Fig. 2a) as described in Subheadings 3.1–3.4 and fill with worm buffer from the outflow port (*see* **Note 52**).
2. Draw animals into worm loading tubing and inject them into the channels as in Subheading 3.5. Ensure the outflow valve is open during worm loading.

3. Once animal traps are filled, close the outflow valve, remove the worm loading tubing, and replace it with a buffer reservoir (*see* **Note 53**). Open the outflow valve to initiate buffer flow.
4. Monitor changes in fluorescent markers (such as position or intensity) over time (Fig. 2b).

3.7.2 Neuronal Imaging Device

Calcium imaging studies determine a neuron's response to a sudden presentation or removal of a chemical stimulus. This device traps a single animal such that its nose protrudes into a channel that is rapidly switched between two fluidic streams. Recent modifications of this design increase the number of animals and stimuli tested per experiment *(*16, 17*)* or enable neural imaging in freely moving animals to correlate neural and behavioral responses *(*16*)*.

1. Prepare the imaging device (Fig. 2c) as described in Subheadings 3.1–3.3 and fill with worm buffer from the outflow port (*see* **Note 52**).
2. Prepare two stimulus reservoirs and tubing as in Subheading 3.4. Typically, one reservoir contains worm buffer (a), and the other a chemical stimulus (b).
3. Prepare a control fluid reservoir connected to the common port of a 3-way actuated microvalve. Connect tubing sets to the normally open (no) and normally closed (nc) ports on the microvalve. Fill the reservoir with worm buffer (*see* **Note 54**) and prime the tubing (*see* **Notes 35** and **36**).
4. Connect all four inlet tubes to the inlet ports via drop-to-drop connections (*see* **Note 55**).
5. Inject one animal into the worm loading port. Observe the animal under the microscope, providing pressure from the worm loading syringe as necessary until it reaches the tapered clamp with its nose just protruding into the fluidic channel as in Fig. 2d.
6. Focus the objective on the neuron of interest under fluorescent excitation (*see* **Note 56**) and set up an acquisition stream (*see* **Note 57**).
7. Acquire video for 30 s to 3 min, actuating the microvalve (*see* **Note 55**) at the desired time and duration to apply a stimulus pulse, as in Fig. 2e.
8. Analyze neuronal fluorescence over time, for example using NeuroTracker *(*16*)* software (*see* **Note 49**). Display results as relative fluorescence change ($\Delta F/F_0$) where F_0 is the baseline fluorescence and $\Delta F(t) = F(t) - F_0$.

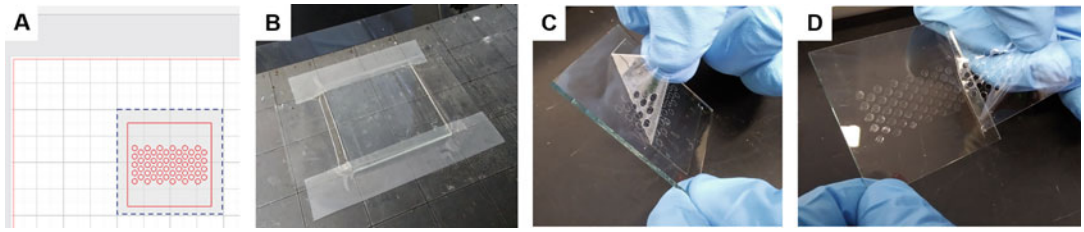

Fig. 11 Preparation of an adhesive tape mold by xurography using an electronic craft cutter. (**a**) Design of a microwell array as it appears in the cutter software. Dotted line indicates the 50 × 50 mm glass slide and cut lines are shown in red. (**b**) Taped glass slide secured to the cutting mat in the position indicated in software (panel a). Note the upper left corner is positioned 2″ from the left edge and 1″ from the upper edge, as in the software image. (**c**) Removal of excess tape from the glass slide by slowly peeling with tweezers or fingers. (**d**) Ensure the excess tape lifts cleanly free of the cut mold features

3.8 Rapid Prototyping of Microfluidic Mold Masters by Xurography

1. Clean hydrophobic TFOCS-treated glass slides (*see* **Note 25**) with 70% ethanol, a lint-free wipe, and compressed clean dry air.
2. Apply one or more layers of adhesive tape to the cleaned slides to build up the desired feature height (*see* **Note 58**). Use a metal ruler or straight edge to apply tape under tension to avoid trapping bubbles.
3. Align the taped glass on the adhesive cutting mat according to the grid marks (Fig. 11a, b). Secure using additional tape around the perimeter.
4. Import a vector graphics file to the craft cutter software or draw the pattern outline using the software tools. Scale the cutting design to desired dimensions and ensure it is located within the grid region that corresponds to the taped glass slide (*see* **Note 59**). Channels 500 μm wide can be reliably fabricated (Fig. 3a).
5. Set the cutting parameters according to the cutting tool model and tape thickness (*see* **Note 60**).
6. Initiate cutting and monitor blade progress. Optionally, perform an initial test cut to check that tape has been cleanly cut, and adjust settings as needed (*see* **Note 61**). Once complete, unload the mat from the rollers and remove the glass substrate.
7. Use tweezers to remove excess tape regions leaving behind the raised microfluidic features (Fig. 11c, d). Tape should separate easily at cut lines with a sharp blade and correct settings (*see* **Note 61**).
8. Place the patterned tape-on-glass master at the bottom of a plastic petri dish and cast PDMS as described in Subheading 3.1 (*see* **Note 62**).
9. Assemble device as in Subheading 3.3 and perform experiment (Fig. 3).

4 Notes

1. All components should be stored clean, dry, and dust-free in closed containers. Rinse microfluidic devices and glass substrates first with deionized water to prevent crystallization of salts within microchannels or tubing. Flush inlet ports using squirt bottles, ensuring the fluid stream passes through each inlet. Next, clean with ethanol, wiping away any debris and smudges with gloved fingers. Finally, rinse with water and quickly blow dry with an air or N_2 gun. This step prevents evaporation which may leave a residual film that is difficult to remove, especially from microfluidic channels. Remove surface dust using tape.
2. Alternative and less expensive PDMS plasma bonding methods have been reported, although they are generally less reliable and more sensitive to exact treatment conditions. These include a hand-held corona discharge wand *(*18*)* and an evacuation chamber placed in a standard microwave oven *(*19*)*.
3. A disposable PDMS casting dish can be made from aluminum foil. We prefer a large 150 mm Petri dish as it has a flat and level bottom, comes with a lid, and protects the wafer from damage during handling and storage.
4. Trim glass slides as necessary to maintain about a 2–5 mm border around the PDMS microfluidic device. Use a 1.5 mm thick glass slide for devices larger than ~25 mm × 25 mm. Align a ruler along the desired cut line, firmly score once with a sharp diamond scribe, and snap the glass by applying evenly distributed pressure on each half. For best results, cut at least 12 mm away from any edge. With proper pressure, the score line should be thin and barely visible.
5. We custom machined a clamp from 1/4″ thick aluminum bar, four 1/4″-20 bolts, and four springs. Two plates each contain a central imaging window and four holes in the corners; the bottom plate holes are tapped with 1/4″-20 threads while the top holes allow free movement of the bolts. The microfluidic device is held between the top and bottom plates by the bolts that compress springs. To assemble, place and center the microfluidic assembly into the device holder, then evenly tighten the four screws in a cross-diagonal manner to prevent uneven point stresses.
6. Our preferred worm buffer is “S Basal” without cholesterol, which sustains healthy animal behavior for long durations and is easy to prepare. A common alternative is “M9 Buffer”: Combine 5.0 g NaCl, 3.0 g KH_2PO_4, 6.0 g Na_2HPO_4, and 0.25 g $MgSO_4{\cdot}7H_2O$ in a beaker and add water to 1 L. Sterilize by autoclave.

7. Some chemicals may react with phosphate buffer, such as copper chloride. Choose an alternate buffered saline solution if necessary.
8. Wild-type worms may be weakly attracted to 1× xylene cyanol in the absence of other stimuli, but the dye usually does not affect behavior to a moderate stimulus. Nonetheless, it is beneficial to repeat experiments without dye to verify minimal contribution of the dye under the conditions tested.
9. Odor dilutions should be freshly prepared each day. IAA is hydrophobic, low density, and weakly soluble in aqueous buffers. Therefore, it rises to the liquid surface and adsorbs to the vial walls and to the pipette tip. We prefer the following dilution procedure: First prepare a $1{:}10^3$ dilution by adding 30 µL IAA to 30 mL worm buffer in a pre-cleaned 40 mL amber vial (ESS Vial). Pipette repeatedly to rinse the pipette tip, then quickly seal and vortex for 30 s. Transfer 3 µL diluted IAA to a second vial containing 30 mL worm buffer for a $1{:}10^7$ final dilution; if desired, include 0.5–1× Dye solution for visualization.
10. Be careful not to gouge the inner surface of the tubing, which may result in a clog or poor seal.
11. Using gloves for improved grip, angle the metal tube into the Tygon tubing until it stretches over the metal tube. Next, push the Tygon tubing down along the metal tube while angling it back and forth. Then grip the Tygon tubing tightly at the metal tube and push firmly down on a cutting mat until it stretches about 1/3 of the way down the 1/2″ long metal tube (4–5 mm).
12. For longer duration experiments, 60 mL syringes or larger reservoirs with Luer fittings may be used.
13. Blocking pins can also be created from stainless steel tubes (NE-1027-12) by applying a droplet of epoxy to one end to seal.
14. Video capture can be performed with a camera attached to the trinocular port of a microscope with a transmitted light base. Alternatively, a less expensive setup can be assembled from a camera, zoom lens, and support stand, for example as described at *albrechtlab.github.io*.
15. This is a messy process. Line the work area with disposable paper, use clean gloves, and be careful to prevent spills, as it is difficult to clean off the highly viscous and oily PDMS materials. We prefer to use a large disposable weigh boat containing 100–120 g PDMS for mixing by hand, as it is easier to ensure complete mixing compared with a cup with tall sides. It is convenient to weigh the viscous base PDMS on a balance,

then tare the balance and pour the curing agent to 1/10 (for 10:1 ratio) of the base weight. An exact 10:1 ratio is not critical, as PDMS will cure properly with a range of base to curing agent from 5:1 to 20:1.

16. We use a transfer pipette or plastic fork to mix PDMS for at least 1 min, using a folding motion to combine the viscous base component with the low viscosity curing agent. Many air bubbles should be generated with proper mixing vigor. Insufficient mixing may result in partially uncured PDMS, an unusable device, and/or a messy mold master.
17. To accelerate this process, briefly vent the chamber and reapply vacuum once or twice.
18. Remove any surface smudges with an isopropanol wash, then water, then air dry. A release agent should be applied to new, untreated mold masters. For silicon wafers, apply TFOCS as described in **Note 25**.
19. To determine the correct PDMS fill volume, first fill with water to the desired depth and note the weight of added water. After drying the mold, add the same weight of PDMS (density 0.97 g/mL is nearly equal to water). For 4–5 mm depth, we fill 85 g PDMS in a 150 mm Petri Dish and 45 g in a 4″ diameter foil boat. To reduce bubble formation, pour quickly with the weigh boat about 1–2 cm above the mold surface while moving linearly across the mold to prevent the PDMS stream from folding upon itself and forming air bubbles.
20. Dust can be carefully removed with a transfer pipette and bubbles on the master surface can be dislodged by gently and slightly tilting the dish back and forth, allowing for viscous shear forces to release the attached bubbles. Bubbles that have risen can be removed by blowing lightly on the PDMS surface.
21. To cleanly cut out the casting, firmly grip the scalpel and insert into the PDMS at an outward angle near the edge of the mold master wafer. Then, making contact between the blade and the wafer, rotate the dish such that the blade follows the perimeter of the wafer making one continuous circumferential cut.
22. If individual devices are outlined with microchannels, it is convenient to align the single-edge razor by feeling for when it falls into the groove. Then, ensure the razor is vertical and press down firmly through to the cutting mat.
23. The punched inlet channel will taper in the direction of punching. Alignment of the inlet hole and the microfluidic features is easier when punching *from* the micropatterned side of the PDMS cast. However, we prefer punching *toward* the micropatterned side for ease of tubing insertions. Tape and mark each inlet with a fine-tip marker on the micropatterned side,

then insert the dermal punch from the opposite side, keeping it straight and vertical. After completely punching through the device, remove the excess PDMS from the punch before pulling it out of the device (Fig. 5g).

24. Holes punched with a damaged or dull dermal punch are prone to leakage. It is advisable to punch a test hole and observe under magnification for smooth round edges free of nicks. Use a new punch if necessary.
25. Render glass and silicon wafer surfaces hydrophobic by vapor deposition of TFOCS. Place cleaned substrates in a vacuum desiccator and add 40 μL of TFOCS to a small aluminum foil dish in the center. Treat for 1 h, then clean with isopropanol, then water, then dry in an air or N_2 stream.

    CAUTION: *TFOCS is corrosive and toxic*. Perform all steps in a fume hood.
26. Mark the drill locations on tape that is applied to the glass, to prevent the ink from washing off during drilling.
27. Hold the glass slide securely on a plastic multi-well (e.g., 96-well) plate, centering the drill hole over a well. This provides support beneath the glass during drilling. Align the bit to the mark, apply water as lubricant and coolant, and apply gentle pressure to grind the glass at 15,000 rpm. Ensure that the bit and glass are wet at all times, or the bit will very quickly become hot and damaged. Continue grinding, releasing pressure every 3–5 s, until the hole is complete.
28. Turn on the vacuum pump and plasma power. After 10–30 s, a plasma should be visible in the vacuum chamber (purple/orange glow). Adjust the gas needle valve until the plasma appears bright red-orange due to the presence of oxygen and nitrogen gases. If no plasma is visible or it appears dim purple, pressure may be too low (open the needle valve slightly to introduce air) or too high (close the needle valve and wait for pressure to decline).
29. Plasma-activated PDMS and glass will form a covalent bond on contact, with no opportunity to reposition them. Carefully align the glass over the microchannel surface, and gently release the glass substrate upon initial contact to prevent internal stresses.
30. Gentle compression for about 10 s ensures a good bond and forces trapped air bubbles out of the device. Test for a successful bond by gently peeling up at each corner of the PDMS; the PDMS device should remain sealed against the glass substrate.
31. Ethanol causes PDMS to swell by 4% *(20)*. Ensure that all ethanol is evaporated by baking at 65 °C for at least 1 h or at room temperature overnight before proceeding with assembly.

Insufficient baking will result in swelling in the center of the device and a poor fluidic seal. Ethanol remaining inside PDMS can be visualized as a cloudy white haze after soaking in water or buffer; if this is observed, additional evaporation time is required.

32. Binder clips should be expanded by bending them open until a 3 mm gap remains at rest (such as by clipping a wide pen barrel and forcing the clip spine toward the barrel). Binder clips should be positioned over a supported PDMS edge without blocking the imaging area, as in Fig. 7. Avoid clamping at an unsupported region of glass or with a clamp that is too small, as stresses may cause the glass to break. The binder clips should grip firmly enough to remain in place but loose enough to be repositioned by sliding along the glass.
33. Fluid will initially flow to the port of least fluidic resistance, often the closest port. It is therefore helpful to block any open ports near the filling port with blocking pins (*see* **Note 13**) to ensure rapid filling of the entire fluidic network.
34. Air bubbles that form upon initial filling of a microfluidic device are absorbed into degassed PDMS. The rate and capacity of bubble absorption increases with time under vacuum and decreases with time outside the vacuum desiccator. Therefore, initial filling of the microchannels with liquid should occur quickly after removing the PDMS device from vacuum, ideally within a few minutes.
35. Remove bubbles from the 3 mL priming syringe by setting the valve to the "Purge" position (Fig. 9), slowly drawing liquid from the 30 mL reservoir, then quickly injecting it back. After 2–3 cycles, all bubbles should be purged.
36. To avoid bubbles trapped in the Luer stub, first ensure that the Luer stub and tubing are dry. Then, rotate the valve to the "Inject" position (Fig. 9) and slowly inject fluid filling the Luer fitting and tubing. If any bubbles are seen in the Luer fitting, flick the fitting while injecting to dislodge and purge them. It is important to remove these air bubbles to avoid their entry into the microfluidic channels during an experiment.
37. It is helpful to set up a vacuum line to aspirate excess liquid on the microfluidic device. Set up a vacuum trap (side-arm flask) and tubing terminated with a male Luer barb and a Luer stub needle (or a 200 μL micropipette tip, trimmed to fit).
38. It is best to flush out loading buffer from the inlet ports, as the Pluronic surfactant reduces the grip between the inlet hole and the metal tube. Flushing will reduce the chance of an inlet tube popping out during an experiment.

39. We advise monitoring the tubing connections and glass substrate for droplet formation indicating a potential leak, especially for a new device. Adjust tubing or clamp tension as needed, or reassemble the device, if a leak occurs.
40. It is convenient to support the microfluidic device on the glass observation stage with four clean scrap PDMS pieces, each about 5 × 10 × 10 mm^3. These supports seal to the stage and to the bottom of the microfluidic device assembly and hold it securely during an experiment (*see* Fig. 1a). Ensure that the scrap PDMS supports are all equal height and do not obscure the arena.
41. To measure total flowrate while all valves are open, briefly remove and reinsert the outflow tubing from the outflow port to introduce a small bubble. Measure the time for the bubble to move 1 cm down the outflow tubing to calculate flowrate (0.02″ ID tubing contains 2 μL/cm of length; a bubble moving 1 cm in 5 s indicates a flowrate of 2 μL/5 s or 0.4 μL/s).
42. Wild-type animals are conveniently picked at L4 larval stage 16–24 h prior to loading as young adult animals.
43. To avoid damaging animals, it is helpful to identify the focal plane of the agar surface with a small mark or bacteria using a worm pick. It is unnecessary to wash animals before transfer to this plate, as any residual bacteria will be washed away in the microfluidic device.
44. Avoid drawing up air bubbles by keeping the metal tube submerged. Ensure that no animals enter into the syringe barrel by drawing up less volume than the tubing (~100 μL for a 50 cm length).
45. After collecting animals into the worm loading tube, deposit them slowly into a small region of the plate and draw them up again into a minimal volume. This process speeds loading of animals into the arena. Draw up enough buffer to observe the last animal in the clear tubing, to ensure that a "drop-to-drop" contact can be made at the worm loading port without losing any animals.
46. Observe animal loading into the arena during gentle injection to ensure they are not damaged. Animals that remain stationary after fast injection may have sustained physical damage.
47. Wild-type animals move slowly for the first 10–15 min in the device. It is beneficial to flush the arena with buffer during this time to wash away any residual bacteria. Verify correct valve positions before initiating the experiment.
48. We typically record at 2 frames per second at a camera resolution of about 40 pixels/mm. Adjust arena position and lens

zoom such that the recorded image is level and fully contained within the video frame. Adjust illumination brightness, camera gain, and/or acquisition time such that animals have good contrast and no pixels are saturated.

49. Commercial and open-source software packages are available for analysis of microfluidic experiment data. For example, ArenaWormTracker quantifies locomotion behavior and NeuroTracker quantifies neural responses, available at *albrechtlab.github.io*
50. Buffer salts that crystallize in tubing may be difficult to remove. Flush with water and air dry by blowing with compressed air after experimentation.
51. Hydrophobic stimuli can leach into PDMS. We submerge devices in a stirred ethanol bath overnight, especially after experiments using high concentrations of hydrophobic stimuli.
52. A surfactant such as Pluronic F127 is not necessary in the loading buffer if the microfluidic channels are narrow. However, Pluronic F127 also prevents protein and bacteria adsorption to the channel surfaces, and should be included if animals are fed bacterial food in the device.
53. Slow buffer flow helps to keep animals in the tapered clamps during time-lapse recordings. For long-term culture in traps, animals may be fed with bacteria.
54. It is useful to add a dye to the control fluid such as 1 μg/mL fluorescein to visualize and confirm proper fluid flow and operation of the valve.
55. When inlets are configured as in Fig. 2c, the animal is subjected to stimulus "a" when the valve is at rest and to stimulus "b" when the valve is energized *(*11*)*.
56. Minimize photodamage and photobleaching by limiting exposure to excitation light and reducing intensity with a neutral density filter.
57. Typical GCaMP recordings are made at ten frames per second. Use a reduced region of interest (ROI) and/or image binning to reduce the acquisition file size.
58. To apply adhesive tape without bubbles, it is convenient to place the clean glass slide(s) on a benchtop, then affix about 5 cm tape directly onto the benchtop, and guide the tape downward onto the glass slide with a ruler or straightedge. Use a blade to free the taped glass from the bench, leaving tabs on the taped glass to help secure it to the cutting mat.
59. Ensure the blade does not travel over the edge of the slide during cutting, otherwise it may become dull.

60. Example parameters for cutting one 50 μm tape layer using the Silhouette Cameo are: force setting 6; speed 3; passes 4; blade depth 6. For two layers (100 μm total), increase force to 9 and passes to 5.
61. If the blade setting is too deep or the force is too high, the blade can dull and scratch the glass. When experimenting with different tape thicknesses and layers, begin with lower settings and iteratively increase the depth and force until a clean cut is produced and cut tape regions separate cleanly.
62. After PDMS has cured and devices are peeled from the mold, check if any tape features came off the mold. Remove any tape in the PDMS channels with tweezers, and prepare new molds as necessary.

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References

1. Chronis N (2010) Worm chips: microtools for C. elegans biology. Lab Chip 10:432–437
2. Muthaiyan Shanmugam M, Subhra Santra T (2016) Microfluidic devices in advanced Caenorhabditis elegans research. Molecules 21: 1006
3. Gupta BP, Rezai P (2016) Microfluidic approaches for manipulating, imaging, and screening C. elegans. Micromachines (Basel) 7:123
4. Cornaglia M, Lehnert T, Gijs MAM (2017) Microfluidic systems for high-throughput and high-content screening using the nematode Caenorhabditis elegans. Lab Chip 17:3736–3759
5. Kamili F, Lu H (2018) Recent advances and trends in microfluidic platforms for C. elegans biological assays. Annu Rev Anal Chem (Palo Alto Calif) 11:245–264
6. Midkiff D, San-Miguel A (2019) Microfluidic technologies for high throughput screening through sorting and on-chip culture of C. elegans. Molecules 24:4292
7. Ben-Yakar A (2019) High-content and high-throughput in vivo drug screening platforms using microfluidics. Assay Drug Dev Technol 17:8–13
8. Levine E, Lee KS (2020) Microfluidic approaches for Caenorhabditis elegans research. Anim Cells Syst (Seoul) 24:311–320
9. Albrecht DR, Bargmann CI (2011) High-content behavioral analysis of Caenorhabditis elegans in precise spatiotemporal chemical environments. Nat Methods 8:599–605
10. Hulme SE, Shevkoplyas SS, Apfeld J et al (2007) A microfabricated array of clamps for immobilizing and imaging C. elegans. Lab Chip 7:1515
11. Chronis N, Zimmer M, Bargmann CI (2007) Microfluidics for in vivo imaging of neuronal and behavioral activity in Caenorhabditis elegans. Nat Methods 4:727–731
12. Tian L, Hires SA, Mao T et al (2009) Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. Nat Methods 6:875–881
13. Bartholomeusz DA, Boutte RW, Andrade JD (2005) Xurography: rapid prototyping of microstructures using a cutting plotter. J Microelectromech Syst 14:1364–1374

14. Speller NC, Morbioli GG, Cato ME et al (2019) Cutting edge microfluidics: Xurography and a microwave. Sensors Actuators B Chem 291:250–256
15. Shrirao AB, Hussain A, Cho CH et al (2012) Adhesive-tape soft lithography for patterning mammalian cells: application to wound-healing assays. BioTechniques 53:315–318
16. Larsch J, Ventimiglia D, Bargmann CI et al (2013) High-throughput imaging of neuronal activity in Caenorhabditis elegans. Proc Natl Acad Sci 110:E4266–E4273
17. Chokshi TV, Bazopoulou D, Chronis N (2010) An automated microfluidic platform for calcium imaging of chemosensory neurons in Caenorhabditis elegans. Lab Chip 10:2758
18. Haubert K, Drier T, Beebe D (2006) PDMS bonding by means of a portable, low-cost corona system. Lab Chip 6:1548
19. Ginn BT, Steinbock O (2003) Polymer surface modification using microwave-oven-generated plasma. Langmuir 19:8117–8118
20. Lee JN, Park C, Whitesides GM (2003) Solvent compatibility of poly(dimethylsiloxane)-based microfluidic devices. Anal Chem 75: 6544–6554