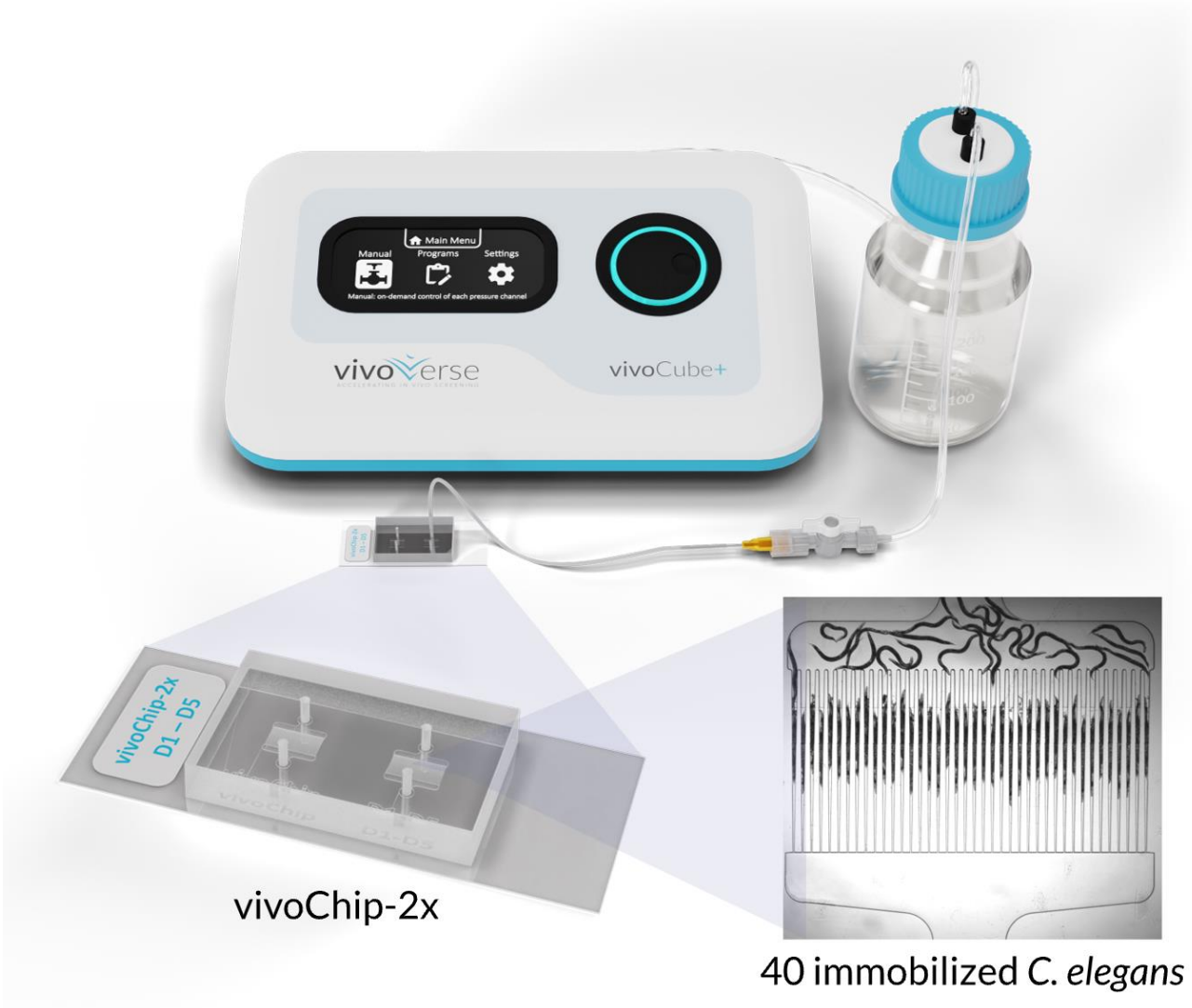
vivoChip®-2x and vivoCube+® Operation Manual™

Version: August 2023



Document Number: VVS-QA-015-02

support@vivoverse.com

I Table of Contents

II Disclaimers and Safety Information

A General Disclaimer

This instruction manual is for the vivoChip-2x system. To ensure safety, obtain optimum performance, and to become sufficiently familiar with the use of this device, any user of the vivoChip-2x should study this manual thoroughly before operating the vivoChip-2x. Retain this instruction manual in an easily accessible place near the vivoChip-2x workstation for future reference. If the device is used in a manner not specified by this manual, the safety of the user and he functional integrity of the vivoChip-2x may be compromised. Always use the vivoChip-2x as outlined in this instruction manual.

B Intended Use

This device has been designed to be used as a microfluidic imaging tool in various routine lab work and research applications. Do not use this device for any purpose other than its intended use. This device is intended solely for research use and is not to be used for any other purposes including, but not limited to, unauthorized commercial purposes (including Contract Research, diagnostics, or manufacture), military purposes, veterinary or clinical use. Contact vivoVerse for full terms and conditions.

C Handling Precautions, Maintenance, and Storage

- The vivoChip-2x is a precision scientific device. Use reasonable care in using, handling, storing, transporting, and disposing of the vivoChip-2x (including by wearing appropriate protective equipment). Ensure it is used only by qualified laboratory personnel who have been trained to use it. Avoid subjecting it to sudden or severe impact.
- Connect any tubing to the vivoChip-2x gently.
- Do not use the vivoChip-2x where it is subjected to dust or strong vibrations.
- To clean fingerprints, dirt, or stains from the cover glass on the vivoChip-2x, use a soft lens tissue to apply a >70% alcohol cleaning solution.
- When disposing of this product, follow any regulations and rules on the disposal of sharps and toxic substances (if used in worm culture) set by your organization and/or the local government.
- Refer to the vivoCube+ User Manual™ (document number VVS-QA-016) for handling precautions, maintenance, and storage guidance for the vivoCube+.

D Warranty

Visit vivoverse.com/salesterms for limited warranty coverage for the vivoChip-2x and vivoCube+.

E Copyright

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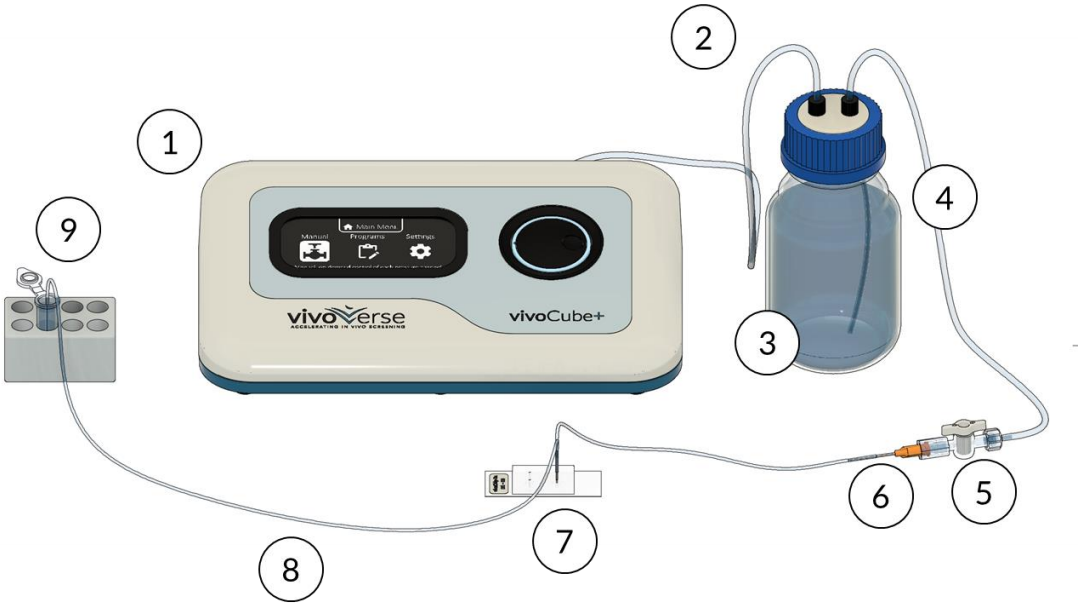
III Complete vivoChip-2x and vivoCube+ System Setup Information

A vivoChip-2x and vivoCube+ System Setup



1. vivoCube+
2. Air tubing
3. GL45 buffer bottle with barbed connectors for buffer
4. Buffer tubing
5. Stopcock valve
6. Luer stub (connected to Input tubing)
7. vivoChip-2x with metal couplers
8. Exit tubing
9. Waste container

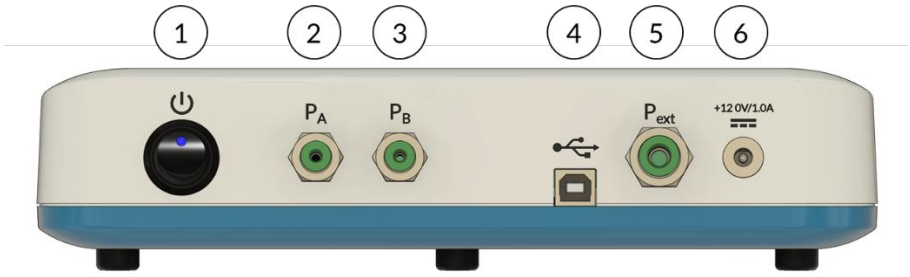
Figure 1: Diagram of complete vivoChip-2x and vivoCube+ system setup with labels

B vivoCube+ Front Panel Schematic

1. OLED screen 2. Multifunction dial

Figure 2: Diagram of the vivoCube+ front panel with labels

C vivoCube+ Rear Panel Schematic



1. Power switch
2. Channel A pressure port (P_A)
3. Channel B pressure port (P_B)
4. USB port
5. External pressure supply port (P_{ext})
6. DC power jack (12V, 1A, ⊖—●—⊕)

Figure 3: Diagram of connectors on the vivoCube+ rear panel with labels

D Electrical Power

The vivoCube+ requires electrical power to function. A power adapter is provided with interchangeable N-, E-, B-, and A-type blades shown in Figure 4, covering most power outlets in North America, Europe, United Kingdom, and Australia, respectively. If you require a different type of power adapter for your electrical power supply, please contact support@vivoverse.com with your country information.

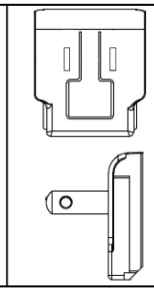
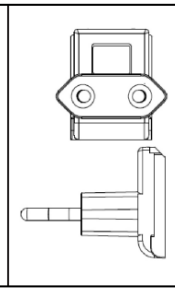
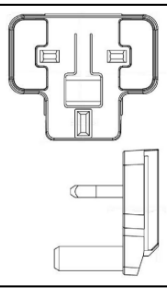
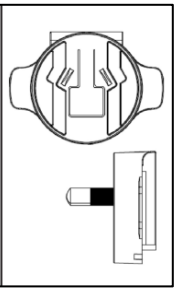
BLADE DESIGNATOR	N	E	B	A
REGION	North America	Europe	UK	Australia
BLADE				

Figure 4: Diagram of the different power blade adapters packaged with the vivoCube+ system

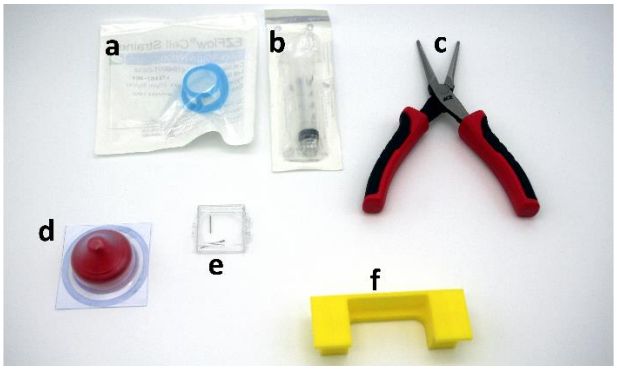
IV Package Contents

The vivoCube+ and vivoChip-2x bundle contains the following items:

vivoCube+
with power supply and USB cable

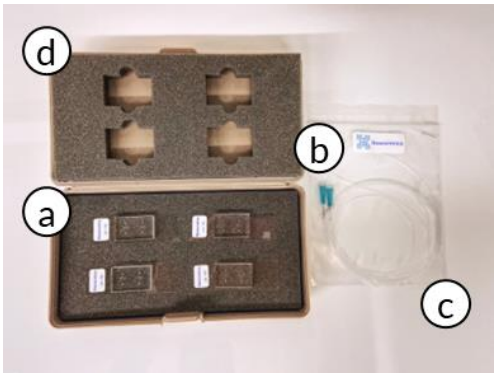
Bottle Kit

Starter Kit



a. 40 µm worm filter
b. 5 mL syringe
c. Needle nose pliers
d. 1.2 µm syringe filter
e. Anesthetic tubing with metal coupler, and metal plug
f. Upright microscope adapter, if included

vivoChip-2x Set



*Number of sets and vivoChip-2x models in each depend on the bundle configuration purchased

a. 4× vivoChip-2x
b. Input tubing, in bag (3’ tubing with green or orange luer stubs and metal coupler)
c. Exit tubing, in bag (2’ tubing with metal coupler and no luer stub)
d. Storage case

V Before You Begin: Preparing Worms, Checking Your Imaging Setup, and Planning Your Study

Follow these tips to obtain the best immobilization, orientation, and imaging performance using the vivoChip-2x system:

- **Grow a synchronized batch of worms ahead of time**: the vivoChip-2x is of no use without worms to immobilize! For best results, grow a batch of age-synchronized *C. elegans* worms following the hypochlorite bleaching protocol given in Appendix 0. Age synchronization ensures the best alignment of the worms within the channels for easiest imaging.
  - The vivoChip-2x can be used with other nematodes (or organisms) besides *C. elegans* as long as the vivoChip-2x model is selected according to the size of the individuals in the population to be loaded. Contact the vivoVerse team at support@vivoverse.com for more information.

- **Grow enough worms**: grow enough worms to load into the vivoChip-2x. Transferring too few worms (<30) into the vivoChip-2x will severely inhibit the immobilization achieved by the vivoChip-2x system. The fewer the number of worms, the worse the immobilization will be.
  - Transferring too many worms will not cause adverse effects.
  - Follow the recommended minimum number for different worm stages/vivoChip-2x models:
    - L1: 150-200
    - L2-L3: 150-200
    - L4-YA: 80-120
    - D1-D5: 80-120

- **Prepare enough buffer**: at least 200 mL of buffer is required to operate the vivoChip-2x system.

- **Use anesthetic to image the finest features (optional)**: if you are interested in imaging fine features at the tip of the head or tail of the worm, you may use anesthetic to further immobilize the worms to improve image quality for longer exposures. Refer to Appendix A.2 for a protocol to load anesthetized worms into the vivoChip-2x.
  - Note that due to the physical immobilization provided by the vivoChip-2x, the concentration of anesthetic required is typically lower than if anesthetic were to applied to worms outside the vivoChip-2x.

- **Have a low magnification imaging setup accessible near your final imaging station**: it is necessary to have a low magnification microscope setup (e.g., a dissecting microscope) accessible during certain steps of the loading process. Having this as part of your final imaging station or at least near it is ideal.

- **Check your imaging setup**: before your first experiment, check that the vivoChip-2x fits within your microscope imaging system. Note that the connected tubing will add required clearance height above the vivoChip-2x.
  - This is especially important with upright microscope systems. An upright adapter is provided with the system for labs planning to use an upright microscope.
  - If you encounter any issues with fit, contact the vivoVerse team at support@vivoverse.com.

First Time Setup

1.1 Ensure the entirety of the components in Section IV Package Contents are present in the shipment you received.

1.2 The buffer bottle and ported cap will need to be assembled before the system is used for the first time. Refer to Figure 5 for these steps.

a Connect the buffer tubing (short tubing with stopcock from the Bottle Kit) to the bottle cap port above the plastic intake tube inside the bottle (right-hand side Figure 5) by screwing the black connector into the white plastic port on the bottle cap. Tighten it until snug.

b Connect the air tubing (long tubing from the Bottle Kit) to the other bottle cap port (left-hand side of the bottle in Figure 5) by screwing the black connector into the other white plastic port on the bottle cap. Tighten it until snug.

c ***Caution:*** *do not bend the translucent plastic barbs at the ends of these black connectors, which can break if handled improperly.*

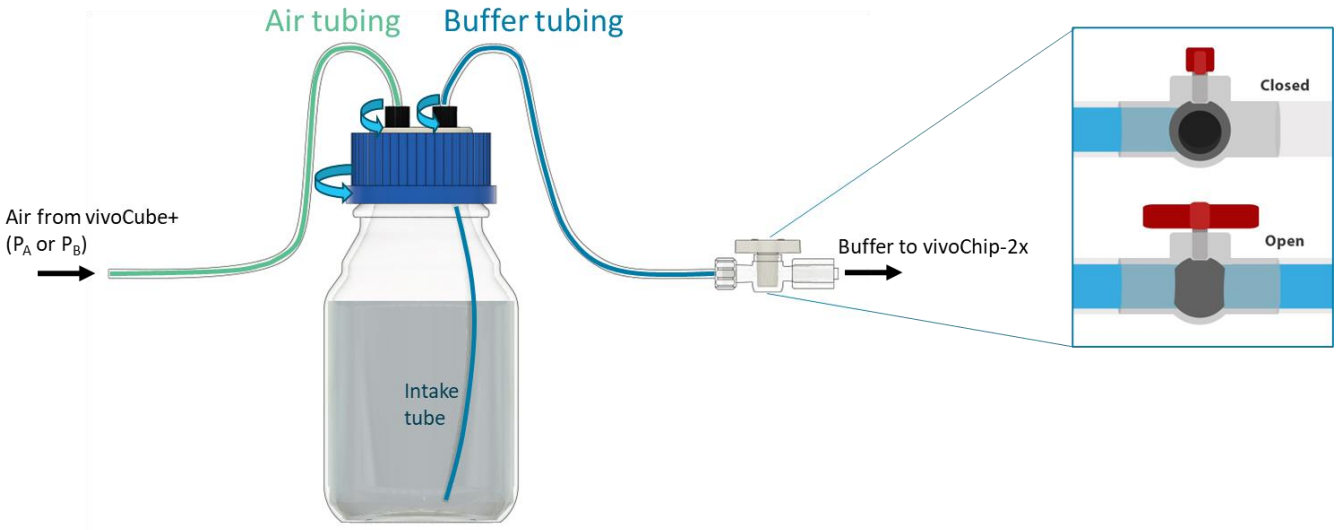


Figure 5: Assembly of the buffer bottle air and buffer tubes

1.3 Familiarize yourself with the open and closed positions of the stopcock shown in Figure 5.

a Turning the stopcock handle to the open position will be referred to as "opening" the valve.

b Turning it to the closed position will be referred to as "closing" the valve.

1.4 Perform the quality inspection on the vivoCube+ upon receiving the shipment according to the vivoCube+ Quality Inspection Document™ (document number VVS-QA-016-F01), if not completed already.

1.5 Before operating the vivoCube+, it is recommended to read the vivoCube+ User Manual™ (document number VVS-QA-016) included with the vivoCube+.

1.6 Remove the luer caps (see Figure 6) from the preconnected tubing attached to the vivoCube+ pressure ports and keep them for storage.

a If your vivoCube+ does not have preconnected tubing attached to the pressure ports, disregard this step.

Figure 6: Image of a luer cap

vivoCube+ and Buffer Bottle Setup, Testing, and Priming

2.1 Fill the buffer bottle with buffer if not already full.

a The buffer bottle should contain at least 200 mL of nematode buffer (such as M9, chemotaxis, S media, S basal, and so on).

b ***Note:*** *If using anesthetic to optionally further immobilize the worms, fill the bottle with at least 200 mL of buffer mixed with the required anesthetic concentration (e.g., 5 mM levamisole). Refer to Appendix A.2 for a protocol to load anesthetized worms into the vivoChip-2x.*

c If the bottle is underfilled, remove the cap by unscrewing it and fill it with the required volume of buffer.

d ***Tip****: to maximize the reusability of the vivoChip-2x device, filter the buffer using the 1.2 µm syringe filter provided in the Starter Kit. This step is optional.*

e Screw the cap back on to seal the bottle shut.

2.2 Check that the cap is screwed tightly onto the buffer bottle. This prevents pressure leakage.

2.3 If your vivoCube+ includes preconnected tubing attached to the pressure ports on the rear panel, connect the air tubing of the buffer bottle to the tubing preconnected to port A (P_A) of the vivoCube+

a Refer to Figure 7 for the twist-lock connection method between the plug/male luer lock connector at the end of the vivoCube+ port A tubing and the socket/female luer lock connector at the end of the air tubing (connected to the buffer bottle).

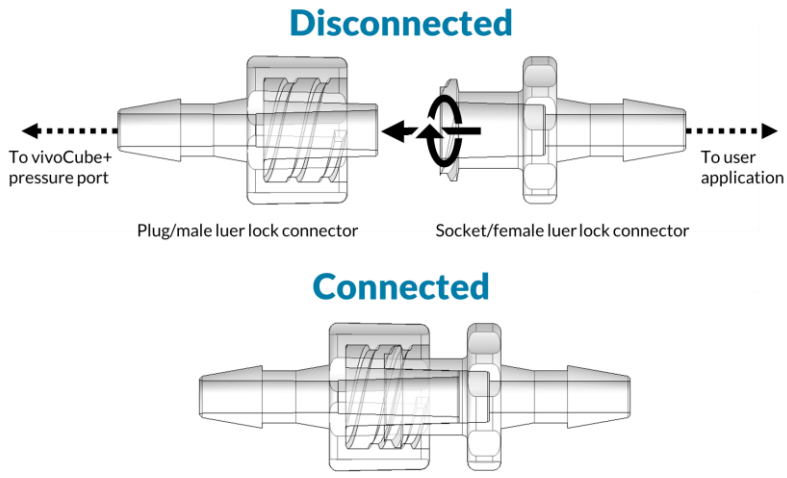


Figure 7: Diagram of plug/male and socket/female luer lock connectors.

2.4 If your vivoCube+ does <u>not</u> include preconnected tubing attached to the pressure ports on the rear panel, connect the air tubing directly to one of the pressure ports of the vivoCube+. In this instruction manual, port A (P_A) is used.

a Insert the end of the air tubing into the one of the ports on the rear of the vivoCube+ (P_A or P_B, shown in Figure 8), firmly pushing the tubing into the green fitting.

b ***Tip****: improper connection of the tubing to the pressure port is a common mistake in setup of the vivoCube+. Double check that the tubing is inserted completely into the fitting. You should feel*

resistance as the tubing is pushed deeper into the fitting as the fitting grips the flexible tubing, and eventually the tubing will be not be able to be pushed in any farther.

c To disconnect the tubing, press and hold the green release ring and then pull the tubing out of the fitting.

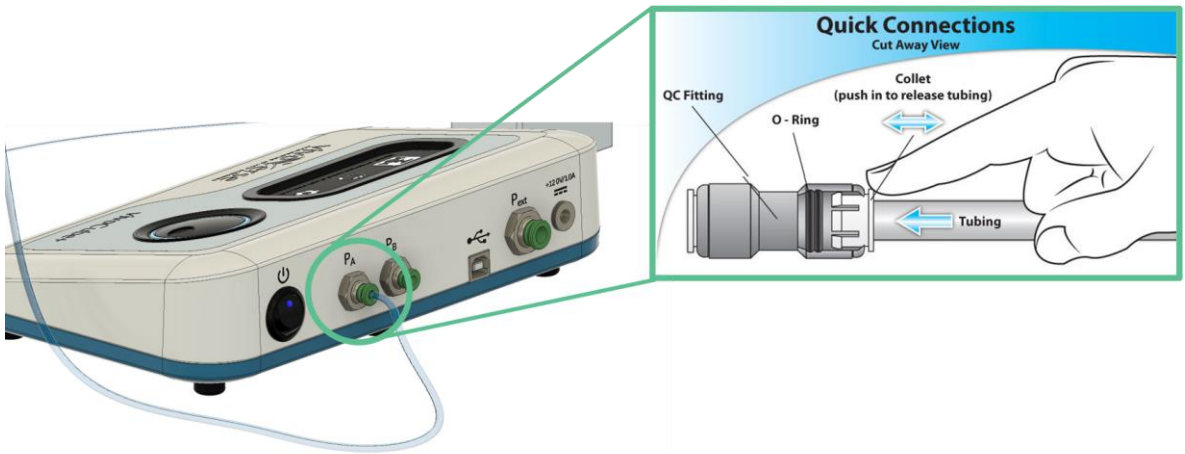


Figure 8: Rear panel of the vivoCube+ with diagram of push-to-connect fitting operation

2.5 Close the stopcock at the end of the buffer tubing (refer to Figure 5 if you are unsure how to do so).

2.6 Connect the vivoCube+ to a power source and turn it on.

a Plug the vivoCube+ power supply into an electrical outlet.

b ***Tip****: typically, the vivoCube+ should be operated adjacent to the final imaging microscope with access to a low magnification objective included or nearby.*

c Connect the barrel jack of the vivoCube+ power supply to the DC power jack (Item 6 in *Figure 3*) on the rear of the vivoCube+.

d Flip the power rocker switch (Item 1 in *Figure 3*) on the rear of the vivoCube+ to the on position (up). The LED light in the rocker switch will illuminate and the display on the front panel of the vivoCube+ will turn on if powered on correctly.

e After the boot sequence completes, the vivoCube+ Home menu will appear on the display, shown in Figure 9.

Figure 9: Depiction of vivoCube+ Home menu with Manual menu option highlighted

2.7 Set the output pressure of channel A to 4.000 psi on the vivoCube+ using the Manual pressure control menu.

a ***Tip:*** *the general operation of the multifunction dial on the front panel of the vivoCube+ (Item 2 in Figure 2) involves turning the dial clockwise or counterclockwise to select the desired option on the display and then pressing/pushing/clicking the dial itself to select that option. In the steps below, the word "select" is used to mean turning the dial to find the desired option on the display and pushing or clicking the dial itself.*

b On the vivoCube+ Home menu, select the Manual menu option.

c Click "Set P(A)" to set the desired pressure for pressure channel A (corresponding to the port labeled P_A on the rear panel of the vivoCube+).

d ***Note:*** *to set the target pressure in "Manual" pressure mode in the Manual menu on the vivoCube+, all four digits of the psi reading (e.g., 4.000, 5.000, etc.) must be set. When setting the digits, the cursor begins on the ones place. Use the multifunction dial to increment or decrement the selected digit and press/push/click the dial itself to set that digit. The cursor then moves to the tenths place. Repeat these steps to set each of the four digits.*

e Rotate the dial to set the pressure to 4.000 psi (setting all four digits as noted above).

f Navigate to "Turn on P(A)" and select it to turn on the pressure output.

g Keep the cursor hovering over "Turn off P(A)."

2.8 Prime the buffer tubing.

a Place the end of the buffer tubing over a waste container (e.g., an open Eppendorf tube or centrifuge tube held in a rack).

b ***Tip****: taping the tubing to the waste container is an easy way to secure it in place.*

c ***Caution:*** *to prevent damage, avoid liquid waste dripping on the vivoCube+ unit.*

d Open the stopcock at the end of the buffer tubing (refer to Figure 5 if you are unsure how to do so).

e Observe the end of the buffer tubing to see when the buffer begins to flow into the waste container.

f As soon as buffer flows through the tubing into the waste container, close the stopcock. The buffer tubing is now primed.

g Look at the live pressure reading. It should read near 4 psi.

h If liquid does not flow through the exit tubing or if the pressure does not read near 4 psi, please see Appendix B to troubleshoot.

i Select "Turn off P(A)" on the vivoCube+ menu.

2.9 If using a vivoChip-2x L1 device or intending to create publication-quality images, perform the steps in Appendix A.1 to prime the vivoChip-2x.

a This process is entirely optional (unless using a vivoChip-2x L1 device) and has no effect on the immobilization and orientation of worms within the channels of the vivoChip-2x device or on the quality of the images of the worms inside the channels. The benefit of priming the vivoChip-2x is that it removes air bubbles from parts of the microfluidic vivoChip-2x device outside the channels.

2.10 Your vivoCube+ system is now ready to use. Proceed to the next step.

Transferring Worms from Growth Plate to Centrifuge Tube

3.1 Fill the 5 mL syringe with 3 mL of buffer.

3.2 Connect the luer stub (Item b in the vivoChip-2x Set in Section IV Package Contents) to the luer lock tip of the 5 mL syringe as seen in Figure 10.

a Do so by screwing the luer stub to the luer connector of the syringe.
b Refer to Figure 7 for a visual representation of the twist-lock mechanism.

Figure 10: Photo of the luer stub screwed onto the 5 mL syringe

3.3 Some steps in the manual have different methods depending on the stage of worm and the vivoChip-2x model used. In steps like this, follow the steps with the label corresponding to the stage/vivoChip-2x model you are using.

vivoChip-2x L1	vivoChip-2x L2-L3	vivoChip-2x L4-YA	vivoChip-2x D1-D5
L1	L2-L3	L4-YA	D1-D5
For Larval 1 worms	For Larval 2 to Larval 3 worms	For Larval 4 to Young Adult worms	For Day 1 to Day 5 Adult worms

3.4 While performing steps 3.5 through 3.11, refer to Figure 11, which outlines the process of transferring worms from the growth plate to the input tubing.

a ***Note:*** C. elegans *worms adhere to plastics so it is essential to use glass pipettes and glass dishes to manipulate or hold worms. If using plastic pipette tips or plastic dishes, you might experience significant loss of worms during transfer steps.*

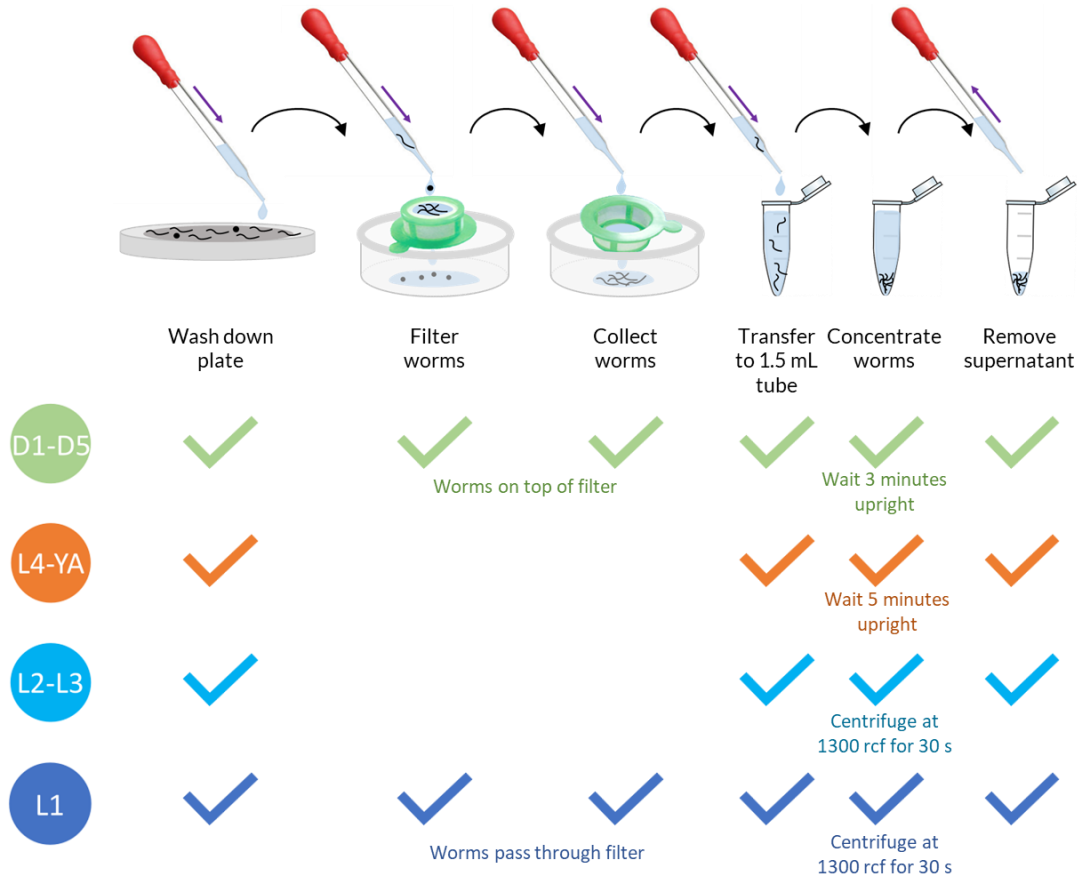


Figure 11: Schematic of plate to tube transfer steps for different worm stages

3.5 Take the following steps depending on which stage and vivoChip-2x model you are working with:

D1-D5

Follow step 3.6 to set up and prime the 40 µm filter. Filtering reduces the number of eggs and other debris entering the vivoChip-2x.
Caution: *neglecting to filter these eggs can inhibit vivoChip-2x performance by blocking channels.*

L4-YA

Filtering is not necessary. Proceed to step 3.7.

L2-L3

Filtering is not necessary. Proceed to step 3.7.

L1

Optionally, follow step 3.6 to set up and prime the 40 µm filter (or a 20 µm filter, not included). Filtering will help remove any large debris such as cuticle fragments or unhatched eggs that might block the narrow channels.

3.6 Set up and prime a 40 µm filter.

a Locate the 40 µm worm filter in the starter kit and place it in a glass dish.

b Wet the filter by pipetting enough buffer onto the bottom of the filter in order to form a hanging droplet on the other side. If necessary, squirt buffer through more vigorously to form the droplet.

3.7 Wash down the growth plate and pipette the worms from it.

a Retrieve the batch of age-synchronized worms (*C. elegans* grown following the hypochlorite bleaching protocol given in Appendix 0).
b ***Note**: it is assumed for this instruction manual that the worms have been grown on an NGM plate.*
c Using a glass pipette, slightly tilt the NGM plate containing the worms and drip 1-2 mL of buffer onto the top end to wash them down.
d Aspirate the buffer containing worms pooled at the bottom of the tilted plate into the glass pipette.

3.8 Take the following steps depending on which stage and vivoChip-2x model you are working with:

D1-D5

a. Refer to Figure 11 for the correct orientation of the worm filter. Flush the buffer containing worms through the bottom of the strainer worm filter. The eggs and particles will pass through the filter while the adult worms will remain on the top side of the filter mesh.
b. Turn the worm filter over and place it in a clean glass dish.
c. Drip fresh buffer through the mesh to wash the worms into the dish.
d. Tilt the dish and aspirate the filtered buffer containing worms pooled at the bottom of the tilted dish into the glass pipette.

L4-YA

Filtering is not necessary. Proceed to step 3.9.

L2-L3

Filtering is not necessary. Proceed to step 3.9.

L1

a. (optional) Flush the buffer containing worms through the bottom of the strainer worm filter. The worms will pass through the filter while the debris will remain on the top side of the filter mesh.
b. (optional) Tilt the dish and aspirate the filtered buffer containing worms pooled at the bottom of the tilted dish into the glass pipette.

3.9 Drip the buffer containing the worms into a 1.5 mL centrifuge tube held upright, e.g., by a tube rack.

3.10 Concentrate the worms at the bottom of the tube into a pellet depending on which stage and vivoChip-2x model you are working with:

D1-D5

Set the tube upright and wait 3 minutes to allow enough time for the worms to fall to the bottom of the tube.

L4-YA

Set the tube upright and wait 5 minutes to allow enough time for the worms to fall to the bottom of the tube.

L2-L3 Seal the tube and centrifuge it at 1300 rcf (or g) for 30 s. Set upright afterward.

L1 Seal the tube and centrifuge it at 1300 rcf (or g) for 30 s. Set upright afterward.

3.11 Without disturbing the pellet, aspirate the supernatant to leave the worms in a 50-100 µL volume of buffer.

a The use of a small, concentrated volume of worms aids the process of transferring and loading worms into the vivoChip-2x in subsequent steps.

Transferring Worms from Centrifuge Tube to vivoChip-2x

4.1 Place the vivoChip-2x on a flat, clean surface near the upright tube containing the concentrated worms.

a ***Caution***: *do not press the vivoChip-2x against an uneven, flexible (such as a paper towel), or dirty surface—doing so can break the bottom glass substrate.*

b Take note of the locations of the inlet and exit holes of the vivoChip-2x as shown in Figure 12.

c ***Note***: *the vivoChip-2x contains 2 duplicate neighboring microfluidic devices. Either can be used.*

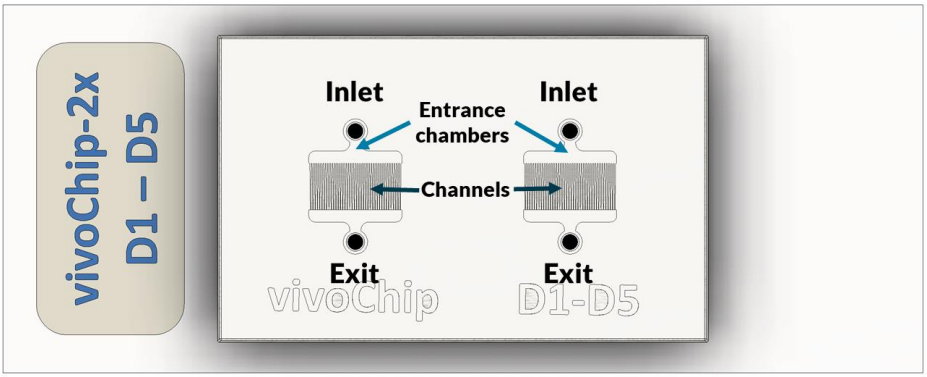


Figure 12: Top-down view of vivoChip-2x showing inlets and exits of microfluidic devices

4.2 While performing steps 4.3 through 4.11, refer to Figure 13, which outlines the process of transferring worms from the growth plate to the input tubing.

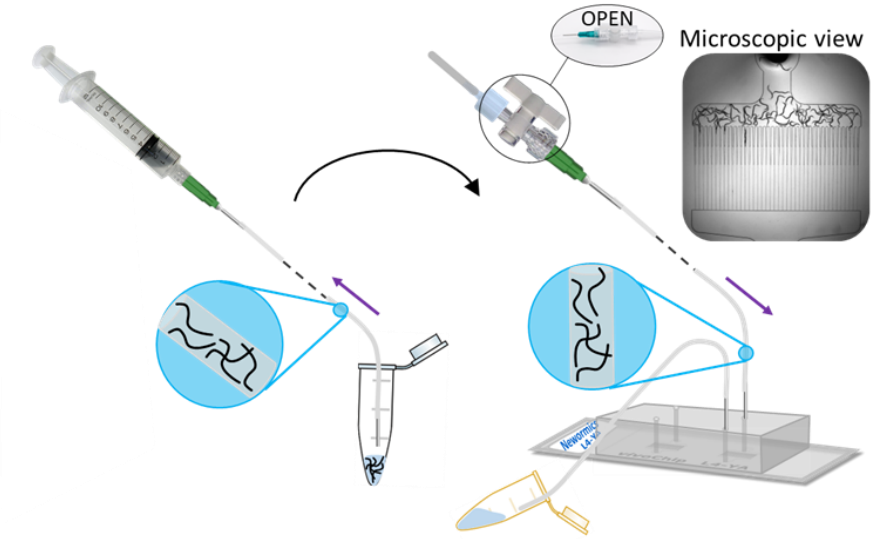


Figure 13: Schematic steps for transfer of worms from 1.5 mL centrifuge tube to vivoChip-2x using included syringe and tubing

4.3 Connect the metal coupler at the end of the exit tubing (to which a metal coupler is attached but no luer stub) to the exit port (see Figure 12) of one of the microfluidic devices (left or right) of the vivoChip-2x.

a Using either your thumb and index finger or the needle nose pliers (Item c in the Starter Kit in Section IV Package Contents), grip the metal coupler.
b Keeping the coupler vertical, gently push the end of the coupler into the vivoChip-2x exit port (see Figure 12), keeping the glass coverslip on the bottom of the vivoChip-2x held against the hard, flat work surface.
c ***Caution****: while pushing the metal coupler into the hole in the device, do not hold the coupler at an angle away from the vertical. Doing so can tear the rubbery part of the vivoChip-2x (made of PDMS material), causing fluid leakage during pressure cycle steps.*
d The coupler should be pressed into at least ¾ of the overall vivoChip-2x thickness (roughly 4 mm) when viewed from the side.

4.4 Prime the input tubing connected to the 5 mL syringe (from step 3.2).

a After positioning the metal coupler at the end of the input tubing over a waste container, slowly and gently depress the plunger of the syringe to drip buffer out of it.
b Once you see flow emerge from the metal coupler at the end of the input tubing, stop pressing the plunger.

4.5 If using anesthetic to optionally further immobilize the worms, refer to Appendix A.2 at this point for a protocol to load anesthetized worms into the vivoChip-2x.

4.6 Read all of steps 4.7 and 4.8 before proceeding. Performing these steps in quick succession ensures no worms are lost in the transfer process.

4.7 Aspirate the worms into only the very end of the metal coupler/input tubing connected to the syringe.

a Dip the metal coupler at the end of the input tubing down to the bottom of the 1.5 mL centrifuge tube containing the pellet of worms.
b Aspirate just enough liquid to pull up the worm pellet from the bottom of the 1.5 mL tube, avoiding bringing the liquid too far into the tubing.
c ***Tip****: the smaller the aspirated liquid volume, the faster and more efficient loading into the vivoChip-2x will be.*
d ***Caution:*** *pulling the syringe plunger too swiftly can bring the worms too far into the tubing and even into the body of the syringe. Doing so will make it difficult to flow them into the vivoChip-2x.*

4.8 Using the method described in 4.3, immediately connect the metal coupler at the end of the input tubing (currently connected to the syringe) to the inlet port of the same microfluidic device of the vivoChip-2x (see Figure 12) connected to in step 4.3.

4.9 Refer to Figure 14 to confirm that the tubing connections to the vivoChip-2x have been made properly.

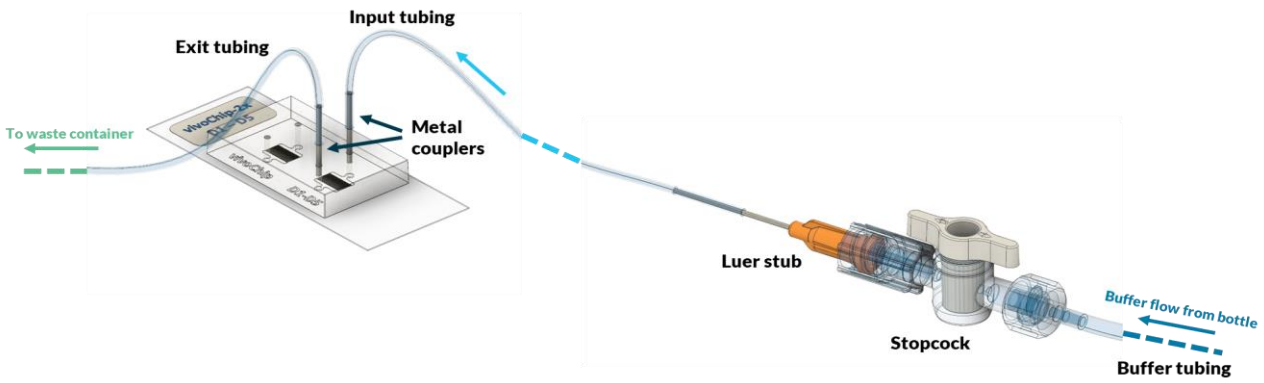


Figure 14: Connection of the input and exit tubing to the vivoChip-2x

4.10 Place the vivoChip-2x on a dissecting stereoscope or microscope with a low magnification objective (e.g., 2× if a stereoscope is not available).

a Viewing the vivoChip-2x at a low magnification is important for the initial worm loading process. If your final imaging station includes a low magnification objective, you can use that.

4.11 Push the worms into the entrance chamber of the vivoChip-2x following the appropriate steps below for your vivoChip-2x type and stage of worms.

D1-D5 & L4-YA

a. While viewing the vivoChip-2x through the stereoscope or microscope, elevate the syringe above the vivoChip-2x and gently squeeze the syringe plunger until you observe the first worm enter the entrance chamber.
b. Once you observe the first worm enter, apply gentle 1-2 second bursts of pressure until you see 40-50 worms enter the entrance chamber.
c. ***Caution:*** *once the worms are inside the vivoChip-2x, do not apply a large or sustained pressure using the syringe plunger. Doing so could push worms into the channels too strongly, which will reduce the effectiveness of the vivoChip-2x for orientation of the worms within the channels and could potentially cause permanent damage to their bodies.*

L2-L3

Proceed to step 4.12.

L1

Proceed to step 4.12.

4.12 Unscrew the luer stub from the syringe and immediately connect it to the one-way stopcock at the end of the buffer tubing (connected to the bottle).

a A quick connection process will prevent air bubbles from being introduced at the tip of the luer stub.
b **Caution:** throughout the remainder of this process, keep the buffer bottle elevated above the vivoChip-2x. Otherwise, a gravity-driven backflow can drag the worms back into the input tubing, causing lower trapping efficiency and poorer vivoChip-2x performance.

4.13 Ensure that the end of the exit tubing is securely placed in a waste container.

Using the vivoCube+ to Immobilize Worms in the vivoChip-2x and Imaging Them

5.1 From the Home menu of the vivoCube+, select the Programs menu (the middle option) shown in Figure 15.

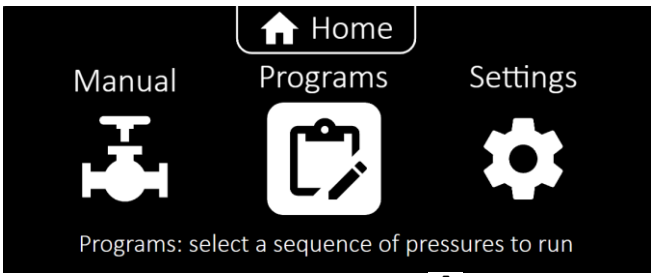


Figure 15: Depiction of vivoCube+ Home Menu with the Programs menu option highlighted

5.2 Within the Programs menu, select the program name corresponding to the vivoChip-2x model you are using. For example, if you are using a vivoChip-2x D1-D5 model, select the "vivoChip-2x D1-D5" program.

5.3 Select the channel corresponding to the pressure port you connected the air tubing to in step 2.3 (Channel A in this manual) in the channel selection menu.

5.4 Select Start (▶) on the Running Program screen to begin the immobilization process.

5.5 Open the stopcock.

D1-D5

Proceed to step 5.6.

L4-YA

Proceed to step 5.6.

L2-L3 & L1

The program begins with a 1.000 psi holding pressure. Periodically observe the vivoChip-2x under the microscope and count the number of worms in the channels. When at least 30 channels have at least 1 worm anywhere in the channel, select Step Forward (⏭).

***Tip**: worms at these stages are small relative to the channels, and you may have to look deep into the channels to find them to count. Figure 16 shows L1 worms immobilized in an L1 vivoChip-2x for reference.*

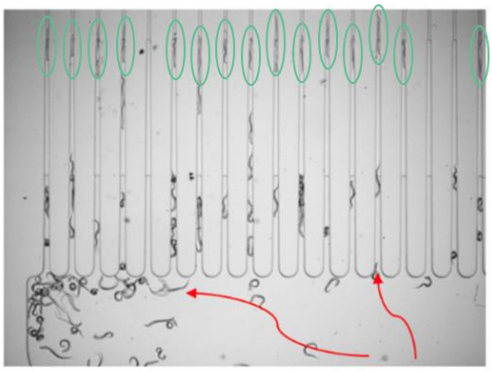


Figure 16: Image of vivoChip-2x showing direction of flow and smaller trapped worms

5.6 The vivoCube+ pressure cycles will automatically load the worms inside the microfluidic channels, orienting them laterally and immobilizing them.

a As the program progresses, the status and duration information will be displayed on the vivoCube+ screen with the Program Progress menu.

b ***Tip****: press Step Back (⏮) to step backwards through the program for additional pressure cycles if needed.*

c At the end of the program, the display will read "COMPLETE!", and the vivoCube+ will maintain a holding pressure to keep the worms immobilized for high-resolution imaging.

d No further action within the vivoCube+ menu is needed. Leave the vivoCube+ on and at this menu for as long as you need to image the immobilized worms.

5.7 Prepare the vivoChip-2x for imaging and set it up in your final imaging station, if different than the imaging station used in steps 4.10-5.4.

a For best imaging results, clean the glass coverslip at the bottom of the vivoChip-2x using a soft lens tissue and a >70% alcohol cleaning solution.

b ***Caution****: the glass coverslip on the bottom of the vivoChip-2x is delicate. Be careful when handling it.*

5.8 If you need to change stations or rooms to perform your final imaging (or if the vivoCube+ needs to be unplugged at any point), it is possible to relocate and set up the system again after immobilization without letting the worms out of the channels in the vivoChip-2x by following these steps:

a Take note of the pressure the vivoCube+ is providing on the "COMPLETE!" screen.

b Power off the vivoCube+ and unplug the power source from the wall.

c ***Caution****: it is critical that while the vivoCube+ is powered off, the buffer bottle is held <u>above</u> the vivoChip-2x. Doing so maintains pressure that keeps the worms from backing out of the channels.*

d Transport the vivoChip-2x and vivoCube+ with all the connected tubing and accessories (for example, by carting it) to the final imaging station.

e Connect the vivoCube+ to a power source and power it on.

f Use the vivoCube+ Manual pressure control menu to set an output pressure equal to the pressure noted in 5.8a (for the pressure channel used) and turn on the pressure.

g ***Tip****: if the need to relocate is frequent, it is recommended to use a portable power bank to maintain power to the vivoCube+ for convenience during relocation.*

5.9 Mount the vivoChip-2x with the immobilized worms on an inverted microscope or stereoscope for imaging.

a If using an upright microscope, use the upright microscope adapter (Item f in the Starter Kit in Section IV Package Contents). Consult Appendix A.4 for more details.

b ***Tip**: the vivoCube+ has a built-in pump for supplying pressure. It is recommended to place the vivoCube+ on a surface separate from your microscope during imaging whenever possible to mitigate any effects the vibration of the pump might have on imaging.*

c ***Caution**: keep the exit tubing inserted in a waste container.*

5.10 Begin observations by focusing on the microfluidic channels/worms using a low magnification (such as 2× as shown in Figure 17) objective before switching to a higher magnification if possible.

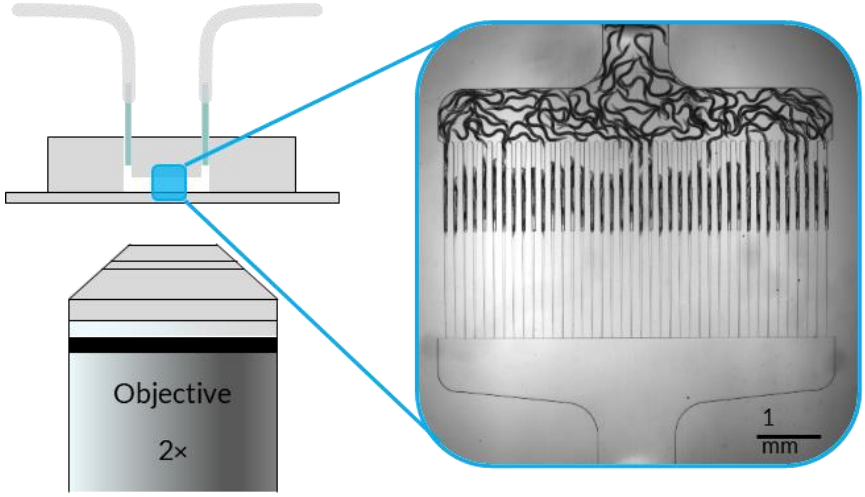


Figure 17: Schematic of an inverted microscope objective viewing the vivoChip-2x with inset showing low magnification of worms trapped in channels

5.11 Proceed with your imaging and observations as you see fit.

Dismount and Clean the vivoChip-2x

6.1 Close the stopcock connected to the buffer tubing.

6.2 Remove the vivoChip-2x from the microscope stage.

a ***Caution****: avoid tugging on the tubing connected to the vivoChip-2x, which can damage the glass coverslip at the bottom of the vivoChip-2x.*

6.3 End the program running on the vivoCube+.

a Return to the vivoCube+ Home menu by selecting the icon in the Program Progress menu.
b Select the Home menu from the Programs menu.
c The pump should turn off when navigating back to the Home menu.

6.4 Remove the input tubing from the vivoChip-2x inlet port, gripping the metal coupler with the needle nose pliers. The recommended pose to do so is shown on the left in Figure 18.

a Place the vivoChip-2x (still connected with metal couplers) on a flat and clean hard surface.
b ***Caution****: keep the thumb and the index finger close to the punched hole in the device as shown in Figure 18. Failure to follow this method can lead to breakage of glass coverslip on the bottom of the vivoChip-2x.*
c Place the thumb and the index finger (of your non-dominant hand) on either side of the metal coupler (directly adjacent to the coupler, see Figure 18) on the rubbery top part of the vivoChip-2x.
d Using the needle nose pliers with your free (dominant) hand, grip the metal coupler.
e Pull the metal coupler up vertically using the pliers (while keeping the glass coverslip at the bottom of the vivoChip-2x flat against the surface) to remove the metal coupler from the inlet port.

Figure 18: Diagram showing improper (left) and proper (right) grip of the vivoChip-2x while removing the metal coupler from the inlet or exit

6.5 Set a constant 6 psi pressure using the vivoCube+ Manual pressure control menu.

a Navigate to the Manual menu from the Home menu.
b Click “Set P(A)” to set channel A pressure.
c Set the pressure to 6.000 psi.
d Turn on the pressure to channel A ("Turn on P(A)").

6.6 Flow buffer through the input tubing into a waste container to clean out any worms left inside.

a Hold the metal coupler of the input tubing over a waste container
b Open the stopcock.
c Let liquid flow out of the tubing into the waste container for 10 seconds to clean out any worms remaining in the input tubing.
d Close the stopcock.

6.7 Turn off the pressure on the vivoCube+ ("Turn off P(A)").

6.8 Remove the exit tubing from the vivoChip-2x exit port following the same steps as 6.4.

6.9 If you wish to discard the vivoChip-2x, you may do so according to the sharps disposal policy of your organization. Otherwise, it is recommended to proceed with the following steps to clean the microfluidic channels of the vivoChip-2x for future reuse.

6.10 Connect the input tubing and exit tubing to the microfluidic device of the vivoChip-2x used earlier but now in reverse.

a Plug the input tubing metal coupler into the vivoChip-2x exit following the same method as step 4.3.
b Plug the exit tubing metal coupler into the input following the same method as step 4.3.
c ***Note**: these connections are now opposite to how they were connected previously.*

6.11 Place the end of the exit tubing (now connected to the vivoChip-2x inlet port) in a waste container and tape it into place if necessary to keep it secure.

6.12 Flow buffer backwards through the vivoChip-2x.

a Confirm that the set pressure for channel A is still 6.000 psi. If not, set it back to 6.000 psi.
b Turn on the pressure to channel A ("Turn on P(A)").
c Open the stopcock.
d After a moment, the buffer will flow into the vivoChip-2x through the exit port and out of the inlet port into the waste container.
e Wait 5 minutes. This process should free most of the immobilized worms from the channels and clean the microfluidic device of other debris.

6.13 Check that the vivoChip-2x microfluidic channels are sufficiently clean.

a With the pressure still on, place the vivoChip-2x on the dissecting stereoscope or microscope with a low magnification objective used previously.
b Look for any leftover worms or debris.
c If needed, wait another 5 minutes and repeat this step.

6.14 If worms or other debris are still stuck in channels after trying step 6.13 multiple times, you may try using the syringe to dislodge what is stuck in the channels.

a Close the stopcock.
b Turn off the pressure on the vivoCube+ ("Turn off P(A)").
c Fill the syringe used earlier completely with buffer.
d Disconnect the luer stub from the stopcock at the end of the input tubing (currently connected to the vivoChip-2x exit port).

e Connect the luer stub back onto the syringe.
f Tap the plunger of the syringe bluntly on the work surface 2-3 times. This can be done with a modest amount of force—not so much that the metal couplers come unattached from the vivoChip-2x.
g Depress the plunger steadily and forcefully for 2-3 seconds.
h Repeat the previous two steps as needed, alternating between the blunt tapping and the slow, forceful push of the syringe.

6.15 Repeat steps 6.12 through 6.14 as many times as desired.

a ***Note****: no microfluidic device, including the vivoChip-2x, is indefinitely reusable. After repeated uses of the vivoChip-2x, eventually some channels will become permanently blocked by debris.*
b ***Tip****: the microfluidic device can still be put to good use despite having channels blocked. For example, a used vivoChip-2x can be reserved as a training tool for new users.*

6.16 Close the stopcock.

6.17 Turn off the pressure on the vivoCube+ ("Turn off P(A)").

6.18 If this vivoChip-2x is to be used within 1 day, store it safely until its next use. If not, proceed to step 6.19.

a It is recommended to leave both the input and exit tubing connected to the vivoChip-2x for this type of short-term storage. This mitigates the likelihood of the liquid in the microfluidic device drying out and leaving precipitate in the channels.

6.19 Disconnect the luer stub connected to the input tubing from the buffer tubing (or syringe if steps 6.14-6.15 were taken).

6.20 If the vivoChip-2x is to be stored for a longer period (more than 1 day), clean the vivoChip-2x microfluidic channels first with alcohol, then distilled water, and finally with air to minimize particulate left inside the device.

a Empty any buffer remaining in the syringe.
b Fill the syringe with 2 mL of >70% alcohol solution.
c Connect the luer stub of the input tubing to the syringe.
d With a light pressure, gradually depress the syringe plunger to dispense the entire 2 mL of liquid through the vivoChip-2x.
e Disconnect the syringe from the input tubing.
f Fill the syringe with 2 mL of distilled water and repeats steps 6.20c-e.
g Empty any water remaining in the syringe, leaving only air (retracting the plunger completely to fill the syringe with air).
h Connect the luer stub of the input tubing to the syringe.
i With a light pressure, gradually depress the syringe plunger to flow the entirety of air in the syringe through the vivoChip-2x.

6.21 Remove both the input and exit tubing metal couplers from the vivoChip-2x following the same steps as 6.4.

6.22 Store the vivoChip-2x and associated accessories for future use.

Notes and Comments

Contact us for further support

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Website: www.vivoverse.com
Email: support@vivoverse.com

ACCELERATING IN VIVO SCREENING

Appendix

A Additional Tips for Improving vivoChip-2x Operation and Performance

A.1 Protocol for Priming the vivoChip-2x

During worm loading and immobilization on a new vivoChip-2x, air bubbles might remain in the corners of the entrance and exit chambers of the vivoChip-2x (see Figure 12 for the location of these chambers). Generally, these bubbles (outside of the channels) have no effect on the immobilization and orientation of worms within the channels of the vivoChip-2x device or on the quality of the images of worms in channels. There are 2 situations in which it is beneficial to remove these bubbles (by priming the vivoChip-2x):

1. When using a vivoChip-2x L1 chip, or
2. When intending to take publication-quality images, especially for lower-magnification images that show areas outside the channels.

Follow this protocol to remove air bubbles in these areas before loading worms in the vivoChip-2x.

1. Connect the input and exit tubing to the vivoChip-2x, following steps 4.1, 4.3, and 4.8 to do so.
2. Connect the luer stub at the end of the input tubing to the stopcock at the end of the buffer tubing.
3. Place the end of the exit tubing in a waste container.
4. Using the Manual menu, apply 2.000 psi to the channel you are using, following the methods in step 2.7.
5. Open the stopcock, letting buffer flow through the vivoChip-2x.
6. Close the stopcock as soon as buffer begins to flow into the waste container.
7. Take the solid metal plug included in the Starter Kit (Item e in the Starter Kit in Section IV Package Contents) and plug the end of the exit tubing with it.
    a. ***Note:*** *push the metal coupler into the end of the tubing with your fingers to connect it. The first time doing so can feel somewhat tight. Subsequent insertions will be easier as the tubing is stretched.*
8. Using the Manual menu, apply 5.000 psi to the channel you are using, following the methods in step 2.7.
9. Open the stopcock.
10. Wait 10 minutes. During this time, the applied pressure will force air through the gas-permeable PDMS material, removing air bubbles.
11. Observe the vivoChip-2x under a low-magnification stereoscope or microscope. If air bubbles remain in the vivoChip-2x, wait 10 more minutes. Repeat this step as necessary.
12. Once the vivoChip-2x is rid of air bubbles, close the stopcock.
13. Remove the metal plug from the exit tubing.
14. Turn off the vivoCube+ pressure by selecting on “Turn off P(A)”.
15. Remove the input tubing from the vivoChip-2x, following step 6.4 to do so.
16. Remove the luer stub (of the input tubing) from the stopcock at the end of the buffer tubing.

A.2 vivoChip-2x Loading and Immobilization Protocol with Anesthetic

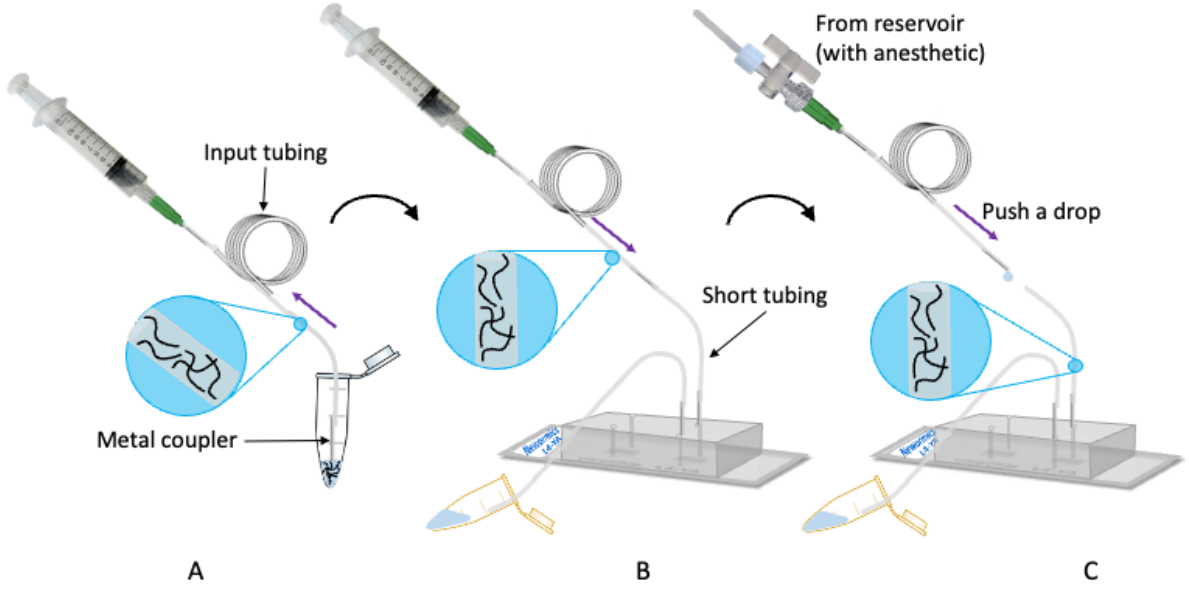


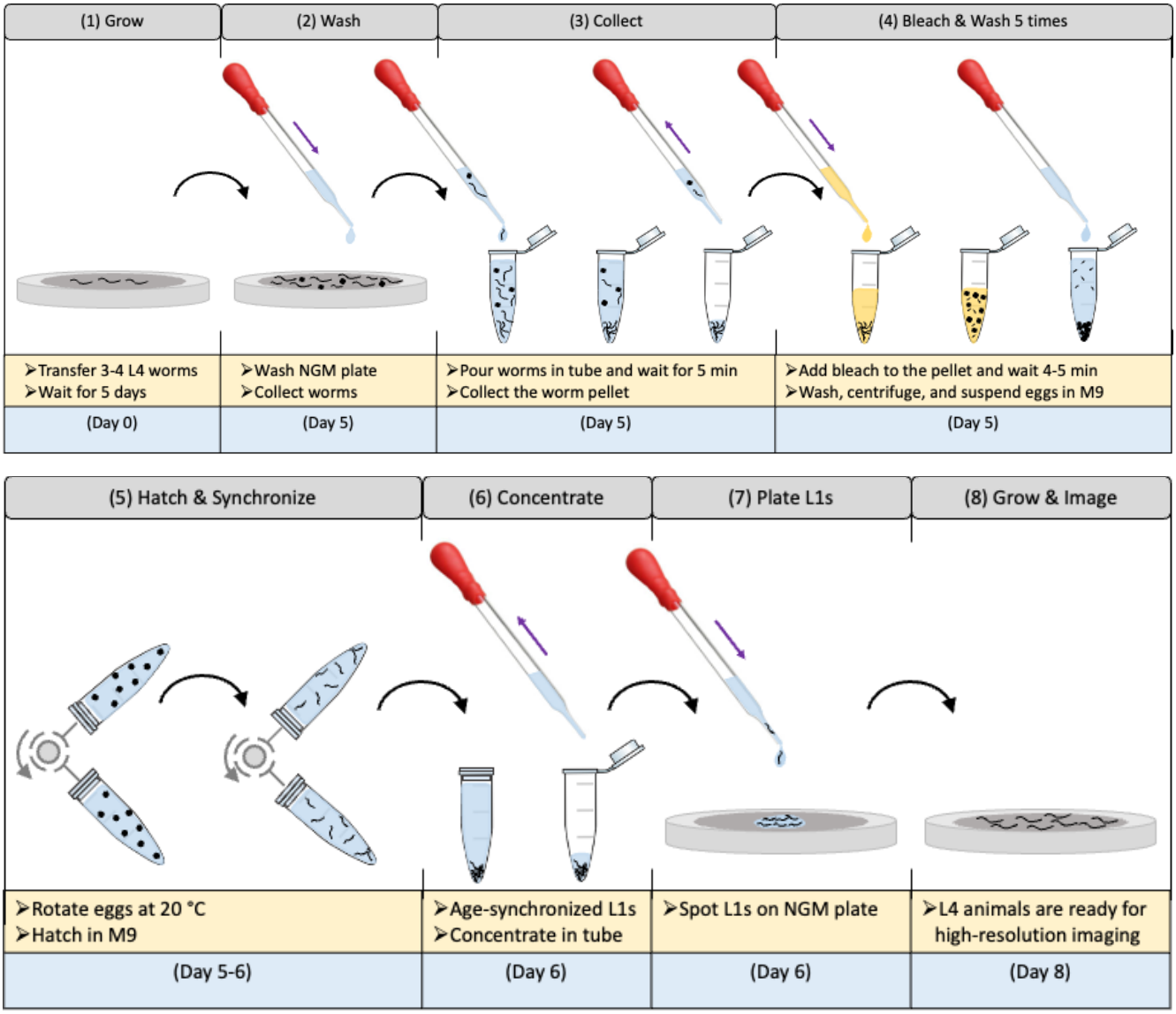
Figure 19: Schematic showing steps of loading anesthetized worms

1. Locate the short (4”) length of tubing with a pre-attached metal coupler (Item e in the Starter Kit in Section IV Package Contents).
2. Ensure the 5 mL syringe is filled with 2-3 mL of buffer **not** containing anesthetic and is connected to the input tubing, as done in steps 3.1-3.2.
3. Insert the metal coupler of the short (4”) length of tubing (following steps 4.1, 4.3, and 4.8 to do so) into the vivoChip-2x inlet.
4. Connect the metal coupler at the end of the input tubing (connected to the syringe) to the short (4”) tubing already connected to the vivoChip-2x inlet.
    a. ***Note:*** *the metal coupler is connected by just pushing the coupler into the end of the tubing with your fingers. The first insertion is a bit tight. Subsequent insertions will be easier as the tubing is stretched.*
5. Gently depress the syringe plunger to flow the buffer through the device, stopping when you see buffer flow out of the exit tubing.
6. Disconnect the long input tubing from the short (4”) length of tubing, keeping the metal coupler at this junction connected to the input tubing.
7. Follow step 4.7 to aspirate the worms through the metal coupler into the short (4”) tubing. This is shown in Panel A of Figure 19.
8. Connect the metal coupler at the end of the input tubing (connected to the syringe) to the short (4”) tubing. This is shown in Panel B of Figure 19.
9. Place the vivoChip-2x to be viewed under a stereoscope or microscope with a low-magnification objective.
10. Gently depress the syringe plunger to push the worms into the vivoChip-2x. Continue until approximately 40-60 worms are pushed into the entrance chamber (see Figure 12).
    a. ***Caution:*** *once the worms are inside the vivoChip-2x, do not apply a large or sustained pressure using the syringe plunger. Doing so could push worms into the channels too strongly, which will*

reduce the effectiveness of the vivoChip-2x for orientation of the worms within the channels and could potentially cause permanent damage to their bodies.

11. Disconnect the luer stub of the input tubing from the syringe.
12. Connect the luer stub to the stopcock at the end of the buffer tubing (also shown in Panel C).
13. Disconnect the long input tubing from the short (4”) length of tubing, keeping the metal coupler at this junction connected to the input tubing.
14. Using the Manual pressure control menu, apply 3.000 psi to the channel you are using.
15. Hold the metal coupler over a waste container and open the stopcock.
16. Once you observe a droplet exit the coupler, wait an additional 5-10 seconds, then turn off the pressure on the Manual menu and close the stopcock.
17. Connect the metal coupler at the end of the input tubing back to the short tubing length connected to the vivoChip-2x entrance.
18. Proceed to step 5.1.

A.3 Age-Synchronized Worm Growth Protocol using Bleach



Notes: the grey bars above the panels correspond to the steps with the yellow bars summarizing the corresponding task. The blue bars indicate what day of the process the step occurs on.

Figure 20: Graphical summary of the synchronization protocol

1. **Grow (Day 0):**
   - Transfer 3-4 larval stage (L4) worms to a 10 cm diameter NGM plate with the food source bacteria of choice.
   - Keep the plate at 20°C for 5 days, after which the mixed population will consist of a mix of gravid worms, small worms, and unhatched eggs.
2. **Wash (Day 5):**

- Wash the NGM plate with worms using buffer and pipette (using a glass pipette) the buffer containing gravid worms, small worms, and unhatched eggs into an upright 1.5 mL centrifuge tube.

3. **Collect (Day 5):**
   - Wait 5 minutes with the tube upright for the large-sized gravid worms to sink to the bottom of the tube. Alternatively, you can centrifuge the tube at 1300 rcf (or g) for 30 seconds.
   - Remove the supernatant (containing the eggs and smaller worms).
   - Leave the gravid worms in ~30-50 µL of buffer at the bottom of the tube.
4. **Bleach & Wash (Day 5):**
   - Make a fresh bleach solution (0.5 mL 10 M sodium hydroxide, 7.5 mL deionized water, and 2 mL sodium hypochlorite bleach). The final sodium hypochlorite concentration should be ~1.2%.
     - ***Note:*** *different brands of bleach have different levels of stability. We recommend keeping the concentrated stock of bleach no longer than six months. Working stocks of bleach solution should be made fresh for each bleaching treatment.*
   - Add 0.5 mL bleach solution in the tube with gravid worms.
   - Wait for 4-5 min. Continuously shake the tube with the worms in bleach solution and vortex it briefly every 60 seconds. Watch the tube in a stereoscope to identify >50% fragmented worms.
   - Immediately, fill the tube with 0.75 mL of buffer solution.
     - ***Caution:*** *do not keep the worms too long in the bleach. Over-bleached eggs are not viable for hatching.*
   - Shake the tube and centrifuge at 1300 rcf (or g) for 30 seconds.
   - Remove the supernatant and leave the egg pellet at the bottom of the tube in ~20 µL of solution.
   - Fill the tube with 1 mL of buffer. Shake the tube and centrifuge again at 1300 rcf (or g) for 30 seconds.
   - Repeat this wash step 4 more times to remove all traces of bleach in the eggs.
   - Fill the tube with 1 mL buffer.
5. **Hatch & Synchronize (Day 5-6):**
   - Leave the tube with unhatched eggs and buffer in a rocker or tube rotator at 20°C for 16-24 hours.
   - All the viable eggs will hatch and grow age-synchronized to larval stage 1 worms (L1s).
6. **Concentrate (Day 6):**
   - Remove the tube with age-synchronized L1s from the shaker.
   - Count the number of L1 worms in a 20 µL sample to approximate the number of worms per mL.
   - Centrifuge the tube at 1300 rcf (or g) for 30 seconds.
   - Remove excess supernatant, leaving the L1s pelleted in the bottom of the tube at a concentration of 200-300 L1s per 100 µL.
7. **Plate L1s (Day 6):**
   - Plate the L1s on a fresh NGM plate with bacteria.
   - Pipette 100 µL of buffer volume containing 200-300 L1s on the plate.
8. **Grow and image age-synchronized worms (Day 6+):**
   - Incubate the NGM plate at 20°C for the appropriate times needed for your desired stage (times may vary depending on strain or culture conditions).

- For L1 through early L2 stage worms (vivoChip-2x L1): 0-24hrs.
- For late L2 through L3 stage worms (vivoChip-2x L2-L3): 24-48hrs.
- For L4 through young adult (YA) stage worms (vivoChip-2x L4-YA): 48-63hrs.
- For D1 through D5 adult worms (vivoChip-2x D1-D5): >70 hrs.
- ***Note:*** *beyond day 1 stage, new progenies are formed on the NGM plates. As the number of worms increase on the NGM plate, they consume more food, and deplete the food amount from the plate. Beyond day 5, it becomes difficult to distinguish the new batch of adult worms (F_1) from the parent population (P_0). To maintain healthy batches of P_0 worms with sufficient food and avoid mixing of parent population with F_1 generation, transfer the parent population to a new plate with food at day 2 stage and day 4 stage.*

A.4 Imaging vivoChip-2x with an Upright Microscope

Locate the plastic upright microscope adapter (Item f in the Starter Kit in Section IV Package Contents), pictured in orange in Figure 21. The input and exit tubing should already be connected to the vivoChip-2x, and worms should already be immobilized within it.

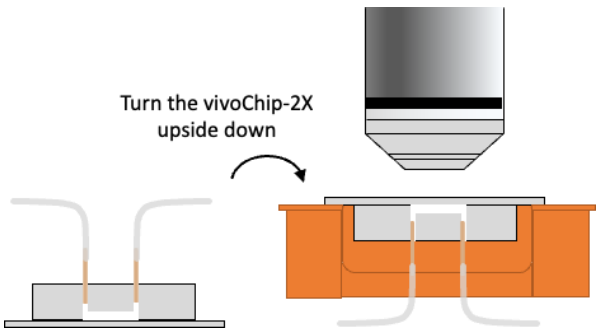


Figure 21: Schematic showing placement of adapter when using upright microscope

Once immobilization complete, turn the vivoChip-2x upside down and mount it on the upright microscope adapter (as shown in Figure 21). Place the adapter holding the vivoChip-2x on the microscope stage. Keep the glass bottom in a fixed position while focusing the objective and performing imaging of worms. Contact the vivoVerse team for additional information about the vivoChip-2x mounting systems for imaging in upright microscopes or for custom adapter solutions if the included adapter is not suitable.

B Troubleshooting

B.1 No Buffer Flow through the Buffer Tubing

If no buffer is flowing from the buffer bottle when connected to the vivoCube+ when you expect it to flow, follow these steps to resolve the issue. Proceed until pressure is applied and buffer flow starts.

1. Close the stopcock at the end of the buffer tubing connected to the buffer bottle
2. Check that the vivoCube+ is powered on. It should be connected to a power outlet, and the power rocker switch should be in the on position.
    a. If the vivoCube+ display does not illuminate, confirm you are using the correct power supply provided with the vivoCube+ (12V, 1A ⊖—C—⊕) and try another outlet.
3. Navigate to the Manual pressure control menu.
4. Set the pressure to 4.000 psi for the pressure channel that your tubing is connected to and turn on the pressure. You should hear the pump running as it turns on.
    a. If the pump does not turn on, try disconnecting the tubing from the push-to-connect fitting at the pressure port of the channel that you are using.
    b. If the pump still does not turn on, navigate to the Settings menu, select “Pressure source,” and set it to built-in pressure source.
5. If your vivoCube+ includes preconnected tubing attached to the pressure ports, disconnect the air tubing from the vivoCube+ pressure port tubing (unscrewing the luer connectors).
6. If your vivoCube+ includes preconnected tubing, take the luer caps (mentioned in step 1.6) from storage and attached them to each pressure port.
7. Check that the tubing is pressed firmly onto the air in/air out connections on the rear of the vivoCube+. This is one of the most likely spots that air can leak.
    a. Remove the tubing from the pressure port and inspect the end of it. If the tubing seems warped, worn out, or otherwise damaged at the end, use scissors to cut a ½” length from the end of the tubing to create a clean end.
    b. Reconnect the tubing to the port See step 2.4 for details on how to connect the tubing to the port.
    c. Push the tubing in farther a few times in a row to make sure it is in all the way.
8. Check if pressure is being applied by the vivoCube+ by looking at the live pressure reading of the pressure channel that you are using.
    a. If the live pressure reading does reach close to the setpoint pressure (4 psi), try using the other pressure port. The other port should be capped either by a luer cap or the sealed buffer bottle (in this case, requiring the air tubing to connected to the other port).
9. If your vivoCube+ includes preconnected tubing, disconnect the luer cap from the preconnected tubing and attach the air tubing from the buffer bottle to the pressure port that you are using.
10. Unscrew the cap of the buffer bottle and inspect the interior of it. It should contain a ring-shaped silicone gasket.
11. Check that the two black color swivel connectors with tubing are screwed tightly to the buffer bottle cap.
    a. Ensure that the swivel connector/tubing with the stopcock (buffer tubing) is screwed into the thread on the cap that connects to the tubing on the interior of the buffer bottle.

12. Screw the cap tightly to the buffer bottle.
13. Check that any other connectors in the fluidic network (such as luer lock fittings) are properly connected.
14. Open the stopcock at the end of the buffer tubing over a waste container. Buffer should flow.
15. Close the stopcock and continue where you left off in the protocol.

B.2 Poor Alignment of Worms in vivoChip-2x

Individuals in a well-synchronized batch of worms will typically be immobilized at similar depths within the microfluidic channels of the vivoChip-2x (see Figure 22, right). Worms not grown according to the synchronization protocols in Appendix A.3 will likely exhibit a wider range of sizes, resulting in a wider, more staggered arrangement within the channels in the vivoChip-2x (see Figure 22, left). Even in a perfectly age-synchronized batch, however, developmental defects (due to chemical exposures, mutations, etc.) can incur a wider distribution of sizes, resulting in more staggered positions of the worms within the channels. Ultimately, this will be application-dependent. To image a more staggered set of worms, additional fields of view may be necessary.

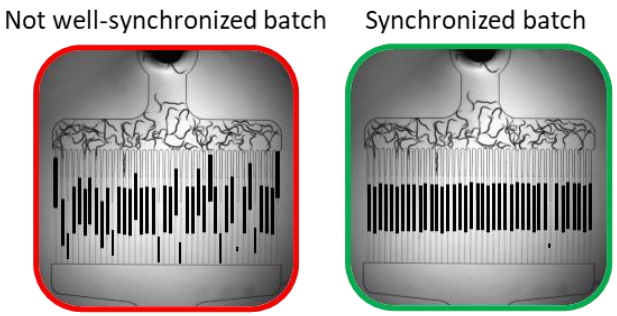


Figure 22: Samples images of a not well-synchronized batch of worms in the vivoChip-2x (left) and a well-synchronized one (right)

B.3 Poor Immobilization due to Small Number of Worms Trapped in Channels

The fluidic design of the vivoChip-2x necessitates that the microchannels be mostly filled with worms. Growing enough worms (see Section V) and transferring enough worms to the vivoChip-2x (see Sections 0 and 0) are steps that are critical to the level of immobilization in the channels in the vivoChip-2x.

C Index of frequently asked questions (FAQs)

- **How many worms should I grow when I want to use the vivoChip-2x?** See Section V.
- **The tips of the heads and tails are still moving, and it is affecting my observation ability. How can I fix this?** See Section V.
- **Can other nematodes or organisms be immobilized in the vivoChip-2x?** See Section V.
- **How do I tell if the stopcock is open or closed?** See Figure 5.
- **What does "priming" mean?** In this manual, we describe the process of flowing liquid through tubing or a vessel to expel out air as priming. For an example of how to prime tubing, see step 2.8.
- **How do I filter buffer containing adult worms?** See steps 3.4 through 3.8.
- **How do I filter buffer containing L1 worms?** See steps 3.4 through 3.8.
- **How do I connect the tubing to the vivoChip-2x?** See step 4.3.
- **How do I disconnect the tubing from the vivoChip-2x?** See step 6.4.
- **My lab where I grow worms is far from a facility where I can image them. How do I handle this?** See step 5.8.
- **How many times can I reuse the vivoChip-2x?** There is no guaranteed number of reuses. To maximize reusability of the vivoChip-2x, follow steps 6.10 through 6.22.
- **I see bubbles not in the channels themselves but in the corners of the microfluidic device in the vivoChip-2x. How do I get rid of these?** See Appendix A.1.
- **How do I synchronize worms?** See Appendix A.3.
- **I have an upright microscope. Can I image the vivoChip-2x in it?** See Appendix A.4.
- **My question is not included in this list or in the manual. How do I get help?** Contact the vivoVerse team at support@vivoverse.com to get in touch.