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Fabrication and Deployment of the In Situ Chemotaxis Assay (ISCA) V.2

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ABSTRACT

Here we outline the process of fabricating and deploying the *In Situ* Chemotaxis Assay (ISCA) and its deployment enclosure.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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MATERIALS

NAME	CATALOG #	VENDOR
Liquid nitrogen		
25% Glutaraldehyde		
Sylgard 184	View	Dow Corning
Microscope Slides (102 x 76 mm)	260230	Ted Pella Inc.
Sterivex Filter (0.2 um)	SVGPL10RC	Merck Millipore
Whatman Anotop Filter (0.02 um)	WHA68093102	Sigma-aldrich
Acrylic Sheet	View	McMaster-Carr
27G Needle		Terumo
18G Blunt End Needle		
BD-20AC Laboratory Corona Treater		Electro-Technic Products
Weld-On 3		SCIGRIP
Falcon Tube (50 mL)		Fischer Scientific
Syringe (10 mL)		Terumo
Syringe (1 mL)		Terumo
KimWipes		Fischer Scientific
20-200 uL Sterile Pipette Tips		
0.2 um Minisart Filter	16534K	Merck Millipore

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Sylgard 184	View	Dow Corning
18G Blunt End Needle		
80% Ethanol		Sigma-aldrich
Microscope Slides (102 x 76 mm)	260230	Ted Pella Inc.
BD-20AC Laboratory Corona Treater		Electro-Technic Products
Acrylic Sheet	View	McMaster-Carr
Weld-On 3		SCIGRIP
Falcon Tube (50 mL)		Fischer Scientific
Sterivex Filter (0.2 um)	SVGPL10RC	Merck Millipore
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KimWipes		Fischer Scientific
20-200 uL Sterile Pipette Tips		
27G Needle		Terumo
Syringe (1 mL)		Terumo
25% Glutaraldehyde		
Liquid nitrogen		

SAFETY WARNINGS

- When using a plasma wand, it is a good idea to do so in a well-ventilated area.
- Acrylic solvents are both flammable and toxic. Handle them with care in a well-ventilated area.
- Always cut away from yourself.

Assembling the ISCA

1 Cast 26g of 1:10 (curing agent:base) polydimethylsiloxane (PDMS aka. Sylgard 184) onto the 3D printed moulds. **Here, it is of critical importance that the PDMS is degassed under vacuum and that there are no bubbles left within the mould.**

We use a process similar to that described here in order to reduce the degassing time: (http://blogs.rsc.org/chipsandtips/2013/10/02/periodic-degassing-of-pdms-to-create-a-perfect-bubble-free-sample/).

Sylgard 184
by Dow Corning
View

2 Cure the PDMS overnight at 40 °C. **The deflection point of the polymer used for 3D printing (VeroGrey) is 48 °C**, so it is best not to approach that value or you may deform your moulds. It is really important here that **the moulds are cured on a level surface** to reduce variability between wells. Generally having no more than 6 moulds curing at a time is preferable, unless you have a large and level area for curing.

40 °C Curing Temperature

12:00:00 Curing Time

3 Cut along the edges of the mould using a scalpel (be gentle and try to cut as close as possible to the edge). **Please note:** do not attempt to cut the PDMS very close to the wells or you will compromise the surface finish of the mould, which will result in improper bonding when the mould is reused. Very gently peel the PDMS layer out of the mould, making sure not to tear the edges.

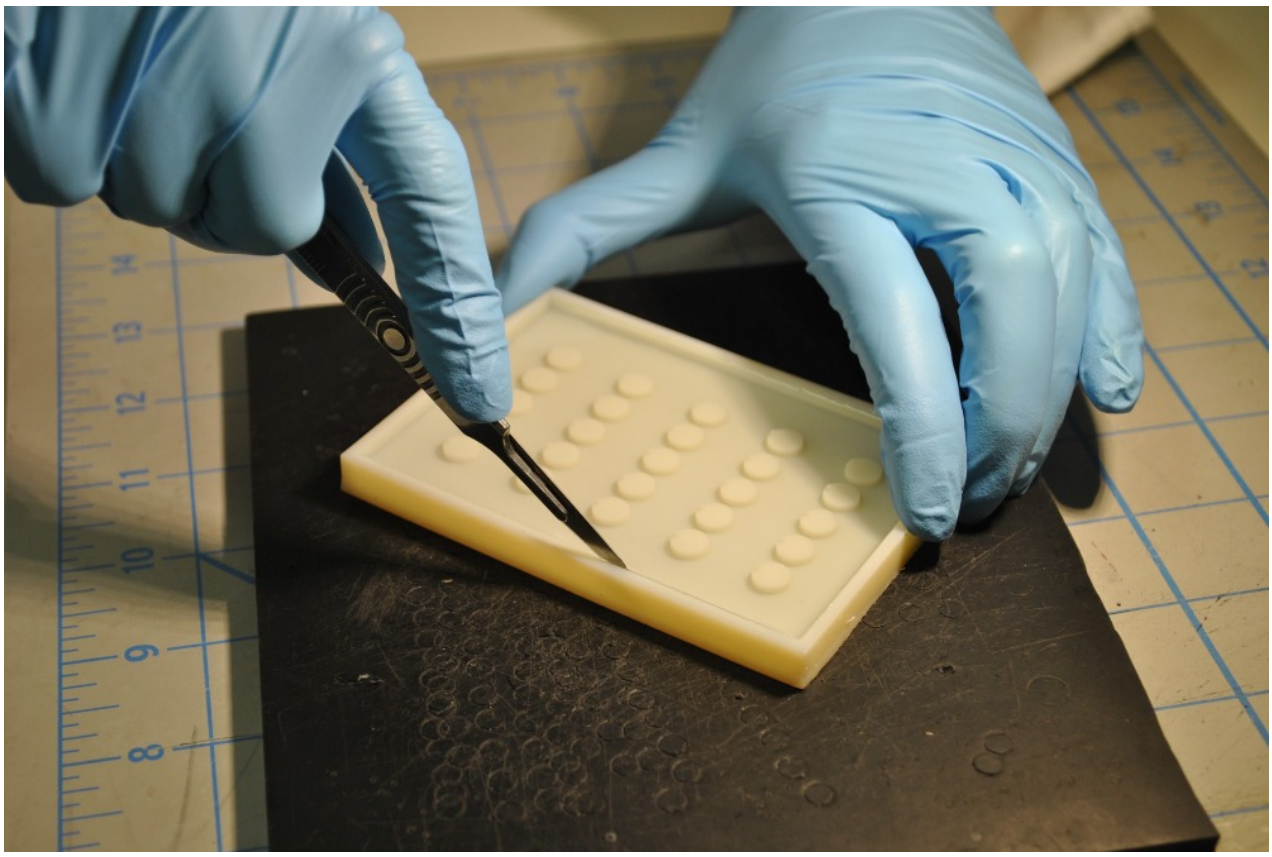

Figure 1. Cut carefully around the cured PDMS layer, near the edge of the mould.

Figure 2. Use the scalpel to carefully peel the edges of the PDMS layer away from the mould.

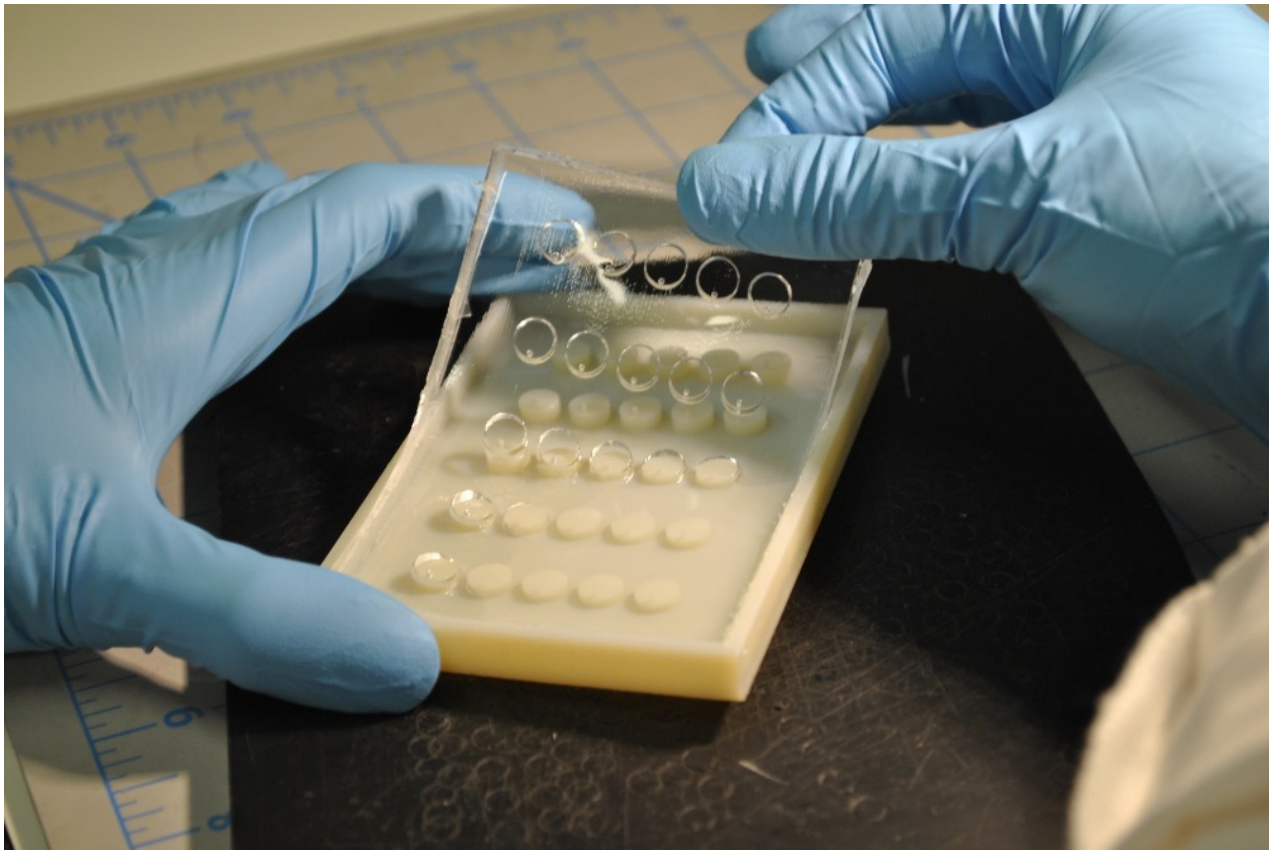

Figure 3. Carefully continue to peel the PDMS layer out of the mould until it completely detaches.

4 Trim the edges of the PDMS slab with a scalpel (approximately 1 cm on each side) to remove the uneven portions at the edge of the PDMS slab.

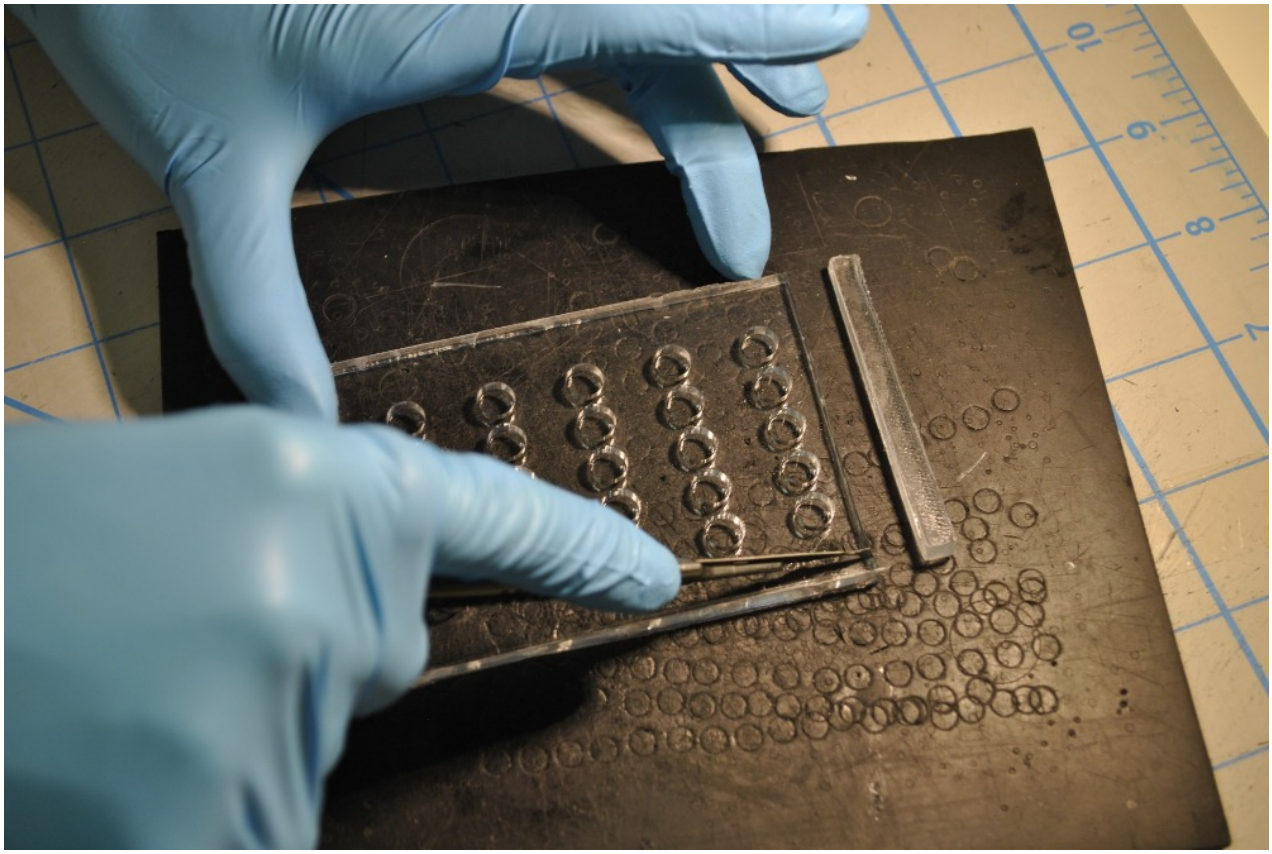

Figure 4. Trim away the rough edges using a scalpel. The surface that will be bonded should face up during this process to reduce accumulation of debris, which impacts bonding.

It should go without saying -- always cut away from yourself. In this step take care.

5 Clean the ISCA ports with an 18G syringe needle. This is a critical step and making sure all the ports are not obstructed has a strong effect on observed variability.
We typically sharpen the edges of the needles down to the correct port diameter. Alternatively, a biopsy punch of the appropriate diameter (0.8 mm) can be used.

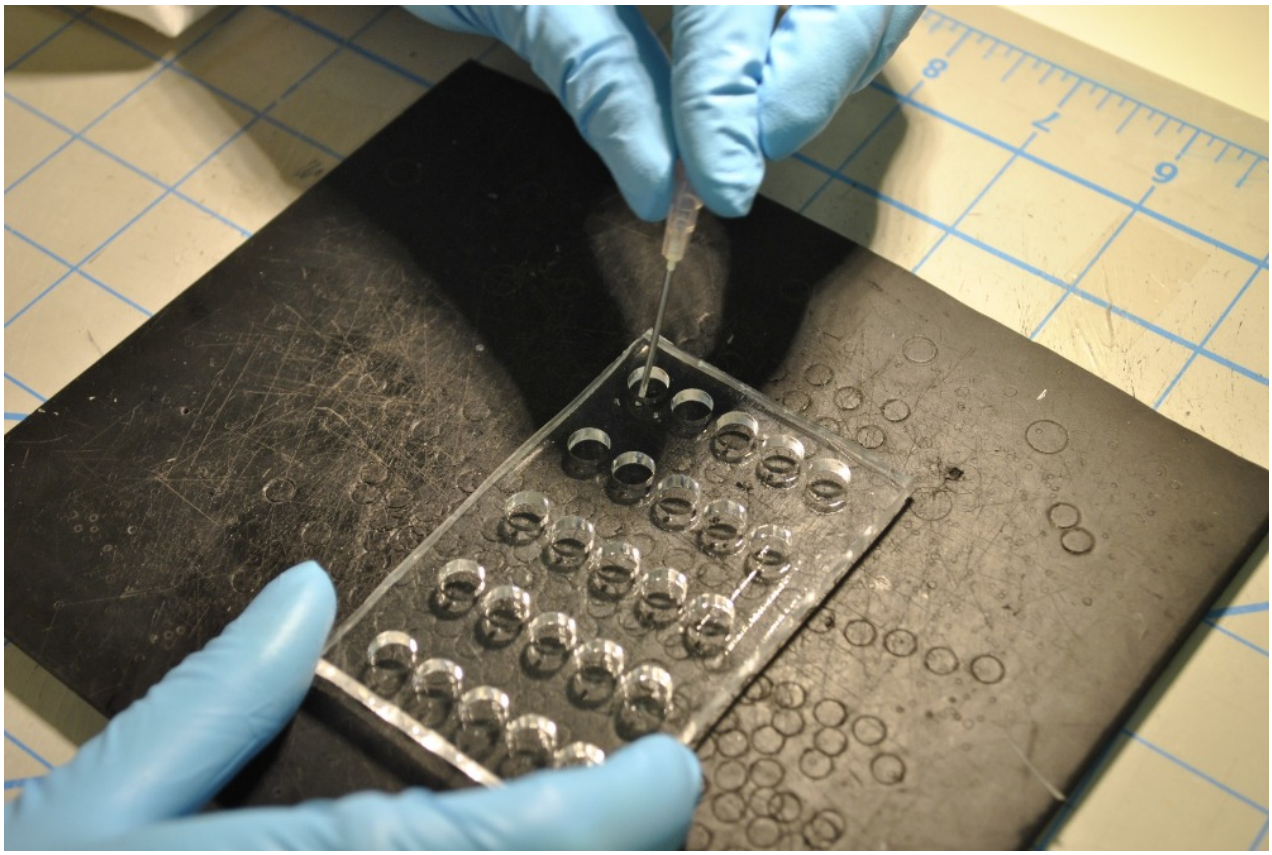

Figure 5. Remove obstructions from the ports using a biopsy punch or syringe tip.

18G Blunt End Needle

6 Clean the PDMS slab using scotch tape. This removes dust and debris from the surface.

7 UV-sterilise the PDMS slab for 30 minutes together with a clean glass slide (use 80% Ethanol, followed by a ddH2O rinse).

80% Ethanol
by Sigma-aldrich

Microscope Slides (102 x 76 mm)
by Ted Pella Inc.
Catalog #: 260230

00:30:00 UV Treatment

8 Plasma-bond the PDMS slab to a glass slide. You can either use a plasma wand or plasma oven for this step. We prefer a plasma oven, as it ensures uniform formation of covalent bonds when the device is assembled.

BD-20AC Laboratory Corona Treater
by Electro-Technic Products

9 Place the devices (glass down) on a hotplate at 80 °C for ~30 minutes after plasma bonding in order to accelerate the formation of covalent bonds.

00:30:00

80 °C

10 Cover the top of the PDMS slab with clean scotch tape. This keeps the top surface clean and prevents dust and debris from entering the ports while the device is stored.

Assembling the Flow-Damping Enclosure

11 Before heading to the field, it is important to build the flow-damping enclosure. In the supplementary information of the paper we provide the .ai file, which can be used to laser-cut the pieces needed to build the enclosure. Again this can be done in house, but you can also outsource this step to a design company.

Acrylic Sheet
by McMaster-Carr
View

12 Once you have the acrylic pieces we generally fasten them together using Weld-On 3 and let the assembly cure overnight. The pieces are designed to fit together in a puzzle-like fashion. A drawing of the assembled enclosure is provided in the supplementary material of the paper.

Weld-On 3
by SCIGRIP

12:00:00

13 Once the bonds have hardened, it's again a good idea to soak the enclosures in deionized water for 24 hrs - prior to use.

24:00:00

Preparing Chemoattractants and Controls

14 Collect water from the field site in a 50 mL Falcon tube. (Immediately prior to experiments)

Falcon Tube (50 mL)
by Fischer Scientific

15 Filter through 0.2 µm Minisart filter.

Figure 6. Filter seawater from the site carefully into a Falcon tube.

0.2 um Minisart Filter
by Merck Millipore
Catalog #: 16534K

16 First filter gently (without applying excessive pressure) through a 0.2 µm Sterivex filter. Use 10 mL sterile syringes for filtration (smaller syringes apply too much pressure and compromise the filtration process).

Sterivex Filter (0.2 um)
by Merck Millipore
Catalog #: SVGPL10RC

Syringe (10 mL)
by Terumo

17 Finally, filter through a 0.02 µm Whatman filter. It is quite difficult to get water through this last filter, so we recommend carefully assessing the amount of water needed for your experiment before starting the last filtration step. This process, when done properly, ensures complete removal of bacterial cells from seawater and provides a safe medium to resuspend your chemoattractants (it also has the same salinity/density and nutrient content as the bulk seawater surrounding the ISCA, providing an ideal negative control for the assay).

Whatman Anotop Filter (0.02 um)
by Sigma-aldrich
Catalog #: WHA68093102

18 Use this water to dilute chemoattractants to the desired final concentration and collect what remains as negative controls (for both flow cytometry and sequencing). Generally, we run dilution series experiments with each chemoattractant to determine the optima before a field campaign.

Deploying the ISCA

19 Fill the wells of the device using a 27G syringe needle and a 1 mL sterile syringe, with the port facing up. This allows fluid to enter the well, while air vents through the port. Fill the well slowly to ensure an even fill, **up to the point where a small droplet comes out of the port on the top surface of the device**. This is really important as it ensures there is no air bubble trapped within the port, which will prevent the diffusion of the chemicals out of the device and therefore the recruitment of bacteria in the wells.

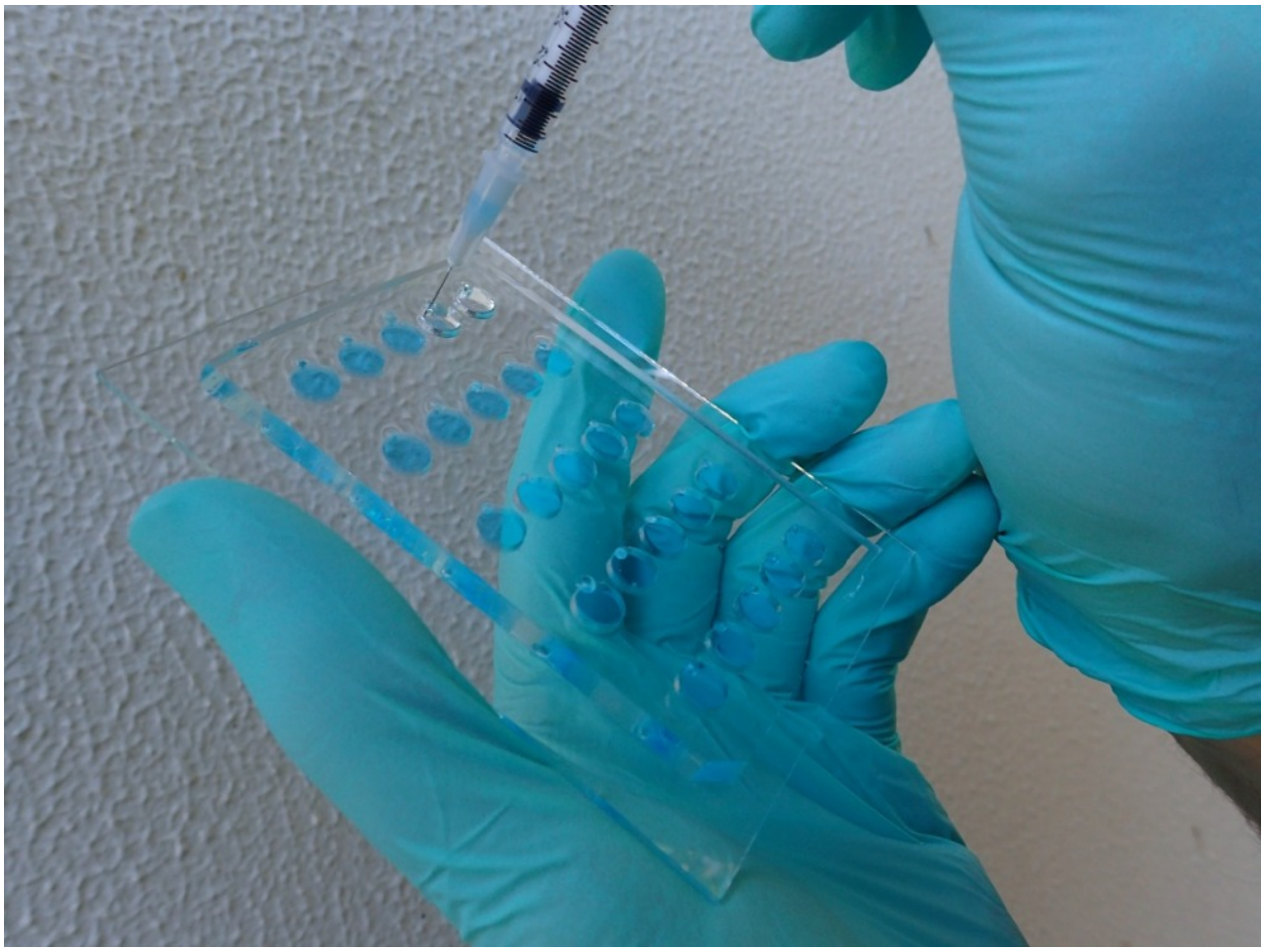

Figure 7. Fill the wells carefully with solutions. Here, angling the device ensures air vents from the port.

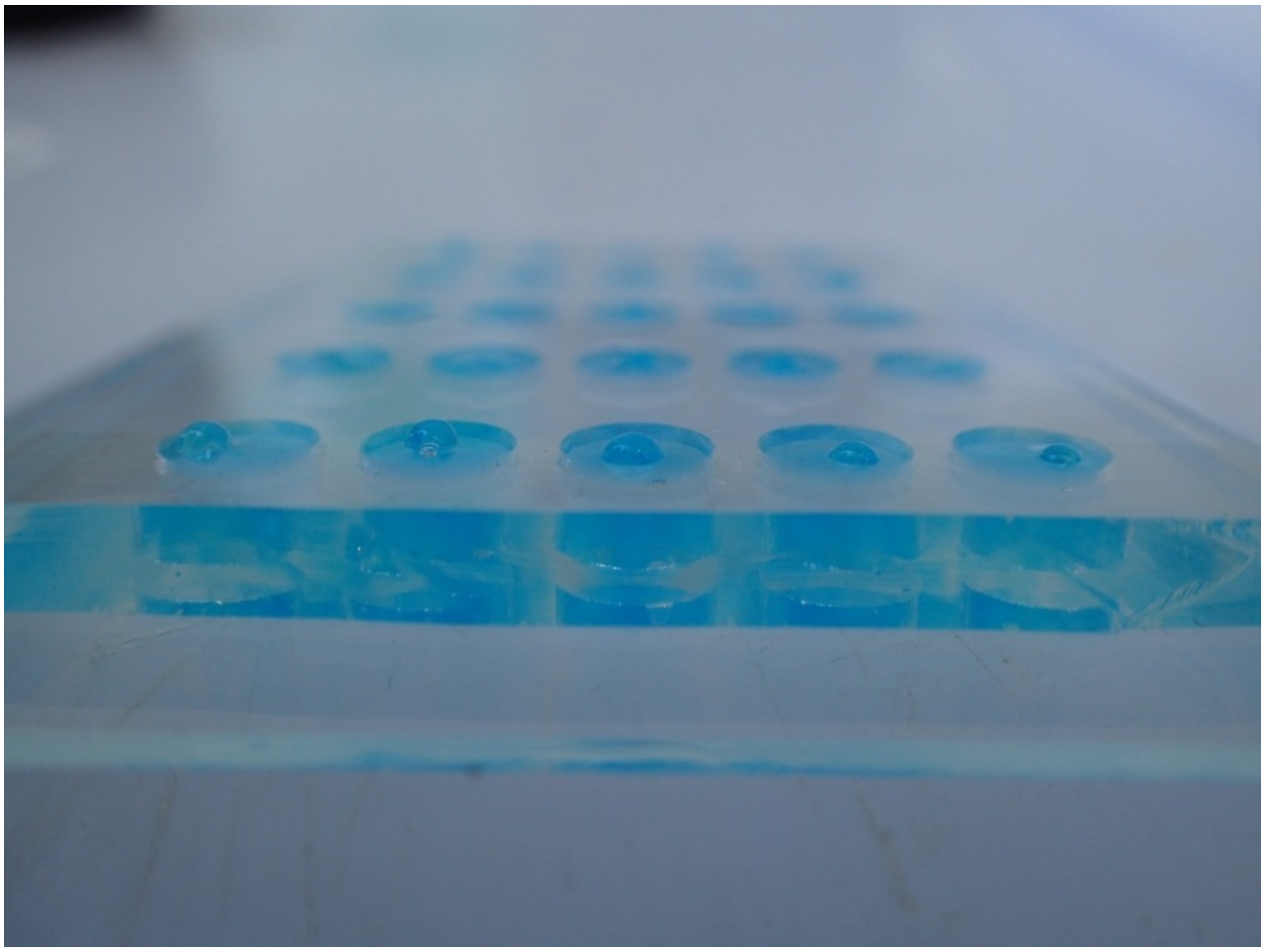

Figure 8. It is very important to leave a droplet above the port when filling. This prevents air bubbles from getting trapped in the port.

27G Needle
by Terumo

Syringe (1 mL)
by Terumo

20 Once all the wells are filled (**remember to use a different syringe and tip for each solute**), place the device into the flow-damping enclosure. The device can be fixed in place using double-sided scotch tape.

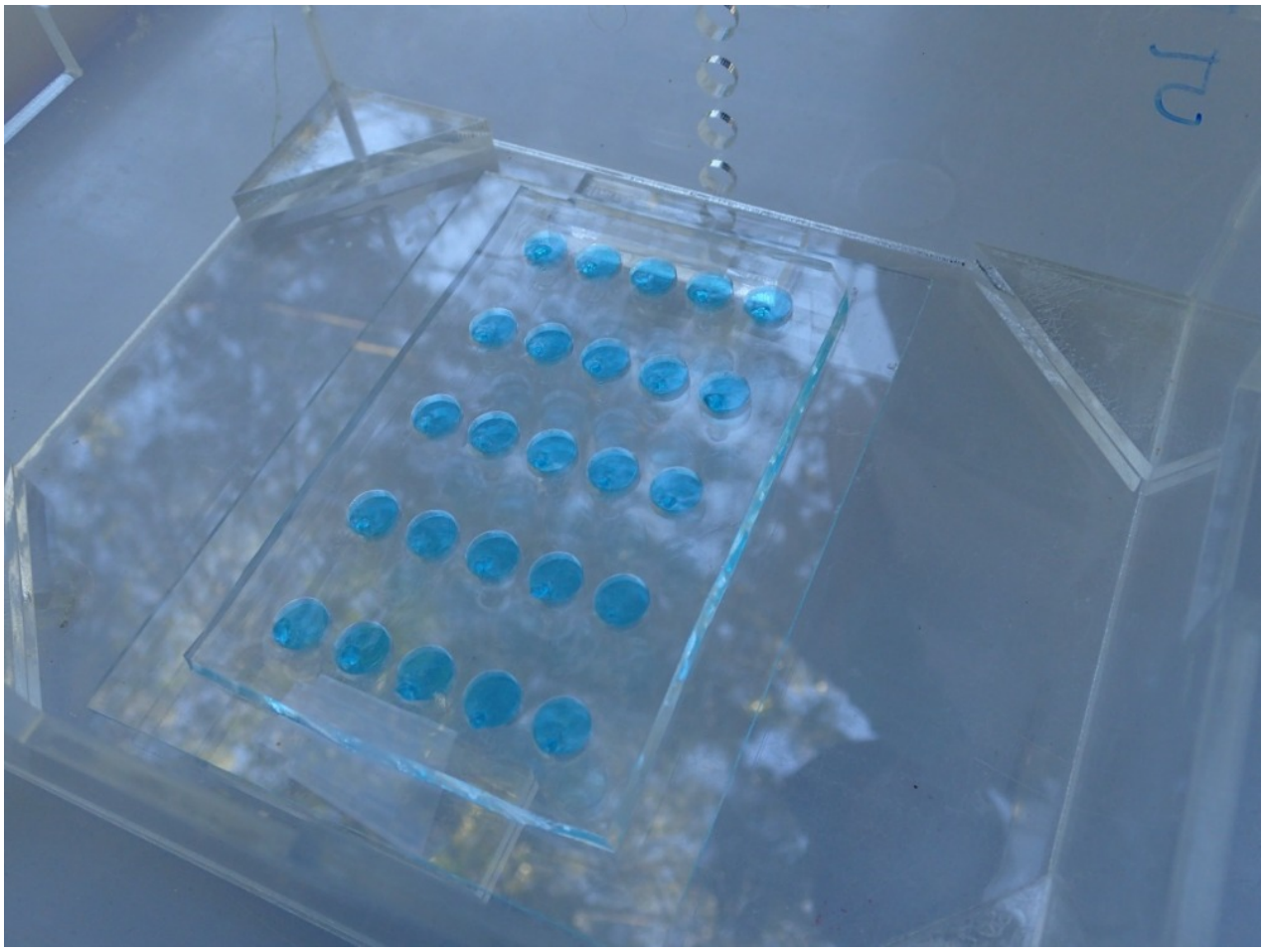

Figure 9. The ISCA taped in place within the flow-damping enclosure.

21 Tape all the seams and holes (except the bottom hole) of the enclosure with scotch tape.

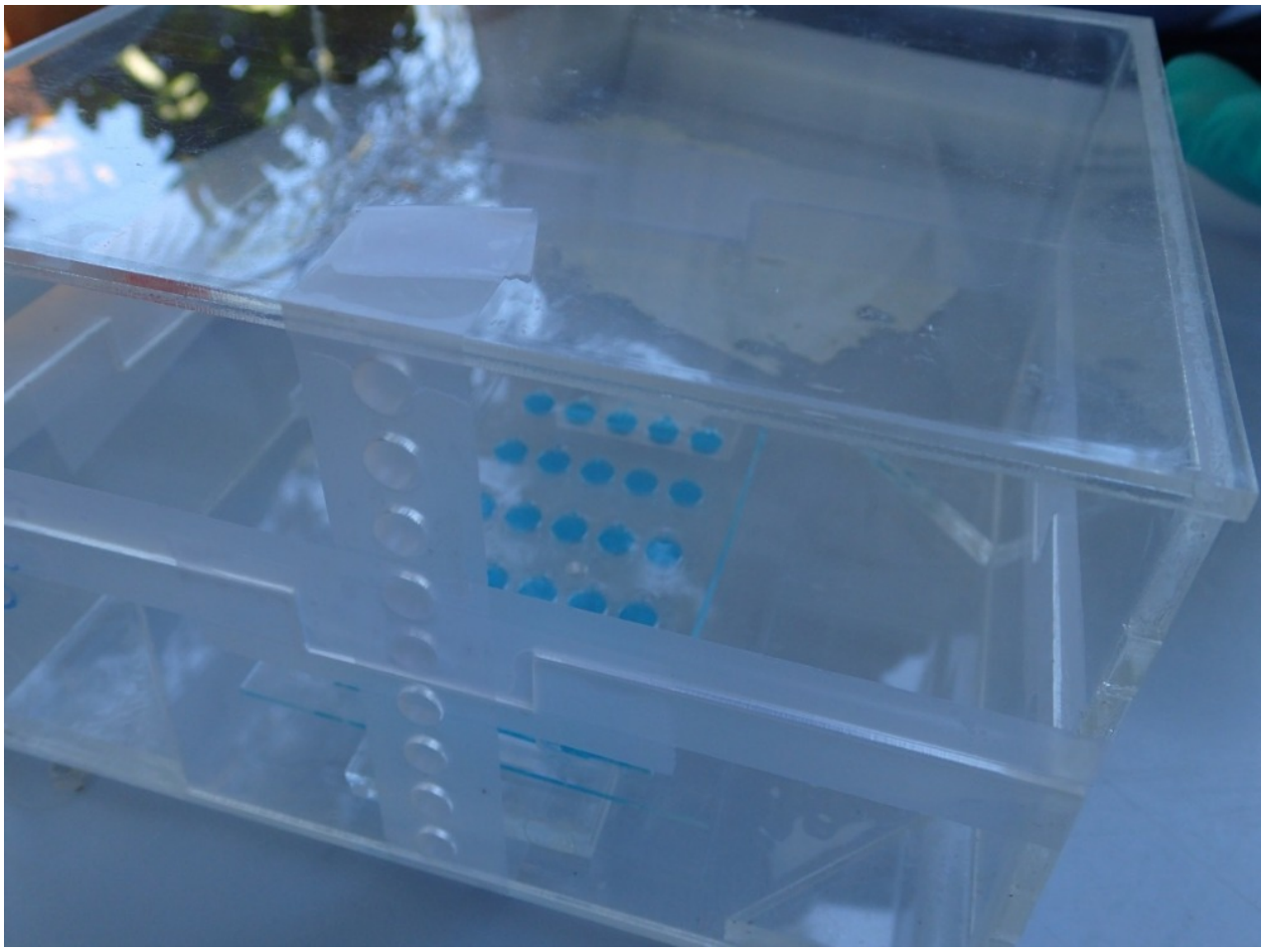

Figure 10. Seal the flow-damping enclosure with scotch tape. Place the vertical strip after securing the top and bottom halves together. This allows slow release of water after deployment.

22 Fix the enclosure onto a stable deployment arm. We use modified wood clamps that can be attached to a solid structure (e.g. wharf, ladder, pillar) and have a small platform for the enclosure (see picture).

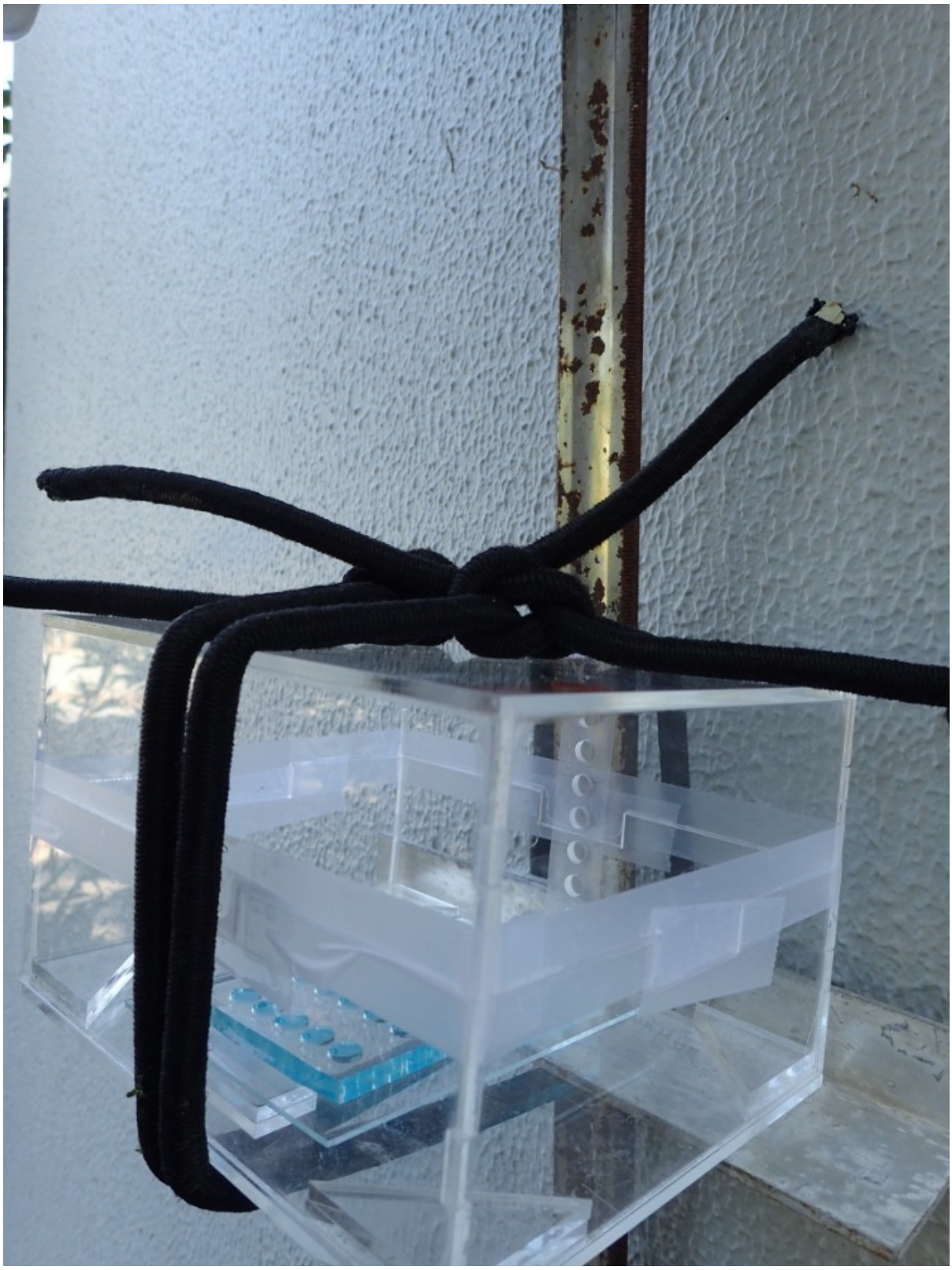

Figure 11. The flow-damping enclosure fixed to the deployment arm. We use bungy cords to fix the enclosure to a modified clamp.

23 Place the box in the water and let the enclosure fill slowly (the slower the better), until all air has escaped from the enclosure. **It is important to vent any air bubbles in the enclosure, as their presence will increase the fluid flow inside the enclosure and could negatively impact the outcome of the experiment**.

Figure 12. Lowering the enclosure into the sea. In this step try to minimize movement of the enclosure during filling.

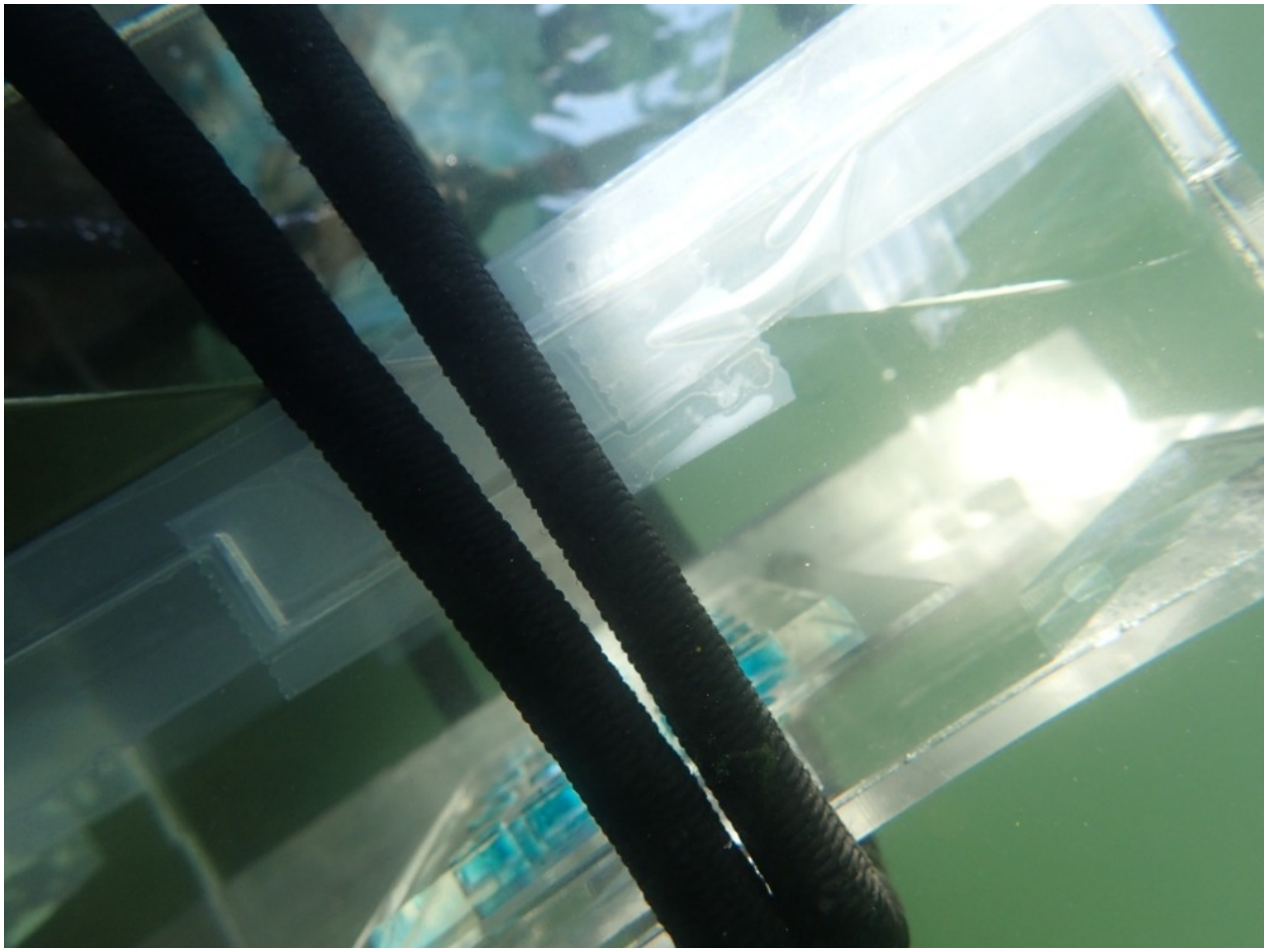

Figure 13. The enclosure should fill slowly while submerged.

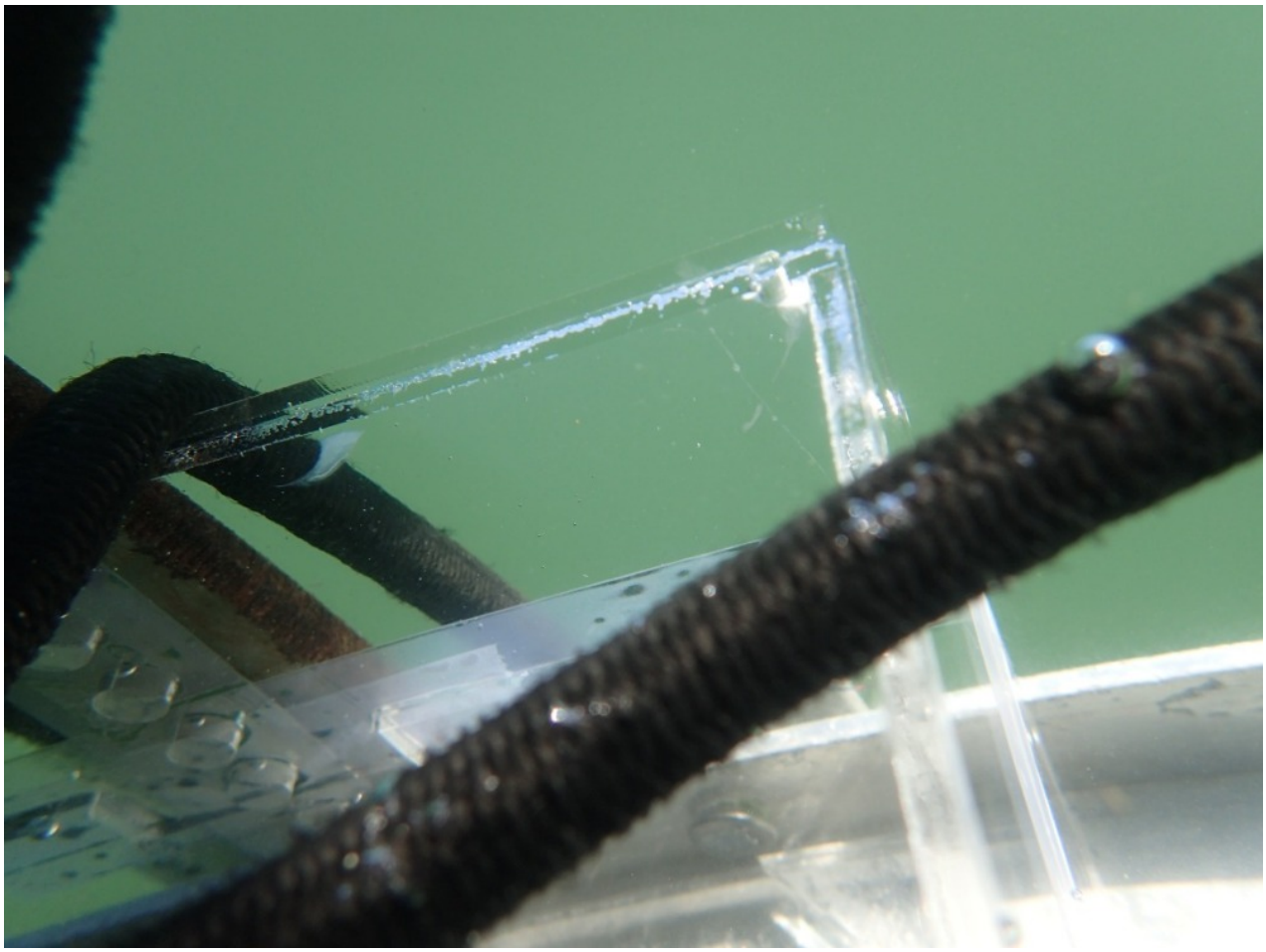

Figure 14. It is very important no bubbles remain within the enclosure. To accomplish this, angle the enclosure so that the air vent (top right of box; shown here) is the highest point.

24 Once the enclosure is full, plug the bottom hole using a 20 µL pipette tip that has been modified to act as a seal (see picture).

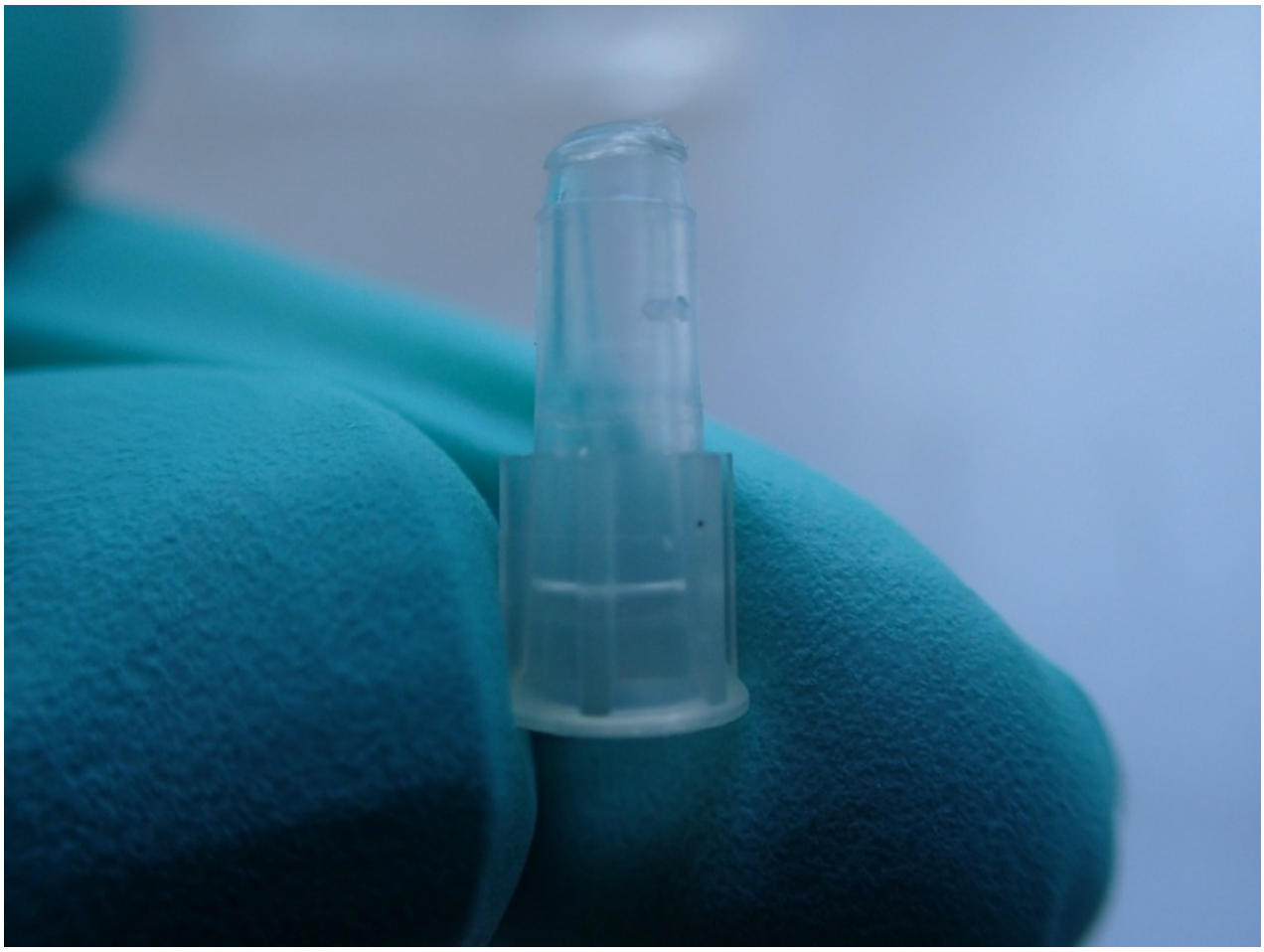

Figure 15. Modified pipette tip used to plug enclosure inlet.

25 Wait. In warm waters we deploy each ISCA for 1 hr.

Figure 16. Fix the deployment arm and wait for the duration of the experiment.

01:00:00 Incubation time

26 Pull the enclosure out of the water and set it on as level of a surface as possible.

27 Progressively unseal (from top-to-bottom) each drain hole. This slowly drains water from the enclosure, (the slower the better). It should take approximately 5 minutes to drain the contents of the box.

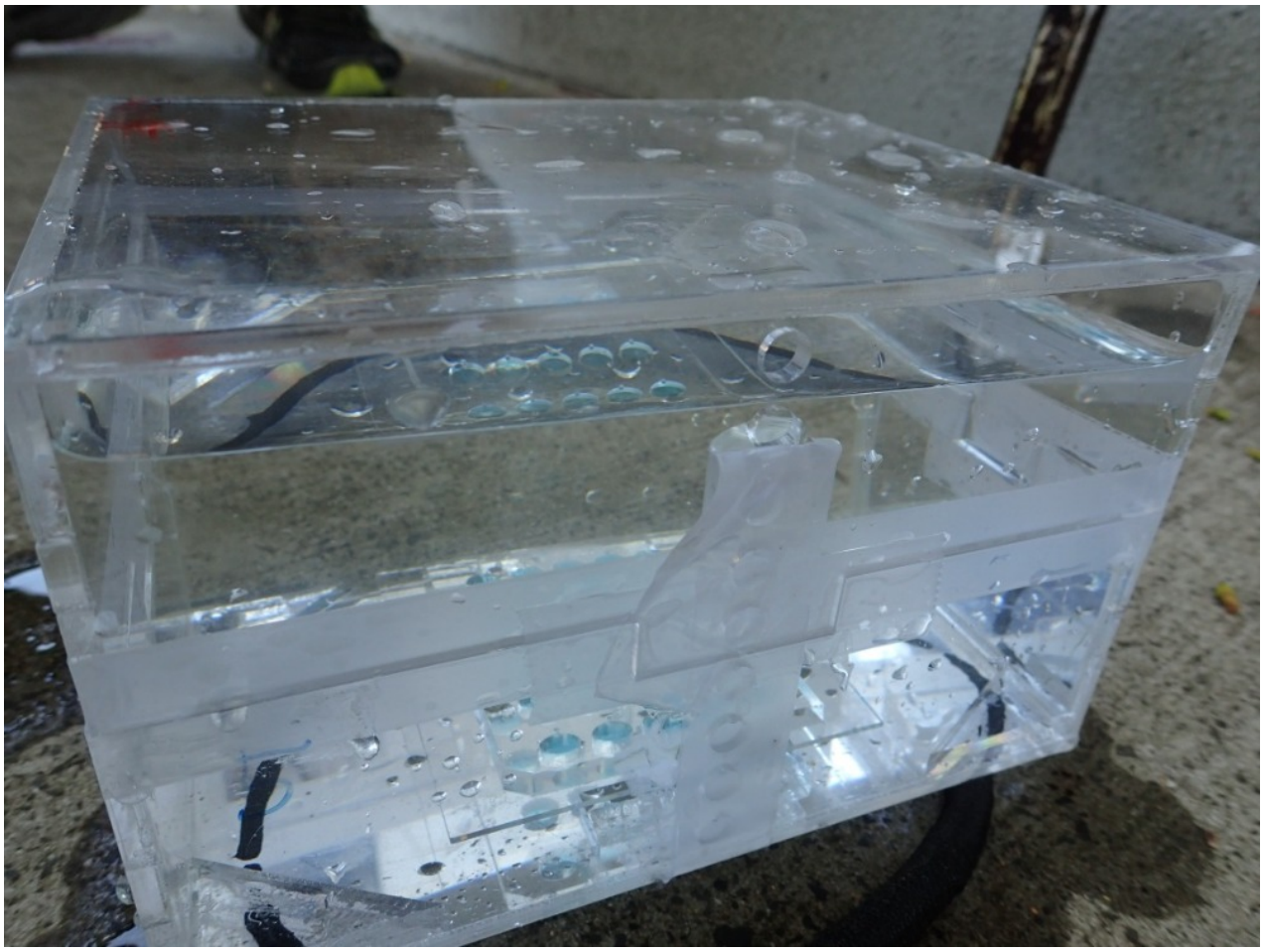

Figure 17. Begin unsealing the drain holes along the side of the enclosure. Carry out this step one hole at a time, allowing the water level to fall to the height of the hole that is uncovered.

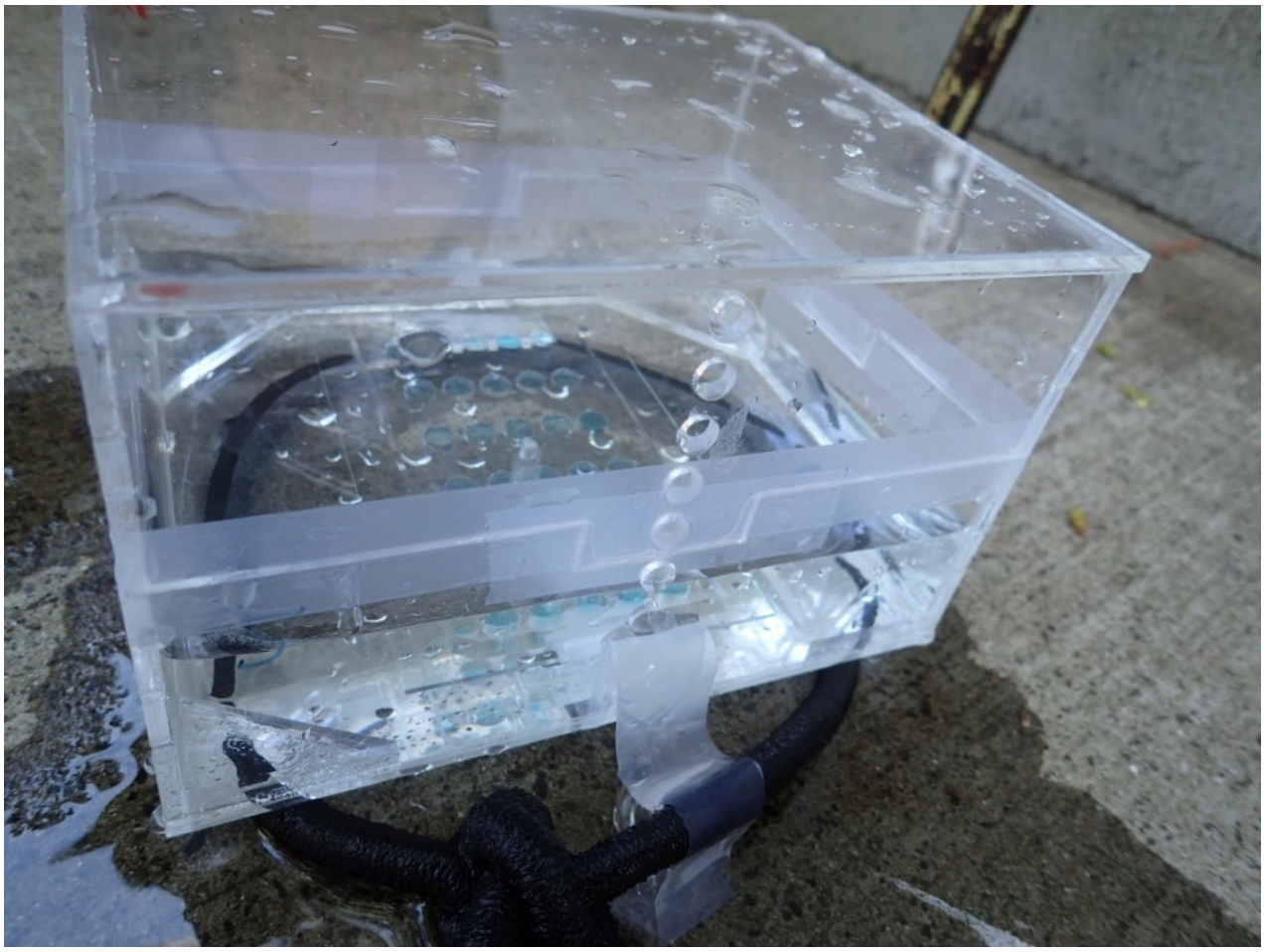

Figure 18. Continue slowly until the enclosure is empty.

28 Remove the ISCA from the drained enclosure and place it on clean Kimwipes to soak excess water from the glass slide. Using a pipette, remove all remaining water droplets from the ISCA top surface, ensuring it is as dry as possible.

KimWipes
by Fischer Scientific

20-200 uL Sterile Pipette Tips

29 Change your gloves and draw well contents slowly with a 1 mL sterile syringe and 27G syringe needle, with the port facing down. Pool the technical replicates (typically 5 ISCA wells/one row) to ensure you get enough material for DNA extraction and cell counts. Out of this pooled volume, 80 µL is typically fixed in glutaraldehyde (2% final concentration) and the remaining volume is flash frozen for subsequent DNA extraction.

27G Needle
by Terumo

Syringe (1 mL)
by Terumo

25% Glutaraldehyde

Liquid nitrogen