Protocol for reproductive span device operation

0. Preparation of the experiment

0.1 The device is designed for loading late L4 worms, so the worms should be maintained on NGM plates for this stage. Place at least 20 L4 worms per strain onto a new NGM plate 10 hours before the experiment.

0.2 UV-killed bacteria will be used to prevent live bacterial proliferation that might potentially form biofilms and block the filters and the channels. Inoculate bacteria the day before the experiment.

1. Fabrication of PDMS microfluidic chip

1.1 Polydimethylsiloxane (PDMS) usually contain two parts, use 10:1 base (big bottle) to catalyst (small bottle) ratio.

A higher base to catalyst ratio such as 15:1 makes the PDMS device softer, and a lower ratio like 5:1 makes the device harder. 10:1 is a standard value, but if you look for a larger deformation of the device, then use a higher ratio.

1.2 Weigh 40g base and 4g catalyst (for 1cm height device on 4'' wafer). Mix for 2 mins at 2000RPM, and then degas for 2 mins at 2200RPM. After that, pour the solution of mixture on the wafer and degas in a vacuum chamber for at least 30 mins until no air bubble can be seen on the wafer surface.

It is important to get rid of all the air bubbles on the surface of the wafer to make the mold reusable. Because if an air bubble is left on top of a small pattern, the pattern will stay in the mold permanently, and will not appear in the device. One way to get rid of extra small air bubbles is to use toothpick.

1.3 Put the solution in 55C oven for at least 3 hours to make it cure.

1.4 Peal the PDMS off the wafer and cut it according to the pattern on it into individual devices. Punch holes on the corresponding place and bond it to the glass slide using oxygen plasma for exciting hydroxyl groups on the surface of glass and PDMS.
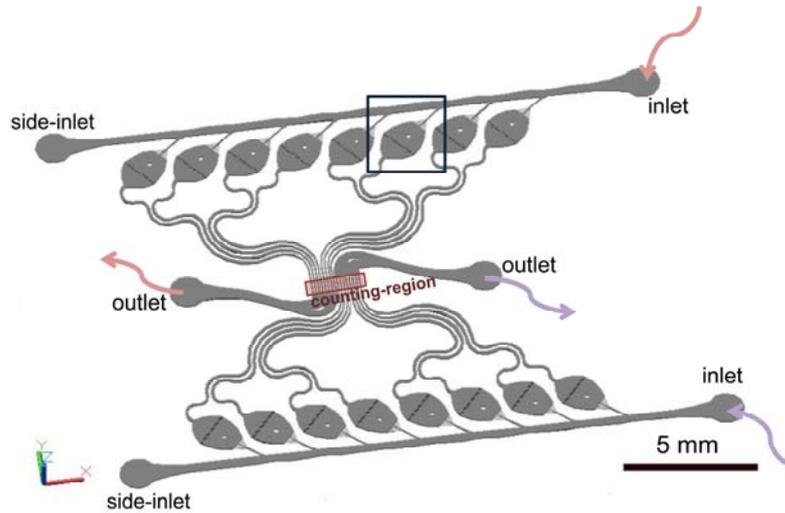
2. Device treatment before worm loading

2.1 Freshly made 5%(w/w) Pluronic F127 solution is used to flow through the device just after bonding and hold for 30mins. This surfactant forms a long-term monolayer on the surface of both the PDMS and the glass and thus prevents bacteria contamination. Here is also the time to get rid of all the air bubbles in the device. If any air bubble is hard to be driven out of the device during this step, a cap of the luer stub adapter is recommended to use by squeezing the top surface of the device to drive the air bubble away. After this step, there will not be any air bubble in the device throughout the whole experiment. Also after the monolayer is formed on the inner surface of the device, for keeping this protective layer intact, no large pressure is suggested in the following steps.

Theoretically, the sooner we do this step after bonding, the better the effect is.

2.2 Use the S-basal buffer to gently wash the device. To make sure there is no air bubble introduced into the device, always use liquid-liquid connection. It is also important when changing syringes in the following steps.

3. Worm loading

3.1 After inoculating overnight, expose the fresh OP50 bacteria to UV light in the gel box for more than 40 minutes to kill the bacteria (the light intensity is also important to completely kill the bacteria and the intensity varies over time, please check). Centrifuge 7500RPM for 5 mins to get the pellet of dead bacteria. Pour the LB solution away, and then add S-basal to vortex.

3.2 The final concentration of the bacteria solution flowed into the device is 10^9 cells per mL in the S-basal solution. The concentration of the bacteria was measured by Nanodrop 2000C. Flow the bacteria through 5um filter and then let it stand for 1 hour to get all the tiny air bubble out of the liquid.

3.3 For each side of the device, two syringes with UV-killed bacteria and one syringe of S-basal are prepared and the tubing is connected from the outlet to a waste container.

3.4 There are two ways to load the worms. One way is to wash off the plate with S-basal buffer and about 10 worms will be drawn into a syringe. Insert the syringe tubing in the inlet, and connect another syringe (with S-basal) into the temporary side-inlet. With the resulting flow, eight worms are aligned in the narrow channels, while the superfluous worms, if any, will remain in one of the two syringes.

The other way is to insert a syringe with UV-killed bacteria into the side-inlet, push the syringe gently until you see a small droplet showing up in the inlet. Then use worm picker to pick 8 worms (for 8 chambers in one side) into the droplet. Due to the gravity, the worms will subside into the device. You can use worm picker to help the worms enter into the inlet.

3.5 After these eight worms are in position, apply a pulse of high pressure (6-10psi) by a syringe to push the worms into their individual chambers, and then the side-inlet is closed by a solid steel pin.

This step needs to be done fast to reduce the mechanical stress applied to the worms. In other words, the worms are not suggested to stay in the 1mm long loading channel for more than 20 seconds.

3.6 Insert a new syringe with UV-killed bacteria into the inlet and fix it in the syringe pump. The experiment begins.

4. Worm capture during the experiment

4.1 Put the device right under the microscope. Adjust the magnification and focus of the scope to make sure the counting region of 16 channels is fully captured by the camera.

4.2 Define three points (the 3 red points in the following figures) for the software to draw the counting region of each of the 16 channel automatically.
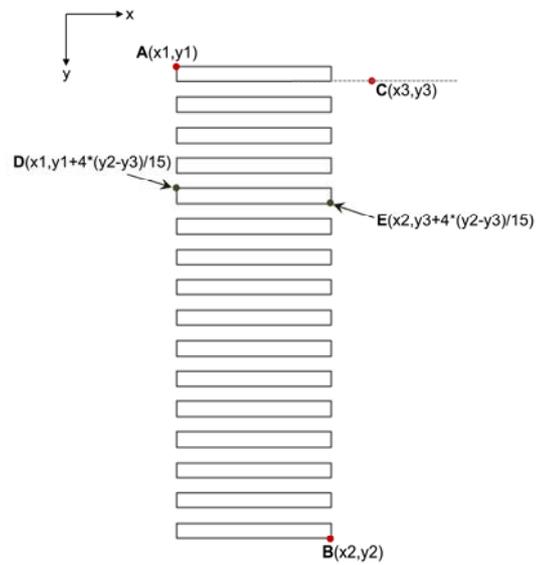
4.3 "Standard deviation" of each channel is analysed in real time and recorded, and the suspected images of worms can also be saved. With these information, we obtain the time information of the progeny of all the worms in a device.