Hydra loading and unloading

BD 10mL Syringe Luer-Lok tip (REF302995)

LS18 18ga x ½” Luer Stubs ([www.instechlabs.com](http://www.instechlabs.com))

Tubing 0.045 ”I.D. x 0.063” O.D. [www.scicominc.com](http://www.scicominc.com) catalog # BB31695-PE/7)

1. Insert (0.045 ”I.D. x 0.063” O.D. [www.scicominc.com](http://www.scicominc.com) catalog # BB31695-PE/7) tygon tubing (attached to syringe with hydra media) into the microfluidic devices and thoroughly wet the chamber with hydra media. Carefully removing all air bubbles.
2. Remove tubing from inlet port and pulling a Hydra few millimeters into the tubing with syringe then inserting the tubing into the inlet port of the microfluidic device.
3. Load hydra into the chamber by applying positive pressure to the inlet syringe. The two opposing syringes can be alternatively used to provide gentle pulses to position the Hydra in the observation chamber. Begin experiments!!!
   1. For chemical perfusion experiments (w hydrostatic flow):
      1. Remove plungers from the syringes connected to perfusion inlet/outlet ports (connected to the spokes on the sides).
      2. Raise one of the syringes approx. 1ft above the device. Keep the second one at device height. This should allow low flow rates for perfusion from the raised syringe.
   2. For chemical perfusion experiment (w syringe pump):
      1. Connect one side of the perfusion port to syringe pump (we have tried 0.02mL/min flow rate) to perfuse chemical.
      2. Use second port as outlet for flow
4. At the end of the experimentation, Hydra can be removed from the microfluidic device gently pulsing the syringes to flow Hydra out of the large inlet port.

Hydra Imaging Platform

Suggested Materials:

Image Acquisition and Enclosure:

USB digital microscope (Dino-lite) or any camera

Dino-lite Camera mount (or any custom camera mount)

Makerbeam XL starter kit + Black foam core (3/16”) OR IKEA bookshelf

Lighting Module:

RBG strip LEDs

Teensey microcontroller

5v power supply

3000 tough Rolux diffuser ([www.Rosco.com](http://www.rosco.com))

Acrylic 3/16” (Custom enclosure for light module)

Image acquisition can be performed with any open source webcam software, such as ispy ([www.ispy.com](http://www.ispy.com)) which allows recording from multiple cameras simultaneously or custom matlab script with image acquisition toolbox.

Acrylic pieces are cut to construct a box to hold the 4x6” board of RGB LEDs and diffuser ~2” away from the LEDs. Teensey microcontrolller is used to programthe RGB LEDs (both intensity and color) with a custom script. LEDs require 5V power supply. The lighting module provides diffused illumination from the bottom of the device for high contrast image capture with sensor from above.

Makerbeam kit (extrusion beams) and foam core are used for making the enclosure for the imaging platform to isolate external lighting.

Microfluidic Device Fabrication:

* Molds can either be fabricated with soft lithography or 3D printed (>200um features)

Behavioral imaging microarenas

fabricate ~660um thick behavioral imaging chambers

Method 1: 3D print:

Design layouts with Autodesk inventor

FormLabs 3d printer (clear resin)

Method 2: Sofft Lithography:

Design layouts with LEdit and print transparencies with CAD/ART services

1. Clean wafer:
   1. Heat 4” Si wafer @250C for 5min,
   2. O2 pClean in cleanroom for 5 min 100Watts
2. Spin coat 3 layers of SU8-2075 for ~220um thickness each (3 layers for ~600um, 2 layers for 440um, etc)

Layer 1

Spin recipe:

Step 1: 300rpm - 100rpm/s - 20s

Step 2: 1150rpm - 500rpm/s - 30s

Pre-Bake:

Programmable hot plate

40 min @ 65C

10c/hr ramp to 85C

45 min @ 85C

cool to RT on plate

Layer 2

Spin recipe:

Step 1: 300rpm - 100rpm/s - 20s

Step 2: 1150rpm - 500rpm/s - 30s

Pre-Bake:

40 min @ 65C

4c/hr ramp to 82C 1hr

5c/hr ramp to 90C for 30 min

cool on hot plate

Layer 3

Spin recipe:

Step 1: 300rpm - 100rpm/s - 20s

Step 2: 1150rpm - 500rpm/s - 30s

Pre-Bake:

40 min @ 65C

4c/hr ramp to 82C 1hr

5c/hr ramp to 90C for 30 min

5c/hr ramp to 60C for 5 min

Cool to RT on plate overnight

1. Expose
   1. 800 mJ/cm2 with UV filter
2. Post-bake

15 min @ 65C (started to see the pattern)

200C/hr ramp to 85C for 10 min

10 min @ 95C

coot to RT

1. Develop

20 minutes in SU-8 developer (took a long time switched out developer once)

IPA rinse

Air dry

1. Hard Bake:

~2 minutes in clean room at 65 then 2 min at 100 cool sligtly

Chemical perfusion chambers

Soft Lith.

1. Clean wafer:
   1. Heat 4” Si wafer @250C for 5min,
   2. O2 pClean in cleanroom for 5 min 100Watts
2. Spin coat SU8-3025 for ~20um thickness

Step 1: 300rpm - 100rpm/s - 20s

Step 2: 3000rpm - 300rpm/s - 30s

1. Prebake:

3 min @ 65C

10min @ 95 C

1. Expose

252 mJ/cm2 with UV filter (mask with perfusion channels/spokes)

1. Post-bake

8 min @ 65C

5 min @ 95C

1. Develop

3 minutes in SU-8 developer

IPA rinse

Air dry

1. Hard Bake:

120C to 150C (5C/30sec ramp)

2min 150C

Ramp to 190

10min @ 190

1. Spin coat SU8-2075 for ~100um thickness

Step 1: 300rpm - 100rpm/s - 20s

Step 2: 1700rpm - 300rpm/s - 30s

1. Prebake:

15 min @ 65C

5C/min ramp

2hr @ 80C

15 min @ 95 C

1. Expose

350 mJ/cm2 with UV filter. Align the mask (without perfusion channels/spokes).

Note: Prev. mask had perfusion channels at ~30um but this mask does not so the perfusion channel height remains 30um after this step but the immobilization chamber will be thicker.

1. Post-bake

10 min @ 65C (ramp 10C/min)

30 min @ 95C

1. Develop

8 minutes in SU-8 developer

IPA rinse

Air dry

1. Hard Bake:

120C to 150C (5C/30sec ramp)

2min 150C

Ramp to 190

10min @ 190

~160um depth at the chamber but only 32 um at perfusion layer

Moulding PDMS

1. Thoroughly Mix PDMS (Sylgard 184) monomer and crosslinker 10:1 (w/w) for 10 mins (~40:4g for one 4”Si wafer)
2. Make walls around the master mold (Si wafer) with Alumuminum foil and pour the PDMS mixture in.
3. Degas in vacuum chamber to remove air bubbles (~ -30mmHg)
4. Cure PDMS in oven (60C for 1-4 hours)
5. Gently peel the hardened elastomer (PDMS) from the mold

Assembling microfluidic devices

1. Using (1mm or 1.25mm) biopsy punches, punch holes for the inlets/outlets ports
2. Clean the microfluidic chips and glass slides (or wafers)
3. Oxygen plasma treat the glass and microfluidic chips with patterned side up (30 sec at 300mTorr)
4. Press the microfluidic chip with pattern side on to the treated side of the glass.
5. Heat cure the device (60c for ~5 mins) to strengthen the adhesion. The device is ready to use!!!

Cleaning microfluidic devices

1. Flush the channels with DI water using syringe fitted with 0.2um filter (~30mL)
2. Submerge the device in DI water and sonicate for ~10 min at RT then heat the DI water (w device) to 160C for 1hr.
3. If the device was used with chemical, soak in DI water overnight (replacing DI water every few hours)
4. Dry the device with compressed air and for complete drying use 60c oven for ~8 hrs