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We use this protocol and it's working

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MAPDH Patterning Protocol

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Multi-Domain Patterning of DNA-Functionalized Hydrogels

Misha Rubanov

DISCLAIMER

ABSTRACT

DNA-functionalized hydrogels are capable of sensing oligonucleotides, proteins, and small molecules, and specific DNA sequences sensed in the hydrogels' environment can induce changes in these hydrogels' shape and fluorescence. Fabricating DNA-functionalized hydrogel architectures with multiple domains could make it possible to sense multiple molecules and undergo more complicated macroscopic changes, such as changing fluorescence or changing the shapes of regions of the hydrogel architecture. However, automatically fabricating multi-domain DNA-functionalized hydrogel architectures, which could enable the construction of hydrogel architectures with tens to hundreds of different domains. We describe a platform for fabricating multi-domain DNA-functionalized hydrogels automatically at the micron scale, where reaction and diffusion processes can be coupled to program material behavior. Using this platform, the hydrogels' material properties, such as shape and fluorescence, can be programmed, and the fabricated hydrogels can sense their environment. DNA-functionalized hydrogel architectures with domain sizes as small as 10 microns and with up to 4 different types of domains can be automatically fabricated using ink volumes as low as 50 µL. We also demonstrate that hydrogels fabricated using this platform exhibit responses similar to those of DNA-functionalized gels fabricated using other methods by demonstrating that DNA sequences can hybridize within them and that they can undergo DNA sequence-induced shape change.

1 Turn on all MAPDH hardware, connect to Micromanager and Pycromanager.

2 Insert the waste tube, 24-inch length of Tygon tubing (0.02"x0.06"), into the outlet of the 5-inlet 100µm-height empty (air-filled) microfluidic chamber.

3 Make four 200 µL ink solutions in four vials in 1x TAEM buffer.

[M] 10 % (v/v) PEGDA 575 [M] 1 Mass Percent LAP

[M] 500 nanomolar (nM) 5Acry_3Cy3_polyT10

Step 3 includes a Step case.

Vial Composition

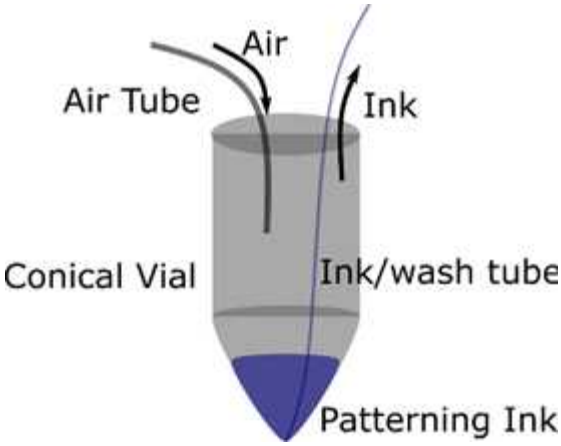
step case

Vial Composition

The composition for each vial can be customized based on the desired hydrogel composition.

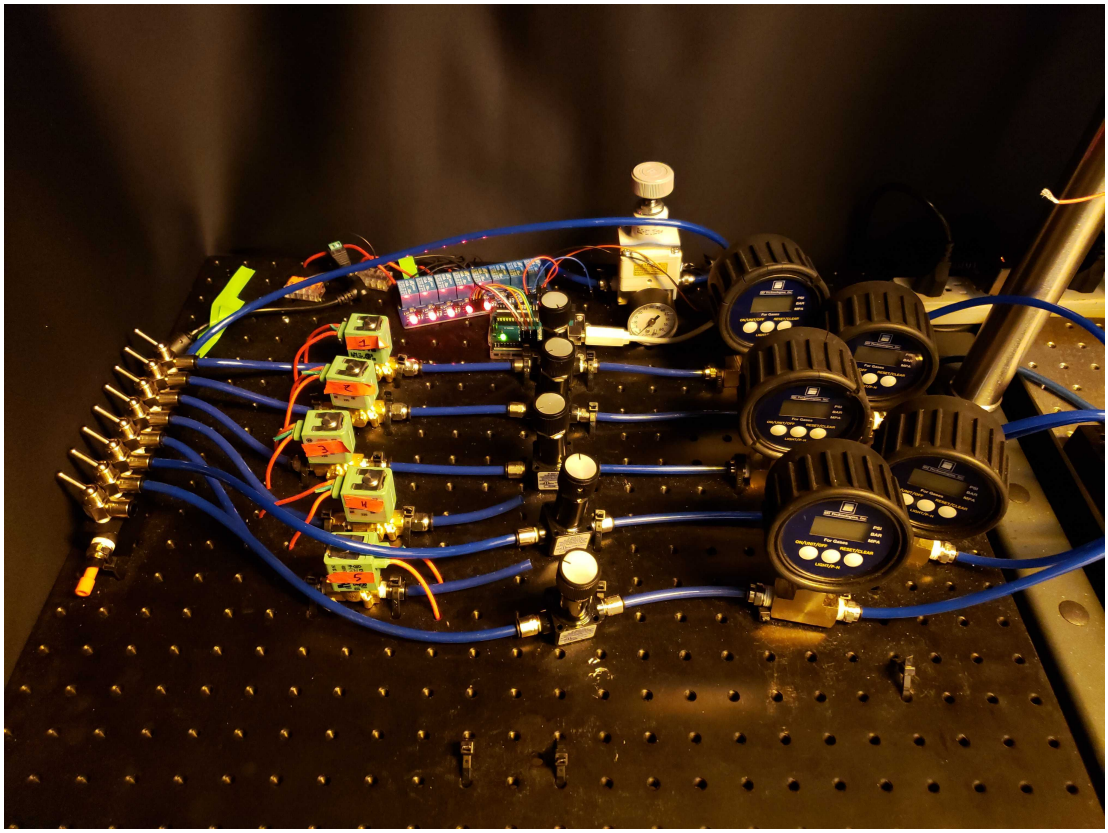
4 Add 800 µL of 1X TAEM (wash solution) into a fifth vial.

5 Screw in each modified cap to its corresponding vial to connect all five vials to the five ink/wash tubes that are connected to the flow controller.



Ink Vial setup

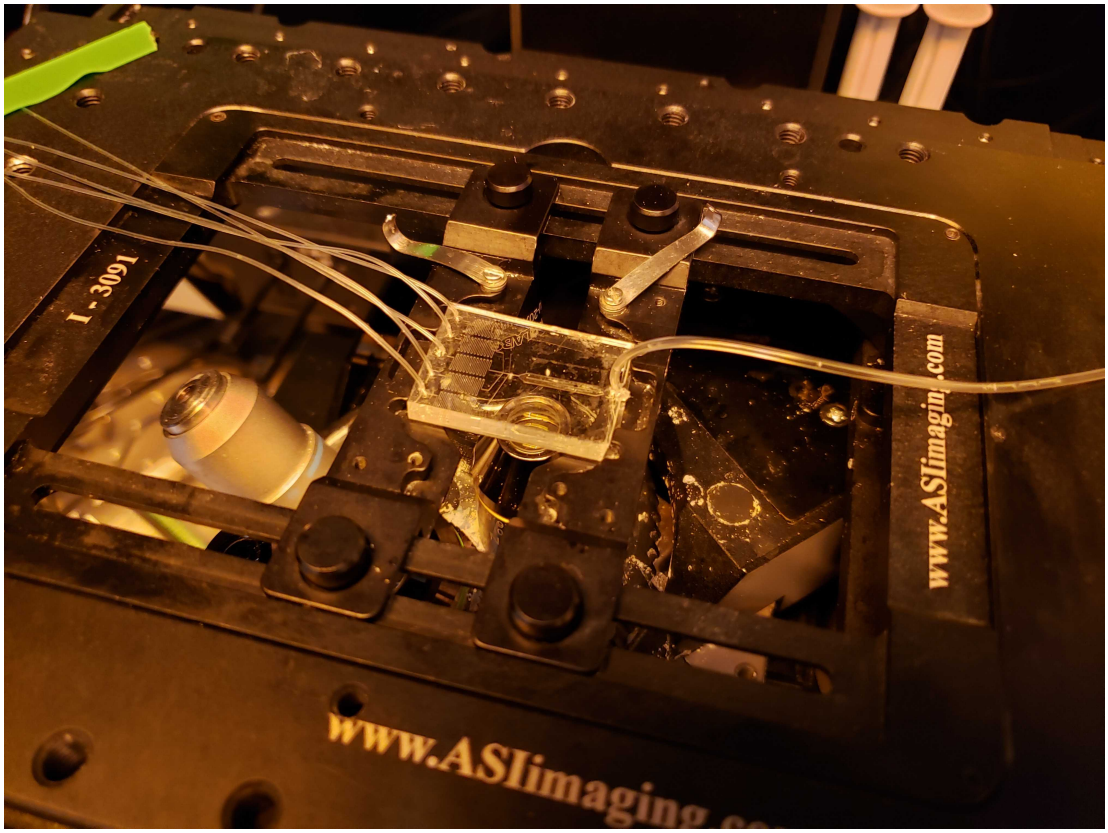
6 Set the pressure for each vial on the low-volume flow controller to 3 PSI



Flow Controller setup. From left to right: manual switches, electronic solenoids, fine pressure gauge, pressure sensor. Right most tubes are fed into ink vials.

7 To prime the first ink tube, run the ink solution through the first ink tube by opening the first valve on the low-volume flow controller.

8 Using a 0.75mm biopsy punch to assist, insert the primed ink tube go to step #7 into the first inlet of the microfluidic chamber.



Microfluidic device on the XY stage after 5 inlet and 1 outlet tubes have been placed.

9 Open the valve, to flow the ink solution through the microfluidic chamber until the ink solution reaches the outlet of the microfluidic chamber, then stop the flow by closing the valve.

10 Repeat steps 7-9 for the solutions in the other four vials, performing these steps for the vial containing the wash solution last.

11 Place the microfluidic flow chamber on the XY Stage, place the vials at a higher height than the microfluidic flow chamber (so that gravity assists rather than impedes flow), and place the end of a waste tube on the rim of a waste receptacle (i.e., a 15 mL Falcon tube). The waste receptacle should be at the same height as the vials.

12 Focus on the surface of the microfluidic flow chamber with the 10X objective.

13 Clamp the waste tubing and all ink tubes except the wash tube.

14 Increase pressure on the flow controller for the wash vial to 5 PSI. Then open the wash valve and leave the wash valve open until all remaining air is removed from the microfluidic flow chamber. To verify all air is removed, look at the microfluidic flow chamber using the microscope.

15 Return the pressure on the flow controller to 3 PSI and stop the flow of the wash solution by closing the valve.

16 Unclamp the waste, ink, and solution tubes.

17 Move field of view of the camera to the patterning channel within the microfluidic flow chamber and refocus if necessary. Then adjust microscope focus to 50% of a full rotation of the focus knob below the channel focal plane for patterning.

18 Run the MAPDH script
Step 18 includes a Step case.
MAPDH scripts

step case 10m

MAPDH scripts

MAPDH scripts can be for patterning different geometries and hydrogel compositions, as well as guides, can be found at https://github.com/MishaRubanov/MAPDH/

19 After patterning is done, flow 1X TAEM wash by opening the wash valve for 00:10:00

20 Take both brightfield and Cy3 micrographs of fabricated hydrogels.