Last Update: March 22, 2023

DNA/RNA-surface preparation (New method, recommended, updated March 2023)

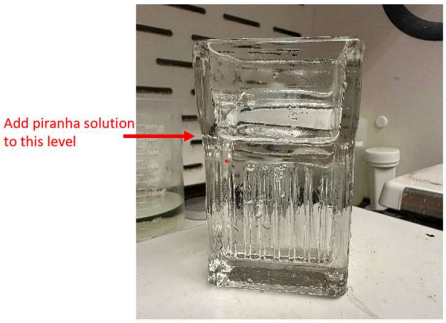
Ref: 1) Jing Pan, Yancheng Du, Hengming Qiu, Luke R. Upton, Feiran Li, and Jong Hyun Choi, *Nano Letters* **2019** *19* (12), 9138-9144, DOI: 10.1021/acs.nanolett.9b04428

1. Etch glass coverslips in Piranha solution (3:1 v/v sulfuric acid: hydrogen peroxide) for 30 minutes.

- Important: Add Sulphuric acid first into the glass container and then add hydrogen peroxide **slowly**. A special glass container is used for this purpose.
- Do this entire process inside the fume hood.
- Stir with a glass rod so that the solution is mixed well, wash the glass rod with running tap water after stirring.
- Be careful while dealing with Piranha solution and use anti acid gloves. Piranha solution can burn any surface within seconds.

Anti acid glove

- Place the glass coverslips into grooves inside the container. You should see bubbles popping from the coverslips due to
- Always prepare coverslips in multiples of 2 as they need to be sandwiched later.



Glass container

Coverslip arrangement

- After 30 minutes, carefully pour the piranha solution in the acid disposal flask inside the fume hood.
- Then open the tap and let water run at medium speed. Fill the container with DI water and then pour the DI water (without moving the coverslips) into running water in the sink. Repeat this 3 times.

2. Wash the coverslips with sufficient DI water and then wash with ethanol.

- Wash each coverslip individually.

- Hold the coverslips using 2 fingers by the sides so that there is minimum area in contact with the fingers.
- Pour DI water on both top and bottom sides. Then rotate it 90 degrees and repeat. Do this until you reach the original side again.
- Next, dry the surface with air stream.
- Do the same with ethanol for each coverslip.

Hold the coverslip like this while washing and drying.

3. Dry the surfaces with filtered air stream.

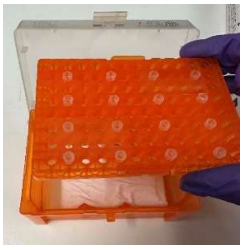
- The drying step after ethanol washing needs to be done quickly.
- Ethanol washing may leave a little residue on the coverslips after drying.
- In that case, wash the surface with ethanol again and dry it with air stream quickly.

4. Add approximately 100 µl 5% (v/v) 11-Azidoundecyltrimethoxysilane in Hexane on one surface. Put another coverslip on top forming a sandwich structure and incubate overnight in the thermocol box.

- Align one side of the second coverslip with the first and gently drop the second coverslip over the first.
- Make sure there are no air bubbles in the center. Air bubbles near the edges may be okay.
- Hexane evaporates very fast. So, prepare the solution quickly.
- Overnight incubation is done at room temperature in dark. Use old pipette tip holder boxes (usually orange in colour) and place 4 pipette tips for 4 corners of the coverslip.
- Add tissues/kimwipes on the bottom surface of the box (below the pipette tip holder) and wet them with Ethanol so that hexane doesn't dry out quickly. If this step is not done, hexane dries quickly, and the surface will not be silanized properly.

Azide silane sealed with paraffin

Coverslips on pipette tip holder box



Tissues on lower surface

5. Wash with **ethanol** to remove excess silane and dry in air stream.

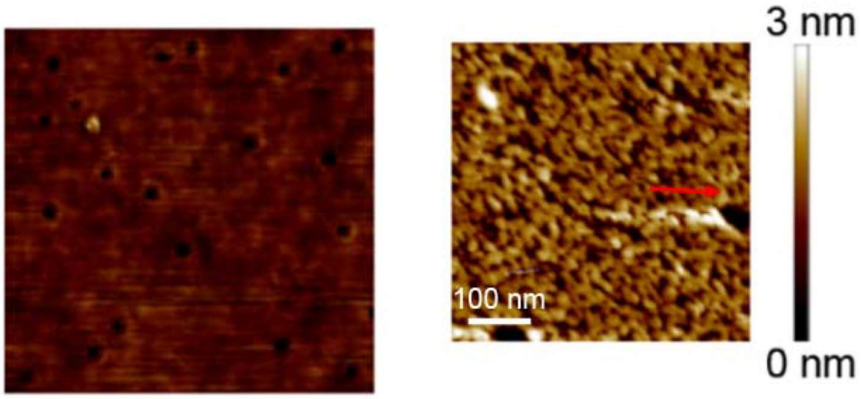
- Make sure to remember the silanized side of the coverslips.

6. Add approximately 100 µl 50 µM DBCO-RNA (prepared according to **DBCO-RNA synthesis)** on the azide modified surface and put another surface on to form sandwich such that the azide-modified surfaces are together inside the sandwich. The surfaces are incubated overnight for DBCO-RNA to click onto azidesilane.

- Calculate the concentration of the prepared DBCO-RNA and dilute accordingly.
- Overnight incubation is done at room temperature in the dark in the thermocol box. This time, wet the bottom side tissues with PBS buffer instead of Ethanol.

7. Wash the RNA-decorated surface with DI water and store in -20°C.

- To ensure that the prepared surfaces are good, characterize them using AFM.
- Also characterize the silanized surface: there should be no aggregations; the black spots in these AFM images are defects of coverslips, which are normal.
- The AFM characterization of RNA surfaces can be used to calculate the RNA density on the surface.



Silanized surface RNA modified surface

Fabrication of microchannel

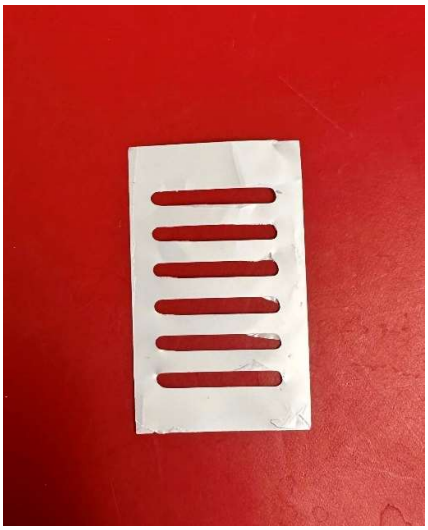
1. Use the double sided adhesive channel to mark positions of intlets and outlets on a glass slide and drill holes with the drills.

2. Bond the Inlet and outlet ports (LabSmith) to the glass slide using epoxy.

- Make sure they are well aligned.
- Use a thin pin to ensure that epoxy isn't blocking the ports.

2. Use the electronic cutter (Silhoutte Curio) to cut 2-mm flow channels on the adhesive sheets.

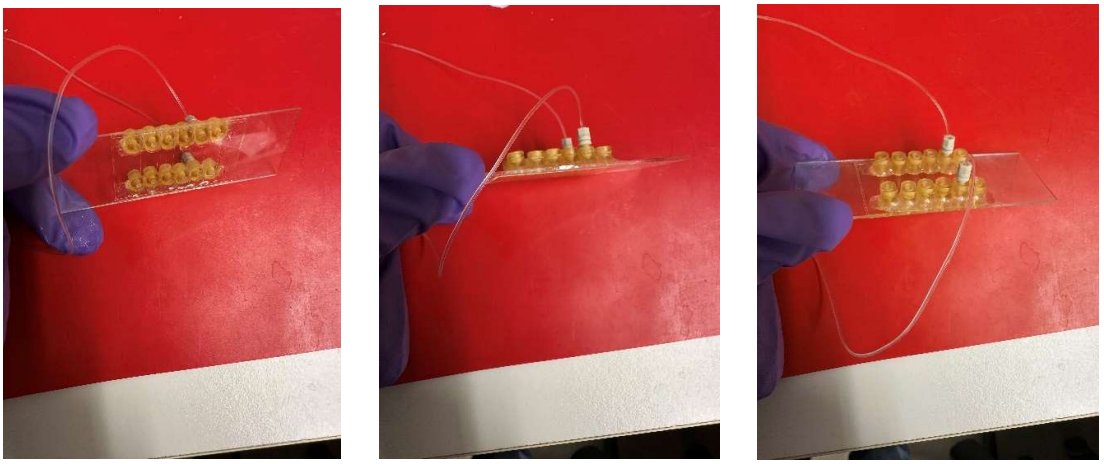
Silhoutte Curio



Double-sided adhesive microchannel

3. Sandwich the channels between the slides and coverslips.

- The adhesive sheets have adhesive surfaces on both sides.
- Peel one section from the double sided adhesive and stick it on the glass slide. Align the channels with the inlet and outlet ports.
- Remove the other side of the double sided adhesive and place the coverslip over it.
- Use a pointed tweezer to press on all places on the coverslip so that there are no air bubbles inside this setup.



Flow channel assembly

DNA/RNA Surface (Old method, not recommended, updated Fall 2017)

1. Expose the coverslips in Piranha solution for 1 hr.
2. Discard the Piranha solution and rinse the coverslips in DI water three times.
3. Dry coverslips with N2 or air stream.
4. Place coverslips and a 200 mL beaker in oven for 30 min under 110C.
5. Take the coverslips and beaker out of oven and let cool to room temperature.

6. Add 142.5 mL hexane and 7.5 mL GPTES into the beaker. Immerse the coverslips in the beaker overnight. (Note: salinization on the coverslip surface relies on the thin water layer on glass surface, thus the reaction takes much longer time compared to ethanol/acid based methods)
7. Rinse the coverslips with hexane several times to remove unbound GPTES.
8. Place the coverslips in the over for 30 min under 110C. This step allow crosslinking of silane molecules. (Curing step)
9. Prepare 100 uM DNA solution in 3X SCC buffer. Add 100 uL per 2 coverslips. Incubate in dark and humid environment for 10 hrs.
10. Rinse the coverslips with DI water.
11. Immerse the coverslips in 50 mM ethanolamine/0.1 % SDS/0.1M Tris pH 9.0 for 30 min.
12. Additional surface passivation with BSA for 10 min before experiment.

DSSTween-20 Surface

1. Scrub off tape residues using acetone from slides.
2. Rinse with DI water, acetone, methanol, Alconox until the slide looks clean.
3. Put slides in a staining jar. Sonicate with 1:1 acetone/methanol to remove tape residues.
4. Rinse with DI water. Dry with N2 gas.
5. Expose the coverslips in Piranha solution for 1 hr.
6. Discard the Piranha solution and rinse the coverslips in DI water three times.
7. Add 5 M KOH to the staining jars and sonicate for 1 hour.
8. Rinse PP staining jar with DI, hexane three times. Dry with N2 gas.
9. Rinse the coverslips with DI water three times. Dry with N2 gas. Put dried coverslips in to staining jar.
10. Add 50 ml hexane and 50ul DDS to the staining jar. Make sure DSS has minimal contact with the air. Tighten the cap.
11. Gently shake the staining jar at room temperature for 1.5 hour.
12. Dump hexane-DSS. Rinse and sonicate the coverslips in fresh hexane for 1min. Repeat this step for 3 times.
13. Dry the coverslips using N2 gas.

PEG Surface

1. Rinse the coverslips thoroughly in DI water.
2. Add acetone to the staining jars. Sonicate for 20 minutes.
3. Discard the acetone and rinse the coverslips in DI water three times.
4. Add 1 M KOH to the staining jars and sonicate for 20 minutes.
5. Rinse the coverslips with DI water three times.
6. Add coverslips to a large beaker. Add sulfuric acid to the beaker containing the coverslips.
7. Add hydrogen peroxide to the sulfuric acid in an acid: hydrogen peroxide ratio of 3:1.
8. Gently stir the solution and leave undisturbed for ~30 minutes.
9. Remove the coverslips and rinse with DI water before putting them into the staining jars.
10. Rinse the coverslips thoroughly with DI water.

11. Prepare 2% v/v solution of silane reagent in methanol. (50ml Methanol, 1.5ml APTES, 2.5 ml acetic acid)
12. Add salinization solution to staining jar and agitate for 20 mins.
13. Rinse with excess amount of water.
14. Dry Coverslips in vacuum desiccator.
15. Prepare a 50:1 methoxy: biotin PEG-NHS mixture in pH7.4 PBS buffer. (100mg PEG, 5mg bioPEG in 500ul Buffer)
16. Pipette 100uL of PEG mixture onto a dry salinized coverslip and place another coverslip on top. Use a spacer between two coverslips. Make sure the entire side of the coverslip is coated.
17. Incubate for 3 hrs. (At least for 2 hr).
18. Wash with excess water. Blow dry with filtered air/Oven dry.
19. Prepare (0.01 mg/ml avidin solution), (1mg/ml BSA), (0.01mg/ml biotin-BSA and 1mg/ml BSA mixture) in Tris-HCl buffer.
20. Flow BSA/biotin-BSA solution through microchannel and incubate for 5 min.
21. Flow Tris-HCl through to wash away excess BSA.
22. Flow avidin solution through and incubate for 1 min.
23. Flow Tris-HCl through to wash away excess avidin.
24. Flow BSA solution through and incubate for another 5 min.
25. Flow Tris-HCl through to wash away excess BSA.
26. Prepare 2.5% v/v (5ul SWCNTs in 195ul TAE) DNA-SWCNTs sample TAE buffer. Flow through microchannel and incubate for 30 mins.
27. Flow TAE buffer through to wash out unbound SWCNTs. (200 flow cell volume)
28. Prepare 2.5% v/v (5ul QD in 195 TAE) DNA-QD sample in TAE buffer. Flow through microchannel and incubate for 30min.
29. Flow TAE buffer through to wash out unbound SWCNTs. (200 flow cell volume)
30. Flow reaction buffer through the channel.