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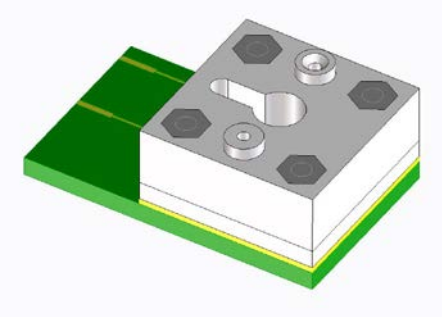
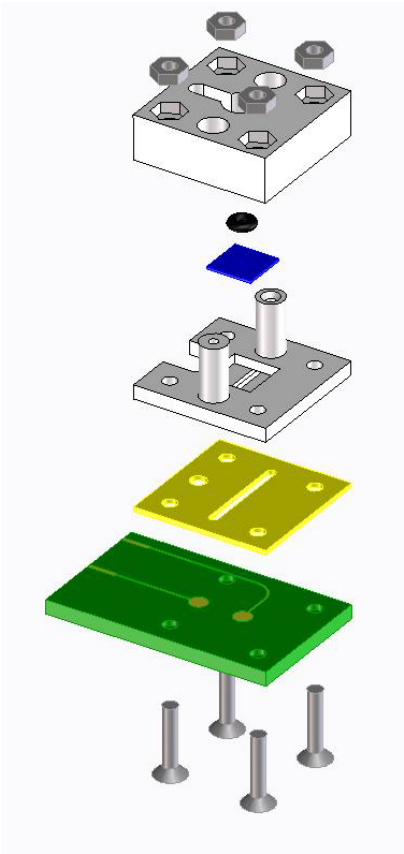
## eNPR – Flow cell for Nanopore Chip assembly and measurement instructions

This short user guide will show how to assembly the flow cell for Nanopore Chip and how to use it for nanopore experiments. The fully assembled **flow cell** looks like this.



### Parts stack



n. 4 M1.6 nuts

Top fluidic with top well

Sealing O-Ring

5x5 mm Nanopore chip (square)

Bottom fluidic with channels to access the bottom well

Gasket

PCB with Ag electrodes

n. 4 M1.6x8mm screws

Two kind of nanopore chips are availbe, both with a SiN layer where the nanopore is drilled, but one type is glass-based and the second type is SiO based. The thickness is different between the two nanopore chip, and it's 200 µm for the glass based and 500 µm for the SiO, so please use the correct bottom fluidic part with the correct nanopore chip thickness.

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## Assembly procedure

For best results, the 5x5mm silicon nitride chip should be treated with oxygen plasma.

**Oxygen Plasma Treatment** [1]:

Expose both sides of the 5x5 mm silicon nitride nanopore chip to oxygen plasma.

The following parameters were established using a Gatan Model 950 Advanced Plasma System.

Time per side - 2-3 minutes
Oxygen flow rate - 40 sccm
Forward RF Power- 30 W

**Alternative method: Piranha Cleaning** (please see reference [2,3]).

Place the nanopore containing silicon nitride chip into a 10 ml Pyrex beaker. Take care not to break the nitride window as it is very delicate. Place the beaker on a hotplate in a fume hood and set to 80°C.

Clean the nanopore chip with piranha solution using great care. First add 3 ml sulfuric acid to the container using a glass pipette. Next, carefully add 1 ml hydrogen peroxide to the sulfuric acid to make piranha solution 14. Please take all precautions.

Allow the nanopore chip to soak in piranha solution for 5 minutes.

Remove the piranha solution from the beaker using a glass pipette. Place it into a proper storage receptacle.

Fill the beaker with the de-gassed, de-ionized water using a clean glass pipette. Empty beaker of water and repeat at least 5 times.

Remove the nanopore chip with clean tweezers and dry it by light suction.

### Flow cell assembly (in case of dry nanopore chip)

1. Chlorinate the silver electrodes on the PCB by adding a drop of bleach on each electrode at least 10 min.

2. Clean the gasket and the O-Ring with Isopropanol or Ethanol, rinse with Milli-Q water and dry.

3. Clean the nanopore chip with Isopropanol or Ethanol, rinse with Milli-Q water and dry.

4. Make sure that the top and bottom fluidic chambers are completely dry.

5. Assembly the flow cell according to the stack above. Load the nanopore chip as normal and tighten the screws securely so that the seal is tight.

6. Fill the bottom well with 30 µl of salt solution.

7. Fill the top well with 60 µl of salt solution.

8. Insert the flow cell into the eNPR reader.

**Testing the chip**

Use the Elements Data Reader (EDR) 3 software.

Set 20 nA as current range, 20 kHz as sampling rate in the EDR control tabs.

In the Analysis, RC estimation tab click on the Start button.

Check that the capacitance of the chip is > 2.5pF.

If measured capacitance is below 2.5 pF, see troubleshooting below.

**Trouble shooting:**

- A capacitance value below 2.5pF indicates the buffer solution is not in contact with the nitride membrane. First inspect the top side of the chip for any air bubbles and remove as necessary.
- If capacitance remains below 2.5pF, thoroughly flush and replace the top and bottom solutions with Milli-Q water.
- Aggressively flush the bottom well with isopropanol or ethanol (this aids in the wetting of the bottom chamber).
- Flush out the isopropanol thoroughly with water several times. Be careful not to add any air bubbles.
- Replace the salt solution on the bottom well, being careful not to add air bubbles.
- Re-measure capacitance.

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**Flow cell assembly (in case of wet SiO nanopore chip)**

1. Chlorinate the silver electrodes on the PCB by adding a drop of bleach on each electrode at least 10 min.

2. Clean the gasket and the O-Ring with Isopropanol or Ethanol, rinse with Milli-Q water and dry.

3. Make sure that the top and bottom fluidic chambers are completely dry.

4. Please be careful with the Si chips, they are more fragile than the glass chips.

5. Remove the chip from the Eppendorf tube with tweezers and place with the "pit" side up onto the chamber (the idea is to keep the flat side of the chip near the bottom solution so that a bubble does not form in the etched "pit" of the chip).

6. Place the o-ring on top of the chip and seal the chamber tightly.

7. Place a small amount of water (10 uL) on the top side of the chip to keep it wet.

8. Flush the bottom of the cell with isopropanol or ethanol.

9. Flush the bottom of the cell with Milli-Q water. Be careful not to introduce bubbles into the chamber. Finally place the salt solution on both sides of the cell.

10. Insert the flow cell into the eNPR reader.

**Testing the chip**

Use the Elements Data Reader (EDR) 3 software.

Set 20 nA as current range, 20 kHz as sampling rate in the EDR control tabs.

In the Analysis, RC estimation tab click on the Start button.

If you do not see a current, check the capacitance of the chip using the amplifier. If solution is in contact with the membrane window, the capacitance should be greater than 4pF (silicon chips). If is less than this value, that means the solution has not made contact with the chip itself. If this is the case, do the following:

- carefully check the top side of the chamber for bubbles and remove with a pipette if observed.
- flush the bottom of the chamber with water.
- aggressively flush the bottom of the chamber with isopropanol or ethanol (this aids in the wetting of the bottom chamber).
- flush out the isopropanol thoroughly with water several times. Be careful not to add any bubbles.
- replace the salt solution on the bottom, being careful not to add bubbles.

**References:**

1. Smeets RMM, Keyser UF, Dekker NH, Dekker C. Noise in Solid-State Nanopores. *Proc. Natl. Acad. Sci.* U.S.A. **2008**;105(2):417–421
2. Wanunu M, Meller A. Selvin, P. R., Ha, T. *Single-molecule techniques: a laboratory manual*. Cold Spring Harbor Laboratory Press. New York. 395-420 (2008).
3. Niedzwiecki DJ, Movileanu L. Monitoring Protein Adsorption with Solid-state Nanopores. *J. Vis. Exp*. **2011**. p. e3560.