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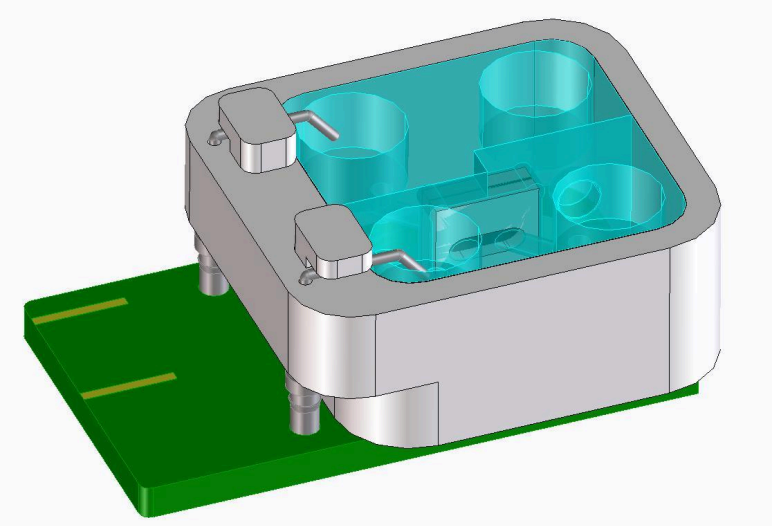
Nanopore Flow cell: assembly and cleaning procedure



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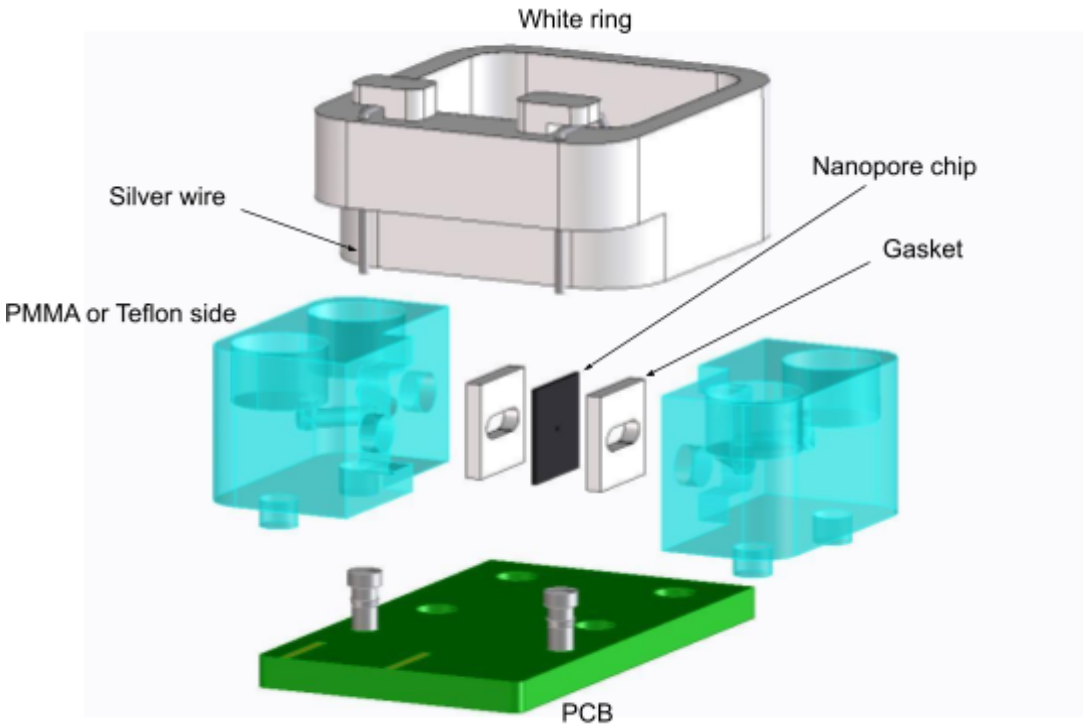
This user guide provides details on the individual components of the Nanopore Flow cell, its proper assembly and describes the cleaning procedure. This flow cell is designed to host 5 x 5 mm nanopore chips and is compatible with a range of nanopore chips thicknesses, namely from about 200 um to 500 um.

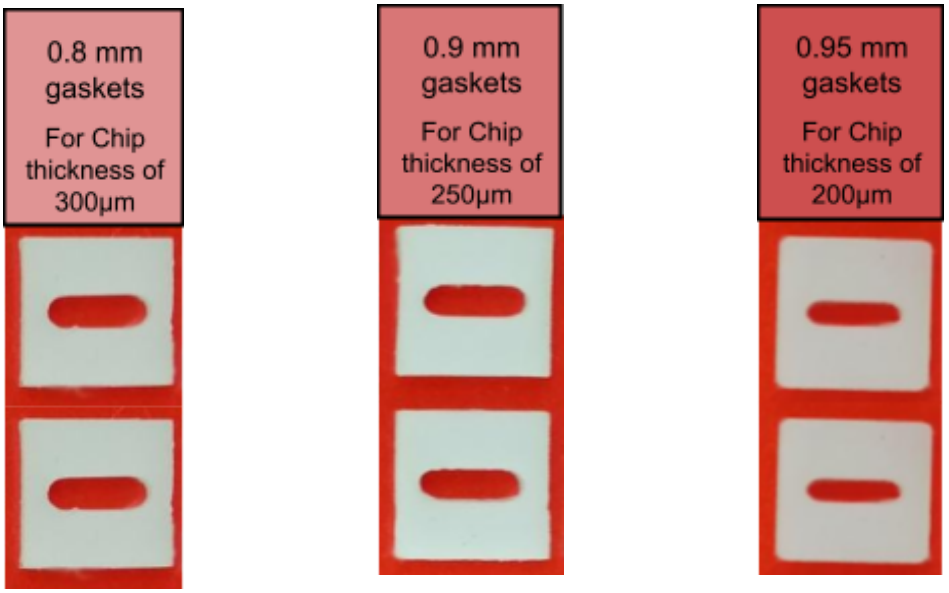
The flow cell consists of two plastic parts made of PMMA or Teflon. When assembled, the two parts form a squared cavity 1.7 mm thick that hosts the nanopore chip. Sets of gaskets of different thicknesses are provided to seal the nanopore inside the cavity, preventing leakage. A white ring holding two silver wires (Ag/Cl electrodes) keeps the two plastic parts in place. The assembled parts sit onto a PCB that fits into the Elements nanopore reader amplifiers.



Items list and description

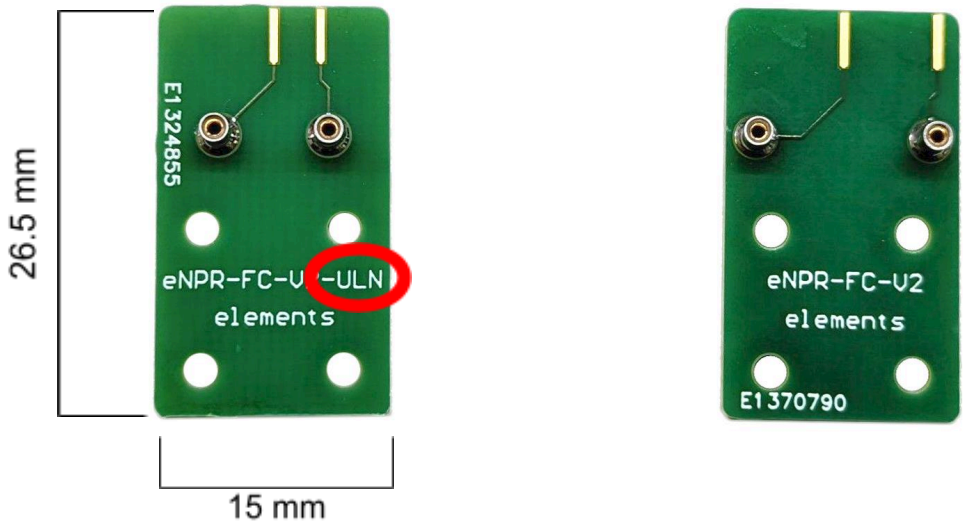
- **Plastic-made sides**: The two plastic sides can be made either of PMMA or Teflon, depending on the purchased flow cell. Each side embeds two fluidic channels that link one side of the nanopore chip to a couple of reservoirs. Most of the P200 tips of laboratory pipettes fit into the fluidic channels to allow the addition of the electrolyte without the formation of bubbles.

- **White ring:** the Teflon made ring is designed to keep in place the two plastic sides when the flow cell is assembled and holds the two silver wires electrodes. The wires can be removed from the ring and replaced any time. The diameter of the wire must be 0.5 mm to properly fit into the PCB sockets.
- **Gaskets**: The thickness of the nanopore chips can vary from a few, to several hundreds of micrometers. Six pairs of gaskets differing in their thickness are provided with the flow cell: 0.7, 0.75 and 0.8 mm in type 1 set or 0.85, 0.9 and 0.95 mm in type 2 set). Choose the gasket with the proper thickness (thicker chips need thinner gaskets) to fit nanopore chips of any thickness in this flow cell. Here below some examples of the combination sizes of gaskets and nanopores chips are shown.



- **The PCB**: If you have purchased the Elements eNPR 100kHz amplifier, you may choose between two different flow cell's PCB: either the low noise (LN) or the ultra low noise (ULN). With the LN configuration the maximum applicable voltage is ± 2000 mV. The ULN configuration allows the RMS noise to be reduced by ~30% but limits the applied voltage to ± 700 mV. The UltraLowNoise PCB is labeled as ULN to be distinguished from the LN one. If the label disappears, the two PCBs can be distinguished by the distance of the gold pads electrodes as shown in the figure below. **Important Note:** The EDR software must be set according to the used PCB by clicking on the dedicated button as described in the dedicated "how-to" guide.

  In the case you have purchased the Elements eNPR 10 MHz amplifier, you must use only the low noise (LN) PCB and no additional settings are required in the software.



Flow cell assembly & cleaning procedure

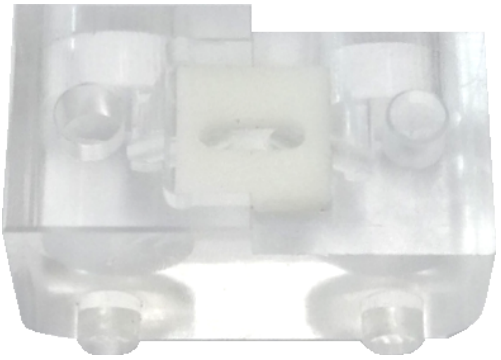
Before assembling the flow cell, check that every part is in good condition. In particular, check for the presence of deep scratches or crevices at the area in contact with the gaskets that may prevent the proper sealing. Just before starting the flow cell assembly, the plastic made sides (PMMA or Teflon) must be cleaned with isopropanol and carefully dried. The gaskets must be cleaned too by soaking them in isopropanol for some seconds. We suggest using a gentle flow of air compressed to dry the parts (including the small fluidic channels in the plastic-made sides). The gaskets may be dried using adsorbent paper that does not leave residues.

1. Set up the white ring by installing the silver wires as shown in the figure below. Before fitting the silver wires into the silver wire holder, they must be chlorinated. The simplest method to chlorinate the silver wires is to immerse them into pure bleach until they become light gray coloured (typically 15 to 30 minutes are enough). The commercial bleach purchased in any supermarket is fine for this purpose.

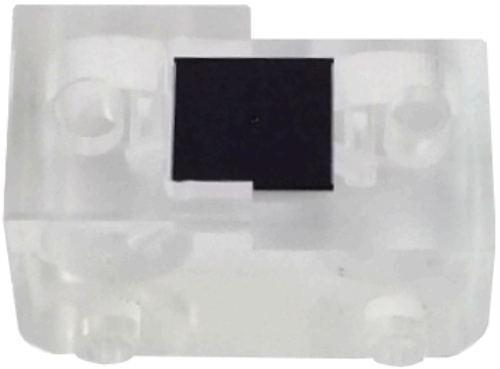
Important note: make sure not to chlorinate the portion of the wire that fits into the sockets on the PCB by keeping them outside the bleach. Before installing the wires in the white ring, rinse them with abundant distilled water.

2. Choose one of the transparent plastic parts and place it on a flat surface as shown below.

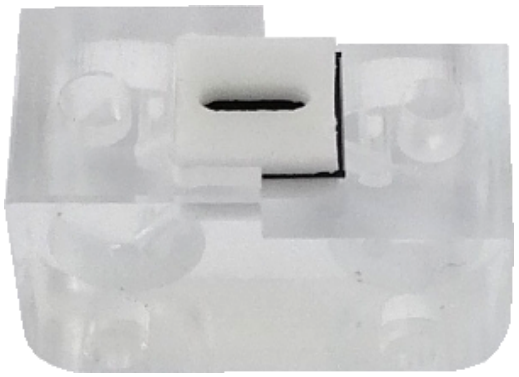
3. After selecting the appropriate gasket (according to the thickness of your nanopore chip), clean the gaskets with Isopropanol for a few seconds, then wash them with deionized water and dry them. Now place one of them on the plastic piece as shown. Important note: to prevent leakage in the flow cell final assembly, make sure the gaskets are perfectly dried before installing.



4. Install the nanopore chip on top of the gasket as shown below (the black square represents the chip). If you treat the chip with a wetting solution like the Piranha, you must dry the nanopore chip before installing it into the flow cell. Installing a wet chip may lead to leakage issues in the final assembly.



5. Next, place the second gasket on top of the nanopore chip.

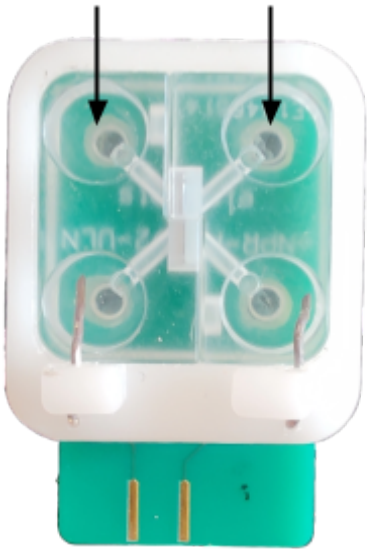


6. By inserting the second transparent plastic piece, you can feel the compression of the gasket. You will have the same shape as a sandwich.

7. Fit the sandwich into the provided white ring

8. Place the sandwich surrounded by the white ring onto the PCB. The four small plastic pins at the bottom of the transparent parts must fit into the corresponding holes drilled in the PCB, serving as a guide for the positioning of the fluidic cell.

10. Your flow cell is now ready, you have two chambers on the left and right, each containing a minimum of 10 and a maximum of 60 microliters. Using either a p200 or p100 pipette, inject your electrolyte solutions through the holes pointed by the blue arrows in the figure below.



10. Insert the flow cell into the eNPR reader and add the electrolyte as soon as possible.

11. At the end of each experimental session, clean the flow cell as described below. Note: the use of any solvents other than those listed above is not recommended. Before using a solvent, check its compatibility with the flow cell material (PMMA, Delrin, Teflon, PC). The cleaning procedure refers to the plastic parts of the flow cell. The gaskets can be cleaned simply by rinsing with distilled water. Do not use solvents such as alcohols acids or bases to clean the gaskets.

PMMA Flow cell cleaning:

Carefully separate the components, rinse the flow cell with DD water. Spray rubbing alcohol (Isopropyl Alcohol) and gently clean the components using a soft bristled toothbrush. You can use compressed air to remove rubbing alcohol (Isopropyl Alcohol) and water until the chip is dry. Once all the components are clean and dry, store them in the dry airtight container.

To remove oligonucleotides such as DNA aptamers that are weakly bound to the flow cell surface, you can use a 0.1 M buffer containing KH_2PO_4, KCl and NaCl salts, pH of 7.4.

If you have used biological substances, you can use 10% bleach solution for 10 minutes and then 70% Isopropyl Alcohol) for 10 minutes.

At the end of any treatment, wash with DD water and let the flow cell dry.

Do not use Ethanol, Acetone or pure bleach to clean the PMMA flow cell. If dishwashing liquid is used, the flow cell hydrophobicity changes.

Teflon™ Flow Cell cleaning

Teflon is the most versatile plastic in terms of chemical compatibility. It is highly resistant to most acids, alcohol, detergent and solvents. In any case, we recommend not to expose the flow cell to solvents for a long period of time. After some minutes, wash with DD water and dry.