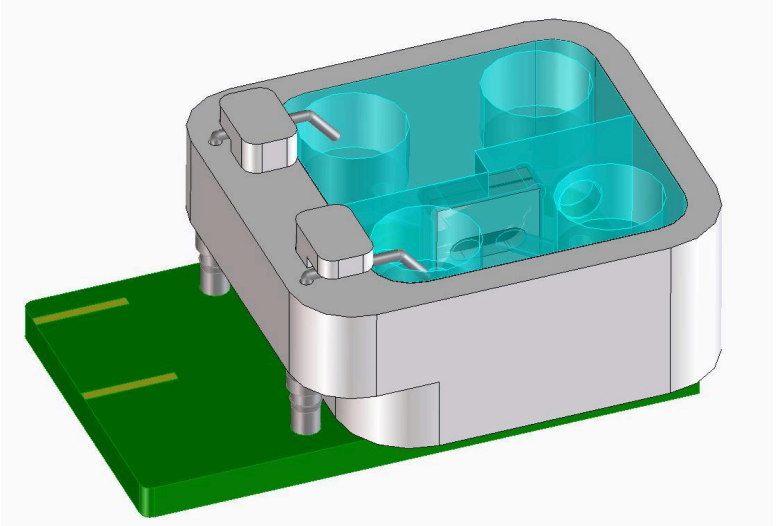
elements
enabling technologies for Life Science

Nanopore Flow cell for 5x5 and 4x4 mm nanopore chips: assembly and cleaning procedure

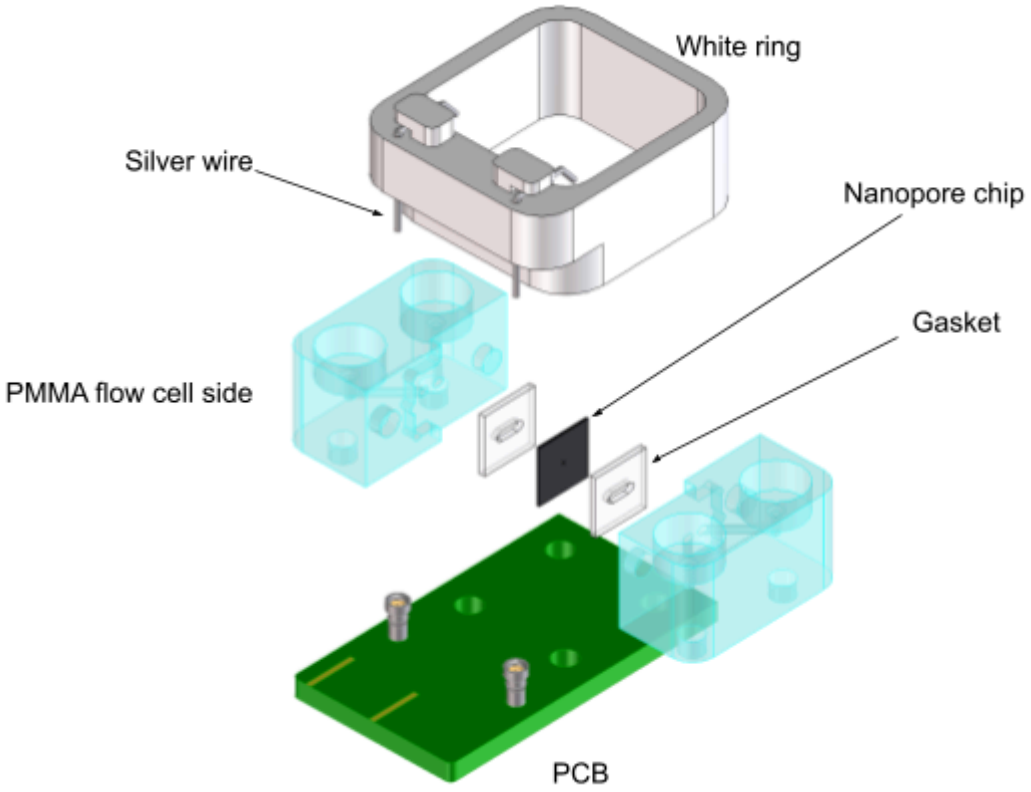


ELEMENTS srl - ITALY - C.F/P.IVA/VAT 04113900403 - www.elements-ic.com

commercial info: info@elements-ic.com - technical support: support@elements-ic.com

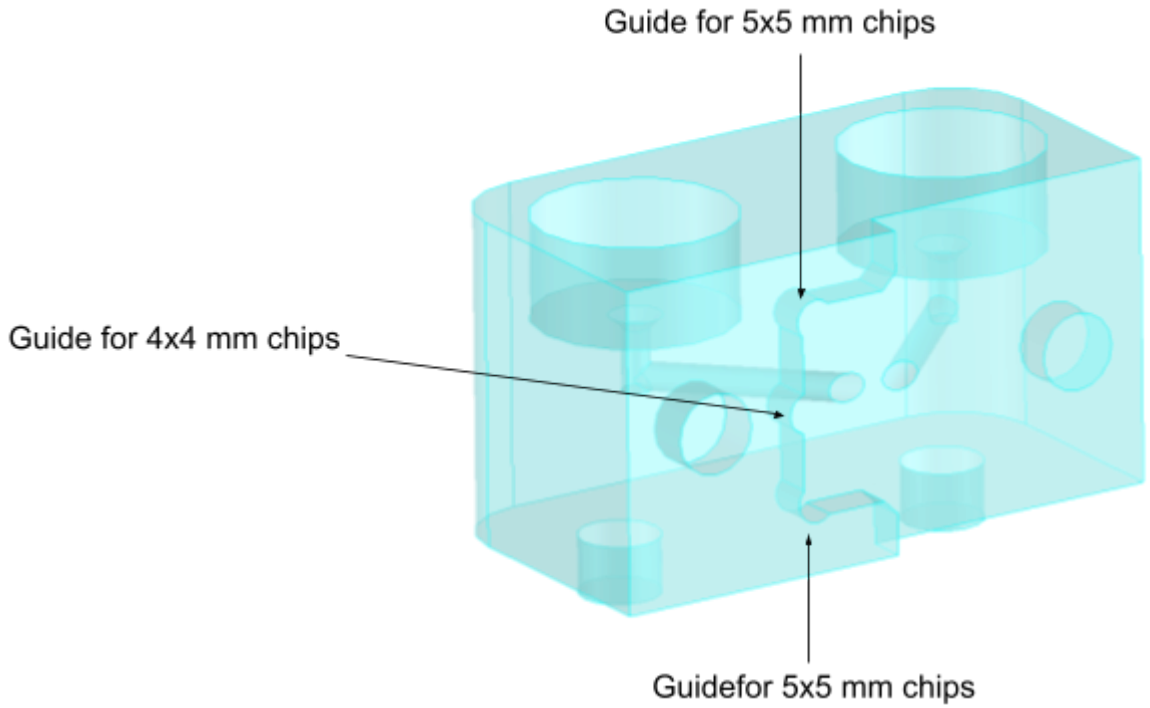
This user guide details the assembly process and cleaning procedure for the Nanopore Flow Cell. Designed to accommodate 5 x 5 mm or 4 x 4 mm nanopore chips with a thickness of 200 µm, this flow cell ensures optimal performance. For compatibility with different chip thicknesses, please refer to our alternative flowcell.

The flow cell comprises two PMMA (Polymethyl methacrylate) plastic components. When assembled, these components create a squared cavity, measuring 1 mm in thickness, designed to accommodate the nanopore chip. A pair of silicon gaskets, each measuring 0.5 mm in thickness, seals the nanopore within the cavity. The structural integrity of the assembly is upheld by a Delrin white ring, housing two silver wire electrodes (Ag/Cl), which securely fasten the plastic components. Finally, the fully assembled unit is mounted onto a Printed Circuit Board (PCB) tailored to integrate with Elements nanopore reader amplifiers.



Items list and description

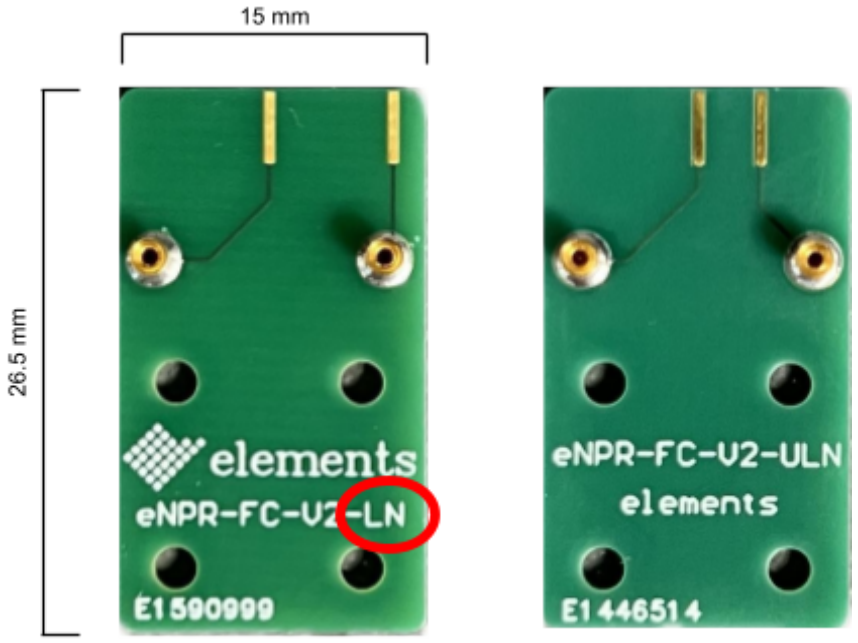
- **PMMA-made sides**: Each side features two fluidic channels that link one side of the nanopore chip to a couple of reservoirs. Most of the P200 tips of laboratory pipettes fit into the fluidic channels to allow the addition of the electrolyte without the formation of bubbles. The plastic side embeds the squared cavity that hosts the nanopore chip. In each plastic side, guides are machined in the squared cavity to help the positioning of the chip during the assembly (see Flow Cell Assembly chapter).



- **White ring:** made of Delrin, it secures the plastic sides and houses the silver wire 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:** Silicon-made, 0.5 mm thick, they ensure optimal sealing of the nanopore chip by filling the squared cavity.

- **PCB**: If you have purchased the Elements eNPR 100kHz amplifier, you have the option to choose between two different PCB configurations: the low noise (LN) or the ultra-low noise (ULN) PCB. The former configuration allows for a maximum applicable voltage of ± 2000 mV. It is suitable for general use and provides reliable performance. The ULN configuration offers approximately 30% reduction in RMS noise compared to the LN configuration. However, it limits the maximum applied voltage to ± 700 mV. This configuration is ideal for applications where minimizing noise is critical. It's important to note that the UltraLowNoise PCB is labeled as ULN to distinguish it from the LN PCB. If the label is not visible, you can differentiate between the two PCBs by examining the distance between the gold pads electrodes, as illustrated in the figure below.
  Important Note: Regardless of the PCB configuration used, it is crucial to set the EDR software according to the PCB in use. This can be done by clicking on the dedicated button within the software interface, as outlined in the corresponding "how-to" guide.

  In the case of purchasing the Elements eNPR 10 MHz amplifier, you should exclusively use the low noise (LN) PCB. Fortunately, no additional settings are required in the software when using this configuration.



Low Noise PCB Ultra Low Noise PCB

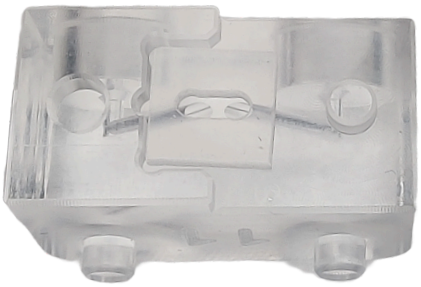
Flow cell assembly & cleaning procedure

Before assembling the flow cell, it's imperative to ensure that every part is in optimal condition. Pay particular attention to checking for the presence of deep scratches or crevices in areas that come into contact with the gaskets, as these could impede proper sealing.

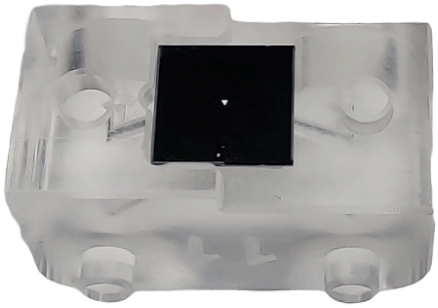
Prior to starting the flow cell assembly, clean the PMMA sides with isopropanol and ensure thorough drying. Similarly, the gaskets should be cleaned by soaking them in isopropanol. It's recommended to use a gentle flow of compressed air to dry all parts, including the small fluidic channels in the PMMA sides. Alternatively, absorbent paper that leaves no residues can be used to dry the gaskets.

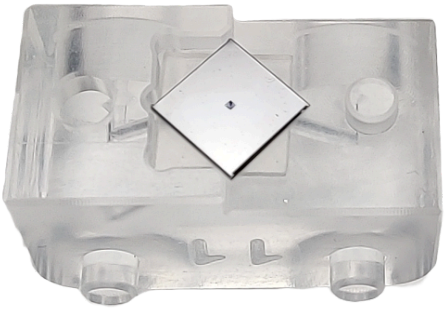
1. Set up the white ring by installing the silver wires according to the provided instructions. Before fitting the silver wires into the holder, they must be chlorinated. Chlorinate the silver wires by immersing them in pure bleach until they turn light gray (typically 15 to 30 minutes). Ensure that only the portion of the wire outside the sockets on the PCB is chlorinated. Rinse the wires with distilled water and dry before installing them into the white ring.

2. Choose one of the transparent plastic parts, clean it with 100% Isopropanol and dry. Place it on a flat and clean surface (e.g. inside a petri dish). Position one of the cleaned gaskets on the plastic piece as shown below. Important note: to prevent leakage in the flow cell final assembly, make sure the gaskets are perfectly dried before installing.

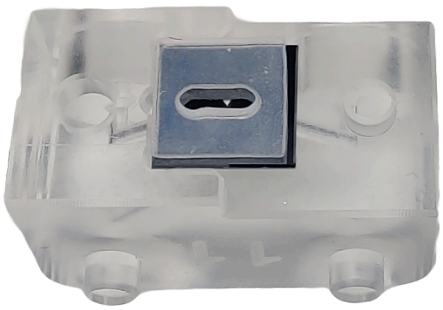


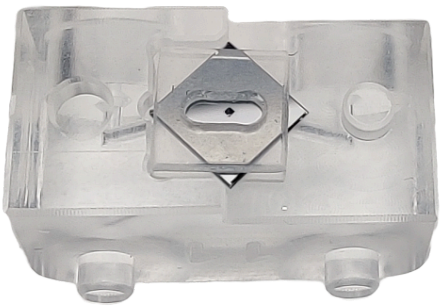
3. Center the nanopore chip on top of the gasket. Ensure proper alignment based on the size of the chip (5x5 mm or 4x4 mm) and its orientation. For 5x5 mm chips, align the perimeter with the one of the gasket (left figure). The 4x4 mm chips must be positioned rotated by 45°, using the dedicated guides (right figure). Important note, if you treat the chip with a wetting solution such as Piranha, ensure thorough drying before installing it into the flow cell. Failure to dry the chip adequately may result in leakage issues during the final assembly.





4. Next, place the second gasket on top of the nanopore chip. Even in this case, note that the alignment is different for 5x5 (left figure) and 4x4 chips (right figure).





5. Sandwich the assembly between the two transparent plastic pieces, feeling the compression of the gaskets.

6. Fit the sandwich into the provided white ring and place it onto the PCB. The alignment pins on the plastic parts should fit into corresponding holes in the PCB, guiding the positioning of the fluidic cell.

7. With the flow cell assembled, you now have two chambers, each containing a minimum of 20 and a maximum of 60 microliters. Inject the electrolyte solutions through the designated holes, as indicated in the provided figure. Finally, insert the PCB into the nanopore reader slot.

The depicted + and - symbols in the figure illustrate the polarity of the voltage when a +100 mV stimulus is administered. For further information please refer to this guide.

Flow cell cleaning:

After each experimental session, it's essential to clean the flow cell thoroughly. 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). The cleaning procedure refers to the plastic parts of the flow cell. The gaskets can be cleaned simply by immersing them in pure isopropanol and rinsing with distilled water.

Separating the components, carefully and rinse the flow cell with deionized water (DD water). Immerse the two PMMA parts in Isopropyl Alcohol (or spray it) and gently clean the components using a soft-bristled toothbrush. Use compressed air to remove rubbing alcohol and water until the parts are dry. Store all components in a dry and clean environment.

To remove weakly bound oligonucleotides or biological substances, consider using specific cleaning solutions:

For oligonucleotides: Use a 0.1 M buffer containing KH2PO4, KCl, and NaCl salts at a pH of 7.4.

For biological substances: Utilize a 10% bleach solution for 10 minutes, followed by a 70% Isopropyl Alcohol soak for 10 minutes.

After any treatment, thoroughly wash with DD water and allow the flow cell to dry completely.

Avoid using Ethanol, Acetone, or pure bleach to clean the PMMA flow cell, as these may damage the material. Additionally, the use of dishwashing liquid may alter the flow cell's hydrophobicity.

By following these detailed assembly and cleaning procedures, you can ensure optimal performance and longevity of your flow cell setup.