User Quick Guide

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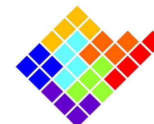
How to paint lipid bilayer membrane with the BLMKit using the paintbrush

Revision History

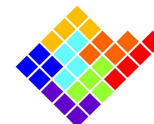
Date	Version	Description
17/01/2024	1.1	Insert new entries on top
17/01/2024	1.0	First version of document



This document provides a detailed guide showing how to paint lipid bilayer membrane with the BLM Kit using the paintbrush. An alternative method to create a lipid bilayer is described in the guide “MM technique in the BLMkit recording chamber”. Please note the Montal-Müller technique works well only in the new “BLM cuvette_MM” recording chamber, distributed from January 2024. It is particularly difficult to apply this procedure in the earlier “BLM cuvette_STD” because of the low position of the septum aperture in the compartment. To learn more about the differences between the “std” and “MM” BLM cuvettes, please see the related guide ““Structural and functional details of the STD and MM BLM cuvettes”.

The guide is divided in the following sections:

1) Instructions for software installation
2) BLM Kit content and setup
3) Step by step instructions to setup an experiment:
    1. Electrodes chlorination
    2. Lipid bilayer formation and troubleshooting
    3. BLM cuvette cleaning
4) Sample data obtained from users with the BLM Kit

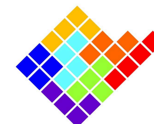


1) Software installation

1) Download EDR4 software from http://elements-ic.com/downloads/. To date (January 2024), you may still control your eONE amplifier with EDR3 software (download from the same link) but support for EDR3 will end soon(except for major bug fixes). Please send an email to support@elements-ic.com and request a free firmware update to make your eONE device compatible with EDR4 (please attach a screenshot of the "device information" window accessible from the "?" menu to the email).

2) Unzip the archive and start the installation following instructions.

3) Plug the eONE amplifier to any USB port of your computer and wait for hardware driver installation.



2) BLM KIT content and setup

The kit is contained into a 16 x 10 x 5 cm (6.3 x 3.9 x 2 in) Faraday cage and includes:

- The amplifier belonging to the eONE family

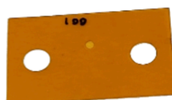
- A recording chamber composed by two Delrin-made cuvettes embedding the holes for the Ag/Cl electrode.
- a set of Teflon-made septa embedding the micro-aperture (50, 80, 110 µm sized) and/or a set of Polyimide (Kapton) septa embedding the micro-aperture (100, 150, 200 µm sized).

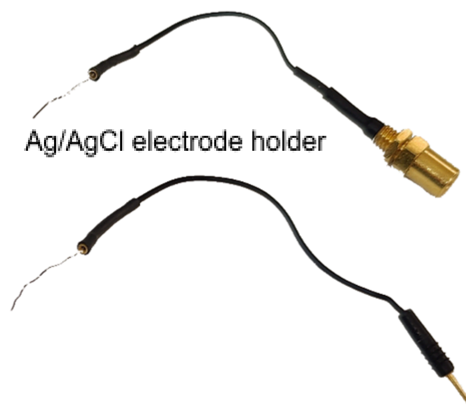
- a paintbrush

- Ag electrodes and input connector to plug the eONE amplifier. Depending on the eONE amplifier you have purchased, the silver wires connectors might be different from the one shown in the picture below.

BLM cuvette_MM

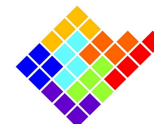
BLM Septum





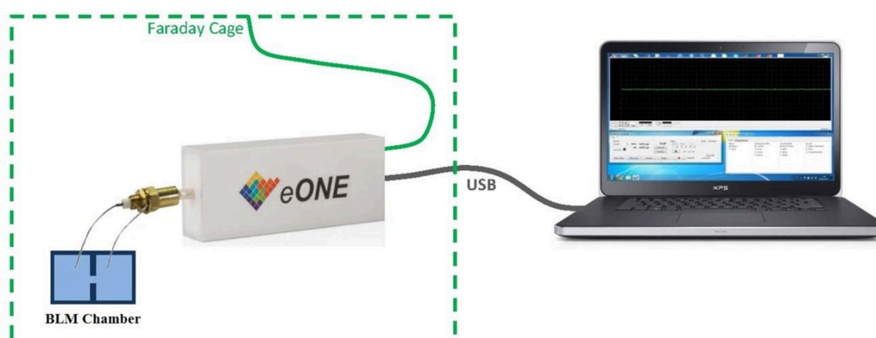
Ag/AgCl electrode holder

Paintbrush



When everything is assembled, the kit looks as shown in the picture below. Please, watch the video shown in the BLMkit webpage to get a detailed view of the BLM kit assembly and connection.

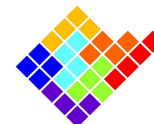
BLM Kit experimental setup example



BLM Kit connections

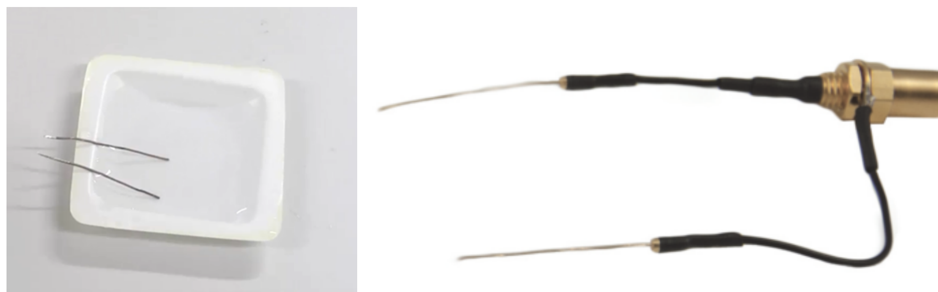
Warning:

Do not short-circuit the electrodes with the Faraday cage!



3) Setting up an experiment

3.1) Instructions for chlorinating the Ag/AgCl electrodes



1) Before chlorinating, clean the Ag wires:

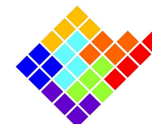
- If it is the first usage, simply clean the wires with ethanol and rinse with double-distilled water.
- If the wires were previously chlorinated, remove the old Ag/AgCl coating by abrading the surface with an extra fine grit sandpaper; then rinse with ethanol and double-distilled water.

2) Chlorinate the silver wires:

- The simplest method to chlorinate the silver wires is to immerse them into pure bleach until they become light gray coloured (it typically takes 15 to 30 minutes). 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 Ag/Cl electrode connector by keeping them outside the bleach, as shown in the figure above.
- An alternative way that requires a little more effort but yields to a deeper and more uniform chlorination is the electroplating method.

  Chlorinating a silver wire using the electroplating is achieved by making it positive relatively to a solution containing NaCl (0.9% w/v) or KCl (1 M) and passing a current at a rate of approximately 1 mA/cm^2 for about 1 minute (or until the wire is adequately plated and becomes gray coloured). For example, chlorination of a 1 cm-long 0.5 mm Ag wire (that is the diameter of the wires used in the kit) requires 0.15 mA of current. While plating, occasionally reverse the polarity for several seconds; this helps to deepen the chloride coating and yields to a more stable electrode.

Chlorination must be performed every time the observed current baseline is not stable overtime. It is recommended to perform a chlorination every 5 hours of continuous experiments.



3.2) Lipid Bilayer Membrane formation (with the paintbrush-based method)

1) **Assemble the BLMkit** and the recording chamber as shown in the video "painting the lipid bilayer membrane with the paintbrush" available in the video section of the BLMkit webpage. The video also provides a detailed view of the next steps described below.

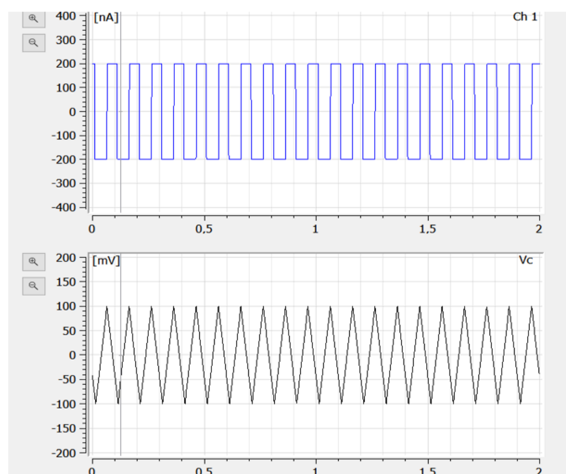
2) **Prepare the lipid solution**: in this example we have dissolved DPhPC lipids at 10 mg/ml in 95% n-decane (or n-octane) and 5% butanol.

3) **Pretreat the aperture of the septum** with 1% hexadecane in n-hexane solution using the provided paintbrush to increase lipids affinity to the septum in the area surrounding the hole. Wait 5 minutes for the solvent evaporation before filling the compartments (step 5).

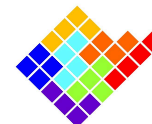
4) **Setup the software**: connect the amplifier and open EDR software. Set the 20 nA range and disable the voltage command filter tool (for more details, please see the EDR related "how to" guides or watch the video). Use the RC compensation tool to apply a triangular wave stimulus and estimate in real time the capacitance of the circuit.

5) **Fill the BLM cuvette**: fill both compartments with 700 ml of electrolyte solution. The top level of the solution must be above the hole of the septum.

Check the proper filling of the hole. A full-scale square wave (due to amplifier saturation) should be seen, as shown in the figure below.

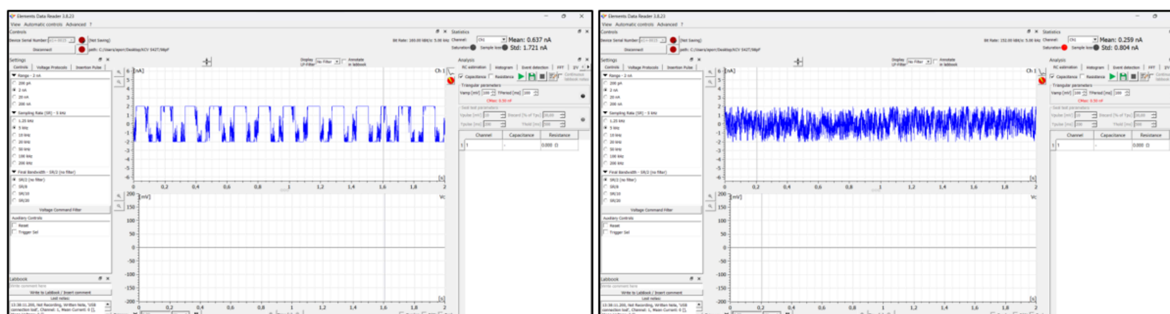


If saturation is not achieved, it means that the aperture is not totally wet. Proceed by removing the buffer solution with a lab pipette and fill the BLM chamber again by pushing



the solution against the aperture of the septum. Another method is to give some brief and gentle mechanical shocks to the septum in order to get the aperture wet.

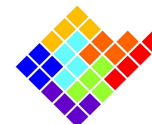
6) **Compensate electrode offsets**: Before proceeding to the compensation, it is convenient to apply a 0 mV constant voltage value to better follow the zeroing of the current signal. After setting the voltage to 0 mV, click on the “voltage offset compensation” button. Depending on the EDR version this button can be found in different positions. Please refer to the EDR related “how to” guides to find it. Once the current offset is compensated (i.e. current fluctuates around 0), disable the Digital Compensation tool before proceeding. If not disabled, any further measurement will be useless.Voltage offset compensation must be performed only when there is no membrane formed in the aperture.

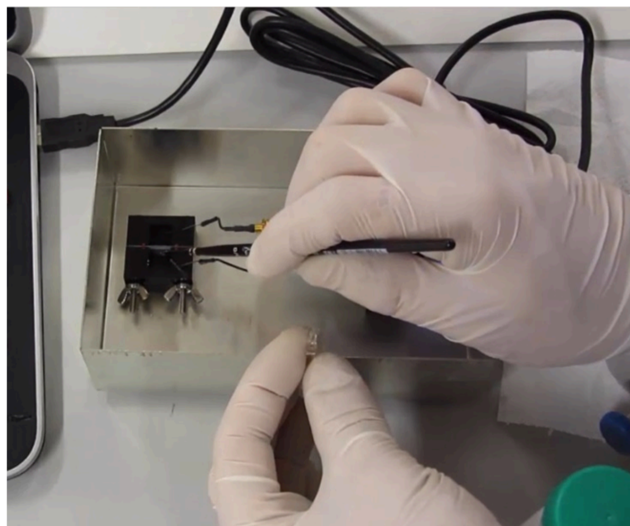


Current signal during the voltage offset compensation

Current signal after disabling the automatic voltage offset compensation tool

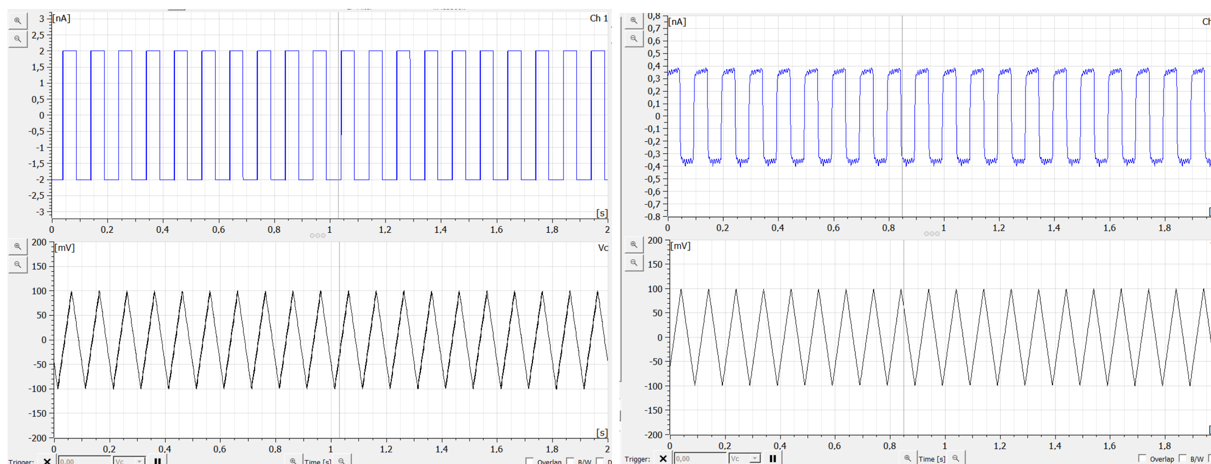
7) **Create the membrane**: start to gently paint the lipid solution over the hole in the Teflon (or Polyimide) septum using the paintbrush: dip the paintbrush in the lipid containing solution and shake it to remove the excess of solution that may cause a lipid plug formation. If a much larger current signal is observed, or the waveform appears as mixed squared-triangular, there is a leak in the recording chamber, possibly due to an incorrect assembly. In this case, disassemble the recording chamber, clean all the components as described at the end of this guide and restart from scratch.

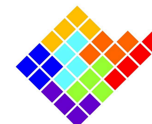




Monitor the membrane formation by looking at the current response:

when no membrane is present, a full-scale square wave due to amplifier saturation is observed (figure below, left). When the membrane forms, a time-dependent squared wave current signal can be seen in the display window; its amplitude increases proportionally to the bilayer capacitance (figure below, right). Check the membrane capacitance estimated in real time in the RC estimation panel.





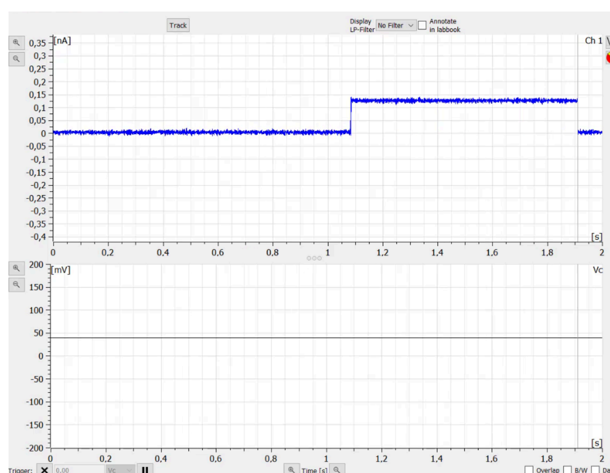
If no membrane forms, keep on adding small quantities of lipids to the septum following these steps:

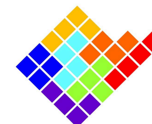
- Clean the paintbrush by dipping it in an ethanol containing-tube;
- Dry the paintbrush using a wipe;
- Dip the paintbrush in the lipids solution and remove the excess by shaking
- Add other lipid solution to the hole of the Teflon (or Polyimide) septum by painting;
- Repeat the procedure until the membrane is formed.

8) **Test the membrane stability**: once the membrane is formed, enable the voltage command filter option to increase the signal to noise ratio and test its robustness overtime by applying a series of constant voltage values. If the membrane is well formed, after a short current peak due to the membrane capacitance, the current must be constant at 0 pA.

9) **Add the pore forming protein**: add the membrane protein/molecule and wait for reconstitution into the lipid membrane. Depending on the expected current amplitude, select the lowest current range available in order to increase the signal to noise ratio.

   As a test bench, we suggest adding a commercially available pore-forming cytotoxin, α-hemolysin (αHL, Sigma Aldrich, SKU H9395-.5MG), which is easy to handle and, when properly stored, it is stable for several months. Briefly, dissolve the lyophilized toxin in MilliQ water to make a stock solution at 0.10 mg/ml that can be stored at 5 °C. The day of the experiment, prepare a 1:500 dilution in the electrolyte recording solution (3M KCl, 20mM TRIS-HCl, pH8). Add small amounts (1 ul) of this solution in the CIS side of the recording chamber and wait for the protein reconstitution into the lipid bilayer. The latter is visible as a current step from 0 pA to about 130 pA at + 40 mV bias voltage (see screenshot below). If multiple steps of the same amplitude are visible, multiple toxins have been inserted into the lipid bilayer. In this case, it is convenient to further dilute the working solution (e.g. try making 1:1000).





Tips to make successful experiments:

- Use clean double distilled or Milli-Q water and fresh filtered (0.2 µm) electrolyte solution.

- To avoid "lipid plugs" the lipid solution can be diluted more (like 5 mg/ml DPhPC in n-nonane or n-decane).

- If you can't see any insertion, try to add more protein into the solution or improve the quality of the lipid bilayer. The latter can be easily evaluated by monitoring the membrane capacitance of the membrane. As a general rule, the larger the capacitance value the higher the quality. As a reference, the typical values obtained using the above-described procedure are shown in the table below (experimental conditions are reported in the caption). Important note: changing the experimental conditions (e.g. different lipids or different electrolyte solution) might result in getting different capacitance values.

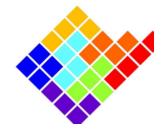
Material	Teflon	Teflon	Teflon	Polyimide	Polyimide	Polyimide
Thickness	50 µm	50 µm	50 µm	50 µm	50 µm	50 µm
Hole size ∅	50 µm	80 µm	110 µm	100 µm	150 µm	200 µm
Typical BLM capacitance (pF)	7 – 9	18 – 28	25 – 50	20 – 40	35 – 90	45 – 140

Membrane capacitances were measured using DPhPC lipids dissolved in n-decane at 10 mg/ml

If the capacitance value is smaller than those reported in the table for a long time (15-20 min) a “lipid plug” has probably formed; so, gently spread the lipids using the paintbrush without adding any lipid solution.

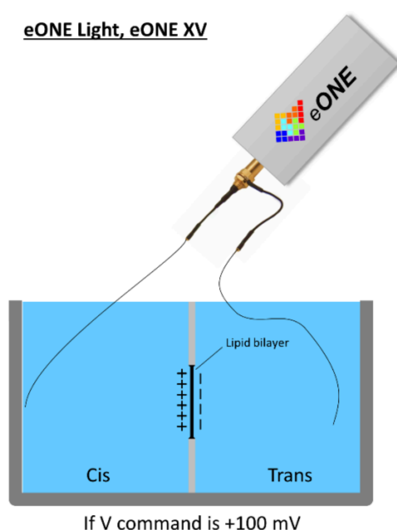
Sometimes, especially at the beginning of the experiment, it is useful to break and form the lipid membrane again to promote BLM thinning and protein insertion and avoid "lipid plug".

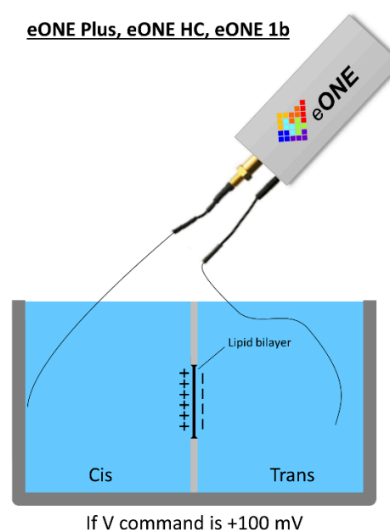
To avoid lipid plugs it is also important to dry with a wipe (or an air flux) as better as possible the paintbrush before dipping it in the lipid containing solution.

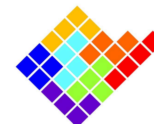


- Adding the protein to the right side of the recording chamber (cis or trans side of the figure below) may be crucial for the protein reconstitution in the lipid bilayer. For example, since αHL is slightly negatively charged in a buffer solution at pH 7, it could be useful to add the toxin in the cis chamber (negative electrode) when a constant positive voltage is applied.

Depending on the eONE amplifier version used, negative charged protein should be added to the cis chamber, according to the scheme shown below:







3.3) Instructions for Teflon (or Polyimide) septum and BLM chamber's cleaning

Warning:

Do not use Acetone to clean Teflon septum

The safest way is to clean the Teflon (or Polyimide) septum by using ethanol to remove lipids; then simply rinse the septum with double-distilled water. Acetone is compatible with the Delrin material and the Polymide (Kapton) septa. Do not use acetone to clean Teflon septa.

Another procedure is to use trisodium phosphate (TSP, Na_3PO_4) solution (40-50 mM), which is a stronger cleaner than ethanol.

In this case you can use the following procedure to clean the supports:

1) Rinse the chambers with double-distilled water to remove the water-soluble components
2) Remove the Teflon (or Polyimide) support from the BLM chamber
3) Clean the aperture in the septum using the trisodium phosphate (TSP) solution;
4) Rinse with dilute HCl solution (0.1% by volume) to remove any residual TPS;
5) Rinse with double-distilled water to remove any remaining HCl;
6) Gently dry the septum using a wipe or a low air jet.

Warning:

Since the Teflon septum is very fragile, be careful to not apply a high pressure on them while rinsing or drying.