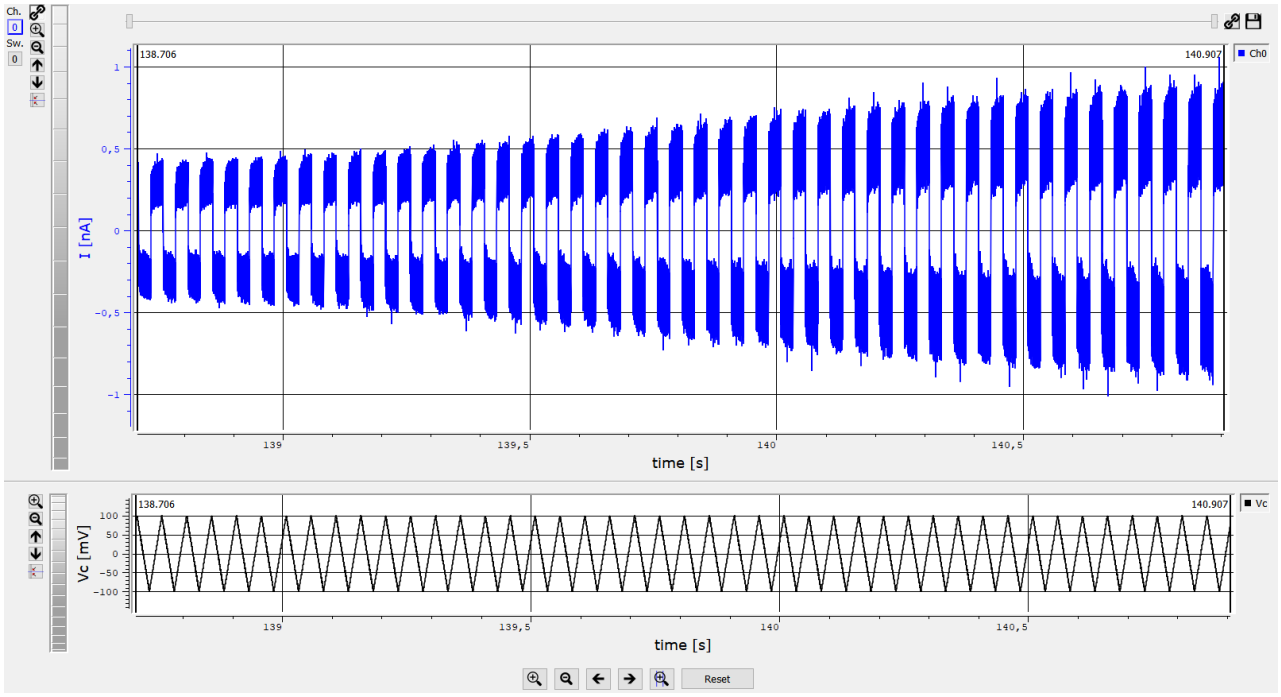
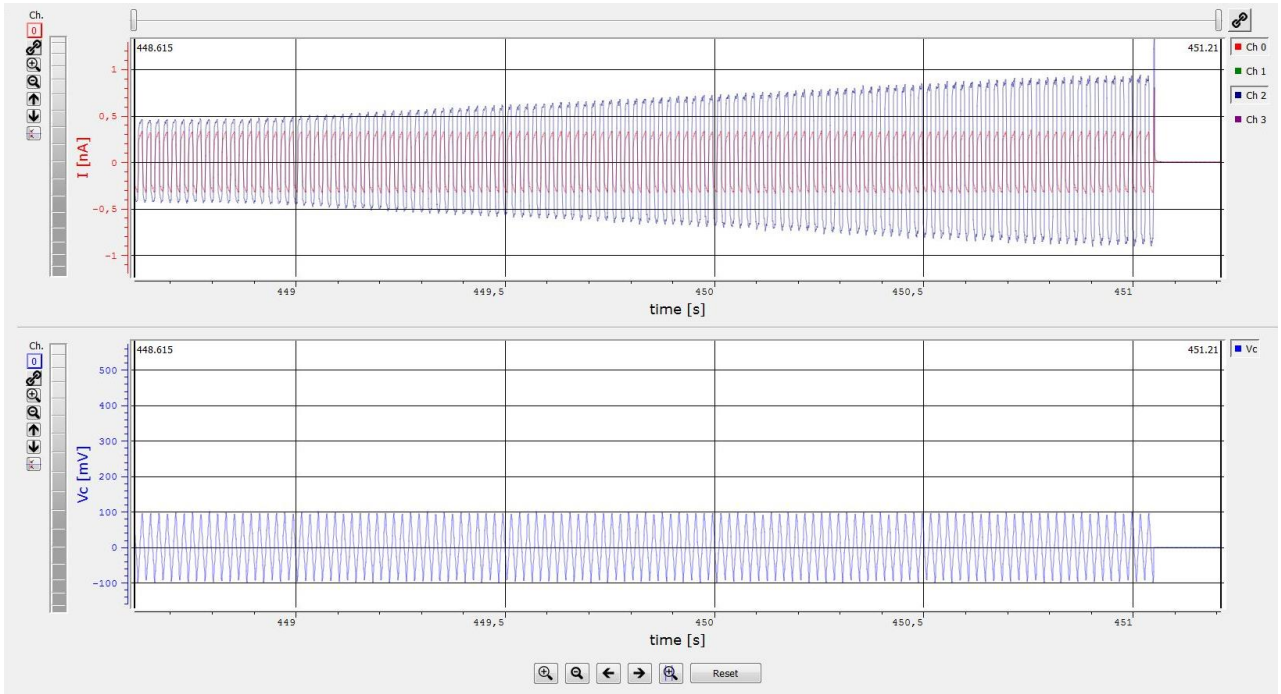
BLM Application note

BLM formation & thinning

Here is an example of typical BLM formation (BLUE) with the square wave amplitude that increases as the lipids thin into the lipid bilayer configuration. The BLM thinning behaviour is also compared to the “lipid plug” situation (RED). Data acquired at 20KHz sampling rate – applying a triangular wave of 200mVpp





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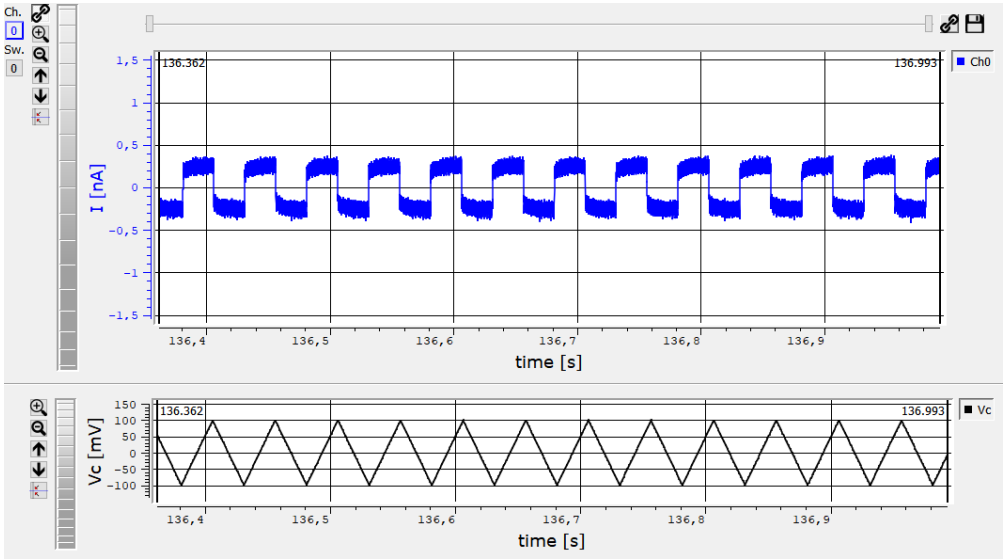
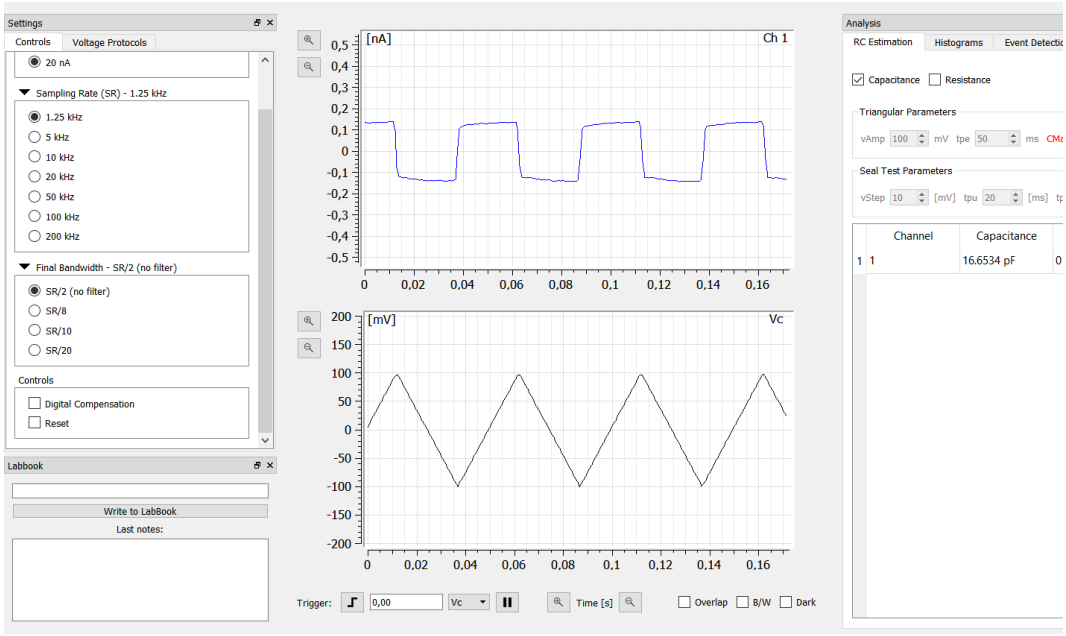
Bilayer Capacitance reference values depending on hole dimensions

Hole diameter (μm)	Hole Area (μm²)	Maximum theoretical Capacitance (pF) (considering 0,5 μF/cm² spec. cap.)	(Experimentally obtained) reference capacitance value for a "good" BLM (pF)
50 (Teflon)	1963.5	9.82	**7 - 9**
80 (Teflon)	5026.55	25.13	**18 - 28**
100 (Polyimide)	7853.98	39.27	**20 - 40**
110 (Teflon)	9503.32	47.52	**25 - 50**
150 (Polyimide)	17671.46	88.36	**35 - 90**
200 (Polyimide)	31415.93	157.08	**45 - 140**

Lipid plug example

When a lipid plug is obtained it is suggested to try to promote BLM thinning painting the solution altready present on the hole without any other addition of lipids. Another method is to give some mechanical shocks to the chamber to break the lipid plug and then try to form the BLM again without any addition of lipid solution.

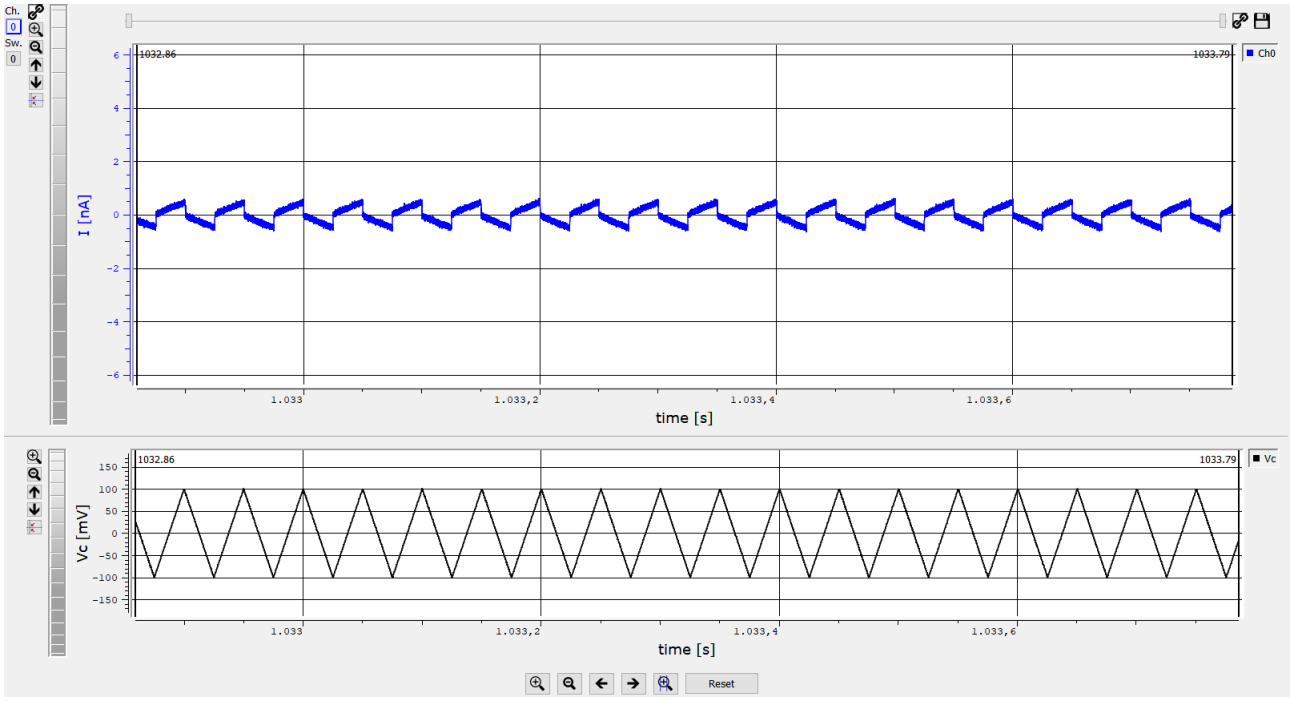


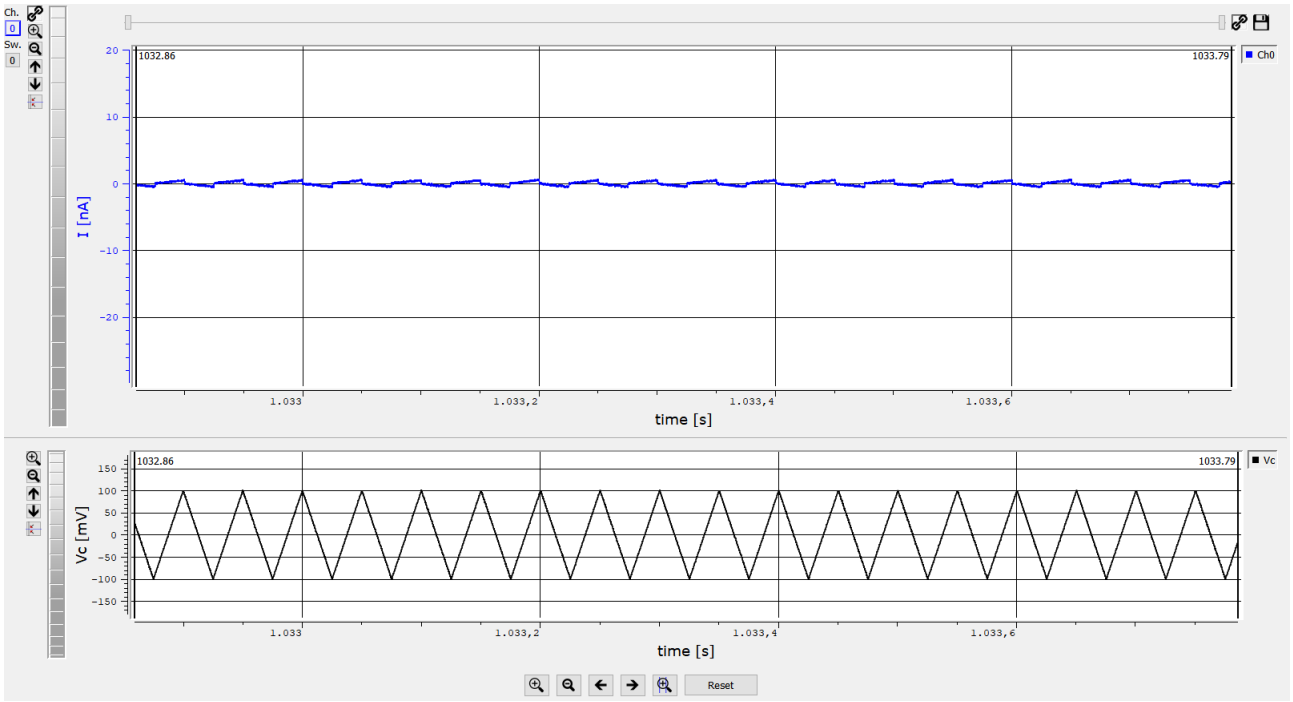


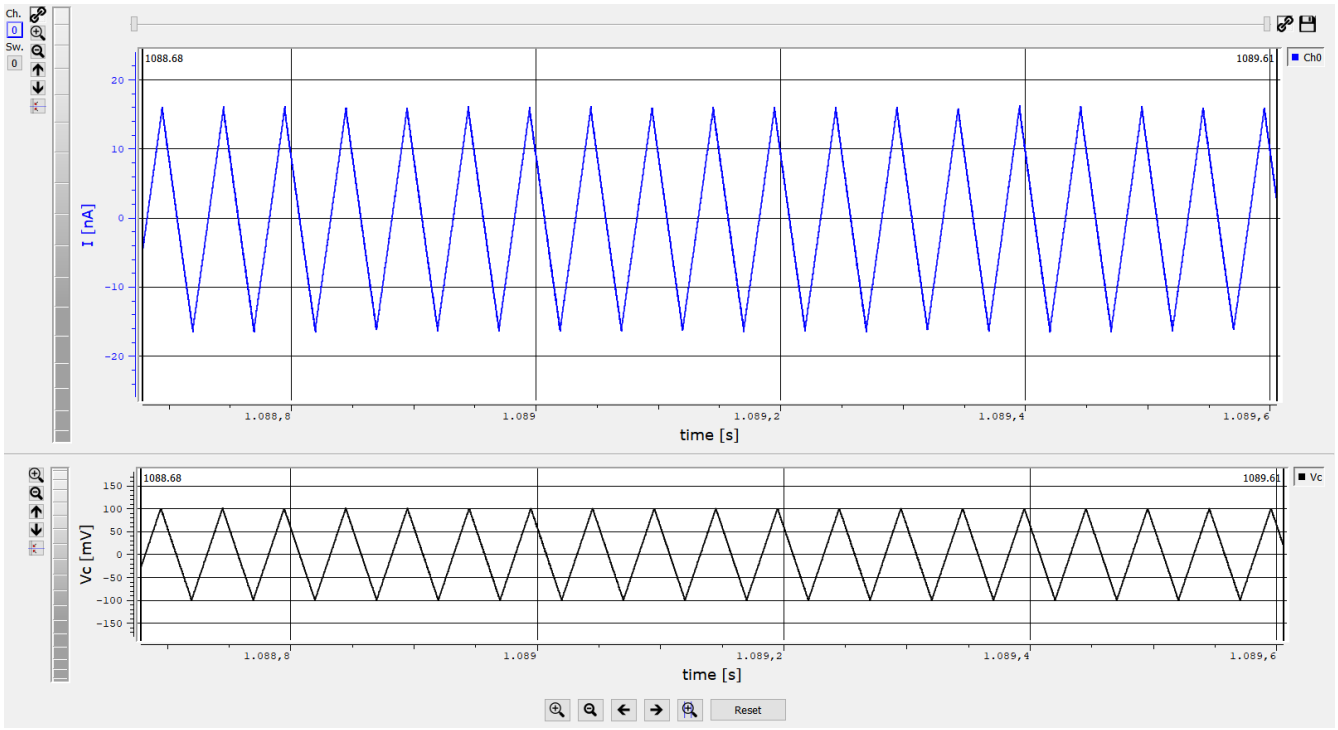
(110 µm hole)

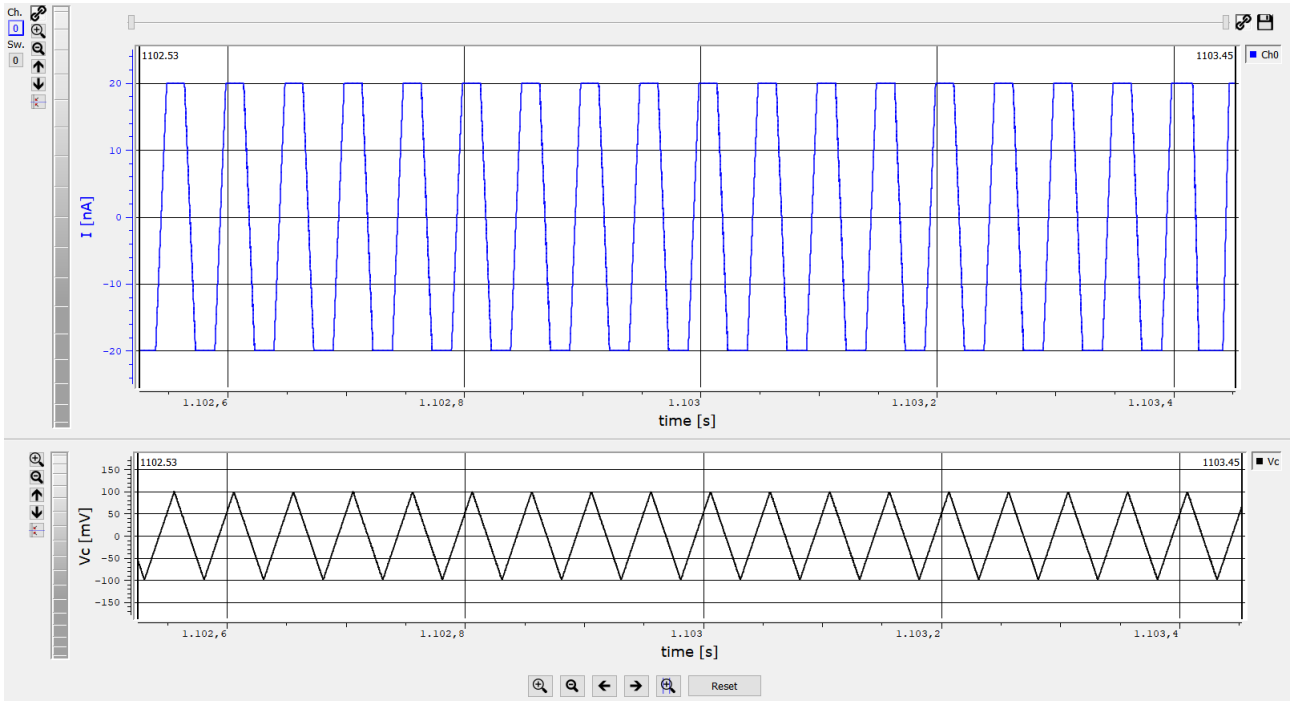
Leaky BLM and progressive membrane breakage

Sometimes it could happen that a progressive membrane breakage occur instead of an instantaneous breakout of the membrane. When a membrane starts to be leaky a current wave in between to a square wave and a triangular wave can be seen as a response to the applied triangular wave voltage protocol.


Ch.
0
Sw.
0
1032.86
1033.79
Ch0
I [nA]
6
4
2
0
-2
-4
-6
1.033
1.033,2
1.033,4
1.033,6
time [s]
1032.86
1033.79
Vc
Vc [mV]
150
100
50
0
-50
-100
-150
1.033
1.033,2
1.033,4
1.033,6
time [s]
Reset


Ch.
0
Sw.
0
1032.86
1033.79
Ch0
I [nA]
20
10
0
-10
-20
1.033
1.033,2
1.033,4
1.033,6
time [s]
1032.86
1033.79
Vc
Vc [mV]
150
100
50
0
-50
-100
-150
1.033
1.033,2
1.033,4
1.033,6
time [s]
Reset

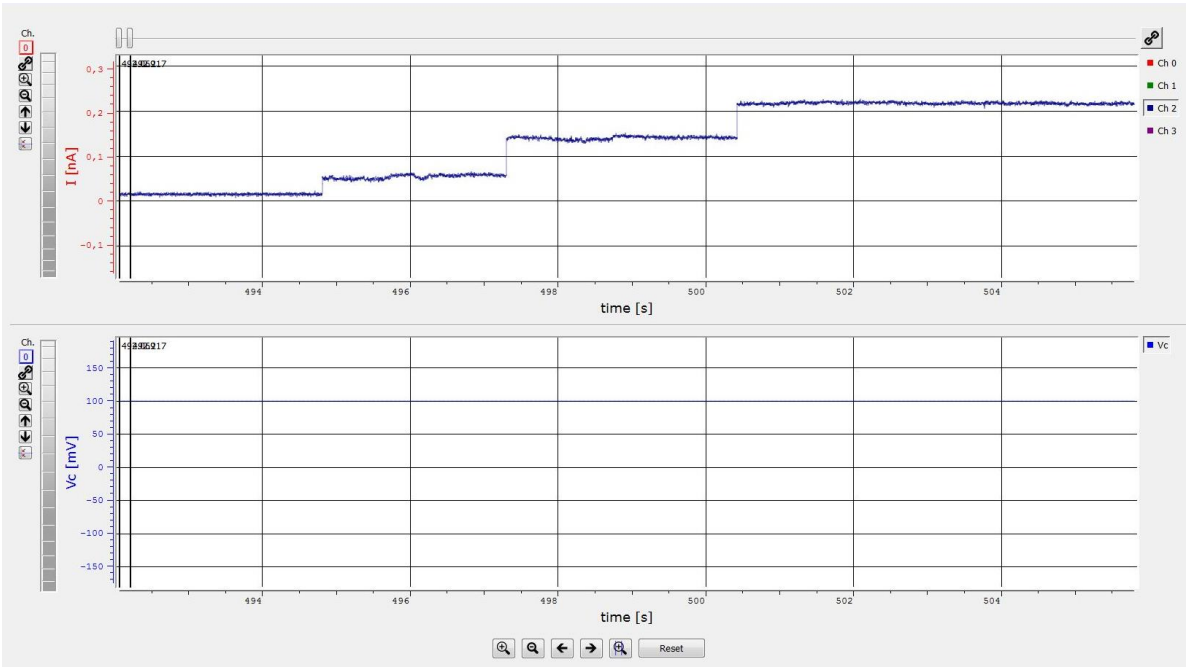




If, after some period of usage and of trials to get a BLM, a square wave cannot be reached and a leakage current is always present when applying a constant voltage, the problem could be given by the damaged septum hole or some leaky path through the BLM cuvette. Sometimes these could be caused by capillarity either if the buffer levels in the chambers are too high, or if the screws are too tigh causing the rubber gaskets being squeezed.

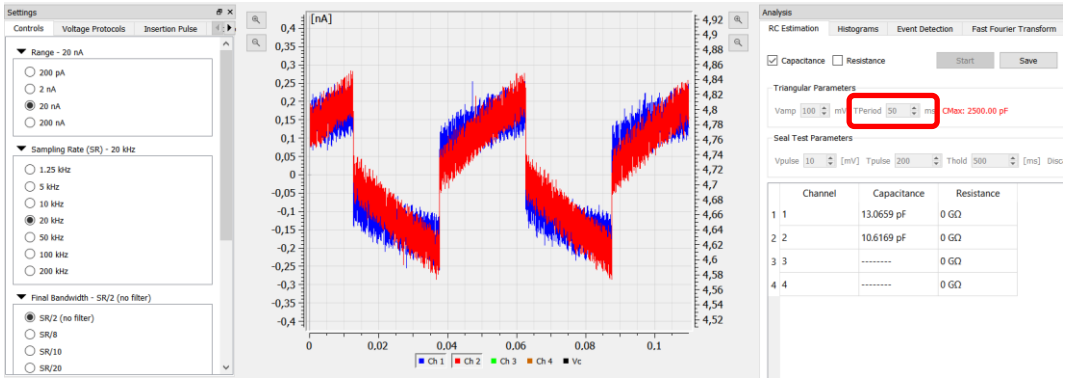
Alpha- Hemolysin insertions

After getting a good BLM, ion channels or pore forming toxins under studies can be added to the chambers. Here is just a proof of concept example of alpha-hemolysin insertions into a BLM membrane using the BLM kit, with 100mV constant voltage applied between the two chambers. Since usually once good BLM start form is easy to . If a BLM is broken, it is possible to keep the constant voltage applied and check the membrane formation by the zero current situation.

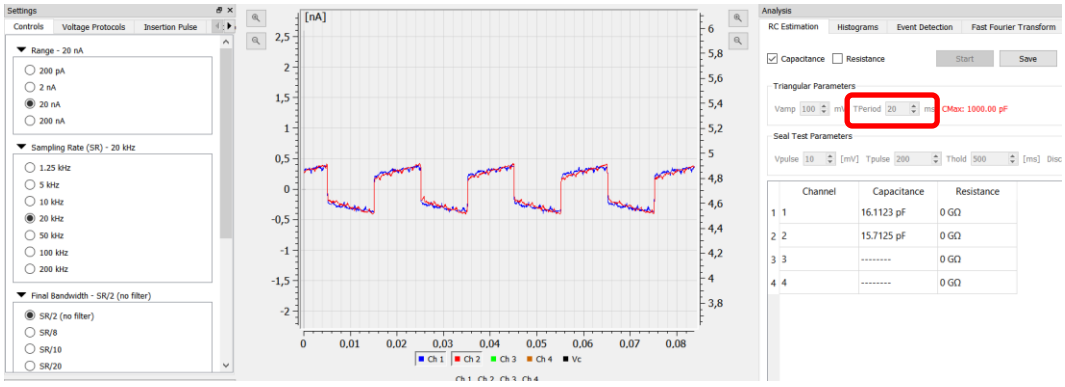


Applied triangular wave frequency effect

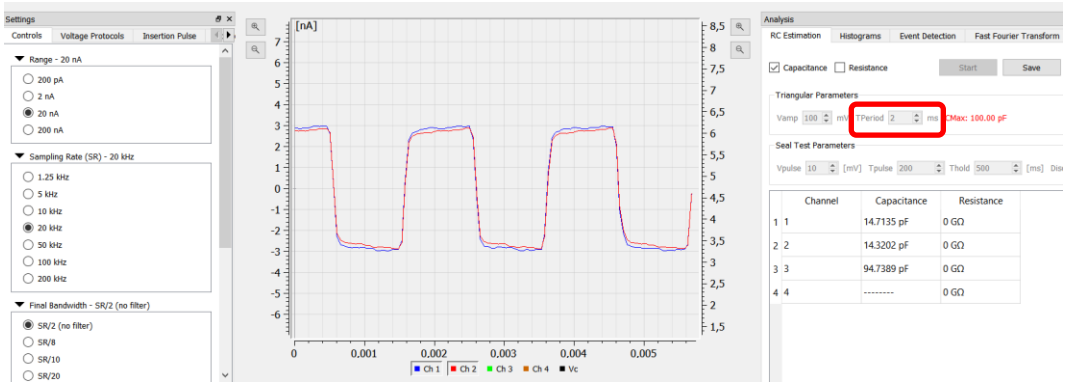
The frequency of the applied triangular voltage wave may also change the current wave topography and in some setups (depending on the stray capacitance of the pierced membrane) a good bilayer (or also a lipid plug) can have a slightly triangular shape, so it is suggested to try to use different square wave frequencies depending on the particular pierced membrane.



Tpe=50ms



Tpe=20ms



Tpe=2ms

In this proof of concept example, the acquired current wave is as more squared as higher is the frequency of the applied triangular wave (lower Tpe).

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