# An Automatic System for Bilayer Lipid Membrane Formation and Monitoring

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***Abstract*— Ion channels are natural nanometric pores formed by proteins across cell membranes. They are responsible of part of cell signaling and a large part of pharmaceutical compounds are interacting with them. Therefore, single ion channel screening is being proposed as a fundamental technique for investigating the function of cell membrane proteins with pharmaceutical compounds. The technique consisting in embedding ion channels in artificial bilayer lipid membranes (BLM) is gaining attention over patch clamp approach due to its characteristics of performing parallel tests over selected classes of channels. However, no valid procedures for automatic formation and real time monitoring of BLM arrays have been presented so far. More specifically, since BLM is based on a manual and time-consuming technique, there is a strong need of automatic systems for forming BLMs in a fully parallel fashion for testing compounds in high throughput screening (HTS) fashion. In this paper, an automatic liquid dispensing system for BLM formation is presented using commercial 3+1 axes movement stepper machine together with a multi-sensor technique for monitoring BLM formation in real time. As proof of this concept, the automatic dispensing system is interfaced with an 8 channel electronic interface where low noise amplifiers are able to automatically sense BLM formations by means of current sensing.**

***Keywords - Bilayer lipid membrane, automatic dispensing system, ion channels, high thoughput screening.***

## I. INTRODUCTION

Bilayer lipid membranes are artificial biological substrates composed of phospholipid mixtures suspended into different organic solvents that self assemble to form bilayers under specific conditions [1]. BLMs are used to host ion channel proteins since they well approximates the natural behavior of cell membranes. Ion channels are natural nanopores that regulates the ions exchange from intra and extra-cellular solution, and they are involved into all the cellular life process [2]. Because of their key role in the physiologic process, ion channels behavior can be altered or compromised from several diseases [3]. For these reasons, simple and versatile systems that allow to verify and screening drugs interaction with ion channels are required to reduce time consuming in the drugs discovery and validation processes [4].

The integration of biological nanopores, such as ion channels with electronics is also a promising approach for the development of novel biosensors that are able to detect low concentrations of target molecules [5], or even identify differences between different DNA bases [6].

BLMs can be used instead of natural cell membrane without significant information loss using channel proteins incorporation techniques [5]. BLMs can be formed by means of different techniques using microfluidic chambers and systems to mechanically support the bilayer.

Various techniques to form BLMs are reported in literature: one of the first developed is the painting method, consisting in applying a small quantity of lipid-solution dissolved in an organic solvent using a borosilicate rod over an aperture immersed in water. The orfice, in the range of tens to hundreds micrometers, is created in a hydrophobic material foil such as Teflon or Delrin [1]. At the lipid-aqueous interface, a lipid monolayer will be constituted thanks to the amphiphilic properties of lipids. The lipid-solvent solution wets the hydrophobic walls of the aperture, resulting in a thinning of the solvent solution in the center of the aperture, until a completely fusion of the two monolayers into a single bilayer. A small quantity of solvent remains at the aperture perimeter (the annulus), increasing the BLM stability and acting as connection between the thick Teflon sheet and the final thickness of the BLM, typically in the nanometric range [1].

Following different approaches, other techniques were developed: some are based on droplets of water inside lipid solution [8], others using solvent evaporation and resulting thinning of the bilayer [9], or based on the liquid insertion through a microfluidic chip which spreads lipids over a small Teflon apertures [10]. These techniques allow the BLMs formation, but they are not suitable to be automated. In contrast, the Montal-Muller approach presents some characteristic procedures so as to to be automated [11]. As illustrated in Figure 1, the Montal-Muller technique consists of the formation of two lipid monolayers above the aqueous surface of two separated chambers by applying a lipid solution in a volatile solvent, such as hexane or chloroform. The chambers are separated by a thin septum where a micro aperture is drilled and, during the monolayer formation, it is kept out from the aqueous surface. After the evaporation time, the micro aperture of the septum is lowered and the two monolayers formed into the chambers aqueous surfaces are folded down against each other, forming a bilayer across the aperture [11].

This paper present first example of a fully automatic system for parallel BLM formation inspired on the Montal Muller approach employing disposable bilayer chambers [12] and an array of sensing amplifiers to continuously

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monitor BLM formation. The system allows testing and optimizing different protocols, offering a large flexibility and a fast characterization of membranes.

Section II will show the system architecture and working principle. Section III will present the implementation of the mechanical control. Section IV will show a real experiment where BLM are formed in parallel fashion and monitored in real time by embedded amplifiers. Finally, Section V will present an application of the apparatus for recording single ion-channel signals.

## II. SYSTEM STRUCTURE AND CALIBRATION

The proposed automatic liquid handling system (Figure 2) is composed of: i) Sutter Instrument MP-285: 3-axis micromanipulator for pipette automatic movements in the 3D space; ii) Newport NSA12: single-axis micromanipulator for multipipette flux control; iii) Anachem Ltd 8-Channel Pipette (20-200 µL); iv) LabVIEW™ control panel.

Sutter Instruments MP-285 micromanipulator offers a range of movement of 25 mm in the 3 directions. It allows also selecting between two submicron movement resolutions of 0.2 mm (coarse range) and of 40 nm (fine range). Newport NSA12 provides a motorized, linear plunger, whose position can be controlled with sub-micron (0.1 µm-µSTEP) resolution over 11 mm of travel, with a minimum incremental motion of 0.3 µm (3 µSTEPs). Each µSTEP is 1/64 of the full-step (FS) of 6.4 µm. The NSA is mounted in a fixed aluminum bracket (screwed in the MP-285 vertical plate) that provides both a rigid coupling between NSA12 plunger and the pipette plunger along the vertical axis and the rigid coupling between the MP-285 and the multipipette. In order to have the maximum pipette volume range, when the NSA12 plunger is fully retracted, the pipette plunger is fully extended and vice-versa. The mechanical interface is compatible with any kind of pipettes.

Calibration is done using a precision balance (±0.1mg resolution) knowing these experimental relationships, which are dependent on the particular pipette tips used:

$$8100\,\mu STEPS = 100\,\mu L \Rightarrow 810\,\frac{\mu STEPS}{\mu L} = 12.66\,\frac{FS}{\mu L} \quad (1)$$

$$1\cdot\frac{mm}{s} = 156.25\cdot\frac{FS}{s}\cdot\frac{\mu L}{12.66\,FS} = 12.35\,\frac{\mu L}{s} \Rightarrow 12.34\,\frac{\mu L}{mm} \quad (2)$$

Using these two relationships, it has been possible to relate the volume with the linear movement of the NSA12 plunger as in equation (1) and its velocity with the infusion/withdrawal flow rate as in equation (2) in order to have a control with a precision of ±1µL.

Both micromanipulator are connected to a PC using RS-232 interface and are controlled by LabVIEW™. Elementary operations are implemented in LabVIEW™ executable in order to obtain modular software, very versatile for different applications as described below.

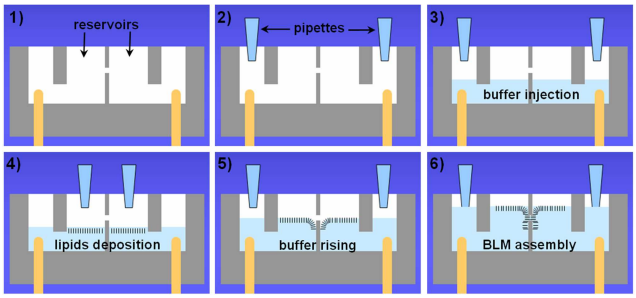


Figure 1. Montal Muller technique for BLM formation on a small hole on a Teflon or Delrin septum separating two chambers. 1) device empty; 2) pipette positioning on the inlet channels; 3) injection of buffer solution; 4) deposition of lipids for monolayer assembly on the two solution surfaces; 5) rising solution level 6) final bilayer assembly.

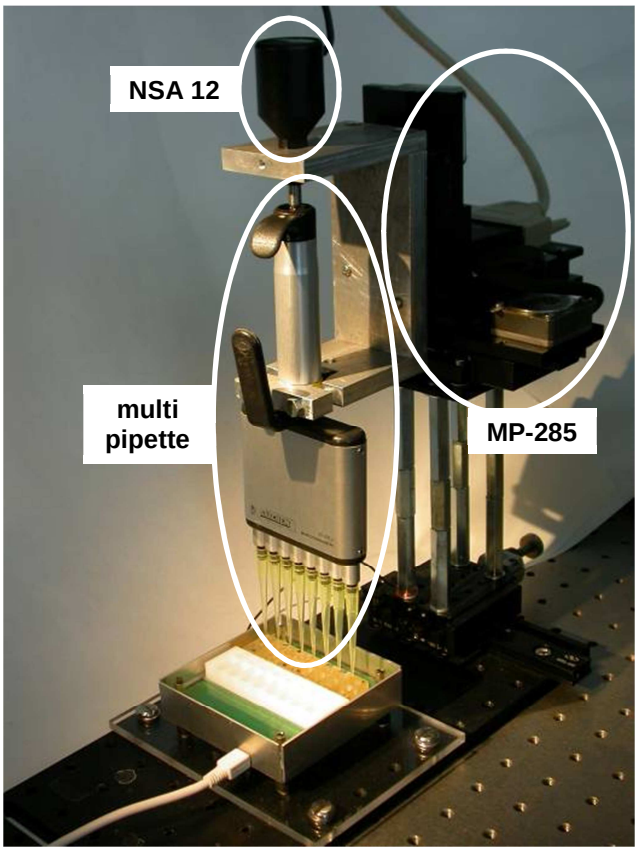


Figure 2. Details of the micromanipulators and multipipette composing the compact automatic liquid handling system.

## III. IMPLEMENTATION OF THE CONTROL

The described system has been designed with the particular aim to create a compact automatic liquid handling robot for BLM formation able to be interfaced with an array platform [13]. The system is controlled by an implemented user-friendly control panel (Figure 3). This has been

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designed following a modular and reusable design, starting from elementary operations, implemented in different subroutines and associated to single independent controls.

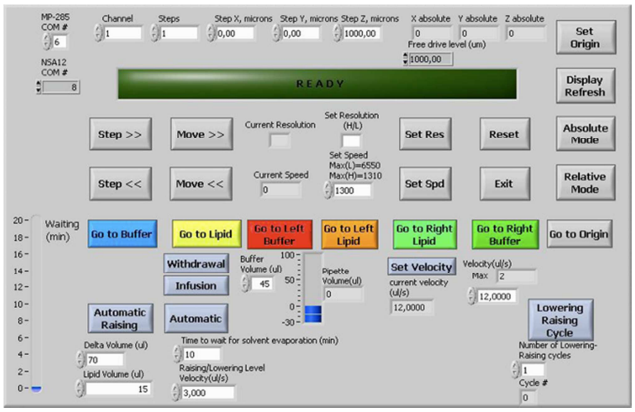


Figure 3. LabVIEW™ Control Panel interface for automatic liquid handling.

The operations are referred to the reference system fixed to the array platform [13]. Elementary operations are available to the user, more specifically: 1) to move the multipipette to buffer reservoir; 2) to move the multipipette to lipid reservoir; 3) to shift left/right the multipipette on the bottom of left/right chamber; 4) to move the multipipette on the bottom of left/right chamber at the air-buffer interface level where lipids are deposited; 5) to return to origin: move the multipipette to the reference system origin; 6) to withdraw a fixed amount of buffer selected by the user (µL); 7) to inject a fixed amount of buffer selected by the user (µL). Furthermore, several important parameters for BLM formation can be set, in order to test different BLM protocols, in particular: a) volume of buffer/lipids injected/withdrawed (µL); b) infusion/withdrawal flow rate (µL/s); c) number of lowering/raising buffer level cycles in both chambers; d) waiting time for lipid solvent evaporation (minutes). Finally, stacking and synchronizing the different elementary operations, two automatic BLM formation protocols (both using the Montal-Müller method) have been implemented.

## IV. MEASUREMENTS ON PARALLEL BLM FORMING BY REAL-TIME MONITORING

As proof of concept, two protocols for automatic BLM formation have been implemented, following the Montal Muller technique [11]. They are called “Automatic Raising” and “Automatic”. I) Automatic raising. The system withdraws a quantity of buffer from the reservoir and then fills the two chambers beneath the microhole (buffer volume ≤ 70µl). Then, a selected quantity of lipids is injected in the two chambers and, after a waiting time for solvent evaporation, the buffer level in the two chambers is raised by a fixed quantity at a fixed velocity. At the end the pipette is moved to the reference system origin. II) Automatic. This sequence is illustrated in Figure 5. The system withdraws a quantity of buffer (1) from the reservoir it fills the two chambers above the microhole (2) (3), in order to allow to use the offset correction (4) functionality implemented in the readout interface. Then a selected quantity (5) of lipid is infused (6) (7) in the two chambers and, after a waiting time for solvent evaporation, the buffer level in the two chambers is lowered and immediately raised (8) (9) by a selected quantity of buffer.

For a better lipid spreading over the microhole, the “lowering/raising cycle” functionality has been implemented. This procedure automatically move alternatively the pipette to both chambers, withdraws and then injects a selected quantity of buffer from the two chambers using a fixed pipette flow rate (selected in “raising/lowering level velocity” option). In order to better distribute the lipid at the air-buffer interface, the user can set several automatic cycles.

To monitor the membrane formation, following a known technique [1], a triangular wave of 80 $mV_{pp}$@6Hz is applied by the electronic system and the current signals coming from the parallel fluidic bilayer chambers are visualized and recorded by means of a PC-based custom graphical user interface (GUI). As shown, in Figure 4, if the lipid bilayer is not formed, the microhole acts as an electrical resistor. Hence, the current follows the same shape as the voltage stimulus. When a BLM is formed, the equivalent circuit element could be modelled as a capacitor and the current is given by the derivative of voltage stimulus, resulting in a square current waveform proportional to the membrane capacitance. This method allows to known the dimensions of the bilayer since different BLM have different square wave amplitudes (Figure 5).

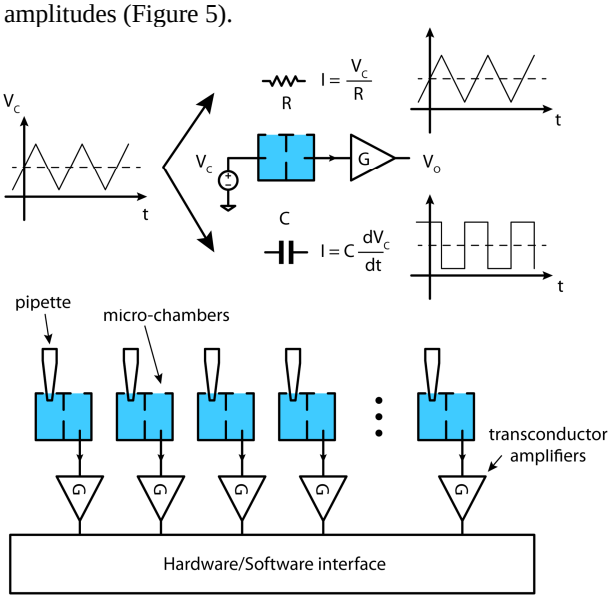


Figure 4. Equivalent electrical circuit of the septum (above). If BLM is not present, the microhole can be represented by a resistor. In the presence of a lipid membrane it can be modeled by a capacitor. The difference is sensed by a low-noise transconductance amplifier placed on each spot of the array (below).

The typical measured current is about 300 $pA_{pp}$, equivalent to a membrane capacitance of about 156 pF,

much higher of the septum strain capacitance (8–10 pF range), which can be neglected.

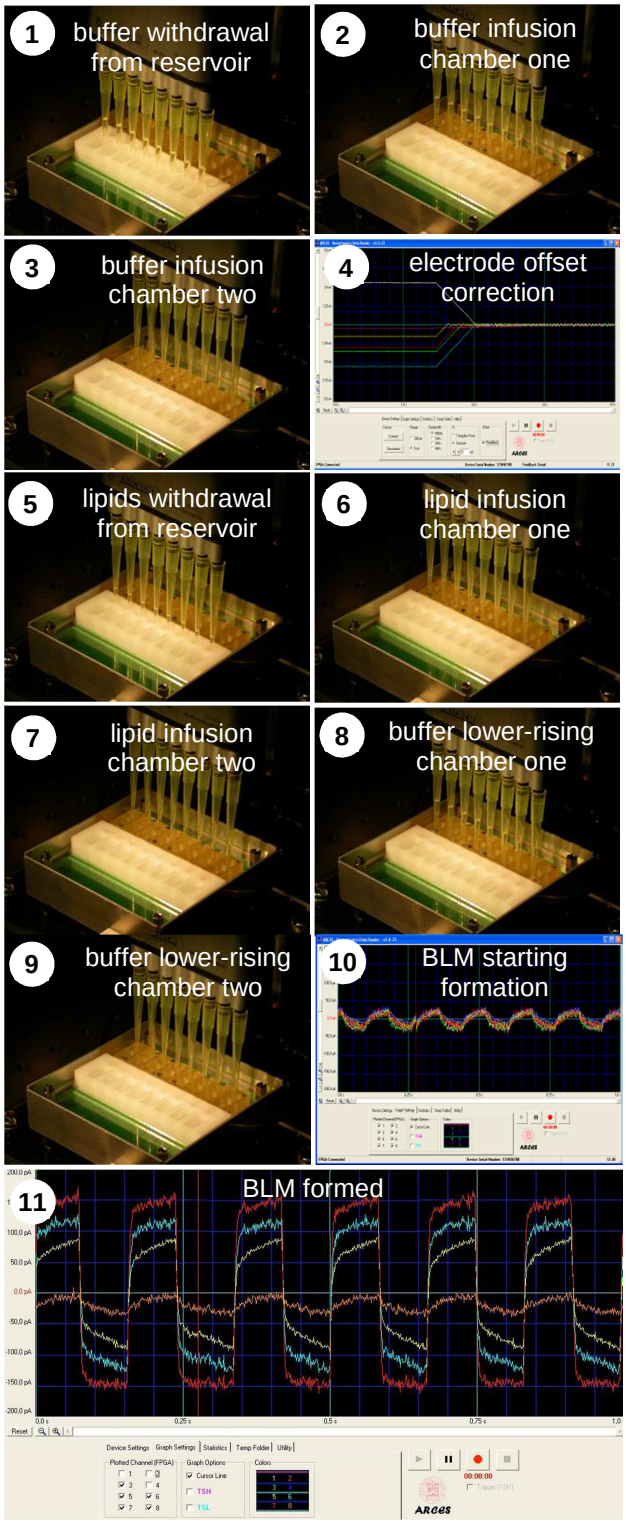


Figure 5. Example of automatic operations sequence implemented in the "automatic" procedure. Steps 4-10-11 are screenshots of the GUI interface. The three square-wave signals (red, cyan and yellow) in step 11 demonstates the concurrent formation of three independent BLMs of different dimensions on different microholes, while brown wave indicates the early stages of membrane formation where a lipid agglomerate is present and acts as a very small capacitance between the two chambers.

## V. APPLICATION TO SINGLE CHANNEL RECORDINGS

As a working proof of formation of artificial lipid bilayer resulting from the proposed approach, a typical single channel recording using artificial lipid bilayer membrane will be shown. In the trans-chamber an α-haemolysin (αHL) protein is embedded with the BLM with a final concentration of 5ng/mL. αHL is an exotoxin secreted by the bacterium *staphylococcus aureus* that forms pores allowing ions and molecules to pass through the BLM.

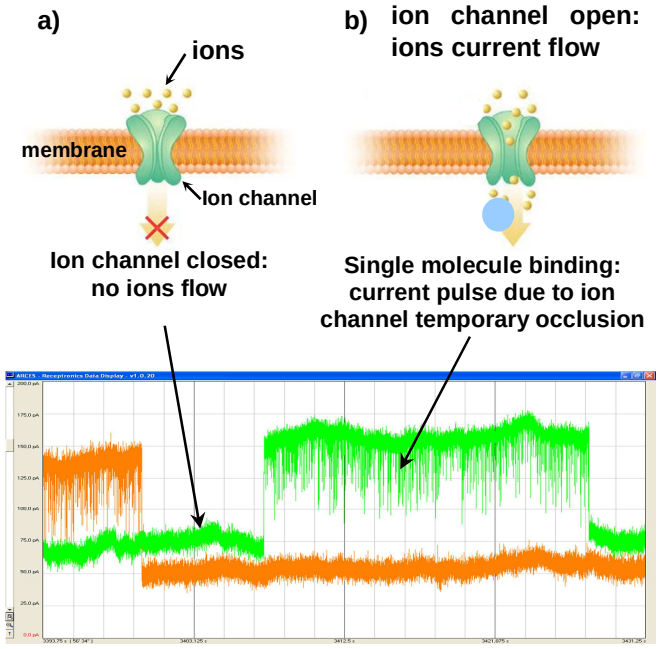


Figure 6. Example of single molecule binding activity over two differents BLM.

When no ion channel is embedded into BLM, no passage of current occurs, due to the high electrical resistance (several Gigaohms) of the membrane with respect to ions. After insertion of a αHL pore into BLM a constant current is detected through the membrane. When a transmembrane potential of -80mV, is applied a current of about -80pA is observed. Finally, we have also introduced β-cyclodextrin (βCD), which binds the α-haemolysin protein and significantly reduces the ionic channel conductance, and thus the measured current. This molecule is able to get into the lumen of the pore from the stem side and to bind to it, partially blocking the channel and causing stochastic current spikes. Figure 6 is illustrating this experiment where current pulses are readout using the same low-noise transconductance amplifiers used for monitoring BLM formation.

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## VI. CONCLUSION

A compact parallel liquid handling system for automatic BLMs formation procedure and real time monitoring is presented. The apparatus shows for the first time a complete automatic setup for parallel BLM formation. A user-friendly interface controlling the micromanipulators has been implemented in order to automatically create BLMs regardless of the user's ability with a high yield rate. As proof of concept, a complete sequence for parallel BLM formation is reported together with single channel recording using the same transconductance amplifiers used to monitor BLM formation.

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