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# Scalable micro-cavity bilayer lipid membrane arrays for parallel ion channel recording

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ABSTRACT

A compact, scalable and high-throughput bilayer ion channel recording platform capable of simultaneous data acquisition from multiple bilayers is presented. Microfluidic chips house micro-cavities over which bilayers are made; each connected to a custom-made compact electronic readout circuit based on ASICs (Application-Specific Integrated Circuits). The micro-cavities are fabricated using a simple dry-film resist process on a glass wafer. Single 15 mm × 15 mm glass chips contain four separately addressable bilayers, each with integrated Ag/AgCl electrodes. The number of bilayers is scaled by increasing the number of ASICs and four-cavity chips. Each chip can be cleaned and re-used many times and the cavity-suspended lipid bilayers are stable for up to 10 days. System performance is demonstrated with simultaneous electrical recordings of the ion channels gramicidin A and α-hemolysin in multiple bilayers.



## 1. Introduction

Ion channel proteins play an important role in physiological processes and as drug targets they are of considerable interest to the pharmaceutical industry [1–3]. Electrophysiological analysis of these proteins provides detailed information about the function of ion channels and their modulation by pharmacological drugs. The two widely used systems for electrophysiological measurements are patch-clamped cells and aperture-suspended or supported lipid bilayers [4]. Patch clamp techniques are usually performed on living cells and require skilled manual operation. However, automated planar patch clamp techniques, based on microfabricated glass aperture chips rather than the conventional glass pipettes, have been developed and successfully commercialized in the past decade [5,6]. These instruments require a significant investment, due in part to the high cost of connecting each measurement site to a single commercial current amplifier, and are not configured for single-channel electrophysiology [5], which is related to problems with obtaining high quality GOhm seals between the cell and the glass aperture, which can lead to significant leakage currents that limit the signal quality [2].

Simplified biomembranes such as aperture-suspended lipid bilayers exhibit low leakage currents and are suitable for recording single-channel current events. The cost per data point is lower and throughput is higher than with patch clamp methods because cell culture and fluidic manipulation are not required [2]. Furthermore, it is relatively easy to obtain high quality single channel current recordings from artificial lipid bilayer systems. The principal limitations of lipid bilayer electrophysiology are (i) difficulty in forming stable long-lifetime bilayers and (ii) incorporation of purified vesicle-reconstituted membrane proteins into the bilayers. Recently it was demonstrated that the stability of artificial bilayer membranes can be significantly increased by using shaped apertures fabricated in negative resists such as SU8 [7], or complex (incline/rotation) exposure technique [8]. In order to increase throughput, bilayer arrays have been developed, for example using stereo-lithography to define chambers around a thin film of a hydrophobic polymer that includes an aperture for bilayer formation [9,10]. Both ease of bilayer formation and stability has been improved through the

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use of small cavities in the range 6–50 μm diameter [11–13]. However, small cavities have small in situ electrodes, which limits the chip lifetime or measurement duration [11,14]. Baaken et al. developed a 4 × 4 array of micro-cavities using a single layer of SU8 [11], and Ogier et al. fabricated micron sized cavities using a combination of SU8 and polymer films [12]. Suzuki et al. fabricated a 96 well planar bilayer structure using stereolithography, with apertures patterned in parylene [13]. Osaki et al. described a PDMS device with tens of micro-cavities as side chambers in a common microfluidic channel but only showed recording from one cavity [15]. Devices have also been made by bonding parylene film with pre-formed apertures to a backplane made using stereolithography [13]; parallel electrical recording was not possible and each well was sequentially switched to a recording amplifier [13]. Parallel recording using large commercial multi-channel (8 channel) amplifiers has been reported [9,11,16]. This approach is costly and the long wires increase electrical noise. To minimize parasitic capacitance a fully integrated platform has been described, which uses a custom made amplifier integrated directly beneath a solid-state nanopore [14]. Although this significantly reduces parasitic capacitance, thereby increasing the bandwidth, it is incompatible with the need for a disposable bilayer chip.

Previously we demonstrated a parallel recording platform that used small Application-Specific Integrated Circuits (ASICs) for continuously recording from several discrete bilayers made using the traditional vertical cup arrangement [17]. Both the digital control and analogue readout circuitry were fabricated on the same PCB, which led to an unacceptable level of noise due to crosstalk. The classical cup configuration also consumes comparatively large amounts of sample and imposes limits on scaling. To address this issue, we have designed a bilayer platform that uses a new design of ASIC and electronics, interfacing with a disposable glass microfluidic chip. The chip has four separate micro-cavities for four bilayers, each with its own integrated Ag/AgCl electrode, as shown in Fig. 1. The system can continuously record from multiple bilayers formed across apertures above cavities as shown in Fig. 1. The cavity has a diameter of 150–200 μm and is made using a layer of dry-film resist, with a thickness of ~55 μm. A second layer of dry-film resist partially covers this cavity, creating a smaller aperture of 20–100 μm diameter for the bilayer. This two-layer structure enables the bilayer and electrode size to be defined independently. A small aperture increases bilayer stability whilst a large cavity and electrode increases recording time. Each bilayer is connected via the integrated electrode to its own amplifier (ASIC) which sits directly next to the chip, interfaced using a simple edge connector. No wires are used to connect the bilayer chip to the electronics, and no electrical multiplexing is necessary, minimizing parasitic capacitance and electrical noise. In addition, the analogue and digital circuits are kept separate from each other, reducing electromagnetic interference and noise. The system is compact and has substantially less noise compared with other multiplexed bilayer platforms that use discrete off-the-shelf amplifiers [9,10]. The entire system is housed in an aluminium box and can be scaled as required; the design described here accommodates three chips (see Fig. 3) with a total of 12 bilayers, all of which can be recorded simultaneously.

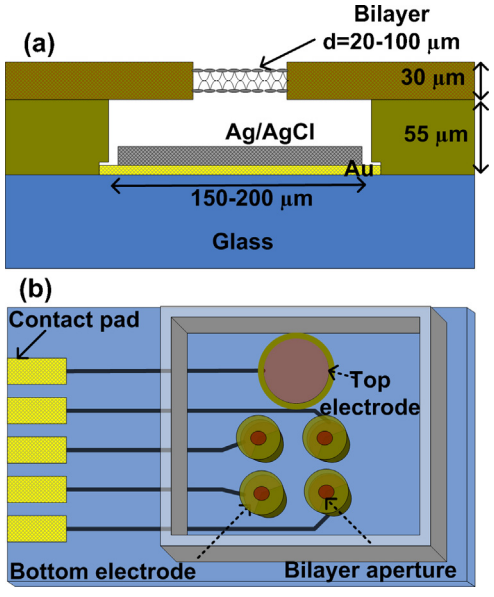


**Fig. 1.** (a) Schematic diagram of a micro-electrode cavity showing the large-area Ag/AgCl bottom electrode and the aperture for the suspended lipid bilayer (not to scale). (b) Diagram of a single chip with four separate cavities for bilayer formation, each with an integrated recording electrode. The chip also contains a common driven electrode that is in contact with the larger shared aqueous compartment on top of the bilayers. Each chip has five contact pads to interface with four ASICs and a common driven electrode.

## 2. Materials and methods

### 2.1. Fabrication of micro-electrode cavity chips

A four-mask process was used to fabricate the device according to the sequence shown in Fig. 2a. The substrate consisted of a 100 mm diameter 700 μm thick glass wafer. The first stage involved deposition and patterning of 200 nm gold (with 20 nm Cr adhesion layer) to define the electrodes and contact pads, using photoresist and wet etching. AZ9260 resist was spin-coated and patterned using a second mask to define areas for silver deposition. Subsequently, silver was electroplated onto the gold using a solution of 0.1 M $AgNO_3$ and 0.5 M $NH_3$. A two-electrode system was used with a large-area silver film as counter electrode. Electrodes were Ag plated for ~6 min at a current density of ~2 mA/cm$^2$, giving a silver thickness of 5–6 μm. After electroplating, a layer of AgCl was formed by immersion in $FeCl_3$ solution for 2 min [18].

After stripping the AZ9260 resist and cleaning, the cavity was defined from a single layer of TMMF S 2055 dry-film resist (S200 series, TOK, Kanagawa, Japan), laminated onto the wafer at 80 °C followed by soft baking for 5 min at 70 °C [19]. The protective foil was removed before soft baking and the resist exposed in a mask aligner (EVG 620) using an i-line filter for 40 sec at 11 mW/cm$^2$. Post exposure baking consisted of ramping to 90 °C for 2 min, with a hold at 90 °C for 3 min, then a ramp down to 30 °C for 5 min. The resist was developed in EC solvent for 8 min. A second layer of TMMF S 2030 was laminated over the first layer to define the aperture on top of the cavity. This was laminated at a temperature of 45 °C, the protective foil was removed from the resist, and the substrate was kept at room temperature overnight. The resist was then exposed for 27 s at 11 mW/cm$^2$ followed by a post exposure bake at 47 °C for 15 min in a convection oven with the resist facing downward, to prevent the unexposed resist falling into the cavity [20]. The resist was then developed in EC solvent for 7–8 min and the wafer was hard baked at 170 °C for 30 min.

Aperture-suspended bilayers require a hydrophobic support material. Therefore the surface of the chip was made hydrophobic by exposure to $CF_4$ plasma at 50 W for 2 min with 2% $O_2$ and 98% $CF_4$ at 50 mTorr pressure. Following this treatment, the TMMF contact angle increased to 115°. Fig. 2b and c shows optical and