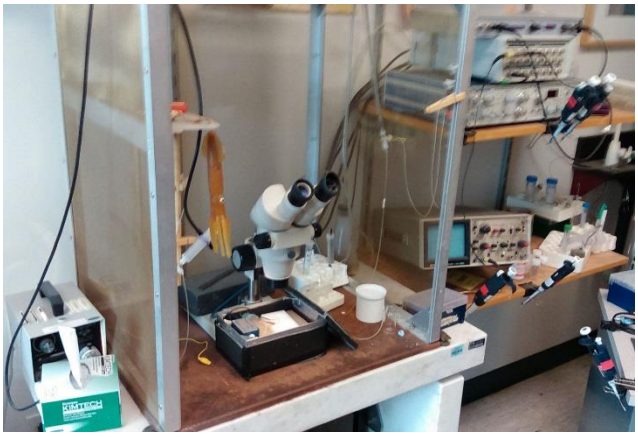
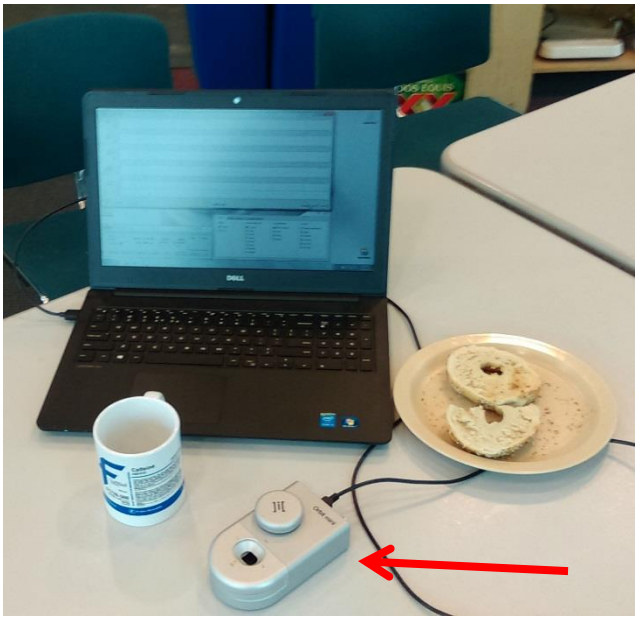
Getting going with the Orbit mini planar bilayer system: A narrative

Chris Miller, Brandeis University

After 40 years of using home-built planar lipid bilayers for recording ion channels from membrane fragments or purified protein preps, I recently purchased the Orbit mini system. This was motivated by my own curiosity regarding the noise-improvement claims made by Nanion, about which I was skeptical, and also by the compactness and mobility of the Orbit-mini. Moreover, with more labs now functionally characterizing purified ion channel proteins in planar bilayers, the standardization offered by this instrument seemed a real advantage to the field in lowering barriers to new users of the technique, barriers that can seem quite daunting in setting up from scratch a home-built system. The purpose of this narrative is to compare my long experience with home-built planar bilayers with our first 2-3 months of adapting the Orbit mini to our current application – a ~10 pS fluoride-selective ion channel that shows rare closing events on the 10-ms timescale. All the work here was done by Dr. Nick Last, a postdoctoral associate not previously trained in electrophysiology. Nick's experience provides an excellent model for a skilled biochemist undertaking planar bilayer channel recording for the first time.



I compare the Orbit mini to our current planar bilayer system, optimized over decades and in daily use here. This consists of a plastic chamber consisting of a horizontal partition with a hand-fashioned hole of ~50 µm diameter on which bilayers are painted, separating "upper" and "lower" aqueous chambers of 0.7 mL and 0.2 mL volume, respectively. Partitions are cut from overhead transparency film (80 µm thickness), are cleaned after use and stored in ethanol, and can be re-used literally for years. The chambers are connected through salt bridges to a standard patch-recording setup (Axopatch 200-series or Warner 505). The chamber and headstage are mounted in a metal box with lid, with all surfaces coated in soundproofing material, on a vibration-damping table. Bilayers are 'painted' while viewing the partition under a stereomicroscope. For highest-quality recordings, I typically use bilayers of 30-40 pF capacitance. To record our 1-pA F^- channel at 100 mV holding voltage, we set the low-pass filter corner-frequency at 200 Hz, where we can clearly discern the ~1-10-ms closing events (rms noise is ~ 0.3 pA). In addition to the headstage voltage-noise amplified through the membrane capacitance, a major source of noise in our bilayers – and one irritating in its variability from bilayer to bilayer and day to day – is microphonic pickup of sound in the room; this is often difficult to filter out because it includes low-frequency components.



Contrary to my initial expectations, the Orbit mini (red arrow) has performed exceptionally well for our application, with vastly improved signal-to-noise characteristics over our best recordings on the home-built system. The improvement in electronic noise is partly due to miniaturization, but I imagine mainly due to the much smaller bilayers (~10 pF) that form on the holes in the chip-partitions (MECA chips, developed by Ionera Technologies). These have lower capacitative and microphonic pickup, and the result is dramatic. To record a 1 pA channel on the Orbit mini, we can now set the filter frequency to 625 Hz to achieve ~0.2 pA rms noise. This represents better than a 3-fold improvement in signal to noise, a really enormous advantage. A mystery remaining in my mind is why these very small bilayers still permit easy insertion of ion channels, which has been our happy experience. In our home-built system, membranes smaller than ~25 pF become extremely refractory to channel incorporation; I suspect that the particular geometry of the holes formed in the MECA chip underlies this felicitous feature.

The compactness of the instrument makes for flexible and convenient placement in the lab. With a tiny footprint, it sits on a table or desk accompanied by only a laptop. In addition, bilayers are painted 'blind' with a teflon wand supplied by Nanion without observing the partition through a microscope. Thus, the entire support-package of microscope, illuminator, Faraday cage, vibration-table, and rig-rack is eliminated. The acquisition software is intuitive and robust, and the data can be stored in formats readable by the Axon analysis programs that we routinely use. Moreover, the four electrically independent holes in the chip-partition means that four bilayers can be monitored simultaneously, thus quadrupling the probability of catching a clean single channel to record. This represents a significant, practical boost in experimental efficiency.

Our learning curve in taming the new Orbit mini was smooth on the whole, but we experienced a few bumps along the road that I list here – problems for which the Nanion technical support staff were very responsive and helpful.

1. **Deterioration of chip-partitions.** Nanion claims that each partition (cost ~$65) can be washed and re-used for 3-5 days without deterioration. But for the first few weeks of work, we could form stable bilayers only on the first day after opening a new MECA chip. We eventually tracked this down to our customary inclusion of 50µg/mL serum albumin in all our solutions (to suppress binding of added proteins such as

antibodies to the chamber walls). We cannot wash this residue away, but if we omit this component, the chip lifetimes are as advertised.

2. **Lipid solution for bilayer formation.** We have always used 10 mg/mL phospholipid in n-decane for forming bilayers, but this does not work well on the MECA partitions. Instead, we find that n-nonane is an excellent lipid solvent in the new system – better than the n-octane recommended. Pre-conditioning of the holes with air-dried lipid – a necessity for the home-made partitions – is not recommended for the Orbit mini.

Some maneuvers often used in our home-built systems that cannot be done with the Orbit mini:

1. **Constraints regarding Cl^-.** Since the Ag-AgCl electrodes are built into the chip-partitions, Cl^- must always be present (preferably at equal concentrations on both sides of the bilayer). It is not possible to use salt bridges to connect the electrodes to Cl^--free solutions. Therefore, all our F^- channel recordings necessarily require 10 mM Cl^- added to both 200 mM NaF solutions – not a problem for us because the inertness of Cl^- for this channel was already established (in our home-built system). But this could be troublesome for anyone studying anion selectivity properties.

2. **Perfusion of trans chamber**. The lower chamber cannot be perfused, and so, as with standard patch-recording, only one side - the upper solution - may be changed during an experiment. This precludes one of the strengths of planar bilayers – straightforward double-sided perfusion while recording a channel.

3. **Application of solute gradients.** Because of the small volume of the lower chamber (~20 pL!!) and the way solution is introduced to the Orbit mini, both chambers must be initially filled with the same aqueous solution. Only after bilayer formation can a solute gradient be applied, and only by changing the upper solution, an easily accomplished maneuver. If the bilayer breaks, any gradient will quickly be dissipated, and the solutions must be entirely replaced and new bilayers formed.