Orbit mini® Getting Started

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The Orbit mini

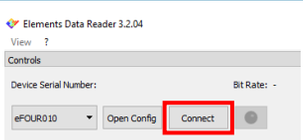
The Orbit mini is explicitly designed to meet the special requirements of experiments on artificial bilayers. Use of Ionera's MECA (micro electrode cavity array) chip technology combined with state of the art low noise amplifiers (Elements S.R.L.) enables the fully parallel recording of four separate lipid bilayers at bandwidths up to 100 kHz with excellent signal-to-noise ratios. The system has been validated with targets as diverse as ligand and voltage gated ion channels, porins and origami DNA constructs, antimicrobial peptides or membrane active toxins. The optional temperature control for the Orbit mini furthermore allows for experiments on temperature sensitive species such as TRP channels or for experiments at physiological temperatures.

1 Material

- Orbit mini connected to a computer via USB
- Element's EDR 3 software installed on the computer
- Nanion Orbit mini test cell
- MECA 4 recording chip, buffer of choice
- Lipid stock solution (e.g. DPhPC, c = 10 mg/ml in octane)
- Instrumentation for painting bilayers (pipette, brush, Teflon piece…)

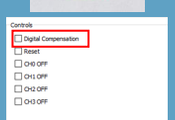
2 Start the software and connect the device

- Connect the Orbit mini to a computer via a mini-USB to USB cable, start the EDR 3 software and connect the device by pressing the 'connect' button.



3 Calibrate the Orbit mini

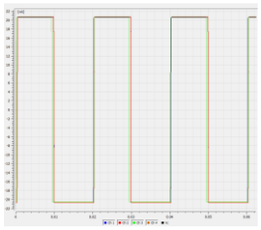
- The Orbit mini needs to be calibrated after each startup of the software
- Choose the 200 pA gain and insert the test cell
- Close both lids and activate the 'digital compensation' by ticking the box under Settings → Controls
- The software will automatically adjust the four signals close to zero; untick the box again afterwards
- Use the 'Voffset correction' to manually finetune each channel to zero
- Untick the 'digital compensation' box and remove the testcell
- The Orbit mini is now calibrated. To avoid miscalibration, please:



DO NOT RUN THE DIGITAL COMPENSATION WITHOUT TESTCELL!

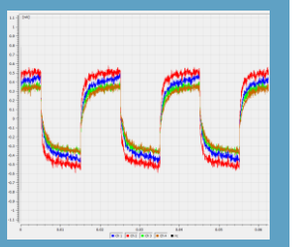
4 Apply MECA 4 chip and fill the cavities

- Place a MECA 4 chip in the Orbit mini and close the lower lid
- apply 150 µl buffer of choice on the MECA 4 chip with a pipette
- Follow the wetting of the cavities e.g. by application of a triangular voltage wave (Protocol 1)
- The resulting signal should alternate between the positive and negative maximum of the chosen gain range (depicted on the right) as the cavities are uncovered and thus enable unhindered ion flux
- If the cavities are not readily wetted, please use the provided wetting tool to gently push the buffer inside the cavities



5 Paint lipid bilayers

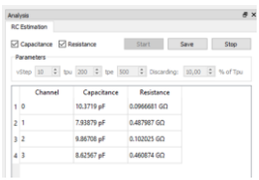
- Manually paint a lipid bilayer on the chip's four cavities by gently spreading lipid solution over them with e.g. one of the provided tools for painting (brush, Teflon piece in holder, glass rod)
- Nanion recommends to paint bilayers via lipid covered air bubbles:
  1. Take e.g. a 10 µl Eppendorf pipette (small white tip)
  2. Cover the tip with lipids by dipping it into the lipid stock solution without aspirating any of it. Due to capillary forces a small amount of lipid solution will remain in the pipette tip - squeeze out any of these residues from the pipette tip
  3. Place the tip on the chip's surface close to a cavity, dispense a small air bubble (2-3 µl) over the cavity and aspirate it back. This results in the formation of a lipid bilayer which can be monitored by following the change in the current response: the signal should not go into saturation anymore and exhibit capacitive properties as shown on the right
- Repeat step 3 on all cavities. If bilayer formation is not successful within a first run start again at step 1.
- Note: Do not throw the tip away afterwards. It can be still used to repaint broken bilayers during the experiment.
- For a short video about this protocol please visit www.nanion.de



6 Judge bilayer quality

- Use the ZAP function (Settings → Voltage Protocols) to judge the status of the bilayers: a thinned out lipid monolayer should get destroyed
- Simply repaint the bilayers afterwards
- Use the RC estimation function (Analysis) to calculate the membrane capacitance C_m via an applied test-pulse
- C_m will vary for different lipid mixtures and/or solvents but the capacitance for freshly painted bilayers can easily be compared to the one found for functional bilayers

ZAP



7 Clean MECA 4 chips

- Clean the MECA 4 chips directly after each experiment
- Use double-distilled water to remove buffer residues
- Use ethanol and/or isopropanol to remove lipid residues
- The chips are quite robust: use precision wipes to manually clean them
- Thoroughly dry the MECA chips before starting a new experiment as you otherwise might encounter offsets in the empty chip signals

8 Optimize your system

- Switch off channels with broken membranes (Settings → Controls) as open cavities might give rise to offsets in the remaining channels
- Make sure that you always get zappable, thinned out membranes
  - If this fails, try different lipid-to-solvent ratios in the range of 1mg to 30 mg lipid(s) per ml solvent
  - Try different solvents like octane, decane, nonane or even hexane until you find the one best suited for your system
- The Orbit mini itself as well as the EDR software and a list of application examples are being constantly updated to provide further options and to increase the ease of use - so make sure to regularly check
  - www.nanion.de
  - www.elements-ic.com
  - www.ionera.de

  for the newest updates and documentation!