Bio Currents Research Center Protocol

<u>Equipment and Software</u>

Introduction

The material below has been adapted from several primary papers and reviews originating from the BRC. These should be referred to in publications and for the original source materials.
References:

1. *Smith, P.J.S., Sanger, R.S. and Messerli, M.A. (2007)* Principles, Development and Applications of Self-Referencing Electrochemical Microelectrodes to the Determination of Fluxes at Cell Membranes. In: Methods and New Frontiers in Neuroscience. Ed. Adrian C. Michael. CRC Press. Ch. 18: 373-405.
2. *Messerli, M.A., Robinson, K.R. and Smith, P.J.S. (2006)* Electrochemical sensor applications to the study of molecular physiology and analyte flux in plants. In: Plant Electrophysiology - Theory and Methods. Ed. Alexander G. Volkov. Springer-Verlag. 73-107.

The equipment and software described below are designed and assembled at the BioCurrents Research Center (Woods Hole, MA). All the development and microelectrode testing described in the protocols has been done using these designs. The basic components required to do self-referencing are the pre-amplifiers designed for potentiometric and amperometric detection, the main amplifier, a suitable PC with an analog to digital board, running the graphic user interface (GUI: IonView: Fig. 1). This software also runs the motion controller interfacing with the 3-axis manipulator.

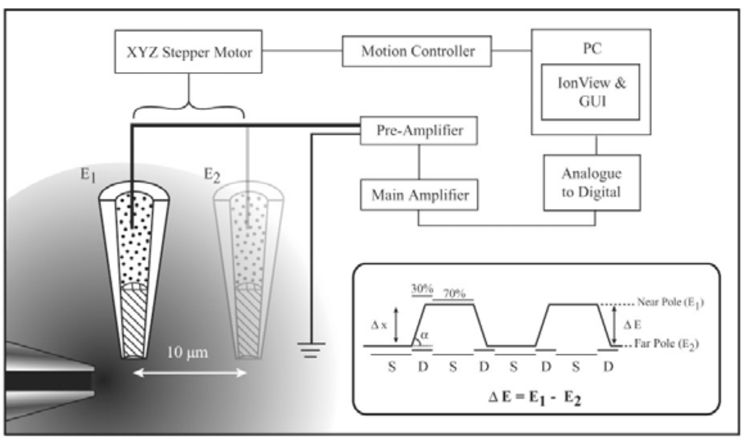

Figure 1: *A schematic illustration of the self-referencing set-up. In this case a electrode is shown near a source of the analyte, moving in a 'square' wave step between two positions 10 µm apart. Data is collected at 1000 points per second with the first 30% collected during and after translation being discarded (D). Movement is frequently set for a 10 µm displacement (Δx) at a frequency of 0.3 Hz, thus data is collected at each pole for approximately 1 second or 70% of the cycle time (S). Comparing, in this case, voltages collected between the two extremes of movement, gives rise to a differential value (ΔE).*

Preamplifiers

Voltage Head Stage - Potentiometric

For potentiometric measurement the active element of the voltage head stage is an AD549 operational amplifier (op-amp: Analog Devices, Norwood, Ma.) configured as a voltage follower. This allows the microelectrode voltage to be sensed while drawing little current.

Current Head Stage - Amperometric

For amperometric measurements the components of the head stage are reconfigured to be a voltage clamp. The microelectrode is held at a command potential and the current that flows through the microelectrode is collected. An AD549 op-amp is configured as a current to voltage converter, having a 1 GO sense resistor. The command voltage is applied to the positive input of the op-amp and the microelectrode is attached to the negative input. An AD620 instrumentation amplifier is used to subtract the command voltage from the output of the op-amp leaving a voltage that is proportional to the microelectrodes current flow, times the value of the sense resistor, or 1V per nA.

Amplifier

Amplification of the position dependant portion of the microelectrode signal must be sufficient for digitizing with reasonable resolution. The analog signal from the electrode must be amplified enough to make the sub-µV changes in voltage resolvable by the analog to digital converter without exceeding the dynamic range of the converter. Currently in use is a 16-bit data acquisition system (Datatranslation, Marlboro, MA) with a dynamic range of ±10 Volts. This gives 65536 possible numbers to represent a voltage in the ± 10 V range, or 305 µV per least significant bit change. To resolve µV changes, a gain of 1000 times is applied, giving 305 nV per step or least significant bit change. The problem here is that the microelectrodes background voltage is frequently high, up to 100 mV. Since the dynamic range is ± 10 V the signal will have to be offset closer to zero volts before the gain is applied. Many methods can be used to do this. Two are currently in use at the BioCurrents Research Center. In the first a resistor and capacitor (RC), having a time constant of 10 sec, generates a value that is subtracted from the non-filtered signal before the gain is applied, thus constructing a high pass filter. This allows signals faster than the time constant to be amplified while the impact of DC levels, and signals slower than the time constant, are minimized. The second method in use is a sample and hold approach. Here the computer, either by timed control or manual control (using a software button), sends a digital signal to a sample and hold component in the amplifier, latching the real time analog voltage to be the new reference or offset value of the live signal. Gain can be applied. In the above example the gain needed, and therefore the maximum offset that can be tolerated without exceeding the dynamic range of the analog to digital converter, is determined by the resolution of the converter (number of bits). If a high enough resolution converter becomes available no nulling of the offset would be necessary.

Motion Control

Microelectrode movement is accomplished by a robotic manipulator and driving electronics. The system designed and used within the BRC comprises 0.9-degree stepper motors (Oriental Motors, Torrance, CA) fitted to Newport 433 and 432 translation stages (Newport Corporation, Irvine CA). The motors drive a 100 pitch lead screw via a zero backlash flexible bellows coupling (Servometer, Cedar Grove, NJ). A motor, in turn, is driven by a 128 micro-step drive. Micro-stepping increases the resolution of motion and smoothness of operation. The software increments or decrements the micro-step count to the motor and the shaft angle follows. Driving the motors with linear potential, rather than using the popular chopper method, reduces electrical interference but at the expense of motor torque. The system is capable up to several hundred microns per second at sub micron resolution and repeatability. [3]

Software

IonView is the name given to the BRC windows program used for data acquisition and motion control. It has a standard graphic user interface (GUI). The current version of IonView provides simple access to data acquisition parameters - choice of RC subtract versus sample and hold, distance and speed of translation, and the choice of signal averaging. Data are processed and presented on screen, in real-time. As the polarity of the signal recorded is entirely dependent on which pole is subtracted from which, by convention one pole of translation is designated the +ve pole, as selected by the angle of movement, and is always the near pole of translation. A separate drop down window handles electrode positioning.

The equipment required for self-referencing is very similar to that found in most electrophysiological laboratories. A complete list of requirements and a cost estimate can be obtained by contacting the BioCurrents Research Center (Email - infobrc@mbl.edu). As part of our dissemination and outreach program the BRC sells and assists in the installation of general and specialized equipment and software.

Literature Cited:

3. *Danuser, G. (1999)* Photogrammetric calibration of a stereo light microscope. J. Microscopy, 193, 62-83.