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A highly versatile and easily configurable system for plant electrophysiology

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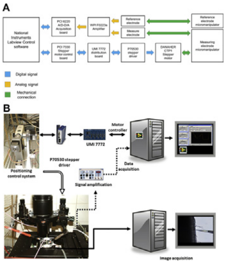
G R A P H I C A L A B S T R A C T



A B S T R A C T

In this study we present a highly versatile and easily configurable system for measuring plant electrophysiological parameters and ionic flow rates, connected to a computer-controlled highly accurate positioning device. The modular software used allows easy customizable configurations for the measurement of electrophysiological parameters. Both the operational tests and the experiments already performed have been fully successful and rendered a low noise and highly stable signal. Assembly, programming and configuration examples are discussed.

The system is a powerful technique that not only gives precise measuring of plant electrophysiological status, but also allows easy development of ad hoc configurations that are not constrained to plant studies.

- We developed a highly modular system for electrophysiology measurements that can be used either in organs or cells and performs either steady or dynamic intra- and extracellular measurements that takes advantage of the easiness of visual object-oriented programming.

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- High precision accuracy in data acquisition under electrical noisy environments that allows it to run even in a laboratory close to electrical equipment that produce electrical noise.
- The system makes an improvement of the currently used systems for monitoring and controlling high precision measurements and micromanipulation systems providing an open and customizable environment for multiple experimental needs.

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Method

System development and assembly

The system consists of different modules: mechanical, electrical, optical and digital. The control center is programmed using Labview object-oriented programming software (National Instruments, USA) to control both the signal acquisition and the micromanipulator movement.

The mechanical part of the system consists of an inverted microscope (Nikon Eclipse TE2000), a couple of positioning micromanipulators (Narishige MM-89, Nikon/Narishige, Japan); one holds the reference electrode and the other holds the measuring electrode The second micromanipulator is coupled to a hydraulically driven high precision micromanipulator (Nikon/Narishige MO-188). The knobs of this micromanipulator corresponding to the x and y axis are coupled to a custom-made retainer that is fixed to the axis of a stepper motor (DANAHER CTP12ELF10MMA00, Danaher Motions, Washington DC, USA) which is electronically controlled by a P70530 stepper driver, connected to a UMI 772 distribution board which in turn is connected to a PCI 7330 dual axis motion controller (all from National Instruments, USA). Detailed characteristics of the steeper motors can be found in the Additional Information section.

The signal acquisition system consists in a WPI FO223a amplifier (World Precision Instruments, London, UK) connected to a PCI 6220 A/D-D/A acquisition board (National Instruments, USA). Both the PCI7330 and PCI 6220 are plugged to a PC computer running windows. Different combinations of operating system and Labview software can be used although in our first development the system was running under Windows XP second edition and Labview 8.6, but we have made successful tests under windows 7 and Labview 11, which shows both the adaptability and compatibility of the system. An exhaustive list of operating system and Labview versions can be seen in Table 1.

A schematic representation of all the connections is displayed in Fig. 1 (A and B) and a detailed photography of the actual sample mounting can be seen in Fig. 7A.

System programming

The system was originally programmed using National Instruments Labview 8.6 software running on a Windows XP operating system, but this is not the only possible working environment, since, as stated above, it can run under several combinations of operating systems and Labview versions (for example. Windows 7 and Labview 11). The core of the programming can be seen in Fig. 2 and consists in a Virtual instrument (VI) featuring a very simple loop. Each iteration extracts a number of samples and by averaging them to reduce spurious noise a smooth signal acquisition is achieved. Each value of the average is sent to the graph display together with the timestamp provided by a time counter and a x-y graphic is displayed in which x-axis corresponds to time and y-axis to the intensity of the signal, measured in mV. From this simple nuclear program, a series of different implementations have been done, each one corresponding to a specific need. The two main configurations can be seen in Figs. 3 and 4. Fig. 3B corresponds to a continuous single channel current measuring (for example membrane potential) and Fig. 4 corresponds to ion fluxes measurement configuration.

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