

Myocyte Calcium & Contractility Recording System (μ Step Configuration)

Complete real-time, turnkey system for
calcium and contractility recording

- 1000 pseudo-ratiometric calcium measurements/
second
- 1000 cell and sarcomere length measurements/
second



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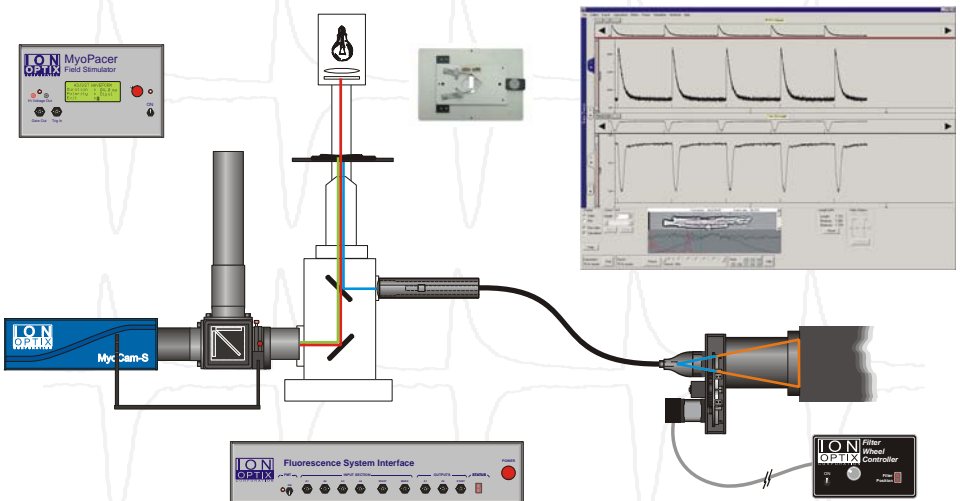
November 2011

Myocyte Calcium & Contractility System Overview

Interest in the physiology and pathology of the heart has led to a substantial increase in the number of laboratories studying the myocardium at the cellular level using isolated cardiac muscle cells or myocytes. Although excitation-contraction (EC) coupling, the process by which electrical stimuli induce a mechanical response in myocytes, has been an area of intense investigation for over half a century, elucidation of the fundamental molecular and biophysical mechanisms remains at the forefront of cardiovascular research. Impairment to the regulatory machinery governing EC coupling correlates strongly with the onset and progression of many myopathies. Cellular dimensioning and intracellular calcium recordings offer an important measure of EC coupling in isolated myocytes and myofibers while also providing key insights into the processes that affect cardiovascular health.

IonOptix developed its **Myocyte Calcium & Contractility Recording System (MMSYS: μ Step Myocyte System)** over many years of collaboration with top cardiovascular researchers. We take pride in a line of precision products that are application driven and built to meet the needs of a demanding research environment. Since its inception in 1990 IonOptix has built and installed hundreds of high performance, turnkey systems in research laboratories worldwide.

A complete system includes everything necessary for simultaneously acquiring and analyzing calcium and cellular geometry data with our new IonWizard 6 software. MMSYS also includes an inverted fluorescence microscope equipped with a calcium photometry objective and brilliant optics, a cell stimulator with a stimulation/superfusion chamber and temperature control, and a suite of A/D analog and digital connections for synchronous data collection.



IonWizard Software Suite

Acquisition of EC Coupling Data in Myocytes

Our complete systems are built from components designed to work seamlessly with one another and our IonWizard core software, providing completely synchronous and accurate data acquisition. IonWizard's core functions are expanded through the **SoftEdge**, **SarLen** and **PmtAcq** acquisition modules to record cell length, sarcomere length and ratiometric fluorescence data. Through these acquisition modules, IonWizard communicates directly with two root devices, the MyoCam-S video acquisition camera and our fluorescence system interface.

IW6 is capable of acquiring up to 4 channels of 1000 Hz analog data. It also supports analog data outputs and digital inputs. IW6 now features a signal generator function for programming voltages to drive and control external hardware.

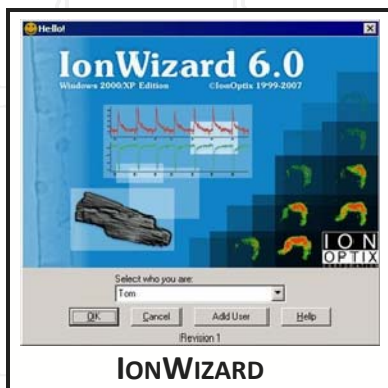
EDQACQ provides the ability to detect the cell boundaries at separate lines for left and right edges. A threshold is then used to locate the edge within the detection window. This dynamic measurement allows up to 1000 length recordings per second when coupled to the MyoCam-S.

SARACQ performs an average of the lines within the area of interest to create a well resolved striation pattern. A fast Fourier transform (FFT) is then calculated and the peak within the power spectrum represents the average sarcomere spacing, measured in real time up to 1000 times per second.

PMTACQ supports PMT-based photometry recordings as well as coordinated wavelength control for dual excitation calcium acquisition.

Features

- Coupled with either a Fluorescence System Interface, SoftEdge, SarLen and PMTACQ form a powerful integrated myocyte recording and analysis system.
- Cell length or sarcomere data are sampled at rates as high as 1000 Hz. Cell calcium levels are simultaneously sampled at 1000 Hz for single excitation dyes, 1000 Hz for dual excitation dyes sampled pseudo-ratiometrically.
- Multiple 'epochs' define separate acquisition regimes. Within each epoch, independent sampling rates for cell length, photometry, and analog data are specified.
- Switches between different epochs occur automatically or via user intervention.



IONWIZARD



SARLEN

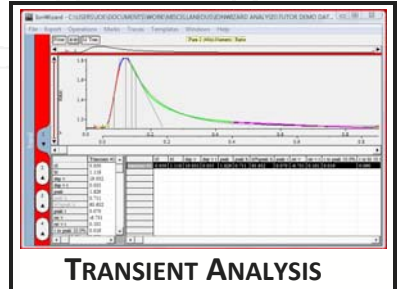
- SoftEdge & SarLen work seamlessly with the IonOptix MyoCam-S™.
- Edge detection is based on either image intensity or the derivative of image intensity.
- Edges are detected from "outside in" on both cell edges.
- Independent left and right edge analysis on separate video lines improves the accuracy of length measurement.
- Fluorescence or cell length data is optionally output as an analog voltage in real-time.

IonWizard Software Suite (cont.)

Analysis of EC Coupling Data in Myocytes

Transient Analysis

IonWizard has extensive transient fitting capacities. Each transient is marked for analysis either automatically or manually via IonWizard's transient mark facility. Subsequently, the transients are fit by a series of high order polynomials to arrive at a mathematical model of the curve. This model is used to determine a variety of characteristic transient parameters (over 30 in total). IonWizard automatically detects upward or downward going transients thus calculating and presenting the parameters appropriately. The user may peruse each transient to confirm the accuracy of the fits. A graphical feedback mechanism permits verification of each parameter. The transient parameters are exported to a file or copied directly into a spreadsheet using the clipboard.



Transient Parameters Found by IonWizard:

Time Zero. The beginning time of the transient. This can be found three ways: via a baseline finding algorithm, by user specification, or keyed off an external mark.

Baseline. The value of the transient at time zero.

Departure/Return Velocity (i.e. dc/dt , dL/dt). The maximum (minimum) velocity reached on the rising and falling phases of the transient. These values can also be presented as a percentage of the baseline value and as a percentage of the transient height. The time of the departure/return velocity is also given.

Peak/Peak Height. The peak is the highest/lowest value reached by the transient. This parameter is also used to calculate peak height. The height can be shown as a percentage of the baseline (i.e. percent shortening for length data). The time of the peak relative to time zero is also given.

Time to % Peak / Time to % Baseline. The user can configure IonWizard to find the time required for the transient to reach up to three different levels on the rising and falling phases. These times may be reported relative to either time zero or to the peak time.

Exponential Fit. A single exponential curve is fit to the trace starting at one of three places: the peak, the time of maximum return velocity, or some user defined % return to baseline. Three parameters are fit: amp, tau, and off. The equation fit is $y = amp * e^{-t/tau} + off$.

Bi-exponential Fit. A bi-exponential curve is fit to the trace starting at time zero. Four parameters are fit: amp, tau rise, tau fall, and off. the equation fit is $y = amp * (e^{-t/tau\ fall} - e^{-t/tau\ rise}) + off$.

Area. The area under the curve (i.e. from baseline to peak) is calculated for two time ranges: from time zero to peak and from the peak to the end of the transient. For upward going transients, the area from the baseline down to zero is also found for both time ranges.

μStep

Dual Excitation Light Source

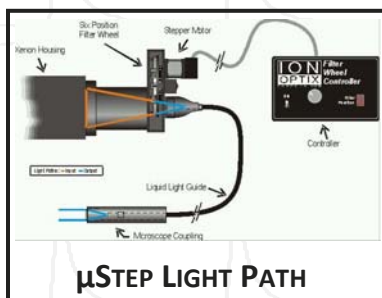
Cellular calcium levels rise and fall on millisecond time scales. For dual excitation indicator dyes such as Fura-2, the fluorescence excitation light source must offer millisecond temporal resolution. The IonOptix μStep also exploits our new filter wheel design that permits not only increased sampling speed (up to 8 ratio pairs per second), but also eliminates the need for a separate electronic shutter (movement to a shuttering position accomplished in 50 ms). It is a flexible device that can be used as part of a complete system or as a stand-alone device with six separate filter positions. The μStep permits a modified sampling technique enabling pseudo-ratio sampling rates of 1000 Hz over short periods of time (visit us on the web for an application note on the interpolated numerator method). The μStep delivers fluorescence illumination to your microscope through an efficient liquid light guide and a microscope-specific adapter.

Features

- Controller with position display.** A microcontroller provides the "intelligence" to drive the filter wheel and display current position. Filter position can be controlled either manually or by computer via a parallel or serial connection.
- Shuttering.** The μStep is designed to use the filter wheel itself as a shutter between sampling periods to minimize light exposure, thereby avoiding dye bleaching and photodamage to the preparation. Separate electronic shuttering system is not required.
- Xenon Arc Sub-system.** Standard arc lamp housing, igniter and power supply serves as source 75 watts of polychromatic light. Usable wavelength range is 300-700nm and is limited only by the availability of band pass filters.
- Excitation Optics.** Excitation filters of your specification are included with each system. Sliders provide allow rapid placement of neutral density filters in the common excitation path, providing a convenient means to select between ND filters (a 5-piece ND set is provided with MUS200).



μSTEP



μSTEP LIGHT PATH

- Emission Filter / Dichroic.** The appropriate emission filter and dichroic mirror is provided for your microscope.
- Light Guide.** A liquid filled light guide delivers the excitation light to the microscope providing vibration and electrical isolation and flexible position options.
- Microscope Coupling.** Connects the light guide to your microscope's epifluorescence port. Couplings are available for all common microscopes or can be custom built for your particular microscope.
- Cables.** Includes cables to connect to the Fluorescence System Interface.

Fluorescence System Interface

System Integration & Control

The IonOptix Fluorescence System Interface, model FSI700, provides all the standard non-video input, output and device control hardware needed for a typical dual-excitation fluorescence system. The FSI700 may be combined with a variety of IonOptix components to create the specific combination of system capabilities that are required in your experiments.

The FSI700 may be connected to external devices using the four analog inputs, the two analog outputs or the start and mark trigger inputs. IonWizard's flexible device configuration allows the experimenter to specify the name and unit scaling of each auxiliary signal for easy-to-read data files.



FSI700

Features

Inputs

- **Dual PMT inputs.** Provides connection to two PMT sensors.
- **Start in.** This TTL signal allows external initiation of data sampling.
- **Mark in.** This TTL signal is recorded during data acquisition to provide event synchronization information.
- **Analog to Digital.** Four channels of 16-bit A/D with input voltage range of $\pm 5V$.

Outputs

- **Start out.** Allows slave stimulators or recorders to be synchronized with fluorescence data recording.
- **Digital to Analog.** Two channels of $\pm 5V$. 12-bit D/A outputs can be configured as monitor or control signals.

Light Source Control

- **IonOptix 25pin.** Provides control signals to/from excitation light source.

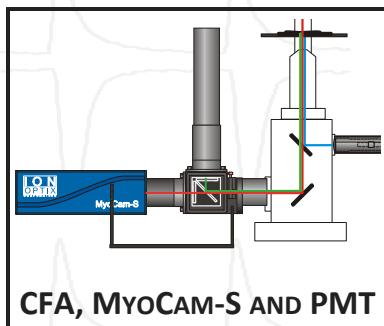
Includes

- Includes half-length, full-height PCI computer interface card and six foot cable.
- IonWizard driver software for Windows 2000, XP.

Fluorescence & Video Detection

Real-time Cellular Dimensioning

For detecting and quantifying fluorescence emission, we furnish systems with one or more photomultiplier tubes (PMTs). The PMT offers broader dynamic range, faster acquisition rates and greater photosensitivity than CCD-based sensors. To enable simultaneous cellular dimensioning with the MyoCam-S, we equip every combined dimensioning and photometry system with our cell framing adapter (CFA). The CFA hosts several optical elements for filtering and directing light to the appropriate device. It holds an aperture for physically framing the image, preventing unwanted extracellular background from contributing to the fluorescent signal. The CFA also comes outfitted with our MyoHandle, a device for mechanically rotating the camera image to align the cell for dimensioning. Coupled with the appropriate optical filters, our CFA, camera and PMT offer precise, simultaneous calcium and contractility measurements.



PMT Sub-System

The new PMT400 sub-system offers true photon counting using an end-on alkali PMT tube with integrated high-voltage power supply and amplifier discriminator. This combined package increases reliability and decreases cost. The PMT400 is directly powered and controlled by the IonOptix model FSI700 Fluorescence System Interface, where it is under software control. For stand-alone applications, a separate module (PTV100) is available that provides power to the PMT400 and a BNC analog "count" output for connection to other devices.



Features

- Integrated Tube, Supply and Amplifier/Discriminator.** Single package holds photon-quality alkali PMT tube (180-600nm, 400nm peak sensitivity), integral, high-voltage power supply pre-set to the photon counting voltage, and amplifier/discriminator.
- C-Mount.** Industry standard C-mount available to provide easy connection to standard microscope adapters.
- IonOptix Coupling and Emission Filter.** An IonOptix to C-mount coupling holds the included emission filter and directly connects to the IonOptix Cell Framing Adapter.
- Cable.** Integrated cable connects directly to Fluorescence System Interface. Standard extension cables allow the cable length to be easily increased.

Fluorescence & Video Detection (cont.)

MyoCam-S: High Speed Digital Video

The IonOptix MCS100 represents the "next generation" of our popular MyoCam. It's an all-digital, variable field rate camera that utilizes the USB 2.0 standard to remove the restrictions of analog video formats and frame grabbers. Its maximum pixel clock rate is twice that of the MyoCam so that our "standard" ¼ field rate jumps to 380Hz; sufficient to capture/characterize the fastest cardiac myocyte contractile transient. The MyoCam-S™ gives you complete control over all aspects of video acquisition to deliver the optimum combination of temporal and spatial resolution needed for your experimental requirements. All analog processing and digitization is done inside the camera to minimize analog noise. The digital data is then transferred to the computer using a standard high-speed USB 2.0 port eliminating the cost of a frame grabber.



MYOCAM-S w CFA300

Features

- **CCD sensor.** 774 pixels wide by 245 lines (progressive) or 490 lines (interlaced).
- **Frame rates (lines).** 97Hz (245 lines), 250Hz (87 lines), 500Hz (36 lines), 1000Hz (10 lines).
- Complete control of camera acquisition window (start pixel & width, start line & height).
- **Selectable pixel clocks.** Standard, 2x high-speed, and low-noise.
- 12-bit A/D converter with 8 or 12 bit read-out.
- Programmable CCD gain and offset.
- Programmable integration time to stop fast movement or increase camera sensitivity.
- Programmable frame interval and external trigger to synchronize with other recording devices.
- Multiple cameras can be exactly synchronized.
- Compatible driver for use with any standard Windows video application
- Single cable to camera controller.

Fluorescence & Video Detection (cont.)

Cell Framing Adapter

The Cell Framing Adapter is used to simplify and optimize cell fluorescence recording using a PMT. An adjustable iris is used to frame a rectangular area of the microscope field of view maximizing selected cell fluorescence while masking background signal. The CCD camera displays the image area that the PMT will record. The CFA's D-Cube holds a dichroic mirror and emitter to reflect and filter fluorescence emission before collection by the PMT. The use of a red filter in the microscope condenser permits the transmitted image to be visualized concurrently with the indicator dye fluorescence; a feature which is exploited by our combined fluorescence recording and dimensioning system. The Cell Framing Adapter comes with the matching mount to connect to the PMT Sub-System.

Features

- **Microscope Coupling.** The microscope coupling attaches to the side port or trinocular head of all common microscopes.
- **D-Cube.** The D-Cube provides convenient access to emission optics. An appropriate dichroic mirror and emission filter for your selected emission band is included.
- **Rectangular Aperture.** Masks signal from cell and debris adjacent to the cell of interest.
- **Magnification coupling.** A range of demagnification couplers are available to optimize the image size presented to the system camera.
- **MyoHandle.** This mechanical element couples the rectangular aperture to the camera so that both elements can be rotated in tandem, facilitating cellular alignment.

Options

- **Option D: Dual Emission.** The CFA optics can be stacked to permit dual emission PMT recording.



Myocyte Fluorescence Microscope

Motic AE31 & Olympus UApo/340 Objective

The IonOptix-configured Motic AE31 inverted microscope provides an ideal platform for combined photometry and dimensioning measurements. It features upscale research functions, such as halogen Koehler illumination and epifluorescence capacity. The AE31 also incorporates Motic's Color Corrected Infinity Optical System [CCIS®] to produce crisp, flat and high contrast images. We equip our microscope packages according to the specific demands of the proposed IonOptix system. For calcium photometry systems, the AE31 comes configured with an Olympus fluorescence objective with high numerical aperture and UV transmission. Properly equipped with an IonOptix fluorescence illumination system, the Motic serves as an exceptional choice for researchers in search of reliable, high fidelity data acquisition at an affordable price.



Features

Motic AE31 Inverted Microscope

- **CCIS Optics.** Color corrected infinity optical system.
- **Brightfield Illumination.** Koehler illumination system w/ true DC 6V-30W output delivers bright, consistent illumination at all optical magnifications.
- **Mechanical Stage.** Precise control of sample position. Comfortable long wand allows user to rest forearm while manipulating stage.
- **Trinocular Head.** Easy access to auxiliary components. Efficient transmission of light for photometry and cellular dimensioning.
- **Microscope Base.** Wide base for strength and rigidity. Inverted "Y" support provides additional lateral support. Ergonomic design provides easy adjustment of focus and stage controls.
- **Epifluorescence Package.** 3 cassette capacity. Easy access to dichroic filter positioning. Direct and efficient fluorescence illumination port.

Olympus UApo/340

- **UV Transmission.** High transmission of 340nm light; ideal for Fura-2 based Ca^{2+} photometry.
- **40X.** Ideal magnification for simultaneously dimensioning sarcomere and cell length
- **0.90 NA.** Non-immersion lens with excellent transmission and collection of light.
- **0.2mm Working Distance.**
- **Water proof and oil proof cap.**

Acute Cell Stimulators

MyoPacer

The IonOptix MyoPacer is a cell stimulator that has been designed specifically around the needs of the myocyte research community. The microcontroller driving the pacer allows for greater protocol versatility. Five frequencies can be pre-programmed to allow easy and rapid frequency changes. Its ability to emit bipolar stimuli or to alternate stimulus polarity greatly reduces problems with electrolysis at the electrodes. Output voltage (± 40 V), frequency (.010 to 99 Hz), and duration (.4 msec to 90 msec) can be easily adjusted from the front panel. All programmed features are saved in non-volatile memory for quick start-up.



Features

- **Digital adjustment of:**
 - frequency (0.010-99Hz)
 - pulse duration (0.4-90 msec)
 - voltage (up to ± 40 V)
- **Software sync.** TTL synchronization with Fluorescence or Data System Interface allows interpretation of stimulation events by IonWizard, enabling event averaging of selected transients.
- Pulses can be uni-polar, bi-polar, or alternate polarity between pulses.

Inputs / outputs

- **High Voltage Out.** ± 40 V.
- **Trigger In.** Trigger a stimulus with a TTL pulse.
- **Gate out.** TTL pulse concurrent with high voltage pulse.

Acute Cell Stimulators

MyoPacer EP

The IonOptix MyoPacer EP cell stimulator incorporates the functionality of our current MyoPacer plus many of the new features requested by users for electrophysiological studies. It was created to give researchers the capability of delivering pre-designed stimulation sequences for obtaining restitution curves and doing arrhythmia and defibrillation studies. It is based on the original MyoPacer and retains original functionality, but adds the ability to generate off beats, delays, and multiple frequency protocols. The MyoPacer EP is designed around the strategy of creating multi-phase protocols. Each protocol can have up to 5 phases. A phase can be defined either as a pulse train or a delay. If it is defined as a pulse train, a period or frequency is selected.

Phases end either after a programmed time/# of pulses, or when a manual or external TTL trigger is received. The end of a phase will immediately initiate the next phase or, if on the last phase, the return to the first phase. As traditional electrophysiological researchers have used the terminology S1, S2... to describe the various phases, the MyoPacer EP will automatically display the appropriate label when looking at a phase.



MYOPACER EP
(w/ FHD CHAMBER)

Unique EP Features

- **Restitution Protocols.** Deliver fixed number of pulses initiated by an external TTL pulse or manual start.
- **Arrhythmia Protocols.** Insert an offbeat pulse at a fixed interval.
- **Exercise Protocols.** Run multiple pulse trains with individually programmable frequency and duration.
- **Irregular Pacing.** Random variation of specified frequency within definable percentage window and guaranteed average effective rate.
- **External Triggering.** Change to next pulse train with TTL 'advance' input and/or trigger individual pulses with TTL 'pulse' input.
- **Programmable TTL Output.** Flexible output options for synchronizing your experiment.

Standard MyoPacer Features

- Maximum current: 240mA.
- Digital adjustment of frequency (0.010-99Hz), pulse duration (0.4-24 msec), and voltage (up to $\pm 40V$).
- Bipolar pulses reduce electrolysis byproducts.
- TTL Gate output is concurrent with high voltage pulse.

Microscope Chamber Systems

FHD

The FHD Microscope Chamber System is a newly designed chamber system to perfuse and stimulate cardiac myocytes on the microscope's stage. The silicone gasket seals disposable coverslips, preventing leakage without the use of sealing grease or vasoline. The FHD chamber system is ideal for use with either freshly isolated or cultured cells.

Features

- **Hinged lid.** Includes all the paraphernalia of solution line, solution height adjustment, ground wires, and platinum stimulation wires.
- **Molded silicone insert.** Forming the flow area of the chamber, the insert is available in a variety of designs and is easily replaced, exchanged, and cleaned.
- **Rapid exchange of cover slips.** Ideal for those researchers using cells cultured or plated on 25mm cover slips. The chamber offers effortless insertion of fresh slips with only a twist of the lid lock.



FHDRC1

- **Very low solution levels.** Rapid solution change with nearly laminar flow and minimal turbulence while separate aspiration port maintains stable solution level.
- **Thermistor.** Independent thermistor assembly with standard resistance for feedback temperature controller/ monitor. Can be used with our TEMPC2 package to monitor bath temperature.

C-Stim

The C-Stim is a new Cell MicroControls (CMC) superfusion chamber system for microscopy studies. The C-Stim utilizes a reusable, autoclavable chamber insert. Its coverslip bottom is glued in place, which nearly eliminates solution leakage into the scope. The chamber holder is designed for quick and easy removal of one chamber insert and replacement. The platinum wire stimulation assembly is also a "snap-in part" and is configured with a built in thermistor to sample bath temperature. The geometry of the inflow/outflow ports permits a stable solution level at low bath volume (<0.5ml) and achieves nearly laminar flow at a flow rate of up to 2ml/min.



CMC C-STIM

Features

- Reusable & autoclavable chamber inserts.
- Integral coverslip bottom prevents solution leakage.
- Low chamber volume and tapered inflow/outflow channels achieve laminar flow; separate aspiration port maintains stable solution level.
- Platinum wire field-stimulation electrode assembly is easy to insert/remove, connects easily to MyoPacer and other field stimulators.
- Stimulation assembly includes a built-in thermistor which works in conjunction with the CMC feedback temperature controller.

Temperature Control

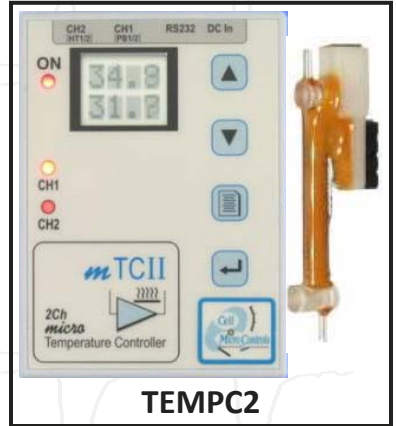
TEMPC2: CMC mTCII & HPRE2

The mTCII micro-Temperature Controller is a versatile 2 channel temperature controller powerful enough to heat small tissue baths, microscope stages, small animal heaters etc. but also able to control miniature heating devices like the included HPRE2. The mTCII uses state-of-the-art microcontrollers to provide a flexible, standalone instrument that allows control over the user interface and instrument function while maintaining temperature accurately. The mTCII has two ways of driving heaters, with a PWM (pulse width modulated 2.5-20kHz) or an analog output for lower noise. In the PWM mode it can control currents up to 1.5Amps provided by a Li-ion battery or 15V power supply. The HPRE2 Pre-heater is a 3 loop capillary heater for pre-heating solution.

Features

mTCII

- Sophisticated PID miniature controller accurately establishes and holds temperature.
- 2 independent channels.
- Compatible with many low voltage heater elements.
- RS232 port for control, logging.
- 2 heating modes, PWM [8W] or analog [2W].
- Maintains setpoint & parameters in non-volatile memory.
- Reprogrammable firmware.



HPRE2

- Dimensions: 45x14x7mm (LxWxH)
- Heater resistance \approx 17 Ω
- Thermistor sensor: 10k Ω NTC
- Plugs into Heater Aux of mTCII

IonOptix Systems

Our goal is to support the scientific research community with an array of systems that meet experimental demands while adhering to our philosophy of designing and developing innovative high-performance products at fair prices. The following are some of the application-driven systems currently available. Visit us at www.IonOptix.com for more information.

Calcium and Contractility/ Diameter

- Myocyte Calcium and Contractility Recording Systems
 - Calcium (HyperSwitch & MuStep)
 - Contractility (Cell & Sarcomere Length)
 - Force (MyoStretcher)
- Vessel Calcium and Diameter Recording System

General Photometry

- Fluorescence Photometry System

Tissue Bath Fluorometry

- FluoroPlex
- FluoroHeart

Cell Pacing

- Cell Culture Pacing

Myocyte Harvesting

- Myocyte Harvesting System

ION OPTIX



“The Barn” — IonOptix Headquarters in Milton, Massachusetts

Company History

IonOptix makes quality ratiometric fluorescence and cell dimensioning data acquisition systems. We have been making reasonably priced, high performance systems since 1990.

IonOptix prides itself on post-sale customer support. Telephone and email support is available on an unlimited basis. More importantly, every system sale includes a one to two day installation visit to set up the system and train the customer. We consider this training to be critical as it gets the customer up and running as quickly as possible. We run experiments with your preparations during the visit to be assured that all technical issues particular to the experimenter's preparations have been covered.

Resellers

Our good friends at Cairn Research are currently our only resellers. They sell our components in conjunction with their fluorescence and electrophysiology systems.



Cairn Research

www.cairn-research.co.uk

Authorized Representatives

In efforts to afford this high level of service to all our customers, IonOptix has entered into agreements with local representatives in several countries. The representatives have been chosen based on their technical and biological expertise, familiarity with our products, and on their customer service skills.



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Japan

www.primetech.co.jp



Commat Ltd.
Turkey

www.commat.com.tr



Bioprobes Ltd.

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www.bioprobeshk.com

www.bioprobeschina.com



Scitech Korea Inc.

Korea

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