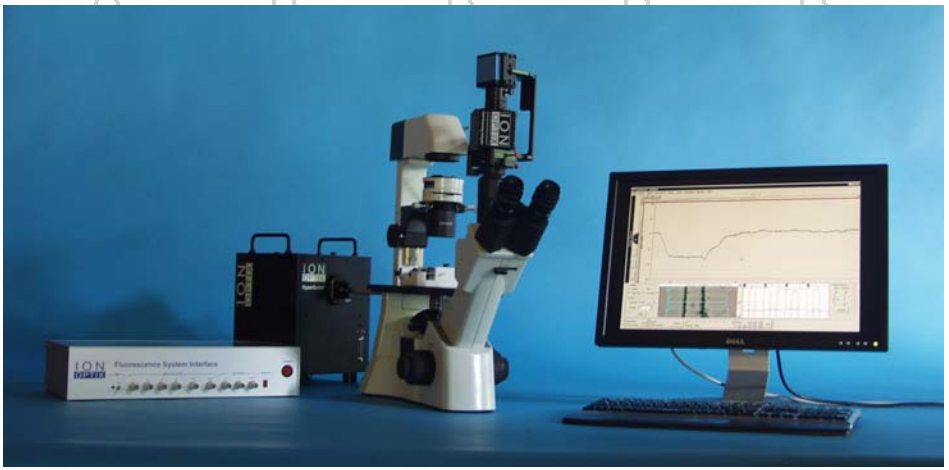
Vessel Calcium, Diameter & Flow Recording System

(HyperSwitch-Configured Vessel System)

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

- 250 ratiometric calcium measurements/second
- Up to 60 vessel diameter measurements/second (outer and luminal diameters, wall thickness, area, media/lumen ratio)
- Real-time flow characterization (vascular resistance, shear stress, Reynolds number)



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ION OPTIX

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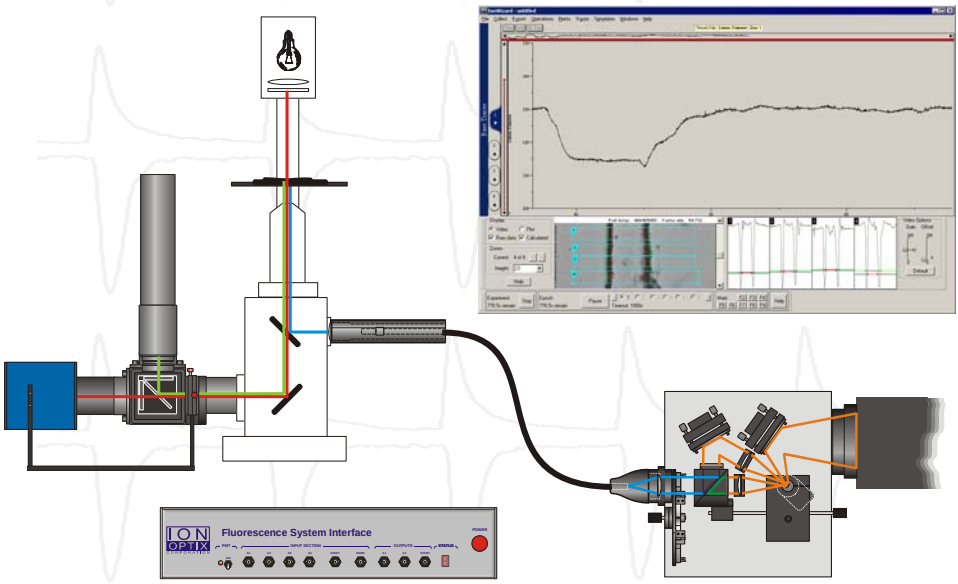
November 2011

Vessel Calcium, Diameter and Flow Recording System Overview

Interest in the physiology and pathology of the circulatory system has led to a substantial increase in the number of laboratories studying isolated and pressurized blood vessels. Although the relationship between intracellular second messengers and vascular constriction/ dilation has been an area of intense investigation for decades, elucidation of the fundamental molecular and biophysical mechanisms remains at the forefront of vascular physiology research. Impairment to the regulatory machinery governing vasodilation and vasoconstriction correlates strongly with the onset and progression of many pathologies, including cardiomyopathies. Arterial **calcium, diameter** and **flow** recordings from arterial segments offer an important physiologic measurement while also providing key insights into the processes that affect vascular health.

IonOptix developed its **Vessel Calcium, Diameter & Flow Recording System (HVSYS: HyperSwitch Vessel System)** over many years of collaboration with top vascular 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.

When coupled to your pressure myography system, HVSYS provides everything necessary for simultaneously acquiring vessel calcium and geometry data with our new IonWizard 6 software. HVSYS also includes an inverted fluorescence microscope equipped with a calcium photometry objective and brilliant optics, a digital dimensioning camera and a suite of analog and digital connections for synchronous data collection.

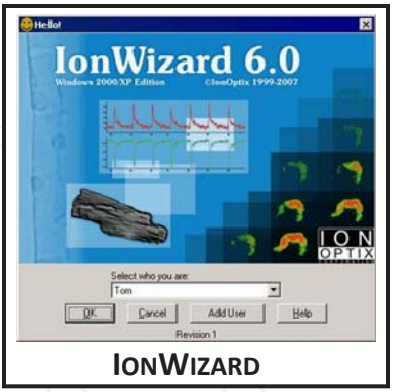


IonWizard Software Suite

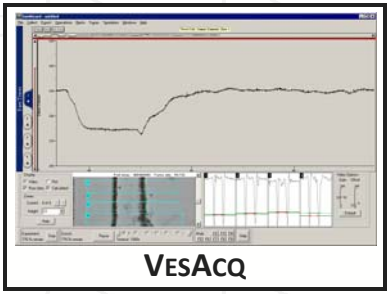
Acquisition of Vessel Calcium, Diameter & Flow

Our IonWizard core software provides completely synchronous and accurate data acquisition through the **VesAcq**, **FloAcq** and **PMTAcq** acquisition modules to record vessel geometry, flow characteristics and ratiometric fluorescence data. IonWizard 6 (IW6) supports a suite of A/D inputs and outputs as well as digital inputs. IW6 now features a signal generator function for programming voltages to drive and control external hardware (*e.g.*, myograph pressure regulators).

Our new VesAcq dimensioning software offers a simpler user-interface, more precise control and enhanced functionality. IonOptix' FloAcq acquisition and recording module provides real-time indicators of arterial flow dynamics, based on raw flow rate and inlet and outlet pressure inputs from your myography system along with dimensioning outputs from our VesAcq software. PMTAcq supports PMT-based photometry recordings as well as coordinated wavelength control for dual excitation calcium acquisition.



IONWIZARD



VESACQ

Features

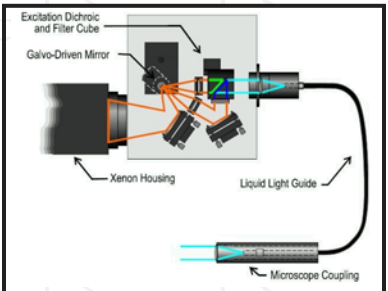
- Cell calcium levels are sampled at 1000 Hz for single excitation dyes, 250 Hz for dual excitation dyes sampled ratiometrically. Dimensioning data is recorded at rates as high as 60 Hz with our VesCam.
- Multiple 'epochs' define separate acquisition regimes. Within each epoch, independent sampling rates for diameter, photometry, and analog data are specified. Switches between different epochs occur automatically or via user intervention.
- Line averaging - unlike convention "edge detection", where contrast information is evaluated on a single line, VesAcq averages contrast over all of the lines within a user-defined region to minimize artifactual contrast (*i.e.*, remnants of blood, fat, folding, etc.), making your measurements simpler and more reliable.
- Multiple ROIs allow measurement of up to four regions along the vessel at the same time.
- Auto-gained contrast - simplifying the user interface, contrast information is automatically maximized within the contrast window to enable precise control of thresholds.
- **VesAcq outputs:**
  - Inner/outer diameter
  - Left/right/average wall thickness
  - Inner/outer/cross-sectional area
  - Media/lumen ratio
- **FloAcq outputs:**
  - **Mean pressure**
  - **Flow velocity**
  - **Vessel wall *shear stress*** - a characterization of the frictional drag exerted on arterial walls during flow. High shear stress triggers vascular dilation in order to regulate the mechanical forces exerted on arterial walls.
  - **Vascular resistance** - a definition of the force opposing the movement of solution through a vessel. A greater vascular resistance will require a greater degree of vasodilation in order to maintain constant vascular pressure.
  - **Reynolds number (Re)** - describes whether the flow is either turbulent or laminar. A large Re suggests turbulence, where nonaxial flow instabilities such as vortices result from high flow volume, high temperature, high flow velocity, branch points, etc. At low Re , where flow is dominated by viscous forces, laminar flow will move in parallel velocities throughout the lumen of the vessel.

HyperSwitch

High Speed 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 switch between excitation wavelengths with speed and precision. The IonOptix HyperSwitch uses a galvanometer-driven mirror to steer light between two light paths. With sub-millisecond switching times, the HyperSwitch offers 250 true ratios per second when driven by our IonWizard core software and the PMTACQ acquisition module. The HyperSwitch comes equipped with a Xenon arc lamp for nearly uniform light intensity in the near ultraviolet and visible spectrum. The HyperSwitch delivers fluorescence illumination to your microscope through an efficient liquid light guide and a microscope-specific adapter.

HYPERSWITCH



HYPERSWITCH LIGHT PATH

Features

- **Fast switching.** Galvanometer-based switching enables sub-millisecond switching. Complete dual excitation ratiometric collection achieved in 4 msec.
- **Xenon Arc Sub-system.** Standard arc lamp housing, igniter and power supply serves as a 75 watt source of polychromatic light. Usable wavelength range is 300-700nm and is limited only by the availability of band pass filters.
- **Light Guide.** A quartz, liquid filled light guide delivers the excitation light to the microscope providing vibration and electrical isolation and flexible position options.
- **Excitation Optics.** Excitation filters of your specification are included with each system. May be externally "flipped" out of light path for conventional fluorescence excitation. A manually controlled, 6-position filter wheel in the common excitation path provides a convenient means to select between neutral density filters (a 5-piece ND set is provided).
- **Emission Filter / Dichroic.** The appropriate emission filter and dichroic mirror and cube are provided for your microscope.
- **Cables.** Includes cables to connect to the Fluorescence System Interface.
- **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.

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. The FSI's inputs are used to collect output data from myograph in real time. 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 ±5V.

Outputs

- **Digital to Analog.** Two channels of ±5V. 12-bit D/A outputs can be configured as a variety of monitor or control signals.

Light Source Control

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

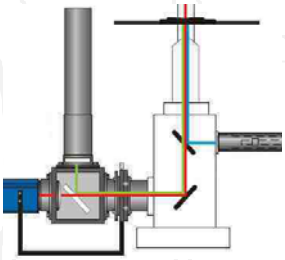
Includes

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

Fluorescence & Video Detection

Simultaneous Vessel Calcium & Diameter

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 simultaneuos vessel dimensioning with the VesCam, we equip 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 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 vessel for dimensioning. Coupled with the appropriate optical filters, our CFA, camera and PMT offer precise, simultaneous calcium and diameter measurements.



CFA, VESCAM AND PMT

PMT Sub-System

The new PMT400 sub-system offers true photon counting using an end-on bialkali 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, an optional photon-to-voltage converter (PTV100) is available that provides power to the PMT400 and a BNC analog "count" output for connection to other devices.

PMT400 W CFA

Features

- **Integrated Tube, Supply and Amplifier/Discriminator.** Single package holds photon-quality bialkali PMT tube (180-600nm, 400nm peak sensitivity), integral, high-voltage power supply preset 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 C-mount coupling directly connects to the IonOptix Cell Framing Adapter (and can hold a 25 mm filter).
- **Cable.** Integrated cable connects directly to Fluorescence System Interface. Standard extension cables allow the cable length to be increased easily.

Fluorescence & Video Detection (cont.)

VesCam: Digital Video Dimensioning

The IonOptix VesCam is a high performance yet economical solution for visualizing vessels. It's an all-digital camera that utilizes the USB 2.0 standard to remove the restrictions of analog video formats and frame grabbers. The camera utilizes a monochromatic Sony CCD progressive scanning sensor. We offer three VesCams with varying sensor sizes and resolutions to suit the needs of the investigation. Exposure times and contrast levels are adjustable to maximize the contrast of vessel features. All analog processing and digitization is done inside the camera to minimize 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.

VESCAM (LENS NOT INCLUDED)

Features

VesCam1.25

- **CCD sensor.** 1/4" sensor chip; 640 pixels wide by 480 lines (progressive); 5.6 µm X 5.6 µm pixel size.
- **Frame rates.** 60, 30, 15, 7.5, 3.75 fps.
- **Sensitivity.** 0.5 lx at 1/30s.

VesCam1.33

- **CCD sensor.** 1/3" sensor chip; 1024 pixels wide by 768 lines (progressive); 4.65 µm X 4.65 µm pixel size.
- **Frame rates.** 30, 15, 7.5, 3.75 fps.
- **Sensitivity.** 0.5 lx at 1/15s.

VesCam1.5

- **CCD sensor.** 1/2" sensor chip; 1280 pixels wide by 960 lines (progressive); 4.65 µm X 4.65 µm pixel size.
- **Frame rates.** 15, 7.5, 3.75 fps.
- **Sensitivity.** 0.5 lx at 1/7.5s.

All models

- Programmable CCD gain.
- Programmable integration time to stop fast movement or increase camera sensitivity.
- Single USB 2.0 cable to camera for power and data transmission.
- **Independent software.** Complete video controls for imaging acquisition included. Can be used to snap single images or generate movies.

Fluorescence & Video Detection (cont.)

Cell Framing Adapter

The Cell Framing Adapter is used to simplify and optimize fluorescence recording using a PMT. An adjustable iris is used to frame a rectangular area of the microscope field of view maximizing selected regional 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.

CFA300 W ADAPTER

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 alignment.

Options

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

Vessel Fluorescence Microscope

Motic AE31 & Olympus UIS2 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.

MOTIC AE31 MICROSCOPE

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 20X or UPLSAPO 10X

- **UV Transmission.** High transmission of 340nm light; ideal for Fura-2 based Ca^{2+} photometry.
- **10X or 20X.** Ideal magnification for simultaneously dimensioning vessel diameter.
- **High NA.** Non-immersion lens with excellent transmission and collection of light.

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 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)
- Vessel Calcium and Diameter Recording System

Tissue Bath Fluorometry

- FluoroPlex
- FluoroHeart

General Photometry

- Fluorescence Photometry System

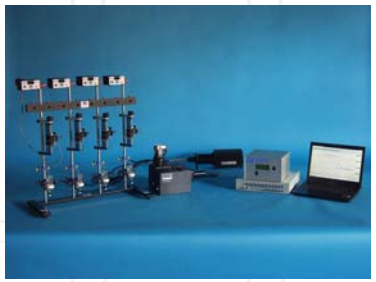
Cell Pacing

- Cell Culture Pacing

Myocyte Harvesting

- Myocyte Harvesting System

CA & CONT



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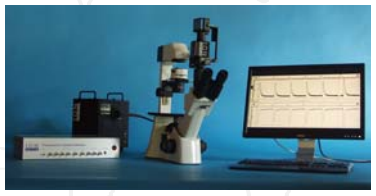

FL PHOTOMETRY

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

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.

Bioprobes Ltd.

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www.bioprobeschina.com

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