



HORIBA



# EasyRatioPro



# Fluorescence Imaging

Explore the future Automotive Test Systems | Process & Environmental | Medical | Semiconductor | Scientific



## PTI gets back to its roots, offering a completely new revolutionary user interface for the collection and analysis of ratiometric imaging data for calcium, pH, and intracellular ion imaging!

PTI has been a leader in the field of quantitative fluorescence imaging for over twenty years. With the EasyRatioPro, we are introducing a bold advance in the field of microscope imaging by offering the most comprehensive high-speed multi-wavelength imaging system of its kind. It offers imaging researchers superb quality and efficiency through one intuitive, integrated production environment. In addition, it revolutionizes the imaging arena by offering a unique hands-on user interface for unparalleled control. This high-resolution system embodies the latest PTI innovations by incorporating cutting-edge technology to deliver unprecedented image quality. Highlights of the EasyRatioPro include direct streaming to hardrive, new high-resolution camera support and peripheral options, abundant wavelength channels and I/O capacity, extensive flexibility, and much more. EasyRatioPro gives you control in imaging like never before that is complementary to photometry options.

## What's in the Box

- DeltaRAM X<sup>™</sup> monochromator
- 2 meter liquid light guide
- Labmaster PCI interface & I/O
- EasyRatioPro software
- Warp Drive Imaging<sup>™</sup> human user interface
- Imaging workstation with 19" flat panel monitor
- Installation and training

EasyRatioPro allows researchers to reclaim control of their imaging research by reducing the learning curve associated with complex general purpose imaging platforms.

## Liquid Light Guide Adapter For All Common Fluorescence Microscopes

- Leica
- Zeiss
- Nikon
- Olympus
- Both inverted and upright configurations
- Epi or direct coupling





## Warp Drive Imaging<sup>™</sup> Features

- Touch sensitive motorized faders
- 60 function keys
- Full size numeric keypad
- Professional transport controls
- LCD and LED displays
- USB and I expansion slot for other optional interfaces

The Warp Drive Imaging<sup>™</sup> is directly linked to the software, so whether you change parameters with the Warp Drive or directly in the software, they will communicate and both will represent the current settings.



Select buttons Record buttons Solo buttons

Mute buttons

Channel exposure

Time faders

## EasyRatioPro Hardware Features

#### Warp Drive Imaging<sup>™</sup> Interface

The PTI Warp Drive is an external human interface device that serves as a control board to complement your EasyRatioPro system. It is geared toward those who prefer the familiar tactile interaction with its innovative and intuitive methods of manually manipulating while visually monitoring your sessions. With typical imaging platforms, your screen is cluttered with multiple windows. The Warp Drive reduces the clutter on-screen by eliminating many of these previously necessary windows. Through traditional controls, the PTI Warp Drive also serves to dramatically accelerate your workflow by freeing you from the limitations of mouse-and-keyboard operation.

The interface device also increases productivity through its many other innovative features. Introducing real-time control of up to 60 programmable function keys. The touch sensitive, motorized faders can control exposure time, binning, gain, and wavelength selection of up to 16 different channels. Optimize your application on the fly!



This space-efficient, full-featured control interface option is suited well for multi-room research facilities. In space challenged or cramped microscopy labs, the small footprint of the Warp Drive interface is appreciable. In addition, its ease of use makes it a great asset to any core facility with its small learning curve.





## EasyRatioPro Hardware Features

#### DeltaRam X<sup>™</sup> Random Access Monochromator

PTI's innovative DeltaRAM X<sup>TM</sup> represents the next bold step in the evolution of light sources. The compact, patented single monochromator design permits the selection of any wavelength in two milliseconds or less. This means it can perform up to 250 ratios per second! It is ideally suited for multi-wavelength applications as well as excitation scanning. The combination of the DeltaRAM's tuneability with two bilateral slits sanction the ultimate in bandpass and throughput flexibility. This allows the user to choose any bandpass desired from 0–24 nm to maximize the dynamic range of ratiometric dyes knowing that filters have a fixed band pass. The DeltaRAM X<sup>TM</sup> features a computer controlled shutter to prevent photobleaching for photosensitive samples. An essential feature of the DeltaRAM X<sup>TM</sup> is that it delivers powerful excitation wavelengths from 250–650 nm under synch-lock computer control. Synch–lock control locks the DeltaRAM X<sup>TM</sup> to the detector's exposure time or camera frame readout.

#### Lamp and Power Supply

PTI lamp power supplies are highly regulated DC units that provide very stable power for your choice of xenon, mercury, or combination mercury-xenon short arc lamps as well as tungstenhalogen lamps. Every imaging system comes standard with a 75 Watt xenon source coupled to the DeltaRAM X<sup>TM</sup>. This delivers the ultimate in application flexibility by providing a broadband source from 200 nm to 2 microns that the DeltaRAM X<sup>TM</sup> monochromator then precisely separates into monochromatic light to meet your requirements.



#### Liquid Light Guides and Microscope adapters

The EasyRatioPro system features a two-meter Liquid Light Guide (LLG) to deliver light to your sample. Typically, a fiber is used for this purpose. However, due to its gel matrix consistency, the flexible LLG delivers 30% more light, and is not susceptible to dead spots or hot spots like traditional fibers. It can be coupled to your choice of any commercially available fluorescence microscope with an Epi or direct port. The microscope adapters we provide are customized to match each microscope for light efficiency and a homogenous sample illumination.

## DeltaRAM X<sup>™</sup> Specifications

- Excitation wavelength range: 250–650 nm
- Wavelength selection speed: < 2 milliseconds
- Wavelength bandwidth: adjustable from 0–24 nm
- Two meter Liquid Light Guide and adapter for user specified or supplied fluorescence microscope

## Power Supply and Lamp Specifications

- 75 Watt compact arc xenon bulb
- 200 nm to 2 microns
- Thermally-matched front surface ellipsoidal reflector for 70% efficiency
- No cooling or ozone venting required

## Liquid Light Guide Specifications

- 2 mm core
- 2 meters long
- Light transmission from 300–650 nm









## Supported CCD Cameras

Photometrics Cameras

- Cascade II
- Cascade 512 & 512B
- Cascade IK
- Coolsnap HQ & HQ2
- Coolsnap ES
- Coolsnap EZ
- Coolsnap CF
- QuantEM:512SC
- Evolve 512SC

## Andor Cameras

- iXon series
- Andor Neo
- Zyla sCMOS

#### Hamamatsu Cameras

- ORCA 2
- 9100 series
- Hamamatsu Orca Flash 4.0

#### PCO Cameras

## EasyRatioPro Hardware Features

#### Choose the ideal camera for your application

PTI has selected a range of high performance cameras to complete your EasyRatioPro highspeed imaging system. You may choose a camera from PTI's own CoolOne series that utilize the latest in CCD and digitization technology. In addition, the EasyRatioPro supports other lines of cameras. So whatever your performance or budget requirements, a camera is available to meet all of your research needs.

## Electron Multiplier CCD Cameras

The principal difference between a charge multiplying CCD and a traditional CCD is the presence of a special extended serial register called the multiplication register. This signal boosting process occurs before the charge reaches the readout amplifier, effectively reducing the CCD readnoise by the on-chip multiplication gain factor, which can be greater than 1000x. The main benefit of the technology is a far better signal to noise ratio for signals below the CCD readnoise floor. EMCCDs show a similar sensitivity to Intensified CCDs (ICCDs), however they are much less expensive. The great thing about EM technology is that it combines the high speed and resolution of traditional CCDs, and the low light level detection of an ICCD—all without sacrificing resolution and without the high cost of an ICCD.

> Applications where EMCCDs are advantageous include: Single molecule fluorescence High-speed calcium imaging Live cell GFP imaging And many more...

#### Traditional CCD Cameras

CCDs have high quantum efficiency across the visible spectrum and into the NIR. They are an excellent choice for monochrome imaging and offer exceptional resolution and performance for their value. There is a wide variety of CCD formats available with TE cooling down as low as -80°C and a selection of readout speeds. EasyRatioPro can couple to whichever camera is right for you.

Select one of these cameras for the following applications:

Live-cell imaging High-speed emission ratio imaging Low-copy gene analysis and gene expression profiling Quantitative FRET, FRAP, FISH Luminescence And many more..



PCO.EDGE (all models)



## About the Software

From fixed cell preparations to dynamic events, EasyRatioPro software represents the ultimate expandable platform for professional live cell imaging applications. There is no other system that delivers the superb quality, flexibility, and creativity options of the EasyRatioPro software.

## Data Handling

PTI's Acquisition Engine (PAE) streams imaging data to hard disk for acquisition and playback. This gives the user the benefit of running experiments without worrying about the internal RAM limitation of the computer. You can high-speed stream up to terabytes of hard disk storage. Competitive systems can only offer RAM based high speed streaming, limited to 2 GB of RAM.

## Data Integrity

PTI knows how valuable your data is, which is why we use non-destructive image processing. Processing plug-ins can be applied during record or playback mode. The raw data integrity is kept, allowing the researcher to experiment with threshold masking to enhance the image data with peace of mind that their data remains in its original form on the hard drive.

#### Future Expansion

EasyRatioPro software also allows for the maximum flexibility for your future needs with our modular software architecture. Plug-ins can be added to the system to increase functionality. This feature allows you to get started inexpensively and then add functionality later as your research grows.

## Other Features of the Software:

- Square, ellipse, freehand, and linear profile drawn area of interest photometry in real-time or in post acquisition.
- Trace math analysis functions such as anti-log, average, combine, XY combine, differentiate, integrate, and linear fit, peak finder.
- Real-time generation of user defined event markers and event journaling.
- Real-time non-destructive plugins for ratio-metric calculation, thresholding, and background subtraction.



EasyRatioPro software represents the ultimate workhorse platform for professional live cell imaging by setting the standard for reliability, efficiency, and integration with a multi-wavelength illuminator.





## Other Features of the Software:

- Export of image data to popular formats such as .avi, .bmp, .jpg, .tif, and .png.
- Control of up to 16 excitation, emission, or derived channels.
- "Warp Drive" human use interface control for dedicated control over exposure, gain, electron multiplier, mute, solo, select, record, window functions, jog/shuttle, play, rewind, fast forward, stop, time code, looping and much more.

#### Peace Of Mind

An experiment session file keeps track of all imaging data on the hard disk RAID or server, allowing you to never worry about where you saved your image data. The session keeps track of all image data, region of interest data, wavelength, channels, exposure times, and all vital controls automatically with each image collected.

#### Day To Day Routines

Session templates for common experiments are provided in addition, you can create your own application templates. Ready to run common experiments "out of the box" with minimal input from the user. Just push record and go!

#### EasyRatioPro Plug-Ins

Plug-Ins are special purpose software components that provide additional image processing functionality to EasyRatioPro. The plug-ins available for the EasyRatioPro provide a comprehensive set of digital image processing functions that include Background Subtract, Fast Image Math, Inverse, Math Operations, ROI Calculation and Threshold. The number and variety of plug-ins that you can use simultaneously in a session are limited by the amount of processing power available on the CPU. The more powerful your computer, the greater the number and variety of plug-ins that you can use simultaneously. Due to this dependence on the CPU or host processing, the more plug-ins you use concurrently in a session, the greater the impact it will have on other aspects of your system's performance such as the acquisition speed, playback speed, number of channels and processing latency.



Color Composite Plug-In



#### Calibration Plug-In



Formula: Y = pH = ( pKa • Beta ) + Log (

X - Rmin

Rniax - X

#### You Need An EasyRatioPro If:

Collection and analysis of ratiometric imaging data for calcium, pH, and intracellular ion imaging are important to you. PTI has poured all of our 20 years of ratiometric imaging expertise into this new package. The modular package is easily expandable and offers plug-ins for different applications. It allows researchers gain back control of their research by reducing the learning curve associated with complex general purpose imaging platforms. This allows users to focus on their research applications rather than get tied up in learning a complex imaging system.



## EasyRatioPro Is Up For The Task Of Many Applications

As a scientist, you don't want to make the wrong decision when buying an imaging system. Many systems on the market claim to be able to do all fluorescence imaging applications. However, this is simply not true. Other systems are complex and offer so many options that will never even be used for ion applications. This clutter can be confusing. EasyRatioPro has been designed for ion imaging applications and it is finely tuned for kinetic imaging. Some applications are listed below. Simply do a Google™ Scholar search for Photon Technology, and you will find thousands of applications where our fluorescence products have been used.

## In-Situ Calibration of Intracellular [Ca\*+],



(This figure Copyright © 1993 The American Physiological Society. Used by permission.)

## In-Situ Calibration of Intracellular [Na<sup>+</sup>]



Harootunian A.T. Kao J.P.Y. Eckert B.K. and Tsien R.Y., J. Biol. Chem. 1989, 264, 19458-19467

## **Applications**

#### Calcium Measurement

- Intracellular free Ca<sup>2+</sup>
- Mitochondrial Ca<sup>2+</sup>
- Endoplasmic reticulum (ER) Ca<sup>2+</sup> pool
- Lysosomal Ca<sup>2+</sup>
- Extracellular near membrane Ca<sup>2+</sup>

#### Other lons

- Intracellular Mg<sup>2+</sup>
- Intracellular Mn<sup>2+</sup>
- Intracellular Zn<sup>2+</sup>
- Intracellular Sr<sup>2+</sup>
- Intracellular Na<sup>+</sup>
- Intracellular Cl<sup>-</sup>
- Cytosolic labile iron pool (LIP): Iron homeostasis

## Membrane Potential

- Plasma membrane Potential
- Mitochondrial Membrane Potential *pH* 
  - Intracellular pH
  - Vacuolar pH
- Lysosomal pH
- GFP & Variants
  - Identifying cells coexpressing GFP for analysis
  - Use mitoGFP to visualize mitochondria and measure mitochondrial calcium [Ca<sup>2+</sup>]m
  - Use ratiometric GFP (redox-GFP) to measure cellular/mitochondria reduce/oxidize reaction
  - HEK-293 cells transfected with the CaSR-pEGFP vector
  - ECFP
  - EYFP
- And Many More!



## Applications

#### FRET

- Donor Excitation Spectroscopy
- Emission intensity
- Donor emission intensity
- Acceptor emission
  intensity
- Ratio of donor/acceptor emission intensity
- Measure molecular proximity
- Binding of enzyme to its substrate
- Measure Endoplasmic reticulum (ER) Ca<sup>2+</sup>

## Other Measurements

- Calcium imaging of Single HEK 293 cell, Taste Bud, Pea Pollen tubes
- Respiratory burst & reactive oxygen species (ROS)
- NAD(P)H
- · Changes in CaM availability
- Demonstration of selective plasma membrane permeabilization by digitonin in HEK293 cells
- Detection by immunofluorescence of PfCRT expression in HEK293 cells
- Receptor-mediated Glutamate release (by following NADH signal)
- Fura-dextra
- Cellular GSH level
- FL-aldosterone efflux
- Latency of response to exogenous ATP or ADP.
- Membrane traffic
- Liposome
- ER membrane
- Cell contractile activity & cell Size
- Cell Volume Measurement
- Phagocytosis
- Cell injury or death by Pl fluorescent
- Metal induced cytotoxity And Many More!

## More Applications

## In-Situ Calibration of Intracellular pH,



Propagated Cytoplasmic Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>cv</sub>) Responses Along a Fluo-4-loaded DVR Wall



Zhang, Q L, et al. Am. J. Physiol. Regul. Intgr. Comp. Physiol. 291:R1688-R1699 2006

## Emission ratio of indo-1 loaded BAECs



A



Changes in CaM availability in BAECs (A) also expressing BSCaM2 (B) Time courses of free Ca<sup>2+</sup>- CaM

Tran, Q, et al. JBC 278 (27): 24247-24250, 2003

В

## Fluorescent Zn<sup>2+</sup> detection in MIN6 cells

Fluorescent Zn<sup>2+</sup> detection in MIN6 cells labeled with FluoZin-3. A, DIC image of MIN6 cell. B, fluorescent image of the same cell incubated with 1 mM glucose before Zn<sup>2+</sup> treatment. C, 10  $\mu$ M Zn<sup>2+</sup> was added to incubation medium, and the image was acquired after incubation for 30 min. D, 250  $\mu$ M KATP channel inhibitor tolbutamide was added to C, and the image was acquired after 15 min.



Gyulkhandanyan, A, et al. JBC 281(14): 9361-9372, 2006



## More Applications

## Intracellular Calcium

Fura-2 imaging for  $[Ca^{2+}]_i$  in hippocampal neurons from old rats before (A) and after NMDA (B)



Brewer, G, et al. Brain Research, 921(1-2): 1-11, 2001.

## Ratio Imaging of GFP expression



Bezprozvanny, I, et al. Neuron, 39 (7): 227-239, 2003.

#### Regenerative Calcium Oscillations



Pessah, I, et al. Biophys J, 79 (5): 2509-2525, 2000.

Rat Medium Spiny Neuron S2 cells loaded with Fura-2.

Extracellular near-membrane Ca2+



Hofer, A. M, et, al. J Cell Sci, 116(pt 8): 1527-38, 2003.

## More Applications

## Calcium-related Measurement

- Ca<sup>2+</sup> Waves
  - Ca<sup>2+</sup> wave quantitation Ca<sup>2+</sup> wave induced by mechanical stimulation Ca<sup>2+</sup> wave induced by mechanical wounding Ca<sup>2+</sup> wave propagation in non-contacting cells over a physical gap
- Ca<sup>2+</sup> Oscillation induced by: Hg<sup>2+</sup> K<sup>+</sup> Heat ATP Dopamine FSH
  - Caffeine
- Ca<sup>2+</sup> mobilization
- Ca<sup>2+</sup> homeostasis
- Propagated cytoplasmic Ca<sup>2+</sup>cyt responses along the DVR wall.
- Simultaneous Measurement of intracellular Ca<sup>2+</sup> and: Cell volume Phygocytosis Cl<sup>-</sup> Oxidase activation Ca<sup>2+</sup>-CaM

And Many More!

Accessories



## **Optional Accessories**

No system would be complete without the ability to add options and accessories. PTI is a onestop shop for upgrades, add-ons and accessories to complement your imaging system.

## Dual C-Port Adapter with Flipping Mirror

The IM-9 is a Dual C-port adapter for your microscope with a flipping mirror allowing the user to select between two different emission detection devices. It is primarily used to mount two optical detectors: one output for a photometer, and another output for a camera. Both ports are identical. The flipping mirror allows easy and rapid switch between the two detectors without hardware change. This is good for sequential but not simultaneous, measurement. It is very useful to retrofit old fluorescence scopes. It attaches directly to the C-mount on a microscope, it provides one input and two standard C-Port outputs.

## Single Channel Photometer

A high sensitivity PMT detector add-on to your imaging system. It is ideal to measure very fast or ultra low light levels. The photometry subsystem includes a photometer with high sensitivity analog or photon counting detection, electronics, and FelixGX software package and ASOC interface. It may require a IM-9 dual C-port adapter, in order to accommodate both the photometer and camera.

- Photon counting mode: for low signal, as in most ratio fluorescence measurement.
- Analog modes: for high signal situations
- Detector wavelengths: UV- NIR with appropriate PMT
- Precise field-of-view control
- A viewing eyepiece for Parfocal viewing.
- An adjustable aperture for selecting the area of interest.
- Easy to switch from 'view' to 'measure' via a flipping mirror.
- Connects to your microscope on any of the available ports via a C-mount adapter.

## Dual Channel Photometer

All of the properties of a single channel photometer, plus it allows for simultaneous detection of two emission wavelengths by means of two independent PMT detectors. This option has a provision for a beam splitter and a dichroic cube for emission wavelength selection. The dichroic assembly is placed within the photometer for selection of the two emission wavelength ranges. Provides high-speed (millisecond) detection for emission-shifted probes with data acquisition rates of up to 1000 ratios per second.

## Dual View Image splitter module

The IM-5B adds popular dual Image splitter modules to your EasyRatioPro system. This allows for emission ratio and FRET imaging applications like Indo-1, JC-1 membrane potential or CFP/ YFP FRET.

#### Fluorescence Lifetime Upgrades

A pulsed laser or a laser diode excitation source and a gated detector can be added to your microscope to measure fluorescence and phosphorescence lifetimes. FelixGX advanced analysis software is used for curve fitting the decay data.





## A Complete Line of Fluorescence Spectroscopy Instruments from PTI



Explore the future