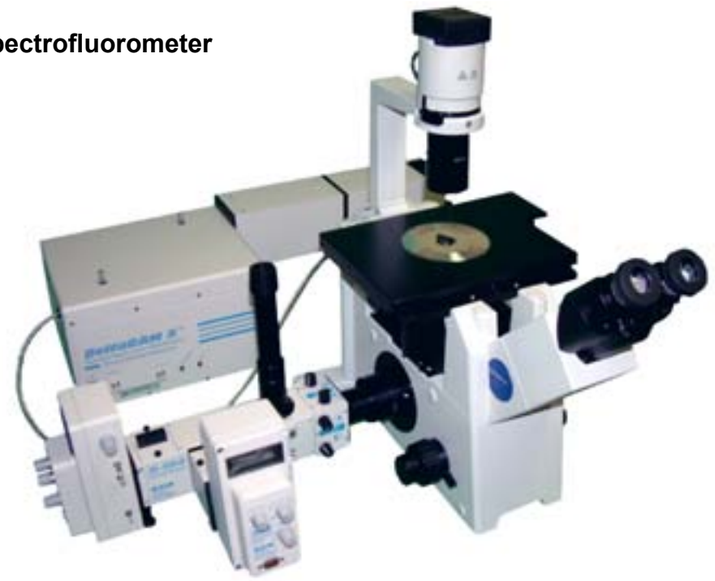
RatioMaster

Microscope-based ratio spectrofluorometer



PTI's time tested RatioMaster™, offering researchers solid, dependable, sensitive detection for the collection and analysis of ratiometric photometry data for calcium, pH, and intracellular ion imaging!

The RatioMaster™ is a microscope-based ratio spectrofluorometer capable of dynamic ratio fluorescence measurements on a millisecond timescale. A xenon arc lamp provides high intensity, continuous broadband illumination. Alternating excitation wavelengths are selected by a computer-controlled high-speed random access monochromator coupled to a inverted fluorescence microscope with a liquid light guide. Emitted light is collected from the sample and passed through a photometer with a bilateral translatable iris and a viewing eyepiece to a switchable analog/photon counting photomultiplier detector. Analog detection is used when emitted light is relatively intense while low light levels are detected by photon counting. All system functions are under computer control. Data is collected and analyzed by proprietary Windows™ based advanced fluorescence software.

What's in the Box

- DeltaRam X™ monochromator
- 2 meter liquid light guide
- D104 microscope photometer
- BryteBox™ acquisition hardware
- FeliX32™ software
- Installation and training

The RatioMaster™ is the the most sensitive solution if you are looking at a single region of intrest

Liquid Light Guide Adapter For All Common Fluorescence Microscopes

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

EasyRatioPro Hardware Features

DeltaRAM X™ Random Access Monochromator

PTI's innovative DeltaRAM X™ 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™ features a computer controlled shutter to prevent photobleaching for photosensitive samples. An essential feature of the DeltaRAM X™ is that it delivers powerful excitation wavelengths from 250 - 650 nm under synch-lock computer control. Synch–lock control locks the DeltaRAM X™ 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 tungsten-halogen lamps. Every RatioMaster™ system comes standard with a 75-Watt xenon source coupled to the DeltaRAM X™. This delivers the ultimate in application flexibility by providing a broadband source from 200 nm to 2 microns that the DeltaRAM X™ monochromator then precisely separates into monochromatic light to meet your requirements.

Liquid Light Guide and Microscope Adapter

The RatioMaster™ 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™ Specifications

- Excitation wavelength range: 330 - 650 nm
- Wavelength selection speed: < 2 milliseconds
- Wavelength bandwidth adjustable from 0 - 24nm
- Two meter Liquid Light Guide and adapter for a 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 300-900 nm

Hardware

RatioMaster™ Photometer

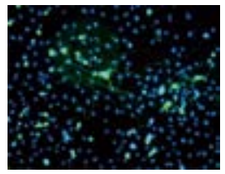
PTI's proprietary D-104 photometer, featuring an analog and digital PMT readout. The D-104 photometer incorporates an eyepiece to visually observe the cells on the microscope stage. By adjusting a pair of vertical and horizontal apertures in the field of view, a small number of cells, a single cell, or even a portion of a cell can be isolated for measurements. An optional camera mount can replace the eyepiece for digital visualization. After identifying the area of interest you wish to measure, simply toggle the built-in flipping mirror from 'view' to 'measure' to direct the emission from the microscope through an interchangeable filter to the photomultiplier tube for photon counting or analog detection.

For dual-emission probes, PTI offers a dual detection solution by mounting a second detector to the D-104 photometer. A dichroic beam splitter is placed in the photometer to direct the appropriate emission wavelengths to each detector for simultaneous dual detection readout.

This detection system excels at applications such as the following:
• Live-cell imaging
• High-speed emission ratio imaging
• Quantitative FRET, FRAP, FISH
• Luminescence
• Electrophysiology
• Many more…

Specifications

• Universal C-mount adapter
• Analog and digital PMT
• LCD readout display
• 1 inch filter holder
• Horizontal and vertical aperature adjustments



Optional Dual Channel detection for emission shifted dyes!

About The Detection

Your application needs may change on a daily basis. PTI recognizes this fact and that is why we offer our standard detection system with both analog and digital detection. Digital, or photon counting, is the most sensitive as individual photon events are recorded. This type of detection is mainly used in low light level applications. Analog counting monitors the current that is being produced through the dynodes of the PMT, and thus is capable of higher intensity recordings than in digital mode. Analog detection is used mainly in high light level applications.

About the Software From fixed cell preparations to dynamic events, FeliX32™ fluorescence software represents the ultimate, expandable platform for professional live cell single region of interest applications. There is no other system that delivers the sensitivity, flexibility, and capability of options as the FeliX32™ software platform.

Smart Electronics PTI's BryteBox™ data acquisition computer streams data to the host computer for acquisition. This gives the user the benefit of running experiments without worrying about host computer interruptions or the limitation of the computer. You can stream high speed data and control TTL inputs and outputs with minimal latency. Competitive systems offer add-in cards or USB modules that become outdated fast, due to the ever changing computers. Not a problem with PTI's BryteBox™ built-in intelligent interface.

Data Integrity PTI knows how valuable your data is, which is why we use a database to archive and store experimental data. The raw data integrity is kept, allowing the researcher to manipulate data with peace of mind that their data remains in its original form on the hard drive.

Open Architecture FeliX32™ software also allows for maximum flexibility for your future needs with our modular software architecture. Components can be added to the system to increase functionality. This feature allows researchers to get started inexpensively and then add functionality later as their research grows.

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

Modular Design If the out of the box acquisition protocols do not quite fit your applications, the PTI Macro editor allows you to create your own specialized experiments by a drag and drop command library into a loop logic. This means you do not have to be a programmer or understand a scripting language to implement user defined macros. You simply select the function you want and then apply it to the loop. This allows the researcher to increment the start excitation wavelength, emission wavelength, number of points per second, increase temperature, add solutions with a titrator and a host of other acquisition modes. The Macro editor is simply one of the easiest to use ever designed by PTI!

Software

• Universal C-mount adapter
• Analog and digital PMT
• LCD readout display
• 1 inch filter holder
• Horizontal and vertical aperature adjustments

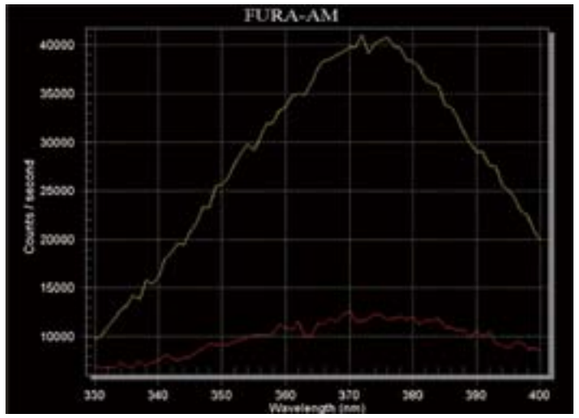
Other Features of the Software:

- Square, 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, linear fit, and peak finder.
- Real-time generation of user defined event markers and event journaling.
- Real-time non-destructive ratiometric calculation, and background subtraction.
- Export of data to popular formats such as .ana, .ang, .spc or text files.
- Control of up to 10 excitation, emission channels
- Unlimited derived channels.

Applications

RatioMaster™ Is Up For The Task Of Many Applications
Excitation spectrum of Fura-AM

Complete hydrolysis of the membrane permeable form of Fura-2 (Fura-2/AM) to Fura-2 is critical for accurate measurement. Fura-2/AM has a different excitation spectrum compared with Fura-2. The scanning property of our monochromator makes it easy to check the status of Fura-2/AM hydrolysis.

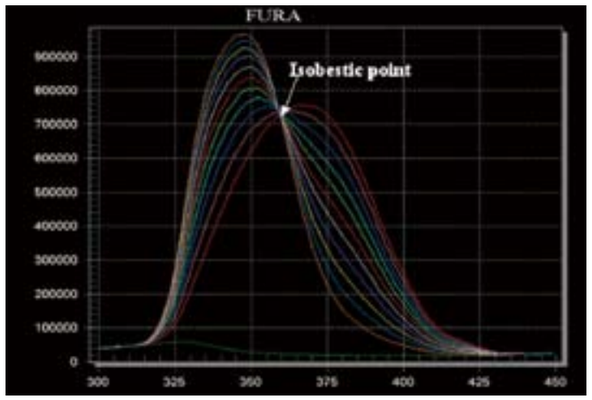


Frequently Used Fluorescence Dyes

Excitation-Shifted	
Application	Dye
Calcium	Fura-2, Fura PE3 FFP1 8 Quin-2 Fura-Red
Sodium	SBFI BCECF HPTS SNAFL® DM-NERF® CI-NERF®
Magnesium	Mag-Fura-2 Mag-Fura-5
Membrane Potential	Di-4-ANEPPS Di-8-ANEPPS

Fura-2 Titration

The fluorescence excitation spectrum of Fura-2 shifts to the lower wavelength upon Ca++ binding. This is a major characteristic of an ion-specific ratio fluorescent dye.



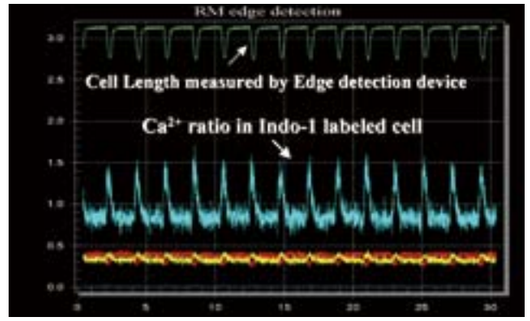
Emission-Shifted	
Application	Dye
Calcium	Indo-1
pH	SNARF-1® SNARF-2®
Magnesium	Mag-Indo-1
Membrane Fluidity	Pyrene Fas PATMAN
Free Fatty Acids	ADIFABTM
Membrane	JC-1
Lipids, phoholipids	Nile Red
Nucleic acids	Acridine Orange

Applications

Simultaneous measurement of Ca2+and myocyte cell length with FeliX32™

The above figure shows data traces from the simultaneous collection of fluorescence and cell length from a cardio myocyte. Myocyte was loaded with Fura2 and excited by alternating 340 nm and 380 nm wavelengths using the RatioMaster™ system. The blue trace shows the calcium ratio increase and decrease with the cell contraction. The contraction data (green trace) can be correlated with sample accuracy with the fluorescence data since both signals were collect simultaneously.

Non-Ratiometric	
Application	Dye
Calcium	Fluo-3 Calcium Green™ Calcium Orange™ Calcium Crimson™ Rhod-2
Chloride	SPQ SPA MQAE
HIV protease	HIV protein substrate
F-actin	phallotoxins
PKC	Fim-1 Rim-1

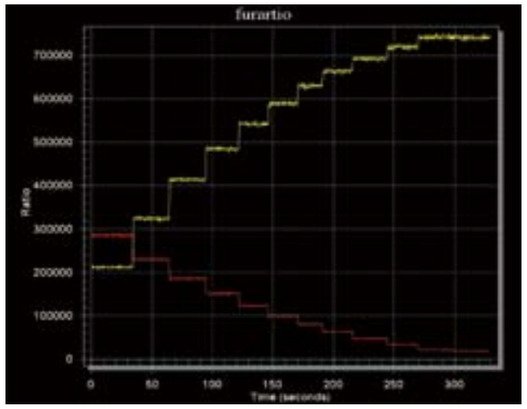


Excitation-Shifted	
Application	Dye
Nucleic acids	DAPI and FITC FITC and APC Fura Red™ and BCECF FITC and Texas Red® DAPI and PE

And Many More!

In-Situ Calibration of Intracellular [Ca2+]i

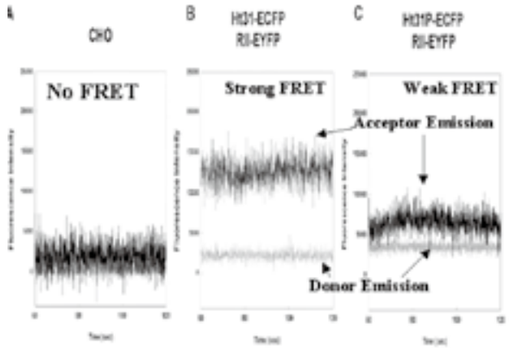
By measuring the ratio of fluorescent intensity at 340/380 nm, [Ca2+]i can be measured over several orders of magnitude and with a high degree of precision.



More Applications

FRET for Binding Assay

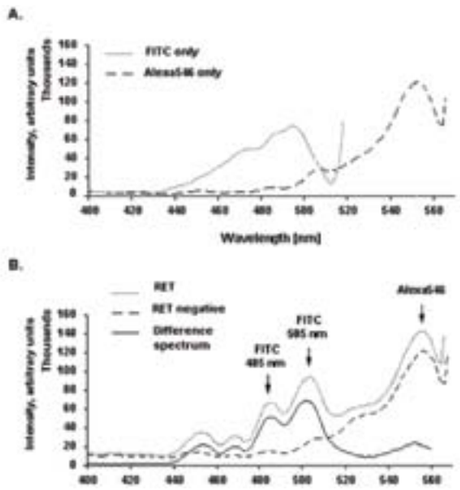
FRET occurs when the EYFP fusing RII protein kinase binds to the ECFP fusing Ht31 protein (kinase anchoring protein). The results of this FRET: the donor (ECFP) emission fluorescence decreases. The acceptor (EYFP) emission fluorescence increases. Instrument: PTI's FRET RatioMaster™ Pro.



Reuhr, M. et al. JBC, 274(46): 33092

FRET for molecular proximity assay

FRET occurs because the seprase and the plasminogen activator receptor co-localize on the membrane of malignant melanoma cells. FRET was detected via a fluorescently labeled antibody targeted to each receptor. The results of this FRET: the acceptor's excitation spectrum gains spectral features of the donor's excitation spectrum.



Artym, V, et al. Carcinogenesis 23 (10), 1593

Common FRET Pairs

EGFP/DSRed2 FRET
CFP/DsRed2 FRET
BFP/DsRed2 FRET
CoralHue M-Cyan/K-Orange
GFP/Rhod2
FITC/CY3 FRET
FITC/TRITC FRET
YFP/TRITC FRET
CY3/CY5 FRET
CFP/YEP FRET
BFP/GFP FRET

Applications

Calcium Measurement
- Intracellular free Ca^{2+}
- Mitochondrial Ca^{2+}
- Endoplasmic reticulum (ER) Ca^{2+} pool
- Lysosomal Ca^{2+}
- Extracellular near membrane Ca^{2+}

Simultaneous Measurement of intracellular Ca^{2+} and:
- Cell volume
- Cell contraction: Edge detection device
- Membrane current: Patch Clamps
- Phygocytosis
- Cl^-
- Oxidase activation
- Ca^{2+}-CaM

Membrane Potential
- Plasma membrane Potential
- Mitochondrial Membrane Potential

pH
- Intracellular pH
- Phagosomal pH
- Vacuolar pH
- Lysosomal pH

And Many More!

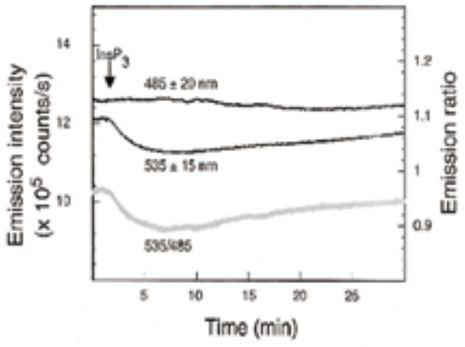
RatioMaster Ratiometric Spectrofluorometer

More Applications

FRET ER Ca^{2+}

FRET occurs when ER Ca^{2+} binds to the "chameleon 3er", causing it to fold, thereby bringing the two dyes in close proximity. The results of the FRET:

- The donor (CFP) emission fluorescence decreases
- The acceptor (YFP) emission fluorescence increases
- The emission ratio of Acceptor/Donor increases
- InsP3 triggers the release of ER Ca^{2+}, thus decrease the level of FRET

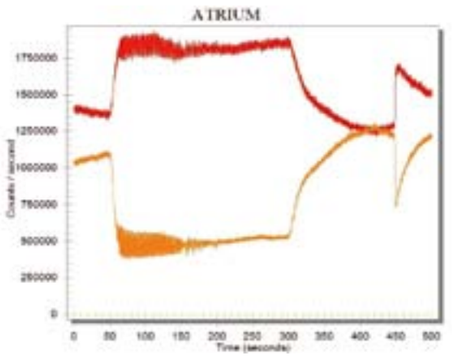


Use ER-targeted FRET indicator, chameleon 3er with CFP at one end and YFP at another end.

Xu, W, et al. JBC, 275 (47), 36676

Sample: freshly isolated rabbit atrium.

Stimulated with 90 mM KCl , nicardipine was added (2 uM) returned to normal Tyrode medium, followed by 10 mM caffeine.



Kim Sung Joon (Seoul Nat'l University)
(Dept. Physiology and Biophysics)

Applications

Calcium-related Measurement
- Ca^{2+} transients
- Ca^{2+} mobilization
- Ca^{2+} homeostasis
- Ca^{2+} waves
- Ca^{2+} oscillation
- Ca^{2+} spikes
- Ca^{2+} entry
- Capacitative Calcium Entry (CCE)

Calcium-related Measurement
- Intracellular Mg^{2+}
- Intracellular Mn^{2+}
- Intracellular Zn^{2+}
- Intracellular Sr^{2+}
- Intracellular Na^+
- Intracellular Cl^-
- Intracellular Ba^{2+}
- Divalent cation influx
- Labile iron pool (LIP)
- Iron homeostasis
- Na^+/H^+ exchange
- H^+/HCO^{3-} exchange
- P_f and PCO_2

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

Other Measurements
- Respiratory burst & reactive oxygen species (ROS)
- NAD(P)H
- Nitric Oxide (NO)
- Intracellular GSH
- Identifying cells coexpressing GFP for analysis
- Use ratiometric GFP (redox-GFP) to measure cellular/mitochondria reduce/oxidize reaction
- Calcein-based cell viability assay
- Oxygen consumption in cultured cells

Accessories

Optional Accessories

No system would be complete without the ability to expand with additional options and accessories. PTI is a one-stop shop for upgrades, add-ons and accessories to complement your photometry system.

Dual C-Port Adapter with Flipping Mirror

The IM-9 is a Dua C-port adapter for the microscope with a flipping mirror allowing the user to select between two different emission detection devices primarily used to mount two optical detectors: one output for the photometer and another output for a camera. Both ports are identical. The flipping mirror allows easy and rapid switching between the two detectors without any hardware change. Good for sequential, not simultaneous, measurement. Very useful to retrofit old fluorescence scopes. Attaches directly to the C-mount on a microscope. Provides one input and two standard C-Port outputs.

Dual C-Port Adapter for Edge Detection, Fluorescence and Electrophysiology

PTI's new Dual C-Port adapters attach directly to the C-Port of any microscope. They provide one input and two standard C-Port outputs. Both ports have access to the full microscope field of view while a red dichroic mirror separates light to two output ports for above and below 620 nm. PTI's model D104 microscope photometer is attached to the port with under 620 nm and CCD camera to the port above 620nm. The aperture on the model D104 is down field from the red dichroic so fluorescence may be collected from a smaller area than the image. The Dual C-Port adapter allows simultaneous viewing of fluorescence and bright field with trans-illuminator on and 660nm long pass filter installed. (NOTE: emission filter for D104 MUST be placed in the D104 NOT in the microscope) Common applications include edge detection, fluorescence, electrophysiology, and any other application where view of the full field during fluorescence measurements is advantageous.

Dual Channel Photometer

All the properties of a single channel photometer, plus:
Allows for simultaneous detection of two emission wavelengths by means of two independent PMT detectors. Has provision for a beam splitter and a dichroic cube for emission wavelength selection. Dichroic assembly is placed within the photometer for selection of the two emission wavelength ranges. Provides high-speed (millisecond) detection for emission-shifted probes. Data acquisition rates of up to 1000 ratios per second are possible.

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. FeliX32™ advanced analysis software is used for curve fitting the decay data.

Adding Imaging

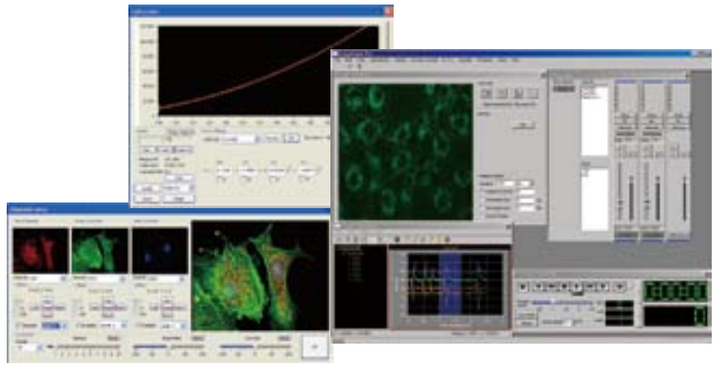
PTI Offers Combination Photometry and Imaging Systems with Ease

EasyRatioPro is the most comprehensive system of its kind. EasyRatioPro offers imaging researchers superb quality and efficiency through one intuitive, integrated production environment. This high-resolution system embodies the latest PTI innovations, incorporating cutting-edge technology to deliver unprecedented image quality and performance in an easy to use package. Featuring dramatic power, high speed streaming Image Acquisition, brand new high-resolution camera support and peripheral options, abundant channel wavelength count and I/O capacity, extensive flexibility and much more. EasyRatioPro gives you control over your imaging world like never before.

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 and flexibility of the EasyRatioPro software.

Calibration Plug-In



Color Composite Plug-In

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

Supported CCD Cameras
Roper Scientific Cameras

- Cascade II
- Cascade 512 & 512B
- Cascade 1K
- Coolsnap HQ & HQ2
- Coolsnap ES
- Coolsnap EZ
- Coolsnap CF
- QuantEM:512SC

Photon Technology Cameras

- CoolOne QE
- Coolone EM

WarpDrive Specifications

- Touch sensitive motorized faders
- 60 function keys
- Full size numeric keypad
- Professional transport controls
- LCD and LED displays
- USB and 1 expansion slot for other optional interfaces

EasyRatioPro

EasyRatioPro with Warp Drive

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

Photon Technology International, Inc. 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 highresolution 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™ monochromator
- 2 meter liquid light guide
- Labmaster PCI interface & I/O
- EasyRatioPro software
- WarpDrive 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

Hardware

EasyRatioPro Hardware Features
Warp Drive 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.

Warp Drive Features

- Touch sensitive motorized faders
- 60 function keys
- Full size numeric keypad
- Professional transport controls
- LCD and LED displays
- USB and 1 expansion slot for other optional interfaces

The Warp Drive 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

Hardware

EasyRatioPro Hardware Features
DeltaRam X™ Random Access Monochromator

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Lamp and Power Supply

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Liquid Light Guides and Microscope adapters

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Liquid Light Guide Specifications

- 2 mm core
- 2 meters long
- Light transmission from 300-900 nm

Power Supply and Lamp Specifications

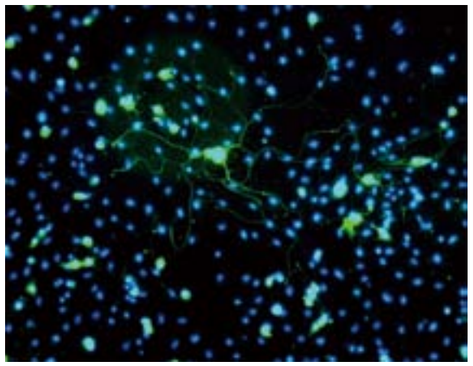
- 75-Watt compact arc xenon bulb 200 nm to 2 microns
- Thermally-matched front surface ellipsoidal reflector for 70% efficiency
- No cooling or ozone venting required

EasyRatioPro Hardware Features
Choose the ideal camera for your application

Photon Technology International has selected a range of high performance cameras to complete your EasyRatioPro high-speed 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 the Roper Scientific line of cameras. Roper is one of the world's leading camera manufacturers. 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 read-noise 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 read-noise 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.



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 -80ºC and a selection of readout speeds. EasyRatioPro can couple to whichever camera is right for you.

Applications where EMCCDs are advantageous include:

- Single molecule fluorescence
- High-speed calcium imaging
- Live cell GFP imaging

and many more…

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...

Software

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

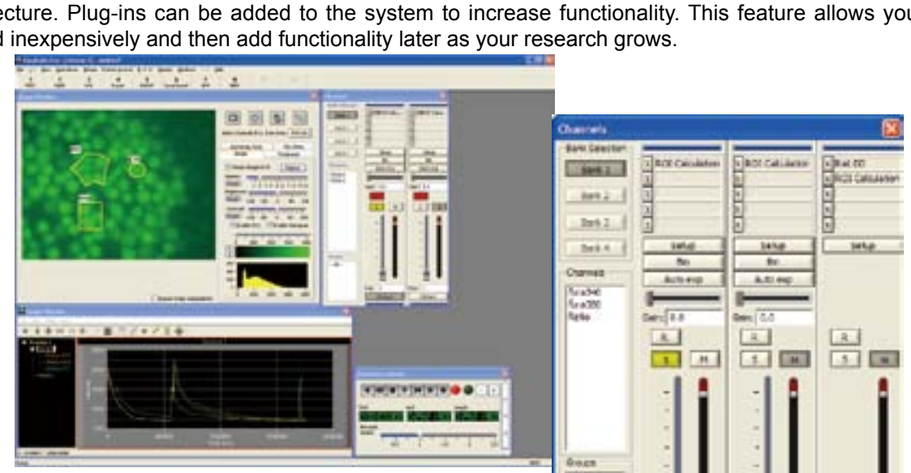
PTI's Acquisition Engine (PAE) streams imaging data to hard disk for acquisition and playback. This gives the user the benefit of running experiments without worrying about the internal RAM limitation of the computer. You can high-speed stream up to terabytes of hard disk storage. Competitive systems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



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:

- 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 plug-ins for ratio-metric calculation, thresholding, and background subtraction.

Software

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.

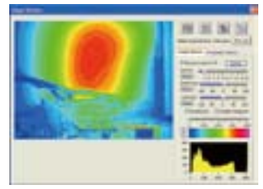
Day To Day Routines

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!

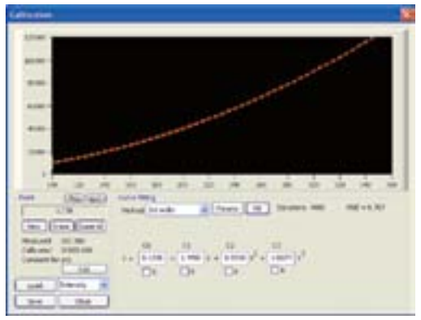
EasyRatioPro Plug-Ins

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



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:

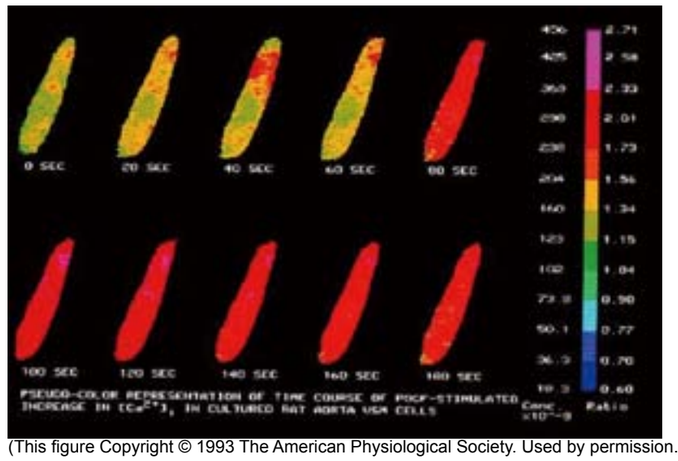
- Export of image data to popular formats such as .avi, .bmp, .jpg, .tif, and .png.
- Control of up to 16 excitation, emission, or derived channels.
- "Warp Drive" human use interface control for dedicated control over exposure, gain, electron multiplier, mute, solo, select, record, window functions,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

Applications

EasyRatioPro Is Up For The Task Of Many Applications

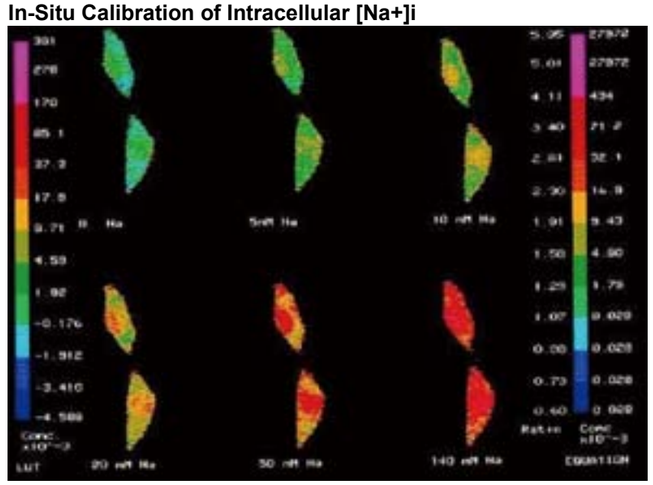
As a scientist, you don't want to make the wrong decision when buying an imaging system. Many systems on the market claim to be able to do all fluorescence imaging applications. However, this is simply not true. Other systems are complex and offer so many options that will never even be used for ion 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++]i



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

In-Situ Calibration of Intracellular [Na+]i



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

Calcium Measurement
- Mitochondrial Ca^{2+}
- Endoplasmic reticulum (ER) Ca^{2+} pool
- Lysosomal Ca2+
- Extracellular near membrane Ca^{2+}

Other Ions
- Intracellular Mg^{2+}
- Intracellular Mn^{2+}
- Intracellular Zn^{2+}
- Intracellular Sr^{2+}
- Intracellular Na^{+}
- Intracellular Cl^{-}
- Cytosolic labile iron pool (LIP): Iron homeostasis

Membrane Potential
- Plasma membrane Potential
- Mitochondrial Membrane Potential

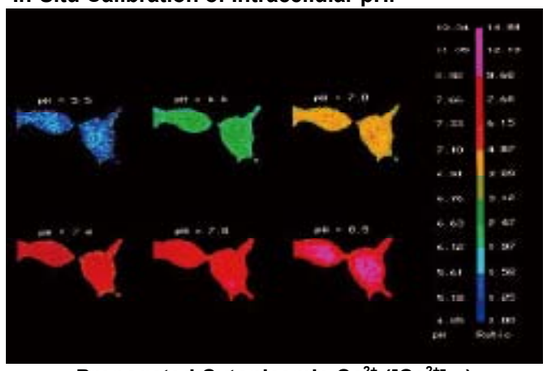
pH
- Intracellular pH
- Vacuolar pH
- Lysosomal pH

GFP & Variants
- Identifying cells coexpressing GFP for analysis
- Use mitoGFP to visualize mitochondria and measure mitochondrial calcium $[Ca^{2+}]m$
- Use ratiometric GFP (redox-GFP) to measure cellular/mitochondria reduce/oxidize reaction
- HEK-293 cells transfected with the CaSR-pEGFP vector
- ECFP
- EYFP

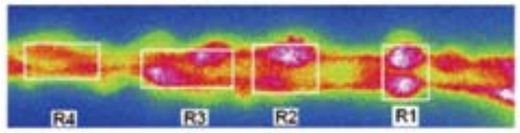
And Many More!

More Applications

In-Situ Calibration of Intracellular pHi

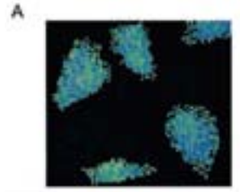


Propagated Cytoplasmic Ca^{2+} ($[Ca^{2+}]_{cyt}$) Responses Along a Fluo-4-loaded DVR Wall



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

Emission ratio of indo-1 loaded BAECs

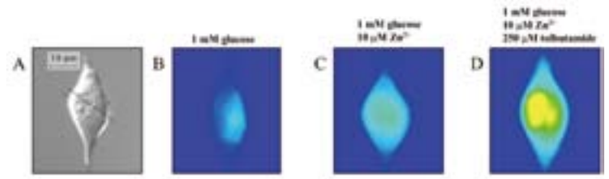


Changes in CaM availability in BAECs (A) also expressing BSCaM2 (B) Time courses of free Ca^{2+}-CaM

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

Fluorescent Zn^{2+} detection in MIN6 cells

Fluorescent Zn^{2+} 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^{2+} treatment. C,10 μM Zn^{2+} was added to incubation medium, and the image was acquired after incubation for 30 min. D, 250 μM 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

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^{2+}

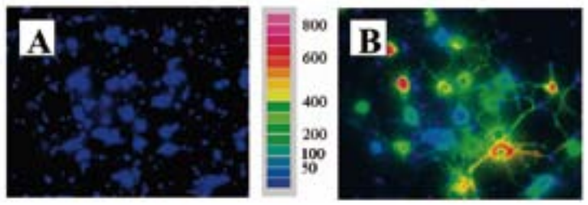
Other Measurements
- Calcium imaging of
  - Single HEK 293 cell
  - Taste Bud
  - Pea Pollen tubes
- Respiratory burst & reactive oxygen species (ROS)
- NAD(P)H
- Changes in CaM availability
- Demonstration of selective plasma membrane permeabilization by digitonin in HEK293 cells
- Detection by immunofluorescence 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 PI fluorescent
- Metal induced cytotoxity

And Many More!

More Applications

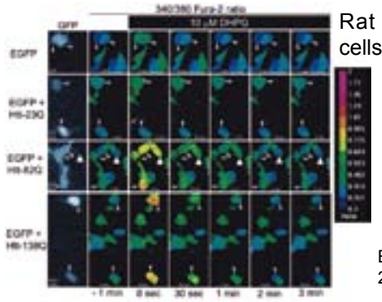
Intracellular Calcium

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



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

Ratio Imaging of GFP expression



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

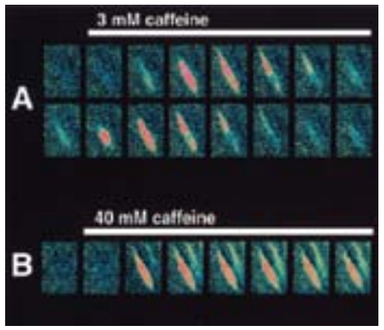
Bezprozvanny , I, et al. Neuron, 39 (7): 227-239, 2003.

Calcium-related Measurement

- Ca^{2+} Waves
  - Ca^{2+} wave quantitation
  - Ca^{2+} wave induced by mechanical stimulation
  - Ca^{2+} wave induced by mechanical wounding
  - Ca^{2+} wave propagation
    - In non-contacting cells
    - Over a physical gap
- Ca2+ Oscillation induced by:
  - Hg^{2+}
  - K^{+}
  - Heat
  - ATP
  - Dopamine
  - FSH
  - Caffine
- Ca^{2+} mobilization
- Ca^{2+} homeostasis
  Propagated cytoplasmic Ca^{2+}cyt responses along the DVR wall.
- Simultaneous Measurement of intracellular Ca^{2+} and:
  - Cell volume
  - Phygocytosis
  - Cl^{-}
  - Oxidase activation
  - Ca^{2+}-CaM

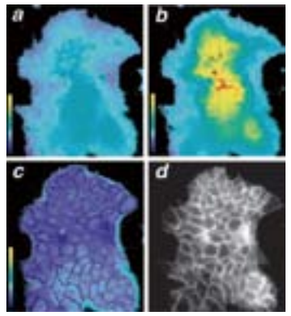
And Many More!

Regenerative Calcium Oscillations



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

Extracellular near-membrane Ca^{2+}



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

Accessories

Optional Accessories

No system would be complete without the ability to add options and accessories. PTI is a one-stop 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 FeliX32™ software package and BryteBox™ interface. 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. FeliX32™ advanced analysis software is used for curve fitting the decay data.