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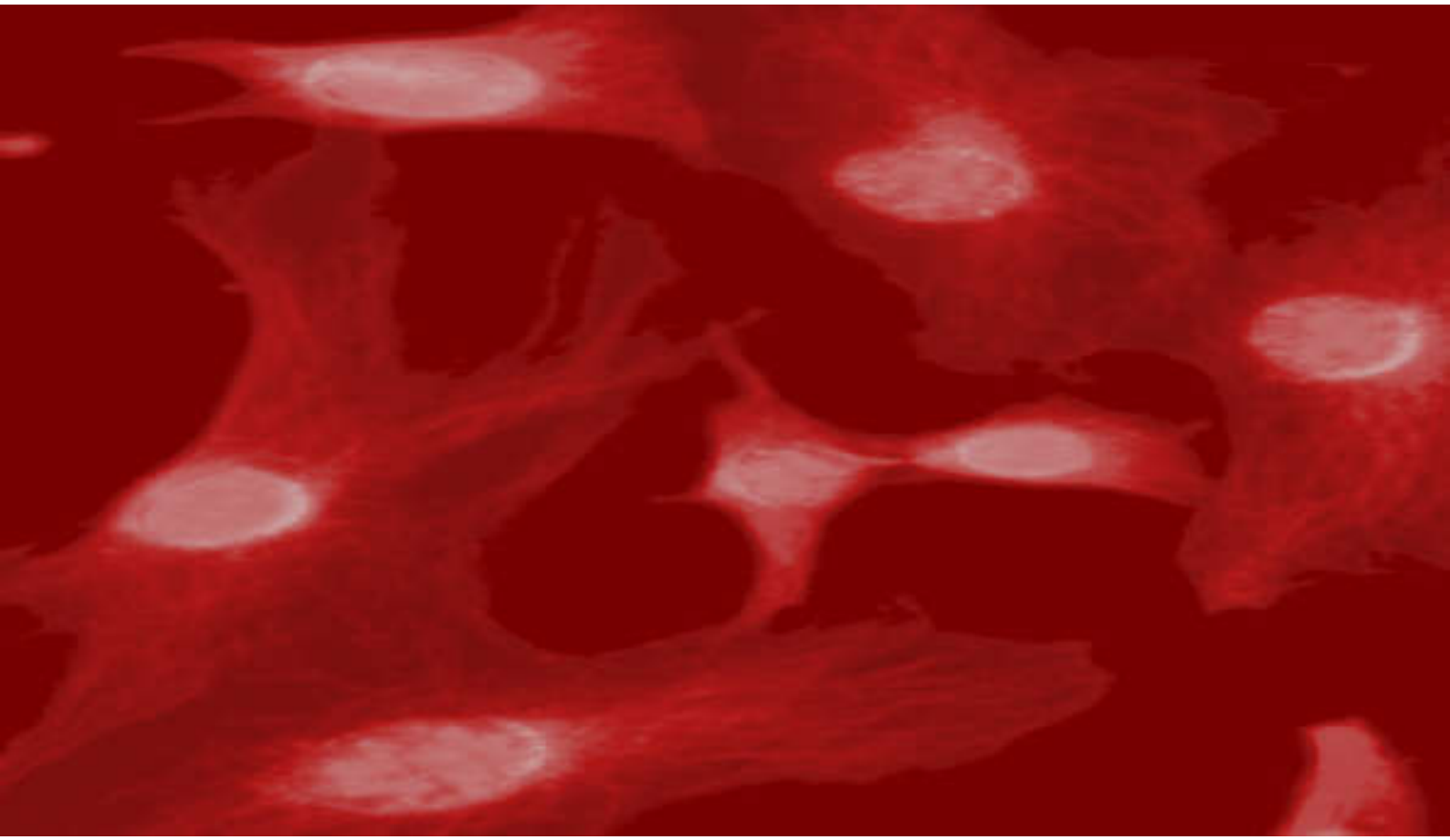
RatioMaster



Fluorescence Microscopy

RatioMaster

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
- D 104 microscope photometer
- FelixGX™ software
- PTI electronics interface
- Installation and training

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

Liquid Light Guide Adapter For All Common Fluorescence Microscopes

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

Hardware

DeltaRAM X™ Specifications

- Excitation wavelength range: 330–650 nm
- Wavelength selection speed: < 2 milliseconds
- Wavelength bandwidth adjustable from 0–24 nm
- 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 300–650 nm

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.

Hardware

RatioMaster™ Photometer

The fluorescence emission from cells on the microscope stage is measured with 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.

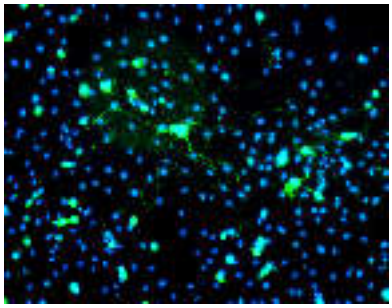
This detection system excels at applications such as the following:

- Live-cell imaging
- High-speed emission ratio imaging
- Quantitative FRET, FRAP, FISH
- Luminescence
- Electrophysiology
- Many more...

Optional Dual Channel detection for emission shifted dyes!

Specifications

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



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.

Software

Software

- Excitation Ratio
- Emission Ratio
- Excitation Scan
- Emission Scan
- Multiple Dyes
- Timebased
- Calculate FRET Parameters
- Determine R_0
- Transform Commands
- Concentration Map
- Concentration Equation
- Lookup Table
- Data Analysis

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.

About the Software

From fixed cell preparations to dynamic events, FelixGX™ 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 FelixGX™ software platform.

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

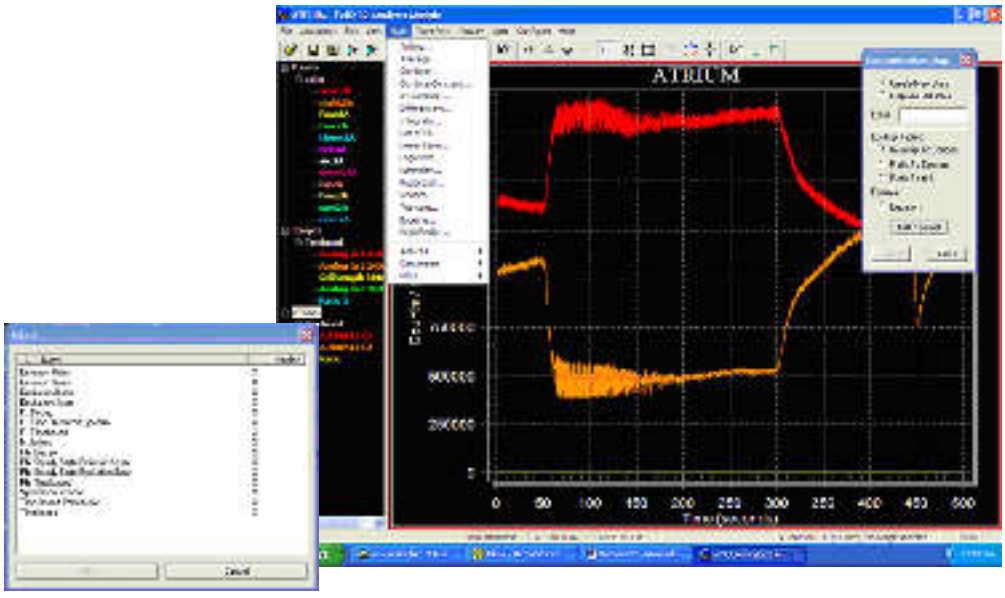
FelixGX™ software also allows for maximum flexibility for your future needs with our modular software architecture. Components can be added to the system to increase functionality. This feature allows researchers to get started inexpensively and then add functionality later as their research grows.

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

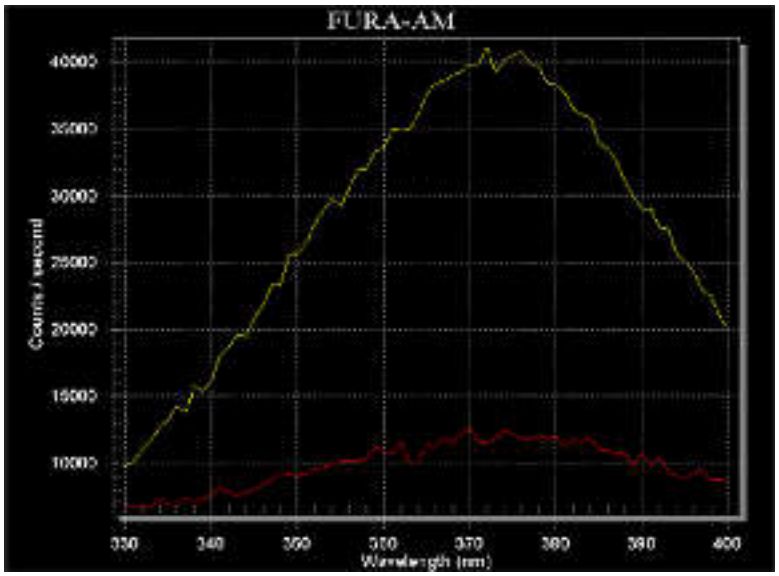
If the out of the box acquisition protocols do not quite fit your applications, the PTI Macro editor allows you to create your own specialized experiments by a drag and drop command library into a loop logic. This means you do not have to be a programmer or understand a scripting language to implement 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 and a host of other acquisition modes. The Macro editor is simply one of the easiest to use ever designed by PTI!



Applications

RatioMaster™ Is Up For The Task Of Many Applications

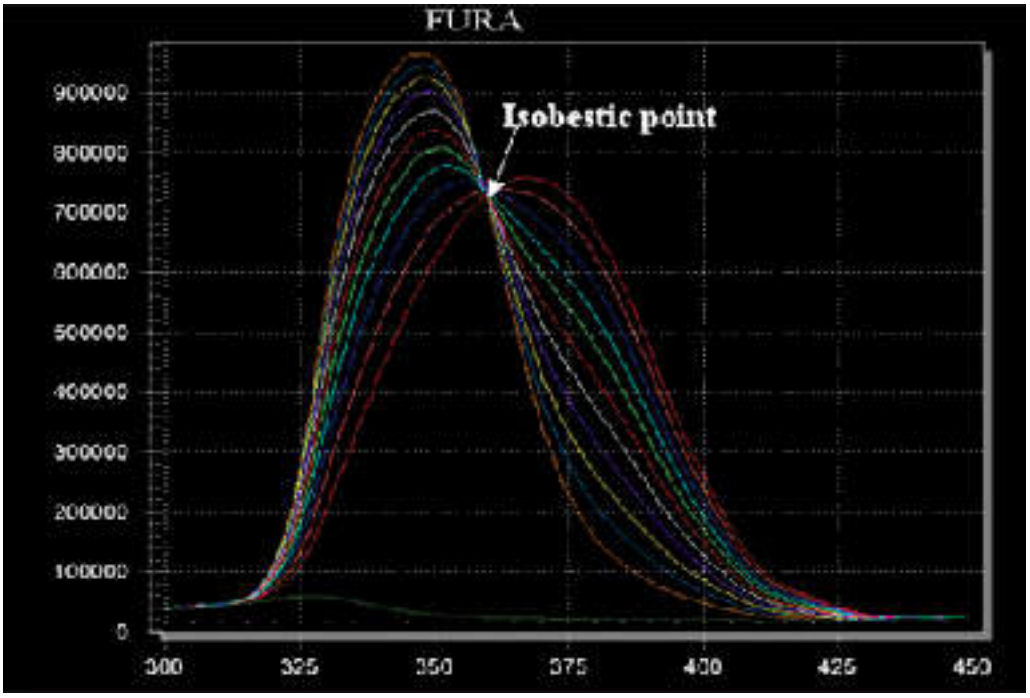
Excitation spectrum of Fura-AM



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.

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.



Frequently Used

Excitation-Shifted

Application	Dye
Calcium	Fura-2, Fura PE3 FFP18 Quin-2 Fura-Red
Sodium	SBFI BCECF HPTS SNAFL® DM-NERF® CI-NERF®
Potassium	PBFI
Magnesium	Mag-Fura-2 Mag-Fura-5
Membrane Potential	Di-4-ANEPPS Di-8-ANEPPS

Emission-Shifted

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

Applications

Fluorescence Dyes

Non-Ratiometric

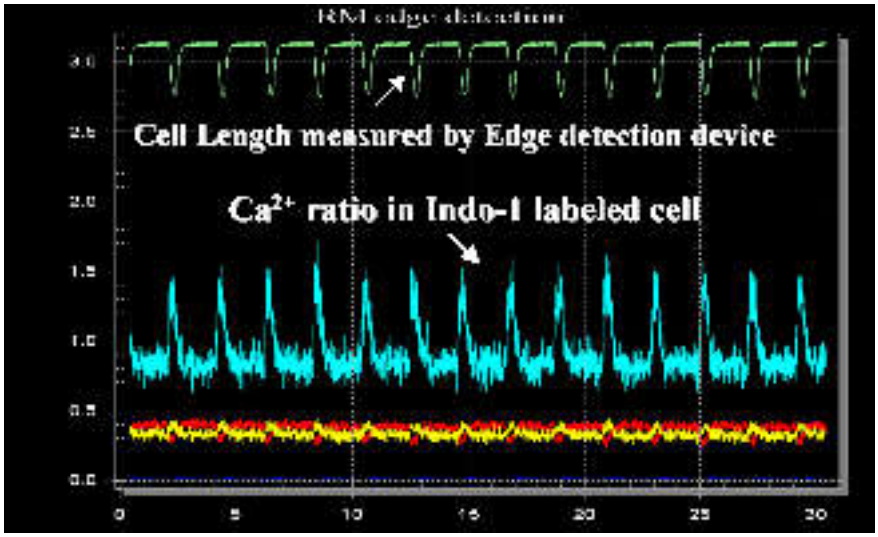
Application	Dye
Calcium	Fluo-3 Calcium Green™ Calcium Orange™ Calcium Crimson™ Rhod-2
Chloride	SPQ SPA MQAE
HIV protease	HIV protein substrate
F-actin	phallotoxins
PKC	Fim-1 Rim-1

Dual Dye

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

And Many More!

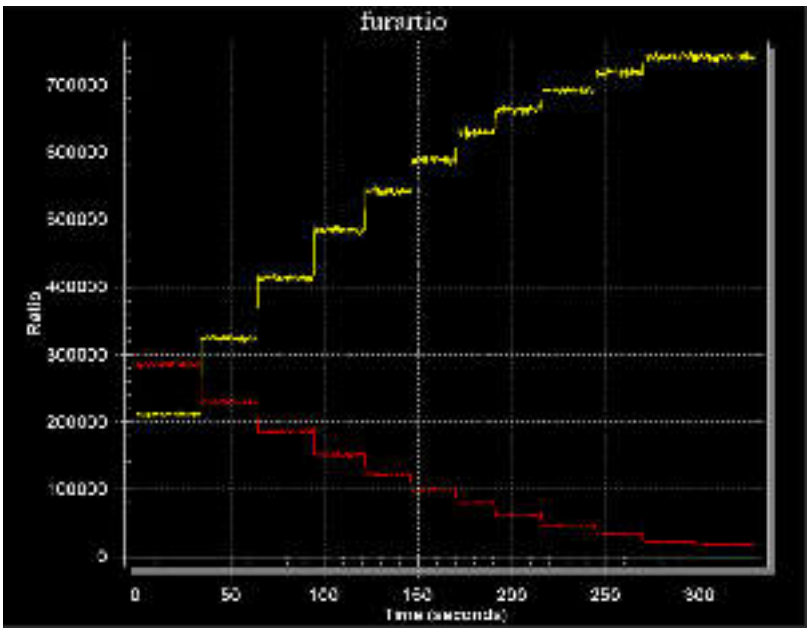
Simultaneous measurement of Ca^{2+} and myocyte cell length



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.

In-Situ Calibration of Intracellular $[Ca^{2+}]_i$

By measuring the ratio of fluorescent intensity at 340/380 nm, $[Ca^{2+}]_i$ can be measured over several orders of magnitude and with a high degree of precision.

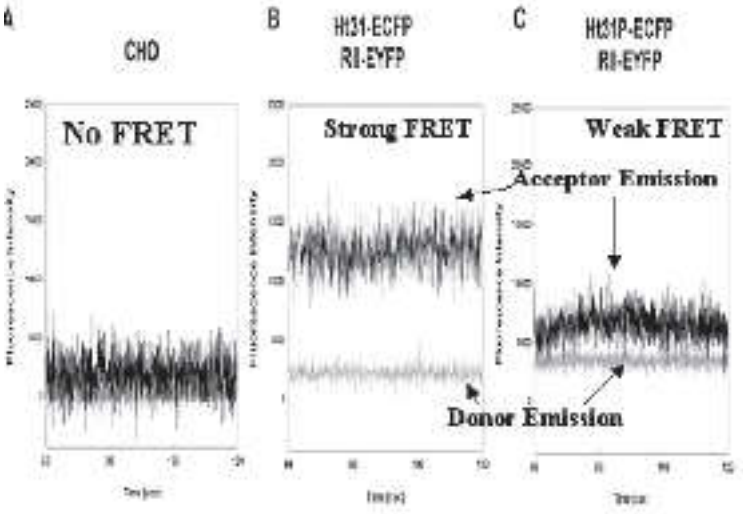


Applications

More Applications

FRET for Binding Assay

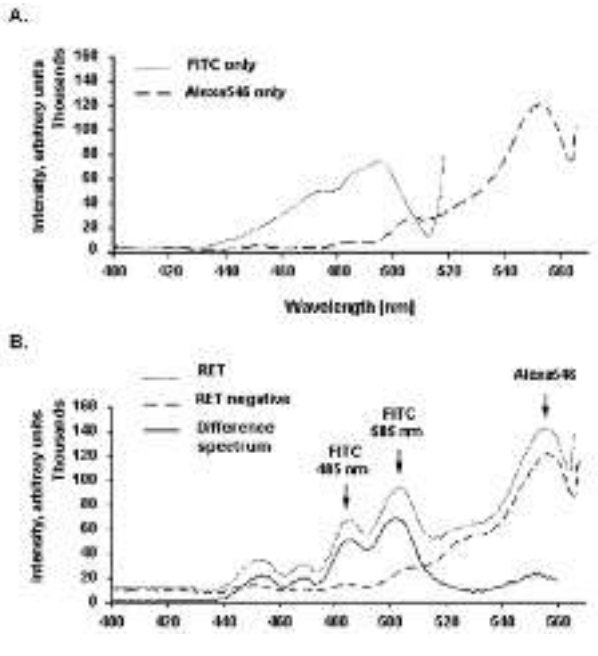
FRET occurs when the EYFP fusing RII protein kinase binds to the ECFP fusing Ht31 protein (kinase anchoring protein). The results of this FRET: the donor (ECFP) emission fluorescence decreases. The acceptor (EYFP) emission fluorescence increases. Instrument: PTI's FRET RatioMaster™ Pro.



Reuhr, M. et al. JBC, 274(46): 33082

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/Rhod 2

FITC/CY3 FRET

FITC/TRITC FRET

YFP/TRITC FRET

CY3/CY5 FRET

CFP/YEP FRET

BFP/GFP FRET

And Many More!

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!

Applications

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)

Other Ions

- 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_i 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

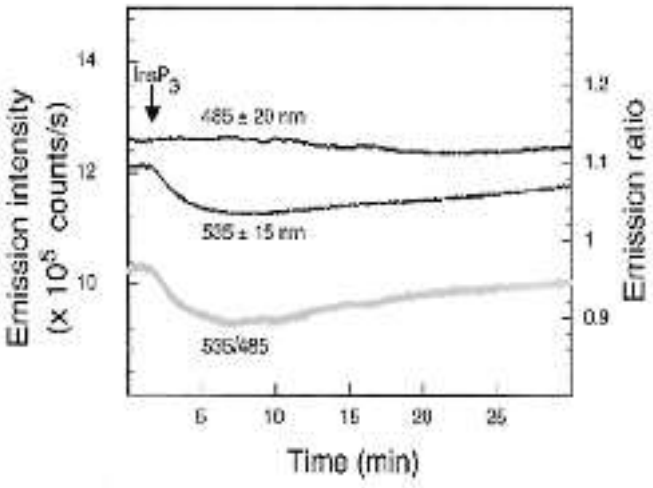
And Many More!

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:

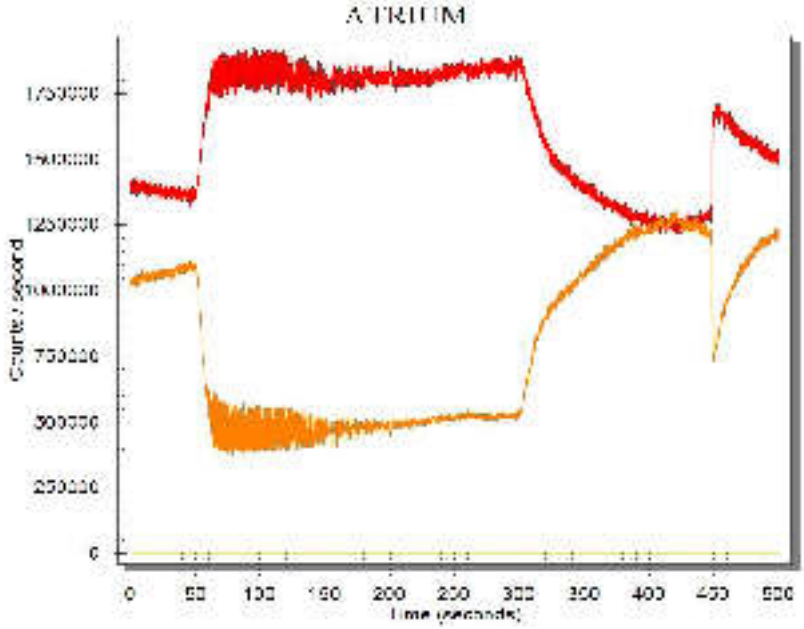
- The donor (CFP) emission fluorescence decreases
- The acceptor (YFP) emission fluorescence increases
- The emission ratio of Acceptor/Donor increases
- InsP3 triggers the release of ER Ca^{2+}, thus decrease the level of FRET



Xu, W, et al. JBC, 275 (47), 38870

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

Excitation Ratio



Kim Sung Joon (Seoul Natl University)
(Dept. Physiology and Biophysics)

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.

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 Dual 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.

Adding Imaging

Supported CCD Cameras

Photometrics Cameras

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

Andor Cameras

- iXon series

Hamamatsu Cameras

- ORCA 2
- 9100 series

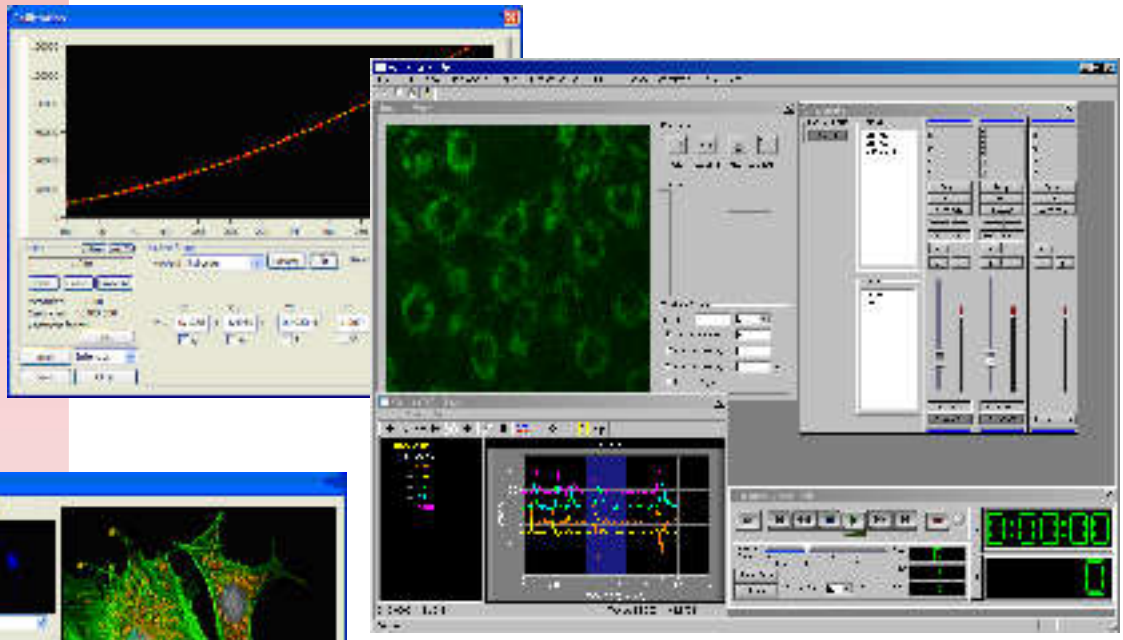
PTI Offers Combination Photometry and Imaging Systems with Ease

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.

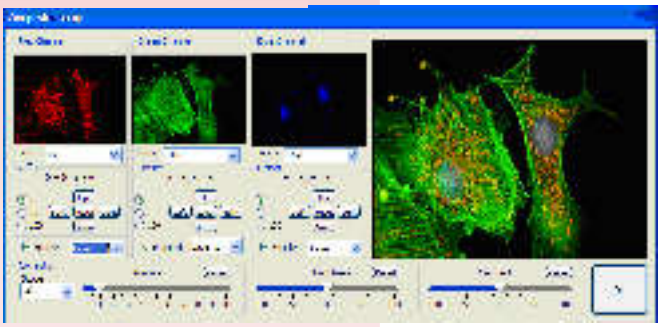
About the Software

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



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.



Color Composite Plug-In

Warp Drive Imaging™ 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

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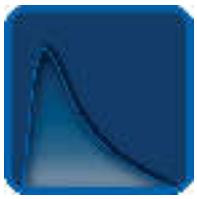
A Complete Line of Fluorescence Spectroscopy Instruments from PTI

QuantaMaster™ Series
Steady State Fluorescence and Phosphorescence Spectrofluorometers



TimeMaster™ Series
Fluorescence Lifetime Spectrofluorometers

RatioMaster™ Series
Fluorescence Microscopy Spectrofluorometers

ImageMaster™ Series
Fluorescence Imaging Systems

FluoDia™

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