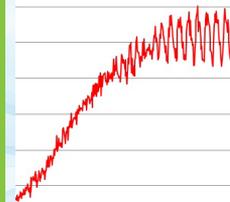
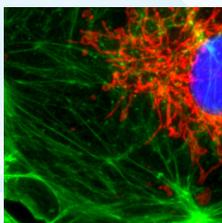
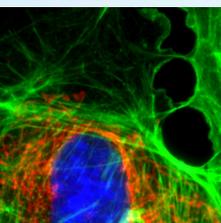
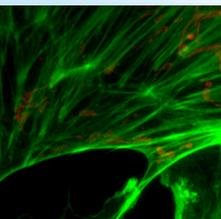
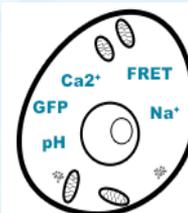
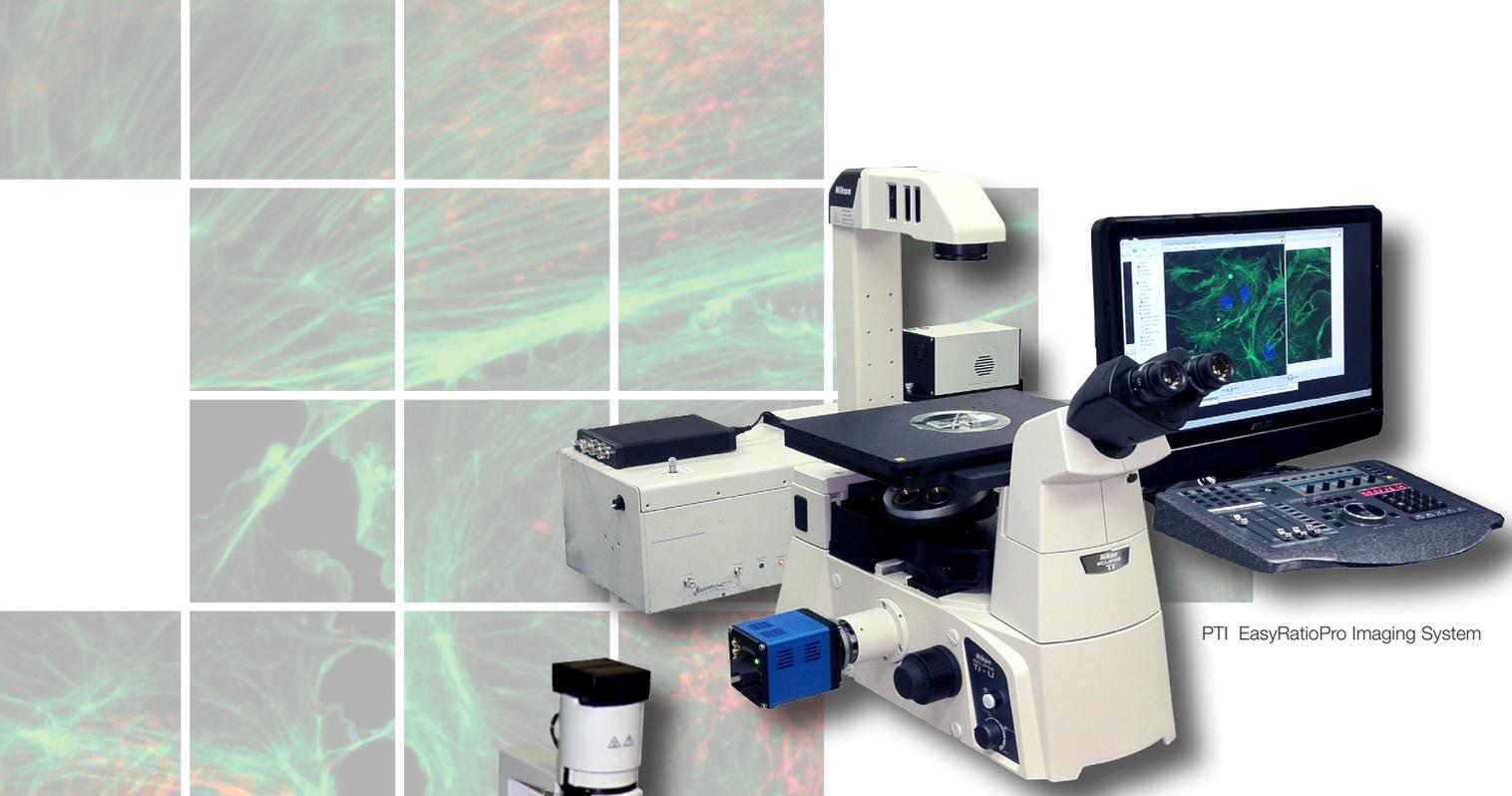


## Intracellular Ion Kinetic Solutions

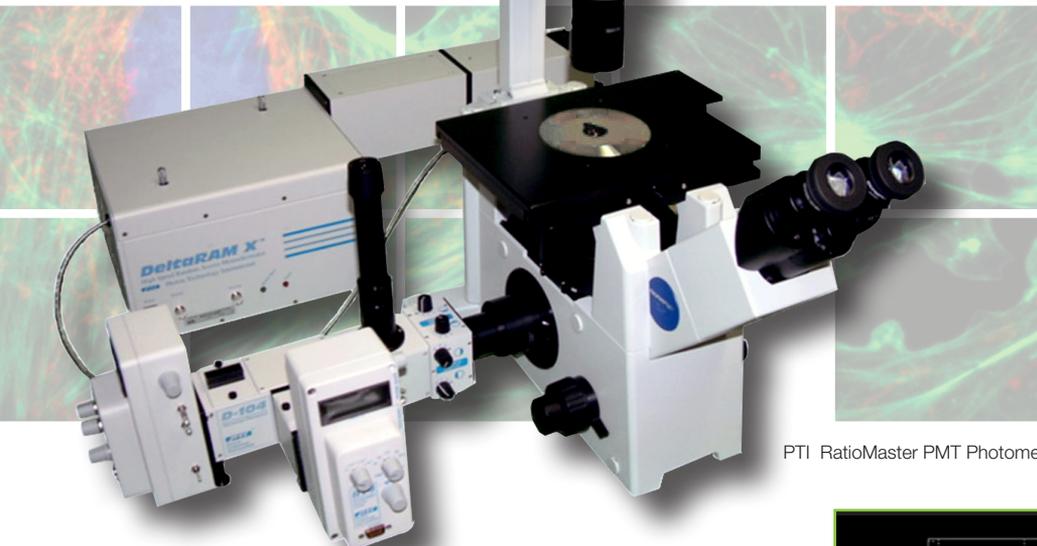
PTI EasyRatioPro, PTI RatioMaster, PTI QuantaMaster™ 800



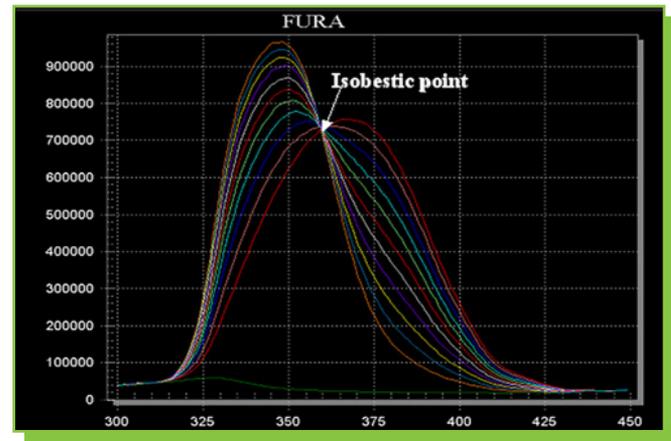
Live cell quantitative intracellular ion kinetics research



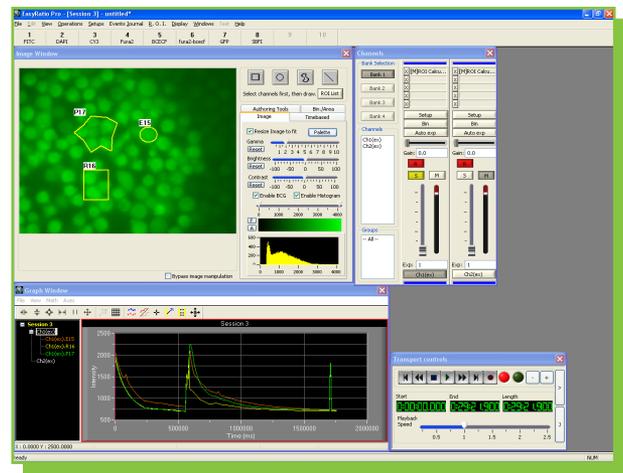
PTI EasyRatioPro Imaging System



PTI RatioMaster PMT Photometry System with Dual Emission Detector



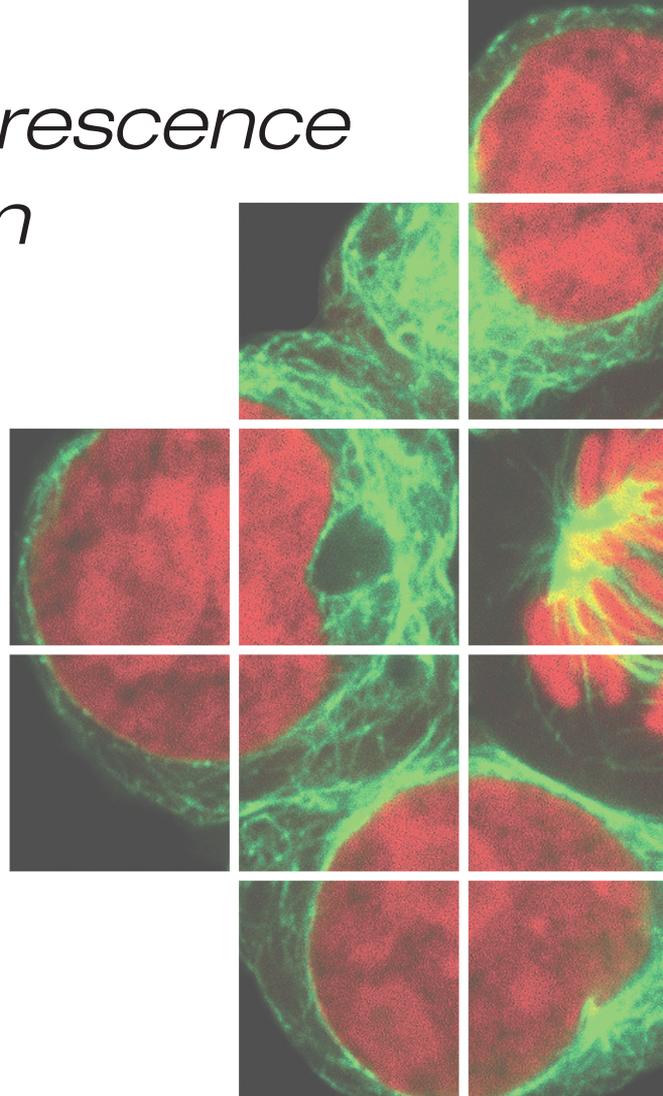
Ratiometric techniques take advantage of the shift in the fluorescence spectrum (e.g. Fura-2) upon binding a specific ion. The extent of the spectral shift is commensurate with the free ion concentration, allowing calibration of a 340/380 nm ratio into ion concentration (in this case intracellular Ca<sup>2+</sup>).



PTI 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

*The suite of Ratio Fluorescence Systems available from HORIBA Scientific has aided breakthrough research in areas such as*

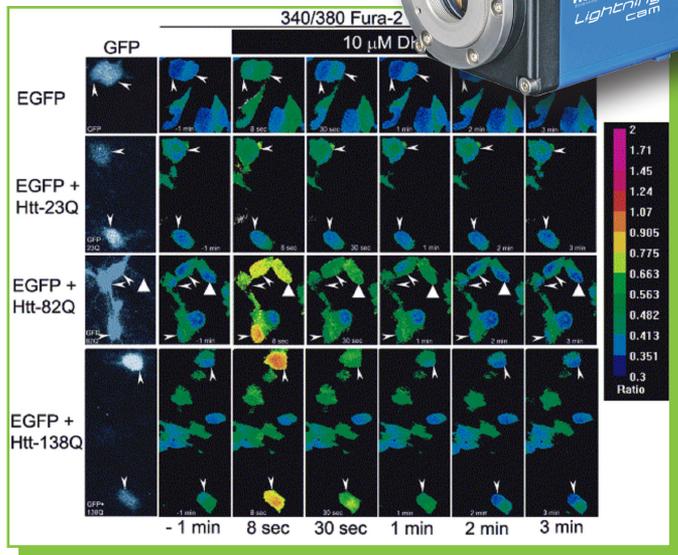
- *Ca<sup>2+</sup> signaling*
- *Cardiac contractility and function*
- *Alzheimer's disease*
- *Huntington's disease*
- *Mitochondrial membrane potential*
- *Fluorescent lipid probes*
- *Neurite outgrowth regulation*
- *Calcium entry pathways*
- *Store operated calcium channels*
- *Diabetes-induced ion fluxes in cardiac cells*
- *Ryanodine receptors*



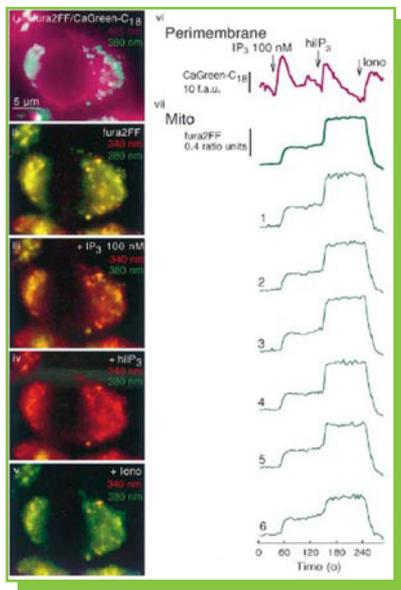
## Ratio Fluorescence Systems

HORIBA Scientific introduces the latest version of the PTI EasyRatioPro system for intracellular calcium imaging research, originally developed by recently acquired Photon Technology International (PTI). PTI was the first to introduce a patented ratio fluorescence system, the DeltaScan, shortly after the first Fura-2 publication in 1984 by Roger Tsien. EasyRatioPro is dedicated to live cell intracellular calcium microscopy, providing a comprehensive instrumentation package incorporating all the necessary hardware and dedicated software tools for fast kinetic imaging of ion transients in live cells. Both excitation-shifted and dual emission techniques are supported with the bulk of modalities involving Fura-2 and FRET/Dual Emission protocols, respectively.

# PTI EasyRatioPro Imaging Systems



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.



Fura-2FF-loaded permeabilized cell

Left: the overlaid images show the distribution of the membrane-bound CaGreen-C18 (image i, purple) and the mitochondrially compartmentalized Fura-2FF (image i, green), and the changes in the Fura-2FF fluorescence (images ii-v, 380 nm green/340 nm red) upon addition of 100 nM IP3 (ii versus iii), 12.5 M IP3 (iii versus iv) and ionomycin (iv versus v).

Right: time courses of the global  $[Ca^{2+}]_{pm}$  response (vi) and the average  $[Ca^{2+}]_m$  response (vii, thick line), and the  $[Ca^{2+}]_m$  responses of the marked (1-6 on image i) individual mitochondria (vii, thin lines).

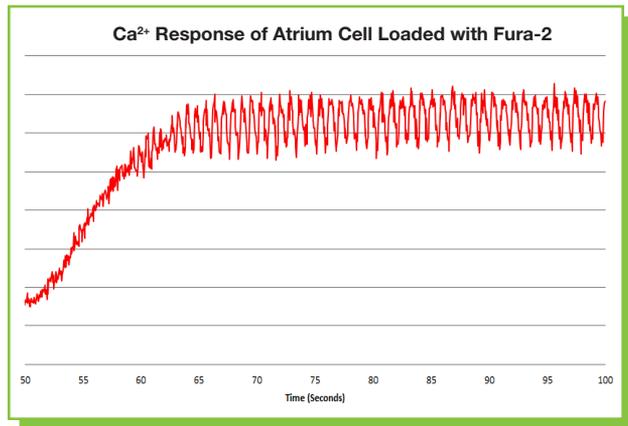
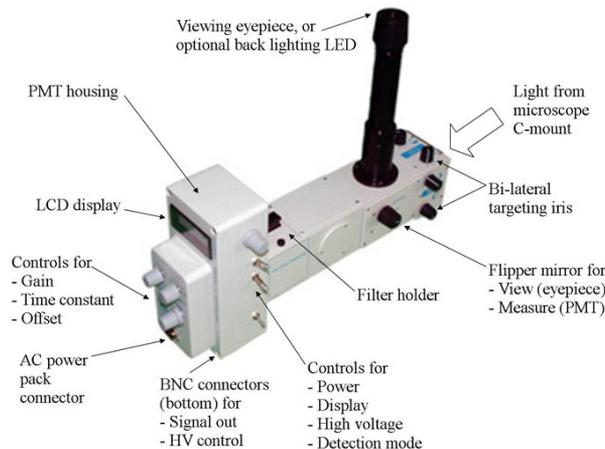
EMBO, 18(1): 96-108,  
Courtesy of Dr. G. Hajnoczky,  
Thomas Jefferson University

# Fluorescence Excitation

All three systems are configured with the patented DeltaRAM Random Access Monochromator that allows changing software-selected wavelengths in as little as 2 milliseconds. This means that these systems can deliver up to 250 ratio measurements per second! Such time resolution is unprecedented in the intracellular calcium research field! For imaging applications, the time response of most cameras is an order of magnitude less due to the necessary exposure time to achieve meaningful results. The PTI EasyRatioPro control software seamlessly synchronizes the DeltaRAM wavelength change with the camera exposure demanded by the application, ensuring perfect data fidelity every time.

# PTI RatioMaster PMT Photometry Systems

Dedicated microscope PMT photometer system or PMT enhancement for an imaging system.



Sample: freshly isolated rabbit atrium  
Stimulated with 90 mM KCl, nifedipine was added (2  $\mu$ M) returned to normal Tyrode medium, followed by 10 mM caffeine.

# PTI QuantaMaster Cell Suspension System

Dedicated cuvette-based ratio fluorometer or cuvette sample compartment enhancement for a microscope based system.



# Fluorescence Imaging Detection

The LightningCam sCMOS camera from HORIBA is an excellent imaging camera for most applications, offering high resolution and very good speed. In addition, the PTI EasyRatioPro system also supports dozens of different camera models from HORIBA's many manufacturing partners. These models can be sCMOS, EMCCDs, CCDs, ICCDs and frame transfer CCDs. Frame rates of these range from a modest dozen to hundreds of frames per second. The choice of camera appropriate for a given experimental protocol is governed by the requirements of the measurement.

In addition to being able to accommodate the fastest image acquisition cameras and provide dynamic wavelength changes for multi-wavelength experiments, EasyRatioPro streams images directly to the hard drive, avoiding data congestion so common to memory-limited instruments. Thus even bursts of high-speed time lapse experiments are recorded easily, without any software or RAM limitations.

# FRET and Dual Emission Imaging Detection

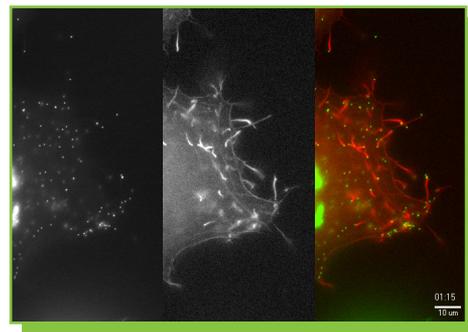
For more advanced detection modalities, dual wavelength detection is utilized with special emission splitter devices. These simultaneously project two images side by side on a single camera chip, by splitting the emission wavelength range into short and long wavelengths using a dichromatic mirror and special emission band-pass filters. Since in this type of application there are no moving parts, the acquisition speed is limited only by the frame rate the camera is capable of. These are passive optical devices that connect between the imaging camera and the microscope.

These modalities are:

- FRET (Fluorescence Resonance Energy Transfer) with the following *Donor-Acceptor pairs*:  
CFP/YFP, CY3/CY5, BFP/GFP, FITC/TRITC, etc.
- Dual probe experiments using two spectrally different probes:  
YFP/mRFP, Fura-Red/BCECF, JC-1 etc.
- Emission-shifted intracellular ion protocols:  
Indo-1, Carboxy SNARF, di-8-ANEPPS, SPQ and derivatives

These dual emission splitter devices can also be configured with:

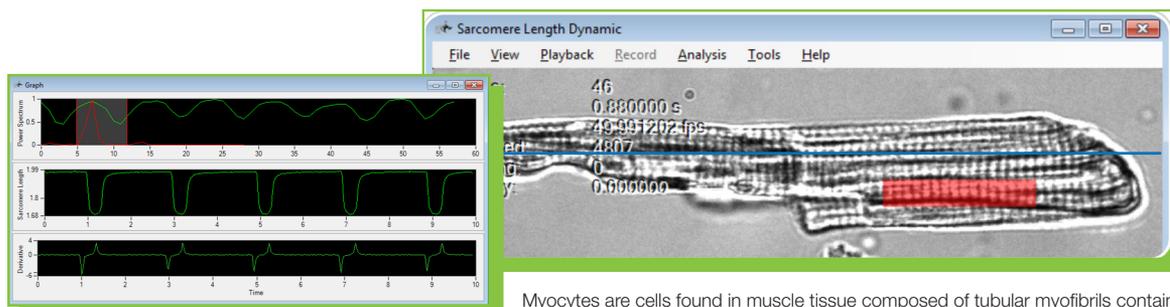
- A polarizing dichroic mirror to provide two orthogonal polarizations in the two channels, enabling real-time monitoring of anisotropy changes typical of membrane fluidity.
- A defocusing lens in one of the channels, enabling detection related to the third (z) dimension for monitoring 3D particle movement through the Z-dimension.



Virus particles (green, labeled with YFP) move on microtubules to the plasma membrane. There they induce actin polymerization (red, labeled with mRFP-actin). The actin tail that forms, helps to propel the virus around and into neighboring cells to spread the infection. (Dual-labeled dataset acquired with Dual View™ dual emission device, Photometrics)  
Courtesy of Dr. Michael Way  
Cellular signaling and cytoskeletal function  
The Francis Crick Institute, Lincoln's Inn Fields Laboratory

# Fluorescence PMT Detection

For microscope photometry systems, HORIBA offers a PMT photometer that optionally can be configured with two highly sensitive detection photomultipliers operating either in photon counting or analog mode, depending on whether the signals are very weak, or of high intensity. The addition of an available video camera enables the simultaneous monitoring of sarcomere length from within the ratio measurement protocol.

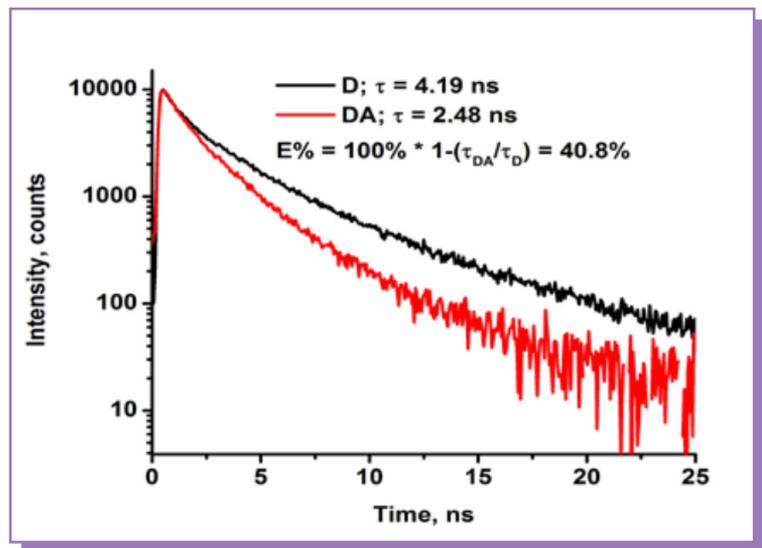


Power spectrum, sarcomere length trace and its derivative for the determination sarcomere length.

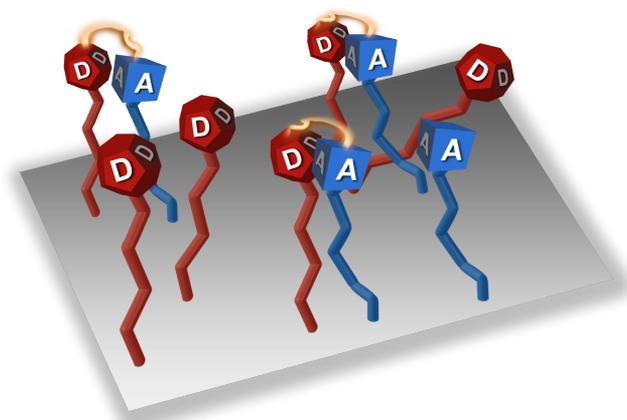
Myocytes are cells found in muscle tissue composed of tubular myofibrils containing repeating sections of sarcomeres which appear as dark and light bands when viewed under a microscope. Sarcomeres, composed of long, fibrous proteins that slide past each other when the muscles contract and relax, form the basic contractile units of these myocytes or muscle cells.

# FLIM Fluorescence Lifetime Imaging Upgrade Option

The PTI EasyRatioPro system can be easily upgraded to be able to perform Fluorescence and Phosphorescence Lifetime measurements through the addition of a laser diode for illumination, and a time correlated single photon counting (TCSPC) gated detector. Such measurements are especially useful since the FRET phenomenon results in noticeable lifetime changes, and the measurement is unhampered by concentration, non-uniform loading or light scattering. The FLIM option operates independently of the ratio fluorescence modality. To learn more, refer to our DeltaMyc brochure.



Decay curves for donor (D) only and donor plus acceptor (DA) for the system above. Samples gratefully acknowledged from M. Barroso, Center for Cardiovascular Sciences, Albany Medical College.



Model of the transferrin molecules bound to polylysine-coated coverslips exhibiting FRET.



# Specifications

# Ratio Fluorescence

## DeltaRAM High Speed Illuminator (Common to all systems)

Excitation Wavelength Range	250–650 nm
Wavelength Selection Speed	< 2 milliseconds point-to-point
Data Acquisition Rate	1 kHz single wavelength
Light Source	High efficiency continuous Xenon arc lamp
Repetition Rate	1 to 250 Hz (software controllable)
Excitation Modes	Single, dual, multi-wavelength time-based or excitation spectral scanning
Bandpass	0–25 nm, continuously adjustable (computer control available)
Wavelength Accuracy	+/- 1 nm
Resolution	0.5 nm
Light Delivery	2 m Liquid Light Guide
Coupling	Adapters available for all fluorescence microscopes or cuvette sample compartment

## High Speed LightningCAM Imaging Camera (Standard camera for PTI EasyRatioPro. Other cameras available)

Camera Type	sCMOS
Resolution	2048 x 2048
Pixel Size	6.5 X 6.5 $\mu$ m
Quantum Efficiency % @ 590 nm Peak	> 82%
Dynamic Range A/D	16 bits
Readout Noise @ 30 fps/100 fps e-RMS	< 1.4 / < 1.5
Imaging Frequency, Frame Rate	100 fast scan/30 slow scan (full frame, faster with subframes)
Exposure Time Rolling Shutter/Global Shutter	10 $\mu$ s to 10 s
Optical Input	C-mount
Software	EasyRatioPro

## Digital PMT Photometer for Low Cost, High Speed, Single Point Microscope Photometry

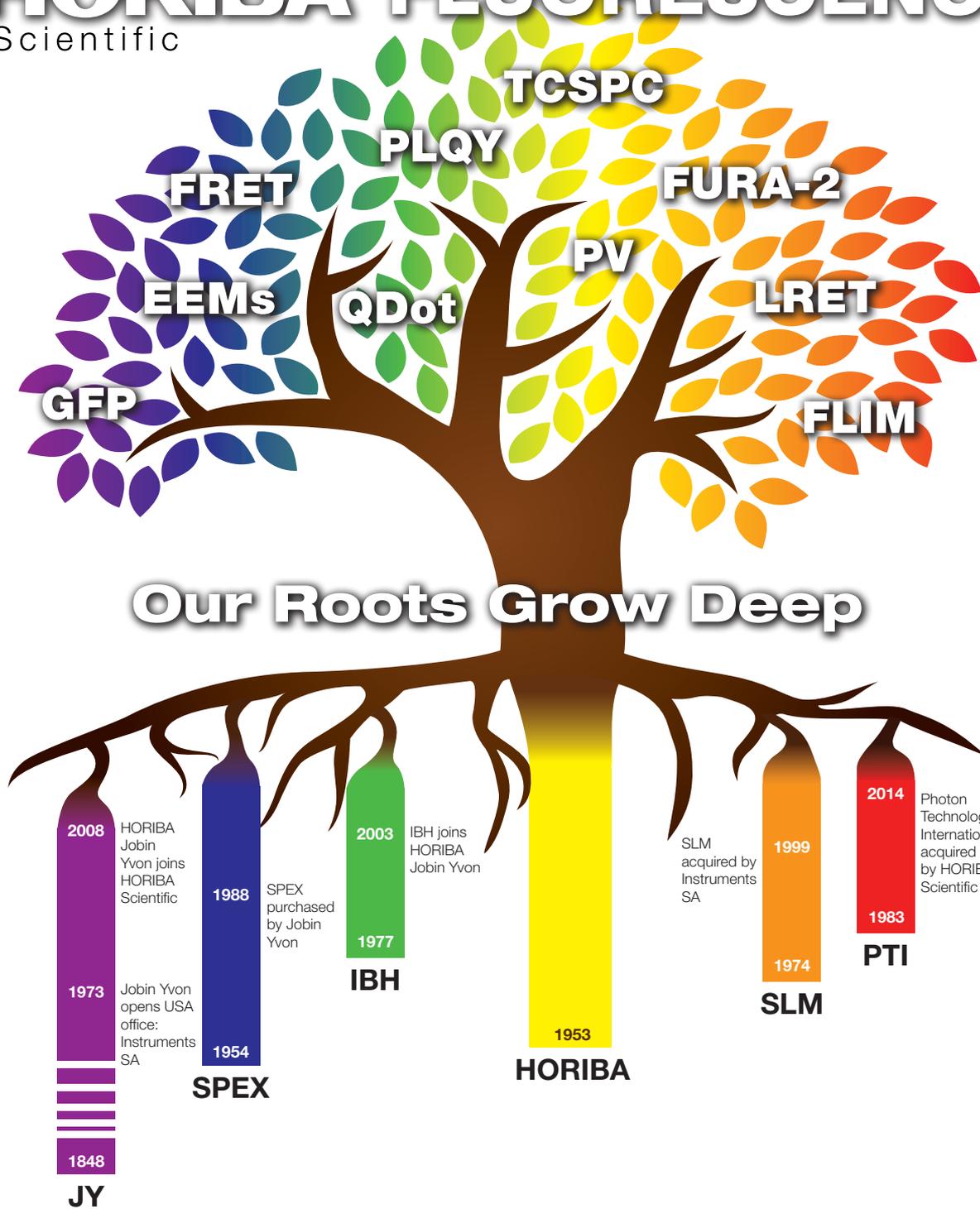
Area of Interest Selection	Variable bilateral adjustable slit
Number of Detection Channels	1, 2 or 3
Detection Mode	Photon counting or analog
Detector Type	Photomultiplier Tube (PMT)
Wavelength Range	185-650 nm (up to 850 nm)
Optical Input	C-mount
Software	FelixGX

## Cuvette Fluorometer

Sample Holder	Thermostated micro-stirred cuvette holder
Emission Monochromator	300 mm, coma-aberration corrected, asymmetrical, 1 to 30 nm bandwidth
Number of Detection Channels	1 or 2
Detection Mode	Photon counting or analog
Detector Type	Photomultiplier Tube (PMT)
Wavelength Range	185-650 nm (up to 850 nm)
Software	FelixGX

# HORIBA FLUORESCENCE

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HORIBA Scientific has a policy of continuous product development, and reserves the right to amend part numbers, descriptions and specifications without prior notice.