

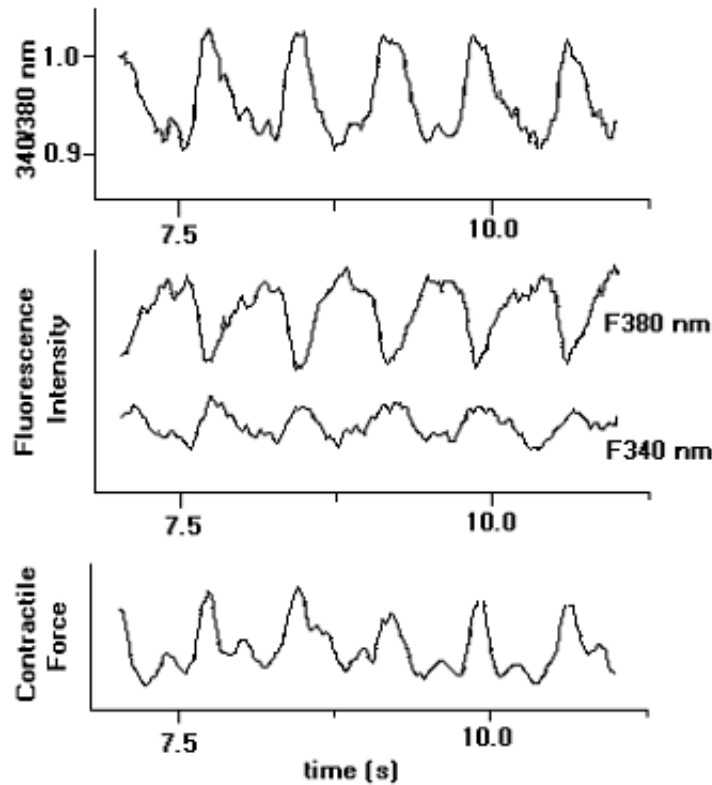
PTI Technical Note

Remote Sampling of Intracellular Calcium Transients on Langendorff-Perfused Mammalian Whole Heart

The measurement of intracellular ion transients concurrent with extrinsic regulation of myocardial function comprising nervous (electrical) and chemical (hormonal) control is made possible by the non-invasive technique of ratio fluorimetry. PTI offers instrumentation configured to perform such measurements and acquire multiple datasets of $[Ca^{++}]_i$, force, pH, temperature, etc., into as many as eight detector input channels.

For the experiment described here, retrograde coronary perfusion with constant coronary perfusion pressure was established with a supply of oxygenated physiological saline circulated using a peristaltic pump. The isolated rabbit heart used in this example was loaded by perfusing with $5 \mu M$ Fura-2 AM (Teflabs, Austin, TX, USA) after careful preparation to avoid circulatory problems.

Hydrolysis of the indicator was assessed by taking the fluorescence excitation spectrum of the intracellularly trapped dye. UV excitation light of 340 nm and 380 nm wavelengths with 3 nm bandpass was delivered to the sample. Fluorescence emission of intracellular Fura-2 was gathered at 510 nm. Intracellular calcium transients and changes in the contractile force in the Langendorff-perfused beating heart were measured to study the effects of various Ca^{++} channel blockers, vasodilators, K^+ channel openers, and catecholamines.

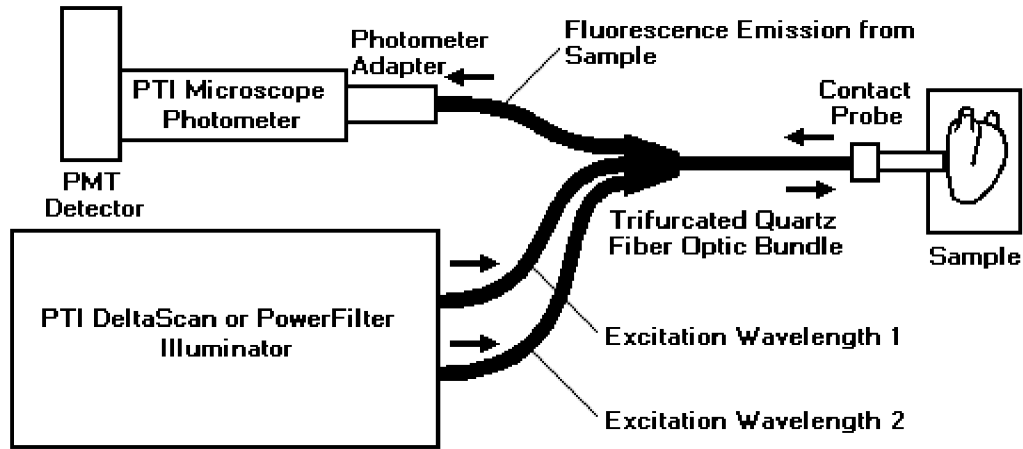


The measurements were performed in collaboration with the Department of Physiology University Medical School, Debrecen Hungary. Some of the experimental data is shown above.

A diagram of the basic system used for the experiment is shown below. The illuminator, a PTI DeltaScan™ high-speed dual-wavelength scanning system, delivers alternating wavelengths of light to the sample via two legs of a custom-designed trifurcated quartz fiber optic bundle. (A PTI PowerFilter™ illuminator can also be used.) The common end of the fiber optic bundle is fitted with a contact probe which delivers the excitation light to the sample. The third leg returns the emission light to a PTI microscope photometer fitted with one or two PTI Model 810 photon-counting photomultiplier detectors. The data is collected and analyzed on a PC-compatible computer with PTI's proprietary Felix™ Fluorescence Analysis Software. Other hardware configurations are possible to accommodate different PTI components as well as to perform dual-emission measurements.

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