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Application Note

Biofluorescence of Calcium

Use in heart muscle slices with Fura-8

Calcium sensing:

A specific target molecule in muscle contraction is Ca^{2+} as key intermediate signaling event between excitation and contraction of muscle fibers, and thus essential for the analysis of the force development in muscles. On the cellular level, force production is therefore directly related to the transient increase in the myoplasmic free calcium concentration. More specifically, the assessment of both parameters simultaneously is therefore critical in the evaluation and interpretation of the force development characteristics. Fluorescence techniques used in conjunction with muscle research systems (like WPI's **SI-MT-L** or **SI-HTB2**) to record muscle force is an **innovative technique** in cardiac muscle and skeletal muscle physiology. WPI's Biofluorometer (**SI-BF-100**) was specifically developed to monitor rapid changes in Ca^{2+} transients, i.e. $\Delta[Ca^{2+}]$.

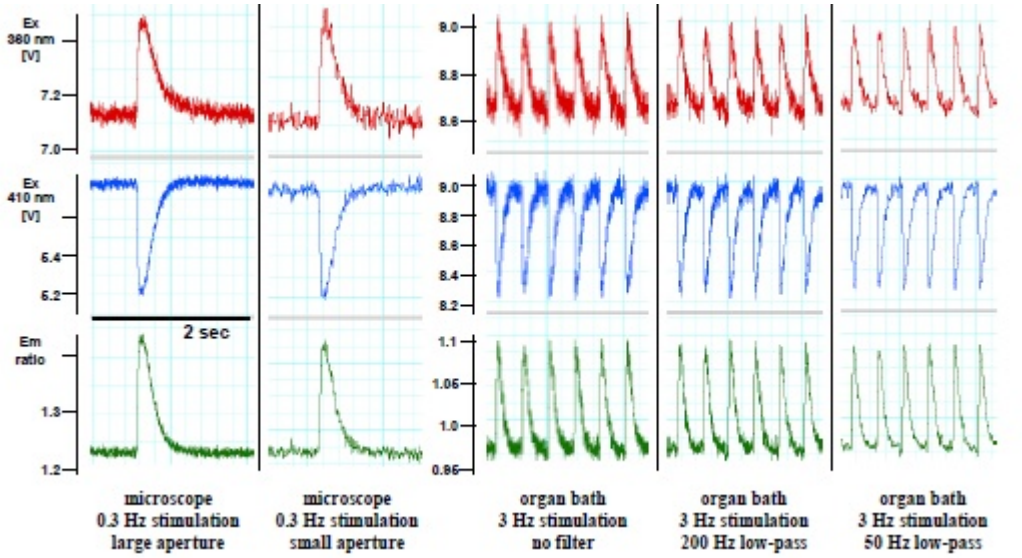

Figure 1. Organ bath setup: Average fluorescence intensities of Fura-8 loaded murine myocardium slices excited at 365/410 nm wavelength and detected at 525 nm. Left, using two apertures for fluorescence detection via the microscope. Right, same experimental condition using optical fiber fluorescence detection with the SI-BF-100SMA in the organ bath, sampled at 1 kHz. The organ bath data are low-pass filtered at 200 Hz or 50 Hz cut-off frequency. Adapted and reprinted with permission from Belz et al., Proc of SPIE Vol 9702, 97020Q-1 - 97020Q-11, 2016.

Advantages using Fura-8 dye:

- More sensitive to calcium than Fura-2
- Higher signal-to-noise ratio than Fura-2
- Simplified dye loading by incubation for 1 hour @ room temperature
- Emission peak response is shifted to longer wavelength (peak @525nm)
- Red-shift dual excitation wavelength (354nm & 415nm)

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Advantages of using the SI-BF-100

- Does not require warm-up time for stability
- Does not require costly excitation and emission mechanical wheel filters
- Optical fiber combiner to mix up to three High-Power (HP) LED modules
- Exchangeable and cost-effective HP LED modules to reduce the optical demands of the setup
- High sample rates to capture with high-fidelity the kinetics of ion transients
    - adjustable from 1Hz to 1000Hz
- Adjustable duty cycle can reduce potential for photobleaching
- High sensitive optical fibers and photomultiplier modules allow for detecting even weak fluorescence signals
- Direct ratiometric measurements to cancel out:
    - possible effects of uneven dye loading
    - movement artifacts that led to inhomogeneous distribution of the dye during contraction and thus the fluorescence light is not detected over the entire preparation/spot or
    - indicator bleaching
- Up to four modes of fluorescence detection of indicator dyes and natural fluorophores:
    - single excitation/single emission
    - dual excitation/single emission
    - single excitation/dual emission
    - dual excitation/dual emission

Application in heart muscle slices:

The Biofluorometer opens a wide field in functional fluorescence research, by studying the fundamental and/or applied aspects of the underlying energetics and signaling aspects of muscle contraction. This is notably useful in:

- Pre-clinical & Toxicological Studies:
    - screening of potential drugs
    - evaluating the side effects of drugs
    - evaluating models of cardiac disease
- Sports & Rehabilitation:
    - disuse vs. overuse
    - muscle damage
    - function for heart transplantation

Biofluorescence of Calcium: Use in heart muscle slices with Fura-8

Tissue sample requirements

- Slices of skeletal muscle
- Slices of cardiac muscle
- Typical tissue sample size 5-10 mm in diameter
- Minimal pre-stretched sample size 2-3 mm in diameter for detection spot.

Single emission probe (WPI #94650) for direct sensing of Ca^{2+} via indicator dye Fura-8

System configuration

The SI-BF-100 setup for detecting Ca^{2+} transients via Fura-8 depends on the excitation and emission wavelengths of the indicator dye Fura-8.

The SI-BF-100 setup depends on your experimental paradigm and the sample size of interest:

- BF-100SMA-C-Fura 8: direct sensing of calcium via direct measurement in tissue sample
- BF-1002XLG-C-Fura 8: direct sensing of calcium via microscope setup in muscle cell or in small tissue sample
- BF-100CAM-C-Fura 8: calcium imaging via microscope setup with camera in muscle cell or in small tissue sample

BF-100SMA-C-Fura 8:

Description	Item #
SI-BF-100 Main Unit with SMA connection	1xSI-BF-100SMA
HP LED 365 nm	1x99209-1
HP LED 420 nm	1x99209-4
Optical Filter 535 nm, 43 nm BP	1x802238
Single Emission Probe	1x94650
Data Sampling Board with Software	1xLABTRAX-MDAC

BF-100CAM-C-Fura 8:

Description	Item #
SI-BF-100 Main Unit with LLG connection	1xSI-BF-100LLG
HP LED 365 nm	1x99209-1
HP LED 420 nm	1x99209-4
Optical Filter 535 nm, 43 nm BP	1x802238
Liquide Light Guide Ø 3mm	3x802407
CM-CAM-2XLG Packaging	1x99259
Data Sampling Board & Software	1xLABTRAX-MDAC

BF-1002XLG-C-Fura 8:

Description	Item #
SI-BF-100 Main Unit with LLG connection	1xSI-BF-100LLG
HP LED 365 nm	1x99209-1
HP LED 420 nm	1x99209-4
Optical Filter 535 nm, 43 nm BP	1x802238
Liquide Light Guide Ø 3mm	2x802407
CM-2XLG Packaging	1x99261
Data Sampling Board with Software	1xLABTRAX-MDAC

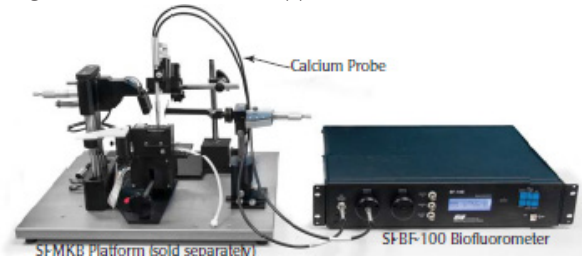
Options

Description	Item #
Options for direct sensing on tissue sample	
Manual Manipulator for securing probe	**M3301**
Magnetic Base	**M10**
Horizontal Tissue Bath with 1 channel	**SI-MT-L**
Horizontal Tissue Bath with 2 channels	**SI-HTB2**
Horizontal Tissue Bath with 4 channels	**SI-HTB4**
Options for imaging/direct sensing via microscope on muscle cells/muscle fibers	
Cell Tester	**SI-CTS200A**
OptiSarc with USB3 camera	**SI-OSARC**
USB camera for sample adjustment with camera	**USBCAM50**
Inverted microscope for BF-1002XLG or BF-100CAM setup	**INV-101**
Any other HP LED with specific wavelength*. WPI offers:	
HP LED 365nm	**99209-1**
HP LED 470nm	**99209-2**
HP LED 532nm	**99209-3**
HP LED 420nm	**99209-4**
HP LED 436nm	**99209-5**
HP LED 488nm	**99209-6**
HP LED 510nm	**99209-7**
HP LED 560nm	**99209-8**
HP LED 589nm	**99209-9**

**ask WPI for further details of the specifications.*

References

- *Belz et al., Fiber Optic Biofluorometer for Physiological Research on Muscle Slices (2016). Proceedings of SPIE, Vol. 9702 in Optical Fibers and Sensors for Medical Diagnostics and Treatment Applications XVI.*



Single channel muscle test system with SI-BF-100SMA-C-Fura 8 setup for Ca^{2+} detection during contraction.

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