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Biofluorometer

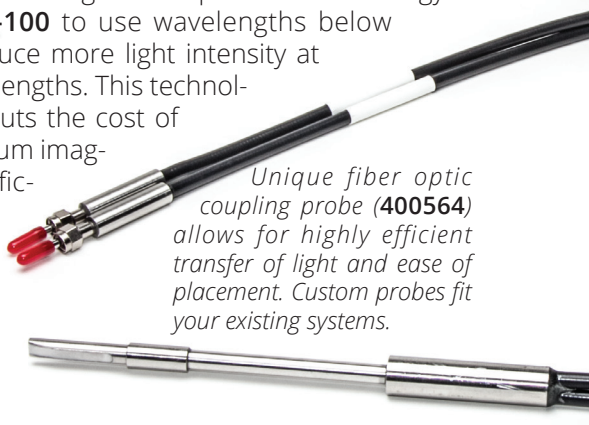
LED-based Fluorometer for Life Science Fluorescene Applications

The new **SI-BF-100** is an LED-based fluorometer for life science applications. It is predominantly used in one of two ways:

- Stand-alone for probe-based applications
- Connected to a microscope for fluorescence imaging

It is ideally suited for ratiometric calcium detection (FURA-8™) and ATPase detection (via NADH fluorescence). With up to three LED modules (wavelengths), the **SI-BF-100** covers many fluorometric applications in neuroscience, muscle physiology and cell biology.

Advancements in optics and LED technology simplify ratiometric calcium imaging, making this equipment more affordable. A breakthrough in WPI patented technology allows the **SI-BF-100** to use wavelengths below 380nm and produce more light intensity at those lower wavelengths. This technology significantly cuts the cost of photometric calcium imaging without sacrificing resolution or quality.



*Unique fiber optic coupling probe (**400564**) allows for highly efficient transfer of light and ease of placement. Custom probes fit your existing systems.*

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WHO

Key Audience

- Muscle Physiology
- Neurophysiology
- Biochemistry/Chemistry
- Biotechnology
- Drug discovery

Markets

- Screening of potential drugs
- Models of cardiac disease
- Functioning of transplanted heart
- Functioning of cultured heart tissue
- Muscle dystrophies/myopathies
- Muscle disuse/overuse or damage
- Pre-clinical & toxicology
- Sports & Rehabilitation

WHAT IT DOES

In-Vitro Applications

Using the **SI-BF-100 Biofluorometer** equipped with high intensity LED modules and a fiber-optic probe, researchers can perform many different types of analysis on intact tissue *in vitro*. Some potential applications include:

- Simultaneously monitor electrical activity and calcium concentration
- Simultaneously monitor force generation and calcium generation

Microscopic Imaging Applications

Using the **SI-BF-100 Biofluorometer** equipped with high intensity LED modules, coupled with an inverted microscope, researchers can perform many different types of analysis on both single cells and small cell cultures. Some potential applications include:

- Simultaneously monitor electrical activity and calcium concentration
- Simultaneously monitor force generation and calcium concentration.
- Integration of fluorescence microscopy onto existing patch-clamp setups

WHY IT WORKS

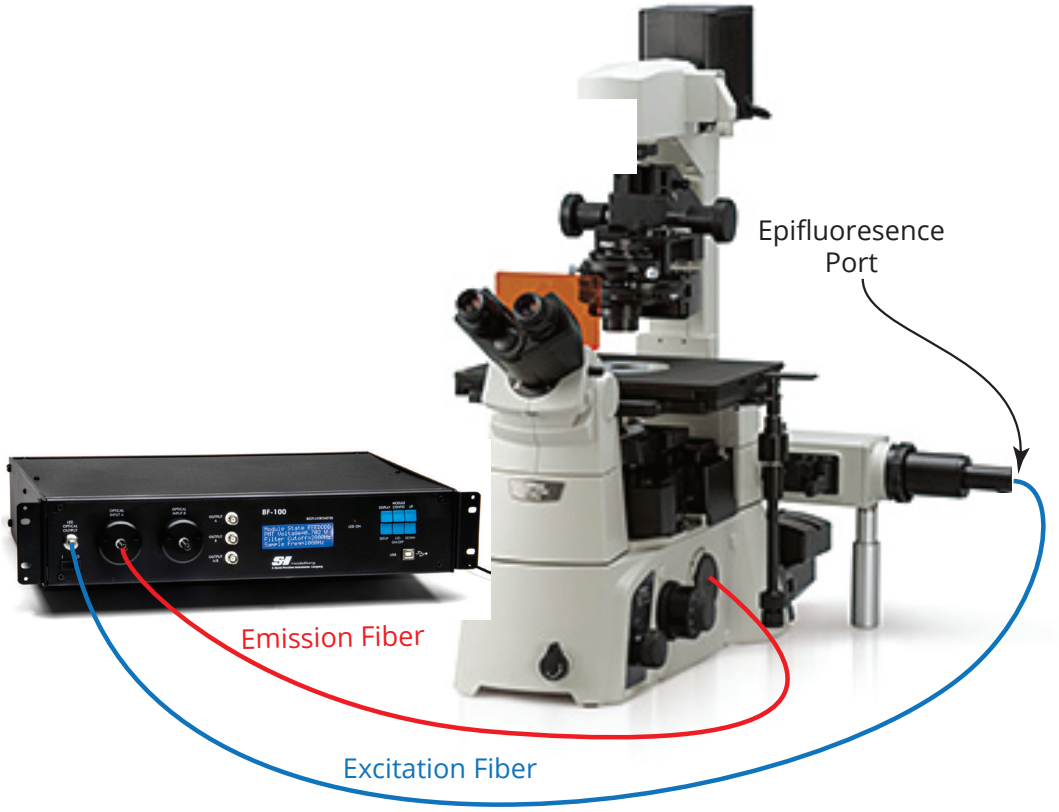
Benefits

- Most accurate and affordable fluorometer on the market today
- LED light source means no warmup time and 10,000 hours of lamp life
- Choice of a single wavelength designed for your specific application
- Eliminates need for costly excitation and emission filters
- Require NO costly lamp replacement
- Includes up to three (3) LED modules
- Electronic controls of the LED modules means no filter wheels required

On this dual excitation probe, the LEDs in the center of the probe are illuminated, while the exterior ring of LEDs is dark.

Features

- LED light sources require less power, give off less heat and are more compact and affordable
- Sampling rates up to 1kHz (1,000 ratios/s max.). At lower speeds, signal averaging is used for noise reduction.
- The warm up time of less than one minute is a dramatic improvement over the common 20–60 minutes required by xenon or mercury light sources
- Using a separate reference channel, ultra-stable, continuous ratio calculations automatically compensate for LED intensity drift–less noise and more accurate measurements
- Application-specific probes available for existing tissue baths and cuvette systems
- Replace the emission filter easily or change the LED modules to transform the **SI-BF-100** into a general purpose fluorometer for many other applications
- Can accept external light source for use of specific wavelengths or intensities
- BNC analog outputs (±10V) relate to the emission intensities for both the individual channels and a ratio



The Biofluorometer can connect to the epifluoresence port of a microscope, and its high intensity LED light source is used for the illumination.

Probe Options

Method	Description	WPI-Supported Application
Single excitation/ Single emission	Sample is excited at one frequency of light. As a result, a single (but different) wavelength of light is emitted and directed to a photomultiplier tube (PMT).	ATPase measurement via NADH. Excitation: 365 nm Emission: 470 nm
Single excitation/ Double emission	Sample is excited at one frequency of light. As a result, two separate wavelengths of light are emitted. The emission light is directed to two different PMTs which measure the light intensities independently.**	GFP based calcium detection Excitation: 460 nm Emission: 480 nm without calcium and 530 nm with calcium
Double excitation/ Single emission	Sample is excited as two different frequencies of light flash alternately. As a result, a single (but different) wavelength of light is emitted and directed to a PMT. The ratio of the emission (as a result of each excitation wavelength) is used to determine calcium concentration.	Calcium measurement with Fura-8™ Excitation: 365 nm and 410 nm Emission : 525 nm
Double excitation/ Double emission	Sample is excited as two different frequencies of light flash alternately. As a result, two separate wavelengths of light are emitted. The emission light is directed to two different PMTs which measure the light intensities independently.	No currently supported examples

**NADH is auto-fluorescent and indirectly measures ATPase activity.

SI-BF-100 Specifications

Ca^{2+} Excitation Wavelength ..... 365 nm, 410 nm
ATPase (NADH) Excitation Wavelength ..... 365 nm
Fiber Optic Light Input/Output ..... LLG or SMA terminated
Bandwidth ..... 1000 ratios/second
Analog Output Range ..... 0–10 V (continuous, equivalent to a ratio 0–10)
Analog Output Impedance ..... 100 W
Power ..... 12 VDC, 0.5 A, (universal power supply, 110/240 VAC)
Warm Up Time ..... <1 minute
Dimensions ..... 3.5"H x 17"W x 13"D (88 x 431 x 330 mm)

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Available LED Modules

The Biolfuorometer comes with three LED Modules for three different wavelegnths.

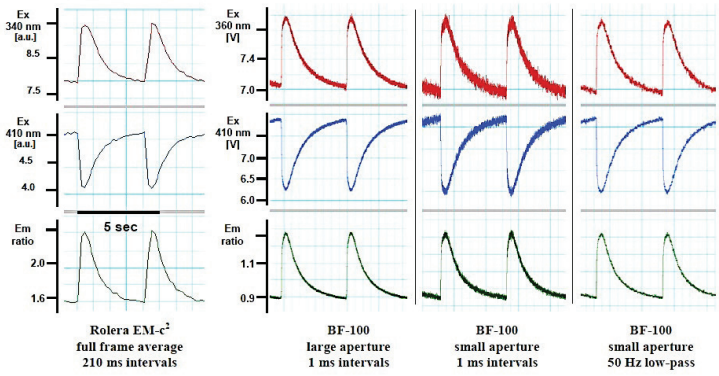
LED Wavelength	365 nm	410 nm	460 nm	520 nm
Power Rating	10 mW	30 mW	50 mW	30 mW
Full-Width Half-Maximum	20 nm	20 nm	20 nm	20 nm
Example Applications	• Indo-1 (Calcium Measurement) • NADH (Cellular Energetics)	• Fura-8™ (Calcium Measurement	• Fluo-3,4,8 (Calcium Measurement) • FAD (Cellular Energetics) • GFP	

NOTE: Ratiometric measurement available in double excitation and single emission mode with 365 nm and 410 nm excitation wavelengths for Fura-8™ use.

References

Fluorescent probes have emerged as indispensable tools for the investigation of cellular physiology. Small molecule dyes are taken up by cells, and alter their fluorescent characteristics in response to ion binding or membrane integration. This enables the determination of various metal ions, such as Na^+, K^+, Ca^{2+}, Mg^{2+} and Zn^{2+}, as well as pH and membrane potential in various cellular compartments. In striated muscle, the interaction between membrane potential (Vm) and intracellular free Ca^{2+} determines the excitation-contraction coupling and is essential for the analysis of force development. Furthermore, fiber optic probes allows collecting fluorescence from a small tissue area, while a relatively high light intensity can be obtained, which may help to reduce the optical demands of the setup. In addition, high sample rates can be used with such a setup, which is mandatory for the correct assessment of the kinetics of ion transients and action potentials.

A fiber optic based Biofluorometer with three LED excitation sources and two PMT based detectors, which can be connected to a fluorescent microscope or fiber optics probes has been developed. The focus of this work was to determine the performance of this instrument in the detection of calcium transients on human and murine myocardium slices in a microscope setup and a horizontal tissue bath, allowing the simultaneous registration of the fluorescence signal and contraction forces. It was shown that the high power LED-based excitation sources in the instrument performs well down to excitation light starting at 365nm wavelength. The two PMT-based fiber coupled detector inputs can measure fluorescent ratios up to a speed of 1000Hz, enabling the observation of calcium transients in stimulated human and murine myocardium tissue in a microscope setup and in a horizontal tissue bath, making the instrument a very versatile research tool.



Microscope setup: Average fluorescence intensities of Fura-8™ loaded human left ventricular slices excited at 340/410 nm or 365/410 nm, respectively and detected at 525 nm. Data represent the calculated ratios (lower traces). Left the imaging data of a Rolera EM-c² camera (left) and right the response of the SI-BF-100LLG using two aperture settings. The fluorescent data collected with the small aperture setting and low-pass filtered at 50 Hz, gives lower noise disturbed data but are still comparable to those data obtained with the large aperture. Note the large time difference in the detection of the fluorescence signal between SI-BF-100LLG detection and the camera based imaging data (1ms vs. 210 ms), allowing the detection of rapidly changing Ca^{2+} transients (adapted from Belz et al., SPIE letters, 2016).

Belz, M., Dendorfer, A., Werner, J., Lambertz, D., & Klein, K.-F. (2016). *Fiber optic biofluorometer for physiological research on muscle slices*. In I. Gannot (Ed.), SPIE BiOS (p. 97020Q). International Society for Optics and Photonics. http://doi.org/10.1117/12.2220291

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