Fiber Optic Biofluorometer for Physiological Research on Muscle Slices

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ABSTRACT

A focus of research in cell physiology is the detection of Ca^{2+}, NADH, FAD, ATPase activity or membrane potential, only to name a few, in muscle tissues. In this work, we report on a biofluorometer using ultraviolet light emitting diodes (UV-LEDs), optical fibers and two photomultipliers (PMTs) using synchronized fluorescence detection with integrated background correction to detect free calcium, Ca^{2+}, in cardiac muscle tissue placed in a horizontal tissue bath and a microscope setup.

Fiber optic probes with imaging optics have been designed to transport excitation light from the biofluorometer's light output to a horizontal tissue bath and to collect emission light from a tissue sample of interest to two PMTs allowing either single excitation / single emission or ratiometric, dual excitation / single emission or single excitation / dual emission fluorescence detection of indicator dyes or natural fluorophores. The efficient transport of light from the excitation LEDs to the tissue sample, bleaching effects of the excitation light in both, polymer and fused silica-based fibers will be discussed. Furthermore, a new approach to maximize light collection of the emission light using high NA fibers and high NA coupling optics will be shown. Finally, first results on Ca^{2+} measurements in cardiac muscle slices in a traditional microscope setup and a horizontal tissue bath using fiber optic probes will be introduced and discussed.

Keywords: fluorescence, optical fibers, free calcium detection, Ca^{2+}, cardiac muscle tissue, horizontal tissue bath, microscope

1. INTRODUCTION

Fluorescent probes have emerged as indispensable tools for the investigation of cellular physiology [1]. 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 [1,2,3]. In striated muscle, the interaction between membrane potential (V_m) and intracellular free Ca^{2+} determines the excitation contraction coupling and is essential for the analysis of force development. The assessment of both parameters by measurement of fluorescence from Ca^{2+}- and V_m-sensitive dyes has therefore evolved as a standard technique in cardiac muscle physiology. Imaging even provides a spatial resolution of these fluorescence signals, thus enabling the analysis of excitation conduction and of local cellular inhomogeneity. Imaging techniques, however, require elaborate biological preparations, such as dye-loaded perfused hearts, and can provide high spatial resolution only when tissue motion is effectively eliminated. In this manner, the imaging approach does not permit a simultaneous registration of fluorescence signals and contraction forces. For many research applications and for drug screening, measurements at the cellular level are not required, while the analysis of cell responses in a defined tissue area would be a more versatile approach. Such measurements can be performed in multicellular tissue preparations kept in well-established setups for muscle contractility determinations, such as a horizontal organ bath. By collecting fluorescence from a small tissue area, a relatively high light intensity can be

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obtained, which may help to reduce the optical demands of the setup and to achieve a high sample rate that is mandatory for the correct assessment of the kinetics of action potentials and ion transients [1, 2].

In this work, we focus on the detection of free calcium, Ca^{2+}, in slices of mouse and human heart tissue using a ratiometric fluorescent measurement approach. The technique is essentially independent of uneven dye loading, sample thickness, photobleaching effects and dye leakage. Indo-1, Fura-2 and the recently developed Fura-8 [3] are commonly used dyes excited by either single or dual wavelength excitation and/or by dual or single emission detection, respectively. Until now, fluorometers for physiological and general biological research were complicated devices with powerful light sources based on discharge lamps, filter wheels that selected the proper wavelengths of excitation light, multiple photomultipliers tuned to detect emissions from bound and unbound indicators and processors that compensated for fluctuations in light intensity, as well as motion artifacts. PMT and high speed filter wheel-based systems allowed the collection of 200-500 ratios per second, whereas camera-based systems allow the detection of 20–50 ratio based frames with a reasonable signal to noise.

We designed a fiber optic based biofluorometer (BF-100, World Precision Instruments, Inc.) targeted at muscle research using exchangeable high power LED modules, photomultiplier modules and optical plastic fiber combiners, avoiding any moving and high-priced parts. Either a fluorescence microscope using an epi-fluorescent input port and a c-mount terminated output port or fiber optic probes detecting fluorescence in a tissue bath can be connected to the instrument using 3 mm liquid light guides or PMMA plastic fibers with a 3 mm core diameter (Figure 1).

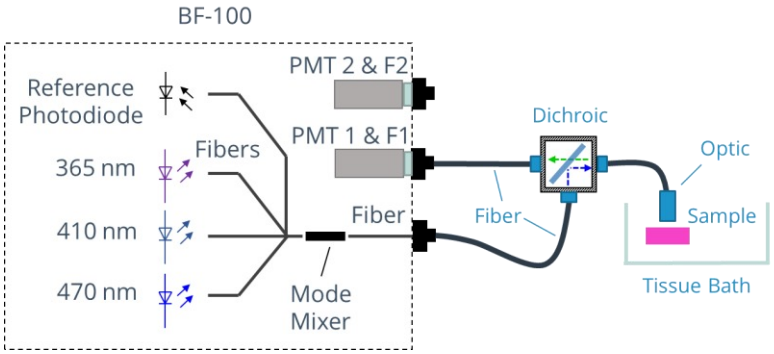

Figure 1: Schematic setup of the LED-based biofluorometer with a fiber optic probe detecting a fluorescence signal from a tissue sample in a horizontal tissue bath. In this setup only one PMT is used.

Several fiber optic probes consisting of fused silica-based fiber optic bundles, plastic fibers or a dichroic mirror based single fiber arrangement were developed for usage on samples of different sizes and geometries in a horizontal tissue bath. Data was collected from the BF-100 with an 8-channel data acquisition system at 5 kHz sample frequency (LabTrax 8/16 with MDAC software, World Precision Instruments, Inc.). For reference, a traditional camera-based microscope setup consisting of a Zeiss Examiner upright microscope, a 10x magnification dip-in objective, and a Rolera EM-c^2 camera (QImaging) with a 0.8 mm x 0.8 mm field of view was used. The design concept of the fluorometer system, usage and limitations of PMMA plastic fiber in the coupling optics, performance of a microscope-based setup and a fiber optic based setup in a horizontal tissue bath measuring free calcium, Ca^{2+}, in human and mouse heart slices are being reported in the following paragraphs.

2. EXPERIMENTAL SETUP

2.1 Design Concept of the BF-100 Fiber Optic Biofluorometer

An entire fluorometer system, consisting of high power UV and VIS LEDs used as excitation light sources for either single or dual wavelength excitation, an optical fiber combiner to combine up to three LEDs, two photomultiplier modules as detectors for either single or dual wavelength detection and a microprocessor-based electronic controller board was designed. In order to avoid a costly dichroic mirror setup, the three LED modules were coupled to an internal mode scrambling and mixing PMMA fiber with a 3 mm core diameter via classical 3 x 1.5 mm core PMMA fiber bundle

(Figure 1). The LEDs are driven by a current source; current (light power), sample frequency and on/off times of each LED can be set to minimize dye bleaching during a measurement cycle [4]. The sensitivity of the instrument is set through the PMT's gain and its noise performance was improved through a 6th order filter after the PMT output, even before the signals are processed. For long-term stability, the unit has a feedback mechanism (Reference Photodiode, see Fig. 1) to monitor the actual excitation light output of the biofluorometer and make required adjustments to the output readings. Furthermore, within the measurement cycle, the background light is measured and subtracted from the fluorescence signal. This was found to be particularly useful with fiber optics probes being sensitive to the pickup of stray light in horizontal tissue bath applications. The biofluorometer records up to 1000 ratios / second, enabling, e.g., the recording of fast calcium transients in stimulated heart tissue of a mouse. In order to reduce bleaching, the ON time of the individual LEDs can be shortened down to 5% of the measurement cycle. A sample and hold circuitry after the PMTs captures the pulsed output and generates a real-time signal at the analog outputs of the unit.

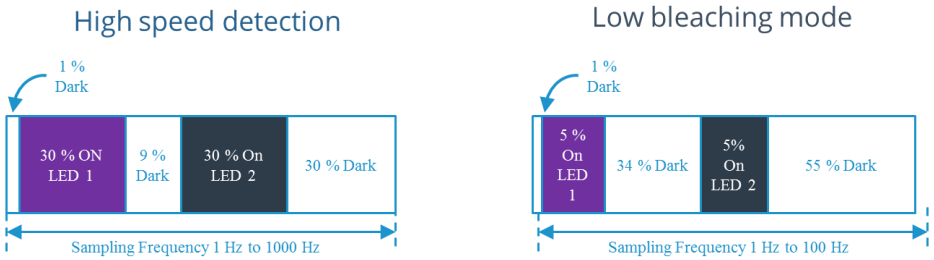

Figure 2: Duty cycle adjustment to reduce photo bleaching for sampling frequencies of 100 Hz and 1000 Hz. Module 1 and Module 2 turned on for 30 % at 1000 Hz and 5% at 100 Hz respectively.

The instrument can record in four different modes. In the first and traditional mode, a fluorophore is excited at one wavelength and the fluorescence signal is detected at a higher wavelength (single excitation, single emission). A typical example would be the detection of free calcium, Ca^{2+}, with Fluo8 [3]. In the second mode, an indicator is excited at two wavelengths and the fluorescent signal is recorded at one wavelength with a PMT. This mode is called dual excitation / single emission mode and is for example used in the detection of free calcium using the indicators Fura-2 or Fura-8, where the concentration of calcium is proportional to the ratio of the two recorded fluorescence signals. In this case, the detection becomes independent of dye concentration and indicator bleaching and was in our case the preferred detection technique. In a third mode, a fluorophore is excited at one wavelength and the fluorescence signal is detected at two wavelength using two PMTs. This case is called single excitation / dual emission mode and used with, e.g., Indo-1, where the ratio of the two fluorescence signal is proportional to the concentration of free calcium (3). Finally, in the fourth mode, dual excitation / dual emission, two separate fluorophores, e.g. NADH and FAD, having different excitation and emission wavelength requirements can be detected simultaneously with high speed.

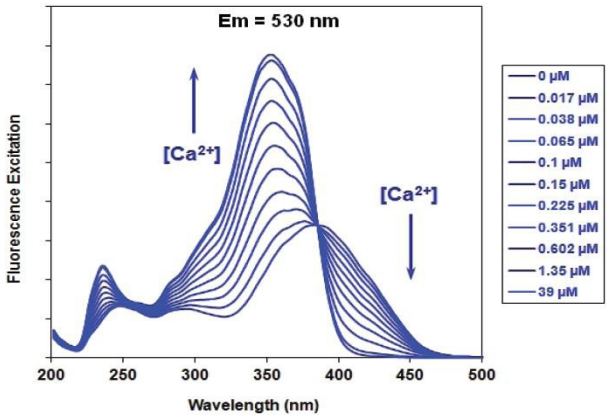

Figure 3: Fluorescence excitation spectra of Fura-8™ in solutions containing 0 to 39 µM free Ca^{2+} [3].

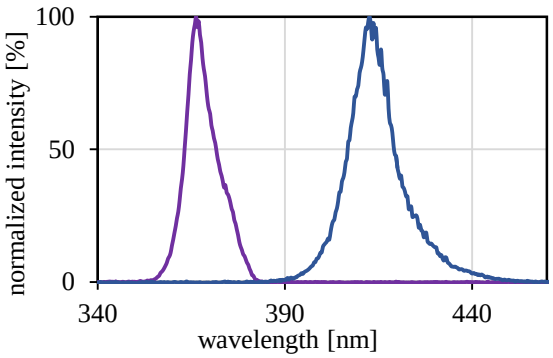

Figure 4: Normalized intensity spectrum of the 365 nm (violet) and 410 nm (blue) high power LED measured with a spectrometer (SpectraUSB, World Precision Instruments).

With the goal of this work being the detection of free calcium, Ca^{2+}, in muscle tissue in a microscope setup and in a horizontal tissue bath, we selected the ratiometric dye Fura-8. Fura-8 is a relatively new dye, but is based on Fura-2, which is a widely preferred calcium indicator, requiring excitation at 340 nm and 380 nm and emitting light at 510 nm. The key advantages of Fura-8 in comparison to Fura-2 in our application is that the dye can be excited at 365 nm and 410 nm wavelength and the emission of the dual excitation / single emission dye can be recorded at 530 nm (see Figure 3). This allows the usage of relatively inexpensive high power LEDs of 365 nm and 410 nm wavelength (see Figure 4). Further, it makes the usage of PMMA fibers possible, which tend to fluoresce when excited below 360 nm, generating a false fluorescence signal during a measurement.

2.2 Attachments of the fluorometer to a microscope and a horizontal tissue bath

Light is coupled to and from the biofluorometer using liquid light guides or optical fibers. An aperture is placed at the image location of the C-Mount output of the microscope (Axio Examiner Z1, Zeiss), which is then imaged either on a camera (not shown) or an optical fiber connected to the fluorometer. Figures 5A, B, C and D show connections to a microscope, a dichroic mirror based single fiber arrangement, a fiber optic probe based on 19 x 300 µm core diameter fibers positioned in a rectangular array of 1 mm x 3.8 mm and 19 x 300 µm fibers positioned in a circle shaped probe with an active bundle diameter of 1.8 mm. A dichroic mirror with a cutoff wavelength of 460 nm (T460LPXR, Chroma) and an emission filter with a center wavelength of 525 nm and a bandwidth of 50 nm were used in the microscope (A) and the probe B setup. Probe B uses a 15 mm focal length lens to image a sample onto a 3 mm liquid light guide. This probe exhibits a relatively large field of depth and is therefore especially useful for moving objects. With probes C and D, the rectangular and the round shaped fiber bundle is placed in close proximity of a sample object and works well with smaller and only slightly moving objects.

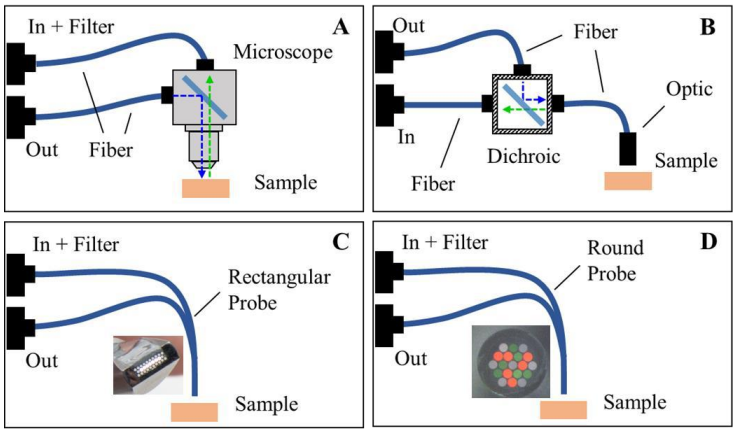

Figure 5: Possible experimental setups for the BF-100. **A** depicts the attachment to a fluorescent microscope, **B** shows a single fiber based detection system with a dichroic mirror and an imaging system at the distal end of a liquid light guide. Dichroic mirrors are used in **A** and **B** to separate excitation and emission light. In **C** a rectangular shaped fiber bundle and in **D** a round shaped fiber bundle is used to deliver excitation light to and pick up fluorescent light from the sample.

2.3 Setup for characterization of PMMA fibers

A key performance question of the biofluorometer in this application was the optical UV transmission and stability of plastic optical fibers (POF), in this case PMMA based fibers, in its internal optical combiner. Figure 6 shows a schematic illustration of two measurement setups to determine the basic attenuation and UV degradation of PMMA fibers. In Figure 6A, a PMMA based fiber is exposed to UV light of a high power LED (365 nm) coupled into the fiber; light power is measured with a power meter (ThorLabs PM100D + S140). The setup in Figure 6B is used to determine the

spectral attenuation using a deuterium / halogen light source (D4H, World Precision Instruments, Inc) and a fiber optic spectrometer (SpectraUSB2, World Precision Instruments, Inc.).

Figure 6: Sketch of the power measurement (**A**) and the spectral loss measurement (**B**) to determine the UV degradation of a PMMA fiber.

The spectral attenuation of a fiber was measured using the standard cut-back method [5, 6]. Based on two different lengths, l_x (x = short or long), with different output powers, P_x, the equation for the spectral attenuation $\alpha(\lambda)$ [dB/m] is as follows:

$$\alpha(\lambda) = (10/\Delta l)*\log\{ P_{short}(\lambda)/P_{long}(\lambda) \} \quad (1)$$

with $\Delta l = l_{long} - l_{short}$ as the length-difference between short and long lengths and $P_{short,long}$ as the output power of short and long fiber.

White light is coupled into the fiber under test. A polychromatic detector records the wavelength-resolved intensity spectrum at the output of the test fiber. (Fig. 6). The detector-system consists of a fiber-optic spectrometer (FOS) with a diode-array detecting system measuring the FOS-signal $S_0(\lambda)$, which is proportional to the output power of the fiber.

UV-light itself can change the transmission of a lightguide. For measuring these UV-induced changes generated by the UV-LED (365 nm), the setup **A** in Fig. 6 can be used; a similar system for UV-damage in all-silica fibers was described in more detail [7]. The PMMA fiber is irradiated by a high power LED at a wavelength of 365 nm, while the output power is measured by a power-meter (ThorLabs PM100D + S140C) for a duration of 24 hours. In addition, the spectral UV-induced loss was measured with setup **B**. The spectral loss L and gain G can be determined by comparing the transmission spectra before and after the irradiation, as follows:

$$L_{UV}\,[dB] = +(10) * \log\{ S_0(\lambda)/S_t(\lambda) \} > 0 \quad (2),$$

$$G_{UV}\,[dB] = -(10) * \log\{ S_0(\lambda)/S_t(\lambda) \} > 0 \quad (3),$$

with FOS-signal S_0 at the beginning (t = 0 s) and time-dependent FOS-signal S_t.

2.4 Tissue preparation and experimental conditions

Viable slices from human and murine myocardium were prepared as described by Brandenburger et al. [1]. In short, heart samples were obtained from C57BL/6 mice or from the failing myocardium of patients who had received a heart transplant. Whole mouse hearts and about 1 cm^3 cubes of left ventricular human tissue were embedded in agarose and cut into 300 µm thick slices using a vibratome. Murine slices were used at the day of the preparation, while human tissue was cultured with a liquid-air interface on organotypic filters [1], and was used for up to 12 days after preparation. Muscle slices were glued at opposing ends to plastic triangles (Histoacryl) and were pinned at relaxed length on a silicone sheet. In this state, they were incubated with 4 µM Fura-8, AM (AAT Bioquest) dissolved in HEPES-buffered M199 medium supplemented with 30 mM BDM and 0.7 % Kolliphor EL (Sigma-Aldrich) at room temperature for 2 - 6 hours. Prior to experimentation, tissues were washed with HEPES-buffered EBSS and incubated for at least an additional 30 min.

For microscopic observation, slices were mounted at 10 % extension of slack length in a custom-made chamber, and were submerged in HEPES-buffered EBSS at 30 °C. The slices were electrically stimulated with monopolar pulses of 3 ms duration and 50 mA current via graphite electrodes. Excitation of Fura-8 fluorescence was performed with a Lambda DG-4 Plus light source (Sutter Instrument) equipped with an ET340x (Chroma) and a 420/10 excitation

bandpass filter (EdmundOptics), or with the 365 nm and 410 nm LED Module of the BF-100 fluorometer. Images filtered with an ET-525/50m emission filter (Chroma) were obtained with a Zeiss Examiner upright microscope, a 10x magnification dip-in objective and a Rolera EM-c^2 camera (QImaging) controlled by VisiView software (Visitron). For the force measurements, slices were mounted to the force transducer of a horizontal organ bath, and were superfused with 37 °C oxygenated Krebs-Ringer solution. Length was adjusted to achieve 0.3 and 1 mN diastolic force for mouse and human tissues, respectively. Contractions were inducted via platinum field electrodes by 1 ms bipolar pulses at 50 % above excitation threshold.

3. EXPERIMENTAL RESULTS & DISCUSSION

3.1 Spectral Attenuation and UV Degradation of PMMA Fibers

Fig. 7 shows the spectral attenuation of two PMMA fibers of 750 and 1500 µm core diameter, measured with set-up B shown in Fig. 6. The 750 µm exhibits a slightly higher basic attenuation of 1.8 dB/m in comparison to 1.3 dB/m of the fiber with a core diameter of 1500 µm. However, below 340nm, the attenuation significantly increases in both fibers. Similar results have been published elsewhere [8]; especially at small core diameters of e.g. 250 µm.

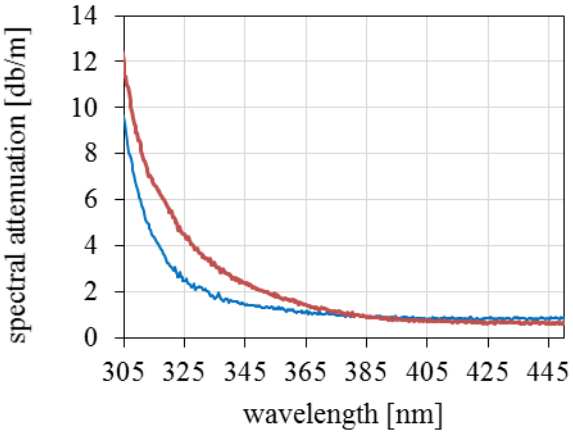

Figure 7: Spectral attenuation of two PMMA fibers with core diameters of 750 µm (red) and 1500 µm (blue) determined with the cutback-method using set-up in Figure 6 (B) [*7*].

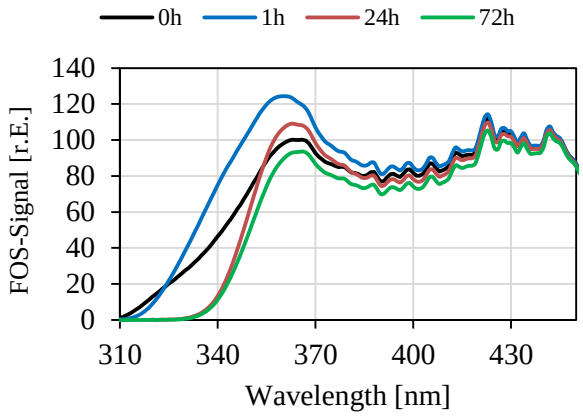

Figure 8: Measured output power of a 3 m long PMMA-based fiber with 1500 µm core diameter determined with the setup **B** in Fig. 6, before and after irradiation with an UV-LED (365 nm) for different time durations (t = 0, 1, 24 and 72 h) using setup **A** in Fig. 6. The results indicate an additional polymerization step leading to a higher transmission (at 1 hour) and an additional degradation for higher UV doses. The input power into the fiber was approx. 80 mW.

Fig. 8 shows the output intensity spectra of a 1500 µm core diameter PMMA fiber of 3 m length before (t = 0s) and after 1, 24 and 72 h of irradiation with a high-power UV-LED of 365 nm wavelength. A custom-tailored coupling system (see Fig. 6), resulted in input power levels as high as 80 mW. The gain of approx. 1.2 dB after 8 hours of 365 nm irradiation or UV-light delivery through the POF was determined at these input power levels. As indicated in [8,9], the degree of polymerization of the PMMA-based fiber is responsible for this effect, especially observed for fibers with smaller core diameter, e.g. below 500 µm. At longer continuous irradiation, light transmission was found to decrease slightly at and below 365 nm wavelength. After approximately two days of continuous transmission at 365 nm, light levels were found to decrease. UV-LED induced losses appeared to increase linearly but only slightly with time [9], thus proving, that PMMA based fibers can be used for light delivery at such wavelengths. Recently, high-power UV-LEDs for shorter wavelengths became available (Thorlabs). As excitation at 340nm is of particular interest to us, preliminary results done with such LEDs indicate that induced losses in a 1 m long PMMA-based fiber was found to be significantly increased by 5 dB and more after a 24 hour period. Details of this study will be presented in the near future.

3.2 Comparison of Camera and PMT based calcium detection with a microscope setup

Figure 9 shows a microscopic analysis of Ca^{2+} transients in human myocardial tissue. A slice of explanted human myocardium was cultured for 12 days, loaded with Fura-8, and stimulated at 0.25 Hz. A high resolution picture of fluorescence at 410 nm excitation and 525 nm emission reveals reasonably uniform labelling of the cardiomyocytes at the tissue surface (Figure 9). The image depicts a 0.8 mm by 0.8 mm field of view and was used to compare the performance of the camera-based and biofluorometer-based detection of free calcium. As described above, an adjustable aperture was placed into the image position of the c-mount terminated output port of the microscope. Figures 10 and 11 show the two image sizes the aperture was adjusted to during the following experiments with the biofluorometer. The diameter of the large aperture was set to 0.8 mm, the diameter of the small aperture was set to 0.25mm.

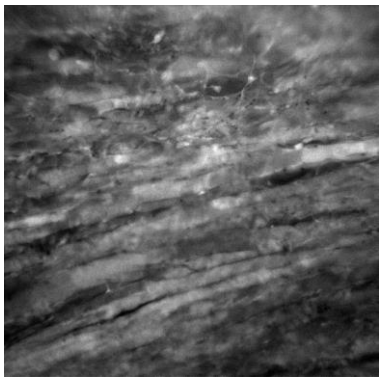

Figure 9: fluorescence image of a Fura-8 loaded slice of human myocardium.

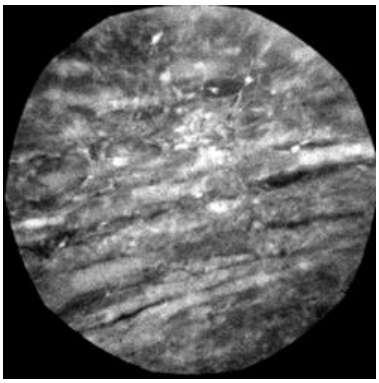

Figure 10: image of Fig. 9, confined by the large aperture setting (0.8 mm)

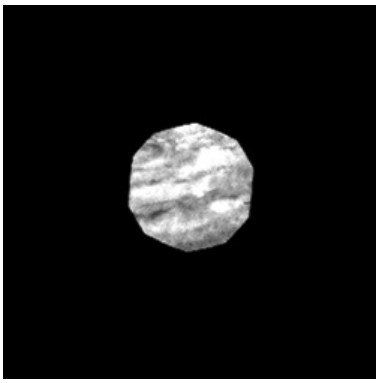

Figure 11: image of Fig. 9, confined by the small aperture setting (0.25 mm)

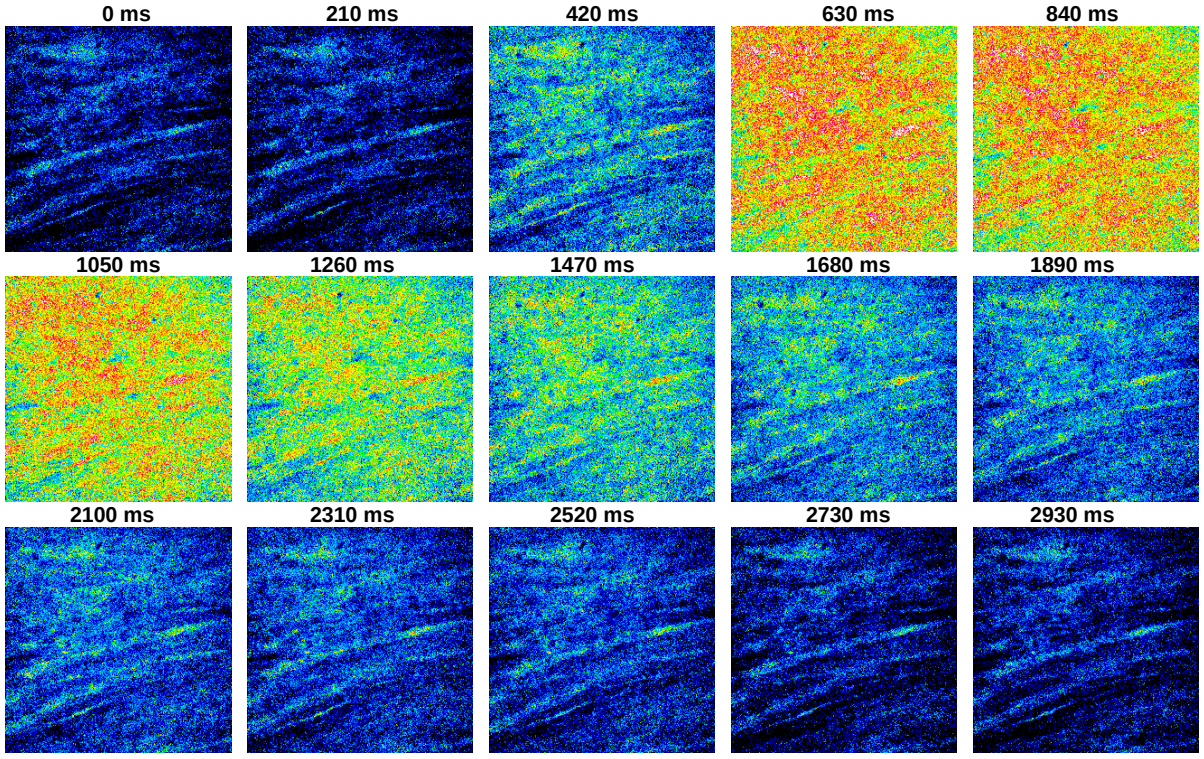

Figure 12: Color coded images of the intensity ratios at 525 nm emission taken at alternating excitation at 340 and 410 nm. Image pairs were recorded over a complete contraction cycle at 210 ms intervals.

Pairs of images were captured at 525 nm with excitation wavelengths of 340 nm and 410 nm at 210 ms intervals. Each image had a 250 x 250 pixel resolution and an integration duration of 30 ms. The image series (Figure 12) depicts the color-coded intensity ratios at 340/410 nm excitation for each pixel during a complete contraction cycle. The time series demonstrates a well synchronized Ca^{2+} transient over the observed tissue area. The average fluorescence intensities calculated from the total image area (Figure 13, leftmost graph) permit quantification of the amplitude of Ca^{2+} transients, but provide too little time resolution for kinetic analysis. On the right side of Figure 13, fluorescence data obtained with the BF-100 is shown. The instrument was set to 1000 ratios/second and signals were recorded at 5000 Hz. Fluorescent light was collected with the same emission filter centered around 525 nm used in the microscope setup, however the Fura-8 stained tissue was excited at 365 nm and 410 nm using high power LED modules of the BF-100, respectively. Ratios were calculated from raw data and not corrected for background fluorescence and are therefore for illustration of the signal performance only.

Data recorded with the BF-100 has a much higher time resolution due to the fact that a ratio is calculated every 1 ms. This can be observed at the rising and falling slopes of the calcium transients. The reduction of signal caused by the small aperture setting was adjusted by increasing the PMT gain slightly. However, this yielded an increase in noise, which was reduced by applying a 50 Hz low pass filter to the recorded signal.

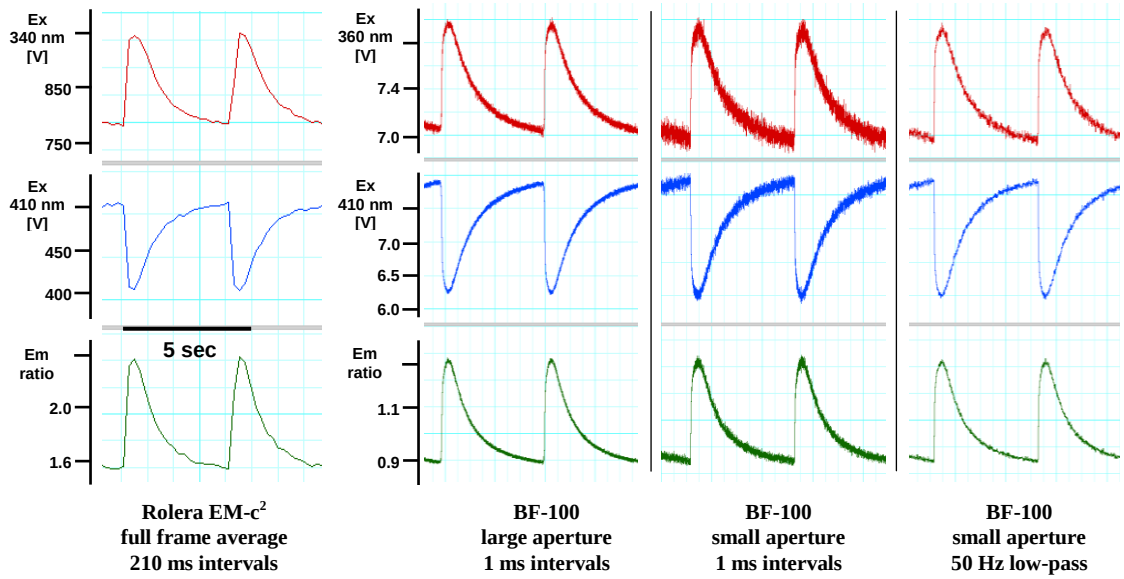

Figure 13: Average fluorescence intensities detected at 525 nm, when excited at 340nm and 410nm respectively and ratios calculated from the imaging data of the Rolera EM-C2 camera (left). Fluorescence response of the BF-100, detected at 525 nm, when excited at 365 nm and 410 nm wavelength using the large and the small aperture setting. Furthermore, fluorescent data collected with the small aperture setting and filtered with a 50 Hz low pass filter is shown.

3.3 Detection of calcium transients in human myocardium in a microscope setup and in a horizontal tissue bath

The next goal of this work was to detect calcium transients in a horizontal tissue bath. Human myocardium slices were cultured for 12 days, loaded with Fura-8 and mounted in a horizontal tissue bath, superfused with 37 °C oxygenated Krebs-Ringer solution and attached to a force transducer at one side, as described above. Length was adjusted to 1 mN diastolic force and contractions were inducted via platinum field electrodes by 1 ms bipolar pulses at 50 % above excitation threshold at 0.5, 1 and 2 Hz. Probe B (Figure 5) was attached to the BF-100 biofluorometer and its distal end positioned 15 mm from the sample tissue, generating an excitation and detection spot of approx. 3 mm diameter at the tissue sample. The instrument was set to 1000 Hz ratio detection and 30% LED ON time (pulse width). In this

experimental setup, light is coupled through a dichroic mirror via a liquid light guide directly to the sample. Fluorescent light is picked through the same fiber but guided to the PMT input at the dichroic mirror. Signals were recorded at a 5000 Hz sampling rate and processed with a 50 Hz low pass filter. The positive inotropic effect of β-adrenergic stimulation and a corresponding increase in the peak levels of intracellular Ca^{2+} can clearly be demonstrated. The preparation responded with a further increase in contractility to an acceleration of beating rates, an effect that was accompanied at 2 Hz electrical stimulation by an incomplete return of cytosolic Ca^{2+} to baseline levels.

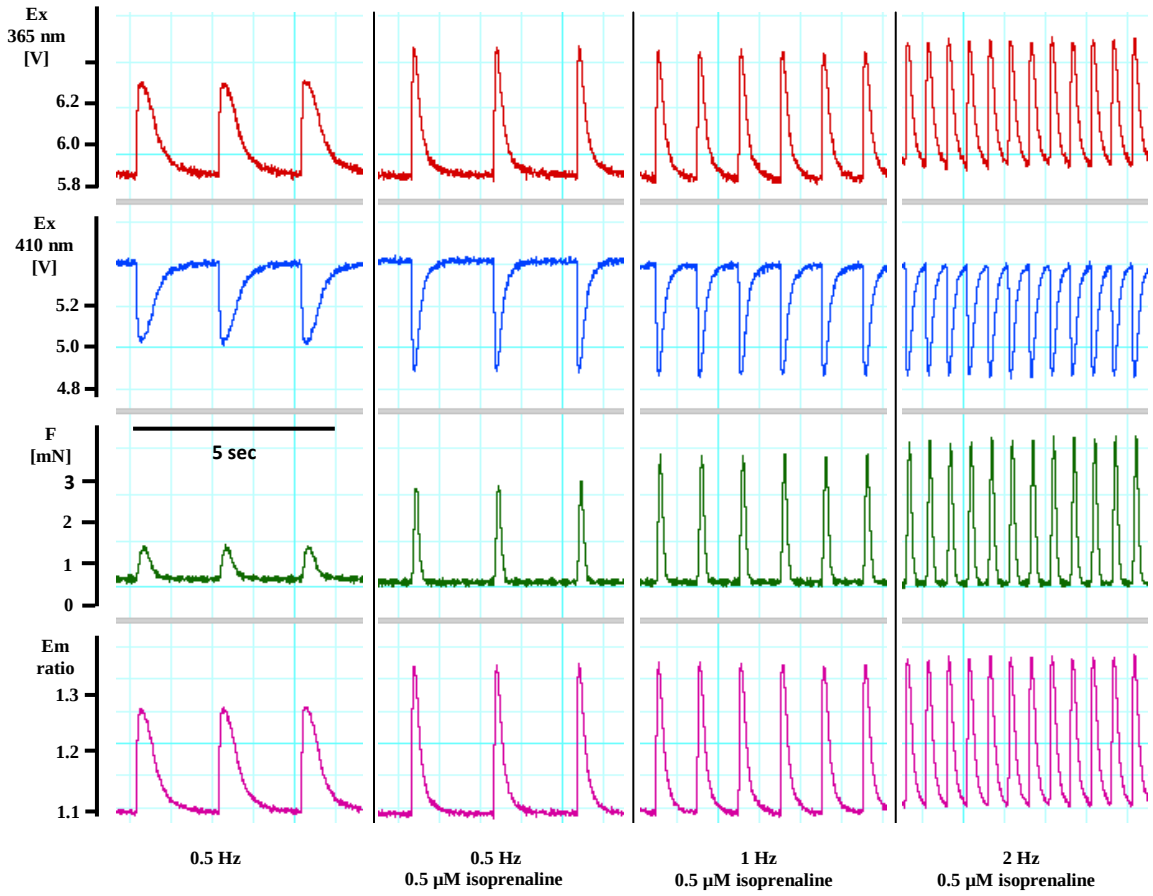

Figure 14: Simultaneous measurement of Ca^{2+}-transients and contraction force of human myocardium. A slice of human myocardium was cultured for 12 days, loaded with Fura-8, and mounted in an organ bath. Fluorescence measurements were performed with probe B (see Figure 5) of the BF-100 biofluorometer. Signals were recorded at 5000 Hz sampling rate and were processed with a 50 Hz low-pass filter.

3.4 Detection of calcium transients in murine myocardium in a microscope setup and in a horizontal tissue bath

Murine myocardium samples are much smaller than the human myocardium samples discussed previously. A typical heart slice has a relaxed diameter of 4-5 mm. During preparation, muscle slices were glued at the posterior and anterior sides of the left ventricular wall to plastic triangles and were pinned at relaxed length on a silicone sheet. In the microscope setup as well as within a horizontal tissue bath, the tissue was stretched to achieve around 0.3 mN diastolic force, which resulted in a sample strand of 2-3 mm width. The samples were superfused with 37 °C oxygenated Krebs-Ringer solution and stimulated via platinum field electrodes by 1 ms bipolar pulses at 50 % above excitation threshold. With the microscope setup, a 0.8 mm x 0.8 mm field of view using the 10x objective allowed the observation of a part of the left ventricular wall, when using the large aperture setting. Using the small aperture setting, an active part of the

tissue could be selected. However, in the horizontal tissue bath, the round fiber optic probe D with a bundle diameter of 1.8 mm (see Figure 5) was used. Although this probe is based on a fiber bundle with separate excitation and emission fibers and does not require a dichroic mirror and has a very small field depth, calcium transients could be observed and are shown in Figure 15. In order to improve the signal to noise, the data was processed through a 200 Hz and a 50 Hz Filter. Thus, the high sampling rate of the BF-100 enabled the recording of calcium transients stimulated at 3 Hz.

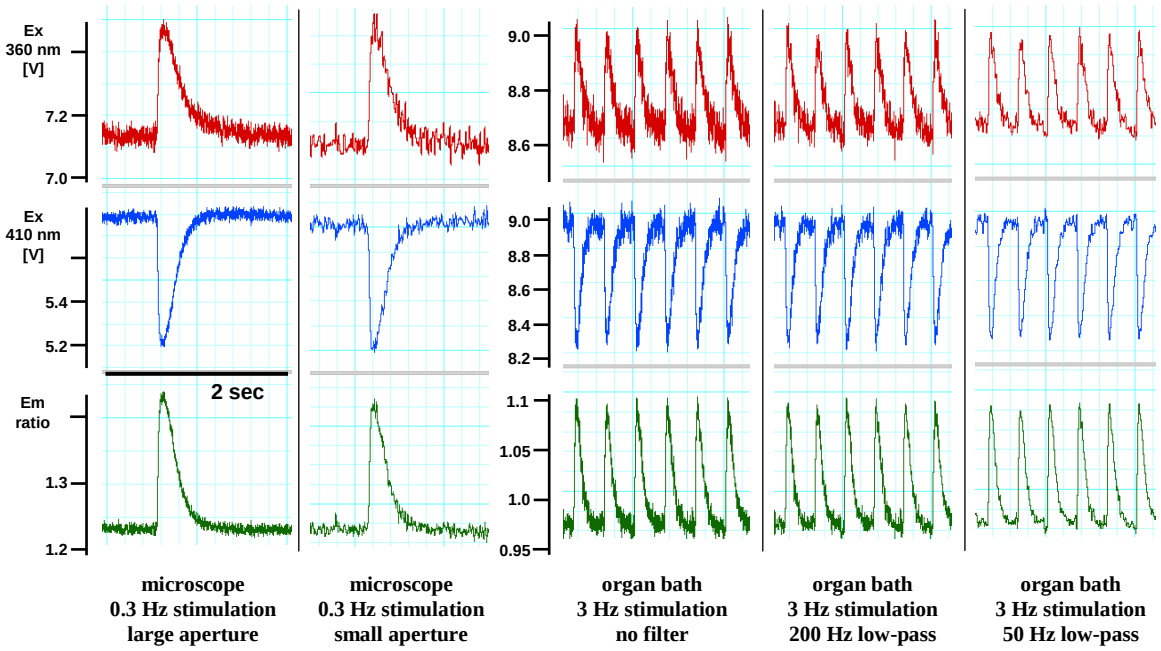

Figure 15: Average fluorescence intensities of Fura-8 loaded murine myocardium slices detected at 525 nm, when excited at 365 nm and 410 nm wavelength using the large and the small aperture setting in the microscope setup and a round fiber optic with a bundle diameter of 1.8 mm in a horizontal tissue bath. Furthermore, the horizontal tissue bath data filtered with a 200 Hz and a 50 Hz low pass filter is shown.

4. SUMMARY AND OUTLOOK

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. It was shown that the PMMA fiber-based combiner used to mix three high power LED-based excitation sources in the instrument performs well down to excitation light starting at 365 nm wavelength. The two PMT-based fiber coupled detector inputs can measure fluorescent ratios up to a speed of 1000 Hz, 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. As the fluorescence signals were not calibrated for 0 µM (absence of free calcium) and 40 µM of free calcium in these experiments, the calculated ratios can only be interpreted qualitatively, but not quantitatively. This will be the focus of future work. In addition, future investigations will focus on whether high power 340 nm LEDs can be used in this instrument. New applications and a more rigorous study of the performance of this new instrument will be published in the near future.

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