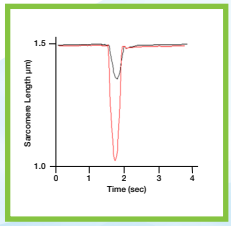
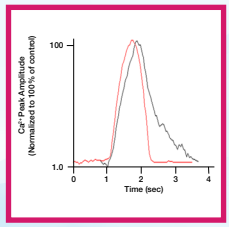
HORIBA Scientific

Fluorescence

Studying heart disease by simultaneous measurement of changes of Sarcomere Length and concomitant intracellular ionic calcium concentration in freshly isolated cardiac myocytes.





Application Note
Life Sciences

FL-2019-07-03

Abstract

From a pathophysiological perspective, the HORIBA Ratiomaster RM-50 with the Sarcomere Length measurement module enables investigators to directly assess whether cardiac dysfunction associated with cardiovascular diseases is mediated by alterations in cardiomyocyte free ionic Ca^{2+} concentration resulting in impaired cardiomyocyte contractility. This may be of particular importance in drug discovery and screening of novel compounds that may activate, or inhibit, specific signaling pathways in the heart and may be useful as a novel therapeutic strategy for improving cardiac function in a variety of cardiomyopathies. The Ratiomaster RM-50 is designed to measure and quantify calcium transients and cell contractility of fura-2 loaded cardiomyocytes. The system is capable of high sensitivity, high speed readout (1000 fps) of contrast edge changes and is based on a sensitive PMT photometer for detection of single photon fluorescence. Thus, Ratiomaster permits long duration kinetic measurements with real-time processing of sarcomere length and calcium ratios with full graph trend charting. High speed tracking of fura-2 loaded and electrically-stimulated cardiomyocytes can be used to study the effect(s) of pharmacological compounds on intracellular free Ca^{2+} concentration $[Ca^{2+}]_i$ and the timing of the Ca^{2+} transients, while simultaneously assessing the effects of the compounds on cardiomyocyte contractile function.

Introduction

Dr. Derek Damron's laboratory at Kent State University (Kent, OH, USA), utilizes freshly isolated, electrically stimulated cardiomyocytes to determine the extent to which intravenous anesthetic agents modify $[Ca^{2+}]_i$ and contractility, as well as to elucidate the signal transduction pathways by which this occurs. Current studies are focused on the screening of novel compounds that may be of potential therapeutic value for the treatment of patients suffering from a variety of forms of heart failure. The first step towards assessing the efficacy of novel therapeutic agents targeted at overcoming the cardiac dysfunction observed in the failing heart involves the use of fluorescence microscopy to examine whether these compounds modify $[Ca^{2+}]_i$ and associated changes in cardiomyocyte contractility. Several of the research group's compounds were tested in the Kent State HORIBA-sponsored Fluorescence Imaging Core lab facility in Kent OH, USA using fura-2 loaded, electrically stimulated cardiomyocytes to assess the extent to which the compounds result in changes in the magnitude as well as the rate of the rise and fall of intracellular Ca^{2+} concentration as a function of time, while simultaneously assessing the same parameters associated with cardiomyocyte contraction and relaxation.[1]

Materials and Methods

Cardiomyocytes were freshly isolated from mouse hearts and plated onto cover slips to be studied in a 37°C temperature-regulated imaging chamber (Warner Instruments) supported by an Olympus IX72 inverted fluorescence microscope. Healthy myocytes were selected and paced at a frequency of 0.3 Hz using a field stimulator (Grass Valley Stimulator). Felix GX software with sarcomere length module (SL-101HS HORIBA/PTI Piscataway, NJ) was used to select a region of the myocyte to track the striation pattern of the cardiomyocytes. A fast fourier transform (FFT) was completed on the optical density trace using the software to extract the changing frequency spectrum. The spectrum was then converted to length and calibrated against the objective magnification. This yielded changes in the contractility as a function of time. Simultaneously, Ratiomaster collected the calcium ratio fluorescence signal from the predefined region of interest to show concurrent changes in the intracellular calcium signals during the contractions and relaxations Figure 1.

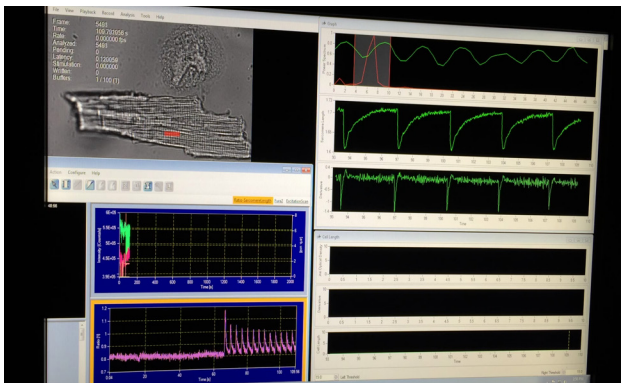


Figure 1: To play the movie of the related experiment click on the provided link: **https://youtu.be/pKOTi0sB0K0**

Results and Discussion

Figures 2 and 3 are representative traces depicting alterations in sarcomere length and $[Ca^{2+}]_i$ in cardiomyocytes before and after exposure to compound X. Individual traces are portrayed in an overlay to demonstrate compound X-induced changes in fractional shortening, sarcomere length, Ca^{2+} amplitudes and decay constants as a function of time.

HORIBA Equipment Used

RM-50 Ratiomaster
SL-101HS Sarcomere Length Option

Conclusions

This application demonstrates the utility of the Ratiomaster systems with the sarcomere length module for assessing experimental agent-induced alterations in fura 2-loaded, electrically stimulated cardiomyocyte kinetics. Real-time measurements of cardiomyocyte contractility and $[Ca^{2+}]_i$ can be used to quantify dynamic modifications in rates of contraction and relaxation, as well as intracellular Ca^{2+} cycling. These fundamental studies provide important insight into the development of novel therapeutic treatment modalities designed to enhance cardiac contractile function, particularly with respect to the contractile dysfunction observed in patients suffering from a variety of forms of heart disease.

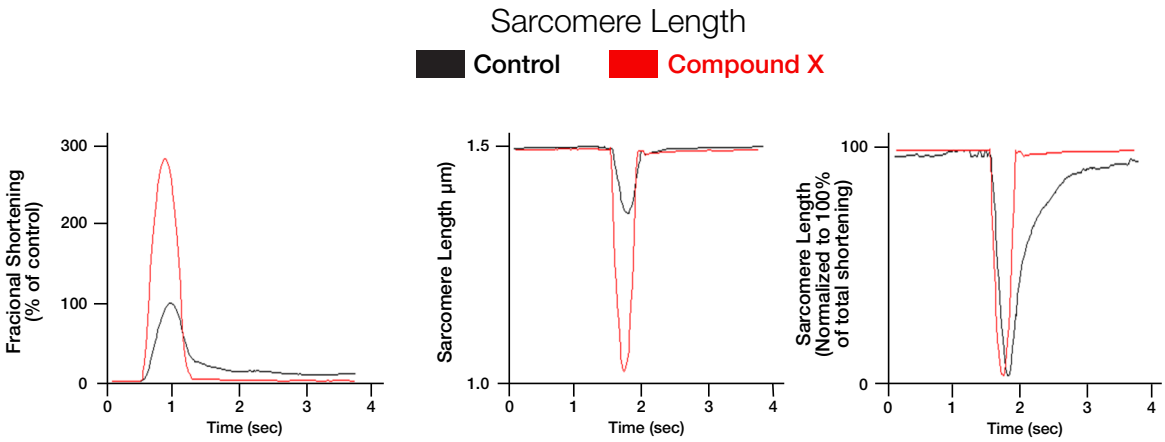


Figure 2

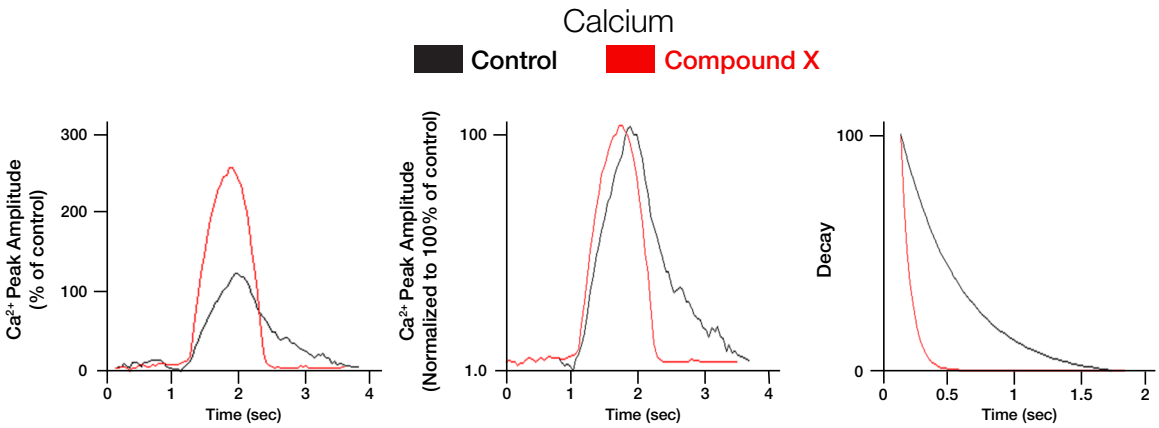


Figure 3

References

1. TRPA1 ion channel stimulation enhances cardiomyocyte contractile function via a CaMKII-dependent pathway. Andrei SR, Ghosh M, Sinharoy P, Dey S, Bratz IN, Damron DS. Channels (Austin). 2017 Nov 2;11(6):587-603. doi: 10.1080/19336950.2017.1365206. Epub 2017 Aug 25.

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