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## **SarcOptiM for ImageJ: high-frequency online sarcomere length computing on stimulated cardiomyocytes**

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1  
2 **SarcOptiM for ImageJ: high frequency online**  
3 **sarcomere length computing on stimulated**  
4 **cardiomyocytes**

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10  
11 **Running Head:**

12 Sarcomere length computing with ImageJ

13  
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31 Abstract

32 Accurate measurement of cardiomyocyte contraction is a critical issue for scientists working on  
33 cardiac physiology and physiopathology of diseases implying contraction impairment.  
34 Cardiomyocytes contraction can be quantified by measuring sarcomere length but few tools are  
35 available for this and none is freely distributed. We developed a plug-in (SarcOptiM) for the  
36 ImageJ/Fiji image analysis platform developed by the National Institute of Health. SarcOptiM  
37 computes sarcomere length via FFT analysis of video frames captured or displayed in ImageJ and thus  
38 is not tied to a dedicated video camera. It can work in real time or offline, the latter overcoming  
39 rotating motion or displacement related artifacts. SarcOptiM includes a simulator and video  
40 generator of cardiomyocyte contraction. Acquisition parameters such as pixel size and camera frame  
41 rate were tested with both experimental recordings of rat ventricular cardiomyocytes and synthetic  
42 videos. It is freely distributed and its source code is available. It works under Windows, Mac or Linux  
43 operating systems. The camera speed is the limiting factor since the algorithm can compute online  
44 sarcomere shortening at frame rates above 10 kHz. In conclusion, SarcOptiM is a free and validated  
45 user-friendly tool for studying cardiomyocyte contraction in all species including human.

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47 Keywords: cardiomyocyte contractility; sarcomere dynamic; video analysis; ImageJ plug-in; fast  
48 Fourier transform.

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## Introduction

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Isolated cardiomyocyte (CM) contractions can be recorded and measured with two main methods: cell shortening and sarcomere shortening. The latter is probably the most common and reliable technique used to characterize isolated CM contractile performance, because it does not depend on cell shape and size (4). Sarcomere shortening technique has applications in different research fields including cardiovascular physiology, pathophysiology such as heart failure, pharmacology and toxicology (2, 3, 6–8, 11).

Under transmission light microscopy, striated muscle cell sarcomeres show a transverse pattern due to the alternation of light (isotropic; I) and dark (anisotropic; A) bands corresponding to the very regular organization of thin filaments of actin associated with regulatory proteins such as tropomyosin and troponin (I band) and thick filaments of myosin (A band). These bands have a profile that can be assimilated to a sinusoidal curve. The frequency of this sinusoid, which represents the distance between the dark bands of the myosin filaments and therefore the sarcomere length (SL), can be extracted from Fourier spectrum analysis of the CM image. The sarcomere shortening method consists of computing the sarcomere spatial frequency and thus SL in each frame of a live or recorded video of a contracting CM. There are commercially available software for the analysis of SL and SL shortening but they are tied to dedicated video cameras and microscope systems (*e.g.*, IonOptix and Aurora Scientific Inc). Several laboratories have developed their own software, but under licensed languages such as LabVIEW™(1, 5) or MATLAB™(8, 10).

An open source algorithm has also been developed in Python to compute sarcomere length (9). However, it is not implemented in a program and requires users to develop their own software to interface their camera with the analysis algorithm. This unfortunately makes that software very difficult to use by people without programming skills.

The aim of our work was to provide scientists working on CM – or any other structure presenting a striated pattern (*e.g.*, transverse tubules, ryanodine receptors distribution, collagen structure, *etc.*) – with powerful, ultrafast and accurate software compatible with most of the video cameras and finally not affected by cell displacement and rotation in the field. Moreover, this software has to be easy and ready to use, free and open source. Thus, we developed SarcOptiM, a plug-in for the open source microscopy image analysis software of the NIH: ImageJ. It has two operating modes. Online mode allows the real time analysis and display of the contraction of a cell along a line (1 pixel thick or more) drawn along its longitudinal axis (*i.e.*, *on cell axis* mode). Offline mode is dedicated to the analysis of pre-recorded videos of contracting CM. This mode can be used in two ways: *on cell axis* mode, as above or *on entire frame*, which extracts data from the entire video image. SL measurements with the *on entire frame* mode avoid artifacts due to rotation and/or movement of the cell.

SarcOptiM has been validated and packaged with a number of control features including a means of constructing synthetic videos of a model cell contracting according to user defined parameters.

SarcOptiM has been tested under Linux, Windows and Mac OS. It is freely available in the plug-in section of ImageJ website and on <http://pccv.univ-tours.fr/ImageJ/SarcOptiM/>.

## 90 **Materials and methods**

### 91 **Isolation of rat ventricular cardiomyocytes**

92 All protocols have been approved by the local ethical committee (Comité d’Ethique en  
93 Expérimentation Animale Val de Loire, Tours, France). Adult male Wistar rats were anesthetized with  
94 pentobarbital (60 mg/kg). The heart was rapidly removed and coronary circulation was retrogradely  
95 perfused through the aorta. Enzymatic digestion was performed by perfusion of 0.1 UI/mL Liberase™  
96 Research Grade in Krebs-Ringer-Bicarbonate solution (in mM: 35 NaCl, 25 NaHCO<sub>3</sub>, 4.75 KCl, 1.19  
97 KH<sub>2</sub>PO<sub>4</sub>, 16 Na<sub>2</sub>HPO<sub>4</sub>, 134 sucrose, 10 HEPES, and 10 glucose, pH adjusted to 7.4) at 37°C. At the end  
98 of the digestion, ventricles were separated and gently mechanically dissociated with Pasteur  
99 pipettes.

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### 101 **Experimental setup and protocol**

102 Cardiomyocytes were placed in a perfusion chamber mounted on an inverted microscope (Nikon  
103 Diaphot 300) for online sarcomere analysis and video recording of contraction at 25°C. Myocytes  
104 were superfused with Tyrode’s solution containing (in mM): 140 NaCl, 5.37 KCl, 1.36 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>,  
105 0.33 NaH<sub>2</sub>PO<sub>4</sub>, 10 HEPES, 11 glucose, at pH 7.4, adjusted with NaOH. Myocytes were electrically field  
106 stimulated at 1 Hz with square wave pulses (80 mA, 2 ms) delivered through a pair of platinum  
107 electrodes.

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### 109 **Recording of cardiomyocyte contraction**

110 The videos of rat left ventricular myocytes contracting under electrical stimulation at 1 Hz were  
111 captured with an IDS UI-1220LE-M-GL camera (Imaging Development Systems, Germany). Online  
112 experiments in ImageJ used the plug-in HF\_IDS\_Cam (available on ImageJ website). Video recording  
113 for subsequent offline analysis used the camera supplier software uEye Cockpit. The camera was  
114 connected to an USB port of a PC workstation (Xeon E3 1241v3 3.5 GHz processor) running under  
115 either Linux or Windows 7 (64 bits) operating systems.

116

### 117 **Synthetic videos of a cardiomyocyte contraction simulation**

118 Each sarcomere measurement mode of SarcOptiM has been validated with synthetic videos of a  
119 modeled contracting CM. These were created with the *Video synthesis* tool which is implemented in  
120 SarcOptiM. In these synthetic videos, frame rate, pixel size and CM features (length, width, resting  
121 SL, sarcomere shortening and contraction/relaxation speed factor), as well as CM movement and  
122 rotation in the video field can be user defined to match particular experimental preparations and test  
123 user hardware. The equation:  $SL_t = SL_{min} \times e^{-\exp(-t \times SF) - t \times SF + 1}$  (with  $SL_t$ : SL at  $t$  time;  $SL_{min}$ : minimum  
124 sarcomere length at maximum peak shortening;  $t$ : time;  $SF$ : contraction/relaxation speed factor  
125 which adjusts the theoretical model to fast or slow twitches) is used to model the CM contraction. In  
126 our examples the standard parameters were as follows: resting SL: 1.8 μm, sarcomere shortening: 5  
127 % of the resting SL, pixel size: 0.3 μm, video frame rate equivalent: 100 Hz, number of frames: 40  
128 (*i.e.*, a total equivalent of 400 ms record), angle of the CM in the frame: 30° from the horizontal,

129 contraction/relaxation speed factor: 1. Example of synthetic CM generated by this module is  
130 available in the Data Supplement (Video\_1.mp4).

### 131 **Setting up and use**

132 To install SarcOptiM in ImageJ:

- 133 1. Unzip the SarcOptiM.zip file;
- 134 2. Put the SarcOptiM folder into the "ImageJ\plugins" folder;
- 135 3. Put the SarcOptiM.ijm file into the "ImageJ\macros\toolsets" folder.

136 The user guide on our institutional website and on the ImageJ website contains more information  
137 and a video demonstration of its use is available in the Data Supplement (Video\_2.mp4). It is  
138 important to note that another plug-in is necessary to acquire video flow from digital, *e.g.*,  
139 HF\_IDS\_Cam and Webcam Capture, or analog *e.g.*, PixelSmart Frame Grabbers cameras.

140

### 141 **Statistics**

142 All boxplots indicate minimum, first quartile, median and mean, third quartile and max values. All  
143 statistics have been performed with R and SigmaStat. For the comparison between groups, ANOVA  
144 tests were performed. Differences are considered significant when the P value is inferior to 0.05.

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## Results

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### Analysis algorithm description and outputs

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In *on cell axis* mode (Figure 1), the sarcomere measurement algorithm searches for a peak in the fast Fourier transform (FFT) of the grey level profile of the myocyte longitudinal axis along the line positioned on the image by the user (Fig. 1A, B). The position of the peak located in the FFT spectrum within the limits for SL values defined by the user is determined (Fig. 1C). Then given the pixel size, the sarcomere length is calculated.

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For an analysis with *on entire frame* mode, the algorithm is similar, except that the FFT is calculated over the entire image and is not confined to the myocyte. The peak is sought with native “find maxima” ImageJ function in a 2D plane within the limits for SL values defined by the user (Fig. 2). Then given the pixel size, the x-y coordinates of the peak and its Euclidian distance from the center of the Fourier spectrum, the SL is calculated.

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At the end of the sarcomere measurement computation, each SL value is plotted *versus* time in a new window (Fig. 1D, 2C). Time is determined by video frame rate. The XY data can be saved as a comma separated values file (.csv) or copied into the clipboard for further analysis.

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For a more complete understanding of the analysis, please consult the source code.

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### Accuracy of the SL measurement with different pixel sizes

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Since the accuracy of sarcomere measurement depends on the spatial resolution of the acquired image, the effect of pixel size upon SL parameters has been investigated with the *on cell axis* mode of SarcOptiM. In this test, we used a synthetic video of a mathematically modeled CM contraction with the standard parameters (see Materials and methods) and six realistic pixel sizes. The sarcomere measurement on each frame during the contraction and relaxation was compared to the value assigned to that point in the model. Absolute differences between each measured and each theoretical value are shown as boxplot in Fig. 3A. For a pixel size between 0.20 and 0.35  $\mu\text{m}$ , the error of the measurement is, in the worst case, inferior or equal to 0.005  $\mu\text{m}$ . For a pixel size of 0.40 and 0.45  $\mu\text{m}$ , the measurement error lies between 0.006 and 0.007  $\mu\text{m}$ . These values are far below the pixel size since there are obtained with a frequency analysis by FFT and not by a simple metric measurement (7).

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### Comparison between on cell axis and on entire frame modes of analysis

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The *on cell axis* mode is much faster than the *on entire frame* mode because the latter requires a two-dimensional FFT calculation instead of a single one. However, it could fail if the contracting cell moves and leaves the measurement axis. Analysis mode *on entire frame* avoids this problem since no measurement axis is required as illustrated by the video of a rat ventricular CM moving and rotating in the field (see Data Supplement (Video\_3.mp4 and Video\_4.avi)). Moreover, multiple dead cells in the analyzed field do not affect the SL measurement. However, this mode restricts application to offline analysis of pre-recorded experimental videos since actual performances of current computer



184 are insufficient to allow online analysis. To compare the quality of analysis in both modes, two  
185 synthetic videos with standard parameters (see Materials and methods) were used. In the second  
186 video, a rotary motion from 30° down to the horizontal was applied to the CM. The first video was  
187 analyzed with *on cell axis* and *on entire frame* modes. The second video was analyzed with the *on*  
188 *entire frame* mode alone. As above, SL data obtained through a contraction-relaxation cycle were  
189 compared to their model values and the results are shown in Fig. 3B. The *on cell axis* mode allows the  
190 best accuracy of measurement with a maximum error in the worst case less than 0.005  $\mu\text{m}$ . With the  
191 *on entire frame* mode, the worst case error is slightly higher since it can reach 0.009  $\mu\text{m}$  due to the  
192 pixel interpolation when the cell axis is not exactly horizontal or vertical. However, with this mode,  
193 the SL measurement is not affected and the maximum error is not significantly increased by the cell  
194 rotation in the visual field (Fig. 3B and 3C). Thus, the *on cell axis* analysis mode is recommended in  
195 standard conditions. The *on entire frame* analysis mode has to be used when the CM moves or  
196 rotates in the field despite this will result in a slightly higher variability of SL measurement.

197 A supplemental tool based on the offline *on entire frame* mode has also been developed to  
198 simultaneously measure contractions of several cardiomyocytes. It should be noted that this tool can  
199 be used only if the cells do not have exactly the same orientation in the video field. This is required  
200 to separate and distinguish the peaks corresponding to each cell in the FFT spectrum.

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## 202 **Effect of the sampling frequency upon the quality of the** 203 **measurement of rat cardiomyocyte contractions under basal** 204 **conditions and following $\beta$ -adrenergic stimulation with** 205 **isoproterenol**

206 Several video capture frequencies were tested to determine the optimal frame rate for sarcomere  
207 measurement of a rat ventricular CM before and after superfusion of 100 nM isoproterenol (known  
208 to increase contraction kinetic and amplitude). Examples of sarcomere measurements (*on cell axis*  
209 mode) in control conditions at video frame rates of 25, 50, 100, 250, 500, 1000 Hz are shown Fig. 4.  
210 The optimal frequency was determined by measuring for each rate the minimum sarcomere length  
211 (minSL) and maximum sarcomere shortening speed ( $d\text{SL}/dt_{\text{max}}$ ; Fig. 5A, B). The acquisition frequency  
212 is considered sufficient when minSL and  $d\text{SL}/dt_{\text{max}}$  vary by less than 5 % from the values determined  
213 at 1000 Hz. In control conditions, 100 Hz is enough to record minSL (1.708 vs. 1.708  $\mu\text{m}$  at 1000 Hz)  
214 and  $d\text{SL}/dt_{\text{max}}$  (-2.26 vs. -2.27  $\mu\text{m}\cdot\text{s}^{-1}$  at 1000 Hz). However, the sampling rate must be increased to  
215 250 Hz in the presence of isoproterenol to obtain an accurate  $d\text{SL}/dt_{\text{max}}$  (minSL: 1.576 vs. 1.576  $\mu\text{m}$  at  
216 1000 Hz and  $d\text{SL}/dt_{\text{max}}$ : -6.68 vs. -6.98  $\mu\text{m}\cdot\text{s}^{-1}$  at 1000 Hz). The effect of 100 nM isoproterenol on the  
217 contraction of a rat CM recorded at 250 Hz is shown in Fig. 5C, D. The increases of sarcomere  
218 shortening and of contraction and relaxation rates are clearly visible (Fig. 5D). Therefore, with this  
219 preparation and under these experimental conditions a frame rate of 250 Hz is recommended for SL  
220 measurements but this parameter has to be optimized for other experimental conditions.

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## 223 **Discussion and conclusions**

224 SarcOptiM is the first open source, easy to use and freely available software plug-in for the analysis  
225 of cardiomyocytes contractions with the sarcomere shortening method. We validated the  
226 measurement algorithms with synthesized videos with defined parameters. Tests were also  
227 conducted on isolated rat ventricular myocytes.

### 228 **Versatility**

229 SarcOptiM works with any analog or digital camera that can be interfaced with ImageJ, Fiji and  
230 Micromanager as shown by examples available on the software download webpage. This is not  
231 necessarily the case for licensed software which may work only with dedicated cameras. A non-  
232 exhaustive list of cameras working with ImageJ is available on the ImageJ website  
233 (Plugins>Acquisition section). Another list for Micromanager is available on micromanager website  
234 device section. Thus, a camera can be chosen to meet the requirements of the experiment, in  
235 particular with regards to the best resolution-sensitivity-speed compromise. Moreover, SarcOptiM  
236 allows sarcomere measurement on pre-recorded videos. This offline analysis is useful for  
237 experiments requiring several analyzes or if the CM moves or rotates in the optical field. Finally, the  
238 total analysis time can be significantly reduced with SarcOptiM. Indeed, the analysis could also be  
239 simultaneously performed on several cells with the offline *multiple cells on entire frame* mode,  
240 provided that cells do not have exactly the same orientation in the video field.

### 241 **Performance**

242 The reliability of the sarcomere measurement provided by SarcOptiM has been demonstrated by  
243 tests on synthetic videos. Measurements can be performed as long as CM sarcomeres are visible on  
244 the video, even in low light conditions. In addition, they are not affected by light oscillations as the  
245 calculations are done on the image FFT and not on their brightness. The limitation is sufficient grey-  
246 scale definition to separate signal-noise in the FFT (Fig. 1C). With these features, the use of mid-  
247 range cameras, such as the IDS UI-1220LE-M-GL, is possible. This camera is able to record sarcomere  
248 shortening at frequencies above 1000 Hz, although we found that a sampling rate of 250 Hz is usually  
249 enough for rat ventricular cardiomyocytes (Fig. 5). For other cell types, such as skeletal muscle, which  
250 require a high sampling rate, the measurement algorithm is not a limiting factor for online  
251 computing: at full speed, it can calculate SL at sampling rates far above 10 kHz.

252 In addition to its free distribution and versatility, SarcOptiM presents several other advantages since  
253 it is integrated into an image processing software. It is thus possible to work on the video as well as  
254 on images, to adjust brightness or contrast, to define areas of interest or other processing. With this  
255 plug-in, sarcomere measurement can be done whatever the orientation of the cell in the video  
256 frame, unlike most licensed software and the Python algorithm (9) which require a perfect horizontal  
257 orientation of the cell. Moreover, it allows an easy adjustment of the measuring axis during the  
258 analysis.

259 A model of myocyte contraction is included in SarcOptiM, which is another asset of the software.  
260 This is used to construct video simulations with user defined parameters. Users can thus test,  
261 evaluate and optimize pixel size, video frame rate, upload time and computer performance for their  
262 own experimental conditions and hardware configuration.

263 SarcOptiM has been developed to work under Windows, Mac OS and Linux operating systems;  
264 however, under identical computing conditions we found that the best performance of the algorithm  
265 was obtained in Linux.

### 266 **Known limits**

267 Native video import in ImageJ is limited to AVI encoded in MJPEG or images series *e.g.*, TIFF series.  
268 However, additional plug-ins can open other video formats *e.g.*, MovieIO. Moreover, given the ability  
269 of ImageJ to analyze video, it is likely that the number of importable video formats will increase. A  
270 limitation of SarcOptiM is the impossibility to receive a synchronization signal, for example to  
271 indicate the moment when electrical stimulation is applied to the cell.

### 272 **Perspectives**

273 This open-source plug-in developed in ImageJ macro language and Java can be modified by anyone to  
274 improve existing functions or add new ones such as analysis with autocorrelation function or  
275 measurement method by edge detection.

276 Analysis with *on entire frame* mode of SarcOptiM cannot currently be used for real time analysis  
277 since there is insufficient processor power for the online computation of the two dimensional Fourier  
278 spectrum (Fig. 2). In the future with improved processor performances, not only should this be  
279 possible but should also allow the analysis of several contracting isolated myocytes at the same time  
280 as well, whatever their orientations in the visual field and without motion artifacts.

281

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287 **Disclosures**

288 No conflicts of interest, financial or otherwise, are declared by the authors.

289 **Author contributions**

290 Author contributions: C.P., F.G., P.B., and V.M. conception and design of research; C.P., F.G., A.Y.  
291 performed experiments; C.P., F.G. analyzed data; C.P. and F.G. interpreted results of experiments;  
292 C.P., F.G., A.Y., C.O.M. prepared figures; C.P. drafted manuscript; C.P., F.G., A.Y., C.O.M., P.B., and  
293 V.M. edited and revised manuscript; C.P., F.G., A.Y., C.O.M., P.B., and V.M. approved final version of  
294 manuscript.

295

296 **Endnote**

297 At the request of the authors, readers are herein alerted to the fact that additional materials related  
298 to this manuscript may be found at the institutional website of one of the authors, which at the time  
299 of publication they indicate is: [<http://pccv.univ-tours.fr/ImageJ/SarcOptiM/>]. These materials are not a  
300 part of this manuscript, and have not undergone peer review by the American Physiological Society  
301 (APS). APS and the journal editors take no responsibility for these materials, for the website address,  
302 or for any links to or from it.

303

## 304 **Figure legends**

305

306 **Figure 1:** Flow diagram of the algorithm for SarcOptiM *on cell axis* analysis. (A) An example of an  
307 input image opened with ImageJ. The white line represents the axis positioned for sarcomere  
308 measurement. (B) Grey level profile along the axis of measurement. (C) Fourier spectrum of the grey  
309 level profile. Double headed arrow indicates the limits between which the algorithm searches for the  
310 peak. This interval is user defined as the minimum and maximum sarcomere lengths set before the  
311 analysis. The vertical arrow shows the detected peak corresponding to sarcomere spatial frequency  
312 in A. (D) Example of output plot representing sarcomere lengths vs. time.

313 **Figure 2:** Flow diagram of the algorithm for SarcOptiM *on entire frame* mode. (A) An example of the  
314 input image opened with image J. (B) 2D Fourier spectrum of the image (left) and 3D surface plot of  
315 the FFT spectrum (right). Rings indicate the limits set for searching the peak defined by the user as  
316 minimum and maximum sarcomere lengths before the analysis. Arrows show the detected peak  
317 corresponding to sarcomere spatial frequency in this frame. (C) Example of output plot representing  
318 sarcomere lengths vs. time

319 **Figure 3:** (A) Absolute error of the sarcomere length measurement corresponding to the difference  
320 between the measured and the model values during a simulated cardiomyocyte contraction were  
321 recorded for different pixel sizes with *on cell axis* mode.  $n = 40$  frames for runs for each pixel size. (B)  
322 Effect of different analysis modes on absolute error of the sarcomere length measurement during a  
323 simulated cardiomyocyte contraction with or without myocyte rotation. ( $n = 40$  frames). L: *on cell*  
324 *axis* mode without cell rotation, F: *on entire frame* mode without cell rotation, FR: *on entire frame*  
325 mode with cell rotation. Pixel size was  $0.3 \mu\text{m}$ . In panels (A) and (B), boxplots indicate minimum, first  
326 quartile, median and mean, third quartile and max values for each condition. \*:  $p < 0.05$  with ANOVA  
327 and Bonferroni post-hoc t-test; NS: non-significant. (C) Example of offline sarcomere length  
328 measurements with both *on entire frame* (grey line) and *on cell axis* (black line) modes during cell  
329 contraction and rotation. The video used for these measurements is available in the Data  
330 Supplement (Video\_4.avi). Note that the *entire frame* mode allows undisturbed sarcomere length  
331 measurement during the cell rotation, especially during the diastole.

332 **Figure 4:** Effect of video frame rate on sarcomere length measurement during contraction of a rat left  
333 ventricular cardiomyocyte electrically stimulated at 1 Hz under basal conditions (*on cell axis* mode).  
334 (A-F) Sarcomere length measurements at frame rates of 25, 50, 100, 250, 500 and 1000 Hz. Pixel size  
335 was  $0.3 \mu\text{m}$ .

336 **Figure 5:** Measurement of rat left ventricular cardiomyocyte contractions electrically evoked at 1 Hz  
337 under basal conditions and following superfusion by 100 nM isoproterenol (iso) with *on cell axis*  
338 mode. (A-B) Effect of video sampling rate on the measurement of minimum sarcomere length  
339 (minSL; A) and maximum sarcomere shortening speed ( $dSL/dt_{\text{max}}$ ; B) in control conditions (grey) and  
340 in the presence of isoproterenol (black). (C-D) Effect of isoproterenol on the contraction recorded at  
341 a video frame rate of 250 Hz and a pixel size of  $0.3 \mu\text{m}$ . (C) Continuous recording of sarcomere  
342 measurement during the superfusion of isoproterenol (bar above the trace). (D) Examples of  
343 sarcomere shortening before and after superfusion of isoproterenol (arrows in C). 's' indicates the  
344 time of electric stimulation.

345 **References**

346

- 347 1. **Ait-Mou Y, Hsu K, Farman GP, Kumar M, Greaser ML, Irving TC, de Tombe PP.** Titin strain  
348 contributes to the Frank–Starling law of the heart by structural rearrangements of both thin- and  
349 thick-filament proteins. *Proc Natl Acad Sci U S A* 113: 2306–2311, 2016.
- 350 2. **Butler L, Cros C, Oldman KL, Harmer AR, Pointon A, Pollard CE, Abi-Gerges N.** Enhanced  
351 characterization of contractility in cardiomyocytes during early drug safety assessment. *Toxicol*  
352 *Sci Off J Soc Toxicol* 145: 396–406, 2015.
- 353 3. **Carillion A, Feldman S, Jiang C, Atassi F, Na N, Mougnot N, Besse S, Hulot J-S, Riou B, Amour J.**  
354 Overexpression of cyclic adenosine monophosphate effluent protein MRP4 induces an altered  
355 response to  $\beta$ -adrenergic stimulation in the senescent rat heart. *Anesthesiology* 122: 334–342,  
356 2015.
- 357 4. **Delbridge LM, Roos KP.** Optical methods to evaluate the contractile function of unloaded  
358 isolated cardiac myocytes. *J Mol Cell Cardiol* 29: 11–25, 1997.
- 359 5. **Fan D, Wannenburg T, de Tombe PP.** Decreased myocyte tension development and calcium  
360 responsiveness in rat right ventricular pressure overload. *Circulation* 95: 2312–2317, 1997.
- 361 6. **Fowler ED, Benoist D, Drinkhill MJ, Stones R, Helmes M, Wüst RCI, Stienen GJM, Steele DS,**  
362 **White E.** Decreased creatine kinase is linked to diastolic dysfunction in rats with right heart  
363 failure induced by pulmonary artery hypertension. *J Mol Cell Cardiol* 86: 1–8, 2015.
- 364 7. **Gannier F, Bernengo JC, Jacquemond V, Garnier D.** Measurements of sarcomere dynamics  
365 simultaneously with auxotonic force in isolated cardiac cells. *IEEE Trans Biomed Eng* 40: 1226–  
366 1232, 1993.
- 367 8. **Llewellyn ME, Barretto RPJ, Delp SL, Schnitzer MJ.** Minimally invasive high-speed imaging of  
368 sarcomere contractile dynamics in mice and humans. *Nature* 454: 784–788, 2008.
- 369 9. **Peterson P, Kalda M, Vendelin M.** Real-time determination of sarcomere length of a single  
370 cardiomyocyte during contraction. *Am J Physiol - Cell Physiol* 304: C519–C531, 2013.
- 371 10. **Sheikh F, Raskin A, Chu P-H, Lange S, Domenighetti AA, Zheng M, Liang X, Zhang T, Yajima T, Gu**  
372 **Y, Dalton ND, Mahata SK, Dorn GW, Brown JH, Heller-Brown J, Peterson KL, Omens JH,**  
373 **McCulloch AD, Chen J.** An FHL1-containing complex within the cardiomyocyte sarcomere  
374 mediates hypertrophic biomechanical stress responses in mice. *J Clin Invest* 118: 3870–3880,  
375 2008.
- 376 11. **Song K, Nam Y-J, Luo X, Qi X, Tan W, Huang GN, Acharya A, Smith CL, Tallquist MD, Neilson EG,**  
377 **Hill JA, Bassel-Duby R, Olson EN.** Heart repair by reprogramming non-myocytes with cardiac  
378 transcription factors. *Nature* 485: 599–604, 2012.

379

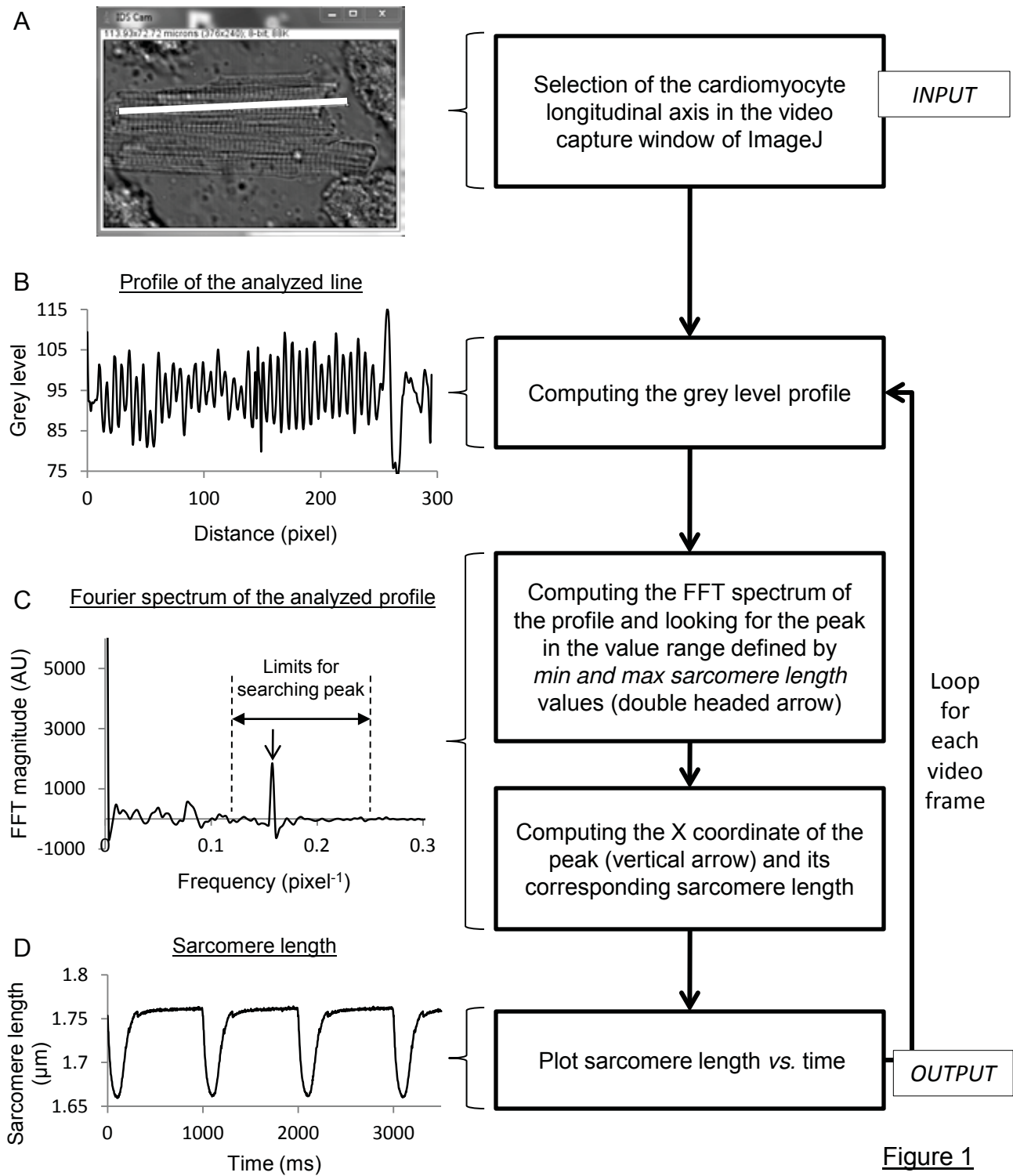


Figure 1

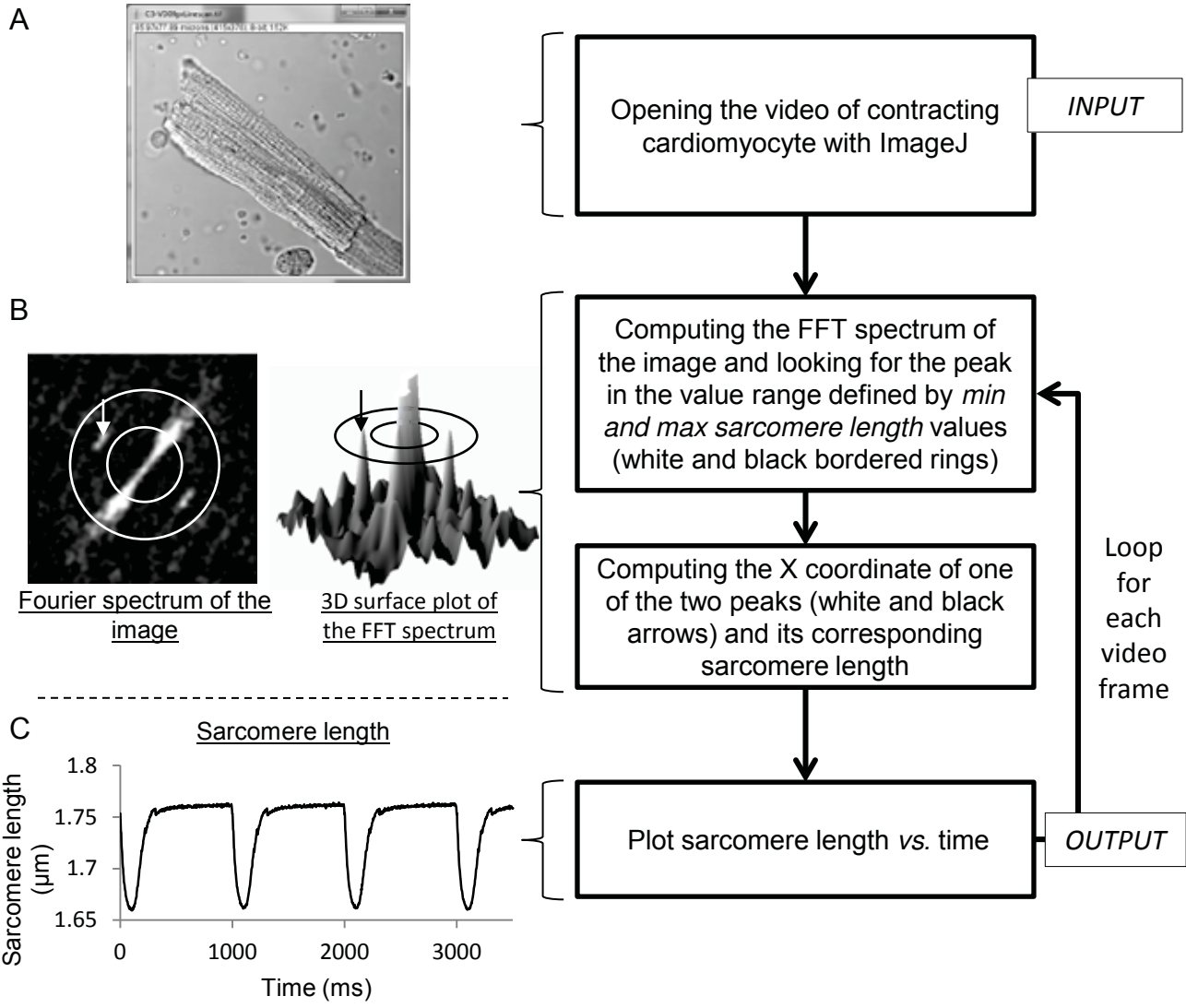
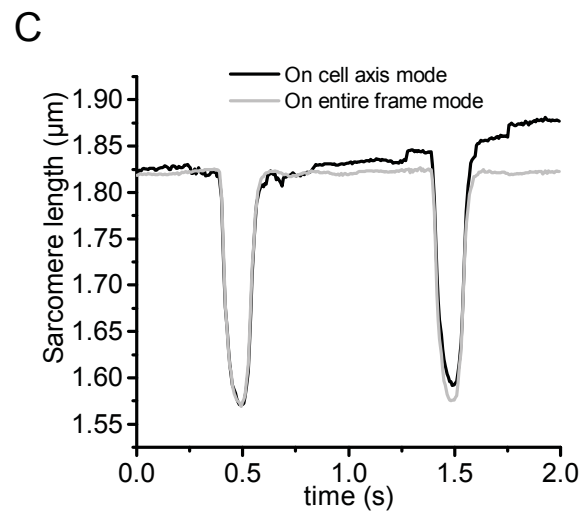
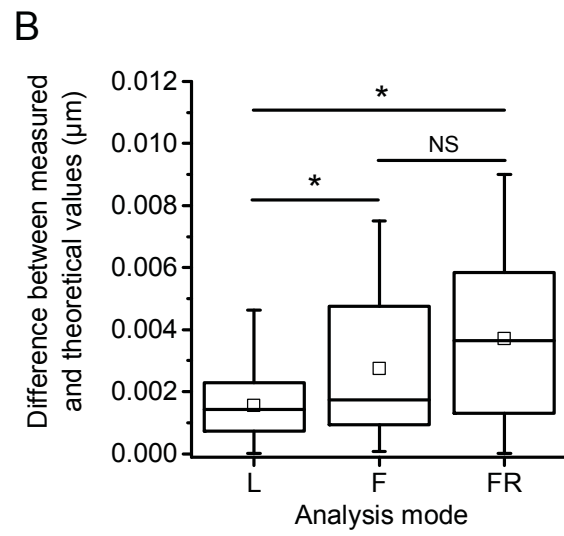
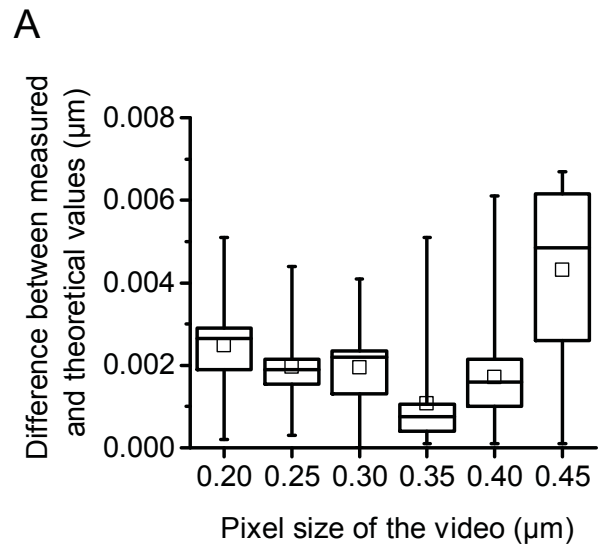
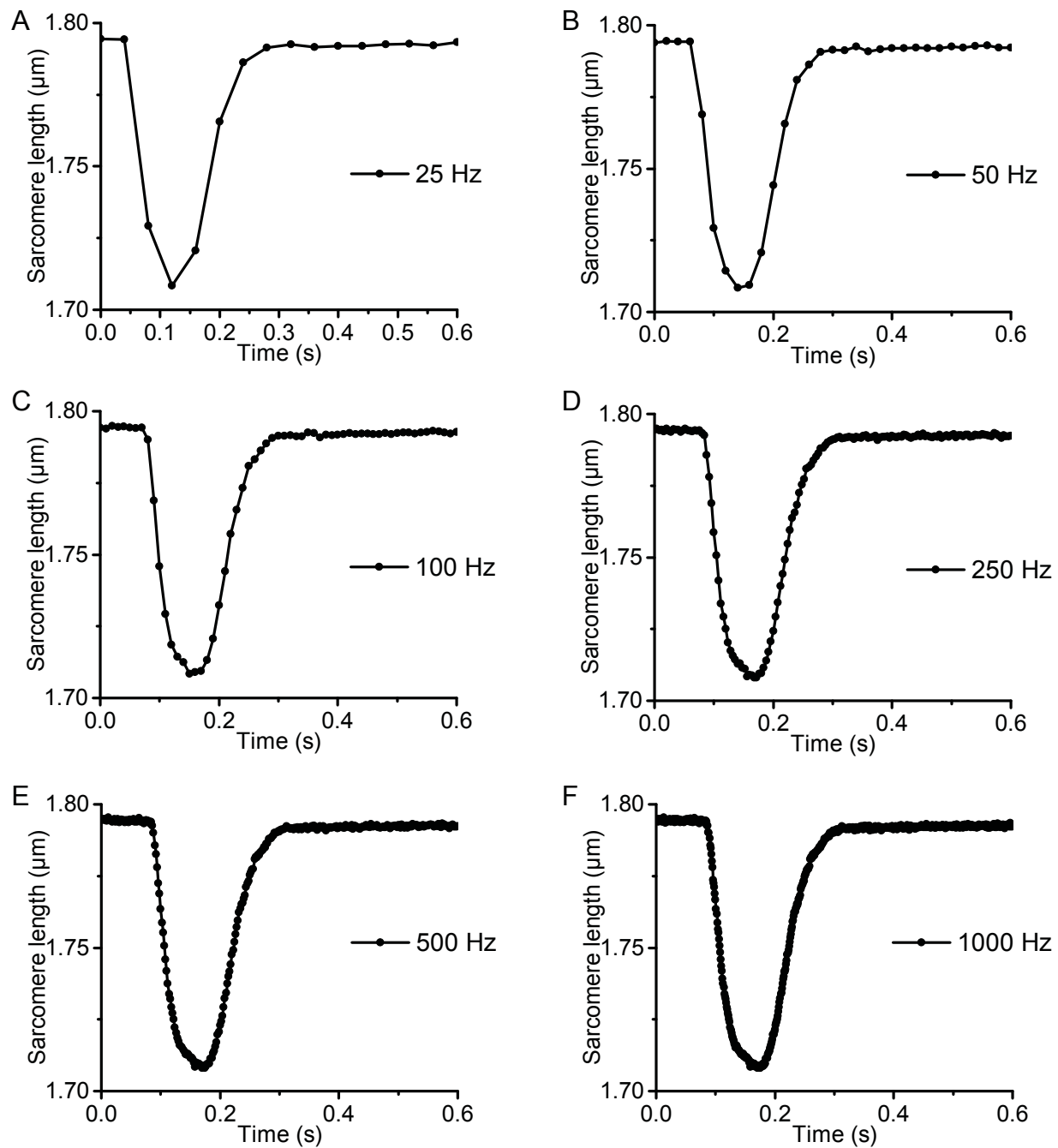


Figure 2

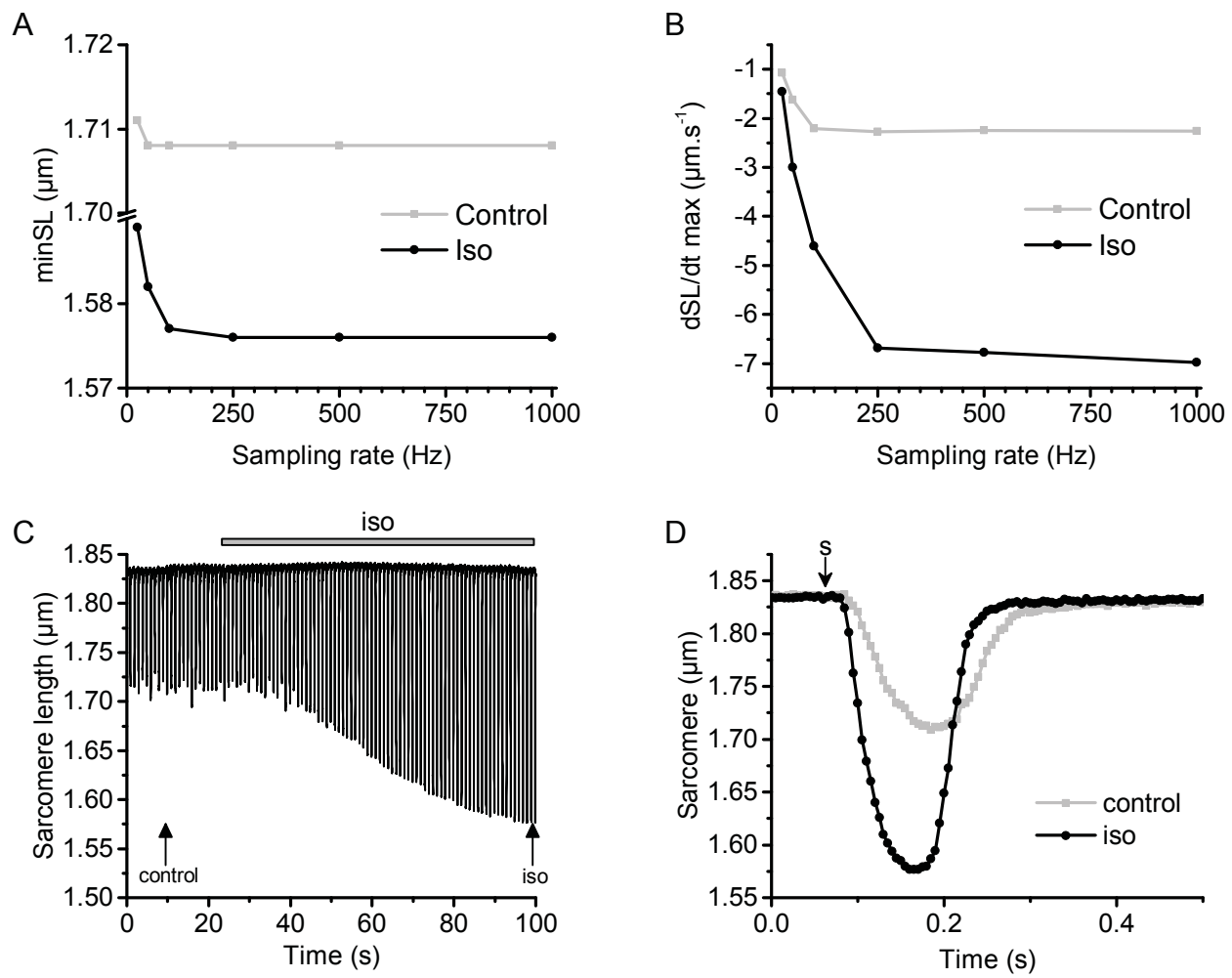




**Figure 3**



**Figure 4**



**Figure 5**