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

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Pasqualin C, Gannier F, Yu A, Malécot CO, Bredeloux P, Maupoil V. SarcOptiM for ImageJ: high-frequency online sarcomere length computing on stimulated cardiomyocytes. Am J Physiol Cell Physiol 311: C277–C283, 2016. First published June 22, 2016; doi:10.1152/ajpcell.00094.2016.—Accurate measurement of cardiomyocyte contraction is a critical issue for scientists working on cardiac physiology and physiopathology of diseases implying contraction impairment. Cardiomyocytes contraction can be quantified by measuring sarcomere length, but few tools are available for this, and none is freely distributed. We developed a plug-in (SarcOptiM) for the ImageJ/Fiji image analysis platform developed by the National Institutes of Health. SarcOptiM computes sarcomere length via fast Fourier transform of video frames captured or displayed in ImageJ and thus is not tied to a dedicated video camera. It can work in real time or offline, the latter overcoming rotating motion or displacement-related artifacts. SarcOptiM includes a simulator and video generator of cardiomyocyte contraction. Acquisition parameters, such as pixel size and camera frame rate, were tested with both experimental recordings of rat ventricular cardiomyocytes and synthetic videos. It is freely distributed, and its source code is available. It works under Windows, Mac, or Linux operating systems. The camera speed is the limiting factor, since the algorithm can compute online sarcomere shortening at frame rates > 10 kHz. In conclusion, SarcOptiM is a free and validated user-friendly tool for studying cardiomyocyte contraction in all species, including human.

cardiomyocyte contractility; sarcomere dynamic; video analysis; ImageJ plug-in; fast Fourier transform

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 troponyosin 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). 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 SL (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 National Institutes of Health: 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 prerecorded 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/

MATERIALS AND METHODS

Isolation of rat ventricular CMs. All protocols have been approved by the local ethical committee (Comité d’Ethique en Expérimentation Animale Val de Loire, Tours, France). Adult male Wistar rats were anesthetized with pentobarbital (60 mg/kg). The heart was rapidly...
removed, and coronary circulation was retrogradely perfused through the aorta. Enzymatic digestion was performed by perfusion of 0.1 UI/ml Liberase Research Grade in Krebs-Ringer-bicarbonate solution (in mM: 35 NaCl, 25 NaHCO₃, 4.75 KCl, 1.19 KH₂PO₄, 16 Na₂HPO₄, 134 sucrose, 10 HEPES, and 10 glucose, pH adjusted to 7.4) at 37°C. At the end of the digestion, ventricles were separated and gently mechanically dissociated with Pasteur pipettes.

**Experimental setup and protocol.** CMs were placed in a perfusion chamber mounted on an inverted microscope (Nikon Diaphot 300) for online sarcomere analysis and video recording of contraction at 25°C. Myocytes were superfused with Tyrode’s solution containing the following (in mM): 140 NaCl, 5.37 KCl, 1.36 CaCl₂, 1 MgCl₂, 0.33 Na₂HPO₄, 10 HEPES, 11 glucose, at pH 7.4, adjusted with NaOH. Myocytes were electrically field stimulated at 1 Hz with square-wave pulses (80 mA, 2 ms) delivered through a pair of platinum electrodes.

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

**Synthetic videos of a CM contraction simulation.** Each sarcomere measurement mode of SarcOptiM has been validated with synthetic videos of a modeled contracting CM. These were created with the video synthesis tool, which is implemented in SarcOptiM. In these synthetic videos, frame rate, pixel size, and CM features (length, width, resting SL, sarcomere shortening, and contraction/relaxation speed factor), as well as CM movement and rotation in the video field, can be user defined to match particular experimental preparations and test user hardware. The equation, SL = SL_{min} × e^{−t × SF} − t × SF + 1 (where SL_{min} is SL at time t; SL_{min} is minimum SL at maximum peak shortening; t is time; and SF is contraction/relaxation speed factor, which adjusts the theoretical model to fast or slow twitches), is used to model the CM contraction. In our examples, the standard parameters were as follows: resting SL, 1.8 μm; sarcomere shortening, 5% of the resting SL; pixel size, 0.3 μm; video frame rate equivalent, 100 Hz; number of frames, 40 (i.e., a total equivalent of 400 ms record); angle of the CM in the frame, 30° from the horizontal; and contraction/relaxation speed factor, 1. An example of synthetic CM generated by this module is available in the data supplement (Supplemental Video S1; supplemental material for this article can be found online at the Journal website).

**Setting up and use.** To install SarcOptiM in ImageJ: 1) unzip the SarcOptiM.zip file; 2) put the SarcOptiM folder into the...

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**Fig. 1. Flow diagram of the algorithm for SarcOptiM on cell axis analysis.** A: an example of an input image opened with ImageJ. The white line represents the axis positioned for sarcomere measurement. B: gray level profile along the axis of measurement. C: Fourier spectrum of the gray level profile. Double-headed arrow indicates the limits between which the algorithm searches for the peak. This interval is user defined as the minimum and maximum sarcomere lengths set before the analysis. The vertical arrow shows the detected peak corresponding to sarcomere spatial frequency in A. D: example of output plot representing sarcomere lengths vs. time.
The user guide on our institutional website (see ENDNOTE) and on the ImageJ website contains more information, and a video demonstration of its use is available in the data supplement (Supplemental Video S2). It is important to note that another plug-in is necessary to acquire video flow from digital, e.g., HF IDS_Cam and Webcam Capture, or analog, e.g., PixelSmart Frame Grabbers cameras.

Statistics. All box plots indicate minimum, first quartile, median and mean, third quartile, and maximum values. All statistics have been performed with R and SigmaStat. For the comparison between groups, ANOVA tests were performed. Differences are considered significant when the \( P \) value is inferior to 0.05.

RESULTS

Analysis algorithm description and outputs. In on cell axis mode (Fig. 1), the sarcomere measurement algorithm searches for a peak in the fast Fourier transform (FFT) of the gray level profile of the myocyte longitudinal axis along the line positioned on the image by the user (Fig. 1, A and 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 SL is calculated.

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 two-dimensional 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.

At the end of the sarcomere measurement computation, each SL value is plotted vs. time in a new window (Figs. 1D and 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.

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

Accuracy of the SL measurement with different pixel sizes. Since the accuracy of sarcomere measurement depends on the spatial resolution of the acquired image, the effect of pixel size on 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 with the value assigned to that point in the model. Absolute differences between each measured and each theoretical value are shown as box plot in Fig. 3A. For a pixel size between 0.20 and 0.35 \( \mu \)m, the error of the measurement is, in the worst case, inferior or equal to 0.005 \( \mu \)m. For a pixel size of 0.40 and 0.45 \( \mu \)m, the measurement error lies between 0.006 and 0.007 \( \mu \)m. These values are far below the pixel size, since they are obtained with a frequency analysis by FFT and not by a simple metric measurement (7).

Comparison between on cell axis and on entire frame modes of analysis. 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 mea-
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, Supplemental Videos S3 and S4). Moreover, multiple dead cells in the analyzed field do not affect the SL measurement. However, this mode restricts application to offline analysis of prerecorded experimental videos, since actual performances of the current computer are insufficient to allow online analysis. To compare the quality of analysis in both modes, two synthetic videos with standard parameters (see MATERIALS AND METHODS) were used. In the second video, a rotary motion from 30° down to the horizontal was applied to the CM. The first video was analyzed with on cell axis and on entire frame modes. The second video was analyzed with the on entire frame mode alone. As above, SL data obtained through a contraction-relaxation cycle were compared with their model values, and the results are shown in Fig. 3B. The on cell axis mode allows the best accuracy of measurement with a maximum error in the worst case < 0.005 μm. With the on entire frame mode, the worst case error is slightly higher, since it can reach 0.009 μm due to the pixel interpolation when the cell axis is not exactly horizontal or vertical. However, with this mode, the SL measurement is not affected, and the maximum error is not significantly increased by the cell rotation in the visual field (Fig. 3, B and C). Thus the on cell axis analysis mode is recommended in standard conditions. The on entire frame analysis mode has to be used when the CM moves or rotates in the field, despite that this will result in a slightly higher variability of SL measurement.

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

Effect of the sampling frequency upon the quality of the measurement of rat CM contractions under basal conditions and following β-adrenergic stimulation with isoproterenol. Several video capture frequencies were tested to determine the optimal frame rate for sarcomere measurement of a rat ventricular CM before and after superfusion of 100 nM isoproterenol (known to increase contraction kinetic and amplitude). Examples of sarcomere measurements (on cell axis mode) in control conditions at video frame rates of 25, 50, 100, 250, 500, and 1,000 Hz are shown Fig. 4. The optimal frequency was determined by measuring for each rate the minimum SL (minSL) and maximum sarcomere shortening speed (dSL/dtmax; Fig. 5, A and B). The acquisition frequency is considered sufficient when minSL and dSL/dtmax vary by < 5% from the values determined at 1,000 Hz. In control conditions, 100 Hz is enough to record minSL (1.708 vs. 1.708 μm at 1,000 Hz) and dSL/dtmax (−2.26 vs. −2.27 μm/s at 1,000 Hz). However, the sampling rate must

![Graph A](http://ajpcell.physiology.org/)

**Fig. 3.** A: absolute error of the sarcomere length measurement corresponding to the difference between the measured and the model values during a simulated cardiomyocyte contraction were recorded for different pixel sizes with on cell axis mode (n = 40 frames for runs for each pixel size). B: effect of different analysis modes on absolute error of the sarcomere length measurement during a simulated cardiomyocyte contraction with or without myocyte rotation (n = 40 frames). L, on cell axis mode without cell rotation; F, on entire frame mode without cell rotation; FR, on entire frame mode with cell rotation. Pixel size was 0.3 μm. In A and B, box plots indicate minimum, first quartile, median and mean, third quartile, and maximum values for each condition. *P < 0.05 with ANOVA and Bonferroni post hoc t-test. NS, nonsignificant. C: example of offline sarcomere length measurements with both on entire frame (shaded line) and on cell axis (solid line) modes during cell contraction and rotation. The video used for these measurements is available in the data supplement (Supplemental Video S4). Note that the on entire frame mode allows undisturbed sarcomere length measurement during the cell rotation, especially during the diastole.
be increased to 250 Hz in the presence of isoproterenol to obtain an accurate dSL/dt (minSL: 1.576 vs. 1.576 µm at 1,000 Hz and dSL/dt max: −6.68 vs. −6.98 µm/s at 1,000 Hz). The effect of 100 nM isoproterenol on the contraction of a rat CM recorded at 250 Hz is shown in Fig. 5, C and D. The increases of sarcomere shortening and of contraction and relaxation rates are clearly visible (Fig. 5D). Therefore, with this preparation and under these experimental conditions, a frame rate of 250 Hz is recommended for SL measurements, but this parameter has to be optimized for other experimental conditions.

DISCUSSION AND CONCLUSIONS

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

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

Performance. The reliability of the sarcomere measurement provided by SarcOptiM has been demonstrated by tests on synthetic videos. Measurements can be performed as long as CM sarcomeres are visible on the video, even in low light conditions. In addition, they are not affected by light oscilla-
Sarcomere length (µm)

Fig. 5. Measurement of rat left ventricular cardiomyocyte contractions electrically evoked at 1 Hz under basal conditions and following superfusion by 100 nM isoproterenol (iso) with on cell axis mode. A and B: effect of video sampling rate on the measurement of minimum sarcomere length (minSL; A) and maximum sarcomere shortening speed (dSL/dtmax; B) in control conditions (shaded lines) and in the presence of isoproterenol (solid lines). C and D: effect of isoproterenol on the contraction recorded at a video frame rate of 250 Hz and a pixel size of 0.3 µm. C: continuous recording of sarcomere measurement during the superfusion of isoproterenol (bar above the trace). D: examples of sarcomere shortening before and after superfusion of isoproterenol (arrows in C). “s” indicates the time of electric stimulation.

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

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

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

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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

ENDNOTE

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

REFERENCES