SoftwareX 12 (2020) 100547

Contents lists available at ScienceDirect

SoftwareX

journal homepage: www.elsevier.com/locate/softx

Original software publication

ContHeart: Software for monitoring isolated cardiomyocyte shortening

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ARTICLE INFO

Article history:
Received 5 December 2019
Received in revised form 15 May 2020
Accepted 12 June 2020

Keywords:
cardiomyocytes
Shortening
Edge detection
Canny method

ABSTRACT

ContHeart is a software designed to analyze cardiomyocyte contractile dynamics using Canny's method for edge detection in recorded videos. It is a tool that allows researchers (users) to post-analyze video usually captured in their work routine, without special capture apparatus. The software has a user-friendly graphical interface in which the user can apply filters and modify parameters to optimize edge detection and shortening measurement. Therefore, the software quickly generates reliable data on the variation of cell dimensions over time, which can be interpreted by the researcher. We believe researchers will find here a powerful tool to enhance the reach of their basic cardiovascular research, allowing them to include the cardiomyocytes shortening analysis on their work. That might increase the scope of knowledge within the field, as the effect of different pathophysiological conditions may be analyzed on cardiomyocyte contraction.

Code metadata (mandatory)

Current code version	*1.0.0*
Permanent link to code/repository used of this code version	https://github.com/ElsevierSoftwareX/SOFTX_2019_373
Code Ocean compute capsule	
Legal Code License	*Apache-2.0*
Code versioning system used	*none*
Software code languages, tools, and services used	*Visual Basic .NET*
Compilation requirements, operating environments & dependencies	*Microsoft visual studio, Emgu CV 3.4.3.3016, .NET Framework 4.6.1*
If available Link to developer documentation/manual	
Support email for questions	jair.goulart@unb.br

Software metadata (optional)

Current software version	*1.0.0*
Permanent link to executables of this version	
Legal Software License	*Apache-2.0*
Computing platforms/Operating Systems	*Microsoft Windows*
Installation requirements & dependencies	*Emgu CV 3.4.3.3016, .NET Framework 4.6.1*
If available, link to user manual – if formally published include a reference to the publication in the reference list	
Support email for questions	jair.goulart@unb.br

1. Motivation and significance

Cardiovascular diseases (CDs) lead to around 17.9 million deaths each year, according to the World Health Organization [1], which corresponds to ∼31% of all deaths in the world. The

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https://doi.org/10.1016/j.softx.2020.100547

high mortality and high costs related to CDs make them a significant research field. The contractility evaluation of isolated cardiomyocytes is an essential tool for cardiovascular research, as it is widely used as an *in vitro* model [2,3]. Studying the contractile function of isolated cardiomyocytes provides valuable information on cardiac excitation–contraction coupling and can be used to investigate the effects of drugs with potential inotropic or lusitropic effect [4].

Several methods may be applied to monitor cardiomyocyte contraction (or shortening). Among the simplest is the edge detection from a video file. Through this method, the researcher applies an algorithm to detect the movement made by the cardiomyocyte during the time course of a recorded video. The current evolution of computational detection methods allows the monitoring of cells with different geometries, regardless the cell shape and contraction axis, allowing not only the shortening measurement in ventricular myocytes, which show a well-determined contraction axis but also in irregular-shaped cells, such as neonatal and cultured cardiac cells [5–7]. Despite the existence of several computational solutions for cardiomyocyte shortening, the implementation of many of them requires the researcher-user to have good knowledge of programming. Many solutions only describe the used algorithm, which must be implemented in a programming language, making it difficult for biomedical researchers to apply such solutions in their research. Some software, such as Video Length Sarcomere (Aurora Scientific, ON, Canada), has a user-friendly interface; however, they use sarcomere – structures present throughout muscle cells – shortening detection technology by fast Fourier transform analysis which requires video signals with high resolutions and high image magnification [8]. Other commercial software, such as and IonOptix system (IonOptix LLC, Westwood, MA, US), offers modules for both sarcomere and cell edge detection. Thus, the technical knowledge needed for the use of the solutions and the costs involved in purchasing commercial software and video capture equipment, keep laboratories with few funds away from this valuable analysis.

We aimed to develop a simple and easy-to-use solution with a user-friendly graphical interface that allows biomedical researchers without programming knowledge to measure the shortening of isolated cardiomyocytes and extract parameters from their contractile characteristics. ContHeart is able to perform the shortening measurement in recorded videos captured in conventional microscopies, even in videos recorded at low resolution and without ideal cell positioning.

2. Software description

ContHeart is a software written in the VB.net language that allows opening video files in various formats (*.mp4, *.avi, *.wmv). The input video containing a contracting isolated cardiomyocyte is then analyzed through the Canny edge detection method [9] to detect the cell and measure parameters of interest for biomedical research. Also, the software generates an output text file (*.txt) containing all results. Goulart et al. (2017) have demonstrated that the algorithm used for edge detection based on Canny's method is efficient for cardiomyocyte detection and cell shortening evaluation [10].

Once the software is running, the user will be able to open a video file from which the first frame is displayed. This frame will be used to define all parameters used to treat the video and detect the edges of the cell. However, if the first frame is not reliable to be used, the user can choose another frame using a trackbar. The parameters that can be chosen to analyze and detect the cell are:

- Cell rotation: the user can rotate the frame to achieve better cell alignment.
- Canny Thresholds: the user sets the thresholds used by the Canny's algorithm to detect the edge of the cell.
- Grayscale: reliable edge detection requires the conversion of each frame in the video to grayscale. The user sets the parameters for the grayscale conversion.
- Image treatment: it allows the user to apply different filters to the frames. The available filters are Blur, Median and Gaussian. The Blur filter smooths the edges of the image, giving it a blurry look, allowing only objects with very sharp edges to be detected, reducing the noise caused by external objects and inner edges of the cell (Fig. 1). The Median filter analyzes the color of a set of pixels and then define the color of each pixel in the set as the color equivalent to the median of the whole set, smoothing the image and removing "salt and pepper" noises. Finally, the Gaussian filter changes the frame so that the color variation follows a Gaussian distribution.
- Factor: it is an important variable that ranges from 0 to 100. It is a percentage value that indicates the threshold to detect rectangles in the frame (more details below).

When the user has set the most appropriate cell detection parameters, the shortening analyzes begin. Since cardiomyocytes are approximately rectangular, the software seeks to detect rectangles from the previously delineated edges (Fig. 2). However, low-resolution videos do not allow the detection of all cell edges in every frame. It makes the cell detection as a contiguous rectangle hard. To solve this issue, ContHeart detects multiple rectangles within the frame, as shown in Fig. 2A, and joins them together to form a larger rectangle, the union rectangle (UR), which would represent the entire cell (Fig. 2B). The software only displays the UR to avoid confusing the user with the small rectangle detection during the software routine.

The software detects all the rectangles in each frame using the rectangle detection algorithm from Emgu CV libraries (.net library wrapper to the OpenCV image processing library). The criterion used to select the rectangles included in UR formation is the Factor chosen by the user. ContHeart only considers for forming the UR rectangles with a perimeter of a minimum ratio of the largest rectangle perimeter. The user defines this minimum perimeter ratio as the Factor variable, e.g., if the user chooses a Factor of 50, ContHeart will only consider rectangles with a perimeter at least half the perimeter of the largest rectangle. Then, the Factor variable ranges from zero to 100; it defines the size of the small rectangle used to form UR. The default value of the Factor is 50, but the user should always test a more suitable value, noting if the detection of UR is in agreement with the cardiomyocyte of interest.

We also draw an ellipse within the UR. The elliptical area can be used to estimate a prolate spheroid, which is an adequate representation of the real area of a cardiomyocyte as well as a rectangle or a three-dimensional representation. Nevertheless, the prolate spheroid representation is optimized for electromagnetic studies, as described by Milan et al. (2019) [11].

ContHeart applies the analysis parameters set in the first frame – or in a representative frame selected by the user – to analyze the first and all subsequent frames. Then, the software records a series of outputs during its operation. The outputted results are, in order of appearance in the exported .txt: the initial X and Y position of the UR, the UR Height and Width, the UR area, the elliptical area drawn within UR, the time position of each frame (in milliseconds) and the frame number. The height, width, and areas of UR and elliptical area are calculated in arbitrary units (in pixels) or nm (if the user performed a calibration step), the unit commonly used in cell measurement. All these data can be used by the researcher-user to evaluate the temporal

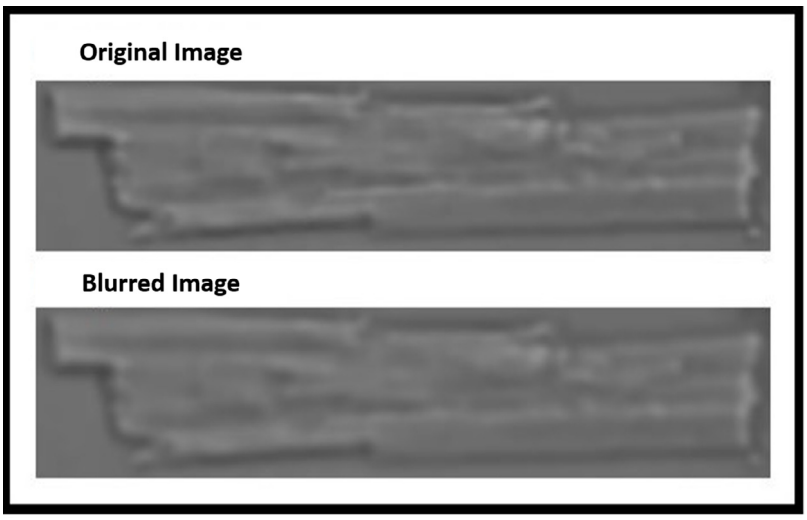

Fig. 1. The uppermost picture shows the original image, and the bottom one shows its blurred version. Even this little blur applied to the bottom image smooths the edges of the cardiomyocyte and makes it more accurately detected by the Canny method.

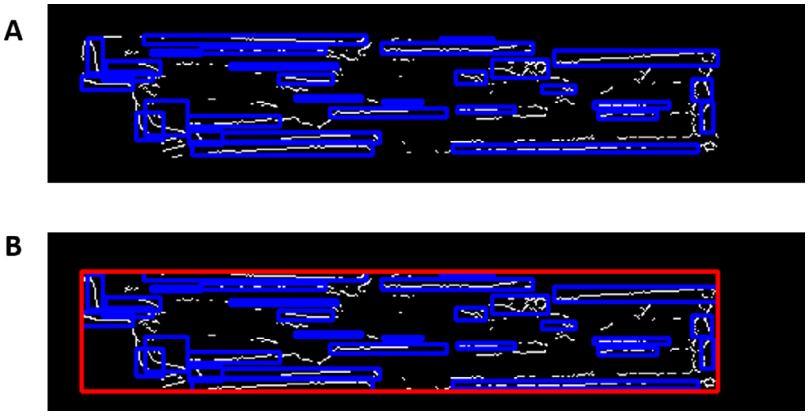

Fig. 2. Edge detection in cardiomyocyte. A. ContHeart uses the Canny method to detect edges in the image; then, rectangles are drawn using the edges. B. The small rectangles (blue) are joined to form a big one that should outline the cell (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

course of the cardiomyocyte contractile activity. Through the data collected the user is able to draw graphs showing the cell shortening, which can be used to estimate important parameters for cardiovascular studies, e.g., contraction amplitude and relaxation time course [10]. In addition, the outputted .txt file present a header showing the path to the video file used, the total duration of the analyzed video in seconds, the video frame count, the initial frame where the analysis was performed, the rotation applied to the frames, and all user-defined parameters, to ensure reproducibility of the performed analysis.

3. Illustrative example

ContHeart allows the user to open a video showing a contractile cell and extract important parameters for cardiovascular research.

Fig. 3 shows the ContHeart main window. The user should click on "Open File" (see 1 in Fig. 3) to choose the video to be analyzed. Once the video is selected, the user will see the first frame of the video on the ContHeart main window (see "Original Frame" space in Fig. 3). We advise users to perform the initial analysis with the standard parameter to get an idea of how to identify the cell. Therefore, the user should click on "Find Contours" (see 2 in Fig. 3). Then, a representation of the detected edges of the original frame will be shown on the right side of the main window (see "Detected Cell" in Fig. 3). Also, the user will see a blue rectangle and a green ellipse that should outline only the cell of interest (Fig. 4). The user can click and drag the mouse over the original frame to select a region (ROI) to be analyzed. ROI selection is especially important if there are several other cells or noise on the original frame. The user can test different parameters to enhance the analysis of the video to get a better detection of the cardiomyocyte (Fig. 4).

In this illustrative example, the analyzed video has two widescreen black bars hindering edge detection (Fig. 4A). Then, we select an ROI around the myocyte (red dashed rectangle in Fig. 4B) to improve the detection. Only this maneuver allowed better detection of the cell (blue rectangle and green ellipse in Fig. 4B). If the cardiomyocyte is tilted, the user might need to use the buttons CCW/CW (see 6 in Fig. 3) to align it. Also, If the first frame is not suitable for initial cell detection, the user can select any other frame using the trackbar (see 15 in Fig. 3). To further improve cell detection in this example (Fig. 4), we change some parameters:

- Factor (see 7 in Fig. 3) was changed to 30;
- Canny thresholds (see 8 in Fig. 3) and grayscale (see 9 in Fig. 3) were unchanged;

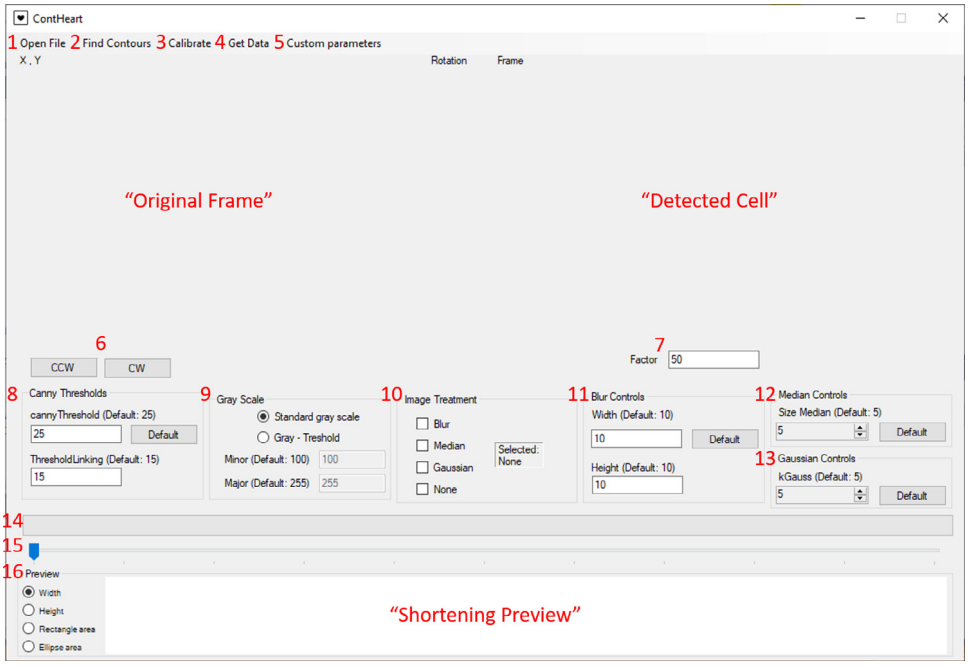

Fig. 3. ContHeart main window. For reference, see red labels indicating essential objects and regions on this screen (see text).

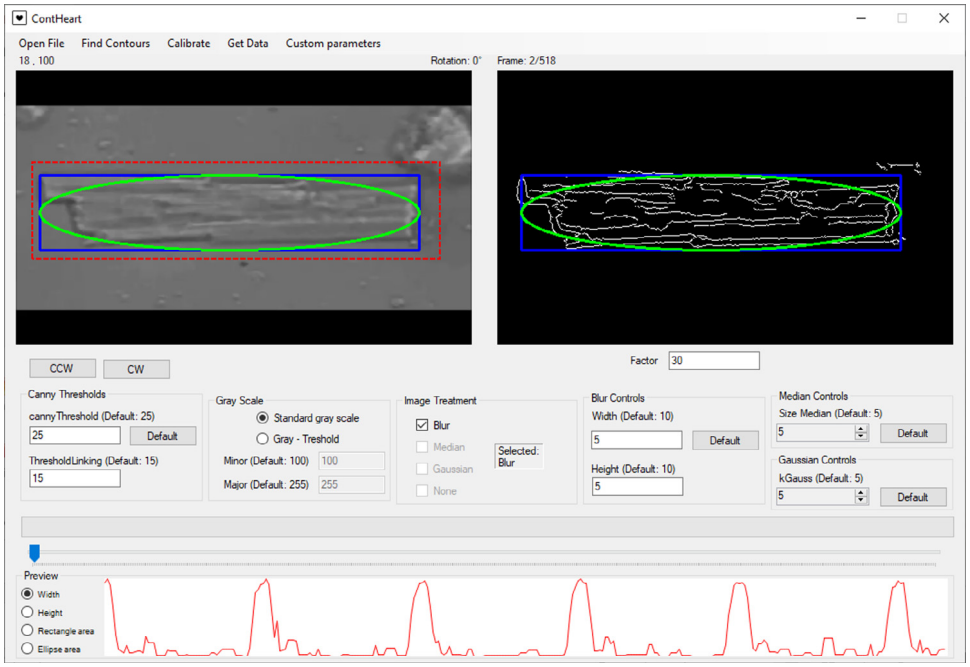

Fig. 4. This picture shows the ContHeart main window with parameters set to detect the cardiomyocyte. The dashed red rectangle indicates the ROI for analysis, blue rectangle, and green ellipse indicate the identified limits of the cell. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

- Blur filter was applied by checking "Blur" in image treatment group box (see 10 in Fig. 3) and blur parameters width and height (see 11 in Fig. 3) were set to 5;
- As the median and the filters were not applied their controls were unchanged (see 12 and 13 in Fig. 3).

After the user finds suitable parameters for detecting the cell, the selected parameters can be saved for use in other analyzes (by clicking on 5 in Fig. 3).

At this point, the user could click on "get data" (see 4 in Fig. 3) to perform a complete analysis of the video. Clicking on "get data", the user will watch each frame being detected at high speed while a progress bar (see 14 in Fig. 3) indicates the progress of the analysis. During the analysis, a line profile is shown as a preview of the detection of cell contraction (Fig. 4). The user can select which parameter will be displayed in the preview: width, height, rectangular and ellipsoidal area (see 16 in Fig. 3). However, If the user performs the shortening evaluation at this point, all results will be generated in arbitrary units, which represent the shortening of cells in pixels of the analyzed frame. The user has to execute a calibration step to obtain the real shortening in a unit of length.

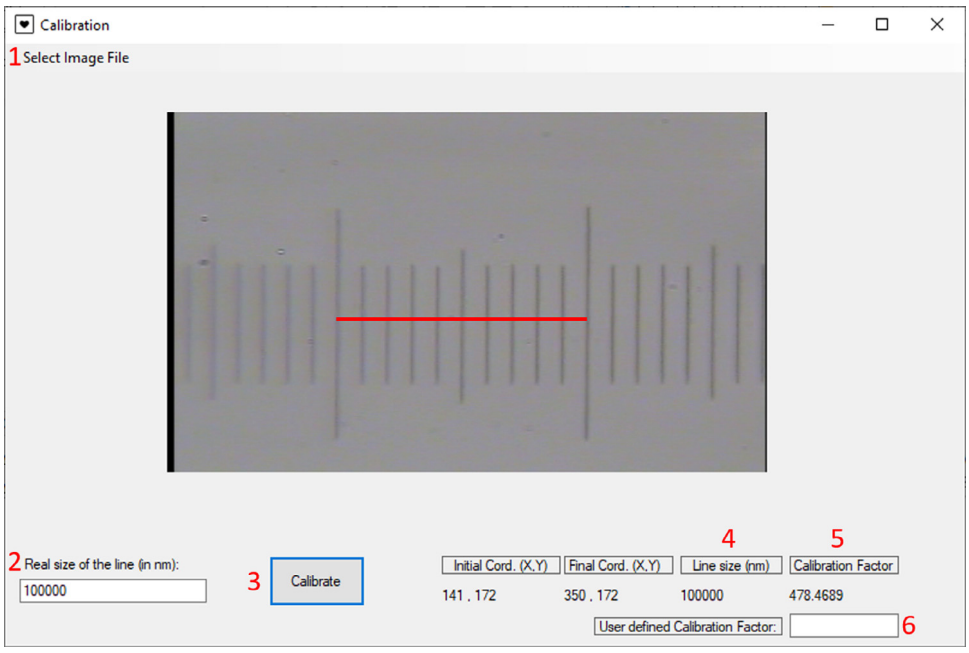

Fig. 5. ContHeart calibration window. For reference, see red labels indicating essential objects and regions on this screen (see text). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

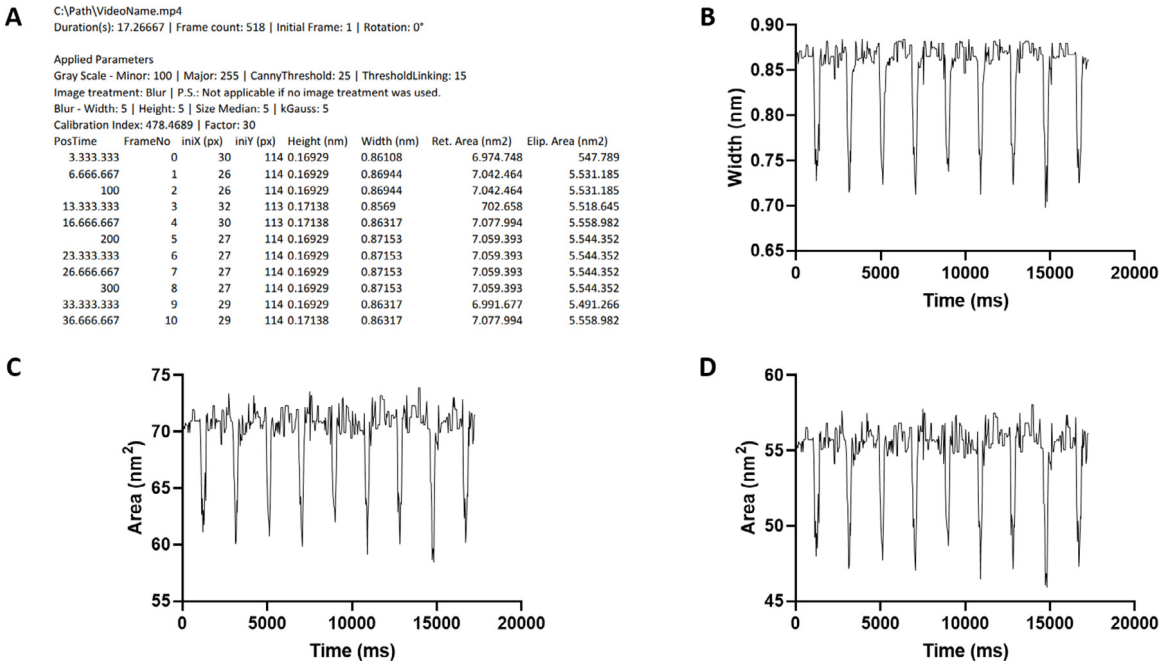

Fig. 6. Example of outputted results from ContHeart. A. Initial segment of the outputted .txt file showing the selected parameters and results. The cardiomyocyte shortening can be obtained from cell width (B), rectangular area (C), and elliptical area (D) variation.

The calibration step requires a reference standard for measurement of length. To proceed with calibration, the user must click "Calibrate" (see 3 in Fig. 3) to open the ContHeart calibration window (Fig. 5). There are two ways to open an image for calibration: 1. if there is a video open on the software main screen, the calibration window will already show that same frame; 2. The user can open a saved image by clicking on "Select Image File" (see 1 in Fig. 5). Also, the user can enter in the appropriated field (see 6 in Fig. 5) a calibration value obtained in a previous calibration. In this illustrative example, we used a graduated graticule (Carl Zeiss, Göttingen, Germany) as a reference (Fig. 5). The calibration step is straightforward, the user needs to draw a line (see red line in Fig. 5) and then insert the real line length in nm (see 2 in Fig. 5). Thereafter, the user must click on the button "Calibrate" (see 3 in Fig. 5). ContHeart will show the real line size and the calibration factor (see 4 and 5 in Fig. 5, respectively). Once the user has performed the calibration, ContHeart will express all the outputted values in nm. Also, it is possible to measure structures in the cell by opening a new image and drawing another line, which may be useful for some research.

The users should click "get data" to finish the analysis when they have already set all the parameters and achieved proper cell detection. ContHeart will analyze the entire video, and it will show a save window. The outputted .txt file (Fig. 6A) has all the

information about the performed analysis, including the path to the video file, its total duration, frame count, the frame where the analysis begins, the rotation applied to the frames, and all other user-defined parameters. The outputted results can be used to draw graphs showing the cell width variation, rectangular area variation, and elliptical area variation (Fig. 6B-D). The outputted .txt file stores data in a tabular structure, i.e., it is a tab-separated values file. Any spreadsheet software supports this file format. Thus, all outputted results can be easily plotted to represent the cardiomyocyte shortening, but the user must define which representation is most suitable for their application. For instance, in this illustrative example, the variation in width (Fig. 6B) showed less noisy shortening measurement than rectangular area variation (Fig. 6C), and elliptical area variation (Fig. 6D). However, this may vary case by case.

4. Impact

The method implemented in ContHeart has been efficient for detection of the amplitude and time course of a typical contraction, as well as for the detection of small unsynchronized contractions, the "contractile waves" [10]. The detection of contractile waves is essential for studies regarding the spontaneous cardiomyocyte activity, which is a predictor of pro-arrhythmic conditions [12–14]. However, this procedure commonly relies on intracellular calcium imaging acquired by confocal microscopy [15, 16], which is an expensive and tricky method. ContHeart also allows the analysis of videos in this condition, allowing the quantification of the waves without the need for intracellular calcium imaging, making it possible to extract contractile information using a more straightforward and cheaper experimental setup. A reliable method to detection of spontaneous contractions might also facilitate the studies of pro-arrhythmic drugs, which are very important to cardiovascular drug development and for evaluation of drug cardiotoxicity.

ContHeart also allows the analysis of asymmetric cells presenting shortening axis not parallel to the major axis of the cardiomyocyte, as occurs in neonatal cells, atrial cardiomyocytes, and cultured cells, for instance. The shortening evaluation of these cells by conventional methods is difficult because they do not have a regular pattern of contraction, poor sarcomere development and irregular shape.

Also, ContHeart is a user-friendly software that can be used by users without programming knowledge. Therefore, ContHeart allows researchers interested in basic cardiovascular research to broaden their scope of analysis using videos recorded in the regular work routine without the need for expensive equipment and software.

5. Conclusions

The analysis of the contractile behavior of cardiomyocytes is a valuable tool for the study of cardiovascular physiology. It allows the understanding of how the cardiomyocyte contraction is affected by different diseases, drugs or environmental conditions. ContHeart is an easy and useful tool that generates relevant data regarding the cardiomyocyte shortening. We expected that ContHeart might support many researchers to extend their field of analysis without the need for expensive software and equipment, or extensive programming expertise.

CRediT authorship contribution statement

Daniel Leal Fagundes: Methodology, Software, Investigation, Writing - original draft. **Jair T. Goulart:** Conceptualization, Methodology, Software, Investigation, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to the scientific initiation program (PIBIC) of the University of Brasilia (UnB), and the research and innovation decanate DPI-UnB.

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