Pulse Video Analysis Manual

Introduction

Pulse applies patented computer vision algorithms [1-2] to measure beating signals and their parameters from videos of beating cardiomyocytes. The web-based platform allows for seamless, scalable deployment that is suitable for high-throughput experiments.

Features

- Analyzes cardiomyocytes seeded at different densities or in 3D culture.
- Analyzes videos captured on any imaging system.
- Measures the contraction relaxation beating signals.
- Measures beat rate, beat duration, max contraction velocity, contraction displacement, prevalence of beatings cells, and more.
- Fully automated, no correcting peaks, no manual ROI delineation.
- Enables scalable and fast high-throughput analysis.
- Optional Windows desktop application automates the upload of videos for large-scale experiments.
- Dedicated user accounts, easy-to-use web interface and secure and efficient data management.

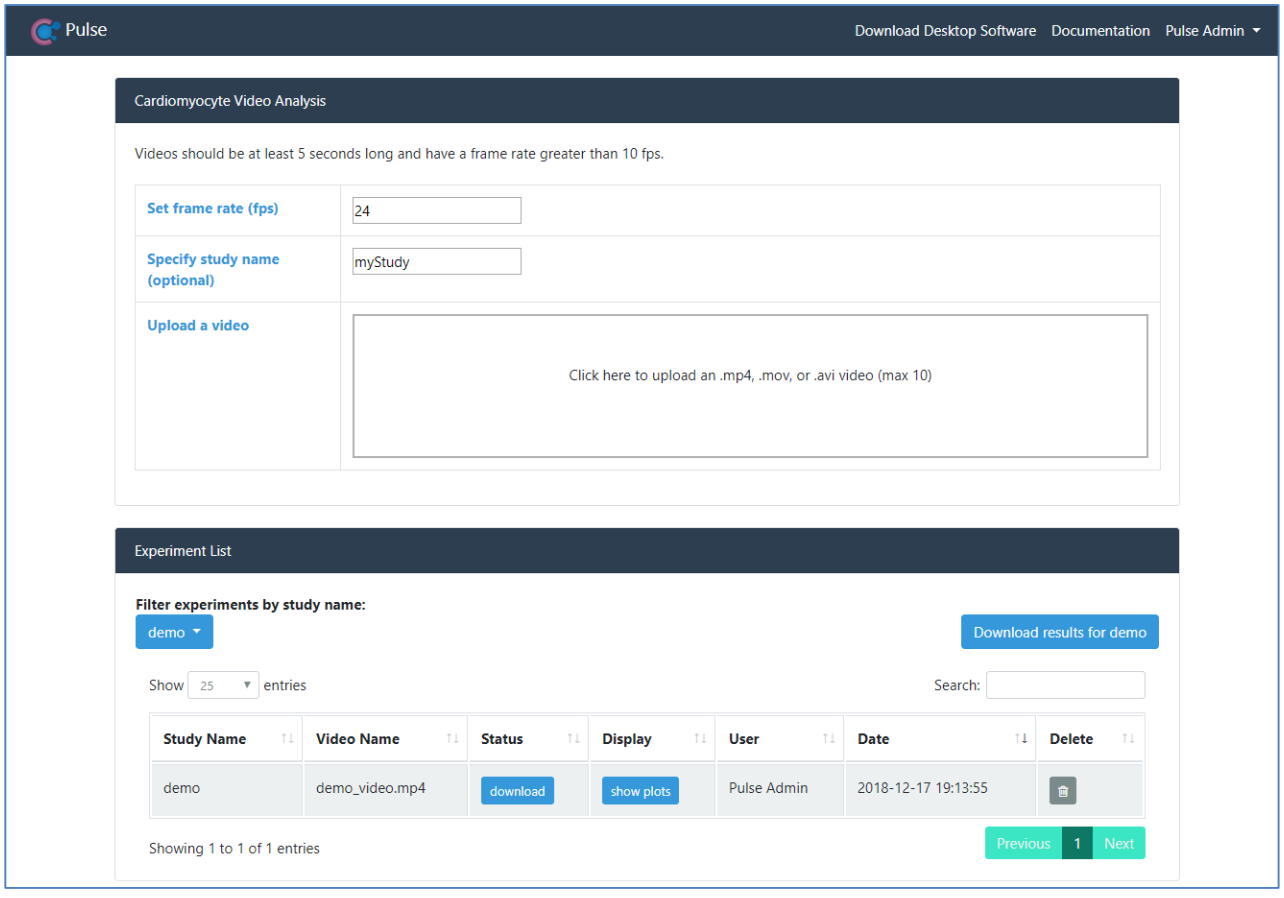
Web Interface

After registration at www.pulsevideoanalysis.com, new users will be directed to the home page as shown below. A demo video has already been uploaded and analyzed to provide an example of the analysis output. In order to see the analysis plots, click on show plots To upload a new video, specify the video frame rate and study name, and click on upload box to select your video(s).

Your video needs to meet the following specifications:

- Format: avi, mp4, or mov
- Frame rate: above 10 frames/second
- Duration: at least 5 seconds

Once the video is uploaded, the analysis is started automatically, and the status will be updated in the Experiment List. Once analysis is finished, a notification email with the results will be sent. You can also download the results for each video by clicking on download or you can download the results for all videos in a study by clicking Download results for demo



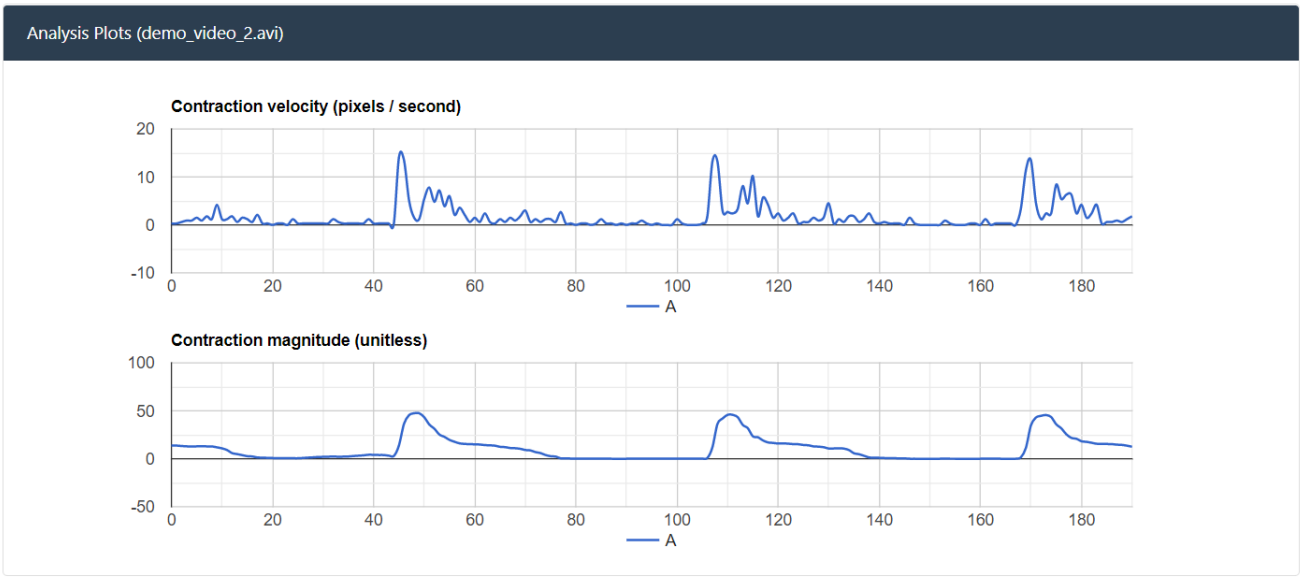
Contractility Analysis – Beating Signals

The Pulse software converts an uploaded video to an image sequence with the specified frame rate. The image sequence is then sent to the Pulse analysis algorithms [3-4], which perform the following:

- Block-wise segmentation of the image sequence.
- Extraction of the motion signal for each block.
- Quantification of the beating signals.
- Noise detection.
- Clustering of the beating signals into a set of unique signals, each representing a region of the culture where cardiomyocytes beat in synchrony.

The figure below shows the measured beating signals for the demo video. The top signal is the estimated contraction velocity measured frame-to-frame, which typically shows the contraction and relaxation as separate peaks. The unit on the y-axis is pixels per second.

The bottom signal is the contraction magnitude, from which all automated measurements are derived except for the maximum contraction velocity. The y-axis for this signal is unitless since it's measured by correlation of pixel intensities of each frame to an estimated resting-state image.



Contractility Analysis – Output Files

The output of the Pulse analysis includes the following files:

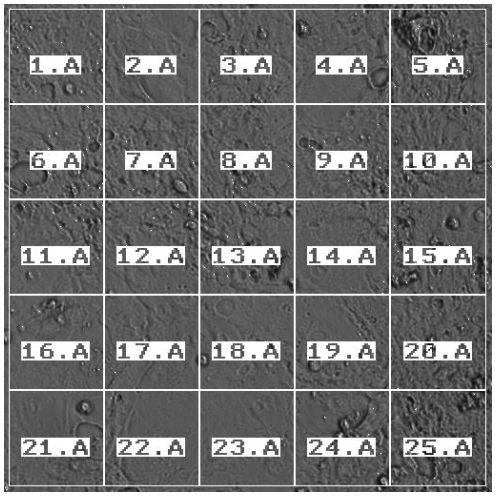
[videoname]_allsignals.csv: includes signals and their measured features

[videoname]_clusters.csv: includes signals and their measured features only for blocks that represent a cluster (a group of blocks beating in sync)

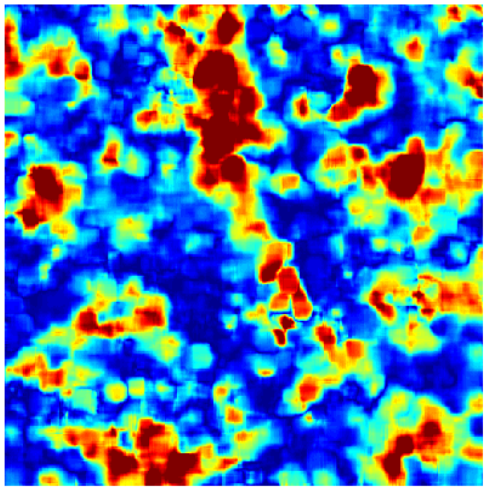
[videoname]_blocklabels.png: shows the block labels (1,2, ..) and cluster labels (A, B, ..)

[videoname].maxVelocity.png: a heatmap of the contraction velocity

Block Labels



Estimated Contraction Velocity Heatmap

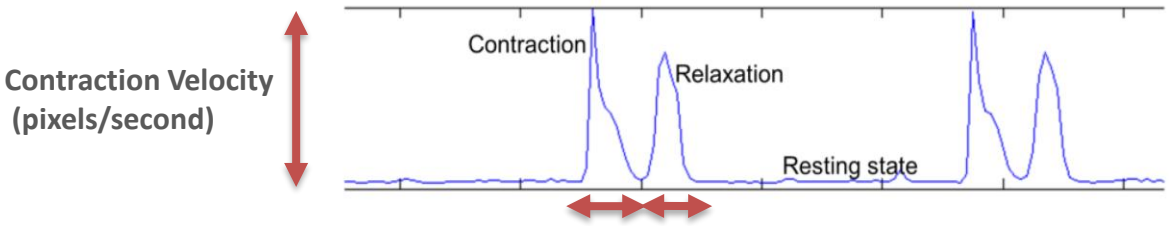


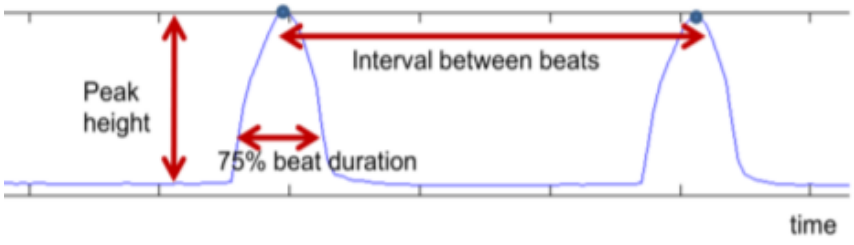
If results are downloaded for a *study,* then summary spreadsheets are generated, with average measurements for each video and each well.

Contractility Analysis – Measurements

The following parameters are measured by Pulse algorithm:

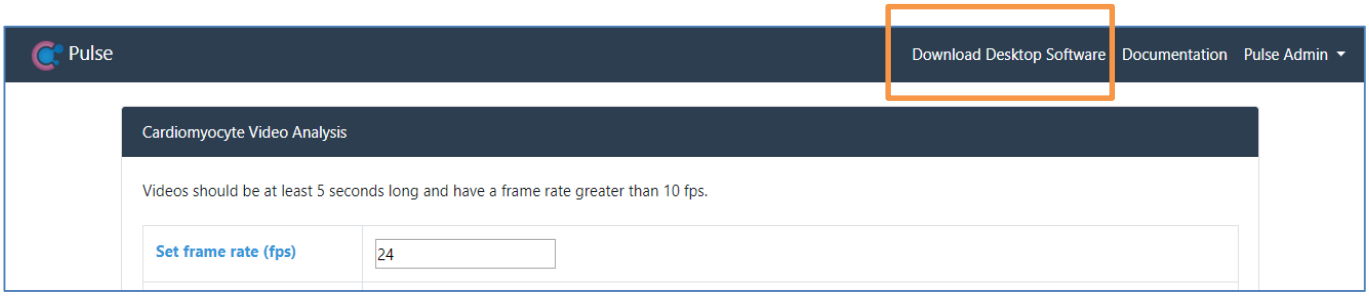
- **Block ID**: The label of a block as shown in [videoname]_blocklabels.png.
- **Cluster Label**: The label of cluster the block is assigned to.
- **Beat Rate**: The Average beat rate calculated over the duration of imaging.
- **Beat Rate Variation**: Standard deviation of beat intervals.
- **Beat Duration (x%)**: Measured at (100-x)% of peak height, reported in seconds.
- **Peak Height:** Unit-less measure of contractile strength.
- **Peak Height Variation:** Standard deviation of peak heights, a measure of beat-to-beat variability.
- **Prevalence:** Percentage of beating cells relative to image area.
- **Max Contraction Velocity:** The maximum contraction velocity in pixels/second.
- **Contraction Displacement:** Maximum contraction in pixels.
- **Contraction Time:** Duration of contraction in seconds.
- **Relaxation Time:** Duration of relaxation in seconds.



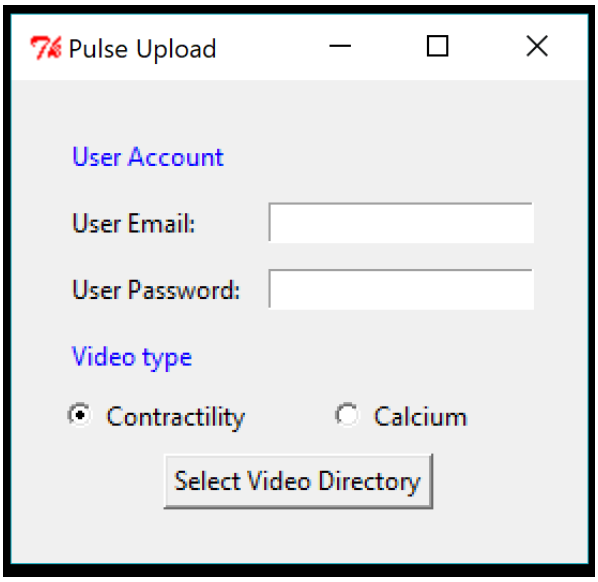


Desktop Application

An optional desktop application is available, which enables analysis of thousands of videos for applications in high-throughput screening. You can download the desktop software from the link on the top bar of the main page as shown below.



You need to download and unzip the file in a preferred location on a Windows machine (typically the computer of the imaging system). To run the upload application, simply double-click on pulseDesktopUpload.exe. You should see a window appear after a few seconds:



First enter your account details (same username and password you use when logging in to the Pulse web service). Then specify the type of video and click on Select Video Directory to choose a directory of your videos. The upload software will then automatically process and upload all videos within the directory. The name of the directory will be used as the study name. After upload you can go back to the Pulse web service to view your videos under the Experiment List.

References

1. US 9,569,845 (2017) Method and system for characterizing cell motion
2. US 9,355,459 (2016) Method and system for characterizing cell motion
3. Maddah M. & Loewke K. Non-Invasive Characterization of Stem Cell-Derived Cardiomyocytes from Phase-Contrast Microscopy. Proceedings of Medical Image Computing and Computer Assisted Intervention (MICCAI), 2014.
4. Maddah M., Heidmann J., Mandegar M., Walker C., Bolouki S., Conklin B. & Loewke K., A Non-invasive Platform for Functional Characterization of Stem-Cell-Derived Cardiomyocytes with Applications in Cardiotoxicity Testing. Stem Cell Reports. 4(4), 621-631, 2015 (Cover Image.)