ContractQuant Analysis Manual

About ContractQuant:
ContractQuant was developed to measure contractile kinetics in stem cell derived cardiomyocytes (iPSC-CMs) in 2D culture by using pixel features at two tracking regions of interest (ROIs) to measure relative displacments of these ROIs during successive contractions. This approach is most effective when the contractile direction is organized primarily in a uniaxial direction as attained in mechanically-decoupled micron-scale 2D cardiac muscle bundles (2DM2Bs) as described in Tsan, et al., *Nature Comm* 2021. In 2DMBs, a variety of contractile parameters are calculated, including contraction/relaxation velocities at different phases of the contractile cycle, contractile acceleration/deceleration, maximum fractional shortening, and contraction frequency. A modified script is also available, as described below, to measure fractional shortening in disorganized (nonpatterned) 2D iPSC-CMs.

Reference: Tsan et al., *Nature Communications* 2021

Install Files:
All scripts written for Matlab R2018a.
Bio-Formats toolbox downloaded and added to the Matlab path

Set Matlab Path:
Set path to include the following folders:
- Bio-formats folder ("bfmatlab" folder)
- Folder containing ContractQuant scripts
- Folder containing files to be analyzed

Microscopy and Image Preparation:
ContractQuant accepts either TIFFs or ND2 files using the Bio-Formats package in Matlab to import. These scripts could be readily modified for other imaging file types. ROIs are set at default locations that result in tracking of the inner 50% of 2DMBs that are centered in the field of view and oriented horizontally. Time series should be obtained with at least 30 fps and ideally 50 fps. Acquisitions should include at least 4 full contractions. The light intensity should be adjusted to the minimal setting at which obvious pixel noise is absent.

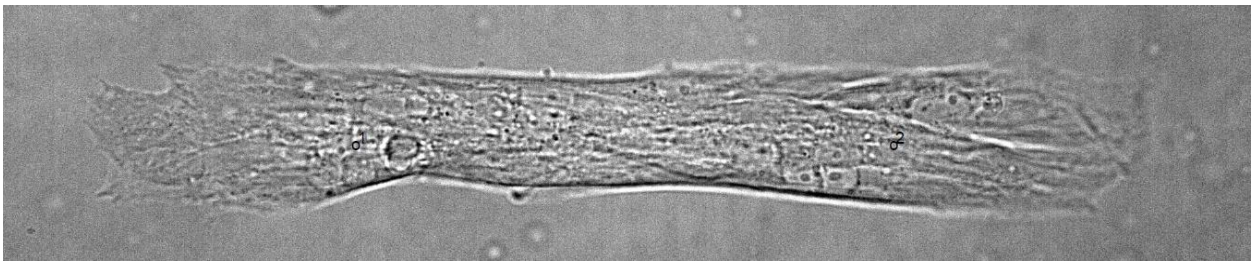
Parameter Setting:
The "Parameter Setting" section of the ContractQuant and/or ContractQuantManual code (line 50) must be edited for the particular microscope and camera set-up.
The default settings are
- PixelDistance = 0.16 (um/pixel)
- SamplingInterval = 0.02 (sec)
- TIF=0 (for ND2 files)
- defaultXs=[580,1460]' (x position in pixels of point 1, x position in pixels of point 2)
- defaultYs=[224,224]' (y position in pixels of point 1, y position in pixels of point 2)
- ROIsize=100

In the ContractQuant script (called by the ContractQuantBatch command), the ROI locations are determined by the defaultXs and defaultYs variables. It is most efficient to always capture time series with the 2DMBs centered and horizontally oriented during imaging so that these values can be hard-coded and analyses can be run in batch. Alternatively, the manual version can be run, for which the user is prompted to click on the desired ROIs. The defaultXs and defaultYs values should be selected to track the inner 50% of the 2DMB. These 2 tracking locations are identified by pixel coordinates and distances are later calculated using the PixelDistance variable. Change the pixel coordinates of tracking points using the defaultXs and defaultYs as needed. The ROIsize is set by default to 100 pixels – change this variable as needed to result in ROIs that are each approximately 5% of the average 2DMB size.

ContractQuantBatch:

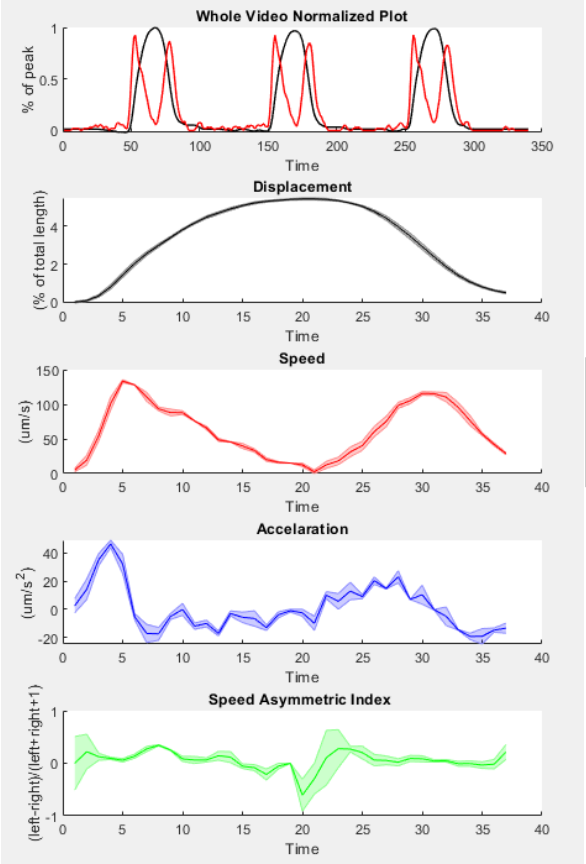
The standard methods for performing analyses with ContractQuant is to use ContractQuantBatch, which runs analyses on all files in a folder. All image files must contain appropriately aligned 2DMBs and the parameter settings must first be adjusted in the ContractQuant script (line 50) to match the microscope and camera settings.

1. Enter at the command line: ContractQuantBatch
2. A dialogue box will open. Navigate to the folder containing all input files. Select any file in this folder (the specific file selected does not matter – only used to extract the folder location).
3. The script will sequentially analyze all image files in the selected folder using the ContractQuant script.
4. All resulting images and spreadsheets of detailed data will be exported to a folder called "ContractionAnalysis" in the same folder as analyzed images. A composite spreadsheet containing results from all files is also generated, "ContractilityResults".
5. Examine the following for each analyzed video:
    a. The _ROI_reference.eps figure should look like this (note small labeled tracking points):



Important: The tracked points occur around 25% of the full tissue width inward from each edge of the tissue. This positioning minimizes the effect of minor differences in the precise location of the tracking points (see Figure 2 and Supplemental Figure 3 from Tsan, et al., *Nature Comm* 2021). Good contractile tracking will be evident in the graphs below. Positioning at the lengthwise edges should be avoided.

    b. The _NorFullTraces figure should look like this:



Whole video normalized plot: should show multiple distinct contractions. Black curve depicts normalized displacement and red curve depicts the absolute value of contraction speed.

Displacement Plot: This will show merged displacement curves from all analyzed contractions with shaded areas depicting 95% confidence intervals. If there is substantial beat-beat variability, large confidence intervals will be present. The baseline should be ~0%.

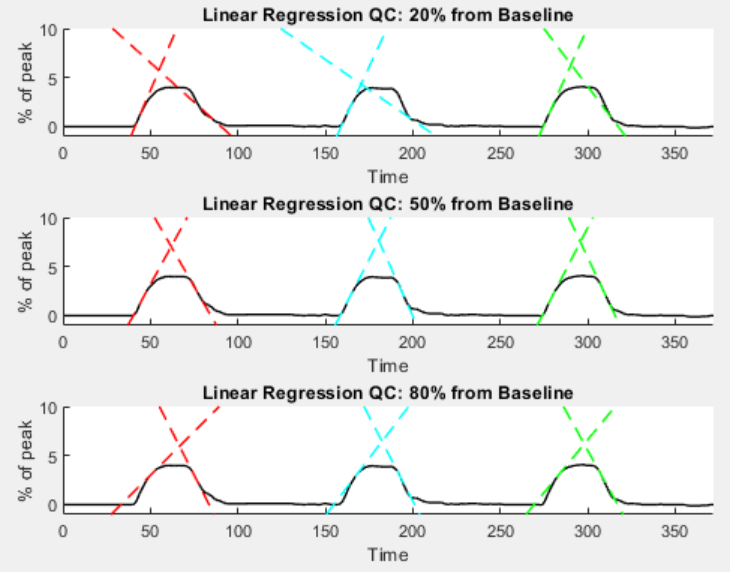
Speed Plot: This will show merged speed curves from all analyzed contractions with shaded areas depicting 95% confidence intervals. The baseline should be ~0.

Acceleration Plot: This will show merged acceleration curves from all analyzed contractions with shaded areas depicting 95% confidence intervals.

Speed Asymmetric Index Plot: This will show merged asymmetric speed index from all analyzed contractions with shaded areas depicting 95% confidence intervals. This is a measure of how uniform the contraction (relative index of contraction speeds from left and right edge of tissue).

**The script will extract the MEDIAN value for each measured parameter. Therefore, outlier contractions that create larger confidence intervals in the merged graphs are acceptable, as long as the median parameters would not be affected.

c. The _Linear_Regressions figure should look like this:



Linear Regerssion at 20% from Baseline: shows regression lines centered at the displacement point at 20% of max contraction (both for contraction and

Linear Regerssion at 50% from Baseline: shows regression lines centered at the displacement point at 50% of max contraction (both for contraction and

Linear Regerssion at 80% from Baseline: shows regression lines centered at the displacement point at 80% of max contraction (both for contraction and

**As above, single outliers are not a problem, since median values will be extracted.

6. If Matlab encounters an error in running any of the individual files, the "ContractilityResults" spreadsheet will have an error message next to the file name rather than exported results. Some figures may be missing for files that encountered errors.

Troubleshooting:

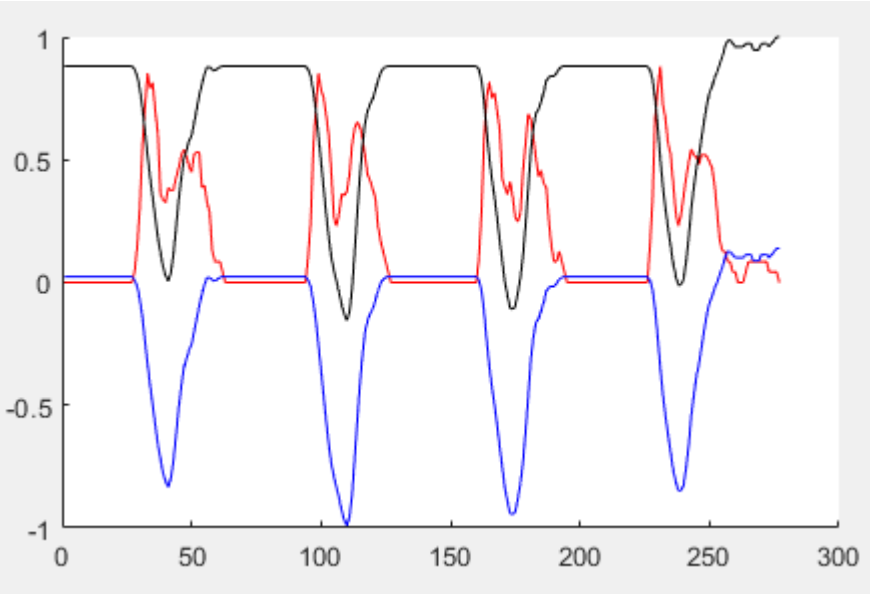
1. If the figures generated do not show good contraction tracking (as in the above), check that the tracking points are correctly located. The locations of these points are shown in the exported ROI_reference.eps image for each analyzed file (as in 5a above) -- these images will be generated even if the analysis encounters an error for that file. If not correctly located, the [x,y] positions can be changed in the Parameter Settings section of the code (line 50) as needed. Alternatively, the **ContractQuantManual** script can be run on files individually with user selection of the ROIs when prompted (see below).
2. Tissues that are contracting very irregularly or have extensive baseline artifact/noise (such as in monolayers) will not run correctly in the standard algorithm. Tissues such as this can be run only with the simplified script **ContractQuantSimpleManual**. This script will only collect a small subset of feasible measurements. See below.
3. If the width of the peri-event traces is either too small or too wide, these can be adjusted by adding an additional input when running the script to adjust the width by a specified number of frames expected per contraction (offsetMargin). A positive number will increase the width by the specified number of frames and a negative number will decrease the width. This is rarely needed, since the script automatically adjusts for the peri-event width, but might be needed under experimental conditions that markedly alter contraction duration.

ContractQuantManual:

This script performs the same analyses as above using user-defined tracking points for the ROIs. The user is prompted to select the tracking points through a GUI. Files are run one at a time.

ContractQuantSimpleManual:

This script is a simplified version that does not do regressions for precise measurements of contractile kinetics at different phases of the contractile cycle, since these measurements are not feasible when there is a lot of variability (as occurs typically with monolayer tissues, or with marked changes in 2DMB tissues with pharmacologic agents). This script prompts for manual selection of the tracking points. Click on 2 locations to analyze the contractile displacement and velocities between these 2 points, then hit enter. This script does not run in batch since manual selection of points is required. A similar distance between points as in the 2DMB approach should be used for comparable results. A single spreadsheet will be generated as below. The whole video normalized plot will show normalization of fractional shortening in two colors – black and blue. Black uses the same normalization that is robust for 2DMB tissues, as above. Blue uses an additional adjustment that should compensate for tissues experiencing stretch (uses a rounded mode value to set the baseline) – this will allow calculation of negative numbers for fractional shortening. The adjusted values as described in the above section account for this latter normalization, so it is important to verify that the baseline adjustment makes sense.



Example of normalized traces for monolayer tissue that exhibits stretch between the 2 selected tracking points. The black plot uses that standard baseline method adjustment as for 2DMBs, which results in an erroneous setting of the baseline near ~1. The blue plot uses a modified baseline adjustment based on the mode (most frequent value) – this correctly identifies the baseline and negative deflections represent stretch. The baseline should be obvious as the regions of the plot that exhibit no motion. All calculations in the ContractQuantSimple are based on the adjusted baseline calculations. Note that the calculated velocities will always be positive since they are absolute values.

Output of ContractQuantSimpleManual:

The excel file contains the following variables in row 2:
TrackingDistance_um = distance between tracking points
PixelDistance = width of pixels in um
ContractionFrequency = frequency of contractions (may not be calculated if video too noisy)
Max_FrShort_All = maximum fractional shortening assuming no stretch
Max_AdjustedFrShort = maximum fractional shortening after adjustment for stretch Min_AdjustedFrShort
Max_Velocity = maximum contraction velocity in the whole acquisition
Velocity_Asymmetry_Index = asymmetry in velocity between tracking points at time of max velocity
median_max_FrShort = median max contraction velocity across multiple beats (may not be calculated if too noisy)
std_max_FrShort = std dev of max fractional shortening across multiple beats (may not be calculated if too noisy)
median_max_Contract_Vel = median of max contraction velocities across beats (may not be calculated if too noisy)
median_max_ContractAccel = median of max contraction accelerations (may not be calculated if too noisy)
median_max_Relax_Vel = median of max relaxation velocities across beats (may not be calculated if too noisy)

Lower rows contain individual contraction results for separate beats (if analyzed).