

Supplementary Data

Supplementary Materials

Description of guided user interfaces

Guided user interface 1: MotionGUI. The MotionGUI interface (Supplementary Fig. S1) is provided to allow users to rapidly generate motion vector fields without the need for programming. The user first uses the Get folder button to identify a folder containing a series of .tiff files. The first image from the .tiff series appears in the GUI, allowing the user to confirm that the right image was selected.

The user then enters the frame rate. Within the Advanced input submenu, users enter the pixel size in μm . If the pixel size is unknown, but the image contains fiduciary markers of known size, the user has the option to measure them to generate the pixel to μm conversion. The user then selects a macroblock size (default 16 pixels) and a maximum detectable motion (default 7 pixels). The user further has the option to scale down the image width to improve processing time.

For image segmentation, the user can check one of several boxes that determine whether there will be an intensity threshold (e.g., for fluorescent reporters), whether intensity will be normalized at each time frame (for imaging motion of GCaMP6f or other indicators of calcium or voltage), and how edge detection will be performed. The user can also set a threshold to be used for the moving foreground detector, if this method is to be used instead of edge detection to segment cardiomyocytes.

After analysis is performed, the user can save the object containing spatiotemporal information about motion vector direction and intensity. The user can also load in previously generated motion vector files (e.g., those generated by batch analysis). The motion vector fields can then be post-processed based on techniques described in the article. Movies and new .tiff series with motion vector quiver maps superimposed over images can be generated, along with plots of time-averaged motion speed. To redefine the x and y

axes (e.g., to determine longitudinal and transverse motion within a tissue), the user can set a custom x -axis.

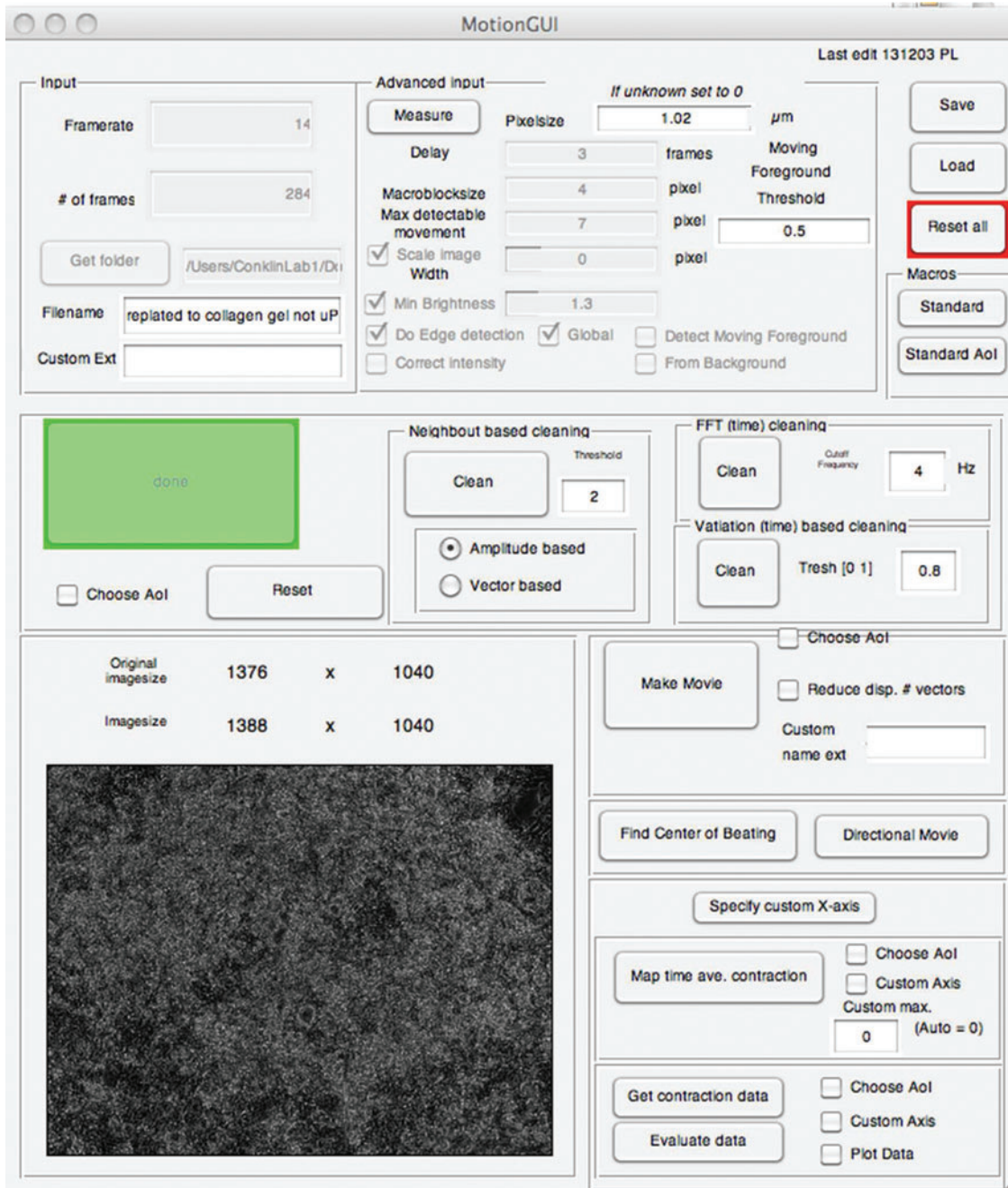
Finally, to generate and evaluate contraction data (either with standard or customized x and y axes), the user uses the Get contraction data button followed by the Evaluate data button.

Guided user interface 1: DataEvaluation. The DataEvaluation interface (Supplementary Fig. S2) is provided to allow users to analyze one-dimensional (1D) tracings of contraction speed, and to generate reports on parameters of these data such as beat rate and maximum contraction velocity. The user first generates motion vector fields with the MotionGUI interface, and the DataEvaluation interface is launched when the user selects Evaluate data.

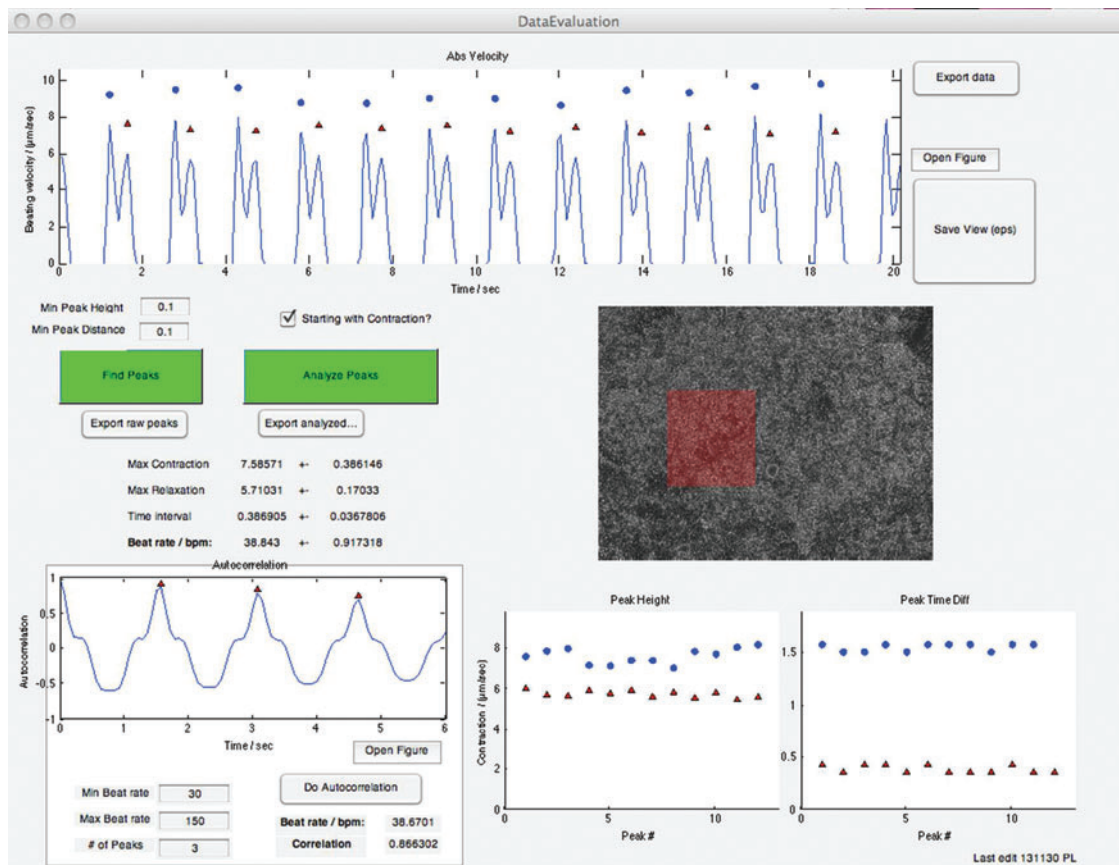
Within DataEvaluation, the user is shown 1D tracings of contraction speed versus time (Supplementary Fig. S2, upper panel) as well as a reference image with the region of interest highlighted. Raw data can be exported with a button to the right of the tracings (to plot in other software such as MS Excel), or a new .fig file can be generated.

To automatically measure beat rate, the user can use a peak finder (Find peaks button) with the option of restricting the height of contraction peaks and the distance between them. When the user selects Analyze Peaks, the peaks are split into contraction (blue) and relaxation (red), and the averaged values of maximum contraction velocity (intensity of the first peak in each doublet), maximum relaxation velocity (intensity of the second peak in each doublet), beat rate, and the time interval between beats. Alternatively, autocorrelation analysis (Do Autocorrelation) provides a robust estimate of beat rate when motion speed is low or signal-to-noise ratio is suboptimal.

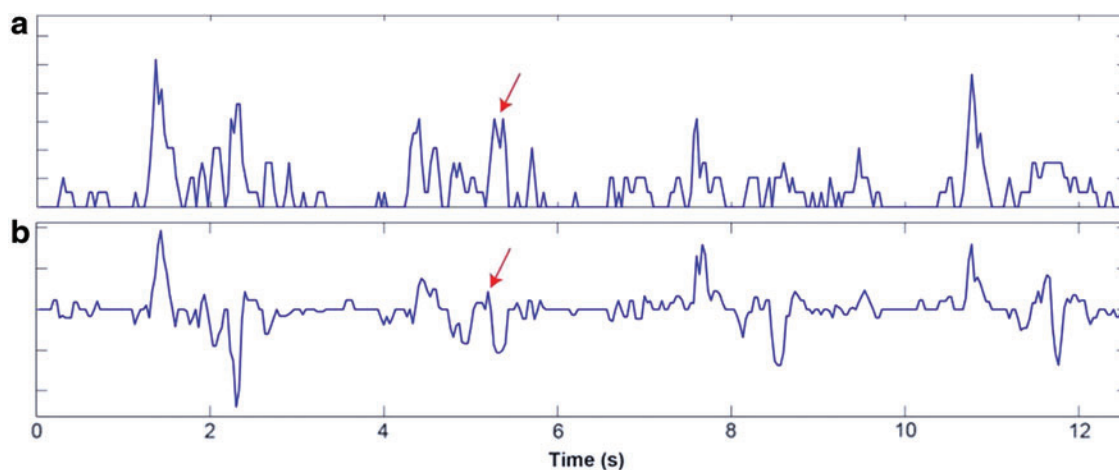
At any point during the analysis, the user has the option to archive the GUI, along with noted tracings, data, and images with analyzed region of interest, with Save View.



SUPPLEMENTARY FIG. S1. Guided user interface for generating motion vectors.



SUPPLEMENTARY FIG. S2. Guided user interface for analysis of one-dimensional tracings of contraction speed versus time.



SUPPLEMENTARY FIG. S3. Utilizing directional motion tracking to detect pathologic motion abnormalities. (a) Tracing of absolute motion for a region that displays potentially aberrant beating patterns. (b) Tracings of the same region showing directional motion, which clearly distinguishes early after depolarization events (red arrow), in which a contraction is followed by a second weak contraction before a full relaxation can occur.