SUPPLEMENTAL MATERIAL

Detailed Methods

All laboratory procedures in this study conform to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health, the Guide for the Care and Use of Laboratory Animals laid out by Animal Care Committee of the University of California (UC). The animal use was approved by the UC Davis Institutional Animal Care and Use Committee.

Cell isolation

Sprague-Dawley rats were purchased from Charles River (http://www.criver.com). The rats were anesthetized with nembutal (100 mg/kg injected IP). After testing for the suppression of reflexes, the hearts were explanted via midline thoracotomy. A standard enzymatic technique was used to isolate the ventricle myocyte. Briefly, the heart was mounted on a Langendorff system and perfused with a modified Tyrode solution containing (in mmol/L) NaCl 135, KCl 4, MgSO4 1.0, NaH_2PO_4 0.34, glucose 15, HEPES 10, taurine 10, pH 7.25 (adjusted with NaOH); the perfusion solution was pre-warmed to 37^{o}C and bubbled with 100% O_2. Then, collagenase B (~ 1 mg/ml, F. Hoffmann-La Roche Ltd, Switzerland), protease type XIV (~0.1 mg/ml), 0.1% BSA and 20 μM Ca^{2+} were added into the perfusion solution, and the heart was enzymatically digested for 15-20 minute. The ventricular tissue was cut down and minced; the remaining tissue was further incubated in the enzyme solution at 37^{o}C for 15-45 minutes, and minced again to collect isolated ventricular myocytes. The cells were used for experiments within 6 hours after isolation.

Cell preparation for Fluo-4 loading and Di8-ANNEPS staining in live cardiomyocytes

Freshly isolated rat VCMs were incubated in Ca^{2+} free Tyrode's solution containing: 145 mM NaCl, 4 mM KCl, 0.33 mM NaH_2PO_4, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose; the pH was adjusted to 7.3 with NaOH. Prior to imaging, the Ca^{2+} concentration in the cell incubation solution was increased to 1 mM in a stepwise manner. The cells were consecutively incubated in Tyrode's solution with 0.2 mM, 0.5 mM and 1 mM Ca^{2+} for 30 min, 20 min and 20 min respectively. The cells were loaded by incubating 0.5 ml of the cell suspension with 0.5 ml of a solution containing Ca^{2+} indicator Fluo-4/AM 2.5 μM (Molecular Probes-Invitrogen, Carlsbad, CA) and 0.75 μM pluronic acid in Tyrode's solution with 1 mM Ca^{2+}, for 30 min at room temperature. To visualize T-tubules, the cells were incubated for 5 min in Tyrode's solution with 1 mM Ca^{2+} and 5 μM Di8-ANEPPS (Molecular Probes-Invitrogen).

Antibody labeling in fixed cardiomyocytes

RyRs of mouse VCMs were labeled with Alexa Fluor 488 conjugated anti-RyR_2 antibodies. The cells were fixed in 1% paraformaldehyde for 5 min and washed in cold phosphate buffered saline (PBS) solution twice. Cell membranes were permeabilized by incubating with 0.1% Triton X-100 solution at RT for 5 min, washed, and blocked. After blocking, the cells were incubated with primary antibody (Anti-RyR_2, mouse monoclonal antibody, MA3-916, Affinity BioReagents, Golden, CO) for 2 hours at RT. They were washed twice with 0.01% Triton X-100 and incubated with secondary antibody (Alexa Fluor 488, Goat anti-mouse IgG, Molecular Probes) for another 2 hours at RT. Finally, the cells were washed twice with 0.01% Triton X-100 and re-suspended in PBS.

Transverse aortic constriction (TAC) surgery and echocardiographic measurements

TAC was performed on mouse (male, 11 week old) as described previously (Rockman et al, ref below), with some modifications. Briefly, after deep anesthesia was induced with 2-4% isoflurane, a small incision was made into the chest cavity in the second intercostal space. The transverse aorta was ligated with suture tied against a 27- gauge wire. Successful constriction of the aorta was confirmed through observation of diminished left carotid artery perfusion. Under 1-4% isoflurane anesthesia

echocardiographic measurements were taken two days before, and 2 weeks and 7 weeks after TAC surgery. Analysis was performed using a Visual Sonic Vevo 2100 system. After confirmation of a decrease of the ejection fraction to less than 50% after 7-8 weeks post TAC, the mouse was used for cardiomyocytes isolation and experimentation.

Calculation of A-band distance pdf using the kernel density methods

The histogram of A-bands distance (Fig.3C) is calculated using the kernel density methods.[36,37] For every measured distance d_i between adjacent A-bands in the linescan image we define the function

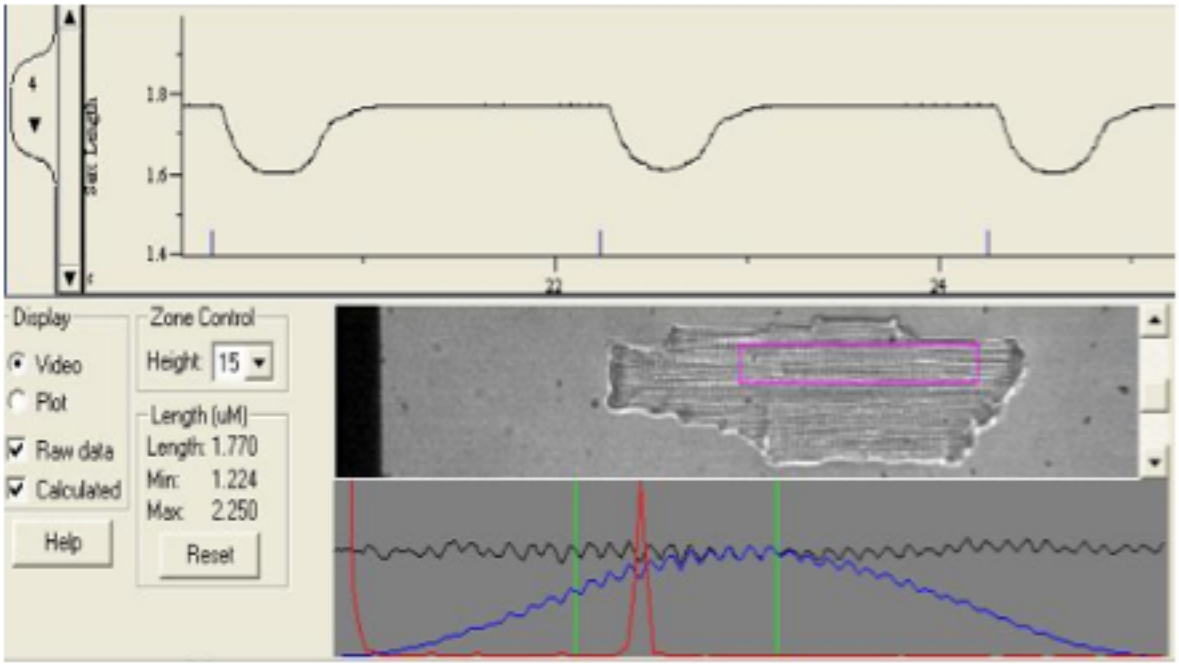
$$h\left(x,d_i\right)=\frac{1}{\sqrt{2\pi}\,wN}\exp\left(-\frac{(x-d_i)^2}{2w^2}\right) \tag{0.1}$$

where N is the total number of distances. w is the bandwidth and, like bin widths used to construct histograms, larger values give smoother pdfs but can lead to loss of resolution. The estimate of the distance pdf $f(x)$ is

$$f(x)=\sum_{i=1}^{N}h(x,d_i)\ . \tag{0.2}$$

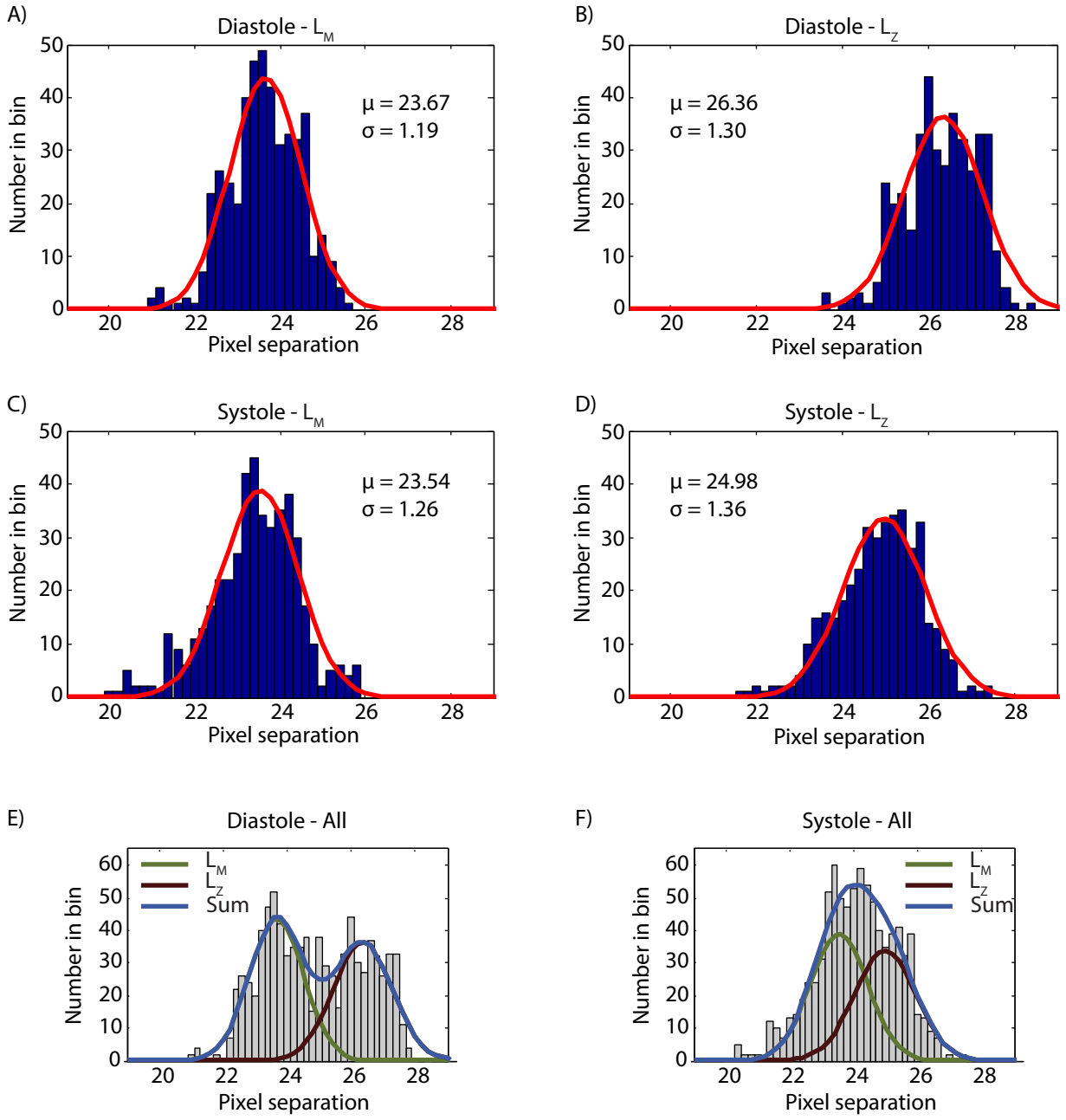
By construction, the integral of $f(x)$ overall x is unity.

Online Figure I



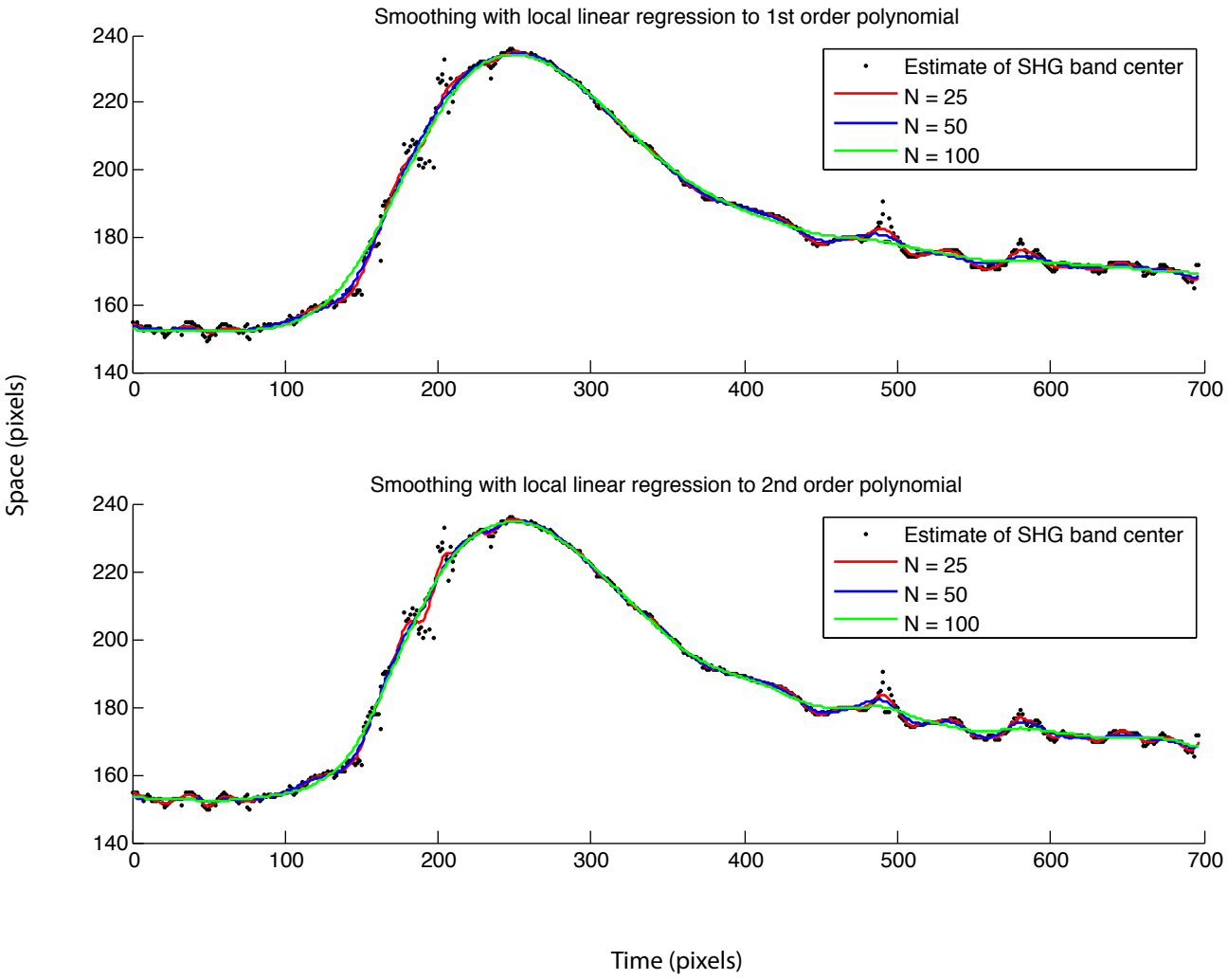
Online Figure I. Sarcomere length measurement using transmission image.
The rat ventricular myocyte shows clear striated sarcomere pattern in the transmission image (middle panel). This pattern in the region of interest (box in magenta color) is recorded as the the periodic change in the light intensity (lower panel, grey line), the FFT spectrum of which is used to calculate the sarcomere length. The change of sarcomere length during cell contraction (upper panel, Sarc Length) show the Sarc Length is about 1.8 μm in diastole and about 1.6 μm in systole.

Online Figure II



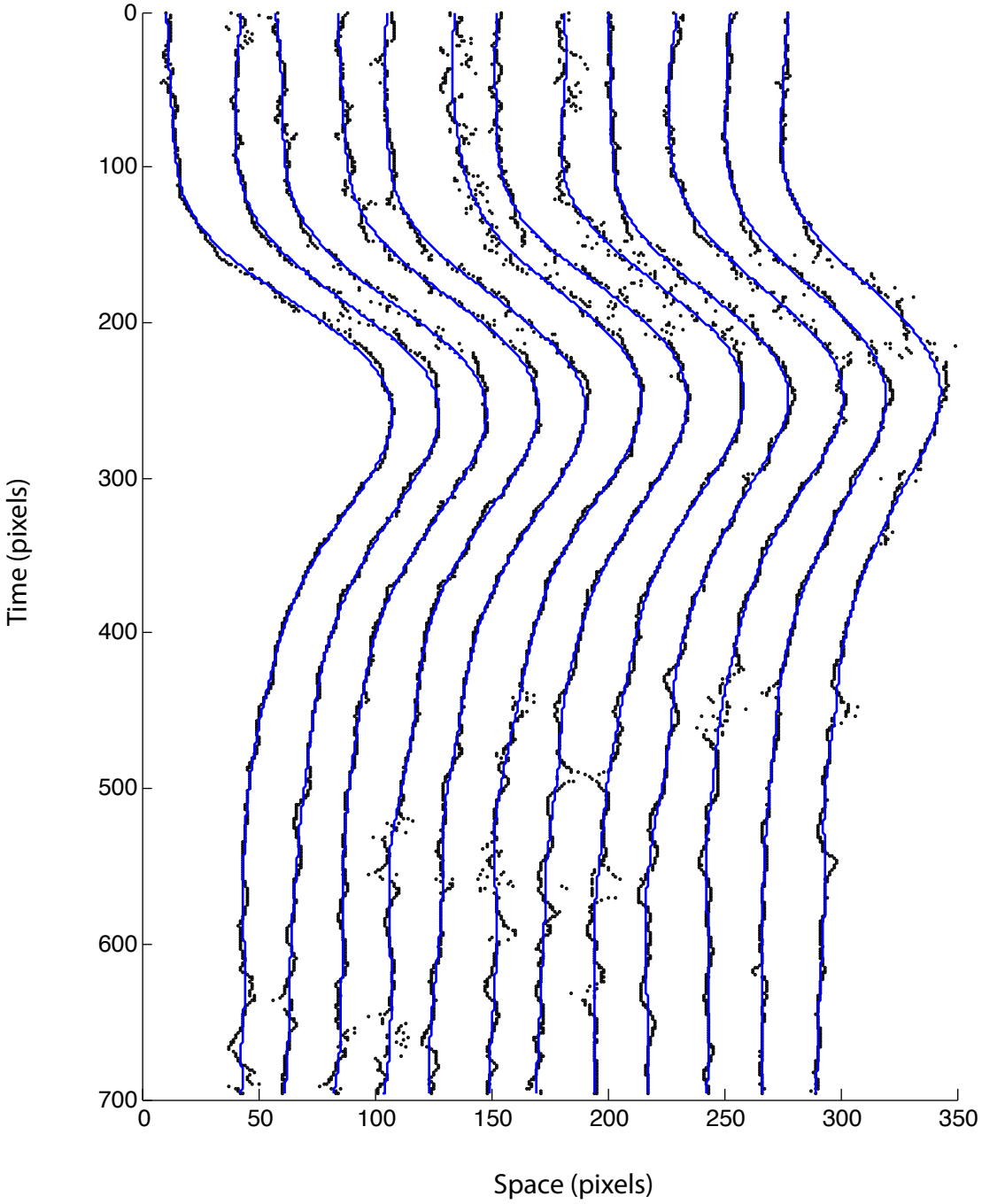
Online Figure II. Histograms of center-to-center distances from Figure 4 of the main text.
Following the data analysis procedure depicted in Figure 4 of the main text, the center-to-center distances between the SHG bright bands were determined and collected into histograms. Alternating distances were binned separately (A-D) and together (E-F) during diastole (A-B) and systole (C-D). Gaussian fits were performed on the histogram data. The alternating distances corresponding to L_M did not shorten during contraction (A vs. C) whereas those corresponding to L_Z did shorten (B vs. D). For Figures 4G-H of the main text (E and F above), all center-to-center distances were binned together (no separation of L_M and L_Z), but the Gaussian fits from A-D above were displayed on the plots to show the relative contributions of L_M and L_Z to the histogram. the "sum" curve in E and F above shows the sum of the displayed Gaussian curve. In these figures, each pixel is approximately 0.033 microns.

Online Figure III



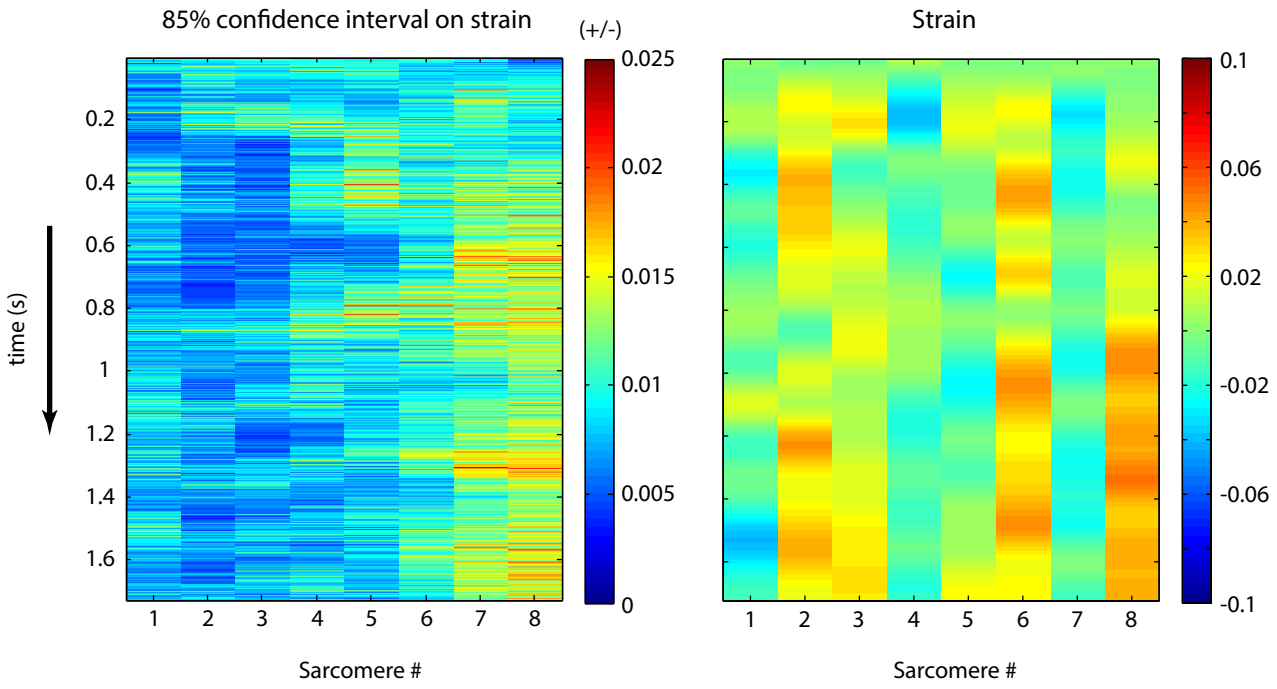
Online Figure III. Smoothing using a local linear regression.
We use a local linear regression to smooth data. In the process, a polynomial is fit to data in a window that is N pixels wide. The window is moved across the data set pixel by pixel. The resulting fits are averaged to obtain the smooth data set. The figure above shows the result of the smoothing process on a single SHG-bright band from the data in Figure 5B of the main text. The figure shows fits using different window sizes (N) and a first degree polynomial (top) or a second degree polynomial. The exact N necessary depends on the signal-to-noise ration in the raw data.

Online Figure IV



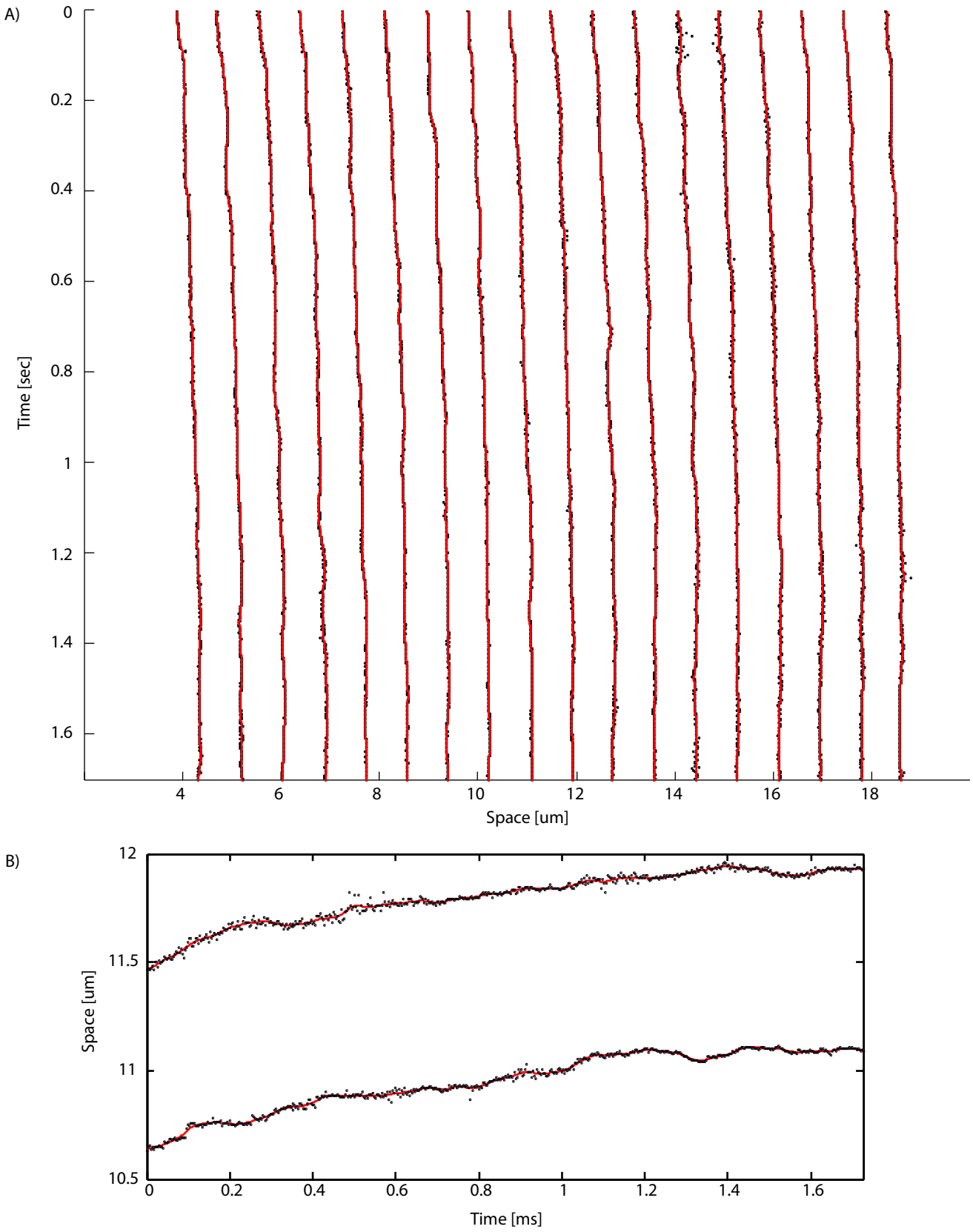
Online Figure IV: Smoothing process applied to a full calcium wave data set.
The result of applying the smoothing process from Online Figure III, using N = 100 and a first order polynomial, to the data used to generate Fig. 5B in the main text, is shown above.

Online Figure V



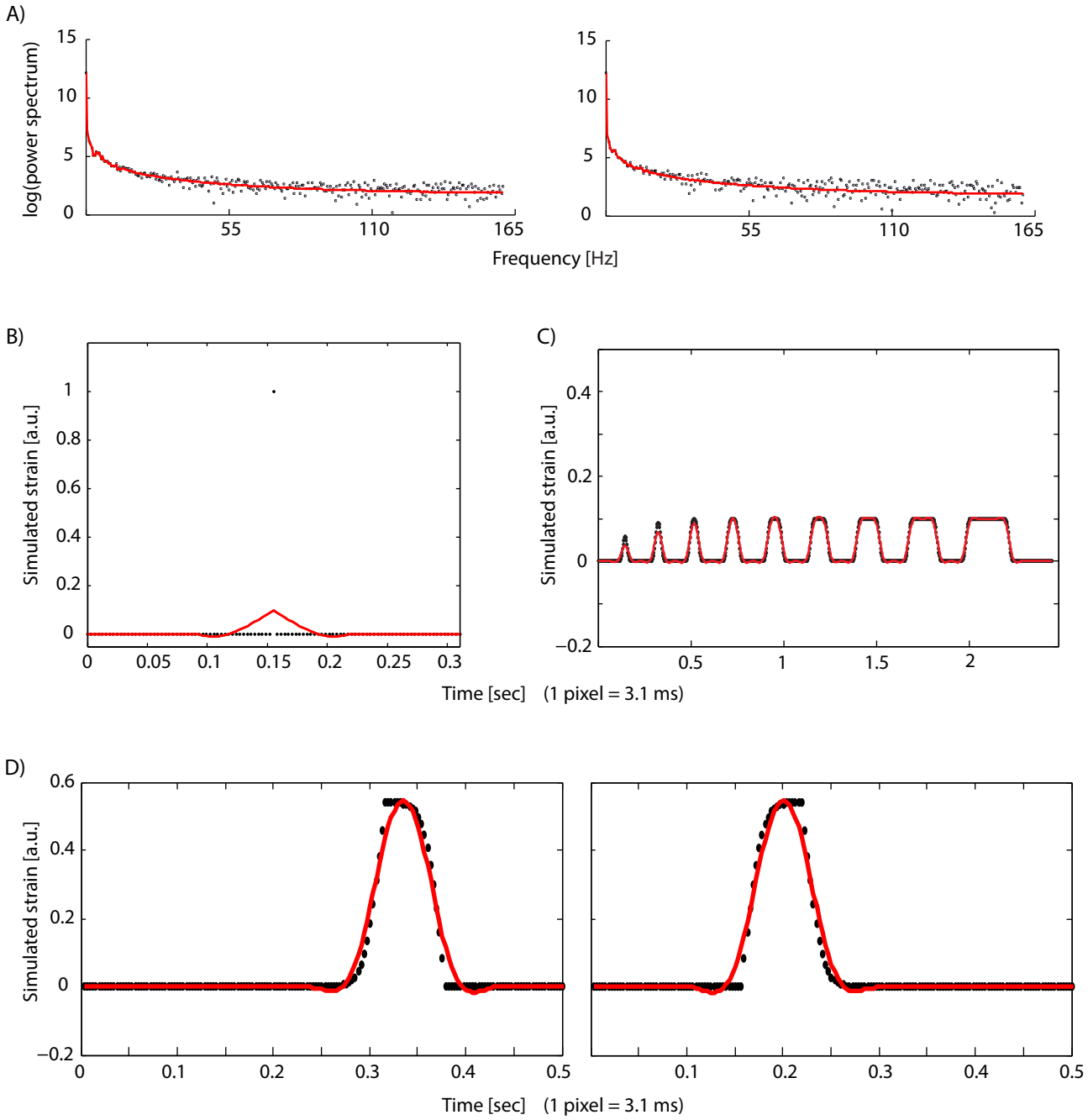
Online Figure V: 85% confidence intervals on peak-finding propagated through calculation of Ca(2+)-spark induced strain. 85% confidence intervals (CIs) were obtained from the Gaussian fitting algorithm (described in Fig. 4 of the main text) that was applied to the calcium spark data underlying Fig. 5C and Fig. 6B-C. The CIs were sub-pixel when displayed over the full set of peaks (e.g. Online Figure VI A). In order demonstrate the ultimate impact of the CIs, they were propagated through the calculation of strain in order to obtain 85% CIs on the local strain events. The CIs are displayed above in a heat map (left panel), next to the strain data (right panel). The CIs were calculated from data before smoothing, whereas the strain map is calculated from post-smoothing data, as described in the main text.

Online Figure VI



Online Figure VI. Smoothing applied to the calcium spark data set.
A) The result of applying the smoothing process from Online Figure III, using N = 20 and a first order polynomial, to the data used to generate Figure 5C in the main text, is shown above. B) Examples of individual bands from (A).

Online Figure VII



Online Figure VII: Effect of smoothing on calcium spark-induced strain.
A) Effect of smoothing on the power sepctra of the bands shown in Online Figure VI B, before (black dots) and after (red line). Smoothing significantly attenuates structure in the power spectra at frequencies of approximately 16-17 Hz and higher. B) Effect of smoothing on a delta function of strain in time (time resolution is 3.1 ms/pixel, as in raw SHG linescan data). C) Effect of smoothing on various smoothed step functions of strain. Smoothing significantly attenuates strain events lasting 60-70 ms and less. D) Simulated strain events with rapid onset/recovery and asymmetry in contraction or relaxation (black dots) were smoothed using the local linear regression algorithm (n=20 pixels, or ~ 60 ms) discussed in the text. Contractile events with fast rise/relaxation times and sharp transitions may be blurred and delayed by the smoothing process. In all plots above, black dots represent data before smoothing algorithm, and red lines represent smoothed data.