Spark number	d(F/Fo)/dt max	FWHM [μm]
1	15.54	2.34
2	12.67	2.08
3	27.91	1.30
4	15.61	1.82
5	19.20	2.08
6	32.29	2.34
7	16.71	2.08
8	27.70	0.52
9	16.91	3.12
10	17.75	2.60
11	13.52	2.08
12	18.46	2.08
13	26.39	1.75
14	16.12	1.04
15	17.42	1.56
16	15.63	2.86

TABLE Supp 1. Analysis of spontaneous sparks measured in rabbit ventricular myocytes. The maximum upstroke velocity and FWHM was 19.36±5.86 %/ms and 1.98±0.66 μm, respectively.

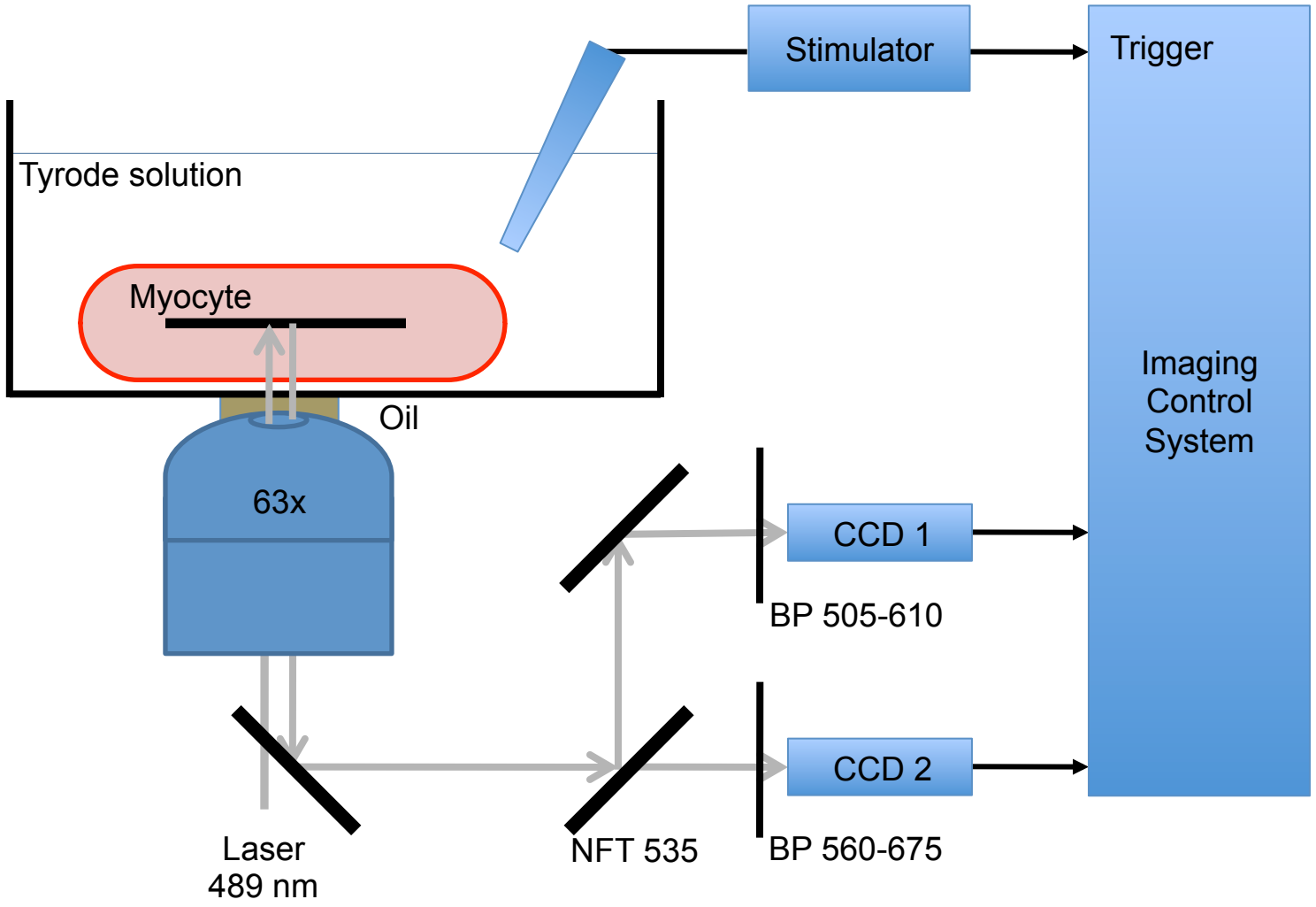

Fig. Supp 1: Sketch of imaging arrangement. Myocytes are superfused with normal bathing solution during imaging using rapid two-dimensional scanning confocal microscopy. The stimulator provides a trigger for the imaging. Fluorescent dyes are excited at 489 nm. Emitted light is filtered through a NFT and BPs before measurement with CCDs. NFT: dichroic mirror; BP: band pass; CCD: Charge coupled line detector.

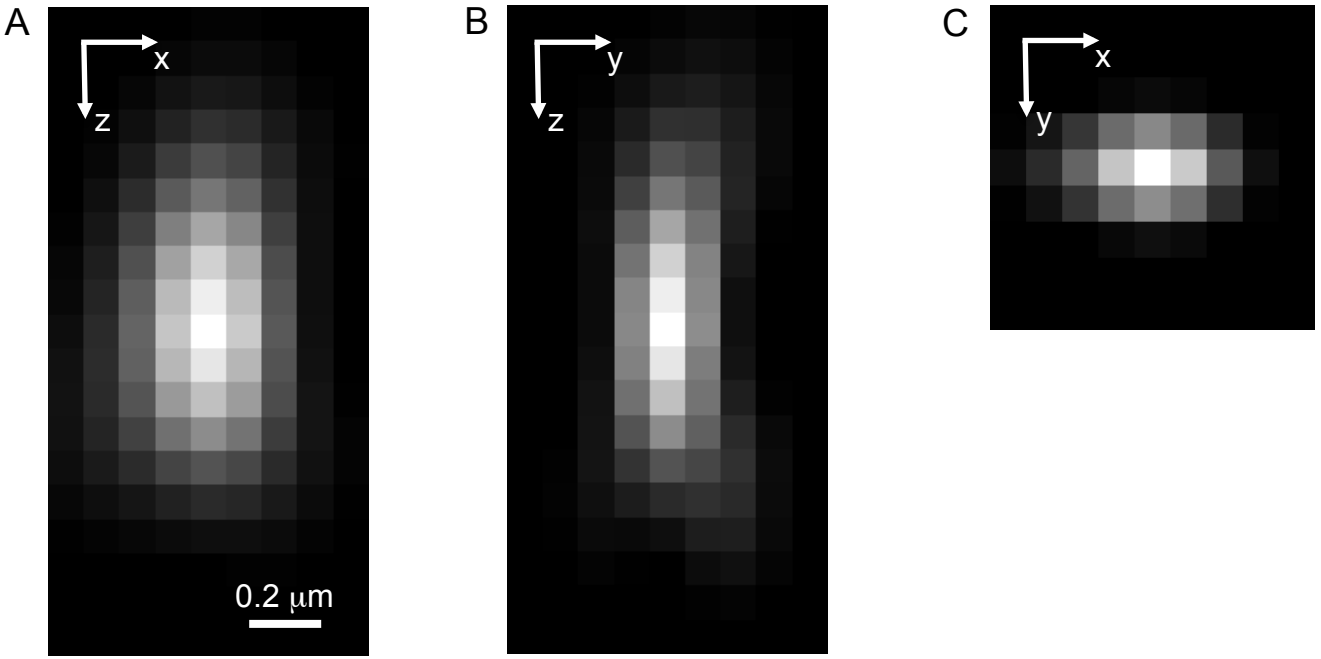

Fig. Supp 2: Point spread function (PSF) for Zeiss LSM 5 Live confocal microscope with 63x oil immersion lens (numerical aperture 1.4). The PSF was created by averaging of a set of PSFs measured within the first 10 μm above the glass slide. The imaging protocol is described in (22). The PSF was sampled at a resolution of 0.1 x 0.1 x 0.1 μm. The full width at half maximum of the PSF was 355, 230 and 720 nm in x-, y- and z-direction, respectively.

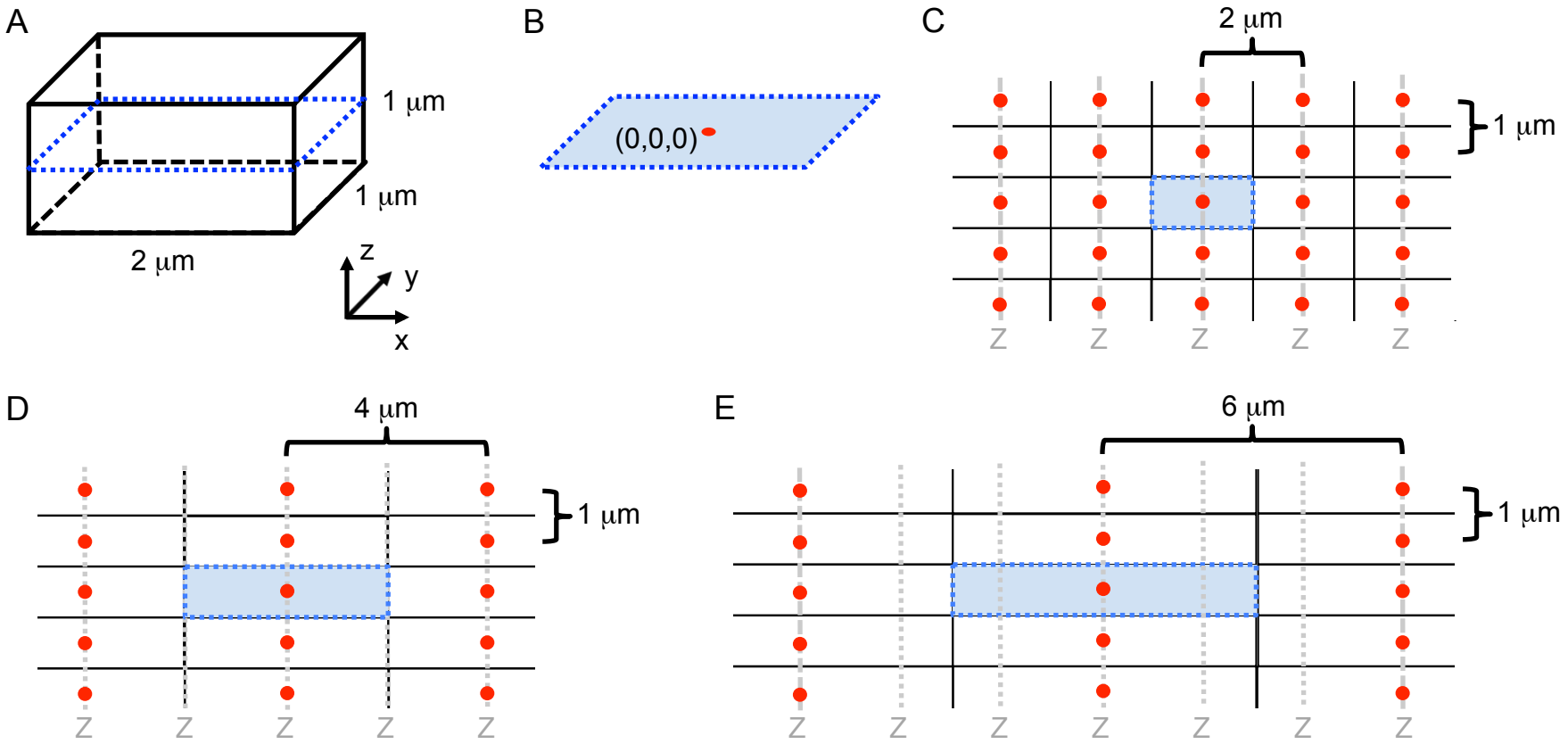

Fig. Supp 3: Modeling of Ca^{2+} signals. (A) The spatial domain is hexahedral and has a size of 2 μm x 1 μm x 1 μm. Neumann boundary conditions were assigned at the surfaces of the hexahedron. (B) Xy cross-section with a central release site (red). The release site is at the origin of the coordinate system. (C) The model assumes an infinite periodic distribution of release sites (red). The sites are in Z lines (grey line) located in the central yz cross-section. While the sketch presents the arrangement in 2D, the developed model assumes a 3D distribution of release sites. The distance between release size in y- and z-direction is 1 μm. Sketch for models with a size of (D) 4 μm x 1 μm x 1 μm and (E) 6 μm x 1 μm x 1 μm.

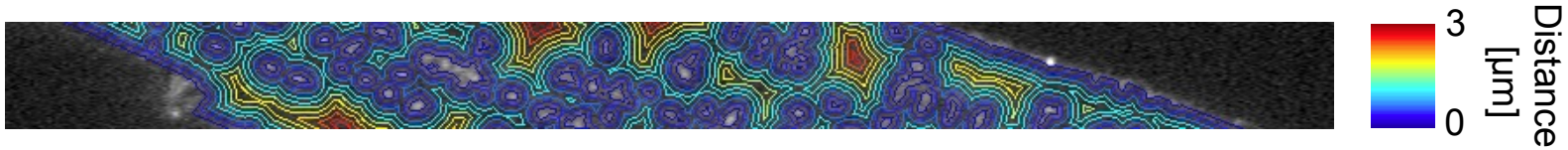

Fig. Supp 4: Distance map calculated from image scans of a representative myocyte (same cell as in Fig. 1). The distance map is shown with isolines overlaid on the di-8-ANEPPS image.

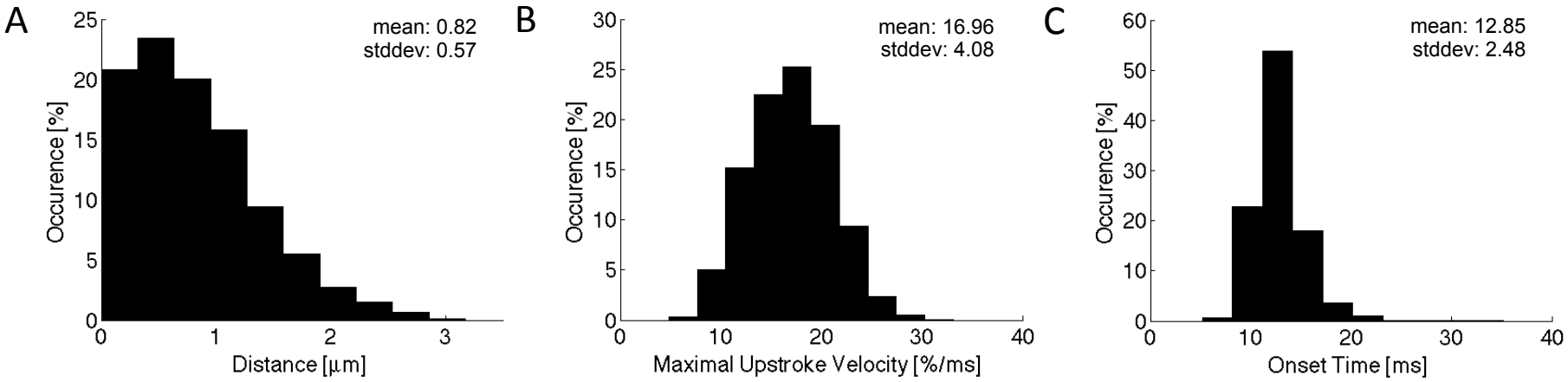

Fig. Supp 5: Statistical analyses of image maps in Fig. 3. (A) The histogram of distances shows that those can range up to 3 μm, but most pixels are in close proximity of the sarcolemma. (B) The histogram provides information on the spread of maximal upstroke velocities. (C) The histogram of onset times indicates that initiation of local Ca^{2+} release is within 5 to 25 ms in this cell.

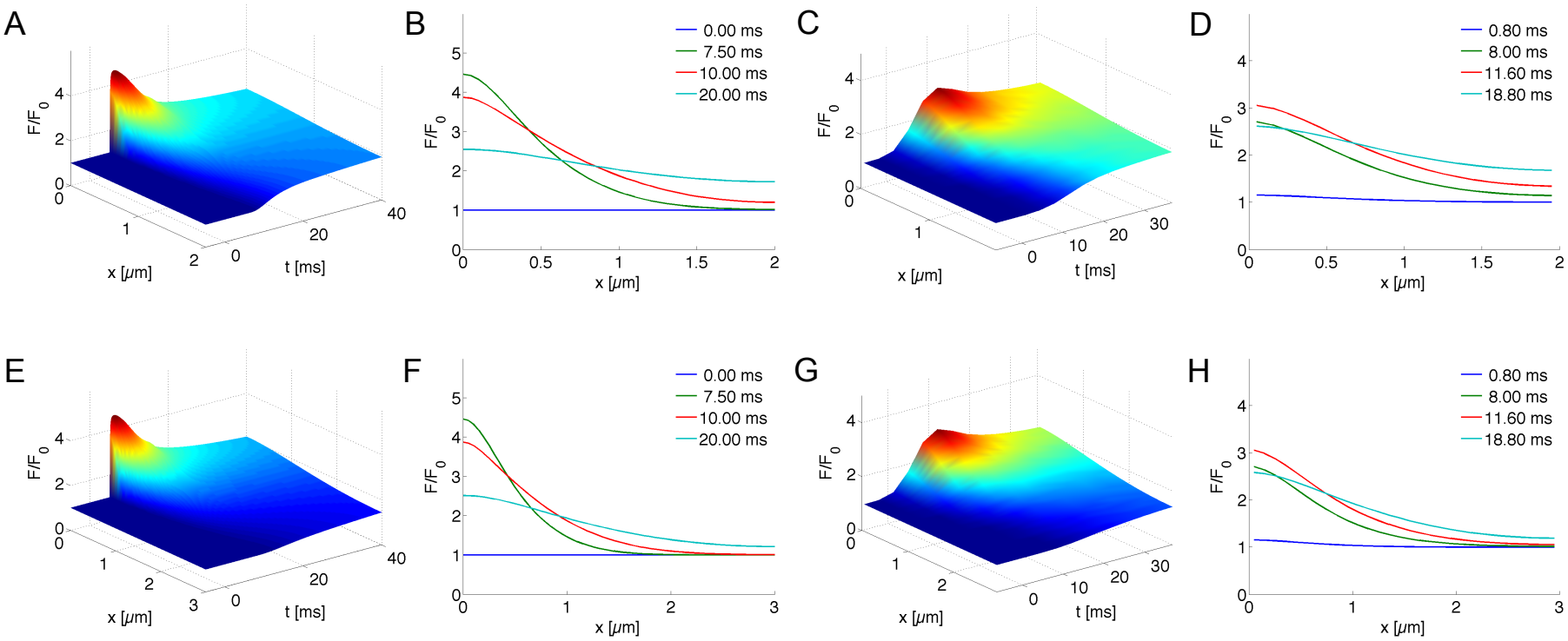

Fig. Supp 6: Modeling and analysis of Ca^{2+} signals. (A) Self-ratioed signal [FLUO-Ca]/ $[FLUO\text{-}Ca]_0$ (F/F_0) calculated from simulation in a domain with size of 4 mm x 1 mm x 1 mm. (B) The signal along the x-semiaxis is shown at different time steps. (C,D) The signal was sampled at 277 Hz, convolved with a PSF and filtered as for processing of measured fluo-4 images. (E-H) Same as (A-D) with a domain size of 6 μm x 1 μm x 1 μm.

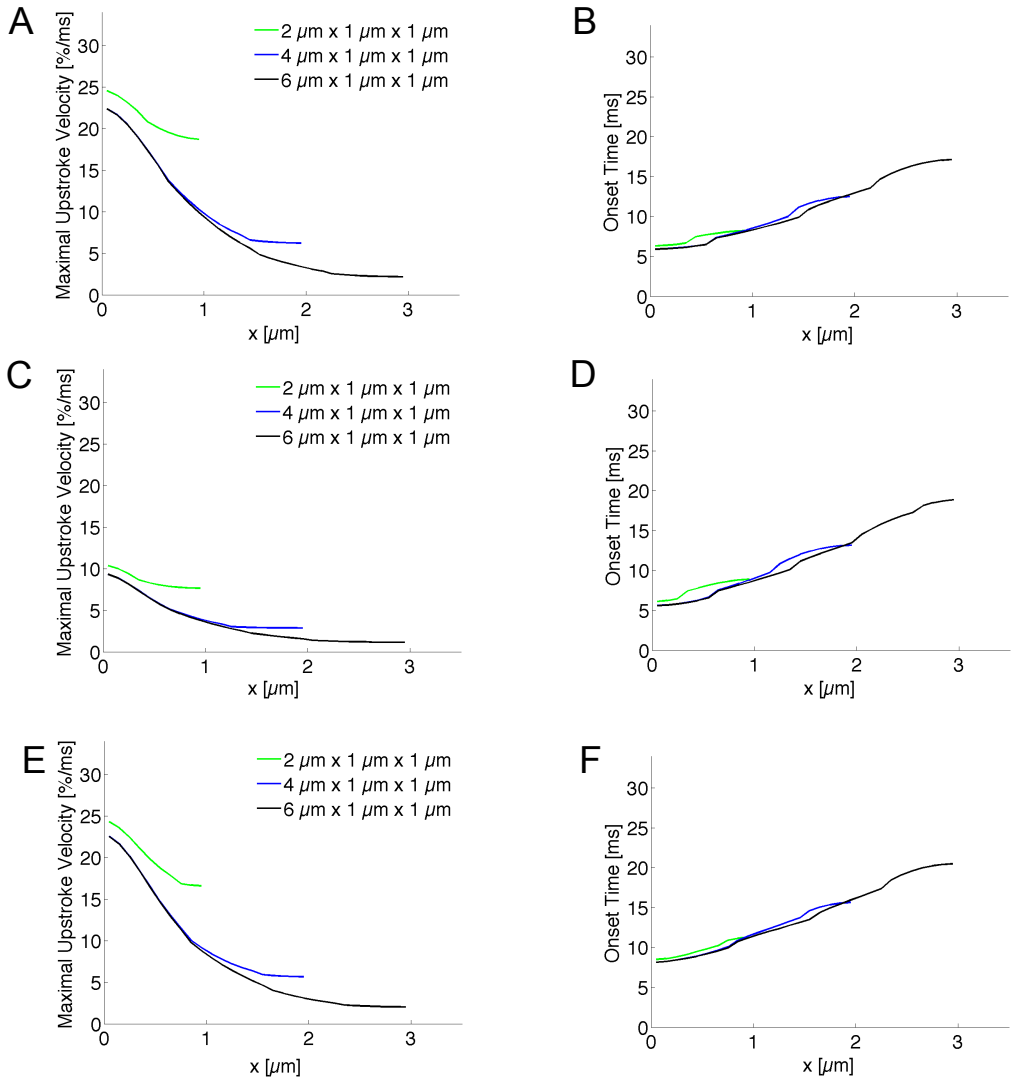

Fig. Supp 7: Analyses of simulated Ca^{2+} signals with varied model parameters. (A) Maximal upstroke velocity and (B) onset time for D_{MYO}=0.6 μm^2/ms. (C) Maximal upstroke velocity and (D) onset time for $[FLUO]_{TOTAL}$=250 μM. (E) Maximal upstroke velocity and (F) onset time for I_{RYR}=2 pA and a release duration of 10 ms.

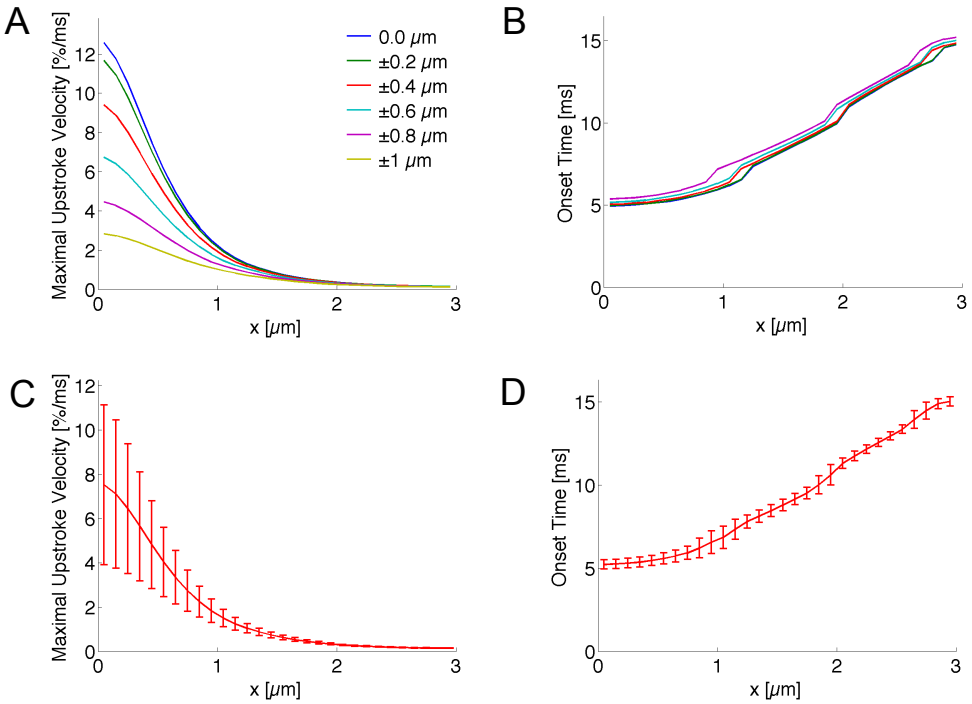

Fig. Supp 8: Analyses of simulated Ca^{2+} signals at different distances to the release site. The distance in z-direction varied between -1 and 1 µm. At each distance, signals along the x-semiaxis were analyzed. (A) Maximal upstroke velocity and (B) onset time versus distance in z-direction. Mean and sttdev of (C) maximal upstroke velocity and (D) onset time.

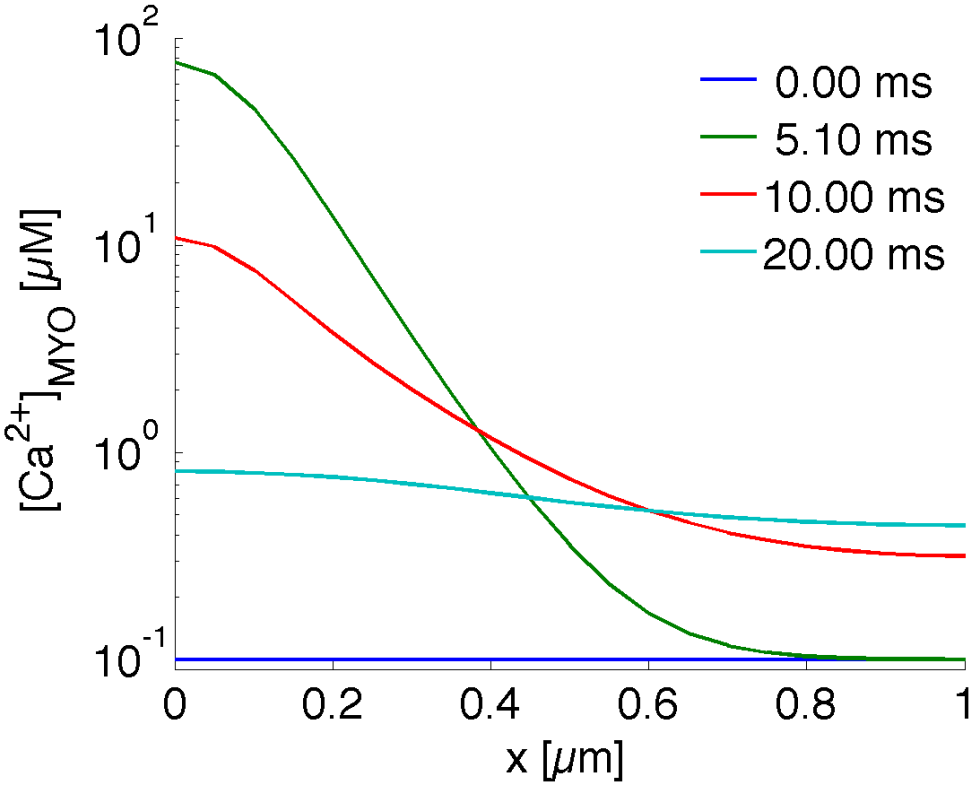

Fig. Supp 9: $[Ca^{2+}]_{MYO}$ profiles at different time points calculated in model with a size of 2 µm x 1 µm x 1 µm. The model was developed to describe the early phase of Ca^{2+} transients. The model neglects removal of Ca^{2+} through the sarcolemma and sarcoplasmic Ca^{2+} uptake.