Supplementary material

Supplementary_Image_Processing_and_Simulation.pdf provides detailed, step-by-step instructions for reproduction of the used image processing procedures. The source (Mathematica notebook) of the shown simulation is also provided.

Raw and step-by-step processed image series are shown for Fig.1A-B, 5C and 6A in QuickTime format (movie1-3).

Supplementary movie 1

ATP-evoked (100 μM at t≈30s) Ca^{2+} rise in the cytosol and mitochondria (depicts Fig. 1A-B). Wide-field fluorescence image series of X-rhod-1 loaded intact rat brain capillary endothelial cell in culture. RAW: raw image sequence of X-rhod-1 loaded endothelial cell stimulated with ATP is shown on the top. Images sequences below were consecutively processed as indicated (for cytosolic and mitochondrial [Ca^{2+}] separately, shown on the left and the right, respectively). LF: lowpass spatial filtering; HF: highpass spatial filtering; ABS: absolute value calculation; SG: spatial Savitzky-Golay kernel smoothing; DE: dilation-erosion smoothing; δf: DF/F_0 normalization; dt: temporal differentiation. OVERLAY: highpass filtered fluorescence differentiated in time shown as δf/t, in pseudocolor (same as dt on the right), overlaid by the cytosolic Ca^{2+} wavefront shown in grayscale (same as dt on the left). The pseudocolor coded image was masked (gated or intensity modulated displayed - IMD) by the HF image. ZOOM: 300% zoom on the OVERLAY image. Playback: slowed down (exposure: 60ms, playback: 10 fps).

Supplementary movie 2

TMRM irradiation induced mitochondrial membrane potential depolarizations at quenching condition (depicts Fig. 5C). Wide-field fluorescence image series of TMRM loaded intact rat brain capillary endothelial cell in culture. Left: raw image series. Right: after differentiation in time (df/dt) in pseudocolor. Red color shows sudden rises (or flickering) of fluorescence. Playback: real time (12.5 fps).

Supplementary movie 3

TMRM irradiation induced mitochondrial membrane potential depolarizations at non-quenching condition (depicts Fig. 6A). Wide-field fluorescence image series of TMRM loaded intact rat brain capillary endothelial cell in culture. RAW: raw image sequence of an endothelial cell exposed to strong illumination is shown on the top. Images below were consecutively processed as indicated. HF: highpass spatial filtering (with absolute value calculation); DE+SG: dilation-erosion smoothing and spatial Savitzky-Golay kernel smoothing; dt: temporal differentiation. The dt image is shown in pseudocolor, gated with the HF (without absolute value calculation) image. CORR: The areas flickering temporally correlated are indicated in different colors. Playback: faster than original (exposure: 80ms, playback: 20 fps).