release flux was observed 3.9 ms after initiation of Ca release when all release sites became active. Ca entry flux was ten times smaller than Ca release flux. When SR Ca release was eliminated after treatment with thapsigargin and caffeine, identical Ca entry flux was observed as in control conditions. In summary, ultra-fast confocal imaging allows investigation of Ca signals with time resolution similar to patch clamp technique, however in a much less invasive fashion.

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Automatic Calcium Spark Detection and Analysis in Time Series of Two-Dimensional Confocal Images

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Calcium sparks are highly localized, brief calcium transients reflecting elementary calcium release from the sarcoplasmic reticulum that can occur either spontaneously or can be triggered during excitation contraction coupling in cardiac myocytes. They hold physiological as well as pathophysiolgical importance. Classically, calcium sparks were almost exclusively analyzed in line scan images because of technical limitations in the acquisition process consequently neglected their 2D spatial properties and distribution inside the cell.

During recent years the increased performance of confocal microscopes enabled high spatial and temporal resolution imaging. This allowed recording of calcium sparks from individual myocytes in time series of two-dimensional confocal images at acquisition rates exceeding 200Hz.

Here, we introduce an automatic three-dimensional approach for such analysis. Following cell border recognition we utilised locally ''derivative-like'' functions for spark-detection allowing the algorithm to analyse the temporal and spatial properties of calcium sparks. Such an approach revealed a highly robust spark-detection process, even when partial or global calcium waves occurred intermittently. 2D-gaussian fits over time were used to quantify such properties including amplitudes, decay time, frequency, spatial position and spread for further analysis. In addition, the algorithm automatically performs cluster analysis of the sparks found in order to identify common spark sites.

In an initial proof-of-concept study, we analysed spontaneous calcium sparks from various origins within the mouse heart (ventricle, left/right aria). The major result of this study was that the sparks display very distinct and characteristic properties that can be linked to physiology of the particular host cell.

Such a novel approach enables us to automatically analyze large populations of local calcium signals and thus fosters the use of such algorithms for novel high-content screening applications.

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Image-Based Monte Carlo Modeling of Ca^{2+} sparks in Ventricular Myocytes

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The geometry of a dyadic calcium release unit (CRU) plays a critical role in regulating intracellular Ca^{2+} spark generation and furthermore the excitation-contraction (E-C) coupling in ventricular myocytes. A number of computational models have showed how the geometries of T-tubules and junctional sarcoplasmic reticulum (jSR) and distributions of L-type Ca^{2+} channels (LCCs) and ryanodine receptors (RyRs) could affect local Ca^{2+} signaling, but almost all of them were based on simple domain geometries such as rectangular or cylindrical shapes. For this reason, incorporating image-based realistic geometric models into mathematical simulation is timely and expected to provide more accurate simulation of many biological processes, in particular, the Ca^{2+} spark formation in ventricular myocytes.

The present study has two goals. First, we adopt a chain of image and geometric processing approaches to construct realistic 3D models (represented by high-quality surface and volumetric meshes) of both T-tubules and junctional SR from electron microscopy images of adult mouse cardiomyocytes. The distributions of LCCs and RyRs are obtained in two ways: random assignment and image-based localization (for RyRs only). The second goal of the present study is the use of Monte Carlo methods to model the randomness of release and diffusion of individual Ca^{2+} ions within a narrow dyadic cleft. To this end, the M-Cell software package, combined with the realistic geometries obtained in the first goal, is used to simulate how Ca^{2+} sparks are generated in a single dyad (or CRU) and how they change temporally and spatially with different geometries of membrane structures and distributions of channeling proteins.

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Markov Chain Models Of Coupled Calcium Channels: Kronecker Representations And Iterative Solution Methods

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Mathematical models of calcium release sites derived from Markov chain models of intracellular calcium channels exhibit collective gating reminiscent of the experimentally observed phenomenon of stochastic calcium excitability (i.e., calcium puffs and sparks). Calcium release site models are stochastic automata networks that involve many functional transitions, that is, the transition probabilities of each channel depend on the local calcium concentration and thus the state of the other channels. We present a Kronecker structured representation for calcium release site models and perform benchmark stationary distribution calculations using both exact and approximate iterative numerical solution techniques that leverage this structure. When it is possible to obtain an exact solution, response measures such as the number of channels in a particular state converge more quickly using the iterative numerical methods than occupation measures calculated via Monte Carlo simulation. In particular, multi-level methods provide excellent convergence with modest additional memory requirements for the Kronecker representation of calcium release site models. When an exact solution is not feasible, iterative approximate methods based on the power method may be used, with performance similar to Monte Carlo estimates. This suggests approximate methods with multi level iterative engines as a promising avenue of future research for large-scale calcium release site models.

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Ca^{2+} Sparks Generate Depolarizing STICs Causing Contraction And Asthmatic Hyperresponsiveness In Airway Smooth Muscle Cells

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Ca^{2+} sparks are well known to be essential for controlling the relaxation of cerebral artery SMCs; however, the functional importance of this local Ca^{2+} signaling in other types of SMCs remains to be determined. Thus, the aim of this study was to investigate the role of Ca^{2+} sparks in airway SMCs. Our data reveal that spontaneous Ca^{2+} sparks could activate spontaneous transient inward currents (STICs) at the resting membrane potential and spontaneous transient outward currents (STOCs) at more positive membrane potentials in mouse airway SMCs. Application of ryanodine to block ryanodine receptors (RyRs) abolished spontaneous Ca^{2+} sparks without altering the whole-cell cytosolic Ca^{2+} levels in single airway myocytes and decreased the resting muscle tension in isolated airway rings, whereas activation of RyRs with a low concentration of caffeine had opposite effects. Iberiotoxin, a selective blocker of big-conductance Ca^{2+}-activated K^+ channels, eliminated STOCs, but did not affect either spontaneous Ca^{2+} spark activity or resting muscle tension. In contrast, NPPB, an inhibitor of Cl^- channels, reduced resting muscle tension. The effect of NPPB was prevented in the presence of the selective voltage-dependent Ca^{2+} channel blocker nifedipine. We have also found that the activity of Ca^{2+} sparks in single asthmatic mouse airway SMCs and in-vivo airway resistance in asthmatic mice were significantly increased. Interestingly, ryanodine caused a stronger relaxation in asthmatic airway smooth muscle. Taken together, these findings suggest that spontaneous Ca^{2+} sparks can activate Ca^{2+}-activated Cl^- channels and then generate STICs, causing membrane depolarization, opening of voltage-dependent Ca^{2+} channels, extracellular Ca^{2+} influx and contraction in airway SMCs. Moreover, Ca^{2+} sparks and attendant STICs are both increased in asthmatic airway SMCs, which may contribute to asthmatic airway hyperresponsiveness.

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Decreased Ca^{2+} Spark Frequency and RyR2 Expression in Cerebral Arteries Following Subarachnoid Hemorrhage

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Ca^{2+} sparks represent local and transient increases in intracellular Ca^{2+} caused by the coordinated opening of ryanodine receptors (RyRs) located in the sarcoplasmic reticulum of muscle. Contrary to the contraction caused by global Ca^{2+} increases, Ca^{2+} sparks promote smooth muscle relaxation via activation of plasmalemmal large-conductance Ca^{2+}-activated potassium (BK) channels leading to membrane potential hyperpolarization and decreased activity of voltage-dependent Ca^{2+} channels. Here, we examined whether impairment of this vasodilator pathway contributes to enhanced cerebral artery constriction associated with subarachnoid hemorrhage (SAH). Using a rabbit SAH model, Ca^{2+}