Automated, derivative-like identification, 3D fitting and phylogenetic tree analysis of Ca\textsuperscript{2+} sparks in ultrafast 2D confocal images series of patient-specific induced pluripotent stem cells (iPSCs) derived cardiac myocytes

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Ca\textsuperscript{2+} sparks are highly localized, brief Ca\textsuperscript{2+} transients reflecting elementary Ca\textsuperscript{2+} release from the sarcoplasmic reticulum that can occur either spontaneously or triggered during excitation contraction coupling in cardiac myocytes. They hold physiological as well as pathophysiological importance. Classically, Ca\textsuperscript{2+} 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 Ca\textsuperscript{2+} 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 (3D) 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 by a phylogenetic tree approach. For a patient-specific stem cell model we recorded in excess of 100,000 Ca\textsuperscript{2+} sparks from human induced pluripotent stem cells (iPSCs) derived from patients carrying catecholaminergic polymorphic ventricular tachycardia (CPVT) a life threatening arrhythmic cardiac disease[1]. In this study we were able to demonstrate for the first time that dantrolene, a drug effective on malignant hyperthermia restored normal Ca\textsuperscript{2+} spark properties and rescued the arrhythmogenic phenotype in diseased myocytes. Our work provides a new in vitro model to study the pathogenesis of human cardiac arrhythmias and develop novel therapies for CPVT only possible by automated analysis of Ca\textsuperscript{2+} sparks.

Such a novel approach also fosters the use of such algorithms for novel high-content screening applications such as drug development and drug screening.


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