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Imaging Calcium Sparks in Cardiac Myocytes

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Abstract

Calcium ions play fundamental roles in many cellular processes in virtually all type of cells. The use of Ca^{2+} sensitive fluorescent indicators has proven to be an indispensable tool for studying the spatio-temporal dynamics of intracellular calcium ($[\text{Ca}^{2+}]_i$). With the aid of laser scanning confocal microscopy and new generation of Ca^{2+} indicators, highly localized, short-lived Ca^{2+} signals, namely Ca^{2+} sparks, were revealed as elementary Ca^{2+} release events during excitation–contraction coupling in cardiomyocytes. Since the discovery of Ca^{2+} sparks in 1993, the demonstration of dynamic Ca^{2+} micro-domains in living cardiomyocytes has revolutionized our understanding of Ca^{2+} -mediated signal transduction in normal and diseased hearts. In this chapter, we have described a commonly used method for recording local and global Ca^{2+} signals in cardiomyocytes using the fluorescent indicator fluo-4 acetoxymethyl (AM) and laser scanning confocal microscopy.

Keywords

Calcium sparks; confocal microscopy; ventricular myocytes; fluorescence; calcium indicators

1. Introduction

Cytosolic free Ca^{2+} ($[\text{Ca}^{2+}]_i$) is a versatile second messenger that can simultaneously regulate multiple processes within an individual cell. In the cardiac cell, cytosolic $[\text{Ca}^{2+}]_i$ ($[\text{Ca}^{2+}]_i$) is actively maintained at a very low level of around 100 nM by Ca^{2+} homeostatic mechanisms, including the SR Ca^{2+} -ATPase (SERCA) and the plasmalemmal $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) and Ca^{2+} -ATPase and by a number of Ca^{2+} buffering molecules (1). During each heart beat, a time-dependent transient increase in intracellular Ca^{2+} concentration (“ $[\text{Ca}^{2+}]_i$ transient”) occurs and is responsible for activating contraction in a process called excitation–contraction coupling. The $[\text{Ca}^{2+}]_i$ transient is triggered by the cardiac action potential (AP) and spreads through the heart as the AP is propagated (2).

$[\text{Ca}^{2+}]_i$ transients during cardiac excitation–contraction (EC) coupling were first described as aequorin luminescence in frog cardiac muscle by David G. Allen (3) and in canine Purkinje fibers by Gil Wier (4) using microinjection of photoprotein into muscle cells. The development of new fluorescent Ca^{2+} indicators, such as fura-2 and indo-1 by Tsien and co-workers (5), had improved $[\text{Ca}^{2+}]_i$ measurements in single cardiac muscle cells. There are two advantages of these dyes. First, the acetoxymethyl (AM) ester derivatives of fura-2 and indo-1 can permeate the cell membrane, which makes the application of these Ca^{2+} indicators much easier comparing to aequorin microinjection. Second, their unique excitation/emission features allow one to be able to make accurate, ratio measurement of intracellular Ca^{2+} concentrations. In conjunction with digital imaging technique, Wier et al. were the first to document spontaneous $[\text{Ca}^{2+}]_i$ waves and different patterns of subcellular Ca^{2+} concentration in quiescent, spontaneously active or hyper-contracting cardiomyocytes

(6). However, the spatial-temporal resolution was not high enough for them to be able to detect the highly localized Ca^{2+} release events in cardiac myocytes as we can observe routinely nowadays.

Advances in Ca^{2+} fluorescence technology (still driven by Tsien and his colleagues) combined with the advent of the laser scanning confocal microscope made it possible for the discovery of Ca^{2+} sparks in cardiac myocytes. Ca^{2+} sparks were firstly reported in quiescent ventricular myocytes by Cheng et al. (7). Since then Ca^{2+} sparks or local Ca^{2+} release events with spark characteristics have been recorded in skeletal muscle (8–10), smooth muscle (11), neurons (12) and more recently in fibroblasts (13). In heart muscle, Ca^{2+} sparks are now well accepted as the elementary events of SR Ca^{2+} release underlying EC coupling, originated from the opening of a cluster of sarcoplasmic reticulum (SR) Ca^{2+} release channels or ryanodine receptors (RyRs). Ca^{2+} spark observed in unstimulated resting single cardiac myocytes represents a local transient increase in intracellular $[\text{Ca}^{2+}]_i$. It has a rapid rise (~10 ms, time to peak) and a moderately quick decay kinetics (~20 ms, half-time of decay) and is confined to an area of ~2.0 μm in diameter or ~8 fl by volume (14). At diastolic $[\text{Ca}^{2+}]_i$ of about 100 nM, Ca^{2+} sparks are spontaneously firing at a very low rate (~100 per second per cell). The occurrence of spontaneous Ca^{2+} sparks does not require Ca^{2+} entry into the cardiomyocyte through L-type Ca^{2+} channels (LCCs) or by other Ca^{2+} pathways across the sarcolemma. However, during cardiac EC coupling, Ca^{2+} influx through LCCs evokes synchronous activation of tens of thousands of Ca^{2+} sparks by a mechanism called Ca^{2+} -induced Ca^{2+} release (CICR) (15, 16). This process is locally controlled within a 12 nm junctional subspace between t-tubular and SR membrane (17). It has been elegantly shown that Ca^{2+} sparks can be triggered by adjacent single LCC openings (16, 18, 19). The summation of numerous Ca^{2+} sparks activated simultaneously all over the myocyte compose a uniform Ca^{2+} transient (see **Fig. 12.1**).

Fluo-3 and fluo-4 have been the indicators of choice in Ca^{2+} spark experiments, because of their unique properties that confer a high signal-to-noise ratio, fast “on” and “off” kinetics and high sensitivity when the indicator responds to $[\text{Ca}^{2+}]$ gradients. Since its introduction in 1989, fluo-3 confocal Ca^{2+} imaging has made significant contribution to our understanding of spatial dynamics of many elementary process of Ca^{2+} signaling in different cell types. Fluo-4, an analog of fluo-3, with higher quantum yield when excited at 488 nm, provides brighter emission signals in response to Ca^{2+} binding when compared to fluo-3. When estimating $[\text{Ca}^{2+}]_i$ from the observed fluorescence signal (F), a common practice is to express the data as the ratio: $R = F/F_0$, where F_0 refers to the baseline fluorescence at resting $[\text{Ca}^{2+}]_i$.

The major disadvantage of fluo-family of dyes (fluo-3/fluo-4), however, is that, upon binding of Ca^{2+} ions, there is little or no shift in its excitation or emission spectrum, which makes it impossible to perform ratiometric measurements of $[\text{Ca}^{2+}]$ (20). Most chemical fluorescent indicators are cell impermeant, therefore many of the fluorescent Ca^{2+} indicators are derivatized with AM ester groups. The AM form of the indicator can diffuse across cell membranes, and once inside the cell, esterases cleave the AM group off the probe leading to a cell-impermeant indicator. Because the AM derivative has low aqueous solubility, some dispersing agents such as Pluronic F-127 are often used to help solubilize large dye molecules in physiological media and facilitate cell loading (20).

2. Materials

1. Fluo-4 AM (10 × 50 μg , F14201, Invitrogen). Store at -20°C .
2. 20% Pluronic F127 in DMSO solution (P-3000MP, Invitrogen). Store at room temperature.

3. Fluo-4 AM loading stock solution: dissolve 50 μg fluo-4 AM with 50 μL 20% Pluronic F-127 DMSO solution. Store stock solution at -20°C .
4. Tyrode's solution with the following composition (mM): 140 NaCl, 5 KCl, 5 HEPES, 1 NaH_2PO_4 , 1 MgCl_2 , 1.8 CaCl_2 and 10 glucose (pH 7.4) adjusted with NaOH. All salts and buffers used for the preparation of normal Tyrode's solution can be purchased from Sigma-Aldrich®. Store at 4°C .
5. Modified Dulbecco's Modified Eagle Medium (DMEM): The basic medium routinely used to keep the isolated adult myocytes is supplemented DMEM (powder purchased from Sigma, catalog #D1152). To make up 50 mL of media for incubating the cells, add 5 mL of inactivated fetal bovine serum, 5 μL insulin (3.66 mg/mL) and 550 μL NaCl (4 M) to 40 mL DMEM solution (made from 0.87 g DMEM powder in 40 mL Milli-Q grade water). Adjust the pH with NaOH to 7.2 and complete the volume. Then keep at room temperature for use on the same day.
6. Electrical stimulator for field stimulation of myocytes, with the capacity to deliver at least 20 V square pulses.
7. Perfusion chamber with attached platinum wires, mounted on the stage of a confocal microscope (*see* Note 1).
8. Confocal microscope equipped with an Argon laser of 488 nm line for fluo-4 excitation and appropriate filters for acquiring emission signals at certain wavelength range (for example, long pass filter that passes emission signals of wavelength >505 nm or band-pass filter that passes emission signals of wavelength between 505 and 550 nm).

3. Methods

3.1. Isolating Ventricular Myocytes from Adult Rat

Adult rat ventricular cells will be prepared by standard methods as previously described in the literature (21). Briefly, male rats weighing between 200 and 300 g will be sacrificed by lethal intraperitoneal injection of pentobarbital sodium (100 mg/kg). The hearts will be rapidly removed and perfused via the Langen-dorff apparatus with Ca^{2+} -free modified Tyrode solution until the blood is washed out. Hearts will then be perfused with Tyrode solution containing 50 μM CaCl_2 along with 1.4 mg/mL collagenase (type 2) and 0.04 mg/mL protease (type XIV) until they are soft (approximately 10 min). The hearts will then be removed from the perfusion apparatus, minced into ~ 1 -mm chunks and stirred for 4 min in Tyrode solution containing 50 μM CaCl_2 , 0.7 mg/mL collagenase and 0.02 mg/mL protease. Cells will be filtered through a 200 μm mesh to remove tissue chunks and extracellular Ca^{2+} concentration is raised to 0.5 mM over 10 min through three centrifuge cycles (0.1 mM Ca^{2+} , 0.2 mM Ca^{2+} , 0.5 mM Ca^{2+}). Finally myocytes will be harvested and stored in modified DMEM until they are used (within 5 h) (22, 23).

3.2. Fluo-4 AM Loading

Add 10 μL fluo-4 AM stock solution to 1 mL of cell suspension (final fluo-4 AM concentration = 10 μM). Cells should remain in the dark at room temperature for 20 min (*see* Notes 2 and 3). Centrifuge the cells (2 min at 200–300 rpm centrifugation), remove the

¹The chamber bottom glass has to be no more than 170 μm thick (#1.5 glass coverslip). High numerical aperture lens (e.g. Zeiss Plan-Apochromat 63 $\times$ oil immersion) have a short working distance of 190 μm . We usually use 120 μm thick #1 glass coverslip).

²Fluo-4 AM dye loading can vary, for example, rabbit or dog myocytes may require longer loading time. Myocytes with an optimal fluo-4 AM loading shall report a baseline fluorescence of 30–40. Too high or too low baseline fluo-rescence levels indicate overloading or underloading of the fluorescent indicator into the cell.

supernatant and gently re-suspend the pellet in indicator-free Tyrode solution. Wait for 20 min to allow for complete de-esterification of AM esters. Then, cells will be ready for Ca^{2+} spark imaging with confocal microscope for up to 2 h (*see* Note 4). The anion-transport inhibitor probenecid (2 mM) may be added to the cell solution to reduce leakage of the de-esterified indicator.

3.3. Ca^{2+} Imaging in Ventricular Myocytes

Rod-shaped myocytes with clear striations and without actively spontaneous contraction (less than one per minute) are considered healthy Ca^{2+} tolerant cells and will be chosen for Ca^{2+} imaging (*see* Note 5). Ca^{2+} transients will be elicited by field stimulation through a pair of platinum electrodes, with a 2 ms supra-threshold square voltage pulse delivered by a commercially available electrical stimulator (such as Myopacer 100, IonOptix Inc.). Cells are normally stimulated at 1 Hz for 15 s to let reach a steady-state condition before recording. An LSM 510 scanning system (Zeiss GmbH, Jena, Germany) equipped with a $\times 63$ oil immersion objective (numerical aperture (NA) = 1.4) will be used for confocal imaging of Ca^{2+} fluorescence (*see* Note 6). Fluo-4 will be excited by 488 nm line of an Argon laser and emission signals over 505 nm will be collected. The brightness of the fluorescent signals represents the relative level of intracellular $[\text{Ca}^{2+}]_i$. For recording Ca^{2+} transients/sparks, a line scan mode is normally utilized. The confocal pinhole is set to render spatial resolutions of 0.4 μm in the horizontal plane and 0.9 μm in the axial direction (*see* Note 7). Ideally, the detector gain is set at around 700 (no digital gain). Line-scan images are acquired at sampling rate of 1.54 or 1.92 ms per line, along the longitudinal axis of the cell. Each line comprises 512 pixels spaced at 0.14 μm intervals. After a sequential scanning, a two-dimensional (2D) image of 512×1000 lines or 512×2000 lines will be generated and stored for offline analysis (*see* below). It is not recommended to scan a cell in the same line region for prolonged time (*see* Note 8).

3.4. Recording Ca^{2+} Transients in Ventricular Myocytes

Fluo-4 AM-loaded cells will be allowed to settle on a coated glass coverslip by gravity (*see* Note 9). Wait for 5–10 min and then turn the perfusion solution on; the cells will be bathed in Tyrode's solution. Cells can be paced with parallel platinum wires connected to an electrical stimulator (Myopacer 100, IonOptix Inc.). Stimulation settings should be as follows: duration: 2 ms; continuous biphasic pulse stimulation; voltage: adjusted to 120% of the threshold voltage that induces Ca^{2+} transients. When cells are stimulated at 1 Hz, the spatially averaged $[\text{Ca}^{2+}]_i$ transient obtained by integrating the line-scan image should be similar to that presented in Fig. 12.1 (*see* Note 10).

³Loading fluo-4 AM may be done at room temperature, rather than at 37°C. Acetoxymethyl ester loading at high temperature may often cause severe subcellular compartmentalization of the indicator and may interfere with the measurement of cytosolic Ca^{2+} concentration.

⁴Cardiomyocytes can last for hours after Fluo-4 AM loading and still provide Ca^{2+} transient data; however, because of the leakage of esterified indicator (although slow), myocytes loaded with fluo-4 AM will exhibit dim fluo-rescence with time. Routinely, we examined cells for 2 h following loading without marked deterioration of Ca^{2+} signals.

⁵Cardiomyocytes suitable for loading and measurement should appear rod shaped, with clear striations and without cytoplasmic protrusions or blebs under phase-contrast light microscopy.

⁶Water immersion objective lens can be used. These objective lens are designed for use with aqueous specimens and immersion medium and can be "corrected" for the unavoidable refractive index mismatch produced by the use of a glass coverslip ($n = 1.51$). Practically, oil immersion lens are often used for the much higher expense of water immersion lens.

⁷The optical resolution of the confocal microscope is 0.4 μm in the horizontal plane and 0.9 μm in the axial direction, as determined by measuring the point spread function of 0.1 μm fluorescent bead (Molecular Probes – Invitrogen Inc.).

⁸Prolonged scanning in the same region may cause photo-bleaching of fluorescent molecules and experimental artifacts induced by photo-damage.

⁹Glass coverslip can be coated with laminin. Laminin promotes cell attachment, preventing stimulated contraction out of the focal plane during imaging. Laminin is diluted to a final concentration of between 1 and 5 $\mu\text{g}/\text{mL}$ in a volume of phosphate-buffered saline or culture medium, which adequately covers the culture surface. Laminin should be applied to the coverslip at least 30 min before plating out cells.

3.5. Recording Ca^{2+} Sparks in Ventricular Myocytes

Spontaneous Ca^{2+} sparks may be recorded using the same con-focal settings used for Ca^{2+} transient imaging (e.g. line scan mode, laser power, pinhole size, detector gain, etc.). During spark recording, cells are kept in Tyrode's solution under resting conditions (non-stimulated). For guinea pig, rabbit, canine and other large mammalian heart cells, a 15-s field stimulation (1 Hz) is required to load the SR prior to spark recording (*see* Note 11). Soon after the halt of field stimulation, a series of line scan images (e.g. 6 sweeps; each sweep image can be 512 pixels $\times$ 1000 lines) will be acquired at a rate of 1.54 or 1.92 ms per line. For rat and mouse heart cells, spontaneous Ca^{2+} sparks may be visualized with or without pre-stimulation. However, it is more accurate to compare Ca^{2+} sparks recorded at steady-state conditions. Figure 12.2 shows a typical confocal line scan image of a Ca^{2+} spark recorded in a control rat ventricular myocyte.

3.6. Image Analyses

Digital image processing will be performed by using custom-devised routines created with IDL programming language (Research Systems, Boulder, CO) (24). The Ca^{2+} level is reported as F/F_0 (or as $\Delta F/F_0$), where F_0 is the resting Ca^{2+} fluorescence. By using the following equation, we can convert the Ca^{2+} fluorescence ratio to a Ca^{2+} concentration (7):

$$[\text{Ca}^{2+}]_i = KR / \{K / [\text{Ca}^{2+}]_{\text{rest}} + 1\} - R\}$$

where K is the dissociation constant of the Ca^{2+} indicator used, R is the fluorescence ratio (F/F_0), $[\text{Ca}^{2+}]_{\text{rest}}$ is the resting Ca^{2+} concentration. Assuming the dissociation constant (K) of fluo-4 AM is 400 nM (*see* Invitrogen Inc.), the resting $[\text{Ca}^{2+}]_i$ of a cardiac myocyte is 100 nM, the amplitude of a typical Ca^{2+} spark is 2; we can then estimate that the peak Ca^{2+} concentration of Ca^{2+} spark is around 270 nM.

Figure 12.1 shows a typical train of Ca^{2+} transients from a normal rat ventricular myocyte. With the aid of computer programming analysis, the rising phase (time to peak, t_{peak}), the amplitude (F/F_0) and the decay kinetics (t_{50} , t_{75} , t_{90}) may be extracted from original Ca^{2+} images (25, 26).

Figure 12.2 displays a typical Ca^{2+} spark and illustrates the analyses of key Ca^{2+} spark parameters: amplitude (F/F_0), duration (FDHM, full duration at half-maximal amplitude) and spatial width (FWHM, full-width at half-maximal amplitude). These parameters represent the basic gating properties of RyRs Ca^{2+} release channels: the release flux (F/F_0) and the gating kinetics of RyRs (FDHM). Ca^{2+} sparks of ventricular myocytes, on average, are about $1.8F/F_0$ in amplitude, 2 μm wide and 25 ms long (27).

References

1. Bers DM. Cardiac excitation-contraction coupling. *Nature*. 2002; 415:198–205. [PubMed: 11805843]
2. Guatimosim S, Dilly K, Santana LF, Saleet JM, Sobie EA, Lederer WJ. Local Ca^{2+} signaling and EC coupling in heart: Ca^{2+} sparks and the regulation of the $[\text{Ca}^{2+}]_i$ transient. *J Mol Cell Cardiol*. 2002; 34:941–950. [PubMed: 12234764]

¹⁰Cells should be field-stimulated at 1.0 Hz for 15 s to reach steady-state Ca^{2+} dynamics before image acquisition.

¹¹Myocytes of large mammals tend to unload their SR Ca^{2+} content, which makes Ca^{2+} sparks hard to be detected at resting conditions. One way to circumvent this problem is to use a conditioning protocol to upload Ca^{2+} into the SR before Ca^{2+} spark recording (28).

3. Allen DG, Blinks JR. Calcium transients in aequorin-injected frog cardiac muscle. *Nature*. 1978; 273:509–513. [PubMed: 307184]
4. Wier WG. Calcium transients during excitation-contraction coupling in mammalian heart: aequorin signals of canine Purkinje fibers. *Science*. 1980; 207:1085–1087. [PubMed: 7355274]
5. Minta A, Kao JP, Tsien RY. Fluorescent indicators for cytosolic calcium based on rhodamine and fluorescein chromophores. *J Biol Chem*. 1989; 264:8171–8178. [PubMed: 2498308]
6. Wier WG, Cannell MB, Berlin JR, Marban E, Lederer WJ. Cellular and subcellular heterogeneity of $[Ca^{2+}]_i$ in single heart cells revealed by fura-2. *Science*. 1987; 235:325–328. [PubMed: 3798114]
7. Cheng H, Lederer WJ, Cannell MB. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science*. 1993; 262:740–744. [PubMed: 8235594]
8. Klein MG, Cheng H, Santana LF, Jiang YH, Lederer WJ, Schneider MF. Two mechanisms of quantized calcium release in skeletal muscle. *Nature*. 1996; 379:455–458. [PubMed: 8559251]
9. Tsugorka A, Rios E, Blatter LA. Imaging elementary events of calcium release in skeletal muscle cells. *Science*. 1995; 269:1723–1726. [PubMed: 7569901]
10. Wang X, Weisleder N, Collet C, et al. Uncontrolled calcium sparks act as a dystrophic signal for mammalian skeletal muscle. *Nat Cell Biol*. 2005; 7:525–530. [PubMed: 15834406]
11. Nelson MT, Cheng H, Rubart M, et al. Relaxation of arterial smooth muscle by calcium sparks. *Science*. 1995; 270:633–637. [PubMed: 7570021]
12. Ouyang K, Zheng H, Qin X, et al. Ca^{2+} sparks and secretion in dorsal root ganglion neurons. *Proc Natl Acad Sci USA*. 2005; 102:12259–12264. [PubMed: 16103366]
13. Wei C, Wang X, Chen M, Ouyang K, Song LS, Cheng H. Calcium flickers steer cell migration. *Nature*. 2009; 457:901–905. [PubMed: 19118385]
14. Cheng H, Lederer WJ. Calcium sparks. *Physiol Rev*. 2008; 88:1491–1545. [PubMed: 18923188]
15. Cannell MB, Cheng H, Lederer WJ. Spatial non-uniformities in $[Ca^{2+}]_i$ during excitation-contraction coupling in cardiac myocytes. *Biophys J*. 1994; 67:1942–1956. [PubMed: 7858131]
16. Cannell MB, Cheng H, Lederer WJ. The control of calcium release in heart muscle. *Science*. 1995; 268:1045–1049. [PubMed: 7754384]
17. Franzini-Armstrong C, Protasi F, Ramesh V. Shape, size, and distribution of Ca^{2+} release units and couplons in skeletal and cardiac muscles. *Biophys J*. 1999; 77:1528–1539. [PubMed: 10465763]
18. Lopez-Lopez JR, Shacklock PS, Balke CW, Wier WG. Local calcium transients triggered by single L-type calcium channel currents in cardiac cells. *Science*. 1995; 268:1042–1045. [PubMed: 7754383]
19. Wang SQ, Song LS, Lakatta EG, Cheng H. Ca^{2+} signalling between single L-type Ca^{2+} channels and ryan-odine receptors in heart cells. *Nature*. 2001; 410:592–596. [PubMed: 11279498]
20. Takahashi A, Camacho P, Lechleiter JD, Herman B. Measurement of intracellular calcium. *Physiol Rev*. 1999; 79:1089–1125. [PubMed: 10508230]
21. Guatimosim S, Sobie EA, dos Santos CJ, Martin LA, Lederer WJ. Molecular identification of a TTX-sensitive Ca^{2+} current. *Am J Physiol Cell Physiol*. 2001; 280:C1327–C1339. [PubMed: 11287346]
22. Mitcheson JS, Hancox JC, Levi AJ. Cultured adult cardiac myocytes: future applications, culture methods, morphological and electrophysiological properties. *Cardiovasc Res*. 1998; 39:280–300. [PubMed: 9798514]
23. Mitra R, Morad M. A uniform enzymatic method for dissociation of myocytes from hearts and stomachs of vertebrates. *Am J Physiol*. 1985; 249:H1056–H1060. [PubMed: 2998207]
24. Cheng H, Song LS, Shirokova N, et al. Amplitude distribution of calcium sparks in confocal images: theory and studies with an automatic detection method. *Biophys J*. 1999; 76:606–617. [PubMed: 9929467]
25. Dias-Peixoto MF, Santos RA, Gomes ER, et al. Molecular mechanisms involved in the angiotensin-(1–7)/Mas signaling pathway in cardiomyocytes. *Hypertension*. 2008; 52:542–548. [PubMed: 18695148]
26. Song LS, Sobie EA, McCulle S, Lederer WJ, Balke CW, Cheng H. Orphaned ryanodine receptors in the failing heart. *Proc Natl Acad Sci USA*. 2006; 103:4305–4310. [PubMed: 16537526]

27. Song LS, Guia A, Muth JN, et al. Ca^{2+} signaling in cardiac myocytes overexpressing the $\alpha(1)$ subunit of L-type Ca^{2+} channel. *Circ Res.* 2002; 90:174–181. [PubMed: 11834710]
28. Song LS, Pi Y, Kim SJ, et al. Paradoxical cellular Ca^{2+} signaling in severe but compensated canine left ventricular hypertrophy. *Circ Res.* 2005; 97:457–464. [PubMed: 16051885]

Fig. 12.1. **a** A train of steady-state Ca^{2+} transients elicited by 1-Hz field stimulation. **b** Spatially averaged Ca^{2+} profile showing the dynamic change of Ca^{2+} signals with time. This panel also depicts the analysis of Ca^{2+} transient amplitude and kinetics.

Fig. 12.2. Typical Ca^{2+} spark recorded from a control rat ventricular myocyte at resting condition, loaded with fluo-4 AM. **a** The analysis of basic Ca^{2+} spark characteristics (amplitude, FDHM and FWHM). **b** A surface plot of the Ca^{2+} spark shown in Panel **a**.