Evidence for Compartmentalized Calcium Dynamics from Scale-Dependent Imaging Measurements

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Abstract

As one of the most essential cell processes, calcium signalling reveals cellular responses to stimuli. The different spatial scales used to measure each signal can impact the activity observed. This study explores whether the measurement scale will impact any activity observed using a high-resolution time-lapse (TIFF) file. Circular regions of interest were used to vary the measurement scale, including radii ranging from 2.0 pixels to 20.0 pixels. To measure change in calcium signal activity, I compared variability ($\Delta F/F_0$) and signal amplitude (signal-to-noise ratio or SNR) across each spatial scale. Smaller spatial scales reflected the highest signal variance (≈ 0.0033), revealing highly localized fluctuations, consistent with stochastic calcium dynamics in cellular microdomains. Larger spatial scales had a lower variance (≈ 0.0003) but also a significantly smaller amplitude (≈ 5.13). The larger scale captured a more stable dynamic but also lost some signal due to averaging with background pixels, revealing a contrasting dynamic in an integrated cellular region. The results showed that calcium signalling is spatially organized, revealing a distinct dynamic in a localized compartment in comparison to an integrated cellular region.

Introduction

Universal messengers, including Ca^{2+} ions, are essential in signalling coordinating pathways like muscle contraction and gene transcription. The dynamic nature of intracellular calcium allows each cell to rapidly respond to an external stimulus and demonstrate a physiological response [1]. However, calcium signalling is not uniform throughout a single cell. Different cellular components and microdomains will demonstrate a specific dynamic, with each local transient having an influential role in a spatially precise signalling event. Calcium microdomains by the plasma membrane are functionally different from those in the cytosol by organelles, allowing information encoding by both amplitude and spatial distribution.

Genetically encoded calcium indicators like GCaMP6 have revolutionized the ability to visualize this process dynamically in cells [1], but data interpretation is dependent on the spatial scale of measurements. When analyzing signals, researchers define a region-of-interest (ROI) to quantify a change in fluorescence ($\Delta F/F_0$) over time. The size of the measurement region determines whether a highly localized transient (spatial stochasticity) was captured or an integrated signal across a larger cellular area [5].

Since calcium dynamics vary across different spatial compartments, I expected that the smaller regions would capture a localized, variable dynamic, while a larger region would capture a more average and stable signal. I hypothesized that calcium signalling would demonstrate spatial heterogeneity: that localized regions in cells would show a distinct dynamic of higher variability due to stochastic fluctuations, while a larger component would show more stable signals because of spatial averaging across multiple signalling sites, and that an intermediate scale would preserve the strongest signal amplitude before background dilution diminishes it. To test

this hypothesis, I systematically varied the spatial scale of measurements (r = 2.0 to 20.0 pixels) across 110 verified active cell sites in a high-resolution GCaMP6 calcium imaging dataset. Through this analysis, the study aims to provide evidence for spatial heterogeneity in calcium imaging, questioning how measurement scale impacts characterization of compartmentalized calcium dynamics.

Methods

Imaging Dataset

Computational analysis was conducted using a high-resolution time-lapse fluorescence calcium image stack (File: 14x2Y.tiff). Image and data processing were performed using Python, utilizing the tifffile, NumPy, scipy, pandas, and scikit-image open-source libraries [2, 4]. Raw pixel intensities were maintained throughout the pipeline to ensure quantitative accuracy.

Motion Correction and Pre-processing

To prevent motion-induced variance, rigid registration using a phase cross-correlation algorithm was used to correct for X-Y drift across all frames in the image stack. Pre-processing was via normalizing the first TIFF frame and applying a Gaussian smoothing filter ($\sigma = 1.5$) to make detected calcium signalling sites more accurate.

ROI Detection and Scaling

Automation of calcium source detection was through the peak_local_max algorithm [4], identifying possible centroids with a minimum distance of 20 pixels and a 0.3 threshold. A 25-pixel border exclusion was used to prevent edge artifacts. To reduce selection bias, an activity-based quality control (QC) filter was applied, requiring a peak $\Delta F/F_0$ at least 2 times the baseline noise floor. This resulted in a final sample of 110 verified active centroids. For each center, concentric circular ROI masks were varied from 2.0 to 20.0 pixels.

Signal Extraction and Neuropil Subtraction

To isolate cellular signals from background contamination, neuropil subtraction was performed for every ROI at each scale [3]. A "background donut" (annulus) was defined 2 to 8 pixels beyond the current ROI boundary. To prevent signal contamination from neighbouring cells, all other detected centroids were masked out of the annulus before calculating the mean neuropil fluorescence ($F_{neuropil}$). The corrected fluorescence F_{corr} was calculated as $F_{corr} = F_{ROI} - (0.7 \times F_{neuropil})$, using the neuropil coefficient referenced by Pachitariu et al. [3].

$\Delta F/F_0$ Processing and Metric Analysis

A sliding 8th percentile filter with a 100-frame window was applied to F_{corr} to define a dynamic baseline and account for photobleaching. The relative change in fluorescence was calculated using the standard $\Delta F/F_0$ formula, with a 1×10^{-3} threshold to prevent division-by-zero errors. Quantitative metrics included the baseline variance for measuring microdomain spatiality and

the corrected Signal-to-Noise Ratio (SNR), which was the amplitude divided by the standard deviation of the baseline to highlight variance from typical background fluctuations.

Statistical Significance

The Standard Error of the Mean (SEM) and p-values were calculated across the 110-cell population to establish statistical significance. Paired t-tests were used to compare SNR values at each radius against the reference (r = 6.0 pixels), with a significance threshold of $\alpha = 0.05$.

Results

Radius Influence on Signal Variance

A non-linear decay relationship is shown between the mean $\Delta F/F_0$ baseline variance and ROI radius from 2.0 to 20.0 pixels. The variance reaches an absolute maximum at the minimum radius (r=2.0), exceeding 0.0030, which indicates a high susceptibility to pixel-level spatial stochasticity when spatial averaging is limited. Following a sharp exponential decay, the curve reaches asymptotic stability at approximately r=10.0 (variance ≈ 0.0005). Beyond this point, further increases in radius toward r=20.0 result in diminishing noise reduction, with variance levels plateauing at approximately 0.0003.

Radius influence on SNR

The mean SNR followed a non-monotonic trend, identifying a definitive peak of ~5.38 at a radius of 6.0 pixels. The SNR of larger radii had a steady, statistically significant decline due to signal dilution from background pixels ($p < 0.01$ at r=10.0). At the maximum tested radius (r=20.0), the SNR dropped to approximately 5.13, representing a notable decrease in signal quality compared to the peak. The SNR measured for a radius smaller than 6.0 was insignificant as per the t-test results.

Statistical Consistency and Power

Based on the sample size of verified active cells (n=110), the Standard Error of the Mean (SEM) for baseline variance remained extremely tight across all tested radii, indicating a highly predictable relationship between ROI scale and noise reduction. While the SNR SEM bars were slightly larger near the 6.0-pixel peak, the overall stability of the 110-cell population has enough statistical power to highlight relatively smaller ROI ($p < 0.01$, r = 6.0) with minimal dilution for this dataset compared to larger radii ($p < 0.001$, r=15.0).

Figures/Tables

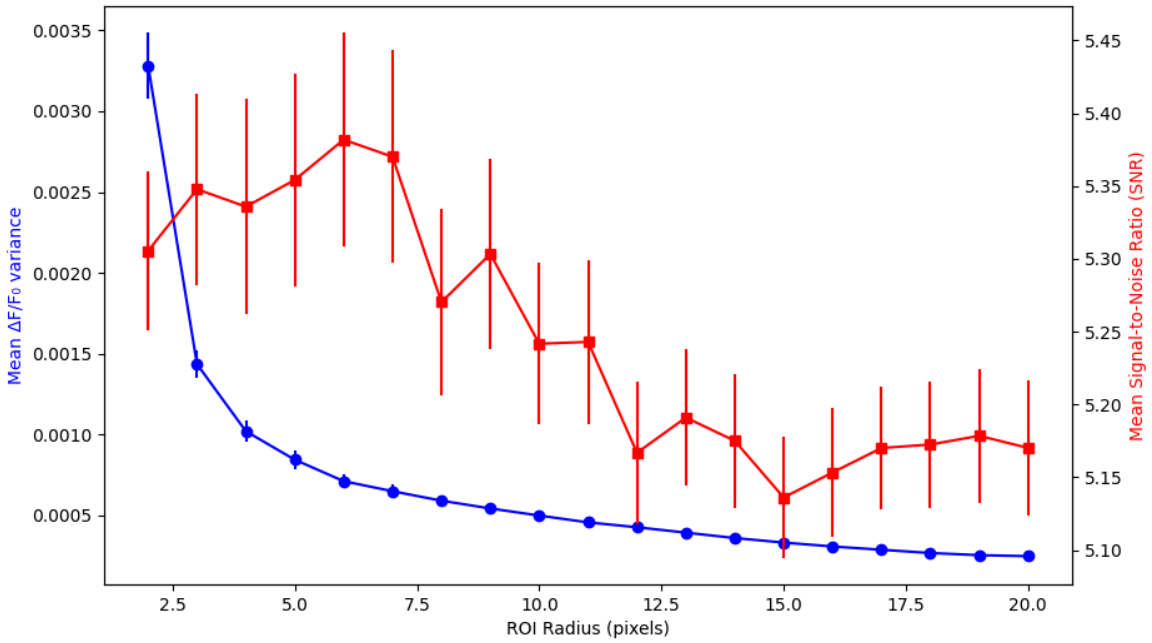

Figure 1. Performance in mean $\Delta F/F_0$ variance and SNR (±1SEM) for various (n=110) ROI radii (2.0 to 20.0 pixels)

Table 1. Statistical summary table comparing the performance in mean $\Delta F/F_0$ variance and SNR (±1SEM) for various ROI radii (2.0, 4.0, 6.0, 8.0, 10.0, 15.0) pixels.

Radius (Pixels)	Mean SNR	Baseline Variance
2.0	~5.30	~0.0033
4.0	~5.34	~0.0010
6.0 (Peak)	**~5.38**	**~0.0007**
8.0	~5.28	~0.0006

10.0	~5.26	~0.0005
15.0	~5.13	~0.0003

Table 2. Paired T-test results summary table for various ROI (2.0, 4.0, 6.0, 8.0, 10.0, 15.0) SNRs against the intermediate radius (6.0px)

Radius (Pixels)	**p-value (vs. 6.0px)**	**Significance**
2.0	0.3269	n.s.
4.0	0.3916	n.s.
6.0	**--**	**Reference**
8.0	0.0154	*
10.0	0.0112	*
15.0	0.0012	**
20.0	0.0124	*

Discussion

The hypothesis was supported and showed that calcium imaging dynamics reflected spatial heterogeneity. Throughout the cell, the calcium signal was not uniform and varied depending on the spatial measurement scale.

Smaller spatial scales ($r < 5.0$) had the highest baseline variance (~0.0033 at $r = 2$). This showed that the microdomain had a highly localized calcium dynamic. Individual calcium channels have stochastic openings and closings, reflecting the variability in signal because of the isolation of measurements in a small functional compartment [1]. The intermediate spatial scale, where $r = 5.0$ to 10.0, had a significantly smaller variance compared to the smaller ROIs ($r=10.0$, variance ~0.0005). The signal amplitude was also the strongest, reaching the maximum value (~5.38 at $r = 6$). A balance between variance and amplitude suggested relative averaging across other local sites, but still preserving the signal quality without dilution through background pixels. These values could represent a specific cellular response reflected by the calcium dynamics. The larger spatial scales, where $r > 10.0$, had the most stable signal with little variance (~0.0003). However, the scale was also observed to have a significantly reduced signal amplitude compared to the baseline. This could be explained by the inclusion of inactive background regions in fluorescent signal calculations, even though no cells were present in the area, creating signal dilution [5]. Overall, the trend in signal variance shows that smaller spatial scales had increased variance from the baseline, possibly due to spatial stochasticity, while larger ROIs had little variation. For signal amplitude, while measurements for smaller ROIs were not significant, the intermediate scale represented the strongest calcium signal amplitude.

Spatial heterogeneity in calcium signalling is supported through the non-monotonic trend I observed, where SNR peaked at an intermediate scale before declining, while variance decreased monotonically with radius. Based on spatial scale, calcium signalling can have different functions in the cell. A localized microdomain could increase precision and control for more specific events like vesicle fusion or ion channel regulation, as they are smaller, confined signalling regions [1]. The high spatial specificity can capture more micro-activity, but also has the risk of capturing microdomain stochasticity. The larger calcium scales could be more beneficial for a broader cellular response, like observing gene expression or metabolism over multiple large regions. Highly spatialized values would represent a more overall dynamic, but also risk missing more local events because of spatial averaging [5]. Therefore, the measurement scale can significantly influence the dynamics observed by calcium signals. The results reveal how calcium signalling does not demonstrate a uniform signal and rather is spatially organized, meaning recording intensity in different regions might reveal a distinct cellular dynamic.

In the study, the standardized annotations and measurements were obtained via circular ROIs to compare each of the different spatial scales. This allowed systematic and consistent comparison between different radii. But biological cells can also often be irregular and non-circular [5]. Since cell components can be influenced by organelle proximity, cytoskeletal structure, and membrane microdomains, a circular ROI may not be completely representative of a true biological cell/signal. In a future study, I could improve accuracy by measuring membrane-specific ROIs and using a geometry-based rather than circular segmentation approach. I also applied rigid motion correction to cells to account for some movement over time, but in an environment with significant movement, the measurement results could have

differed from the actual activity of the cell, especially with a smaller ROI that is more prone to signal loss. This could explain why the SNR data appeared insignificant for ROIs with a radius smaller than 6.0 pixels. Dynamic ROI tracking, as implemented in Suite2p [3], could improve measurement accuracy in future work. Lastly, the genetically encoded calcium indicator I used in this study was GCaMP6, a green fluorescent indicator [1]. A different indicator, however, with different signal kinetics, dynamic range and subcellular targeting could reveal different measurement results. A future study could also investigate, for example, red-shifted calcium indicators, voltage-sensitive indicators and organelle-targeted calcium sensors.

This study showed that spatial context and considering spatial heterogeneity are important for the accurate interpretation of calcium imaging data [5]. Changing the measurement scale directly influences the observed signal characteristics in the calcium cells. These could explain discrepancies in calcium imaging data between different studies. Therefore, researchers must carefully consider the spatial measurement scale, whether the data is highly localized or generalized over a larger area. Small measurements are more sensitive to any calcium microdomains, but may be increasingly inaccurate for larger domains because of stochastic fluctuations. Large scales reduce variability, making data more stable, but could underestimate the strength of a signal of a smaller domain due to dilution from averaging background pixels. Insofar as analysis choices, they influence whether localized or generalized calcium dynamics are reflected in the results.

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