SoftwareX 32 (2025) 102435

Contents lists available at ScienceDirect

SoftwareX

journal homepage: www.elsevier.com/locate/softx

Original software publication

NeuroSpikeX: Comprehensive detection and characterization of neuronal calcium dynamics

J.A. Sergay [a,b,*], A. Hai [a], C. Franck [b]

[a] Department of Biomedical Engineering, University of Wisconsin-Madison, United States of America
[b] Department of Mechanical Engineering, University of Wisconsin-Madison, United States of America

ARTICLE INFO

Keywords:
Calcium imaging
Spike detection
Neural networks
MATLAB software

ABSTRACT

NeuroSpikeX is a user-friendly tool for the quantitative analysis of neuronal calcium dynamics. It provides robust calcium spike detection, comprehensive network metrics, and intuitive graphical interfaces. NeuroSpikeX seamlessly integrates into existing workflows using outputs from the established algorithm NeuroCa, enhancing accuracy and reproducibility. The code effectively analyzes calcium dynamics across numerous *in vitro* datasets containing multiple experimental time points. NeuroSpikeX facilitates detailed cell and network analyses in large datasets, making rigorous calcium transient characterization accessible to researchers with minimal coding expertise.

Code metadata

Current code version	v1.0.0
Permanent link to code/repository used for this code version	https://github.com/ElsevierSoftwareX/SOFTX-D-25-00521
Permanent link to Reproducible Capsule	
Legal Code License	MIT
Code versioning system used	git
Software code languages, tools, and services used	MATLAB 2024b or later
Compilation requirements, operating environments & dependencies	Signal Processing Toolbox, Image Processing Toolbox, Statistics and Machine Learning Toolbox, Wavelet Toolbox, Curve Fitting Toolbox, Parallel Computing Toolbox
If available Link to developer documentation/manual	https://github.com/FranckLab/NeuroSpikeX/blob/main/README.md
Support email for questions	cfranck@wisc.edu

1. Motivation and significance

Quantitative analysis of neuronal calcium dynamics is essential for understanding network connectivity at single-cell resolution across diverse experimental models [1,2]. However, many existing tools require steep learning curves, misidentify calcium transients under certain experimental conditions, or struggle to scale to large datasets. Improving spike detection and network population metrics is therefore critical for revealing how cellular and circuit dynamics respond to experimental manipulations.

Established software such as CaImAn [3] and Suite2p [4] provide powerful analysis pipelines but typically demand complex parameter tuning and coding expertise. More user-friendly MATLAB-based programs, including NeuroCa [5], FluoroSNNAP [6], and Detect [7], have substantial limitations: they often struggle to process large-scale *in vitro* datasets or lack comprehensive export options for downstream analyses.

The contribution of NeuroSpikeX is to bridge this gap by providing an open, scalable, and accessible platform for calcium imaging analysis. During development, NeuroSpikeX was iteratively refined using a wide range of datasets, with hundreds to thousands of cells, to ensure that the software could reliably handle diverse experimental conditions. It has also been tested with both chemical (Fluo-4 AM) and genetically encoded (GCaMP6s) indicators, confirming that its functionality is not limited to a single imaging approach and can efficiently examine calcium dynamics.

* Corresponding author.
E-mail addresses: sergay@wisc.edu (J.A. Sergay), ahai@wisc.edu (A. Hai), cfranck@wisc.edu (C. Franck).

https://doi.org/10.1016/j.softx.2025.102435
Received 22 July 2025; Received in revised form 22 September 2025; Accepted 29 October 2025
Available online 6 November 2025

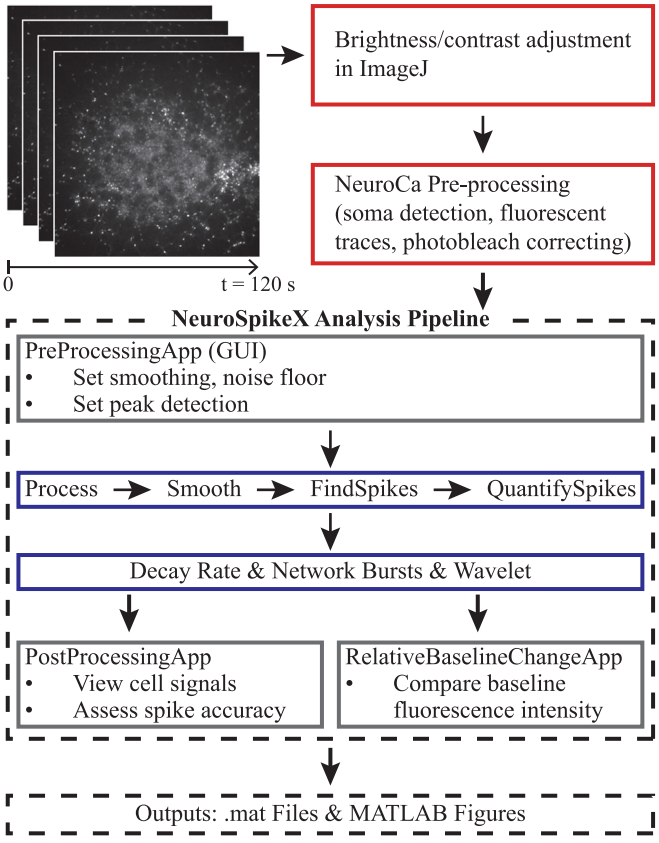

Fig. 1. Flow of NeuroSpikeX analysis pipeline.

2. Software description

2.1. Software architecture

NeuroSpikeX is a MATLAB-based software package for analyzing calcium imaging data preprocessed with ImageJ and NeuroCa. Fig. 1 summarizes the NeuroSpikeX pipeline. The workflow begins with raw timelapse recordings that are formatted in ImageJ for NeuroCa compatibility. NeuroCa pre-processes imaging data by identifying somas, extracting fluorescence traces, and correcting for photobleaching [5]. From NeuroCa, three outputs are required: normalized fluorescence ($\%\frac{dF}{F_o}$; `fdata`), cell soma locations (`center`), and soma sizes (`radii`) [5]. Data processing includes importing NeuroCa outputs, smoothing calcium signals, detecting transients, and performing quantitative analyses. NeuroSpikeX evaluates and compares these data across multiple experimental time points.

2.2. Software functionalities

NeuroSpikeX is structured around a main controller script (`Main_Calcium_Code`) that automates most steps by interfacing with modular functions. Parameter values are first defined in the Pre-Processing App. The main script then executes the full analysis pipeline, which includes artifact removal, time axis extraction, signal smoothing, transient peak and decay detection, and calculation of metrics such as spike rate and intensity at both the cell and network levels. In addition to these computations, the pipeline generates visual outputs — including scalograms (continuous wavelet transforms), raster plots, and histograms of network activity — to aid interpretation. After spikes are identified, synchronous network bursts are detected and annotated on raster plots. Finally, two companion applications extend the analysis by providing additional visualization and validation tools.

2.2.1. Pre-processing app

The Pre-Processing App (Fig. 2) lets users save signal-processing parameters. The settings are then automatically integrated into the rest of the software.

In Tab 1 (Fig. 2A), users load data by selecting the folder for each time point. A standardized analysis duration is set to ensure comparability across samples with different recording lengths. Next, users determine the noise floor, defined as the amplitude range of baseline noise. Noise floors can be applied globally or calculated per sample by selecting noise regions from randomly chosen cell signals (Fig. 3). Calculated noise floors are averaged across time points and conservatively rounded up to improve spike detection reliability. Finally, users adjust the smoothing factor. NeuroCa-derived normalized fluorescent intensity signals are smoothed before peak detection using an exponentially weighted moving average filter. The smoothing coefficient α is calculated as $\frac{2}{n+1}$, where n is user-defined.

Tab 2 (Fig. 2B) focuses on transient peak (spike) detection. Users can apply signal bounds to reduce artifacts, especially with indicators prone to noise. The key parameter is minimum peak prominence, which sets the required rise above background for a peak to be considered valid [8]. Table 1 gives example parameter values for different calcium indicators.

2.2.2. Transient detection

The main script smooths NeuroCa fluorescence traces to reduce high-frequency noise, then identifies spikes as local maxima exceeding the prominence threshold set in the Pre-Processing App. To avoid amplitude underestimation from smoothing, spike intensities are measured from the unsmoothed normalized trace at each detected peak. For each cell, spike intensities are averaged and spike rates are calculated as spikes per second. Network values are obtained by averaging across all active cells in the sample.

2.2.3. Decay rate constant

For each detected transient, the code identifies the end of the decay by calculating the derivative of the smoothed signal and locating the point where it crosses from negative to positive after the peak. The decay window, spanning from the peak to this endpoint, is shifted to start at $t = 0$ and end at $y = 0$. A single-term exponential function, ae^{-bx}, is then fit to the segment, where a represents the transient amplitude and b the decay rate constant. Rate constants are stored for each transient and subsequently averaged at both the cell and sample level for downstream analysis.

2.2.4. Post-processing app

The Post-Processing App (Fig. 4) can be run after the main analysis of any sample and provides two functions: visualization and validation. The Visualize Cell Analysis tab (Fig. 4A) displays active and inactive normalized cell traces for any experimental time point, along with detected peaks and fitted decays.

The Manual Accuracy Check tab (Fig. 4B) supports two validation modes. In the first binary mode, users classify a defined percentage of cells as either containing transients or noise. Standard performance metrics (sensitivity, specificity, accuracy, false-positive/negative rates, and Youden's J) are calculated. In the second, manually counted transients are compared with NeuroSpikeX detections. Accuracy metrics include mean absolute error, root mean square error, systematic bias (mean error and distribution of differences), exact match rate, accuracy within ±1 spike, and symmetric mean absolute percentage error. All validation metrics are automatically saved.

To demonstrate the validation module, we manually counted spikes in four control Fluo-4 AM recordings. NeuroSpikeX achieved a mean accuracy (within ±1 spike) of 70.7%, compared with 58.4% for NeuroCa, with similar proportions of over- and under-counted spikes. These results underscore the importance of NeuroSpikeX's validation module. Calcium signal quality can vary substantially by indicator and experimental setup, so users must be able to quantify accuracy and bias on their own data to ensure the analysis is appropriate for their conditions.

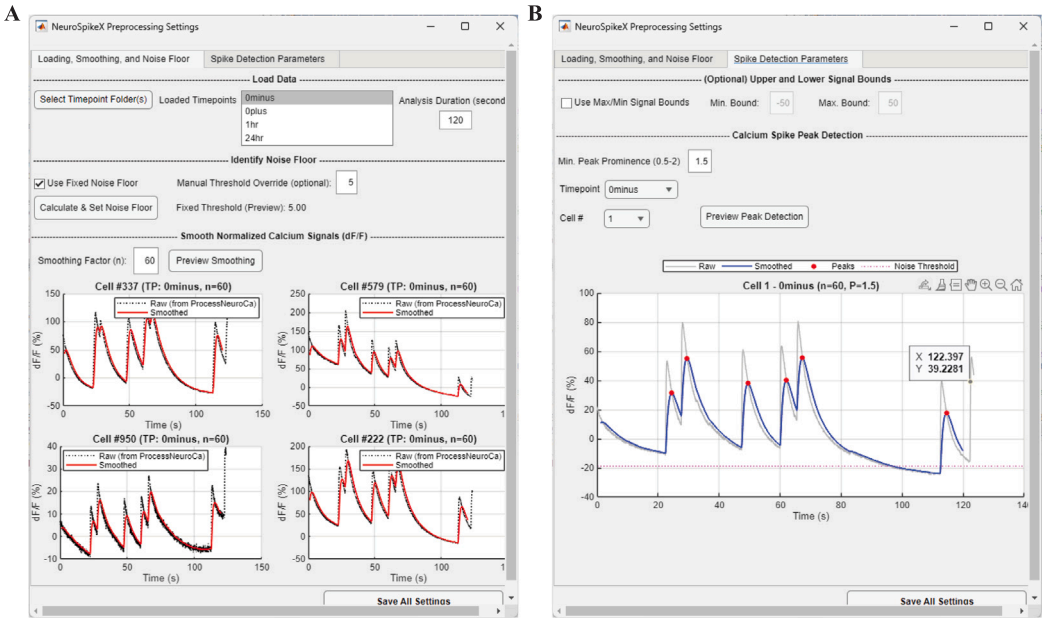

Fig. 2. Pre-Processing App. (A) Tab 1—load data from all time points, set the noise floor threshold, and define the signal smoothing factor. (B) Tab 2—optionally set signal bounds and adjust minimum peak prominence for calcium spike detection.

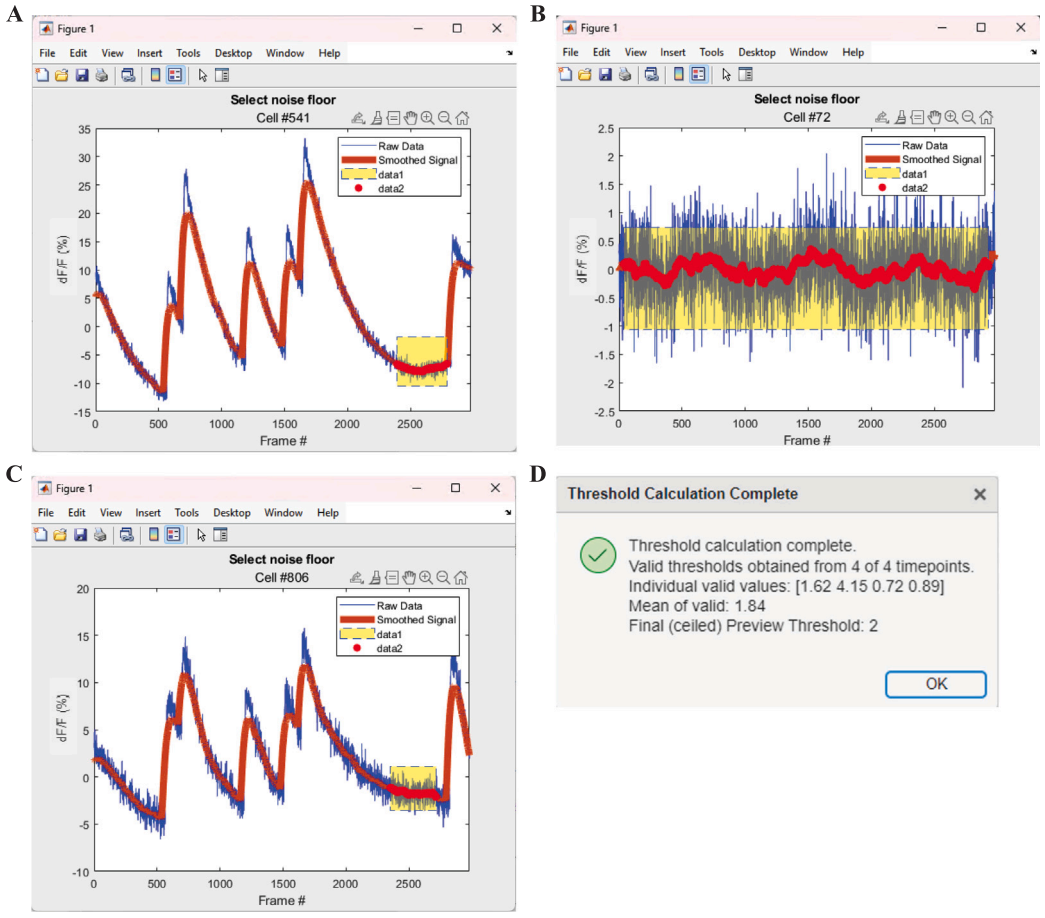

Fig. 3. (A–C) Noise floor selection using interactive boxes and (D) averaged threshold calculation.

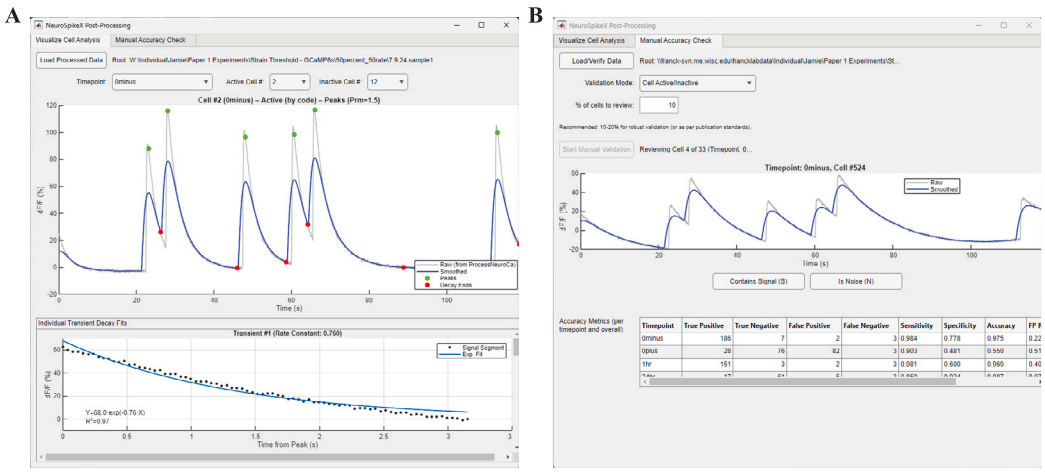

Fig. 4. Post-Processing App. (A) Visualize Cell Analysis Tab—review cell signals, detected peaks, and transient decay fits. (B) Manual Accuracy Tab—manually verify signals and calculate accuracy metrics compared to NeuroSpikeX results.

2.2.5. *Relative baseline intensity change app*

The Relative Baseline Intensity Change (RBIC) App compares baseline fluorescence between two experimental time points to estimate intracellular calcium changes due to injury or treatment. It has three tabs: frame selection, optional radii optimization, and analysis (Fig. 5).

In Tab 1 (Fig. 5A), users select frames that reflect baseline fluorescence. In Tab 2 (Fig. 5B), users can optimize soma and background fluorescence settings. NeuroCa-derived soma coordinates and radii are used by default, but these can be recalculated at baseline intensity for greater accuracy. Background subtraction is performed using an annulus defined by inner and outer factors relative to the soma radius ($r_i = f_{inner} \cdot r_{soma}$ and $r_o = f_{outer} \cdot r_{soma}$). Adjusting these factors refines background estimation. This optimization is beneficial when NeuroCa radii — typically defined at peak brightness — do not represent baseline conditions.

In Tab 3 (Fig. 5C–D), users run the baseline change analysis. Fluorescence change is calculated as $intensity_{time2}/intensity_{time1}$ and reported as $\log_2(fold\_change)$, where 0 indicates no change, +1 a doubling, and –1 a halving of intensity. Two analysis modes are available. Control mode processes all control samples together to establish a baseline distribution of fluorescence variability. The middle 95th percentile of these values defines the "no change" boundary, ensuring that random fluctuations are not misclassified as meaningful changes. Experimental mode then applies these control-derived thresholds when analyzing individual samples, allowing changes in fluorescence to be interpreted relative to the variability observed in controls. Results are saved within each sample directory.

3. Illustrative examples

In vitro co-cultures expressing hsyn-GCaMP6s or loaded with Fluo-4 AM were analyzed using NeuroSpikeX. The samples represented in Fig. 6 were first used in the Pre-Processing App to adjust and save parameters optimized for their calcium signals. Fig. 6A shows a neuron-specific genetically encoded indicator (AAV1-hsyn-GCaMP6s) imaged at 10X with widefield microscopy. Fig. 6B shows a culture stained with Fluo-4 AM, which labels both neurons and astrocytes, under the same imaging conditions. Timelapses from both samples were preprocessed in ImageJ and NeuroCa before analysis in NeuroSpikeX.

Table 1 lists the user-set parameters confirmed in the Pre-Processing App for each calcium indicator. Fluo-4 AM required additional signal bounds and per-sample noise floors due to its higher variability. The parameter values provide a reference framework for users when configuring their own analyses.

Fig. 7 highlights the main graphical outputs of NeuroSpikeX. The data were collected from an *in vitro* co-culture expressing hsyn-GCaMP6s. The sample was stretched to 0.5 axial strain at 50 strain/s under simple tension, and two-minute timelapses were captured at four time points: immediately before stretch (0-), immediately after stretch (0+), one hour after (1hr), and 24 h after (24hr).

Fig. 7A shows the raster plot at 0- with synchronous bursts highlighted in orange. Fig. 7B presents the average network scalogram at 0–. Figs. 7C and 7D are histograms illustrating distribution shifts across four recording times for cellular spike rates and decay rate constants. Finally, Figs. 7E and 7F are outputs from the Relative Baseline Intensity Change App, showing changes in baseline fluorescence between 0– and 0+.

4. Impact

NeuroSpikeX expands the potential for studying calcium signal dynamics within large neural networks subjected to diverse perturbations. Accurate and comprehensive quantification of calcium events is essential for linking experimental conditions to network responses [9–14]. NeuroSpikeX provides a practical framework for extracting both established and underutilized metrics.

Traditional outputs such as spike rate, intensity, and network bursts yield insight into action potential firing frequency, connectivity, and network maturation [1,2,15,16]. NeuroSpikeX extends this analysis by incorporating decay rate constants, which are rarely implemented in other tools and can highlight differences in firing kinetics or calcium clearance mechanisms [17,18]. In addition, it quantifies baseline fluorescence shifts across time points, offering a measure of intracellular calcium homeostasis independent of transient activity [19].

By making these analyses accessible to users with minimal coding experience, NeuroSpikeX lowers technical barriers while promoting reproducibility. This enables researchers to not only characterize network responses under different experimental conditions, but also to explore previously underexamined features of calcium dynamics, advancing studies of disease, injury, and circuit function.

5. Conclusions

NeuroSpikeX broadens the scope of calcium imaging analysis, combining flexible spike detection, transient characterization, and intuitive visualization tools.

The distinctive contributions of this software are: (i) visual interfaces that allow users to customize filtering and detection parameters to match their experimental needs, (ii) a post-processing app for rapid validation of automated classifications, (iii) inclusion of both decay rate constant and baseline intensity analyses alongside traditional spike

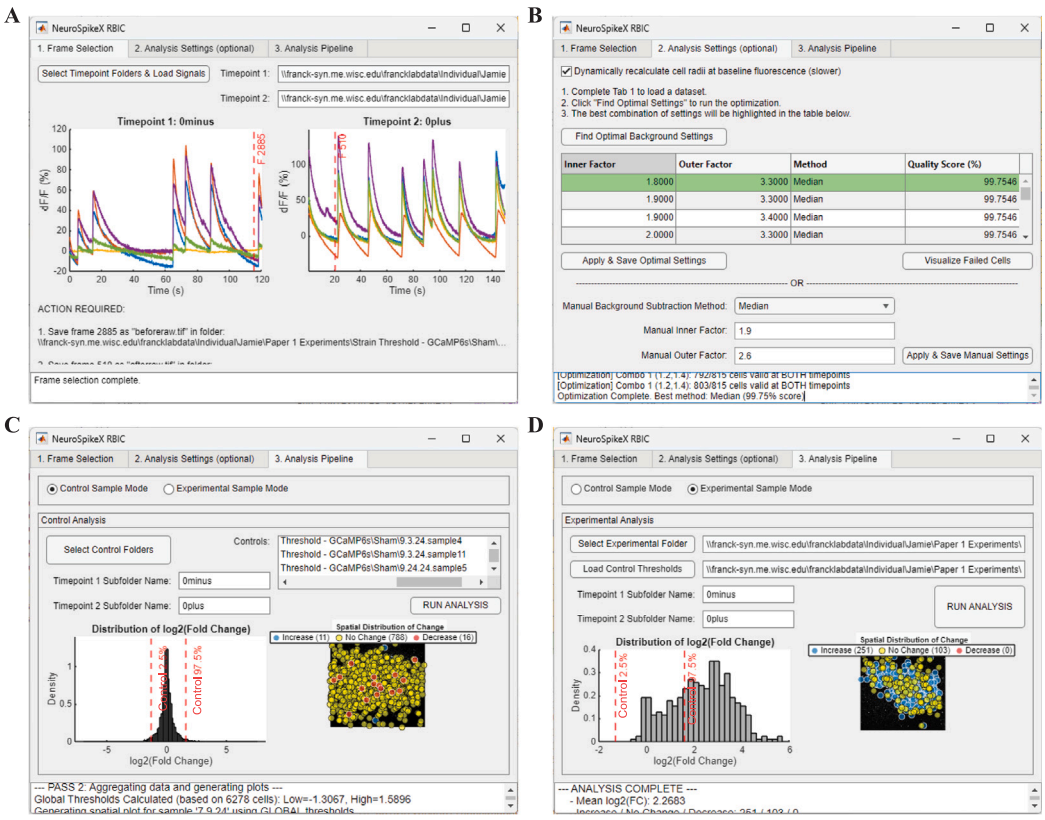

Fig. 5. Relative Baseline Intensity Change (RBIC) App. (A) Frame Selection Tab—select baseline frames. (B) Analysis Settings Tab—optimize soma and background parameters for improved baseline accuracy. (C–D) Analysis Pipeline Tab—control mode defines thresholds from all control samples; experimental mode analyzes individual samples.

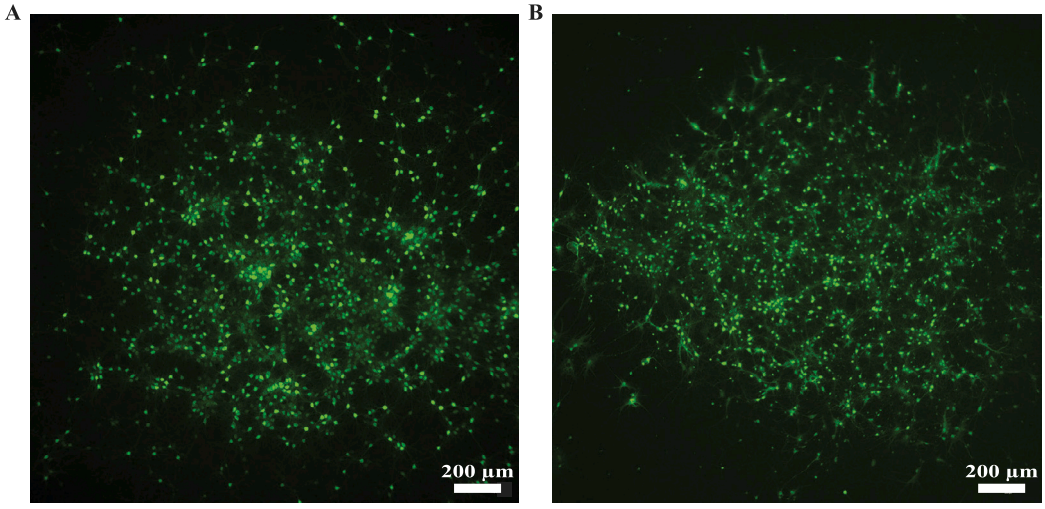

Fig. 6. Experimental images. (A) Culture transfected with AAV1-hsyn-GCaMP6s. (B) Culture stained with Fluo-4 AM.

Table 1
Parameters used for calcium signal analysis across indicators.

Calcium indicator	Smooth Factor (n)	Noise floor	Use Min./ Max. Bounds	Signal bounds	Min. Peak Prominence
AAV1-hsyn-GCaMP6s	60	5%	no	–	1.5%
fluo-4 AM	40	1%–2%	yes	[−50 50]%	0.7%

metrics, and (iv) scalability for large datasets across multiple time points with automated export for downstream use.

As with any analysis pipeline, limitations remain. NeuroSpikeX is currently optimized for in vitro datasets and relies on NeuroCa preprocessing. Extending compatibility to in vivo recordings, alternative preprocessing workflows, and additional calcium indicators would broaden its applicability. Future developments may also integrate machine learning-based classification to further improve accuracy.

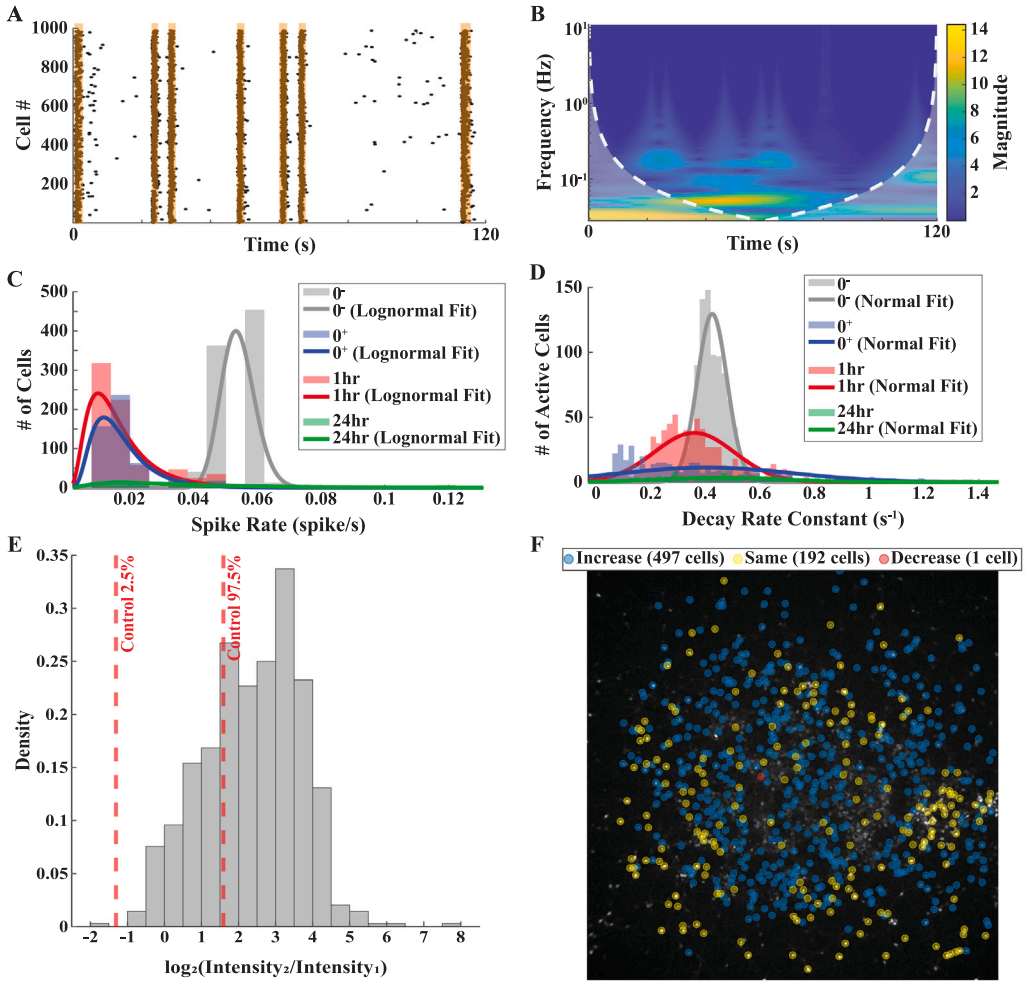

Fig. 7. Graphical outputs. (A) Raster plots, (B) average network scalograms, (C) spike rate distributions, (D) decay rate constants, (E) baseline intensity changes, and (F) spatial distribution of intensity changes.

The distinguished functions of NeuroSpikeX establish a versatile platform for advancing calcium imaging studies and uncovering new dimensions of network behavior.

CRediT authorship contribution statement

J.A. Sergay: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **A. Hai:** Writing – review & editing, Supervision. **C. Franck:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Noah Meltzer for his guidance with signal processing and code review. This material is based upon research supported by the U.S. Office of Naval Research under PANTHER award number N00014-22-1-2828 and N00014-23-1-2006 through Dr. Timothy Bentley. During the preparation of this work, the author used ChatGPT to check grammar and clarity. Google AI Studio and ChatGPT were utilized to refine the NeuroSpikeX code for efficiency and aid in creating the apps. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

References

[1] Grienberger C, Konnerth A. Imaging calcium in neurons. Neuron 2012;73(5):862–85.
[2] Sun Z, Südhof TC. A simple Ca2+-imaging approach to neural network analyses in cultured neurons. J Neurosci Methods 2021;349(109041):109041.
[3] Giovannucci A, Friedrich J, Gunn P, Kalfon J, Brown BL, Koay SA, et al. CaImAn an open source tool for scalable calcium imaging data analysis. Elife 2019;8:e38173.
[4] Pachitariu M, Stringer C, Dipoppa M, Schröder S, Rossi LF, Dalgleish H, et al. Suite2p: beyond 10 000neurons with standard two-photon microscopy. 2016, p. 061507, bioRxiv.
[5] Jang MJ, Nam Y. NeuroCa: integrated framework for systematic analysis of spatiotemporal neuronal activity patterns from large-scale optical recording data. Neurophotonics 2015;2(3):035003.
[6] Patel TP, Man K, Firestein BL, Meaney DF. Automated quantification of neuronal networks and single-cell calcium dynamics using calcium imaging. J Neurosci Methods 2015;243:26–38.
[7] Desai NS, Zhong C, Kim R, Talmage DA, Role LW. A simple MATLAB toolbox for analyzing calcium imaging data in vitro and in vivo. J Neurosci Methods 2024;409(110202):110202.
[8] Prominence - MATLAB & simulink. 2025, https://www.mathworks.com/help/signal/ug/prominence.html. [Accessed 19 June 2025].
[9] Hansen KR, DeWalt GJ, Mohammed AI, Tseng H-A, Abdulkerim ME, Bensussen S, et al. Mild blast injury produces acute changes in basal intracellular calcium levels and activity patterns in mouse hippocampal neurons. J Neurotrauma 2018;35(13):1523–36.

[10] Cramer SW, Haley SP, Popa LS, Carter RE, Scott E, Flaherty EB, et al. Wide-field calcium imaging reveals widespread changes in cortical functional connectivity following mild traumatic brain injury in the mouse. Neurobiol Dis 2022;176:105943.
[11] Fröhlich A, Olde Heuvel F, Rehman R, Krishnamurthy SS, Li S, Li Z, et al. Neuronal nuclear calcium signaling suppression of microglial reactivity is mediated by osteoprotegerin after traumatic brain injury. J Neuroinflammation 2022;19(1):279.
[12] Adams AA, Li Y, Kim HA, Pfister BJ. Dorsal root ganglion neurons recapitulate the traumatic axonal injury of CNS neurons in response to a rapid stretch in vitro. Front Cell Neurosci 2023;17:1111403.
[13] Yoo S, Mittelstein DR, Hurt RC, Lacroix J, Shapiro MG. Focused ultrasound excites cortical neurons via mechanosensitive calcium accumulation and ion channel amplification. Nat Commun 2022;13(1):493.
[14] Chen Z, Chen X, Shimoda S, Huang Q, Shi Q, Fukuda T, et al. A modular biological neural network-based neuro-robotic system via local chemical stimulation and calcium imaging. IEEE Robot Autom Lett 2023;8(9):5839–46.
[15] Chen T-W, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, et al. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 2013;499(7458):295–300.
[16] Ali F, Kwan AC. Interpreting in vivo calcium signals from neuronal cell bodies, axons, dendrites: A review. Neurophotonics 2020;7(1):011402.
[17] Eisner D, Neher E, Taschenberger H, Smith G. Physiology of intracellular calcium buffering. Physiol Rev 2023;103(4):2767–845.
[18] Zhang Y, Rózsa M, Liang Y, Bushey D, Wei Z, Zheng J, et al. Fast and sensitive GCaMP calcium indicators for imaging neural populations. Nature 2023;615(7954):884–91.
[19] Goshi N, Morgan RK, Lein PJ, Seker E. A primary neural cell culture model to study neuron, astrocyte, microglia interactions neuroinflammation. J Neuroinflammation 2020;17(1):155.