MEDICAL UNIVERSITY OF VIENNA

Bringing data closer to intuition

A comprehensive software for analysis, visualization, organization, and fast interaction with large microscopy recordings

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Objective

A typical experiment involving imaging of pancreatic slices in our lab concerns a single field of view showing up to hundreds of cells, in a recording of at least several, often dozens, gigabytes. Current tools (i.e. ImageJ) rely on loading the recording, or its part, into memory, for viewing, analysis, and processing. It also requires laborious and long human engagement. We have developed a set of interdependent tools to automatize as much as possible the analysis pipeline.

Analysis Pipeline

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The main elements of our pipeline are the following (Fig.1):

- Phase and motion correction at the subpixel level;
- Automatic segmentation based on a template image;
- Sequential filtering ROI time traces across all relevant frequencies (with correction for filtering distortions);
- Quantification for each trace (height, AUC, or halfwidth statistics, event rate...).
- Quantification of the effect magnitude for acute pharmacological manipulations, and/or different experimental branches (different mice, or diet, of genetic manipulations).

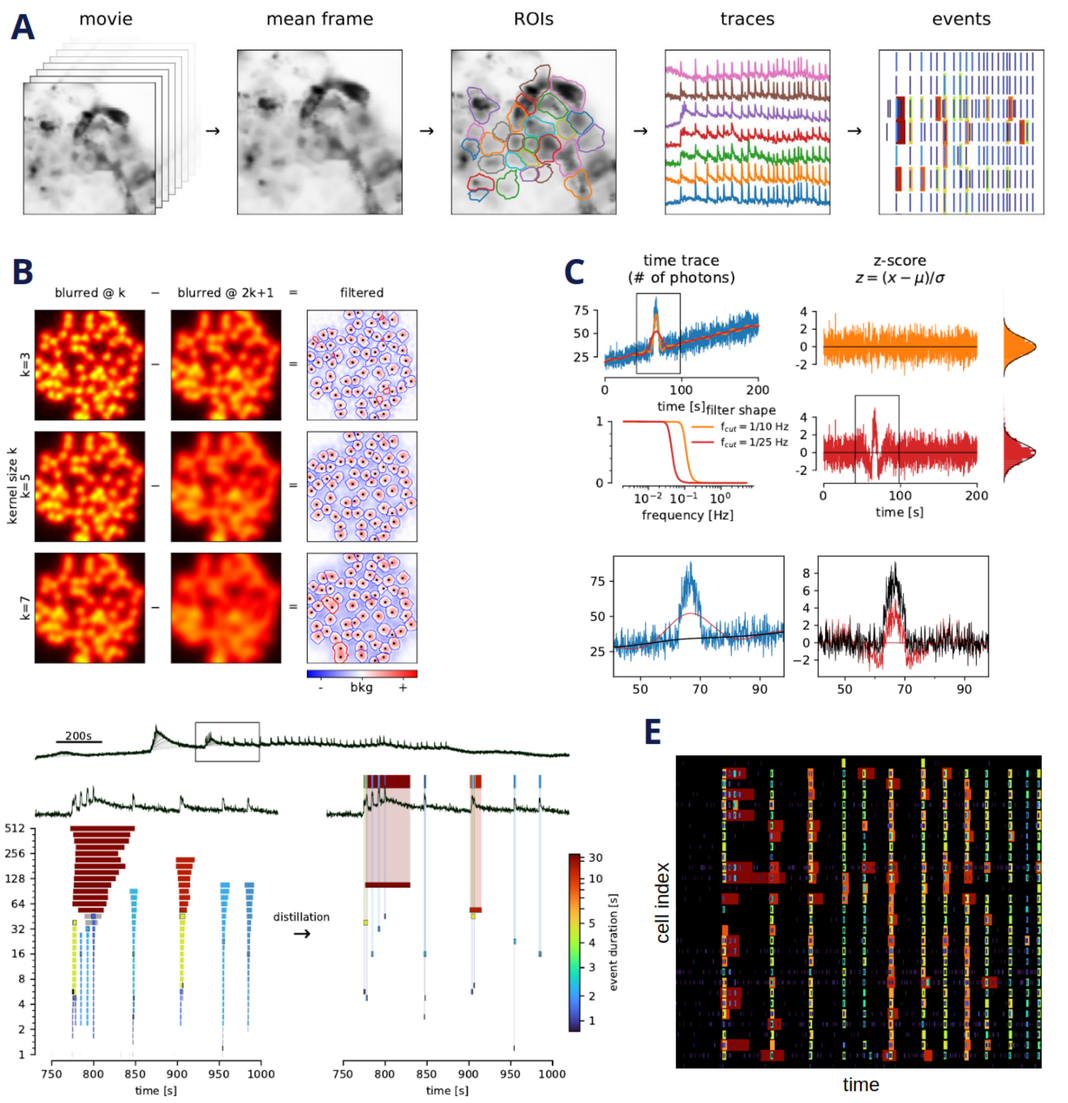

Figure 1: A Schematic of our analysis pipeline. **B** Our segmentation algorithm is based solely on detection of local intensity peaks upon narrow band filtering of the template image. The width of a Gaussian filter needs to correspond to the dimensions of the underlying cells. **C** Detection of a transient event depends on filtering. Transformation to of a signal into *z*-scores enables correction of the typical filtering distortions. **D** For a comprehensive inventory of events at timescales ranging from subsecond to hundreds of seconds, we perform filtering with a hierarchy of log-equidistant frequencies. Each filtering detects some events, which we subsequently cluster and distill to minimize the possibility of double-counting and other artifacts. Finally, a trace is represented by a list of events. **E** Raster representation of all events detected in all cells.

Deployment

We deploy our solution on a jupyterhub server rented from MUW ITSC (128GB RAM, 16 x 2.7GHz Intel Xeon). We use it for storage (currently >20TB), processing, analysis, and visualization. For faster interaction, we developed an array of dedicated dashboards. Further examples on `github.com/szarma/Physio_Ca`

code and examples

Results

Our software led us to deeper insight into physiology of islet cells. We showed, that under physiological conditions [Ca^{2+}] transients appear three timescales: in sub-second, second and tens of seconds time range (Fig 1), with slower events a temporal superposition of the faster ones. We also show that activation of intracellular Ca^{2+} receptors is both sufficient and necessary for glucose-like stimulation, and that a subset of [Ca^{2+}] events could be triggered even in the absence of Ca^{2+} influx across the plasma membrane. In aggregate, our experimental and analytical platform was able to readily address the involvement of intracellular Ca^{2+} receptor in shaping the heterogeneity of [Ca^{2+}] responses in endocrine cells in situ.

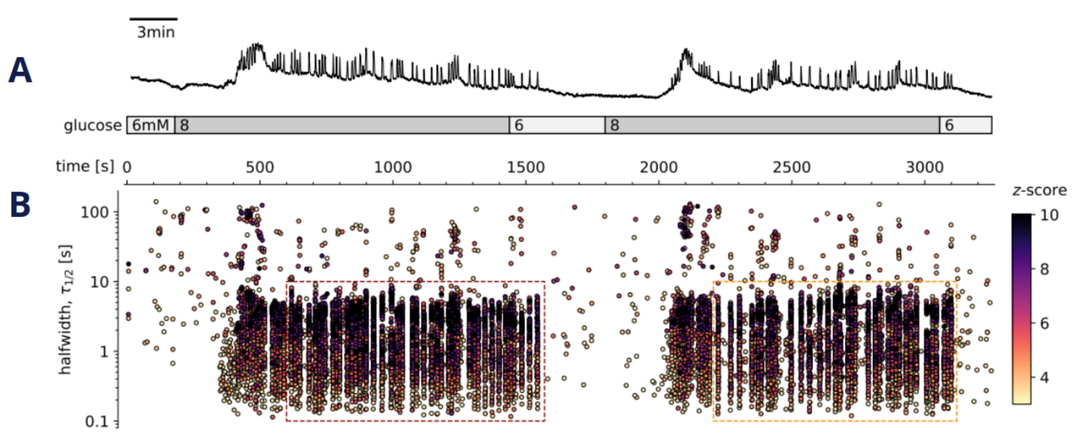

Figure 2: Quantification and analysis of Ca^{2+} dynamics in physiological stimulatory glucose. A Time course of the representative trace (highest correlation with the average trace, c = 0.835) . **B** Events' halfwidths though time. Note the range of halfwidths, the events' synchronicity, and the reproducibility of the phenotype. Color indicates the statistical significance in terms of z-score. **C** In a *z*-score vs halfwidth density plot, the events clearly separate into three groups, which we name ultra-short, short and long. **D** Evidence that the long events are temporal summation of the short ones. Some of the long events are results of phenomena not not necessarily connected to insulin secretion, such as movement, cilia protrusion, metabolism, drift of the objective of the microscope. These effects are less likely to contain substructure of short events, and more likely to appear at the longer timescales, which explain some of the points in the upper left corner.

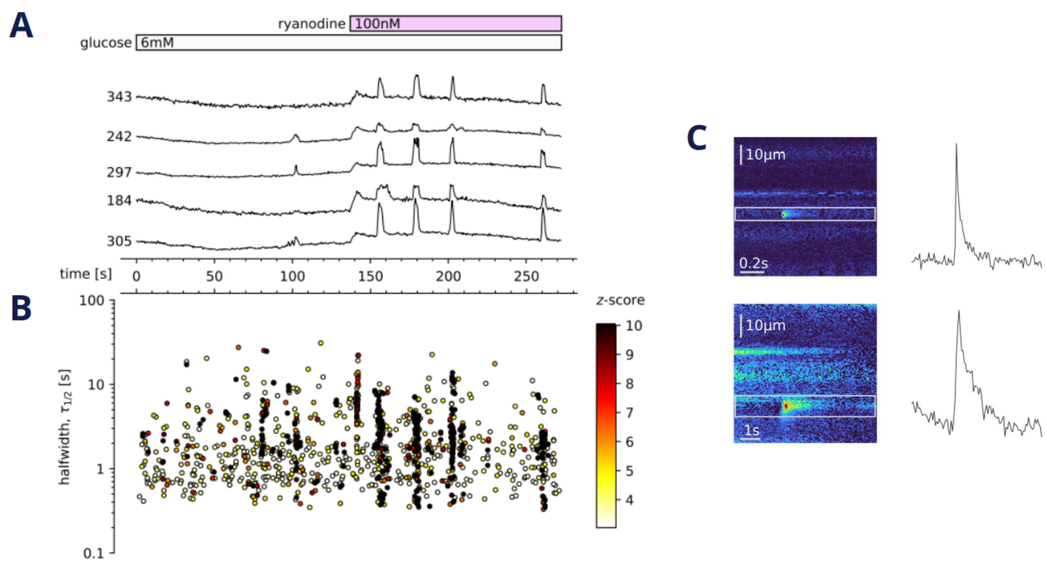

Figure 3: Ryanodine can stimulate islet in substimulatory glucose. A Time course of the several representative traces. **B** Events' halfwidths though time. Ryanodine in low concentrations induces transients qualitatively similar to those observed in glucose. **C** Line scans performed in the same preparation after recordings in A and B. We highlighted events at 100ms range (top) and 1s range (bottom).

video

Preprint

Sandra Postić, Srdjan Sarikas, Johannes Pfabe, Viljem Pohorec, Lidija Križančić Bombek, Nastja Sluga, Maša Skelin Klemen, Jurij Dolenšek, Dean Korošak, Andraž Stožer, Carmella Evans-Molina, James D Johnson, Marjan Slak Rupnik
Intracellular Ca2+ channels initiate physiological glucose signaling in beta cells examined in situ
bioRxiv 2021.04.14.439796; doi: https://doi.org/10.1101/2021.04.14.439796

bioarxiv