

OSSI proposal: Suite2p and Cellpose

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Many biological applications require the segmentation of cell bodies, membranes and nuclei from microscopy images. Computational methods are essential to process these large imaging datasets. We have developed two widely adopted imaging processing pipelines: Suite2p [[paper](#)][[website](#)][[code](#)] and Cellpose [[paper](#)][[website](#)][[code](#)], and are requesting support to maintain and further develop these packages for Janelia and the scientific community.

Software overview

Suite2p is a fast, accurate and complete pipeline written in Python that registers raw movies, detects active cells, extracts their calcium traces and infers their spike times. Suite2p runs on standard workstations, operates faster than real time, and recovers ~2 times more cells than the previous state-of-the-art methods. Its low computational load allows routine detection of ~25,000 cells simultaneously from recordings taken with standard two-photon resonant-scanning microscopes. In addition to its ability to detect cell somas, the detection algorithm can detect axonal segments, boutons, dendrites, and spines. Suite2p has an extensive graphical user interface (GUI) which allows the user to explore their data, and is currently the only fully-functional pythonic GUI for calcium imaging data. Software developers have integrated Suite2p into their packages, such as those for multi-day cell alignment and photostimulation experiments.

Cellpose is a generalist, deep learning-based segmentation algorithm written in Python, which can precisely segment cells from a wide range of image types and does not require model retraining or parameter adjustments. Cellpose can be applied to 2D and 3D imaging data without requiring 3D-labelled data. To support community contributions to the training data, we developed GUI software for manual labeling and for curation of the automated results. We have retrained the model on community-contributed data to ensure the continual improvement of Cellpose. Software developers have integrated Cellpose into their own image processing software, such as CellProfiler, ImagePy, ImJoy, and aPeer. We also developed a Napari plugin for Cellpose ([cellpose-napari](#)).

Significance

Cellpose and Suite2p are widely used in the biology community. Cellpose garners around [4,000 downloads per month](#) and has been cited [192 times](#) since its release last year. Suite2p garners around [2,000 downloads per month](#) and has been cited [256 times](#), 92 times in 2021 alone. Currently, I am the primary developer for Suite2p and Cellpose, and therefore cannot handle all the feature requests, from within Janelia and from the community. Additionally, keeping the software up-to-date with its underlying open-source dependencies requires several hours of work per month or two. Also, adapting the software to the various Janelia workflows often

requires work, and some labs at Janelia are not using Suite2p or Cellpose in part due to the lack of support.

Request

We are requesting support for (1) the maintenance of Suite2p and Cellpose, (2) its integration in processing pipelines for Janelia labs, (3) the development of new features for labs at Janelia that would be made available for the entire community, and (4) the widening of the current documentation. We do not think outreach is an essential component of this project, but we think workshops for the software are useful for the community, and are happy to help with the organization and teaching. If this proposal is accepted, to the parties involved, we will explain the software in depth over a few days, meet once a week as work continues and answer questions via slack continuously as needed.

For examples of new features to develop (3), here are features that have been requested by and/or are in progress in collaboration with Janelia labs:

- 3D cytoplasm segmentation in large volumes from in situ RNA sequencing experiments
- Simultaneous segmentation of nuclei and cytoplasm for improved segmentation for in situ experiments
- Multi-day registration and cell detection from calcium imaging experiments for studying plasticity
- Online cell detection for optogenetic photostimulation experiments
- 3D registration and anatomical cell detection in the fly mushroom body
- Demixing functional activity from dense, overlapping cells in hippocampus and zebrafish
- Anatomically-assisted functional segmentation of neural activity in zebrafish recordings
- Bleed-through correction for multi-channel imaging in fly mushroom body and cortical recordings