

cellfinder: fully automated 3D cell detection and registration of whole-brain images

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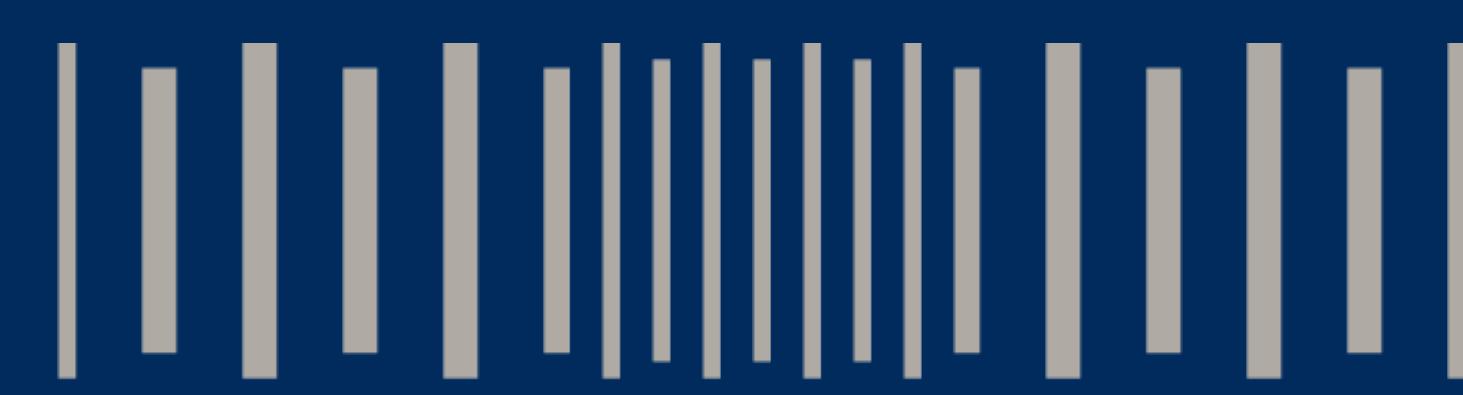
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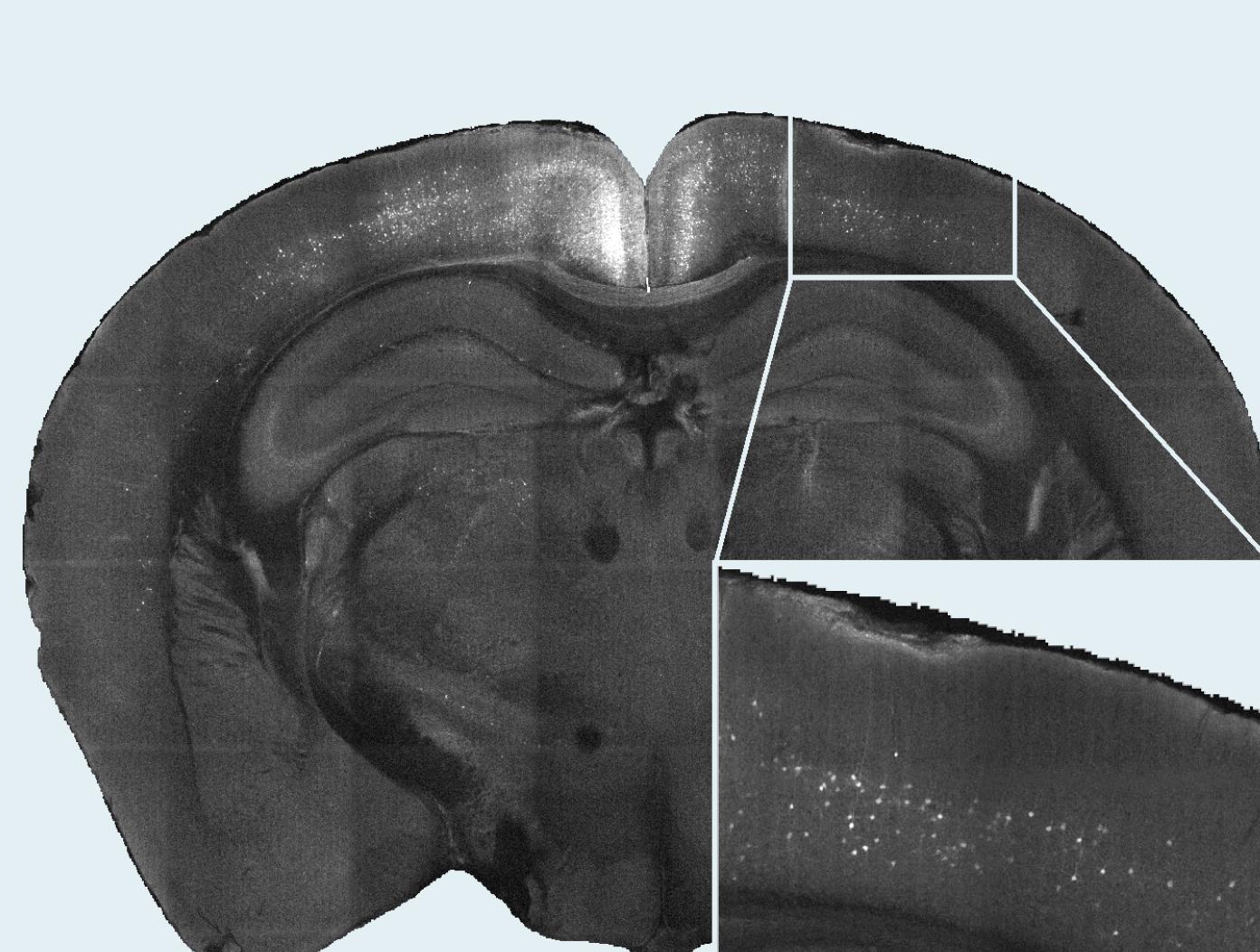


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1. Introduction

Whole-brain microscopy (e.g. serial two-photon & lightsheet) allows neurons to be imaged throughout an entire rodent brain. The scale of the images means that manually mapping these cells is prone to bias and often impractically time consuming.

We present **cellfinder** (github.com/SainsburyWellcomeCentre/cellfinder), an open-source Python software package for fully automated cell detection, segmentation, visualisation and analysis in standard space.

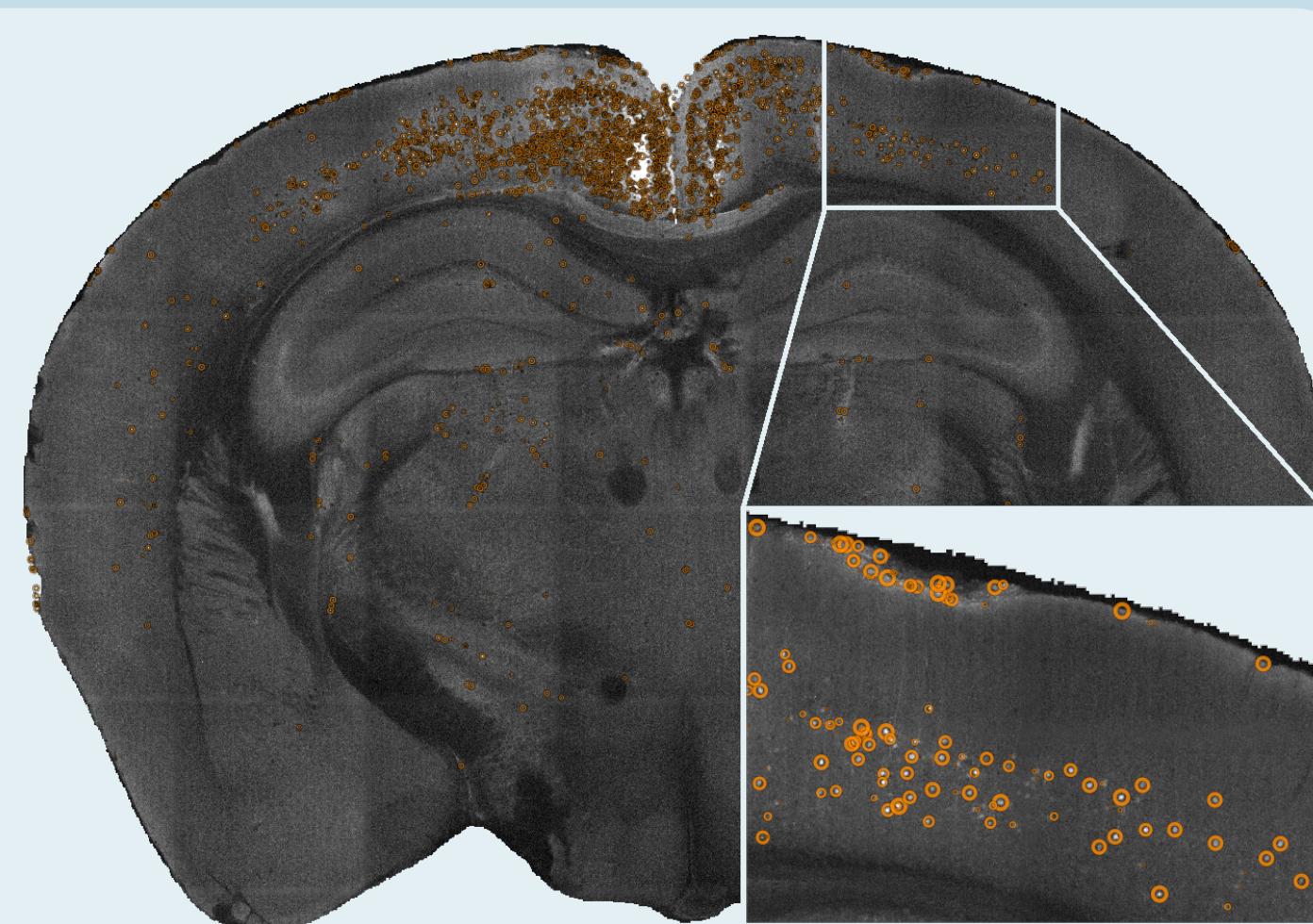


Coronal section from a serial two-photon mouse brain dataset showing labelled cells traced from retrosplenial cortex. Insert shows a region of cortex enlarged for clarity.

2. Cell candidate detection

Initially classical image analysis (using SciPy¹) is used to find cell-like objects:

- 2D - Median filter, Laplacian of Gaussian filter, intensity threshold
- 3D - Spherical filter, merging/splitting

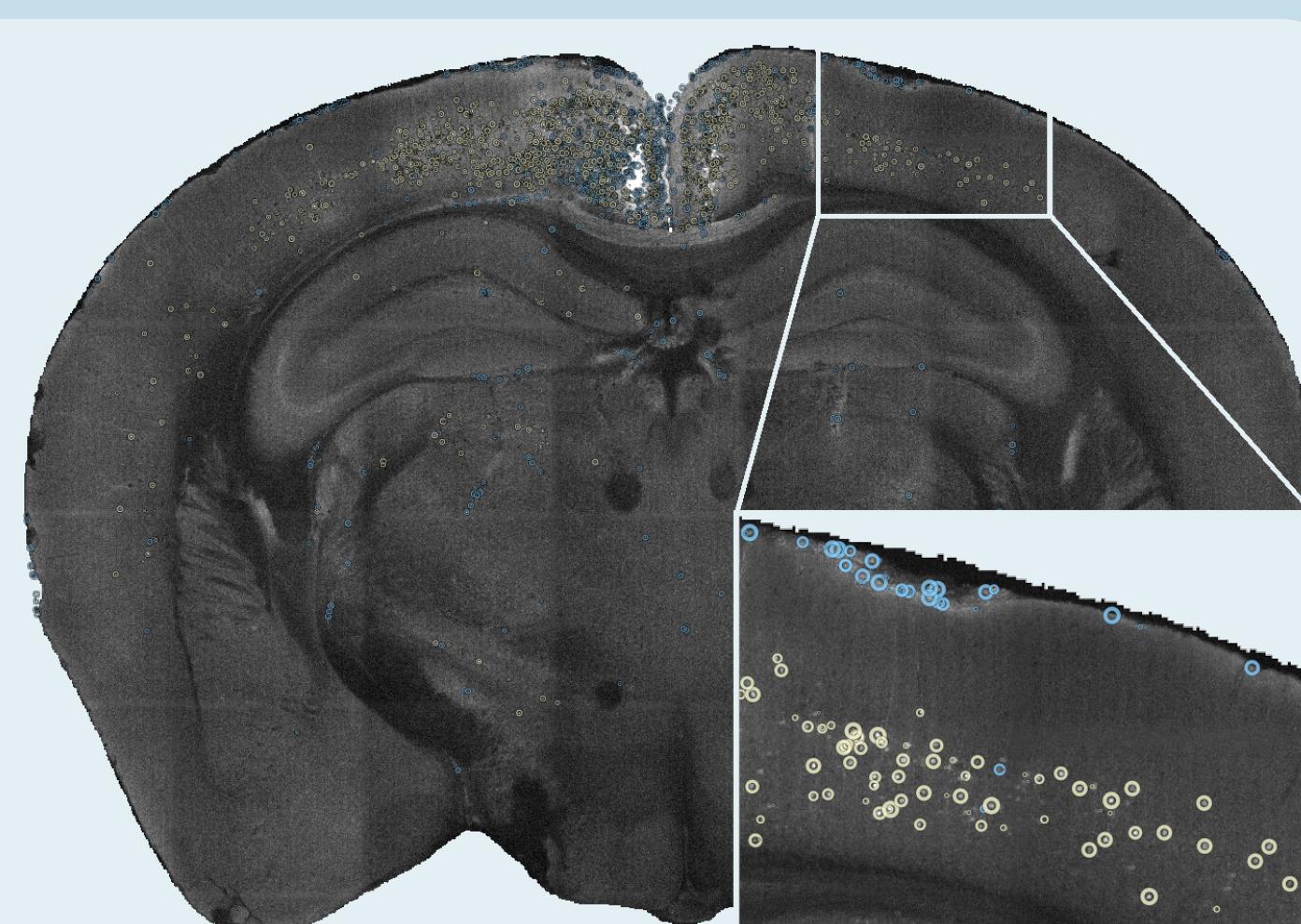


Detected cell-like objects highlighted in orange. Includes cells and cell-like artefacts.

3. Cell candidate classification

A convolutional neural network is then used to remove false positives (implemented in Keras²):

- Extract 50 µm x 50 µm x 100 µm cubes (50 x 50 x 20 voxels) from signal and background channels
- Use a 3D ResNet³ to classify as cell or artefact



Detected cell-like objects classified by the deep learning network into cells (yellow) and artefacts (blue)

4. Registration and segmentation (amap)

For fully automated analysis, we ported the validated amap⁴ pipeline to Python (github.com/SainsburyWellcomeCentre/amap-python⁵).

amap uses NiftyReg⁶ to register a template brain and atlas annotations (e.g. the Allen Reference Atlas⁷, ARA) to the sample, assigning a brain region to detected cells. This transformation can be inverted, allowing detected cells to be transformed to a standard anatomical space.



ARA overlay on sample image showing segmented brain regions

References

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- [2] Chollet, F. et al. Keras. <https://keras.io>, 2015
- [3] He, K. et al. (2015) Deep Residual Learning for Image Recognition. arXiv:1512.03385
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- [5] Tyson, A.L. et al. (2019). amap: automatic atlas propagation. doi:10.5281/zenodo.3582162
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- [7] Lein, E.S. et al. (2007) Genome-wide atlas of gene expression in the adult mouse brain. *Nature*, 445(7124):168–176
- [8] napari contributors (2019). napari: a multi-dimensional image viewer for python. doi:10.5281/zenodo.3555620
- [9] Claudi F. et al. (2020). Brainrender. A python based software for visualisation of neuroanatomical and morphological data. bioRxiv
- [10] connectivity.brain-map.org
- [11] github.com/slicereg/slicereg
- [11] github.com/brainglobe/bg-atlasapi

Installation: pip install cellfinder

Website: cellfinder.info

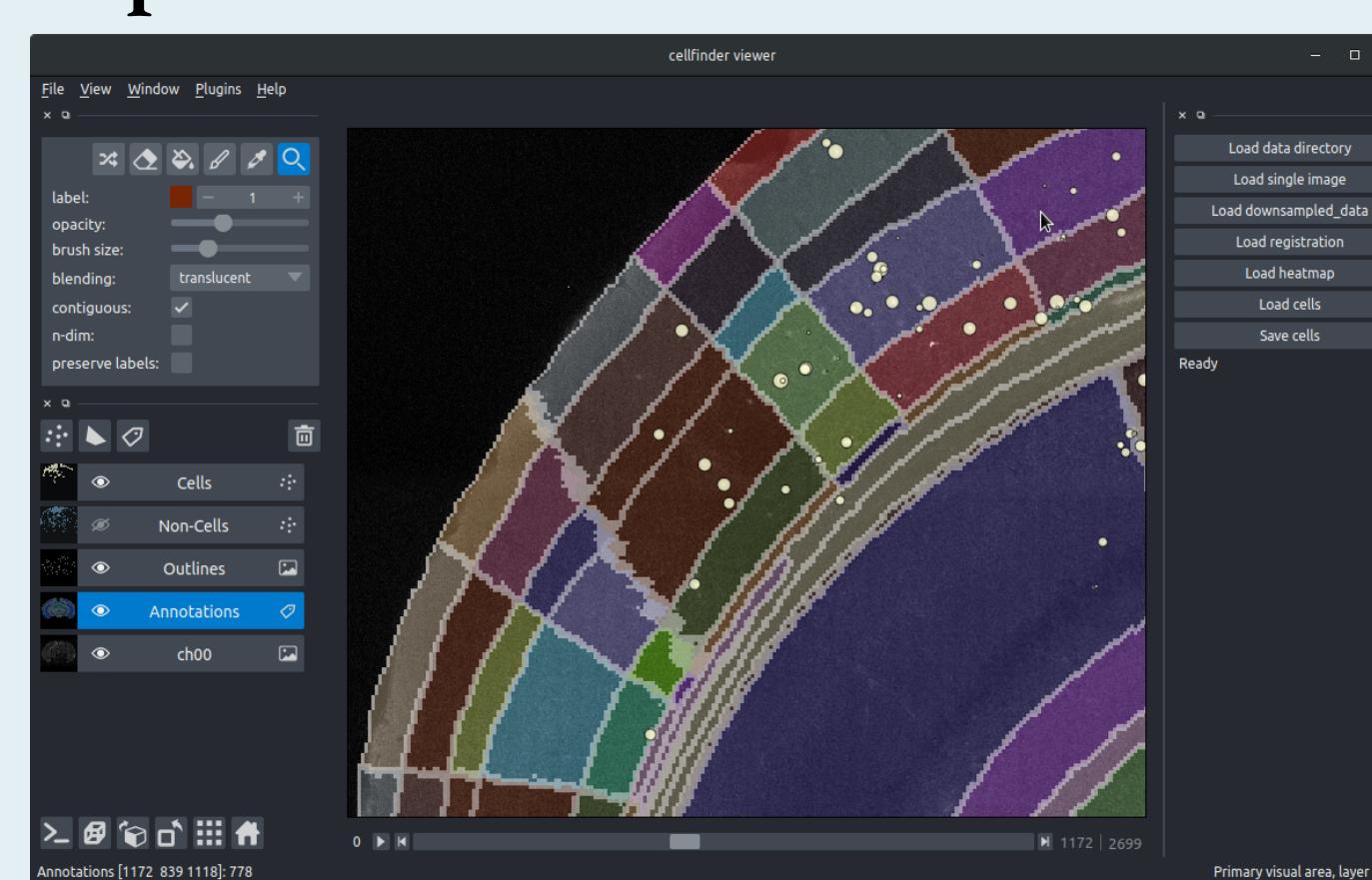
Documentation: docs.cellfinder.info

Repository: bit.ly/cellfinderrepo

5. Visualisation

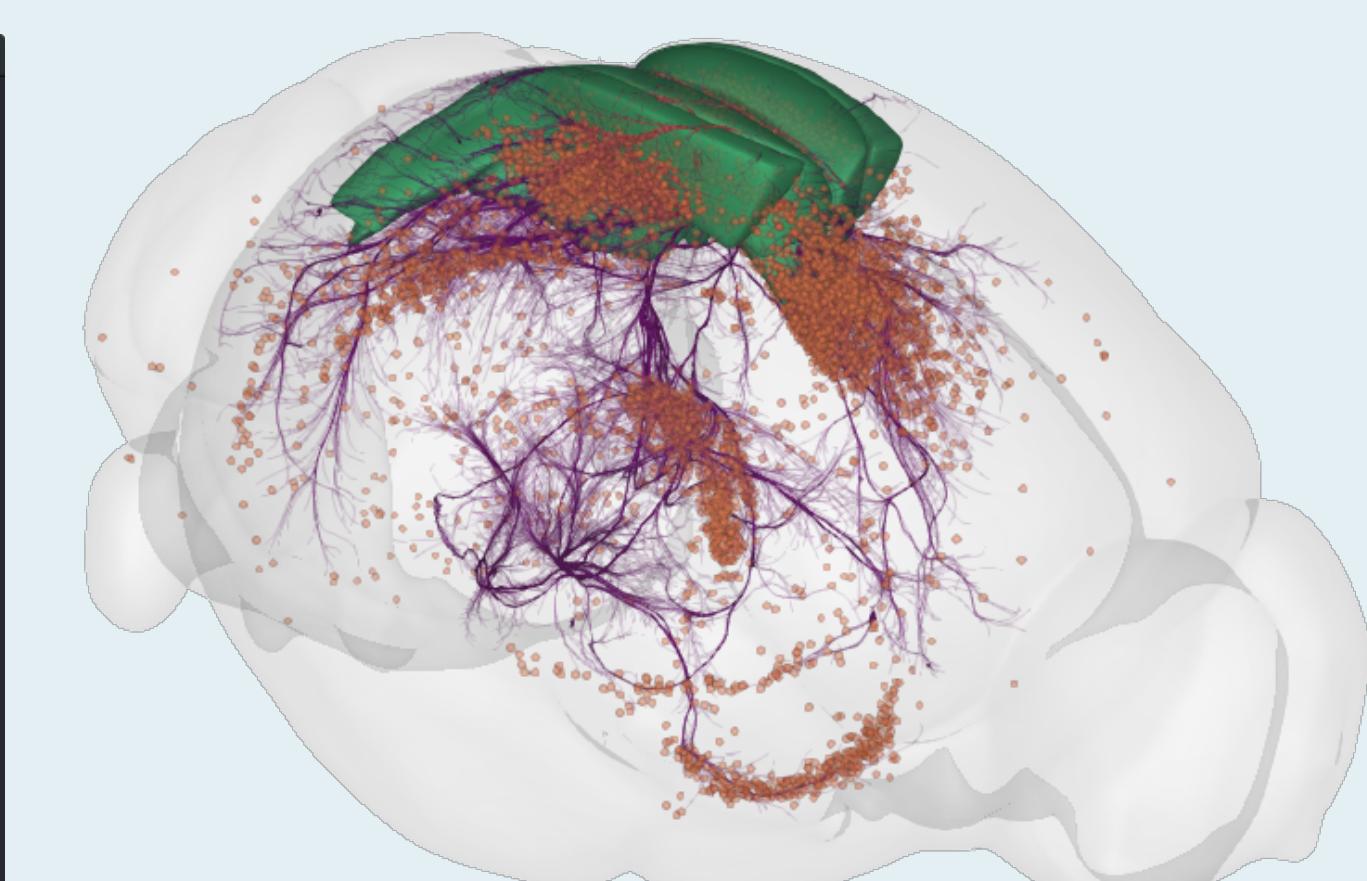
cellfinder uses napari⁸ for exploration of single-subject data and generating training data and brainrender⁹ for exploring multiple-subject data in a common anatomical space along with data from other sources (e.g. mouse connectome project¹⁰).

napari



cellfinder viewer
showing detected cells and registration results

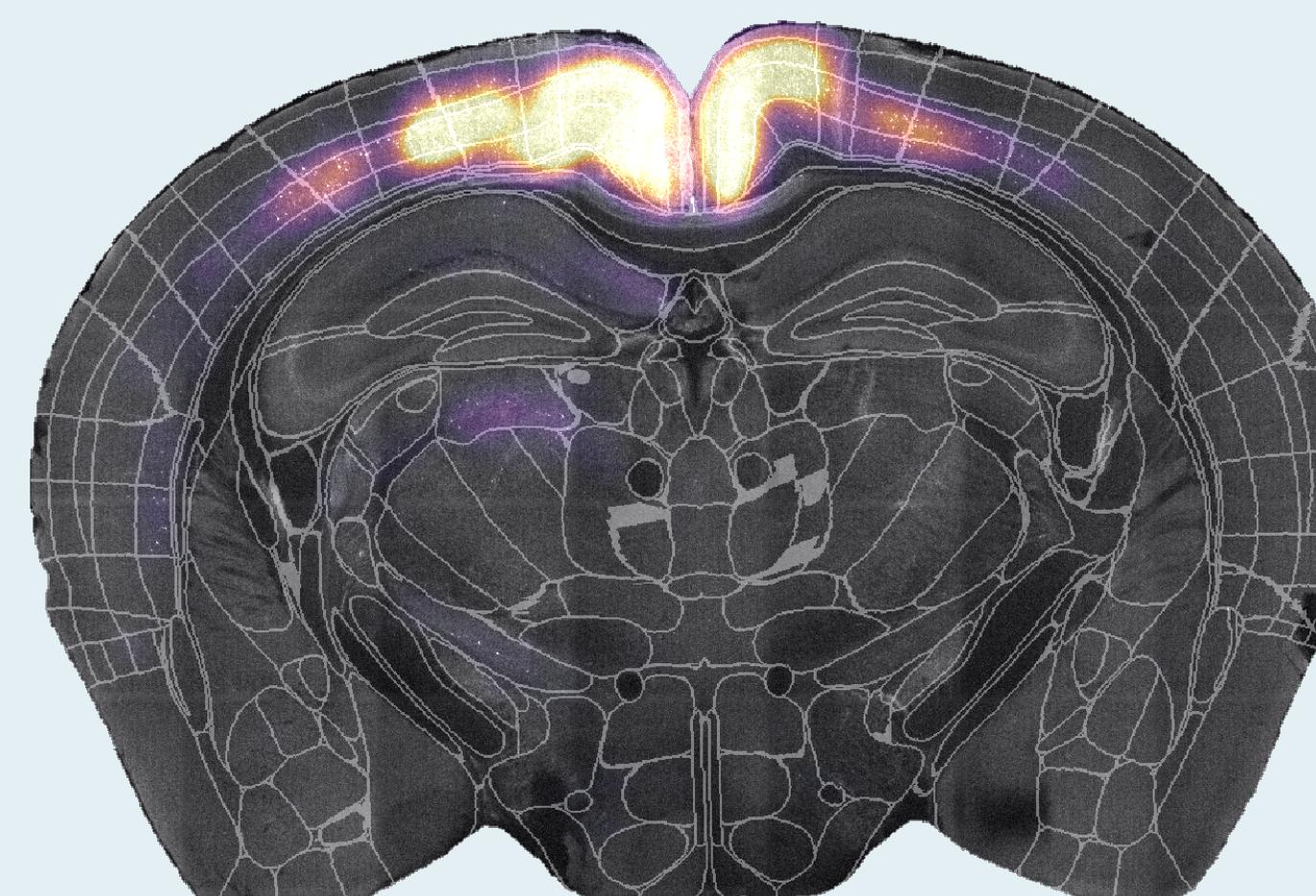
brainrender



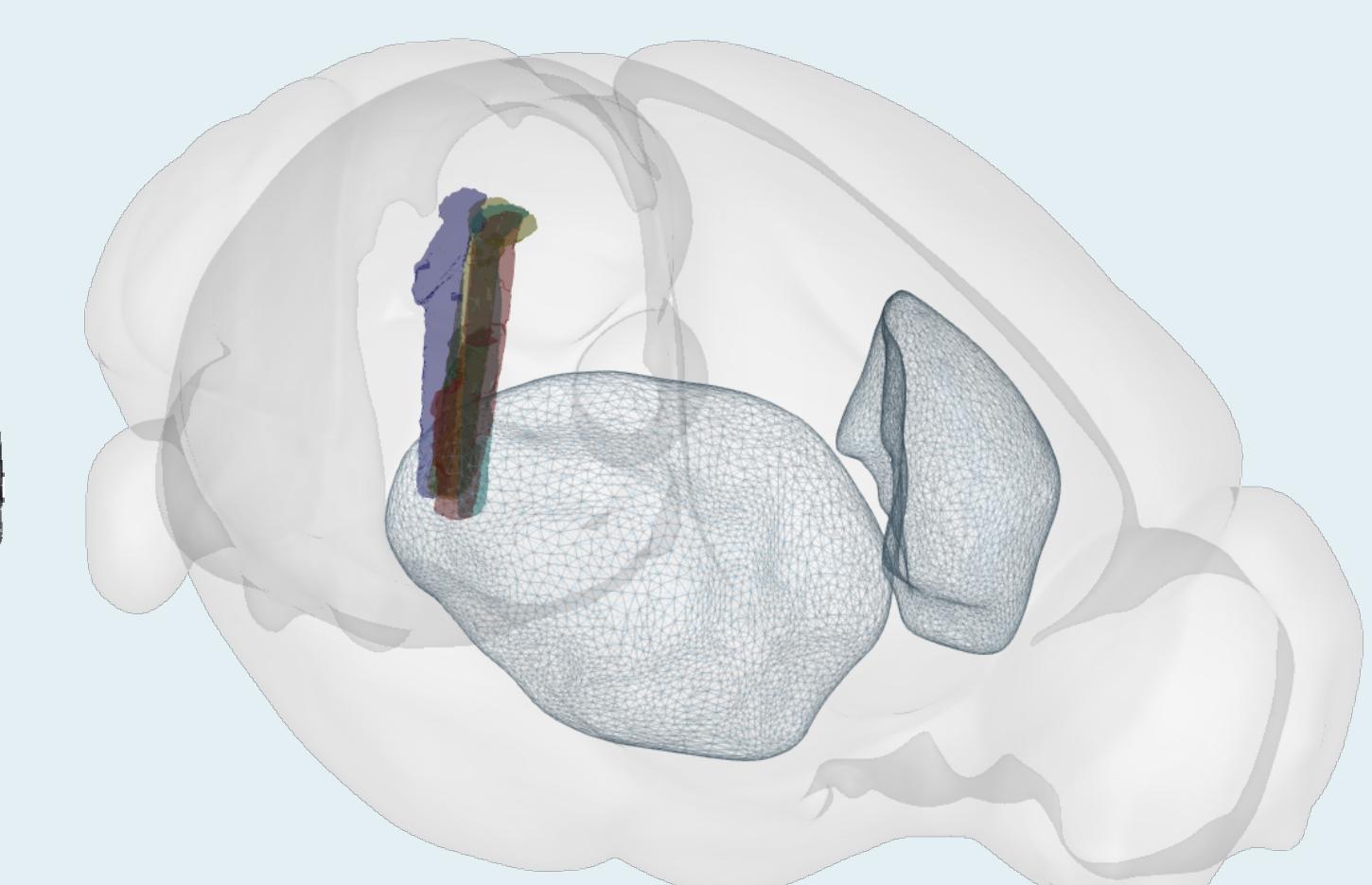
coral - detected cells
green - retrosplenial cortex (RSP)
purple - RSP connectivity

6. Additional tools

Packaged with cellfinder, and under development at github.com/SainsburyWellcomeCentre/neuro, the Python package **neuro** provides further tools.



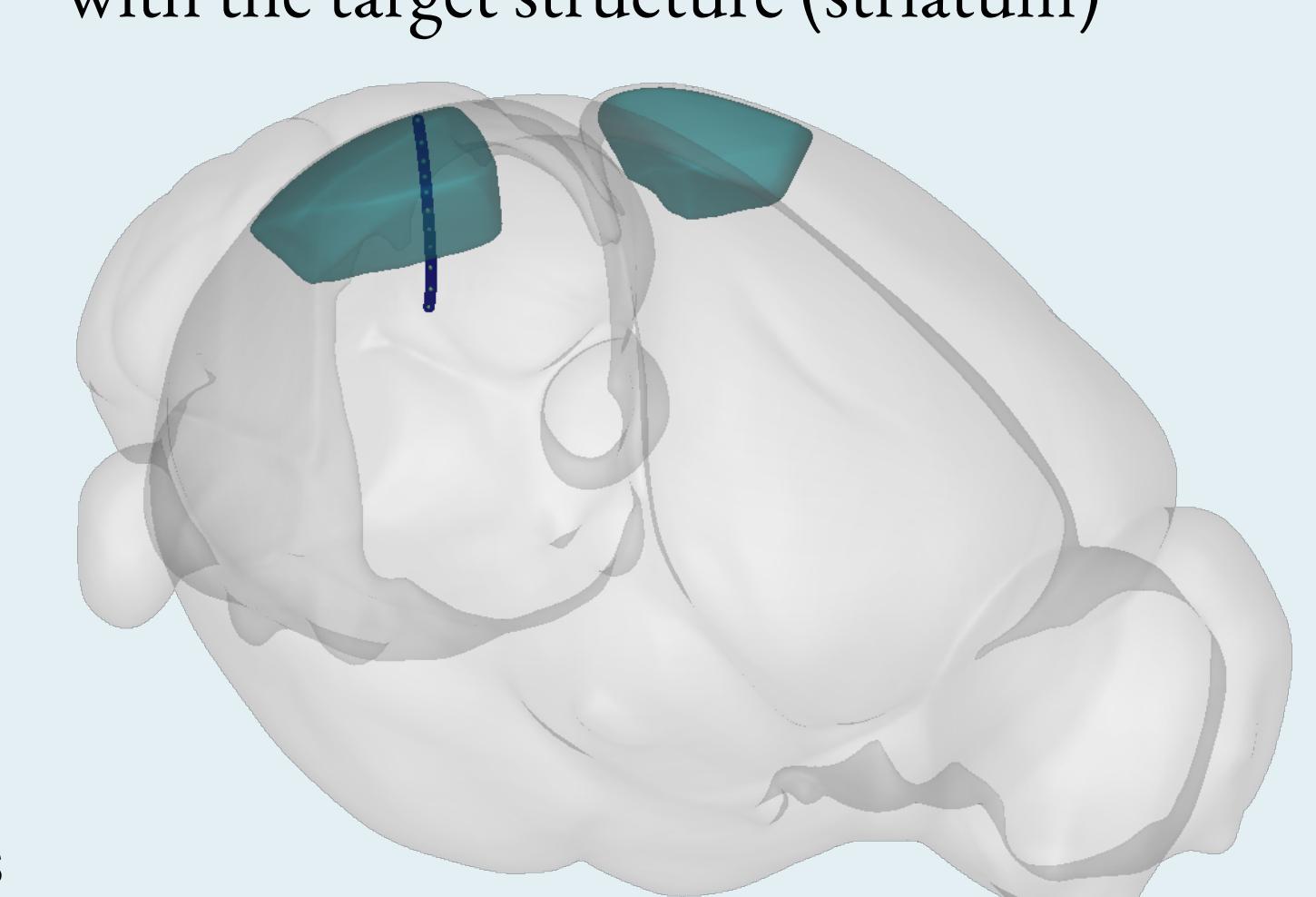
a) Heatmap of cells detected by cellfinder with ARA atlas annotations overlaid



b) Segmented fibre photometry probe lesion sites aligned in ARA space, along with the target structure (striatum)



c) Segmented AAV2r-CRE-eGFP virus injection site in a Flex-tdTomato mouse line visualised within the superior colliculus



d) Traced Neuropixels probe, along with the target structure (primary visual cortex)

7. Summary

- Integrated pipeline for analysis of whole-brain microscopy data
- Efficient 3D cell detection
- Template registration and atlas-based segmentation of brain regions
- Graphical user interface for data exploration
- Installation and use with a single command
- Pre-trained cell classification models and brain atlases provided
- Compatibility with other imaging modalities¹¹, model species and atlases¹² in development

Supported by:

