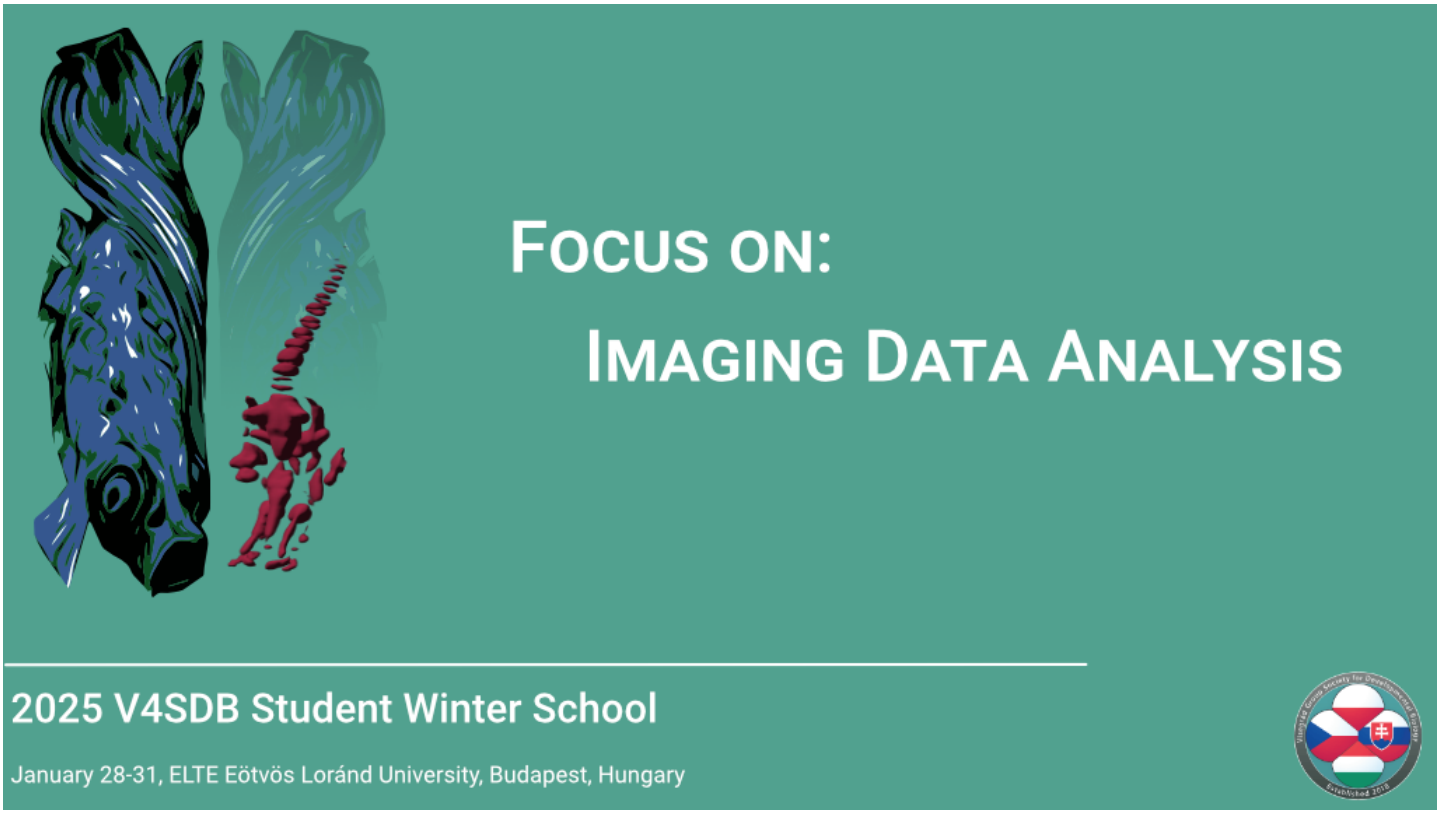
2025 V4SDB Student Winter School
Focus On: Imaging Data Analysis
Materials

Máté Varga (ELTE Eötvös Loránd University, Budapest, Hungary)



Funding

NATIONAL RESEARCH, DEVELOPMENT AND INNOVATION OFFICE
HUNGARY

PROGRAM FINANCED FROM THE NRDI FUND

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EÖTVÖS LORÁND UNIVERSITY

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2025 V4SDB Student Winter School Information

The course focused on the computational analysis of various large imaging datasets. During the course we had five practical sessions, when the participants had the opportunity to gain hands-on experience in the *in silico* processing of large imaging datasets (e.g. volume electron microscopy, calcium imaging, lightsheet microscopy, cell tracking).

As part of the course we also had a **public lecture about the best practices in Open Science by Gáspár Jékely** (see: https://danio-elte.github.io/2025V4SDBStudentWinterSchoolMaterials/open_science.html).

Our target audience were graduate students (both at MSc and PhD level).

Thanks to generous funding by the "Mecenatúra" Program of the Hungarian National Research, Development and Innovation Office (NRDI/NKFIH - MEC_SZ-149306) and the ELTE Excellence Fund this event was generously subsidised with no registration fees.

Lecturers

- Gáspár Jékely - Centre for Organismal Biology, Heidelberg, Germany
- Joanna Pylvänäinen - Åbo Akademi University, Turku, Finland
- Isaac Bianco - University College London, London, UK
- Kenzo Ivanovitch - University College London, London, UK

- Giulia Bertolin -Insitute of Genetics and Developmentof Rennes, Rennes, France
- Adriana Nagy-Dăbâcan - Transylvanian Institute of Neuroscience, Cluj-Napoca, Romania

Organizer:

Máté Varga - ELTE Eötvös Loránd University, Budapest, Hungary

Chapter 1

2025V4SDB Winter School Programme

Tuesday, January 28

Time	Activity
13:00–14:00	Registration
14:00–15:00	**"Open Science in (In)Action: Why, What, and How"** Public Lecture by Gáspár Jékely
15:00–16:00	Coffee Break, Registration
16:00–17:30	Flash Talks by Students
20:00–23:00	Social Program (at The Grund)

Wednesday, January 29

Time	Activity
09:00–11:00	Morning Session I (Gáspár Jékely)
11:00–11:30	Coffee Break
11:30–13:00	Morning Session II (Gáspár Jékely)
13:00–14:30	Lunch (buffet)
14:30–16:30	Afternoon Session I (Joanna Pylvänäinen)
16:30–17:00	Coffee Break
17:00–18:30	Afternoon Session II (Joanna Pylvänäinen)

Thursday, January 30

Time	Activity
09:00–11:00	Morning Session I (Isaac Bianco)
11:00–11:30	Coffee Break
11:30–13:00	Morning Session II (Isaac Bianco)
13:00–14:30	Lunch (buffet)
14:30–16:30	Afternoon Session I (Kenzo Ivanovitch)
16:30–17:00	Coffee Break
17:00–18:30	Afternoon Session II (Kenzo Ivanovitch)

Friday, January 31

Time	Activity
	———
11:00–11:30	Coffee Break
11:30–13:00	Morning Session II (Adriana Nagy-Dăbâcan)
13:00–13:30	Closing remarks, Lunch (take away)

Ĺ Programme notes

- All sessions included breaks to maintain engagementand provide networking opportunities
- A social program was scheduledfor the first evening at The Grund
- Giulia Bertolin's session on Thursday morning had to be cancelled due to vis maior situation

Location

The Winter School took place on the premises of the Faculty of Science of ELTE Eötvös Loránd University (Pázmány Péter stny. 1/A, 1117 Budapest), in room 0.83 ("Eötvös terem").

Chapter 2

Analysis of the *Platynereis dumerilii* connectome using the CATMAID web interface and the API

Instructor:

Gáspár Jékely (Centre for Organismal Studies, Heidelberg University) gaspar.jekely@cos.uni-heidelberg.de

2.1 Workshop Overview

Neuronal connectomics relies on ever increasing datasets of volume electron microscopy (vEM) data that are analysed either manually (skeletonisation) or by AI-assisted segmentation tools, followed by extensive proofreading. The workshop will give an introduction into the web-based collaborative tracing and annotaton platform CATMAID used for connectome analysis. The first part will explore the CATMAID web interface and introduce into the core functions. We will analyse a large dataset from our laboratory containing the whole-body connectome of the larval stage of the marine annelid Platynereis dumerilii, our model organism. In the second part, we will try to repeat some of the analysis by using RStudio and the R package Natverse. This package accesses the same CATMAID webside via the API (application programming interface) and can fetch data from our connectome server. The data can then be analysed in

standard R workflows of network analysis, plotting, statistics, figure assembly and paper writing. I will also give a brief introduction into this open science workflow, as it is used in our lab.

2.2 Software

2.2.1 First Part

CATMAID - The Collaborative Annotation Toolkit for Massive Amounts of Image Data

(Web-based software, no installation needed)

We will use the public EM resources of the Jékely lab and look into the whole-body connectome of the *Platynereis dumerilii* larva.

2.2.2 Second part

Accessing CATMAID via the API from RStudio.

Install **RStudio**:

- An integrated development environment (IDE) to work with R and Python

Install **necessary packages** from RStudio, including Natverse/Catmaid:

- R tools for quantitative neuroanatomy, a package that provides access to the CATMAID API for R users. https://github.com/natverse/rcatmaid

Install the **Tidyverse**:

- a set of packages that work in harmony because they share common data representations and API design

We will use the following GitHub repo: https://github.com/JekelyLab/ELTE_Catmaid_course_25

Here are detailed instructions about what to do.

Repo of the *Platynereis* connectome paper, including the code for all analyses and figures: https://github.com/JekelyLab/Platynereis_3D_connectome_2024

2.3 References

Schneider-Mizell, C. M., Gerhard, S., Longair, M., Kazimiers, T., Li, F., Zwart, M. F., Champion, A., Midgley, F. M., Fetter, R. D., Saalfeld, S., & Cardona, A. (2016). **Quantitative neuroanatomy for connectomics in Drosophila.** eLife, 5, e12059. https://doi.org/10.7554/eLife.12059

Bates, A. S., Manton, J. D., Jagannathan, S. R., Costa, M., Schlegel, P., Rohlfing, T., & Jefferis, G. S. (2020). **The natverse, a versatile toolbox for combining and analysing neuroanatomical data.** eLife, 9, e53350. https://doi.org/10.7554/eLife.53350

Verasztó, C., Ueda, N., Bezares-Calderón, L. A., Panzera, A., Williams, E. A., Shahidi, R., & Jékely, G. (2017). **Ciliomotor circuitry underlying whole-body coordination of ciliary activity in the Platynereis larva.** eLife, 6, e26000. https://doi.org/10.7554/eLife.26000

CHAPTER 2. ANALYSIS OF THE *PLATYNEREIS* CONNECTOME USING CATMAID

Chapter 3

Object Tracking and Track Analysis using TrackMate and CellTracksColab

Instructor:

Joanna Pylvänäinen (Åbo Akademi University)
joanna.pylvanainen@abo.fi

3.1 Workshop Overview

In life sciences, tracking objects within movies is crucial for quantifying the behaviour of particles, organelles, bacteria, cells, and entire organisms. However, tracking multiple objects across numerous movies and analysing the objects' movements can be challenging. This workshop aims to demonstrate the effective utilization of **TrackMate** for object tracking across multiple movies through hands-on exercises. Additionally, participants will learn how to compile, analyse, and explore the acquired tracking data using the **CellTracksColab** platform. Both tools offer user-friendly interfaces tailored to life scientists without coding experience.

By the end of this workshop, participants will be familiar with TrackMate's tracking capabilities, will have analyzed tracking data using CellTracksColab, and will know how to export and visualize their results for further research.

Workshop slides available on the webpage: https://danio-elte.github.io/2025V4SDBStudentWinterSchoolMaterials/cell_tracking.html

3.2 Software:

TrackMate: - An open-source FIJI/ImageJ plugin for tracking cells/particles in 2D microscopy images.

CellTracksColab: - A cloud-based platform for analyzing tracking data.

3.3 Hands-on with TrackMate and CellTracksColab

3.3.1 If you want to test the tools during the workshop please do these before the workshop:

1. Download these datasets:

Link to Zenodo

- 0_Tracking_settings.zip
- 1_TrackMate_batcher_input.zip
- 2_CellTracksColab_input.zip

2. Download and prepare Fiji

- Download Fiji
- if new to Fiji here are some training materials on Zenodo with step-by-step exercises
- Open Fiji and activate **TrackMate Helper** update site. Restart Fiji.

3. You need to have access to GoogleDrive (personal or create one for the purpose of this course)

- Unzip the 2_CellTracksColab_input.zip and upload it to your GoogleDrive
- Create a new folder on you GoogleDrive called Results

3.4 Workshop step-by-step:

1. Cell Tracking with TrackMate

- Open Fiji and open the image_for_tracking_settings image from the 0_Tracking_settings folder.
- Define tracking parameters in the **TrackMate interface**.
- **Save traking settings** as XML for batch processing.
- Open the TrackMate batcher to **Batch process** all images from the 1_TrackMate_batcher_input folder.

- **Export tracking data** (spot tables, track tables, movies).

2. **Analyze TrackMate output in CellTracksColab.**

- Upload your tracking results to your Google Drive. Make sure that the folders are correctly organized.
- You can also use the pre-made CellTracksColab input from the 2_CellTracksColab_input folder
- Create a new folder for results
- Open The CellTracksColab TrackMate notebook and make a copy to your drive.
- Run all cells to visualize tracks and to generate plots.
- Bonus: Utilize advanced dimensionality reduction techniques to understand your data using Dimensionality Reduction notebook

Check out the video from I2K: https://www.youtube.com/embed/fIE4i3G7L9Y

3.5 References

TrackMate Ershov, D., Phan, M. S., Pylvänäinen, J. W., Rigaud, S. U., Le Blanc, L., Charles-Orszag, A., Conway, J. R. W., Laine, R. F., Roy, N. H., Bonazzi, D., Duménil, G., Jacquemet, G., & Tinevez, J. Y. (2022). **TrackMate 7: integrating state-of-the-artsegmentation algorithms into tracking pipelines.** Nature Methods, 19(7), 829–832. https://doi.org/10.1038/s41592-022-01507-1

CellTracksColab Gómez-de-Mariscal, E., Grobe, H., Pylvänäinen, J. W., Xénard, L., Henriques, R., Tinevez, J. Y., & Jacquemet, G. (2024). **CellTracksColab is a platform that enables compilation, analysis, and exploration of cell tracking data.** PLOS Biology, 22(8), e3002740. https://doi.org/10.1371/journal.pbio.3002740

T-Cell Dataset Guillaume Jacquemet. (2023). **T cell dataset for CellTracksColab -2** [Data set]. Zenodo. https://doi.org/10.5281/zenodo.8420011

Chapter 4

Analysis of Calcium Imaging Data

Instructor:

Isaac Bianco (University College London)
i.bianco@ucl.ac.uk

4.1 Workshop Overview

In vivo imaging of calcium dynamics can shed light on a broad range of biological processes from cell migration and tissue morphogenesis to multiple aspects of neuronal activity. In neuroscience, a combination of modern genetically encoded calcium sensors alongside advances in imaging technology are enabling the activity of tens of thousands of neurons to be monitored in behaving animals. In parallel, advances in data processing and analysis are enabling researchers to make sense of vast population activity datasets.

In this workshop, we will:

1) introduce the key principles of calcium imaging;
2) learn how to process raw calcium imaging data and extract estimates of neuronal activity;
3) examine ways to explore large population activity datasets and relate neural dynamics to sensory stimuli, task variables, and animal behaviour.

Workshop slides will be available here

4.2 Software

Fiji: – An open–source image processing package.

Suite2p

- follow the instructions under Local Installation
- be sure to install the GUI version: `python -m pip install suite2p[gui]`
- on the Mac you may need to use: `python -m pip install 'suite2p[gui]'`

Rastermap

- follow the instructions under Local Installation
- again, be sure to install the GUI version
- it should be fine to install it in the same suite2p environment as used above

4.3 Datasets

1. a **small calcium recording** that we will look at in FIJI link (You will need to click on the *Download raw file* button to download the dataset.)

2. a **large calcium imaging movie** for Suite2p testing

    - download any one of the TIFF stacks in this folder link

3. a **zebrafish calcium imaging movie** for Suite2p testing link

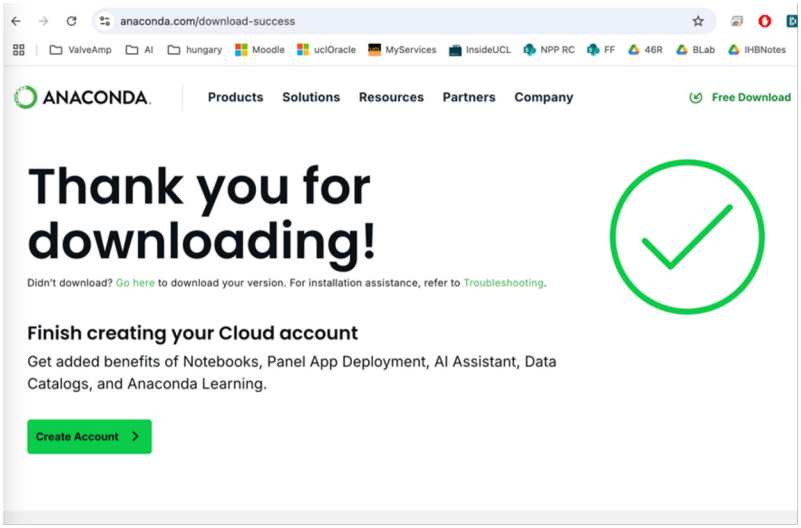
4. a **zebrafish activity dataset** for Rastermap testing link

    - plus some extra files to help interpret the fish dataset link

4.4 Install the Python environment

1. **Install** an Anaconda distribution of **Python**

2. Using either the command line or the Anaconda Navigator, **create a new environment**
    a. Command line: `conda create --name suite2p python=3.9` **or**
    b. Anaconda Navigator, see pic below


anaconda.com/download-success
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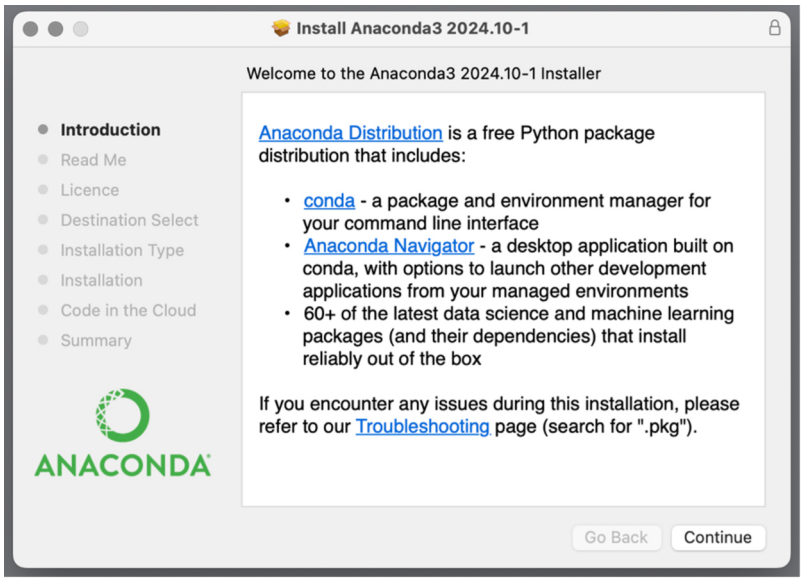
Thank you for downloading!
Didn't download? Go here to download your version. For installation assistance, refer to Troubleshooting.
Finish creating your Cloud account
Get added benefits of Notebooks, Panel App Deployment, AI Assistant, Data Catalogs, and Anaconda Learning.
Create Account


Install Anaconda3 2024.10-1
Welcome to the Anaconda3 2024.10-1 Installer
Introduction
Read Me
Licence
Destination Select
Installation Type
Installation
Code in the Cloud
Summary
ANACONDA
Anaconda Distribution is a free Python package distribution that includes:
conda - a package and environment manager for your command line interface
Anaconda Navigator - a desktop application built on conda, with options to launch other development applications from your managed environments
60+ of the latest data science and machine learning packages (and their dependencies) that install reliably out of the box
If you encounter any issues during this installation, please refer to our Troubleshooting page (search for ".pkg").
Go Back
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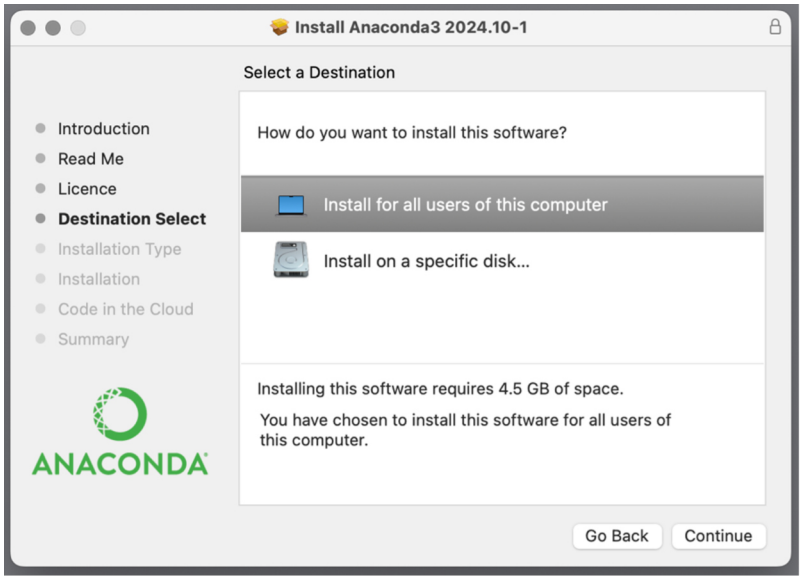
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Introduction
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Summary
ANACONDA
How do you want to install this software?
Install for all users of this computer
Install on a specific disk...
Installing this software requires 4.5 GB of space.
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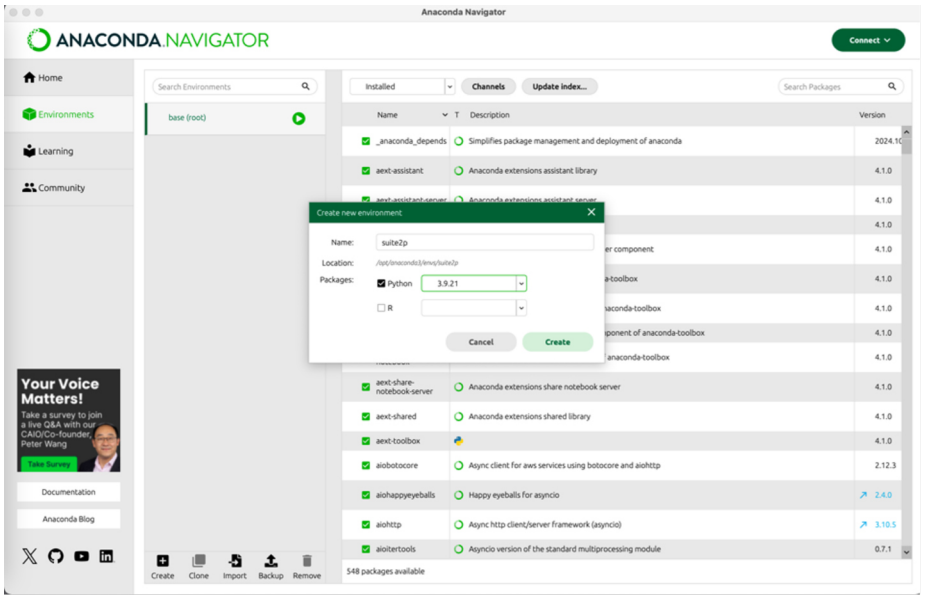
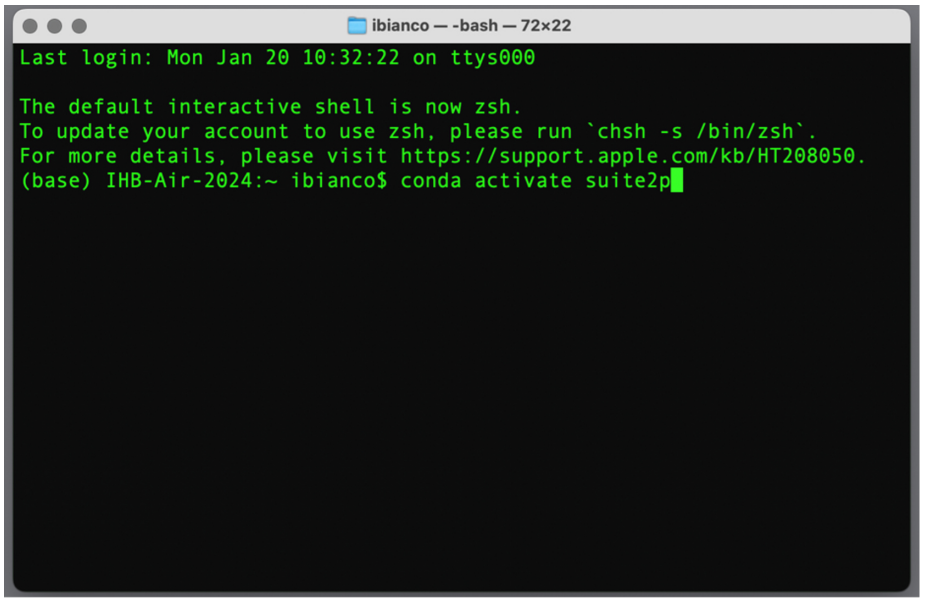
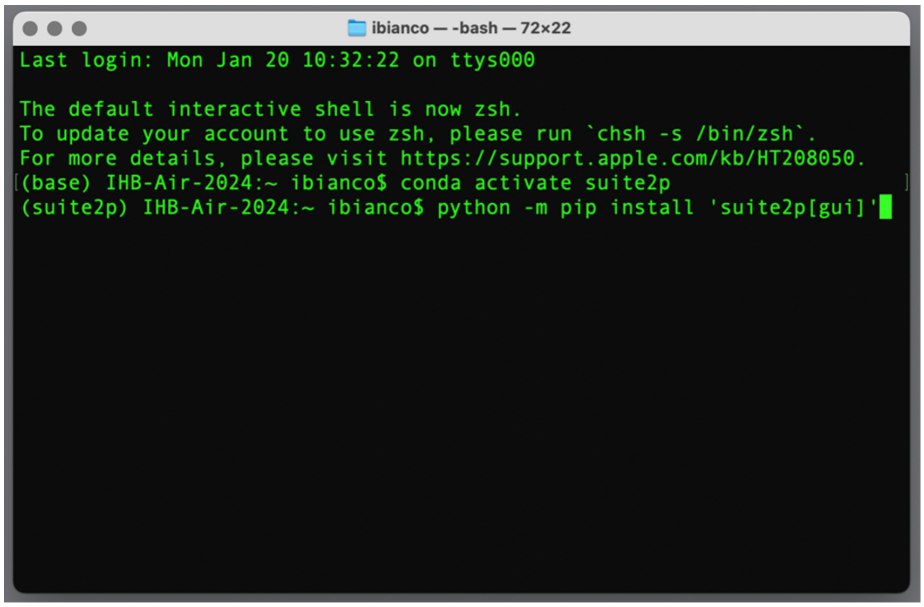


3. Using the command line (open "Terminal" on the Mac), **activate the suite2p** environment: `conda activate suite2p`

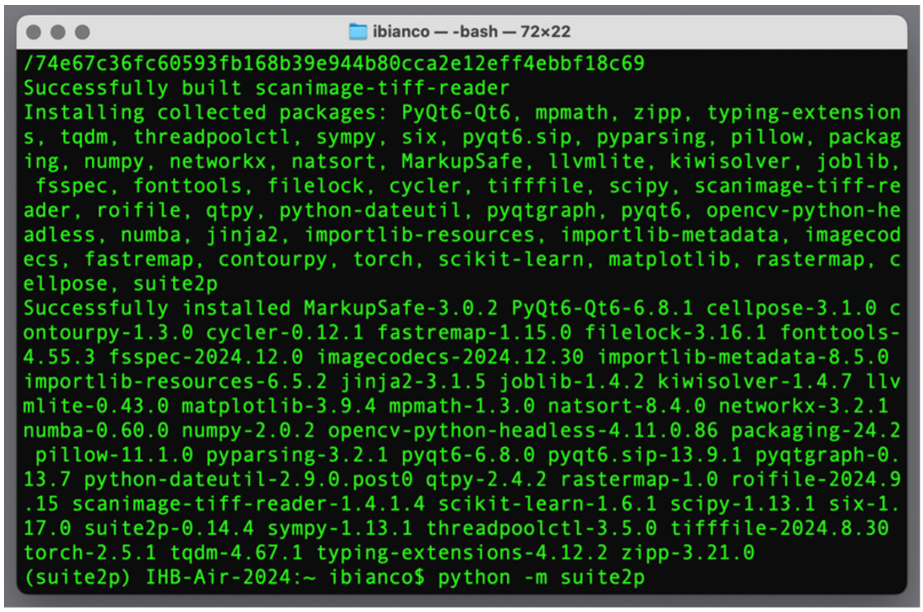


4. Now **install suite2p** by typing: `python -m pip install suite2p[gui]`

   or on the Mac you might need: `python -m pip install 'suite2p[gui]'` (notice the ')



5. Once the download is finished, **launch suite2p**: `python -m suite2p`



All done!

4.5 References

Suite2p Pachitariu M, Stringer C, Dipoppa M, et al. (2017)**Suite2p: beyond 10,000 neurons with standard two-photon microscopy.** bioRxiv https://doi.org/10.1101/061507

Suite2p documentation: https://suite2p.readthedocs.io/en/latest/

OASIS Friedrich, J., Zhou, P., & Paninski, L. (2017). **Fast online deconvolution of calcium imaging data.** PLOS Comp Bio, 13(3), e1005423. https://doi.org/10.1371/journal.pcbi.1005423

Rastermap Stringer, C., Zhong, L., Syeda, A., Du, F., Kesa, M., & Pachitariu, M. (2025). **Rastermap: a discovery method for neural population recordings.** Nat Neurosci, 28(1), 201–212. https://doi.org/10.1038/s41593-024-01783-4

Chapter 5

Introduction to Bonsai

Instructor:

Adriana Nagy-Dăbâcan (Transylvanian Institute of Neuroscience) dabacan@tins.ro

See Github link here.

5.1 Workshop Overview

During the session, we will present Bonsai as a reactive programming tool usefull when designing and analyzing behavioral experiments. We will go through the potential applications for Bonsai and its most relevant usecases. Then, we will get familiar with this tool by implementing a simple workflow combining animal tracking with closed loop control. We will showcase basic programming principles and will open the door for addressing more complex needs in experimental design.

By the end of the workshop, participants will be familiar with Bonsai as a tool for behavioral experimental control and will understand its basic principles of operation. They will have a good source of documentation and will be able to join the community for further support.

Workshop slides are available on the webpage: https://danio-elte.github.io/2025V4SDBStudentWinterSchoolMaterials/bonsai.html

5.2 Software

Bonsai:

- An open-sourcevisual reactive programming software for interactive systems.
- built to work with .NET frameworkin Windows 7 or later.
- for Linux installation, see pointers here.

5.3 Prepare the field

- Download and install Bonsai.
- Install the Starter Pack from the Package Manager.
- Check out the Bonsai Gallery and make sure the example workflows work on your station.
- Download the video file for this workshop.

5.4 Workshop step-by-step

1. Open and visualize video file.
2. Detect animal using simple image processing functions.
3. Compute centroid coordinates in arena.
4. Implement simple conditionals using Logical operators.
5. Integrate python scripting for more complex logics.
6. Trigger some digital line/speaker when animal leaves predetermined home area.

5.5 Bonsai Resources

- Documentation
- Github Forum
- Public Discord Server
- Learning Resources

5.6 References

Lopes, G., Bonacchi, N., Frazão, J., Neto, J. P., Atallah, B. V., Soares, S., ...& Kampff, A. R. (2015). **Bonsai: an event-basedframework for processing and controlling data streams.** *Frontiers in Neuroinformatics**, 9, 7. https://doi.org/10.3389/fninf.2015.00007

Chapter 6

Introduction to live analysis of gastrulation and heart development in mouse

Instructor:

Kenzo Ivanovitch (University College London)
k.ivanovitch@ucl.ac.uk

6.1 Workshop Overview

Investigating the collective behaviors and cell fate decisionsthat drive tissue and organ development requires live-imaging technologies capable of bridging molecular and organ scales, while also supporting long-term imaging over periods ranging from tens of hours to days. In this tutorial, I will present our research on heart development and demonstrate how light sheet microscopy, combined with advanced image analysis, can help answer fundamental questions in developmental biology.

Workshop slides will be available here

6.2 Software

Fiji:

- An open-sourceimage processing package.

Bigsticher

- The BigStitcher is a software package that allows simple and efficient alignment of multi-tile and multi-angle image datasets, for example acquired by lightsheet, widefield or confocal microscopes.

MaMuT

- A Fiji plugin for the annotation of massive, multi-view data.

> Ď Tip
>
> Within Fiji: Help › Update..., click *Manage update sites* and select BigStitcher and MaMut in the list. After applying the changes and restarting Fiji, BigStitcher and MaMut will be available under Plugins › BigStitcher › BigStitcher and Plugins › MaMut

RStudio:

- An integrated development environment (IDE) to work with R and Python https://posit.co/downloads/

Anaconda

6.3 Data

This is the data directory.

6.4 Workshop step by step:

6.4.1 1. Movie registration

We will be using BigStitcher to register the embryo in both space and time. This step is crucial, particularly if your goal is to track cells and analyse, for example, their migratory behavior. Without this registration, your results could be confounded by the movement of the embryo during acquisition.

6.4.2 2. Generation of Lineage tree

You will manually track cells using MaMut to generate lineage trees. This process is essential for addressing when cells become restricted in their lineage potential during development.

6.4.3 3. Representation of the Trajectories in 3D

You will reconstruct the 3D trajectories of cells, enabling you to assess how migratory behavior relates to cell fate specification.

6.5 References

Bigstitcher Hörl, D., Rojas Rusak, F., Preusser, F., Tillberg, P., Randel, N., Chhetri, R. K., Cardona, A., Keller, P. J., Harz, H., Leonhardt, H., Treier, M., & Preibisch, S. (2019). **BigStitcher: reconstructing high-resolution image datasets of cleared and expanded samples.** Nature Methods 16(9), 870–874. https://doi.org/10.1038/s41592-019-0501-0

MaMuT Wolff, C., Tinevez, J. Y., Pietzsch, T., Stamataki, E., Harich, B., Guignard, L., Preibisch, S., Shorte, S., Keller, P. J., Tomancak,P., & Pavlopoulos, A. (2018). **Multi-view light-sheet imaging and tracking with the MaMuT software reveals the cell lineage of a direct developing arthropod limb.** eLife, 7, e34410. https://doi.org/10.7554/eLife.34410

Shayma Abukar, Peter A. Embacher, Alessandro Ciccarelli, Sunita Varsani-Brown, Isabel G.W. North, Jamie A. Dean, James Briscoe, Kenzo Ivanovitch (2023) **Live-imaging reveals Coordinated Cell Migration and Cardiac Fate Determination during Mammalian Gastrulation.** bioRxiv 2023.12.19.572445. https://doi.org/10.1101/2023.12.19.572445