

User Guide

Barley 4D Spatial Transcriptomics Pipeline

This pipeline processes Visium SpaceRanger output to generate clustering as well as transcript abundance plots. It requires the following files from SpaceRanger

- *.h5 file
- filtered_feature_bc_matrix/features.tsv
- spatial (a directory)
 - scalefactors_json.json
 - tissue_lowres_image.png
 - tissue_positions_list.csv

Download the pipeline

To access the pipeline, download it from this [repository](#)

After downloading, you will find the following directories:

- **pipeline:** Contains the R scripts and notebook that form the pipeline.
- **container:** Includes the Dockerfile, script to run the notebook's container (runJupyterDocker.sh), and R package version file (renv.lock).
- **utils:** Contains the necessary scripts to ensure the visualization application displays all required plots.

Install Docker

To run the pipeline, you'll need Docker. You can either install [Docker Desktop](#) or just the [Docker CLI](#).

Docker Desktop includes both the CLI and a graphical user interface, while the CLI version provides only the command-line tools.

Once installed, you'll be able to build and run the container for the pipeline.

Build the Docker container

Before using the Docker container, you need to build it. Follow the steps below:

```
# Navigate to the directory containing the Dockerfile
cd JupyterNotebook/container

# Build the Docker container
sudo docker build . -t analysis-pipeline:1.1.0
```

This will build the container based on Dockerfile and tag it as analysis-pipeline:1.1.0.

How to run the pipeline:

1. Navigate to the JupyterNotebook/container directory
2. Edit the runJupyterDocker.sh script by updating the following variables with the absolute path:
 - DATA_DIR: Directory containing your input data
 - PIPELINE_DIR: Directory where the pipeline is stored
 - UTILS_DIR: Directory containing the necessary scripts for the visualization application
3. Run the Docker container: `sudo bash runJupyterDocker.sh`
4. If you're running the pipeline on a remote Virtual Machine, set up SSH tunneling:

```
# Run the following command to forward the required port:
bash
ssh -L 888:localhost:8888 user@remote_server.com
```

5. Access the Jupyter Notebook: Copy the URL that appears when the container starts and paste it into your browser.

```
[I 2024-11-13 17:11:17.011 ServerApp] jupyter_server_terminals | extension was successfully loaded.
[I 2024-11-13 17:11:17.014 LabApp] JupyterLab extension loaded from /opt/conda/lib/python3.11/site-packages/jupyterlab
[I 2024-11-13 17:11:17.014 LabApp] JupyterLab application directory is /opt/conda/share/jupyter/lab
[I 2024-11-13 17:11:17.015 LabApp] Extension Manager is 'pypi'.
[I 2024-11-13 17:11:17.027 ServerApp] jupyterlab | extension was successfully loaded.

JupyterLab

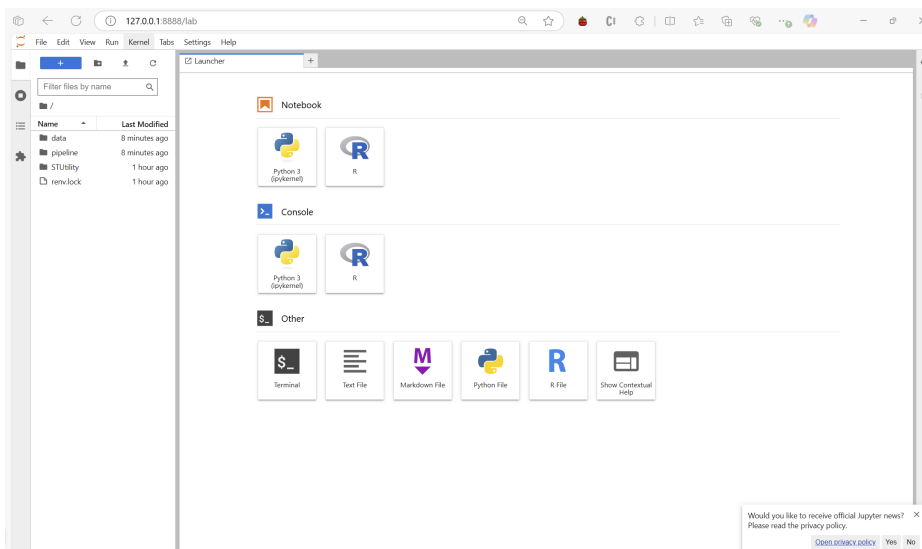
Read the migration plan to Notebook 7 to learn about the new features and the actions to take if you are using extensions.
https://jupyter-notebook.readthedocs.io/en/latest/migrate_to_notebook7.html

Please note that updating to Notebook 7 might break some of your extensions.

[I 2024-11-13 17:11:17.038 ServerApp] nbclassic | extension was successfully loaded.
[I 2024-11-13 17:11:17.041 ServerApp] notebook | extension was successfully loaded.
[I 2024-11-13 17:11:17.041 ServerApp] Serving notebooks from local directory: /tmp/work
[I 2024-11-13 17:11:17.041 ServerApp] Jupyter Server 2.12.5 is running at:
[I 2024-11-13 17:11:17.041 ServerApp] http://eeb3b8dc389b:8888/lab?token=d12b45b8991042ea24cbdced77ed04cd2e5005140ea5f0a8
[I 2024-11-13 17:11:17.041 ServerApp] http://127.0.0.1:8888/lab?token=d12b45b8991042ea24cbdced77ed04cd2e5005140ea5f0a8
[I 2024-11-13 17:11:17.041 ServerApp] Use Control-C to stop this server and shut down all kernels (twice to skip confirmation).
[C 2024-11-13 17:11:17.044 ServerApp]

To access the server, open this file in a browser:
file:///home/jovyan/.local/share/jupyter/runtime/jpserver-55-open.html
Or copy and paste one of these URLs:
http://eeb3b8dc389b:8888/lab?token=d12b45b8991042ea24cbdced77ed04cd2e5005140ea5f0a8
http://127.0.0.1:8888/lab?token=d12b45b8991042ea24cbdced77ed04cd2e5005140ea5f0a8
[I 2024-11-13 17:11:17.925 ServerApp] Skipped non-installed server(s): bash-language-server, dockerfile-language-server, javascript-typescrip
t-language-server, jedi-language-server, julia-language-server, pyright, python-language-server, python-lsp-server, r-languageserver, sql-language-server
, texlab, typescript-language-server, unified-language-server, vscode-css-languageserver-bin, vscode-html-languageserver-bin, vscode-json-languagese
rver-bin, yamll-language-server
```

You should now see the landing page:



6. Navigate to the baseNotebook.ipynb:

Inside the pipeline directory, you will find the baseNotebook.ipynb file. This notebook contains detailed instructions and descriptions for each step of the pipeline.