

# User Guide

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## Barley 4D Spatial Transcriptomics Pipeline

This pipeline processes Visium SpaceRanger output to generate clustering as well as transcript abundance plots. It requires the \*.h5 file and filtered\_feature\_bc\_matrix/features.tsv from SpaceRanger.

### Download the pipeline

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

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 ZhejiangUniversity/Containers

# Build the Docker container
sudo docker build . -f Jupyter-R-432_Seurat440_Dockerfile -t analysis-
pipeline:1.1.0
```

This will build the container based on the specified Dockerfile (Jupyter-R-432\_Seurat440\_Dockerfile) and tag it as analysis-pipeline:1.1.0.

How to run the pipeline:

1. Navigate to the ZhejiangUniversity/DEV directory
2. Edit the runJupyterDocker.sh script by updating the following variables:
  - 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 DEV/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

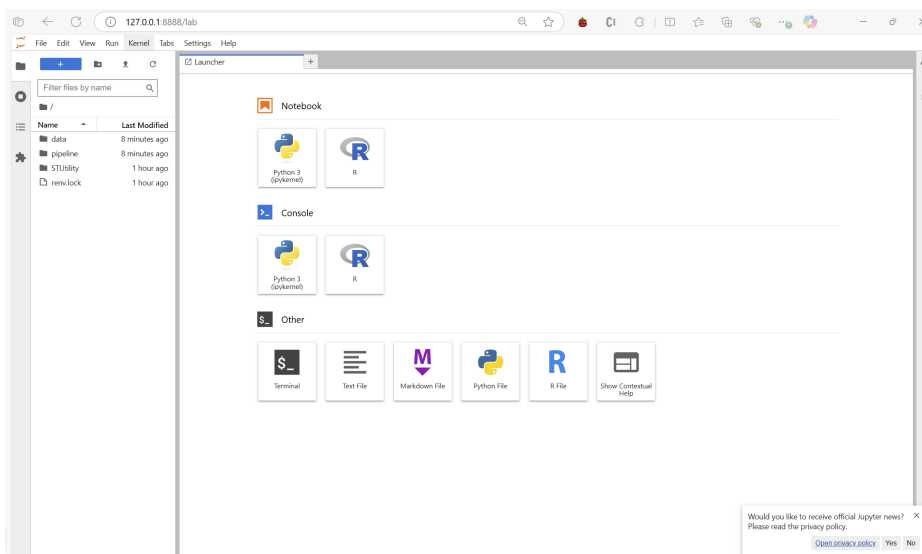
Read the migration plan to Notebook 7 to learn about the new features and the actions to take if you are using extensions.
https://jupyter-notebook.readthedocs.io/en/latest/migrate_to_notebook7.html

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=d12b45b8991042ea24cbdc77ed04cd2e5005140ea5f0a8
[I 2024-11-13 17:11:17.041 ServerApp] http://127.0.0.1:8888/lab?token=d12b45b8991042ea24cbdc77ed04cd2e5005140ea5f0a8
[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).

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=d12b45b8991042ea24cbdc77ed04cd2e5005140ea5f0a8
http://127.0.0.1:8888/lab?token=d12b45b8991042ea24cbdc77ed04cd2e5005140ea5f0a8
[I 2024-11-13 17:11:17.925 ServerApp] Skipped non-installed server(s): bash-language-server, dockerfile-language-server-nodejs, javascript-typescript-languageserver, 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-languageserver-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.