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# **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 \*.h5 file and filtered\_feature\_bc\_matrix/features.tsv from SpaceRanger.

## 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 cor 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:
- 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

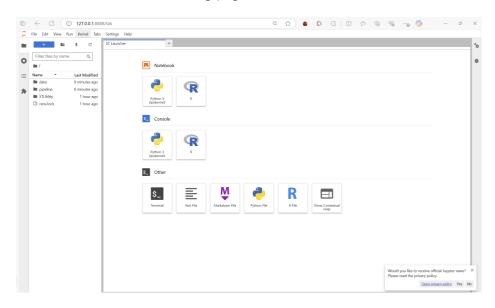
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- 3. Run the Docker container: sudo bash runJupyterDocker.sh
- 4. If you're running the pipeline on a remote Virtual Machine, set up SSH tunneling:

```
# 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.

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.