

Tutorial

Overview

The screenshot shows the stLearn interface with the following components:

- Current page (purple):** Homepage
- Available analysis:** Preprocessing, Ligand-Receptor Interaction, Cell-Cell Interaction, SMC Clustering, PSTS Analysis, DEA Analysis, Gene Plot, Annotation Plot, Cluster Plot, LR Plot.
- Unavailable analysis:** (current stage of analysis make page unavailable)
- Save current analysis state to .h5ad (see uploads/folder for output):** SAVE DATA
- Main page:** ST LEARN
- Upload files:** Documentation, docx, download, h5ad, h5, pdf, zip, license

2 Loading data

The screenshot shows the 'Upload Files' page with two options:

- Option 1: Open Visium data**: Please select the path to your Visium folder. It should include: filtered_feature_bc_matrix.h5 and the spatial folder. Choose folder: No file chosen. UPLOAD FOLDER.
- Option 2: Open AnnData object**: Please select the path to the AnnData.h5ad file. Choose file: No file chosen. UPLOAD FILE.

3 Loading data from Visium folder

The screenshot shows the 'Upload Files' page after successful Visium data upload:

- Select the visium data folder, not any files**
- Note the files within the folder required for successful upload**
- Visium data uploaded successfully!**
- At any time, can be saved to .h5ad: got to step 4 to see how to reload.**
- Perform preprocessing next to unlock additional analyses (Step 5)**

4 Loading AnnData

The screenshot shows the 'Upload Files' page after successful AnnData loading:

- Select .h5ad file**
- After data loading, state of anndata detected**
- Based on anndata state, different analyses/visualisation become available.**
- Save any changes to anndata copy (see uploads/ folder for output)**

5 Preprocessing

The screenshot shows the 'Preprocessing' page with the following parameters:

- Typical form layout in stLearn interactive to set analysis parameters**
- Spot Quality Control Filtering**: Minimum counts per spot: 200, Maximum counts per spot: 300.
- Gene Quality Control Filtering**: Minimum spots per gene: 3, Maximum spots per gene: 5.
- Normalisation, Log-transform, & Scaling**: Normalise total, Log base: 10, Scale: 10.

6 Downstream analyses

The screenshot shows several downstream analysis pages:

- Clustering**: Number of PCs: 50, stSME normalisation, Cluster method: Leiden, Resolution: 1.0, Neighbours (for Louvain/Leiden): 15.
- Manual spot annotation**: Spot alpha: 1, Tissue alpha: 1, Spot size: 5.
- Cluster/Annotation visualisation**: Cluster plot, Annotation plot.
- Gene plotting**: Gene selection: GPC3, Spatial CCI plot.
- Pseudo-space-time (PSTS) inference**: Interactive implementation coming soon...
- Ligand-receptor cell-cell interaction (LR-CCI) analysis**: Ligand-receptor interaction analysis, Ligand-receptor significant interactions plot.

7 Wrap up! Don't forget to save!!