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Version 2

Designing smart spatial omics experiments with S2-omics V.2

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S2-omics



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This protocol is associated with our paper describing S2-omics. Paper link:
<https://www.biorxiv.org/content/10.1101/2025.09.21.677634v1>

ReadTheDocs page: <https://s2omics.readthedocs.io/en/latest/>

Github page: <https://github.com/ddb-qjwang/S2Omics>

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Protocol status: Working

We use this protocol and it's working

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Keywords: spatial molecular profiles across entire tissue section, resulting spatial omics data, using histology image, spatial omics data, smart spatial omics experiments with s2, colorectal cancer tissue section, spatial omics experiment, histology image, designing smart spatial omics experiment, making spatial omics study, spatial omics study, spatial molecular profile, tissue section, preserving critical spatial molecular variation, omic, 10x visiumhd experiment, entire tissue section, critical spatial molecular variation, smart spatial omic

Abstract

A protocol describing the application of S2-omics on a colorectal cancer tissue section, designing 10x VisiumHD experiment.

S2-omics is an end-to-end workflow that automatically selects regions of interest for spatial omics experiments using histology images. Additionally, S2-omics utilizes the resulting spatial omics data to virtually reconstruct spatial molecular profiles across entire tissue sections, providing valuable insights to guide subsequent experimental steps. Our histology image-guided design significantly reduces experimental costs while preserving critical spatial molecular variations, thereby making spatial omics studies more accessible and cost-effective.

We demonstrate S2-omics's utility in selecting a 6.5mm × 6.5mm ROI and predicting the cell-type labels for the whole slide image after conducting VisiumHD experiment on the selected ROI.

Materials

he-raw.jpg: Raw histology image.

pixel-size-raw.txt: Side length (in micrometers) of pixels in he-raw.jpg. This value is usually between 0.1 and 1.0. For an instance, if the resolution of raw H6E image is 0.2 microns/pixel, you can just create a txt file and write down the value '0.2'.

annotation_file.csv(optional): The annotation and spatial location of superpixels, should at least contain three columns: 'super_pixel_x', 'super_pixel_y', 'annotation'. This file is not needed for ROI selection. For an instance, the first row of this table means the cell type of 267th row (top-down) 1254th column (left-right) superpixel is Myofibroblast.

Troubleshooting

Before start

Install Python. We recommend using conda, miniconda, or mamba for this.

To run the demo, first please download the demo data and pretrained model checkpoints file from:

google drive: https://drive.google.com/drive/folders/1z1nk0sF_e25LKMHyJxJVMtROFjuWet2G?usp=sharing

Please place both 'checkpoints' and 'demo' folder under the 'S2Omics' main folder.

In this demo, we mimic the situation that we need to select a 6.5 mm*6.5 mm ROI for Visium HD experiment from a colorectal cancer tissue section. To run the ROI selection (takes about 25 minutes with GPU),



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Install the package and run the ROI selection demo

- 6 # Download S2-omics package
 - 6.1 `git clone -b main https://github.com/ddb-qiwang/S2Omics`
 - 6.2 `cd S2Omics`
- 7 # We recommend using Python 3.11 or above
 - 7.1 `conda create -n S2Omics python=3.11`
 - 7.2 `conda activate S2Omics`
 - 7.3 `pip install -r requirements.txt`



8 # If your server has a very old version of GCC, you can try:

8.1 # pip install -r requirements_old_gcc.txt

9 # Before execution, please write privileges to the .sh files

9.1 chmod +x run_*

9.2 ./run_roi_selection_demo.sh

Run the cell type prediction demo

10 Now, suppose we've obtained the Visium HD data based on which we annotate the superpixels inside the ROI with cell types (annotation_file.csv). To broadcast the cell type information inside the ROI to the whole tissue slide, we can run the following codes (takes about 20 hours with GPU),

10.1 ./run_label_broadcasting_demo.sh

Data format

11 **he-raw.jpg:** Raw histology image.

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13 **annotation_file.csv(optional):** The annotation and spatial location of superpixels, should at least contain three columns: 'super_pixel_x', 'super_pixel_y', 'annotation'. This file is not needed for ROI selection. User can refer to the demo for more detailed input information.

License

- 14 For commercial use of open source software S2-omics, please contact Musu Yuan (musu990519@gmail.com) and Mingyao Li (mingyao@penmedicine.upenn.edu).