STOmics®

Stereo-seq Solution

Revealing TRUE nanoscale resolution to answer your spatial biology questions with STOmics

01 About STOmics and Stereo-seq

STOmics developed the world-leading spatial biology technology Stereo-seq. By achieving multi-centimeter field of view combined with nanoscale resolution for unbiased *in situ* whole transcriptome studies of organisms and tissues, Stereo-seq is poised to revolutionize life sciences research and clinical applications.

Built on DNA Nanoball (DNB) technology, Stereo-seq (SpaTial Enhanced REsolution Omics-sequencing) technology offers researchers a novel tool to explore spatial biology with unprecedented field-of-view, high throughput and subcellular resolution, enabling simultaneous transcriptome study and analysis at tissue-, cellular-, subcellular- and molecular-level. Stereo-seq helps you to establish a solid research foundation for further understanding the relationship between gene expression morphology of cells and local environment.

02 How it works

Stereo-seq Chips come in different sizes, ranging from 0.5cm by 0.5cm to 2cm by 3cm, which are patterned with grids of probes containing spatial coordinates. Upon interaction with a tissue section, cDNA is synthesized *in situ* from mRNA captured by the chip probes. Sequencing the cDNA with their spatial coordinates allows *in silico* reconstitution of the spatial transcriptomic profile of the tissue section, allowing easy visualization and analysis.

03 Highlights

Centimeter-scale field of view

High resolution with ultra-high accuracy

Easy to manage operation time

Subcellular spatial localization biomolecules

Improved chemistry bringing robust and powerful performance

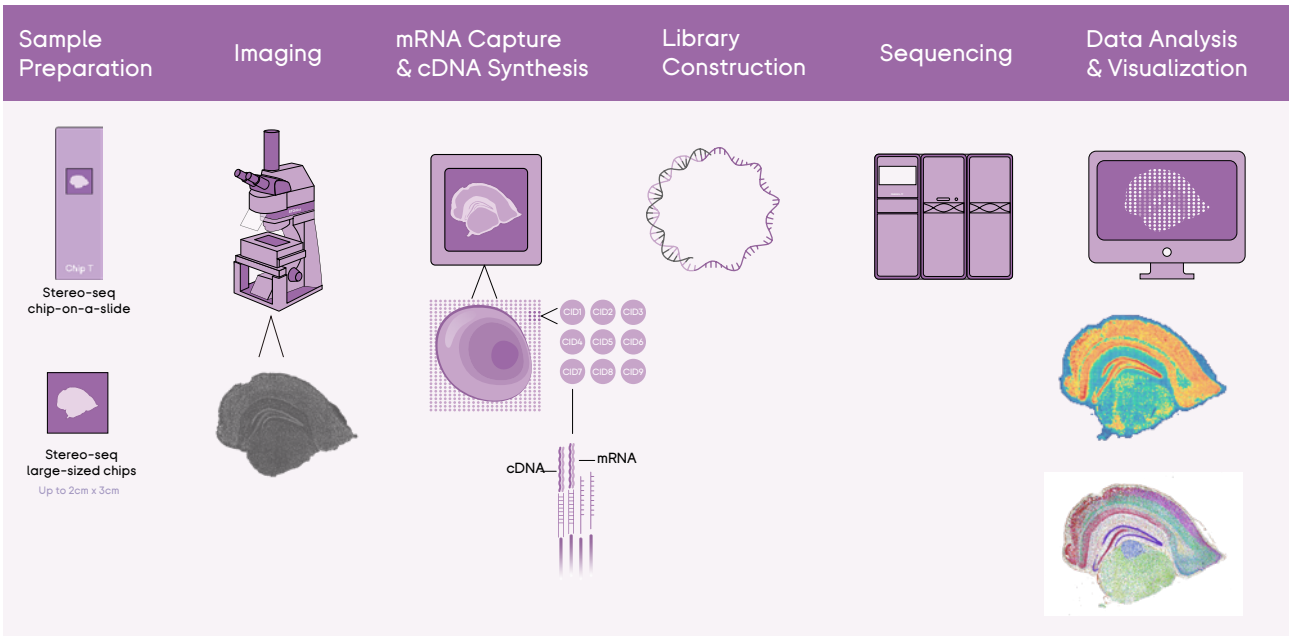


Figure 1. Stereo-seq Tissue-to-data Workflow

04 Application Examples

Empowered with Stereo-seq's robust chemistry, high resolution features and large field-of-view, researchers were able to uncover tissue regeneration and developmental processes as a whole.

Stereo-seq was recently employed in studying Axolotl brain development and regeneration. Over different time points across brain development and brain regeneration post-injury, scientists discovered an induced progenitor cell population that directs brain healing through a cell state transition process mirroring neurogenesis.

With the use of our high resolution Stereo-seq technology, researchers generated a group of spatial transcriptomic data of telencephalon sections that covered six developmental and seven injury-induced regenerative stages. The data at single-cell resolution enabled them to identify 33 cell types present during development and 28 cell types involved in brain regeneration.

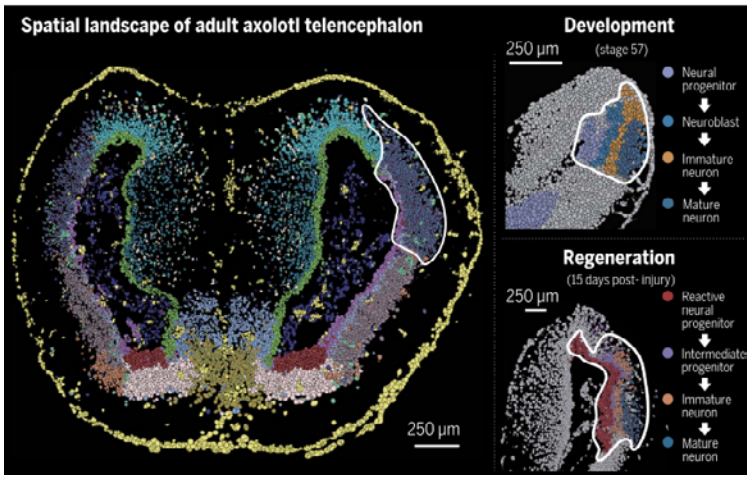


Figure 1. Development and regeneration of axolotl telencephalon. The spatially resolved single-cell transcriptome of the adult axolotl telencephalon as determined by Stereo-seq analyses (left). Upon brain injury in the highlighted lateral pallium region of the left hemisphere, a neural progenitor subpopulation at the wound site was rapidly induced and subsequently replenished lost neurons (bottom right) through a process that partially resembles neurogenesis during development (top right)

Provide robust and reliable tools to understand disease progression mechanisms, discover effective treatments and redefine disease.

Cervical cancer is the fourth most common cancer affecting women's health around the world. Although vaccines and radical hysterectomy are effective measures for preventing and treating cervical cancer, we still lack effective treatments for eliminating advanced cervical cancer. Combined with single-nucleus RNA sequencing (snRNA-seq), researchers employed Stereo-seq to investigate the immunological microenvironment of cervical squamous cell carcinoma (CSCC). Spatial information is critical for understanding cell–cell interactions in tissues, which is unfortunately missing from the snRNA-seq data. By using Stereo-seq to acquire *in situ* gene expression profiles, researchers gained deeper insight into the differences of viral gene expression, immune response, and energy metabolism within cancer tissues and provided initial guidance on achieving better treatment outcomes.

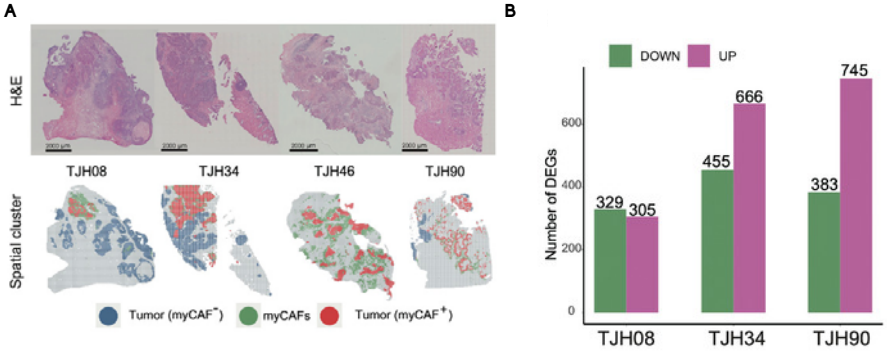


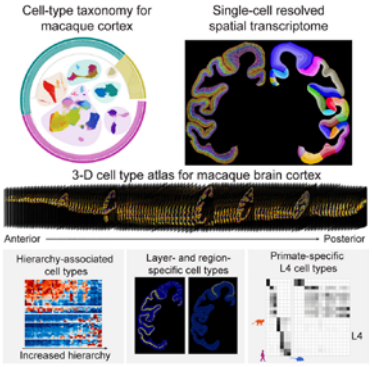
Figure 2. Spatial and functional characterization of myCAFs in CSCC.
A) Spatially projected myCAFs in representative Stereo-seq slides. The projected area was determined based on the MIA score of myCAFs, POSTN expression pattern, and the IHC staining results of POSTN (see Figure S5B,C, Sup- porting Information).

B) Bar plot showing the numbers of up- and down-regulated genes in three Stereo-seq samples. The myCAF+ tumors were compared to the myCAF– tumors.

World's first single-cell resolution spatial mapping of macaque brain cortex.

- A comprehensive cell-type taxonomy is constructed for the entire macaque cortex.
- Stereo-seq reveals the global distribution of 264 cell types and their marker genes.
- Regional density and composition of cell types are coupled with cortical hierarchy.
- Cross-species analysis revealed primate-specific cell types enriched in layer 4.

Elucidating the cellular organization of the cerebral cortex is critical for understanding brain structure and function. Using large-scale single-nucleus RNA sequencing and spatial transcriptomic analysis (Stereo-seq) of 143 macaque cortical regions, researchers obtained a comprehensive atlas of 264 transcriptome-defined cortical cell types and mapped their spatial distribution across the entire cortex.



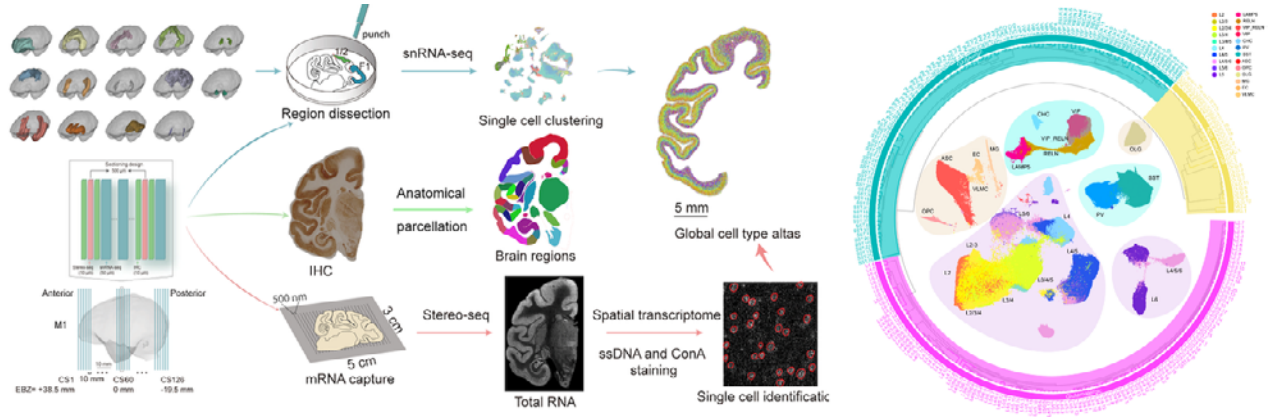
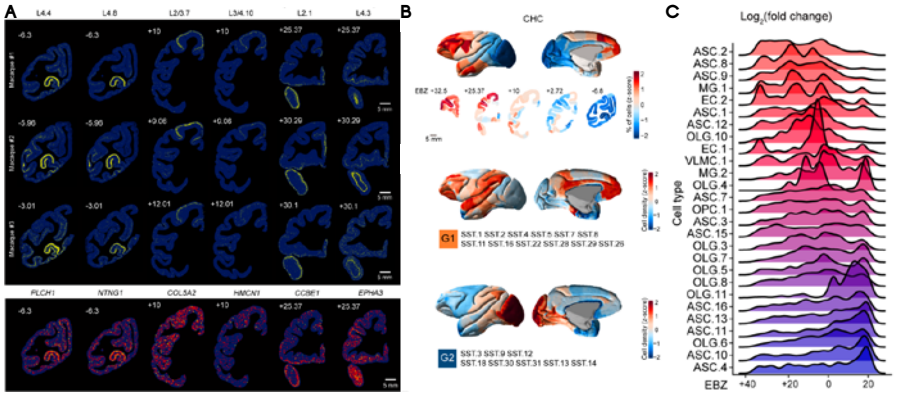


Figure 3. Stereo-seq reveals the global distribution of 264 cell types and their marker genes. A three-level taxonomy tree was further constructure using the hierarchical clustering of these cell types.

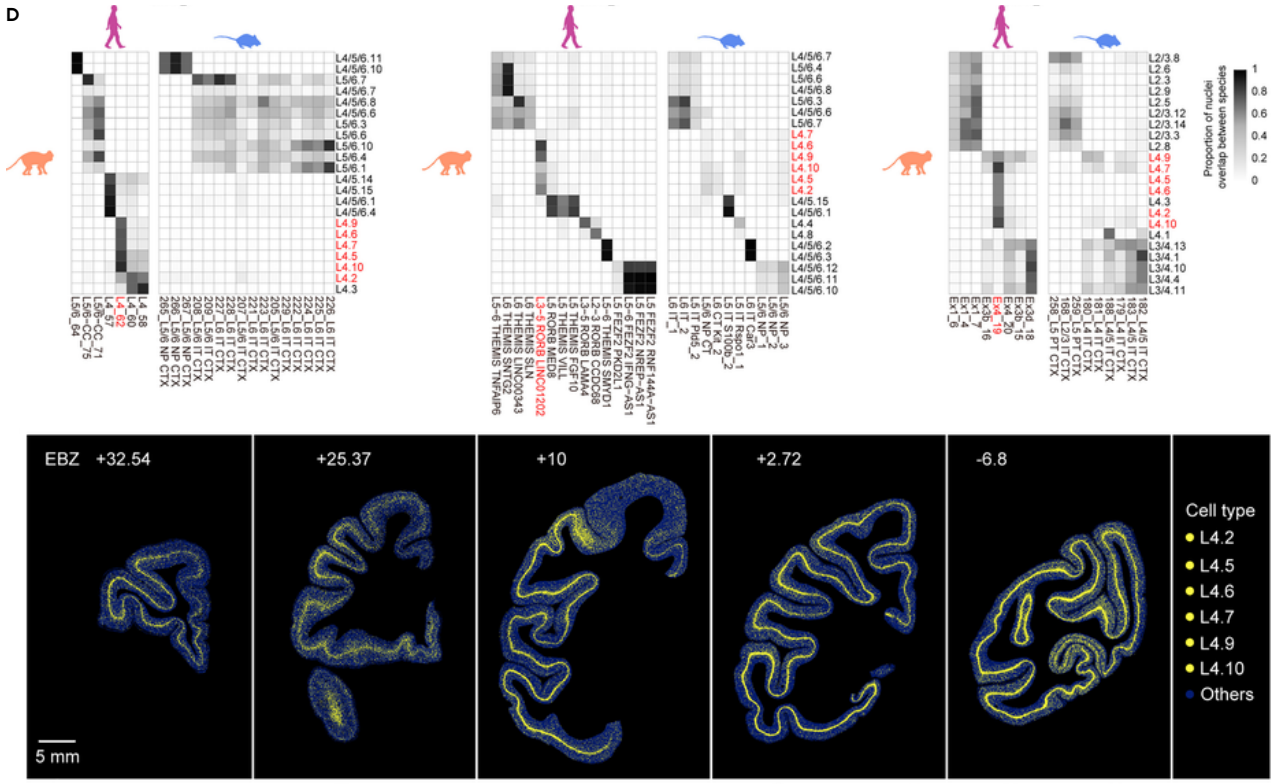
By characterizing the cortical layer and region preferences of glutamatergic, GABAergic, and non-neuronal cell types, as well as regional differences in cell-type composition and neighborhood complexity, a relationship between the regional distribution of various cell types and the region's hierarchical level in the visual and somatosensory systems was discovered.

A) Excitatory neurons
B) GABAergic (inhibitory) neurons
C) Non-neuronal cells



Cross-species comparison of transcriptomic data from human, macaque, and mouse cortices further revealed primate-specific cell types that are enriched in layer 4, with their marker genes expressed in a region-dependent manner. The data provided a cellular and molecular basis for understanding the evolution, development, aging, and pathogenesis of the primate brain.

D) Primate-specific L4 cell types were revealed after further cross-species comparison analysis



05 Recent Publications

1. Single-cell Stereo-seq reveals induced progenitor cells involved in axolotl brain regeneration, *Science* 2022 https://www.science.org/doi/10.1126/science.abp9444
2. Spatiotemporal transcriptomic atlas of mouse organogenesis using DNA nanoball-patterned arrays, *Cell* 2022 https://www.cell.com/cell/fulltext/S0092-8674(22)00399-3
3. A cellular hierarchy in melanoma uncouples growth and metastasis, *Nature* 2022 http://doi.org/10.1038/s41586-022-05242-7
4. Single-cell spatial transcriptome reveals cell-type organization in the macaque cortex, *Cell* 2023 https://doi.org/10.1016/j.cell.2023.06.009

06 Our Products

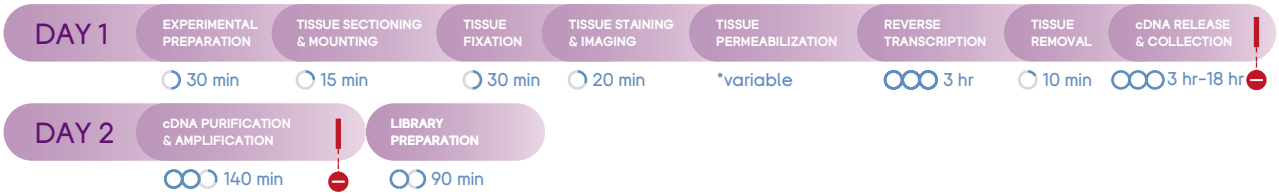
Perform Stereo-seq in your own laboratory with Stereo-seq Kits.

Stereo-seq Permeabilization Set for Chip-on-a-slide				
Part Number	Product		Specification	Description
211SP118	Stereo-seq Permeabilization Kit	111KP118	8 RXN	For determining permeabilization parameters to optimize mRNA capture
	Stereo-seq Chip P Slide (1cm * 1cm)	210CP118	8 EA	
	STOmics Accessory Kit	1000033700	5 PCS	

Stereo-seq Permeabilization Set Workflow TOTAL TIME: ~4 HRS

Stereo-seq Transcriptomics Set for Chip-on-a-slide				
Part Number	Product		Specification	Description
211ST114	Stereo-seq Transcriptomics T Kit	111KT114	4 RXN	For generating a spatially-resolved 3' mRNA library from biological tissue sections
	Stereo-seq Chip T Slide (1cm * 1cm)	210CT114	4 EA	
	STOmics Accessory Kit	1000033700	5 PCS	
Stereo-seq Transcriptomics Set for Chip-on-a-slide (0.5cm * 0.5cm)				
Part Number	Product		Specification	Description
211ST004	Stereo-seq Transcriptomics T Kit	111KT114	4 RXN	For generating a spatially-resolved 3' mRNA library from biological tissue sections
	Stereo-seq Chip T Slide (0.5cm * 0.5cm)	210CT004	4 EA	
	STOmics Accessory Kit	1000033700	5 PCS	

Stereo-seq Transcriptomics Set Workflow TOTAL TIME: ~1.5 DAYS



Stereo-seq Library Preparation Kit			
Part Number	Product	Specification	Description
111KL114	Stereo-seq Library Preparation Kit	4 RXN	For constructing STOmics Library

Stereo-seq PCR Adaptor			
Part Number	Product	Specification	Description
301AUX001	Stereo-seq PCR Adaptor	2 EA	Compatible with PCR thermal cycler as a heating unit

Stereo-seq Permeabilization Set (1cm * 2cm)				
Part Number	Product	Specification	Qty	Description
111SP124	Stereo-seq Permeabilization Kit, 4 RXN 111KP004	4 RXN	1	For determining permeabilization parameters to optimize mRNA capture
	Stereo-seq Chip P (1cm * 2cm) 110CP122	2 EA	2	
Stereo-seq Permeabilization Set (2cm * 2cm)				
Part Number	Product	Specification	Qty	Description
111SP224	Stereo-seq Permeabilization Kit, 4 RXN 111KP004	4 RXN	1	For determining permeabilization parameters to optimize mRNA capture
	Stereo-seq Chip P (2cm * 2cm) 110CP222	2 EA	2	
Stereo-seq Permeabilization Set (2cm * 3cm)				
Part Number	Product	Specification	Qty	Description
111SP234	Stereo-seq Permeabilization Kit, 4 RXN 111KP004	4 RXN	1	For determining permeabilization parameters to optimize mRNA capture
	Stereo-seq Chip P (2cm * 3cm) 110CP232	2 EA	2	

07 Product Features

1. Up to 20mm by 30mm sized chips provide more area coverage for your samples.
2. Patterned probes in nanoscale gives access to subcellular-level details of the tissue section.
3. Support a wide range of tissue types and species, including animals and human.
4. An efficient workflow generates sequencing-ready whole transcriptome library from tissue within 10 hours.
5. Powerful and efficient bioinformatic tools for analyzing and mining single-cell level spatial gene expression patterns to uncover biological phenomena in cancer, developmental biology and neuroscience research.

08 Upstream & Downstream Analysis

ImageStudio → **SAW** → **Stereopy** → **StereoMap**

Image pre-processing | Upstream Analysis | Open source tools | Desktop visualization platform

ImageStudio

Image pre-processing

ImageStudio is an image processing desktop application software designed to **provide intuitive manual tools for manipulating key image functions** that ultimately affect the outcome of Stereo-seq data. It provides assistance with image quality assessment, image stitching re-adjustment, image tissue segmentation and image cell segmentation to facilitate the completion of further bioinformatics analysis.

Key Features

1. Image quality control - assessing the quality of nuclei stained images.
2. Manual image adjustment - provide image adjustment tools for a more accurate registration of gene expression heatmap with the image later in SAW.

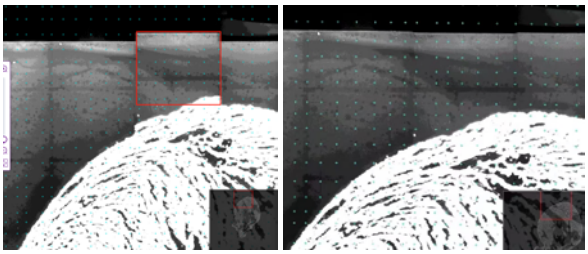
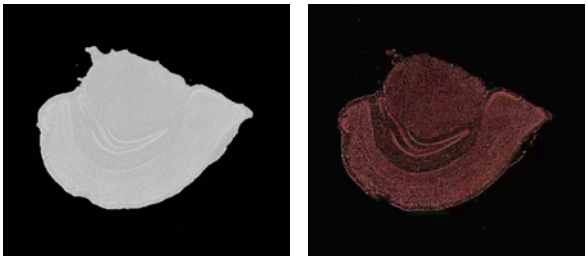


Image with stitching error | Image without stitching error after adjustment

3. Image tissue & cell segmentation - equipped with automatic algorithms to help users identify the contour of tissue and cellular structures.



Tissue Segmentation | Cell Segmentation

SAW (Stereo-seq Analysis Workflow)

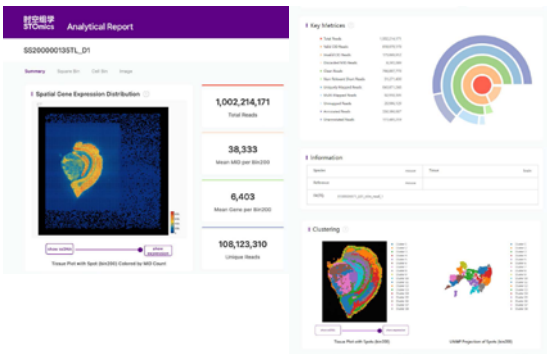
Upstream (basic) Analysis

01100101 01110011 01101110 00100000 **Data to Information**

Stereo-seq Analysis Workflow (SAW) software suite is a set of pipelines bundled to **position sequenced reads to their spatial location** on the tissue section, to quantify spatial gene expression and to visually present single-cell gene expression distribution. SAW processes the sequencing data of Stereo-seq to generate spatial gene expression matrices, and then users could take these files as the starting point to perform downstream analysis.

Key Features

1. Efficient processing of massive data with large field of view and ultra-high resolution.
2. Positioning sequencing data back to tissue space location *in situ*, making it possible to visualize the spatial distribution of gene expression and achieve spatially resolved transcriptomics information.
3. Establish linkage between gene expression at the microscopic level and tissue imaging phenotype at the macroscopic level.
4. Proprietary cell segmentation algorithm makes single-cell level data obtainable.



Stereopy

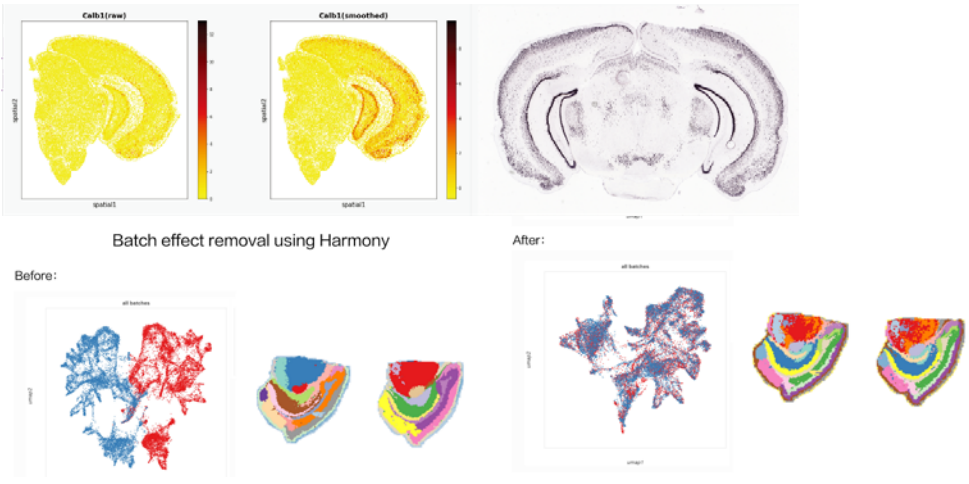
Open source tools

Information to Knowledge

Stereopy is an **open-sourced** python toolkit specifically for **data mining and basic visualization of spatial transcriptomic data**. It provides a series of analyses of spatial omics data, such as clustering, spatial gene pattern, image segmentation and so on. The StereoExpData data format is designed to support efficient parallel computing, which can satisfy the computing needs of multidimensional and massive data generated by Stereo-seq with high resolution and large FOV.

Key Features

1. Support efficient reading and writing (IO), pre-processing, and standardization of multiple spatial transcriptomic data formats.
2. Self-developed Gaussian smooth models, tissue and cell segmentation algorithm models, and cell correction algorithms.
3. Integrate various functions of dimensionality reduction, spatiotemporal clustering, cell clustering, spatial expression pattern analysis, etc.
4. Developed interactive visualization functions based on features of stereo-seq workflow.
5. Achieving cellular type identification with single-cell precision. (which can be applied to inferring the evolutionary trajectory of tumor cells, studying cell fate regulation mechanisms and cell lineage tracing, etc).
6. Spatial clustering integration for identifying cellular characteristics across multiple datasets.



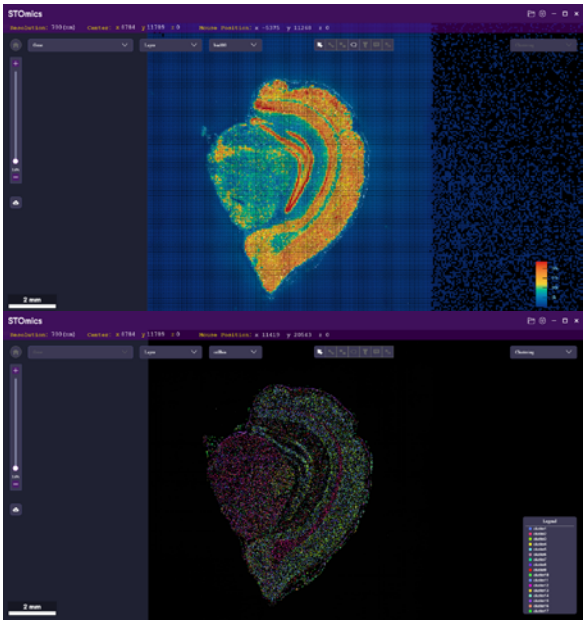
StereoMap

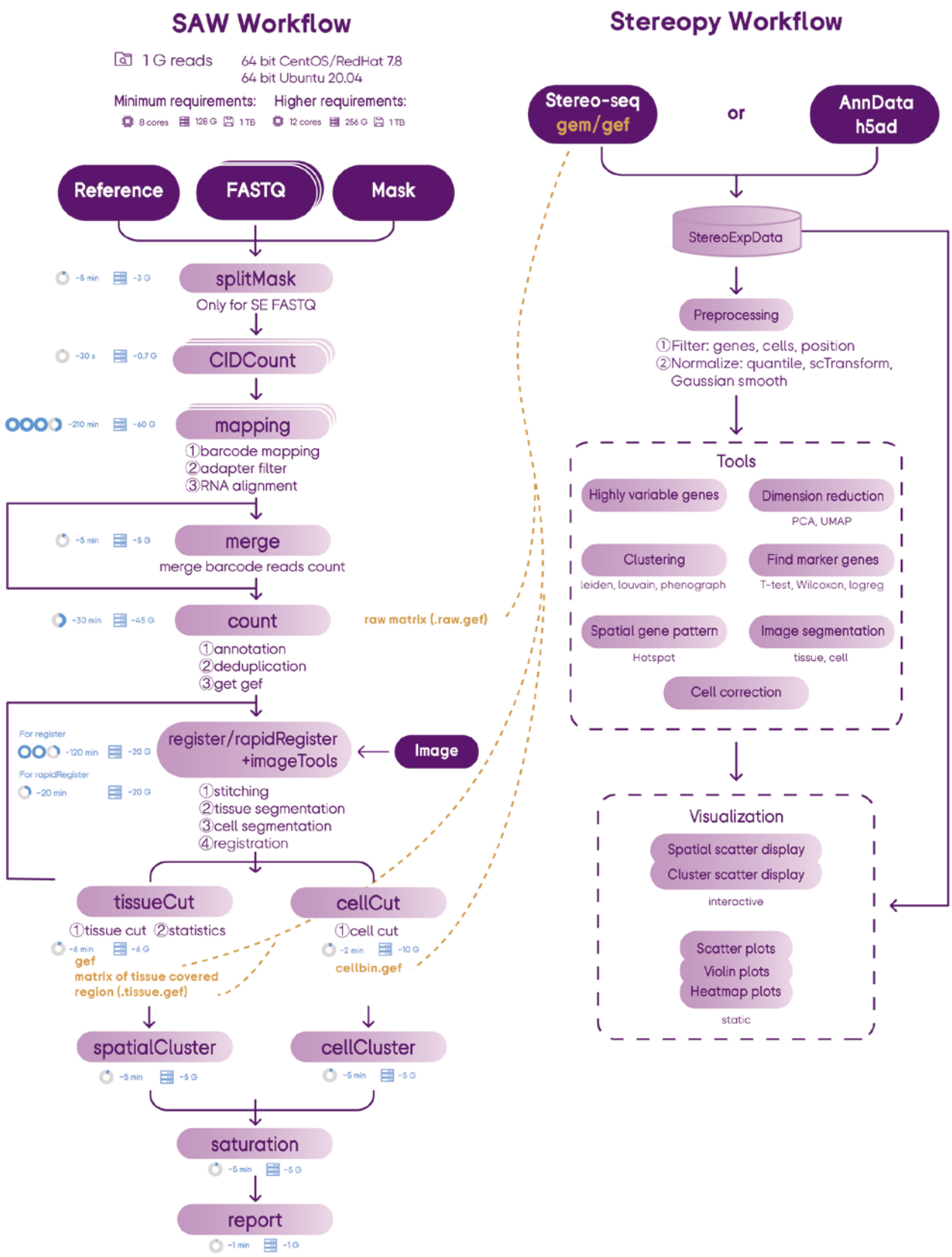
Desktop Visualization Platform

StereoMap is a powerful **high-definition visualization platform** that provides intuitive visualization features in HD to support the display and exploration of Stereo-seq data.

Key Features

1. Visualization of spatially resolved Stereo-seq gene expression heatmap and clustering results.
2. Smart and easy-to-use interactive display of high resolution transcriptomics data.
3. Support efficient download and rendering of publication-level HD images.




SAW Workflow
1 G reads
64 bit CentOS/RedHat 7.8
64 bit Ubuntu 20.04
Minimum requirements:
8 cores 128 G 1 TB
Higher requirements:
12 cores 256 G 1 TB
Reference
FASTQ
Mask
~5 min ~3 G
splitMask
Only for SE FASTQ
~30 s ~0.7 G
CIDCount
~210 min ~60 G
mapping
①barcode mapping
②adapter filter
③RNA alignment
~5 min ~5 G
merge
merge barcode reads count
~30 min ~45 G
count
raw matrix (.raw.gef)
①annotation
②deduplication
③get gef
For register
~120 min ~20 G
For rapidRegister
~20 min ~20 G
register/rapidRegister
+imageTools
Image
①stitching
②tissue segmentation
③cell segmentation
④registration
tissueCut
①tissue cut ②statistics
~6 min ~6 G
gef
matrix of tissue covered region (.tissue.gef)
cellCut
①cell cut
~2 min ~10 G
cellbin.gef
spatialCluster
~5 min ~5 G
cellCluster
~5 min ~5 G
saturation
~5 min ~5 G
report
~1 min ~1 G
Stereopy Workflow
Stereo-seq
gem/gef
or
AnnData
h5ad
StereoExpData
Preprocessing
①Filter: genes, cells, position
②Normalize: quantile, scTransform,
Gaussian smooth
Tools
Highly variable genes
Dimension reduction
PCA, UMAP
Clustering
leiden, louvain, phenograph
Find marker genes
T-test, Wilcoxon, logreg
Spatial gene pattern
Hotspot
Image segmentation
tissue, cell
Cell correction
Visualization
Spatial scatter display
Cluster scatter display
interactive
Scatter plots
Violin plots
Heatmap plots
static

Reach out to us to learn more:

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