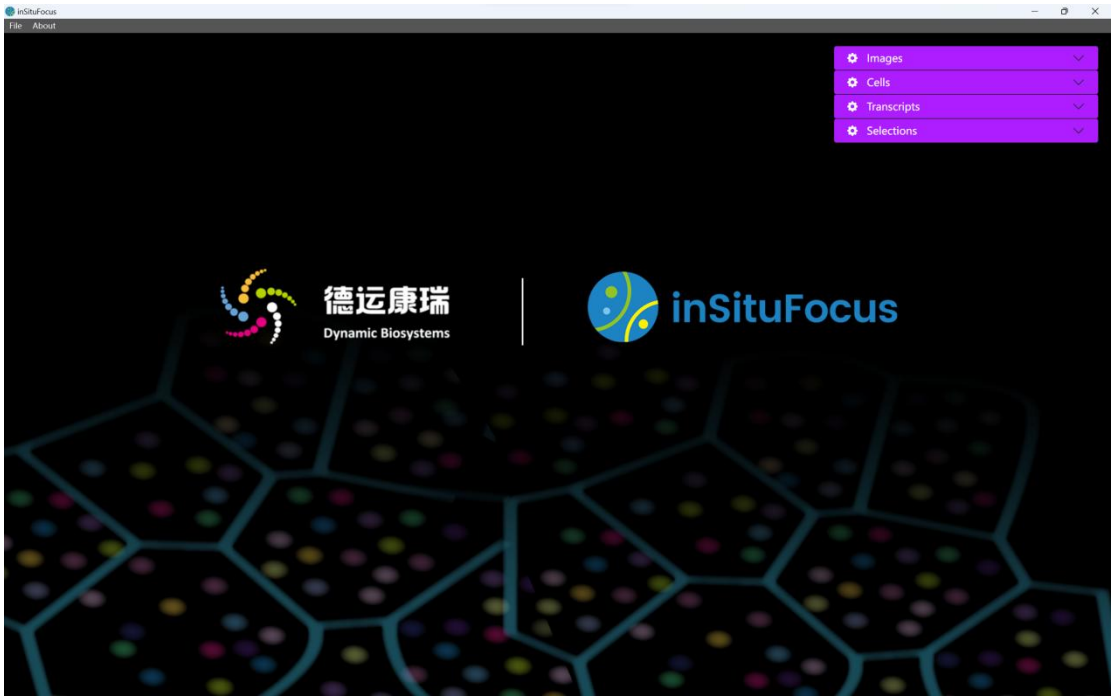
inSituFocus Operation Manual

1. Software Installation and Project Initiation

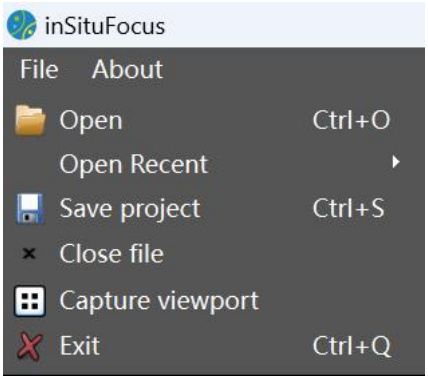
Double click to run the "inSituFocus Installer. exe" file to install the software. You need to set the software installation path and choose whether to create a desktop shortcut.

After installation is complete, double click the shortcut to start inSituFocus. The interface after startup is as follows:

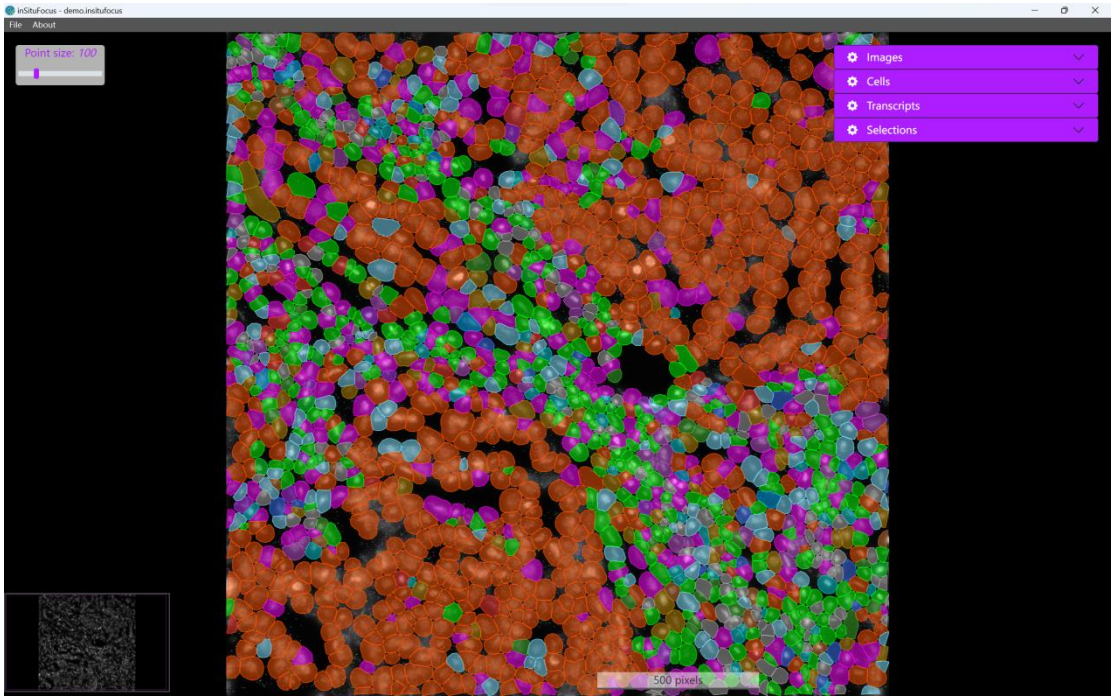


Taking the "inSituFocus demo data" we provide as an example, an inSituFocus project consists of the following files: project configuration file "demo.insitufocus", image "DAPI.tif", ".insitufocus" folder (which contains the converted DAPI image, which can be converted using our tool "ImageConvert.py"), cell file "Cells.geojson", cell dimensionality reduction file "CellUMAP.csv", and gene transcript signal point file

"GeneCoord.csv". The above project files must be located in the same folder and generated by the in situ sequencing data analysis process of Dynamic Biosystems.

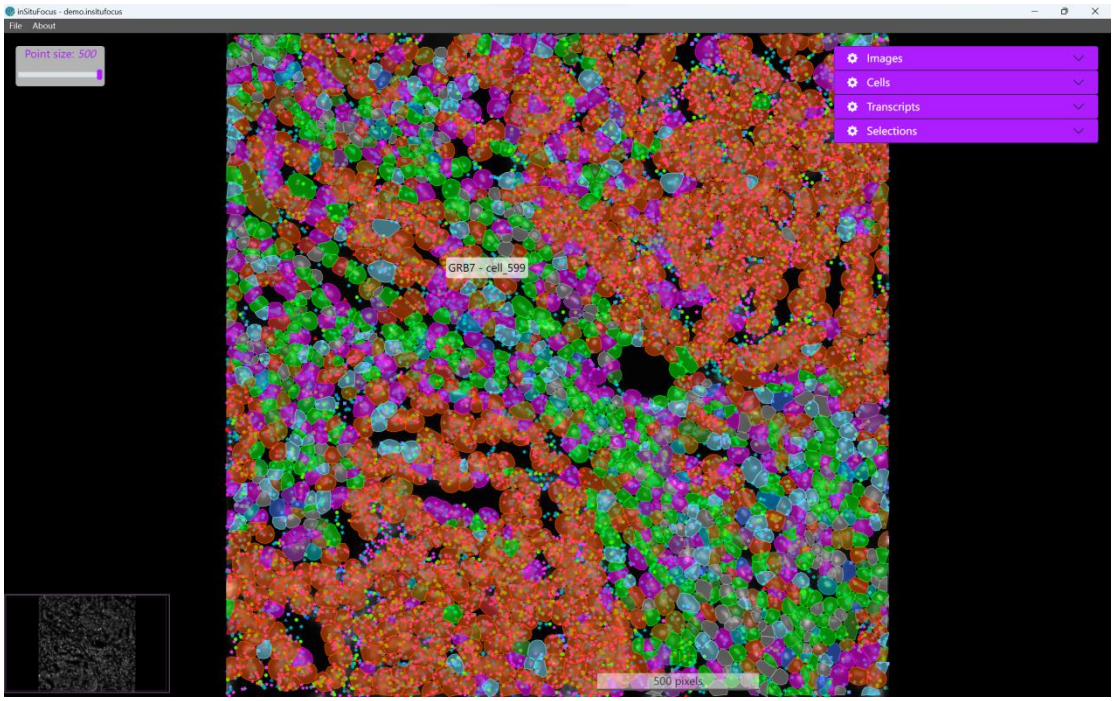
There are two ways to open a project: one is to click "File>>Open" in the menu bar 

, and then select the project configuration file "demo.insitufocus". The second is to double click the "demo.insitufocus" file directly. After the project is launched, cells and signal points are displayed on top of DAPI image.



The mouse can drag the image and zoom in/out, and the transcript signal point size of the gene can be changed by sliding the "Point size" slider.

Clicking on the transcript signal point can display the gene and cell name to which the signal point belongs.

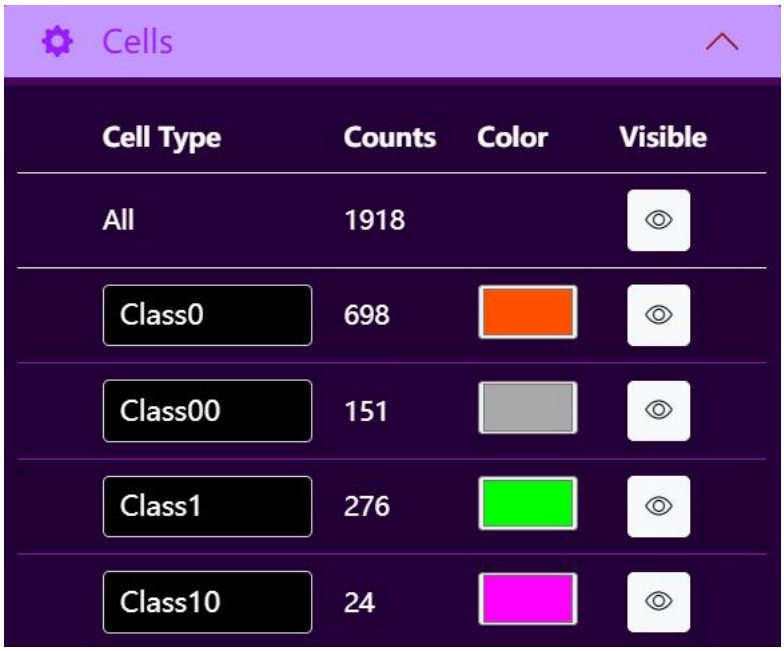


2. Images Module

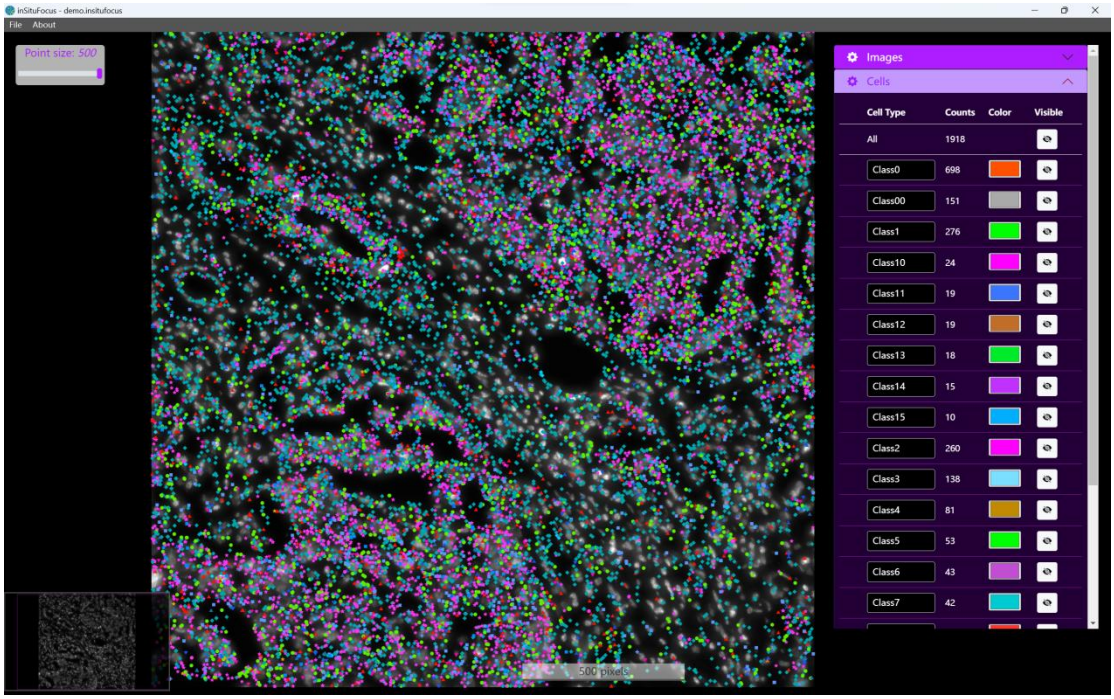
In the images module, you can adjust the visibility, opacity, saturation, brightness, and contrast of the background DAPI image. Click the "Add image layer" button to add a background image layer.

3. Cells Module

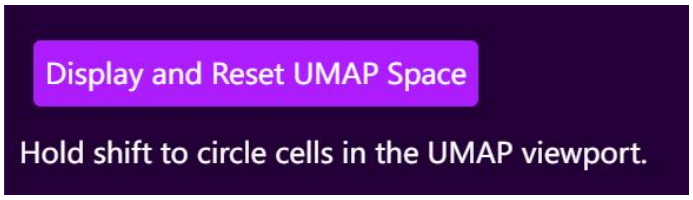
The cells module displays cell type and cell counts, and you can adjust the color and visibility of the cell type.



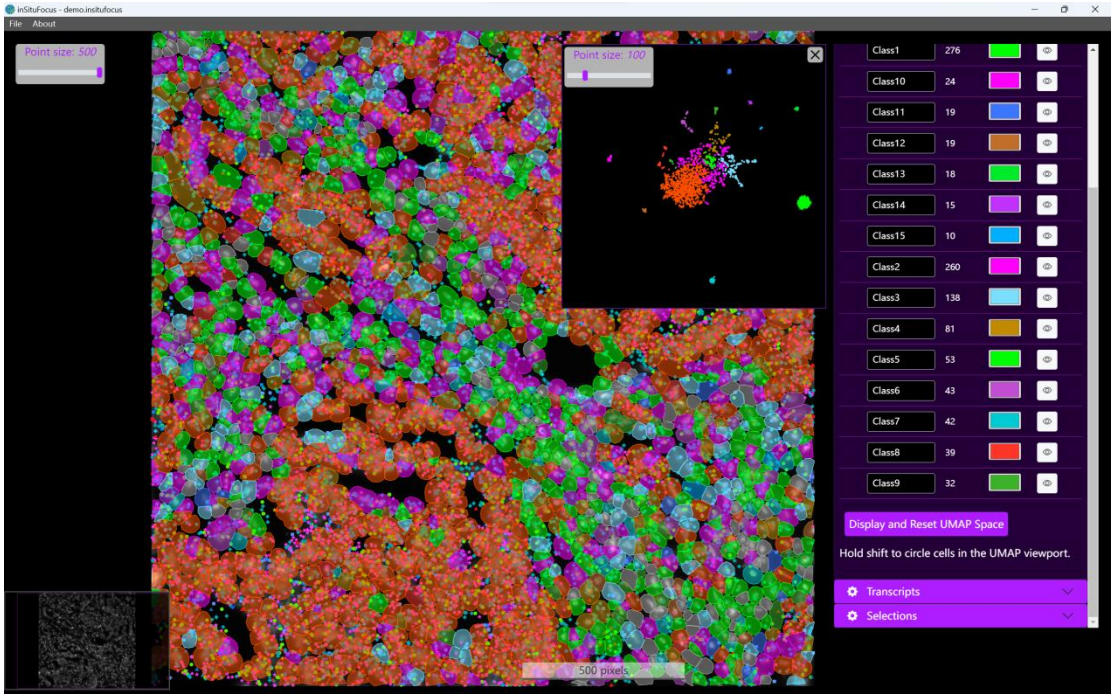
All cells do not display:



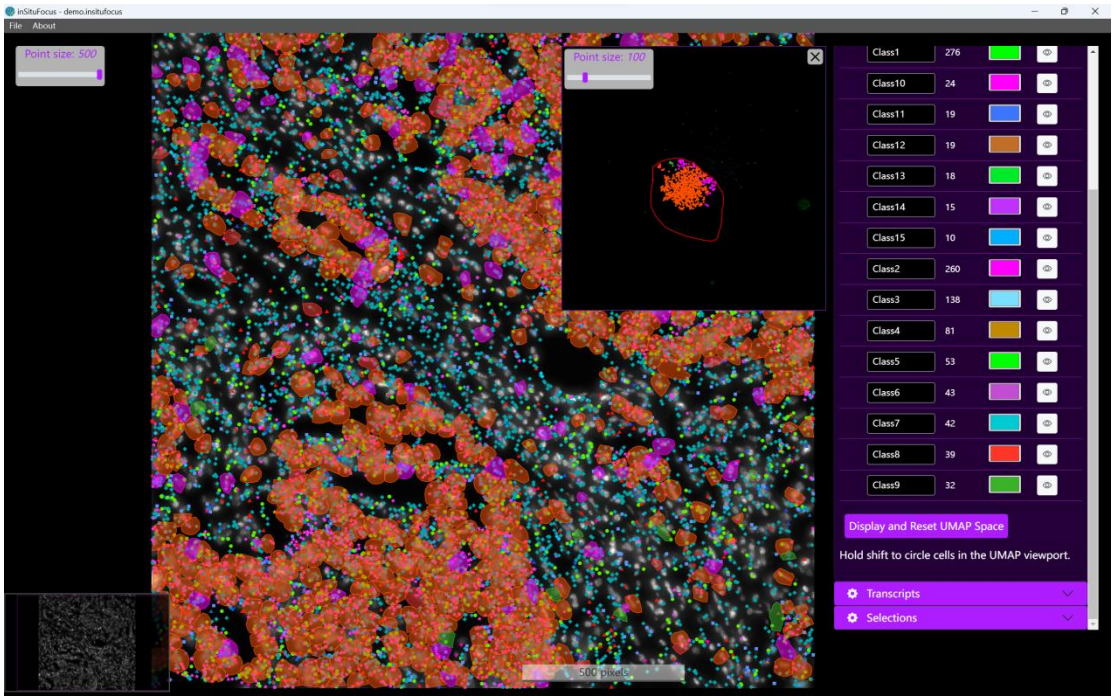
The bottom button of the cells module can control the display of the cell dimensionality reduction map:



Click the "Display and Reset UMAP Space" button to display the dimensionality reduction map. The mouse can drag the image and zoom in/out. The size of the cell point can be changed by sliding the "Point size" slider. Clicking on the cell point in the dimensionality reduction map can display the cell type and name to which the cell point belongs.

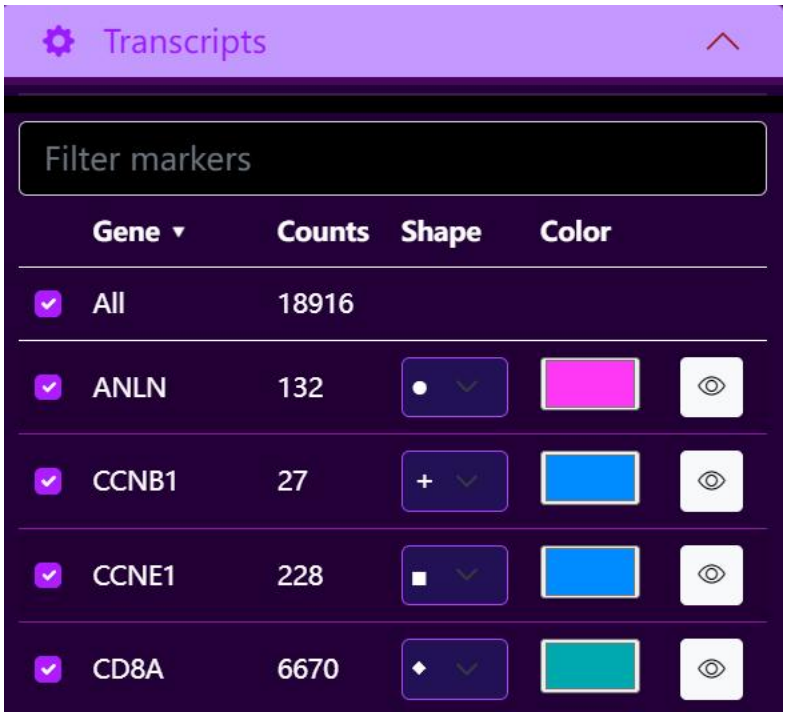


Holding down the shift key allows you to circle cells in the dimensionality reduction map. After selecting, the corresponding cells will be displayed in the main view. Then click the "Display and Reset UMAP Space" button and the cells module visibility button to reset them.。

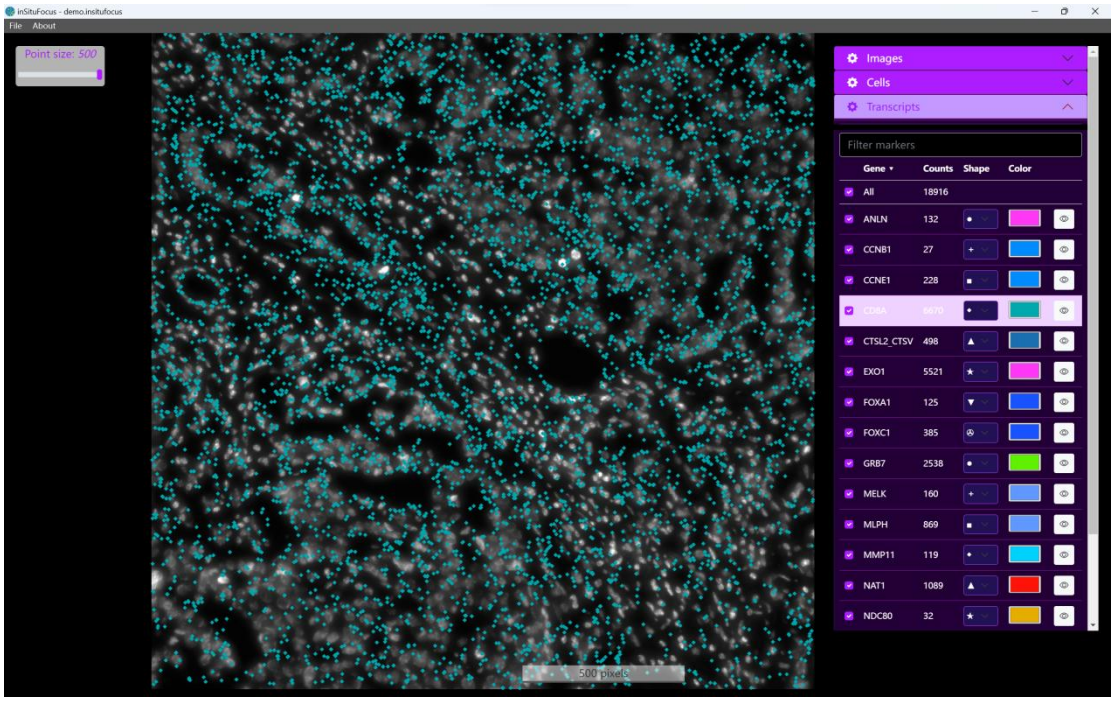


4. Transcripts Module

The Transcripts module displays the genes and counts of the transcript signal points. You can adjust the shape and color of the signal points. Enter the gene name in the "Filter markers" text box to retrieve the gene.

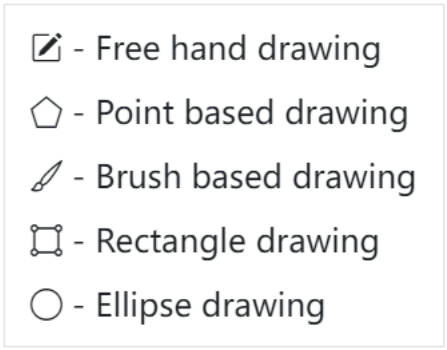


Hovering the mouse over the visibility button  can display the corresponding transcript signal points.



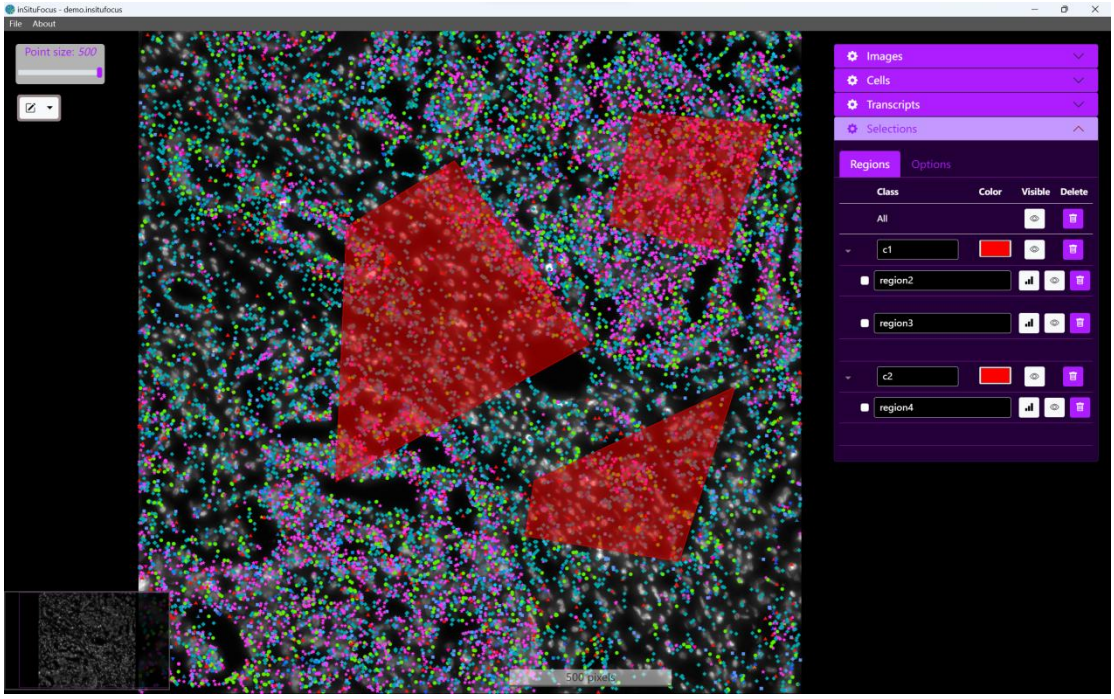
5. Selections Module

The selections module implements three functions: region selection, region analysis, and spot clustering.

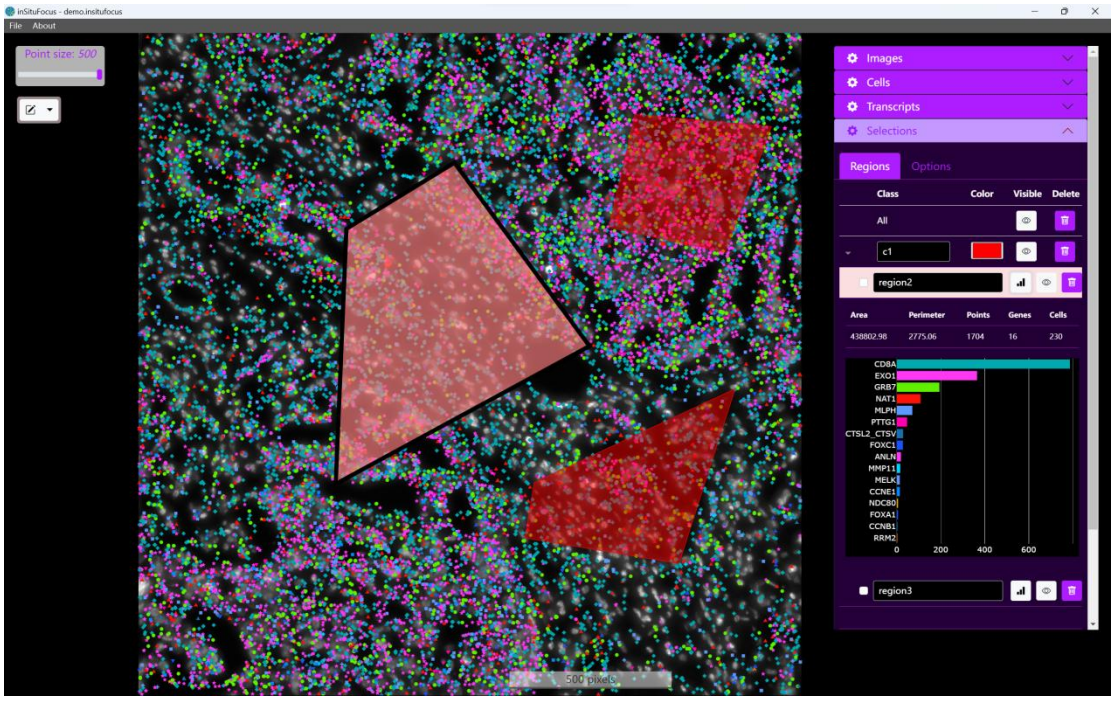
After clicking on the selections module, a region selection tool button  will appear below the "Point size" slider. There are 5 types of region selection tools 

to choose from: free hand drawing, point based drawing, brush based drawing, Rectangle drawing, Ellipse drawing. After selecting a region, a region will be

generated in the regions table. It is necessary to name the region class in the "class" text box, and all regions will be displayed according to the class. The region class name and region name can be modified, and the color and visibility of the region can be adjusted.



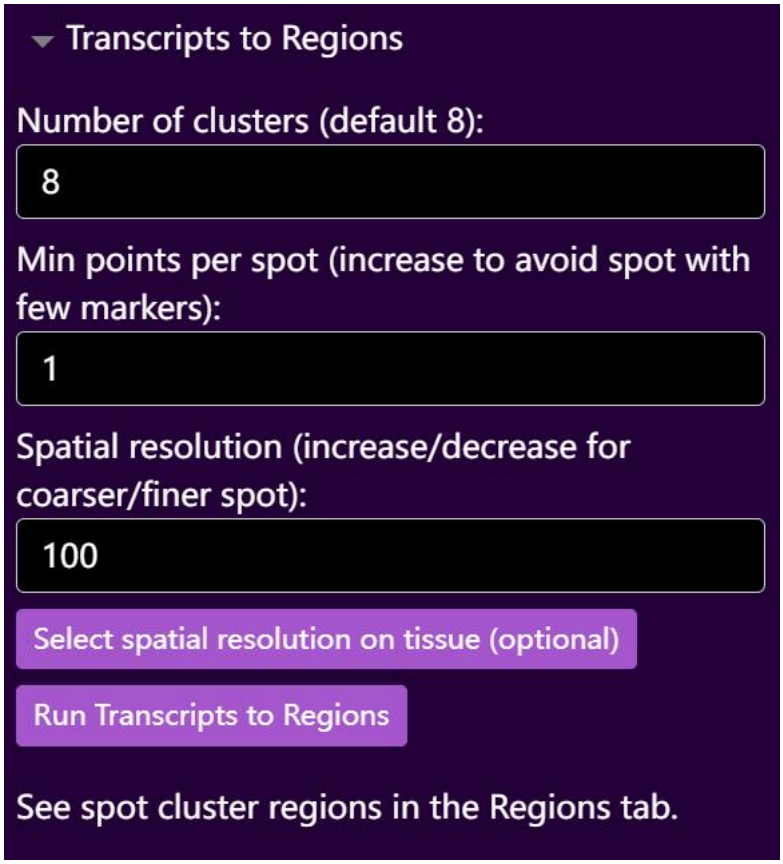
Clicking on the analysis button  of the region will generate region analysis results, including: area, perimeter, transcript signal point counts, gene counts, cell counts, and gene expression histogram.



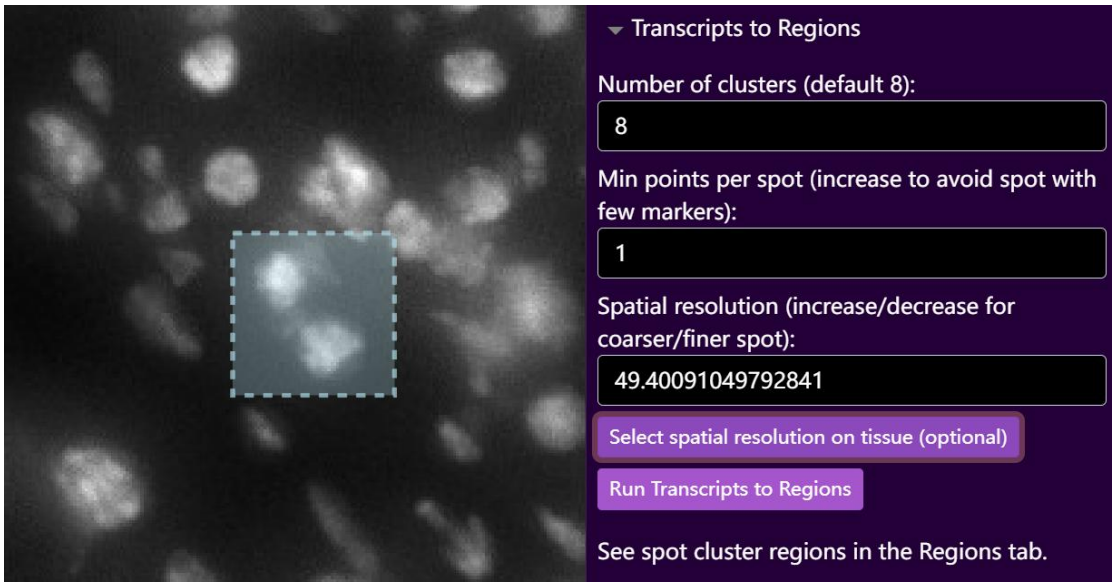
In the options table, you can export the region as a JSON file or a gene expression CSV file, and the saved region JSON file can be imported again (see the demo data file "select_regions.json").

In the options table, spot clustering can be performed by setting three parameters: number of clusters (default 8), min points per spot (default

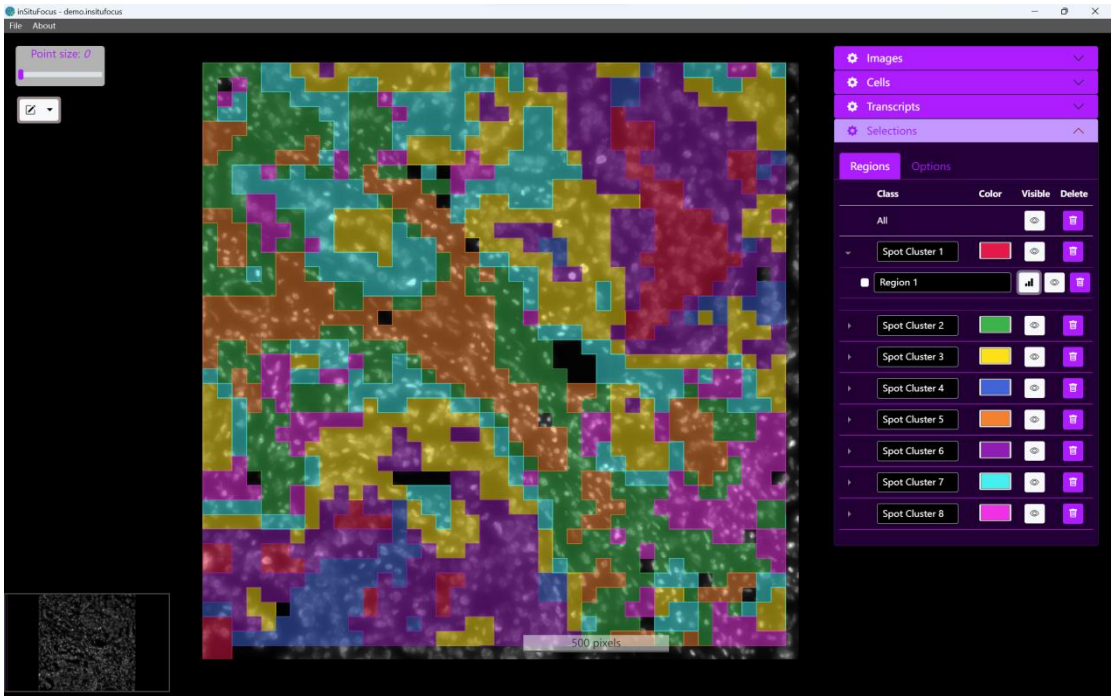
1), and spatial resolution (default 100).



Spatial resolution refers to the pixel size of a spot. You can first click the "Select spatial resolution on issue (optional)" button, and then use the mouse to select the appropriate size in the image. The value in the input box will change in real time.



After setting the parameters, click the "Run Transcripts to Regions" button to perform spot clustering. Note that after the project starts, the system will automatically load the python interpreter. The "Run Transcripts to Regions" button is not available until it is loaded, and the loading time may be related to computer performance. The algorithm clusters spots into a specified number of regions based on the transcript signal point counts of spots gene expression, with the region class name prefixed with "Spot Cluster".


inSituFocus - demo.insitufocus
File
About
Point size: 0
500 pixels
Images
Cells
Transcripts
Selections
Regions
Options
Class
Color
Visible
Delete
All
Spot Cluster 1
Region 1
Spot Cluster 2
Spot Cluster 3
Spot Cluster 4
Spot Cluster 5
Spot Cluster 6
Spot Cluster 7
Spot Cluster 8