SpaceFocus Operation Manual

1. Software Installation and Project Initiation

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

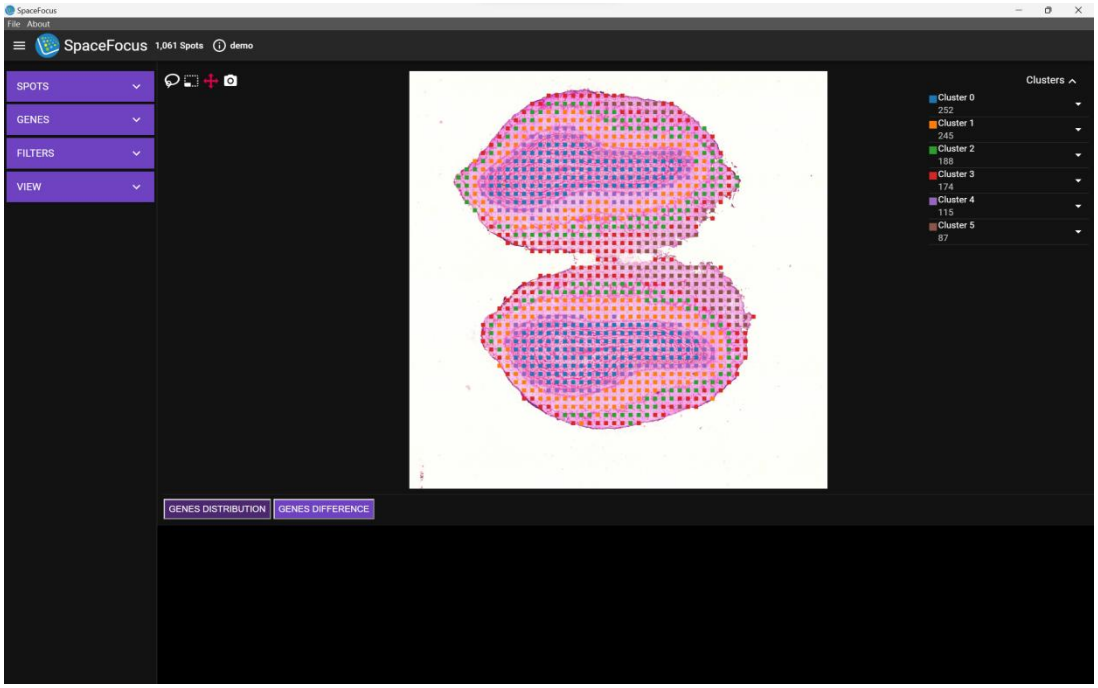
After installation is complete, double click the shortcut to start SpaceFocus. The interface after startup is as follows:

Taking the SpaceFocus demo data we provide as an example, a SpaceFocus project consists of the h5ad file "demo. h5ad" and the image folder "spatial". These project files must be located in the same folder, and any platform's spatial transcriptome data can refer to the following code to generate an h5ad file that is compatible with SpaceFocus:

```python
import scanpy as sc
import re
adata = sc.read_visium("./",count_file ="./filtered_feature_bc_matrix.h5")
adata = adata[:, ~adata.var_names.isin([gene for gene in adata.var_names if re.match("^mt-|^MT-
|^RPL|^RPS|^MRPL|^MRPS|^Rpl|^Rps|^Mrpl|^Mrps", gene)])]
adata.var_names_make_unique()
sc.pp.calculate_qc_metrics(adata, inplace=True)
sc.pp.filter_genes(adata, min_cells=100)
sc.pp.normalize_total(adata, inplace=True)
sc.pp.log1p(adata)
sc.pp.highly_variable_genes(adata, flavor="seurat", n_top_genes=3000)
sc.pp.pca(adata)
sc.pp.neighbors(adata)
sc.tl.umap(adata)
sc.tl.leiden(adata, key_added="Clusters", resolution=0.7)
adata.obs["Clusters"] = adata.obs["Clusters"].apply(lambda x: "Cluster " + str(x))
sc.tl.rank_genes_groups(adata, "Clusters", method="wilcoxon")
adata.obs = adata.obs[["n_genes_by_counts", "log1p_n_genes_by_counts", "total_counts", "log1p_total_counts", "Clusters"]]
adata.obsm["PCA"] = adata.obsm["X_pca"]
adata.obsm["UMAP"] = adata.obsm["X_umap"]
del adata.obsm["X_pca"]
del adata.obsm["X_umap"]
adata.write("./demo.h5ad")
```

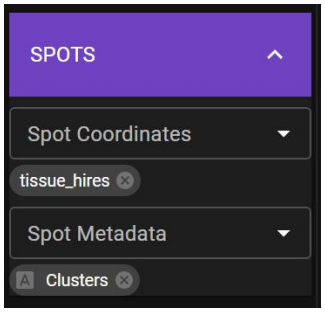
Import the project by clicking "File>>Open file" in the menu bar 

, and then selecting "demo. h5ad" in the project file. After the project starts, the default display is HE image and spot clusters.

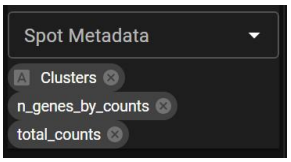


2. SPOTS Module

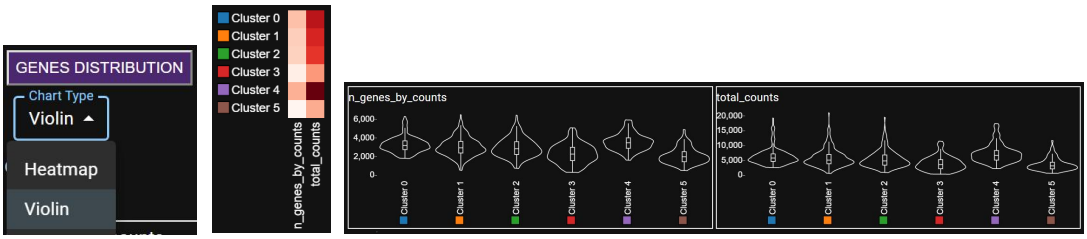
The SPOTS module is used to display the spatial coordinates and metadata of spots, with two selection boxes: Spots Coordinates and Spot metadata.



In the Spots Coordinates selection box, users can select tissue_hires (displaying spots and HE image in the space dimension), UMAP (displaying spot dimensionality reduction map), and spatial (displaying only spots in the space dimension).

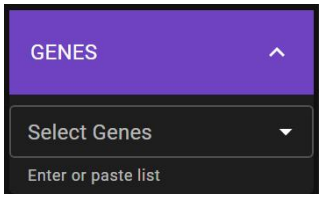
In the Spots Metadata selection box, users can select Clusters (display spot clusters), n_genes_by_counts (display number of spot genes), log1p_n_genes_by_counts (display number of spot genes as log1p), total_counts (display total number of spot UMIs), log1p_total_counts (display total number of spot UMIs as log1p). The selected indicators will appear in the form of small labels below the Spots Metadata selection box 

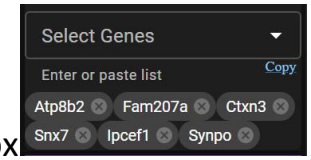
, and clicking on the labels can switch display. To ensure the completeness of other module functions, it is important to select at least one spot category indicator similar to Clusters. If Clusters and other statistical indicators are selected, clicking the GENES DISTRIBUTION button can display heatmaps and violin charts of statistical indicators in each category.



3. GENES Module

The GENES module is used to display the spatial expression of genes, with a selection box called Select Genes.

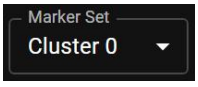


In the Select Genes selection box, users can select genes or input gene names to search. The selected genes will appear in the form of small tags below the Select Genes selection box 

, Clicking on the tags can switch display.

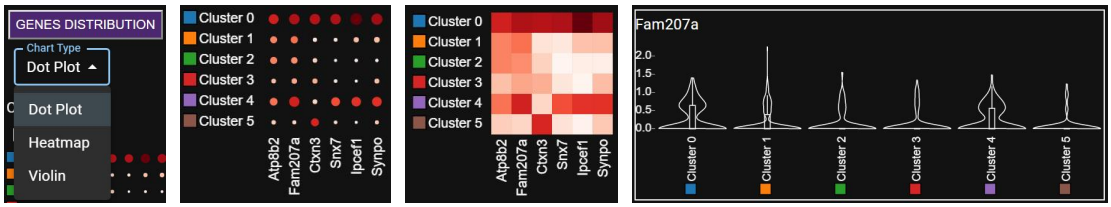
Users can click on the GENES DIFFERENCE button to display the marker gene difference table. Click on the header to sort the table, click on the gene name to add the genes in the table to the small labels below the Select Genes selection box, and display the spatial expression of genes.

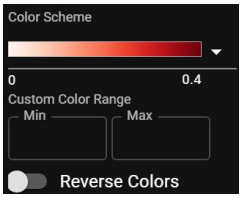
Gene Symbol	Cluster 0:logfoldchanges	Cluster 0:pvals_adj	Cluster 1:logfoldchanges	Cluster 1:pvals_adj	Cluster 2:logfoldchanges	Cluster 2:pvals_adj	Cluster 3:logfoldchanges	Cluster 3:pvals_adj	Cluster 4:logfoldchanges	Cluster 4:pvals_
Tmem88b	1.8	0	-1.45	0.43	-3.72	0.15	-0.26	0.63	0.71	0.44
Kcnb2	2.38	0	-0.68	0.52	-3.56	0	-3.24	0	1.05	0.02

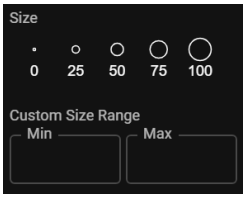
Select a certain cluster in the Marker Set selection box 

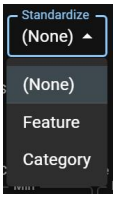
and display the marker genes of that spot cluster (pvals-adj<0.01 and

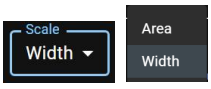
logfoldchanges>1). Click to download the differential gene result file Gene_Difference.tsv for all clusters. If Clusters and Genes indicators are selected, clicking on the GENES DISTRIBUTION button can display dotplot charts, heatmaps, and violin charts of genes in each category.



The dot size of the dotplot chart represents the proportion of spots expressing this gene in the spot category, while the color of the dotplot chart and heatmap represents the average expression level of this gene in the spot category. Users can adjust the color bar and color range 

, dot size range 

, matrix standardization 

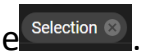
(None not standardized, Feature standardized by gene, Category standardized by category label) below the dotplot chart and heatmap， and violin chart standardization 

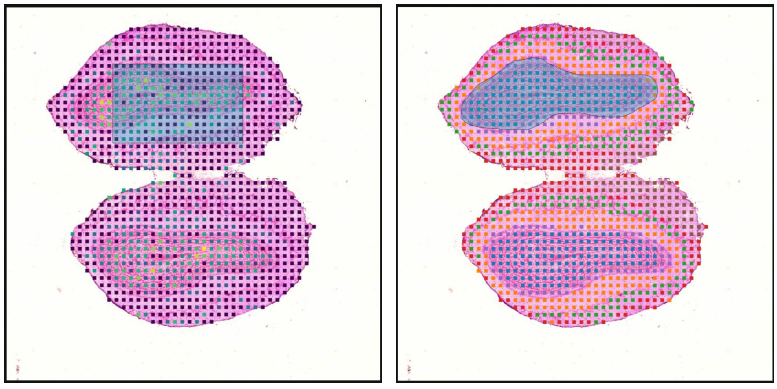
(Width standardized, each violin chart has the same width, and Area standardized, each violin chart has the same area) below the violin chart.

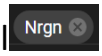
4. FILTERS Module

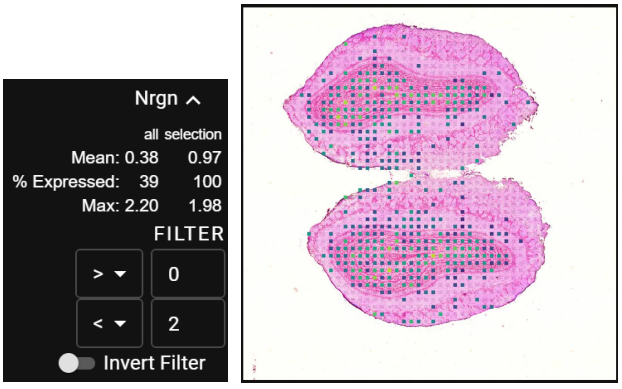
The FILTERS module is used to filter spots in three ways: manual

selection, gene expression range filtering, and category selection.

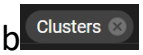
Manual selection requires selecting the selection tool,  can select rectangular areas,  can select irregular areas, both tools can select multiple times. After selection, the Selection label will appear in the FILTERS module  .



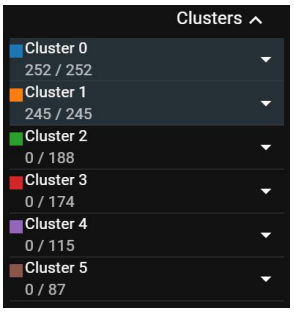
Filtering spots through gene expression range requires setting the upper and lower limits of gene expression, and gene that has been set to an expression range will have a label  in the FILTERS module. Statistical indicators such as total_counts in the SPOTS module can also be filtered in a similar way.

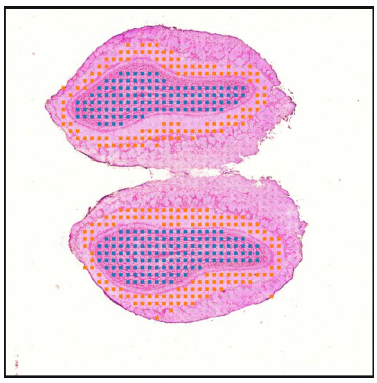


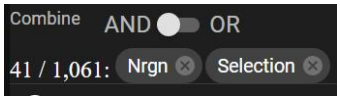
Category selection requires first clicking on the Clusters tab in the SPOTS module, and then selecting a category in the category view (hold down

Ctrl+Alt to select multiple categories). After category selection, the Clusters tab 

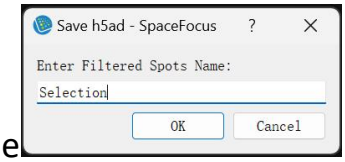
will appear in the FILTERS module.

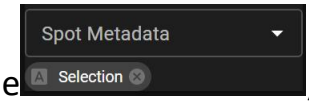




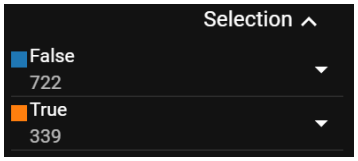
The FILTERS module will logically connect multiple spots filtering labels (AND or OR, default to AND, can be switched to OR by sliding the Combine button) and display the number of spots filtered by the logical connection 

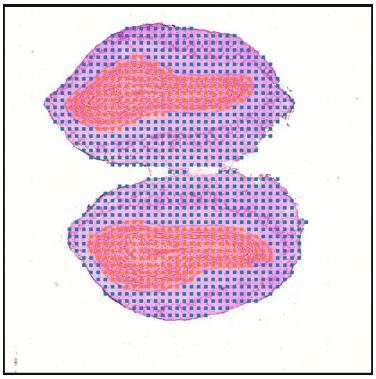
. In the spatial map, only the spots filtered by the logical connection will be displayed.

Users can delete  all filtered tags or export  the filtered spots list file Filtered_Spots.txt. When exporting, you will be asked if you want to save the filtered spots to the h5ad file 

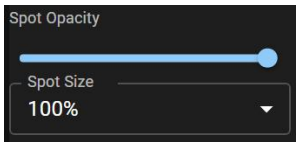
. If necessary, you can enter the category name (default Selection) and click OK. After a moment, the software will automatically restart the project. After restarting the project, the category indicators defined earlier can be found in the SPOTS module 

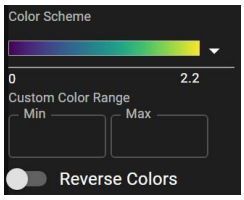
, and the filtered spots will be displayed as True in the spatial map.





5. VIEW Module

The VIEW module is used to adjust the opacity and size of spots in the main view 

, The color bar and color range of spots 

(only effective for visualizing gene expression in the GENES module and statistical indicators such as total_counts in the SPOTS module).