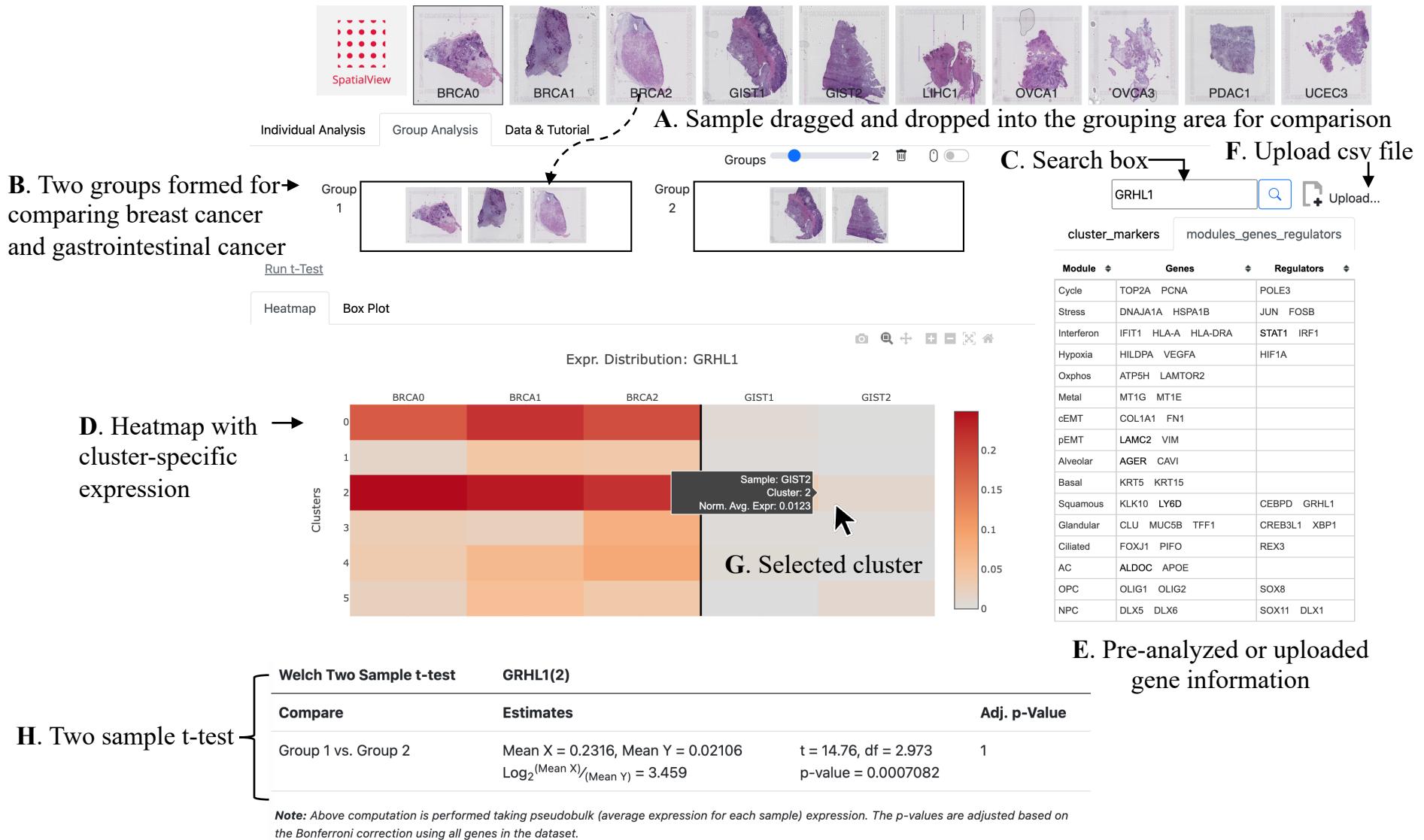


A screen shot of SpatialView. (A) The top panel of SpatialView shows H&E stain thumbnail images from all samples. (B) For single-sample visualization, a user may click to choose any sample from the top panel. For that sample, an H&E image with spot-specific cluster information is shown (C). Upon mouse over of any spot (D), SpatialView provides a zoomed-in H&E image (E) along with spot-specific information (cluster membership, top five gene expressions) (F) and a heatmap of marker gene expression at that spot (G). Sample metadata such as tissue name and tissue type are also shown (H). A user may choose to highlight, show or hide spots belonging to particular clusters by using the cluster-specific toggle buttons (I). Spot-specific size, opacity, and H&E image brightness can be adjusted using the sliders (J). If marker genes are provided, then they can be listed using the ‘markers’ link (K) and the cluster specific markers will be visible in the right panel (L). On click of a gene name or by searching a gene (M), the expression of the gene across the spots can be visualized (Fig. S1). Documentation about a gene in genecards.org can be accessed by a single click on a gene in the heatmap or by double click on a gene name in the list of markers.



Visualization and analysis of multiple samples. The top panel of SpatialView shows H&E stain thumbnail images from all samples (A). In the ‘Group Analysis’ tab, samples can be dragged-and-dropped into groups (B). Here, two groups are shown (Supplementary Figure S2 shows visualization with four groups). A user may specify a gene of interest in the search box (C) and cluster-specific expression is displayed as a heatmap (D) or box plots (Figure S2). If a user has conducted an analysis to identify differentially expressed (DE) genes, or some other genes of interest, they can be presented by SpatialView in the right-side panel (E). For example, information from Fig. 1F (Barkley et al., 2022) is shown in the tab ‘modules_gene_regulators’. User can also upload (F) any csv file containing gene names and SpatialView will present it as a sortable table (Figure S2). On hovering over the heatmap rows (clusters), details about the sample specific average expression of the selected gene are shown (G), and a two-sample t-test is carried out (provided at least three samples are present in one group and two in another) (H).

A. Multiple samples

B. Selected sample

C. Clusters 2 and 3 are selected, cluster 3 is highlighted

D. Search for a gene or metadata

E. Interactive main window: H&E image overlaid with expression of the searched gene

F. Selected spot

G. High resolution H&E image at selected spot

H. Expression at selected spot for the searched gene

I. Cluster-specific marker genes

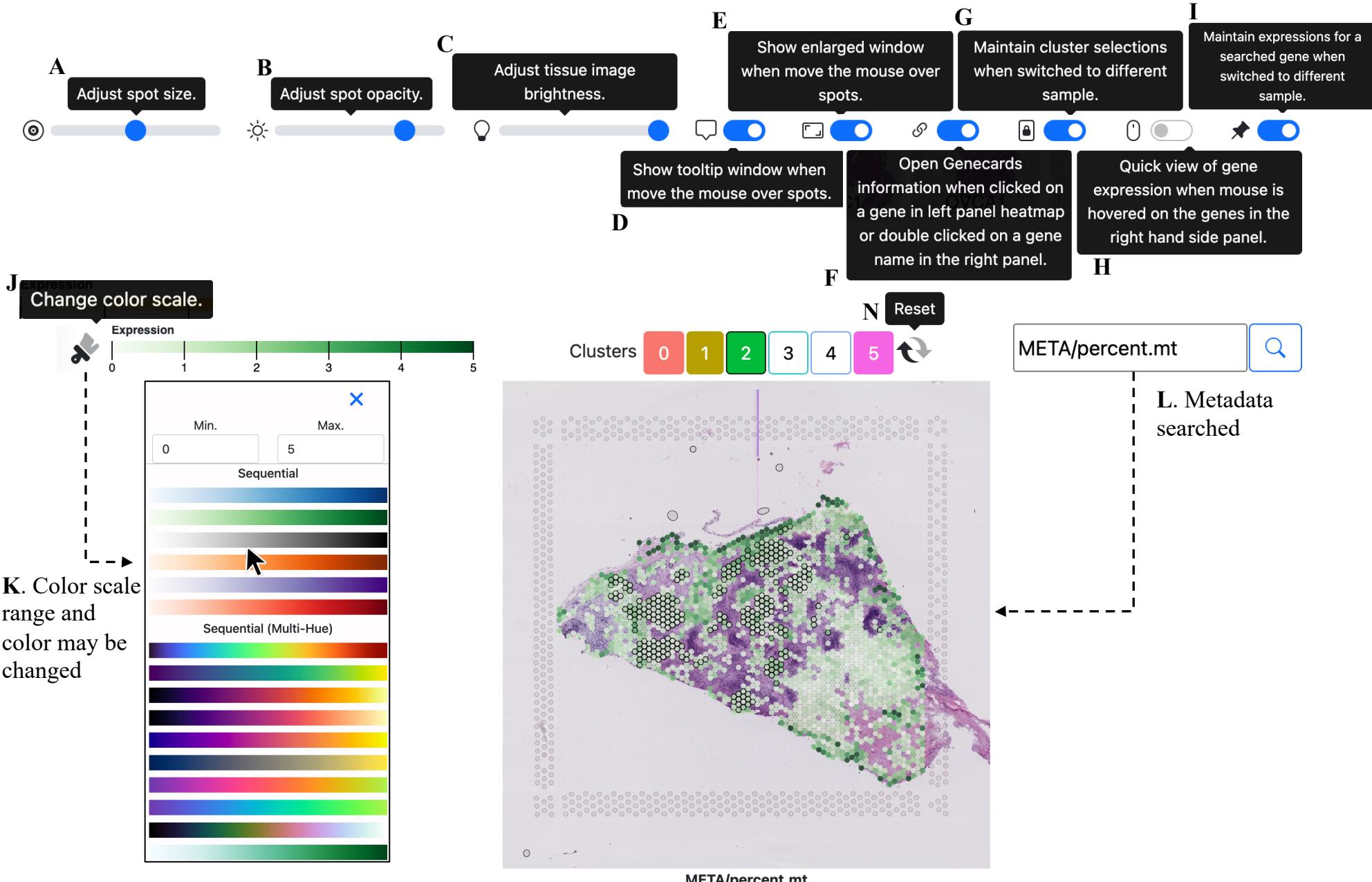
J. Heatmap of marker gene expression at selected spot

| Sample | Type |
|--------|--------|
| BRCA0 | Breast |

Expression

Normalized expressions of markers at the selected spot. Color scale clipped at expression [0,5]

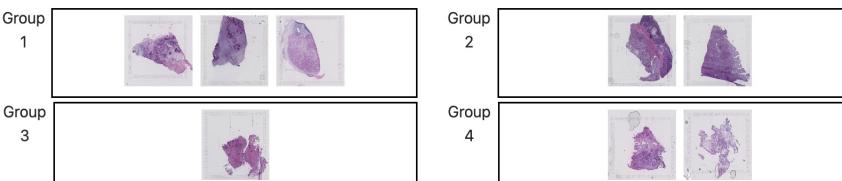
A screen shot of SpatialView. The top panel of SpatialView shows H&E stained thumbnail images from all samples (A). For single-sample visualization, a user may click to choose any sample from the top panel (B). A user can choose interested cluster specific spots using the toggle buttons (C). After searching a gene name in the search box (D), its expression across the selected cluster specific spots will be shown in the interactive panel (E). On mouse over a spot (F), a zoomed-in H&E image is shown (G); spot-specific information includes expression of the searched gene at the selected spot (H). Marker genes are shown in the right-side panel (I) and heatmap showing expressions of markers at the selected spot is shown in the left-side panel (J).



Visualization controls. Sliders for controlling spot size in the main interactive panel (A), spot opacity (B), and Image brightness (C). Toggle buttons for controlling mouseover tooltip (D), zoomed H&E image window (E), genecards pop-up window (F), cluster selection across samples (G), quick view on mouse move over the gene names (H), and retain searched gene/metadata when samples are switched (I). Color scale can be changed using the brush icon (J-K). When metadata is searched in the search bar (L), the interactive window displays the numeric metadata information using the updated color scale. The cluster selections can be reset using the reset button (N).

A. Four groups formed for comparing samples from breast, gastrointestinal, liver, and ovarian cancer

Groups



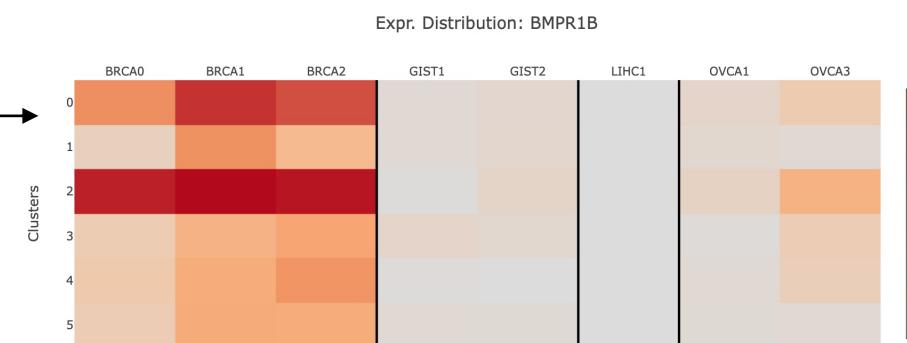
B. Running genome-wide t-test for the formed groups

[Run t-Test](#)

Heatmap

Box Plot

F. Heatmap with → cluster-specific expression



C. Filters may be used before running genome-wide t-test

Min. Avg. Expression
Min. Log 2-Fold Change
[Run and Download](#)

Note: t-Test will be carried out using pseudobulk (average expression for each sample) expressions. Minimum 3 samples required in a group and minimum 2 samples required in other group. The p-values are adjusted based on the Bonferroni correction using all genes in the dataset.

Warning: This process may take a while and browser may be frozen during processing.

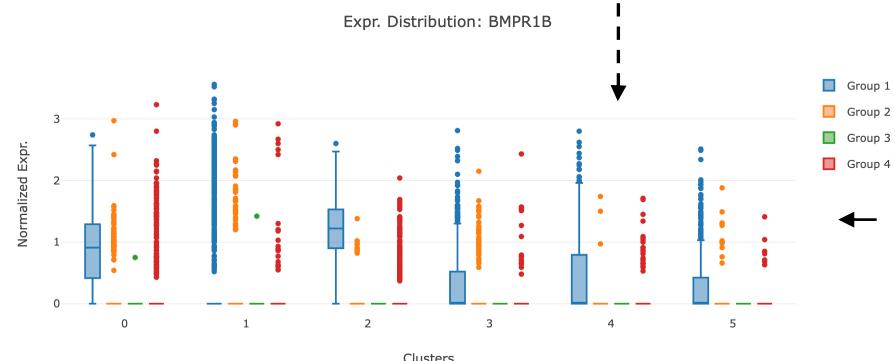
D. Upload .csv file with gene names

Search gene...

cluster_markers modules_genes_regulators [Upload](#)

| Gene | Cluster | Compared Groups | Mean X | Mean Y | Log2FC | P-value | Adj. p-value |
|---------------|---------|---------------------|---------|----------|--------|-----------|--------------|
| XIST | 0 | Group 1 vs. Group 4 | 0.5552 | 1.868 | -1.75 | 0.0002105 | 1 |
| BMPR1B | 2 | Group 1 vs. Group 2 | 1.2 | 0.04659 | 4.687 | 0.0006799 | 1 |
| ENSA | 2 | Group 1 vs. Group 2 | 1.466 | 0.5056 | 1.536 | 0.0006993 | 1 |
| PTMA | 1 | Group 1 vs. Group 4 | 1.431 | 3.013 | -1.074 | 0.001036 | 1 |
| CP | 2 | Group 1 vs. Group 4 | 0.01766 | 1.354 | -6.261 | 0.00113 | 1 |
| HNRNPDL | 1 | Group 1 vs. Group 4 | 0.4436 | 1.283 | -1.532 | 0.001131 | 1 |
| EMILIN1 | 5 | Group 1 vs. Group 2 | 0.1588 | 1.88 | -3.565 | 0.001526 | 1 |
| TFF1 | 2 | Group 1 vs. Group 4 | 2.933 | 0.004049 | 9.501 | 0.001593 | 1 |
| APOL1 | 3 | Group 1 vs. Group 2 | 0.3414 | 1.031 | -1.595 | 0.00167 | 1 |
| XIST | 2 | Group 1 vs. Group 4 | 0.42 | 1.914 | -2.188 | 0.001876 | 1 |
| IL6ST | 0 | Group 1 vs. Group 4 | 1.033 | 0.3953 | 1.386 | 0.001941 | 1 |

E. Selected gene →

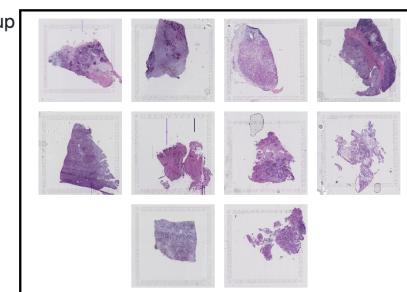


G. Box plots with cluster-specific expression

Four-group comparative analysis. Four groups are formed representing four cancer types (breast, gastrointestinal, liver, and ovarian cancer) by dragging and dropping the corresponding samples into the grouping area (A). After forming the groups, a user may search for a gene of interest in the search box to visualize that gene's expression in any cluster, as described in Fig. 2. Additionally, instead of investigating individual genes, a user may initiate a genome-wide pairwise t-test using the 'Run t-Test' link (B). Before running the test, additional filters may be applied to refine the set of genes considered (C). After completion of the tests, a results file will be saved; for visualizing results, the file can be uploaded using the upload link (D). After uploading the results file, an additional tab with a sortable table will appear in the right-side panel. SpatialView automatically detects the gene names from the uploaded file and allows them to be searched interactively. On mouse over of the *BMPR1B* (E), we see in the table that its expression is higher in group 1 than in group 2 (log 2-fold change 4.687); cluster wise expressions of *BMPR1B* across the groups is also shown in a heatmap (F) and box plots (G).

Groups 1

A. Single group formed → using all the samples



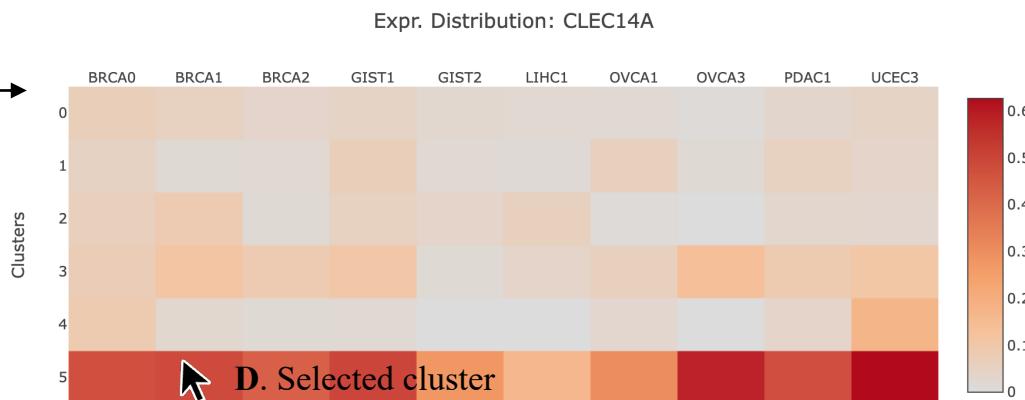
B. Running genome-wide t-test for the formed groups

[Run t-Test](#)

Heatmap

Box Plot

C. Heatmap with → cluster-specific expression



E. Welch t-test comparing cluster 5 vs. rest of the clusters combined

| Welch Two Sample t-test | | CLEC14A(5) | Adj. p-Value |
|-------------------------|---|---|--------------|
| Compare | Estimates | | |
| Cluster 5 vs. Rest | Mean X = 0.4202, Mean Y = 0.0456 $\text{Log}_2(\text{Mean } X)/(\text{Mean } Y) = 3.204$ | t = 8.469, df = 9.255 p-value = 0.00001173 | 0.19348635 |

Note: Above computation is performed taking pseudobulk (average expression for each sample) expression. The p-values are adjusted based on the Bonferroni correction using all genes in the dataset.

One-group analysis with comparisons across clusters. For this analysis, a single group is formed and samples of interest are dragged-and-dropped into the grouping area (A). Once the group is formed, a user may search for a gene of interest (not shown here) or perform a genome-wide search as described in Figure 2 and Figure S2. In the case of one-group, when genome-wide t-tests are carried out using the 'Run t-test' link (B), tests are conducted for one cluster vs. the rest of the clusters. This is also the case on mouse-over of any row (cluster) of the heatmap (C, D).

Customization:

SpatialView can easily be customized by overwriting the human readable config.json (a key-value paired file) located at <application dir>/config/app_config.json and <application dir>/config/data_location.html

The keys and the values in config.json are self-explanatory. To override the default values, user may

- Overwrite the values in the <application dir>/config/app_config.json using any text editor.
- Alternatively, pass a named list to the ‘config.list’ parameter in *prepare10x_from_SpatialExperiment* or *prepare10x_from_Seurat* function in SpatialViewR, and like wise for SpatialViewR a dictionary object may be passed to ‘config_list’ option in *prepare10x_from_scampy* function.

1. Toggle controls:

| Feature | Usage | Key | Default Value | Options |
|---|--|--------------------|---------------|---------------|
|  <input checked="" type="checkbox"/> | Show tooltip window when move the mouse over spots. | tooltip_on | true | [true, false] |
|  <input checked="" type="checkbox"/> | Show enlarged window when move the mouse over spots. | enlarged_window_on | true | [true, false] |
|  <input checked="" type="checkbox"/> | Open Genecards information when clicked on a gene in left panel heatmap or double clicked on a gene name in the right panel. | genecards_link_on | true | [true, false] |
|  <input checked="" type="checkbox"/> | Maintain cluster selections when switched to different sample. | cluster_lock_on | true | [true, false] |
|  <input checked="" type="checkbox"/> | Quick view of gene expression when mouse is hovered on the genes in the right-side panel. | quick_mouseover_on | false | [true, false] |
|  <input checked="" type="checkbox"/> | Maintain expressions for a searched gene when switched to different sample. | pin_search_on | true | [true, false] |

2. Spatial data

| Key | Default value | Description |
|-----------------------------------|------------------------------|--|
| data_file_name_expressions | expression_matrix.csv | 'expression_matrix.csv' should contain the gene expressions. SpatialView prefers sparse encoding and looks for expression_matrix.csv if 'expression_matrix_sparse.txt' is not located. |
| data_file_name_expressions_sparse | expression_matrix_sparse.txt | Expressions in sparse column0oriented (CSC) format. |
| data_file_name_genes | genes.csv | Gene names with gene names in a row. |
| data_file_name_barcodes | barcodes.csv | Barcodes with each barcode in a row. |
| data_file_name_cluster_info | cluster_info.csv | csv file with clusters and associated genes. Each row represents a row. |
| data_file_name_metadata | metadata.csv | csv file with meta data information, analogs to Seurat metadata. |
| data_file_name_scalefactor | scalefactors_json.json | A json file output from 10x Spaceranger. |
| data_file_name_sample_info | sample_info.csv | csv file for sample metadata information. Each row represents for a sample. |
| image_file_name_high_resolution | tissue_hires_image.png | High resolution image file that SpatiaView uses. |
| data_cluster_column | seurat_clusters | Column name in the metadata csv file that contains cluster names for each barcode. |

3. Citation

A SpatialView: An interactive web application
for visualization of multiple samples in spatial
transcriptomics experiments

B Chitrasen Mohanty, Aman Prasad, Lingxin Cheng,
Lisa M. Arkin, Bridget E. Shields, Beth Drolet,
Christina Kendziorski

C <https://doi.org/10.1101/2023.06.13.544836>

| Feature | Key | Default value | options |
|---------|--------------|--|-----------------|
| A | cite_title | SpatialView: An interactive web application for visualization of multiple samples in spatial transcriptomics experiments | Any text string |
| B | cite_authors | Chitrasen Mohanty, Aman Prasad, Lingxin Cheng, Lisa M. Arkin, Bridget E. Shields, Beth Drolet, Christina Kendziorski | Any text string |
| C | cite_link | https://doi.org/10.1101/2023.06.13.544836 | A valid url |

4. Data

The details of the study and the links for the associated data section (in the ‘Data and Tutorial’ tab) can be updated by replacing the appropriate information in the <application dir>/config/**data_location.html** file.