

Supplementary note

TissuePlot

User Guide

Version: 1.0.0

Explore, visualize, and understand spatial transcriptomics data through an interactive web interface.

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1 What is TissuePlot?

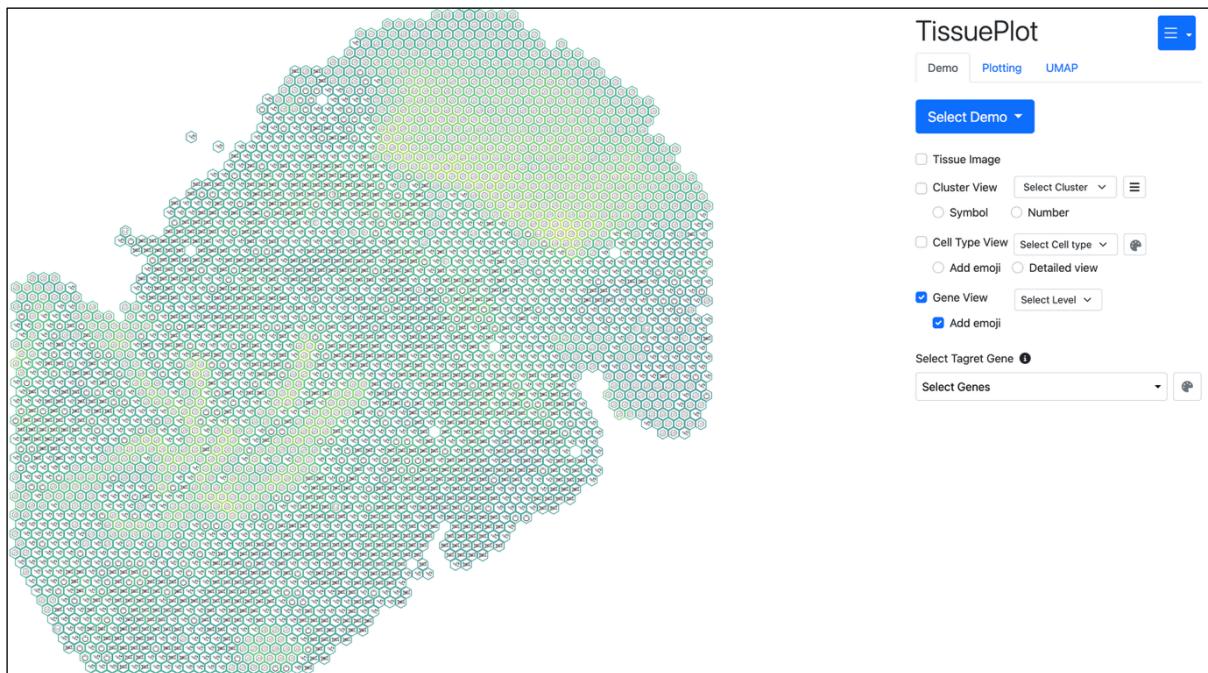
TissuePlot is an interactive web tool (<https://sailem-group.github.io/TissuePlot/>) for visualizing spatial transcriptomics and cell composition data. It displays gene expression, cell type proportions, and spot clustering of tissue samples, optionally overlaying the underlying tissue image for added biological context. Every spot is visualized as a hexagon, making complex tissue structures easy to interpret.

Note: Because it's a client-based application, TissuePlot runs locally, ensuring your data remains private and secure. Most importantly, it does not need coding experience and provide a great way to communicate results in an interdisciplinary team.

2 Application Overview

The TissuePlot window consist of a Main Canvas and a sidebar, which consist of three major sections (tabs):

- Demo Tab – Try different example datasets.
- Plotting Tab – Upload your own spatial datasets and visualize it.
- UMAP Tab – View 2D clustering of your spots using UMAP.



2.1 Main Canvas

All visualization in tissue plot appears in the Main Canvas regardless of where you are using demo data or uploading your own data.

Each spot on the tissue is rendered as hexagon and the visualization updates based on the selected view i.e., Gene View, Cell type view or Cluster view.

This Canvas supports:

- Hovering hexagon to view tooltip with cell type breakdown, and cluster-specific information in the sidebar
- Clicking hexagon to view Spot information as well as Global information (like Distribution of Cell type, Distribution of Cluster, Gene Expression per Cluster, Gene Expression per Cell type, Cell type composition per Cluster,).
- Zoom in and out for closer tissue interaction.
- Synchronized highlighting with UMAP (if enabled).

2.2 Loading your data

2.2.1 Demo Tab – Explore Example Data

The Demo tab allows users to experience the platform using preloaded datasets.

- How to use:
 - Click Select Demo and choose a sample.
 - The spatial layout will be displayed instantly inside the Main Canvas.
 - Now, you can select different views as described below.

2.2.2 Plotting Tab - Upload Your Data

Tissue plot allows to visualize your own spatial transcriptomics data using two flexible methods.

Upload Files Individually:

Use to upload files one by one manually.

Required Files:

File Type	Purpose
Spot Positions File (.csv)	Mandatory. Contains barcode and their x, y coordinates with radius.
Cell Proportions File (.csv)	Mandatory. Contains barcode, cell type proportions and cluster info.
Gene Expression File (.csv)	Optional. Provides gene expression per spot.
Tissue Image (.png)	Optional. Supported format - .png only
UMAP Coordinates File (.csv)	Optional. For dimensionality reduction visualization.
Cell Type Images (.svg)	Optional. Cell type emojis/icons only available once Gene Expression File is available. (File name should match with the Cell types available in Cell proportion file.)

After uploading the necessary files, click "Generate" to build your plot. Data will appear on the same main Canvas used in Demo tab, with all interactive options available.

Upload Folder:

Use this to upload multiple examples with a pre-organized folder with all required files.

Folder Structure:

ExampleData/

```
└── YourExampleName1/
    ├── SpotPositions.csv
    ├── SpotClusterMembership.csv
    ├── TopExpressedGenes.csv (optional)
    ├── SpotUMAP.csv (optional)
    ├── tissue_image.png (optional)
    └── CellTypeA.svg, CellTypeB.svg (optional)

└── YourExampleName2/
    ├── SpotPositions.csv
    └── SpotClusterMembership.csv
```

All files in this folder will be automatically grouped and parsed. Missing or mismatched files (especially .svg) will trigger clear validation errors.

After uploading the folder, click "Generate" to build. Data will appear on the same main Canvas used in Demo tab, with all interactive options available. Multiple examples uploaded via folder, will appear in a dropdown labeled "**Select Example**", allowing user to easily switch between examples and visualize them.

The figure displays two side-by-side screenshots of the TissuePlot application's Plotting tab. Both screenshots show the same interface with different file selection states.

Left Screenshot (Individual File Upload):

- Choose Upload Method:** Radio buttons for "Upload Files Individually" (selected) and "Upload Folder".
- Upload Positions File (.CSV only):** Input field with "Choose file" and "No file chosen".
- Upload Cell Proportions (.CSV only):** Input field with "Choose file" and "No file chosen".
- Upload Genes File (.CSV only):** Input field with "Choose file" and "No file chosen".
- Upload Image (.png only):** Input field with "Choose file" and "No file chosen".
- Upload UMAP file (.CSV only):** Input field with "Choose file" and "No file chosen".
- Upload Cell Type Images (.svg only):** Input field with "Choose files" and "No file chosen".
- Generate:** A blue button at the bottom.

Right Screenshot (Folder Upload):

- Choose Upload Method:** Radio buttons for "Upload Files Individually" and "Upload Folder" (selected).
- Upload Folder:** Input field with "Choose files" and "No file chosen".
- Expected files inside:** SpotPositions.csv, SpotClusterMembership.csv, TopExpressedGenes.csv, tissue_image.png, SpotUMAP.csv, and optional SVGs.
- Generate:** A blue button at the bottom.

Figure: Plotting tab showing two upload modes individual file upload and folder upload.

Uploading Notes:

- Tissue Image must be a .png file.
- Positions, Values, Gene, UMAP files must be .csv files.
- Cell type icons must be .svg files, and match expected cell type names exactly.
- If files are missing or incorrectly formatted, error message will be shown accordingly.

Troubleshooting Tips. 1) Ensure your CSV headers are correctly formatted. 2) Check that SVG file names match your actual cell type labels. 3) UMAP data must match the number of barcodes. 4) Use normalized names (no spaces, lowercase) for better compatibility. All sidebar features available in the Demo tab are also available when you upload your own data under the Plotting tab.

2.3 Visualization Features (Right Sidebar)

Once the demo is loaded or data files are Uploaded, the data will be processed and rendered in the Main Canvas. The right sidebar can be used to select the different views or display options as follow:

2.3.1 Cell Type View

Purpose:

See which cell types dominate each spot and how multiple cell types are distributed spatially with two different options: emoji and Detailed view.

Details:

- The border color of each hexagon indicates the most abundant cell type.
- If you chose option to Add emoji under cell type view, emojis related to the dominant cell type will appear at the center of the hexagons.
- As you select Detailed View option under cell type view, additional hexagons appear, showing the second and third most abundant cell types. If no other cell type is present at a particular spot, only one hexagon will be drawn.
- To view one or more cell type, please use the “Select Cell Type” dropdown next to the “Cell Type View” checkbox. This will show only the spots related to the selected cell types on the Main Canvas.
- To change the color scale, click on the “Color Palette”  button located next to the “Select Cell Type” dropdown. This lets you choose a different color scheme for better visualization.

Tip. Hover over any spots to see a breakdown of 1) all available cell types and their values. 2) Cluster legend (with click functionality) and 3) Associated cluster types with cluster number.

2.3.2 Cluster View

Purpose:

Highlight clusters (groups of similar spots) defined using gene expression or cell type profiles or any other orthogonal data.

Details:

- When enabled, unique shapes or numbers are plotted at the spot centre, depending on their cluster and option selected under the Cluster view.
- Support up to 99 clusters when plotting cluster identity number directly.
- The legend is shown on the right detailing the symbol or number representing each cluster.
- Next to “Cluster view” checkbox you can find the “Select Cluster” dropdown which allow selecting one or more clusters. This helps to show only those spots related to the selected cluster on the main Canvas.
- The user can upload multiple clustering definitions (based on gene expression or copy number inference in the spot membership file. To change the cluster type, click on the button  next to “Select Cluster” dropdown.

Additional Interactive Feature:

- Click a cluster shape in the legend to filter the view to only show spots belonging to that cluster.
- Click again to remove the filter and see all spots again.

2.3.3 Gene View

Purpose:

Visualize the expression level of a one or more genes across all spatial spots.

Details:

- When activated, a dropdown menu appears to choose gene(s) to be plotted.
- To filter spots based on gene expression levels, please use the “Select Level” dropdown next to the “Gene View” checkbox. This allows you to show only the spots with high or low gene expression on the main Canvas, depending on your selection.
- Hexagons are colored by the average gene expression for the selected number of genes from “Select Genes” dropdown.
- The color gradient can be selected (Viridis, Plasma, Turbo) from color palette button  next to “Select Genes” dropdown.
- The intensity of colors can be adjusted using a slider for better visual contrast available inside the color palette button.
- Optionally, cell type emoji's can be overlaid with Add emoji option. (Cell type emojis represent the top cell type at each spot using a small SVG image instead of a plain hexagon.)

Selecting Multiple Genes and Adjusting Intensity:

- Selecting multiple genes from “Select Genes” dropdown:
 - On Mac → hold ⌘ Command and click with your mouse.
 - On Windows → hold Ctrl and click with your mouse.
- Adjusting intensity scale: Use the intensity slider for better visibility. Click and drag the blue dot forward or backward to increase or decrease the intensity scale.

Note: When Gene View is selected

- 1) You can toggle between Cluster View (symbol/number) and Cell Type View.
- 2) You cannot use Cell Proportion View and Gene View at the same time only one can be active at a time.
- 3) However, Cluster View can be combined with Cell Type View or Gene View
- 4) When all views are unchecked, the tissue image will automatically be displayed on the main Canvas.

2.3.4 Tissue Image with Alignment Tool

Purpose:

Overlay various TissuePlot views on top of the Tissue image (e.g., image of H&E stained slide).

Tip. 1) Adjust Stretch and Offset to align the tissue image with the corresponding spot locations. 2) Reduce opacity to improve the hexagons visibility on top of the tissue.

2.3.5 UMAP Tab - Dimensionality Reduction Visualization

Purpose:

View the overall structure of the data to see how spots are grouped in lower-dimensional space based on gene expression or proportions.

Features:

- Plots the data in a 2D UMAP where each point represents one spot.
- Hovering on a point shows the top 3 most dominant cell types inside the tooltip with the cluster information.
- Select cluster from dropdown to highlight specific cluster(s) which is next to Cluster view checkbox.
- Select cell type from dropdown to highlight specific cell type(s) which is next to Cell type view checkbox.
- When you hover over a spot on the main Canvas, the corresponding point in the UMAP plot will be highlighted and vice versa.
- In Gene View, after selecting a gene from the dropdown, the points in the UMAP plot will be colored based on the average expression values of the selected gene, helping you visualize gene expression patterns.

2.3.6 Exporting figures

You can easily save figures from TissuePlot as SVG files for use in presentations or publications.

TissuePlot Canvas:

In the top-right corner of the canvas, click the menu icon (three horizontal lines) . From the menu, select “Export as SVG.” This will download the canvas exactly as it appears on your screen.

Graphs (e.g., Spot Information bar chart, UMAP visualization):

When you move your mouse over a graph, a small “camera icon”  will appear. Click this icon to download the graph as an SVG file.

This way, you can export high-quality, scalable images directly from the application.

3 Walkthrough Example

3.1 Exploring HGSOC-P8 GSM6506117

Let's walk though how to explore the data using **TissuePlot** step by step using Demo P8 which is tissue section from High Grade Serous Ovarian Cancer¹. This guide will help you understand how to interact with the app and explore different biological layers.

Step 1: Access the application with URL <https://sailem-group.github.io/TissuePlot/>

- Go to the Demo tab at the top.
- Click the “Select Demo” dropdown (by default HGSOC-8 GSM6506117 will be selected).
- Choose any demo from the list.
- As soon as you select the demo option, the main Canvas will show a tissue layout made up of hexagons. By default, Gene View will be enabled with emoji option.

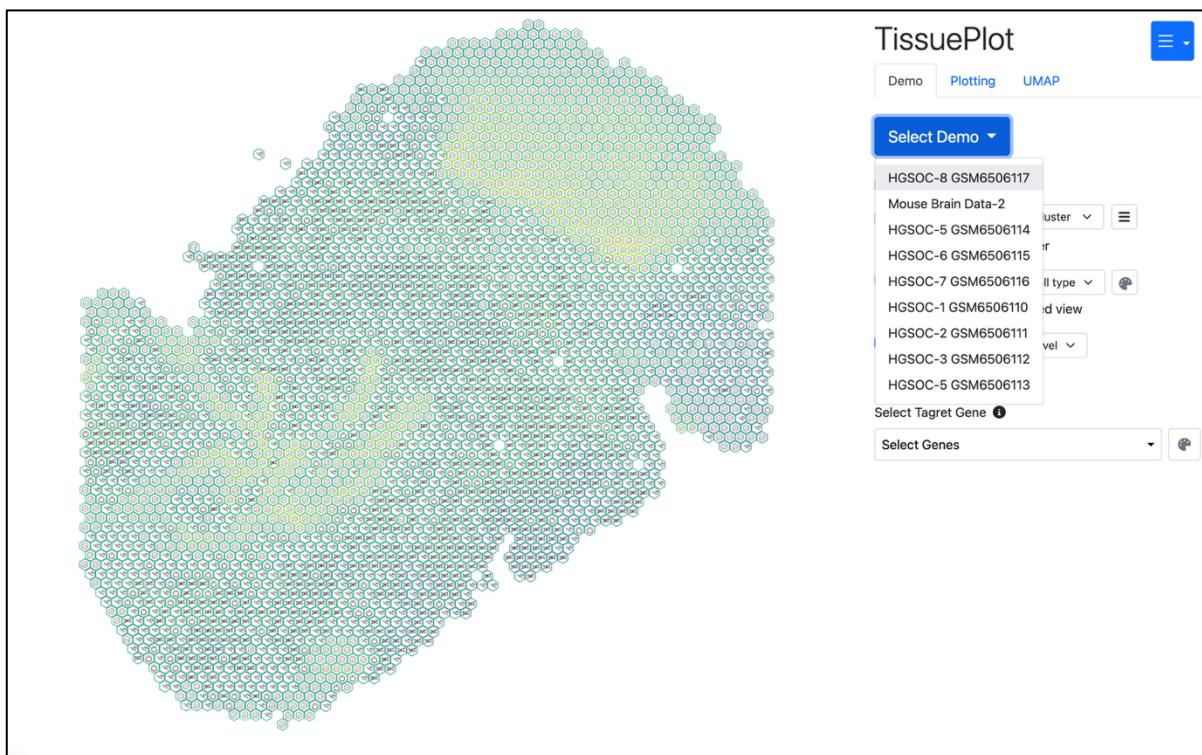


Figure: Selecting Demo and Main Canvas

Step 2: View Cell Proportions

- On the right sidebar, select the “Cell Proportion View”.
- Instantly, you will see each hexagon outlined with a color representing the most dominant cell type at that spot.

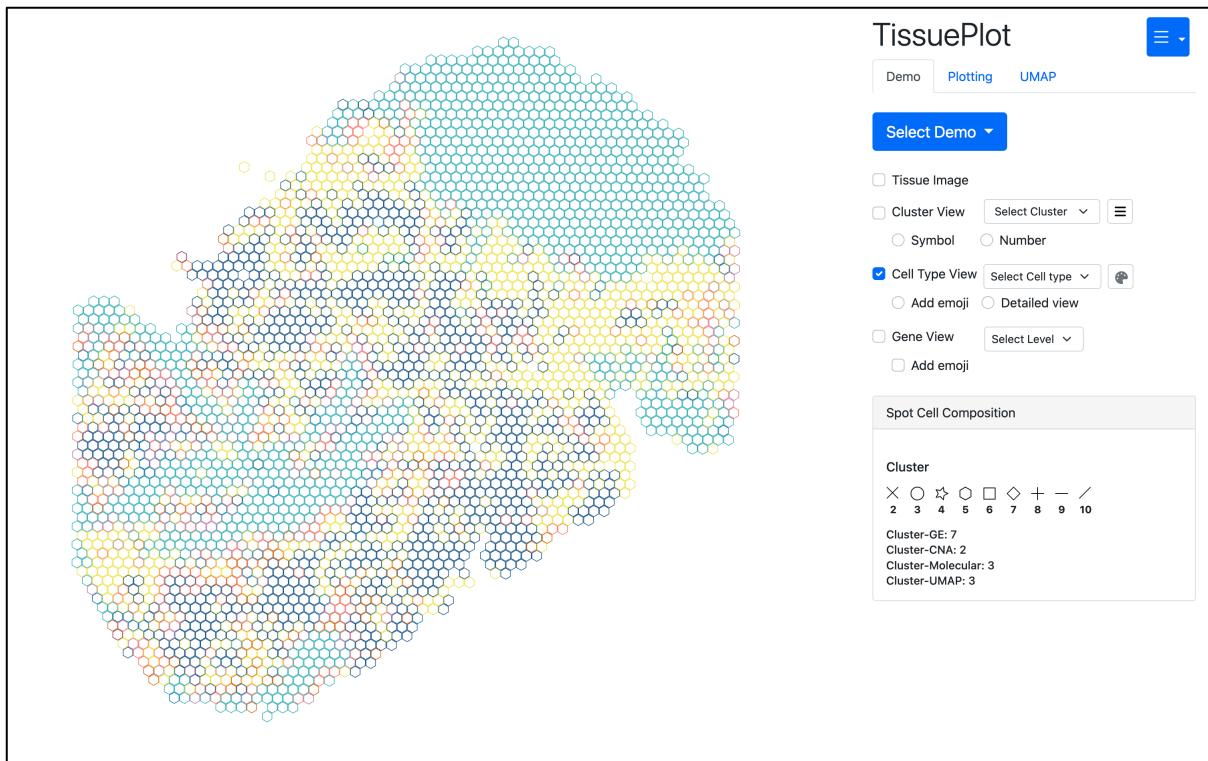


Figure: Cell Proportion View

- With the help of right most button “Color Palette” you can change the color map.

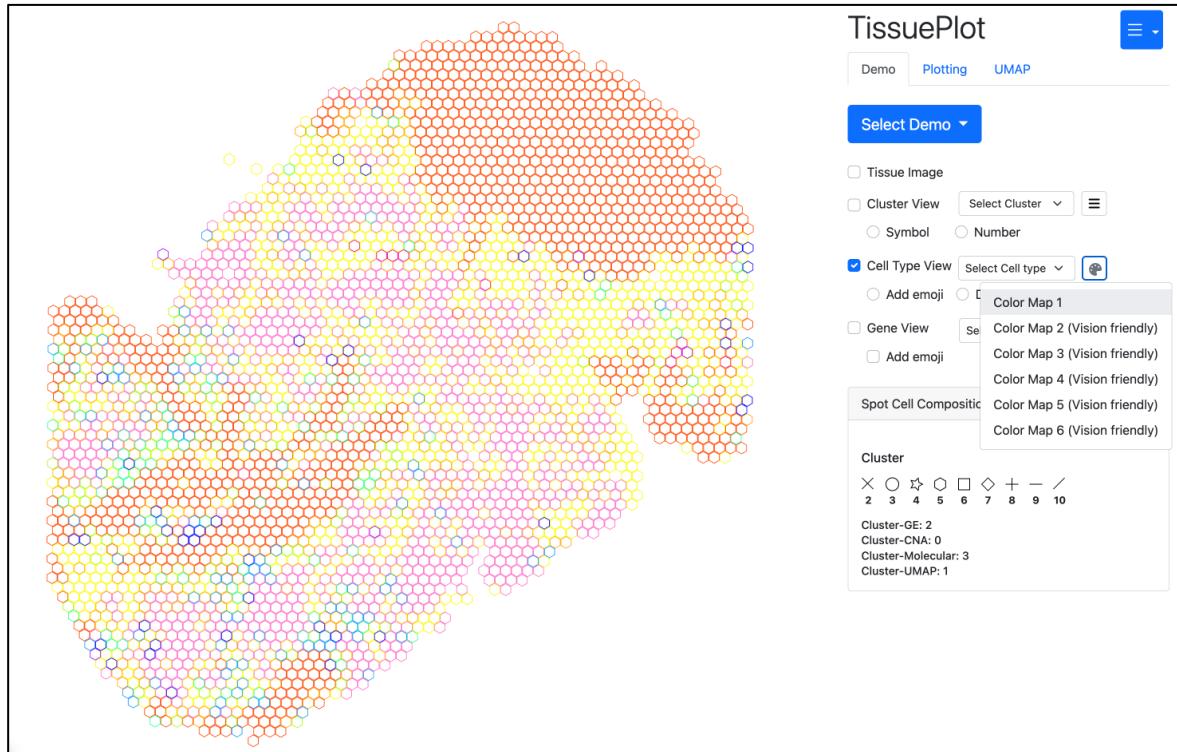


Figure: Cell Proportion View with color scale option

- Turn on the “Add emoji” toggle to see the emoji associated with most abundant cell type inside the spot.

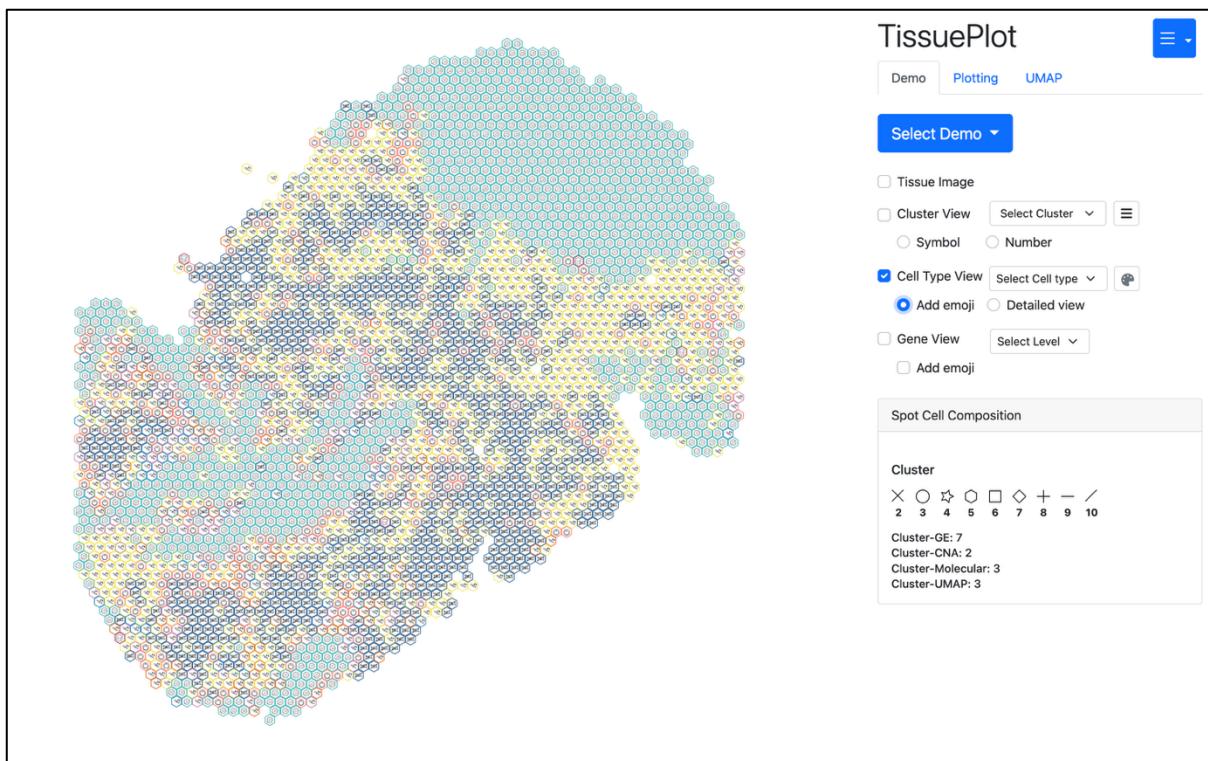


Figure: Cell Proportion View with Cell type Emoji

- Turn on the “Detailed view” toggle to see second and third most abundant cell types inside the same spot.

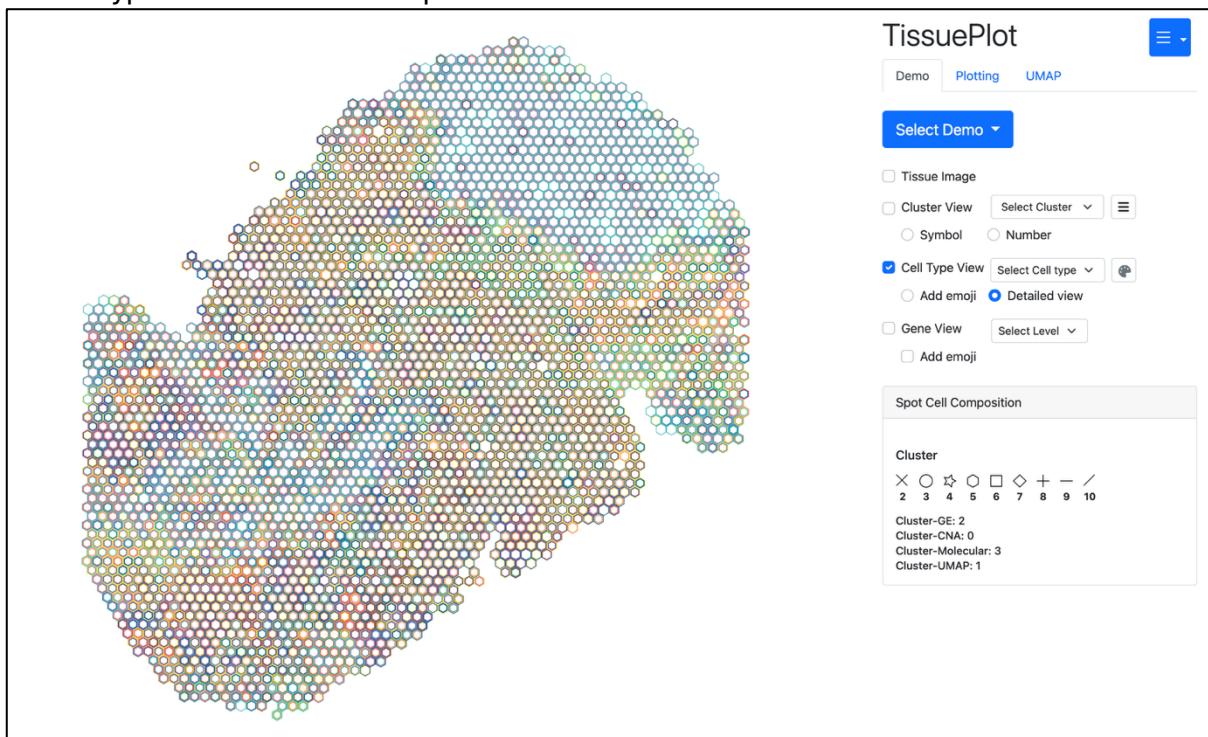


Figure: Cell Proportion View with Detailed view

- Hover over any hexagon to see a tooltip showing all detected cell types and their proportions value with cluster related information.

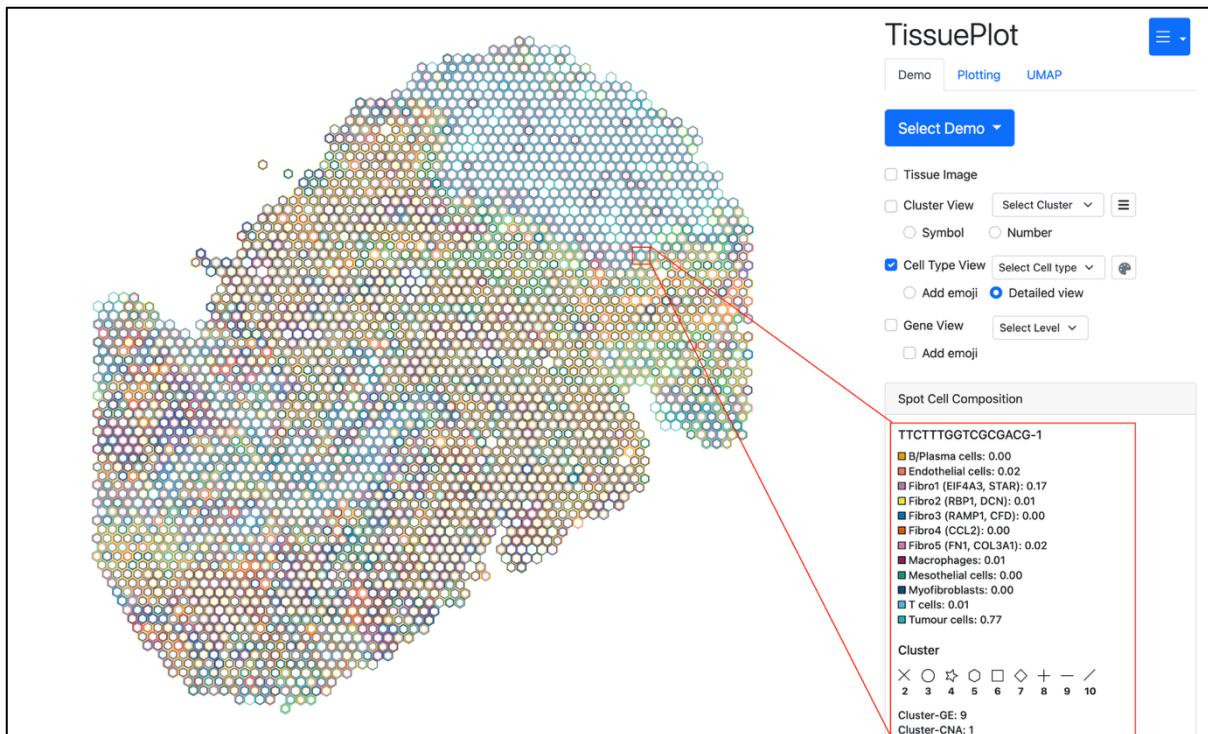


Figure: Information tooltip when hover over spot

- Click on hexagon to view visualization of spot related information and global information (This feature available with all the views).

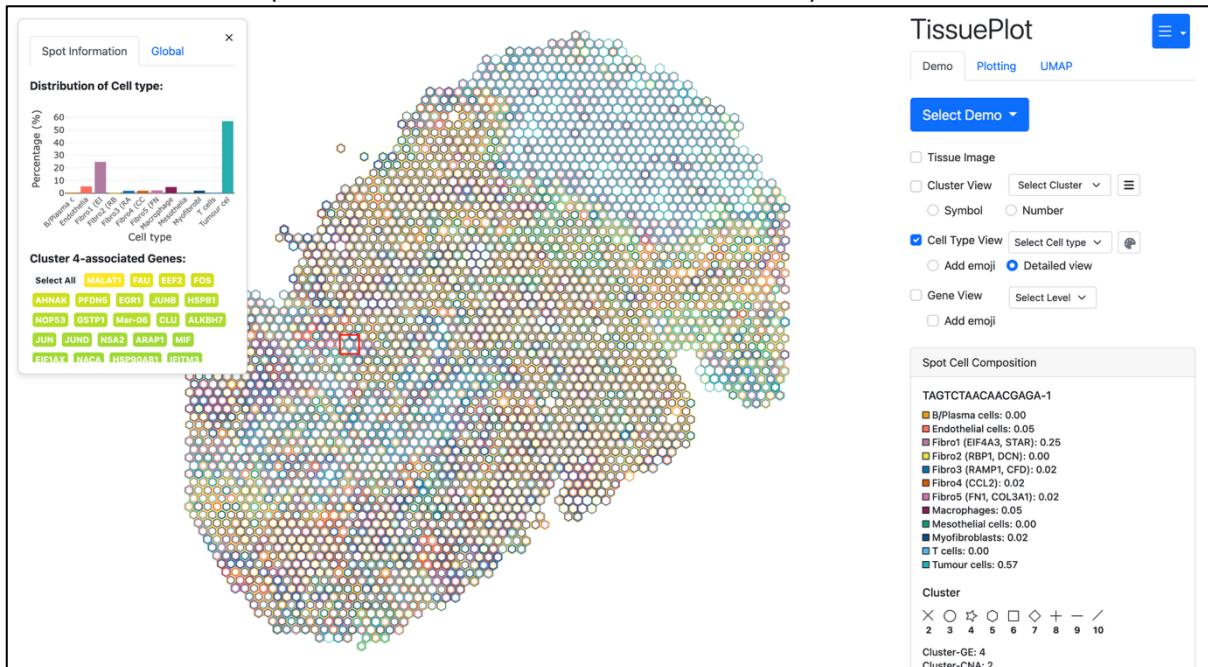


Figure: Spot information popup

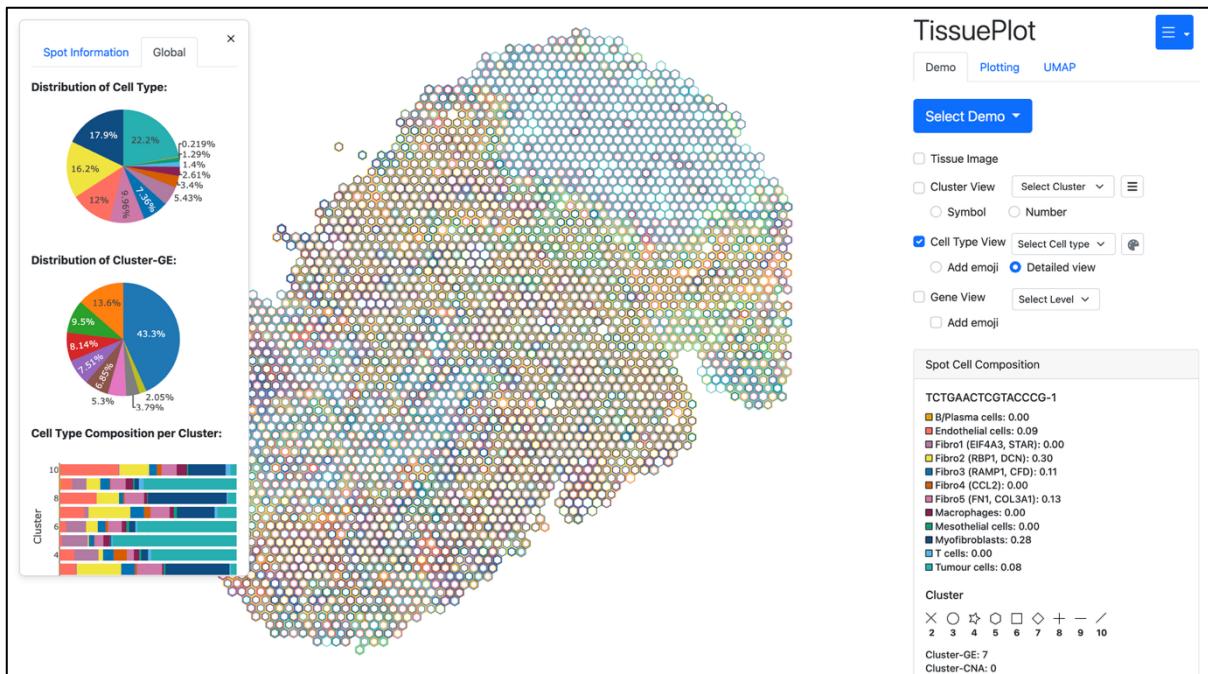


Figure: Global information popup

Step 3: View Clusters

- In the right sidebar, turn on the “Cluster View” option.
- You’ll now see that each hexagon has either a symbol or a number, showing which cluster it belongs to. These clusters are based on either gene expression or cell composition.
- By default, symbols are shown. You can switch between Symbol and Number view using the toggle next to the Cluster View option.

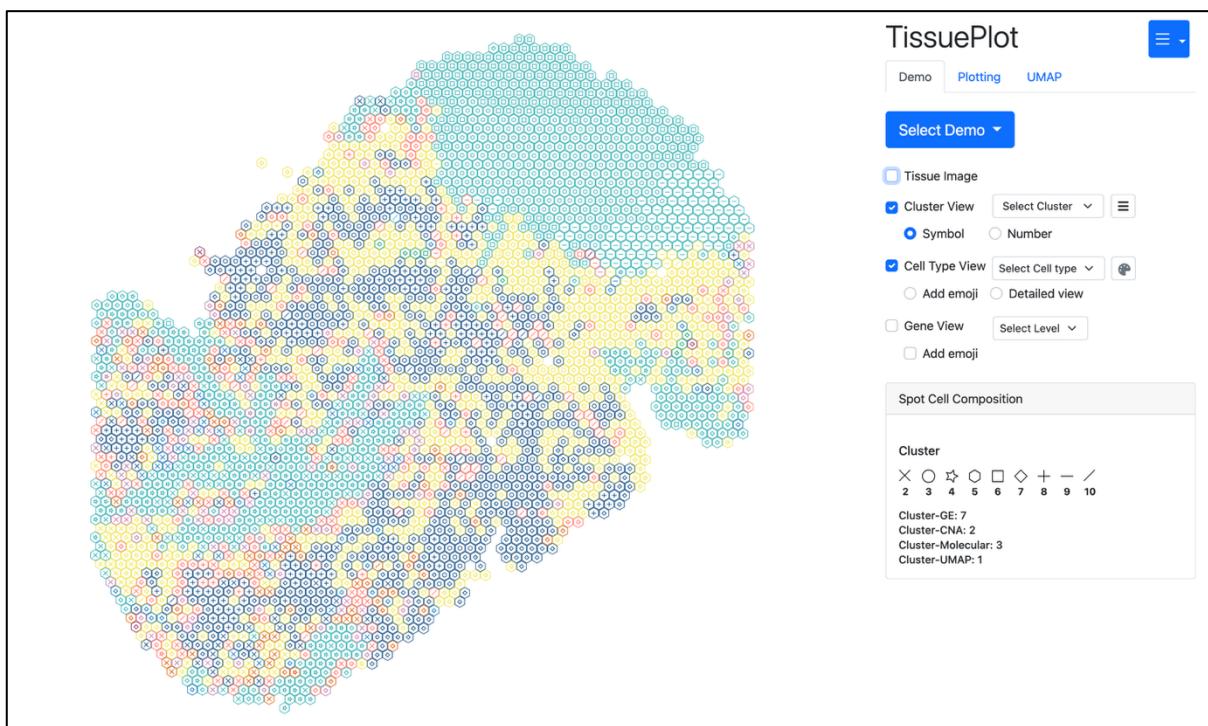


Figure: Cluster View with Symbols

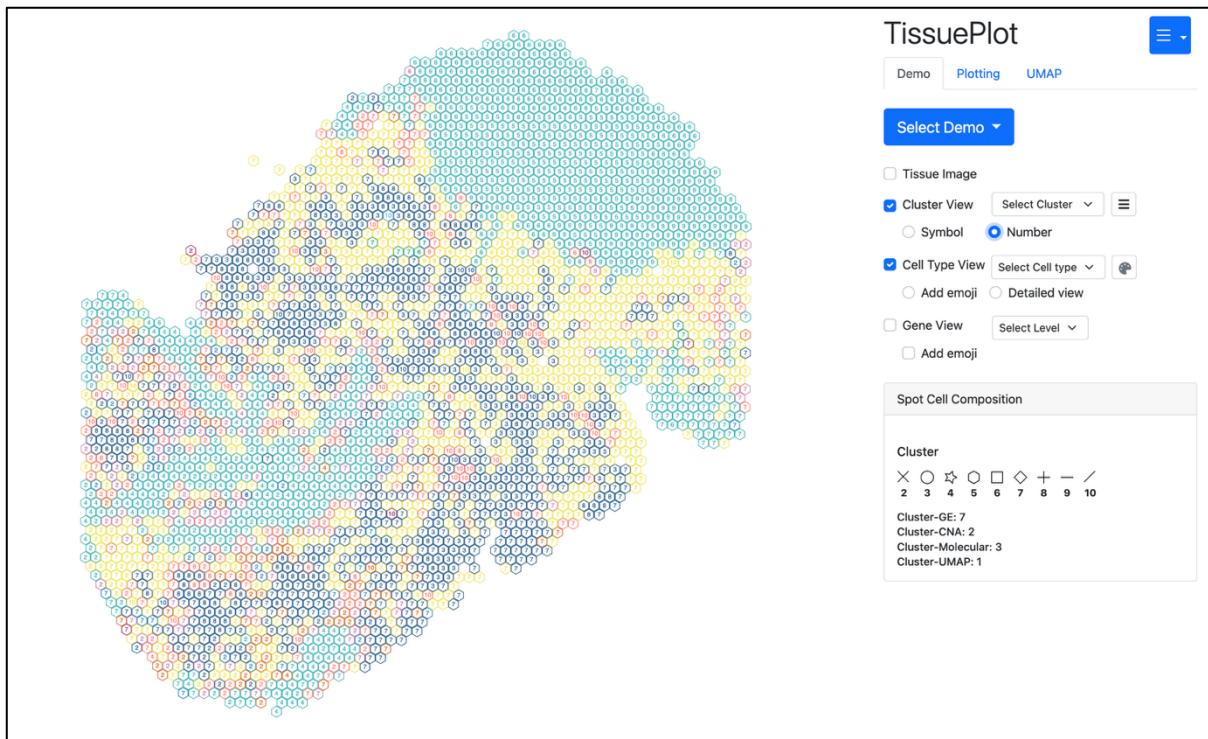


Figure: Cluster View with Number

- On the right side of the screen, you'll see a legend under the Spot cell information box showing all the clusters.
- Click on any symbol or number in the legend to filter the view. For example, click on Cluster 2 to show only spots from that cluster.
- Click the same symbol or number again to reset and show all clusters.

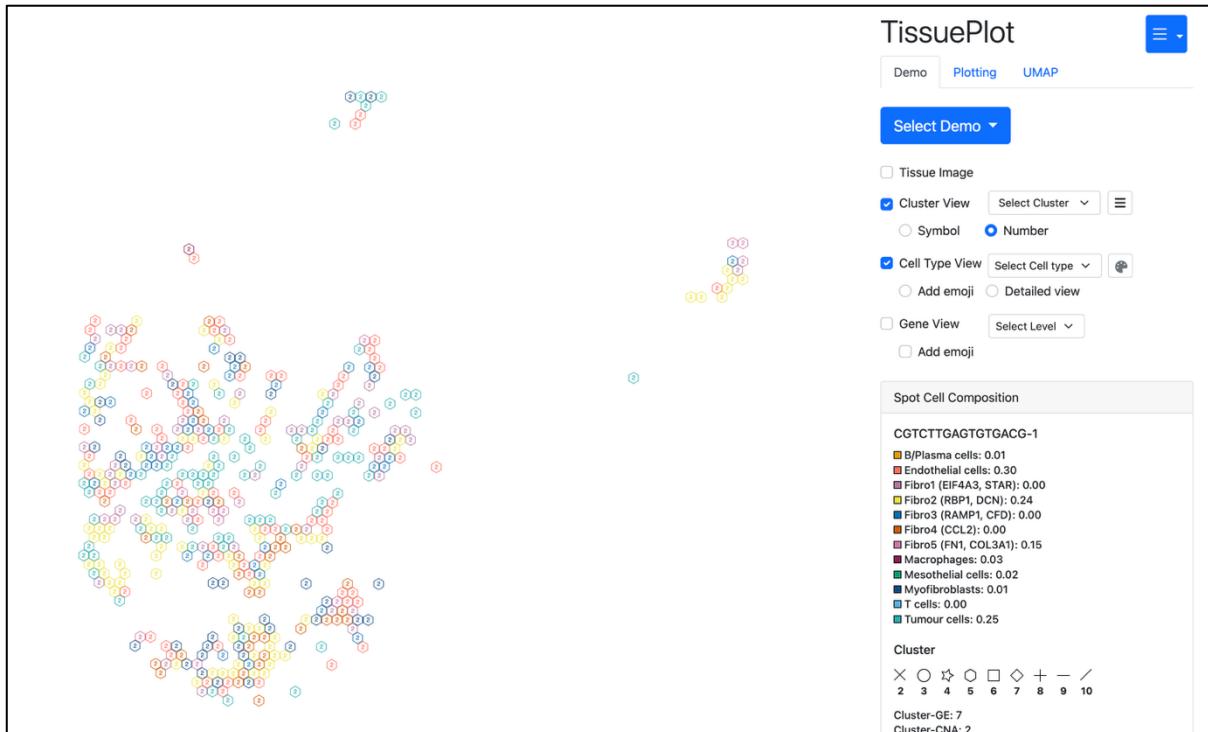


Figure: Interactive cluster filtering using the legend

Step 4: Explore Gene Expression

- Go to the right sidebar and click on “Gene View” to activate it.
- A dropdown will appear where you can choose which genes to visualize.
- By default, all genes are selected, but you can click “Select All” to deselect all genes and pick the one you want by clicking on them. To select multiple genes click ctrl + click on gene to select.
- Now, select the genes A1BG, AAMDC, and AARS from the dropdown list.

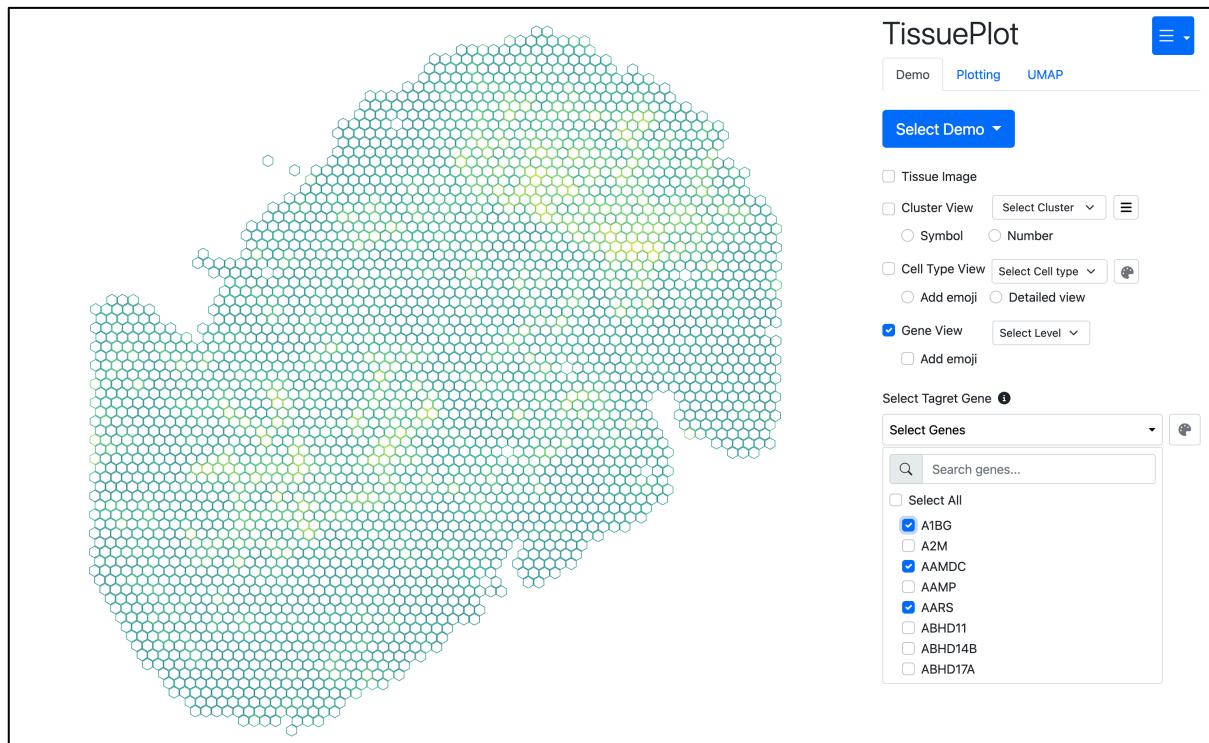


Figure: Exploring Gene Expression

- Just next to the gene dropdown, you'll see a “Color Palette” button.
- Click it to choose from different color maps: Viridis, Plasma, or Turbo.
- Try selecting Plasma to see how the colors change.
- Above the colors, use the intensity slider to adjust how strong the colors look.
- For example, increase the intensity from 1.0 to 1.5 to make the expression levels stand out more.
- Once you make changes to the color scale or intensity, a preview of the new scale will appear just below the slider so you can see exactly how your settings affect the visualization.

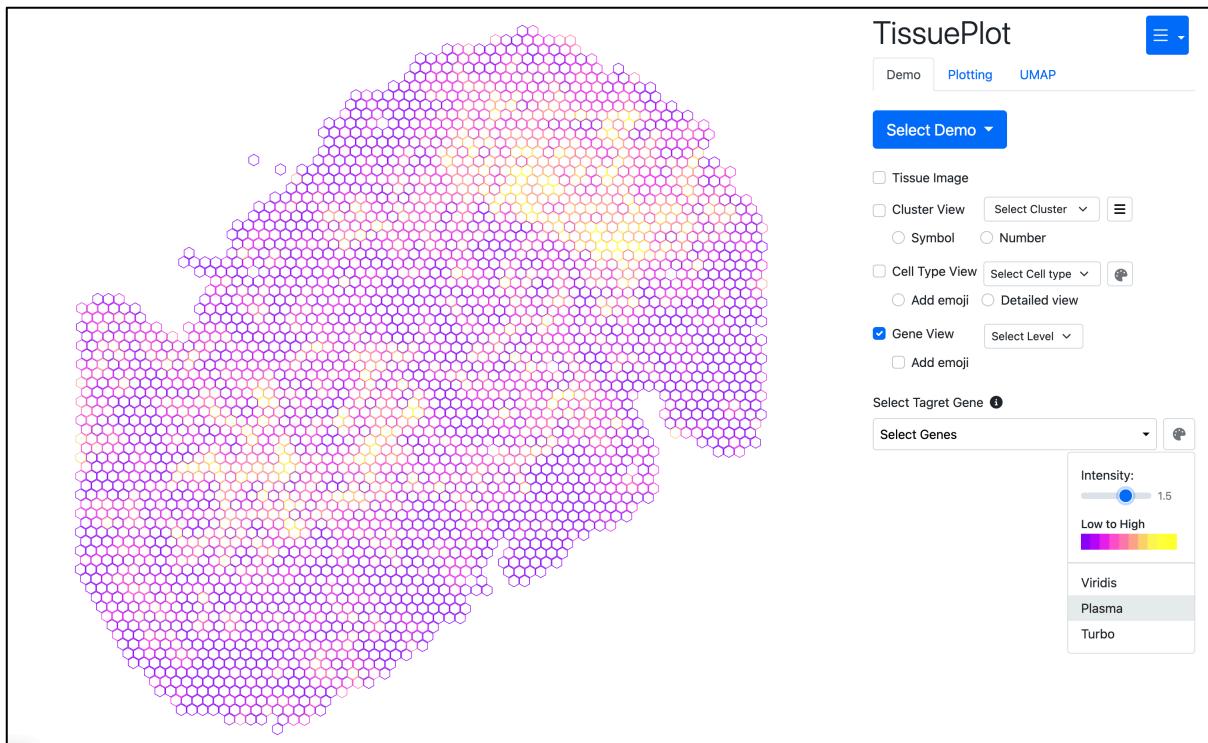


Figure: Gene View – Customization of Color Scale

Step 5: Tissue Image

- In the right sidebar, look for the “Tissue Image” checkbox and turn it on.
- As soon as you check it, the tissue image will appear underneath the hexagon spots on the Canvas.

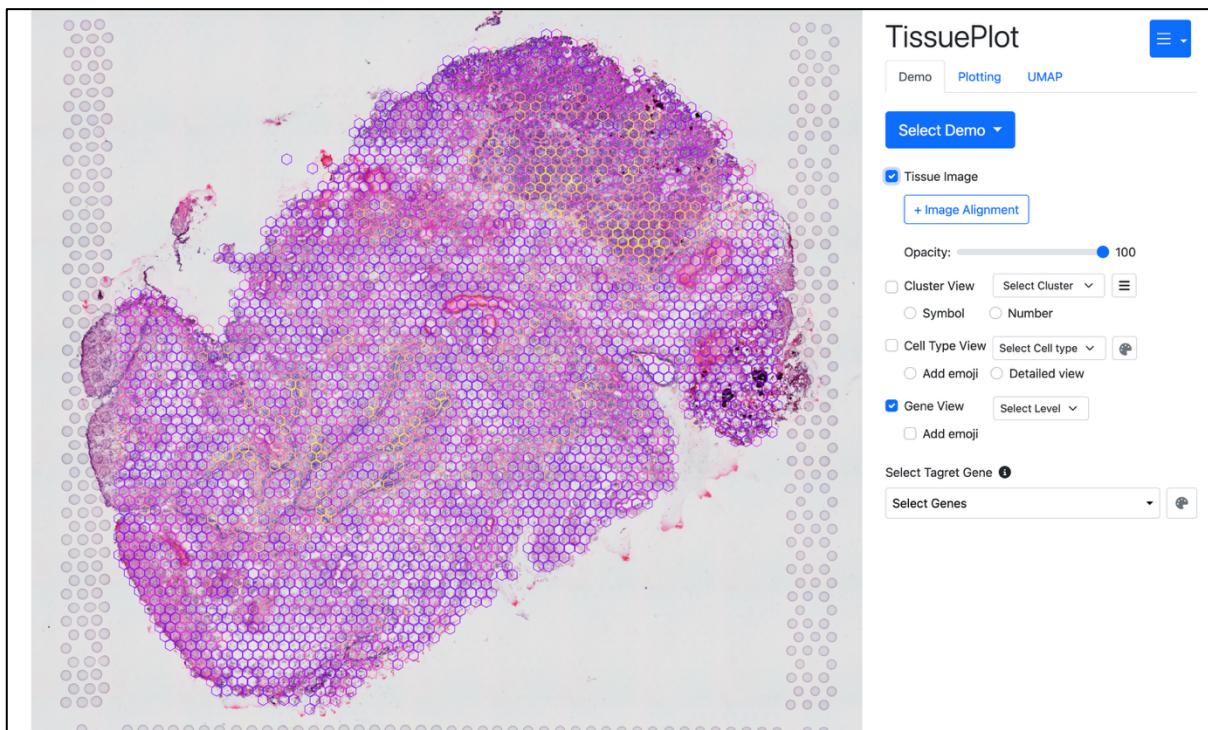


Figure: Tissue Image

- For demo datasets, the image is already aligned properly with the spots, so you should see everything matched up right away.
- After turning on the “Tissue Image” checkbox in the right sidebar, you’ll see an option called “Image Alignment” just below it.
- Click on it as this will expand the section and show you sliders to adjust the image alignment.
- To fine-tune the alignment or improve visibility use the opacity slider below the “Image Alignment” to make the tissue image lighter or darker. This helps you clearly see the data (hexagons) on top of the image. For example, decrease the opacity value from 100% to 50 % to see difference.

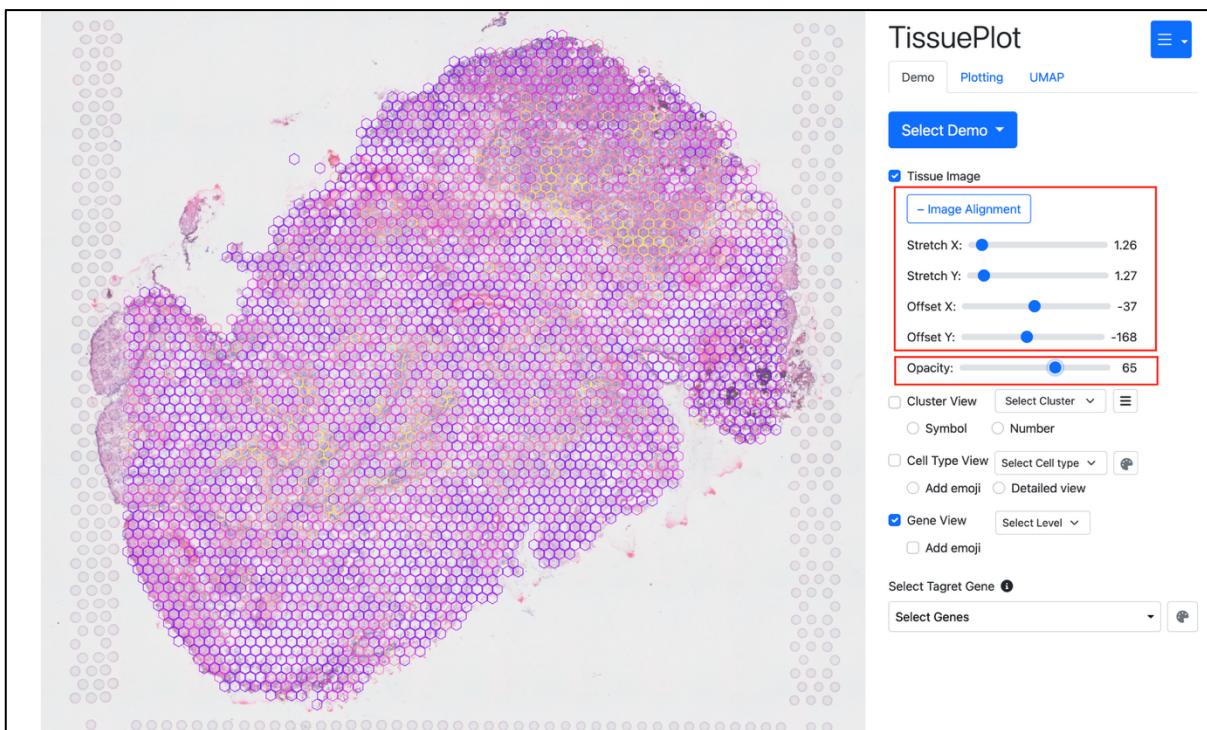


Figure: Customization of Image Alignment and Opacity

- Use the offset sliders to move the image left, right, up, or down.
- Use the scale sliders to stretch or shrink the image so it fits exactly under the hexagon layout.

Step 6: UMAP Tab for more insights

- Click on the UMAP tab at the top-right side of the page.
- A 2D scatter plot will appear, each dot represents a spot from the tissue.
- Hover over any dot to see a tooltip showing:
 - Associated cluster types with cluster number
 - The top 3 cell types

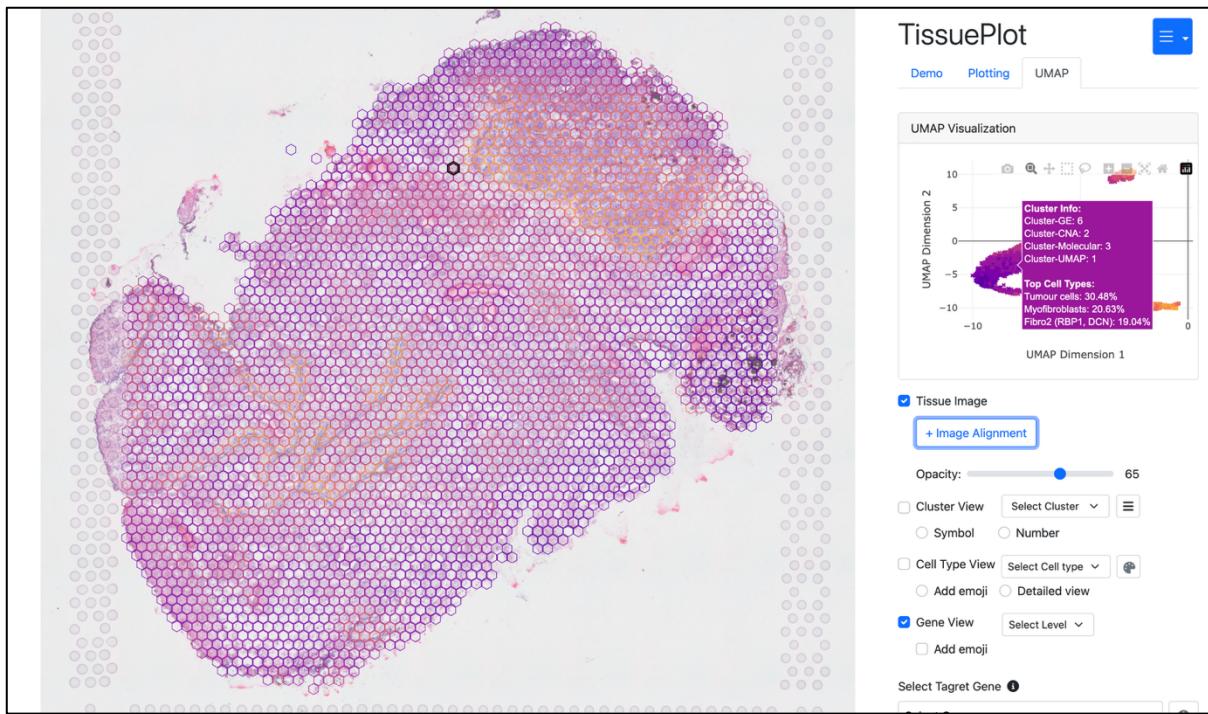


Figure: Interacting with UMAP

- Click on the “Select Cluster” dropdown. You’ll see a list of all available clusters. Now, try selecting clusters 2, 5, and 9.
- Only the spots from these selected clusters will be colored in the UMAP plot. The rest will appear faded making it easy to focus on specific spots.

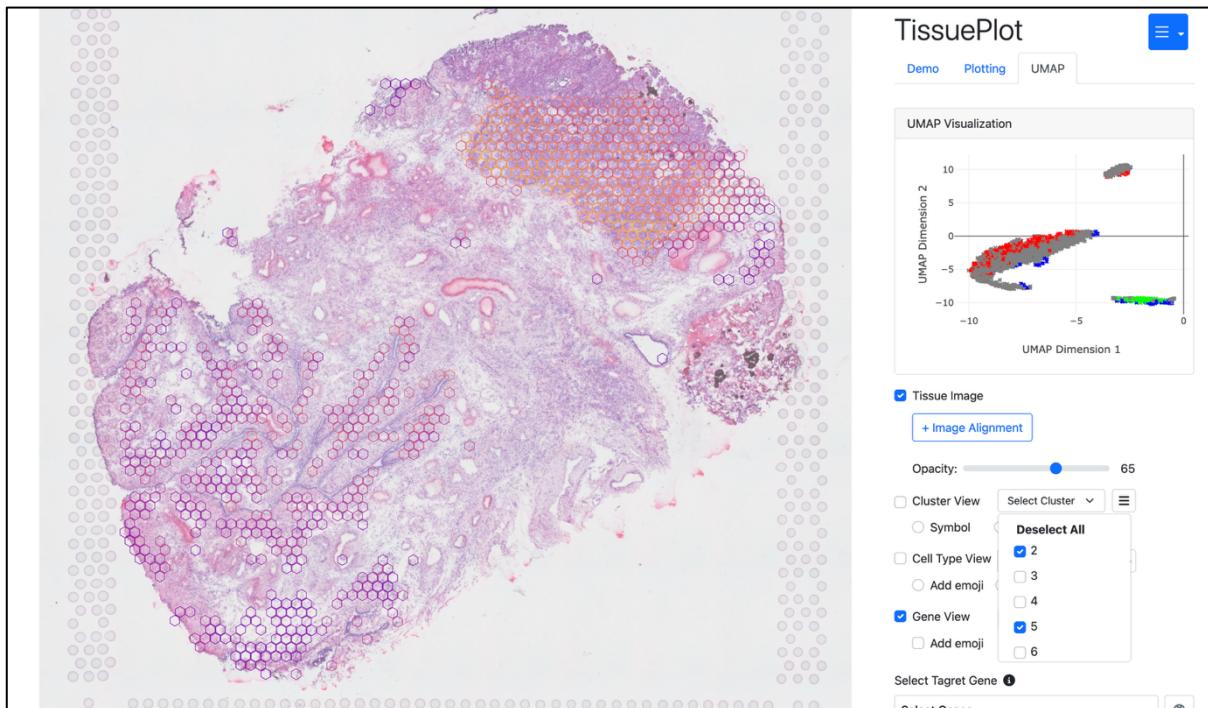


Figure: Highlighting Clusters in UMAP and Main Canvas

- Similar to highlighting Clusters based on selection from “Select Cluster” dropdown, you can also highlight the UMAP plot and main Canvas with specific cell type from “Select Cell type” dropdown.

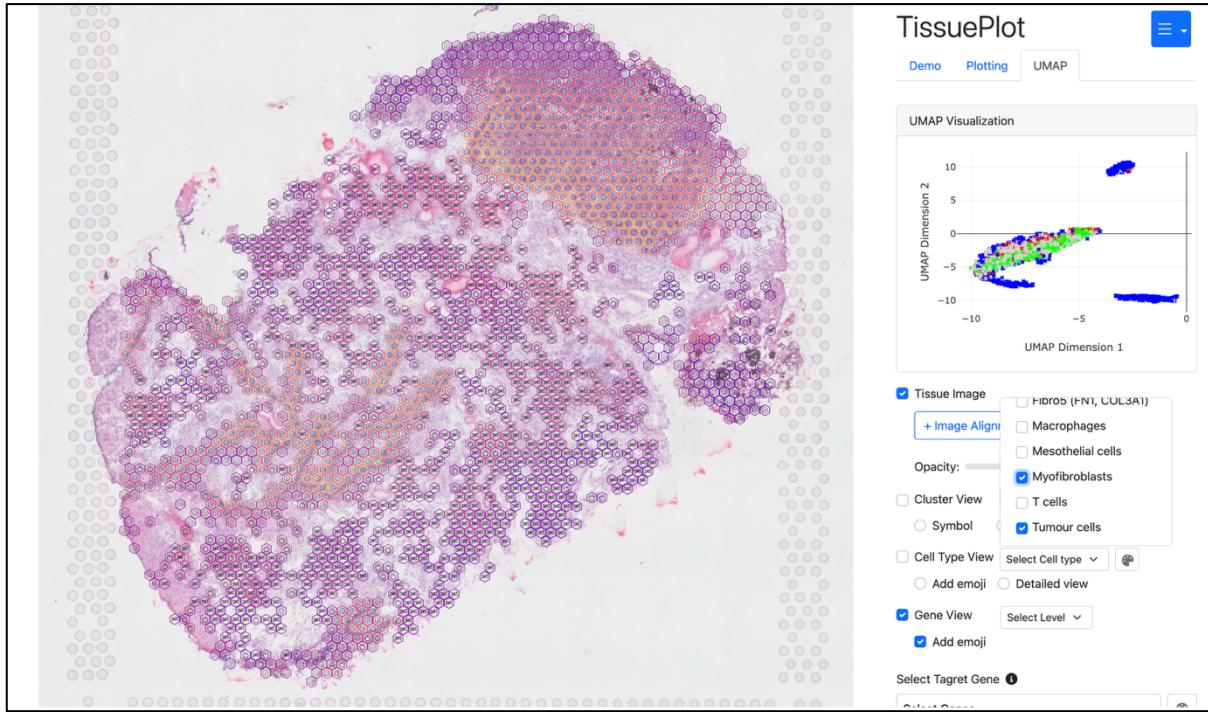


Figure: Highlighting Cell type in UMAP and Main Canvas

- Now, come to the main Canvas view. Hover over any spot on the tissue, that same spot will be highlighted in the UMAP plot, helping you connect the two views and vice versa. (This works in both Cell Composition View and Gene View)

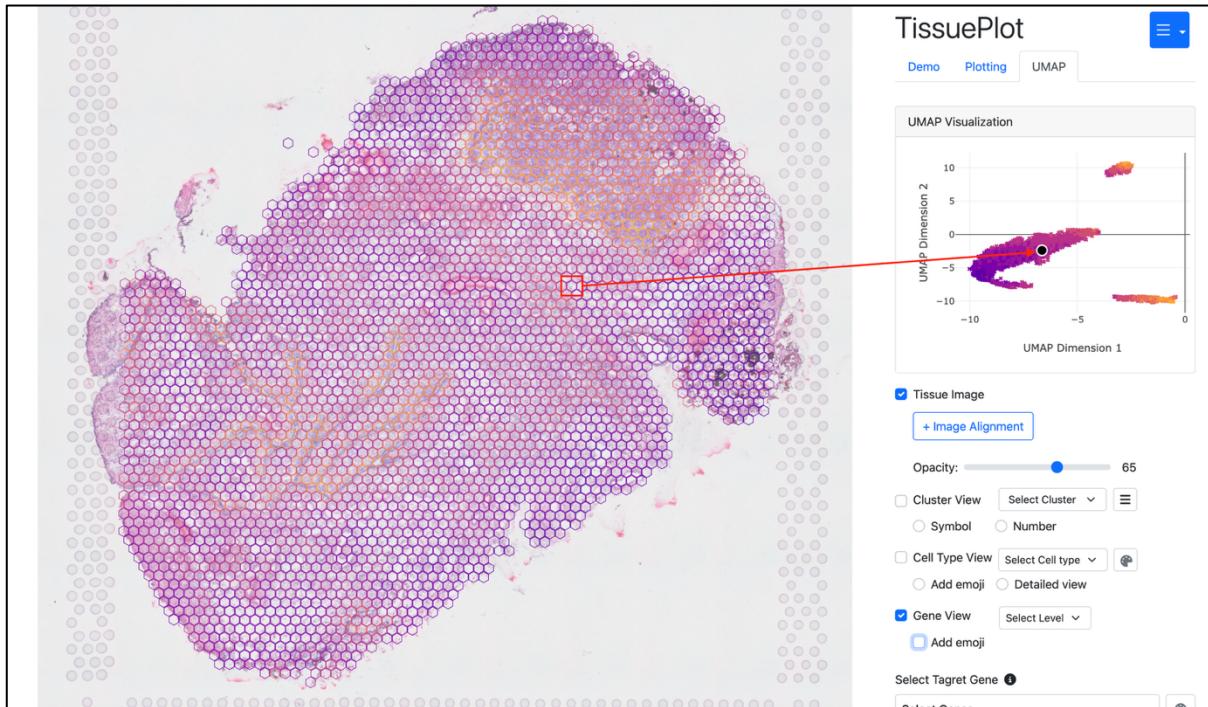


Figure: Linking UMAP to Main Canvas

- Try changing the color map in Gene View (for example, switch from Viridis to Plasma) and hover over the spot from main Canvas view.
- The UMAP plot will update instantly with the new colors, showing gene expression levels across spots.

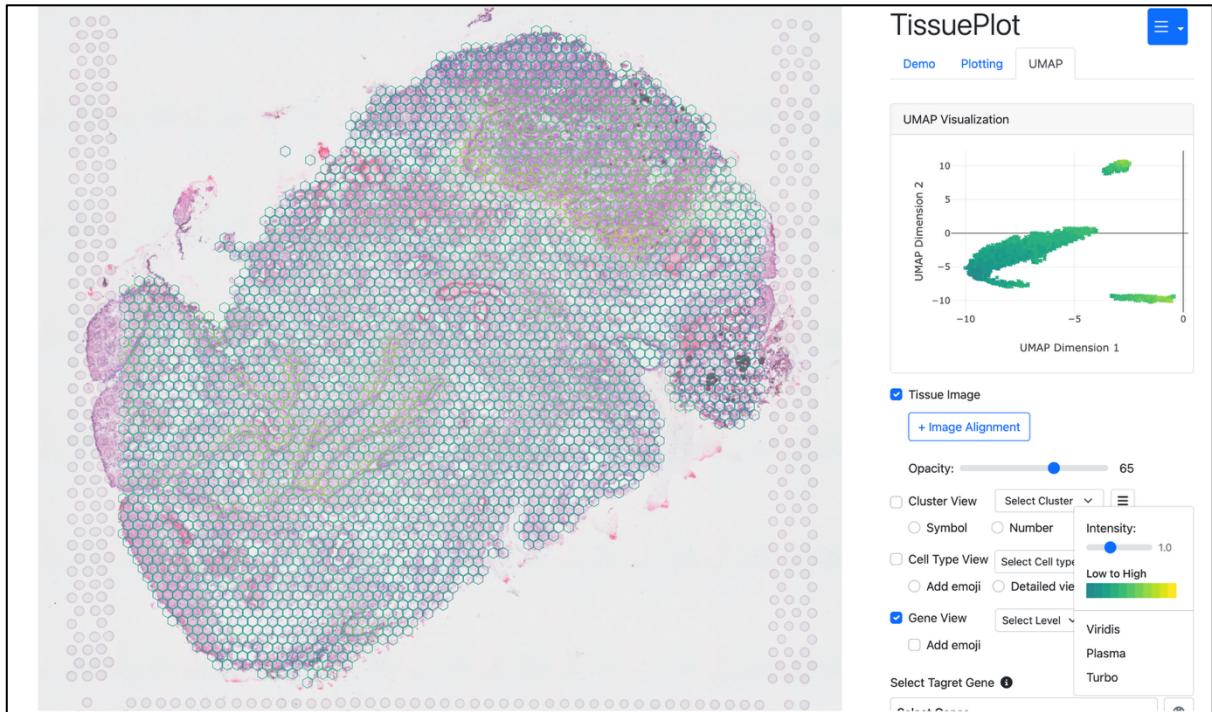


Figure: Color Coding UMAP by Gene Expression

4 Summary

TissuePlot empowers researchers to explore complex spatial biology datasets interactively.

It bridges the gap between raw numbers and spatial understanding by offering:

- Easy upload,
- Flexible viewing,
- Powerful filtering,
- Gene exploration,
- Cluster dynamics visualization,
- Tissue integration,
- UMAP space reduction,
- And customizable color design.

With TissuePlot, understanding the hidden structure of tissue samples becomes faster, easier, and beautifully intuitive.

5 Contact us

For any queries, please contact heba dot sailem at kcl.ac.uk

6 References

1. Denisenko, E. et al. Spatial transcriptomics reveals discrete tumour microenvironments and autocrine loops within ovarian cancer subclones. *Nat Commun* **15**, 2860 (2024).