

Classify cells from multiple samples using t-SNE

- [Multiple single-sample t-SNE plots](#)
- [One multi-sample t-SNE plot](#)

t-SNE (t-distributed stochastic neighbor embedding) is a visualization method commonly used to analyze single-cell RNA-Seq data. Each cell is shown as a point on the plot and each cell is positioned so that it is close to cells with similar overall gene expression. When working with multiple samples, a t-SNE plot can be drawn for each sample or all samples can be combined into a single plot. Viewing samples individually is the default in Partek® Flow® because sample to sample variation and outlier samples can obscure cell type differences if all samples are plotted together. However, as you will see in this tutorial, in some data sets, cell type differences can be visualized even when samples are combined.

Using the t-SNE plot, cells can be classified based on clustering results and differences in expression of key marker genes.

Multiple single-sample t-SNE plots

Prior to performing t-SNE, it is a good idea to reduce the dimensionality of the data using principal components analysis (PCA).

- Click the **Filtered counts** data node after the *Filter features* task
- Select **PCA** from the *Exploratory analysis* section of the task menu (Figure 1)

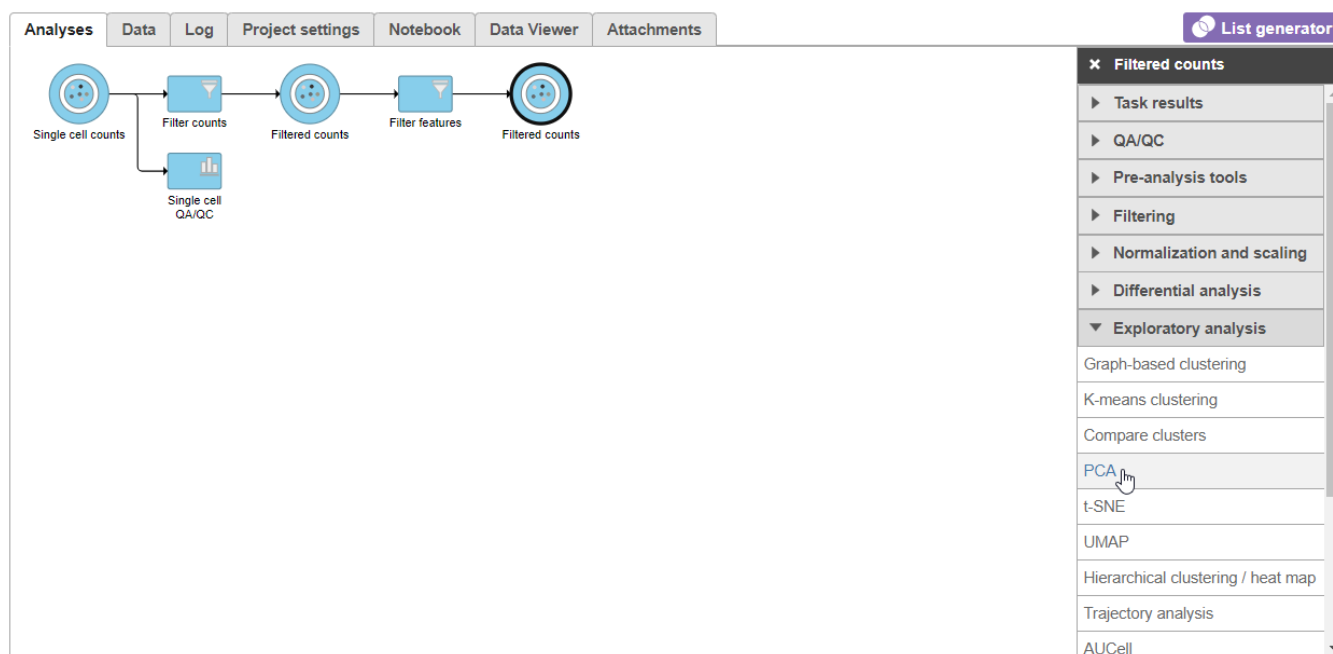


Figure 30. Select the PCA task from the Exploratory analysis menu

- Click **Finish** to run PCA with default settings (Figure 2)

Note, the default settings include the *Split by sample* checkbox being selected. This means that the dimensionality reduction will be performed on each sample separately.

Home > Glioma (multi-sample) > PCA

PCA

Number of principal components 

☐ All

☒ 100  

Features contribute 

☐ equally ☒ by variance

Grouping

Split by sample 

Back

Finish

Figure 31. PCA task set up page with default settings

PCA task and data nodes will be generated.

- Click the **PCA** data node
- Select **t-SNE** from the *Exploratory analysis* section of the task menu (Figure 3)

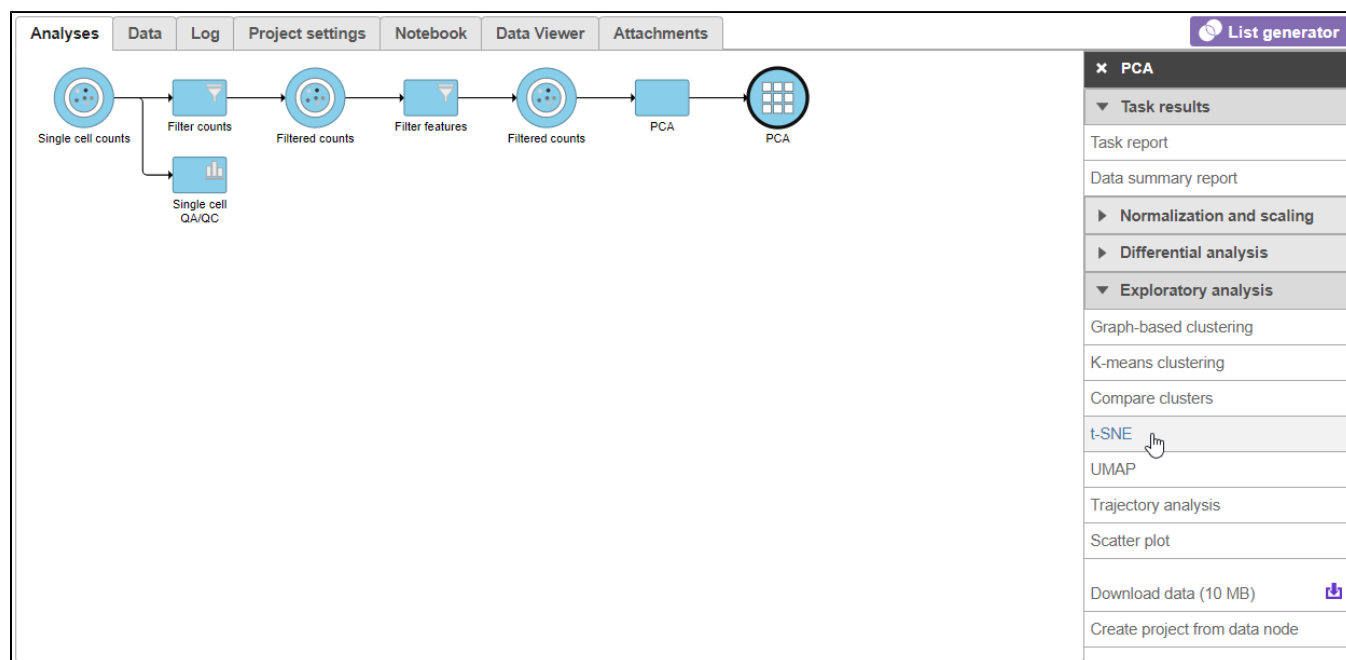


Figure 32. Invoking t-SNE from the task menu

- Click **Finish** from the *t-SNE* dialog to run t-SNE with the default settings (Figure 4)

[Home](#) > [Glioma \(multi-sample\)](#) > [t-SNE](#)

PCA

Number of principal components 

☐ All

☒

Advanced options

Option set

-- Default --

[Configure](#)

[Back](#)

[Finish](#)

Figure 33. t-SNE task set up with default settings

Because the upstream PCA task was performed separately for each sample, the t-SNE task will also be performed separately for each sample. t-SNE task and data nodes will be generated (Figure 5).

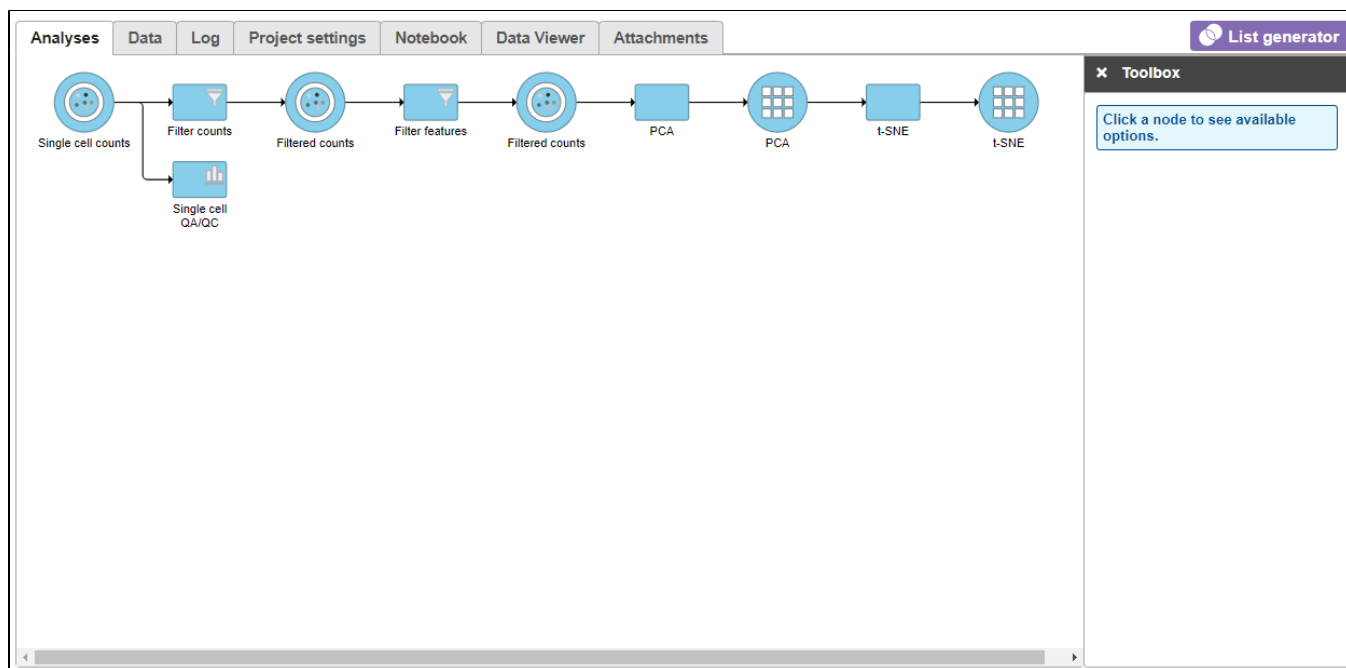


Figure 34. t-SNE task node

Once the t-SNE task has completed, we can view the t-SNE plots

- Click the **t-SNE** node
- Click **Task report** from the task menu or double click the *t-SNE* node

The t-SNE will open in a new data viewer session. The t-SNE plot for the first sample in the data set, MGH36 (Figure 6), will open on the canvas. Please note that the appearance of the t-SNE plot may differ each time it is drawn so your t-SNE plots may look different than those shown in this tutorial. However, the cell-to-cell relationships indicated will be the same.

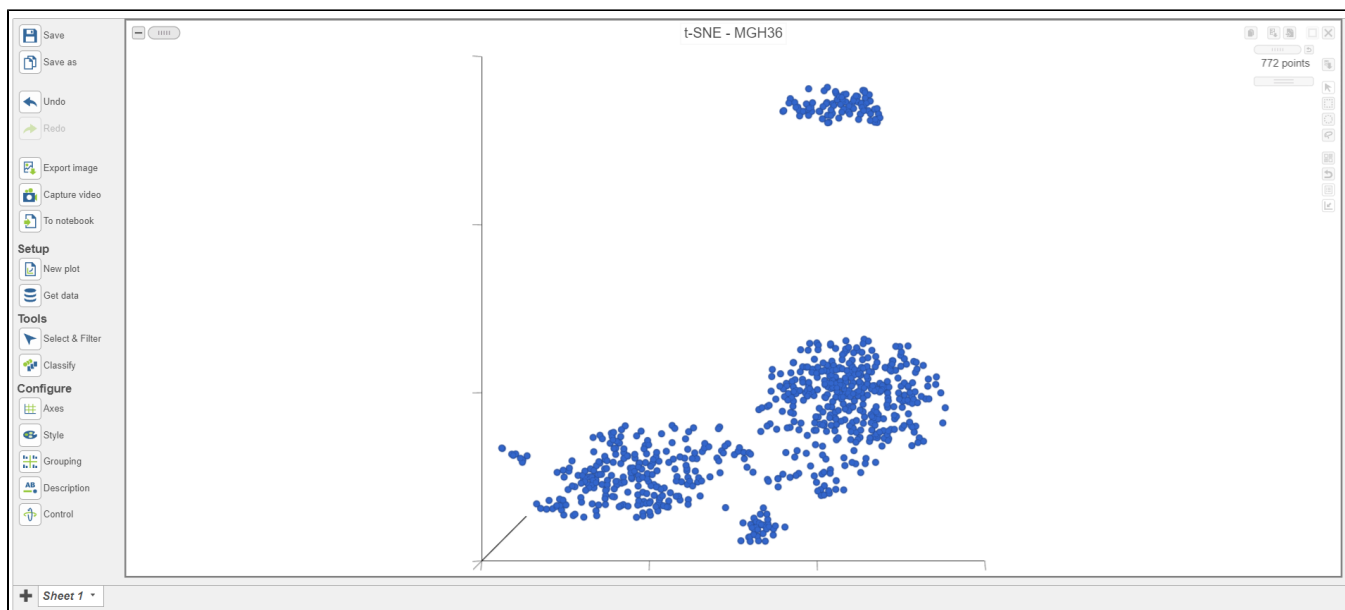



Figure 35. Viewing t-SNE plot of a single sample

The t-SNE plot is in 3D by default. To change the default, click your avatar in the top right > *Settings* > *My Preferences* and edit your graphics preferences and change the default scatter plot format from 3D to 2D.

You can rotate the 3D plot by left-clicking and dragging your mouse. You can zoom in and out using your mouse wheel. The 2D t-SNE is also calculated and you can switch between the 2D and 3D plots on the canvas. We will do this later on in the tutorial.

Each sample has its own plot. We can switch between samples.

- Open the **Axes** icon on the left under *Configure* (Figure 7)
- Navigate to *Misc*
- Select the  icon below the *Sample* name to go to the next sample

The t-SNE plot has switched to show the next sample, MGH42 (Figure 7).

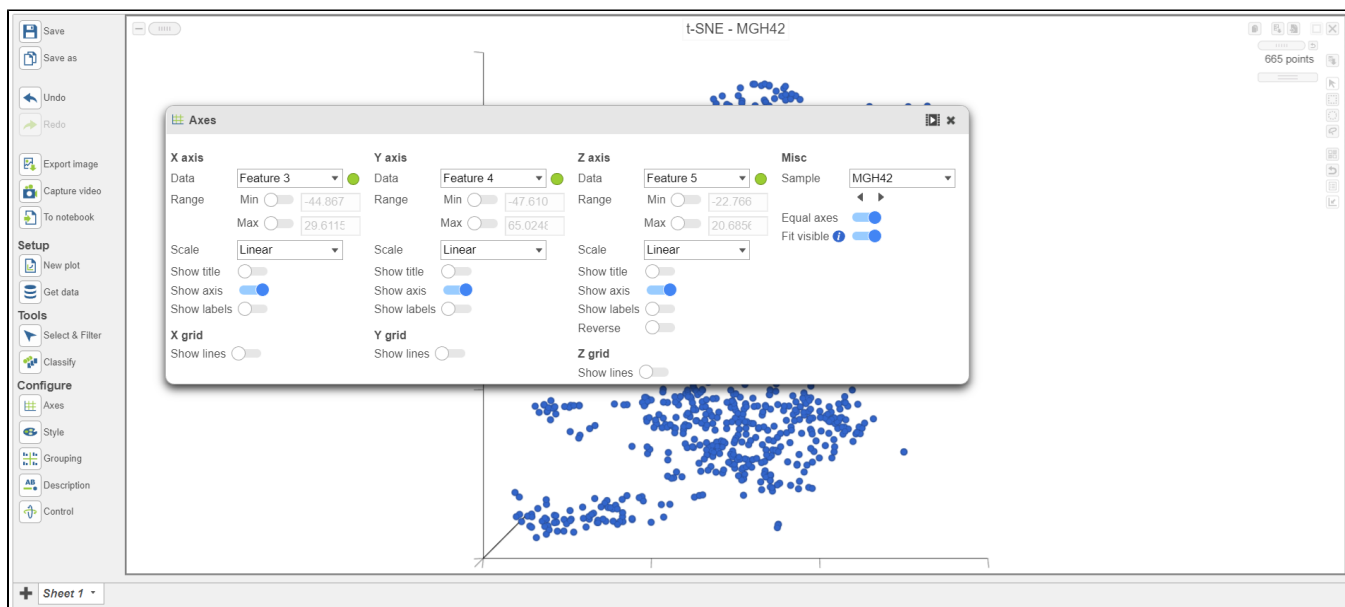


Figure 36. Viewing t-SNE plot of MGH42

The goal of this analysis is to compare malignant cells from two different glioma subtypes, astrocytoma and oligodendroglioma. To do this, we need to identify the malignant cells we want to include and which cells are the normal cells we want to exclude.

The t-SNE plot in Partek Flow offers several options for identifying, selecting, and classifying cells. In this tutorial, we will use the expression of known marker genes to identify cell types.

To visualize the expression of a marker gene, we can color cells on the t-SNE plot by their expression level.

- Select any of the count data nodes from **Get data** on the left (Single cell counts, or any of the Filtered counts, Figure 8)
- Search for the **BCAN** gene
- Click and drag the BCAN gene onto the plot and drop it over the **Green (feature)** option

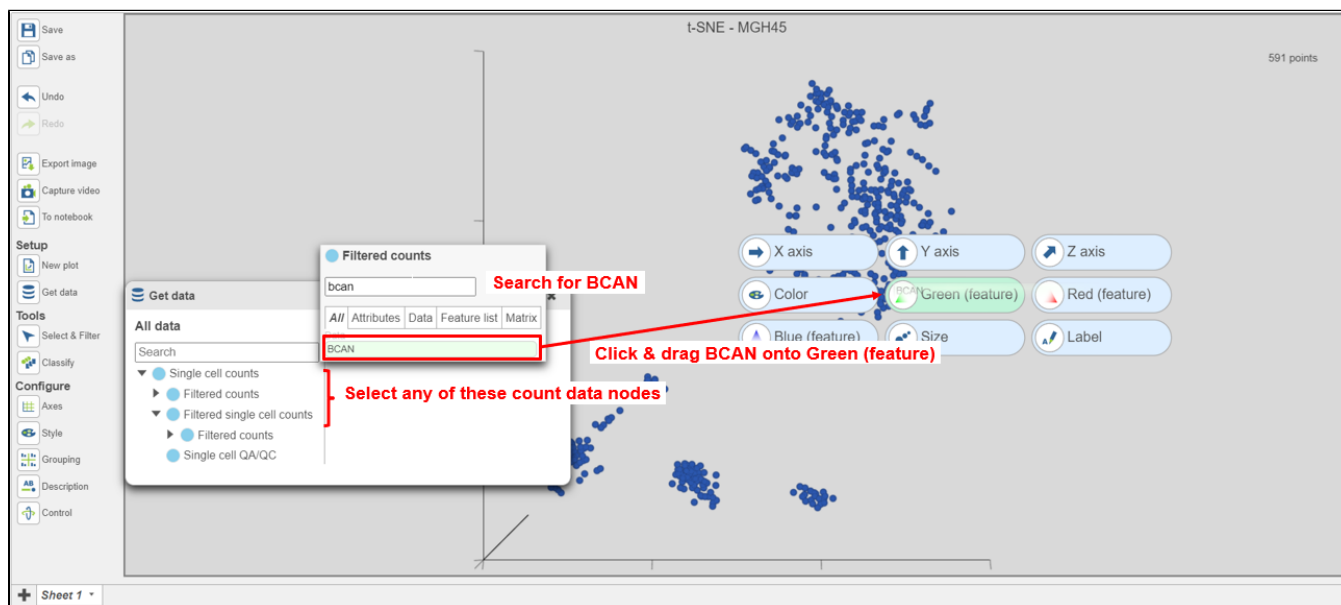


Figure 37. Coloring cells by BCAN expression

The cells will be colored from black to green based on their expression level of BCAN, with cells expressing higher levels more green (Figure 9). BCAN is highly expressed in glioma cells.

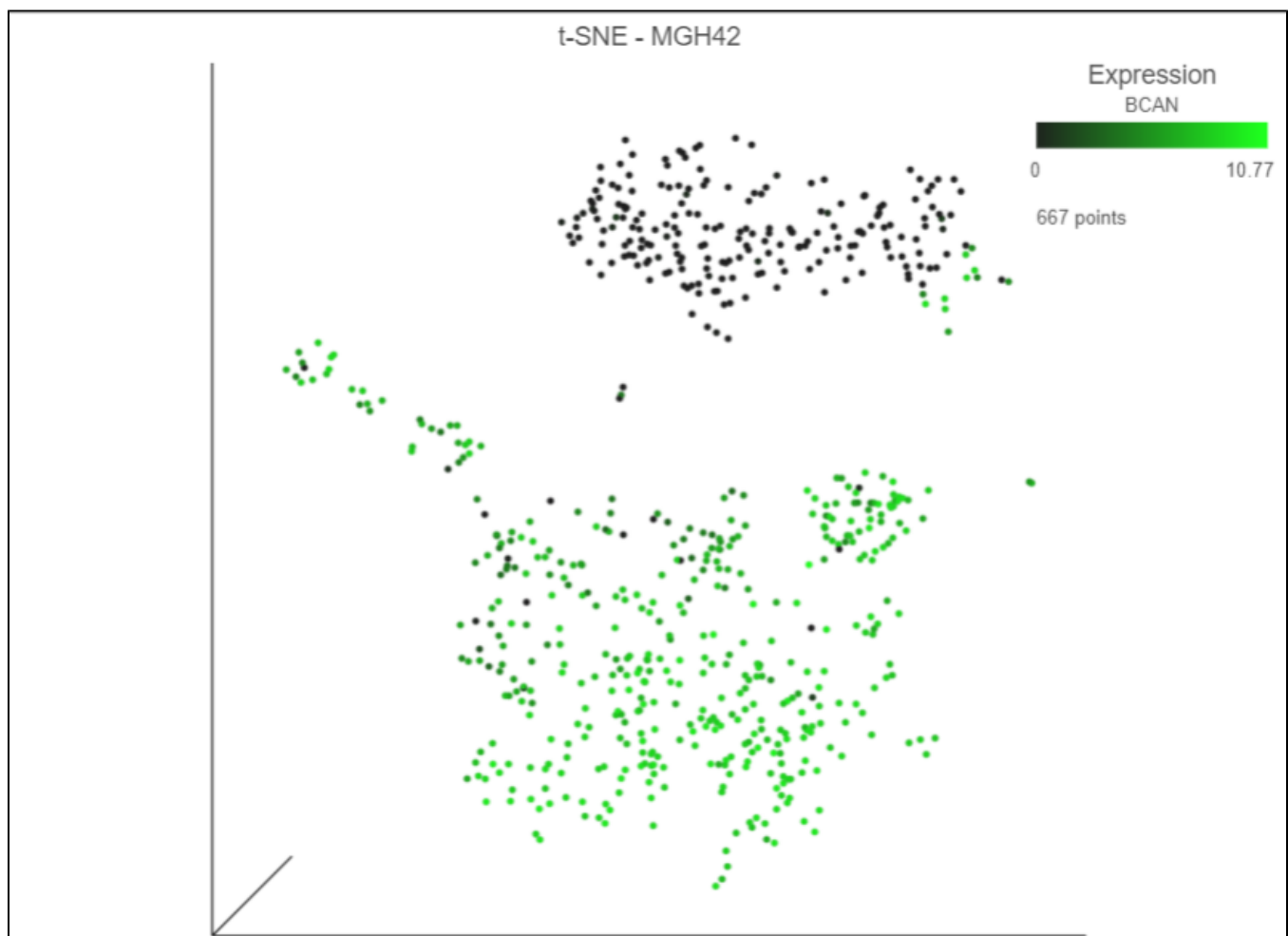


Figure 38. Cells colored by BCAN expression

In Partek Flow, we can color cells by more than one gene. We will now add a second glioma marker gene, GPM6A.

- Select any of the count data nodes from the *Data* card on the left (Single cell counts, or any of the Filtered counts)
- Search for the **GPM6A** gene
- Click and drag the GPM6A gene onto the plot and drop it over the **Red (feature)** option

Cells expressing GPM6A are now colored red and cells expressing BCAN are colored green. Cells expressing both genes are colored yellow, while cells expressing neither are colored black (Figure 10).

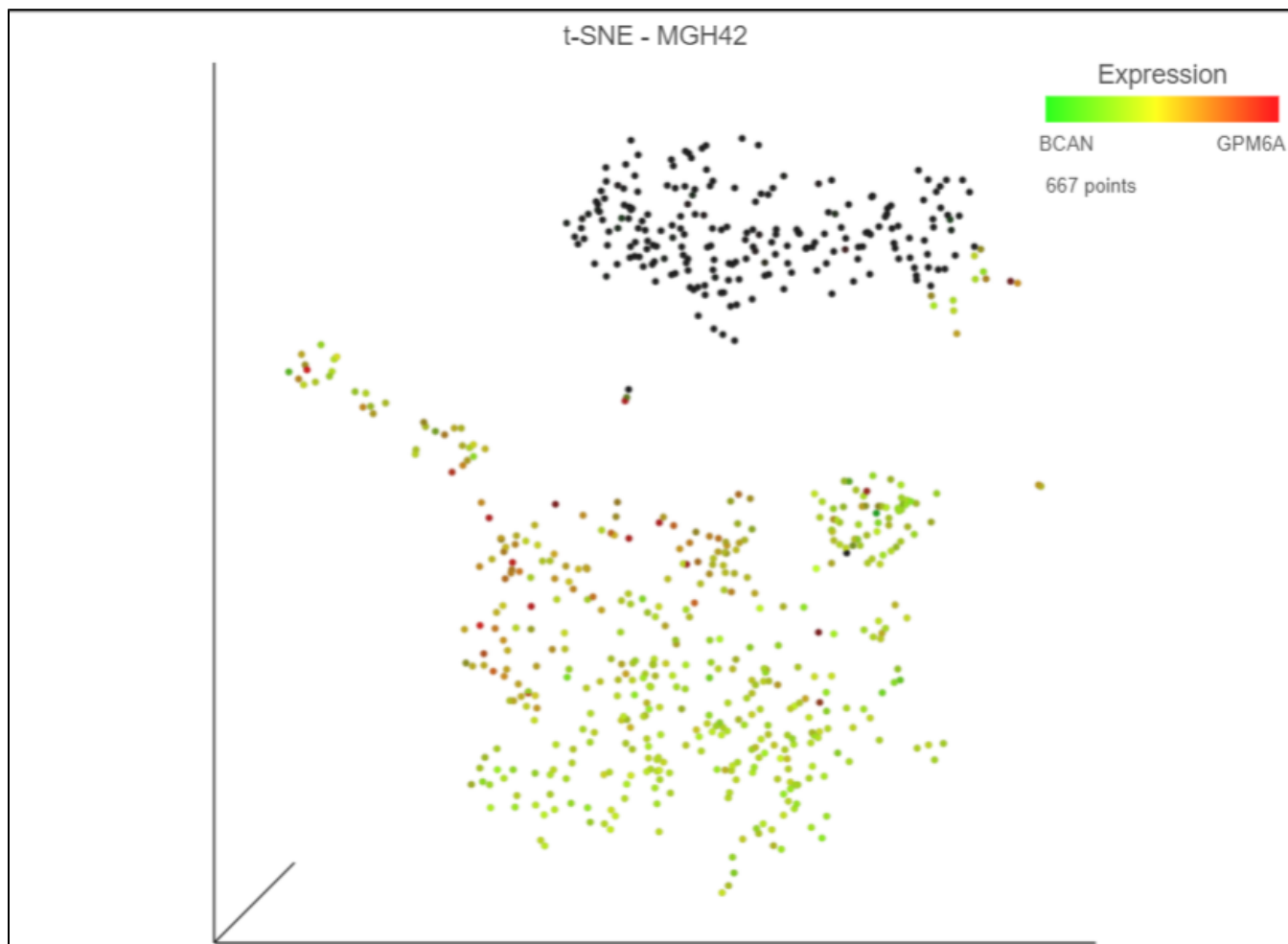


Figure 39. Coloring cells by BCAN and GPM6A

Numerical expression levels for each gene can be viewed for individual cells.

- Switch to pointer mode by clicking  in the top right corner of the plot
- Select a cell by pointing and clicking

The expression level for that cell is displayed on the legend for each gene. Expression values can also be viewed by mousing over a cell (Figure 11).

- Deselect the cell by clicking on any blank space on the plot

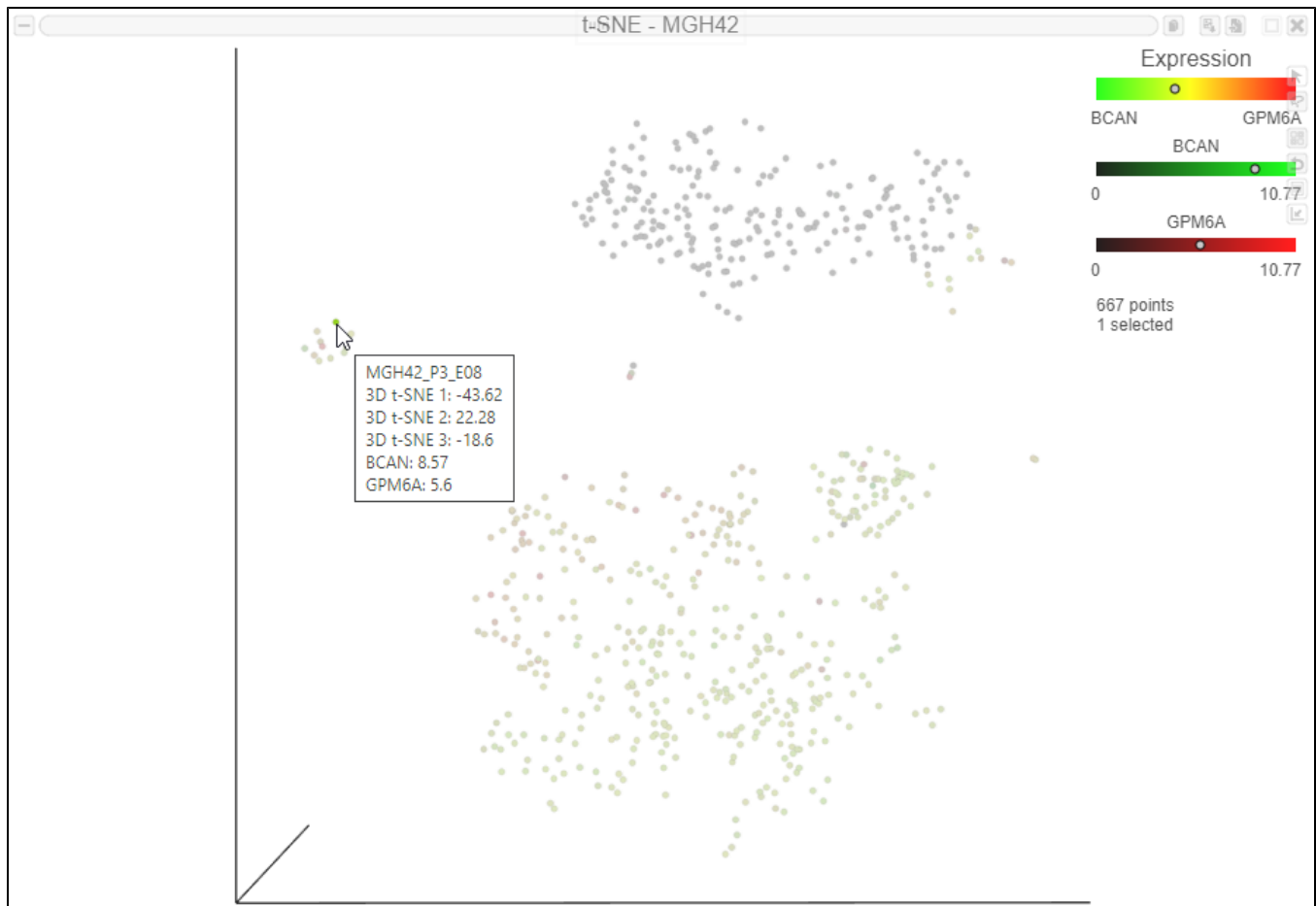



Figure 40. Viewing expression levels for an individual cell. The dots on the legend indicate the expression level of the selected cell. The expression levels at

Now that cells are colored by the expression of two glioma cell markers, we can classify any cell that expresses these genes as glioma cells. Because t-SNE groups cells that are similar across the high-dimensional gene expression data, we will consider cells that form a group where the majority of cells express BCAN and/or GPM6A as the same cell type, even if they do not express either marker gene.

- Switch to lasso mode by clicking  in the top right of the plot
- Draw the lasso around the cluster of green, red, and yellow cells and click the circle to close the lasso (Figure 12)

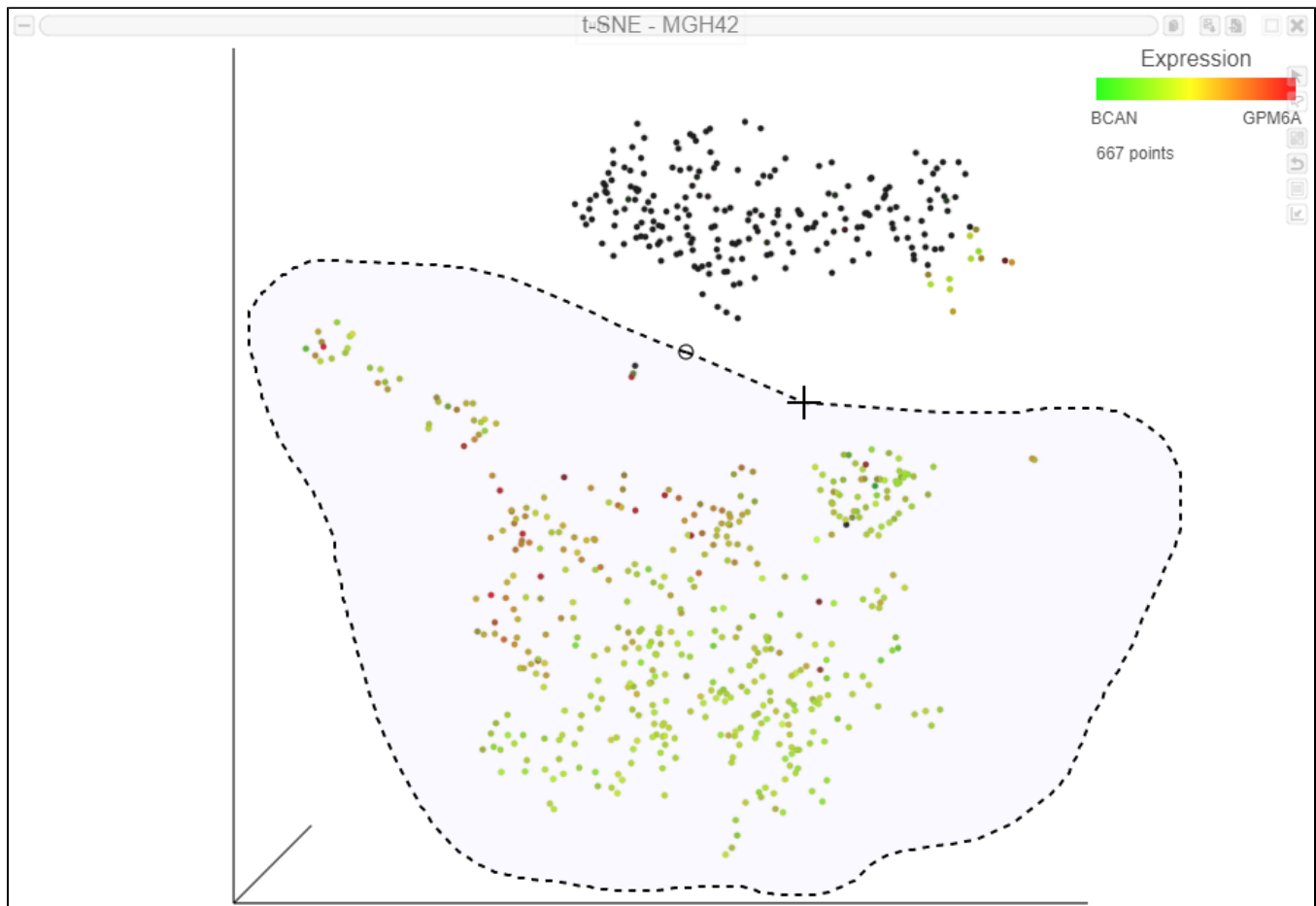


Figure 41. Selecting a group of cells using the 3D lasso tool

Selected cells are shown in bold and unselected cells are dimmed. The number of selected cells is indicated in the figure legend. The cells are plotted on the color scale depending on their relative expression levels of the two marker genes (Figure 13)

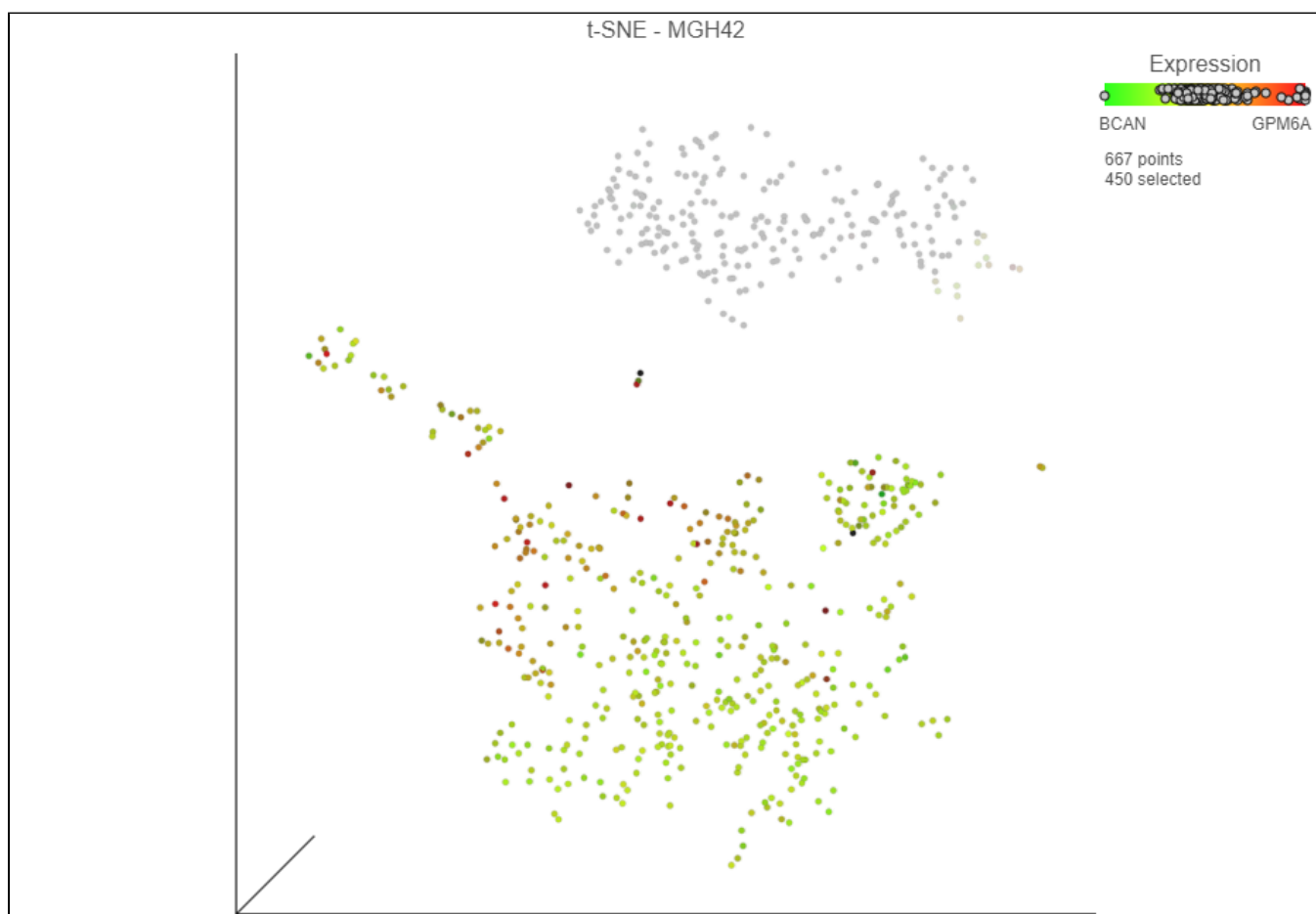


Figure 42. Viewing expression levels for a group of cells

- Click **Classify selection** in the **Classify** icon under *Tools*

A dialog to give the classification a name will appear.

- Name the classification **Glioma**
- Click **Save** (Figure 14)

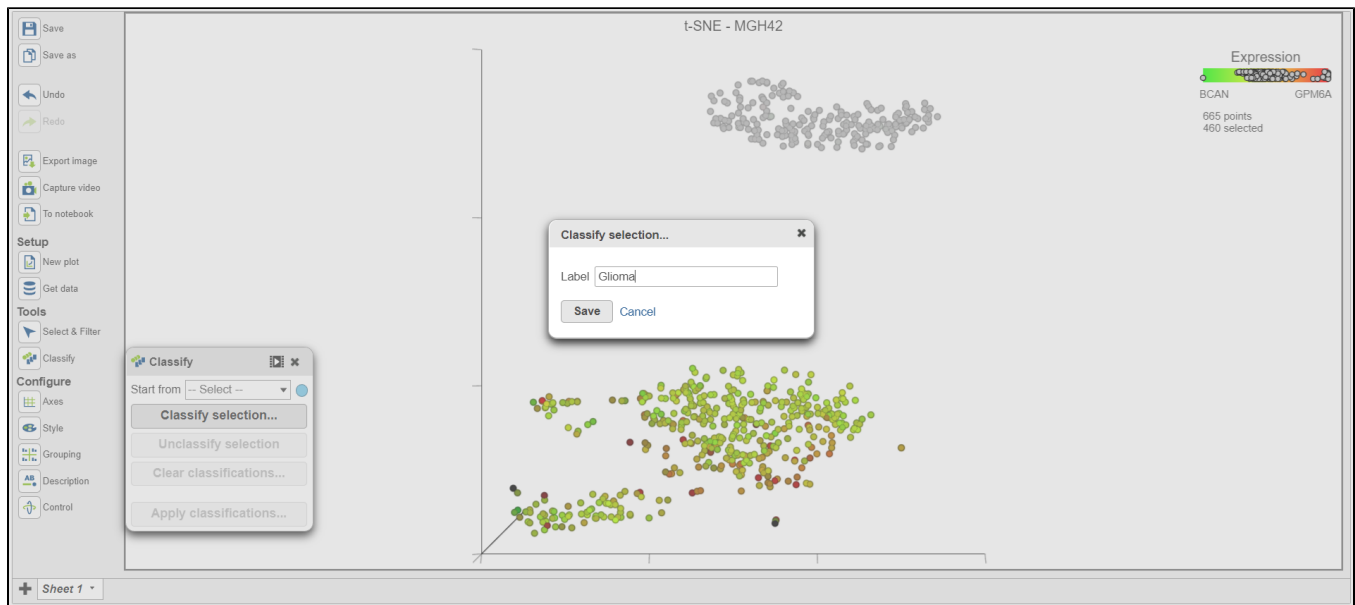


Figure 43. Classifying selection

Once cells have been classified, the classification is added to **Classify**. The number of cells belonging to the classification is listed. In MGH42, there are 460 glioma cells (Figure 15).

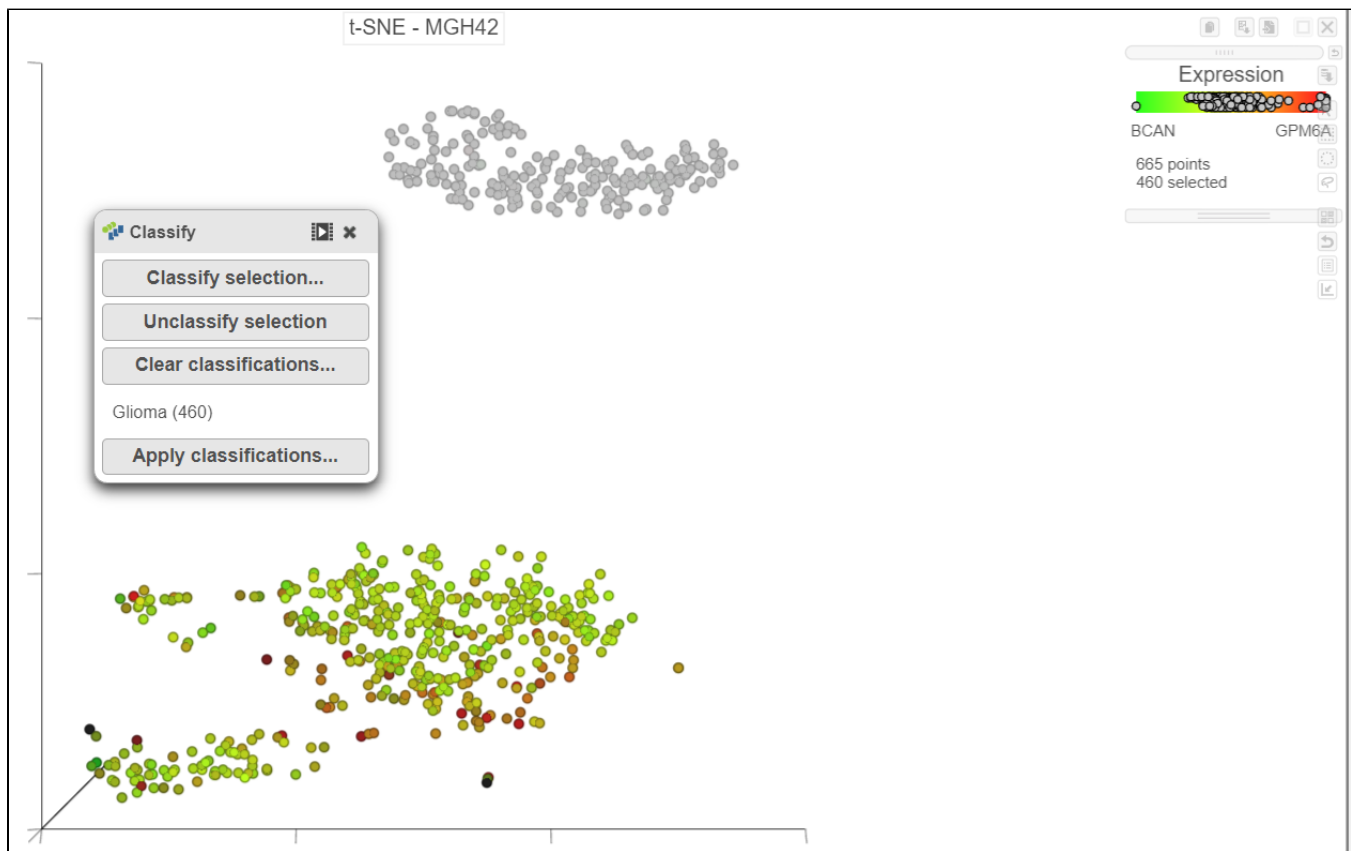






Figure 44. The number of cells in each classification is displayed

Classifications made on the t-SNE plot are retained as a draft as part of the data viewer session. In this tutorial, we will classify malignant cells for each

sample before we save and apply the classifications, but if necessary, you can save the data viewer session by clicking the  **Save** icon on the left to retain all of the formatting and draft classifications. The data viewer session will be stored under the *Data viewer* tab and can be re-opened to continue making classifications at a later time.

- Switch to pointer mode by clicking  in the top right corner of the plot
- Deselect the cells by clicking on any blank space on the plot
- Open **Axes** and navigate to *Sample* under *Misc*
- Select the  icon below the sample name to go to the next sample, MGH45
- Rotate the 3D t-SNE plot to get a better view of cells from the green, red, and yellow cluster
- Switch to lasso mode by selecting  in the top right corner of the plot
- Draw the lasso around the cluster of colored cells and click the circle to close the lasso (Figure 16)

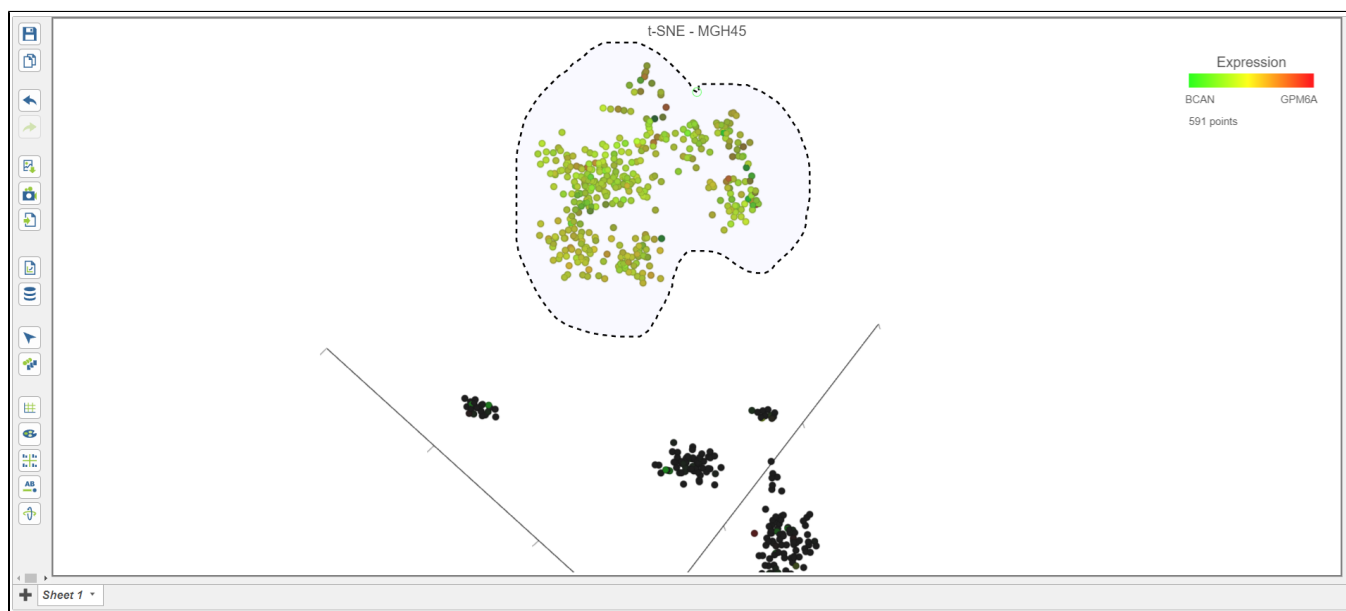


Figure 45. Classifying malignant cells in sample MGH45

- Select **Classify selection** in the **Classify** icon
- Type **Glioma** or select **Glioma** from the drop-down list (Figure 17)
- Click **Save**

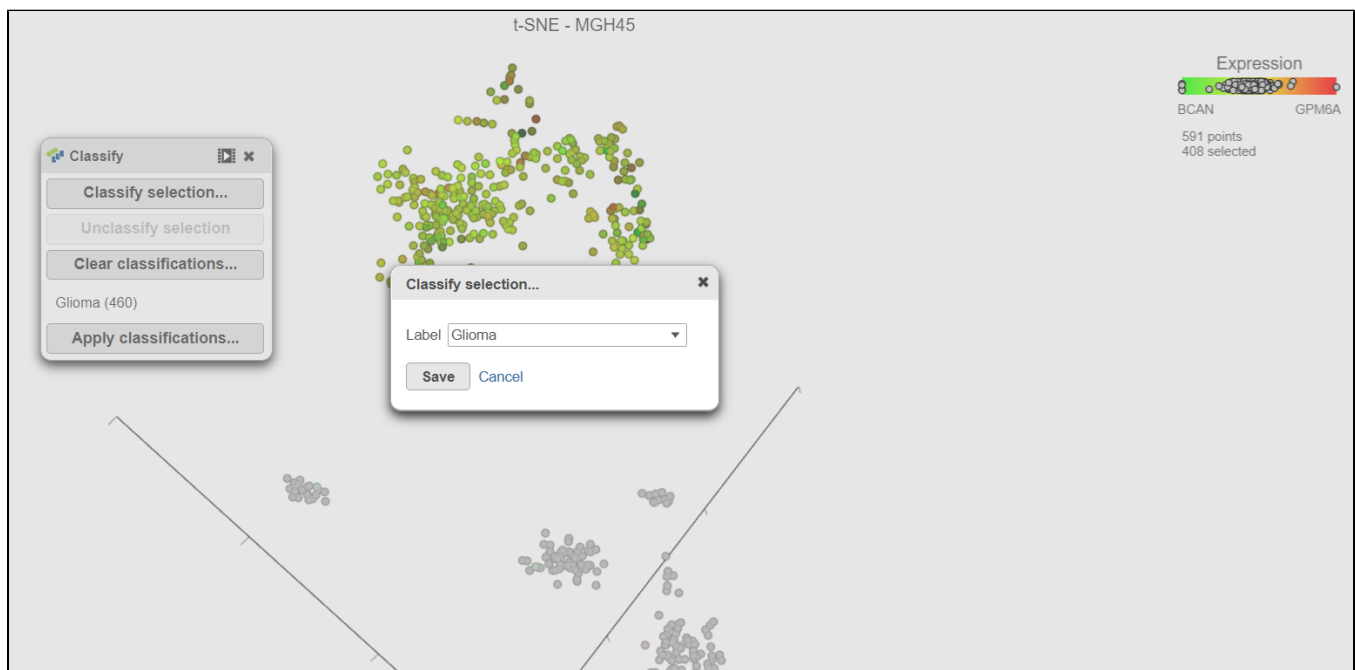


Figure 46. Adding cells in a second sample to an existing classification

- Repeat these steps for each of the 6 remaining samples. Remember to go back to the first sample (MGH36) to classify the glioma cells in that samples too.

There should be 5,322 glioma cells in total across all 8 samples.

- The classification name can be edited or deleted (Figure 18).

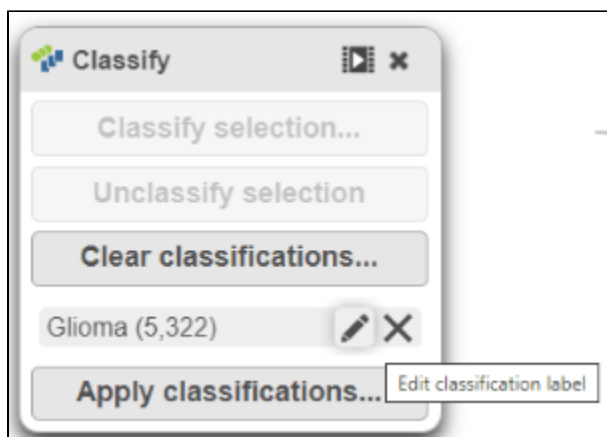


Figure 47. Edit or delete the classification

With the malignant cells in every sample classified, it is time to save the classifications.

- Click **Apply classifications** in the **Classify** icon
- Name the classification attribute **Cell type (sample level)**
- Click **Run** (Figure 19)

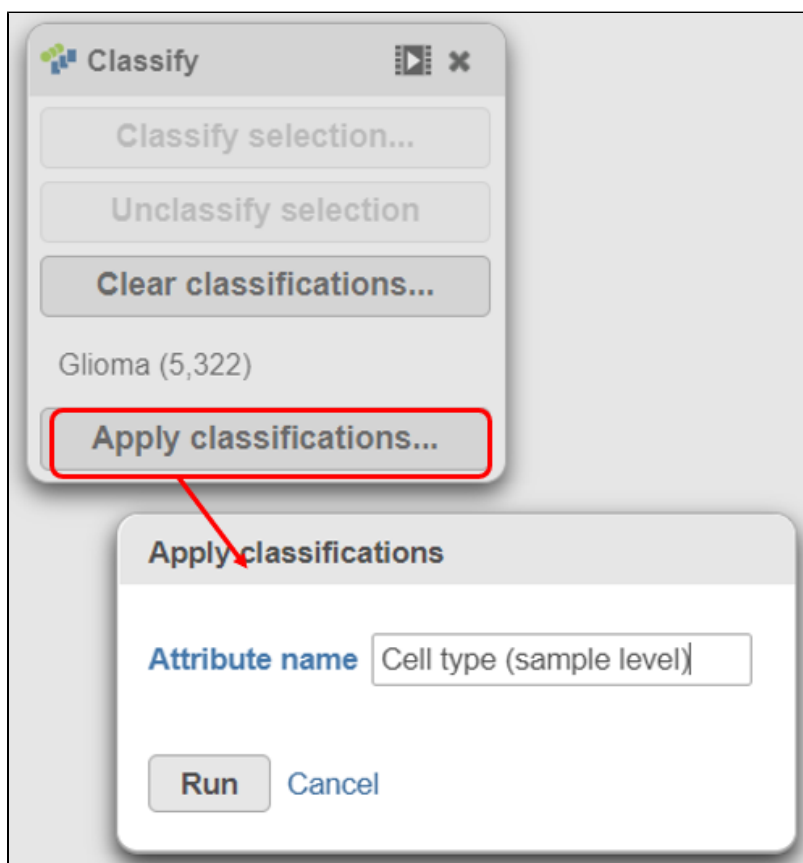


Figure 48. Name the cell-level attribute

The new attribute is stored in the *Data* tab and is available to any node in the project.

- Click on the **Glioma (multi-sample)** project name at the top to go back to the *Analyses* tab
- Your browser may warn you that any unsaved changes to the data viewer session will be lost. Ignore this message and proceed to the *Analyses* ta

One multi-sample t-SNE plot

For some data sets, cell types can be distinguished when all samples can be visualized together on one t-SNE plot. We will use a t-SNE plot of all samples to classify glioma, microglia, and oligodendrocyte cell types.

- Click on the **Glioma (multi-sample)** project name at the top to go back to the *Analyses* tab
- Click the **Filtered counts** data node after the *Filter features* task
- Click **PCA** in the *Exploratory analysis* section of the task menu
- Uncheck the **Split by sample** checkbox (Figure 22)
- Click **Finish**

[Home](#) > [Glioma \(multi-sample\)](#) > [PCA](#)

PCA

Number of principal components 

☐ All

☒ 100

Features contribute 

☐ equally ☒ by variance

Grouping

Split by sample ☐

Back

Finish

Figure 49. Combine all cells into one plot by unchecking the *Split by sample* box

The PCA task will run as a new green layer.

- Click the new **PCA** data node
- Select **t-SNE** from the *Exploratory analysis* section of the task menu
- Click **Finish** to run the t-SNE task with default settings

The t-SNE task will be added to the green layer (Figure 23). Layers are created in Partek Flow when the same task is run on the same data node.

[Home](#) > [Glioma \(multi-sample\) \(Project owner\)](#)

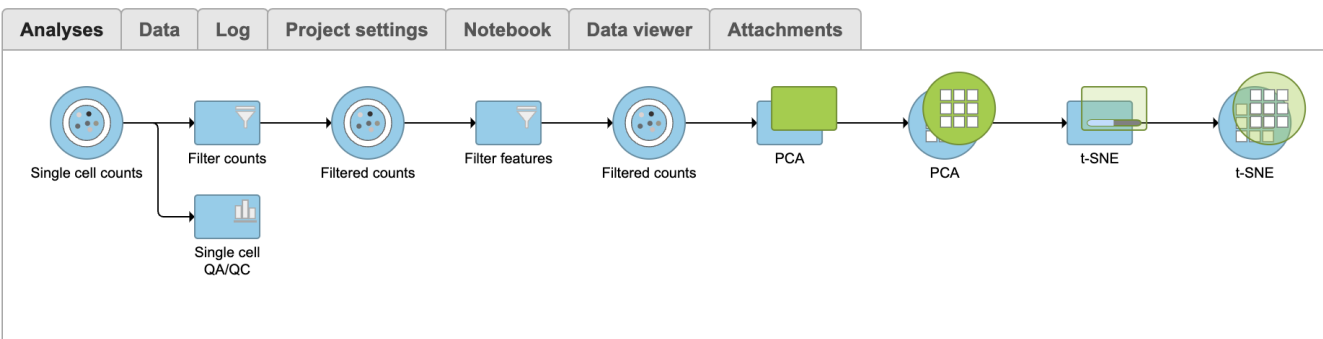


Figure 50. Multi-sample PCA and t-SNE tasks are added as a new layer

Once the task has completed, we can view the plot.

- Double-click the **green t-SNE** data node to open the t-SNE scatter plot
- Click and drag the **2D scatter plot** icon onto the canvas and **replace** the 3D scatter plot (Figure 24)

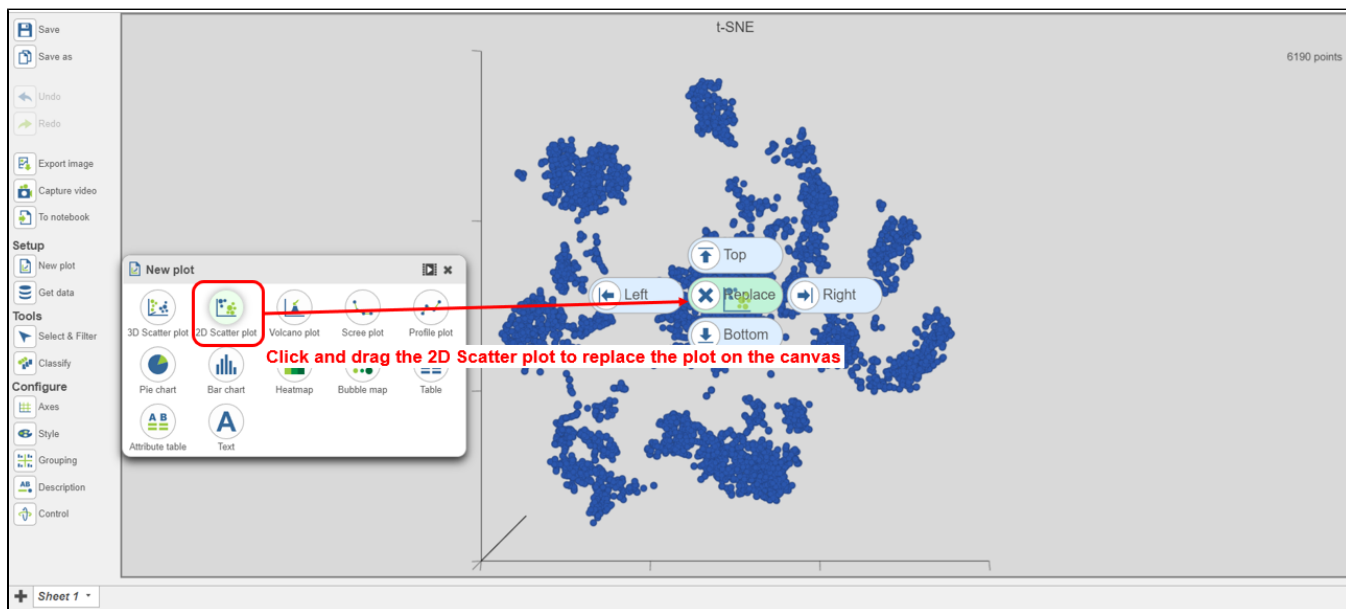


Figure 51. Viewing the multi-sample t-SNE plot

- Search for and select **green t-SNE** data node (Figure 25)



Figure 52. Select the green multi-sample t-SNE data node to draw the 2D t-SNE plot

- In the **Style** icon, choose **Sample name** from the *Color by* drop-down list under *Color*

Viewing the 2D t-SNE plot, while most cells cluster by sample, there are a few clusters with cells from multiple samples (Figure 26).



Figure 53. Viewing the multi-sample t-SNE plot in 2D

Using marker genes, BCAN (glioma), CD14 (microglia), and MAG (oligodendrocytes), we can assess whether these multi-sample clusters belong to our known cell types.

- Select any of the count data nodes from the *Data* card on the left (Single cell counts, or any of the Filtered counts)
- Search for the **BCAN** gene
- Click and drag the BCAN gene onto the plot and drop it over the **Green (feature)** option
- Search for the **CD14** gene
- Click and drag the CD14 gene onto the plot and drop it over the **Red (feature)** option
- Search for the **MAG** gene
- Click and drag the MAG gene onto the plot and drop it over the **Blue (feature)** option

After coloring by these marker genes, three cell populations are clearly visible (Figure 27).

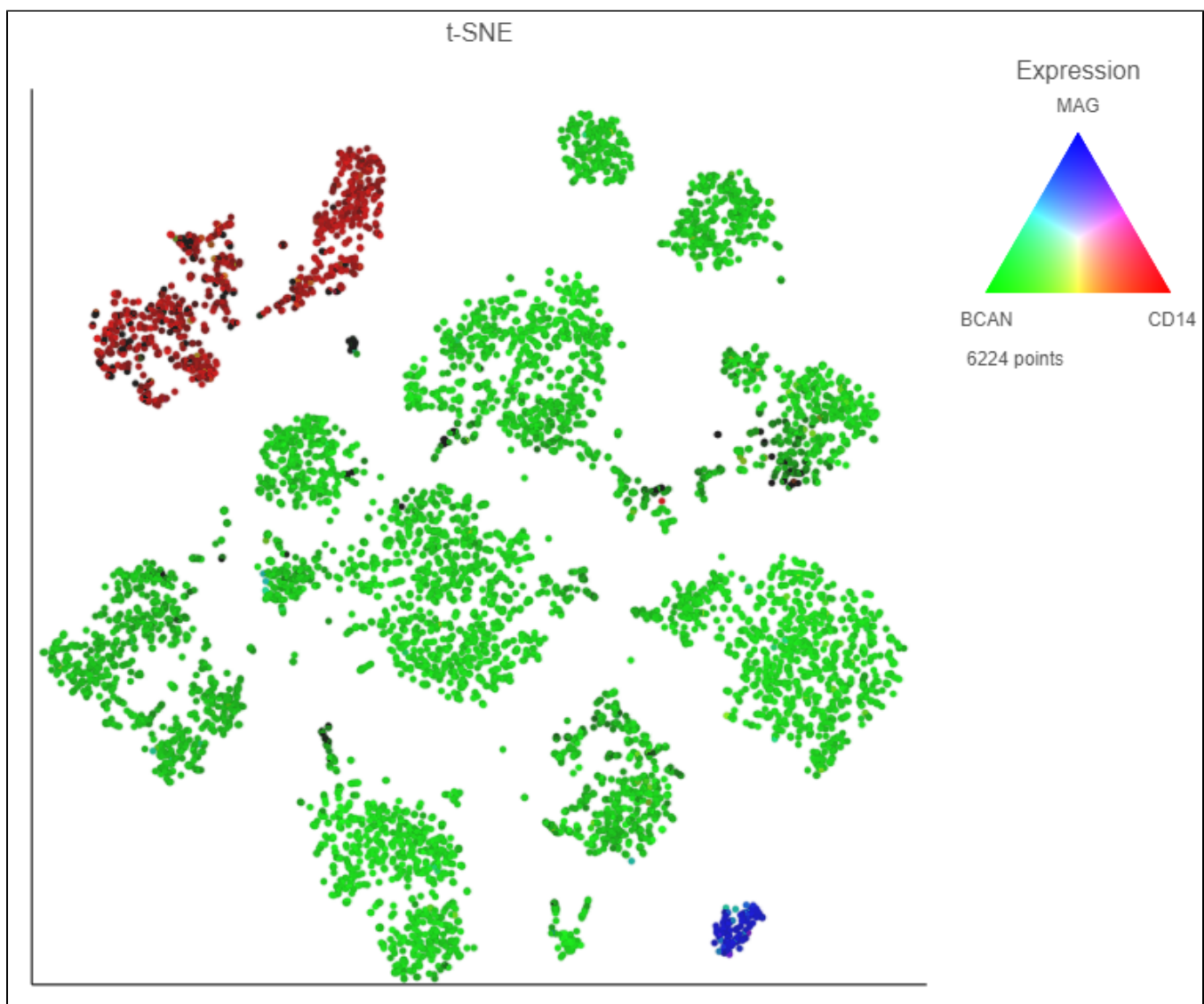


Figure 54. Overlaying marker gene expression on the multi-sample t-SNE plot

The red cells are CD14 positive, indicating that they are the microglia from every sample.

- Switch to lasso mode by clicking the  icon in the top right of the plot

- Draw the lasso around the cluster of red cells and click the circle to close the lasso (Figure 28)
- Open the **Classify** tool and click **Classify selection**
- Name the classification **Microglia**
- Click **Save**

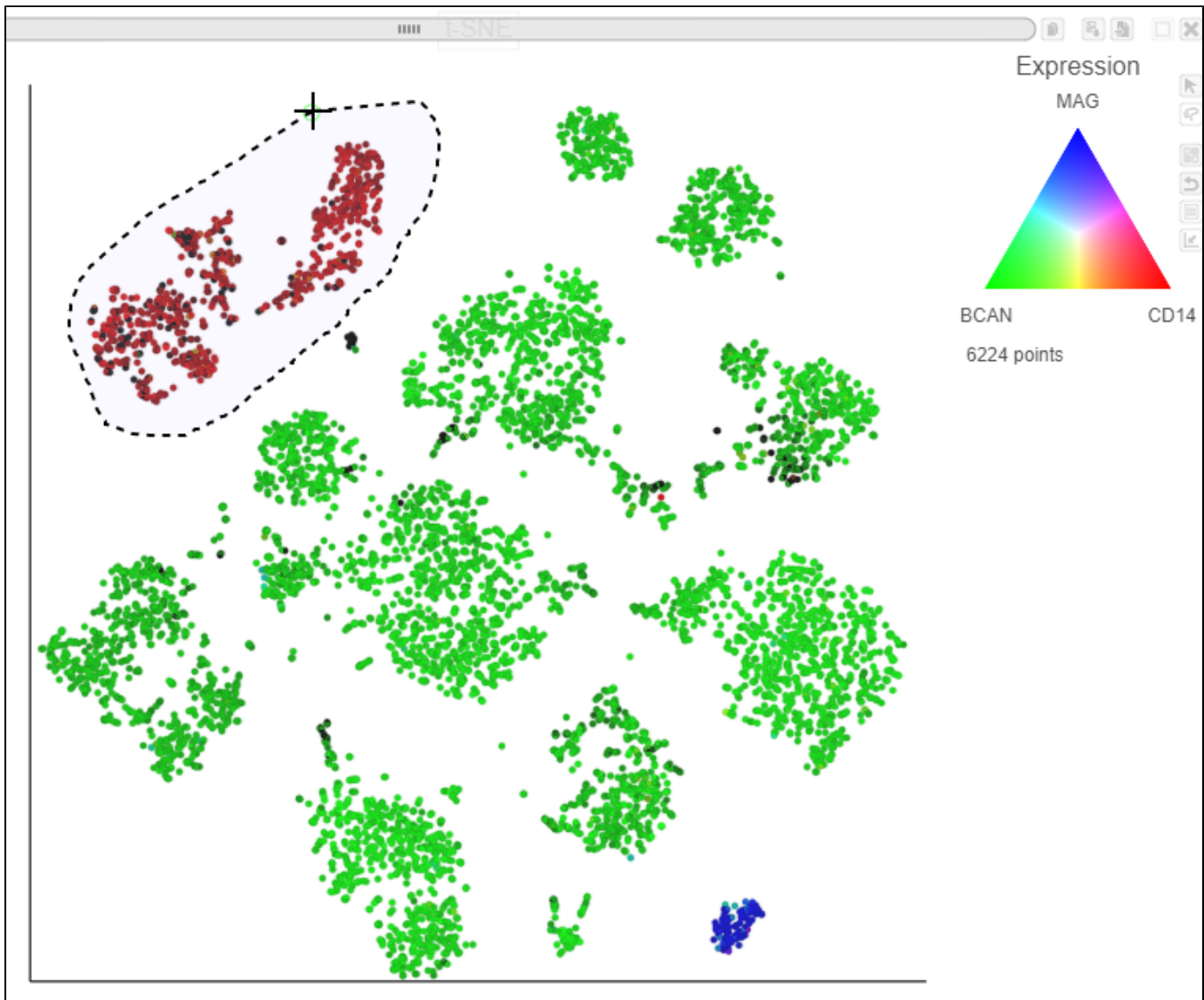






Figure 55. Classifying microglia (red)

The blue cells are MAG positive, indicating that they are the oligodendrocytes from every sample.

- Switch to pointer mode by clicking  in the top right corner of the plot
- Deselect the cells by clicking on any blank space on the plot
- Switch to lasso mode again by clicking the  icon in the top right of the plot
- Draw the lasso around the cluster of blue cells and click the circle to close the lasso
- Open the **Classify** tool and click **Classify selection**
- Name the classification **Oligodendrocytes**
- Click **Save**

Finally, we will classify the BCAN expressing cells on the plot as glioma cells from every sample.

- Switch to pointer mode by clicking  in the top right corner of the plot
- Deselect the cells by clicking on any blank space on the plot
- Switch to lasso mode again by clicking the  icon in the top right of the plot
- Draw the lasso around the cluster of green cells and click the circle to close the lasso
- Open the **Classify** tool and click **Classify selection**

- Name the classification **Glioma**
- Click **Save**
- Switch to pointer mode by clicking  in the top right corner of the plot
- Deselect the cells by clicking on any blank space on the plot

The number of cells classified as microglia, oligodendrocytes, and glioma are shown in **Classify** (Figure 29)

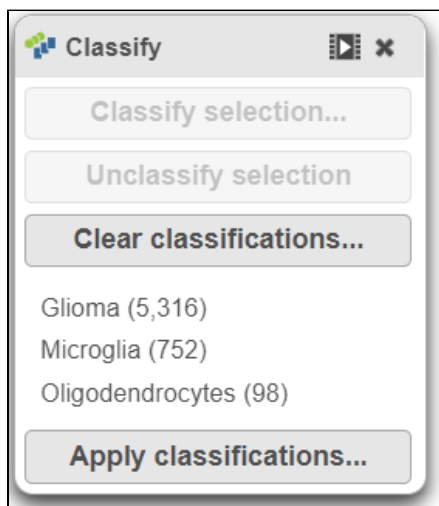


Figure 56. The number of cells for each cell type

- Click **Apply classifications** in the **Classify** icon (Figure 30)

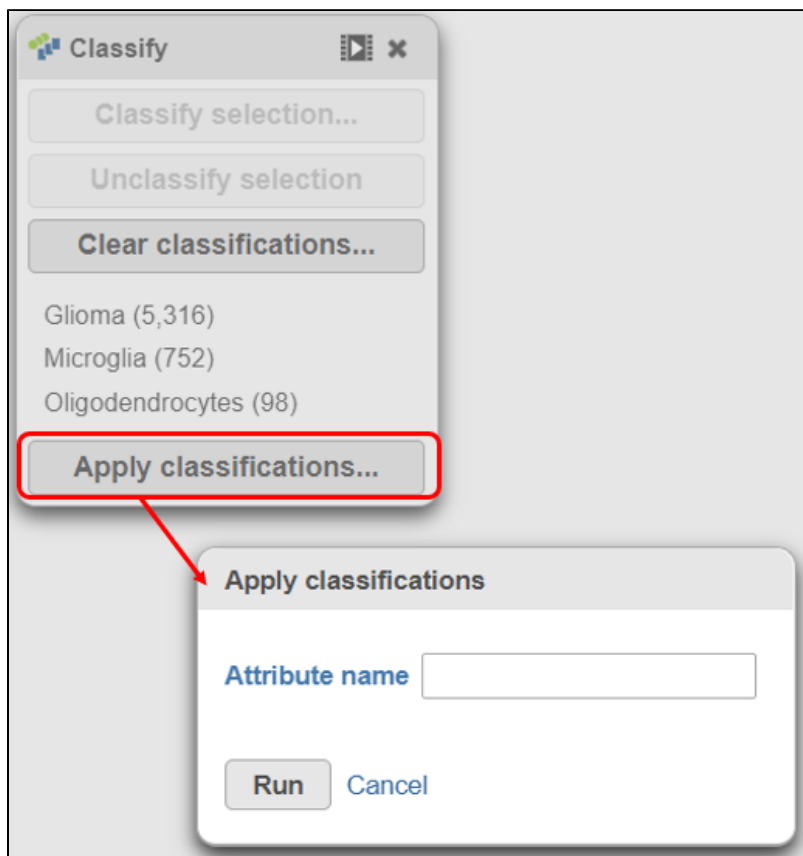
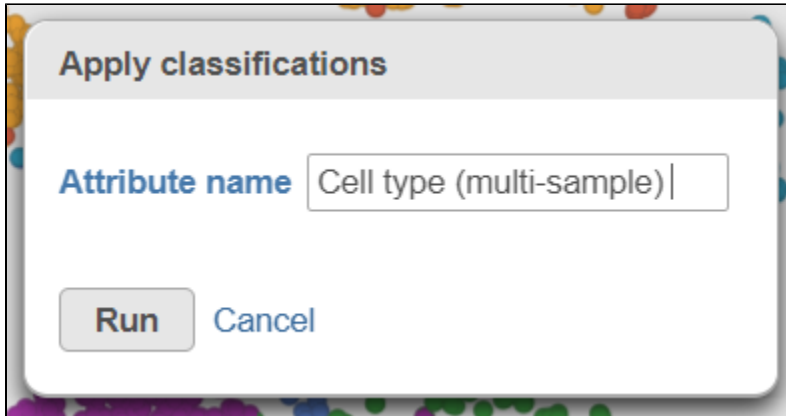


Figure 57. Apply classifications to the data project

- Name the classification attribute **Cell type (multi-sample)** (Figure 31)

- Click **Run**



Apply classifications

Attribute name

Run **Cancel**

Figure 58. Name the cell-level attribute

The new attribute is now available for downstream analysis.

- Click on the **Glioma (multi-sample)** project name at the top to go back to the *Analyses* tab
- Your browser may warn you that any unsaved changes to the data viewer session will be lost. Ignore this message and proceed to the *Analyses* ta

[« Getting started with the tutorial data set](#) [Compare expression between cell types with multiple samples](#) »

Additional Assistance

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