Perform Exploratory analysis

- Use Principle Components Analysis (PCA) to reduce dimensions
 Classify cells based on a marker for expression

Use Principle Components Analysis (PCA) to reduce dimensions

- Click the Normalized counts data node
- Expand the Exploratory analysis section of the task menu
- Click PCA

		🥏 Venn diagram	
x Normalized cou	nts		Â
Craph-based cluste	ring		
K-means clustering	ang		
Compare clusters			
PCA			
t-SNE Visualize	our results using prin	cipal component analy	sis
UMAP			
Hierarchical cluster	ng / heatmap		
AUCell			
SVD			
Scatter plot			

In this tutorial we will modify the PCA task parameters, to not split by sample, to keep the cells from both samples on the PCA output.

- Uncheck (de-select) the Split by sample checkbox under Grouping
- Click Finish

PCA
Number of principal components
The number of principal components to calculate.
Al
Features contribute
Feature expression can be standardized prior to PCA so that the contribution of each feature does not depend on its variance. Choose "by variance" if you prefer to take variance into account and focus on most variable features.
 equally
Grouping
Split by sample
Back Finish

• Double-click the circular PCA node to view the results



From this PCA node, further exploratory tasks can be performed (e.g. t-SNE, UMAP, and Graph-based clustering).

Classify cells based on a marker for expression

- Choose Style under *Configure*Color by and search for *fasn* by typing the name
 Select *FASN* from the drop-down



The colors can be customized by selecting the color palette then using the color drop-downs as shown below.

		Customize colors ×
		Two-color numeric
		The two-color numeric association of the second of the second s
& Style		Maximum 🔳 🗸 Minimum 🔳 🗸
Color	Shape	Reset to default colors
Color by FASN 🔻	Shape by Fixed shape	
Range override Min 🔵 0	Customize colors Shaded	Save Cancel
Max () 19.9:	Border size 🔿	
Fog	Labeling	
Opacity	Label by 👔 None	
Size		
Size by Fixed size 🔻 🔵		
Point size	6	

Ensure the colors are distinguishable such as in the image above using a blue and green scale for Maximum and Minimum, respectively.

- Click FASN in the legend to make it draggable (pale green background) and continue to drag and drop FASN to Add criteria within the Select & Filter Too/
- · Hover over the slider to see the distribution of FASN expression



Multiple gene thresholds can be used in this type of classification by performing this step with multiple markers.



• Drag the slider to select the population of cells expressing high FASN (the cutoff here is 10 or the middle of the distribution).

- Click Classify under Tools
- Click Classify selection



• Give the classification a name "FASN high"



• Under the Select & Filter tool, choose Filter to exclude the selected cells

► Select & Filter	× 10
Select Deselected points O Dim O Gray O None Selection mode O Manual O Criteria Add criteria Select V	Filter Clear filters Exclude selected points A Deservation miter Apply feature filter
Criteria FASN X 10 Invert Pin histogram	

Exit all Tools and Configure options

- Click the "X" in the right corner
- Use the rectangle selection mode on the PCA to select all of the points on the image





This results in 147538 cells selected.



- Open Classify
- Click Classify selection and name this population of cells "FASN low"
- Click Apply classifications and give the classification a name "FASN expression"



Now we will be able to use this classification in downstream applications (e.g. differential analysis).

« Process Xenium data Make comparisons using Compute biomarkers and Biological interpretation »

Additional Assistance

If you need additional assistance, please visit our support page to submit a help ticket or find phone numbers for regional support.

