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Space Ranger output

The Visium spatial gene expression solution from 10X Genomics allows us to spatially resolve RNA-Seq expression data in individual tissue sections. For the analysis of Visium spatial gene expression data in Partek Flow, you will need the following output files from the Space Ranger *outs*[1] subdirectory:

- The filtered count matrix -- either the .h5 file (one file) or feature-barcode matrix (three files).
- spatial – Outputs of spatial pipeline (Figure 1) (Spatial imaging data)

```
Outputs:
- Run summary HTML:                         /home/jdoe/runs/sample345/outs/web_summary.html
- Outputs of spatial pipeline:              /home/jdoe/runs/sample345/outs/spatial
- Run summary CSV:                          /home/jdoe/runs/sample345/outs/metrics_summary.csv
- BAM:                                      /home/jdoe/runs/sample345/outs/possorted_genome_bam.bam
- BAM index:                                /home/jdoe/runs/sample345/outs/possorted_genome_bam.bam.bai
- Filtered feature-barcode matrices MEX:    /home/jdoe/runs/sample345/outs/filtered_feature_bc_matrix
- Filtered feature-barcode matrices HDF5:   /home/jdoe/runs/sample345/outs/filtered_feature_bc_matrix.h5
- Unfiltered feature-barcode matrices MEX:  /home/jdoe/runs/sample345/outs/raw_feature_bc_matrix
- Unfiltered feature-barcode matrices HDF5: /home/jdoe/runs/sample345/outs/raw_feature_bc_matrix_h5.h5
- Secondary analysis output CSV:            /home/jdoe/runs/sample345/outs/analysis
- Per-molecule read information:            /home/jdoe/runs/sample345/outs/molecule_info.h5
- Loupe Browser file:                    /home/jdoe/runs/sample345/outs/cloupe.cloupe
```

Figure 7. The content in outputs directory from Space Ranger. The folder and file highlighted in red are the required files

We recommend import count matrix file using .h5 file format, which allow you to import multiple samples at the same time. You need to rename the file names and put all the files in the sample folder to import in one go.

The *spatial* subdirectory contains image related files, they are:

tissue_hires_image.png

tissue_lowres_image.png

aligned_fiducials.jpg

detected_tissue_image.jpg

tissue_positions_list.csv

scalefactors_json.json

The folder should be compressed in one .gz or zip file when you upload to Flow server. You can pick the file for each sample from the Partek Flow server, your local computer, or a URL using the file browser .

Annotate Visium image

To run Annotate cells:

- Click a Single cell counts data node
- Click the **Annotation/Metadata** section in the toolbox
- Click **Annotate Visium image**

You will be prompted to pick a Spatial image file for each sample you want to annotate (Figure 2).

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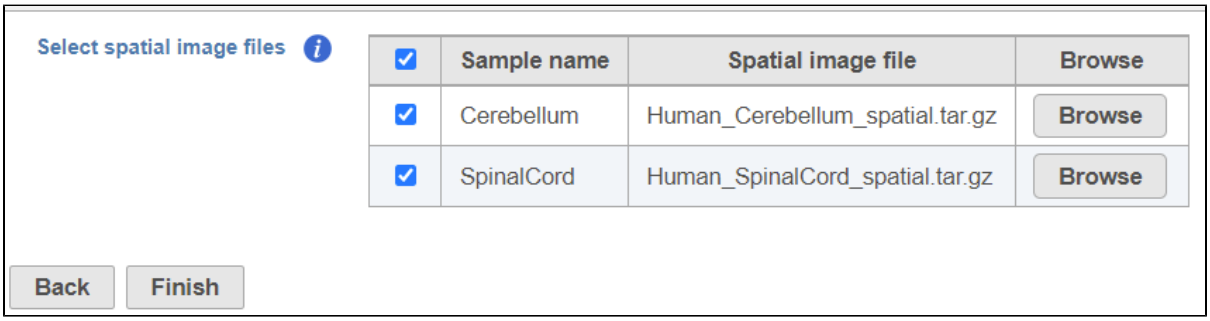

Figure 8. Select the zipped image folder for each sample. The image zip file should contains 6 files including image files and tissue position text file with a scale factor json file

- Click **Finish**

A new data node, Annotated counts, will be generated (Figure 3).

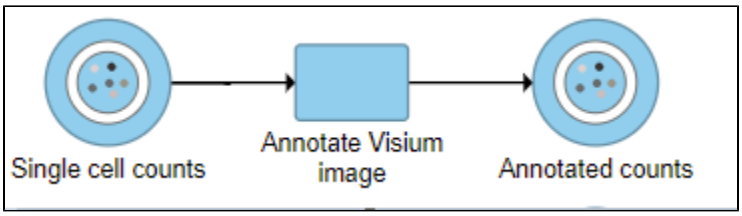

Figure 9. The Annotated counts output node is Split by sample. This means that any tasks performed from this node will also be split by sample. Invoke tasks from the Single cell counts node to combine samples for analyses.

When the task report of the annotated counts node is opened (or double click on the Annotated counts node), the images will be displayed in data viewer (Figure 4)

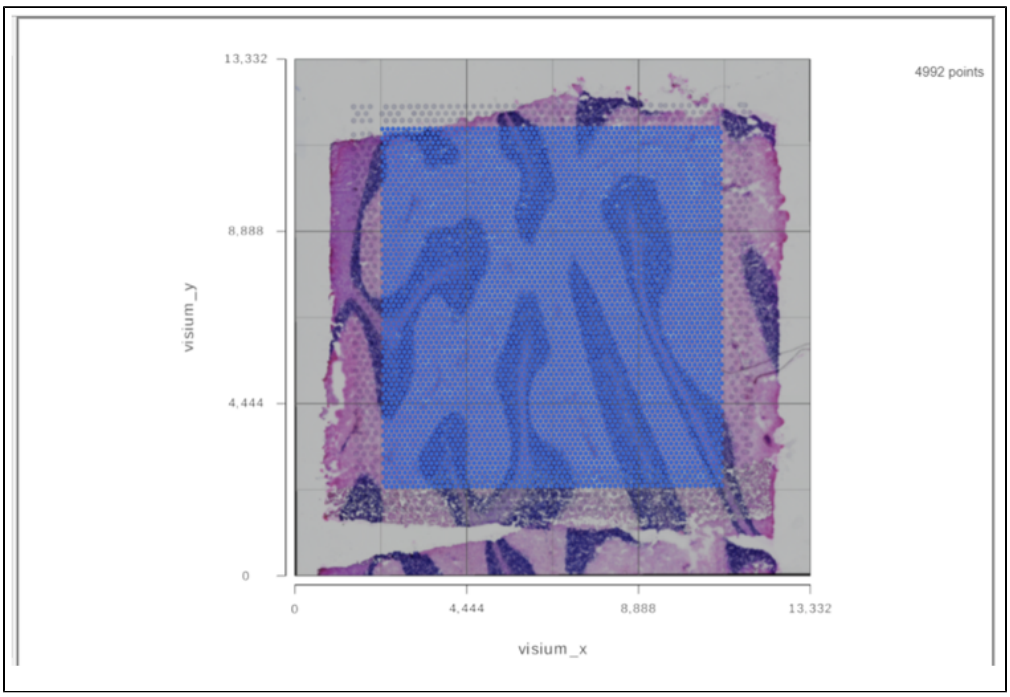

Figure 10. Tissue image displayed in data viewer

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It is a 2D plot and XY axes are the tissue spot coordinates. Tissue spots are on top of the slide image. The opacity of the tissue spots can be changed using the slider to show more of the image under **Configure>Style>Color**.

To view different samples in the Data viewer, navigate to **Axes** under *Configure* Click on the ▶ button under **Content** in the left panel (Figure 5).

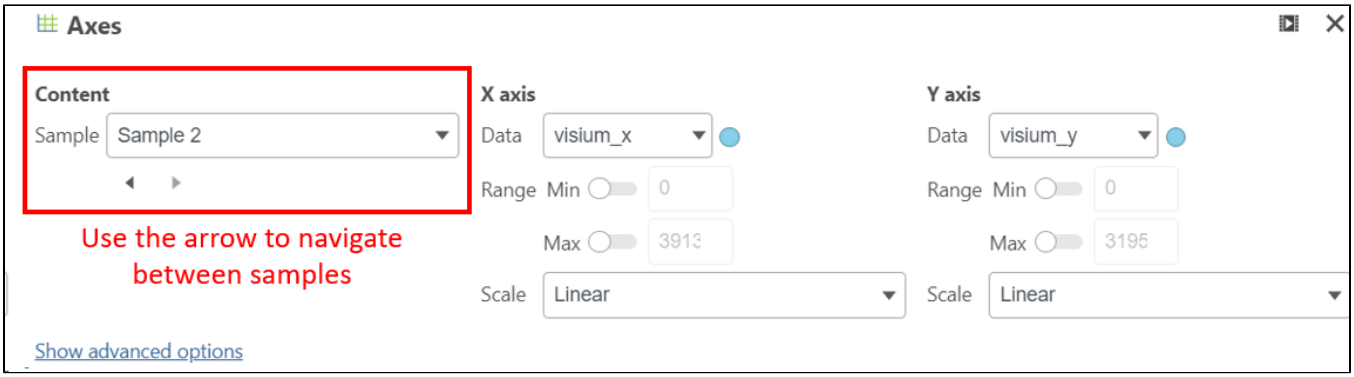

Figure 11. View different samples in the data viewer

From the **Configure>Background>Image** drop-down list in the Data viewer, different formats of the image can be selected (Figure 6).

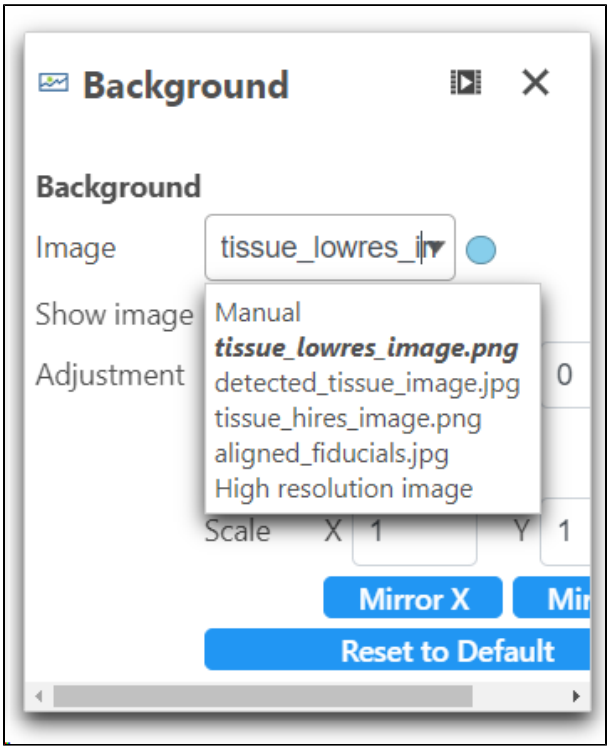

Figure 12. Select different format of slide image as background

Click on **Show image** ⬭ to turn on or off the background image.

Note that the "Annotate Visium image" task splits by sample so all of the downstream tasks will also do this (e.g. if the pipeline is built from this node all of the downstream tasks will also be split and viewed per sample). To generate plots with multiple samples on one plot, build the pipeline from the "Single cell counts" node.

References

[1] https://support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/output/overview

Additional Assistance

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

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