

Space Ranger

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What is Space Ranger?

Space Ranger is a set of analysis pipelines that process Visium Spatial Gene Expression data with brightfield and fluorescence microscope images (1).

Space Ranger in Partek Flow

Space Ranger 2.0.1 has been wrapped in Partek Flow as *Space Ranger* task. The task takes .fastq and .jpeg/.tiff files as input and performs alignment, filtering, barcode counting, and UMI counting. The output is gene expression count matrix in a .h5 format (both raw and filtered are available for download via *Task Details*), as well as a .zip file with spatial files (image). Note that the *Space Ranger* task in Partek Flow does not include all the options and uses cases covered by the Space Ranger pipelines of 10x Genomics.

Note: when using the *Space Ranger* task in Partek Flow, there are more restrictions on the sample name-- sample name can only contain letters, digits, underscores and dashes. Please Edit the sample names on Data tab in Partek Flow to remove any other characters, e.g. space etc.

Running Space Ranger in Partek Flow

To run Space Ranger in Partek Flow select **Unaligned Reads** node on the *Analysis* tab and then select the **Space Ranger** task in the toolbox (Figure 1).

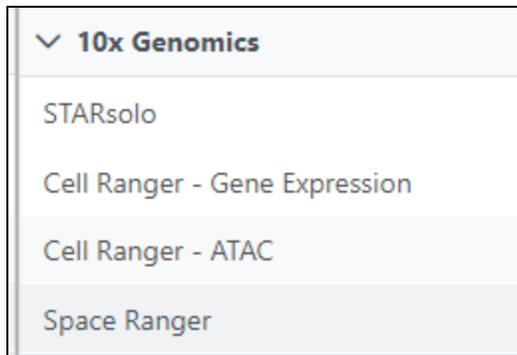


Figure 11. *Space Ranger* task in the toolbox

Select the 10x assay type. Choose CytAssist gene expression if you are using the Visium CytAssist gene expression library.

For both *Space Ranger* or *Cell Ranger* tasks, a *Reference assembly* is required (Figure 2). To define the *Reference assembly*, first select the *Genome build* for the organism of interest, then select the annotation *Index*. In this manner, custom libraries can be created (e.g. keep the *Genome build* but change the annotation *Index*).

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Assay type

10X assay type
The selected data node must have fastq files

Spatial gene expression CytAssist gene expression

Reference assembly

Assembly
Select genome, then select annotation index.

Mus musculus (mouse) - mm10

Index

Ensembl Transcripts release 98 (Administrator)

Image and barcode files

Sample files
For Spatial GEX, image files are single H&E brightfield images in TIFF or JPG format; For CytAssist GEX, image files are CytAssist instrument captured eosin stained Brightfield tissue image with fiducial frame in TIFF format. Probe set files are optional CSV files specifying the probe set used. Formalin-fixed paraffin-embedded (FFPE) image file requires probe set file.

Sample name	Image file	Browse image file	Probe set file	Browse probe set file
CytAssist_FreshFrozen_Mouse_Brain_Rep1_S1	CytAssist_FreshFrozen_Mouse_Brain_Rep1_image.tif	<input type="button" value="Browse"/>	CytAssist_FreshFrozen_Mouse_Brain_Rep1_probe_set.csv	<input type="button" value="Browse"/>

Advanced options

Use slide serial number file
Select a file specifying samples' slide and area information

Slide parameter
Set this if the slide serial number and area identifier are unknown. The value passed to this depends of the slide version. Possible values for CytAssist enabled workflow are visium-2 or visium-2-large. Setting this will cause Space Ranger to use default spot positions.

visium-2-large

Option set

-- Default --

Figure 12. Space Ranger setup dialog. If no Cell Ranger reference is present, one needs to be created first

To add a new species genome, choose *New assembly* from the drop down for *Genome build* which will open a new window with configuration options to edit, then click **Create** (Figure 3).

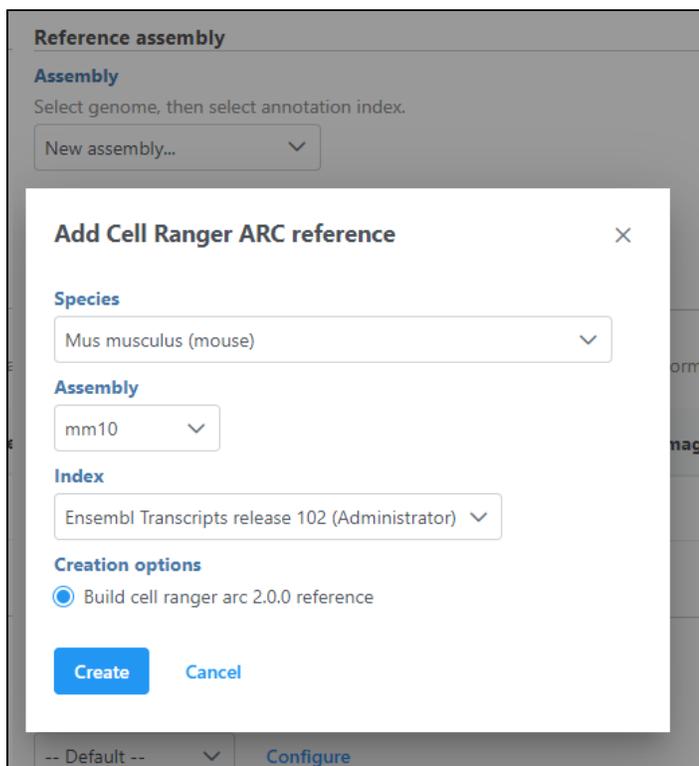


Figure 13. Downloading a Cell Ranger reference. Supported references appear in the drop down menu

The sample table under *Input options* has one row per sample (Figure 2). *Image file* is required, and that is a single hematoxylin and eosin brightfield image in either .jpg or .tiff format. Click on the **Browse** button under *Browse image file* and the file browser will come up. Point to the image file and push **Continue**. Formalin-fixed paraffin-embedded (FFPE) image files require the *Probe set file* otherwise it is optional; it is a .csv file specifying the probe set used (=target panel).

If you want to specify the sample's slide and area information, **check** the box by the *Use slide serial number file* under *Advanced options* and then click **Browse** to point to the file. The file should be tab-delimited with samples on rows. The first column is the sample name, the slide name is on the second column, slide area is on the third column.

If the *Slide serial number* is not available for CytAssist samples, the *Slide parameter* should be specified where *visium-2* corresponds to a 6.5 x 6.5 mm capture area and *visium-2-large* corresponds to a 11 x 11 mm capture area.

If necessary, click on the **Configure** link in the *Advanced options* section to open the *Advanced Options* dialog (Figure 4). Use *R1 length* to hard trim the R1 reads to specified length; use *R2 length* to hard trim the R2 reads to specified length.

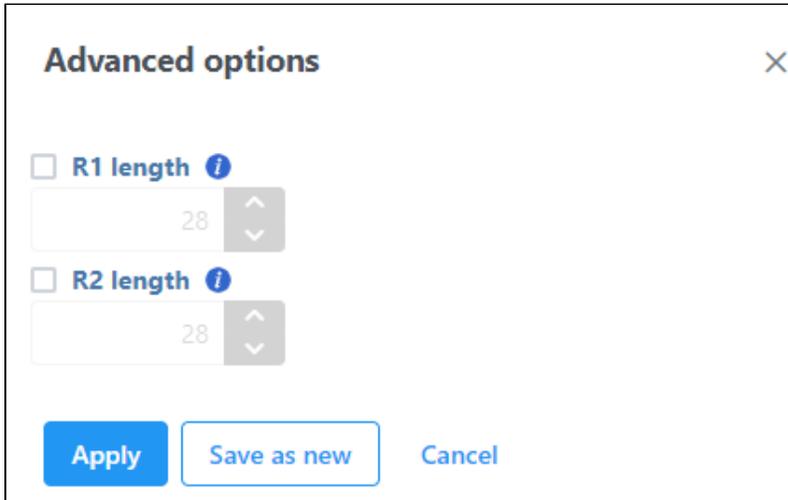


Figure 14. Advanced options of Space Ranger

Space Ranger task report

The result of *Space Ranger* task is the *Single cell counts* data node, which contains the gene expression data. Double click the **Single cell counts** node to invoke the task report (Figure 5) which is the same as the 'Summary HTML' from the original 10x Genomic pipelines. Task report is sample based. You can use the dropdown list in top left to switch samples (not shown in Figure 6). Each report consists of two pages: *Summary* and *Analysis*. For details, please visit [10x Genomics web page](#).

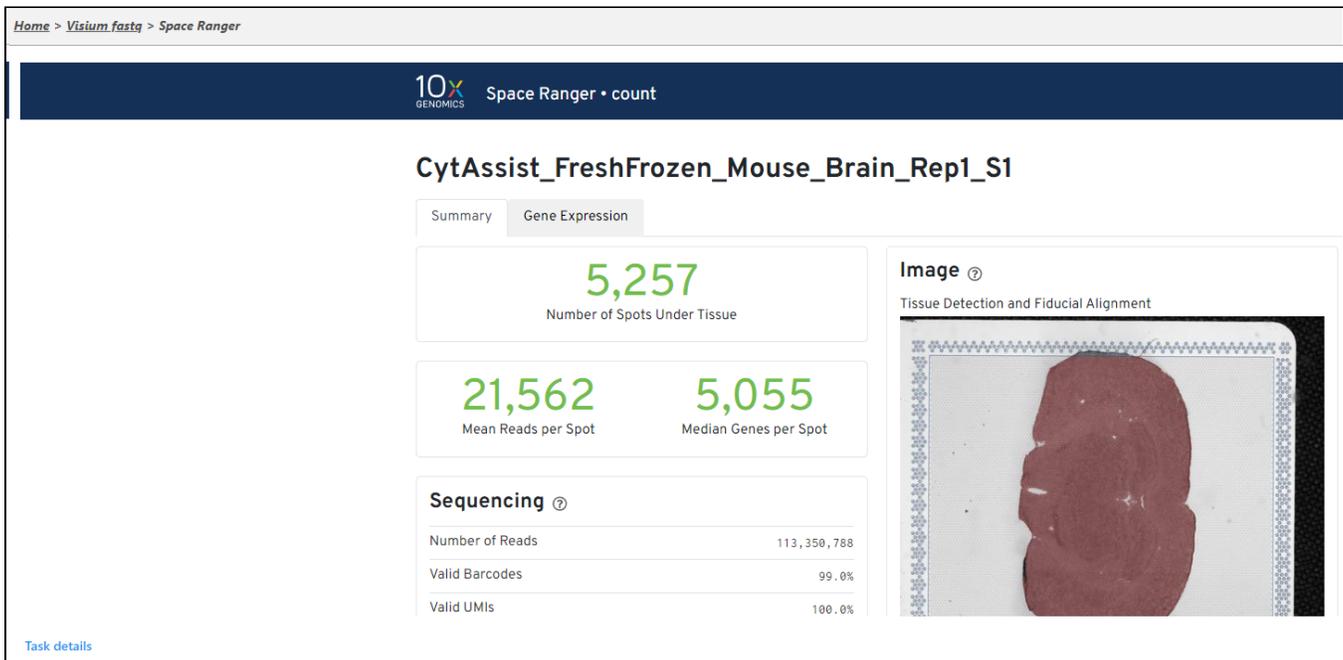


Figure 15. Space Ranger report. The report matches the Summary HTML from the Space Ranger pipeline by 10x Genomics. Each sample is shown as a sample selector will be displayed in top left

Annotate Visium image

After creating the *Single cell counts* node, the next step is to associate the microscopy image with the expression data. To start, select the **Single cell counts** data node and then go to **Annotate Visium image** in the toolbox (Figure 6).

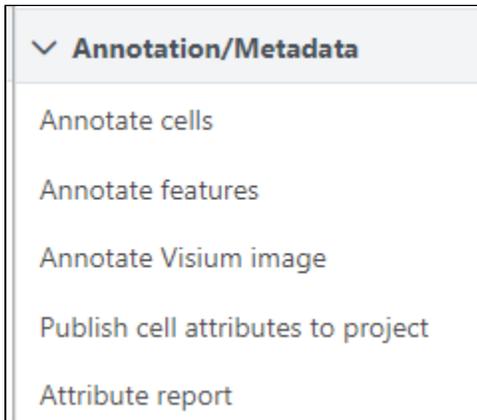


Figure 16. Annotate Visium image task associates a microscopic image with expression data

The setup page shows the sample table (one sample per row; Figure 7). Click on the **Browse** button to open the file browser and point to the file **<project-name>_spatial.zip**, created by the *Space Ranger* task. After that, click on **Finish** to launch the *Annotate* task.

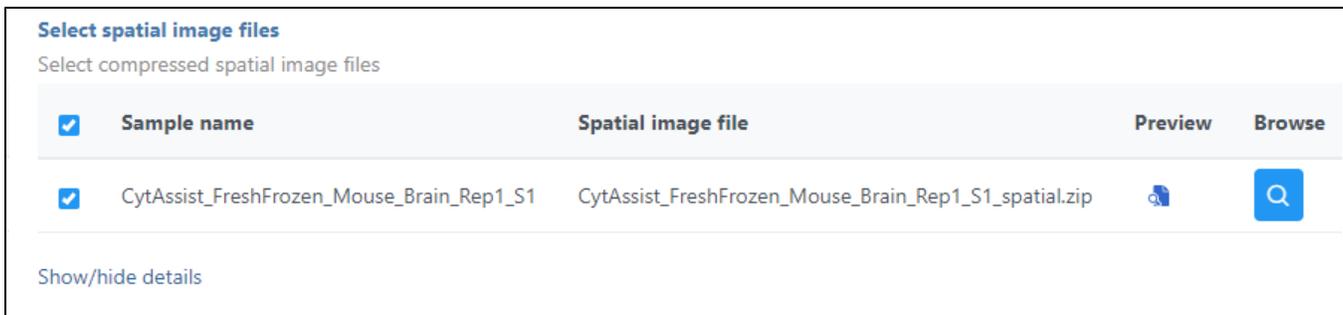


Figure 17. Annotate Visium image setup page

You can find the location of the *<project-name>_spatial.zip* file using the following steps. Select the **Space Ranger** task node (i.e. the rectangle) and then click on the **Task Details** (toolbox). Click on the **Output files** link to open the page with the list of files created by the *Space Ranger* task. **Mouse over** any of the files to see the directory in which the file is located. Figure 8 shows the path to the .zip file which is required for *Annotate Visium image*.

Output files

File name	Result	Created	Size
count.col.annotation	Single cell counts	12 Dec 2023, 01:46 PM CST	2.29 MB
count.mat.matrix	Single cell counts	12 Dec 2023, 01:46 PM CST	58.30 MB
count.oids	Single cell counts	12 Dec 2023, 01:46 PM CST	41.07 KB
count.col.matrix	Single cell counts	12 Dec 2023, 01:46 PM CST	127.59 KB
count.transcripts	Single cell counts	12 Dec 2023, 01:46 PM CST	1003.81 KB
count.row.annotation	Single cell counts	12 Dec 2023, 01:46 PM CST	203.93 KB
count.effective_lib_sizes.txt	Single cell counts	12 Dec 2023, 01:46 PM CST	73.12 KB
count.observations	Single cell counts	12 Dec 2023, 01:46 PM CST	15.40 KB
count.v3.col.metadata	Single cell counts	12 Dec 2023, 02:02 PM CST	1.31 KB
CytAssist_FreshFrozen_Mouse_Brain_Rep1_S1_spatial.zip	Spatial files	12 Dec 2023, 01:46 PM CST	31.92 MB
CytAssist_FreshFrozen_Mouse_Brain_Rep1_S1_filtered_feature_bc_matrix.h5	HDF5 file	12 Dec 2023, 01:34 PM CST	28.81 MB
CytAssist_FreshFrozen_Mouse_Brain_Rep1_S1_raw_feature_bc_matrix.h5	HDF5 file	12 Dec 2023, 01:34 PM CST	38.98 MB
CytAssist_FreshFrozen_Mouse_Brain_Rep1_S1_spatial_enrichment.csv	Spatial enrichment files	12 Dec 2023, 01:37 PM CST	1.88 MB
CytAssist_FreshFrozen_Mouse_Brain_Rep1_S1_web_summary.html	Summary	12 Dec 2023, 01:46 PM CST	4.14 MB
flowtasklog-spaceranger201count.log	Task logs	12 Dec 2023, 01:46 PM CST	66.95 KB
Total size of output files 167.82 MB			

Figure 18. Mousing over a file on the Output files page shows a balloon with the file location

A new data node, *Annotated counts*, will be generated (Figure 9).

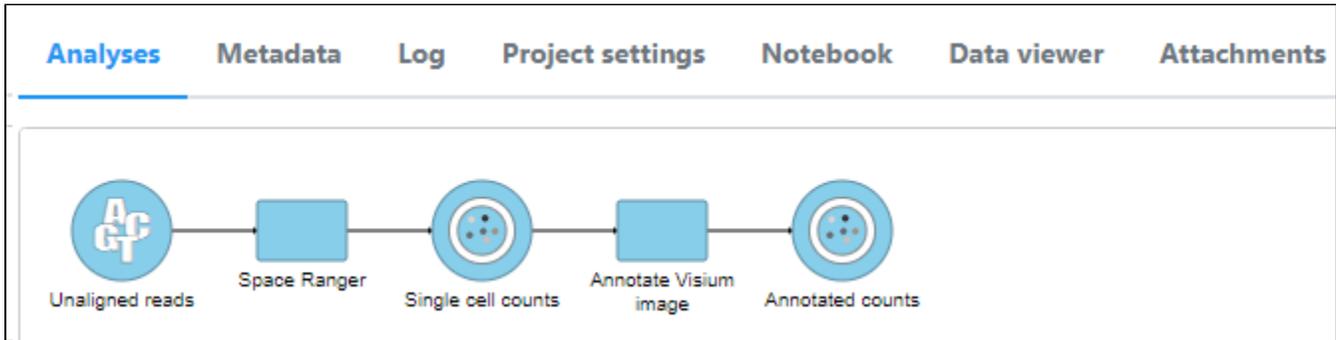


Figure 19. A new Annotated counts data node is generated

The *Annotated counts* 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.

Annotate Visium image task creates a new node, *Annotated counts*. Double click on the **Annotated counts** node to invoke the *Data Viewer* showing data points overlaid on top of the microscopy image (Figure 10).

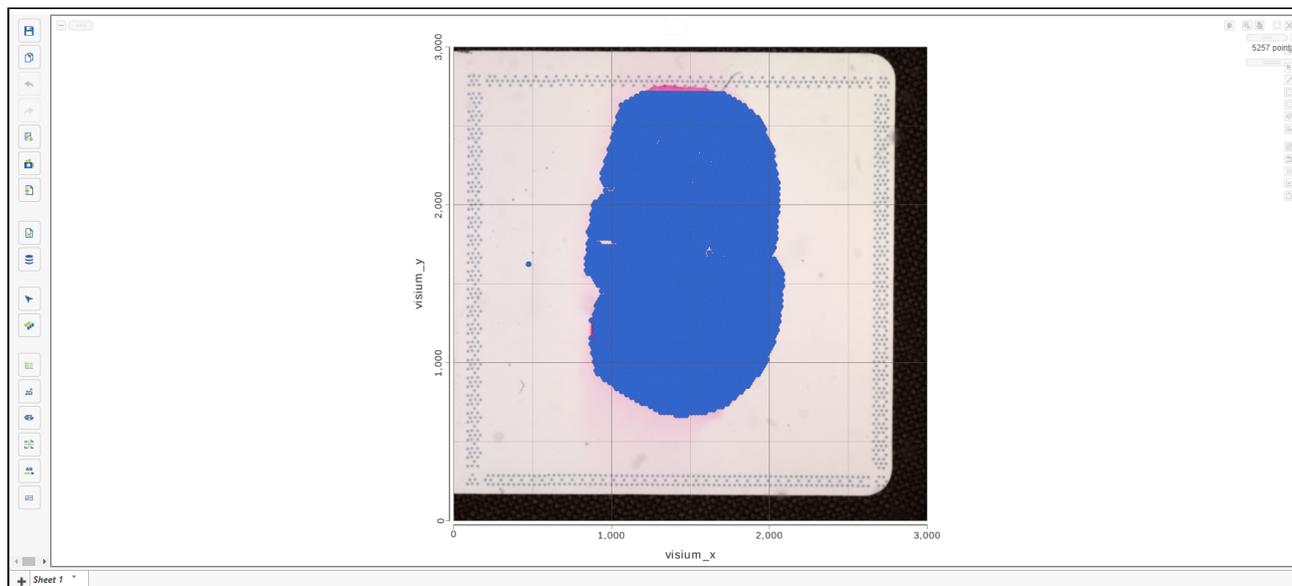


Figure 20. Data Viewer session as a result of opening an Annotated counts data node. Each data point is a tissue spot

Resources

1. <https://support.10xgenomics.com/single-cell-gene-expression/software/overview/welcome>