SINGULAR
GENOMICS

G4X VIEWER

G4X FILE OUTPUTS

Understanding G4X File Outputs Names

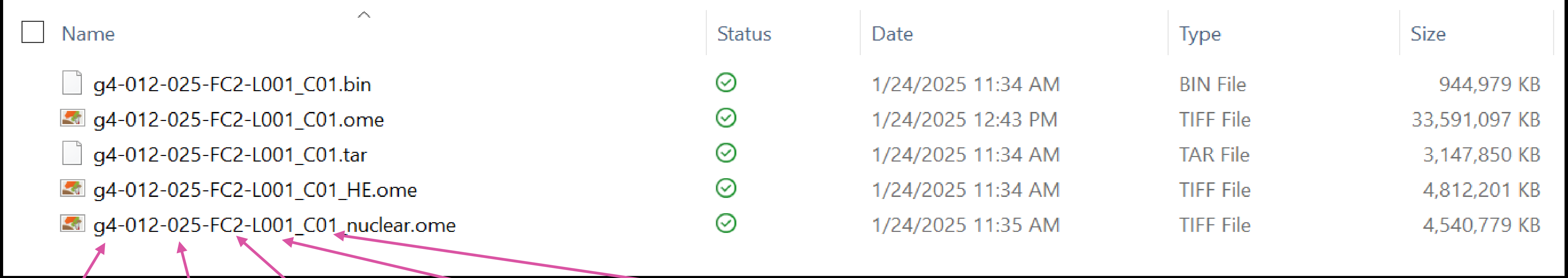
Possible Tissue Locations

4-lane flow cell

A1	B1	C1	D1	E1	F1	G1	H1
A2	B2	C2	D2	E2	F2	G2	H2
A3	B3	C3	D3	E3	F3	G3	H3
A4	B4	C4	D4	E4	F4	G4	H4

2-lane flow cell

A1	B1	C1	D1	E1
A2	B2	C2	D2	E2



☐ Name	Status	Date	Type	Size
g4-012-025-FC2-L001_C01.bin	✓	1/24/2025 11:34 AM	BIN File	944,979 KB
g4-012-025-FC2-L001_C01.ome	✓	1/24/2025 12:43 PM	TIFF File	33,591,097 KB
g4-012-025-FC2-L001_C01.tar	✓	1/24/2025 11:34 AM	TAR File	3,147,850 KB
g4-012-025-FC2-L001_C01_HE.ome	✓	1/24/2025 11:34 AM	TIFF File	4,812,201 KB
g4-012-025-FC2-L001_C01_nuclear.ome	✓	1/24/2025 11:35 AM	TIFF File	4,540,779 KB

Instrument name/ number

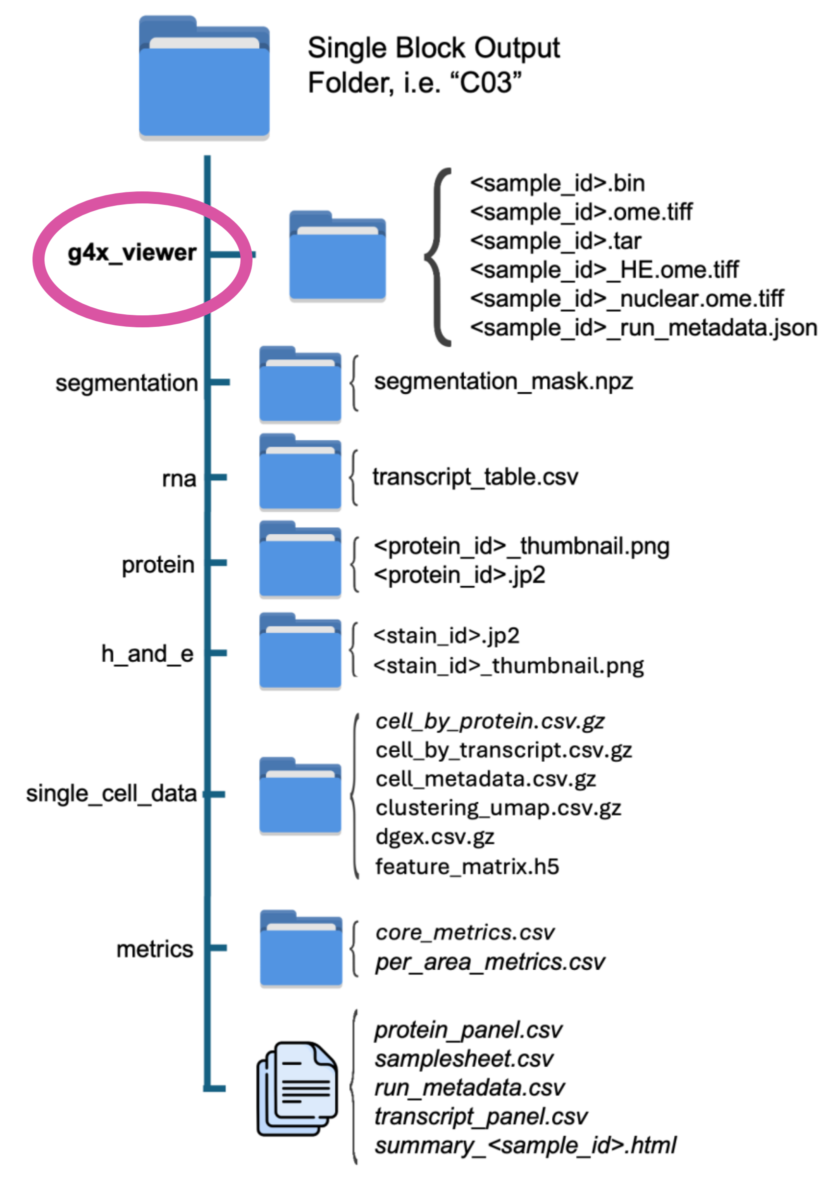
Run number

Flow cell number
* The nest can take FC1, FC2, FC3, FC4 depending on where the sample is placed

Lane number

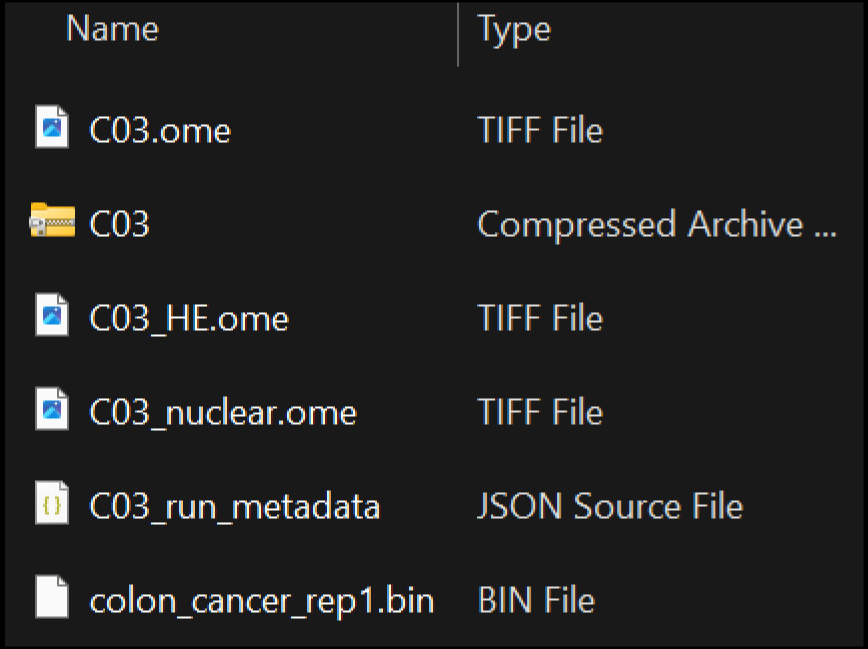
Tissue location on the lane

G4X File outputs



Single Block Output Folder, i.e. "C03"

- g4x_viewer
  - <sample_id>.bin
  - <sample_id>.ome.tiff
  - <sample_id>.tar
  - <sample_id>_HE.ome.tiff
  - <sample_id>_nuclear.ome.tiff
  - <sample_id>_run_metadata.json
- segmentation
  - segmentation_mask.npz
- rna
  - transcript_table.csv
- protein
  - <protein_id>_thumbnail.png
  - <protein_id>.jp2
- h_and_e
  - <stain_id>.jp2
  - <stain_id>_thumbnail.png
- single_cell_data
  - *cell_by_protein.csv.gz*
  - cell_by_transcript.csv.gz
  - cell_metadata.csv.gz
  - clustering_umap.csv.gz
  - dgex.csv.gz
  - feature_matrix.h5
- metrics
  - *core_metrics.csv*
  - *per_area_metrics.csv*
- *protein_panel.csv*
- *samplesheet.csv*
- *run_metadata.csv*
- *transcript_panel.csv*
- *summary_<sample_id>.html*

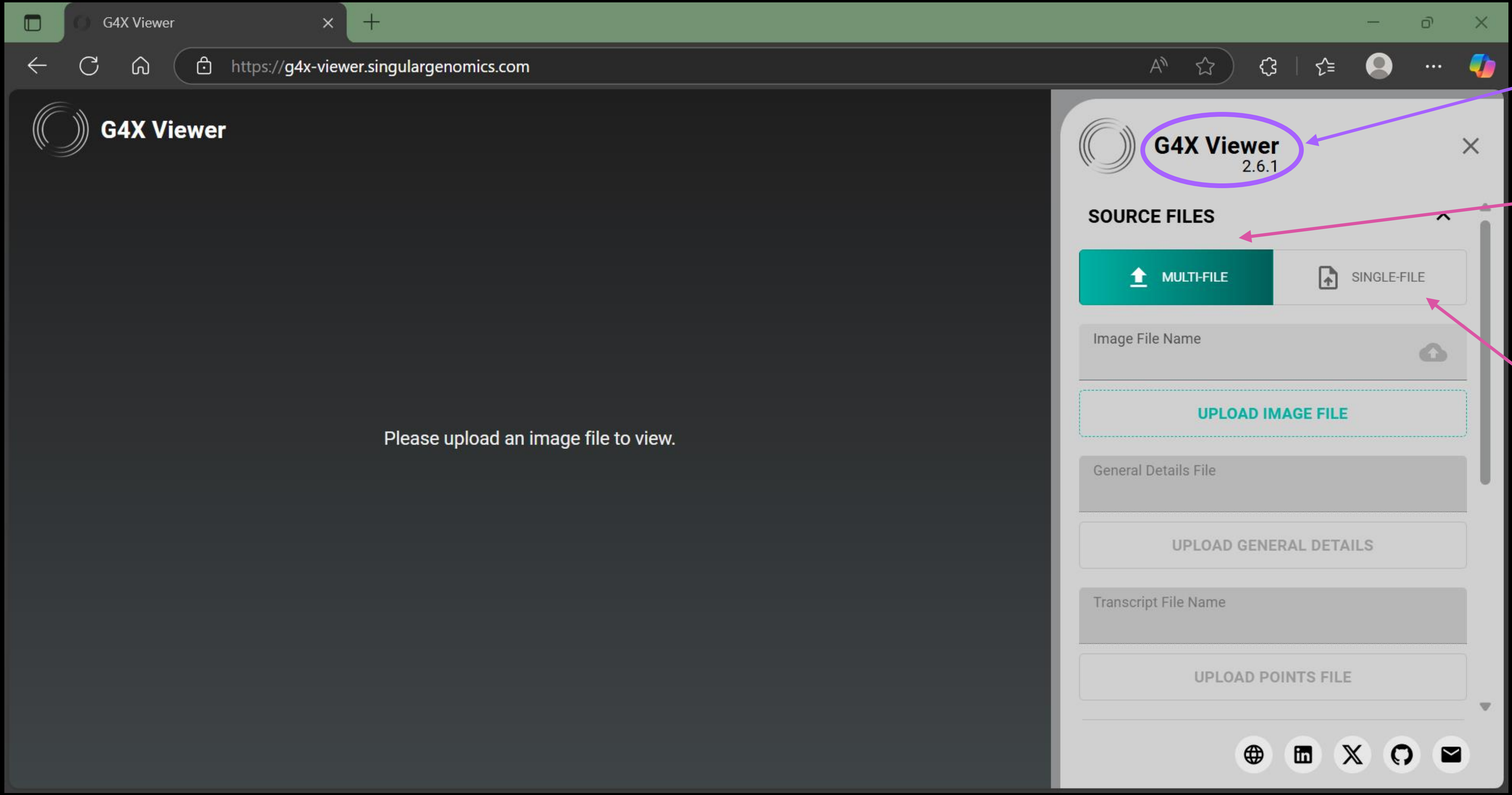


Name	Type
C03.ome	TIFF File
C03	Compressed Archive ...
C03_HE.ome	TIFF File
C03_nuclear.ome	TIFF File
C03_run_metadata	JSON Source File
colon_cancer_rep1.bin	BIN File

File output extension	Data type
<sample_1>.bin	Cell segmentation mask
<sample_1>.ome	Proteomics data
<sample_1>.tar folder	Transcriptomics data
<sample_1>_HE.ome	fH&E images
<sample_1>_nuclear.ome	Fluorescent version of the fH&E
<sample_1>_metadata.json	General Details File

G4X VIEWER

G4X HOMEPAGE



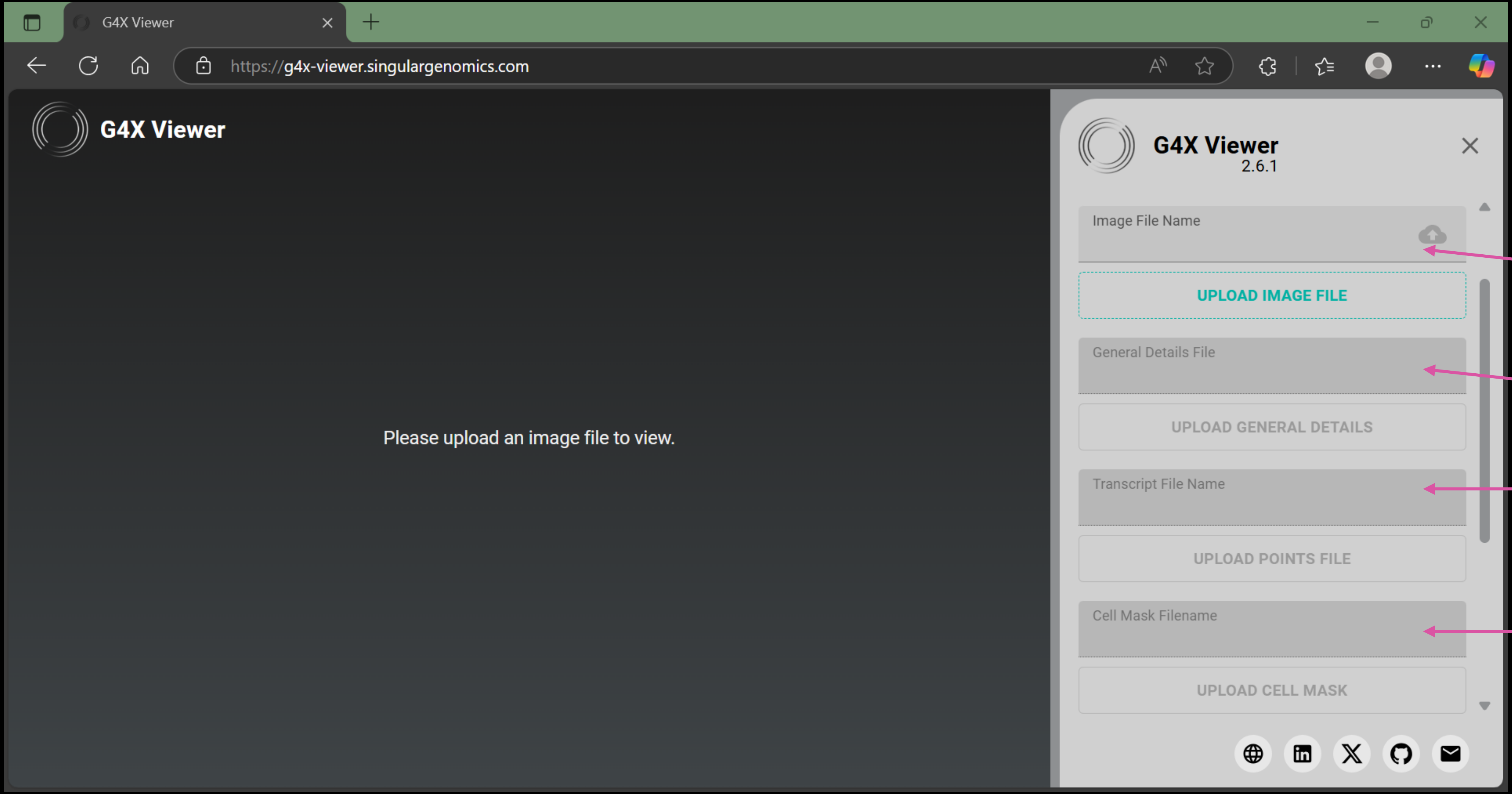
G4X Viewer Version

Multifile Upload
Upload each individual file

Single file Upload
requires a compressed .tar folder

html-based G4X viewer : https://g4x-viewer.singulargenomics.com/

UPLOADING FILES



Proteins images file*
Ome.tiff

Metadata JSON file
.json

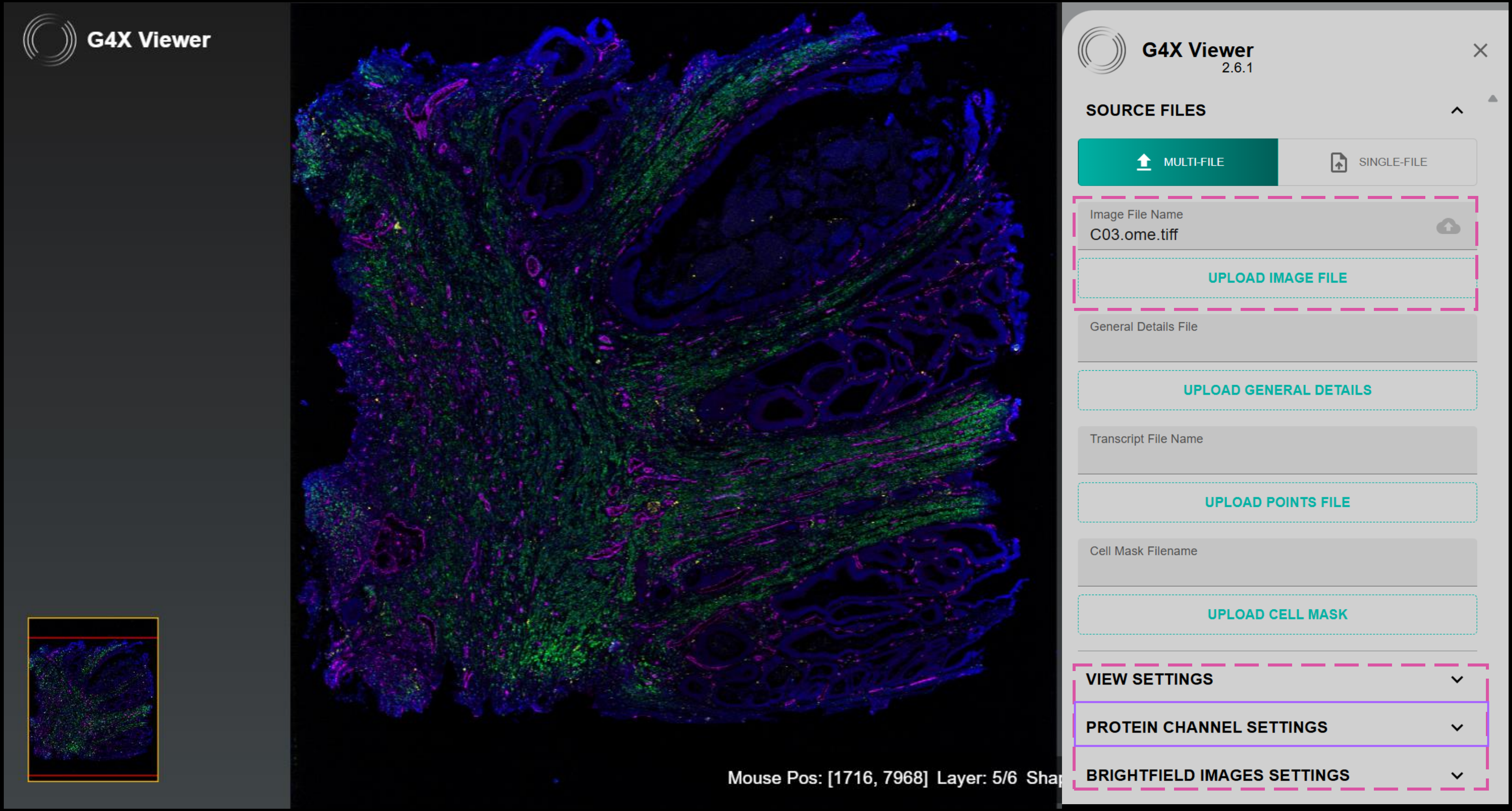
Transcripts Images Folder
.tar

Cell Segmentation Mask
.bin

*can also be used to visualize the fH&E images- HE.ome.tiff

PROTEOMICS

PROTEIN CHANNEL SETTINGS



G4X Viewer

G4X Viewer
2.6.1

SOURCE FILES

Image File Name
C03.ome.tiff

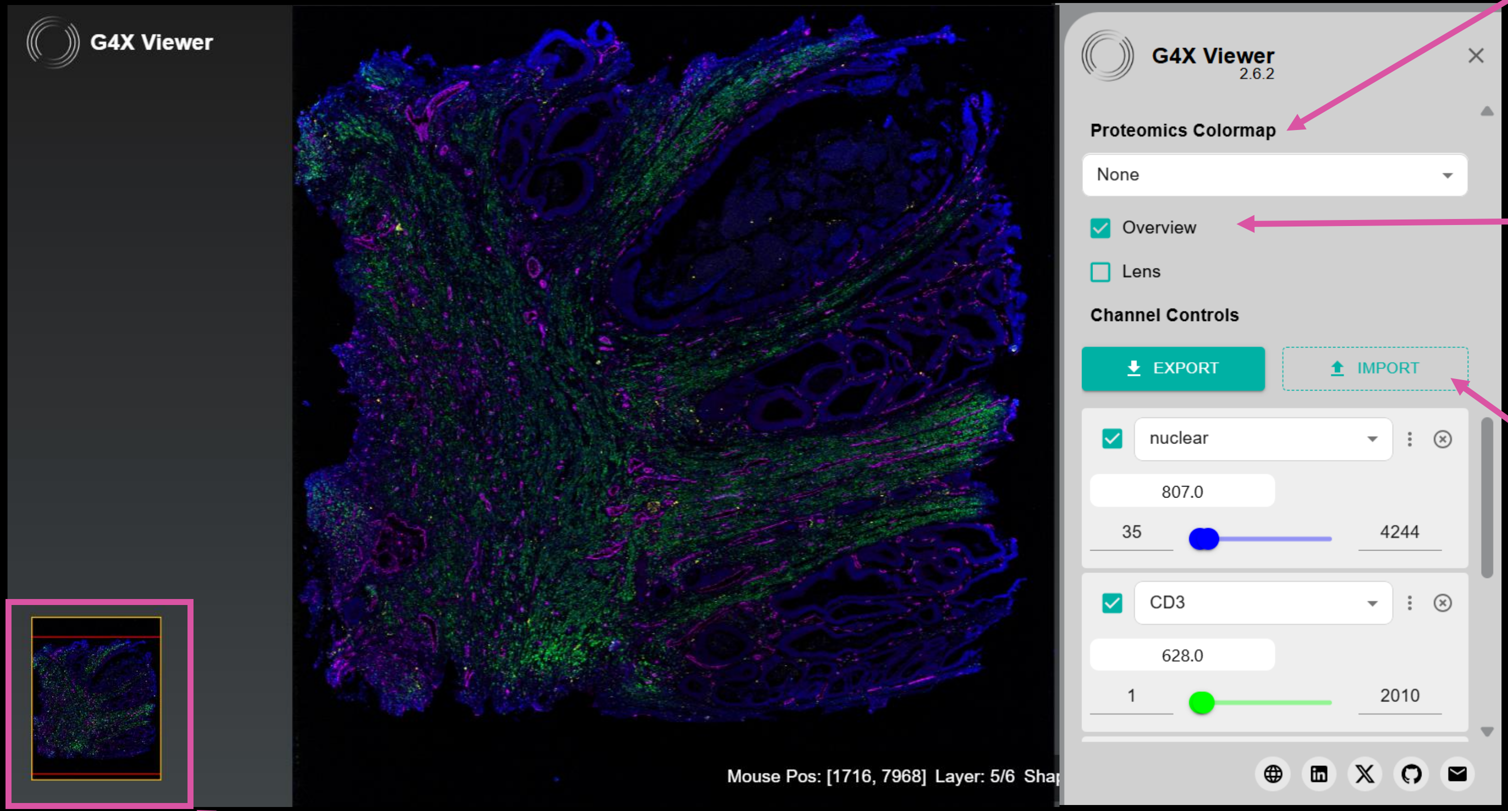
General Details File

Transcript File Name

Cell Mask Filename

Mouse Pos: [1716, 7968] Layer: 5/6 Sha

PROTEIN CHANNEL SETTINGS



Proteomics Colormap

None

☑ Overview

☐ Lens

Channel Controls

☑ nuclear
807.0
35 4244

☑ CD3
628.0
1 2010

Mouse Pos: [1716, 7968] Layer: 5/6 Sha

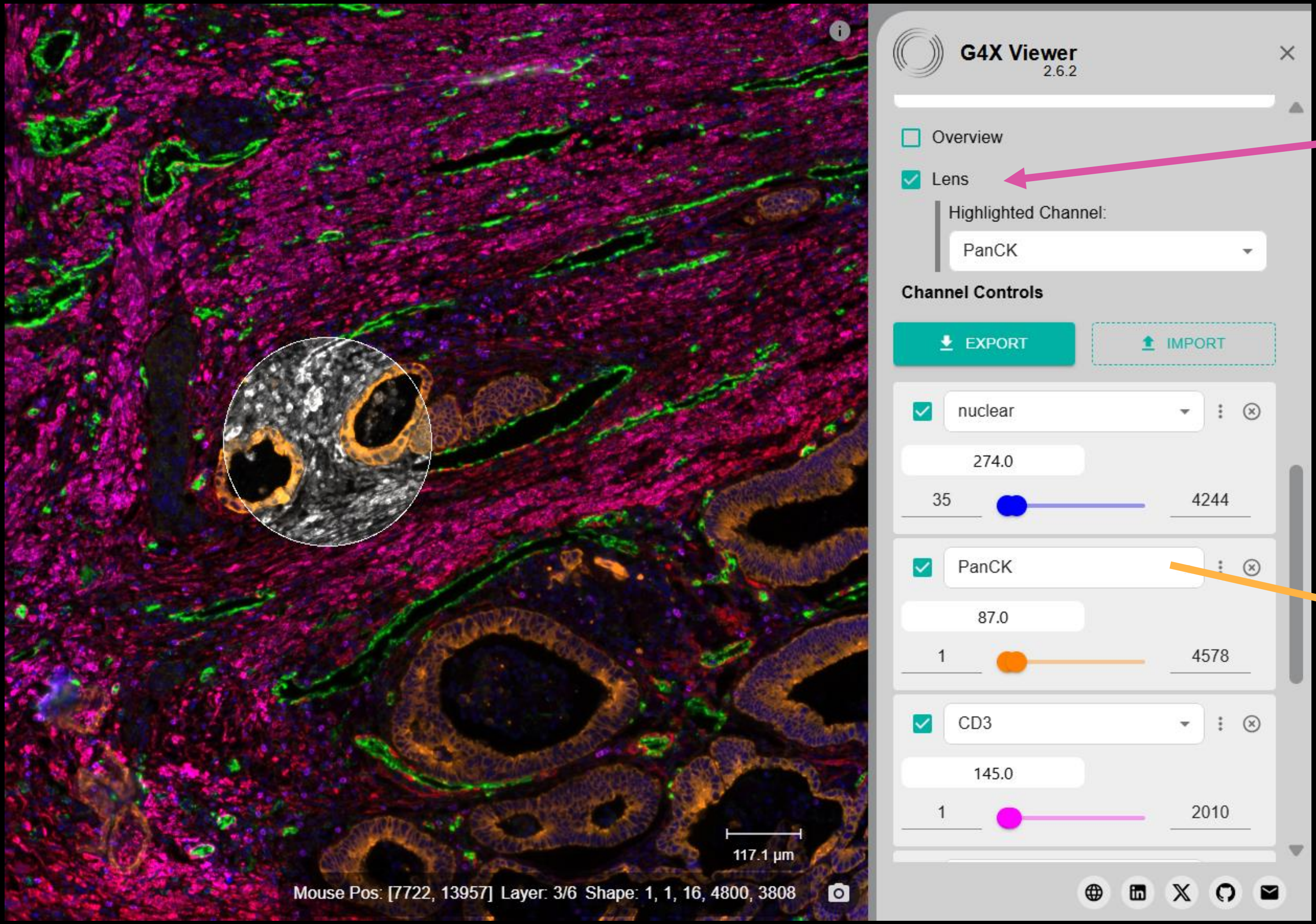
Choose a Proteomics Color Map to visualize the proteins selected in a heat map to show protein density

Overview window at the bottom left – especially useful when visualizing a zoomed in anatomical region on the tissue

You can export and import your protein color and contrast settings to reuse or share for consistency across datasets or publications.

Overview window

LENS FUNCTION



G4X Viewer
2.6.2

☐ Overview

☑ Lens

Highlighted Channel: PanCK

Channel Controls

EXPORT IMPORT

☑ nuclear
274.0
35 4244

☑ PanCK
87.0
1 4578

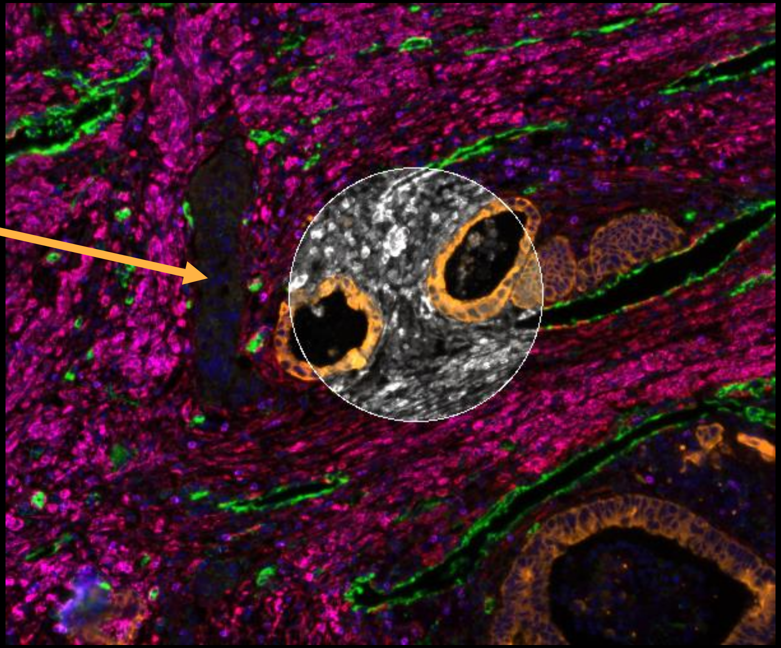
☑ CD3
145.0
1 2010

117.1 µm

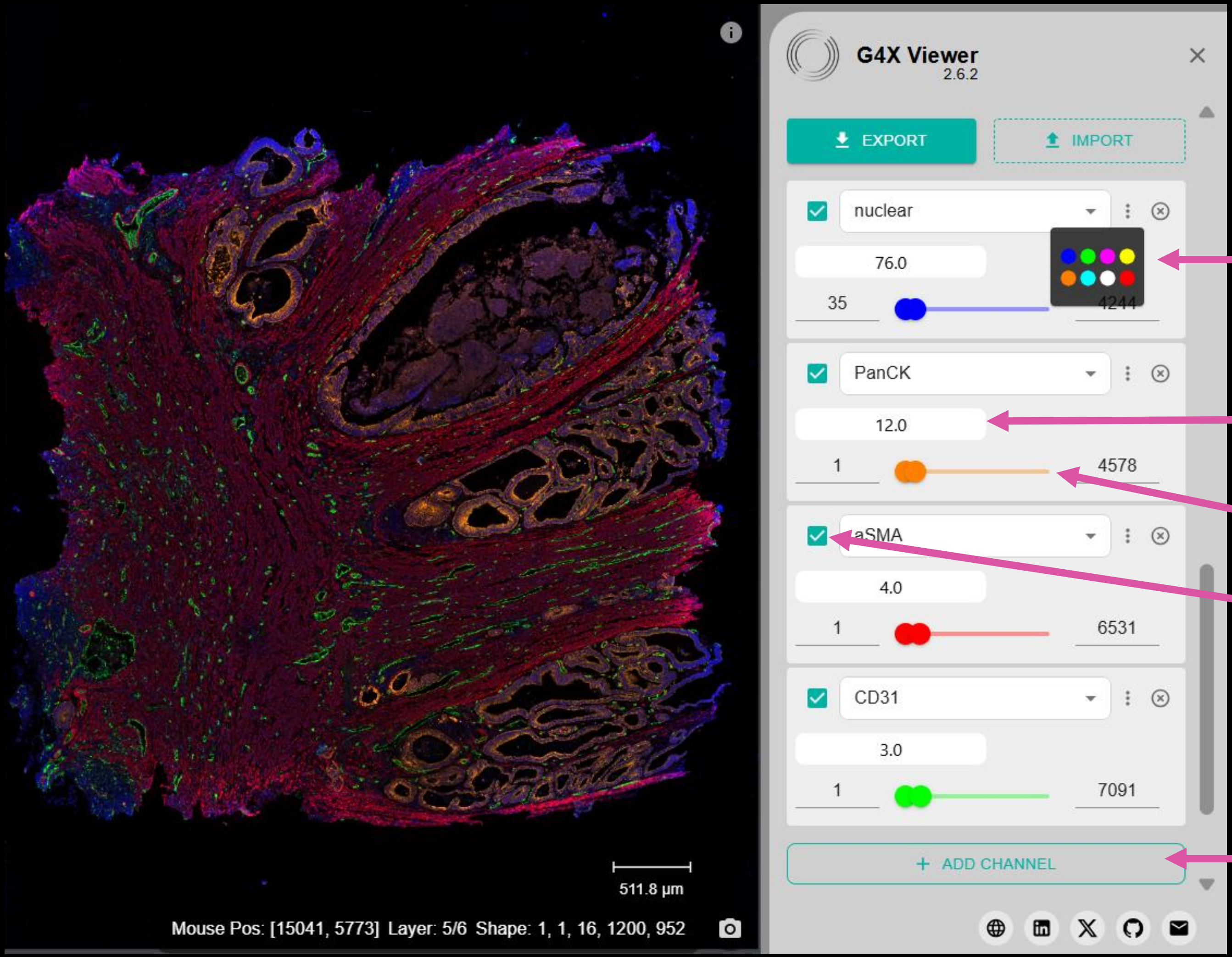
Mouse Pos: [7722, 13957] Layer: 3/6 Shape: 1, 1, 16, 4800, 3808

Can be turned on for a specific marker of interest to visualize individually on the tissue

Greys out all proteins except for the one highlighted



PROTEOMICS CHANNEL SETTINGS



☑ nuclear
76.0
35 — 4244

☑ PanCK
12.0
1 — 4578

☑ aSMA
4.0
1 — 6531

☑ CD31
3.0
1 — 7091

Mouse Pos: [15041, 5773] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

Change Color of individual markers

Pixel intensity of individual channel

Adjust contrast

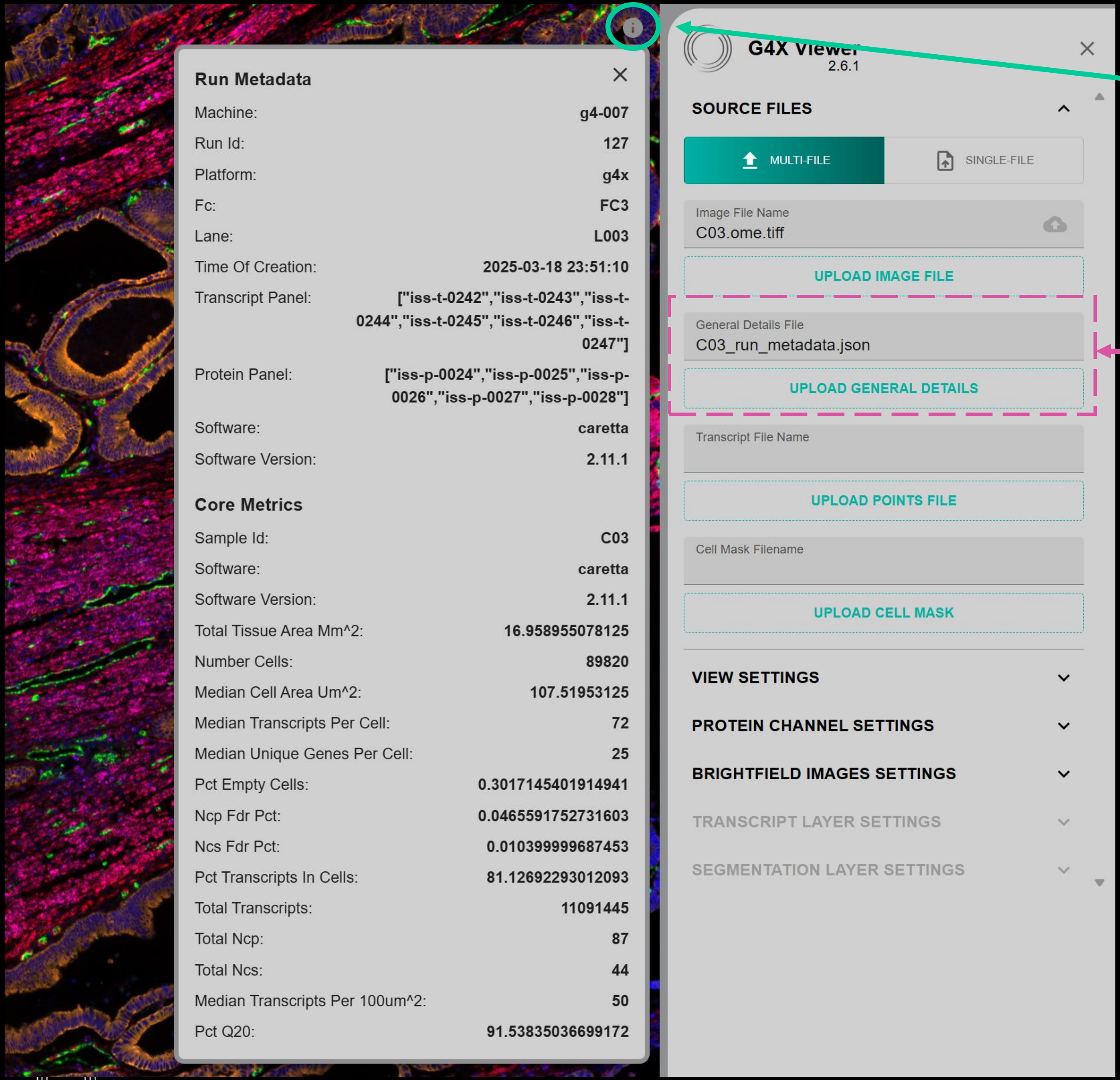
Turn on and off individual channels

Visualize up to 6 Channels

SINGULAR
GENOMICS

GENERAL DETAILS FILE

GENERAL DETAILS FILE



Run Metadata

Machine:	g4-007
Run Id:	127
Platform:	g4x
Fc:	FC3
Lane:	L003
Time Of Creation:	2025-03-18 23:51:10
Transcript Panel:	["iss-t-0242","iss-t-0243","iss-t-0244","iss-t-0245","iss-t-0246","iss-t-0247"]
Protein Panel:	["iss-p-0024","iss-p-0025","iss-p-0026","iss-p-0027","iss-p-0028"]
Software:	caretta
Software Version:	2.11.1

Core Metrics

Sample Id:	C03
Software:	caretta
Software Version:	2.11.1
Total Tissue Area Mm^2:	16.958955078125
Number Cells:	89820
Median Cell Area Um^2:	107.51953125
Median Transcripts Per Cell:	72
Median Unique Genes Per Cell:	25
Pct Empty Cells:	0.3017145401914941
Ncp Fdr Pct:	0.0465591752731603
Ncs Fdr Pct:	0.010399999687453
Pct Transcripts In Cells:	81.12692293012093
Total Transcripts:	11091445
Total Ncp:	87
Total Ncs:	44
Median Transcripts Per 100um^2:	50
Pct Q20:	91.53835036699172

Image File Name: C03.ome.tiff

General Details File: C03_run_metadata.json

Transcript File Name:

Cell Mask Filename:

Once the metadata.json file is uploaded, the info icon becomes active, showing assay and sample details

Upload the metadata.json file

TRANSCRIPTS

TRANSCRIPTS: UPLOAD .TAR FOLDER

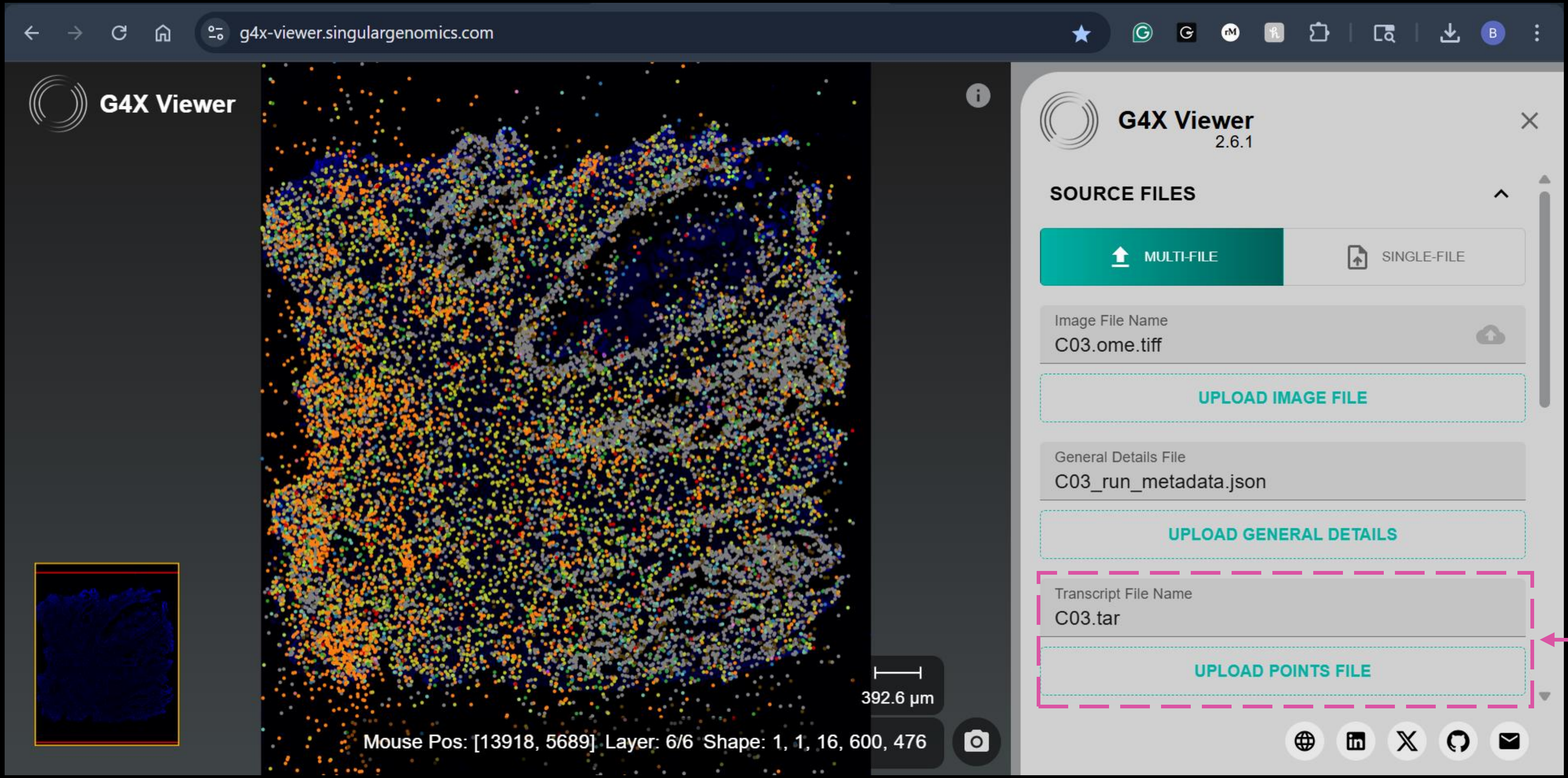
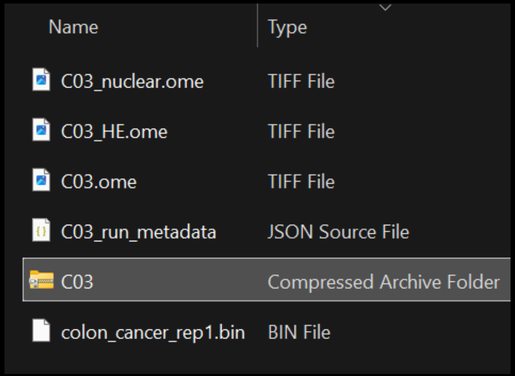


Image File Name
C03.ome.tiff

General Details File
C03_run_metadata.json

Transcript File Name
C03.tar

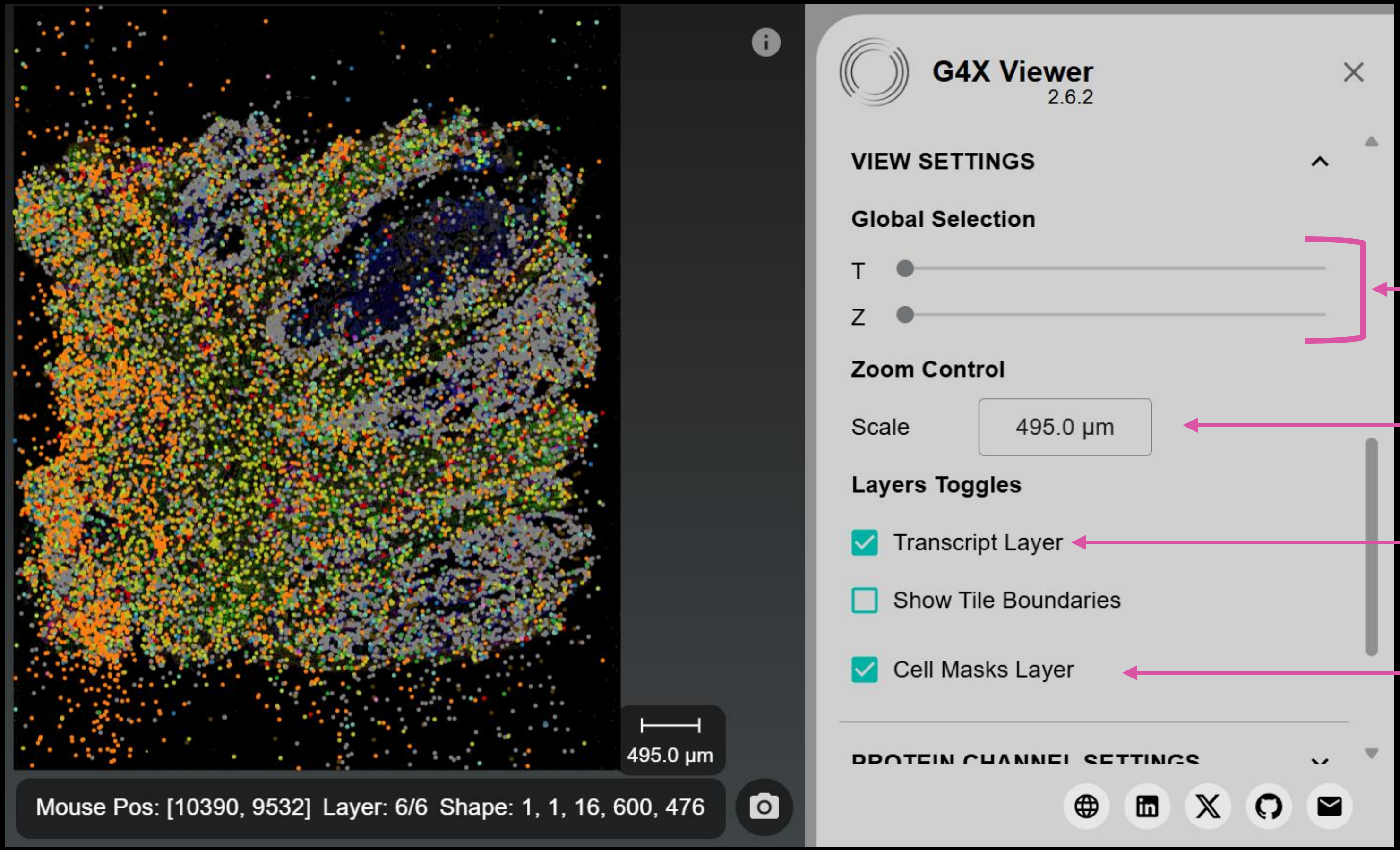
Mouse Pos: [13918, 5689] Layer: 6/6 Shape: 1, 1, 16, 600, 476



Name	Type
C03_nuclear.ome	TIFF File
C03_HE.ome	TIFF File
C03.ome	TIFF File
C03_run_metadata	JSON Source File
C03	Compressed Archive Folder
colon_cancer_rep1.bin	BIN File

Upload .tar compressed folder

VIEW SETTINGS



Scale: 495.0 µm

☑ Transcript Layer

☐ Show Tile Boundaries

☑ Cell Masks Layer

Mouse Pos: [10390, 9532] Layer: 6/6 Shape: 1, 1, 16, 600, 476

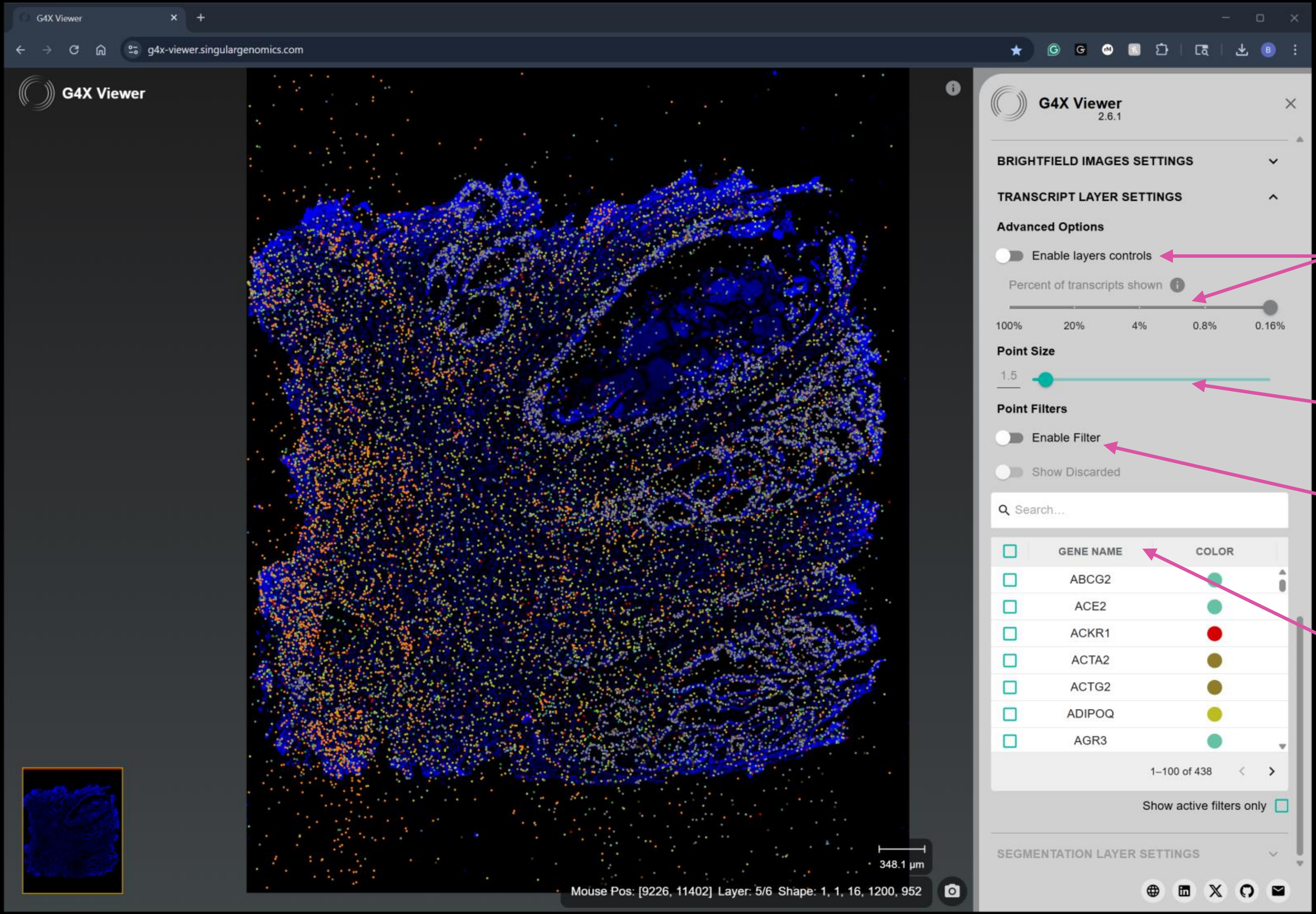
In version 2.6.2 T and Z are not functional but will be updated in later versions

Can set the scale manually

Toggle the transcript layer on and off

Toggle the cell mask layer on and off

TRANSCRIPT LAYER SETTINGS



Advanced Options

Enable layers controls

Percent of transcripts shown: 100% · 20% · 4% · 0.8% · 0.16%

Point Size

1.5

Point Filters

Enable Filter

Show Discarded

Search...

☐	GENE NAME	COLOR
☐	ABCG2	●
☐	ACE2	●
☐	ACKR1	●
☐	ACTA2	●
☐	ACTG2	●
☐	ADIPOQ	●
☐	AGR3	●

1–100 of 438

Show active filters only ☐

348.1 µm

Mouse Pos: [9226, 11402] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

Enable Layers allows you to choose the percent of transcripts you want to view

Change the point size visible genes

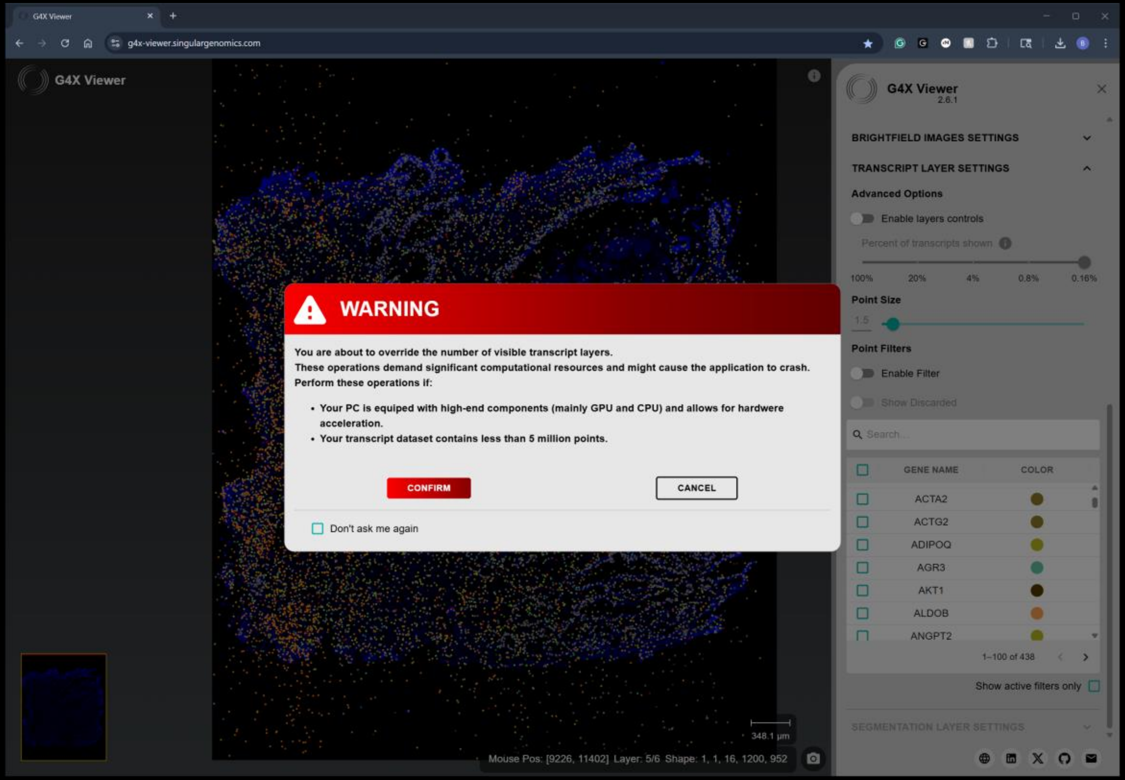
By default, you are viewing all genes. By enabling filter, you can select only the genes you want to view

List of genes and the color they are being displayed

*Note the default is 0.16% of all transcripts. When you zoom in the percent of transcripts visible increases. The max zoom shows 100% of transcripts

SINGULAR GENOMICS

TRANSCRIPTS: ENABLE LAYERS CONTROL



WARNING

You are about to override the number of visible transcript layers.
These operations demand significant computational resources and might cause the application to crash.
Perform these operations if:

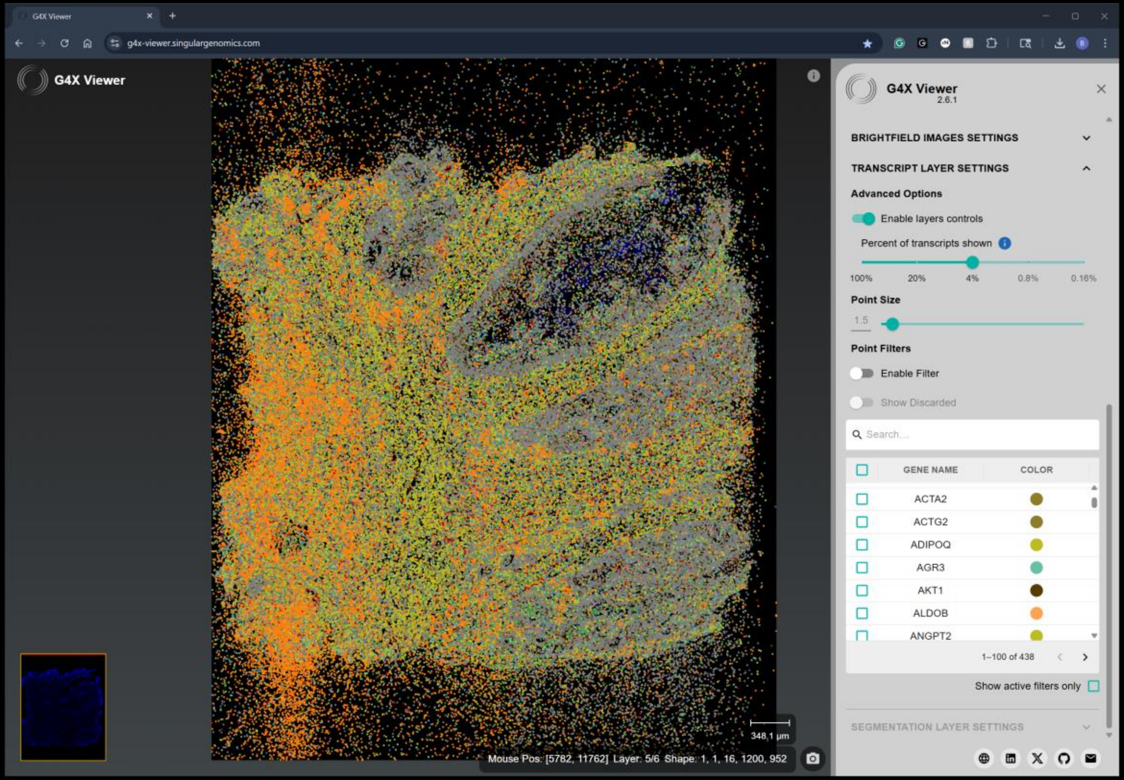
- Your PC is equiped with high-end components (mainly GPU and CPU) and allows for hardwere acceleration.
- Your transcript dataset contains less than 5 million points.

☐ Don't ask me again

Mouse Pos: [9226, 11402] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

When Enabling layers controls a waring message will pop up to let you know that viewing 100% of transcripts can crash the application

You can confirm to continue



● Enable layers controls

Point Size 1.5

○ Enable Filter

☐	GENE NAME	COLOR
☐	ACTA2	●
☐	ACTG2	●
☐	ADIPOQ	●
☐	AGR3	●
☐	AKT1	●
☐	ALDOB	●
☐	ANGPT2	●

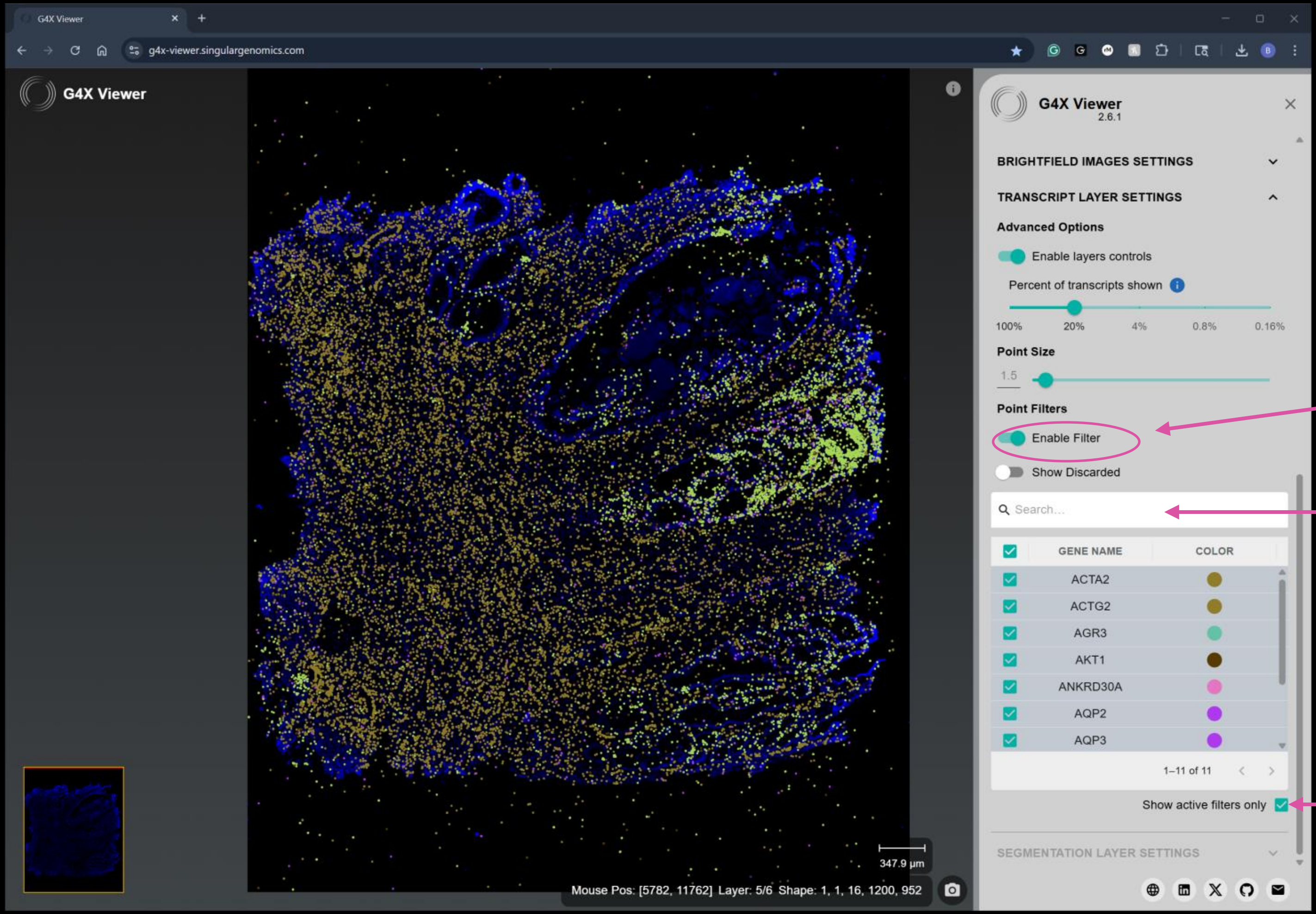
1–100 of 438

Mouse Pos: [5782, 11762] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

Now you can choose the percent of genes you want to view. Note that it does take time to render

SINGULAR GENOMICS

TRANSCRIPTS: ENABLE FILTER



Mouse Pos: [5782, 11762] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

BRIGHTFIELD IMAGES SETTINGS

TRANSCRIPT LAYER SETTINGS

Advanced Options

- Enable layers controls (on)
- Percent of transcripts shown: 100% 20% 4% 0.8% 0.16%

Point Size: 1.5

Point Filters

- Enable Filter (on)
- Show Discarded (off)

Search...

☑	GENE NAME	COLOR
☑	ACTA2	●
☑	ACTG2	●
☑	AGR3	●
☑	AKT1	●
☑	ANKRD30A	●
☑	AQP2	●
☑	AQP3	●

1–11 of 11

Show active filters only ☑

SEGMENTATION LAYER SETTINGS

Enable filter shows only selected genes

Search for specific genes to visualize

Select to view only genes you have selected

SINGULAR GENOMICS

For Research Use Only. Not For Use In Diagnostic Procedures. Product In Development.

fH&E

BRIGHTFIELD IMAGES SETTINGS

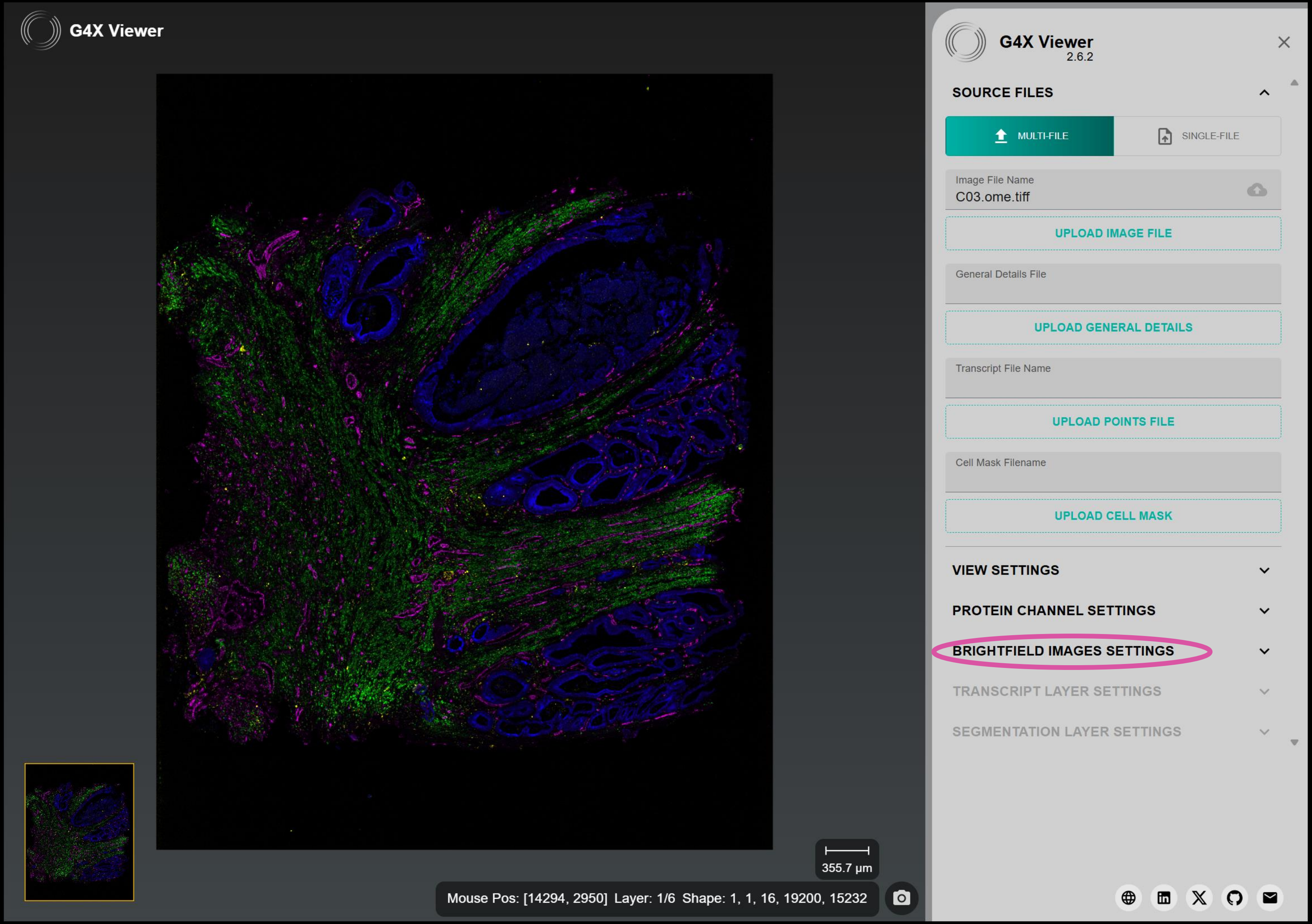


Image File Name: C03.ome.tiff

Mouse Pos: [14294, 2950] Layer: 1/6 Shape: 1, 1, 16, 19200, 15232

BRIGHTFIELD IMAGES SETTINGS: UPLOAD fH&E

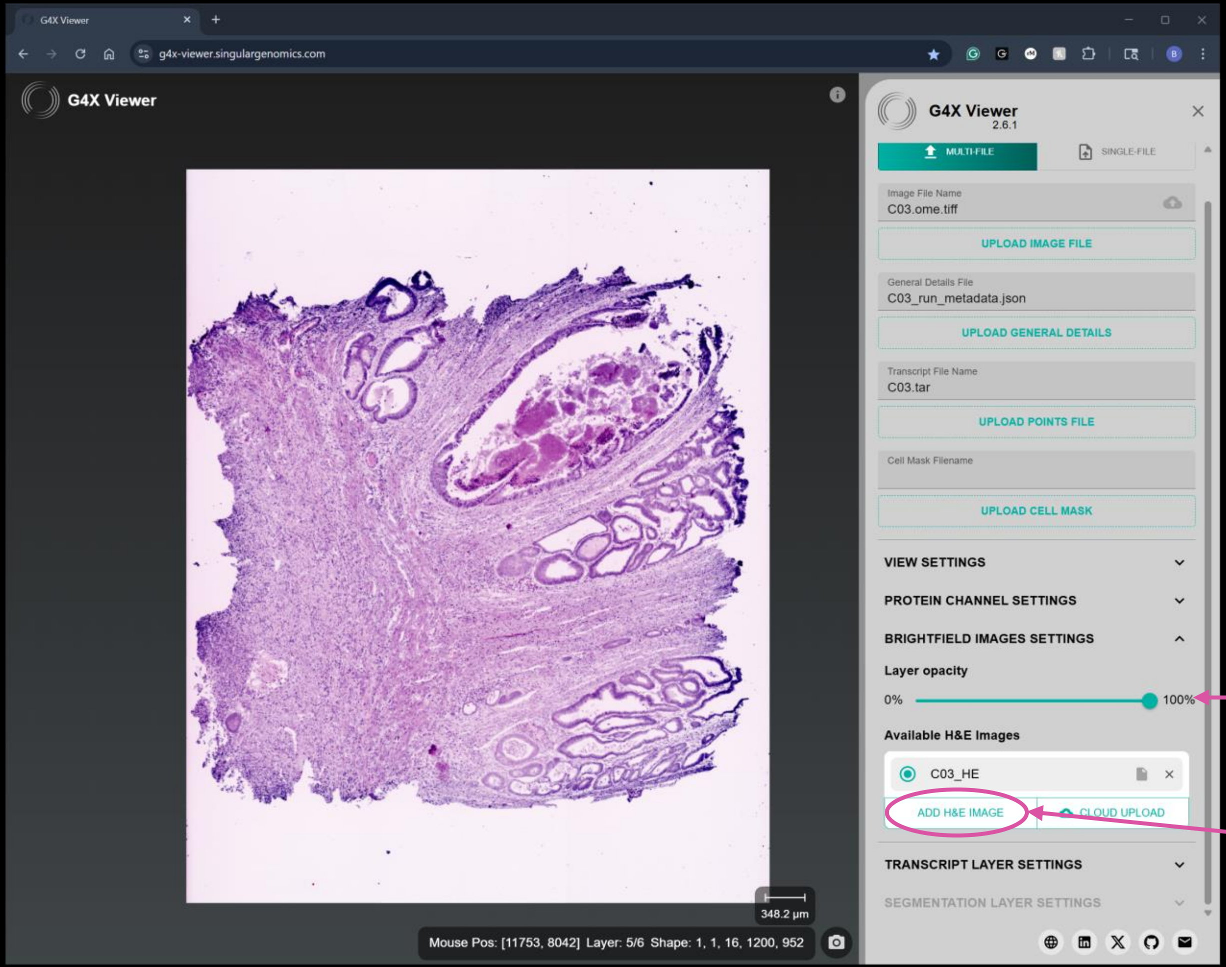


Image File Name: C03.ome.tiff

General Details File: C03_run_metadata.json

Transcript File Name: C03.tar

Cell Mask Filename:

Layer opacity: 0% — 100%

Available H&E Images: ● C03_HE

Mouse Pos: [11753, 8042] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

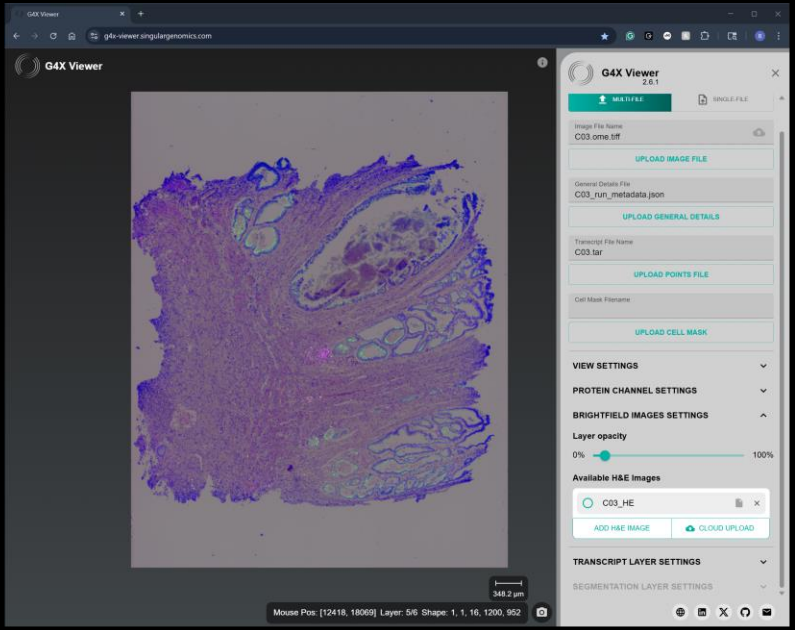


Image File Name: C03.ome.tiff

General Details File: C03_run_metadata.json

Transcript File Name: C03.tar

Cell Mask Filename:

Layer opacity: 0% — 100%

Available H&E Images: ○ C03_HE

Mouse Pos: [12418, 18069] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

The opacity of the fH&E image can be adjusted from here to make it easier to switch back and forth between proteins and fH&E

Upload fH&E

*fH&E image can be visualized on top of the proteins and the transcripts data

SINGULAR GENOMICS

CELL SEGMENTATION

CELL LAYER SETTINGS: UPLOAD.BIN FILE

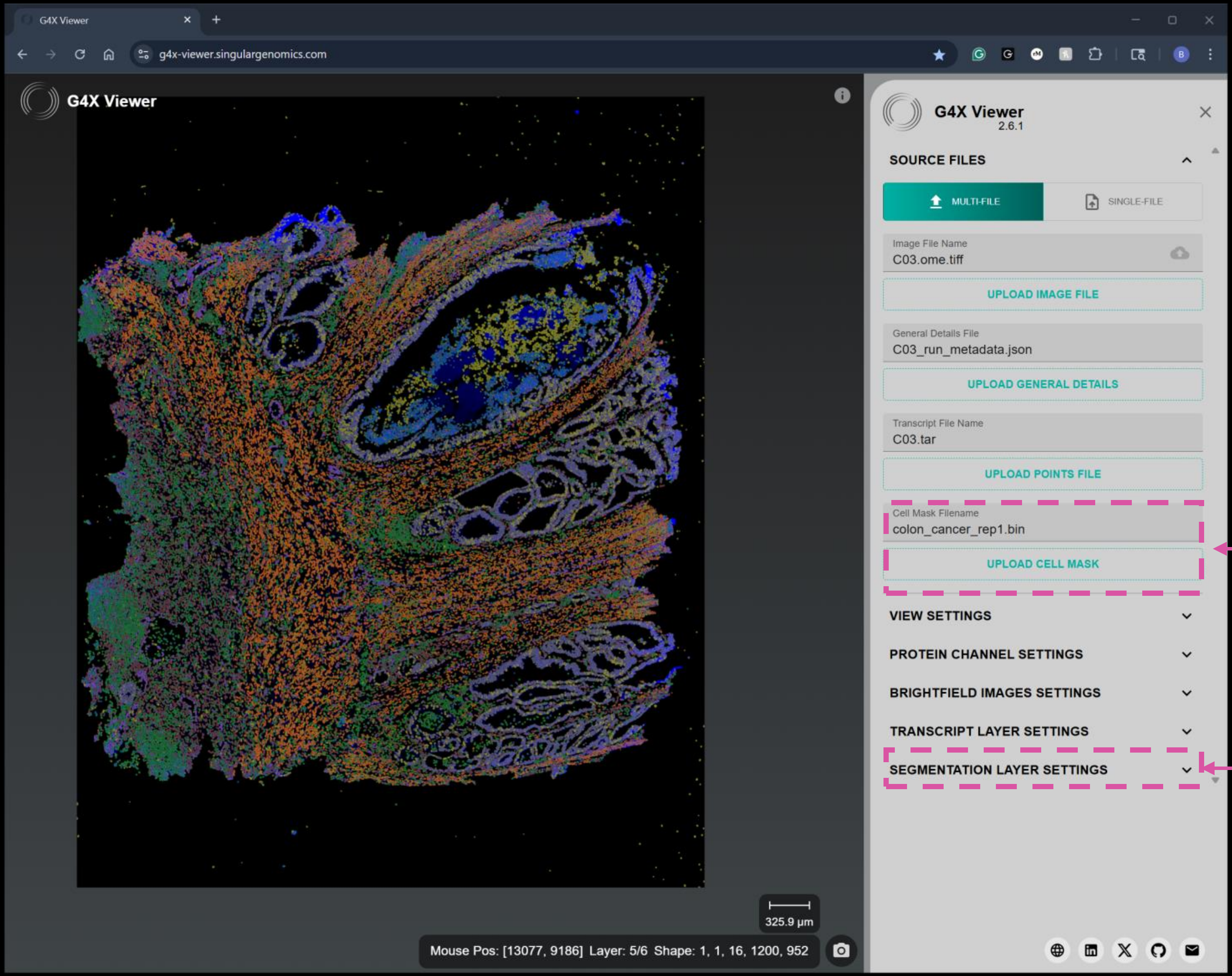


Image File Name
C03.ome.tiff

General Details File
C03_run_metadata.json

Transcript File Name
C03.tar

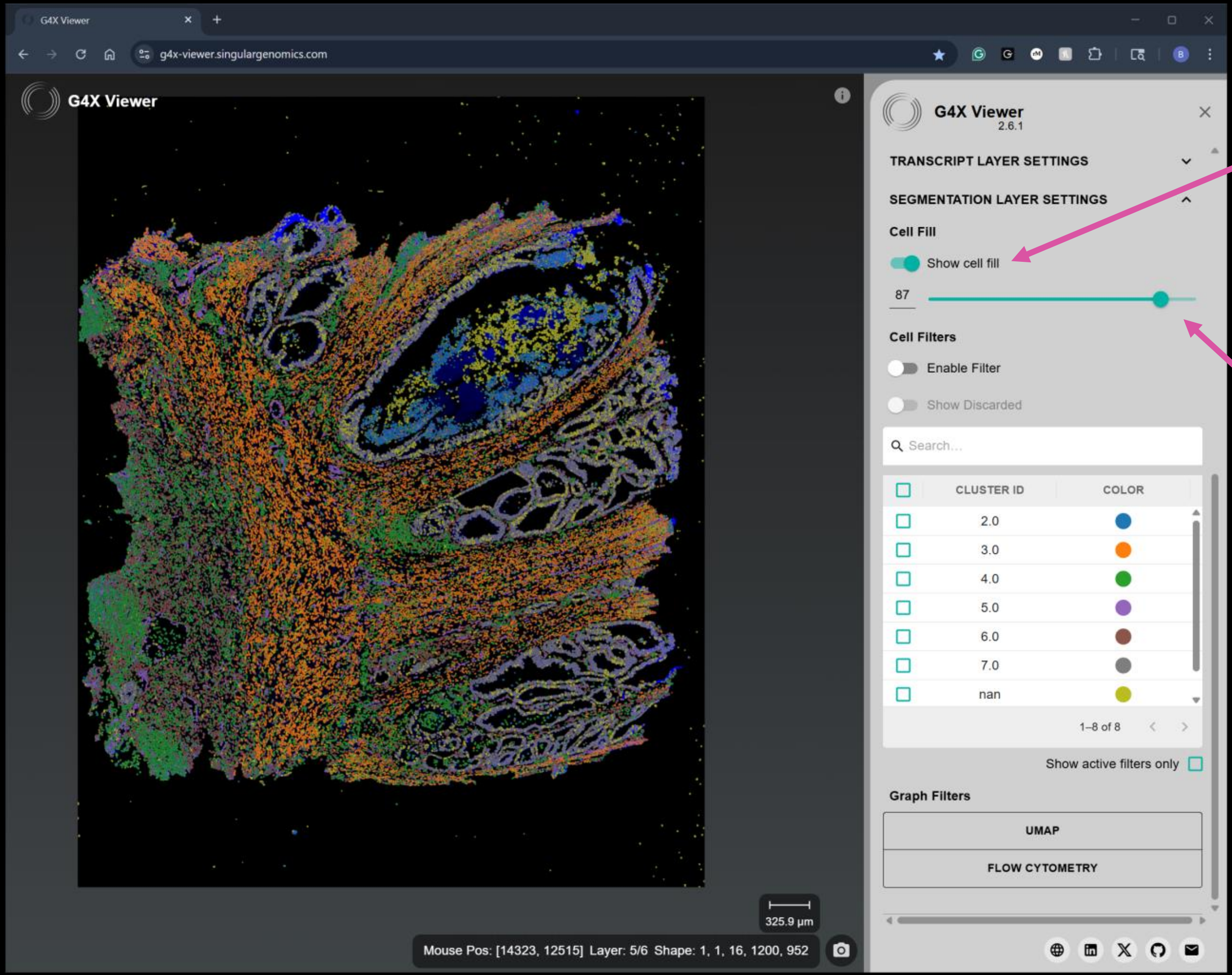
Cell Mask Filename
colon_cancer_rep1.bin

Mouse Pos: [13077, 9186] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

Upload cell segmentation mask - .bin file

Segmentation Layer Settings

CELL LAYER SETTINGS



G4X Viewer

g4x-viewer.singulargenomics.com

G4X Viewer 2.6.1

TRANSCRIPT LAYER SETTINGS

SEGMENTATION LAYER SETTINGS

Cell Fill

● Show cell fill

87

Cell Filters

○ Enable Filter

○ Show Discarded

Search...

☐	CLUSTER ID	COLOR
☐	2.0	🔵
☐	3.0	🟠
☐	4.0	🟢
☐	5.0	🟣
☐	6.0	🟤
☐	7.0	●
☐	nan	🟡

1–8 of 8

Show active filters only ☐

Graph Filters

UMAP

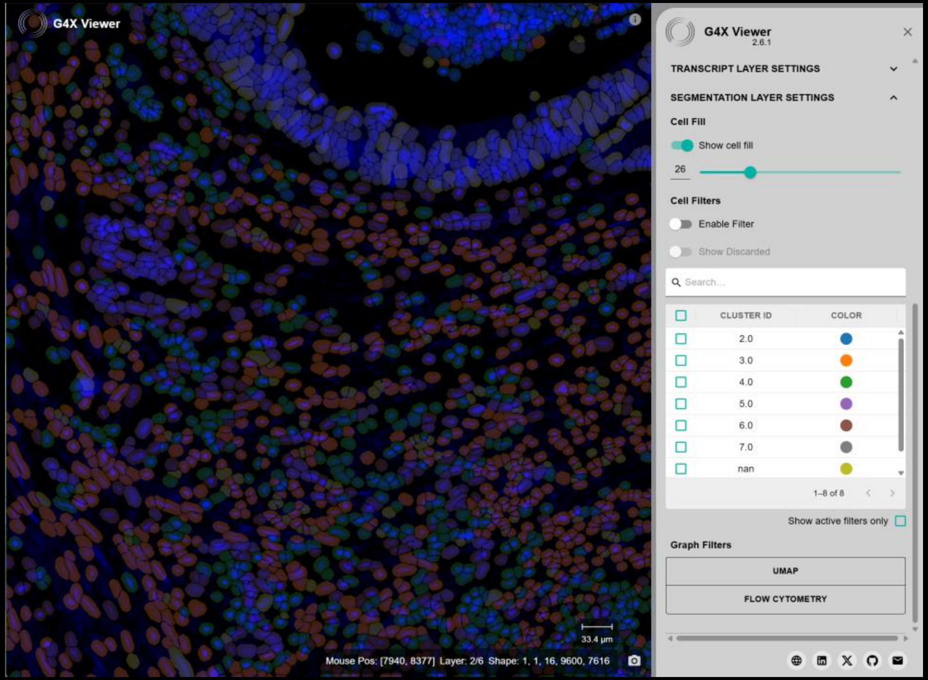
FLOW CYTOMETRY

325.9 µm

Mouse Pos: [14323, 12515] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

Toggle on and off cell segmentation

Can adjust the opacity of the cell mask



G4X Viewer 2.6.1

TRANSCRIPT LAYER SETTINGS

SEGMENTATION LAYER SETTINGS

Cell Fill

● Show cell fill

26

Cell Filters

○ Enable Filter

○ Show Discarded

Search...

☐	CLUSTER ID	COLOR
☐	2.0	🔵
☐	3.0	🟠
☐	4.0	🟢
☐	5.0	🟣
☐	6.0	🟤
☐	7.0	●
☐	nan	🟡

1–8 of 8

Show active filters only ☐

Graph Filters

UMAP

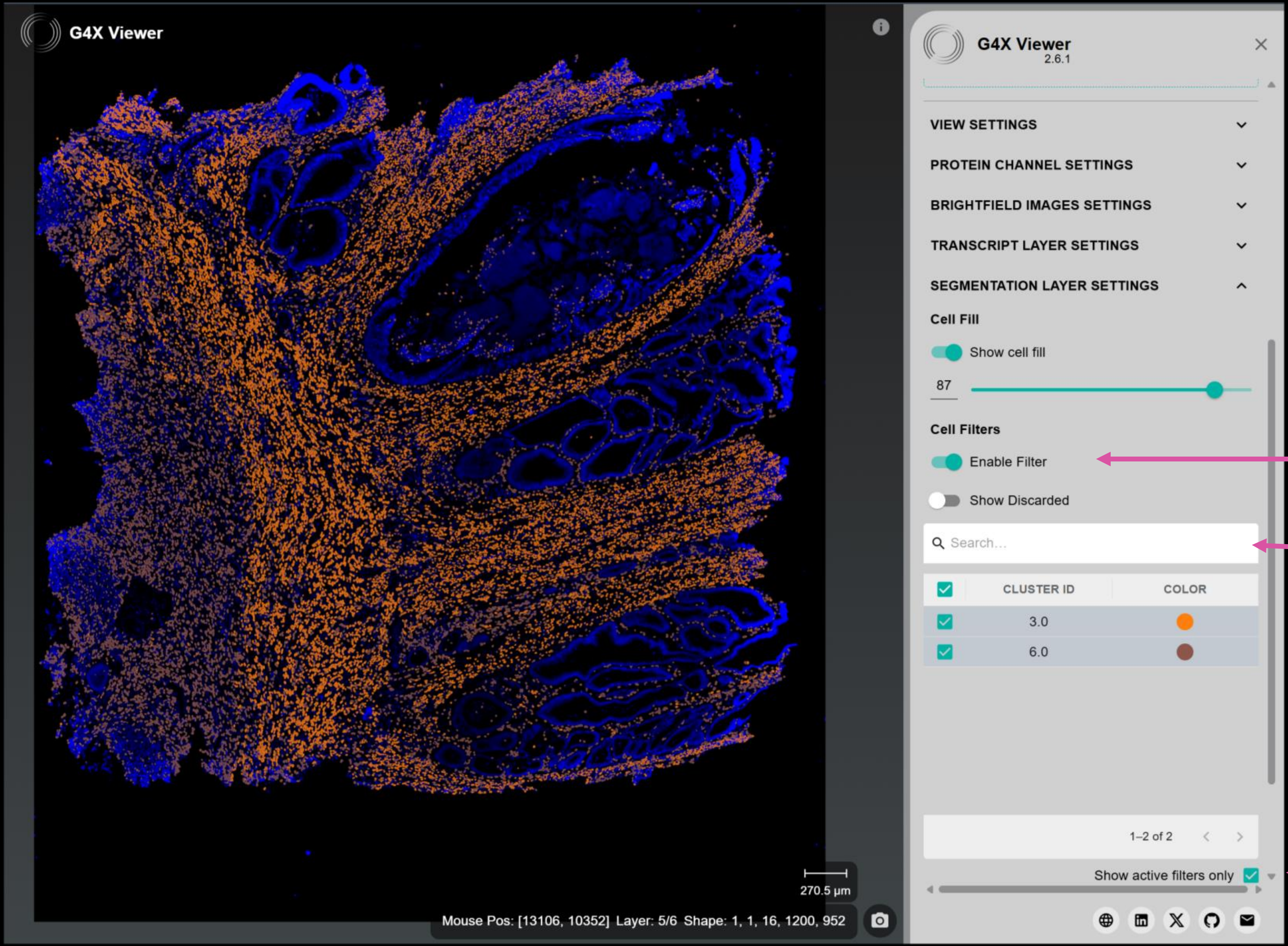
FLOW CYTOMETRY

33.4 µm

Mouse Pos: [7940, 8377] Layer: 2/6 Shape: 1, 1, 16, 9600, 7616

Image zoomed in to see cell mask

CELL LAYER SETTINGS: ENABLE FILTER



Cell Fill

● Show cell fill

87

Cell Filters

● Enable Filter

○ Show Discarded

☑	CLUSTER ID	COLOR
☑	3.0	●
☑	6.0	●

Show active filters only ☑

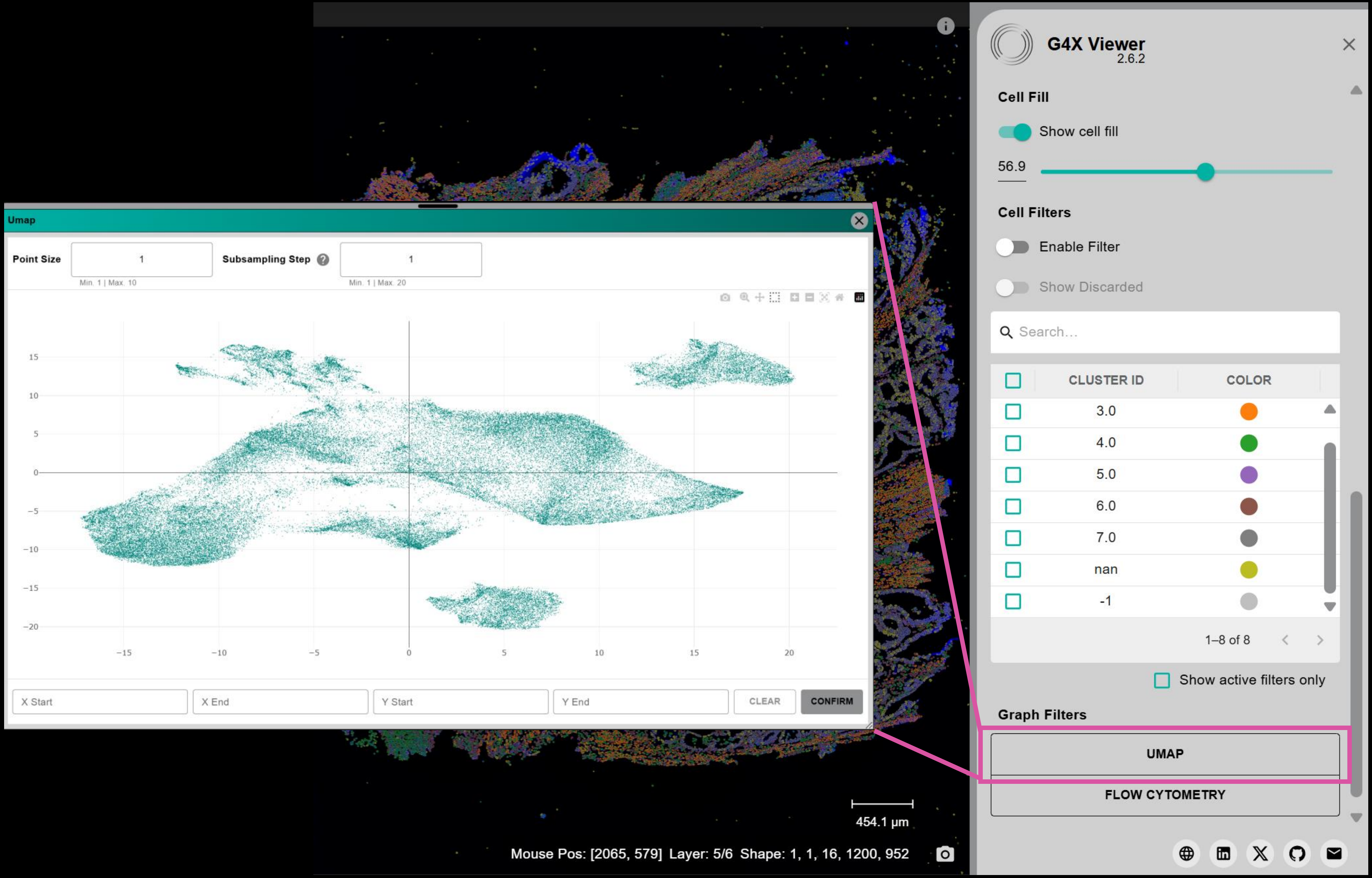
Mouse Pos: [13106, 10352] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

Enable filters to only view selected clusters

Search cluster IDs

Only view selected clusters

CELL LAYER SETTINGS: UMAP



Point Size 1 (Min. 1 | Max. 10)

Subsampling Step 1 (Min. 1 | Max. 20)

G4X Viewer 2.6.2

Cell Fill

● Show cell fill

56.9

Cell Filters

○ Enable Filter

○ Show Discarded

Search...

☐	CLUSTER ID	COLOR
☐	3.0	🟠
☐	4.0	🟢
☐	5.0	🟣
☐	6.0	🟤
☐	7.0	⚫
☐	nan	🟡
☐	-1	⚪

1–8 of 8

☐ Show active filters only

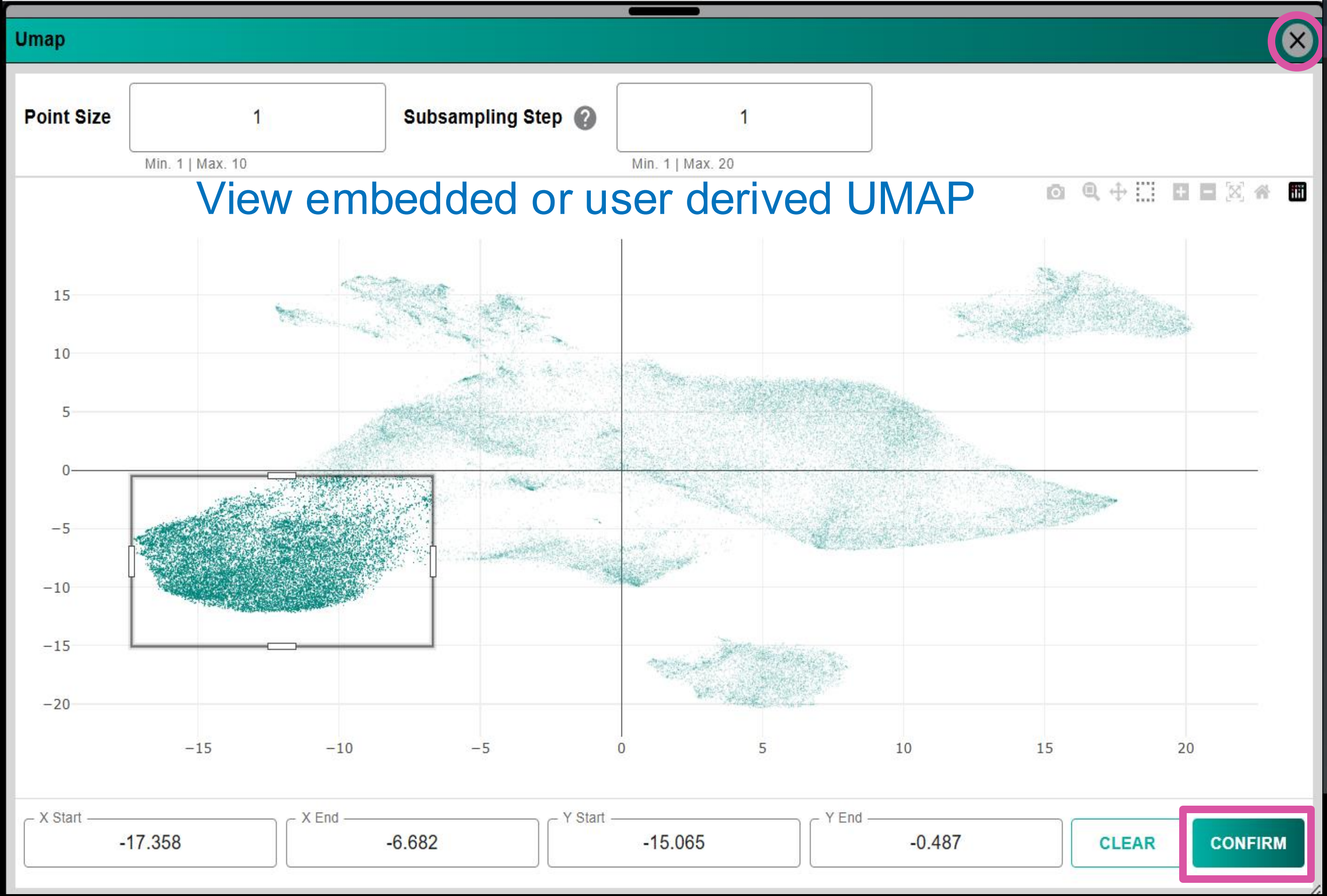
Graph Filters

Mouse Pos: [2065, 579] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

In addition to visualizing individual cell clusters, you can utilize the UMAP selector tool to select a specific ROI on the interactive UMAP embedding and plot the spatial localization of selected cell.

This can be useful for exploring cluster- and sub-cluster level spatial pattering of cells in your tissue.

CELL LAYER SETTINGS: UMAP FILTER



Point Size: 1 (Min. 1 | Max. 10)

Subsampling Step: 1 (Min. 1 | Max. 20)

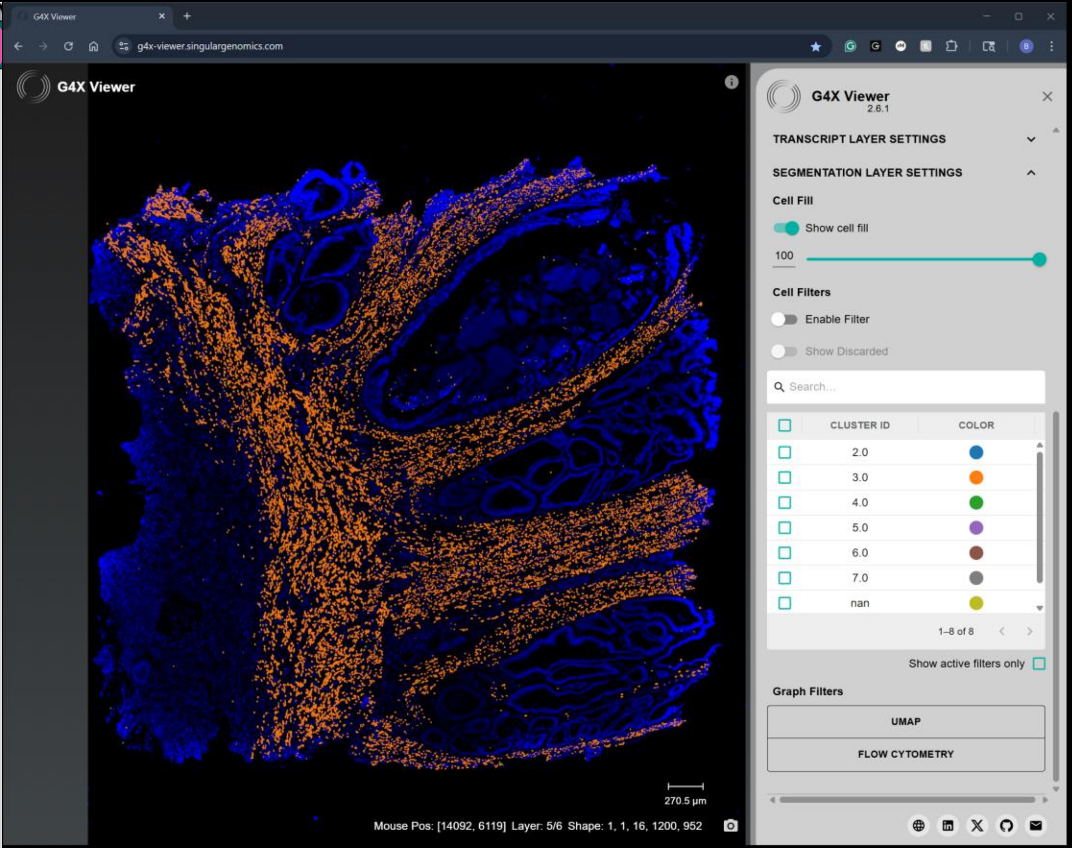
View embedded or user derived UMAP

X Start: -17.358

X End: -6.682

Y Start: -15.065

Y End: -0.487



Mouse Pos: [14092, 6119] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

TRANSCRIPT LAYER SETTINGS

SEGMENTATION LAYER SETTINGS

Cell Fill

Show cell fill: on

100

Cell Filters

Enable Filter: off

Show Discarded: off

Search...

☐	CLUSTER ID	COLOR
☐	2.0	●
☐	3.0	●
☐	4.0	●
☐	5.0	●
☐	6.0	●
☐	7.0	●
☐	nan	●

1–8 of 8

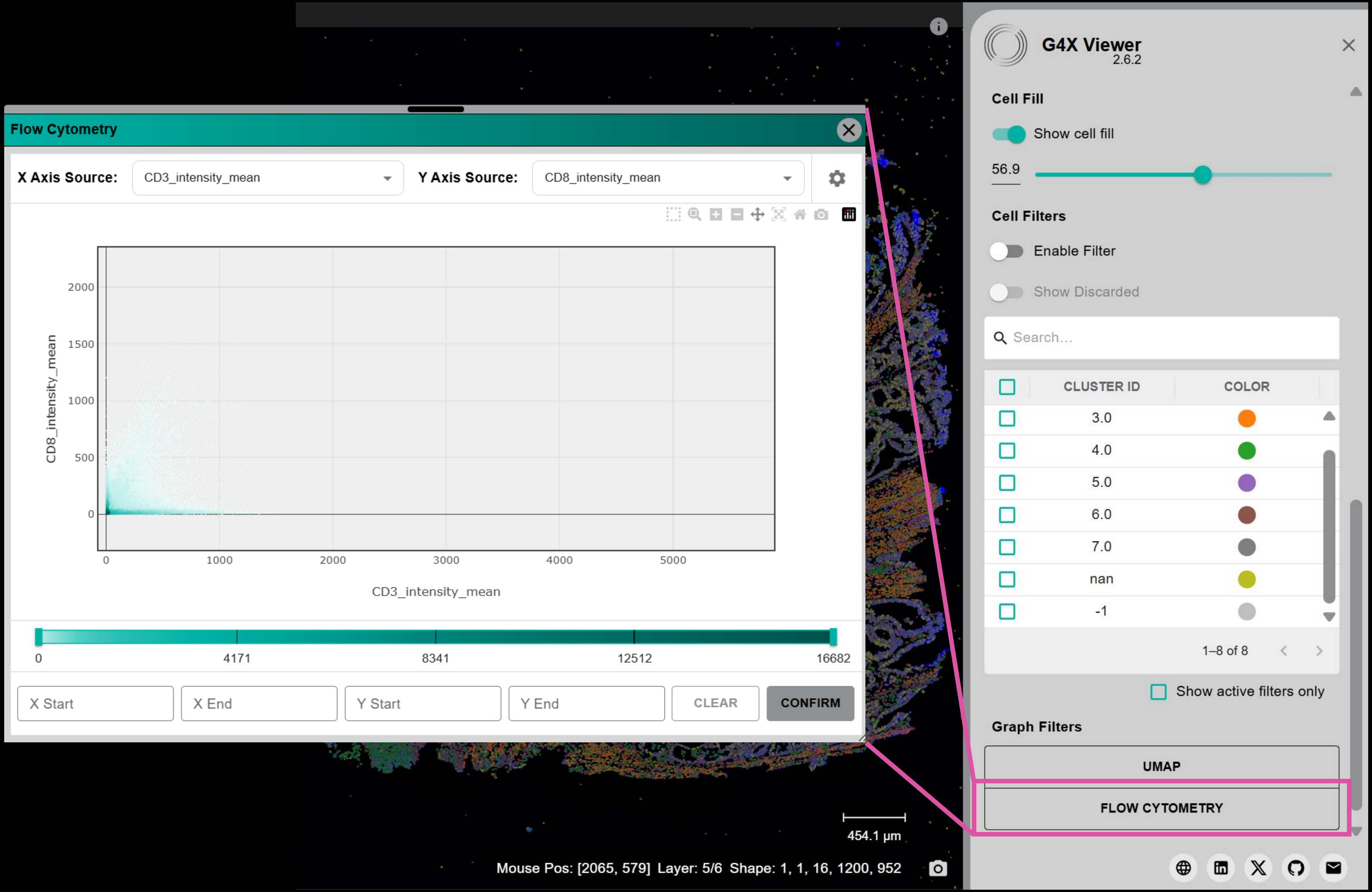
Show active filters only ☐

Graph Filters

Spatial plotting of cells from selected ROI

- To use the selector tool, use the cursor to select a specific group of cells within the UMAP you want to spatially plot
- Once you have selected an ROI, hit confirm and close out of the window to update the viewer

CELL LAYER SETTINGS: FLOW CYTOMETRY



X Axis Source: CD3_intensity_mean

Y Axis Source: CD8_intensity_mean

Mouse Pos: [2065, 579] Layer: 5/6 Shape: 1, 1, 16, 1200, 952

● Show cell fill

56.9

○ Enable Filter

○ Show Discarded

☐	CLUSTER ID	COLOR
☐	3.0	●
☐	4.0	●
☐	5.0	●
☐	6.0	●
☐	7.0	●
☐	nan	●
☐	-1	●

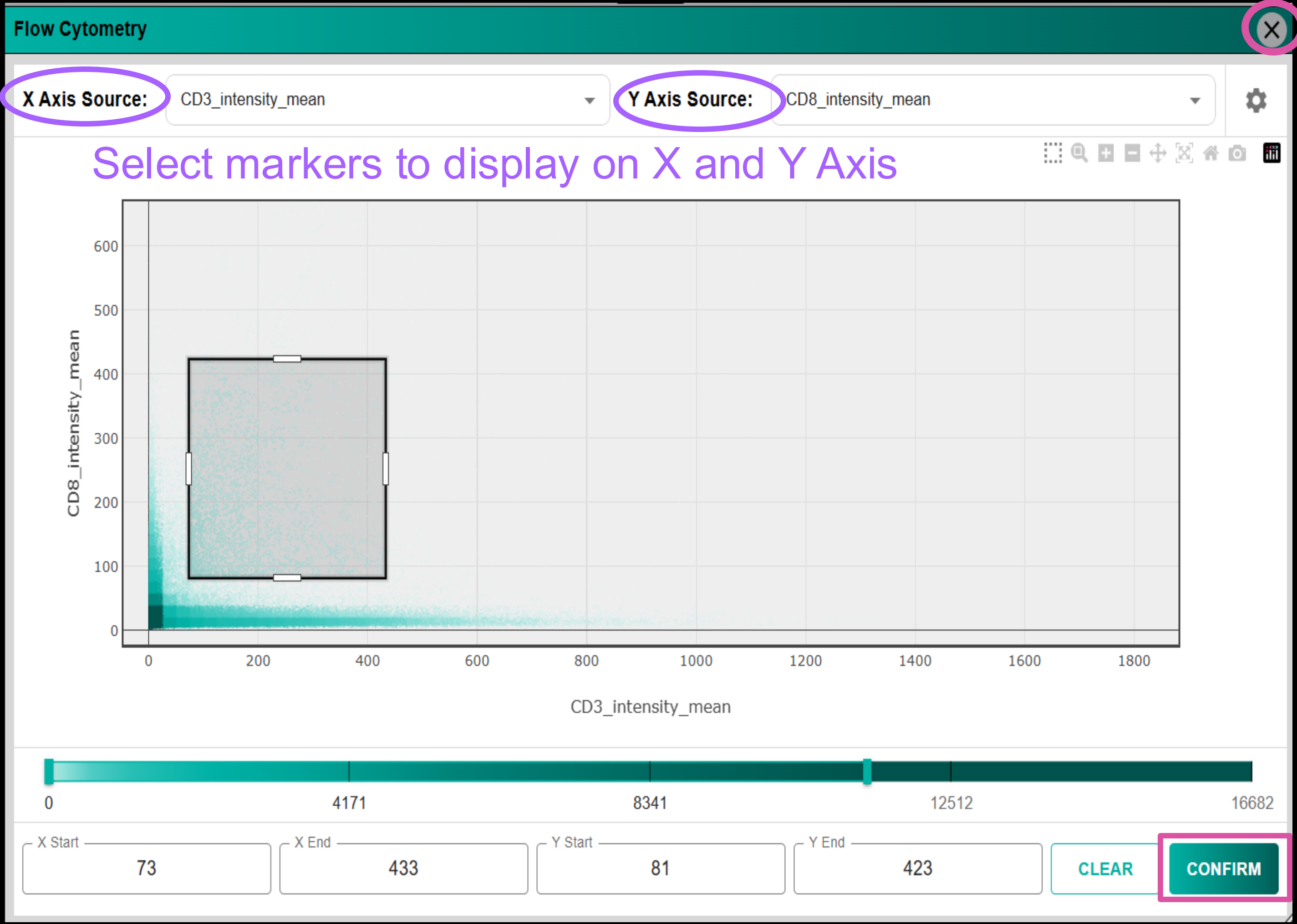
☐ Show active filters only

A Flow Cytometry ROI filter can also be applied to the data, enabling you to select cells based on protein expression levels. This filter can be applied independently or in conjunction with others like the UMAP ROI filter.

*When you first open the Flow Cytometry window, it is in the linear scale

SINGULAR GENOMICS

CELL LAYER SETTINGS: FLOW CYTOMETRY FILTER



Flow Cytometry

X Axis Source: CD3_intensity_mean

Y Axis Source: CD8_intensity_mean

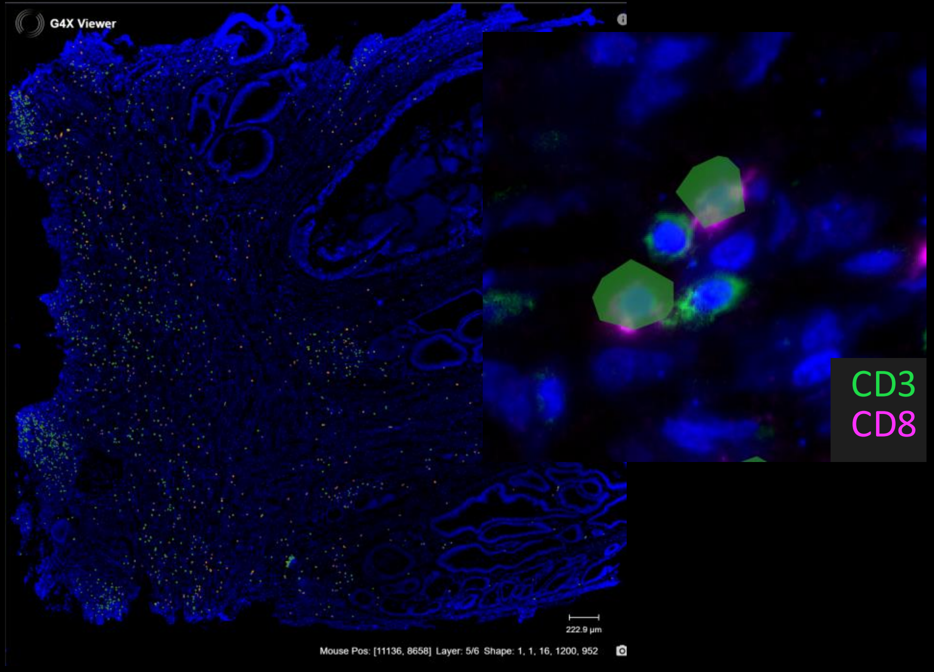
Select markers to display on X and Y Axis

X Start: 73

X End: 433

Y Start: 81

Y End: 423

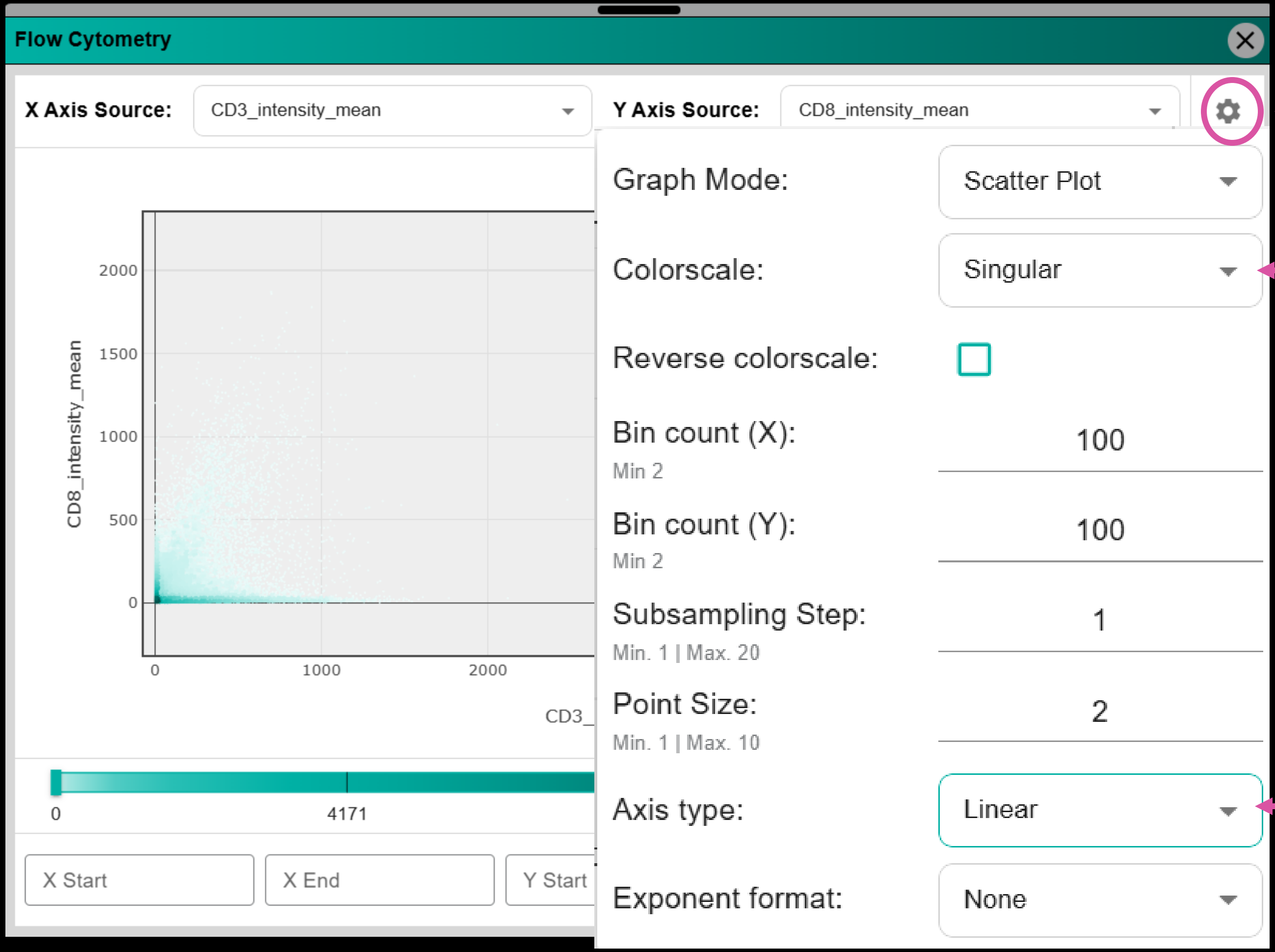


Confirm and close after making your selection to visualize cells

*If you did not clear the UMAP this will only show cells selected within the UMAP filter

You can also select an ROI with the flow cytometry selection tool. Here, we are plotting all cells which are CD3 and CD8 positive.

CELL LAYER SETTINGS: FLOW CYTOMETRY FILTER SETTINGS



X Axis Source: CD3_intensity_mean

Y Axis Source: CD8_intensity_mean

Graph Mode: Scatter Plot

Colorscale: Singular

Reverse colorscale: ☐

Bin count (X): 100
Min 2

Bin count (Y): 100
Min 2

Subsampling Step: 1
Min. 1 | Max. 20

Point Size: 2
Min. 1 | Max. 10

Axis type: Linear

Exponent format: None

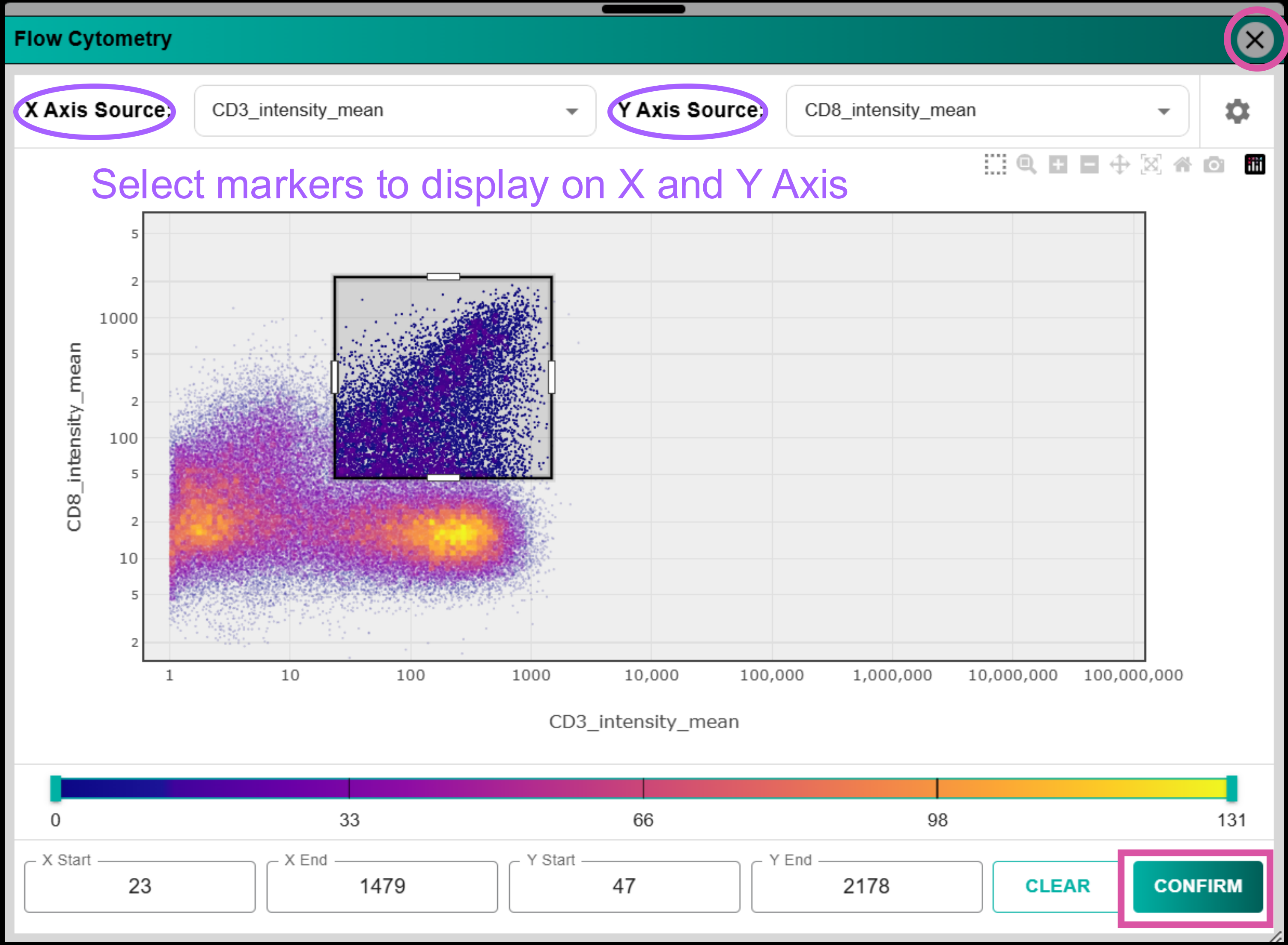
Change graph settings

Change Color

Can change Axis Type

SINGULAR GENOMICS

CELL LAYER SETTINGS: FLOW CYTOMETRY FILTER LOG SCALE



X Axis Source: CD3_intensity_mean

Y Axis Source: CD8_intensity_mean

Select markers to display on X and Y Axis

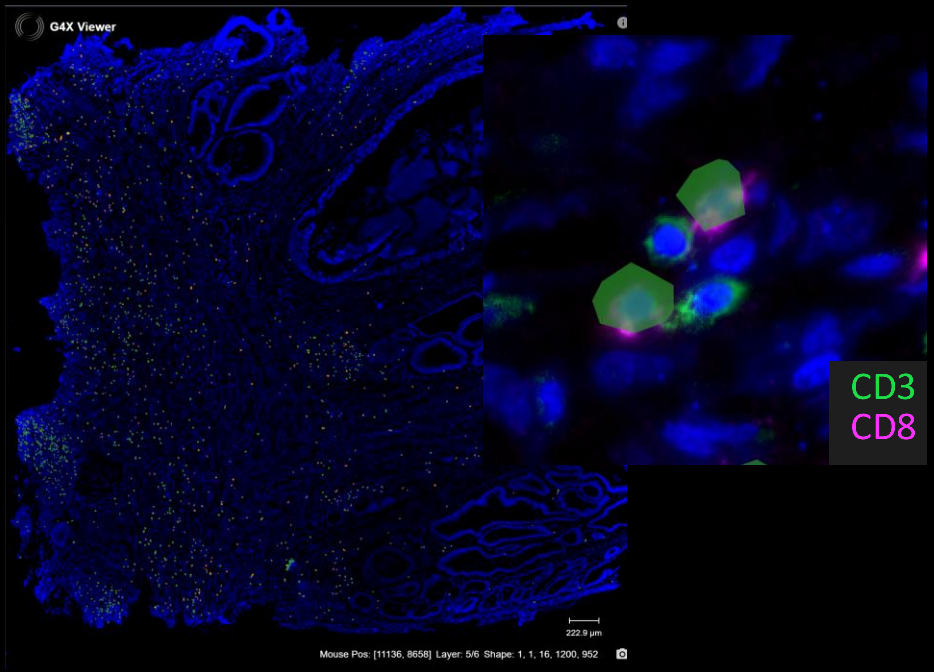
X Start: 23

X End: 1479

Y Start: 47

Y End: 2178

Select cells of interest with selection tool.
Here we chose CD3 and CD8 positive cells

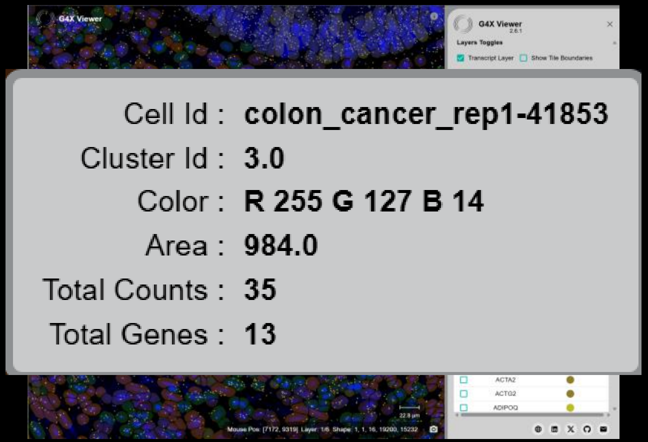


Close and confirm to visualize cells selected

*If you did not clear the UMAP this will only show cells selected within the UMAP filter

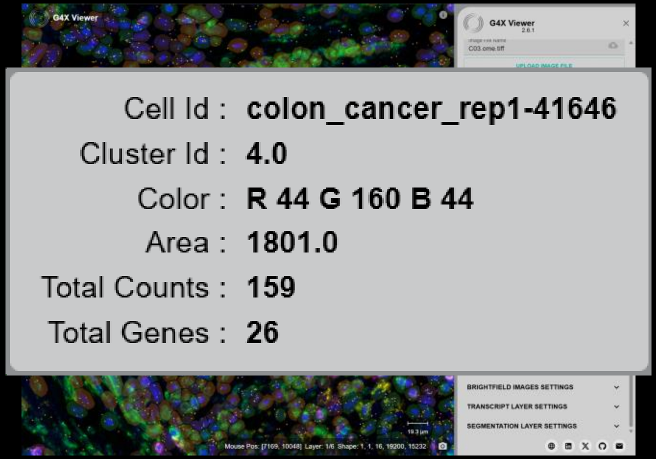
OTHER FEATURES

OTHER FEATURES



Cell Id : **colon_cancer_rep1-41853**
Cluster Id : **3.0**
Color : **R 255 G 127 B 14**
Area : **984.0**
Total Counts : **35**
Total Genes : **13**

When cell mask is enabled
Hover over a cell to reveal detailed information



Cell Id : **colon_cancer_rep1-41646**
Cluster Id : **4.0**
Color : **R 44 G 160 B 44**
Area : **1801.0**
Total Counts : **159**
Total Genes : **26**

When the transcript layer is enabled
Hover over a single transcript to reveal detailed information

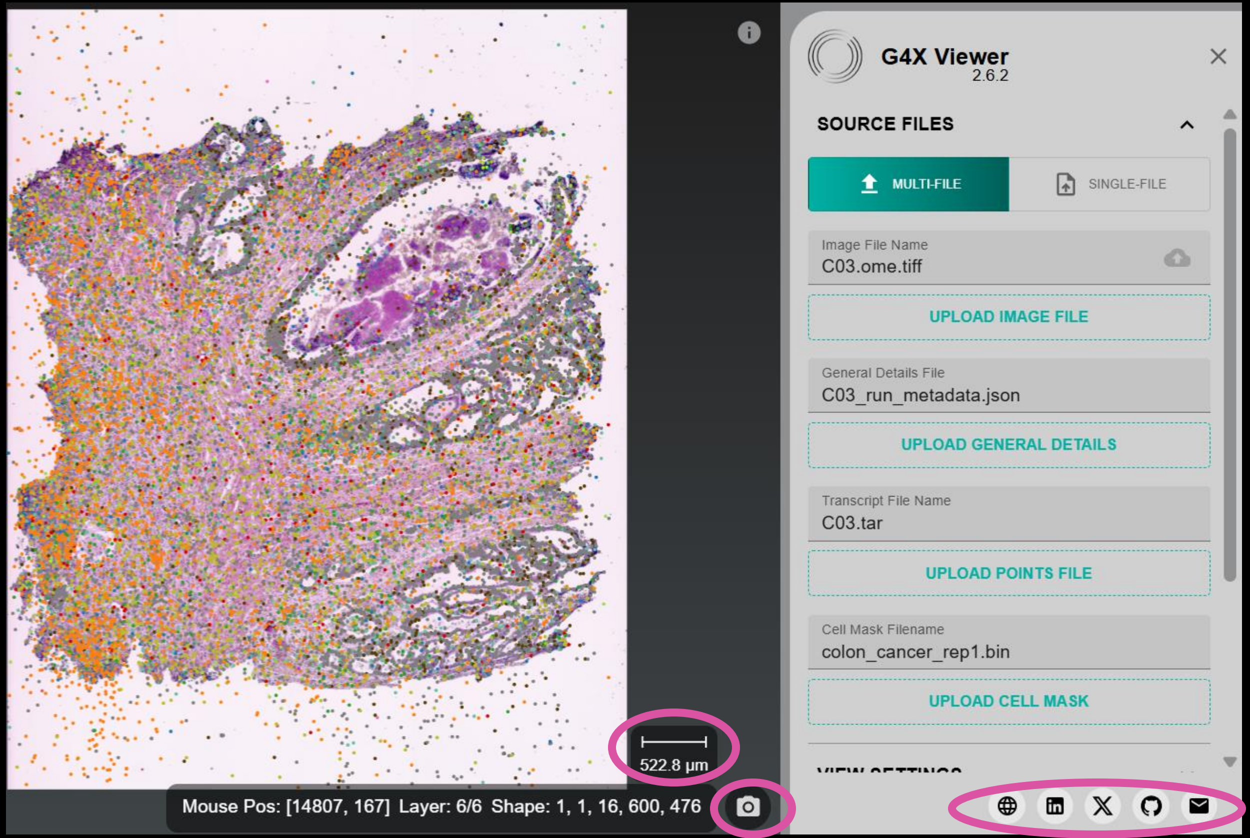


Image File Name: C03.ome.tiff
General Details File: C03_run_metadata.json
Transcript File Name: C03.tar
Cell Mask Filename: colon_cancer_rep1.bin

```
Mouse Pos: [14807, 167]  Layer: 6/6  Shape: 1, 1, 16, 600, 476
```

The scale bar and screenshot matched current resolution of view

For more information find us online with the links in the bottom right corner

G4X VIEWER

QUESTIONS?

YOU CAN CONTACT THE CARE TEAM AT
CARE@SINGULARGENOMICS.COM