SMI Data File ReadMe

Transcript File

| fov | cell_ID | cell | x_local_px | y_local_px | x_global_px | y_global_px | z | target | CellComp |
|-----|---------|----------|------------|------------|-------------|-------------|---|---------|----------|
| 1 | 80 | c_1_1_80 | 761 | 730 | 101383 | 730 | 6 | AQP4 | None |
| 1 | 80 | c_1_1_80 | 714 | 731 | 101430 | 731 | 4 | ATP9A | None |
| 1 | 80 | c_1_1_80 | 730 | 731 | 101414 | 731 | 5 | PLP1 | None |
| 1 | 80 | c_1_1_80 | 740 | 731 | 101404 | 731 | 3 | NBL1 | None |
| 1 | 80 | c_1_1_80 | 744 | 731 | 101400 | 731 | 6 | ST8SIA1 | None |
| 1 | 80 | c_1_1_80 | 717 | 732 | 101427 | 732 | 4 | SCARB2 | None |
| 1 | 80 | c_1_1_80 | 717 | 732 | 101427 | 732 | 5 | RBM5 | None |
| 1 | 80 | c_1_1_80 | 747 | 732 | 101397 | 732 | 2 | MBP | None |
| 1 | 80 | c_1_1_80 | 760 | 732 | 101384 | 732 | 2 | CYP2R1 | None |
| 1 | 80 | c_1_1_80 | 706 | 733 | 101438 | 733 | 5 | ABCA2 | None |

· Column descriptions:

- fov
- The Field Of View (FOV) the transcript is in. All FOVs from the sample are contained within this transcript file.
- o cell_ID
- Unique identifier for a single cell within a given FOV. To make a unique identifier for a cell within the whole sample use both the
 "fov" and "cell_ID" columns. Transcripts not assigned to cells have a value of 0.
- x_local_px
 - The x position of this transcript within the FOV, measured in pixels. To convert to microns multiply the pixel value by 0.12 um
 per pixel. For FOV-local coordinates, the origin (0,0) is the top left of the FOV.
- y_local_px
 - Same as "x_local_px" but for the y dimension. Due to differences in Cartesian and imaging plotting frames of reference, the
 local y-axis coordinates should be inverted to align the transcript positions to images.
- x_global_px
 - See "x_local_px" description. The global positions describes the relative position of this transcript within the large sample
 reference frame. For global coordinates, the origin (0,0) is the bottom left of the capture area.
- y_global_px
- Same as "x_global_px" but for the y dimension
- The Z-slice image number. This has an arbitrary unit.
- target
- The gene detected. HUGO gene symbols are used.
- CellComp
 - The subcellular compartment (Nuclear, Membrane, Cytoplasmic, or None) in which the transcript was detected as determined by cell segmentation algorithm. In this dataset, subcellular locations were not calculated.

Cell Expression File

S3_exprMat_file.csv

| | fov | cell_ID | cell | A1BG | A2M | AAAS | AAK1 | NegPrb1 | NegPrb10 | NegPrb11 | FalseCode1 | FalseCode10 | FalseCode100 |
|----|-----|---------|----------|------|-----|------|------|---------|----------|----------|------------|-------------|--------------|
| 2 | 3 | 86 | c_1_3_86 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 3 | 87 | c_1_3_87 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 3 | 88 | c_1_3_88 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 3 | 89 | c_1_3_89 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 3 | 90 | c_1_3_90 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 3 | 91 | c_1_3_91 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 3 | 92 | c_1_3_92 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 3 | 93 | c_1_3_93 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 3 | 94 | c_1_3_94 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | 3 | 95 | c_1_3_95 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

- Column descriptions:
 - fov
 - The Field Of View (FOV) the transcript is in. All FOVs from the sample are contained within this Cell Expression file.

- o cell_ID
 - Unique identifier for a single cell within a given FOV. To make a unique identifier for a cell within the whole sample use both the
 "fov" and "cell. ID" columns. All transcripts not assigned to a cell are show with a "cell. ID" value of 0.
- Gene expression target (AATK, ABL1, ..., etc)
- The number of transcripts observed for a given gene target for a given cell.
- Negative probes (i.e. NegPrb##)
 - Negative probes, which do not match any sequence within the transcriptome or genome, are included. These can be used to assess background levels.
- False codes (i.e. FalseCode##)
- False codes, which represent barcodes that are not included in the probe mix.

Cell Metadata File

S3 metadata file.csv

| slide_ID | Run_Tissue_name | e fov | cell_ID | AspectRatio | CenterX_local_px | CenterY_local_px | CenterX_global_px | CenterY_global_px | Width | Height | t Area | Mean.Histone | Max.Histone | Mean.G | Max.G | Mean.rRNA | Max.rRNA | Mean.GFAP | Max.GFAP | Mean.DAPI | Max.DAPI | cell |
|----------|-----------------|-------|---------|-------------|------------------|------------------|-------------------|-------------------|-------|--------|--------|--------------|-------------|--------|-------|-----------|----------|-----------|----------|-----------|----------|----------|
| 1 | S3 | 1 | 2 | 1.64 | 492 | 34 | 492.07794 | 93597.99 | 113 | 69 | 5760 | 2190 | 8824 | 98 | 1440 | 4342 | 15388 | 7123 | 55904 | 406 | 1264 | c_1_1_2 |
| 1 | S3 | 1 | 3 | 0.90 | 734 | 38 | 734.11628 | 93593.99 | 69 | 77 | 4433 | 22881 | 45304 | 21 | 344 | 1465 | 7948 | 1522 | 37744 | 548 | 1308 | c_1_1_3 |
| 1 | S3 | 1 | 4 | 1.98 | 2566 | 21 | 2566.40651 | 93610.99 | 85 | 43 | 2743 | 9047 | 29624 | 3 | 288 | 1151 | 7148 | 891 | 21056 | 119 | 440 | c_1_1_4 |
| 1 | S3 | 1 | 5 | 2.18 | 3358 | 19 | 3358.53198 | 93612.99 | 85 | 39 | 2609 | 13435 | 38648 | 48 | 896 | 2175 | 6812 | 9699 | 46224 | 111 | 512 | c_1_1_5 |
| 1 | S3 | 1 | 6 | 0.94 | 3869 | 69 | 3869.61293 | 93562.99 | 132 | 140 | 7551 | 9807 | 43080 | 222 | 1504 | 2113 | 7164 | 3246 | 33648 | 154 | 680 | c_1_1_6 |
| 1 | S3 | 1 | 7 | 0.91 | 3812 | 58 | 3812.60390 | 93573.99 | 105 | 116 | 7657 | 8957 | 28200 | 215 | 4120 | 1406 | 4536 | 2222 | 25472 | 216 | 720 | c_1_1_7 |
| 1 | S3 | 1 | 8 | 1.00 | 66 | 78 | 66.01046 | 93553.98 | 77 | 77 | 4565 | 6881 | 14648 | 73 | 956 | 1342 | 8444 | 2606 | 24848 | 606 | 1260 | c_1_1_8 |
| 1 | S3 | 1 | 9 | 0.82 | 296 | 75 | 296.04689 | 93556.98 | 45 | 55 | 1955 | 261 | 1364 | 23 | 356 | 288 | 4784 | 13069 | 55904 | 409 | 1032 | c_1_1_9 |
| 1 | S3 | 1 | 10 | 0.85 | 138 | 91 | 138.02186 | 93540.98 | 61 | 72 | 2824 | 13185 | 26168 | 49 | 2860 | 1441 | 5280 | 2711 | 41696 | 457 | 992 | c_1_1_10 |
| 1 | S3 | 1 | 11 | 2.06 | 4189 | 111 | 4189.66362 | 93520.98 | 132 | 64 | 5896 | 9077 | 20344 | 245 | 1344 | 5583 | 28780 | 922 | 14800 | 293 | 712 | c_1_1_11 |

- · Column descriptions:
 - slide_ID
 - The slide the cell is on.
 - Run_Tissue_name
 - An annotation for the tissue.
 - fov
 - The Field Of View (FOV) the cell is in. All FOVs from the sample are contained within this Cell Metadata file.
 - cell_ID
 - Unique identifier for a single cell within a given FOV. To make a unique identifier for a cell within the whole sample use both the
 "fov" and "cell ID" columns.
 - Aspect Ratio
 - width divided by height.
 - CenterX_local_px
 - The x position of this transcript within the FOV, measured in pixels. The pixel edge length is 120nm. Thus, to convert to microns
 multiply the pixel value by 0.12 um per pixel. For FOV-local coordinates, the origin (0,0) is the top left of the FOV.
 - CenterY_local_px
 - Same as "CenterX_local_px" but for the y dimension. Due to differences in Cartesian and imaging plotting frames of reference, the local y-axis coordinates should be inverted to align the cell positions to images.
 - CenterX_global_px
 - See "CenterX_local_px" description. The global positions describes the relative position of this transcript within the large sample reference frame. For global coordinates, the origin (0.0) is the bottom left of the capture area.
 - CenterY_global_px
 - Same as "CenterX_global_px" but for the y dimension
 - Width
 - Cell's maximum length in x dimension (pixels)
 - Height
 - Cell's maximum length in y dimension (pixels)
 - Area
 - Number of pixels assigned to a given cell.
 Mean.Histone
 - Weari.mistor
 - Mean fluorescence intensity within a given cell.
 - Max.Histone
 - Maximum fluorescence intensity within a given cell.
 Mean.G
 - Mean fluorescence intensity within a given cell.
 - This channel was left blank in this experiment.
 Max.G
 - Maximum fluorescence intensity within a given cell.
 Mean.rRNA
 - Mos
 - Mean fluorescence intensity within a given cell.
 - MeanMax.rRNA
 - Maximum fluorescence intensity within a given cell.
 - Mean.GFAP
 - Mean fluorescence intensity within a given cell.
 - Max.GFAP
 - Maximum fluorescence intensity within a given cell.
 - Mean.DAPI
 - Mean fluorescence intensity within a given cell.

- Max.DAPI
 - Maximum fluorescence intensity within a given cell.
- - Identifier for the cell that comprises the slide ID, fov. and cell ID

FOV Positions File

This file is used to reference each FOV within the global structure of the sample.

S3 fov positions file.csv

| Slide | FOV | X_mm | Y_mm |
|-------|-----|----------|-----------|
| 1 | 1 | 12.28598 | 0.0000000 |
| 1 | 2 | 12.28598 | 0.5119162 |
| 1 | 3 | 12.28598 | 1.0238316 |
| 1 | 4 | 12.28598 | 1.5357473 |
| 1 | 5 | 12.28598 | 2.0476630 |
| 1 | 6 | 12.28598 | 2.5595787 |
| 1 | 7 | 12.28598 | 3.0714943 |
| 1 | 8 | 12.28598 | 3.5834100 |
| 1 | 9 | 12.28598 | 4.0953257 |
| 1 | 10 | 12.28598 | 4.6072414 |

- · Column descriptions:
 - Slide
 - The slide number.
 - FOV
 - The Field Of View (FOV) number.

 - The relative x position of this the FOV, measured in millimeters. The FOV coordinate corresponds to the top right of the FOV. · Y_mm
 - See "X_mm" description, but in for the y dimension.

Cell Polygons File

This file is a simple polygon descripton of cell boundaries. These are provided as a graphical convenience but are only approximations of the cell boundries, which are fully defined by the cellLabels mask images.

S3-polygons.csv

| fov | cellID | cell | x_local_px | y_local_px | x_global_px | y_global_px |
|-----|--------|---------|------------|------------|-------------|-------------|
| 1 | 2 | c_1_1_2 | 542 | 0 | 101602 | 0 |
| 1 | 2 | c_1_1_2 | 545 | 1 | 101599 | 1 |
| 1 | 2 | c_1_1_2 | 547 | 3 | 101597 | 3 |
| 1 | 2 | c_1_1_2 | 548 | 6 | 101596 | 6 |
| 1 | 2 | c_1_1_2 | 548 | 22 | 101596 | 22 |
| 1 | 2 | c_1_1_2 | 543 | 37 | 101601 | 37 |
| 1 | 2 | c_1_1_2 | 539 | 45 | 101605 | 45 |
| 1 | 2 | c_1_1_2 | 525 | 59 | 101619 | 59 |
| 1 | 2 | c_1_1_2 | 509 | 67 | 101635 | 67 |
| 1 | 2 | c_1_1_2 | 506 | 68 | 101638 | 68 |

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 - - The Field Of View (FOV) number.
 - · cellID
 - Unique identifier for a single cell within a given FOV. To make a unique identifier for a cell within the whole sample use both the "fov" and "cell_ID" columns.
 - - Identifier for the cell that comprises the slide_ID, fov, and cell_ID
 - x_local_px
 - The x position of this transcript within the FOV, measured in pixels. To convert to microns multiply the pixel value by 0.12 um per pixel. For FOV-local coordinates, the origin (0,0) is the top left of the FOV.
 - - Same as "x_local_px" but for the y dimension. Due to differences in Cartesian and imaging plotting frames of reference, the local y-axis coordinates should be inverted to align the cell positions to images.
 - x_global_px

- The relative x position of this the FOV, measured in pixels. To convert to microns multiply the pixel value by 0.12 um per pixel. For global coordinates, the origin (0,0) is the bottom left of the capture area.
- y_global_px

 See "x_global_px" description, but in for the y dimension.