Swiss Institute of
Bioinformatics

INTRODUCTION TO SEQUENCING-BASED SPATIAL TRANSCRIPTOMICS DATA ANALYSIS

Space-ranger and SpatialExperiment

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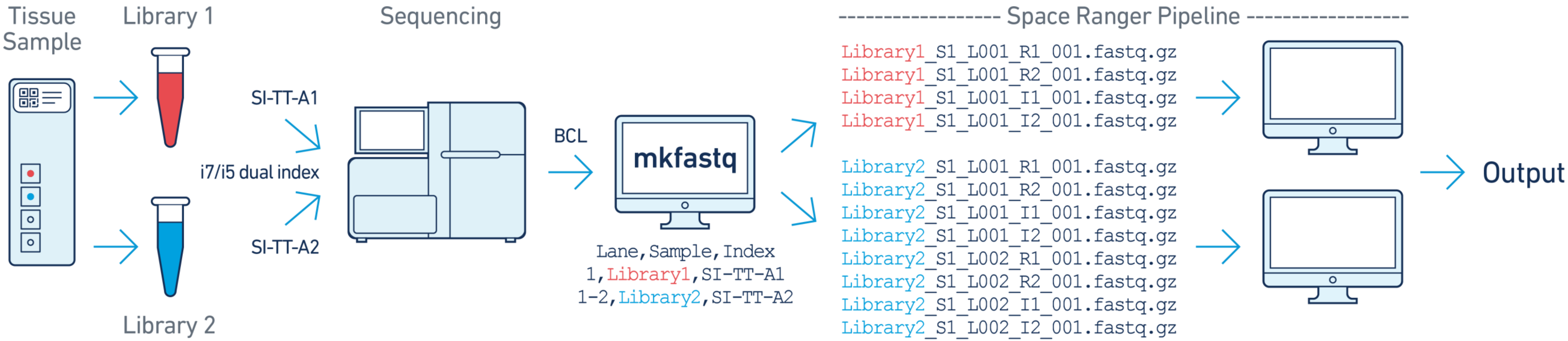
SWITZERLAND

What is the pipeline (with tools) to obtain counts by features metrics for single-cell transcriptomics data?

Workflow of obtaining read counts



Tissue Sample

Library 1

Library 2

SI-TT-A1

i7/i5 dual index

SI-TT-A2

Sequencing

BCL

mkfastq

```
Lane,Sample,Index
1,Library1,SI-TT-A1
1-2,Library2,SI-TT-A2
```

------------------ Space Ranger Pipeline ------------------

```
Library1_S1_L001_R1_001.fastq.gz
Library1_S1_L001_R2_001.fastq.gz
Library1_S1_L001_I1_001.fastq.gz
Library1_S1_L001_I2_001.fastq.gz

Library2_S1_L001_R1_001.fastq.gz
Library2_S1_L001_R2_001.fastq.gz
Library2_S1_L001_I1_001.fastq.gz
Library2_S1_L001_I2_001.fastq.gz
Library2_S1_L002_R1_001.fastq.gz
Library2_S1_L002_R2_001.fastq.gz
Library2_S1_L002_I1_001.fastq.gz
Library2_S1_L002_I2_001.fastq.gz
```

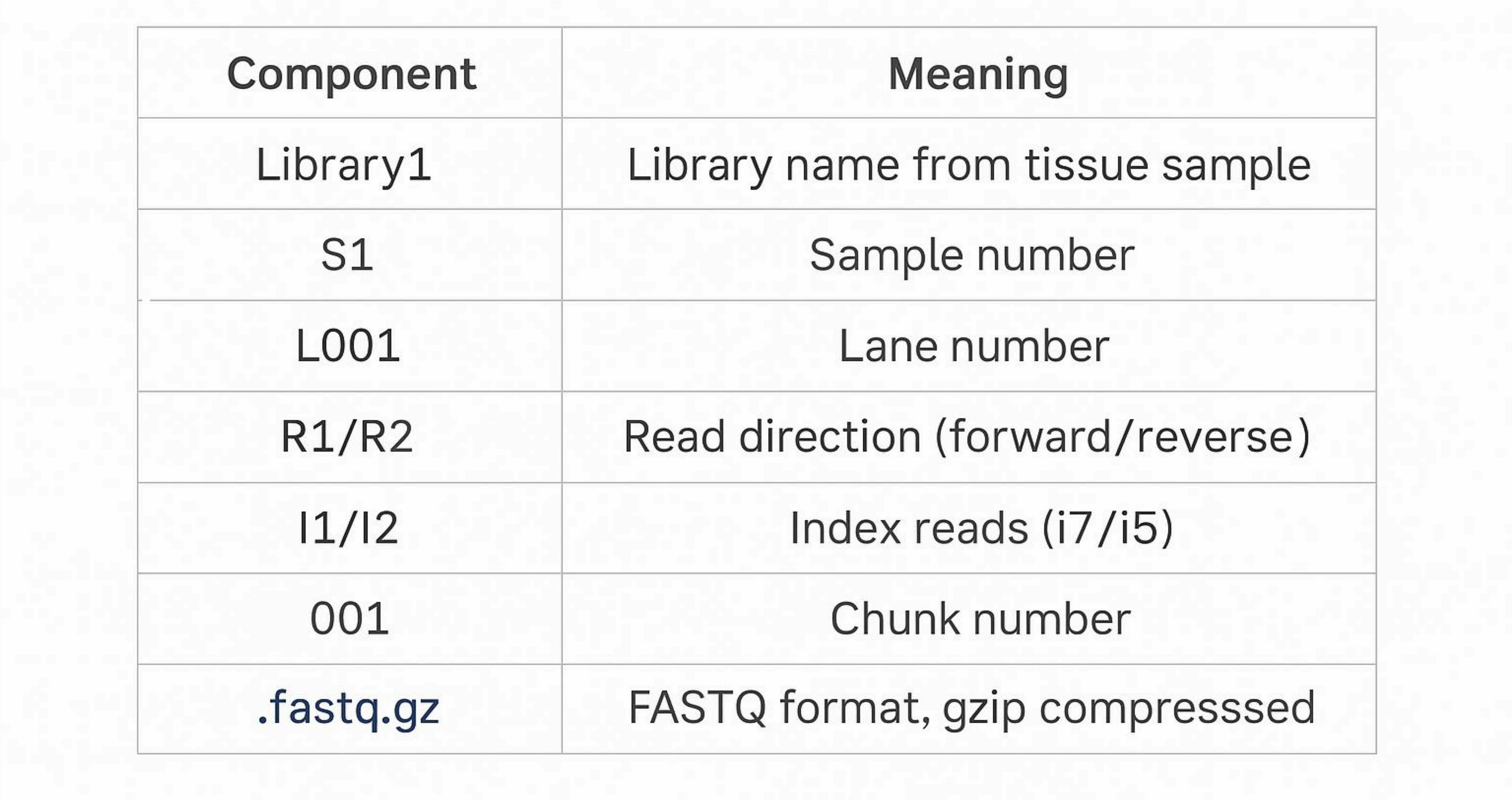
Output

https://www.10xgenomics.com/support/software/space-ranger/latest

What does this sample name mean?

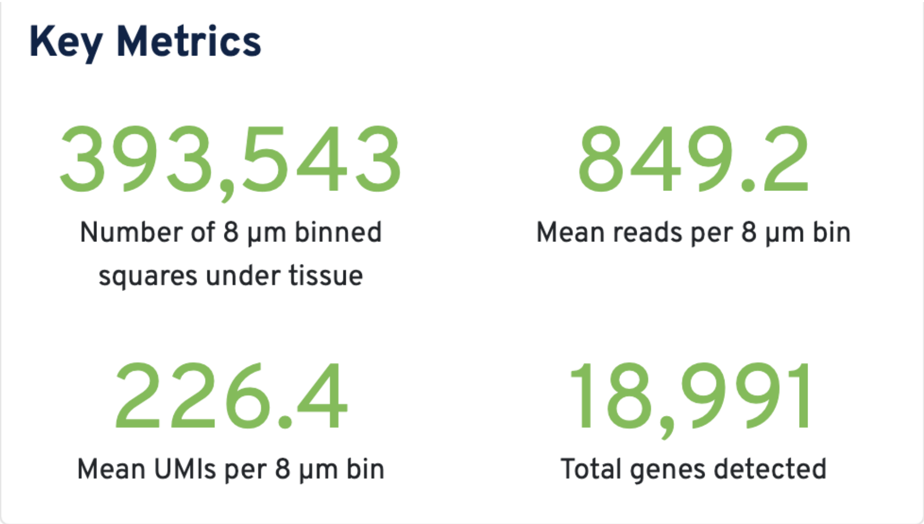
`Library1_S1_L001_R1_001.fastq.gz`

Library1_S1_L001_R1_001.fastq.gz



Component	Meaning
Library1	Library name from tissue sample
S1	Sample number
L001	Lane number
R1/R2	Read direction (forward/reverse)
I1/I2	Index reads (i7/i5)
001	Chunk number
.fastq.gz	FASTQ format, gzip compresssed

Space-ranger



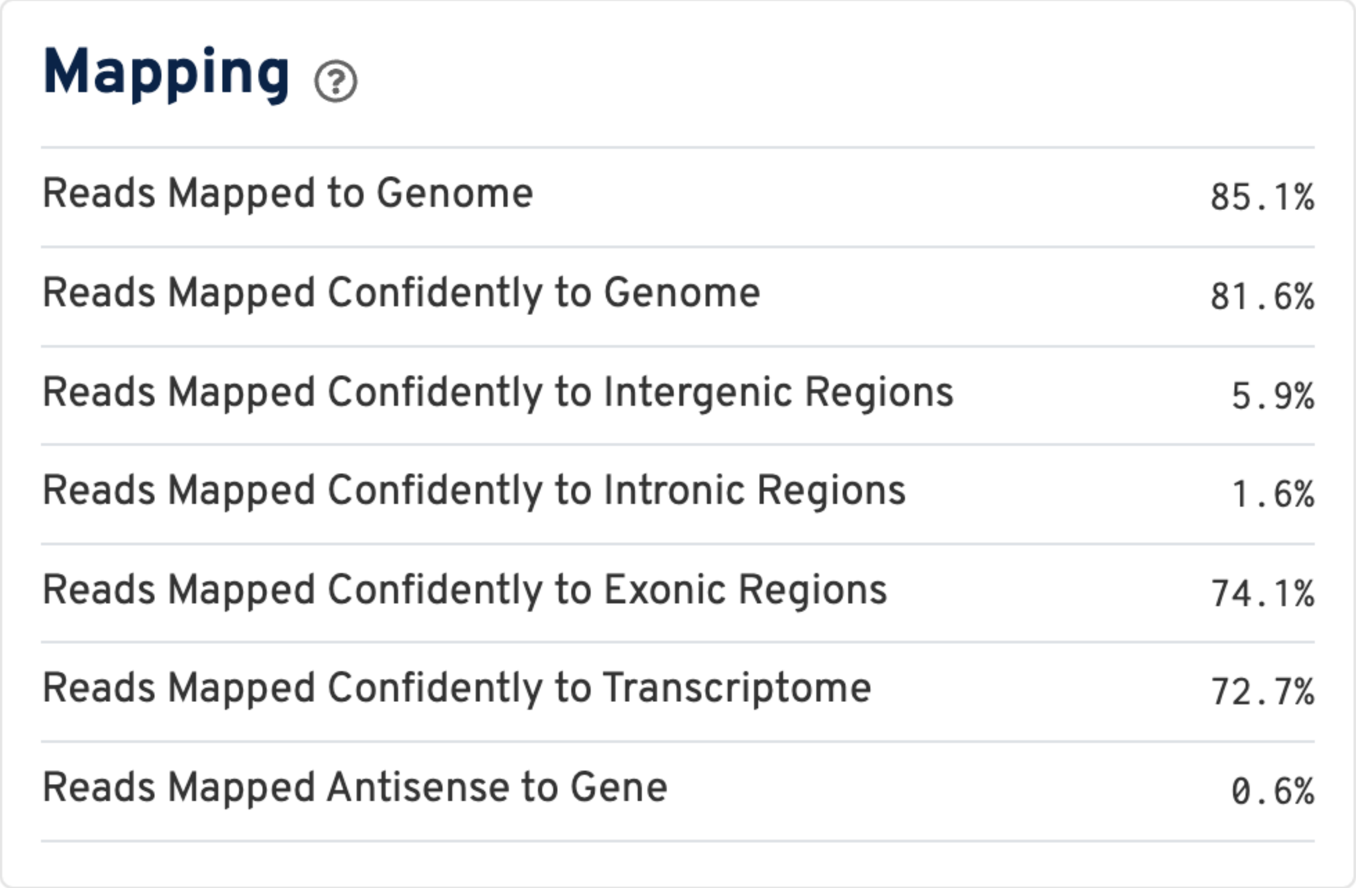
Key Metrics

393,543
Number of 8 µm binned squares under tissue

849.2
Mean reads per 8 µm bin

226.4
Mean UMIs per 8 µm bin

18,991
Total genes detected



Mapping

Reads Mapped to Genome	85.1%
Reads Mapped Confidently to Genome	81.6%
Reads Mapped Confidently to Intergenic Regions	5.9%
Reads Mapped Confidently to Intronic Regions	1.6%
Reads Mapped Confidently to Exonic Regions	74.1%
Reads Mapped Confidently to Transcriptome	72.7%
Reads Mapped Antisense to Gene	0.6%

Space-ranger

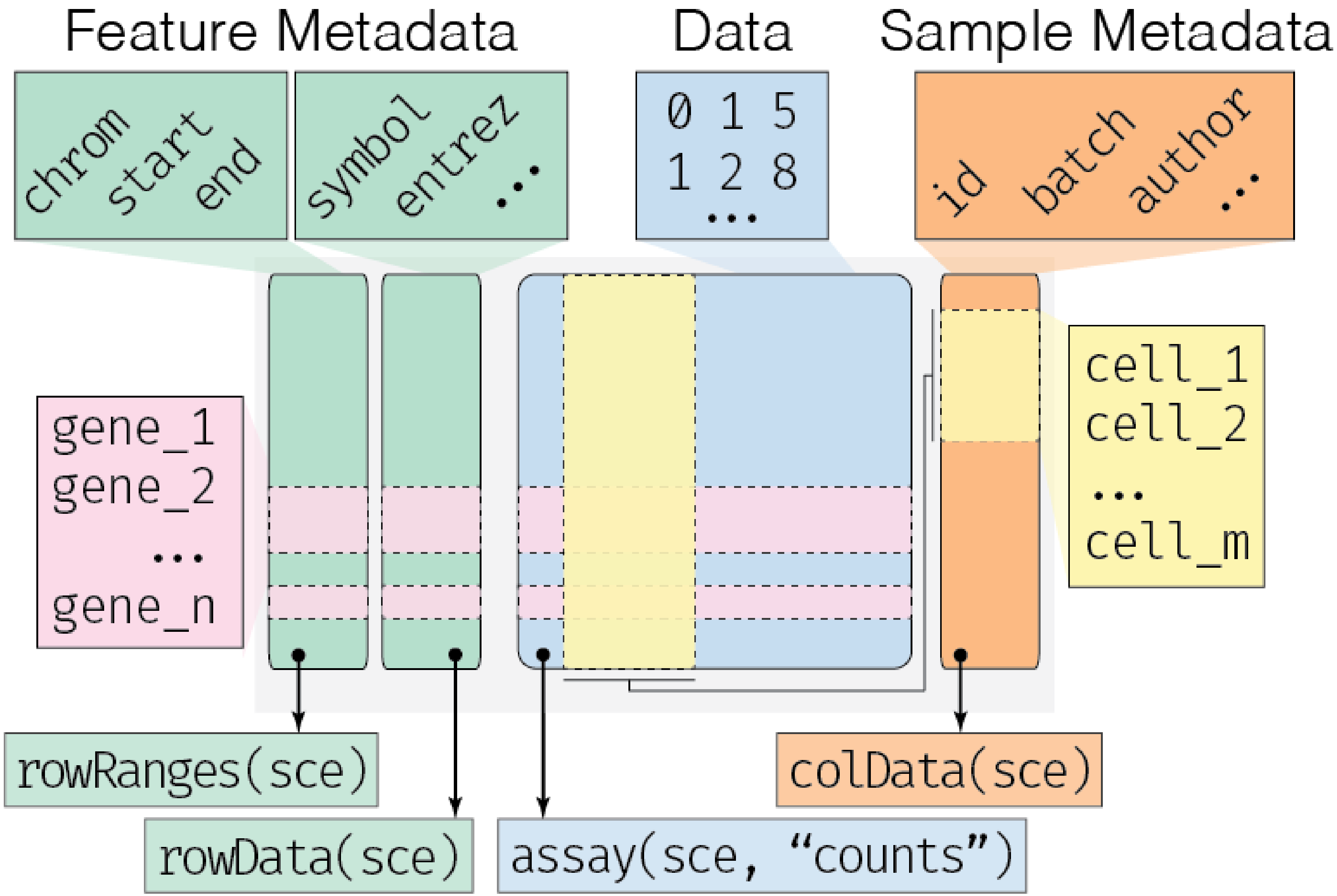
```
binned_output :
├ square_002um
├ square_008um
├ square_016um
├──── analysis
│     ├──── clustering
│     ├──── diffexp
│     ├──── pca
│     ├──── tsne
│     └──── umap
├──── cloupe.cloupe
├──── filtered_feature_bc_matrix
│     ├──── barcodes.tsv.gz
│     ├──── features.tsv.gz
│     └──── matrix.mtx.gz
├──── filtered_feature_bc_matrix.h5
├──── spatial
│     ├──── LV123
│     │     ├──── scalefactors_json.json
│     │     ├──── tissue_hires_image.png
└──── web_summary.html
```

Which framework have you used for single-cell transcriptomics data analysis?

Which framework have you used for single-cell transcriptomics data analysis?

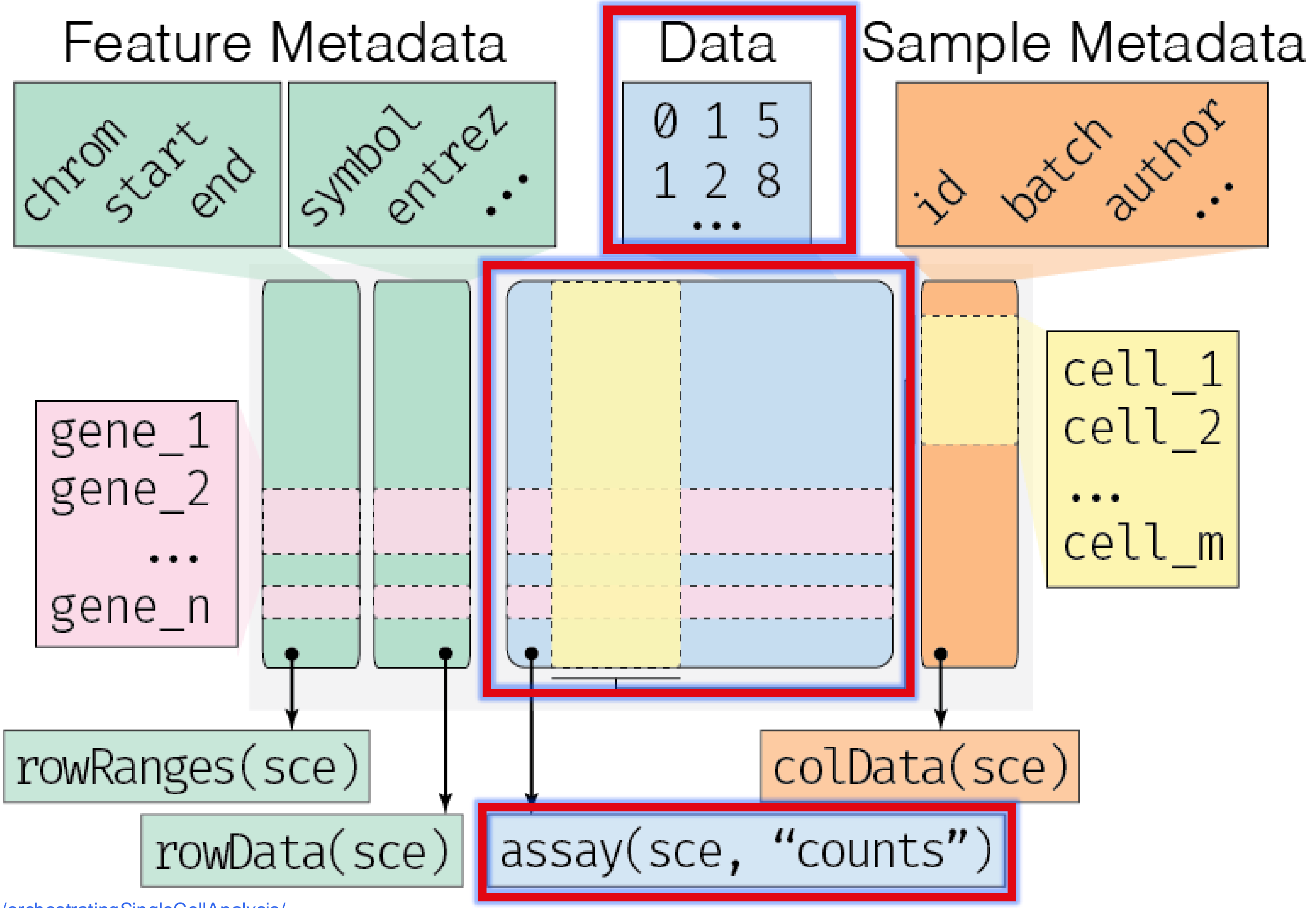
`Seurat`?

SingleCellExperiment



https://robertamezquita.github.io/orchestratingSingleCellAnalysis/

SingleCellExperiment



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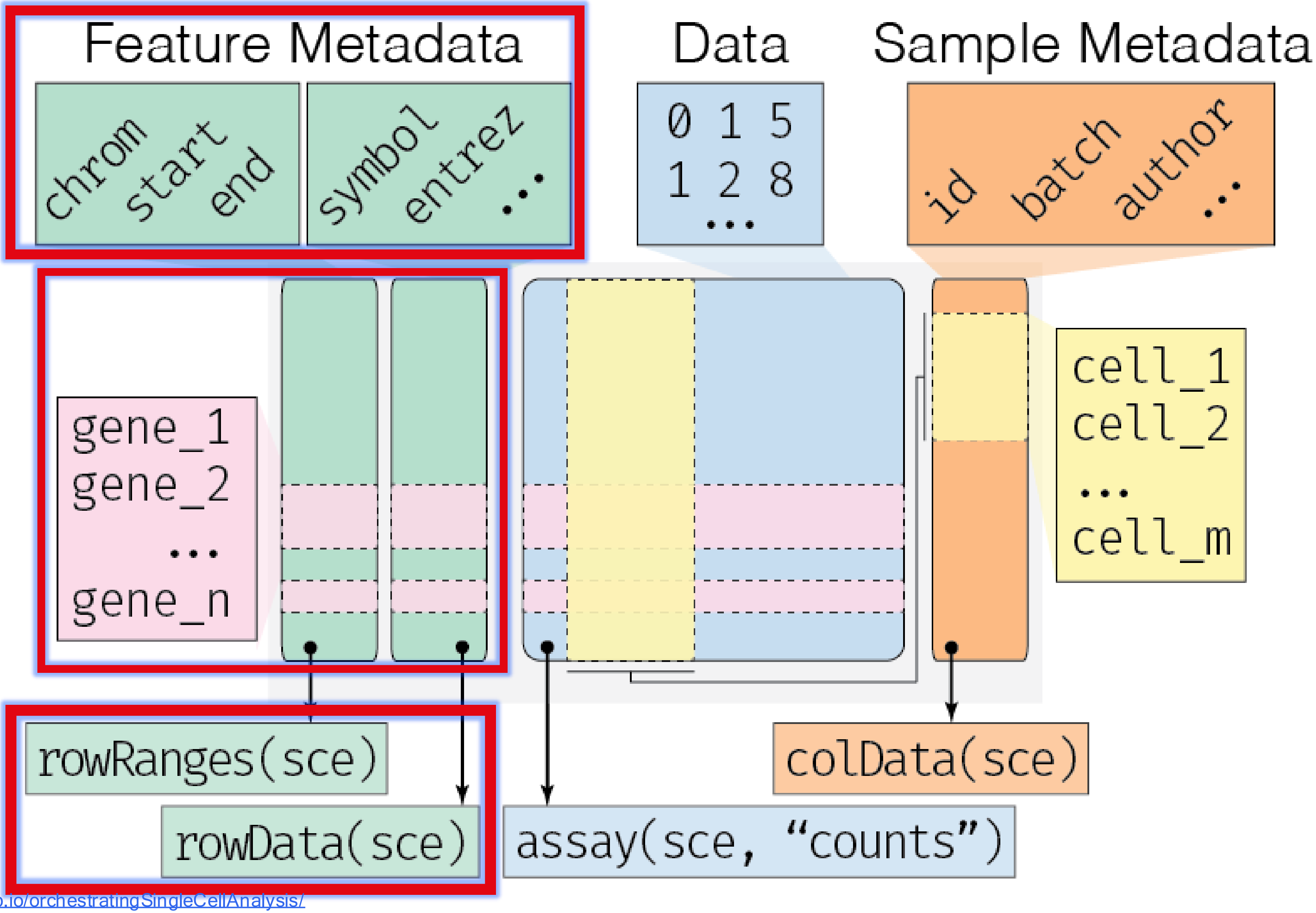

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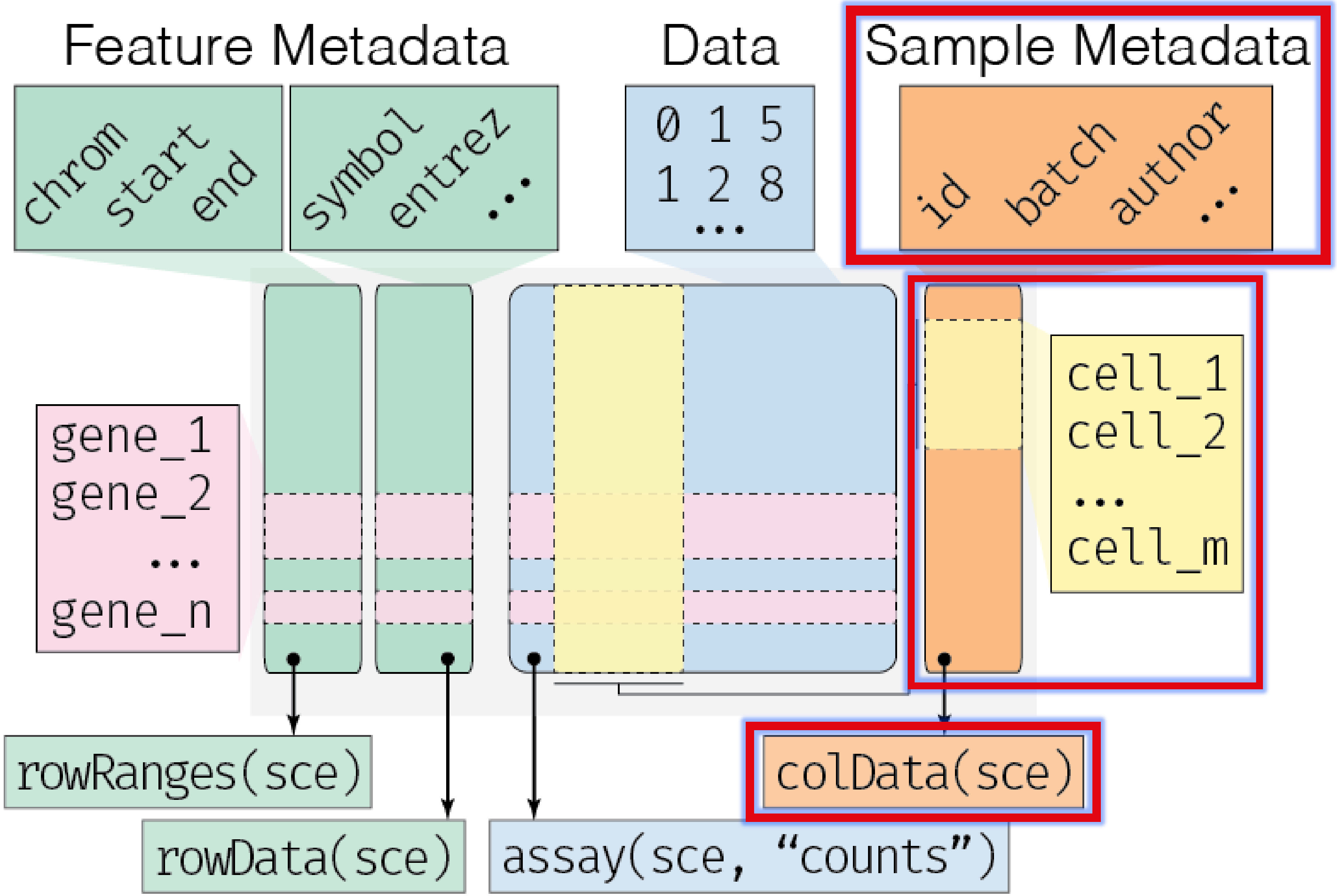

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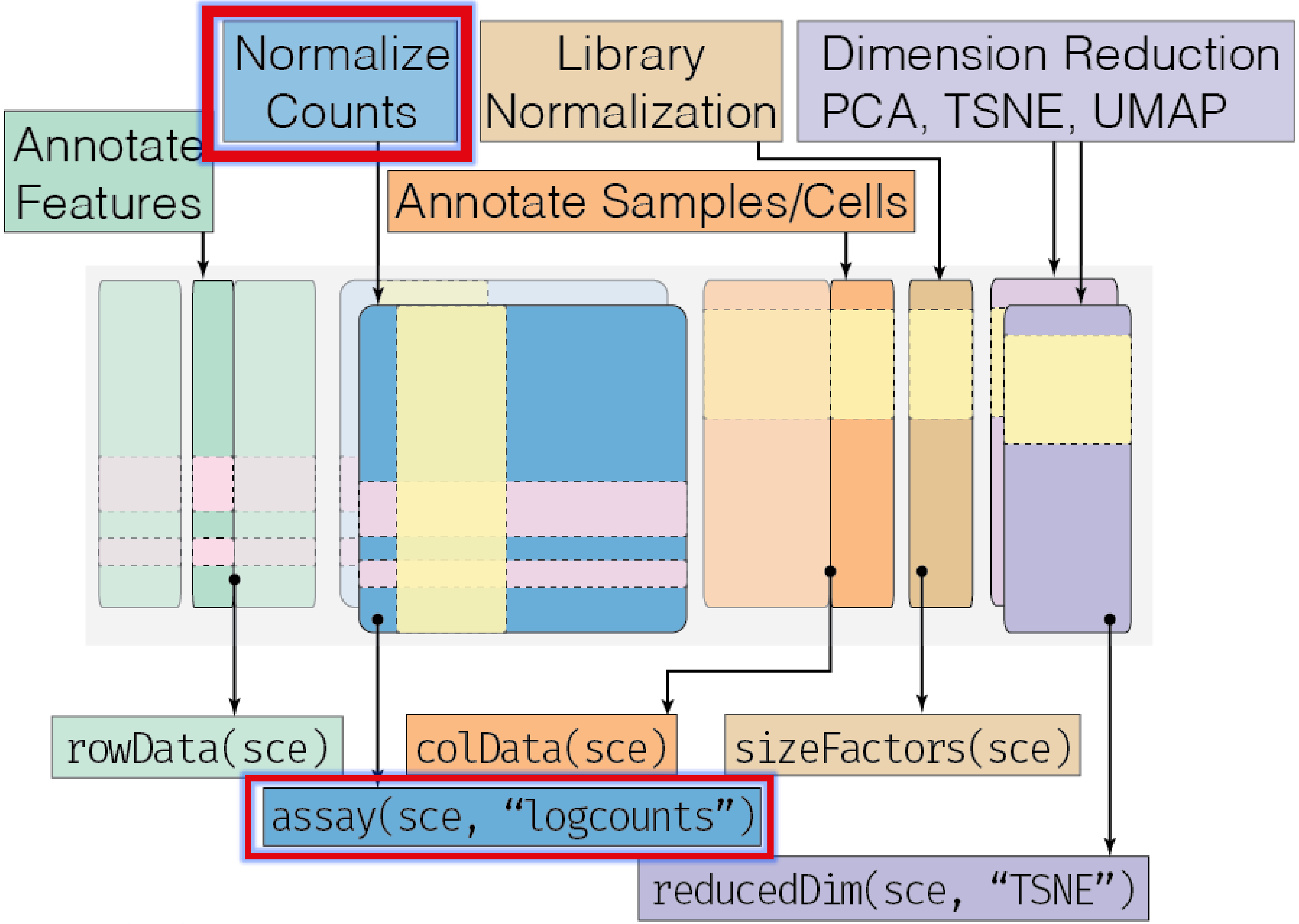
Extending `SingleCellExperiment`



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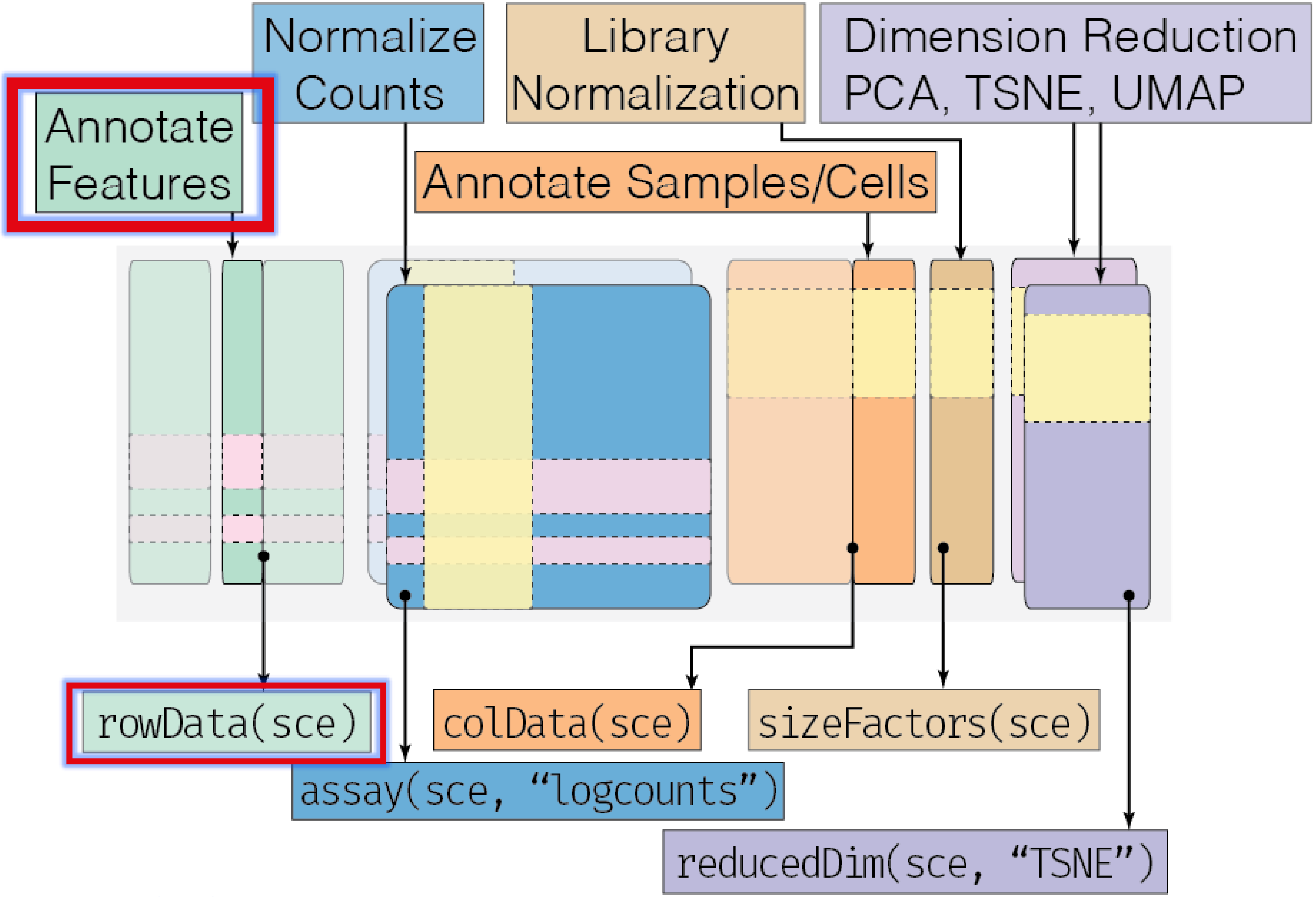

Normalize Counts

Library Normalization

Dimension Reduction PCA, TSNE, UMAP

Annotate Features

Annotate Samples/Cells

rowData(sce)

colData(sce)

sizeFactors(sce)

assay(sce, "logcounts")

reducedDim(sce, "TSNE")

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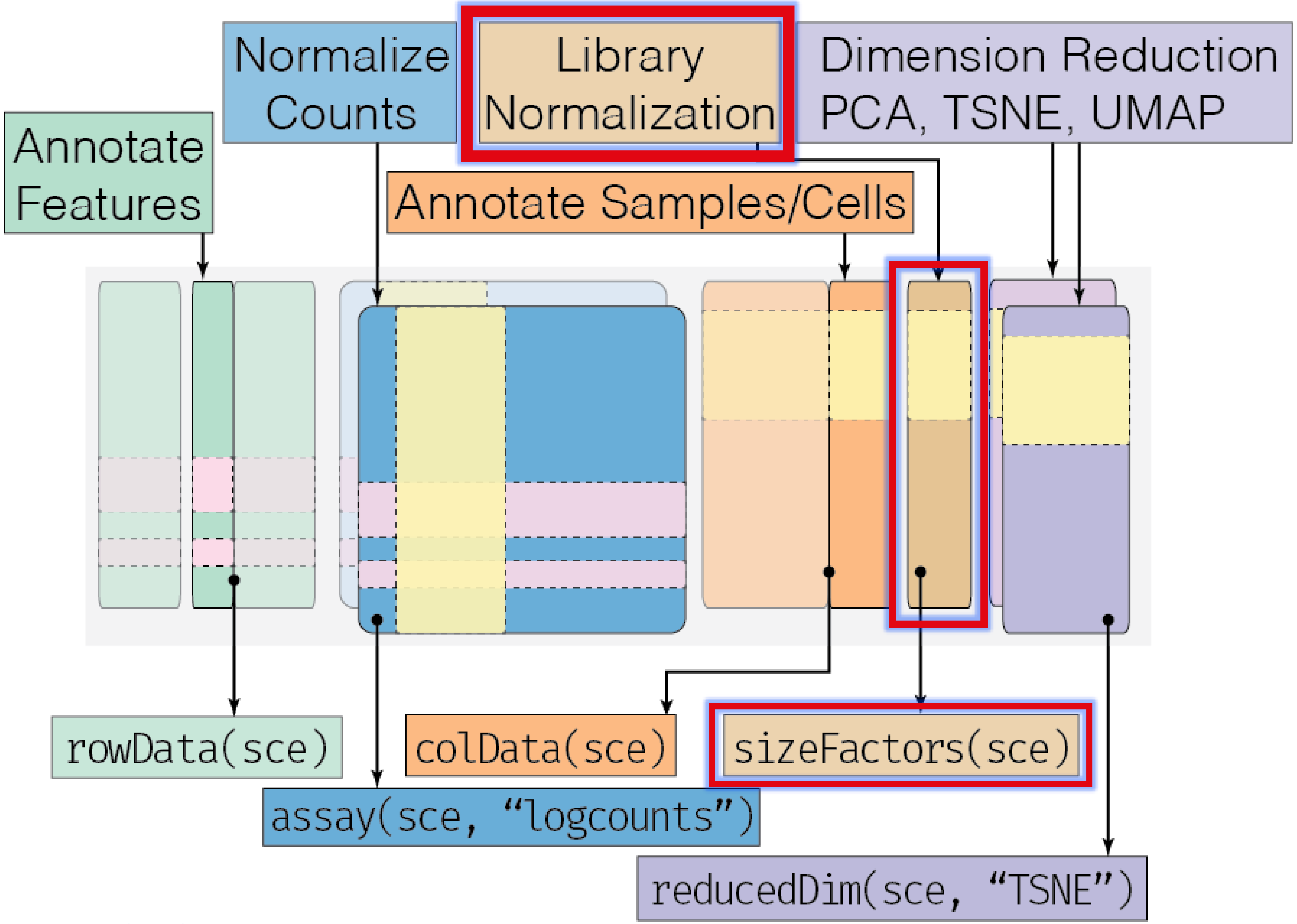
Extending `SingleCellExperiment`



Normalize Counts

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`rowData(sce)`

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`sizeFactors(sce)`

`assay(sce, “logcounts”)`

`reducedDim(sce, “TSNE”)`

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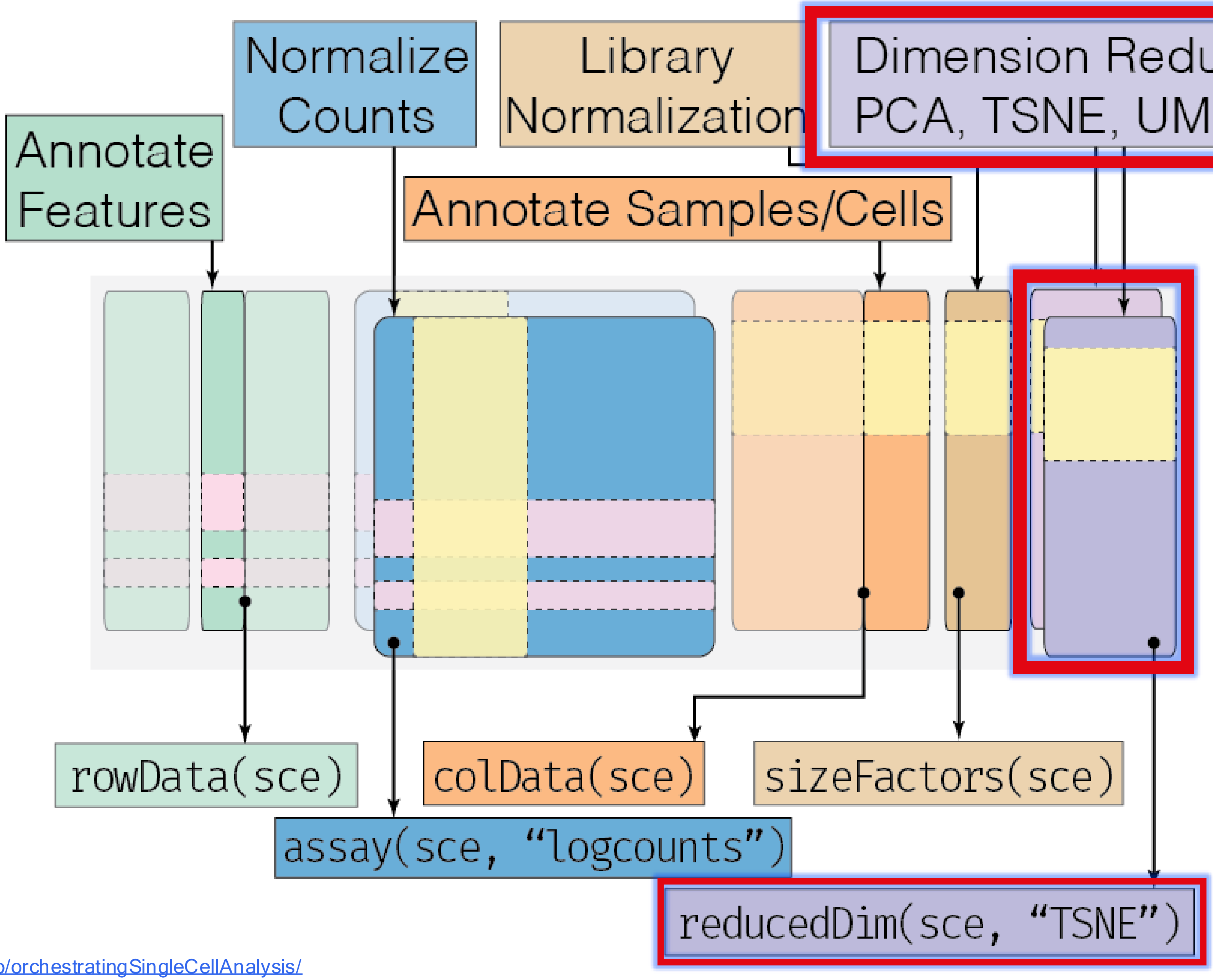
Extending `SingleCellExperiment`



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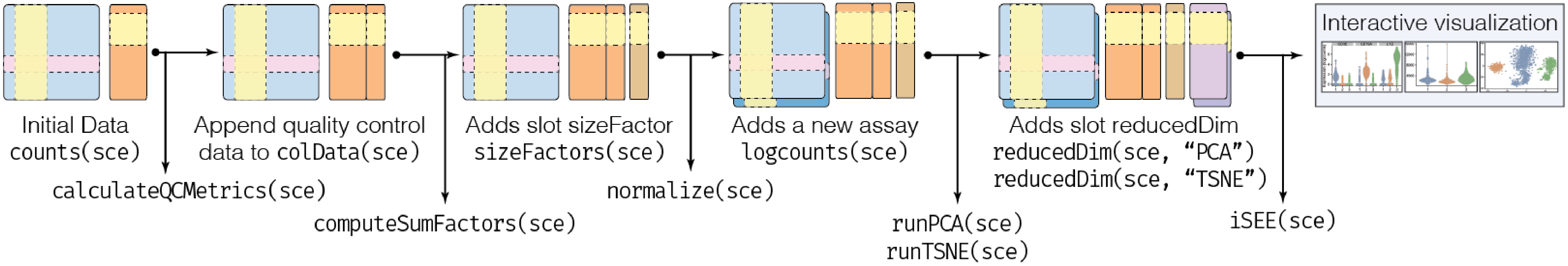
Example workflow of `SingleCellExperiment` extension



Initial Data
`counts(sce)`

`calculateQCMetrics(sce)`

Append quality control data to `colData(sce)`

`computeSumFactors(sce)`

Adds slot sizeFactor
`sizeFactors(sce)`

`normalize(sce)`

Adds a new assay
`logcounts(sce)`

`runPCA(sce)`
`runTSNE(sce)`

Adds slot reducedDim
`reducedDim(sce, “PCA”)`
`reducedDim(sce, “TSNE”)`

`iSEE(sce)`

Interactive visualization

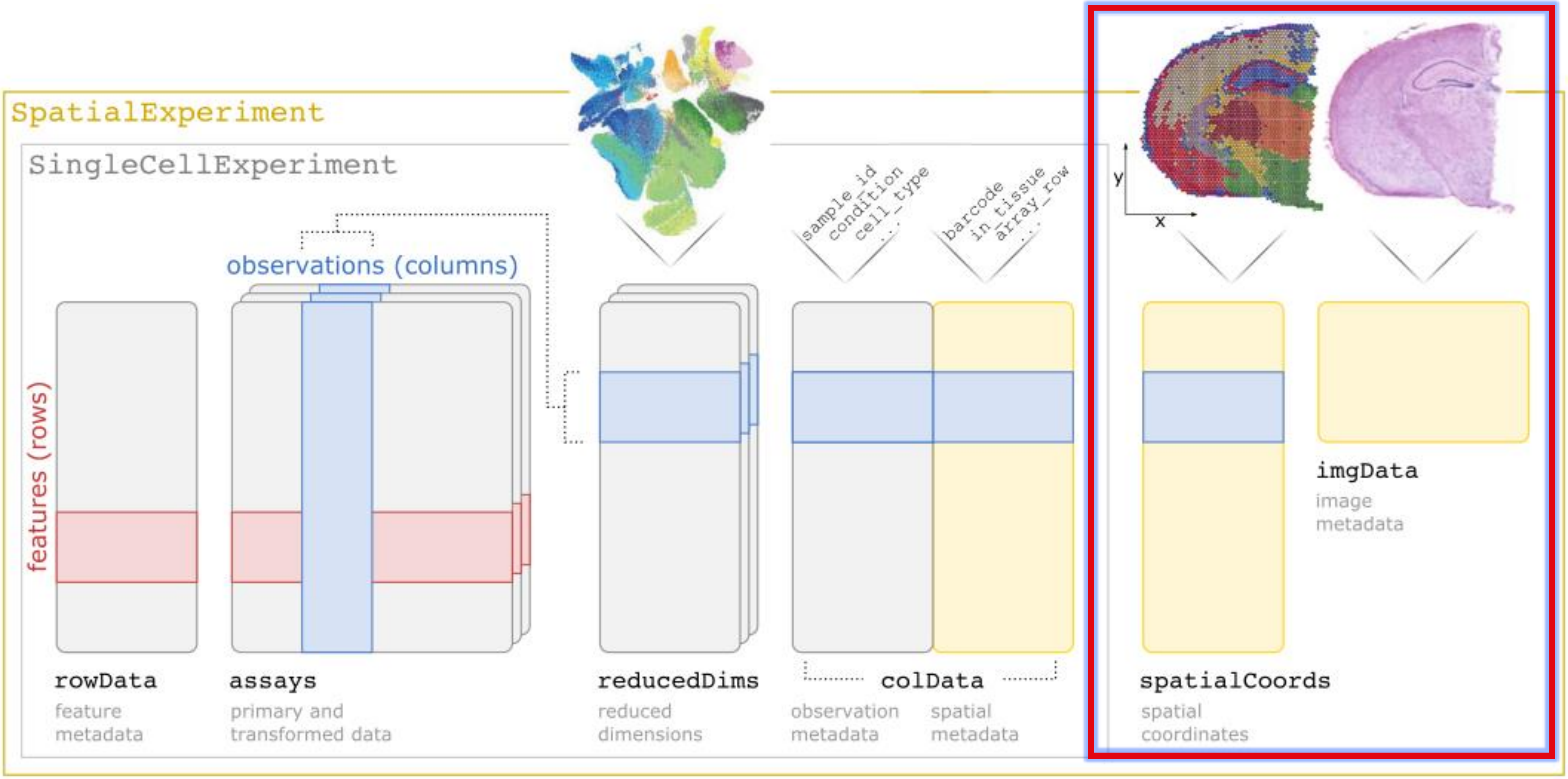
https://robertamezquita.github.io/orchestratingSingleCellAnalysis/

Can we use

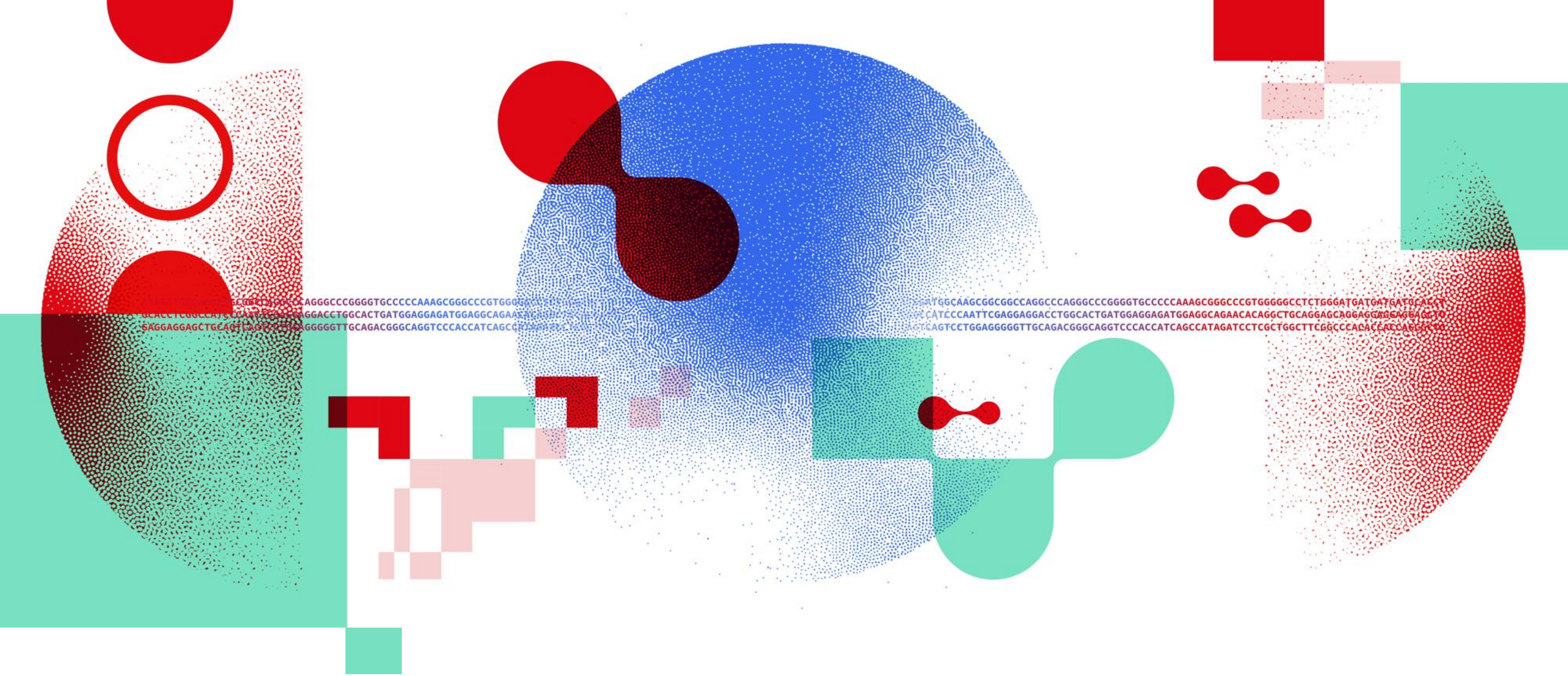
`SingleCellExperiment`

for spatial trascriptomics data?

SpatialExperiment



https://www.bioconductor.org/packages/release/bioc/html/SpatialExperiment.html



Thank you

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