



Swiss Institute of
Bioinformatics

INTRODUCTION TO SEQUENCING-BASED SPATIAL
TRANSCRIPTOMICS DATA ANALYSIS

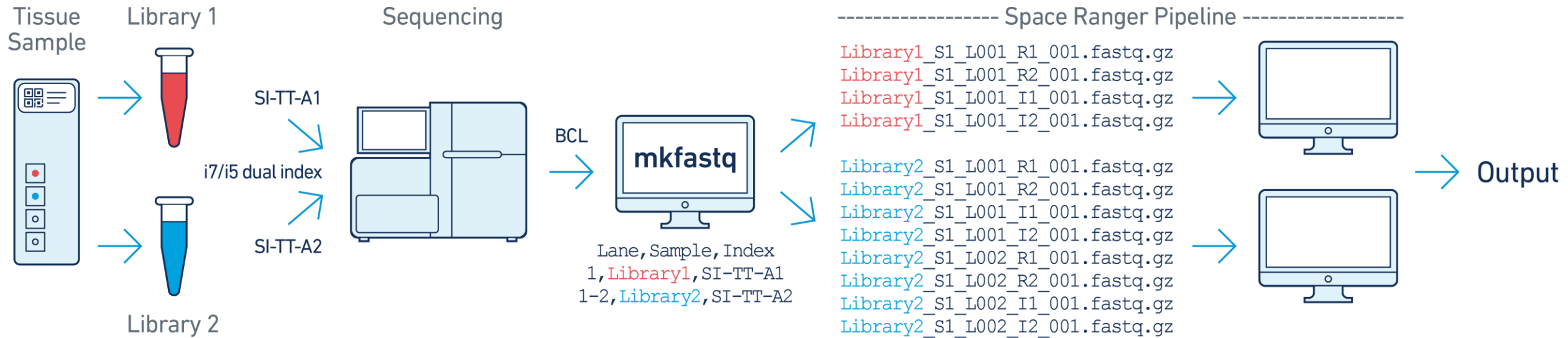
QC, Data Structure, normalization, and scaling

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

Workflow of obtaining read counts



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 compressed

Space-ranger

Key Metrics

393,543

Number of 8 μ m binned
squares under tissue

849.2

Mean reads per 8 μ m bin

226.4

Mean UMIs per 8 μ m 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

```

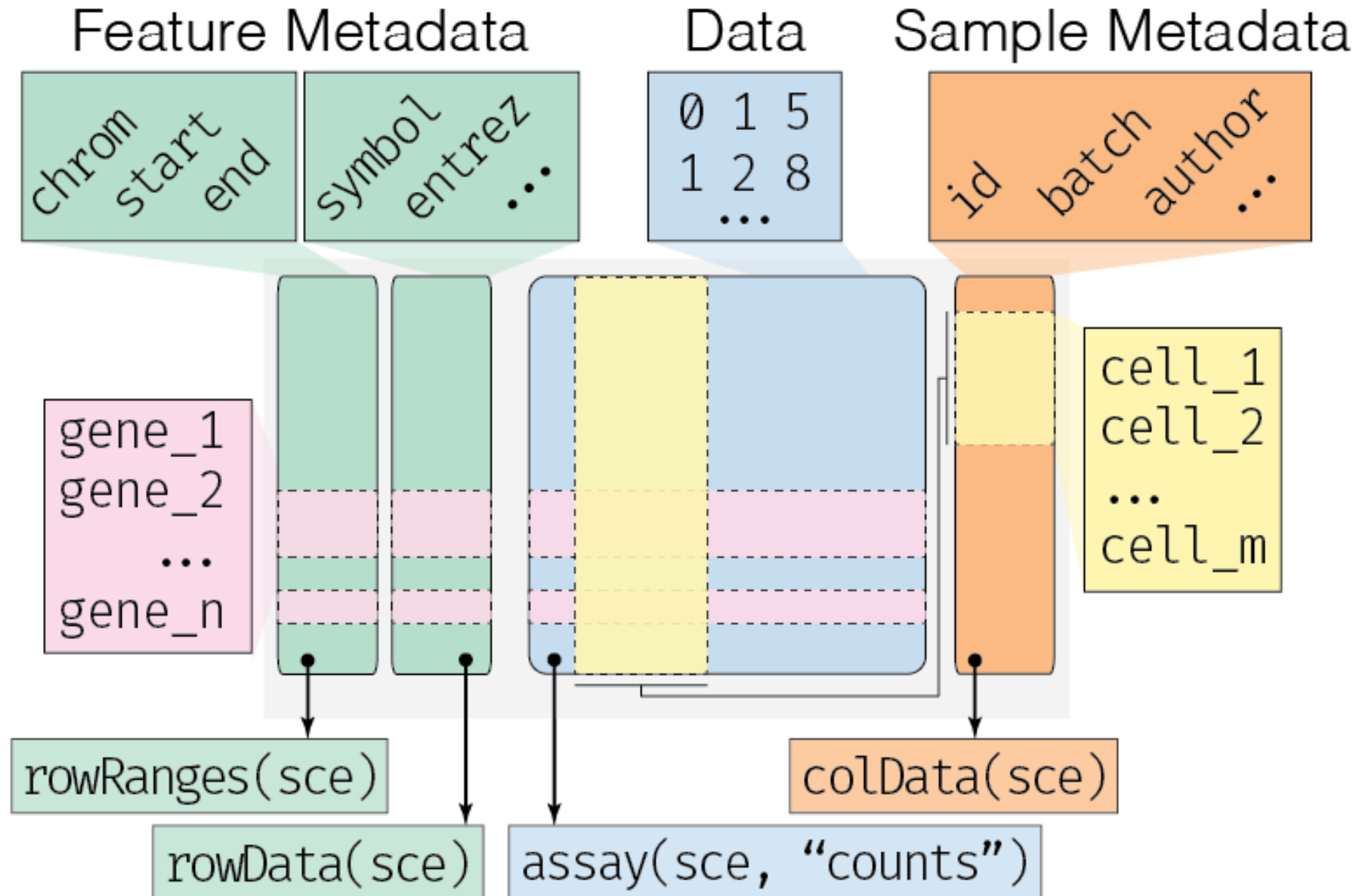
binned_output :
├─ square_002um
├─ square_008um
├─ square_016um
├─ analysis
│   ├── clustering
│   ├── diffexp
│   ├── pca
│   ├── tsne
│   └─ umap
├─ cloupe.cloupe
├─ filtered_feature_bc_matrix
│   ├── barcodes.tsv.gz
│   ├── features.tsv.gz
│   └─ matrix.mtx.gz
├─ filtered_feature_bc_matrix.h5
├─ spatial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

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

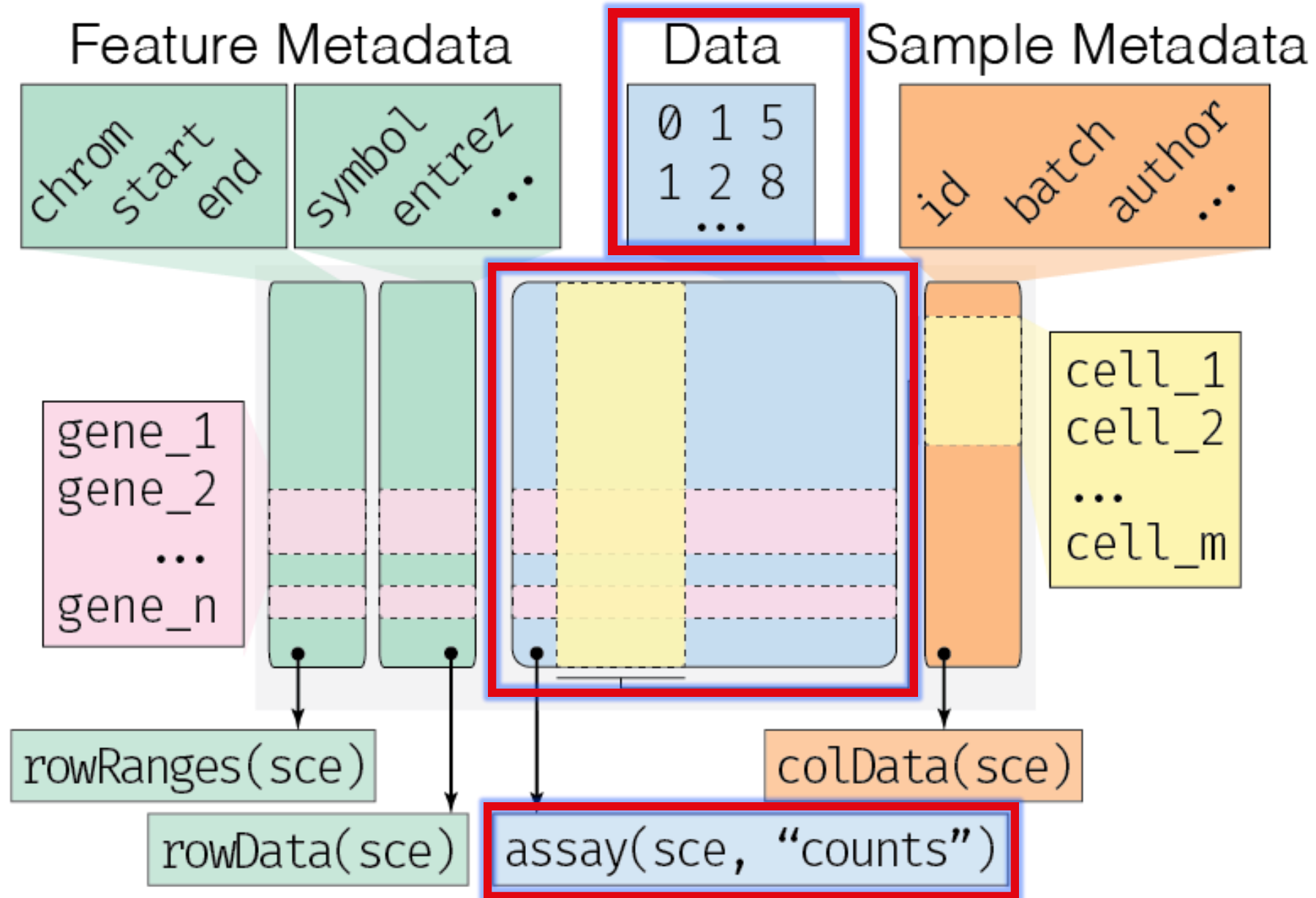
**Which framework have you used
for single-cell transcriptomics
data analysis?**

Seurat?

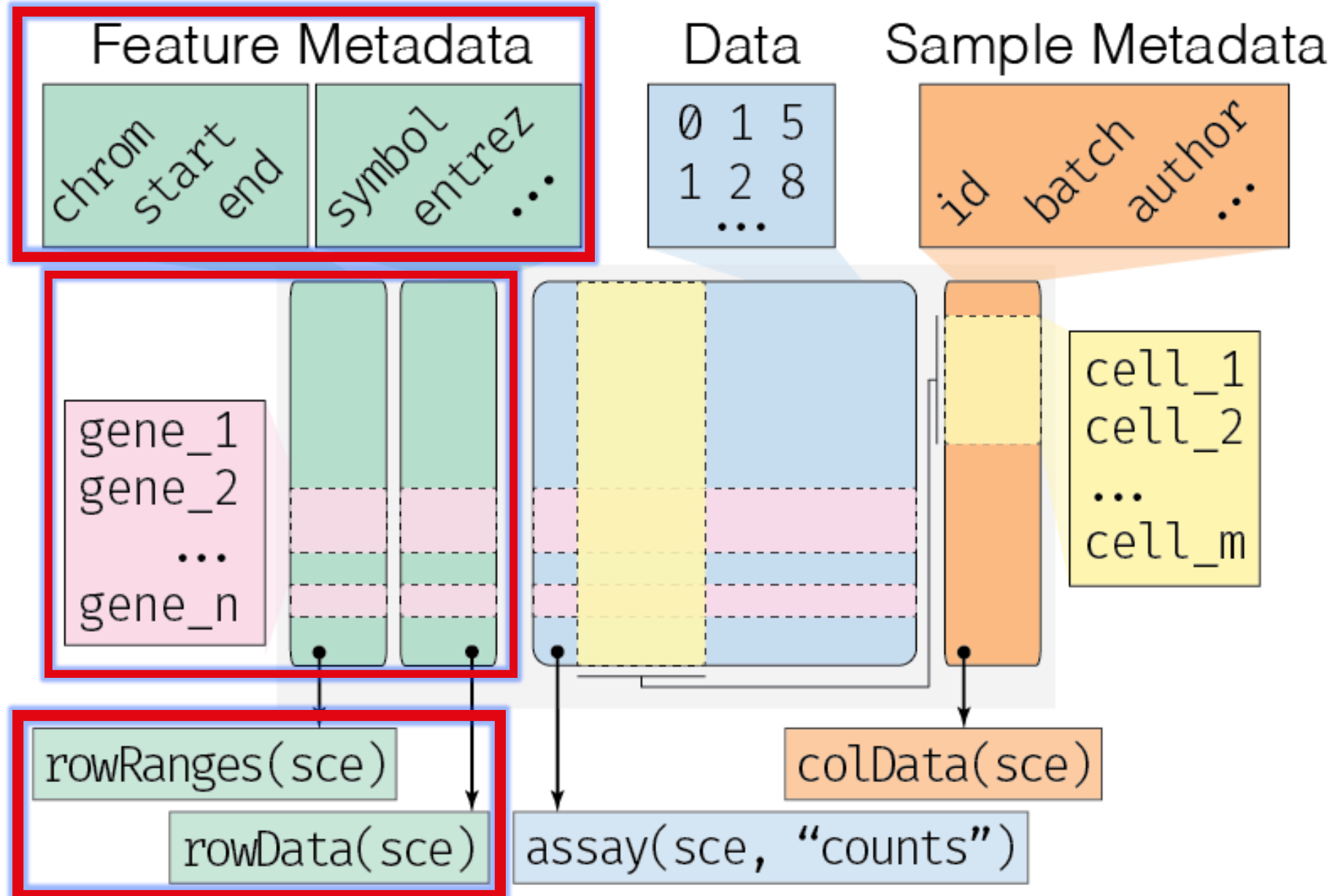
SingleCellExperiment



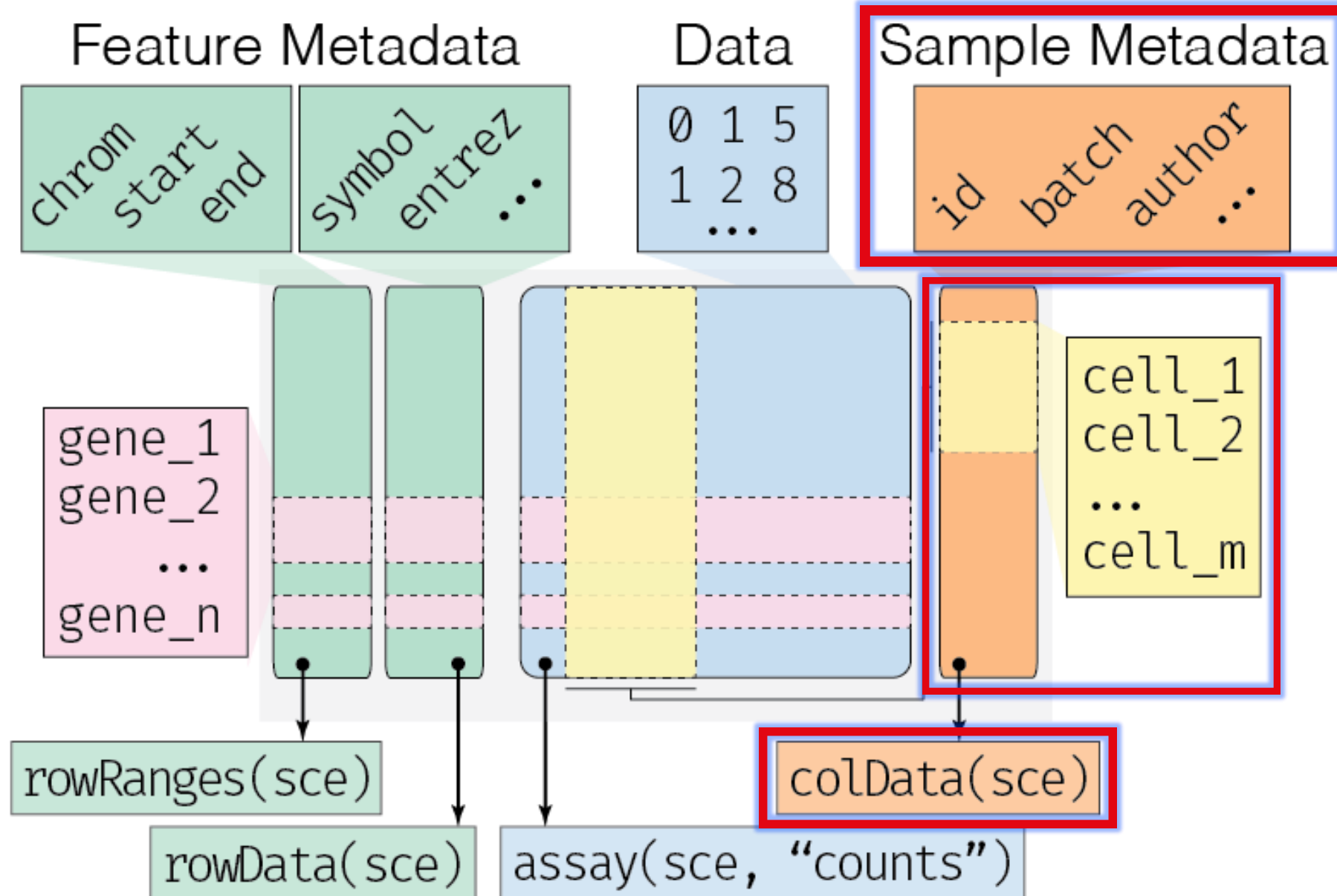
SingleCellExperiment



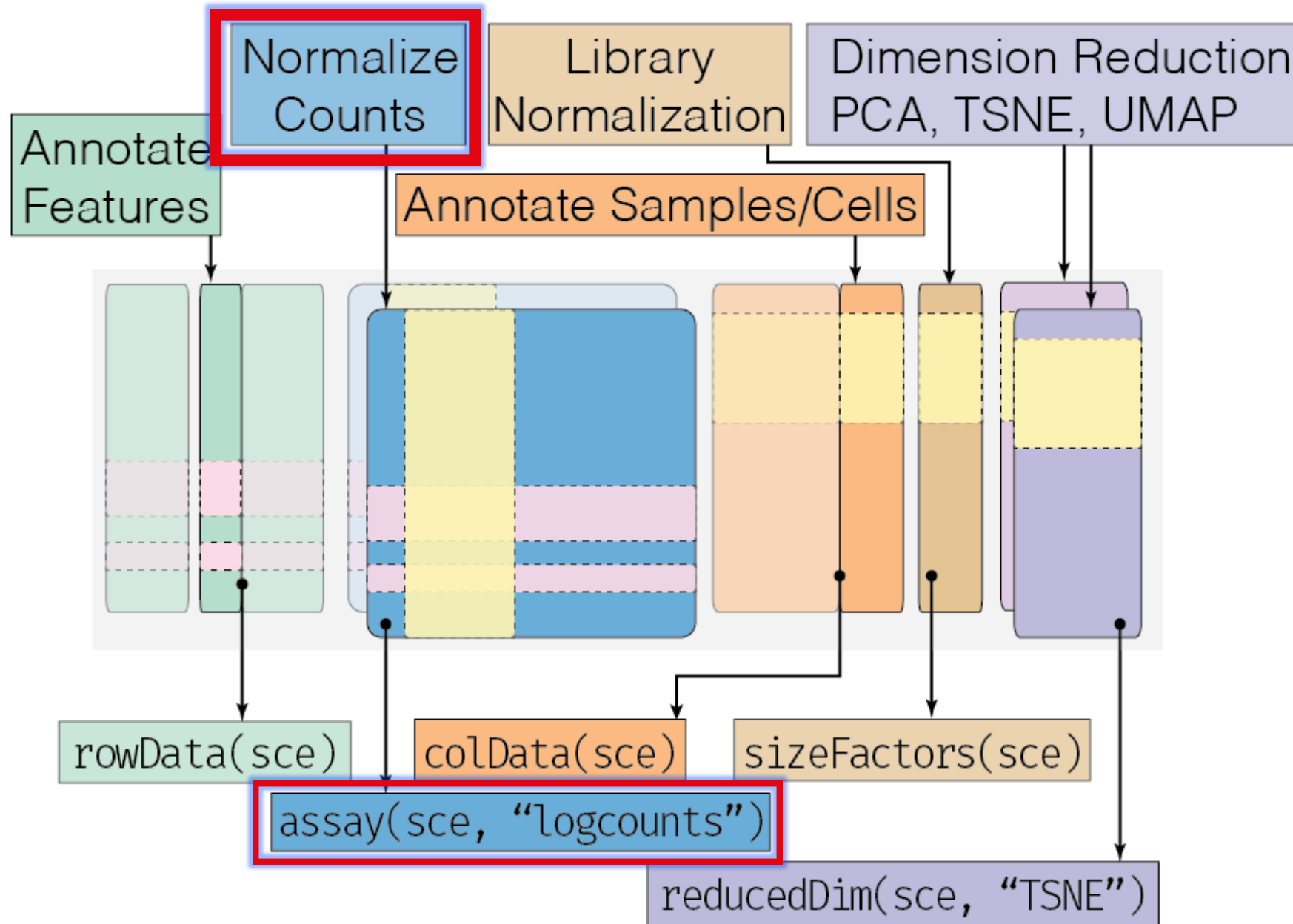
SingleCellExperiment



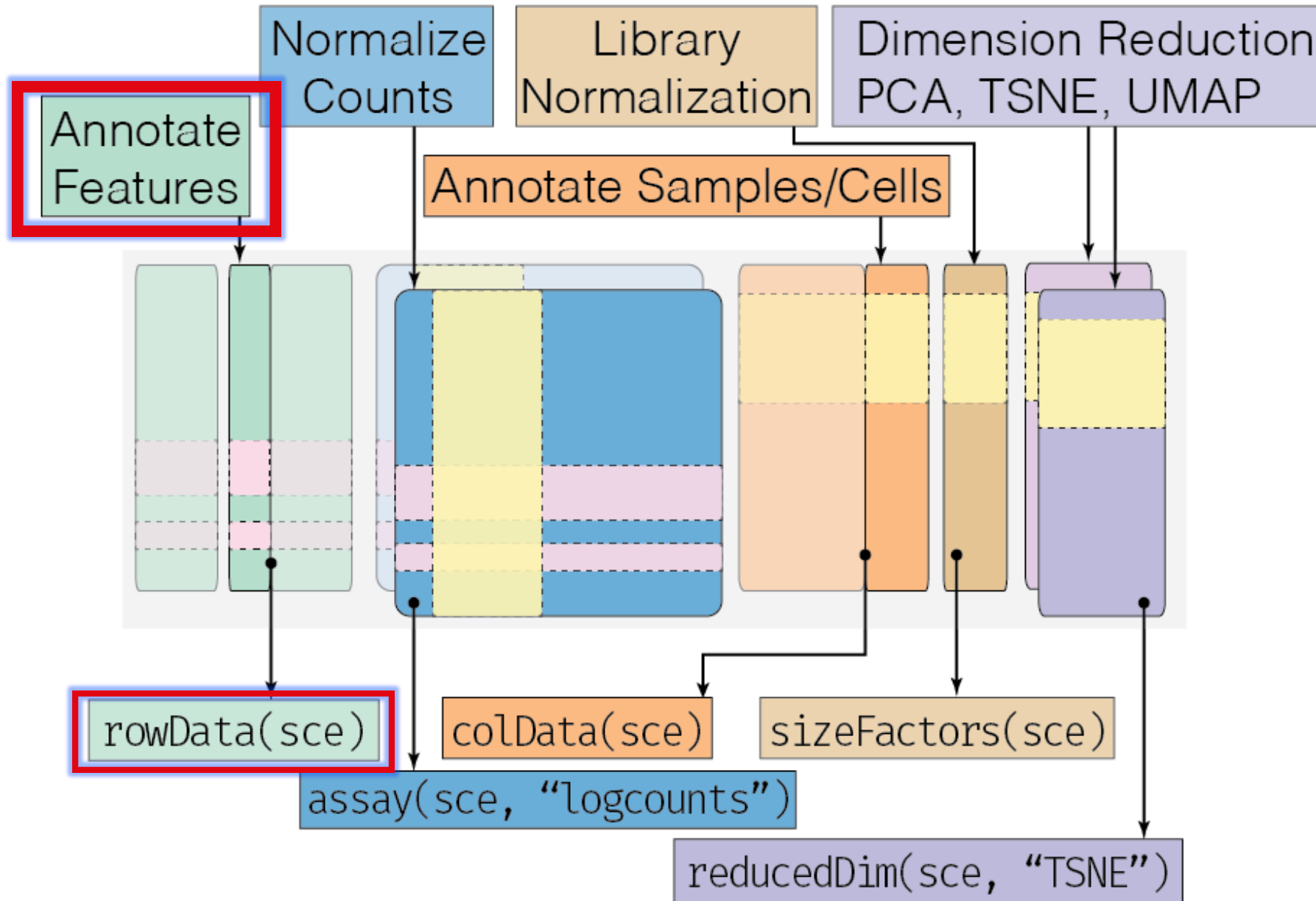
SingleCellExperiment



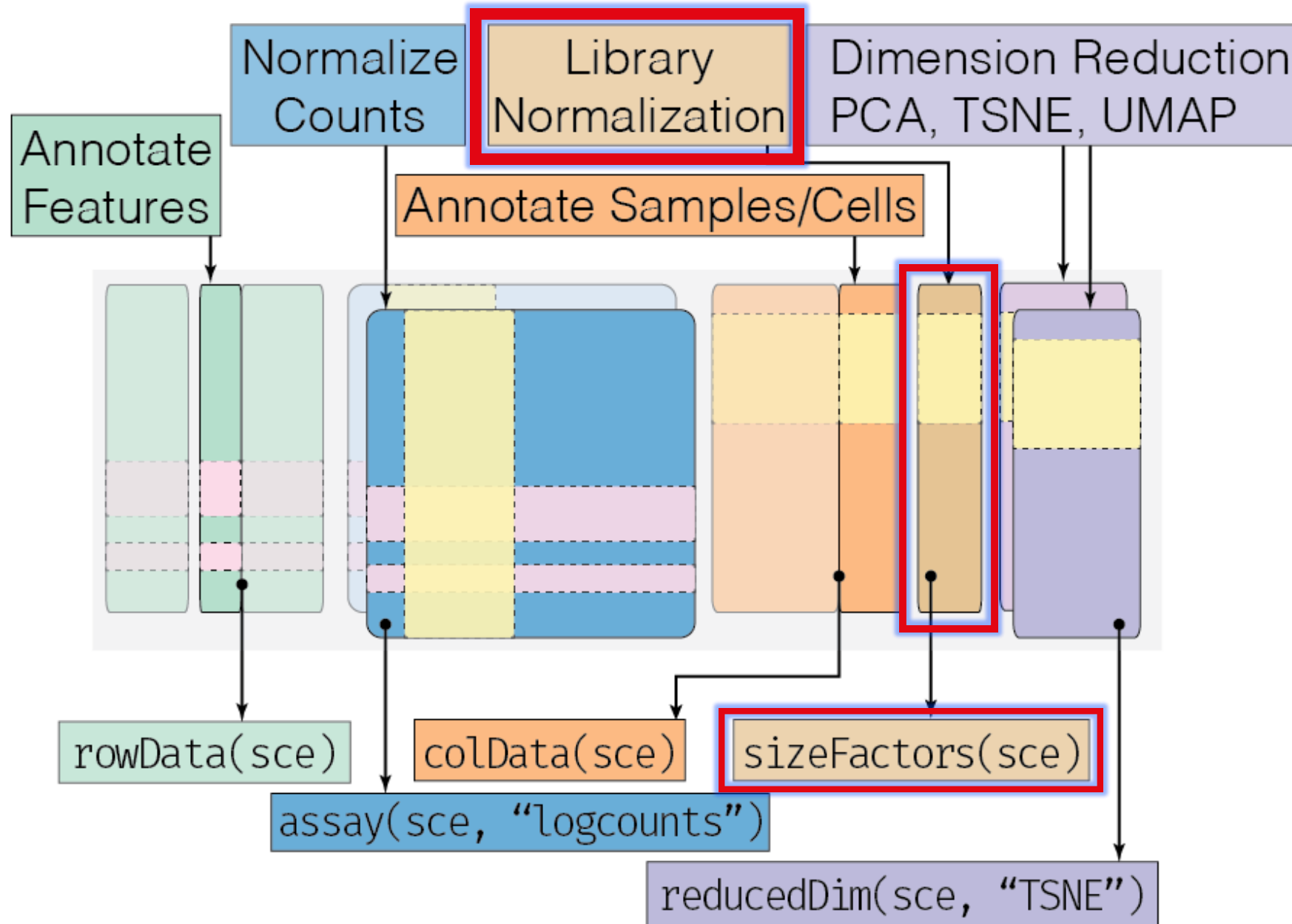
Extending SingleCellExperiment



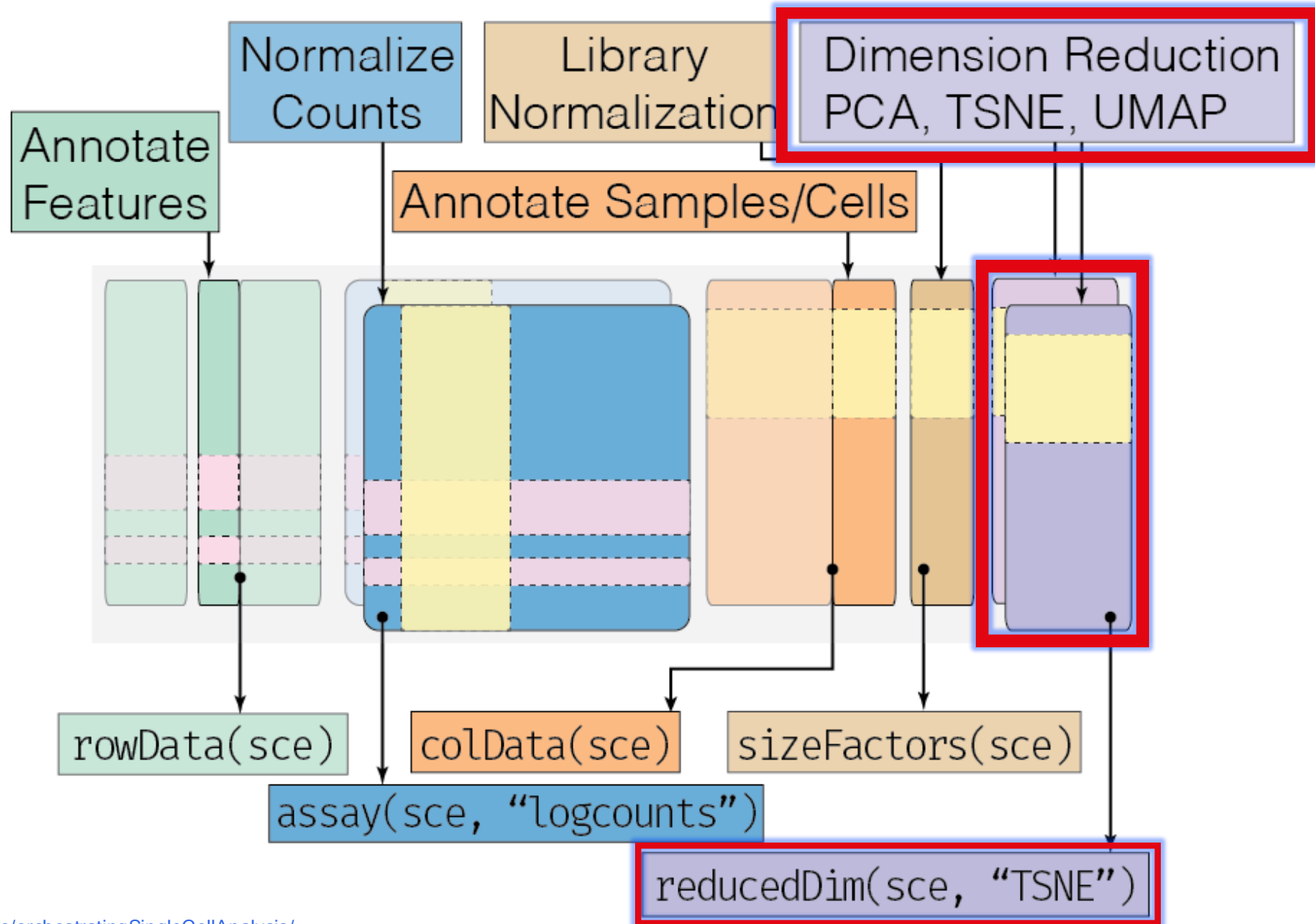
Extending SingleCellExperiment



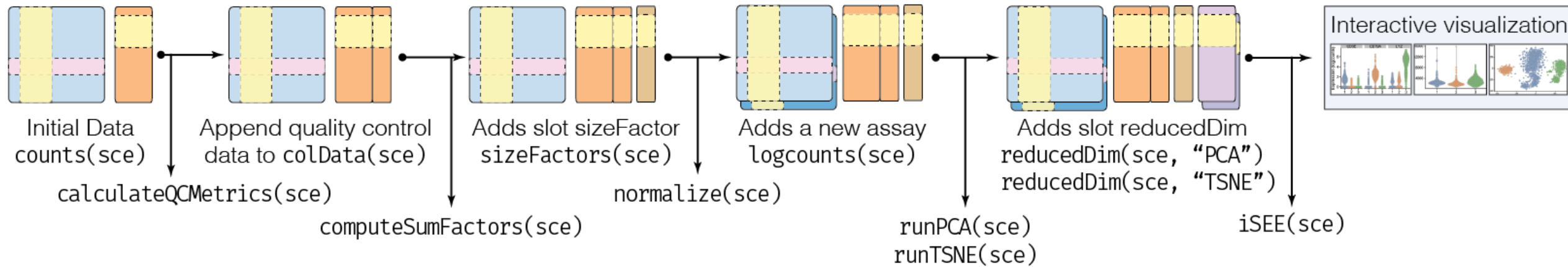
Extending SingleCellExperiment



Extending SingleCellExperiment



Example workflow of SingleCellExperiment extension



Can we use
SingleCellExperiment
for spatial transcriptomics data?

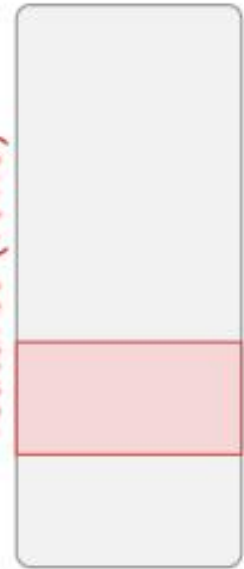
SpatialExperiment

SpatialExperiment

SingleCellExperiment

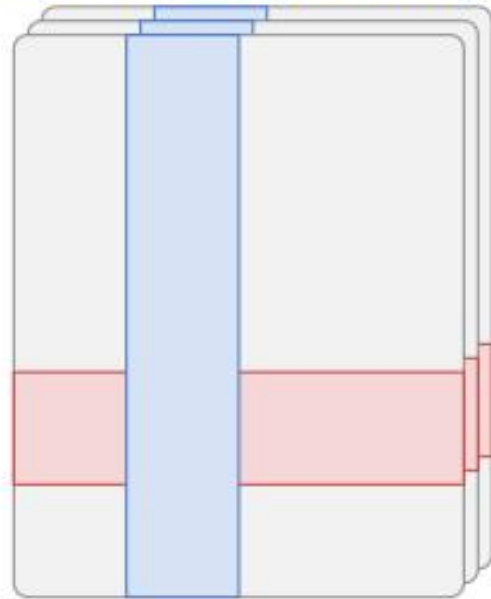
observations (columns)

features (rows)



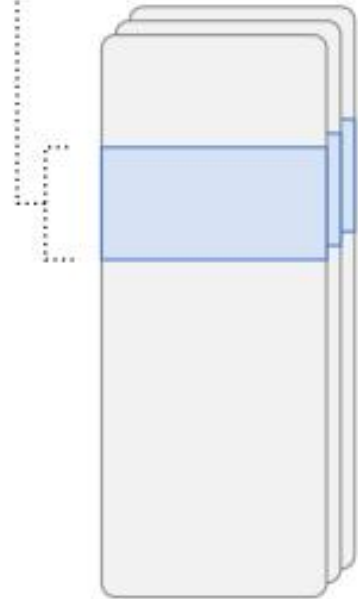
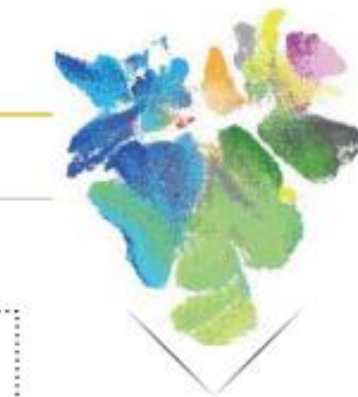
rowData

feature
metadata



assays

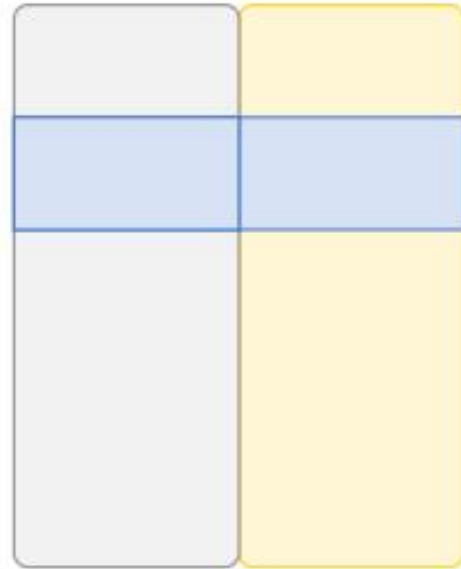
primary and
transformed data



reducedDims

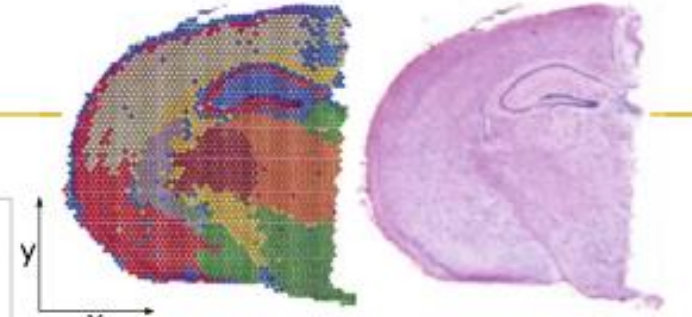
reduced
dimensions

sample id
condition
cell_type
...
barcode
in tissue
array_row
...



colData

observation
metadata spatial
metadata



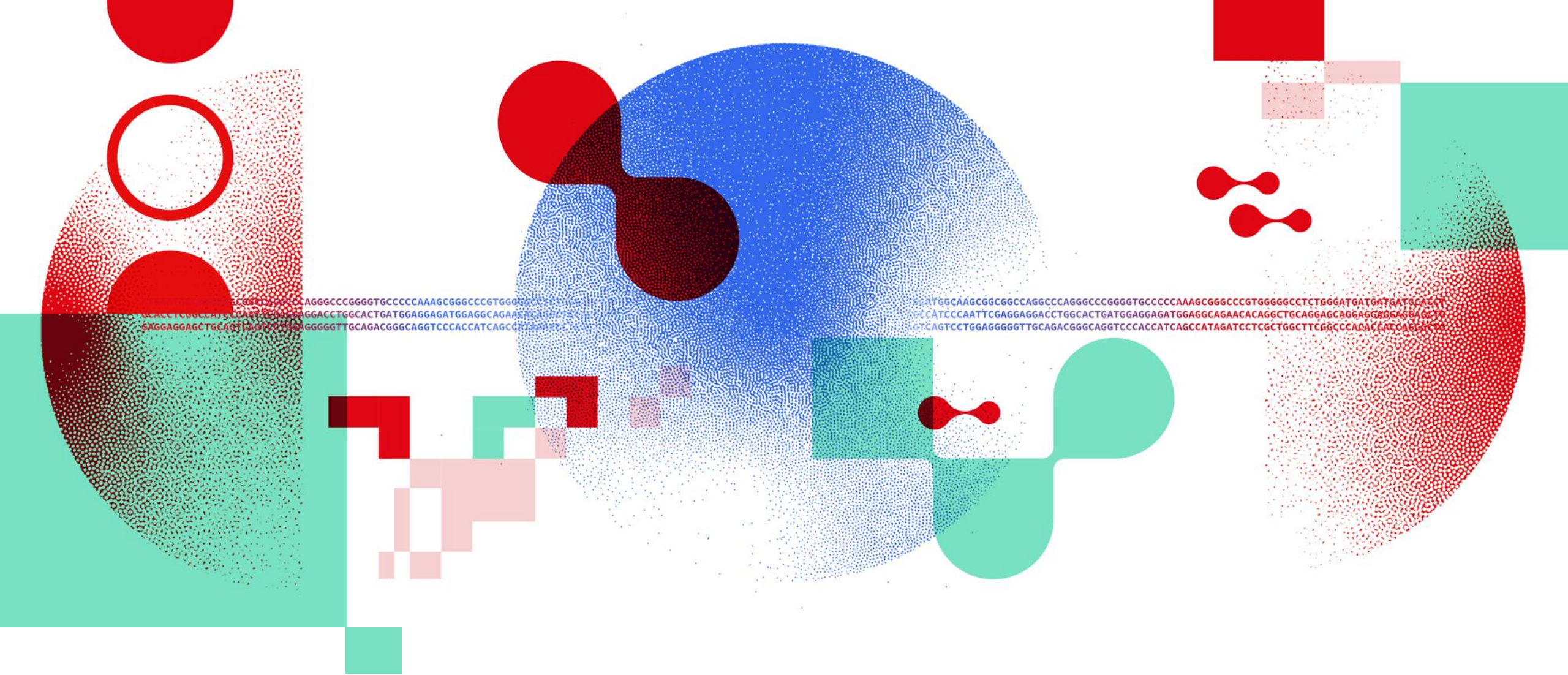
spatialCoords

spatial
coordinates



imgData

image
metadata



Thank you

DATA SCIENTISTS FOR LIFE

sib.swiss