BestPracticesSTBiocBook

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Welcome

Published: September 27, 2024

This is the website for the online book **'Best Practices for Spatial Transcriptomics Analysis with Bioconductor'**.

This book provides discussion and interactive examples on best practices for computational analysis workflows for spatial transcriptomics data, using the Bioconductor framework within R. The chapters contain details on individual analysis steps as well as complete example workflows, with interactive example datasets and R code.

The book is organized into several parts, including introductory materials, analysis steps, and example workflows.

Additional details on analysis workflows for non-spatial single-cell data as well as further introductory materials on R and Bioconductor can be found in the related book Orchestrating Single-Cell Analysis with Bioconductor (OSCA).

Preamble

Package: BestPracticesSTBiocBook **Authors:** Lukas M. Weber [aut, cre], Leonardo Collado-Torres [aut], Stephanie C. Hicks [aut] **Compiled:** 2024-09-27 **Package version:** 0.99.0 **R version:** R version 4.4.1 (2024-06-14) **BioC version:** 3.20 **License:** CC BY 4.0

Docker image

A `Docker` image built from this repository is available here:

ghcr.io/lmweber/bestpracticesstbiocbook

Get started now

You can get access to all the packages used in this book in $<$ 1 minute, using this command in a terminal:

Listing 0.1 `bash`

```
docker run -it ghcr.io/lmweber/bestpracticesstbiocbook:devel R
```

RStudio Server

An RStudio Server instance can be initiated from the `Docker` image as follows:

Listing 0.2 `bash`

```
docker run \
    --volume <local_folder>:<destination_folder> \
    -e PASSWORD=OHCA \
    -p 8787:8787 \
    ghcr.io/lmweber/bestpracticesstbiocbook:devel
```

The initiated RStudio Server instance will be available at https://localhost:8787.

Session info

Click to expand

Part I

Introduction

1 Introduction

1.1 Overview

Bioconductor

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1.3 Scope and who this book is for

Visium Data

Preprocessing

1.4 Bioconductor

Bioconductor

1.5 Additional resources

- Orchestrating Single-Cell Analysis with Bioconductor (OSCA)

- R for Data Science
- Data Carpentry Software Carpentry

- detailed guide
  YouTube videos
- Visium Data Preprocessing

1.6 Contributions

GitHub issues

References

2 Spatial transcriptomics

2.1 Overview

Method of the Year 2020

2.2 Sequencing-based platforms

2.2.1 10x Genomics Visium

10x Genomics Visium

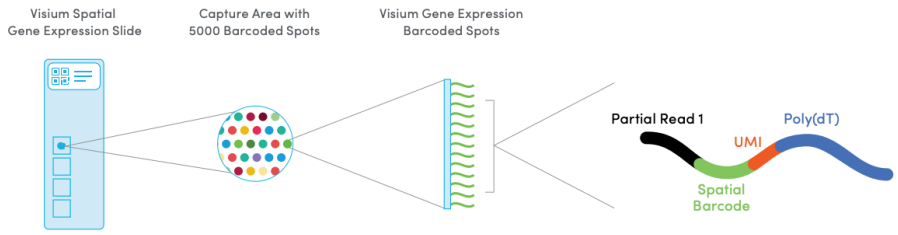

Figure 2.1: Schematic illustrating the 10x Genomics Visium platform. Source: 10x Genomics Visium

2.2.2 10x Genomics Visium HD

10x Genomics Visium HD

2.2.3 Curio Seeker

Curio Seeker

2.3 Molecule-based platforms

2.3.1 10x Genomics Xenium

10x Genomics

2.3.2 Vizgen MERSCOPE

Vizgen

2.3.3 NanoString CosMx

NanoString

References

3 Bioconductor data classes

3.1 Overview

3.2 SpatialExperiment class

SpatialExperiment

SingleCellExperiment

Bioconductor vignette

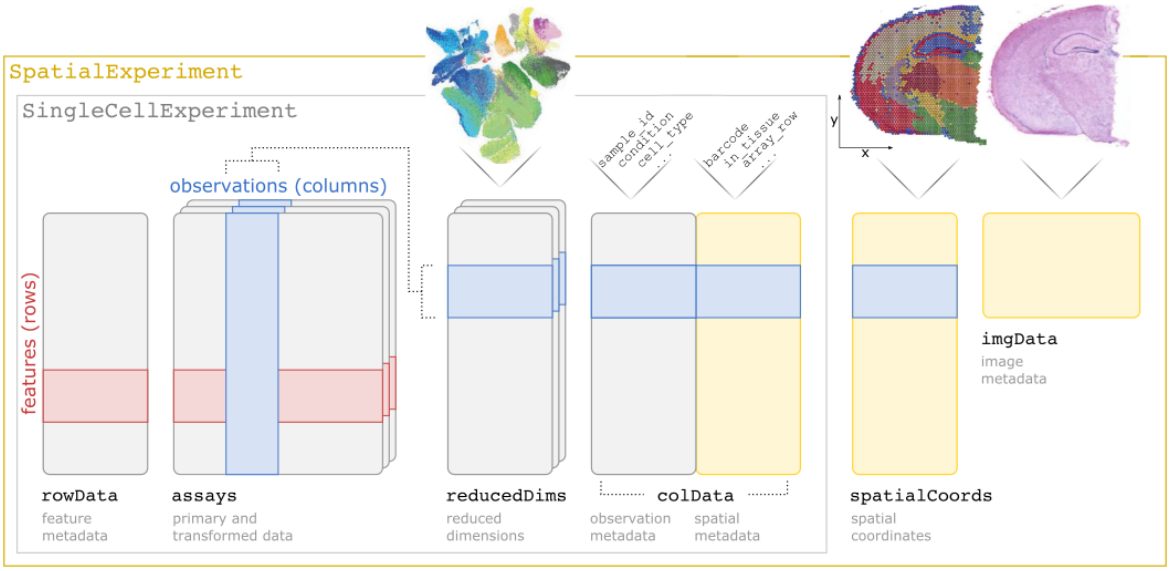

Figure 3.1: Overview of the `SpatialExperiment` data class for storing and manipulating spatial transcriptomics datasets within the Bioconductor framework.

3.3 Molecule-based data

3.3.1 MoleculeExperiment

Bioconductor package

3.3.2 SpatialFeatureExperiment

Bioconductor package

References

Part II

Analysis steps

4 Analysis steps

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4.1 Save data objects for re-use in later chapters

4.1.1 Human DLPFC dataset

```
# LOAD DATA

library(SpatialExperiment)
library(STexampleData)
spe <- Visium_humanDLPFC()

# save object
saveRDS(spe, file = "spe_load.rds")
```

```r
# QUALITY CONTROL (QC)

library(scater)
# subset to keep only spots over tissue
spe <- spe[, colData(spe)$in_tissue == 1]
# identify mitochondrial genes
is_mito <- grepl("(^MT-)|(^mt-)", rowData(spe)$gene_name)
# calculate per-spot QC metrics
spe <- addPerCellQC(spe, subsets = list(mito = is_mito))
# select QC thresholds
qc_lib_size <- colData(spe)$sum < 600
qc_detected <- colData(spe)$detected < 400
qc_mito <- colData(spe)$subsets_mito_percent > 28
qc_cell_count <- colData(spe)$cell_count > 10
# combined set of discarded spots
discard <- qc_lib_size | qc_detected | qc_mito | qc_cell_count
colData(spe)$discard <- discard
# filter low-quality spots
spe <- spe[, !colData(spe)$discard]

# save object
saveRDS(spe, file = "spe_qc.rds")
```

```r
# NORMALIZATION

library(scran)
# calculate logcounts using library size factors
spe <- logNormCounts(spe)

# save object
saveRDS(spe, file = "spe_logcounts.rds")
```

```r
# FEATURE SELECTION

# remove mitochondrial genes
spe <- spe[!is_mito, ]
# fit mean-variance relationship
dec <- modelGeneVar(spe)
# select top HVGs
top_hvgs <- getTopHVGs(dec, prop = 0.1)

# save object
```

```
saveRDS(spe, file = "spe_hvgs.rds")
saveRDS(top_hvgs, file = "top_hvgs.rds")
```

```
# DIMENSIONALITY REDUCTION

# compute PCA
set.seed(123)
spe <- runPCA(spe, subset_row = top_hvgs)
# compute UMAP on top 50 PCs
set.seed(123)
spe <- runUMAP(spe, dimred = "PCA")
# update column names
colnames(reducedDim(spe, "UMAP")) <- paste0("UMAP", 1:2)

# save object
saveRDS(spe, file = "spe_reduceddims.rds")
```

```
# CLUSTERING

# graph-based clustering
set.seed(123)
k <- 10
g <- buildSNNGraph(spe, k = k, use.dimred = "PCA")
g_walk <- igraph::cluster_walktrap(g)
clus <- g_walk$membership
colLabels(spe) <- factor(clus)

# save object
saveRDS(spe, file = "spe_cluster.rds")
```

References

5 Load data

5.1 Overview

3

Visium Data

Preprocessing

STexampleData

5.2 Dataset

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5.3 Load data

STexampleData

```
library(SpatialExperiment)
library(STexampleData)

# load object
spe <- Visium_humanDLPFC()
```

5.4 SpatialExperiment object

[3]

```
# check object
spe
##  class: SpatialExperiment
##  dim: 33538 4992
##  metadata(0):
##  assays(1): counts
##  rownames(33538): ENSG00000243485 ENSG00000237613 ... ENSG00000277475
##    ENSG00000268674
##  rowData names(3): gene_id gene_name feature_type
##  colnames(4992): AAACAACGAATAGTTC-1 AAACAAGTATCTCCCA-1 ...
##    TTGTTTGTATTACACG-1 TTGTTTGTGTAAATTC-1
##  colData names(8): barcode_id sample_id ... reference cell_count
##  reducedDimNames(0):
##  mainExpName: NULL
##  altExpNames(0):
##  spatialCoords names(2) : pxl_col_in_fullres pxl_row_in_fullres
##  imgData names(4): sample_id image_id data scaleFactor

# number of genes (rows) and spots (columns)
dim(spe)
##  [1] 33538  4992

# names of 'assays'
assayNames(spe)
##  [1] "counts"

# row (gene) data
head(rowData(spe))
##  DataFrame with 6 rows and 3 columns
```

```
##                          gene_id   gene_name    feature_type
##                      <character> <character>     <character>
##  ENSG00000243485 ENSG00000243485 MIR1302-2HG Gene Expression
##  ENSG00000237613 ENSG00000237613     FAM138A Gene Expression
##  ENSG00000186092 ENSG00000186092       OR4F5 Gene Expression
##  ENSG00000238009 ENSG00000238009  AL627309.1 Gene Expression
##  ENSG00000239945 ENSG00000239945  AL627309.3 Gene Expression
##  ENSG00000239906 ENSG00000239906  AL627309.2 Gene Expression

# column (spot) data
head(colData(spe))
##  DataFrame with 6 rows and 8 columns
##                             barcode_id     sample_id in_tissue array_row
##                            <character>   <character> <integer> <integer>
##  AAACAACGAATAGTTC-1 AAACAACGAATAGTTC-1 sample_151673         0         0
##  AAACAAGTATCTCCCA-1 AAACAAGTATCTCCCA-1 sample_151673         1        50
##  AAACAATCTACTAGCA-1 AAACAATCTACTAGCA-1 sample_151673         1         3
##  AAACACCAATAACTGC-1 AAACACCAATAACTGC-1 sample_151673         1        59
##  AAACAGAGCGACTCCT-1 AAACAGAGCGACTCCT-1 sample_151673         1        14
##  AAACAGCTTTCAGAAG-1 AAACAGCTTTCAGAAG-1 sample_151673         1        43
##                       array_col ground_truth   reference cell_count
##                       <integer>  <character> <character>  <integer>
##  AAACAACGAATAGTTC-1          16           NA          NA         NA
##  AAACAAGTATCTCCCA-1         102       Layer3      Layer3          6
##  AAACAATCTACTAGCA-1          43       Layer1      Layer1         16
##  AAACACCAATAACTGC-1          19           WM          WM          5
##  AAACAGAGCGACTCCT-1          94       Layer3      Layer3          2
##  AAACAGCTTTCAGAAG-1           9       Layer5      Layer5          4

# spatial coordinates
head(spatialCoords(spe))
##                       pxl_col_in_fullres pxl_row_in_fullres
##  AAACAACGAATAGTTC-1                3913               2435
##  AAACAAGTATCTCCCA-1                9791               8468
##  AAACAATCTACTAGCA-1                5769               2807
##  AAACACCAATAACTGC-1                4068               9505
##  AAACAGAGCGACTCCT-1                9271               4151
##  AAACAGCTTTCAGAAG-1                3393               7583

# image data
imgData(spe)
##  DataFrame with 2 rows and 4 columns
```

```
##        sample_id    image_id   data scaleFactor
##      <character> <character> <list>   <numeric>
##  1 sample_151673      lowres   ####   0.0450045
##  2 sample_151673       hires   ####   0.1500150
```

5.5 Build object

SpatialExperiment

```
# create data
n_genes <- 200
n_spots <- 100

counts <- matrix(0, nrow = n_genes, ncol = n_spots)

row_data <- DataFrame(
  gene_name = paste0("gene", sprintf("%03d", seq_len(n_genes)))
)

col_data <- DataFrame(
  sample_id = rep("sample01", n_spots)
)

spatial_coords <- matrix(0, nrow = n_spots, ncol = 2)
colnames(spatial_coords) <- c("x", "y")

# create SpatialExperiment object
spe <- SpatialExperiment(
  assays = list(counts = counts),
  colData = col_data,
  rowData = row_data,
  spatialCoords = spatial_coords
)
```

5.6 Molecule-based data

[3]

References

6 Quality control

6.1 Overview

-
-
-
-

6.2 Load data from previous steps

4

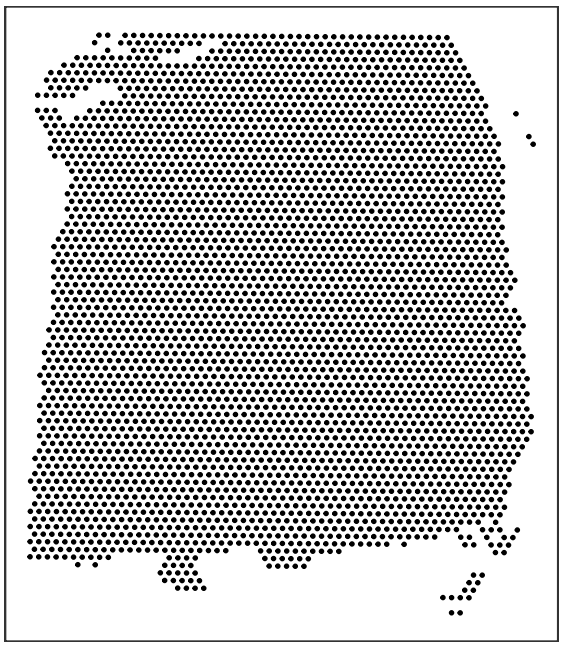
```
library(SpatialExperiment)
spe <- readRDS("spe_load.rds")
```

6.3 Plot data

ggspavis

```
library(ggspavis)
```

```
# plot spatial coordinates (spots)
plotSpots(spe)
```



6.4 Calculate QC metrics

```
library(scater)
```

```
# subset to keep only spots over tissue
spe <- spe[, colData(spe)$in_tissue == 1]
dim(spe)
##  [1] 33538  3639
```

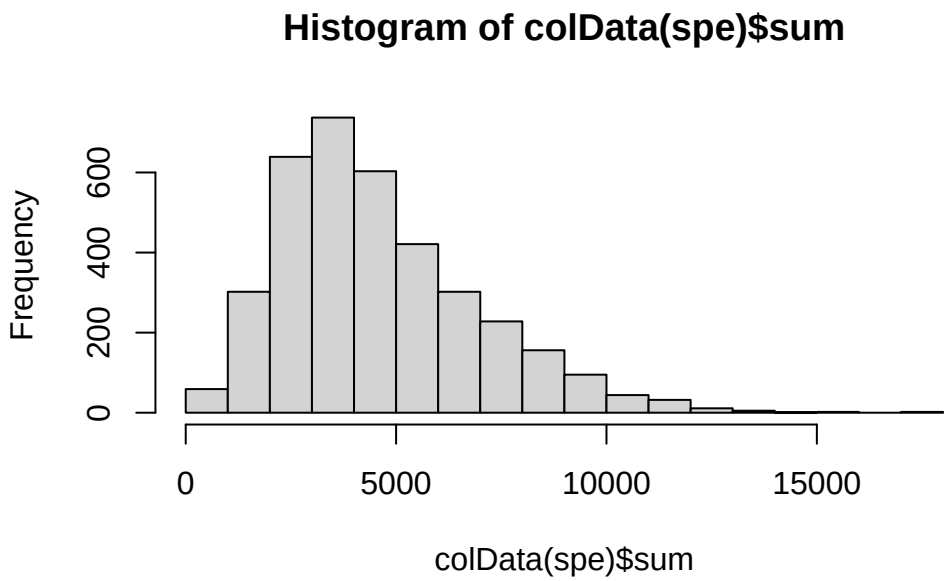
```
# identify mitochondrial genes
is_mito <- grepl("(^MT-)|(^mt-)", rowData(spe)$gene_name)
table(is_mito)
##  is_mito
##  FALSE  TRUE
##  33525    13
rowData(spe)$gene_name[is_mito]
##   [1] "MT-ND1"  "MT-ND2"  "MT-CO1"  "MT-CO2"  "MT-ATP8" "MT-ATP6" "MT-CO3"
##   [8] "MT-ND3"  "MT-ND4L" "MT-ND4"  "MT-ND5"  "MT-ND6"  "MT-CYB"

# calculate per-spot QC metrics and store in colData
spe <- addPerCellQC(spe, subsets = list(mito = is_mito))
head(colData(spe))
##  DataFrame with 6 rows and 14 columns
##                            barcode_id     sample_id in_tissue array_row
##                           <character>   <character> <integer> <integer>
##  AAACAAGTATCTCCCA-1 AAACAAGTATCTCCCA-1 sample_151673         1        50
##  AAACAATCTACTAGCA-1 AAACAATCTACTAGCA-1 sample_151673         1         3
##  AAACACCAATAACTGC-1 AAACACCAATAACTGC-1 sample_151673         1        59
##  AAACAGAGCGACTCCT-1 AAACAGAGCGACTCCT-1 sample_151673         1        14
##  AAACAGCTTTCAGAAG-1 AAACAGCTTTCAGAAG-1 sample_151673         1        43
##  AAACAGGGTCTATATT-1 AAACAGGGTCTATATT-1 sample_151673         1        47
##                     array_col ground_truth   reference cell_count       sum
```

```
##                     <integer>  <character> <character>  <integer> <numeric>
##  AAACAAGTATCTCCCA-1       102       Layer3      Layer3          6      8458
##  AAACAATCTACTAGCA-1        43       Layer1      Layer1         16      1667
##  AAACACCAATAACTGC-1        19           WM          WM          5      3769
##  AAACAGAGCGACTCCT-1        94       Layer3      Layer3          2      5433
##  AAACAGCTTTCAGAAG-1         9       Layer5      Layer5          4      4278
##  AAACAGGGTCTATATT-1        13       Layer6      Layer6          6      4004
##                      detected subsets_mito_sum subsets_mito_detected
##                     <numeric>        <numeric>             <numeric>
##  AAACAAGTATCTCCCA-1      3586             1407                    13
##  AAACAATCTACTAGCA-1      1150              204                    11
##  AAACACCAATAACTGC-1      1960              430                    13
##  AAACAGAGCGACTCCT-1      2424             1316                    13
##  AAACAGCTTTCAGAAG-1      2264              651                    12
##  AAACAGGGTCTATATT-1      2178              621                    13
##                     subsets_mito_percent     total
##                                <numeric> <numeric>
##  AAACAAGTATCTCCCA-1              16.6351      8458
##  AAACAATCTACTAGCA-1              12.2376      1667
##  AAACACCAATAACTGC-1              11.4089      3769
##  AAACAGAGCGACTCCT-1              24.2223      5433
##  AAACAGCTTTCAGAAG-1              15.2174      4278
##  AAACAGGGTCTATATT-1              15.5095      4004
```

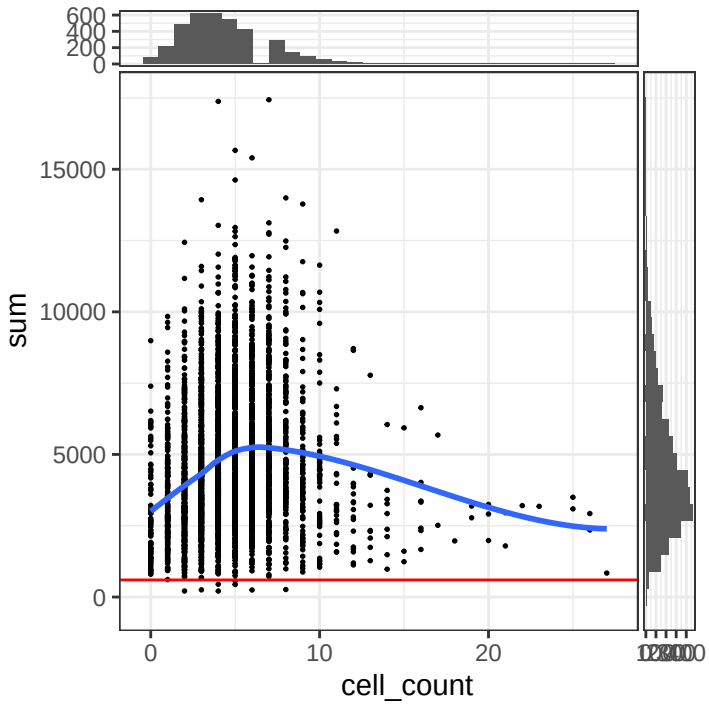
6.5 Selecting thresholds

6.5.1 Library size

```r
# histogram of library sizes
hist(colData(spe)$sum, breaks = 20)
```

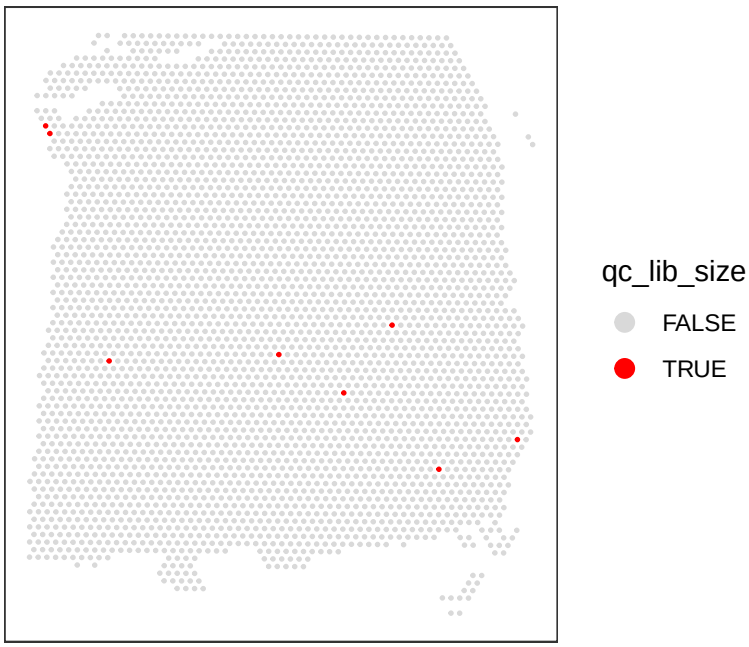



```r
# plot library size vs. number of cells per spot
plotSpotQC(spe, plot_type = "scatter",
           x_metric = "cell_count", y_metric = "sum",
           y_threshold = 600)
##  `geom_smooth()` using formula = 'y ~ x'
##  `stat_xsidebin()` using `bins = 30`. Pick better value with `binwidth`.
##  `stat_ysidebin()` using `bins = 30`. Pick better value with `binwidth`.
```

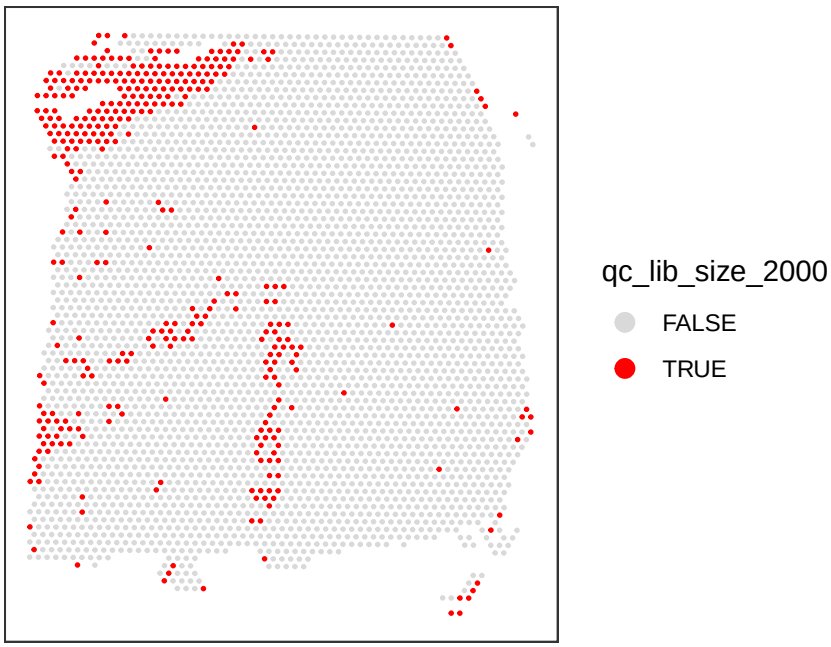



```
# select QC threshold for library size
qc_lib_size <- colData(spe)$sum < 600
table(qc_lib_size)
##  qc_lib_size
##  FALSE  TRUE
##   3631     8

colData(spe)$qc_lib_size <- qc_lib_size
```

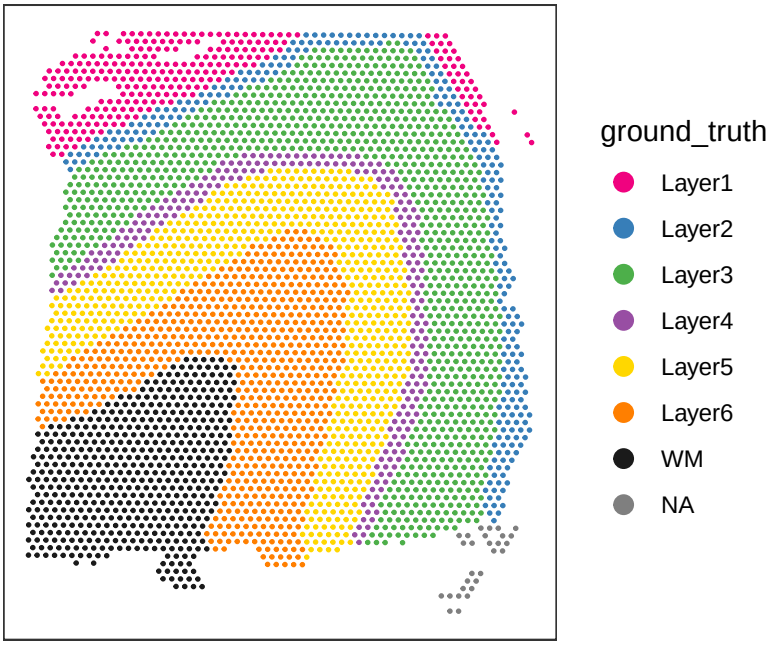
```
# check spatial pattern of discarded spots
plotSpotQC(spe, plot_type = "spot",
           annotate = "qc_lib_size")
```




```
# check spatial pattern of discarded spots if threshold is too high
qc_lib_size_2000 <- colData(spe)$sum < 2000
colData(spe)$qc_lib_size_2000 <- qc_lib_size_2000
plotSpotQC(spe, plot_type = "spot",
           annotate = "qc_lib_size_2000")
```

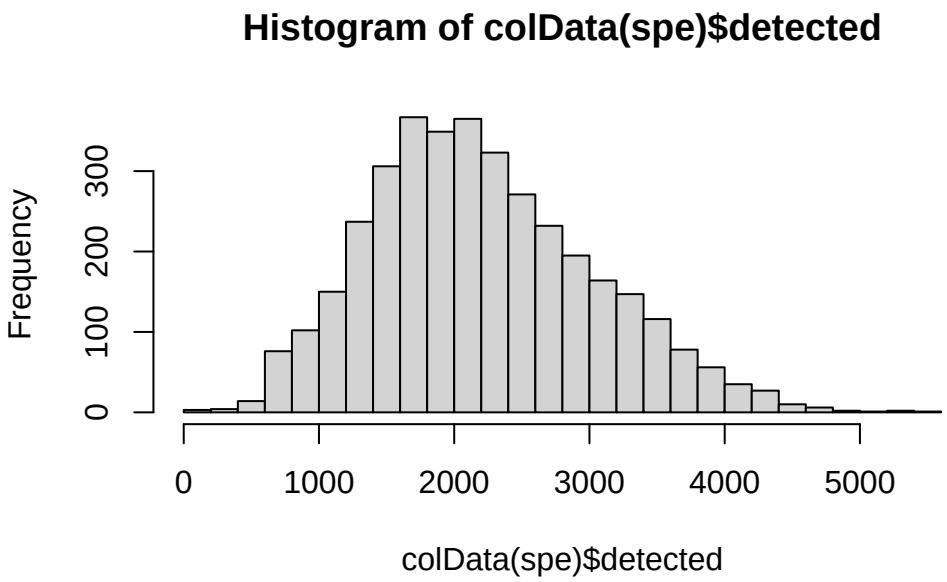



```
# plot ground truth (manually annotated) layers
plotSpots(spe, annotate = "ground_truth",
          pal = "libd_layer_colors")
```

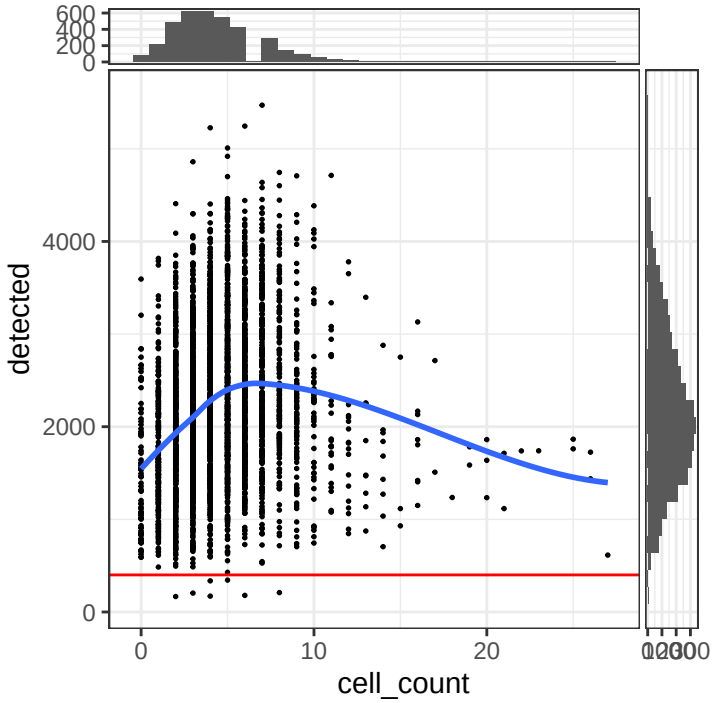


6.5.2 Number of expressed features

```
# histogram of numbers of expressed genes
hist(colData(spe)$detected, breaks = 20)
```

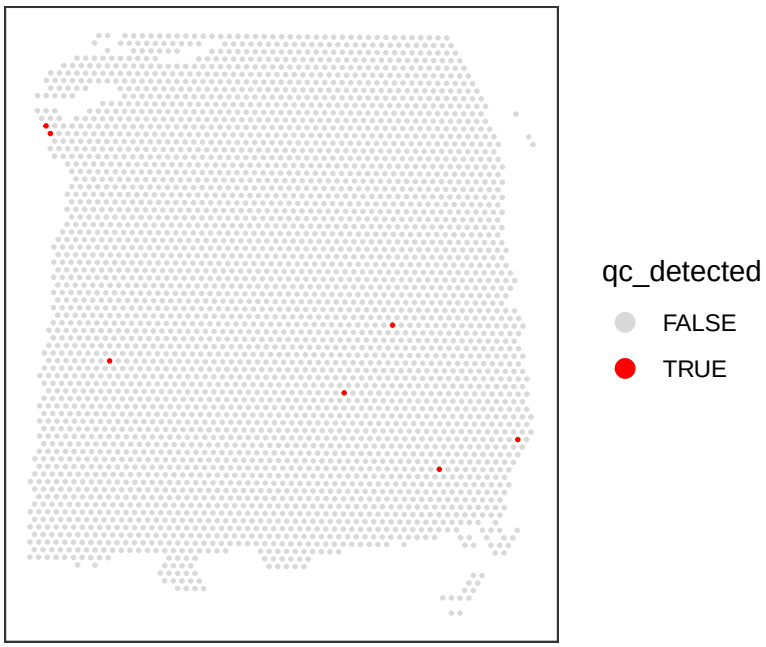



```
# plot number of expressed genes vs. number of cells per spot
plotSpotQC(spe, plot_type = "scatter",
           x_metric = "cell_count", y_metric = "detected",
           y_threshold = 400)
##  `geom_smooth()` using formula = 'y ~ x'
##  `stat_xsidebin()` using `bins = 30`. Pick better value with `binwidth`.
##  `stat_ysidebin()` using `bins = 30`. Pick better value with `binwidth`.
```

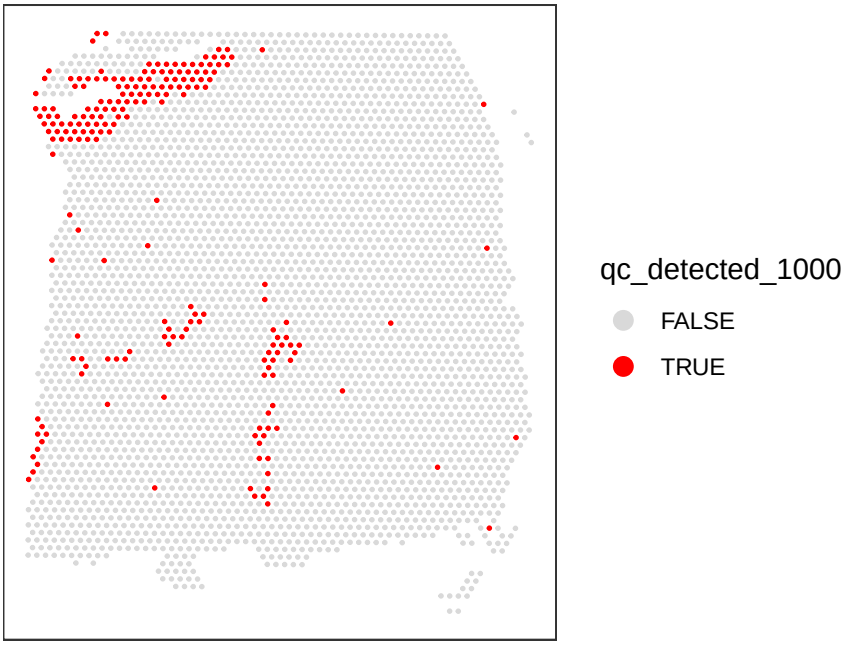



```
# select QC threshold for number of expressed genes
qc_detected <- colData(spe)$detected < 400
table(qc_detected)
##  qc_detected
##  FALSE  TRUE
##   3632     7

colData(spe)$qc_detected <- qc_detected
```

```
# check spatial pattern of discarded spots
plotSpotQC(spe, plot_type = "spot",
           annotate = "qc_detected")
```

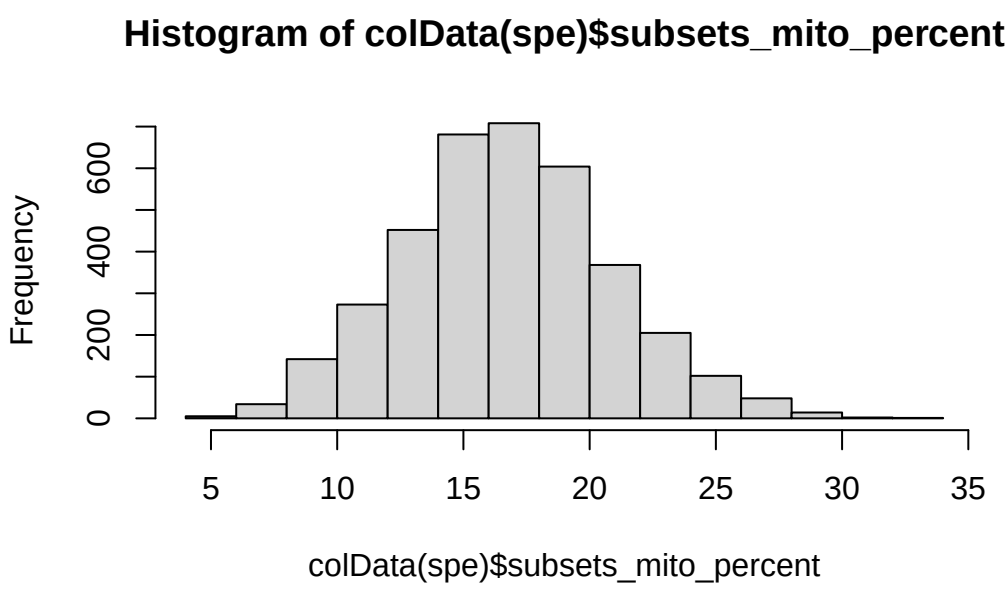



```
# check spatial pattern of discarded spots if threshold is too high
qc_detected_1000 <- colData(spe)$detected < 1000
colData(spe)$qc_detected_1000 <- qc_detected_1000
plotSpotQC(spe, plot_type = "spot",
           annotate = "qc_detected_1000")
```

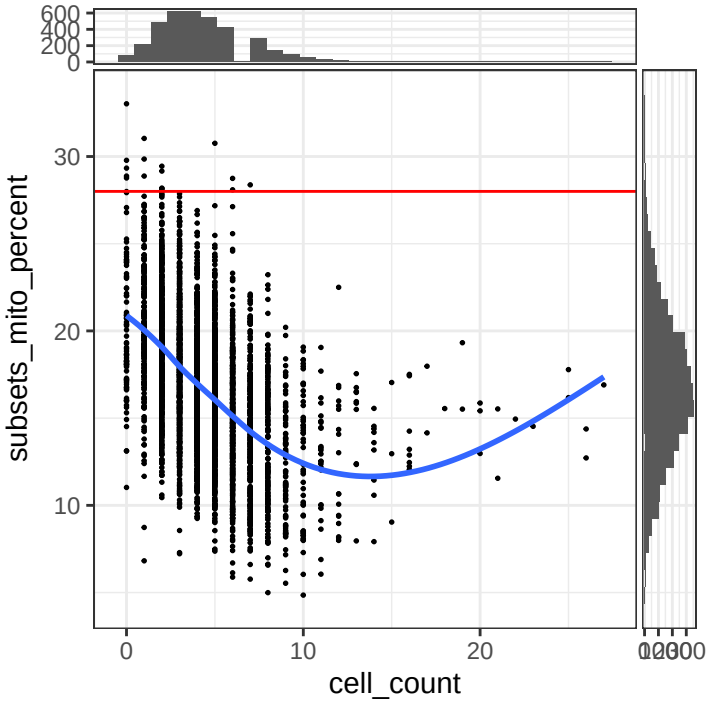


6.5.3 Proportion of mitochondrial reads

```
# histogram of mitochondrial read proportions
hist(colData(spe)$subsets_mito_percent, breaks = 20)
```

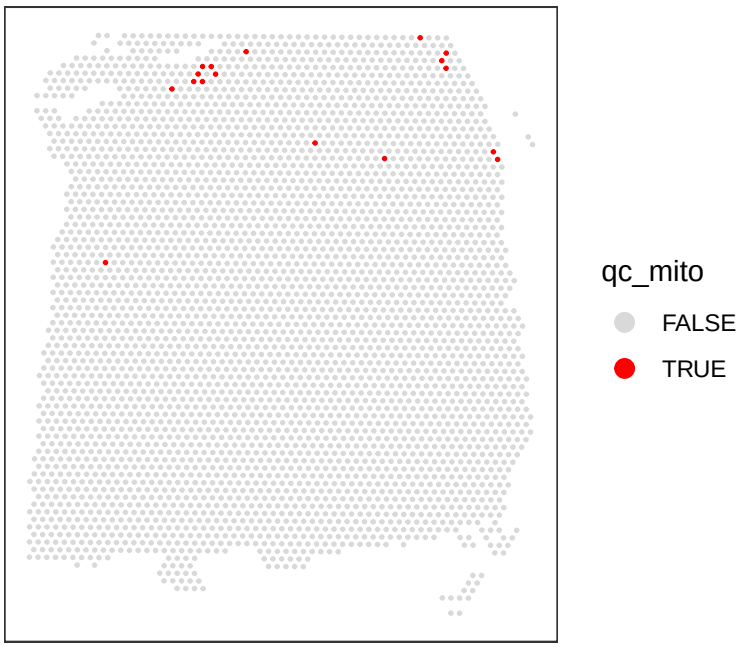



```
# plot mitochondrial read proportion vs. number of cells per spot
plotSpotQC(spe, plot_type = "scatter",
           x_metric = "cell_count", y_metric = "subsets_mito_percent",
           y_threshold = 28)
##  `geom_smooth()` using formula = 'y ~ x'
##  `stat_xsidebin()` using `bins = 30`. Pick better value with `binwidth`.
##  `stat_ysidebin()` using `bins = 30`. Pick better value with `binwidth`.
```

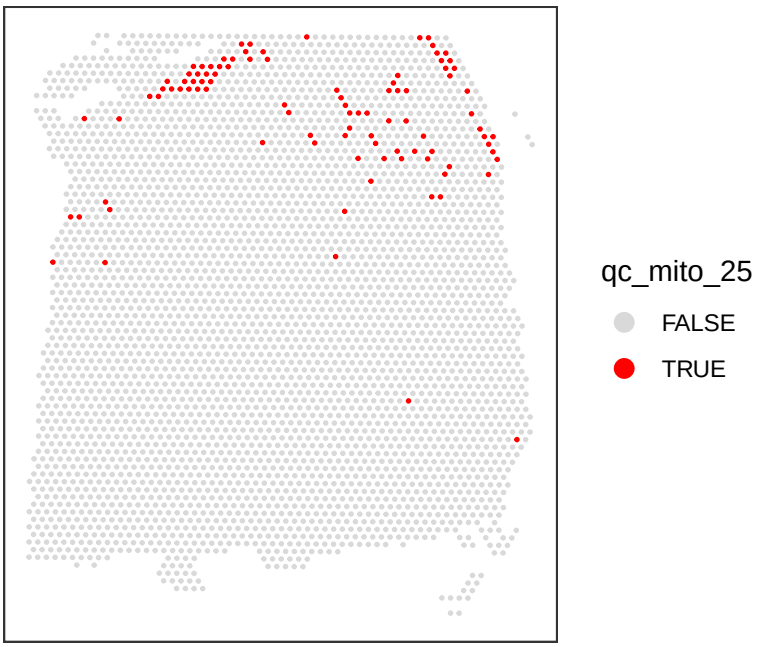



```
# select QC threshold for mitochondrial read proportion
qc_mito <- colData(spe)$subsets_mito_percent > 28
table(qc_mito)
##  qc_mito
##  FALSE  TRUE
##   3622    17

colData(spe)$qc_mito <- qc_mito
```

```
# check spatial pattern of discarded spots
plotSpotQC(spe, plot_type = "spot",
           annotate = "qc_mito")
```

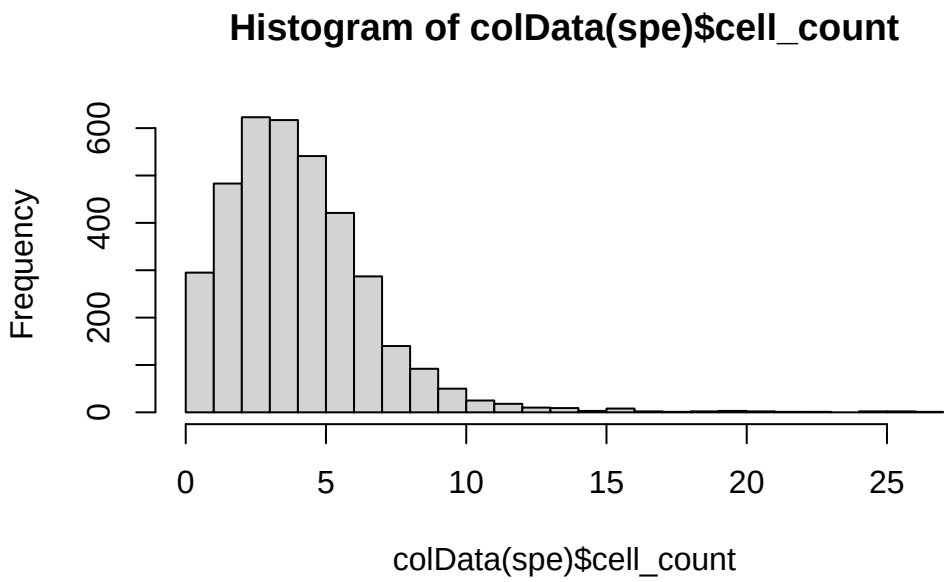



```
# check spatial pattern of discarded spots if threshold is too high
qc_mito_25 <- colData(spe)$subsets_mito_percent > 25
colData(spe)$qc_mito_25 <- qc_mito_25
plotSpotQC(spe, plot_type = "spot",
           annotate = "qc_mito_25")
```



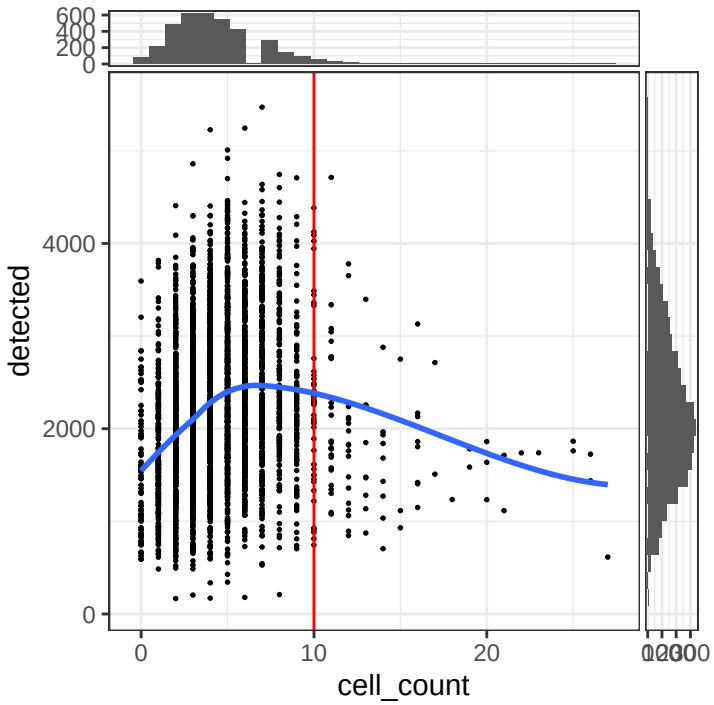
6.5.4 Number of cells per spot

```
# histogram of cell counts
hist(colData(spe)$cell_count, breaks = 20)
```



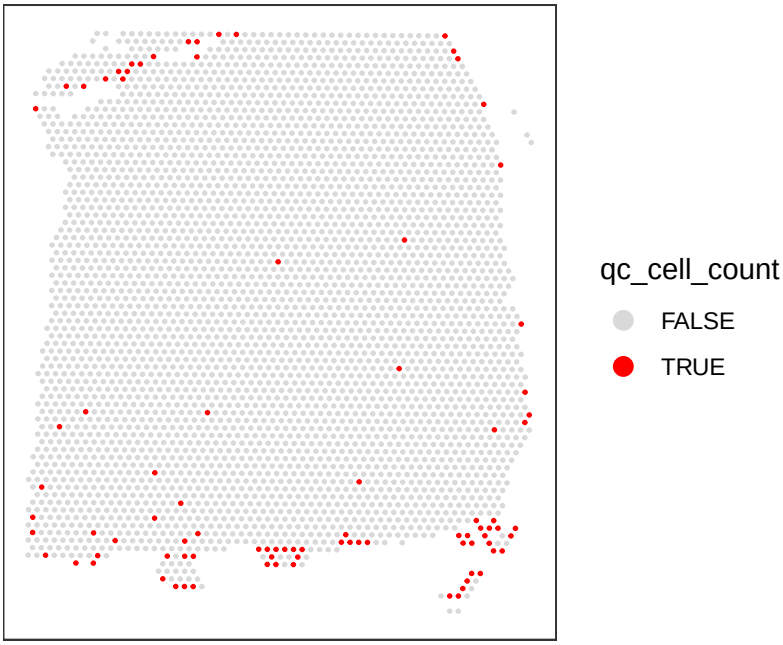

```
# distribution of cells per spot
tbl_cells_per_spot <- table(colData(spe)$cell_count)
```

```
# plot number of expressed genes vs. number of cells per spot
plotSpotQC(spe, plot_type = "scatter",
           x_metric = "cell_count", y_metric = "detected",
           x_threshold = 10)
##  `geom_smooth()` using formula = 'y ~ x'
##  `stat_xsidebin()` using `bins = 30`. Pick better value with `binwidth`.
##  `stat_ysidebin()` using `bins = 30`. Pick better value with `binwidth`.
```




```
# select QC threshold for number of cells per spot
qc_cell_count <- colData(spe)$cell_count > 10
table(qc_cell_count)
##  qc_cell_count
##  FALSE  TRUE
##   3549    90

colData(spe)$qc_cell_count <- qc_cell_count
```

```
# check spatial pattern of discarded spots
plotSpotQC(spe, plot_type = "spot",
           annotate = "qc_cell_count")
```

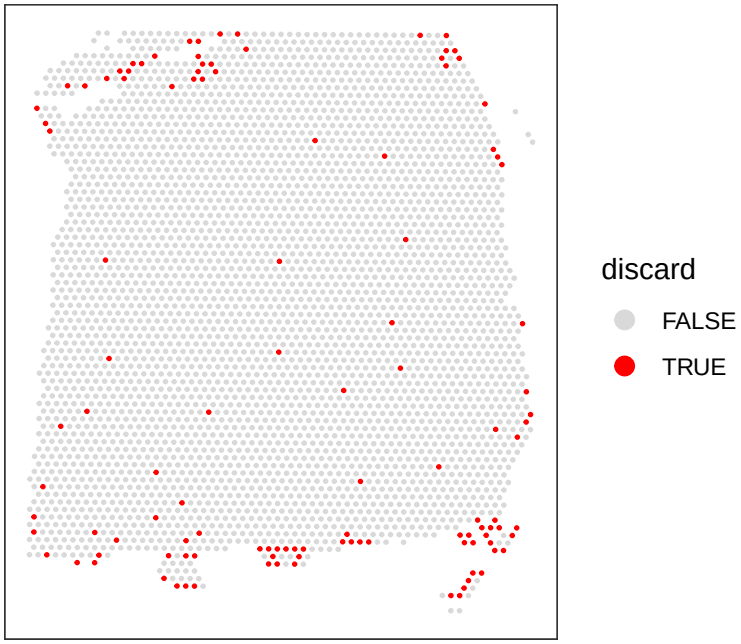


6.5.5 Remove low-quality spots

```
# number of discarded spots for each metric
apply(cbind(qc_lib_size, qc_detected, qc_mito, qc_cell_count), 2, sum)
##    qc_lib_size   qc_detected       qc_mito qc_cell_count
##              8             7            17            90

# combined set of discarded spots
discard <- qc_lib_size | qc_detected | qc_mito | qc_cell_count
table(discard)
##  discard
```

```
##  FALSE  TRUE
##   3524   115

# store in object
colData(spe)$discard <- discard
```

```
# check spatial pattern of combined set of discarded spots
plotSpotQC(spe, plot_type = "spot",
           annotate = "discard")
```




```
# remove combined set of low-quality spots
spe <- spe[, !colData(spe)$discard]
dim(spe)
##  [1] 33538  3524
```

6.6 Zero-cell and single-cell spots

```r
# distribution of cells per spot
tbl_cells_per_spot[1:13]
##
##   0   1   2   3   4   5   6   7   8   9  10  11  12
##  84 211 483 623 617 541 421 287 140  92  50  25  18

# as proportions
prop_cells_per_spot <- round(tbl_cells_per_spot / sum(tbl_cells_per_spot), 2)
prop_cells_per_spot[1:13]
##
##    0    1    2    3    4    5    6    7    8    9   10   11   12
## 0.02 0.06 0.13 0.17 0.17 0.15 0.12 0.08 0.04 0.03 0.01 0.01 0.00
```

6.7 Quality control at gene level

[8]

References

7 Normalization

7.1 Overview

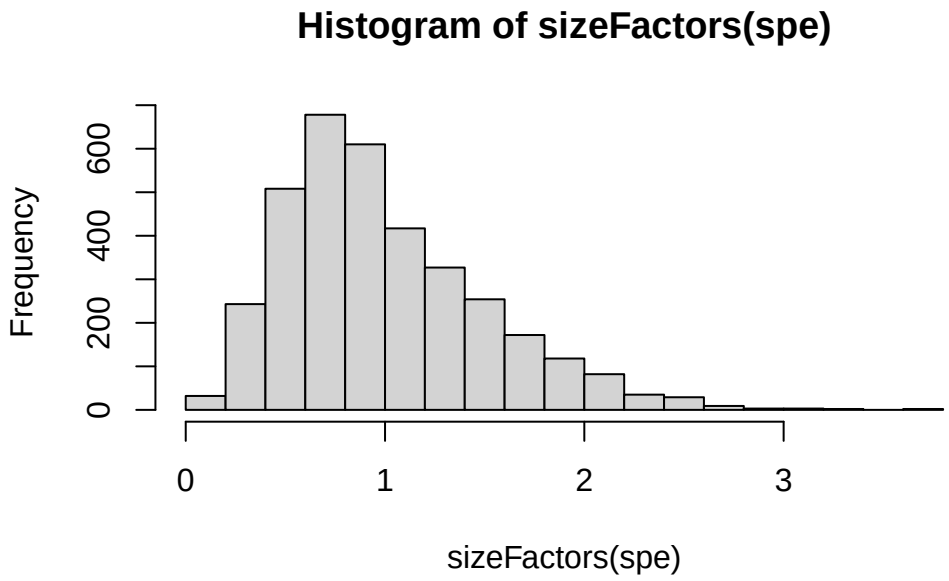
7.2 Load data from previous steps

4

```
library(SpatialExperiment)
spe <- readRDS("spe_qc.rds")
```

7.3 Logcounts

```
library(scran)

# calculate library size factors
spe <- computeLibraryFactors(spe)

summary(sizeFactors(spe))
##     Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##   0.1321  0.6312  0.9000  1.0000  1.2849  3.7582
hist(sizeFactors(spe), breaks = 20)
```




```
# calculate logcounts and store in object
spe <- logNormCounts(spe)

# check
assayNames(spe)
##  [1] "counts"    "logcounts"
dim(counts(spe))
##  [1] 33538  3524
dim(logcounts(spe))
##  [1] 33538  3524
```

References

8 Feature selection

8.1 Overview

8.2 Load data from previous steps

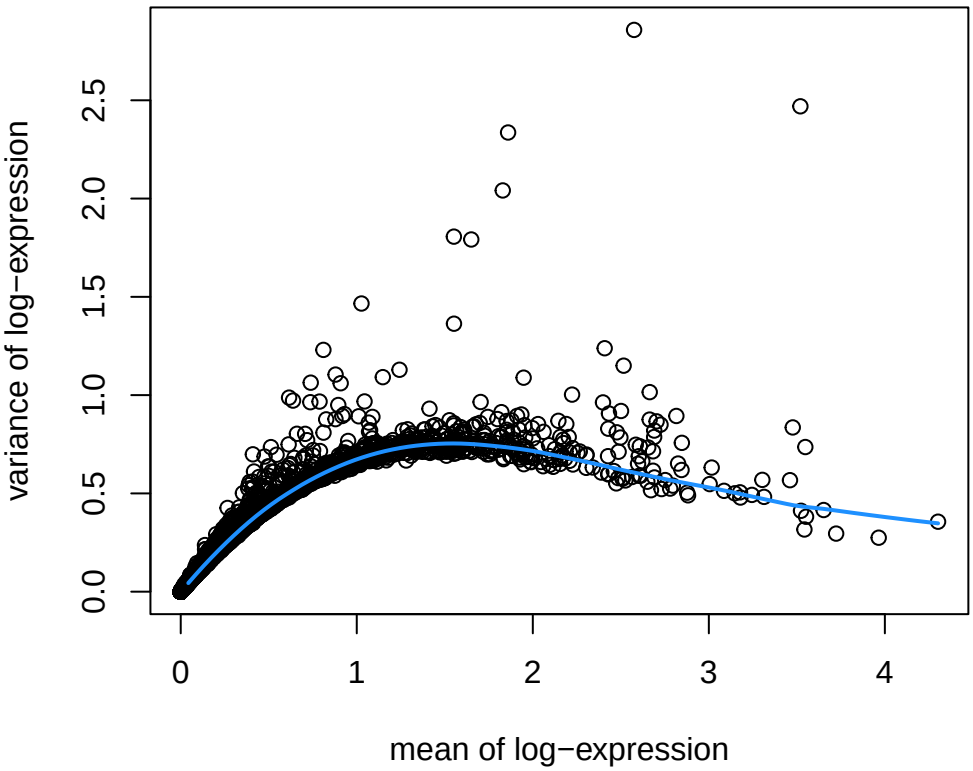
4

```
library(SpatialExperiment)
spe <- readRDS("spe_logcounts.rds")
```

8.3 Highly variable genes (HVGs)

```
# identify mitochondrial genes
is_mito <- grepl("(^MT-)|(^mt-)", rowData(spe)$gene_name)
table(is_mito)
##  is_mito
##  FALSE  TRUE
##  33525    13

# remove mitochondrial genes
spe <- spe[!is_mito, ]
dim(spe)
##  [1] 33525  3524
```

```
library(scran)

# fit mean-variance relationship
dec <- modelGeneVar(spe)

# visualize mean-variance relationship
fit <- metadata(dec)
plot(fit$mean, fit$var,
     xlab = "mean of log-expression", ylab = "variance of log-expression")
curve(fit$trend(x), col = "dodgerblue", add = TRUE, lwd = 2)
```




```
# select top HVGs
top_hvgs <- getTopHVGs(dec, prop = 0.1)
length(top_hvgs)
##  [1] 1438
```

8.4 Spatially variable genes (SVGs)

- Bioconductor
- GitHub
- GitHub
- GitHub

Moran's I Geary's C

8.4.1 nnSVG

nnSVG

```
library(nnSVG)
```

```
# subsample spots for faster runtime in this example
# note: skip this step in full analysis
n <- 100
set.seed(123)
ix <- sample(seq_len(n), n)
spe_nnSVG <- spe[, ix]

# filter low-expressed and mitochondrial genes
# using stringent filtering for faster runtime in this example
# note: use default filtering in full analysis
spe_nnSVG <- filter_genes(
  spe_nnSVG, filter_genes_ncounts = 10, filter_genes_pcspots = 3
)
##  Gene filtering: removing mitochondrial genes
##  removed 0 mitochondrial genes
##  Gene filtering: retaining genes with at least 10 counts in at least 3% (n = 3) of spatia
```

```
##  removed 33353 out of 33525 genes due to low expression

# re-calculate logcounts after filtering
spe_nnSVG <- logNormCounts(spe_nnSVG)
```

```
# run nnSVG
set.seed(123)
spe_nnSVG <- nnSVG(spe_nnSVG)
```

```
# investigate results

# show results
head(rowData(spe_nnSVG), 3)
##  DataFrame with 3 rows and 18 columns
##                          gene_id   gene_name    feature_type subsets_mito
##                      <character> <character>     <character>    <logical>
##  ENSG00000074800 ENSG00000074800        ENO1 Gene Expression        FALSE
##  ENSG00000171603 ENSG00000171603      CLSTN1 Gene Expression        FALSE
##  ENSG00000162545 ENSG00000162545     CAMK2N1 Gene Expression        FALSE
##                    sigma.sq    tau.sq       phi    loglik   runtime      mean
##                   <numeric> <numeric> <numeric> <numeric> <numeric> <numeric>
##  ENSG00000074800 0.00840777  0.518011  15.82454  -109.808     0.016   1.75820
##  ENSG00000171603 0.43102027  0.298698  18.12310  -123.212     0.019   2.07433
##  ENSG00000162545 0.15373440  0.547736   2.38046  -119.576     0.014   2.63576
##                       var     spcov   prop_sv loglik_lm   LR_stat      rank
##                   <numeric> <numeric> <numeric> <numeric> <numeric> <numeric>
##  ENSG00000074800  0.530929 0.0521523 0.0159716  -109.735 -0.145454       167
##  ENSG00000171603  0.741077 0.3164987 0.5906665  -126.409  6.394009        86
##  ENSG00000162545  0.702846 0.1487576 0.2191603  -123.760  8.368951        70
##                       pval      padj
##                   <numeric> <numeric>
##  ENSG00000074800 1.0000000 1.0000000
##  ENSG00000171603 0.0408845 0.0817690
##  ENSG00000162545 0.0152302 0.0374227

# number of significant SVGs
table(rowData(spe_nnSVG)$padj <= 0.05)
##
##  FALSE  TRUE
##     96    76

# show results for top n SVGs
```

```
rowData(spe_nnSVG)[order(rowData(spe_nnSVG)$rank)[1:6], ]
##  DataFrame with 6 rows and 18 columns
##                          gene_id   gene_name    feature_type subsets_mito
##                      <character> <character>     <character>    <logical>
##  ENSG00000197971 ENSG00000197971         MBP Gene Expression        FALSE
##  ENSG00000123560 ENSG00000123560        PLP1 Gene Expression        FALSE
##  ENSG00000109846 ENSG00000109846       CRYAB Gene Expression        FALSE
##  ENSG00000173786 ENSG00000173786         CNP Gene Expression        FALSE
##  ENSG00000131095 ENSG00000131095        GFAP Gene Expression        FALSE
##  ENSG00000160307 ENSG00000160307       S100B Gene Expression        FALSE
##                   sigma.sq    tau.sq       phi    loglik   runtime      mean
##                  <numeric> <numeric> <numeric> <numeric> <numeric> <numeric>
##  ENSG00000197971   3.92430  0.175336   0.93014  -118.372     0.019   3.78073
##  ENSG00000123560   3.23544  0.461590   1.03983  -140.269     0.016   2.86143
##  ENSG00000109846   1.89968  0.281795   1.88555  -126.692     0.015   1.86058
##  ENSG00000173786   2.28793  0.402524   1.02675  -129.206     0.015   1.79558
##  ENSG00000131095   2.23709  0.461297   2.49083  -149.004     0.016   1.94543
##  ENSG00000160307   1.24179  0.155737   5.76279  -129.722     0.014   1.82695
##                       var     spcov   prop_sv loglik_lm   LR_stat      rank
##                  <numeric> <numeric> <numeric> <numeric> <numeric> <numeric>
##  ENSG00000197971   2.76535  0.523969  0.957231  -192.250  147.7556         1
##  ENSG00000123560   3.02555  0.628613  0.875146  -196.746  112.9549         2
##  ENSG00000109846   1.88123  0.740785  0.870824  -172.988   92.5906         3
##  ENSG00000173786   1.97274  0.842396  0.850388  -175.363   92.3137         4
##  ENSG00000131095   2.71281  0.768823  0.829047  -191.291   84.5739         5
##  ENSG00000160307   1.46946  0.609953  0.888562  -160.636   61.8281         6
##                         pval        padj
##                    <numeric>   <numeric>
##  ENSG00000197971 0.00000e+00 0.00000e+00
##  ENSG00000123560 0.00000e+00 0.00000e+00
##  ENSG00000109846 0.00000e+00 0.00000e+00
##  ENSG00000173786 0.00000e+00 0.00000e+00
##  ENSG00000131095 0.00000e+00 0.00000e+00
##  ENSG00000160307 3.75255e-14 1.07573e-12

# identify top-ranked SVG
rowData(spe_nnSVG)$gene_name[which(rowData(spe_nnSVG)$rank == 1)]
##  [1] "MBP"
```

8.4.2 Downstream analyses

10

References

9 Dimensionality reduction

9.1 Overview

9.2 Load data from previous steps

4

```
library(SpatialExperiment)
spe <- readRDS("spe_hvgs.rds")
top_hvgs <- readRDS("top_hvgs.rds")
```

9.3 Principal component analysis (PCA)

```
library(scater)
```

```r
# compute PCA
set.seed(123)
spe <- runPCA(spe, subset_row = top_hvgs)

reducedDimNames(spe)
##  [1] "PCA"
dim(reducedDim(spe, "PCA"))
##  [1] 3524   50
```

9.4 Uniform Manifold Approximation and Projection (UMAP)

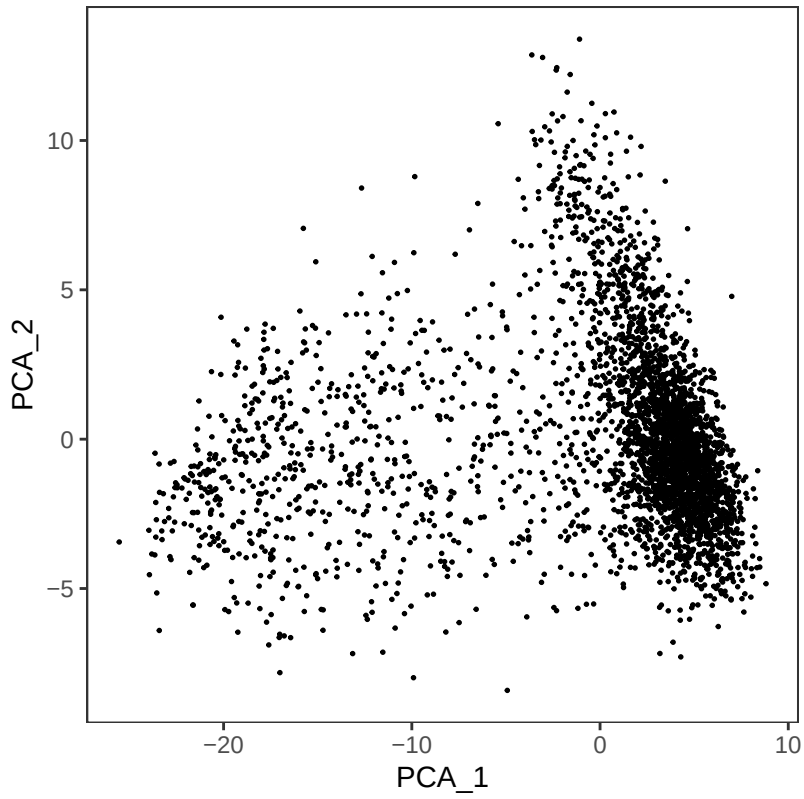
```r
# compute UMAP on top 50 PCs
set.seed(123)
spe <- runUMAP(spe, dimred = "PCA")

reducedDimNames(spe)
##  [1] "PCA"  "UMAP"
dim(reducedDim(spe, "UMAP"))
##  [1] 3524    2

# update column names for easier plotting
colnames(reducedDim(spe, "UMAP")) <- paste0("UMAP", 1:2)
```

9.5 Visualizations

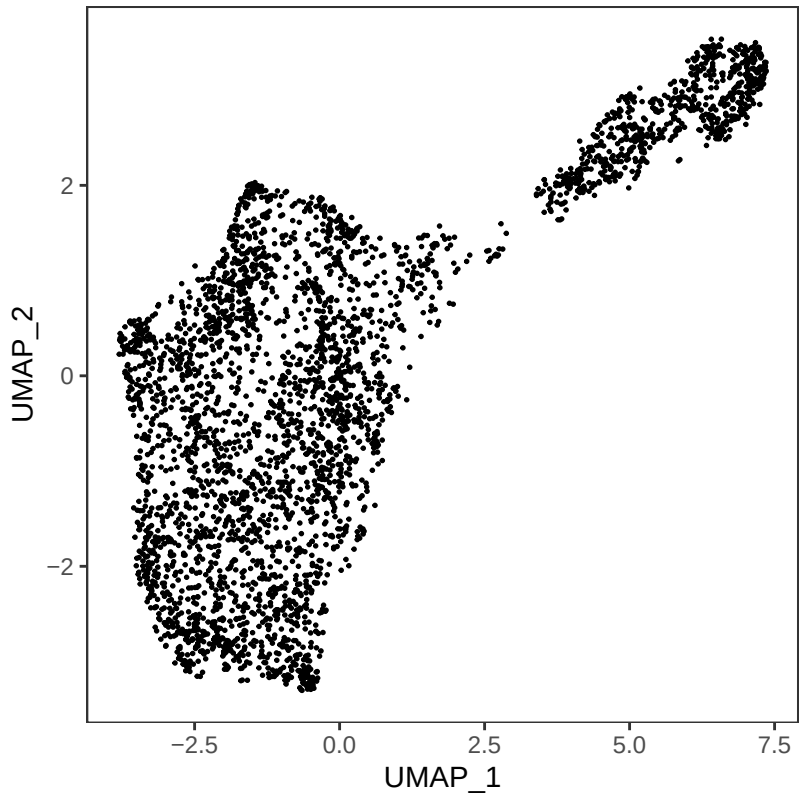
ggspavis

```r
library(ggspavis)
```

```r
# plot top 2 PCA dimensions
plotDimRed(spe, plot_type = "PCA")
```




```
# plot top 2 UMAP dimensions
plotDimRed(spe, plot_type = "UMAP")
```



References

10 Clustering

10.1 Overview

10.2 Load data from previous steps

4

```
library(SpatialExperiment)
spe <- readRDS("spe_reduceddims.rds")
spe_full <- readRDS("spe_logcounts.rds")
```

10.3 Non-spatial clustering

10.3.1 Clustering using HVGs

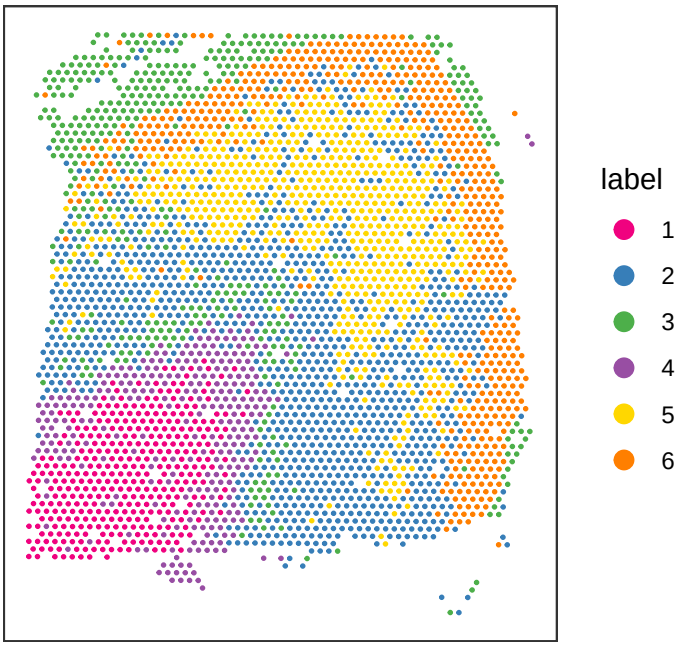
```
library(scran)
```

```
# graph-based clustering
set.seed(123)
k <- 10
g <- buildSNNGraph(spe, k = k, use.dimred = "PCA")
g_walk <- igraph::cluster_walktrap(g)
clus <- g_walk$membership
table(clus)
##  clus
##    1    2    3    4    5    6
##  359 1187  447  291  693  547

# store cluster labels in column 'label' in colData
colLabels(spe) <- factor(clus)
```

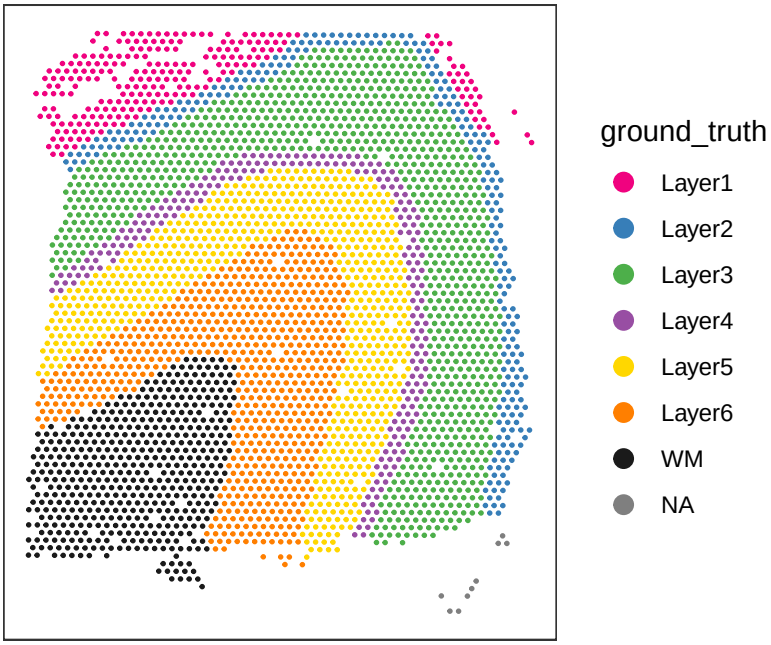
ggspavis

```
library(ggspavis)
```

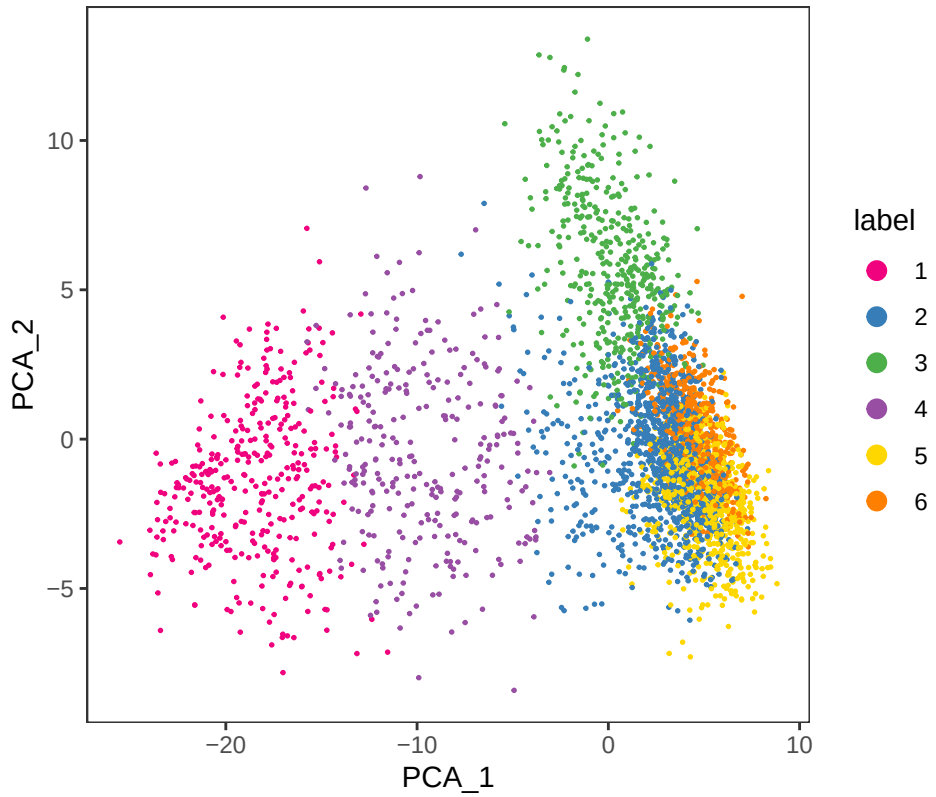
```
# plot clusters in spatial x-y coordinates
plotSpots(spe, annotate = "label",
          pal = "libd_layer_colors")
```



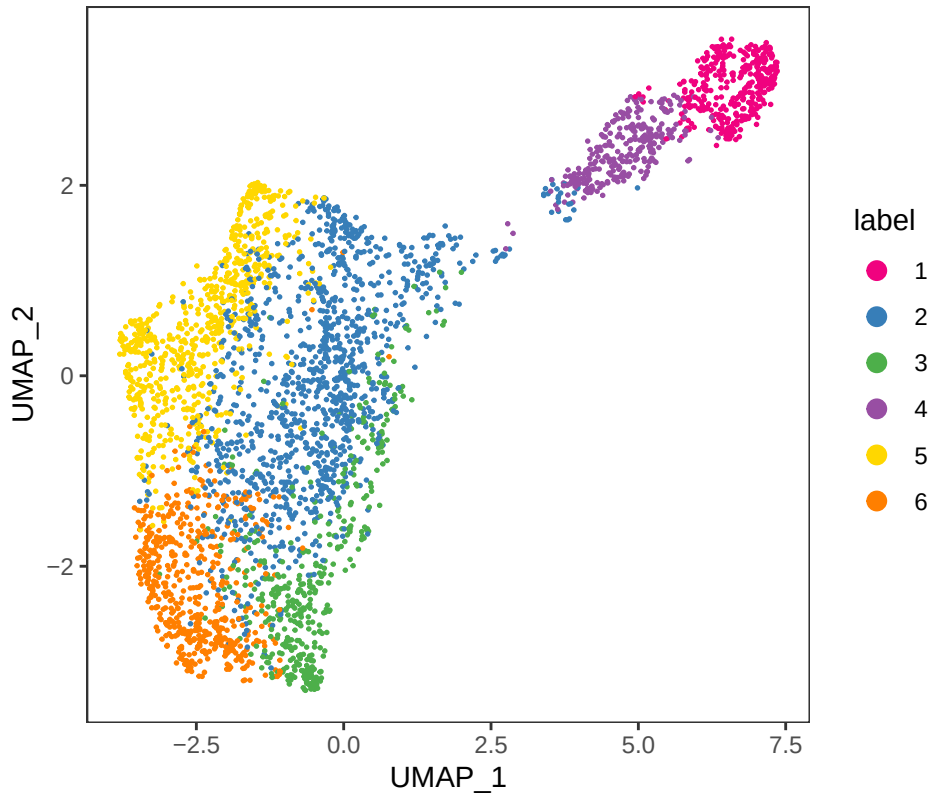

```
# plot ground truth labels in spatial coordinates
plotSpots(spe, annotate = "ground_truth",
          pal = "libd_layer_colors")
```




```
# plot clusters in PCA reduced dimensions
plotDimRed(spe, plot_type = "PCA",
           annotate = "label", pal = "libd_layer_colors")
```




```
# plot clusters in UMAP reduced dimensions
plotDimRed(spe, plot_type = "UMAP",
           annotate = "label", pal = "libd_layer_colors")
```



10.4 Spatially-aware clustering

10.4.1 Clustering using SVGs

8

nnSVG

8

```
library(nnSVG)
```

```
# subsample spots for faster runtime in this example
# note: skip this step in full analysis
n <- 100
set.seed(123)
ix <- sample(seq_len(n), n)
spe_nnSVG <- spe_full[, ix]  ## note: using full object from logcounts step

# filter low-expressed and mitochondrial genes
# using stringent filtering for faster runtime in this example
# note: use default filtering in full analysis
spe_nnSVG <- filter_genes(
  spe_nnSVG, filter_genes_ncounts = 10, filter_genes_pcspots = 3
)
##  Gene filtering: removing mitochondrial genes
##  removed 13 mitochondrial genes
##  Gene filtering: retaining genes with at least 10 counts in at least 3% (n = 3) of spatia
##  removed 33353 out of 33525 genes due to low expression

# re-calculate logcounts after filtering
spe_nnSVG <- logNormCounts(spe_nnSVG)
```

```
# run nnSVG
set.seed(123)
spe_nnSVG <- nnSVG(spe_nnSVG)
```

```
# select top SVGs
# note: using small subset in this example
# use larger set (e.g. top 1000 genes) in full analysis
n_top <- 50
```

```
ix_top <- order(rowData(spe_nnSVG)$rank)[1:n]
top_svgs <- rowData(spe_nnSVG)[ix_top, "gene_id"]
```

```
library(scater)
library(scran)
```

```
# dimensionality reduction
# compute PCA
# note: using small number of components in this example
# use larger number (e.g. ncomponents = 50) in full analysis
set.seed(123)
spe_nnSVG <- runPCA(spe_nnSVG, ncomponents = 10, subset_row = top_svgs)
```

```
# graph-based clustering
set.seed(123)
k <- 10
g <- buildSNNGraph(spe_nnSVG, k = k, use.dimred = "PCA")
g_walk <- igraph::cluster_walktrap(g)
clus <- g_walk$membership
table(clus)
##  clus
##   1  2  3  4  5
##   6  6 53 24 11

# store cluster labels in column 'label' in colData
colLabels(spe_nnSVG) <- factor(clus)
```

10.4.2 Clustering using concatenated features

10.4.3 Spatially-aware clustering algorithms

- Bioconductor
- version 3.9 onwards
- GitHub
- CRAN

References

11 Spot deconvolution

11.1 Overview

6 10

11.2 Previous steps

11.3 Load data from previous steps

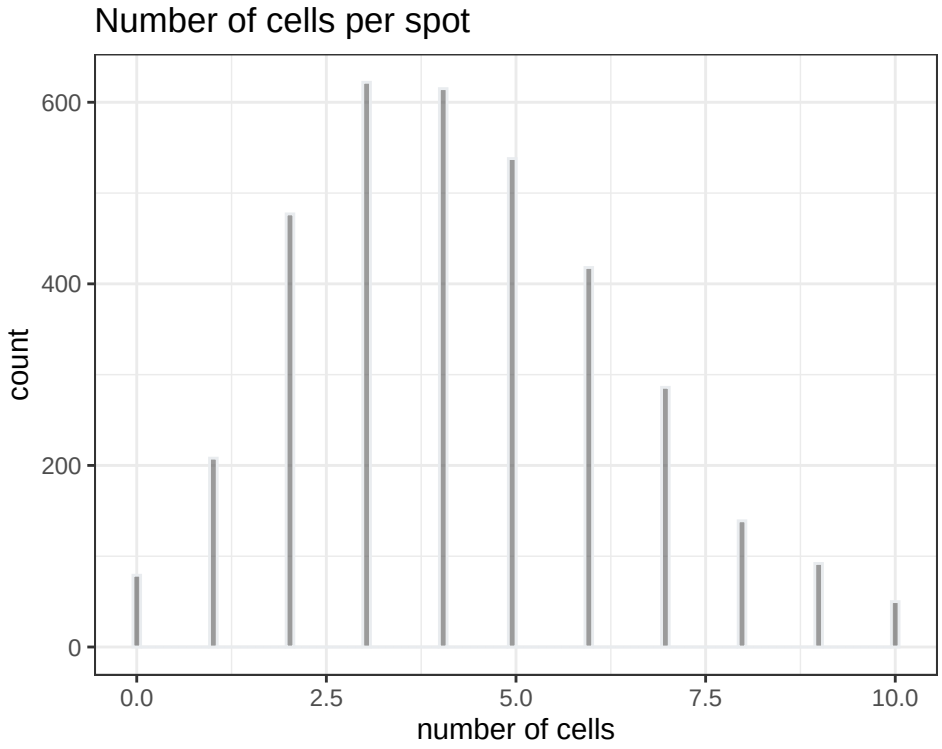
4

```
library(SpatialExperiment)
spe <- readRDS("spe_cluster.rds")
```

11.4 Number of cells per spot

ggspavis

```r
library(ggspavis)

# plot number of cells per spot
plotSpotQC(spe, plot_type = "histogram", x_metric = "cell_count") +
  xlab("number of cells") +
  ggtitle("Number of cells per spot")
```



References

12 Spatial registration

12.1 Overview

11

10

13 Differential expression

13.1 Overview

13.2 Load data from previous steps

4

```
library(SpatialExperiment)
spe <- readRDS("spe_cluster.rds")
```

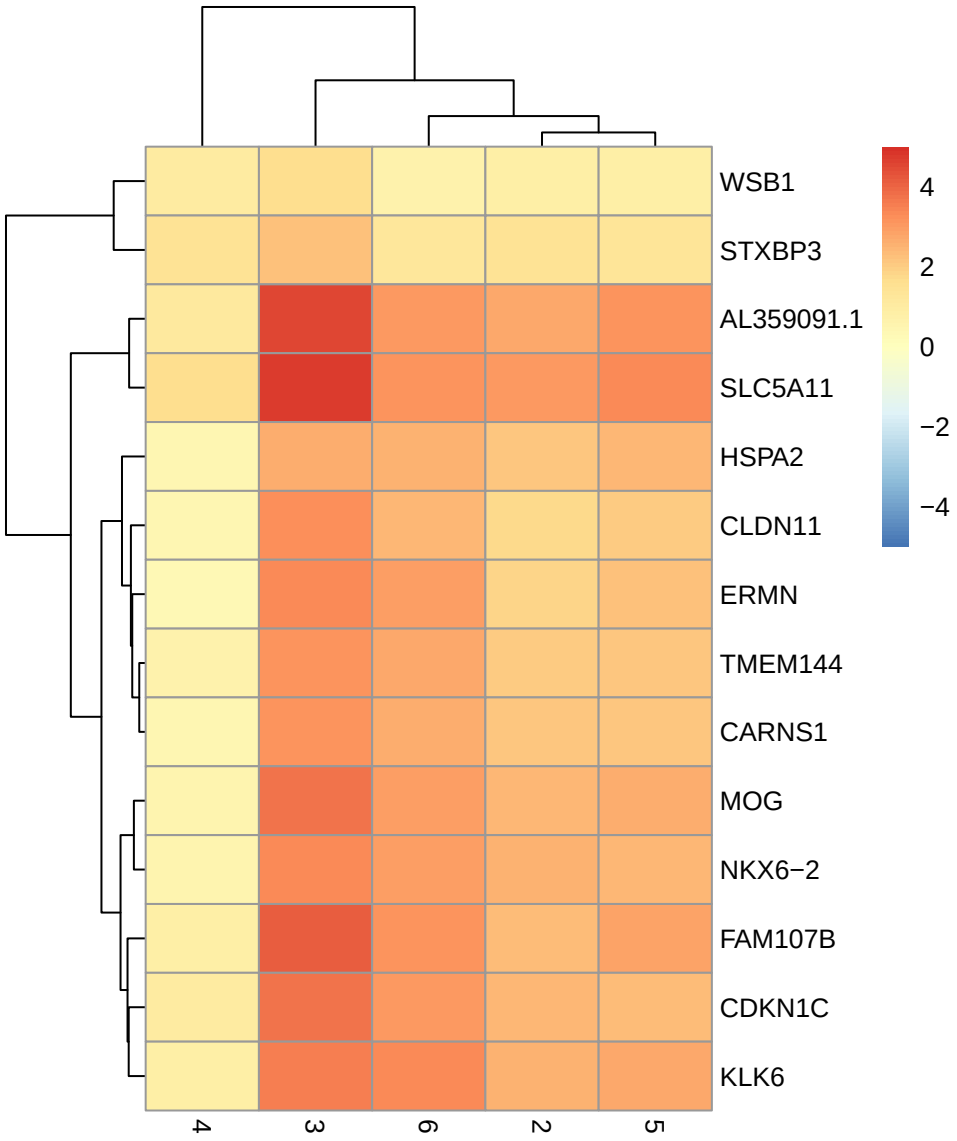
13.3 Differential expression testing

```
library(scran)
library(scater)
library(pheatmap)
```

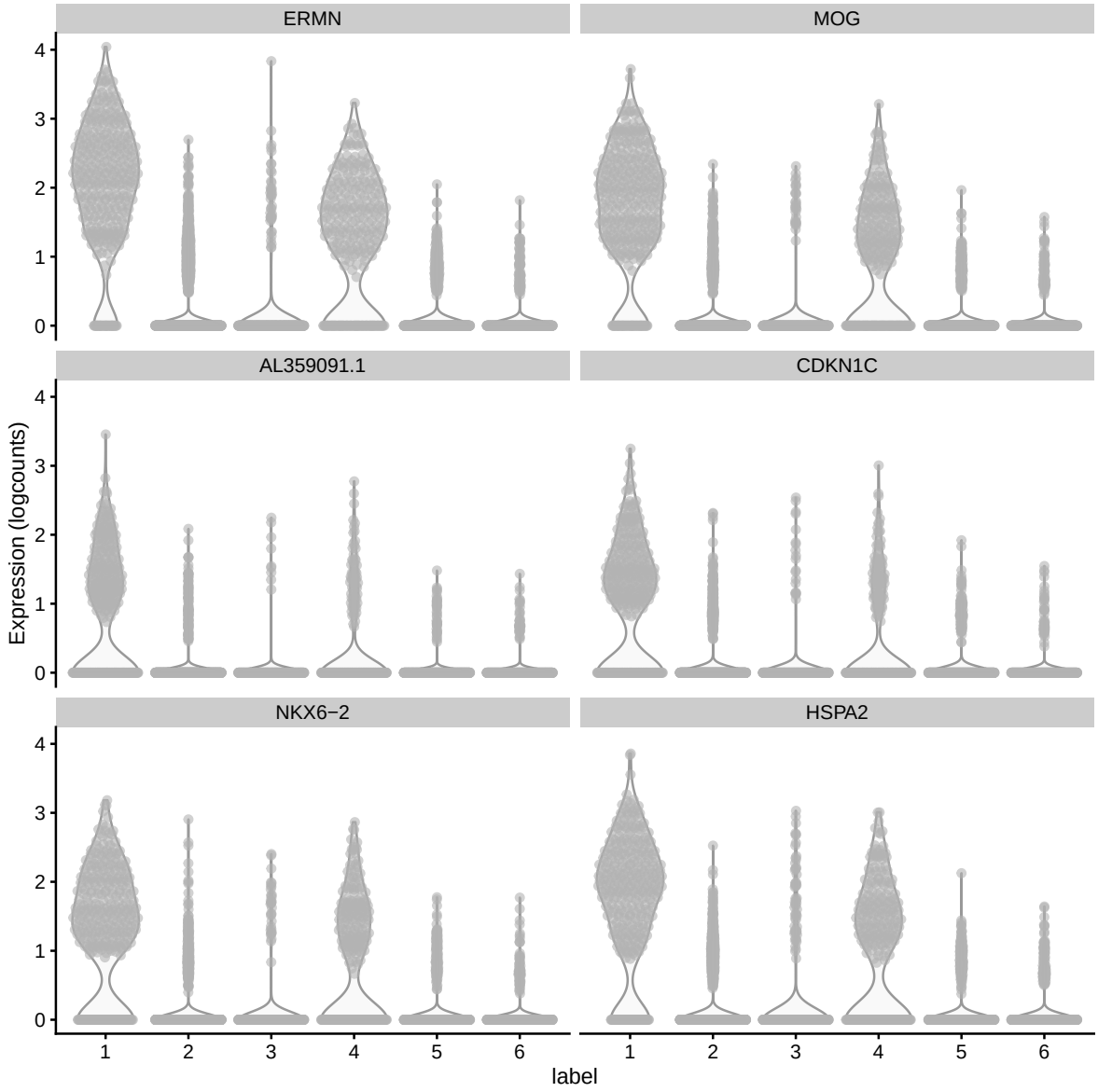
```
# set gene names as row names for easier plotting
rownames(spe) <- rowData(spe)$gene_name

# test for marker genes
```

```r
markers <- findMarkers(spe, test = "binom", direction = "up")

# returns a list with one DataFrame per cluster
markers
##  List of length 6
##  names(6): 1 2 3 4 5 6
```

```r
# plot log-fold changes for one cluster over all other clusters
# selecting cluster 1
interesting <- markers[[1]]
best_set <- interesting[interesting$Top <= 5, ]
logFCs <- getMarkerEffects(best_set)

pheatmap(logFCs, breaks = seq(-5, 5, length.out = 101))
```




```
# plot log-transformed normalized expression of top genes for one cluster
top_genes <- head(rownames(interesting))

plotExpression(spe, x = "label", features = top_genes)
```



13.4 Pseudobulking

References

14 Multiple samples

14.1 Overview

15 Spatial co-localization

15.1 Overview

15.2 Load data from previous steps

4

15.3 Spatial co-localization of cell types within a single sample

spicyR

References

Part III

Workflows

16 Workflows

4

17 Human DLPFC workflow

17.1 Overview

17.2 Description of dataset

spatialLIBD
spatialLIBD Shiny web app

17.3 Load data

STexampleData

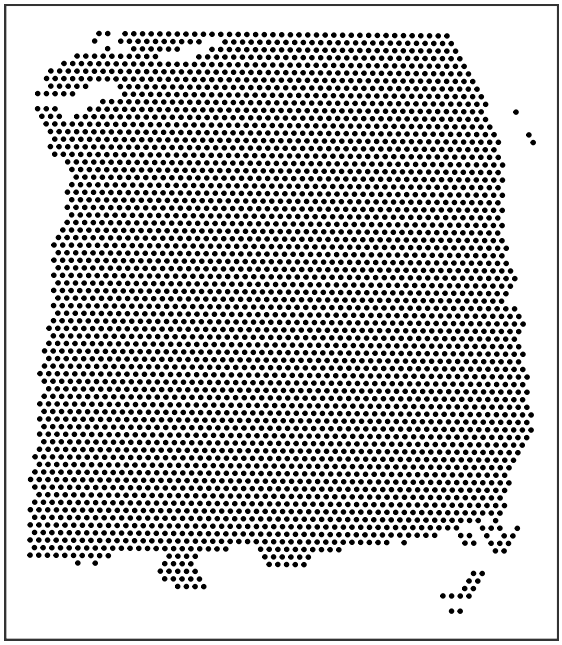
```
library(SpatialExperiment)
library(STexampleData)

# load object
spe <- Visium_humanDLPFC()
spe
##  class: SpatialExperiment
##  dim: 33538 4992
##  metadata(0):
##  assays(1): counts
##  rownames(33538): ENSG00000243485 ENSG00000237613 ... ENSG00000277475
##    ENSG00000268674
##  rowData names(3): gene_id gene_name feature_type
##  colnames(4992): AAACAACGAATAGTTC-1 AAACAAGTATCTCCCA-1 ...
##    TTGTTTGTATTACACG-1 TTGTTTGTGTAAATTC-1
##  colData names(8): barcode_id sample_id ... reference cell_count
##  reducedDimNames(0):
##  mainExpName: NULL
##  altExpNames(0):
##  spatialCoords names(2) : pxl_col_in_fullres pxl_row_in_fullres
##  imgData names(4): sample_id image_id data scaleFactor
```

17.4 Plot data

ggspavis

```
library(ggspavis)
```

```
# plot spatial coordinates (spots)
plotSpots(spe)
```



17.5 Quality control (QC)

```
# subset to keep only spots over tissue
spe <- spe[, colData(spe)$in_tissue == 1]
dim(spe)
##  [1] 33538  3639
```

6

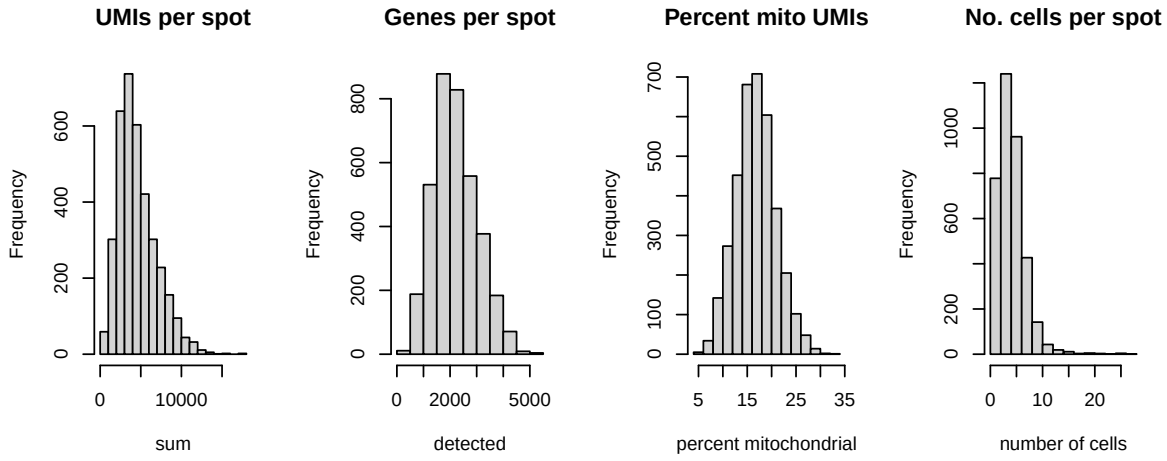
```
library(scater)
```

```
# identify mitochondrial genes
is_mito <- grepl("(^MT-)|(^mt-)", rowData(spe)$gene_name)
table(is_mito)
##  is_mito
##  FALSE  TRUE
```

```
##  33525    13
rowData(spe)$gene_name[is_mito]
##   [1] "MT-ND1"  "MT-ND2"  "MT-CO1"  "MT-CO2"  "MT-ATP8" "MT-ATP6" "MT-CO3"
##   [8] "MT-ND3"  "MT-ND4L" "MT-ND4"  "MT-ND5"  "MT-ND6"  "MT-CYB"

# calculate per-spot QC metrics and store in colData
spe <- addPerCellQC(spe, subsets = list(mito = is_mito))
head(colData(spe), 3)
##  DataFrame with 3 rows and 14 columns
##                            barcode_id     sample_id in_tissue array_row
##                           <character>   <character> <integer> <integer>
##  AAACAAGTATCTCCCA-1 AAACAAGTATCTCCCA-1 sample_151673         1        50
##  AAACAATCTACTAGCA-1 AAACAATCTACTAGCA-1 sample_151673         1         3
##  AAACACCAATAACTGC-1 AAACACCAATAACTGC-1 sample_151673         1        59
##                     array_col ground_truth   reference cell_count       sum
##                     <integer>  <character> <character>  <integer> <numeric>
##  AAACAAGTATCTCCCA-1       102       Layer3      Layer3          6      8458
##  AAACAATCTACTAGCA-1        43       Layer1      Layer1         16      1667
##  AAACACCAATAACTGC-1        19           WM          WM          5      3769
##                      detected subsets_mito_sum subsets_mito_detected
##                     <numeric>        <numeric>             <numeric>
##  AAACAAGTATCTCCCA-1      3586             1407                    13
##  AAACAATCTACTAGCA-1      1150              204                    11
##  AAACACCAATAACTGC-1      1960              430                    13
##                     subsets_mito_percent     total
##                                <numeric> <numeric>
##  AAACAAGTATCTCCCA-1              16.6351      8458
##  AAACAATCTACTAGCA-1              12.2376      1667
##  AAACACCAATAACTGC-1              11.4089      3769
```

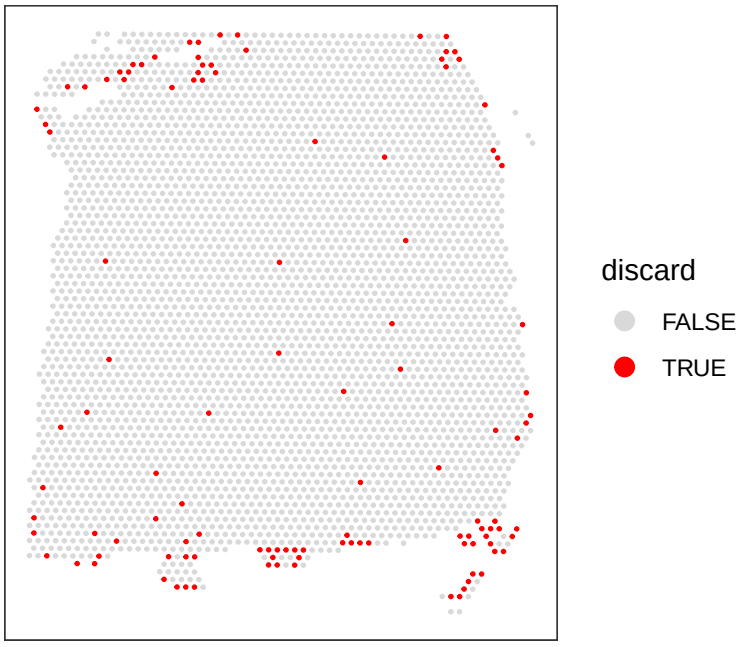
6

```
# histograms of QC metrics
par(mfrow = c(1, 4))
hist(colData(spe)$sum, xlab = "sum", main = "UMIs per spot")
hist(colData(spe)$detected, xlab = "detected", main = "Genes per spot")
hist(colData(spe)$subsets_mito_percent, xlab = "percent mitochondrial", main = "Percent mito
hist(colData(spe)$cell_count, xlab = "number of cells", main = "No. cells per spot")
```




```
par(mfrow = c(1, 1))

# select QC thresholds
qc_lib_size <- colData(spe)$sum < 600
qc_detected <- colData(spe)$detected < 400
qc_mito <- colData(spe)$subsets_mito_percent > 28
qc_cell_count <- colData(spe)$cell_count > 10

# number of discarded spots for each metric
apply(cbind(qc_lib_size, qc_detected, qc_mito, qc_cell_count), 2, sum)
##    qc_lib_size   qc_detected       qc_mito qc_cell_count
##              8             7            17            90

# combined set of discarded spots
discard <- qc_lib_size | qc_detected | qc_mito | qc_cell_count
table(discard)
##  discard
##  FALSE  TRUE
##   3524   115

# store in object
colData(spe)$discard <- discard
```

```
# check spatial pattern of discarded spots
plotSpotQC(spe, plot_type = "spot", annotate = "discard")
```



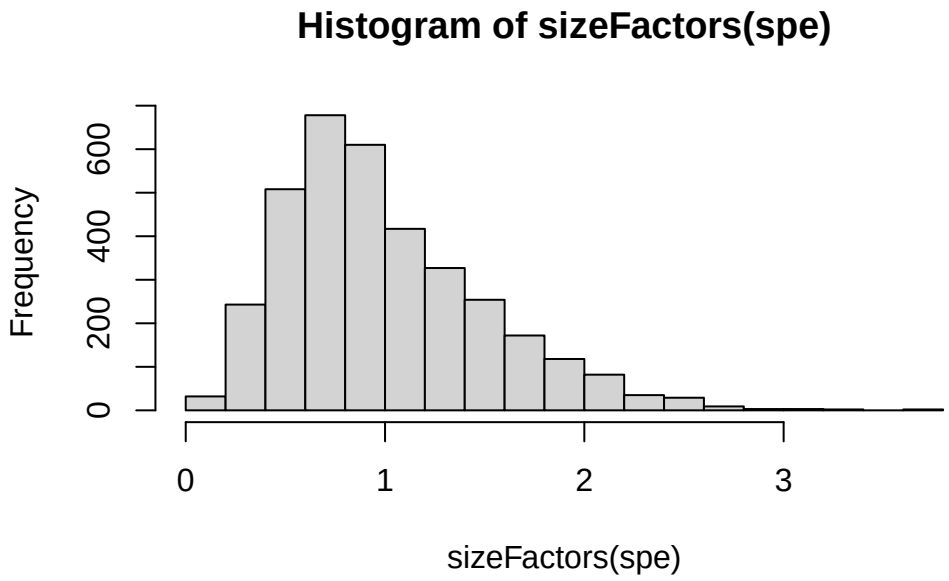

```
# filter low-quality spots
spe <- spe[, !colData(spe)$discard]
dim(spe)
##  [1] 33538  3524
```

17.6 Normalization

7

```
library(scran)
```

```
# calculate library size factors
spe <- computeLibraryFactors(spe)

summary(sizeFactors(spe))
##     Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##   0.1321  0.6312  0.9000  1.0000  1.2849  3.7582
hist(sizeFactors(spe), breaks = 20)
```



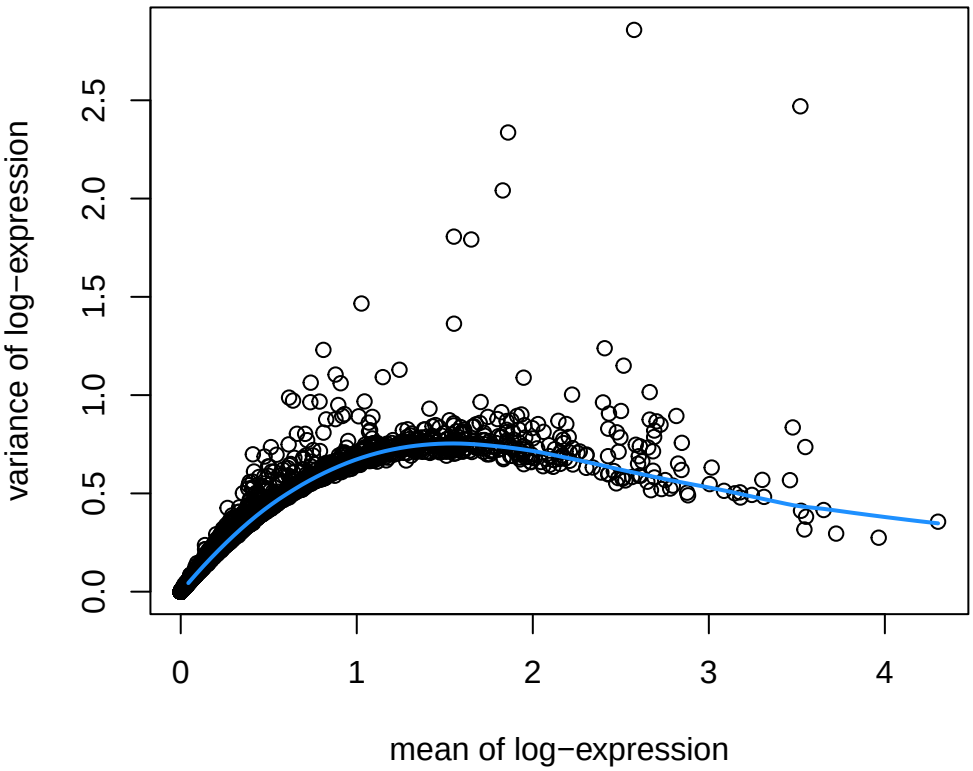

```
# calculate logcounts and store in object
spe <- logNormCounts(spe)

assayNames(spe)
##  [1] "counts"    "logcounts"
```

17.7 Feature selection

8

```
# remove mitochondrial genes
spe <- spe[!is_mito, ]
dim(spe)
##  [1] 33525  3524
```

```
# fit mean-variance relationship
dec <- modelGeneVar(spe)

# visualize mean-variance relationship
fit <- metadata(dec)
plot(fit$mean, fit$var,
     xlab = "mean of log-expression", ylab = "variance of log-expression")
curve(fit$trend(x), col = "dodgerblue", add = TRUE, lwd = 2)
```




```
# select top HVGs
top_hvgs <- getTopHVGs(dec, prop = 0.1)
length(top_hvgs)
##  [1] 1438
```

17.8 Spatially-aware feature selection

nnSVG

```
library(nnSVG)
```

```
# subsample spots
n <- 100
set.seed(123)
ix <- sample(seq_len(n), n)

spe_nnSVG <- spe[, ix]

# filter low-expressed and mitochondrial genes
# using very stringent filtering parameters for faster runtime in this example
# note: for a full analysis, use alternative filtering parameters (e.g. defaults)
spe_nnSVG <- filter_genes(
  spe_nnSVG, filter_genes_ncounts = 10, filter_genes_pcspots = 3
)
##  Gene filtering: removing mitochondrial genes
##  removed 0 mitochondrial genes
##  Gene filtering: retaining genes with at least 10 counts in at least 3% (n = 3) of spatia
##  removed 33353 out of 33525 genes due to low expression

# re-calculate logcounts after filtering
# using library size factors
spe_nnSVG <- logNormCounts(spe_nnSVG)

# run nnSVG
# using a single core for compatibility on build system
# note: for a full analysis, use multiple cores
set.seed(123)
spe_nnSVG <- nnSVG(spe_nnSVG, n_threads = 1)

# investigate results

# show results
head(rowData(spe_nnSVG), 3)
##  DataFrame with 3 rows and 18 columns
##                          gene_id   gene_name    feature_type subsets_mito
##                      <character> <character>     <character>    <logical>
##  ENSG00000074800 ENSG00000074800        ENO1 Gene Expression        FALSE
##  ENSG00000171603 ENSG00000171603      CLSTN1 Gene Expression        FALSE
##  ENSG00000162545 ENSG00000162545     CAMK2N1 Gene Expression        FALSE
```

```
##                     sigma.sq    tau.sq       phi    loglik   runtime      mean
##                    <numeric> <numeric> <numeric> <numeric> <numeric> <numeric>
##  ENSG00000074800 0.00840777  0.518011  15.82454  -109.808     0.016   1.75820
##  ENSG00000171603 0.43102027  0.298698  18.12310  -123.212     0.018   2.07433
##  ENSG00000162545 0.15373440  0.547736   2.38046  -119.576     0.013   2.63576
##                         var     spcov   prop_sv loglik_lm   LR_stat      rank
##                    <numeric> <numeric> <numeric> <numeric> <numeric> <numeric>
##  ENSG00000074800  0.530929 0.0521523 0.0159716  -109.735 -0.145454       167
##  ENSG00000171603  0.741077 0.3164987 0.5906665  -126.409  6.394009        86
##  ENSG00000162545  0.702846 0.1487576 0.2191603  -123.760  8.368951        70
##                        pval      padj
##                    <numeric> <numeric>
##  ENSG00000074800 1.0000000 1.0000000
##  ENSG00000171603 0.0408845 0.0817690
##  ENSG00000162545 0.0152302 0.0374227

# number of significant SVGs
table(rowData(spe_nnSVG)$padj <= 0.05)
##
##  FALSE  TRUE
##     96    76

# show results for top n SVGs
rowData(spe_nnSVG)[order(rowData(spe_nnSVG)$rank)[1:6], ]
##  DataFrame with 6 rows and 18 columns
##                          gene_id   gene_name    feature_type subsets_mito
##                      <character> <character>     <character>    <logical>
##  ENSG00000197971 ENSG00000197971         MBP Gene Expression        FALSE
##  ENSG00000123560 ENSG00000123560        PLP1 Gene Expression        FALSE
##  ENSG00000109846 ENSG00000109846       CRYAB Gene Expression        FALSE
##  ENSG00000173786 ENSG00000173786         CNP Gene Expression        FALSE
##  ENSG00000131095 ENSG00000131095        GFAP Gene Expression        FALSE
##  ENSG00000160307 ENSG00000160307       S100B Gene Expression        FALSE
##                     sigma.sq    tau.sq       phi    loglik   runtime      mean
##                    <numeric> <numeric> <numeric> <numeric> <numeric> <numeric>
##  ENSG00000197971   3.92430  0.175336   0.93014  -118.372     0.019   3.78073
##  ENSG00000123560   3.23544  0.461590   1.03983  -140.269     0.016   2.86143
##  ENSG00000109846   1.89968  0.281795   1.88555  -126.692     0.014   1.86058
##  ENSG00000173786   2.28793  0.402524   1.02675  -129.206     0.015   1.79558
##  ENSG00000131095   2.23709  0.461297   2.49083  -149.004     0.021   1.94543
##  ENSG00000160307   1.24179  0.155737   5.76279  -129.722     0.013   1.82695
##                         var     spcov   prop_sv loglik_lm   LR_stat      rank
```

```
##                  <numeric> <numeric> <numeric> <numeric> <numeric> <numeric>
##  ENSG00000197971   2.76535  0.523969  0.957231  -192.250  147.7556         1
##  ENSG00000123560   3.02555  0.628613  0.875146  -196.746  112.9549         2
##  ENSG00000109846   1.88123  0.740785  0.870824  -172.988   92.5906         3
##  ENSG00000173786   1.97274  0.842396  0.850388  -175.363   92.3137         4
##  ENSG00000131095   2.71281  0.768823  0.829047  -191.291   84.5739         5
##  ENSG00000160307   1.46946  0.609953  0.888562  -160.636   61.8281         6
##                       pval        padj
##                  <numeric>   <numeric>
##  ENSG00000197971 0.00000e+00 0.00000e+00
##  ENSG00000123560 0.00000e+00 0.00000e+00
##  ENSG00000109846 0.00000e+00 0.00000e+00
##  ENSG00000173786 0.00000e+00 0.00000e+00
##  ENSG00000131095 0.00000e+00 0.00000e+00
##  ENSG00000160307 3.75255e-14 1.07573e-12

# identify top-ranked SVG
rowData(spe_nnSVG)$gene_name[which(rowData(spe_nnSVG)$rank == 1)]
##  [1] "MBP"
```

17.9 Dimensionality reduction

```
# compute PCA
set.seed(123)
spe <- runPCA(spe, subset_row = top_hvgs)

reducedDimNames(spe)
##  [1] "PCA"
dim(reducedDim(spe, "PCA"))
##  [1] 3524   50
```

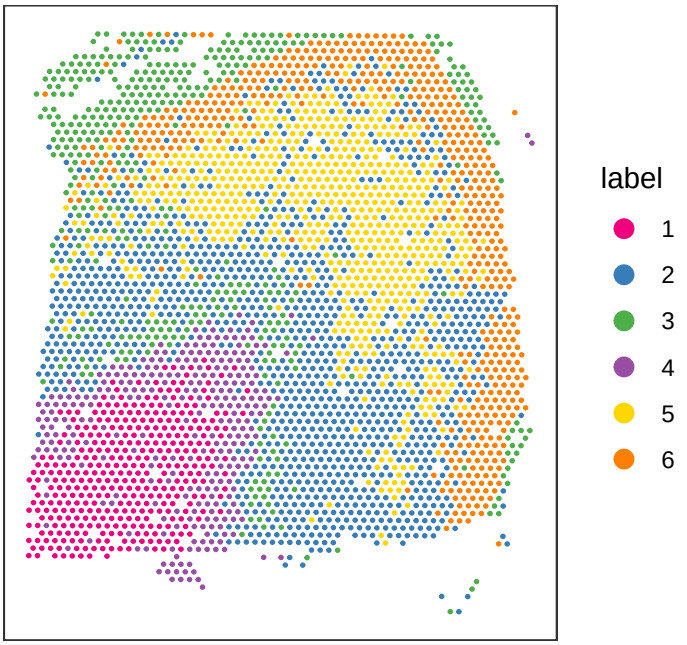
```
# compute UMAP on top 50 PCs
set.seed(123)
spe <- runUMAP(spe, dimred = "PCA")

reducedDimNames(spe)
##  [1] "PCA"  "UMAP"
dim(reducedDim(spe, "UMAP"))
##  [1] 3524    2

# update column names for easier plotting
colnames(reducedDim(spe, "UMAP")) <- paste0("UMAP", 1:2)
```

17.10 Clustering

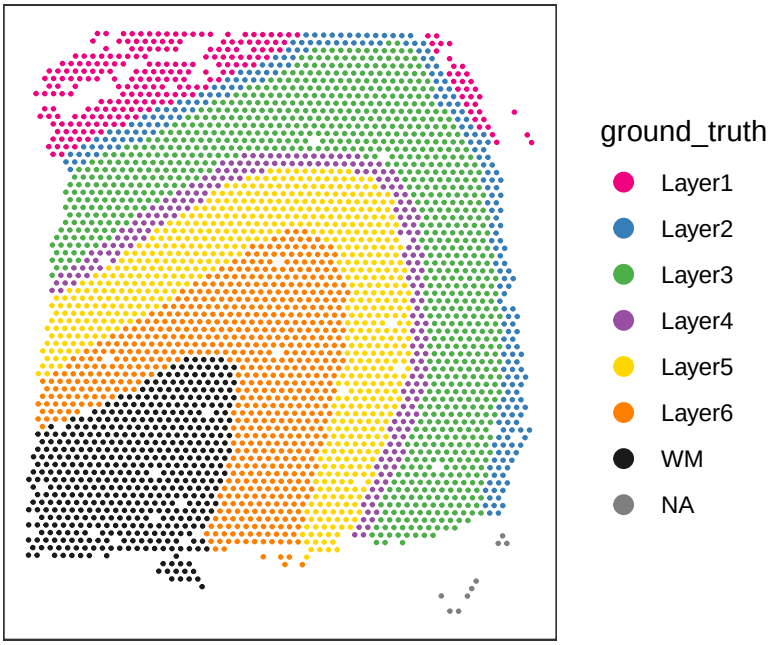
10

```
# graph-based clustering
set.seed(123)
k <- 10
g <- buildSNNGraph(spe, k = k, use.dimred = "PCA")
g_walk <- igraph::cluster_walktrap(g)
clus <- g_walk$membership
table(clus)
##  clus
##    1    2    3    4    5    6
##  359 1187  447  291  693  547

# store cluster labels in column 'label' in colData
colLabels(spe) <- factor(clus)
```

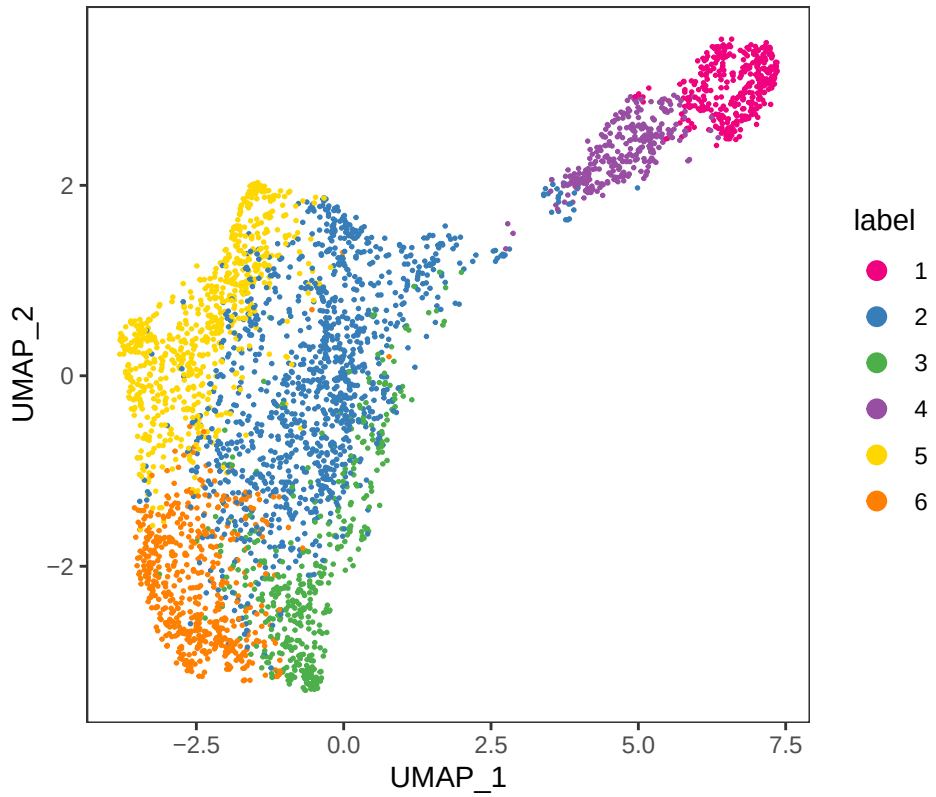
```
# plot clusters in spatial x-y coordinates
plotSpots(spe, annotate = "label",
          pal = "libd_layer_colors")
```




```
# plot ground truth labels in spatial coordinates
plotSpots(spe, annotate = "ground_truth",
          pal = "libd_layer_colors")
```




```
# plot clusters in UMAP reduced dimensions
plotDimRed(spe, plot_type = "UMAP",
           annotate = "label", pal = "libd_layer_colors")
```

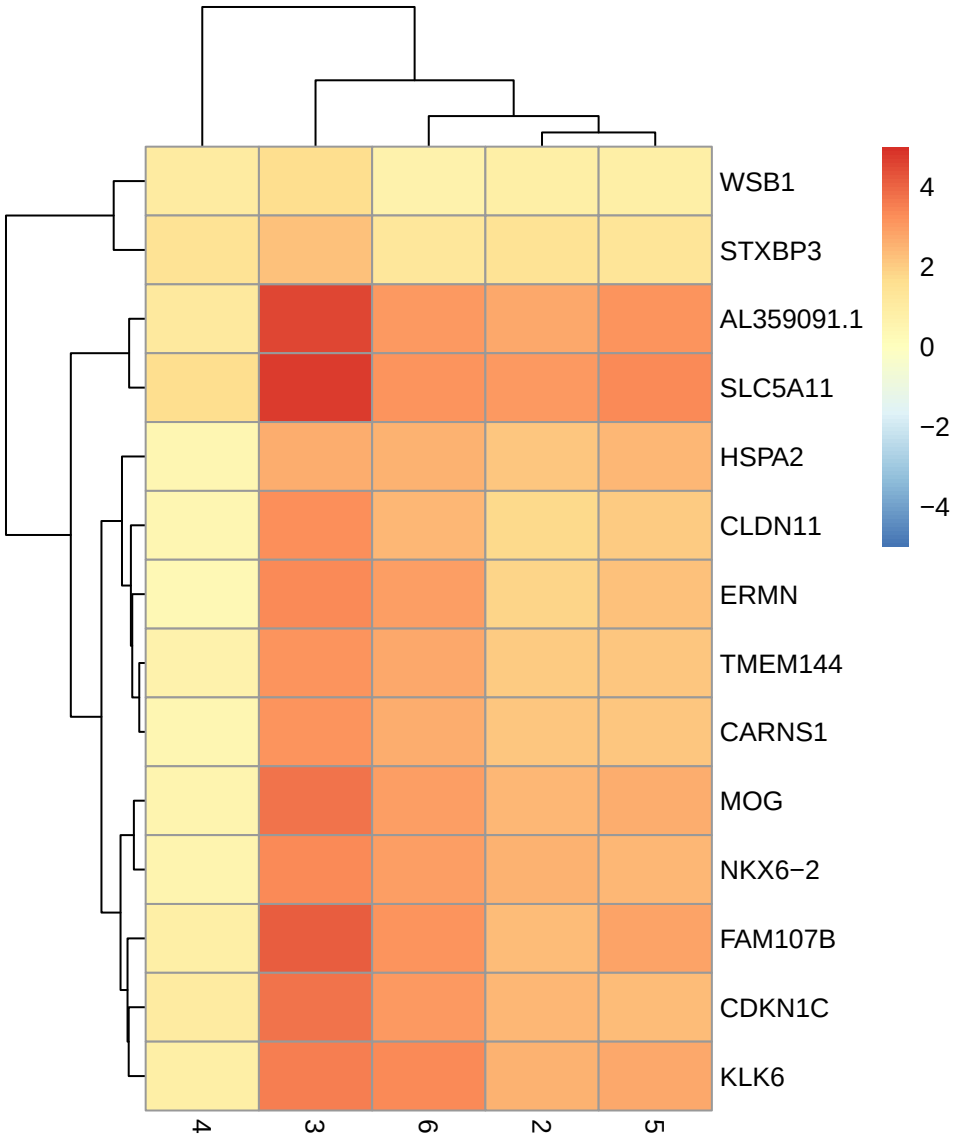


17.11 Differential expression

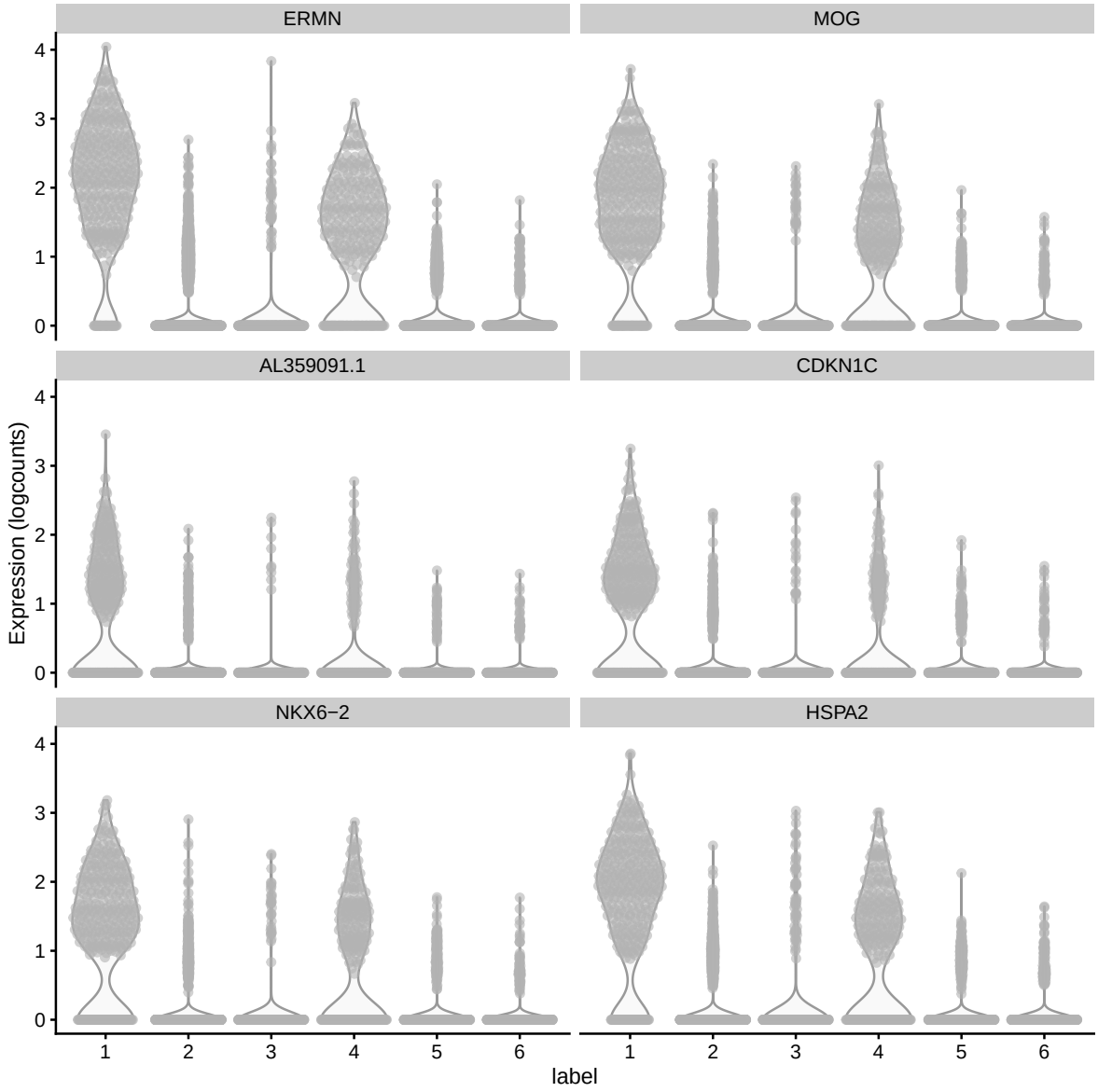
```
# set gene names as row names for easier plotting
rownames(spe) <- rowData(spe)$gene_name

# test for marker genes
markers <- findMarkers(spe, test = "binom", direction = "up")

# returns a list with one DataFrame per cluster
markers
##  List of length 6
##  names(6): 1 2 3 4 5 6
```

```
library(pheatmap)
```

```
# plot log-fold changes for one cluster over all other clusters
# selecting cluster 1
interesting <- markers[[1]]
best_set <- interesting[interesting$Top <= 5, ]
logFCs <- getMarkerEffects(best_set)

pheatmap(logFCs, breaks = seq(-5, 5, length.out = 101))
```




```
# plot log-transformed normalized expression of top genes for one cluster
top_genes <- head(rownames(interesting))

plotExpression(spe, x = "label", features = top_genes)
```


ERMN
MOG
AL359091.1
CDKN1C
NKX6−2
HSPA2
Expression (logcounts)
label
0
1
2
3
4
5
6

References

18 Mouse coronal workflow

18.1 Overview

10x Genomics website

18.2 Description of dataset

10x Genomics website

18.3 Load data

STexampleData

```
library(SpatialExperiment)
library(STexampleData)

# load object
spe <- Visium_mouseCoronal()
spe
##  class: SpatialExperiment
##  dim: 32285 4992
##  metadata(0):
##  assays(1): counts
```

```
##  rownames(32285): ENSMUSG00000051951 ENSMUSG00000089699 ...
##    ENSMUSG00000095019 ENSMUSG00000095041
##  rowData names(3): gene_id gene_name feature_type
##  colnames(4992): AAACAACGAATAGTTC-1 AAACAAGTATCTCCCA-1 ...
##    TTGTTTGTATTACACG-1 TTGTTTGTGTAAATTC-1
##  colData names(5): barcode_id sample_id in_tissue array_row array_col
##  reducedDimNames(0):
##  mainExpName: NULL
##  altExpNames(0):
##  spatialCoords names(2) : pxl_col_in_fullres pxl_row_in_fullres
##  imgData names(4): sample_id image_id data scaleFactor
```

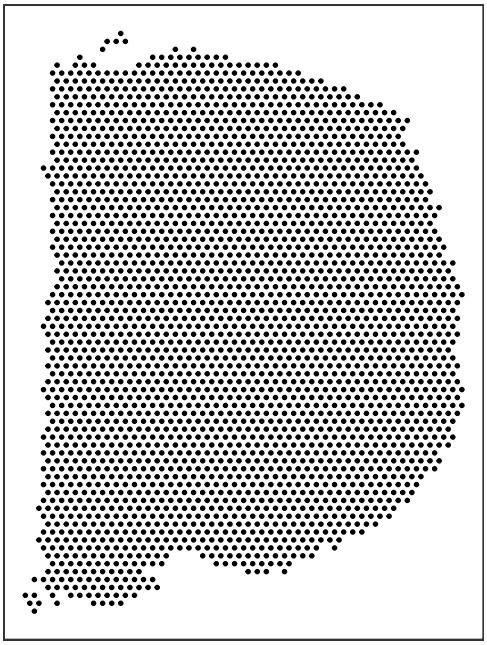
18.4 Plot data

10x Genomics website

ggspavis

```
library(ggspavis)
```

```
# plot spatial coordinates (spots)
plotSpots(spe)
```



18.5 Quality control (QC)

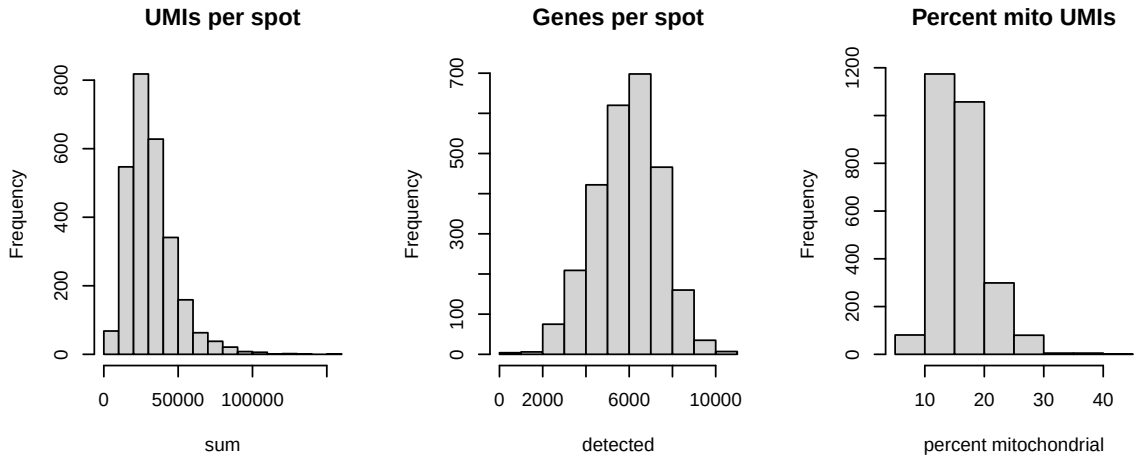
```
# subset to keep only spots over tissue
spe <- spe[, colData(spe)$in_tissue == 1]
dim(spe)
##  [1] 32285  2702
```

```
library(scater)
```

```
# identify mitochondrial genes
is_mito <- grepl("(^MT-)|(^mt-)", rowData(spe)$gene_name)
table(is_mito)
##  is_mito
##  FALSE  TRUE
##  32272    13
rowData(spe)$gene_name[is_mito]
```

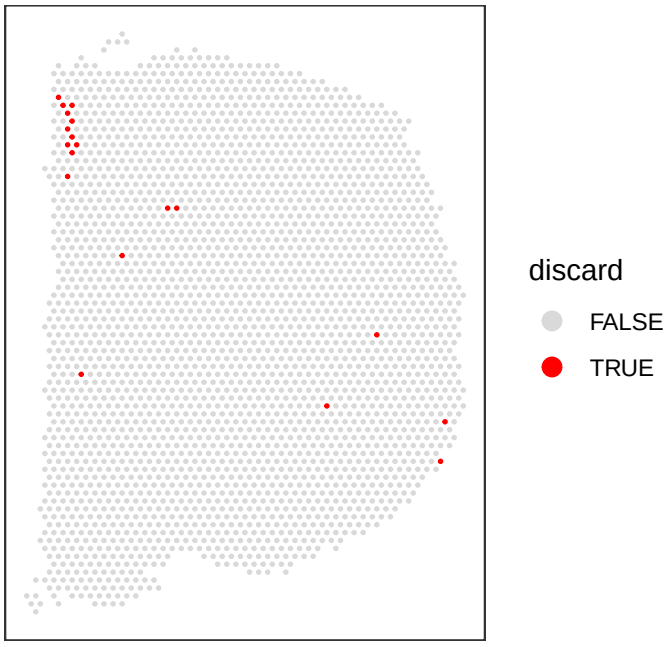
```
##   [1] "mt-Nd1"  "mt-Nd2"  "mt-Co1"  "mt-Co2"  "mt-Atp8" "mt-Atp6" "mt-Co3"
##   [8] "mt-Nd3"  "mt-Nd4l" "mt-Nd4"  "mt-Nd5"  "mt-Nd6"  "mt-Cytb"

# calculate per-spot QC metrics and store in colData
spe <- addPerCellQC(spe, subsets = list(mito = is_mito))
head(colData(spe), 3)
##  DataFrame with 3 rows and 11 columns
##                            barcode_id   sample_id in_tissue array_row
##                           <character> <character> <integer> <integer>
##  AAACAAGTATCTCCCA-1 AAACAAGTATCTCCCA-1    sample01         1        50
##  AAACAATCTACTAGCA-1 AAACAATCTACTAGCA-1    sample01         1         3
##  AAACACCAATAACTGC-1 AAACACCAATAACTGC-1    sample01         1        59
##                     array_col       sum  detected subsets_mito_sum
##                     <integer> <numeric> <numeric>        <numeric>
##  AAACAAGTATCTCCCA-1       102     20935      5230             4036
##  AAACAATCTACTAGCA-1        43     14789      3646             3419
##  AAACACCAATAACTGC-1        19     34646      6272             5068
##                     subsets_mito_detected subsets_mito_percent     total
##                                 <numeric>            <numeric> <numeric>
##  AAACAAGTATCTCCCA-1                    13              19.2787     20935
##  AAACAATCTACTAGCA-1                    13              23.1185     14789
##  AAACACCAATAACTGC-1                    13              14.6280     34646
```

```
# histograms of QC metrics
par(mfrow = c(1, 3))
hist(colData(spe)$sum, xlab = "sum", main = "UMIs per spot")
hist(colData(spe)$detected, xlab = "detected", main = "Genes per spot")
hist(colData(spe)$subsets_mito_percent, xlab = "percent mitochondrial", main = "Percent mito
```




```
par(mfrow = c(1, 1))

# select QC thresholds
qc_lib_size <- colData(spe)$sum < 5000
qc_detected <- colData(spe)$detected < 1000
qc_mito <- colData(spe)$subsets_mito_percent > 30

# number of discarded spots for each QC metric
apply(cbind(qc_lib_size, qc_detected, qc_mito), 2, sum)
##  qc_lib_size qc_detected     qc_mito
##            9           4          11

# combined set of discarded spots
discard <- qc_lib_size | qc_detected | qc_mito
table(discard)
##  discard
##  FALSE  TRUE
##   2683    19

# store in object
colData(spe)$discard <- discard
```

```
# check spatial pattern of discarded spots
plotSpotQC(spe, plot_type = "spot", annotate = "discard")
```

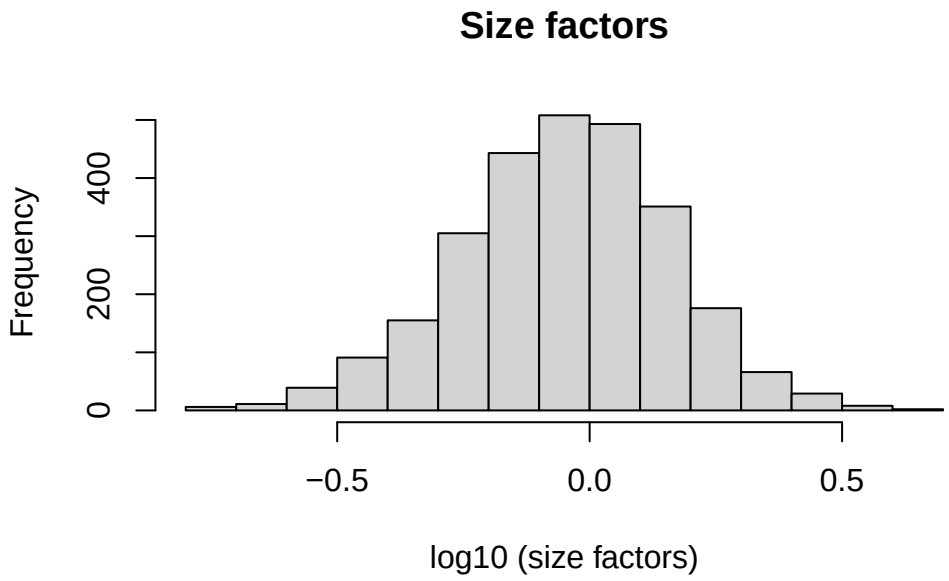



```
# filter low-quality spots
spe <- spe[, !colData(spe)$discard]
dim(spe)
##  [1] 32285  2683
```

18.6 Normalization

```
library(scran)
```

```
# calculate library size factors
spe <- computeLibraryFactors(spe)

summary(sizeFactors(spe))
##     Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##   0.1615  0.6597  0.9083  1.0000  1.2342  4.8973
hist(log10(sizeFactors(spe)), xlab = "log10 (size factors)", main = "Size factors")
```

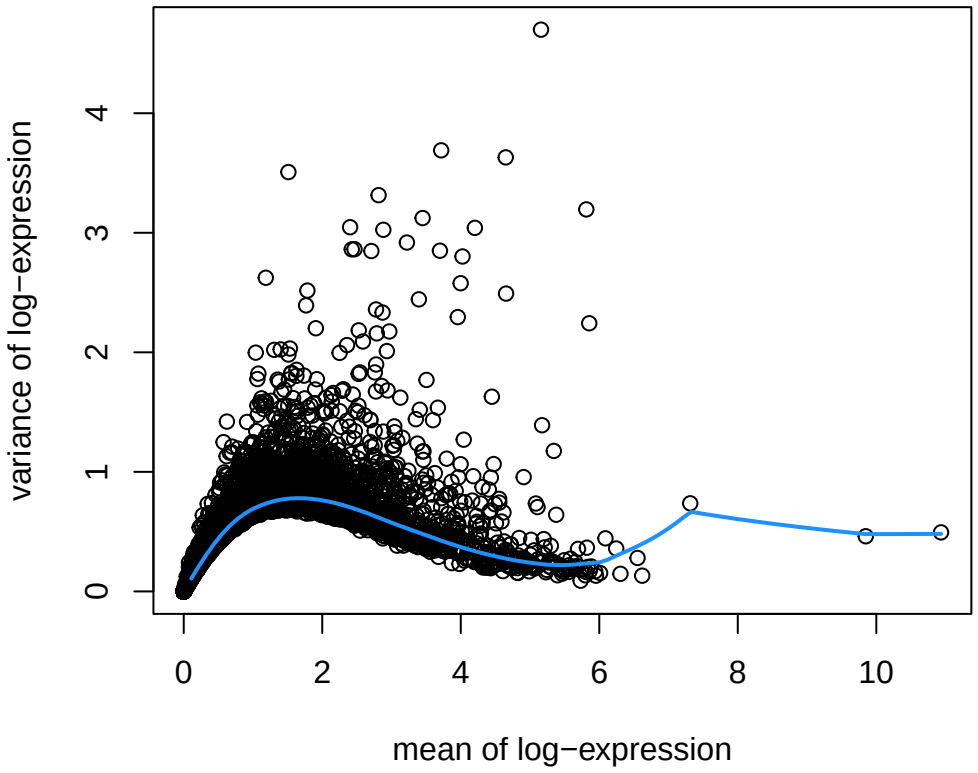



```
# calculate logcounts and store in object
spe <- logNormCounts(spe)

assayNames(spe)
##  [1] "counts"    "logcounts"
```

18.7 Feature selection

```
# remove mitochondrial genes
spe <- spe[!is_mito, ]
dim(spe)
##  [1] 32272  2683
```

```
# fit mean-variance relationship
dec <- modelGeneVar(spe)

# visualize mean-variance relationship
fit <- metadata(dec)
plot(fit$mean, fit$var,
     xlab = "mean of log-expression", ylab = "variance of log-expression")
curve(fit$trend(x), col = "dodgerblue", add = TRUE, lwd = 2)
```



```
# select top HVGs
top_hvgs <- getTopHVGs(dec, prop = 0.1)
length(top_hvgs)
##  [1] 1274
```

```
# identify outlier genes
rev(sort(fit$mean))[1:3]
##  ENSMUSG00000115783 ENSMUSG00000098178 ENSMUSG00000024661
##           10.934946           9.847532           7.313407
outlier_ids <- names(rev(sort(fit$mean))[1:3])

rowData(spe)[outlier_ids, ]
##  DataFrame with 3 rows and 4 columns
##                                gene_id   gene_name    feature_type
##                            <character> <character>     <character>
##  ENSMUSG00000115783 ENSMUSG00000115783         Bc1 Gene Expression
##  ENSMUSG00000098178 ENSMUSG00000098178     Gm42418 Gene Expression
##  ENSMUSG00000024661 ENSMUSG00000024661        Fth1 Gene Expression
##                     subsets_mito
##                        <logical>
##  ENSMUSG00000115783        FALSE
##  ENSMUSG00000098178        FALSE
##  ENSMUSG00000024661        FALSE
```

18.8 Spatially-aware feature selection

nnSVG

```
library(nnSVG)
```

```
# subsample spots
n <- 100
set.seed(123)
ix <- sample(seq_len(n), n)

spe_nnSVG <- spe[, ix]

# filter low-expressed and mitochondrial genes
# using very stringent filtering parameters for faster runtime in this example
# note: for a full analysis, use alternative filtering parameters (e.g. defaults)
spe_nnSVG <- filter_genes(
  spe_nnSVG, filter_genes_ncounts = 50, filter_genes_pcspots = 5
)
##  Gene filtering: removing mitochondrial genes
##  removed 0 mitochondrial genes
##  Gene filtering: retaining genes with at least 50 counts in at least 5% (n = 5) of spatia
##  removed 32074 out of 32272 genes due to low expression

# re-calculate logcounts after filtering
# using library size factors
spe_nnSVG <- logNormCounts(spe_nnSVG)

# run nnSVG
# using a single core for compatibility on build system
# note: for a full analysis, use multiple cores
set.seed(123)
spe_nnSVG <- nnSVG(spe_nnSVG, n_threads = 1)

# investigate results

# show results
head(rowData(spe_nnSVG), 3)
##  DataFrame with 3 rows and 18 columns
##                                 gene_id   gene_name    feature_type
##                             <character> <character>     <character>
##  ENSMUSG00000061518 ENSMUSG00000061518       Cox5b Gene Expression
##  ENSMUSG00000073702 ENSMUSG00000073702       Rpl31 Gene Expression
##  ENSMUSG00000046330 ENSMUSG00000046330      Rpl37a Gene Expression
##                     subsets_mito  sigma.sq    tau.sq       phi    loglik
##                        <logical> <numeric> <numeric> <numeric> <numeric>
```

```
##  ENSMUSG00000061518        FALSE 0.0210665 0.1202984  13.53194  -43.6472
##  ENSMUSG00000073702        FALSE 0.0688871 0.0860842   5.84110  -40.3003
##  ENSMUSG00000046330        FALSE 0.0549986 0.1133114   6.47579  -48.2811
##                       runtime      mean       var     spcov   prop_sv
##                     <numeric> <numeric> <numeric> <numeric> <numeric>
##  ENSMUSG00000061518     0.021   4.98425  0.142977 0.0291204  0.149022
##  ENSMUSG00000073702     0.013   4.83432  0.143616 0.0542917  0.444515
##  ENSMUSG00000046330     0.015   5.35620  0.166734 0.0437844  0.326770
##                     loglik_lm   LR_stat      rank      pval      padj
##                     <numeric> <numeric> <numeric> <numeric> <numeric>
##  ENSMUSG00000061518  -44.1377  0.981135       184 0.6122789 0.6588653
##  ENSMUSG00000073702  -44.3607  8.120691       147 0.0172431 0.0232254
##  ENSMUSG00000046330  -51.8235  7.084636       151 0.0289462 0.0379559

# number of significant SVGs
table(rowData(spe_nnSVG)$padj <= 0.05)
##
##  FALSE  TRUE
##     46   152

# show results for top n SVGs
rowData(spe_nnSVG)[order(rowData(spe_nnSVG)$rank)[1:6], ]
##  DataFrame with 6 rows and 18 columns
##                              gene_id   gene_name    feature_type
##                            <character> <character>     <character>
##  ENSMUSG00000046447 ENSMUSG00000046447     Camk2n1 Gene Expression
##  ENSMUSG00000053310 ENSMUSG00000053310        Nrgn Gene Expression
##  ENSMUSG00000018593 ENSMUSG00000018593       Sparc Gene Expression
##  ENSMUSG00000032532 ENSMUSG00000032532         Cck Gene Expression
##  ENSMUSG00000070570 ENSMUSG00000070570     Slc17a7 Gene Expression
##  ENSMUSG00000024617 ENSMUSG00000024617      Camk2a Gene Expression
##                     subsets_mito  sigma.sq      tau.sq       phi    loglik
##                        <logical> <numeric>   <numeric> <numeric> <numeric>
##  ENSMUSG00000046447        FALSE   1.88342 6.28372e-02   2.05206   -99.021
##  ENSMUSG00000053310        FALSE   3.17506 2.47772e-01   3.26420  -151.225
##  ENSMUSG00000018593        FALSE   2.04163 7.63186e-02   3.68722  -124.793
##  ENSMUSG00000032532        FALSE   2.98145 2.98145e-08   5.07869  -147.464
##  ENSMUSG00000070570        FALSE   2.64081 3.20107e-01   4.15601  -155.620
##  ENSMUSG00000024617        FALSE   1.41249 9.79965e-02   3.74573  -113.269
##                       runtime      mean       var     spcov   prop_sv
##                     <numeric> <numeric> <numeric> <numeric> <numeric>
##  ENSMUSG00000046447     0.017   5.35338   1.50820  0.256357  0.967714
```

```
##  ENSMUSG00000053310     0.019   4.85871   3.71912  0.366737  0.927612
##  ENSMUSG00000018593     0.019   3.61331   1.87664  0.395442  0.963966
##  ENSMUSG00000032532     0.017   4.19282   2.72400  0.411820  1.000000
##  ENSMUSG00000070570     0.015   3.62933   3.00604  0.447757  0.891889
##  ENSMUSG00000024617     0.022   4.11652   1.17870  0.288711  0.935123
##                     loglik_lm   LR_stat      rank        pval        padj
##                     <numeric> <numeric> <numeric>   <numeric>   <numeric>
##  ENSMUSG00000046447  -161.937  125.8321         1 0.00000e+00 0.00000e+00
##  ENSMUSG00000053310  -207.066  111.6804         2 0.00000e+00 0.00000e+00
##  ENSMUSG00000018593  -172.866   96.1453         3 0.00000e+00 0.00000e+00
##  ENSMUSG00000032532  -191.496   88.0644         4 0.00000e+00 0.00000e+00
##  ENSMUSG00000070570  -196.423   81.6050         5 0.00000e+00 0.00000e+00
##  ENSMUSG00000024617  -149.612   72.6864         6 1.11022e-16 3.66374e-15

# identify top-ranked SVG
rowData(spe_nnSVG)$gene_name[which(rowData(spe_nnSVG)$rank == 1)]
##  [1] "Camk2n1"
```

18.9 Dimensionality reduction

```
# compute PCA
set.seed(123)
spe <- runPCA(spe, subset_row = top_hvgs)

reducedDimNames(spe)
##  [1] "PCA"
dim(reducedDim(spe, "PCA"))
##  [1] 2683   50
```

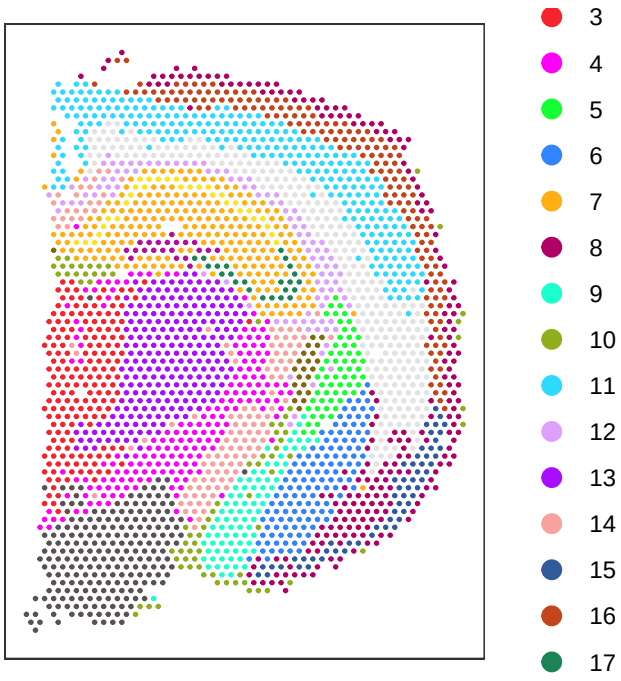
```
# compute UMAP on top 50 PCs
set.seed(123)
spe <- runUMAP(spe, dimred = "PCA")

reducedDimNames(spe)
##  [1] "PCA"  "UMAP"
```

```r
dim(reducedDim(spe, "UMAP"))
##  [1] 2683    2

# update column names for easier plotting
colnames(reducedDim(spe, "UMAP")) <- paste0("UMAP", 1:2)
```

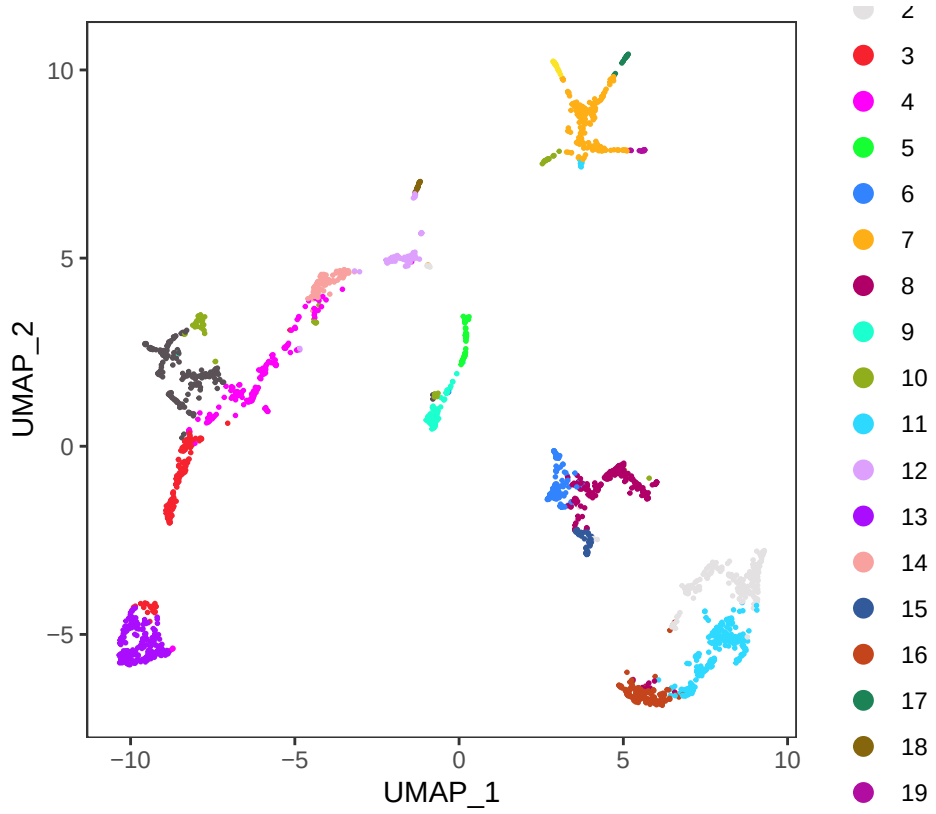
18.10 Clustering

```r
# graph-based clustering
set.seed(123)
k <- 10
g <- buildSNNGraph(spe, k = k, use.dimred = "PCA")
g_walk <- igraph::cluster_walktrap(g)
clus <- g_walk$membership
table(clus)
##  clus
##    1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17  18  19
##  218 255 176 179  60 127 240 217  83  77 271 100 241 125  57 160  23  22  21
##   20
##   31

# store cluster labels in column 'label' in colData
colLabels(spe) <- factor(clus)
```

```r
# define custom color palette
colors <- unname(palette.colors(palette = "Polychrome 36"))

# plot clusters in spatial x-y coordinates
plotSpots(spe, annotate = "label",
          pal = colors)
```




```
# plot clusters in UMAP dimensions
plotDimRed(spe, plot_type = "UMAP",
           annotate = "label", pal = colors)
```

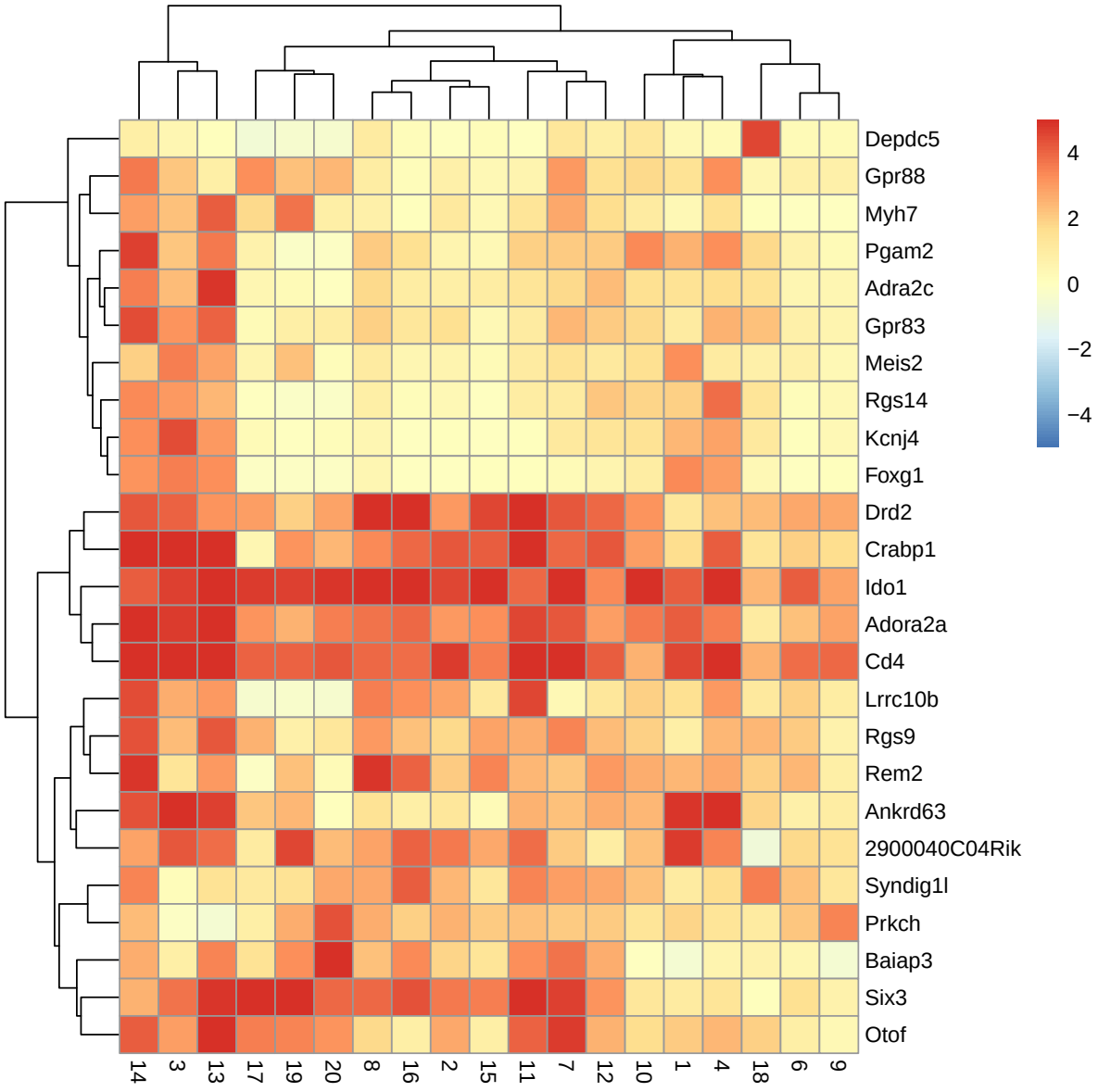


18.11 Differential expression

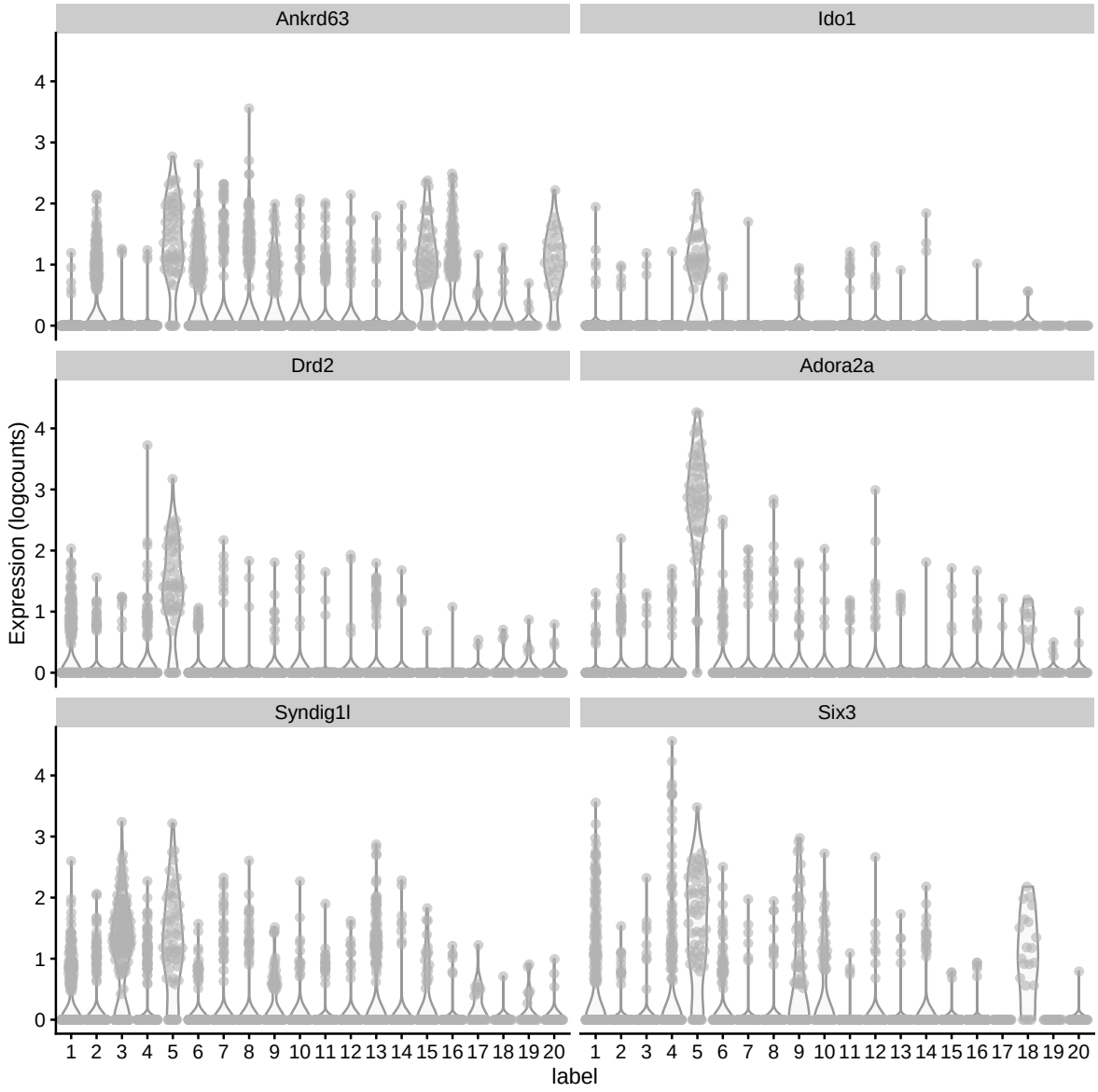
```
# set gene names as row names for easier plotting
rownames(spe) <- rowData(spe)$gene_name

# test for marker genes
markers <- findMarkers(spe, test = "binom", direction = "up")

# returns a list with one DataFrame per cluster
markers
##  List of length 20
##  names(20): 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
```

```
library(pheatmap)
```

```
# plot log-fold changes for one cluster over all other clusters
# selecting cluster 5
interesting <- markers[[5]]
best_set <- interesting[interesting$Top <= 5, ]
logFCs <- getMarkerEffects(best_set)

pheatmap(logFCs, breaks = seq(-5, 5, length.out = 101))
```




```
# plot log-transformed normalized expression of top genes for one cluster
top_genes <- head(rownames(interesting))

plotExpression(spe, x = "label", features = top_genes)
```


Ankrd63
Ido1
Drd2
Adora2a
Syndig1l
Six3
Expression (logcounts)
label

References

19 spatialLIBD workflow

19.1 Overview

[17]

`spatialLIBD`

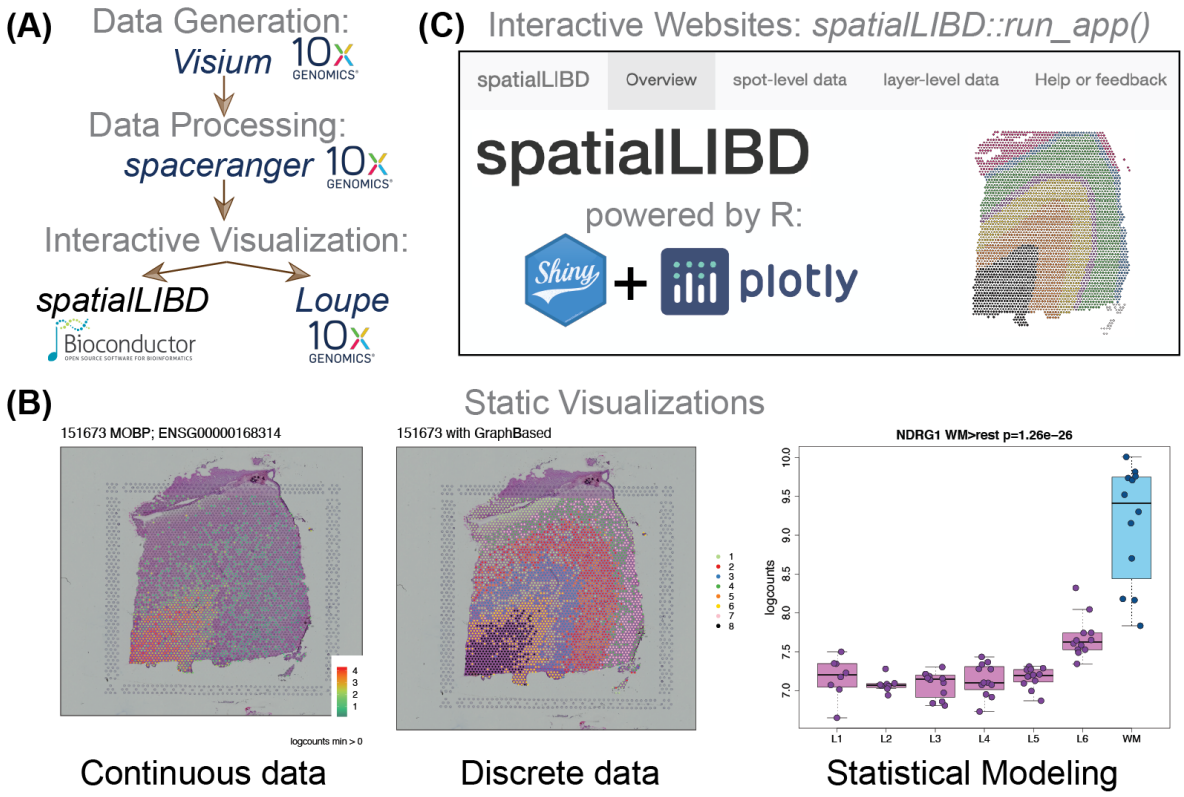


Figure 19.1: `spatialLIBD` overview. Source: @Pardo2022.

19.2 spatialLIBD

19.2.1 Why use spatialLIBD?

-
-

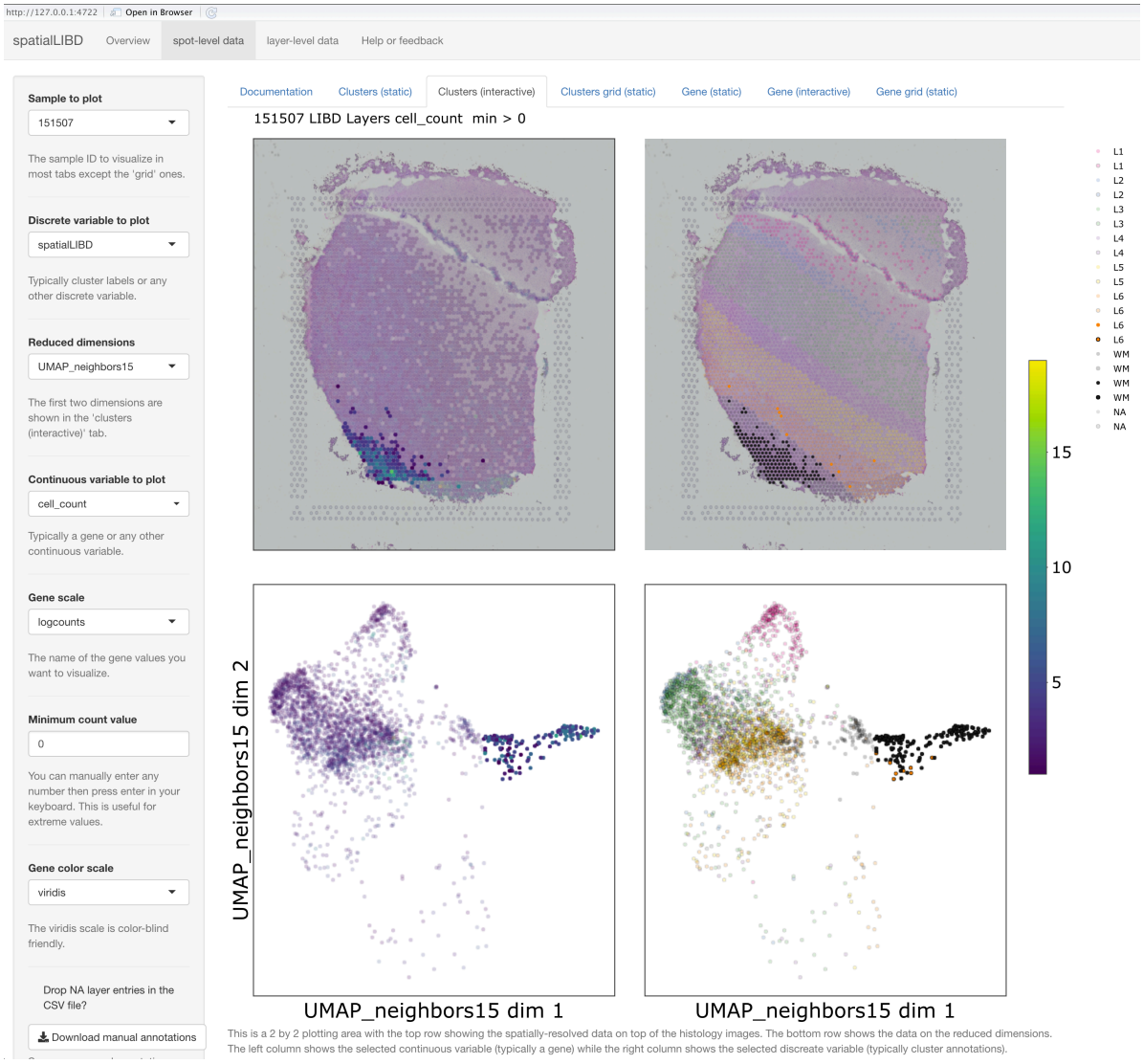


Figure 19.2: Screenshot of the 'clusters (interactive)' section of the 'spot-level data' panel created with the full spatialLIBD dataset. The website was created with `spatialLIBD::run_app(spatialLIBD::fetch_data('spe'))` version 1.4.0 and then using the lasso selection, we selected a set of spots in the UMAP interactive plot colored by the estimated number of cells per spot (`cell_count`) on the bottom left, which automatically updated the other three plots.

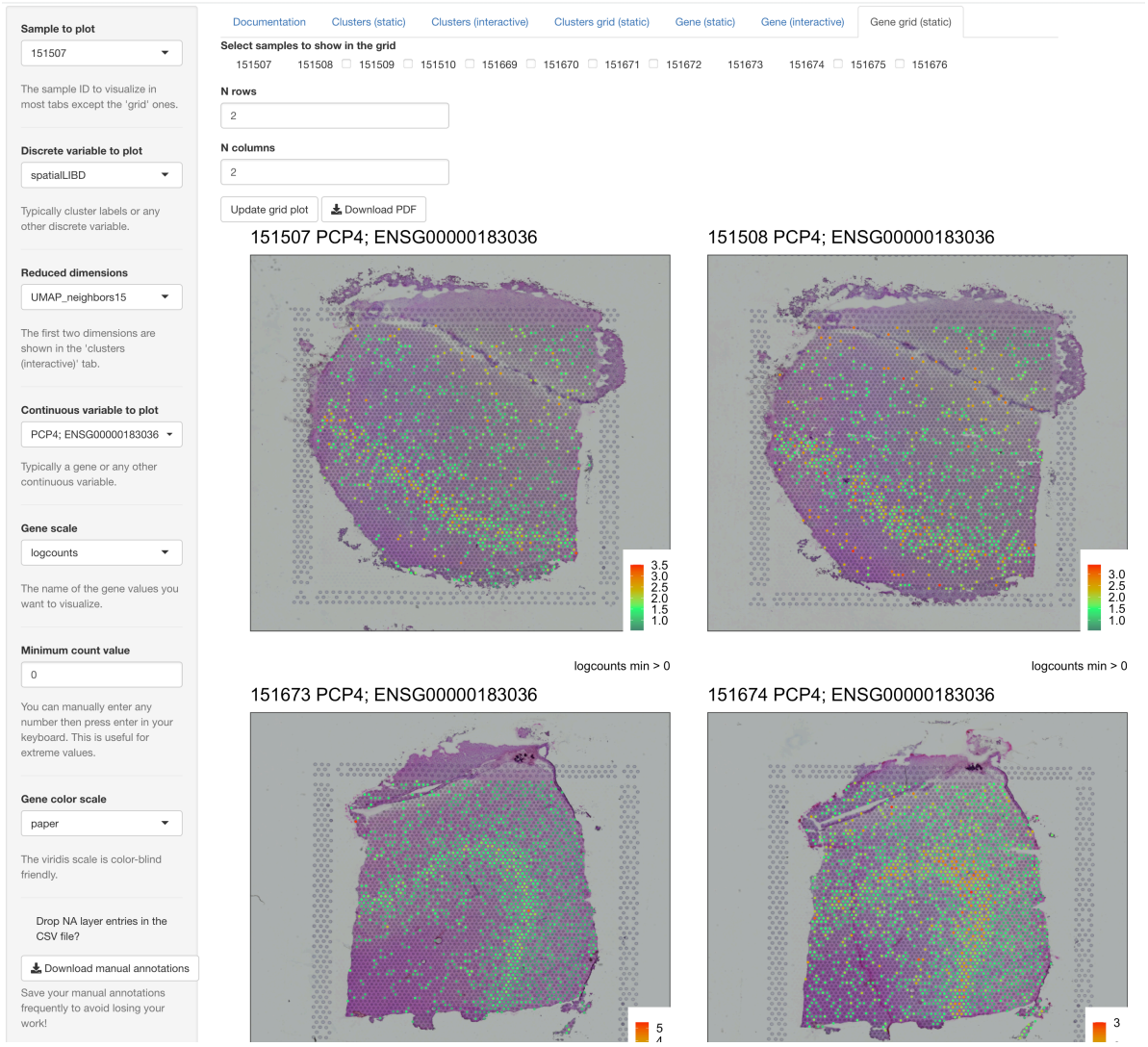


Figure 19.3: Screenshot of the 'gene grid (static)' section of the 'spot-level data' panel created with the full spatialLIBD dataset. The website was created with `spatialLIBD::run_app(spatialLIBD::fetch_data('spe'))` version 1.4.0, selecting the *PCP4* gene, selecting the *paper* gene color scale, changing the number of rows and columns in the grid 2, selecting two pairs of spatially adjacent replicate samples (151507, 151508, 151673, and 151674), and clicking on the *upgrade grid plot* button. Note that the default *viridis* gene color scale is color-blind friendly.

it on your browser [1]

19.2.2 Want to learn more about spatialLIBD?

`spatialLIBD`

Bioconductor landing page `pkgdown` documentation website

- Introduction to spatialLIBD
- Using spatialLIBD with 10x Genomics public datasets

```
citation("spatialLIBD")[1]
##  Pardo B, Spangler A, Weber LM, Hicks SC, Jaffe AE, Martinowich K,
##  Maynard KR, Collado-Torres L (2022). "spatialLIBD: an R/Bioconductor
##  package to visualize spatially-resolved transcriptomics data." _BMC
##  Genomics_. doi:10.1186/s12864-022-08601-w
##  <https://doi.org/10.1186/s12864-022-08601-w>,
##  <https://doi.org/10.1186/s12864-022-08601-w>.
##
##  A BibTeX entry for LaTeX users is
##
##    @Article{,
##      title = {spatialLIBD: an R/Bioconductor package to visualize spatially-resolved tran
##      author = {Brenda Pardo and Abby Spangler and Lukas M. Weber and Stephanie C. Hicks a
##      year = {2022},
##      journal = {BMC Genomics},
##      doi = {10.1186/s12864-022-08601-w},
##      url = {https://doi.org/10.1186/s12864-022-08601-w},
##    }
```

19.2.2.1 Recordings

[2]

[1] Check https://github.com/LieberInstitute/spatialLIBD#shiny-website-mirrors in case you need to use a mirror. `shiny`-powered websites work best on browsers such as Google Chrome and Mozilla Firefox, among others.

[2] Originally available at https://github.com/LieberInstitute/spatialLIBD/blob/master/inst/app/www/documentation_spe.md#sli and-videos.

LIBD rstats club

19.3 Code prerequisites

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- SpatialExperiment
- STexampleData
- scater
- scran
- igraph
- BiocFileCache
- rtracklayer
- lobstr

documented by Bioconductor

[3]

Bioconductor docker images

```
if (!requireNamespace("BiocManager", quietly = TRUE)) {
    install.packages("BiocManager")
}

## Check that you have a valid installation
BiocManager::valid()

## Install the required R packages for this workflow
BiocManager::install(c(
    "SpatialExperiment",
    "STexampleData",
    "scater",
```

[3] This book is under development, so right now you actually need to use `bioc-devel`. Once this book is submitted to Bioconductor, then it'll work with `bioc-release`. TODO: remove this comment when the book is available on `bioc-release`.

```
    "scran",
    "igraph",
    "BiocFileCache",
    "rtracklayer",
    "lobstr",
    "spatialLIBD"
))
```

17

```
## Load packages required for the
## "Visium human DLPFC workflow"
library("SpatialExperiment")
library("STexampleData")
library("scater")
library("scran")
library("igraph")
library("BiocFileCache")
library("rtracklayer")
library("lobstr")
library("spatialLIBD")

## Start tracking time
time_start <- Sys.time()

# load object
spe <- Visium_humanDLPFC()

# subset to keep only spots over tissue
spe <- spe[, colData(spe)$in_tissue == 1]

# identify mitochondrial genes
is_mito <- grepl("(^MT-)|(^mt-)", rowData(spe)$gene_name)

# calculate per-spot QC metrics and store in colData
spe <- addPerCellQC(spe, subsets = list(mito = is_mito))

# select QC thresholds
qc_lib_size <- colData(spe)$sum < 600
qc_detected <- colData(spe)$detected < 400
qc_mito <- colData(spe)$subsets_mito_percent > 28
```

```r
qc_cell_count <- colData(spe)$cell_count > 10

# combined set of discarded spots
discard <- qc_lib_size | qc_detected | qc_mito | qc_cell_count

# store in object
colData(spe)$discard <- discard

# filter low-quality spots
spe <- spe[, !colData(spe)$discard]

# calculate logcounts using library size factors
spe <- logNormCounts(spe)

# remove mitochondrial genes
spe <- spe[!is_mito, ]

# fit mean-variance relationship
dec <- modelGeneVar(spe)

# select top HVGs
top_hvgs <- getTopHVGs(dec, prop = 0.1)

# compute PCA
set.seed(123)
spe <- runPCA(spe, subset_row = top_hvgs)

# compute UMAP on top 50 PCs
set.seed(123)
spe <- runUMAP(spe, dimred = "PCA")

# update column names for easier plotting
colnames(reducedDim(spe, "UMAP")) <- paste0("UMAP", 1:2)

# graph-based clustering
set.seed(123)
k <- 10
g <- buildSNNGraph(spe, k = k, use.dimred = "PCA")
g_walk <- igraph::cluster_walktrap(g)
clus <- g_walk$membership

# store cluster labels in column 'label' in colData
```

```
colLabels(spe) <- factor(clus)

# set gene names as row names for easier plotting
rownames(spe) <- rowData(spe)$gene_name

# test for marker genes
markers <- findMarkers(spe, test = "binom", direction = "up")

## Find the interesting markers for each cluster
interesting <- sapply(markers, function(x) x$Top <= 5)
colnames(interesting) <- paste0("gene_interest_", seq_len(length(markers)))
rowData(spe) <- cbind(rowData(spe), interesting)

## How long this code took to run
time_prereqs <- Sys.time()
time_prereqs - time_start
##  Time difference of 1.533987 mins
```

19.4 Prepare for spatialLIBD

using spatialLIBD with 10x Genomics public datasets

19.4.1 Basic information

```
## Add some information used by spatialLIBD
spe$key <- paste0(spe$sample_id, "_", colnames(spe))
spe$sum_umi <- colSums(counts(spe))
spe$sum_gene <- colSums(counts(spe) > 0)
```

19.4.2 Gene annotation

add the gene annotation data from Gencode

```
## Download the Gencode v32 GTF file and cache it
bfc <- BiocFileCache::BiocFileCache()
gtf_cache <- BiocFileCache::bfcrpath(
    bfc,
    paste0(
        "ftp://ftp.ebi.ac.uk/pub/databases/gencode/Gencode_human/",
        "release_32/gencode.v32.annotation.gtf.gz"
    )
)

## Show the GTF cache location
gtf_cache
##                                                                      BFC1
##  "/root/.cache/R/BiocFileCache/6c63cebea55_gencode.v32.annotation.gtf.gz"

## Import into R (takes ~1 min)
gtf <- rtracklayer::import(gtf_cache)

## Subset to genes only
gtf <- gtf[gtf$type == "gene"]

## Remove the .x part of the gene IDs
gtf$gene_id <- gsub("\\..*", "", gtf$gene_id)

## Set the names to be the gene IDs
names(gtf) <- gtf$gene_id

## Match the genes
match_genes <- match(rowData(spe)$gene_id, gtf$gene_id)
table(is.na(match_genes))
##
##  FALSE  TRUE
##  33267   258

## Drop the few genes for which we don't have information
spe <- spe[!is.na(match_genes), ]
match_genes <- match_genes[!is.na(match_genes)]

## Keep only some columns from the gtf
mcols(gtf) <- mcols(gtf)[, c("source", "type", "gene_id", "gene_name", "gene_type")]

## Save the "interest"ing columns from our original spe object
```

```
interesting <- rowData(spe)[, grepl("interest", colnames(rowData(spe)))]

## Add the gene info to our SPE object
rowRanges(spe) <- gtf[match_genes]

## Add back the "interest" coolumns
rowData(spe) <- cbind(rowData(spe), interesting)

## Inspect the gene annotation data we added
rowRanges(spe)
##  GRanges object with 33267 ranges and 11 metadata columns:
##                     seqnames            ranges strand |   source     type
##                        <Rle>         <IRanges>  <Rle> | <factor> <factor>
##    ENSG00000243485     chr1       29554-31109      + |   HAVANA     gene
##    ENSG00000237613     chr1       34554-36081      - |   HAVANA     gene
##    ENSG00000186092     chr1       65419-71585      + |   HAVANA     gene
##    ENSG00000238009     chr1      89295-133723      - |   HAVANA     gene
##    ENSG00000239945     chr1       89551-91105      - |   HAVANA     gene
##                ...      ...               ...    ... .      ...      ...
##    ENSG00000160298    chr21 46300181-46323875      - |   HAVANA     gene
##    ENSG00000160299    chr21 46324141-46445769      + |   HAVANA     gene
##    ENSG00000160305    chr21 46458891-46570015      + |   HAVANA     gene
##    ENSG00000160307    chr21 46598604-46605208      - |   HAVANA     gene
##    ENSG00000160310    chr21 46635595-46665124      + |   HAVANA     gene
##                          gene_id   gene_name      gene_type gene_interest_1
##                      <character> <character>    <character>       <logical>
##    ENSG00000243485 ENSG00000243485 MIR1302-2HG         lncRNA            TRUE
##    ENSG00000237613 ENSG00000237613     FAM138A         lncRNA            TRUE
##    ENSG00000186092 ENSG00000186092       OR4F5 protein_coding            TRUE
##    ENSG00000238009 ENSG00000238009  AL627309.1         lncRNA            TRUE
##    ENSG00000239945 ENSG00000239945  AL627309.3         lncRNA            TRUE
##                ...             ...         ...            ...             ...
##    ENSG00000160298 ENSG00000160298    C21orf58 protein_coding           FALSE
##    ENSG00000160299 ENSG00000160299        PCNT protein_coding           FALSE
##    ENSG00000160305 ENSG00000160305       DIP2A protein_coding           FALSE
##    ENSG00000160307 ENSG00000160307       S100B protein_coding           FALSE
##    ENSG00000160310 ENSG00000160310       PRMT2 protein_coding           FALSE
##                    gene_interest_2 gene_interest_3 gene_interest_4
##                          <logical>       <logical>       <logical>
##    ENSG00000243485            TRUE            TRUE            TRUE
##    ENSG00000237613            TRUE            TRUE            TRUE
##    ENSG00000186092            TRUE            TRUE            TRUE
```

```
##    ENSG00000238009            TRUE            TRUE            TRUE
##    ENSG00000239945            TRUE            TRUE            TRUE
##                ...             ...             ...             ...
##    ENSG00000160298           FALSE           FALSE           FALSE
##    ENSG00000160299           FALSE           FALSE           FALSE
##    ENSG00000160305           FALSE           FALSE           FALSE
##    ENSG00000160307           FALSE           FALSE           FALSE
##    ENSG00000160310           FALSE           FALSE           FALSE
##                    gene_interest_5 gene_interest_6
##                          <logical>       <logical>
##    ENSG00000243485            TRUE            TRUE
##    ENSG00000237613            TRUE            TRUE
##    ENSG00000186092            TRUE            TRUE
##    ENSG00000238009            TRUE            TRUE
##    ENSG00000239945            TRUE            TRUE
##                ...             ...             ...
##    ENSG00000160298           FALSE           FALSE
##    ENSG00000160299           FALSE           FALSE
##    ENSG00000160305           FALSE           FALSE
##    ENSG00000160307           FALSE           FALSE
##    ENSG00000160310           FALSE           FALSE
##    -------
##    seqinfo: 25 sequences from an unspecified genome; no seqlengths
```

```
## Add information used by spatialLIBD
rowData(spe)$gene_search <- paste0(
    rowData(spe)$gene_name, "; ", rowData(spe)$gene_id
)

## Compute chrM expression and chrM expression ratio
is_mito <- which(seqnames(spe) == "chrM")
spe$expr_chrM <- colSums(counts(spe)[is_mito, , drop = FALSE])
spe$expr_chrM_ratio <- spe$expr_chrM / spe$sum_umi
```

19.4.3 Extra information and filtering

filter the object

```
## Add a variable for saving the manual annotations
spe$ManualAnnotation <- "NA"

## Remove genes with no data
no_expr <- which(rowSums(counts(spe)) == 0)

## Number of genes with no counts
length(no_expr)
##  [1] 11596

## Compute the percent of genes with no counts
length(no_expr) / nrow(spe) * 100
##  [1] 34.85737
spe <- spe[-no_expr, , drop = FALSE]

## Remove spots without counts
summary(spe$sum_umi)
##     Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##      537    2414    3466    3870    4938   15862

## If we had spots with no counts, we would remove them
if (any(spe$sum_umi == 0)) {
    spots_no_counts <- which(spe$sum_umi == 0)
    ## Number of spots with no counts
    print(length(spots_no_counts))
    ## Percent of spots with no counts
    print(length(spots_no_counts) / ncol(spe) * 100)
    spe <- spe[, -spots_no_counts, drop = FALSE]
}
```

```
## Run check_spe() function
spatialLIBD::check_spe(spe)
```

```
##  class: SpatialExperiment
##  dim: 21671 3524
##  metadata(0):
##  assays(2): counts logcounts
##  rownames(21671): ENSG00000243485 ENSG00000238009 ... ENSG00000160307
##    ENSG00000160310
##  rowData names(12): source type ... gene_interest_6 gene_search
##  colnames(3524): AAACAAGTATCTCCCA-1 AAACACCAATAACTGC-1 ...
##    TTGTTTCCATACAACT-1 TTGTTTGTGTAAATTC-1
##  colData names(23): barcode_id sample_id ... expr_chrM_ratio
##    ManualAnnotation
##  reducedDimNames(2): PCA UMAP
##  mainExpName: NULL
##  altExpNames(0):
##  spatialCoords names(2) : pxl_col_in_fullres pxl_row_in_fullres
##  imgData names(4): sample_id image_id data scaleFactor

## End tracking time
time_end <- Sys.time()

## How long this code took to run
time_end - time_prereqs
##  Time difference of 32.32455 secs
```

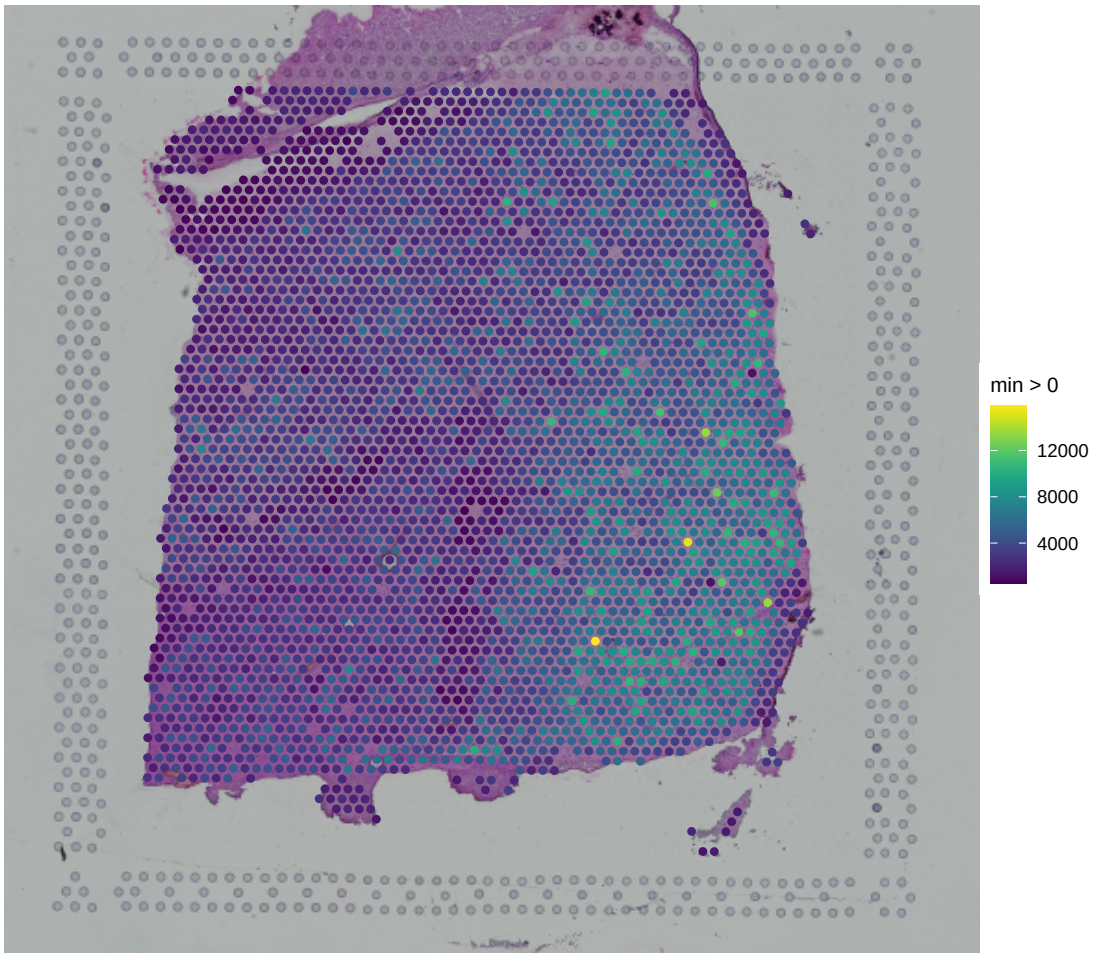
```
saveRDS(spe, file = "spe_workflow_Visium_spatialLIBD.rds")
```

```
spe <- readRDS("spe_workflow_Visium_spatialLIBD.rds")
```

19.5 Explore the data

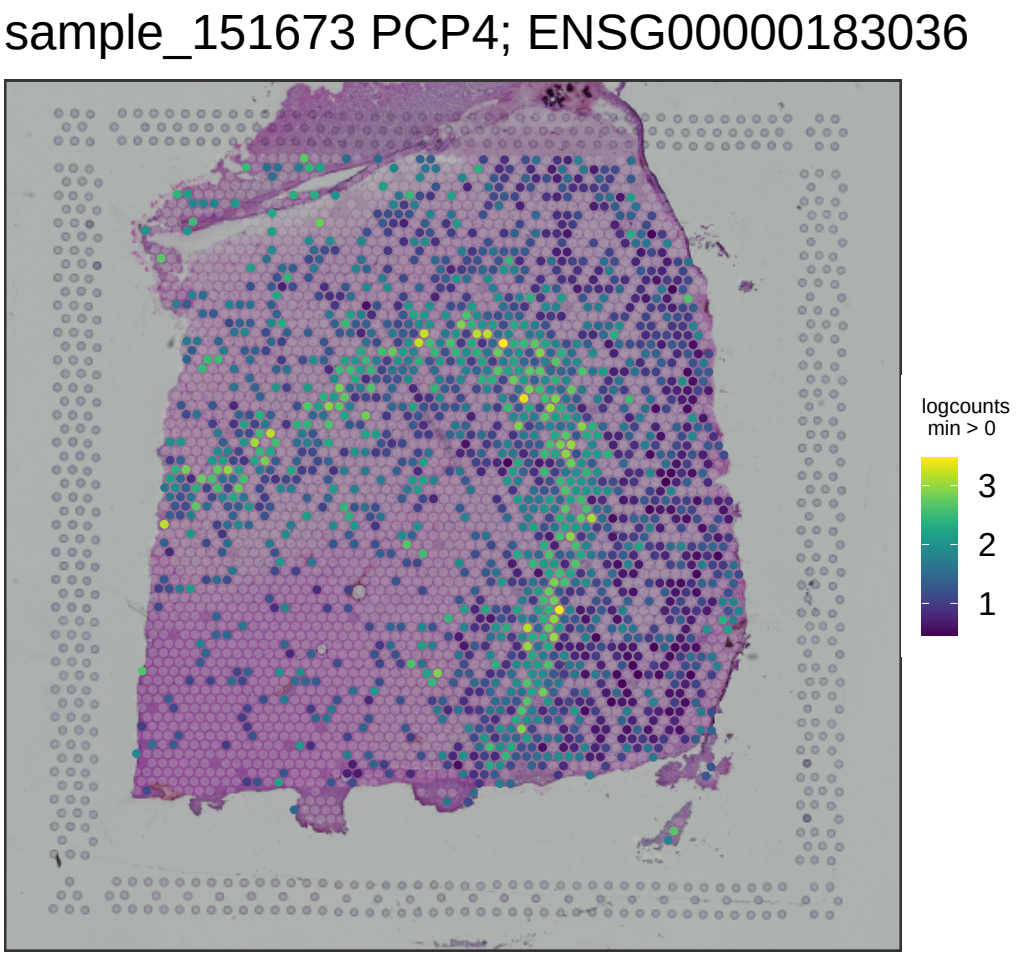
```
## Sum of UMI
spatialLIBD::vis_gene(
    spe = spe,
    sampleid = "sample_151673",
    geneid = "sum_umi"
)
```

sample_151673 sum_umi




```
## PCP4, a layer 5 marker gene
spatialLIBD::vis_gene(
    spe = spe,
    sampleid = "sample_151673",
```

```
    geneid = rowData(spe)$gene_search[which(rowData(spe)$gene_name == "PCP4")]
)
```



visualize the data interactively

```
## Explore all the variables we can use
colData(spe)
##  DataFrame with 3524 rows and 23 columns
##                              barcode_id     sample_id in_tissue array_row
```

```
##                          <character>   <character> <integer> <integer>
##  AAACAAGTATCTCCCA-1 AAACAAGTATCTCCCA-1 sample_151673         1        50
##  AAACACCAATAACTGC-1 AAACACCAATAACTGC-1 sample_151673         1        59
##  AAACAGAGCGACTCCT-1 AAACAGAGCGACTCCT-1 sample_151673         1        14
##  AAACAGCTTTCAGAAG-1 AAACAGCTTTCAGAAG-1 sample_151673         1        43
##  AAACAGGGTCTATATT-1 AAACAGGGTCTATATT-1 sample_151673         1        47
##  ...                              ...           ...       ...       ...
##  TTGTTGTGTGTCAAGA-1 TTGTTGTGTGTCAAGA-1 sample_151673         1        31
##  TTGTTTCACATCCAGG-1 TTGTTTCACATCCAGG-1 sample_151673         1        58
##  TTGTTTCATTAGTCTA-1 TTGTTTCATTAGTCTA-1 sample_151673         1        60
##  TTGTTTCCATACAACT-1 TTGTTTCCATACAACT-1 sample_151673         1        45
##  TTGTTTGTGTAAATTC-1 TTGTTTGTGTAAATTC-1 sample_151673         1         7
##                     array_col ground_truth   reference cell_count       sum
##                     <integer>  <character> <character>  <integer> <numeric>
##  AAACAAGTATCTCCCA-1       102       Layer3      Layer3          6      8458
##  AAACACCAATAACTGC-1        19           WM          WM          5      3769
##  AAACAGAGCGACTCCT-1        94       Layer3      Layer3          2      5433
##  AAACAGCTTTCAGAAG-1         9       Layer5      Layer5          4      4278
##  AAACAGGGTCTATATT-1        13       Layer6      Layer6          6      4004
##  ...                      ...          ...         ...        ...       ...
##  TTGTTGTGTGTCAAGA-1        77       Layer5      Layer5          3      3966
##  TTGTTTCACATCCAGG-1        42           WM          WM          3      4324
##  TTGTTTCATTAGTCTA-1        30           WM          WM          4      2761
##  TTGTTTCCATACAACT-1        27       Layer6      Layer6          3      2322
##  TTGTTTGTGTAAATTC-1        51       Layer2      Layer2          5      6281
##                      detected subsets_mito_sum subsets_mito_detected
##                     <numeric>        <numeric>             <numeric>
##  AAACAAGTATCTCCCA-1      3586             1407                    13
##  AAACACCAATAACTGC-1      1960              430                    13
##  AAACAGAGCGACTCCT-1      2424             1316                    13
##  AAACAGCTTTCAGAAG-1      2264              651                    12
##  AAACAGGGTCTATATT-1      2178              621                    13
##  ...                      ...              ...                   ...
##  TTGTTGTGTGTCAAGA-1      1982              789                    13
##  TTGTTTCACATCCAGG-1      2170              370                    12
##  TTGTTTCATTAGTCTA-1      1560              314                    12
##  TTGTTTCCATACAACT-1      1343              476                    13
##  TTGTTTGTGTAAATTC-1      2927              991                    13
##                     subsets_mito_percent     total   discard sizeFactor
##                                <numeric> <numeric> <logical>  <numeric>
##  AAACAAGTATCTCCCA-1              16.6351      8458     FALSE   1.822839
##  AAACACCAATAACTGC-1              11.4089      3769     FALSE   0.812282
```

```
##  AAACAGAGCGACTCCT-1              24.2223      5433     FALSE   1.170902
##  AAACAGCTTTCAGAAG-1              15.2174      4278     FALSE   0.921980
##  AAACAGGGTCTATATT-1              15.5095      4004     FALSE   0.862928
##  ...                                ...       ...       ...        ...
##  TTGTTGTGTGTCAAGA-1              19.89410      3966     FALSE   0.854739
##  TTGTTTCACATCCAGG-1               8.55689      4324     FALSE   0.931894
##  TTGTTTCATTAGTCTA-1              11.37269      2761     FALSE   0.595041
##  TTGTTTCCATACAACT-1              20.49957      2322     FALSE   0.500429
##  TTGTTTGTGTAAATTC-1              15.77774      6281     FALSE   1.353660
##                        label                       key   sum_umi  sum_gene
##                     <factor>               <character> <numeric> <integer>
##  AAACAAGTATCTCCCA-1        2 sample_151673_AAACAA..       7051      3573
##  AAACACCAATAACTGC-1        1 sample_151673_AAACAC..       3339      1947
##  AAACAGAGCGACTCCT-1        6 sample_151673_AAACAG..       4117      2411
##  AAACAGCTTTCAGAAG-1        2 sample_151673_AAACAG..       3627      2252
##  AAACAGGGTCTATATT-1        2 sample_151673_AAACAG..       3383      2165
##  ...                     ...                     ...        ...       ...
##  TTGTTGTGTGTCAAGA-1        2 sample_151673_TTGTTG..       3177      1969
##  TTGTTTCACATCCAGG-1        1 sample_151673_TTGTTT..       3954      2158
##  TTGTTTCATTAGTCTA-1        1 sample_151673_TTGTTT..       2447      1548
##  TTGTTTCCATACAACT-1        4 sample_151673_TTGTTT..       1846      1330
##  TTGTTTGTGTAAATTC-1        6 sample_151673_TTGTTT..       5290      2914
##                      expr_chrM expr_chrM_ratio ManualAnnotation
##                      <numeric>       <numeric>      <character>
##  AAACAAGTATCTCCCA-1          0               0               NA
##  AAACACCAATAACTGC-1          0               0               NA
##  AAACAGAGCGACTCCT-1          0               0               NA
##  AAACAGCTTTCAGAAG-1          0               0               NA
##  AAACAGGGTCTATATT-1          0               0               NA
##  ...                       ...             ...              ...
##  TTGTTGTGTGTCAAGA-1          0               0               NA
##  TTGTTTCACATCCAGG-1          0               0               NA
##  TTGTTTCATTAGTCTA-1          0               0               NA
##  TTGTTTCCATACAACT-1          0               0               NA
##  TTGTTTGTGTAAATTC-1          0               0               NA

## Run our shiny app
if (interactive()) {
    spatialLIBD::run_app(
        spe,
        sce_layer = NULL,
        modeling_results = NULL,
```

```
        sig_genes = NULL,
        title = "OSTA spatialLIBD workflow example",
        spe_discrete_vars = c("ground_truth", "label", "ManualAnnotation"),
        spe_continuous_vars = c(
            "cell_count",
            "sum_umi",
            "sum_gene",
            "expr_chrM",
            "expr_chrM_ratio",
            "sum",
            "detected",
            "subsets_mito_sum",
            "subsets_mito_detected",
            "subsets_mito_percent",
            "total",
            "sizeFactor"
        ),
        default_cluster = "label"
    )
}
```

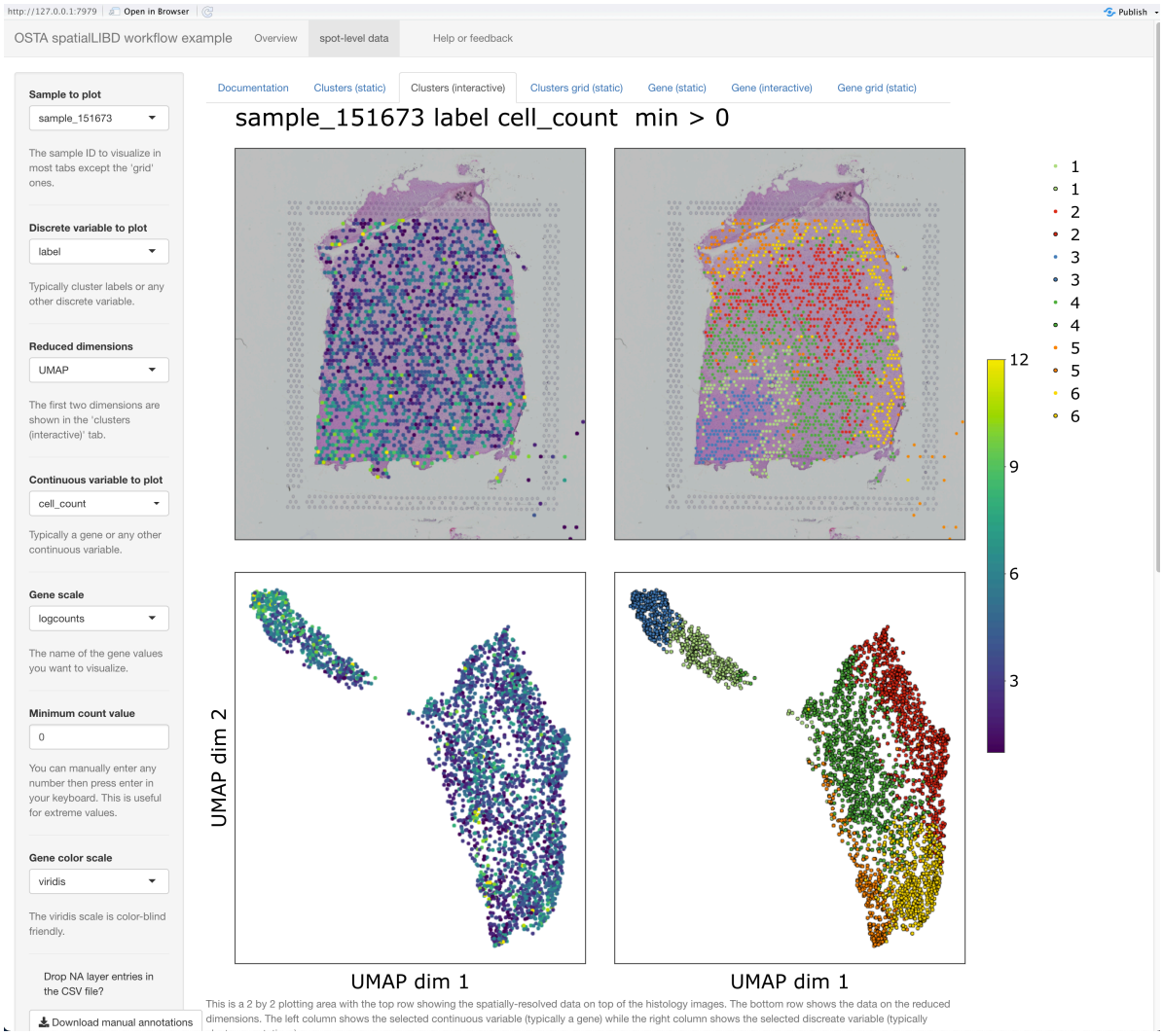


Figure 19.4: Screenshot of the 'clusters (interactive)' section of the 'spot-level data' panel created with with the data from this workflow.

19.6 Sharing your website

```
## Object size
lobstr::obj_size(spe) / 1024^2 ## Convert to MB
##  293.07 B
```

shinyapps.io

on the `spatialLIBD_demo` directory

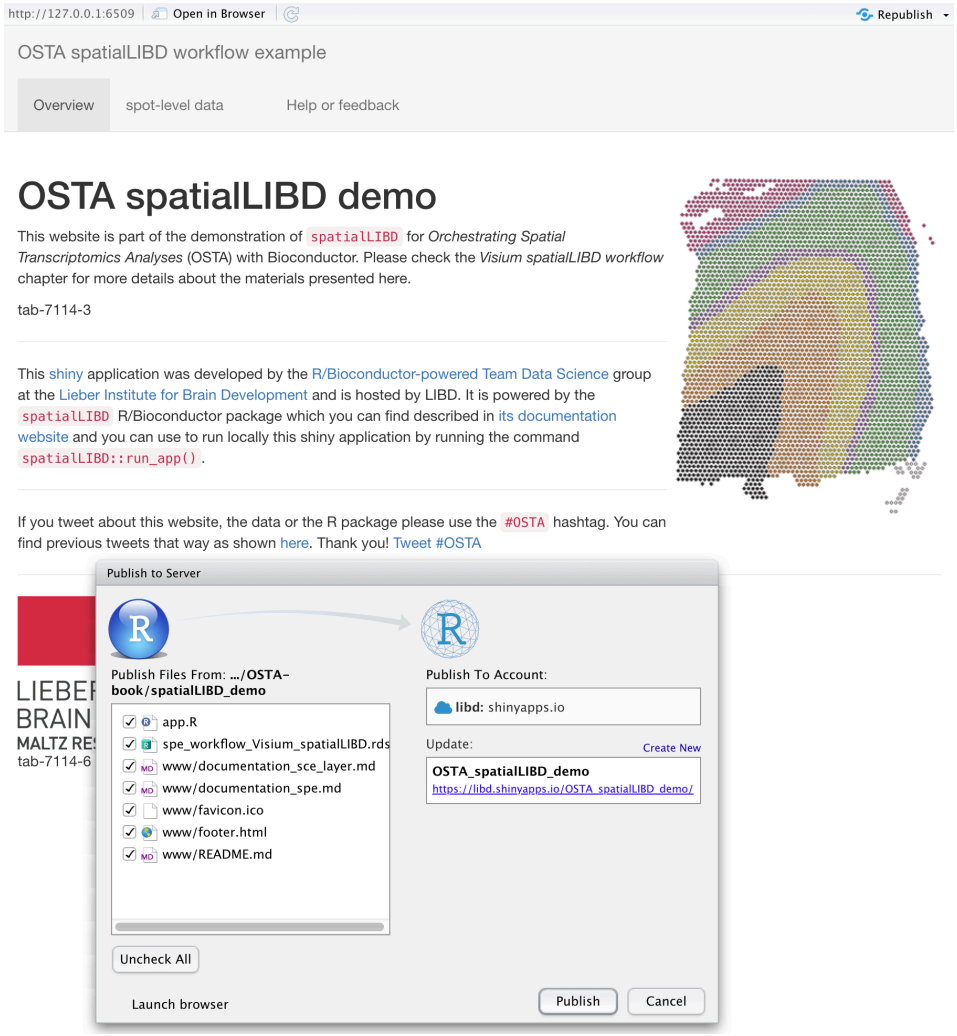


Figure 19.5: Screenshot of the RStudio window for publishing your spatialLIBD-powered website to shinyapps.io

`www` directory files from the `spatialLIBD` repository

adapted them to our liking

just like this one

-
-
-

error configuration files

19.7 Wrapping up

-
- 17
-
-
-

R session information

```
options(width = 120)
sessioninfo::session_info()
##  - Session info -------------------------------------------------------------------------------
##   setting  value
##   version  R version 4.4.1 (2024-06-14)
##   os       Ubuntu 22.04.5 LTS
##   system   x86_64, linux-gnu
##   ui       X11
##   language (EN)
##   collate  C
##   ctype    en_US.UTF-8
##   tz       Etc/UTC
##   date     2024-09-27
##   pandoc   3.4 @ /usr/bin/ (via rmarkdown)
##
```

```
##  - Packages ------------------------------------------------------------------------------
##   package              * version   date (UTC) lib source
##   abind                  1.4-8     2024-09-12 [2] RSPM (R 4.4.0)
##   AnnotationDbi          1.67.0    2024-05-01 [2] Bioconductor 3.20 (R 4.4.0)
##   AnnotationHub        * 3.13.3    2024-08-19 [2] Bioconductor 3.20 (R 4.4.1)
##   attempt                0.3.1     2020-05-03 [2] RSPM (R 4.4.0)
##   beachmat               2.21.6    2024-09-05 [2] Bioconductor 3.20 (R 4.4.1)
##   beeswarm               0.4.0     2021-06-01 [2] RSPM (R 4.4.0)
##   benchmarkme            1.0.8     2022-06-12 [2] RSPM (R 4.4.0)
##   benchmarkmeData        1.0.4     2020-04-23 [2] RSPM (R 4.4.0)
##   Biobase              * 2.65.1    2024-08-28 [2] Bioconductor 3.20 (R 4.4.1)
##   BiocFileCache        * 2.13.0    2024-05-01 [2] Bioconductor 3.20 (R 4.4.0)
##   BiocGenerics         * 0.51.1    2024-09-03 [2] Bioconductor 3.20 (R 4.4.1)
##   BiocIO                 1.15.2    2024-08-23 [2] Bioconductor 3.20 (R 4.4.1)
##   BiocManager            1.30.25   2024-08-28 [2] CRAN (R 4.4.1)
##   BiocNeighbors          1.99.1    2024-09-22 [2] Bioconductor 3.20 (R 4.4.1)
##   BiocParallel           1.39.0    2024-05-01 [2] Bioconductor 3.20 (R 4.4.0)
##   BiocSingular           1.21.4    2024-09-22 [2] Bioconductor 3.20 (R 4.4.1)
##   BiocVersion            3.20.0    2024-05-01 [2] Bioconductor 3.20 (R 4.4.1)
##   Biostrings             2.73.2    2024-09-26 [2] Bioconductor 3.20 (R 4.4.1)
##   bit                    4.5.0     2024-09-20 [2] RSPM (R 4.4.0)
##   bit64                  4.5.2     2024-09-22 [2] RSPM (R 4.4.0)
##   bitops                 1.0-8     2024-07-29 [2] RSPM (R 4.4.0)
##   blob                   1.2.4     2023-03-17 [2] RSPM (R 4.4.0)
##   bluster                1.15.1    2024-09-06 [2] Bioconductor 3.20 (R 4.4.1)
##   bslib                  0.8.0     2024-07-29 [2] RSPM (R 4.4.0)
##   cachem                 1.1.0     2024-05-16 [2] RSPM (R 4.4.0)
##   cli                    3.6.3     2024-06-21 [2] RSPM (R 4.4.0)
##   cluster                2.1.6     2023-12-01 [3] CRAN (R 4.4.1)
##   codetools              0.2-20    2024-03-31 [3] CRAN (R 4.4.1)
##   colorspace             2.1-1     2024-07-26 [2] RSPM (R 4.4.0)
##   config                 0.3.2     2023-08-30 [2] RSPM (R 4.4.0)
##   cowplot                1.1.3     2024-01-22 [2] RSPM (R 4.4.0)
##   crayon                 1.5.3     2024-06-20 [2] RSPM (R 4.4.0)
##   curl                   5.2.3     2024-09-20 [2] RSPM (R 4.4.0)
##   data.table             1.16.0    2024-08-27 [2] RSPM (R 4.4.0)
##   DBI                    1.2.3     2024-06-02 [2] RSPM (R 4.4.0)
##   dbplyr               * 2.5.0     2024-03-19 [2] RSPM (R 4.4.0)
##   DelayedArray           0.31.11   2024-08-04 [2] Bioconductor 3.20 (R 4.4.1)
##   digest                 0.6.37    2024-08-19 [2] RSPM (R 4.4.0)
##   doParallel             1.0.17    2022-02-07 [2] RSPM (R 4.4.0)
##   dotCall64              1.1-1     2023-11-28 [2] RSPM (R 4.4.0)
```

```
##   dplyr                1.1.4     2023-11-17 [2] RSPM (R 4.4.0)
##   dqrng                0.4.1     2024-05-28 [2] RSPM (R 4.4.0)
##   DT                   0.33      2024-04-04 [2] RSPM (R 4.4.0)
##   edgeR                4.3.16    2024-09-22 [2] Bioconductor 3.20 (R 4.4.1)
##   evaluate             1.0.0     2024-09-17 [2] RSPM (R 4.4.0)
##   ExperimentHub      * 2.13.1    2024-07-31 [2] Bioconductor 3.20 (R 4.4.1)
##   fansi                1.0.6     2023-12-08 [2] RSPM (R 4.4.0)
##   farver               2.1.2     2024-05-13 [2] RSPM (R 4.4.0)
##   fastmap              1.2.0     2024-05-15 [2] RSPM (R 4.4.0)
##   fields               16.2      2024-06-27 [2] RSPM (R 4.4.0)
##   filelock             1.0.3     2023-12-11 [2] RSPM (R 4.4.0)
##   FNN                  1.1.4.1   2024-09-22 [2] RSPM (R 4.4.0)
##   foreach              1.5.2     2022-02-02 [2] RSPM (R 4.4.0)
##   generics             0.1.3     2022-07-05 [2] RSPM (R 4.4.0)
##   GenomeInfoDb       * 1.41.1    2024-05-24 [2] Bioconductor 3.20 (R 4.4.0)
##   GenomeInfoDbData     1.2.12    2024-06-24 [2] Bioconductor
##   GenomicAlignments    1.41.0    2024-05-01 [2] Bioconductor 3.20 (R 4.4.0)
##   GenomicRanges      * 1.57.1    2024-06-12 [2] Bioconductor 3.20 (R 4.4.0)
##   ggbeeswarm           0.7.2     2023-04-29 [2] RSPM (R 4.4.0)
##   ggplot2            * 3.5.1     2024-04-23 [2] RSPM (R 4.4.0)
##   ggrepel              0.9.6     2024-09-07 [2] RSPM (R 4.4.0)
##   glue                 1.7.0     2024-01-09 [2] RSPM (R 4.4.0)
##   golem                0.5.1     2024-08-27 [2] RSPM (R 4.4.0)
##   gridExtra            2.3       2017-09-09 [2] RSPM (R 4.4.0)
##   gtable               0.3.5     2024-04-22 [2] RSPM (R 4.4.0)
##   htmltools            0.5.8.1   2024-04-04 [2] RSPM (R 4.4.0)
##   htmlwidgets          1.6.4     2023-12-06 [2] RSPM (R 4.4.0)
##   httpuv               1.6.15    2024-03-26 [2] RSPM (R 4.4.0)
##   httr                 1.4.7     2023-08-15 [2] RSPM (R 4.4.0)
##   igraph             * 2.0.3     2024-03-13 [2] RSPM (R 4.4.0)
##   IRanges            * 2.39.2    2024-07-17 [2] Bioconductor 3.20 (R 4.4.1)
##   irlba                2.3.5.1   2022-10-03 [2] RSPM (R 4.4.0)
##   iterators            1.0.14    2022-02-05 [2] RSPM (R 4.4.0)
##   jquerylib            0.1.4     2021-04-26 [2] RSPM (R 4.4.0)
##   jsonlite             1.8.9     2024-09-20 [2] RSPM (R 4.4.0)
##   KEGGREST             1.45.1    2024-06-17 [2] Bioconductor 3.20 (R 4.4.0)
##   knitr                1.48      2024-07-07 [2] RSPM (R 4.4.0)
##   labeling             0.4.3     2023-08-29 [2] RSPM (R 4.4.0)
##   later                1.3.2     2023-12-06 [2] RSPM (R 4.4.0)
##   lattice              0.22-6    2024-03-20 [3] CRAN (R 4.4.1)
##   lazyeval             0.2.2     2019-03-15 [2] RSPM (R 4.4.0)
##   lifecycle            1.0.4     2023-11-07 [2] RSPM (R 4.4.0)
```

```
##   limma                3.61.11   2024-09-26 [2] Bioconductor 3.20 (R 4.4.1)
##   lobstr             * 1.1.2     2022-06-22 [2] RSPM (R 4.4.0)
##   locfit               1.5-9.10  2024-06-24 [2] RSPM (R 4.4.0)
##   magick               2.8.5     2024-09-20 [2] RSPM (R 4.4.0)
##   magrittr             2.0.3     2022-03-30 [2] RSPM (R 4.4.0)
##   maps                 3.4.2     2023-12-15 [2] RSPM (R 4.4.0)
##   Matrix               1.7-0     2024-04-26 [3] CRAN (R 4.4.1)
##   MatrixGenerics     * 1.17.0    2024-05-01 [2] Bioconductor 3.20 (R 4.4.0)
##   matrixStats        * 1.4.1     2024-09-08 [2] RSPM (R 4.4.0)
##   memoise              2.0.1     2021-11-26 [2] RSPM (R 4.4.0)
##   metapod              1.13.0    2024-05-01 [2] Bioconductor 3.20 (R 4.4.0)
##   mime                 0.12      2021-09-28 [2] RSPM (R 4.4.0)
##   munsell              0.5.1     2024-04-01 [2] RSPM (R 4.4.0)
##   paletteer            1.6.0     2024-01-21 [2] RSPM (R 4.4.0)
##   pillar               1.9.0     2023-03-22 [2] RSPM (R 4.4.0)
##   pkgconfig            2.0.3     2019-09-22 [2] RSPM (R 4.4.0)
##   plotly               4.10.4    2024-01-13 [2] RSPM (R 4.4.0)
##   png                  0.1-8     2022-11-29 [2] RSPM (R 4.4.0)
##   prettyunits          1.2.0     2023-09-24 [2] RSPM (R 4.4.0)
##   promises             1.3.0     2024-04-05 [2] RSPM (R 4.4.0)
##   purrr                1.0.2     2023-08-10 [2] RSPM (R 4.4.0)
##   R6                   2.5.1     2021-08-19 [2] RSPM (R 4.4.0)
##   rappdirs             0.3.3     2021-01-31 [2] RSPM (R 4.4.0)
##   RColorBrewer         1.1-3     2022-04-03 [2] RSPM (R 4.4.0)
##   Rcpp                 1.0.13    2024-07-17 [2] RSPM (R 4.4.0)
##   RCurl                1.98-1.16 2024-07-11 [2] RSPM (R 4.4.0)
##   rematch2             2.1.2     2020-05-01 [2] RSPM (R 4.4.0)
##   restfulr             0.0.15    2022-06-16 [2] RSPM (R 4.4.0)
##   rjson                0.2.23    2024-09-16 [2] RSPM (R 4.4.0)
##   rlang                1.1.4     2024-06-04 [2] RSPM (R 4.4.0)
##   rmarkdown            2.28      2024-08-17 [2] RSPM (R 4.4.0)
##   Rsamtools            2.21.2    2024-09-26 [2] Bioconductor 3.20 (R 4.4.1)
##   RSQLite              2.3.7     2024-05-27 [2] RSPM (R 4.4.0)
##   rsvd                 1.0.5     2021-04-16 [2] RSPM (R 4.4.0)
##   rtracklayer        * 1.65.0    2024-05-01 [2] Bioconductor 3.20 (R 4.4.0)
##   S4Arrays             1.5.9     2024-09-26 [2] Bioconductor 3.20 (R 4.4.1)
##   S4Vectors          * 0.43.2    2024-07-17 [2] Bioconductor 3.20 (R 4.4.1)
##   sass                 0.4.9     2024-03-15 [2] RSPM (R 4.4.0)
##   ScaledMatrix         1.13.0    2024-05-01 [2] Bioconductor 3.20 (R 4.4.0)
##   scales               1.3.0     2023-11-28 [2] RSPM (R 4.4.0)
##   scater             * 1.33.4    2024-07-21 [2] Bioconductor 3.20 (R 4.4.1)
##   scran              * 1.33.2    2024-09-06 [2] Bioconductor 3.20 (R 4.4.1)
```

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##   scuttle              * 1.15.4    2024-08-14 [2] Bioconductor 3.20 (R 4.4.1)
##   sessioninfo            1.2.2     2021-12-06 [2] RSPM (R 4.4.0)
##   shiny                  1.9.1     2024-08-01 [2] RSPM (R 4.4.0)
##   shinyWidgets           0.8.7     2024-09-23 [2] RSPM (R 4.4.0)
##   SingleCellExperiment * 1.27.2    2024-05-24 [2] Bioconductor 3.20 (R 4.4.0)
##   spam                   2.10-0    2023-10-23 [2] RSPM (R 4.4.0)
##   SparseArray            1.5.40    2024-09-26 [2] Bioconductor 3.20 (R 4.4.1)
##   SpatialExperiment    * 1.15.1    2024-06-20 [2] Bioconductor 3.20 (R 4.4.0)
##   spatialLIBD          * 1.17.8    2024-07-25 [2] Bioconductor 3.20 (R 4.4.1)
##   statmod                1.5.0     2023-01-06 [2] RSPM (R 4.4.0)
##   STexampleData        * 1.13.3    2024-05-21 [2] Bioconductor 3.20 (R 4.4.0)
##   SummarizedExperiment * 1.35.1    2024-06-28 [2] Bioconductor 3.20 (R 4.4.1)
##   tibble                 3.2.1     2023-03-20 [2] RSPM (R 4.4.0)
##   tidyr                  1.3.1     2024-01-24 [2] RSPM (R 4.4.0)
##   tidyselect             1.2.1     2024-03-11 [2] RSPM (R 4.4.0)
##   UCSC.utils             1.1.0     2024-05-01 [2] Bioconductor 3.20 (R 4.4.0)
##   utf8                   1.2.4     2023-10-22 [2] RSPM (R 4.4.0)
##   uwot                   0.2.2     2024-04-21 [2] RSPM (R 4.4.0)
##   vctrs                  0.6.5     2023-12-01 [2] RSPM (R 4.4.0)
##   vipor                  0.4.7     2023-12-18 [2] RSPM (R 4.4.0)
##   viridis                0.6.5     2024-01-29 [2] RSPM (R 4.4.0)
##   viridisLite            0.4.2     2023-05-02 [2] RSPM (R 4.4.0)
##   withr                  3.0.1     2024-07-31 [2] RSPM (R 4.4.0)
##   xfun                   0.47      2024-08-17 [2] RSPM (R 4.4.0)
##   XML                    3.99-0.17 2024-06-25 [2] RSPM (R 4.4.0)
##   xtable                 1.8-4     2019-04-21 [2] RSPM (R 4.4.0)
##   XVector                0.45.0    2024-05-01 [2] Bioconductor 3.20 (R 4.4.0)
##   yaml                   2.3.10    2024-07-26 [2] RSPM (R 4.4.0)
##   zlibbioc               1.51.1    2024-06-05 [2] Bioconductor 3.20 (R 4.4.0)
##
##   [1] /tmp/RtmplzBduz/Rinst8c3c9f946d
##   [2] /usr/local/lib/R/site-library
##   [3] /usr/local/lib/R/library
##
##  ------------------------------------------------------------------------------------------
```

References

A Related resources

A.1 Overview

A.2 Data preprocessing procedures for the Visium platform

- Visium data preprocessing

A.3 Resources for other spatial omics platforms

- Analysis workflow for IMC data

- VectraPolarisData

A.4 Data structures

3

- **AnnData** scverse
- **SpatialData**

A.5 Statistical concepts

- **Modern Statistics for Modern Biology**

  chapter on image

  data and spatial statistics

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B.1 Contributors

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- Leonardo Collado-Torres
- Stephanie C. Hicks

GitHub contributors

B.2 Additional acknowledgments

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- Bioconductor Bioconductor Core Team

References