Standardised and reproducible analysis of mass spectrometry-based single-cell proteomics data

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Outline

State of the art in MS-SCP

MS-SCP: Mass spectrometry-based single-cell proteomics
MS-SCP consist of shotgun proteomics at single-cell level

- SCoPE2 quantifies thousands of proteins x thousands single-cells
- Full protocole available
- Full analysis script available

BUT

Lack of standardized analysis software

Our objectives

Provide a suite of software package dedicated to MS-SCP that fulfill:

- User-friendly
- Computationaly efficient
- Modularity: integrate other software packages
- Promote reproducibility
- Platform-independent
- Free of charge

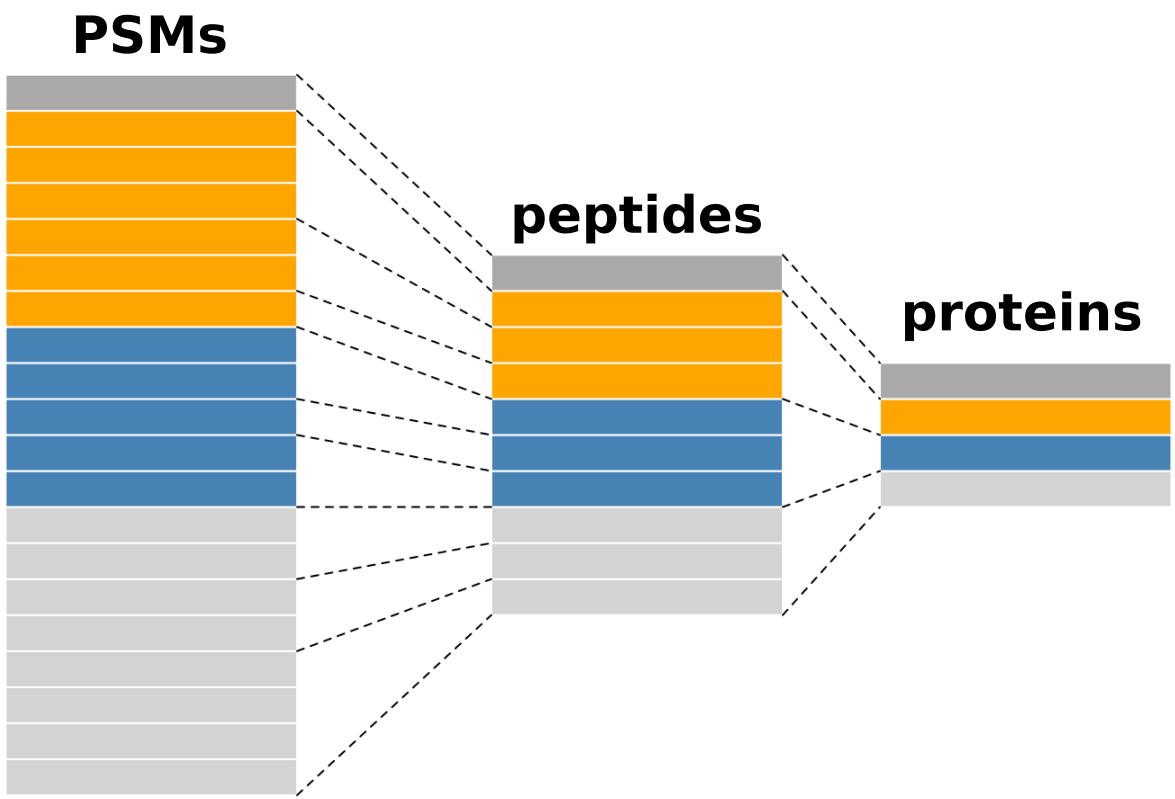
R/Bioconductor is an ideal environment

Outline

Data infrastructure: QFeatures[1]

scp package

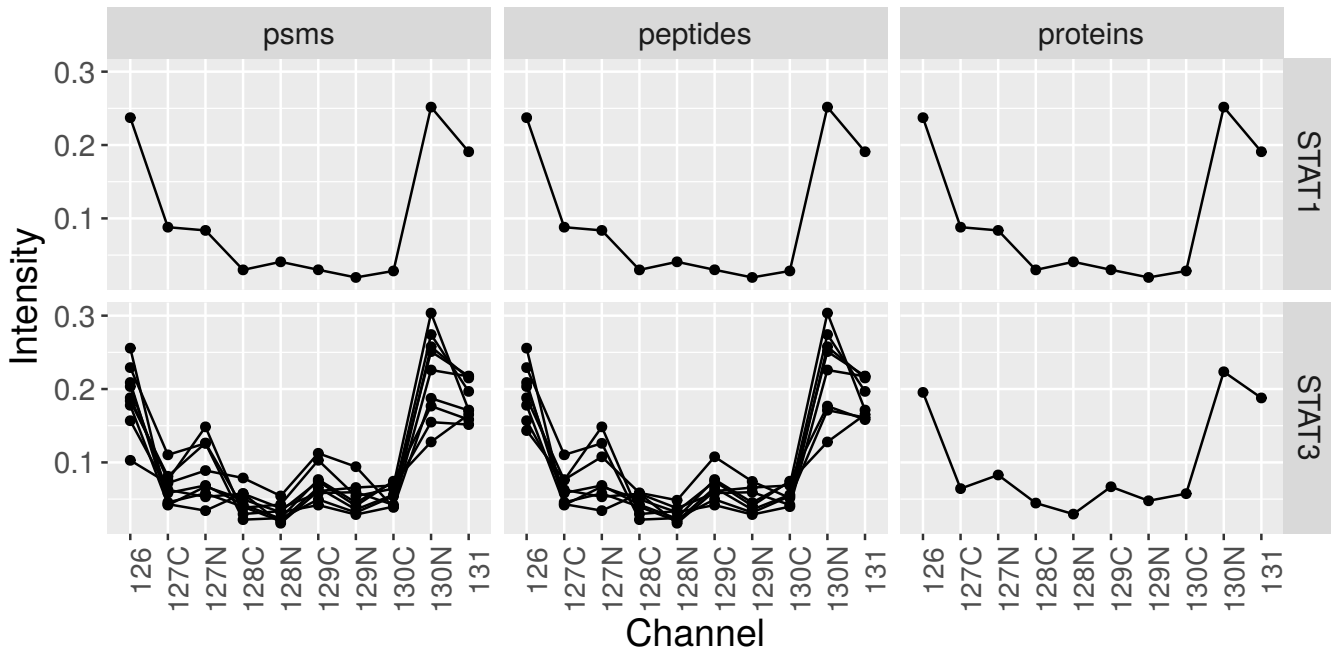
`QFeatures`: data framework dedicated to manipulate and process MS-based quantitative data.



[1] Gatto (2020)

Data infrastructure: QFeatures[1]

`QFeatures`: data framework dedicated to manipulate and process MS-based quantitative data.

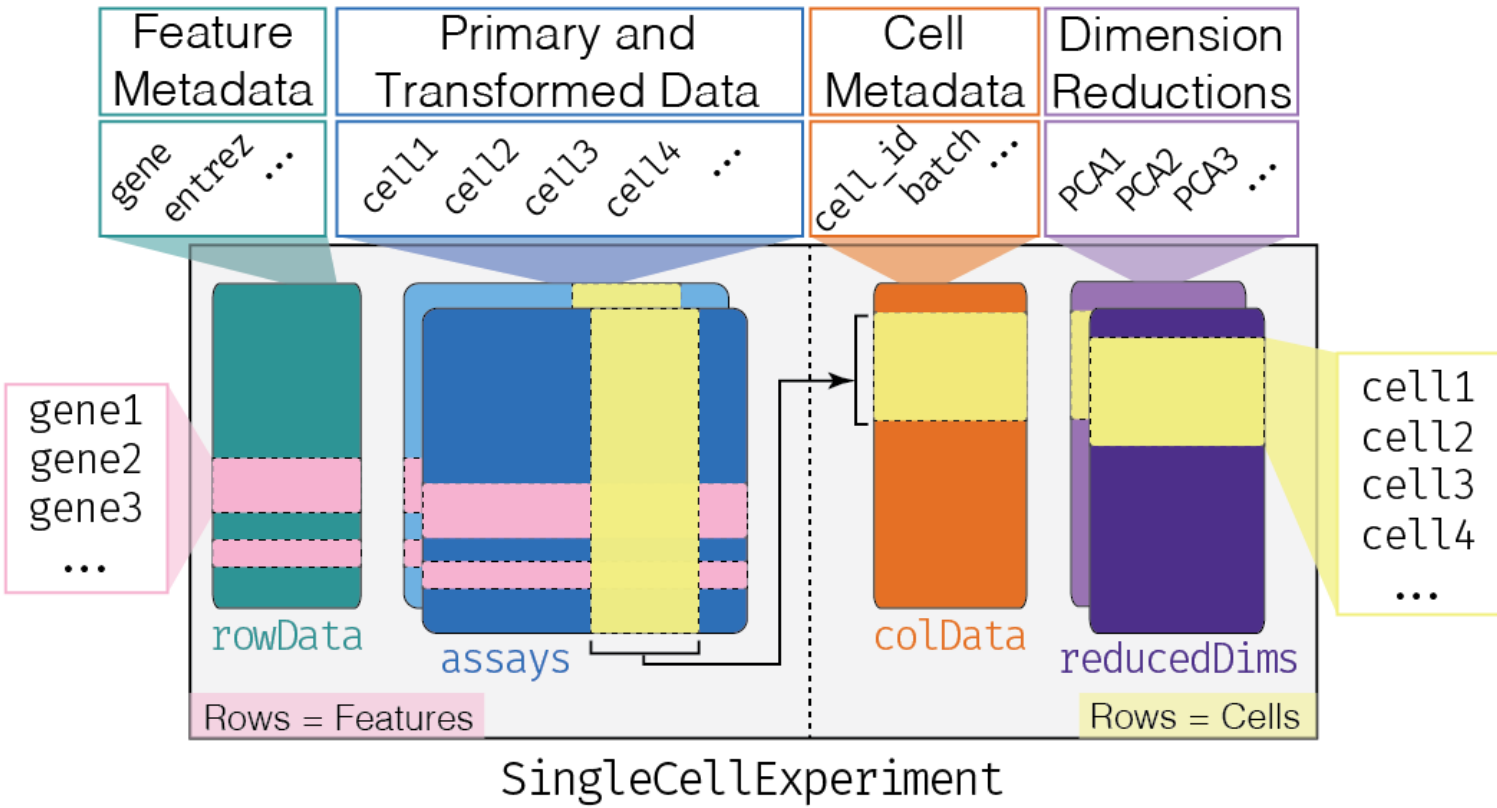


[1] Gatto (2020)

Data infrastructure: SingleCellExperiment[2,3]

scp package

`SingleCellExperiment`: provides dedicated framework for single-cell data analysis.

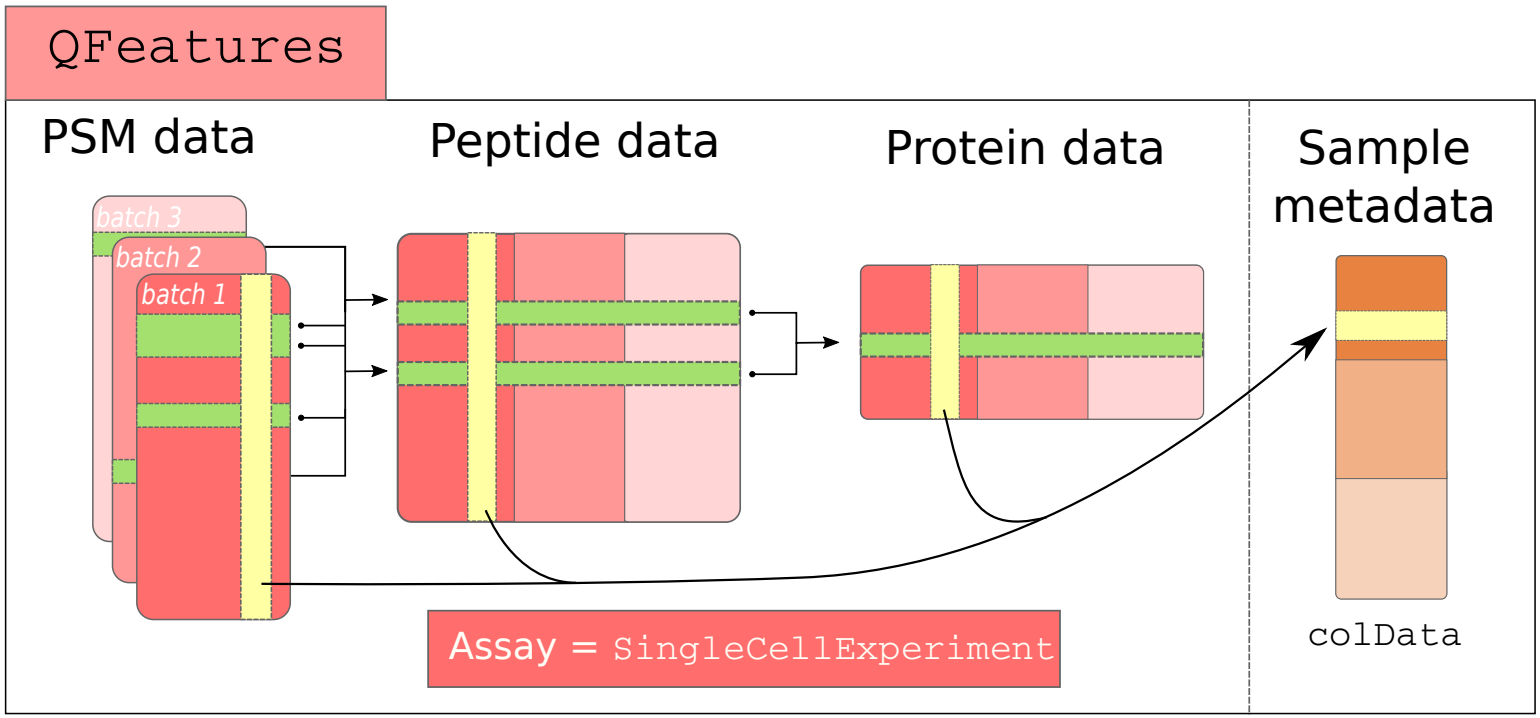


[2] Lun and Risso (2020)

[3] Amezquita et al. (2019)

Data infrastructure (3)

`scp` = `SingleCellExperiment` + `QFeatures`



Load data

Load the SCoPE2 dataset called `specht2019v2`

```
library(scpdata)
data("specht2019v2")
```

Dataset overview

```
show(specht2019v2)
```

```
An instance of class QFeatures containing 179 assays:
 [1] 190222S_LCA9_X_FP94AA: SingleCellExperiment with 2823 rows and 11 col...
 [2] 190222S_LCA9_X_FP94AB: SingleCellExperiment with 4297 rows and 11 col...
 [3] 190222S_LCA9_X_FP94AC: SingleCellExperiment with 4956 rows and 11 col...
 ...
 [177] 191110S_LCB7_X_APNOV16plex2_Set_9: SingleCellExperiment with 4626 r...
 [178] peptides: SingleCellExperiment with 9208 rows and 1018 columns
 [179] proteins: SingleCellExperiment with 2772 rows and 1018 columns
```

Dataset generated from MaxQuant output file using `readSCP()`

Metadata

`colData` stores sample metadata for **all assays** in one table

Set	Channel	SampleType	lcbatch	sortday	digest
190222S_LCA9_X_FP94AA	RI1	Carrier	LCA9	s8	N
190222S_LCA9_X_FP94AA	RI2	Reference	LCA9	s8	N
190222S_LCA9_X_FP94AA	RI3	Unused	LCA9	s8	N
190222S_LCA9_X_FP94AA	RI4	Macrophage	LCA9	s8	N
190222S_LCA9_X_FP94AA	RI5	Macrophage	LCA9	s8	N
190222S_LCA9_X_FP94AA	RI6	Macrophage	LCA9	s8	N
...	...	...	...	...	...

Analysis workflow

1. Load data

PSM data

```
[1] 190222S_LCA9_X_FP94AA: SingleCellExperiment with 2823 rows and 11 columns
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```

2. PSM filtering
3. Expression channel by reference channel division
4. PSM to peptides aggregating
5. Single cells filtering based on median CV
6. Normalization
7. Removal of highly missing peptides
8. Log-transformation

Peptide data

```
[178] peptides: SingleCellExperiment with 9208 rows and 1018 columns
```

Analysis workflow

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Peptide data

```
[178] peptides: SingleCellExperiment with 9208 rows and 1018 columns
```

9. Peptides to proteins aggregation
10. Normalization
11. Imputation
12. Batch correction

Peptide data

```
[179] proteins: SingleCellExperiment with 2772 rows and 1018 columns
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Analysis workflow

1. Load data

PSM data

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```

2. **PSM filtering**
3. Expression channel by reference channel division
4. PSM to peptides aggregating
5. **Single cells filtering based on median CV**
6. Normalization
7. **Removal of highly missing peptides**
8. **Log-transformation**

Peptide data

```
[178] peptides: SingleCellExperiment with 9208 rows and 1018 columns
```

9. **Peptides to proteins aggregation**
10. Normalization
11. Imputation
12. **Batch correction**

Peptide data

```
[179] proteins: SingleCellExperiment with 2772 rows and 1018 columns
```

Outline

PSM filtering

Filter out features based on the feature metadata

Example: filter out reverse hits. The filter is applied to the `Reverse` field in the feature metadata

```
filterFeatures(specht2019v2,
               ~ Reverse != "+")
```

Source code in `QFeatures`

QC metrics (1)

Interesting metrics for MS-SCP quality control:

- Sample to carrier ratio: ratio of the carrier channel intensity signal over the sample channel intensity
- Peptide FDR[4]: expected rate of wrongly assigned features to a given peptide
- Cell median CV[5]: reliability of the protein quantification summarized over each cell.

Example:

```
computeMedianCV(specht2019v2,
                i = "peptides",
                proteinCol = "protein",
                peptideCol = "peptide",
                batchCol = "Set")
```

Source code in `scp`

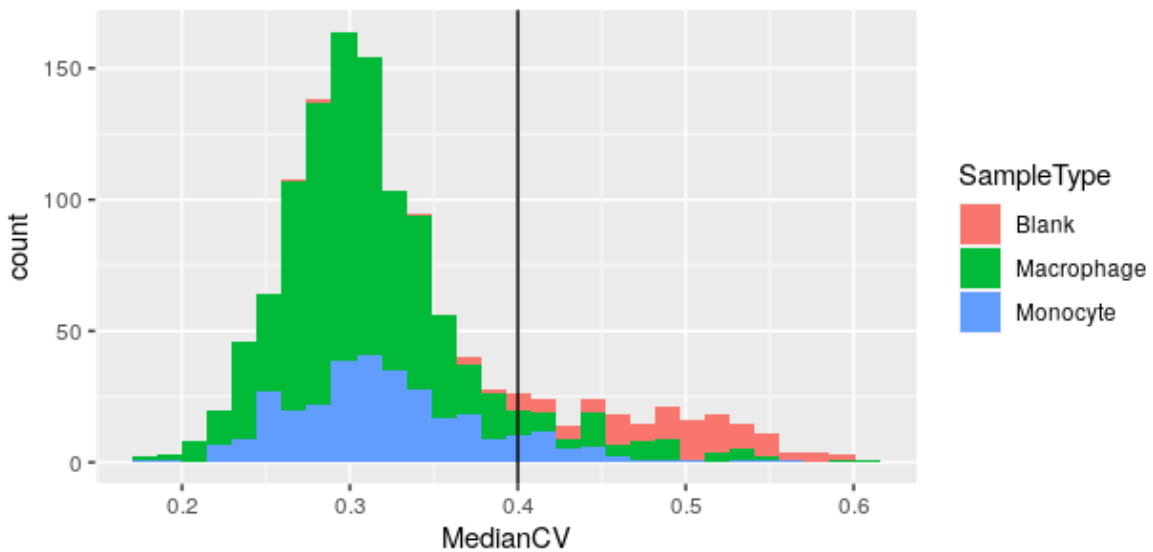
[4] false discovery rate

[5] coefficient of variation

QC metrics (2)

QC metrics are stored in the data set for plotting or subsetting

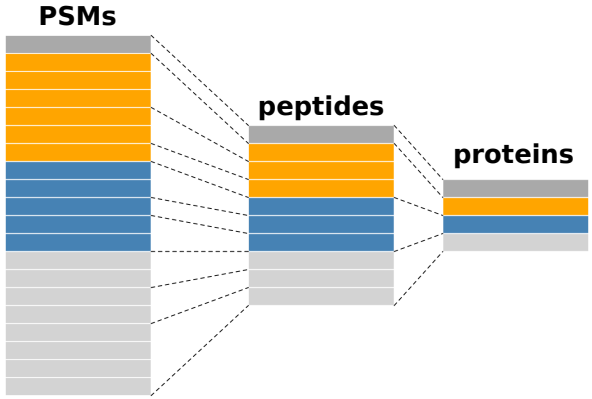
```
library(tidyverse)
specht2019v2[["peptides"]] %>%
  colData %>%
  data.frame %>%
  ggplot(aes(x = MedianCV,
             fill = SampleType)) +
  geom_histogram() +
  geom_vline(xintercept = 0.4)
```



Feature aggregation

Feature aggregation includes 2 steps:

- Combine the quantiative data from multiple features to a single aggregated features
- Store the relationship between the parent features and the aggregated features



Example: aggregate peptides to proteins

```
aggregateFeatures(specht2019v2,
                  i = "peptides",
                  name = "proteins",
                  fcol = "protein",
                  fun = colMedians, na.rm = TRUE)
```

Source code in `QFeatures`

Managing missingness

`0`'s can be either **biological** or **technical** zero. They are better relaced by `NA`'s.

```
zeroIsNA(specht2019v2,
         i = "peptides")
```

Features containing too many missing data (e.g. $>= 99$ %) should be removed

```
filterNA(specht2019v2,
         i = "peptides",
         pNA = 0.99)
```

Source code in `QFeatures`

Data transformation

Common data transformation can easily be applied:

- Normalization
- Log-transformation
- Imputation

Example: log_2-transformation:

```
logTransform(specht2019v2,
             i = "peptides",
             base = 2,
             name = "peptides_log")
```

Source code in `QFeatures`

Custom functions

Some custom function can be applied to the data set too. Example: batch correction using `sva::ComBat`. First, extract the data

```
sce <- specht2019v2[["proteins"]]
```

Build the correction matrix and apply the ComBat algorithm

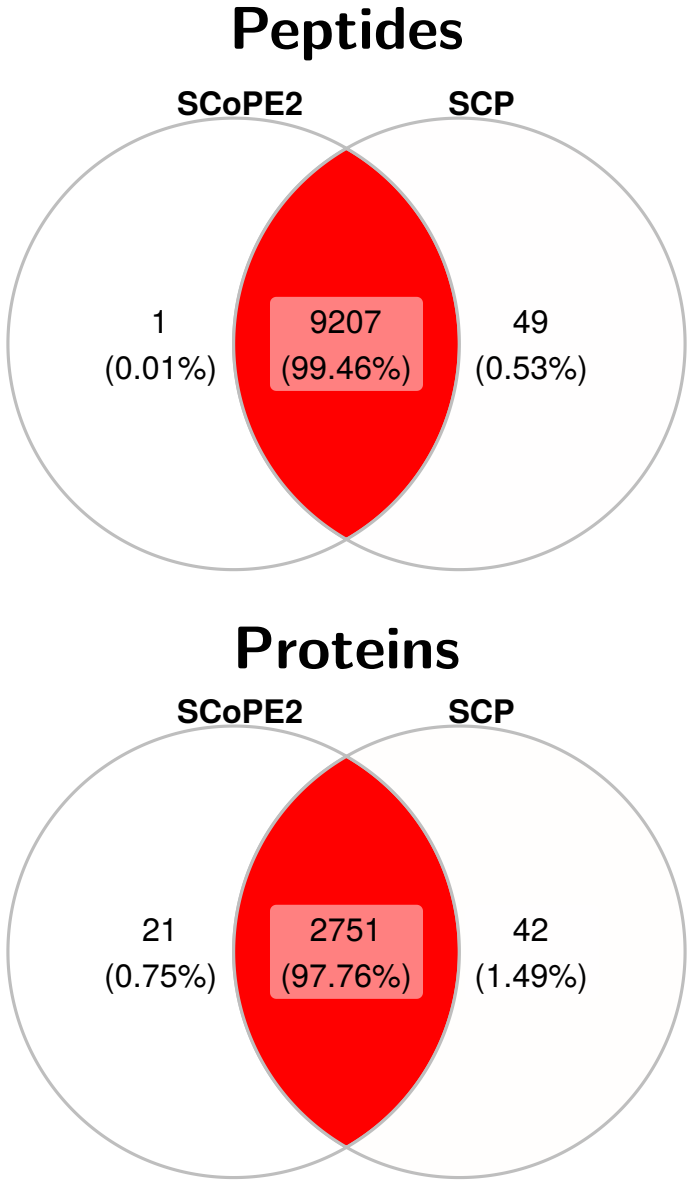
```
batch <- colData(sce)$Set
model <- model.matrix(~ SampleType, data = colData(sce))
assay(sce) <- ComBat(dat = assay(sce),
                     batch = batch,
                     mod = model)
```

Add the corrected protein to the dataset and keep feature relationships

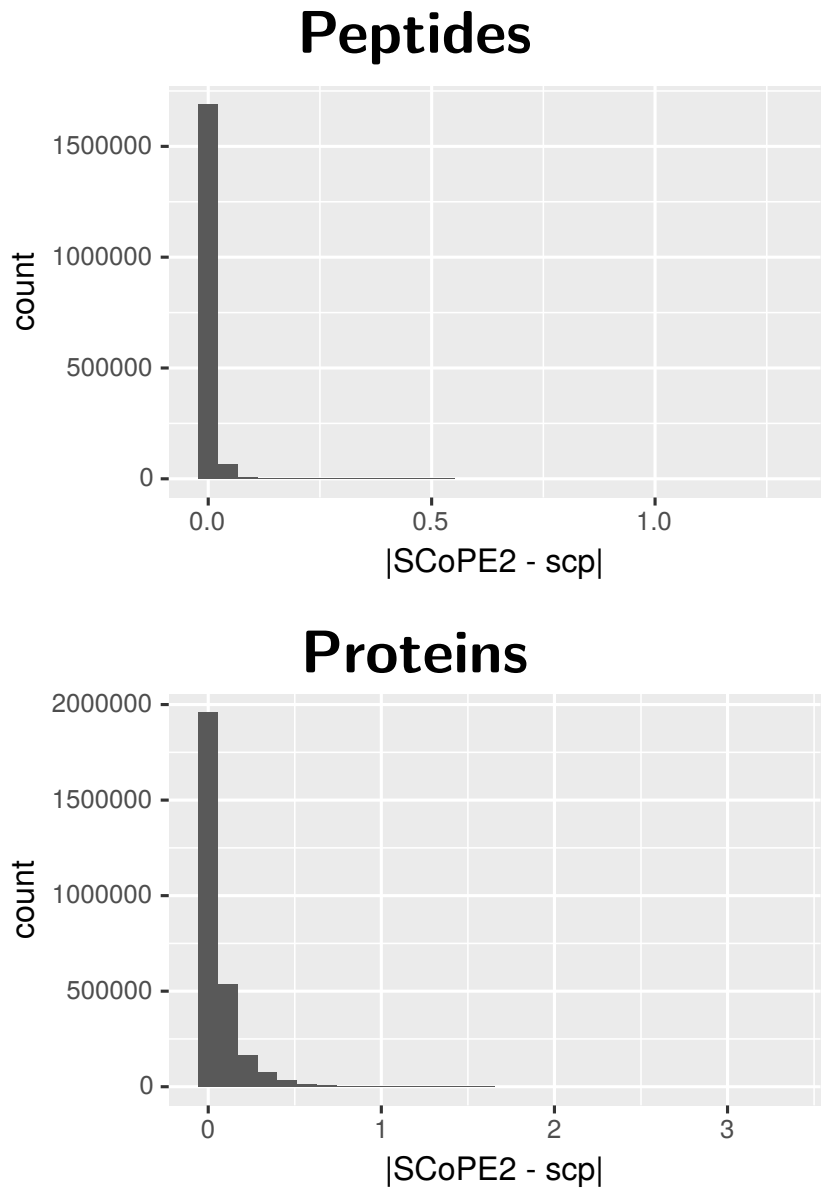
```
addAssay(specht2019v2,
         sce,
         name = "proteins_batchC") %>%
addAssayLinkOneToOne(from = "proteins",
                     to = "proteins_batchC")
```

Outline

Selected features

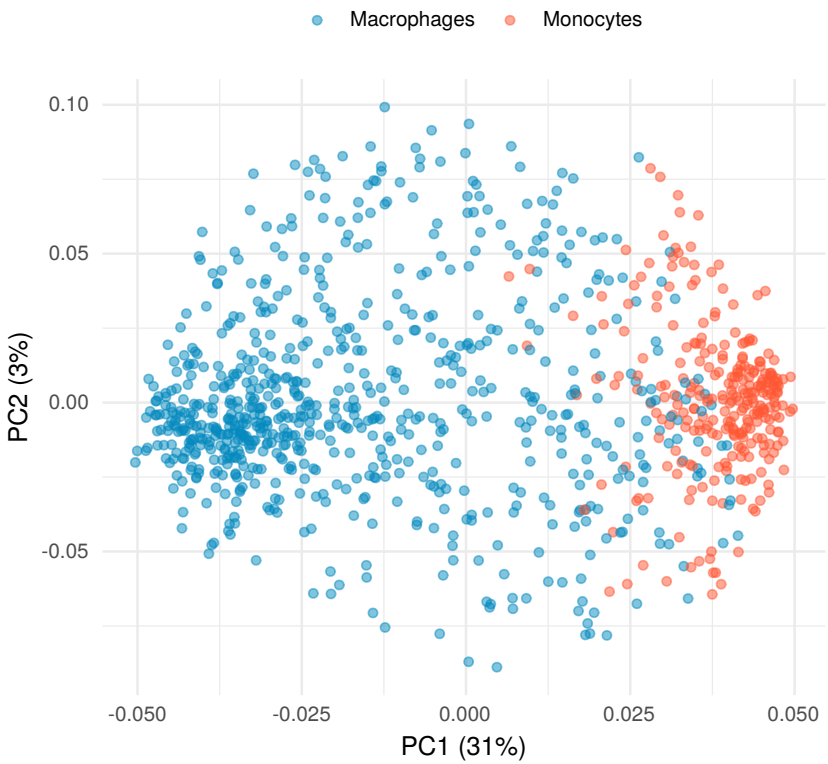


Numerical comparison

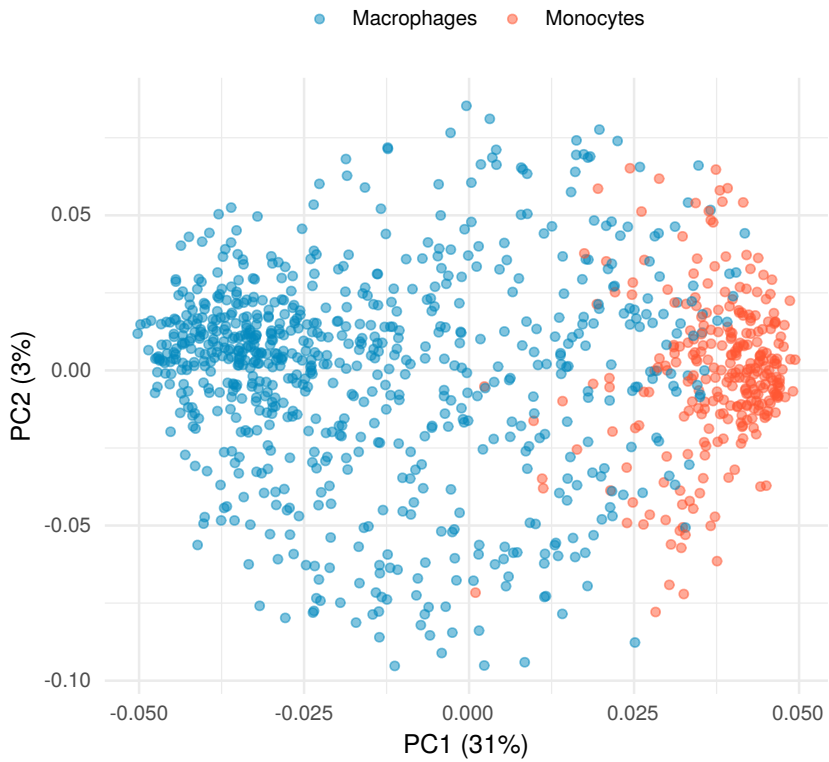


Replicate weighted PCA

SCoPE2



scp

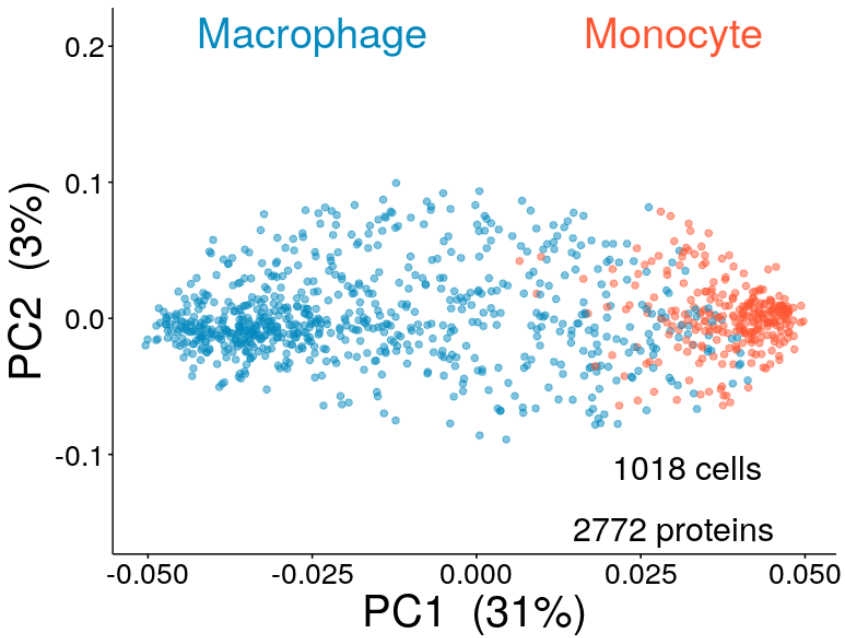


Note about replication

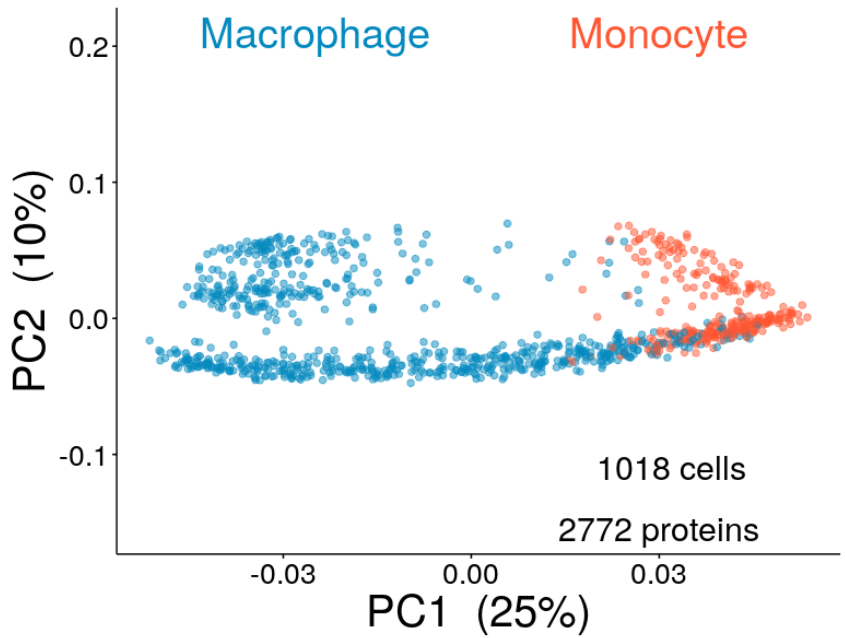
Versioning is essential for replication

Example: batch correction using the `ComBat` algorithm from `sva`

`sva 3.34.0`



`sva 3.36.0`



`scp` is an ideal framework for combining SCP data with other omics measures. Example: SCP data + scRNA-Seq data.

```
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 [178] peptides: SingleCellExperiment with 9208 rows and 1018 columns
 [179] proteins: SingleCellExperiment with 2772 rows and 1018 columns
 [180] rna_processed: SingleCellExperiment with 2205 rows and 1018 columns
```

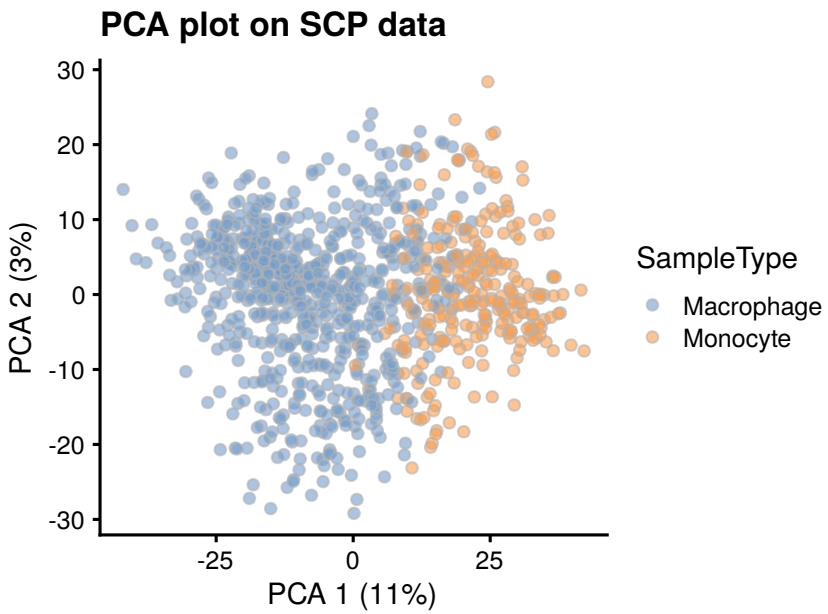

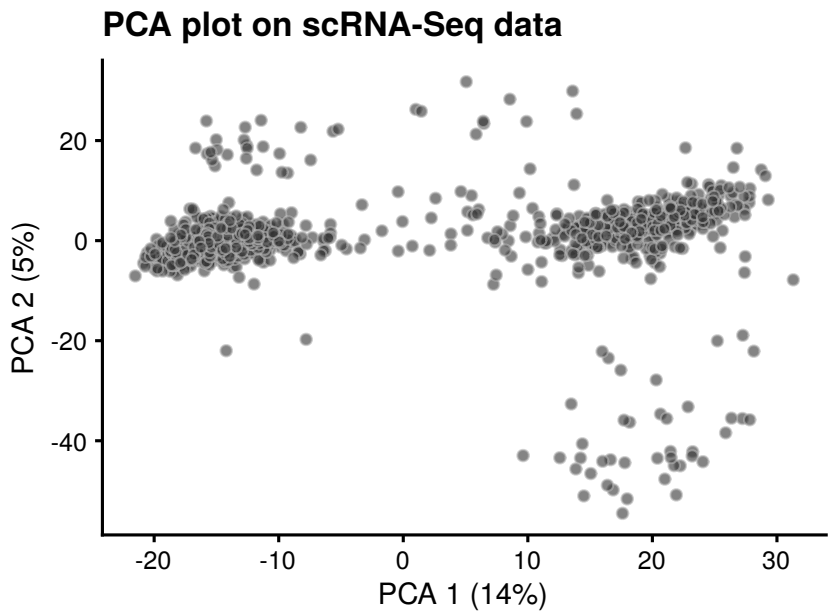

Outline

Missingness

Take home message

- `scp` package suite provides a standardized environment for performing MS-SCP data analysis
- Flexibly reproduce existing analyses from different groups or protocoles (multiplex vs label free)

Advantages:

- Allow automation of the analysis
- Facilitate new computational developments
- Promotes reproducibility
- Increases field visibility
- Include other modalities: scRNA-Seq, ATAC-Seq, etc

Resources

Packages

- `scp`: GitHub repository `UClouvain-CBIO/scp`
- `scpdata`: coming soon
- `QFeatures`: GitHub repository `rformassspectrometry/QFeatures`
- `SingleCellExperiment`: Bioconductor

SCoPE2 reproduction vignette

Available at...

Slides and source code

Available at...

Acknowledgements

References I

Robert A Amezquita, Aaron T L Lun, Etienne Becht, Vince J Carey, Lindsay N Carpp, Ludwig Geistlinger, Federico Martini, Kevin Rue-Albrecht, Davide Risso, Charlotte Soneson, Levi Waldron, Hervé Pagès, Mike L Smith, Wolfgang Huber, Martin Morgan, Raphael Gottardo, and Stephanie C Hicks. Orchestrating single-cell analysis with bioconductor. *Nat. Methods*, pages 1–9, December 2019.

Laurent Gatto. *QFeatures: Quantitative features for mass spectrometry data*, 2020. URL `https://github.com/RforMassSpectrometry/QFeatures`. R package version 0.7.0.

Aaron Lun and Davide Risso. *SingleCellExperiment: S4 Classes for Single Cell Data*, 2020. R package version 1.10.1.