

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

Replication of the SCoPE2 analysis by Specht et al. 2019

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18 August 2020

Outline

Introduction

scp package

scp showcase

Replication results

Conclusion

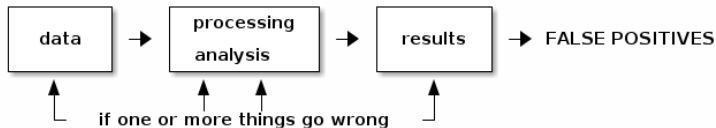
► Expectation



► Expectation



► Reality



- ▶ **Replication-based development** agreement between the developer, the data producer and the user.

Replication is the first step to define sound data infrastructure and principled analysis.

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

- ▶ Contribute a standardised and principled data and analysis that is broadly applicable.
- ▶ Open, transparent and reproducible computational infrastructure to further improve data analysis and interpretation.
- ▶ R and Bioconductor (Huber et al. (2015)) offer an ideal environment to attain these goals.

Implemented in the `scp` package.

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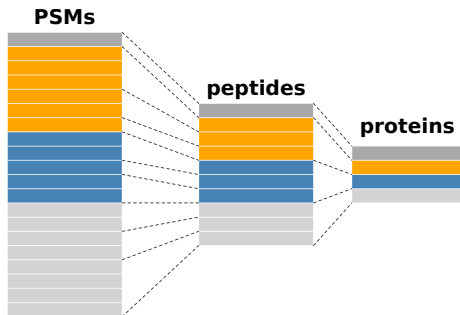
Replication results

Conclusion

Data infrastructure: QFeatures¹

scp package

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

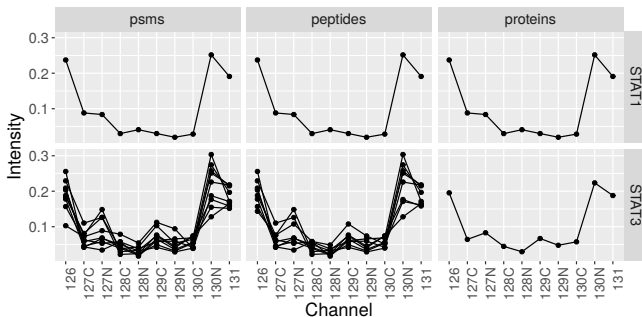
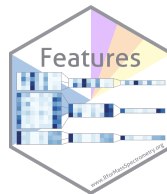


¹Gatto (2020)

Data infrastructure: QFeatures¹

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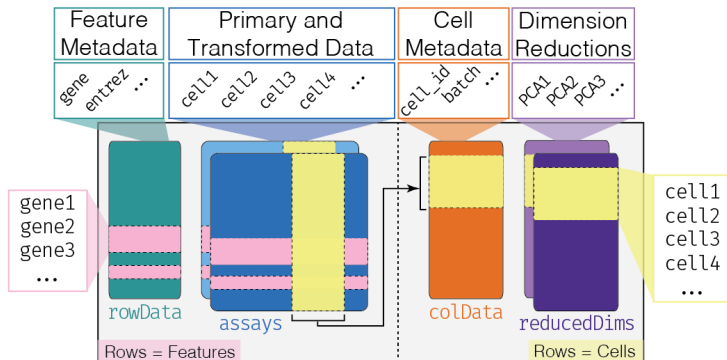
¹Gatto (2020)

Data infrastructure: SingleCellExperiment^{2,3}

scp package



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

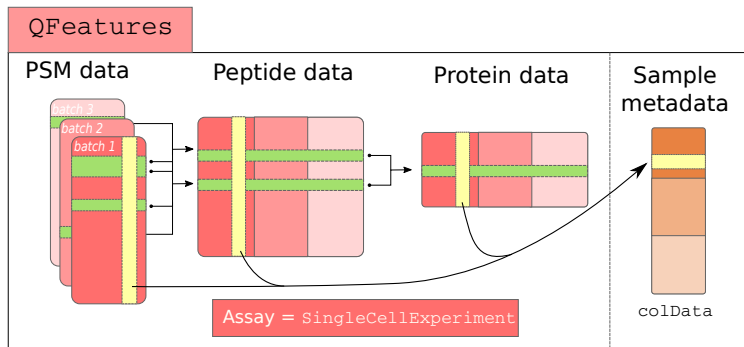


SingleCellExperiment

²Lun and Risso (2020)

³Amezquita et al. (2019)

`scp` = `SingleCellExperiment` + `QFeatures`



AND functions dedicated to processing and analyzing SCP data.

Load the SCoPE2 dataset called `specht2019v2`⁴

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

Dataset overview

```
1 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
```

Tabular data (generated by MaxQuant, ProteomeDiscoverer, ...) can be converted to `QFeatures` using the `readSCP()` function.

⁴Specht et al. (2019)

Sample metadata common to **all assays** are stored in a single table, the `colData`

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
...

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 aggregation
5. Joining sets
6. Single cells filtering based on median CV
7. Normalization
8. Removal of highly missing peptides
9. Log-transformation

Peptide data

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12. Batch correction

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

Conclusion

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

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

Source code in `QFeatures`

Some QC metrics are not computed by MaxQuant:

- ▶ Sample to carrier ratio: discard samples with intensities higher than expected
- ▶ Peptide FDR: expected proportion of features wrongly assigned to a given peptide
- ▶ Cell median coefficient of variation: reliability of the protein quantification in a cell.

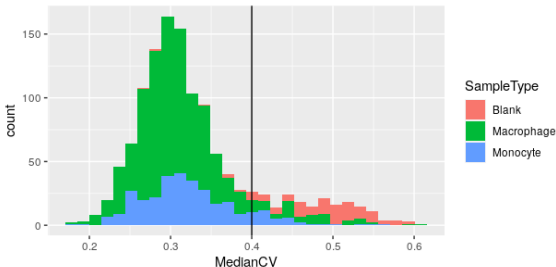
Example:

```
1 computeMedianCV(specht2019v2,
2                 i = "peptides",
3                 proteinCol = "protein",
4                 peptideCol = "peptide",
5                 batchCol = "Set")
```

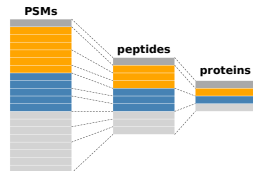
Source code in scp

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

```
1 library(tidyverse)
2 specht2019v2[["peptides"]] %>%
3   colData %>%
4   data.frame %>%
5   ggplot(aes(x = MedianCV,
6             fill = SampleType)) +
7   geom_histogram() +
8   geom_vline(xintercept = 0.4)
```



Example: aggregate peptides to proteins



- ▶ Combine the quantitative data from multiple peptides to a single protein
- ▶ Store the relationship between the protein and the aggregated peptides

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

Source code in `QFeatures`

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

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

Remove highly-missing features (e.g. $\geq 99\%$)

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

Impute missing data

```
1 impute(specht2019v2,  
2       i = "proteins",  
3       method = "knn",  
4       k = 3)
```

Source code in `QFeatures`

Common data transformation can easily be applied such as **log-transformation** or **normalization**.

Example: \log_2 -transformation:

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

Source code in `QFeatures`

Custom function can be applied to the data set, for example batch correction using 'ComBat'. Three-step procedure:

1. Extract the assay to process

```
1 x <- specht2019v2[["proteins"]]
```

2. Apply the custom function

```
2 assay(x) <- ComBat(assay(x), ...)
```

3. Add the processed assay in the dataset

```
3 addAssay(specht2019v2, x, name = "proteins_batch_corrected")
```

Outline

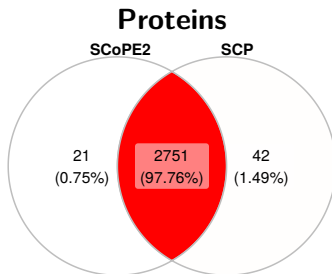
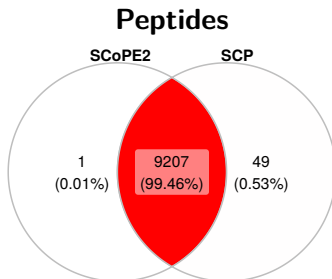
Introduction

scp package

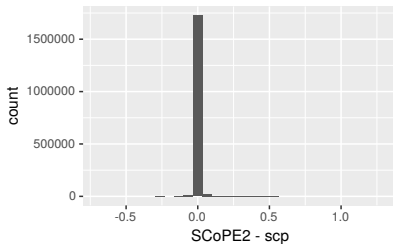
scp showcase

Replication results

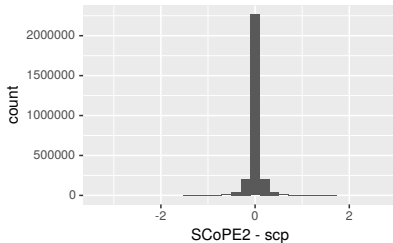
Conclusion



Peptides



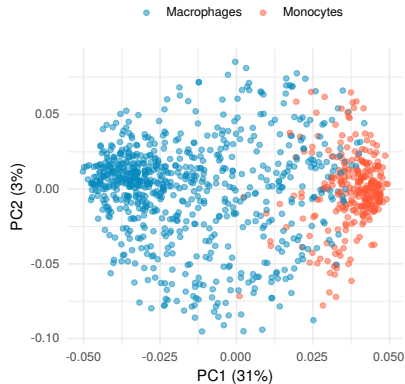
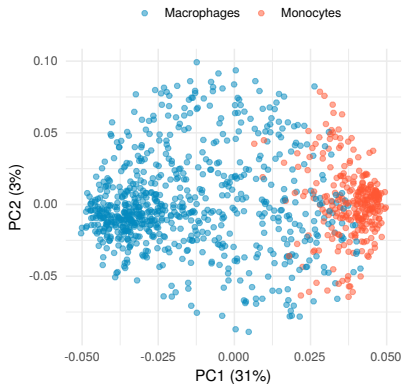
Proteins



Weighted PCA on the **protein** data

SCoPE2 (Figure 3a in preprint)

scp

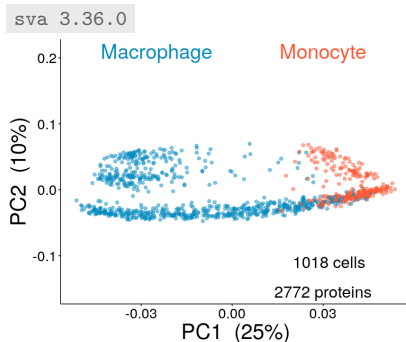
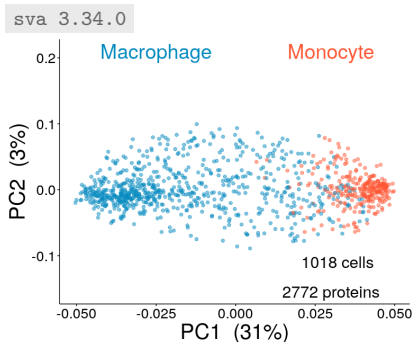


Note about reproducibility

Replication results

Good software development includes continuous maintenance and improvement **BUT** might impact reproducibility

Example: batch correction using 2 versions of the **ComBat** algorithm
(**sva** package)



Documenting software version is **essential** for reproducible work

Outline

Introduction

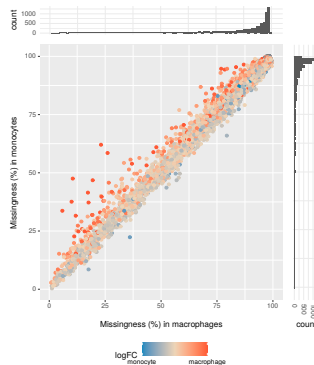
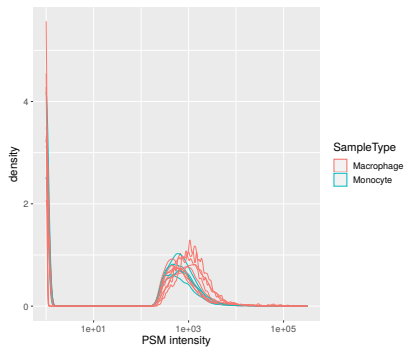
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Conclusion

- ▶ MS-based single cell proteomics: young field, with many challenges and great progress. `scp` to address the need for principled and reproducible data analysis.
- ▶ `scp` isn't specific to SCoPE2/TMT data, applicable to other LF protocols such as nanoPOTS (Williams et al. (2020); Cong et al. (2020)).
- ▶ `scp` and `SingleCellExperiment`: same infrastructure for single cell proteomics and RNA sequencing.
- ▶ Tool for novel computational developments.



Resources

- ▶ `scp`: <http://UClouvain-CBIO.github.io/scp>
- ▶ `scpdata`: coming soon
- ▶ `QFeatures`: <http://rformassspectrometry.org>
- ▶ `SingleCellExperiment`: Bioconductor
- ▶ **Slides**: <http://bit.ly/2020SCP> under CC-BY SA

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Acknowledgements

- ▶ Nikolai Slavov, Harrison Specht, Ed Emmott.
- ▶ Fonds National de la Recherche Scientifique (FNRS)
- ▶ **Thank you for your attention**

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Sarah M Williams, Andrey V Liyu, Chia-Feng Tsai, Ronald J Moore, Daniel J Orton, William B Chrisler, Matthew J Gaffrey, Tao Liu, Richard D Smith, Ryan T Kelly, Ljiljana Paša-Tolić, and Ying Zhu. Automated coupling of nanodroplet sample preparation with liquid Chromatography-Mass spectrometry for High-Throughput Single-Cell proteomics. *Anal. Chem.*, July 2020.