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

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Summary

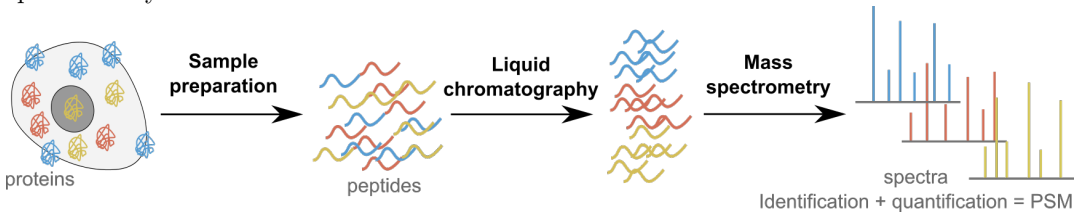
Recent advances in sample preparation, processing and mass spectrometry (MS) have enabled the emergence of MS-based single-cell proteomics (SCP). We have developed a computational framework by means of two Bioconductor packages to standardize SCP data analysis. It will facilitate the development of dedicated algorithms, for instance to deal with missingness and batch effects that are characteristic of this type of data, and will promote the reproducibility of further SCP data analyses.

Take home message

The amount of available MS-based SCP data is rapidly increasing and so is the need for dedicated analysis software. We offer two R/Bioconductor packages. The first package, `scpdata`, disseminates curated SCP data sets for method development and benchmarking. The second package, `scp`, implements functions to streamline the analysis of SCP data. This work provides the ground for reproducible and rigorous development and benchmarking of new state-of-the-art methods.

SCP technology

SCP is enabled by recent advances in sample preparation, liquid chromatography and mass spectrometry.

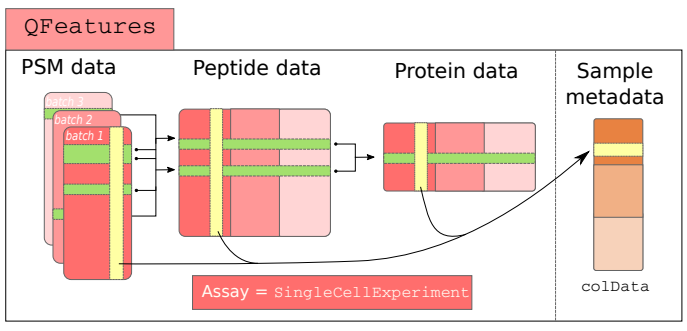


- **Label-free acquisition**: **low throughput** (∼ 15 samples/day) and **low identification** rate, but **accurate quantification** (*e.g.* Zhu et al. 2019 [1])
- **Multiplexed acquisition**: **high throughput** (∼ 200 samples/day), but label **cross-contamination** (*e.g.* SCoPE2 by Specht et al. 2020 [2])

Both methods generate data with high **missingness** and considerable **batch effects**.

SCP data framework

SCP data framework = `SingleCellExperiment` [3] + `QFeatures` [4]



`scp`

`scp` implements functions to streamline the analysis of SCP data. This code chunk partially reproduces the SCoPE2 analysis by Specht et al. 2020 [2].

```
readSCP(quantTable = quantData, metaTable = metaData,
        channelCol = "Channel", batchCol = "Set") %>%
    zeroIsNA(i = 1:4) %>%
    filterFeatures(~ Reverse != "+" & Potential.contaminant != "+") %>%
    subsetByAssay(dims(.)[1, ] > 150) %>%
    computeSCR(i = 1:3, colDataCol = "SampleType",
               carrierPattern = "Carrier",
               samplePattern = "Macrophage|Monocyte") %>%
    filterFeatures(~ !is.na(.meanSCR) & .meanSCR < 0.1) %>%
    aggregateFeaturesOverAssays(i = 1:3, fcol = "peptide",
                                name = paste0("peptides_", names(.)),
                                fun = robustSummary) %>%
    joinAssays(i = 4:6, name = "peptides") %>%
    computeMedianCV(i = "peptides_filter1", proteinCol = "protein",
                    peptideCol = "peptide", batchCol = "Set") %>%
    normalize(i = "peptides", name = "peptides_norm",
              method = "median", na.rm = TRUE) %>%
    logTransform(i = "peptides_norm", name = "peptides_log",
                 base = 2) %>%
    aggregateFeatures(i = "peptides_log", name = "proteins",
                      fcol = "protein", robustSummary) ->
    scp
```

`scpdata`

`scpdata` disseminates curated SCP data sets for method development and benchmarking.

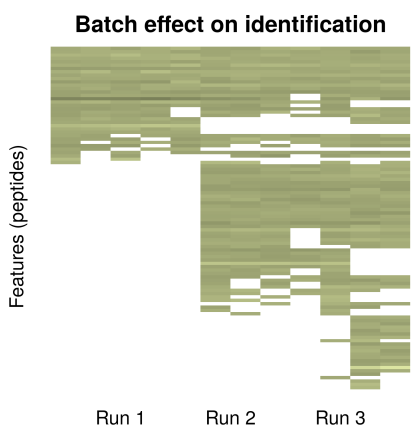
	Title	Description	Species	Date	# Assays
1	specht2019v2	Specht et al. 2019: macrophage...	Homo sapiens	2019-12-05	179
2	specht2019v3	Specht et al. 2019: macrophage...	Homo sapiens	2019-10-04	179
3	dou2019_lysates	Dou et al. 2019: HeLa lysates ...	Homo sapiens	2019-10-15	3
4	dou2019_mouse	Dou et al. 2019: single cells ...	Mus musculus	2019-10-15	13
5	dou2019_boosting	Dou et al. 2019: testing boost...	Mus musculus	2019-10-15	7
6	zhu2018MCP	Zhu et al. 2018 (Mol. Cel. Pro...	Rattus norvegicus	2018-09-01	1
7	zhu2018NC_hela	Zhu et al. 2018 (Nat. Comm.): ...	Homo sapiens	2018-02-28	1
8	zhu2018NC_lysates	Zhu et al. 2018 (Nat. Comm.): ...	Homo sapiens	2018-02-28	1
9	zhu2018NC_islets	Zhu et al. 2018 (Nat. Comm.): ...	Homo sapiens	2018-02-28	1
10	cong2020AC	Cong et al. 2020 (Ana. Chem.):...	Homo sapiens	2020-01-02	9
11	zhu2019EL	Zhu et al. 2019 (eLife): chick...	Gallus gallus	2020-11-04	62

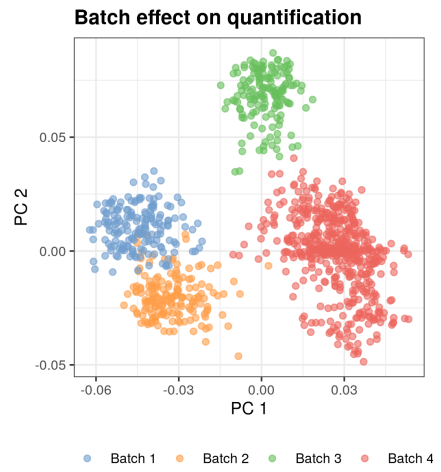
Batch effects

Batch effects are inherent to SCP data since many samples have to be distributed across **different MS runs**.

- Multiplexed acquisition: 1 run ∼ 4-10 cells
- Label-free acquisition: 1 batch = 1 cell

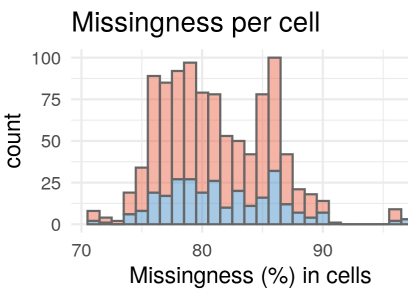
Batch effects impact the peptide **identification** (missingness) and **quantification**

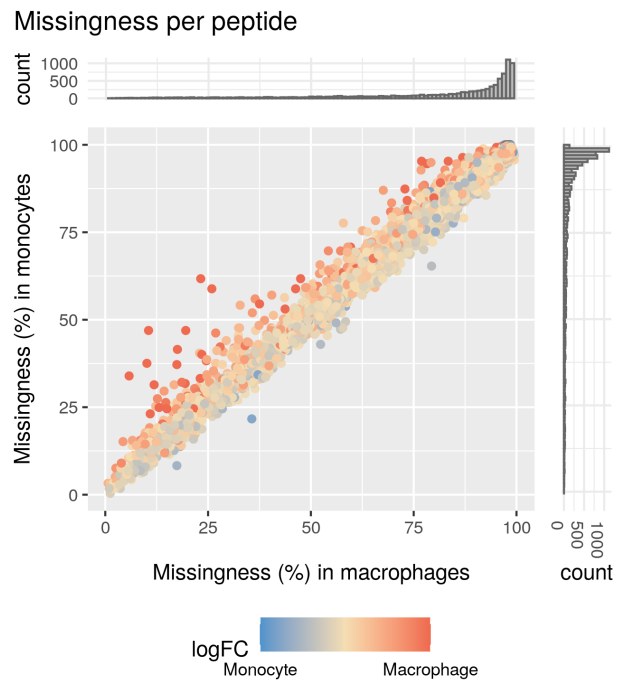




Missingness

- **Biological missingness**: some proteins are not expressed in all cells
- **Technical missingness**: low amounts of sample material, instrument sensitivity and batch effects
- **Both**: some proteins are harder to identify (low expression) in one cell type





Reproducibility

`scp` provides a **standardized** pipeline for **unified** and **reproducible** analysis of SCP data. We used `scp` to reproduce 2 published analyses:

- The analysis by Specht et al. [2]: **multiplexed** quantification of 1018 single-cells, either human macropages or monocytes. **Good documentation** allowed for good replication.
- Label-free SCP analysis by Zhu et al. [1]: **label-free** quantification of 28 single-cells from chicken utricles. **Poor documentation** did not allow for replication.

References

[1] Y. Zhu, M. Scheibinger, D. C. Ellwanger, J. F. Krey, D. Choi, R. T. Kelly, S. Heller, and P. G. Barr-Gillespie, "Single-cell proteomics reveals changes in expression during hair-cell development," *Elife*, vol. 8, Nov. 2019.

[2] H. Specht, E. Emmott, A. A. Petelski, R. Gray Huffman, D. H. Perlman, M. Serra, P. Kharchenko, A. Koller, and N. Slavov, "Single-cell proteomic and transcriptomic analysis of macrophage heterogeneity." Oct. 2020.

[3] R. Amezquita, A. Lun, E. Becht, V. Carey, L. Carpp, L. Geistlinger, F. Marini, K. Rue-Albrecht, D. Risso, C. Soneson, L. Waldron, H. Pages, M. Smith, W. Huber, M. Morgan, R. Gottardo, and S. Hicks, "Orchestrating single-cell analysis with bioconductor," *Nature Methods*, vol. 17, pp. 137–145, 2020.

[4] L. Gatto and C. Vanderaa, *QFeatures: Quantitative features for mass spectrometry data*, 2020. R package version 0.99.3.

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