

Quantitative single cell proteomics experiments with SCoPE2

HARRISON SPECHT

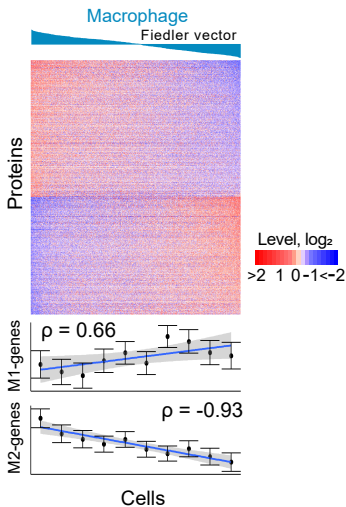
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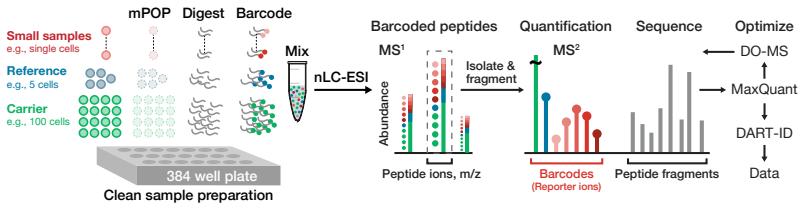
Application

- Emergence of macrophage heterogeneity
- Absent exogenous polarizing cytokines
- *in vitro*



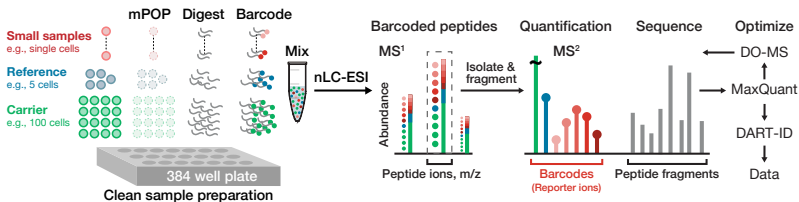
SCoPE2 workflow

Single Cell Proteomics by Mass Spectrometry (SCoPE2)



SCoPE2 workflow

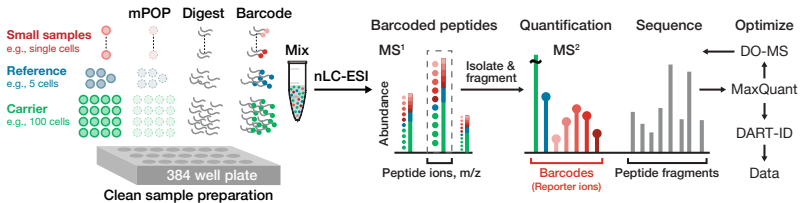
Single Cell Proteomics by Mass Spectrometry (SCoPE2)



- Carrier: Enough material to reduce losses, aid identification

SCoPE2 workflow

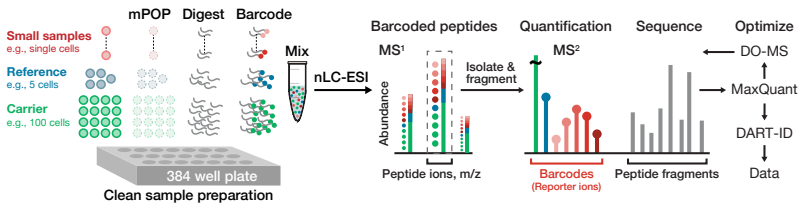
Single Cell Proteomics by Mass Spectrometry (SCoPE2)



- Reference: A consistent denominator in same linear range of quantitation as single cells, for comparing relative protein levels across sets

SCoPE2 workflow

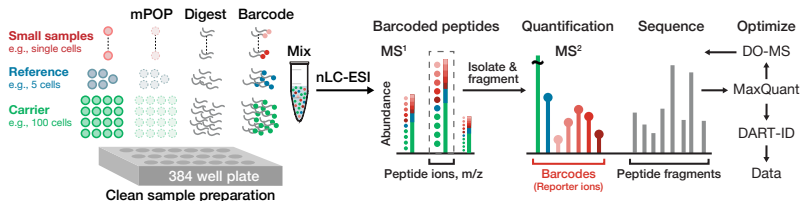
Single Cell Proteomics by Mass Spectrometry (SCoPE2)



- Set: Carrier, reference, and as many single cells or control wells as unique barcodes allow

Protocol summary 1/4

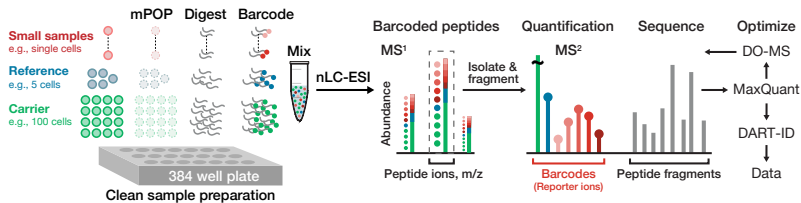
Single Cell Proteomics by Mass Spectrometry (SCoPE2)



1. Prepare 384-well plates with 1uL of 25 pmol Waters MassPrep in HPLC water.
2. Sort single cells into the 384-well plates.
3. Freeze plates at -80C until ready to proceed.

Protocol summary 2/4

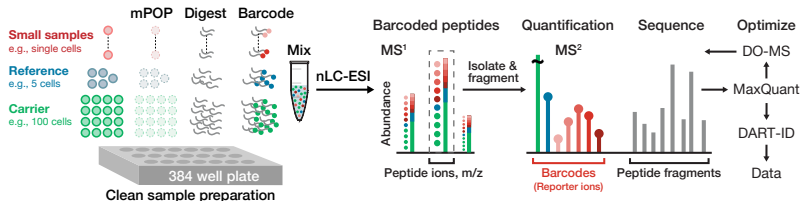
Single Cell Proteomics by Mass Spectrometry (SCoPE2)



4. Rapidly transfer plate to thermal cycler, heat samples to 90C for 10 minutes.
5. Spin down and cool plates, sonicate 5 minutes, spin again.
6. Add 0.2 uL mastermix to each well, to a final concentration of 100mM TEAB, 10ng/uL Trypsin and 0.25 units/uL benzonase.

Protocol summary 3/4

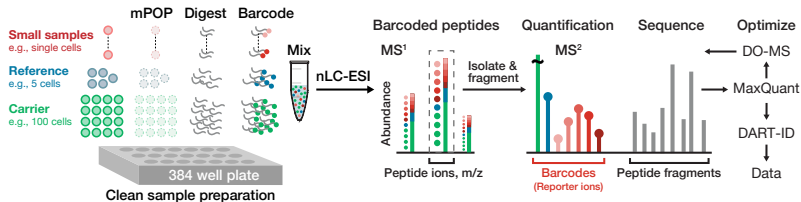
Single Cell Proteomics by Mass Spectrometry (SCoPE2)



7. Incubate plate at 37C for 3h.
8. Add 0.5uL of 22mM TMT to each sample. Incubate RT, 1hr.
9. Add 0.2uL of 0.5% hydroxylamine to each sample. Incubate RT, 30min.

Protocol summary 4/4

Single Cell Proteomics by Mass Spectrometry (SCoPE2)



10. Combine samples by passing 5uL Carrier/Ref mixture through each sample well. Follow with 50% acetonitrile.

11. Dry sample and resuspend in 1.2uL of 0.1% formic acid in glass autosampler vial or store dry at -80C.

12. Analyze by LC-MS/MS.

Protocol, required equipment

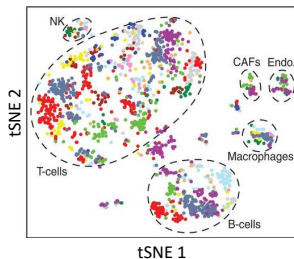
1. Means of isolating single cells: by hand, flow cytometry/FACS, et cetera
2. Means of heating 384-well plate, preferably with heated lid to minimize evaporation.
3. Automated liquid dispensing helps, but all volumes used can be accomplished with multichannel.

Protocol, tips and tricks

1. Clean trypsin is key.
2. TEAB is mass spec compatible.
3. Small volume injections.
4. Inject directly onto the analytical column to avoid losses.
5. Glass to minimize peptide losses.
6. Turning off voltage or dripping solvent to keep quenched TMT from dirtying MS.

Design considerations

- How many cells
- How many genes (proteins)
- Power calculations



¹Tirosh et al. Science. 2016.

Murphy's law...

- Assume everything that can go wrong, will go wrong!
- Whatever goes wrong, will have disastrous consequences!

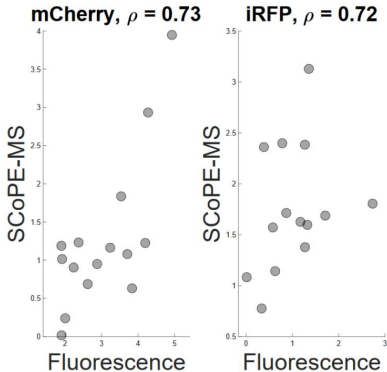
How do we design experiments?

1. Did we succeed? Can we trust our data?
2. If not, how did we fail? Design to diagnose!

Verification of success

- External comparisons
 - Fluorescent proteins
 - Bulk measurement

Example: fluorescent proteins

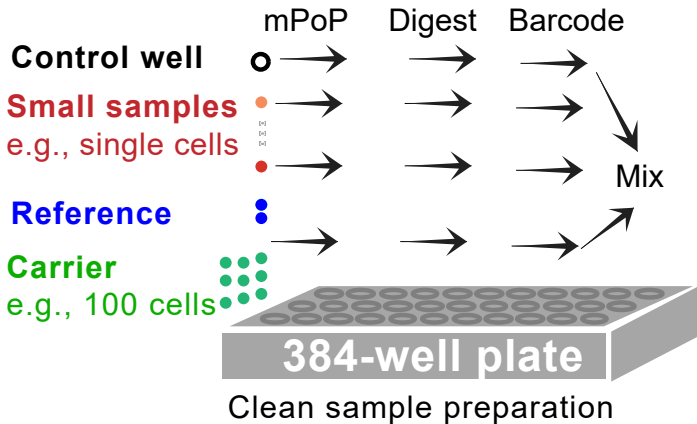


³Budnik et al. Genome Biology. 2018.

Verification of success

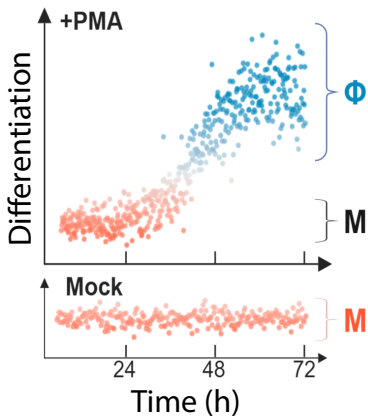
- Internal validation
 - Control wells
 - Relative reporter intensity (rRI)
 - Consistency of protein quantification

Control wells



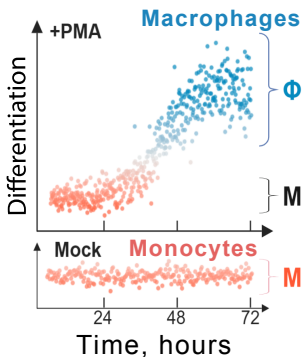
Example experiment

Monocyte (**M**) to Macrophage (**Φ**) differentiation



Design to avoid barcode impurity

Differentiation

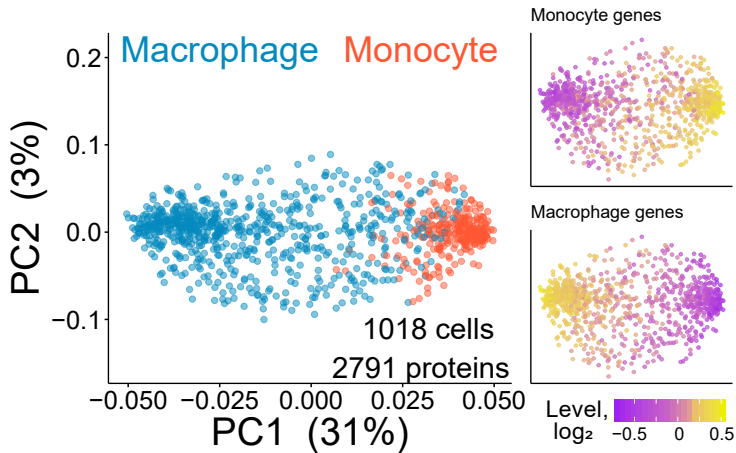


Sets Design

169 SCoPE2 Sets

| | | Samples | Barcode (RI) |
|------------|-----------|---------|--------------|
| Randomized | Carrier | | 126C |
| | Reference | | 127N |
| | Unused | | 127C |
| | 1- Φ | | 128C |
| | Control | | 129N |
| | ■ | ■ | |
| | ■ | ■ | |
| | 1-M | | 134N |

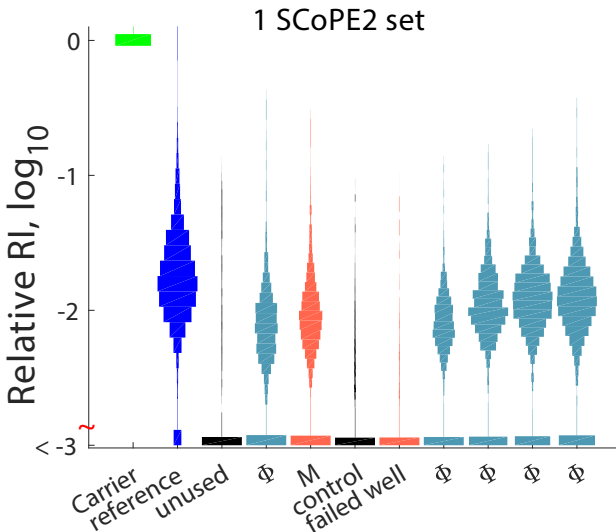
Results!



Example experiment

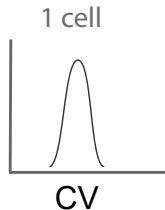
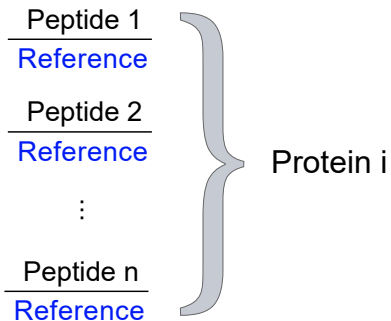
1. Did we succeed? Can we trust our data?

Internal validation

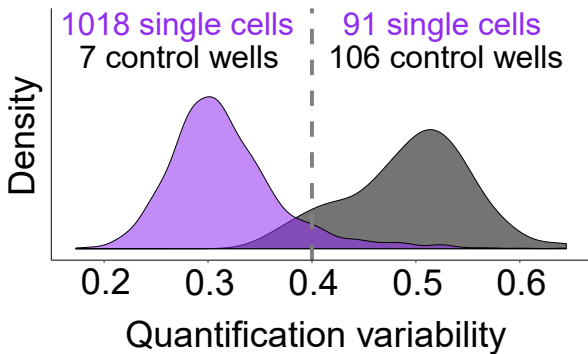


Consistency of quantification

$$\text{Coefficient of variation (CV)} = \frac{\sigma}{\mu}$$



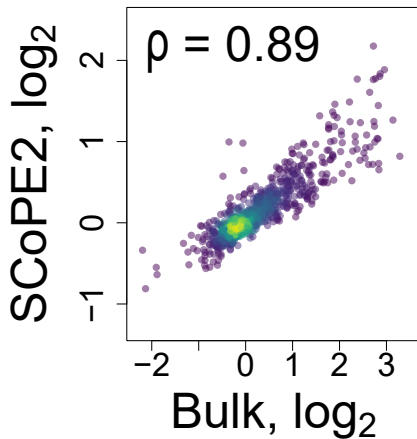
Internal validation



External comparison

$$\begin{array}{c}
 \text{Protein}_1 \\
 \vdots \\
 \text{Protein}_x
 \end{array}
 \begin{array}{c}
 \text{Mean}\left(\frac{M_1}{\text{Ref}_1}, \frac{M_2}{\text{Ref}_2}, \dots, \frac{M_i}{\text{Ref}_i}\right) \\
 \hline
 \text{Mean}\left(\frac{\Phi_3}{\text{Ref}_3}, \frac{\Phi_4}{\text{Ref}_4}, \dots, \frac{\Phi_n}{\text{Ref}_n}\right) \\
 \\
 \text{Mean}\left(\frac{M_1}{\text{Ref}_1}, \frac{M_2}{\text{Ref}_2}, \dots, \frac{M_i}{\text{Ref}_i}\right) \\
 \hline
 \text{Mean}\left(\frac{\Phi_3}{\text{Ref}_3}, \frac{\Phi_4}{\text{Ref}_4}, \dots, \frac{\Phi_n}{\text{Ref}_n}\right)
 \end{array}$$

External comparison

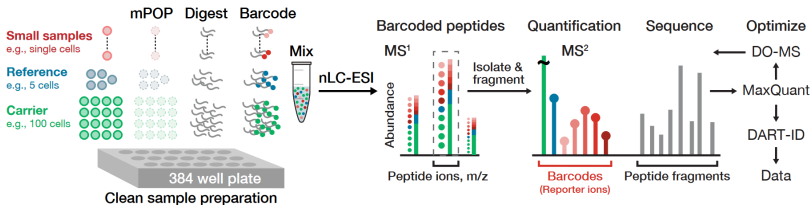


Example experiment

2. How could we have failed ?

What to diagnose in SCoPE2

Single Cell ProtEomics by Mass Spectrometry (SCoPE2)



Sample preparation

Plate position
Digestion
Labeling

Chromatography

Sample carryover
Peak sampling

Mass spectrometry

Low sensitivity
Dirty instrument
Coisolation

Data analysis

Combining sets

Optimizing LC-MS/MS



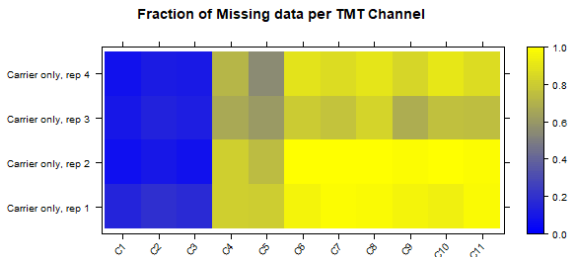
100xM standard

| RI | samples |
|------|----------------------|
| 126 | 5,000 T-cells |
| 127N | 5,000 monocytes |
| 127C | not used |
| 128N | not used |
| 128C | 100 T-cells |
| 129N | 100 monocytes |
| 129C | 100 T-cells |
| 130N | 100 monocytes |
| 130C | 100 T-cells |
| 131N | 100 monocytes |
| 131C | not used |

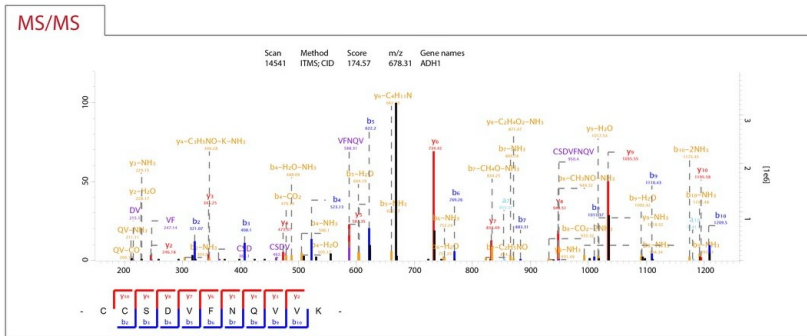
Is the LC clean?

100xM standard

| RI | samples |
|------|-----------|
| 126 | Carrier |
| 127N | Reference |
| 127C | Unused |
| 128N | Unused |
| 128C | Unused |
| 129N | Unused |
| 129C | Unused |
| 130N | Unused |
| 130C | Unused |
| 131N | Unused |
| 131C | Unused |



Coisolation

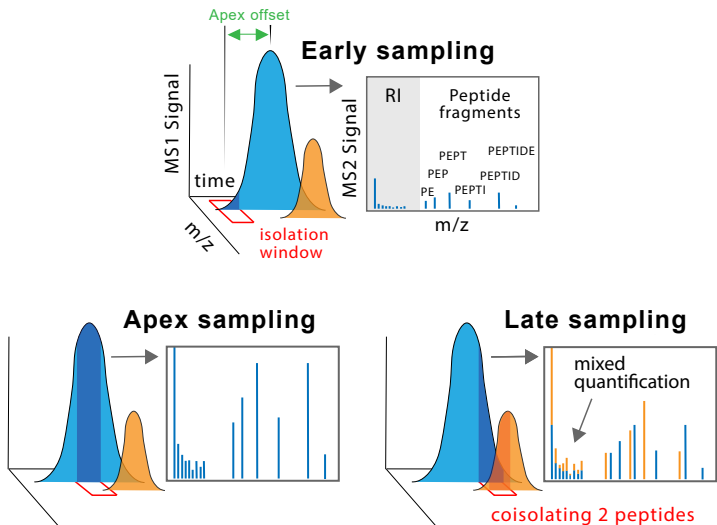


⁴Tyanova et al. Proteomics. 2015.

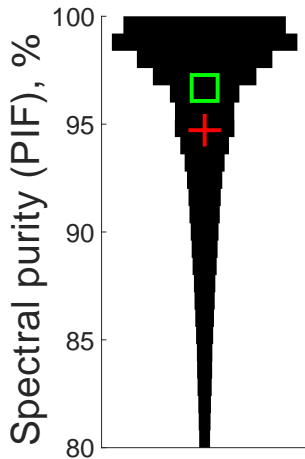
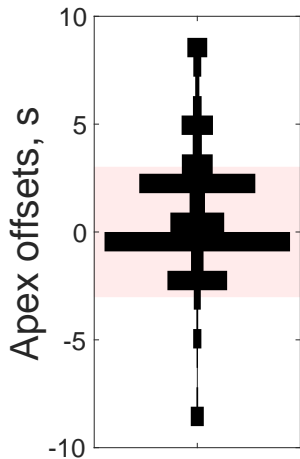
Harrison Specht

Single cell proteomics experiments

Increasing probability of apex sampling



Results!

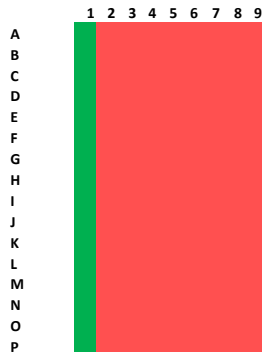
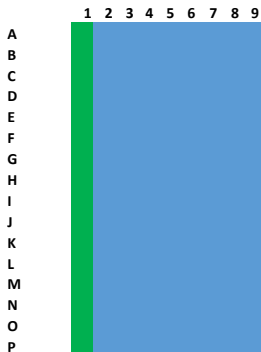


More...

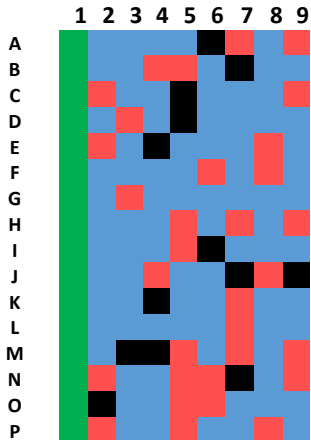
Issues beyond LC-MS/MS:

Batch effects

- Separate treatment and control from potential batches

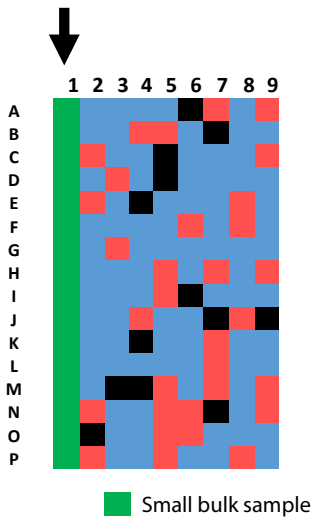


Randomized plates



Further diagnosis

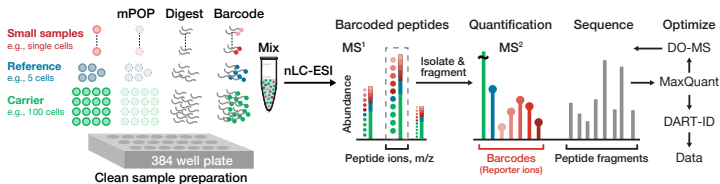
- Digestion efficiency
- Labeling efficiency



Summary

- 1. Protocol + tips
- 2. Experimental design

Single Cell Proteomics by Mass Spectrometry (SCoPE2)



SCoPE2

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- [Single-cell proteomics](#)

SCoPE2:

Single Cell Proteomics by Mass Spectrometry (Version 2)

Quantifying proteins in single cells at high-throughput with mass spectrometry

[SCoPE2 Preprint](#) [GitHub](#) [JPR Perspective](#) [Science Perspective](#)

Abstract

The fate and physiology of individual cells are controlled by protein interactions. Yet, our ability to quantitatively analyze proteins in single cells has remained limited. To overcome this barrier, we developed SCoPE2. It lowers cost and hands-on time by introducing automated and miniaturized

Workshop videos



Design of single-cell proteomics experiments | Harrison Specht | SCP2019

Nikolai Slavov

1:00:24



Data integration and analysis. Standards for benchmarking quantification | Nikolai Slavov | SCP2019

Nikolai Slavov



Sample preparation for single-cell MS analysis | Edward Emmott | SCP2019

Nikolai Slavov



Optimizing LC-MS/MS analysis with DO-MS | Gray Huffman | SCP2019

Nikolai Slavov

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R. Gray Huffman

Nikolai Slavov

Merck Exploratory Sciences

David H. Perlman

Hendrick Wesseling

Erik Hett

Harvard Medical School

Marco Serra

Peter Kharchenko

MIT

Antonius Koller



Questions?

SCoPE2: scope2.slavovlab.net

SCoPE-MS: bit.ly/SCoPE-MS

harrisonspecht.com

Appendix

| Benchmark | SCoPE-MS | SCoPE2 | Relevant figure |
|--|----------|---------|-----------------|
| Correlation to benchmark fold-changes | 0.2 | 0.89 | 3c |
| Purity of ions isolated for quantification | 79% | 97% | 2d |
| Single-cell protein measurements / hour | 610 | 4,630 | 2f |
| Sample preparation: | | | |
| - Time, hours / cell | < 1 | < 0.03 | 2a |
| - Cost, USD / cell | < 10 | < 1 | 1a |
| LC-MS/MS: | | | |
| - Time, hours / cell | 0.50 | 0.12 | 2a |
| - Cost, USD / cell [†] | 48 - 96 | 10 - 20 | 1a |