Extending Coverage in Multiplexed Single-Cell Proteomics

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ABSTRACT

Here we evaluated the Thermo Scientific™ Orbitrap Ascend™ Tribrid™ mass spectrometer (MS) for the application of Single Cell Proteomics by Mass Spectrometry (SCoPE-MS) and real-time search (RTS) assisted acquisition methods thereof. Furthermore, we evaluated the µPAC™ Neo Low-Loads column for high-throughput single-cell analysis. The results show a similar performance between Thermo Scientific™ Orbitrap Eclipse™ MS and Orbitrap Ascend MS and a considerable improvement when using the µPAC Neo Low-Loads column, visible as higher spectra identification rates and signal-to-noise ratios (S/N) in the single-cell channels. Furthermore, the results show a large improvement in identified proteins per cell using RTS Enhanced Quant of Single Cell Spectra (RETICLE), due to higher spectra identification rates and improved S/N distribution across the measured proteins.

INTRODUCTION

Multiplexed single-cell proteomics using the SCoPE-MS approach was rapidly adopted in the field for being accessible, sensitive, and enabling high single-cell throughput. This is enabled by the key concepts of isobaric multiplexing of single cells using Thermo Scientific™ TMTpro™ and adding a carrier channel with a 200-cell equivalent. However, the long ion accumulation times required for sampling enough ions from the single-cell channels limit the number of quantified peptides during the LC-MS/MS analysis. Here we evaluated the Orbitrap Ascend™ Tribrid™ MS for applying SCoPE-MS and RTS-assisted acquisition strategy RETICLE that uses fast real-time searched linear ion trap scans to preselect MS^1 peptide precursors for quantitative MS^2 Orbitrap acquisition. Furthermore, in combination with the latest generation of µPAC™ Neo Low-Loads columns for limited sample analysis, we demonstrated significant improvements in single-cell proteome coverage for high-throughput single-cell analysis.

MATERIALS AND METHODS

The Orbitrap Ascend Tribrid MS was compared to the Orbitrap Eclipse Tribrid MS using a diluted cell lysate composed of FACS-isolated cells from the OCI-AML8227 cell-culture model and labeled with TMTpro™ 16plex. The sample was measured using a DDA MS^2 OT/OT method. The same LC setup was used to compare the Orbitrap Eclipse to the Orbitrap Ascend (Evosep Whisper100 20SPD, 1h gradient). To evaluate the µPAC Neo Low-Loads column, a gradient with the same length and flow rate of 65 nL/min was used in a direct injection setup on a Vanquish Neo UHPLC system.
Real single-cell samples using isolated cells from the same OCI-AML8227 cell-culture model were measured using the µPAC Neo Low-Loads column on the Orbitrap Ascend MS (Fig. 1) to compare the performance of classical MS^2 acquisition to an RTS-assisted RETICLE method using two different injection times.

Figure 1. The Orbitrap Tribrid Ascend MS coupled to a Thermo Scientific™ FAIMS Duo Pro interface and Thermo Scientific™ EASY-nano ion source, and a Thermo Scientific™ Vanquish™ Neo UHPLC system.

RESULTS

1) Performance comparison using a diluted cell lysate

Diluted lysates for FACS isolated cells from the OCI-AML8227 cell-culture model (leukemic stem cells, progenitors, and differentiated blasts) and labeled with TMTpro™ 16plex, as shown in Fig. 2, were measured in triplicate using a DDA MS^2 OT/OT method with three different instrument setups. The same chromatographic setup was used to compare the Orbitrap Eclipse MS to the Orbitrap Ascend MS. For evaluating the µPAC Neo Low-Loads column, a gradient with the same length and flow rate was used in a direct injection setup. The results in Fig. 3 show a similar performance between Orbitrap Eclipse MS and Orbitrap Ascend MS using the same packed column and different maximum injection times. A considerable improvement in quantified spectra, identified quantified spectra, average single-cell proteins, and S/N was observed when using the µPAC Neo Low-Loads column in the Orbitrap Ascend MS compared to the packed column.

Figure 2. The TMTpro™ 16plex contains a 200-cell equivalent carrier channel and the single-cell equivalent of three different cell types FACS sorted from the OCI-AML8227 leukemia culture model.

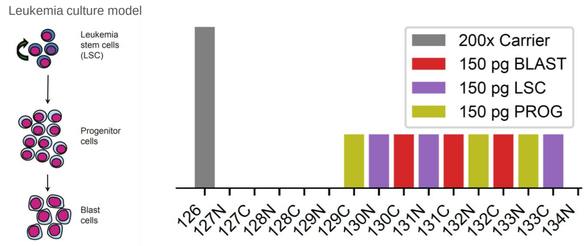
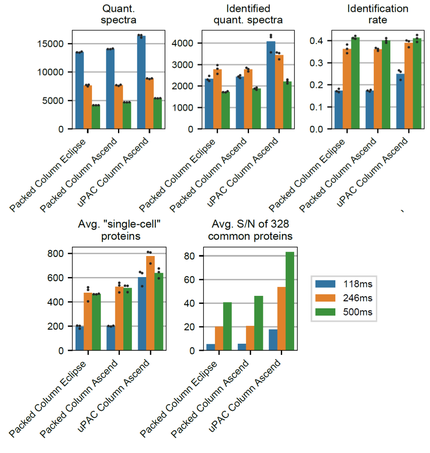


Figure 3. Results of triplicate measurements for each method. For each LC-MS/MS setup, three different MS^2 OT ion injection times were tested.



2) RETICLE performance on real single-cell samples

Real single-cell samples isolated from the OCI-AML8227 cell-culture model were TMTpro 16plex labeled and measured using the µPAC Neo Low-Loads column setup on the Orbitrap Ascend MS. Table 1 shows the results comparing the performance of the classical MS^2 acquisition to RTS-assisted method RETICLE using two maximum injection times. The results in Fig. 4 show a large improvement in proteins per cell using RETICLE, due to higher spectra identification rates and improved S/N distribution across the measured proteins (Fig. 5). Additionally, a throughput of 272 cells per day was obtained with the RETICLE method, double the throughput previously published using a similar setup on Orbitrap Eclipse Ms with comparable proteome coverage (Furtwängler *et al.*, 2022).

Table 1. Results summary of the real single-cell datasets generated using the µPAC Neo Low-Loads column with the Orbitrap Ascend. Classical MS^2 acquisition was compared to RTS-assisted acquisition using RETICLE in two different injection times.

	MS^2 500 ms	MS^2 750 ms	RETICLE 500 ms	RETICLE 750 ms
Quantification spectra	37,586	31,128	20,961	18,367
Identification rate of quantification spectra	0.236	0.222	0.660	0.675
Cells	83	81	82	82
Proteins shared found in at least 10 cells	764	646	1,445	1,331
Proteins with >70% coverage	371	290	655	562

Figure 4. Boxplot of the number of proteins identified per cell with an S/N value in the respective single-cell channel, i.e., quantified proteins, comparing classical MS^2 acquisition to RTS-assisted acquisition using RETICLE in two different injection times.

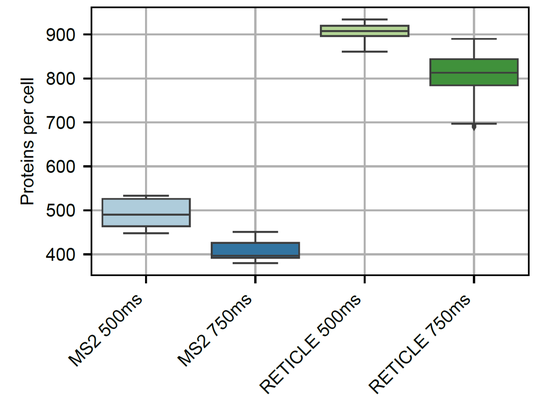
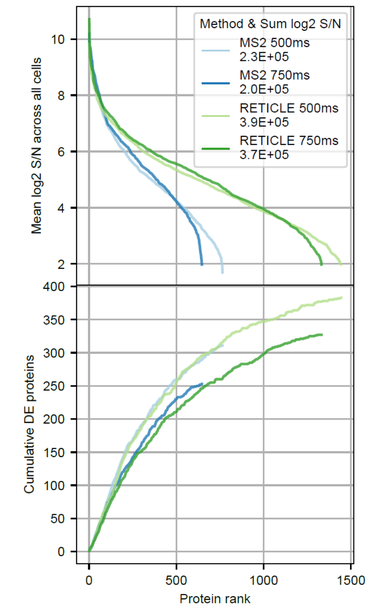


Figure 5. Top: Comparison of the S/N distributions per identified protein in each single-cell dataset. RETICLE acquires a more evenly distributed S/N profile across proteins. Bottom: Cumulative distribution of proteins detected as differentially expressed between BLAST and LSC. RETICLE enables the detection of more differentially expressed (DE) proteins.



CONCLUSIONS

- The Orbitrap Ascend Tribrid MS is well suited for single-cell proteomics.
- The µPAC Neo Low-Loads column provides an enhanced signal resulting in higher proteome coverage for single-cell analysis.
- RTS-assisted acquisition using RETICLE outperforms classical MS^2 methods on real single-cell sample analysis.

TRADEMARKS/LICENSING

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