Sensitive and robust high-throughput workflow for qualitative and quantitative single-cell/single cell like analysis

Aaron Robitaille[2], Tabiwang N. Arrey[1], Fernanda Salvato[2], Jeff Op De Beeck[3], Nicolaie Eugen Damoc[1], Bernard Delanghe[1]; 1Thermo Fisher Scientific, Bremen, Germany, [2]Thermo Fisher Scientific, San Jose, California, [3]Thermo Fisher Scientific - Belgium, Ghent, Belgium

Introduction

In recent years single cell analysis has profited from advances in LC-MS based proteomics approaches\tools. Nevertheless, there are still challenges in this field of application. To address some of these challenges, new technological developments, as well as improvements on existing LC-MS-based proteomics workflows are a necessity. Here, we evaluated the use of a novel HRAM mass spectrometer, nano-flow UHPLC and solid silicon micro-pillar array column technology for high-throughput single cell applications.

Materials and methods

Sample Preparation

Individual HeLa cells were isolated using CellenONE® system from Cellenion, followed by reduction, alkylation and trypsin digestion as per manufacturer's instructions. For the dilution Pierce™ HeLa digest (10 ug) was reconstituted by adding 200 µL of 0.1% FA, sonicated for 5 min, then dilution in 0.1% FA to 1 ng/µl.

Methods

Single cell digests and the diluted standard HeLa digest samples were analyzed using a Thermo Scientific™ Orbitrap Astral MS with Thermo Scientific™ FAIMS Pro™ interface coupled to a Thermo Scientific™ Vanquish™ Neo UHPLC system. Separation was performed on a Thermo Scientific™ µPAC™ Neo HPLC 50 cm column. During the separation, the flow rate is ramped down from a starting 750 nl/min to as low as 65 nl/min at a point where the peptides start eluting the column. An active gradient of 11 min was used, the time from injection to injection was 18 min (80 samples per day). With this we make use of the increased ionization efficiency associated with low flows. The Orbitrap Astral MS was operated in DIA mode with 20 Th windows. The FAIMS Pro interface was set to -50 compensation voltage (CV). The Orbitrap analyzer was operated at 240.000 resolution in MS1.

Data Analysis

All data was processed using Spectronaut 18 in directDIA mode and with Proteome Discoverer 3.1

Figure 1. Base peak chromatogram of a 50 pg HeLa dilution.

Results

Hela dilution series

HeLa standard dilution series from 50 pg to 5000 pg were analysed in triplicates using this 80 SPD workflow, resulting in on average more than 2700 to more than 6650 protein groups quantified.

For the 250 pg HeLa standard more than 4400 protein groups were quantified on average for 3 replicates (Figure 3).

Figure 2. Overview of quantified protein groups and unique peptides for the diluted HeLa standard when processing all files together with Proteome Discoverer 3.1.

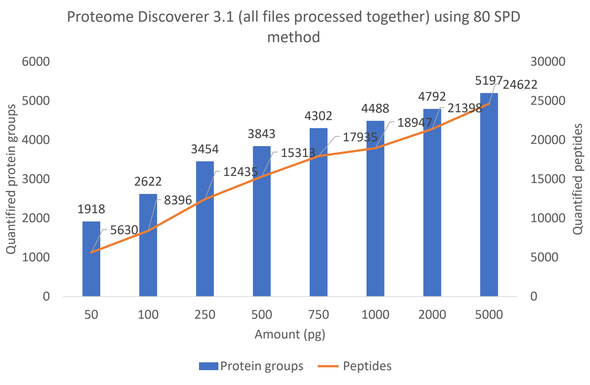


Figure 3. Overview of quantified protein groups and unique peptides for the diluted HeLa standard when processing the replicates of different amounts separately with Spectronaut 18.

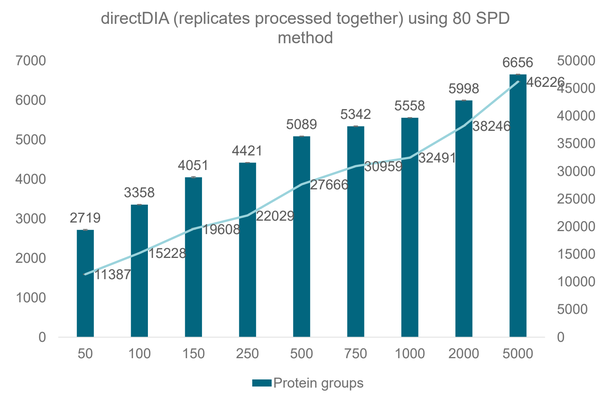
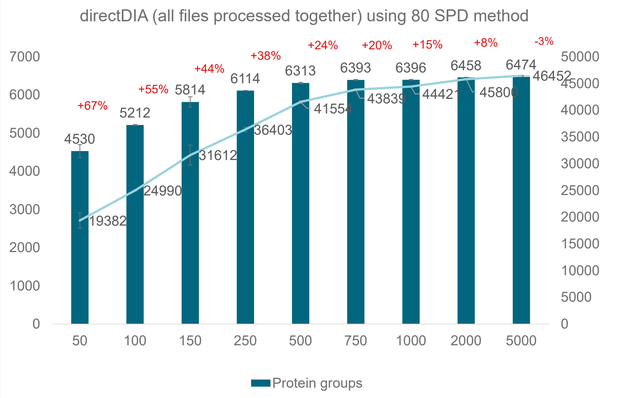
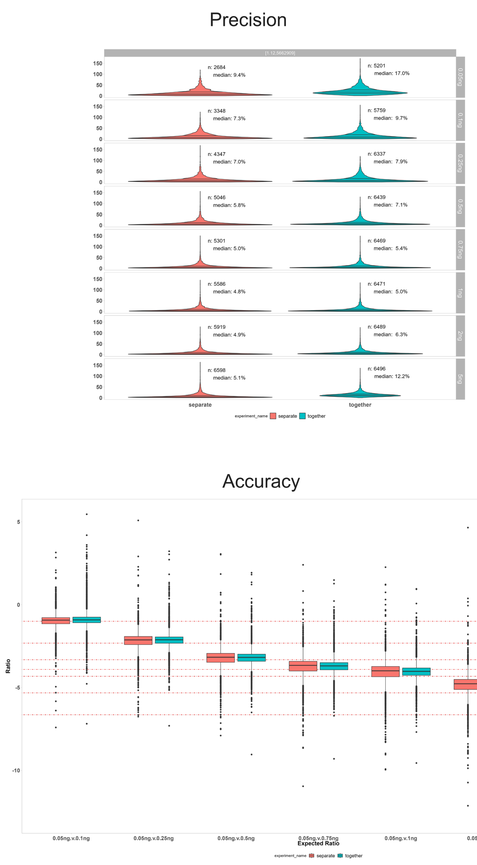


Figure 4. Overview of quantified protein groups and unique peptides for the diluted HeLa standard processing all files together with Spectronaut 18.



Processing the whole dilution series together results in an increase of quantified proteins. For the 250 pg HeLa standard this results in an increase of almost 40%, to more than 6100 proteins quantified (Figure 4).

Figure 5. Precision (top) and Accuracy (bottom) of the HeLa dilution series, comparing processing replicates separately with processing all files together.

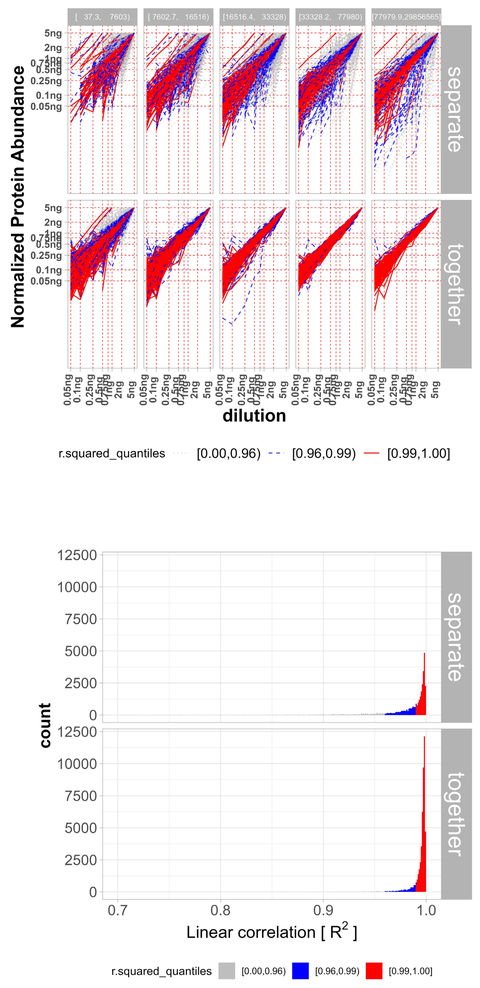


When the replicates are processed separately, precision of the dilution series is excellent, with median cv below10 %. When processing the all the files together a slight increase of median cv is observed for all amounts, except for the 50 pg where the increase is larger (median cv 17%, Figure 5). This can be explained by the high increase (~ 70 %) of low abundant proteins quantified for the 50 pg HeLa dilution.

Processing all files together is improving the accuracy for all ratios as showed in the box and whisker plot (figure 5).

In figure 6, calibration curves are drawn for all the protein groups, divided by abundance in 5 quantiles. The linearity of all the curves are excellent. Also here, we can notice that the linear correlation coefficients are improved by processing all the files together, which clearly can be seen in the density plot of the linear correlation coefficients (Figure 6 bottom).

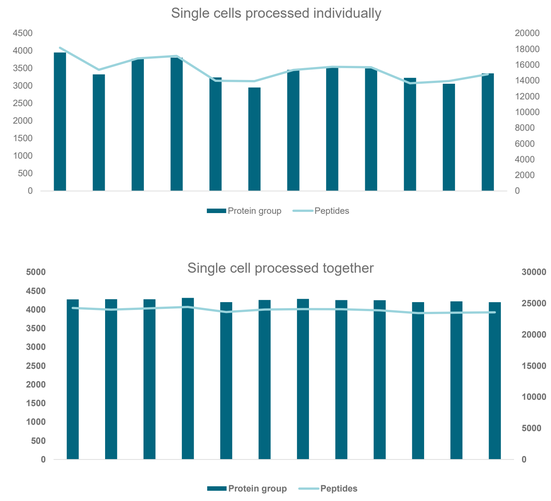
Figure 6. Calibration curves of all the proteins in 5 abundance quantiles (top) with density plots of linear regression coefficients for all the proteins (bottom), comparing processing replicates separately with processing all files together.



Single cell data

12 individual HeLa cells were analysed using the same workflow as described for the HeLa dilution series (Figure 7 top). On average ~3400 proteins were quantified. A higher variance is observed for the single cells, which is reflecting the different cell cycle state of those cells.

Figure 7. Average protein groups end unique peptides for the individual single cells, processed separately (top) or together (bottom) with Spectronaut 18 using the 80SPD method.



Processing all 12 files together increased the number of quantified protein groups per single cell to more than 4250 (Figure 7 bottom).

Another way of processing raw files is using libraries.

2 libraries were created using Spectronaut, one of 12 single cells, another one using 3 replicates of 20 cells.

Figure 8. Average protein groups end unique peptides for the individual single cells together with Proteome Discoverer 3.1 using the 80SPD method.

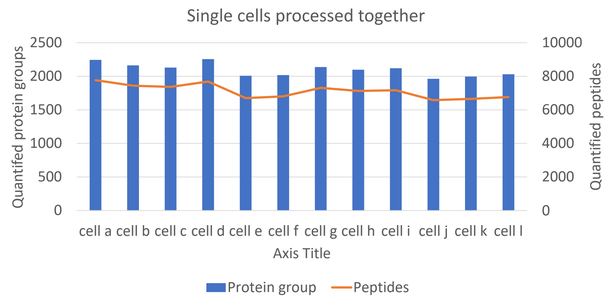


Figure 9. Average protein groups of the single cells using different ways of processing the raw files using Spectronaut 18.

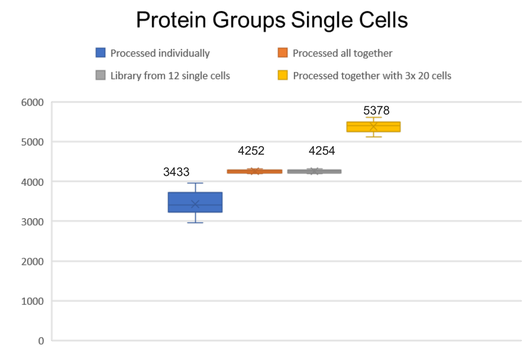
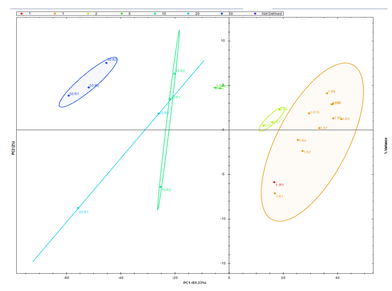


Figure 10. Principal component analysis of the single cells.



The result of the different ways of processing the single cell raw files are summarized in Figure 9. There is not much difference between processing all files together and using a spectral library of 12 cells. On average ~ 4250 protein groups are quantified.

Using a library of 20 cells increases the average number of quantified protein groups for a single cell to nearly 5400.

Conclusions

- At high sample throughput the Orbitrap Astral mass spectrometer has higher sensitivity to deliver accurate and precise quantitation of low input loads, including single cells.
- At 80 SPD more than 6000 proteins are quantified on average for a 250 pg HeLa standard.
- At 80 SPD more than 5350 proteins were quantified on average for a single HeLa cell.

Acknowledgment

We would like to thank Anjali Seth and David Hartlmayr from Cellenion for supplying the prepared single cell samples.

Trademarks/licensing

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.

Learn more at thermofisher.com/singlecellproteomics