APPLICATION NOTE 65714

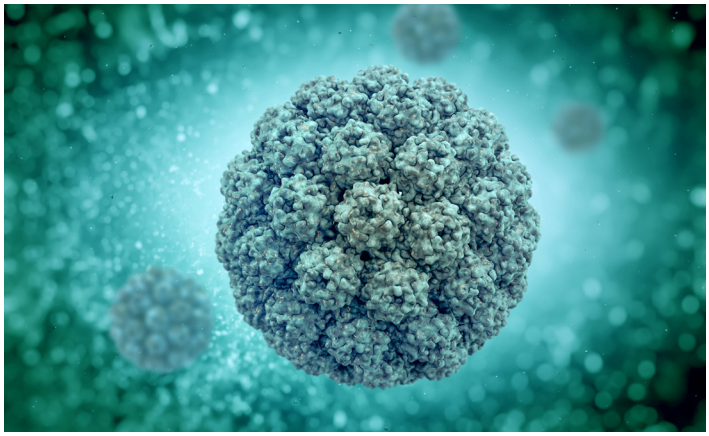
High-throughput single-cell proteomics analysis with nanodroplet sample processing, multiplex TMT labeling, and ultra-sensitive LC-MS

Authors: Khatereh Motamedchaboki[1], Maowei Dou[1], Yongzheng Cong[2], Romain Huguet[1], Aaron M Robitaille[1], Yufeng Shen[3], Geremy Clair[4],Charles Ansong[4], Daniel Lopez-Ferrer[1], Ryan T. Kelly[2,4], and Ying Zhu[4]

[1]Thermo Fisher Scientific, San Jose, CA, [2]Brigham Young University, Provo, UT, [3]CoAnn Technologies LLC, Richland, WA, [4]Pacific Northwest National Laboratory, Richland, WA, USA

Abstract

Understanding heterogeneity at the single-cell level is of great interest for biomedical research. Mass spectrometry (MS) based proteomics is a promising technique for single-cell analysis by enabling the identification and quantification of thousands of proteins in an unbiased manner. However, due to inefficiencies in single-cell isolation, large sample losses during sample preparation and low throughput, the extension of proteomics to single-cell studies has been largely ineffective. To address these challenges, we combined nanoPOTS (Nanodroplet Processing in One-pot for Trace Samples) technology with Thermo Scientific™ Tandem Mass Tag™ (TMT™) isobaric labeling to efficiently process and analyze single-mammalian cells containing <0.2 ng total proteins using the Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer with Real-Time Search to improve single-cell proteome coverage and enhance quantification accuracy.



Materials and methods

Single cells were isolated from cultured murine cells via fluorescence-activated cell sorting and the nanoPOTS platform[1]. Single cells and 5 ng boost sample[2] were digested and labeled with Thermo Scientific™ TMT10plex™ isobaric labeling reagents on the nanoPOTS chip. A Thermo Scientific™ UltiMate™ 3000 RSLCnano system was used with 30 µm i.d. × 30 cm C18 column with integrated electrospray emitter (CoAnn Technologies) at 20 nL/min coupled to the Orbitrap Eclipse Tribrid mass spectrometer[3]. MS[2] and SPS MS[3] with Real-Time Search[4] analysis was performed on 5 single-cell batches (total 40 cells from three different cell types, epithelial, endothelial and raw immune cells). Thermo Scientific™ Proteome Discoverer™ 2.4 software was used for data analysis. Thermo Scientific™ TMT quantifications were evaluated with a focus on proteomics analysis throughput, protein coverage and improvement in quantification accuracy.

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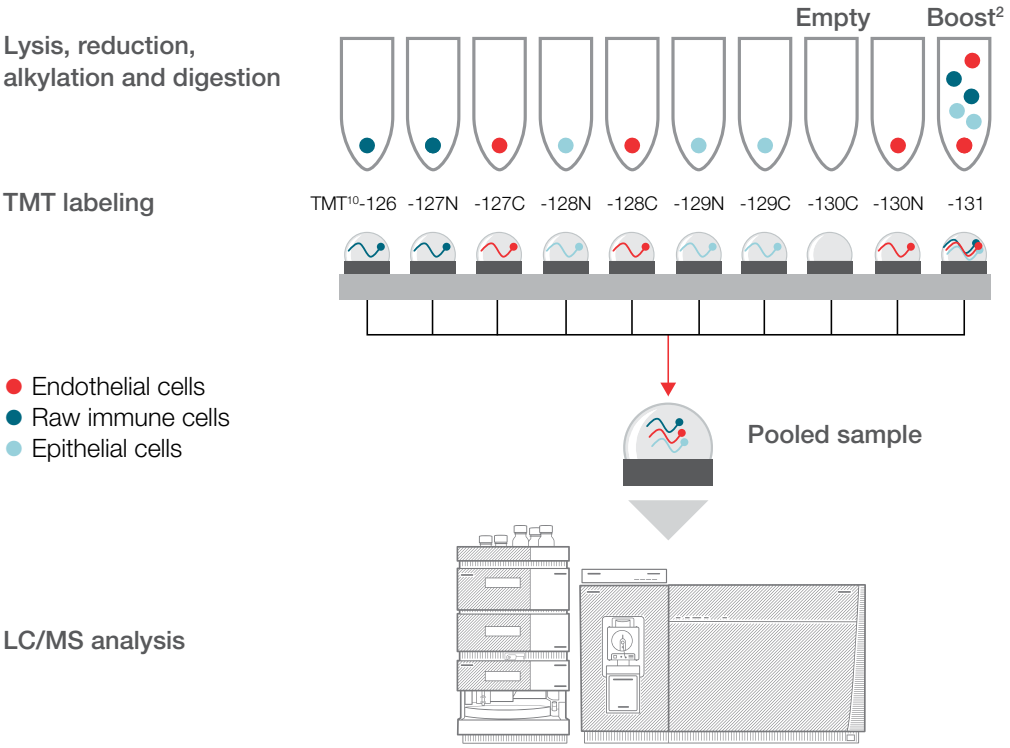

Figure 1. Illustration of the nanoPOTS Sample Preparation. The nanoPOTS-based single-cell proteomics sample preparation including tryptic digestion and TMT mass tagged peptide labeling in lower than typical digestion volume (200 nL), minimizing the surfaces that samples are in contact with to avoid sample loss in low amount of protein samples like single cell.

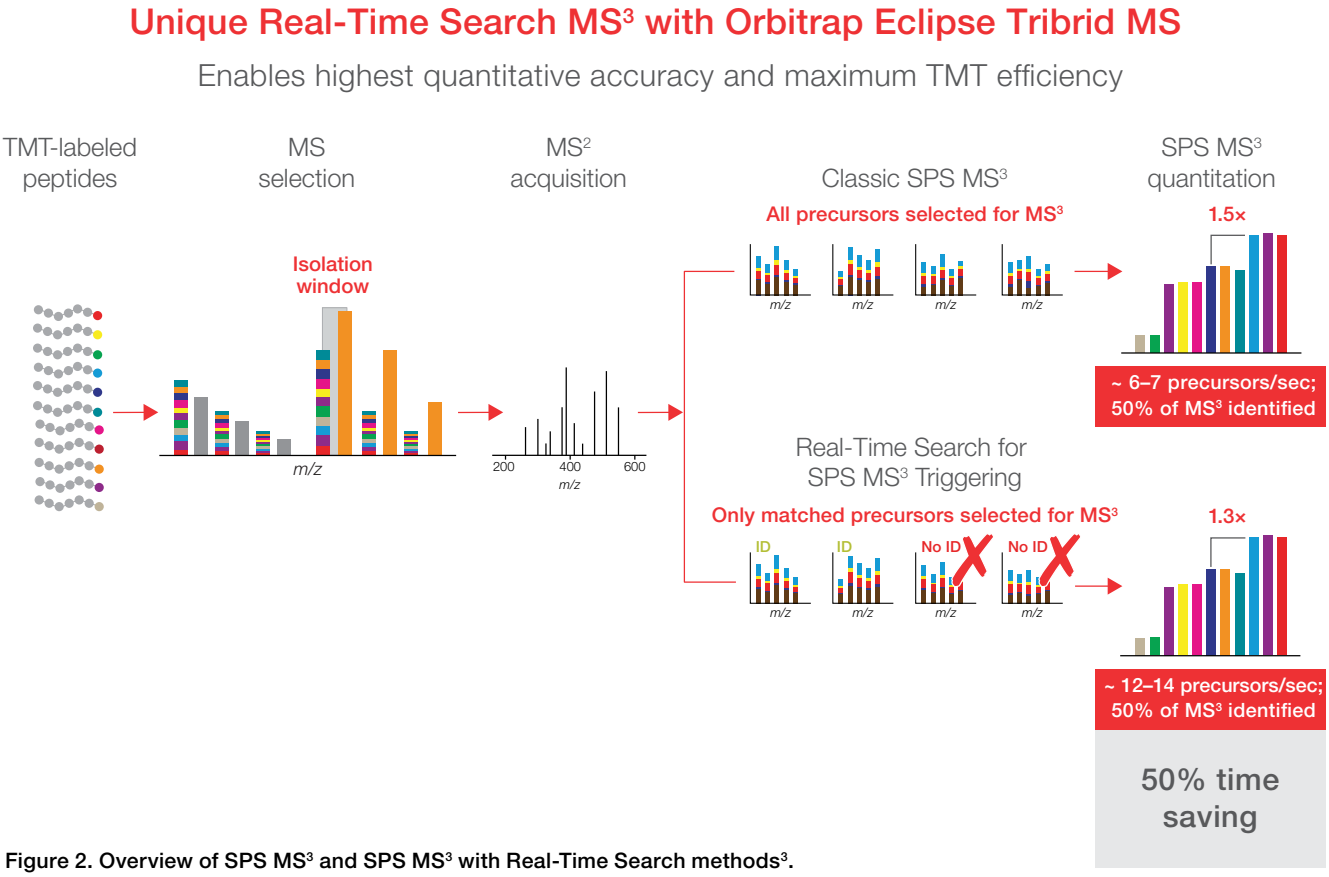

Figure 2. Overview of SPS MS³ and SPS MS³ with Real-Time Search methods³.

High-throughput single-cell protein quantitation

To understand the biology of the cells in single-cell level, we will need to analyze many cells hence there is a need in proteomics for a method that offers higher throughput and multiplexing capability for single-cell analysis. The MS^2 level based single-cell proteomics is a well established workflow[2]. Here we have evaluated the MS^2 and MS^3 level TMT single-cell multiplexed LC-MS approaches on Orbitrap Eclipse Tribrid MS and optimized the method to improve quantitation accuracy without compromise on protein coverage.

Results

The TMT10plex analysis of the three cultured murine-cell populations (C10, SVEC and Raw immune cells) enabled identification of 2346 proteins and quantification of 1300 proteins among 40 single cells. We have demonstrated that the single-cell proteome can be quantified using TMT by combing nanoPOTS with Orbitrap Eclipse Tribrid mass spectrometer, enabling researchers to investigate cell heterogeneity as well as rare cells.

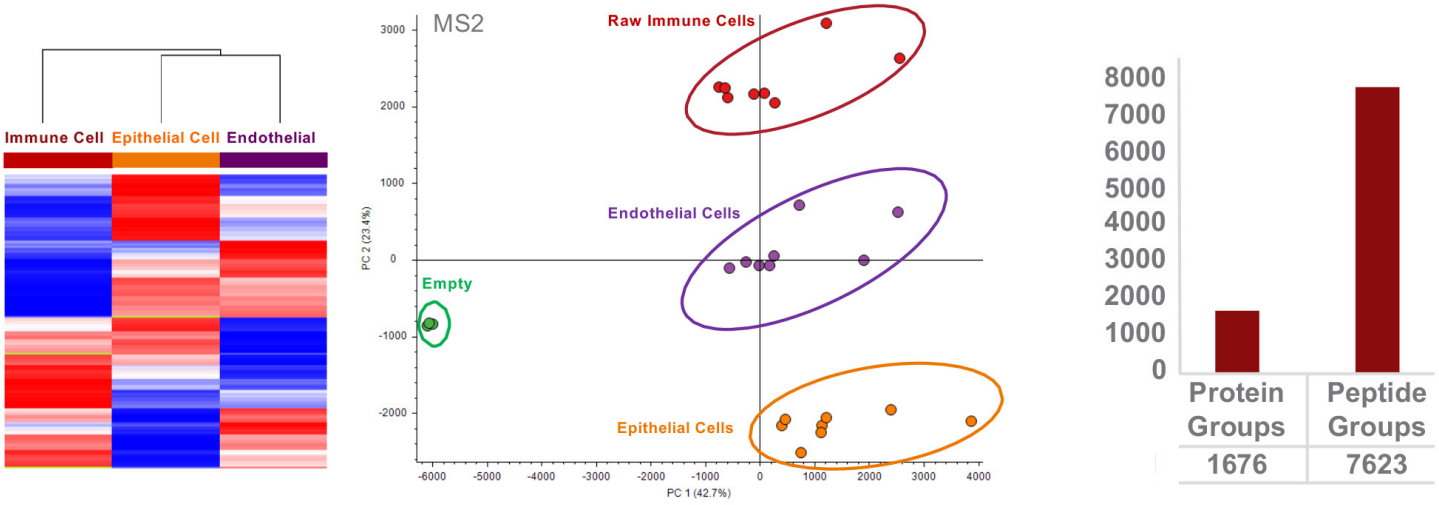

Figure 3. High-throughput murine-cell classification using TMT MS^2 approach. A total of 24 single cells were processed on three nanoPOTS chips and analyzed in three replicate LC-MS runs. The heat map (left) and PCA analysis (middle) of TMT10plex data show clear differentiation between the three different cell types (raw immune cells, epithelial and endothelial cells). A total 1676 protein and 8234 peptide groups were identified with TMT MS^2 approach.

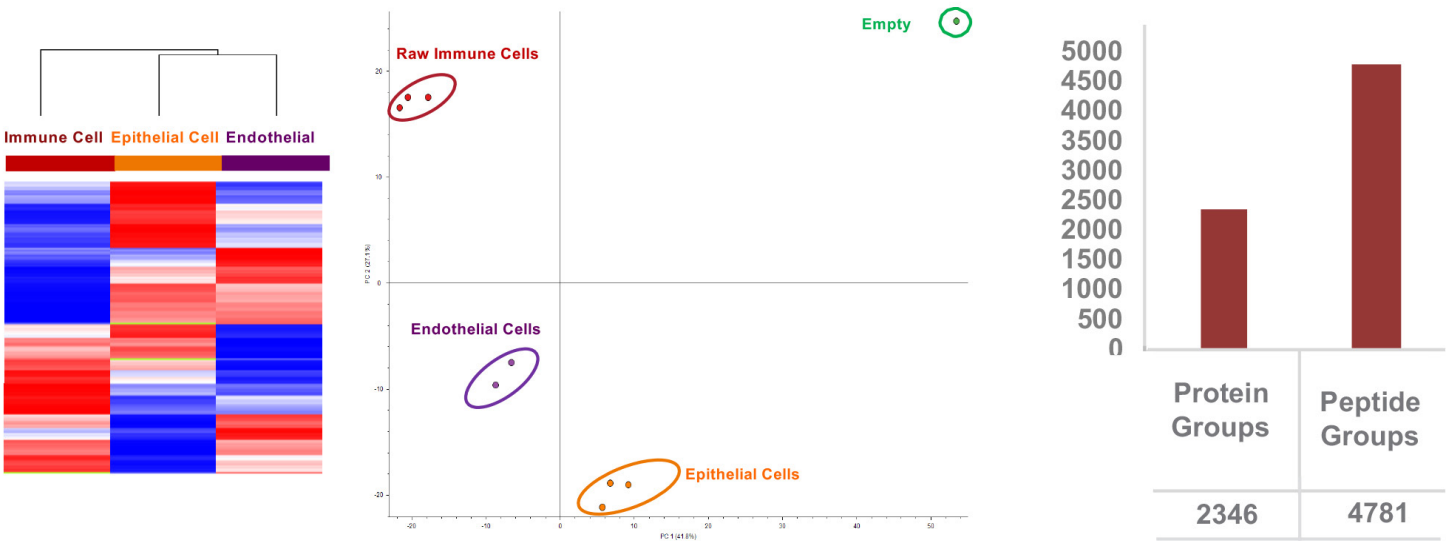

Figure 4. High Throughput murine-cell classification with Real-Time Search for SPS MS^3. A total of 16 single cell were processed on two nanoPOTS chips and analyzed in two LC-MS runs. The heat map (left) and PCA analysis (middle) of the TMT10plex experiments show clear differentiation between the three different cell types (raw Immune cells, epithelial and endothelial cells). A total of 2346 protein and 4781 peptide groups were identified (right) with improved quantitative accuracy and protein coverage with Real-Time Search for SPS MS^3.

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TMT analysis of two different cell types showed improve coverage of differentially expressed proteins when using SPS MS^3 with Real-Time Search as compared to MS^2 based TMT analysis.

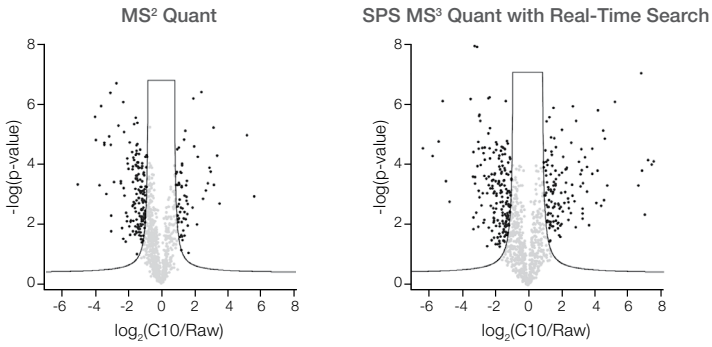

Figure 5. Volcano plot of TMT10plex analysis using TMT MS^2 and SPS MS^3 with Real-Time Search on Orbitrap Eclipse Tribrid MS. TMT SPS MS^3 with Real-Time Search method provided improved coverage of differentially expressed protein between the three cell types and improved quantitative accuracy without compromising in total number of proteins identified.

Conclusions

- The nanoPOTS platform combined with TMT multiplexed isobaric labeling provides a robust, high-throughput proteomic preparation method for handling extremely small biological samples like single cells.
- Reproducible quantitative proteome measurement with coverage of 2,000 protein groups was achieved among a total of 40 single cells obtained from cultured murine cell populations.
- NanoPOTS integrated with multiplexed isobaric labeling represents a highly promising platform towards; single-cell typing, understanding of stem cell development, proteomic studies of isolated clinical specimens (circulating tumor cells) and proteome imaging of tissue heterogeneity.

REFERENCES

1. Zhu Y., et. al. *Angew. Chemie - Int. Ed.* 57, 12370 (2018)
2. Budnik B., et. al. *Genome Biol.* 19, 161 (2018)
3. Brian K. E., et. al. *J Proteome Res.*, 18, 1299 (2019)

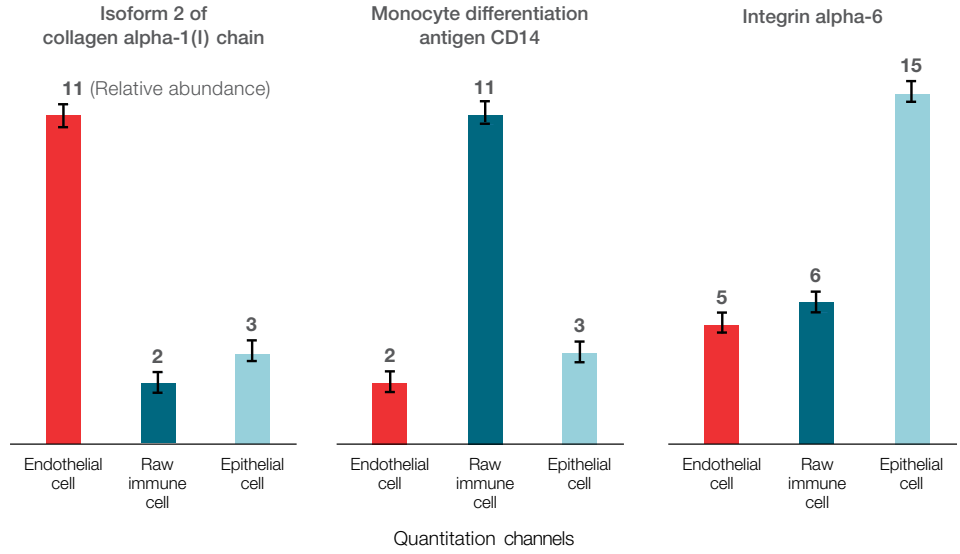

Figure 6. Differential quantitation of cell type biomarkers in single cells. Confirmation of overexpression of known protein biomarkers in epithelial, endothelial and raw immune individual cells validate the inherent sensitivity of this high-throughput single-cell quantitation workflow.

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