Sensitivity and High-throughput Single-cell Proteomics Workflow on New Quadrupole-ion trap-Orbitrap Mass Spectrometer with FAIMS Separation

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INTRODUCTION

Traditionally, proteomics experiments are applied to large populations of cells, representing the average protein expression under given biological conditions. However, understanding the cellular heterogeneity provides insights that cannot be gained from bulk studies, such that the analysis of single-cell protein expression is of growing interest. Current LC-MS-based proteomics workflows have not been widely applicable to single cell analysis, mainly due to large sample losses during sample preparation, limited analytical sensitivity and low throughput. To address these challenges, we have combined nanopOTS (Nanodroplet Processing in One-pot for Trace Samples) technology with tandem mass tag (TMT) labeling to analyze single mammalian cells containing ~0.2 ng total proteins on the Thermo Scientific™ Orbitrap Eclipse™ Trüeb™ mass spectrometer with real-time search and the Thermo Scientific™ FAIMS Pro™ interface to improve single cell proteome coverage and enhance quantitation accuracy. The FAIMS-enhanced label-free workflow resulted in the identification of an average of 829 protein groups from single HeLa cells with high-confidence MS² spectra. This was 3-fold higher than without FAIMS. The Thermo Scientific™TMT10plex™ analysis of these cultured murine cell populations were compared with MS² and SPS MS² method with Real Time Search. We have demonstrated that single cell proteomes can be quantified using label-free or TMT workflows by combining nanopOTS with the Orbitrap Eclipse Trüeb™ mass spectrometer, and the FAIMS Pro™ Interface, enabling researchers to investigate cell heterogeneity as well as rare cells in an ultra-sensitive, high-throughput LC-MS analysis.

MATERIALS AND METHODS

Sample Preparation: Single cells were isolated from cultured murine and HeLa cells via fluorescence-activated cell sorting and cells were labeled with TMT multiplexing reagents on nanopOTS chip1. Thermo Scientific™ Pierce™ HeLa Protein Digest Standard was dissolved in sample loading buffer containing 2% Acetonitrile in 0.1% TFA and 0.1% FA with 30 min of vortexing and spinning down in concentration range of 0.5 ng/µL (equivalent to ~2 HeLa cells) for method development.

Methods: Single cell tryptic digest and single cell HeLa dig (0.5 ng) were individually transferred to a short (4 cm) capillary tube and peptides were loaded to a 5 cm solid phase extraction (SPE) trap for peptide trapping with minimum sample loss followed by analytical peptide separation on analytical column (20 µm i.d. 3, 5, 50 µm for label-free analysis and 30 µm i.d. 17, 30 µm from CoAnn Technologies for TMT10plex analysis) on a Thermo Scientific™ Ultima™ 3000 QTOF system coupled to a PRI-TO2 Sonation column (sonation lab solutions) and new Orbitrap Eclipse Trüeb™ mass spectrometer with FAIMS Pro Interface. The ultra low nanoLC flow rate of 20nmn for single cell analysis was achieved through split flow set up1.

Data Analysis: Single cell and single cell level data files were processed using Thermo Scientific™ Proteome Discoverer™ 2.2 software with 2-stage SEEKREST search parameter including tryptic and semi tryptic search and parcolator was used between each search to calculate the false discovery rate (FDR) and only those spectra with q-values lower than 0.01 were sent to the subsequent search filter and MaxQuant software for match between runs to estimate proteins in the blank sample analysis.

RESULTS

The performance of this ultra-sensitive LC-MS workflow was evaluated using the oribitr and the ion trap for detection of CID and fragmentation selectivity. HeLa cell digest was first optimized using 0.5 ng aliquots of Thermo Scientific Pierce HeLa Protein Digest Standard and later evaluated with single HeLa cell digests on nanopOTS chip.

Figure 1. Label-Free Single Cell MS Method Optimization. MS² and MS³ in high resolution Orbitrap with HCD fragmentation shown to provide sensitivity and selectivity for analysis of single cell proteomes. With a 2 hours gradient and two CV (compensation voltage) switching, 2032 protein groups and 8571 peptide groups were identified from 0.5 ng HeLa dig with FDR rate of 1% or better.

Table 1. LFG Performance Enhancement with FAIMS Pro Interface. FAIMS enhances proteome coverage in single cell level and allows lower abundant peptide detection by MS².

<table>
<thead>
<tr>
<th>Separation</th>
<th>Initial Workflow</th>
<th>Optimized Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Spectrometer</td>
<td>250000 µm/min TMT10plex MS²</td>
<td>150000 µm/min TMT10plex MS²/FAIMS Pro Interface</td>
</tr>
<tr>
<td>Data Analysis</td>
<td>MaxQuant</td>
<td>ProTeome Discoverer 2.2 Software</td>
</tr>
</tbody>
</table>

FAIMS Pro interface provides performance gains required for improved protein coverage in LFG proteomics workflow. ~3000 peptides and ~830 protein groups were identified from a single HeLa cell alone. This is the first example of ~1000 proteins being identified from single mammalian cells with LFG proteomics approach.

High-throughput Single Cell Protein Quantitation

To understand the biology of the cells in single cell level, we need to analyze many cells hence there is a need to a proteomics method that offers higher throughput for single cell analysis. The MS² level single cell proteomics analysis on Thermo Scientific™ Orbitrap™ mass spectrometers is a well-established approach. Here we have evaluated the MS² and MS³ level TMT single cell multiplexed LC-MS analysis on Orbitrap Eclipse Trüeb™ MS and optimized a method to improve quantitation without compromise on protein coverage.

Figure 3. High-throughput murine cell classification with TMT isotopic labeling. Total 16 single cell processed on two nanopOTS chips were analyzed with SPS MS² with Real-Time Search method and total of 24 single cells were analyzed with MS³ method. The PCA analysis (left) of TMT10plex analysis shows clear differentiation between the three different cell types (Raw Immune, Endothelial and Epithelial Cells) with both methods with SPS MS² with Real-Time Search providing improved accuracy with better separation between cells with different types without compromising total coverage. Total 2346 protein and 4761 peptide groups were identification by TMT isotopic labeling (right) with improved quantitative accuracy and differential protein coverage with Real-Time Search for SPS MS³.

CONCLUSIONS

• This ultra-sensitive low nanoflow LC-MS method, with FAIMS Pro Interface and high resolution Orbitrap Eclipse Trüeb mass spectrometer’s ion trap sensitivity has significantly improved single cell proteome coverage.
• The FAIMS Pro Interface has become a valuable tool for low cell single cell proteomics analysis.
• The nanopOTS platform combined with TMT multiplexed isotopic 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 2000 protein groups was achieved among a total of 40 single cells obtained from cultured murine cell populations.
• nanopOTS integrated with multiplexed isotopic labeling represents a highly promising platform towards single cell typing, understanding of stem cell development, proteomic studies of isolated clinical samples (circulating tumor cells) and proteome imaging of tissue heterogeneity.

REFERENCES


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