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Ultra-sensitive LC/MS workflow for in-depth label-free analysis of single mammalian cells with nanodroplet sample processing

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ABSTRACT

In the last decade, single cell RNA sequencing has advanced our understanding of transcript heterogeneity. Currently, there are strong efforts to enable single cell proteomic analysis using mass spectrometry (MS)-based workflows. While the analysis of single-cell-sized aliquots from bulk-prepared tryptic digests has been demonstrated, only recently have label-free strategies been reported for profiling hundreds of proteins from single mammalian cells. Further development in sample processing, separations, MS and data analysis are necessary to realize single cell proteomics with greater depth of coverage and quantitative accuracy. Here we introduce an improved LC separation coupled to the new Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometers with a Thermo Scientific™ FAIMS Pro™ interface to increase proteome coverage for single mammalian cells.

MATERIALS AND METHODS

Single cells were isolated and processed on nanoPOTS (nanodroplet Processing in One-pot for Trace Samples) platform[1]. Solid phase extraction (SPE) and analytical columns ranging from 20 to 30 µm i.d. were used for peptide trapping and separation. A Thermo Scientific™ UltiMate™ 3000 RSLCnano system coupled to an Orbitrap Eclipse Tribrid MS with a FAIMS Pro interface were used for this ultra-sensitive workflow. Raw data files were processed using Thermo Scientific™ Proteome Discoverer™ 2.4 software and MaxQuant software.

Figure 1. Single cell picking and protein digestion with nanoPOTS: Viable cultured HeLa cells were imaged using an inverted microscope incorporated into the nanoPOTS platform. A glass capillary tip was used to aspirate individual targeted cells and dispense them into nanoPOTS nanowells for sample processing.

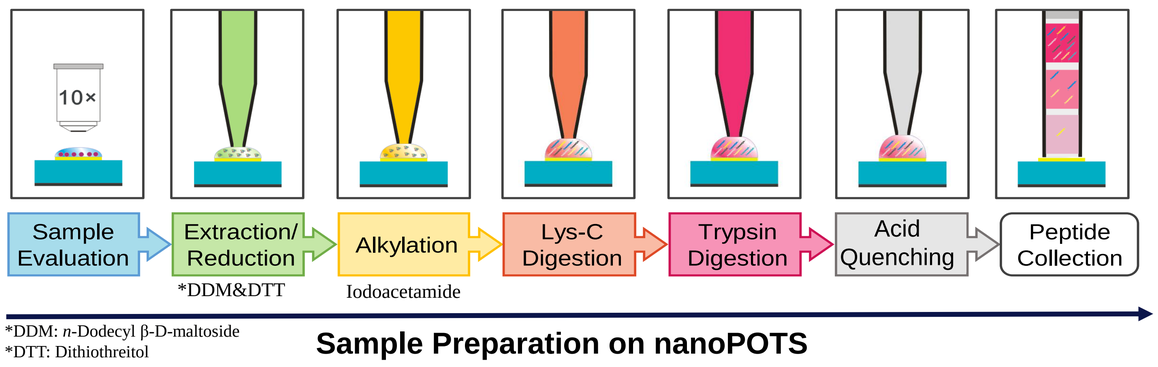
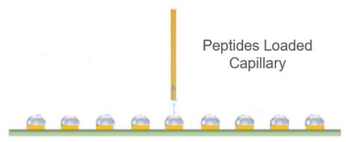


Table 1. Miniaturizing the digestion volume. Processing samples in lower than typical digestion volume minimizes the surface samples are in contact with, thereby, helping to avoid sample loss in low amount of protein samples in a single cell level.

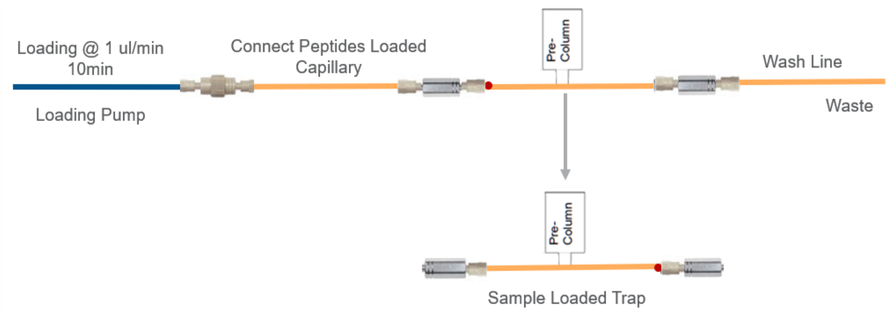
	Tube/Micro Well Method	nanoPOTS Method
Reaction Volume	**100 µL**	**200 nL**
Surface	**127 mm²**	**0.8 mm²**
Digestion Kinetics	**Low**	**High**

Figure 2. Ultrasensitive low nanoflow LC-MS workflow for single cell analysis. The three steps loading, and LC setup are shown below; this provides direct introduction of tryptic digested single cell proteins to analytical column for peptide separation and MS analysis.

Step 1 Loading Peptides Into Short Capillary Tube



Step 2 Loading Peptides Into the SPE Column



Step 3 Connect the SPE online and start 160 min LC-MS Analysis

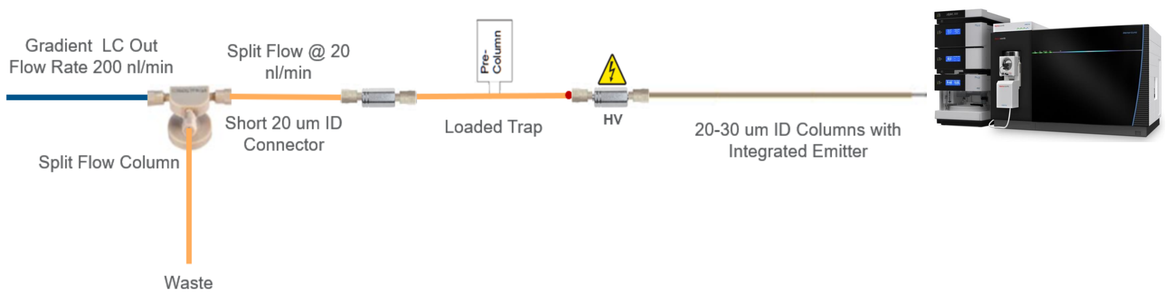


Figure 3. Unique peptides and protein groups identified from isolated HeLa cells with MS and MS/MS analysis on an Orbitrap Eclipse Tribrid MS with HCD fragmentation without FAIMS Pro interface. Isolated single HeLa cells, (uniform and large), pooled HeLa cells 20 and 100 cells and blank injection were digested and analyzed. Protein and peptide group identifications reported bellow are based on MaxQuant with MS/MS spectra and > 1% FDR. MaxQuant match between run (MBR) was against 20 and 100 identified spectra.

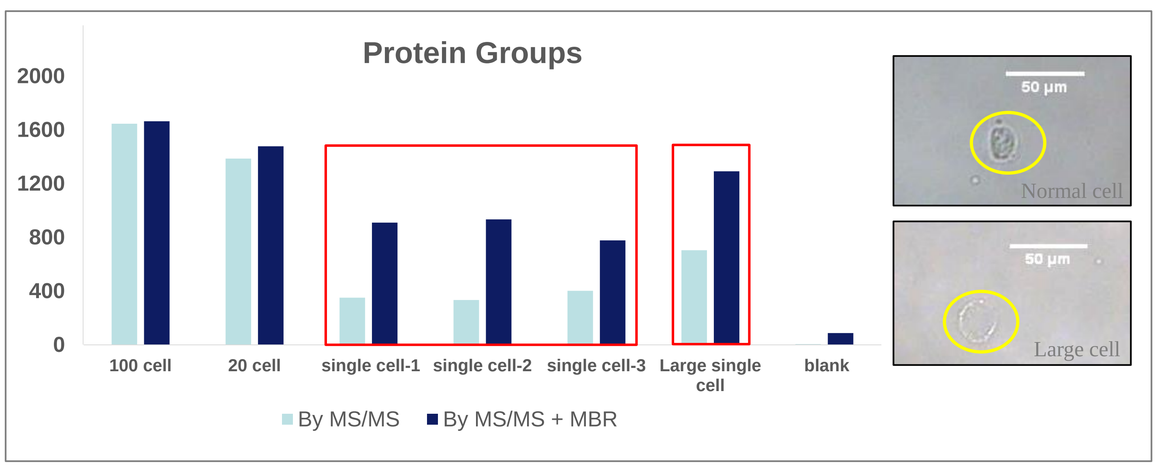


Figure 4. FAIMS Pro interface. The next generation differential ion mobility, FAIMS Pro interface enabled more proteins to be identified by reducing chemical noise and matrix interferences, resulting in improved assay robustness and increased sensitivity[2]. The FAIMS Pro interface offers separation based on a combination of factors, such as charge state, shape, conformation, and size of gas phase ions. It also improves dynamic range and peak capacity, which are particularly beneficial in proteomics applications like single cell analysis. Side view of ion spray source, FAIMS electrodes and transfer tube to the mass spectrometer (A), and schematic overview of the FAIMS Pro interface; electrode assembly with 1.5mm gap between outer and inner electrode (B) is shown.

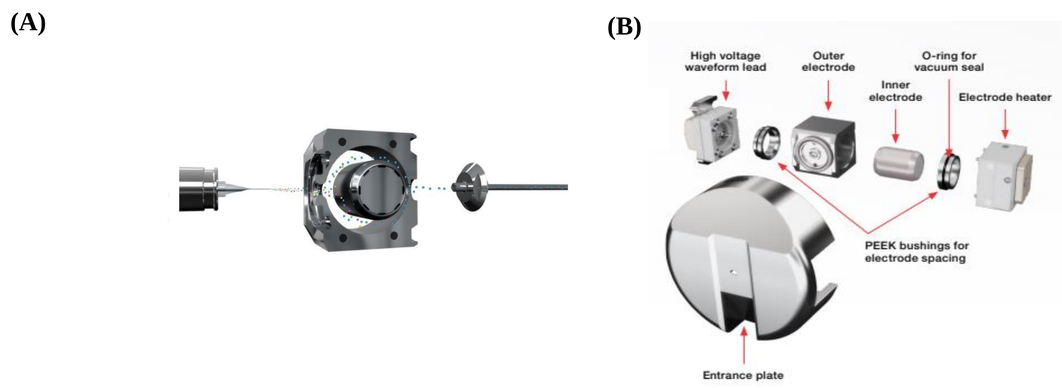


Figure 5. FAIMS Pro interface enhances proteome coverage in singe cell level by removing +1 charged ions; suppressing multiply charged peptides ions from single cell, allowing lower abundant peptides to be detected by MS.

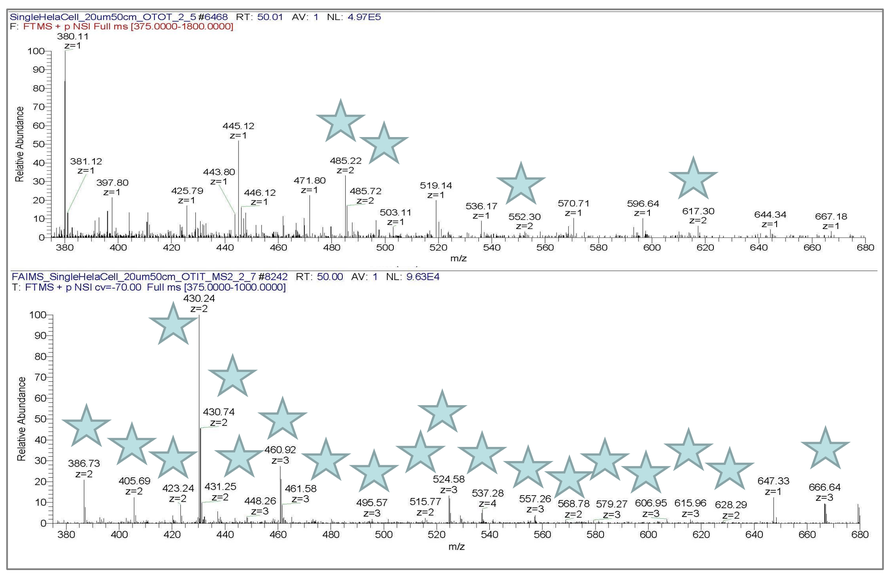
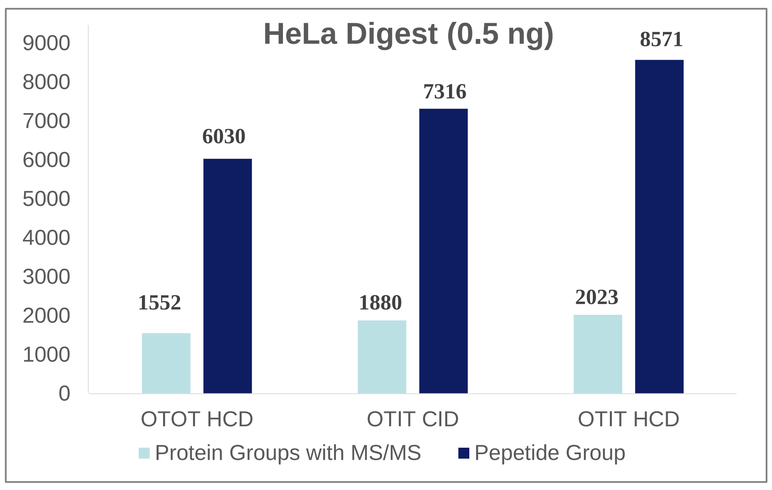


Figure 6. FAIMS Pro Method Performance. The performance of this ultra-sensitive LC-MS workflow was evaluated and optimized using 0.5 ng aliquots of Thermo Scientific™ Pierce™ HeLa Protein Digest Standard. MS and MS/MS in high resolution Orbitrap with HCD fragmentation shown to provide sensitivity and selectivity required for analysis of single cell proteomics. With a 2 hr gradient and two CV (compensation voltage) switching, 2023 protein groups and 8571 peptide group were identified from 0.5 ng HeLa digest with MS/MS spectra and FDR rate of 1% or better.



RESULTS

Application of FAIMS Pro in LC-MS acquisition for single cell analysis provides performance gains required for improved protein coverage in a label free proteomicsworkflow. ~3000 peptides and ~830 protein groups were identified by MS/MS from a single HeLa cell alone, and match between runs identifications increased identifications to ~1300 protein groups and ~5800 peptide groups. This is the first example of >1000 proteins being identified from single mammalian cells with a label-free proteomics approach**.**

	Initial Workflow	Optimized Method
Sample Preparation	nanoPOTS	nanoPOTS
Separation	30 µm i.d. column	20 µm i.d. column
Mass Spectrometer	Lumos	Eclipse+FAIMS Pro
Data Analysis	MaxQuant	Proteome Discoverer
Single HeLa Cell Protein Group ID by MS/MS (MS/MS+MBR)	211 (669)	829 (1300)
100 HeLa Cells Protein Group ID by MS/MS	2109	3067

CONCLUSIONS

- The nanoPOTS nanodroplet processing platform dramatically enhance proteomic sample processing and analysis for small cell populations and single cells.
- Our ultra sensitive low nano flow LC-MS method with FAIMS Pro interface and Orbitrap Eclipse mass spectrometer's ion trap sensitivity with high resolution has significantly improved single cell proteome coverage.
- This MS-based proteomics workflow has become a valuable tool for label-free single cell proteomics analysis.

REFERENCES

1. Zhu, Y. et. al. Nat. Commun. 9, 882, (2018)
2. Schweppe D.K. et. al. Anal. Chem. 91,6, 4010-4016 (2019)

TRADEMARKS/LICENSING

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