

IMPROVED QUALITATIVE AND QUANTITATIVE ANALYSIS OF THE HUMAN MITOCHONDRIAL PROTEOME BY HYBRID ACQUISITION

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INTRODUCTION

Mitochondria are essential organelles for the regulation of cell life and death. Literature suggests the involvement of mitochondrial dysfunction in many human diseases, some strictly linked to mutations in the mitochondrial genome, and others someway connected to mitochondrial functionality by proteins sequence defects. At the same time, proteins possibly related to mitochondrial function await validation at the transcript and protein level. A novel hybrid acquisition mode, named Multi-Mode Acquisition, which is the product from combining of DDA and DIA in a single experiment, and associated analysis tools, are described for the analysis of the mitochondrial complement. A dramatic fragmentation spectra quality increase was observed, which in turns improves confidence and coverage of identifications compared to conventional acquisition and processing modes.

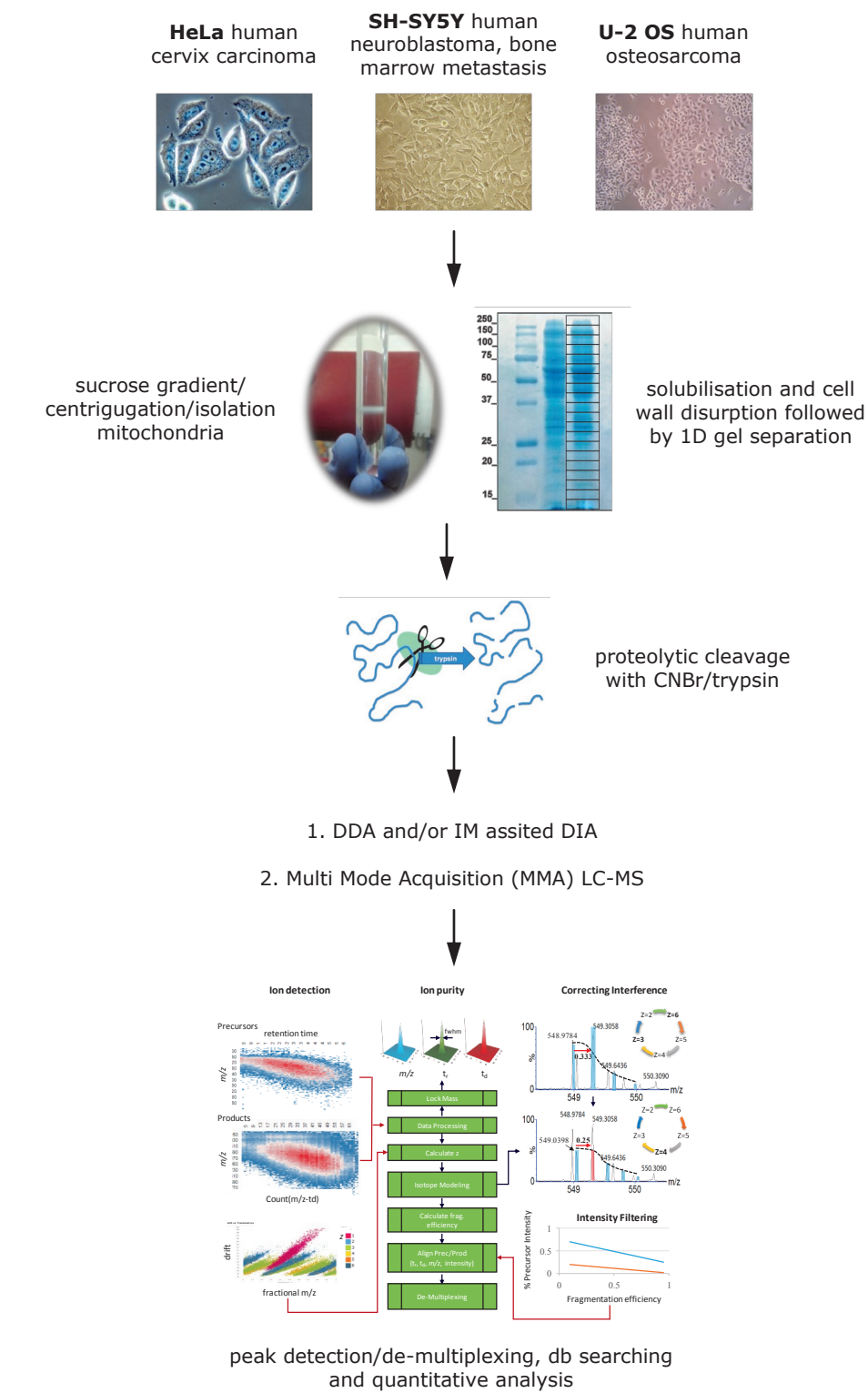


Figure 1. Experimental design and acquisition strategy.

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METHODS

Sample preparation

Mitochondria were isolated from various human cell line sample as shown in Figure 1. Following solubilisation and wall disruption in the presence of Rapigest, proteins were reduced/alkylated and trypsin digested. The following 1D gel fractions were analysed in detail, HeLa fractions 9 to 12, U2OS fractions 7, 8 and 9, and SHSY fractions 6, 7 and 9 (all centering around ~ 50 KDa protein Mw).

LC-MS conditions

Nanoscale LC separation of tryptic peptides was conducted with a trap column configuration using an M-class system and a 2 h gradient from 5-40% ACN (0.1% FA) at 300 nl/min using a BEH 1.7 μ m C18 reversed phase 75 μ m x 20 cm nanoscale LC column. MS data were acquired in top 20 data dependent analysis (DDA) and in ion mobility enabled data independent analysis mode (LC-IM-DIA-MS ((U)(H)DMS⁵) using a Synapt G2-Si instrument (Waters Corporation, Wilmslow, UK).

Informatics

Data were processed and deconvoluted with research software and searched with Mascot Server v2.5.1 (Matrix Science, London, UK) as illustrated by the right hand (green) components of the Multi-Mode Acquisition (MMA) workflow shown in Figure 2. De-multiplexed IM-DIA spectra were visualized with xiSPEC (University of Edinburgh, United Kingdom). The de-multiplexing process using spectral information from different parts of the MMS data streams and complementary samples is explained in Figure 3.

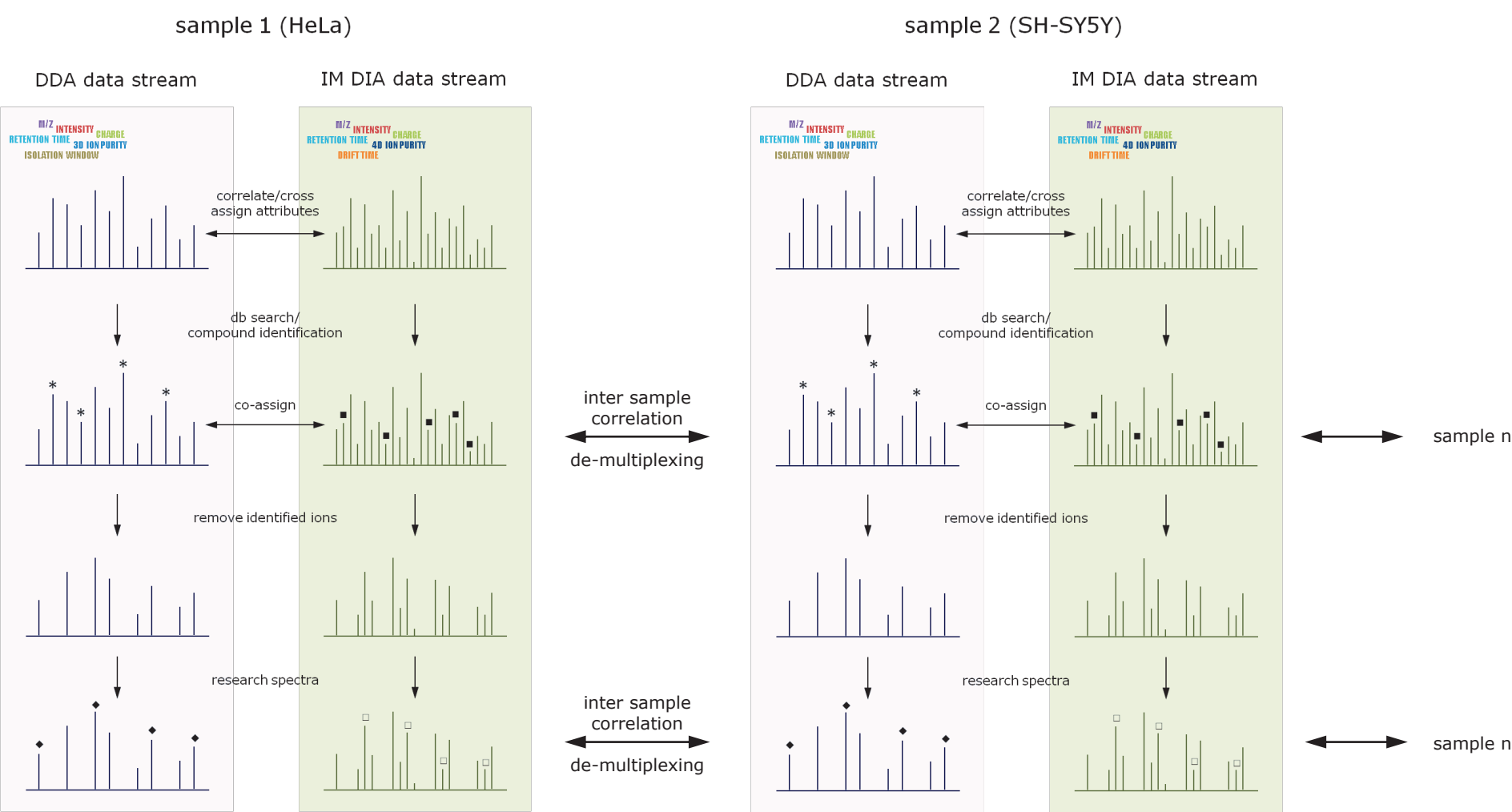


Figure 3. De-multiplexing process MMA data streams. De blue and green sections represent the DDA and IM-DIA part of the acquisition schema, each with specific attributes that can be cross-correlated to multiplex across data streams and samples.

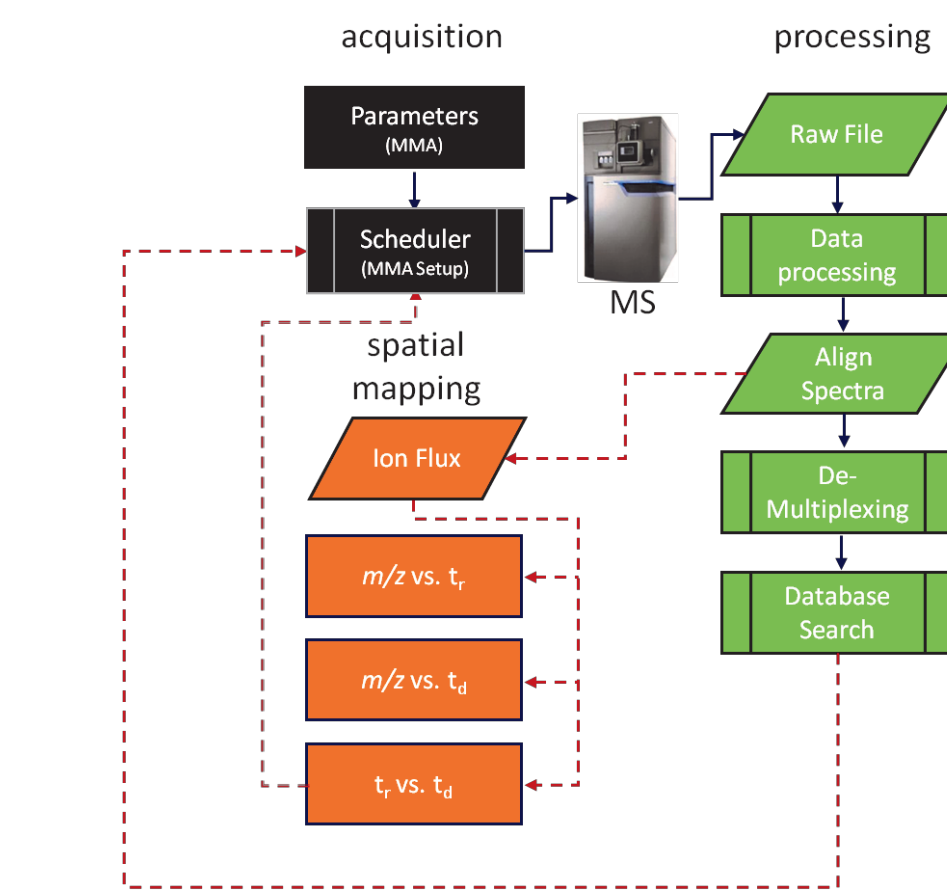


Figure 2. Acquisition and processing details MMA workflow.

RESULTS

A detailed example of the de-multiplexing process for a single protein is show in Figure 4, showing from top the bottom the original spectra identified from the data streams searched independently and additional IM-DIA spectra identified by inter/intra combination and multidimensional (t_r , t_d , m/z and intensity) de-multiplexing DDA and IM-DIA data.



Figure 4. De-multiplexing and identification process MMA illustrated for selected peptides from Coiled-coil domain-containing protein 51 (CCD51_HUMAN) by contrasting MMA data streams and product ion spectra from multiple cell line samples. The top part illustrates the individual identified peptides with different data streams and/or samples. The lower pane is complemented with reconstructed IM-DIA spectra based on complementary ion detections and identifications, providing improved coverage and quantitative precision. $t_{r,a}$ = adjusted retention time.

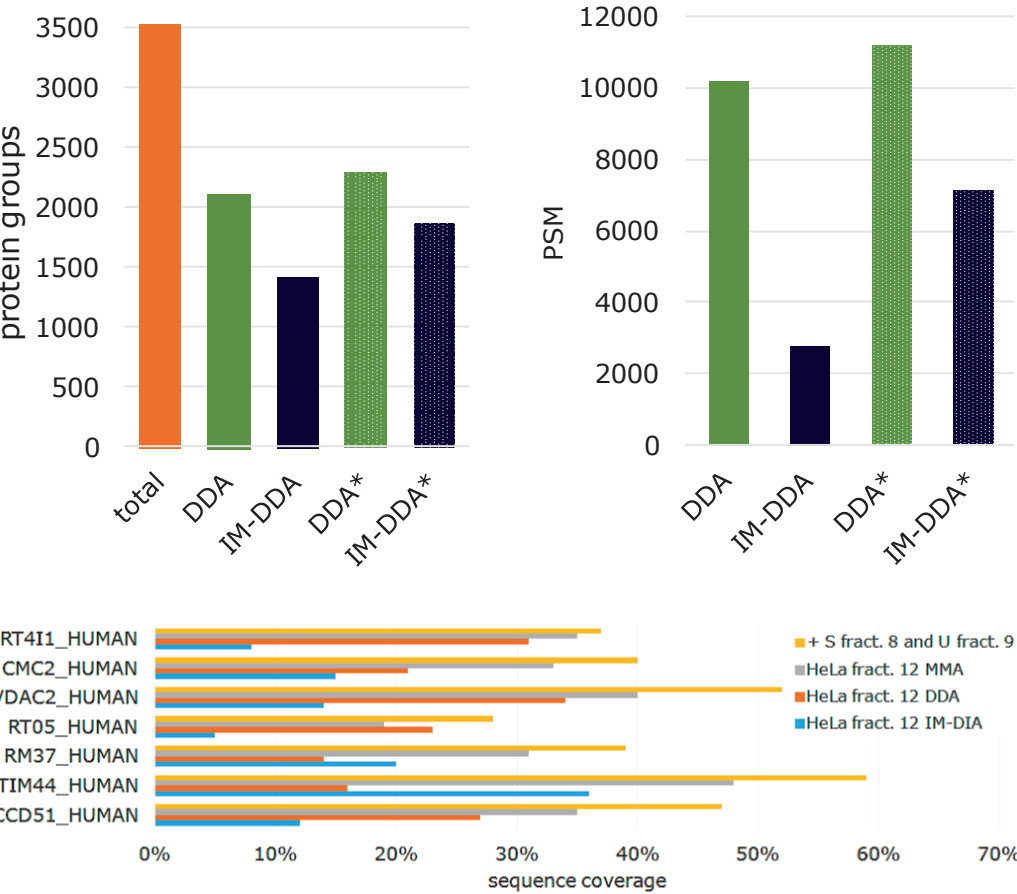


Figure 5. Number of proteins (groups), peptides (PSMs) and coverage for HeLa 1D fractions 9 to 12 acquired in DDA and MMA modes of acquisitions, and complementary analyses across all data streams and different human cell lines. DDA* = MMA processed DDA; IM-DIA* = MMA processed IM-DIA. The MMA software tools allows for DDA product ion spectra to be swept across the IM-DIA data and vice versa. Inclusion of the secondary data streams from the SHSY (S) and U2OS (U) cell line samples, following the same process, allows for a further increase in sequence coverage.

CONCLUSION

- Multi-mode acquisition methods afford the ability to assign multi-dimensional ion properties across data dependent and data independent data streams
- Enhanced multi-mode acquisition processing tools allow for improved charge state assignment, ion interference reduction, and the de-multiplexing of chimeric data dependent and data independent spectra
- Qualitative sequence coverage and quantitative accuracy were both enhanced using the collective ion properties from all data streams and samples
- On average a 2.1 fold increase in number of peptides, a 1.7 fold increase in number of proteins, and a 50% reduction in AUC quantitation was observed

References

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