Application Note

ionopticks

Aurora Series 5cm RAPID UHPLC packed emitter column

High-throughput Proteomics: Complex Samples

DATA-DEPENDENT ANALYSIS

More than **3600** proteins per run dda-PASEF

180 times per day

Integrated nanoZero® fitting

Featured products:

5cm Aurora Series packed emitter column with CaptiveSpray Insert
(5cm x 150µm ID, 1.6µm C18)
Part No. AUR2-50150C18A-CSI

5cm Aurora Series packed emitter column
(5cm x 150µm ID, 1.6µm C18)
Part No. AUR2-50150C18A

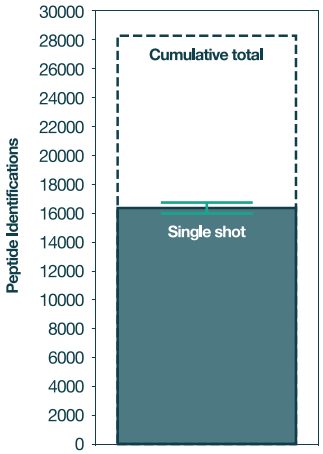


Fig 1 Number of unique peptide IDs

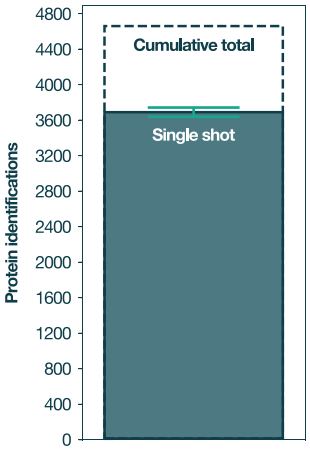


Fig 2 Number of unique protein IDs

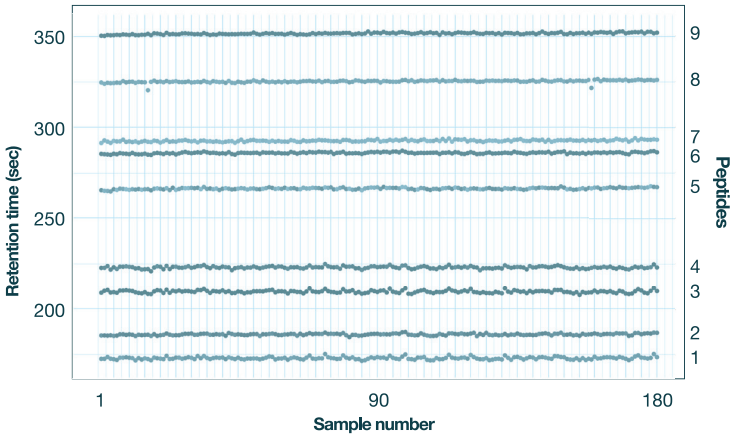


Fig 3 Retention time comparison for 9 different peptides across 180 samples

Ultra-sensitive, high-throughput proteomics

IonOpticks' 5cm Aurora series packed emitter columns featuring our revolutionary nanoZero® fitting, allow for simplified plug-and-play high-throughput proteomics. These columns are capable of analysing 180 samples per day with a 5 min gradient.

Tryptic digested HeLa cell lysate was injected and separated using our 180 samples per day method and data dependent acquisition using PASEF resulting in 3,600 protein identifications (16,000 unique peptide identifications) [Fig1, Fig 2] with highly reproducible peptide retention times between runs [Fig 3].

Robust reproducibility

- **High-throughput analysis**
- **Maximum protein IDs**
- **Minimum peak widths**
- **Consistent performance over large sample cohorts**

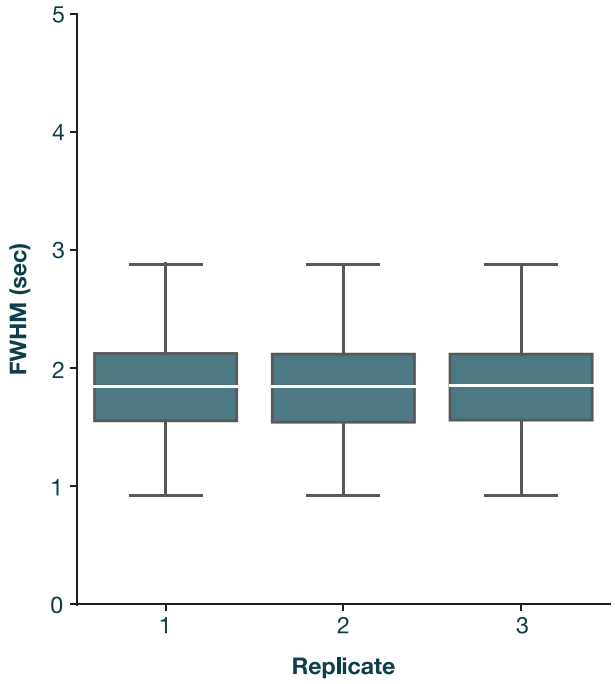


Fig 4 Median and standard deviation of FWHM for peptides

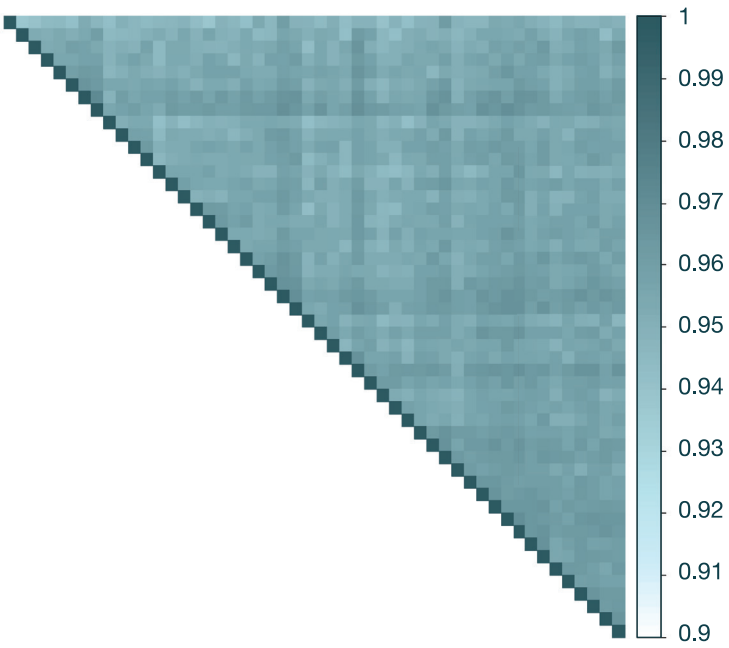


Fig 5 Pearson correlation plot of peptide intensities

1.9
seconds
Full width
Half maximum

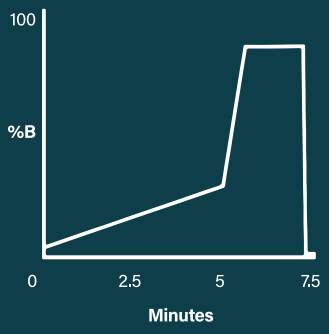
>0.95
mean Pearson correlation

across
180
samples

5cm Column
5min gradient

Time (min)	Composition (% Buffer B)	Flow Rate (µl/min)
0	5	2
5	34	2
5.5	85	2
6.5	85	2
6.8	0	2
7.5	0	2

Example gradient:



Methods

HeLa Protein tryptic digest standard (Pierce, Thermo Fisher) was reconstituted in 2% acetonitrile/1% formic acid in MilliQ water to a concentration of 80 ng/µL. Samples were analysed on a M-class (Waters, USA) coupled to a timsTOF Pro (Bruker) equipped with a CaptiveSpray source. 80ng (1µl) of peptides were separated on a 5cm X 150µm Aurora column, using a constant flow rate of 2µl/min. The column was maintained at room temperature. Sample was injected into a sample loop which takes approximately 0.5min. Mobile phase at 100% buffer A continues to flow over the analytical column during this period facilitating column equilibration. The sample loop was switched on-line for 1min at 100% buffer A. A recommended sample gradient is outlined in Figure 6.

Separately, 20µg of a Hela tryptic digest (Pierce, Thermo Fisher) was resuspended in 10mM Ammonium Formate pH 10. Peptides were separated into 12 fractions using a stage-tip containing 4 X C18 plugs. Fractions were lyophilised to dryness using a CentriVap (Labconco) before reconstitution in 2% ACN, 1% FA prior to analysis. Fractions were analysed using the same LC-MS method as the single shot samples.

Raw data processing and analysis. Data was analyzed by MaxQuant software using the integrated Andromeda search engine and searched against the human Uniprot Reference Proteome and matched to a high pH fractionation library using the Match-between-runs feature.

Further reading:

Simplified high-throughput methods for deep proteome analysis on the timsTOF Pro.

Aurora Series UHPLC packed emitter column user guide

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