

# Data Independent Analysis Leveraging Artificial Intelligence

## Introduction

Data-Independent Acquisition (DIA) is a bottom-up technique for generating mass spectrometry (MS) proteomic data. Precursor ions are isolated in pre-defined windows and then fragmented. These isolation windows can be defined either by  $m/z$  range, or by entry time into the instrument (called broadband DIA). In each of these isolation windows, all precursor ions are fragmented and then analyzed in the second stage of tandem MS. This contrasts with Data-Dependent Acquisition (DDA), where a fixed number of precursor ions are selected and analyzed in the second stage of tandem MS. Data-Independent Acquisition methods allow for improved detection of low-abundance peptides, increased specificity, and better reproducibility compared to DDA.

## Analysis of Data-Independent Acquisition using Artificial Intelligence

While DIA methods produce more comprehensive data than DDA methods, analysis of these runs pose a challenge because the resulting fragment ion spectra are highly multiplexed. Furthermore, the relationship between the precursor ion and its fragments are lost since a fragment could result from any precursor ion present in the isolation window. Conventional approaches use a sample specific spectral library generated from a previous DDA run to search the multiplexed spectra. This strategy effectively nullifies the advantages of DIA, because DDA must be run first. Deep learning can be leveraged to generate these spectral libraries by making MS/MS and RT predictions, which eliminates the need for a DDA spectral library. This simplifies the workflow of analyzing DIA data greatly and preserves its advantages over DDA methods. Spectrus is a participant in this new paradigm and has developed its own model to generate spectral libraries for DIA analysis. This allows Spectrus to unlock the full potential of DIA methods with the entire workflow handled in house.

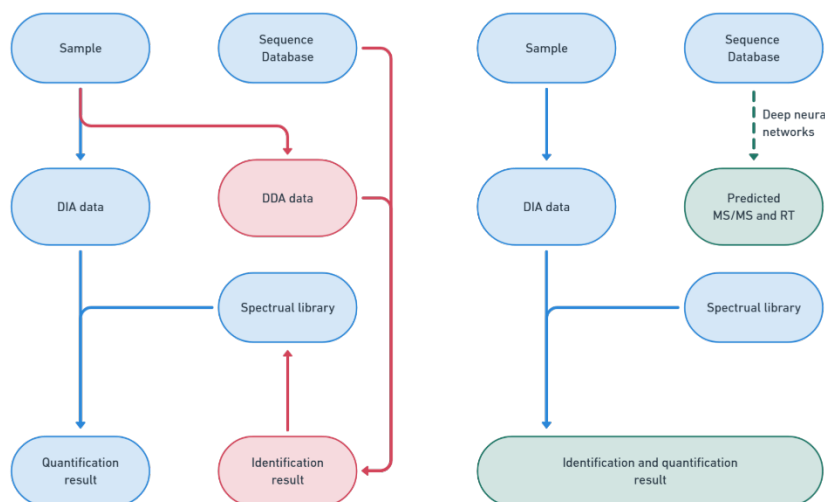


Fig.1 (Left Graph) Conventional DIA analysis in which DIA data is searched against a DDA generated spectral library. (Right Graph) DIA analysis utilizing deep neural networks to generate a spectral library

## Sample DIA Results

### HeLa Digest Identifications for DDA (7/3/24) v DIA (7/7/24)

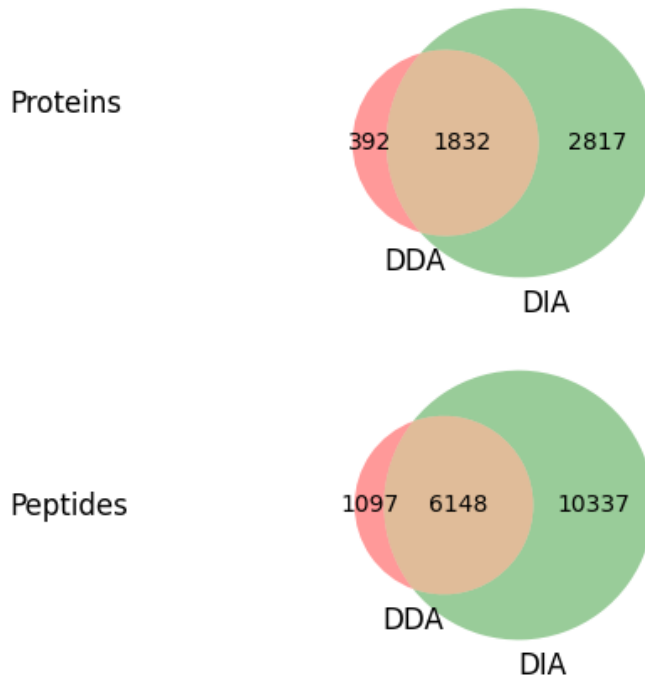


Fig. 2 Quantity of proteins (top) and peptides (bottom) identifications in HeLa digest sample ran in both DIA and DDA modes

Above is the quantity of proteins and peptides identified in a HeLa digest sample — run in both DIA and DDA modes. DIA modes identify almost twice as many proteins and peptides as DDA modes. This is because DIA methods fragment all precursor ions within an isolation window, while DDA only fragments specific ones. Running MS with DIA methods leads to a much more comprehensive and complete analysis of the sample.

## Why Spectrus is right for Data Analysis?

Spectrus' in-depth analysis allows clients to easily draw conclusions from their results. Leveraging Artificial Intelligence and report scripting, Spectrus is able provide timely, accurate, and reliable reports. Analysis and generation of reports by hand is time consuming and leads to batch effect bias as well as operator bias; both are minimized by scripting. This gives researchers and scientists quick and accurate analyses they can rely on.