BIOGNOSYS
NEXT GENERATION PROTEOMICS

Spectronaut Pulsar X

Maximize proteome coverage and data completeness
by utilizing the power of Hybrid Libraries

More versatility in proteomics research

Spectronaut™ has delivered highest performance in protein analysis for years, specifically for discovery proteomics applications. Spectronaut™ Pulsar X offers seamless combination and integration of some of the previously separate workflows, extending the applications of the Spectronaut™ software suite.

> **“Spectronaut™ Pulsar X integrates DDA and DIA data for Hybrid Library generation, which yielded excellent results in our hands and opened up exciting possibilities for novel data acquisition strategies.”**
>
> **Florian Meier**
> Max Planck Institute of Biochemistry, Martinsried, Germany (Matthias Mann Group)

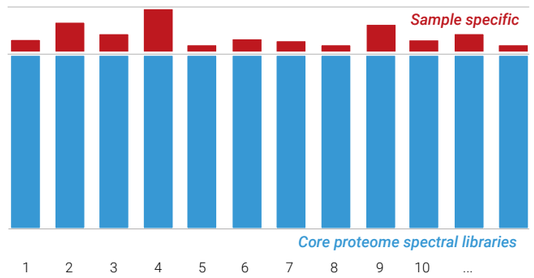
Data independent acquisition (DIA) has become the method of choice for discovery proteomics workflows. Spectronaut™ has been able to achieve the highest protein coverage and data completeness through its cutting-edge data analysis algorithms including integrated search engine Pulsar.

Spectronaut™ Pulsar X offers novel workflows to significantly extend the number of research applications:

- **Hybrid library generation:** By combining core proteome libraries (project or resource libraries) with sample specific libraries (directDIA), the number of quantifiable proteins can be increased
- **Spike-in workflow:** In combination with Biognosys’ new PQ500 reference peptide kit, targeted proteins can be label-free quantified using a comprehensive reference calibration curve spanning five orders of concentration
- **Host-cell proteins:** Calibration carry-over allows for higher precision in identification and quantification of low abundance host-cell proteins

Spectronaut™ Pulsar X also includes new features that facilitate the data analysis through improved performance and visualization:

- **Extensive spectral libraries:** Increased performance and memory efficiency allows processing of very large experiments
- **Data match plot:** Filters to choose which matched ions to visualize
- **Protein-coverage plot:** More classes of peptides annotated with streamlined run overview
- **Temporary storage:** Robust framework to prevent conflicts from Spectronaut™ running in multiple instance



TECHNICAL INFORMATION

SUPPORTED INSTRUMENTS

- Thermo Scientific™ Q Exactive™ Series
- Thermo Scientific™ Orbitrap Fusion™ Series
- SCIEX TripleTOF® Series (5600, 5600+, 6600)
- Bruker Q-TOF Series
- Waters Xevo T-QS

RECOMMENDED SYSTEM REQUIREMENTS

Windows 7 x64 or higher, CPU Intel Core i7 4770, 3.4 GHz (octa core) or more, HDD 500 GB free space or more, Memory 16 GB or more, Software .NET 4.5 or higher.

SUPPORTED METHODS

Spectronaut™ Pulsar X analyzes raw data from a large variety of different methods:

- DIA
- WiSIM-DIA
- SWATH™
- SWATH™ 2.0
- SONAR™

Methods should acquire MS1 and MS2 or MS2 only scans. Cycle time of the method should be in the range of 1-3 seconds depending on your average peak width. Gas phase fractionation is supported.

Hybrid Libraries

More identifications with less (or no) overhead

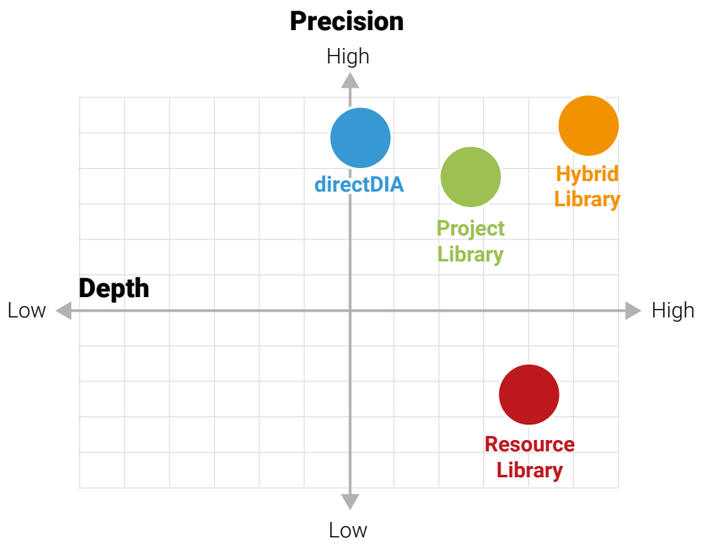
A data independent acquisition (DIA) experiment usually delivers a highly convoluted data set that requires a spectral library to extract the fragments of individual peptides hidden in the data. Libraries can be made directly from the samples that are analyzed (project specific library) or from data repositories (resource library) that may have been generated with different samples or a different instrument type.

In 2017, Biognosys introduced a library-free method that generates libraries on-the-fly from the DIA data (directDIA), which provides a third option. In any case, the quality of a spectral library is critical for the quality of the result.

The main criteria for a good library are:

Depth: Only peptides that are contained in the library can be identified in DIA data. In practice larger libraries allow for the identification of more peptides, which translates to more proteins being identified.

Precision: The better the retention times and fragment intensities in the spectral library are matched to the actual data from a specific sample; the better peptides are correctly identified. Precision also relates to the content of the library; searching for plasma proteins in a huge cancer tissue library will not identify all possible plasma proteins.



The challenge: All three library methods come with trade-offs

Project specific libraries are very precise but they are often limited in depth because the measurement time to generate a deep library may not be feasible. In addition, these libraries are typically made with pooled or individual samples. Proteins that are present in only a few samples can therefore be diluted out and missed. This is especially the case in large sample sets with high biological variance.

A resource library may be deeper but is usually less precise. It may still be limited in depth and miss relevant proteins if the sample source was not exactly the same as in the experimental samples. Different sample preparation methods, (e.g. digestion time, extraction buffer), can also result in different identifiable peptides with preparation specific peptides being unidentified despite a large resource library.

DirectDIA method provides precise libraries from each single sample. However, the depth is lower compared to project specific libraries, which are usually generated from multiple fractionated samples.

The solution: Hybrid Libraries lead to best results because they are deep and precise

Spectronaut™ Pulsar X supports a new method of library generation that combines the strengths of all library types and avoids their limitations. The underlying concept is to cover the **core proteome** with a resource library (or project specific library) and complement the library with directDIA identifications that cover the **sample specific proteome**.

Spectronaut™ Pulsar X achieves this result through correct statistical treatment of both libraries independently. We call this method **Hybrid Libraries**.

In developing the Hybrid Library technology, our focus was to maximize the number of quantifiable proteins. However, it also comes with a reduction in experimental overhead. If a resource library is used in combination with directDIA, no instrument time is required for library generation.

The power of a Hybrid Library in lung cancer data set

To showcase the capabilities of a Hybrid Library workflow, we have analyzed a set of samples from 12 individuals (cancerous and healthy tissue), acquired in DIA mode using Thermo Scientific™ Q Exactive™ HF-X. Pooled fractionated samples (30 runs) were acquired in DDA mode for the deep project specific library.

The data was deconvoluted using a project specific library, a resource library (Kim et al.), and a Hybrid Library that includes resource library and on-the-fly DIA based library (directDIA). Results show that the number of identified peptide precursors and protein groups using a Hybrid Library outperforms other two approaches in this experiment.

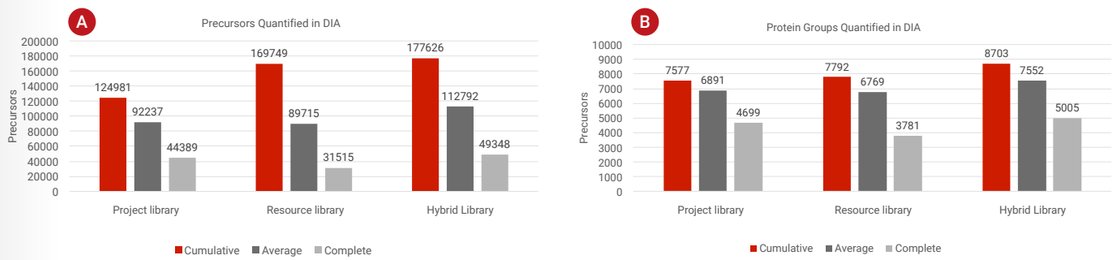
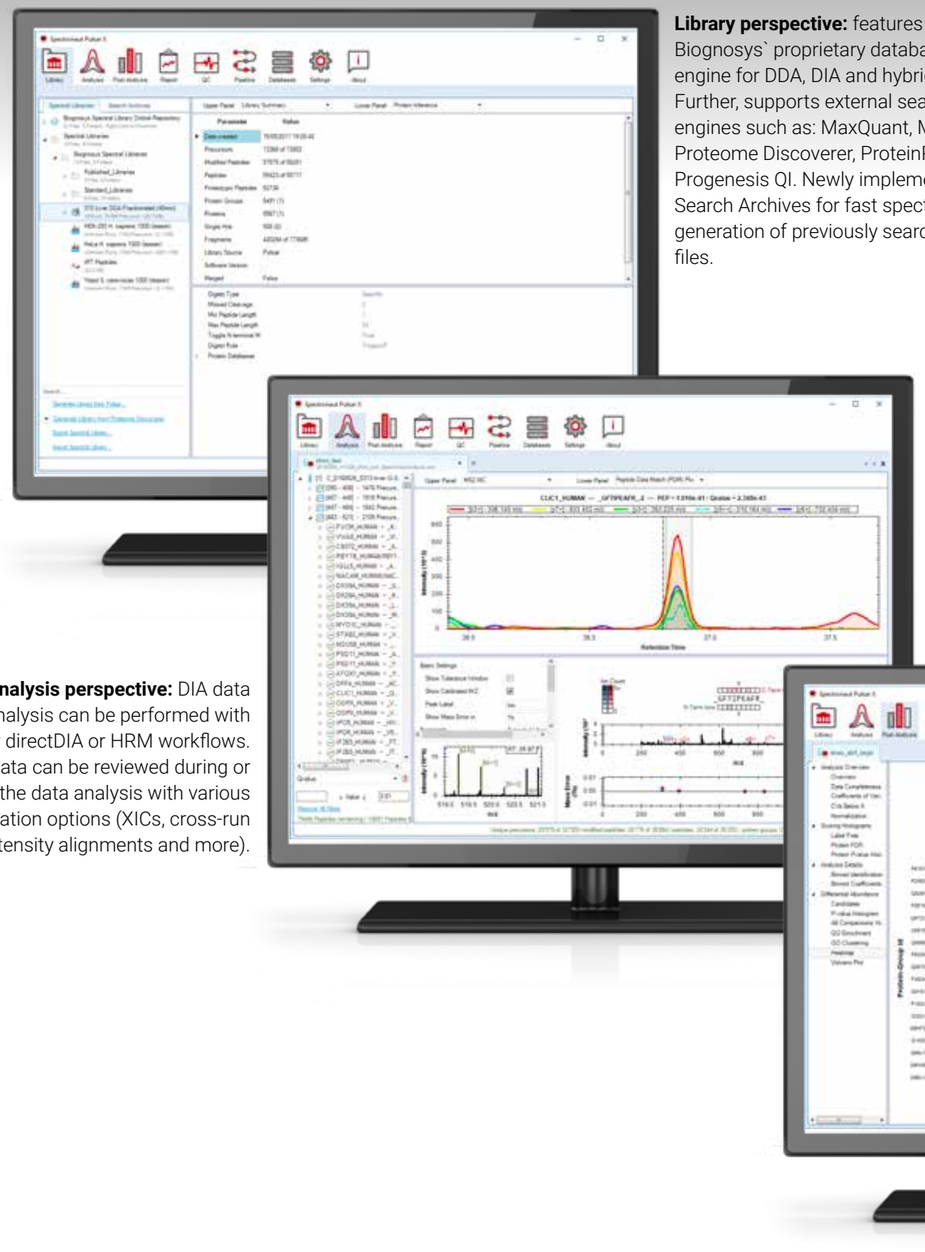


Figure: Identified peptide precursors A and protein groups B in lung cancer tissues using a DIA workflow. **Cumulative:** number of unique peptide precursor or protein group identified across all runs; **Average:** average number of peptide precursors or protein groups identified per run; **Complete:** number of peptide precursors or protein groups identified in every run of the experiment

Reference: *Kim MS et al. A draft map of the human proteome. Nature. 2014 May;509(7502);575-81.*

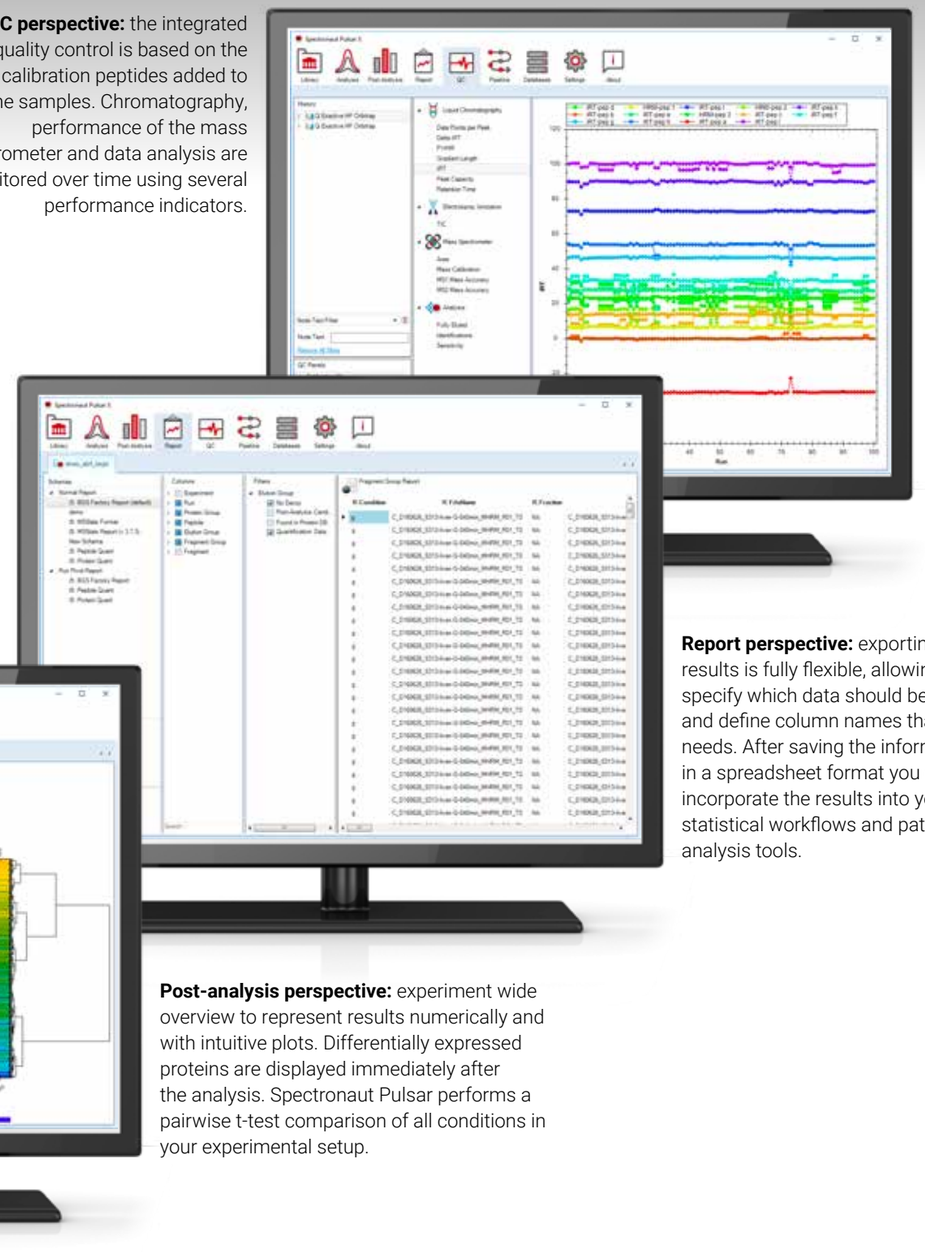
Software features

Spectronaut™ Pulsar X is specifically developed for the analysis of DIA data using multiple data deconvolution strategies. It is organized in modules called perspectives that follow the DIA analysis flow.



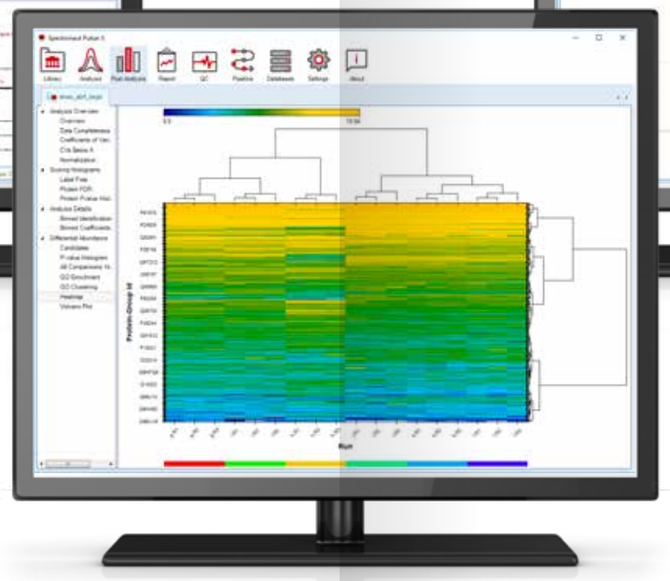
Library perspective: features Pulsar Biognosys` proprietary database search engine for DDA, DIA and hybrid libraries. Further, supports external search engines such as: MaxQuant, Mascot, Proteome Discoverer, ProteinPilot and Progenesis QI. Newly implemented are Search Archives for fast spectral library generation of previously searched raw files.

Analysis perspective: DIA data analysis can be performed with either directDIA or HRM workflows. Raw data can be reviewed during or after the data analysis with various visualization options (XICs, cross-run intensity alignments and more).



QC perspective: the integrated quality control is based on the iRT calibration peptides added to the samples. Chromatography, performance of the mass spectrometer and data analysis are monitored over time using several performance indicators.

Report perspective: exporting the results is fully flexible, allowing you to specify which data should be exported and define column names that fit your needs. After saving the information in a spreadsheet format you can incorporate the results into your own statistical workflows and pathway analysis tools.



Post-analysis perspective: experiment wide overview to represent results numerically and with intuitive plots. Differentially expressed proteins are displayed immediately after the analysis. Spectronaut Pulsar performs a pairwise t-test comparison of all conditions in your experimental setup.

How to get

Spectronaut™ Pulsar X

If you're interested in learning more about Spectronaut™ Pulsar X or to get a free trial don't hesitate to contact us at order@biognosys.com. We would be pleased to schedule a meeting and support you in implementing next generation proteomics solutions in your lab.

ABOUT BIOGNOSYS

Biognosys is a leader in next generation proteomics, dedicated to transforming life science research by making the most advanced proteomics tools available to researchers. The company offers a suite of products and services to decode the proteome and equip researchers from all fields with a deep view of protein expression in biological systems.

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