

Release Notes

MS/MS^{ALL} with SWATHTM Acquisition MicroApp

Add-in for PeakView[®] Software
Version 2.0.1

Introduction

The MS/MS^{ALL} with SWATHTM Acquisition MicroApp workflow provides a method for collecting MS/MS of all species in a sample on an LC-time scale using the SCIEX TripleTOF[®] 5600/5600+ systems, the TripleTOF[®] 4600 system, or the TripleTOF[®] 6600 system. The SWATH Acquisition MicroApp generates extracted ion chromatograms (XIC) of fragment ions from MS/MS spectra of targeted proteins and peptides and integrates the peak areas from the SWATH data files.

New Enhancements in Version 2.0.1

This release addresses minor compatibility issues with the new recommended hardware configuration (Dell T7910, running Microsoft Windows 7 64-bit operating system) and provides support for Microsoft .NET 4.5.1 framework.

Note: The MS/MS^{ALL} with SWATHTM Acquisition MicroApp version 2.0 supports PeakView[®] software versions 2.1 and later.

Requirements

- Make sure that the PeakView[®] software version 2.2 is installed and is closed. Only the PeakView 2.2 software is supported by the MS/MS^{ALL} with SWATHTM Acquisition MicroApp version 2.0.1
- Minimum operating system requirement: 64-bit Windows 7 OS SP1
- Microsoft Excel 2010 or Excel 2013 software (for the SWATH Processing Export All feature)
- Administrative-level user permission is required to install the MS/MS^{ALL} with SWATH Acquisition MicroApp 2.0.1. However, after installation, all Windows 7 Standard users can use the software.
- Microsoft Visual C++ 2010 SP1 Redistributable Package (x86) is required. If this is not detected during the installation process, the SWATH Acquisition MicroApp installer installs it automatically.

NOTE: If Microsoft Visual C++ 2010 SP1 Redistributable Package (x86) files are installed during the SWATH Acquisition MicroApp installation process, then Microsoft Visual C++ 2010 SP1 Redistributable Package (x86) is not listed under Add or Remove Programs.

Installation Instructions

Access the MS/MS^{ALL} with SWATH™ Acquisition MicroApp 2.0.1 for installation from:

- the MS/MS^{ALL} with SWATH™ Acquisition MicroApp 2.0.1 Software DVD
- the SCIEX Downloads Web site at <http://sciex.com/software-downloads>

Install from the Software DVD

Prerequisite: Make sure that PeakView® version 2.2 software is installed and closed.

1. Insert the **MS/MS^{ALL} with SWATH™ Acquisition MicroApp 2.0.1 Software DVD**.
2. Double-click the **Swath.msi** file, and then follow the instructions.
3. Exit and then start the PeakView software.

Install from the SCIEX Downloads Web Site

Prerequisite: Make sure that PeakView® version 2.2 software is installed and closed.

1. Go to <http://sciex.com/software-downloads> and then select the **MS/MS^{ALL} with SWATH™ Acquisition MicroApp 2.0.1** software from **Other Software Downloads**.
2. Save the software download zip file to the computer desktop.
3. After the zip file is downloaded, double-click the file and extract all files to the computer.
4. Locate and then double-click the **Swath.msi** file.
5. Follow the instructions.
6. Exit and then start the PeakView software.

What's New in 2.0

Streamlined UI workflow and settings

The SWATH interface has been streamlined and reorganized based on user feedback. All peptide and XIC filter settings are now in one dialog, accessed from the **Processing Settings** menu option. The user can select various view options, which the SWATH Acquisition MicroApp remembers when opening a new session. This includes window sizing and pane selection.

New columns and improved calculations

New columns on the **Peptides** table show **Expected RT**, **Observed RT**, **Score**, **FDR**, and **% Gaps** values. These columns reflect improved SWATH Processing calculations and data. For more information and procedures for use, refer to the *MS/MS^{ALL} with SWATH™ Acquisition MicroApp User Guide*.

Sample Type conversion when importing

When importing labeled workflows, users can select the label format from drop-down lists for both **Sample Type** and **Convert Sample Type To** type.

Processing Settings dialog consolidates peptide and XIC filter options

Users can now select and apply all peptide and XIC filter criteria in one dialog, including **Number of Peptides per Protein**, **Number of Transitions per Peptide**, **Peptide Confidence Threshold**, **False Discovery Rate Threshold**, **XIC Extraction**

Window, and **XIC width**. The SWATH Acquisition MicroApp remembers the filter selection criteria and applies it to the current and future sessions. Users can clear manually selected criteria at any time and return the settings to default selection criteria.

False Discovery Rate Threshold filter and new FDR column

Users can set a False Discovery Rate (FDR) Threshold filter value on the **Processing Settings** dialog. This value determines the threshold that provides a measure of confidence of the identified peak groups by performing false discovery rate analysis on checked peptides. Only the peptides that meet the FDR threshold set here are reported in the exports. The default FDR threshold value is 1% (which means that a maximum of 1% false positive peak groups is exported), with a range from 0 to 100%. The **FDR** column in the **Peptides** table shows all FDR values after they are processed.

View Settings dialog

Users can choose which panes are visible during a session by selecting **View Settings** from the SWATH menu and then selecting the check boxes next to the panes to view (Protein, Peptide, and Fragment response panes). The SWATH Acquisition MicroApp remembers the previous settings selected and then shows the same panes when the next session is opened.

Additional Save Ion Library options

In addition to the **Save Ion Library** option available in the SWATH Acquisition MicroApp 1.0, users can now select the **Save Selected Ion Library** option, to save only those peptides that are automatically or manually selected. Users can also select **Save Selected Ion Library with Updated RT**, an option that is available if RT calibration has been performed or if the data has been fully processed. This option saves only those peptides that are selected and have had the RT updated.

Show Selected Proteins only

When reviewing proteins, users can now select the **Show Selected Only** check box at the top of the **Proteins** table to show only the proteins and the associated peptides that match selection criteria. This check box is selected by default.

Fix Rank processing setting

A **Fix Rank** check box is available on the **Processing Settings** dialog for users to select to maintain the peptide ranking when importing a .txt ion library. Fix Rank enables users to set the rank for which peptides are used for quantitation. This applies to text files only.

If an ion library text file (as opposed to a .group or .mzid file) is imported with values in the **Rank** column, this field is selected by default and the software uses the imported rank. If the box is not selected, then the SWATH Acquisition MicroApp uses its own ranking mechanism.

Rank column added to Peptides table

A **Rank** column on the **Peptides** table displays the ranking for each peptide for the selected protein. This is the order in which peptides have been chosen by the algorithm for use in quantitation. If sorting has been done on other columns, this column can be used to return to the original sort order.

For ion libraries in .txt format, if the **Fix Rank** check box is selected, then the value in the **Rank** column of the ion library is shown and used. The lowest Rank value reflects the highest priority.

SCIEX iTRAQ® reagent ion library can now be imported

A SCIEX iTRAQ reagent ion library can be imported. Users must convert the data to a sample type recognized by the SWATH Acquisition MicroApp.

Extracted data might not appear in XIC and Spectrum panes

If different gradients are used in IDA and SWATH, then extracted data might not appear in the XIC and Spectrum panes. To resolve this issue, select peptides from across all retention times and perform an RT-calibration. Even if no extracted XIC is visible, select peptides from the Expected RT column without reviewing data quality in XIC and Spectrum panes and perform an RT calibration. Following RT calibration, extracted data is visible in the XIC and Spectrum panes.

Duplex workflows and settings are now available

When using Duplex workflows, additional settings and options are available for Heavy/Light pairs, including:

- Rank setting is shared for both Heavy/Light and Light/Heavy pairs.
- Export options include Ratio Light/Heavy and Ratio Heavy/Light.
- Graph options for duplex XICs display the peptide form (either Heavy or Light) selected in the Peptides Table on the top of the graph and the bottom peptide is shown on the other form.

Peaks not detected using a noise-based approach are estimated only

If a peak corresponding to one of the forms of a Heavy/Light pair is not detected using a noise-based approach, then the noise in the empty channel is estimated for the ratio calculation.

Text Search now available for peptides

Users can now use **Text Search** for both peptides and proteins. If both Text Search fields have content, then combined search results are displayed. If a Peptide Text Search string is entered, then all of the proteins containing that sequence are also listed in the Proteins pane. The proteins that contain the Text Search string for both the protein and peptide sequence are displayed in black. The proteins that contain the sequence but not the Text Search string are displayed in grey.

Intensity column added to Peptides list

An **Intensity** column is now shown on the **Peptides** list to indicate the different intensities of otherwise duplicate peptides found in the ion library.

SWATH metrics displayed

Summary information is shown at the top of the SWATH Processing window. This information includes the detection rate (the number of found peptides/the number of targeted peptides multiplied by 100), at a set FDR, over the number of samples analyzed.

Retention Time calibration

A new **Retention Time** dialog can be used to perform retention time calibration for all peptides in the ion library. Users can perform either an automatic or a manual retention time calibration.

The user first selects the peptides to use for calibration. If the **RT Cal Protein** check box is selected, then the calibration is automatically done. Alternatively, users can select **Edit RT Cal** to calculate the FIT curve and apply the calibration manually.

For more information, refer to the *MS/MS^{ALL} with SWATHTM Acquisition MicroApp User Manual*.

New SWATH session file saved as compressed package

A complete SWATH session file is saved as a compressed package that contains both the original ion library (.txt file) and the .swath file (which is actually an .xml file with all the results - s/n, score, updated RT). To access the .xml file or the original ion library in .txt format, first rename the .swath file with a .zip extension and then extract the individual files.

New ion library templates available in Help folder

The following two templates are now available for use:

- SWATHTM Ion Library Labeled EXAMPLE.txt (for labeled library)
- SWATHTM Ion Library EXAMPLE.txt (for unlabeled library)

Support for windows down to 1 Da SWATH Processing window width

SWATH Processing can support data with variable window widths down to 1 Da.

Known Issues and Limitations

SWATH Acquisition MicroApp versions are not compatible

SWATH Acquisition MicroApp 1.0 cannot be used to open SWATH 2.0 session files and SWATH Acquisition MicroApp 2.0 cannot be used to open SWATH 1.0 session files.

Transitions are ignored when they fall outside the MS/MS m/z range

Only fragment ions within the MS/MS m/z range can be manually or automatically selected.

Need to convert before importing a SCIEX iTRAQ[®] reagent ion library

The user must convert to an unlabeled or other duplex workflow before importing a SCIEX iTRAQ reagent ion library.

The final score for Heavy and Light peptides in Duplex workflows can vary

Heavy and light peptides in duplex workflows are scored as a group. However, their final score can vary if the two are found at slightly different retention times, because the noise in these two areas can be slightly different.

Associated proteins not selected even if peptides selection criteria met

Manually selected peptides that meet **Processing Settings** criteria are selected, but associated proteins are not automatically selected. The user must manually select proteins as well.

Data files must be accessed on a local hard drive

The SWATH Acquisition MicroApp requires all data files (*.wiff files, *.group files, *.mzid files, etc.) to be accessed on a local hard drive. Attempting to add data files stored on networked drives might result in errors.

Selecting y-98 or b-98 ions from *.group or *.mzid files requires manual changes

The ProteinPilot™ software has the ability to identify and label ions that show the 98 loss on the fragment ions (y and b) on Serine and Threonine phospho-peptides, but the SWATH Acquisition MicroApp does not consider these ions when choosing fragments to extract, even when they are the dominant ions. Users can, however, import a text file (*.txt) with these types of ions and they can be used by the SWATH Acquisition MicroApp.

When importing *.group or *.mzid files, save the ion libraries, then edit the data using spreadsheet software, and then reload the ion library. The notation for these types of fragments in the **frag_type** column of the *.txt file must be "y-98" or "b-98."

Select All feature does not update automatically if Peptide filters or selection changes

If the **Select All** check box in the **Proteins** group is selected, then all of the proteins and associated peptides are initially selected. However, if any peptide filter options are changed (**Number of Peptides, Peptide Confidence, Exclude Modifications, Exclude Shared**), then all of the peptides in the updated Peptides list are not automatically selected. After changing any filter options, the user must clear and then select the **Select All** check box again.

Failure can occur when processing large sets of SWATH data files

Processing a large number of SWATH data files at a time (for example, more than 30 files) can result in failure in the PeakView® software processing. SWATH data files must be processed in smaller batches.

Text search does not search for numeric values

The user cannot search for numeric values (for example, specific *m/z* values) in the SWATH Acquisition MicroApp.

Protein information is exported as Accession number only

The text (*.txt) **Export** option in the SWATH Acquisition MicroApp exports the protein **Accession** number in the **Protein** column, not the **Protein Name**.

Error message appears when reducing SWATH window width to 1 Da

If users attempt to reduce the window width to 1 Da without editing the related Config file, the following error message is shown:

"SWATH window widths below 4 Da are not supported. Please contact Support@ABSciex.com for more information."

Contact SCIEX support if this message appears.

Not enough peptides can lead to invalid FDR results

Invalid FDR results can occur if too few peptides are used.

mzIdentML support

The SWATH Acquisition MicroApp 2.0 supports mzIdentML version 1.1 and version 1.2 candidate. However, the **Maximum Number of Proteins to Import** value applies only to .group files. When mzIdentML (.mzid) files are used, all of the proteins are imported. Because of this difference, when a Paragon Algorithm search is used as the source of the ion library, it is recommended that data be imported directly from a ProteinPilot™ software *.group file. If the data is imported from another search engine using mzIdentML, then make sure that only one protein per group is included in the mzIdentML file. For example, in the Mascot Export settings, users can select to include only one protein per group. Refer to the *MS/MS^{ALL} with SWATH™ Acquisition MicroApp User Guide*.

mzIdentML use of optional Protein Name attribute

Some mzIdentML files have an optional **Protein Name** attribute. If this is present, then the Protein Name is shown in the **Name** column. However, if this optional attribute is not present, then the SWATH Acquisition MicroApp shows the protein **Accession** number in the Name column. In this case, both the Accession and the Name columns show the Accession number.

The N value for the list of Proteins in the Proteins table might not be in sequence

If there are no peptides or ions matching the SWATH Acquisition MicroApp selection criteria (for example, **Peptide Confidence**, **Exclude Modifications**, **Exclude Shared**) set by the user, then the **N** value for proteins in the **Proteins** table might not be in sequence.

LC time-scale must be the same for all SWATH (*.wiff) files

The SWATH Acquisition MicroApp 2.0 requires that all of the SWATH (*.wiff) files have the same LC time-scale (for example, a 60-minute gradient). In addition, the SWATH acquisition method for all SWATH (*.wiff) data files must be the same.

SWATH Acquisition MicroApp is compatible with only ProteinPilot™ software 4.5 (and higher) .group files

The SWATH Acquisition MicroApp 2.0 is compatible with only ProteinPilot™ Software 4.5 (and higher) .group files that have been run using identification and supported sample types.

Session or Ion Library must be reloaded if Proteins and Peptides view deleted

To continue a session if the **Proteins** and **Peptides** lists are deleted, the saved session must be reloaded or the saved ion library must be reimported. To hide the lists from view rather than delete them, use the **View Settings** menu.

No peptide information is shown after loading a mzIdentML file

If an mzIdentML file is loaded that is missing a <fragmentation> element, then the ion library is loaded but no peptide information is shown. Reload the mzIdentML with the required element.

Modifications containing amino acids with B, J, O, U, X, or Z are ignored

If an imported ion library includes peptides containing amino acids with B, J, O, U, X, or Z, these peptides are ignored by the SWATH Acquisition MicroApp and are not shown in the SWATH ion library.

Interrupted processing sessions are only partially processed

If a processing session is interrupted, then the session must be deleted and reprocessed.

Where to Get Help

Customer Training and Documentation

- *MS/MS^{ALL} with SWATHTM Acquisition MicroApp User Guide*
- *PeakView[®] Software Reference Manual.*

Contact Us

- Email: support@sciex.com
- Web: www.sciex.com

Document Revision History

Revision	Description of Change	Date
A	First release	August 2014
B	Revision of mzIdentML support details	August 2014
C	Updated branding, added computer information, changed Microsoft .NET Framework 4.0 to Microsoft .NET Framework 4.5	February 2016

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