

MarkerView

This document describes version 1.3 of the MarkerView™ software, a stand-alone application for generating aligned peak lists from Analyst® software LC/MS data files, and other data, and for performing subsequent data processing using PCA (principal components analysis), PCA-DA (PCA using discriminant analysis), or t-tests. Typical applications include: metabolomics, biomarker discovery, metabolite identification, and impurity profiling.

Version 1.3.0.1

Where to Get Help

Click **Help** in the main menu to open the *User Guide* or the *Reference Guide*.

The *User Guide* provides a tutorial introduction to all of the main features of the program. If you have not previously used the MarkerView™ software, it is suggested that you start with this document.

The *Reference Guide* document describes all of the features of the program in detail. Unlike the *User Guide*, this document does not provide examples of work but does describe the functionality of the various menu items and options.

SCIEX Support

- sciex.com/contact-us
- sciex.com/request-support

Requirements

- The program has been tested with both 32- and 64-bit versions of Microsoft Windows 7 operating system. Newer versions of Microsoft Windows have not been formally tested.
- Microsoft Office 2013 or later is required to view and print Microsoft Word reports and Microsoft Excel data spreadsheets.

Install the Software

1. Log on to the computer as a user with Administrator privileges.
2. If a previous version of the MarkerView™ software is already installed on the computer, then make sure that the software is closed.

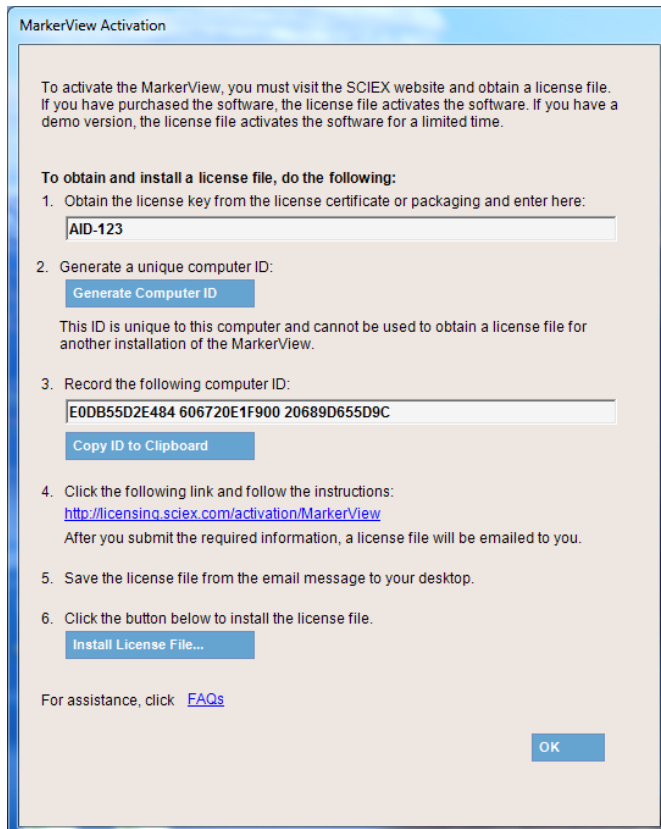
Note: You can remove the previous version or leave it installed. To remove the previous version, use the 'Uninstall a program' wizard in the Control Panel. Click on the old version of the software in the list and then click Uninstall.

3. Download the required .zip file from the SCIEX Web site (www.sciex.com/software-downloads-x2110).

Tip! To prevent potential installation issues, save the file to a location other than the computer desktop.

4. After the download is complete, right-click the downloaded file and then click **Extract All**.

5. Navigate to the extracted files or to the DVD and then double-click **MarkerView 1.3 Software.exe**.
6. The installation script launches and then the installer Welcome Screen opens.
7. Follow the instructions to install the software. It is usually not necessary to change the default installation location.
8. After the program has been installed, open it either from the shortcut on the desktop or from the Windows Start menu.
9. The first time the program is started, the following window opens.

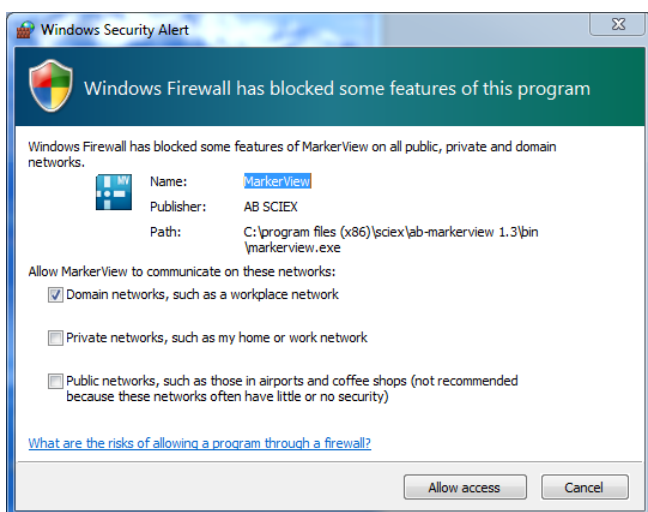


10. Type or paste the license key, exactly as shown, in the appropriate field. If a license key is not available then contact the SCIEX sales or a support representative.
11. Click **Generate Computer ID**. This action creates a unique identifier for the computer.
12. Click **Copy ID to Clipboard**.
13. Click the SCIEX licensing link and then paste the copied ID in the Computer ID field.
14. Follow the instructions to obtain the license.

Note: Internet access is required to obtain the license. If the computer does not have Internet access, then make a copy of the generated computer ID. On a computer with Internet access, go to the licensing page of the SCIEX web site and then follow the instructions to obtain a license.

15. After the required information is submitted, then a license file is sent to all e-mail addresses provided.
16. When the e-mail containing the license file is received, copy the license file to the computer desktop.

17. Click **Install License File** in the software activation dialog.
18. A Browse dialog allowing you to select the license file opens. Browse to and then select the license file and then click **Open**.
A message opens indicating that the license was successfully installed.
19. Click **OK** and then the MarkerView™ software will open.
The first time MarkerView™ software is opened after installation the following message might open.



20. If the Windows Security Alert dialog opens then accept the default option of 'Domain networks, such as a workplace network', and then click **Allow access** so that external utilities included with the MarkerView™ software can properly communicate with it.

Remove the Software

To remove MarkerView™ software, use the 'Uninstall a program' wizard in the Control Panel. Click on the MarkerView™ software in the list and then click Uninstall.

Known Issues and Limitations

- When processing a large number of LC/MS files (or a smaller number of very large files) on a 32-bit computer then the program might run out of memory. It is recommended that all peaks files for the samples are generated in smaller batches and then generate the Peaks Table using these peaks files. This process is described in both the *User Guide* and the *Reference Guide*.
- If the MarkerView™ software Activation window shown in step 4 of the installation was cancelled, then the add-on utilities cannot connect to the MarkerView™ software. This can be corrected using the Windows Firewall control panel. Contact support for more information.
- MarkerView™ software cannot process LC/MS data from QTRAP® or other triple-quadrupole instruments that was acquired in centroid mode. Profile mode data acquisition must be used.

Appendix: New Features

New Features in Version 1.3

- A wizard has been added that combines many of the previously separate steps into a single workflow.
- Data created in the new .wiff2 format by the X500 System is fully supported.
- Infused MS/MS of Everything data is now supported.
- Box-and-whiskers plots are now available.
- A new normalization technique known as most-likely-ratio (MLR) normalization has been added.
- When MS/MS^{ALL} with SWATH[®] acquisition data are processed, it is now possible to view MS/MS spectra for selected peaks (similar to what was previously possible for IDA data).
- An option has been added to the t-test to use the *Welch* variant.
- The Samples Table and Scores Table now has the ability to add custom columns which you can populate with arbitrary data.
- A new add-in 'Set Names' is available that allows names to be assigned to peak rows for targeted workflows.
- Previously assignment of PCVG groups was available using an add-in script. This functionality is now integrated and the parameter setting has been automated.
- When creating a t-test, a Profile Plot and a Box-and-Whiskers plot are automatically generated.
- A new option is added to limit the time range for XIC calculation to speed processing.
- The peak-finding process has been made faster when multiple samples are being processed on computers that contain more than one CPU or core.
- A licensing approach is being used that does not require manual copying-and-pasting and e-mailing of computer IDs.

New Features in Version 1.2.1

- For MS and LC/MS data, charge state and isotope status are automatically assigned for each peak. The default plotting symbols for peaks are based on the isotope status. When opening data processed with version 1.0 or 1.1 of the program, users can assign this information using the command.
- The t-test Table has been considerably reworked to show additional metrics and plots.
- For LC/MS data, 'Peak List Spectra' are available. These are 'virtual' mass spectra constructed using the list of detected peaks and a retention time window, as opposed to the raw profile data.
- A new Peak Info Table is available that contains general information for each peak.

- Mass spectra and XICs originating from the Analyst® software .wiff files can be sent directly to the Analyst® software. This is useful so that the data can be explored further using the various tools available.
- It is now possible to define specific plotting symbols for variables as opposed to just samples.
- To potentially save having to define many custom plot symbols for different group names, define plot symbols once for groups named '1', '2', '3', and so on to use these symbols when plotting if a specific symbol is not otherwise defined.
- The 'Enhance' LC/MS peak-finding algorithm has been modified to calculate centroid masses in the same way as the Analyst® software spectral peak finding algorithm. The net result is that spectra from wiff files will have more accurate mass-to-charge values reported. In addition, the algorithm was modified to allow it to more reliably find closely eluting isomers as separate peaks. Note that for these reasons the results obtained with this version of the application will not exactly match those obtained with previous versions.
- If the MS data has been processed for a series of peptide IDA runs, a Mascot .mgf file can be created containing a peak list for each of the available MS/MS spectra for each peak added to the interest list.
- A new normalization option, Normalize Using Total Area Sums, has been added.
- Use the Shift and Ctrl keys to select multiple files or samples in the Select Samples dialog.
- For greater ease of use, the command to sort Profile Plots has moved from the context menu to the toolbar and has some additional functionality and the command to generate Profile Plots for selected peaks is now available on the toolbar as well as the context menu.

The following functionality was added for the minor version 1.2 to 1.2.1 upgrade:

- It is no longer necessary to install a version of the program that corresponds to a specific version of the Analyst® software. The Analyst® software is not required to process LC/MS data in .wiff files.
- Processing of MRM data (especially data acquired with the *Scheduled* MRM™ algorithm) has been enhanced. Also refer to working with group names, calculating group area ratios, and selecting peaks matching a given pattern in the name.
- A shortcut list of recently saved files is available.
- The 'Peak Name' field in the Peaks Table is now editable.

New Features in Version 1.1

- The *Use area integrated from raw data, not from original peak finding* option of the *Alignment & Filtering* dialog can lead to more accurate peak areas for low-level components. This is true for quadrupole data as compared to time-of-flight data.
- The *Baseline Subtraction* option is useful when importing linear-mode MALDI spectra because this type of data often has a positive baseline offset.

- The *4x00 LC-MALDI Peak Lists* import command is used to create a Peaks Table from a series of 4700 or 4800 LC-MALDI runs. This is done by importing peak lists that were previously exported from the 4700 or 4800 system by using the Peaks Explorer program.
- The *MRM Chromatograms* import command allows MRM data to be directly imported into the program – a chromatographic peak finder is used to find peaks in each MRM chromatogram.
- The *Process peak lists* option of the *Process Spectra Options* dialog is used to import spectral peak lists (from text files) as opposed to profile spectra.
- Processed MS data for a series of IDA runs can be shown for product spectra corresponding to specific MS peaks of interest. These spectra are automatically zoomed to exclude 'empty' regions at the start or end of the acquired mass range.
- The group name, shape, size, color, and so on that are set for the plot symbol information can be exported and subsequently re-imported. This is useful if plot symbols defined on one computer are to be used on a second computer.
- Custom colors can be used for plotting symbols.
- The PCA-DA option when performing PCA can be very useful for improving sample separation when there are known groups or classes.
- The *Normalize Using Median Peak Ratios* command is useful for normalizing sample response when internal standards are not available.
- The *Normalize Using Manual Scale Factors* command is used when you have specific normalization factors, most likely obtained from an analysis that was not based on using a mass spectrometer.
- The *Ratio Responses to Selected Sample* command allows you to directly examine 'fold changes' with respect to a reference sample.
- The status bar shows information for the active data set in the pane.
- The mass-to-charge and retention times for all peaks can be saved in the Interest List and in a format suitable for directly importing as an Analyst® software IDA exclusion list. The intention is to allow product spectra to be acquired for these peaks during a second run.
- Peak labeling for Scores Plots can be disabled.
- Both the {x, y} data points and their corresponding peak lists can be exported to tab-delimited text files for spectra and XICs. For the new IDA product spectral plots, users can also export in Mascot .mgf format.

Revision History

Revision	Description	Date
A	First release at software version 1.3	April 2016

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