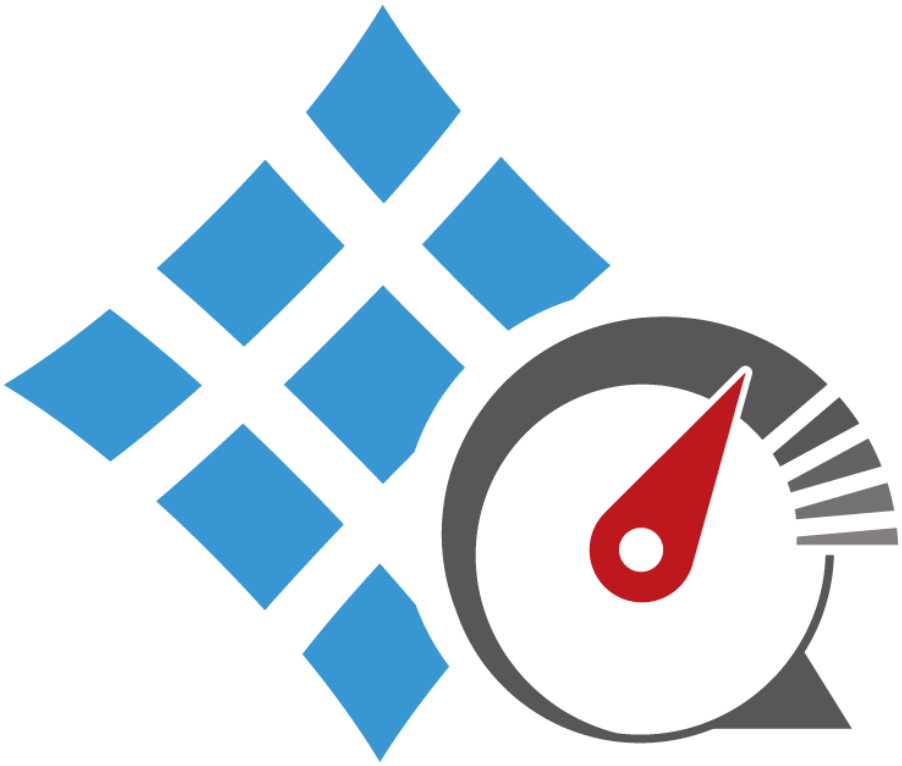

QuiC 1.0 (Owens)

User Manual

1 Contents

2 General Information

2.1 Computer System Requirements

Minimal

Operating System:	Windows 7, x64
CPU:	Intel ® Core™ CPU, 2.7 GHz (quad-core) or similar
Hard drive:	200 GB free space
Memory:	8 GB
Software:	.NET 4.6.1 or higher

Recommended

Operating System:	Windows 7 or higher, x64
CPU:	Intel Core i7 4770, 3.4 GHz (octa-core) or similar
Hard drive:	500 GB free space, solid state drive (SSD)
Memory:	16 GB or more
Software:	.NET 4.6.1 or higher

2.2 Scope of QuiCSoftware

QuiC is a software tool for quality control (QC) analysis and visualization of mass spectrometry-based proteomics runs. It supports the analysis of DDA, DIA, MRM and PRM runs from different vendors (to date, ThermoFisher and Sciex).

2.3 QuiC

QuiC is free of charge. A free license can be requested at info@biognosys.com. QuiC requires the iRT Kit or the HRM Calibration Kit to be spiked into each sample.

2.4 QuiC Release Features

2.4.1 QuiC 1.0

- QC run analysis pipelines
- Support for MRM, PRM, DIA and DDA
- Support for Thermo Fisher and Sciex
- Automated folder monitoring
- Various LC and MS proteomics QC readouts based on the iRT peptides
- Support for additional background peptides

2.5 Supported Data Acquisition Methods

QuiC analyzes a large variety of different QC data formats from DDA, DIA, MRM and PRM. Minimal requirements are a reversed phase chromatography with a linear or nonlinear gradient. All samples need to contain the iRT calibration mix to perform quality control analysis with QuiC.

In case you experience technical problems with the software or if you have feature suggestions please contact support@biognosys.com.

3 Getting Started

3.1 Getting QuiC

Free licenses for QuiC software are available for all users. The software package can be downloaded at www.biognosys.com/shop. After successful registration you will receive a download link together with a license key to activate your software.

3.2 QuiC Activation

When you start QuiC for the first time, you will be asked to activate your software. You should have received a license key with the download link for QuiC. If you do not have a license key yet, you can obtain one by registering yourself at www.biognosys.com/shop.

If your computer has access to the internet, activation will be automatic once you have pasted your license key into the QuiC activation dialogue. In case your QuiC computer does not have an internet connection or the connection is blocked by a firewall, you can also activate your software using email. The respective instructions will appear after a few seconds if online activation was not successful. Save the registration information file on your computer and send this file to support@biognosys.com. You will receive a license file usually within a workday. To activate QuiC using a license file, click on the "Browse License File…" button in the QuiC Activation dialogue.

3.3 Calibration Kit

To enable fully automated and sensitive QC with QuiC, we developed the calibration kit (iRT kit) that is spiked into each sample before the measurement. The calibration kit contains a mix of non-naturally occurring synthetic peptides. For DDA and DIA a pre-specified discovery library is additionally targeted for QC analysis. The default background library bundled with QuiC contains Human (HeLa) peptides.

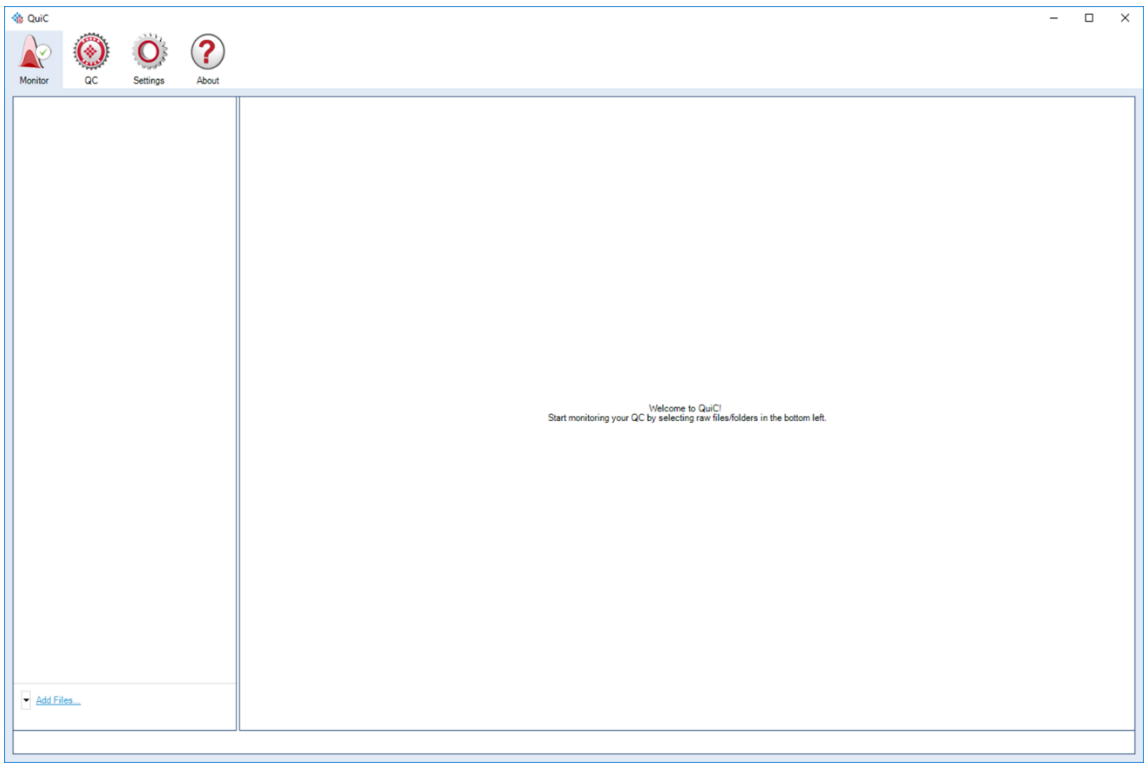

Figure 1. QuiC quality control is based on the peptides in the iRT kit or HRM calibration kit. Chromatography, mass spectrometer performance and analysis can be monitored in every run and over time for DDA, DIA, MRM and PRM using several performance indicators.

3.4 Settings

It is recommended to pre-configure QuiC after the first start to enable optimal performance.

3.4.1 QC

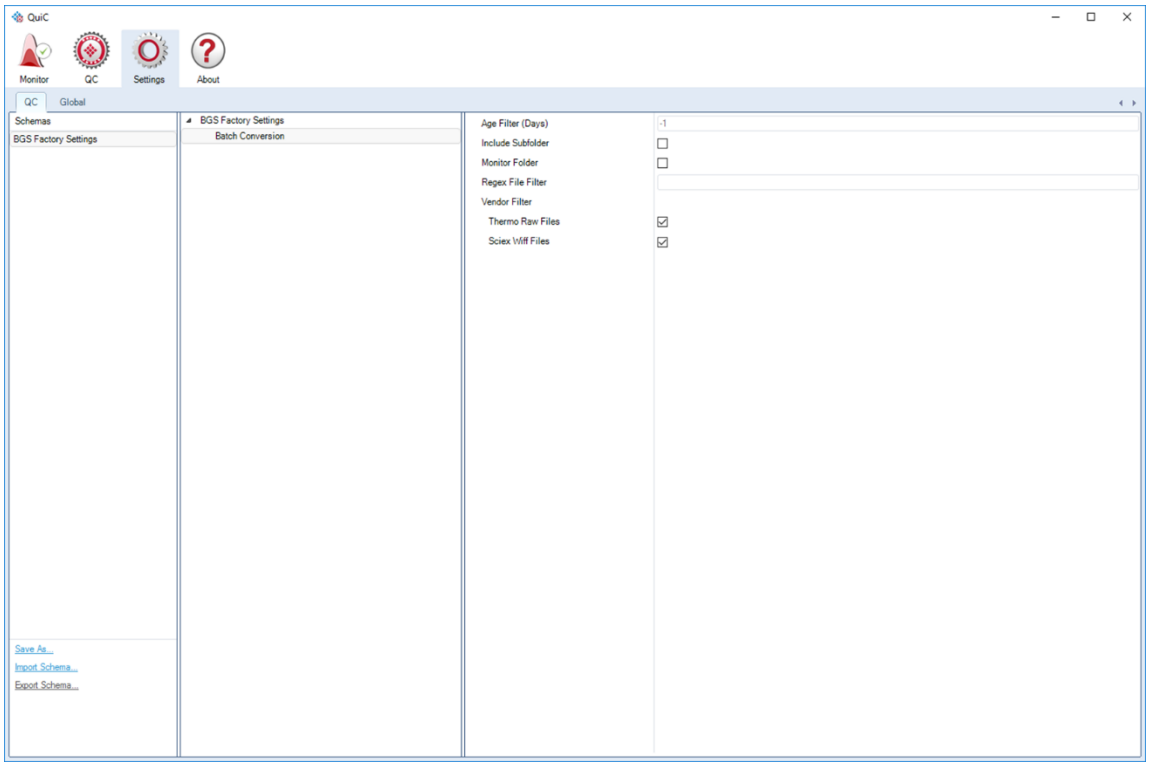

Figure 2. Analysis schemas can be created to be used and re-used in your analysis pipelines.

In the *QC* page of the Settings perspective, you can define analysis schemas that contain the parameters that will be passed to the QC analysis pipeline. These settings can be saved to be re-used across multiple analyses. You can create as many schemas as you want as well as import/export them. There are two categories pertaining to you schema: “Analysis” and “Batch Processing”. Analysis settings affect the results of an analysis of a single run, while batch processing settings control things like what files in a folder should be considered for analysis. The criteria currently available for your schemas are as follows.

3.4.1.1 Batch Processing

Age Filter (Days)

Only raw files that have been acquired within the specified number of days will be considered. If all are to be considered, this field should be set to “-1”.

Include Subfolder

The batch analysis will recursively walk the entire subdirectory hierarchy of the selected folder and add any runs in the hierarchy.

Monitor Folder

This places a “monitor” on the folder. Whenever a new run is added to a monitored folder, it will automatically be added to the task queue for processing.

Regex File Filter

This allows you to specify a regular expression pattern to consider only files whose names match the pattern to be analyzed. More information regarding regular expressions and their syntax can be found here.

Vendor Filter

Only runs associated with a particular vendor will be considered.

3.4.1.2 Analysis

Background Path

For DDA and DIA analyses, the runs are targeted using a predefined spectral library. This is used to generate richer statistics in the "Mass Spectrometer" and "Discovery" sections of the QC perspective. By default, this library is a standard HeLa library that is bundled with the software. Currently, we only accept libraries that are in the Biognosys *.kit* format. Contact us at support@biognosys.com if you would like more information about generating your own kits.

3.4.2 Global

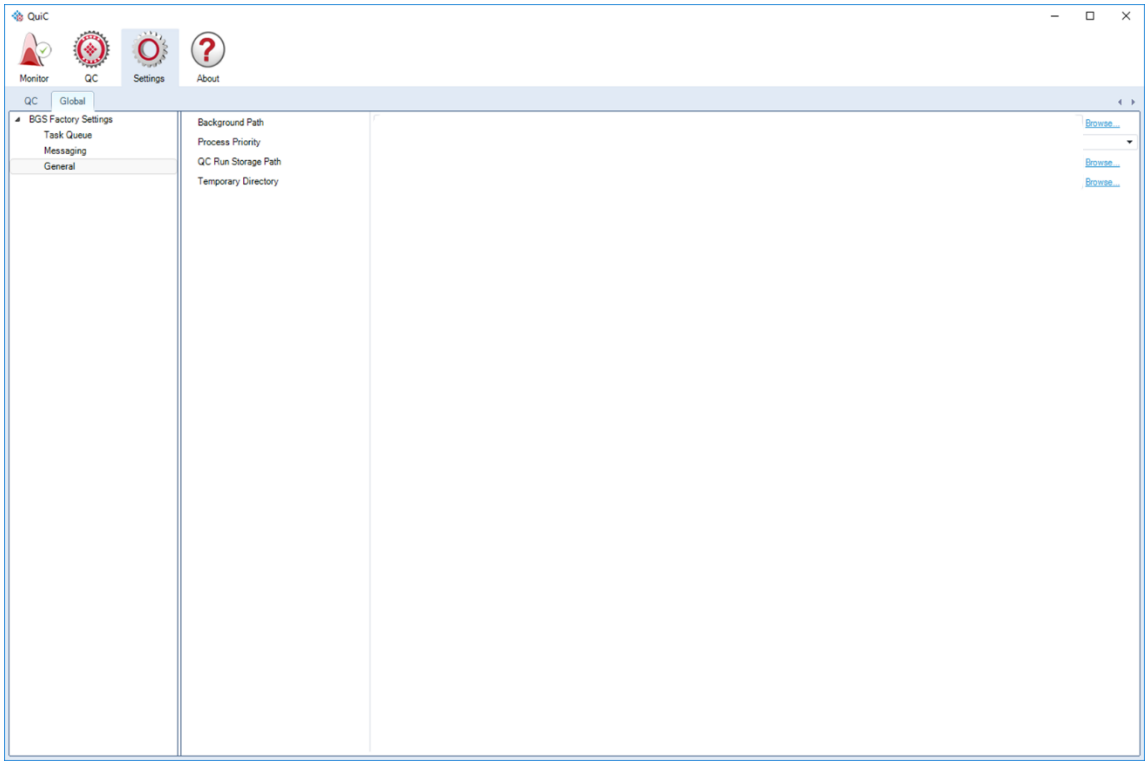

Figure 3. The *Global* page of the Settings perspective allows you to specify options that will apply universally to all QC analyses. Things like analysis task auto-deletion, notification options and storage paths can be set here.

The *Global* page of the Settings perspective allows you to specify options that will apply universally to all QC analyses. Things like analysis task auto-deletion, notification options and storage paths can be set here.

3.4.2.1 Task Queue

Task Queue options pertain to how individual run processing tasks are automatically handled in the Monitoring perspective. The parameters to be set are described as follows.

Automatic Task Removal

If you expect the task queue to grow substantially over time, you can opt to have tasks removed automatically. Note that removing a *task* does not remove your *QC run data* that is displayed in the QC perspective. Task removal will simply keep your analysis queue cleaner and easier to navigate. It also will keep memory usage to a minimum.

Periodic task removal will remove all completed task at user-defined time increments. Cleanup can occur every N days, hours, or minutes where N is specified in the "Value" field. You can specify whether you would like successfully completed tasks, skipped tasks or erroneous tasks to be removed.

Tasks can also be removed "immediately", meaning as soon as completed. Again, successful, skipped and/or erroneous tasks can be selected from removal.

Setting Automatic Task Removal to "Never" will prevent any tasks from being removed.

Select Running Task

The currently running task in the queue will be selected automatically, guaranteeing that you will always see the most recent runs in the queue.

3.4.2.2 Messaging

You can opt for desktop notifications of important events in the analysis pipeline. Here you can specify whether you want notifications when an error occurs during a task, when a task was successfully completed, or when a task completed successfully but warnings were triggered.

3.4.2.3 General

All miscellaneous settings can be found in "General". They are summarized as follows.

Check for Updates on Startup

Tick this box if you'd like QuiC to automatically check our server for newer versions of QuiC.

CPU Affinity

Here you can control what cores will be used in your analyses. If you are running multiple compute-intensive applications, for example, you may want to reduce the amount of cores used by QuiC.

Instrument Acquisition Buffer [mins]

If you are monitoring a folder in QuiC that contains files that are actively being written to by the mass spectrometer, you may need to concern yourself with this parameter. Certain mass spectrometers will leave a certain amount of time between when the spectral data is written to the file and when the final footer data is appended at the end of acquisition. This duration is difficult to detect and so we leave it as a parameter for the user to control.

NOTE: At the time of writing this manual, QuiC has only been thoroughly tested on a live Thermo setup. It has not been tested in a setting where a monitor is set on a folder having files that are partially written by a Sciex instrument.

QC Run Storage Path

You can select where you'd like permanent QC history data to be stored. This can be useful if you'd like to store your history on a network so that multiple PCs can view it.

Temporary Directory

QuiC's internal pipelines write large intermediate files to disk. If disk space is limited, you can set the path to which temporary files are written to a separate drive or partition.

4 QuiC Usage

QuiC can perform QC analysis in near real time by monitoring the folder in which mass spectrometric data is stored. Alternatively, files can actively be added to perform QC analysis.

4.1 Monitor Perspective

The Monitor perspective in QuiC allows you to manage the QC analysis of different files from DDA, DIA, MRM and PRM from ThermoFisher and Sciex.

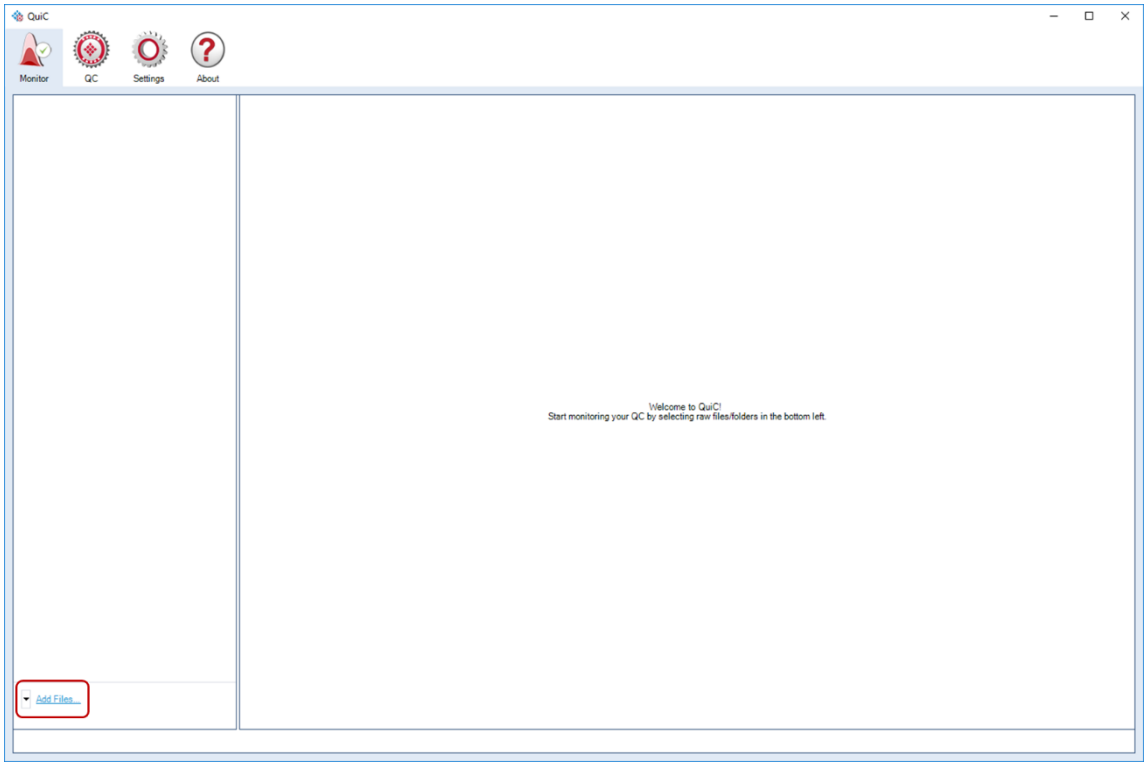

Figure 4. The monitor perspective allows you to set up a task queue of QC runs. As runs are processed, extracted metadata and visualizations will be made available on-the-fly in QC perspective. You can add individual files or entire folders to the queue.

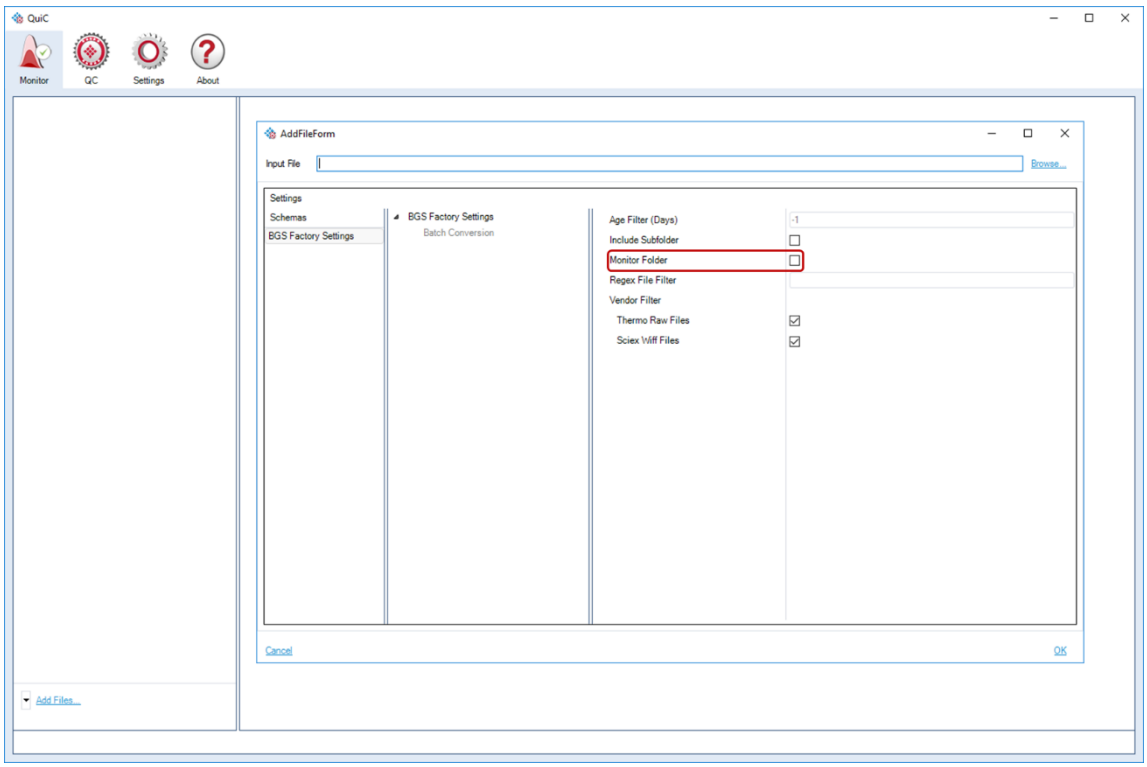

Figure 5. One powerful feature of QuiC is the ability to "monitor" folders. Files that are newly added to a monitored folder will automatically be added to the task queue.

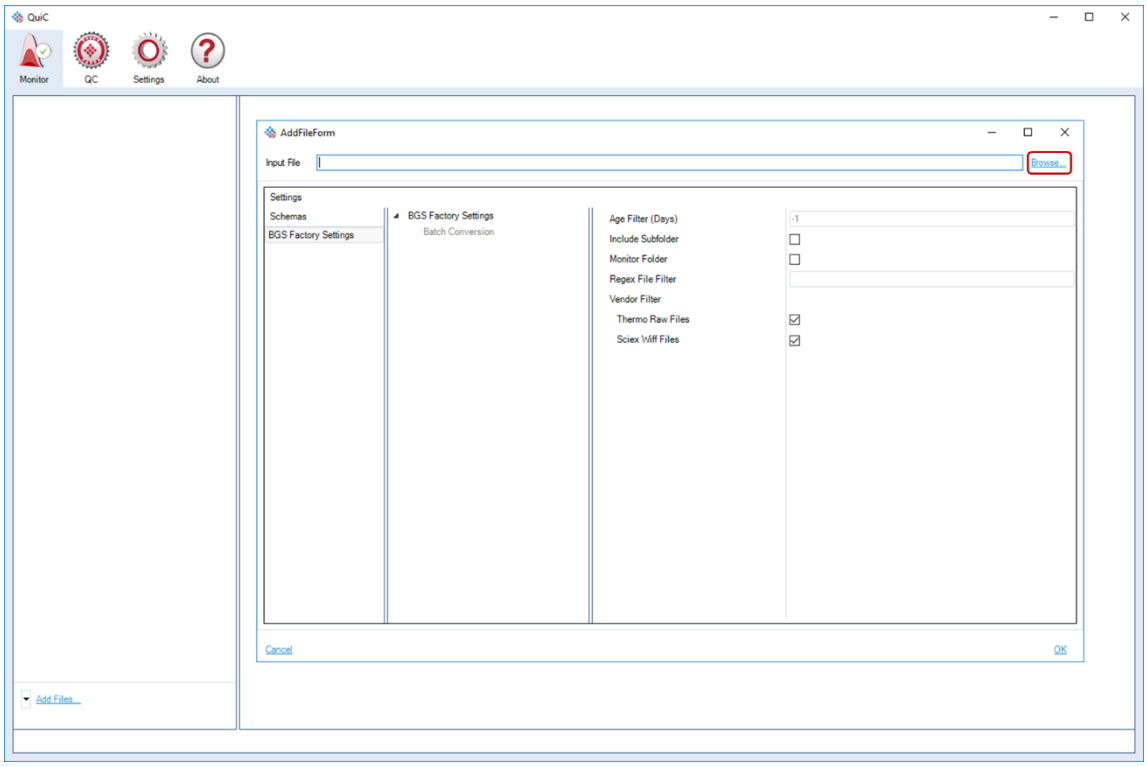

Figure 6. Select "Browse" to browse for a supported vendor raw file.

4.2 QC Perspective

QuiC stores all of the necessary information of the QC analysis and provides the review of the summarized extracted values. This data review step is done in the QC Perspective in the form of run-based visualizations.

Under *History,* all of the mass spectrometers from which the data was acquired serve as root elements of the run history hierarchy. Each instrument node can be expanded to reveal the different workflows used. The data is further subdivided into acquisition month, analysis time and, finally, individual run. By modifying your selection in the History tree, the displayed data in the plotting panel on the right changes dynamically. You can select multiple nodes in the tree by control-clicking or shift-clicking.

QuiC provides histograms for individually selected runs or box plots to compare distributions across multiply selected runs. In some cases, two plot types can be selected and compared as scatter plots across axes (e.g. try selecting a single run in the History tree and control-click “iRT” and “RT” to see the scatter plot comparison). Depending on which method is selected in History, the following plots can be viewed.

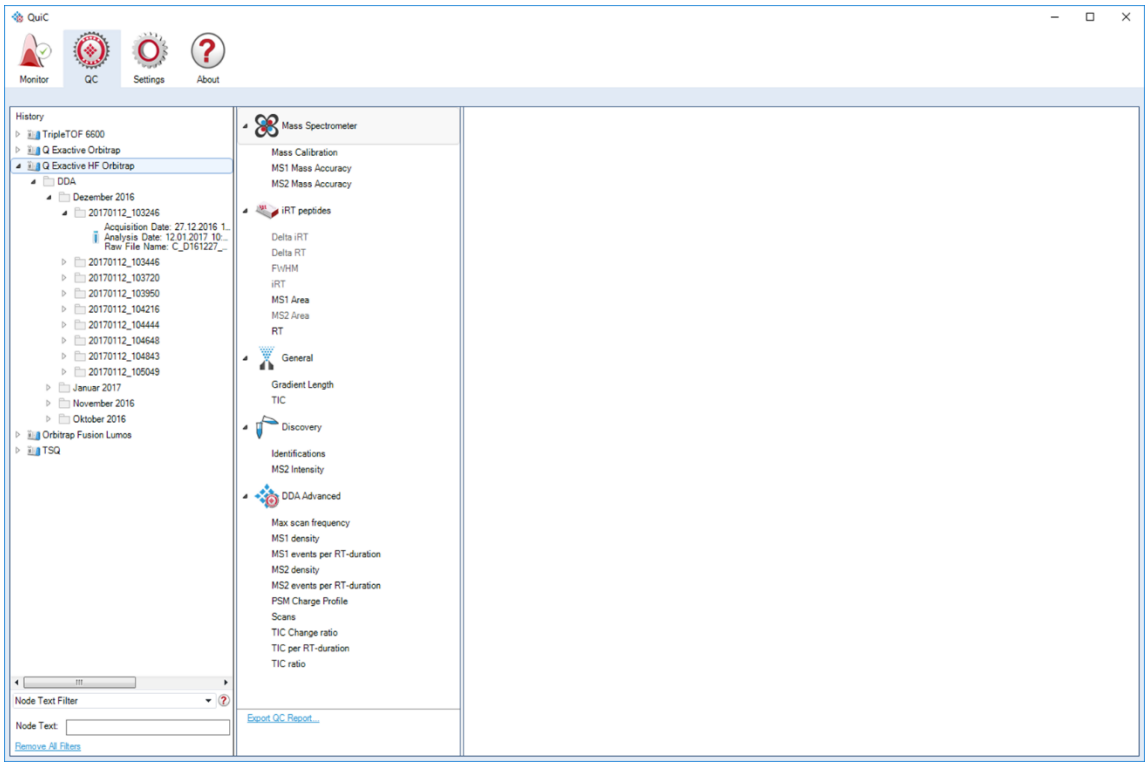

Figure 7. You can select one or multiple runs in the History tree. Runs are organized by instrument, method, acquisition date, and analysis date.

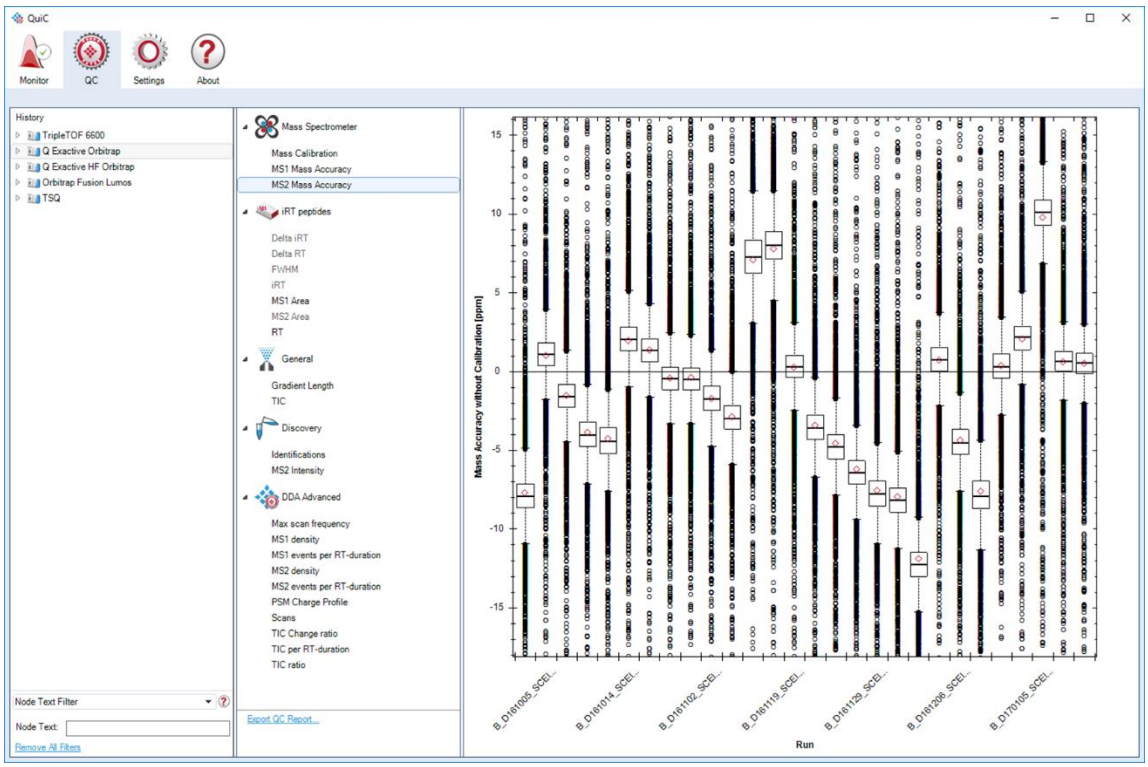

Figure 8. Selecting multiple runs allows you to compare the runs within a single QC plot. For example, here we see the MS2 mass accuracy represented as a single box plot per run. Note the plots always ensure that runs are ordered chronologically by acquisition date so as to better track instrument performance over time.

4.2.1 Mass Spectrometer

Instrument QC is displayed in this category for all peptides in the background library.

QuiC Mass Calibration

Absolute MS1 and MS2 mass accuracy (systematic shift) in ppm before QuiC's calibration as compared to the precision (noise) after calibration. The average is computed across identified peptides in the background library. Note that the calibration is used in Biognosys' other products and has shown to provide a substantial boost to our identification power.

MS1 (MS2) Mass Accuracy

Uncalibrated mass errors that were averaged in the Mass Calibration plot above.

4.2.2 iRT Peptides

Plots in this category only display QC data extracted from the identified iRT kit peptides. The plot types are summarized as follows.

Delta iRT

Delta iRT as given by the difference between the peptide's theoretical iRT and its empirical iRT, computed from the linear iRT/RT regression function.

Delta RT

Delta RT as given by difference between the peptide's theoretical RT, computed from the linear iRT/RT regression function, and its empirical RT.

FWHM

The full width at half maximum (FWHM) precursor peak width. Fragment ion intensities are summed up across fragments at every RT increment to create a single peak. FWHM is computed on this peak by taking half of the apex intensity and calculating the difference between two RTs associated with this intensity.

iRT

Empirical iRTs computed from the iRT/RT regression.

MS1 Area

The sum of all isotopic peak areas.

MS2 Area

The sum of all fragment ion peak areas.

RT

Measured apex retention times.

4.2.3 General

General QC information.

Gradient Length

LC gradient lengths estimated from the data.

TIC

Total Ion Chromatogram (TIC), which provides insight into instrument stability and amount of sample injected.

4.2.4 Discovery

These plots provide QC for discovery-based acquisitions.

Identifications

The number of identified background precursors and protein groups per run.

MS2 Intensity

Total fragment ion current computed as the sum of all MS2 intensities per scan.

4.2.5 DDA Advanced

These plots pertain only to DDA data and were first presented in (Ma et al., 2012).

Max Scan Frequency

The fastest frequency [scans per minute] for MS1 (MS2) collection in any minute.

MS1 Density

The RT at which each quartile of the MS1 scan peaks have occurred.

MS1 Events per RT-duration

The RT interval for each quartile of all MS1 events divided by RT-Duration.

MS2 Density

The RT at which each quartile of the MS2 scan peaks have occurred.

MS1 Events per RT-duration

The RT interval for each quartile of all MS2 events divided by RT-duration.

PSM Charge Profile

A breakdown of all of the peptide spectrum matches (PSMs) with a clear feature having charges 1, 2, 3, 4, 5, >5. Also considered are peaks originating from the precursor window that lack known charge but appear to have charge 1 or charge >1.

Scans

The number of MS1 (MS2) scans that were collected.

TIC Change Ratio

The TIC Change Ratio is computed as follows: For each consecutive scan pair, the TIC fold change is computed. These fold changes are then sorted in increasing order. The TIC Change Ratio for quartile N is given as the ratio of the N-th quartile fold change to the (N-1)-th quartile fold change.

TIC per RT-duration

The RT interval for which each quartile of the TIC accumulates divided by RT-duration.

TIC Ratio

The TIC ratio for the N-th quartile is given as the log ratio of the area under N-th intensity-sorted quartile to that of the (N-1)-th quartile.

5 References

Escher, C., Reiter, L., MacLean, B., Ossola, R., Herzog, F., Chilton, J., ... & Rinner, O. (2012). Using iRT, a normalized retention time for more targeted measurement of peptides. *Proteomics*, *12*(8), 1111-1121.

Ma, Z. Q., Polzin, K. O., Dasari, S., Chambers, M. C., Schilling, B., Gibson, B. W., ... & Tabb, D. L. (2012). QuaMeter: multivendor performance metrics for LC–MS/MS proteomics instrumentation. *Analytical chemistry*, *84*(14), 5845-5850. http:// http://pubs.acs.org/doi/pdf/10.1021/ac300629p