

Application Note

Trace Peak Settings for Intact and Chromatography Workflows

Customer Success Science Team

Introduction

Customization of elution peak settings automate peak picking and streamline workflows to accommodate for a variety of sample types. Both Intact and Chromatography Workflows allow the analyst to customize elution peak picking settings.

Summary

- Elution Peak Settings During Project Creation
 - Importing Traces: Embedded and Non-Embedded Traces
 - Selecting Trace Type and Trace Peak Options
 - Customizing Trace Peak Options
 - Editing Global Trace Peak Filters
- Elution Peak Settings After Project Creation
 - Editing Elution Peaks Globally
 - Editing Elution Peaks Manually
 - Editing Global Trace Peak Filters

Elution Peak Settings During Project Creation

Importing Traces: Embedded and Non-Embedded Files

After dragging and dropping the raw files into the Samples Table, the Traces Table will automatically populate with any embedded traces that are in that file (Figure 1).

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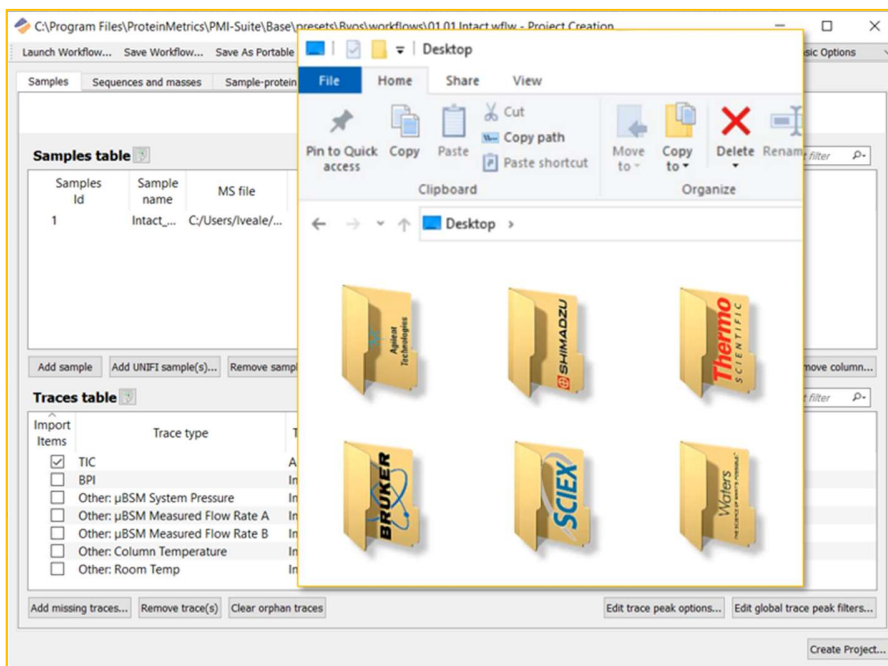


Figure 1. Automatic Population of the Traces Table for Embedded Traces

If a trace is not captured by the MS control software, it is still possible to annotate this data in the Byos project. To do so, export the non-embedded traces to X, Y (intensity, time) data in a CSV format. Add this CSV file to the appropriate line on the Samples tab as shown below (Figure 2).

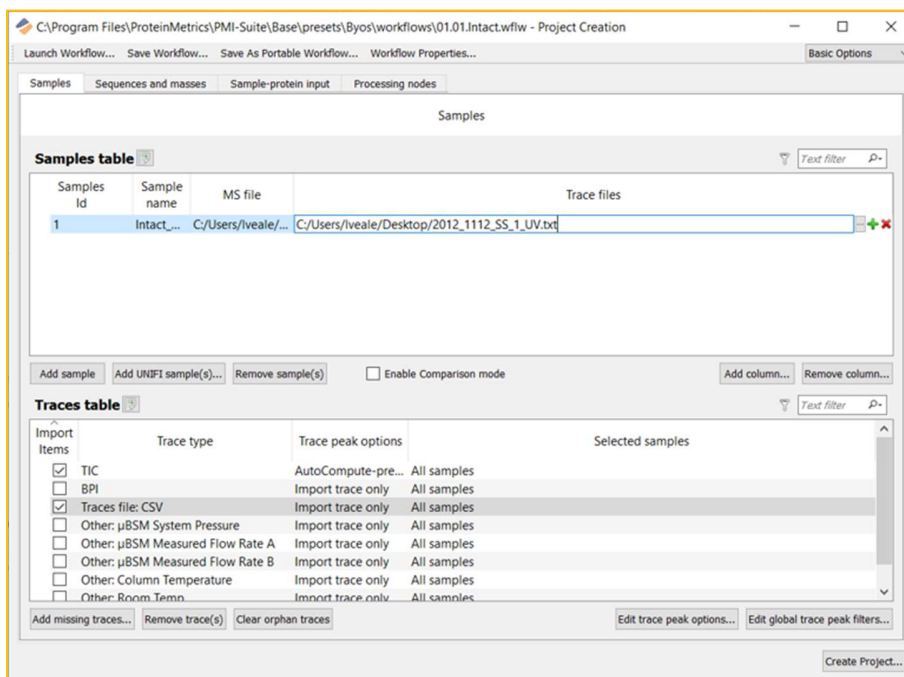


Figure 2. Population of the Traces Table for Non-Embedded (Exported) Traces

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Selecting Trace Type and Trace Peak Options

Selecting the traces for deconvolution or importation to the project is simply done by checking the box to the left of the trace type under the Import Items column, and choosing the Trace Peak Option that is to the right of the Trace Type. There are several trace peak options for deconvolution that can be performed. These options consist of SingleSlice, AutoCompute, CustomSlice(s), FixedWidthSlice(s), or AutoComputeByApex. The last option, ImportOnly, allows for importation of the trace without deconvolution. More information about customizing these peak picking settings are in the following section.

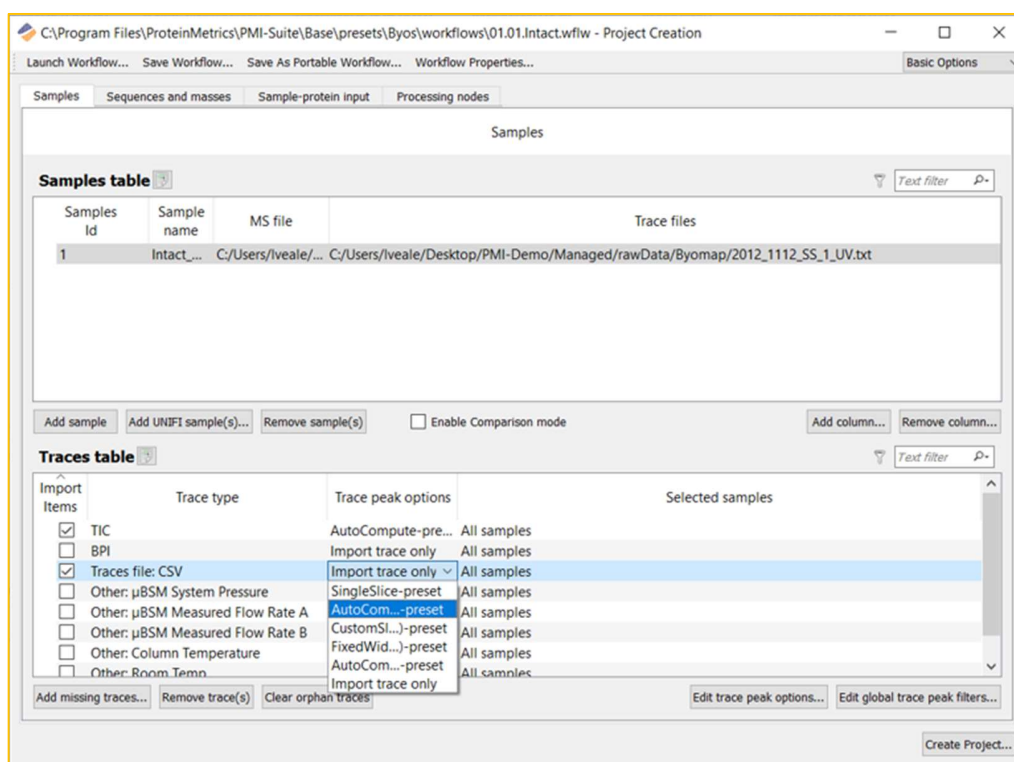


Figure 3. Selecting Trace(s) for Deconvolution or Importation to Project

Edit Trace Peak Options: Customize Settings

Customization of the trace peak options can be found under the “Edit trace peak options...” button. Clicking this button opens the pop-up menu for customization to occur.

For each of these following settings, the baseline may be customized for Auto or Flat. Under the Auto baseline, a smoothing width can be set. A larger smoothing value here can merge trace peaks, while a smaller value may split peaks.

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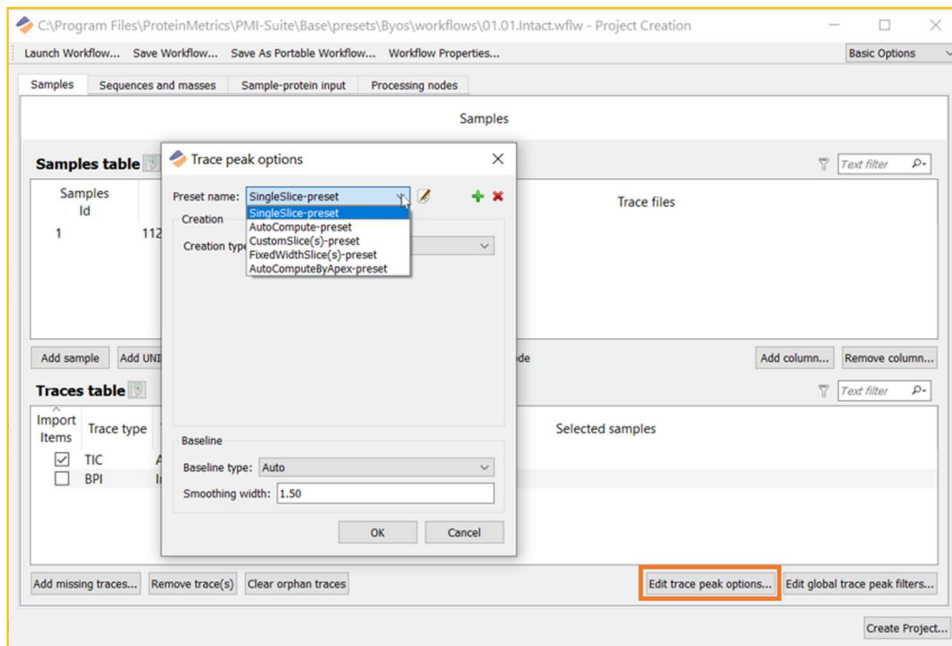


Figure 4. Customize Trace Peak Options

Customizing Trace Peak Options: Single Slice

The SingleSlice trace peak option processes the entire sample as a single peak, rendering one deconvolved spectrum per file. This setting is particularly useful for simple samples, or for a “quick first look” at your data.

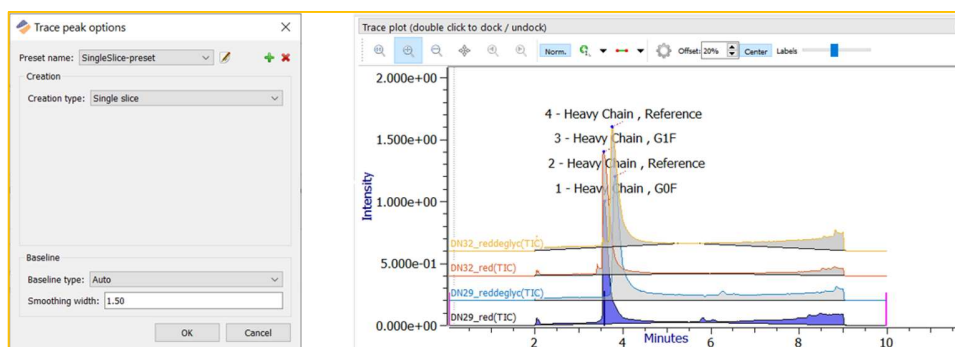


Figure 5. Single Slice Setting with Trace Plot Example

Customizing Trace Peak Options: AutoCompute

The AutoCompute trace peak option is our default peak picking algorithm. This peak picking algorithm determines the trace peaks based on their minimum width after smoothing. This setting will render a deconvolved spectrum per peak.

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Customization of the smoothing with and minimum width may be needed for optimal peak picking. In the example shown below (figure 6), the smoothing width setting will look for an inflection point every 0.1 minutes. The minimum width setting states that peaks will be 0.5 minutes wide. For wide, choppy, peaks these settings should be increased. Likewise, for thin, pointy peaks, these settings may need to be decreased.

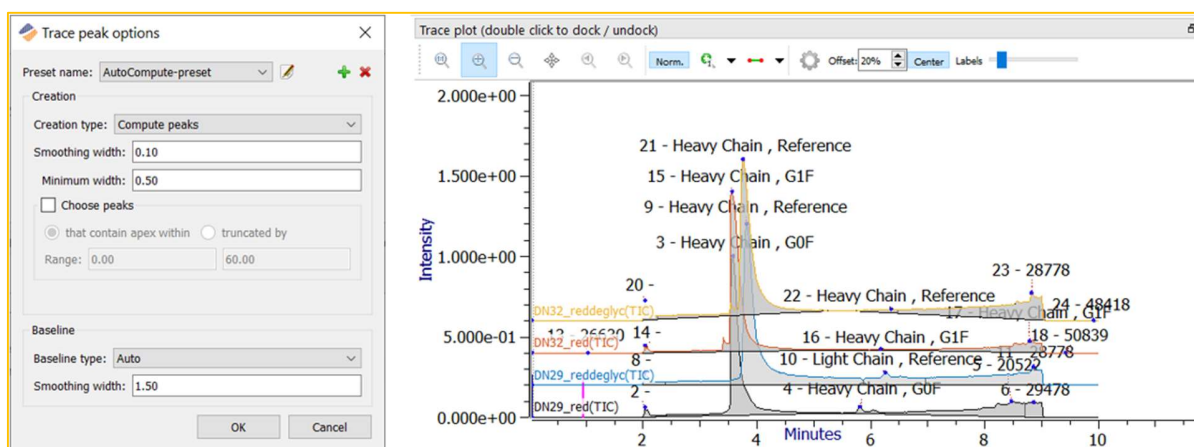


Figure 6. AutoCompute Setting with Trace Plot Example

Customizing Trace Peak Options: CustomSlice(s)

The CustomSlice(s) trace peak option allows for analyst defined start and end time points, rendering a deconvolved spectrum per each time point. This setting is particularly useful for IdeS digests, or samples in which peaks are expected to elute within particular time windows.

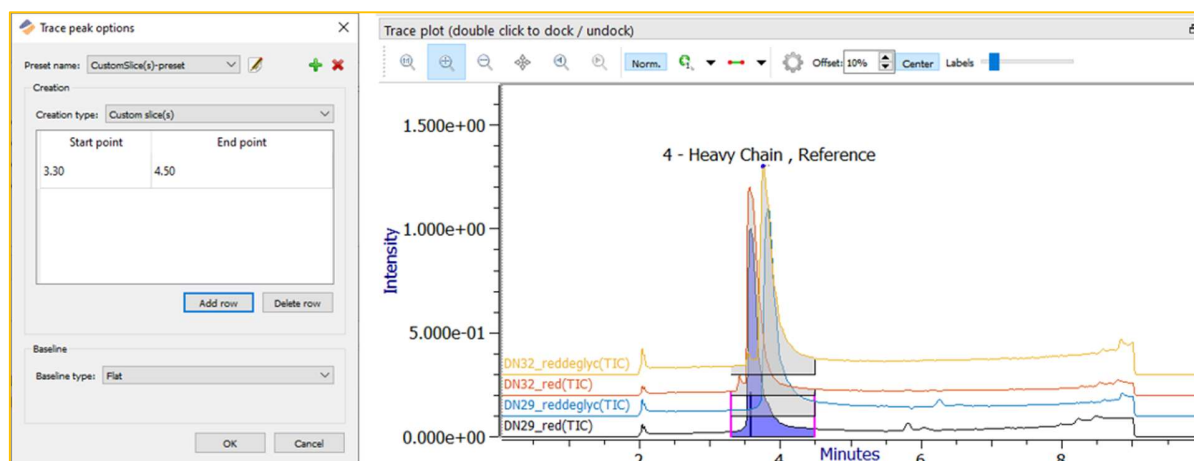


Figure 7. CustomSlice(s) Setting with Trace Plot Example

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Customizing Trace Peak Options: FixedWidthSlice(s)

The FixedWidthSlice(s) trace peak option divides the trace peak(s) by a regular interval. This setting will render a deconvolved spectrum for each interval. FixedWidthSlice(s) are particularly useful for samples with poor chromatographic separation, such as AAVs.

In the example below (figure7), The first slice (interval) was set from 3.25 minutes to 3.50 minutes. The step between slices was set to 0.25 minutes, or the same duration as the first slice as to not overlap the slices. Keep in mind that decreasing the step in between slices for some overlap may be beneficial for particularly complex samples. Finally, the optional setting, Number of slices, was set to 10. This equates to 10 slices, at 0.25 minutes a piece, or slices for 2.5 minutes.

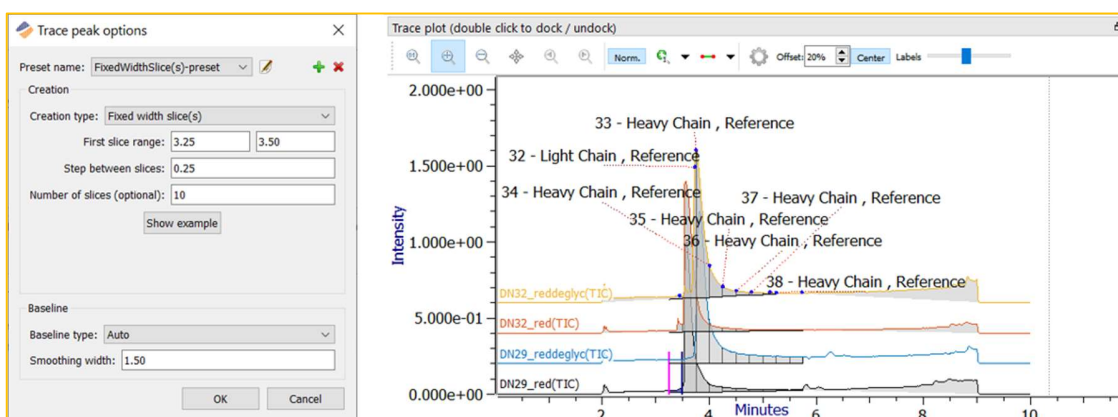


Figure 8. FixedWidthSlice(s) Setting with Trace Plot Example

Customizing Trace Peak Options: AutoComputeByApex

The AutoComputeByApex trace peak option operates in the same way as our default (AutoCompute) peak picking algorithm. Please see the above section for peak picking customization details.

This setting allows for finer control of the chromatographic time range. The two options are “that contains apex within” or “truncated by”.

The “that contains apex within” will select the entire peak if a shoulder or tail falls before or after the time range indicated (figure 9a).

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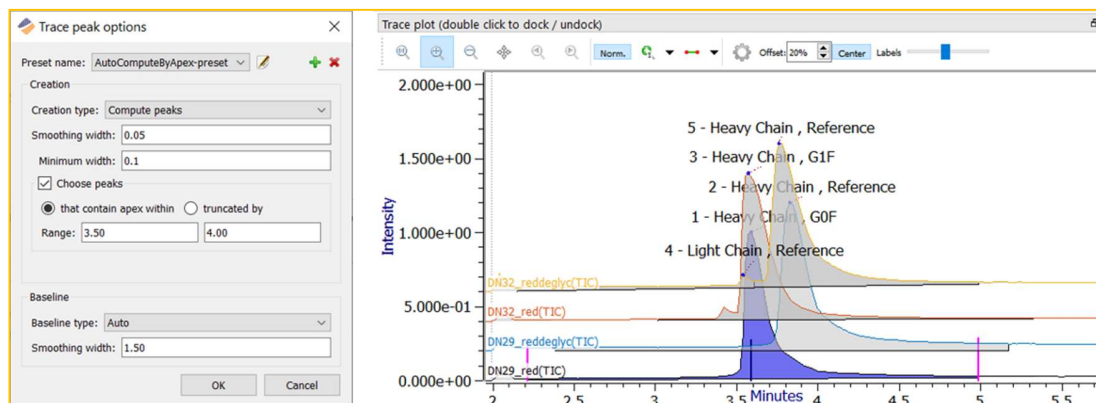


Figure 9a. AutoComputeByApex Setting- that contains apex within- with Trace Plot Example

Alternatively, the “truncated by” setting will perform a hard cutoff and exclude any shoulder or tail falls before or after the time range indicated (figure 9b).

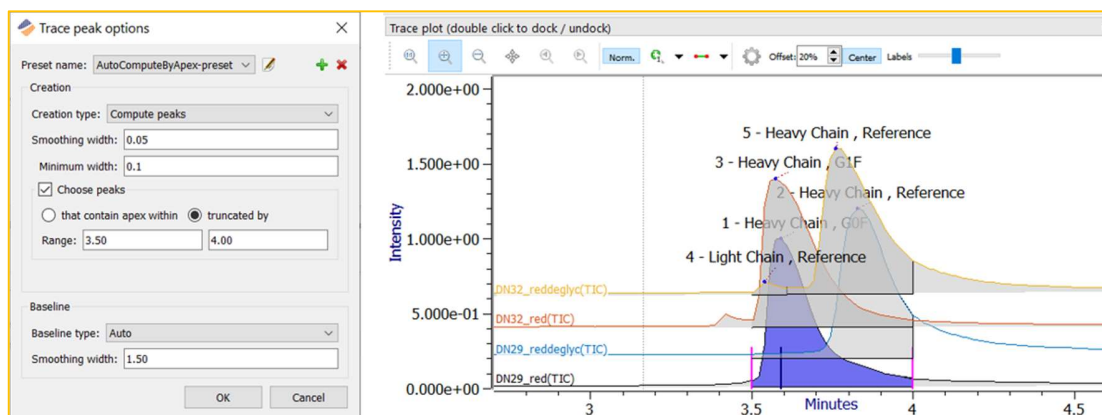


Figure 9b. AutoComputeByApex Setting- truncated by- with Trace Plot Example

Edit Global Trace Peak Filters

The “Edit global trace peak filters” button on the bottom right corner of the screen allows for more control of the trace peaks.

The peak options portion, allows for an optional time cutoff. Additionally, a minimum peak area threshold can be applied to skip low abundant peaks. By default, this threshold is set to 5.00%. If you find that you are missing the low abundant peak(s) or slice(s) this filter can be adjusted to a lower percentage or set to zero.

The peak comparison options portion of the pop-up is for comparison projects. By default, any comparison trace’s peak that falls above or below the reference peak by a ratio 10 will be highlighted in red.

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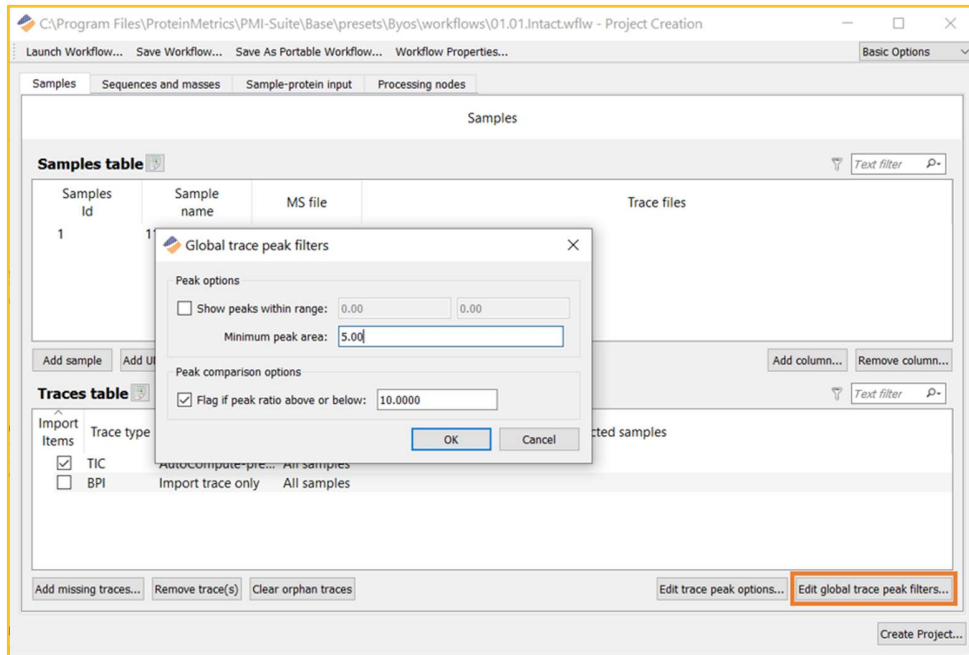


Figure 10. Global Trace Peak Filters

Elution Peak Settings Adjustments After Project Creation

Editing Elution Peaks Globally

The quickest and easiest way to adjust the elution peaks for all samples after project creation is under Edit> Recompute trace peaks and baseline... (figure 11). The popup for customizing the trace peaks will open for adjustment. After globally editing the trace peaks, go to Edit> Auto compute and assign masses to globally deconvolute your traces.

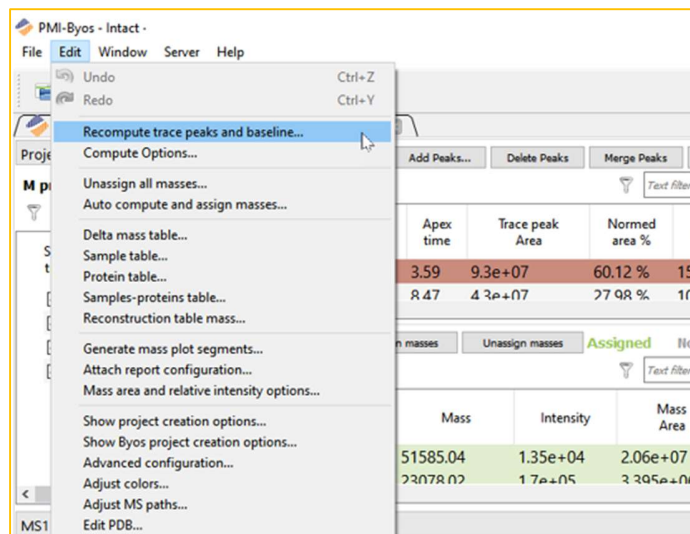


Figure 11. Accessing the Recompute trace peaks and baseline menu after project creation.

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Editing Elution Peaks Manually

In some cases, globally adjusting the trace peaks is necessary. Above the trace peaks table, buttons to Add, Delete, Merge, and Split peaks are available. Start and end times can be edited for a peak by clicking and editing the times in the trace peaks table, or by dragging the pink bars on the trace plot chromatogram.

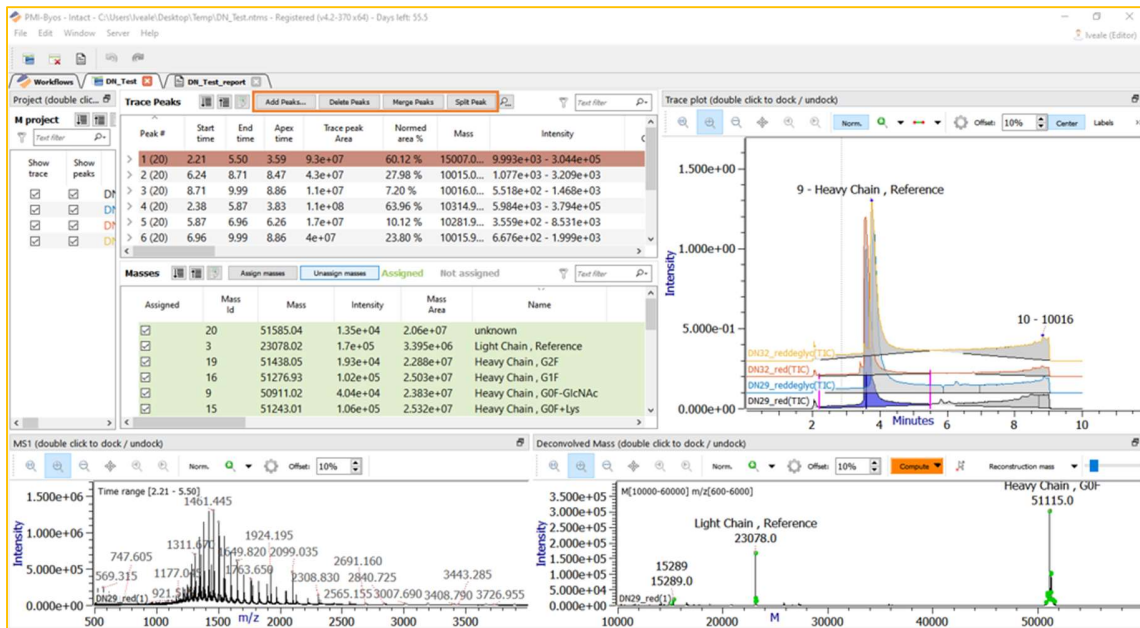


Figure 12. Editing the Elution Peaks Manually

Application Note

Editing Global Trace Peak Filters

The global trace peak filters menu can be accessed after project creation after by clicking on the magnifying glass button above the trace peaks table. Here, the optional time range, minimum peak area threshold, and comparison project ratio flagging can be edited.

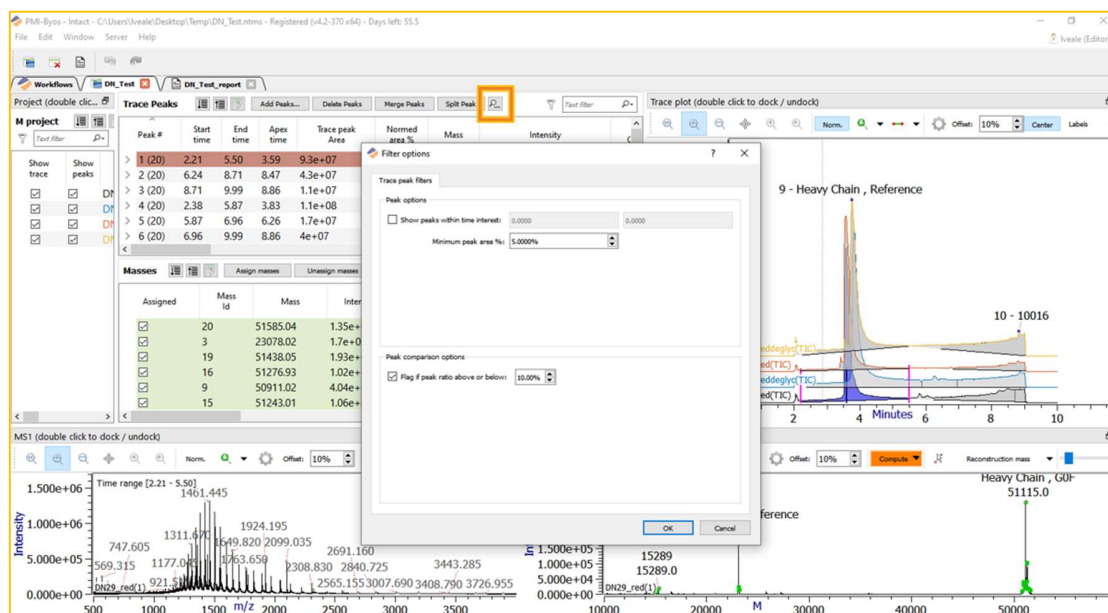


Figure 13. Editing Global Trace Peak Filters

For more resources and background on Intact and Chromatography data analysis workflows, visit <https://www.proteinmetrics.com/workflows/> or contact the Customer Success Team at support@proteinmetrics.com.

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