icIEF-UV+MS Data Analysis for Intabio ZT System

Biologics Explorer Software 6.0 Guidelines

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icIEF-UV+MS Data Analysis for Intabio ZT System

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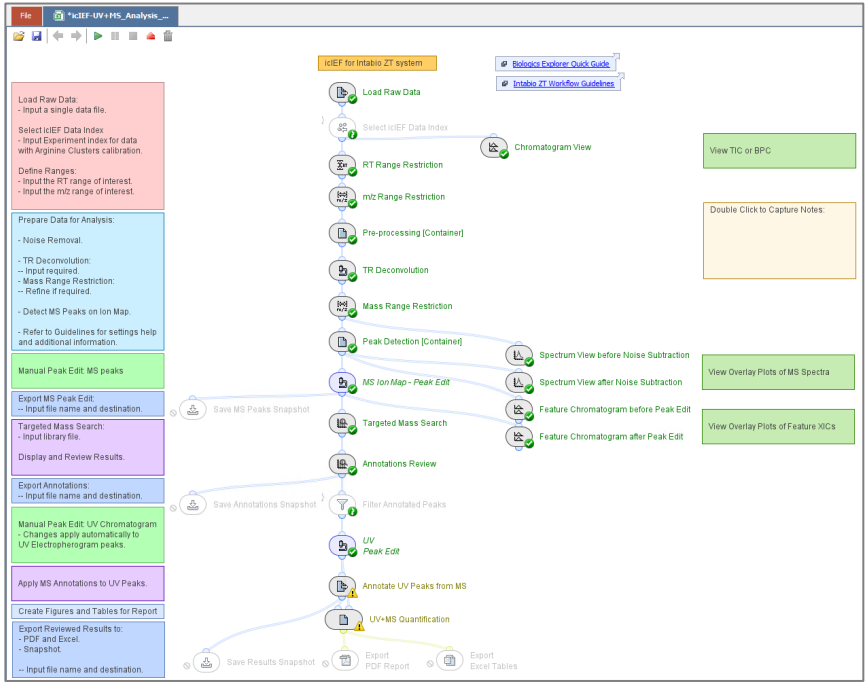
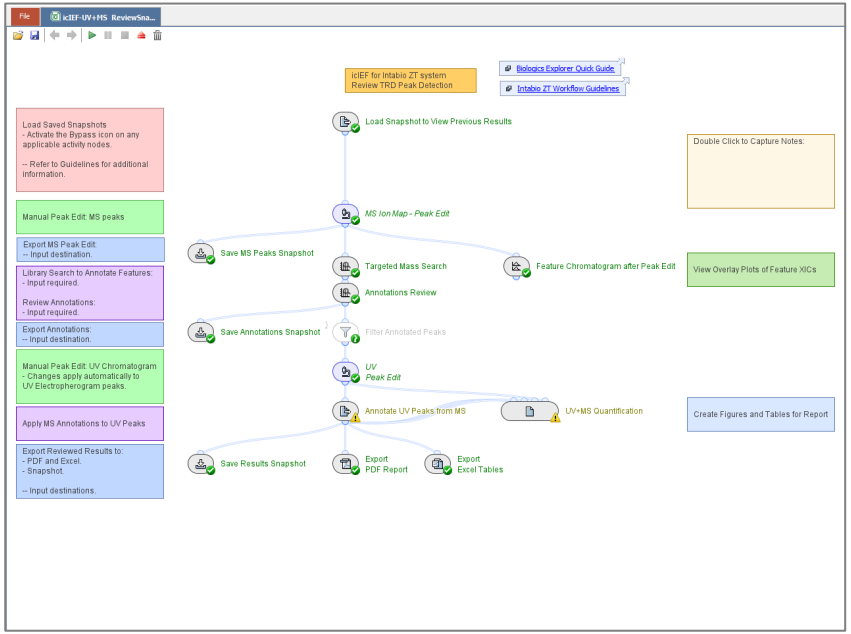
Overview of the Workflows

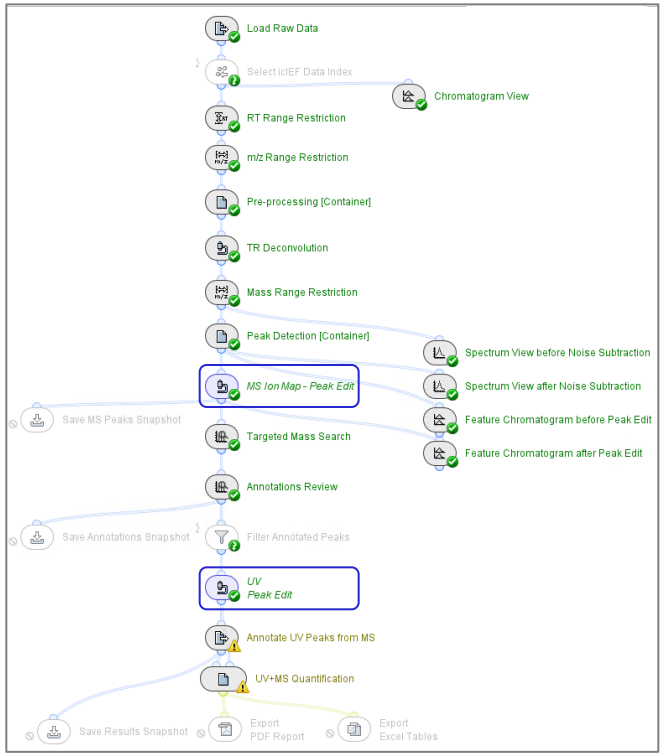


icIEF-UV+MS_Analysis_Be6.0



icIEF-UV+MS_ReviewSnapshots_Be6.0

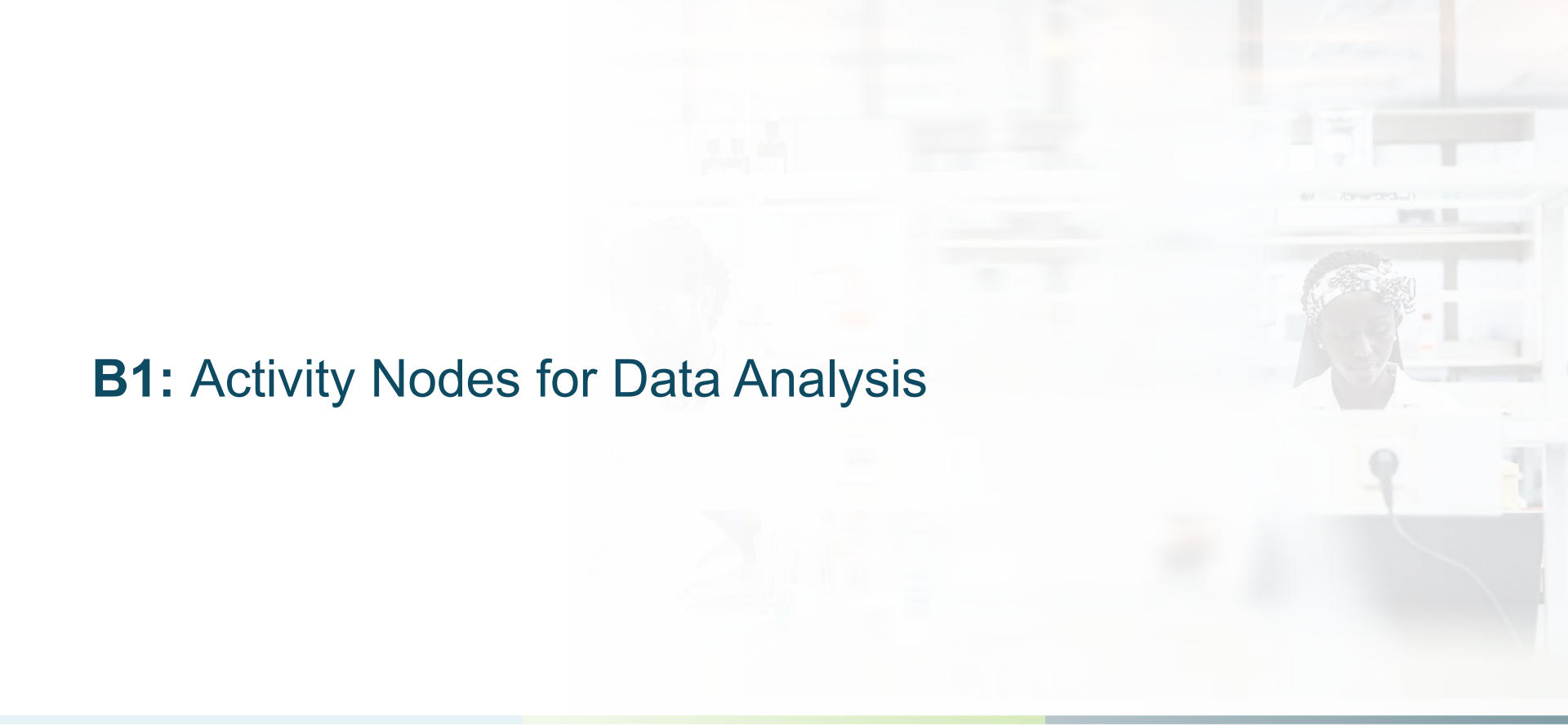
How to Use the icIEF-UV+MS Workflows



- Use the workflows to analyze a single sample from an icIEF-UV separation with the Intabio ZT system.
- The main path of the workflow processes the data.
- The branched activity nodes on either side contain tools to:
  - Visualize data before and after data processing.
  - Create tables and figures for the *Export Report* activity nodes.
  - Save intermediate data and final results as Snapshots (sbf files).
- The blue activity nodes require manual input to continue.
- Use the *Export Excel Report* for intermediate data analysis and quick access to results for optimization.
- Use the *Export PDF Report* for final data results.
  - Excel tables are also exported by the *Export PDF Report*.
- Use the icIEF-UV+MS_ReviewSnapshots workflow to continue analysis of saved sbf files.

B: Guidelines for icIEF-UV+MS Workflows

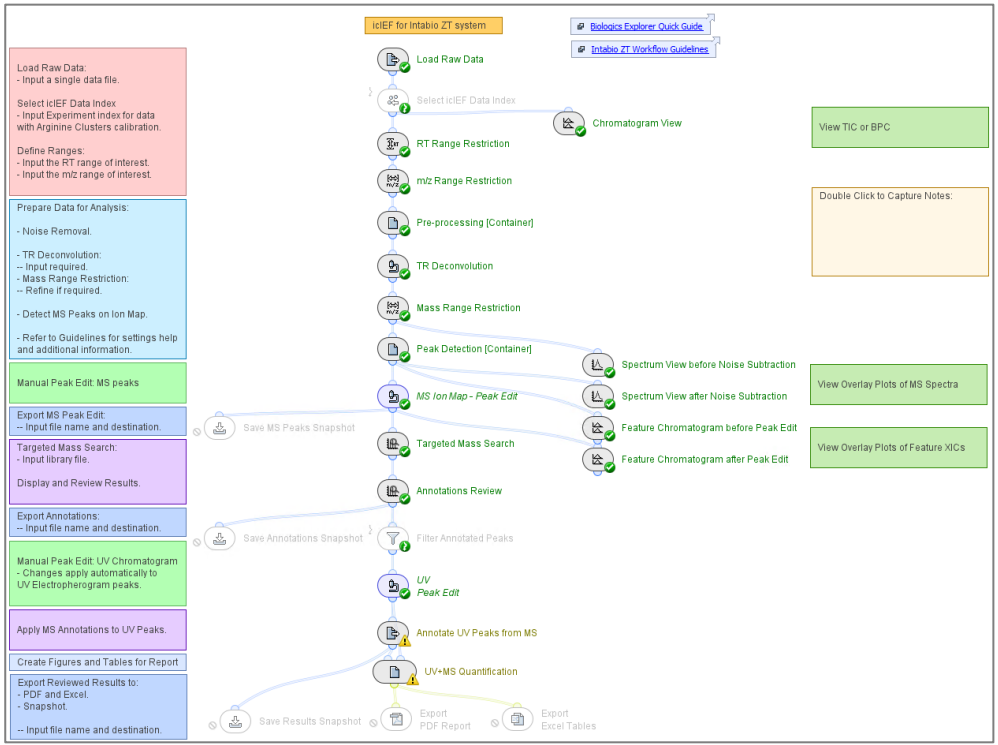
B1: Activity Nodes for Data Analysis



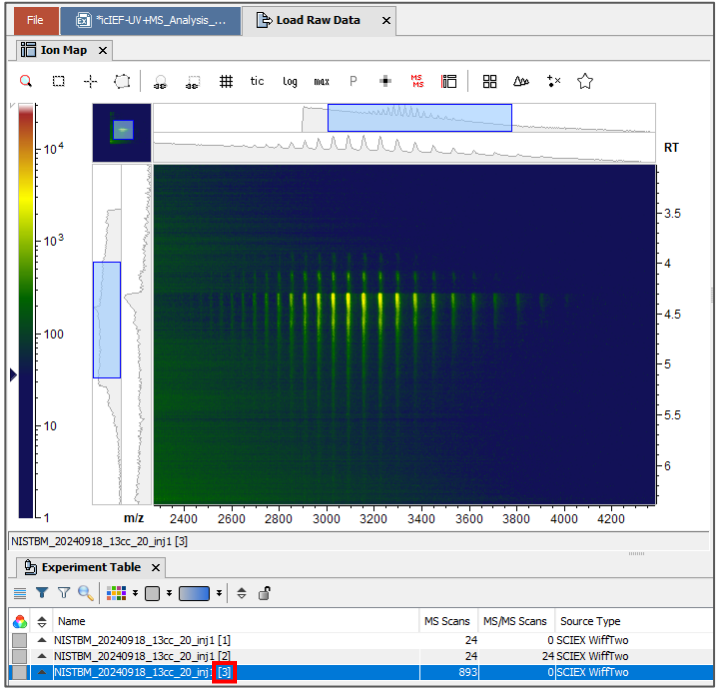
icIEF-UV+MS_Analysis Workflow

- This section contains information about these activity nodes of interest:
  - *Select icIEF Data Index*
  - *Spectrum Baseline Subtraction*
  - *Chemical Noise Subtraction*
  - *TR Deconvolution*
  - *Peak Detection*
  - *MS Ion Map - Peak Edit*
  - *Targeted Mass Search*
  - *Annotations Review*
  - *Filter Annotated Peaks*
  - *UV Peak Edit*
  - *Annotate UV Peaks from MS*

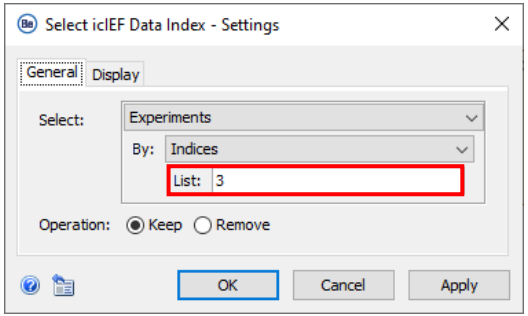
Note: For information about activity nodes that are used in all workflows, for example *Load Raw Data*, refer to the document: *Biologics Explorer Quick Guide*.



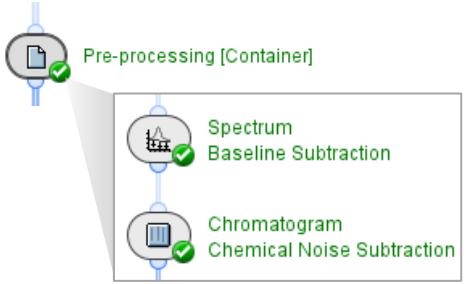
Select icIEF Data Index



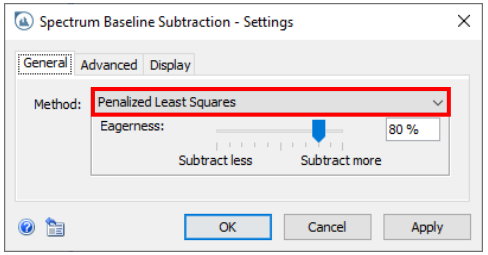
- To analyze data acquired with the Arginine Clusters calibration, deactivate the **Bypass** icon on *Select icIEF Data Index*.
  - Identify the applicable the **Experiments: Indices** value for the entry in the **Experiment Table** that contains the sample data.



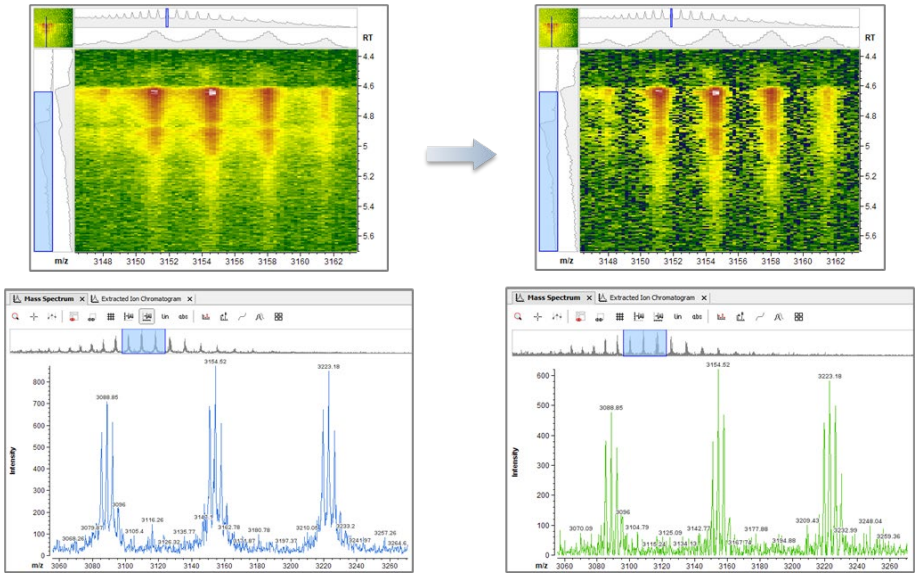
Spectrum Baseline Subtraction



- Use *Spectrum Baseline Subtraction* before deconvolution to remove background noise and decrease the intensity of satellite peaks in the deconvoluted data.
- Use *Spectrum Baseline Subtraction* after deconvolution to optimize peak detection.



- **Penalized Least Squares** subtraction has an effect on low intensity signals only.
- **Quantile** subtraction has an effect on all signals.
  - **Quantile** subtraction should be used with care for the analysis of intact proteins.

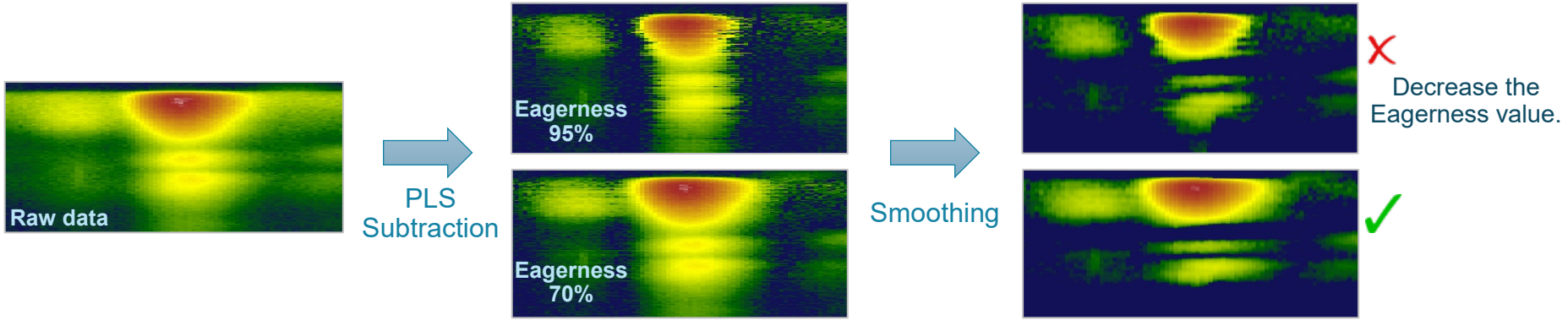


Before *Spectrum Baseline Subtraction*

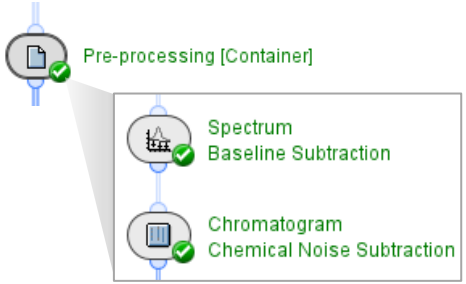
After *Spectrum Baseline Subtraction*

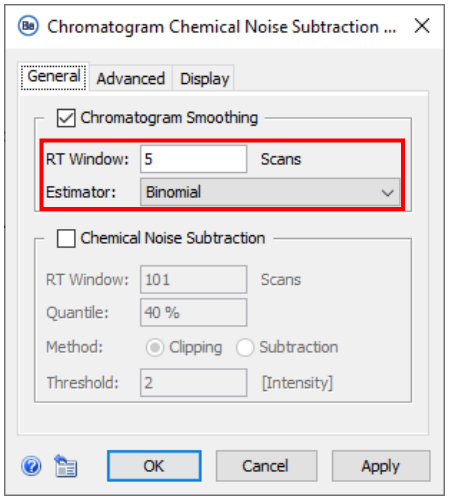
Spectrum Baseline Subtraction: Penalized Least Squares

- **Penalized Least Squares** decreases the valley height between large peaks, which decreases the intensity of satellite peaks in deconvoluted spectra.
  - High **Eagerness** values (greater than 90%) require extensive **Smoothing** in *Chromatogram Chemical Noise Subtraction*.
  - If features in the ion map have irregular borders after smoothing, then decrease the **Eagerness** value.



Chromatogram Chemical Noise Subtraction

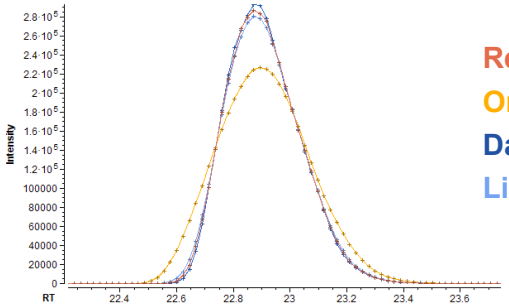




Chromatogram Smoothing is used to improve the RT profile of peaks.

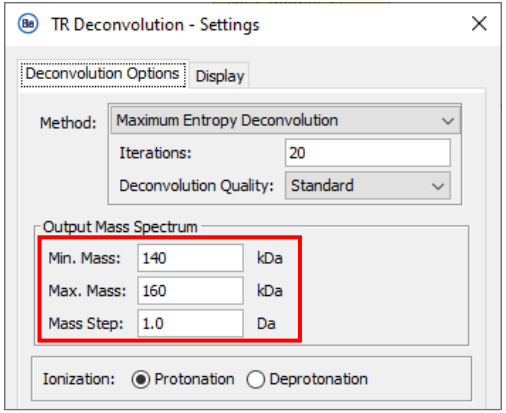
- Use **Chromatogram Smoothing** after **Penalized Least Squares** (in *Spectrum Baseline Subtraction*), especially if a high **Eagerness** value was used.

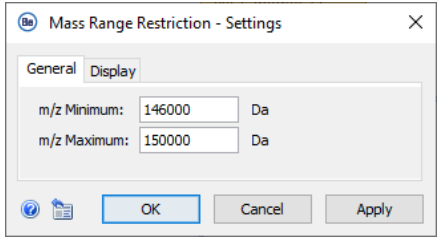
- **Estimator:**
  - **Moving Average** replaces the intensity of each data point with the mean average intensity of the data points in the **RT Window**. High values cause peak widths to increase, but peak volume is conserved.
  - **Binomial** is an iterative form of **Moving Average** that has less effect on peak widths if a large **RT Window** (large number of scans) is used.



Red: Moving Average (5 scans)
Orange: Moving Average (15 scans)
Dark Blue: Binomial (5 scans)
Light Blue: Binomial (15 scans)

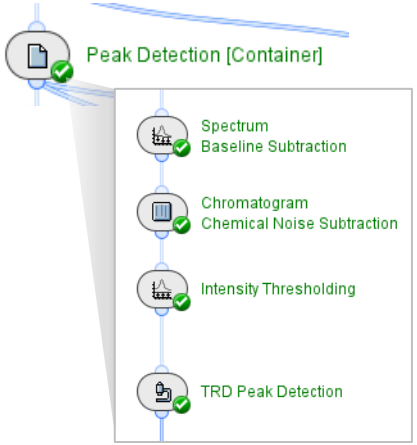
TR Deconvolution

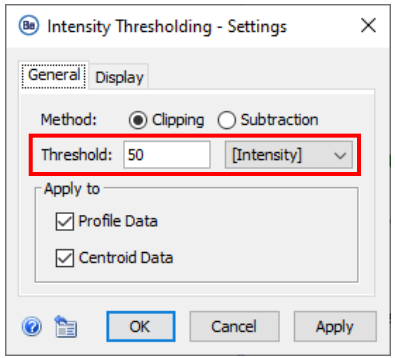




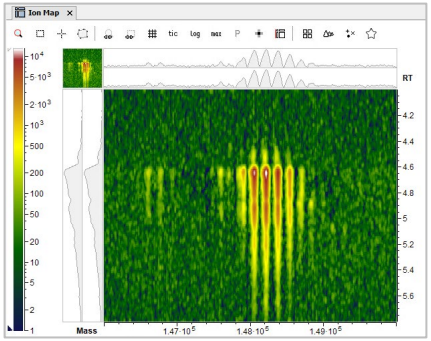
- *TR Deconvolution*: **Time-Resolved Deconvolution** occurs over the full RT range to create a deconvoluted ion map.
  - **Maximum Entropy Deconvolution**:
    - **Iterations**: Increase to 50 to increase peak definition.
    - **Deconvolution Quality**: The number of data points used for deconvolution.
      - Select **High** for isotopically-resolved data.
      - Select **Standard** for lower-resolution data.
  - **Min. Mass** and **Max. Mass:**
    - Use a wide mass range to reduce the number and intensity of harmonic peaks.
  - Optimize the **Mass Step**:
    - Set a value that keeps the peak resolution of the data.
      - 0.1 Da to 0.2 Da for isotopically-resolved data.
      - 1 to 2 Da for lower-resolution data.
- *Mass Range Restriction*: Enter the mass range that will be shown in the ion map.

Peak Detection: *Intensity Thresholding*

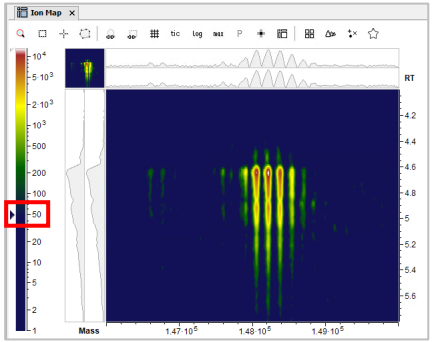




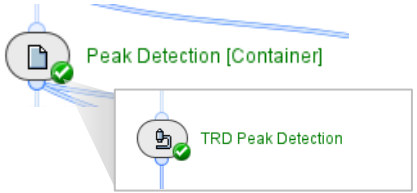
- To optimize peak detection after deconvolution, use *Spectrum Baseline Subtraction*.
  - **Chromatogram Smoothing** is required after **Penalized Least Squares** subtraction.
- To control the level of background noise, use *Intensity Thresholding*.
  - Optimize the **Threshold** value for each dataset.
    - Make sure that sufficient noise is removed but the lowest-intensity proteoform of interested is detected.
  - Use the intensity slider on the ion map to find the correct value.

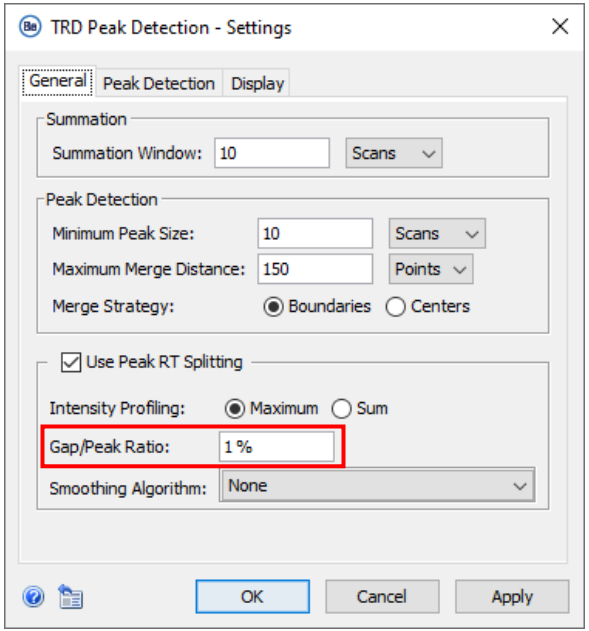


Visualize noise removal in the ion map with the intensity slider.

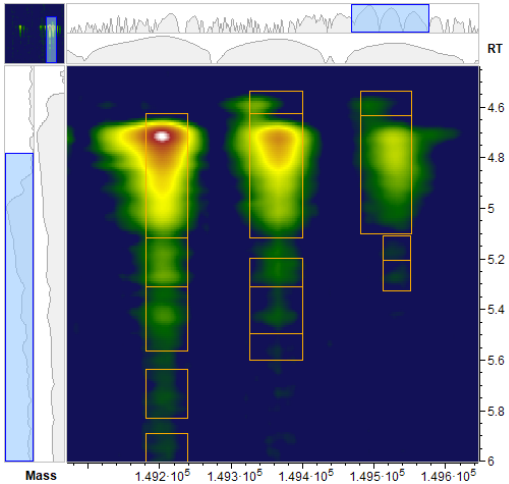


TRD Peak Detection: RT

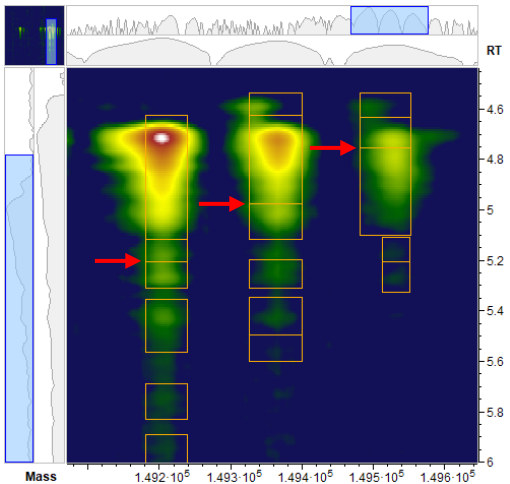




- To control peak splitting in the RT direction, optimize the **Gap/Peak Ratio**.
  - The percentage value specifies the minimum height difference between two closely eluting peaks that is required for them to be detected as separate peaks.

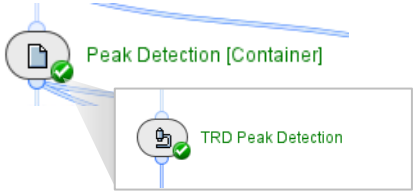


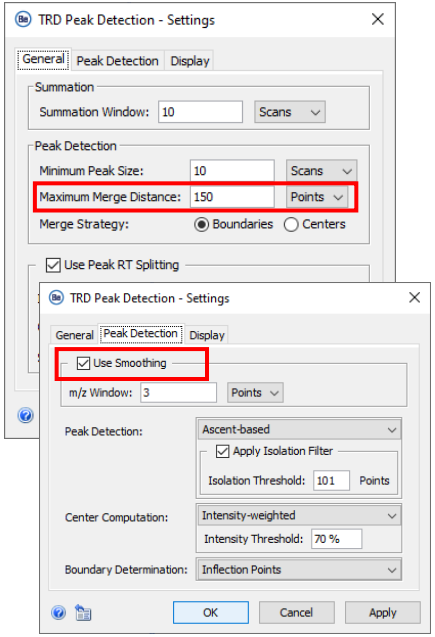
Gap/Peak Ratio: 10%



Gap/Peak Ratio: 1%

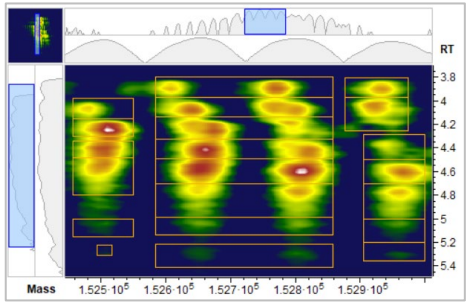
TRD Peak Detection: m/z





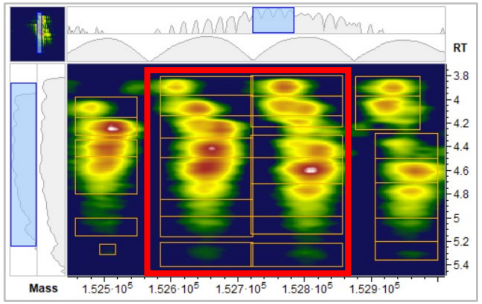
- To control peak splitting in the *m/z* direction, optimize **Use Smoothing** and **Maximum Merge Distance**.
- The following settings can improve *m/z* splitting for some samples:
  - Decrease the **Maximum Merge Distance** on the **General** tab (see **B** below).
  - Do not select **Use Smoothing** on the **Peak Detection** tab (see **C** below).

A



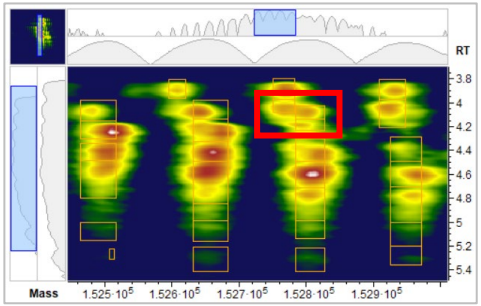
Maximum Merge Distance: 150 Points
Use Smoothing: Selected
m/z Window: 3 Points

B



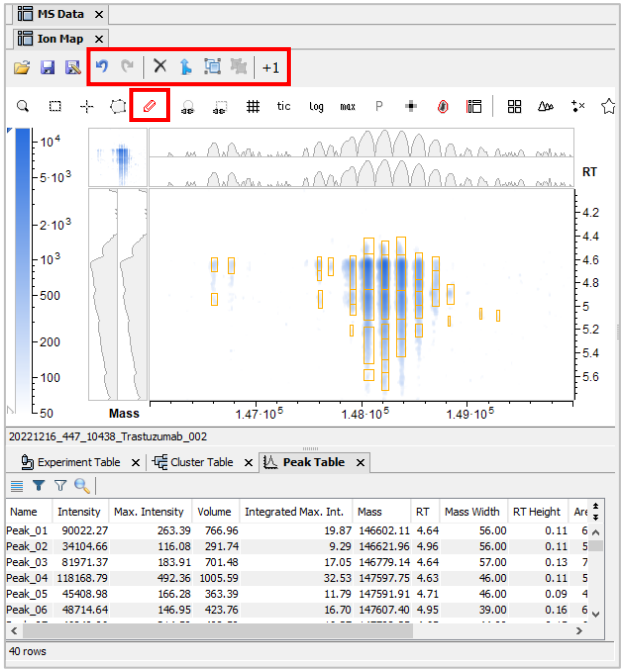
Maximum Merge Distance: 100 Points
Use Smoothing: Selected
m/z Window: 3 Points

C

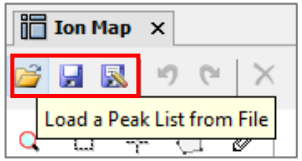


Maximum Merge Distance: 150 Points
Use Smoothing: Not selected

MS Ion Map - Peak Edit

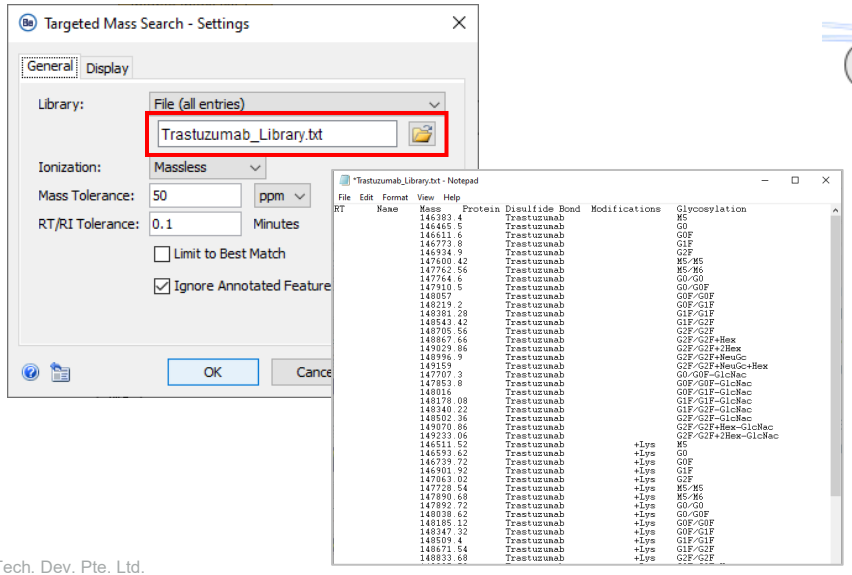


- To manually change the peaks in the ion map that were detected in *TRD Peak Detection* after deconvolution, use *MS Ion Map - Peak Edit*.
  - Select the **Edit Mode** icon to:
    - Move the peak boundaries.
    - Merge or split selected peaks.
    - Delete peaks.
    - Draw new peaks.
  - Changes can be saved and the **Peak List** loaded again.
    - The saved **Peak List** keeps all characteristics of the original peaks.
    - Peak boundaries and peak centers (mass and RT coordinates) are not automatically updated.
    - If peak boundaries are changed, then the peak centers will be calculated again.



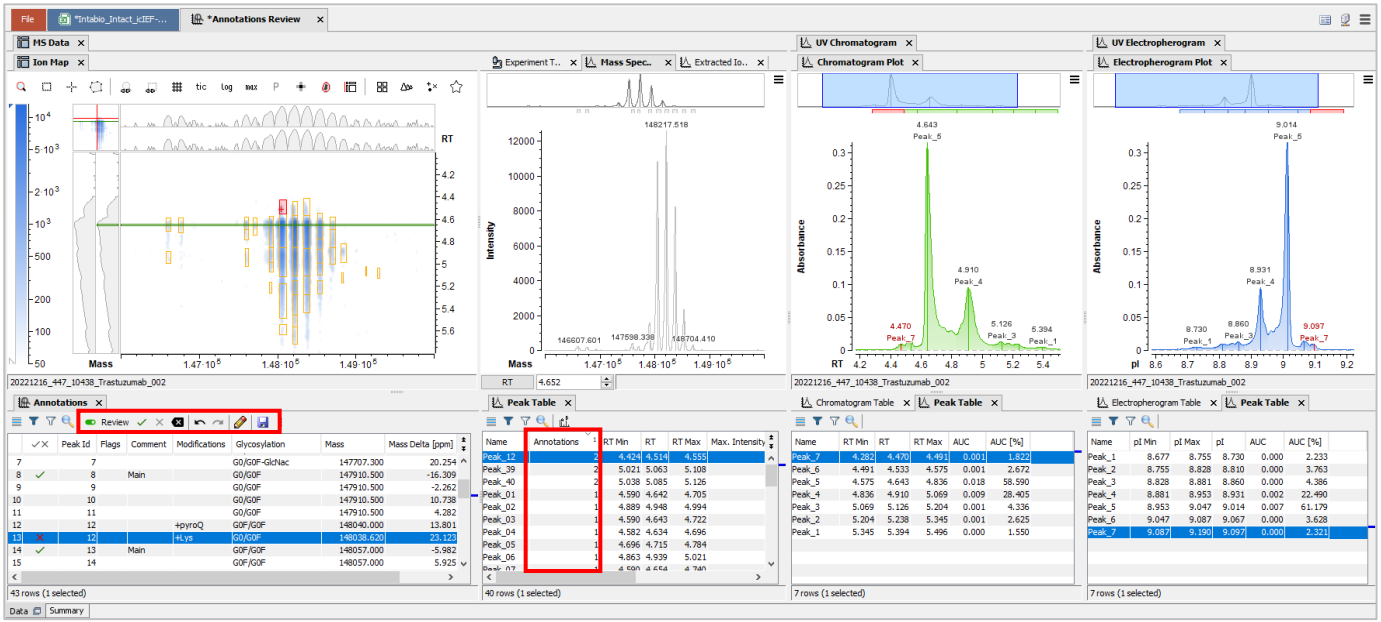
Targeted Mass Search

- The input library for *Targeted Mass Search* must be a tab-separated txt file.
  - The tab-separated txt file can be edited in Excel.
- To supply additional information, add columns to the library as required.
  - To control the columns that are shown in the final report, use the *MS Quantification* activity node, in the *UV+MS Quantification* container.



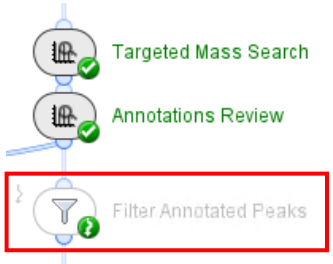
Annotations Review

- To **Accept** or **Reject** annotations, activate the **Review** mode.
  - To add a comment, either type in the applicable row in the **Comment** column, or use the icon to add the same comment to multiple rows.
- To apply the changes, click the **Save** icon, and then select **Save and Reload**.



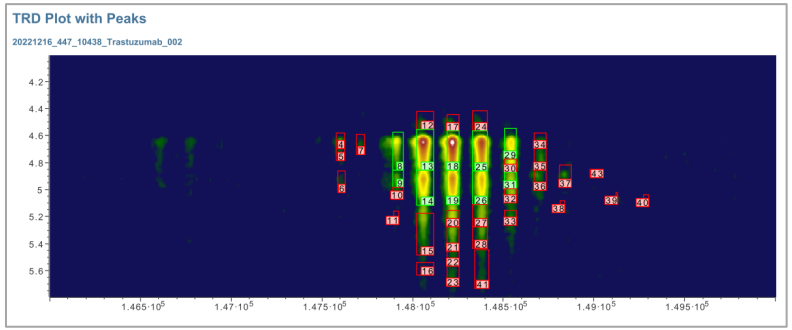
- Click on the **Annotations** column header to order by entry value.

Filter Annotated Peaks

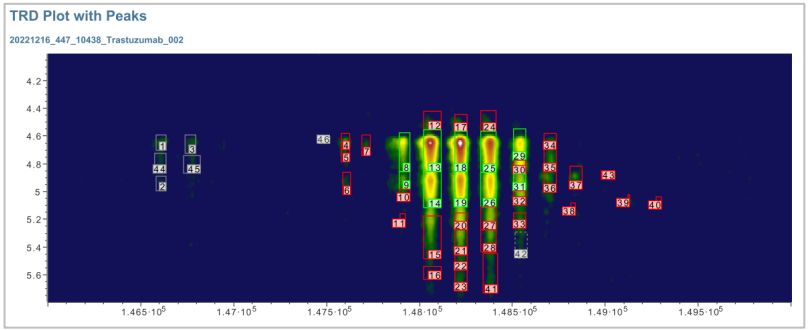


- Use *Filter Annotated Peaks* to remove peaks that are not annotated from the final results that are reported by *TRD Plot with Peaks*.
  - To use *Filter Annotated Peaks*, deactivate the **Bypass** icon.

With *Filter Annotated Peaks*:

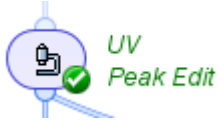


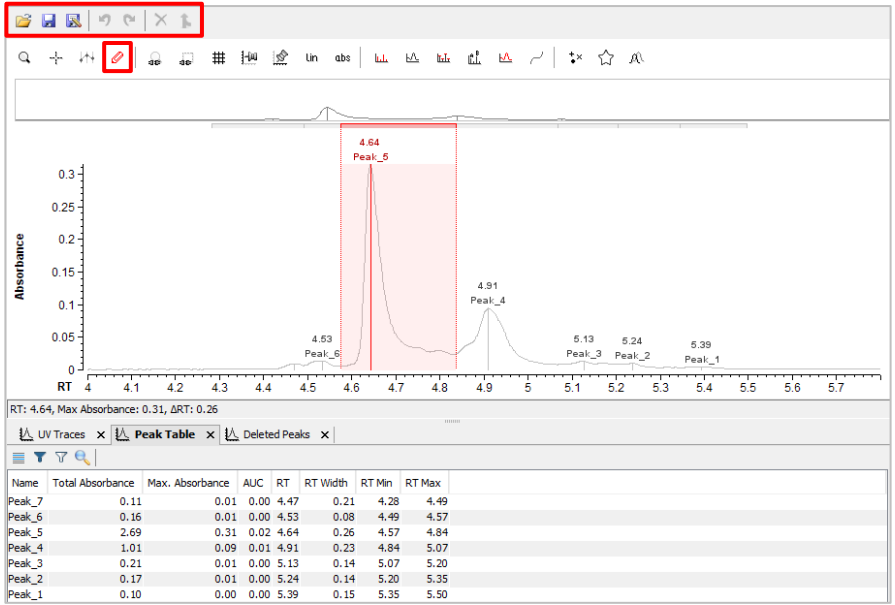
Without *Filter Annotated Peaks*:



- Solid **green** boundary + peak number in green shading = Peak was accepted.
- Solid **gray** boundary + peak number in gray shading = Peak was rejected.
- Solid red boundary + peak number in red shading = Peak is annotated but unreviewed.
- Dashed **gray** boundary + peak number in gray shading = Peak is unannotated and unassigned.

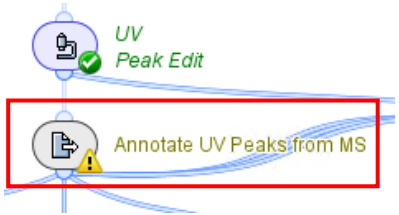
UV Peak Edit



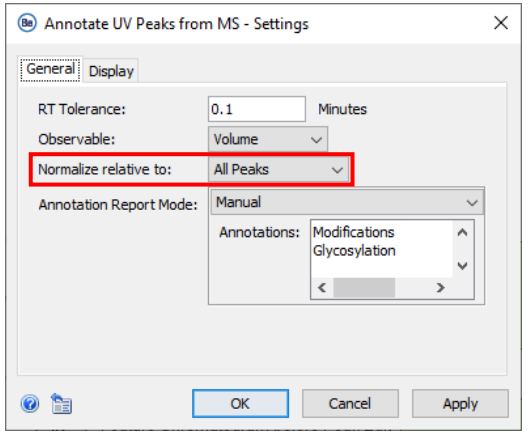


- To manually change the UV peaks that were defined in the ibo file supplied by the Intabio ZT system, use *UV Peak Edit*.
- Select the **Edit Mode** icon to:
  - Move the peak boundaries.
  - Merge selected peaks into a single peak
  - Delete peaks.
  - Draw new peaks.
- Changes made to the **UV Chromatogram** are also applied to the **UV Electropherogram**.
- Changes can be saved and applied to other data.

Annotate UV Peaks from MS



- This activity node uses MS peak information from *Annotations Review* to annotate the related peaks in the **UV Chromatogram** and **UV Electropherogram**.
  - A related peak must be detected in the specified **RT Tolerance**.



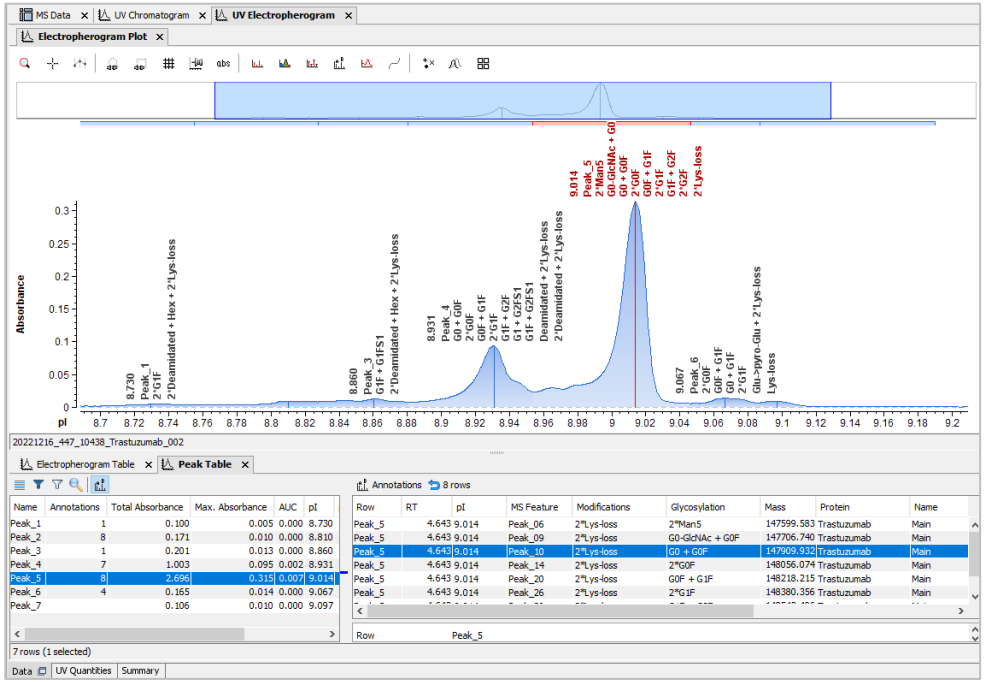
- **Normalize relative to**:
  - **All Peaks**: Relative UV absorbance is calculated across all detected peaks.
  - **Annotated Peaks**: Relative UV absorbance is calculated across all annotated peaks
- **Annotation Report Mode: Manual**:
  - Select the information about the annotated features that is included in the result table.

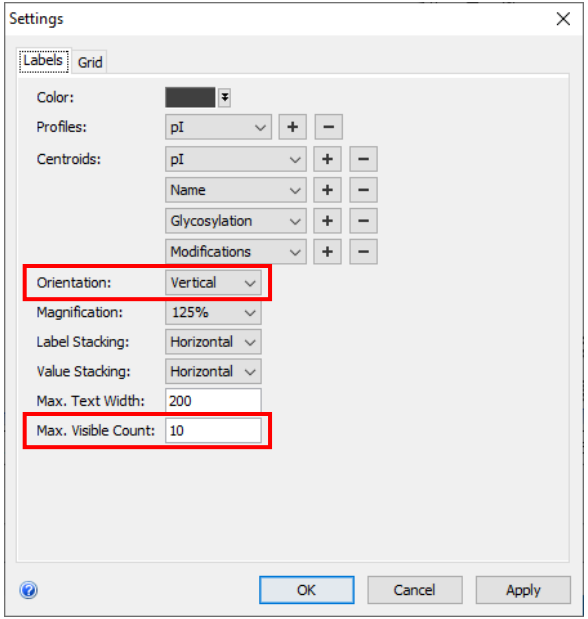
Note: If normalized UV abundances cannot be calculated, then the activity node shows a **yellow warning**.

Status	Suspicious
Message	The normalized UV absorbances could not be computed due to missing and/or duplicated annotations.

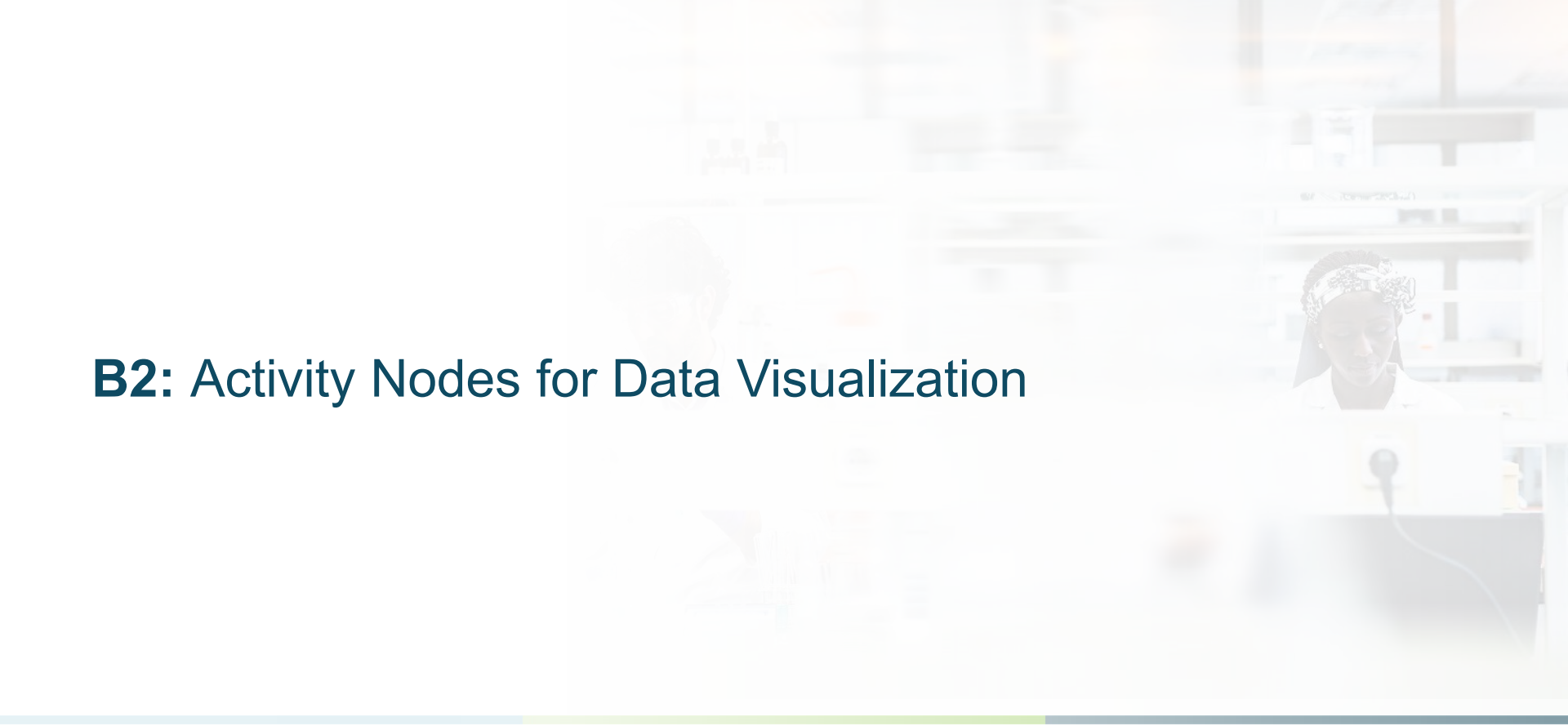
Annotate UV Peaks from MS

- To add labels to UV peaks, right-click the **Electropherogram Plot**, and then click **Settings**.
  - To see all MS identifications for each electropherogram peak, select **Orientation: Vertical** and increase the **Max. Visible Count**.





B2: Activity Nodes for Data Visualization



UV Electropherogram and UV Chromatogram Plots

- The results of most of the activity nodes in the workflow contain the **UV Electropherogram Plot** and **UV Chromatogram Plot**.
  - Use the **Save Layouts** icon to save a preferred location for each window.

    Note: For more information, refer to the document: *Biologics Explorer Quick Guide*.

UV Electropherogram Plot

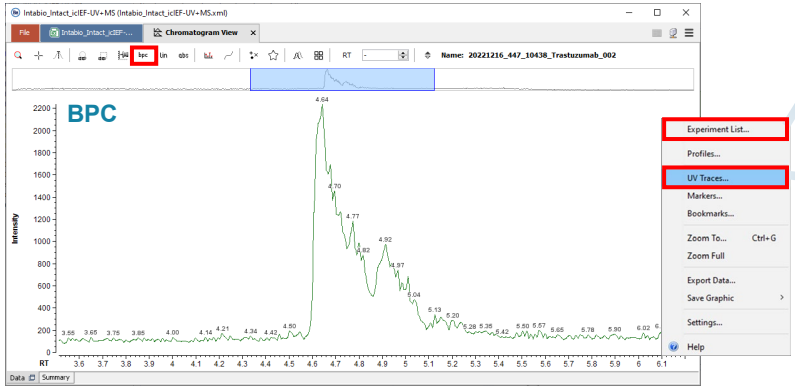
- Shows the icIEF image after separation, before mobilization.
- Data is in the pI scale.

UV Chromatogram Plot

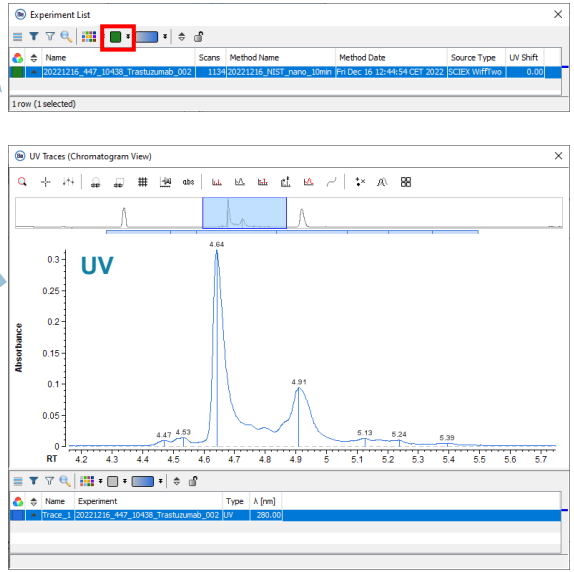
- Shows the icIEF image after manual alignment in the Intabio software.
- Data is in the RT scale.

Chromatogram View

- *Chromatogram View* shows the total ion chromatogram (TIC), or base peak chromatogram (BPC), of the data before pre-processing.



To change between the **TIC** and **BPC**, use the icon in the Tool bar.



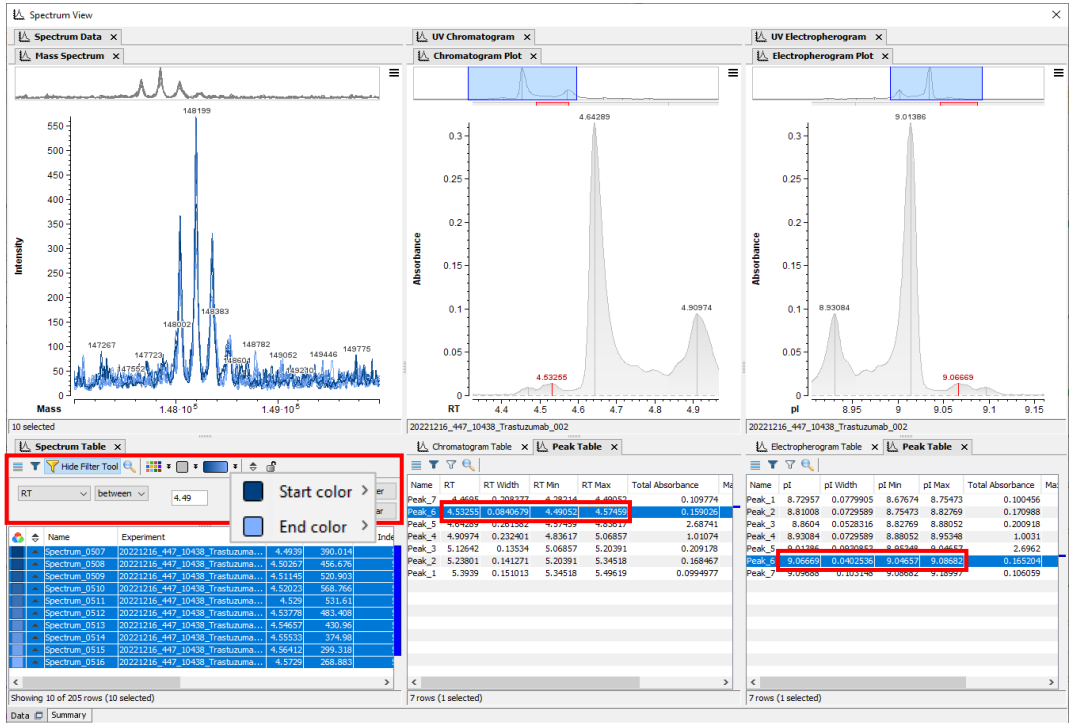
To open the **Experiment List** and **UV Chromatogram**, right-click the plot.

Spectrum View

Spectrum View before Noise Subtraction

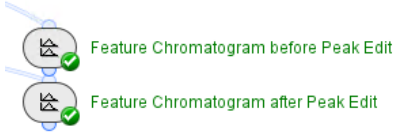
Spectrum View after Noise Subtraction

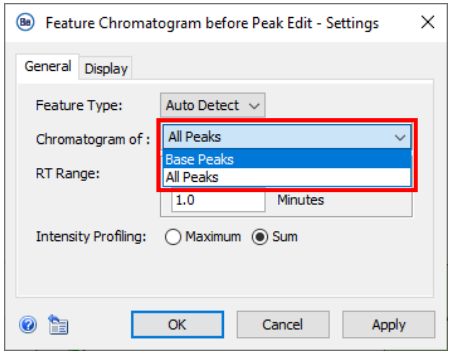
- To see a deconvoluted mass spectrum for each MS scan before and after noise subtraction, use the *Spectrum View* activity nodes.



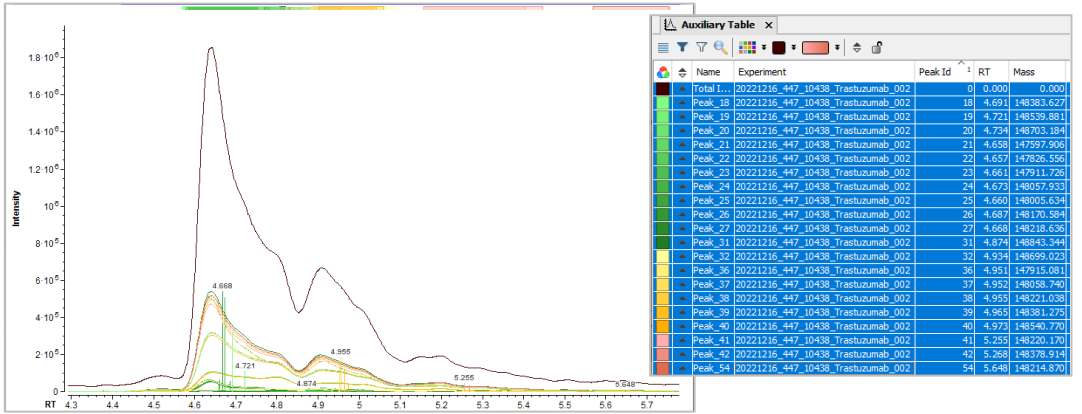
- Use the **Filter Tool** in the **Spectrum Table** to select the time range of interest for each UV peak.
  - The applicable scans are shown in the **Spectrum Table**.
- Use the **Peak Table** of the **UV Electropherogram** tab to view the pI information of each peak.

Feature Chromatogram



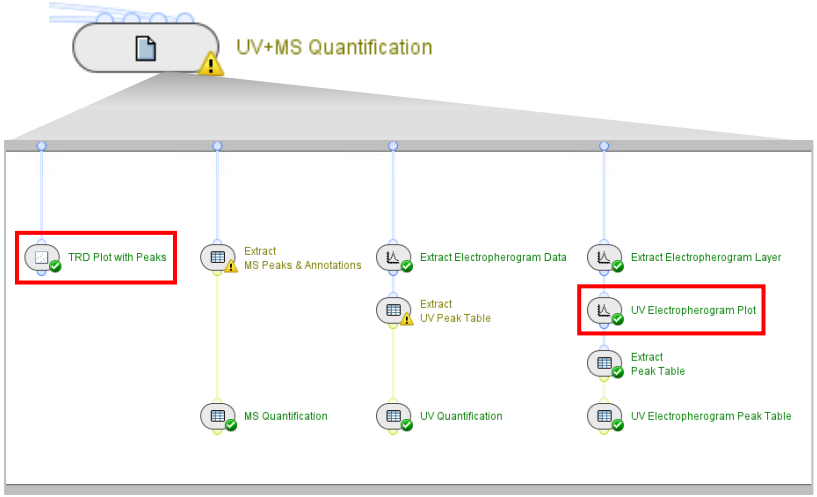


- To see the deconvoluted extracted mass spectrum for each feature before and after *MS Ion Map - Peak Edit*, use the *Feature Chromatogram* activity nodes.
  - To show the BPC, select **Base Peaks** in the **Chromatogram of** field in the settings.
  - Visually align the results of this activity node with the UV Electropherogram to select the correct peak boundaries in the *MS Ion Map - Peak Edit* activity node.



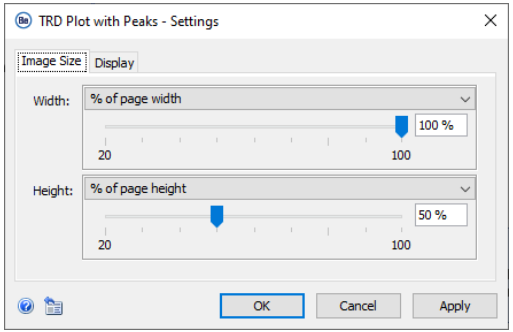
B3: Activity Nodes to Report and Export Results

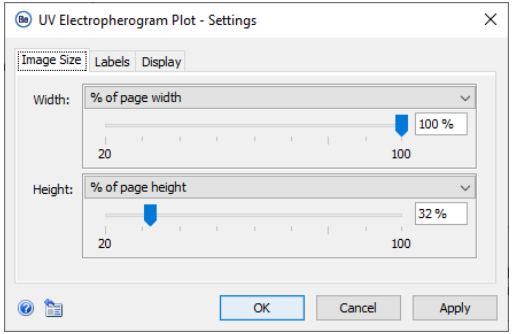
Extract Report Elements: *UV+MS Quantification*



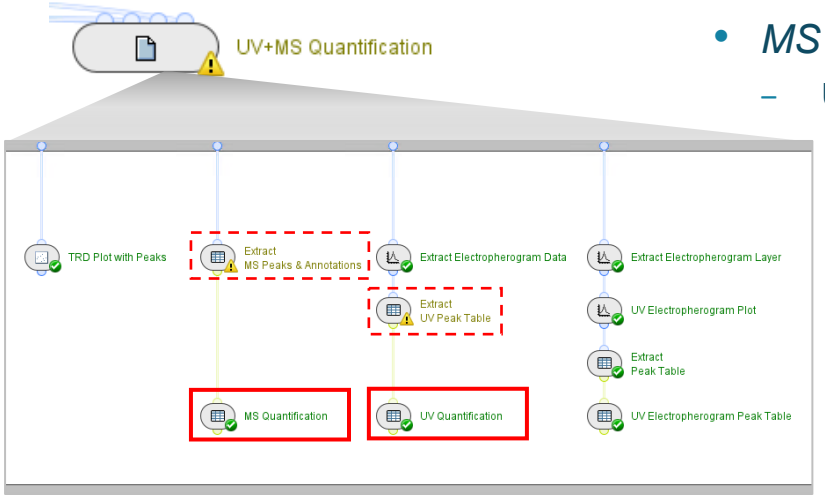
- Use the activity nodes in *UV+MS Quantification* to specify what is included in the exported *PDF Report* or *Excel Tables*.
  - *TRD Plot with Peaks*
    - Select the size of the image in the PDF Report.

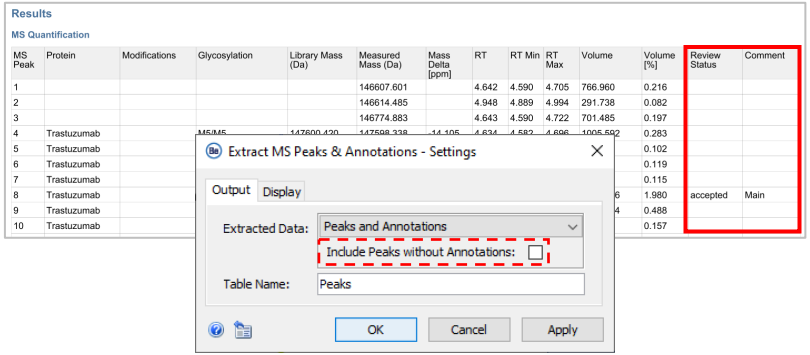
    Note: For more information, refer to the page: *Filter Annotated Peaks*.
  - *UV Electropherogram Plot*
    - Select the size of the image in the PDF Report.



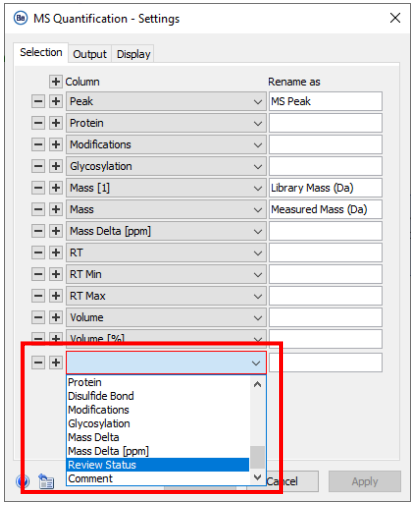


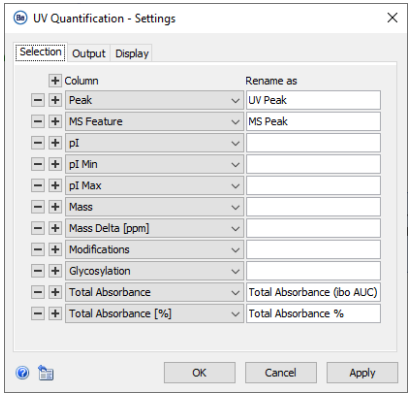
Extract Report Elements: *UV+MS Quantification*





- *MS Quantification* and *UV Quantification*
  - Use the +/- icons to select the columns to include in the PDF Report.



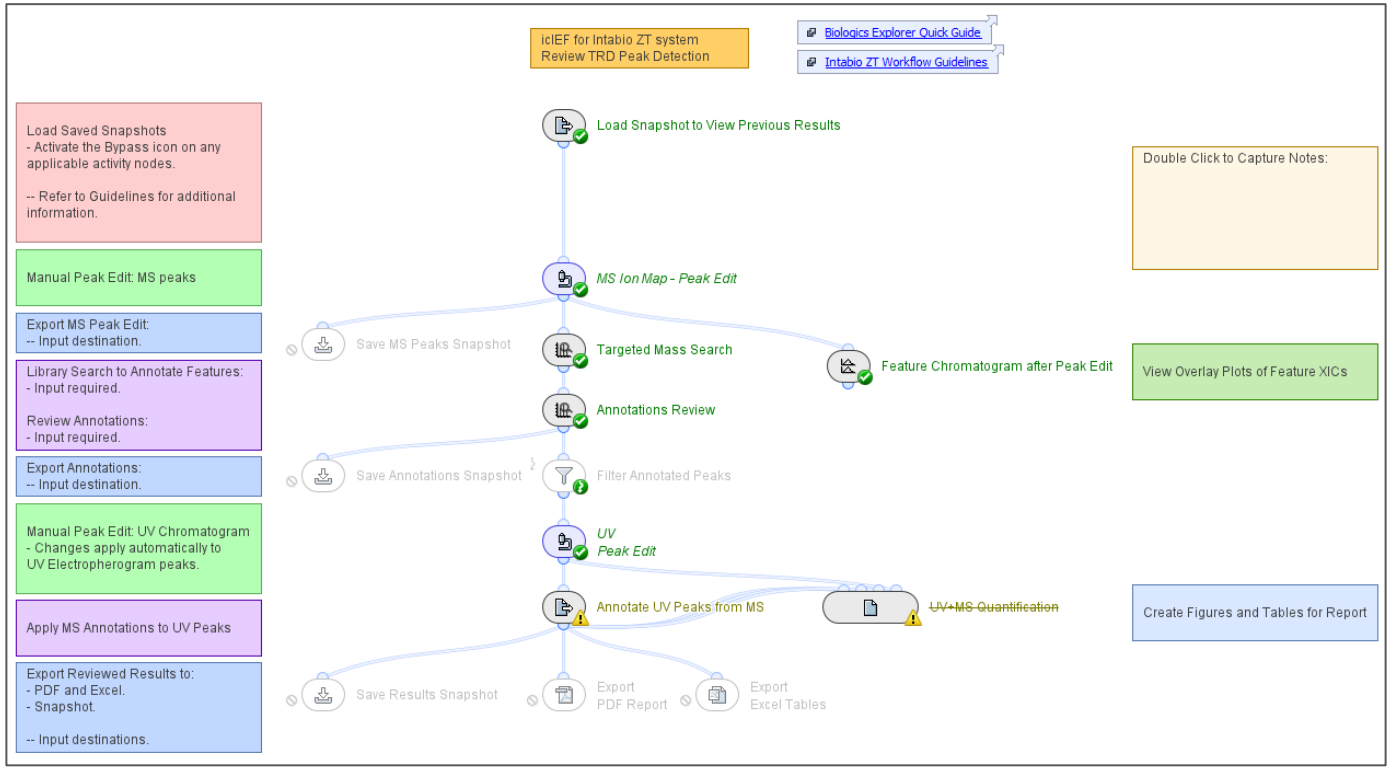


Note: If **Review Status** is selected, but there are no accepted identifications, then the activity node shows a **yellow warning**.

- **Review Status** and **Comment** do not apply to rejected peaks.
- To remove unannotated and rejected peaks from the reported tables, do not select **Include Peaks without Annotations** in the *Extract Peaks* activity nodes.

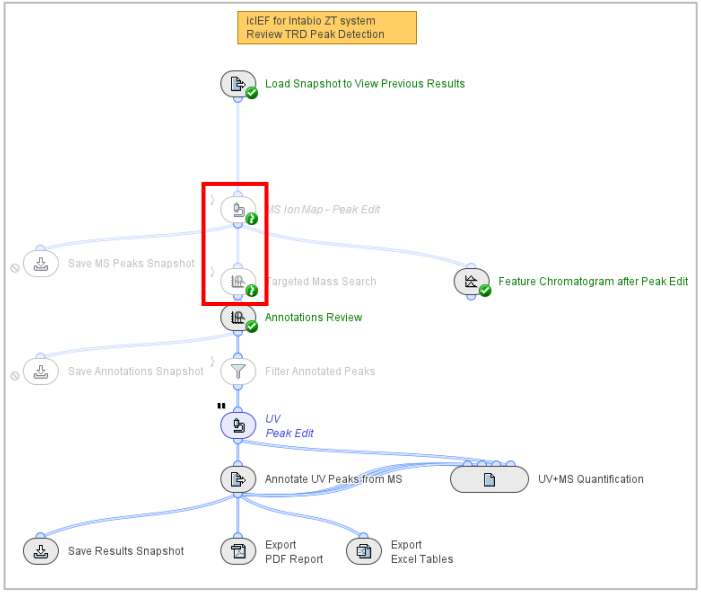
B4: How to Use the icIEF-UV+MS_ReviewSnapshots Workflow

icIEF-UV+MS_ReviewSnapshots Workflow



How to Use the Review Snapshots Workflow

- Use the Review Snapshots workflow to:
  - Open a saved Snapshot (sbf) file created using an icIEF-UV+MS_Analysis Workflow.
  - If required, then continue to edit the peak detection on the ion map.



- To use intermediate results in a saved sbf file:
  1. Use the icIEF-UV+MS_ReviewSnapshots workflow.
  2. Select the sbf file in *Load Snapshots to View Previous Results*.
  3. Activate the **Bypass** icon on the activity nodes that are before the sbf file was saved.
     - For example: To load an sbf file from *Save Annotations Snapshot*, activate the **Bypass** icon for the activity nodes between *Load Snapshot to View Previous Results* and *Annotations Review*.

- The activity nodes in this workflow are used exactly as described for the icIEF-UV+MS_Analysis workflow.

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