

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

iFAMS v. 6.3 Quant Tutorial

File: -

Positive Ion Mode



iFAMS v. 6.3 Quant Tutorial

CONTENTS

SLIDE

□ GUI Overview	4
□ STFT Deconvolution – NIST mAb series	15
□ Quantitation and Calibration – NIST mAb series	42
□ Gabor Slicer – multi-protein sample	68
□ Deconvolution Refinement – multi-protein sample	95
□ Isotopic Distribution Calculator – multi-protein sample	120

iFAMS v. 6.3 Quant Tutorial

Term definitions and explanations and further details on iFAMS menu options and functions can be found in the iFAMS v. 6.3 manual.

In this tutorial...

Essential actions are in **red text**

Supplementary information is in **blue text**

(additional windows can be closed once they are no longer present in the tutorial)

GUI Overview

Required data: none

- Descriptions of the iFAMS GUI general menus and tools

Note: Keep GUI file/executable in the same location as the “iFAMS Fun” folder

Displays the file path of the last loaded data

Displays current ion mode used in the iFAMS analysis

Toggles between positive and negative modes through the Settings menu

Zoom tool is quite handy for finding signal in the spectrogram

Remember to deselect Zoom to resume drawing boxes on the spectrogram to select signal

Helpful tools for adjusting the displayed figures

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Saves the iFAMS screen as a PNG, EPS, etc.

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

- Load Ordinary FT
- Load STFT
- Plot STFT From Clipboard
- Load Deconvolved or IFFT Spectra
- Load Batch Parameters
- Batch Data Files
- Batch Deconvolved Spectra
- Export Data
- Export Batch Parameters

Menu for loading and saving spectra/processing data

“Home” resets any zoom functions performed.

If an action leads to unsatisfactory results and no undo-type action is provided, it is best to reload the file.

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File Settings Fourier Analysis Tools STFT

Load Ordinary FT

Loads MS data from a CSV or TXT

Load STFT

Loads MS data copied to the clipboard

Plot STFT From Clipboard

Loads processed spectra and displays additional saved content if processed from iFAMS v6.3

Load Deconvolved or IFFT Spectra

Loads processing settings from a “batch_params” file

Load Batch Parameters

Batch Data Files

Uses stored processing settings to deconvolve and/or integrate a set of MS or deconvolved files

Batch Deconvolved Spectra

Saves recent deconvolved spectrum, integrated peaklist, and other results and processing parameters

Export Data

Saves processing parameters to a “batch_params” file

Export Batch Parameters

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File **Settings** Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

- ✓ Positive Ion Mode
- Adjust Data Domain
- Show Interpolated MS
- Normalize Abundances
- Smooth Isotope Resolution

General pre-processing and display settings

File: -

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File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

File Settings Fourier Analysis Tools ST

✓ Positive Ion Mode

Adjust Data Domain

Show Interpolated MS

Normalize Abundances

Smooth Isotope Resolution

Toggle for the ion mode setting. Determines the mass of the charge carrier used in charge correction

Pre-processing tool for adjusting data x-value range and sampling density

Toggle for displayed MS data (raw v. interpolated)

Toggles for displayed deconvolved spectra

File: -

Positive Ion Mode



File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

- Plot Absolute Data
- Plot Real Data
- Open iFAMS Maxima Peak Finder
- Manually Enter Charge States and Submass
- Run iFAMS Analysis
- Run Mass Defect Analysis

Tools for deconvolving a spectrum using ordinary FT

File: -

Positive Ion Mode



File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

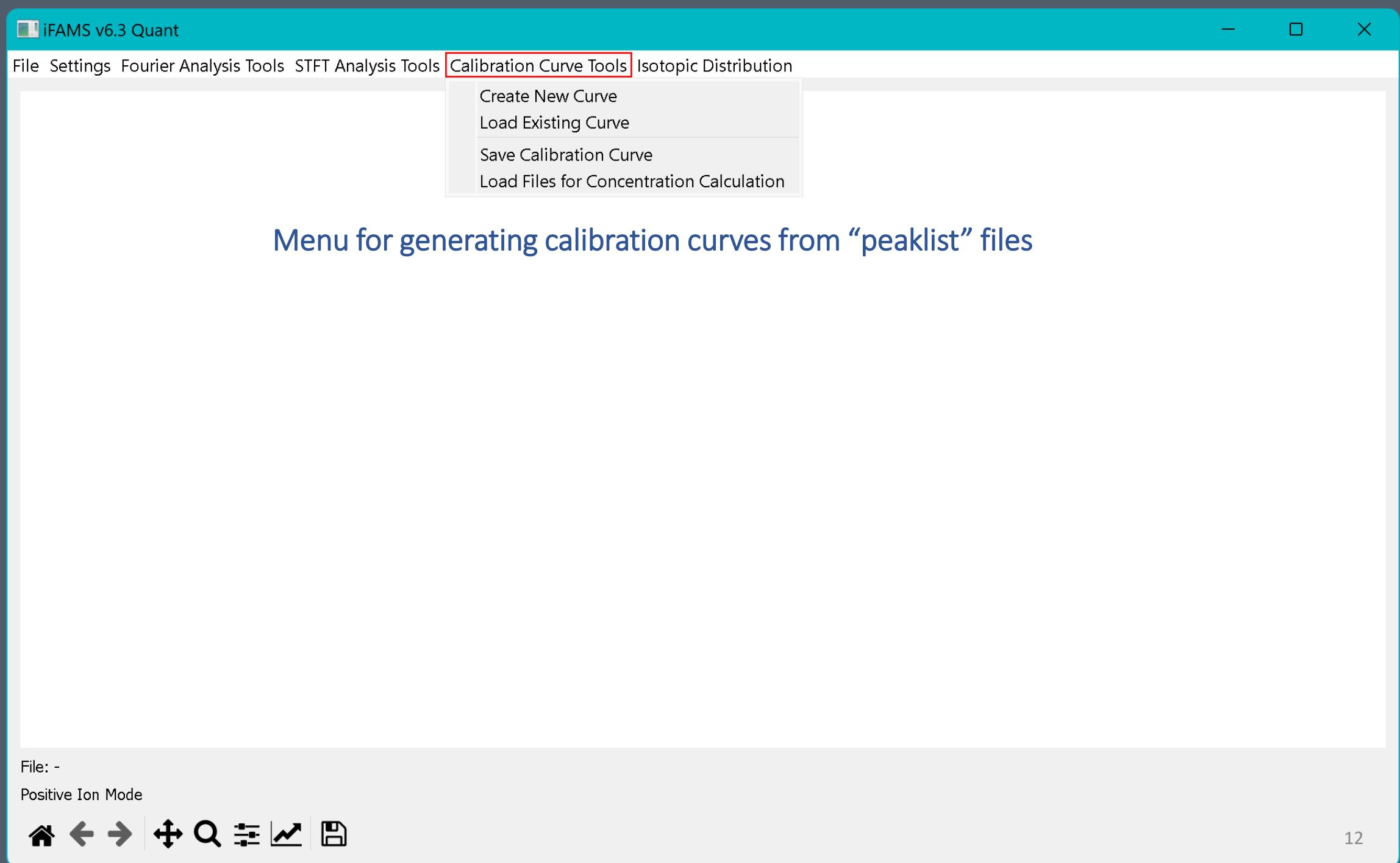
- Change STFT Parameters
- Open Gabor Slicer
- Run Guided Search
- Add Gabor Selection Ctrl+E
- Save Gabor Selections Ctrl+S
- Delete Previous Gabor Selection Ctrl+Del
- Open Harmonic Finder
- Run iFAMS Analysis
- Adjust Charge State Assignments
- Open Quantitative Peak Integrator
- Run Mass Defect Analysis
- Show Only Spectrogram
- Open Noise Calculator

Tools for deconvolving a spectrum using STFT which includes the Gabor spectrogram

File: -

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iFAMS v6.3 Quant

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

- Calculate Distribution
- Overlay Data Reconstruction
- Export Calculated Distribution

Isotope calculator for generating a theoretical mass distribution from the analyte's sequence (amino acid or nucleotide)

File: -

Positive Ion Mode

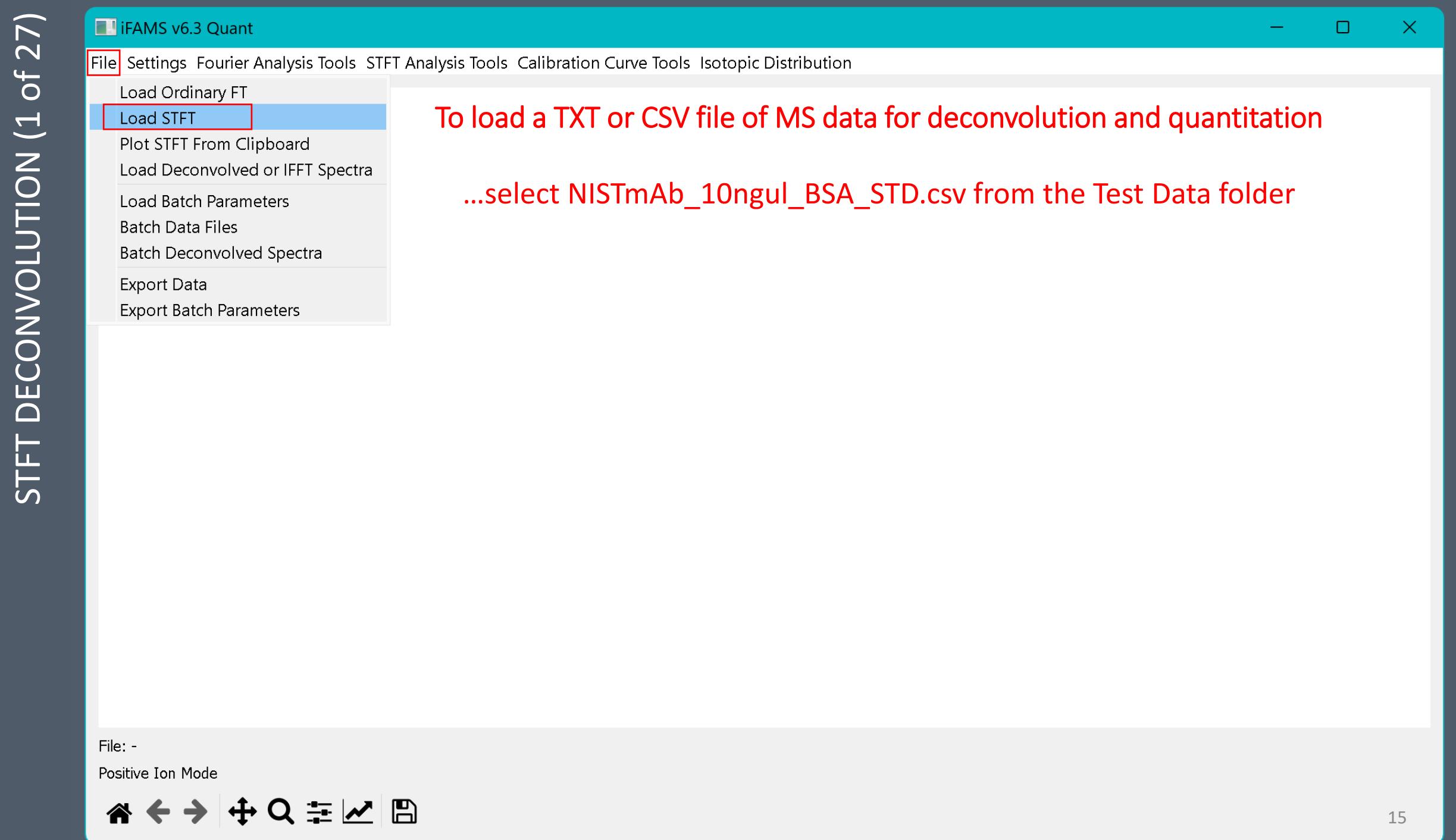
Home Back Forward Search Plot Histogram

13

STFT Deconvolution

Required data: NIST mAb series

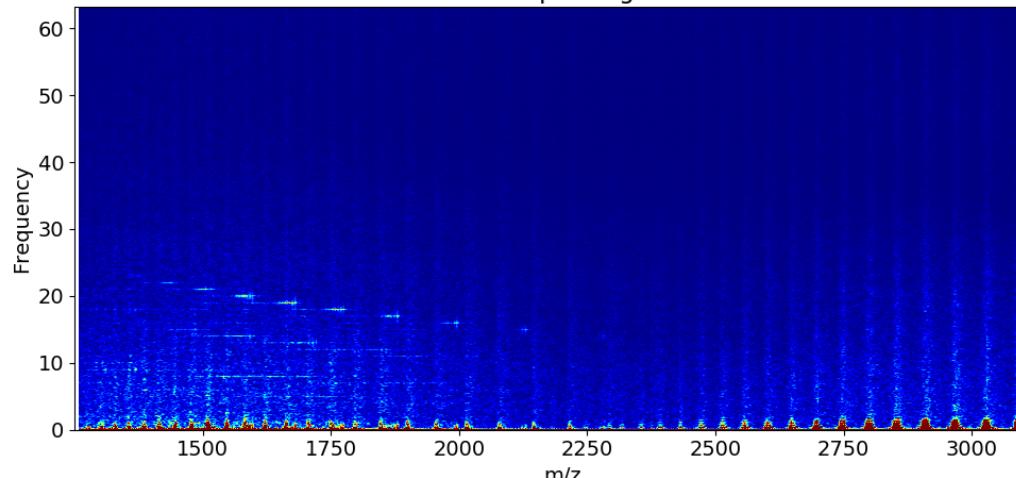
- STFT parameters adjustment
- Multiple protein data selection and deconvolution with the Guided Search tool
- Adjustment of automatic charge state assignments



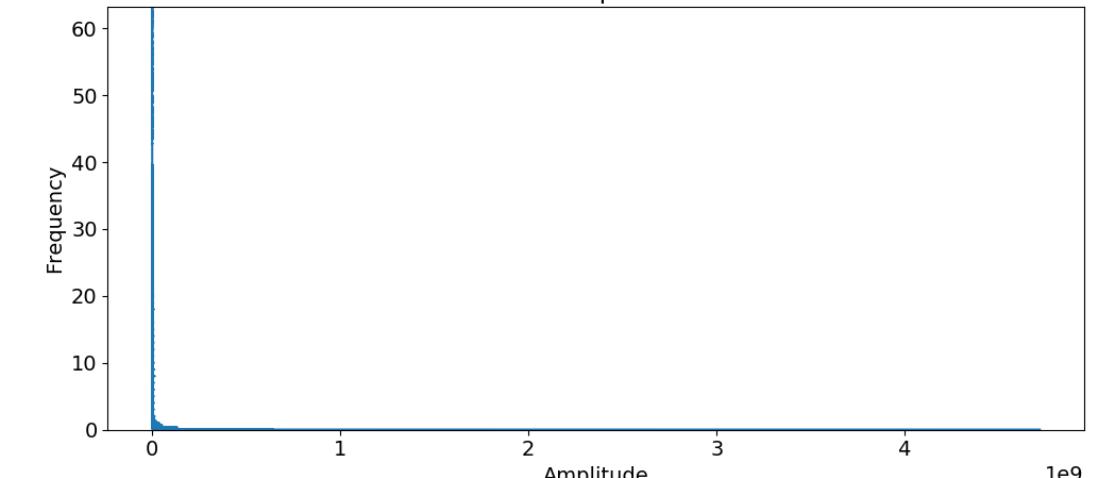
File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

Processed as STFT...

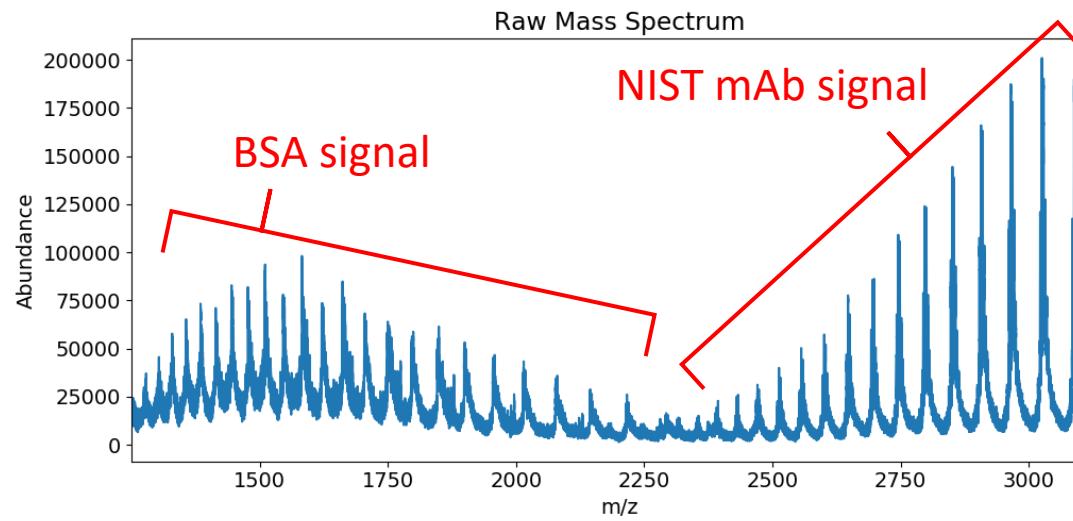
Gabor Spectrogram



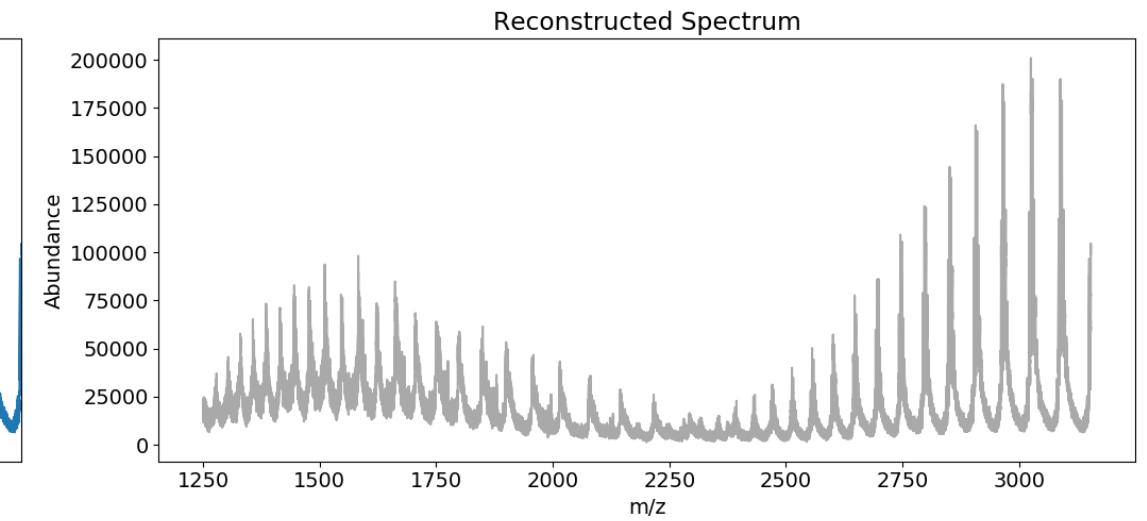
Fourier Spectrum



Raw Mass Spectrum

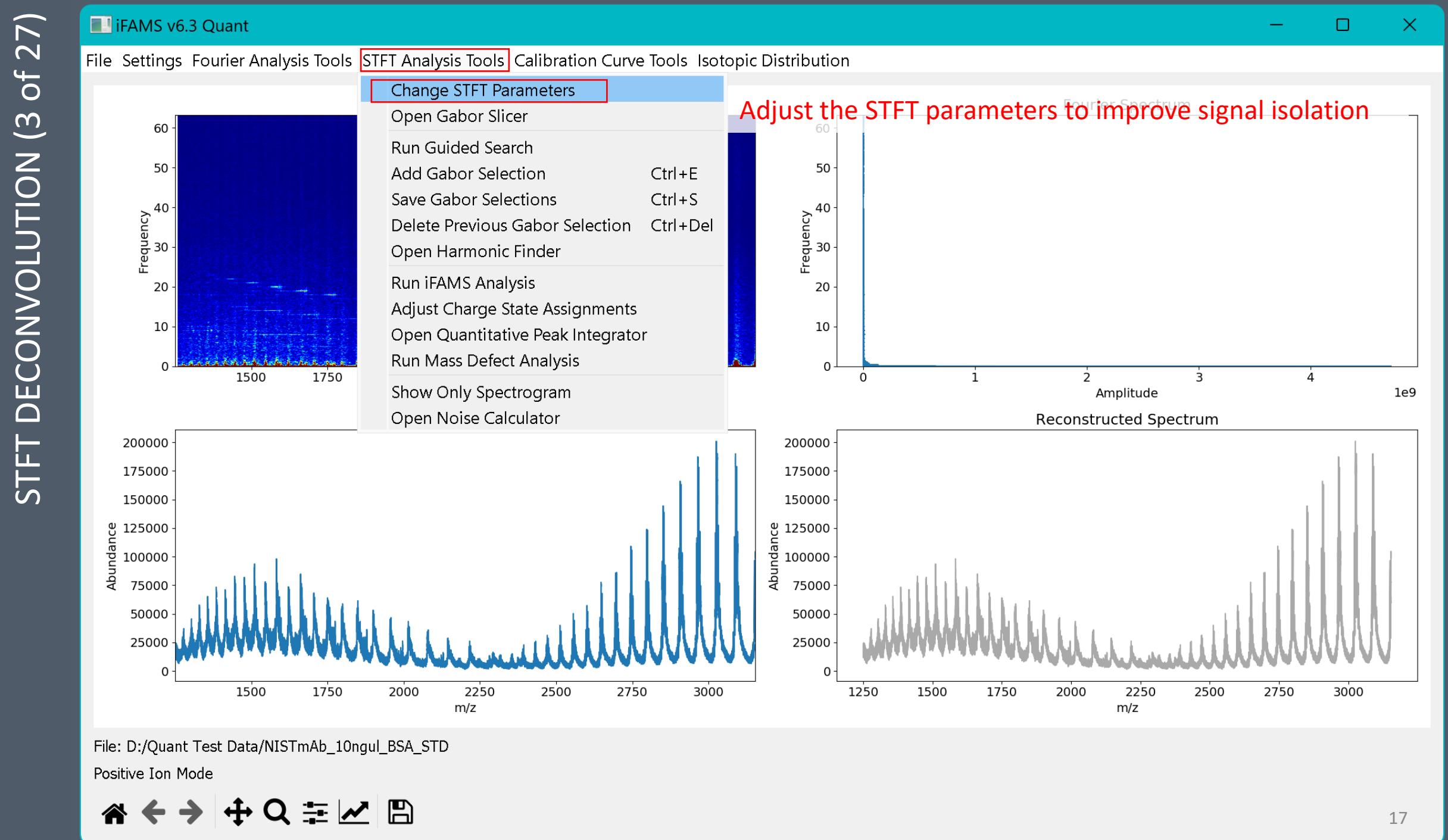


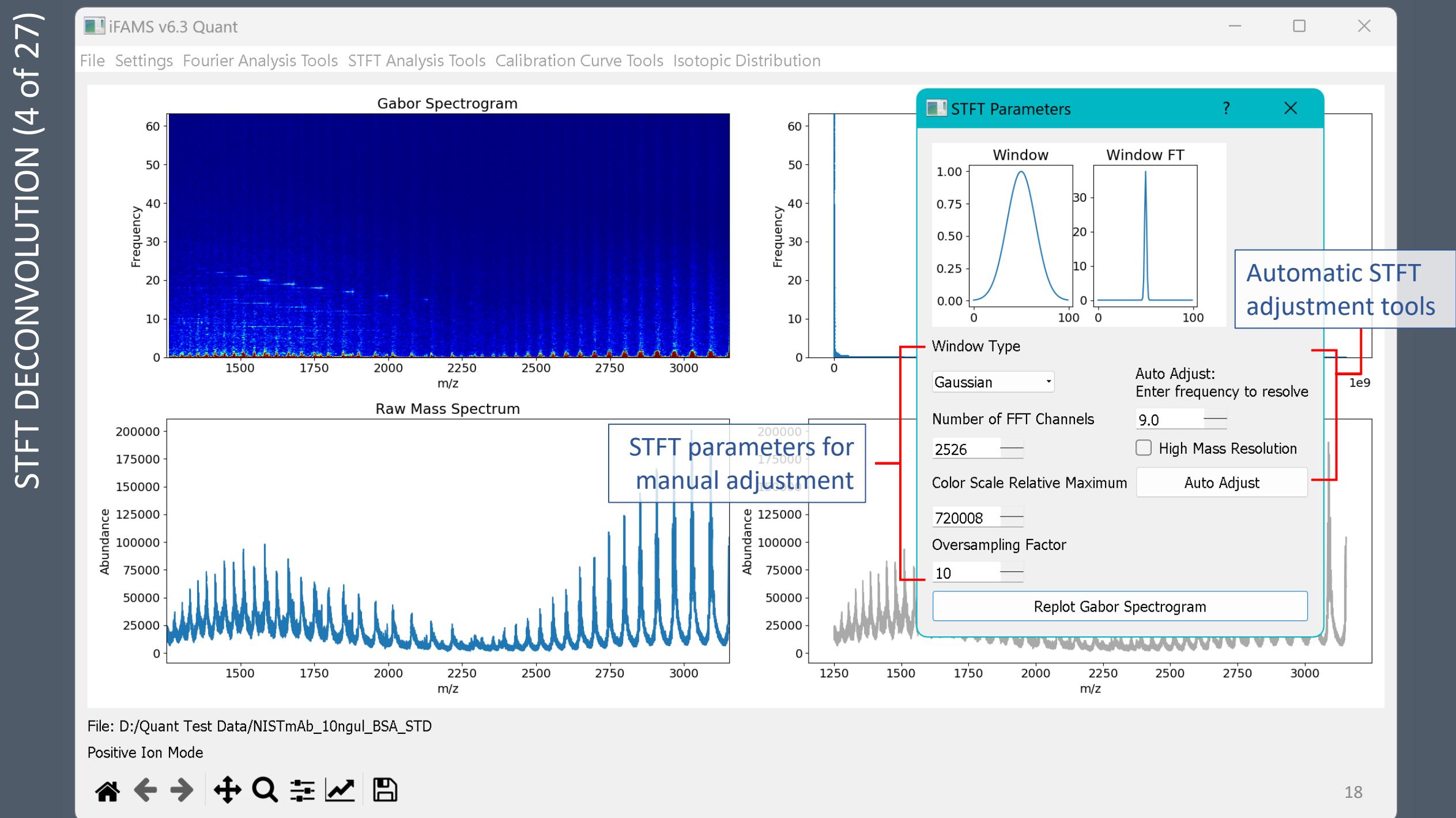
Reconstructed Spectrum

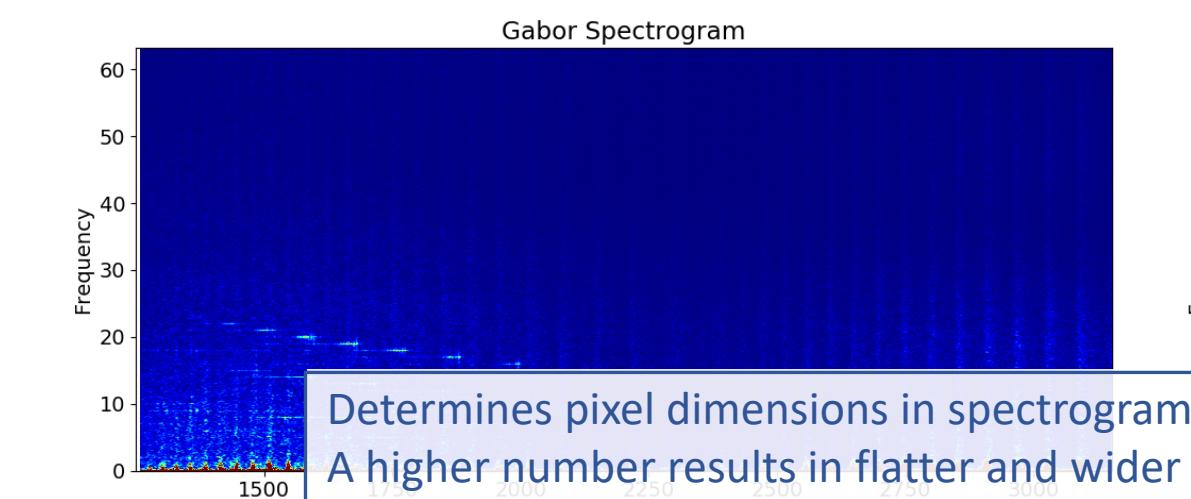


File: D:/Quant Test Data/NISTmAb_10ngul_BSA_STD

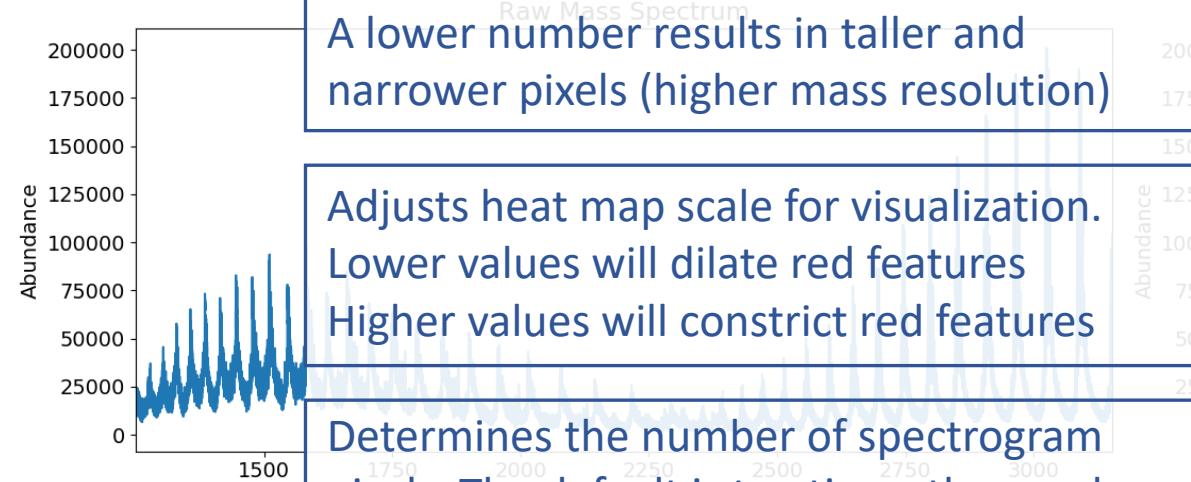
Positive Ion Mode



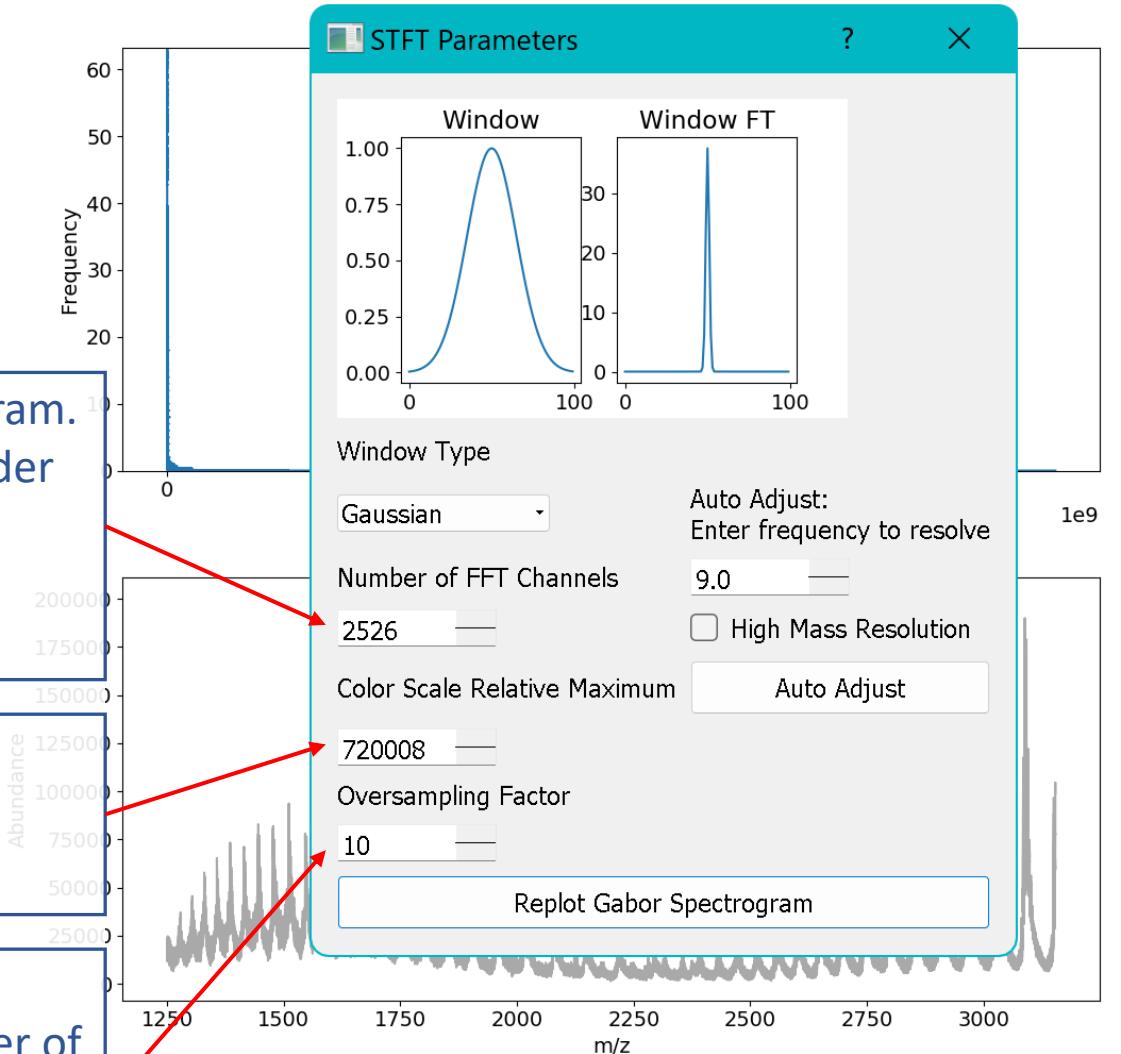


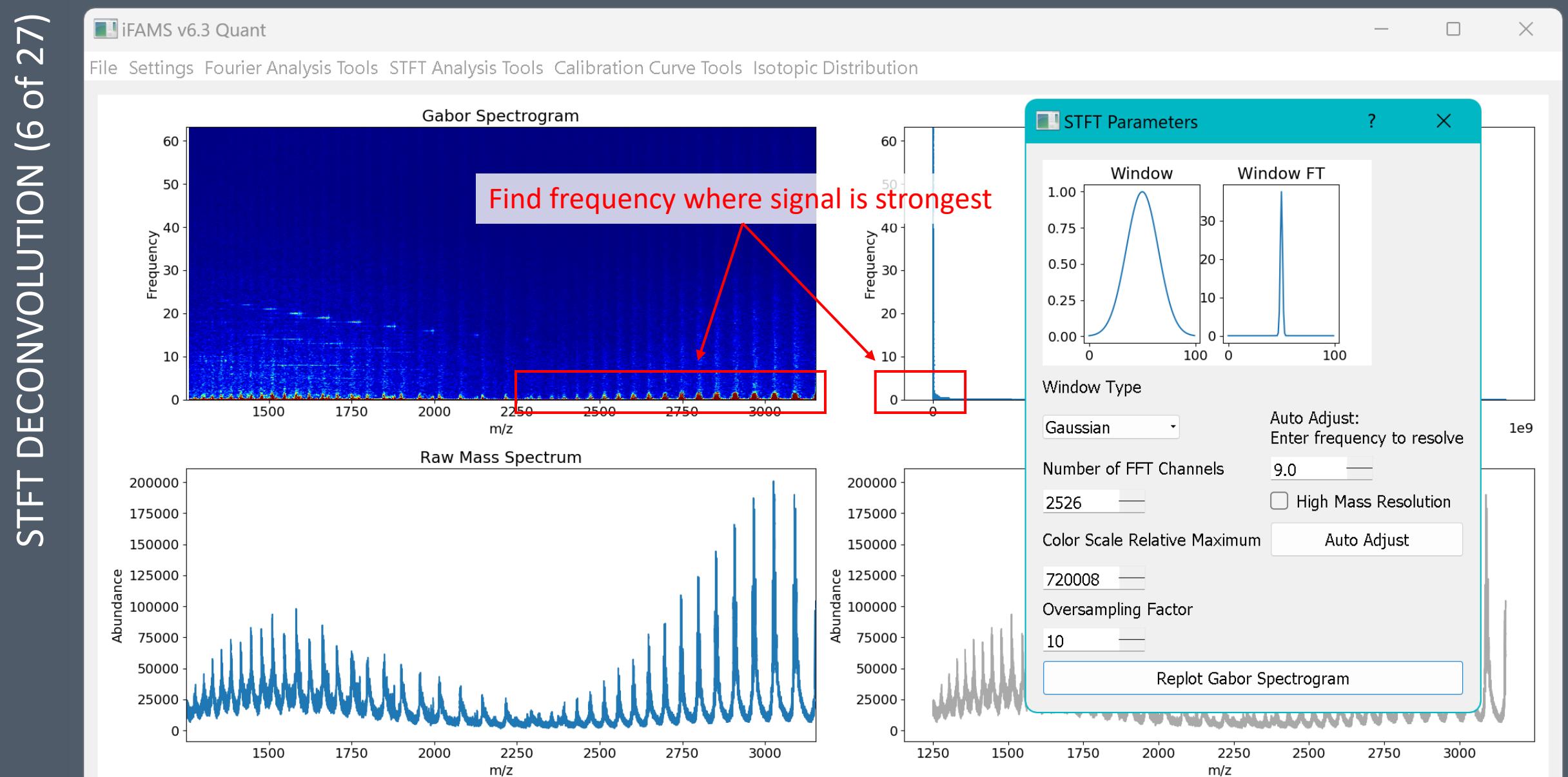


- Determines pixel dimensions in spectrogram
- A higher number results in flatter and wider pixels (higher frequency resolution)
- A lower number results in taller and narrower pixels (higher mass resolution)



Determines the number of spectrogram pixels. The default is ten times the number of data points. Higher values increase the number of pixels which results in a “smoothing” effect on the spectrogram



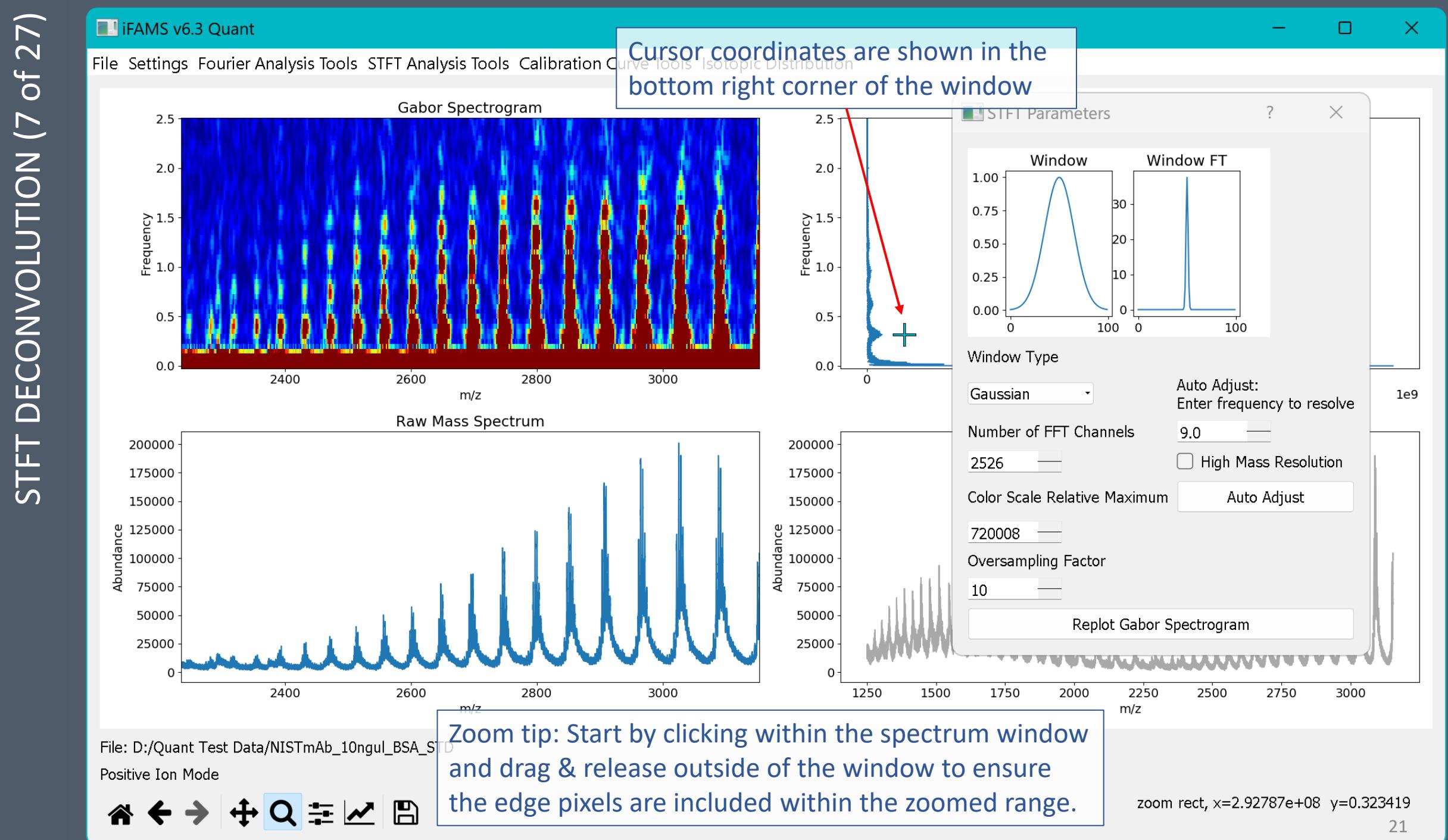


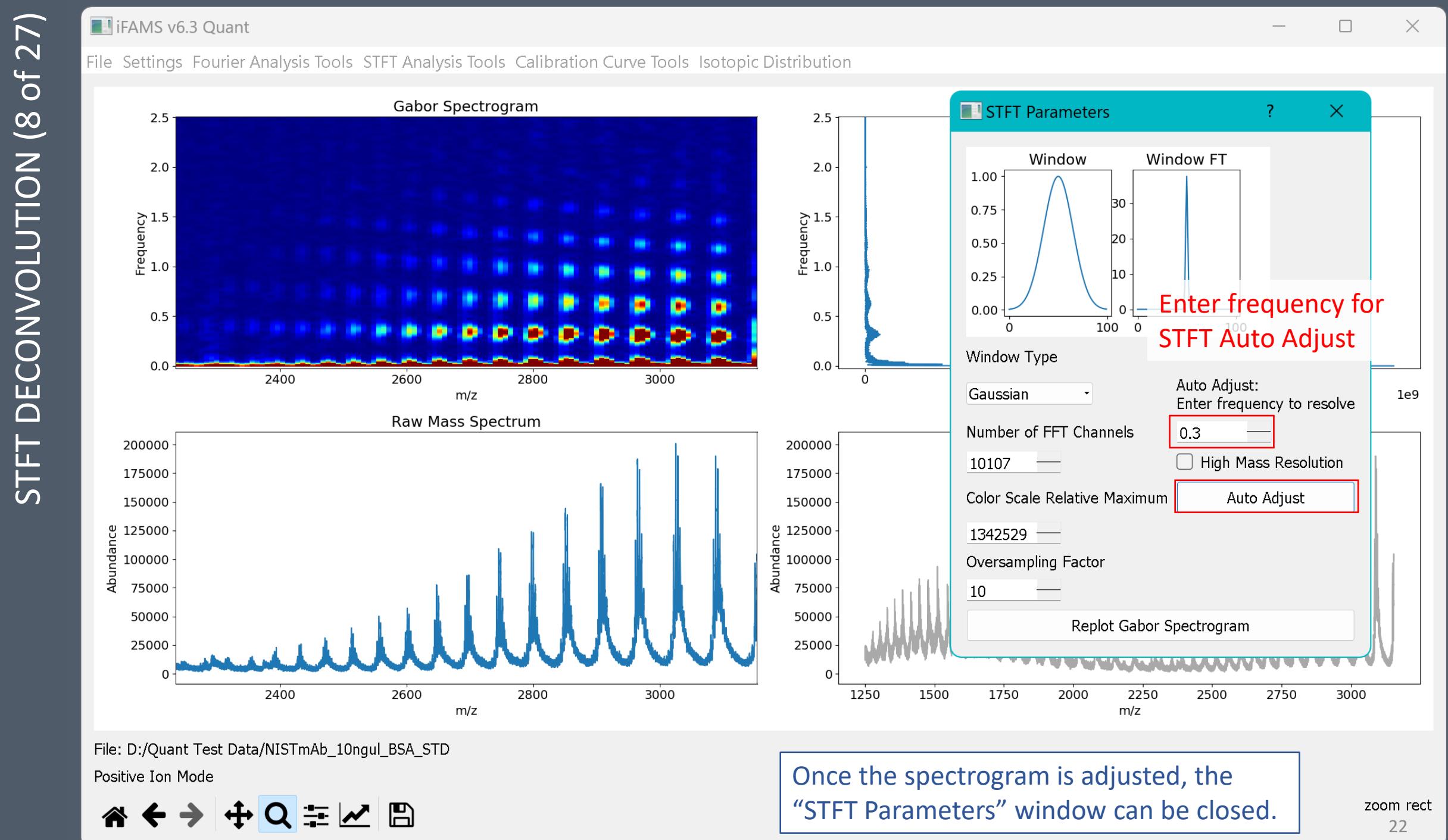
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Positive Ion Mode

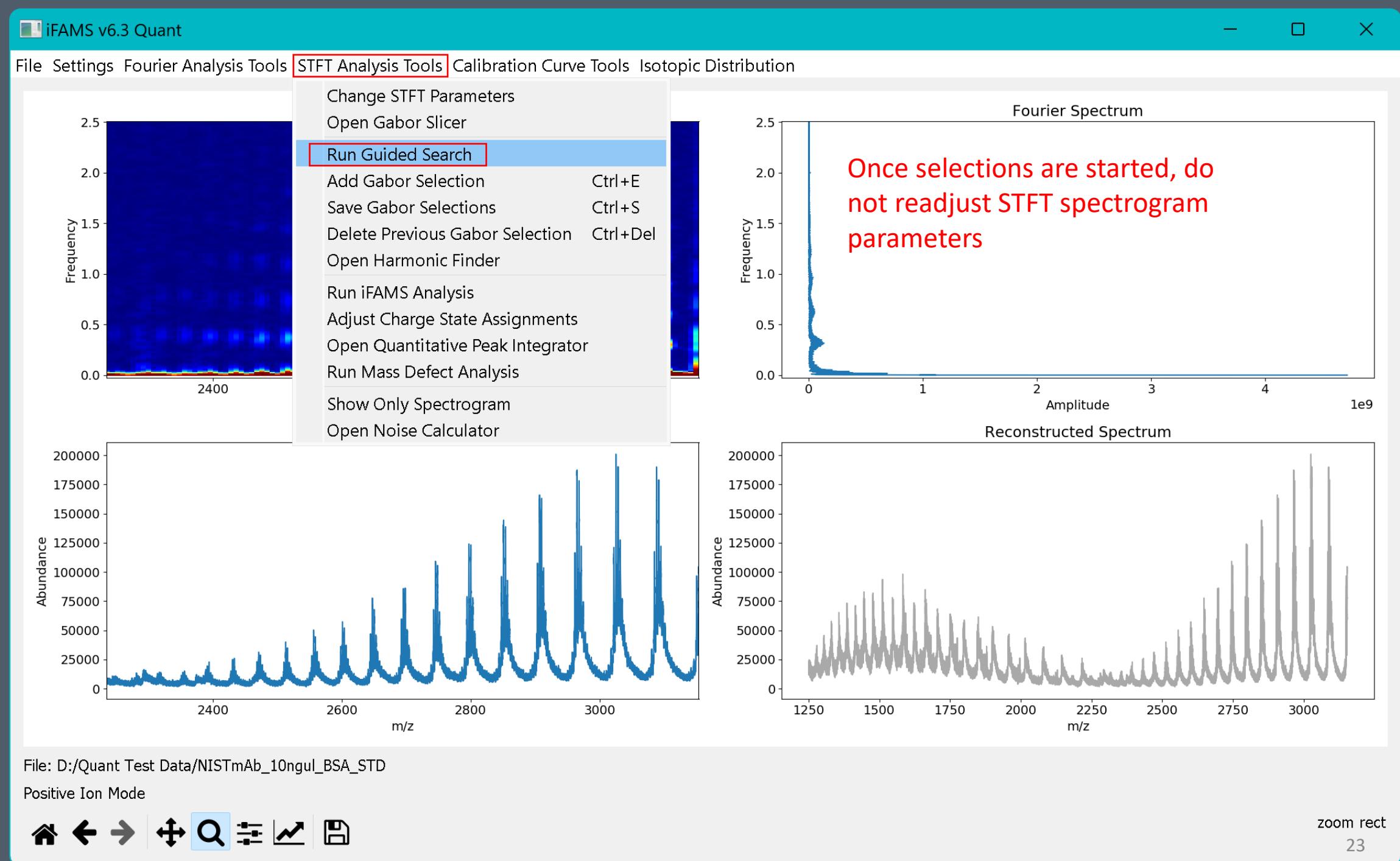
Use the Zoom tool to check lower frequencies

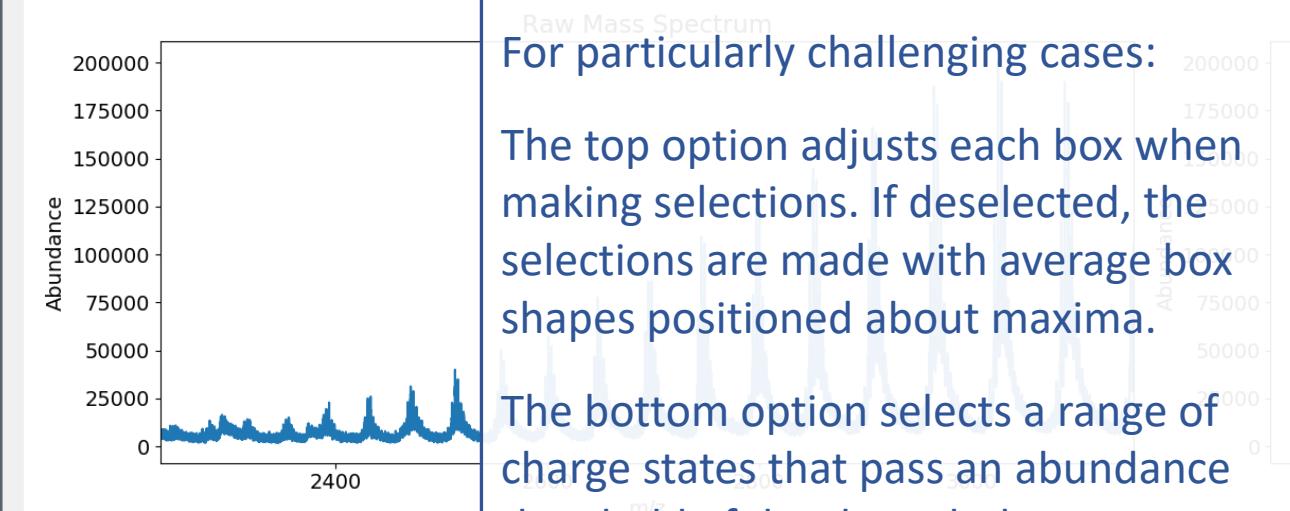
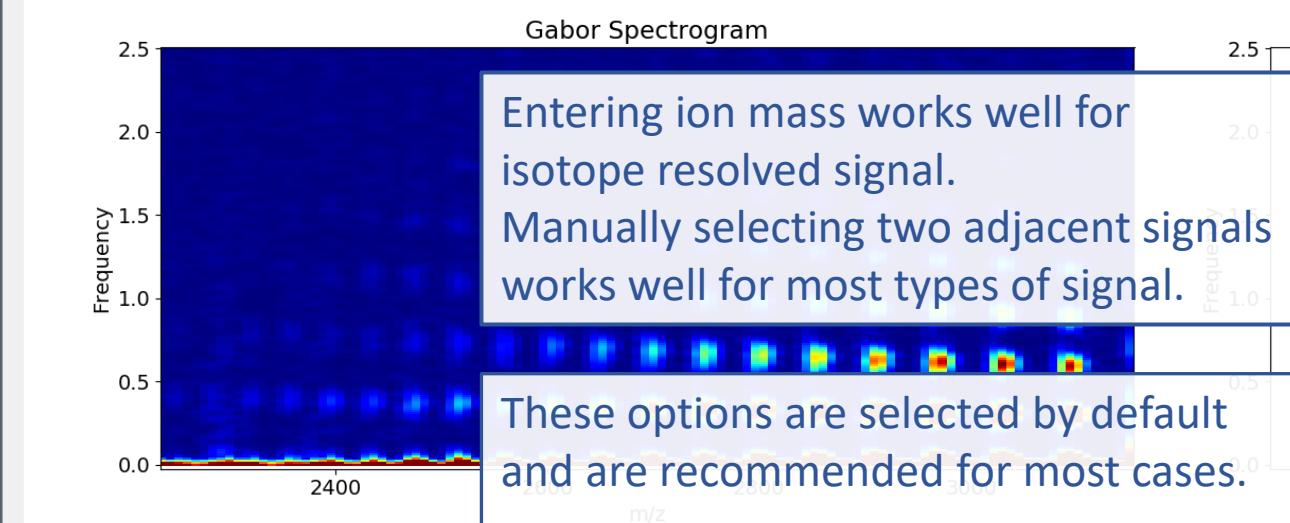
(see next slide for zoom recommendations)





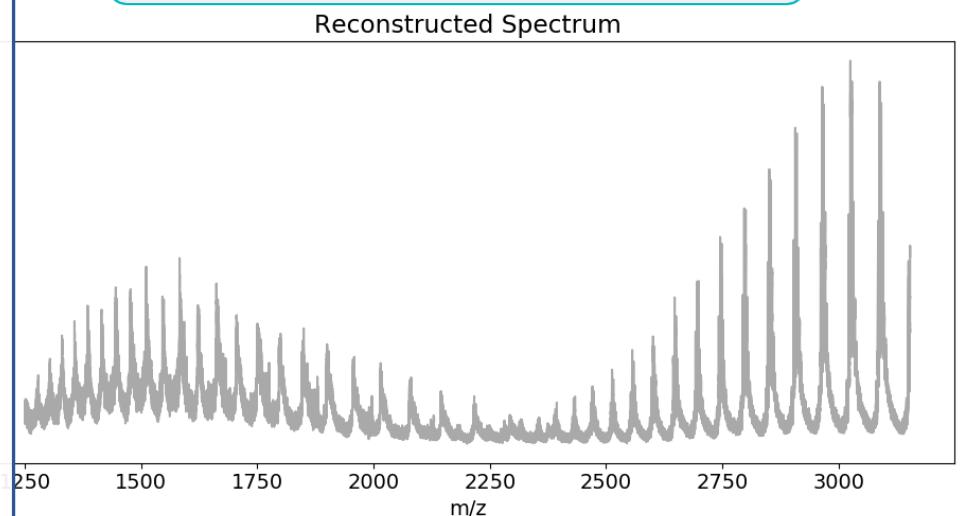
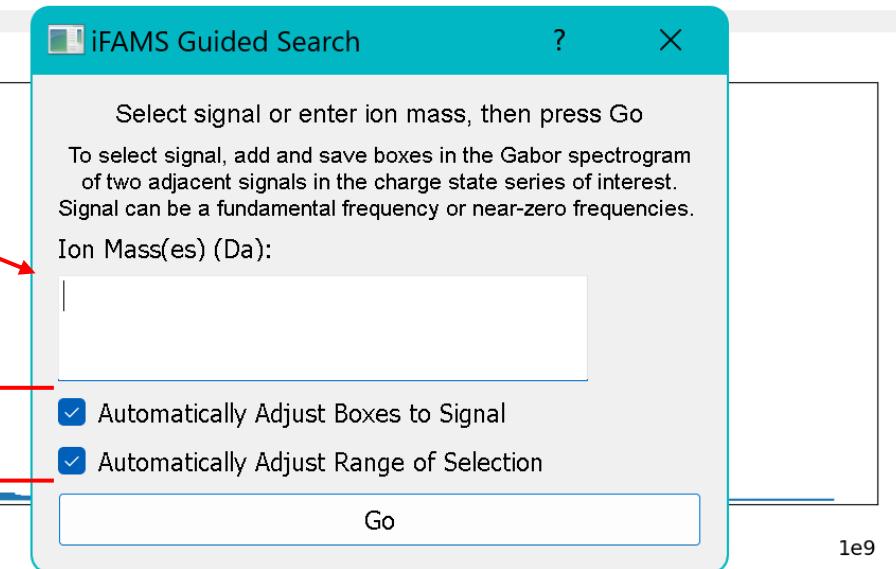
STFT DECONVOLUTION (9 of 27)

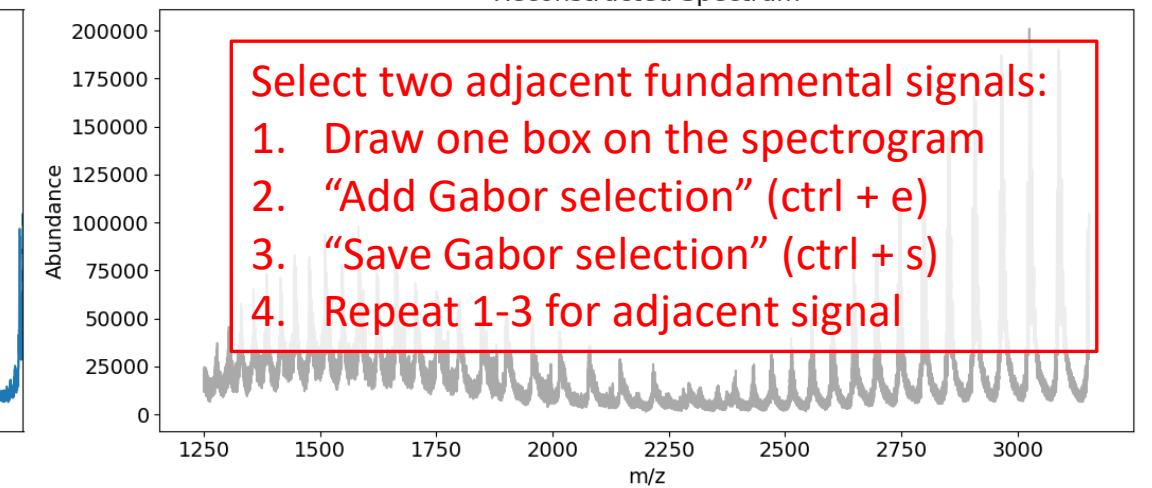
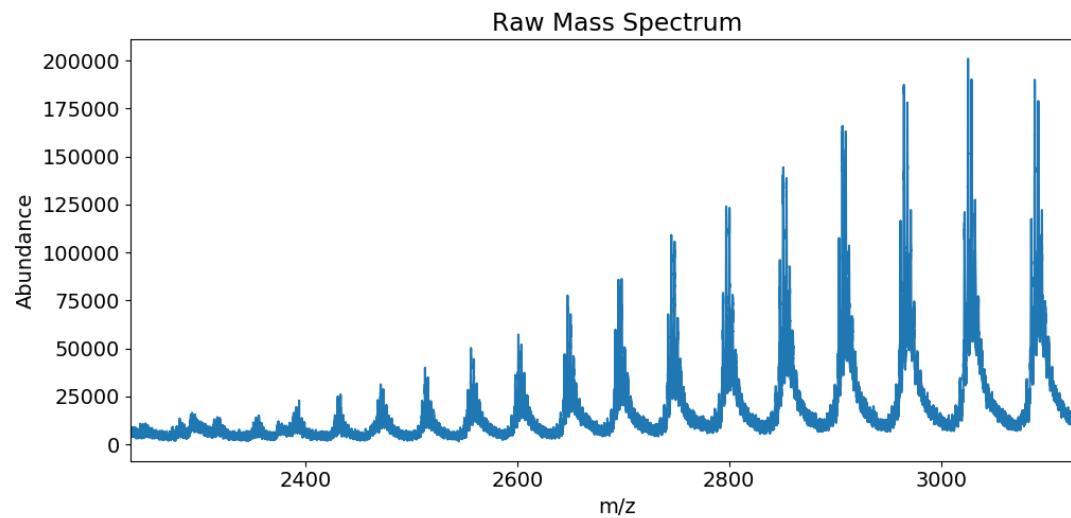
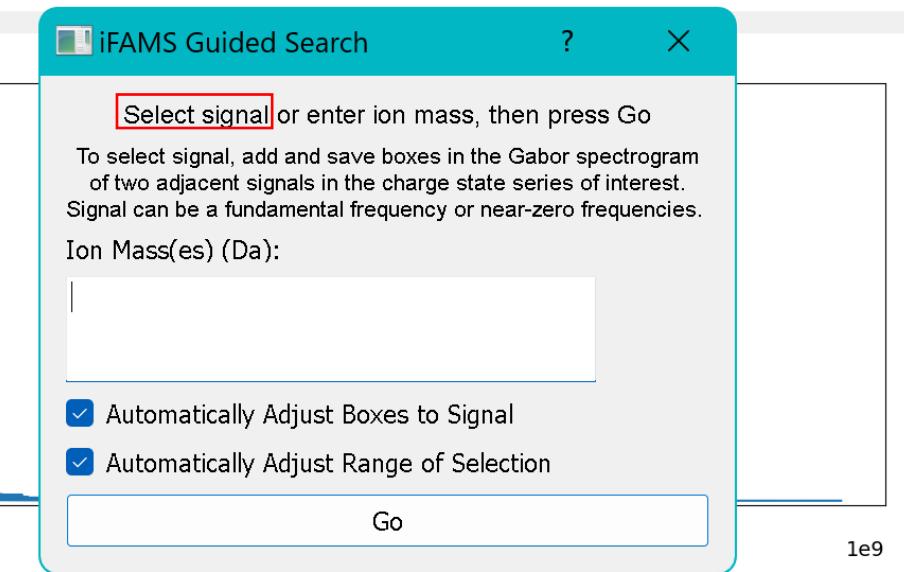
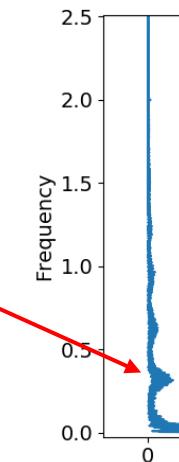
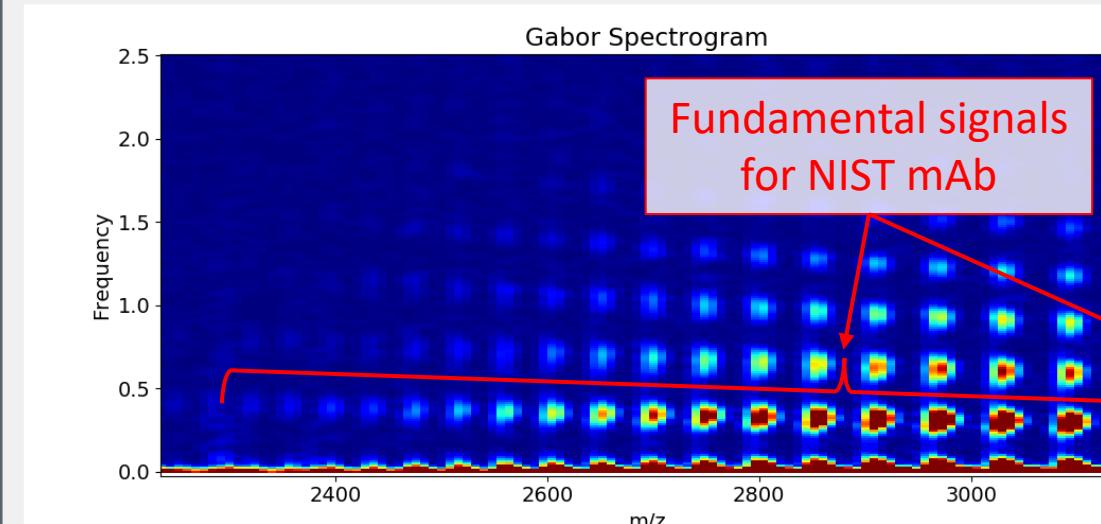




File: D:/Quant Test Data/NISTmAb_10ng

Positive Ion Mode





File: D:/Quant Test Data/NISTmAb_10ngul_BSA_STD

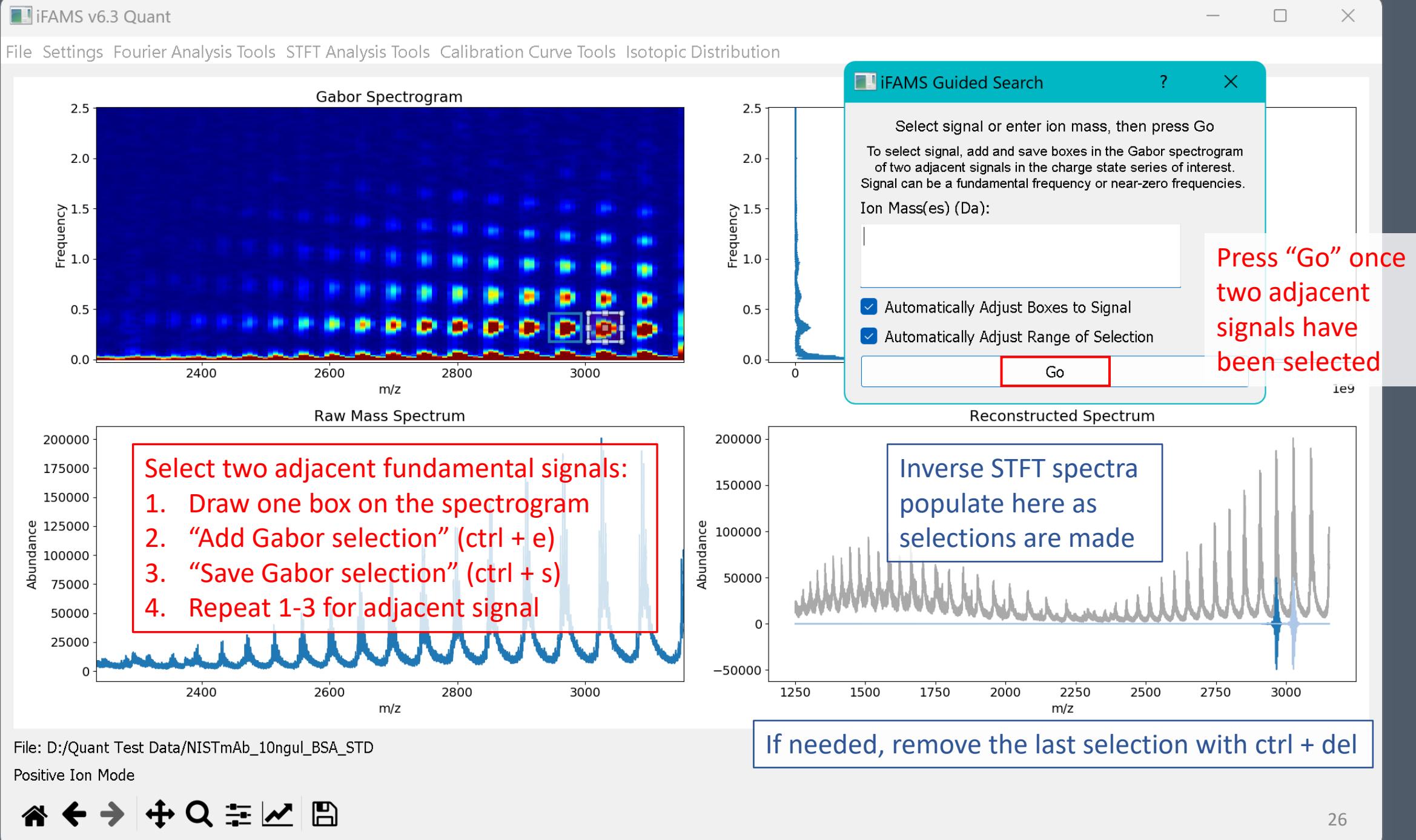
Positive Ion Mode



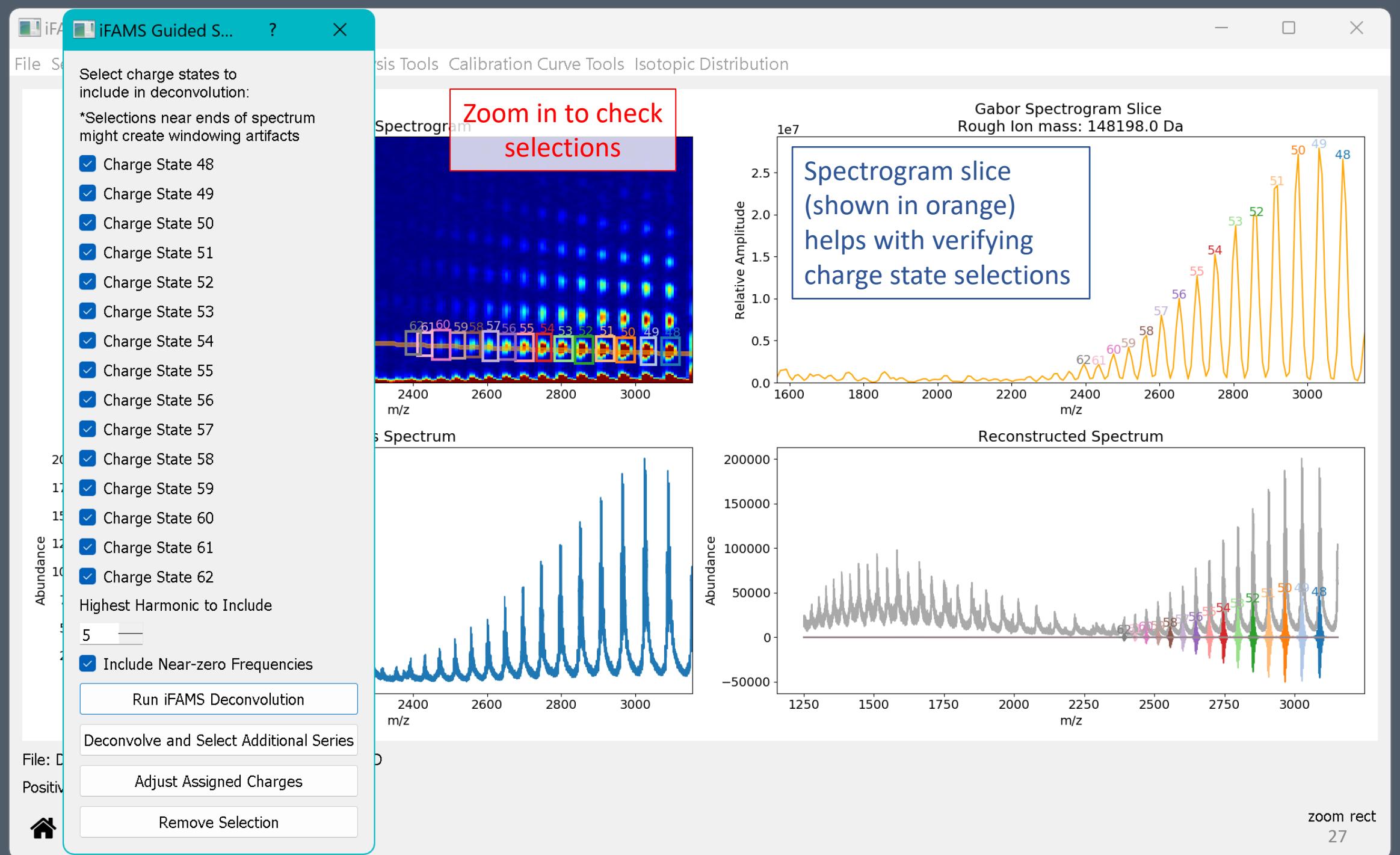
If needed, remove the last selection with ctrl + del

(see next slide for example selections)

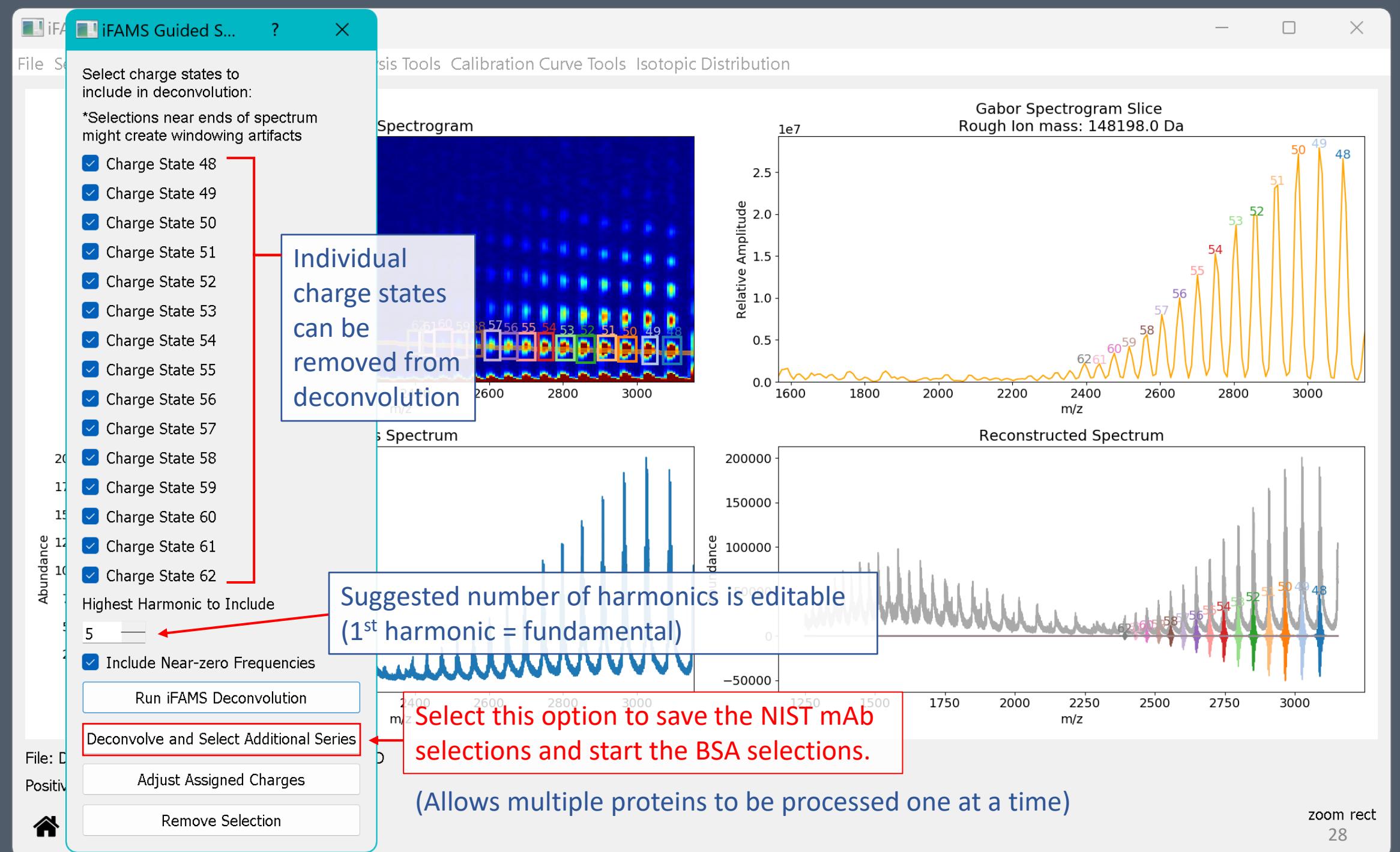
STFT DECONVOLUTION (12 of 27)

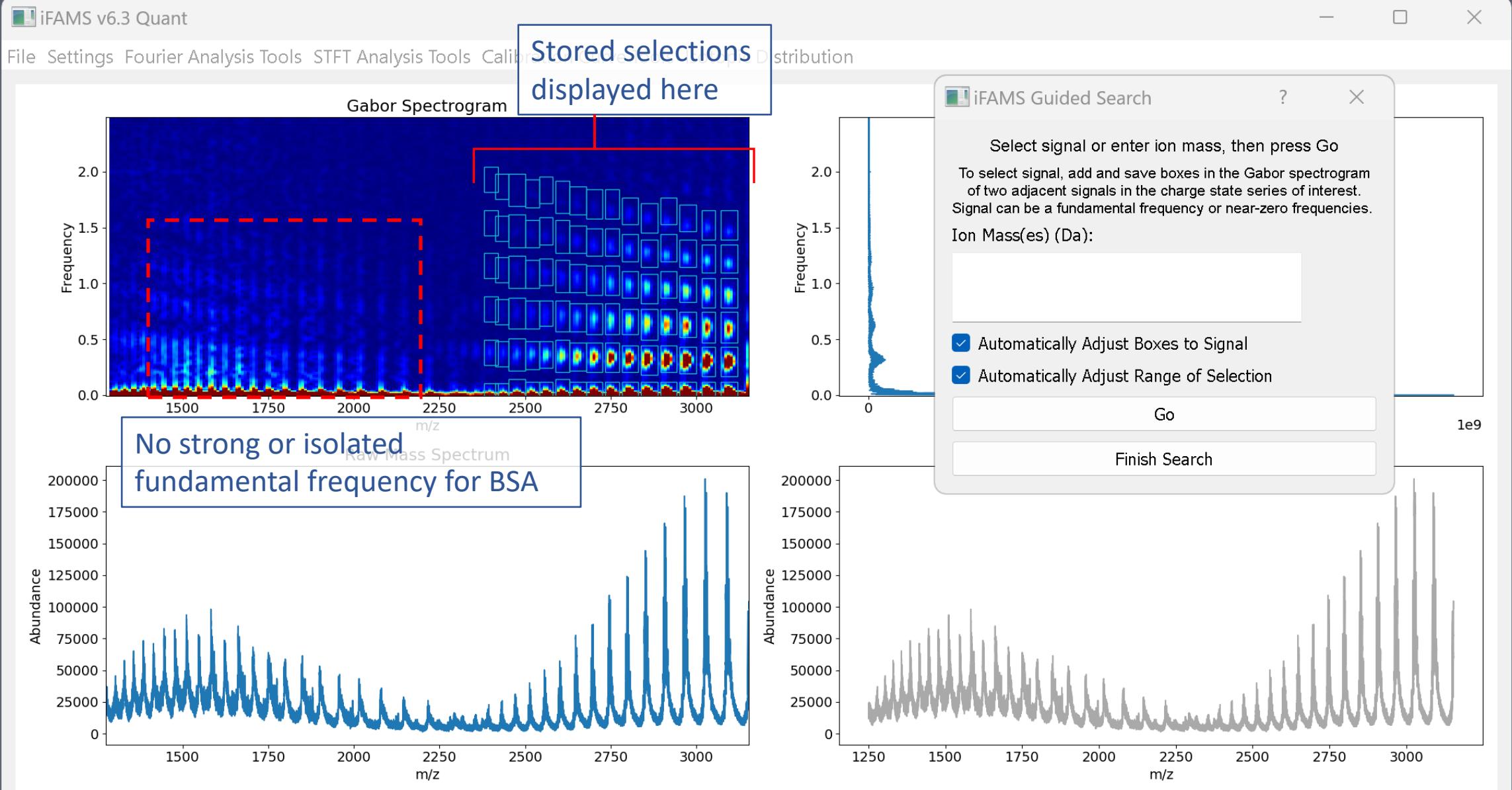


STFT DECONVOLUTION (13 of 27)



STFT DECONVOLUTION (14 of 27)





File: D:/Quant Test Data/NISTmAb_10ngul_BSA_STD

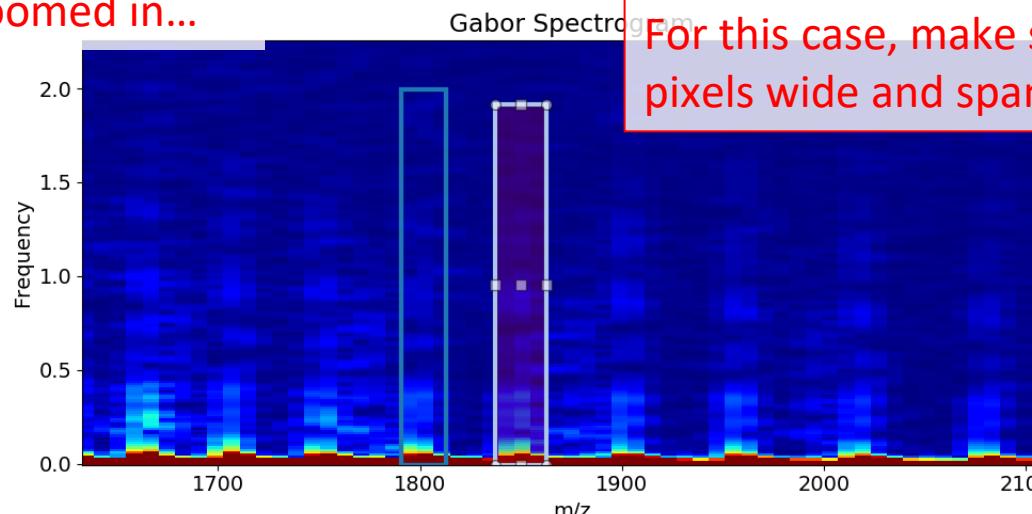
Positive Ion Mode



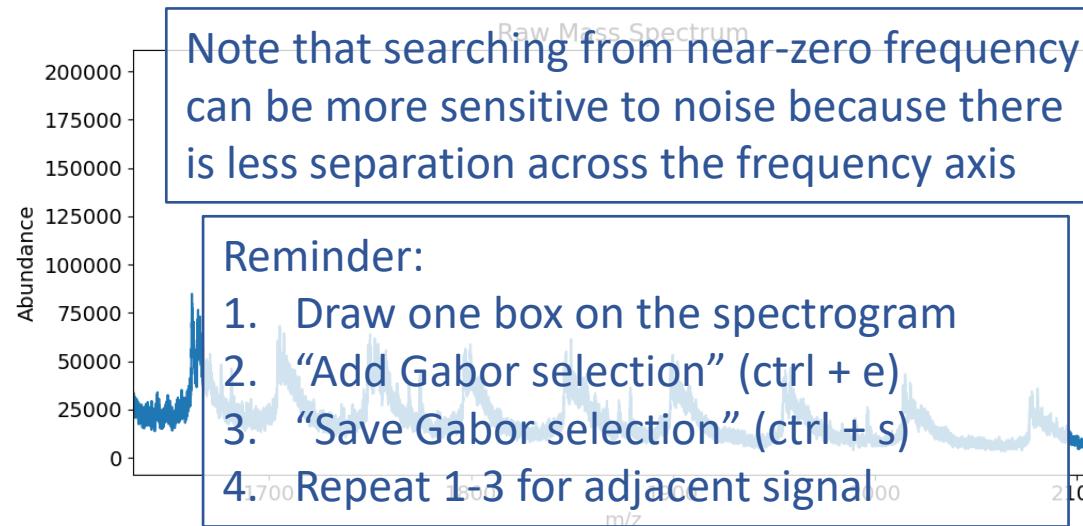
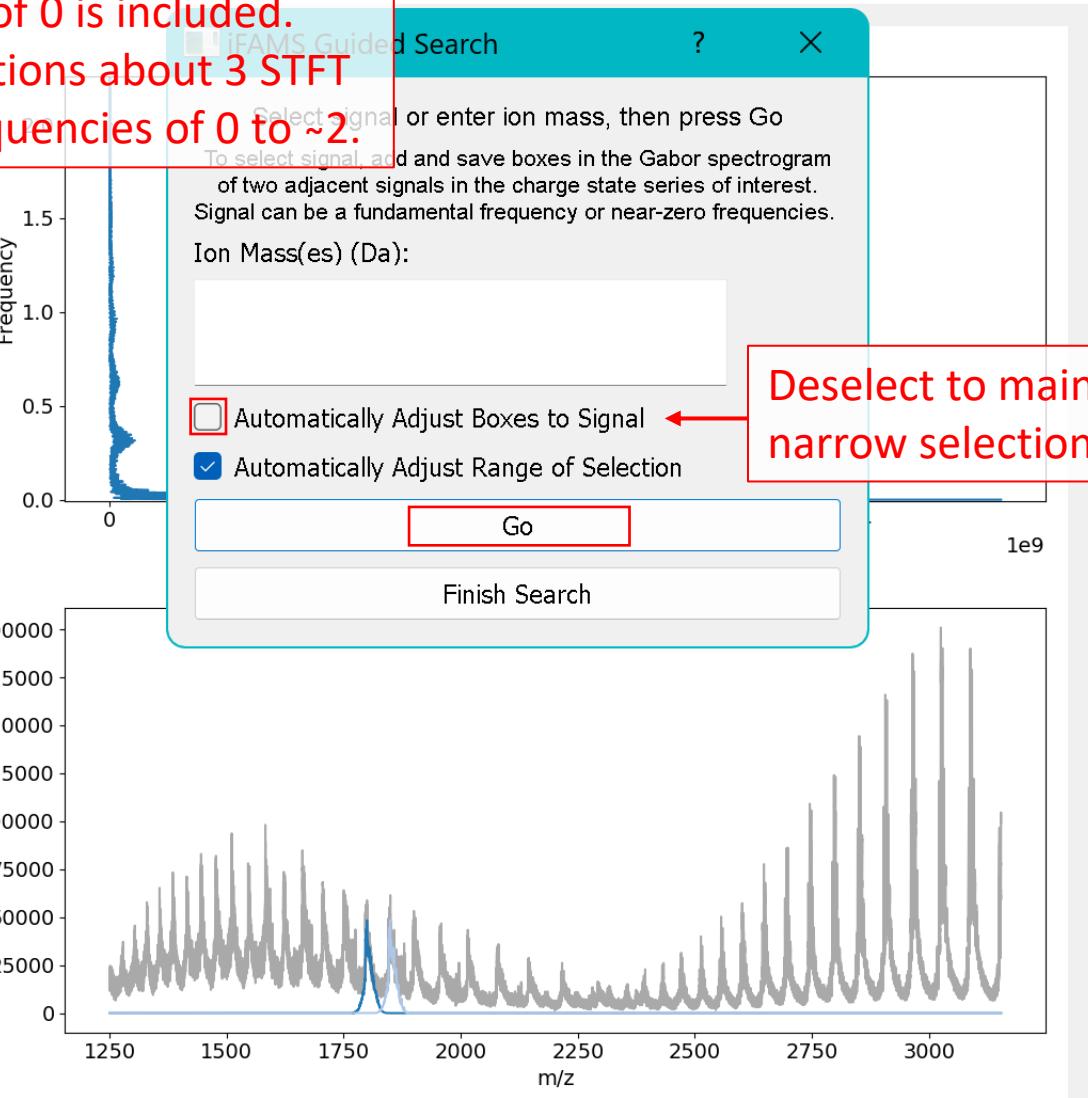
zoom rect

29

Zoomed in...



Select the “near-zero frequency” ensuring that frequency of 0 is included.
For this case, make selections about 3 STFT pixels wide and span frequencies of 0 to ~2.

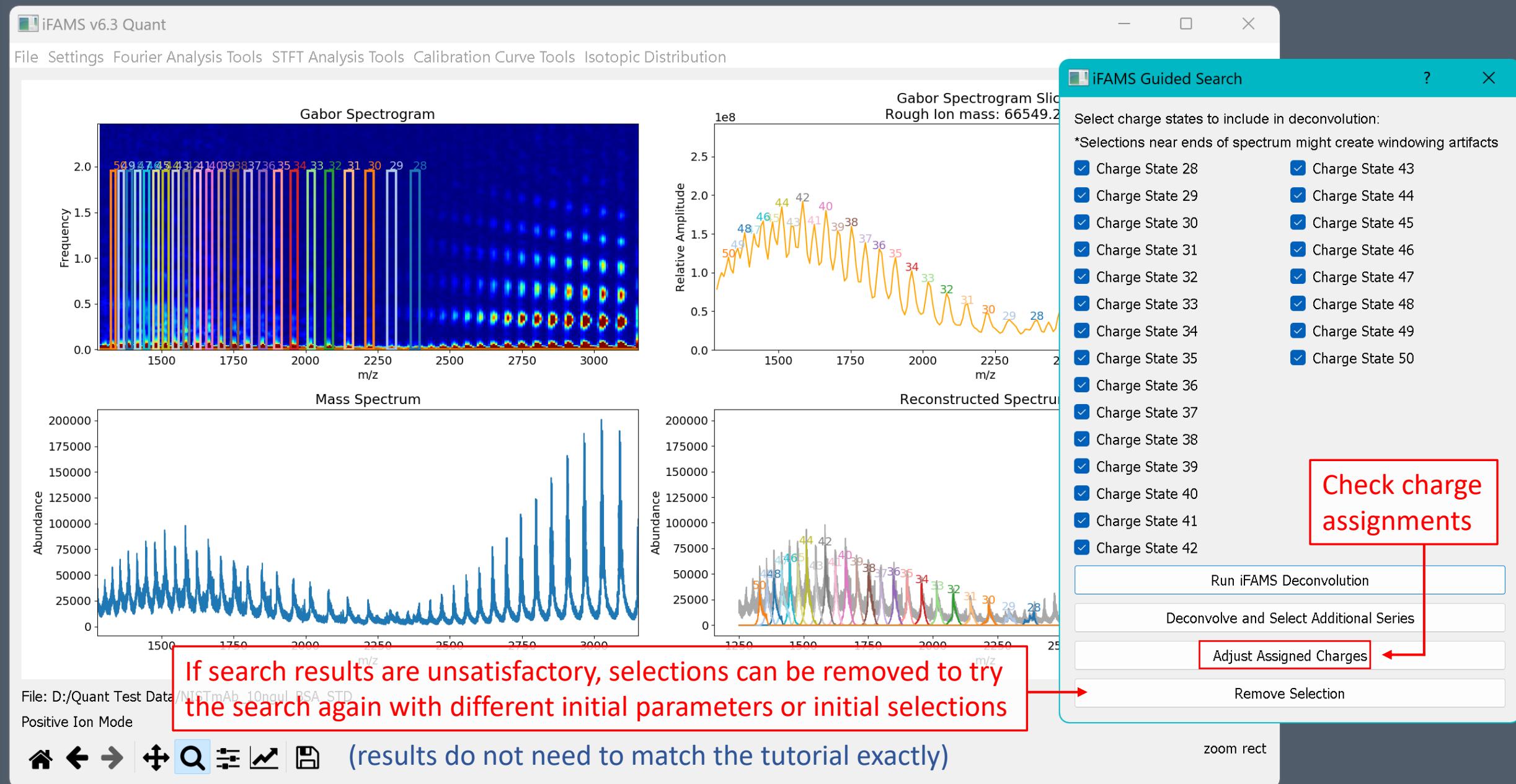


File: D:/Quant Test Data/NISTmAb_10ngul_BSA_STD

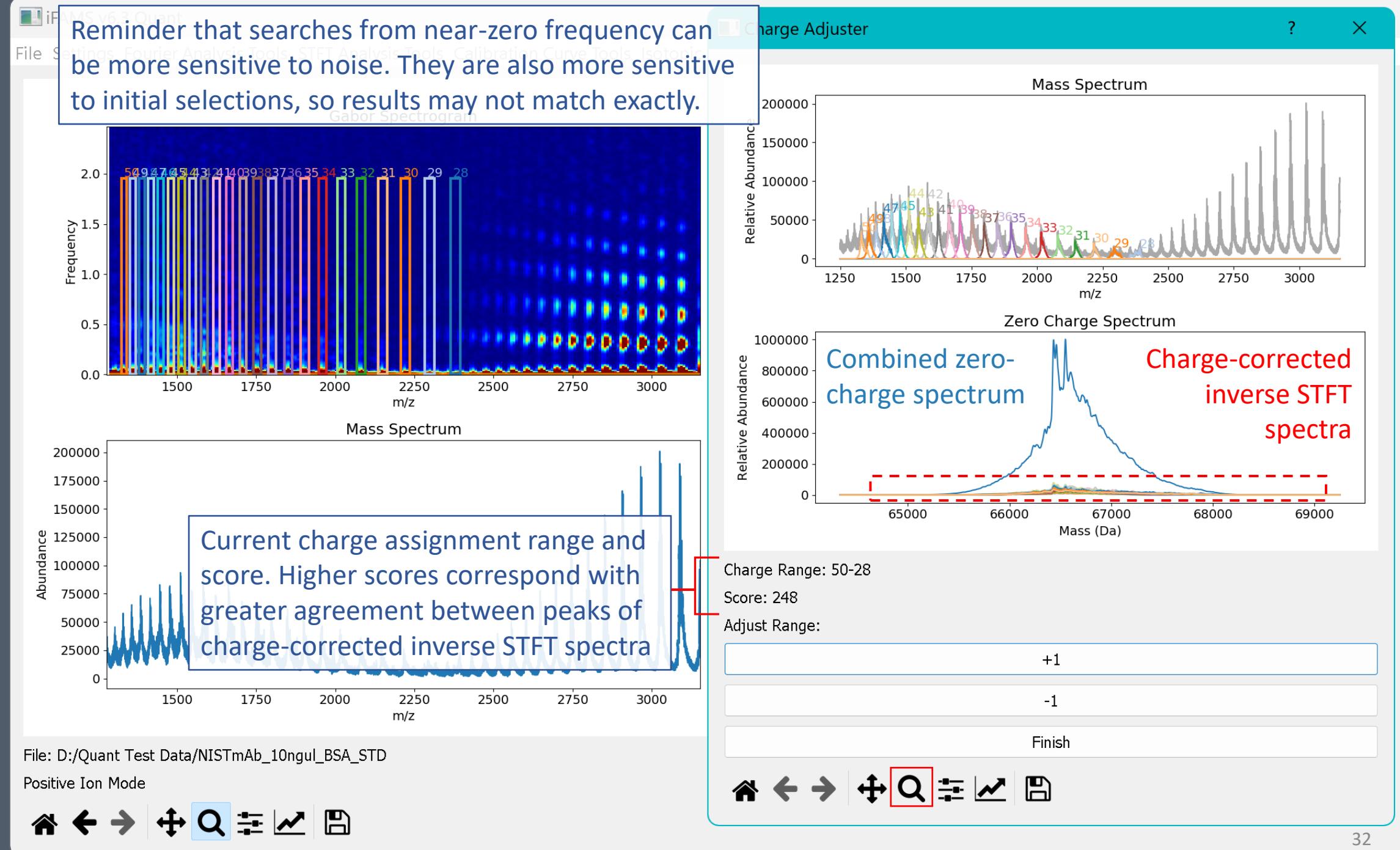
Positive Ion Mode

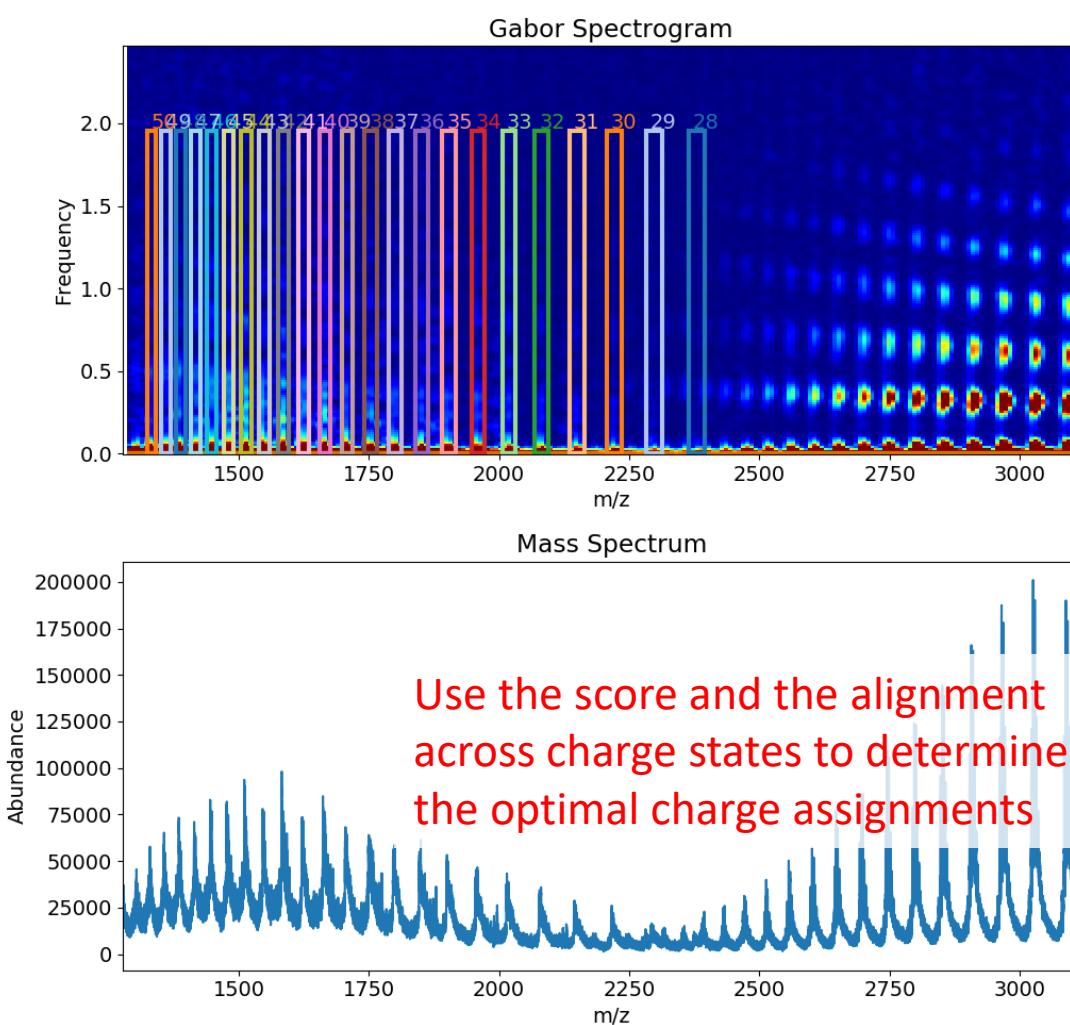


It can be helpful to use this tool to slide the spectrogram to make a larger buffer around frequency of 0 and allow for more room while drawing boxes



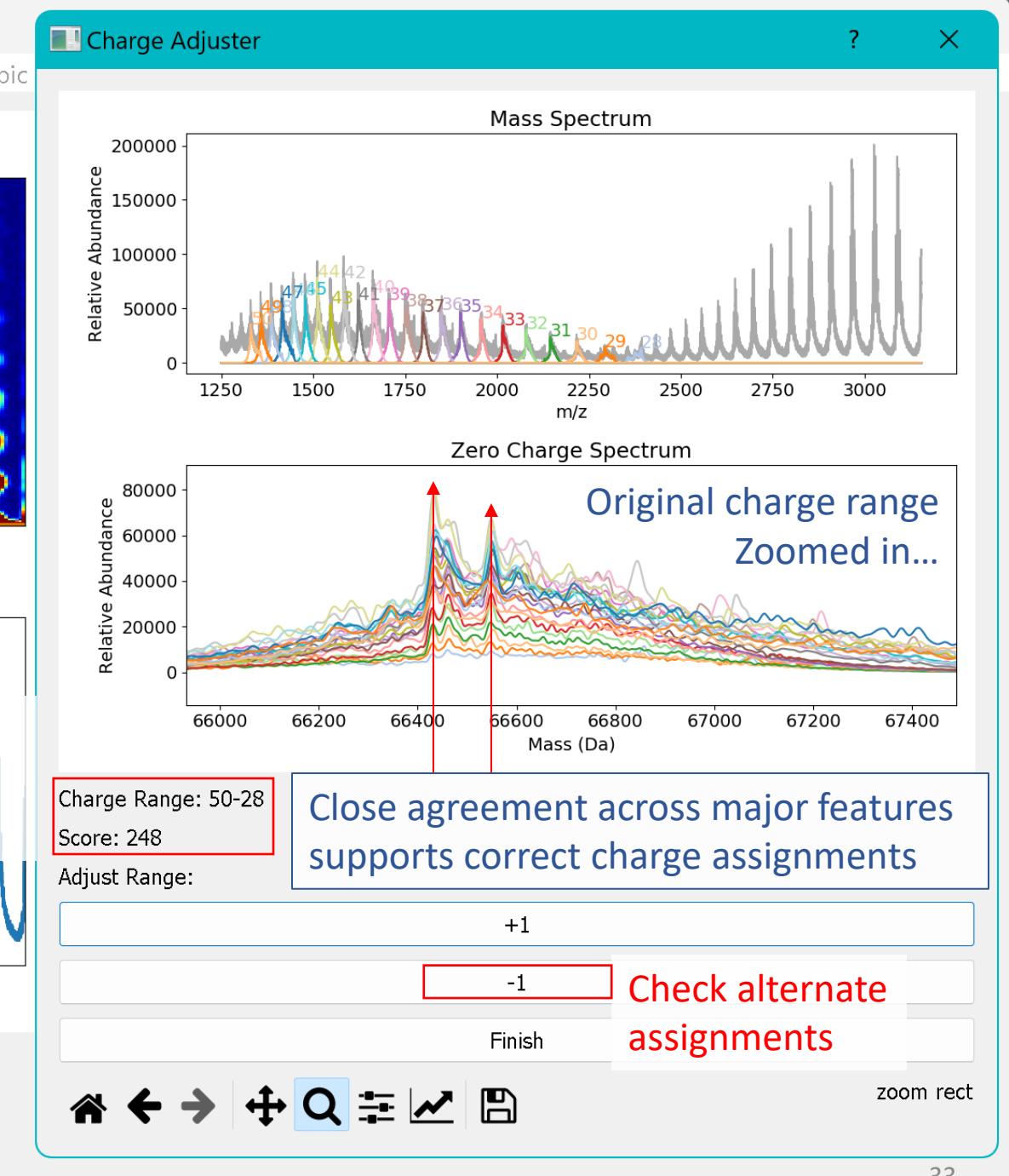
STFT DECONVOLUTION (18 of 27)

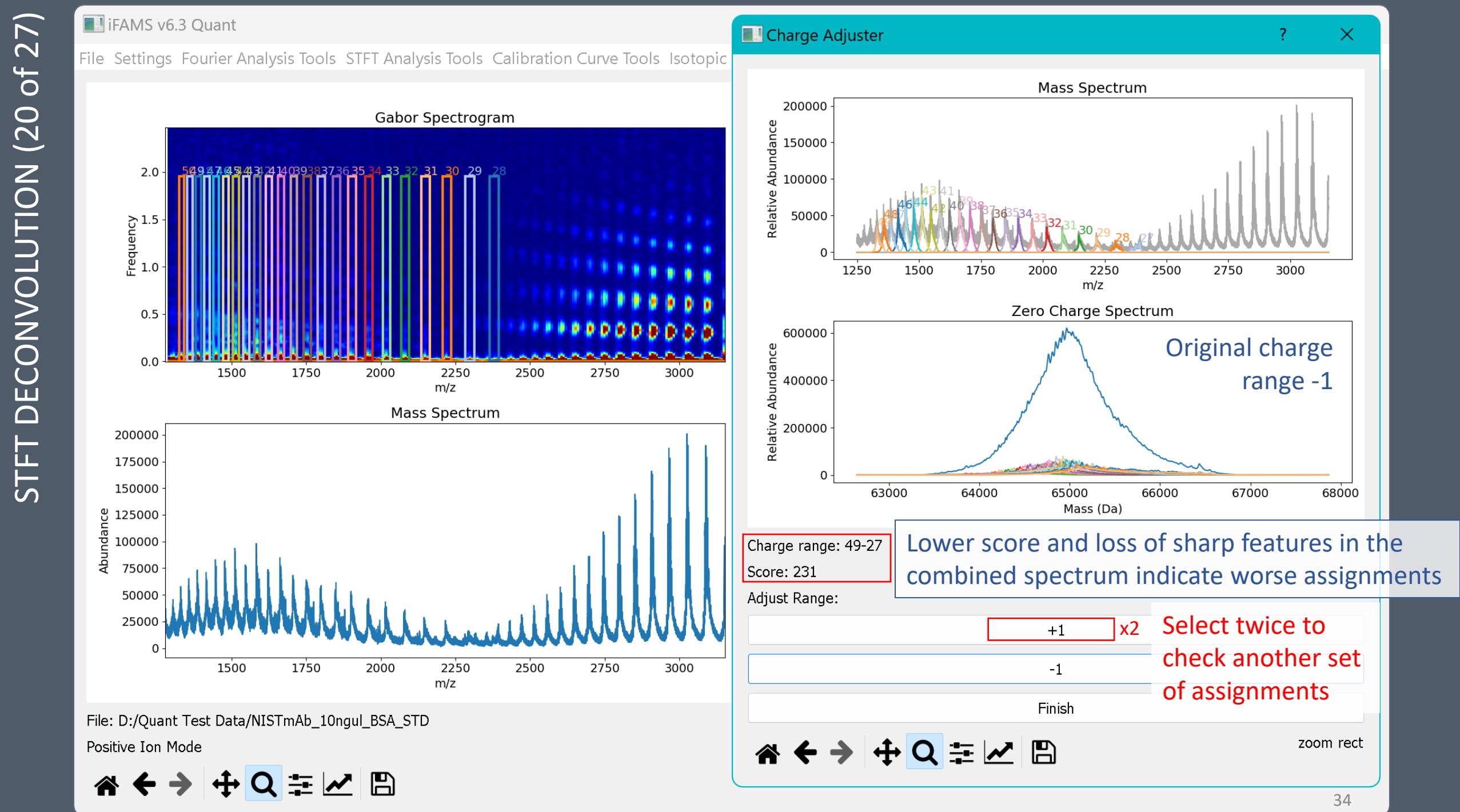


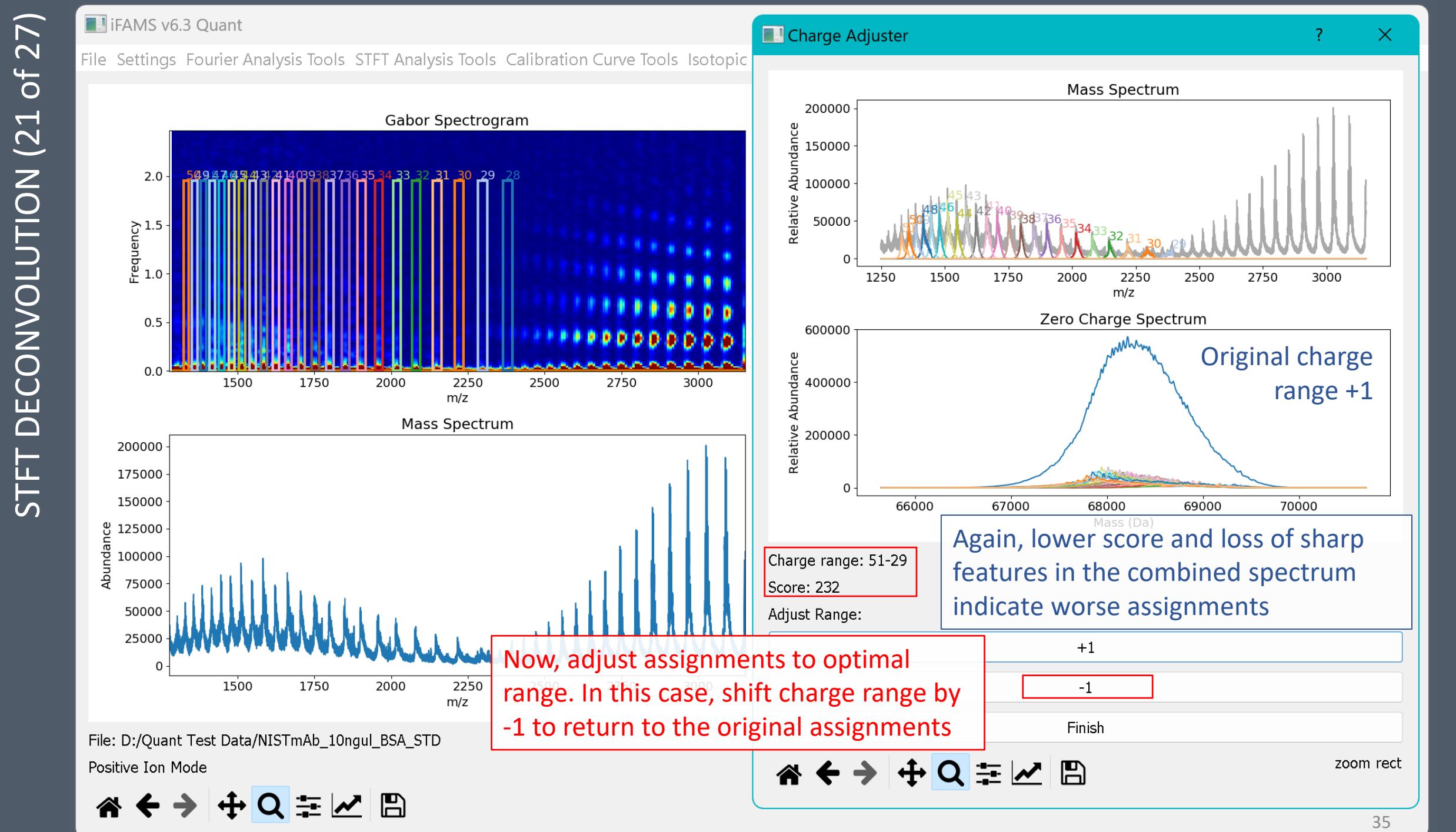


File: D:/Quant Test Data/NISTmAb_10ngul_BSA_STD

Positive Ion Mode







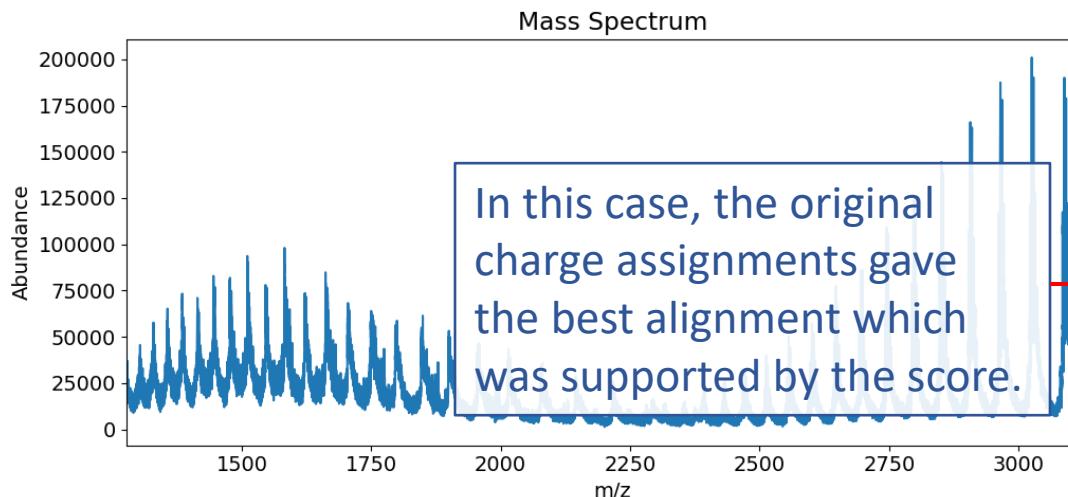
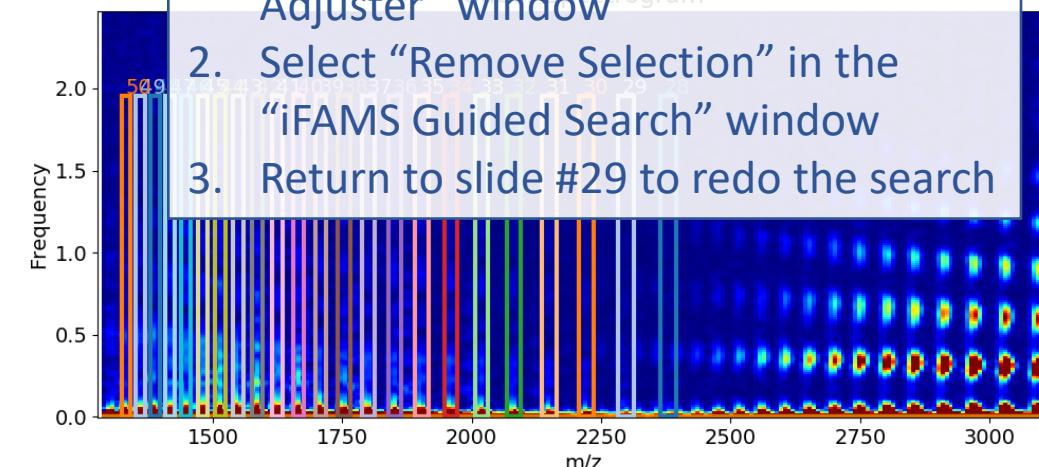
STFT DECONVOLUTION (22 of 27)

iFAMS v6.3 Quant

File Settings Fou

If no charge range seems to align peaks:

1. Select “Finish” in the “Charge Adjuster” window
2. Select “Remove Selection” in the “iFAMS Guided Search” window
3. Return to slide #29 to redo the search



File: D:/Quant Test Data/NISTmAb_10ngul_BSA_STD

Positive Ion Mode



Charge Adjuster

Mass Spectrum

Relative Abundance

m/z

Zero Charge Spectrum

Original charge range

Relative Abundance

Mass (Da)

Charge Range: 50-28

Score: 248

Adjust Range:

+1

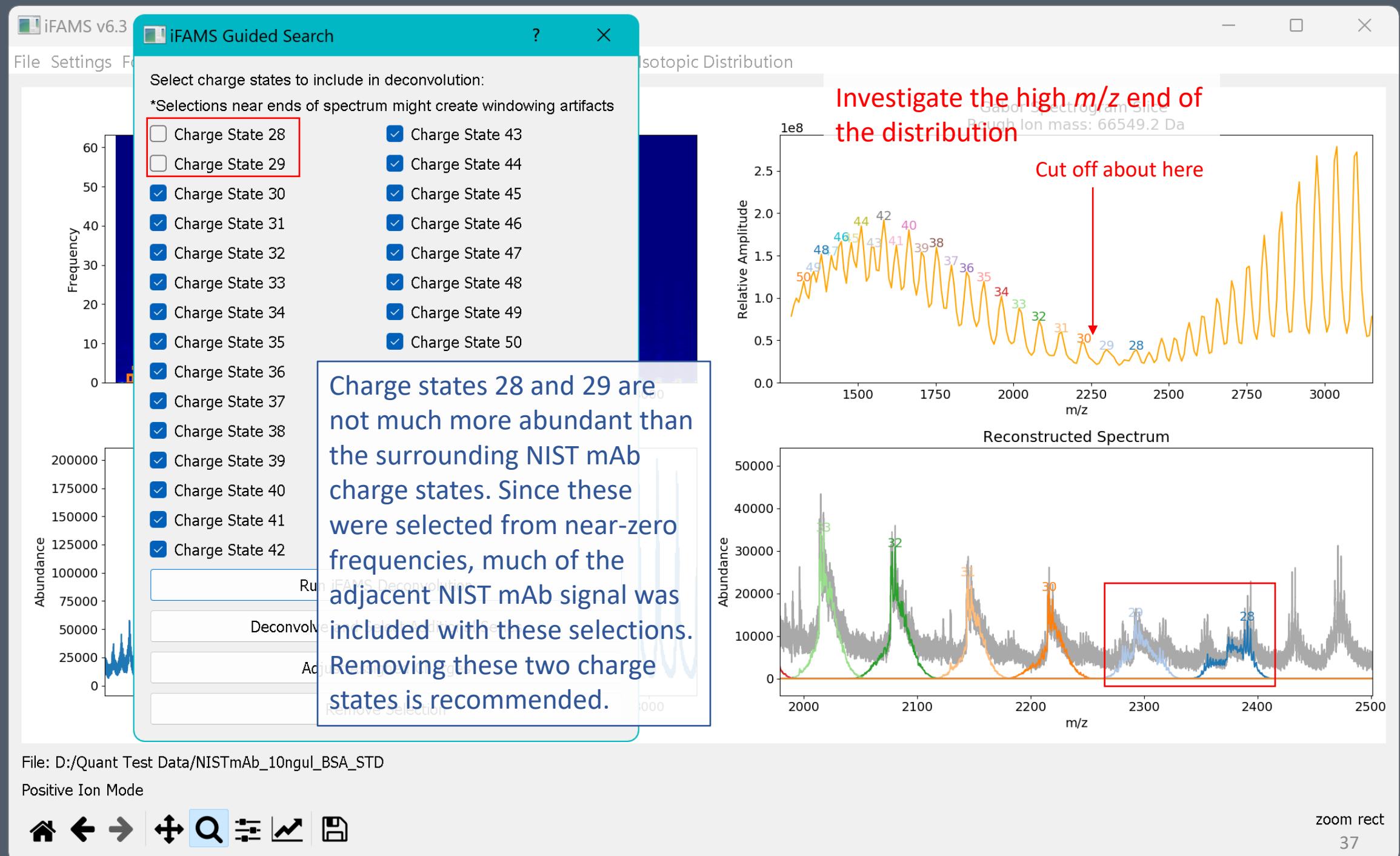
-1

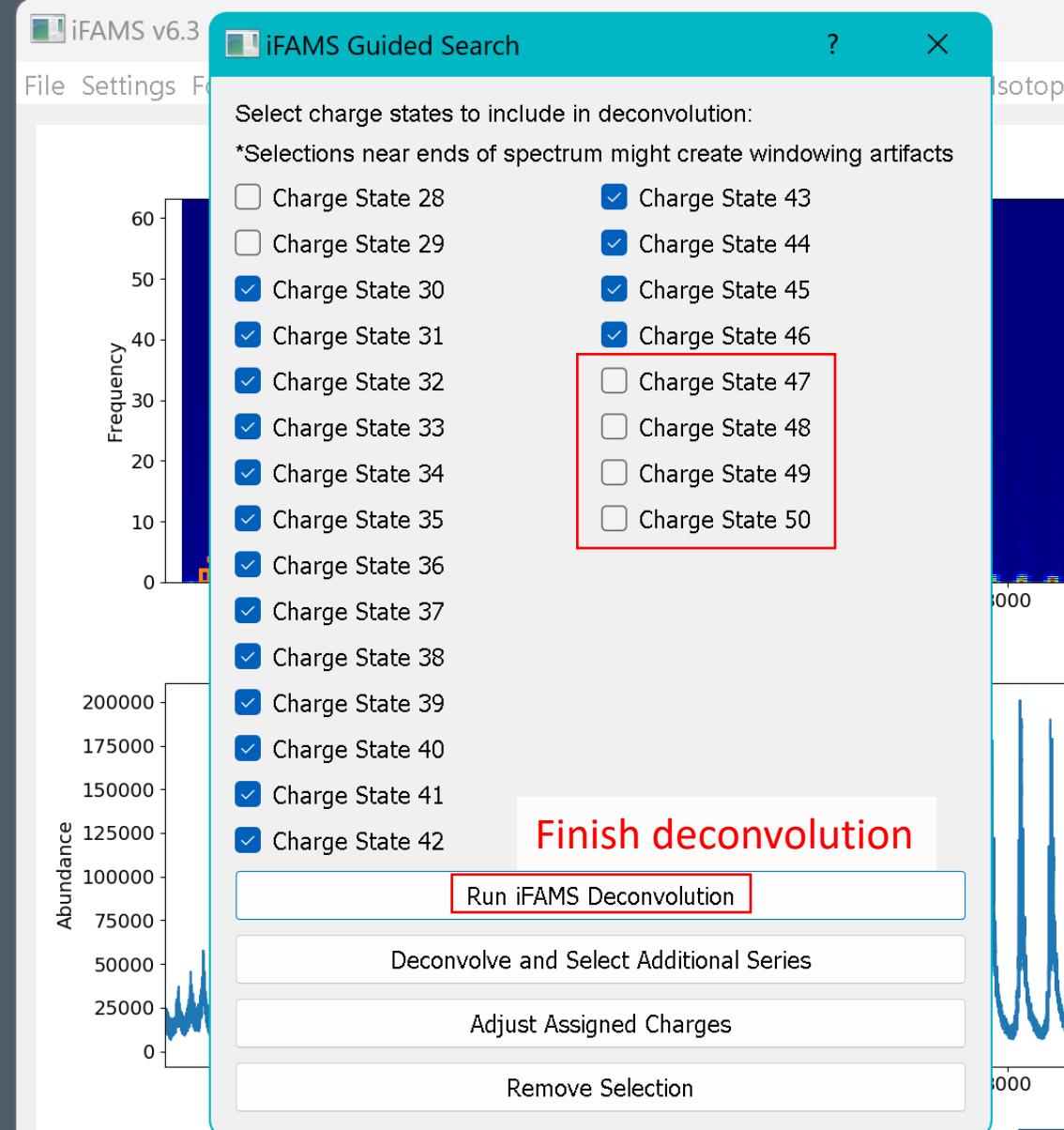
Select “Finish” to keep the current charge assignments

Finish

Home Back Forward Magnifying glass Histogram Save

36



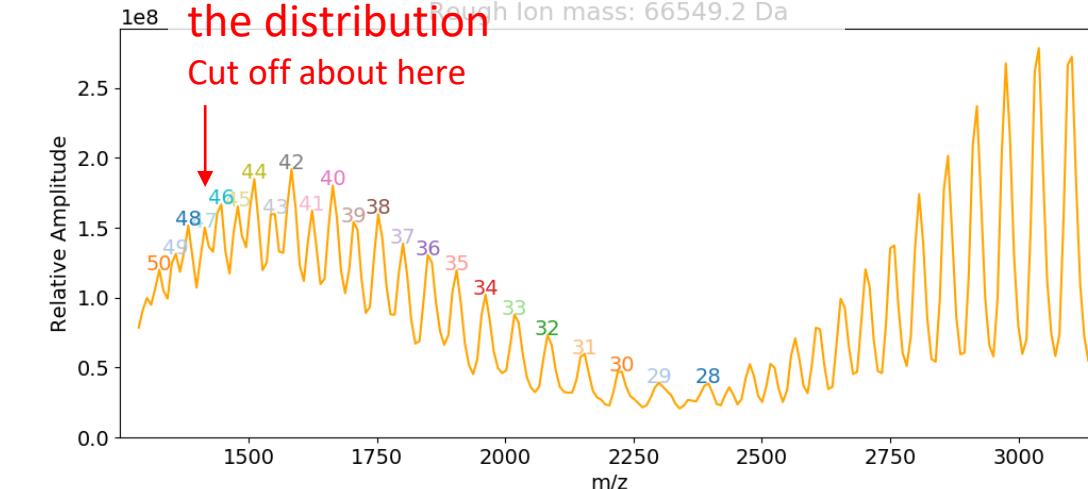


File: D:/Quant Test Data/NISTmAb_10ngul_BSA_ST

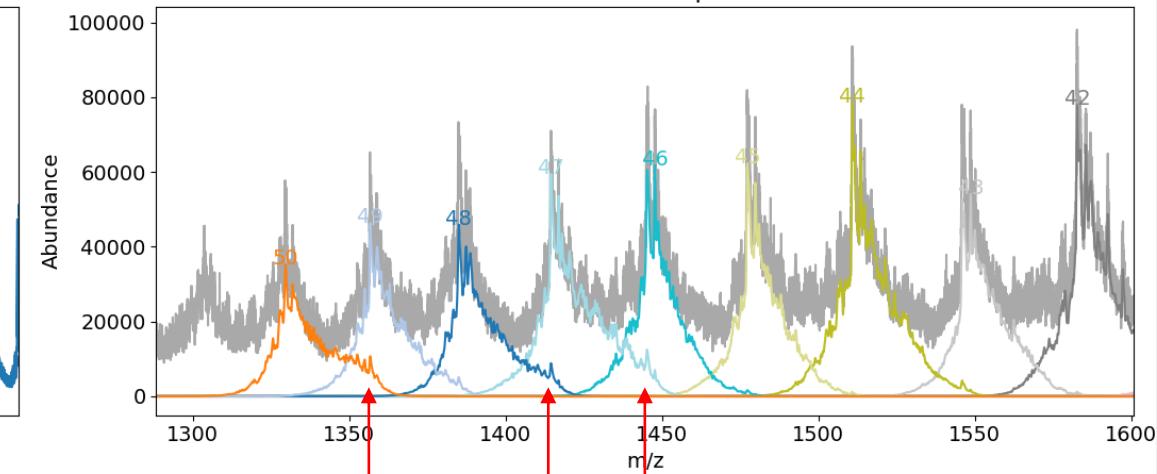
Positive Ion Mode

Investigate the low m/z end of the distribution

Cut off about here



Reconstructed Spectrum



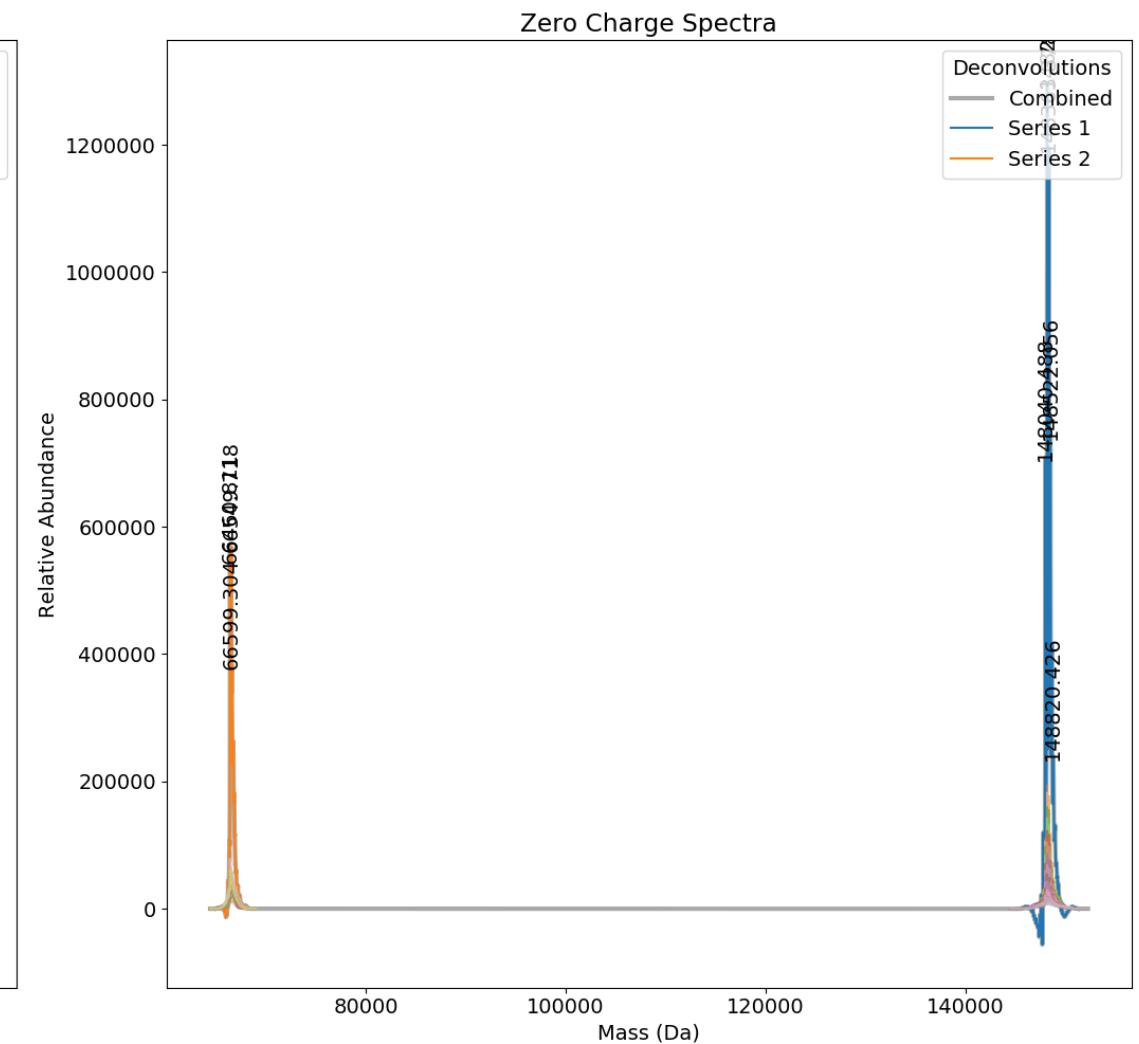
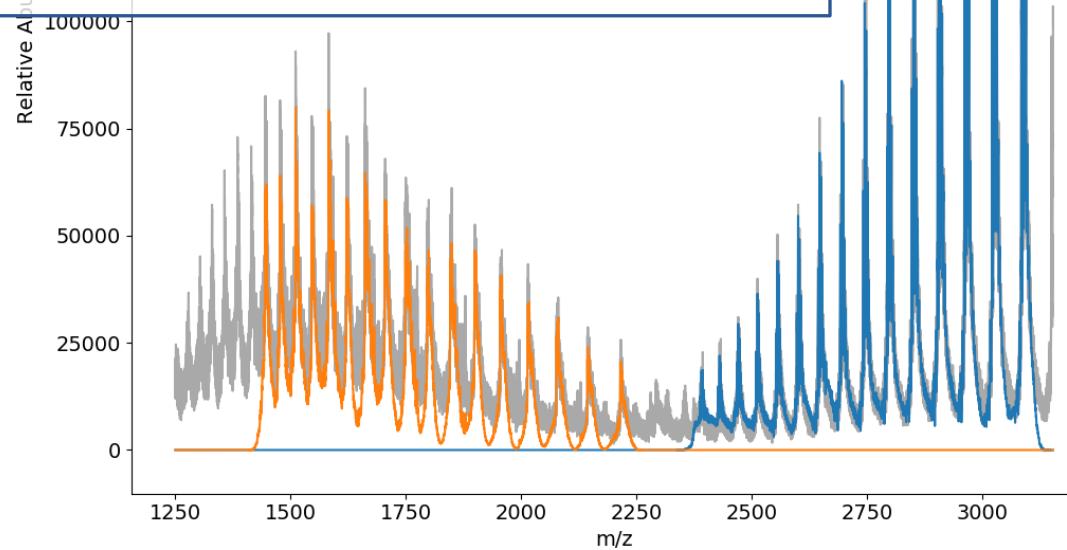
Watch out for overlapping selections at high charge states.



STFT DECONVOLUTION (25 of 27)

The iFAMS deconvolution corrects each inverse STFT spectra by the charge and sums them together for the zero-charge spectrum.

A baseline correction is applied to each combined zero-charge spectrum to account for artifacts that arise from the Gabor selection.

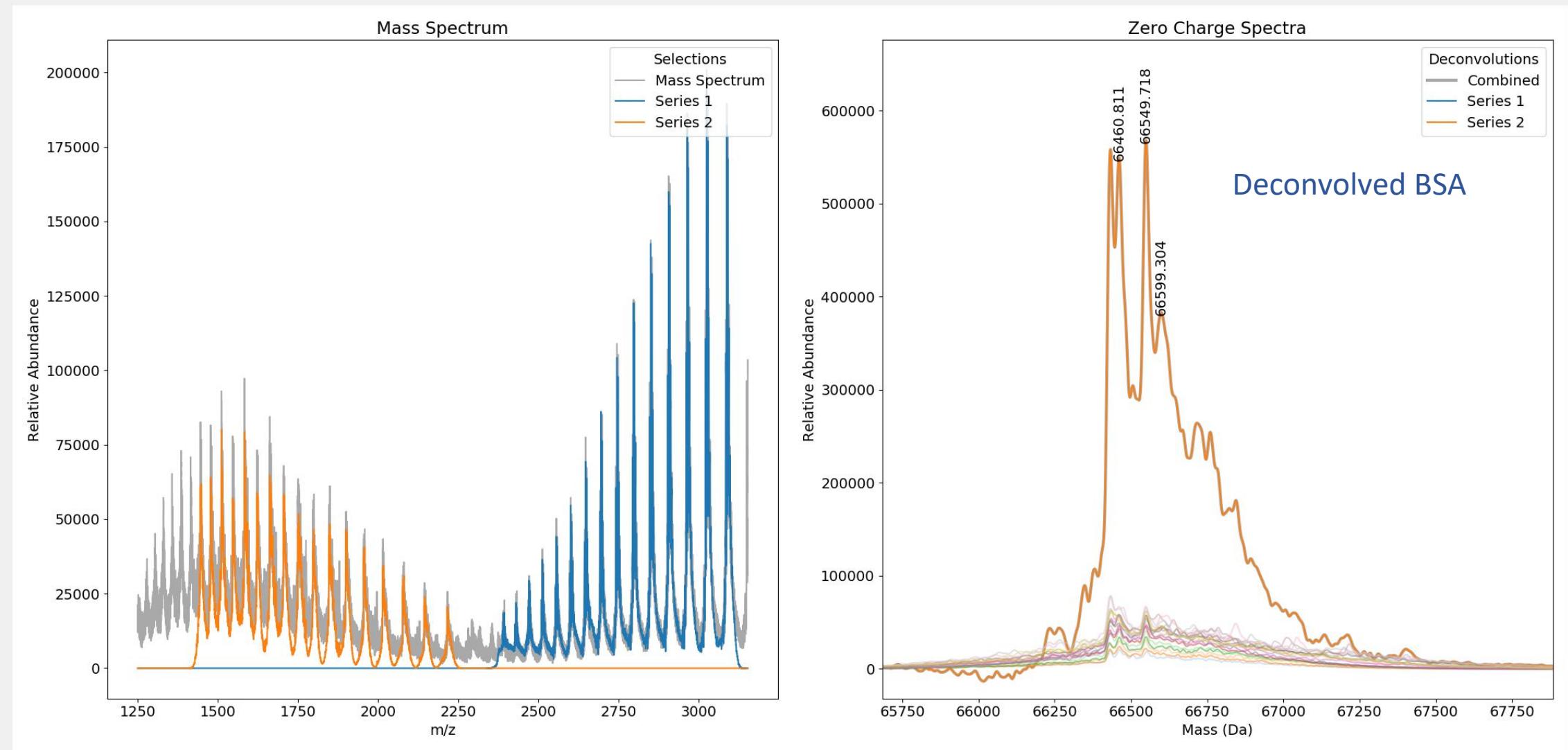


File: D:/Quant Test Data/NISTmAb_10ngul_BSA_STD

Positive Ion Mode



File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

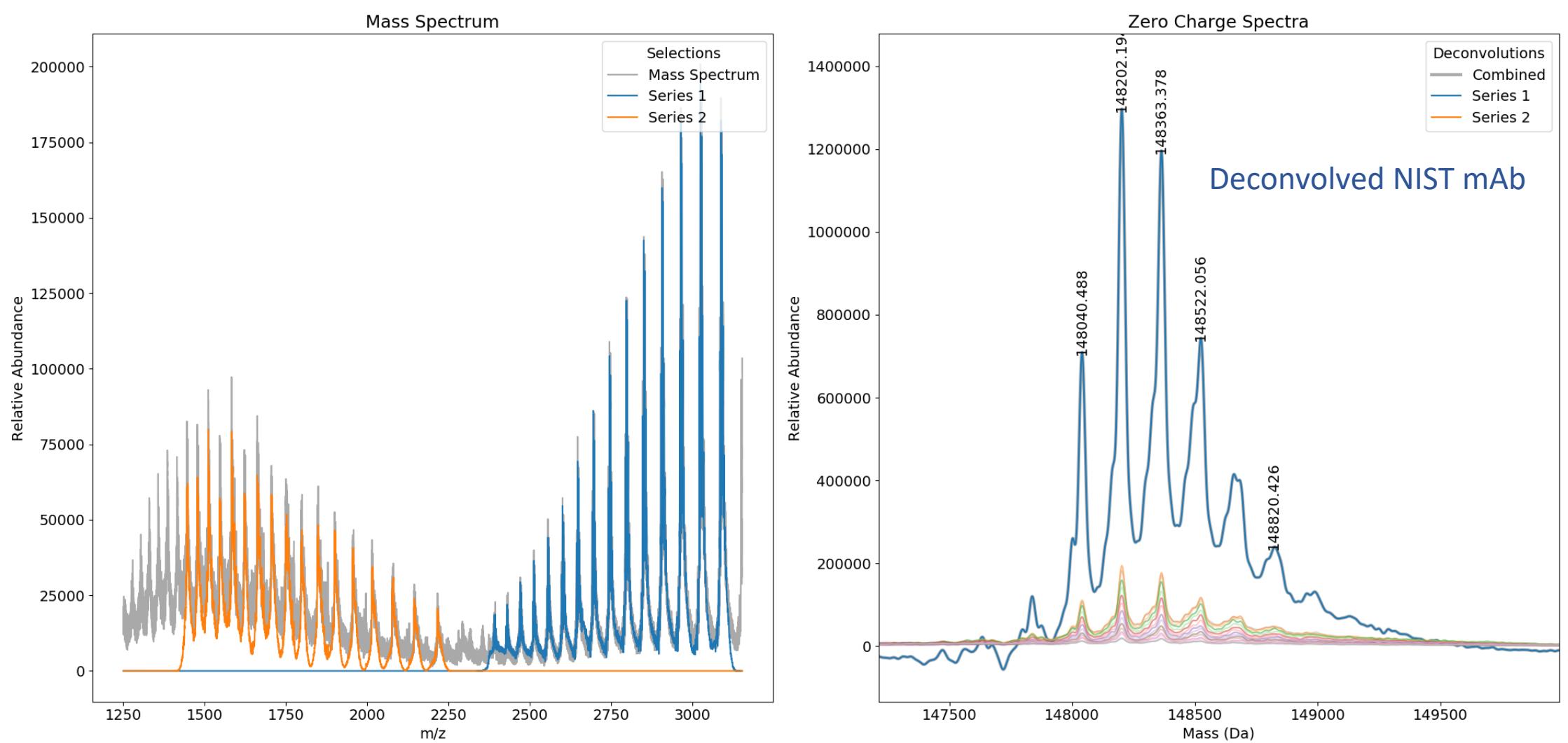


File: D:/Quant Test Data/NISTmAb_10ngul_BSA_STD

Positive Ion Mode



zoom rect
40

[File](#) [Settings](#) [Fourier Analysis Tools](#) [STFT Analysis Tools](#) [Calibration Curve Tools](#) [Isotopic Distribution](#)

File: D:/Quant Test Data/NISTmAb_10ngul_BSA_STD

Positive Ion Mode



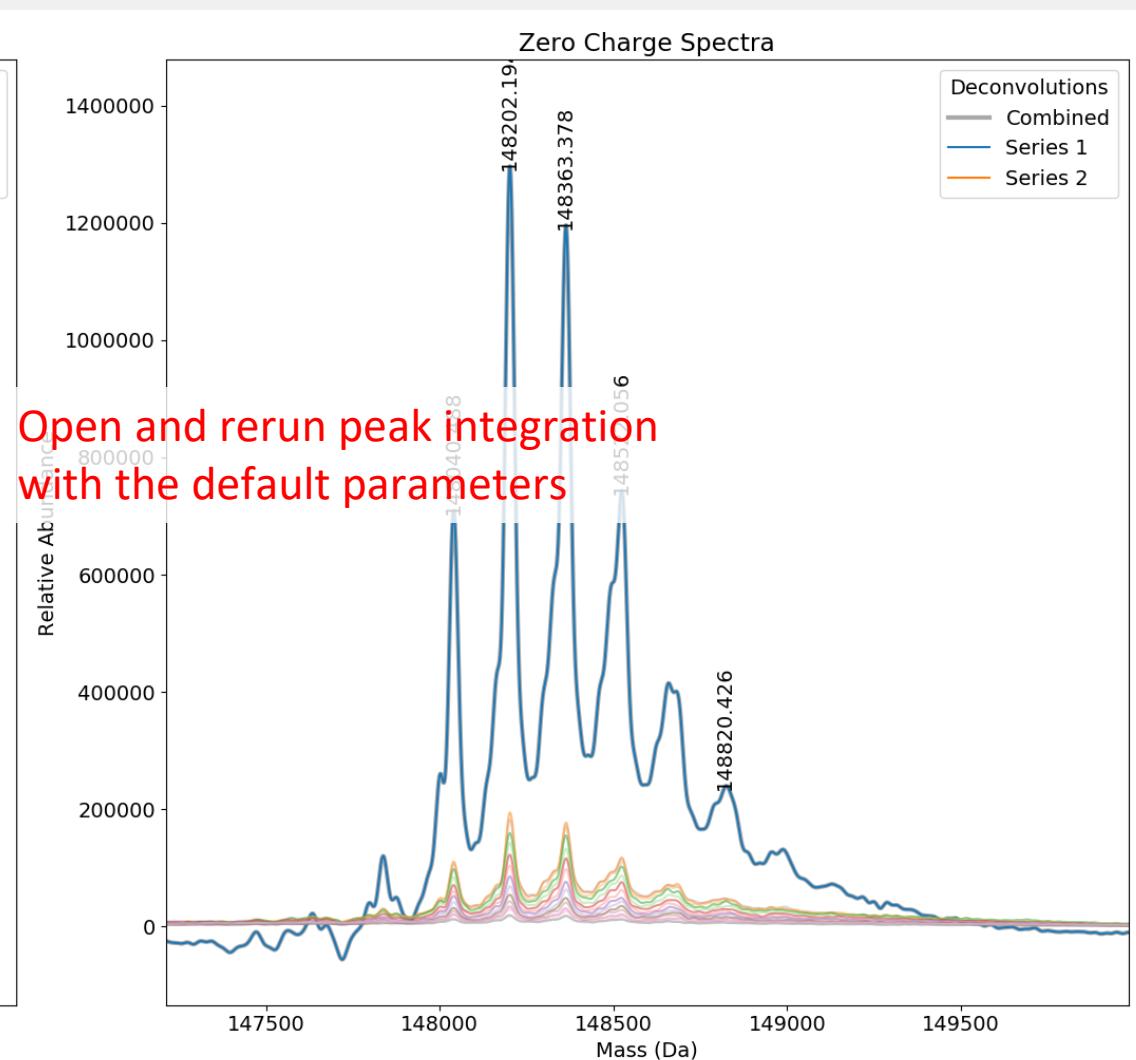
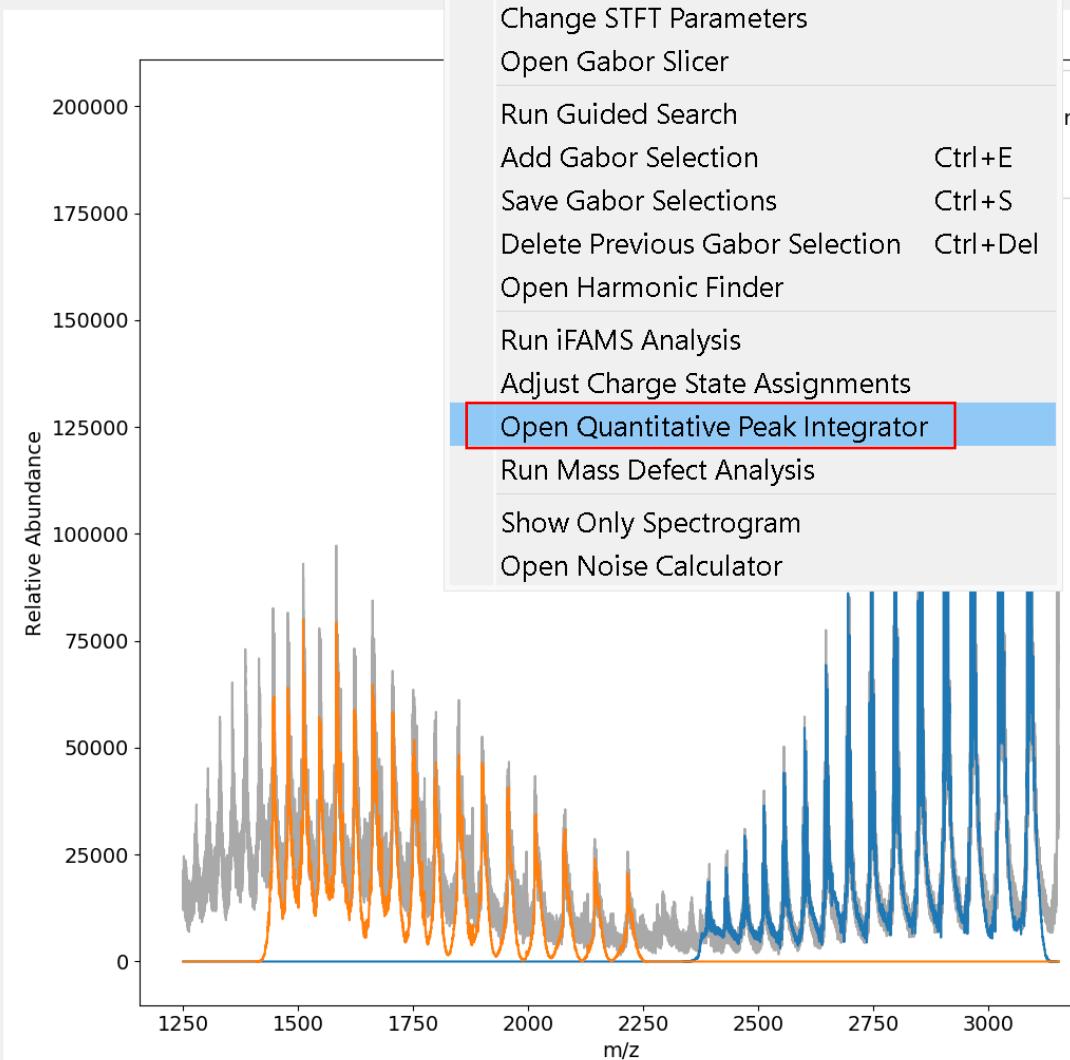
Quantitation and Calibration

Required data: NIST mAb series

- Peak integration options
- Batch deconvolution
- Calibration curve generation & optimization and concentration calculation

QUANTITATION AND CALIBRATION (1 of 25)

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

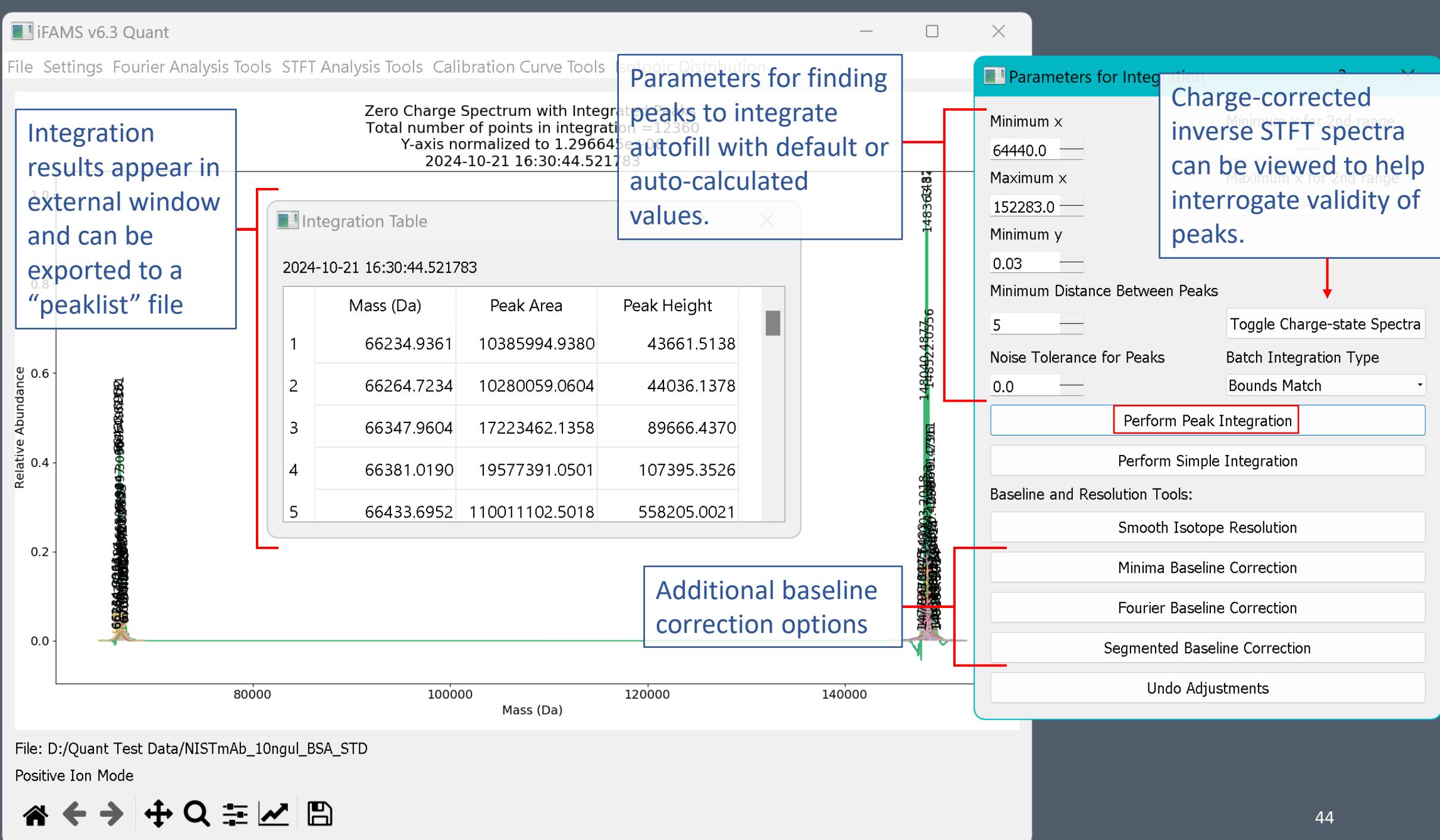


File: D:/Quant Test Data/NISTmAb_10ngul_BSA_STD

Positive Ion Mode



QUANTITATION AND CALIBRATION (2 of 25)



QUANTITATION AND CALIBRATION (3 of 25)

iFAMS v6.3 Quant

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

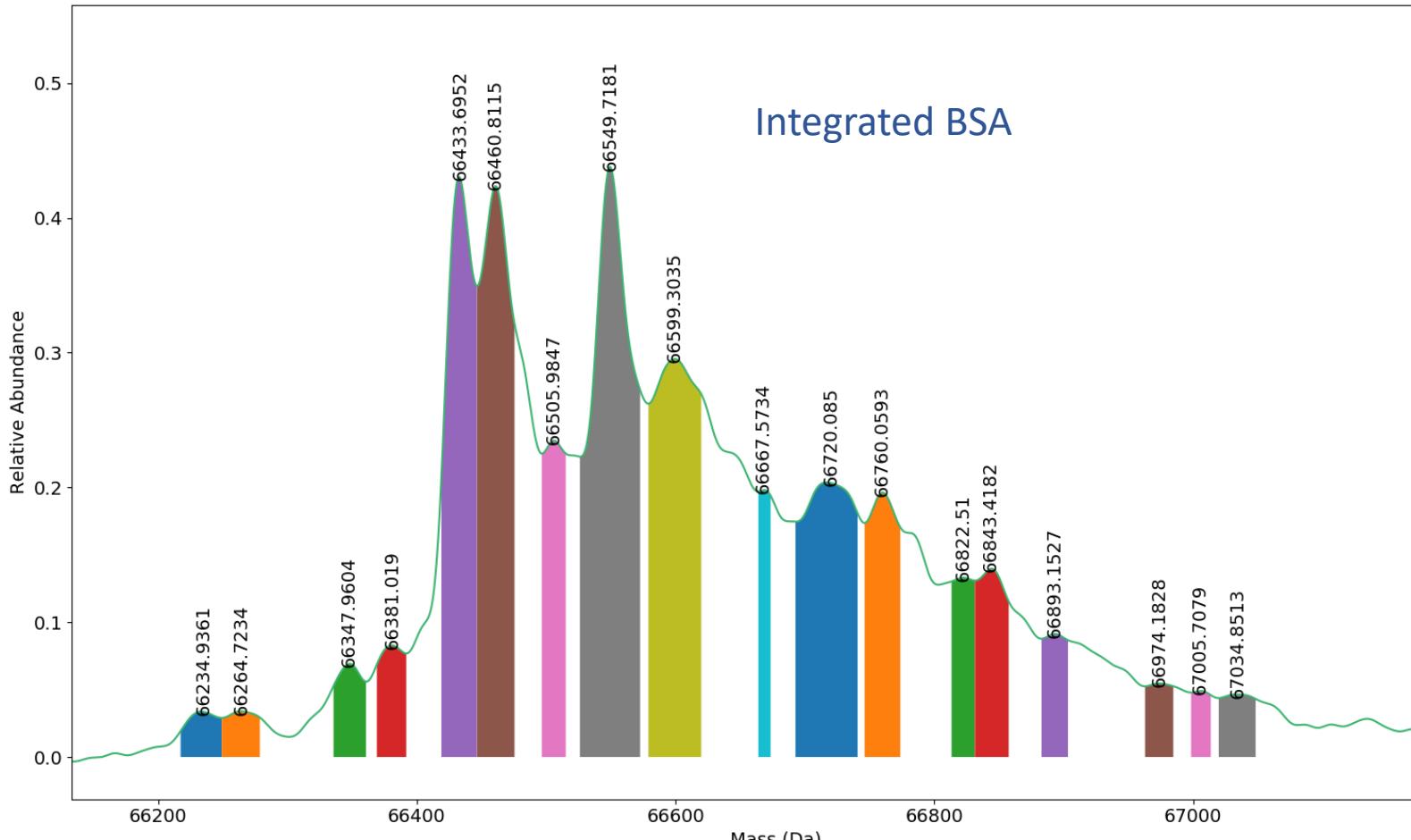
Parameters for Integration

?

X

Check integration results...

Zero Charge Spectrum with Integrated Peaks
Total number of points in integration = 12360
Y-axis normalized to 1.296645e+06



Minimum x

64440.0

Minimum x for 2nd range

—

Maximum x

152283.0

Maximum x for 2nd range

—

Minimum y

0.03

Minimum Distance Between Peaks

5

Toggle Charge-state Spectra

Noise Tolerance for Peaks

0.0

Batch Integration Type

Bounds Match

Perform Peak Integration

Perform Simple Integration

Baseline and Resolution Tools:

Smooth Isotope Resolution

Minima Baseline Correction

Fourier Baseline Correction

Segmented Baseline Correction

Undo Adjustments

File: D:/Quant Test Data/NISTmAb_10ng/uL_BSA_STD

Positive Ion Mode



Integration Table

?

X

2024-10-21 16:30:44.521783

Mass (Da)

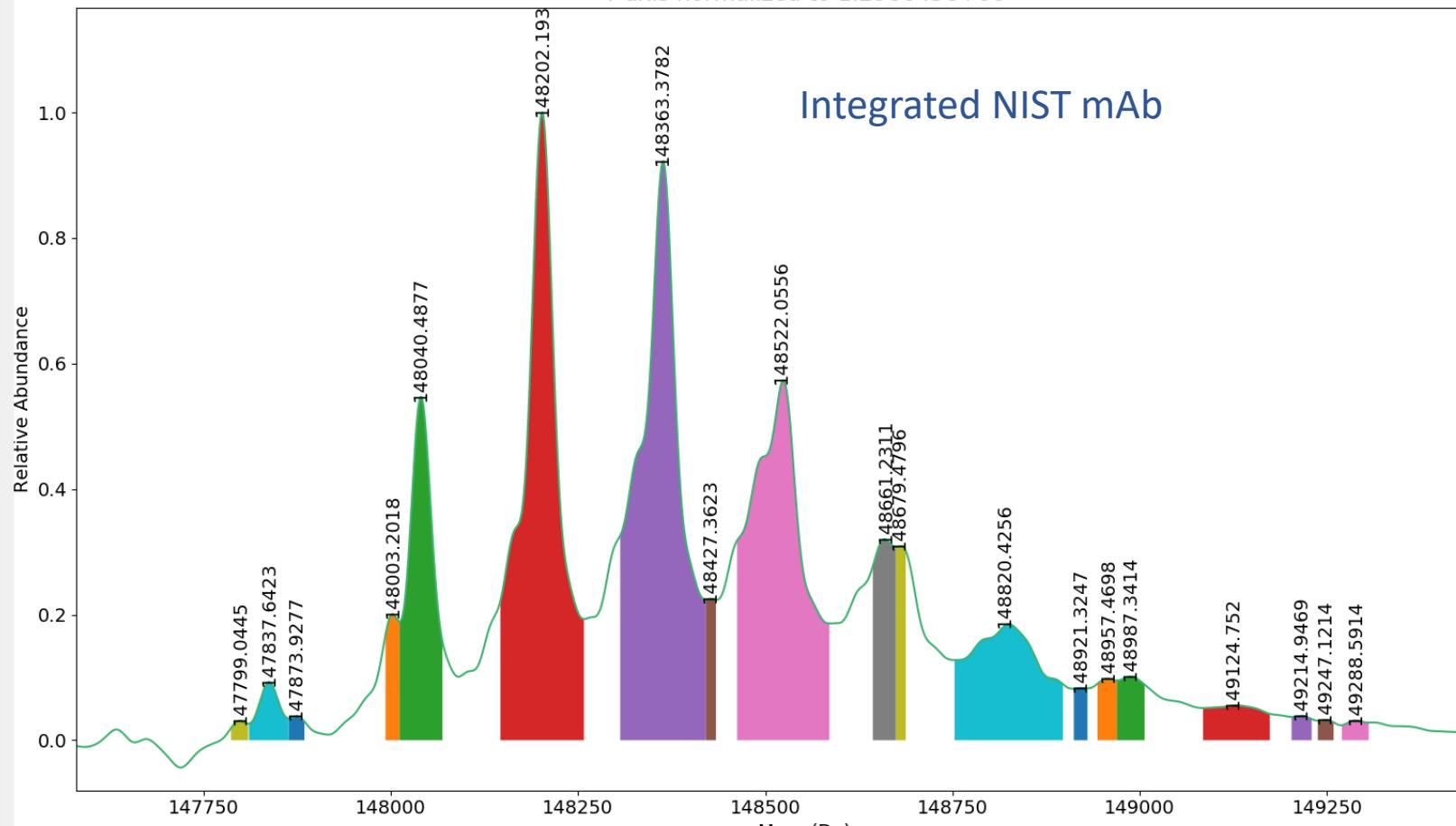
Peak Area

Peak Height

zoom rect

Check integration results...

Zero Charge Spectrum with Integrated Peaks
 Total number of points in integration =12360
 Y-axis normalized to 1.296645e+06



File: D:/Quant Test Data/NISTmAb_10ngul_BSA_STD

Positive Ion Mode



Parameters for Integration

Minimum x Minimum x for 2nd range

64440.0

Maximum x Maximum x for 2nd range

152283.0

Minimum y

0.03

Minimum Distance Between Peaks

5

Noise Tolerance for Peaks

0.0

Baseline and Resolution Tools:

Integration Table

2024-10-21 16:30:44.521783

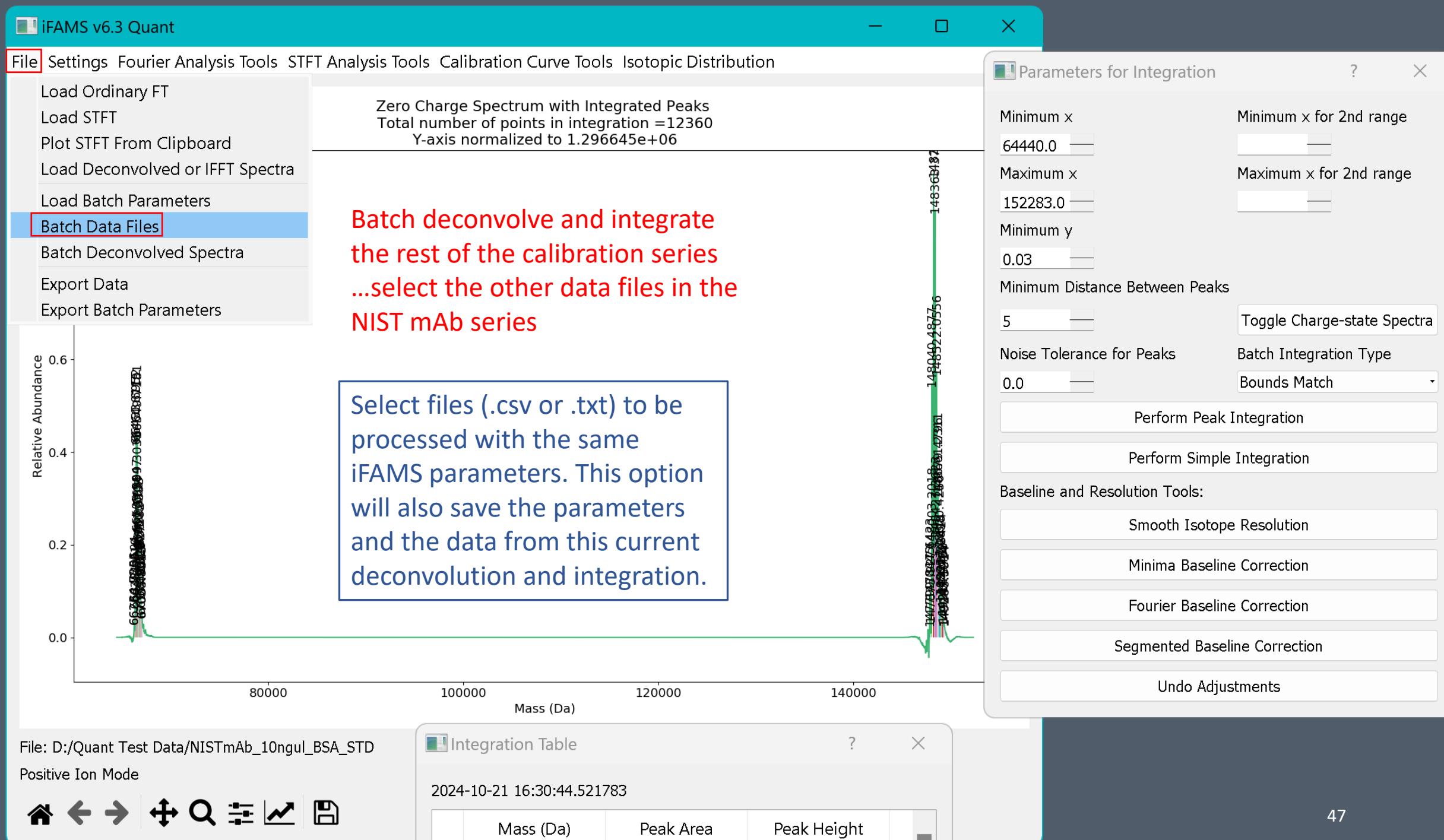
Mass (Da)

Peak Area

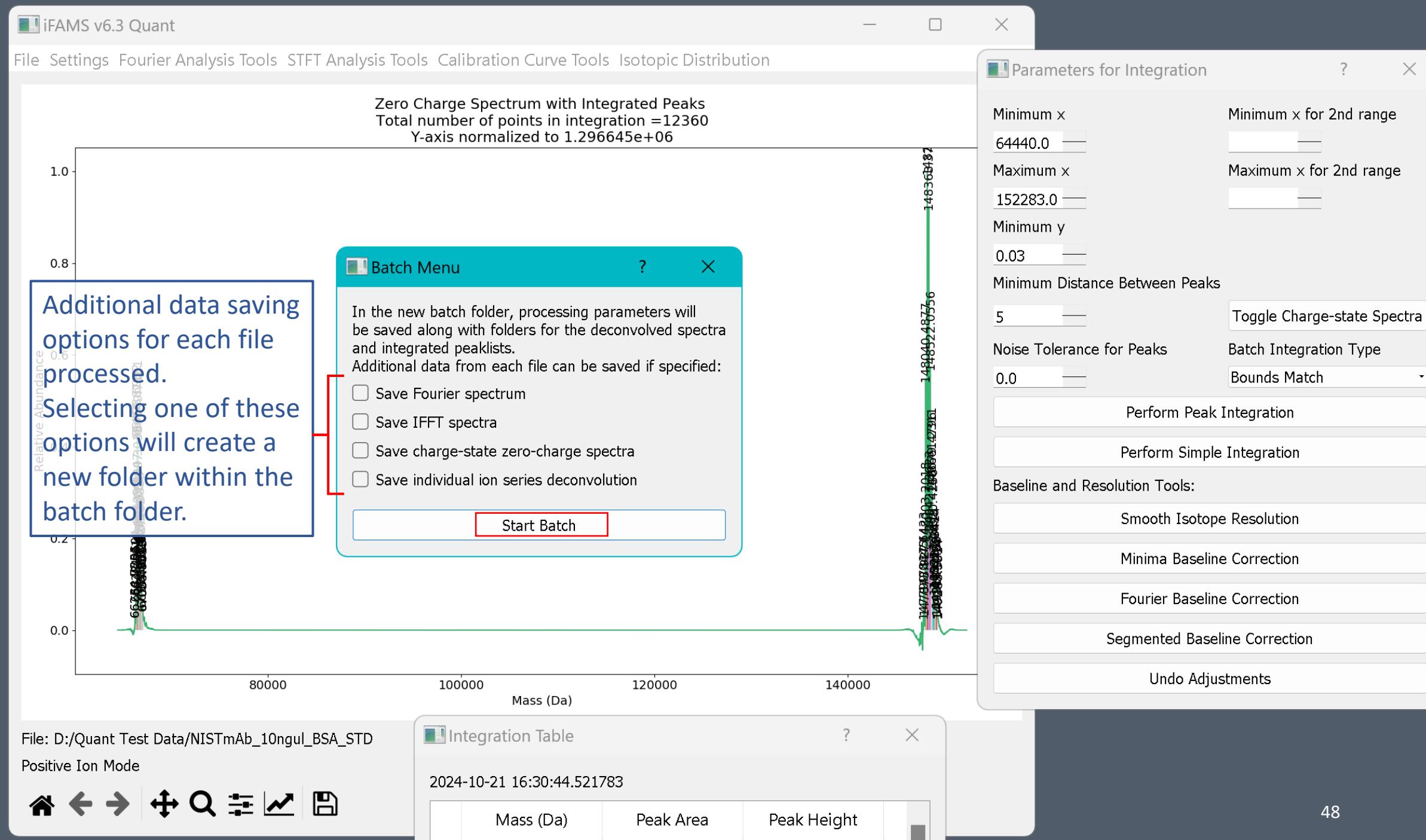
Peak Height

zoom rect

QUANTITATION AND CALIBRATION (5 of 25)



QUANTITATION AND CALIBRATION (6 of 25)



QUANTITATION AND CALIBRATION (7 of 25)

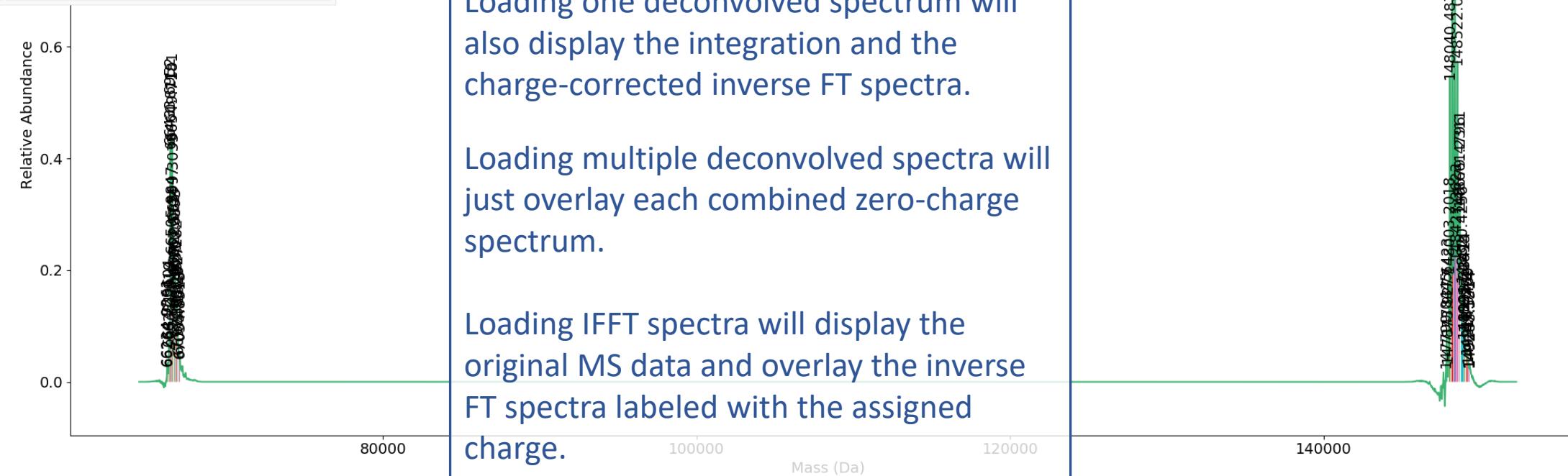
The screenshot shows the iFAMS v6.3 Quant software interface. At the top, there is a menu bar with options: File, Settings, Fourier Analysis Tools, STFT Analysis Tools, Calibration Curve Tools, and Isotopic Distribution. Below the menu is a toolbar with various icons. The main area is a file browser window titled "241021_Batch1". The path in the address bar is: DATA (D:) > Quant Test Data > 241021_Batch1. The search bar contains the text "Search 241021_Batch1". The browser includes standard navigation buttons (back, forward, up, down) and a refresh button. Below the address bar are buttons for New, Cut, Copy, Paste, Find, Delete, Sort, View, and Details. The file list table has columns for Name, Date modified, Type, and Size. The table shows the following items:

Name	Date modified	Type	Size
Deconvolved_spectra	10/21/2024 4:59 PM	File folder	
Peaklists	10/21/2024 4:59 PM	File folder	
241021_batch_params_from_NISTmAb_10ngul_BSA_STD	10/21/2024 4:56 PM	Microsoft Excel Co...	28 KB

In the left sidebar, the "DATA (D:)" drive is selected. Other drives listed are "Creative Cloud Files", "This PC", "Windows (C:)", "Data Pot (E:)", "Secondary SSD (S:)", and "Data Pot (E:)". A note in the center of the screen states: "The batch folder is created in the same location as the processed file and labeled with the date and batch number."

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

- Load Ordinary FT
- Load STFT
- Plot STFT From Clipboard
- Load Deconvolved or IFFT Spectra**
- Load Batch Parameters
- Batch Data Files
- Batch Deconvolved Spectra
- Export Data
- Export Batch Parameters

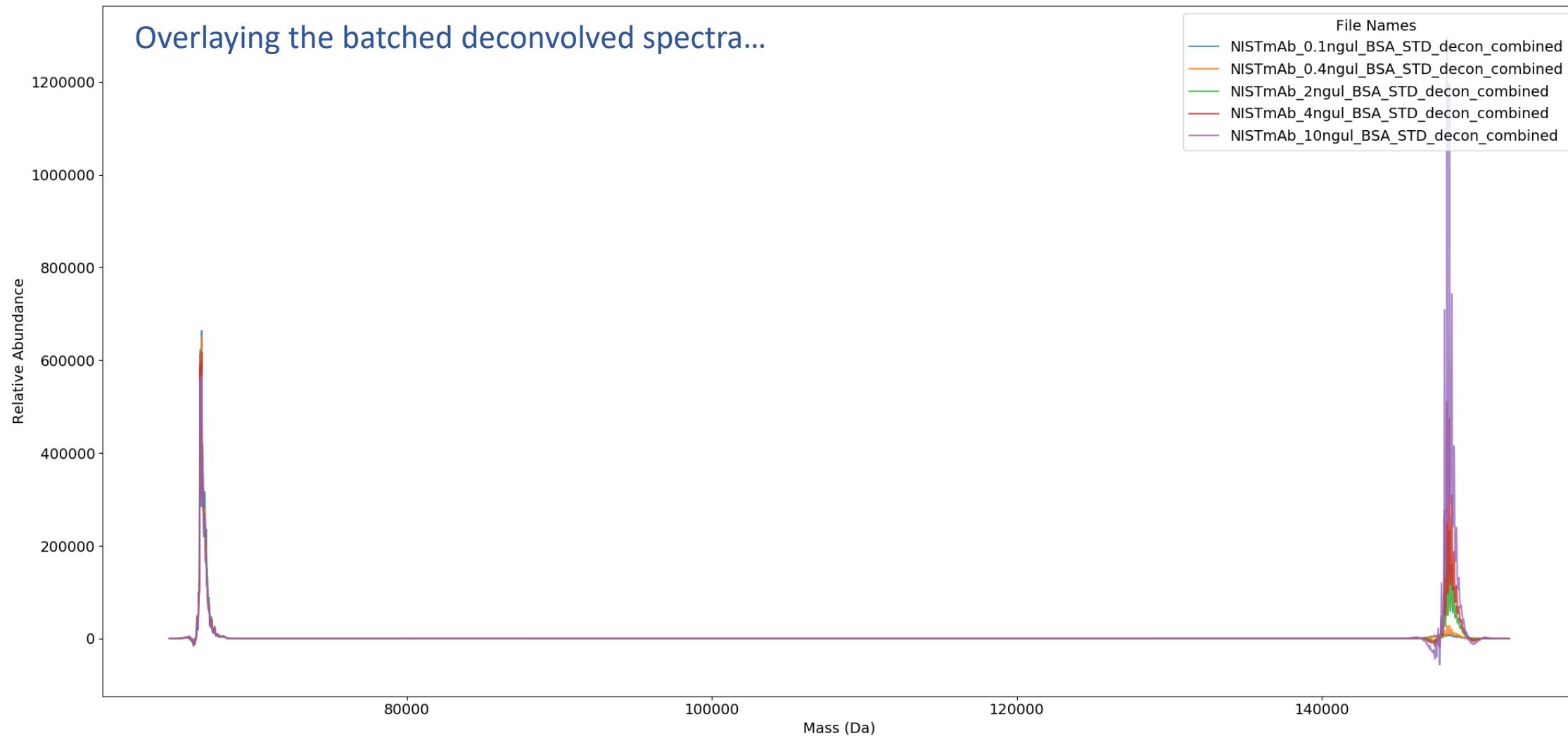


File: D:/Quant Test Data/NISTmAb_10ng/uL_BSA_STD

Positive Ion Mode

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

Overlaying the batched deconvolved spectra...



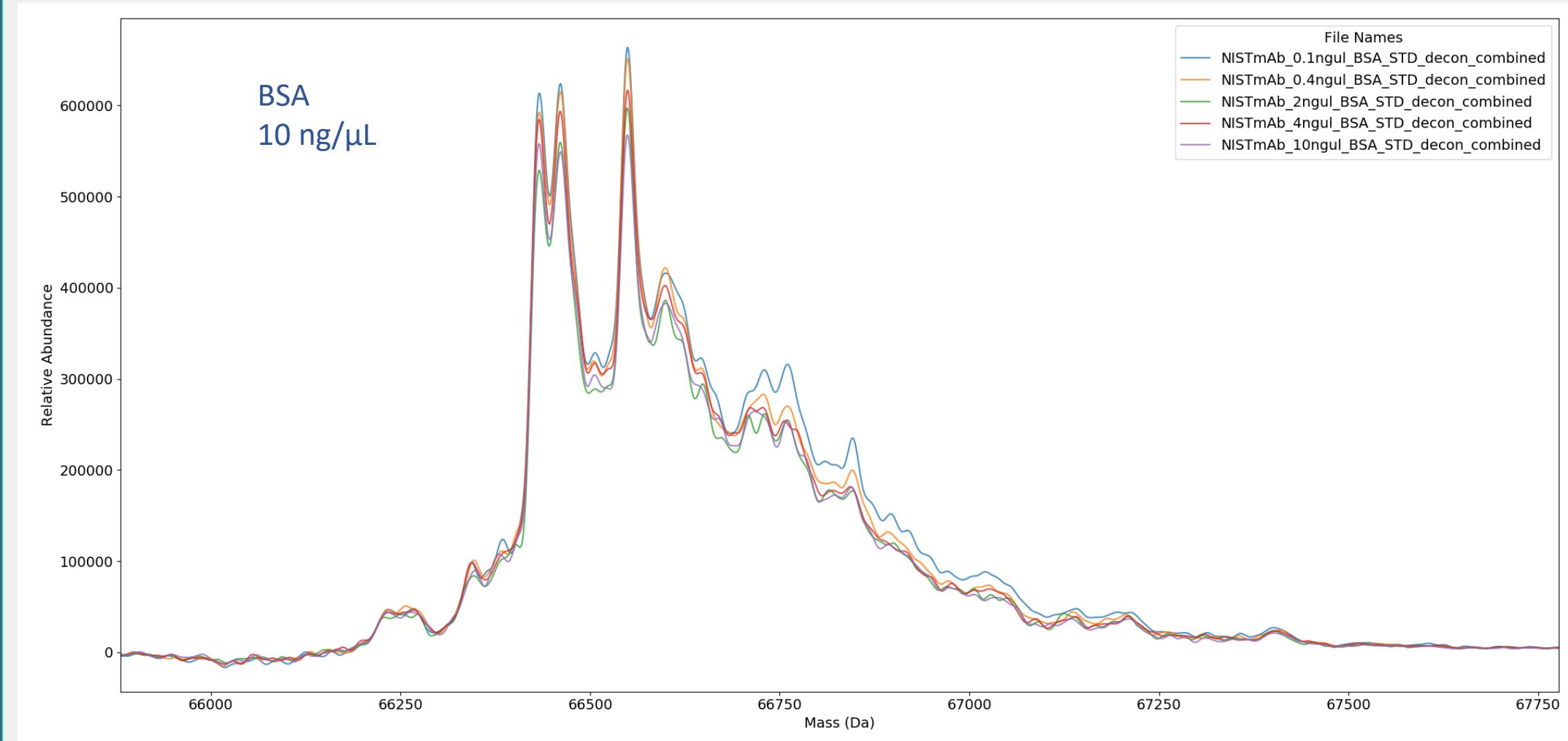
File: D:/Quant Test Data/241021_Batch1/Deconvolved_spectra/NISTmAb_0.1ngul_BSA_STD_decon_combined

Positive Ion Mode



zoom rect
51

QUANTITATION AND CALIBRATION (10 of 25)

[File](#) [Settings](#) [Fourier Analysis Tools](#) [STFT Analysis Tools](#) [Calibration Curve Tools](#) [Isotopic Distribution](#)

File: D:/Quant Test Data/241021_Batch1/Deconvoluted_spectra/NISTmAb_0.1ngul_BSA_STD_decon_combined

Positive Ion Mode



zoom rect

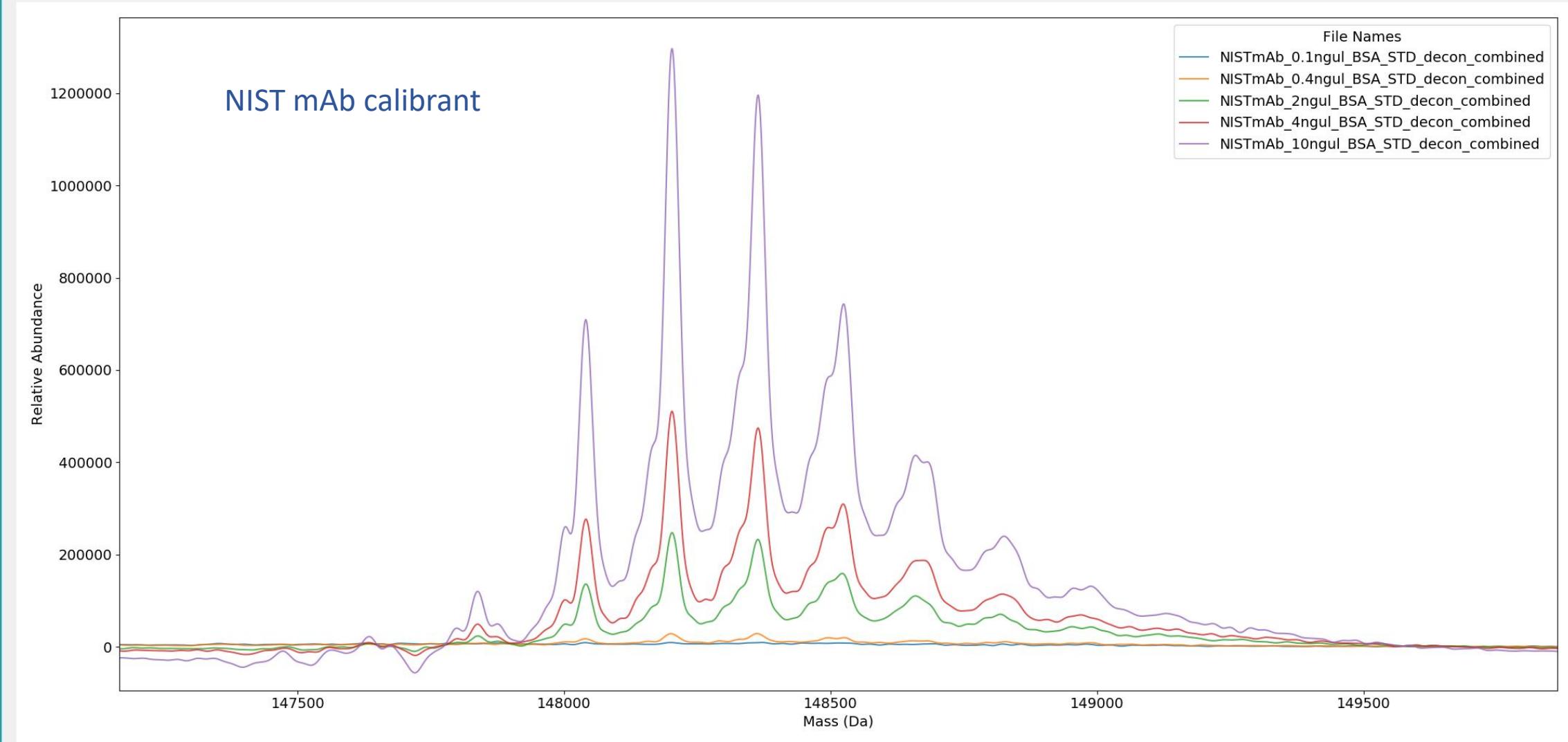
52

QUANTITATION AND CALIBRATION (11 of 25)

iFAMS v6.3 Quant

- X

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution



File: D:/Quant Test Data/241021_Batch1/Deconvolved_spectra/NISTmAb_0.1ngul_BSA_STD_decon_combined

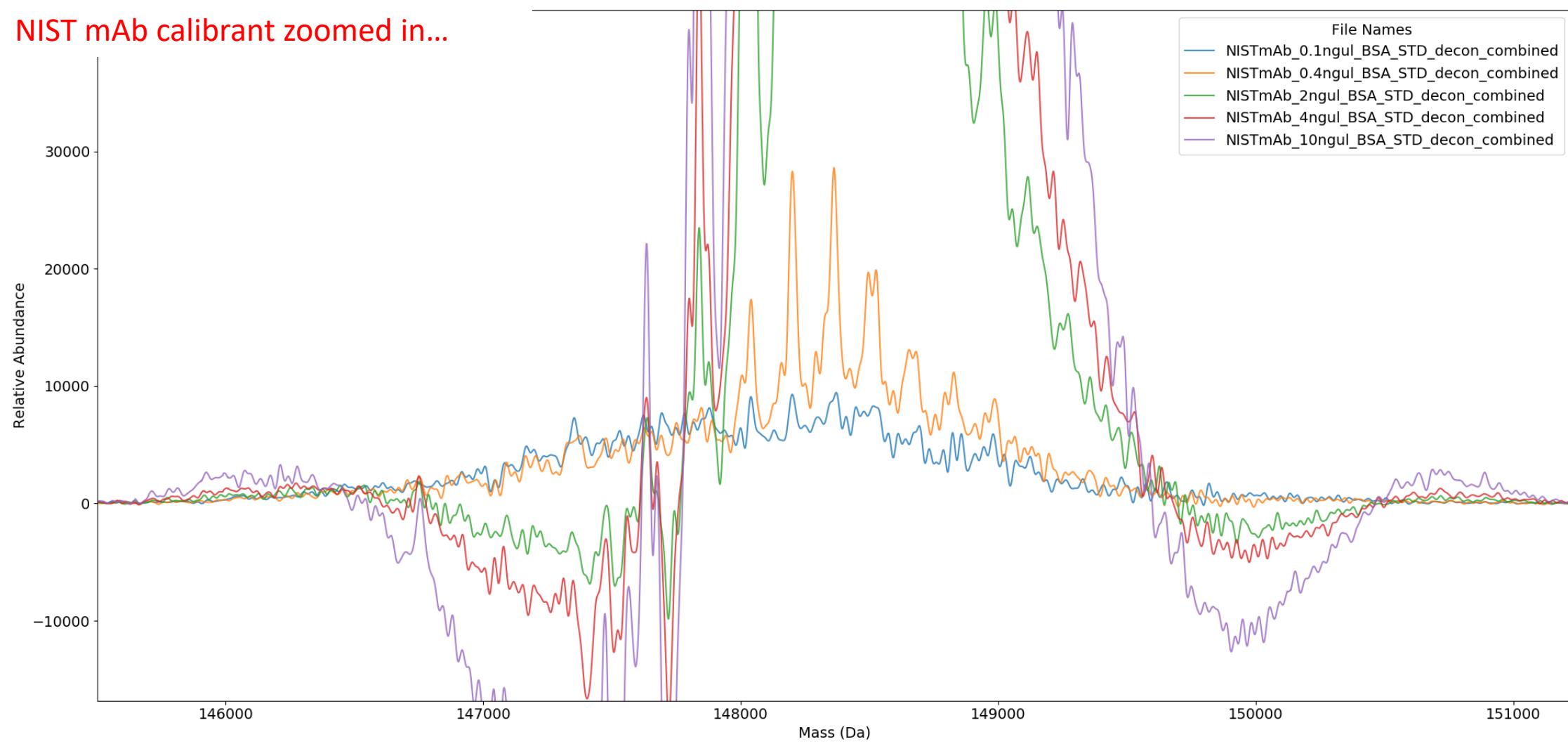
Positive Ion Mode



zoom rect
53

QUANTITATION AND CALIBRATION (12 of 25)

NIST mAb calibrant zoomed in...



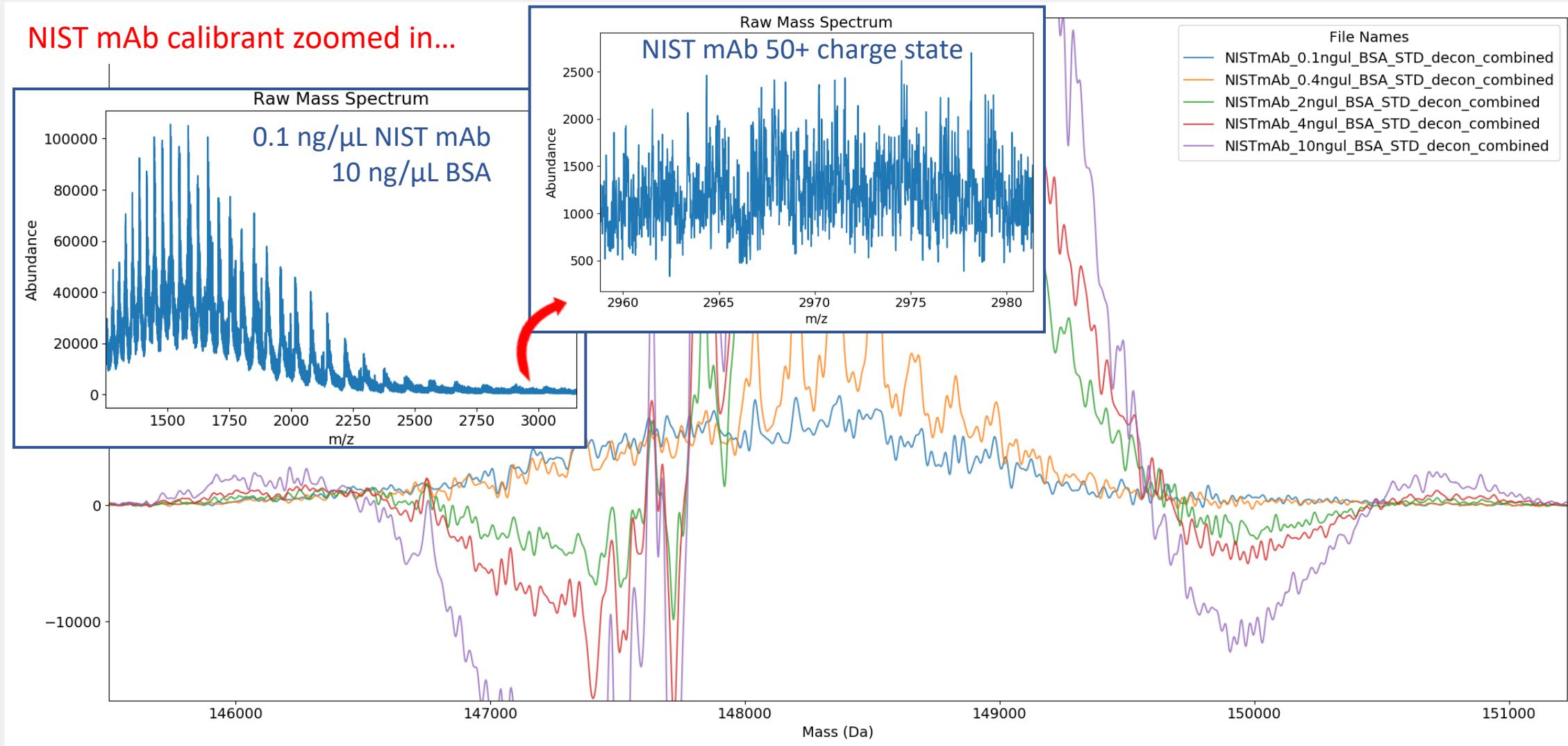
File: D:/Quant Test Data/241021_Batch1/Deconvolved_spectra/NISTmAb_0.1ngul_BSA_STD_decon_combined

Positive Ion Mode



zoom rect
54

QUANTITATION AND CALIBRATION (13 of 25)



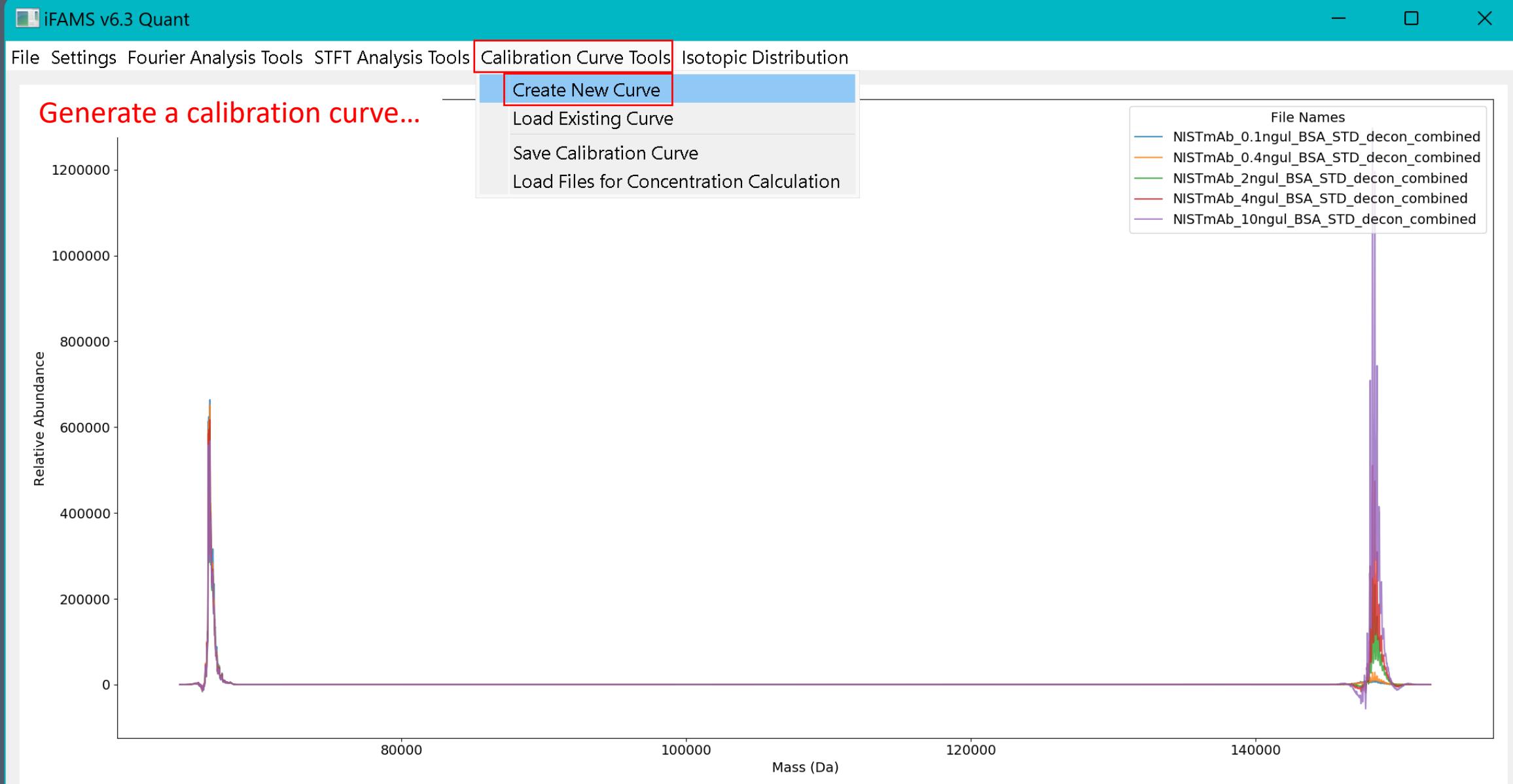
File: D:/Quant Test Data/241021_Batch1/Deconvolved_spectra/NISTmAb_0.1ngul_BSA_STD_decon_combined

Positive Ion Mode



zoom rect
55

QUANTITATION AND CALIBRATION (14 of 25)



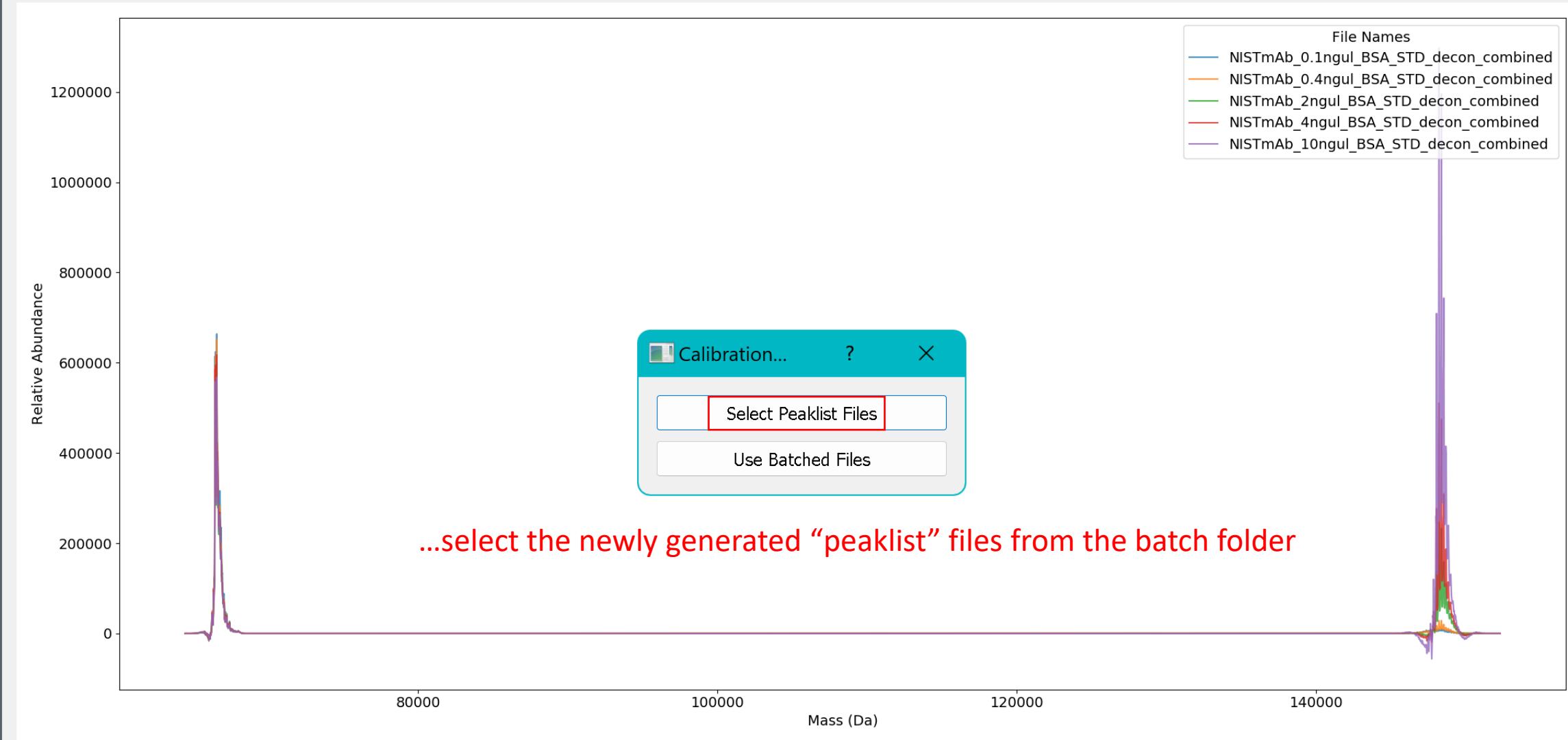
File: D:/Quant Test Data/241021_Batch1/Deconvolved_spectra/NISTmAb_0.1ngul_BSA_STD_decon_combined

Positive Ion Mode

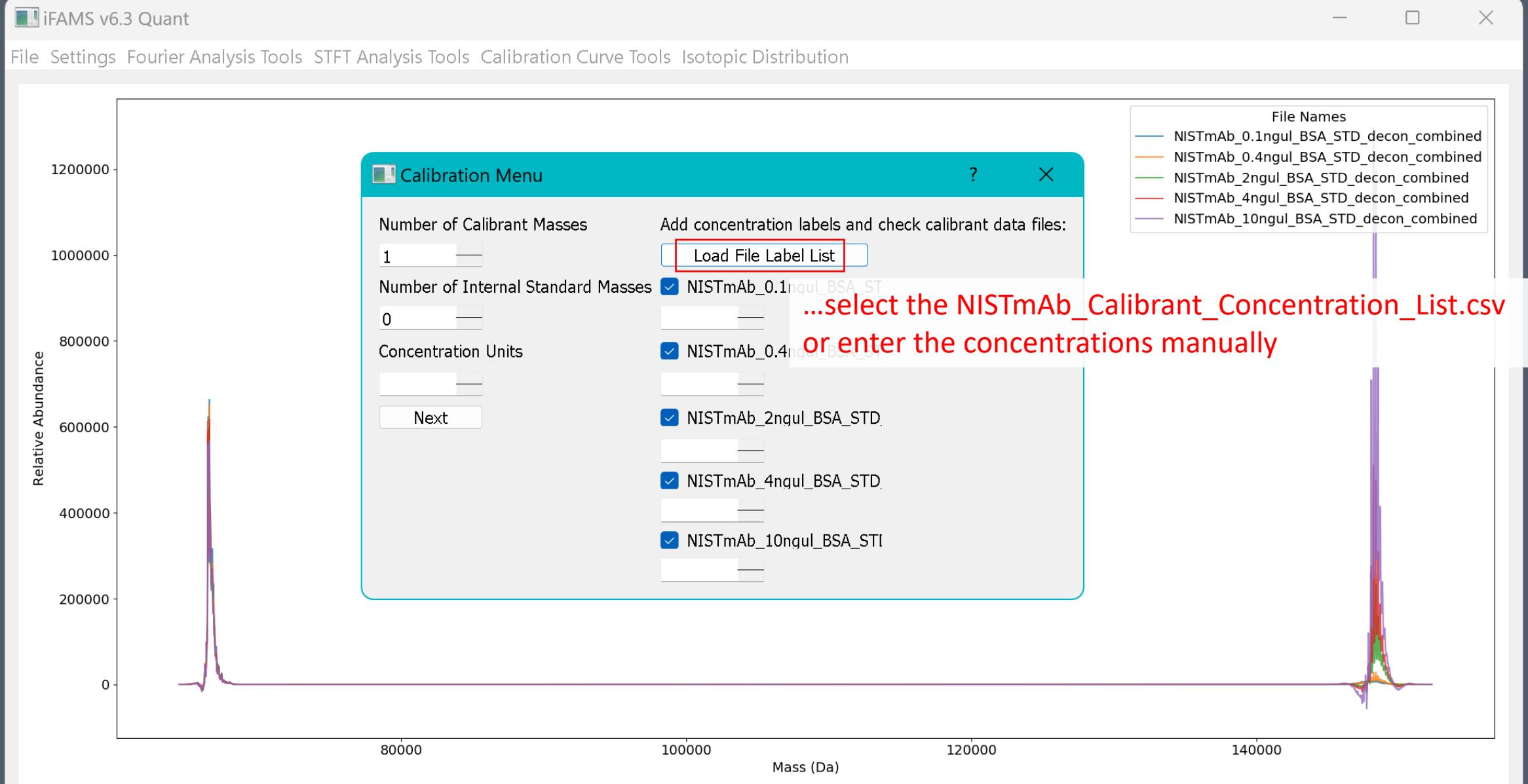


zoom rect
56

QUANTITATION AND CALIBRATION (15 of 25)



QUANTITATION AND CALIBRATION (16 of 25)

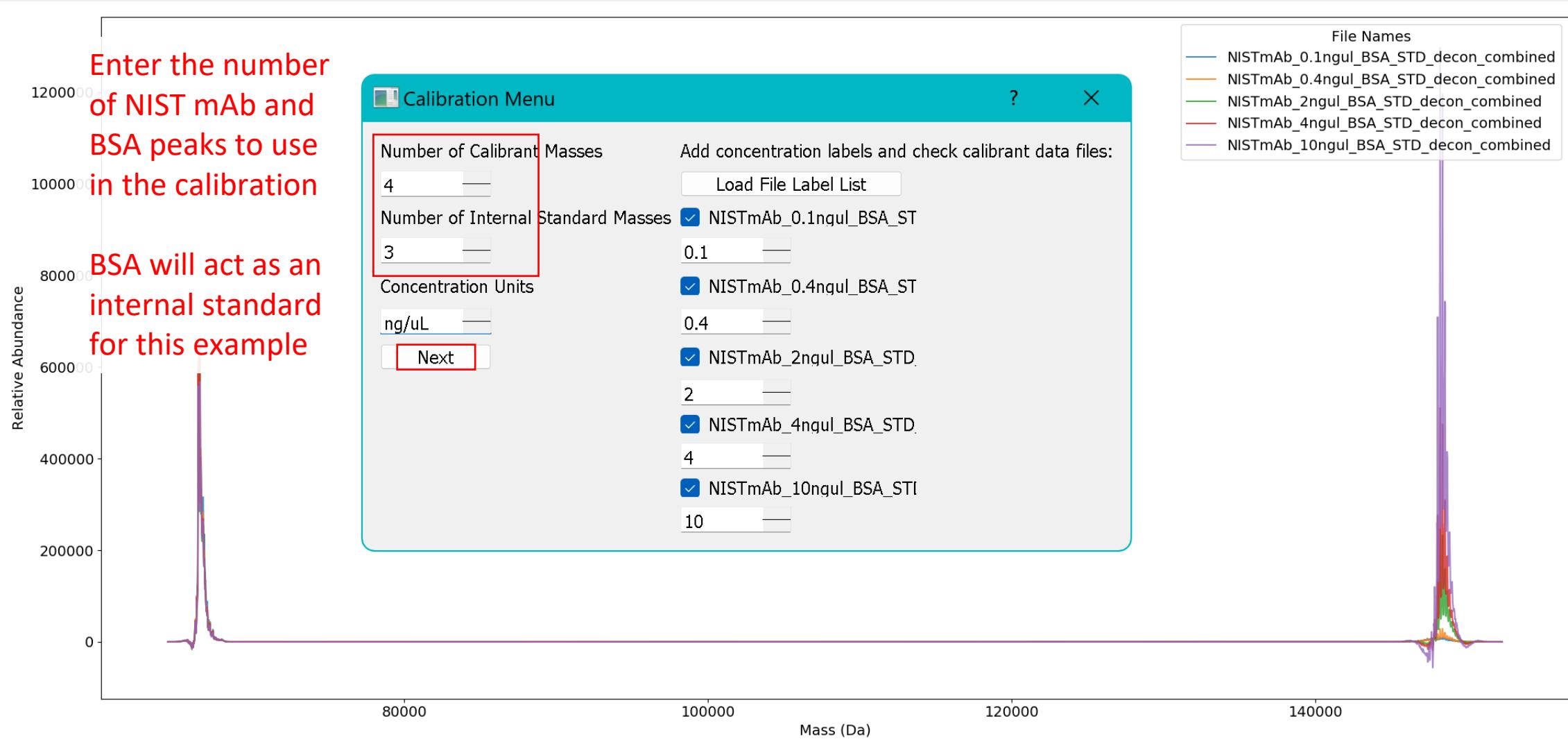


File: D:/Quant Test Data/241021_Batch1/Deconvolved_spectra/NISTmAb_0.1ngul_BSA_STD_decon_combined

Positive Ion Mode



QUANTITATION AND CALIBRATION (17 of 25)



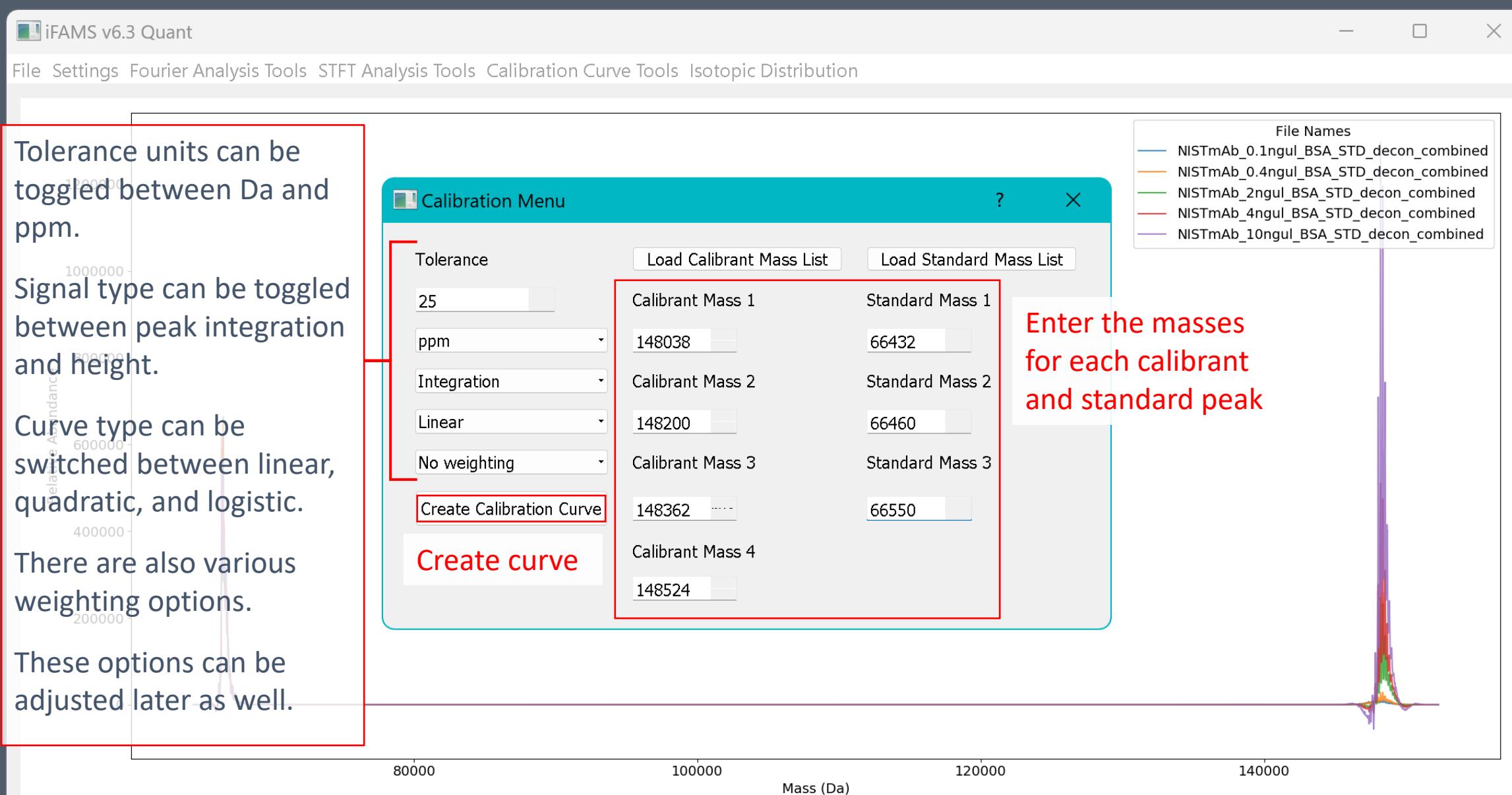
File: D:/Quant Test Data/241021_Batch1/Deconvolved_spectra/NISTmAb_0.1ngul_BSA_STD_decon_combined

Positive Ion Mode



zoom rect
59

QUANTITATION AND CALIBRATION (18 of 25)

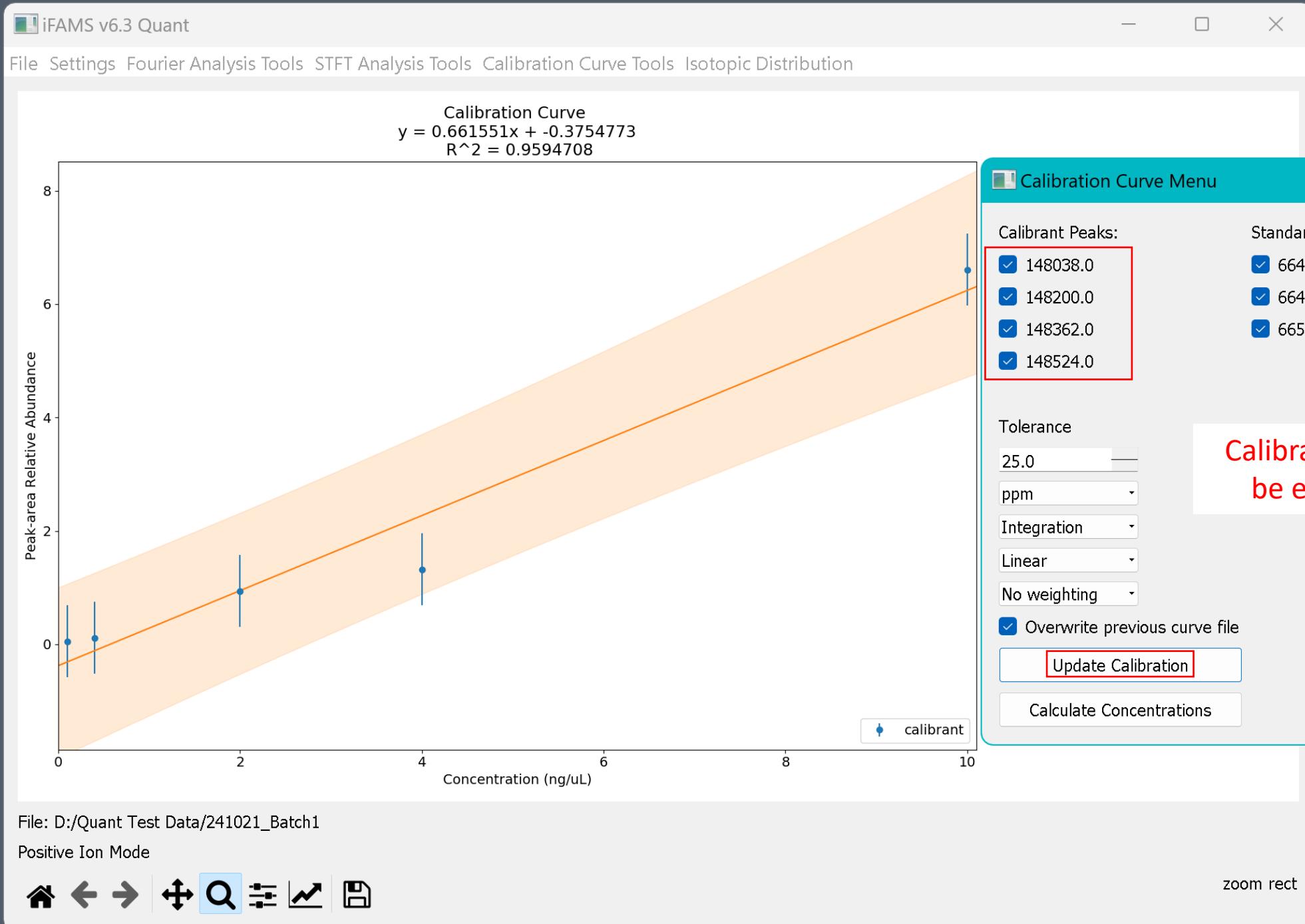


File: D:/Quant Test Data/241021_Batch1/Deconvolved_spectra/NISTmAb_0.1ngul_BSA_STD_decon_combined

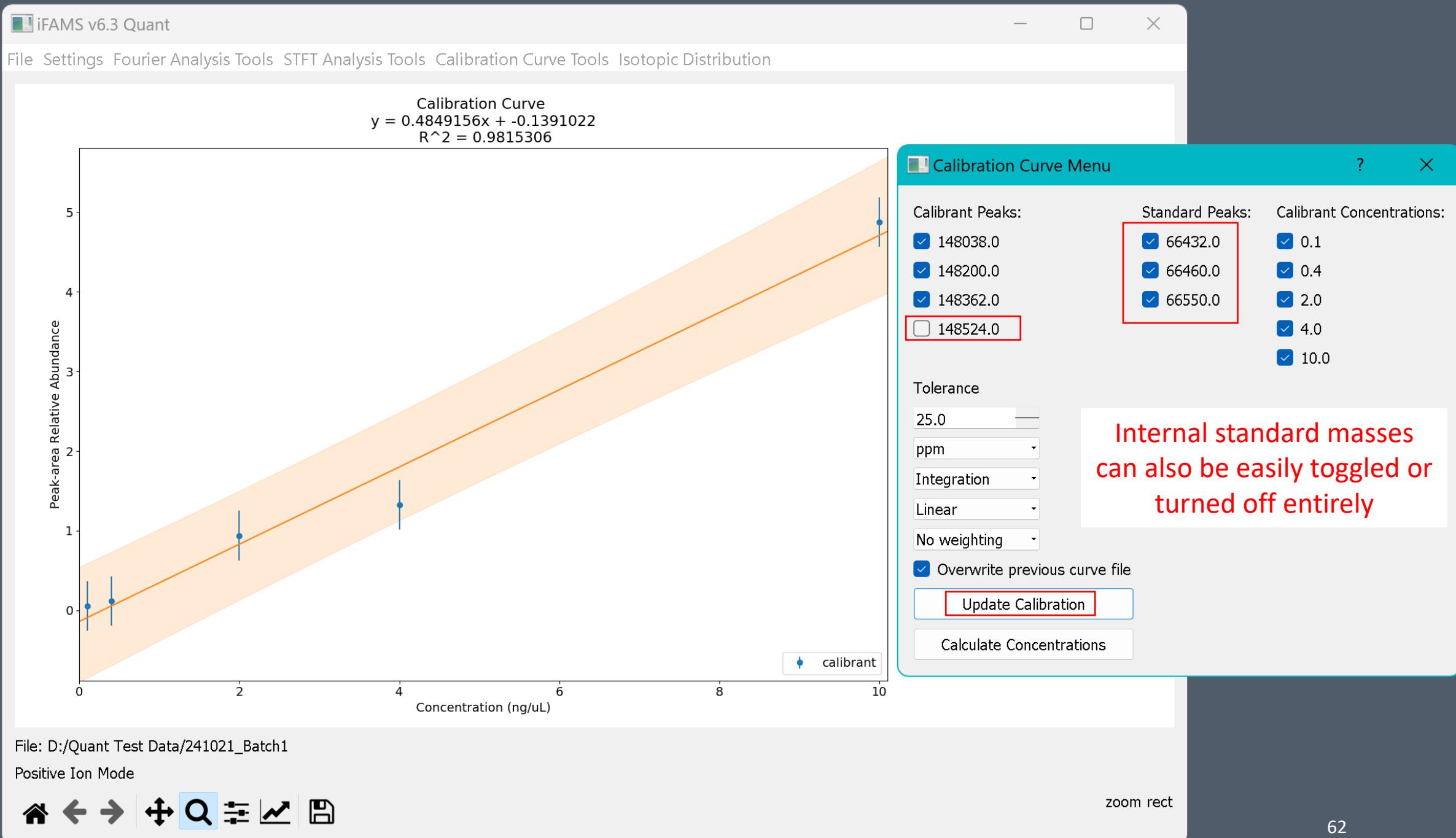
Positive Ion Mode



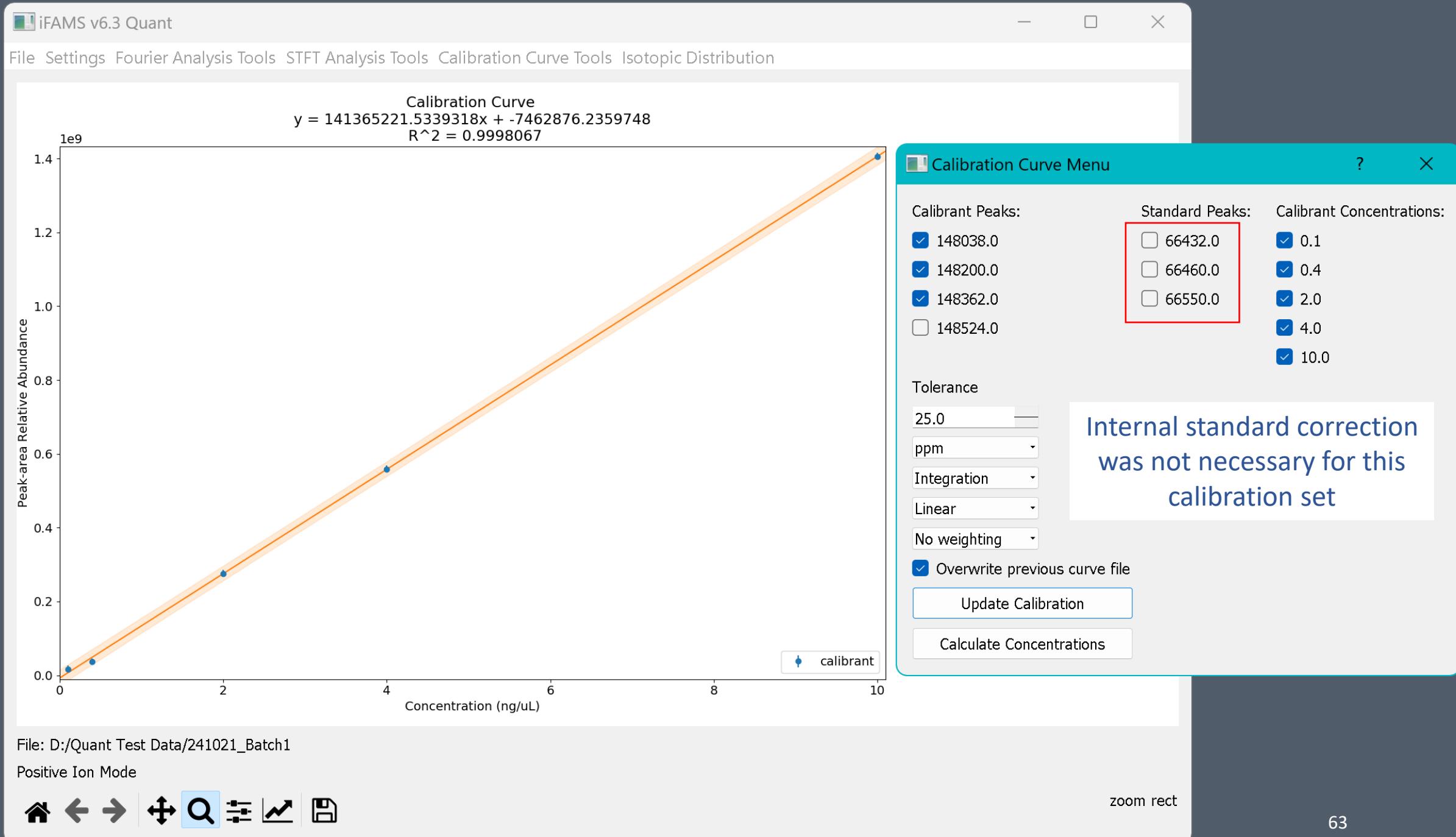
zoom rect
60



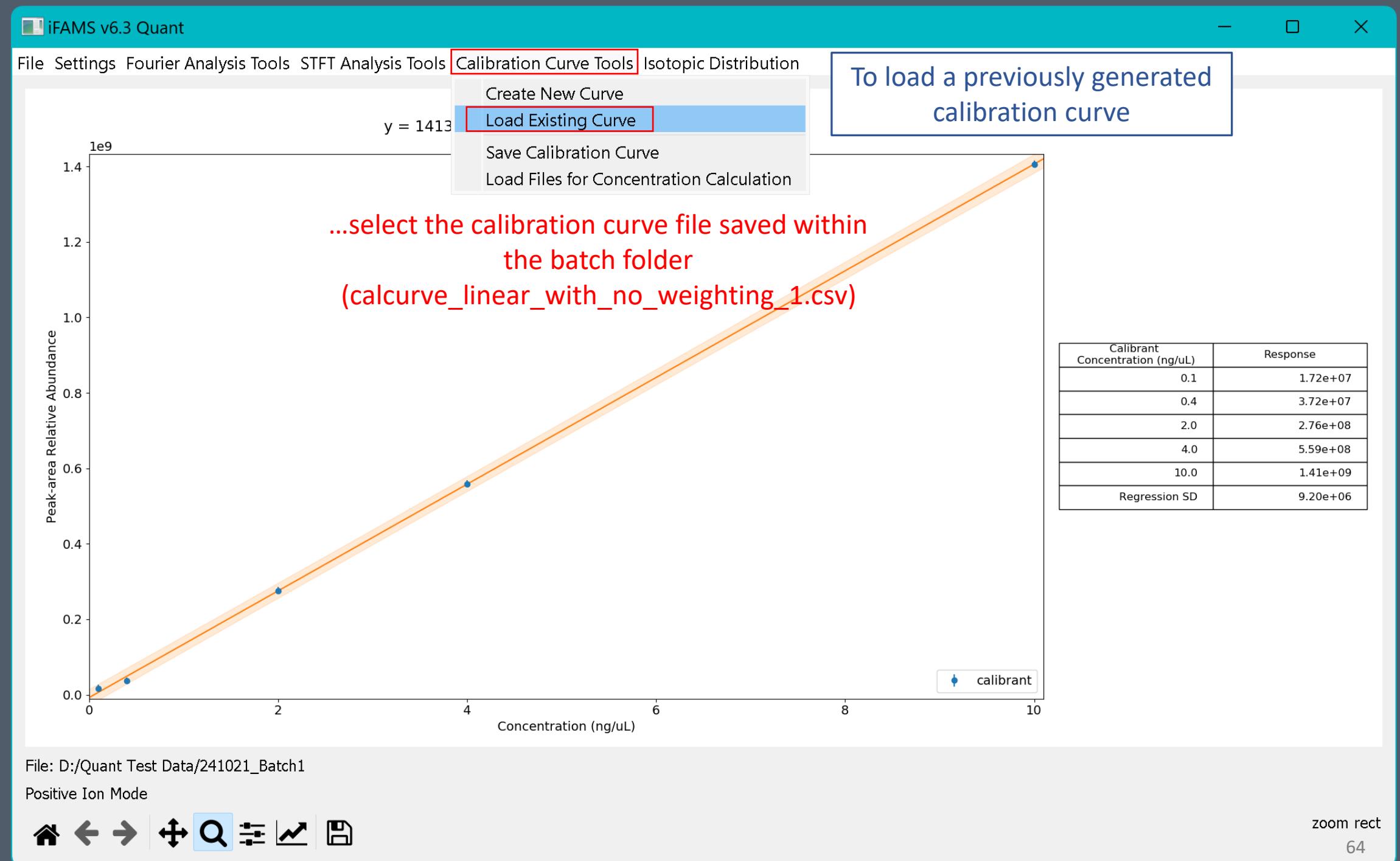
QUANTITATION AND CALIBRATION (20 of 25)



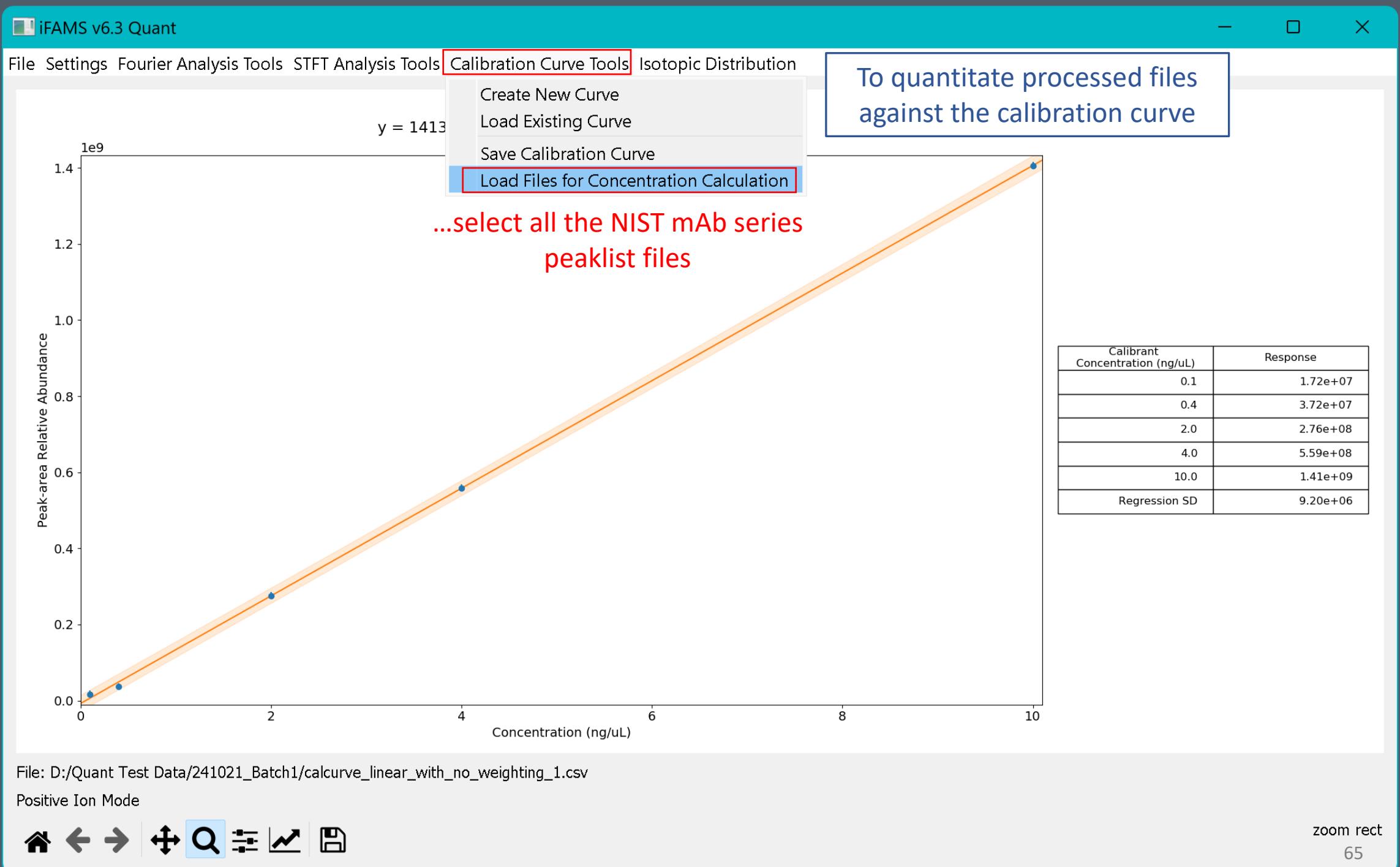
QUANTITATION AND CALIBRATION (21 of 25)



QUANTITATION AND CALIBRATION (22 of 25)



QUANTITATION AND CALIBRATION (23 of 25)

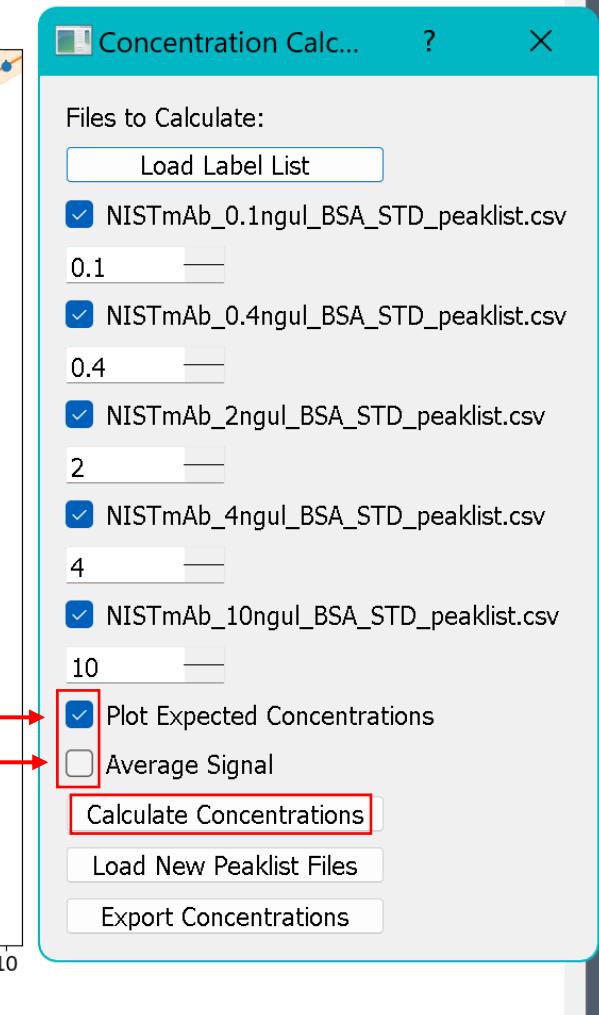
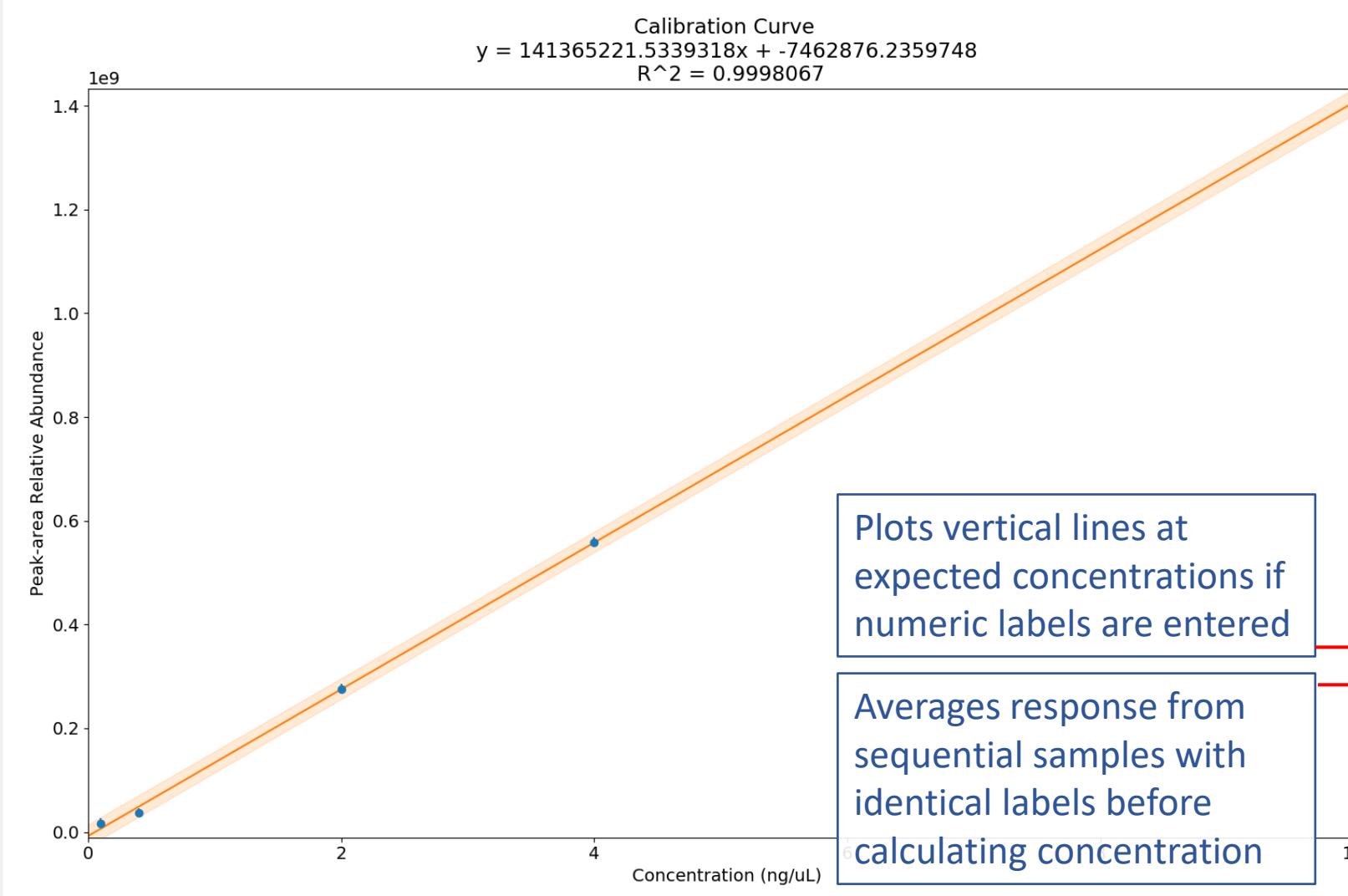


QUANTITATION AND CALIBRATION (24 of 25)

iFAMS v6.3 Quant

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

Enter concentrations and adjust settings



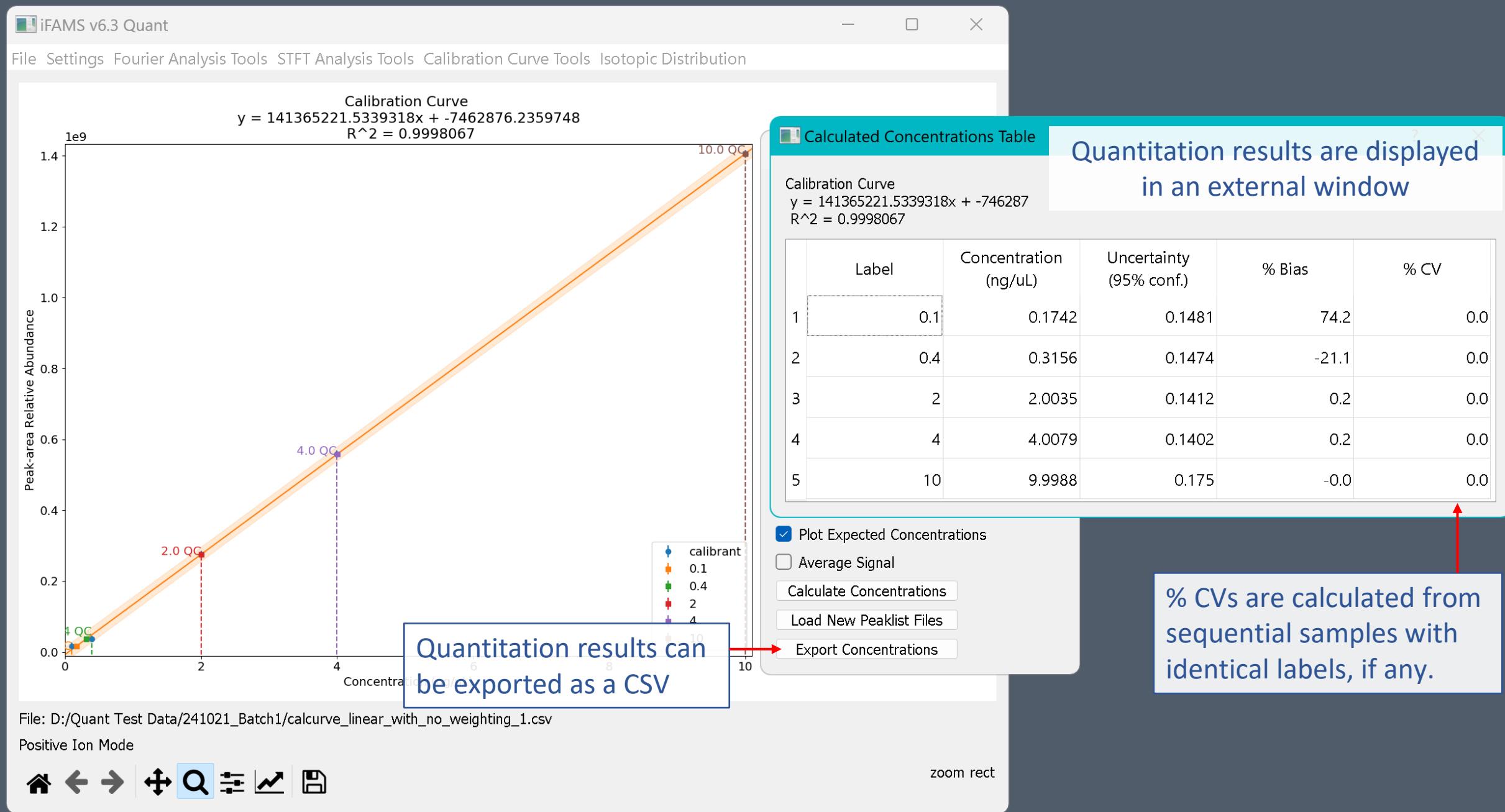
File: D:/Quant Test Data/241021_Batch1/calcurve_linear_with_no_weighting_1.csv

Positive Ion Mode



zoom rect

QUANTITATION AND CALIBRATION (25 of 25)



Gabor Slicer

Required data: Multi-protein sample

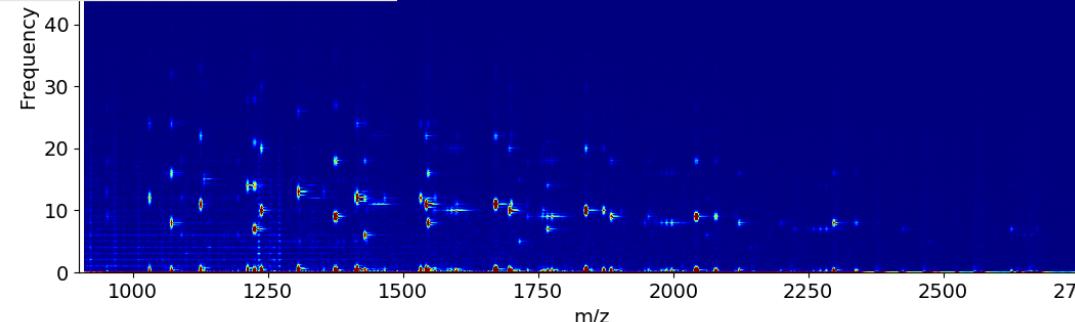
- Data domain adjustment
- Ion mass estimation with the Gabor Slicer
- Multiple protein deconvolution by mass input

Positive Ion Mode Adjust Data Domain

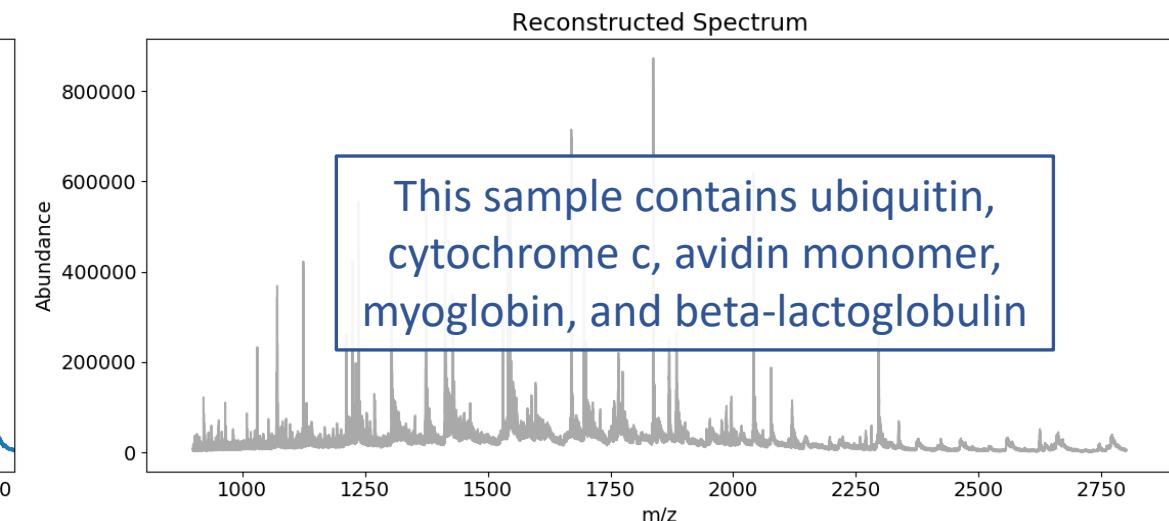
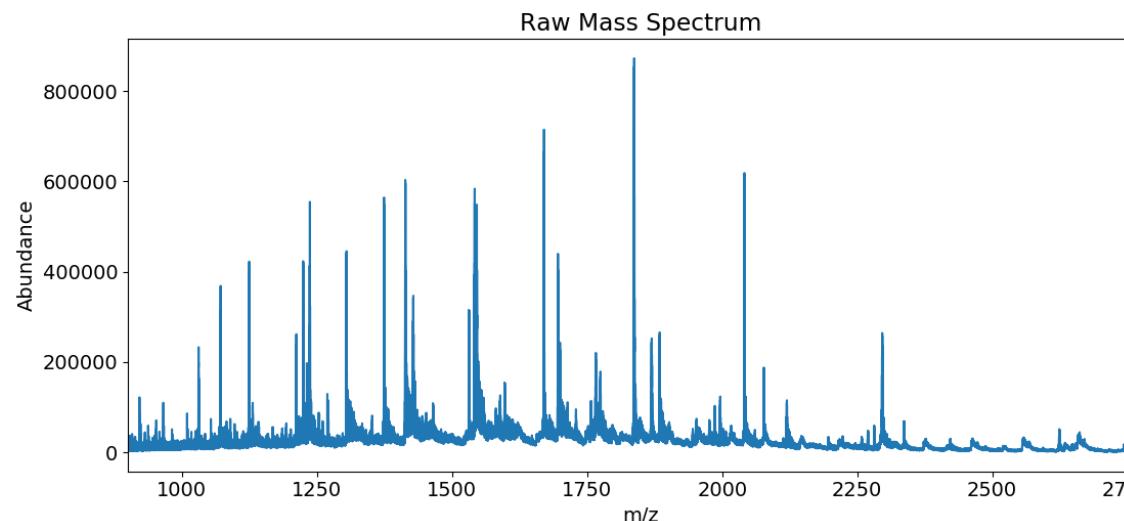
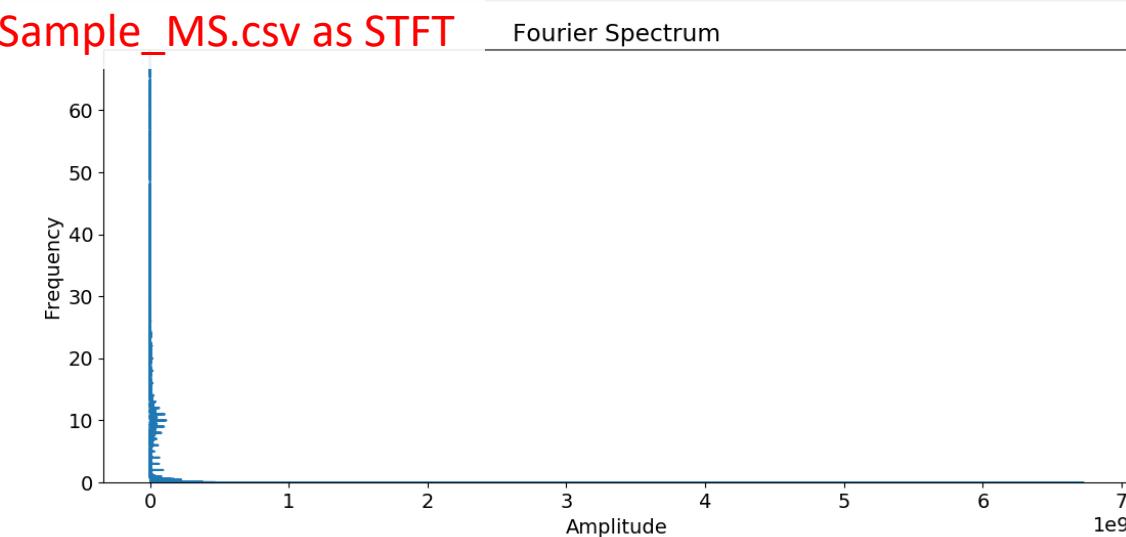
Show Interpolated MS

Normalize Abundances

Smooth Isotope Resolution

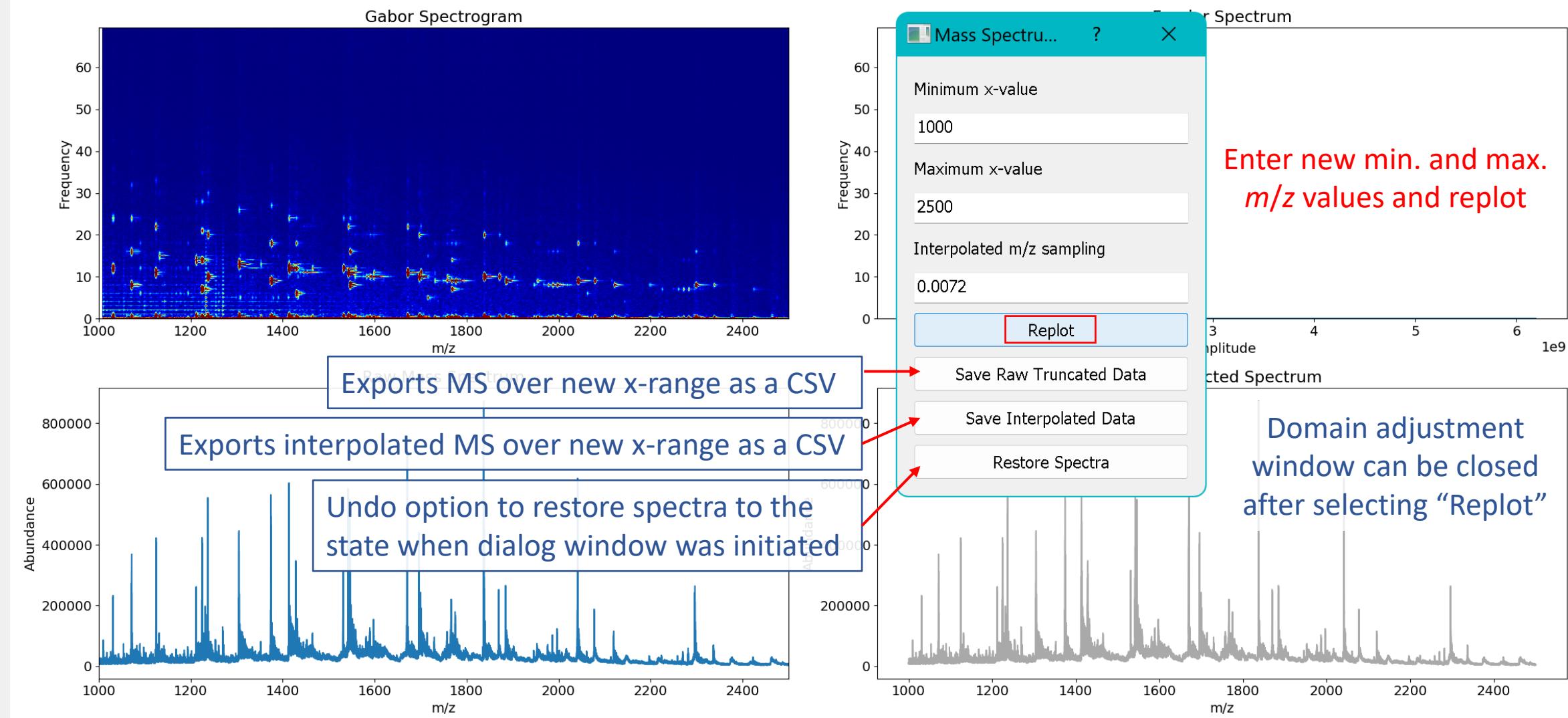


Load iFAMS_Quant_Multi-Protein_Sample_MS.csv as STFT

Adjust the *m/z* range

File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

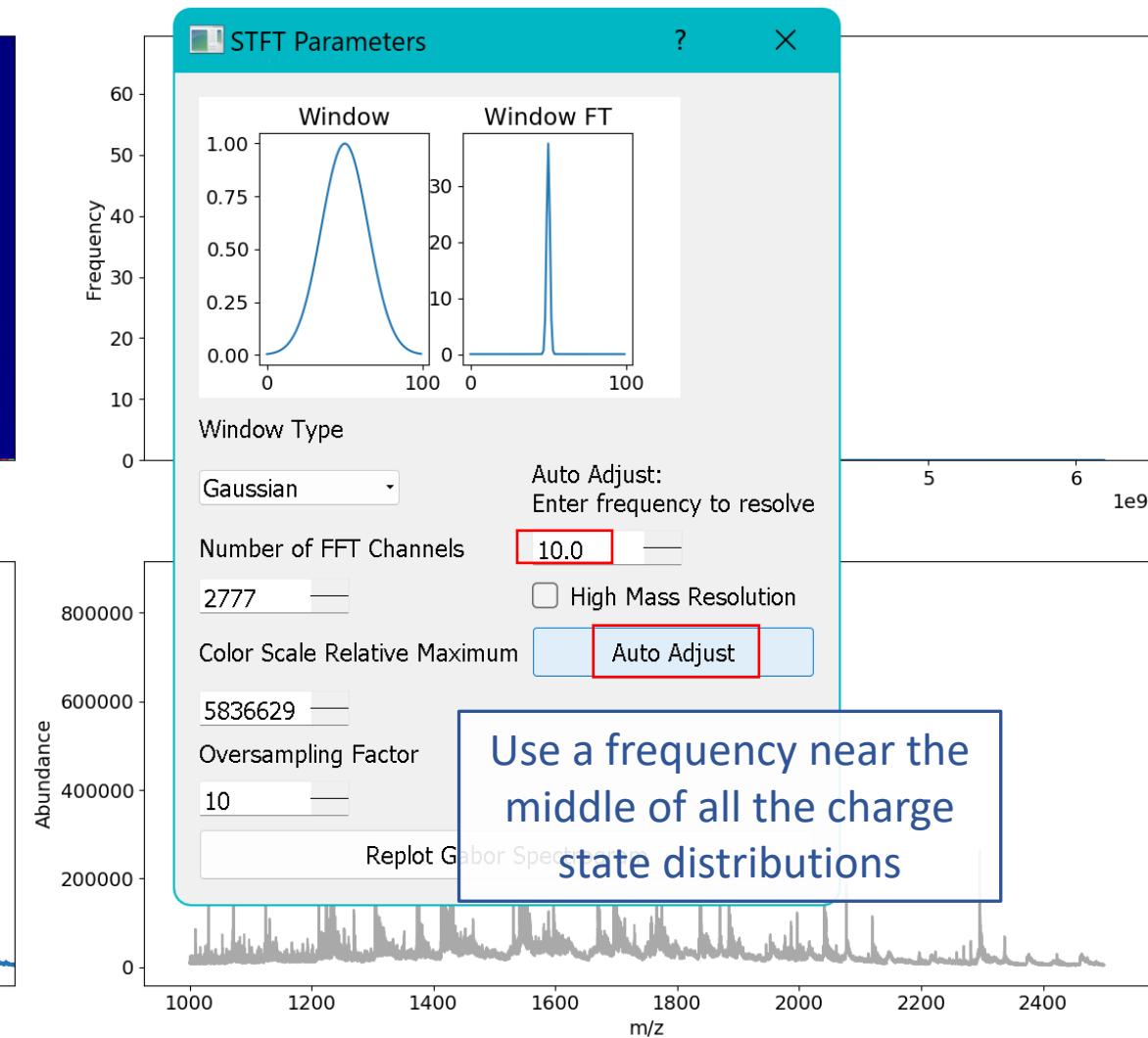
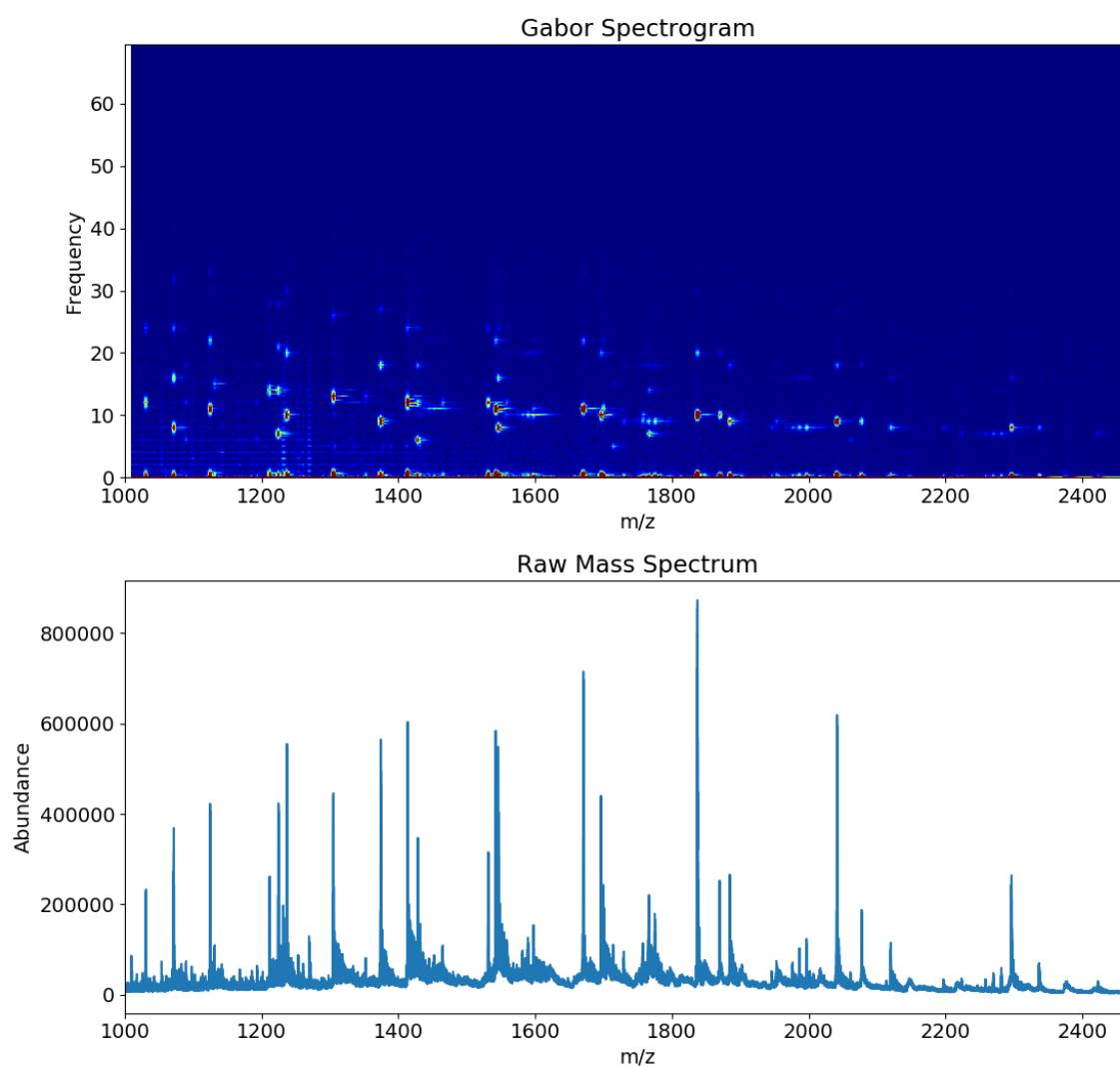
Positive Ion Mode



File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode

Re-adjust the STFT parameters



File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode



Change STFT Parameters

Open Gabor Slicer

Run Guided Search

Add Gabor Selection

Ctrl+E

Save Gabor Selections

Ctrl+S

Delete Previous Gabor Selection

Ctrl+Del

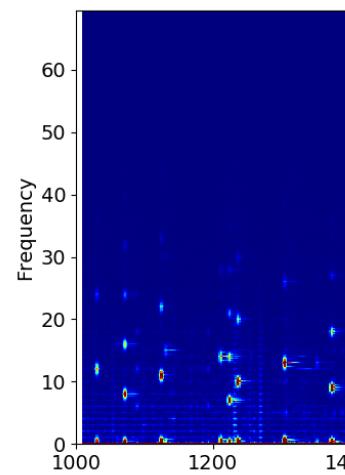
Open Harmonic Finder

Run iFAMS Analysis

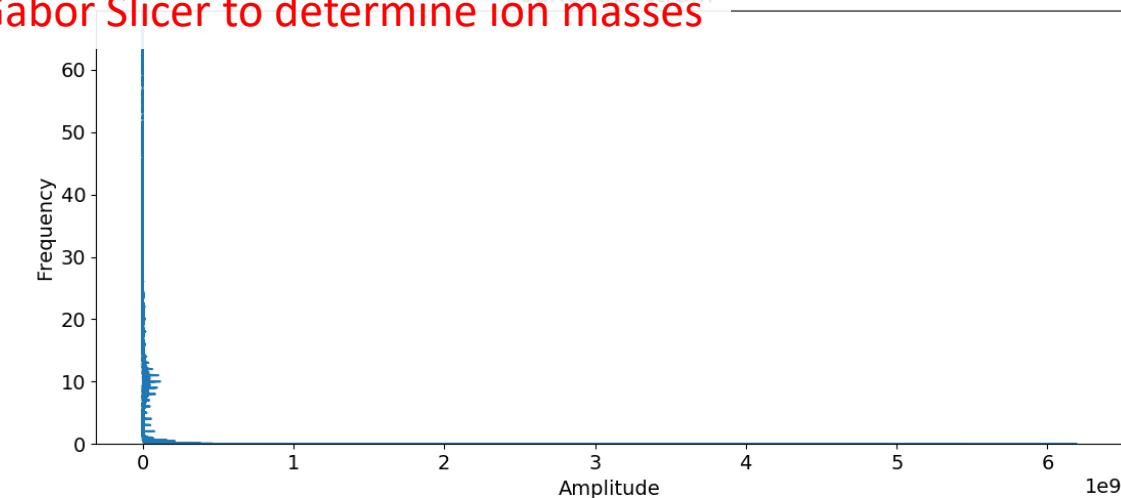
Adjust Charge State Assignments

Open Quantitative Peak Integrator

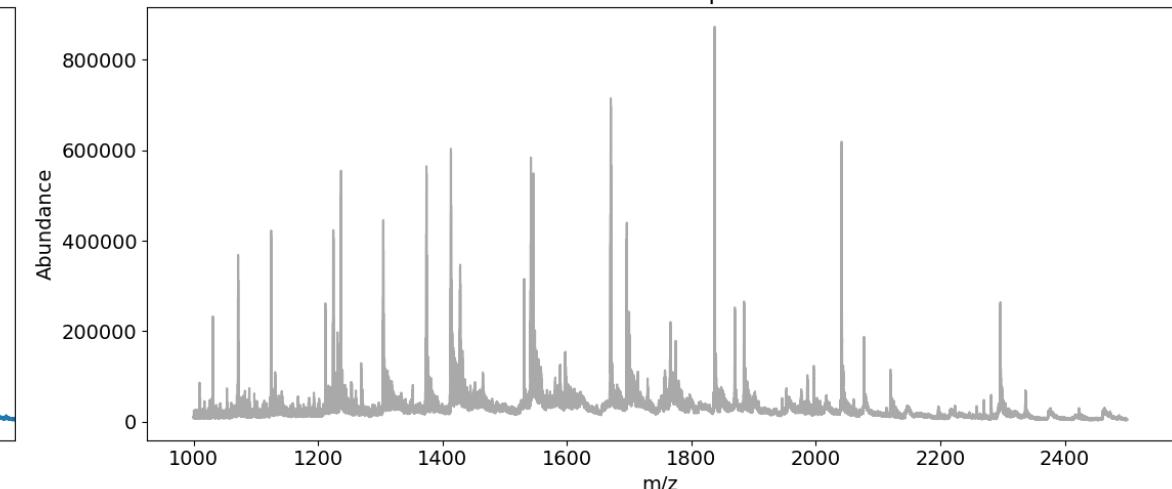
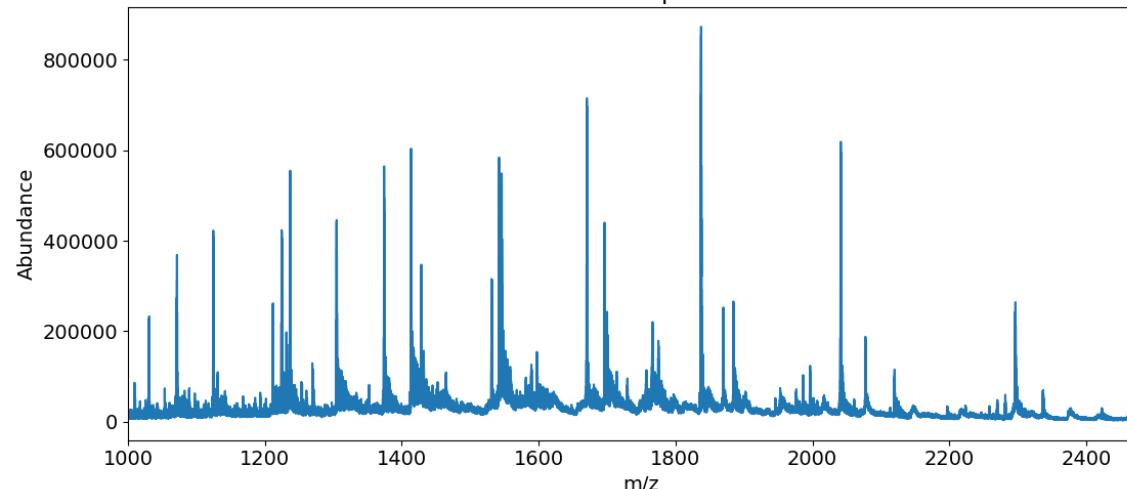
Run Mass Defect Analysis



Raw Mass Spectrum

Open Gabor Slicer to determine ion masses

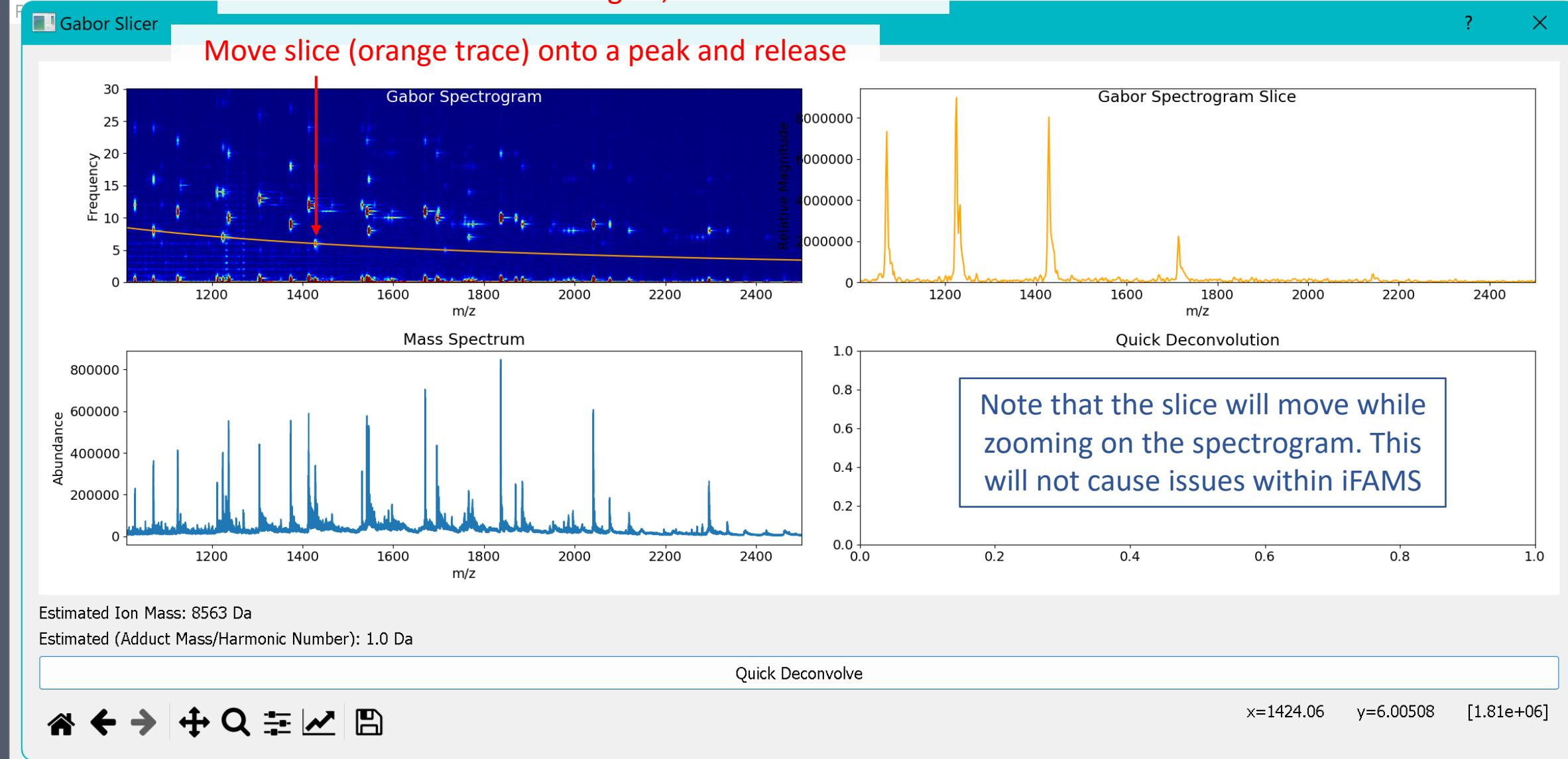
Reconstructed Spectrum



File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode

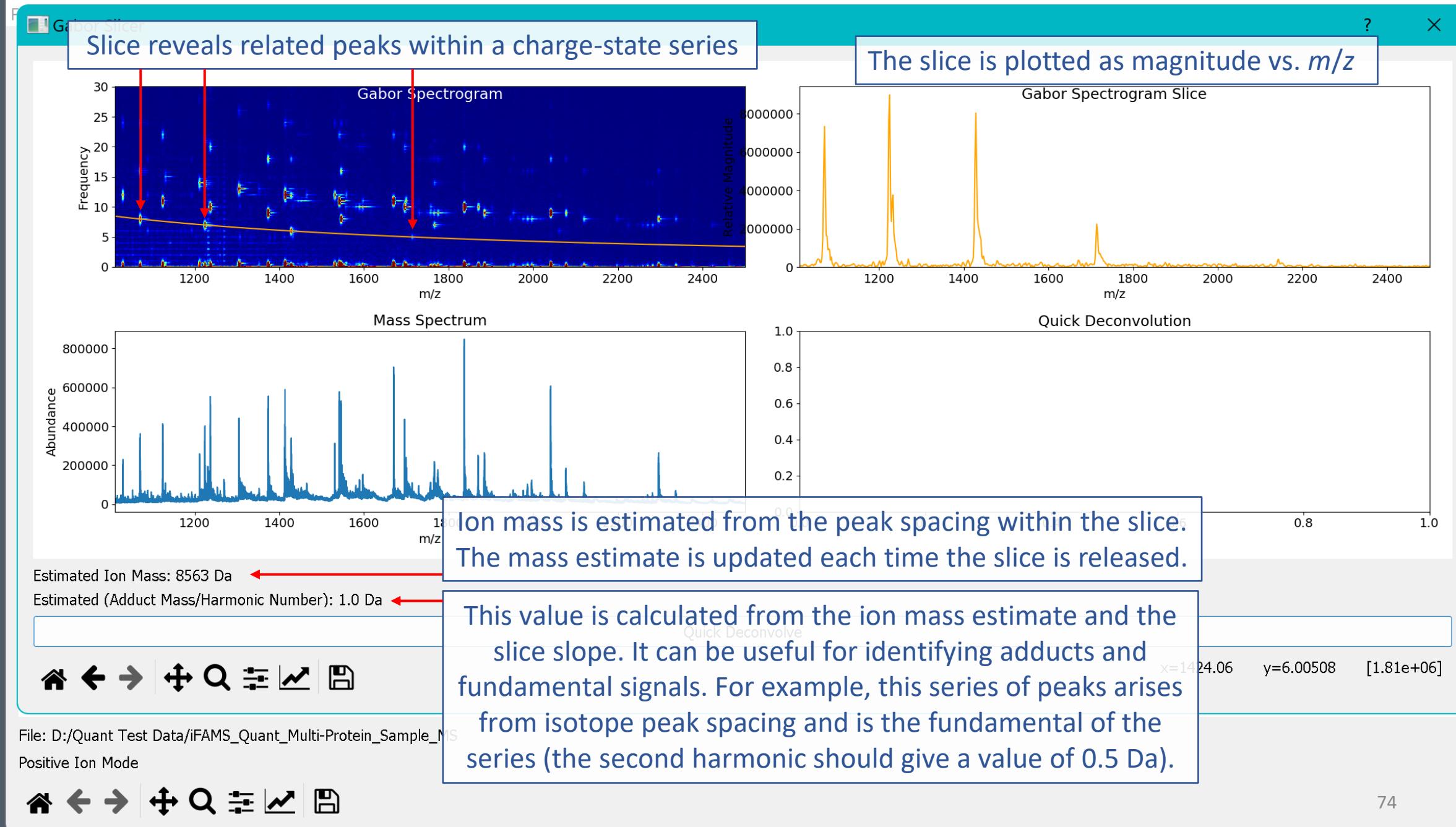
Zoom in on most abundant signal, then deselect zoom

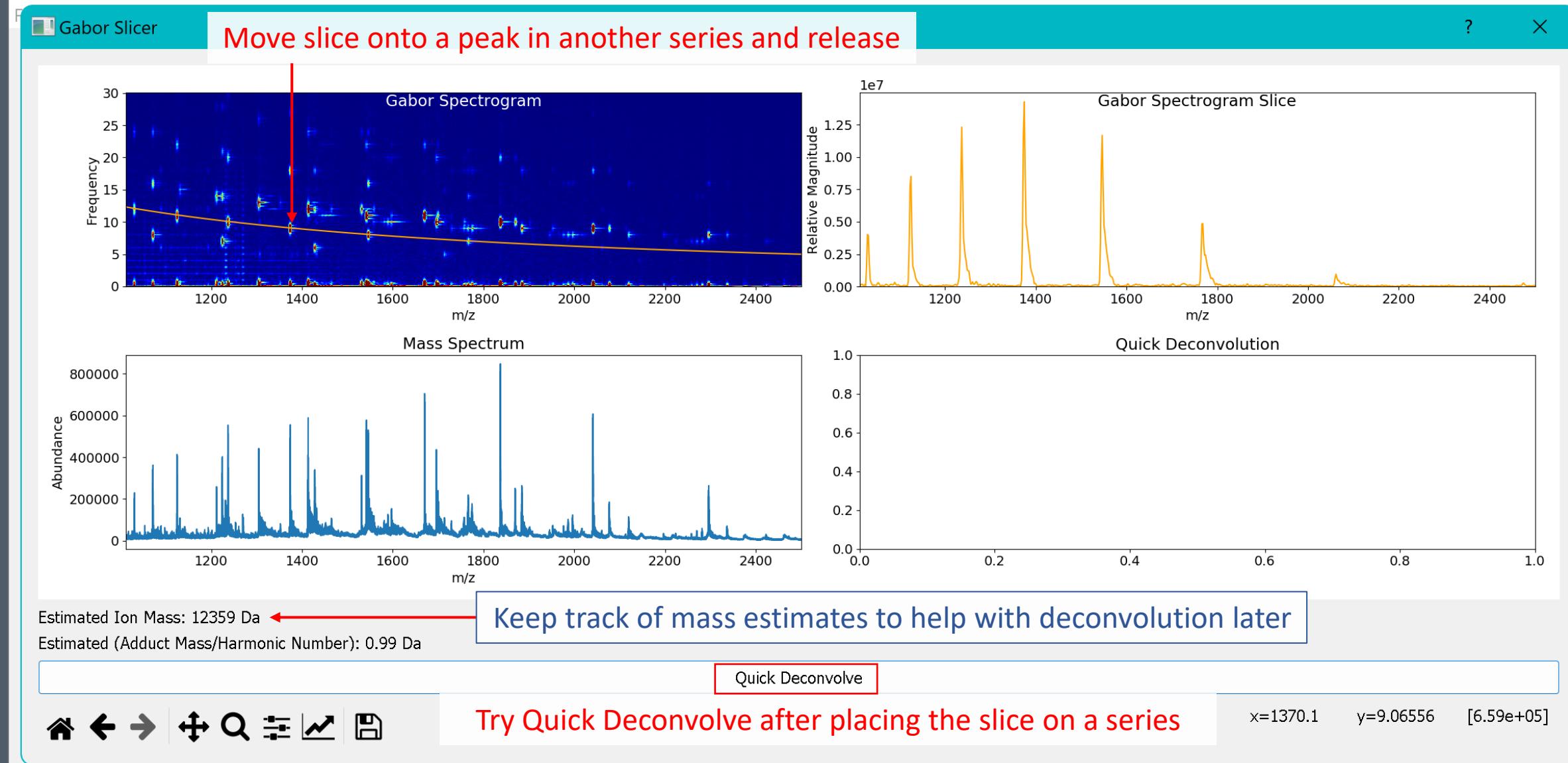


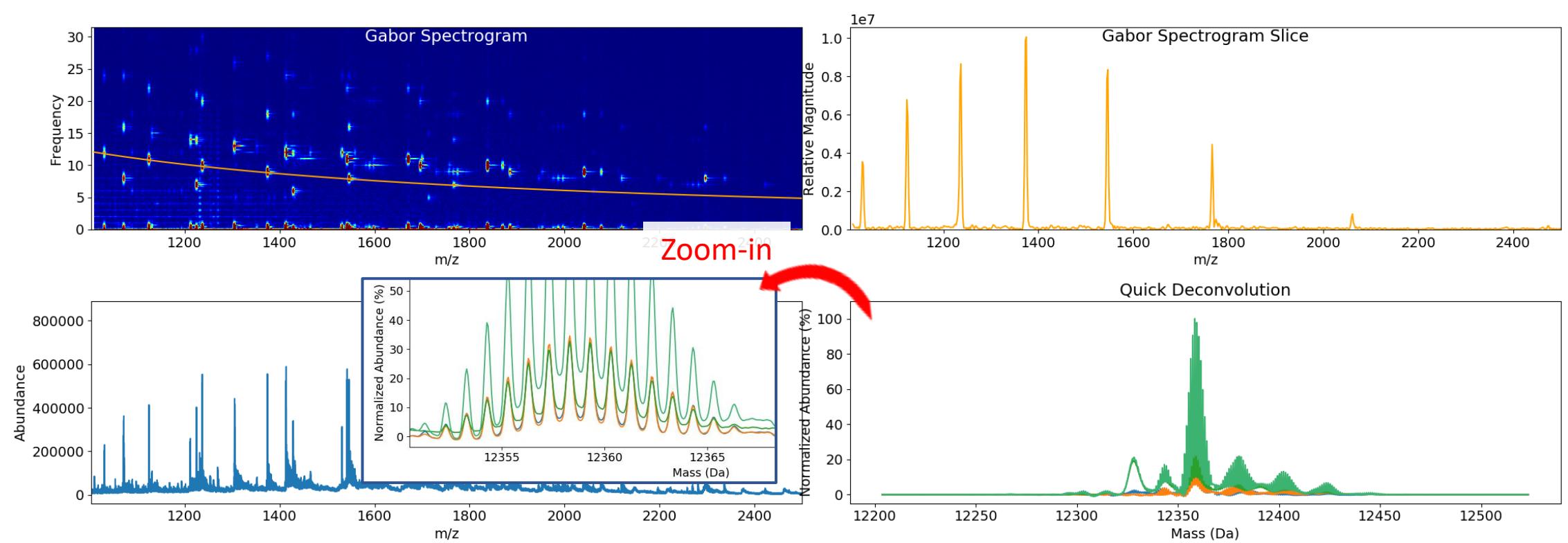
File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode







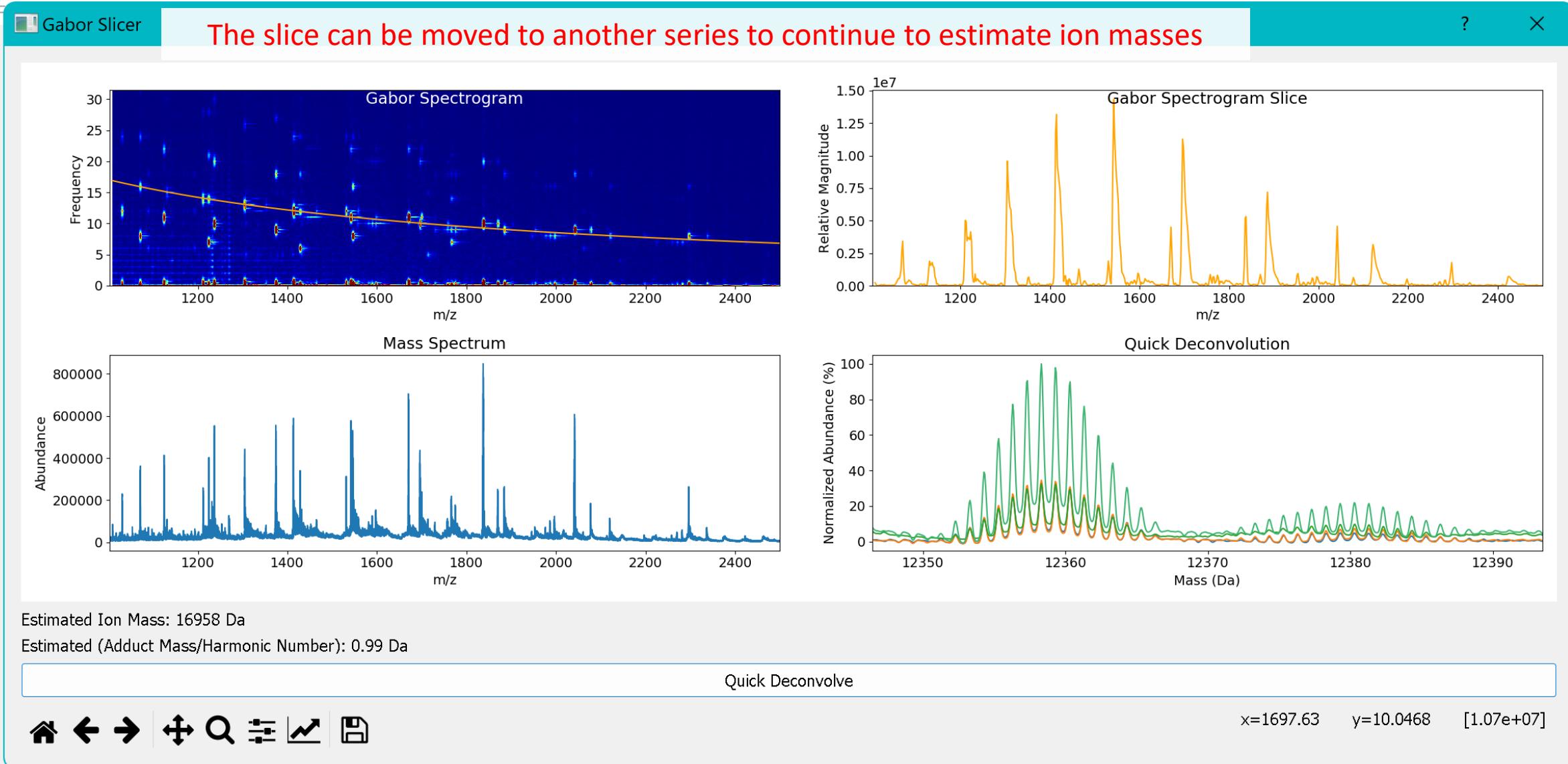


Estimated Ion Mass: 12359 Da

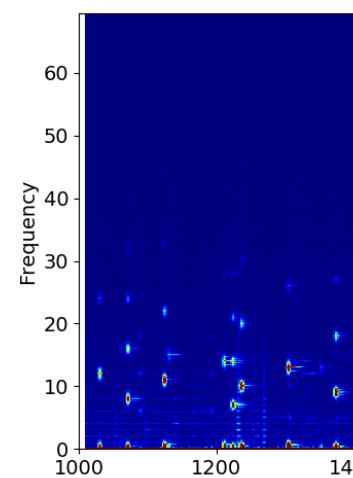
Estimated (Adduct Mass/Harmonic Number): 1.0 Da

Quick Deconvolve

This tool can help verify the ion mass estimate as well as identify some closely related peaks (such as sodium adduction). However, the results from this tool cannot be exported or carried through to other features of iFAMS because this rough deconvolution prioritizes speed over quality. In this example, only three charge states were used although best practices would recommend using six charge states in this case.



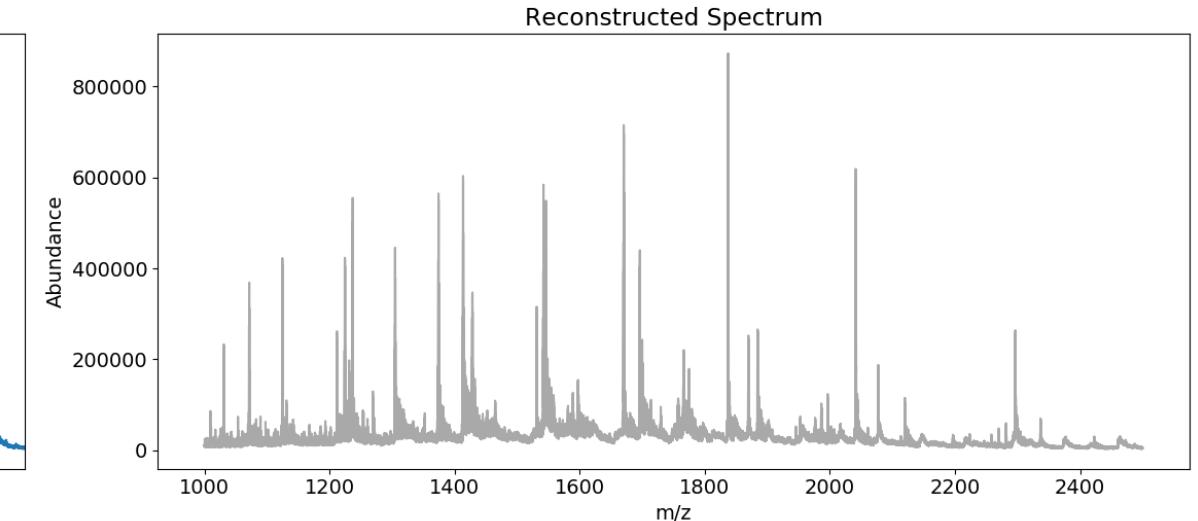
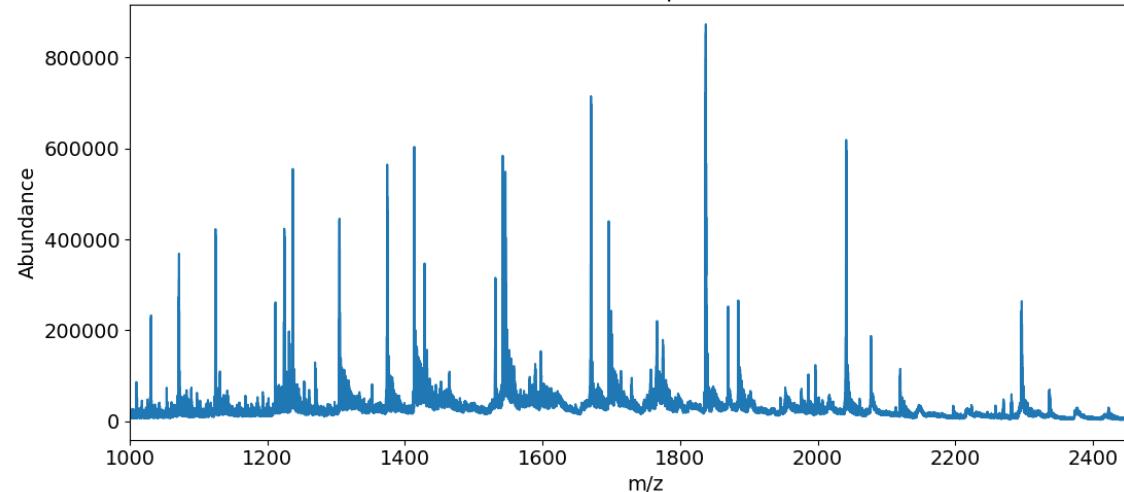
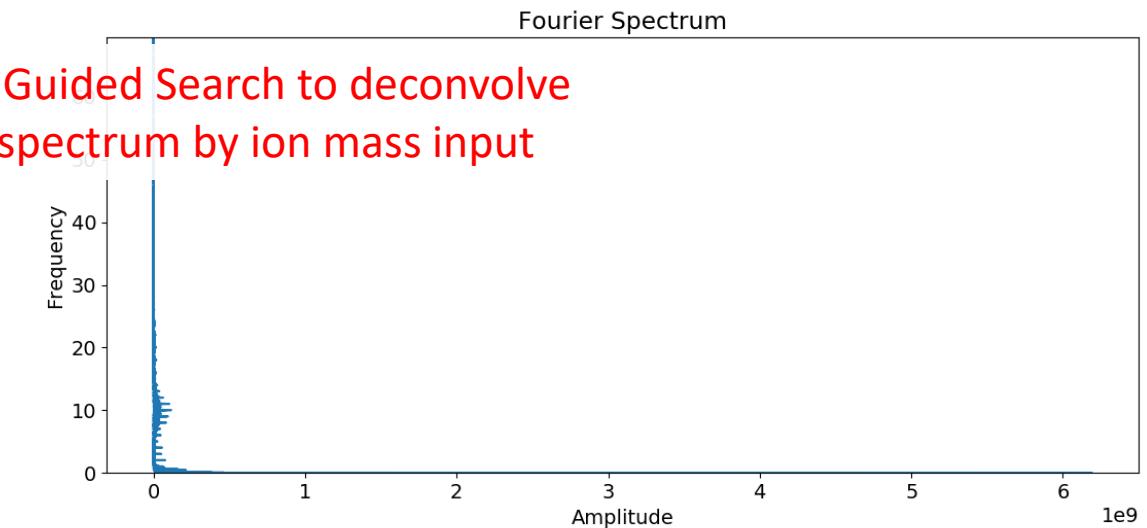
File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution



- Change STFT Parameters
- Open Gabor Slicer
- Run Guided Search**
- Add Gabor Selection Ctrl+E
- Save Gabor Selections Ctrl+S
- Delete Previous Gabor Selection Ctrl+Del
- Open Harmonic Finder
- Run iFAMS Analysis
- Adjust Charge State Assignments
- Open Quantitative Peak Integrator
- Run Mass Defect Analysis
- Show Only Spectrogram
- Open Noise Calculator



Open Guided Search to deconvolve
the spectrum by ion mass input

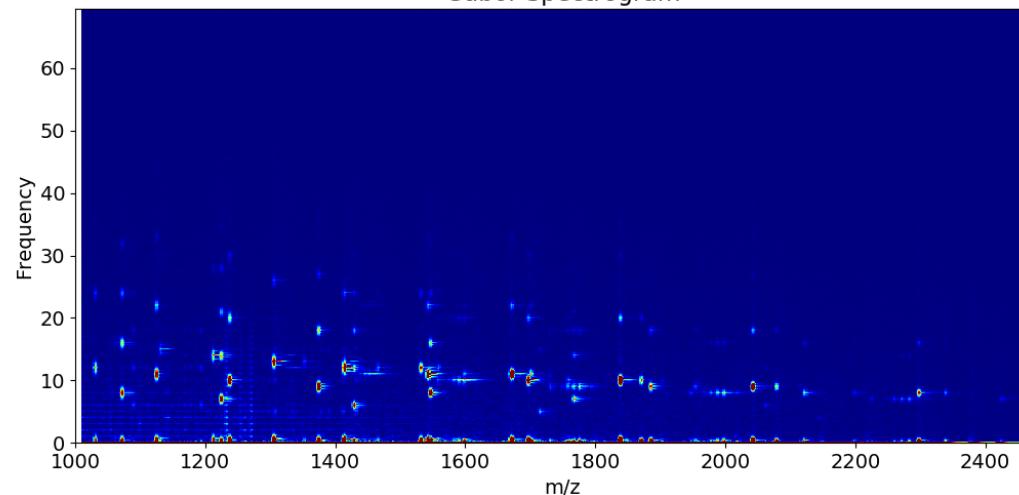


File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

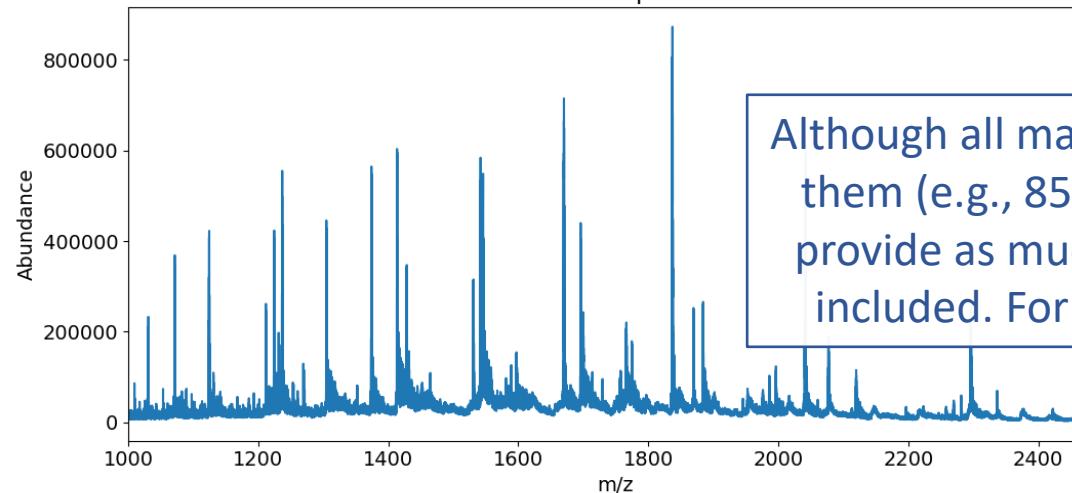
Positive Ion Mode



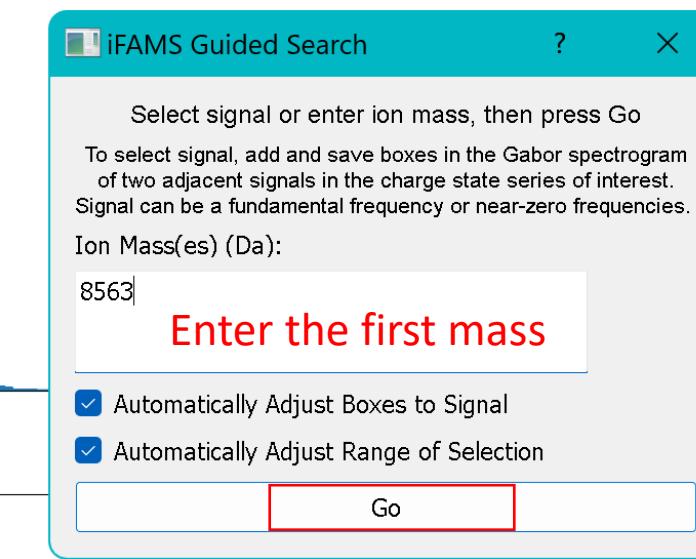
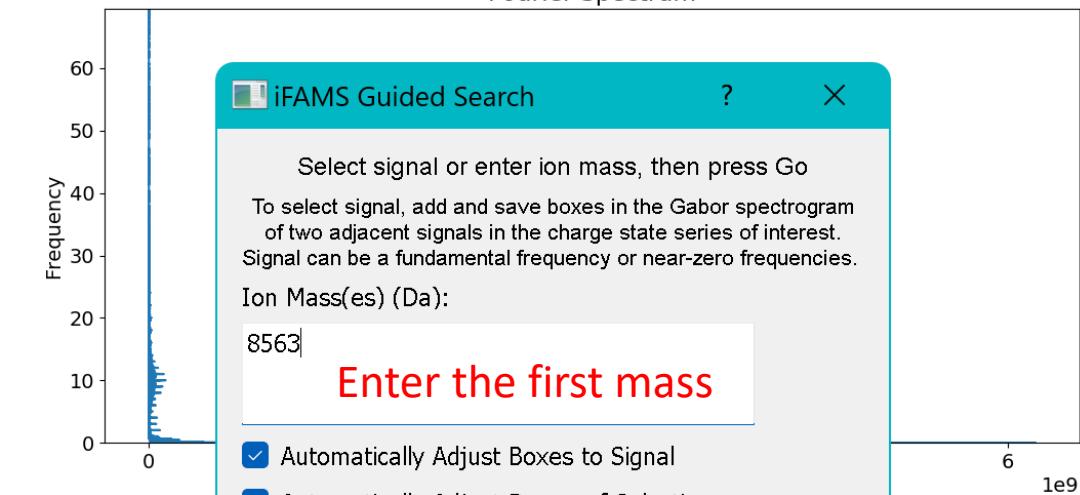
Gabor Spectrogram



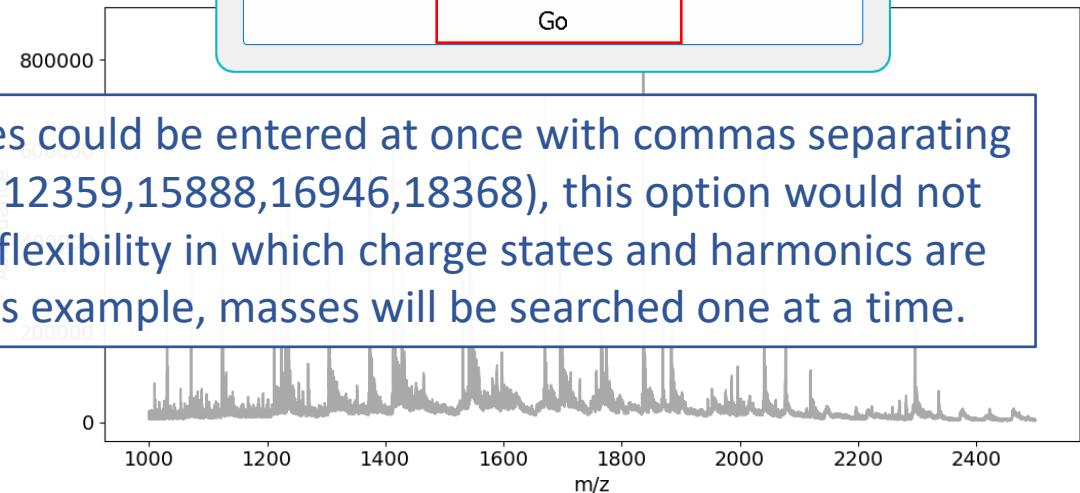
Raw Mass Spectrum



Fourier Spectrum



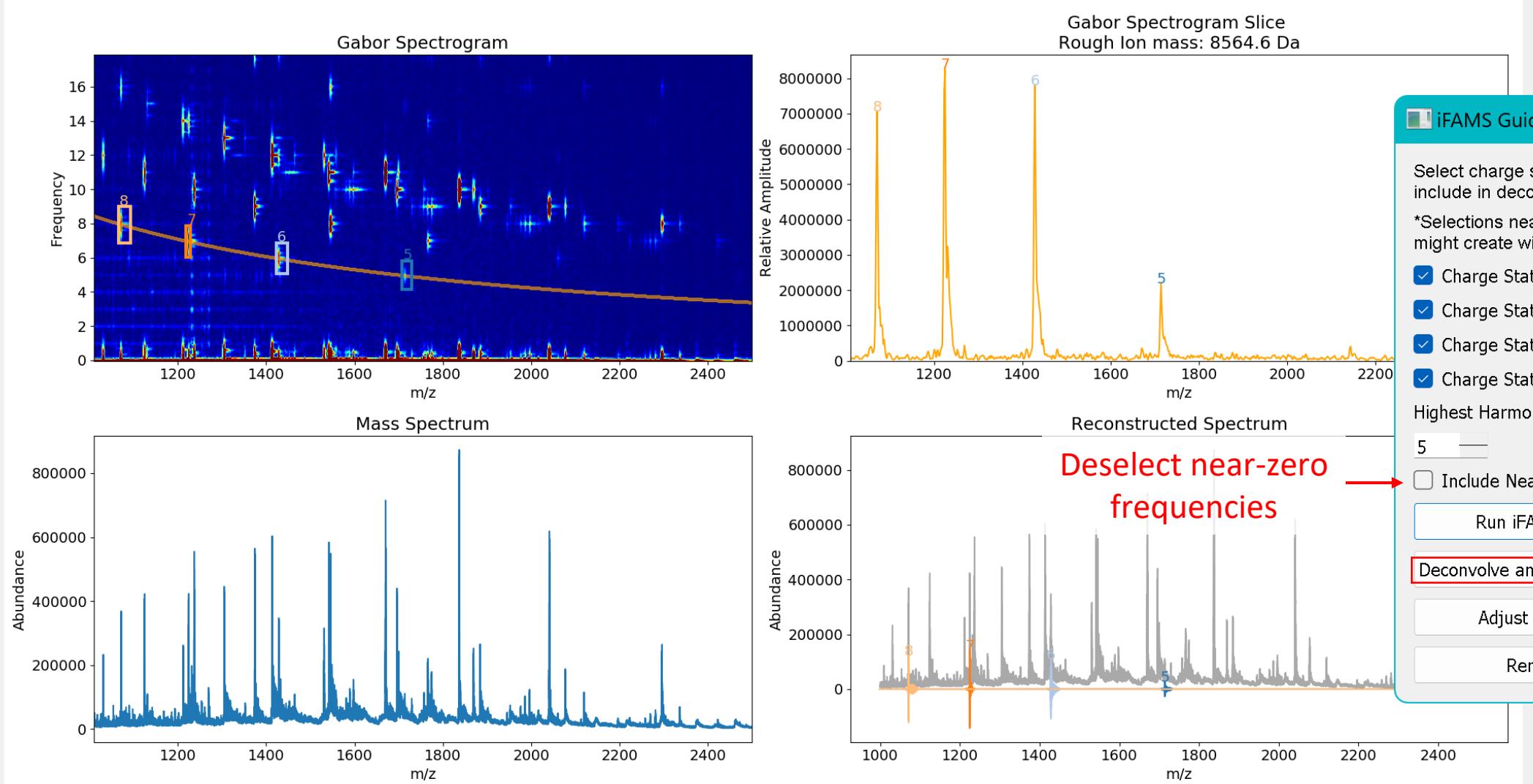
Although all masses could be entered at once with commas separating them (e.g., 8563,12359,15888,16946,18368), this option would not provide as much flexibility in which charge states and harmonics are included. For this example, masses will be searched one at a time.



File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode

Mass estimate is flexible and is updated by search results



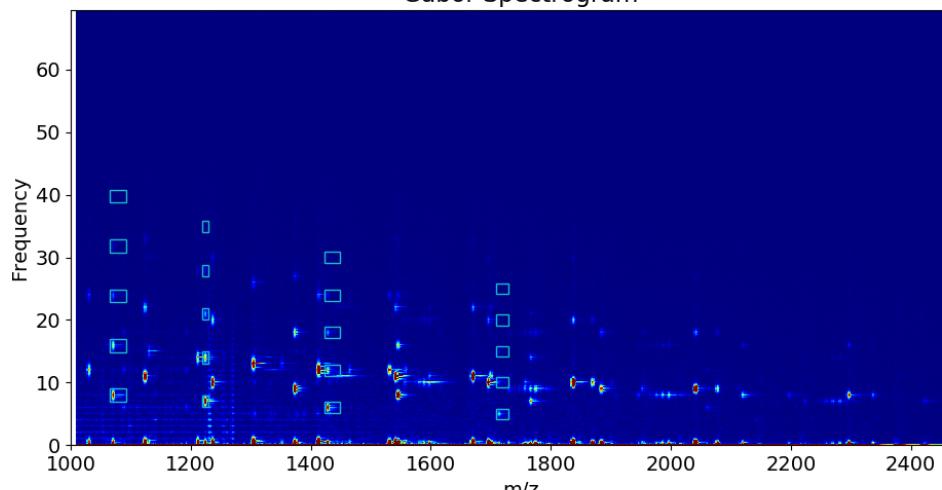
File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode

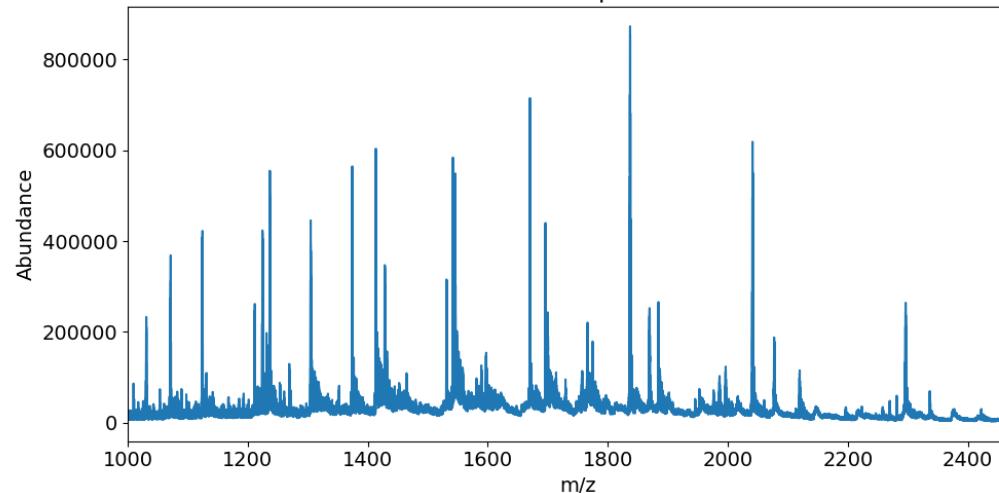


Since several of the charge state series have peaks in the Gabor spectrogram with overlapping m/z ranges, the near-zero frequencies should be excluded to avoid overlapping selections leading to misassigned signal

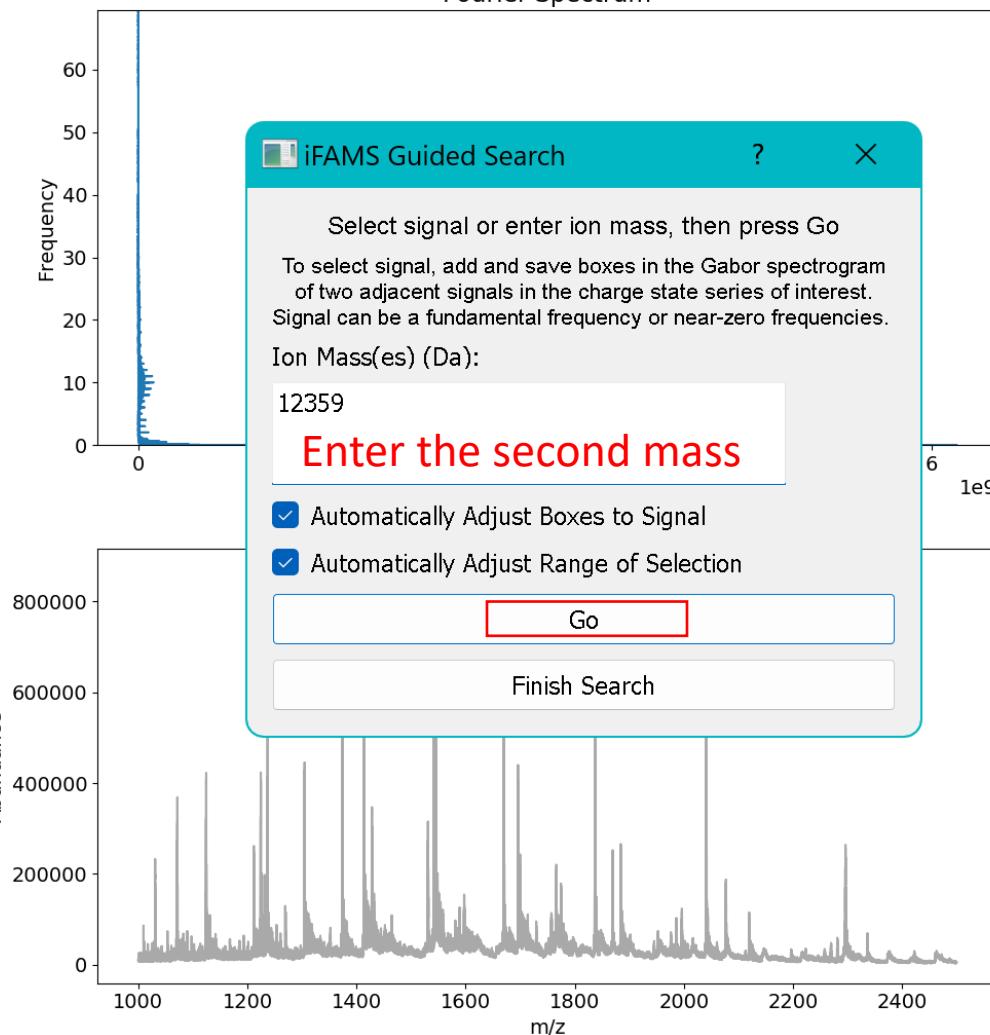
Gabor Spectrogram



Raw Mass Spectrum



Fourier Spectrum



File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode

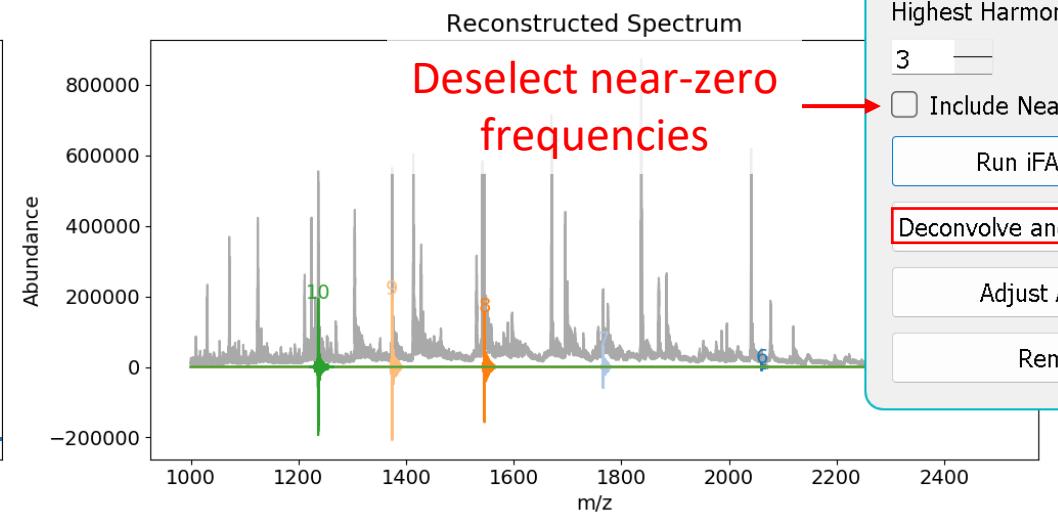
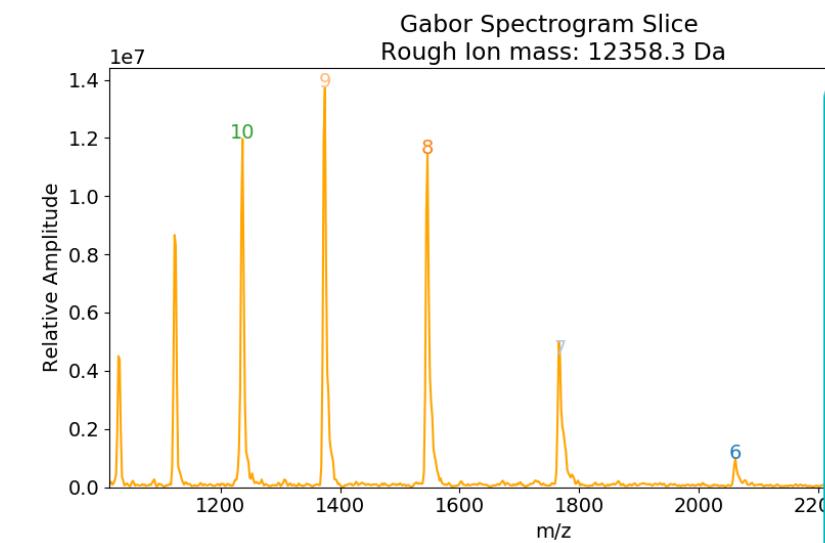
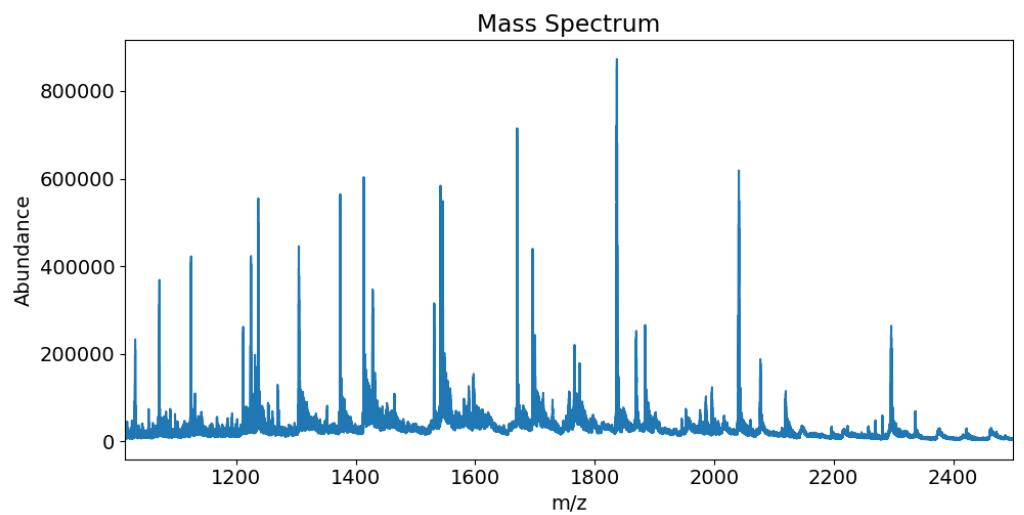
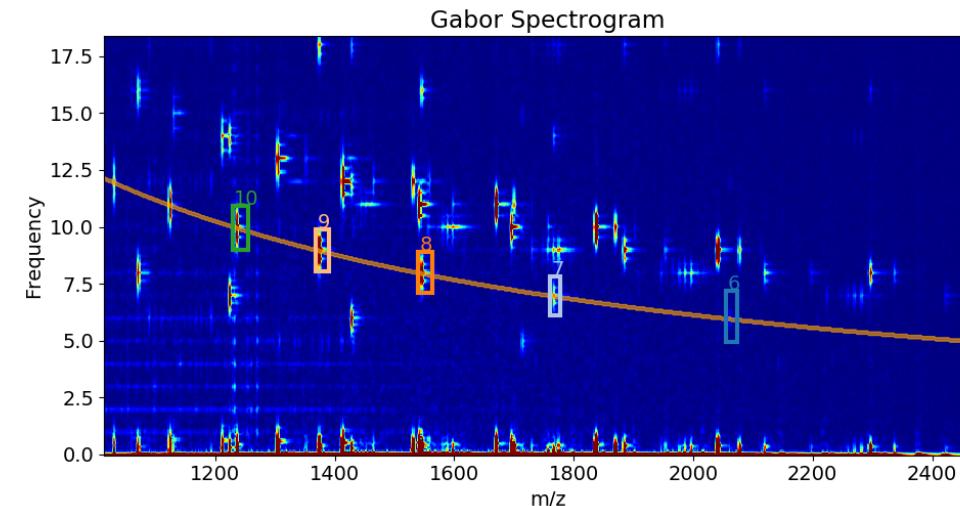


zoom rect

GABOR SLICER (14 of 26)

iFAMS v6.3 Quant

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution



iFAMS Guided S... ? X

Select charge states to include in deconvolution:

*Selections near ends of spectrum might create windowing artifacts

Charge State 6

Charge State 7

Charge State 8

Charge State 9

Charge State 10

Highest Harmonic to Include

3

Include Near-zero Frequencies

Run iFAMS Deconvolution

Deconvolve and Select Additional Series

Adjust Assigned Charges

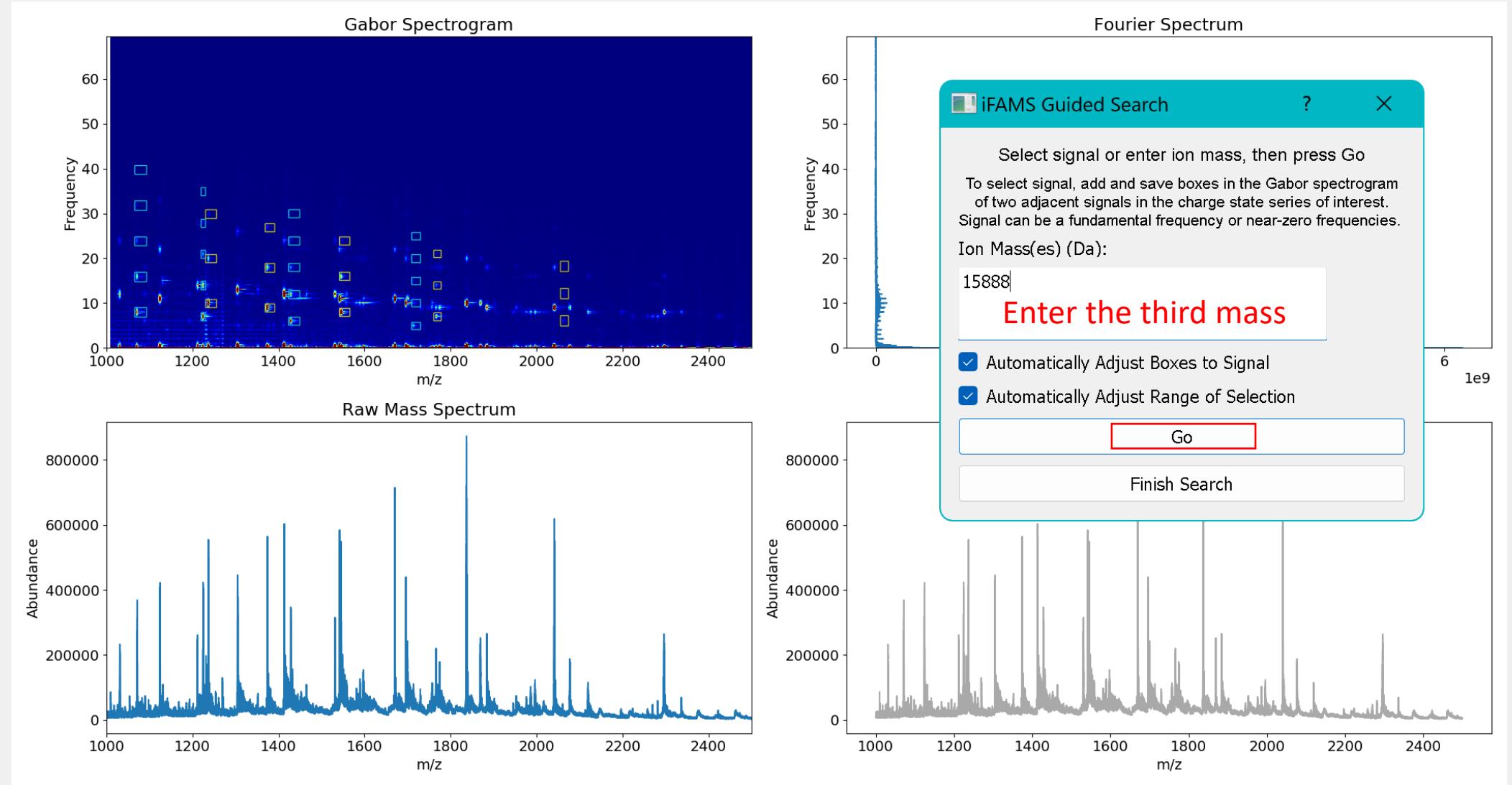
Remove Selection

File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode



zoom rect

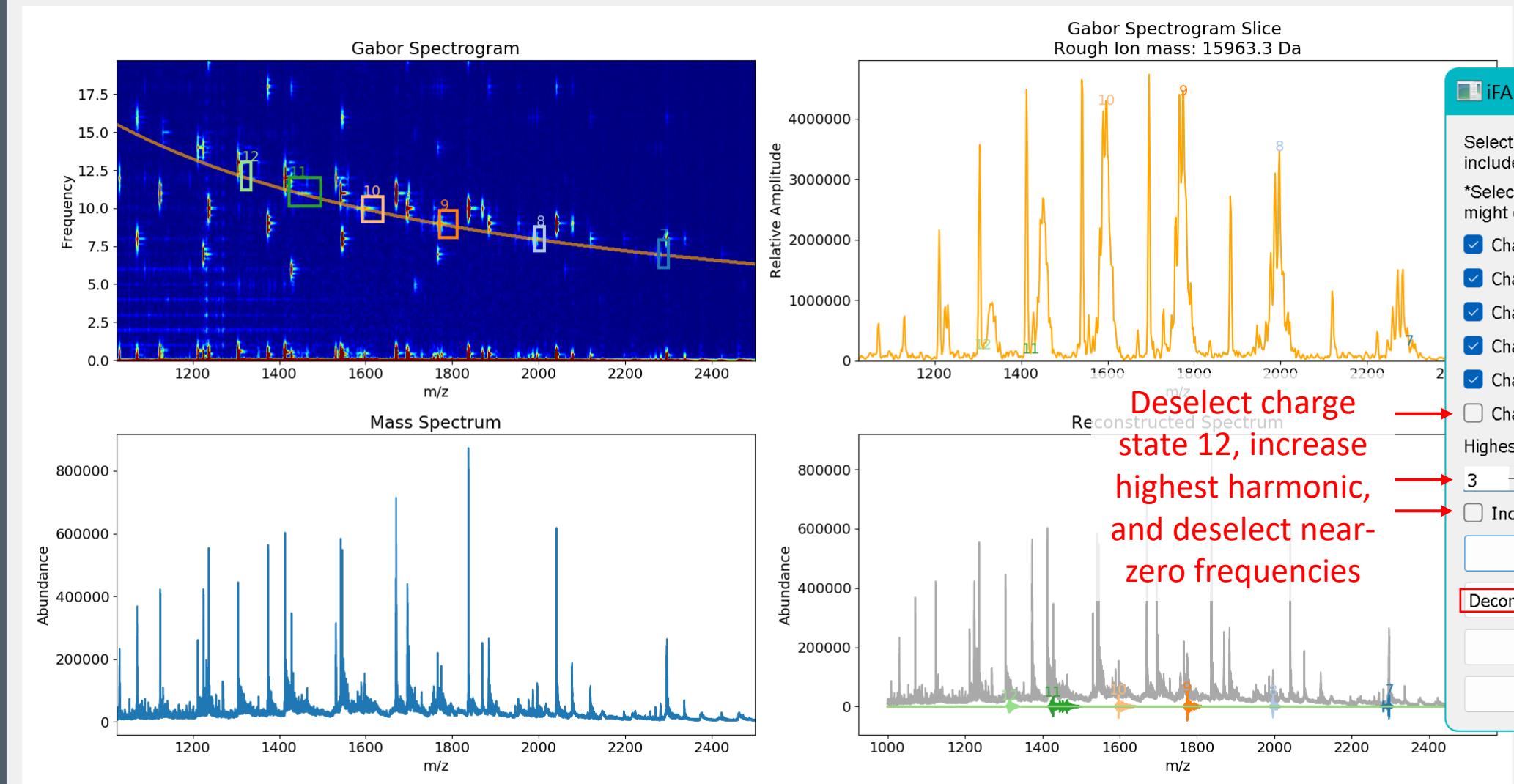


File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode



zoom rect



iFAMS Guided S... ? X

Select charge states to include in deconvolution:

*Selections near ends of spectrum might create windowing artifacts

Charge State 7
 Charge State 8
 Charge State 9
 Charge State 10
 Charge State 11
 Charge State 12

Highest Harmonic to Include

3

Include Near-zero Frequencies

Run iFAMS Deconvolution

Deconvolve and Select Additional Series

Adjust Assigned Charges

Remove Selection

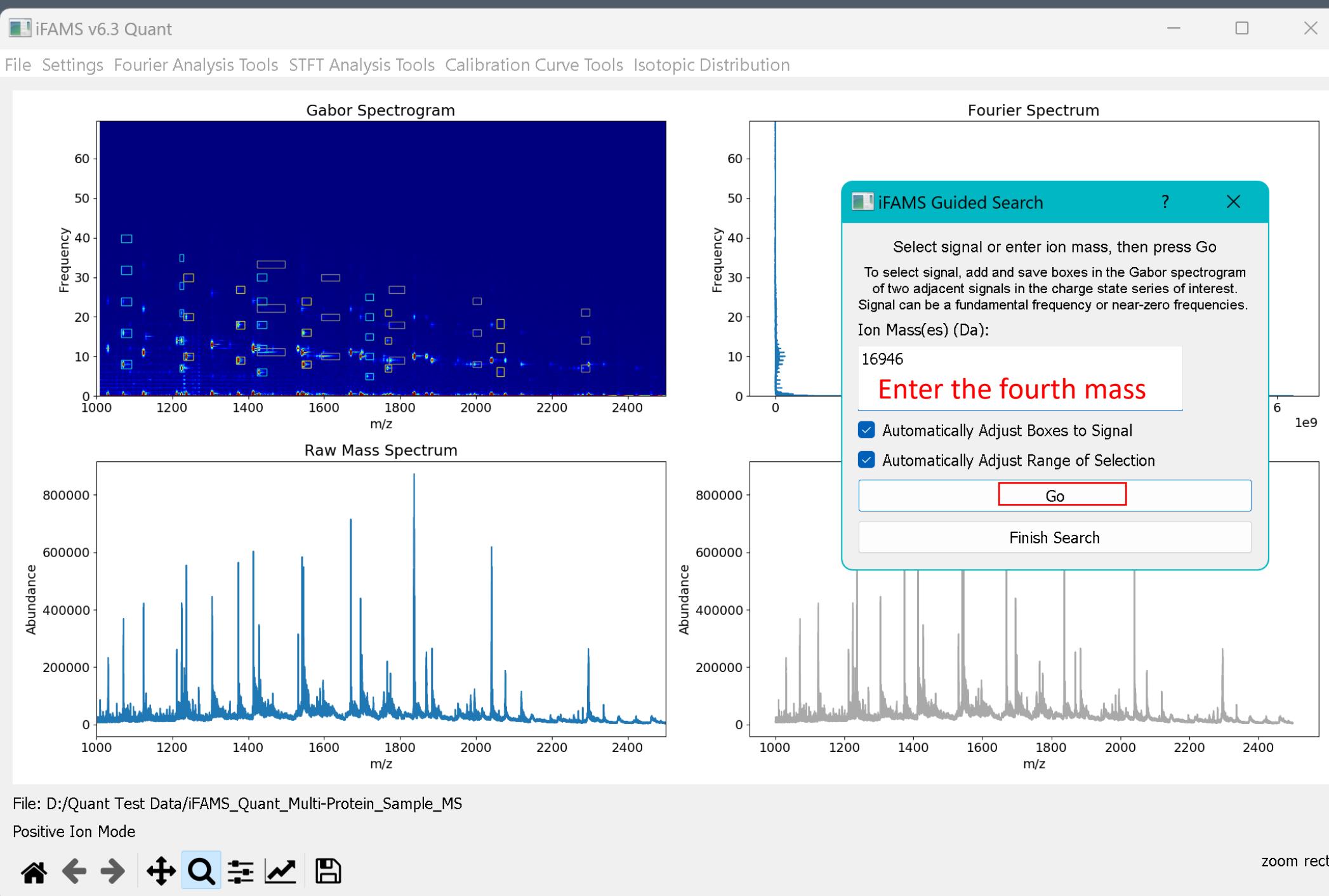
Deselect charge state 12, increase highest harmonic, and deselect near-zero frequencies

File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode



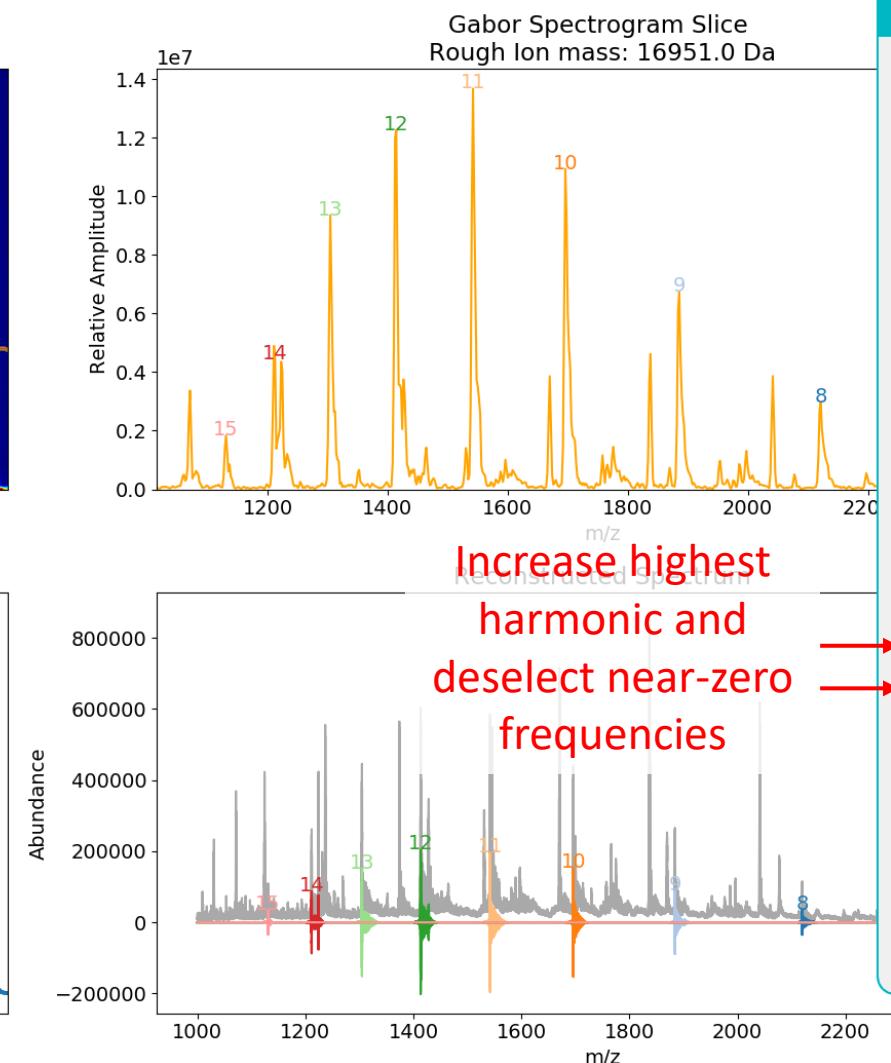
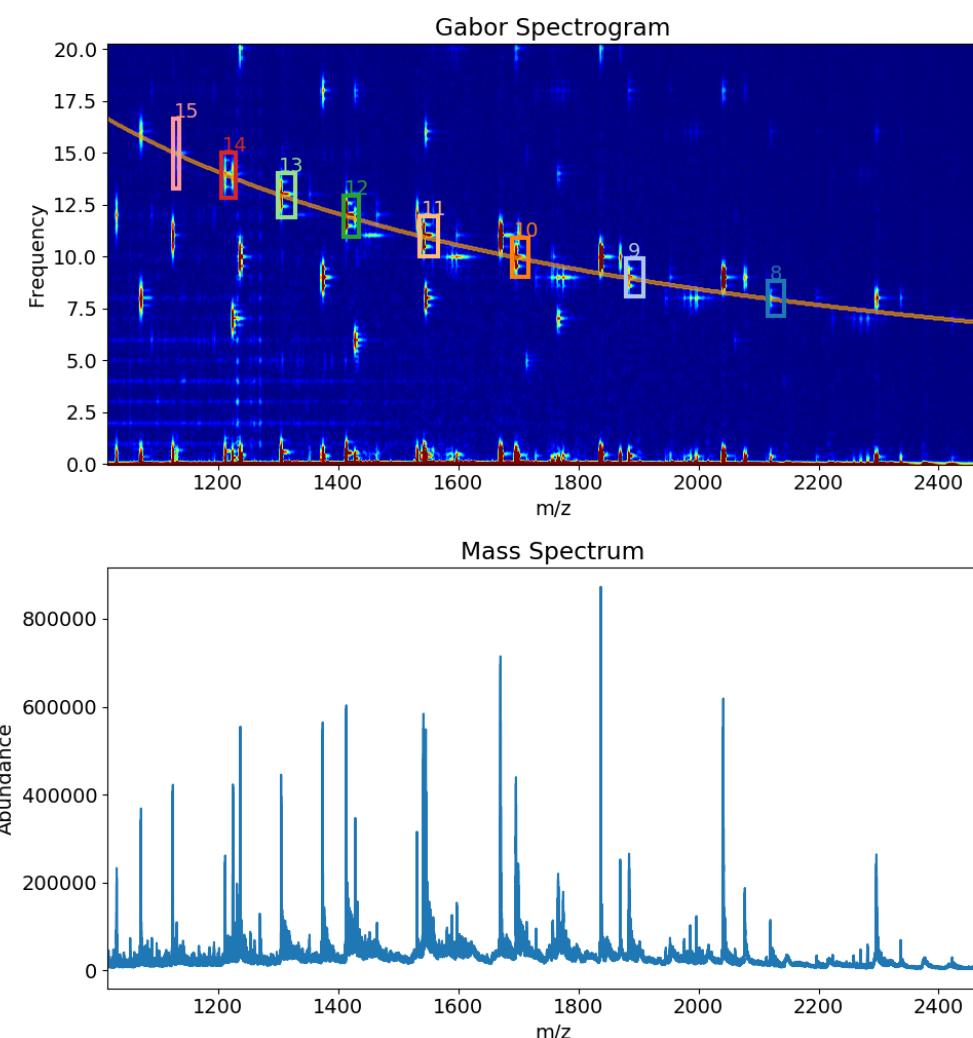
zoom rect



GABOR SLICER (18 of 26)

iFAMS v6.3 Quant

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution



iFAMS Guided S... ? X

Select charge states to include in deconvolution:

*Selections near ends of spectrum might create windowing artifacts

Charge State 8
 Charge State 9
 Charge State 10
 Charge State 11
 Charge State 12
 Charge State 13
 Charge State 14
 Charge State 15

Highest Harmonic to Include

3

Include Near-zero Frequencies

Run iFAMS Deconvolution

Deconvolve and Select Additional Series

Adjust Assigned Charges

Remove Selection

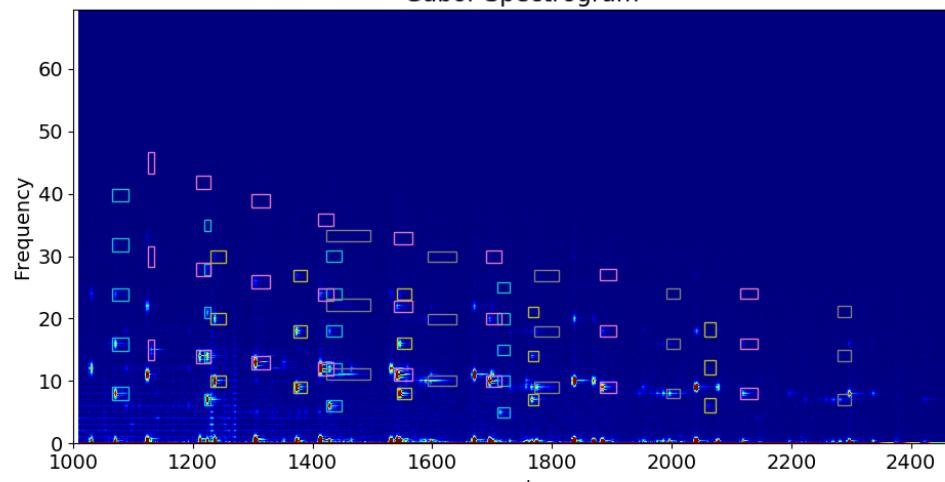
File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode

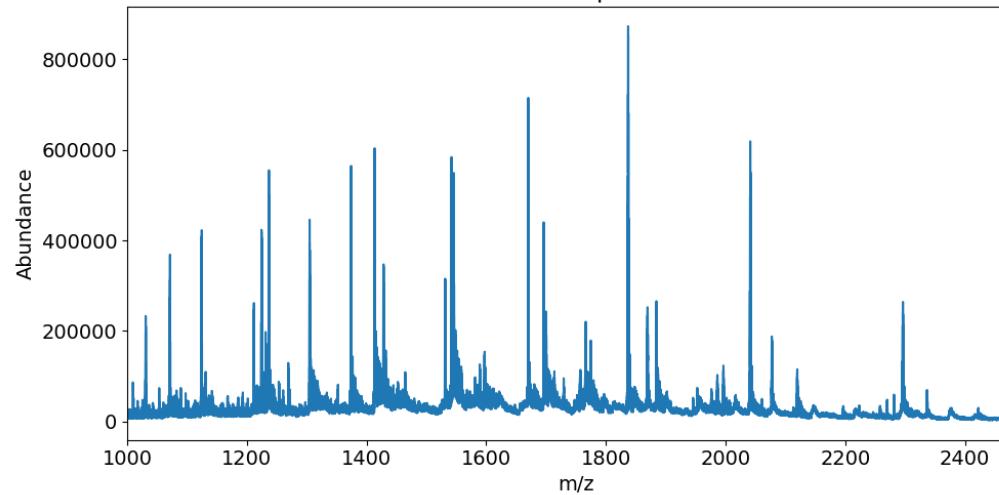


zoom rect

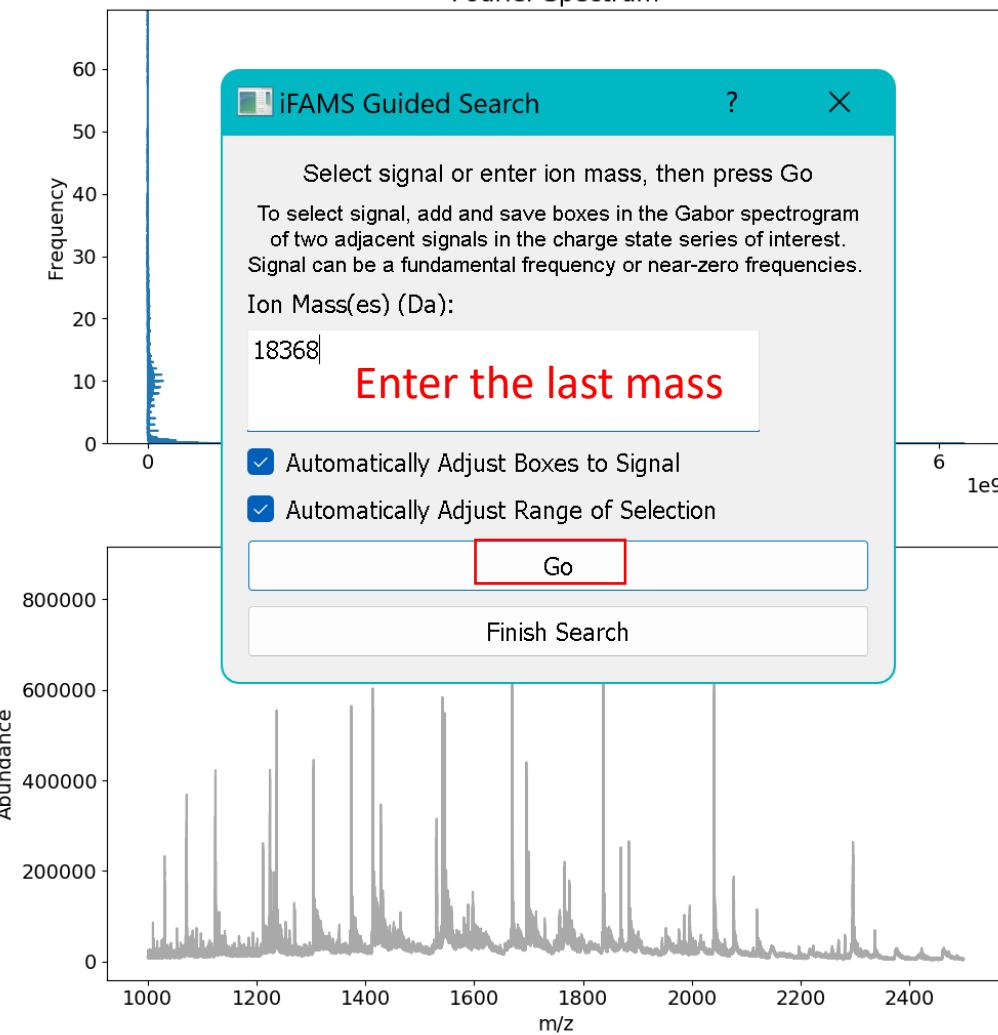
Gabor Spectrogram



Raw Mass Spectrum



Fourier Spectrum



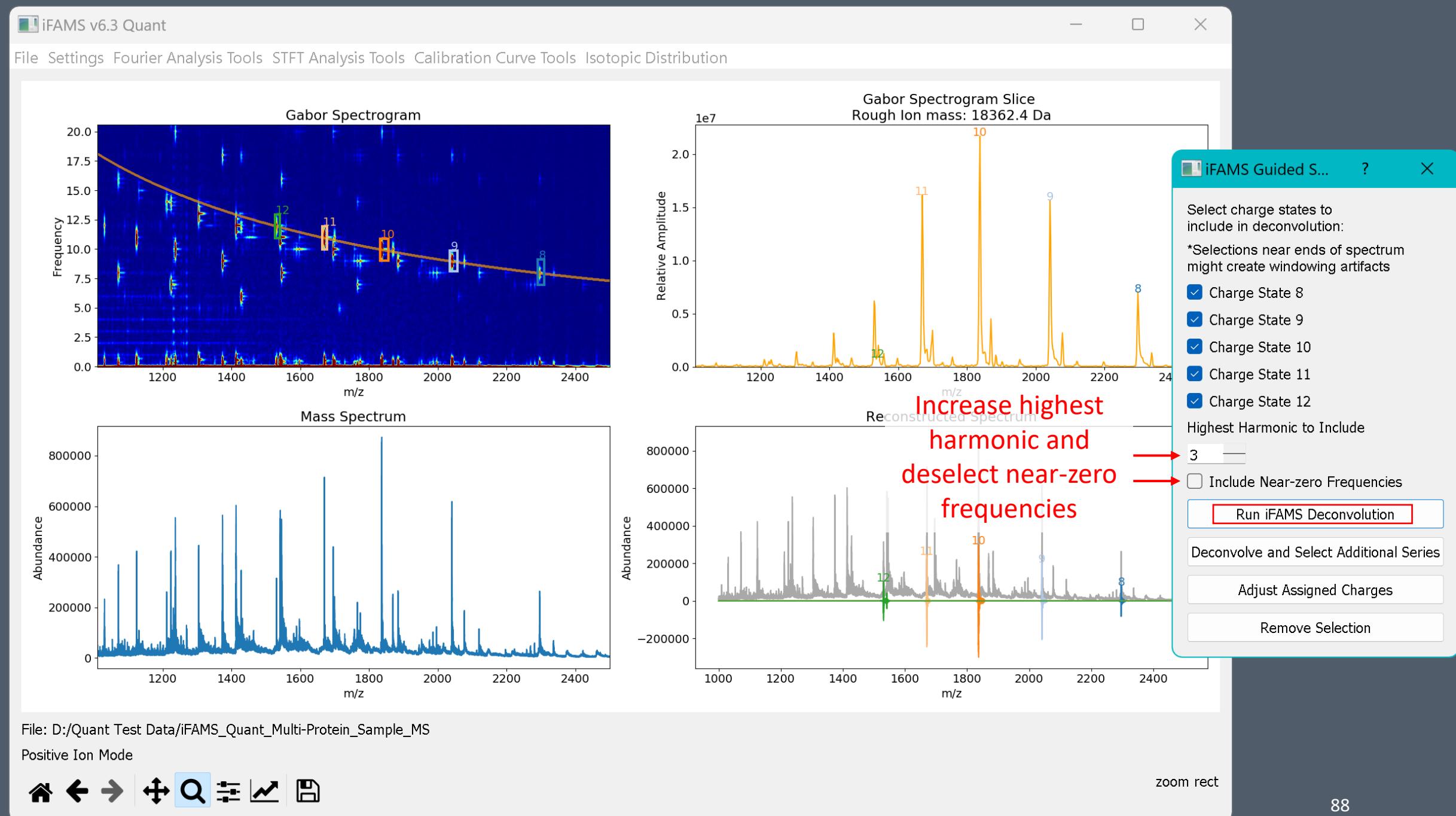
File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode

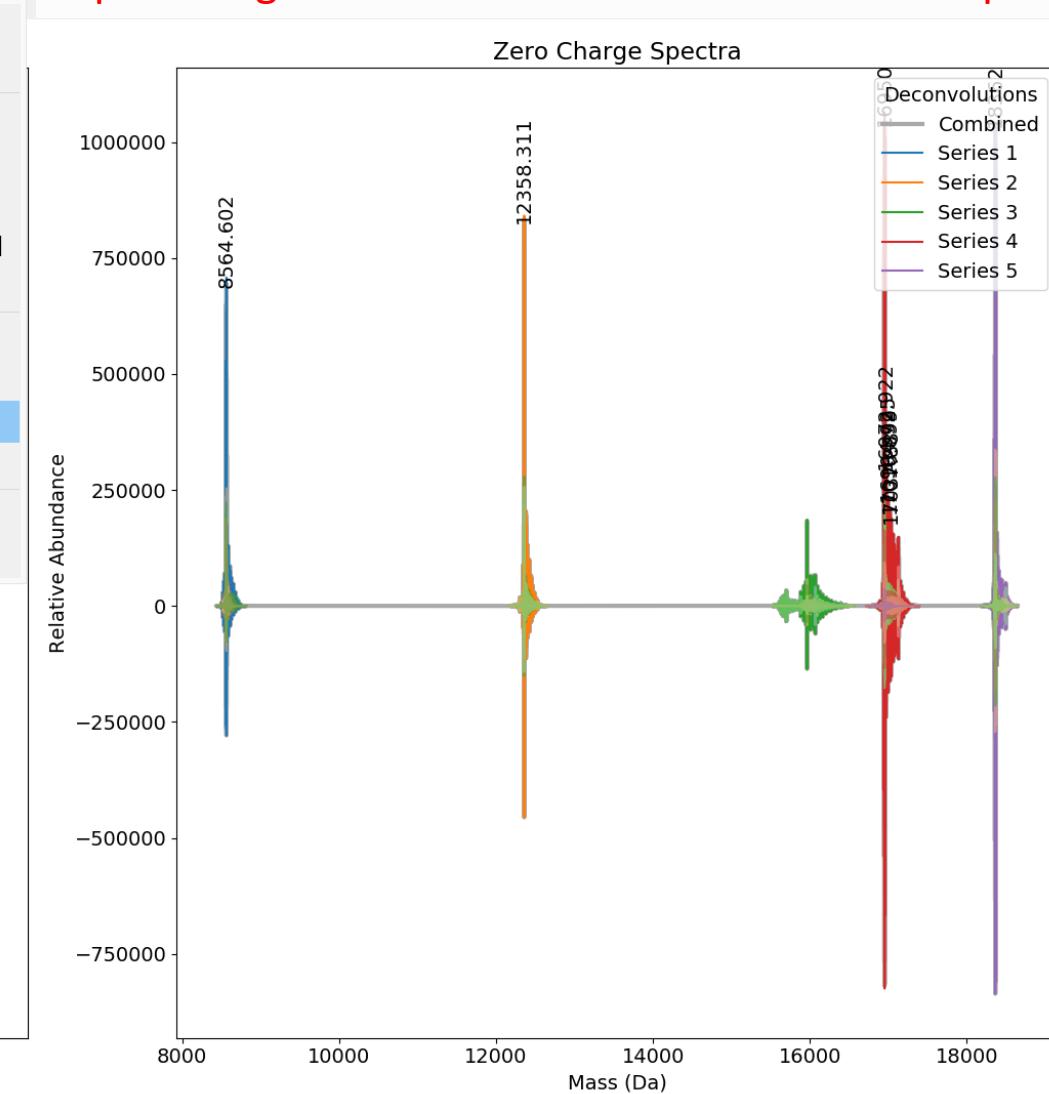
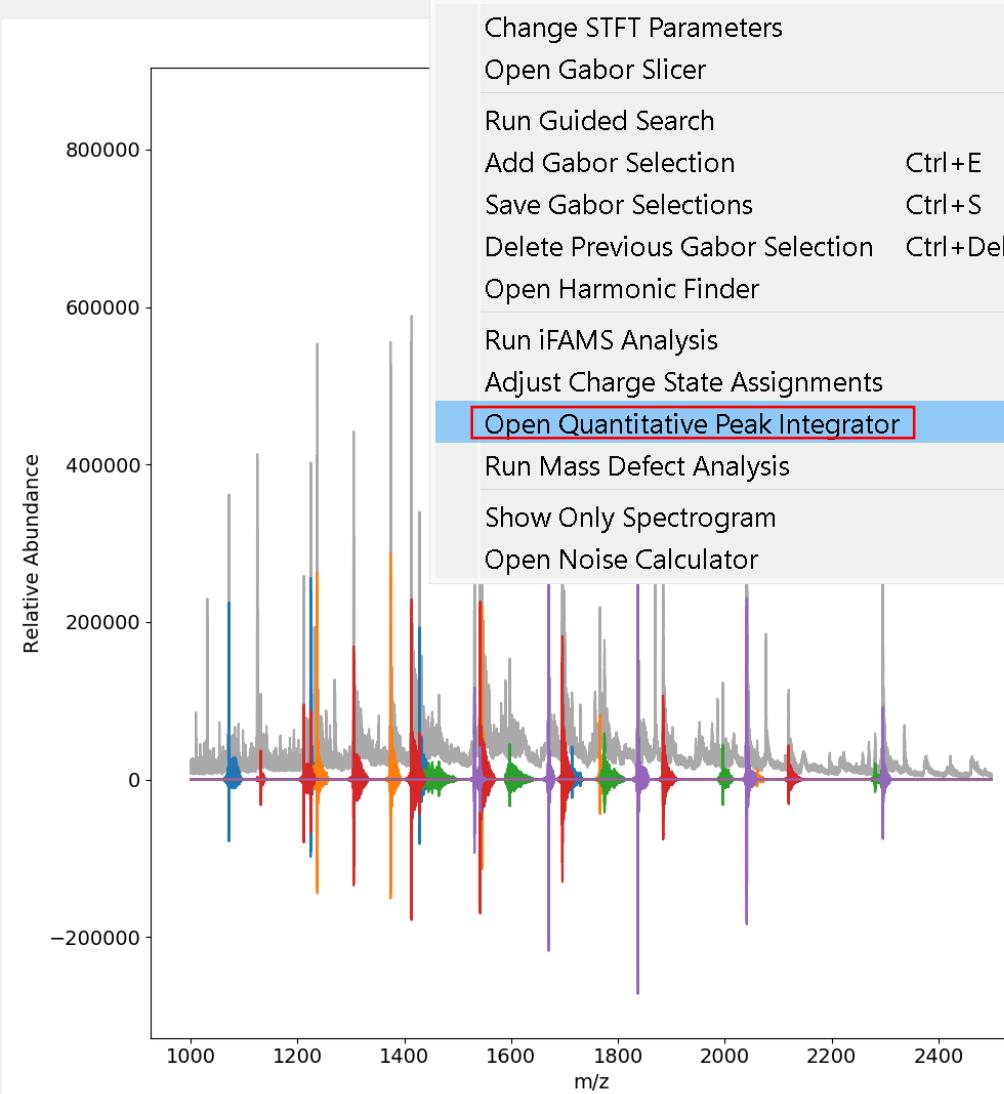


zoom rect

GABOR SLICER (20 of 26)



File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Pattern Tools Open Integrator to access baseline correction options



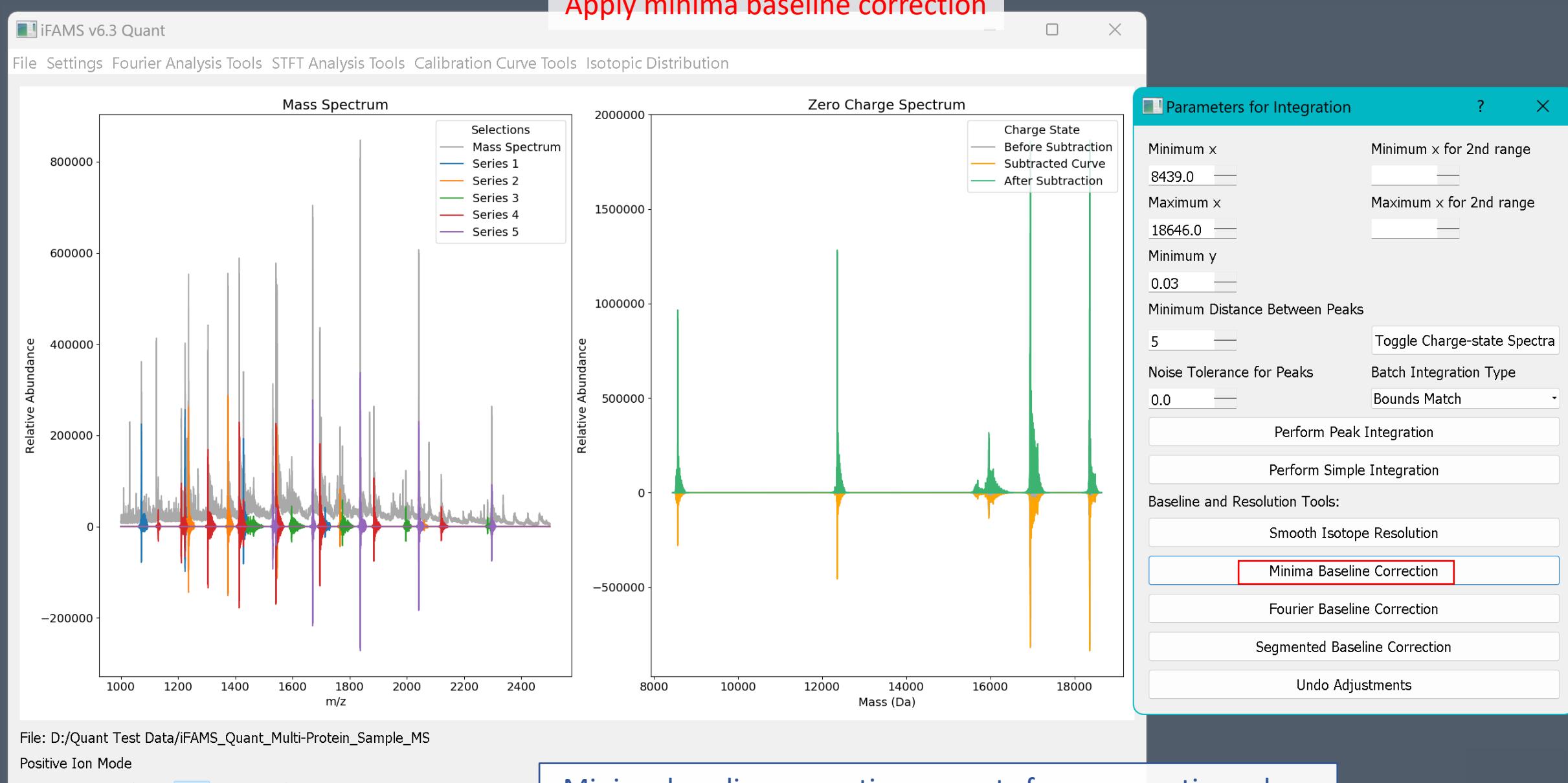
File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode

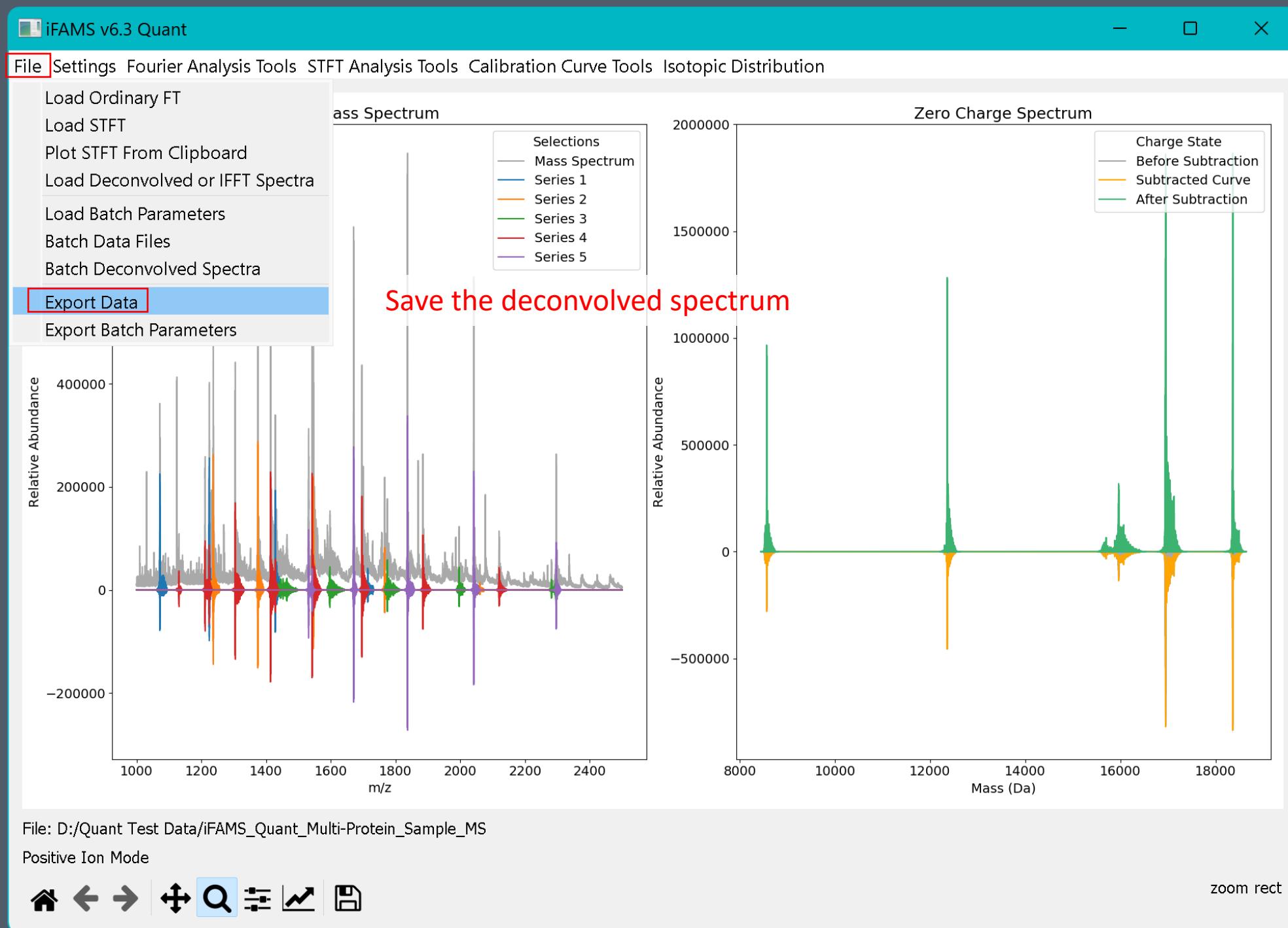


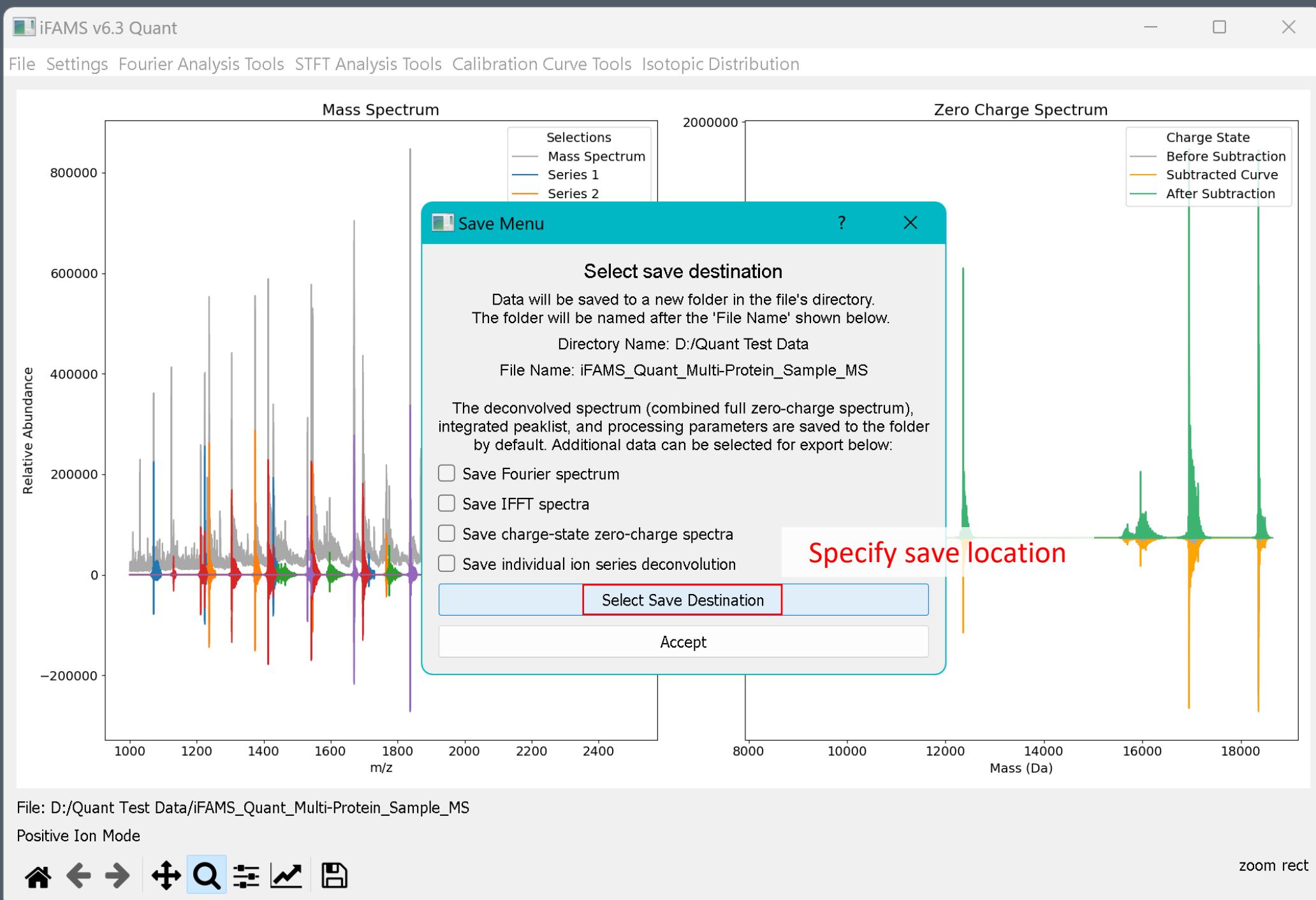
Without near-zero frequencies, deconvolved signal will oscillate about 0.

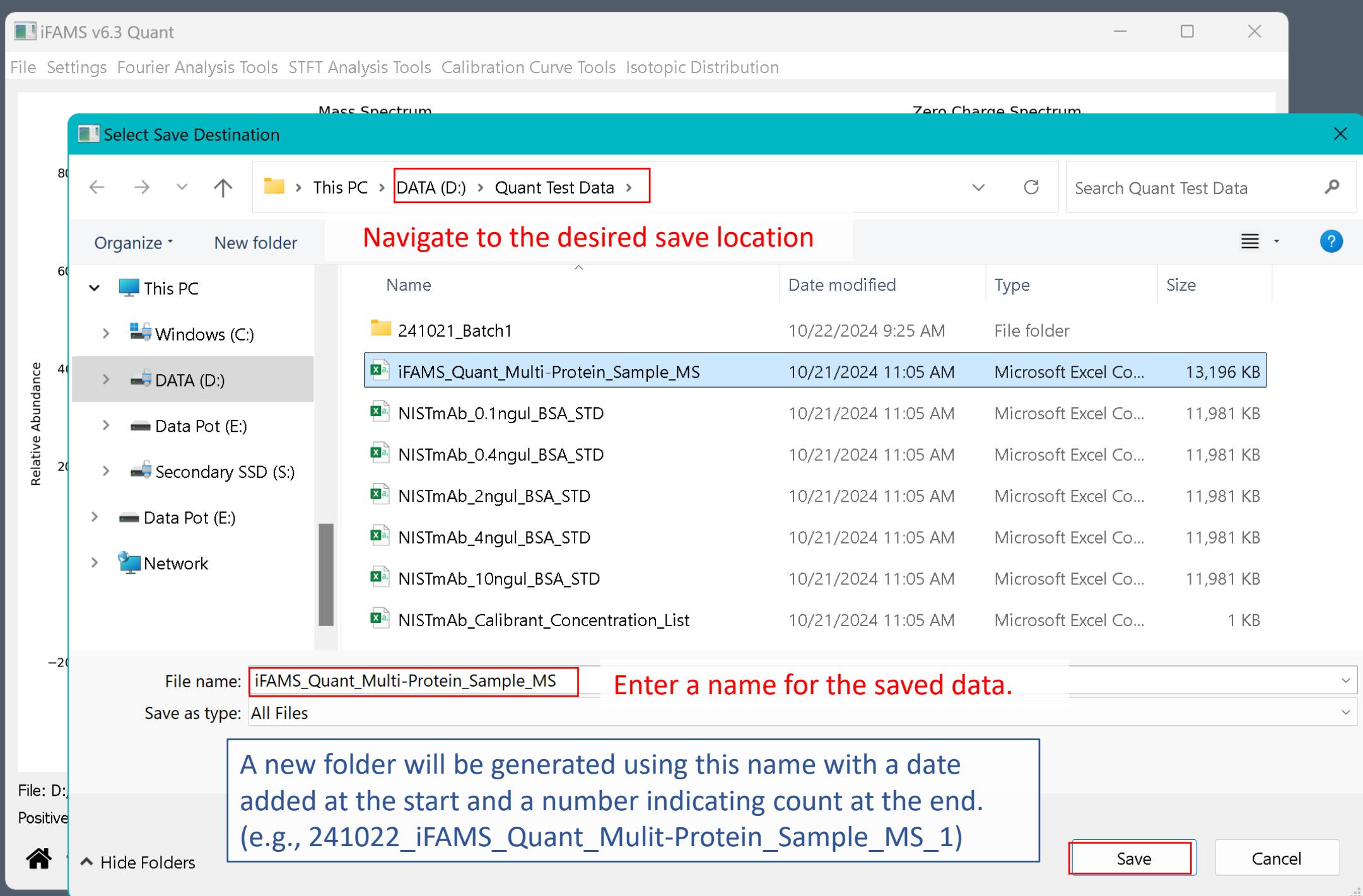
zoom rect

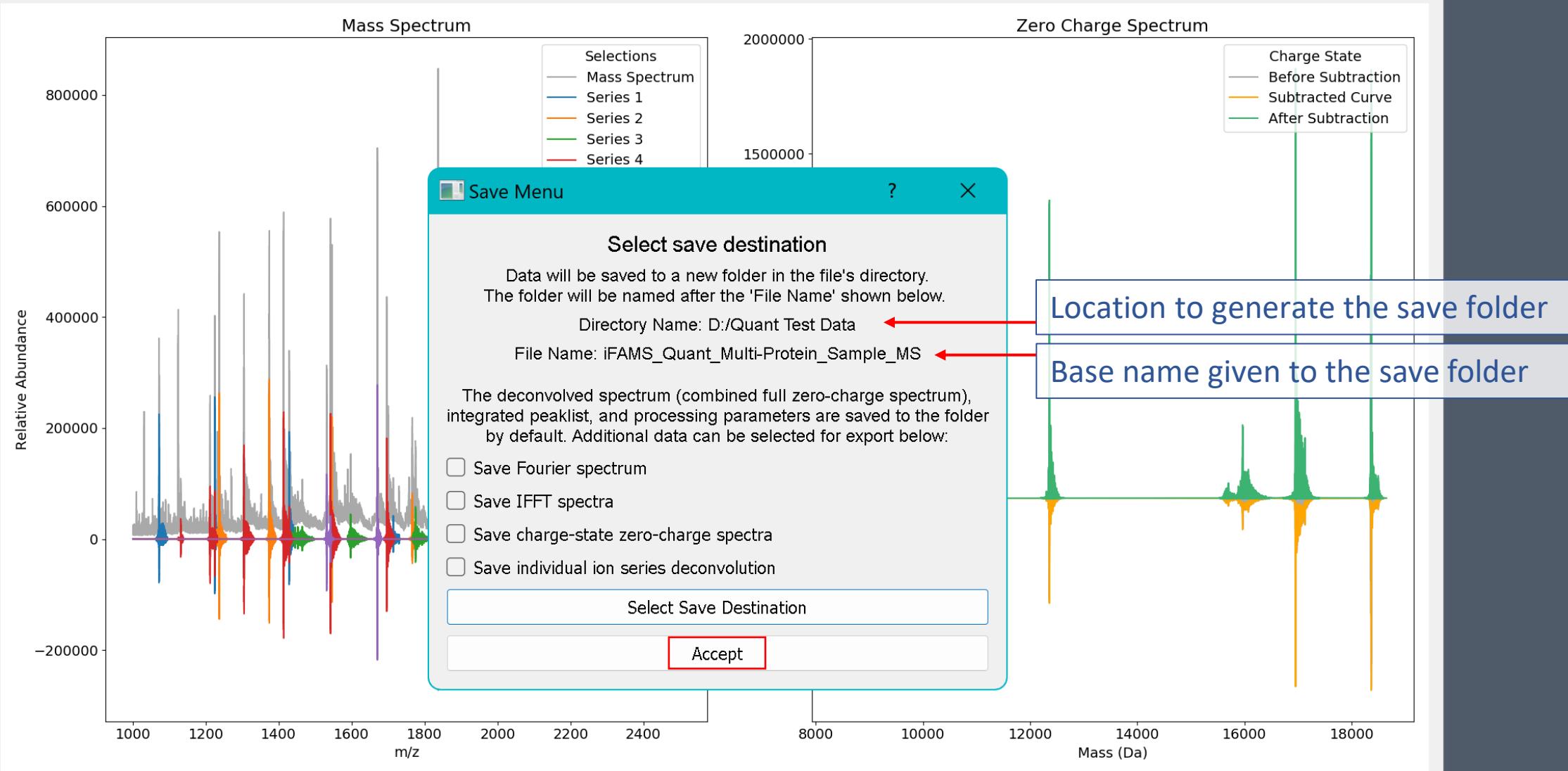


Minima baseline correction corrects for any negative values and is recommended if near-zero frequencies are excluded









File: D:/Quant Test Data/iFAMS_Quant_Multi-Protein_Sample_MS

Positive Ion Mode



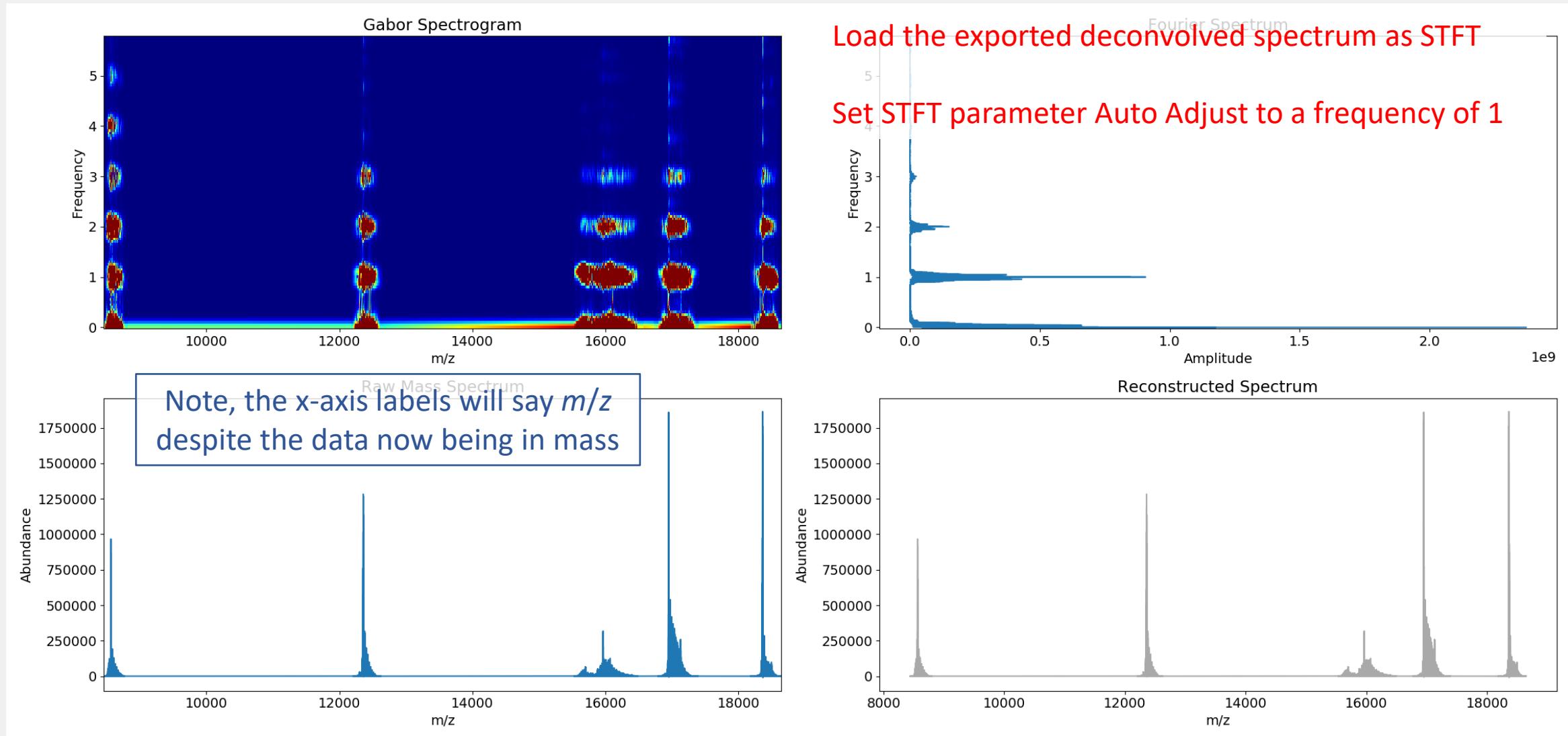
zoom rect

Deconvolution Refinement

Required data: Multi-protein sample

- Refinement of preliminary deconvolution by secondary STFT filtration

DECONVOLUTION REFINEMENT (1 of 24)

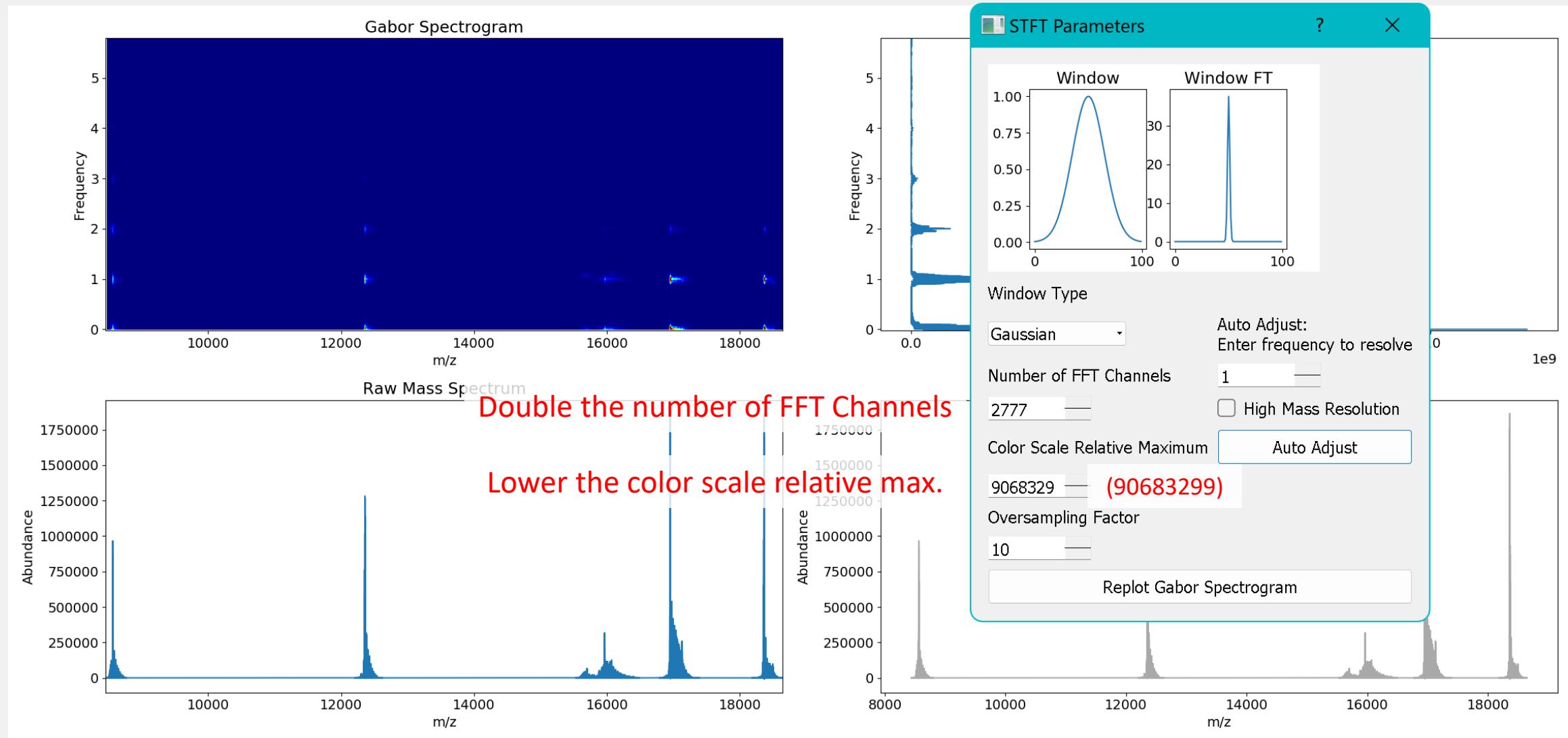


File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode



DECONVOLUTION REFINEMENT (2 of 24)



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/1_iFAMS_Quant_Multi-Protein_Sample_MS_decon_combine.nex

Positive Ion Mode

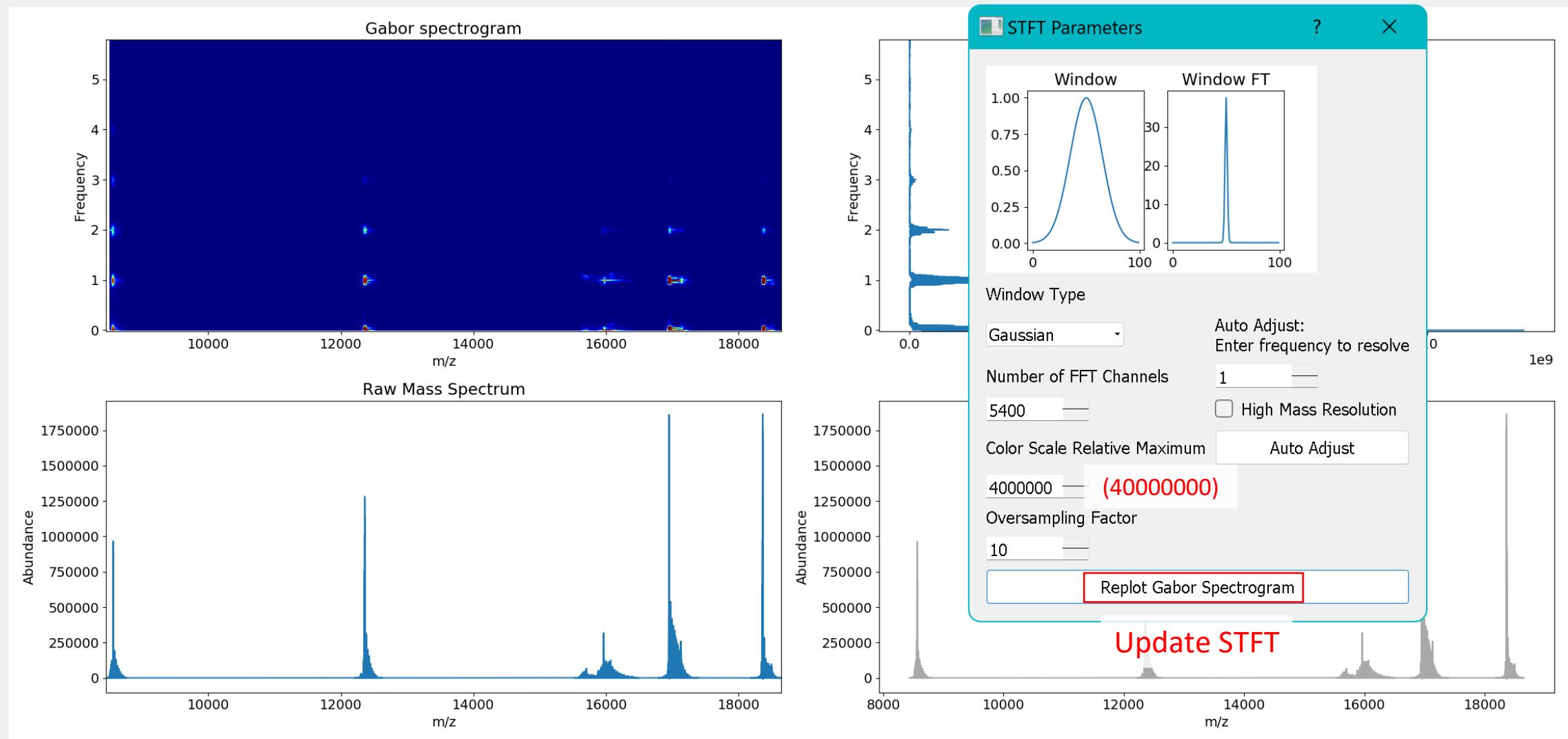


zoom rect
97

DECONVOLUTION REFINEMENT (3 of 24)

iFAMS v6.3 Quant

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution



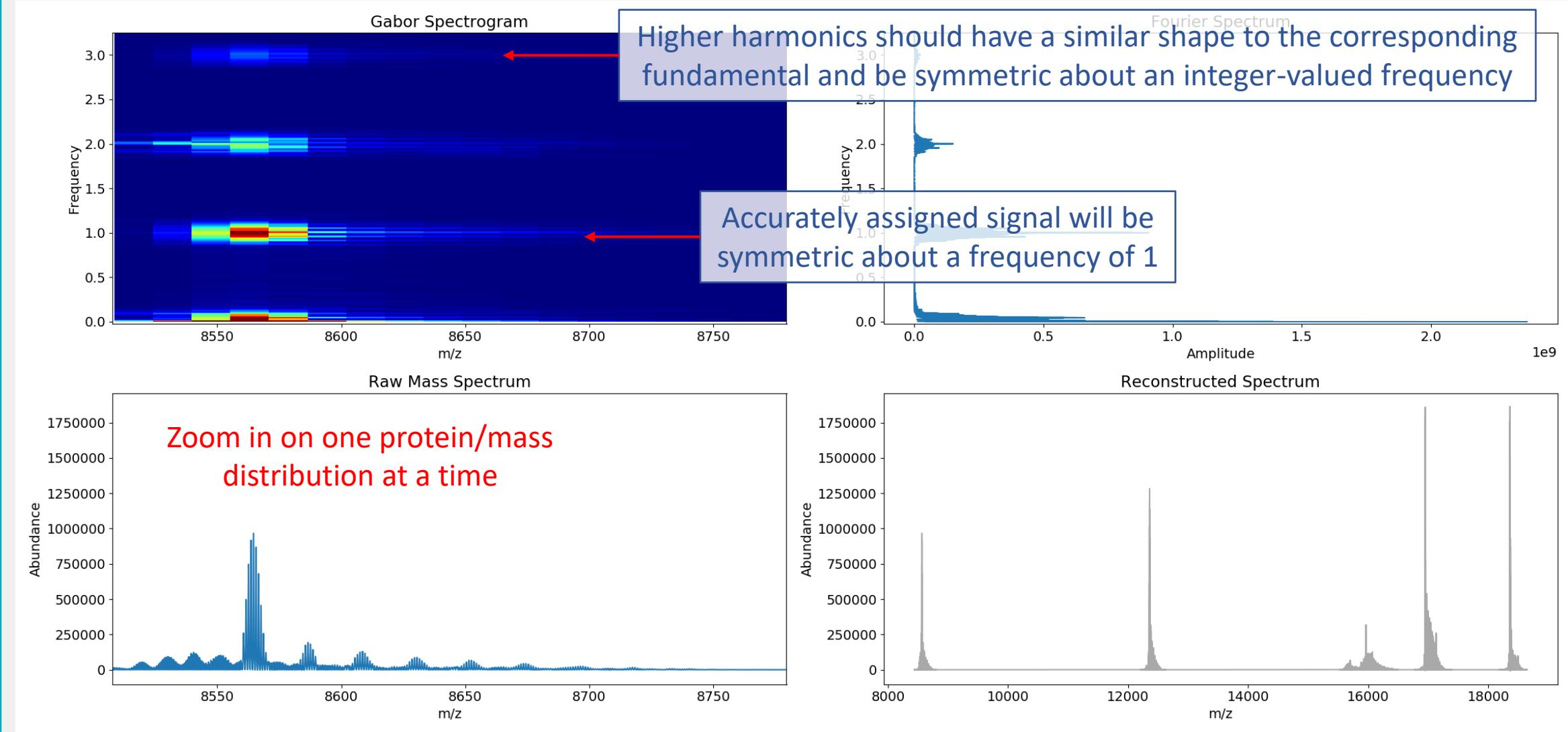
File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode



zoom rect
98

DECONVOLUTION REFINEMENT (4 of 24)



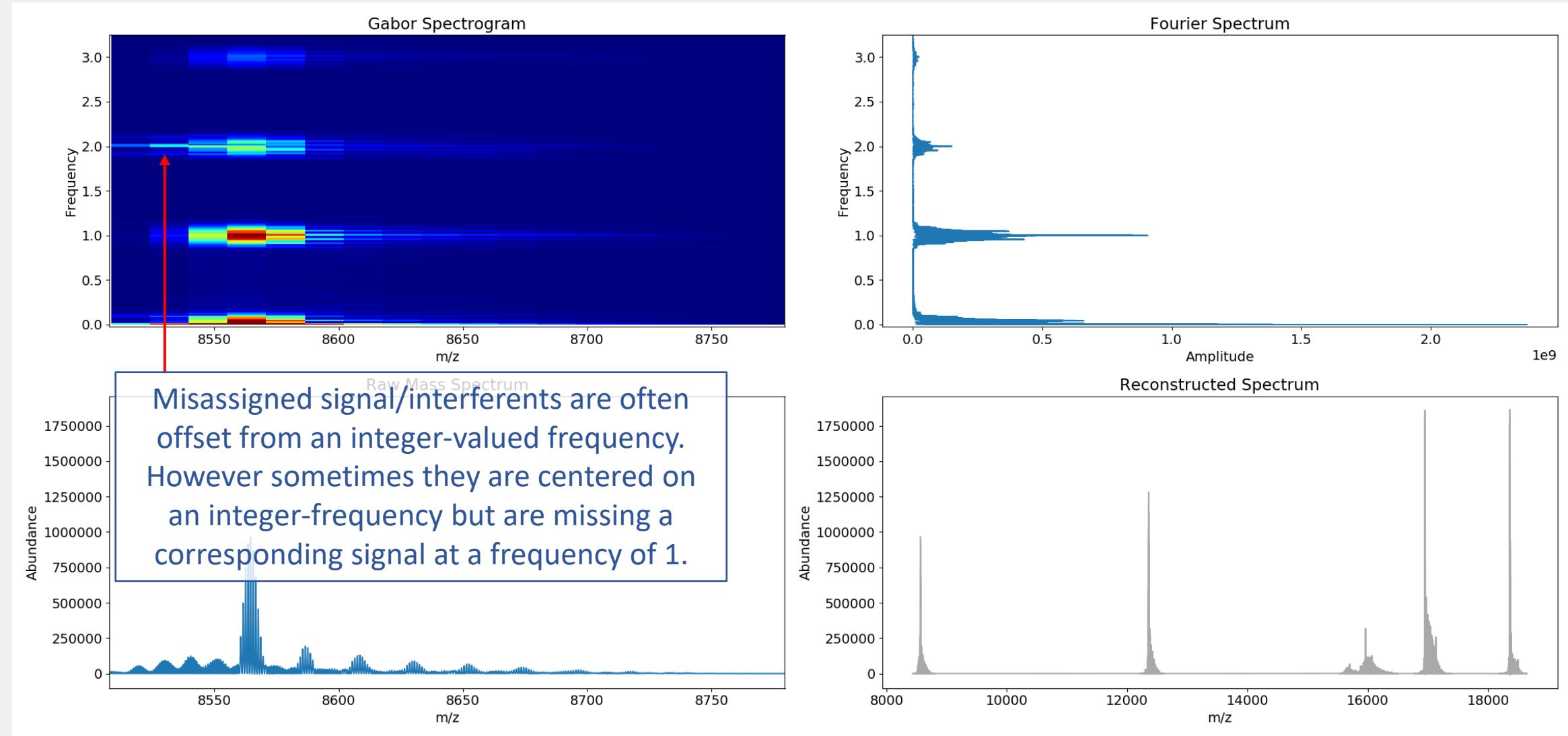
File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode



zoom rect
99

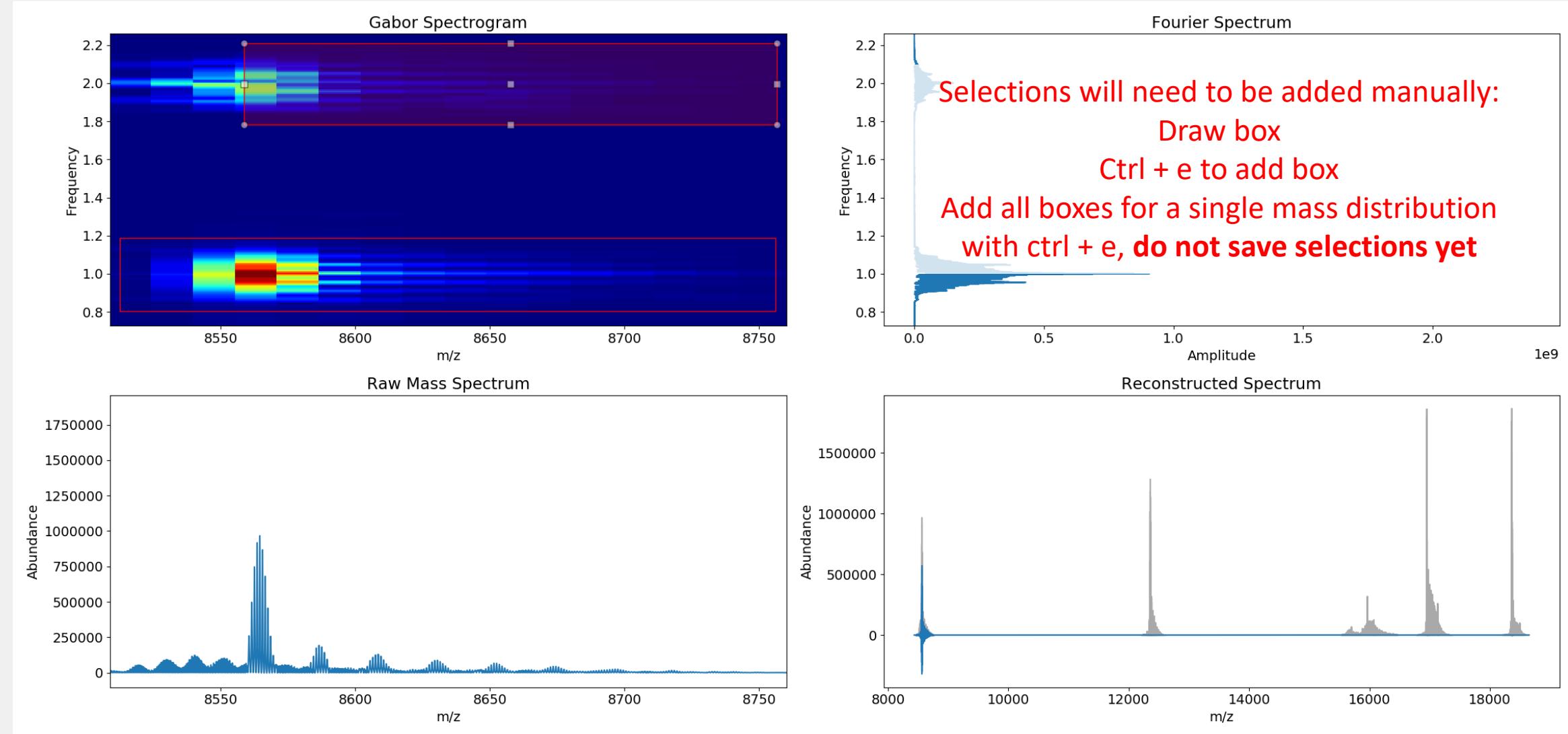
DECONVOLUTION REFINEMENT (5 of 24)



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

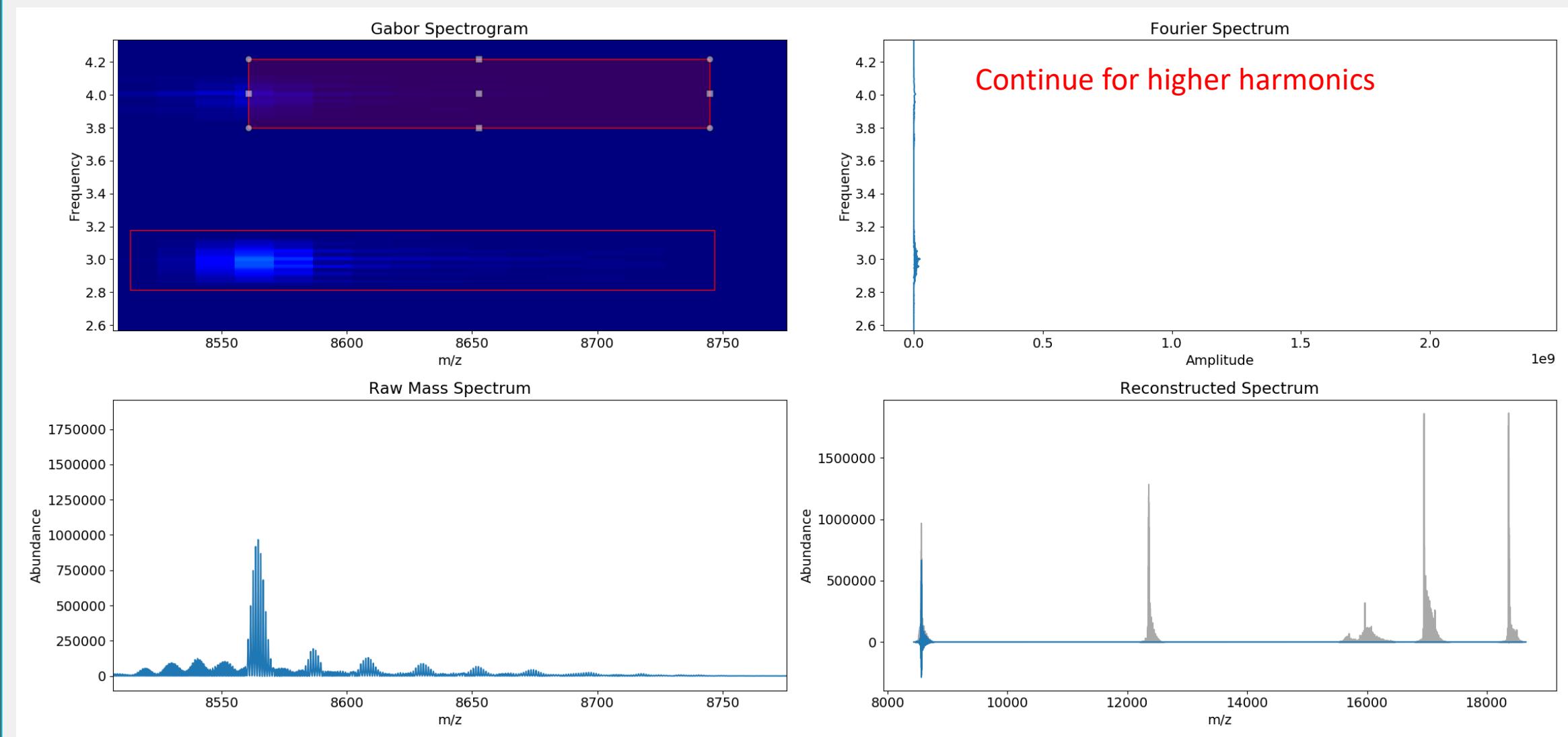
DECONVOLUTION REFINEMENT (6 of 24)



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

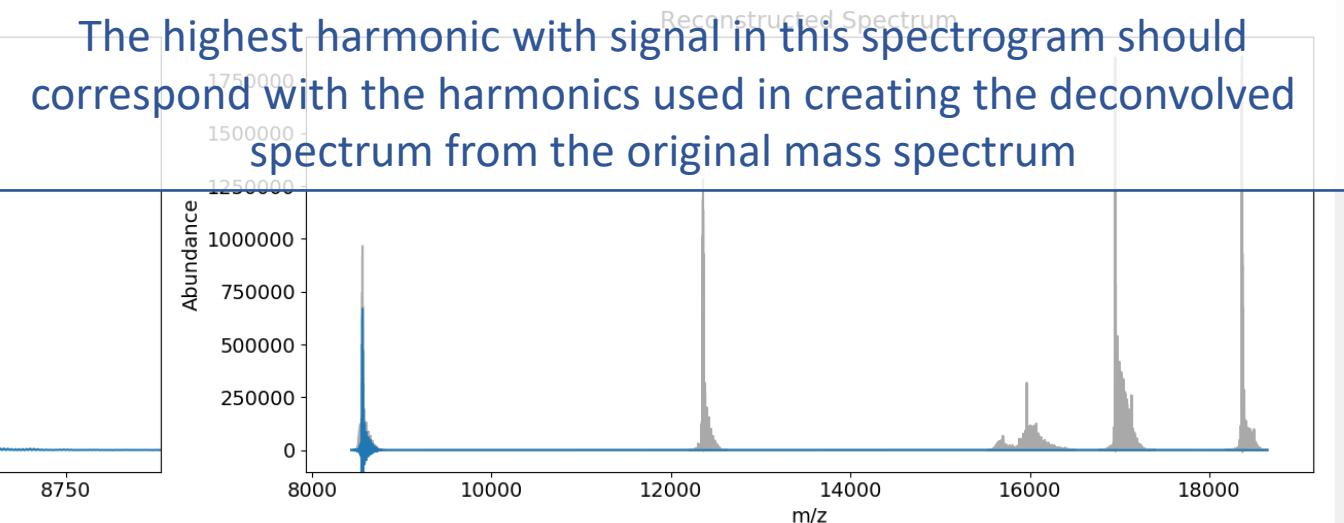
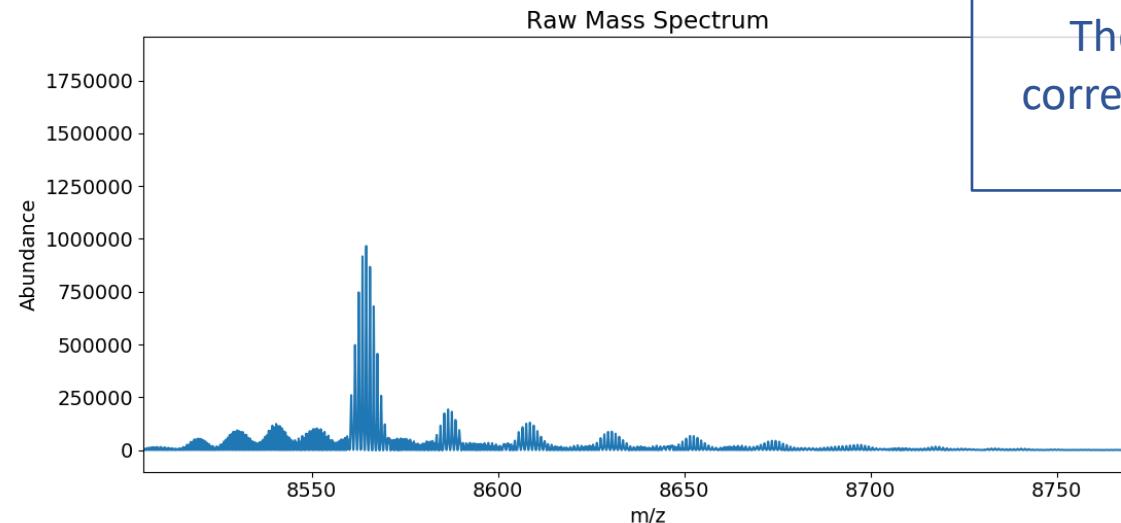
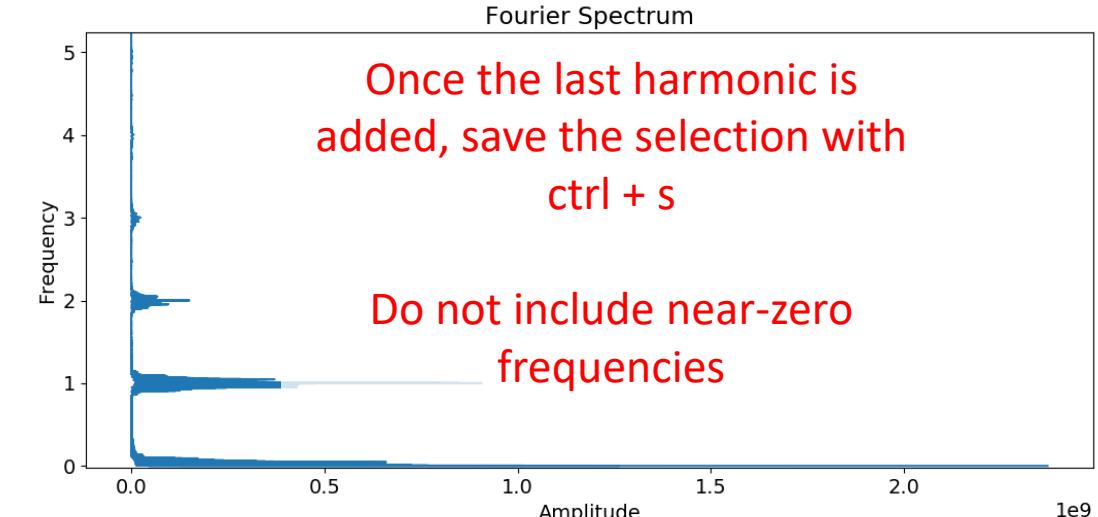
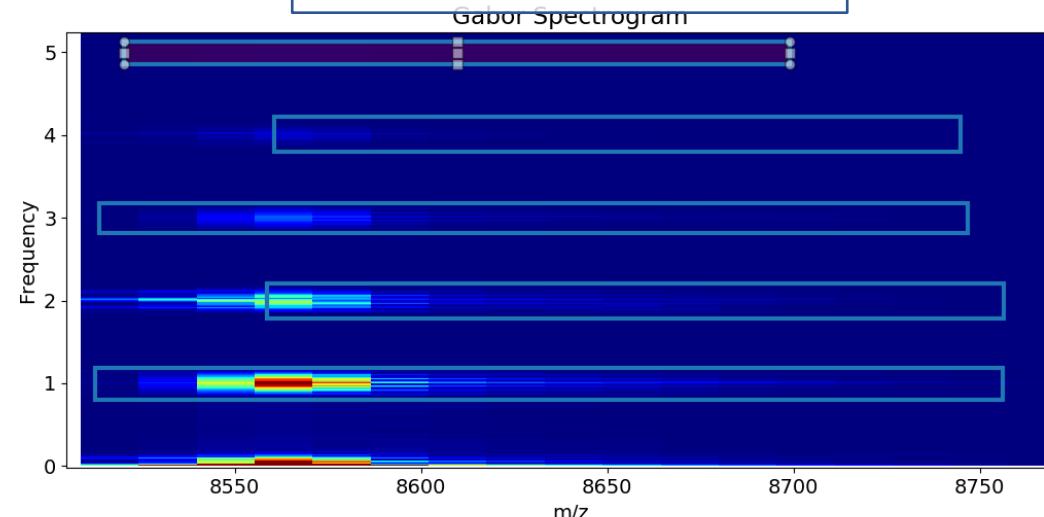
DECONVOLUTION REFINEMENT (7 of 24)



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

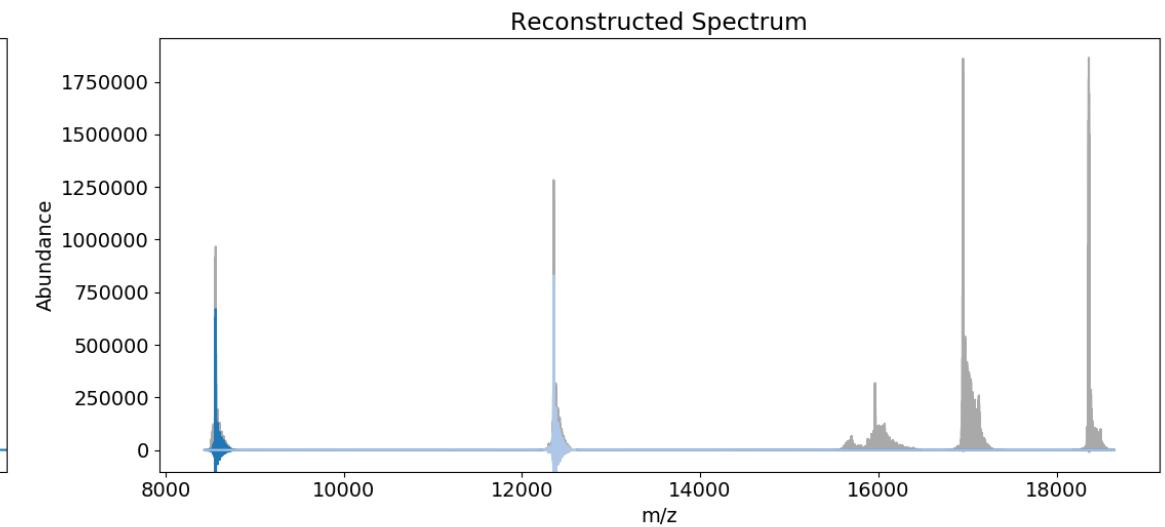
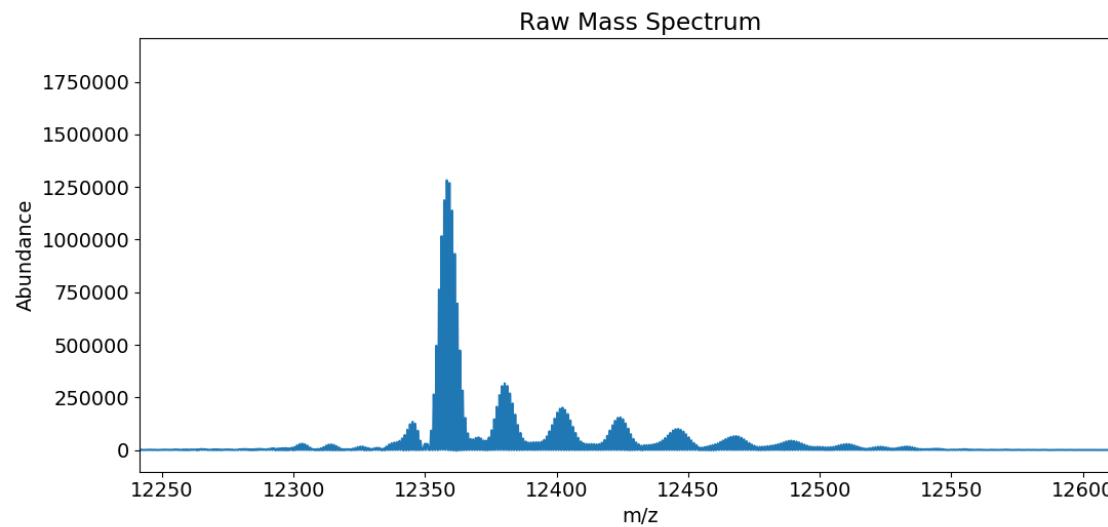
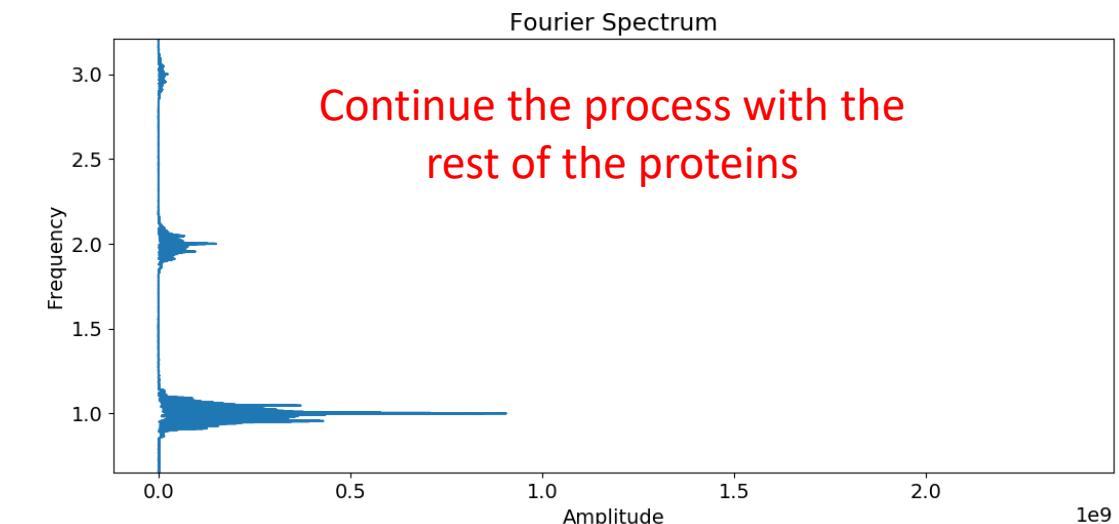
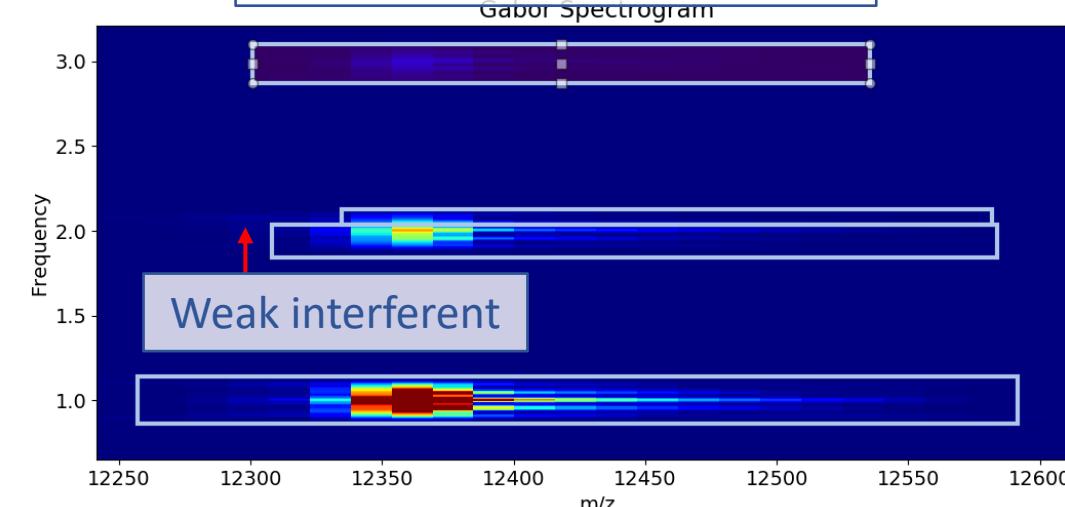
Selections for ubiquitin



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

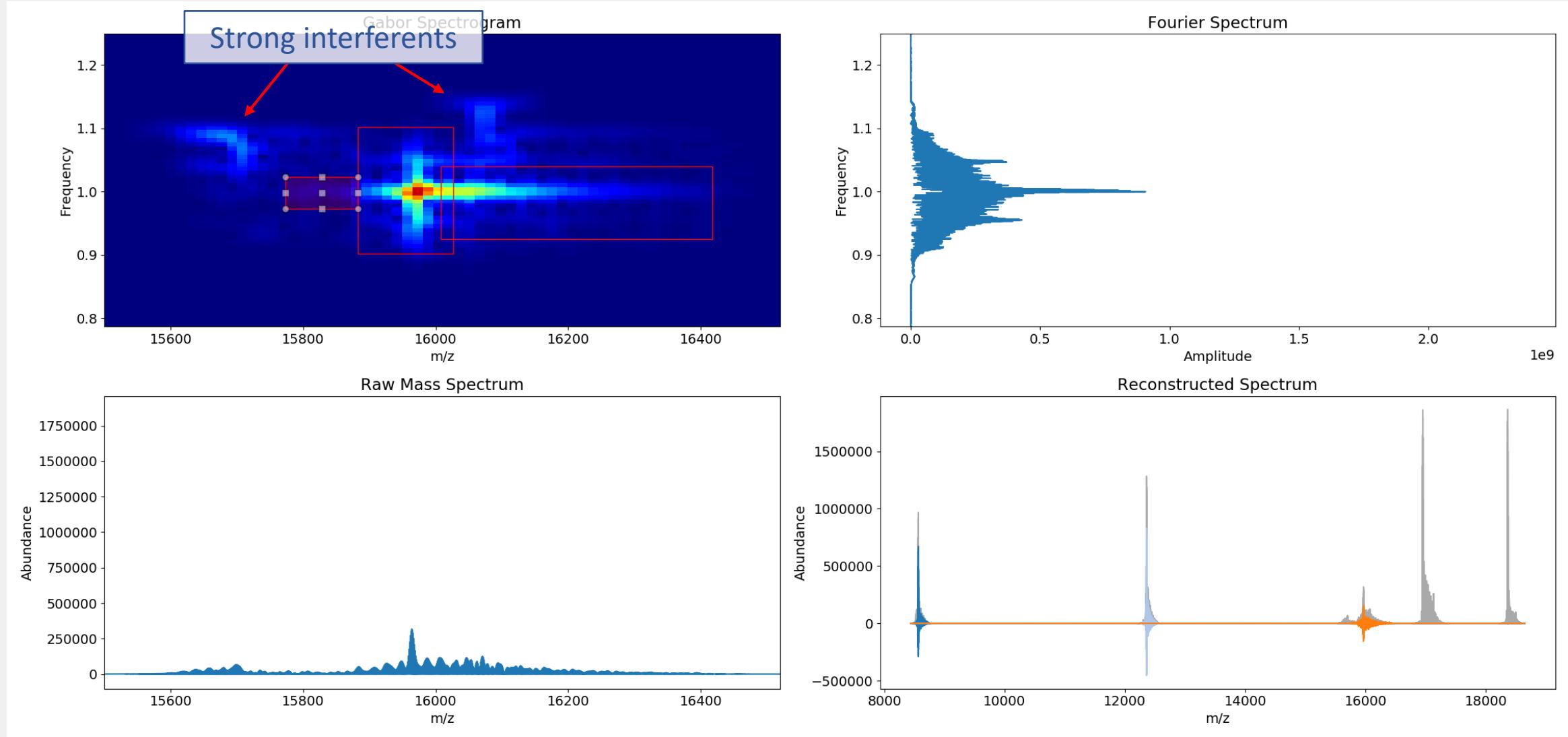
Selections for cytochrome c



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

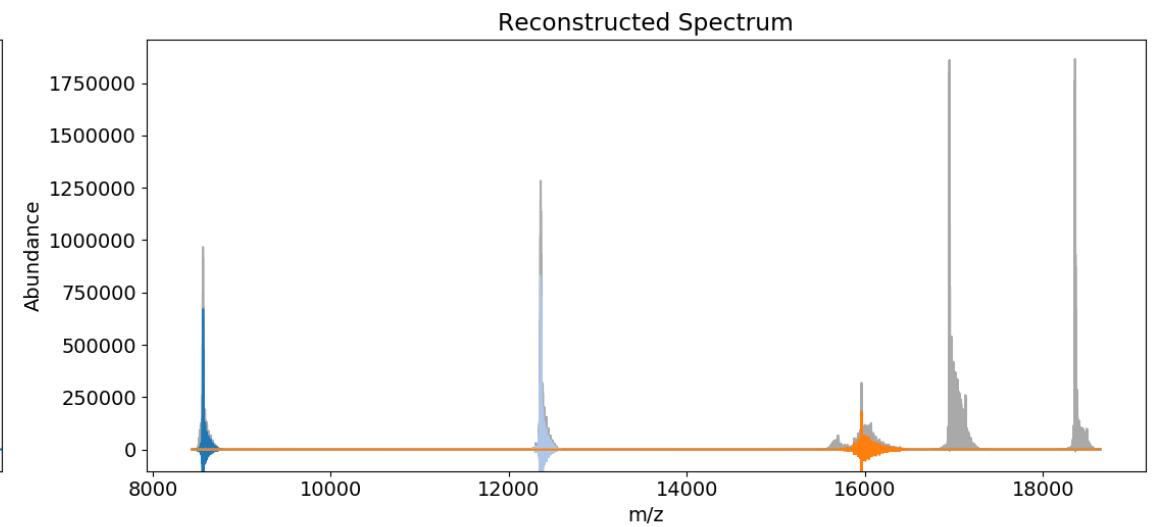
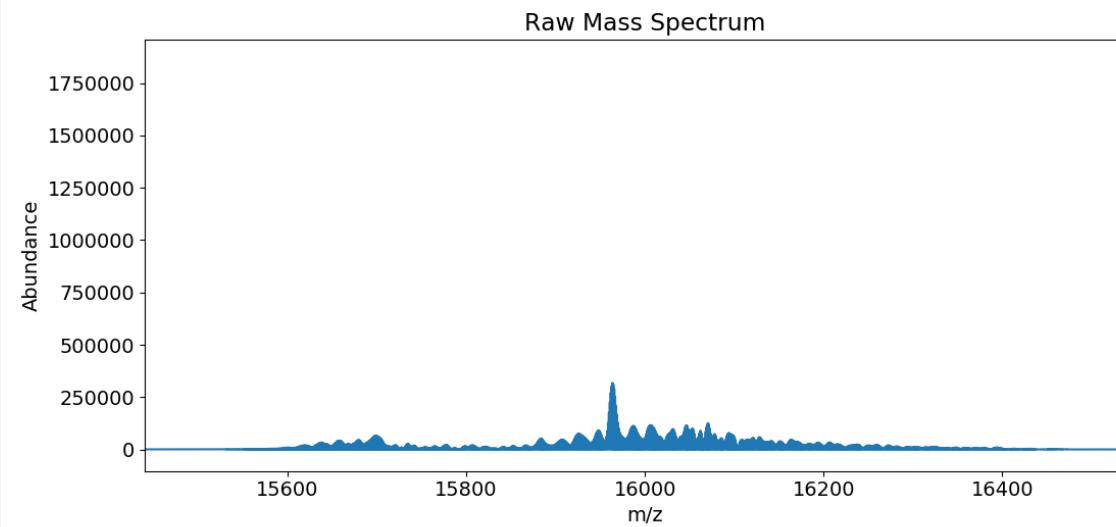
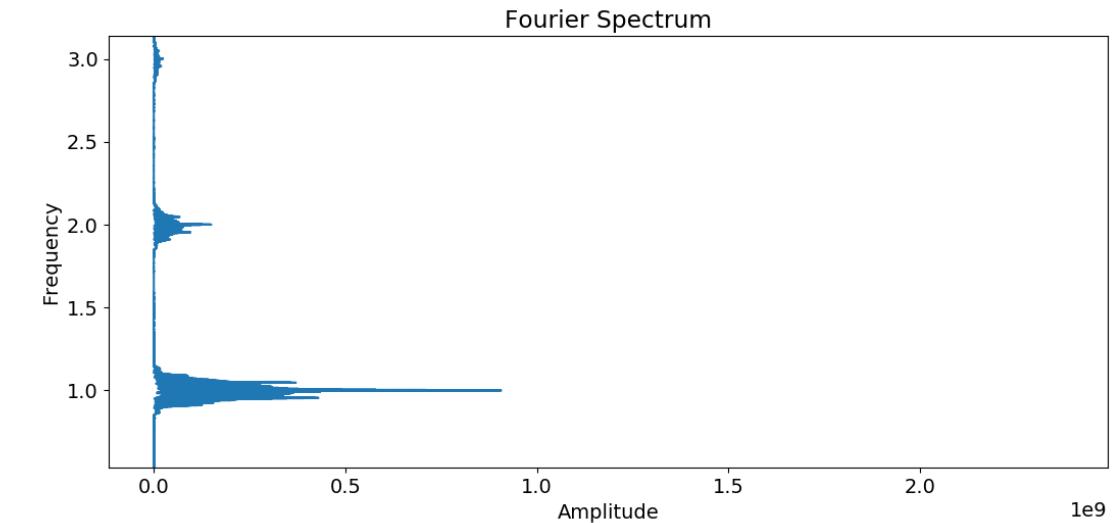
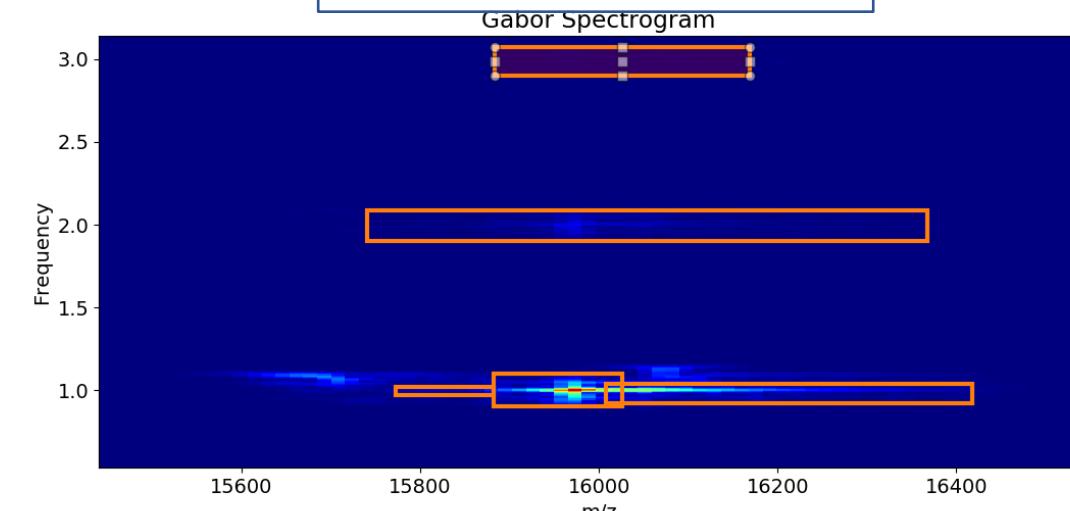
DECONVOLUTION REFINEMENT (10 of 24)



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

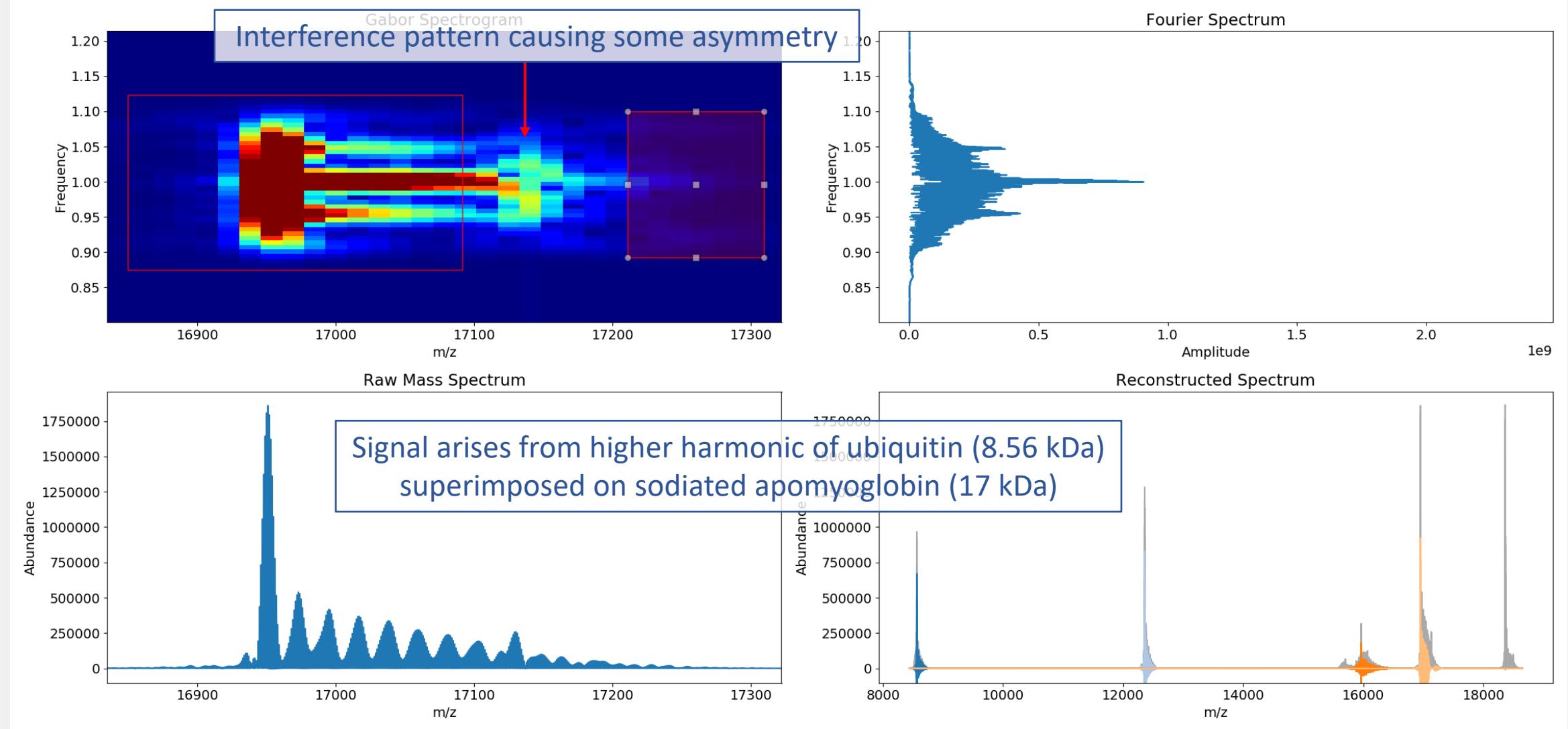
Selections for avidin



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

DECONVOLUTION REFINEMENT (12 of 24)



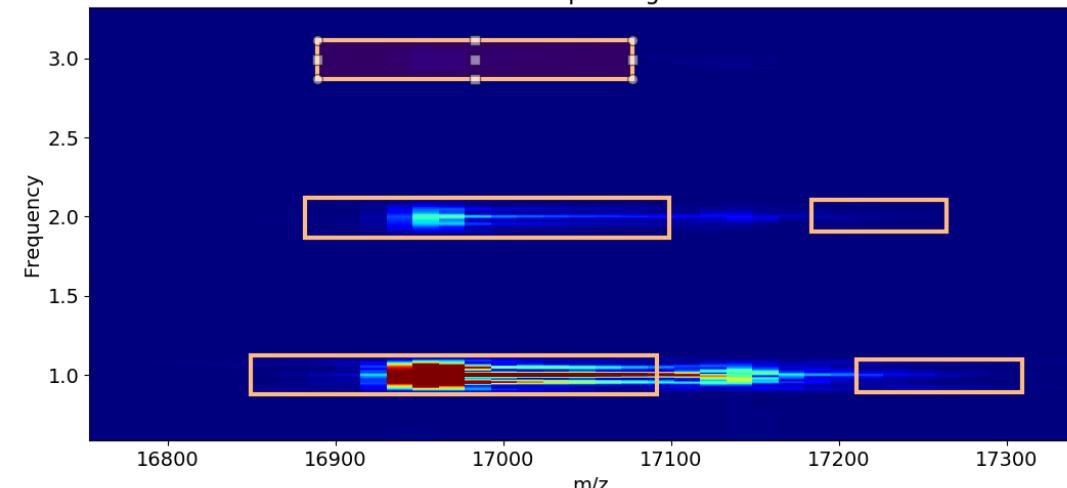
File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

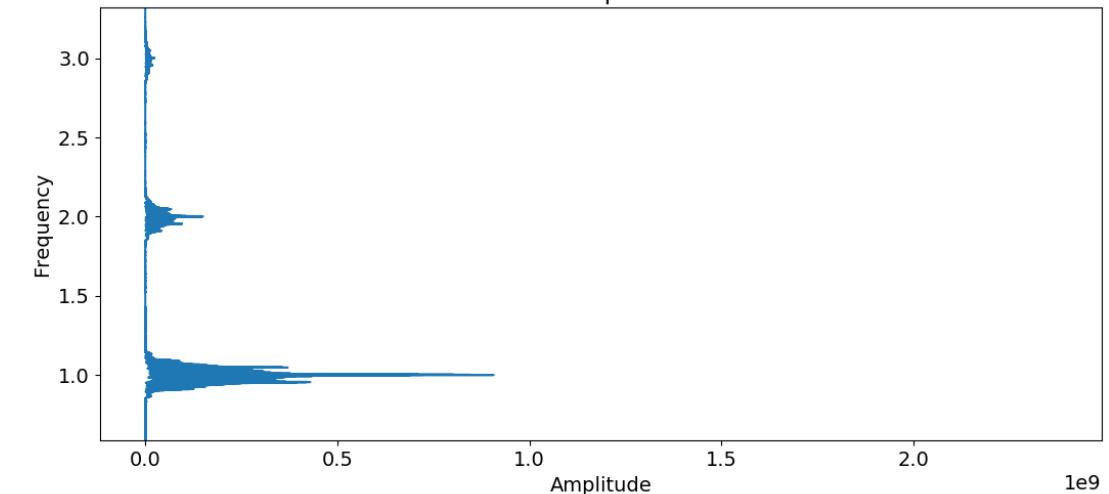


Selections for myoglobin

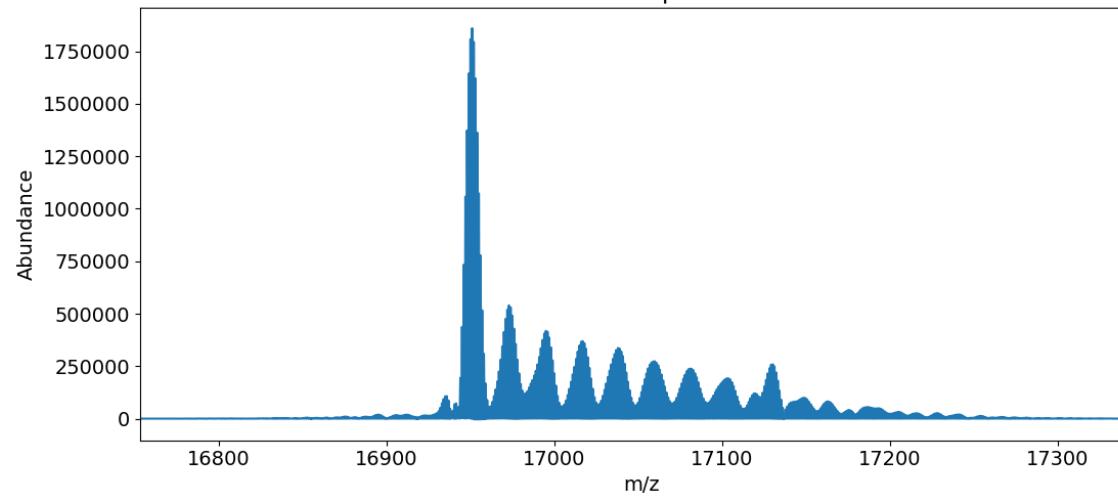
Gabor Spectrogram



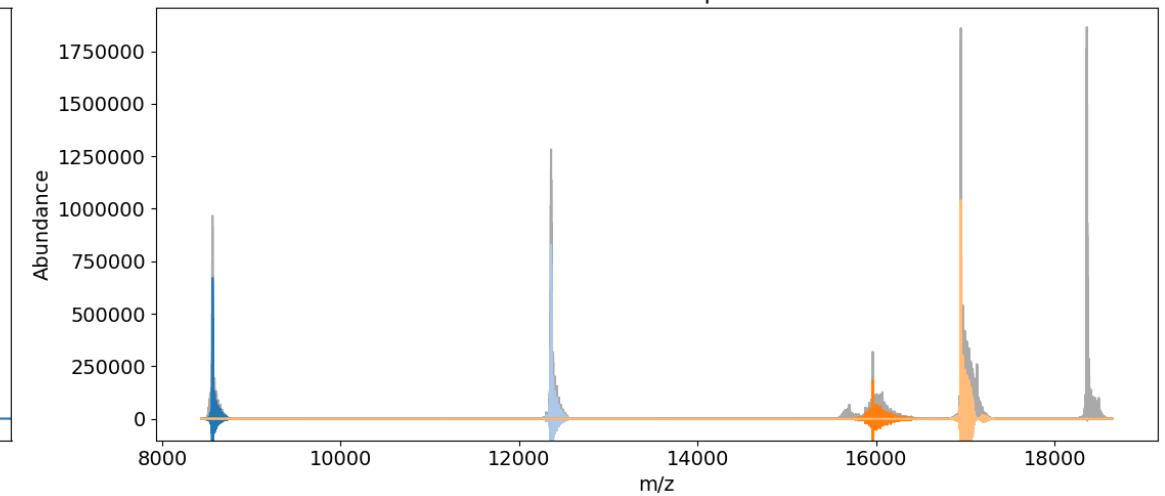
Fourier Spectrum



Raw Mass Spectrum



Reconstructed Spectrum

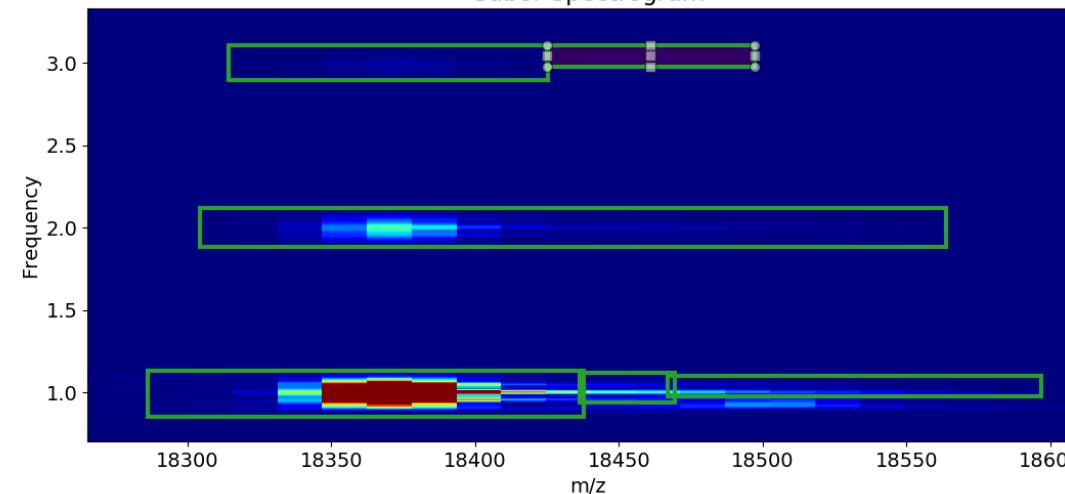


File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

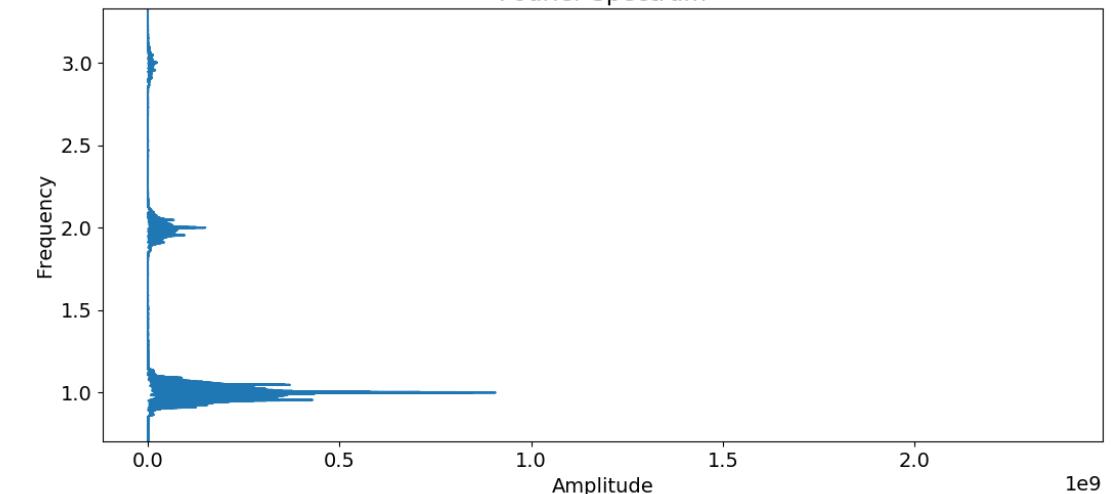
Positive Ion Mode

Selections for beta-lactoglobulin

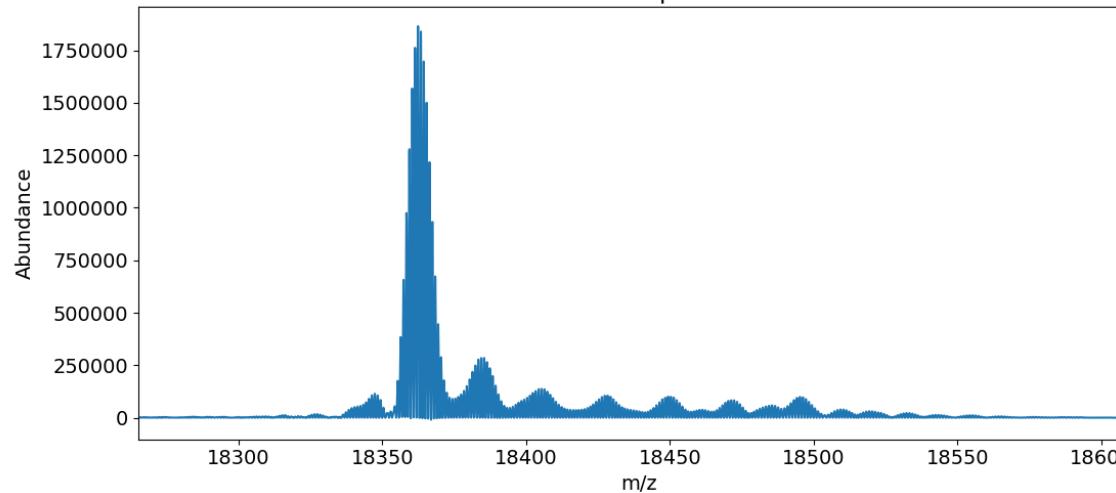
Gabor Spectrogram



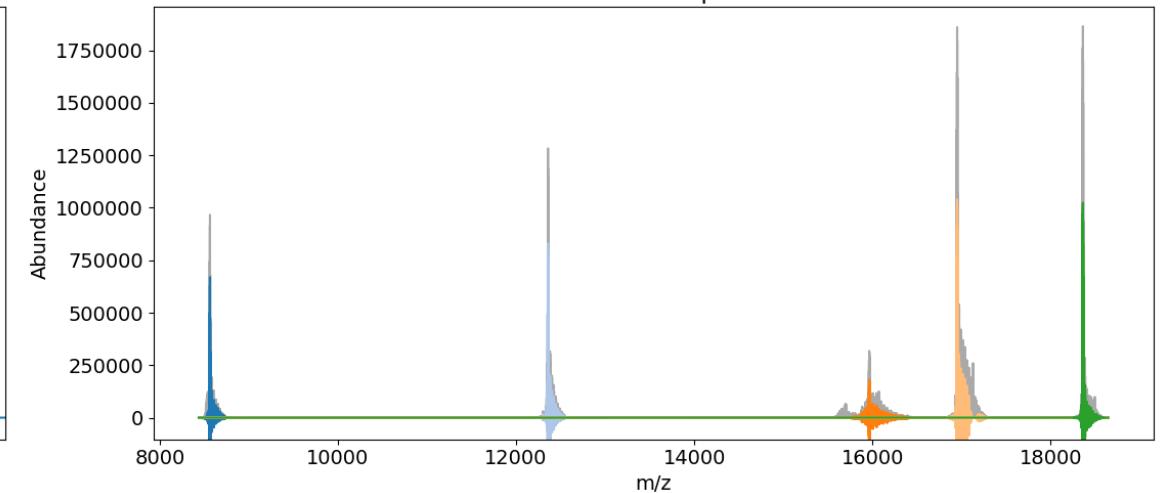
Fourier Spectrum



Raw Mass Spectrum

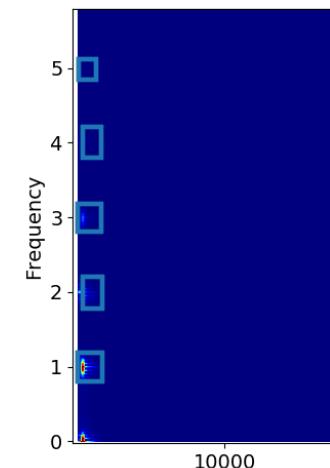


Reconstructed Spectrum

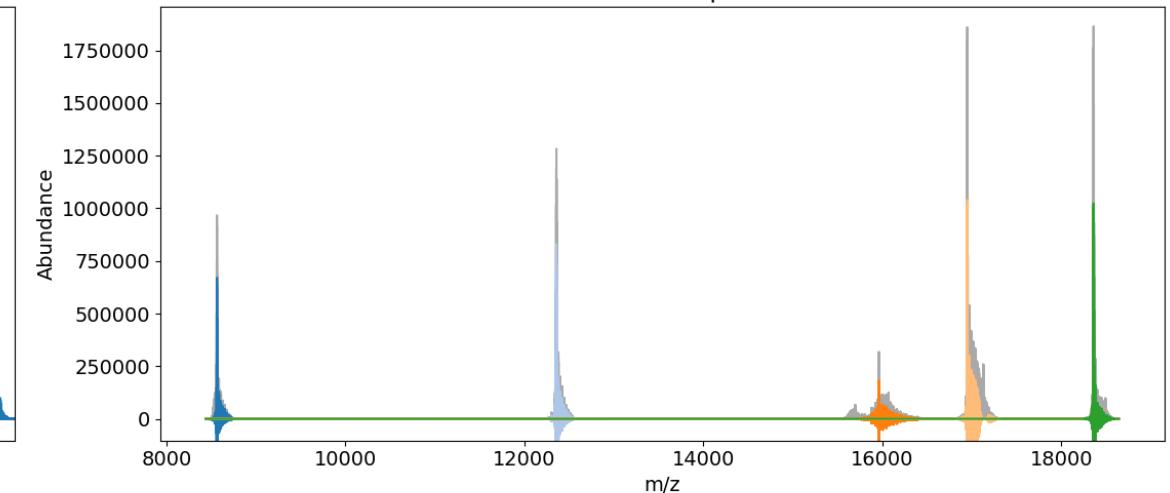
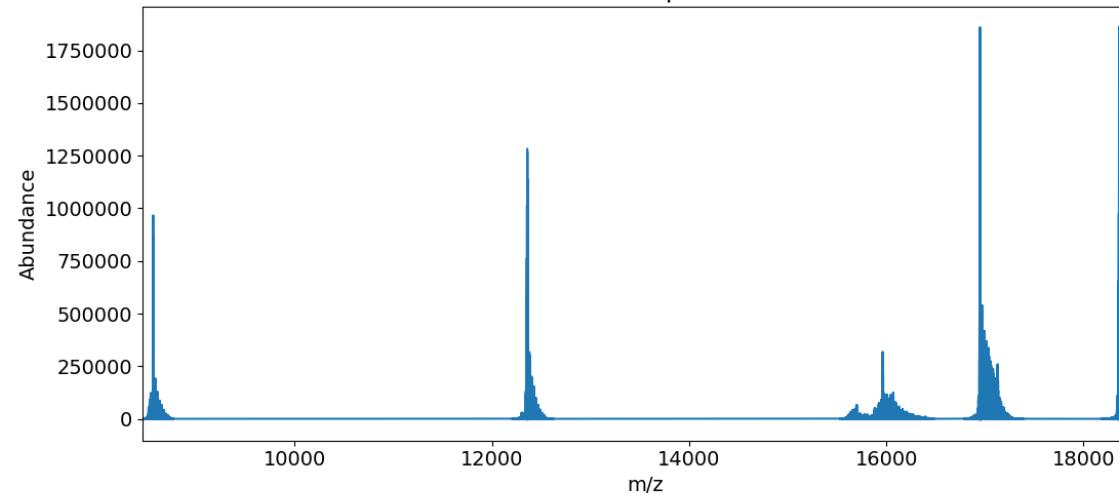
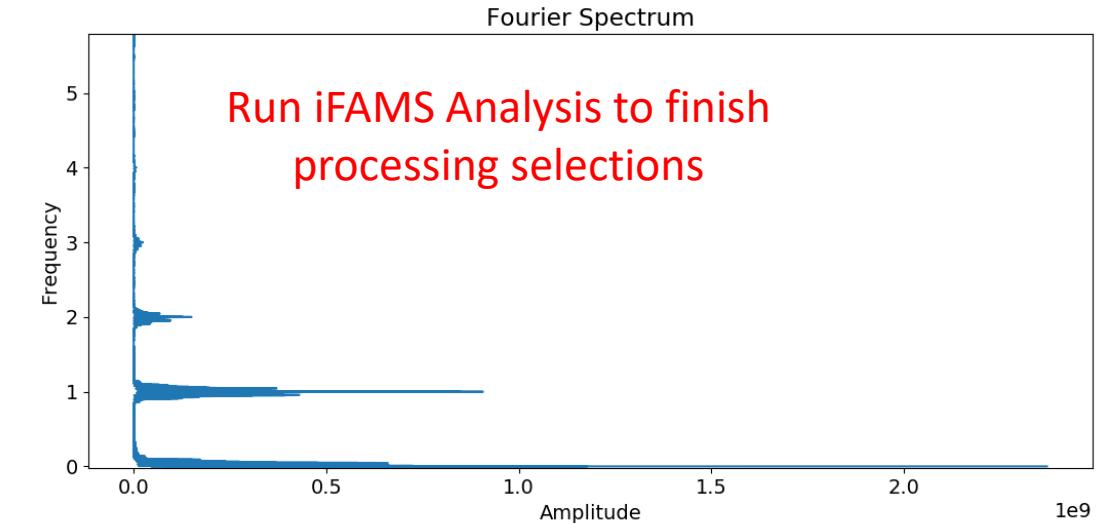


File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode



- Change STFT Parameters
- Open Gabor Slicer
- Run Guided Search
- Add Gabor Selection Ctrl+E
- Save Gabor Selections Ctrl+S
- Delete Previous Gabor Selection Ctrl+Del
- Open Harmonic Finder
- Run iFAMS Analysis**
- Adjust Charge State Assignments
- Open Quantitative Peak Integrator
- Run Mass Defect Analysis
- Show Only Spectrogram
- Open Noise Calculator



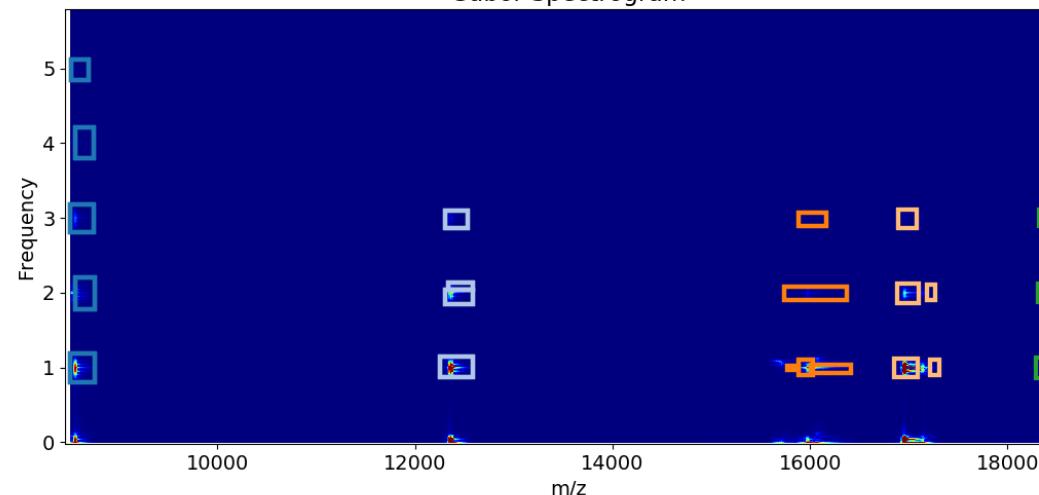
File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

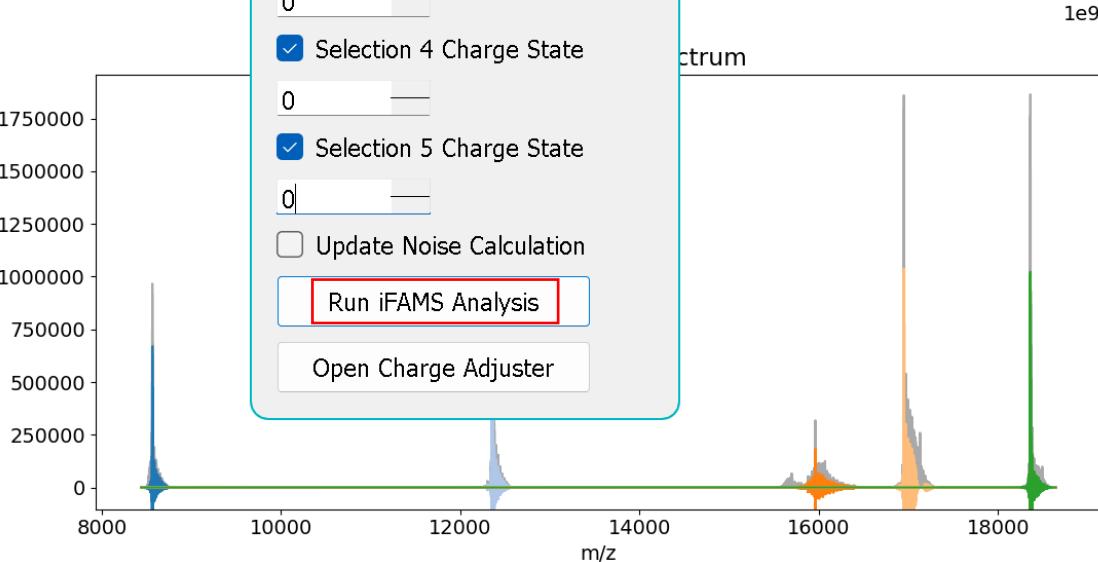
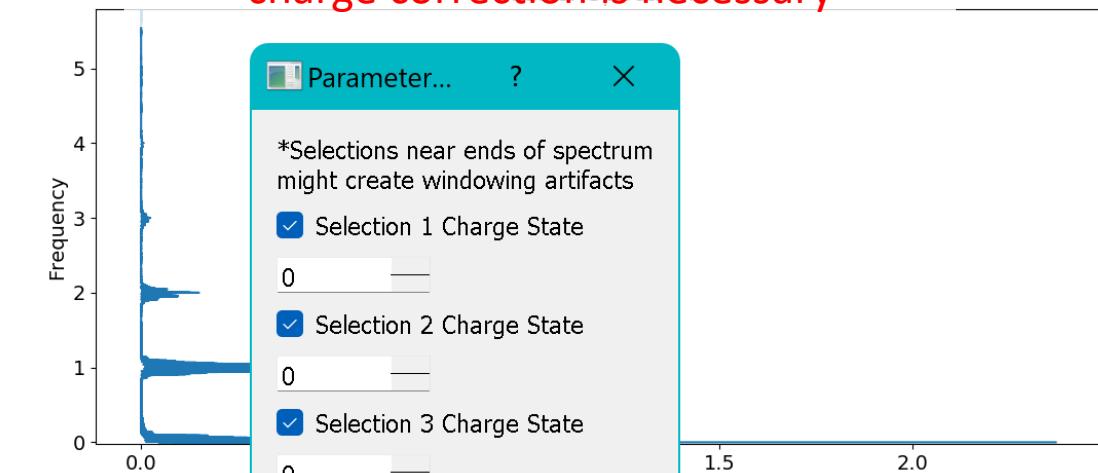
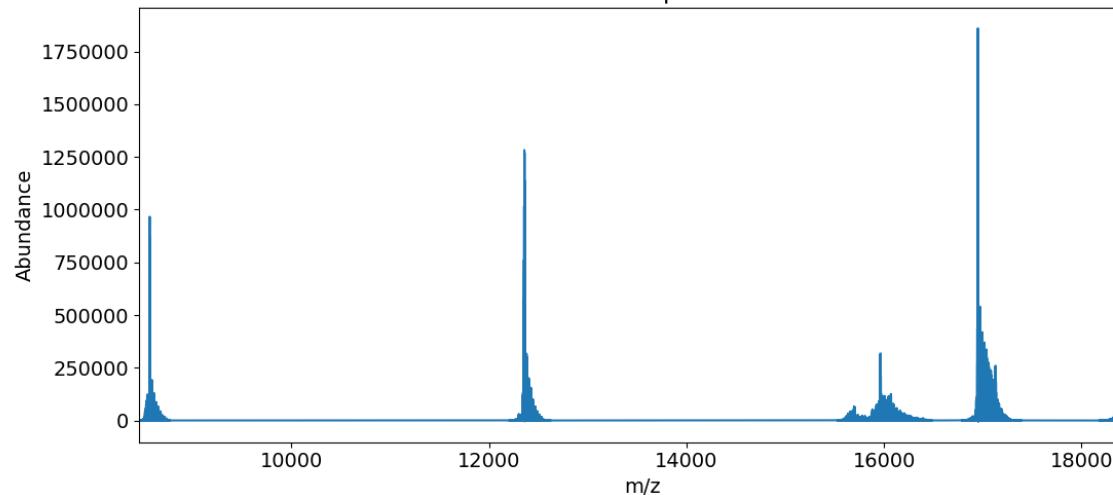
DECONVOLUTION REFINEMENT (16 of 24)

Enter 0 for every “charge state” since no charge correction is necessary

Gabor Spectrogram

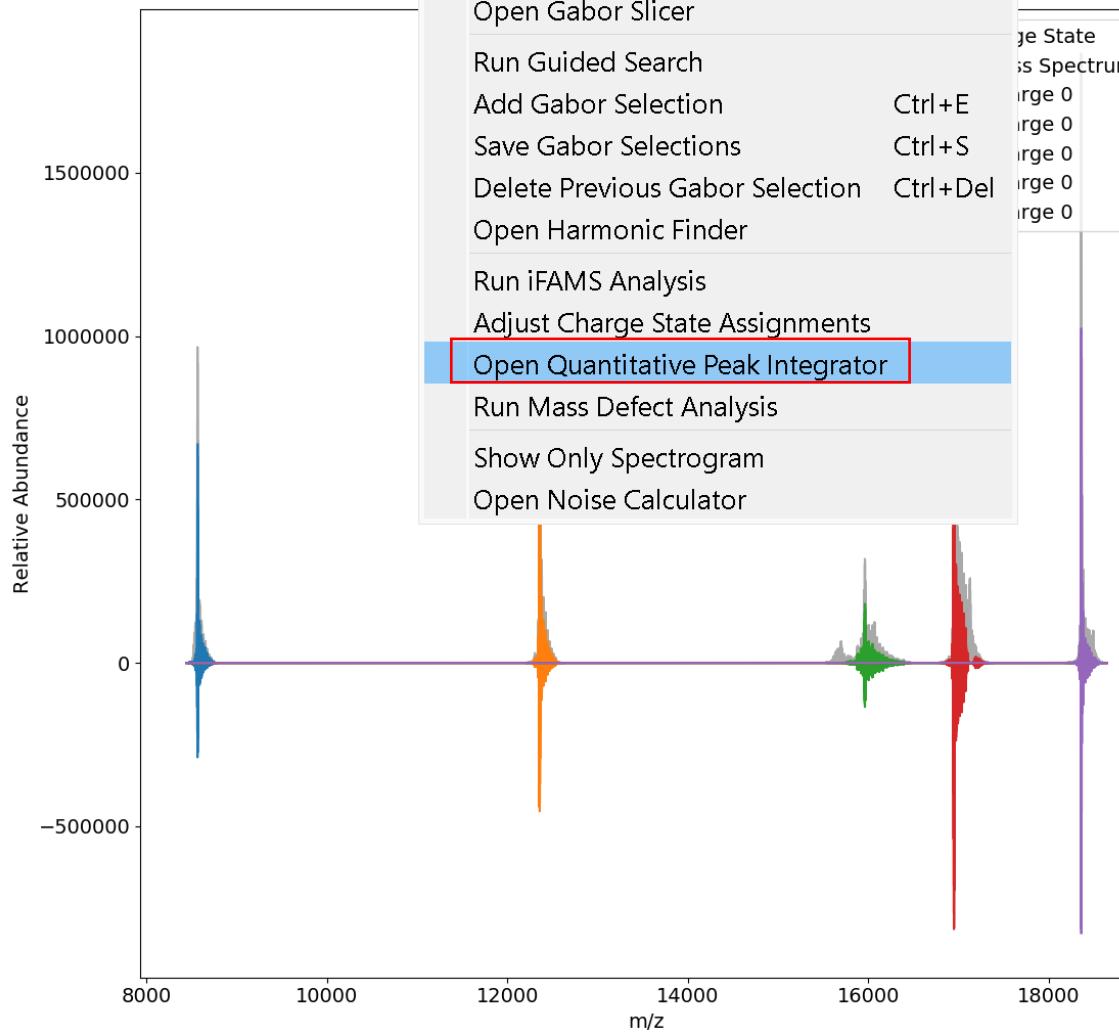


Raw Mass Spectrum



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode



Change STFT Parameters

Open Gabor Slicer

Run Guided Search

Add Gabor Selection

Ctrl+E

Save Gabor Selections

Ctrl+S

Delete Previous Gabor Selection

Ctrl+Del

Open Harmonic Finder

Run iFAMS Analysis

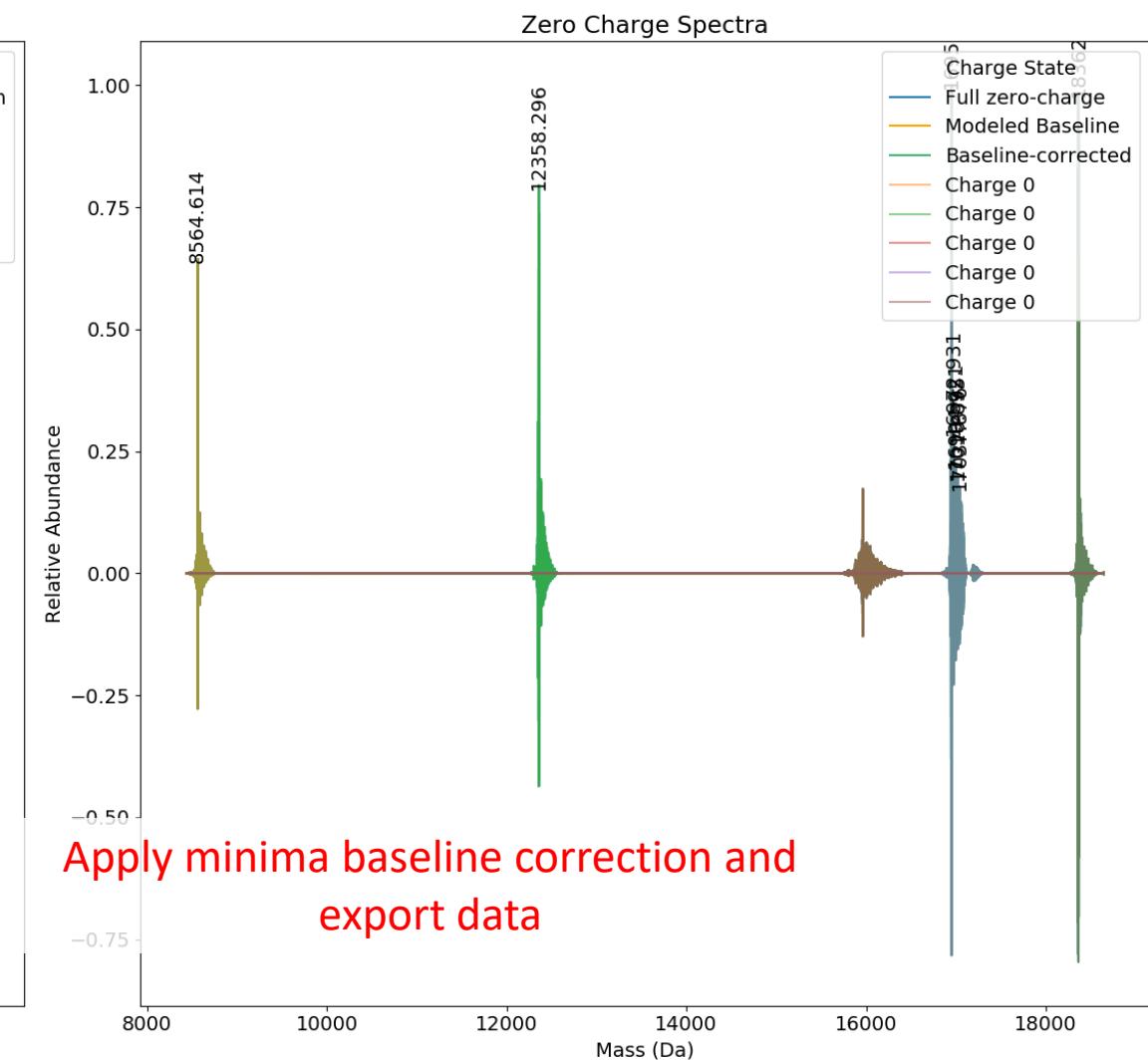
Adjust Charge State Assignments

Open Quantitative Peak Integrator

Run Mass Defect Analysis

Show Only Spectrogram

Open Noise Calculator



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_1.ms

Positive Ion Mode

The data should be exported as a new folder created within the last export folder

DECONVOLUTION REFINEMENT (18 of 24)

Load Ordinary FT

Load STFT

Plot STFT From Clipboard

Load Deconvolved or IFFT Spectra

Load Batch Parameters

Batch Data Files

Batch Deconvolved Spectra

Export Data

Export Batch Parameters

Mass Spectrum

Charge State

Mass Spectrum

Charge 0

Charge 1

Charge 2

Charge 3

Charge 4

Charge 5

Charge 6

Charge 7

Charge 8

Charge 9

Charge 10

Charge 11

Charge 12

Charge 13

Charge 14

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Charge 292

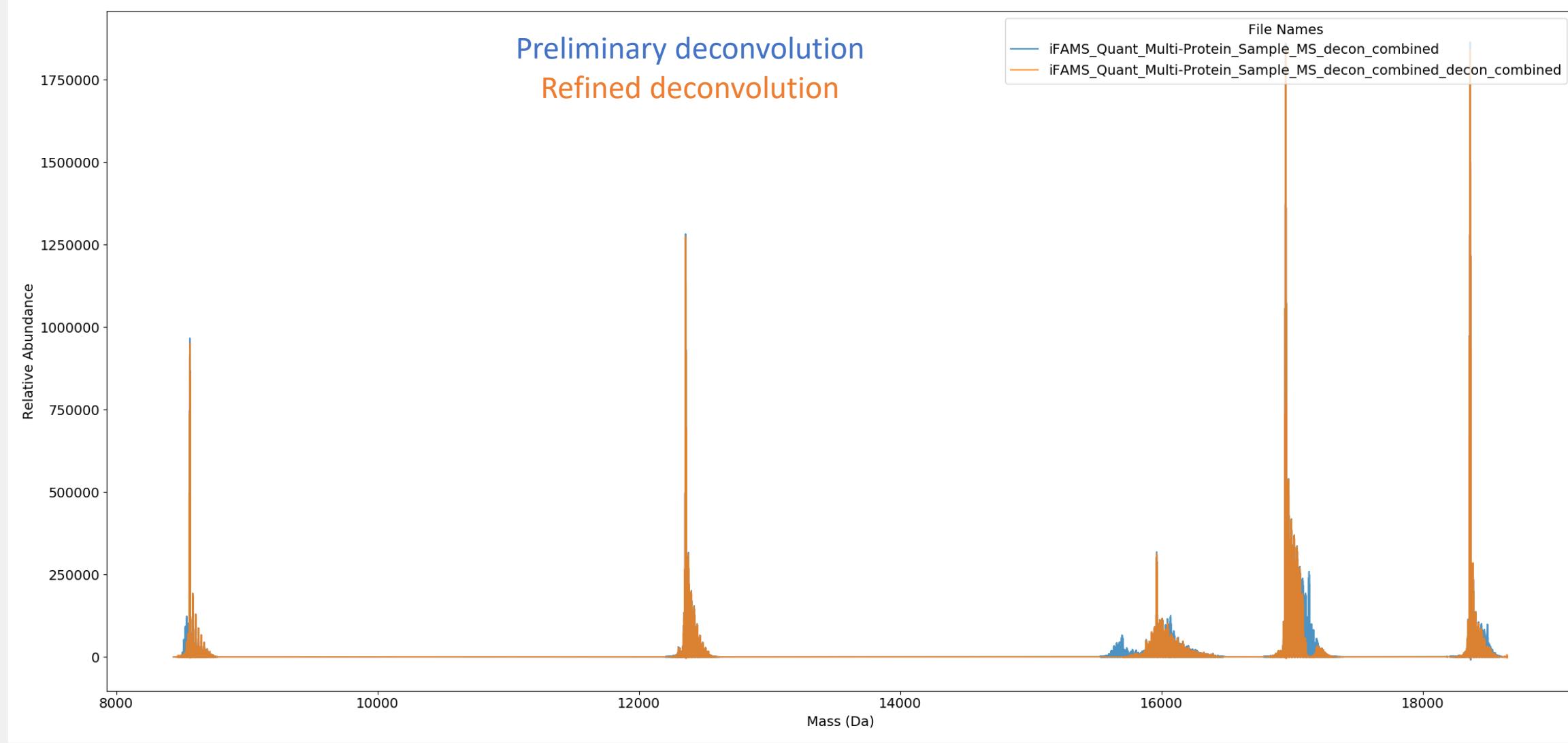
Charge 293

Charge 294

Charge 295

Charge 296

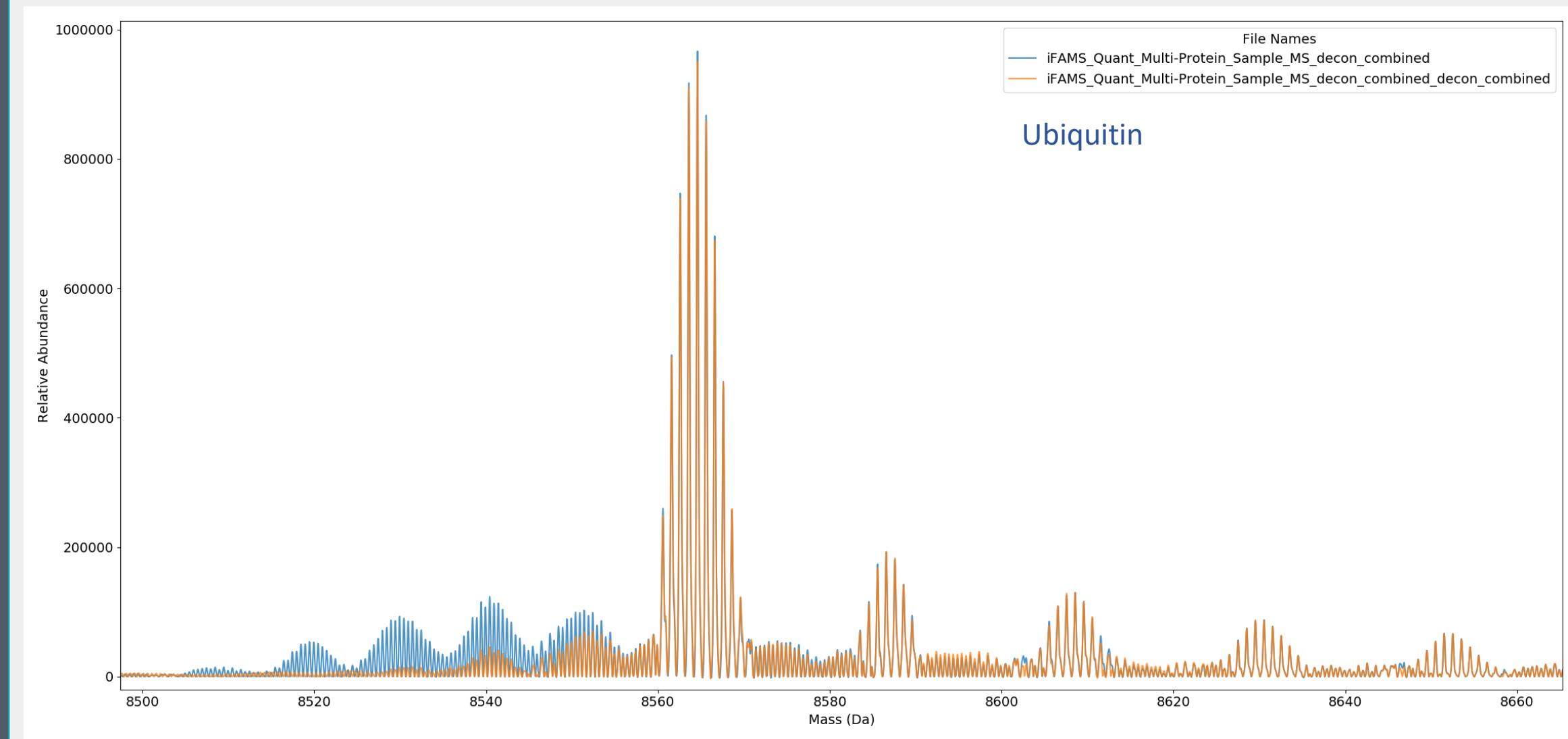
Charge 297



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

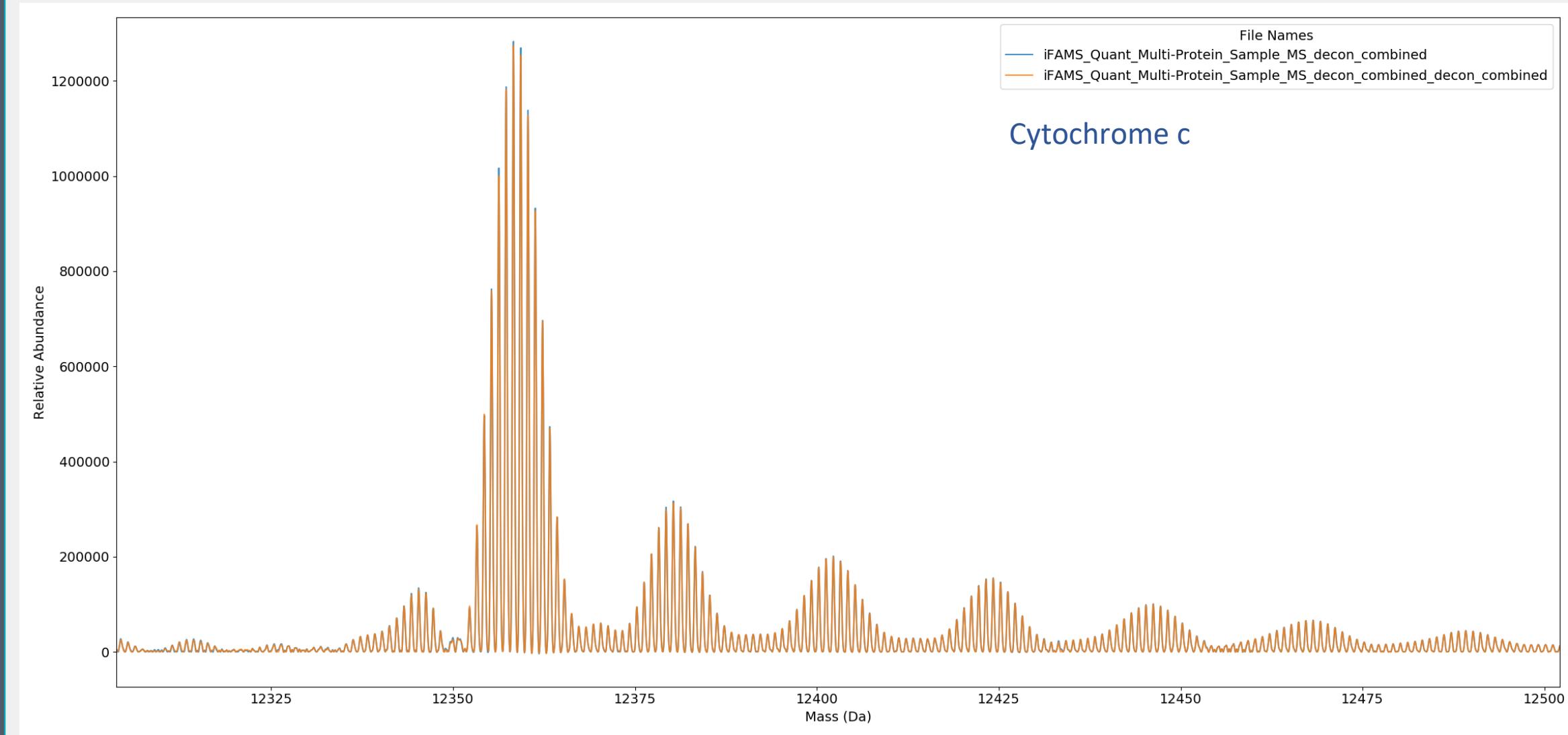
Positive Ion Mode



zoom rect

115

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution



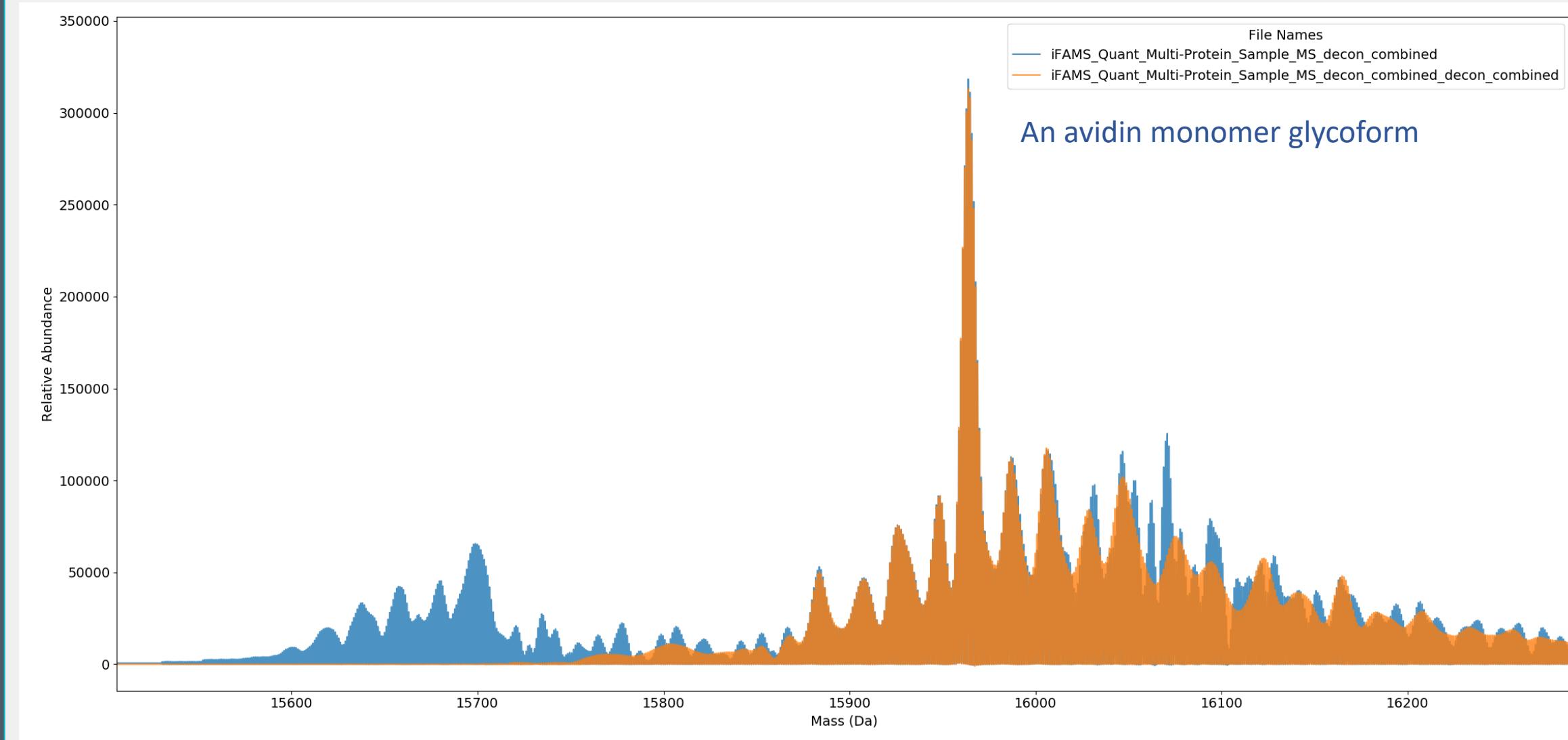
File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode



zoom rect

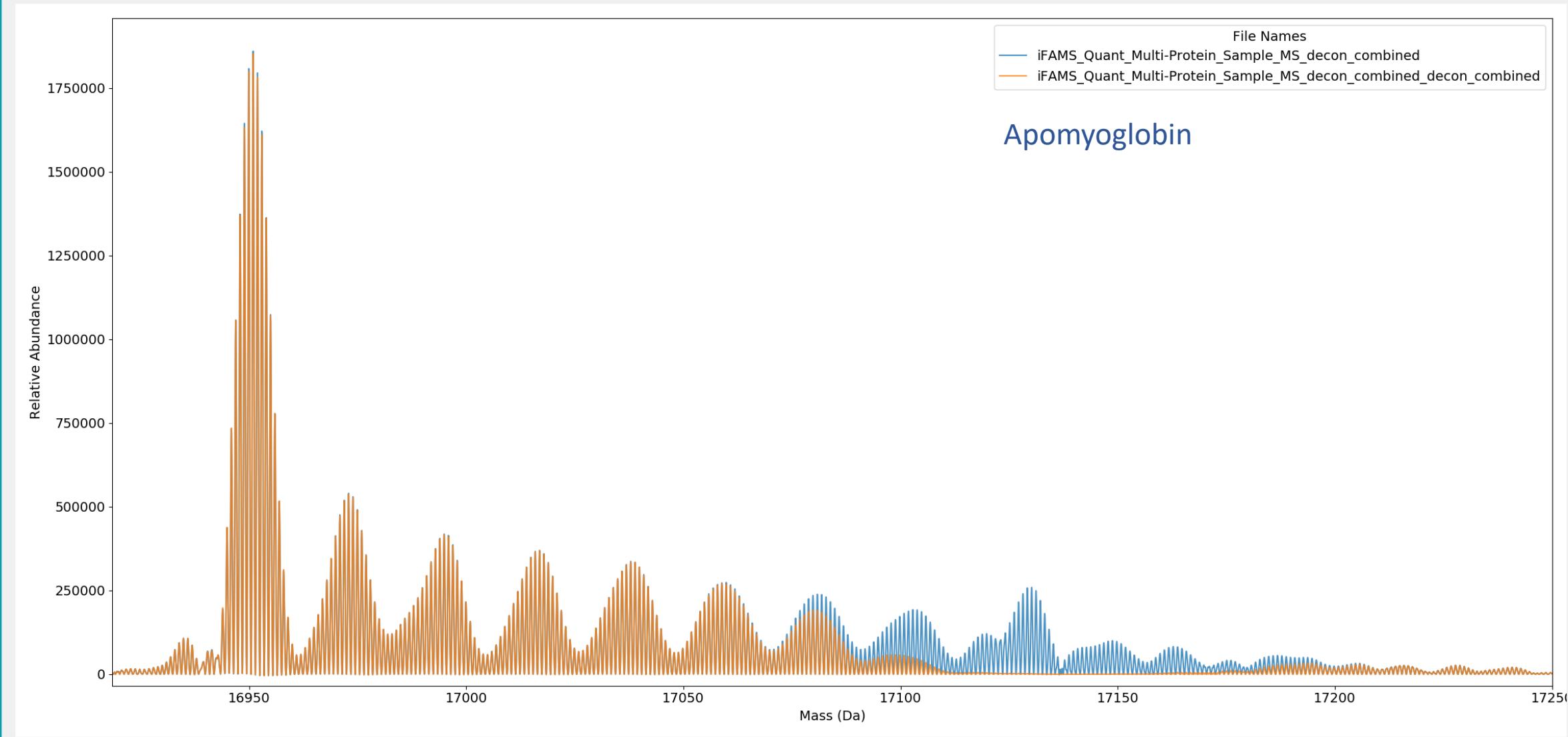
File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

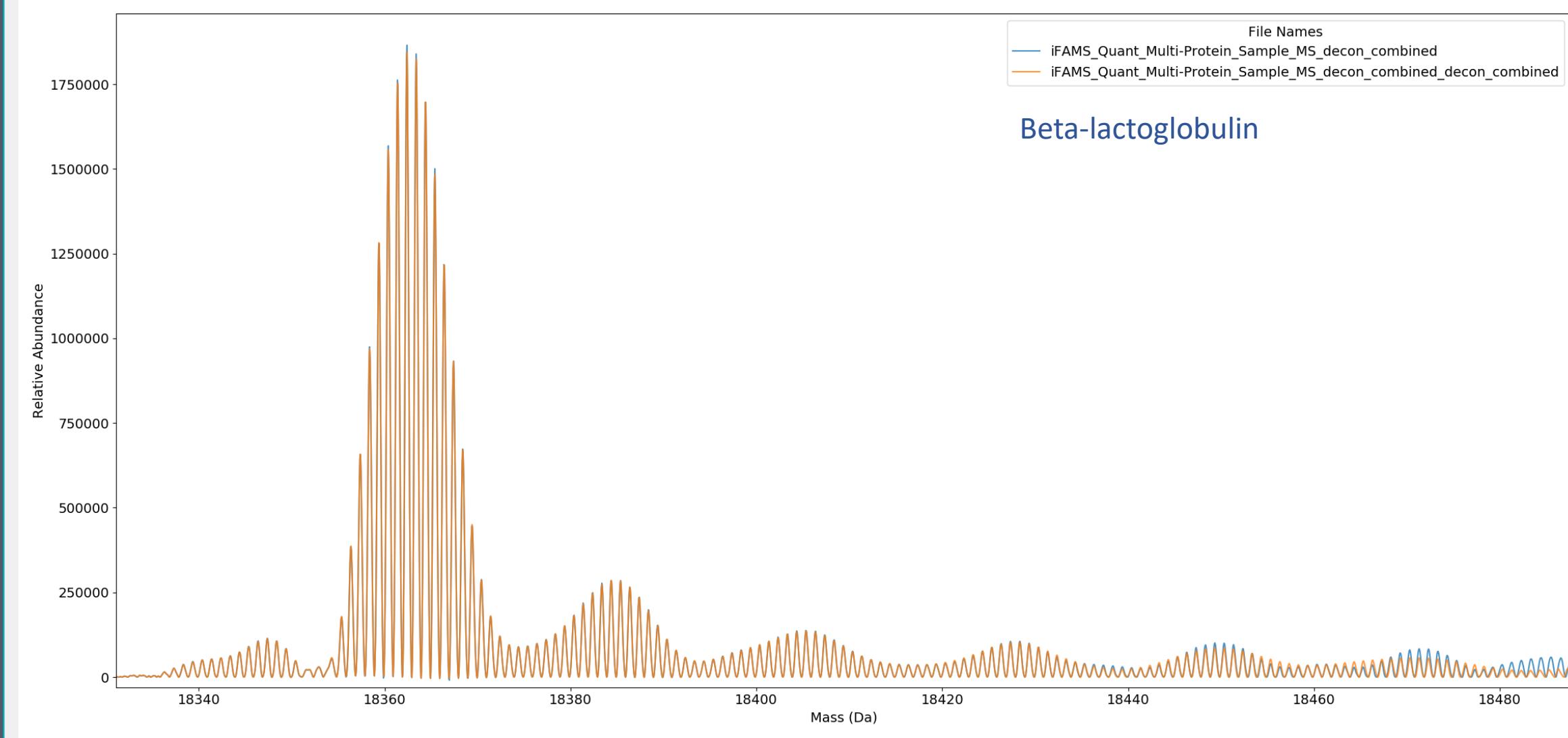


File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode



zoom rect
118

[File](#) [Settings](#) [Fourier Analysis Tools](#) [STFT Analysis Tools](#) [Calibration Curve Tools](#) [Isotopic Distribution](#)

File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

zoom rect
119

Isotopic Distribution Calculator

Required data: Multi-protein sample

- Theoretical isotopic distribution calculation using an amino acid sequence
- Visual comparison of theoretical and empirical mass distributions
- Adjustment of theoretical mass distribution to account for post-translational modifications

ISOTOPIC DISTRIBUTION CALCULATOR (1 of 12)

iFAMS v6.3 Quant

File Settings Fourier Analysis Tools STFT Analysis Tools Calibration Curve Tools Isotopic Distribution

Calculate Distribution

Overlay Data Reconstruction

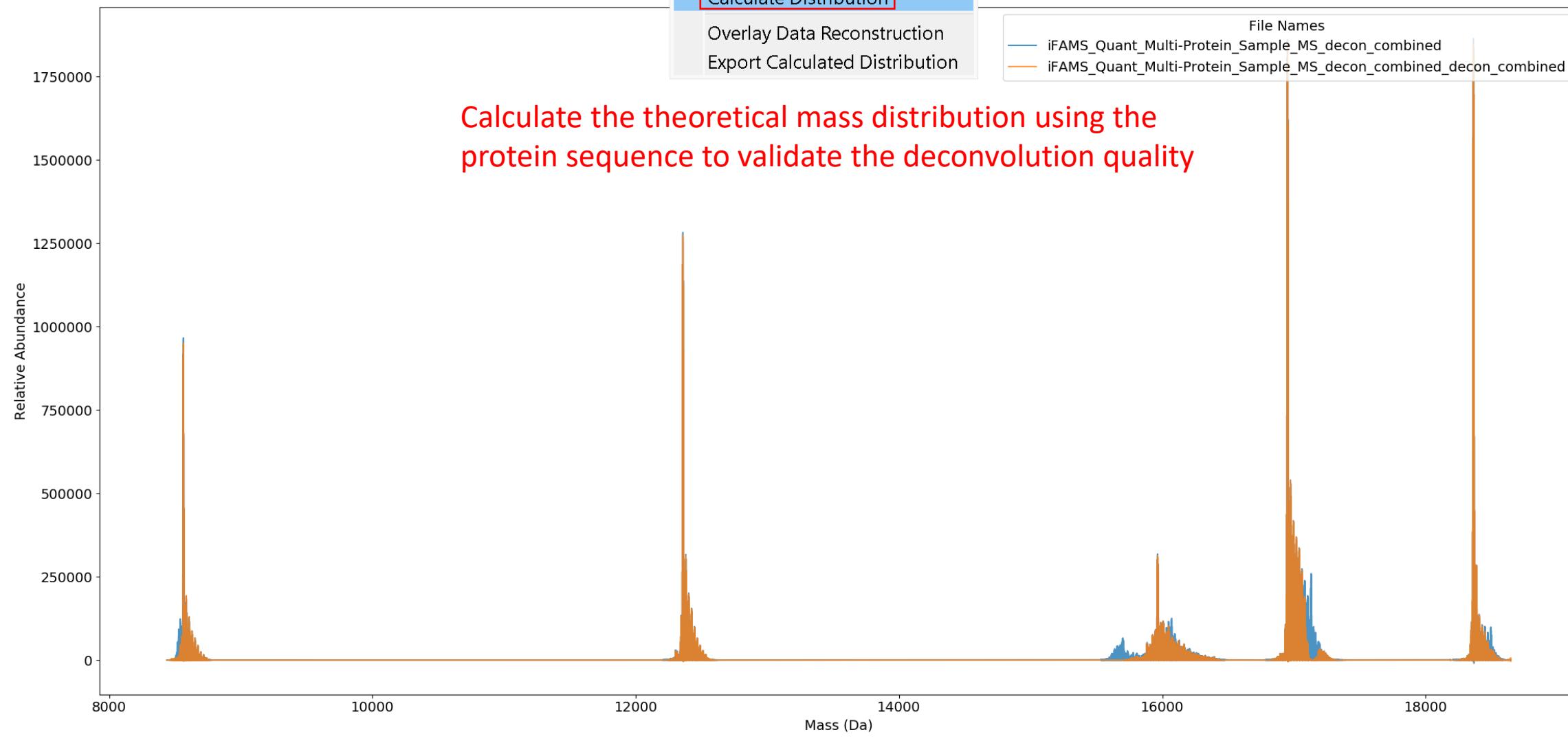
Export Calculated Distribution

File Names

iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined_decon_combined

Calculate the theoretical mass distribution using the protein sequence to validate the deconvolution quality



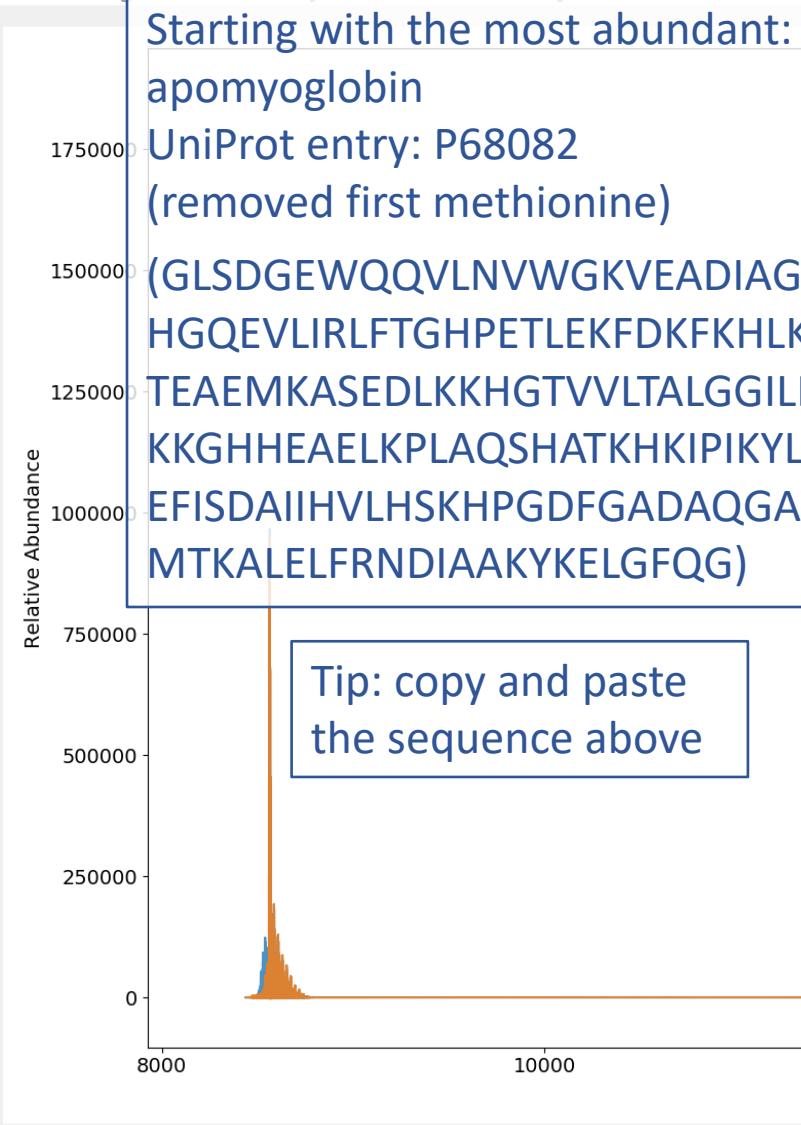
File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode



zoom rect

121



Molecular System Parameters

Protein/Nucleotide Sequence
[amino acid sequence (UPPERCASE)]
[RNA/DNA sequence (r/d + lowercase)]

**GLSDGEWQQQLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFHKLKTEAE
MKASEDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAII
HVLHSKHPGDFGADAQGAMTKALELFRNDIAAKYKELGFQG**

Oligomeric State
1

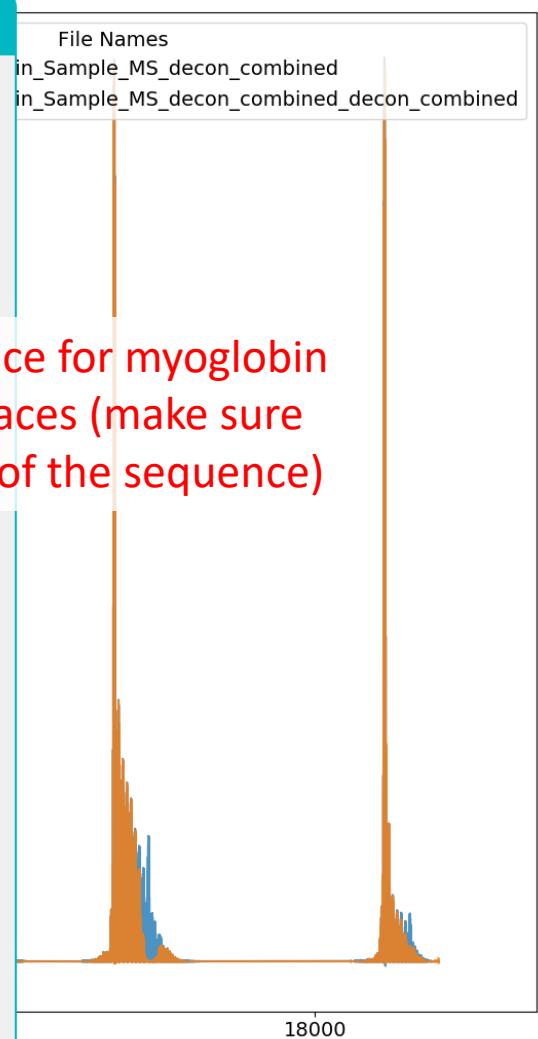
Adduct Composition
(e.g. "Na 1 Cl 1")

Adduct Density (per monomer)
1

Set Resolution Width
0.1

Calculate Mass Distribution

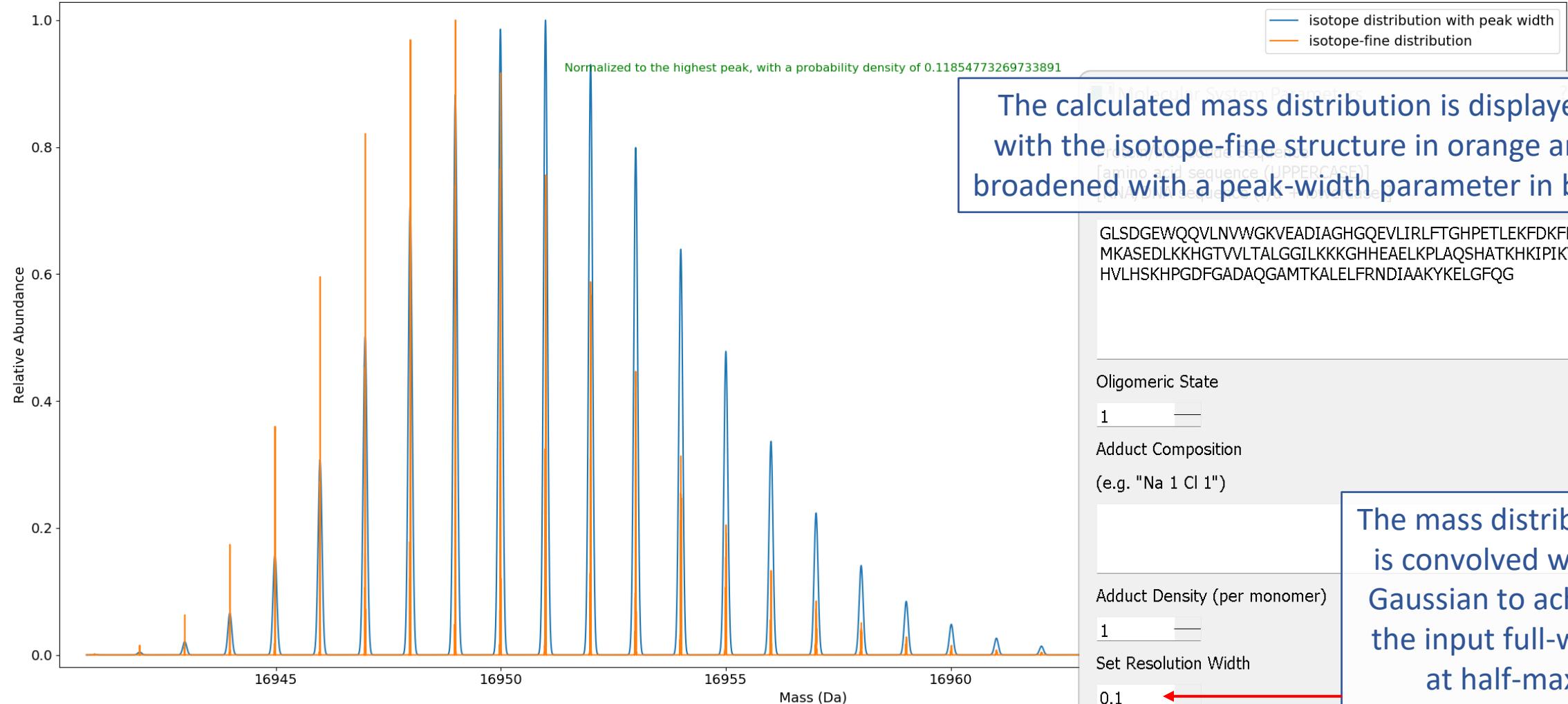
Export Mass Distribution



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode

Zero Charge Spectrum



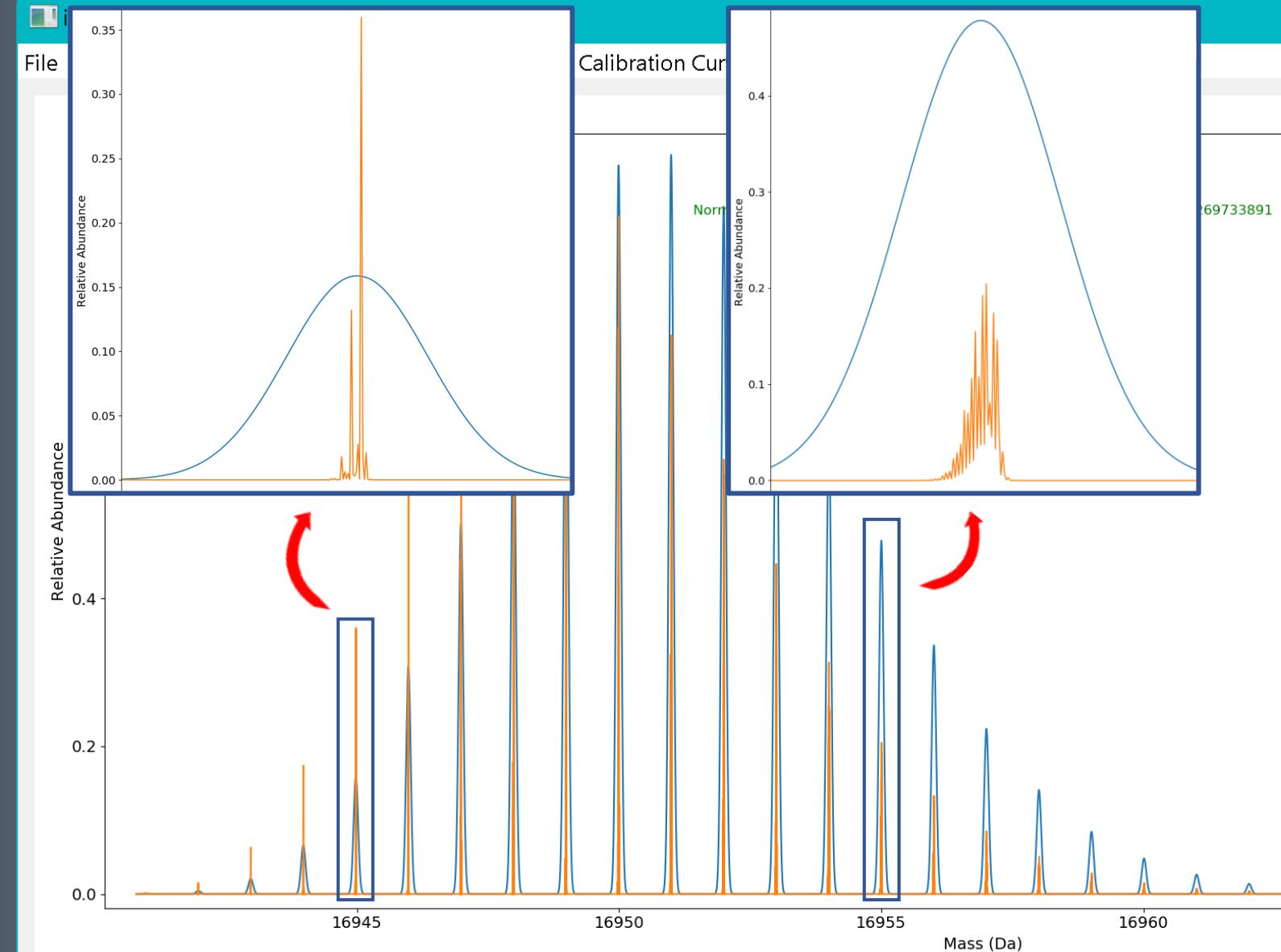
File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode



Calculate Mass Distribution

Export Mass Distribution



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined

Positive Ion Mode



Note that broadening combines multiple isotope-fine peaks and may seem to shift the theoretical distribution. Due to this, a non-zero "Resolution Width" may better represent empirical data with finite resolution

Protein/Nucleotide
[Amino acid sequence (UPPERCASE)]
[RNA/DNA sequence (1/u + lowercase)]

GLSDGEWQQQLVNWGKVEADIAGHGQEVIRLFTGHPETLEKFDKFHLKTEAE
MKASEDLKKHGTVLTLGGILKKKGHHEALKPLAQSHATKHKIPIKYLEFISDAII
HVLHSKHPGDFGADAQGAMTKALELFRNDIAKYKELGFQG

Oligomeric State

1

Adduct Composition

(e.g. "Na 1 Cl 1")

Adduct Density (per monomer)

1

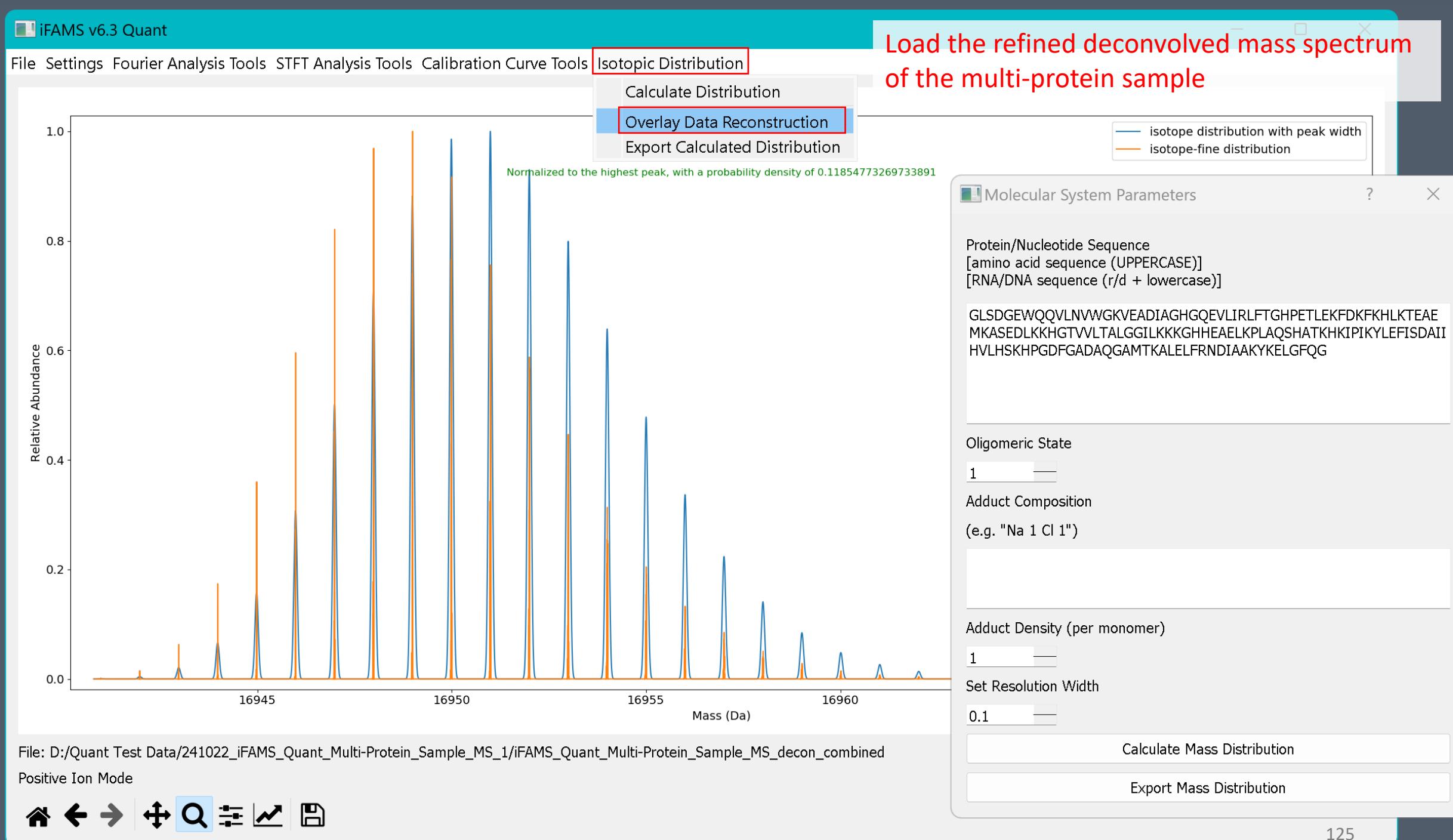
Set Resolution Width

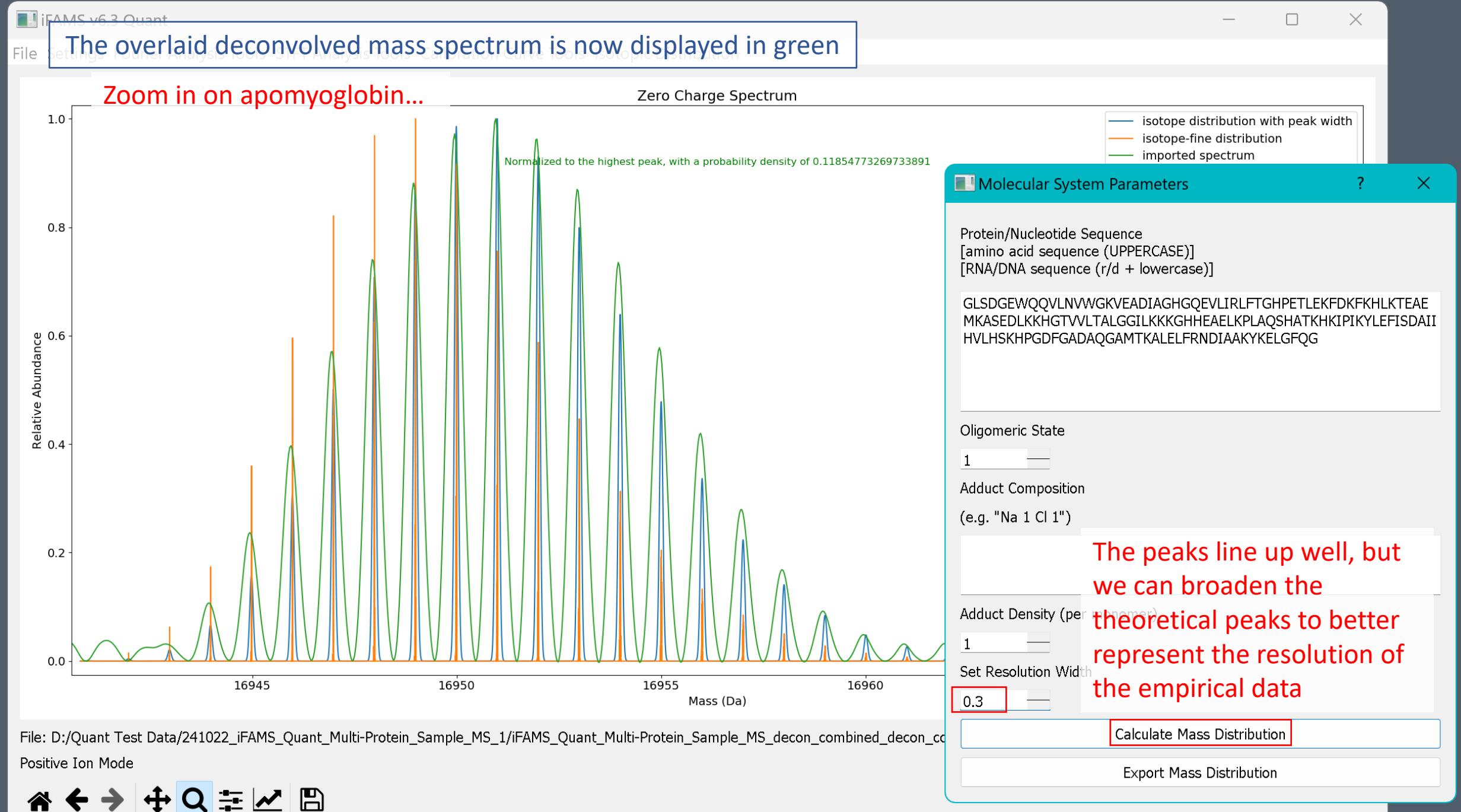
0.1

Calculate Mass Distribution

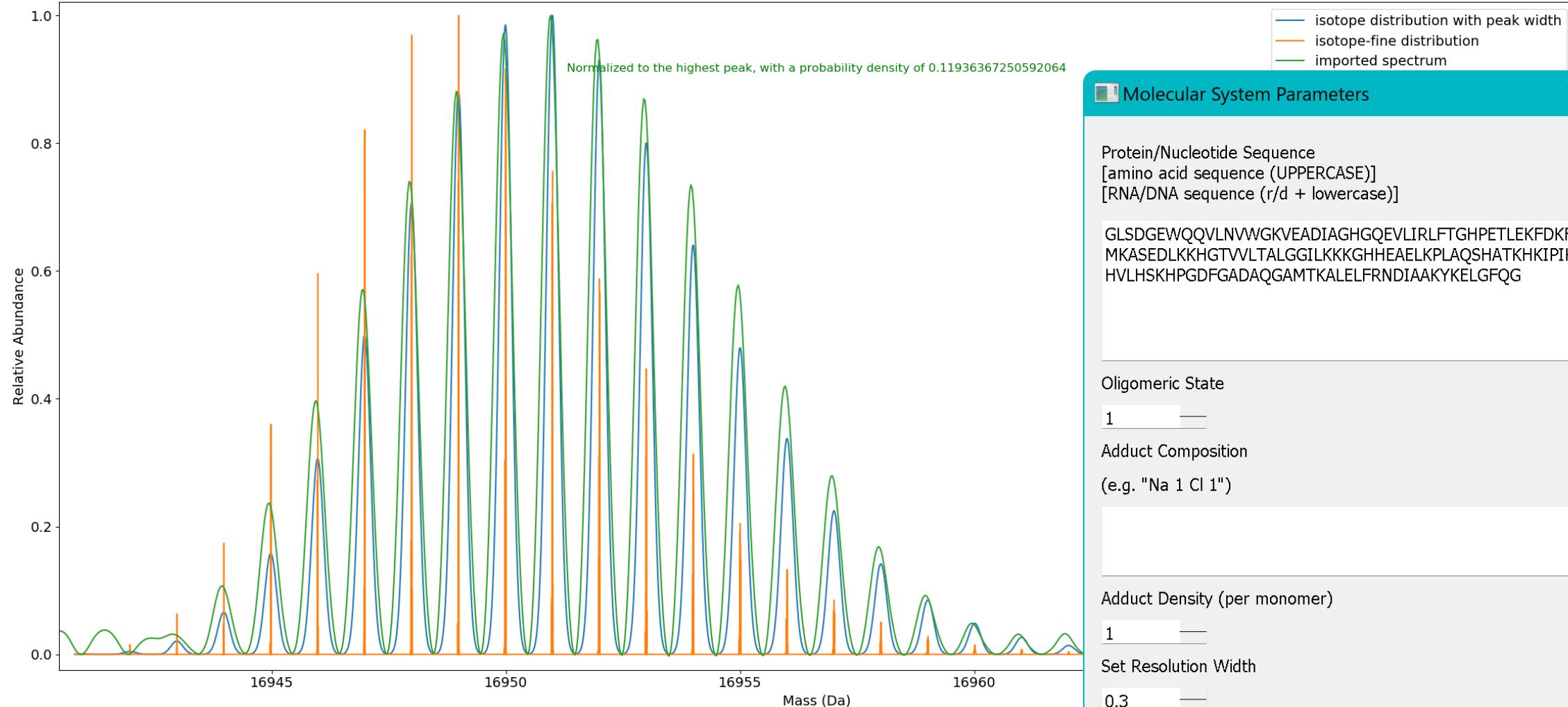
Export Mass Distribution

ISOTOPIC DISTRIBUTION CALCULATOR (5 of 12)





Zero Charge Spectrum



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi_Protein_Sample_MS_1/iFAMS_Quant_Multi_Protein_Sample_MS_1/exported/decon_c

Positive Ion Mode



Now, the broadened theoretical mass distribution
can be exported for downstream calculations.

Molecular System Parameters

Protein/Nucleotide Sequence
[amino acid sequence (UPPERCASE)]
[RNA/DNA sequence (r/d + lowercase)]

GLSDGEWQQVLNVWGKVEADIAGHGQEVIRLFTGHPETLEKFDKFHLKTEAE
MKASEDLKKHGTVLALGGILKKKGHHEALKPLAQSHATKHKIPIKYLEFISDAII
HVLHSKHPGDFGADAQGAMTKALELFRNDIAAKYKELGFQG

Oligomeric State
1

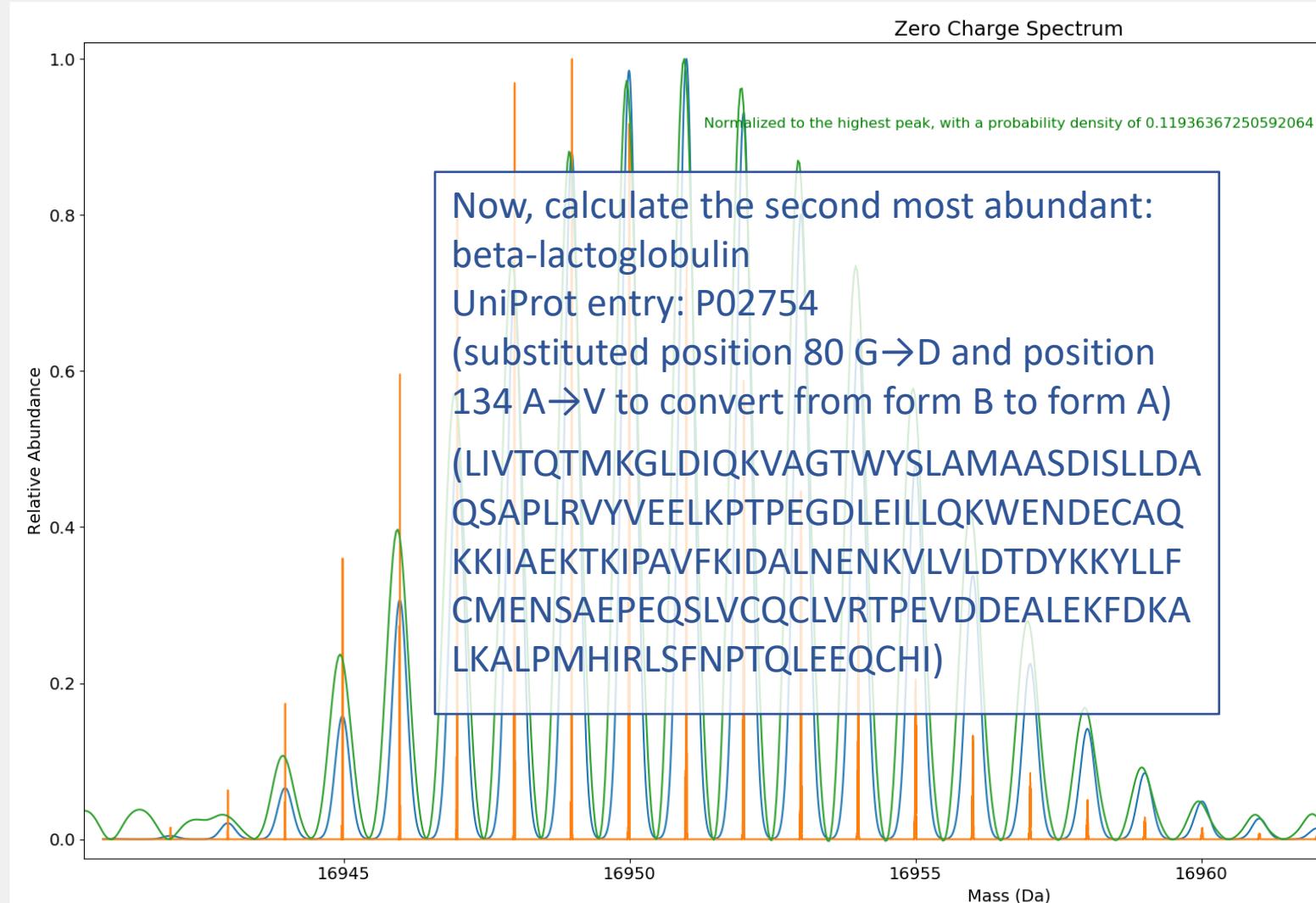
Adduct Composition
(e.g. "Na 1 Cl 1")

Adduct Density (per monomer)
1

Set Resolution Width
0.3

Calculate Mass Distribution

Export Mass Distribution



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined_decon_co

Positive Ion Mode



Molecular System Parameters

Protein/Nucleotide Sequence
[amino acid sequence (UPPERCASE)]
[RNA/DNA sequence (r/d + lowercase)]

LIVTQTMKGDIQKVAGTWYSLAMAASDISLLDA
QSAPLRVYVEELKPTPEGDLEILLQKWENDECAQ
KKIIAEKTKIPAVFKIDALNENKVLVLDTDYKKYLLF
CMENSAEPEQSLVCQCLVRTPEVDDEALEKFDKA
LKALPMHIRLSFNPTQLEEQQCHI

Enter the amino acid sequence
for beta-lactoglobulin

Oligomeric State: 1

Adduct Composition
(e.g. "Na 1 Cl 1")

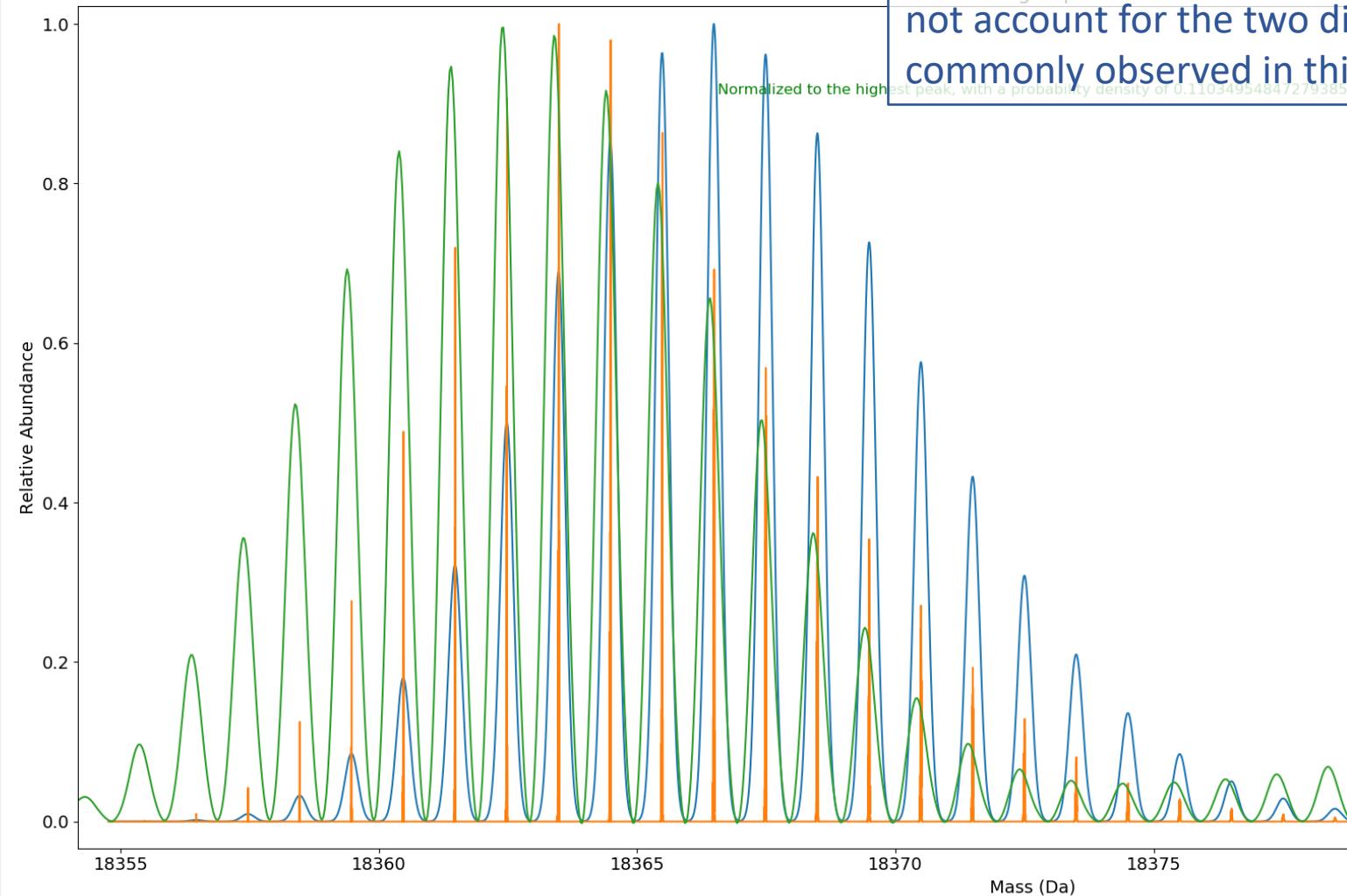
Adduct Density (per monomer): 1

Set Resolution Width: 0.3

Calculate Mass Distribution

Export Mass Distribution

The theoretical distribution is heavier
because the amino acid sequence does
not account for the two disulfide bonds
commonly observed in this protein



File: D:/Quant Test Data/241022_iFAMS_Quant_Multi-Protein_Sample_MS_1/iFAMS_Quant_Multi-Protein_Sample_MS_decon_combined_decon.co

Positive Ion Mode



Molecular System Parameters

Protein/Nucleotide Sequence
[amino acid sequence (UPPERCASE)]
[RNA/DNA sequence (r/d + lowercase)]

LIVTQTMKGLDIQKVAGTWYSLAMAASDISLLDAQSAPLRYVEELKPTPEGDLEI
LLQKWENDECAQKIIAEKTKIPAVFKIDALNENKVLVLDTDYKKYLLFCMENSAEP
EQSLVCQCLVRTPEVDDEALEKFDKALKALPMHIRLSFNPTQLEEQCHI

Oligomeric State
1

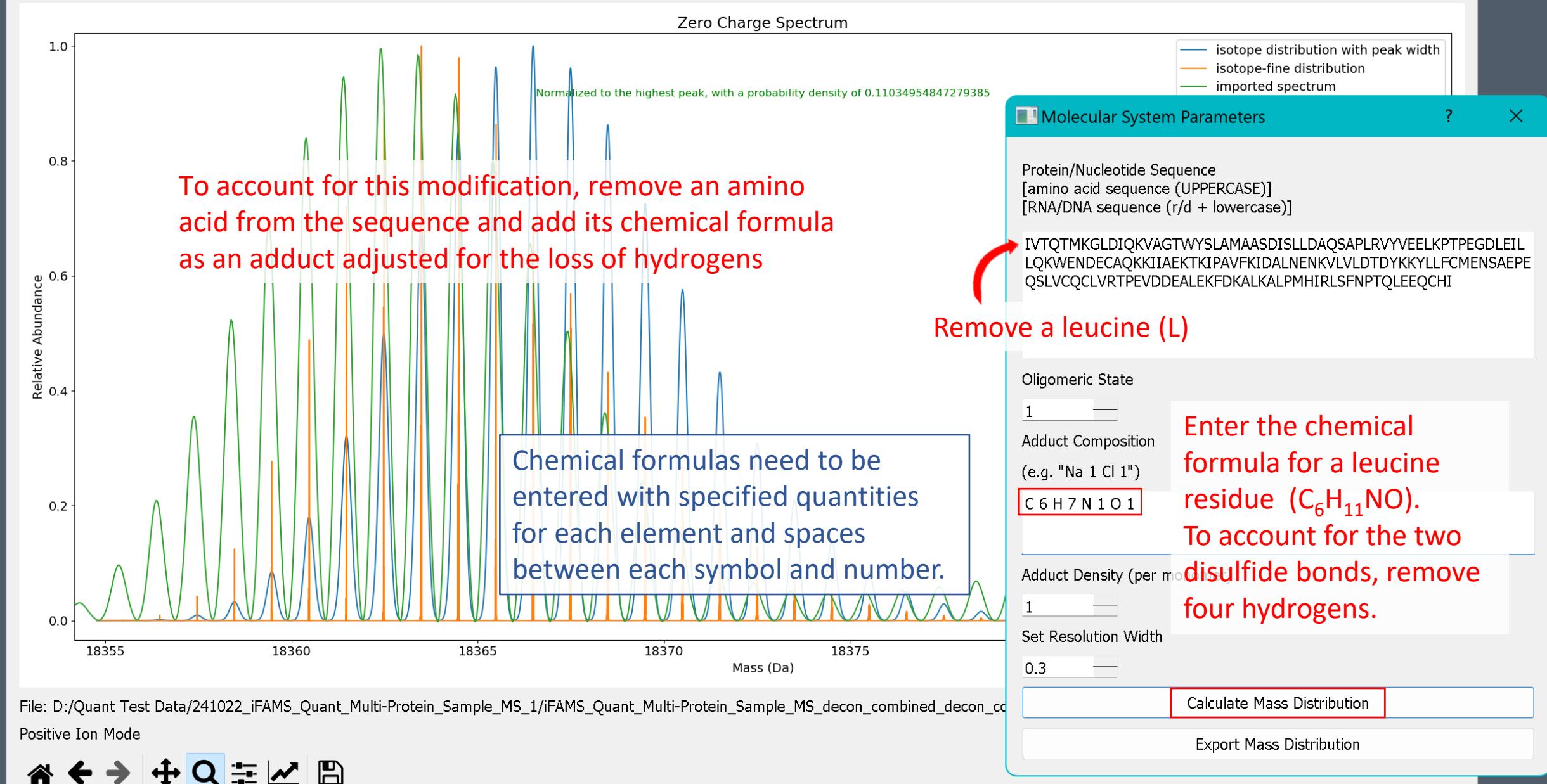
Adduct Composition
(e.g. "Na 1 Cl 1")

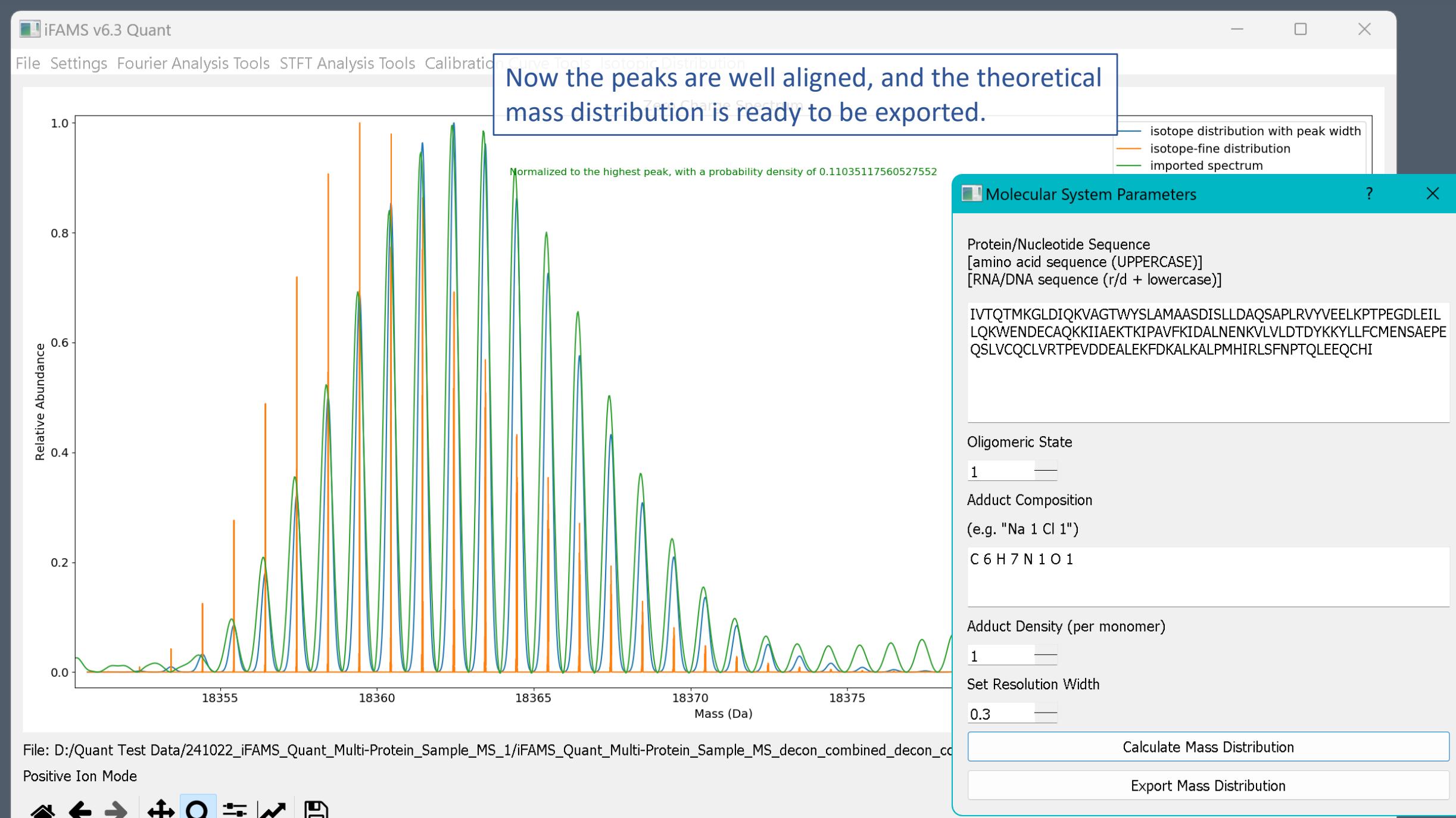
Adduct Density (per monomer)
1

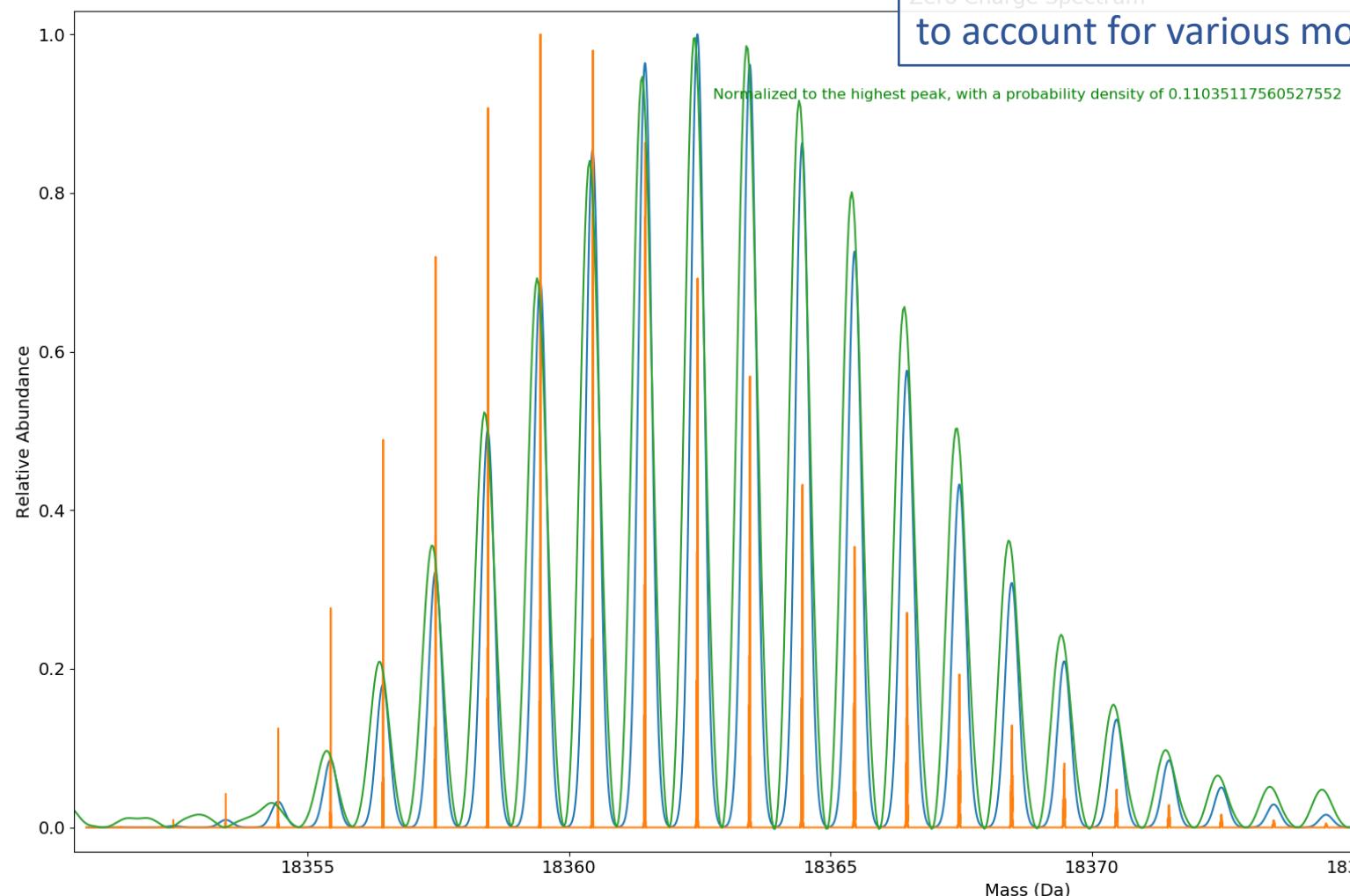
Set Resolution Width
0.3

Calculate Mass Distribution

Export Mass Distribution







Alternatively, the entire sequence can be entered as a chemical formula which can aid with adjusting the theoretical distribution to account for various modifications.

Molecular System Parameters

Protein/Nucleotide Sequence
[amino acid sequence (UPPERCASE)]
[RNA/DNA sequence (r/d + lowercase)]

Oligomeric State
1

Adduct Composition
(e.g. "Na 1 Cl 1")
C 821 H 1318 N 206 O 250 S 9

Adduct Density (per monomer)
1

Set Resolution Width
0.3

Calculate Mass Distribution

Export Mass Distribution

File: D:/Quant Test

Positive Ion Mode



The elements and quantities used in each calculation are output in the command line. This can be useful for converting an amino acid or nucleotide sequence into a chemical formula