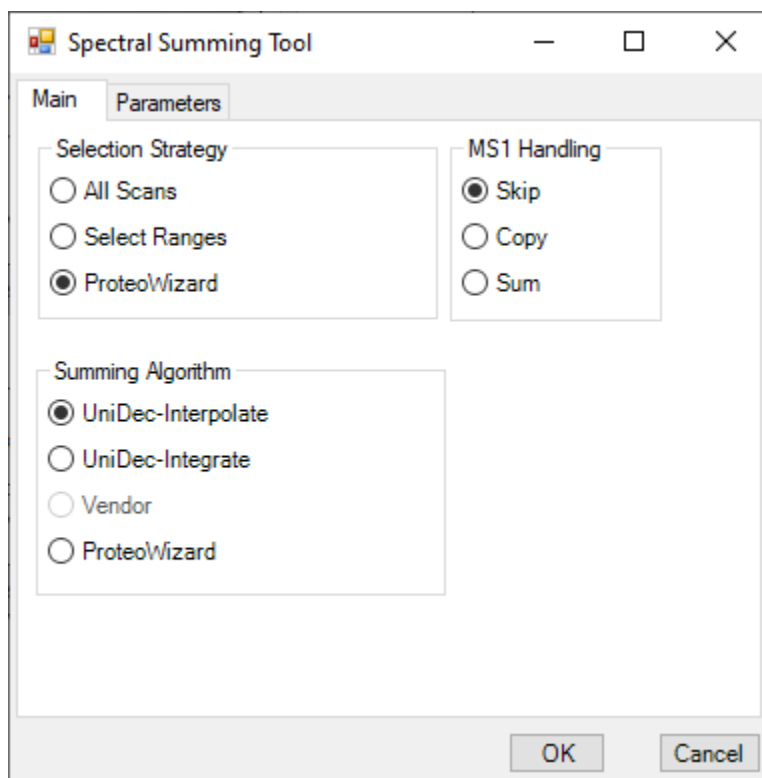


# Best Practices for Spectral Summing in MASH Native

## Version 1.1

### Introduction

Spectral summing aids interpretation of mass spectrometry (MS) data by increasing signal-to-noise of spectral features and reducing data file sizes through combination of multiple input scans to a single output scan. Spectral summing can improve sequence coverage, fitting scores, and speed up any post-summing data processing. While certain MS applications may sum scans during acquisition using vendor-specific summing techniques, others rely on post-acquisition summing before any data processing will occur, a common approach in liquid chromatography-MS (LC-MS) workflows. However, many vendor software packages only contain a single summing option, which may or may not suit user's individual needs. MASH Native now supports spectral summing through the new "Spectral Summing Tool", providing a variety of summing options to suit user's needs (Figure 1). Users can define their scan selection strategy, chosen summing algorithm, and desired MS1 file handling.



**Figure 1.** The main spectral summing interface.

The selection strategy defines which scans should be summed, all MS2 scans in the data file, a specific range of scans within the file, or summed by precursor ion using ProteoWizard selection. The chosen summing algorithm can affect summing results based on the intended use

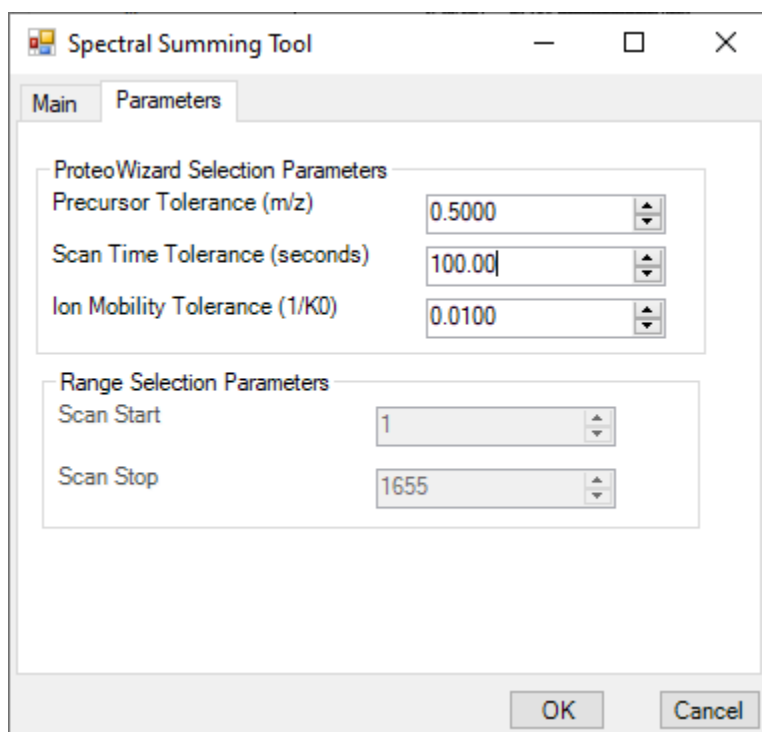
or design of the algorithm. There are four summing algorithms available, each with different strengths:

- UniDec-Interpolate: designed to oversample the data, best for spectra with low peak density  $m/z$  space.
- UniDec-Integrate: best for spectra with high peak density  $m/z$  space.
- Vendor: Enables vendor-specific averaging for thermo data files.
- Proteowizard: Provides simple, unweighted spectral averaging.

After the selection and summing algorithm have been chosen, users can determine handling for any MS1 scans in the data file, with options to skip, copy or sum the scans:

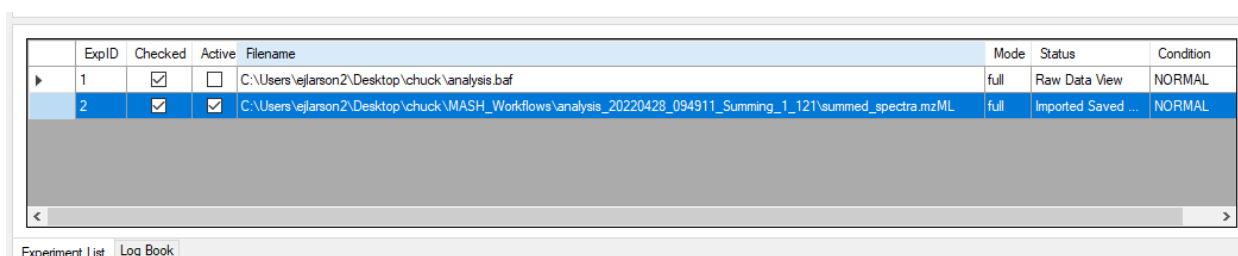
- Skip: Does not move any MS1 scans into the results file.
- Copy: Moves all MS1 scans into the results file unchanged.
- Sum: Generates a simple average of all MS1 scans as a single scan in the results file.

The Parameters tab in the Spectral Summing Tool window allows users to further define selection parameters (Figure 2).



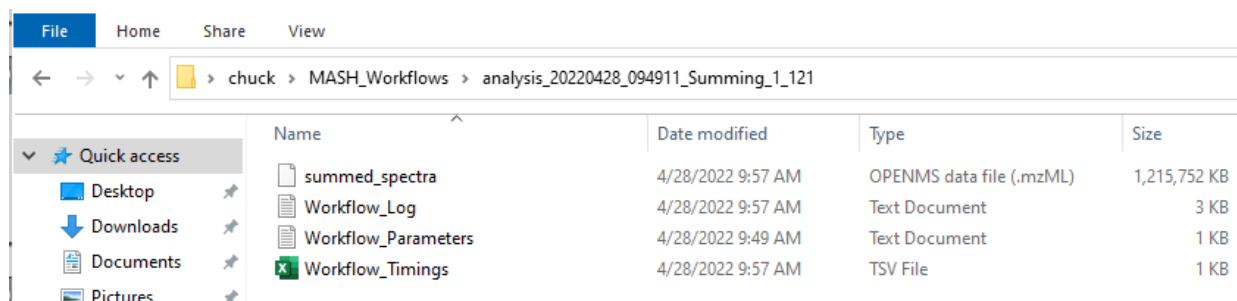
**Figure 2.** Selection parameters for spectral summing in MASH Native.

Note that while ProteoWizard selection lists precursor mass tolerance, scan time tolerance, and ion mobility tolerance, the first two parameters are currently active in MASH Native for ProteoWizard scan selection. Correct setting of the parameters is important to achieve the best possible summing results. Setting precursor tolerance (m/z) to the isolation width ensures that peaks picked from the same isotopic cluster are not counted as separate precursors. Additionally, setting scan time tolerance (s) to the average chromatographic peak width is recommended. After successful summing, an \*.mzML file was generated with the original non-summed file and located in the same file path as the raw data file (Figure 3, 4).



ExpID	Checked	Active	Filename	Mode	Status	Condition
1	<input checked="" type="checkbox"/>	<input type="checkbox"/>	C:\Users\ejlarsen2\Desktop\chuck\analysis.baf	full	Raw Data View	NORMAL
2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	C:\Users\ejlarsen2\Desktop\chuck\MASH_Workflows\analysis_20220428_094911_Summing_1_121\summed_spectra.mzML	full	Imported Saved ...	NORMAL

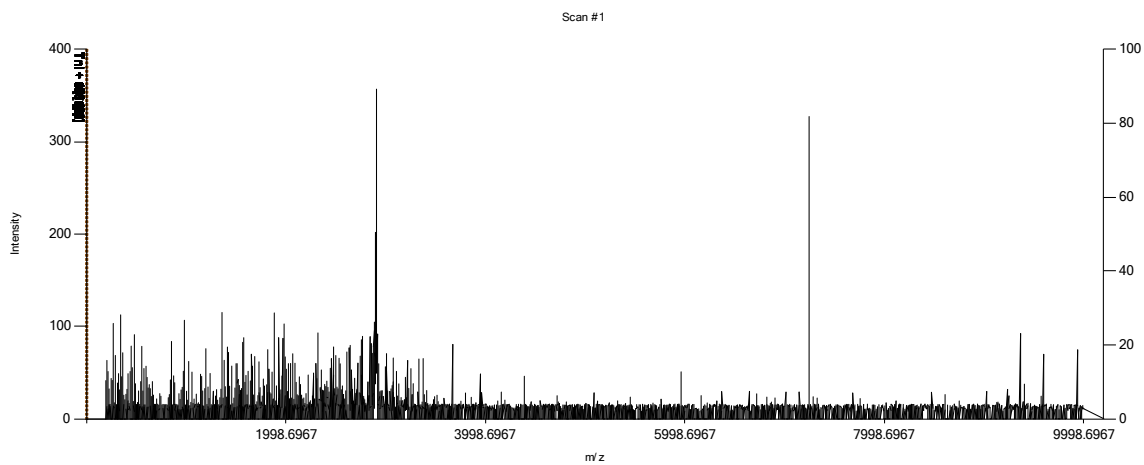
**Figure 3.** Summed spectra are output as a \*.mzML file.



Name	Date modified	Type	Size
summed_spectra	4/28/2022 9:57 AM	OPENMS data file (.mzML)	1,215,752 KB
Workflow_Log	4/28/2022 9:57 AM	Text Document	3 KB
Workflow_Parameters	4/28/2022 9:49 AM	Text Document	1 KB
Workflow_Timings	4/28/2022 9:57 AM	TSV File	1 KB

**Figure 4.** Summed spectra output file path.

The output spectra may then be searched or processed normally and should offer faster, higher S/N results than raw data (Figure 5).



**Figure 5.** Summed spectral results.