Converting Raw LC-HRMS/MS Files into mzML files

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In order to use the data analysis tools, all files must be converted into *.mzML files. In order to convert proprietary vendor files, all users *must* download ProteoWizard MSConvert tool, which can be acquired by going to https://proteowizard.sourceforge.io/ and downloading the most recent version of ProteoWizard.

Once downloaded and install, follow the next steps to convert the raw file(s) to *.mzML format.

1) Run ProteoWizard MSConvert program

Select the program from the start Menu > Proteowizard > MSConvert

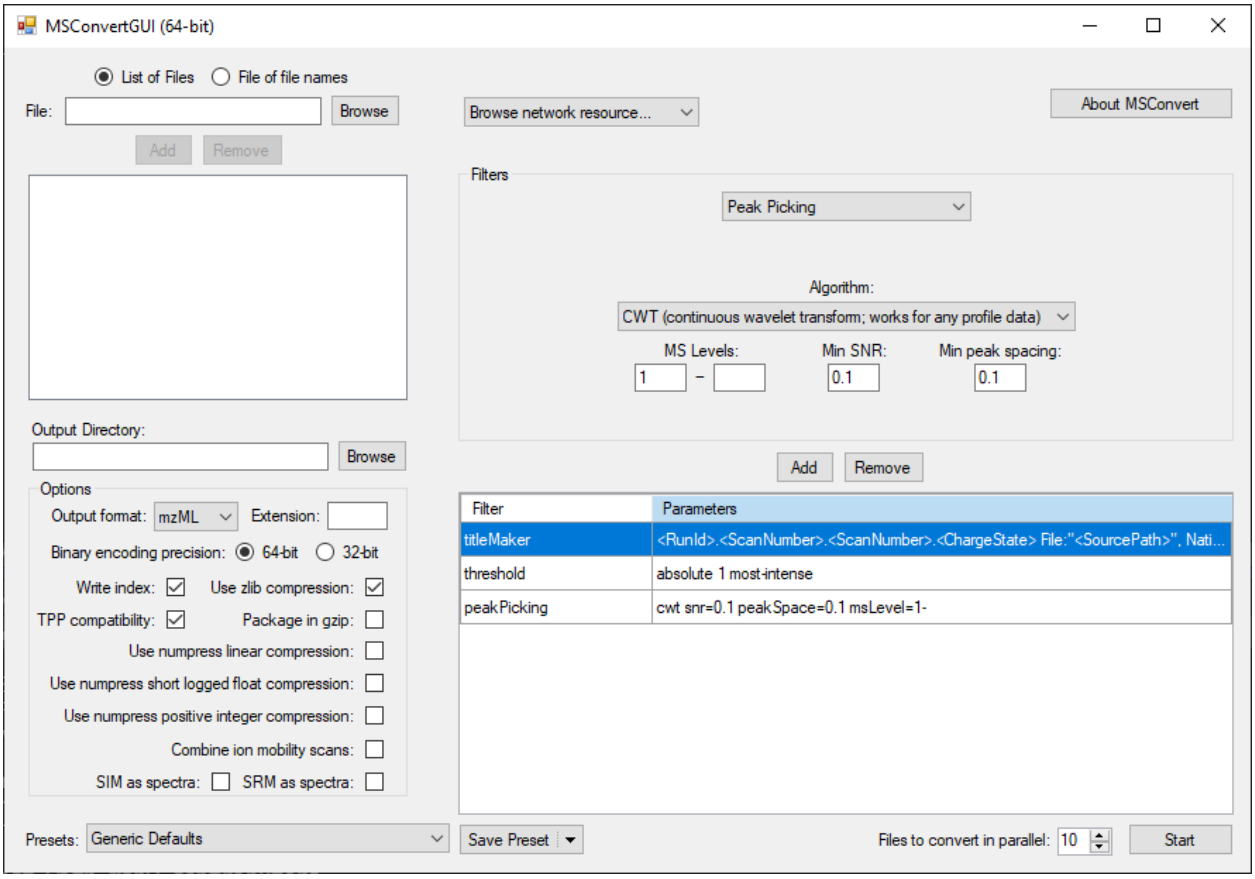

Figure 1: Initial screen when running MSConvert

2) Select the files to be converted

The MSConvert software can convert the following vendors:

Thermo (*.raw), Waters (*.raw), SCIEX (*.wiff2), Agilent (*.D), Shimadzu (*.LCD,) Bruker (*.D)

- Select the files using the **Browse** button.

Note: If selecting only one file at a time, you must press the *Add* button to include the file in the list.

- Select the *Output Directory* Folder

Note: It will default to the same directory as the original file.

After loading the file, the program should look like the below image.

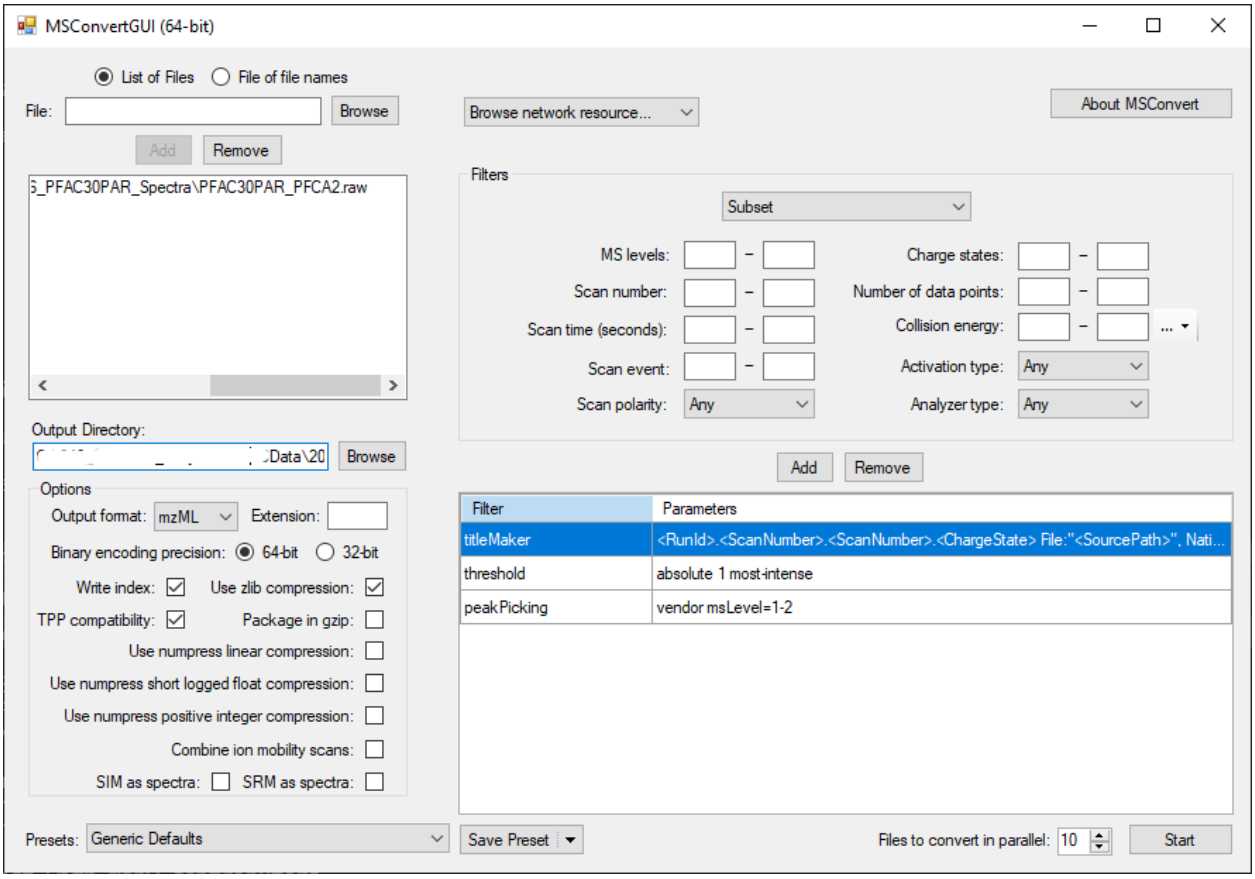

Figure 2: MSConvert with data file loaded

3) Adjust the mzML file parameters

- Select **mzML** from the *Output format* input and make sure the *Extension* input is blank
- Select **64-bit** under *Binary encoding precision*
- Check the box next to *Write Index*, *TPP Compatibility*, and *Use zlib compression* and leave all other boxes unchecked.

See the image above for the proper selection

4) Add the conversion filters

To use the data analysis tool the following filters *must* be used, after selecting the proper parameters click the **Add** button:

Select the *Peak Picking* filter and include the following parameters:

- **Algorithm**: Vendor or CWT

Note: Vendor filter does not work for Waters files, you must use CWT

- **MS Levels**: 1 -

If using CWT:

- **Min. SNR**: 0.1
- **Min. peak spacing**: 0.1

Parameters should look as follows

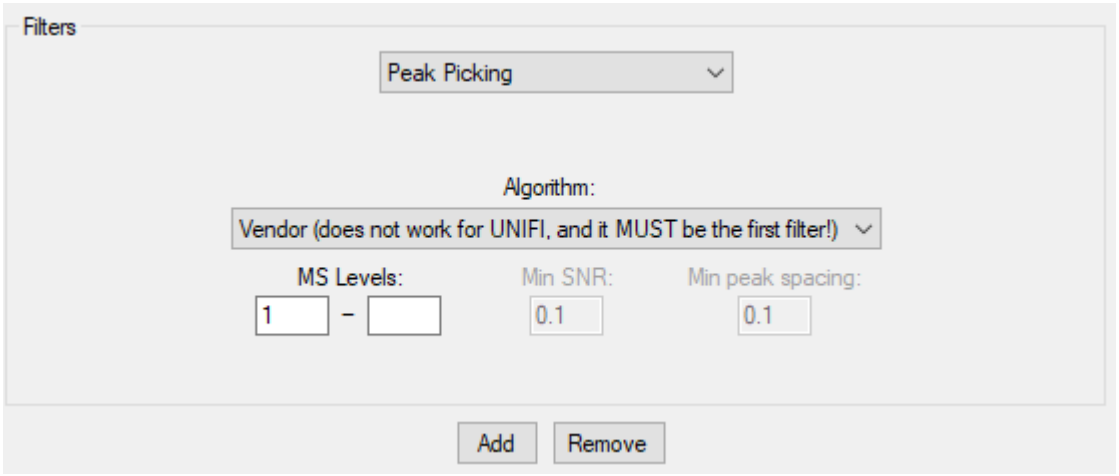

Figure 3: Peak picking filter parameters

Press the **Add** Button

Select the *Threshold Peak Filter* and include the following parameters:

- **Threshold type**: Absolute intensity
- **Orientation**: Most intense
- **Value**: 1

Parameters should look as follows

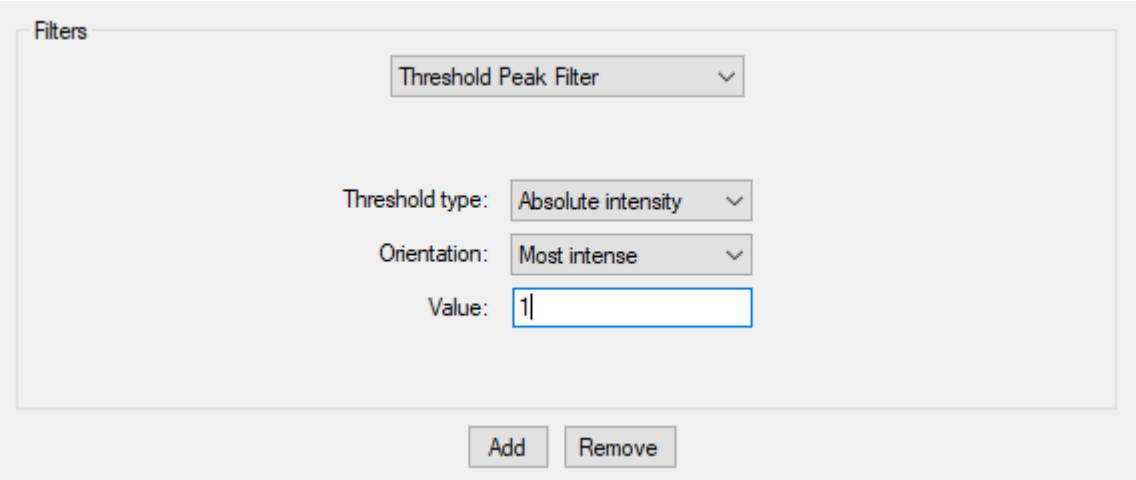

Figure 4: Threshold peak filter parameters

Press the **Add** Button

If the raw data is from a Waters Corporation mass spectrometer, you must use the *Lockmass Refiner*

- **Reference m/z**: use the m/z of the lockmass, for Leucine-Enkephalin (most common), the negative ionization m/z is 554.2615 and the positive ionization m/z is 556.2771.
- **m/z Tolerance**: 0.1

The parameters should look as follows:

Press the **Add** Button

Note: The TitleMaker filter shows up upon opening MSConvert every time, this can be included and will not affect the data analysis. But peakPicking must still be the first line in the filters.

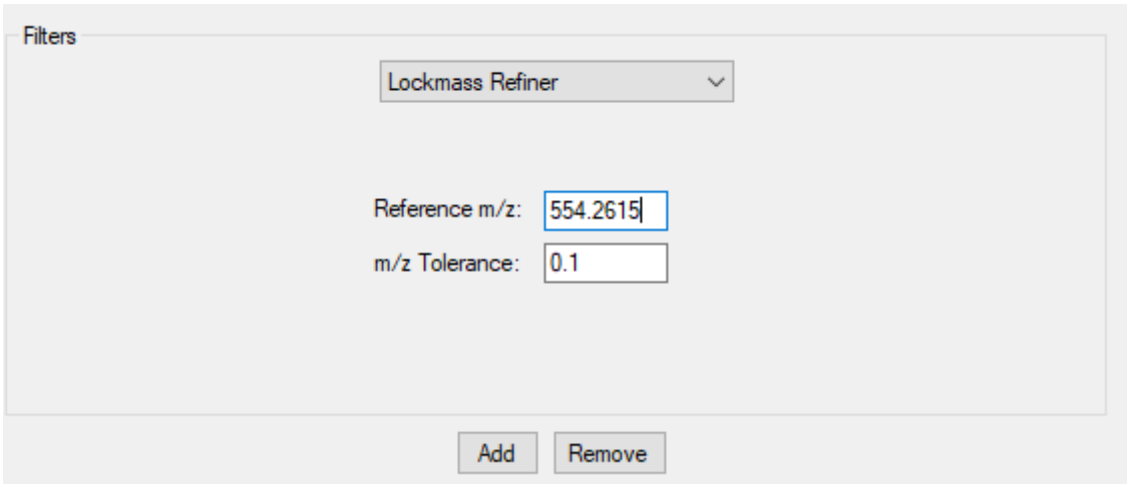

Figure 5: Lockmass refiner parameters

5) Press Start Button

This will convert all added files to *.mzML format and put them in the assigned Output directory.

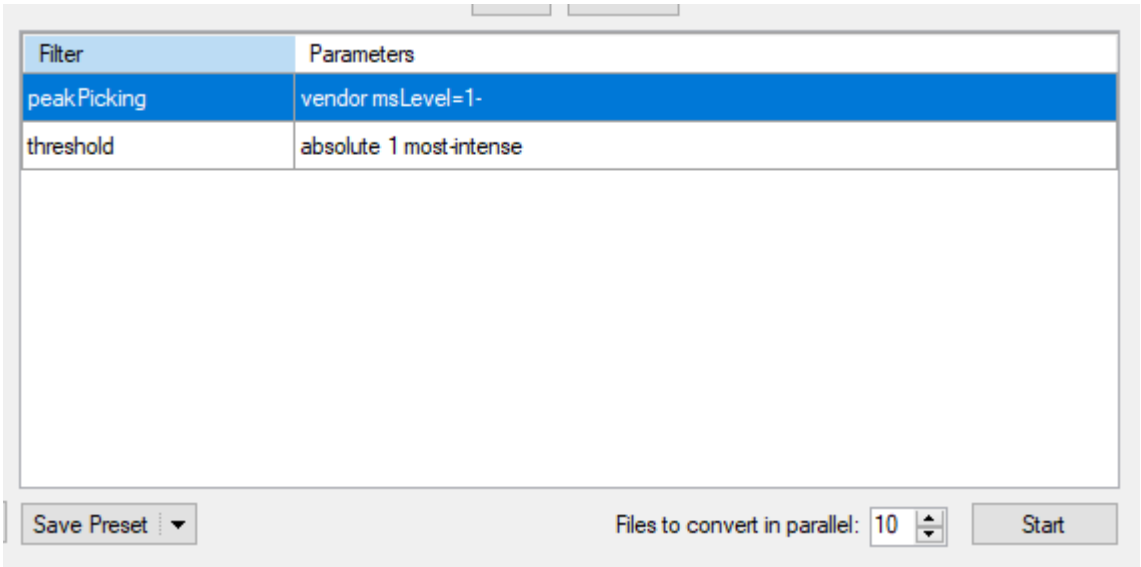

Figure 6: MSConvert Start button

Note: Waters Corporation instruments should have the lockmass refiner filter in the as well.

6) Save the settings as a preset using the *Save Preset* dropdown menu (optional)

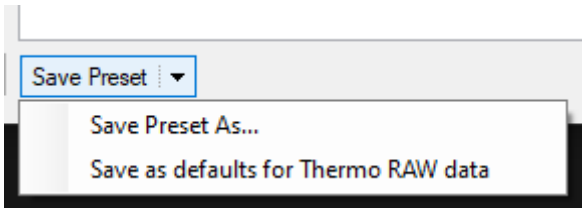

Figure 7: MSConvert presets menu