# Important Notes regarding the Spectrum Analyzer Software:

## Installation

To "install" the Software, simply copy all the files contained in this folder into a folder on your PC. The program will not work, if either of the dlls (MathNet.Iridium.dll or XRawfile2[\_x64].dll) is missing.

## Functionality

The Spectrum Analyzer Software was designed to analyze .raw files to provide complete information about the spectra. The following data is collected:

* **General Spectrum Information:**
  + Spectrum Number
  + Retention Time
  + Precursor Spectrum Number
  + Precursor m/z
  + NCE
  + Collision Energy in eV
  + Isolation width
  + Low mass
  + High mass
  + Cycle Time
  + Scans after last MS1 – scan
  + Cycle time
* **Precursor Information (for both MS1 and MS2 scans):**
  + Ion Injection Time
  + TIC (Total Ion Current)
  + Intensity
  + Noise
  + Signal to Noise
  + Total Intensity in isolation window
  + Total intensity in the whole scan
  + PIF (Precursor Intensity Fraction):  
    MS1: Precursor Intensity / Sum of Intensities in isolation window  
    MS2: Precursor Intensity / Sum of Intensities in whole MS2 scan
  + Precursor Isolation Efficiency:  
    = Precursor intensity in MS2 spectrum / Precursor intensity in MS1 spectrum.   
    This only yields useful results if the precursor is not fractioned.
* **Reporter Ion Information:**
  + Deviation in ppm
  + Intensity
  + Noise
  + Signal to Noise
  + Number of reporter ions found
  + Total intensity of the reporter ions
* **Elution Profile Information:**
  + Area
  + FWHM
  + Apex Intensity
  + Apex Retention Time

The collected data is output in a .txt file (comma separated values), which can easily be loaded into calculation software, such as Excel or R, where it can be further processed.  
For each input .raw file an output file with the name **[.raw file name]-analysis.txt** is created.

## Usage

Click on **Select RAW File(s)** in the **RAW Files** – Tab (see Figure 1) to choose the files you want to analyze.



Figure : RAW Files

In the **General Settings**– Tab (see Figure 2) you can make the following settings:

* **Search Tolerance:**Defines the maximally allowed tolerance with which certain ions (rep. ions and precursor) that are searched in the spectra may deviate from their expected mass.
* **Apply MS1 Precursor Interpolation:**  
  If this is checked the MS1 – scan related values used for calculations in an MS2 – scan are linearly interpolated between the preceding and the following MS1 – scan.  
  The idea is to obtain an approximation of the actual current values.  
    
  In general, the values are taken from the previous MS1 – scan.
* **Replace Precursor Information From Previous Scan:**If this is checked, the values from the previous MS1 – scan are completely replaced by the interpolated values in the output. Otherwise, additional columns labeled **Previous MS1 - …** are inserted in the output, where these values can still be found.
* **Output Status Log Information:**Not fully implemented yet!!  
  At the moment, checking this will additionally output the **Spray Current** and the **Spray Voltage**.



Figure : General Settings

The **Quantitation Options** – Tab (see Figure 3) offers the following settings:

* **Perform Quantitation Analysis:**The reporter ion information is only gathered, if this box is checked. Otherwise, no respective columns will be generated in the output file
* **Select Quantitation Method:**  
  Choose the quantitation method to determine the reporter ions that should be searched for in the spectra.
* **Reporter Ion Filter Settings:**  
  Allows the definition of Intensity and Signal to Noise thresholds for the reporter ions. I.e., if an ion does not exceed both thresholds it is considered not present in the spectrum.



Figure : Quantitation Options

Finally, the **Peak Shape Options** – Tab offers the possibility to perform an elution profile analysis. I.e. the respective parameters are added to the output.

**Please note that this step can dramatically increase the runtime of the analysis!**

It should therefore only be applied, if the information is indeed needed.



Figure : Peak Shape Options