

## MsXelerator™ High Resolution Peak Picking and Isotope Pattern Filtering using the IPF II Algorithm - A Quick Starting Guide -

**Introduction:** this quick starting guide teaches you how to perform High Resolution Peak Picking based on Isotopic Signatures. The IPF II algorithm can use any isotopic pattern for peak detection. In its most basic form, the algorithm is used to detect peaks having a  $^{12}\text{C}/^{13}\text{C}$  isotopic pattern. Special Patterns are e.g. Cl, Br, unlabeled/labeled GSH,  $2^+$ ,  $3^+$  charge ions or more complicated patterns. The IPF II algorithm is available from both the MPeaks and the IPeaks modules. IPF II finds co-eluting peaks having a specific mass difference in a specific ratio. Margins on the mass accuracy and ratios can be set by the user.

- Data Loading/Importing:** start the Browser and Import or open a data file. **Attention:** the High Resolution Peak Picking algorithm IPF II can only use centroided data. Be sure to convert to centroided data during import if your data are in profile mass mode.
- Checking a few Basic Parameters:** using the Browser, you need to determine 3 basic parameters: width of relevant Peaks, accuracy of your instrument and an estimate of the noise level. To Plot Accurate EICs enter the accurate mass value in the m/z box or click any mass peak from the spectrum. Be sure direct accurate plotting is activated (Item under Options). Select or plot a few EIC's of some relevant peaks and measure/estimate the following: the width of the peak. You can make your scans visible by pressing the **Scans Visible Icon** button. Next use the Peak Width Measurement tool to estimate the width in minutes and scans. Try to measure just above the baseline. From the EIC's and the Mass Spectra try to estimate the noise level, e.g. Orbitrap noise level  $\sim 10.000$  counts. Using high resolution EIC plotting decide what mass accuracy is applicable, e.g. for Orbitrap you can typically use  $\pm 0.005$  Da to extract accurate chromatograms.
- Start MPeaks or IPeaks:** if you want to detect charge  $1^+$  peaks, start MPeaks from the Browser. For any other isotopic signature start IPeaks. When using MPeaks select from the menu: "High Res. Special > High Resolution Peak Picking". When using IPeaks press the "IPF II" button. The following GUI on the right will be displayed. The different options and settings are explained below.

- As a default, always use the "Full Search" option.
- Select the Mass and Time Range you want to use. Select "Full" to return to the maximum mass and time ranges of your data file.
- Select the Isotopic Pattern from the Predefined Delta m/z List. This will set the mass difference. Optionally you can fill in any mass difference you want to use.
- The mass difference related to the pattern will be displayed. Decide on the accuracy of your instrument. For Orbitrap Data you can use an accuracy of  $\pm 0.005$  Da. For lower resolution instruments use a

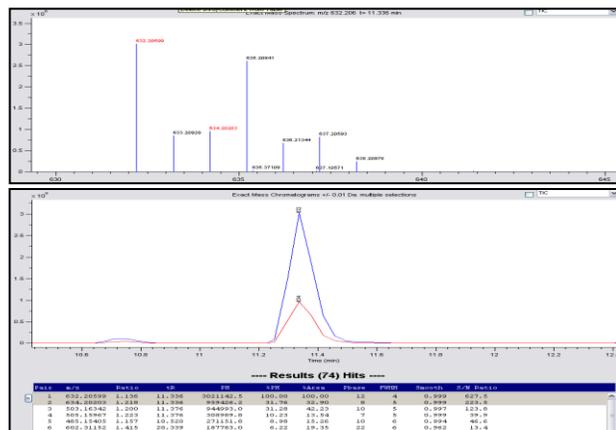


higher value. The low/high ratios between the light and heavy isotope peaks are selected based on the predefined pattern. Change these values if necessary. You can use the Browser to overlay accurate EICs of light and heavy isotope peaks. From the Task items, you can calculate the ratio between both peaks.

- Leave the “Check and Filter on ratios” option checked.
  - When detecting  $1^+$  ions, you probably don’t want to add the second isotope peaks to the table. Uncheck the option “Add Second Isotope Peak to Result Table”. When using specific isotope patterns, you do want to check this option. In this case you can plot both isotope peaks in overlay directly from the result table.
  - In the field “Window Width”, you can enter the estimated peak width in scans.
  - In the “Intensity Threshold” field you will have to enter the estimate of the noise level, or the threshold for peak detection. The threshold applies to the first isotope peak.
  - Depending on the application you may want to use special weighing for the mass range below  $m/z$  300. Sometimes there can be an enormous amount of ions in the low mass region. If this range is not so much of interest you can apply a higher threshold for this region.
  - Always use the “Check Co-Elution” option. Specify the width in minutes for calculating the correlation. As a default the algorithm uses a correlation threshold of 0.8. Use lower levels to allow more sensitive peak detection.
  - Check the “Remove  $^{13}\text{C}$  Isotopes” Option, to automatically remove  $^{13}\text{C}$  peak hits from the list.
  - When using very sensitive settings, the algorithm might find small peaks only 1-2 scans wide. Check this option to remove very narrow hits. Optionally you can smooth the extracted ion currents before calculating the correlation.
  - Press “Load Defaults” to use default settings for GSH detection (labeled/unlabeled GSH, ratio 1:1).
4. **Press Run to start the Calculations:** IPF II will show a number of intermediate results regarding the number of ions it has to process in each step. All full Scan MS Spectra will be checked. If the number of ions in the second step is very high, e.g. larger than 50.000, please consider different settings. You might want to change the noise threshold or use a smaller mass range or skip the first or last part of the chromatogram. These regions often have large amounts of ions, not related to your specific questions and take a lot of processing time.

5. **View and Check your Results:** Check and plot overlays of light and heavy accurate EIC’s and the MS spectrum at the detected retention time for each pair. The displayed spectrum will show the specified Isotope Peaks marked in red.

6. **Additional Processing Options:** both the MPeaks and IPeaks module contain a large number of additional processing algorithms to further reduce the list. E.g. apply differential analysis using a control sample, check which peaks are co-eluting or which peaks from the list might be adducts. You can further check which peaks are inside a specific Mass Defect Window (MDF). Start the Identification Engine to search against different data bases or use the list of common Biotransformation (Drug Profiling).



7. **Problematic Data Files:** IPF II is a very fast, sensitive and powerful algorithm. However, sometimes processing can be relative slow. This might be the case when the isotopic pattern is not very specific (e.g.  $^{12}\text{C}/^{13}\text{C}$  pattern), or when background ions are present eluting over the full retention time range (with same isotopic pattern). In these cases the calculation can take a long time to complete. Consider different settings as mentioned in 4. As an alternative you can use the IPF I algorithm in IPeaks or use Nominal Peak Picking followed by conversion to accurate mass in MPeaks.

**Full Document References:**

1. MsX Differential Analysis two samples 2011.pdf
2. MsX Reactive Metabolite Detection\_Tutorial\_IPF\_II and Postprocessing Filters.pdf
3. MsX Searching 2+ Ions using IPF II.pdf