

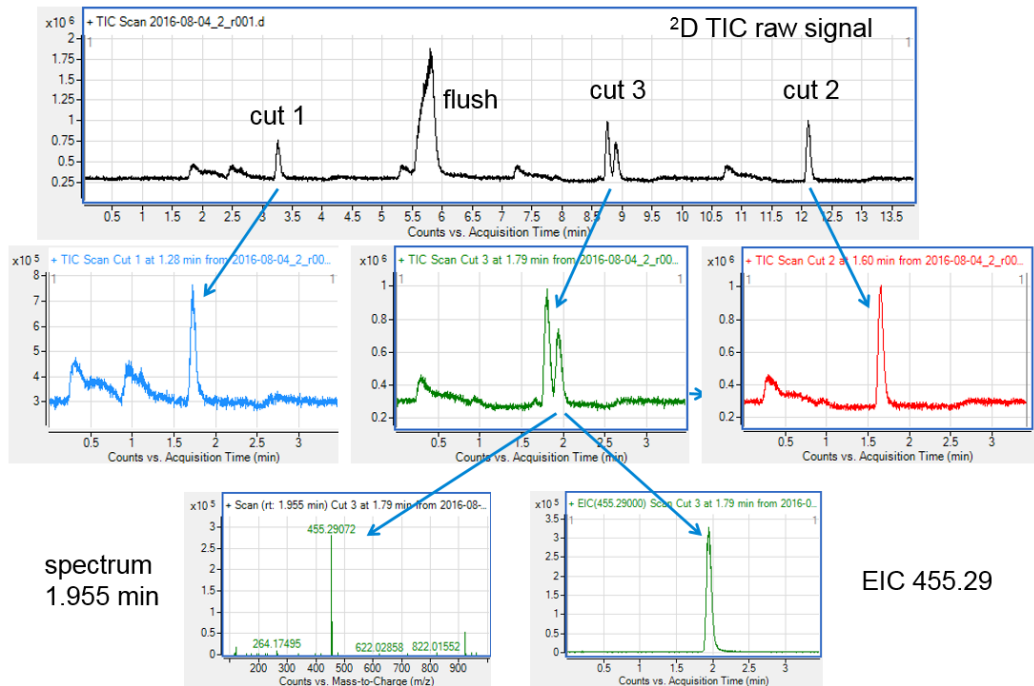


# <sup>2</sup>D Chromatogram Creator for MassHunter

## Introduction

<sup>2</sup>D Chromatogram Creator for MassHunter provides an easy access to 2D-LC measurements for Agilent MassHunter users. Similar to the 2D-LC Viewer, it generates <sup>2</sup>D chromatograms (abundance over time, usually the TIC) for cuts created in **Multiple Heart-Cutting or High-Resolution Sampling 2D-LC** for display and data analysis in MassHunter.

Comprehensive 2D-LC measurements can be displayed and analyzed with GC Image LCxLC Edition Software, which is described in the 2D-LC system manual, that can be found in folder documentation of the 2D-LC Software.



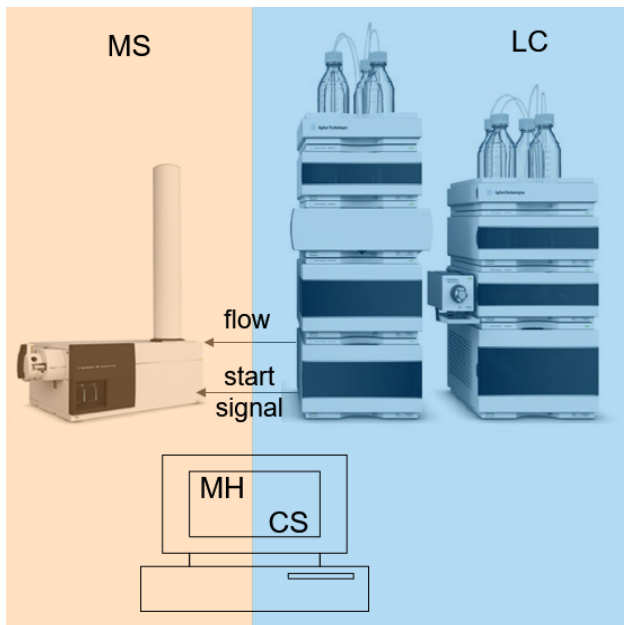
**Figure 1** Splitting the raw data file into 2D chromatograms

The raw MS TIC signal continuously measures MS data of the <sup>2</sup>D eluent. <sup>2</sup>D Chromatogram Creator for MassHunter creates one <sup>2</sup>D chromatogram per cut made in the first dimension of a multiple heart-cutting or high-resolution sampling measurement. These chromatograms can be used for further data analysis in MassHunter.



## Function

For running 2D-LC/MS measurements, this is a typical system:

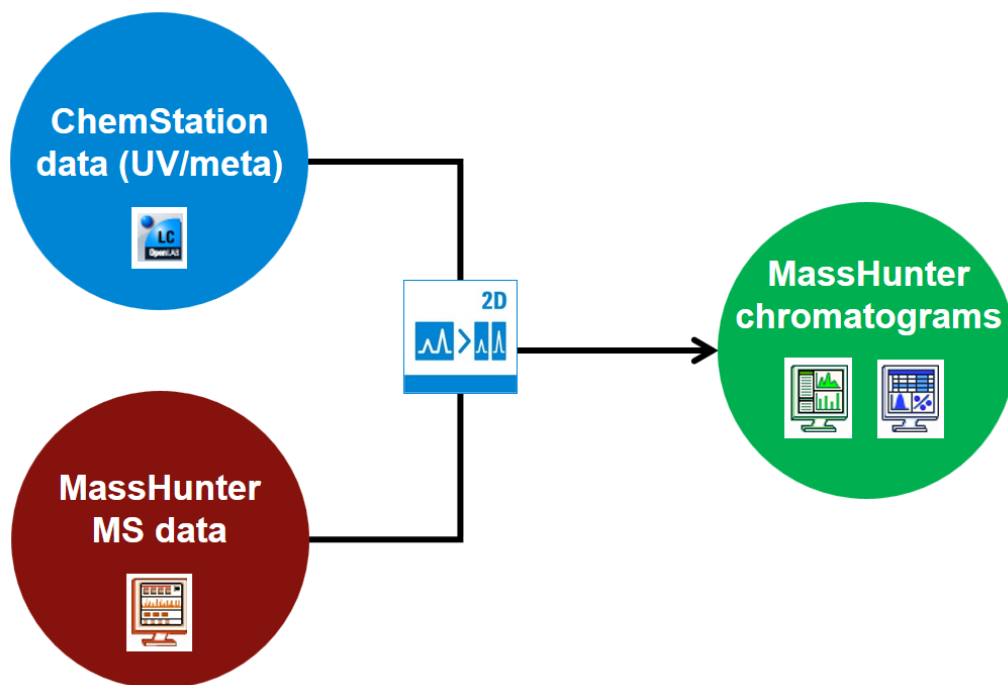


**Figure 2** Typical system setup

The LC part is an LC system as described in the 2D-LC system manual. A typical system uses a UV detector in the first dimension and an optional UV detector in the second dimension. The 2D-LC instrument control and UV signal acquisition is done with OpenLAB CDS ChemStation (CS) Edition.

The MS detector, a TOF, Q-TOF or Triple Quadrupole, is used in the second dimension. It is controlled by MassHunter (MH) Acquisition Software, which acquires the MS signal.

For synchronizing signals of LC and MS, an APG remote cable connects both parts of the system, which communicates start and stop signals.



MassHunter has no knowledge about the size, order and position of cuts created by OpenLAB CDS ChemStation Edition. Therefore, <sup>2</sup>D Chromatogram Creator for MassHunter is used for combining UV signals and meta data of cuts in OpenLAB CDS ChemStation Edition with the MS signal acquired by MassHunter.

As output, it creates following MassHunter chromatograms:

- A series of <sup>2</sup>D chromatograms, one per cut including MS and optional UV signals
- If applicable, a <sup>1</sup>D UV chromatogram (for a typical but optional <sup>1</sup>D UV detector) in MassHunter data format including cut data
- An optional raw <sup>2</sup>D UV signal and a
- <sup>2</sup>D MS raw signal

## Compatibility

### NOTE

MassHunter LC/MS Data Acquisition Version 10.0 requires 2D Chromatogram Creator rev. 1.1.20.

<sup>2</sup>D Chromatogram Creator for MassHunter, OpenLAB CDS ChemStation Edition and MassHunter have been tested for co-execution (i.e. installed and running on same PC) with respect to parallel data acquisition in following configuration:

- <sup>2</sup>D Chromatogram Creator 1.1.20

- OpenLAB CDS ChemStation Edition C.01.07 SR3 (Workstation)
- Agilent OpenLAB 2D-LC Software A.01.04
- MassHunter Q-TOF B.08.00
- MassHunter LC/QQQ B.08.02
- MassHunter Data Analysis B.08.00
- Operating system: Windows 10 Professional 64-Bit

### Modules:

- 1290 Infinity II modules supported with LC Driver A.02.16
- QQQ or Q-TOF

### PC Hardware:

- MassHunter bundle PC HP Z440 or equivalent

<sup>2</sup>D Chromatogram Creator for MassHunter has also been tested with OpenLAB CDS ChemStation Edition C.01.07 SR2, MassHunter Acquisition for TOF and QTOF B.06.01 SP1, MassHunter Acquisition for QQQ B.08.00 SP1, MassHunter Qualitative Software B.07.00 SP1 and SP2 and MassHunter Quantitative Software B.07.01. No co-execution of OpenLAB CDS ChemStation Edition and MassHunter was tested in this configuration.

MassHunter Qualitative B.07.00 is known to display misleading signal names.

## Installation

Install the 2D-LC/MS system as outlined above. The 2D-LC system installation is described in the 2D-LC system manual. The installation of the MS instrument and its hydraulic and electronic connection to an LC system is described in the MS manual. Installations of OpenLAB CDS ChemStation Edition and MassHunter software packages are described in respective user manuals.

Install <sup>2</sup>D Chromatogram Creator for MassHunter by double-clicking setup.exe in folder software of the 2D-LC Software DVD. For simple file handling, <sup>2</sup>D Chromatogram Creator for MassHunter should preferably be installed on the PC with MassHunter data analysis software.

## Example Data

Folder "example Data\2D Chromatogram" on the 2D-LC software CD contains two subfolders MassHunter and ChemStation with example data which can be used for trying out this software. It uses a sequence/worklist with 3 measurements of 3 compounds:

**Table 1** Example Data

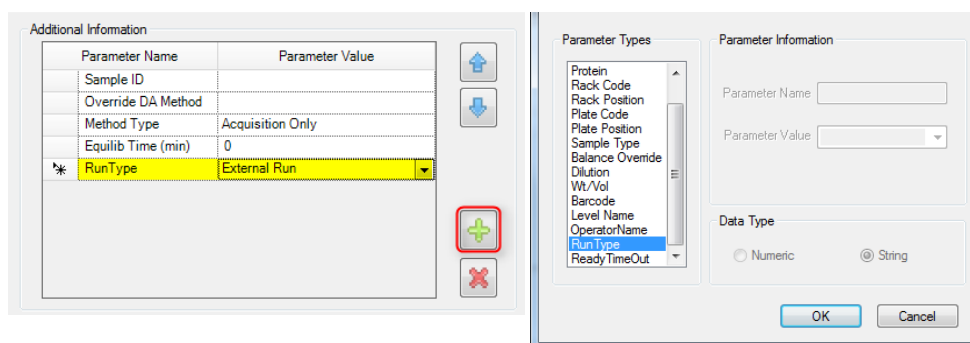
#	Compound	m/z	<sup>1</sup> D retention time
1	Imipramine	280.407	1.33 min
2	Protriptyline	263.37	1.66 min
3a	Nortriptyline	263.38	1.84 min
3b	Verapramile	454.6	1.84 min

I.e. compounds 3a and 3b are co-eluting in <sup>1</sup>D and are separated in <sup>2</sup>D. Compounds 2 and 3a have the same mass but are separated in the <sup>1</sup>D chromatogram. See also figures 1 and 13.

## Workflow for a Single Measurement

For a 2D-LC/MS measurement, methods need to be set up in both OpenLAB CDS ChemStation Edition and MassHunter. Set up the 2D-LC method as described in the 2D-LC system manual. Set the stop time in the <sup>2</sup>D pump. Then create a method in MassHunter for the MS.

For synchronizing run times (i.e. starting and stopping measurements simultaneously), please use “External start” as a run parameter:

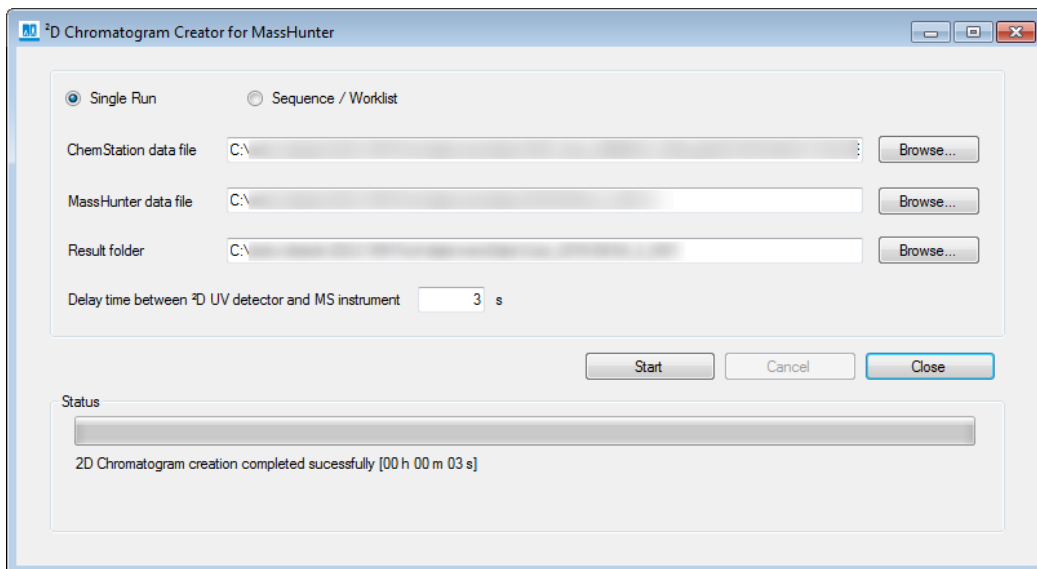


**Figure 3** Using run type external start

In section “Additional information”, add parameter “run type” and choose option “external run”. Set stop time “no limit” in MassHunter.

Then start the run in OpenLAB CDS ChemStation Edition, which will automatically start the MassHunter run. Both chromatographic data systems will create data files, which will be used for combined data analysis. Make OpenLAB CDS ChemStation Edition and MassHunter data files available through a network share or copy data to the PC with <sup>2</sup>D Chromatogram Creator for MassHunter. Having both files on one PC is faster, more secure and recommended.

Start <sup>2</sup>D Chromatogram Creator for MassHunter.



**Figure 4** User interface for single data files

Use mode “Single Run”.

Provide data files for OpenLAB CDS ChemStation Edition and MassHunter. A result folder is proposed and can be changed as needed.

If an optional UV detector is used in <sup>2</sup>D, a delay time can be set between this detector and the MS detector. This value is used for aligning MS versus UV data by compensating the time offset.

Click the start button for starting the file processing. This can take some time depending on the file size.

## Workflow for a Sequence/Worklist

Create methods in both OpenLAB CDS ChemStation Edition and MassHunter.

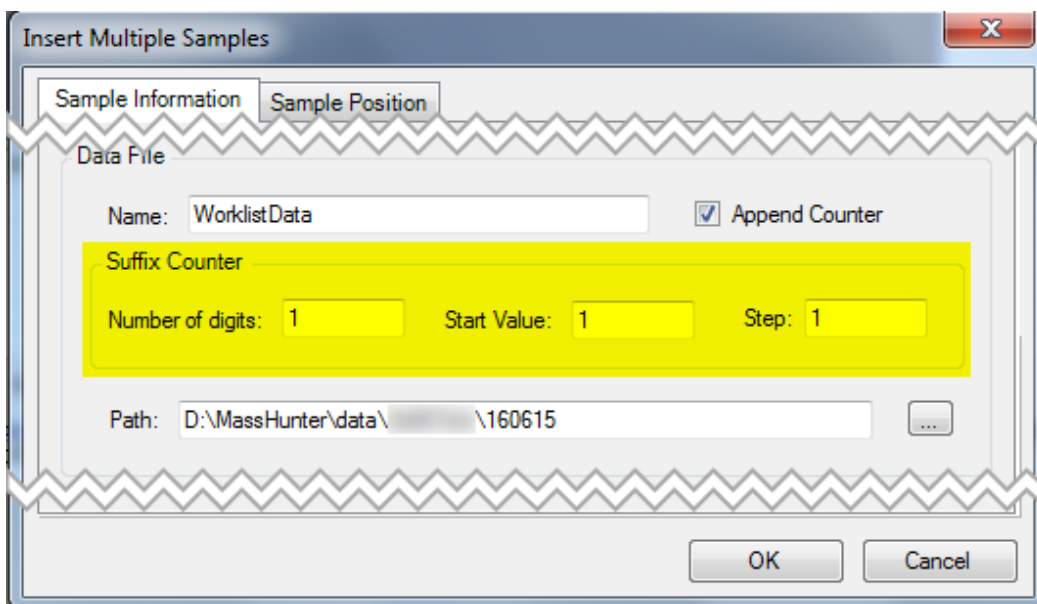
Set up a sequence in OpenLAB CDS ChemStation Edition.

The screenshot shows the 'Sequence parameters' dialog box with the 'Sequence output' tab selected. Under the 'Data file' section, the 'Path' is set to 'C:\Chem32\1\Data\' and the 'Subdirectory' is empty. The 'Prefix/Counter' radio button is selected, with a prefix of 'mySample' and a counter of '001'. The 'Name Pattern' section shows a pattern '<SeqLine><SampleLoc><SampleName>' resulting in the file name '042-P1-A1-Example.D'.

**Figure 5** Using counters for data files in OpenLAB CDS ChemStation Edition

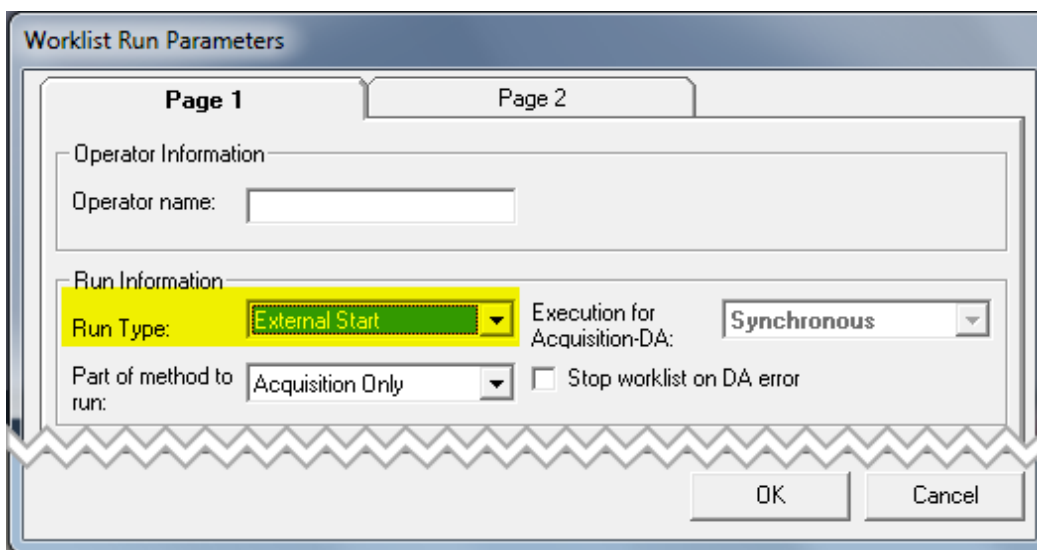
In sequence parameters, use an incremental counter for the data file names. This counter is the recommended way for later linking OpenLAB CDS ChemStation Edition data files to MassHunter data files.

Similarly set up a worklist in MassHunter and use an incremental counter. There are various possibilities for applying a counter, e.g. inserting multiple samples using a counter.



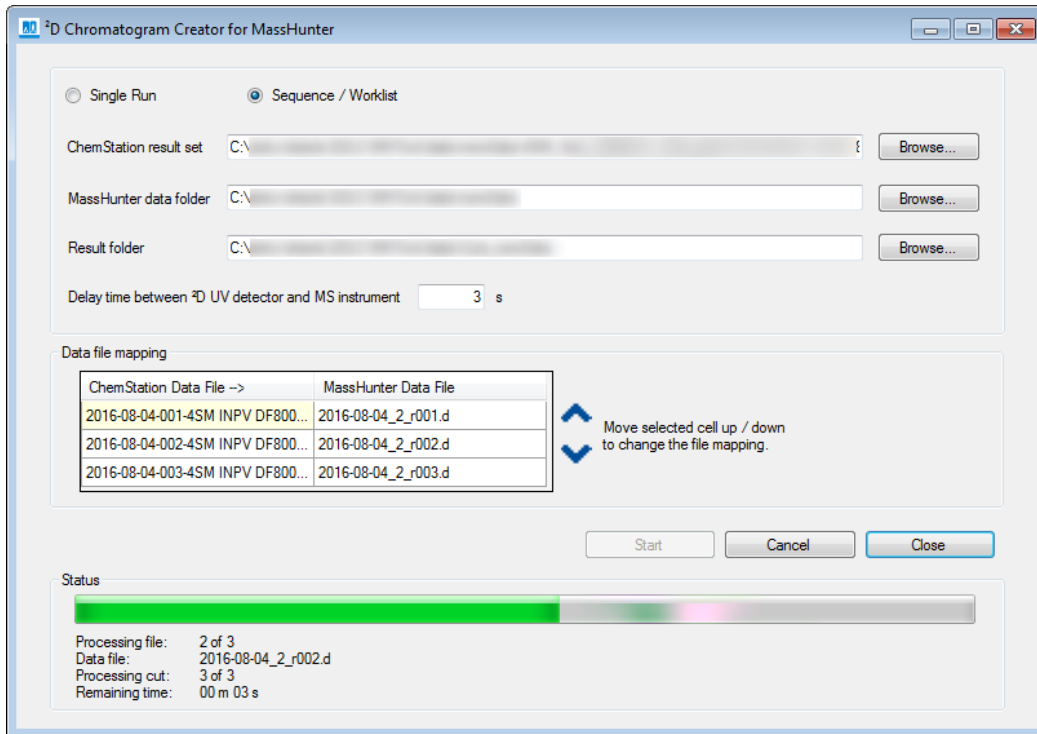
**Figure 6** Using counters for data files in MassHunter Worklists

Set “External Start” as run type in “Worklist Run Parameters” for synchronizing runs.



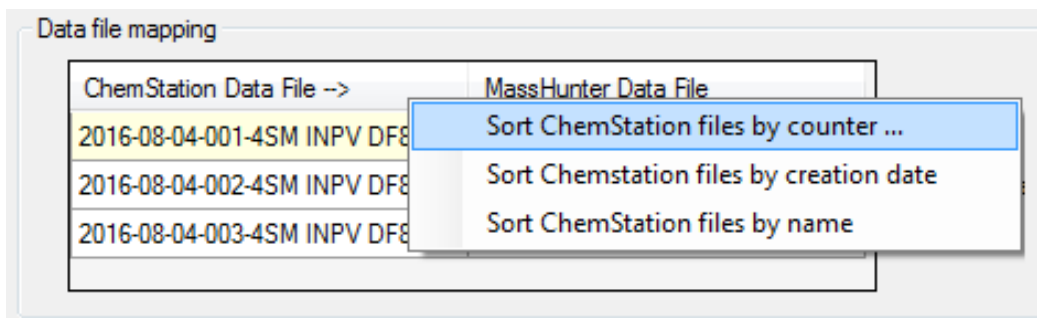
Start <sup>2</sup>D Chromatogram Creator for MassHunter with option “Split multiple data files”:

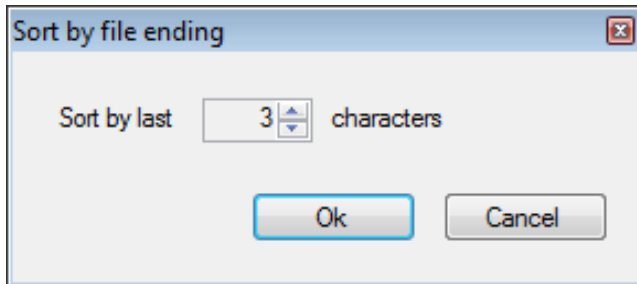




**Figure 7** Data file mapping

In table “Data file mapping” OpenLAB CDS ChemStation Edition versus MassHunter data files are matched. Each line lists the OpenLAB CDS ChemStation Edition data file and the corresponding MassHunter data file. Please verify if the default linkage is correct, which sorts data files for both software editions by their creation date (same chronologic order is applied). However, this date may change when copying files. Sorting can be adjusted by right-clicking on the headers of the data file mapping table. Different sorting options are available for both OpenLAB CDS ChemStation Edition and MassHunter:



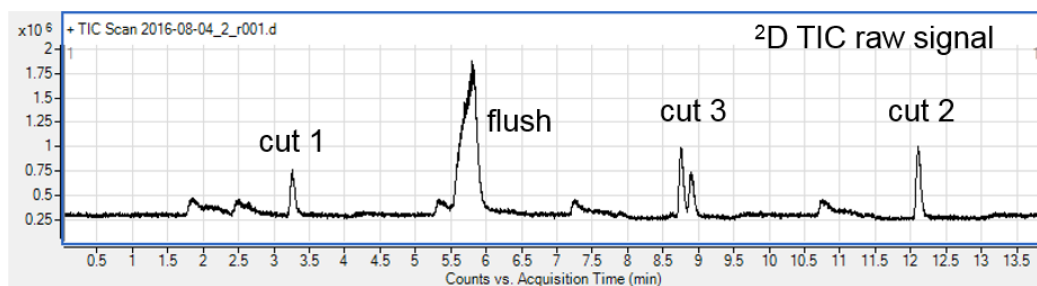


**Figure 8** Sorting options

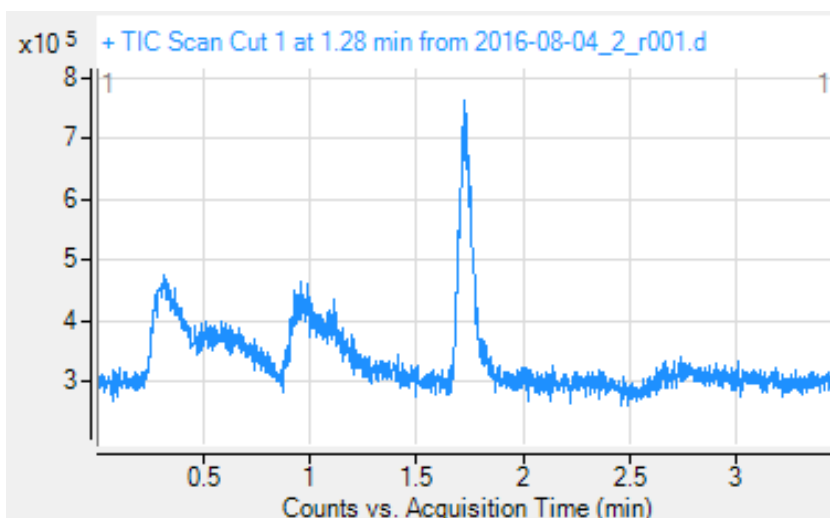
Sorting by file ending using the counter defined previously is the best user-defined way for linking data files.

## Extracting and Displaying Result Data

Result files are stored to the result folder set above. Data for cuts can be imported as single files or by loading all data files at once. Each cut contains data for the <sup>2</sup>D signals (MS, optional UV) and the <sup>1</sup>D UV signal. By default, the <sup>2</sup>D TIC is displayed:



**Figure 9** Raw <sup>2</sup>D MS signal (TIC)

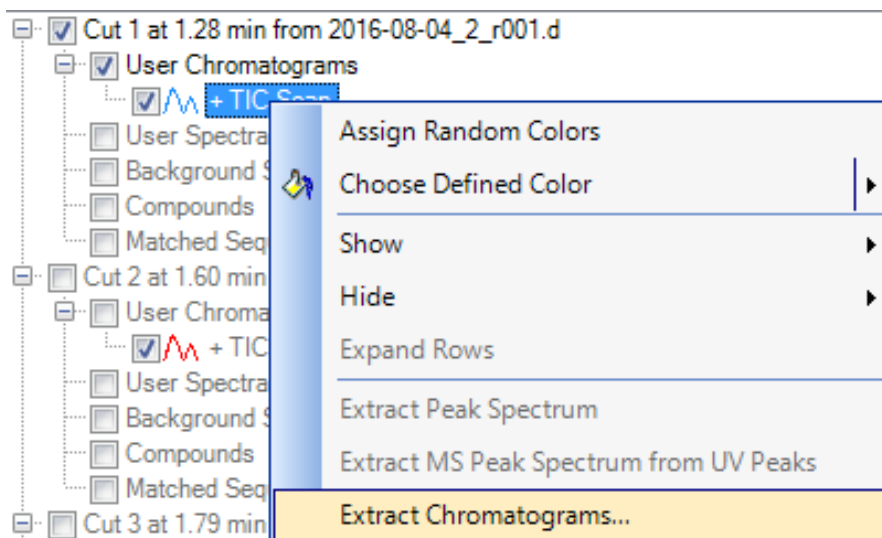


**Figure 10** TIC for cut 1 at 1.28 minutes in <sup>1</sup>D

Figure 10 shows an example for cut 1. <sup>2</sup>D Chromatograms are named by their cut number, the first dimension retention time and the original MassHunter data file. This example uses a <sup>2</sup>D gradient time of 3.5 minutes corresponding to the length of that <sup>2</sup>D chromatogram.

In case of high-resolution sampling measurements, series of adjacent cuts can be defined. Then, cuts are numbered by the series and an incremental counter, i.e. cut 1-3 is the third cut of the first series.

The optional <sup>2</sup>D UV chromatogram can be extracted by right-clicking the TIC scan and using function “Extract Chromatograms...”, see Figure 11.



**Figure 11** Extracting chromatograms

Use Type Other Chromatograms and choose the <sup>2</sup>D detector (in this example DAD2) and the signal with the wavelength of interest, see Figure 12.

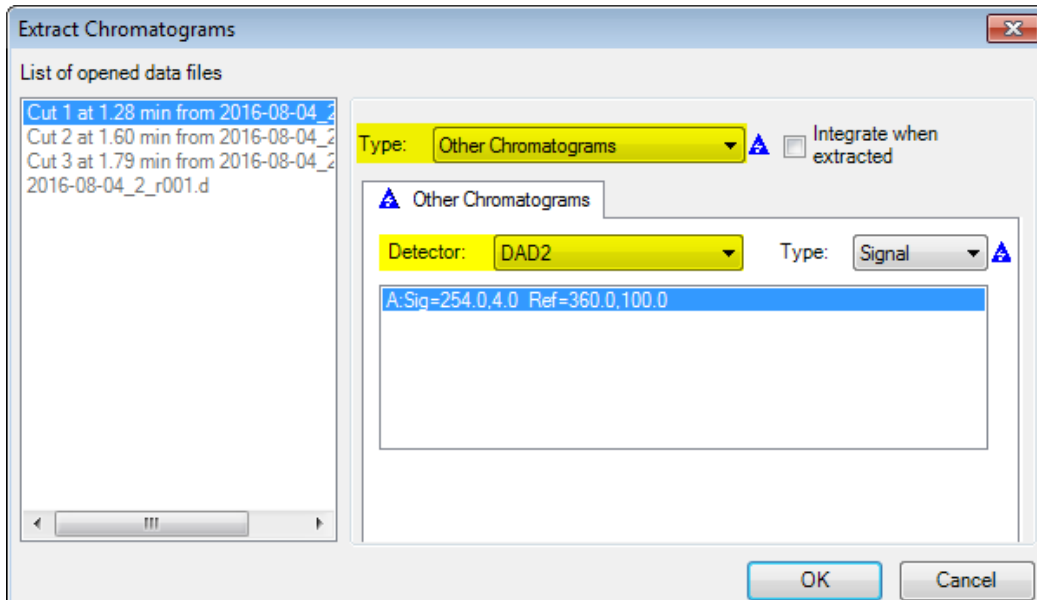


Figure 12 Available chromatograms

Similarly, chromatograms can be extracted from the original <sup>2</sup>D MS data file, which has been enriched by 2D-LC and UV data. Available signals are

- <sup>1</sup>D UV signal
- <sup>2</sup>D UV signal
- Cuts in <sup>1</sup>D

Similar to the 2D-LC Software, these data files can be used for displaying the <sup>1</sup>D measurement with cuts (here in red), see Figure 13.

All MS Chromatograms can then be processed further in MassHunter as usual, e.g. for extracting ion chromatograms, spectra and data analysis.

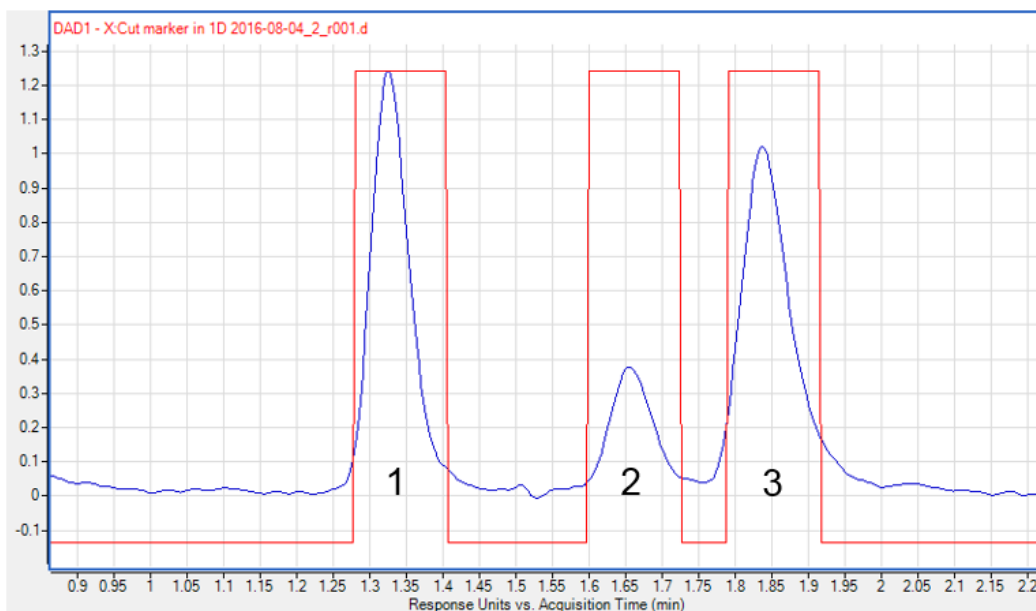


Figure 13 1D UV Chromatogram with cuts

