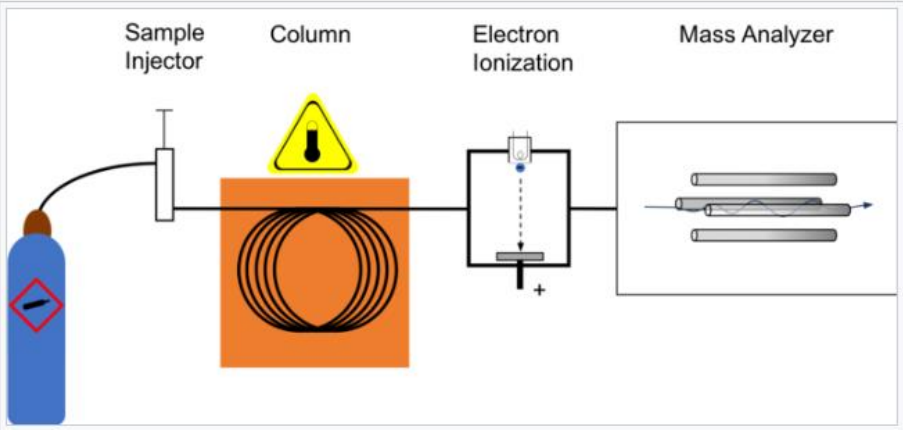
Free NIST GC-MS Software Lab for Universities:

Part 4: Processing GCMS Data with AMDIS



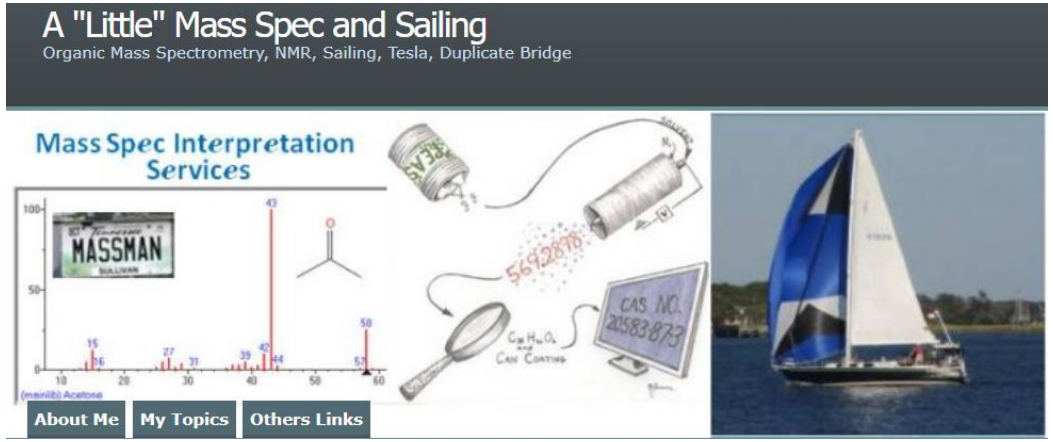
James Little
August 29, 2023
38 years Eastman Chemical Company
7 years Mass Spec Interpretation Services
https://littlemsandsailing.wpcomstaging.com/

Link to GCMS Schematic Above

Link to University Logos

Free NIST GC-MS Software Lab for Universities

Part 1: Very Basic Theory of GCMS Analyses
Part 2: Installation of Software
Part 3: Library Searches
Part 4: Processing GCMS Data with AMDIS
Part 5: Understanding EI Fragmentation with MS Interpreter
Part 6: Structure Searches with Input from ChemSketch
Part 7: Creating a User Library
Part 8: Advanced Processing with NIST Software

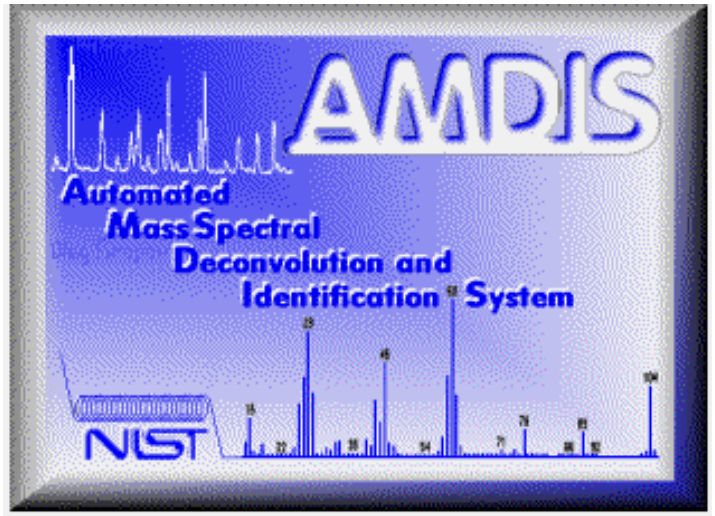


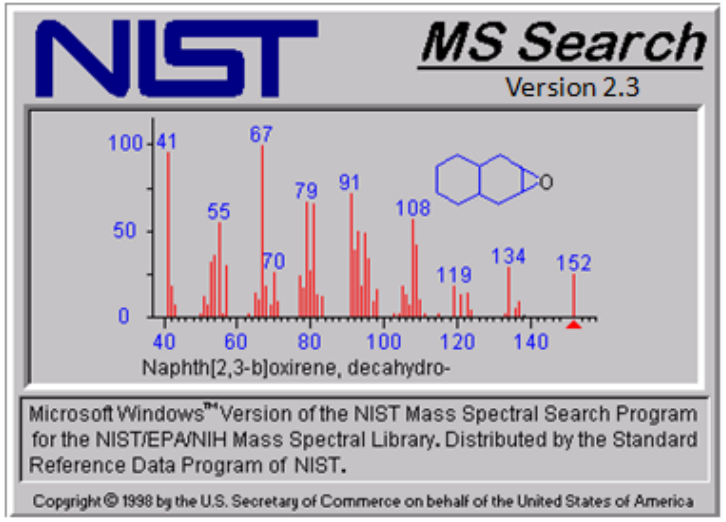
Link to Training Website

*Modified** Basic Instructions for Using AMDIS with MS Search

By James L. Little, O. David Sparkman
Input from Gary Mallard
*9/6/2020 (Many additional slides on many topics added by JL)

See AMDIS Manual for Detailed Instructions





What is AMDIS?

Automated **M**ass spectral **D**econvolution and **I**dentification **S**ystem

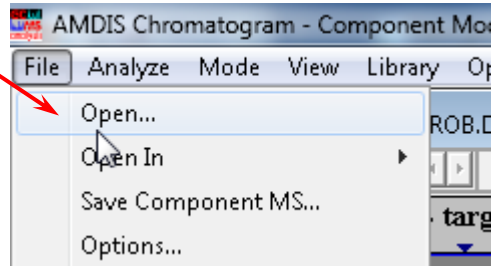
Developed to automatically detect chemicals in violation of Chemical Weapons Convention

- Software to ***automatically*** separate (deconvolute) chemical background in GC/MS data from signal for sample components
- Deconvoluted spectra can be ***sent*** to the NIST Mass Spectral Search Program for identification
- If necessary, manual background subtraction performed

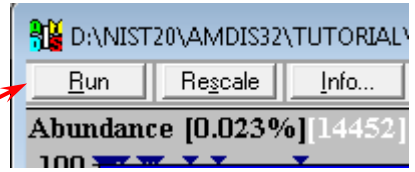
AMDIS Essentials

- ***Must always*** open a data file and run deconvolution ***before*** sending a spectrum to ***external*** NIST 2.4 Search Program
- ***Three ways*** to ***obtain a spectrum*** for searching: automatically, manually by ***LMB*** on spectrum, or manually with background subtraction
- AMDIS extracts the mass spectra of individual **Components** from chromatograms, these are symbolize with a ▼ on top of the chromatogram at the point of elution

Must always first

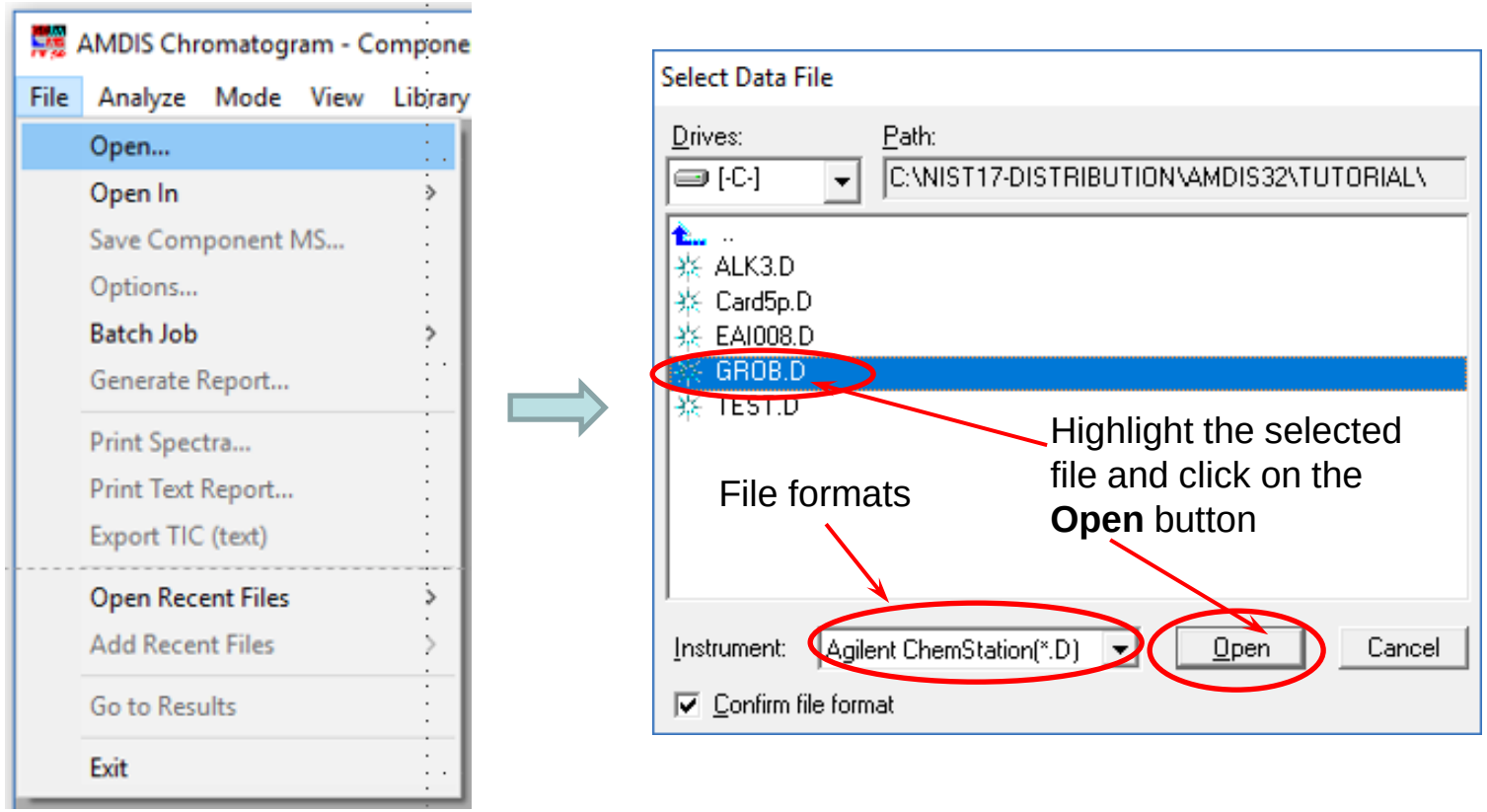


then



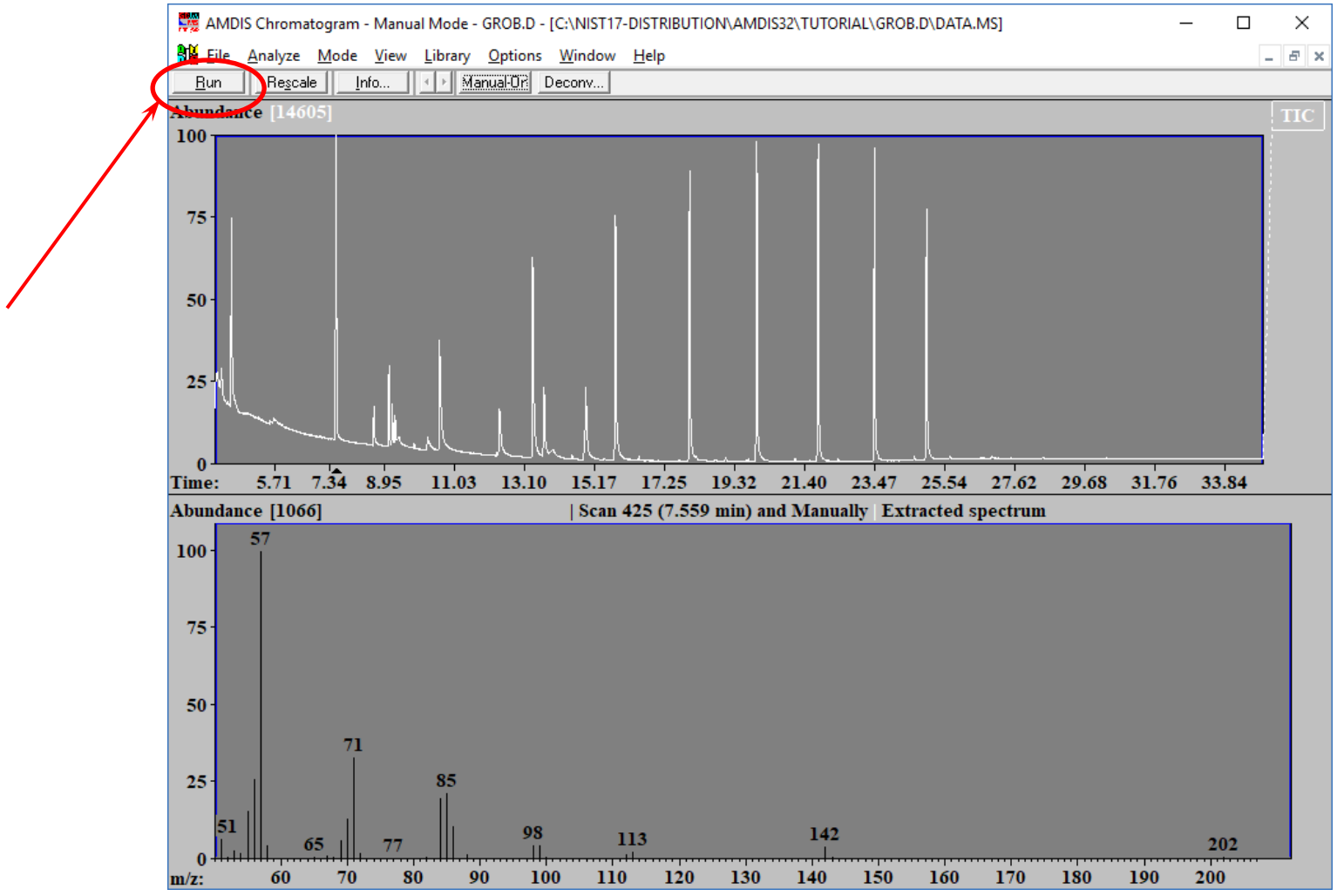
Opening File with AMDIS

- Can process many different file types with AMDIS including Agilent, netCDF, etc.
- Many manufacturers supply utility programs for conversion of files in their native format to the “standard” netCDF format
- File formats accessed by “pull down” menu
- ***Before*** sending components to library search, ***must*** open and run the file to get background corrected spectra



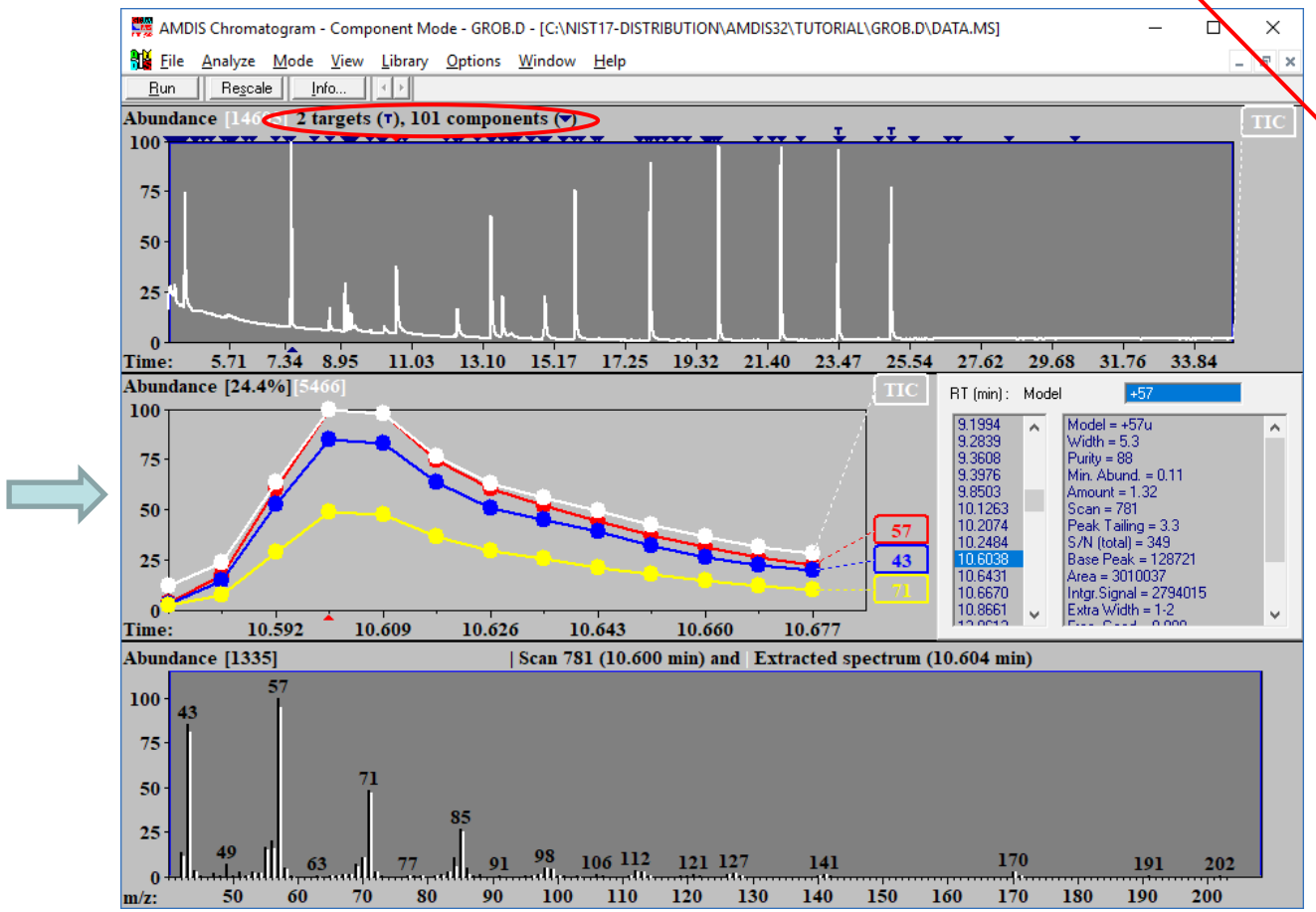
Deconvoluting Spectra

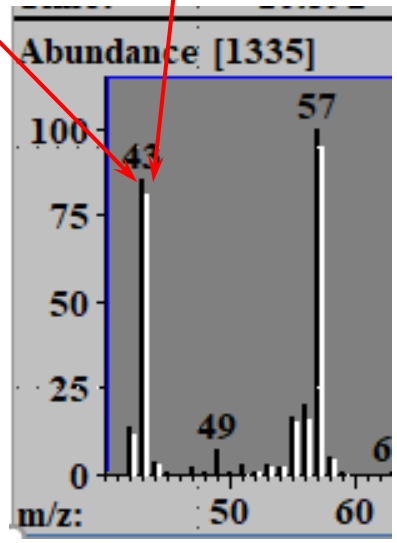
- First click the **LMB** with the **Pointer** on the **Run** button to deconvolute the file
- The computer plots a chromatogram from every *m/z* value in the data file
- Then “looks” at the stacked plots to determine which ions “belong” with each other and subtracts out ions from air, column bleed, other nearby components, etc.



Evaluating Deconvoluted Results

1. Note the number of **Components** found (101)
2. Note the little blue upside-down triangles (▼), left click on any one to see deconvoluted spectrum
3. After selecting one blue triangle, can step through by using up or down arrows ***on your keyboard***
4. The left middle window shows what ions were “modeled” to define your spectrum
5. The right middle window show you the associated parameters for each peak
6. The bottom window shows the unsubtracted spectrum in black and the deconvoluted in white



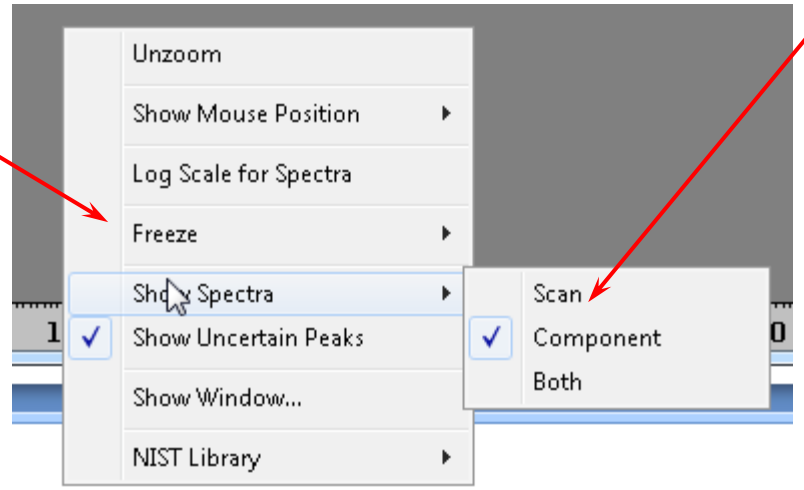


Evaluating Deconvoluted Results (continued)

- Can just show the **Component** (white peaks), the Scan (black peaks), or Both, but best to get accustomed to looking at both
- When the black matches the white, you probably have a good spectrum of a major **Component**
- For minor **Components**, possibly coeluting with a major **Component**, the white will be different than black and in many cases smaller
- With default “deconvolution parameters”, AMDIS will sometimes ID too many components
- The “deconvolution parameters” need to be adjusted to minimize this
- Very dependent on having a good stable signal from the instrument, but in my experience, just tends to do that without using the appropriate filters for processing (*more on that later*).

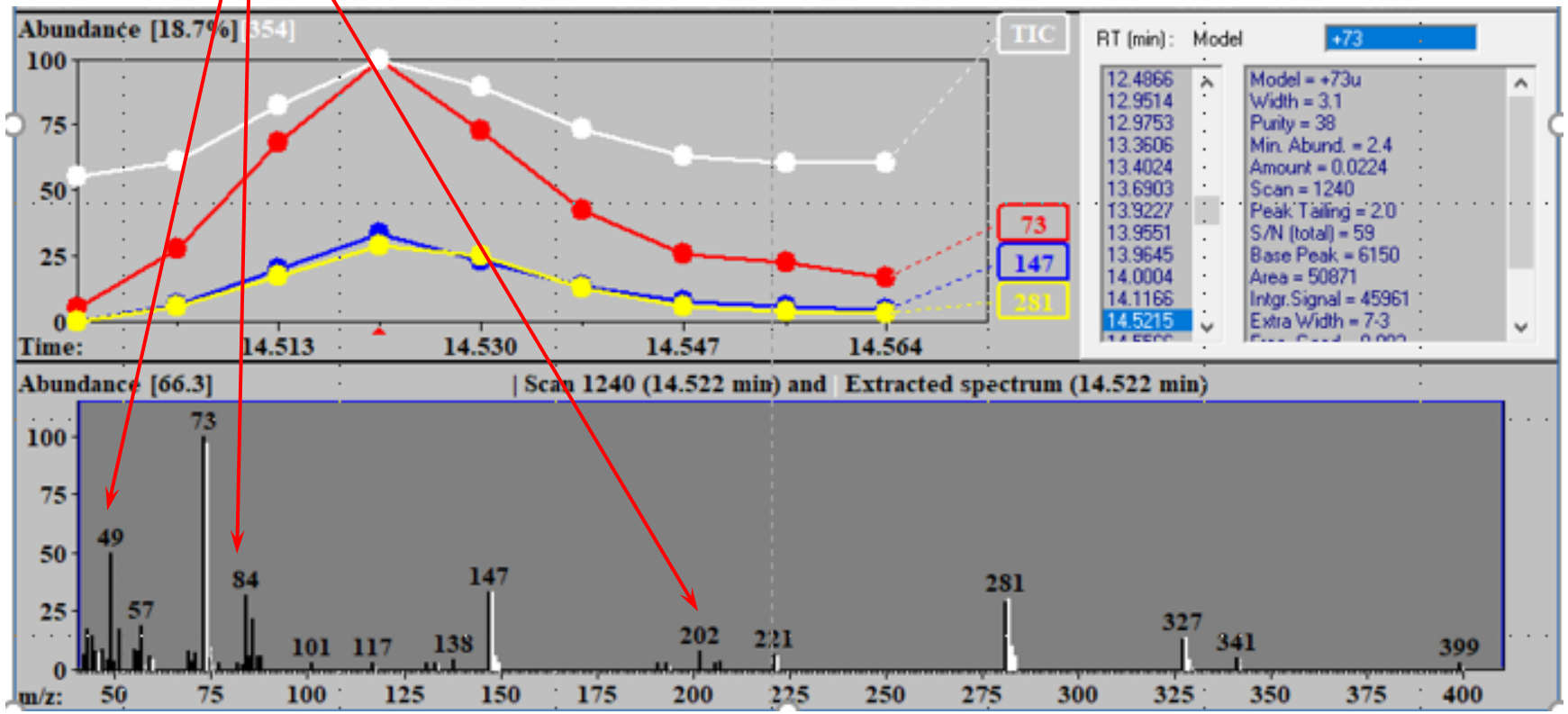
RMB Menu displayed by placing the **Pointer** on the Spectrum window and clicking the **RMB**

Which peaks will be displayed in the Spectrum window.



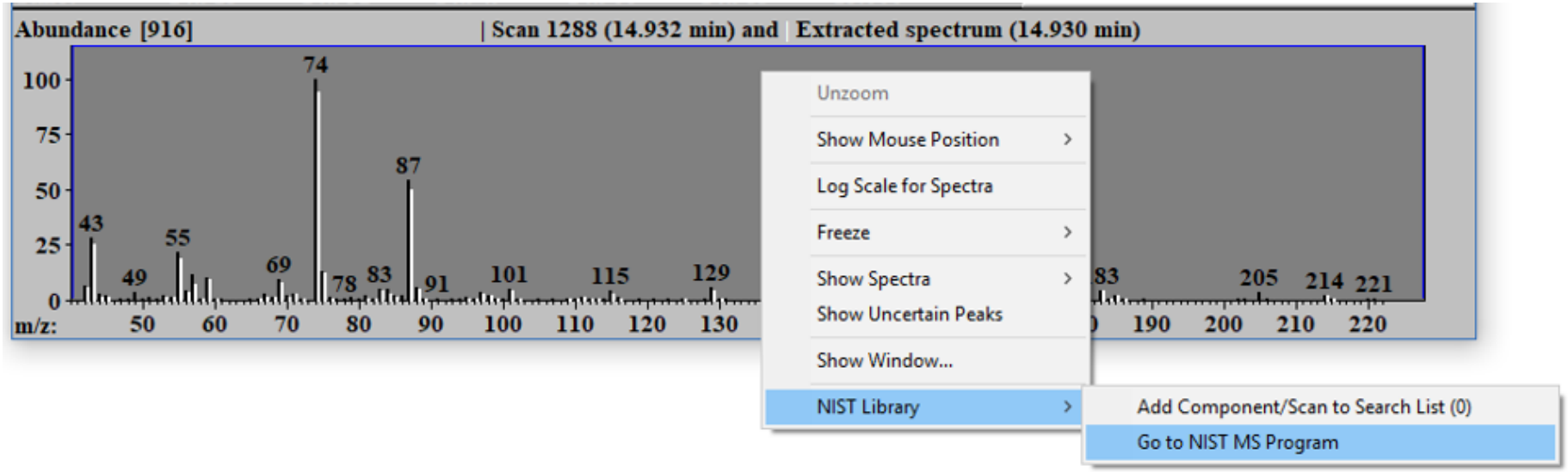
Evaluating Deconvoluted Results (continued)

- Note black (uncorrected peak with background)
- White is spectrum corrected for back ground and all non tracking ions removed



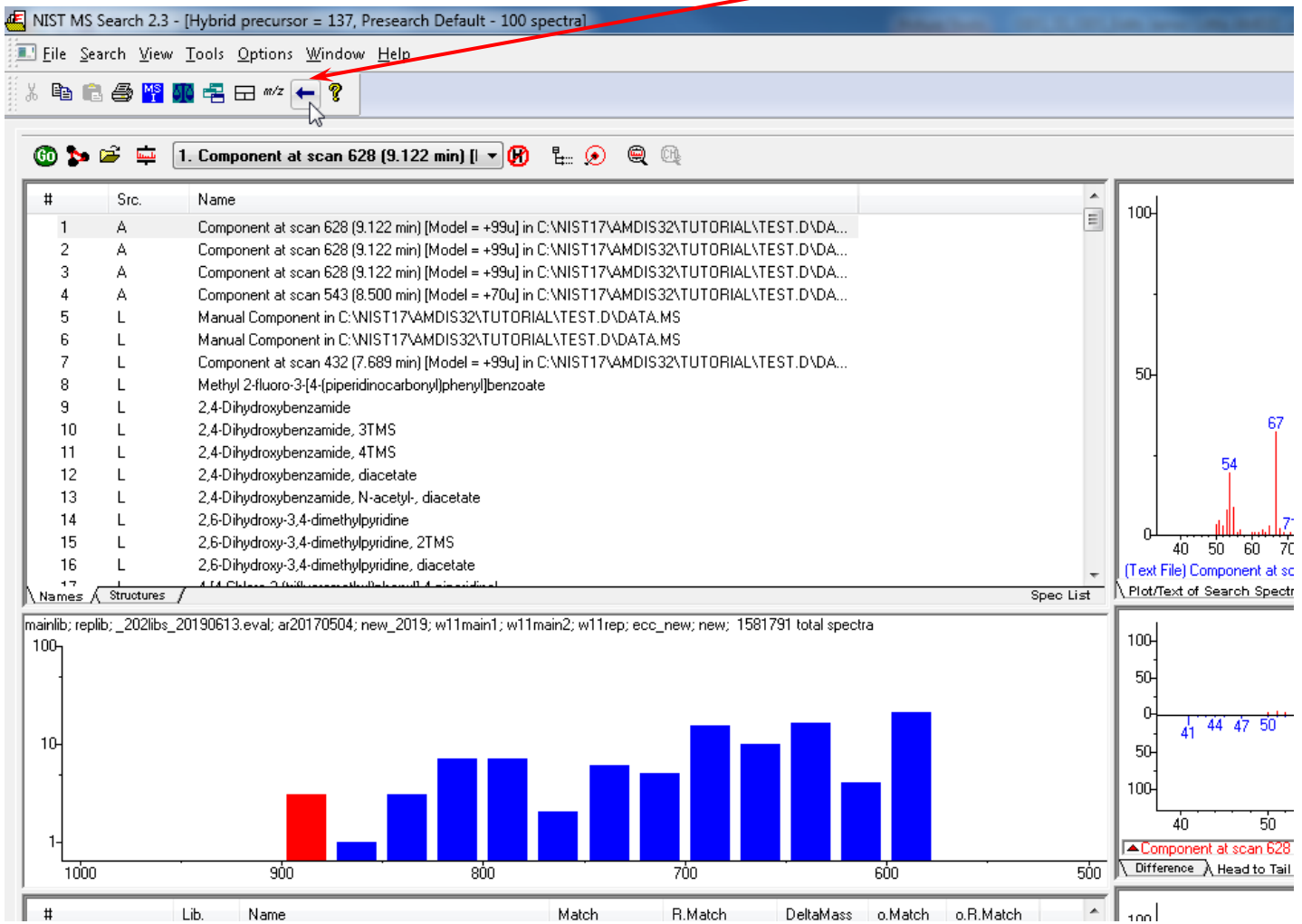
Sending Deconvoluted Spectra to NIST Search Program

- To send an individual mass spectrum to the NIST MS Search Program, click the **RMB** with the **Pointer** on the spectrum to display the **RMB** menu
- Select **Go to NIST MS Program**
- The spectrum will be sent to the NIST MS Search Program, if the Program is active; and, if not active, it will be started and the spectrum then sent
- If **Automation** is checked in the **Library Search Option's Search** tab, the search will occur automatically and the results will be displayed in the MS Search Program
- ***Tip:*** Can just LMB on chromatogram and obtain ***manual spectrum*** (*no background correction*) and send to MS Program for searching



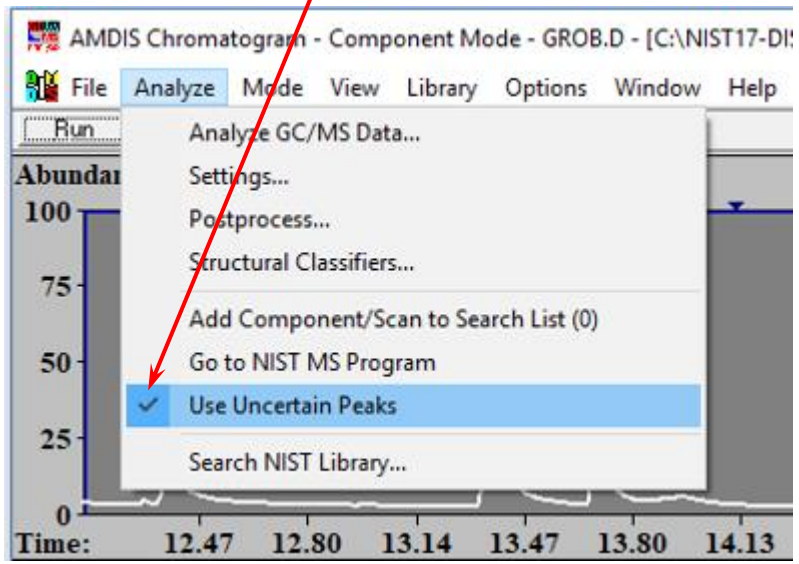
Returning to AMDIS Window after NIST Search

- After NIST search, return to AMDIS window by putting the **Pointer** on “Switch to Caller” button and click the **LMB.**

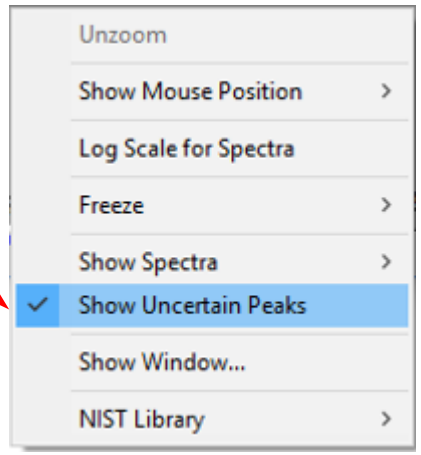


Uncertain Peaks, Dashed Lines, in Deconvoluted Spectrum

- Sometimes the AMDIS "decides" that some peaks "***might***" be associated with the deconvoluted spectrum, but it is not sure; you will need to change the basic settings if you want to use them
- These "***uncertain peaks***" are shown as dashed white lines in the spectrum
- To use them and send them for library searching, the Analyze settings have to be changed
- First, click the **RMB** with the **Pointer** on the spectrum to cause the display of the **RMB** menu and select **Show Uncertain Peaks**. Once selected, this will remain until changed.
- Then go to top of the **Analyze** menu, displayed from the Main Menu, and select **Use Uncertain Peaks**



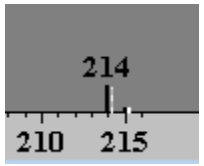
Analyze Menu



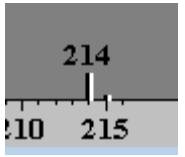
Right Mouse-button Menu with Pointer on Spectrum

Avoiding Uncertain Peaks in a Spectrum

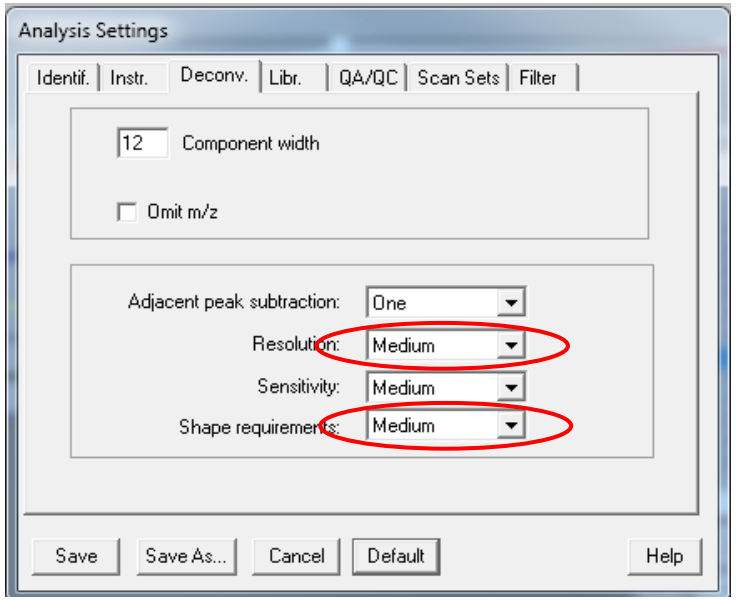
- Often uncertain peaks ***can be avoided*** by changing the default settings for Resolution and/or Shape Requirements in the ***Analysis Settings*** menu
- ***Alert!*** Internal library searches ***do not use uncertain peaks***, so best results obtained by avoiding their formation!
- Of course, uncertain peaks ***not*** a concern with spectra obtained manually

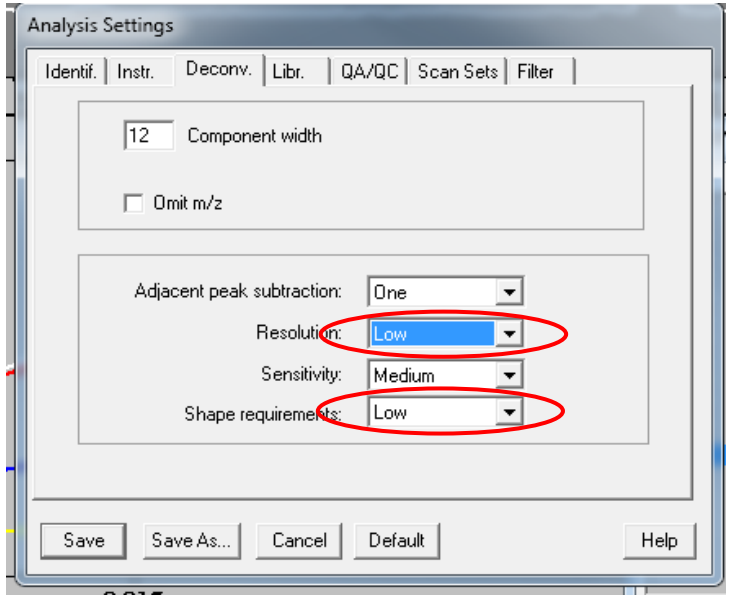


Default Settings



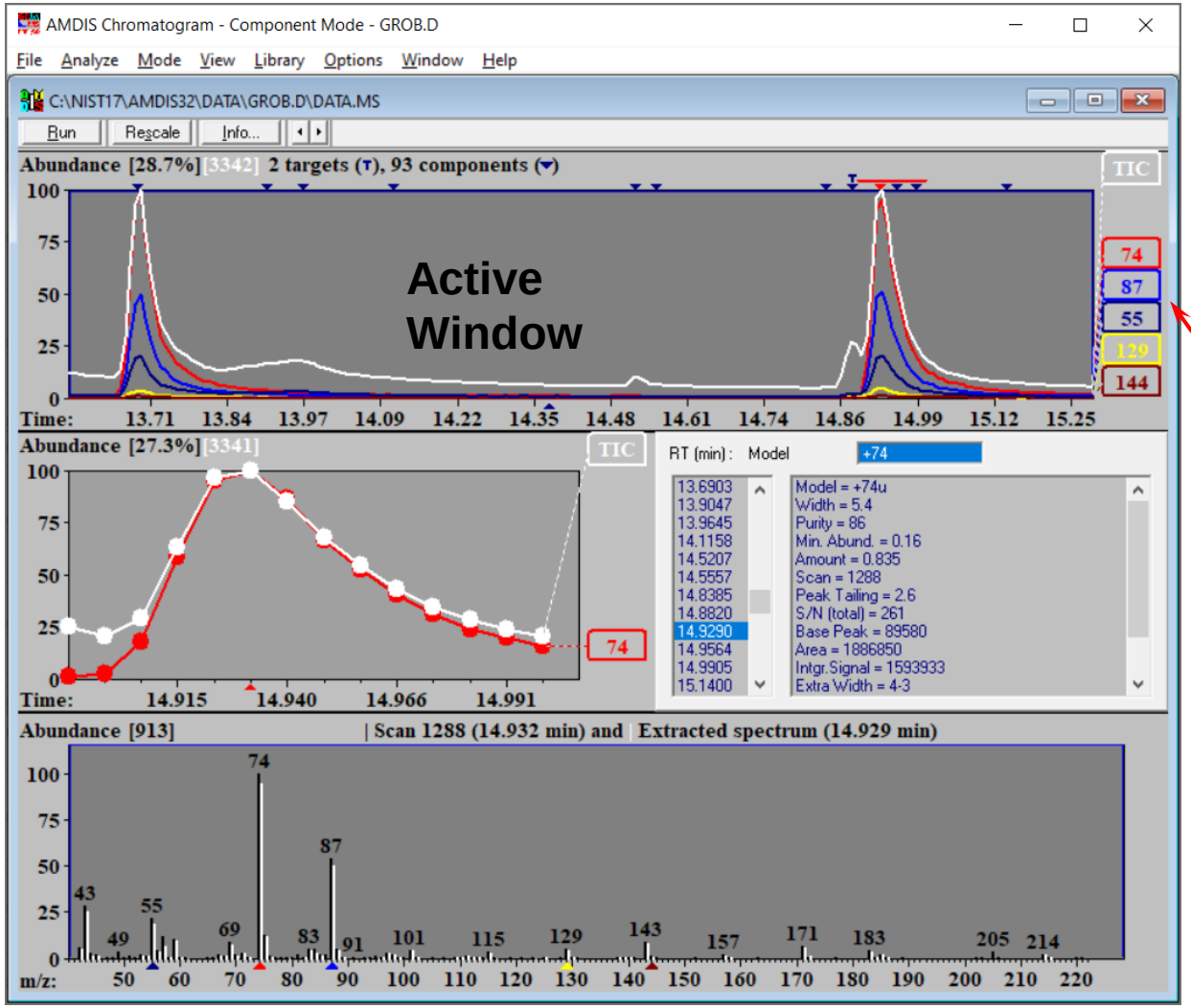
Modified Settings





Plotting Single (or Extracted) Ion or (Mass) Chromatograms

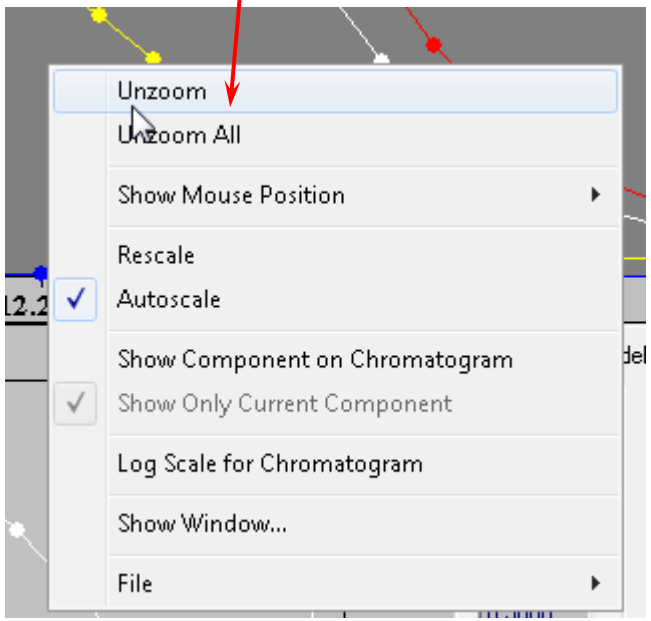
- To plot ion current *vs.* time (i.e., a mass chromatogram), just click the **LMB** with the **Pointer** on the peak representing the ion in the spectrum window, and the mass chromatogram will ***immediately*** be displayed in a different color in the active window. The intensity of the peak produced by the 1st selected ion is set to be 100%. If a subsequent ion is more abundant than that 1st selected ion, its plot will be off scale

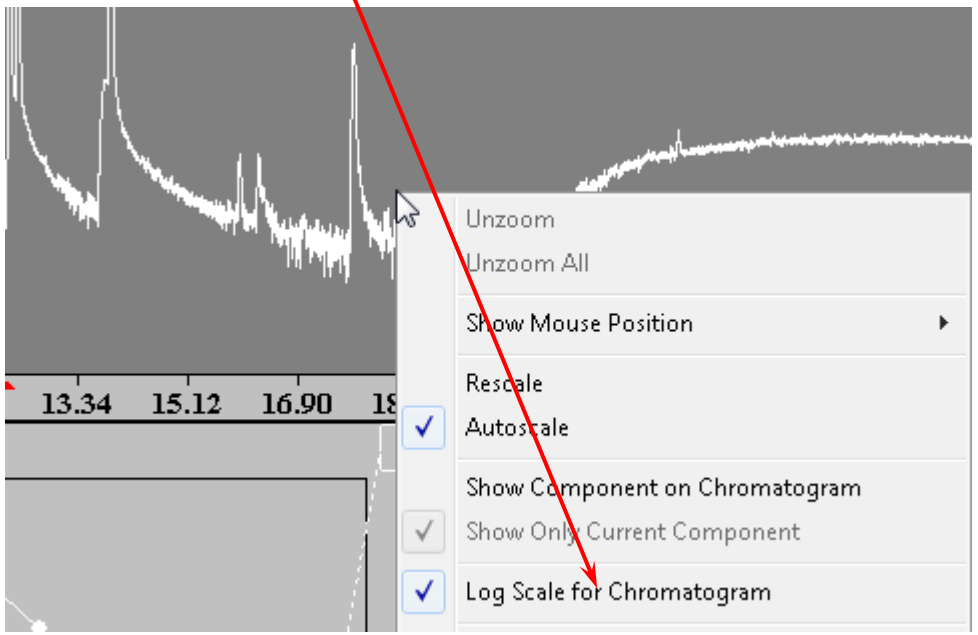


- Either the chromatogram (top) window of the model (middle left) window can be the active window
- To select the active window, put the **Pointer** on the bar above the window and click the **LMB**.
- The active window is dark gray
- To delete that mass chromatogram, just click the **LMB** with the **Pointer** on its box to the right of the top chromatogram
- ***Tip:*** the TIC (total ion chromatogram) box can be toggled off for easier viewing of low intensity mass chromatograms ***or*** use log scale as describe on next slide

Expanding Chromatograms or Plotting in Log Scale to See Small Peaks

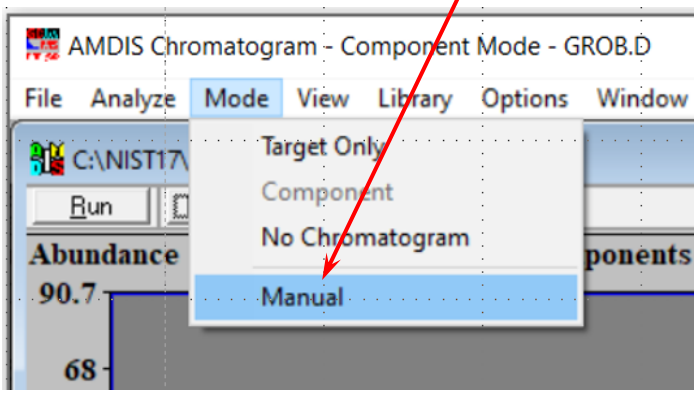
- To expand the chromatogram or spectrum, just hold down the **LMB** and drag (Drag-n-Drop)
- To unzoom, right click in the window and select **Unzoom** or **Unzoom All** from **RMB** menu
- Another way to see small peaks is to put Mouse-pointer on the chromatogram (or spectrum) window, click the **RMB**, and select **Log Scale for Chromatogram** or **Log Scale for Spectra** from the **RMB** menu

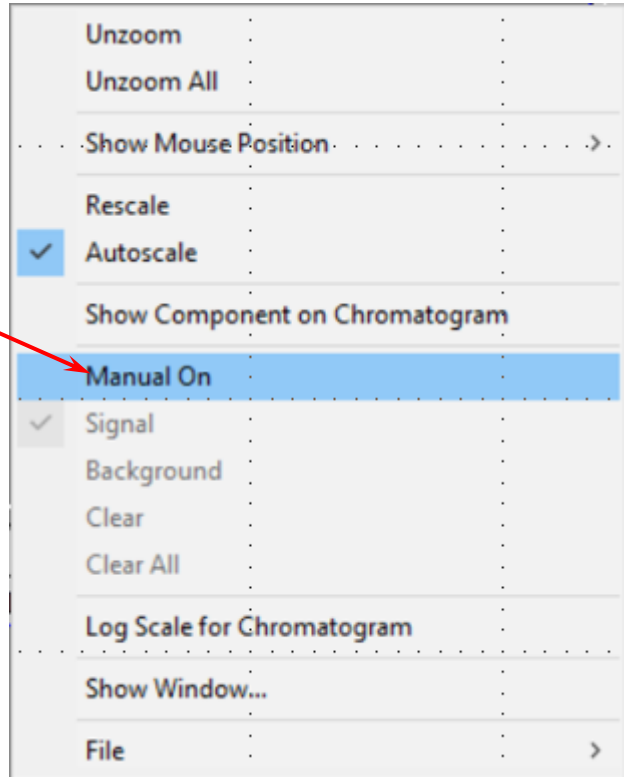




Manually Processing File in AMDIS

- If you just want an non-deconvoluted (uncorrected) spectrum of the background, click the **LMB** with the **Pointer** on the scan of interest, it can then be sent to the MS Search Program and searched against the NIST and/or other libraries
- AMDIS can produce a manual background-subtracted spectrum, typical of other MS software
- Often helpful for broad or peaks with excessive tailing
- First, go to top bar and select **Manual** from the **Mode** menu on the Main Menu bar
- Second, display the **RMB** menu and select **Manual On**

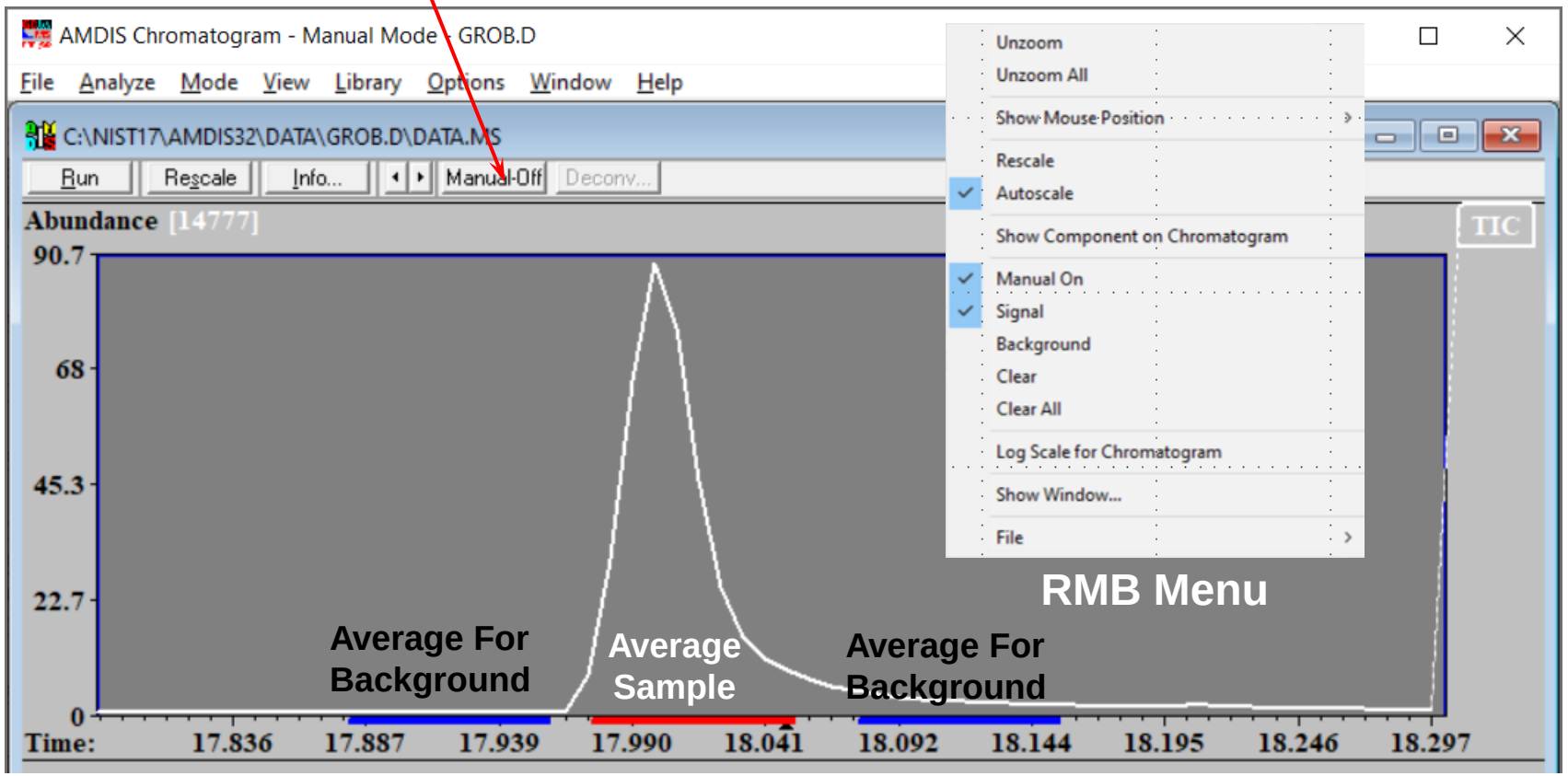




RMB Menu displayed by putting **Pointer** on Chromatogram window and clicking the **RMB**

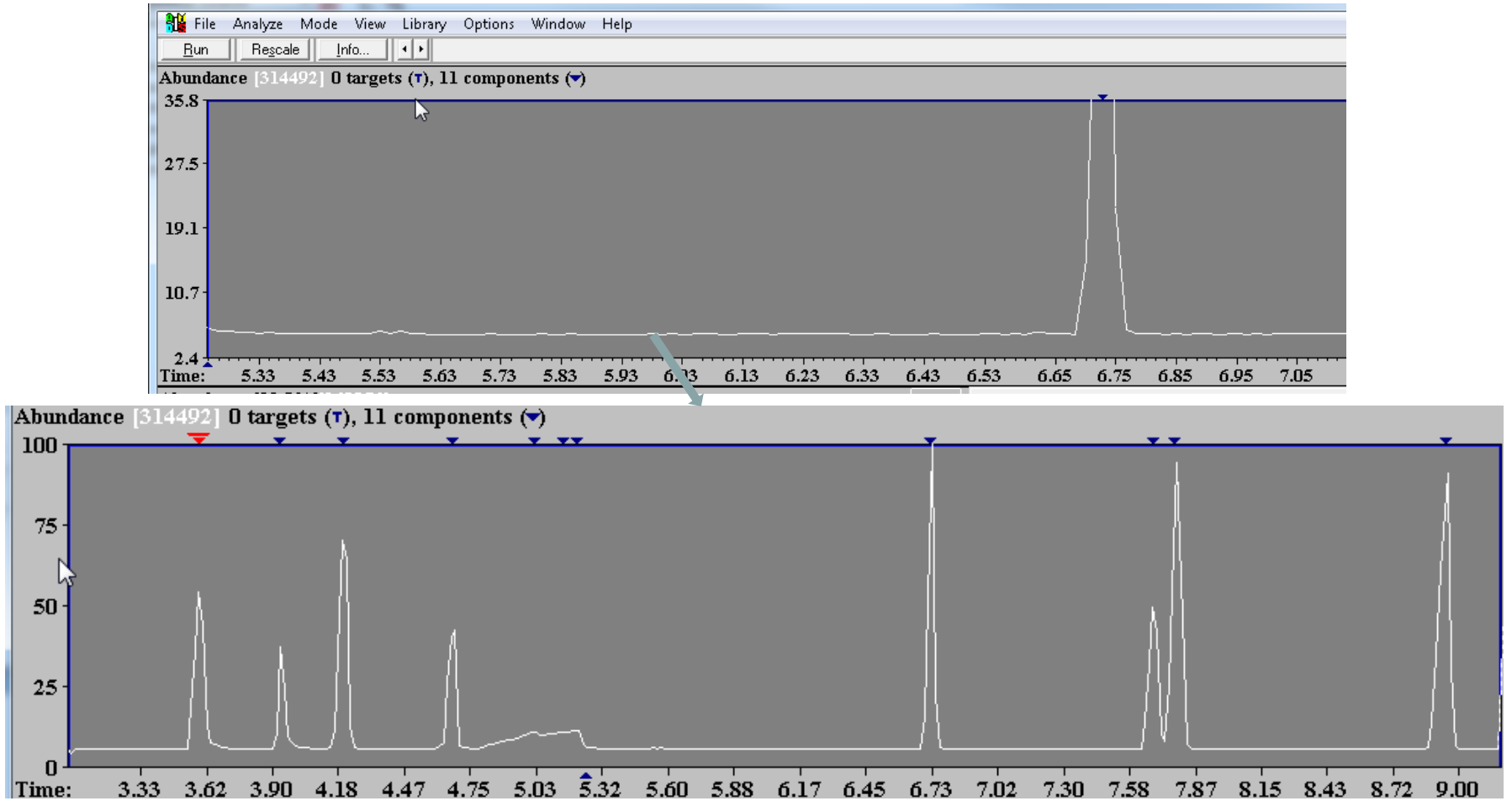
Manually Processing File in AMDIS (continued)

- From **RMB** menu displayed with **Pointe**r on the chromatogram window select (***one at a time***) in a sequence, **Signal** (one or more ranges to average) and **background** (one or more ranges)
- The manually background spectrum is shown in the spectrum window, (bottom of the two displayed windows; the model window (middle), used in deconvolution, is no longer present
- The chromatogram window can be unzoomed using the **RMB** menu; but, to zoom requires **LMB** clicking on the **Manual Off** button above the chromatogram turning it to **Manual On**
- The spectrum obtained can be sent to MS Search using the **RMB** menu



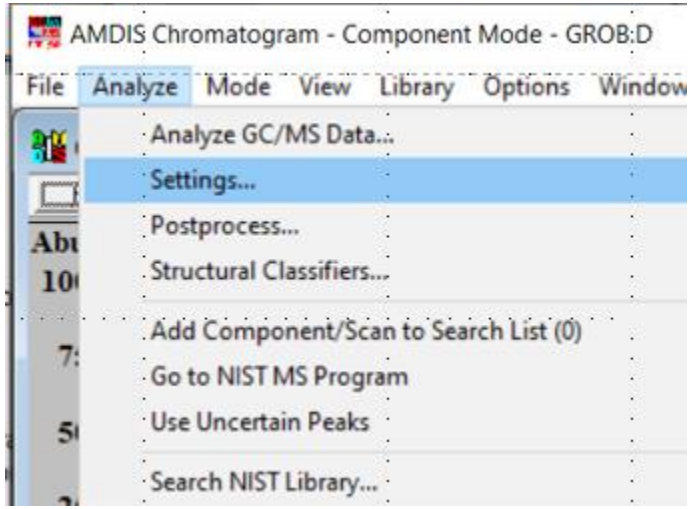
Adjusting Parameters for Optimized Peak Detection

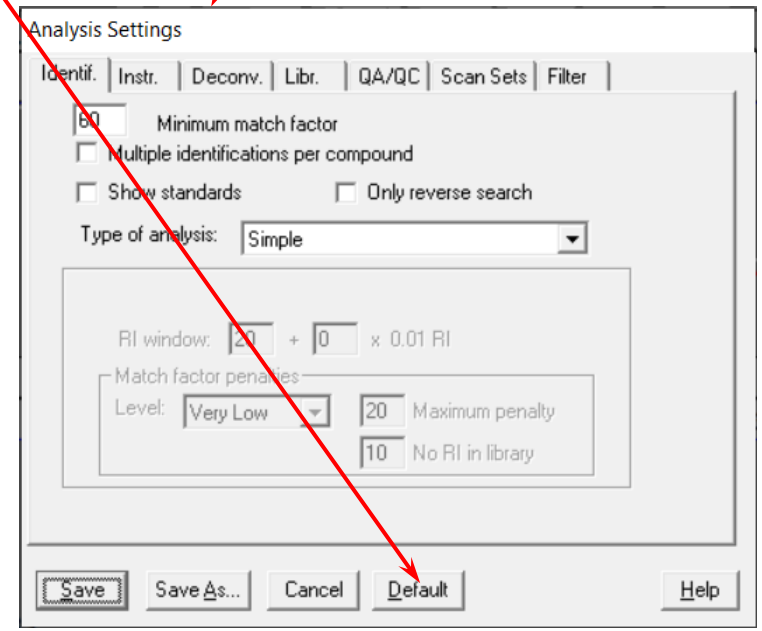
- When trying to determine proper parameters, expand the chromatogram to only show the most difficult areas
- Change to parameter and only the area shown will be reanalyzed
- After getting all the parameters as desired, then show the whole chromatogram and **Run** (Reanalyze) again
- This will greatly speed the process!
- ***Tip:*** NIST wrote 3 part series on suggested parameters for deconvolution[23]



Minimizing Marking Components in Chromatogram

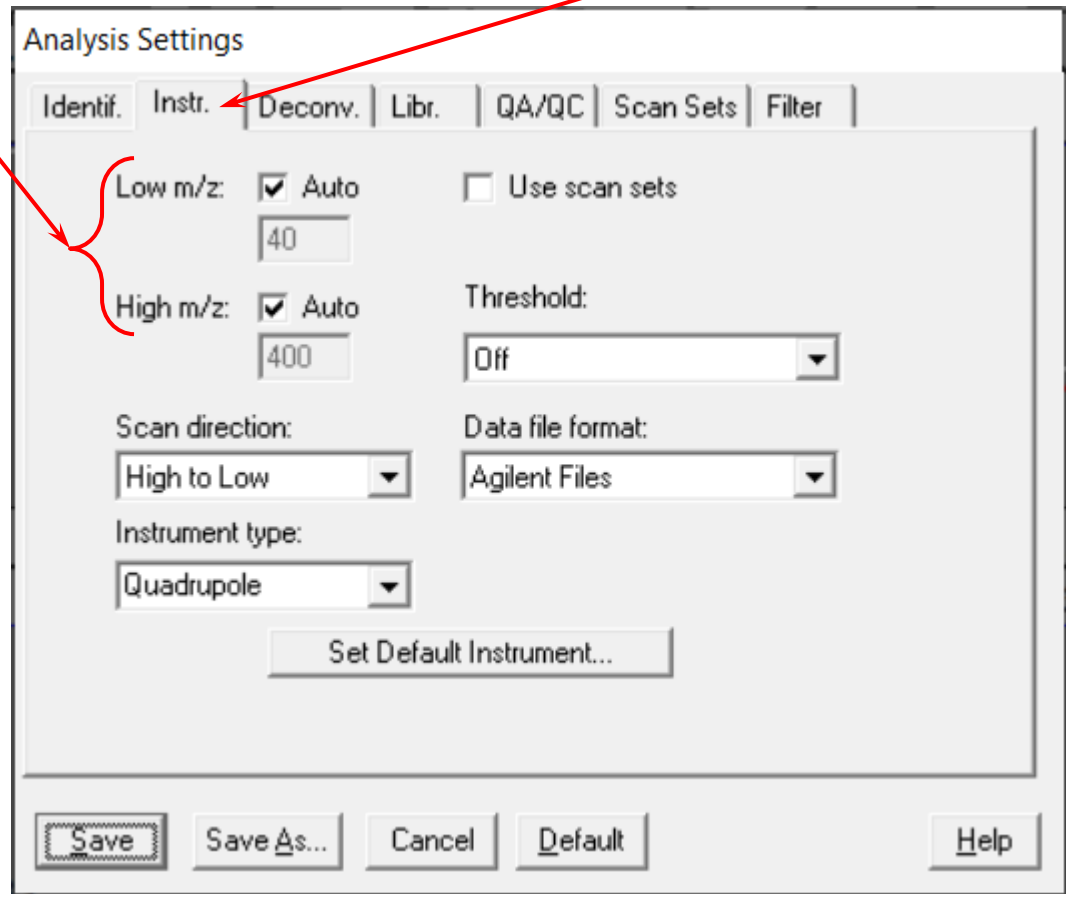
- The “multi-marking” of **Components** due to noise or instrument scanning irregularities can be annoying
- Almost all instruments under varying conditions tend to have this problem
- This can be minimized by adjusting some parameters In the **Analysis Settings** dialog box
- Note that there are multiple tabs with many parameters in this dialog box
- It is easy to restore the program’s **Default** settings





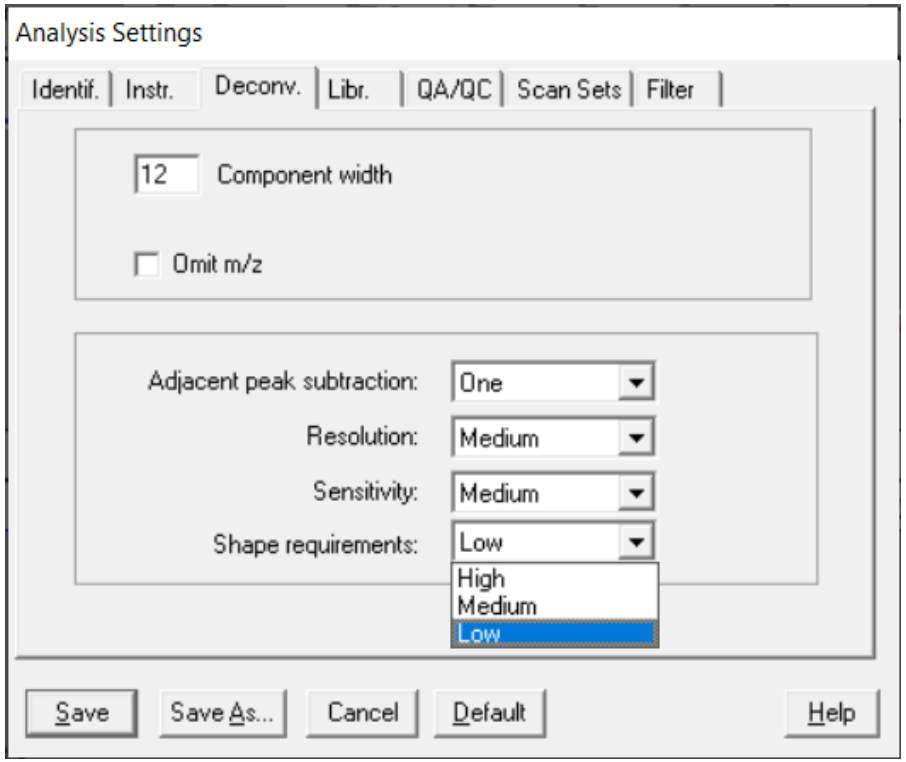
Minimizing Marking Components in Chromatogram (cont'd)

- Setup the processing parameters based on the instrument and its scan function (**Instr** tab)
- Can set the low and high m/z manually, or just automatically use those determined by AMDIS from the file



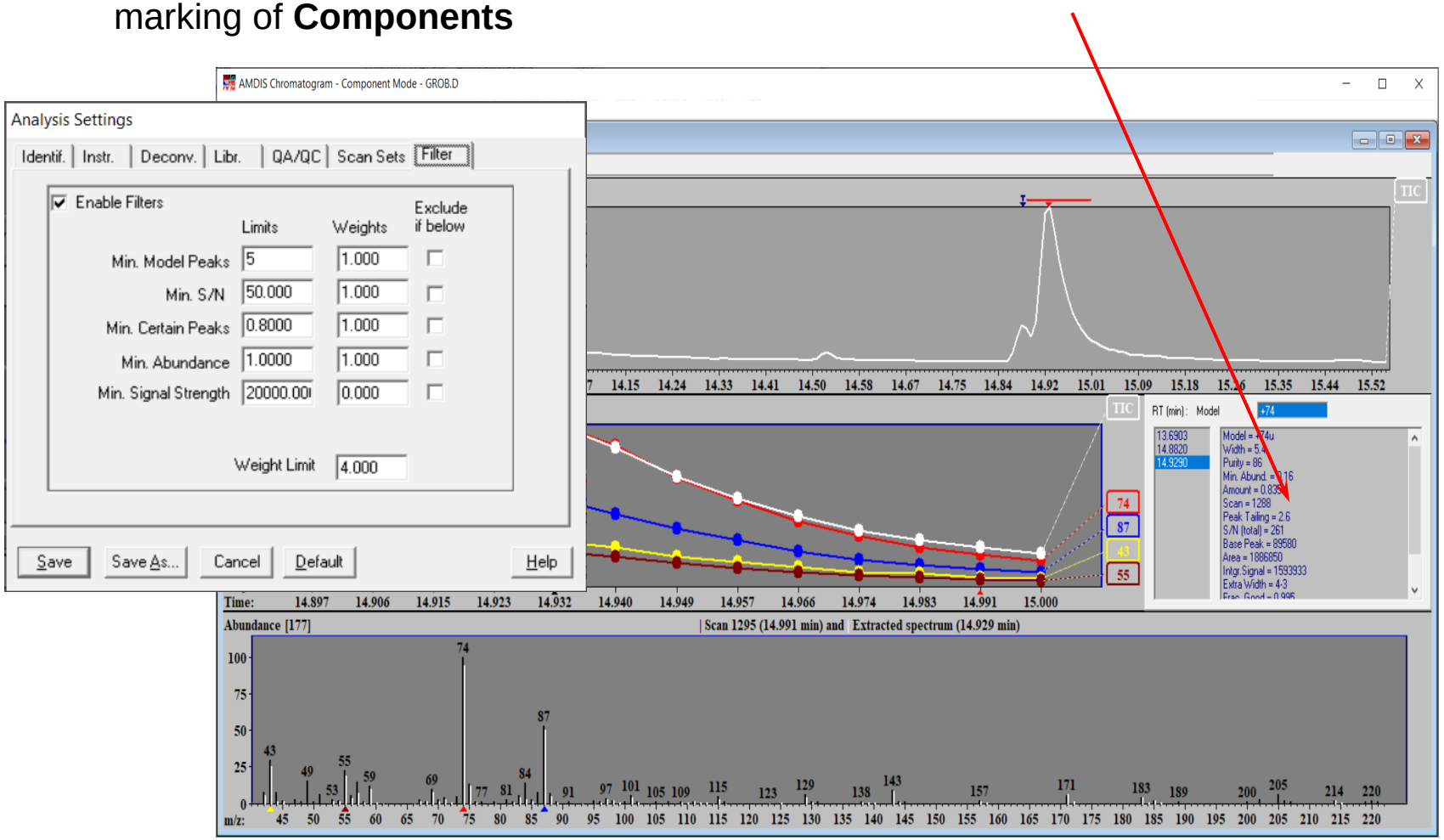
Minimizing Marking Components in Chromatogram (cont'd)

- The ***Deconvolution t***ab can be set to get rid of some peaks
- In particular, for tailing peaks, might want to set the **Shape** requirements to Low
- The default for **Shape** requirements is Medium
- The values show below are the **Defaults**
- In general, the **Filter** tab (*see slides 36-37 of this handout*) usually minimizes the multi-marking of **Components**



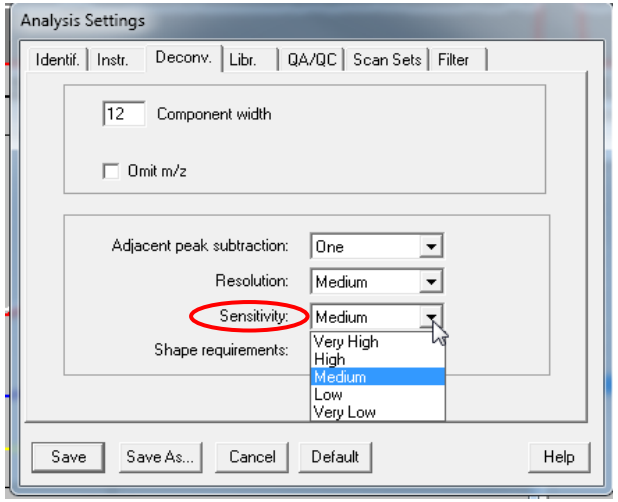
Minimizing Marking Components in Chromatogram (cont'd)

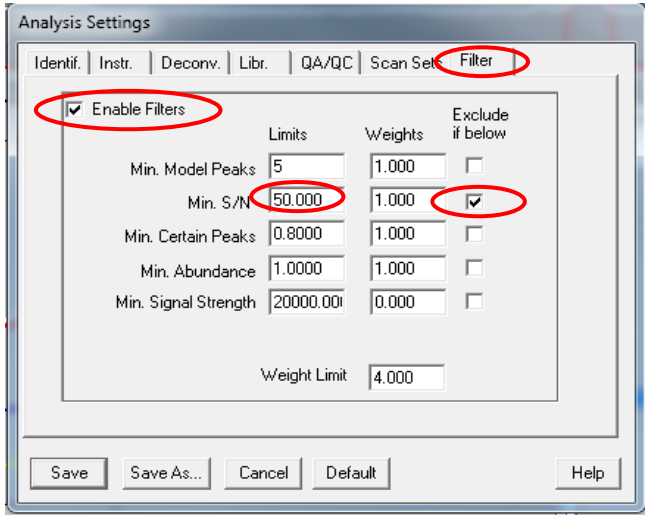
- The default filter settings are shown below; the default settings do NOT have the **Enable Filters** check box selected; unless checked, the fields are grayed
- The values associated with a particular **Component** can be viewed in the window next to the Model (middle-left) window
- Looking at these values gives an idea of how to limit parameters to minimize the marking of **Components**

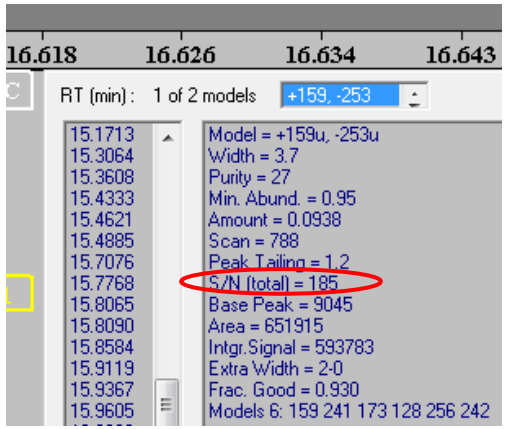


Minimizing Number of Components Detected

- The number of components detected can be minimized by many approaches
- One approach is to change the **Sensitivity** setting in the **Analysis Settings**
- Another approach is to use the filter settings and **Exclude if below** a specified **Min S/N**
- To optimized, expand the chromatogram and find a suitably sized “small” peak to find an appropriate S/N to mark the number of components detected







Problem No Chromatogram/Spectrum Window!

1. AMDIS initially designed to only yield report
2. If you inadvertently select “Go to Results”
3. Then “Analyze”
4. You could see “NO TARGET CHEMICALS FOUND!”
5. Select “Confirm” button to return to chromatogram/spectrum window normally used for qualitative analyses