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chemistry software solutions

BiologicalsHOS: an opportunity for NMR

The Mestrelab team in collaboration with Bruker

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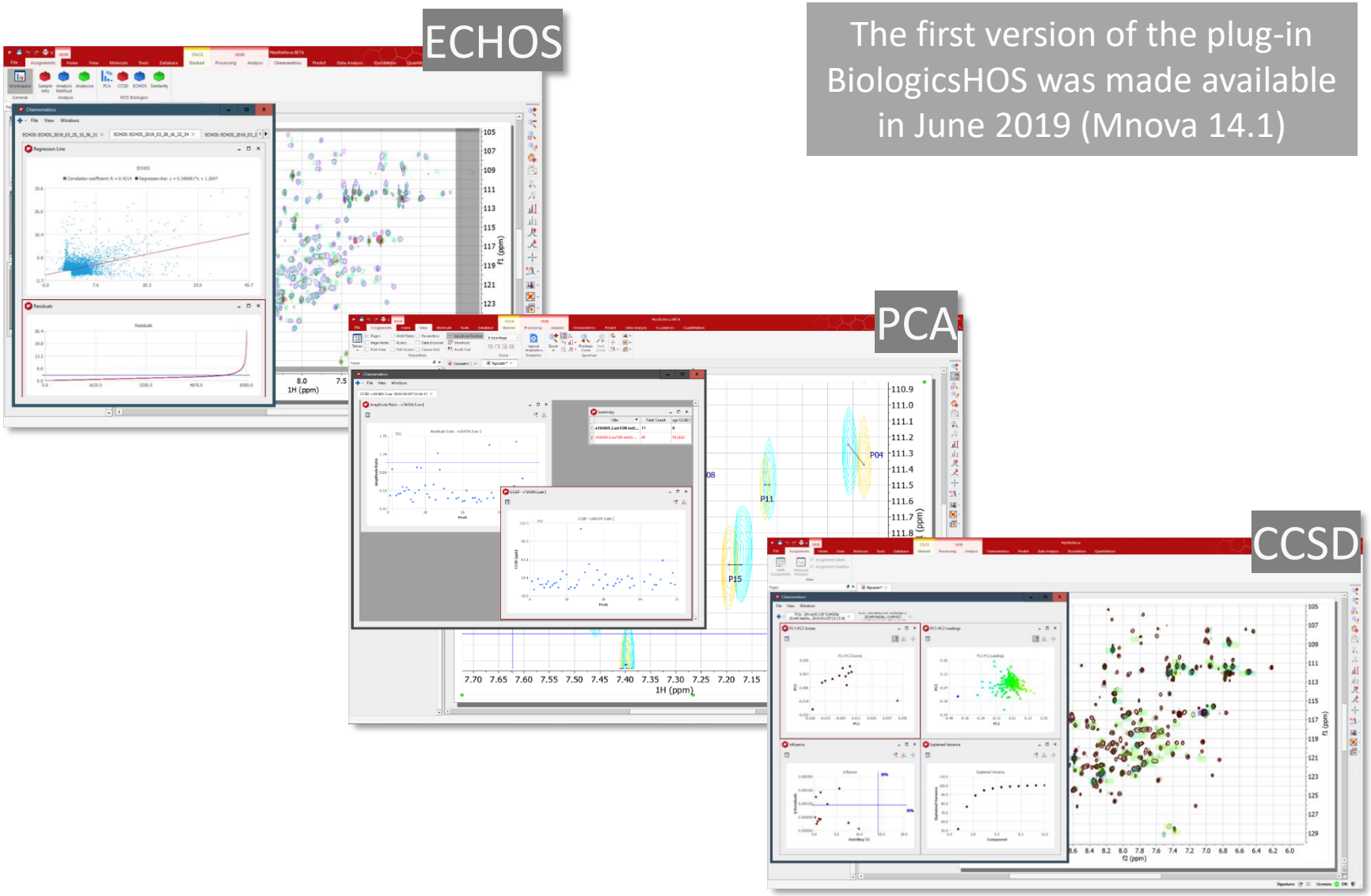

The first version of the plug-in BiologicsHOS was made available in June 2019 (Mnova 14.1)

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Preamble

The use of NMR to evaluate the higher order structure (HOS) of medium to high MW species has been well established as a sensitive, robust analytical tool.

Mnova has long been a favourite data processing and analysis tool, having ease-of-use and powerful capabilities.

Building on this solid foundation, specialised tools are now available to facilitate the analysis of 2D NMR data for HOS evaluation.

We describe the use of Mnova to:

- Process 2D NMR data
- Prepare for analysis
- Apply ECHOS analysis (data points)
- Apply CCSD analysis
- Apply PCA analysis

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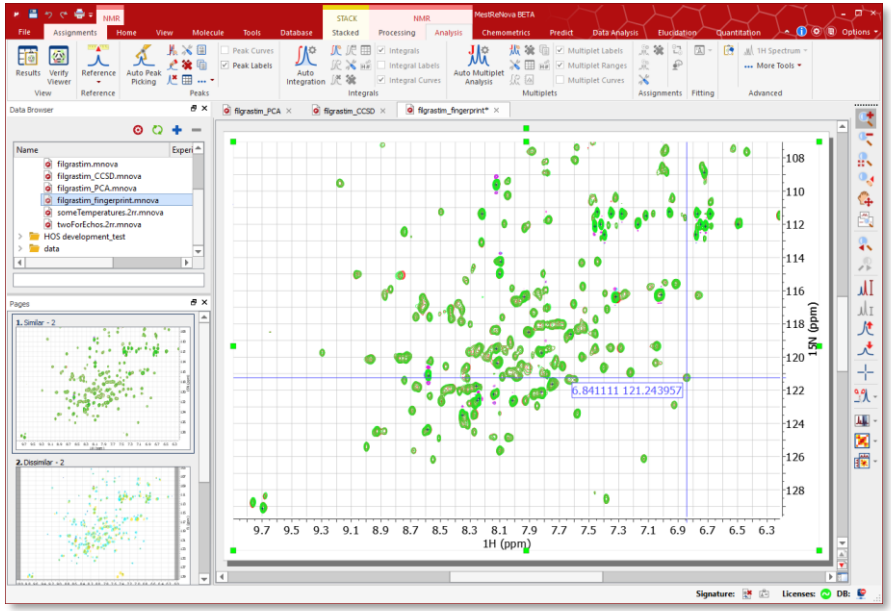
Basics



UI fundamentals:

- Powerpoint-like interface
- Ribbon interface (contextual)
- Multiple documents each with 1 or many spectra

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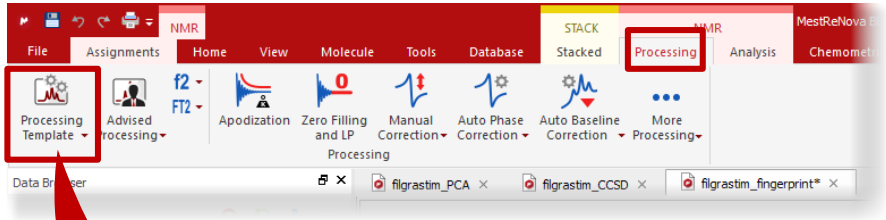
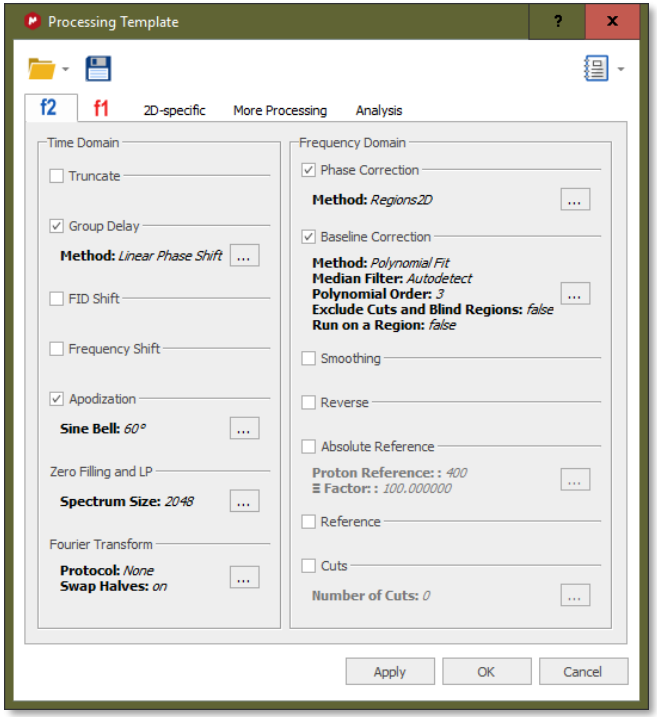
Basics - processing





Most processing settings will be imported together with the NMR data from your spectrometer. The majority are applied automatically, or you can easily save your favourite processing for it to be reused (“template”).

There is, however, every opportunity to reprocess using a wide range of important steps:

- NUS
- Apodization
- Zero-filling
- Phasing
- Baseline correction
- Compression and denoising

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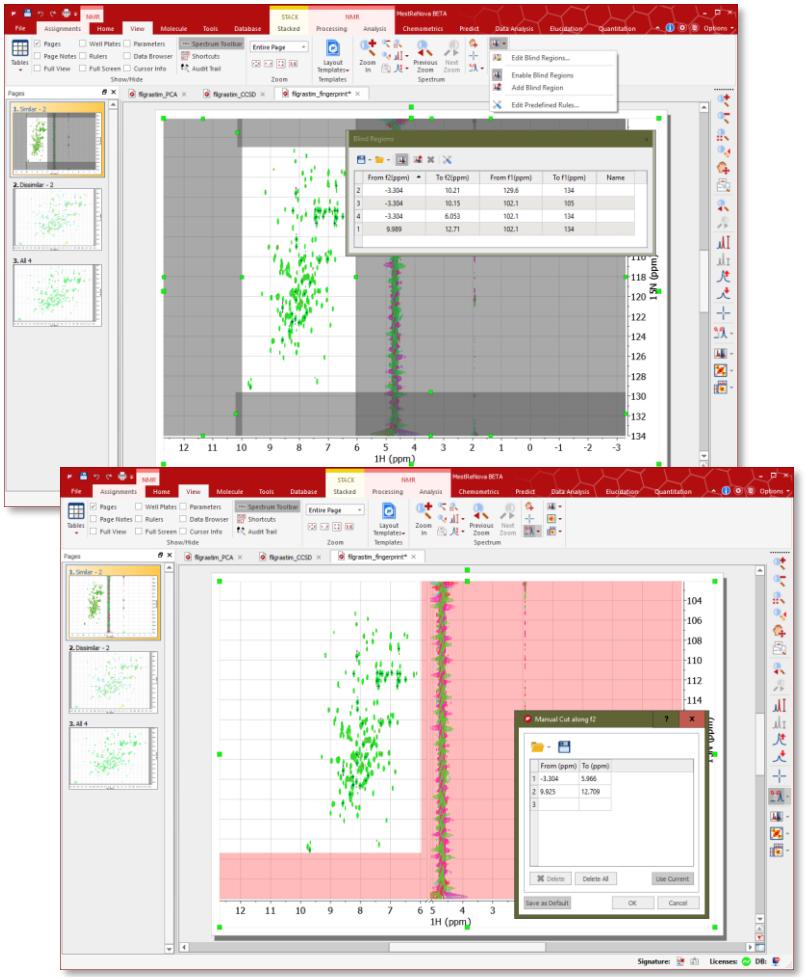
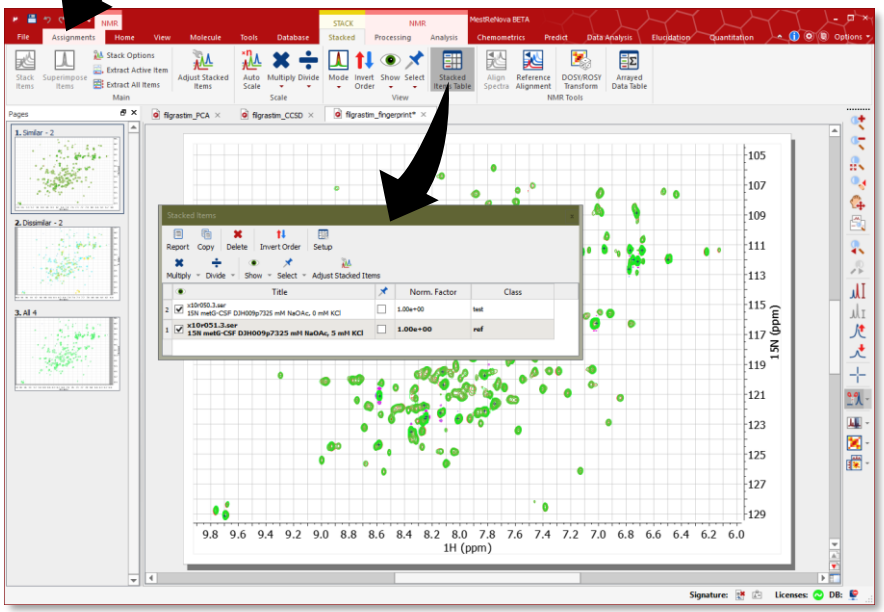
Basics – restricting the ROI



- You can use *blind regions* or *cuts* to force Mnova to only use one or more region(s) of interest (ROI).
- Spectral regions outside of the ROI will not be analysed. It is important to exclude regions of noise.
- These can be saved and applied automatically upon import

Basics – stacked spectra

Select spectra (pages) and *superimpose*



Two spectra are *superimposed*

- Most analyses start with 2 or more spectra that are *stacked.*
- Select 2 or spectra in the “Pages” window (Ctrl/Shift + click), and click on the “Superimpose items” button.
- These can be easily viewed, selected, classified, and coloured in many ways.

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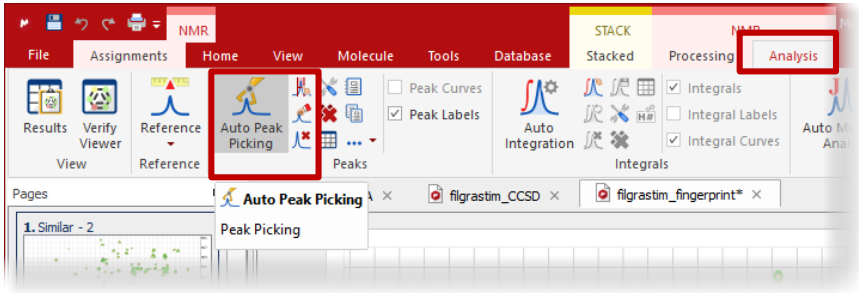
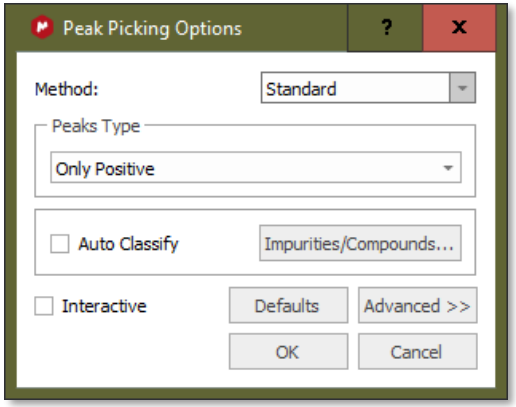
Basics – peak picking





CCSD analysis requires peaks to be picked.

- This can be performed either automatically or manually
- The result will depend heavily on the vertical threshold that is set before peak picking is applied

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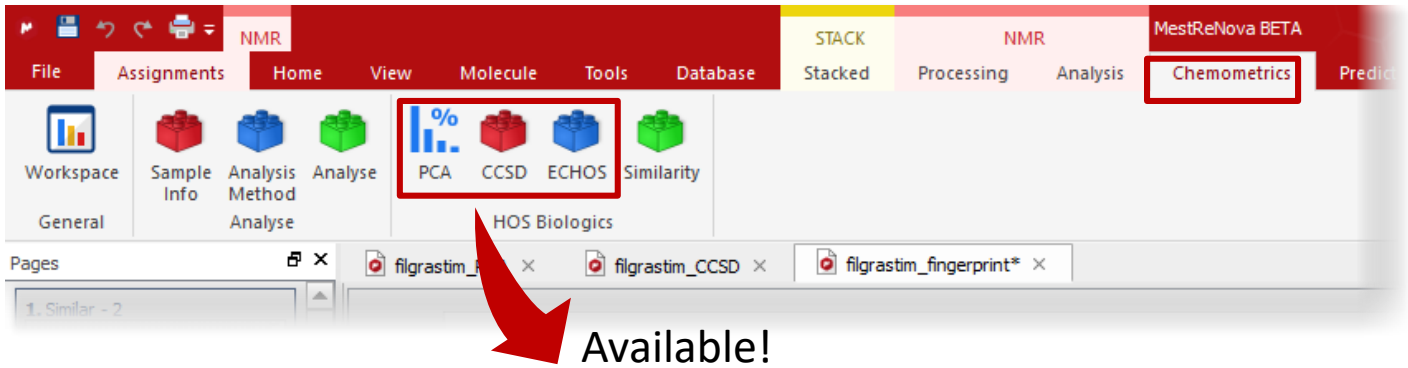
MBioHOS



- **MBioHOS***
- Mnova has a plug-in that will help you evaluate HOS of mAbs using these literature methods.
- Through an ILC, it has been shown that these methods are robust and fit for purpose.

Available analyses:

- ECHOS
- CCSD
- PCA

* Sold by Bruker as “BiologicsHOS”.

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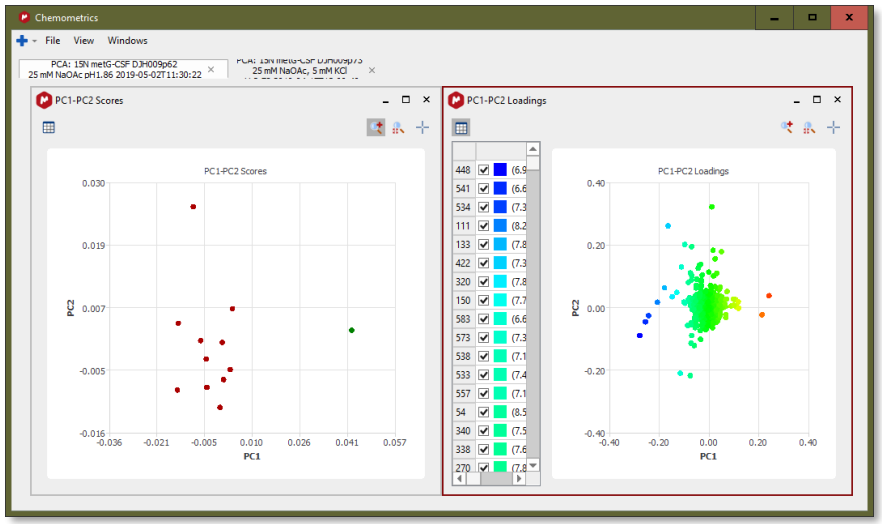
MBioHOS - Workspace



- The “workspace” window is where you interact with the results from an HOS analysis
- The content is contextual to the analysis
- Previous analyses can be accessed through tabs
- Access tabular data and use for selection and colouring
- Where appropriate, cursors can be correlated
- Zoom capability, window arrangement

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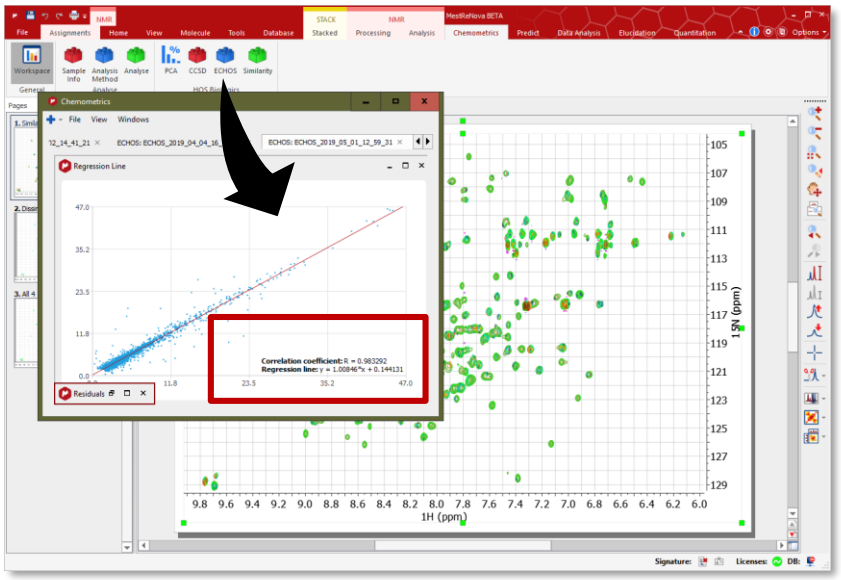
ECHOS - I



- ECHOS is a pointwise intensity comparison of a reference- with test spectrum/spectra.
- The spectra must be stacked.
- The compared points are plotted as a scatter graph.
- The line fit's R-value is an indication of spectral similarity. The closer to a value of 1.0 the better the fit.

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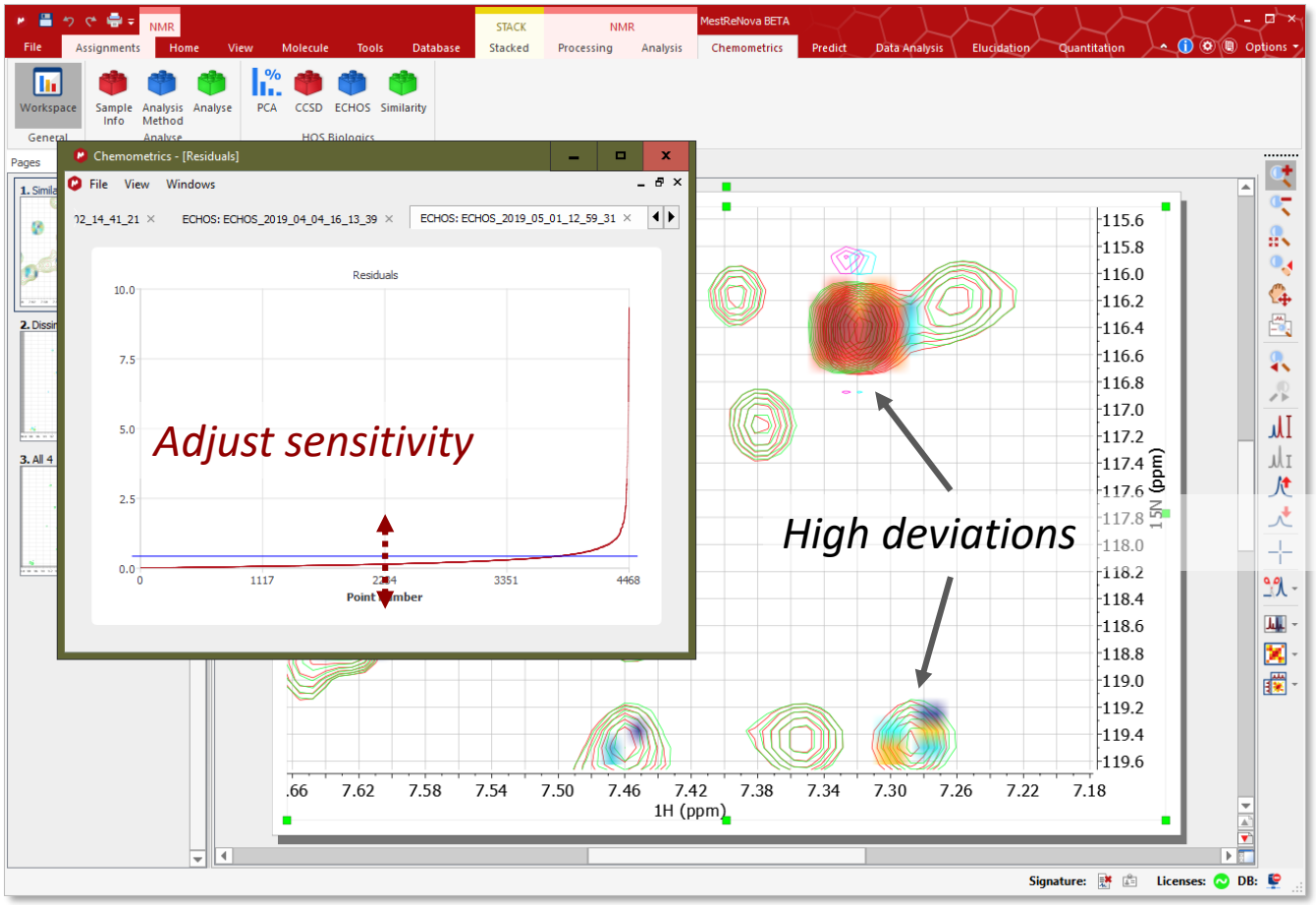
ECHOS - II

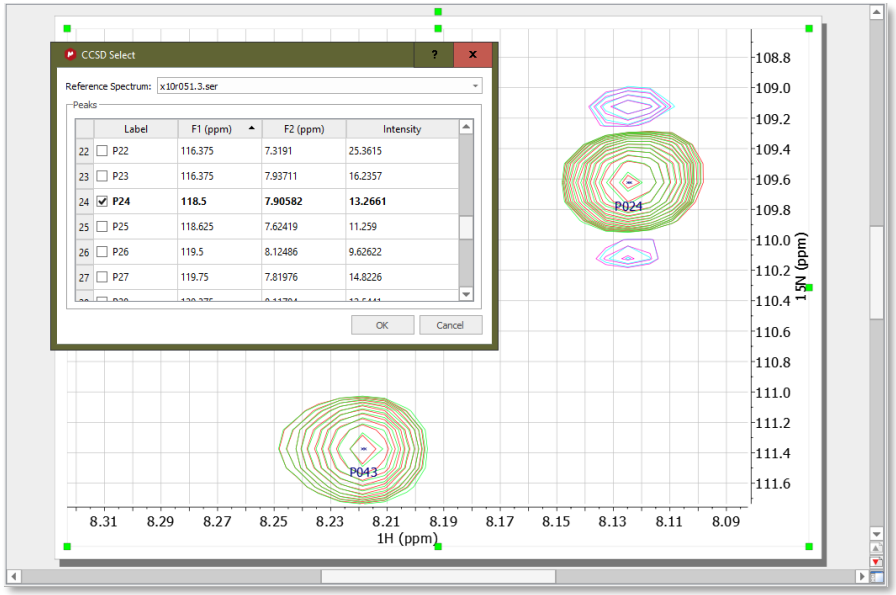
The Residuals shows the spectral regions where the spectra differ.

The deviation is shown as a heat map bitmap, superimposed on the 2D contour plots of the spectra that are being compared.



CCSD - I

Reference peak



Combined **C**hemical **S**hift **D**ifferences measure the amount that peaks are shifted between the compared spectra.

We also measure the amplitude changes.

The reference spectrum should be peak picked

Normalisation

A peak in the reference spectrum for which no height or chemical shift change is expected must be identified.

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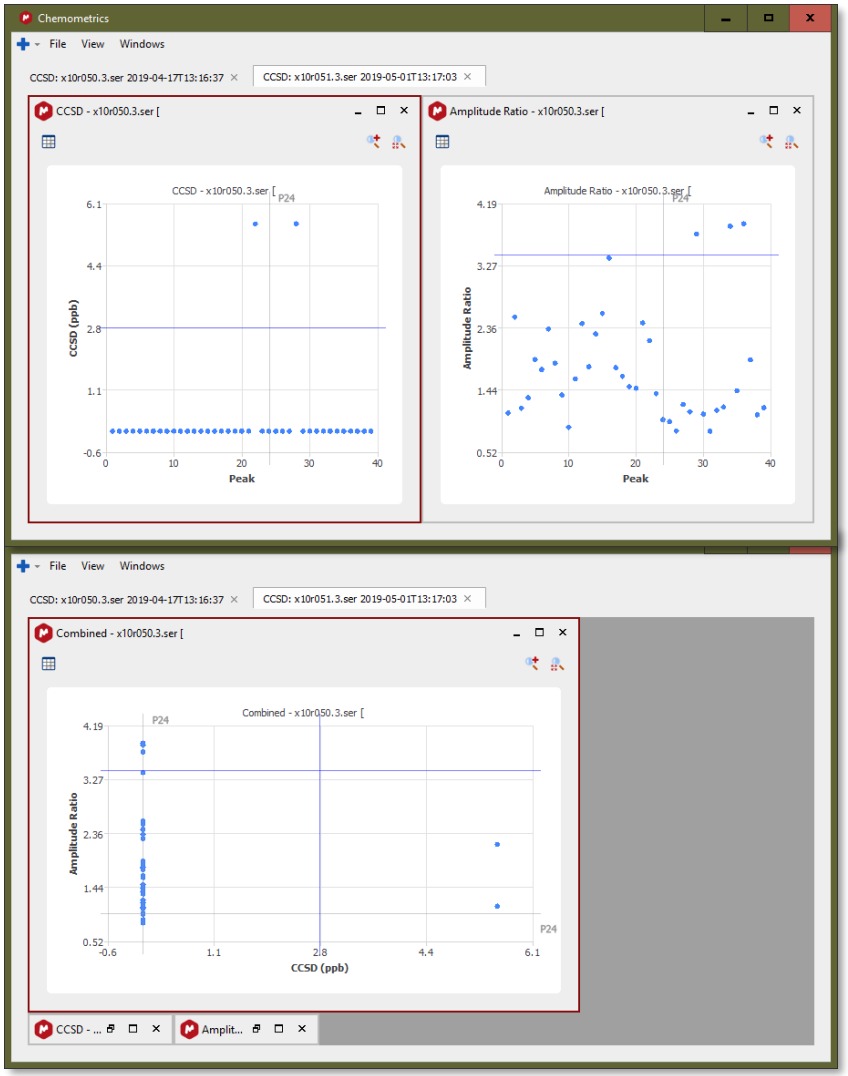
CCSD - II



The CCSD is automatically shown for peak movement and amplitude changes.

- CCSD plot
- Amplitude ratio plot
- Combined plot (CCSD vs amplitude ratio)

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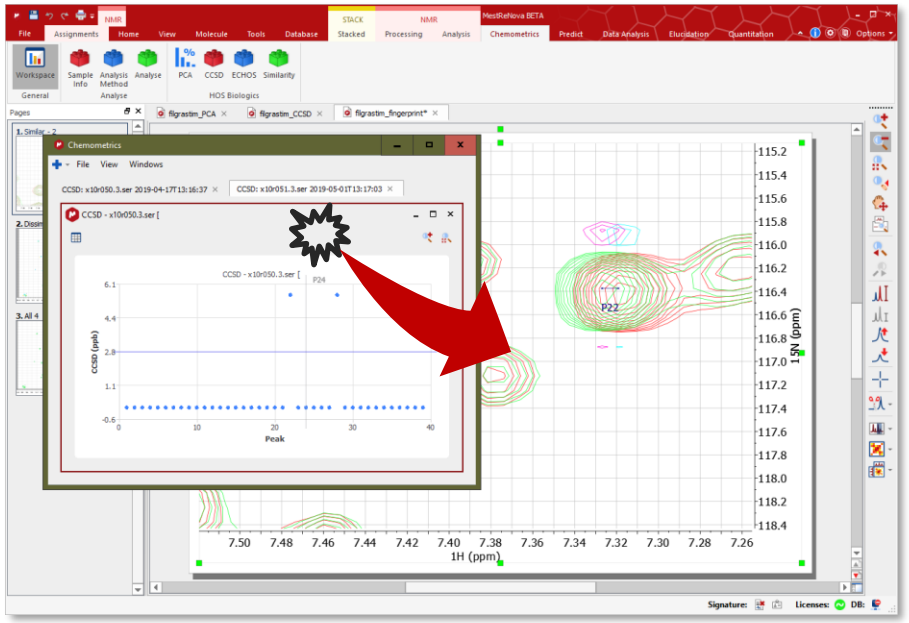
CCSD - III

Peak #22 is shown to have moved



Interactivity with the spectrum

- Click on a point to expand and show the spectral region with the corresponding peak.
- Use this to decide whether or not a peak movement is real or artefactual.

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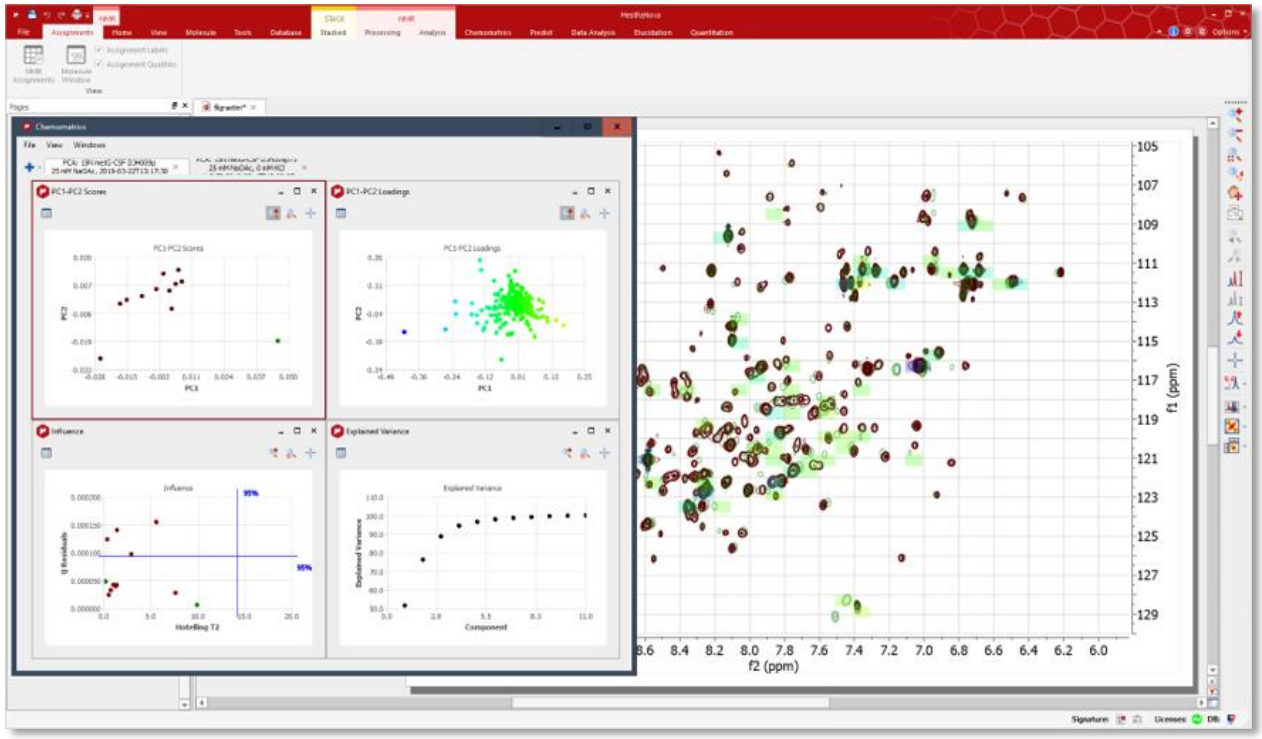
PCA



- **P**rincipal **C**omponent **A**nalysis is a popular way to show whether or not spectra are similar, and display the parts of the spectra where they differ.
- Each spectrum is reduced to a single spot in the components plots.

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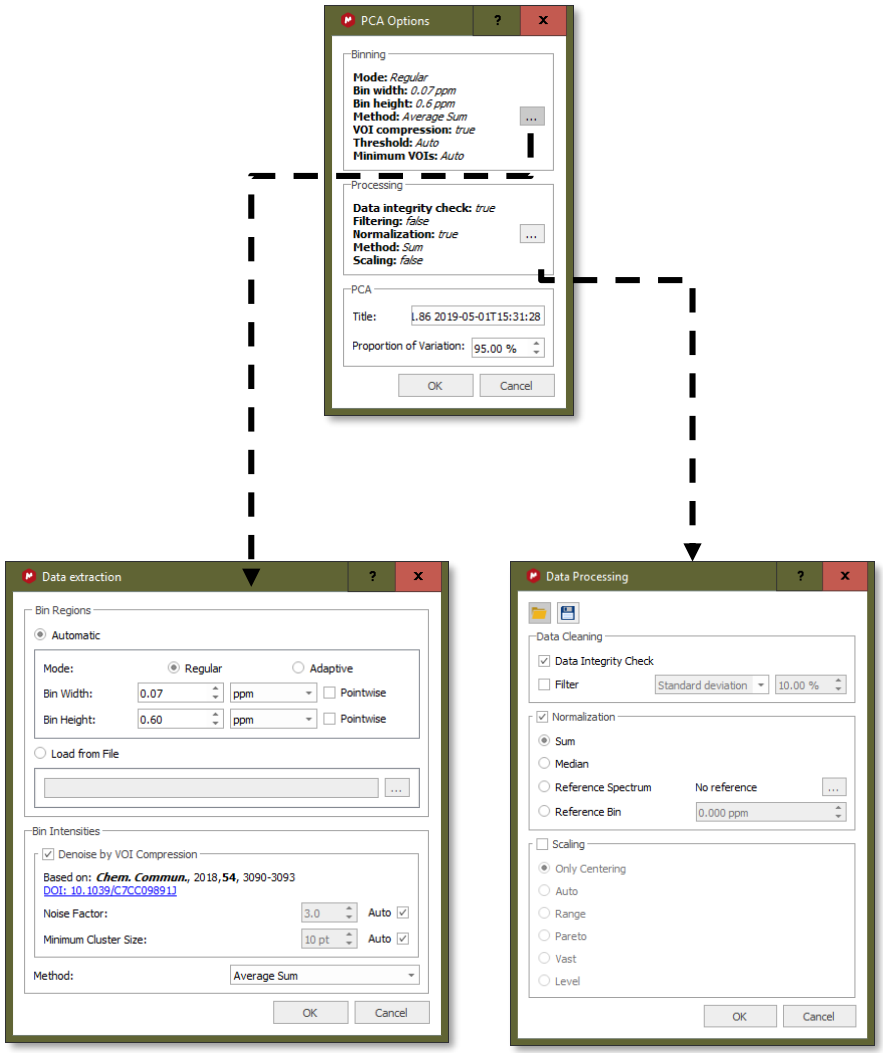
PCA – binning and data pretreatment



- You will need to select PCA options that result in you reference data clustering well in the loadings plots (e.g., PC1-PC2).
- This process is largely “trial and error”, but once the correct conditions have been found they can be reused with confidence.

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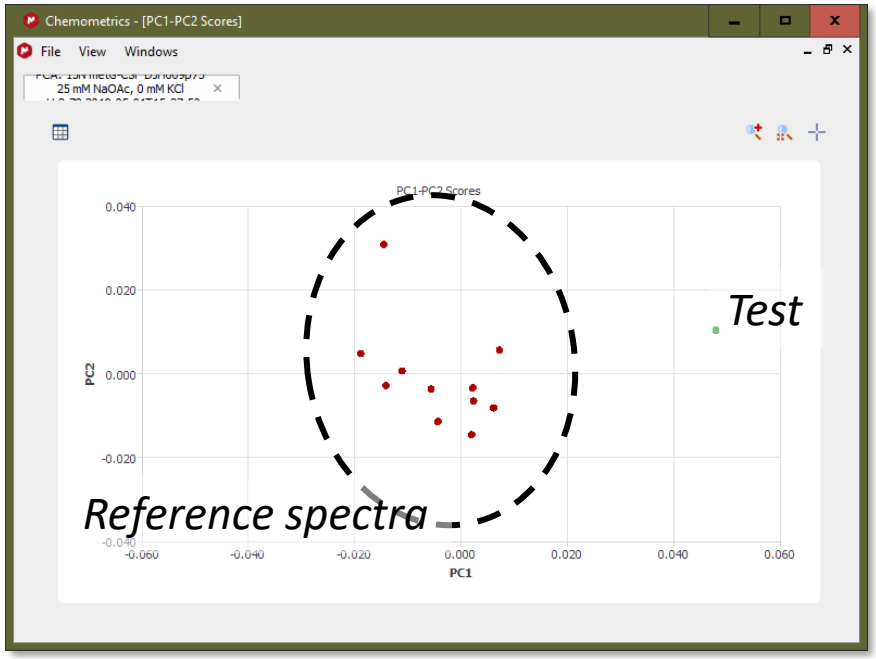
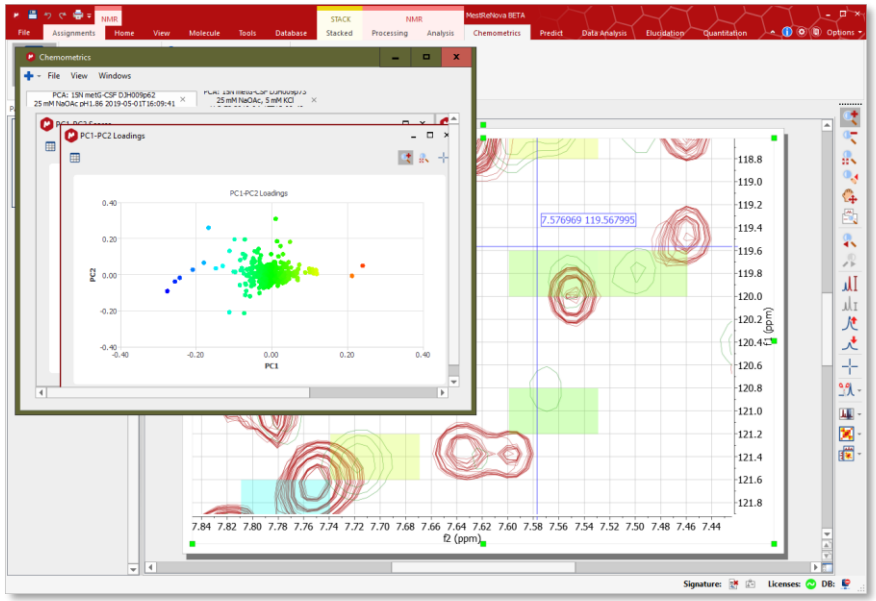
PCA – Loadings plots



Scores plots

- We can see whether or not spectra are similar, based on whether or not they *cluster*.
- Reference spectra should be similar, and whether or not a test spectrum is, too, is indicated by the closeness of the test spectrum dot to the cluster of reference spectra.

PCA – Loadings plots



Loadings plots

- These allow you to see which bins in the spectral regions cause one spectrum to be different from another.

- There is full interactivity between the points on the scores plot and the NMR spectra.

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thank you