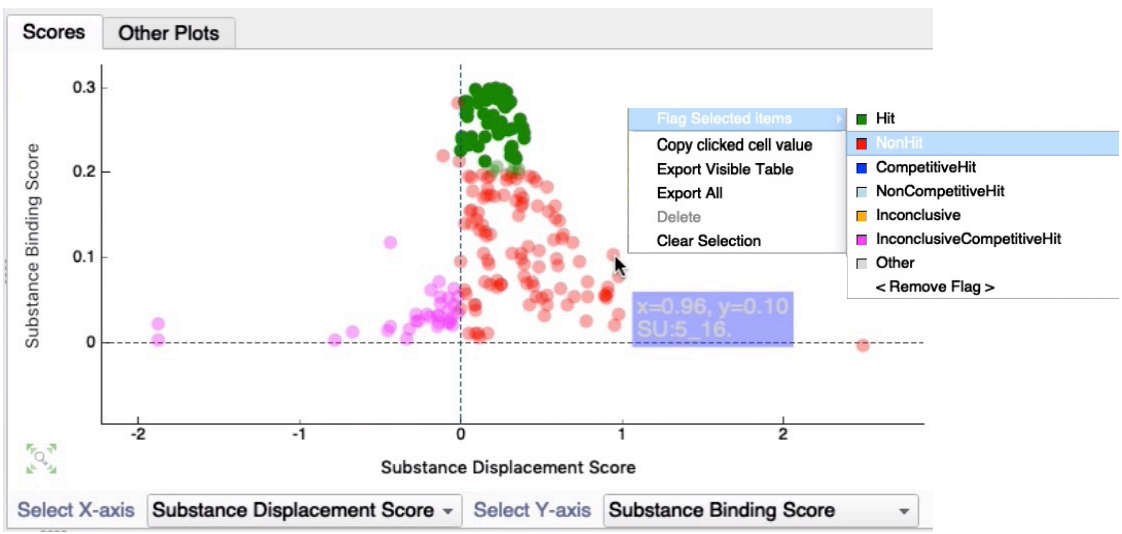
CCPN

CcpNmr AnalysisScreen Version 3

AnalysisScreen
Hit Analysis Tutorial



Introduction

This tutorial will show the general usage of the Hit-Analysis module in CcpNmr AnalysisScreen Version **3.0.4**.
It is assumed that you have some basic familiarity with the program, e.g., from having completed our Beginners Tutorial.

You will need to use the data located in the **/data/AnalysisScreen_Tutorial** directory of the CcpNmr V3 examples data which you can download from: https://www.ccpn.ac.uk/v3-software/tutorials/tutorial-data-and-examples.

Contents

Start CcpNmr Analysis V3

- Apple users by running Screen on the Launcher
- Unix users by using the terminal command: *bin/screen*
- Windows users by double-clicking on the *screen.bat* file

Disclaimer

Datasets used for this tutorial are randomly generated and don't have any biological significance.
All spectra shown are synthetic and for demonstration purposes only.
All compound names are randomly chosen and might have incorrect chemical properties or not be represented by the linked spectra.
Please note that the images shown are only representative and you may encounter minor differences in your setup.

Introduction

Getting started, basic operations

Sidebar

All data contained in a project, such as spectra and peak lists are located in the sidebar. **Double-clicking** on an item will open its properties popup.

Display

A display can contain multiple overlaid spectra which share the same axes. To show/hide a single spectrum, click on its toolbar button. If you close a display, you can open a spectrum by **dragging and dropping** it into the drop area from the sidebar or by **right-clicking** on a sidebar item and selecting **Open as module**. You can also add additional spectra to a spectrum display module or drag several spectra into the drop area together to open them simultaneously.

Mouse

- Pan -> **Left-drag** in display
- Zoom in/out -> **Scroll wheel** in display
- Context menu -> **Right-click**
- Select a peak -> **Left-click** on a peak symbol "X"
- Move a peak -> select first, then **middle-click and drag**

Two-Letter Shortcuts

Press the first letter on your keyboard e.g., **M**, followed by the second letter, e.g., **K** (case insensitive). Press **Esc** to cancel the first letter.

Common in this tutorial:

SE -> Snap to Extremum the selected peaks

HA -> Open the Hit Analysis GUI Module

PI -> Open the Pipeline GUI Module

MC -> Clear all marks

Space-Space -> Open the Python console GUI Module

For more commands and operations

Main Menu ⟶ Help ⟶ Tutorials ⟶ Beginners Tutorial

OR

Main Menu ⟶ Help ⟶ Show Shortcuts

Introduction

CcpNmr Analysis Screen Nomenclatures

Sample
A CcpNmr object containing information about the NMR physical sample, e.g., pH, ionic strength etc
CcpNmr links: Sample component, Spectrum (e.g.: the spectrum Control, Target...)

Sample component
A CcpNmr object containing information about the Substance in the NMR physical sample, (e.g., concentration)
CcpNmr links: Substance

Substance
A CcpNmr object containing information about a biological molecule, (e.g., the small molecule and its general properties such as: SMILES, MW etc.)
CcpNmr links: Sample component, Spectrum (e.g.: the Singleton Spectrum)

SpectrumGroup
A CcpNmr object containing a collection of spectra.
CcpNmr links: Spectra

Control (spectrum)
The spectrum recorded at time X for a sample containing one or multiple substances prior the addition of a biological target
CcpNmr links: Sample

Target (spectrum)
The spectrum recorded at time X for a sample containing one or multiple substances plus a biological target
CcpNmr links: Sample

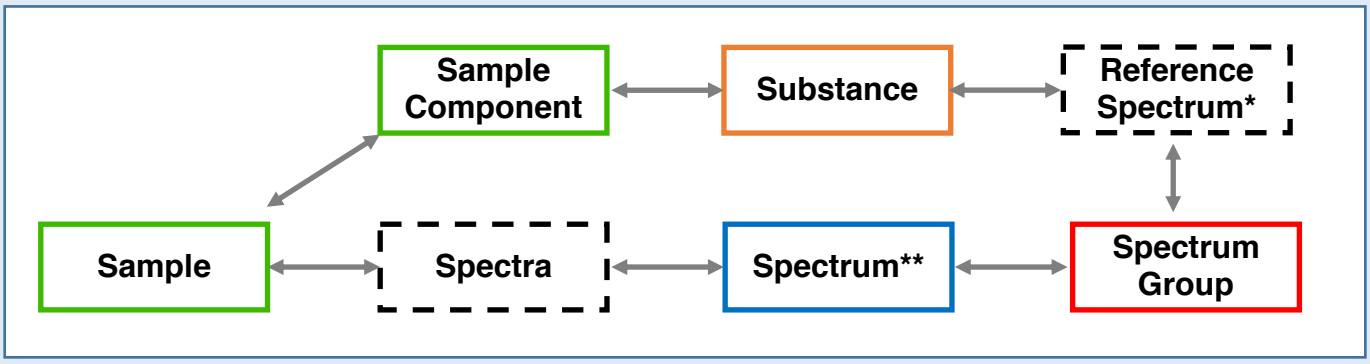
Displacer (spectrum)
The spectrum recorded at time X for a sample containing one multiple substances plus a biological target and a known binder. Also named as "competitor"
CcpNmr links: Sample

Reference Mixture
The spectrum recorded for a sample containing multiple substances. Its peaks and their annotations are used as a template and to identify substances in future screening analyses
CcpNmr links: Substances

Reference Singleton
The spectrum recorded for only one substance
CcpNmr links: Substance

Binding Substance
The substance linked to a spectrum (reference) whose peaks have been matched to the spectral peaks (Control – Target) denoting a binding event

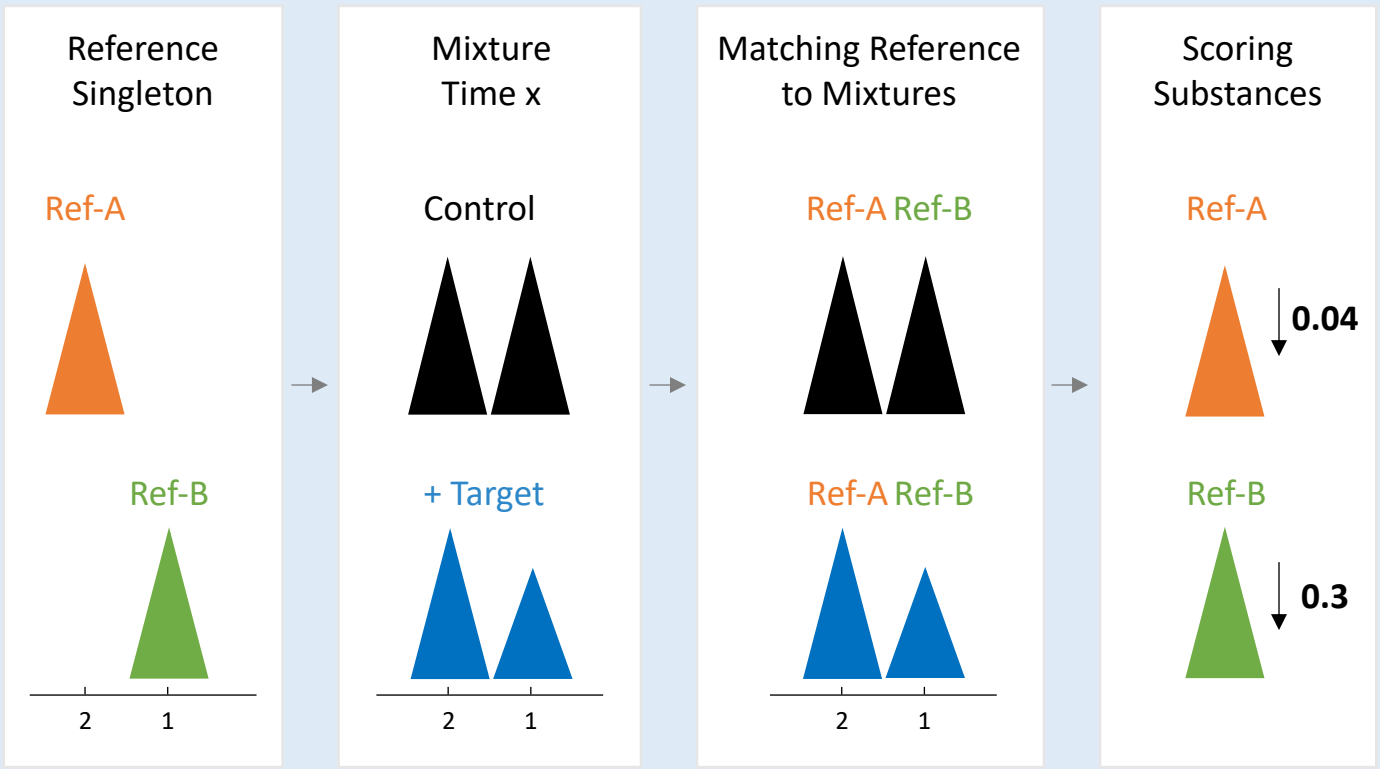
Peak Match
The virtual linkage between a Reference – Control – Target (– Displacer) peaks in a screen Dataset



The schematic diagram shows how objects are linked in CcpNmr Analysis.
* *Reference Singleton*; ** *Control, Target, Displacer, Reference Mixture*

Introduction

Schematic representation of the Screen analysis workflow



i. ii. iii. iv.

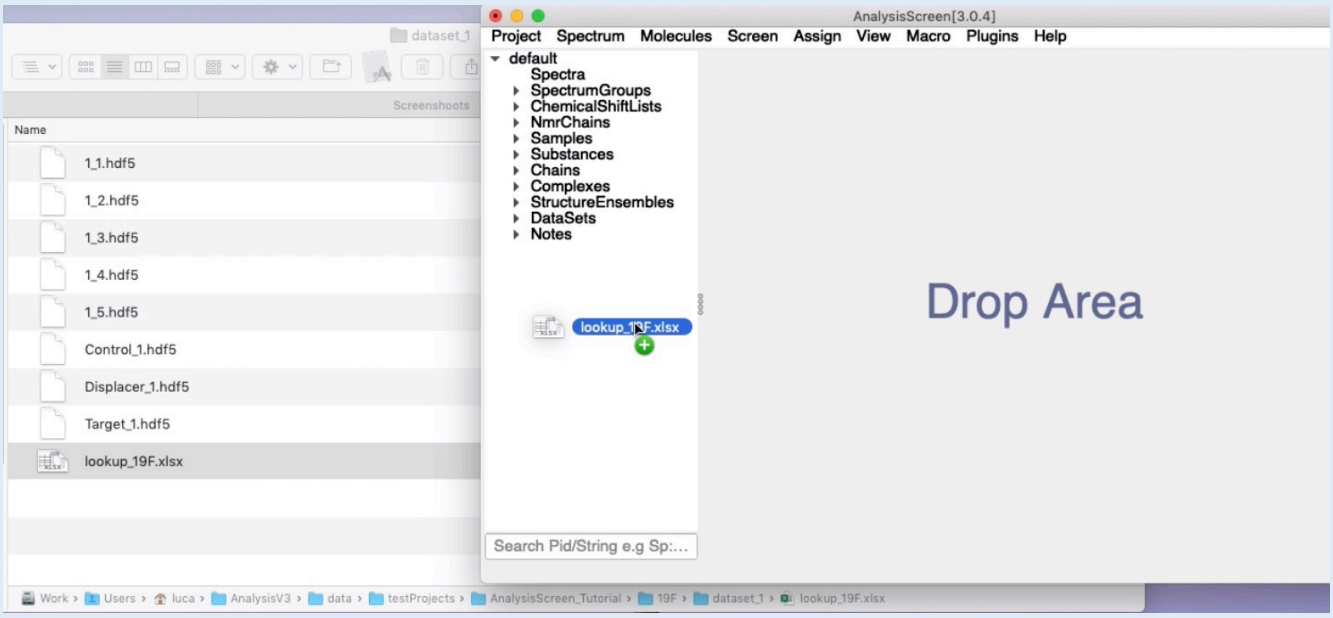
i. The *Ref–A* and *Ref–B* cartoons represent the two Reference Singleton spectra recorded for the Substance Ref–A and Ref–B

ii. The *Control* and *+Target* cartoons represent spectra recorded at a time X for samples containing only the components (*Control*) and plus a biological target (*+Target*)

iii. The Reference Singleton signals are matched to the Control and Target spectral signals based on chemical shift position

iv. Spectral differences between the Control and Target are scored using different calculation methods. Scores are then transferred to the matching Substances as indication of their binding activity

1 Part 1: Manual And Semi-Automatic Analysis



By loading an Excel file, correctly formatted, all the necessary links are automatically established ensuring an optimal functioning of the screening tools.
See the “HowTos_SidebarObjects” for more information.

1A Drag & drop the file lookup_19F.xlsx into the program

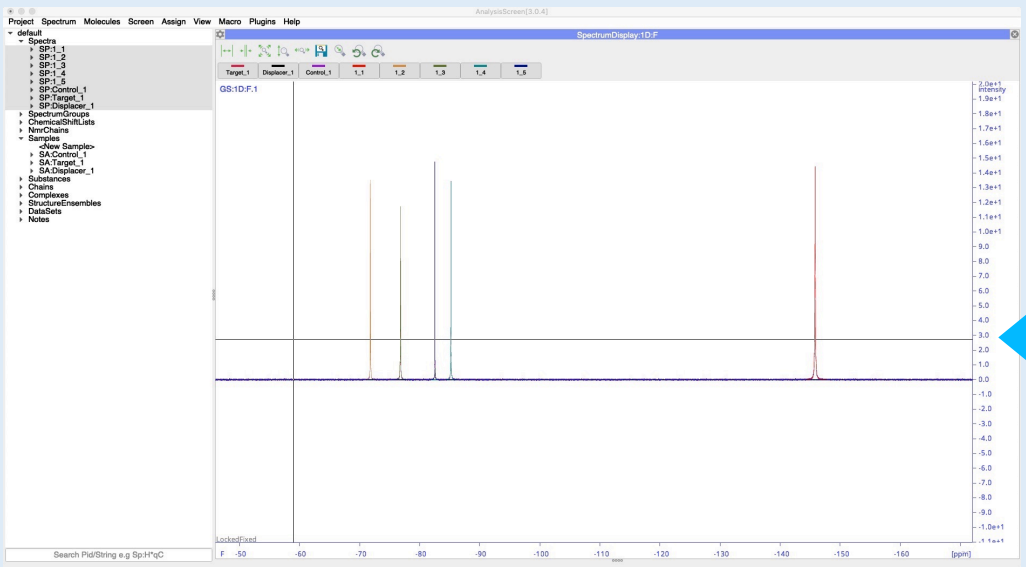
- Locate the demo dataset in the AnalysisScreen tutorial folder in AnalysisV3/data/testProjects/AnalysisScreen_Tutorial/19F/dataset_1
- Drag & drop the Excel file lookup_19F.xlsx into the sidebar or drop area.
  See the “HowTos_ImportDataFromExcel” for creating excel files

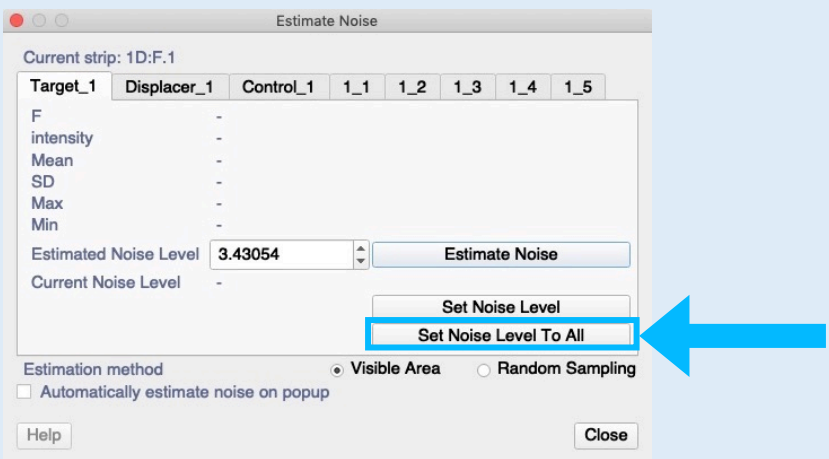
1B Open all spectra

- select all spectra from Sidebar, drag and drop them onto the Drop Area.

You can reorder the spectra on toolbar, by placing the mouse cursor over the spectrum-button and holding-down the middle mouse button; an alias image will appear, drag it to a new position then release it

1 Prepare Data





1C Set noise level

On display:

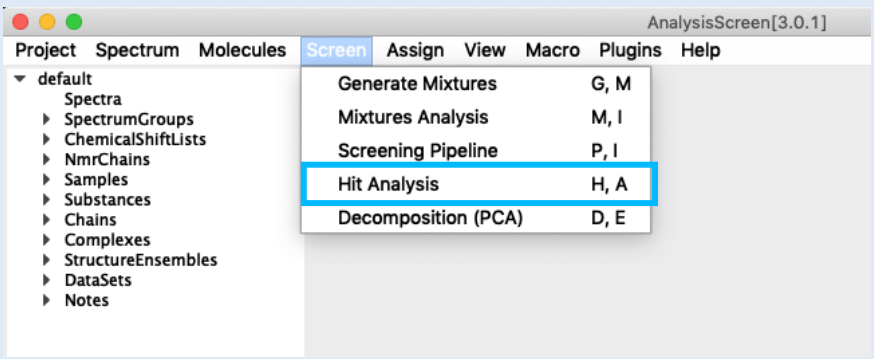
- place the mouse cursor at a position where you want to set the noise level, for example, at ~ 3 in the Intensity axis.
- right click -> **Estimate Noise** -> **Set Noise Level To All** (displayed spectra)

1D Stack Spectra

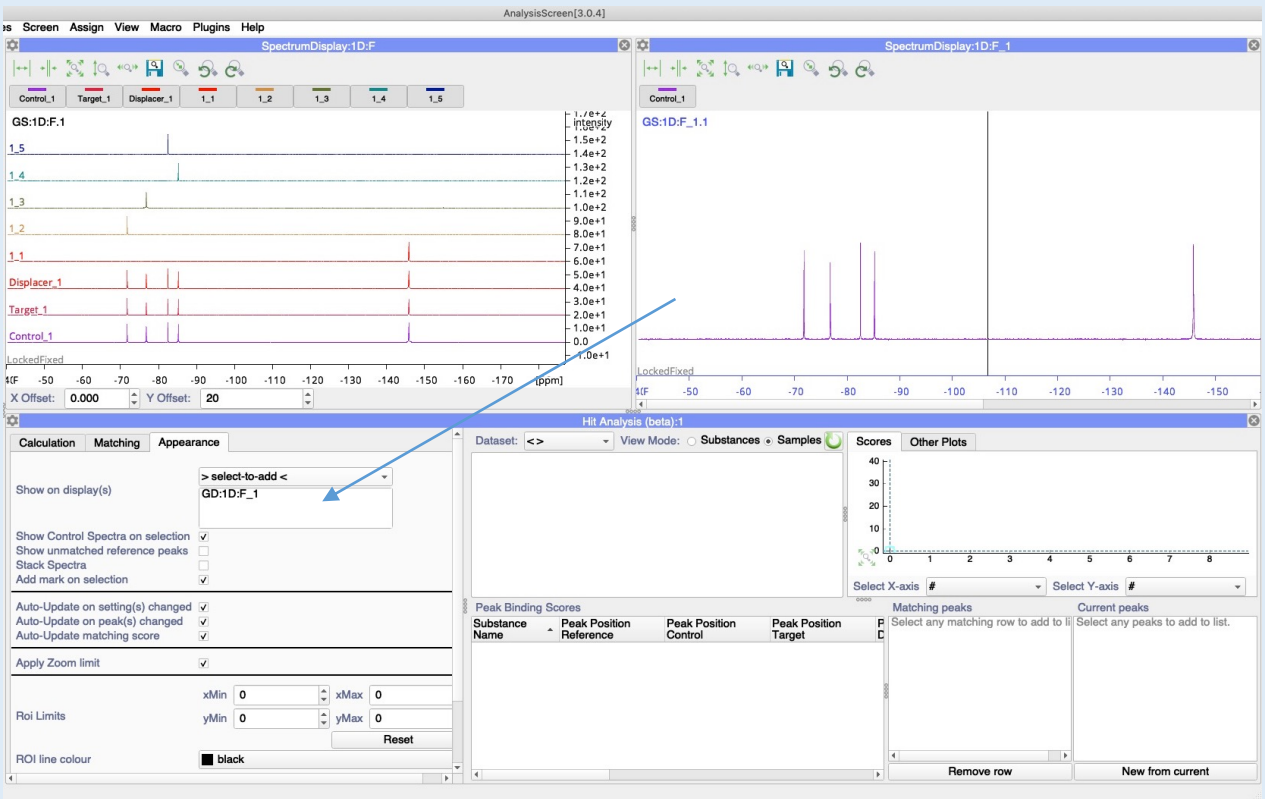
On display:

- right click -> **Stack Spectra** (shortcut **SK**)
- **Y Offset:** 20
- Zoom in-out as required to show all spectra in the display
- Scroll mouse-wheel over the Intensity axis to adjust the Y-range

2 Hit Analysis Module setup



Shortcut
"HA"



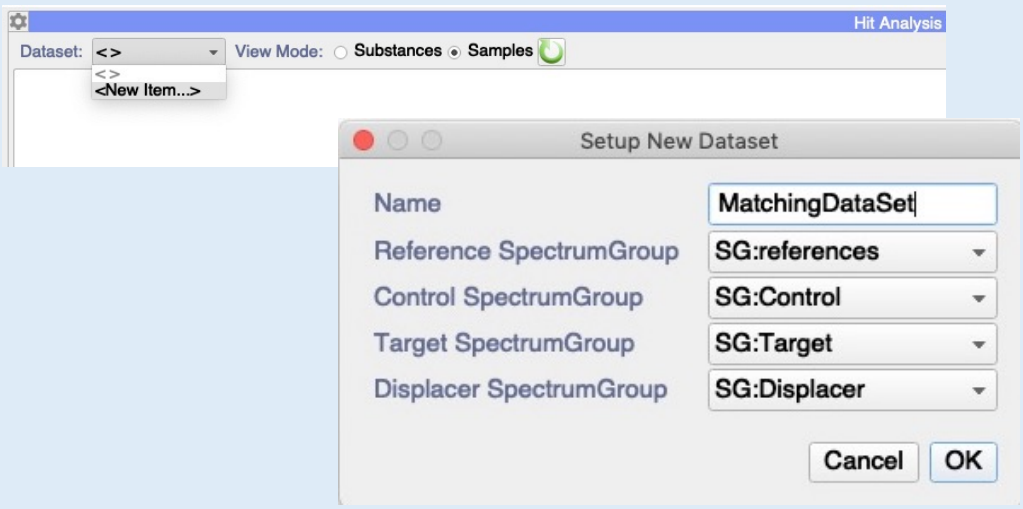
2A Open the Hit Analysis Module

- Go to **Main Menu ⟶ Screen ⟶ Hit Analysis** (shortcut **HA**)

2B Open a second Spectrum Display and re-arrange layout

- Open any spectrum next to the already opened stacked display
- Click on the **Hit Analysis** module **Settings** gear icon :
- **Appearance tab**
- **Show on display(S) -> right click -> remove all**
- Add the new unstacked **Spectrum Display** (e.g. GD:1D:F_1) to the list
  The module will perform soon a series of dynamic actions on this display.
- Close the settings
- re-arrange the Hit Analysis Module below the Spectrum Displays so to show all its widgets on the screen.

2 Hit Analysis Module setup



The **Hit Analysis** module contains two views: by Substance and by Sample. This selection determines the behaviour of the two main tables.

The top table contains a list of the substances or samples depending on the selected view. The lower table contains a list of all the peaks for the single substance reference spectrum or all the substances present for a selected sample.

Tips:

You can sort the tables by any column by **clicking** on the **column heading**. **Click** on the **column heading** a second time to reverse the sort order.

To show/hide columns in the tables:

- **Right-click** on the table header and select **Columns Settings…** .
- Check/Uncheck the columns as required.

The module contains also plots and matching editing widgets and they are described in more details in the pipeline section.

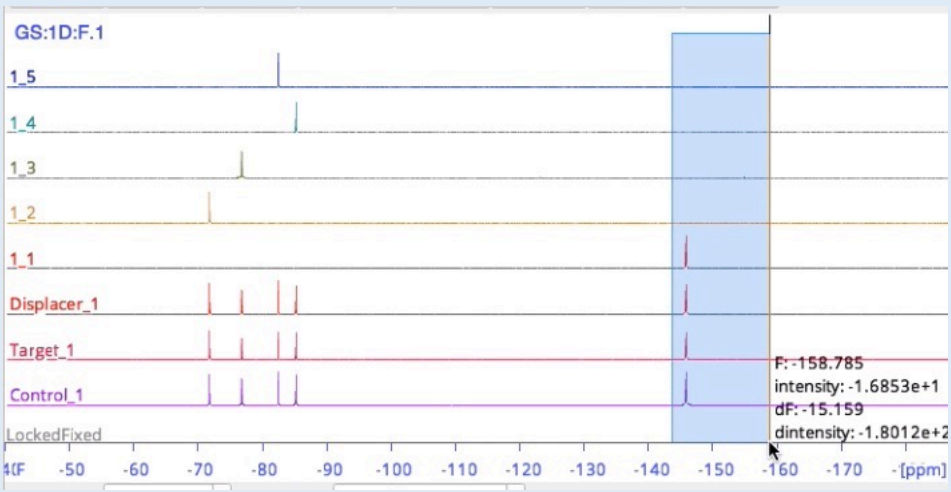
2c Create new Screening Dataset

Hit Analysis module

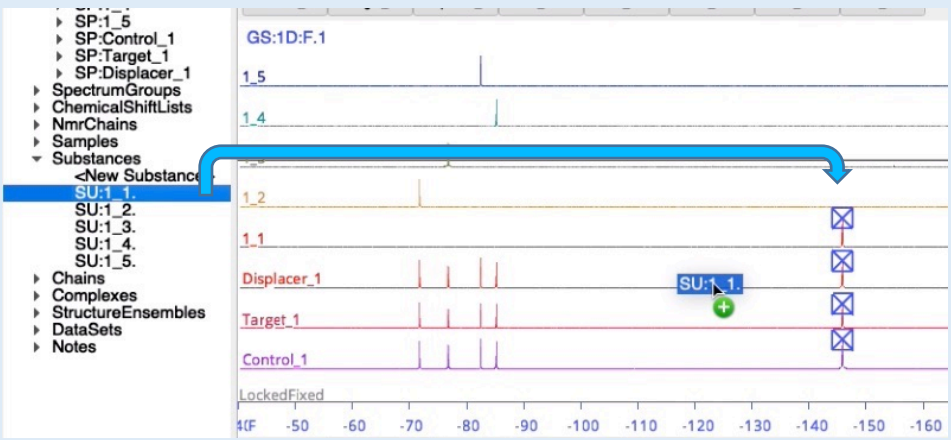
- Select <**New Item**…> on the **Dataset** pulldown
- Change name or keep default
- SpectrumGroups pulldowns are automatically selected if the SGs start with names: References, Control, Target, Displacer.
- Press Ok to proceed

Note the module will also work without the Reference and Displacer SpectrumGroups

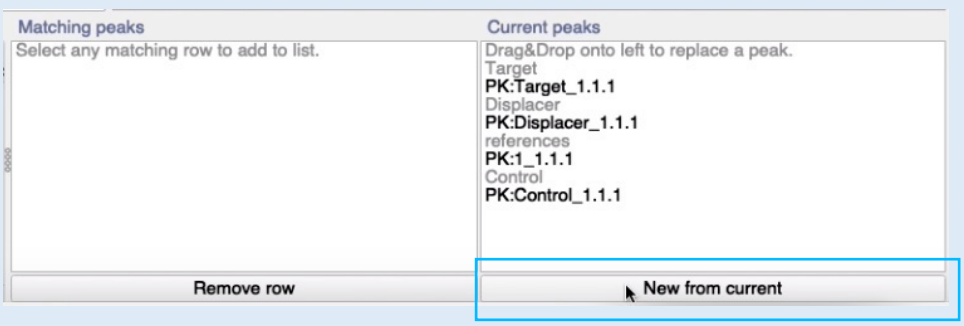
2 Hit Analysis Module



Pick peaks



D&D a Substance 1_1 on peaks



Click New

2D Pick and match

On the stacked display 1D:F.1:

- Pick peaks at ppm -145.8

  use CTRL (or CMD for Mac) + SHIFT + Left-drag to create a (blue) picking region so to include all spectral signals around -145 ppm.

  This signal correspond to the Substance 1_1

On Sidebar:

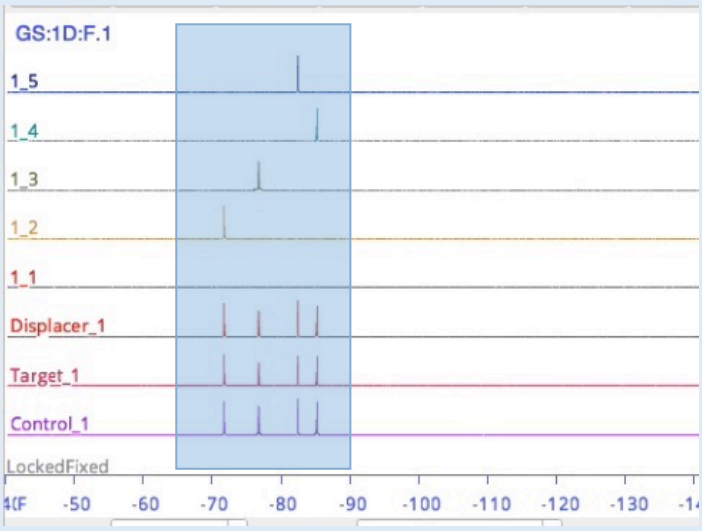
- locate and expand the Substances branch
- Drag & Drop SU:1_1 to the selected peaks, this annotates the peaks with the substance name for an easy visual inspection

On the Hit Module:

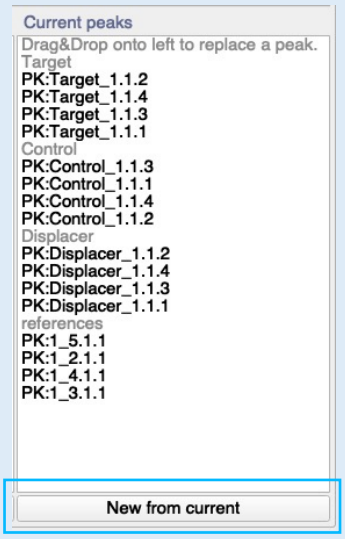
- the **Current peaks** will appear at the bottom-right corner
- Press **New from current**

Hit Analysis Module

Multi-picking and semi-auto peak matching



Pick peaks



Click New

2E Semi-automatic matching

If you don't need to annotate peaks, you can add multiple matches at once.

On the stacked display 1D:F.1:

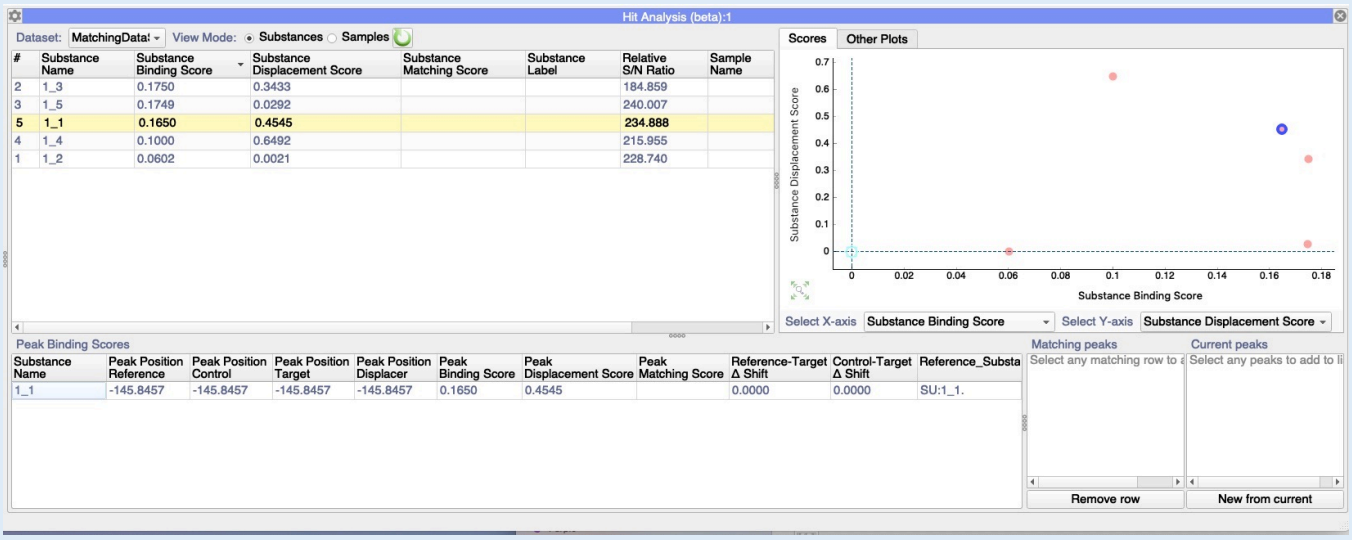
- Pick the remaining spectral signals by creating a larger picking region with the shortcut CTRL (or CMD for Mac) + SHIFT + Left-drag

On the Hit Module:

- the **Current peaks** will appear at the bottom-right corner, make sure there is an even number for each category, e.g.: 4 Reference peaks – 4 Control Peaks etc... in any order.
- Press **New from current**
- Tables will update accordingly

(For larger dataset, this process can be fully automated, including annotation and matching, see the Pipeline section on this manual)

2 Hit Analysis Module



2F Scoring Engines

On the Hit Module:

- Select Substances in the View Mode to list all Substances on the top table

The **Substance Binding Score** gives an indication of the spectral changes between the Control-Target spectrum for the peaks matched to the Substance reference spectrum, therefore, it can be used to assess the substance binding quality.

This score is given by the calculation engine selected in the settings.

- Change the default from the calculation tab. By hovering over the labels for each option, a tip-text window will show the equation used
- You can also define your equation in the free entry box:

  use V1 and V2 to define the variables for the calculation matrix

  - V1 represent each Ligand signal in the presence of the target (Target)
  - V2 represent each Ligand signal in the absence of the target (Control)

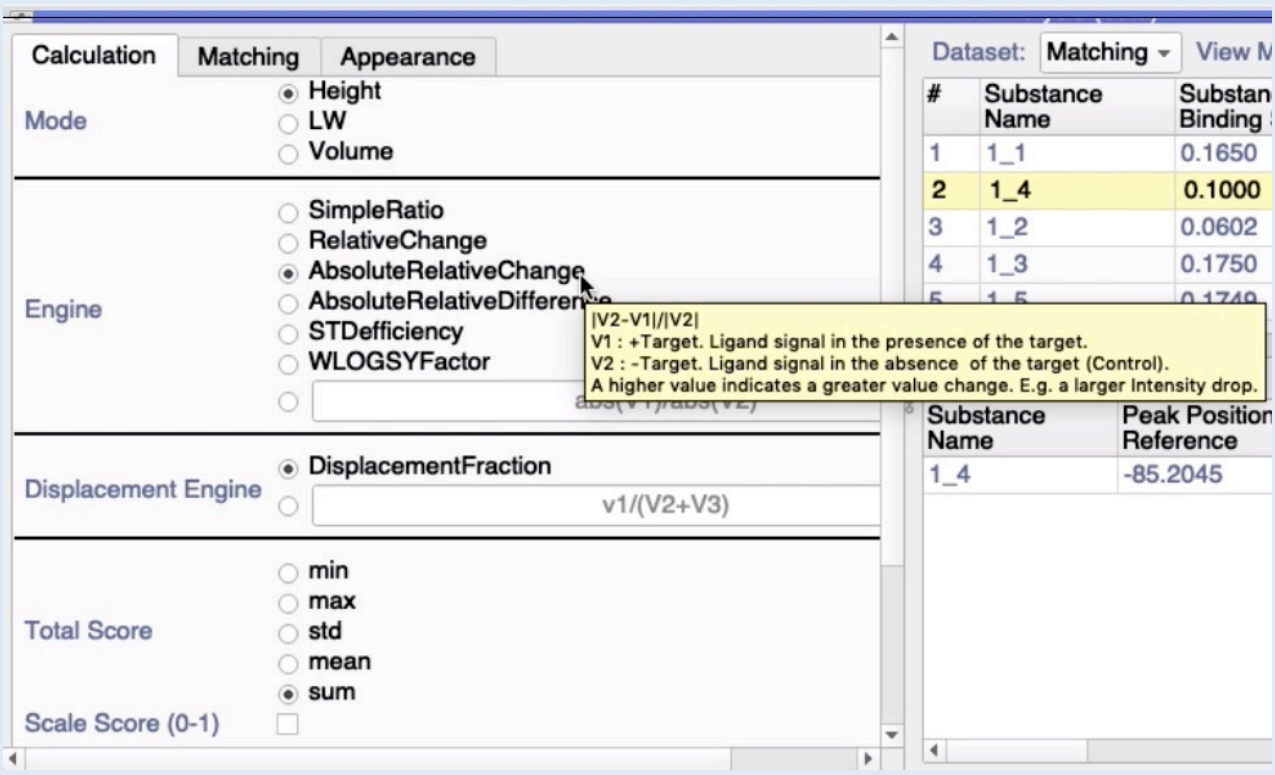
  The following arithmetic operations are supported:

  ``+``, ``-``, ``*``, ``/``, ``**``, ``%``, ``//``

Same rule applies for the **Displacement Engine,**

When building your own equation, define the Displacer signal with the variable V3

Hit Analysis Module



Scoring Engines (continued)

Furthermore, this score is the result of all the single peak-matches contributions.

In this 19F demo dataset, only one observation is recorded per reference spectrum.

However, if multiple peaks per substance are present, the total Substance Binding score will be given by one of :

Min, Max, std, mean, or sum

of all the single peak binding scores.

- Select this option from the Settings **Total Score**

Whereas, if **Samples** is selected in the View Mode, the Sample Binding Score is given by all the Substances binding scores.

By changing settings, the whole module will update and recalculate automatically all scores. For larger datasets this can be time-expensive, disable this feature from:

- Appearance tab
- Uncheck Auto-Updates on settings(s) changed

2 Hit Analysis Module

Selections

This module has multiple dynamic selections

Selecting a row on **the Substance table**, it will:

1. List all the contributing matches in the Peak Binding Scores table below.
2. Display all the spectra associated to the binding match
3. Select a relative item in the Scores scatter plot

Selecting a row on the **Samples table**, it will:

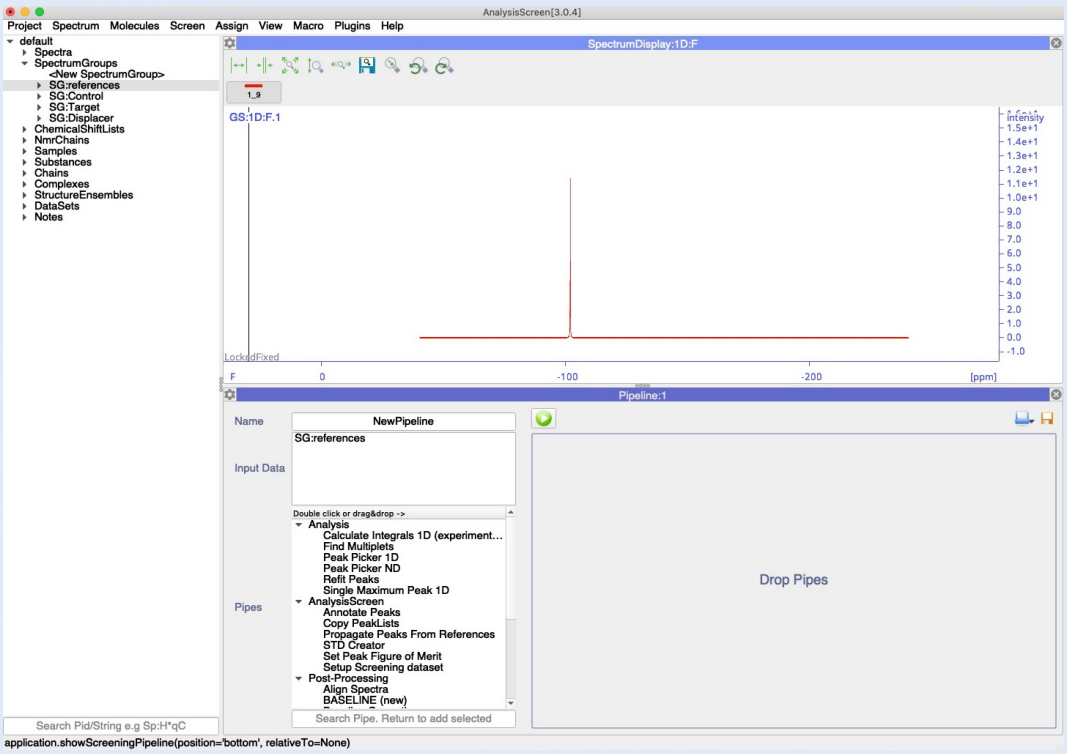
1. List all the contributing matches in the Peak Binding Scores table for all the substances present in the sample
2. Display all the spectra associated to the binding match, including all references spectra.

Selecting a row on the **Peak Binding Scores table**, it will:

1. Select all peaks included in the match
2. Navigate to Peak Position
3. Populate the Matching/Current Peaks lists widgets

Double-clicks on tables will re-execute the single selection.

3 Part 2: Automatic Analysis with Pipelines



Automatic peak matching and binding analysis

In this section, a pipeline is built to inspect spectral changes between simulated samples recorded for mixtures of substances with and without a target, and with target and displacer (competition analysis).

3A Drag & drop the file lookup_19F_dataset2.xlsx into the program

- Locate the data of dataset_2 inside the folder:
  AnalysisV3/data/testProjects/AnalysisScreen_Tutorial/19F/dataset_2/data

- Drag & drop the file lookup_19F_dataset2.xlsx into the sidebar or drop area.
  See the “HowTos_ImportDataFromExcel” for creating excel files

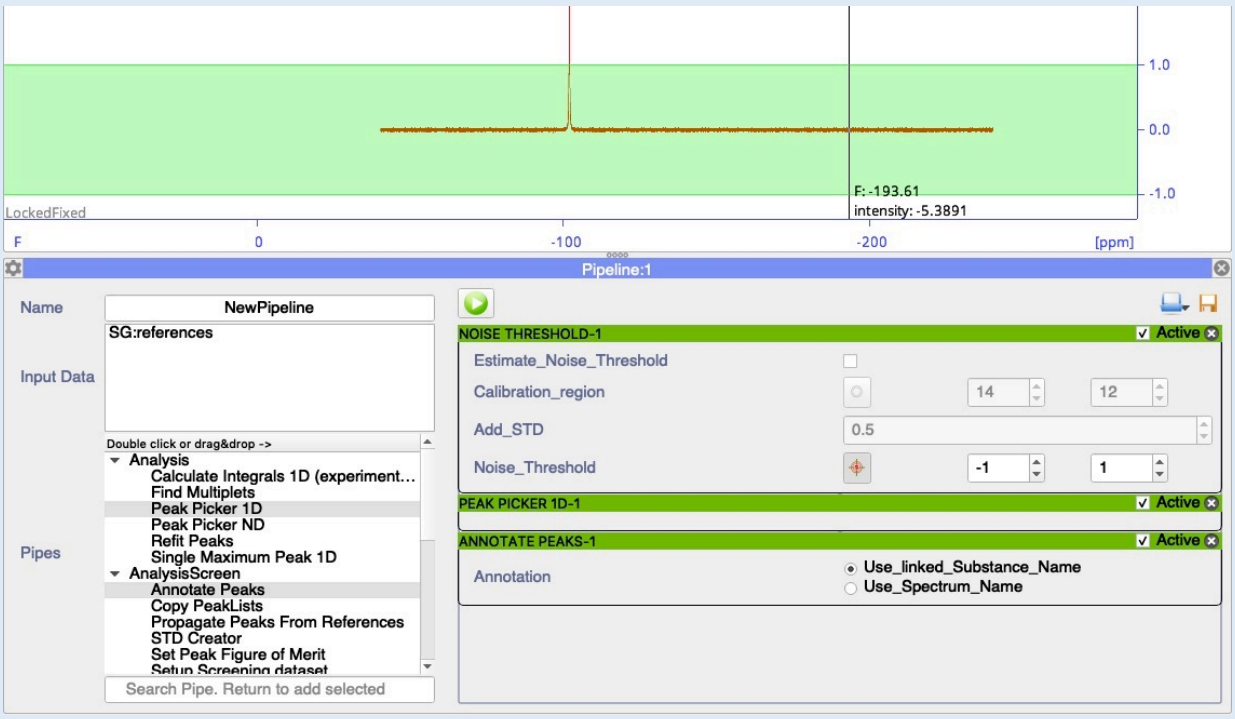
3B Open the first reference spectrum

- select the first spectrum on Sidebar, drag and drop it onto the Drop Area.

3C Open the pipeline module, shortcut PI

- Open the pipeline module from the main menu:
  Menu > Screen > Pipeline
  or use the shortcut PI
- Expand the Sidebar branch for SpectrumGroups
- Select **SG:References**, drag & drop it into **the Input Data** of the Pipeline

3 Pipelines



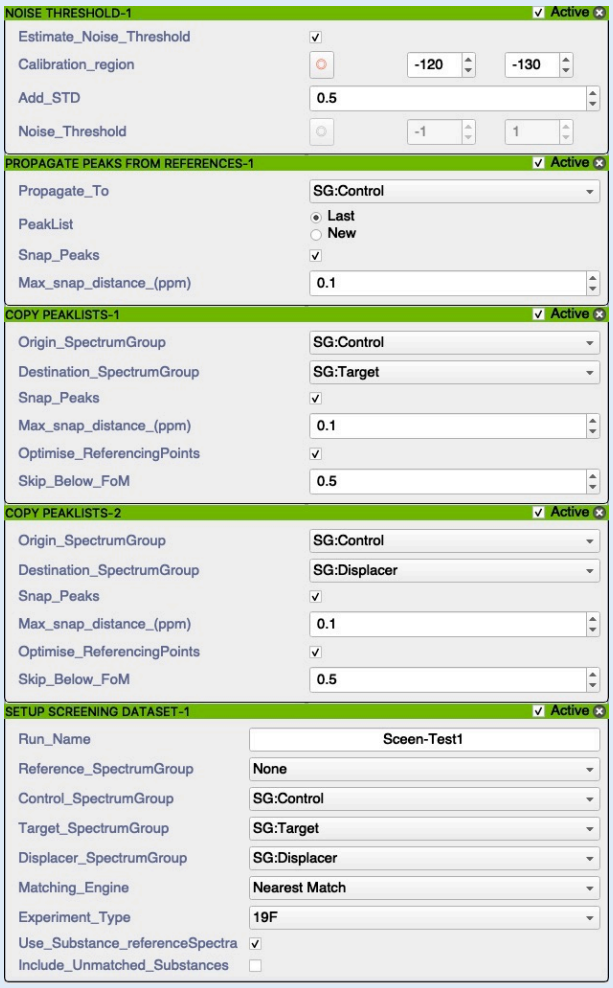
Multiple Pipes can be added and re-order by holding and dragging the green top bar. See “HowTos_Pipelines” for more information

3D Pick peaks on SG:References

- On the Pipes list widget search and add to the pipeline area by double clicking the pipe name or via drag and drop:
    1. **Noise Threshold**
        - Uncheck **Estimate Noise threshold**
        - Click on the Target button 
        - Insert the **Noise Threshold** values -1, 1
          either by dragging the green lines that appears on displays or inserting the values in the entries
    2. **Peak Picker 1D**
    3. **Annotate Peaks**
        - Check **Use linked Substance Name**

- Run the pipeline using the green play button

3 Pipelines



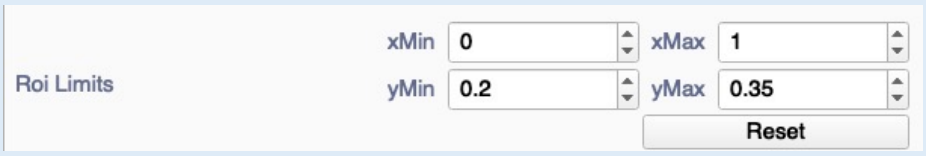
3E Setup screening pipeline

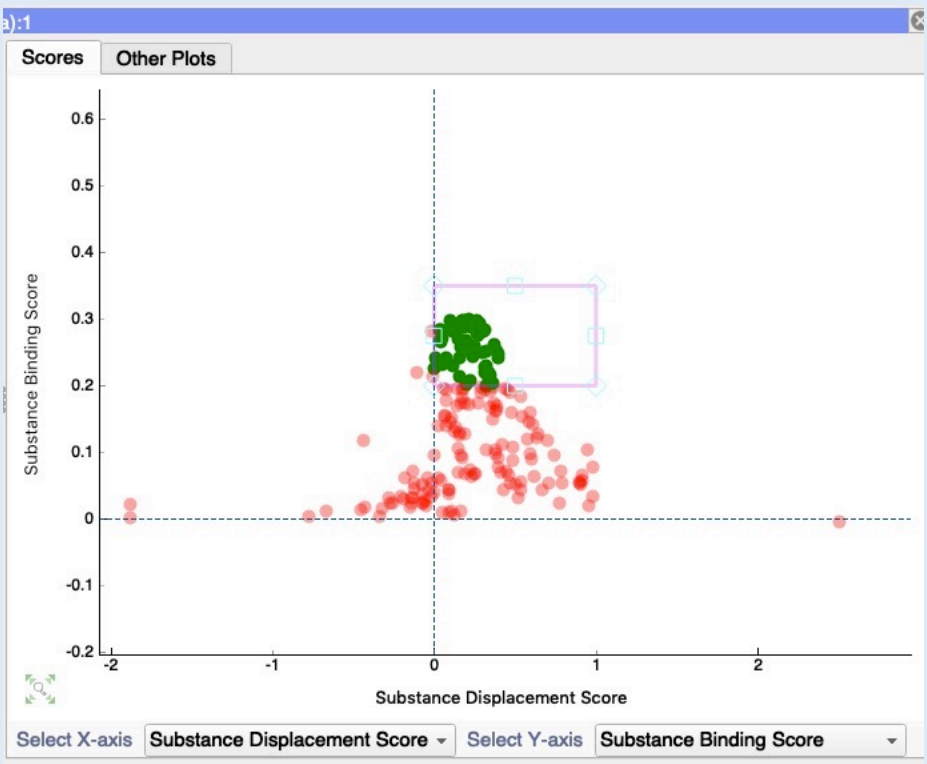
- Clear the input data
- On sidebar, multiselect 'SG:Control', 'SG:Target', 'SG:Displacer'
- drag and drop on the pipeline **Input Data**
- close all pipes (right click on any pipe header -> close all)
- On the Pipes list widget search and add to the pipeline area:

1. **Noise Threshold**
   - Calibration region: -120, -130 ppm
2. **Propagate Peaks from References**
   - Propagate to: SG:Control
3. **Copy PeakLists (1)**
   - Origin SpectrumGroup: SG:Control
   - Destination SpectrumGroup: SG:Target
4. **Copy PeakLists (2)**
   - Origin SpectrumGroup: SG Control
   - Destination SpectrumGroup: SG:Displacer
5. **Setup Screendataset**
   - Run name: 19F_Pipeline
   - Reference SpectrumGroup: **None**
   - SG:Control, SG:Target, SG:Displacer on their respective entries
   - Matching engine: **Nearest Match**
   - **Check** Use Substance ReferenceSpectra

- Run the pipeline using the green play button

4 Hit Analysis Module – Filters





Before flagging Substances, always inspect the matches with the table selections. Snap peaks with the shortcut SE, or correct matches from the two list widgets at the bottom right corner.

4A Define Binding Hits

- Main Menu ⟶ Screen ⟶ Hit Analysis (shortcut HA)
- Select Dataset: 19F_Pipeline
- View mode: Substances
- Scatter Plot:

  X-axis: Substance Displacement Score

  Y-axis: Substance Binding Score

Open settings:

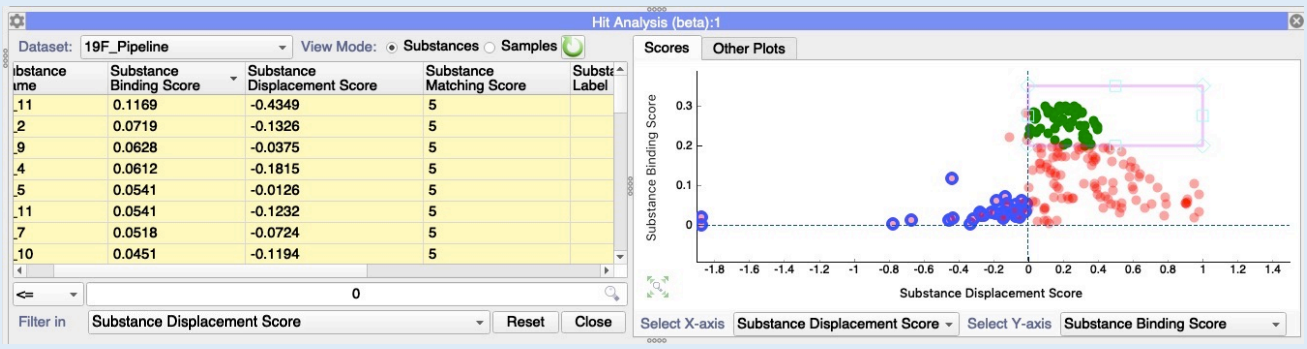
- Calculation tab
  - Engine: AbsoluteRelativeChange
- Appearance tab
  - Roi Limits: click reset
  - xMin: 0 xMax: 1
  - yMin: 0.2 , yMax: 0.35

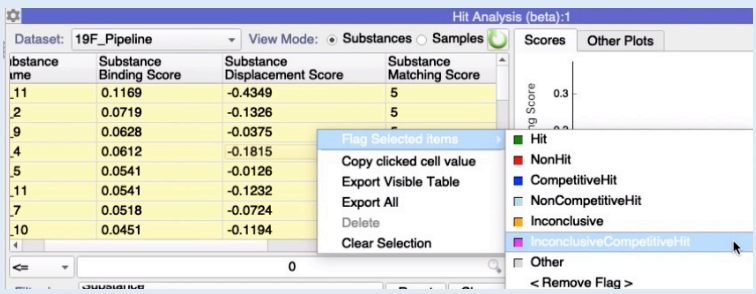
  this will create a Region of interest on the scatter plot

On the scatter plot:

- right click (over an item) -> Select within ROI
- right click (over an item) -> Flag Selected Items -> Hit

4 Hit Analysis Module – Filters





The Hit analysis module has several filters for defining hits based on dynamically set threshold limits.

4B Filter Tables

Another way of defining hits is by applying one or multiple filter from the substance table:

- Right click on the Substance Table header -> **filter**

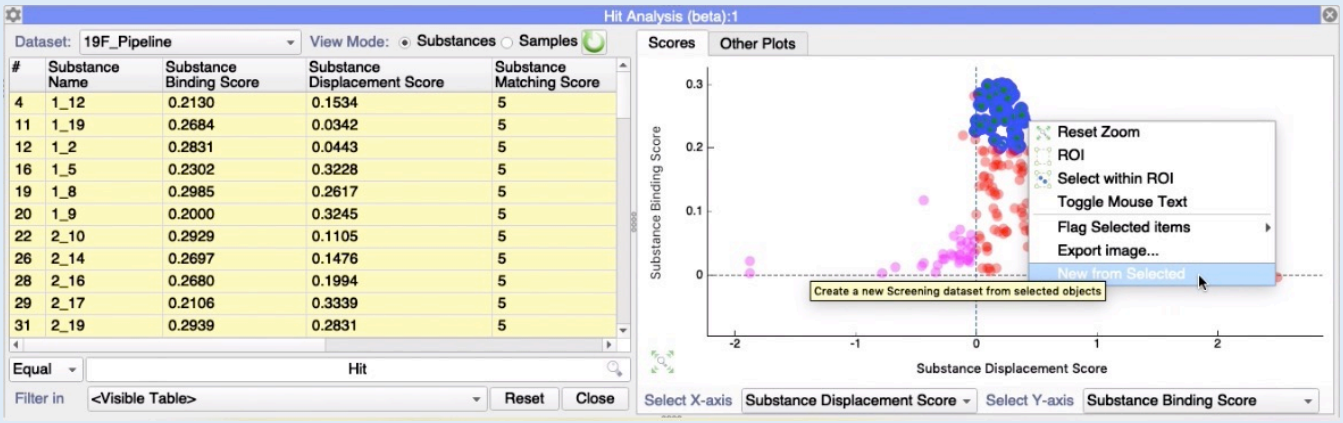
  first filter:

  - select: "<= " 0.2 (less than)
  - filter in: Substance Binding Score
  - press the search button

  second filter:

  - select: "<= " 0
  - filter in: Substance Displacement Score
  - press the search button
- Select all rows
- if a display is opened, a warning will pop up: Click **No** to don't add all the selected spectra on the current Spectrum Display
- right click on a row: Flag Selected Items -> Inconclusive Competitive Hit

4 Hit Analysis Module – Filters



#	Substance Name	Substance Binding Score	Substance Displacement Score	Substance Matching Score
4	1_12	0.2130	0.1534	5
11	1_19	0.2684	0.0342	5
12	1_2	0.2831	0.0443	5
16	1_5	0.2302	0.3228	5
19	1_8	0.2985	0.2617	5
20	1_9	0.2000	0.3245	5
22	2_10	0.2929	0.1105	5
26	2_14	0.2697	0.1476	5
28	2_16	0.2680	0.1994	5
29	2_17	0.2106	0.3339	5
31	2_19	0.2939	0.2831	5

4C Extract and export

- Sort the substance table by Substance Label
  or filter by **Equal** "**Hit**" in Visible Table or Substance Label (**reset** any previous filter first)
- Select all the rows for Substances flagged as Hit
- Move to the scatter plot, right click -> **New from selected**
  This will create a new dataset containing only this subsets, named from the time-stamp. You can rename the dataset from the sidebar.
- Select the newly created dataset on the Hit Analysis module,
- Either inspect the substances as shown in Section 2 or export the table
- Right click on a Substance Table row -> **Export All**

4D Export raw data

To get the raw data which is used to build the Hit Analysis Module

- Right click on a Substance Table row -> E**xport Raw Data**
- On the file dialog, Save as: type a name plus the extension, e.g.: .xlsx, .csv, .tsv or .json

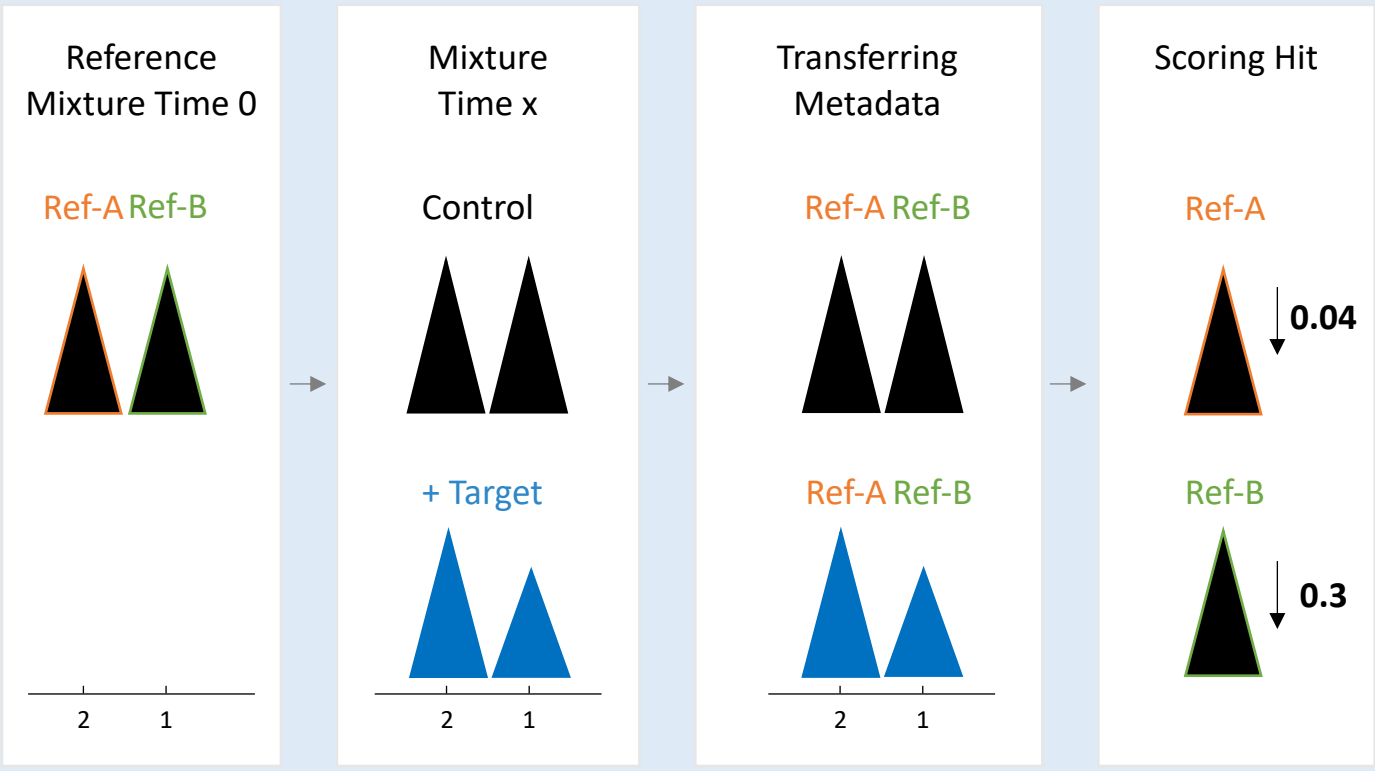
This will create a table which contains all peak metadata, including peak ppm position, height etc for further inspection or macros.

5 Part 3: Recurring Analyses

Schematic representation of a recurring Screen analysis workflow

When screening same mixtures of compounds against different targets, information about the reference spectra can be transferred across projects using **NEF** files.

Annotated peaks from singleton reference spectra can be transferred to a Reference Mixture. These will be used for matching future screening without the need of loading and peak picking again large reference libraries, therefore reducing the analysis time and potential errors.



i. ii. iii. iv.

i. The *Reference Mixture Time 0* represents a spectrum recorded at a time 0 for a sample containing only the components *Ref-A* and *Ref-B* . These are labeled in the peak annotation and contain links to the original Substance objects (see-next slides)

ii. The *Control* and *+Target* cartoons represent spectra recorded at a time X for samples containing only the components (*Control*) and plus a biological target (*+Target*)

iii. The *Reference Mixture Time 0* peaks and labels are transferred to the Control and Target spectral signals and peaks are refitted to the new spectra

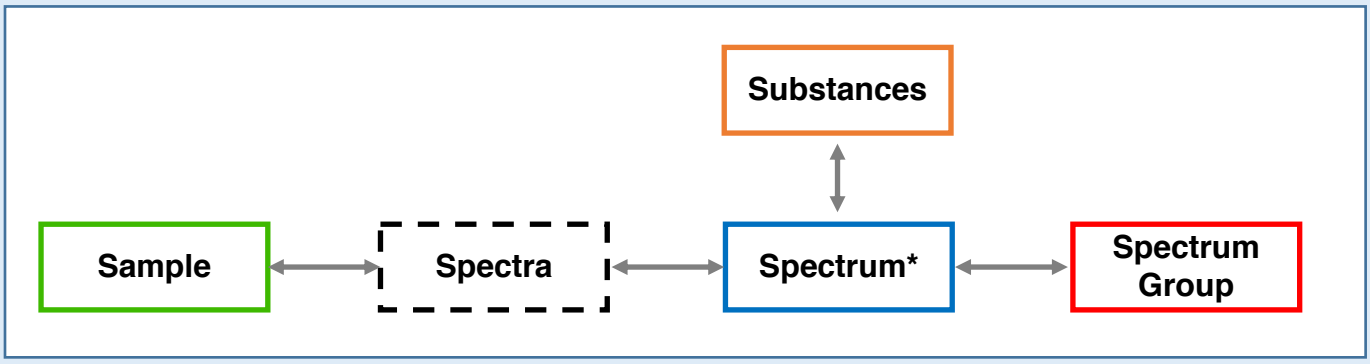
iv. Spectral differences between the Control and Target are scored using different calculation methods. Scores are then transferred to the linked Substances as indication of their binding activity

5 Create NEF

In a NEF files are stored all the information regarding Samples, Substances, Spectra and Spectrum Groups

A NEF file can be created from an existing screening dataset with few modifications. These include **renaming** Samples and Spectrum Group from "Control" to "ReferenceMixture" (or similar), to avoid name-clashes; **deleting** the singleton reference spectra and finally **linking** the Substances to the Reference mixtures .

The pipe **LinkSubstancesToSpectrum** connects all substances presents in a mixtures to a Reference Mixture Spectrum



* Reference Mixture Spectrum at Time 0

5A Load and prepare Data

- Load the project 19F_referencesPicked.ccpn from the dataset_3 directory, .../AnalysisScreen_Tutorial/19F/dataset_3/19F_referencesPicked.ccpn
- Open the Pipeline module
- Add 'SG:RefMix' on **Input Data**
- Add the pipe **Propagate Peaks from References**
    - select Propagate to: 'SG:RefMix' and keep the rest as default
- Add the pipe **LinkSubstancesToSpectrum**
- **Run**

5B Correct peaks (optional for this tutorial)

Because the NEF file will function as a template for future screening analyses, it is wise to inspect all Reference Mixtures, ensuring all peaks are correctly annotated to the respective Reference Substance

- Open the first sample, 'SA:Control_1', right click -> Open Linked spectra or drag and drop it to the dropping area.
- inspect peaks on the Control_1 are correctly positioned from the references, or use the shortcut "SE" to re-snap to extremum the selected peak(s)
  (You can change snapping limits from Preferences, Spectrum Tab, **1d Search Box Widths)**
- on sidebar, use the shortcut Ctrl (or Cmd)+up/down directional keys to visualise the next/previous sample and associated spectra.

5 Handle spurious signals

Peak annotation

When using NEF files as template for screening calculations, Substances are tracked in the Reference Mixture signals through the peak annotations.

Peak annotation name can be made by three parts:

Prefix	substance name	The exact substance name	Mandatory
Separator	_	Underscore	Mandatory if Suffix
Suffix	Any	Any single word tag that can help identify the signal; e.g.: impurity, TFA, Salt, a serial number, an atom name etc.	Optional

Peak annotation examples: “Compound1_CF3, Compound2_Salt, Unknown”

Peaks with figure of merit of 0 are excluded from screening calculations

5C Add extra annotations for impurities, solvents etc and exclude them from calculations

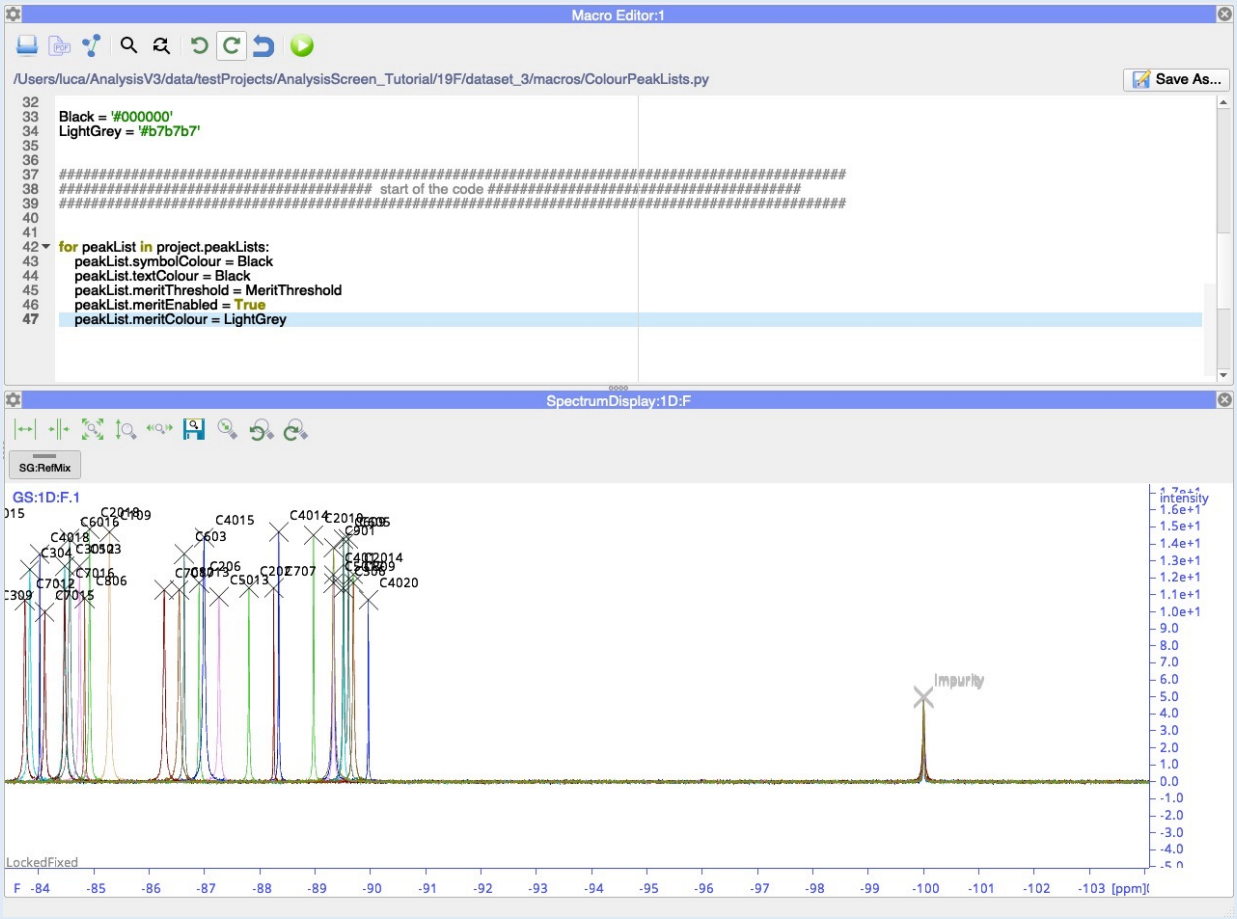
- Close all GUI Modules
- Drag and drop the ‘SG:RefMix’ on the dropping area
- Pick/Select all peaks in the region -99.9, -100.1 ppm
- open the Python Console from Menu > View or shortcut “Space-Space”
- Run the command:

```
tag = 'Impurity'
for peak in current.peaks:
    peak.annotation = '_'.join(filter(None, set([peak.annotation, tag])))
    peak.figureOfMerit = 0
```

Find the code as a Macro in the tutorial directory:

.../19F/dataset_3/macros/AnnotateCurrentPeaks.py

5 Handle spurious signals



5D Change colours for peaks

Change symbol/text colours so that real signal peaks (black) are graphically distinguishable from excluded (light grey)

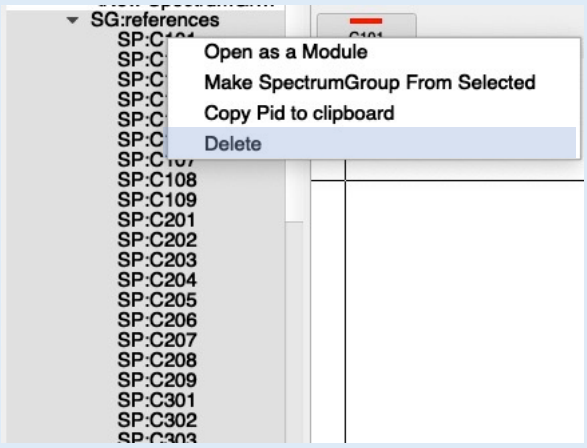
- on the Python run the command:

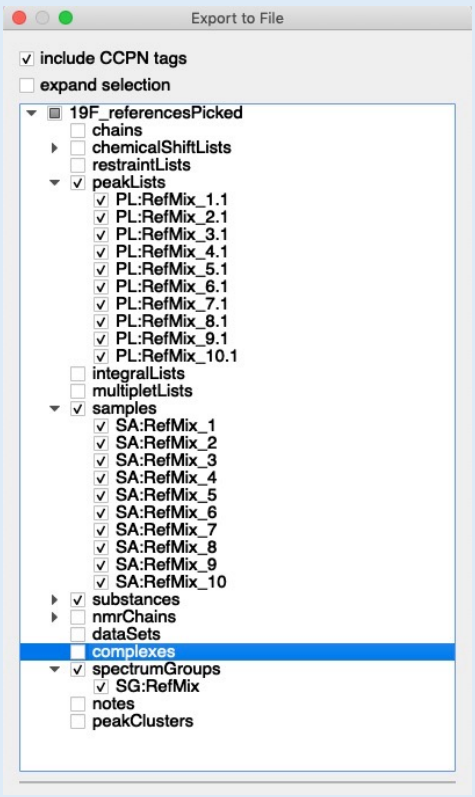
```
black = '#000000'
lightGrey = '#b7b7b7'
for peakList in project.peakLists:
    peakList.symbolColour = black
    peakList.textColour = black
    peakList.meritThreshold = 0.5
    peakList.meritEnabled = True
    peakList.meritColour = lightGrey
```

Find the code as a Macro in the tutorial directory:

.../19F/dataset_3/macros/ColourPeakLists.py

5 Export to NEF





5E Delete Reference Spectra

On Sidebar:

- Expand the SpectrumGroup tree for References
- Select all spectra in the 'SG:references', including the SpectrumGroup 'SG:references' : right click -> **Delete**

Or run the command:

```
sg = get('SG:references')
project.deleteObjects(*list(sg.spectra)+[sg])
```

5F Export to NEF

Export metadata related to the Reference Mixtures

- Main Menu -> Project -> Export -> NEF File
- On the NEF Dialog Set checked:

  General:

  ☑ include CCPN Tags,

  Project tree:

  ☑ PeakLists

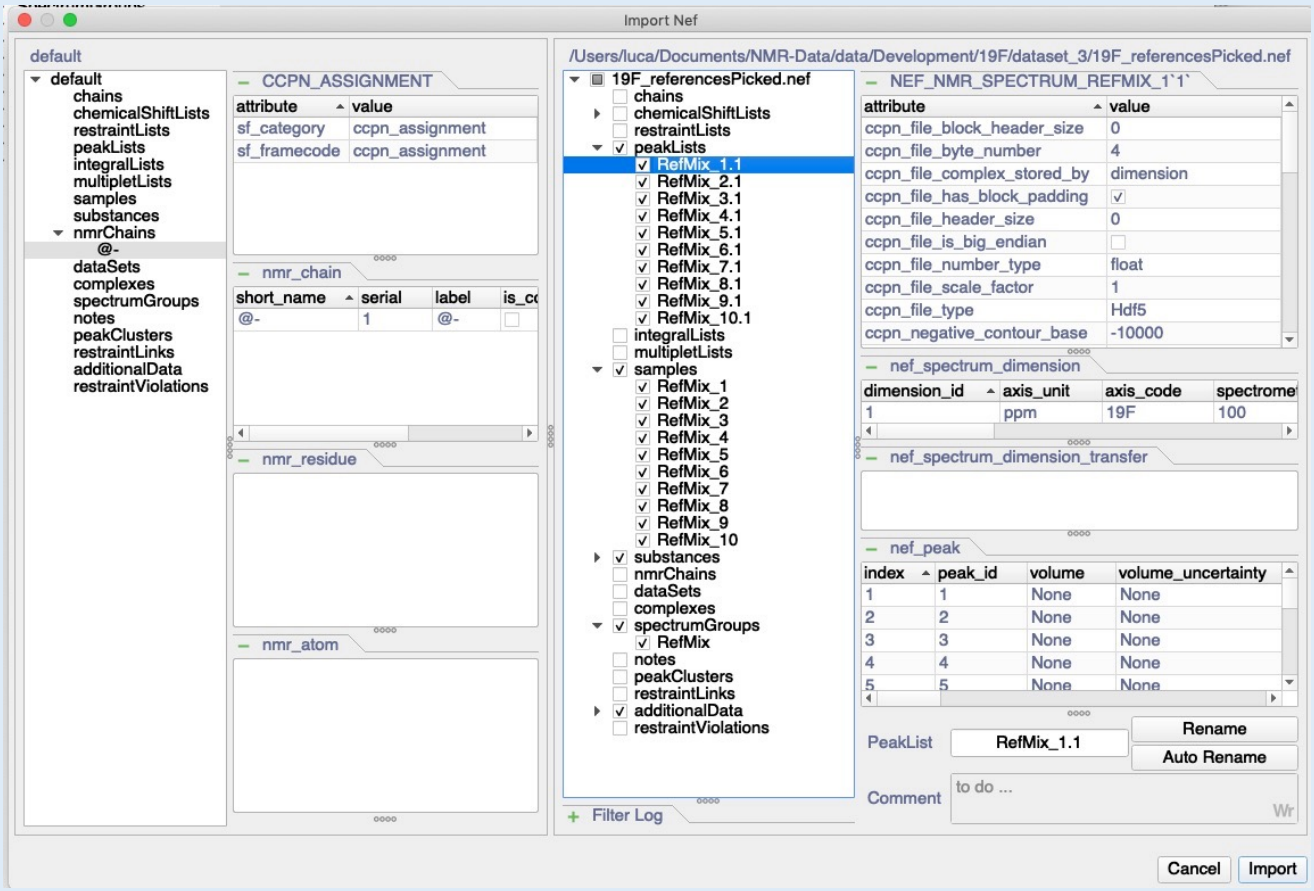
  ☑ Samples

  ☑ Substances

  ☑ SpectrumGroups

- Save to your local disk

6 Screen From NEF



6A Import From NEF

- Start a new project
- Main Menu -> Project -> Import -> NEF File
- Select the lately created NEF file or the file provided in the tutorial data directory
- On the NEF Dialog Set checked:

  Project tree:

  ☑ PeakLists

  ☑ Samples

  ☑ Substances

  ☑ SpectrumGroups

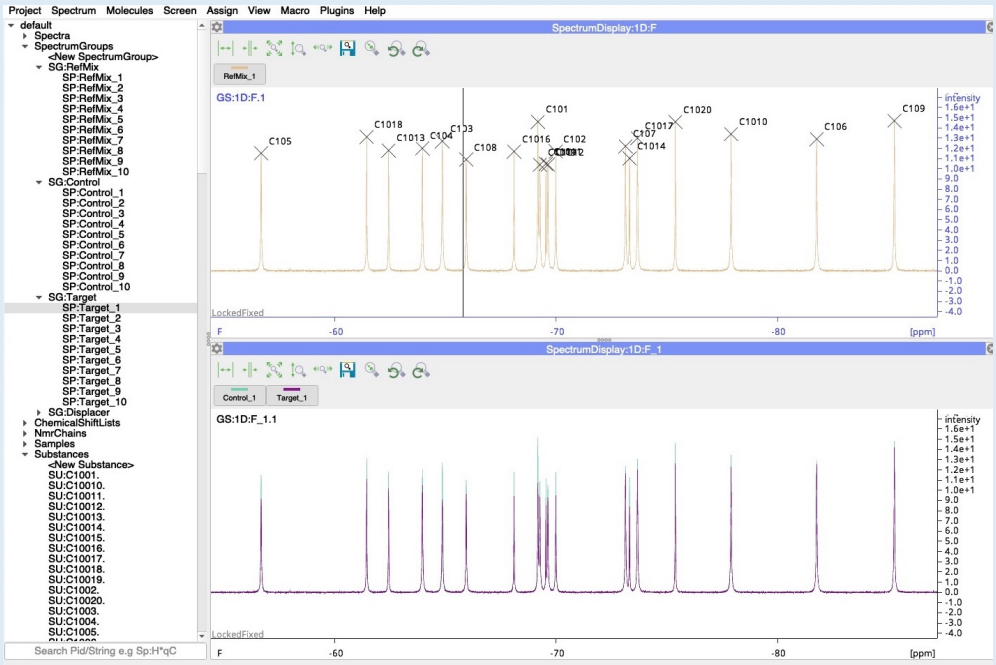
  ☑ AdditionalData
- Import

Make sure there are not name clashes

If there are empty peak lists, run on PythonConsole:

```
toDelObjs = [pl for pl in project.peakLists if not pl.peaks]
project.deleteObjects(*toDelObjs)
```

6 Screen From NEF



Sometimes your latest dataset might be slightly offset compared to the Reference Mixtures in the NEF files. In that case and before copying peaks, you could re-reference the X-axes using the pipe **Align Spectra** and the Y-axes using the pipe **Scale spectra**.

6B Import Data from Excel

Import the latest screening data from the excel file

- Load the excel file from the tutorial data folder:
  .../19F/dataset_3/Data_Time_x/lookup_19F_TimeX.xlsx

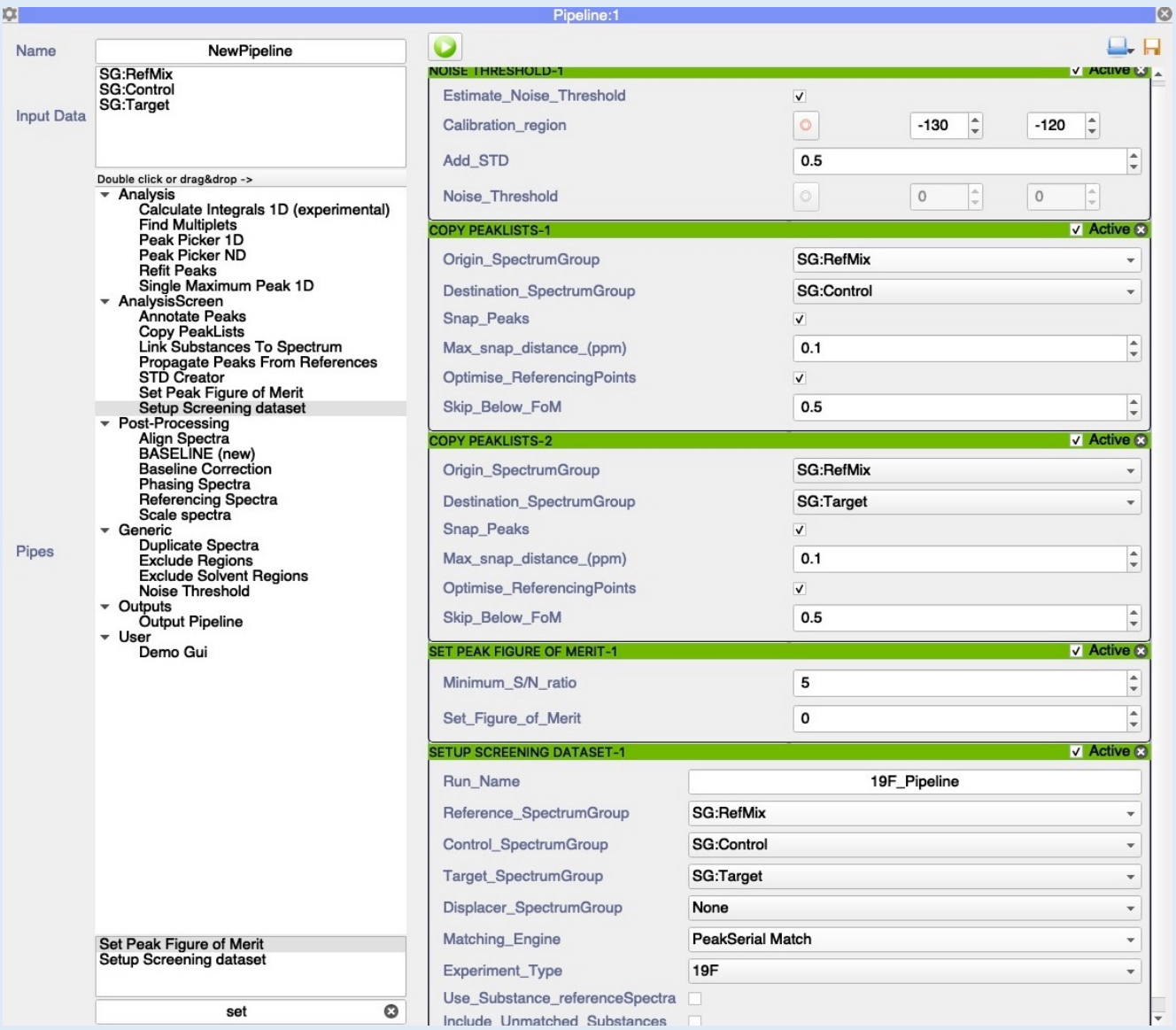
Note how this lookup only contains the Sample sheets without the SampleComponents field and the Substances sheet. This information has been carried over from the NEF file.

6C Setup screening pipeline

- Open the Pipeline (Menu -> Screen -> Pipeline or shortcut PI)
- On sidebar, multiselect 'SG:Control', 'SG:Target', 'SG:RefMix'
- drag and drop on the pipeline **Input Data**
- On the Pipes list widget search and add to the pipeline area:
  1. **Noise Threshold**
     - set Checked Estimate Noise Threshold
     - Calibration region -130, -120
  2. **Copy PeakLists (1)**
     - Origin SpectrumGroup: SG:RefMix
     - Destination SpectrumGroup: SG:Control

... Continues Next

6 Screen From NEF



6c ...*Continued*

add the following pipes

3. **Copy PeakLists (2)**
   - Origin SpectrumGroup: SG:RefMix
   - Destination SpectrumGroup: SG:Target
4. **Set Peak Figure of Merit**
   - Minimum S/N ratio: 5
   - Merit 0
5. **Setup ScreenDataset**
   - Run name: 19F_Pipeline
   - Reference SpectrumGroup: SG:RefMix
   - SG:Control, SG:Target on their respective entries
   - Matching engine: **Peak Serial Match**
   - Uncheck Use_Substance_ReferenceSpectra

- Run the pipeline
- Check results on the Hit Analysis Module as in Section 4

Screen From NEF

#	Substance Name	Substance Binding Score	Substance Displacement Score	Substance Matching S
80	C409	0.3002	0.0996	5
58	C307	0.2957	0.1009	5
62	C4010	0.2955	0.3224	5
53	C3020	0.2955	0.1354	5
50	C3018	0.2943	0.0508	5
151	C8019	0.2940	0.2890	5
118	C607	0.2940	0.3218	5
200	C1009	0.2931	0.2555	5
1	C101	0.2903	0.2354	5

Substance Name	Peak Position Reference	Peak Position Control	Peak Position Target	Peak Position Displacer	Peak Binding Score	Peak Displacement Sco
C409	-54.1434	-54.1434	-54.1434	-54.1434	0.3002	0.0996

6D Completed Project

An example of a completed project is available in the dataset_3 directory: .../19F/dataset_3/19F_NEF_Completed.ccpn

Hit Analysis Module

On selection of Substance or Sample items from the main tables, you may notice how the Singleton Reference Spectra are replaced by the Reference Mixture spectra. The peak annotations will now provide a visual reference to the matching Substance.

Contact Us

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Issues and bug reports:
https://www.ccpn.ac.uk/forums

Cite Us

Mureddu, L. et al. CcpNmr AnalysisScreen, a new software programme with dedicated automated analysis tools for fragment-based drug discovery by NMR. J. Biomol. NMR (2020)

Skinner, S. P. et al. CcpNmr AnalysisAssign: a flexible platform for integrated NMR analysis. J. Biomol. NMR 66, (2016)