UNIVERSITY OF LIVERPOOL

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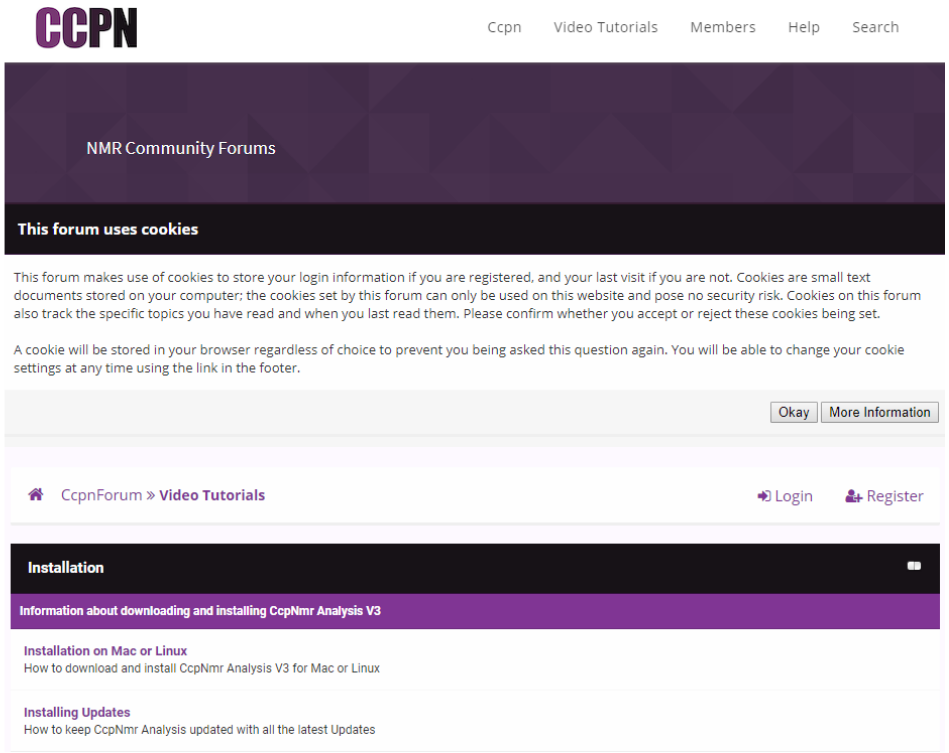
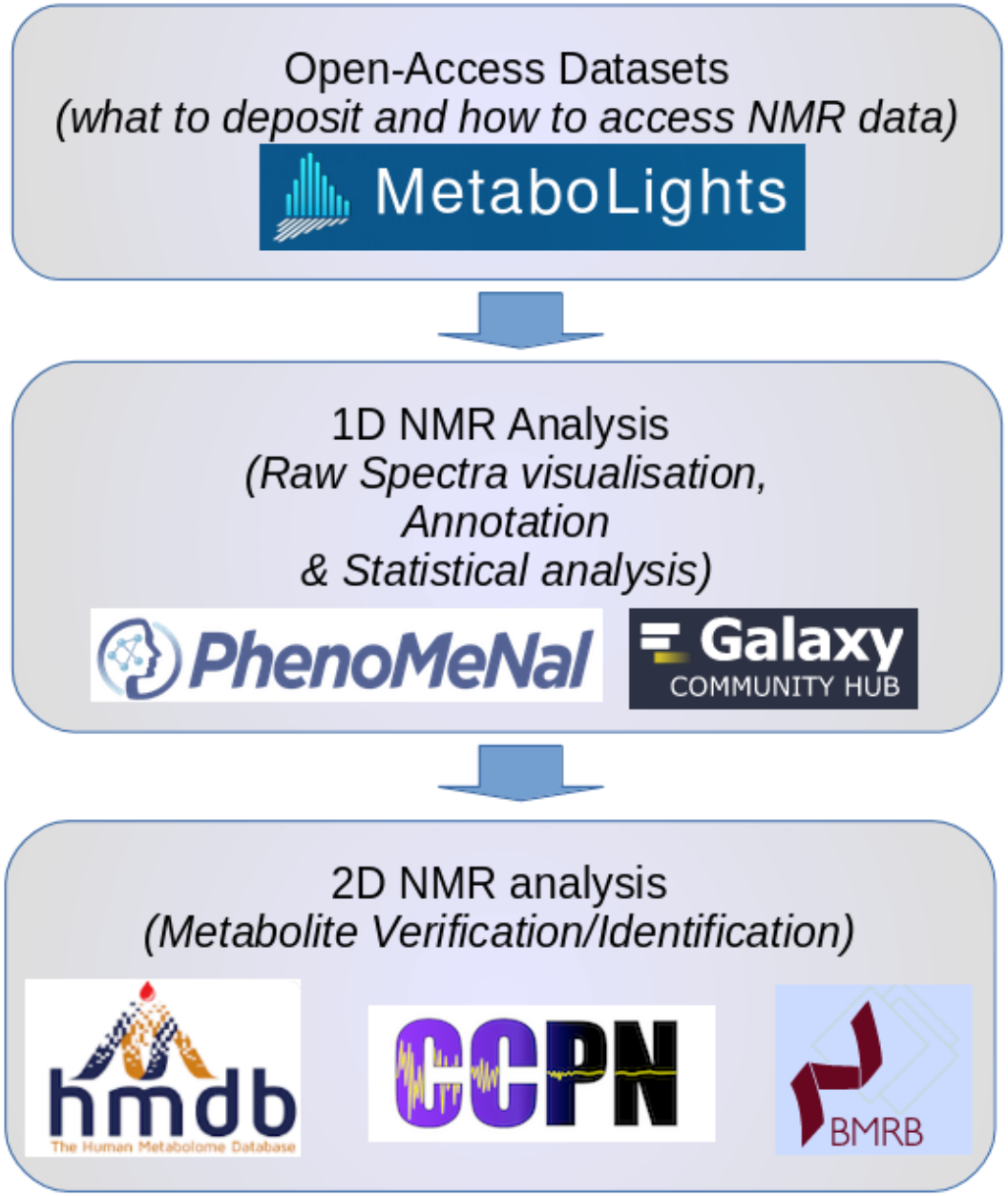
NMR Metabolomics via CCPN: Integrating 1D and 2D NMR from repositories and standard libraries

https://www.ccpn.ac.uk
https://www.ccpn.ac.uk/forums



CCPN

Workshop Structure



Section 3. Analysing 2D NMR via Collaborative Computing Project for NMR (CCPN). This includes download of 2D NMR standards from BMRB (Biomolecular Resonance Bank)/ HMDB and overlay with CCPN metabolite library.

How do we verify metabolites?

2D NMR
- Homonuclear
- Heteronuclear

Spiking samples

Orthologous method:
- Mass Spectrometry
- Infrared Spectroscopy

Heteronuclear Two-Dimensional NMR

Advantages:

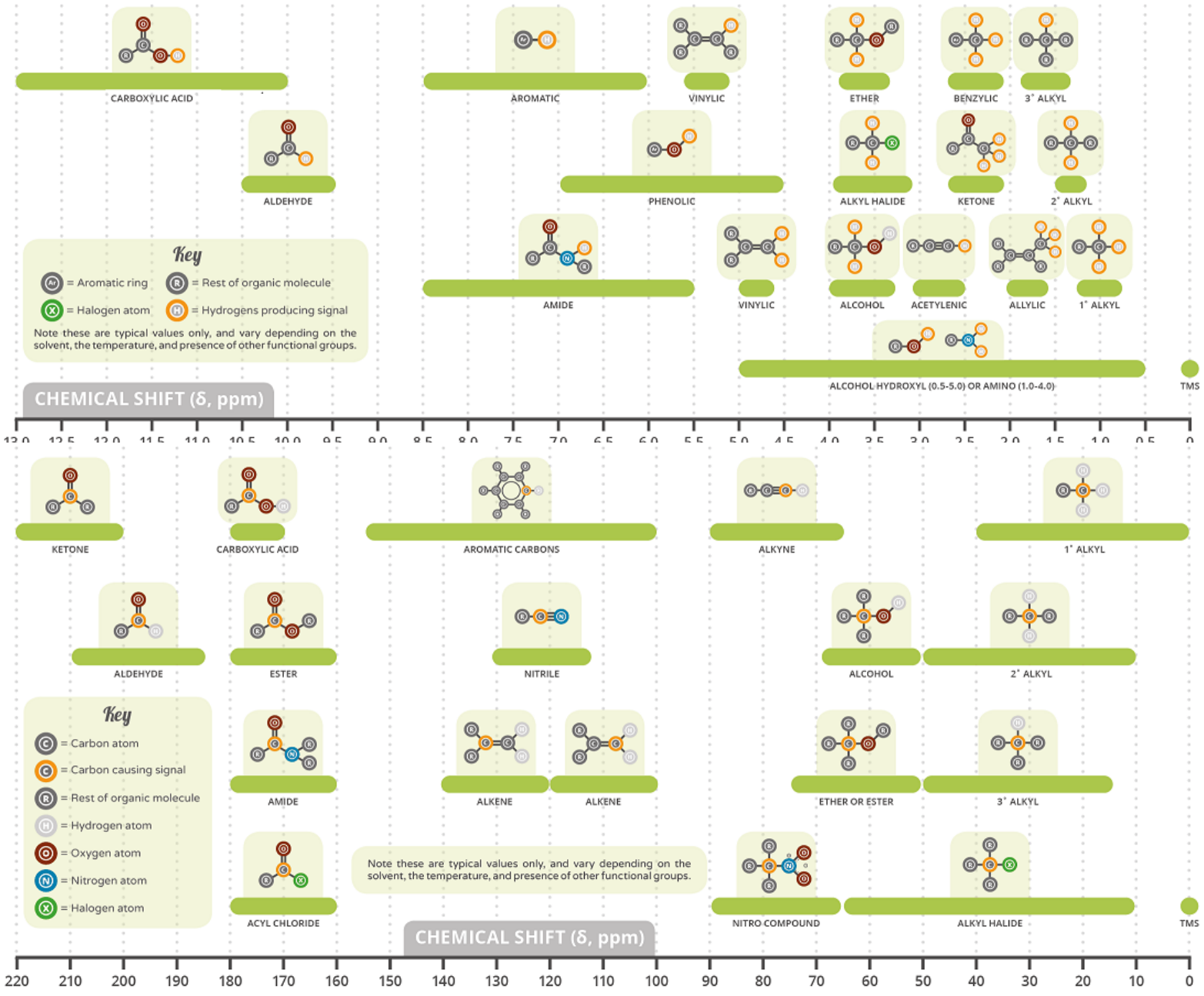
- Additional Dimension – reduces Ambiguity
- Decoupling reduces complexity
- 13C chemical shift range more informative than 1H
- 13C can be used to monitor metabolic flux

Limitations:

- Sensitivity Reduced
  - (13C only 1.1% natural abundance)
- Increased time (collect multiple spectra to build up 2D)
- Less standard metabolite spectra available
- Does not integrate easily metabolomics tools

Chignola *et al* The CCPN Metabolomics Project: a fast protocol for metabolite identification by 2D-NMR. *Bioinformatics*. **2011** Jan 6; 27(6): 885-886

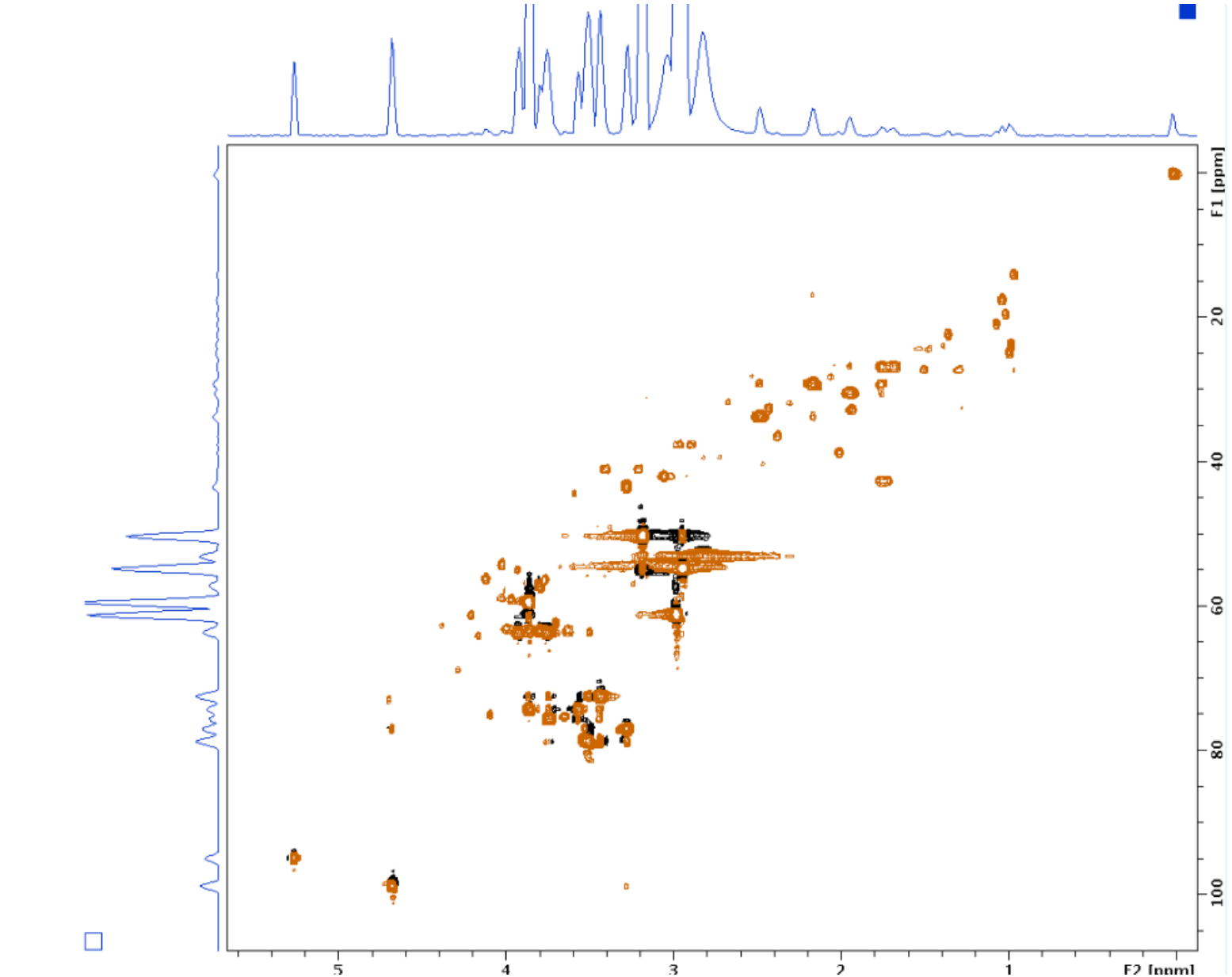
Heteronuclear Two-Dimensional NMR



1H NMR chemical shift range 0-12ppm

13C NMR chemical shift range 0-250ppm

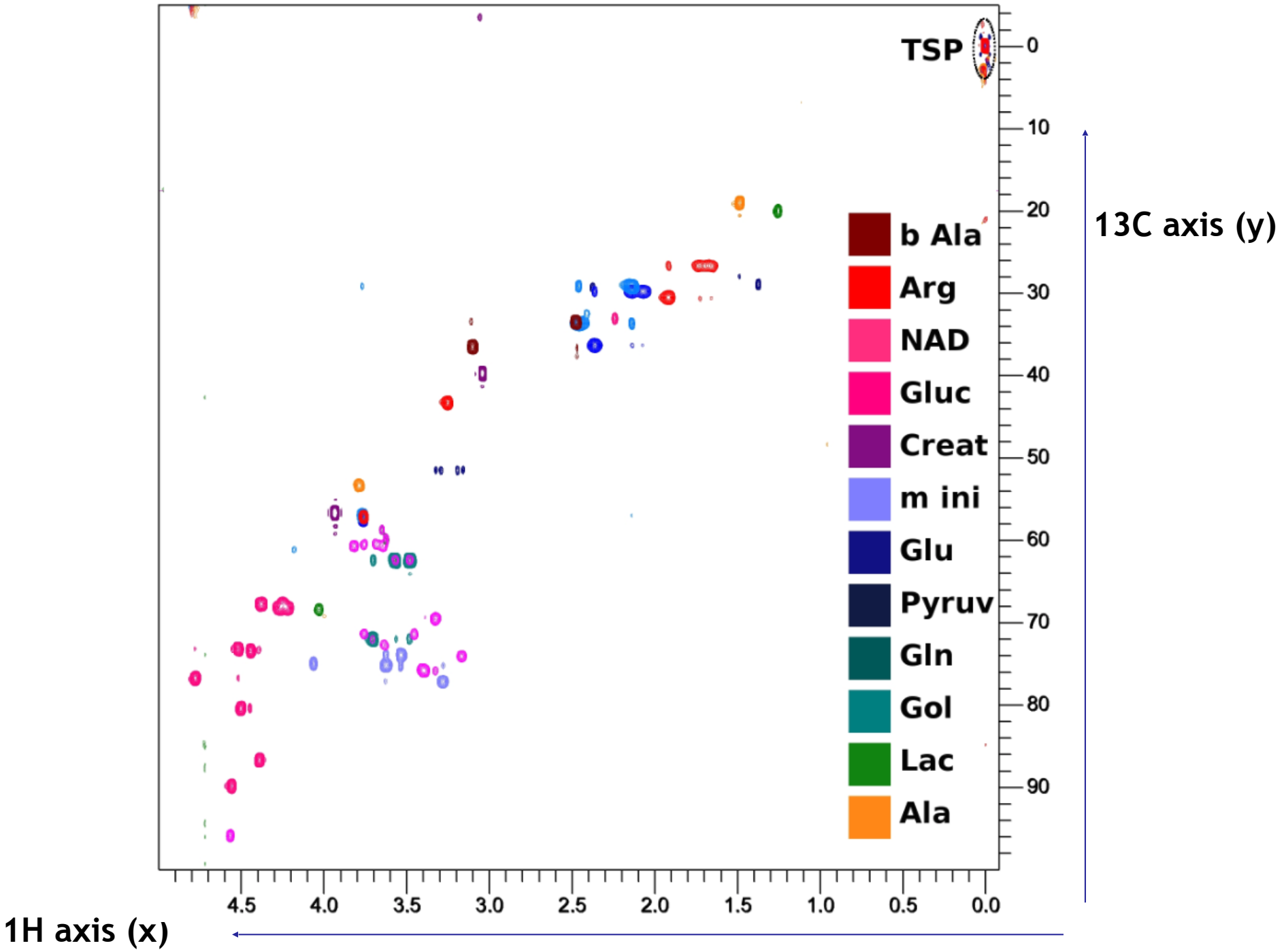
How do we verify metabolites?



13C axis (y)

1H axis (x)

How do we verify metabolites?



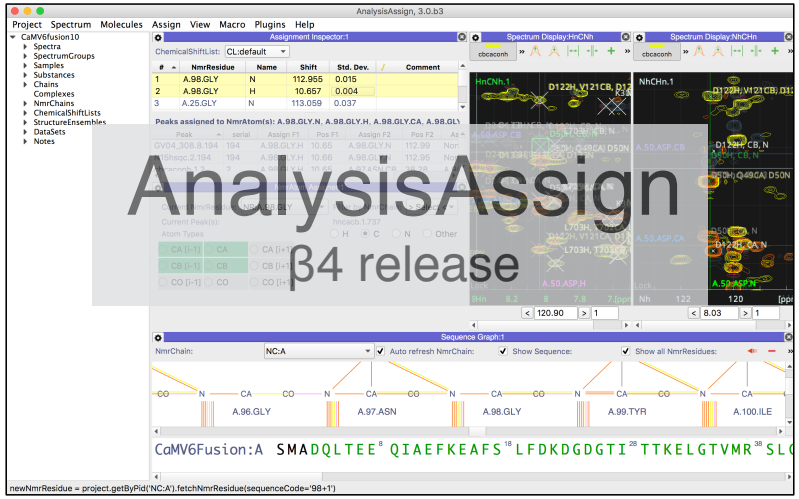
CcpNmr Analysis version 3 software

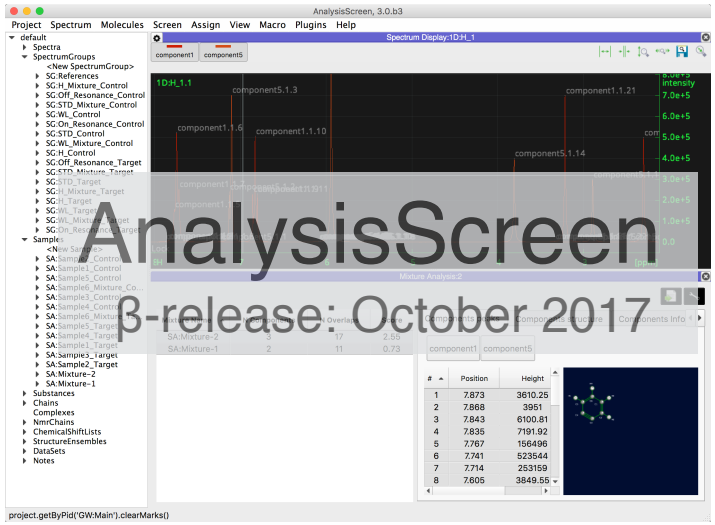
- Collaborative Computational Project for NMR (CCPN): exists since 2000
- MRC funded partnership (28 partners) network (2013-2016, 2016-2019)

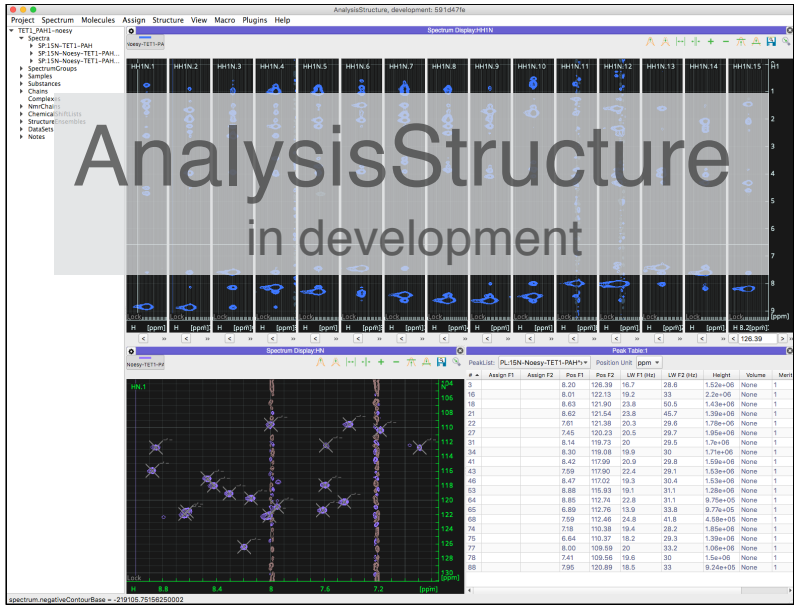
- Support for software development, promoting best practices, knowledge sharing and collaboration

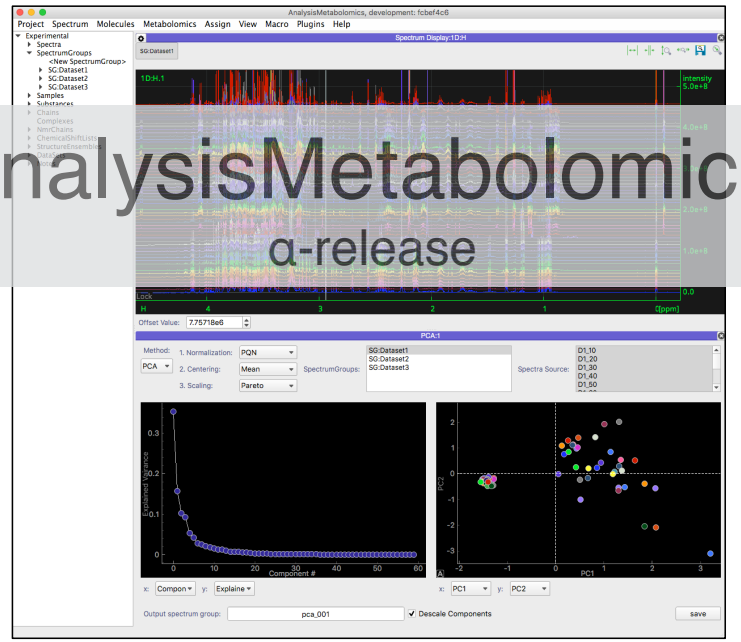
CcpNmr Analysis version 3 software

Suite of '4' (+1) programs:









CcpNmr AnalysisAssign / MetaBolomics

Interface for nD assignment and data analysis:

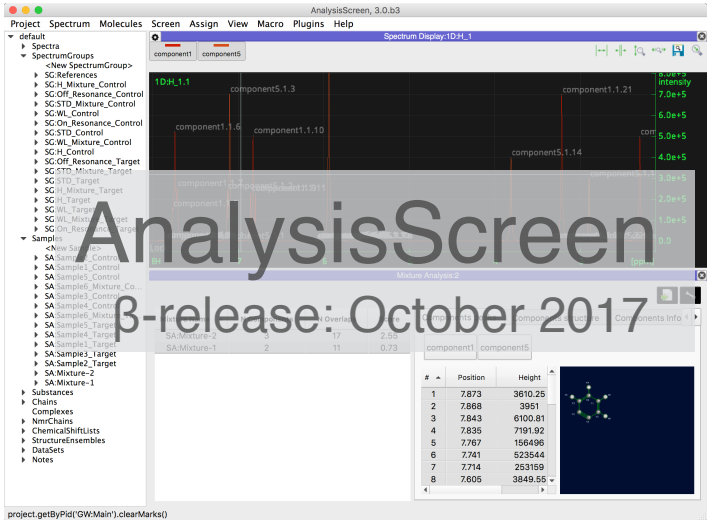
- Drag and drop
- Adaptable and Customisable
- Flexible (easy python macro's)
- Scientific (e.g. Numpy, Scipy libraries)

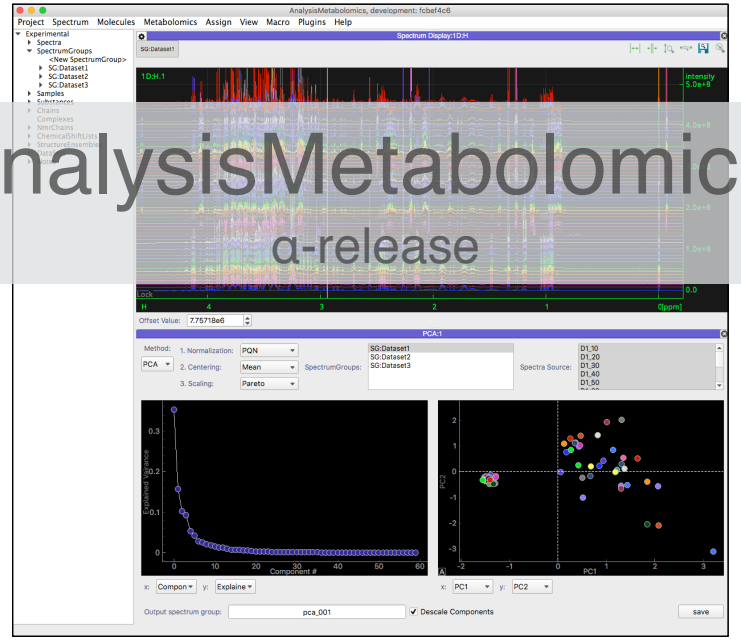
- Fully V2 compatible (automatic conversion)
- Fully NEF compatible (import/export)
- Sparky importer

- SVG/PDF exporter for high quality plots

CcpNmr version 3 software

- AnalysisScreen: interface for NMR-based small-molecule screening
- AnalysisMetabolomics: interface for NMR-based metabolomics
- Extensive 1D capabilities (and nD of course) → also suitable for other small molecule NMR applications!
- Internal analysis/processing routines that function as ‘pipes’
- Ability for complex operations, such as PCA and other multi-variate methods





CCPN Assign Demonstration

Lets visualise some 2D spectra from the metabolights repository (MTBLS626) using CCPN analysis

→ go to www.ebi.ac.uk/metabolights/MTBLS626

→ check study protocols tab (are there 2D spectra described in the NMR assay)
→ yes!

→ go to files tab (download m_ a_ i_ s_ files as described in part 1 in order to check what spectra to download)

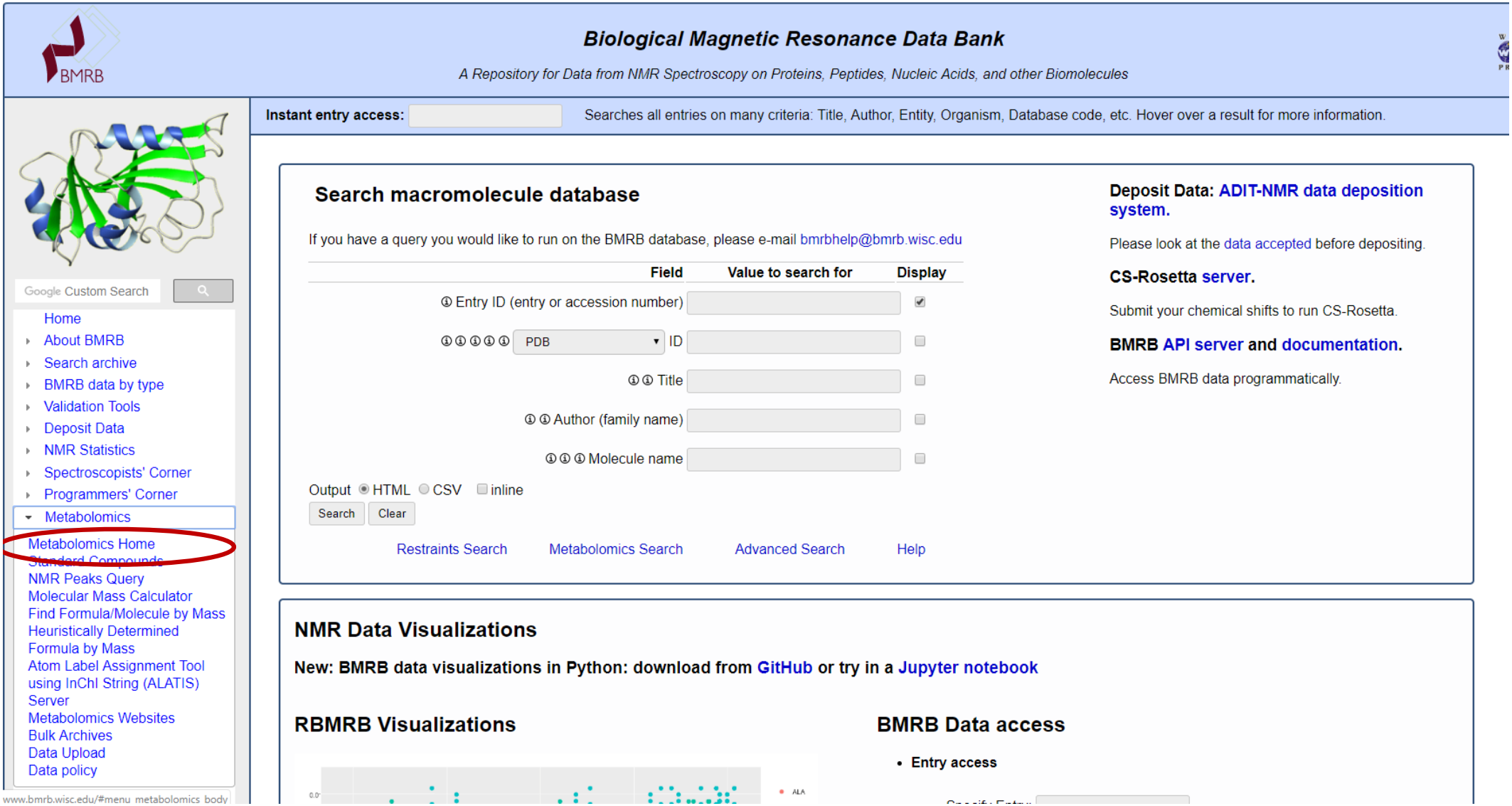
→ we selected:

→ download to zip

→ use CCPN assign to visualise spectra

http://www.bmrb.wisc.edu/

BMRB



CCPN Assign Demonstration

1 – getting started & Importing Spectra

2 – creating a stacked plot (optional)

2a – rename via python

3 -printing pdf (optional)

4 – pipeline processing (optional)

5 – PCA (optional)

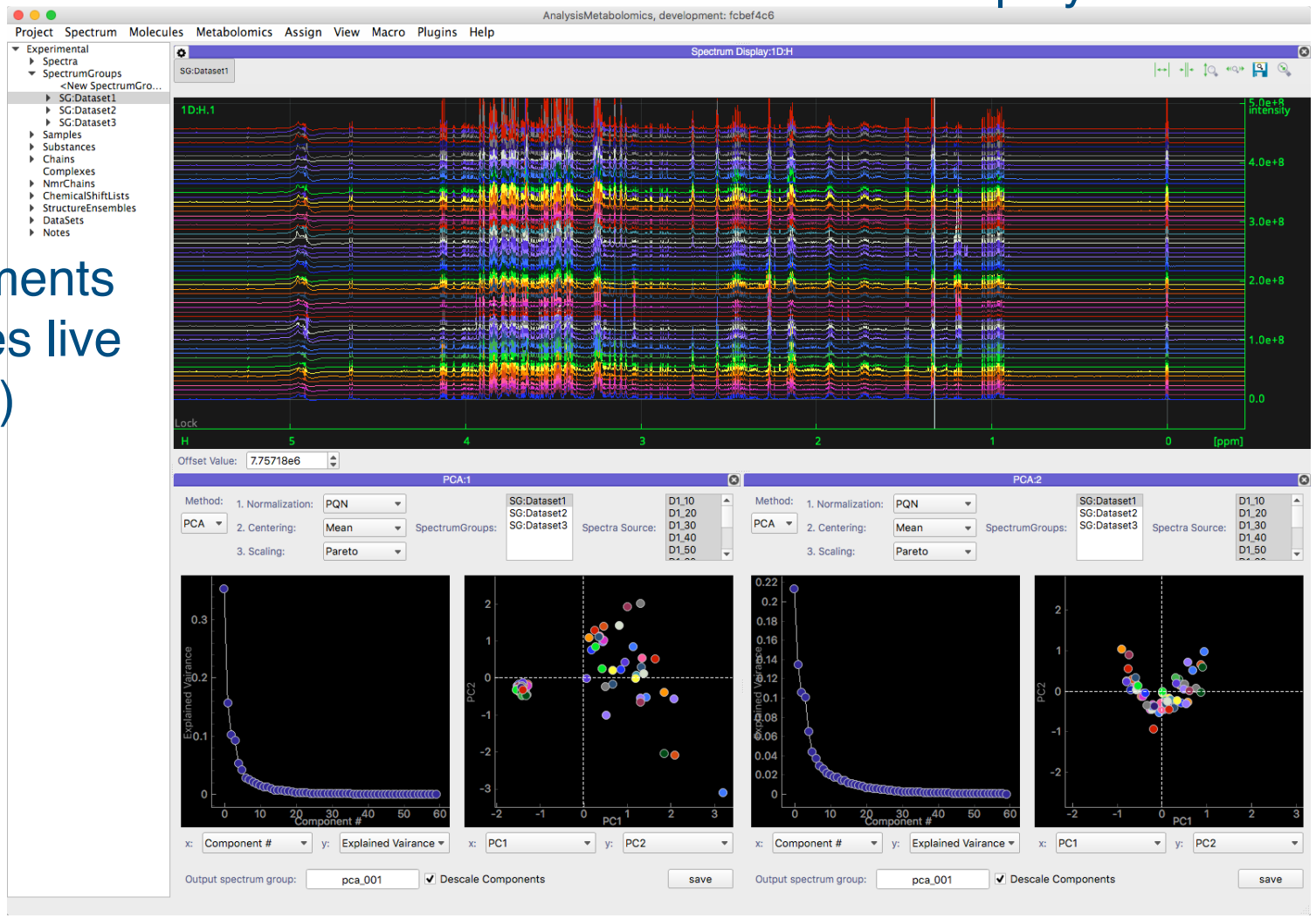
CcpNMR (CCPN) Tutorials:
https://youtube.com/channel/UCKyPsbC0mgaXAn03Owah50w

CcpNmr AnalysisMetabolomics

‘Modules’ contain the functionalities to display data

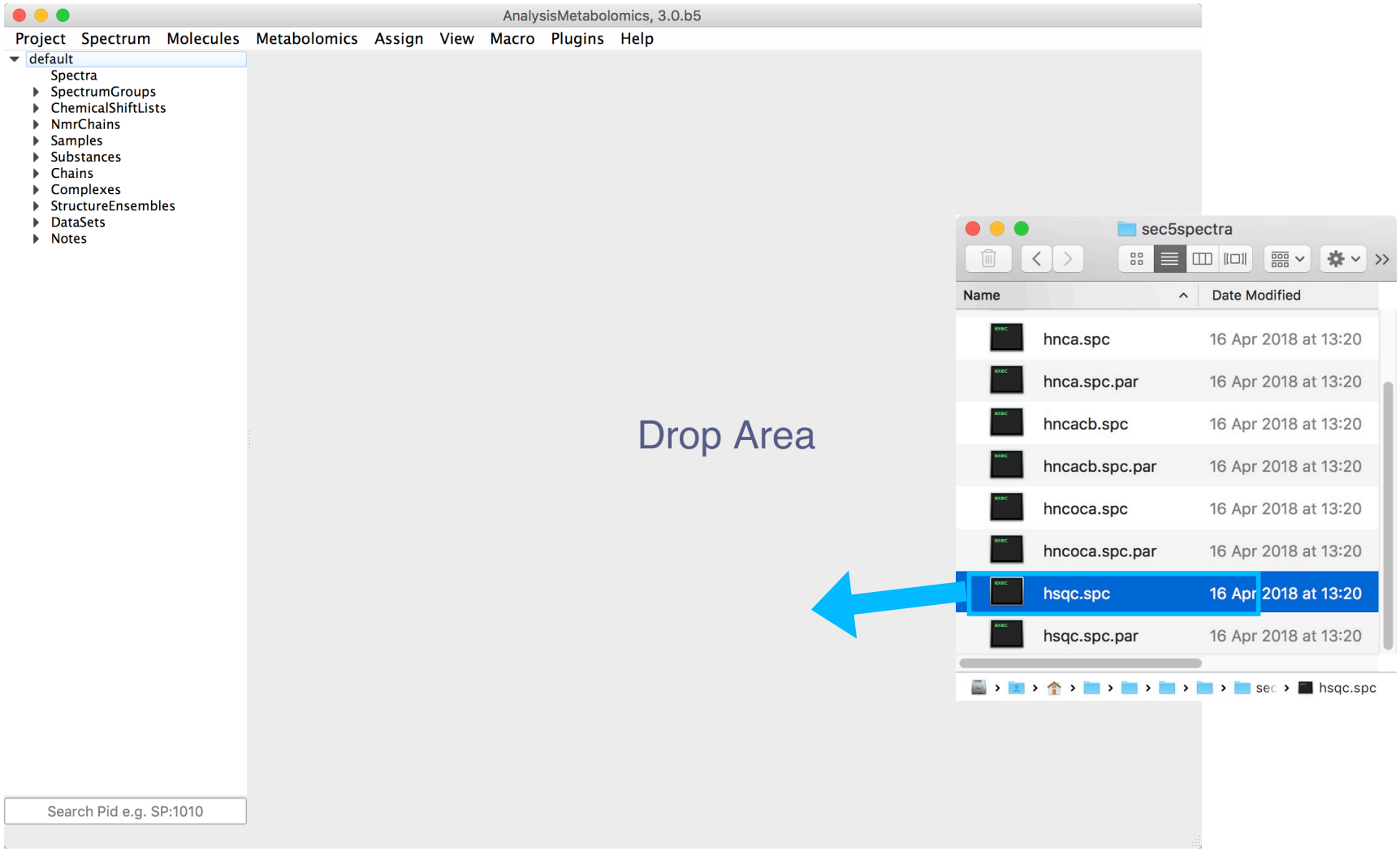
display ‘Modules’ (spectra)

data elements (properties live here)

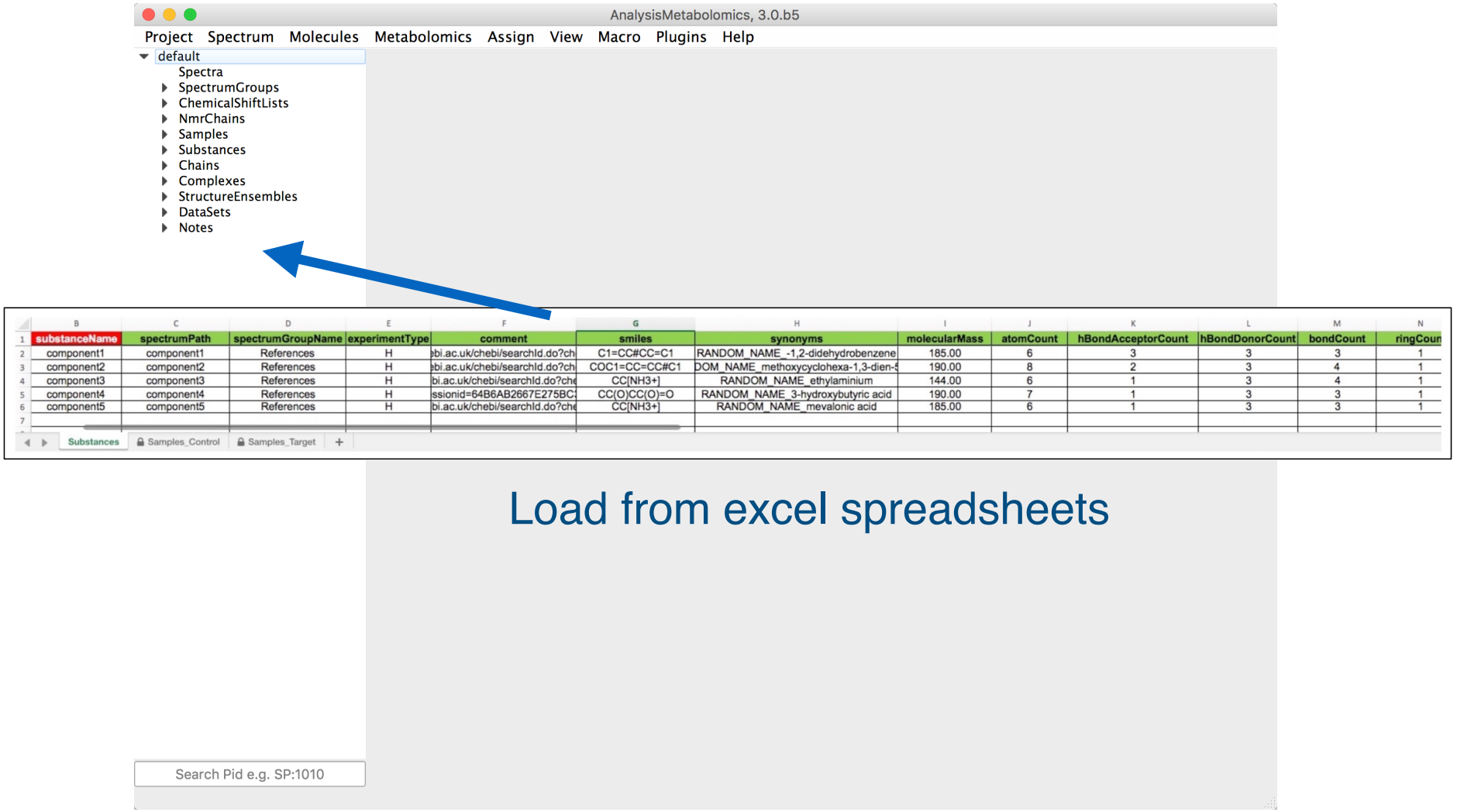


PCA

CcpNmr AnalysisMetabolomics



CcpNmr AnalysisMetabolomics

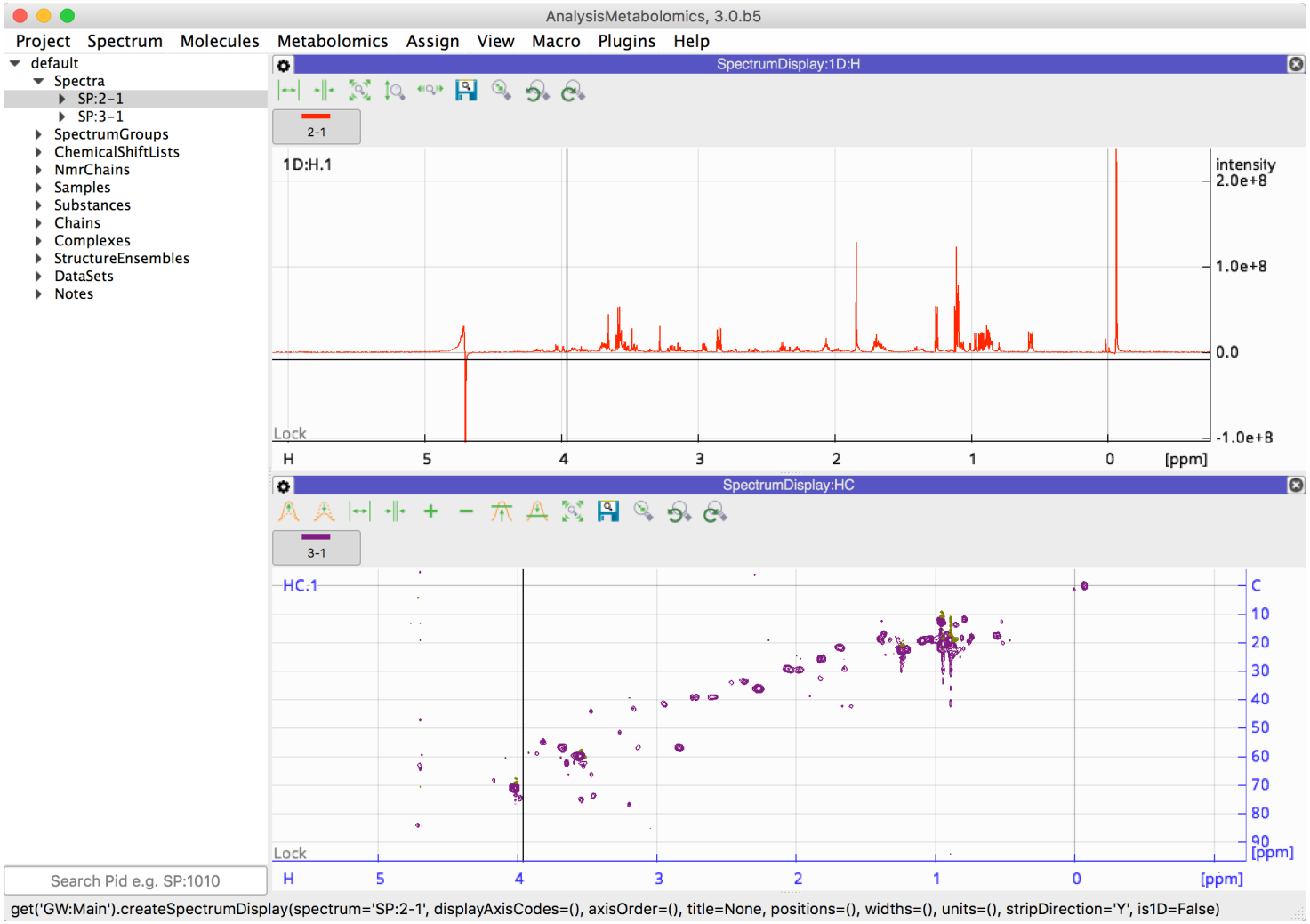


substanceName	spectrumPath	spectrumGroupName	experimentType	comment	smiles	synonyms	molecularMass	atomCount	hBondAcceptorCount	hBondDonorCount	bondCount	ringCoun
component1	component1	References	H	bi.ac.uk/chebi/searchId.do?ch	C1=CC#CC=C1	RANDOM_NAME_-1,2-didehydrobenzene	185.00	6	3	3	3	1
component2	component2	References	H	bi.ac.uk/chebi/searchId.do?ch	COC1=CC=CC#C1	DOM_NAME_methoxycyclohexa-1,3-dien-	190.00	8	2	3	4	1
component3	component3	References	H	bi.ac.uk/chebi/searchId.do?che	CC[NH3+]	RANDOM_NAME_ethylaminium	144.00	6	1	3	4	1
component4	component4	References	H	ssionid=64B6AB2667E275BC	CC(O)CC(O)=O	RANDOM_NAME_3-hydroxybutyric acid	190.00	7	1	3	3	1
component5	component5	References	H	bi.ac.uk/chebi/searchId.do?che	CC[NH3+]	RANDOM_NAME_mevalonic acid	185.00	6	1	3	3	1

Substances | Samples_Control | Samples_Target

Load from excel spreadsheets

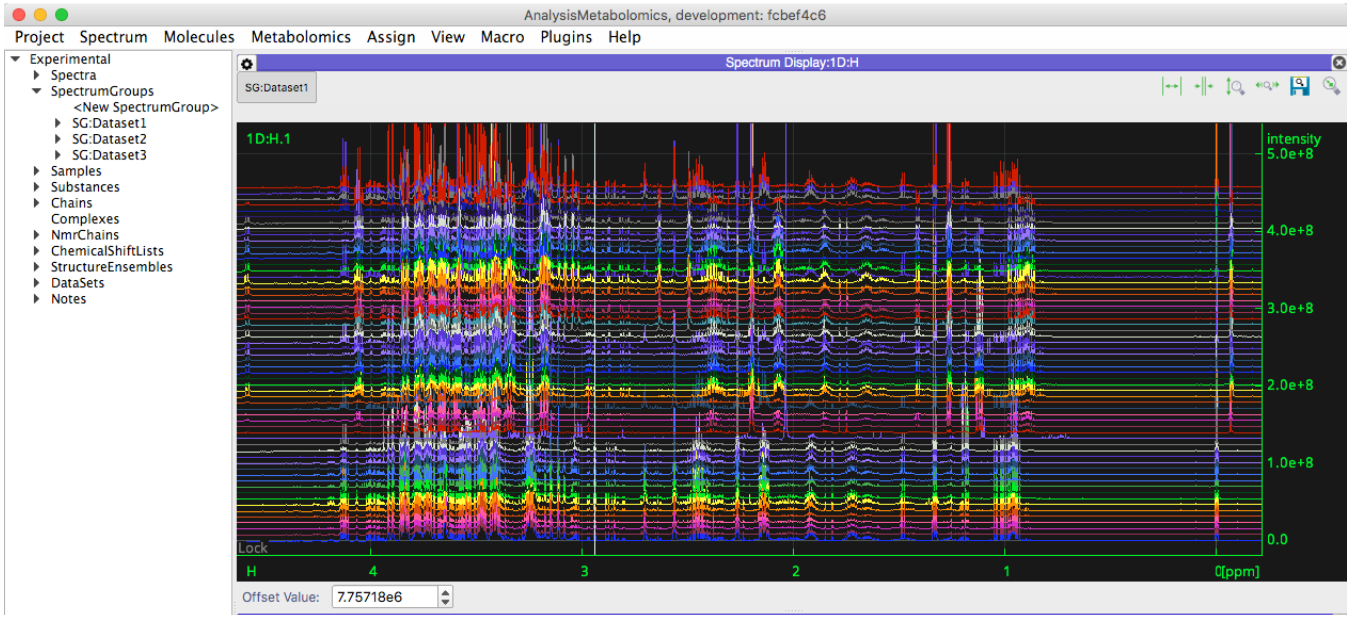
CcpNmr AnalysisMetabolomics



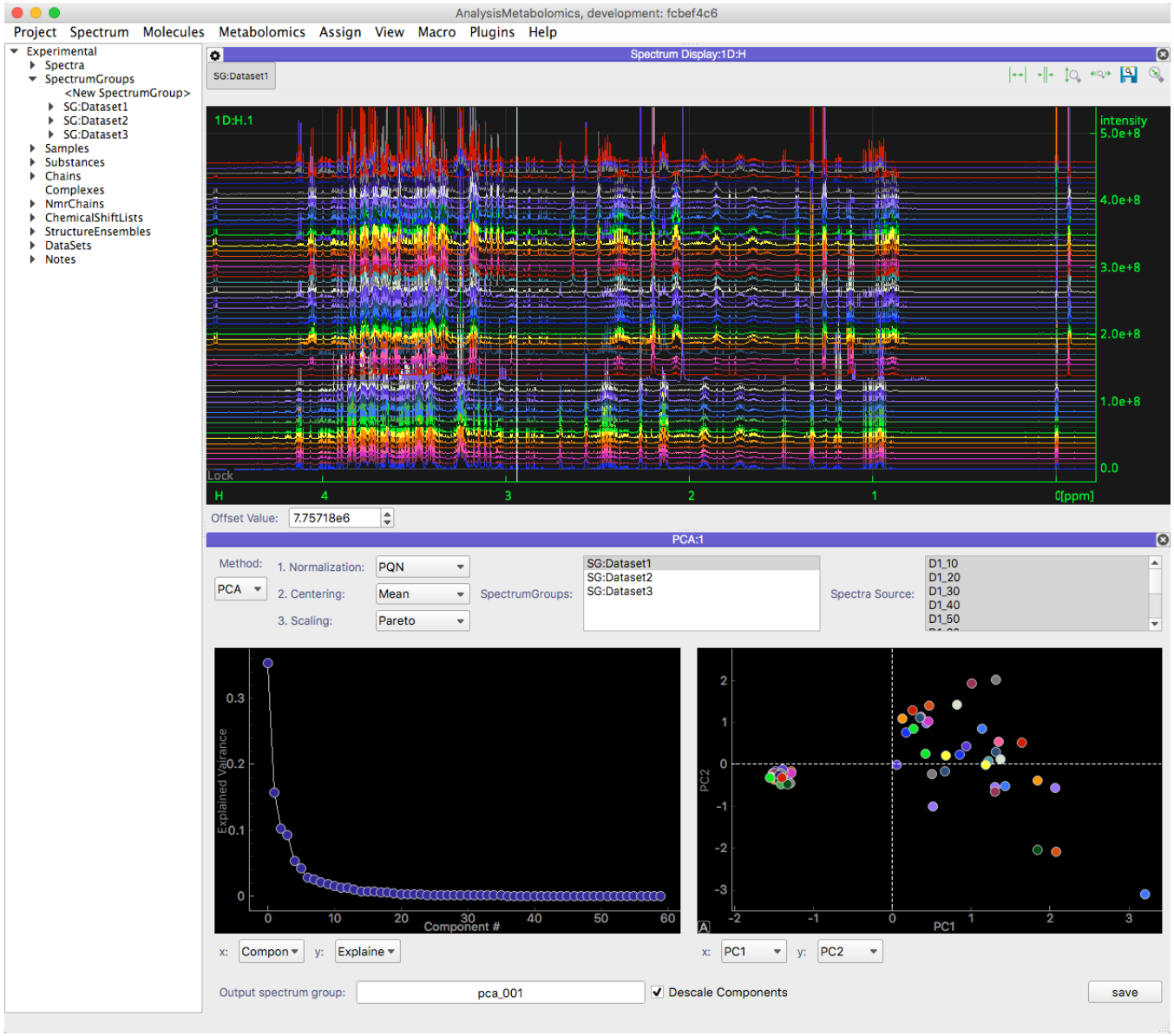
1D's

2D (nD)

CcpNmr AnalysisMetabolomics



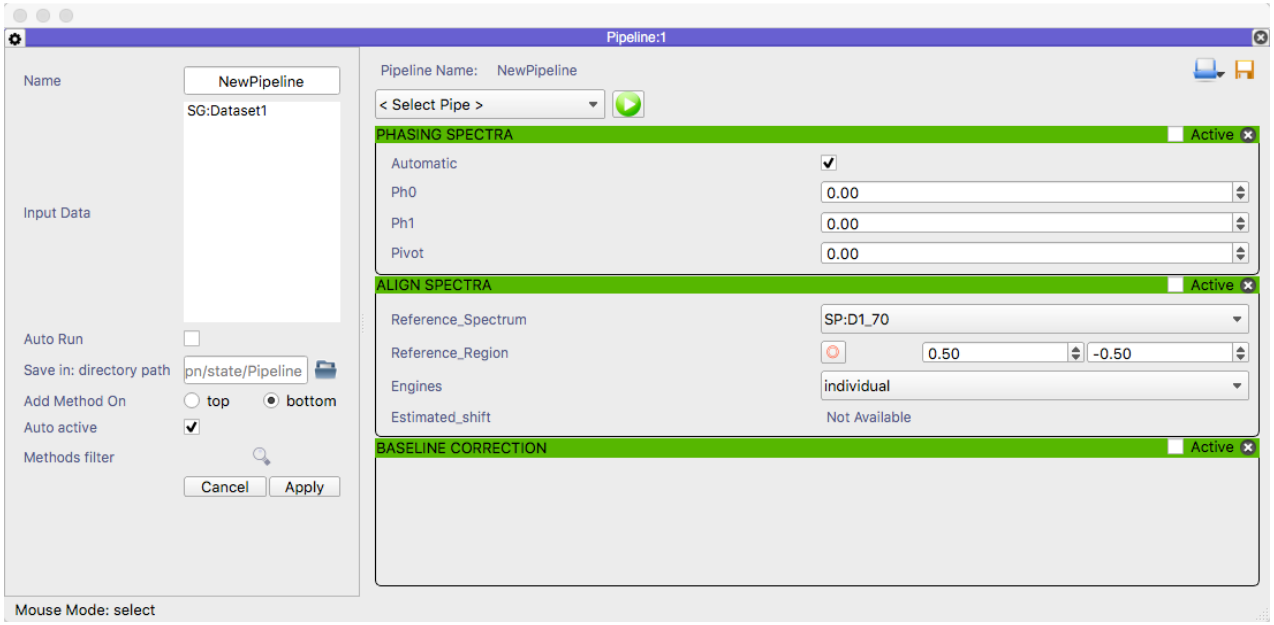
CcpNmr AnalysisMetabolomics



PCA

CcpNmr AnalysisMetabolomics

Processing pipes:
flexible way for data processing and analysis

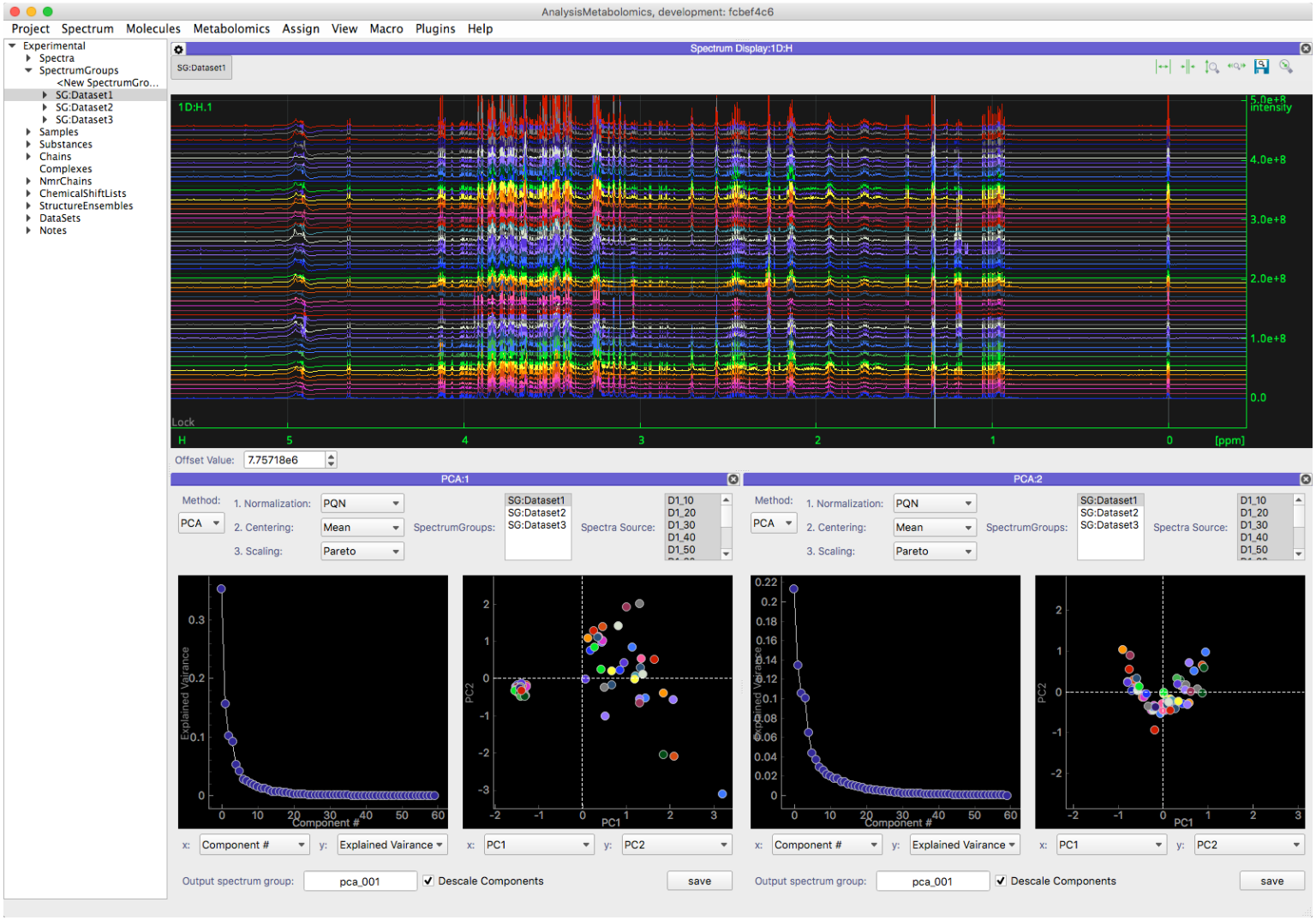


Automatic phasing

Aligning spectra

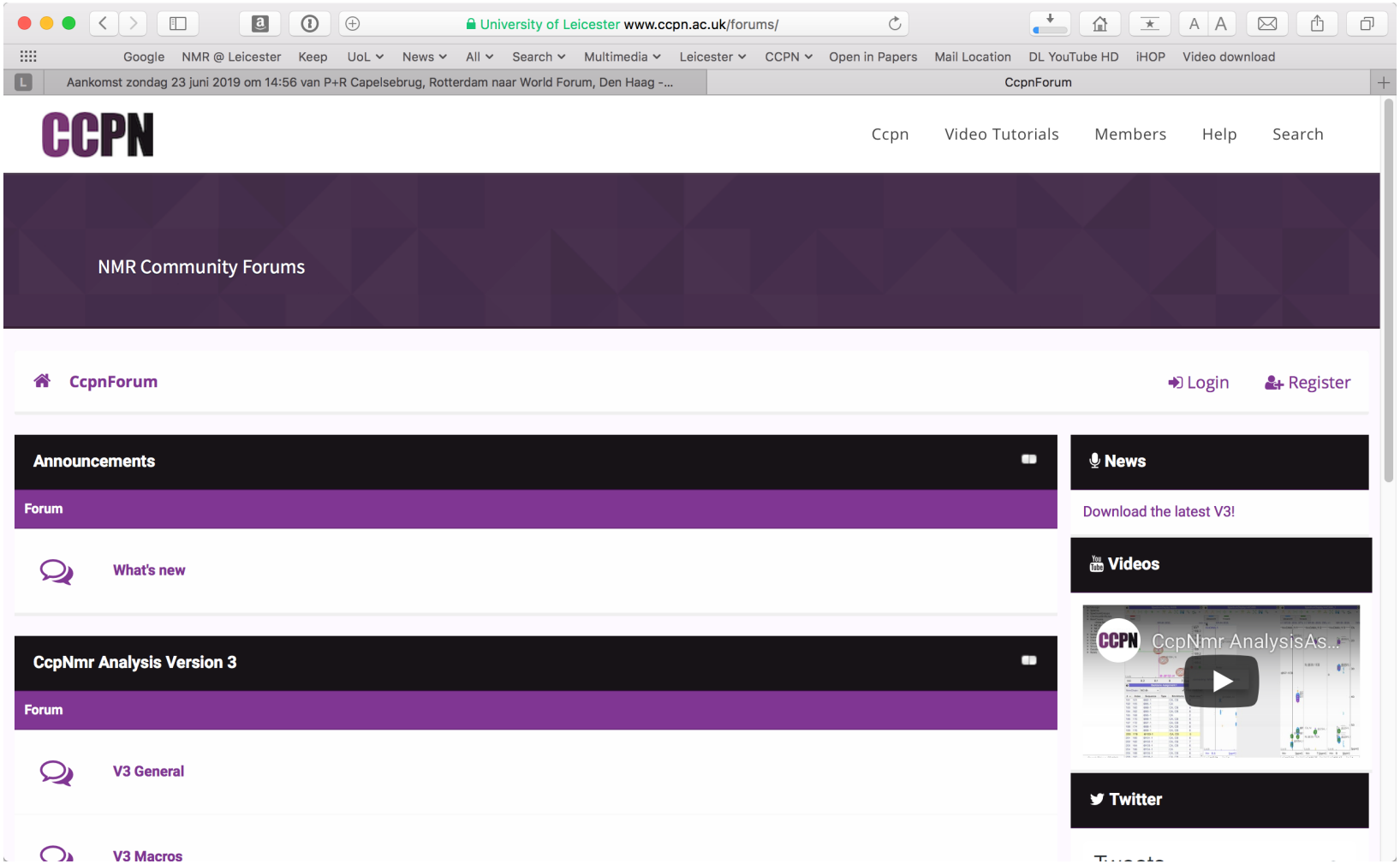
Baseline correction

CcpNmr AnalysisMetabolomics



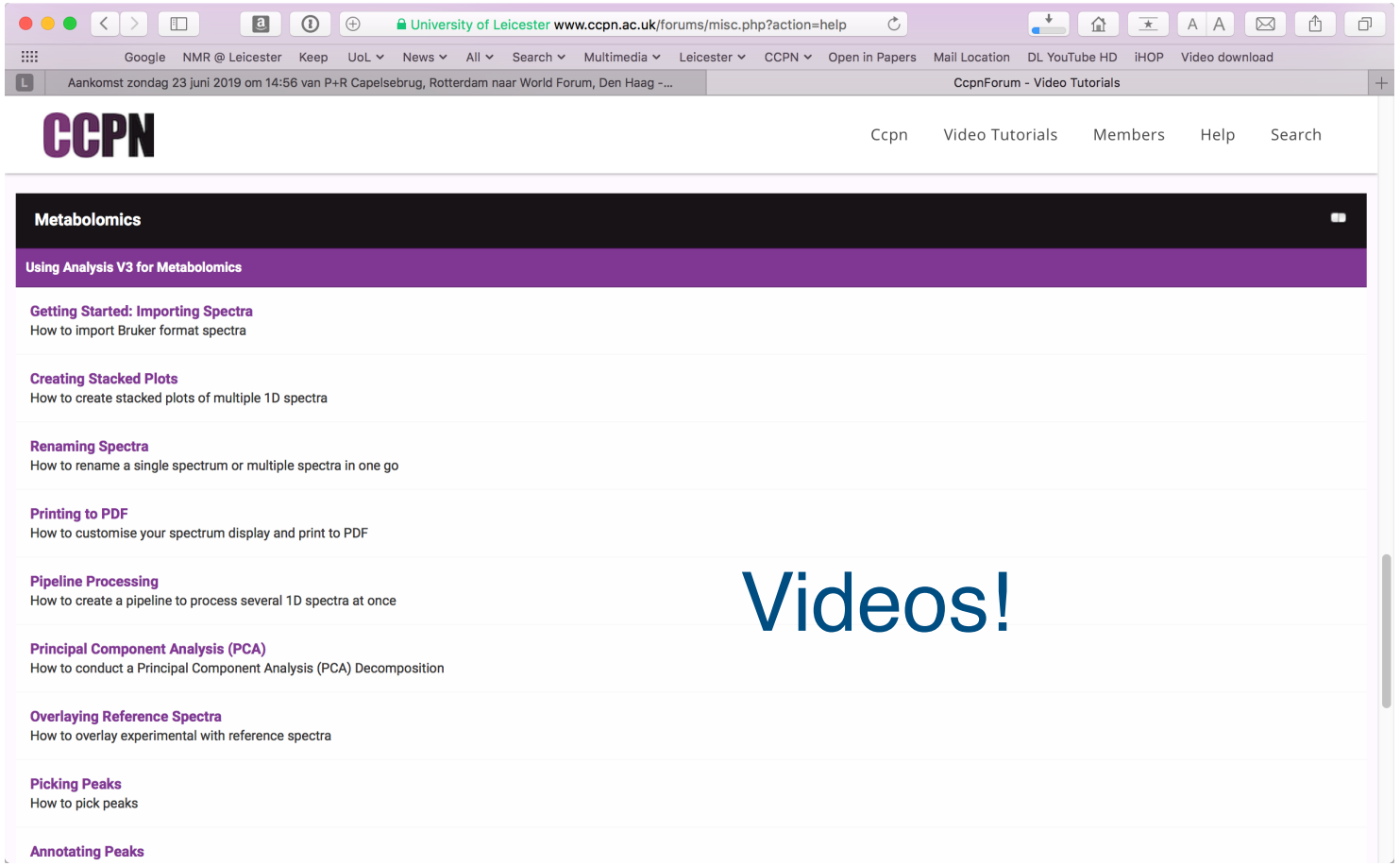
PCA (before) PCA (after)

CcpNmr forum



www.ccpn.ac.uk/forums

CcpNmr forum



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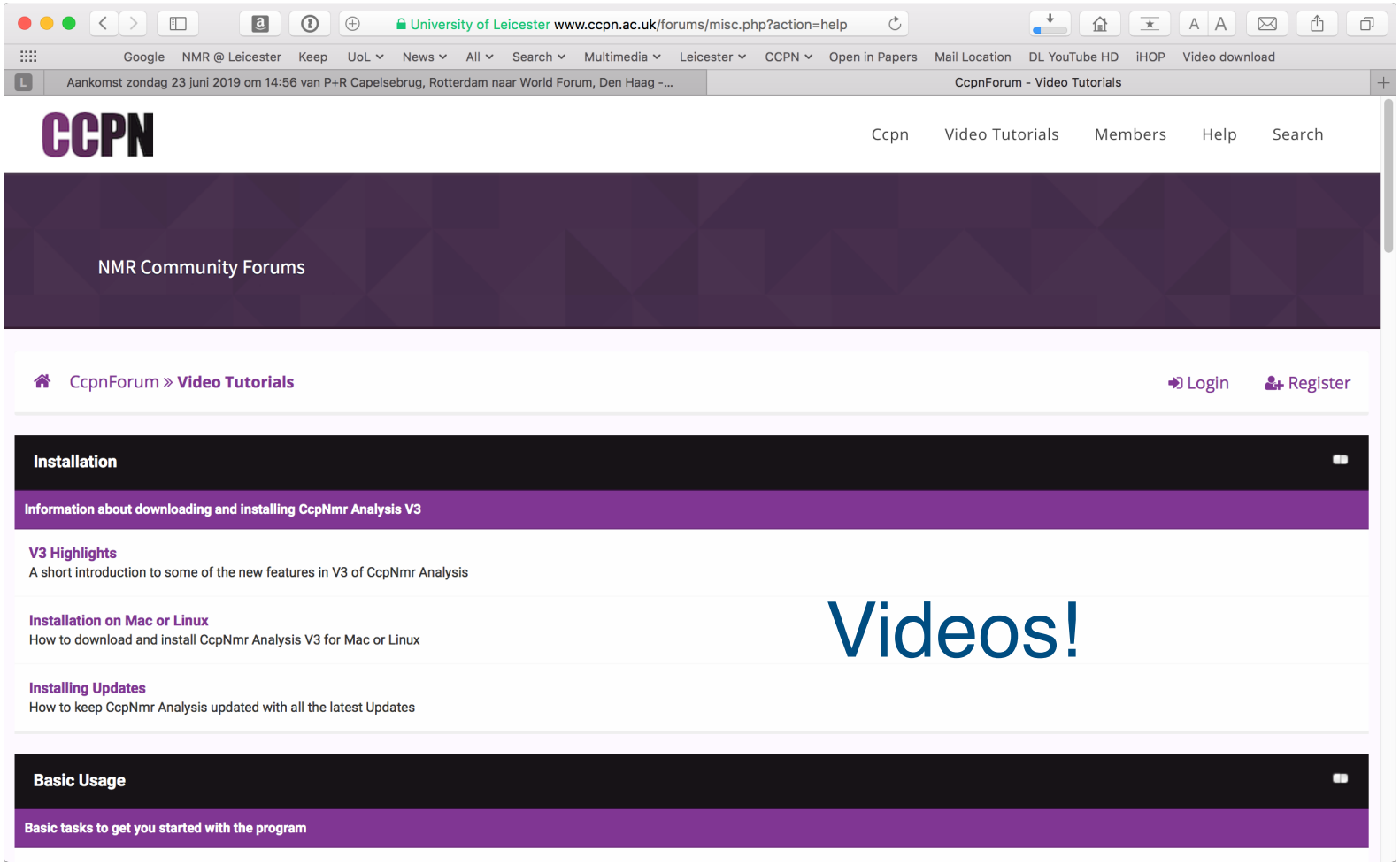
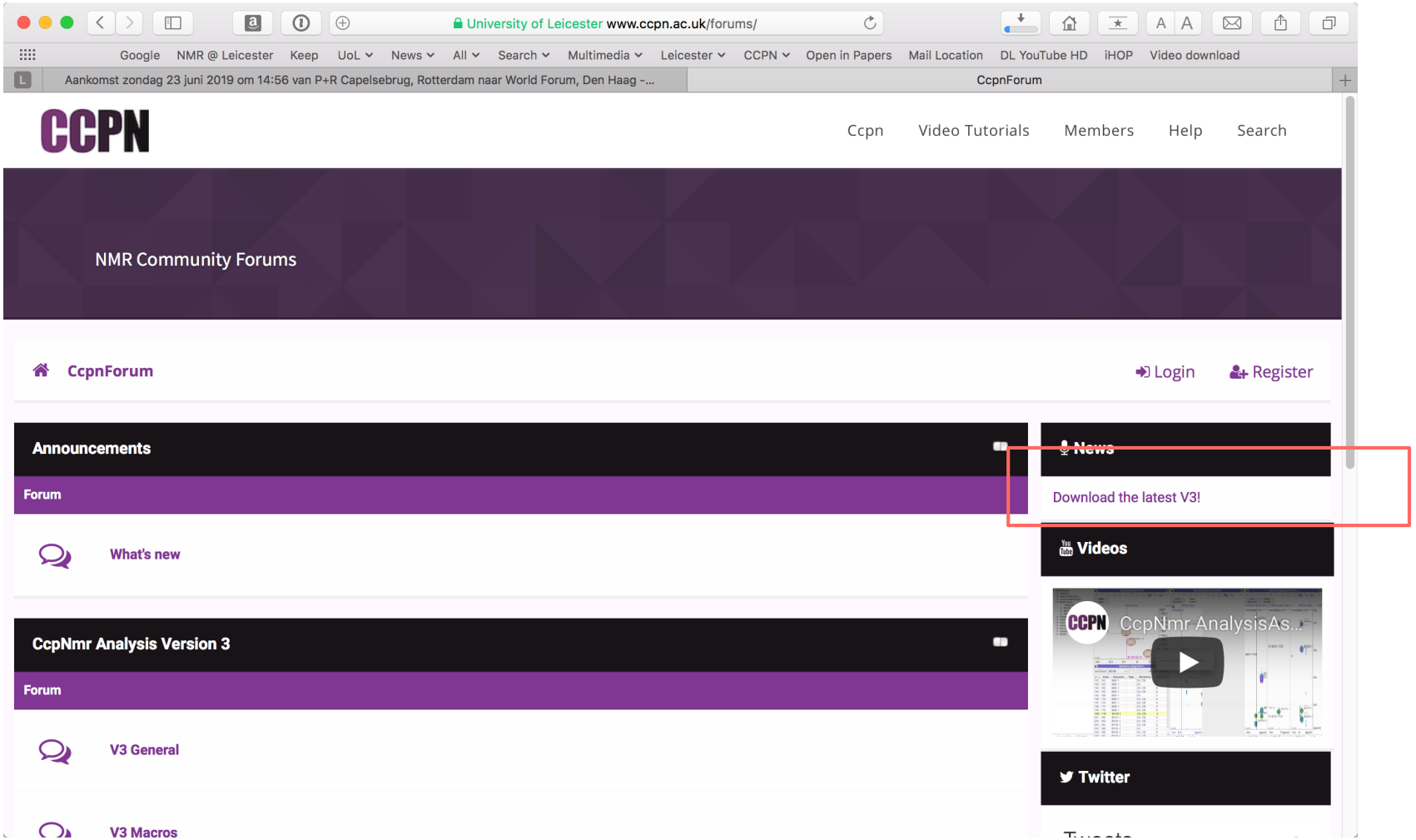
CcpNmr forum



Videos!

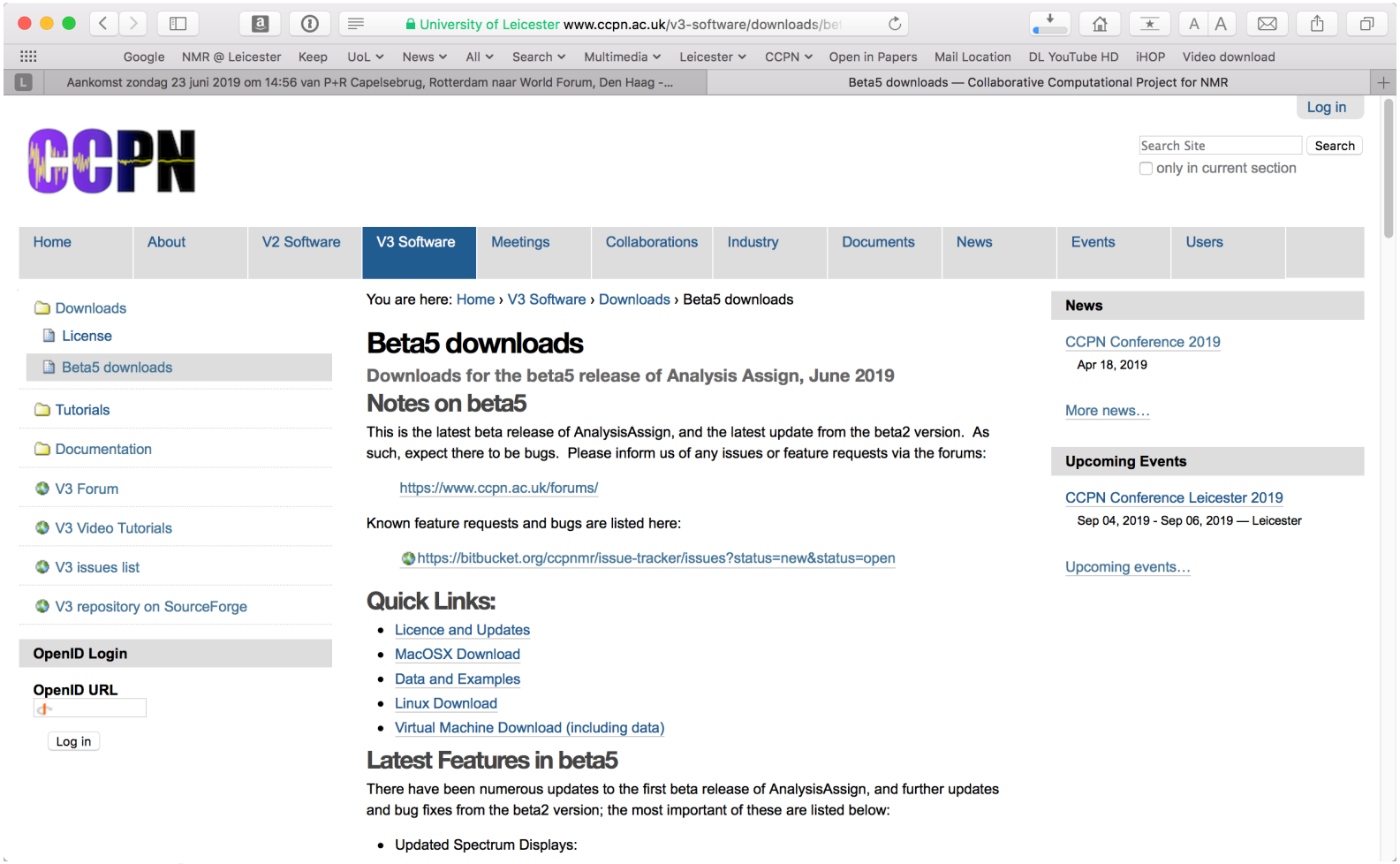
www.ccpn.ac.uk/forums

CcpNmr forum: installation



www.ccpn.ac.uk/forums

CcpNmr forum: installation



Bootable Linux
OSX install (USB)
Linux install (USB)

CcpNmr forum: installation

OSX installation issues