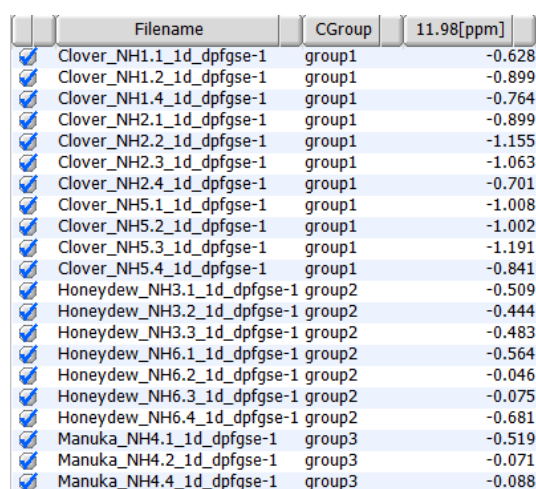


Delta Chemometrics for PCA analysis

- Install R software and have the program open on your desktop. If you get the error “cannot find RScript.exe “ Close the R software.
- Open Delta processing window click analyse and in the drop-down menu click chemometrics.
- The chemometrics window should open, if there is an error message that’s because the R packages haven’t been loaded. Follow separate instructions for loading these but only install the packages that the error window specifies.
- Once loaded and delta has been restarted there should be no error
- Click on file, click open, find the NMR spectra data that you have processed. These spectra should have been previously processed by reference to the solvent, machine phasing, and baseline correction.
- You can select multiple files by holding down the “Ctrl” key while clicking on the files you want.
- Once all files selected press the green tick box to load the spectra.
- In the pre-processing window ensure ‘Process data’ is ticked. In the list section click the open file button to find your bucket integration processing list that you constructed previously. Once the file is selected click the green tick to submit it.
- Tick base line correction and referencing, ensure to type in the solvent reference. The tolerance can be 0.4 ppm.
- In the bucket integration section, input all the exact parameters that you used for the bucket integration process list you made previously.
- Leave the ‘Group’ box as the default ‘SAMPLE.CGroup’.
- Click the process button with the blue arrow symbol. Your data should come up in the central window, with each file in separate rows.
- If you did not alter the ‘Group’ box there will be an empty column ‘CGroup’. Here you must fill in the column manually to specify the respective groups your samples are associated with. For example, if you had a few different types of honey that you were sampling you should assign each type of honey their own group. ‘R’ and Delta will only recognise the grouping if you type it in the format ‘group1’. This is the only way that you will be able to discern the different samples in the PCA score graph output. See the attached image below for an example (Figure 1).



Filename	CGroup	11.98[ppm]
Clover_NH1.1_1d_dpfge-1	group1	-0.628
Clover_NH1.2_1d_dpfge-1	group1	-0.899
Clover_NH1.4_1d_dpfge-1	group1	-0.764
Clover_NH2.1_1d_dpfge-1	group1	-0.899
Clover_NH2.2_1d_dpfge-1	group1	-1.155
Clover_NH2.3_1d_dpfge-1	group1	-1.063
Clover_NH2.4_1d_dpfge-1	group1	-0.701
Clover_NH5.1_1d_dpfge-1	group1	-1.008
Clover_NH5.2_1d_dpfge-1	group1	-1.002
Clover_NH5.3_1d_dpfge-1	group1	-1.191
Clover_NH5.4_1d_dpfge-1	group1	-0.841
Honeydew_NH3.1_1d_dpfge-1	group2	-0.509
Honeydew_NH3.2_1d_dpfge-1	group2	-0.444
Honeydew_NH3.3_1d_dpfge-1	group2	-0.483
Honeydew_NH6.1_1d_dpfge-1	group2	-0.564
Honeydew_NH6.2_1d_dpfge-1	group2	-0.046
Honeydew_NH6.3_1d_dpfge-1	group2	-0.075
Honeydew_NH6.4_1d_dpfge-1	group2	-0.681
Manuka_NH4.1_1d_dpfge-1	group3	-0.519
Manuka_NH4.2_1d_dpfge-1	group3	-0.071
Manuka_NH4.4_1d_dpfge-1	group3	-0.088

Figure 1. Snippet from the data window indicating group assignments.

- In the ChemPack analysis window write out a suitable name for your PCA output so that you can identify it afterwards. A name that uses symbols will be rejected.

- In PCA processing window make the 'option' 'classical' with 'scaling' set to 'auto scale'. Tick 'Save CSV'.
- In 'Plots' tick the 'Score' box, the 'Loading' box, and the 'Diagnosis' box. In 'Score' tick the box for 'Labels' and make this 100%. Tick the box for 'Ellipse' and make this 'Classical'. Tick the box for 'Legend' and choose your preferred position for it.
- In 'Diagnosis' ensure the 'Both' option is selected and tick the 'Save diagnosis to CSV' box.
- In order to actually get graphs, you must click graph options and change the 'Output Graph To' section to either PDF or PNG, but PDF is preferred.
- Once this is set, click the 'PCA' button with the blue arrow symbol.
- This will output the results to Documents > JEOL > Delta 6.1 > Reports. See the attached image for an example of what the final result would look like (Figure 2).

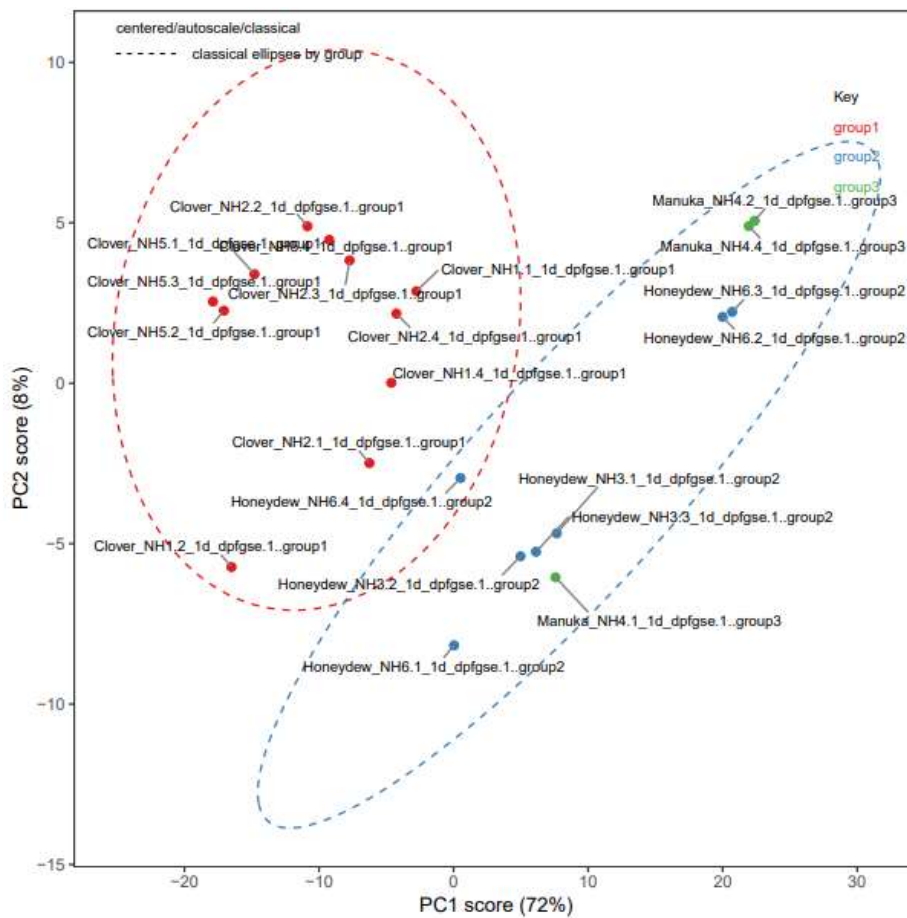


Figure 2. PCA score output for honey samples.