Instructions for installing '2D NMR Analysis v1p03'

Once ImageJ is installed (available online at http://rsb.info.nih.gov/ij/index.html) download BrukerOpener.jar and NMR_2D_analysis_v1p03.jar and save in the ImageJ 'Plugin' folder. You will also need to download HandleExtraFileTypes.class and save it in the 'Input-Output' sub-folder in the 'Plugin' folder. Once these are all downloaded, restart ImageJ.

Note the most recent version of BrukerOpener on the ImageJ website does not appear to open out 2rr files correctly. If you also have this problem download an older version of rr2iiOpener.class available from the same site as our plugin

Instructions for using 2D_NMR_Analysis_v1p03

Opening Files:

1. To open 2rr files click 'Open Files'and type in the number of images (i.e. 2rr files) you wish to open. In the browsing window that will then appear, select the folder containing the experiments you wish to open. You will then prompted to enter the experiment numbers you wish to analyse. (If the window requesting experiment numbers is too long to fit on the screen, you will not see the 'OK' button. In this case, chose a smaller number of experiments to open. You will then need to create two or more stacks to combine to create the 'master' stack).
2. Each of the spectra you have chosen will then open on your desktop as individual images. Combine the individual spectra into a single 'stack' by clicking 'Build Stack'. (In the case that you may have had to create two or more 'stacks', these can be simply combined in the same manner).
3. Scale the stack using the 'Scale Stack' option to change the image dimensions.
4. Images are usually inverted from their original appearance and can be flipped using the 'Flip Images Vertically' option.
5. Can remove negative points that create background colour if required (enter value of 0)
6. To change the colour scheme of the image use either the 'LUT NMR' or 'LUT Fire' buttons. Alternatively, there are many other colouring options built into ImageJ (Image → Lookup Table).
7. To alter the intensity of the chosen colouring scheme, use the 'Adjust LUT' button and select 'auto adjust' or the individual sliders.
8. Individual spectra can now be scrolled through using the slider at the bottom of the stack.
9. To save the stack for later use, ensure it is saved as a .tif in the main ImageJ window (File → Save As → Tiff)

Calibration of spectra

When a stack is created, as above, the ppm values of the spectra are not transferred. While individual peaks are visible, the ppm values cannot be directly read off the stack using the cursor. If desired, the ppm values can be set using the 'Calibrate' option in the plugin. This will ask for the maximum and minimum ppm values in the x and y dimensions. Once this is done, if the cursor is placed on the image, the ppm values are shown beneath the image. Note that integration cannot be carried out when the stack is calibrated. In order to remove the calibration, simply click on 'Reset Calibration'.

Selection of regions of interest (ROI)

To integrate peaks they must first be selected.

1. Click on ‘Start ROI Manager’
2. Select a region encompassing the first peak (use rectangular, oval, polygon or freehand selection tools in the main ImageJ window)
3. Click 'Add' in ‘ROI Manager’ window
4. Repeat steps 2 and 3 for as many peaks as desired
5. All selected regions can be shown using
   ROI Manager window → Show all
6. When all regions (peaks) are selected remove slice info
   ROI Manager window → More… → Remove Slice Info (Note that ‘slice info’ must be removed prior to integration, otherwise the program hangs)
7. ROIs can be saved in a .roi file for later use or modification if desired
   ROI Manager window → Save
   ROI Manager window → Open – (for later use)

Integration of peaks

1. To Integrate peaks select either ‘Integrate Selected Peaks’ or ‘Integrate Pixels’ (These are two different numerical techniques that should yield the same results. If in doubt use the latter).
2. A window will appear prompting the user to specify the ‘threshold’ level at which to integrate each peak i.e. only if a pixel in the ROI has a value above the specified threshold will it be included in the integration.
3. A results window will open with the sum (integral) for each selected region for each slice in the stack. This data can be saved and opened as a spreadsheet in programs such as Excel.