Tutorial 2D-FLCS

This document explains stepwise procedure for 2DFLCS analysis.

Requirements

1. Confocal microscope (Leica sp8x).
2. TCSPC module (PicoQuant).
3. MATLAB software (Parallel computing toolbox)
4. ImageJ software. (optional)

List of Codes in the folder

1. Step1_extract_ptu_to_CSV.m
2. ptu_read_in_loop.m
3. Step2_make_partition.m
4. All_frames_partition.m
5. Partition_2D_core.m
6. GetParentFolder.m

Preliminary

1. Transfer raw TCSPC data to folder of your choice. PicoQuant stores data in *.ptu formats.
2. Check if you have parallel computing toolbox installed (web link)
3. For further analysis, we can use ImageJ integration in MATLAB. (MATLAB Scripting (imagej.net))

Procedure

1. Open the folder with codes (“/Tutorial_2DFLCS”)
2. Converting Raw data (.ptu) to CSV.
    a. Run “**Step1_extract_ptu_to_CSV.m”** in MATLAB.
    a. A dialog box will appear where you should select the folder containing raw data.
    b. After completion CSV files will be created from the raw *.ptu files.
3. Partitioning data to generate 2DFD slices.
    a. Run “**Step2_make_partitions.m**”
    b. Note: This process might take a while depending on total photons in the data.
    c. After completion, the slices will be saved as “Partitioned[folder_name].mat” in the folder containing data.
4. Shrink Partitions
    a. Run **‘’Step3_shrink_partitions.m”**
    b. You will see a new .mat file generated with name (‘shrinked_new_[partition].mat’)below the partitioned data
5. 2D ILT analysis
    a. Run “Step4_ILT_analysis.m”
    b. Specify the required inputs

c. If it is the first time analyzing the current dataset, the variable “IRF_1D_shift_range” should be set to a range of integers to figure out the best IRF time shift appropriate for the data.
d. After finding out the best IRF shift, you can input just the minimum value to continue to 2D analysis.
e. The data is stored as frames in a folder named ‘batch_processed_xxxx’ (xxxx being the input file name.)

6. Extracting the correlation functions from the 2D ILT frames (This part is subjective and one can use whatever analysis methods accessible. I have found an easy way but it includes some specific actions which might not be desirable for some)
    a. This part will require the ImageJ plugin in MATLAB activated. (See MATLAB Scripting (imagej.net))
    b. Open ImageJ matlab using the command “ImageJ”. In case of a not defined error, you need to add the ImageJ path to matlab Using the command “addpath('~\fiji-win64\Fiji.app\scripts')” where ~ is the path to the local installation of ImageJ.
    c. Load the ILT_frames_xxxx.mat file in matlab workspace and display it using the command (IJM.show(‘ILT_frame’)).
    d. An ImageJ window will open where 2D-ILT frames are shown as different slices.
    e. To extract the correlation function of a specific peak, draw an appropriate rectangle over the peak and in the ImageJ window, select Image>>> Stacks >>> Plot Z-axis Profile.
    f. From the plot window, click on “List” and copy the contents. Also click on “Live” so that you can see the profile while adjusting the rectangle.
    g. Paste the data on the clipboard into a variable in matlab (for a 2x2 peaks spectra, you can use variable names like c11, c12, c21, c22)
    h. In the ImageJ window, slide the rectangle to the next peak and repeat steps f. and g.
    i. If the stored variable names are in the form, c11,c12,c21,c22, you can run the script “step5_process_corrs_after_imageJ.m”. Else, specify the variable names by editing the code file appropriately.
    j. This generates a plot of correlation functions extracted above. These can be fit to extract transition timescales of chemical exchange process.