Hokkaido University

Raman Analyzer

User Guide (for version 1.7)

Lei Zhao
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Contents

Installation

- For Windows, run the app installer named ‘RA_Installer_Web’ (Fig. 1) as administrator and follow the on-screen instructions.
- If MATLAB Runtime was not detected by the installer, it will be downloaded and installed during the installation process.
- Mac OS is currently not supported.

Figure 1: Raman Analyzer installation file icon.

Start the Raman Analyzer

- Please run the installed Raman Analyzer app as administrator.
- The launcher window (Fig. 3) will be displayed after initialization.

Figure 2: Splash screen during initialization.

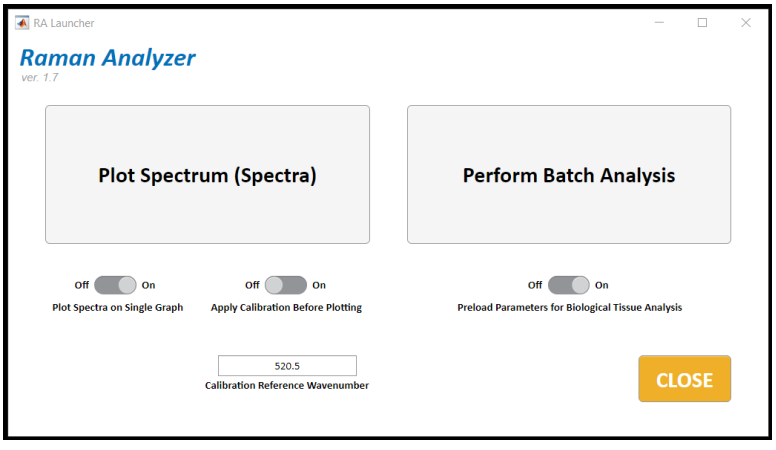

Figure 3: Raman Analyzer launcher window.

File Type, Data Format, and File Placement

File type and data format

- Data to be process by the Raman Analyzer app should be in .txt file format.
- Each .txt file should only contain data for a single spectrum, with wavenumber (x-axis) in the first column and intensity (y-axis) in the second column.
- Data can have no header line or one line of header txt. (Fig. 4)

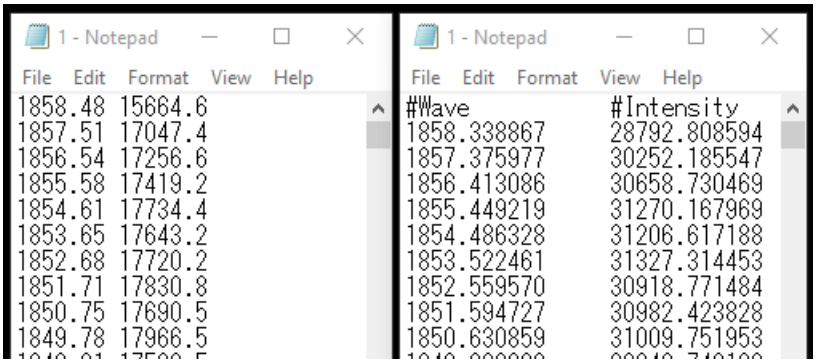

Figure 4: Acceptable file format.

File placement

- .txt files to be batch analyzed should be placed in the same folder, and the calibration file should be placed in a separate directory. (Fig. 5)

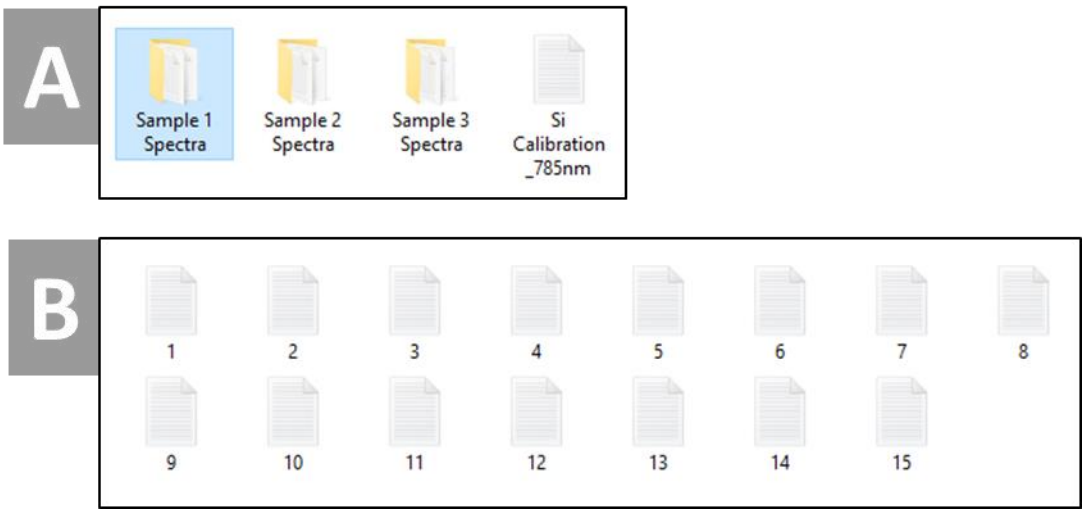

Figure 5: Placement of spectra files. Spectra from the same sample should be placed in a single folder, and the calibration file should be placed separate (A). The example here shows 15 .txt files corresponding to 15 spectra in the 'Sample 1 Spectra' folder (B).

Plot Raman Spectra

- The left half of the launcher window provides controls for plotting and previewing Raman spectra. (Fig. 6)

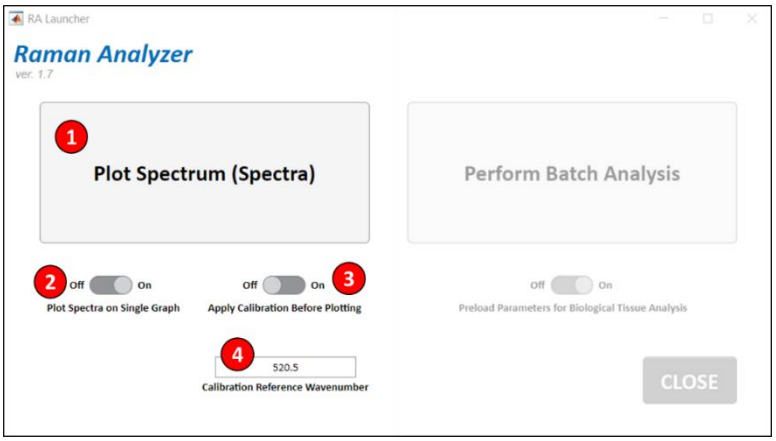

Figure 6: Controls for plotting Raman Spectra. Select files for plotting [1]; Plot spectra on single/multiple graph [2]; Apply calibration before plotting [3]; Reference wavenumber to be used for the calibration [4].

Select files to plot

- Press the 'Plot Spectrum (Spectra)' button ([1] in Fig. 6) to select file. Multiple files can be selected by holding down 'Ctrl' or 'Shift' Key. Then, click 'Open' to confirm. (Fig. 7)

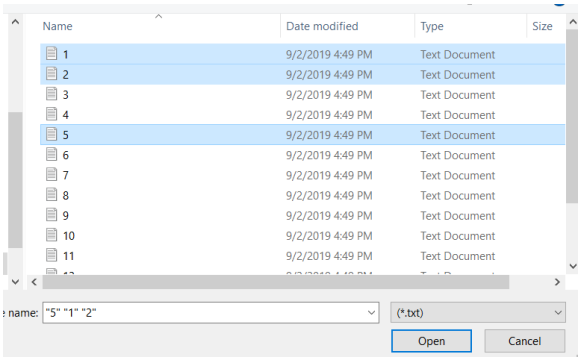

Figure 7: Select files for plotting.

Plot spectra on single/multiple graph

- This can be controlled by the 'Plot Spectra on Single Graph' toggle switch ([2] in Fig. 6). The default switch position is 'On', so toggle the switch to the 'Off' position to have spectra plotted on separate graphs.
- File names of the spectra will be displayed in the legend if toggle switch was set to 'On', otherwise the file name will be displayed in the graph title. (Fig 8)

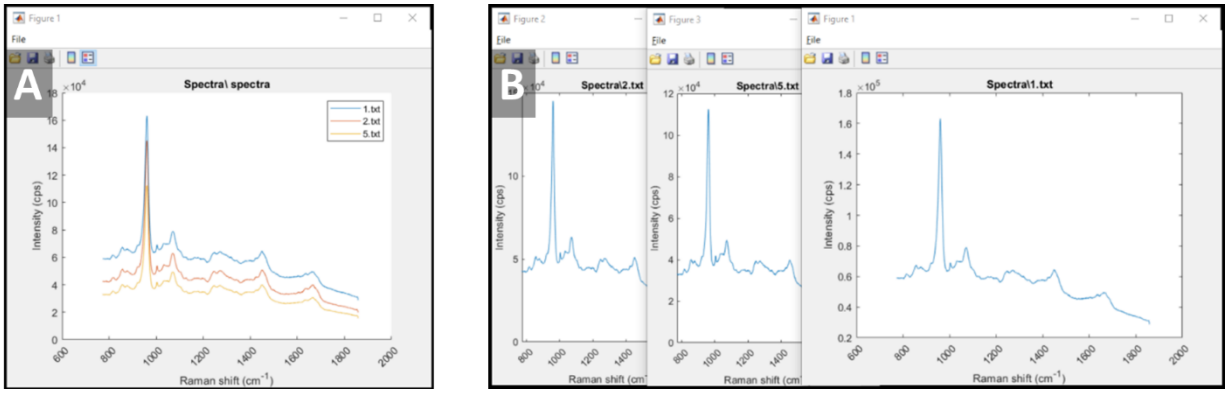

Figure 8: Plot with 'Plot Spectra on Single Graph' toggle switch 'On' (A) and 'Off' (B).

Apply calibration before plotting

- This is controlled by the 'Apply Calibration Before Plotting' toggle switch ([3] in Fig. 6). The default switch position is set to 'Off'.
- When the switch is toggle on, user will be promoted to import a calibration .txt file after selecting files for plotting. (Fig. 9)
- The calibration .txt file should contain spectrum data collected from a pure element with known peak wavenumber. An example is shown in (Fig. 10)

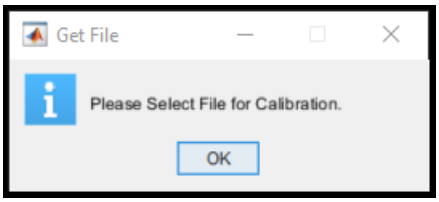

Figure 9: Prompt for importing calibration file.

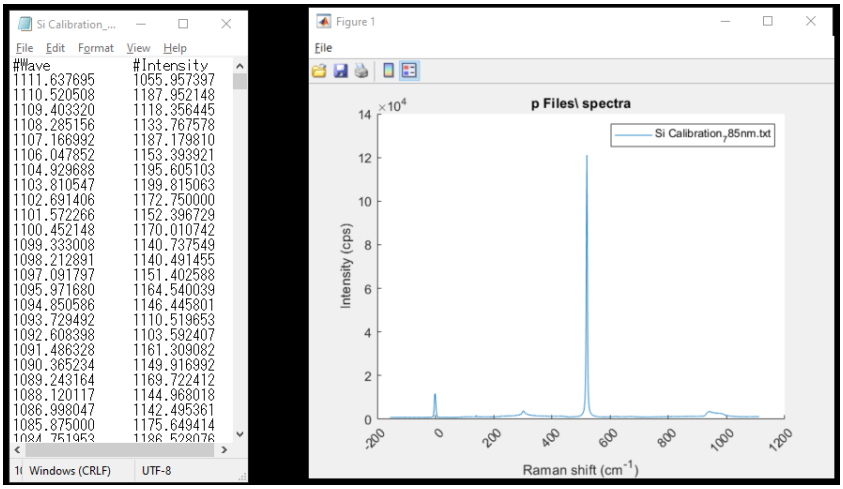

Figure 10: Calibration .txt file and spectrum.

- The default calibration material is set to Silicon with a reference peak wavenumber of 520.5. This can be changed by modifying the 'Calibration Reference Wavenumber' input box. ([4] in Fig. 6)

Spectra Analysis

- The right half of the launcher window provides controls for analyzing Raman spectra data. (Fig. 11)
- Analysis can be started by clicking the 'Perform Batch Analysis button. ([1] in Fig. 11) The launcher window will close automatically upon clicking the button, and it will be relaunched after analysis is complete.

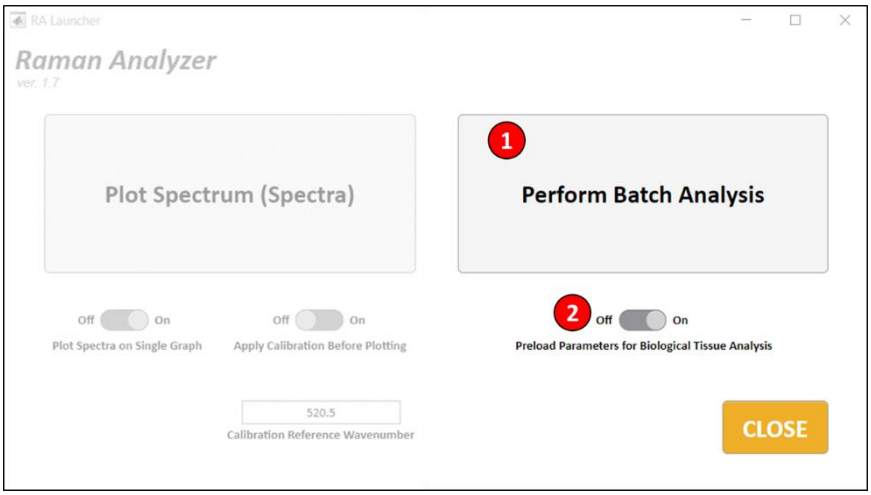

Figure 11: Controls for analyzing Raman spectra data.

Set working directory

- User will be prompted to select working directory at the beginning of the analysis process, (Fig. 12) within which, all analysis results will be saved.
- To avoid warnings during the analysis, it is recommended to keep all files to be analyzed and the calibration file under the working directory.
- After click 'OK' on the prompt window, use the file explorer window to navigate to desired directory and click the 'Select Folder' button to confirm.

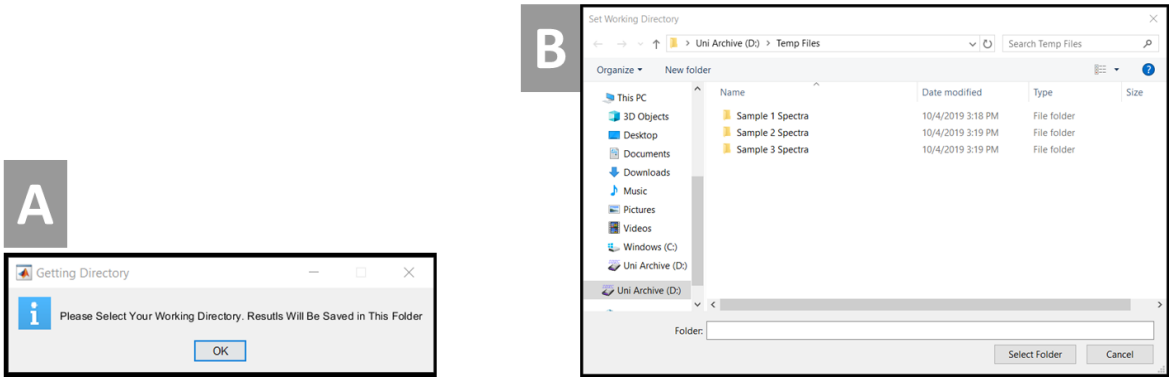

Figure 12: Select working directory. Prompt for selecting working directory (A); File explorer window for setting directory (B).

Control panel

- The control panel will be opened automatically after the working directory is set. The default control panel is preloaded with parameters suitable for analyzing biological tissues. (Fig. 13A) A general version of the control panel without preloaded parameters (Fig. 13B) is also available by switching the 'Preload Parameters for Biological Tissue Analysis' toggle on the launcher window ([2] in Fig. 11) to the 'Off' position before starting analysis.

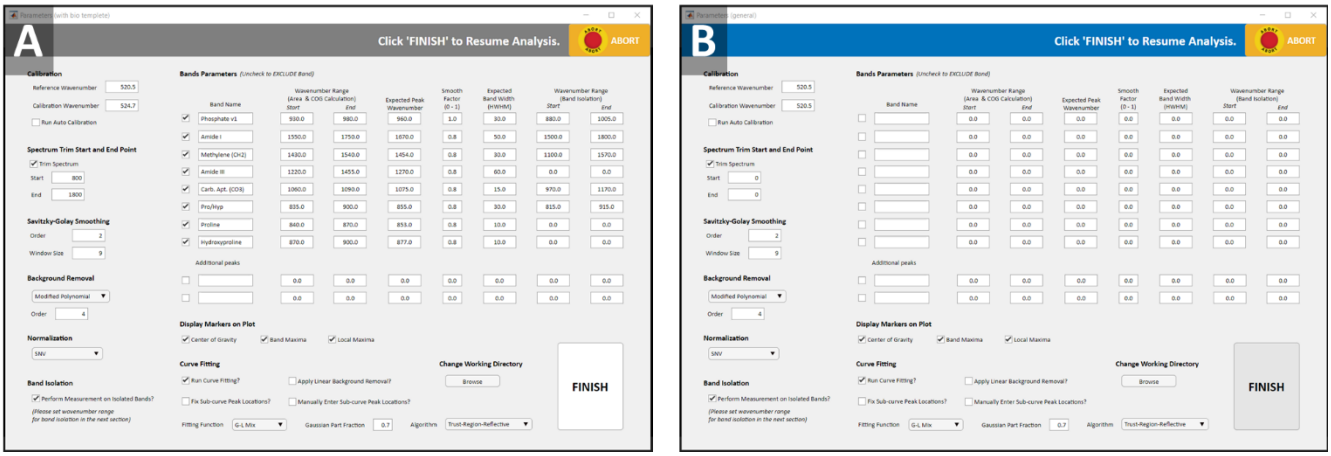

Figure 13: Control panel for biological tissues (A) and general materials (B) analysis.

Calibration

- Apply calibration using a .txt file is set as default. (Fig. 14A)
- The calibration wavenumber can also be entered manually by unchecking the 'Run Auto Calibration' checkbox. (Fig. 14B)
- Calibration can also be turned off by unchecking the 'Apply Calibration' checkbox. (Fig. 14C)

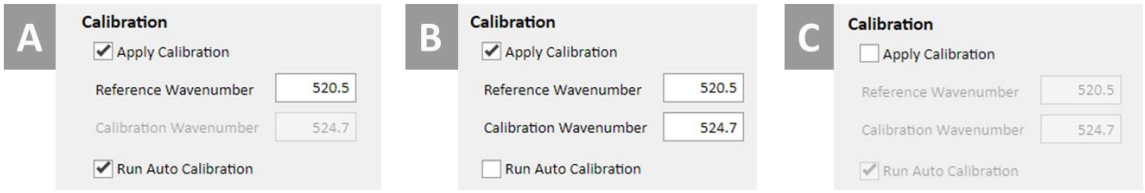

Figure 14: Calibration settings.

Data trimming

- Data trimming is turned on by default when using the control panel for biological tissues. (Fig. 15)
- Enter the start and end wavenumber into the 'Start' and 'End' input box to define trimming range.
- Trimming can be turned off by unchecking the 'Trim Spectrum' checkbox.

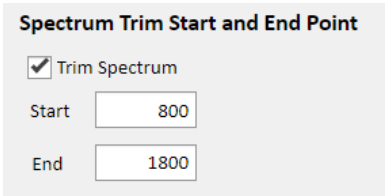

Figure 15: Data Trimming.

Smoothing

- Savitzky-Golay smoothing is used to reduce data noise.
- The order and window size can be modified using the 'Order' and 'Window Size' input boxes. (Fig. 16)

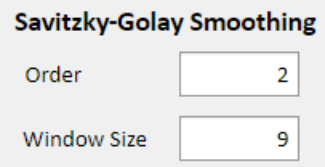

Figure 16: Savitzky-Golay smoothing.

Background removal

- The default background removal method is 4th order modified polynomial. (Fig. 17A)
- The background removal method and polynomial order can be changed by using the dropdown box (Fig. 17B) and the 'Order' input box.

Figure 17: Background removal method.

Normalization

- Four spectrum normalization methods are provided and can be selected from the 'Normalization' dropdown box. (Fig. 18A)
- If 'Reference Peak' option is selected, user will be asked to manually input the peak by mouse-click during the normalization process. (Fig.18B)
- User can also select the 'No Normalization' option to skip the normalization process.

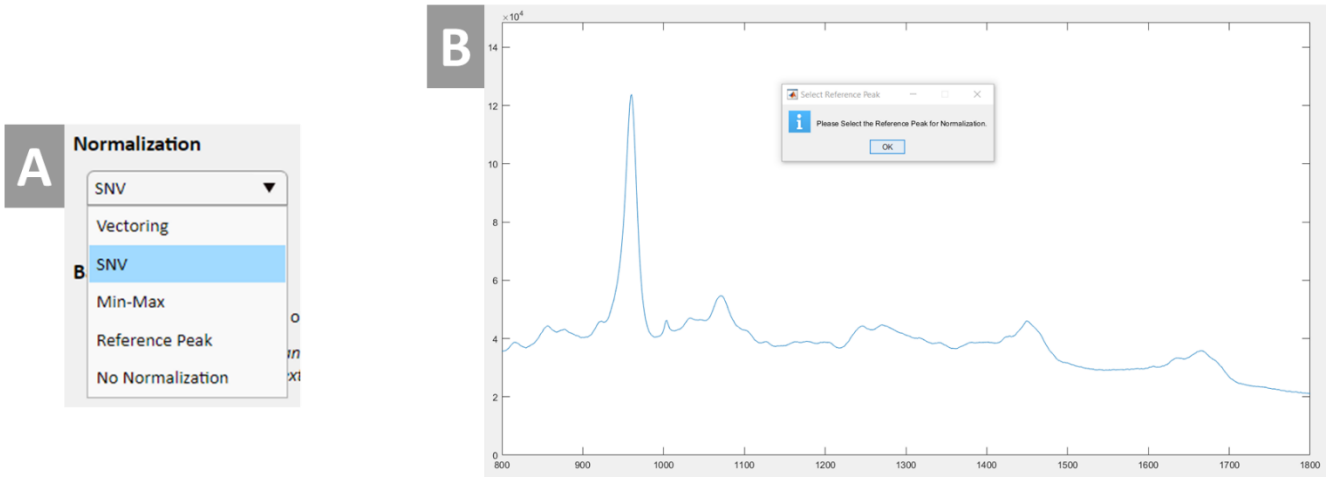

Figure 18: Normalization methods. SNV stands for Standard Normal variate).

Band isolation

- The band isolation option provides more accurate measurements by applying trimming and linear background removal to each band defined in the 'Bands Parameters' section of the control panel (bands with checked checkbox).
- When Band isolation is enabled, start and end wavenumbers of each band need to be defined in the 'Bands Parameters' section for applying data trimming.

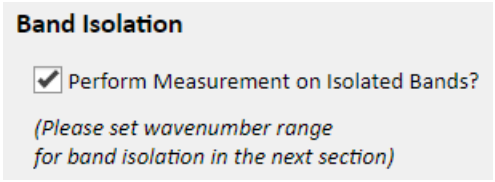

Figure 19: Band isolation checkbox.

Bands parameters

- Parameters for each band to be analyzed are entered in this section. Raman Analyzer will only perform measurements on bands with checked checkbox.
- Parameters required for each band are (from left to right): Band Name; Start and End wavenumber for calculating the band area and center of gravity; Expected Band Peak Wavenumber; Smooth Factor for noise reduction; Band Width in terms of half width at half maximum; Start and End wavenumber for data trimming if 'Band Isolation' option was selected in the previous section.

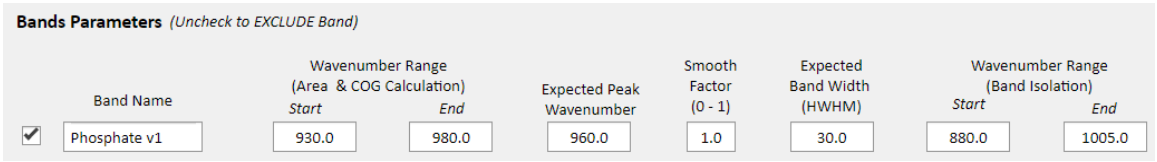

Figure 20: Parameters for Raman bands.

Display markers on plot

- Markers for the center of gravity, band maxima (calculated band peak using derivative), and local maxima (peak intensity at user entered peak wavenumber) can be displayed on the final spectrum by checking the options in the 'Display Markers on Plot' section. (Fig. 21)

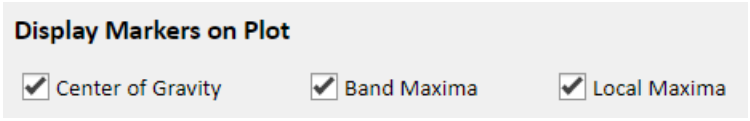

Figure 21: options for marker display.

Options for curve fitting

- Curve fitting allows user to fit multiple curves to selected spectrum bands. The option is turned on by default. Uncheck the 'Run Curve Fitting' checkbox to turn off curve fitting during the analysis process.
- Check the 'Apply Linear Background Removal' checkbox to perform additional linear background removal before applying curve fitting.
- The wavenumber of fitted curves can be fixed by mouse-click on plot (check the 'Fix Sub-Curve Peak Locations' checkbox) or by manual enter (check the 'Manually Enter Sub-Curve Peak Location' checkbox).

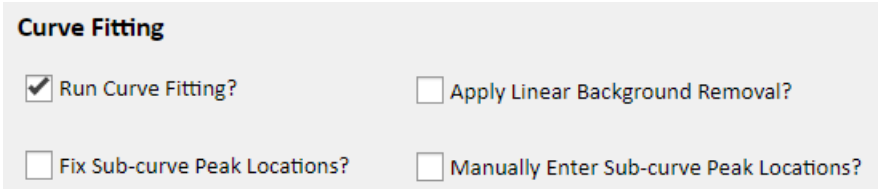

Figure 22: Options for curve fitting.

- Three fitting functions, Gaussian, Lorentzian, Gaussian-Lorentzian Mixture (G-L Mix), can be selected using the dropdown box. (Fig. 23A)
- When the 'G-L Mix' function is selected, the fraction of Gaussian part can be defined using the 'Gaussian Part Fraction' input box. (Fig. 23B)
- Two fitting algorithms, Trust-Region-Reflective and Levenberg-Marquardt, are available for the fitting process. The algorithm can be changed using the 'Algorithm' dropdown box. (23C)

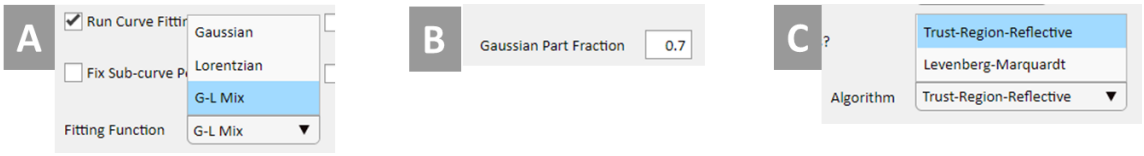

Figure 23: Curve fitting functions and algorithms.

Change working directory

- working directory can be changed by clicking the 'Browse' button under the 'Change Working Directory' option. (Fig. 24)

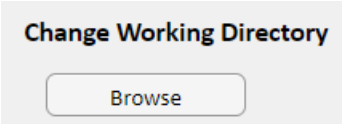

Figure 24: Change working directory.

Cancel analysis

- The spectra analyzing process can be canceled by clicking the 'ABORT' button on the right top corner of the control panel. (Fig. 25)

Figure 25: Abort button for cancelling the analysis.

Proceed to the next step

- After finishing setting parameters on the control panel, click the 'FINISH' button (Fig. 26) or simply close the control panel window to proceed to the next step.

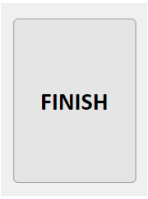

Figure 26: Click the 'FIHISH' button to proceed to the next step.

Import Files

- Select the folder containing only spectrum files to be analyzed and click 'Select Folder' to confirm. (Fig. 27A)
- If the selected folder is outside of the working directory, user will be promoted to: (Fig. 27B)

- Confirm selection ('YES'): Raman Analyzer will temporarily change working directory to the selected folder during analysis and switch back to the original working directory upon completion.
- Change directory ('CHANGE DIRECTORY TO CURRENT FOLDER'): Raman Analyzer will change working directory permanently to the selected folder.
- Select new directory ('SELECT NEW DIRECTORY'): let user select a new working directory.

- If the 'Run Auto Calibration' option was selected on the control panel, user will be promoted to select a calibration file prior to the folder selection step. (Fig. 27C)

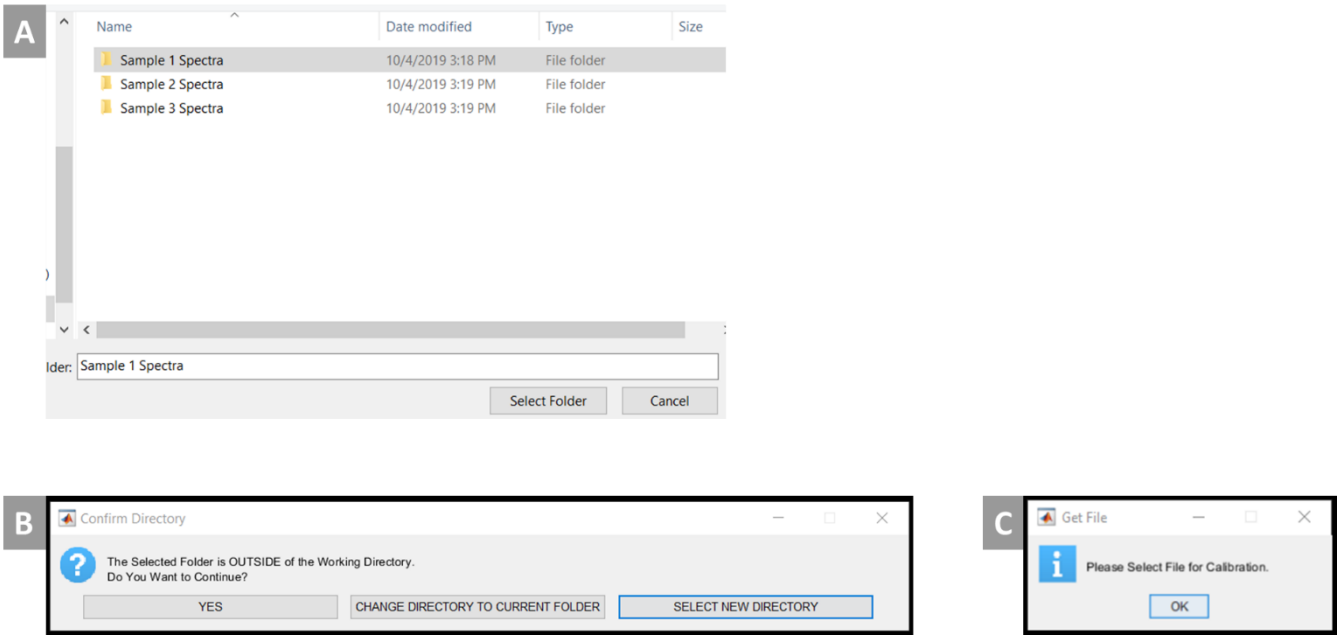

Figure 27: Import files for analysis.

Cosmic ray removal

- The cosmic ray removal process is activated after importing files for analysis. Each imported spectrum will be plotted and displayed in a window. Use will be asked to input file numbers for cosmic ray removal. (Fig. 28)
- Enter the plot number of the spectrum affected by cosmic ray (black arrows in Fig. 28) into the input box and click 'OK'.
- If more than one spectrum is affected, separate the plot numbers by space or comma. Eg. (7 9 or 7,9)
- If no entry is needed, leave the input box empty and click 'OK' or 'Cancel' to proceed to the next step.

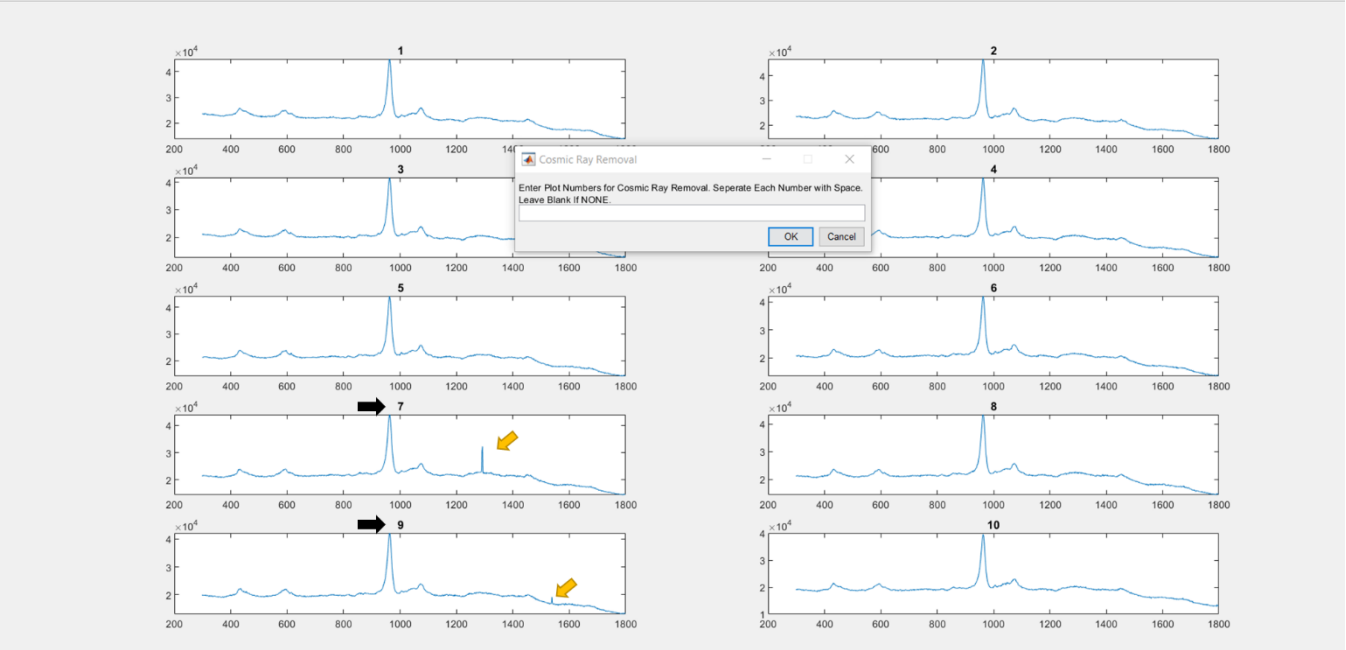

Figure 28: Cosmic ray removal. Yellow arrows indicate spikes caused by cosmic ray; black arrows indicate plot numbers that need to be entered the input box.

- After selecting affected spectra, use will be asked to input the spike location with mouse-click. (Fig. 29A)
- Click once on the spike and press 'Enter' on the keyboard when finish. The selected spike location will be numbered and marked by a vertical dash line (Fig. 29B)
- If more than one spike is presenting, click once on each spike and press 'Enter' on the keyboard when finish.
- Repeat the process on the next spectrum if more than one spectrum were selected.

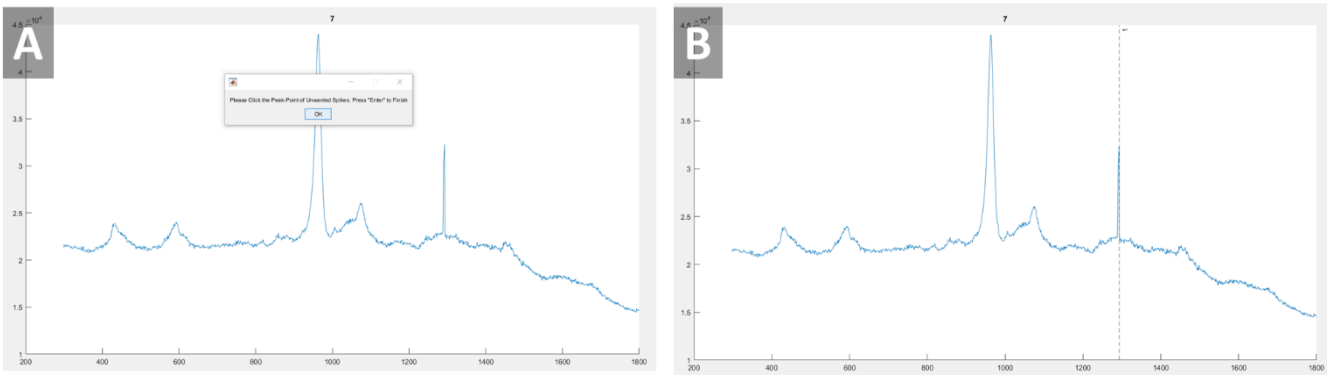

Figure 29: Prompt for input spike location for cosmic ray removal (A) and selected spike (B)

- Spectra after cosmic ray removal will then be presented to the user. (Fig. 30A) If additional cosmic ray removal is need, click 'Yes' on the prompt box and enter the plot number again to repeat the process. (Fig. 30B)
- Click 'No' or 'Cancel' to proceed to the next step.

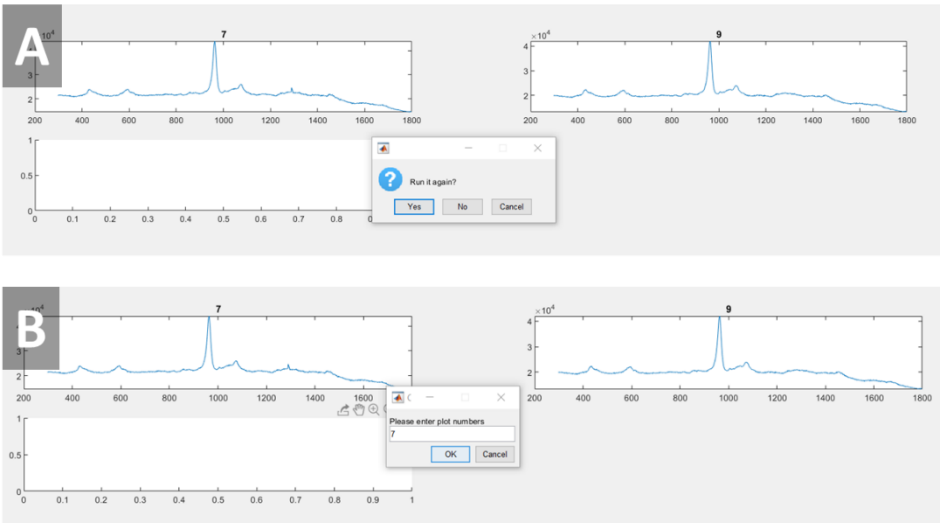

Figure 30: Check cosmic ray removal results (A), and the process can be repeated if additional process is required (B).

Spectra analysis

- The analysis process is fully automated, and no user input is required.
- Background removal result (Fig. 31A) and the final averaged spectrum with marked measurement results (Fig. 31B) will be displayed on screen as figures.

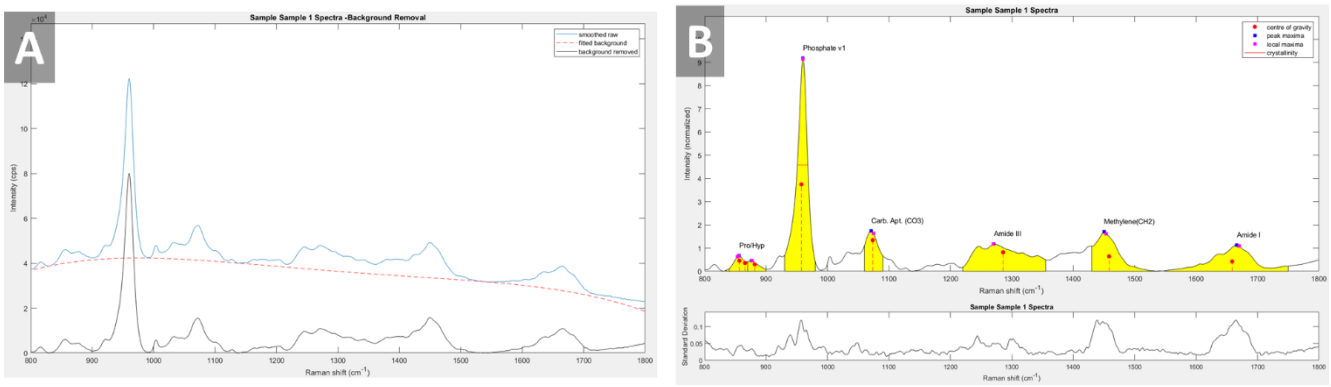

Figure 31: Background removal result (A) and measurement results (B) from the analysis process.

- If the 'Band Isolation' option was selected on the control panel, additional figures showing individual bands will also be displayed as figures. (see Fig. 32 for examples)

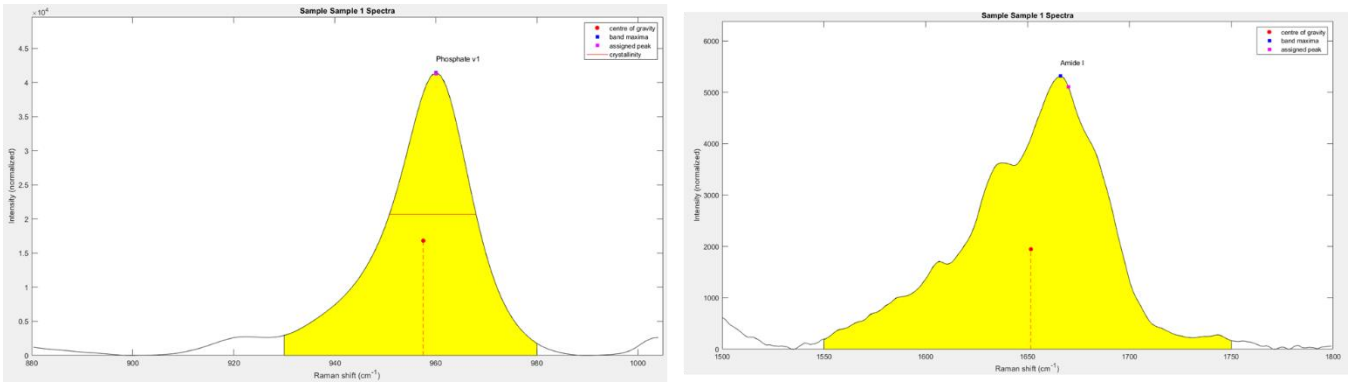

Figure 32: Examples of isolated bands analysis.

- A dialogue box will be displayed upon completion ask for user input (Fig. 33), and user can choose from the following three options:
    - ‘YES’: repeat the analysis on a different sample using the same settings.
    - ‘YES (Update Settings)’: perform another analysis with updated settings. This option will open the control panel and set all settings to default.
    - ‘No (Finish)’: finish analysis. This option will finish the analysis process and relaunch the launcher window.

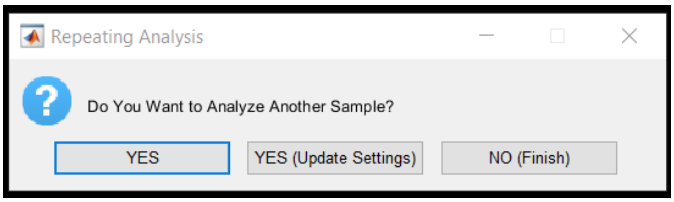

Figure 33: Request for user input after analysis completion.

Curve fitting

Isolate spectrum band

- If the ‘Curve Fitting’ option was selected on the control panel, the process will start immediately after the spectra analysis step. (Fig. 34A)
- User will be asked to select the start and end point of a spectrum band using mouse-clicks. (Fig. 34B)
- Figure 35 demonstrates the difference between with and without the ‘Apply Linear Background Removal’ option turned on.

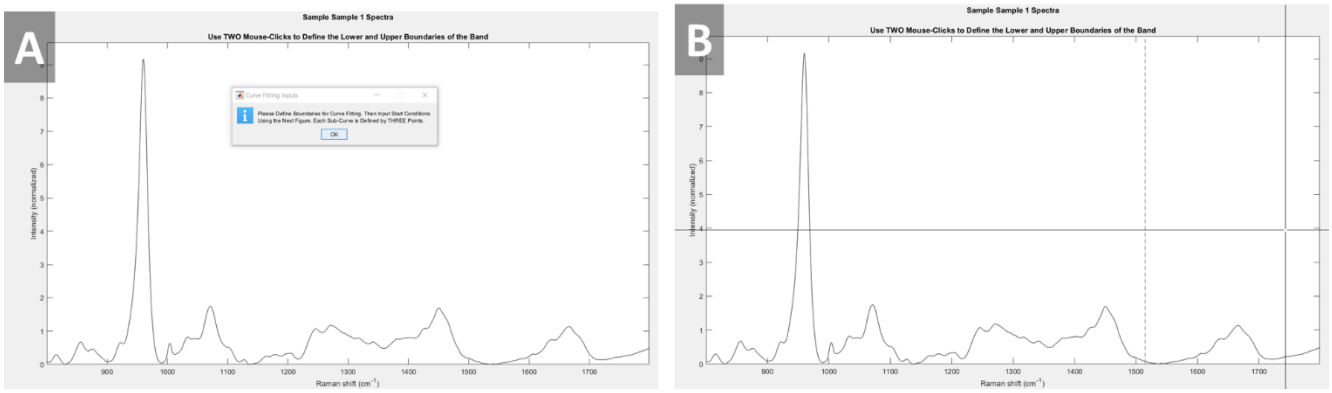

Figure 34: Prompt for define start and end point for curve fitting (A). Two mouse-clicks to isolate a spectrum band (B).

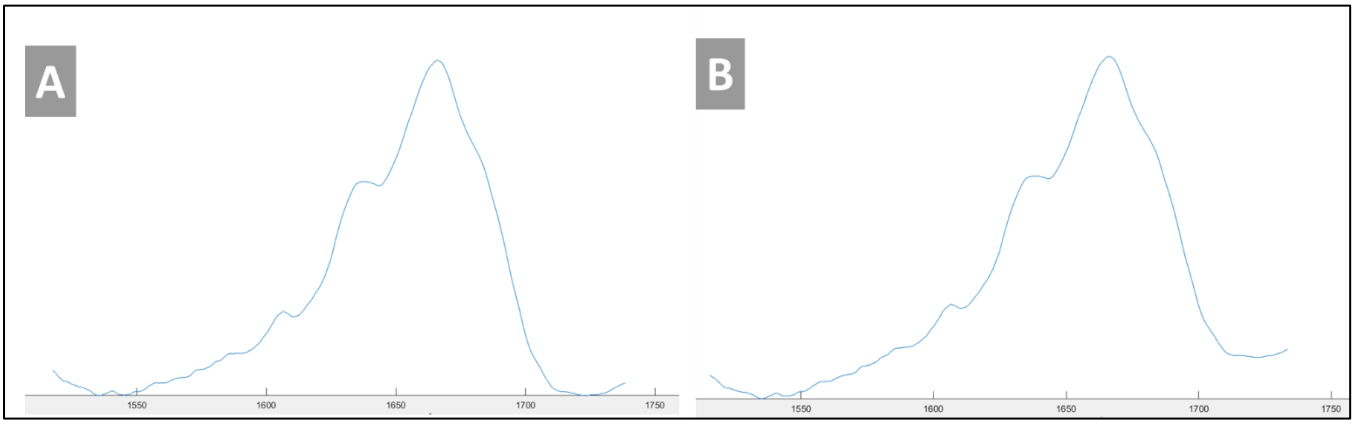

Figure 35: Isolated band with (A) and without (B) turning on the ‘Apply Linear Background Removal’ option.

Input start condition

- A start condition is required for the curve fitting process.
- Each curve is defined by three mouse-clicks: (Fig. 36A)
    - The approximate mid-point of the left slop.
    - The approximate peak of the curve.
    - The approximate mid-point of the right slop.
- User can define a maximum of 50 curves using this three-point system. (Fig. 36B shows 6 curves defined for the Amide I band)
- Press the 'Enter' key on the keyboard to start curve fitting.

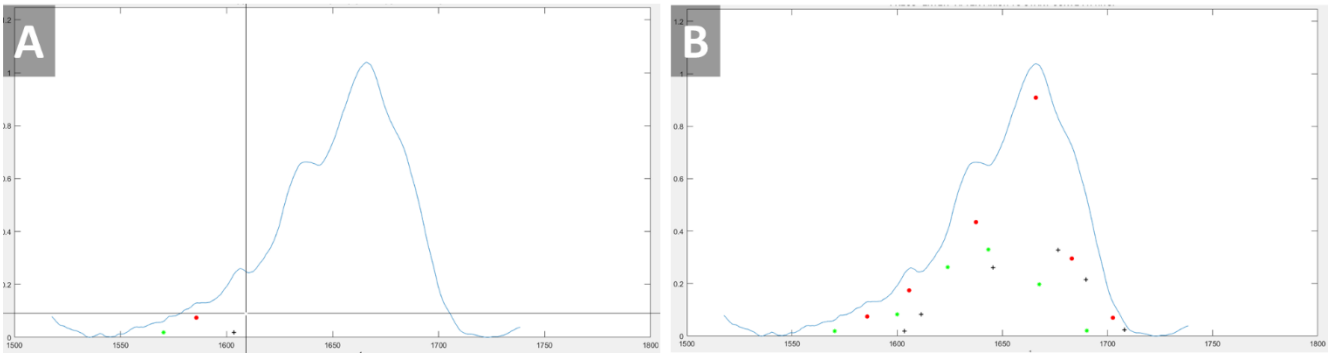

Figure 36: Use the three-point system to define a curve (A). An example of six curves defined for the Amide I spectrum band (B).

- If the 'Manually Enter Sub-Curve Peak Locations' option was selected on the control panel, user will be asked to input peak wavenumber for each defined curve after pressing the 'Enter' key. (Fig. 37) Please separate each peak wavenumber by space or comma.

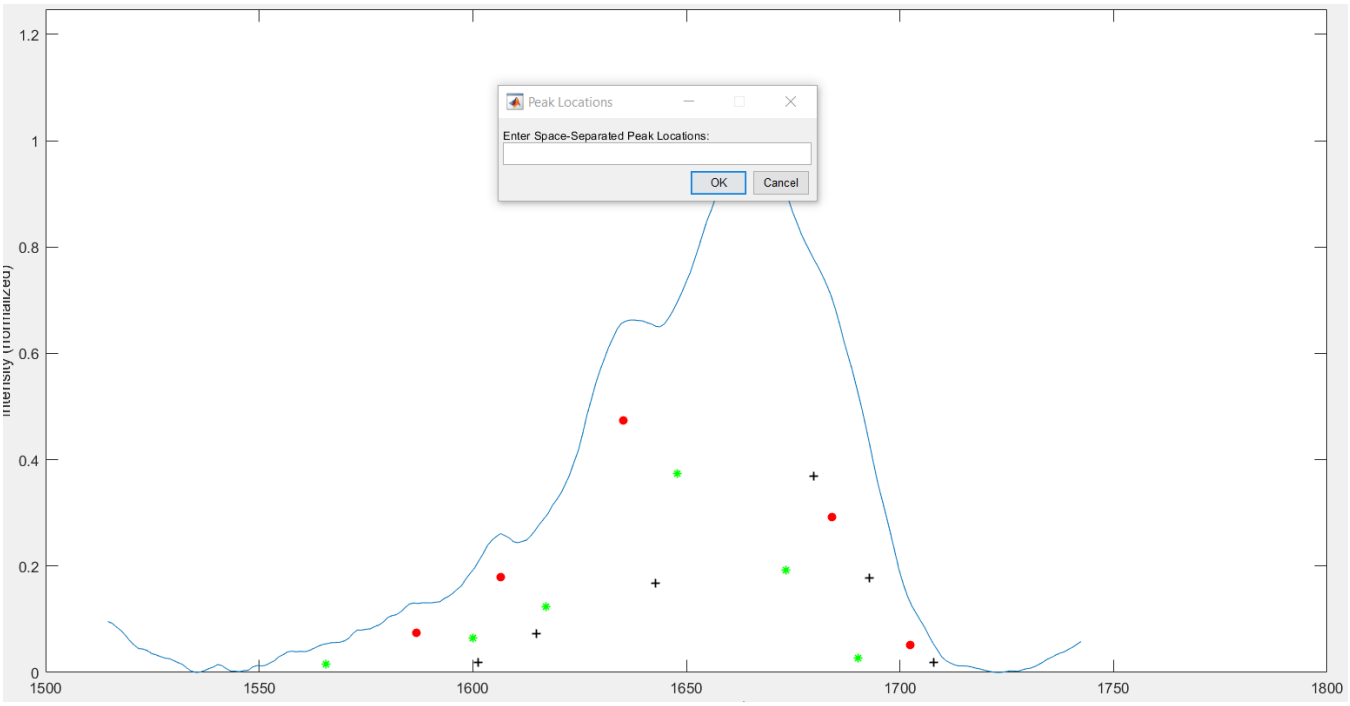

Figure 37: prompt for inputting curve peak wavenumbers.

Generate start condition and curve fit

- The start condition (Fig. 38A) and final fitted curves (Fig. 38B) will be displayed on screen as two separate figures.
- If the 'Fix Sub-Curve Peak Locations' or 'Manually Enter Sub-Curve Locations' option was selected on the control panel, the curve peak wavenumber will not be changed during the fitting process.

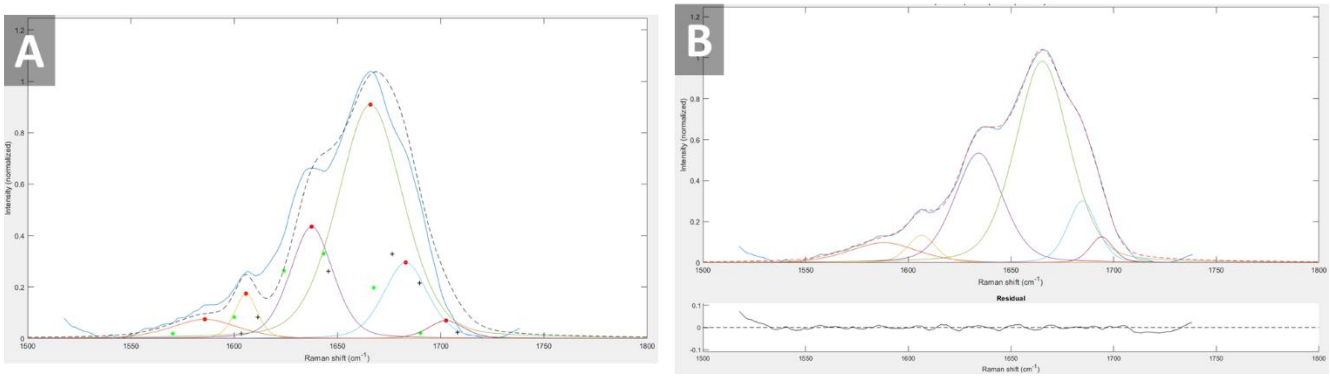

Figure 38: Curve fitting. Start condition (A) and final fit (B).

Analysis results

- Analysis results will be saved under the working directory with a folder name of 'Results_*imported folder name*'.
- The following file, in .xlsx format, will be saved in the results folder:
    - FinalSpectrum: data of the final averaged spectrum with standard deviation information.
    - FittedCuvers (when curve fitting is selected): all information related to the curve fitting process. Detailed description of each fitted curve.
    - FittingResidual (when curve fitting is selected): information on curve fitting residuals.
    - Measurements: measured values of all bands defined on the control panel.
    - Parameters: control panel settings used for the analysis.

Exit Raman Analyzer

- To exit Raman Analyzer, click the 'CLOSE' button on the launcher window (Fig. 39) or simply close the launcher window.

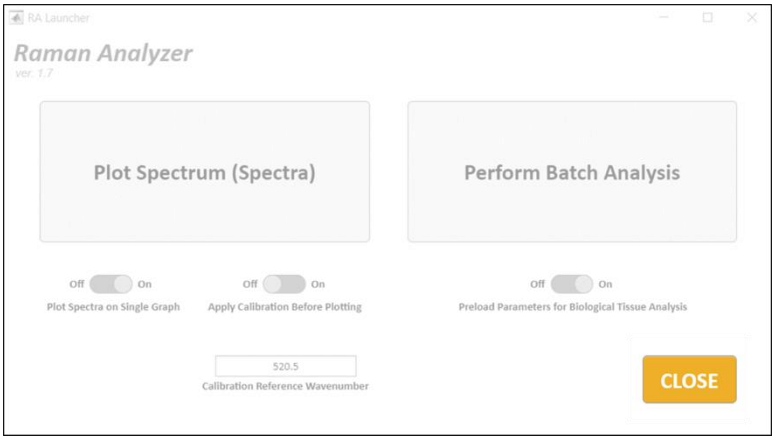

Figure 39: Click the 'CLOSE' button in the launcher window to exist Raman Analyzer.