FORAM®x3

Raman Spectral Comparator

User Manual

(Applications)

foster+freeman

(0083-2-01)
June 2013

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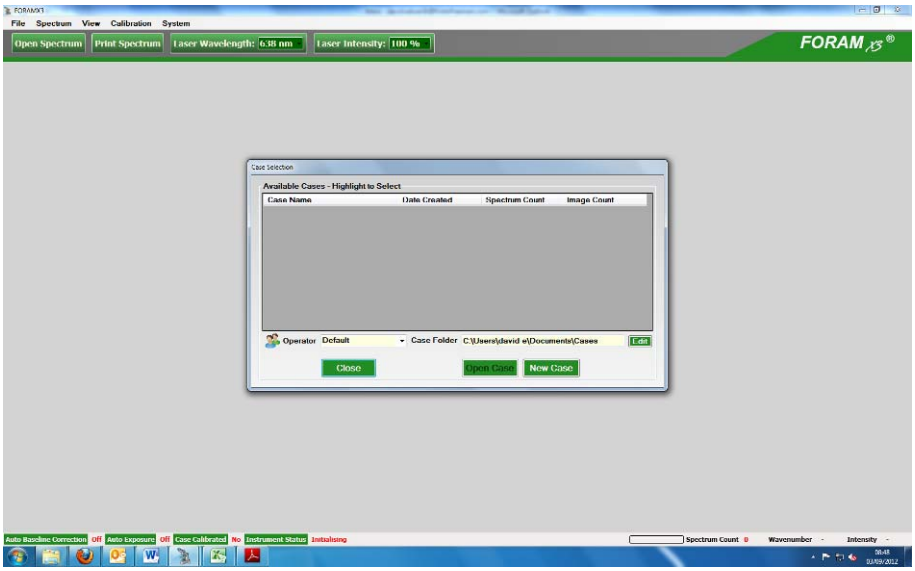
1: TURNING ON THE FORAMX3

The FORAMx3 should be switched on in the following order to ensure correct communication between the instrument modules.

1. Switch on the computer and wait for the Windows desktop to load.
2. Switch on the FORAMx3 (the power switch is found at the front of the module).
3. Start the FORAMx3 software using the shortcut icon on the PC Desktop.

4. The FORAMx3 log-in screen should now be displayed:



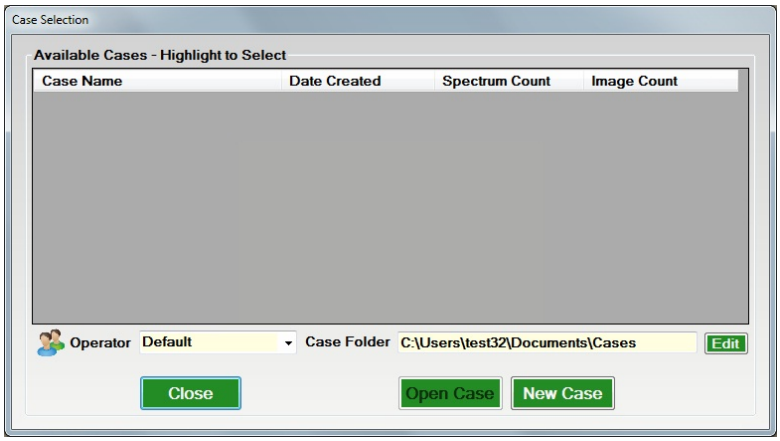
5. For optimum performance, the FORAMx3 should be allowed to warm up for at least 10 minutes before use.

2: LOGGING IN TO THE FORAMX3 SOFTWARE

When the software opens the user must choose whether to create a new case, open an existing case or work in a research (or non-casework) mode.

a. Using the Research (Non-Casework) Mode

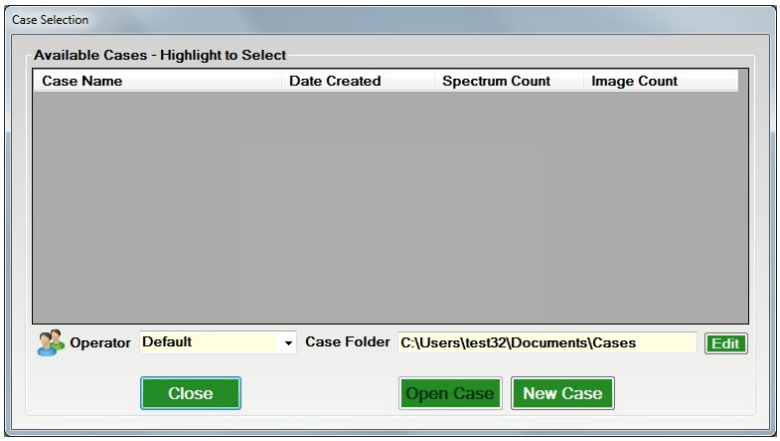
1. At "Case Selection" select *Close*. There will be no case-details or calibration log for this selection.



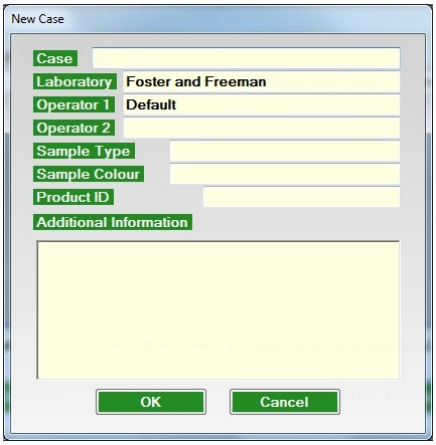
b. Creating a new case

1. Check that the Operator field is correct. Depending on the software set-up this may be 'Default' or the user's name.

2. At "Case Selection" choose select *New Case.*

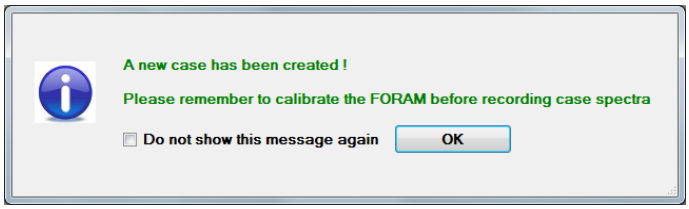


3. The user must then enter the case details:



4. The *Case* and *Operator 1* fields are mandatory. *Operator 1* is automatically populated with the Operator in use at "Case Selection".
5. Once all the relevant case details have been entered select *OK.*

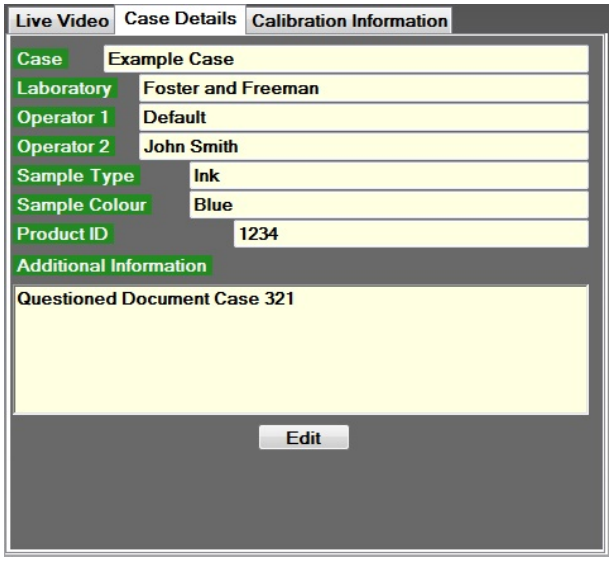
6. If *New Case* was selected by mistake select *Cancel.*
7. Once a New Case has been created the user will be reminded to calibrate the FORAMx3. Select *OK.*



8. This reminder will appear after every new case is created. If it is not required select the tick-box: *Do not show this message again.*

Viewing and Editing Case Details

1. Case Details can be viewed and edited after case creation.
2. Select *Case Details* to view the case details:

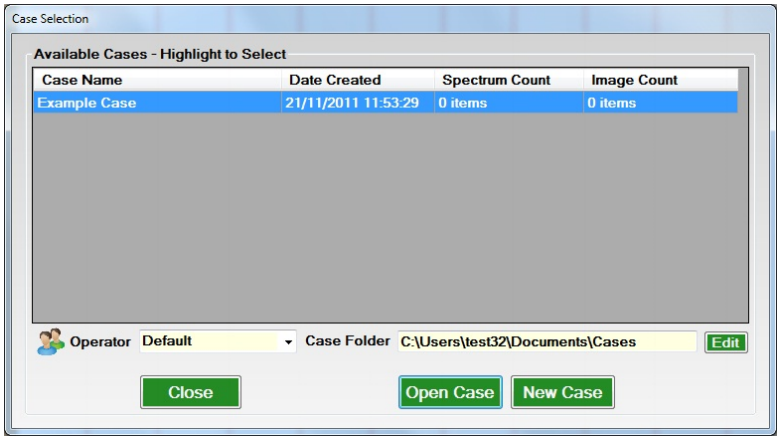


3. To edit the details select *Edit.*
4. To complete the process, select *Save*, which will have replaced the *Edit* button during editing.

c. Opening an existing case

1. Check that the Operator field is correct. Depending on the software set-up this may be ‘Default’ or the user’s name.

2. At “Case Selection” select the required case so that it is highlighted in blue.

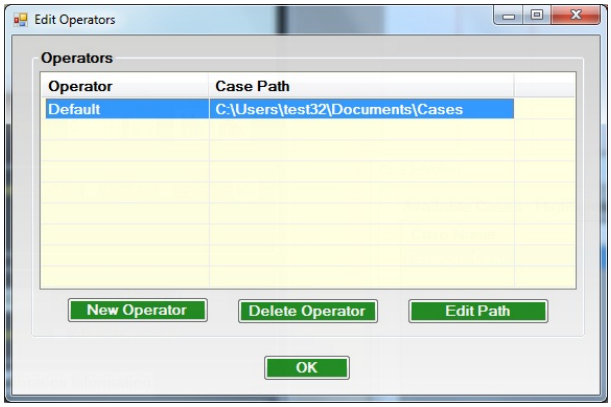


3. Select *Open Case.*

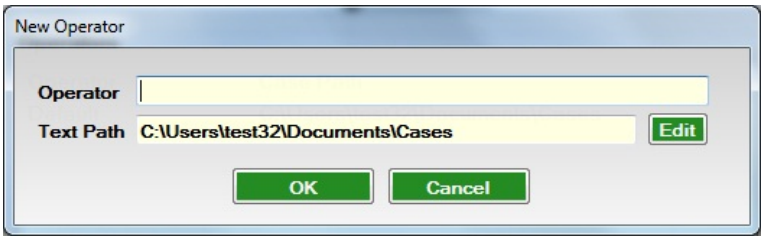
d. Creating a New Operator

1. Select *Edit* at “Case Selection”.

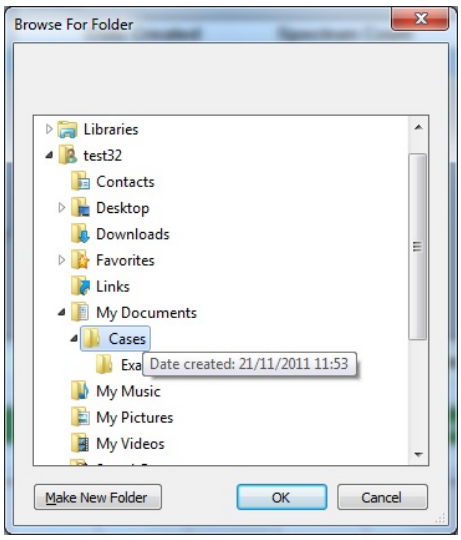
2. To create a new operator, select *New Operator.*



3. Enter an operator name in the *Operator* field:

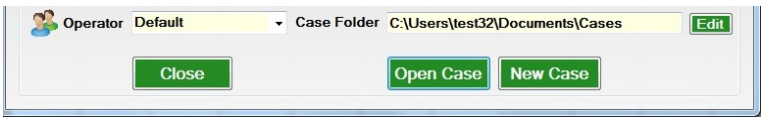


To choose an alternative save location, choose Edit next to the Text Path field and either specify a new field by choosing a folder to save to or choose Make New Folder to create a new save location. When finished select OK.

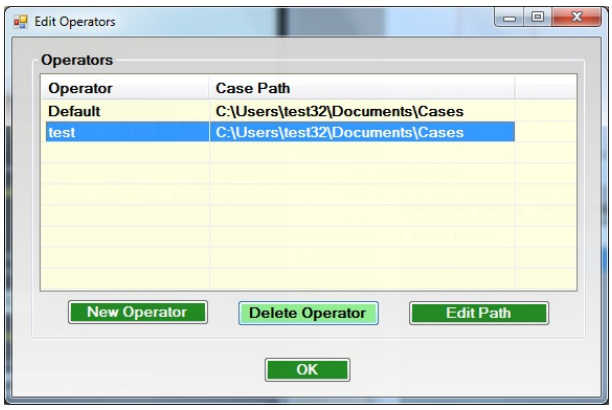


e. Deleting Operators

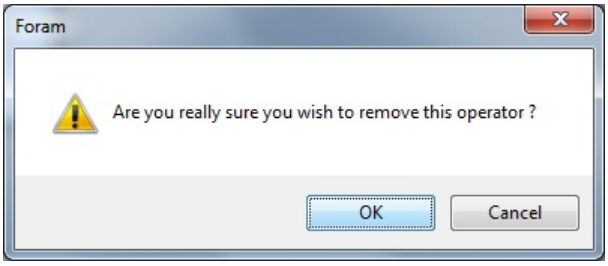
1. Select *Edit* at “Case Selection”.



2. To delete an operator select the operator to be removed so that the operator name is highlighted in blue. Then select *Delete Operator*.



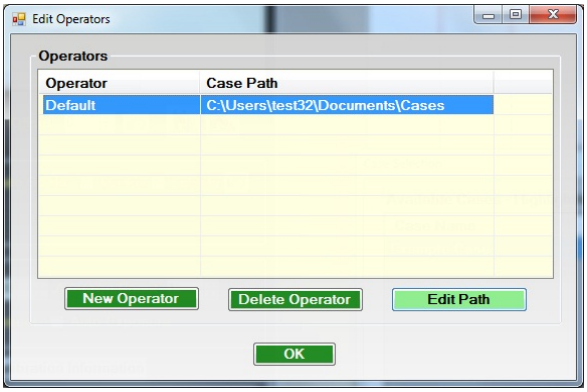
3. For the action to be completed select *OK* for confirmation.



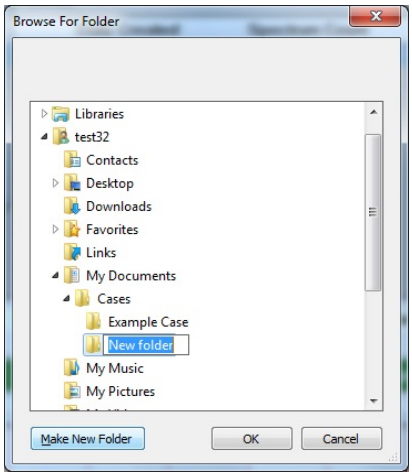
f. Changing Save Location

1. Select *Edit* at "Case Selection".

2. To change save location select the desired operator so that the operator name is highlighted in blue. Then select *Edit Path*.



3. Specify a new field by choosing a folder to save to or choose *Make New Folder* to create a new save location. When finished select *OK*.



3: SETTING UP THE FORAMx3

The following instructions may all be performed whilst the FORAMx3 is warming up.

a. Objective Lens Selection

1. The FORAMx3 has three objective lens: 5x, 10x, 20x and 40x. The objective lens in use is the one facing directly downwards.
2. To change between objective lenses rotate the turret to the desired lens.

Lens Magnification	Colour
x5	Red
x10	Yellow
x20	Green
x40	Blue

Please note that the precision of the technique will depend on the objective used. Selecting an objective with a higher magnification will allow the user to be more precise.

b. Sample Placement and Stage Manipulation

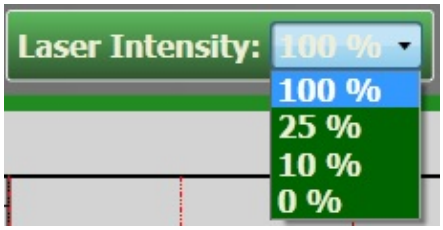
1. Place the sample directly in line with the illumination.
2. Focus on the sample using the Z control of the microscope manipulation stage.
3. Fine position the sample using the X and Y controls of the microscope manipulation stage.

N.B. The FORAMx3 is sensitive to ambient light. For best results ensure that the work is performed in a darkened room.

c. Laser Power Control

The power of the laser is one of the variables that can affect the Raman signal that is measured by the FORAMx3.

1. The laser power is adjusted using software control. To adjust the laser power, select the desired intensity from the drop down list.



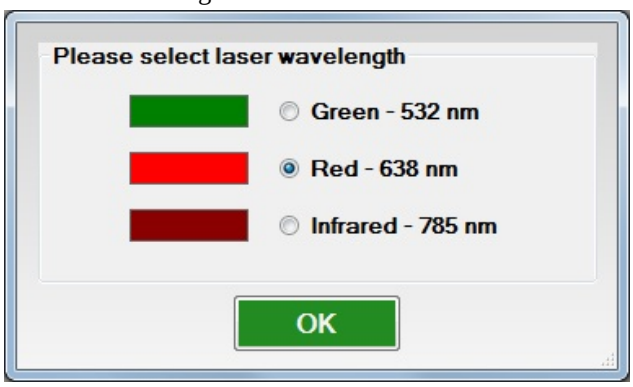
When examining dark or coloured samples it is recommended to take measurements at 10% laser power due to the sample potentially melting or photo-degrading at higher laser power settings.

d. Selecting Laser Wavelength

The FORAMx3 contains three separate lasers. Each laser operates at a different wavelength and the user can switch between the lasers using software control.

The user is prompted to select the initial laser following closing the log-in window, opening a case or creating a new case.

1. Following log-in select laser wavelength.



2. Select OK

The laser wavelength can be changed at any time from within the FORAMx3 software by selecting the desired laser wavelength from the drop-down list at the top of the screen.

e. Camera Control Options

White Balance

On occasion the camera image may not give a true representation of the colour of the sample. This can be rectified at any time by the following steps.

1. Place a piece of white paper under the microscope objective.
2. Select *White Balance* (under the camera image).

Crosshairs

Crosshairs can be used to aid the focusing of the laser spot.

1. Select the *Crosshairs* button to turn the crosshairs on and off.

Digital Zoom

A digital zoom function can be used to be more precise in the positioning of the laser spot.

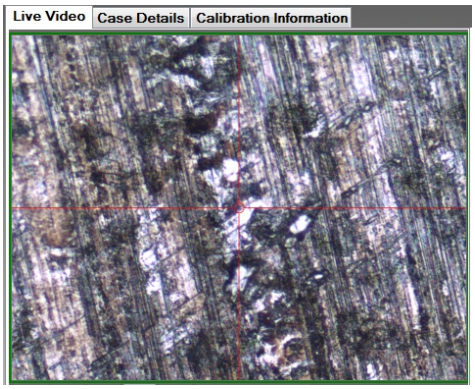
1. Move the *Zoom* slider bar to increase/decrease zoom.

Adjusting the Camera Position

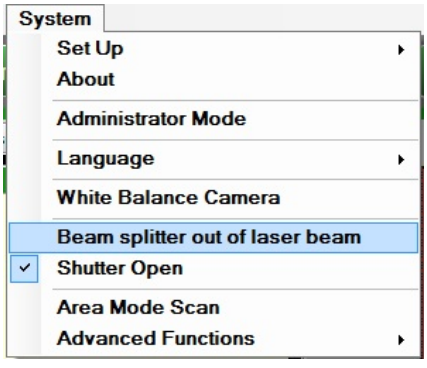
Each laser wavelength needs to be independently centred to align with the crosshairs seen on the live video image.

1. Adjust the laser power to *10%*.

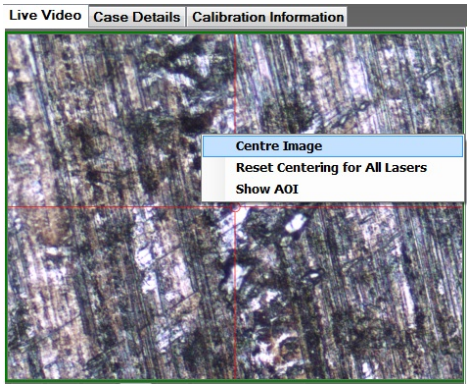
2. Place a piece of metal or foil under the microscope objective and focus on the surface of the foil.



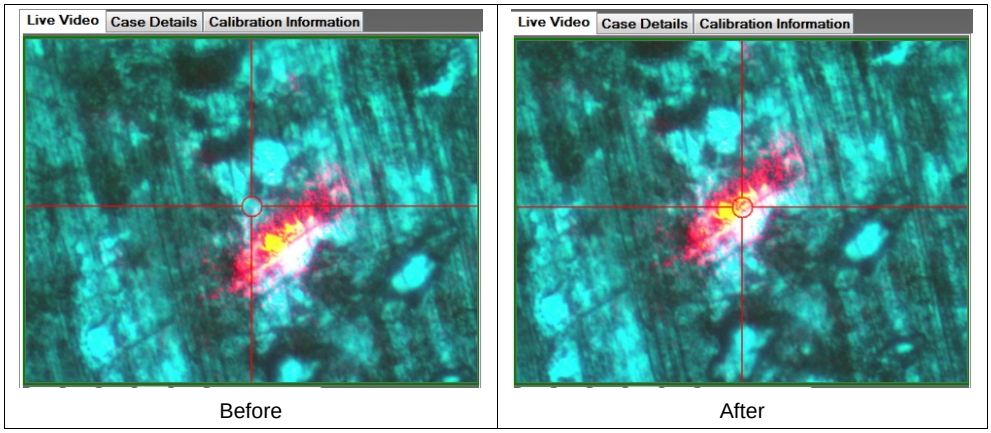
3. Select *Beam splitter out of laser beam*:



4. The laser spot of the selected laser should now be visible. Using the mouse right-click on the *Live Video* image.



5. Select *Centre Image*.
6. Adjust the position using the *arrows* until the laser spot is in position.



7. Select *Close.*

N.B. This process will need to be completed for each laser wavelength. Once the first laser is finished, change laser wavelength and repeat the above process.

Please note that all positions can be reset using the *Reset Centering for All Lasers*.

4: CALIBRATING THE FORAMX3

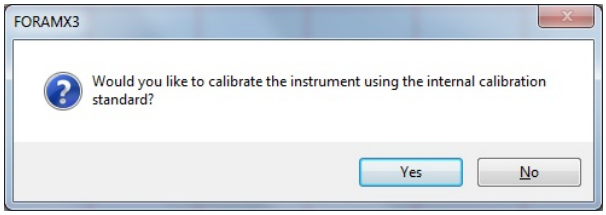
The FORAMx3 is calibrated during production. For regular wavelength calibration checks the "Internal Polystyrene Standard" is used. Polystyrene produces a Raman spectrum with peaks at characteristic wavelengths. Polystyrene was selected as the calibration standard in accordance with the ASTM International Standard **E 1840 – 96 Raman Shift Standards for Spectrometer Calibration.**

The FORAMx3 wavelength should be calibrated each time it is used. Each time a new case is created there will also be a prompt to calibrate the case for case integrity. An automatic calibration carried out when a case is open will have its information added to a calibration log.

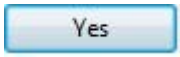
The FORAMx3 also has photometric calibrations for the 532 nm and 785 nm laser wavelengths. Two calibration standards, NIST 2242 and NIST 2241 are supplied for calibrating the 532 nm and 785 nm wavelengths respectively. Photometric calibrations should be checked on a weekly basis.

Internal Raman Shift Calibration

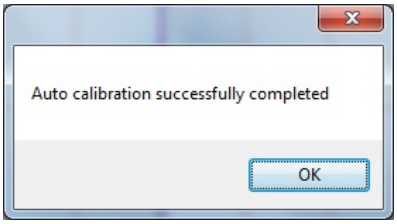
Upon entering the FORAMx3 software or whenever the laser wavelength is changed the software will prompt the user to calibrate the system using the newly selected laser.



1. Select *Yes* to calibrate the FORAMx3 using the internal standard



2. Once the system has recorded a spectrum and calibrated the system the following dialogue box will appear. Select *OK* to confirm the internal calibration.

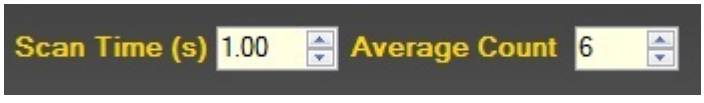


b. Manual Raman Shift Calibration

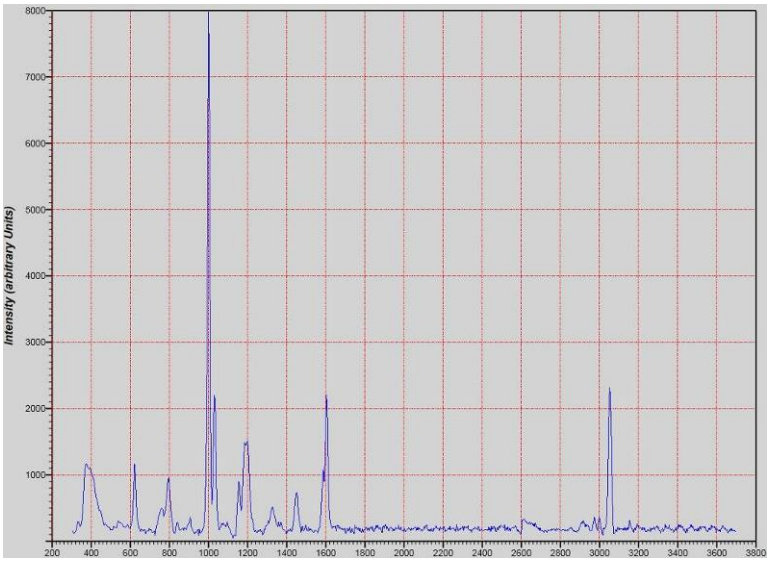
1. Position a polystyrene bead directly under the laser.
2. Focus on the polystyrene bead and fine tune the position using the microscope translation stage. Start with the surface of the polystyrene bead should be clearly seen in the live video. Once this has been achieved make a quarter turn of the focus wheel to focus in to the sample.

Focusing in to the polystyrene sample will provide a more intense spectrum.

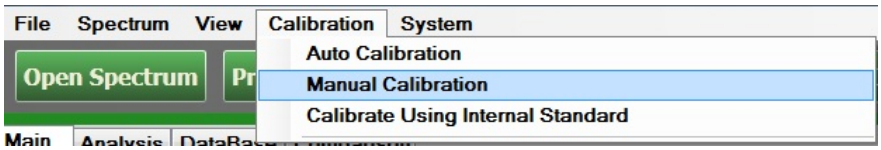
3. Set the *scan time* to 1 second *average count* to 6.



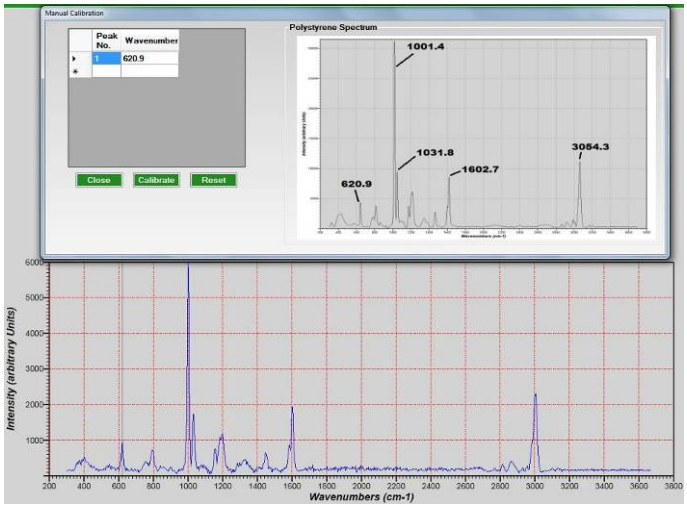
4. Record a spectrum of the polystyrene bead.



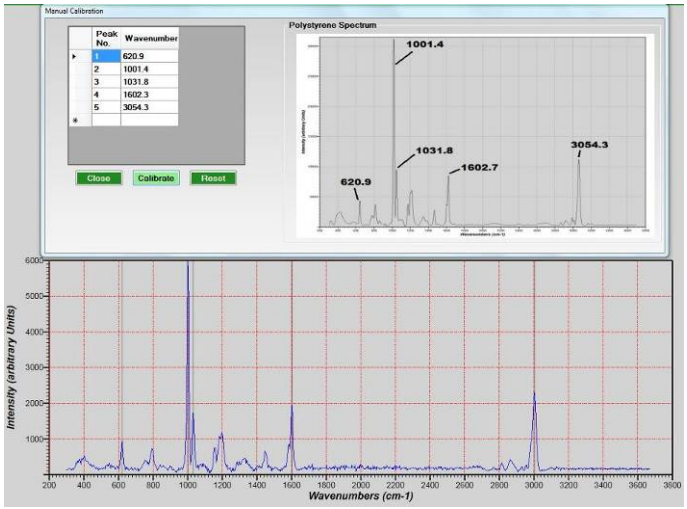
5. Navigate to the Calibration menu and select *Manual Calibration.*



6. Select the peaks as highlighted in the Manual Calibration window. This will populate the fields for peak number and position.



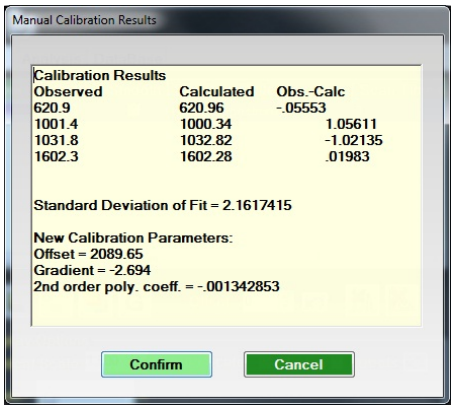
Starting manual calibration



All peaks selected

7. If a mistake was made during peak selection, select *Reset.*

8. Following peak selection select *Calibrate.*
9. Select *Confirm* to confirm the manual calibration.



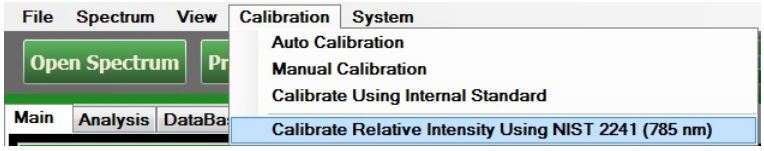
10. Select *Close* to complete the manual calibration process.

Please note that during Manual Calibration peaks must be selected in the correct order. Failure to do so will not allow the system to calibrate.

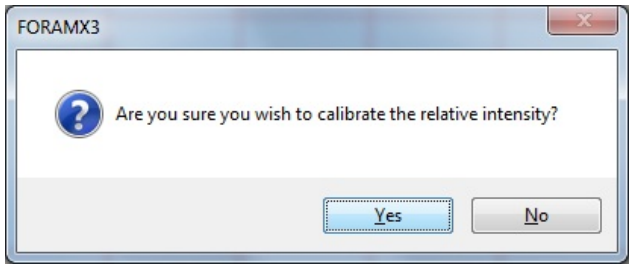
c. Photometric Calibration of FORAMx3

1. Select the *532 nm* or *785 nm* laser wavelength.

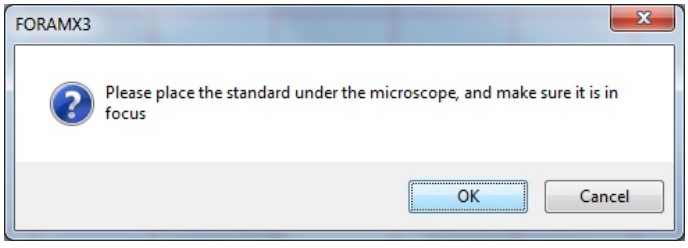
2. Delete all displayed spectra.
3. Focus on the NIST calibration standard.
4. Select *Calibrate Relative Intensity Using NIST (standard number and laser wavelength change depending on laser wavelength selected).*



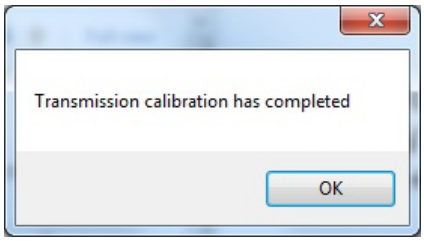
5. Select *Yes* to confirm the calibration process:



6. Select *OK* to confirm that the sample is in position:



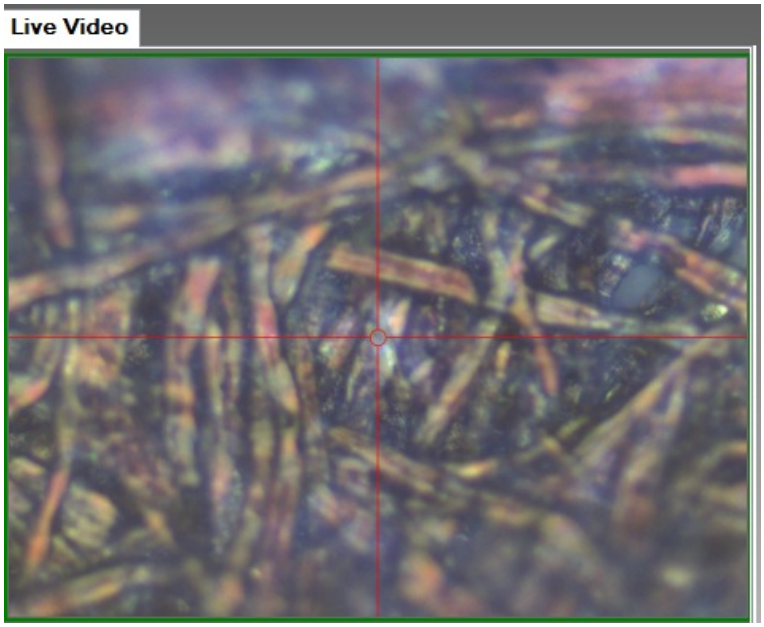
7. Following completion select *OK*:



5: RECORDING SPECTRA AND IMAGE CAPTURE

a. Recording and Saving Individual Spectra

1. Place the sample under the objective lens and bring the object image in to focus using the microscope stage.

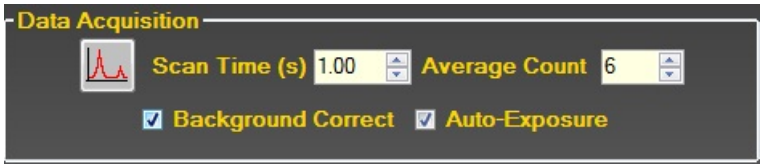


2. Enter the number of *Average Counts* for the sample being analysed. Increasing the *Average Count* improves the signal-to-noise ratio but also increases the data acquisition time.

Scan Time (s)	Minimum Recommended Average Count
> 1	6
0.01 – 1	12

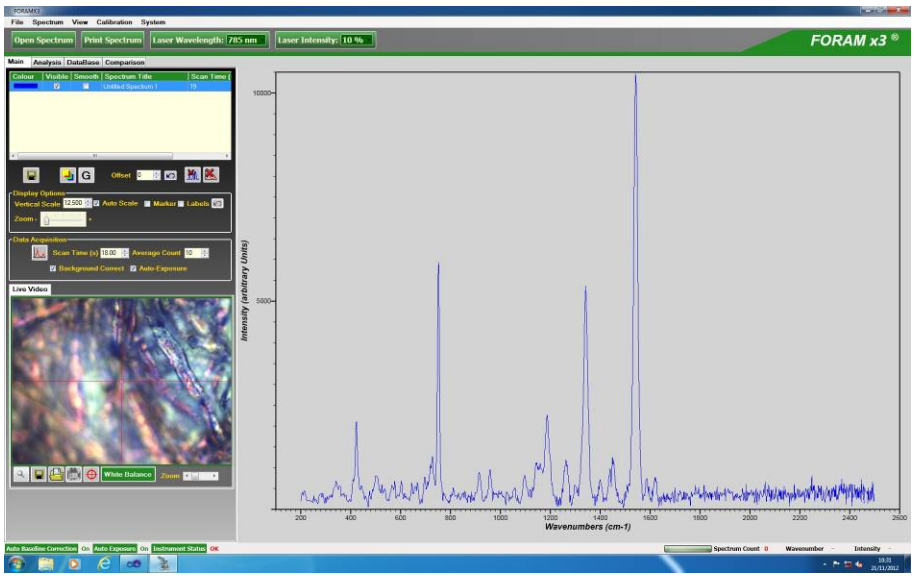
3. Select the *Auto Exposure* tick box. *Auto Exposure* will automatically determine the optimum scan time for the sample being analysed.

4. Select the B*ackground Correct* tick box. *Background correct* will automatically background correct the spectrum being recorded to allow for better spectra comparison.

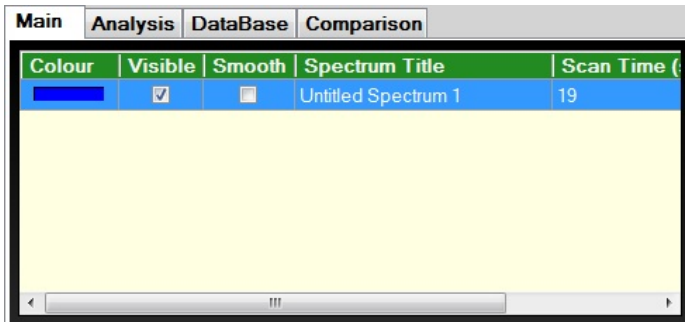


5. Record the spectrum of the target object using by selecting the *Record Spectrum* button.

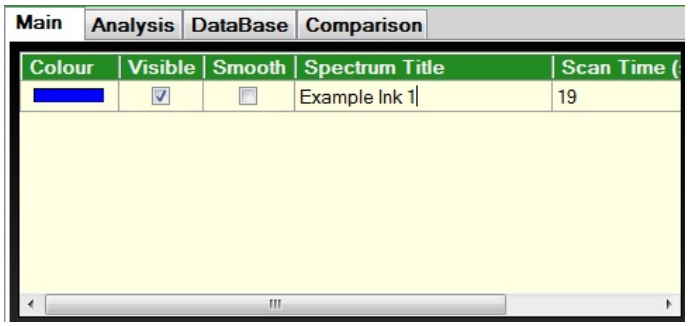
6. The spectrum of the target object is displayed on the spectral graph to the right of the screen; the image updates as each new count is acquired.



7. When the spectrum acquisition is finished, the spectrum details appear under the *Main* tab.

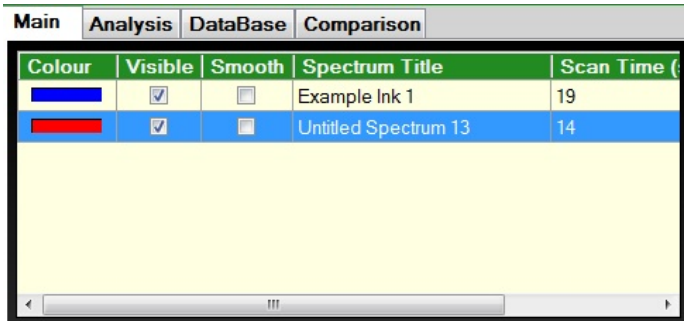


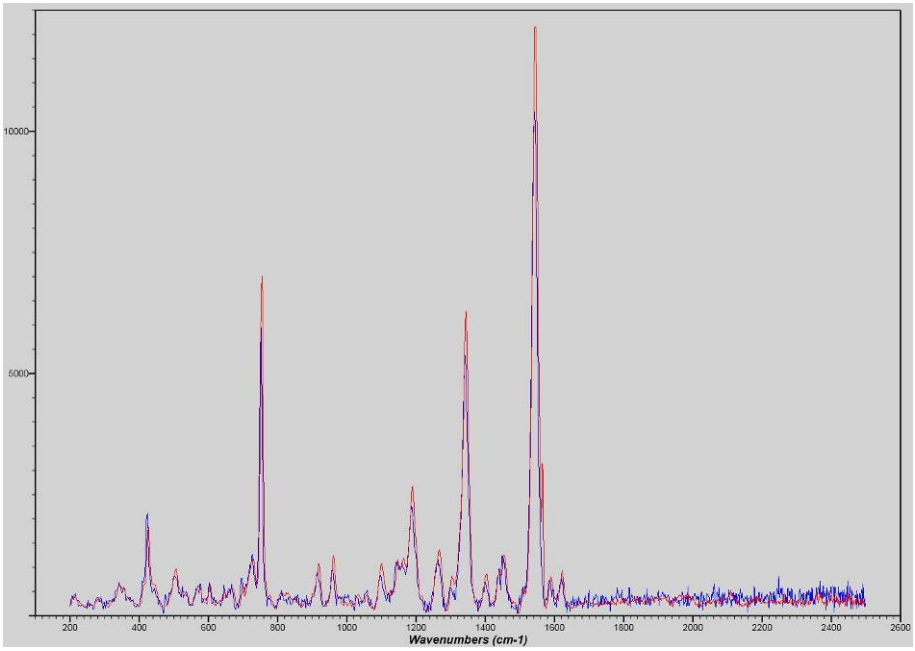
8. Select the *Spectrum Title* field and enter an appropriate file name in accordance with your local laboratory procedure.



9. Press the *Return* key on the keyboard to complete the renaming process.
10. Select *Save Spectrum* and store in an appropriate location on the computer.

11. Repeat steps 1 - 9 to record the spectrum of any further samples in the case.
12. Each new spectrum is shown on the spectral graph in a different colour and the same colour is used to identify the record in the *Main* tab table.



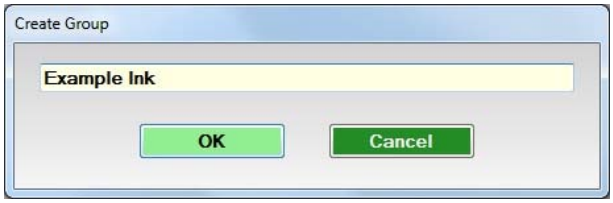


b. Recording group spectra

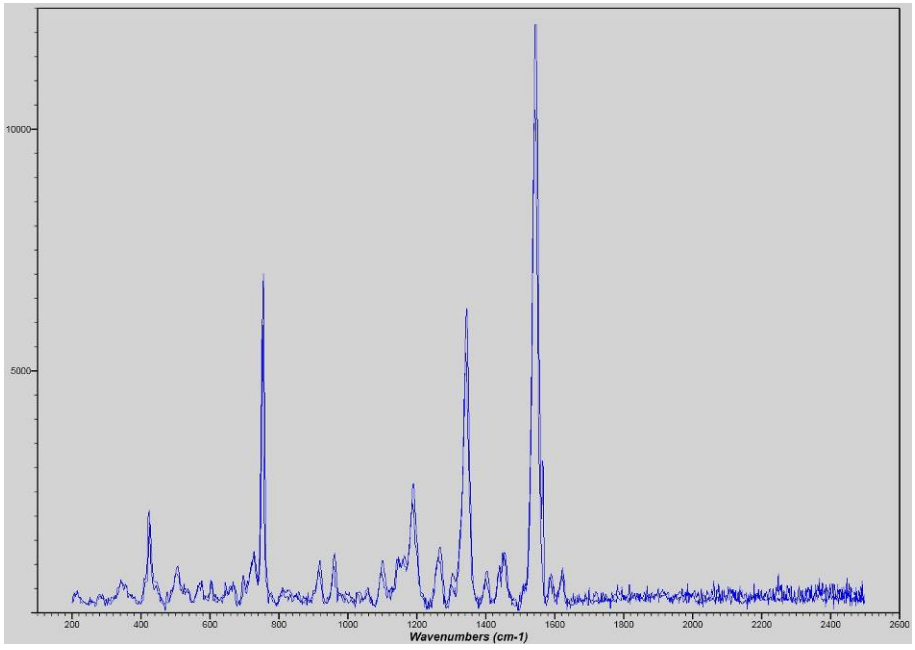
To save time entering sample details for each individual measurement, it is possible to *Create a Group* for samples from a specific source.

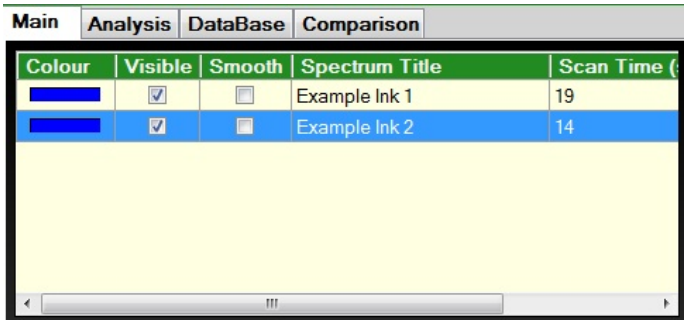
1. Before recording any spectra, select the *Create Group* tool button.

2. Enter the required *Group* name into the pop up window displayed.



3. Record the Raman spectrum of the sample using the method detailed in Steps 1 – 7 of *Recording and Saving Individual Spectra* (Focus on target object => *Record Spectrum*).
4. Each new spectrum is shown on the spectral graph using the same colour and is identified by the *Group* name and a sequentially numbered value (1, 2, 3, ... , etc.) under the *Main* tab.





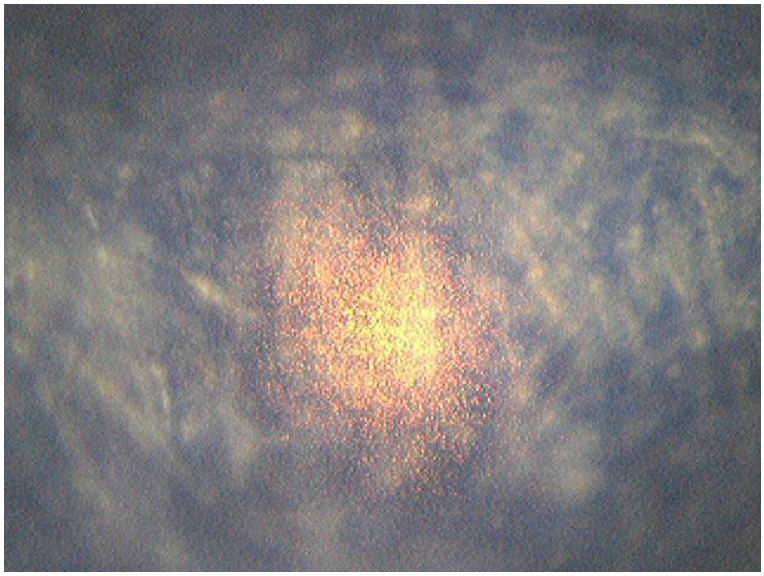
5. Highlight the relevant spectrum by selecting it using the left mouse button so that the spectrum is highlighted in blue. Select *Save Spectrum* and store the record in an appropriate location on the computer in accordance with your local laboratory procedure.

6. The *Group* name will be assigned to each new sample analyzed until the *Create Group* function is used to set up a new group or the FORAMx3 software is closed. If a sample from a different source is accidently analyzed whilst the Group function is active, it's possible to save the record using the correct identifier by selecting the relevant *Spectrum Title* and entering the appropriate details.

c. Saving a Sample Image

In addition to saving the spectrum of an analysed sample, the FORAMx3 software also enables the operator to save the image of the analysed sample seen on the *Live Video* screen. Select the *Save Image* tool button below the Live Video screen.

The saved image shows the sample from which the measurement was obtained without measurement area cross-hairs superimposed.



6: SPECTRAL DISPLAY OPTIONS

Loading Spectra for Analysis

Previously stored spectra are opened using the *Open* command from the *File* menu or using the shortcut tool button on the toolbar.

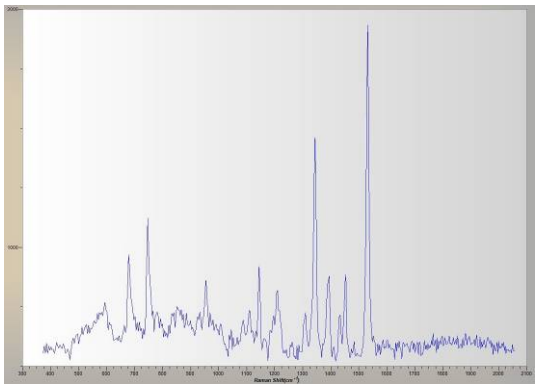
Changing Spectrum Display Colour

This option avoids confusion arising due to spectra from different sources have been assigned the same default display colour when originally recorded.

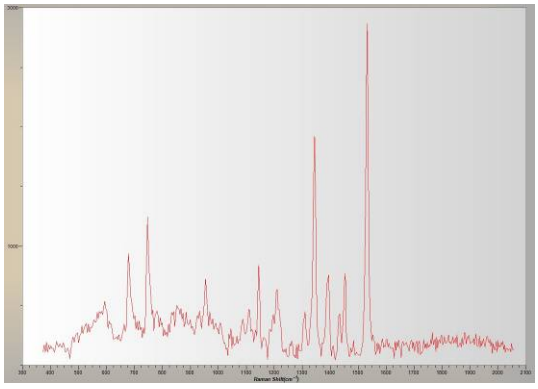
1. To change the display colour of a spectrum, select the required spectrum from the Main tab menu and either *Change Colour of Selected Spectrum* from the Main tab right-click menu or using the *Change Colour* tool button.

2. Select a suitable colour from the pop up window shown and select *OK*.

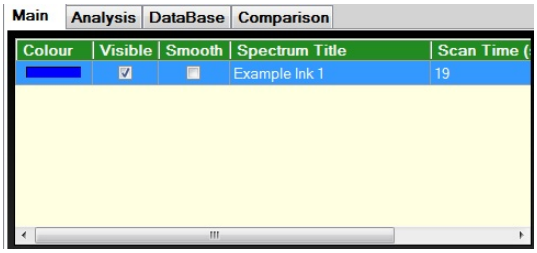
3. The selected spectrum's colour is updated on the spectral graph and in the *Main* tab table.



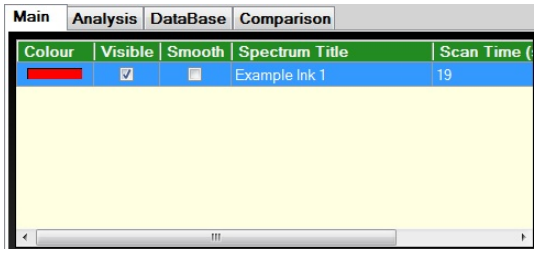
a) Original Display Colours



b) New Display Colours



a) Original display colour

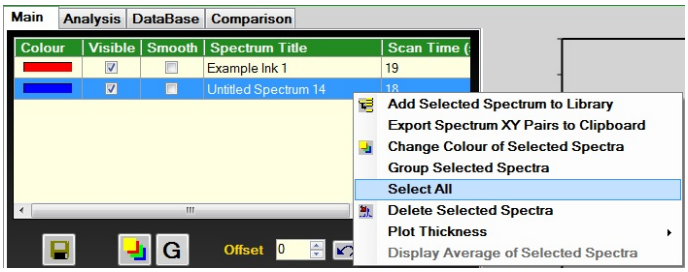


b) New display colour

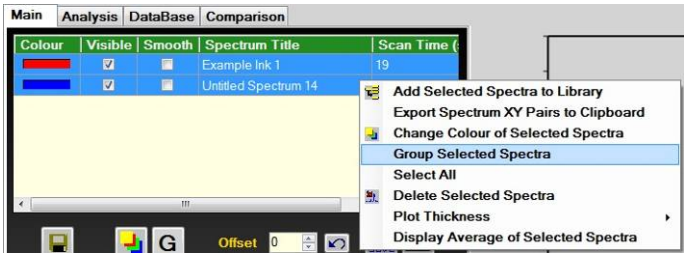
Group Selected Spectra

This option enables the spectra from different samples from the same source to be grouped together. Grouped spectra are shown on the screen the same colour and treated by the FORAMx3 software as coming from a single source.

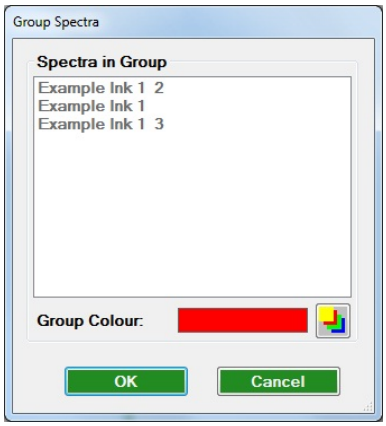
1. Select the records from the *Main* tab table to be Grouped using the *Select All* option from *Main* tab mouse menu or by using the cursor and the standard Microsoft features using the <CTRL> key to select individual records or the [SHIFT] key to select a run of record.



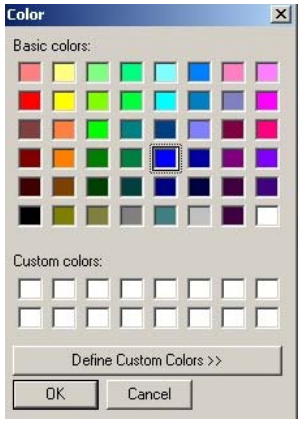
2. Right click on the *Main* tab table again and select *Group Selected Spectra.*



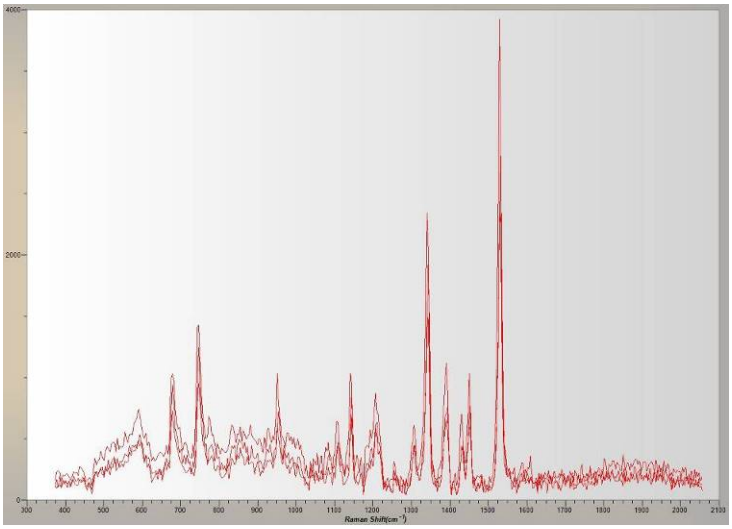
3. The following message is shown:



4. Select the *Group Colour* tool button, pick a suitable colour from the pop up window shown and select *OK.*



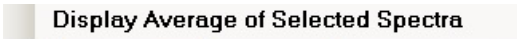
5. The chosen colour will appear in the *Group Spectra* window. Select *OK* to confirm or use the *Group Colour* tool button to make a different selection.
6. The selected spectra and associated records in the *Main* tab table will also be shown in the same colour.



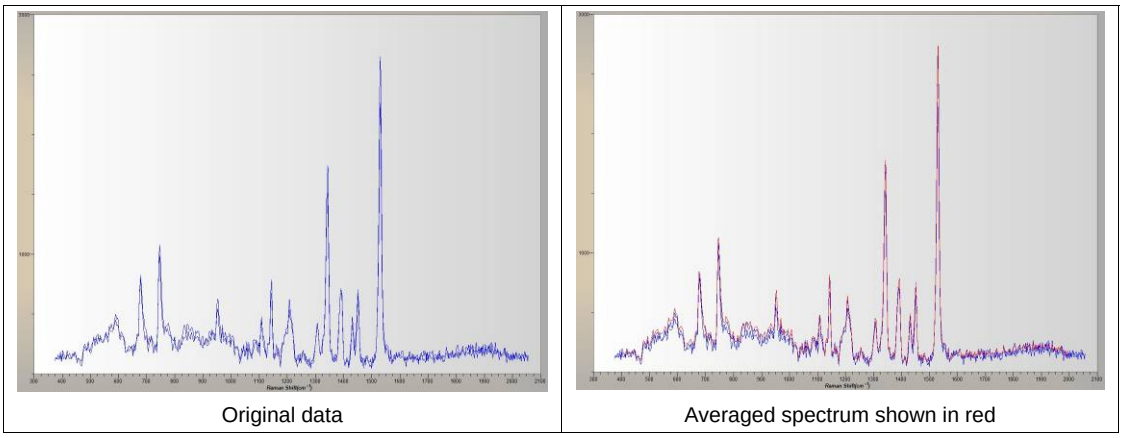
Displaying Averaged Spectra

This option generates an averaged spectrum from two or more selected spectra. The function does not alter any of the original data files used in the averaging calculation. If comparisons are carried out using averaged spectra it is recommended that the original data files used to generate the average spectrum are saved so that the source data is available for review if required.

1. Select the records to be averaged from the *Main* tab table.
2. Select the *Display Average of Selected Spectra* option from the Main tab right-click menu.



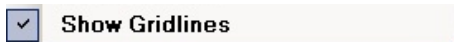
3. The *Averaged Spectrum* is displayed in a new colour on the spectral graph and added to the records listed in the *Main* tab table.

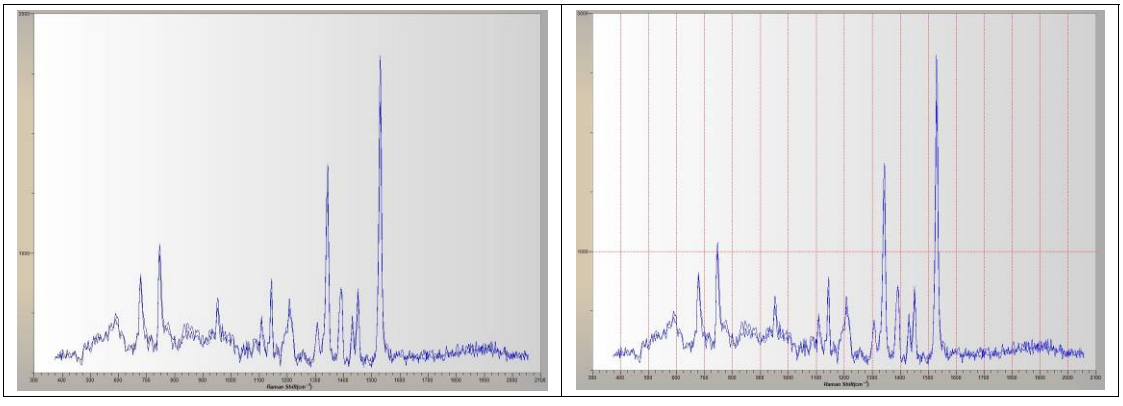


Original data | Averaged spectrum shown in red

Gridlines

Gridlines are automatically shown on the spectral graph. To removes these lines from the display and printed report, deselect the *Show Gridlines* option on the *View* menu.



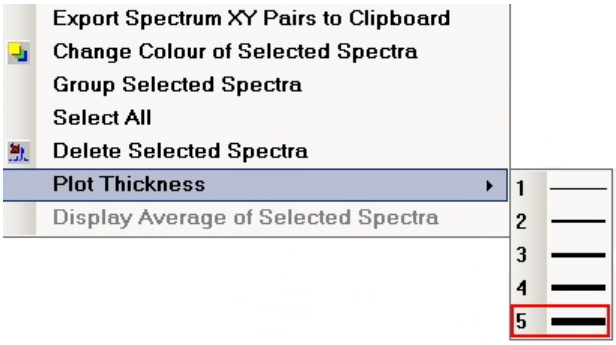


The colour and appearance of the *Gridlines* may also be changed to suit individual preferences using the *Set Up* option from the *System* menu.

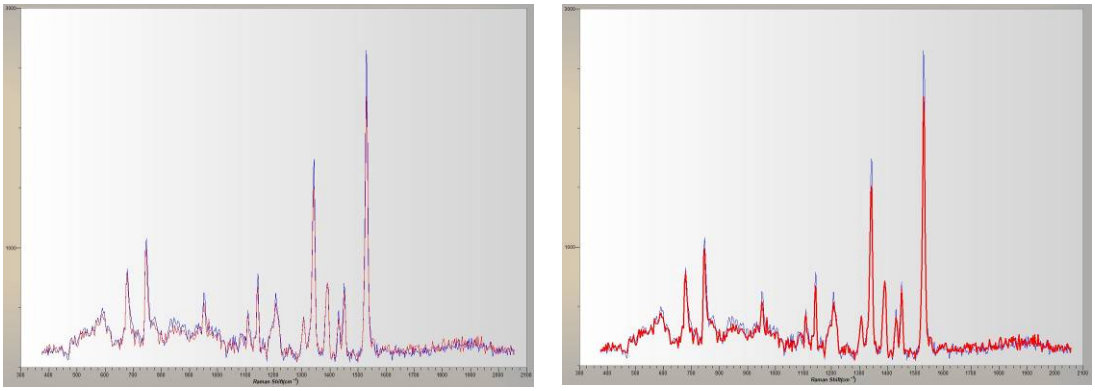
Plot Thickness

This option allows users to change the thickness line used on the spectral graph for the selected spectrum or spectra. It may be used to highlight a specific spectrum where spectra are overlapping and not clearly distinguishable by colour or where use of the *Offset* function is not desired.

1. Select the spectrum or spectra from the *Main* tab table.
2. Right click on the *Main* tab table again and select the *Plot Thickness* required from the menu shown.



3. The graphical screen is updated to show the new spectrum.

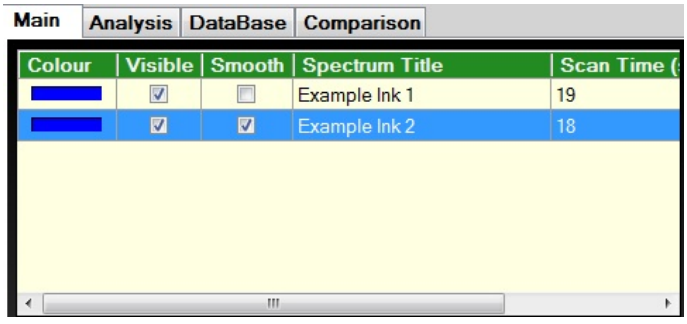


Smoothing spectra

Although the *Average Count* function will smooth spectra by removing noise there is a supplementary software function for smoothing spectra.

Within the FORAMx3 software the smoothing is carried out using a 5-point Savitsky-Golay filter.

To smooth spectra select the *Smooth* tickbox in the *Main* tab Spectra Details window:



7: BACKGROUND CORRECTION

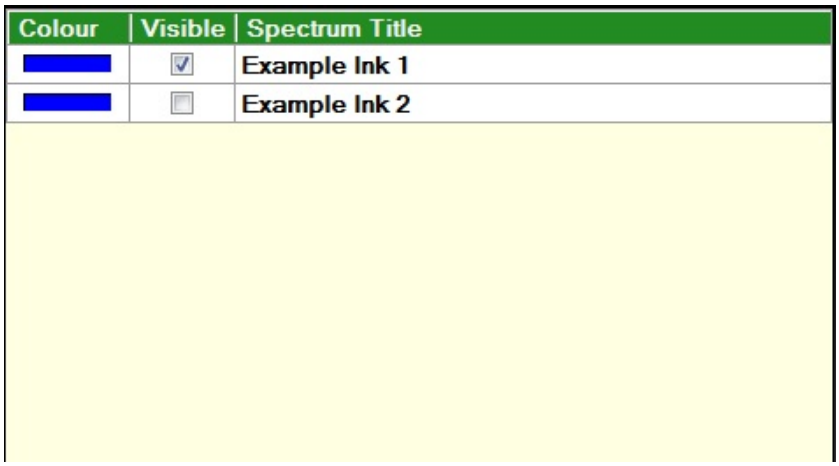
For easier spectral comparisons it is recommended that spectra are background corrected. Background correction allows for spectra to be overlaid, spectra to be offset in a more presentable style and finally smaller peaks will become more visible as a result of removing the unused area beneath the spectrum baseline.

Background correction can be carried out automatically and manually.

a. Automatic Background Correction

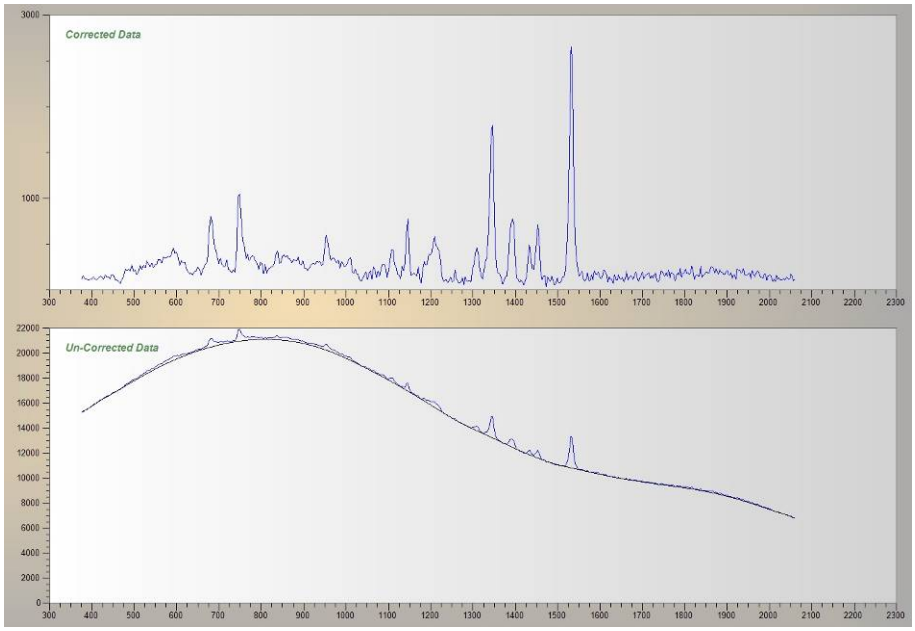
1. Either record a spectrum as detailed in **Recording Spectra** or open a spectrum as detailed in **Spectral Display Options**.
2. Select the *Analysis* tab.

3. Select the spectrum to be background corrected by selecting the corresponding tick box.



4. *Automatic Background Correction* is selected by default.
5. Select the *Automatically Correct Background* button.

The uncorrected spectrum will display with the background correction line shown. The background corrected spectrum is also shown.



6. To remove an existing background correction, select the *Remove Background Correction* button.

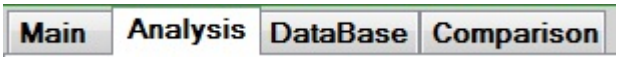
7. After one spectrum has been background corrected all remaining spectra can also be corrected by selecting the *Apply to All Spectra* button:

The *Apply to All Spectra* function will create the best background correction for each spectrum, rather than using the background correction created for the first spectrum and applying that to all remaining spectra.

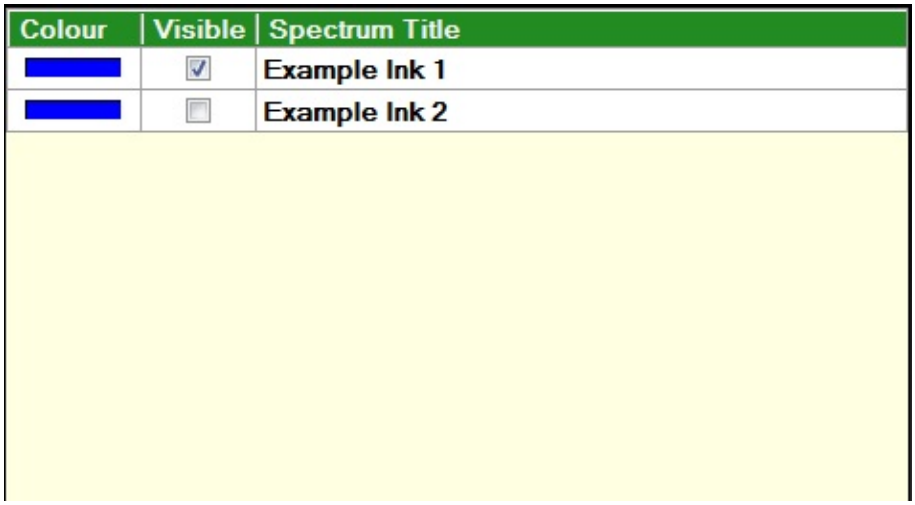
8. If more than one spectrum has been background corrected it is possible to remove all background corrections. This is done by selecting the *Remove from All Spectra* button:

b. Manual Background Correction

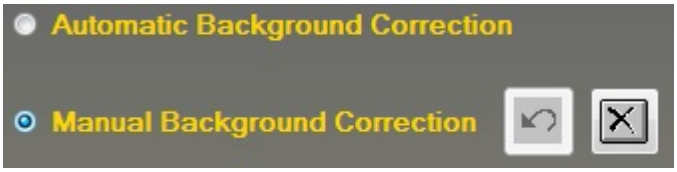
1. Either record a spectrum as detailed in **Recording Spectra** or open a spectrum as detailed in **Spectral Display Options**.
2. Select the *Analysis* tab.



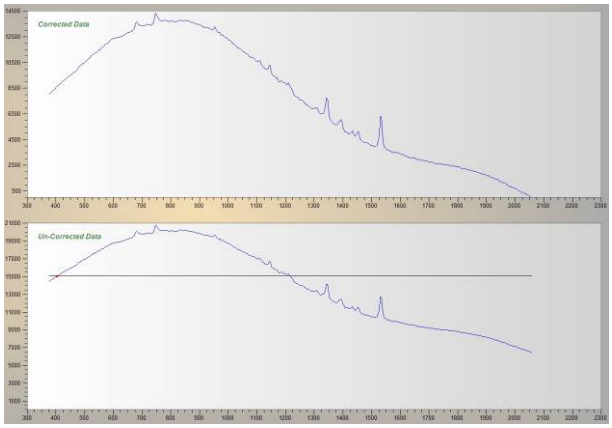
3. Select the spectrum to be background corrected by selecting the corresponding tick box.



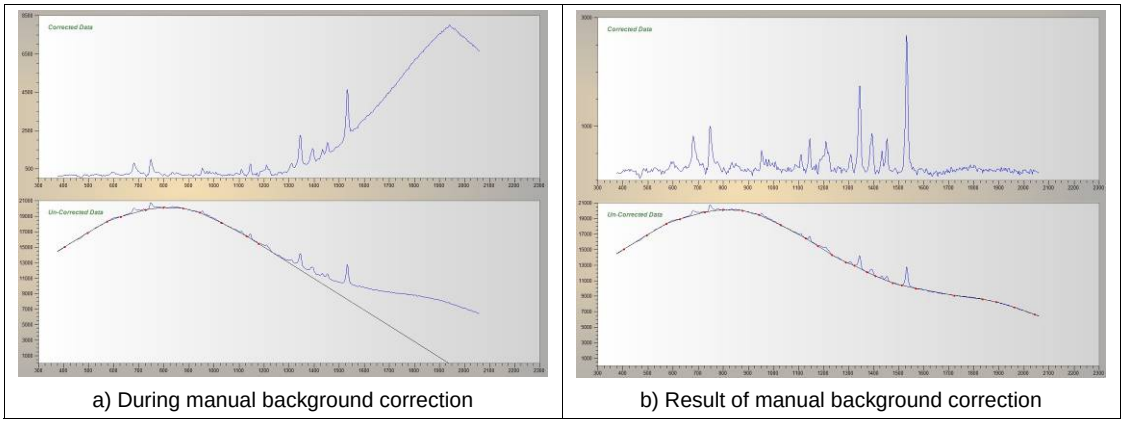
4. Select the *Manual Background Correct* radio button.



5. Select points on the baseline of the *Uncorrected Data* to start background correcting the spectrum.



6. As points are added to the baseline of the *Uncorrected Data* the *Corrected Data* screen will update showing the current result of the manual background correction.



a) During manual background correction

b) Result of manual background correction

7. To remove an existing background correction, select the *Remove Background Correction* button.

8. After one spectrum has been background corrected all remaining spectra can also be corrected by selecting the *Apply to All Spectra* button.

The *Apply to All Spectra* function will create a background correction for the other spectra using the same point positions that the user selected. It is not recommended to use this function in *Manual Background Correction*.

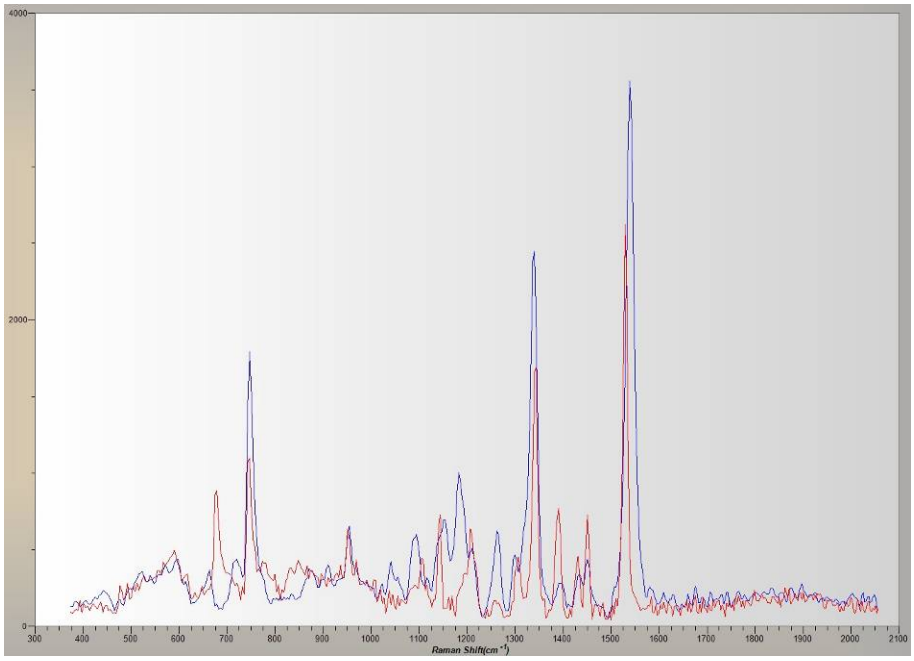
9. If more than one spectrum has been background corrected it is possible to remove all background corrections. This is done by selecting the *Remove from All Spectra* button.

8: SPECTRAL COMPARISON OPTIONS

a. Visual Comparisons

Overlay of Spectra

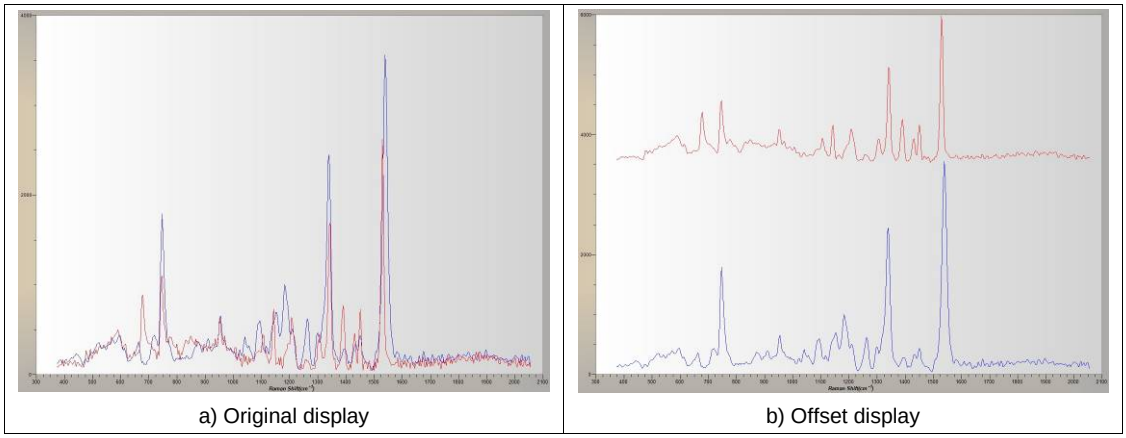
Once spectra have been baseline corrected the spectra will be overlaid (on top of each other). This allows for easier examination for presence and absence of peaks between spectra.



Offset of Spectra

This option enables the vertical positions of selected spectra to be increased by a fixed amount across the full wavelength range so altering their position displayed on screen.

1. Select the required spectrum or spectra from the *Main* tab table.
2. Use the  buttons to increase / decrease the *Offset* as required.
3. The spectral graph is updated as the offset is changed.



a) Original display

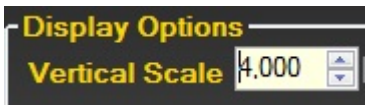
b) Offset display

4. A second spectrum may now be selected and a different *Offset* applied if desired.
5. The *Reset Offset* tool button is used to remove all offsets applied.

Vertical Scale

The spectral graph vertical scale is automatically scaled to show the acquired spectra in full. This option enables the user to disable the *Auto Scale* function and manually set the vertical scale.

1. Use the  buttons to increase / decrease the vertical scale as required. The *Auto Scale* function is automatically disabled.

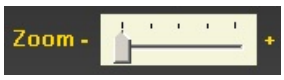


2. To reactivate the automatic scaling, select *Auto Scale* check box:

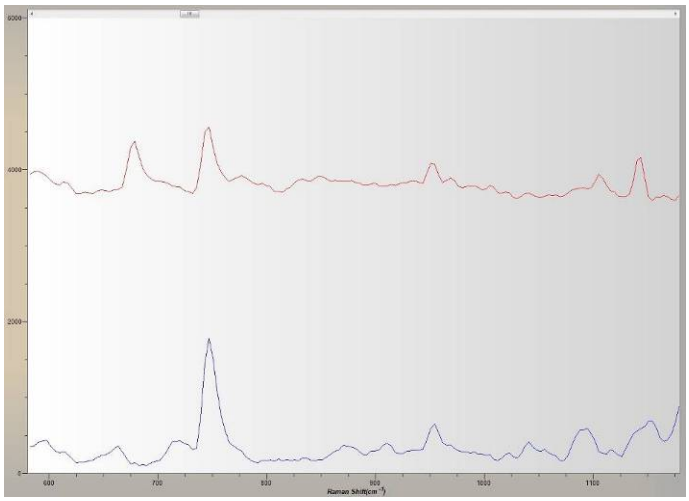
Horizontal Zoom

The *Zoom* function enables users to magnify the spectral graph from the full, default range from 300cm^{-1} to 2100cm^{-1} to a maximum zoom range of 450cm^{-1}. The scroll bar at the top of the spectral graph is used to scan through the different wavelengths in the collected spectra. Unlike *Zoom Window* the spectra shown are not normalised when displayed.

1. Move the slider on the *Zoom* bar to the desired magnification:



2. Use the scroll bar at the top of the spectral graph to scan through the wavelength range.



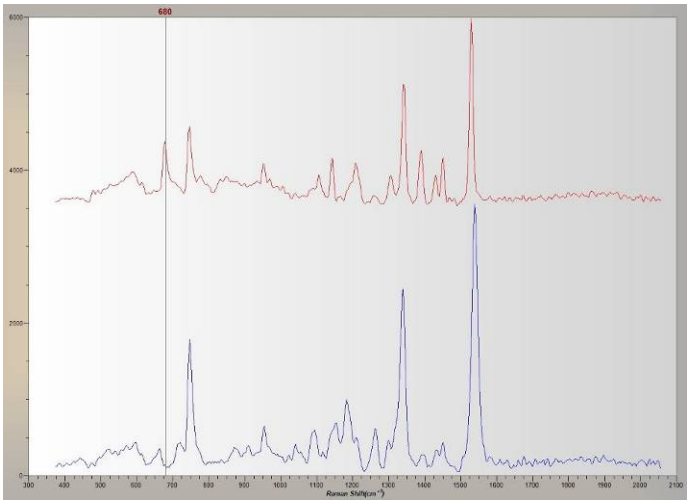
Please note these spectra have also been offset.

Marker

This option places a vertical line on the spectral graph. The marker line can be dragged to any position throughout the wavelength range shown to help users align spectral features at a given wavelength.

1. Select the *Marker* check box in *Display Options*; the marker line is automatically shown at 1000cm^{-1}.

2. Use the mouse to drag the marker to any position required desired in the spectral graph; the relevant wavelength is automatically shown at the top of the screen.

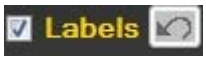


Please note that the marker is not printed.

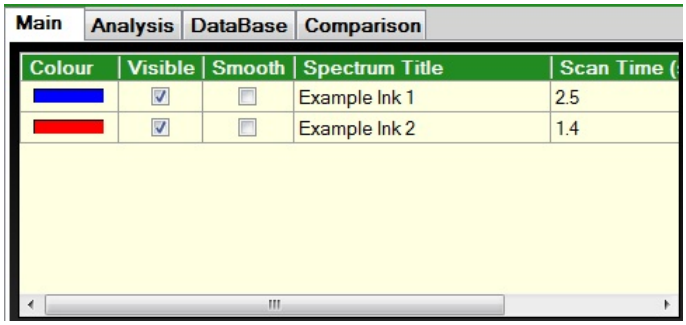
Labels

This option provides users with a means of placing labels on the recorded spectra.

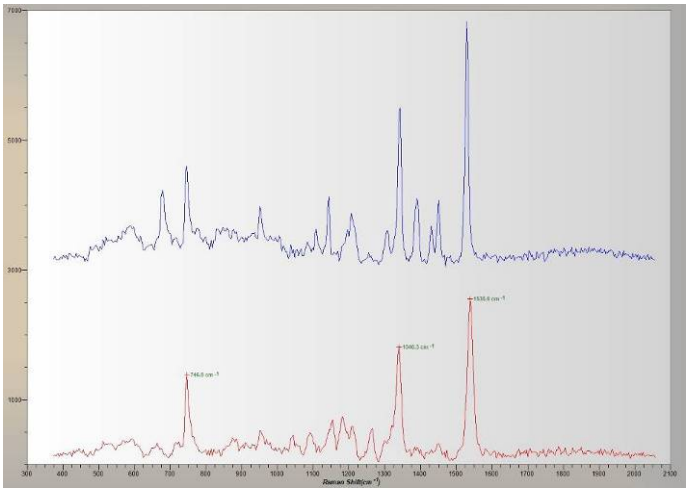
1. Select the *Labels* check box in *Display Options.*



2. Select the spectrum in the *Spectra Details* that you wish to add labels to. Once the spectrum has been selected it will be highlighted in blue.



3. Using the mouse left click on the peak you want to label. The label will then appear.



4. To remove a label, right click with the mouse. This will remove the last added label.
5. To remove all labels select the *Clear Labels* button.

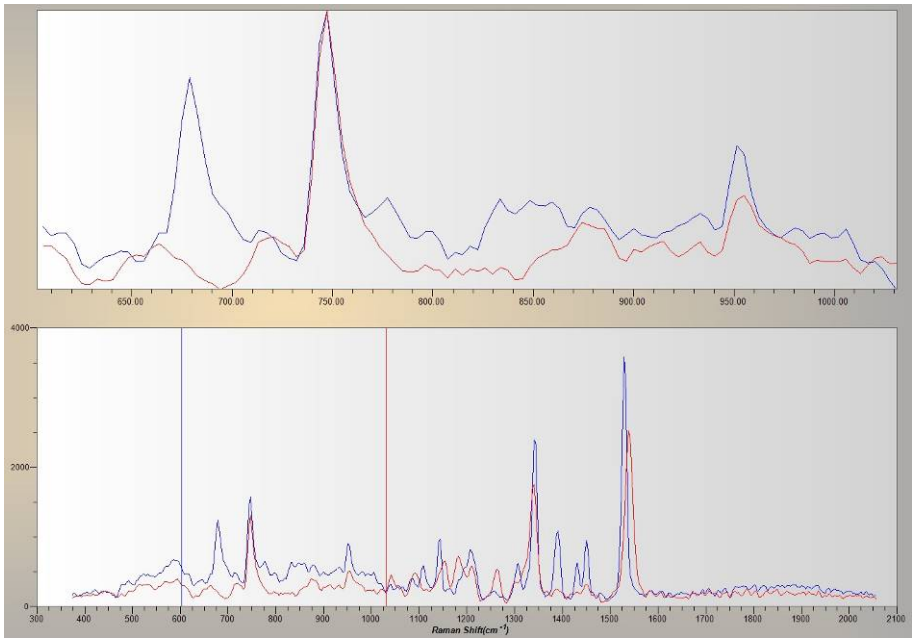
The colour and appearance of the *Gridlines* may also be changed to suit individual preferences using the *Set Up* option from the *System* menu.

Zoom Window

The *Zoom Window* provides users with a magnified view of a selected area of the overall wavelength range enabling more detailed comparisons to be made. The function also normalises the spectra displayed in the upper, *Zoom Window* making it easier to compare the significant spectral features between samples with significantly different depths of colour.

1. To open the *Zoom Window*, select *Zoom Window* from the *View* Menu.

2. This opens a second window above the original spectrum/spectra in which only the data from the wavelengths between the blue and red markers are shown.



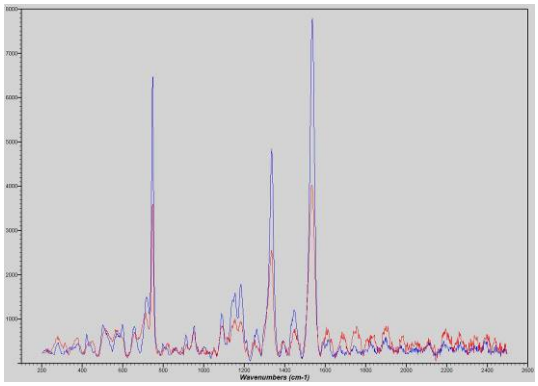
3. To change the zoomed wavelength range shown, drag the blue and red markers using the mouse cursor to the new positions required.

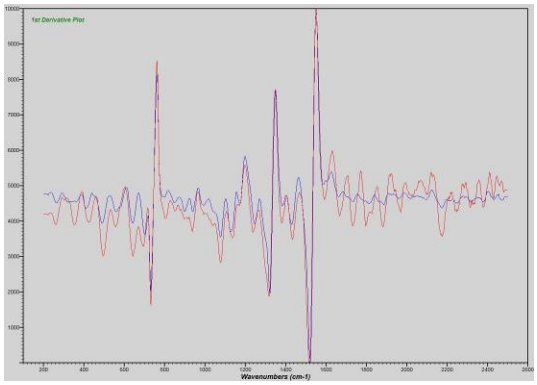
Statistical Comparisons

1st and 2nd Derivatives

Raman spectra often have many peaks that can be used to compare different samples. Where the spectra obtained contain very few prominent features, discrimination of the samples is far more difficult as minor differences are more difficult to detect by eye. Calculating the 1st derivative of a spectrum, which shows the change in slope of the spectrum with wavenumber, can often yield more useful information than the original data in this situation as 1st derivative function enhances subtle differences in the original spectrum such that features which were not readily identifiable in the original spectrum are clearly observed in the transformed data.

In the following images the spectra obtained from 2 samples have been used to illustrate the effect of using the 1st Derivative function.





The function must, however, be used with caution as differences in the 1st derivative spectrum may be observed where samples originate from the same source. These false discriminations are most likely to occur where there is a wide variation in the intensity of the results obtained for individual samples. The spectra in the example above highlight these differences.

The 2nd derivative is, as the name suggests a derivative of the derivative. This may be used to highlight differences in cases for which the 1st derivative does not provide sufficient information.

1. Load the required data files into the FORAMx3 software
2. Select the *Comparison* tab:

3. Select the *First Derivative* or *Second Derivative* tool button from *Display Options*:

4. The spectral graph will update to show the *First Derivative/Second Derivative* spectra for the data files shown in the *Comparison* tab menu.
5. To return to the original spectrum display, select *Clear*:

Grouping of Spectra

The FORAMx3 software provides two spectral corrections (**Multiplicative Scatter Correction** and **Standard Normal Variate)** that can allow the user to interpret the data more easily. The two corrections are similar in their function. It is recommended that the two methods are tried in turn to determine which gives optimum results.

Multiplicative Scatter Correction Plot

1. Select the *Comparison* tab.
2. Select the *MSC* from *Display Options*:

Standard Normal Variate Plot

1. Select the *Comparison* tab:

2. Select the *SNV* from *Display Options*:

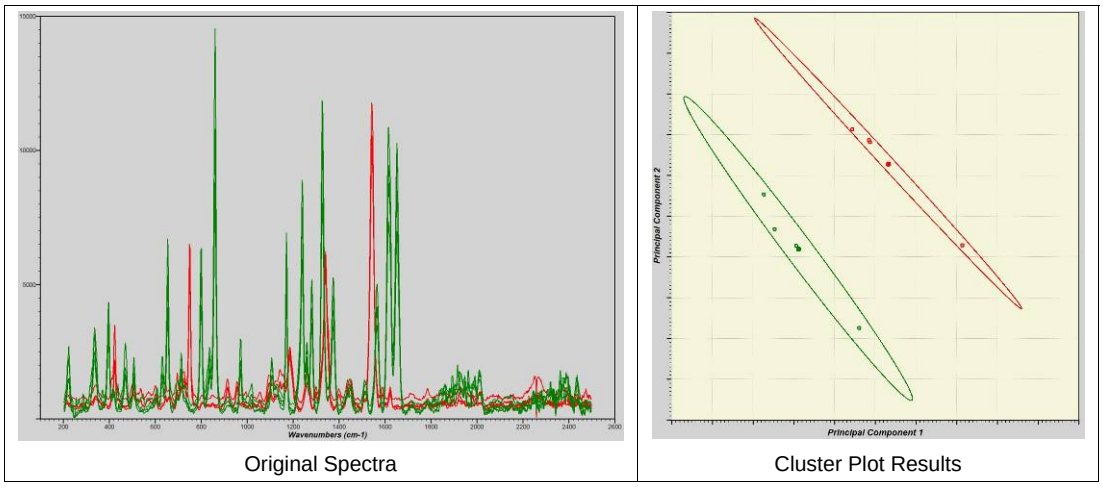
Statistical Comparisons

The FORAMx3 software also provides two statistical methods for objectively examining the results (**Principle Component Analysis (PCA)** and **Polar Quantification Plot (PQS)**) that allow the user to interpret the data more easily.

Both options require that the user group sets of spectra from each sample. The software will then compare the groups of spectra using either of the two statistical methods to aid the user in discriminating the samples. This can be achieved by using *Cluster Plots*.

PCA and ***PQS*** analysis can only be used when four or more spectra are present in each sources being compared and the *Group* function has been applied to the results so that the set of spectra from each source are identified for the statistical calculations.

PCA

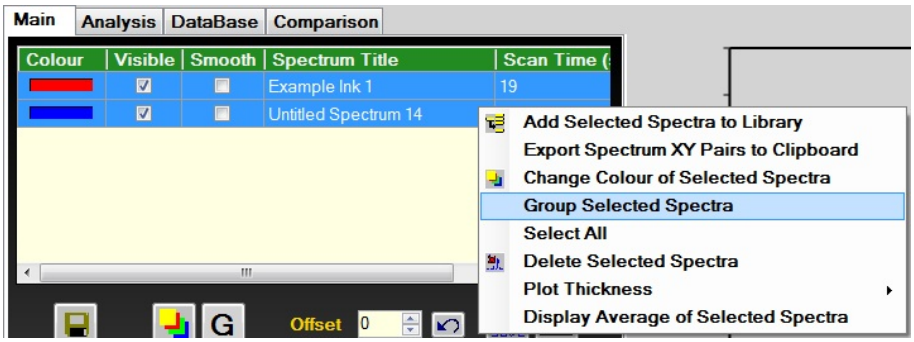


Original Spectra | Cluster Plot Results

The x/y axes represent Principal Component 1 and 2 that have been calculated by the ***PCA*** algorithm. Whilst values are associated with each of these axes, they are not shown on the graph because the absolute differences between sources are not important, only whether or not the confidence limit ellipses overlap or not.

1. Load the required data files into the FORAMx3 software.

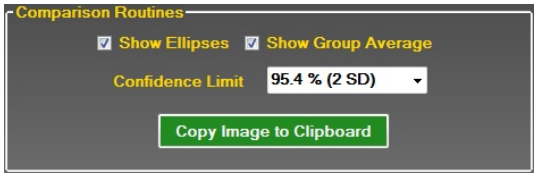
2. From the *Main* tab screen, *Group* the spectra from each source using the *Group Selected Spectra* function.



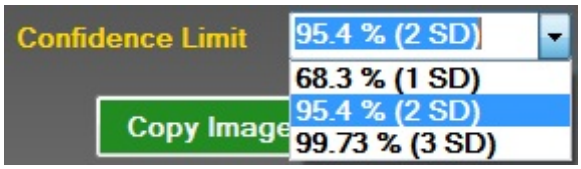
3. Select the *Comparison* tab.

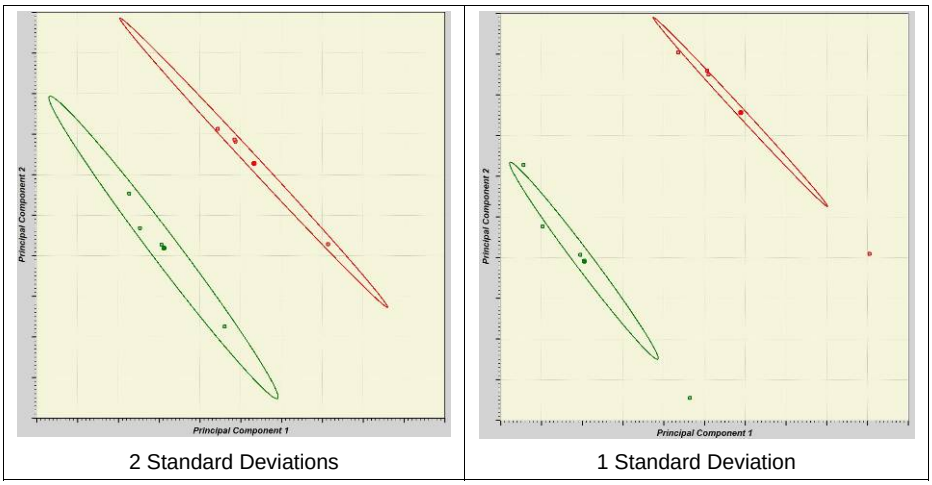
4. Select *PCA Plot.*

5. The spectral graph is updated to show the results of the comparison performed.
6. If desired select the options to show *Confidence Ellipses, Show Group Average.*



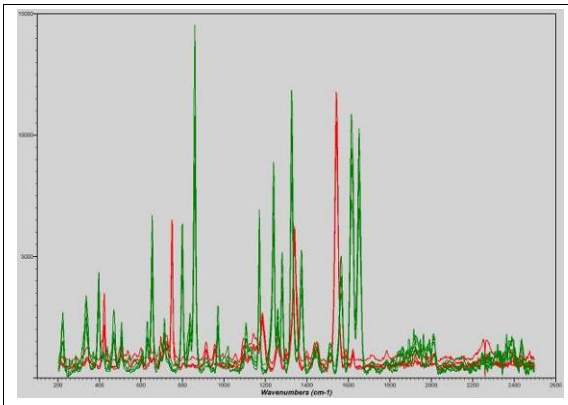
7. If desired change the *Confidence Limits.*



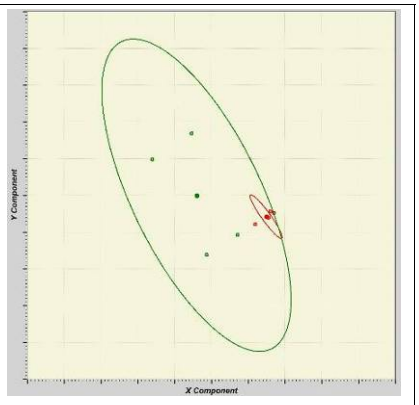


2 Standard Deviations | 1 Standard Deviation

PQS



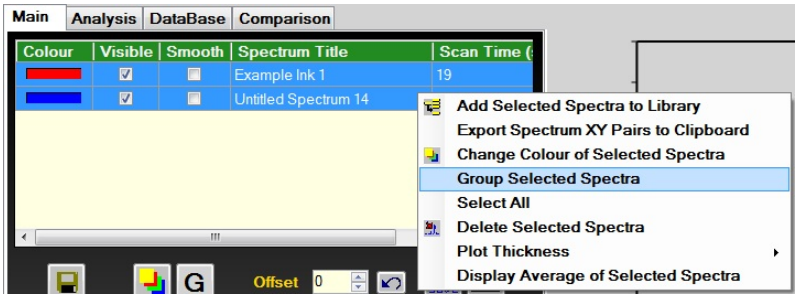
Original Spectra



Cluster Plot Results

The x/y axes represent the X Component and the Y component that have been calculated by the ***PQS*** algorithm. Whilst values are associated with each of these axes, they are not shown on the graph because the absolute differences between sources are not important, only whether or not the confidence limit ellipses overlap or not.

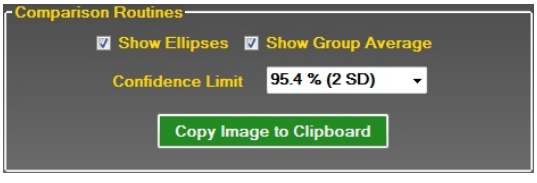
1. Load the required data files into the FORAMx3 software.
2. From the *Main* tab screen, *Group* the spectra from each source using the *Group Selected Spectra* function.



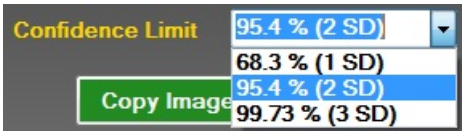
3. Select the *Comparison* tab.

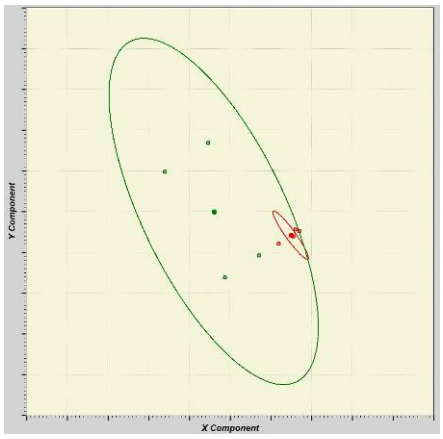
4. Select *PQS Plot.*

5. The spectral graph is updated to show the results of the comparison performed.
6. If desired select the options to show *Confidence Ellipses, Show Group Average.*

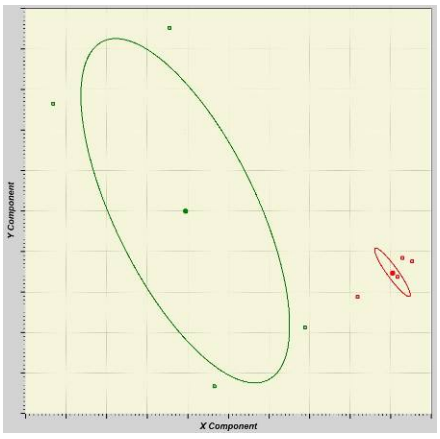


7. If desired change the *Confidence Limits.*





2 Standard Deviations

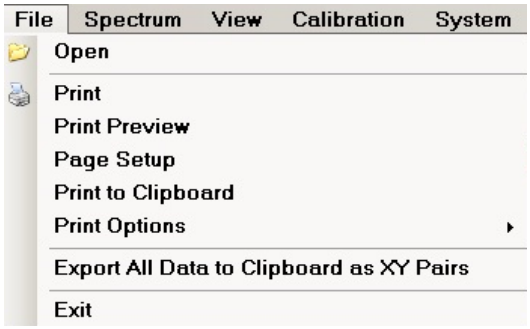


1 Standard Deviation

9: EXPORTING DATA AND PRINTING RESULTS

The FORAMx3 software provides the user with a range of options for exporting or printing the spectral data obtained during examinations. Data can be exported in text or graphical format and copied into local laboratory formats for further processing or reporting. An overview of the options available is provided below.

File Menu



Print

Prints the information currently displayed in the window to the right of the screen. On the *Main* tab screen the spectral graph will be printed; on the *Analysis* tab screen the selected spectrum in its uncorrected form with baseline will be printed; and on the *Database* tab screen the search and match spectrum will be printed.

Print Preview

Generates a preview of the information to be printed.

Page Set Up

Enables the user to select paper size, orientation and margin size for printed reports.

Print to Clipboard

Copies spectral graphs to the Windows Clipboard enabling the user to paste the results into supported applications such as Microsoft Word.

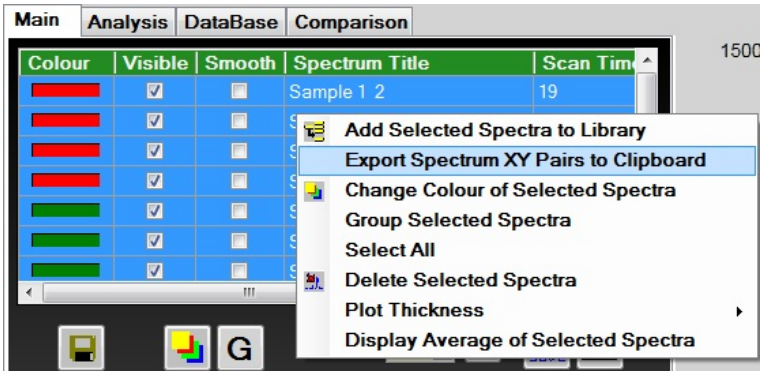
Print Options

Enables the user to determine whether the Spectrum report includes: a) the spectral graph and associated data file names or b) the spectral graph, data file names and the *Scan Time* and *Average Counts* used to acquire the data.

Export All Data to Clipboard as XY Pairs

Exports the intensity value at every other wavenumber recorded for all data files in the *Main* tab table to the Windows Clipboard to be pasted into supported applications such as Microsoft Word and Excel.

The XY pairs for an individual spectrum can be exported by using the right mouse click menu on the *Main* tab and selecting *Export Spectrum XY Pairs to Clipboard.*



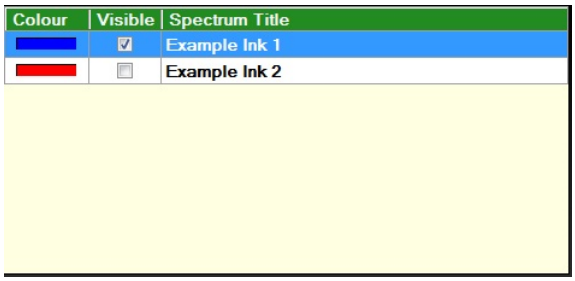
10: DATABASE SEARCHING

The FORAMx3 software includes the functionality to create databases and search against them. In the future the FORAMx3 software will also include the functionality to incorporate commercial libraries and search against them.

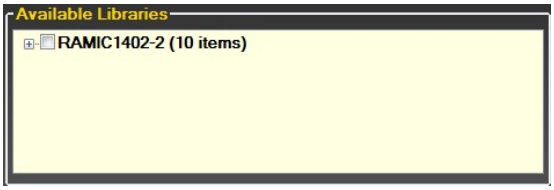
Searching against databases is accessed through the *Database* tab:

a. Searching Against a Database/Library

1. A spectrum must be available to be used to search against a database/library. Either record a spectrum as detailed in **Recording Spectra** or open a spectrum as detailed in **Spectral Display Options**.
2. Select the spectrum you wish to search by selecting it using the corresponding tick box. The selection of a spectrum can be confirmed by the spectrum name being highlighted in blue

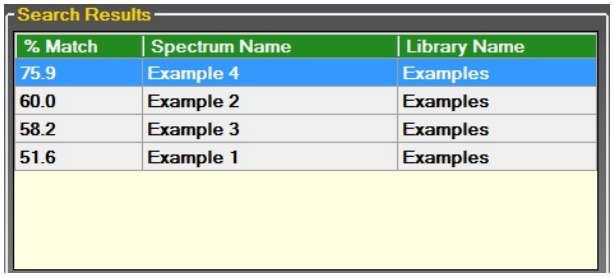


3. Select the library/database(s) that you wish to search against by selecting from the *Available Libraries*.

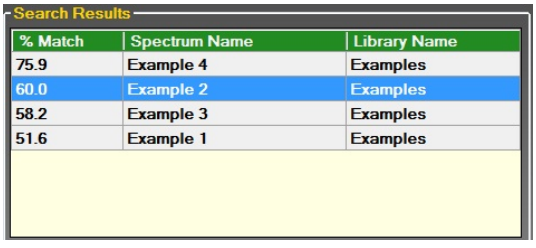


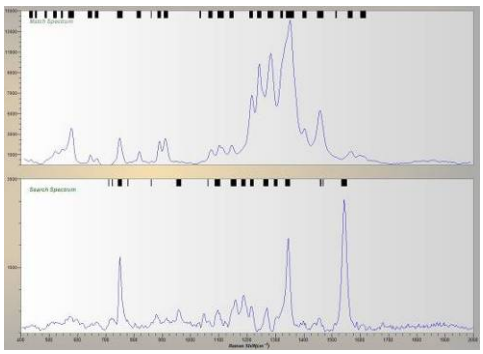
4. Search against the library/database(s) by selecting the *Search* button.

5. The results of the search are shown in the *Search Results*. The results are sorted from the highest % Match to the lowest % match. The spectrum name and the library/database name are also shown.



6. To compare the search spectrum with other database spectra select the required spectrum name from the *Search Results*. The *Match Spectrum* will then update with the selected spectrum.





7. By default only spectra with a % Match over 50% will be displayed. This threshold can be adjusted to show spectra with different % Match. The *Search Threshold* ranges from 1% to 100% and is adjusted by selecting the appropriate arrow buttons to increase or decrease the *Search Threshold*.

8. The results can be removed by selecting the *Clear* button.

The search algorithm, as shown by the Search Mode, is Pearson's Correlation of the first derivative. This a standard algorithm used in spectral searching/matching.

b. Creating a New Database/Library

1. A spectrum must be available to be used to add to a database/library. Either record a spectrum as detailed in **Recording Spectra** or open a spectrum as detailed in **Spectral Display Options**.
2. Select the *Add Spectra to Database* button.

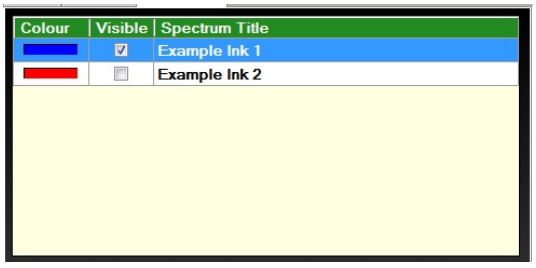
3. Select the *Create New Library* button.

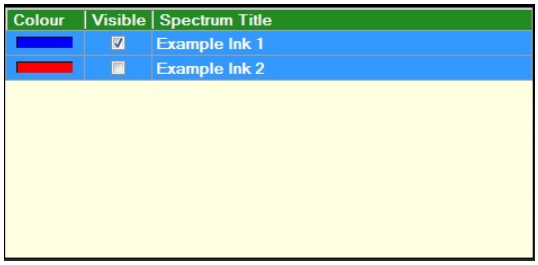
4. Enter the name for the new database/library.

5. Select *OK* to confirm the name. Select *Cancel* to return to the previous window without creating a new menu.
6. To complete the creation of a new database/library select *Close*. The *Available Libraries* in the *Database* tab will now be updated showing the new database/library.

c. Adding a Spectrum to a Database/Library

1. A spectrum must be available to be used to add to a database/library. Either record a spectrum as detailed in **Recording Spectra** or open a spectrum as detailed in **Spectral Display Options**.
2. Select the spectrum you wish to search by left mouse clicking on its name. The selected spectrum name will be highlighted in blue. More than one spectrum can be selected by holding the left mouse button and dragging over the names to be selected. The <Shift> key and <Ctrl> can be used in conjunction with the clicking on spectra names to select multiple spectra.

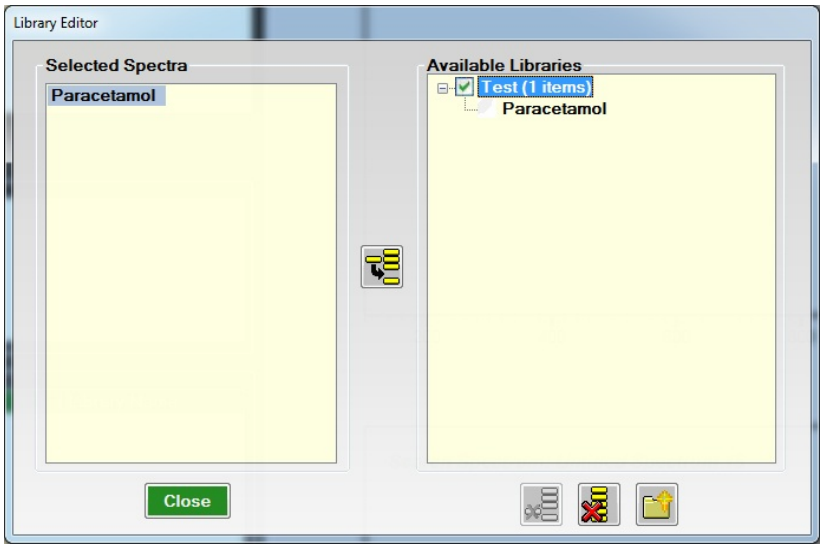




3. Select the *Add Spectra to Database* button.

4. Select the spectrum/spectra to be added to a library/database.

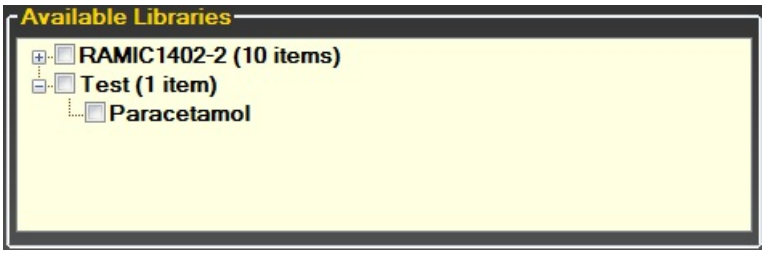
5. Select the library/database you wish to add the spectrum/spectra to by selecting the relevant tick box.



6. Select the *Add Selected Spectra to Selected Library* button.

7. To complete the process select *Close*.

The *Available Libraries* in the *Database* tab will now be updated with the relevant database/library containing the added spectra.



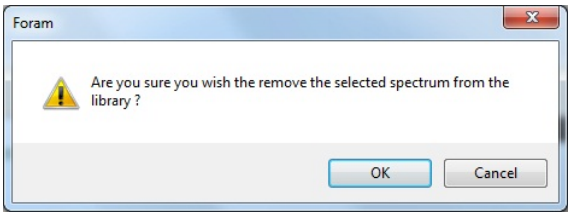
d. Deleting Spectra from a Database/Library

1. A spectrum must be available to access the *Library Editor*. Either record a spectrum as detailed in **Recording Spectra** or open a spectrum as detailed in **Spectral Display Options**.
2. Select the *Add Spectra to Database* button.

3. Expand the database/library you wish to delete a spectrum from by selecting the + next to the database/library name.

4. Select the spectrum you wish to delete.
5. Select the *Delete Selected Spectra* button.

6. To confirm the deletion of the spectrum click *OK*.

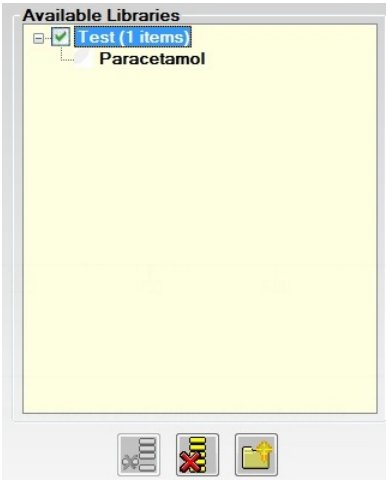


7. To complete the process select *Close*. The *Available Libraries* in the *Database* tab will now be updated with the deleted spectra removed.

e. Deleting a Database/Library

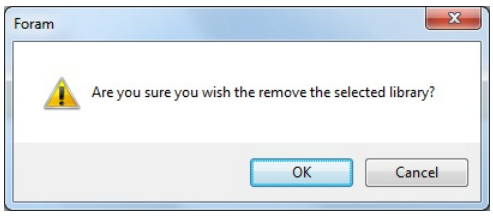
1. *A spectrum must be available to access the Library Editor. Either record a spectrum as detailed in* **Recording Spectra** or open a spectrum as detailed in **Spectral Display Options**.
2. Select the *Add Spectra to Database* button.

3. Select the database/library you wish to delete.

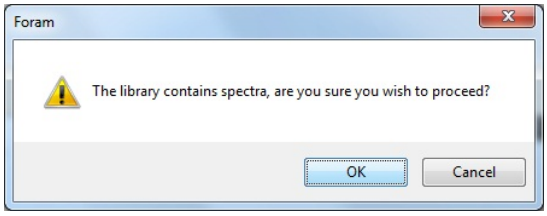


4. Select the *Delete Selected Library* button.

5. To confirm the deletion of the database/library click *OK*.



6. To confirm the deletion of the database/library click *OK*.



Deleting a database/library is confirmed twice. The first instance confirms the deletion of the library. The second instance gives notification that the database/library contains spectra which will be deleted with the deletion of the database/library.

7. To complete the process select *Close*. The *Available Libraries* in the *Database* tab will now be updated with the deleted database/library removed.

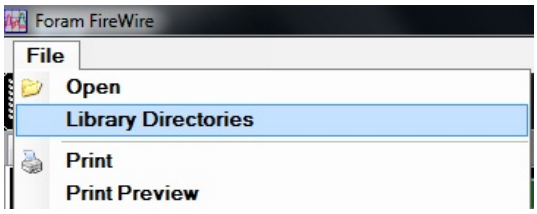
f. Adding a Commercial Library or Database

The FORAMx3 software supports commercial libraries. Typically the commercial libraries will be purchased with the FORAMx3. To use the libraries they must be added to the software by the user.

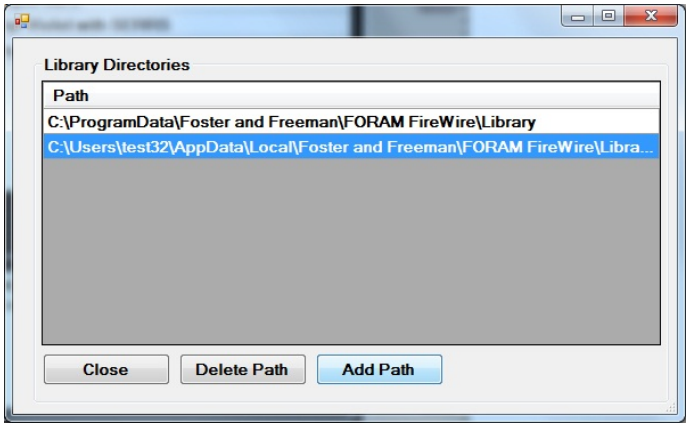
To add the library:

1. Check that the *Database* tab is selected.

2. Select *Library Directories* from the *File* menu.

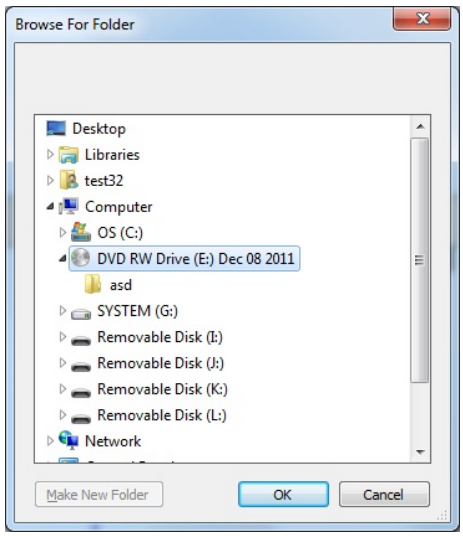


3. The *Library Directory* dialogue window will open

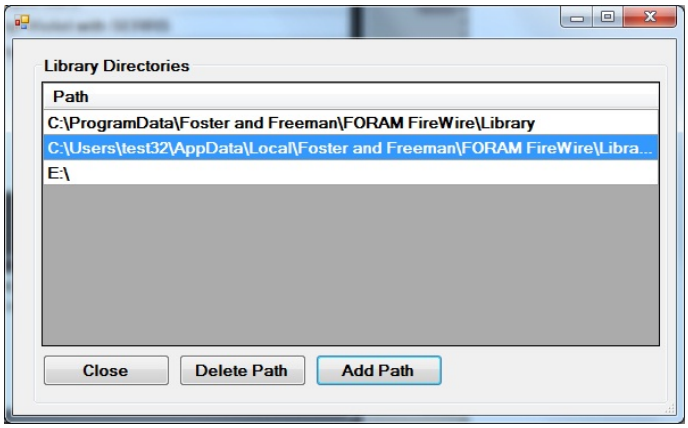


4. Select *Add Path*

5. Browse to the Library location. This can be a location on the hard disk (C:) or a temporary file location (CD/DVD Drive):



6. The *Library Directory* dialogue window will now be updated displaying the new directory location



7. To finish the process select *Close.*

   If required a path can be removed by highlighting the relevant path so that it is highlighted in blue and then select *Delete Path*.

8. The *Available Libraries* within the *Database Tab* will now be updated to show the included library.

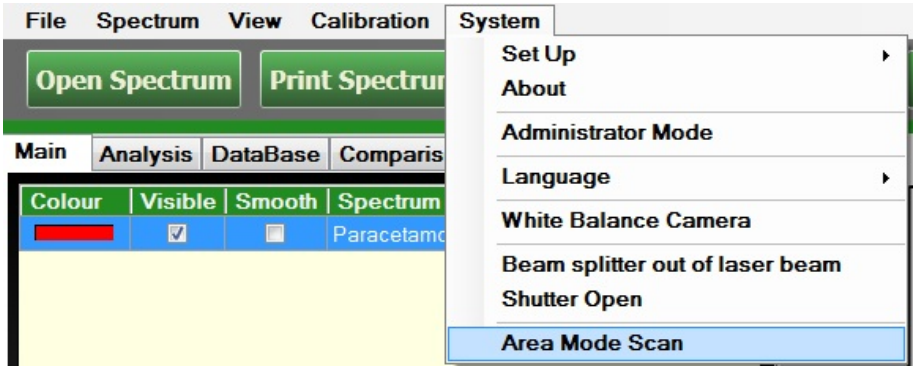
Note: If a temporary file location (for example the CD/DVD Drive) was selected during this process when the CD/DVD is removed it will not be possible to search against the libraries on that CD/DVD until the CD/DVD is replaced in the CD/DVD Drive.

11: ADVANCED OPTIONS

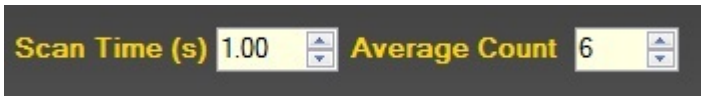
Area Mode Scan

The Area Mode Scan allows a confocal spectrum of a sample to be taken. For example if the target material is upon a fluorescent substrate the Area Mode Scan can be used to ensure that the fluorescent substrate is not recorded.

1. Select *Area Mode Scan* from the *Settings* menu.



2. Set the *Scan Time* and *Average Count* as required.



3. Place the sample under the microscope objective and focus on the surface of the sample.
4. Select *Record Spectrum.*

APPENDIX A: HINTS/TIPS FOR OBTAINING GOOD SPECTRA

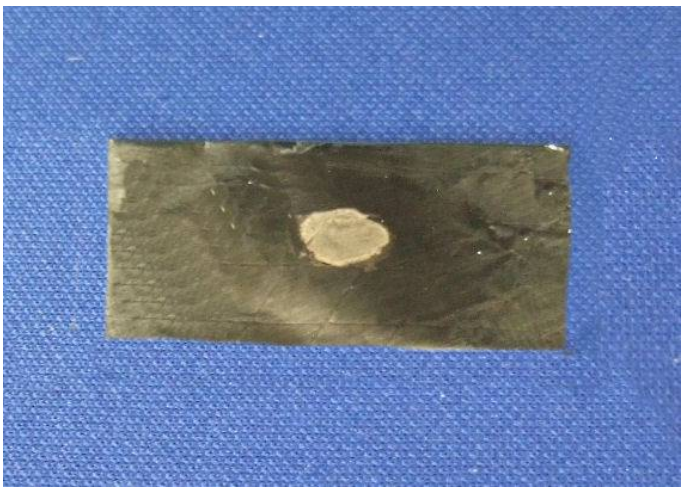
The FORAMx3 is a powerful tool for comparing a variety of forensic materials. To obtain results from the FORAMx3 that enable the user to achieve the best interpretation of the findings, there are a few basic rules when obtaining sample spectra.

- All samples in a case must be prepared using the same materials and preparation methods.
- Select samples from each source for comparison that represent the full range of variation present.
- Thin film samples can be attached to aluminium foil on a glass microscope slide to allow for better positioning and focus.
- Avoid mounting transparent samples on glass slide, as glass can weakly fluoresce and mask the Raman signal
- Select an area of the sample that, wherever possible, is clean and free from damage.
    - The presence of dirt and/or damage can have a significant effect on the sample spectrum obtained due to diffraction of the light passing through the sample.
- Before recording spectra the sample should be placed and focused so that a circular laser spot can be seen close to the centre of the live video image.
- Ensure that no ambient light is allowed through to the detector. Ambient light will cause positive/negative peaks to be observed that are not caused by the sample. Ambient light can be blocked by:
    - Working in a dark room environment with lights switched off and using curtains to cover windows.
    - [Place a cloth over the FORAMx3 hardware unit].
- Although 100% laser power will produce a result in the quickest time it is sometimes advantageous to use a lower laser power of 25% or 10% with a longer scan time. This allows more information to be collected by the spectrometer and provide better results. This is true for highly absorbing samples, which may burn or melt on the highest laser power setting.
- Set the *Average Count* to a minimum of six (6). Using the *Average Count* function will reduce the amount of noise observed in the final result.
- Obtain a minimum of six (6) measurements from each sample to observe any variation.

Extraction Method

It has been observed that results can be improved (notably for laserjet toners) by extracting the sample from the document. The method is carried out as follows:

1. Take a small portion of the sample (for toner, this would be an area of toner on a piece of paper).
2. Place the portion of the sample in a 350µl vial.
3. Submerge the sample in acetone (typically 40µl).
4. Shake the vial vigorously for a minimum of ten (10) seconds. The acetone may become discoloured if the sample was toner.
5. Place a strip of aluminium foil on a microscope slide.
6. Pipette the sample ‘extract’ solution from the vial using a micropipette and place a drop of the solution on the foil.
7. Allow the extract solution to dry.
8. Repeat *Step 6* in the same position to concentrate the sample area.
9. Record a minimum of six (6) spectra of different regions of the dried sample extract using the FORAMx3.
10. Repeat the method for other samples.



The final sample should appear as a white film on the foil

APPENDIX B: ROUTINE QUALITY ASSURANCE CHECKS

Quality Assurance checks are performed on the FORAMx3 to:

- Check that the instrument is operating correctly before use.
- Demonstrate that the instrument's performance is consistent over a period of time.
- Check that the supplementary materials are still providing accurate results.

The checks are designed to demonstrate:

a) The wavenumber accuracy of the instrument.

b) The reproducibility of the instrument.

c) The reproducibility of the associated materials.

The following information details performance standards that are achievable using the Foster & Freeman FORAMx3 and which may be used to help develop local Quality Assurance protocols.

Each laboratory must develop and document a system of checks appropriate to their local working practices and quality requirements.

Calibration Check

Frequency:	DAILY
Q.A. Test:	Polystyrene standard check
Acceptable Result:	Successful calibration
Photometric Response:	Weekly

APPENDIX C: LASER SPOT SIZES AND FIELDS OF VIEW

The size of the laser spot is fixed, so the area of the sample from which the spectrum is obtained is determined by the magnification of the microscope objective used.

The table below provides details for the field of view and laser spot size for each of the microscope objectives supplied with the FORAMx3.

Objective lens	Field of View (VSC Suite)		Laser spot size (µm)
	X (mm)	Y (mm)	
x 5	1.52	1.14	20
x 10	0.76	0.57	10
x 20	0.38	0.29	5
x 40	0.19	0.14	2.5

APPENDIX D: ADVANCED TECHNICAL NOTES

This Appendix provides details of Multiplicative Scatter Correction, Standard Normal Variate, Principle Component Analysis and Polar Qualification System.

Grouping Functions

Multiplicative Scatter Correction (MSC)

MSC is useful for correcting for effects of specular and diffuse Raman and Fluorescence signals in samples which tend to scatter light strongly e.g. white powders. In Raman spectroscopy, specular signal is Raman or Fluorescence signal that arises from the surface particles, whilst diffuse signal is Raman or Fluorescence signal that arises from the sample after penetrating the sample particles. Samples with small efficiently packed particles give rise to more specular signal. This can give rise to baseline offsets.

MSC attempts to correct for this by making a simple linear regression of each spectrum point against a reference spectrum. The mean of the spectra is used as the reference. The effect of the correction is to more closely group spectra that arise from the same sample, thus enabling the observation of small differences between samples which have similar spectra.

Standard Normal Variate (SNV) Transform

The SNV transform is similar to MSC described above. However in this case, the mean of the group of spectra is subtracted from each spectrum and the length normalised to 1. The results of the SNV transform are often similar to MSC, and again the effect of the transform is to more closely group spectra that arise from the same sample.

References

Practical Guide to Chemometrics – Edited by Paul Gemperline. CRC Press 2006, ISBN 978-1-57444-783-5.

Clustering Functions

Polar Qualification System (PQS)

PQS is a mathematical method for discriminating groups of spectra which have subtle difference in their spectra features. It involves normalising the spectra and plotting each one in spherical polar coordinates. The centre of mass of the resulting shape is then calculated giving rise to an X and Y coordinate for each spectrum. These coordinates are then plotted, and can reveal subtle differences in spectra.

References

Qualifying Pharmaceutical Substances by Fingerprinting with NIR Spectroscopy and PQS, Cornelis van der Vlies, Karoly J. Kaffka and Wim Plugge. Pharmaceutical Technology Europe, April 1995.

PQS (Polar Qualification System) The New Data Reduction and Product Qualification Method. K.J.Kaffka and Zs. Seregely, Acta Alimentaria. Volume 31 (1) pp 3 – 20 (2002).

Principal Component Analysis (PCA)

PCA is multivariate mathematical method for discriminating groups of spectra which have subtle difference in their spectra features. It is used routinely in analytical chemistry for the discrimination of groups of samples exhibiting subtly different spectra, but also has other uses such as de-noising and image processing.

It involves a pre-processing step, in this case taking the first derivative of each spectrum, and auto-scaling.

A matrix is then created from the pre-processed intensity values of each spectrum. The rows of the matrix are the pre-processed intensity values whilst the columns are the spectrum number. The next step involves a mathematical transform to approximate the original matrix by the product of two smaller matrices, the score (which contains the weighting of the principal components) and loading matrix (which contains the principal components themselves). This transform is accomplished by a linear algebra technique called Singular Value Decomposition (SVD). The first few principal components contain the most significant information in the spectra, like common features, whilst the higher components are typically noise.

In a similar fashion to PQS above, we can plot the scores in a 2 dimensional plot, in our case the first and second component. These plots can often reveal small differences in groups of spectra.

References

Practical Guide to Chemometrics – Edited by Paul Gemperline. CRC Press 2006, ISBN 978-1-57444-783-5.

Chemometrics – Matthias Otto. Wiley 2007, ISBN 978-3-527-31418-8

Forensic Chemistry – Susan Bell. Pearson Prentice Hall (2006) ISBN 0-321-56657-2

APPENDIX E: APPLICATION NOTES

Application Note 1: Blue gel pens

Application Note 2: Toners

Application Note 3: Printer inks

foster+freeman

APPLICATION NOTE 1

(0080-51-05) November 2011

FORAM®: DISCRIMINATING BLUE GEL PENS

FORAM®: Raman Spectral Comparator

In recent years, gel pens have become more commonly used by the general public, in preference to traditional ball point and liquid ink pens. Gel pens present new challenges to document examiners since many employ inks which are based on pigments, rather than dyes, which cannot easily be extracted for analysis by thin layer chromatography (TLC).

Several scientific studies have been published reporting the use of Raman spectroscopy to discriminate between gel pens. Mazella and Buzzini [1] have applied Raman spectroscopy using two different excitation wavelengths to give a discrimination rate of 68% for pigmented blue gel pens. Zieba-Pulus et al [2] utilised a combined Raman/µXRF instrument to analyse a range of materials of forensic interest including blue gel pens.

In this Application Note, we demonstrate the potential of the Foster + Freeman Raman Spectral Comparator (FORAM) to differentiate blue gel pens.

Raman spectroscopy involves the scattering of laser light from a target material, the analysis of which provides the user with a spectral "fingerprint" of the molecular composition of the material.

Gel pens

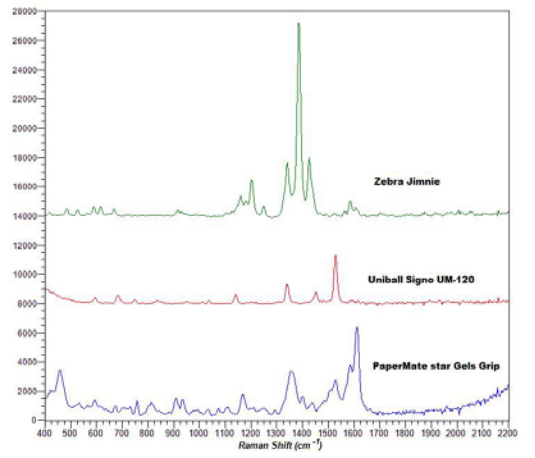
The study reported here involved subjecting inks from 13 different types of blue gel pen to analysis using the FORAM. Separate Raman spectra were recorded from each of the inks using each of three laser excitation wavelengths: 532 nm, 685 nm and 785 nm. Spectra were baseline-corrected using software containing a propriety fluorescence filter.

Ref.	Name	Ink type
1	Faber Castell	Unknown
2	Paper Mate Star Gels Grip	Unknown
3	Pentel Hybrid K230	Pigment
4	Pentel Hybrid KN706	Pigment
5	Pilot G-1 0.7	Dye
6	Pilot G-2 10	Dye
7	Pilot P-500	Pigment

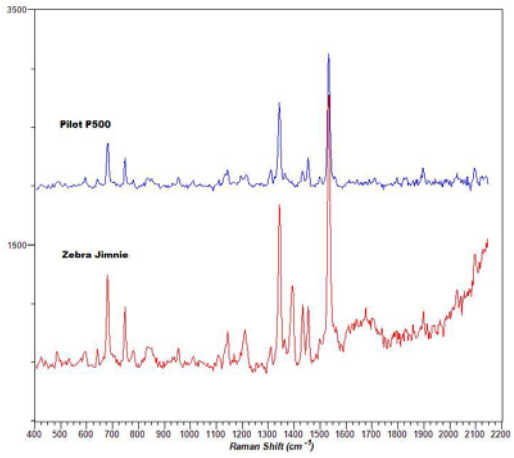
Ref.	Name	Ink type
8	Stabilo PointVisco	Unknown
9	Uniball Jetstream SX-210	Pigment
10	Uniball Signo UM-120	Pigment
11	Zebra Jimnie	Pigment
12	Zebra J-Roller RX	Pigment
13	Zebra Sarasa	Pigment

Results and Discussion

FORAM®: Raman spectra (Blue gel pens)



Laser wavelength = 532 nm



Laser wavelength = 685 nm

Discrimination rate

Many of the spectral pairs showed clear differences, yielding the following visual discrimination rates:

Number of sample pairs in the study = 13 x 12/2 = 78		
Laser wavelength (nm)	Number of pairs discriminated	Discrimination rate (%)
532	57	73
685	55	70
785	56	72
Combined	59	76

Note that whilst spectra obtained with longer wavelength excitation can provide additional discrimination, the intensity of the Raman emission becomes progressively weaker as the excitation wavelength lengthens.

Conclusions

The FOR spectrometer has the ability to discriminate between different types of blue gel pens. The use of a number of excitation wavelengths can improve the overall discrimination rate. The instrumentation is cost effective, compact and almost free of maintenance.

References

[1] W.D. Mazzella and P.Buzzini, Forensic Science International 152 (2005), pp. 241-247.

[2] J. Zieba-Palus, R.Borusiewicz and M.Kunicki, Forensic Science International 175 (2008), pp. 1-10.

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APPLICATION NOTE 2

(0080-52-05) November 2011

FORAM®: DISCRIMINATING TONERS

FORAM®: Raman Spectral Comparator

The discrimination of laser printer and photocopiers toner present the document examiner with particular challenges. Conventional analytical techniques, such as visible/IR absorption, which are useful in ink examination are not applicable to toners. Other techniques, such as FTIR (Fourier Transform Infrared) spectroscopy, are either quite destructive to the document or are time consuming and expensive. The various FTIR techniques which may be applied to toners have been described elsewhere: Merrill et al [1]. In this Application Note, we demonstrate the potential of the Foster + Freeman FORAM Raman Spectral Comparator to discriminate toners. Initially, we attempt to discriminate toner in-situ on the document. Subsequently, we extract the acetone soluble components from the toner and deposit the solute onto aluminium foil. The solute is then subjected to Raman analysis in the same way.

Raman spectroscopy involves the scattering of laser light from a target material, the analysis of which provides the user with a spectral "fingerprint" of the molecular composition of the material

Toner samples

The study reported here involved subjecting different types of toner to analysis using the FORAM (Laser excitation wavelength = 685 nm). Spectra were baseline-corrected using a proprietary software fluorescence filter.

Ref.	Name	Colour after extraction
1	HP 98X	Colourless
2	HP Color LaserJet 4600 PCL6	Colourless
3	HP Color LaserJet 9500 PCL6	Colourless
4	HP LaserJet 6P	Colourless
5	HP LaserJet 1022 Series	Grey
6	HP LaserJet 1160	Grey
7	HP LaserJet 1200	Grey
8	HP LaserJet 2300	Colourless

Ref.	Name	Colour after extraction
9	HP LaserJet 4300tn	Grey
10	HP LaserJet P3005 PCL6	Colourless
11	Kodak 235	Black
12	Konica 3340	Black
13	Lanier M6765 6755	Grey
14	Minolta CF 900	Yellow
15	Mita DC1860	Black

Toners contain a variety of components: Fusible resin, Iron oxide (Fe_3O_4), Carbon black, Dyes/Pigments, Surfactants, Charge control agents [2]. Typical resins include the following compounds: Styrene/butadiene copolymer, Polyester, Styrene ethylhexylacrylate, Styrene n-butylacrylate, Other copolymers. The colour of the toner may be modified by the addition of dyes: Nigrosine, Victoria blue, Methyl violet, Pthalocyanines, Azo-pigments, Quinacridones. The charge control agents are often complex organometallic compounds, which also act as dyes, or quaternary ammonium salts (both aromatic and aliphatic): [3].

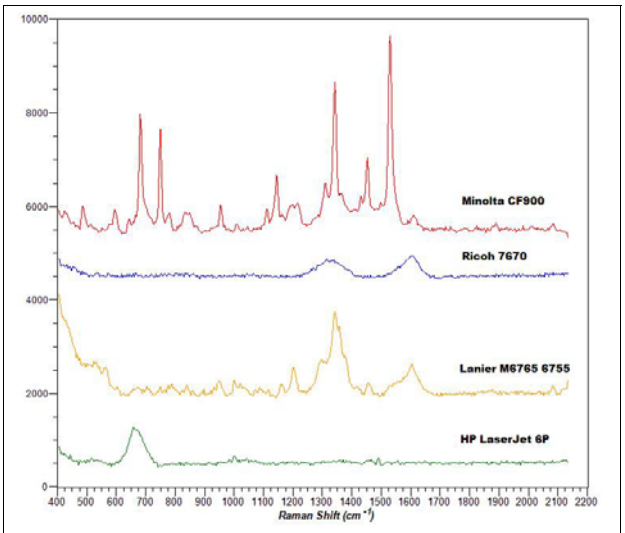
Components of the toner were extracted by immersing a small area of the document (~5 mm2) in 2 ml of acetone (Chromasolv Plus, Sigma Aldrich 650501-1L) for several hours. Approximately 0.3 ml of the resulting solution was then applied to a microscope slide covered with aluminium foil and allowed to dry.

Spectra of the remaining residue were recorded using the FORAM in the usual way. The aim of the extraction process was to concentrate the soluble components (resins and dyes) whilst removing possible interference from the insoluble components (carbon black and iron oxide).

Results and discussion

Number of sample pairs in the study = 15 x 14/2 = 105.

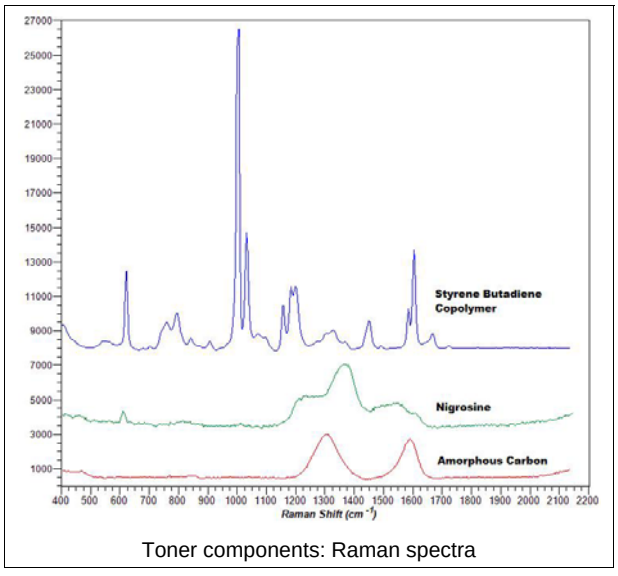
Toner (In situ): Discrimination rate = 72%



Toners: Raman spectra

Most of the spectral pairs showed clear differences. Overall visual discrimination rate = 72% (76 pairs).

Toner components



Toner components: Raman spectra

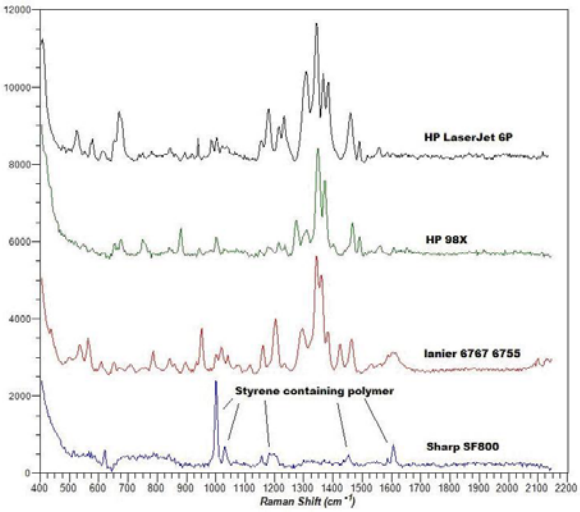
Many of the components either yielded no Raman spectrum or fluoresced too intensely to enable one to be obtained.

Note that the spectrum of Ricoh 7670 correlates well with that of amorphous carbon. It is surprising, however, that despite the toner containing as much as 60% resin, no spectral peaks corresponding to the resin component are observed. It is likely that the peak at 668 cm-1 in the spectrum of HP 6P LaserJet arises from magnetite: [4].

Toner residue: Discrimination rate = 84%

Most of the spectral pairs showed clear differences. Overall visual discrimination rate = 84% (88 pairs).

Note that spectral peaks corresponding to styrene are observed in the spectrum of the extract from Sharp SF800 toner. It is assumed that the styrene is present in a copolymer.



Toner extract: Raman spectra

Conclusions

The FORAM spectrometer has the ability to discriminate between different types of toner, both in situ on the document, and after extraction into acetone. Discrimination rates of 72% and 84% were achieved. Since the FORAM spectrometer requires only extremely small amounts of material to obtain a spectrum, the method for extracting and concentrating the toner extracts could be optimised further, thereby reducing the amount of material removed from the document. The instrumentation is cost effective, compact and almost free of maintenance.

References

[1] R.A.Merrill, E.G.Bartick and W.D.Mazella, Journal of Forensic Sciences, Vol.41, No.2 March 1996.

[2] “Advances in the forensic analysis and dating of inks”, R.L.Brunelle and K.R.Crawford, published by Charles C Thomas, Springfield, Illinois 2003, pp. 41 – 44.

[3] “Electro-photography and Development Physics”, L.B.Schein, Laplacian Press 1996, pp. 85 – 87.

[4] A.Zoppi, C.Lofrumento, E.M.Castellucci and M.G.Migliorini, Spectroscopy Europe 14/5 (2002), pp. 16 - 20.

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APPLICATION NOTE 3

(0080-53-05) November 2011

FORAM®: DISCRIMINATING PRINTER INKS

FORAM®: Raman Spectral Comparator

The low cost and ready availability of inkjet printers has greatly increased the frequency with which documents produced by these machines are encountered by document examiners. Conventional analytical techniques, such as visible/IR absorption, which are normally useful in the examination of inks are not as effective with printed documents produced by inkjet printers. Other techniques, such as chromatography, are not ideal as they often involve the destruction of a small portion of the document.

Whilst the application of Raman and SERRS spectroscopy to the analysis of questioned documents is widely discussed in the scientific literature [1, 2, 3, 4], the application of these techniques to the analysis of black inkjet inks is somewhat limited.

SERRS = Surface Enhanced Resonance Raman Scattering

Littleford et al [4] have used SERRS spectroscopy to probe the structural changes of the chromophore present in black inkjet inks when deposited onto paper. They also give examples of the types of dye that are likely to be found in inkjet inks.

In this Application Note, we demonstrate the potential of the Foster + Freeman FORAM Raman Spectral Comparator to discriminate black inkjet inks when used in conjunction with the SERRS technique.

Raman spectroscopy involves the scattering of laser light from a target material, the analysis of which provides the user with a spectral “fingerprint” of the molecular composition of the material

Ink samples

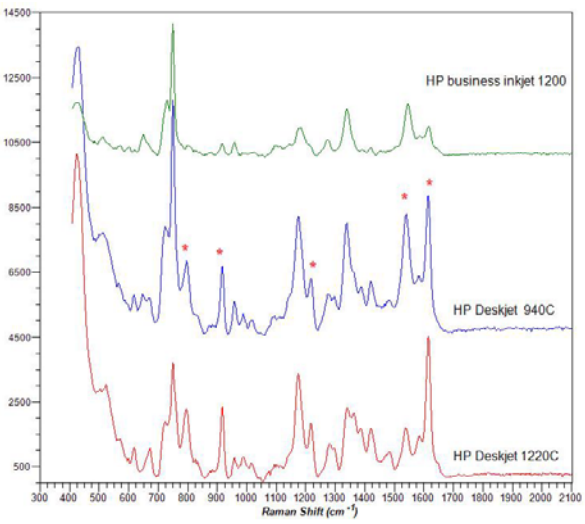
The study reported here involved subjecting different types of ink to analysis using the FORAM. (Laser excitation wavelength = 685 nm). The SERRS technique was effected by applying poly-L-lysine (Sigma-Aldrich) and gold colloid (British Biocell) to the ink mark on the document [5] prior to recording each Raman spectrum. Spectra were baseline-corrected using a proprietary software fluorescence filter.

Ref.	Inkjet printer
1	Canon M610
2	Canon Pixma MP520
3	Epson Stylus C64
4	Epson Stylus C66
5	Epson Stylus DX7450
6	HP Business Inkjet 1200
7	HP C4180
8	HP Deskjet 820 Cxi

Ref.	Inkjet printer
9	HP Deskjet 940c
10	HP Deskjet 1220c
11	HP Deskjet 6122
12	HP Deskjet F2180
13	HP Photosmart 7150
14	Lexmark 4300
15	Lexmark Black Inkjet Cartridge #70

Results and discussion

Number of sample pairs in the study = 15 x 14/2 = 105. Most of the spectral pairs showed clear differences. Overall visual discrimination rate = 84% (88 pairs).



Raman spectra (SERRS): Ink samples

Brunelle and Crawford [6] describe the various types of dyes, solvents, dye complexing agents and surfactants typically found in inkjet inks. The dye component, which is the component expected to give rise to the spectra shown above, is frequently an azo-dye with a very broad visible absorption profile. The differences between the dyes are often due to modification or addition of side chain groups [4] to improve properties such as light fastness or solubility. Although it has not been possible to identify the dyes giving rise to the different spectra shown, the small spectral differences observed are consistent with the assertion that the dye molecules have a similar basic molecular structure, but have different side chain groups. Further work is needed to prove this assertion.

Conclusions

The FORAM spectrometer has the ability to discriminate between different types of printer ink when the SERRS technique is applied. Overall visual discrimination rate = 84%. The instrumentation is cost effective, compact and almost free of maintenance.

References

[1] T.Andermann, Problems of Forensic Sciences, Volume 46 (2001), pp. 335 – 344.

[2] W.D. Mazzella and P.Buzzini, Forensic Science International 152 (2005), pp. 241-247.

[3] M.Kunicki, Problems of Forensic Science, Volume 51 (2002), pp. 56 – 70.

[4] R.E.Littleford, M.P.Hughes, G.Dent, D.Tackley and W.E.Smith, Applied Spectroscopy, Volume 57, Number 8 (2003), pp. 977 – 983.

[5] E.Wagner and S.Clement, Problems of Forensic Sciences, Volume 46 (2001), Pages 437 – 441.

[6] "Advances in the forensic analysis and dating of inks", R.L.Brunelle and K.R.Crawford, published by Charles C Thomas, Springfield, Illinois 2003, pp. 41 – 44.

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