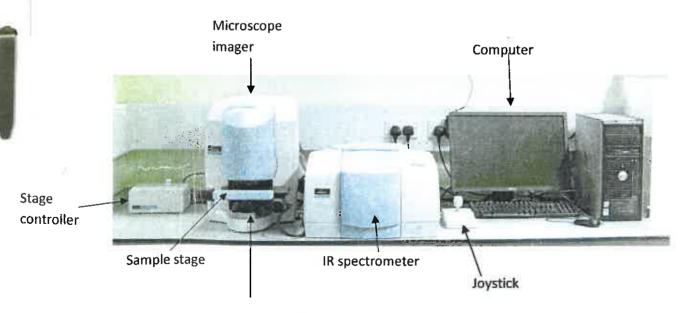
# A Guide to using the IR Microscope in the CAF

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## Model:

PerkinElmer Spectrum 100 FT-IR Spectrometer attached to a Spotlight 400 Imaging System



Lower cassegrain

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or more information than is displayed in this guide, four books on the use of the instrument are kept n the cupboard underneath.

**lote on changing the desiccant:** There is a guide to changing the desiccant in the instrument on p39 of he Spectrum 100 Series Getting Started Guide. An example order form is provided at the back of this Juide.

## 1. Starting a session

1.1 If the computer is off, turn it on and press return on the windows log-in screen.

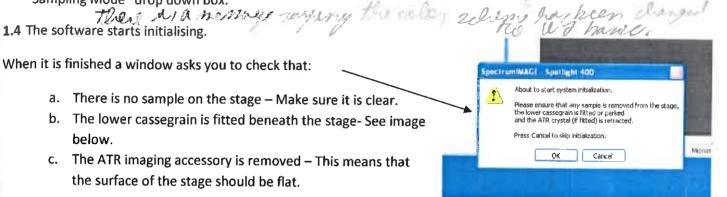
Note: DO NOT use your own log-in name and password (if this has been done turn to the troubleshooting section).

- 1.2 Open the "SpectrumIMAGE" Software from the shortcut on the desktop.
- 1.3 In the window that opens type your name.

Select "attached instrument" from the "Instrument" drop down box. Select "Point mode" from the "Sampling Mode" drop down box.

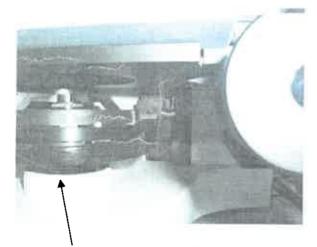
When it is finished a window asks you to check that:

- a. There is no sample on the stage Make sure it is clear.
- b. The lower cassegrain is fitted beneath the stage- See image below.
- c. The ATR imaging accessory is removed This means that the surface of the stage should be flat.



Note: The instrument should always be left in this state when it has finished being used.

Click "OK" and the stage will begin to move to the four corners at the limits of its thread. The "Monitor Visible" screen will then show the aperture calibration as it is carried out.



Lower cassegrain underneath the sample stage

It says aperture calobrakeen has completed successfully at the end

### 2. Basic Information

### 2.1 Screen Layout

### SpectrumIMAGE has 3 main windows:-

Monitor Visible Window – A live camera "C" and the background "B" camera recording of the sample well as any images recorded.

Control Window – A live camera recording of the sample stage.

Control Window – Allows you to control settings on the instrument.

## 2.2 Transmittance or reflectance mode?

hese can be selected in the **Control** Window under "Sampling Mode". The instrument will click and make a slight thirring sound when changing between these modes.

### Transmittance mode:

f the sample is thin enough, the IR beam can be passed through it e.g. fibres and microtomed samples or small ystals that have been mounted on IR transparent media (e.g the silicon chip in the cardboard box).

### Reflectance mode:

the sample is too thick for transmittance measurements and not too strongly absorbing, then the IR beam can reflected from the surface e.g. biological samples or mineral samples mounted on glass slides (non-IR ansparent).

## 2.3 Using the Joystick to focus on a sample

- To move the sample stage left, right, forwards or backwards lean the joystick to the sides.
- To move the stage up or down and focus on the sample twist the joystick.
- An image of the sample surface will be seen in the Monitor visible window.

## 3. Adding Liquid nitrogen

### WARNINGS!

DO NOT lean the liquid nitrogen dewer on the funnel or it may push the detector out of alignment.

DO WEAR the yellow cryogenic gloves and goggles.

DO ATTEND a liquid nitrogen training course.

If you have NOT been on the course then you must ask a member of staff or a trained research student to complete this procedure for you.

DO NOT overfill the microscope detector or the microscope optics may be damaged.

**3.1** Remove the liquid nitrogen dewer from the cupboard and fill with liquid nitrogen from the Chemistry loading bay.

Note: Fill dewer to half full for half a day of instrument use or ¾'s full for 1 day of use.

- 3.2 Open the lid on the IR microscope imager.
- 3.3 Remove the black stopper.
- 3.4 Slot the funnel into place.
- 3.5 Put the clips on the sides of the dewer up as this makes it easier to pour.
- 3.6 Tip the liquid nitrogen carefully into the funnel until it is  $\frac{1}{2}$ 's full.
- 3.7 Put the dewer down on the table and wait for the funnel to empty and for the gas cloud to disperse.

Note: It is important to wait or pressure may build up inside the microscopes internal dewer.

- 3.8 Once the cloud has cleared add another ¾ funnel of liquid nitrogen and again wait for the cloud to clear.
- 3.9 Once this has cleared then add three more funnels worth (¾'s full) of liquid nitrogen.
- **3.10 DO NOT** overfill the internal dewer. When it is almost full the nitrogen will drain through the funnel more slowly.

## 4. Recording a visible image

- 4.1 In the Stage Control window drag a box around the cross with a "C" next to it (this is the same as the cross in the Monitor visible window) by holding down the left-hand mouse button.
- 4.2 Double click in the box to focus on that region.
- 4.3 Repeat this until only the area to be imaged is shown. The coordinates along the edges of this window record the size of the area.

**Note:** The larger the area shown in the **Stage Control** window, the longer the time needed record the visible image.

- 4.4 In the Stage Control window select the "Survey" tab and "Visible Image Survey". The stage moves as the image is recorded.
- 4.5 This can be saved as a bitmap file by selecting "Save as" in the Stage Control window and save to your personal file.

**Note:** This will not record any crosses or boxes marking the locations of point spectra or maps. To record an image including these, press the "Print Screen" button on the keyboard, open the "Paint" programme from the computers Start menu, paste in the image and save the file.

**4.6** To go back to the previous scale and choose another area go to the **Stage Control** window, select the "view" tab and "Previous scale" or "Default Scale".

## 5. Analysing a thick sample

- 5.1 Leave the "Operation mode" as "point mode".
- 5.2 In the Control window click "Zero".
- 5.3 Move the sample stage to its highest position using the joystick
- 5.4 Turn the silver locking lever that holds the lower cassegrain in position until it is loose, but **DO NOT** unscrew it completely (see figure 7.1).
- 5.5 Gently slide out the lower cassegrain and place it in the foam in the black box labelled "Box 1".
- 5.6 In the **Control** window click "Park", wait while the image (figure 7.2) in the **Control** window says "moving" and click "Park" again. Wait once more (about 20 seconds). This process moves the lower cassegrain stub to its lowest position.

Note: You must press "Park" twice here and wait or you may damage the instrument.

5.7 Lower the stage carefully using the joystick and secure the sample in position.

Note: Be careful not to drop anything through the stage as it may damage the optics now exposed below.

- 5.8 To refit the lower cassegrain, use the joystick to raise the stage to its highest position.
- 5.9 In the control window, click "zero" and the lower cassegrain stub will move upwards.
- 5.10 Slide the lower cassegrain all the way back into the dovetail connector and tighten the silver screw.

**Note:** Make sure the lower cassegrain sits correctly in position as sometimes it is difficult to push it all the way back into the connector.

### 6. Point Mode

### 6.1 Recording a background IR spectrum

Note: All of the parameters needed for the sample spectrum must be the same as in the background.

- 6.1.1 In the Control window, check that the "Operation mode" is selected as "Point mode".
- **6.1.2** Select either reflectance or transmittance mode in the **Control** window.
- 6.1.3 When using transmittance mode ensure the stage is clear.
- 6.1.4 When using reflectance mode:
  - a. Click the "Load position" button in the **Stage Control** window. The stage moves down so that samples can be positioned more easily.



b. Place the black sample holder onto the stage and rest the brass disc (in the cardboard box) securely on top.



- c. Click the load position button again and the stage will return to the central position.
- d. Adjust the "Illumination" to 15% using the double arrowed bar in the Control window.
- e. Focus on the surface of the brass disc. An image can be seen in the **Monitor visible** window.
- **6.1.5** Adjust the aperture size\* in the **Control** window, under "Aperture", "Change...". This is the area of the sample that will be analysed and it is shown by the size of the red box in the **Monitor Visible** window.



**Note:** There should be no space in this box that does not cover the sample or the spectrum recorded will contain a lot of noise.

**6.1.6** Click the "Scan" tab in the **Stage Control** window and then "Background". Check the settings\*, click "Start" and a new window appears showing the background as it is scanned.

\*As a starting point use the following settings: aperture size  $100x100\mu m$ , wavelength range  $4000-500cm^{-1}$ , 64 repeat scans, interval  $2.00cm^{-1}$ .

#### nmary:

nsmittance mode – Select transmittance mode, check the stage is empty, choose the aperture size, in the stage arel window click the "scan" tab and select "background", check the settings, click "start".

ectunce mode – Select reflectance mode, click the "load position" button, place the brass disc on the sample let on the stage, click "load position", change illumination to 15%, focus on the brass disc, choose the aperture in the stage control window click the "scan" tab and select "background", check the settings, click "start".

A typical background spectrum

## 6.2 Recording an IR spectrum from a sample

- 6.2.1 In the Control window, check that the "Operation mode" is selected as "Point mode".
- 6.2.2 Click the "load position" button to drop the stage.
- **6.2.3** Place the sample on the stage and secure in position. If working in reflectance mode remove the brass disc first.
- 6.2.4 Click the "load position" button to return the stage to the centre.
- **6.2.5** Check that the correct "Sampling Mode" is selected in the **Control** Window transmittance or reflectance.
- 6.2.6 Check that the aperture size is correct (Control window, "Aperture", "Change...").
- 6.2.7 Focus on the sample surface using the joystick.
- 6.2.8 Illuminate at an appropriate percentage.
- **6.2.9** Use the joystick to move the stage and position the red square in the **Monitor Visible** window over the area to be analysed.
- 6.2.10 Click the "Scan" tab in the Stage Control window, select "sample ratio..." and check the settings. \*
- 6.2.11 Click "Start" and a new window opens showing the spectrum as it is scanned.
- **6.2.12** Save each point spectrum as it is recorded or it may be lost if the computer crashes.
- \*As a starting point use the following settings: aperture size  $100x100\mu m$ , wavelength range  $4000\text{-}500\text{cm}^{-1}$ , 64 repeat scans, interval  $2.00\text{cm}^{-1}$ .

#### nmary:

eck the appropriate "Sampliny mode", click "Loud position", place sample on stage, click "Load position", focus, he stage control window, click the "scan" tab and select "sample ratio…", check the settings, click start save spectrum.

## 7. Image Mode

## 7.1 Recording a background IR map

**Note:** This does not need to be done for each experiment. If a background has already been recorded with the appropriate combination of settings then it can be re-used by the computer.

- 7.1.1 In the Control window, change the "Operation mode" to "Image mode".
- 7.1.2 Select either reflectance or transmittance mode in the Control window.
- 7.1.3 When using transmittance mode ensure the stage is clear.
- 7.1.4 When using reflectance mode:
  - a. Click the "Load position" button in the **Stage Control** window. The stage moves down so that samples can be positioned more easily.



b. Place the black sample holder onto the stage and rest the brass disc (in the cardboard box) securely on top.



- c. Click the load position button again and the stage will return to the central position.
- d. Adjust the "Illumination" to 15% using the double arrowed bar in the Control window.
- e. Focus on the surface of the brass disc. An image can be seen in the Monitor visible window.
- 7.1.5 In the Stage Control window select the "Scan" tab and click "Start Image Background...".
- 7.1.6 Select the appropriate settings.\*

**Note:** The pixel size will depend upon the level of detail required in the IR map. There is a choice of three (6.25, 25,  $50\mu m$ ). A smaller pixel size will increase the time taken to record the map. If unsure, select the  $25\mu m$  pixel size to start with.

## 7.1.7 Click the "start" button.

\*As a starting point use the following settings; wavelength range 4000-680cm<sup>-1</sup>, 16 repeat scans, resolution 8.0cm<sup>-1</sup>, scan speed 1.0cms<sup>-1</sup>, 25µm pixel size.

#### mmary:

insmittance mode - Select transmittance mode, in the control window under "operation mode" select "image ide, check the stage is empty, in the stage control window select the "scan" tab and select "start image ikground...", check the settings, click "start".

Hectance mode — Select reflectance mode, in the control window under "operation mode" select "image de"; click the "load position" button, place the brass disc on sample holder on the stage, click "load position", inge illumination to 15%, focus on the brass disc, in the stage control window select the "scan" tab and select the "stan" tab and select the "scan" tab and select the "scan" tab and select the settings, click "start".

A typical background spectrum

## 7.2 Recording an IR map from a sample

- 7.2.1 In the Control window, check that the "Operation mode" is selected as "Image mode".
- 7.2.2 Click the "load position" button to drop the stage.
- **7.2.3** Place the sample on the stage and secure in position. If working in reflectance mode remove the brass disc first.
- 7.2.4 Click the "load position" button to return the stage to the centre.
- **7.2.5** Check that the correct "Sampling Mode" is selected in the **Control** Window transmittance or reflectance.
- 7.2.6 Focus on the sample surface using the joystick and find the area to be analysed.
- 7.2.7 Illuminate at an appropriate percentage.
- **7.2.8** It may help to record a visible image of this area of the sample before an IR map is recorded (see section 4).
- **7.2.9** In the **stage control** window drag a box around the area to be mapped by holding down the left-hand mouse button.
- 7.2.10 Click the "Start Image" button.
- **7.2.11** The "Start Image" dialog box appears. Enter a file name and check that the settings\* are the same as for the background. Give the map a name and browse for the correct file to save it to.

**Note:** Check the time predicted for the completion of the image scan as certain parameters can make it very large and the instrument will require enough liquid nitrogen to cool the detector for this entire period.

- **7.2.12** Select "Start Image" and an infrared map of the surface of the sample will be recorded in a new window.
- 7.2.13 Top up the internal dewer with three funnels (¾'s full) every 2 hours to ensure that the map is completed.

**Note:** If the liquid nitrogen runs out before the map is completed, it will leave a stripe across the image where no spectral data has been recorded. This affects the way the data is processed by the computer.

\*As a starting point use the following settings; wavelength range 4000-680cm<sup>-1</sup>, 16 repeat scans, resolution 8.0cm<sup>-1</sup>, scan speed 1.0cms<sup>-1</sup>, 25µm pixel size.

**Note:** The pixel size will depend upon the level of detail required in the IR map. There is a choice of three (6.25, 25,  $50\mu m$ ). A smaller pixel size will increase the time taken to record the map. If unsure, select the  $25\mu m$  pixel size to start with.

#### mary:

Teck the appropriate "Sampling mode", theck that "Image mode" is selected, click "Load position", place the aple on stage, click "Load position", focus, adjust illumination, (record a visible image), click the "start image" con, check the settings, click "start".

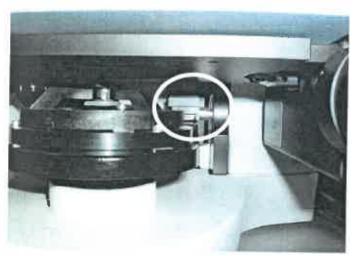
## 8. ATR Imaging Mode

te: This mode involves contact between the sample and the ATR crystal, so soft pliable samples such as bank tes, or completely flat hard samples are the most suitable. This technique demonstrates better spatial resolution and can be used for samples that are too thick or opaque for transmission mode or too strongly absorbing for flectance.

### 1 Setting up

- 8.1.1 Leave the "Operation mode" as "point mode" for the moment.
- 8.1.2 In the Control window click "Zero".
- 8.1.3 Move the sample stage to its highest position using the joystick.
- **8.1.4** Turn the silver locking lever that holds the lower cassegrain in position until it is loose, but **DO NOT** unscrew it completely (figure 7.1).

8.1.5



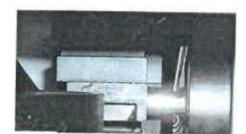


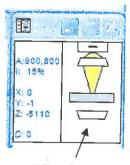
Figure 7.1

- 8.1.6 Gently slide out the lower cassegrain and place in the foam in the black box labelled "Box 1".
- 8.1.7 In the Control window click "Park", wait while the image (figure 7.2) in the Control window says "moving" and click "Park" again. Wait once more (about 20 seconds). This process moves the lower cassegrain stub to its lowest position.

Note: You must press "Park" twice and wait or you may damage the instrument.

- **8.1.8** Use the joystick to move the stage to its lowest position and bring it forwards, towards you as far as it will go.
- **8.1.9** Unscrew the six screws that secure the flat stage plate (circled in white in figure 7.3). Place in the box labelled "Screws". **DO NOT** lose them.

Figure 7.2



Will say "moving" here



Figure 7.3

**8.1.10** Remove the ATR stage plate from "Box 2" and swap with the flat stage plate. The upright black plastic goes to the back (see figure 7.4).



Figure 7.4

- **8.1.11** Tighten the two black thumbscrews (back left and bottom right of stage plate circled in white in figure 7.4).
- **8.1.12** Slide the crystal arm (from "Box 2") onto the two pillars on the ATR stage plate, with the larger part of the crystal arm to the right. Remember to press the brake lock (blue circle in figure 7.5).

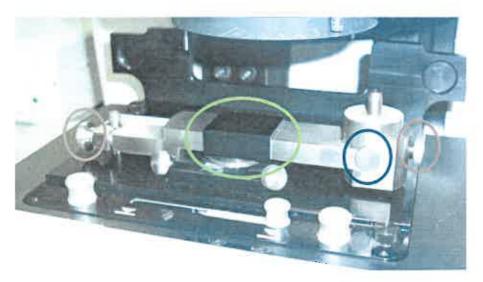


Figure 7.5

8.1.13 Tighten the silver screws at either end of the crystal arm (red circles in figure 7.5).

- 8.1.14 Slide the black dustcover to the right to expose the crystal (green circle in figure 7.5).
- 8.1.15 In the Control window, change the "Operation mode" to "ATR Imaging".
- **8.1.16** A window entitled "ATR Imaging Mode" may open. Click "OK" if the instructions to this point have all been completed.
- **8.1.17** A warning message may then be displayed checking that the lower cassegrain has been removed. If this is correct then click "OK".
- **8.1.18** A new window entitled "ATR Register Crystal Position Wizard" will open to register the crystal position.
- **8.1.19** Following the on-screen instructions, move the stage so that the scribe mark (on the metal arm to the right of the crystal) can be seen in the **Monitor visible** window.
- 8.1.20 Focus on it using the joystick.
- 8.1.21 Use the joystick to move along the scribe mark and focus on the edge of the crystal well.
- 8.1.22 Click the "Move to ATR Crystal Position" button.
- 8.1.23 Centre the dotted pattern and focus.
- 8.1.24 Click "Finish".

### ! Recording a background

- 8.2.1 In the stage control window, select the "scan" tab and select "Start ATR Background".
- **8.2.2** A window opens. There is no need to **Go to Last Known Crystal Position** as this has already been found in the previous steps.
- 8.2.3 Select the resolution and pixel size to be used.

**Note:** 16cm<sup>-1</sup> and 1.56µm are a good starting point if unsure.

8.2.4 Click "Finish" and a background is recorded.

**Note:** Steps 7.2.1 – 7.2.4 need to be repeated if a different resolution or pixel size is needed.

### Recording a map

- 8.3.1 Select "Start ATR Image" from the "Scan" tab in the stage control window.
- 8.3.2 A new window entitled "ATR Imaging Wizard" opens.
- 8.3.3 Enter an experiment name and browse for the correct file to save data to.
- 8.3.4 Click "Next".
- 8.3.5 Make sure that only "Baseline Offset Correction" is selected. Do not select anything else.

- 8.3.6 Click "Next".
- 8.3.7 Follow the on screen instructions for mounting a sample.

**Note:** The metal discs in "**Box 2"** may need to be used to make the sample higher so that contact can be made with the crystal.

**Note:** Do not apply too much pressure with the base of the crystal on the sample or it may cause damage to the sample and the crystal.

8.3.8 At the end of the instructions click "Next" and chose the appropriate image parameters.

**Note:** Check the estimated duration of the scan and top up the internal dewer with three funnels (¾'s full) every 2 hours to ensure that the map is completed.

- 8.3.9 Click "Finish" and then "OK".
- 8.3.10 An infrared map of the surface of the sample will be recorded in a new window.

## 4 Ending an ATR Imaging session

**Note:** If you have been working in ATR **Imaging** mode then conduct the proper procedure to return the equipment to normal point or image mode as other users may not be trained in using ATR imaging mode.

- 8.4.1 Lower the sample stage and bring forwards, towards you.
- 8.4.2 Slide the black dust cover back across the crystal to protect it.
- 8.4.3 Undo the silver screws and lift the crystal arm away from the stage (pressing the brake lock).
- 8.4.4 Place back into "Box 2".
- 8.4.5 Loosen the black thumb screws and lift away the ATR stage plate, again replacing it in "Box 2".
- 8.4.6 Replace the flat stage plate and screw into place.
- 8.4.7 Use the joystick to raise the stage to its highest position.
- 8.4.8 In the control window, click "zero" and the lower cassegrain stub will move upwards.
- 8.4.9 Slide the lower cassegrain all the way back into the dovetail connector and tighten the silver screw.

**Note:** Make sure that the lower cassegrain sits correctly in position as sometimes it is difficult to push it all the way back into the connector.

- 8.4.10 In the Control window, change the "Operation mode" back to "point mode".
- 8.4.11 Click the cross button and log out of the SpectrumIMAGE software.

## 9. Data Processing

### 1 Point spectra

- **9.1.1** Use the "Spectrum" software in "Work Offline" mode to process point spectra. There is a shortcut on the desktop.
- 9.1.2 "File", "Open" the .sp file.
- 9.1.3 Select the "View" tab and click on "Label Peaks". The wavenumbers of the most prominent peaks will automatically be added to the spectrum.
- 9.1.4 Click the "Vertical Cursor" button and a vertical green line appears on the screen.
- 9.1.5 Hover over the green line with the mouse, left-click, hold and drag the line over the spectrum to a peak. Release the mouse button and double click to label the peak.
- 9.1.6 Repeat on the other peaks.
- **9.1.7** Zoom into an area by holding down the left hand mouse button, dragging a square over the area and double clicking inside it.
- **9.1.8** To zoom out and view the whole spectrum press either of the "Previous scale" or "default scale" buttons.

### .2 IR maps

ote: These are some basic instructions to for processing IR maps. There are several other ways to process.

- **9.2.1** Open SpectrumIMAGE in "Work Offline" mode. Do not try to process spectra whilst running an experiment as it may cause the computer to crash.
- 9.2.2 "File", "Open" the .FSM file.

## 9.2.3 Viewing the IR spectrum for each data point in a map

- observe the IR spectrum found at each data point or pixel in the IR map:
  - a. Right-click on the image of the map
  - b. Select "view spectrum"
  - c. A new **Spectrum** window opens showing the IR spectrum recorded at that particular point in the map.
  - d. Hold the cursor over the red cross that appears and it changes from an arrow to 4 arrows joined at the centre.
  - e. Hold down the left-hand mouse button and drag the red cross over the IR map.
  - f. The spectrum in the Spectrum window changes as the cursor crosses each data point.

g. Let go of the mouse button and the **Spectrum** window displays the IR spectrum for the new location of the red cross on the map.

## 9.2.4 Principal Component Analysis

To conduct a principal component analysis on the IR map and manipulate the results:-

- a. Check "Average Absorbance" and click "OK".
- b. The map opens in a new window. The colours on this map represent the average area underneath the whole spectrum at each point and so they are not very helpful in interpreting the map.
- c. Click the "process" tab and select "show structure".
- d. Wait for the software to conduct the principal component analysis.

Note: If an error message is displayed keep pressing "OK" until the next window opens.

e. A new window entitled Layer Manager opens and the image of the IR map changes to show the results of Principal Component Analysis (Scores 1-3).

**Note:** The new IR map image picks out the areas that have different IR spectra in different colours. Areas of the same colour have similar spectra.

- f. 8 score files are recorded by the instrument. To add these to the IR map image, select the score (4,5,6,7 or 8) in the "List" box of the **Layer Manager** window and uncheck the "hide" box.
- g. Alternatively, to view each of the scores alone, select "single" instead of "mixed" in the "view" box and then select each score one at a time. The image of the map will change.

**Note:** From the "help" tab select "contents" and search for "principal component analysis" to find out more about what this type of analysis displays.

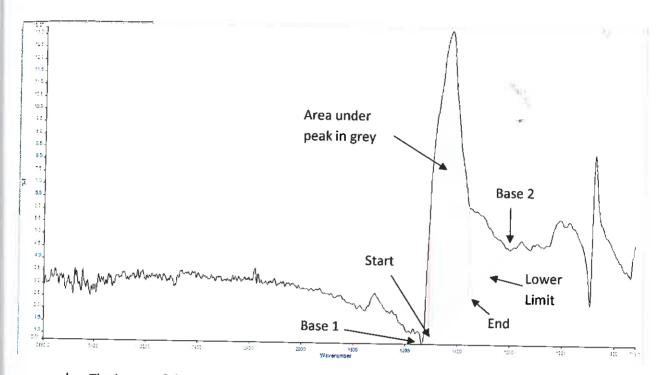
## 9.2.5 ChemiMaps

nap a particular peak (or functional group) across the IR map:-

- a. Check "ChemiMap" and click "OK".
- b. Click "OK" in the ChemiMap View window.
- c. Check the settings. \*
- d. Click "OK".
- e. An image of the IR map appears in a new ChemiMap window.
- f. Right click anywhere on the map.

g. A new **Spectrum** window appears showing the IR spectrum for that point. It also contains red lines and an area between them coloured in grey. The area in grey is the area that is being measured by the software (see the image below).

**Note:** The spectrum shown below is from a map recorded in reflectance mode, so the peaks are reversed.



h. The image of the IR map now changes. The redder coloured areas show where this peak (or functional group) is present and the bluer areas where it is not present at all. In this case the red areas would show the presence of dolomite, whilst the blue would show its absence.

**Note:** From the "help" tab select "contents" and search for "ChemiMaps" to find out more about what this type of analysis displays.

- i. To change the colour of the ChemiMap, click the "view" tab in the **ChemiMap** window and select "Format...".
- j. Under "Colour Scheme" select a new colour from the drop down box and click "OK". It can be useful to select a different colour for each peak (functional group) selected in the map, to make it easier to view the different distributions.
- k. To change the Display format from a false colour image to another format such as a contour map, click the "View" tab in the ChemiMap window and select "Display Type", then check the new format and the image of the IR map will change.
- I. To look at the new ChemiMap parameters (ie. the positions of the base, start and end points), click the "View" tab in the ChemiMap window and select "ChemiMap parameters...". A new window opens showing that the new point values have been added to the boxes.

a starting point use the following settings; Type= "Area", check "2 base points", leave the "Base1", "Base 2", "It" and "End" values as they are, if map was recorded in reflectance mode then check "Reflectance".

## 10. Ending a session

- 10.1 Leave the instrument and the computer ON when a session is finished.
- 10.2 If you will be the next user stay logged into spectrumIMAGE, if not then log out, but leave the computer and the instrument ON.
- 10.3 Always remove any samples from the stage and replace the lower cassegrain if you have removed it for any reason.
- 10.4 If you have been working in ATR Imaging mode then conduct the proper procedure to return the equipment to normal point or image mode as other users may not be trained in ATR imaging mode.

- once more has kinished some as intensity image
- File (Topleft) open, search for file, double chick.
- open as Chemimas
- view (Chemimap window) spectrum
  - Ho shange colours of chemi map bottom corner bottom corner eblour scheme
- -to save cheminap, save as, (Image)
  imp (open as chemin
  images)

## 11. Troubleshooting

The most common problem is the communication between the computer and the instrument.

This can manifest in different ways. The computer may give different error messages or the microscope may not respond to commands.

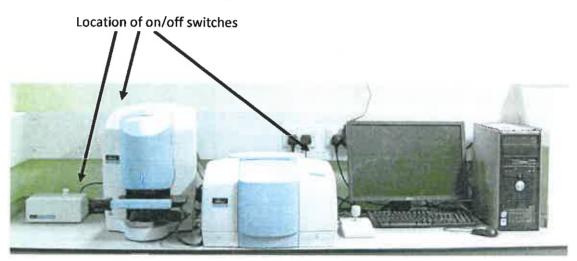
This is likely to happen each new day the instrument is used.

The only way to solve this is to turn everything off and on.

However, note that by doing this you will lose any data that you have just recorded (e.g. images, positions of data points, spectra) so try to save these before you resort to turning the instrument off.

nplete the following instructions:-

- 11.1 Exit the spectrumIMAGE software.
- 11.2 Shut down the computer.
- 11.3 Switch off the instrument using the three blue switches behind the equipment; the spectrometer, the microscope and the joystick controller (see image below).



11.4 Turn the computer back on and press return on the log in screen **DONOT** use your own log in name and password! If this has been done then use the following details:-

Username: Customer Password: (leave blank and press enter) Log-on to: SCPC299 (this computer)

- 11.5 Wait for the screen to load up fully.
- 11.6 Turn on the three instrument switches Always leave the instrument ON.
- 11.7 Wait for 10-15 minutes before logging into spectrumIMAGE to allow the instrument to start-up and connect to the computer. Hovering over the pictures on the bar at the bottom of the screen will show if they have connected properly.
- 11.8 Now log into spectrumIMAGE and start normally.
- 11.9 If this does not work, repeat the procedure from steps 1-8. This may need to be done 2 or 3 times to start the instrument working properly.