LabRAM

User Guide

An Introduction to the Software and Hardware



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Section 1

Introduction to the LabRAM Hardware and Software



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Introduction to the LabRAM Hardware

The following diagram of a LabRAM HR system shows the typical layout. The arrangement of stems and accessories is identical for the smaller LabRAM 300 instrument. The instrument can be considered in four parts:

- 1. Lasers the HeNe (633nm) laser is internal, whilst other lasers are external, mounted on an extended chassis at the back of the system.
- 2. Microscope sampling is carried out through a standard optical microscope.
- 3. Spectrometer dispersing the Raman signal into its constituent parts for detection by detector (usually CCD, but other formats can be used).
- 4. Optics for coupling the lasers to the sample, and carrying the Raman signal through to the spectrometer.



MICROSCOPE

(with sampling accessories, such as XY stage, Z motor, heating cooling stages etc)

Introduction to the LabRAM Hardware - continued

To provide the greatest flexibility and versatility, the LabRAMs include a number of simple stems (or push-pull bars) to allow fast switching between different functionalities.



	Present on LabRAM	Present on LabRAM HR	Function	
Stem 1	\checkmark	\checkmark	Operates camera beamsplitter. DOWN = camera UP = Raman	
Stem 2	0	0	LabRAM 300 – operates switching mirror for point mode (IN) and line scan (OUT). LabRAM HR – operates switching mirror for vis-NIR (IN) and UV-vis (OUT), or point mode (IN) and line scan (OUT).	
Stem 3	0	0	Operates switching mirror for microscope (IN) and fibre entrance (OUT)	
Stem 4	0	Ο	LabRAM 300 – operates grating turret (in the LabRAM HR this is motorised). IN = high resolution (grating 1) OUT = low resolution (grating 2) LabRAM HR – operates switching mirror for two detectors. IN = side detector OUT = top detector	

 \checkmark = always present

O = sometimes present

Introduction to the LabRAM Hardware - continued

In conjunction with the software, the LabRAM control box provides an interface between the user and the instrument. An explanation of the switches on the front panel is given below.



For more information on the hardware, including optical layout, please see the LabRAM User Manual.



Introduction to the LabSpec Software

The LabSpec software provides a complete data acquisition and analysis package for use with the LabRAM.

The LabSpec 4.XX folder on the desk top contains four icons:



The main LabSpec screen can be divided up into a number of regions, as shown below:



In the rare event of a software crash, it is recommended that the software is exited, and then double click on the **Software Reset** icon in the main LabSpec folder on the desk top. Click *OK*, and then re-open the software with the **LabSpec 4.XX** icon.

Note that LabSpec can also be used for simple data analysis (no instrument control) on other computers. See page 63 for details on how to install LabSpec to do this.

The Hardware Toolbar

The hardware tool bar at the bottom of the screen controls settings such as the laser, filter wheel, confocal hole, slit, spectrometer and gratings.





The Hardware Toolbar - continued





White Light Illumination – Image Acquisition

The LabRAM is equipped with standard white light illumination of the sample, by reflection and/or transmission. A colour camera linked to the software allows the sample to be visualised, and the image captured on the computer and saved.

1. Put the camera beam splitter into place.

DOWN = camera UP = Raman

2. Turn on the white light illumination.



- 3. In the software, click on the **video** icon to start the camera read out.
- 4. To stop the continuous readout, click on the STOP icon on the top right hand side of the screen.
- 5. <u>Remember</u> to turn off the white light illumination, and take the camera beam splitter out (=UP) before starting a Raman measurement.

To illuminate by transmission only, remove the white light fibre optic from the top left port of the microscope:







STOR

Section 2

The LabSpec Software for Data Acquisition



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Acquiring a spectrum

The LabSpec software provides the user with a number of methods for acquiring a single spectrum.

Real Time Spectrum Adjustment

This method is primarily designed for a fast display of the spectrum on screen corresponding to a single shot window. The update is continuous, providing a useful way of adjusting the focus position to maximise the Raman signal, and quickly monitoring the stability of the spectrum.

Each spectrum displayed replaces the previously displayed spectrum – there is no averaging or accumulation of the spectra, and no extended coverage possible. To do this you must use **Spectrum Accumulation** (see below).

- 1. Ensure the central spectrograph position has been correctly selected (remember, the position typed into the "spectro." window corresponds to the central position of the resulting spectrum).
- 2. Choose the integration time required
- 3. Click on the **Real Time Spectrum** Adjustment icon.
- 4. To stop the continuous readout, click on the STOP icon on the top right hand side of the screen.

– Laser – Filtr – Filtr	- Hole	Spectro	- Time	- Options
632.817 nm	300 µm	1500 cm-1		1800 - 7 ×100 - ~
1 <u>00</u>			Ū	
STOP				

Spectrum Accumulation

This method allows spectra to be acquired with multiple accumulations and averaging, and/or with coverage over extended regions.

Click on the Spectral Windows icon.

This allows you to define whether the acquisition is to be over a single shot window or an extended range.

The options from the drop down box are:

- SIMPLE = single shot
- MULTIWINDOW = extended range (1)
- CREST = extended range (2)





Simple

This method allows a single shot spectrum to be acquired, with user defined integration time and averaging.

- 1. Click on the Spectral Windows icon and choose SIMPLE from the drop down menu.
- 2. Ensure the central spectrograph position has been correctly selected.
- integration 3. Choose the time required
- 4. Choose the number of accumulations.
- 5. Click on the Acquisition Options icon to select a cosmic ray (random spike) removal algorithm. See page 24 below.
- 6. Now click on the Spectrum Accumulation icon to start the acquisition.
- 7. The acquisition can be stopped at any time by clicking on the STOP icon (top right of screen).

Multiwindow (extended range 1)

This method allows a spectrum to be acquired over an extended range, with a defined integration time and averaging. The extended range is covered by taking a number of individual single shot windows and 'gluing' these together. This procedure is fully automated through the software.

- 1. Click on the Spectral Windows icon and choose MULTIWINDOW from the drop down menu.
- 2. WINDOW In the Spectral PARAMETERS section, type in the Start ("From") and Stop ("To") positions.
- 3. Choose what COMBINING MODE options are required (see below).









- 4. Select the integration time and number of accumulations from the tool bar at the bottom of the screen.
- 5. Click on the **Acquisition Options** icon to select a cosmic ray (random spike) removal algorithm. See page 24 below.
- 6. Click on the **Spectrum Accumulation** icon to start the acquisition.
- 7. The acquisition can be stopped at any time by clicking on the STOP icon (top right of screen).

COMBINING MODE

The basic operation of MULTIWINDOW acquisition involves the acquisition of a number of discrete spectral windows, covering the chosen extended range. In order to simplify the process, it is possible to instruct the software to combine the individual spectra automatically, resulting in just one spectrum over the chosen extended range.

- Combination after acquisition checking this box will result in the individual spectra being automatically combined immediately after the acquisition.
- Remove combinated objects checking this box will cause the individual spectral to be deleted following the combination process, leaving just the final combined spectrum.
- Adjust objects intensity in order to remove any possible artefacts (such as steps) being introduced into the combined spectrum, checking this box will cause the baselines of the individual spectra to be adjusted prior to combination, in order to give the best possible result.
- Overlap this defines the overlap between the individual windows, in pixels. A typical value of 50 will give good results.

Note that individual windows can also be manually combined after acquisition simply be clicking on the COMBINE button. The *Remove combinated objects* and *Adjust objects intensity* check boxes will still function.









CREST (extended range 1)

The Continuous Rapid Extended Scanning Technique involves moving the grating in a number of very small steps, allowing the spectrum to be slowly built up step by step. The advantage of this method is that any pixel to pixel variation in response can be averaged out over the spectrum.



- 1. Click on the **Spectral Windows** icon and choose CREST from the drop down menu.
- 2. In the SPECTRAL WINDOW PARAMETERS section, type in the Start ("From") and Stop ("To") positions.
- Set the ACCUMULATION NUMBER. A good starting value for this is 10, but higher or lower values can be used as desired. For more information see below.
- 4. Set the SUB-PIXEL FACTOR to 1, and SMOOTHING (%) to 0. Again, for more information see below.
- 5. Select the integration time and number of accumulations from the tool bar at the bottom of the screen. Note that the accumulation number set here is **in addition to** the CREST ACCUMULATION NUMBER already specified.



 Click on the Acquisition Options icon to select a cosmic ray (random spike) removal algorithm. See page 24 below.



8. The acquisition can be stopped at any time by clicking on the STOP icon (top right of screen).

ACCUMULATION NUMBER, SUB-PIXEL FACTOR, and SMOOTHING (%)

Accumulation number – this value represents the number of individual acquisitions contributing to any particular data point in the final spectrum. The higher this number the better will be the signal to noise, the smaller the step size, and the more pixel to pixel averaging there is.

Sub-pixel factor – setting this number to anything other than one will cause the spectrometer to step by an amount less than a whole pixel value. The result is to increase the number of data points defining a band (ie, 2 = twice the number of data points, 3 = three times the number etc). Note that this will increase the total acquisition time, and it is suggested that sub-pixel acquisition is only used over limited ranges.



Smoothing (%) – this allows control over the algorithms used for the CREST sub-pixel acquisition, and relates to the response profile of an individual pixel to light. Typically this value can be set to 0.





Separate spectral regions and independent accumulation times

As well as defining a complete extended range for acquisition, a number of discrete ranges can be acquired, for example, 200-1000 cm⁻¹ and 2500-3500 cm⁻¹. In this way, only regions of interest need be acquired, and regions with no (useful) information can be ignored, thus saving time. Additionally, the separate ranges can be acquired with independent integration times, so that the resulting signal to noise can be optimised for each window.

- 1. Click on the **Spectral Windows** icon and choose MULTIWINDOW or CREST from the drop down menu.
- Check the boxes in the SPECTRAL WINDOW PARAMETERS section to select the number of individual windows required, and type in the Start ("From") and Stop ("To") positions for each region.
- 3. For CREST set the ACCUMULATION NUMBER, SUB-PIXEL FACTOR and SMOOTHING (%) values.

Note that for MULTIWINDOW acquisition the automatic combine function does not operate when acquiring discrete spectral regions. However. the regions can be combined manually after acquisition, by clicking on the Combine button.

- 4. If independent accumulation times are required for the individual regions, check the *Independent Accumulation Times* box, and set the integration time for each region.
- 5. Set the number of averages in the toolbar at the bottom of the page.
- If using independent accumulation times for each region, it is useful to set the intensity scale to "counts per second" (via the OPTIONS menu in the toolbar).









 Click on the Acquisition Options icon to select a cosmic ray (random spike) removal algorithm. See page 24 below.



8. Click on the **Spectral Accumulation** icon to start the acquisition.



9. The acquisition can be stopped at any time by clicking on the STOP icon (top right of screen).

Note that the automatic combine function does not operate when acquiring discrete spectral regions by MULTIWINDOW, but the regions can be combined manually after acquisition.

For other acquisition functions which can be used in conjunction with those discussed here, please see the **Other Acquisition Functions** section on page **24**.



Acquiring Mapped Images and Profiles

Introduction

With suitable accessories on the LabRAM, it is possible to acquire XY mapped images, and line (X or Y), depth (Z), time and temperature profiles.

The principal of these measurements involves acquiring an array (2D or 1D) of spectra, with each spectrum acquired with a particular varied parameter (for example, position, or time).

<u>Variation</u>	<u>Description</u>	Hardware requirement
X and Y position	тар	motorised XY stage
X or Y position	line profile	motorised XY stage
Z position	depth or Z profile	motorised Z stage/piezo
X, Y and Z position	volume	motorised XY and Z stage/piezo
Time	time profile	no accessory required
Temperature	temperature profile	heating/cooling sample stage

In all cases, the spectral array (3D for volume, 2D for map, 1D for depth, line, time, and temperature profiles) is saved as a single file, allowing fast and easy analysis of the data.

The example image below shows the point by point nature of a Raman mapped image.





Acquiring Mapped Images and Profiles - continued

Setting up a mapping/profiling experiment

With suitable accessories on the LabRAM, it is possible to acquire XY mapped images, and line (X or Y), depth (Z), time and temperature profiles.

- 1. On the microscope select the objective to be used for the mapping experiment, and select the *same* objective in the software.
- 2. If an XY map, XYZ volume, or line profile is to be acquired, obtain a white light image of the sample, and define the area/line to be analysed.





Discrete points across a surface (right mouse click to add and delete points)	
Horizontal (X) line ———	-
Vertical (Y) or sloped (XY) line ———	\mathbb{Z}
Rectangular (XY) area	
Polygonal (XY) area	8
Circular (XY) area	0

If a depth (Z), time or temperature profile is to be acquired, simply ensure that the sample is correctly positioned beneath the microscope.

3. Click on the **Acquisition Data Parameters** icon in order to define the mapping parameters.

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Acquiring Mapped Images and Profiles - continued

4. For each specific property, set up the experiment by clicking on the relevant icon. This will give you a choice for how the parameters are defined:

Don't use - do not use that property

Defined size – defines the data array size

Defined step – defines the step size in s, $^{\circ}$ C, or μ m.

The examples on the right show an XY map set up, either by defined size (indicating a final array of 20 x 20 points), or defined step (indicating a step size of 10 μ m in both X and Y directions). If *size* is defined, *step* will adjust accordingly, and *vice versa*.



Defined size



Defined step

-Use-	- Size -	Step-	Variation
* 🔁			
т			
0)			
z			
¥ r	37	10	80.7 -> 438.5 μm
×🛃	49	10	100.6 > 576.9 µm
F AA	1014	1	10 > 1023 pix

For area mapping X and Y dimensions need to be defined. For volume, define the Z dimension as well. Line profile For horizontal line profile, X dimension is defined only. For sloped line profile, X and Y dimensions are active, but size and step are defined only through the X dimension. **Time profile** For time profile, define time only. For time profile, define time only. **Depth profile** For temperature profile, define **Temperature profile** temperature only.

Area/Volume mapping

Acquiring Mapped Images and Profiles - continued

4. (continued)

Further specific details:

For <u>time profiles</u>, the step size is in seconds, and defines the time between the start of each acquisition. Note that the overall acquisition time per data point must be less than the step size.

For <u>depth (Z) profiles</u>, a negative number in the **variation** box implies moving the analysis point *into* the sample (ie, $-20 \rightarrow 20 \ \mu m$ will move 20 μm below the start position, and step its way up to 20 μm above the start position). The start position is always defined as 0 μm .

For temperature profiles, a simple ramp (ie, Defined size or Defined doesn't include step) а dwell (equilibration) time at each temperature. In order to do this. choose Arbitrary and click on Set Up to define the required temperature profile with dwell times.





Other Acquisition Functions

Cosmic Ray (Random Spike) Removal

The CCD detectors such as those used on the LabRAMs are sensitive to other forms of radiation, and in particular to random events known as 'cosmic rays'. These can interfere with acquisition of spectra by registering as very sharp and strong bands in the spectrum.

Since the occurrence of a cosmic ray is random, it is extremely unlikely that a cosmic ray will occur in exactly the same part of the spectrum in two or more consecutive accumulations. Hence, it is possible to use a simple algorithm to detect random events in a spectrum (as opposed to constant features, such as a Raman peak).

In LabSpec there are two algorithms to choose from.

Multi accum This is the more robust algorithm, and works by comparing two (or more) accumulations within an acquisition. If a random cosmic ray spike is observed, the spike will be removed. For this algorithm to work, the accumulation number (averaging) must be set to greater than 2.

Single accum This algorithm attempts to locate a spike in a single accumulation acquisition (ie, accumulation number = 1), by analysing bands for sharpness (width) and intensity. This algorithm needs to be used with care, for very sharp Raman bands can easily be modified. However, the algorithm works very well when looking at broad features (such as Raman bands of amorphous materials, photoluminescence etc).

Note that both algorithms can be used for SIMPLE, MULTIWINDOW, and CREST, single point, mapping, profiling etc.

1. Click on the **Acquisition options** icon.





3. Ensure that the accumulation number (averaging) for the acquisition is set appropriately:



Multi accum \rightarrow 2 or more

Single accum \rightarrow 1



Autosave

The **Autosave** function (via <u>OPTIONS</u> in the menu bar) automatically saves each spectrum/mapped image/profile immediately the acquisition finishes.





Autofocus

The Autofocus ensures an optimal focus is reached before an acquisition.

The Autofocus can work in three ways, depending upon the instrument configuration:

- Monitor total scattered light intensity with a pin diode maximum = focus position
- Monitor laser spot size on video smallest, brightest spot = focus position
- Monitor Raman intensity on CCD maximum intensity = focus position

Click on the **Acquisition Options** icon ². Check the box to activate the autofocus, and click on the *Autofocus* button to set up the autofocus function.



Autoexposure

The Autoexposure function allows the software to automatically adjust the integration time to ensure a good signal to noise. An initial acquisition is made, using the standard acquisition time set in the hardware tool bar. The quality of this spectrum is then compared to preset autoexposure parameters, and if necessary the acquisition time is increased or decreased to fit the required limits.

The **Autoexposure** is activated by checking the box in the main hardware tool bar.



Once activated, click on the **Autoexposure** icon

to adjust the parameters.

🗑 Auto expo 🛛	- 🗆 🗵		
× Saturation control			
Threshold value	9500		
Minimal time	1		
× Low level control			
Threshold value	2500		
Desired value 3500			
Maximal time	60		

Saturation control - sets the upper limit for acquisition eg. 9,500 counts. If this limit is reached it will calculate the acquisition time required to bring the spectrum below this threshold. A minimum time value can be set so that the system does not try to continually adjust this ad-infinitum.

Low level control - sets the values for low signals. The threshold value here defines the minimum difference between the highest and lowest intensity data points. With any measurement below this threshold value the software will calculate the increased time required to achieve a suitable acquisition (to meet the desired threshold value if entered) and set this as the resultant acquisition Again, a maximum acquisition time is specified here, to prevent the time. software attempting to make an infinitely long acquisition (for example, if there is no signal.



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Extended white light video imaging

With a motorised XY stage on the LabRAM, it is possible to montage a number of individual white light images together, in order to obtain an image from a much large area than would normally be possible.

- 1. Set up the LabRAM for white light illumination.
- 2. Ensure the correct objective is specified in the hardware tool bar.
- 3. In the software, click on the **Extended video acquisition** icon.
- 4. Set the parameters for the extended image acquisition.

<u>Number of images</u> = total number of images in X and Y dimensions to be montaged.

<u>Max size (pix)</u> = the maximum pixel size of the *final* extended image.

<u>Overlap (pix)</u> = individual images are overlapped in order to ensure seamless montaging. Input the size of overlap regions to be used.

<u>Total size (μm) </u> = display of the total size of the final extended image.

5. Click on the *Start* button to start the acquisition.

Note that once an extended image has been acquired all the standard map/profile experiments can be performed using this image.









Section 3

The LabSpec Software for Data Analysis and Display



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Data Analysis Functions

The LabSpec software contains a wide range of data analysis functions, which are briefly described here.



Arithmetic

Allows spectra to be have a constant value added/subtracted, or be multiplied/divided by a constant value.

Additionally, two spectra can be added/subtracted/multiplied/divided.





Baseline Correction

Allows removal of a background (eg, fluorescence) from a spectrum.



Once the baseline has been correctly approximated, click on *Sub* to subtract the baseline from the spectrum.





Correction

Allows normalisation, zeroing, and correction of a spectrum.



In order to normalise to a particular band, activate a spectrum containing the band, and click on *Get*. Adjust the two green cursors in the **Correction** window to select the band to be used for normalisation.

Clicking on *Norma* will now normalise the active spectrum so that the integrated area of the selected band = 100.







Profile

Allows a series of spectra to be bundled together into one file – a spectral profile. Examples of profiles include line analysis, time and temperature profiles, analysis of a series of slightly differing materials etc. Compiling spectra into one profile can simplify subsequent analysis.

In addition, a profile in the X or Y direction can quickly be generated from a Raman mapped image using this tool.

Creating a profile from individual spectra

- 1. Open the **Profile** window.
- 2. Activate the first spectrum to be added to the profile.
- 3. Click on Add in the Profile window.



- 4. Repeat the process with additional spectra to be added into the profile.
- 5. Once complete, close the **Profile** window.
- 6. Three windows will now be open:

Spectral_profile – overlapped data of all spectra

Profile – the profile displayed according to intensities between the three cursors (R, G, and B).

Spectrum – spectrum at current cursor position

Creating a profile from a mapped image

1. Place the map cursor in the correct X (horizontal) or Y (vertical) position to select the precise row/column from which to create the profile.

2. Click on *Horizontal* to create a profile across a row of the map (X dimension), or *Vertical* to create a profile down a column of the map (Y dimension).

3. A new window will be created showing the profile.



Smoothing and Filtering

Allows a spectrum to be smoothed in order to reduce noise, and other filtering algorithms (such as spike removal) to be applied.

Once the parameters are correctly set (see below), click on the *Filtr* button to apply the algorithm to the spectrum.





Fourier Transformation

Applies Fourier transform processing to the active spectrum. Using the IFT functionm allows particular frequencies contributing to the spectrum can be removed (for example, removing high frequency can reduce the random pixel to pixel noise apparent in a spectrum).





Peak Labelling and Band Fitting

Allows peaks within a spectrum to be quickly labelled, and for particular bands to be fitted in order to accurately calculate band position, width, amplitude and integrated area.

Peak Labelling

To activate the peak labelling algorithm, either click on the Search button, or adjust the Height and Neighbour scroll bars. These two scroll bars control parameters used within the searching algorithm for locating peaks in terms of the peak size and proximity to other peaks. Adjusting these scroll bars will allow the labelling to be optimised.

	🧑 Peaks & Bands	
Real time refresh of labelling on the spectrum so that the effect of adjusting <i>Height</i> and <i>Neighbour</i> can be monitored.	Peaks searching Height Neighbour Refresh Yes No Fitting Iteration number 10 Deviation χ^2 1.5362e+08 View Label format X Peak label X Band shape X Attachment	Search Fit Resume Approx Del Clear the labels from the spectrum. Save Bands Func Var
Check boxes allow the displa	y to be controlled:	Label format – allows the number of decimal places to be set (1 = X.X, 2 = X.XX etc)

Peak label – display the peak label Band sum – display the complete band sum used in the fitting Band shape - display individual band shape for each labelled peak Attachment – attach the peak label to the peak, or float at top of window.

Note: if a spectrum is saved with peak labels, the peak label information will be saved with the spectrum for future reference.



Peak Labelling and Band Fitting (continued)

Band Fitting

1. Click on *Func* in order to define the algorithm to be used for the band fit.



- Click on the peak Label icon, and mark which peaks are to be fitted. (left click = add, right click = delete).
- 4. Once all the peaks have been marked, click on *Approx.* and then *Fit.*



Peaks and Ban	da .	
None		0
Gauss/Lorentz	a"(g"gauss(1.41421"(x-p)/w)+(1-g)"kr	۲
Gauss	a*gauss(1.41421*(x-p)/w)	0
Lorentz	a"loren([x-p]/w)	0
AssimGauss	a"(step(x-p)"gauss(1.4"(x-p)/w)+(1-ste	0
AssimLor	$\label{eq:a} a"(step(x\cdot p)"loven)[x\cdot p]/w] * (1 \cdot step(x\cdot p)$	0
Baseline		
Line	n+c'y	Add
Polynom2	n+c'y+d"[[y-g]^2]	Add
Polynom3	n+c'y+d'[[y-g]^2]+c'y+e'[[y-m]^3]	Add



🮯 Peaks & Bands	_ 🗆 🗙
Peaks searching	Casada
Height I	R
Neighbour 🔸 🗾 🕨	Resume
Refresh 🔍 Yes 🔿 No	Approx
Fitting	Dei
Iteration number 10	Clear
Devision 1	Save
Deviauuri	Bands
χ ² 1.5362e+08	Func
View	Var
Label format	
× Peak label × Band sum	
X Band shape X Attachment	Close

5. The results will be displayed on the screen.

Note: if a spectrum is saved with the resulting band fit, the information will be saved with the spectrum for future reference.

	🧑 Peaks & Bands		Opens a new window
Fit parameters:	Peaks searching Height	Search	the band fit for baselines
Iteration number =	Neighbour 🚺 🗾 🕨	Resume	, and peaks. (p=position,
number of iterations of the	Refresh 💿 Yes 🔿 No	Approx	/ w=full width half max.,
fitting procedure to be	Fitting	Del	a=amplitude or max.
completed before	Iteration number 10	Clear	intensity, g=Gaussian
stopping.	Deviation 1	Bands	contribution, s=integrated
Deviation – if the x^2 value	χ ² 1.5362e+08	Func	area)
Deviation = If the χ^2 value		Var	
changes by less than this	Label format . 1		Allows initial start
amount, the fit procedure	🗙 Peak label 🛛 🕱 Band sum		parameters to be
will be considered	🕱 Band shape 🕱 Attachment	Close	specified and/or fixed
complete.			



Band Integration

Allows a fast integration of a band area.

1. Select the green pair of cursors



- 2. If the cursors aren't visible on screen, click on the **Cursor normalisation** icon.
- 3. With the cursors on screen, click on the **Integral** icon.



4. The area between the cursors will be shaded, and the integration results displayed.

The integrated area is divided into two parts; the top section (corresponding to the peak itself), and the bottom section (approximating background contribution). The values displayed correspond to the total integrated area, and the area of the top and botom sections:





Object dimensions

Allows extraneous data to be removed from a spectrum/map/profile. For example, if a spectrum includes data from 200 cm⁻¹ to 3500 cm⁻¹, but the region of interest is only between 400 cm⁻¹ and 800 cm⁻¹, it is possible to delete the data outside of this region.

- 1. Activate the spectrum.
- 2. Zoom in on the region that is to be retained ('extracted').
- 3. Click on the *Extract* button.



4. Note that this change is permanent if the file is saved, so it is recommended that the extracted data is saved with a new file name.

> The spectrum/map/profile can also be rescaled in all dimensions by manually typing in the new limits and clicking on *Scale*. The effect of this can be reversed by clicking on *Expand*.





Object generation

Controls visibility and behaviour of cursor components for map and profile analysis by cursor and models.

If a particular component is deleted by accident, it can be returned by unchecking and then rechecking the particular box in the **Object generation** window.





ASCII multi-file save

Saves all open files (spectra/mapped images/profiles) in text format.





Custom Units

Allows units other than cm⁻¹ and nm to be used. eV and kbar are preset, but custom defined formulae can also be used.

		🗑 Custom units			
		- Configuration			
Drop down box containing —		eV eV			
		Unit name eV		◀	This name will be displayed in OPTIONS in
		- Functions F(x)			the menu bar.
Function defining the	the	1 eV = A/x		◀	x = wavelength in nm
custom unit	ſ	1 nm = A/x		◀	x = custom unit
	Ċ	- Variables			
	(A 1240 B			
Variables used in the		C			
function formulae above.		E			
	•	ОК	Cancel		

Note that whatever unit is chosen from the **Custom Units** drop down box will be displayed in <u>OPTIONS</u> (in the menu bar).

	Multi	
<	nm ✔ cm-1 eV	>
	✓ Count Count/s	
	✓ Device bar XY(Z) stage	
	Auto save	



Analysing Mapped Images and Profiles

Before any analysis is made it is recommended that the data is saved. In all cases, ensure the window containing all the overlapped spectra (**Spectral_Image** or **Spectral_Profile**) is highlighted, and save in the normal way.

A spectral image or profile is initially displayed with three active windows as below:



Spectrum – the spectrum at the current cursor position

Note: the following descriptions are based upon analysis of a Raman mapped image, but apply equally to analysis of a line, temperature, time or depth profile.

Analysing with Cursors

The initial method for analysing a mapped image or profile is to use three cursors (\mathbf{R} , \mathbf{G} and \mathbf{B}) to define regions. The image is generated by displaying the spectral intensity between these cursors. For example, in the above diagram, the image can be seen to comprise three coloured regions, which correspond to peak intensities in regions defined by the cursors.

The three cursors are simply selected from the three coloured cursor icons on the bottom right hand side of the **Spectral_image** and **Spectrum** windows.

If one or more of the cursors aren't currently displayed on screen, they can be returned by clicking on the **Cursor normalisation** icon the toolbar.

Using the cursors provides a fast method of getting useful information from the data. However, in order to distinguish different components this method does require that there are distinct, non-overlapping peaks which can analysed through the cursors.

Analysing with Modelling

With the modelling functionality, it is possible now to distinguish spectral components that have a large number of overlapping bands, and look at the distribution of any number of species (with the cursors, this is limited to just three – one for each cursor).

The modelling algorithms are based upon correlation fitting of known reference spectra (the models) to the raw data. These model spectra can in fact be obtained separately (ie, run spectra of known raw materials) or taken from the mapping data itself.

- 1. With the map data open, click on the **Model** icon on the toolbar and set if for *Full search tree*.
- 2. If you wish to load in model spectra which you have already acquired separately, open up these spectra.
- 3. Activate the spectrum you wish to use as the first model component.

If you wish to take this model component directly from the mapping data, use the map cursor to find the spectrum you wish to use, and then activate the raw data spectrum (light blue) in the **Spectrum** window.

- 4. Click on *Get* in the **Model** window.
- 5. A new window will open up which contains the model spectrum. The purple cursors define which region of the spectrum is to be used for the correlation fitting.
- 6. This process can now be repeated as desired. Remember, if model spectra are being taken from the map itself, ensure the <u>light blue</u> spectrum in the **Spectrum** window is highlighted before clicking *Get*.







The **Image** window now contains both the information from the cursors and the models.

If the data is in overlay mode (through FORMAT > VIEW in the toolbar), the image will either display all the cursor intensities, or all the model intensities, but not both at the same time. Which is displayed can simply be chosen by using the mouse to activate any cursor indicator or any model indicator.



The **Spectrum** window now shows not only the raw spectrum at the current cursor position but also information about the correlation fit.

The coloured legends in the top right hand corner indicator the contribution of each component to the correlation fit to provide a match with the raw spectrum.

The coloured spectra displayed comprise:



Displaying a map of band position, width, area etc

The data contained within a mapped image or profile can be analysed by a band fitting routine, and the results can then be plotted to give a map showing band position, width, area etc. Such analysis can be of use when investigating changes in phase, stress/strain in materials and other such applications.

- 1. With the map data open, in the **Spectral_image** window use the zoom tool to select just the area which contains the bands which are to be fitted.
- 2. Click on the **Data objects** icon and then the *Extract* button, in order to remove all extraneous data, and leave just the region of interest. It is best that this extracted data is now saved with a different file name, so that the original map with complete data is not lost.
- 3. Open the **Peaks & Bands** window.
- 4. Choose the band function you wish to use for the fitting procedure, by clicking on the *Func.* button. A baseline can also be added at this point.
- 5. With a function selected, now select the peak **Label** tool from the left hand tool bar.
- 6. On the **Spectral_image** window, mark which peaks you wish to fit.
- 7. Click on *Approx.* and then *Fit* to start the fitting procedure.

<u>Note</u>: this could take a few minutes to complete for a large map.













8. Once the fit is complete, click on *Bands.*



9. A new window will open, containing details of the band parameters resulting from the fit.

These include information on the baseline (if present) and all the bands in the fit procedure. The parameters are as follows:

p = peak position (cm⁻¹)

a = amplitude (max. intensity)

w = full width half max (cm^{-1})

g = gaussian contribution (1=max)

s = integrated area of band

The band parameters shown are for the spectrum at the current cursor position.

10. Clicking on one of the parameter labels will generate a new mapped image, giving information about that parameter.

For example, a map could be generated showing the exact peak position of a band, according to the coloured scale (for example, dark regions \rightarrow low wavenumber position, bright regions \rightarrow high wavenumber position).





Data Display Functions

FORMAT in the menu bar allows a number of display settings to be adjusted.



View



Overlay behaviour (single, multi, and multi

Display spectra, all selection, 100% or

Colours/Axes

Superimposition

light image.

Check boxes allow axes. titles, and gridlines to be displayed. Colour palettes allow their colours to be changed if desired.

Axis titles (default values are shown)



Display Functions - continued

3D Option – only accessible when the 3D View mode is chosen (FORMAT>VIEW).

Note that the only single components can be shown in 3D display.



Adjust the angle of the 3D display.

Scale



Other Functions

Multi

The **Multi** function (via <u>OPTIONS</u> in the menu bar) allows whatever process is performed on the active spectrum to be performed on *all* open spectrum.

For example, if a constant value is added to one spectrum, with **Multi** selected, all the open spectra will have that constant value added to them. This can be a useful way to save a number of files – with **Multi** selected, a save dialog window will appear for each open spectrum, in the order they are displayed in the **Objects** list.

Axe + Text

If this option is checked (via \underline{E} DIT in the menu bar), data saved as text format will include both the X and Y axis data. With this option unchecked, only the Y (intensity) axis data will be saved.

✔ Axe+Text	
Axe+Text	

X and Y axis data saved as text format.

Y axis data **only** saved as text format.

Copy and Paste

The standard **Copy** and **Paste** functions (via \underline{E} DIT in the menu bar) are slightly expanded in LabSpec.

COPY TEXT = copy the spectrum in text format (one or two columns of data) to paste into another application (Excel...)

COPY PICTURE = copy the spectrum window as an image to paste into another application (Word, Powerpoint...)

COPY DATA = as COPY TEXT.

XY(Z) Stage

By checking this option (via \underline{O} PTIONS in the menu bar) an additional window will appear in the hardware toolbar, showing information about the current XY(Z) stage position.





Other Functions - continued

Units

Units for the X axis can be specified as cm⁻¹, nm and *custom*, and for the Y axis units can be specified as counts or counts/s. Via <u>OPTIONS</u> in the menu bar.

Custom units are defined and chosen using the **Custom units** icon in the toolbar – see page 39 for more details.

More Objects

Up to 6 spectra can be chosen and activated through the coloured radio tags at the edge of the spectrum window. However, **More objects** (via <u>OBJECTS</u> in the menu bar) provides a full list of all open spectra, with coloured radio tag and file name.





Other Functions - continued

Page Set Up

Page Set Up (via <u>FILE</u> in the menu bar) allows the print page to be configured, including the spectrum itself, information from the acquisition, logo, and comments.





Section 4

A Summary of Software Icons



Explore the future



A Summary of Icons



Delete

Deletes active spectrum/mapped image/profile.



File Open

Open a file (spectrum, mapped image, profile, colour image, text, spc...).



File Save

Save a file (spectrum, mapped image, profile, colour image, text, spc...).



Cursor Normalisation

Bring the cursors back into window.



Rescale Window Rescale the display that the entire spectrum (or all spectra) are displayed.



Print – see page 49

Print the current spectrum/mapped image/profile.



Information

Displays acquisition parameters relating to the active spectrum.



Spectral Windows – see page 13

Set the spectral range to be acquired (single shot, multiwindows, CREST).



Arithmetic – see page 30 Add/subtract/multiply/divide spectra.

M

Baseline Subtraction – see page 30 Model and subtract a background from a spectrum.

A Summary of Icons - continued



Correction – see page 31

Zero or normalise spectra.



Profile – see page 32

Create a profile from a number of spectra, or from a mapped image.



Filtration – see page 33 Apply smoothing and filtering algorithms to the active spectrum.



Fourier Transform – see page 33 Apply DFT and IFT processing to the active spectrum.



Peaks and Bands – see page 34 Label peaks, and apply band fitting to spectra and mapped images.



Integral - see page 36

Calculate the integrated area between two cursors.



Palette

Adjust the colour/contrast/brightness settings for images.



Object Dimensions – see page 37

Extract a region of interest from the active spectrum.



Object Generation – see page 38

Control visibility and behaviour of cursors and models for mapping analysis.



Modelling – see page 41

Analyse maps and profiles using correlation fitting of model components.

A Summary of Icons - continued



Video Readout - see page 11

Acquire a white light image of the sample.



Extended Video Acquisition – see page 28

Montage a number of white light images together for larger coverage.



Real Time Spectrum Adjustment – see page 13

Real time update of spectrum, with defined accumulation time - no averaging



Spectrum Accumulation – see page 13 Allows spectra to be acquired with multiple accumulations and averaging.



CCD Readout Display the CCD image, with defined accumulation time.



Array Acquisition – see page 20 Acquire a mapped image, or line/time/depth/temperature profile.



Acquisition Data Parameters – see page 20 Set the parameters for an array acquisition (ie, step size, array size...).



Acquisition Options Set up parameters for autofocussing, line scan and cosmic ray removal.



Detector Parameters Parameters for CCD detector read out – includes temperature read out.



Scale Parameters – see pages 60 and 61

Set parameters for calibrating camera image and laser spot on screen.

Page 54

A Summary of Icons - continued



Spectral ID – see separate user manual

One touch link to Spectral ID database searching module.



ASCII Multi-file Save - see page 38

Save a batch of files in text format – for spectra, mapped images and profiles.



Custom Units - see page 39

Use custom units for X axis, including eV and kbar.



Stop Acquisition Stop acquisition of spectrum/mapped image/profile.



Cursor

Use the cursors with the mouse.



Noise Filtration

Use the mouse to smooth a particular part of a spectrum.



Shape Correction

Amend a peak, or edit out a cosmic ray (random spike) with the mouse.



Zoom Drag out the area to zoom with the mouse.



Horizontal Shift Shift the X axis with the mouse.



Vertical Shift

Shift the Y axis with the mouse.

A Summary of Icons - continued



Intensity

Rescale the intensity scale with the mouse.



Shift

Shift the X and Y axis scales with the mouse.



Add Constant – see page 30

Add/subtract a constant value to the active spectrum.



Multiply Constant – see page 30 Multiply/divide the active spectrum by a constant value.



Label – see page 34

Label a peak, or identify a band for band fitting.



Move Peak Maximum – see page 34 Adjust the position of a labelled/marked peak.



Fit Peak Width – see page 34

Adjust the band width of a labelled/marked peak.



Section 5

Maintenance and Calibration Procedures



Explore the future



Before acquiring a spectrum, the LabRAM needs to be calibrated. This is a simple procedure involving two software parameters

- **ZERO** This is a number used to define the position of the zero order of the spectrograph and can be thought of as the number of motor steps to move away from a mechanical calibration sensor.
- **KOEFF** This can be thought of as the number of nm moved per motor step.

Part 1 - Calibrating the Zero order position

1. Begin by selecting the grating you wish to calibrate and move the spectrograph to **zero order** using the sicon in the *Spectro.* section of the hardware toolbar.

Note that the two gratings are calibrated individually.

- 2. Set the instrument up as follows:
 - Hole = 300 μm
 - Slit = 150 μm
 - Turn on the white light by reflection.
 - Ensure Camera beamsplitter is selected
 - Change UNITS to nm (via Options in toolbar)
- 3. Now use the spectrum adjustment icon $\frac{1}{100}$ to take a spectrum adjusting the acquisition time or white light intensity until the signal level is around 10,000 counts.
- 4. Press 💷 .

Use the **RED** cursor to measure position of the band, remember that it is highly unlikely to read exactly 0 nm. The band should be within +/- 1 pixel of Zero.

The way to measure the nm value of a pixel is to slowly move the RED cursor and watch the position value increment. The value of one increment is the value of 1 pixel, e.g. For a HR with a 1800 g/mm grating 1 pixel = 0.02nm.

- 5. If the band is not at zero open the calibration window using the icon in . Now adjust the ZERO parameter and watch the band position move, adjust until the band is within +/- 1 pixel of 0 nm.
- 6. Resend the spectrograph to zero order and retake the spectra. This will make sure that your calibration changes have taken effect. Check the zero order position again, and if necessary, make further adjustments.
 - **NOTE:** The ZERO parameter should be changed in small increments e.g. +5 or -5 at a time.

Calibrating the LabRAM - continued

Part 2 - Calibrating the Raman spectrum

NOTE: This part of the calibration can also be done using a known emission line from a Mercury or Neon lamp.

- 7. Now change the UNITS to wavenumber (cm⁻¹) and move the spectrograph to a position at which you can monitor the Si Raman band (520.07cm⁻¹).
- 8. Insert your standard silicon sample and focus the sample in the normal way.
- Acquire a spectrum of the silicon sample (remember to remove the camera beamsplitter and turn off the white light illumination) using the spectrum adjustment icon again you should now be able to see the Silicon Raman peak. Press .
- Again use the RED cursor to measure the position of the band. It should within +/- 1pixel of 520.07 cm⁻¹. If it is not within this limit then adjust the KOEFF value to move the position of the band.

Adjust the KOEFF by a small amount only (start by changing just the least significant digit), and retake a spectrum each time you change the KOEFF to monitor the effect.

NOTE: If the KOEFF is altered by a large amount it is advisable to recheck the zero order.

10. Once you are satisfied with the calibration, close the calibration window and ensure that you save the changes when prompted.

	Calibration	<u>_ </u>	
	- Mfluo	Bands	
	Zero 2970		
	Koeff 0.0042923000		
7	Error		∕ Mfluo save
Zero and Koeff	- Options		Click hore to
values	Test 🛈 Hole 🔿 Slit		
	Shutter 🛈 Close 🔿 Open	- Spectrograph	save line
	Unit 🛈 Spectr 🦳 Pixel	S1 0	in the boxes
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	From To Number	\$3 585.25	
	Hole 1 900 23	S4 621.73	Mfluo restore
	Depth 0 0 1	S5 659.90 🛨 👀	Click here to
	C	reload the	
			f previous Zero and Koeff values.

Changing Laser Wavelength

- 1. Open the top panel of the LabRAM, and remove the dust cover, in order to access the top optics.
- 2. Adjust/replace the items as displayed in the picture below:



- 3. Replace the dust cover, and close the top panel.
- 4. Ensure all the push-pull bars are correctly chosen for the particular laser wavelength to be used.
- 5. Adjust the laser wavelength in the software:



Calibrating the XY Stage Movement

- 1. Insert the graticule, and focus on the markings .
- 2. Ensure the same objective as is being used is specified in the software.
- 3. Capture an image of the graticule
- 4. Using the "square/rectangle" mapping selection cursor (selected from the bottom right of the white light image), set the X dimension to match exactly that of a known distance on the graticule.
- In the scale parameters box input the correct X size. Having typed in the number, make sure you press return, and then click "SET".

 Repeat for the Y size (you may need to acquire another white light image). Remember again to press return having typed in the correct number, and then click on "SET".

7. This procedure needs to be repeated for each objective being used (ie, 10x, 50x, 100x).

If you are using a macro lens/objective (x4, x5) it is likely that the graticule will be too fine to use. A simple ruler with 1mm markings should be sufficient in this case.













Calibrating the Laser Spot Position

- 1. Send the spectrograph to the reference diode position by clicking on the right hand arrow.
- 2. Open the confocal hole to 300 $\mu m.$
- 3. Insert the standard silicon sample under the microscope, and focus in the normal way.
- 4. Turn off the white light illumination, but keep the camera running.
- 5. On the main control box, turn the reference diode **on** using the left hand switch (marked "diode"). [up = on down = off]
- 6. You should now see the red diode spot on the camera image. Adjust the microscope focus to bring the spot to a tight focus.
- Close the confocal hole down to between 50 μm and 100 μm (ensuring you can still see the diode spot on the camera image). Adjust the focus again.
- 8. Stop the camera running.
- 9. On the still image centre a single cursor point
 - Click on the discrete point cursor
 - Normalise









Calibrating the Laser Spot Position - continued

- 10. Drag the cursor square to the centre of the diode spot (it is best to zoom in on the spot using the zoom icon first)
- 11. Open the scale parameters box
- 12. Click on "SET" for the "Centr (xy)" row.

13. Click on "YES" to confirm the change to the scale parameter.

	Length X (pm)	0
.1.		
🗑 Scale paramet	ers	>

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Close

Set

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Centr (x,y)

Amplitude

Warning	×
Scale parametrs will be changed	
Yes No Cance	





- 🗆 ×

Installing LabSpec Software for Data Analysis

1. Double click on the "LabSpec4XX.exe" to start the installation procedure.

2. Click on "next"

 The Installer will automatically choose a location to save the LabSpec programme files. Click on "continue". If you wish you can amend the file location, and then click on "continue".

4. The programme files are copied to the specified location.









📇 The LabSpec



Installing LabSpec Software for Data Analysis - continued

5. The installer now searches for previously installed LabSpec versions on the computer. If it finds a previous version, it asks: "Would you like to use the configuration files of the previous LabSpec version?"

Click on "no".

5. The installation will be completed. Click on "Finish".

6. A **Config** window now opens. Click on "treatment".

7. At the prompt, click on "OK".





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Installing LabSpec software for data treatment - continued

8. Click on "Close".

9. When prompted, save the changes to the configuration file by clicking on "Yes".

10. A new folder labelled **LabSpec 4.XX** will appear on the desktop, containing the following icons.

Using Spectral ID and Ascii Dump with LabSpec data treatment software

• Exit the LabSpec software.

HORIBAJOBIN YVON

- In the LABSPEC > DRIVERS directory on the hard drive, copy the files "toascii.dll" and "search32.dll" and paste these into the LABSPEC > PLUGINS.
- Click on "init" or "software reset" within the LabSpec folder on the desktop (and click "OK" to confirm).
- Restart the LabSpec software two icons for Spectral ID () and Ascii File Dump () will now be visible on the toolbar.

Config Software LabSpec 4.16 Uninstall Reset



Co



Section 6

Contact Details



Explore the future



Contact Details for Horiba Jobin Yvon

For further information in the UK please contact:

RAMAN SERVICE DEPARTMENT

For all enquiries concerning software, hardware, maintenance and service issues, including lasers, heating/cooling stages, and other accessories.

RAMAN SALES OFFICE

For all enquiries about software and applications, upgrades, new accessories and new instruments.

HORIBA Jobin Yvon Ltd., 2, Dalston Gardens,

Stanmore,

Middlesex,

HA7 1BQ

Tel: 020 8204 8142 Fax: 020 8204 6142

http://www.jobinyvon.co.uk

н	0	RI	RA	1	OR	N	VV	ON	
				10	OD	IN	IV	UN	

 USA:
 HORIBA Jobin Yvon Inc, 3880 Park Avenue, Edison, NJ 08820-3012, Toll-Free: +1-866-jobinyvon Tel: +1-732-494-8660, Fax: +1-732-549-5125, E-mail: info@jobinyvon.com, www.jobinyvon.com

 France:
 HORIBA Jobin Yvon S.A.S., 16-18, rue du Canal, 91165 Longjumeau Cedex, Tel: +33 (0) 1 64 54 13 00 Fax: +33 (0) 1 69 09 93 19, www.jobinyvon.fr

 Japan:
 HORIBA Ltd., JY Optical Sales Dept, Higashi-Kanda Daliji Building, 1-7-8 Higashi-Kanda, Chiyoda-ku, Tokyo 101-0031, Tel: +81 (0) 3 3861 8231, www.jyhoriba.jp

 Germany:
 +49 (0) 89 462317-0, taly: +39 0 2 57603050, UK: +44 (0) 20 8204 8142, +86 (0) 10 6849 2216

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