



## LabSpec 6

### Spectroscopy Suite

Reference Manual

Applies to version: 6.4  
Doc. UM-LabSpec6/Eu  
Part Number: 1131 089 151



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We have been at the forefront of Raman spectroscopy since its infancy, introducing ground breaking innovations such as the first Raman microscope and the world's first commercial remote Raman sampling probe.

HORIBA Scientific's Raman Division today comprises the combined expertise of the HORIBA Jobin Yvon, Dilor and SPEX companies and is proud of having more than 3000 Raman systems installed worldwide. Well equipped applications labs and full technical support enable HORIBA Scientific to provide services right through from the earliest proof of principle investigations. With a broad range of technologies available, HORIBA Scientific can uniquely provide solutions for analytical, research and industrial Raman analyses.

If you have any questions regarding the installation or the maintenance of your system, please contact one of our representatives:

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**QR Code:**



## HORIBA Scientific

With its long experience in the research and manufacture of scientific instruments (since 1819), Raman instrumentation from HORIBA Scientific uses state of the art technology and design for the optical, mechanical, electronic and computer components within each System.

The result is unequalled performance, flexibility and ease of use, with less human error and greater productivity. The level of precision and reproducibility for measurements is perfectly matched to the demands of today's laboratory and production facilities.

Last but not least, HORIBA Scientific instruments are designed and manufactured with complete user safety in mind.

**Our Internet Site:** <http://www.horiba.com/scientific/products/raman-spectroscopy/>

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The **LabSpec6 Spectroscopy Suite** represents the latest generation software for HORIBA Scientific's Raman, photoluminescence (PL) and cathodoluminescence (CL) instruments, providing easy computer controlled operation for quality control, analytical and research applications alike. It maintains the market leading performance for which LabSpec is known, with flexibility and performance to provide the most comprehensive data acquisition, processing and display tool available.

Your LabSpec6 Software is delivered according to your specific order details and (where appropriate) is ready to control a system. In case of specific needs or if you have any questions concerning the software and equipment delivered, please contact your HORIBA Scientific Service Team or your local representative.

- **About LabSpec6**

**LabSpec6** is a fully configured data acquisition and analysis software designed for HORIBA Scientific's range of Raman, photoluminescence (PL) and cathodoluminescence (CL) spectrometers and microscopes.

It enables a full range of experiment types including single spectrum acquisition, multidimensional spectral array acquisition (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes), video image capture and automated multiwell interrogation.

Many commonly used data analysis and processing routines are also available, including peak labelling and fitting, smoothing, noise reduction, baseline subtraction, linear and non-linear filtering and multivariate analysis techniques such as CLS, PCA, MCR, HCA, DCA and PLS. LabSpec6 is also compatible with Microsoft Visual Basic scripting (VBS), allowing specific acquisition and analysis routines to be written by the user. In addition, third party software routines can call LabSpec6 through ActiveX modules.

- **Use of this manual**

This manual must be used in conjunction with the relevant hardware manual accompanying each HORIBA Scientific spectrometer.

It is advisable that specific hardware/software training is provided to new users by either a trained HORIBA Scientific service or application engineer, or by an experienced user within the institution.

Please note that not all systems will have all available accessories and options installed, and thus the screen shots and images in this manual may not directly correspond to software installed with the specific system you are using. If in doubt, please contact your local HORIBA Scientific office or representative to receive more information about available accessories and options.

- **Terminology used**

For clarity, explanations on key terms and conventions used throughout this manual are given below.

***Instrument, equipment, system, spectrometer***

These terms refer to the main Raman hardware, which typically comprises laser(s), microscope, monochromator(s), and detector(s).

***Accessory***

This refers to optional devices such as motorized sample stage, or temperature stage.

***Dialog window***

This refers to a window which appears during the operation/set-up of a specific function.

***Tabs***

The «>» symbol indicates a section within a tab, with the tab name displayed first, and then the section (for example, Processing > Correction, or Analysis > Peaks).

***[button]***

Text in square brackets (for example, [OK] or [Cancel]) refers to a software button in the dialog window currently being described.

***{radio button}***

Text in curved brackets (for example {Video On} or {Video Off}) refers to a radio button in the dialog window currently being described.

***<enter ↵>***

This refers to the keyboard 'enter' or 'return' button.

***Multidimensional spectral array***

This refers to a spectral 'map' or 'profile', including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes.

- **Instruments controlled by LabSpec6**

A wide range of HORIBA Scientific instruments are controlled and operated by LabSpec6 software, including the following:

- LabRAM HR and LabRAM HR Evolution
- LabRAM ARAMIS
- LabRAM IR and IR2
- XploRA
- XploRA PLUS
- XploRA ONE
- XploRA INV
- HE spectrograph
- Modular Raman Systems (including iHR and micro-HR spectrometers)
- AccuRA

Chapter 2

# Installation

Your Raman System is controlled by LabSpec6 software developed by HORIBA. If the system has been delivered with a computer, LabSpec6 has been already installed and tested with your system. If not, the HORIBA Installation Team or your local representative will install it.

However, you may need to install software on your own computer for offline data analysis and processing of data acquired with the instrument. In this case, follow the installation procedure described below:

## 2.1 Procedure

- 1 Insert the LabSpec CD inside the CD drive of your computer.
- 2 Usually, the installer window appears automatically. If not, from the Windows Explorer interface, double-click on the setup\_6\_X\_XXX.exe file.

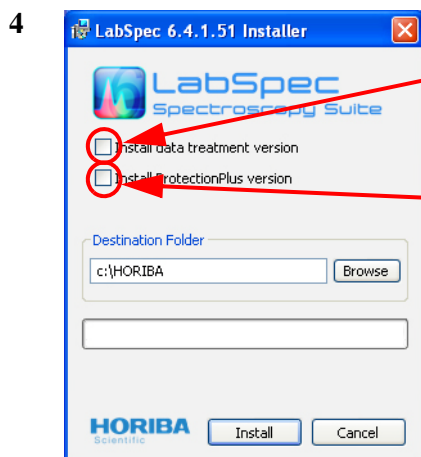


If using Windows 7 you may need to right click and select «Run as administrator».



**Read the HORIBA Scientific Software license Agreement then click «I agree» button to continue the installation process.**

- The default installation folder is C:\HORIBA. It is recommended that this default location is used, without modification.
- Tick the box «Install data treatment version» if LabSpec6 should be installed for data treatment only (no instrument control). This will create the desktop shortcut with the «treatment» key, which stops all communication with hardware, and hides the Acquisition tab.



**IMPORTANT:** tick the box «Install Treatment version». This will by-pass hardware detection, and install LabSpec6 for data processing and analysis only.

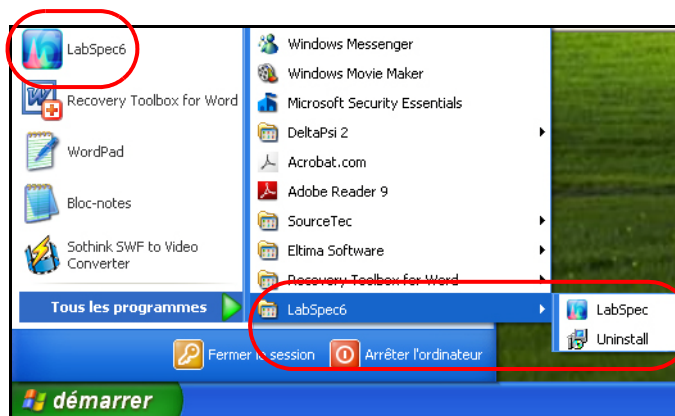
Tick the box «Install ProtectionPlus Version» to install LabSpec6 with enhanced security and data integrity features for regulatory compliance (including 21 CFR 11). ProtectionPlus requires separate purchase and licensing. **DO NOT INSTALL UNLESS YOU HAVE LEGITIMATELY PURCHASED THE MODULE.**

- 5 Click on the *[Install]* button to launch the installation. Once done, click on *[Exit]*, and LabSpec is ready to run.

If a previous version of LabSpec is found on the PC, you will be prompted to use the configuration of that previous version:

- Click [Yes] if you want to transfer the configuration and user files from the previous version. This means that all user settings and hardware configuration will be immediately available in the newly installed version.
- Click [No] if you do not want to transfer the configuration and user files from the previous version. This means that the new installation will not include any user settings and hardware configuration files.

- 6 From the Windows **Start>Program**, search the LabSpec software and launch it. Here below is an example of Windows XP Start window.



XP example for launching LabSpec

As part of the installation, shortcuts are created in the Start > All Programs, and on the desktop.

## 2.2 Uninstalling LabSpec6

Each installation folder includes an «uninstall.exe» program. Double click (or right click, and select «run as administrator») to uninstall LabSpec6.

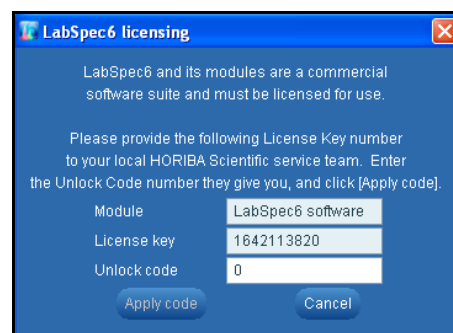
Uninstalling LabSpec6 will delete all original install files in the program folder (C:\HORIBA\LabSpec6\_X\_XXX) and any shortcuts with original names («LabSpec6»).

## 2.3 Licensing LabSpec6

LabSpec6 is a commercial software package, and must be licensed for use. Licensing is done using software unlock codes, which are specific for each individual PC.

When LabSpec6 is first run, you will be prompted to license the software. The prompt window will display the name of the module which must be licensed, and a license key number.

This key must be sent to your local HORIBA Scientific representative. Once accepted, your local representative will send you an unlock code. Enter this code in the related field, then click on the *[Apply code]* button. LS6 is ready to run.



### IMPORTANT NOTE:

If you are running on Windows 7, it is recommended to run LabSpec6 with full administrator rights when you carry out the above licensing procedure. To do this, right click on the LabSpec6 shortcut and select «run as administrator».

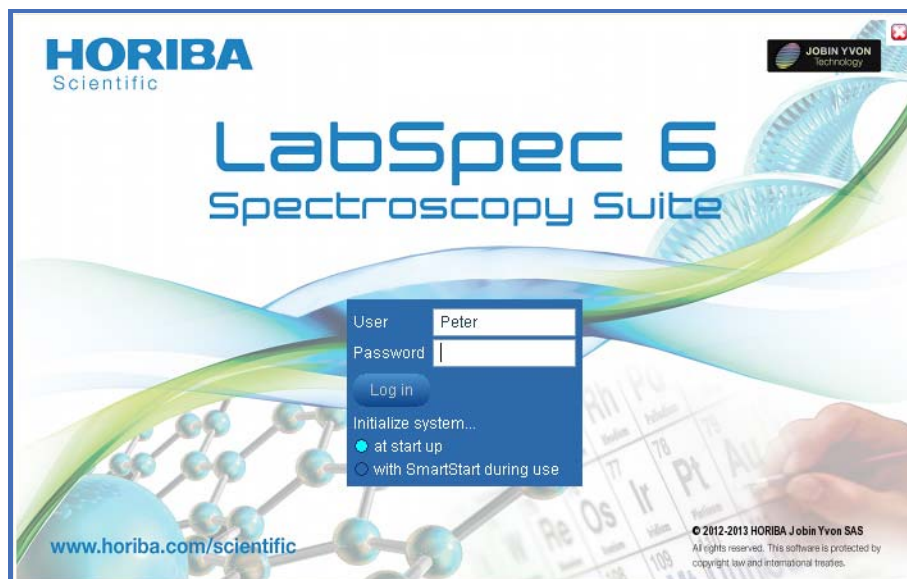
If this is not done, it is possible that the license will only be temporary, and will be requested again when you start the next session.

This only needs to be done during licensing. For normal use the software can be started by running LabSpec6 in the normal way.

## 2.4 User Accounts

LabSpec6 includes a User Accounts module, to allow individual user login name/password for each operator. If this module is active, then each time LabSpec6 is launched, a «Login» window is displayed. Enter your login name/password to run your LabSpec settings.

The User Accounts module is administered by the system Administrator. If you need an account to be created, or your password to be reset then please contact the Administrator.



**NOTICE:** Depending on your LabSpec6 version, this window can be slightly different. Running the version connected to a system (i.e., not in standalone data treatment mode), you will have the option to select whether the instrument is initialized at start up (immediately the software is launched) or using a SmartStart procedure where initialization is done in the background.

## 2.4.1 User levels

LabSpec6 offers four user levels. If the User Accounts module is active, you will be assigned to one of the following levels. The assignment is done by the system Administrator. If you need additional rights, then please contact the Administrator, who will be able to change your access level:

**Operator (basic):** reduced graphical user interface, and data acquisition for single spectra only.

**Operator (full):** reduced graphical user interface, and full data acquisition capabilities.

**Expert:** full interface, and full data acquisition capabilities.

**Administrator:** full interface and full data acquisition capabilities. Access to Configuration and Service tabs, and User Accounts set up.

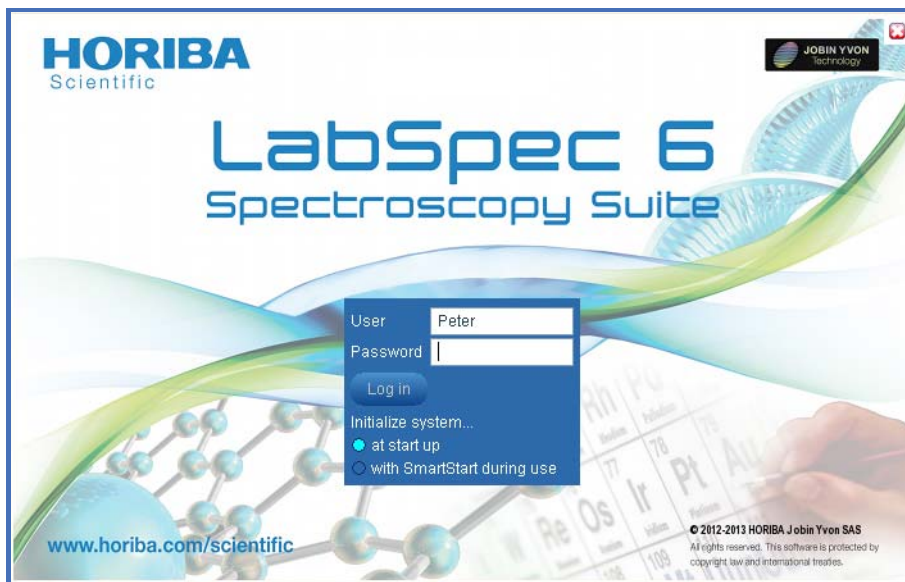
**NOTICE:** please note that each user has his own workspace which preserves individual settings and options.

## 2.4.2 New User Log In

Once a user account has been created, it becomes possible for a User to run LabSpec6 under his own personal environment. The procedure below explains how to log in for the first time when you have received a temporary 3-digit key from your administrator.

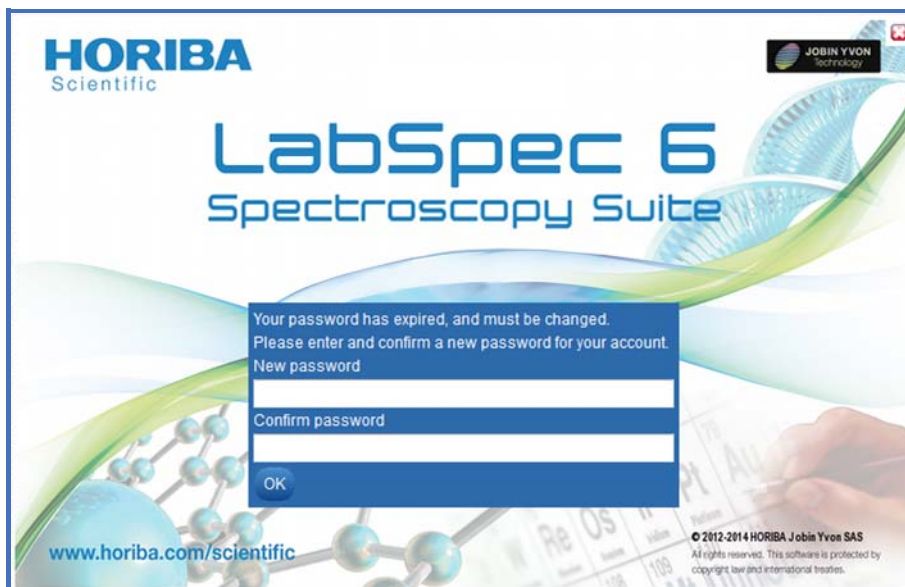
- 1 Launch LabSpec6,

2 The following window will be displayed:



- Enter your User name,
- Enter as the password, the 3-digit temporary key delivered by your administrator,
- Click on the [Log in] button.

3 This next screen will be displayed:

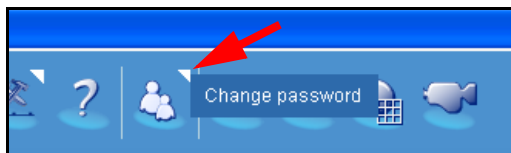


- Enter now your new personal password then click on [OK] to validate. A return receipt popup window is displayed. Click on [OK] to validate: LabSpec6 will now launch.

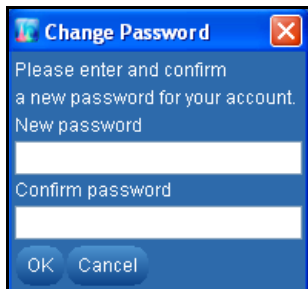
### *Changing the password*

Every user can change his own password by using the procedure below:

- 1 Click on the small triangle located on the «User login» icon bar, then click on «Change password».



- 2 The following popup window will be displayed. Enter twice the new password then click «OK» to validate.





Chapter 3

# Key Concepts of LabSpec6

To get the most out of LabSpec6, you should be familiar with some of its general operating concepts. The following information should help you to understand LabSpec6’s working model in a few key areas.

## 3.1 Data handling when working with LabSpec6

All data opened or acquired within LabSpec6 is stored within a temporary «workspace» file. It is this file which stores all manipulations/modifications to data - so the original file remains unaltered until you choose to save any changes to the local individual data file.

## 3.2 Using Workspace Files

The complete workspace of LabSpec6 can be saved to a workspace (.bin) file. This file type stores all settings for LabSpec6 including:

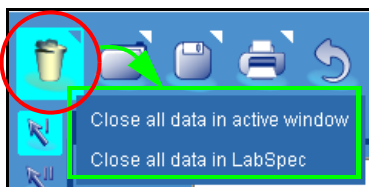
- 1) All open data
- 2) All interface settings and preferences
- 3) All current parameters and configurations

To save or open a full workspace (.bin) file, use the standard File Save and File Open tools, and select the file type to be «User workspace (.bin)».

Saving and loading workspaces can be used to work with complete projects, including multiple data files and specific settings. It can also be used to retain user preferences (for example, display settings and option) but in this case a better method would be to activate and use the User Accounts module.

## 3.3 Icon right click menus

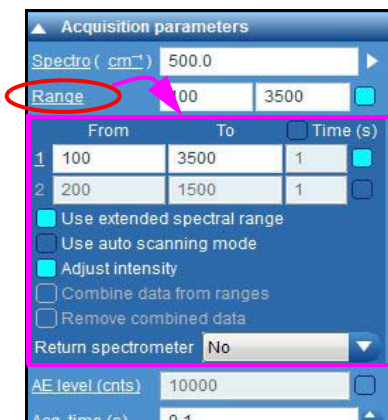
Any icon marked with a white arrow in its top right hand corner has a right click menu which offers further functionality and controls. See “[Icon right click menus](#)” on page 28 for full details.



06/23/2015

### 3.4 Hyperlink text

Any text in the Control Panel which is underlined acts like a hyperlink. Left click on the text to access additional menus or controls.



### 3.5 Customization and Automation with LS6

HORIBA Scientific's LabSpec6 software platform offers virtually every possible function for data acquisition, processing and display that a typical spectroscopist will require. This includes acquiring multidimensional spectral arrays, ranging from simple 1D time profiles, through to 3D XYZ confocal volumes, and beyond. However, despite these comprehensive capabilities we acknowledge that sometimes users want to do things in their own way.

LabSpec6 includes three modules which make complete customization and automation of a user's Raman experiment a reality. The recordable **Methods module** (see “[Methods Tab](#)” on page 90) allows routine multi-step tasks to be stored and recalled at will; **visual basic scripting** (VBS) allows complete customization and automation of almost every possible LabSpec function via user scripting (see “[Scripts](#)” on page 66); and finally the ActiveX module allows LabSpec6's functionality to be called remotely from third party software. Separate documentation for VBS and ActiveX is available.

## Chapter 4      **Looking at the working area**

LabSpec6 covers the complete needs for a spectroscopic experiment. Usually, there are five main steps for such a study: Preparing the sample, performing the acquisition, processing and then analyzing the data, and finally displaying the results. LabSpec6 offers advanced capabilities for all five stages.

Please note that each user has its own workspace.

This chapter is meant to be a brief introduction to the various navigation and functional controls of this simple – yet powerful – program. Here we will concentrate on the major interface elements that are common throughout LabSpec. Parts of the program that require more detailed description will be covered in subsequent chapters.

### **4.1 Workspace Overview**

The LabSpec6 workspace uses dynamic icon bars and menus, which adapt themselves depending on the particular window you are working on, or the operation you are performing.

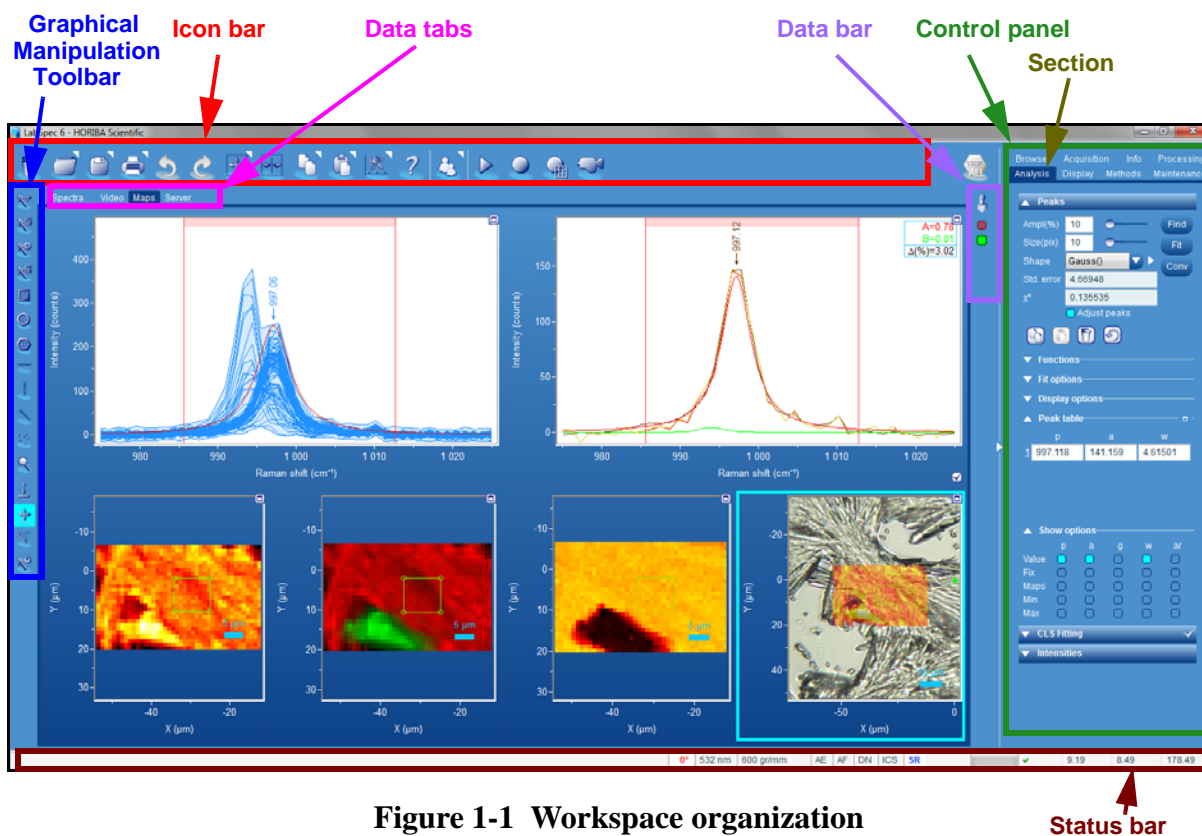
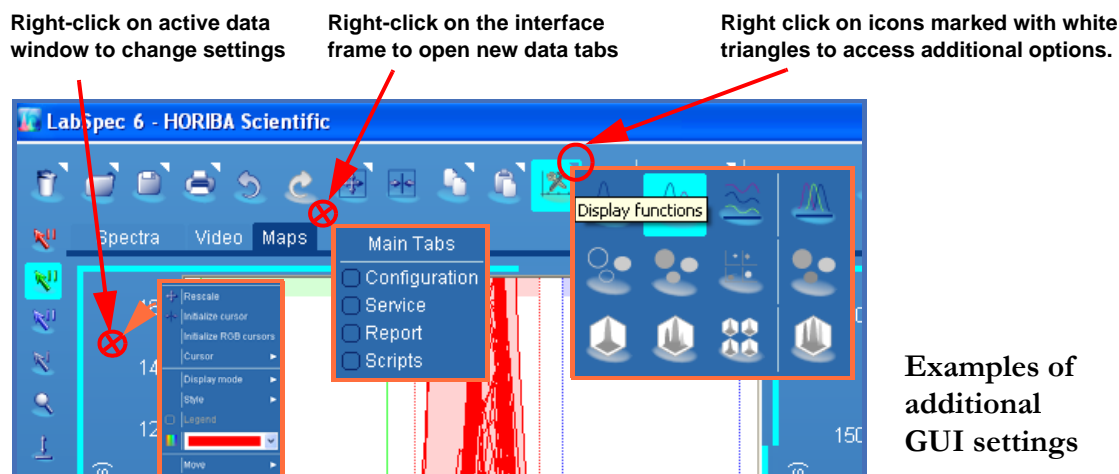
#### **4.1.1 Environments**

The LabSpec6 environment refers to the arrangement of various workspace components in the main application window.

- **Menus and bars**

Many of LabSpec6's most commonly used functions are represented as icons within bars, which appear around the main interface. These icons give you instant access to data manipulation tools, file handling functions, viewing options, and more, at the press of a button.

The design of LabSpec6 intentionally limits drop-down menus and pop up windows to simplify its interface.



**Figure 1-1 Workspace organization**

Figure 1-1 shows the menus and area organization on the main application window. Eight different areas have been defined for dedicated actions. They are listed below:

> **Icon bar**

The Icon bar is located on the top left hand side of the main application screen. It groups general functions which are most often used. Several icons has additional right click menu: for details see section “Icon right click menus” on page 28.

06/23/2015



Remove active data from LabSpec.  
Additional: right-click on the icon to display additional menu



Open files  
Additional: right-click on the icon to display additional menu.



Save As  
Additional: right-click on the icon to display additional menu to list the options: see page 28.



Print active window  
Additional: right-click on the icon to display additional menu.



Undo last action (Ctrl+Z)  
NOTE: undo functions are stored individually for each data file open in LabSpec6. In the case of a group operation select each individual data file to undo the operation.



Redo last undone action



Rescale axes  
Additional: right-click on the icon to display additional menu.



Initialize the cursors (to restore them to the center of the active window)



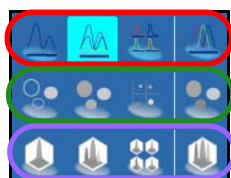
Copy selected data to clipboard (Ctrl+C)  
Additional: right-click on the icon to display additional menu.



Paste data from clipboard to active window (Ctrl+V)



Data display mode



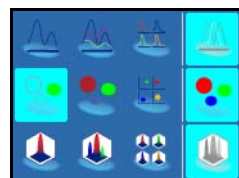
**1D graphics options**

**2D graphics options**

**3D graphics options**



Data display mode changes when the graphics are normalized.



**Example of normalized graphics**



Launch the LabSpec6 context help.  
Additional: right-click on the icon to display additional menu.

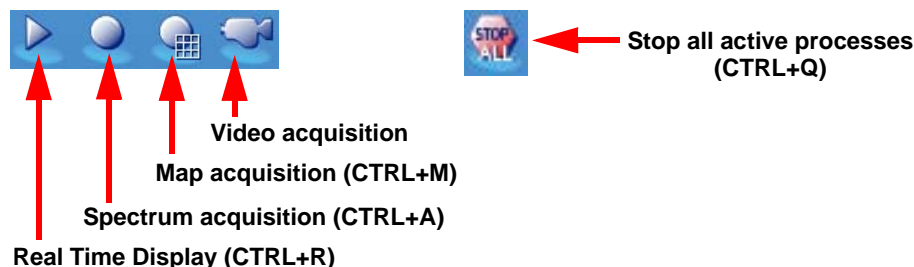


Launch the spectrum database searching



Login access  
Additional: right-click on the icon to display additional menu.

The buttons shown below activate various acquisitions modes (RTD real time display, acquire spectrum, acquire map and acquire video)



## > Data tabs

LabSpec6 offers three major Data Tab workspaces: **Spectra**, **Video** and **Maps**. LabSpec6 treats and displays data files according to their type.

The **Spectra** Data Tab is used to display and manipulate individual spectra.

The **Maps** Data Tab is used to display and manipulate multidimensional spectral arrays (maps), such as line/temperature/time profiles, XY surfaces, XZ/YZ slices, or XYZ volumes.

The **Video** Data Tab is used to display optical video images, either in live readout of the camera, or static, captured images.

The optional **Scripts** tab is used for full Visual Basic Script (VBS) editing and control. This tab is accessed by right clicking on the tab labels, and selecting «Scripts».






The optional **Report** tab displays customizable report template page, which will be used for quick printing. This tab is accessed by right clicking on the tab labels, and selecting «Report».











The **3D** tab (only available if the 3D Surface and Volume Rendering module is activated) is used for full 3D representation of surface and volume images.

## > Graphical Manipulation Toolbar




The commonly used functions for data display and processing related to the Data Tabs are grouped as icons within the Graphical Manipulation Toolbar, which appear on the left hand side of the main interface. This toolbar is adaptive, which means it changes according to the working context.

### General Toolbar for spectra




-  Activate single line cursor; intensity and wavenumber axis values will be displayed in the Status bar
-  Activate double-line cursor; the center point between the cursors is indicated on the spectrum, and the position of each cursor and width between them will be displayed in the Status bar.
-  Activate the «crosshair» cursor. Useful to follow the intensity and wavenumber of a spectrum.
-  Peak characterization: activate area and peak position calculations between the cursors
-  Zoom tool - click and drag on the spectrum to zoom in. Right click > Rescale to return to full view.

-  Move selected spectrum inside the window; axes are not modified
-  Scale intensity data; can be used with the mouse scroll wheel. In this case, it remains active while using the other tools
-  Launch a pen editor
-  Launch a spike remover/corrector
-  Shift the selected spectrum up or down (i.e., add/subtract a constant value to the data)
-  Multiply the selected spectrum by a constant factor (i.e., scale the data to increase or decrease its intensity)
-  Add baseline points using the mouse. These points will be used for the baseline subtraction
-  Add a peak label and/or mark a peak to be used for peak fitting
-  Activate a color/size/thickness setup popup window of a selected object (spectrum, units, cursor.)
-  When many spectra are displayed, by default, they are superimposed. To shift horizontally and vertically each spectrum, relative to each other, move the two sliders located on the bottom left hand side of the interface.  
Upper slider for stack vertical shift (%)  
Lower slider for stack horizontal shift (%)

### Specific Toolbar tools for Maps

-  Activate the red cursor range; cursor positions and width between cursors will be displayed in the Status Bar.
-  Activate the green cursor range; cursor positions and width between cursors will be displayed in the Status Bar.
-  Activate the blue cursor range; cursor positions and width between cursors will be displayed in the Status Bar.

### Specific Toolbar tools for map display

-  Activate the cross cursor; the spectrum corresponding to the cursor position will be displayed in the Cursor Spectrum window.
-  Activate the rectangle cursor; the average spectrum from within the cursor area will be displayed in the Cursor Spectrum window.
-  Activate the circle cursor; the average spectrum from within the cursor area will be displayed in the Cursor Spectrum window.



Activate the polygon cursor; the average spectrum from within the cursor area will be displayed in the Cursor Spectrum window.



Activate an X-line. To change the number of points, enter the appropriate X-size value in the Acquisition>Map table.



Activate an Y-line. To change the number of points, enter the appropriate Y-size value in the Acquisition>Map table.



Activate a diagonal-line. To change the number of points, enter the appropriate X-size value (Y-size is greyed) in the Acquisition>Map table.



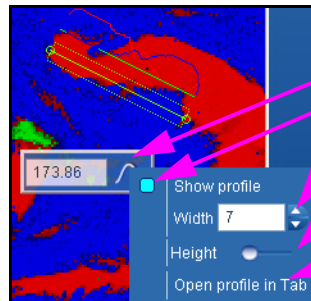
Activate points everywhere on the sample. The order of acquisition will follow the point marking. Right-click on a point then select **show index** to see the numbering.



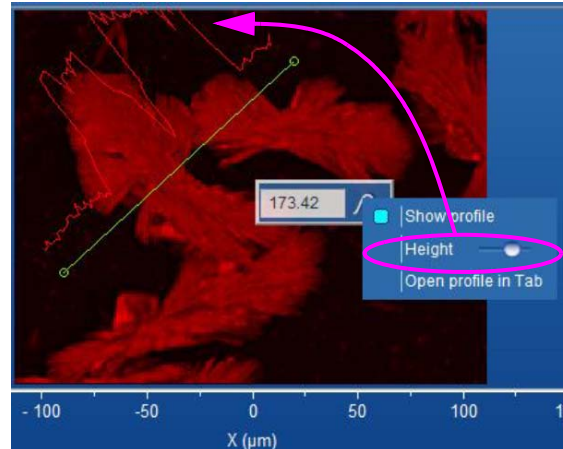
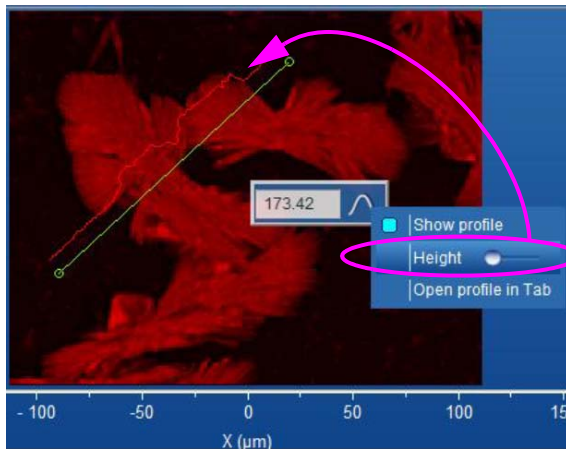
Activate the Intensity scale adjustment tool. Once activated, left-click and move the mouse up/down.



Activate the measurement tool. Once activated, the measurement and line profile tool is displayed on the image. Set the position of the line by moving the end points then click on the Profile icon to display the options (see below).



- Activate the Profile options
- Tick to show the profile
- Set width of the line which will be used to create the intensity profile
- Use the Height slider to adjust the display size of the line profile (see example below)
- Open the profile in a new tab



Remark: once the profile is displayed, moving the line will update the profile curve

### Specific Toolbar tools for 3D image display



Activate the 3D rotation cursor for the selected 3D view

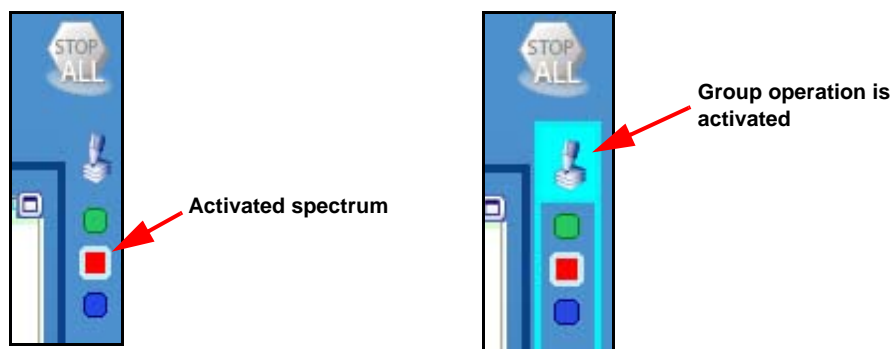
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## > Data Bar

Any data which is displayed in one of the Data tabs will be identified by a colored radio button. To activate a spectrum click on its corresponding data button; once active any manipulation you make will be done to this spectrum.

If the **Group Operation** icon is turned on, then the manipulation you make will be applied to all spectra displayed in the current window. This allows you to batch process multiple data files with a single operation.



## > Control panel

The Control panel is separated into individual tabs, organized according to general function:

- **Browser:** displays all data files loaded into LabSpec6, and allows you to choose whether to hide/show the data within their corresponding data tabs.
- **Acquisition:** control instrument set up and acquisition parameters.
- **Info:** obtain information about the currently active data, including acquisition parameters, custom information provided by the user, and a history of file manipulations.
- **Processing:** functions to manipulate data which result in the original data being changed.
- **Analysis:** functions to obtain information from the data; the original data is not changed by these functions.
- **Display:** functions to control data display and general LabSpec6 options.
- **Methods:** specific tab to create and use custom Methods for automated data acquisition and analysis within LabSpec6.
- **Maintenance:** tab for access to user maintenance functions including instrument calibration and AutoCalibration sections.

## > Status bar

The Status Bar runs along the very bottom of LabSpec's main window. It provides information about system status (e.g., progress of an acquisition), temperature of the detector (green indicates the detector is at working temperature; red indicates the detector is above the working temperature and should not be used), active options (e.g., intensity correction, or extended range settings), hardware status (Laser On/Off, ND filters value, Autofocus On/Off etc.) and cursor positions.

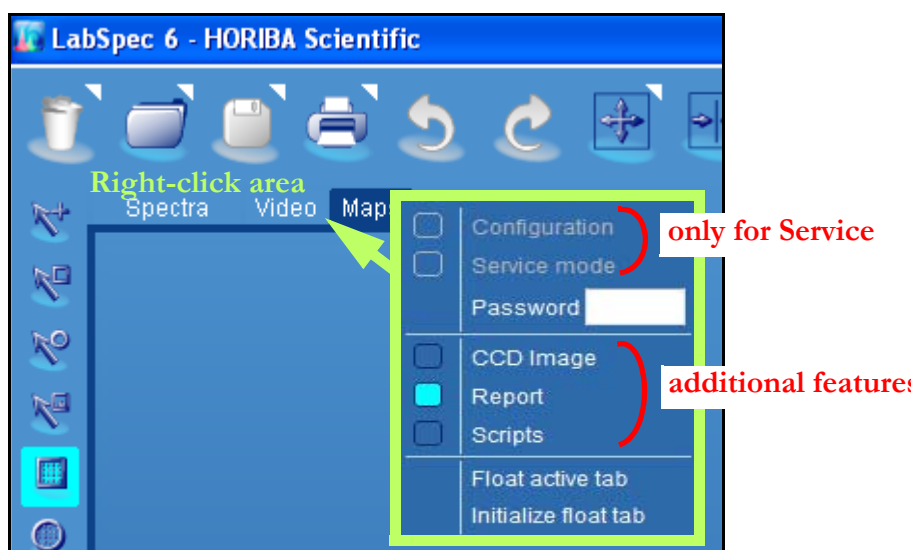
Note that most indicators in the Status Bar can be used to choose an option (laser, grating etc.) or switch a device ON or OFF - left click on the Status Bar indicator to adjust or select.

## 4.1.2 Setup and Saving Environment

This chapter explains how to define, choose and save an interface environment. First of all, as a rule, each time you exit LabSpec6 the full environment including the currently loaded data is saved. If large amounts of data are present in LabSpec6, the save process could take several seconds - an indicator will show that LabSpec6 is closing. Running LabSpec6 later will load the data and the environment of the previous LabSpec6 session. When User accounts are active, the environment for each individual user will be saved and reloaded, whenever that user logs in.

### Changing the «Data tabs» and activating the «Settings Panel»

By default, three data tabs are visible: **Spectra**, **Video** and **Maps**. To activate or deactivate additional tabs, right-click on the general interface area then tick on/off the choices which appear in the selection box (see figure below).



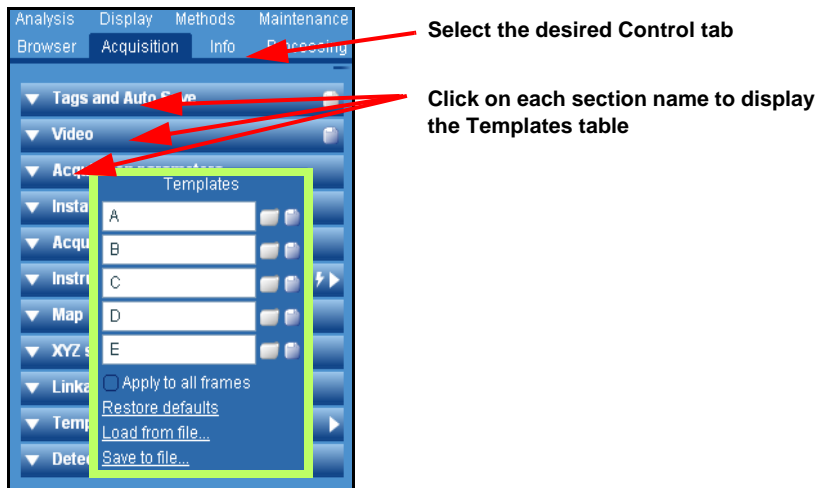
**NOTE:** the **Configuration** and **Service** tabs are not available to Operator level users. The Configuration tab and Service mode require a password to access.

### Changing the «Control Panel» environment

By default, the **Control Panel** is composed of eight tabs: Browser, Acquisition, Info, Processing, Analysis, Display, Methods and Maintenance.

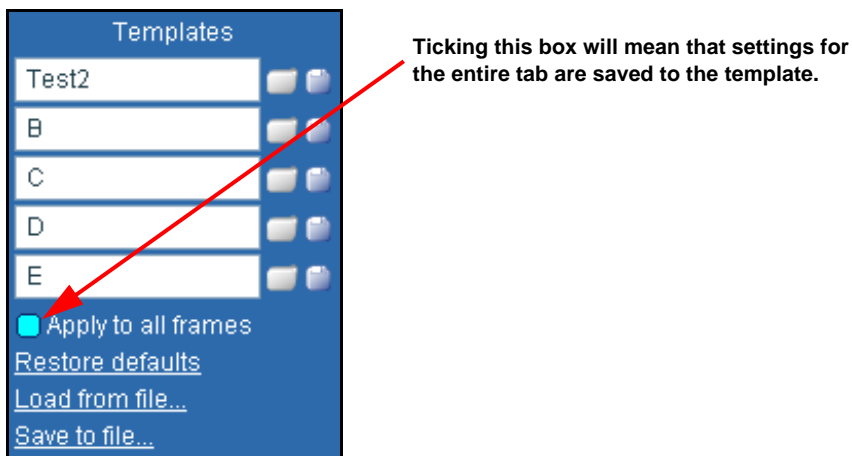
The Control Panel set up can be saved using the Templates table, either for individual sections in each tab, or for an entire tab. See detailed explanation [“Templates Management” on page 32](#).

- **Saving the Control Panel environment**

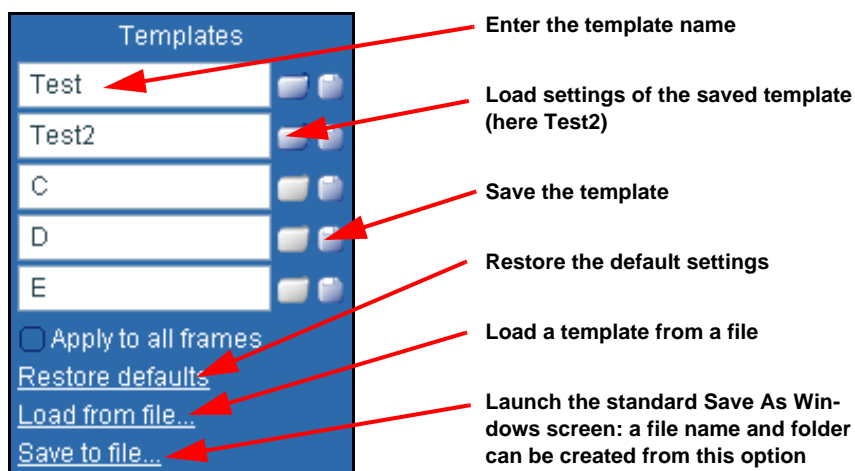


LabSpec6 allows the Control panel templates to be saved either for an individual section within a tab (e.g., Acquisition Parameters), or for an entire tab (e.g., Acquisition). First set up the tab or the section as you desire it, and then use the Templates table to save the set up.

- **Template features:** before any action on the template, you have to choose if the template will be used on the selected section only or on the entire tab. To activate this choice, use the [*Apply to all frames*] tick box (see figure below).



- All of the current settings can be saved and retrieved later using this feature. The figure below shows all the options.

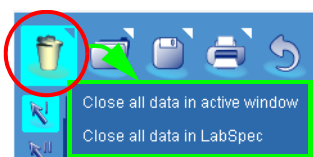


**NOTICE:** On the figure above the template creation or loading will only concern a single section and not the complete control tab, because {Apply to all frames} is unticked.

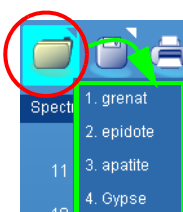
### > Icon right click menus

LabSpec6 offers a number of standard right click menus to provide fast access to commonly used functions.

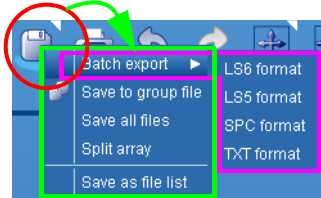
In addition, icons marked with a small white triangle located in their top right corner have a right click menu offering further functions and controls. These are described below.



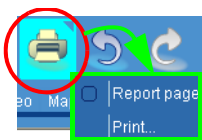
Two **Close** options are available:  
 - Close the data of the current active window  
 - Close all the opened data in LabSpec6



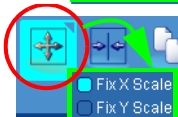
The window shows the four last opened data. Click on the listed data file to load it again.



Several choices are available:  
 - Batch export saves all open data in the active window to the chosen folder. Moreover the export format can be chosen: LS6, LS5, SPC or TXT format.  
 - Save to group file: save all open data in the active window to a single data file. When this file is opened, all data will be available for individual processing and analysis.  
 - Save all files: save all the opened data file to their current file location, with their existing names. If a file does not have a file location (because it has not yet been saved) a File Save dialog window will be displayed.  
 - Split array: save all spectra of an array (map) to separate spectrum files.  
 - Save as file list: This creates a list file of each open file and its file location. When the file list is opened within LabSpec6, all data included in the list will be opened. This allows a «project» file to be created, with multiple files from multiple locations.



Two choices are available: print or open the Report page window to setup the presentation before printing.



Two choices are available: fix the X and/or the Y axis. If they are unticked, the axes will be rescaled automatically.



**Data:** Copy the active data as real data, retaining its 'data type' identifier.

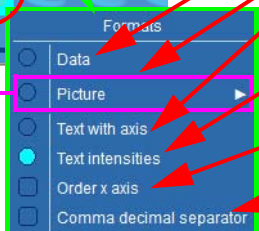
**Picture:** Copy the active window as an image.

**Text with axis:** Copy the active data to text (ASCII) format, including the axis information.

**Text intensities:** Copy the data to text (ASCII) format, without the axis information.

**Order x axis:** if this option is ticked, then text files will always be saved with the spectrum axis in «low» to «high» order.

**Comma decimal separator:** if this option is ticked, data will be copied using a comma decimal separator (i.e., 1000,00) rather than the default point decimal separator (i.e., 1000.00)



Choose whether the screen cursors will be included in the copy image.

Choose whether the copy image will be gray scale only.

Select what color background will be copied

**White:** white background with black text

**Transparent:** transparent background with black text

**Screen:** background exactly as displayed in LabSpec6

**Compatibility mode:** select this mode to make the copy function compatible with older software which do not support modern display functions. In this mode «Transparent background» copying is disabled.

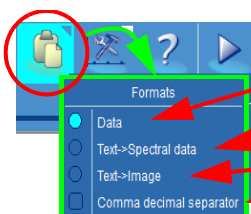
Exclude «empty» display space when images are displayed with correct aspect ratio.



Define the precise size of the image to copy.

Define the text size, relative to that shown in the native LabSpec6 display.

Open a **File Save** dialog window to save the copy image in .wmf, .jpg or .bmp format.



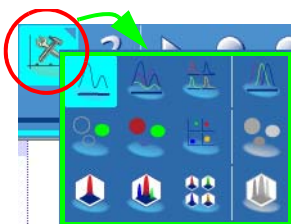
Paste data or text file (ASCII) information into LabSpec6.

**Data:** paste data which has been copied from within LabSpec6, retaining its 'data type' identifier.

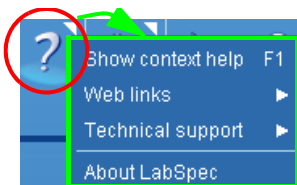
**Text->Spectral data:** paste text (ASCII) data which represents a spectrum.

**Text->Image:** paste text (ASCII) data which represents an image.

**Comma decimal separator:** if this option is ticked, data pasting will be compatible with comma decimal separators (i.e., 1000,00) rather than the default point decimal separator (i.e., 1000.00)



**Display mode:** the choices are applied to the current selected window. The options allow 1D, 2D and 3D data display to be shown in SINGLE mode, OVERLAY/STACK mode, or TILE mode. The right hand column activates a Normalized Display mode, where the highest and lowest intensity values for each data object are displayed to the same scale.

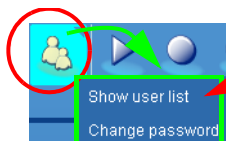


A **context help** is activated with the F1 key.

A **web links** list is displayed for fast access to useful HORIBA internet sites - note that these will only be accessible if the computer is connected to the internet.

The **Technical Support** section offers the **Save configuration report** feature, which will create an automatic report file which can help the HORIBA support team to diagnose issues with the hardware or software. The **Local contacts** link opens a HORIBA web page with listings of local offices and agents around the world.

**About LabSpec** displays the current LabSpec version information.



**Show User list:** this choice is only visible to users with LabSpec6 Administrator rights. Once selected the User Accounts tab appears in the Data Tabs

**Change password:** select this choice to change the current password

# Getting Started

Normally LabSpec6 is delivered already installed on the System computer. The software interface will only display functions and options which relate to installed options/peripherals on your System. This adaptability to a dedicated System creates a cleaner working interface. Note, however, that some parameters detailed in this manual could be different from those displayed for your System.

## 5.1 What can I do?

LabSpec6 enables a full range of experiment types including single spectrum acquisition, multidimensional spectral array acquisition (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes), video image capture and automated multiwell interrogation.

Many commonly used data analysis and processing routines are also available, including peak labelling and fitting, smoothing, noise reduction, baseline subtraction, linear and non-linear filtering and multivariate analysis techniques such as CLS, PCA, MCR, HCA, DCA and PLS.

LabSpec6 is also compatible with Microsoft Visual Basic scripting (VBS), allowing specific acquisition and analysis routines to be written by the user. In addition, third party software routines can call LabSpec6 through ActiveX modules.

The software offers full control on the motorized hardware components, including:

- Spectrometer entrance slits
- Neutral density (ND) filter wheels
- Confocal pinhole aperture
- Diffraction grating
- Laser rejection filters
- CCD detector binning and readout
- Video Image capture and digital storage

### Peripheral functions:

- Motorized XY and Z axis sample stages
- Fast feedback Autofocus
- DuoScan adaptive optics
- Additional detection systems
- External triggering
- Many third party accessories (e.g heating/cooling stages)

In addition, LabSpec6 includes direct data links with other third party softwares. This provides an automated «one click» link between LabSpec6 and the other software platform, allowing data to be immediately transferred from one to the other. Examples include:

- Eigenvector's Solo+MIA multivariate analysis package
- Bio-Rad's KnowItAll® informatics suite for spectrum database searching and Raman functional group analysis.

## 5.2 Open/Save data files

Data files (including spectra, «map» multidimensional spectral arrays and videos) can be opened and saved using the respective icons in the Icon bar.





## 5.3 Startup options

This chapter describes the startup options necessary to handle LabSpec6.

**Select the «Display» tab from the Control Panel**

The «Color & Text» section groups the «cosmetic» parameters for data, axes, background etc. At the startup, use the default settings but keep in mind that they can be modified.

Choose the font and font size for the Graphics User Interface (GUI) and the data to suit personal preferences.

Select the appropriate interface language

Select any optional modules you wish to activate. Note that most of these optional modules must be purchased before they can be unlocked and used. Please contact HORIBA Scientific or your local representative.

Select the toolbars icon size. Result depends on your monitor resolution.

Treatment Preview: when enabled, pressing the Ctrl key + hovering the mouse over data treatment arrows (such as smoothing, baseline correction etc) will show a quick preview of the results.

Display Preview: when enabled, pressing the Ctrl key + hovering the mouse over the «Display functions» will show a quick preview of the results.

## 5.4 How can I acquire a spectrum?

### • Requirements

System must have the complete acquisition part, ready for acquisition. The warm-up period must be done (according to your specific System requirements).

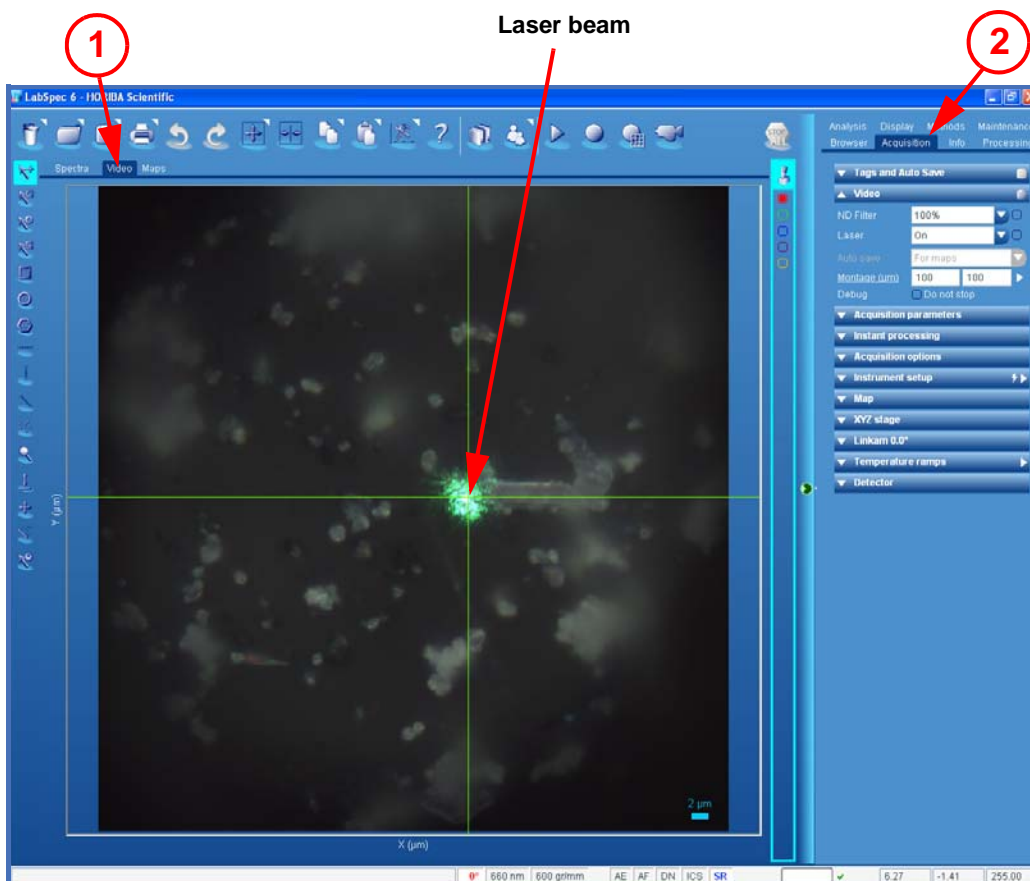
**Optional:** the video camera for sample visualization helps the user setup his sample for complete control through LabSpec6.

• **General Procedure**

- 1 Place a sample on the Microscope Stage or in the Macro Chamber. Depending on your System the sample's size can be large or microscopic.
- 2 Use a visualization device to correctly position microscopic samples. As a minimum, optical binocular lenses of the microscope must be used. An optional Video Camera can be added and will highly increase the positioning precision and ease of use.




If a Video Camera is linked to the PC, use the procedure shown in figure 1-2. This figure shows the sample illuminated by a laser beam. Follow the action procedure:

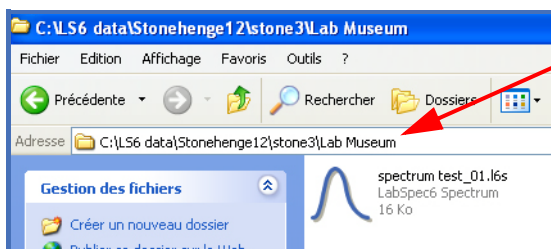
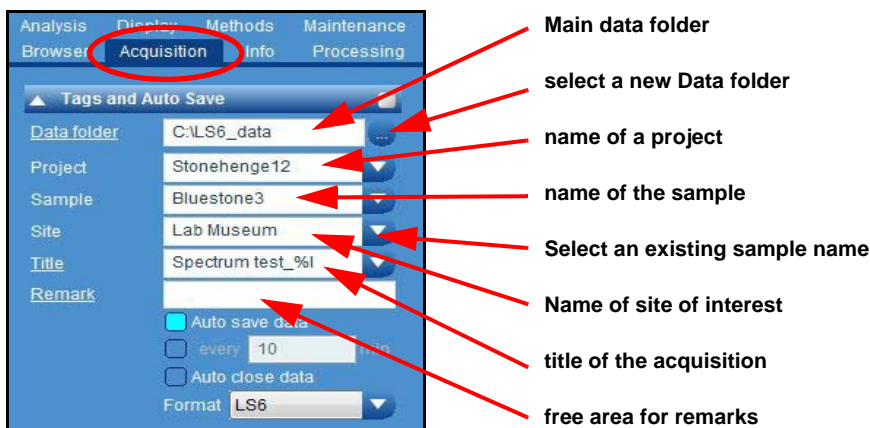
- **Marker 1:** switch to **Video** Data Tab; the video camera image is displayed in the main window. Depending on the system being used, the sample and/or the laser beam is visible.
  - **Marker 2:** the **Acquisition** Control Panel is activated. This is done to control the acquisition parameters, particularly **Acquisition > Video** section.
- 3 The sample is visible through the video camera or the microscope binocular lenses. Using the sample stage (manual or automatic), move the sample to select the analysis position. If a Macro Chamber is used, the sample adjustment is made at sight.




**Figure 1-2 Sample visualization using a video camera**

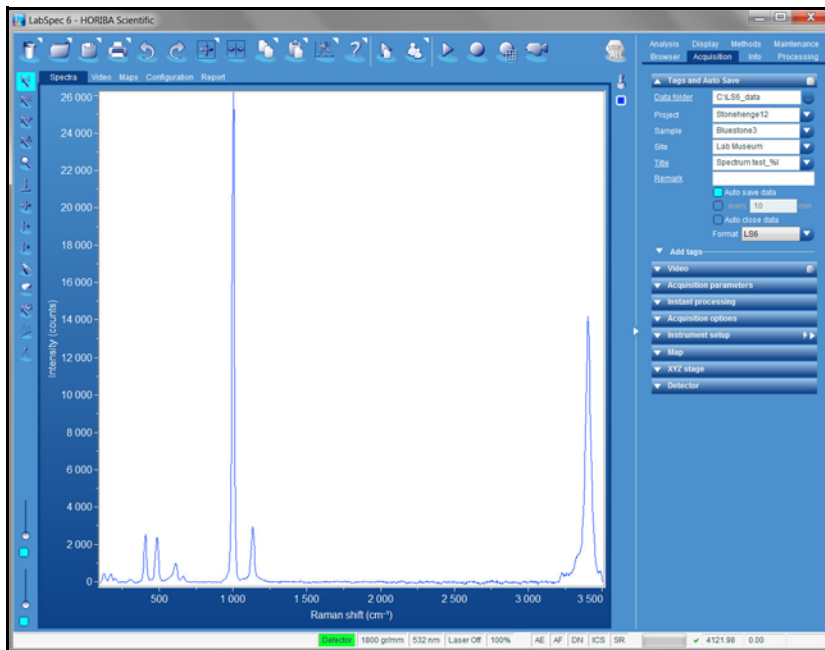
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- 4 Once the sample adjustment is done, stop the Video by pressing the  icon.  
[Remove the visualization optics, if this function is not automated]
- 5 Select **Spectra** from the Data Tabs menu, then press the  button to launch the **Real Time Display**. According to the selected parameters, real time acquired spectra will be continuously displayed. This will help you to improve the sample positioning or acquisition parameters like the Acquisition time(s) and other System settings.
- 6 Once optimization is complete through the Real Time Display acquisition click on the  icon to stop the RTD. The final acquisition can be now performed, but before launching it, you may want to define the project. The figure below shows the labels which will be added from the root folder to the sample name. If the [Auto save data] tick box is not ticked, then these project tags will simply be added to the data information shell.
- 7 If the [Auto save data] tick box is ticked and active, then the data will be saved automatically into the corresponding folder structure:
- 8 Data folder \ Project \ Sample \ Site



This is an example of a generated path based on the entered labels shown above. This tree is shown from the Windows Explorer.

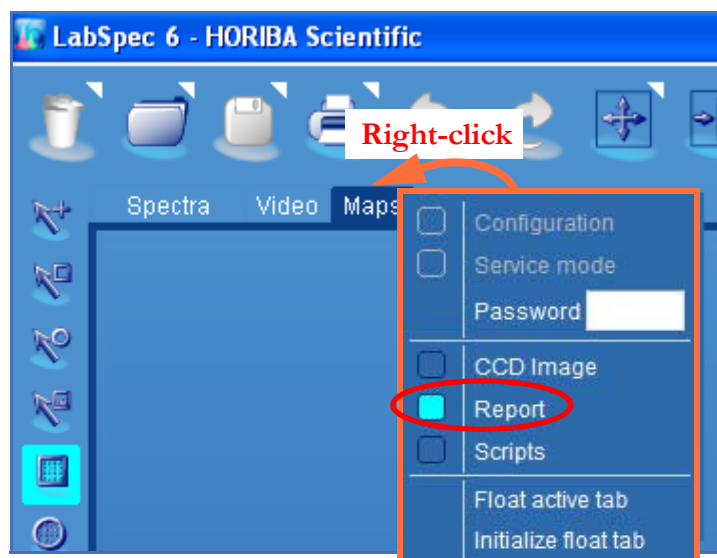
- 9 Then, press the Spectrum Acquisition  icon: this action launches the acquisition according to the defined parameters. The results are automatically displayed and data recorded on the hard disk (if *Auto save data* box is enabled).



## 5.5 How to generate a report

When the acquisitions have been done, it is important to print the results including a graph or image with the measured parameters. To create a report page follow the procedure described below:

- 1 If not yet done, add the **Report** tab to the **Data Tabs Menu**: Right-click on the Data Tabs Menu to display the options window. Tick on the **Report** choice. The Report tab is then created.



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- 2 Using the mouse, select a data window.
- 3 Click on the **Report Data Tabs Menu**: the previously selected data window is automatically retrieved to the report area. The report template can be edited, saved, opened according to user requirements, as described below. Note that each data tab (Spectra, Video, Map) has its own individual report template.
- 4 Right-click to display the page properties. Figure below details the choices.

**Print** ← Print the reporting page

**Format...** ← displays the layout properties of the reporting page.

**Load...** ← Load/Save a report

**Save...** ← Restore the default report

**Default** ←

**Properties** ← Displayed Properties depend on the activated element (see below)

**Options** ←

**Gray scale** ← Gray scale: display and print the report in gray scale only.

**Inverse images**

**Add** ←

**Remove** ← Remove the selected element

**Activate frame** ← the Up/Down arrow buttons select the framed elements of the report page

**Zoom 25 %**

**Zoom 50 %**

**Zoom 100 %**

**Zoom to fit**

**Page** ←

**Font Size** 10

**Frame**

**Active Graph**

Parameters

Information

Logo

Header

Footer

Image

Text

Spectra

Video

Maps - All spectra

Maps - Cursor spectrum

Maps - Cursor image

Maps - Scores image

Maps - Peak fit image

Maps - Extra image

Maps - MVA info

Maps - Video

**Select the element to add to the report**

**Zoom xx% changes the size of the reporting page. Zoom to fit choice resizes the report to the monitor space available.**

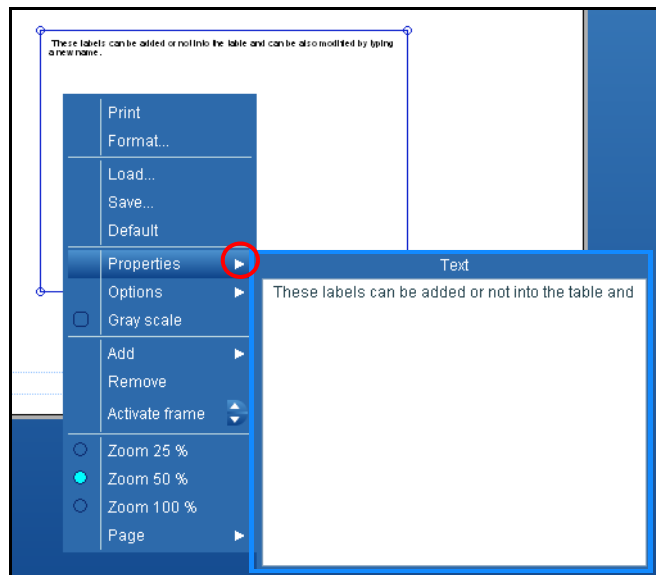
**Insert before**

**Insert after**

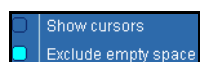
**Remove**

**Insert or Remove a report page**

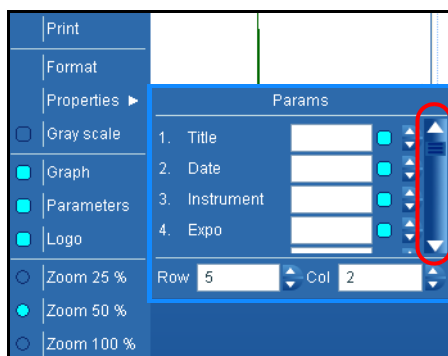
The displayed **Properties** parameters depend on the activated element on the report page.



If the selected element is a text box, the Properties will show a text editor.



If the selected element is a spectra, a map or a 3D Image, the Properties will display two parameters: show or not the cursors and exclude or not empty space.



If the selected element is a table, the properties table list up to 50 parameter labels which can be added according to the used sample. These labels can be added or not into the table and can be also modified by typing a new name. Do not forget to re-dimension (row and column) for a better presentation.

**IMPORTANT:** When an image is added to the report, an empty frame is displayed on the report. Select it then click on **Properties** to launch the data browser to locate the image file which is to be inserted. A .jpg or .bmp format image is required.

- 5 Print the reporting page using *Print button* in the main icon bar, or the right click option (Print) on the report template.

Chapter 6

# Control Panel

The right hand Control Panel contains tabs and sections to set up all necessary data acquisition, processing, analysis and display functions.

## 6.1 Templates Management

Templates can be used to save interface settings and options for each section and each entire tab within the Control Panel. This allows the user to quickly recall a complete set up and then repeat a process with identical conditions.

A typical example is to save a template for the entire Acquisition tab of the Control Panel. This allows the user to quickly re-apply a complete hardware and acquisition configuration, and then run the measurement from the required acquisition icon.

**Click on every header**

**A quick list of five templates is available for each parameters family.**

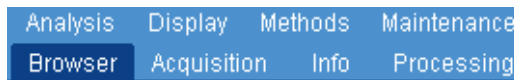
**How to use it?**

1. Set up the section (or full tab) with the settings as desired,
2. Click on a section header (here Tags)
3. Enter a name in the title field
4. Click on the save icon to save it
5. To load a previously saved template, click on the folder icon

**IMPORTANT:** select this option if you want to save a template for the entire tab (frame), rather than for the specific section only.

- Restore the factory default template
- Load a template file
- Save a template in a file

## 6.2 Browser Tab

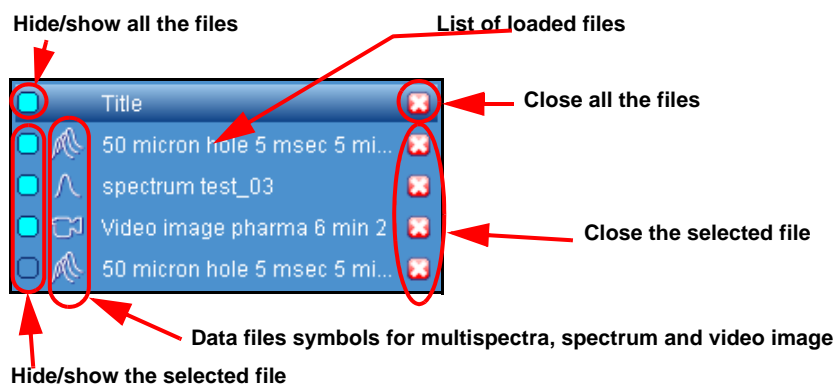


The Browser Tab displays the loaded data files.

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### > How to use the Browser?

Each time a data file is loaded, it appears in this list. The list allows you to hide or show a data file by ticking off or on the box located in front of the file, or close this file by clicking on the close icon

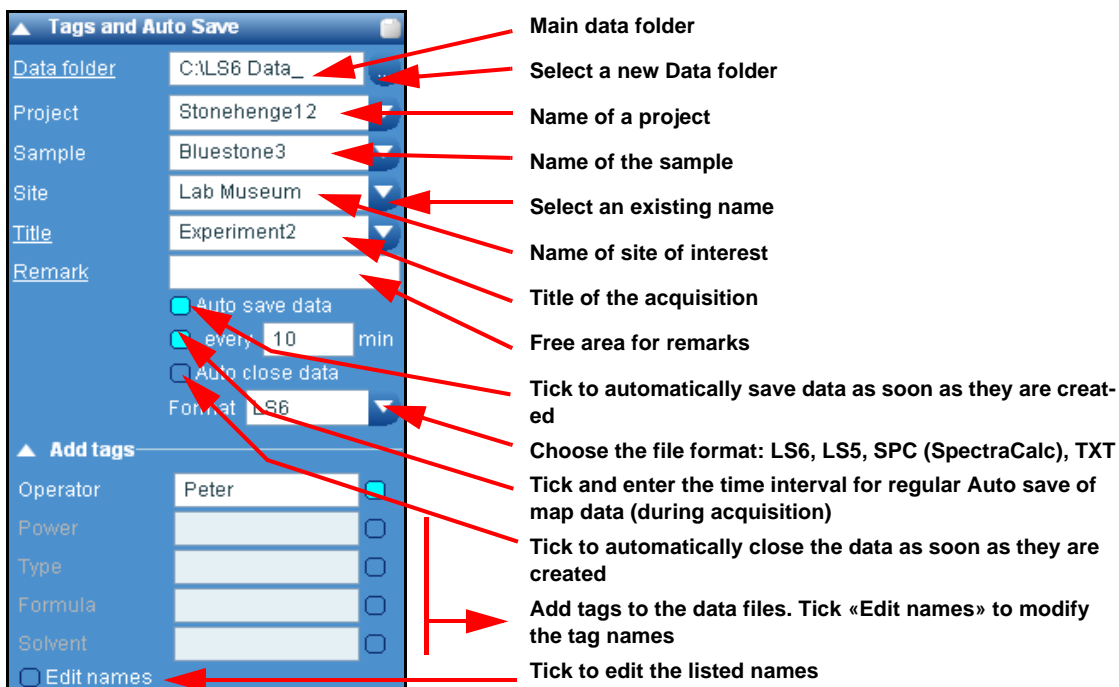


## 6.3 Acquisition Tab



### > Tags & Auto Save

Before starting a new acquisition session, it is important to fill up the Project Tags. This feature defines the complete names and project folders paths. Fill in each field and follow a logical strategy for easy data recovery. The figure below shows an example of the created structure according to the entered tags.



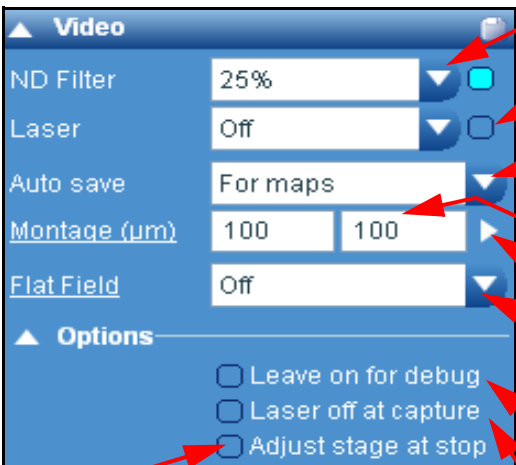
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The Auto save feature only concerns the data (e.g. spectra or maps) which are currently being acquired. Existing data open within LabSpec is not affected.

## > Video

The video image is displayed in both the video and map Data Tabs..



**Select laser filter value (100% means full laser power), and tick the box to have the filter automatically loaded whenever the camera is run.**

**Select the laser to be «on» or «off» and tick the box to have the laser automatically turned on/off when the camera is run**

**Activates the video automatic save feature (for maps, spectra or both). This requires the Auto Save function to be activated in the "Tags and Auto Save" section**

**Enter the minimum video size for automated video montaging.**

**Start the video montage**

**Select to apply Flat Field non-uniformity correction to the video readout and capture. Flat Field can be used to improve image quality in cases where the illumination is not uniform or uneven on the sample (see below).**

**Click to permanently run and display the video during acquisition. Note that the camera optics will be inserted into the Raman beam path, resulting in less signal. This mode can be useful to verify sample stage movement during acquisition.**

**Click to force automatic laser switch off for final image capture. If the laser is turned on during video RTD, it will be switched off, then the final image will be captured.**

**Click to activate the automatic backlash correction for XY stages. When active, when the video readout is stopped, the stage will perform a fast backlash correction before final image capture.**

### Using the Flat Field Correction

The **Flat Field** corrector file must be saved before use. To do this, use a reference sample which has no visible features.

Acquire the video image, making sure that Flat Field is turned off; then click on **Flat Field > Save reference**. Reference files are stored individually for each objective and camera on the system.

Flat Field correction can be applied to non-corrected data after acquisition by clicking on **Flat Field > Apply correction**.

Flat Field example:

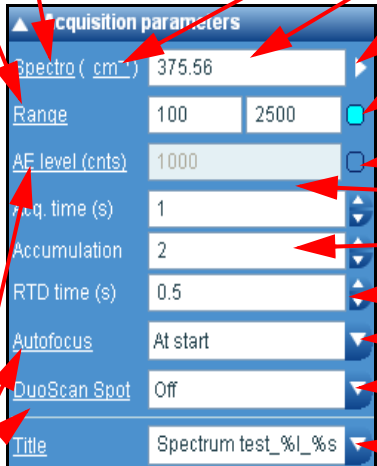


## > Acquisition parameters

The Acquisition parameters section groups the most important parameters of an acquisition. The figure below details the main parameters.

**Acquisition range: tick on the related box to adjust it**

click on «Spectro» to move to zero order or to initialize the position or set the precision digits



Select the working spectral unit

Enter the spectrograph wavelength position

Move the spectrograph to the entered wavelength position

Activate the spectral range function, to acquire full range (or custom range) data. If this is not turned on, a limited spectrum will be acquired - the exact range will depend on the spectrometer, grating and laser.

Tick this box to activate the Autoexposure; enter the Autoexposure level value.

Integration Time in sec.

Number of accumulations

Select the Real Time Display cycle time

Select the Autofocus mode (if available)

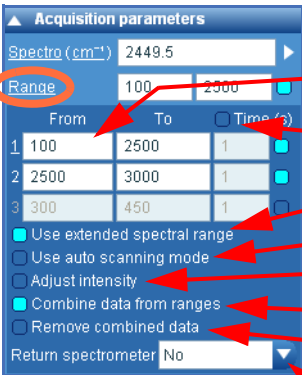
Turn on the DuoScan adaptive optics scanning mode, and click on «Duoscan Spot» to select the scanning shape.

The spectrum title entered in the Tags can be also changed here. Click on «Title» to choose a title preset.

Open additional menu (see below)



Notice: When Auto Exposure is active, the Acq. Time parameter is greyed out.



«Range» additional parameters:

Click on the «Range» word to open the Range table. Enter in this table the spectral ranges. On the right of each range, tick on the box to activate the range.

Tick on this box to assign a different integration time for each spectral range

Activate the multiple window mode

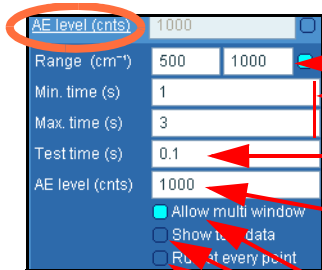
Activate the auto scanning continuous spectrum readout mode

Adjust baselines automatically for seamless stitching.

Tick this box to combine spectral data from individual ranges

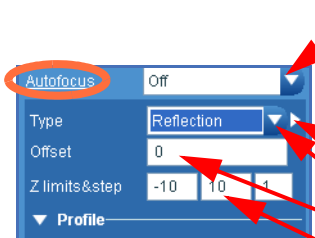
When ticked, the original individual spectral data will be removed, leaving just the combined data on screen

Select whether, at the end of an acquisition, the spectrometer will remain where it is, move to the start position of the specified range (First window), or move to the position where it was before the acquisition was started (Origin).



«Auto Exposure» additional parameters:

- Set the spectral range on which the Auto Exposure will be effective
- Enter the limits of the acquisition time
- Set the initial test acquisition time, in seconds (s). Typically an acquisition time between 0.3s and 1s is suitable, although it can be increased if most samples are very weak Raman scatterers
- Auto Exposure level intensity; the Auto Exposure function will automatically calculate an acquisition time (within the Min. and Max. time limits) in order to reach the specified signal level.
- Should be activated by default (see paragraph below)
- Displays the test data
- Auto Exposure is performed at each individual point of a hyperspectral map. In this case, display the map with «counts/s» intensity units to see correct relative intensities across the map.



Select the Autofocus mode: choice are «At each XY point», «At start», «Tilt at limits», «Tilt at midway» and «At every point».

«Autofocus» additional parameters:

- Start the autofocus device
- Enter the type of autofocus device: reflection or spectral
- Enter the autofocus offset
- Enter the Z autofocus limits and the autofocus step
- If the «spectral» autofocus is chosen, select the active spectrum range and the acquisition time.

### About Auto Exposure?

The Auto Exposure function calculates the necessary acquisition time to acquire a spectrum with a maximum signal level specified by the user. It is useful to ensure a good quality spectrum is acquired regardless of the inherent signal strength of the sample.

The Auto Exposure function initially acquires a test spectrum with a fast «**Test time**» acquisition time, and calculates the maximum signal level in the spectrum. Based on this data a new final acquisition time is calculated to yield a maximum signal equal to the specified «**AE level**», and the acquisition is made. The option «**Allow multi window**» should be activated by default for optimized Auto Exposure behavior when a wide spectrum range is specified. Uncheck this option only if you need to compare an acquisition performed with an earlier version of software (LS6.2 or LS6.3). «**Min. time**» and «**Max. time**» acquisition times are also set by the user, to prevent the software attempting an infinitely short or long measurement.

### About Autofocus

Autofocus is an optional device which performs automatic focusing on a sample. The Autofocus can be selected to operate in different ways.

- At each XY point: Autofocus will only be performed when the X,Y coordinate changes. For example, in an XY map focusing will take place at each point in the map.

- At start: Autofocus is performed at the start of a measurement only. For example, in an XY map, focusing will take place at the beginning of the map only, and not at each individual XY position.

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- Tilt at limits: this mode is only available for XY mapping experiments; five Autofocus points are defined at the four corners and center of the map area, which are used to calculate the tilt of the sample. During the mapping, focus is continually adjusted to compensate for the tilt.
- At every point: Autofocus is made at every point of a map, including time, temperature and X,Y coordinates.
- Tilt at midway: four Autofocus points are defined at the midway between the corners and the center of the map area and the last one on the center itself. This mode is optimized for circular samples.

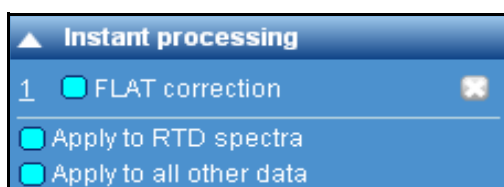
According to the System and the installed Autofocus device(s), two main types are available; Autofocus by reflection or by spectrum intensity. Each type operates with the same general principle. The sample is moved through a certain Z (focus) range, and a signal is monitored to detect whether the sample is in focus. Once the Z movement has completed, the focus position is calculated and the sample is moved to this position. The spectrum is then acquired at this point:

- By **reflection**: this mode monitors the Raman laser beam with a reflection detector; the optics are set up so that maximum signal is detected when the sample surface is at focus.
- By **spectral** intensity: This mode directly monitors the Raman spectrum intensity detected in the normal way. The focus point is chosen to be where the Raman spectrum intensity is greatest.

### What are the differences in Autofocus type?

The «**Reflection**» autofocus specifically locates the sample surface. The «**spectral**» autofocus focuses based on spectrum intensity, and the final «focus» position may not necessarily be the sample surface. For example, if the sample is heterogeneous in the Z axis, it is possible to set the autofocus to work based on a specific part of the Raman spectrum. In this way, the auto-focusing can be made to locate a specific component, even if it is not located at the surface of the sample.

## > Instant processing



The Instant Processing (IP) module allows the customer to apply data processing functions automatically immediately after acquisition.

In normal operation, with Instant Processing turned off, the acquisition sequence follows these steps:

- Start Acquisition
- Finish Acquisition
- Software reads raw data spectrum from CCD detector
- Software displays raw data spectrum on screen

With Instant Processing, an additional processing step is added

- Start Acquisition
- Finish Acquisition
- Software reads raw data spectrum from CCD detector
- Software applies «**Instant processing**» data treatment
- Software displays processed data spectrum on screen

The «**FLAT correction**» is a default IP method which comes with LabSpec6. This is a baseline subtraction method, which allows the user to have instantly baselined data.

In addition, from the Methods tab, the user can also then save any of his own methods so that it can be used by IP (see Method “Instant Processing” on page 94).




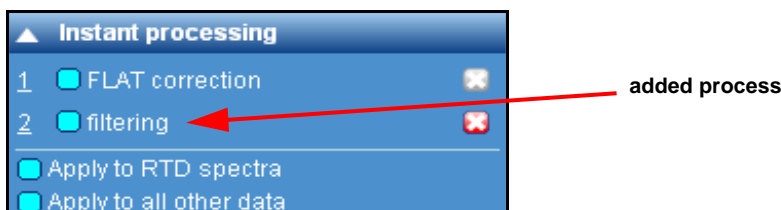
- Setup method in the normal way,
- In the bottom «Instant processing» section of the Method tab, type in a name for the method, and click on the “green arrow” button to validate.
- The method has now been added to Acquisition > Instant Processing.
- From the Acquisition > Instant Processing section the user can activate any method in the list (and also delete any “custom” methods – but not FLAT, since this is a default LabSpec6 method).
- Tick the box «Apply to RTD spectra» for the IP method to be applied to data recorded with the RTD  button.
- Tick the box «Apply to all other data» for the IP method to be applied to data recorded with the «Acquire Spectrum»  and «Acquire Map»  buttons.

Figure below shows an added «Filtering» process created from the Methods feature.



## > Acquisition options

▲ Acquisition options	
Delay time (s)	0
Binning	1
Readout mode	Signal
Shutter mode	Auto
Spike filter	Multiple accum.
Denoiser	Off
Laser mode	Auto
<u>ICS correction</u>	Off
<u>Dark correction</u>	Off

Enter the delay time between clicking the «acquire spectrum» button, and when the actual measurement is started. The delay time is useful to include a constant stabilization or photo-bleaching time for each measurement.

Enter the binning factor: see explanation below

Enter the readout mode: **Signal only (Signal)**, **Signal with background subtraction (Signal-Dark)**, **Background only (Dark)** and **permanent signal with background subtraction S-D (Always)**

The detector Shutter mode controls the behavior of the detector shutter. «Auto» means that shutter will open/close for measurements which are greater than 0.5s, and will be permanently open for measurements which are less than 0.5s. «Open/Close» means the shutter will always open and close between measurements. «Always open» means that the shutter will always be open throughout the measurements.

During the acquisition, this filter removes the spikes (cosmic rays) from acquired spectra; two options are available: «Single accumulation» uses a specific algorithm to remove spikes. Do not use it for spectra with narrow lines. For those spectra «Multiple accumulations» is more adapted as 2 acquisitions are done and compared to remove the spikes (see explanation below)

Two Denoiser levels can be selected to denoise acquired spectra (see explanation below).

Enable dark correction - automatic correction for detector response and pixel variation

**ICS correction: Intensity Correction System** corrects the instrument spectral range sensitivity. This is done by comparison with a calibrated source. This feature is delivered with each system and by default the ICS correction is set to ON. Optionally the ICS Client module allows you to record your own calibration reference correction. Thus, when an additional device is added to the instrument, a new ICS correction can be created. If the ICS correction file is missed, this parameter is displayed in red color.

Clicking «ICS correction» will display three choices. Read explanation «About the ICS Correction» on page 48.

### What is the binning factor?

The binning factor co-adds a specific number of adjacent pixels.

#### **Binning Factor = 1 (default value)**

When the «Binning Factor» is set to 1, the full number of data points is used to create the spectrum. The exact number depends on the specific configuration and set up of the Raman system.

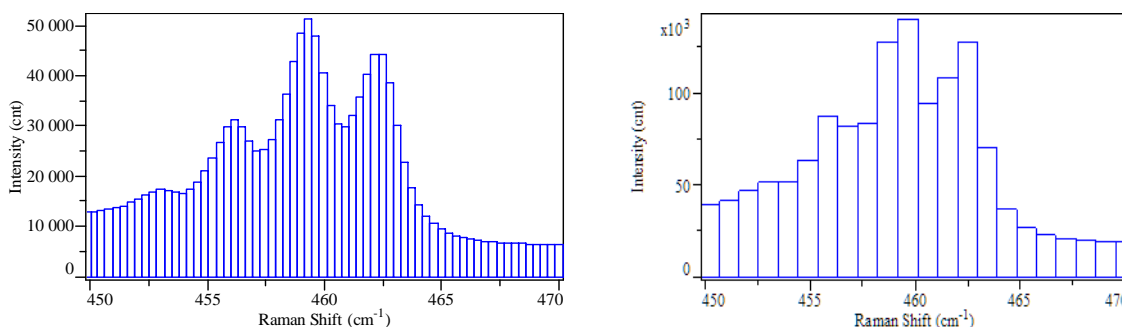
The default value for «Binning Factor» is 1.

#### **Binning Factor > 1**

When the «Binning Factor» is set to >1, the number of data points used to create a spectrum is reduced, by co-adding adjacent pixels on the detector. Increasing the «Binning Factor» results in spectra which have less data points, and result in smaller file sizes; in addition, the spectral quality (e.g., signal to noise ratio) will be increased. However, spectral resolution will be correspondingly reduced, which may result in detailed spectral features being lost.

Typically using a «Binning Factor» > 1 is only suitable for specialized applications. If in doubt, use the default value of 1.

Figure below shows an example of an acquired spectrum with a «Binning Factor» = 1 (left graph) and «Binning Factor» = 3 (right graph).



Note how there are 3x less data points, but the maximum signal level is 3x higher, resulting in a spectrum with increased spectral quality (e.g., signal to noise ratio). However, the detail of the spectrum (such as peak shape) is reduced.

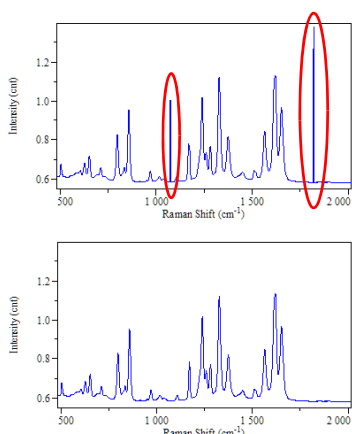
### How does the spike filter work?

The **Spike Filter** is a sophisticated algorithm designed to remove random spikes (also known as cosmic rays) which can appear in any spectrum. These spikes are entirely natural, and do not indicate a fault with your system. Without the «Spike Filter» it is difficult to know whether an observed peak is real (e.g., Raman) or a spike artefact. With the **Spike Filter** active you are assured that all peaks in your spectrum are real.

The **Spike Filter** uses two alternative algorithms:

- Single accum. - works for a single spectrum accumulation only, and locates spikes based on the spike size (width) selected. Left click on the **Spike Filter** label to select the desired size. The Single spike filter can also be applied post-acquisition - see information about Correction processing functions.
- Multiple accum. - works for multiple spectrum accumulations (i.e., number of accumulations is  $\geq 2$ ). The multiple spectra are compared, to identify real peaks and spikes. The comparison method ensures that real peaks can never be incorrectly deleted.

The spectrum shown below (top graph) was acquired without the «Spike Filter» - the marked peaks are random spikes, which are artefacts unrelated to the sample. The other spectrum (bottom graph) was acquired with the «Spike Filter» active. This time all peaks in the spectrum are Raman peaks, and there are no spikes visible.



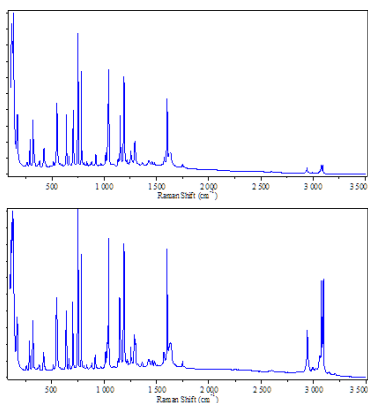
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### About the ICS Correction

The LabSpec intensity correction algorithm allows true comparison of spectra acquired using different laser wavelengths and optics. Without intensity correction, spectra acquired using different laser wavelengths and optics can show significant differences in relative peak intensities. These differences are caused by varying performance of the instrument components (including microscope objectives, laser rejection filters, diffraction gratings and CCD detectors) at different wavelengths.

The spectrum shown below (top graph) for 4-acetyl salicylic acid was acquired using a 785 nm near infra-red laser. In this case the CCD detector sensitivity decreases as the Raman shift increases. Thus peak intensities at high Raman shift positions are observed to be significantly weaker than they really are. The observed spectrum is not correct, since it is perturbed by instrumental factors.

By applying the ICS correction factor the perturbed Raman spectrum peak intensities can be restored, as illustrated in the corrected spectra shown (bottom graph).



### How is the ICS Correction Factor Calculated?

The ICS correction factor is created individually for each instrument at the time of manufacture.

The output from a calibrated white light source is passed through the system, and the recorded «response» spectrum is compared with the calibrated «source» spectrum of the white light source. The correction factor is calculated as

$$\text{correction} = [\text{source}] / [\text{response}]$$

The obtained correction factor is a multiplicative factor, which means that each spectrum acquired is multiplied by the correction factor to yield the corrected spectrum.

A correction factor is created for each and every pairing of laser wavelength and diffraction grating available on the instrument (e.g., 532nm-600gr/mm; 532nm-1800gr/mm; 633nm-600gr/mm; 633nm 1800gr/mm etc). When the automatic intensity correction is activated, the software automatically applies the appropriate correction factor based on the active laser wavelength and diffraction grating pair.

### About the Denoiser filter

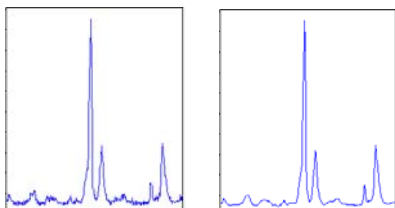
The Denoiser function is a unique noise reduction algorithm which can be used to significantly enhance spectrum quality without losing subtle spectral information. When Denoiser is activated, all data acquired will be processed with the Denoiser function - this includes real



time display (RTD) acquisition, spectrum acquisition and multidimensional spectral array acquisition.

Standard smoothing functions can result in loss of peak shape and position, and subtle features (such as weak shoulders on a strong band) can be lost. The Denoiser function ensures that all this important information is retained, whilst still reducing noise in the spectrum.

The spectra shown below illustrate the effect of the Denoiser function.



Two main Denoiser algorithms are available from the «Denoiser» drop down box:

- **Standard:** recommended for spectra with signal to noise  $\geq 20$ ,
- **Lite:** recommended for very noisy spectra with signal to noise  $\leq 20$ .

Denoiser can be applied post-acquisition using the [Processing>Smoothing & Filtering>Type> Denoiser](#).

## > Instrument Setup

The Instrument Setup section allows selection of key system hardware, including the detector, diffraction grating, laser and other hardware options (such as objective, filter, slit, hole etc). Only installed devices are available in the drop down lists.

**Instrument initialization**

Parameter	Value
StageXY	Marzhauser
StageZ	Marzhauser
Objective	x100
Grating	1800 gr/mm
ND Filter	100%
Laser	532 nm
Slit	150
Hole	70
AFM	Off
Collection	Microscope

- Activate the current parameters
- Select the installed XY Stage
- Select the installed Z Stage
- Select the objective magnification. Note that on Systems without motorized microscope nose pieces, the objective turret will need to be manually moved to select the correct objective. This selection in LabSpec6 is used for the software to correctly scale video images.
- Select the grating
- Select the ND filter level: choose the attenuation to avoid CCD detector saturation and/or sample burning
- Select the laser wavelength to use
- Moving to the entered value
- Type in the slit value in  $\mu\text{m}$  with correct symbol
- Type in the confocal hole diameter value in  $\mu\text{m}$
- Switch to the AFM optical path, for combined Raman-AFM and TERS measurements (this option is only available on AFM systems).
- Select the signal collection location (typically the microscope, external fiber coupled device, or transmission Raman accessory).

Tick this box to enable automatic adjustment of the spectrometer position when the active laser and grating are changed

Adjust spectro

Load current

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## > Map

This section defines additional parameters for a map. First at all, select the **Video** from the Data Tabs Menu and choose a mapping profile from the **Graphical Manipulation Toolbar**. Then, from the Maps section (see below), select the mapping size by entering the XYZT sizes and limits of the pattern and the step sizes for each acquisition point. The acquisition pattern is then immediately displayed. Please notice that you can resize the pattern directly using the mouse on the video image; in this case the sizes in the table are automatically updated.

The screenshot shows the 'Map' configuration window with the following settings and annotations:

	From	To	Size	Step	
Z	0.00	100.00	11	10.0	<input type="checkbox"/>
Y	-1760.59	1760.59	11	352.	<input checked="" type="checkbox"/>
X	-1848.03	1848.03	11	369.1	<input checked="" type="checkbox"/>
t	0.00	100.00	1000	0.1	<input checked="" type="checkbox"/>

Map type: Polygon  
 Total data points: 0  
 Estimated time: 0 sec  
 SWIFT mode for L Acq. time(s) 0.1  
 Turn laser off during pauses  
 Skip center (%) 0    
 Extra images: With spectra  Alone   
 Z   
 Aux. device

**Annotations:**

- Mapping sizes properties (points to the table)
- Currently selected map shape (points to Polygon)
- Total data points (points to 0)
- Estimated total acquisition time (points to 0 sec)
- SWIFT feature used for ultra fast mapping (optional) (points to SWIFT mode)
- Activate the laser pause in case of long waiting time between acquisitions (points to Turn laser off)
- Exclude the central circular/elliptical region for circle/ellipse and polygon map shapes (points to Skip center)
- Extra images (see below) (points to the Extra images section)

- **SWIFT feature (option):** SWIFT allows ultra-fast Raman mapping, by using sophisticated communication methods between the sample mapping stage and detector. It is generally suitable for acquisition times ranging from <1ms through to 1s (depending on the system detector). When the box is unticked, the map will be acquired in the standard 'point by point' mode, which is suitable for acquisition times greater than 0.5s.
- **Extra images:** the Extra Images section allows non-spectral images to be generated, either on their own (using the dedicated RTD and Acquire buttons) or in combination with spectrum acquisition via standard mapping acquisition. Only certain configured devices will be available, which are typically the Z-motor (optional), and autofocus diode (optional). Other devices may be available, depending on instrument configuration

## > XYZ Stage

This section allows the operator to move a motorized stage by entering a position. A fine adjustment has been added for the Z axis.

Enter the XYZ positions

The screenshot shows the 'XYZ stage' configuration window with the following settings and annotations:

XY	0.0	0.0	<input type="button" value="↶"/>	<input type="button" value="↷"/>
Z	0.0	<input type="text"/>	<input type="button" value="↶"/>	<input type="button" value="↷"/>

**Annotations:**

- Set the stage origin: once done, the current XY and Z position becomes the stage origin and XY and Z position is displayed (0.0). If the active stage is DuoScan, then clicking this icon will move the DuoScan mirrors to their central 0,0 position. (points to the origin icon)
- Move to the entered XY positions (points to the XY move buttons)
- Move to the Z position (points to the Z move buttons)
- Z fine adjustment (points to the Z input field)

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## > Linkam

The Linkam section allows heating and cooling sample stages manufactured by Linkam Scientific to be controlled directly by LabSpec6.

The screenshot shows the Linkam 0.0° control interface with the following fields and controls:

- Temp. (°C):** A text input field containing '20' and a 'Free' button.
- Limits (°C):** Two text input fields labeled 'Min' and 'Max' containing '-50' and '50' respectively.
- Speed (°C/min):** Two text input fields labeled 'Heating' and 'Cooling' both containing '10'.
- Time (s):** Two text input fields labeled 'Heating' and 'Cooling' both containing '10'.
- Stabilization Time (s):** A text input field containing '10'.
- Always hold temperature:** An unchecked checkbox.
- LNP Pump:** Two radio buttons labeled 'Auto' (selected) and 'Maximum'.
- Data Path:** A text input field.

Red arrows point from descriptive text labels to these fields:

- Enter a desired temperature
- Deactivate the temperature - the sample will return to ambient temperature naturally
- Activate temperature control, so that the sample will be held at the desired temperature
- Enter the temperature limits of the experiment
- Enter the desired temperature ramp rate that will be used by LabSpec6 during an automated temperature measurement
- Enter the desired time (in seconds) that will be used to move from one temperature to another. The necessary ramp rate will be automatically calculated by LabSpec6
- Enter sample equilibration/stabilization time according to the diagram below
- Tick the box to hold target temperature
- LN<sub>2</sub> pump activation according to the experiment needs
- Select the data storage path; data acquired using the Linkam Temperature Ramps function will be saved to this location.

The Linkam device can be used in three main ways:

- 1 Move to a single temperature position; manually start a Raman acquisition.
- 2 Set up a single temperature ramp, by defining start/stop temperatures and step size. Raman acquisitions are automatically made at each temperature once it is reached. This is done using the LabSpec6 **Acquisition > Maps** section.
- 3 Set up multiple ramps, each of which can be defined with start/stop temperatures, step, heating/cooling rate and stabilization hold times. This is done using the HORIBA Scientific Linkam Script which must be loaded in the Linkam section. The ramps are defined in the **Acquisition > Temperature Ramps** section (see below).

The Linkam section is used to set up default parameters for the heating/cooling stage, and also set a single temperature to be reached.

Once these parameters are set, temperature ramp experiments can then be set up in the Maps section, where the heating/cooling rates and stabilization time used will be those specified in the Linkam section.

## > Temperature ramps

The **Temperature ramps** section is a dedicated interface for the **Linkam** heating/cooling stages which allows fully customizable temperature ramps to be defined and acquired.

If an experiment requires several temperature steps, the additional parameters are grouped in the following table.

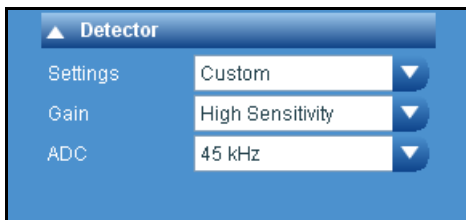
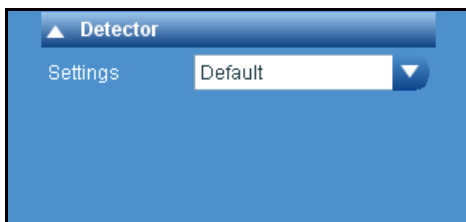
▲ Temperature ramps						
#	From	To	Speed	Step	Hold	
1	0	0	0	0	0	<input checked="" type="checkbox"/>
2	0	100	10	10	5	<input checked="" type="checkbox"/>

Spectrum acq  Video acq

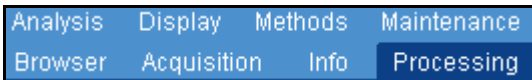
click on this arrow to start the temperature ramp measurement

## > Detector

The Detector section shows key parameters relating to the current detector (which is selected in the Instrument Setup section). Usually the «Default» settings are used, but it is possible to choose other available options by opting for the «Custom» settings. The menu options available for «Custom» settings will depend on the specific detector and its configuration.





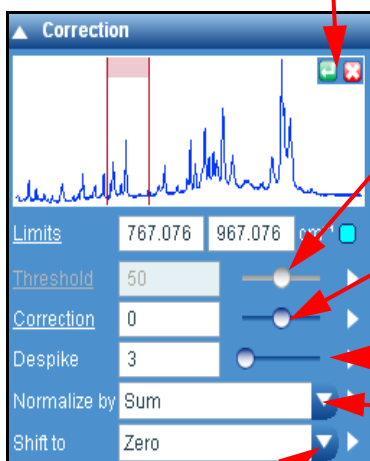
## 6.4 Processing Tab



### > Correction

The **Correction** feature allows the user to apply spectral normalization and correction to both single spectra, and multidimensional spectral arrays.

Click on  icon to load the active spectrum in the main LS6 graphical user interface (GUI) into the «Correction» window. Click on  icon to clear the «Correction» window, and remove the spectrum displayed there.





Click on **[Zero]** to automatically subtract a constant intensity value from the active spectrum or multidimensional spectral array, so that the lowest intensity pixel is at zero. When applied to a multidimensional spectral array, each spectrum in the array is zeroed independently.

**[Zero (noise)]** is similar to **[Zero]** except that it first calculates the average noise on the spectrum. It then shifts the spectrum to zero, correcting for the noise, so that the average position of the spectrum is at zero.



If the cursor is not visible in the Correction window, enter manually the Limits parameters.

#### How to use it:

- 1 Open a spectrum from the Open file icon located in the Icon bar (see “Icon bar” on page 20),
- 2 Click on  to load the active spectrum into the «Correction» window. Click on  to clear the «Correction» window, and remove the corrector spectrum displayed there.

**Threshold** the active multidimensional spectral array, to remove spectrum which have intensities above or below a set threshold value. The threshold function will be applied individually to each spectrum in the array, and will convert any thresholded spectrum to have zero intensity throughout its spectral range.

See below for configuration details for this function.

**Correct** the active spectrum or multidimensional spectral array, to subtract a specified amount of the corrector spectrum. This function is useful if a substrate, diluent or contaminant spectrum needs to be removed from a sample spectrum.

See below for configuration details for this function.

**Despike** the active spectrum or multidimensional spectral array. Adjust the width of the spike to remove.

**Normalize** each spectrum according to:

{Sum} - normalize so that the sum of the spectrum intensity is 100.

{Area} - normalize so that the area of the spectrum is 100

{Maximum} - normalize so that the maximum intensity point in the spectrum is 100.

{Unit vector} - this treats a spectrum as a multi-dimensional vector, the intensity at nth spectral point being the nth element of the vector. It is normalized so that the norm (or inner product between itself) is 1.

{Normal variate} - this treats a spectrum as a collection of n (intensity) values. It normalizes so that the standard variation over n values is 1.

If the **Limits** tick box is ticked, the correction functions (Threshold, Correct, Normalize and Shift) will be calculated within the limit range only, but applied to the full spectrum.

A corrector spectrum only needs to be loaded if the Subtract (Correction) function is going to be used. Other functions (Threshold, Normalize, Shift to) can be directly applied to active data within LabSpec6.

### 3 Limits:

Set the spectral range where the correction will be calculated/applied by ticking the «Limits» box, and typing the limit values.

The limits can also be set by adjusting the position of the cursors in the Correction window which are continuously updated.

### 4 Setting up Threshold:

Click on «Threshold» to access set up controls for thresholding.

**Remove:** choose to threshold (remove) spectra which have an intensity above or below the threshold limit.

**Mode:** choose if the threshold value for each spectrum is calculated from the maximum spectrum intensity, or as the average spectrum intensity across the spectrum.

**Value:** choose if the threshold value set in the main interface (or with the slider) corresponds to an absolute intensity value, or is a % value, relative to the maximum intensity across the entire multidimensional array.

**Limits:** set the minimum and maximum values for the Threshold slider.

### 5 Setting up Correction:

Click on «Correction» to access set up controls for Correction.

**Value:** choose if the correction amount set in the main interface (or with the slider) corresponds to an absolute multiplier (for example, subtraction amount = {value}\*spectrum) or is a % value, relative to the intensity of each spectrum to be corrected..

**Limits:** set the minimum and maximum values for the Correction slider.

### 6 Despiking

The Despiking function is a filter which removes spikes according to the width of these spikes. This function is active within the defined spectral limits. It works for single spectra and maps files.

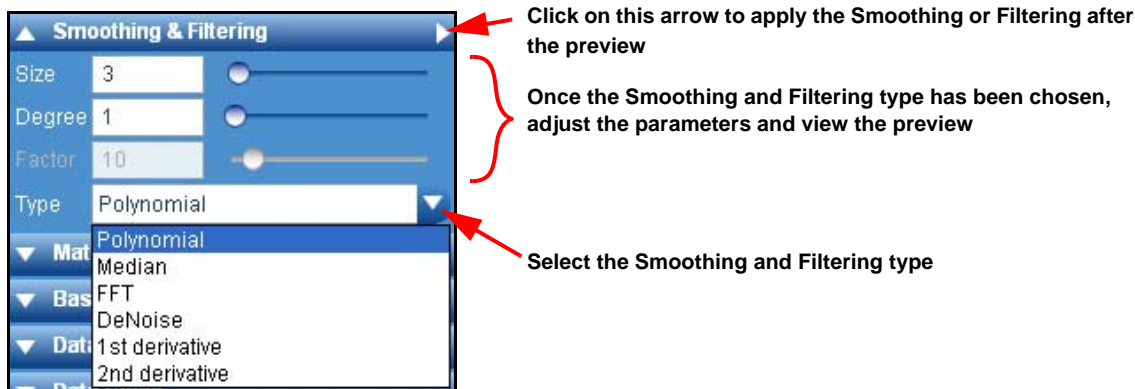
The “Size” 3, 4, or 5 is related to the pixel width of the spike. For example, if a spike has a width of 4 pixels, then it will be removed with a «Size» of 4 but not of 3.

## > Smoothing & Filtering

The smoothing or filtering functions allow spectra to be smoothed or converted to first and second derivative functions. Typically these functions allow spectral quality to be improved after acquisition. LabSpec6 uses several algorithms for smoothing acquisition data.

Note that in some cases smoothing can remove or alter features in a spectrum. Smoothing should be used with care.

In practice, select a smoothing or filtering function, then, using the mouse, adjust the slider to view a preview of the result. If the result is acceptable, click on the white «apply» arrow button to apply the smoothing/filtering.



### Polynomial

LabSpec6 Polynomial option uses the Savitzky-Golay smoothing algorithm which removes local signal noise while preserving the shape of the signal.

Savitzky-Golay smoothing fits a polynomial function of a specified «Degree» through a range («Size») of adjacent pixels, and replaces those pixels with the polynomial curve. The window where this operation is applied is moved across the entire spectrum. The «Degree» and «Size» must be set to an appropriate level.

Typically the smaller the «Degree» and the larger «Size» the more significant the smoothing.

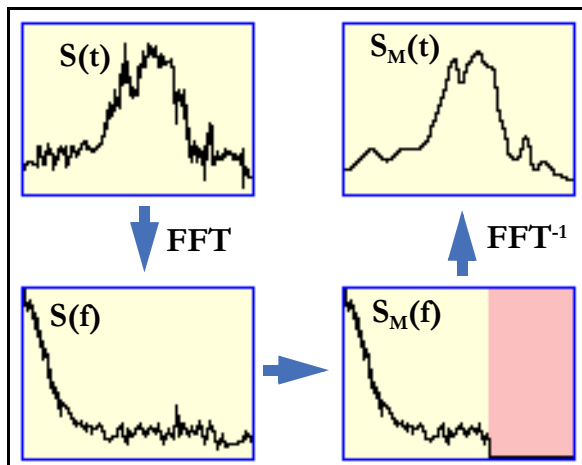
### Median

The median smoothing algorithm also uses a sliding window to smooth data.

The median filter is an effective method that can suppress isolated noise without blurring sharp edges. Specifically, the median filter replaces a data point by the median of all data points in the window. Typically the larger the «Size» the more significant the smoothing.

### FFT

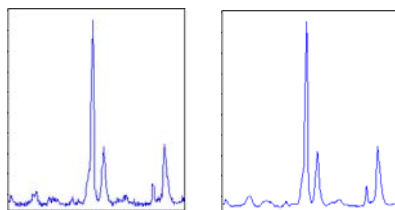
The spectrum smoothing using Fast Fourier Transforms (FFT) is based on mathematical manipulations. The spectrum is first converted from wavenumber (or wavelength) space to frequency space by applying a Fourier transform. In Fourier space, the high frequency components are discarded, then the spectrum is converted back to wavenumber space. Signal smoothing is performed by removing the frequency components (corresponding to noise), using the **Factor** slider, while the useful (corresponding to Raman peaks) low frequency components are retained. The process is depicted schematically below (the pink area represents the range of removed frequencies):



Typically the larger the «Factor» value the more significant the smoothing.

### Denoise

The Denoise function is a unique noise reduction algorithm which can be used to significantly enhance spectrum quality without losing subtle spectral information.



Standard smoothing functions can result in loss of peak shape and position, and subtle features (such as weak shoulders on a strong band) can be lost. The Denoise function ensures that all this important information is retained, whilst still reducing noise in the spectrum.

The spectra shown left illustrate the effect of the Denoiser function.

Typically the larger the «Size» and «Factor» the more significant the smoothing.

### 1st Derivative

Select [1st derivative] to convert the active spectrum to its first derivative function. At each pixel position the derivative is calculated using a defined range («Size») of pixels either side of it.

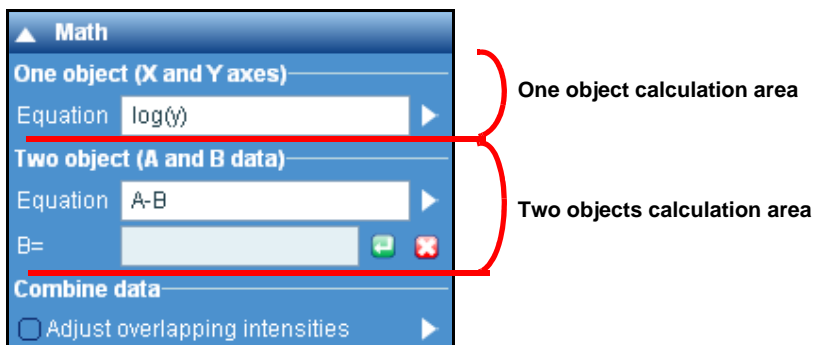
### 2nd Derivative

Select [2nd derivative] to convert the active spectrum to its second derivative function. At each pixel position the second derivative is calculated using a defined range («Size») of pixels either side of it.



## > Math

Commonly used mathematical formulae can be applied to a data object. The Math functions can be performed on both single spectra, and multidimensional spectral arrays.



The Math section is divided into two sections: one object and two objects calculations.

### One object: application of mathematical functions on axis values

Enter the desired mathematical conversion. Y means the Y-axis (intensity) values, and X means the X-axis spectral values (e.g., 500  $\text{cm}^{-1}$ , 501  $\text{cm}^{-1}$ ).

#### For example,

Equation (Y) =  $\log_{10}(Y)$  converts the spectrum to have a logarithmic intensity scale.

### Two objects: application of mathematical functions between two objects

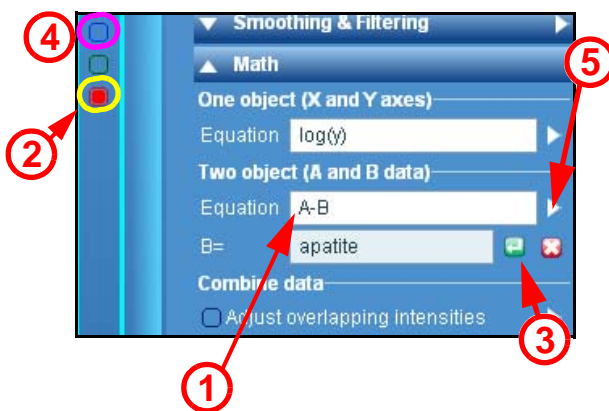
Enter the desired mathematical conversion. A means the active data object, and B means a second data object.

#### For example,



Equation =  $A + B$  adds data object A to data object B.

Standard mathematical functions can be used within the equation, including +, -, \*, /, ^, exp, log10, ln, sin, asin, cos, acos, tan, atan, abs, sqrt etc.

This function can include two objects A and B which are single spectra, and multidimensional spectral arrays. The step procedure is explain below:



- 1 Enter the mathematical equation linking the A and B data

- 2 Select a data in the data bar that you want to assign as B
- 3 Assign it as B data by clicking on  icon. The name of the data will be automatically displayed in the «B=» field.
- 4 Select the second data in the data bar which will be, by definition, the A data
- 5 Click on the «Apply equation» button  to perform the calculation.

### Combine data

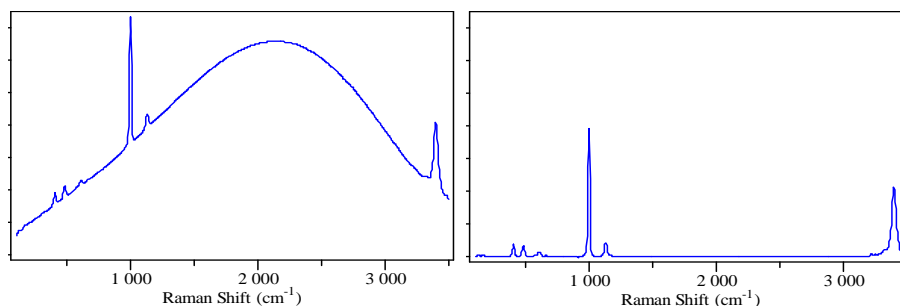
Click on the **Combine data** arrow  to average all open data.

Tick the «Adjust overlapping intensities» for spectra with different spectral coverage. The overlapping regions will be adjusted so that their baselines coincide. This helps to avoid stitching problems, and yields a seamless stitch.

## > Baseline correction

Baseline correction allows a high background in a spectrum to be subtracted, to yield a spectrum with a flat, zero baseline. The correction can be applied to a single spectrum or a multidimensional spectral array of spectra (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes).

LabSpec6 baseline correction is based on a 20<sup>th</sup> degree polynomial.



*Figures illustrate an example of a spectrum before and after baseline correction.*

### How to select the degree (order) of the polynomial

Low order polynomials (i.e., degree=1 or 2) have limited flexibility, and cannot easily adapt to highly shaped backgrounds. They are useful for simple backgrounds. High order polynomials (i.e., degree > 10) can adopt complex shapes, and are suitable for complicated backgrounds.



**A generalized approach is to start with a middle-of-the-range value, like order 4. If the baseline, after correction, follows the spectrum envelope, there is no need for a different solution.**

**Baseline correction**

- Type:  Line  Poly
- Degree: 7
- Max points: 64
- Noise points: 10
- Attach to curve
- Correct noise
- Color: [Green]

Buttons: Fit, Sub

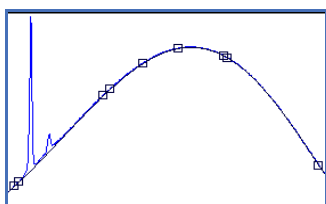
Annotations:

- Select the algorithm used to define the baseline curve
- Launch the Fit
- After the Fit calculation, subtract the resulting fitted curve from the spectrum
- Slider for Degree selection
- Select the amount of baseline data points
- see explanation below
- Select the baseline color

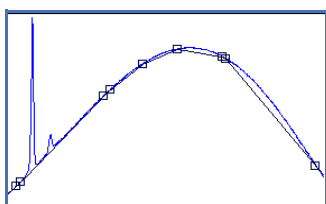
**Options:**

- **Type**

Select the algorithm used to define the baseline curve.



**Polynomial:** fits a polynomial curve through the baseline points set on the spectrum. The degree of the polynomial is set from the «Degree» field/slider.



**Lines:** fits a straight line between baseline points set on the spectrum.

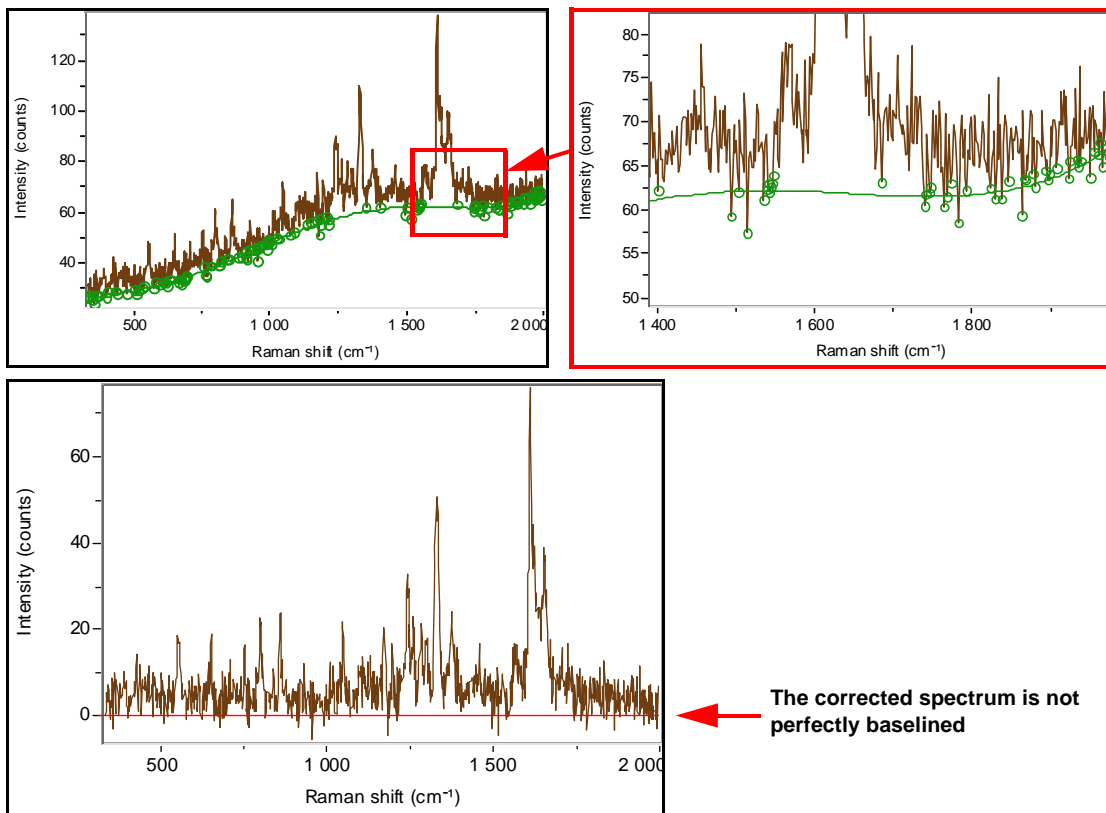
- **Degree**

Select the degree of polynomial equation used to create the baseline curve - when the baseline «Type» is set to «Poly».

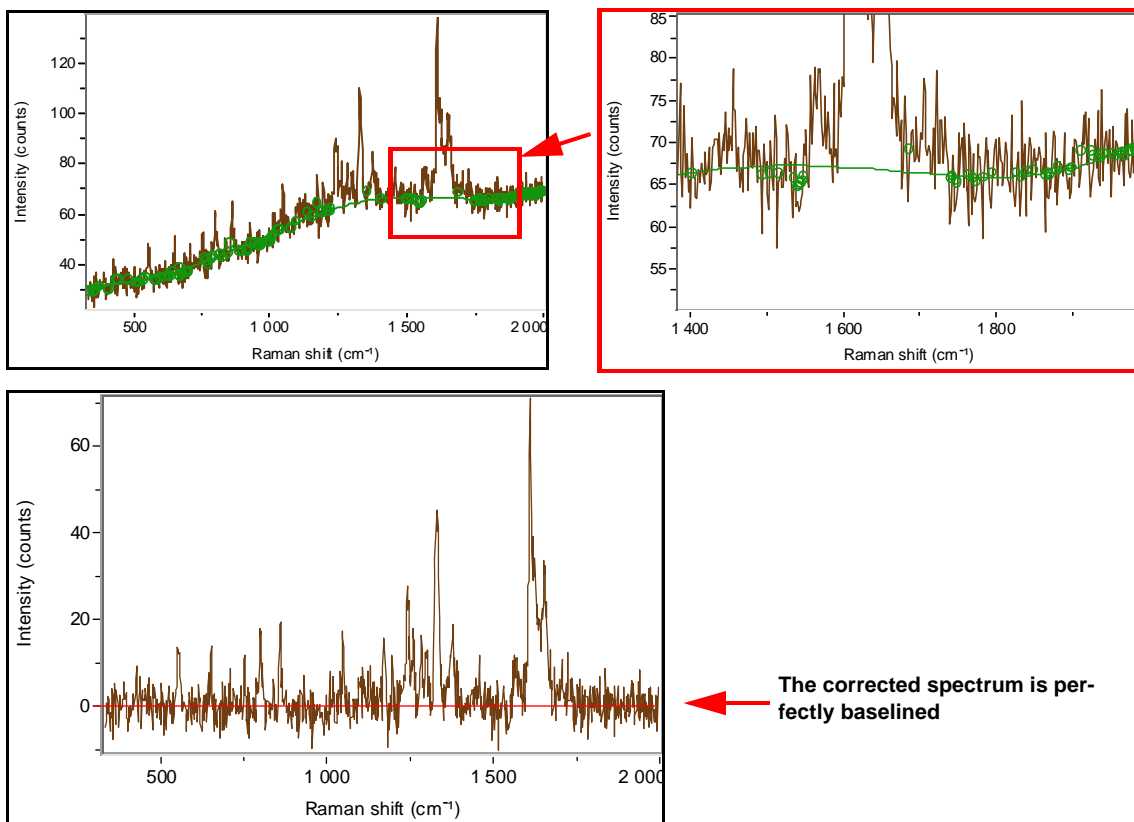
To select the polynomial degree, move the **Degree** slider to adapt the baseline. In general, the higher the degree of the polynomial the more curved will be the baseline.

- **Noise points**

This additional parameter corrects the baseline calculation. Without the noise correction, the baseline points are generally located at minimum pixel intensities as shown on the figure below:



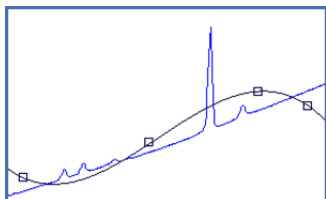
When using the Noise correction, baseline points are correctly located in the center of the noise (see figure below).



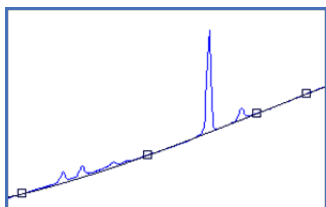
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- **Attach to curve**

Select the «Attach the curve» mode for manual definition of baseline points used to define the baseline curve.



**Not Selected:** baseline points are set at the exact intensity and spectral position ( $\text{cm}^{-1}$  or nm) specified by the user.



**Selected:** baseline points are forced to sit on the spectrum, at the spectral position ( $\text{cm}^{-1}$  or nm) specified by the user.



The «Attach to curve» mode is particularly important when defining a baseline to be used for correction of multidimensional spectral array data. In this case, «Attach to curve» should be selected to ensure that the baseline points will adapt to the varying intensities of the spectra within the multidimensional spectral array.

## > Data Calibration

The Data Calibration module allows easy post-acquisition calibration and adjustment of data. Three different procedures are available according to the type of data and calibration requirements:

### Shift Spectrum

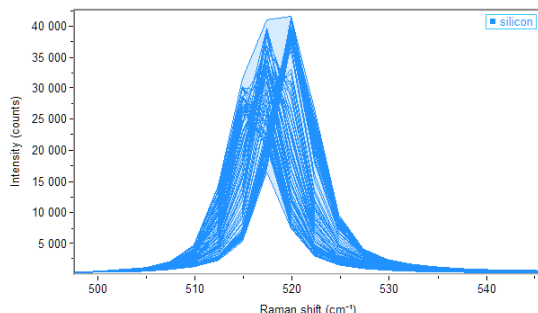


Enter the shift increment

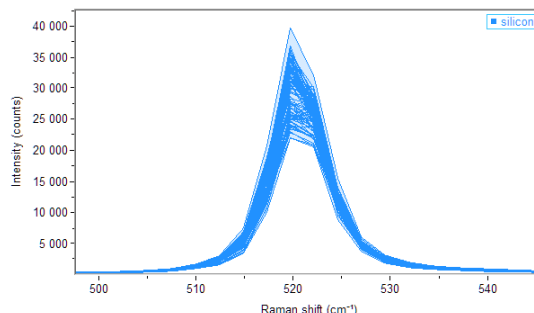
Move the spectrum to the right or left direction

This offers a fast, basic data calibration tool, to manually shift a spectrum up or down the spectrum axis. When applied to the «All spectra» window of map files, all spectra will be uniformly shifted by the same amount.

### Calibrate Spectrum



Raw map data showing thermal drift



Map data after calibration to reference peak

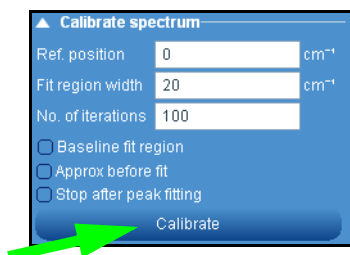
The **Calibrate spectrum** function performs a peak fit on a known reference peak within the spectrum, and then automatically shifts the spectrum so that the known reference peak is precisely and correctly positioned. This function is particularly useful to compensate for thermal drifts caused by unstable lab environments.

When applied to the “All spectra” window of a multidimensional array, each spectrum is individually corrected. Note that the final calibrated array data file will be truncated at the extreme spectral limits, according to the amount of calibration shift applied to each spectrum.

To apply the calibration, set the known reference peak position (**Ref. position**) and the fitting region (**Fit region width**). The maximum peak shifting relative to the reference position must lie within the fit region width. The fitting region will be defined as  $[\text{Ref. position}] - \frac{1}{2}[\text{Fit region width}]$  to  $[\text{Ref. position}] + \frac{1}{2}[\text{Fit region width}]$ . Set the number of peak fitting iterations and click on [Calibrate] to start the process.

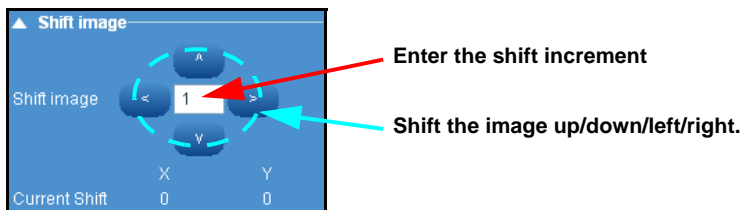
Additional tools are provided for optimized calibration:

- **Baseline region:** a second order polynomial baseline correction will be made in the fit region prior to the peak fitting. This can be useful to improve calibration results in cases where the reference peak is lying on a sloping background. Note that the baseline correction is only applied during the peak fitting, and the final calibrated data will remain with the full background present.
- **Approx. before fit:** a fast optimization of the peak fit starting positions will be performed prior to peak fitting. This is particularly useful if there is large miscalibration, with the reference peaks
- **Stop after peak fitting:** this options will stop the calibration process after the peak fit, but before shifting the spectra. This can be useful to check the peak fitting process and confirm it is fully optimized for the specific data. If the results are acceptable, then uncheck the “Stop after peak fitting” button, and re-run the calibration.



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## Shift Image



The Shift Image function can be used on both images (such as videos) and multidimensional arrays.

## > Data Range

The Data Range section displays complete information about the range of a selected data object. It can be used to modify the data ranges (for example, increase or decrease number of data points, or extract a portion from the original data object).

Parameters displayed include:

The **Data Range** section displays information for the following key parameters:

X - spatial axis (X) for spectral arrays

Y - spatial axis (Y) for spectral arrays

Z - spatial axis (Z) for spectral arrays

T - temperature axis for spectral arrays

t - time axis for spectral arrays

S - spectral axis (nm/eV/cm-1) for spectra

I - image intensity

	Size	Step	From	To
Y	48	0.9815	4.9036	52.017
X	43	0.9804	-27.570	14.5911
S	49	1.1126	1022.6	968.11
I			-8.5917	305

### How does it work?

The table initially shows the full data range parameters of the currently selected data. It can be used to change the data range, step size, or to extract a small portion from the original data file.

**[Extract]:** This allows the user to extract a small portion of data from the original complete data file. To use this, first either use the Zoom button to locate the region to be extracted, or manually modify the «From» and «To» parameters for each axis. Then click [Extract]. This will delete all data outside of the specified region, and just leave the small portion that was

selected. Note that when the data file is saved, the original full range data will be lost. It is recommended to make a back up of the original data first.

**[Scale]:** this function allows the data range to be rescaled. The data will retain the same number of data points, but it can be stretched/skewed to fit across a new data range.

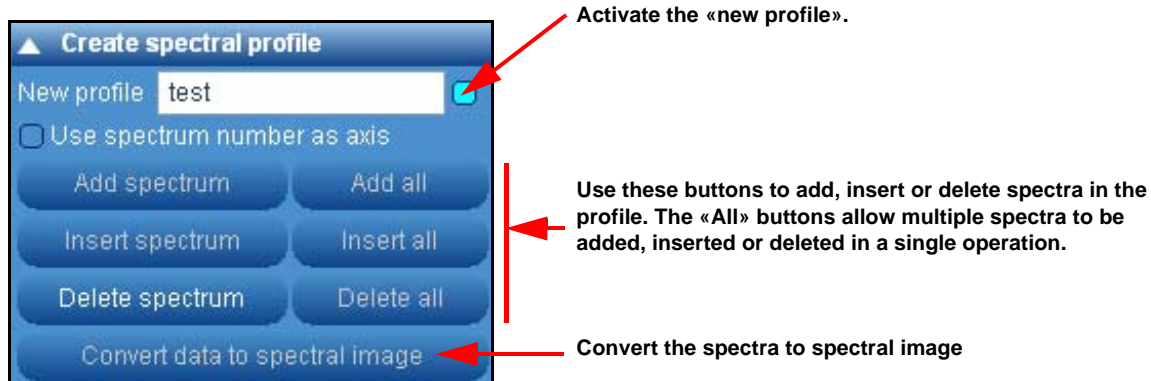
**[Size]:** this function allows a data file to be resized, to increase or decrease the number of data points within the file. The resizing will be done using automated polynomial interpolation. For example, an image comprising 100 data points in the X dimension could be resized to have 200 data points in the X dimension. To use this function, modify the values in the Size box, and then click on [Size].

**[Bin]:** this function allows the number of data points to be reduced by co-adding adjacent data points. To use this function, modify the values in the Size box, and then click on [Bin]. Note that binning can only be made by an integer factor (i.e., to co-add every 2 data points, every 3 data points, etc). If the specified size value does not fit this requirement, it will be automatically modified to the nearest possible value.

## > Create Spectral Profile

The Spectral Profile dialog window allows a 1D multidimensional spectral array to be created from individual spectra, and for spectra to be added to, deleted from, and inserted into an existing 1D multidimensional spectral array. This function is useful to create profiles from spectra where there is sequential change in some experimental parameter (e.g., reaction temperature, or concentration).

To activate the feature, tick the box located next to the «New profile» title. Type the desired name for a new profile.





**General Procedure:**

- Give a name for the resulting spectral profile.
- Activate the first spectrum that is to be added to the spectral profile.
- Click on **Add** to create the spectral profile.
- Continue to activate additional spectra, and add or insert them into the profile. Spectra can be removed from the profile using the **Delete** button. Multiple spectra can be added, inserted or deleted using the **Add all**, **Insert all** and **Delete all** buttons.

When the profile is created, the standard window associated with a multidimensional spectral array will be displayed. The profile can now be analyzed in the normal way, using cursors and fitting.

**Add spectrum**

Click on [Add spectrum] to add the active spectrum to the end of the spectral profile.

**Adding Spectra to a Spectral Profile**

To add a spectrum into an existing Spectral Profile, use **Open file** icon  to open the spectrum which is to be added.


Select the spectrum, and click on [Add spectrum] to automatically add the spectrum at the end of the spectral profile. For example, if the profile already has spectra at positions 1, 2 and 3, a new spectrum added to the profile will be at position 4.

**Add all**

Same as above but all of the opened spectra are used to create the spectral profile.

**Insert spectrum**

Click on [Insert spectrum] to add the active spectrum to the spectral profile at the position shown by the cursor in the «Maps» window.

To insert a spectrum into an existing Spectral Profile, use **Open file** icon  to open the spectrum which is to be inserted.

From the «**Maps**» Data tabs window, activate the cursor and select the position at which the new spectrum is to be inserted.

Select the spectrum which is to be inserted, and click on [Insert spectrum] to insert it at the position indicated by the cursor. In a default spectral profile, the other spectra originally at and above this position will be shifted upwards in the profile.

**Insert all**

Same as above but all of the opened spectra are inserted in a specific position indicated by the cursor.

**Delete spectrum**

Click on [Delete spectrum] to delete a spectrum from the spectral profile. The spectrum at the position shown by the cursor in the «Maps» Data tabs window will be deleted.

**Delete all**

Delete all the spectra of a profile.

## > Scripts

### Visual Basic Scripting (VBS)

Visual Basic Scripting (VBS) is an active scripting language, designed as a lightweight programming tool within the Microsoft® environment. Within LabSpec6 it can be used to create customized scripts which access nearly all functions available to a standard user within the interface - in combination with the standard VBS commands and functions it offers a real programming capability directly within LabSpec6. In essence, VBS provides automation to a level beyond the Methods module, described below - it does however require a degree of background knowledge and understanding in VBS, so is not suitable for all users.

The library of LabSpec6 objects for VBS include functions to set parameters/values within the interface (i.e., to set up measurement conditions), and acquire, process and display data. In addition, the native VBS functions such as logical decisions, looping, waiting etc, allow scripts to be generated which can encompass complete experiment protocols, with feedback of results and adjustment of parameters through the experimental process.

One potential drawback of VBS is speed - scripts are traditionally slow to run. In LabSpec6 this does not present a problem. The script is used to set up processes, but the actual process is then carried out using LabSpec6's native, high end compiled software modules. Thus, even time dependent functions such as detector read out, motor movement, and ultra-fast mapping can all be reliably controlled with VBS.

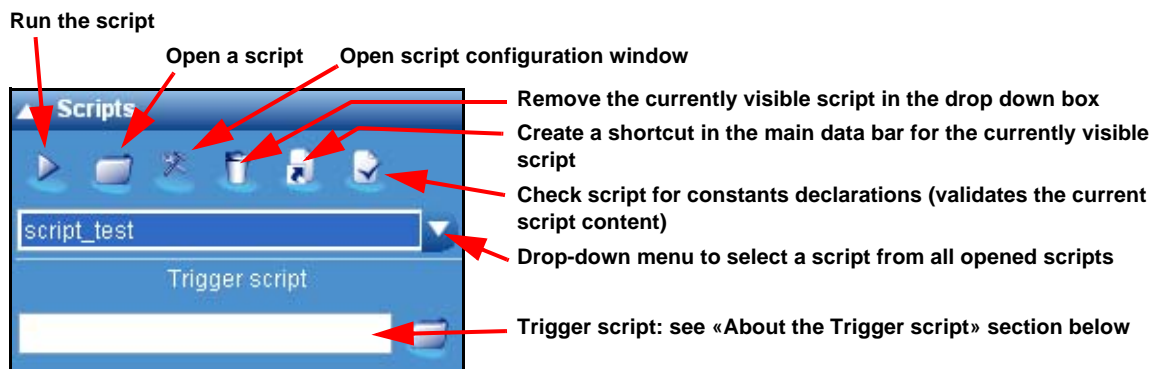
To open up access to VBS for less experienced users, and to speed up scripting for experienced programmers, LabSpec6 includes a library of script «building blocks». These are pre written functions which can be called and inserted into a user script very quickly using LabSpec's fully integrated script editor. This module acts as an ideal tutor in scripting for beginners, since they have at immediate disposal fully functioning script examples to start working with. For more experienced programmers, these «building blocks» will ensure ultra-fast programming.

### How does it work?

The Script module has a 2-level control depending on the user requirements.

#### Level 1

From the Control Panel, select **Processing > Scripts**. The following screen will be displayed:

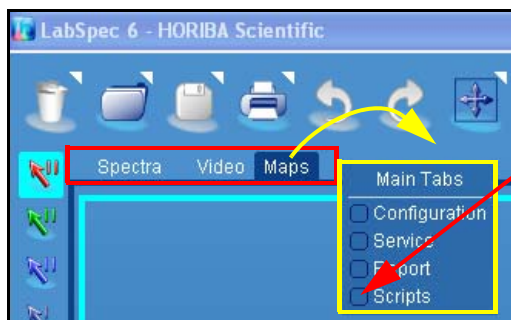


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This window is appropriate for a ready-to-run script. Open a script, then run it by pressing the *[run]* button.

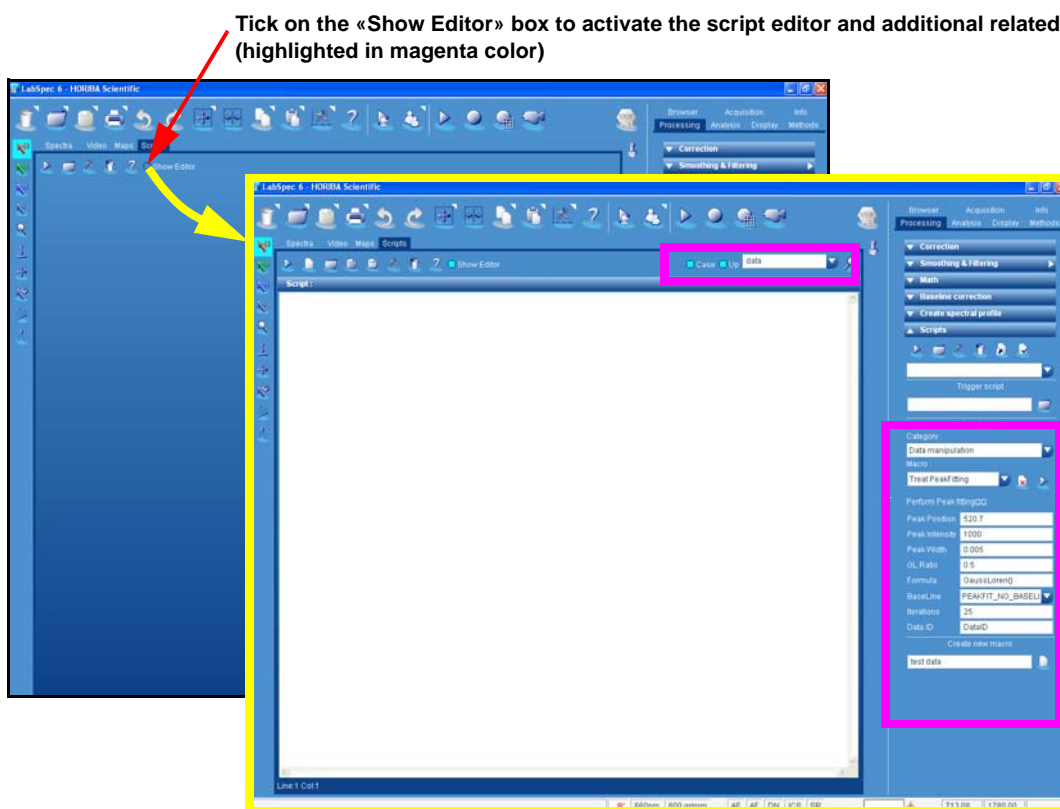
## Level 2

Right-click somewhere in the **data bar** area to display the additional menu choice. Tick the «Scripts» box to add the Scripts data bar (see figure below).



Tick on the Scripts box to display the additional menu choice. Note that this option will not be available to users with Operator rights.

The following screen will then be displayed:



Tick on the «Show Editor» box to activate the script editor and additional related menus (highlighted in magenta color)

Once the «Scripts» menus are fully deployed, the user can create, modify and run scripts within LabSpec6. The **Insert Macro** part of the **Control Panel Scripts** allows the user to easily create a new script.


## Extended Scripts menu

The figure below shows the extended scripts menu located in the **Control Panel > Processing**.

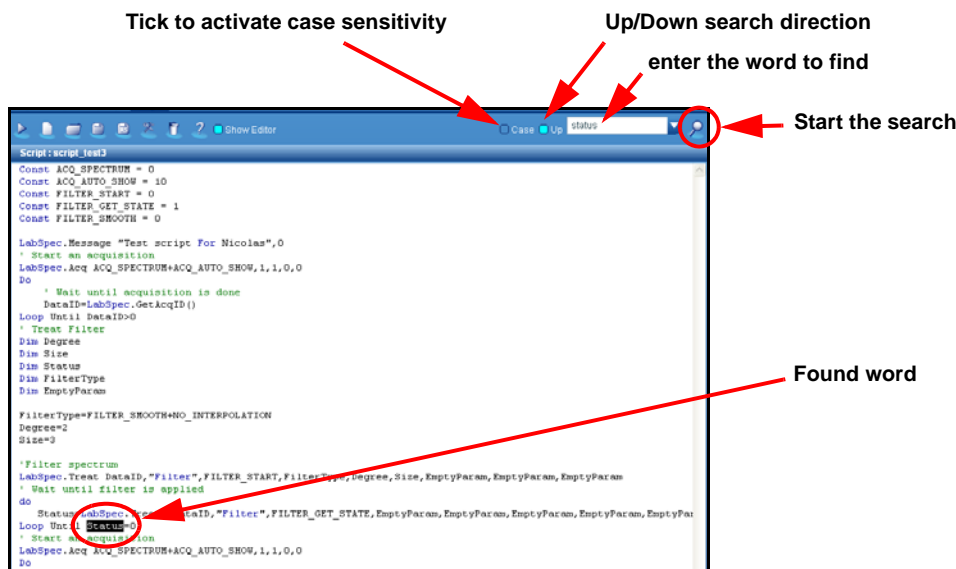
The **Insert Macro** section allows pre-defined script modules (macros) to be inserted into the script editor, for fast script development.

The **Create new macro** section is used to create custom script macros from a highlighted section of the script displayed in the Script Editor.

1. Choose the script macro category.
2. Select a specific macro in the chosen category. Then underneath you will see all the possible options for this script, which can be simply set up in the boxes.
3. Once built, insert the macro to the editor
4. Enter a name for a new custom script macro.
5. Create the macro from the selected (highlighted) script text in the Script Editor window. This new macro will now be available in the Custom category.


A complete on-line library detailing all available VBS commands (with examples) is available by pressing the Scripts Help  icon in the main Scripts tab.

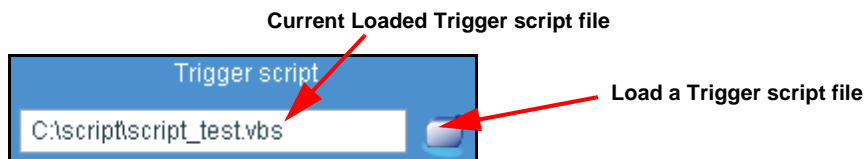
The Script Editor includes a search utility to find a specific word. The option «Case» allows the search to be case sensitive. If the «Up» box is ticked, the search will be performed up from the current cursor position (normally it will search down from the current cursor position).



### About the Trigger script

The **Control panel > Scripts** includes a section called *Trigger script*. The Trigger script is in fact a Visual Basic Scripting program (.vbs) which can be automatically run before or after data acquisition. This trigger script allows automated specific data processing (filtering, smoothing...) but can also control external hardware like a laser commutation or the control of non-HORIBA hardware.

The commands of the Trigger script are explained in the LabSpec Automation on-line help, by clicking the Help  icon. Then, select the **GetTriggerMode** from the **Collect Data** section. Once the script has been created, select the Trigger script file by clicking on the Load Trigger script icon (see figure below).



The loaded Trigger script will be executed according to the commands located in the main script (execution timing) and the trigger definition within the script. Note that only one trigger script can be defined, but it can include multiple processes occurring before and after acquisitions.

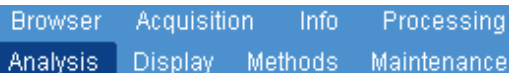
## &gt; FTIR



The FTIR module provides a number of data conversion tools for use with FTIR data. This allows data to be converted between «Transmission» and «Absorbance», and transformed according to ATR, Kramers-Kronig and Kubelka-Monk algorithms.

This module must be activated in **Display > LabSpec options > Modules** – it is a free module which can be used without licensing.

## 6.5 Analysis Tab

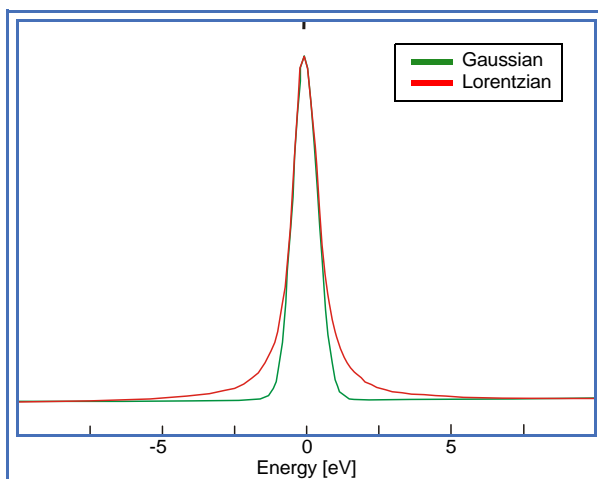


### > Peaks

The Peaks Fitting module allows peaks in a spectrum to be automatically labeled by their position, and full peak fitting can be carried out to fully characterize peak parameters such as position, amplitude, full width at half maximum height (FWHM) and area. Overlapping peaks can be fully deconvoluted through the peak fitting routine.

The Peak Fitting functions can be performed on both single spectra, and multidimensional spectral arrays.

The peak detection is based on mathematical equations using symmetric and asymmetric **Gaussian or Lorentzian Shapes distribution**. The figure below shows the Gaussian and Lorentzian shapes.



The **Lorentzian** shape appears primarily in spectral applications, and like the **Gaussian** it arises from several of the molecular phenomena that broaden spectral lines, including collisions between the atoms that emit the radiation. Spectral scientists usually work primarily with Lorentzian and Gaussian peak functions or with functions based upon them. For example, **GaussLor** consists of a convolution of Gaussian and Lorentzian peaks. In some cases peak shapes are not symmetric, and in this case the asymmetric shapes (indicated by the «A» prefix) can be used.

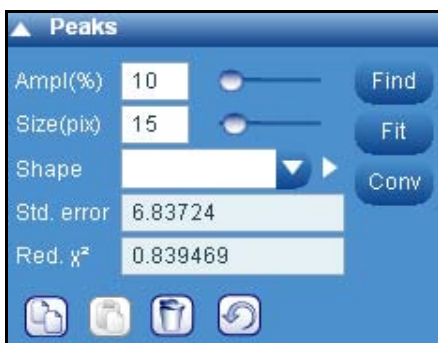
- **Find option**

The **Find** routine locates local intensity maxima, and assigns these as peaks.

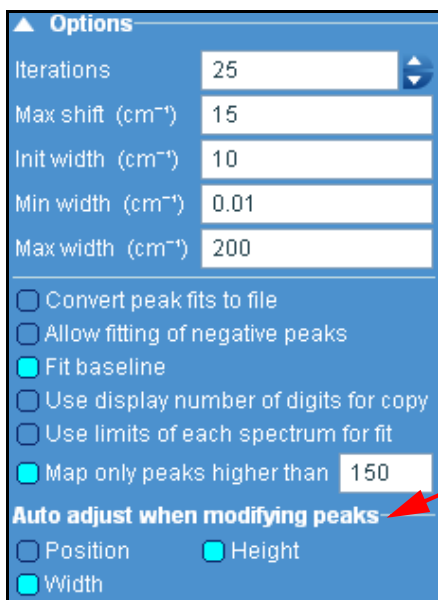
A local maximum must be greater than a certain percentage of the maximum intensity in the whole spectrum. The «Ampl(%)» parameter defines this percentage of maximum spectral intensity. Typically, as the «Ampl(%)» is increased, only the most intense peaks will be identified. If low intensity peaks need to be identified «Ampl(%)» should be reduced.

A local maximum is assumed to exist within a finite number of adjacent data points. The «Size (pix)» parameter defines this number. Typically, as the «Size (pix)» is increased, only peaks which are widely separated will be identified. If close lying peaks need to be identified «Size (pix)» should be reduced.

The «Ampl(%)» and «Size(pix)» values can be set by typing a value in the appropriate box, or using the slider. The peak labelling displayed on the active spectrum will update continuously, so that the result can be monitored.



## > Fit options



If «Position», «Height» and/or «Width» of Auto Adjust peaks are ticked, then when a peak is manually placed on the spectrum an automatic peak shape (Position, Height or Width) adjustment will be made, in order to quickly match the raw data profile.

This routine uses a Levenberg-Marquardt non-linear peak fit algorithm, and iteratively adjusts all peak parameters to minimize the standard error «Std. error».

The maximum number of iterations can be set by typing an appropriate number in the «Iteration» field. Typically the larger the iteration number the more accurate the final fit result will be, but the longer the process will take.

«Max shift (cm<sup>-1</sup>)» limits the maximum X-axis shift that can be made to a peak during the fitting procedure.

«Init. width (cm<sup>-1</sup>)» is the initial peak width that will be used when a peak is located, or manually placed on a spectrum. Generally it is useful to have an appropriate peak width before peak fitting.

«Min» «Max, width (cm<sup>-1</sup>)» limit the minimum and maximum peak widths that can be used during the fitting procedure.

Convert to file: this feature is linked to the [Conv]  button.



- If «Convert peak fits to file» = OFF (unticked), then clicking on the [Conv] button will transform the current spectrum into the sum of the fitted peaks. This spectrum of the peak fit sum can now be saved/modified.
- If «Convert peak fits to file» = ON (ticked), then clicking on the [Conv] button will launch a File “Save as” dialog window. Define a file name (for example “peak”) and save location, then click on the [Save] button. LabSpec will save the files «peak.16s» (sum spectrum from the peaks), “peak\_1.16s” (spectrum of peak #1), “peak\_2.16s” (spectrum of peak #2) etc. The individual peaks from the peak fitting can now be opened from further analysis or inspection.

When «Negative peak» is ticked, the fitting routine will work with negative intensity peaks. When unticked, it will only allow peaks to have positive intensity.

Click on the **Fit** button to launch the fitting process. The result is estimated by the Standard error and reduced  $\chi^2$  values. The smaller the Standard Error the better the fit result. A good quality fit is indicated by the reduced  $\chi^2$  value tending to 1.

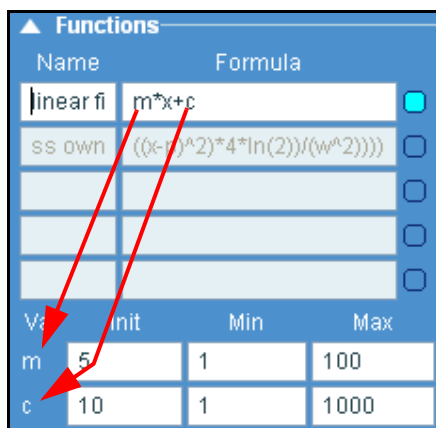
If the «Fit Baseline» box is ticked the peak fitting routine will additionally fit the specified baseline. The baseline must be specified first, using the **Baseline Correction** dialog window (see “**Baseline correction**” chapter).

- «**Use display number of digits for copy**»: When the peak fit results are copied to text, and then pasted into an external program, a default numerical precision of 6 significant digits will be used. When «Use display number of digits for copy» is enabled (ticked), then the peak information will be copied with the same number of decimal places as specified in the Peaks «Display options» section.

- «**Use limits of each spectrum for fit**»: By default this option should be enabled (ticked). When doing peak fitting on hyperspectral map data, fitting limits will be individually optimized according to each spectrum in the map before fitting starts. Uncheck this option only if you need to compare map peak fit results performed with an earlier version of software (LS6.2 or LS6.3).

- «**Map only peaks higher than**»: Use this option to set a display threshold for peak fit maps. When enabled (ticked), only peaks which have an amplitude greater than the defined value will be displayed in the peak fit map. Other low intensity peaks will not be displayed. This option is useful when displaying map peak fit images where some spectra do not have any peaks.

## > Functions



06/23/2015

The **Functions** section can be used to define custom peak shape and fitting algorithms.

- 1 Type in a name for the new custom function: *linear fit* on the figure above,
- 2 Type in the formula for the custom function.

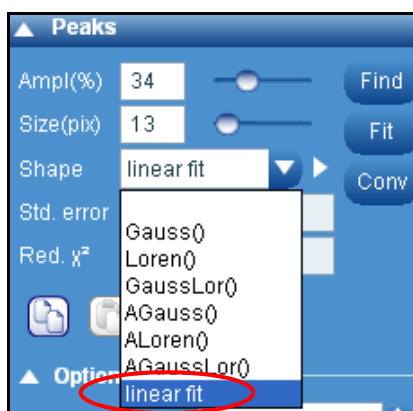
Standard mathematical symbols and operators can be used, including:

**+**, **-**, **\***, **/**, **^**, **exp()**, **log10()**, **ln()**, **sin()**, **asin()**, **cos()**, **acos()**, **tan()**, **atan()**, **abs()**, **sqrt()**.

Four LabSpec 6 symbols are also available: **x** (X-axis spectral position), **p** (peak position), **w** (peak full width at half maximum) and **a** (peak amplitude).

All other symbols/letters added will be considered to be a variable, and will be listed in a table. The figure above shows an example where the variables are *m* and *c*. The table lists each new variable, and includes boxes for the key variable parameters to be set: “**Init**” (initial starting value when “peak” is first added to the spectrum), “**Min**” (minimum allowable value) and “**Max**” (maximum allowable value) should be input. Variable values after fitting will be listed in the peak table.

- 3 Once a formula has been added, it will be included in the “Shape” drop down box. Select the custom shape, and run the fitting in the normal way.



## > Display options



06/23/2015

The Display options window offers a choice of visual parameters like colors and font sizes of the displayed Labels, Peaks, Sum and Residuals.

Specific features:

**Sum:** once activated (ticking the box), creates a curve which matches the sum of all the individual peaks.

**Res (Residual):** Once activated (ticking the box), the residual curve is displayed. The residual curve is the difference between the raw data and the sum spectrum. With perfect fitting, raw data = sum, and therefore residuals = 0 at all points in the spectrum. In reality normally raw data and sum are similar but not identical. In this case, residuals should be a random, flat line. If residuals are strongly patterned (e.g., big peaks) this suggests that the peak fit is not good. Hence, residuals can be used to decide whether the peak fit is good or bad.

### > Peak table and Show options

The **Peak table** window displays peak position, amplitude, full width at half maximum height (FWHM) and area for all peaks labelled on a spectrum. The parameters can be manually adjusted and fixed so that the parameter is not varied during the fitting routine.

	p	a	w
1	124.335	465.699	20.8589
2	405.93	2551.73	20.8589
3	1000.41	26195.7	20.8589
4	1455.83	73.826	6.95313

The Peak table parameters are only displayed if the related symbols [p], [a], [g], [w], [t] and [ar] are ticked in the Show options window.

Each row displays the parameters for a single peak:

	p	a	g	w	ar
Value	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Fix	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Map	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Min	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Max	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Precision	<input checked="" type="radio"/> 6 digits <input type="radio"/> 15 digits				

**[p]** Peak position, in units as displayed on the spectrum's X axis, typically Raman shift ( $\text{cm}^{-1}$ ) or nanometers (nm).

**[a]** Peak amplitude, in units as displayed on the spectrum's Y axis, typically counts (cnt), or counts per second (cnt/s).

**[g]** Gaussian contribution, ranging from 1 (fully Gaussian) to 0 (fully Lorentzian) for a mixed GaussLor peak shape.

**[w]** Peak full width at half maximum height (FWHM) in units as displayed on the spectrum's X axis, typically Raman shift ( $\text{cm}^{-1}$ ) or nanometers (nm).

**[t]** Asymmetry factor for asymmetric peak functions.

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**[ar]** Area of the peak, in area units based on the units displayed on the spectrum's X and Y axes.

**Fix** Once selected from the **Show options** window, the «Fix» tick boxes to the right of each of the p, a, g, w and ar parameters allows a parameter to be fixed. A fixed parameter will not be varied during the peak fitting routine. When a box is ticked the parameter is fixed. When a box is unticked the parameter will be varied during the fitting routine.

**Map** The «Map» tick boxes (which are only displayed for a multidimensional spectral array) to the right of each of the p, a, g, w and ar parameters allows a profile or map image to be generated based on the parameter. For example, it is possible to create an image based on peak position, illustrating how the peak position varies across the map area. To display a profile/image based on a peak parameter first perform peak fitting on the entire data set, and tick the appropriate box. A new map profile/image will be created. To close the map peak fit profile/image, untick the box.

#### **Min/Max**

When a **Min/Max** box for a parameter is ticked from the **Show options** window, the **Peak table** displays additional field(s) near to the related parameter. By default, the Min/Max fields displays the initial parameters used in the peak fitting procedure, and the minimum and maximum values they can take during the fitting procedure. In most cases the default values are suitable for general peak fitting routines, but in specific cases the initial parameters and their minimum and maximum values can be manually adjusted as required.

#### **Precision**

Select the number of digits for peak table display. For typical results, 6 digits is sufficient. In specialized cases 15 digits may be useful, but the relevance of the least significant digits should be assessed according to the data and experiment conditions.

## > **Map Characterization**

The Map Characterization section displays the positions and settings for the «Red», «Green» and «Blue» cursors which are used to create profiles and images from multidimensional spectral arrays (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes). The positions and settings can also be manually configured in this section. Up to three spectral regions can be used to generate Raman chemical images using the «Red», «Green» and «Blue» cursors. In addition, the ratio of the «Green» and «Blue» cursors can also be imaged.

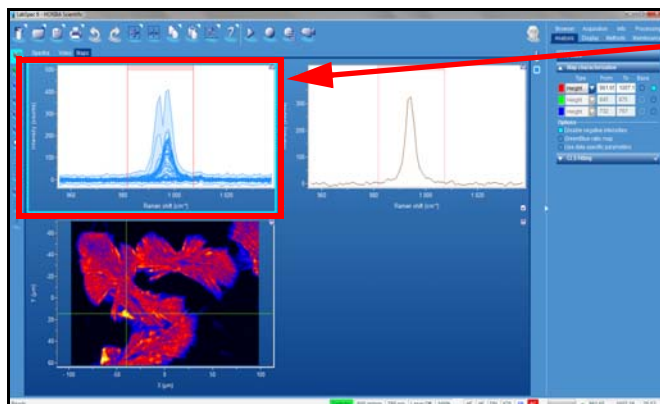
### **Creating maps from selected spectral region**

**Preliminary:** a mapping acquisition should have been performed and the data loaded in the **Maps** workspace.

**Purpose:** Up to 3 spectral regions can be selected to analyze and display a map image/profile showing the variation of spectral intensity within these regions.

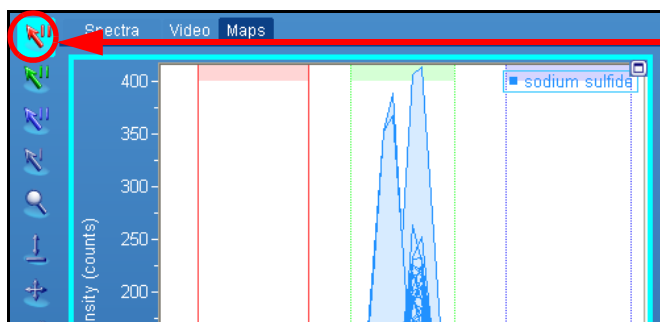
**Description:** the figures below show the steps for intensity selection

1



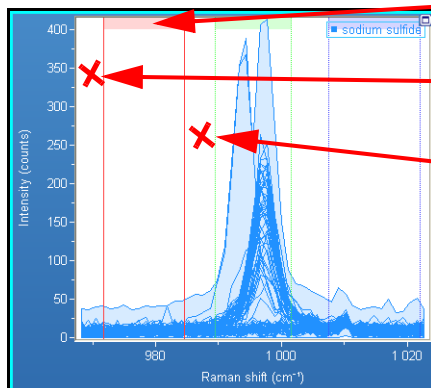
Click on the map «All Spectra» window to select it: a light blue frame is highlighted around this area

2



Click on the red cursor icon to activate the red cursor

3



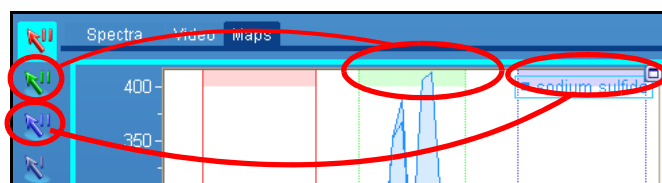
Click on the top shaded area to move the cursor with fixed width

Click outside the left region limit, and drag the mouse to move the left red limit

Apply the same action for the right limit

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 be isolated with the cursors

4



If necessary, proceed the same way to select two new spectral regions with the green and blue sets. Cursors can also be selected by clicking on the top shaded part of the cursors in the data window. Click once, and then the cursor is selected and active.

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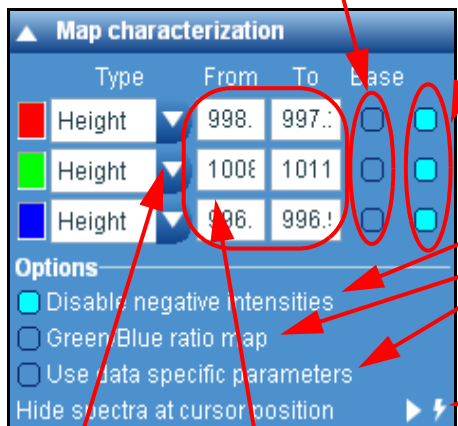
**NOTICE:** to initialize the cursors, click on this icon



located in the Icon bar.

**5** Base parameter: activate the baseline subtraction.

If the «Baseline» box is ticked for a set of cursors («Red», «Green» or «Blue») then the cursor region is first baselined before calculation of the cursor parameter. This mode is useful to ensure that the image created truly reflects a Raman peak and not general background intensity (perhaps from fluorescence or photoluminescence).



Activate the cursors:

If the box is ticked for a set of cursors («Red», «Green» or «Blue») then a profile or image will be generated for the cursor.

Tick this option to disable negative intensities.

If the «Green/Blue» box is ticked an additional profile/image is displayed, showing the ratio of the «Green» and «Blue» cursors (i.e., [Value\_GREEN] / [Value\_BLUE]). This display is useful to visualize a change in peak ratios within a multidimensional spectral array.

When «Use data specific positions» is ticked, the map cursor settings will be saved with the data file. When reloaded, the exact same settings will be used, in order to display the exact same images as previously viewed.

From the Map images (cursor, scores, peak fit) choose a specific pixel (with the 'cross' cursor) or area of pixels (with the 'rectangle', 'ellipse' or 'polygon' cursors). Click on the white arrow to hide the selected pixel(s); To undo and restore the pixel(s) for viewing, click on the lightning symbol. For areas, left click on the underlined Hide spectra inside cursor region or Hide spectra outside cursor region to select which mode should be applied.

Step 3 can be replaced by directly typing the ranges of the Red, Green, Blue cursors

Map Characterization can be made on:

**Height:** maximum intensity value in the cursor region

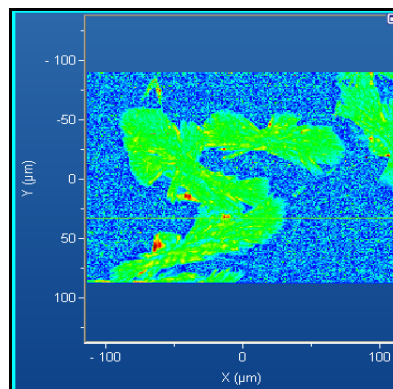
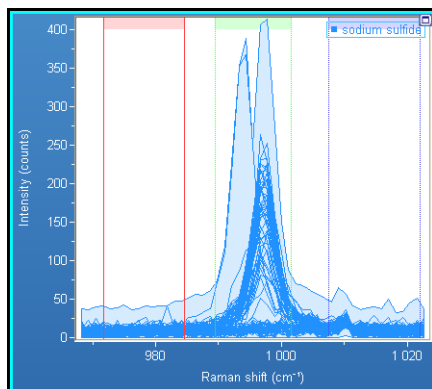
**Area:** peak area (intensity x spectral width) of the cursor region

**Position:** spectral position of the maximum intensity pixel in the cursor region

**Width:** approximate FWHM of the peak in the cursor region

**Dominant:** calculate the spectral dominant wavelength according to the CIE1931 color space. This mode is available only if the PL module has been activated from Display>LabSpec options>Modules

**6** Once the intensity cursor is selected, the related intensity map is displayed. The figure below shows an example.



**NOTE:** steps 1 to 3 can be adjusted during viewing and the results will be seen instantly.

06/23/2015

## > CLS Fitting

The CLS Fitting section is used to set up and perform the multivariate Classical Least Squares (CLS) fitting procedure on single spectra and multidimensional spectral arrays (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes) using a set of reference component spectra. This method is a supervised multivariate decomposition technique.

This procedure can be used to either calculate the contribution of reference spectra within a mixed component single spectrum, or to identify the distribution of reference component spectra within a spectral array to create a profile/image based on the component distribution. The reference spectra must be manually selected (from previously saved spectra, or from within the spectral array).

With the fitting functionality, it is possible 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 CLS Fitting Procedure

- **Single spectrum**


In the CLS Fitting Options section, tick the «Use for spectrum» box.

Click on the «Open loadings» icon, and select the spectra you wish to use as loadings for the CLS fitting.

As soon as the spectra are loaded, the fitting calculation will be made, and the results will be displayed in the Spectra data tab.

- **Spectral array analysis**

At each position within the multidimensional spectral array the CLS fitting procedure finds a linear combination of the reference component spectra which best fits the raw data. The resulting profile/image is created by showing the contribution (score) of each component (loading) as a profile/image.

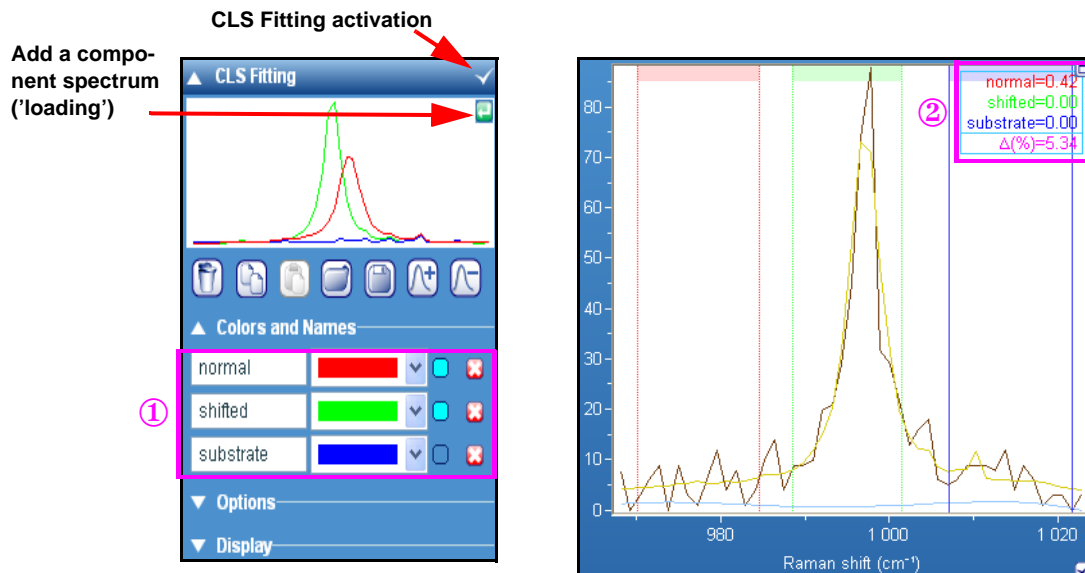
To add a component spectrum (loading), select it first from the Spectra data tab, or Cursor Spectrum map window then click on the [add] button . Proceed similarly for the other components.



For example, if there are three loadings ① with scores ②, the sum, S, of the linear combination is represented by:



$$S = [\text{score1} * \text{loading1}] + [\text{score2} * \text{loading2}] + [\text{score3} * \text{loading3}]$$

The loadings are, for example, reference spectra of pure components.

The scores are the 'amount' of each loading necessary so that S matches the raw data.

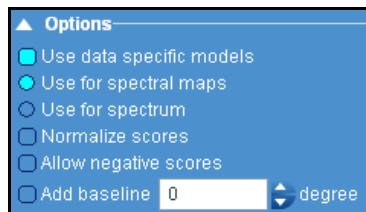


The «Sum/Subtract selected models to/from Spectral Image» buttons   are used to generate a new dataset based on the CLS fitting data:

- Sum selected models : the selected models will be used to create a new dataset made up from the selected loading spectra multiplied by their scores at each pixel.
- Subtract selected models : a new dataset will be created by subtracting from the original dataset the selected loading spectra multiplied by their scores at each pixel.

### Options

The **Options** parameters offers additional optimizations to improve the CLS Fitting results.



- **Use data specific models**

If this option is ticked, the loadings and scores will be saved with the main data file. When you next open the file, the loadings and scores will also be opened.

If this option is not ticked, then the loadings are treated as separate, independent data within LabSpec6. They will not be saved with the data file. When a new data file is opened, scores will be automatically calculated based on the loadings which are displayed in the CLS Fitting section.

- **Use for spectral maps or spectrum**

CLS Fitting can be performed on either a single spectrum (to calculate the contribution of 'pure spectra' within a mixed component spectrum) or with a spectral array «map» (to calculate a profile/image of component distribution across the map. Choose the desired mode using the radio buttons.



- **Normalize scores**

The scores are normalized so that the combination of scores add up to 100%. If «Normalize scores» is turned off the scores are shown as their true values.

- **Allow negative scores**

If this box is ticked, then scores are allowed to be negative during the fitting process. If this box is not ticked, then scores will always take positive values throughout the fitting process.

- **Add baseline**

If this box is ticked, an individual baseline is created for every spectrum in the Raman map, and the parameters of the baseline are fitted as part of the total CLS fitting procedure.

The baseline is defined as  $Y=A+B*X + C*X^2 + \dots$

For degree 1,  $Y=A + B*X$ ,

For degree 2,  $Y=A + B*X + C*X^2$  etc.

When the «Add baseline» is ticked, the baseline and the peaks (from the reference loading spectra) are fitted at the same time. This provides the most accurate baseline correction, and the most accurate CLS fitting for the peaks.

## Display

The Display window allows additional components to be displayed.



Tick the «Error» box to display the Error value with the other score values, and to display the error spectrum.

Tick the «Sum» box to display the sum spectrum. This allows you to compare the fit results (Sum) with the raw data.

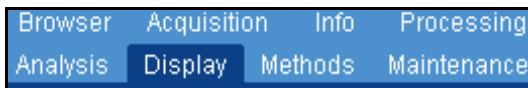
Tick the «Residual» box to display the residual curve. The residual of an observed value is the difference between the observed value and the estimated function value.

If «Show error map» is ticked an additional score profile/image will be displayed based on the error between the sum of the linear combination and the raw data. Regions of high error (bright intensity) indicate a bad fit, and could be caused by a missing reference component spectrum.

To remove the error profile/image untick the «Show error map» box.


Tick the «Base» box the display the calculated baseline.

## 6.6 Display Tab



The Display section includes additional parameters related to data and interface display. Thus the graphics and text color, the spectra and image visualization, axis scale and legends, and language choice of the graphical user interface can be changed and personalized.

### > Scales

The display scale of a graphic presentation can be changed from the **Graphical Manipulation Toolbar** (see [figure 1-1](#)) by using the **Zoom** button  or from the **Scales** section.

When a graphic presentation is selected (by using the mouse), the **Scales** section is immediately updated using the parameters of the current presentation. You can also use this Scales section to redefine a new range for the axes X, Y and the intensity (I). Do not forget to tick the **Fix** box if you want stop the window autoscaling when new data is added.

If the Zoom button is used, the scale will be automatically fixed, until the Rescale button is clicked, or the «Fix» boxes are unticked in the Scales section.

The Scales section displays information for the following key parameters:

- X - spatial axis (X) for spectral arrays
- Y - spatial axis (Y) for spectral arrays
- Z - spatial axis (Z) for spectral arrays
- T - temperature axis for spectral arrays
- t - time axis for spectral arrays
- S - spectral axis (nm/eV/cm-1) for spectra
- I - image intensity.

The screenshot shows the 'Scales' section of the software interface. It contains a table for defining axis ranges and several checkboxes for scaling options. Red arrows point to specific elements with explanatory text:

	From	To	Fix
X	967.549	1023.21	<input type="checkbox"/>
Y	-28.0722	15.093	<input type="checkbox"/>
I	-8.59172	45	<input type="checkbox"/>

Annotations:

- Enter the new X range (points to the X 'From' field)
- Enter the new Y range (points to the Y 'To' field)
- Tick a Fix box to freeze the defined range (points to the X 'Fix' checkbox)
- Enter the Intensity scale range (points to the I 'To' field)
- Enter a region of interest on which the zero intensity, and/or maximum scale and/or minimum scale will be ignored (points to the Region fields)
- Ignore region with zero intensity (points to the first checkbox)
- Ignore region for max scale (points to the second checkbox)
- Ignore region for min scale (points to the third checkbox)
- Region: 988.185 1004.09 (points to the Region input fields)
- Stacks section (points to the Stacks header)
- Image, Vertical, Horizontal (points to the sliders and checkboxes in the Stacks section)
- Axes expansion (points to the right side of the Stacks section)

**NOTE:** greyed parameters appears if the selected window is not set to «stack» layout

### > Axes

The Axes section groups a large choice of presentation options. The table below describes the parameters:

### Units

The Frequency (or wavelength) and Intensity units displayed on the selected window can be chosen from a set list.

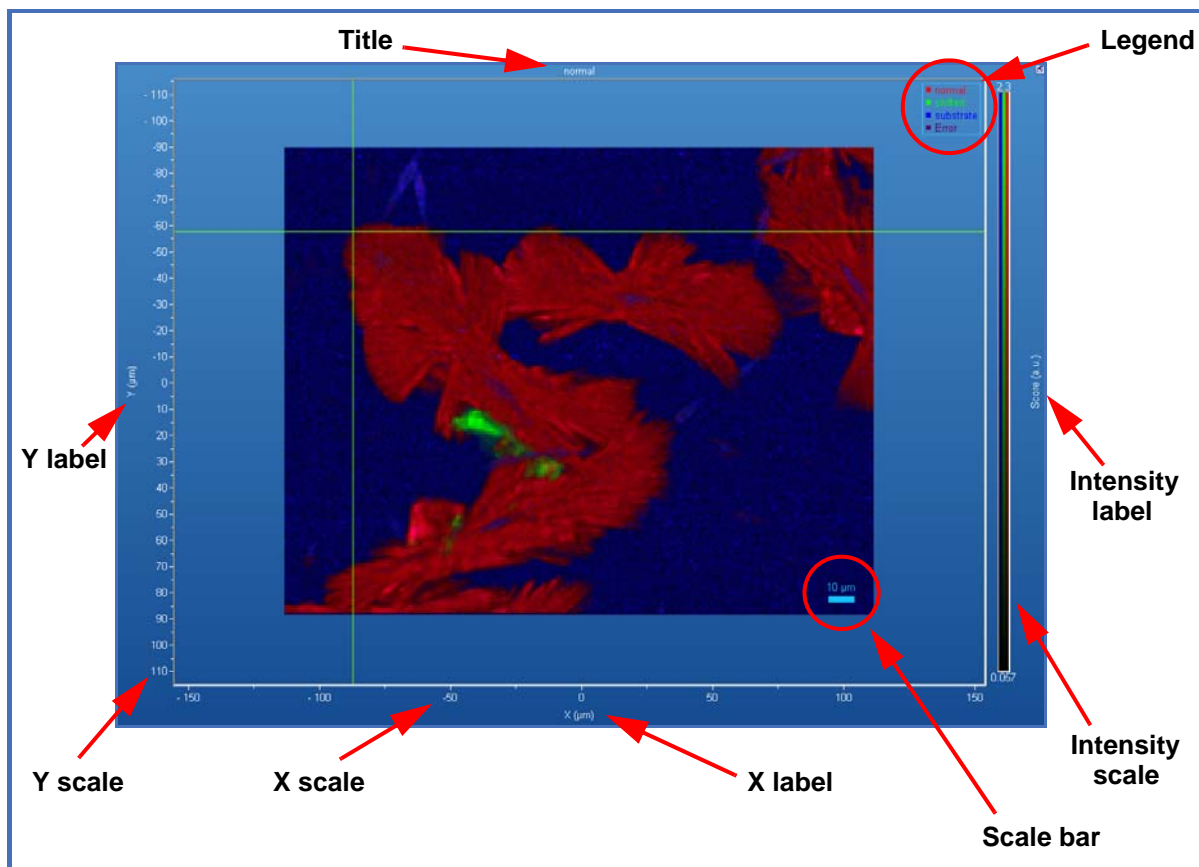
### Style

The Style/Options parameters allows the user to customize data presentation.

	Show	Tick	Name	Custom
X	Show X axis scale and label	Show axis tick marks	Show X Label	Enter the new X label name (replace the default)
Y	Show Y axis scale and label	Show axis tick marks	Show Y Label	Enter the new Y label name (replace the default)
Z	Show Z axis scale and label	Show axis tick marks	show Z Label	Enter the new Z label name (replace the default)
I	Show intensity scale and label	Show intensity axis tick marks	Show intensity Label	Enter the new intensity label name (replace the default)

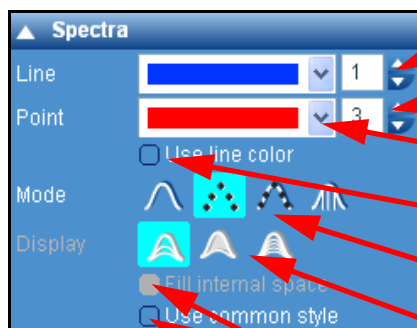
### Options

<b>Grid X</b>	X Grid On/Off (not applicable for 2D and 3D display)	
<b>Grid Y</b>	Y Grid On/Off (not applicable for 2D and 3D display)	
<b>Legend</b>	Displays the legend, with a choice of a fixed «framed» legend in the top right corner, or a stacked legend (to match stacked data) in left, center or right positions. Click on the Legend text to select the format and position.	
<b>Title + Type</b>	Displays the data title and the type above the graphic presentation	
<b>Preserve X/Y</b>	Maintain the X/Y ratio of images (i.e., maintain aspect ratio). (not applicable for 1D display).	
<b>Scale bar</b>	The scale unit indicator can be turned on and off; click on the Scale bar text to select in which corner the bar will be displayed.	
<b>3D frame</b>	Use a 3D style frame around each window.	
<b>Log scale</b>	Switch the selected window intensity scale from linear to logarithmic.	
<b>Use common style and options</b>	If this box is ticked, then each data window type (1D - spectra; 2D - images; 3D - images) will use a common style that you select. For example, if you turn on the «Title» in the Spectra tab, the «Title» will also be displayed on all other spectra windows (such as a map «All Spectra» or «Cursor Spectra» windows).	



## > Spectra

This parameters section allows the user to change the graphical presentation mode of a selected spectra window.



- Type in the line thickness: only active if the Line or Line Point presentation is chosen
- Enter the point thickness: only active if the Point or Line Point presentation is chosen
- Point color: when Point or LinePoint presentation is selected (see below), it becomes possible to apply a new color to these points
- Use line color: if ticked, the point color will be matched to the chosen line color. The point color palette (above) will become inactive
- Mode: select the spectrum display mode from Line, Point, Line Point, and Histogram.
- Display: select the display mode for the «All Spectra» window of spectral arrays. Partial will display a small percentage of all spectra in the array. Limits will display only the minimum and maximum intensity values, and All will display all spectra in the array.
- Tick to fill the internal space of the selected spectrum (a).
- Tick «Use common style» so that all spectra are displayed in the same style, as selected in the Spectra section. If this option is unticked, each spectrum can be individually formatted.

> Images 2D & 3D

The Images 2D & 3D section gives control over image display, including color palettes, smoothing, brightness/contrast, and histograms. The Images 2D & 3D section gives control over image display, including color palettes, smoothing, brightness/contrast, and histograms.

**Choose the appropriate color palette. The True Colors palette displays each image component in its own single color (e.g., an image created with the «Red» cursor will be displayed red). All other palettes in this section are false color palettes, where different colors correspond to different intensities. These palettes should only be used when a single image component is viewed on its own.**

**Mixing color mode; with Mixed mode overlapping colors in a single pixel are displayed as a combined color (for example, overlapping red and green will be displayed as orange/yellow). In Unmixed mode, only the dominant color is displayed (for example, if a pixel has 55% red, and 45% green, it would be displayed in the image as being only red).**

**Smoothing effects: 4 modes are available: no effect, horizontal, vertical and complete smoothing**

**Smoothing slider: moving the slider will change the degree of image smoothing.**

**Area**

	Area %	Area ( $\mu\text{m}^2$ )
<span style="color: red;">■</span>	15.7	326.082
<span style="color: green;">■</span>	4.1	84.4783
<span style="color: blue;">■</span>	80.3	1668.28

When the Unmixed color mode is chosen, the Area section shows the area % and area ( $\mu\text{m}^2$ ) for each color component in the image.

**Brightness/Contrast adjustments**

**RGB selection for Brightness/Contrast adjustment of individual R, G and B components.**

**Reset adjustments**

**Hue/Lightness/Saturation adjustments**

**The image histogram displays a chart of number of pixels (Y axis) and intensity value (X axis) for the selected image.**

**If the «Limits» box is ticked, the image will only be displayed with pixels within the limits range. The limits range can be selected manually or using the cursors in the histogram window.**

**«Bins» is the color pallet limit.**

**«Mean» indicates the average level of the selection with the chosen Bins.**

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The 3D section groups specific parameters relating to 3D display of 2D images. Selections in this section will only take effect if the image is displayed in 3D mode.



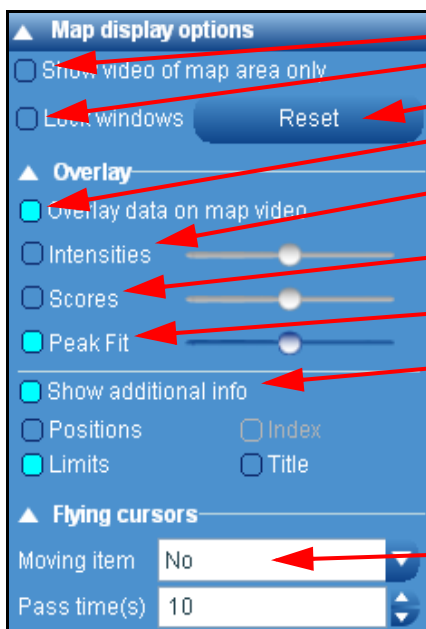
Tick the box to activate the related graphics parameter.

Select the image display mode.

The «Reset axes» parameter resets the image display to the default 3D presentation.

## > Map display options

The Map display options section allows control of the map windows, and how map data is overlaid onto the video window.



Tick the box to display the map area of the video

Tick the box to lock the map window layout in the GUI

If unlocked, reset the default window size and layout

Once ticked, the overlay feature is activated; then choose the data to overlay.

Tick the box to overlay the intensities results on the video. Moving the slider will change the overlay level.

Tick the box to overlay the scores data. Moving the slider will change the overlay level.

Tick the box to overlay the Peak fit data. The slider adjust the overlay level.

Additional information can be added by ticking the following boxes:

**Positions:** displays the data points on the map

**Limits:** displays the limit frame of the data points

**Index:** displays the data points coordinates

**Title:** display the title

This feature sweeps the selected cursor across the full spectrum/image range. Select the desired cursor (including map characterization R,G,B spectrum cursors, and image cursors), and set the pass time, which is the time it will take for the cursor to make a full single sweep of the range. The cursor movement will continue until "Moving item" is set to "No".

## > Color & Text

The **Color & Text** section contains parameters which change the visual display of LabSpec6: each window can be graphically configured by color, background, legend and text size. You can apply these parameters by choosing first a window (graphic, spectrum or image).

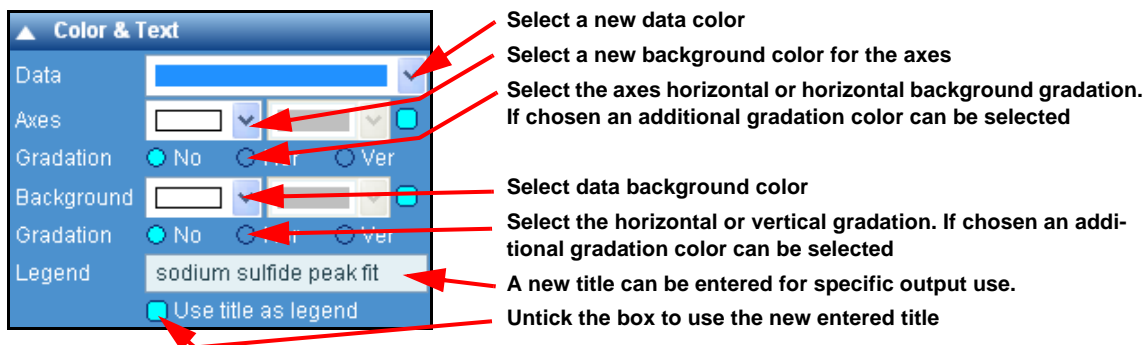
### • Changing the Legend and Color

By default, the data file name (title) will be used as the Legend. Untick the «Use title as legend» box, and type in the desired legend text.

By default, the title displayed for a spectrum or a map is retrieved from the **Acquisition > Tags and Auto Save** section you have completed. If several acquisitions have been performed during the same session, the default Title name is followed by an incremented number. These data file name will can never be changed. As these name appear on the top of the

graphical presentations, it is useful to change the title which will appear on a printed output. To use customized Legend text, just click on a graphic area and type in the new title and validate it by unticking the «Use title as legend» box., untick the «Use title as legend» box, and type in the desired legend text.

In some situations, it could be useful to change the color, for example, of a yellow spectrum. Just click on the graphic area or the one of the color circle of the data clipboard and choose the desired color.



## > LabSpec options

This section includes general setup or options necessary for the optimal use of the software.

**LabSpec options**

**Fonts**

GUI	MS Sans Serif	12
Data	Arial	10

**Languages**

English     Français  
 Deutsch     日本語  
 中文     Русский

**Modules**

User accounts	<input type="checkbox"/>
* MultiWell	<input type="checkbox"/>
* 3D	<input type="checkbox"/>
Solo+MIA data link	<input type="checkbox"/>
FTIR	<input type="checkbox"/>
KnowItAll data link	<input type="checkbox"/>
* Licenser	<input type="checkbox"/>
* Multivariate	<input checked="" type="checkbox"/>
* ParticleFinder	<input type="checkbox"/>
PL	<input type="checkbox"/>
Spectral ID data link	<input type="checkbox"/>
* Licensed module	<input type="checkbox"/>

**Options**

Buttons: Big

- Treatment preview (mouse +CTRL)
- Display preview (mouse +CTRL)
- Apply preview scale
- Enable legend quick formatting
- Enable Undo for display settings
- Use not a number (NaN) in txt files

**Annotations:**

- According to your graphics display, select the font and font sizes for the Graphics user interface (tab and section labels/text) or the data labels (axis labels and units)
- Select the interface language
- The modules section shows optional modules for LabSpec6. Note that many of these are commercial modules, and a license will need to be purchased before they can be used. Commercial modules requiring licensing are indicated with a \*
- According to your graphics display, select the best Tool bar buttons size.
- Treatment preview allows treatment functions to be previewed by pressing CTRL key and hovering the mouse over a button. As soon as the mouse moves away from the button, the preview is stopped.
- Display preview allows display functions to be previewed by pressing CTRL key and hovering the mouse over a button. As soon as the mouse moves away from the button, the preview is stopped.
- Tick this box to rescale data display to match the treatment/display preview.
- Tick the box to enable quick legend formatting - when active left click on the legend to set spectrum color.
- Tick this box to enable «Undo» (Ctrl-Z) for display settings
- Tick this box to use «NaN» identifiers in text files when there is no data present.

## 6.7 Info Tab



### > Parameters

The **Parameters** table displays the acquisition settings of the System, custom information and a history of data manipulations. With this information, the user can verify exactly what condi-



tions were used for a measurement, and if any data processing has been applied to the data since it was acquired.

All of this information is saved with the data file, and therefore can be recalled and viewed at any point, on any PC.

**Acq parameters:** the acquisition parameters and System configuration are displayed when a data object is selected.

**Custom parameters:** These parameters are those entered from the Acquisition>Tags and Auto Save just before an acquisition. They can also be edited directly from this window.

**History parameters:** Each time a data manipulation/processing is performed, LS6 records the type of processing with a date/time stamp.

**Tick the box to display the column with the applied parameters.**

Name	Date	Time
Base	18.05.2011	
Filter:Polynom	Size=19,Dε	09.01.2014
Math:XY	Formula=Y	09.01.2014
Base:Fit	Degree=1,I	09.01.2014

## > Data range

The Data range section displays complete information about the range of a selected data object. It can be used to modify the data ranges (for example, increase or decrease number of data points, or extract a portion from the original data object).

Parameters displayed include:

The **data range section** displays information for the following key parameters:

- X - spatial axis (X) for spectral arrays
- Y - spatial axis (Y) for spectral arrays
- Z - spatial axis (Z) for spectral arrays
- T - temperature axis for spectral arrays
- t - time axis for spectral arrays
- S - spectral axis (nm/eV/abs.cm-1) for spectra
- I - image intensity

▲ Data range				
	Size	Step	From	To
Y	48	0.9815	4.9036	52.017
X	43	0.9804	-27.570	14.5911
S	49	1.1126	1022.6	968.11
I			-8.5917	305

Note that for spectra the Step box shows the average data step through the spectrum. A typical Raman spectrum will have uneven data spacing (i.e., the data spacing varies at different spectral positions).

## 6.8 Methods Tab




### > Working with Methods

The LabSpec6 Methods function provides a powerful tool for customized and/or repetitive data acquisition and processing functions. It allows the user to record a sequence of processes within LabSpec6, and then replay the sequence as and when desired. A «process» means acquiring a spectrum, spectral array (map) or video, or manipulating the data (for example, smoothing, baselining, normalizing etc). If the system includes a motorized XY or XYZ stage, the sequence can include an XYZ positioning to acquire spectra on specific points of a sample. Then, specific acquisitions and processes using a custom mapping can be programmed.

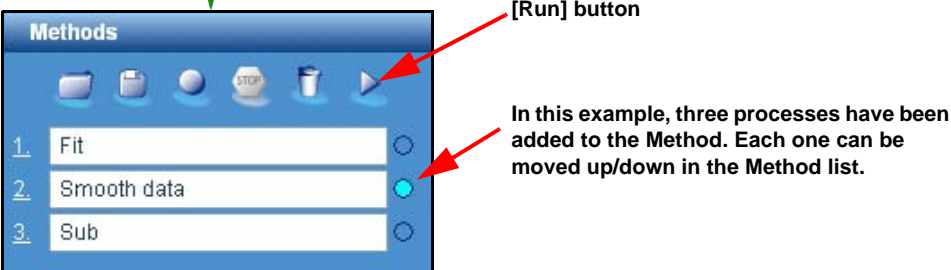
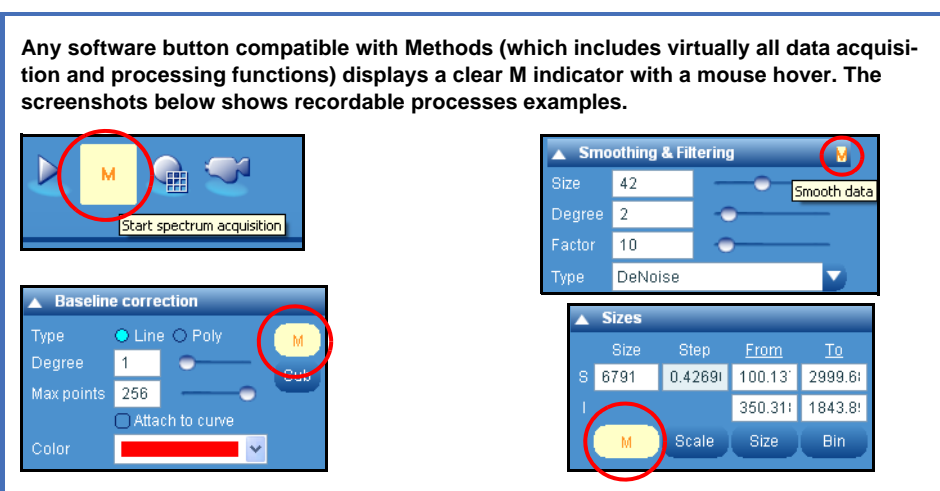
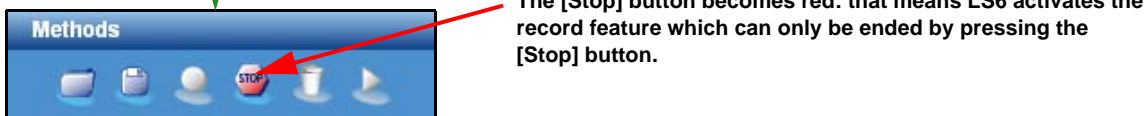
#### Recording a Method

Follow the steps shown below to record and create a Method.

The general principle is to start recording, and then click on any data acquisition or data processing button. Any software button compatible with Methods (which includes virtually all data acquisition and processing functions) displays a clear **M** indicator with a mouse hover. When a button is clicked, its function will be added to the method. Please note that during Method recording, clicking on a process button will not start that process. You will not be able to acquire or process data until recording is stopped.

Repeat these steps until the Method is complete, and all necessary processes have been added to it. Once done, stop the Method recording by clicking the  icon.

The processes in the Method can now be automatically run, in sequence, by running the method.



### Recording a method with XYZ positioning

Methods can be recorded with full XYZ positioning, allowing data acquisition (spectra, maps, videos) to be made at specific positions across a sample. The following procedure should be used.

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- Run video
- Start Methods recording
- Add data acquisition to method. Title shows XYZ positions
- Move sample
- Add data acquisition to method
- Continue adding points
- Stop recording Method

When the Method is run, data acquisition will be done at the specified XYZ positions.

**CAUTION:** the XYZ coordinate positions included for the Method correspond to those shown in the Acquisition > XYZ Stage section. If the stage position is initialized (reset to zero), the Method positions will no longer be valid.

**CAUTION:** at the end of Method recording, the XYZ stage will return to its original position (i.e., the sample position prior to recording the method).

### How to edit a method

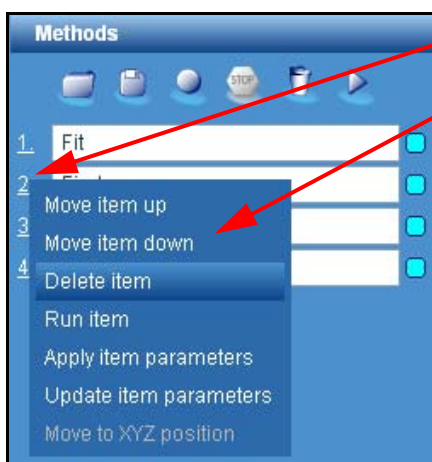
Once created, the Method can be edited and rearranged. Methods can also be saved and loaded for a later use.

## > Editing and rearranging a Method

When a Method has been created, left-click on the underlined number index of each process in the Method to perform an action. The selected item can be moved up/down in the sequence, deleted from the method, individually run or applied/updated to/from the current configuration.

The **Apply item parameters** will update the Acquisition tab in the Control Panel to reflect the experiment conditions stored for Method process. This is a useful way to review the experiment parameters which will be used during the Method.

**Update item parameters** will store the acquisition parameters currently displayed in the Acquisition tab of the Control Panel to the selected process. The type of process (acquire spectrum, acquire map, acquire video etc) cannot be changed.



Left-click on the process number

and select the action to apply:

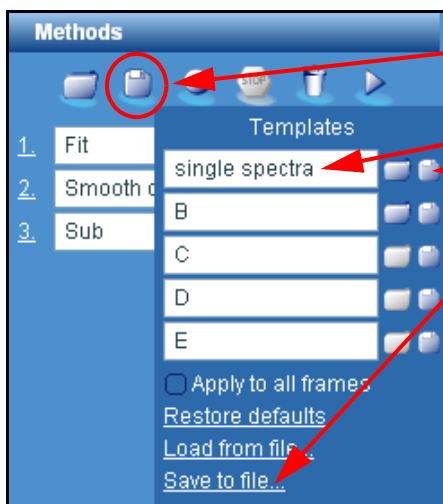
- Up/Down: move the preset through the list
- Delete: delete the item
- Run: run the item
- Apply item parameters to the current Control Panel settings
- Update item parameters from the current Control Panel settings
- Move to XYZ position: move to the XYZ position stored with the process.

«Use current parameters» tick box located at bottom of Method tab:

When ticked, the Method will use the parameters currently set in the Control Panel tabs, in place of the parameters individually stored for each process. This can be useful to make repeated multi-position (XYZ position) acquisitions with varying experiment parameters.

### > Saving a Method

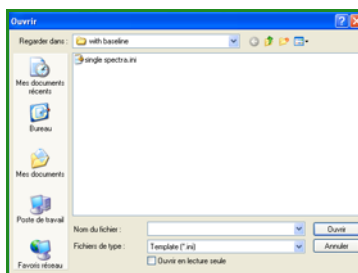
Each method can be saved, using the standard Template window.



Click on the [Save] button to open the options.

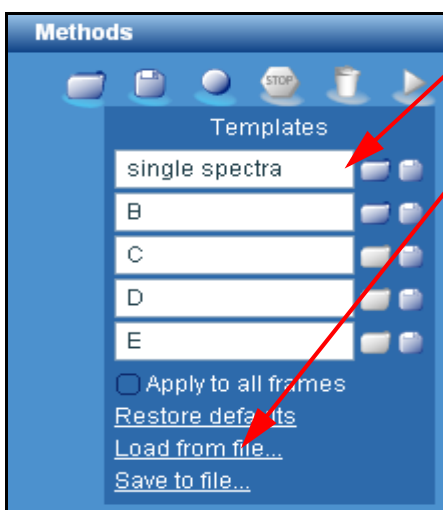
**Quick save:**  
Enter a template name  
Click on «Save» icon

**Save to file:**  
Click on the «Save to file» link to open the standard File save dialog. Select the folder and save the Method.



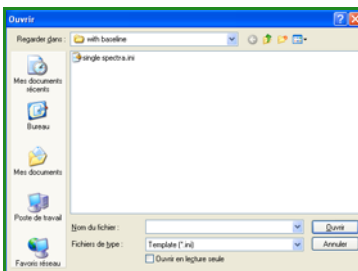
### > Loading a Method

A saved method can be re-loaded using the standard template load window.



**Quick load:**  
Click on the «Load» icon

**Loading a file:**  
Click on the «Load from file» link to open the standard File open dialog window. Select the folder then click on the file to load.



### > Clear Method



Click on the **Clear Method** icon to remove the method, and clear the list of processes.

## > Instant Processing

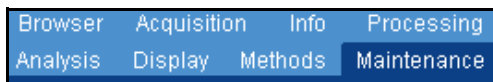
When a process has been created using the Method feature, it can be saved by entering a name in the «New method» area, then clicking on the green arrow. The name immediately appears in the «Instant processing» section in the Acquisition tab (see “Instant processing” on page 41)



Validate a created method to a preset «Instant processing»

Enter a section name which will be displayed in the «Instant processing» section

## 6.9 Maintenance Tab



The Maintenance tab includes functions which are used for daily maintenance and checking of the instrument.

### > Instrument Calibration

The Instrument calibration section allows the spectrometer to be manually calibrated (to ensure that the recorded peak positions in a spectrum are correct). This section is only available to Administrator and Expert users.

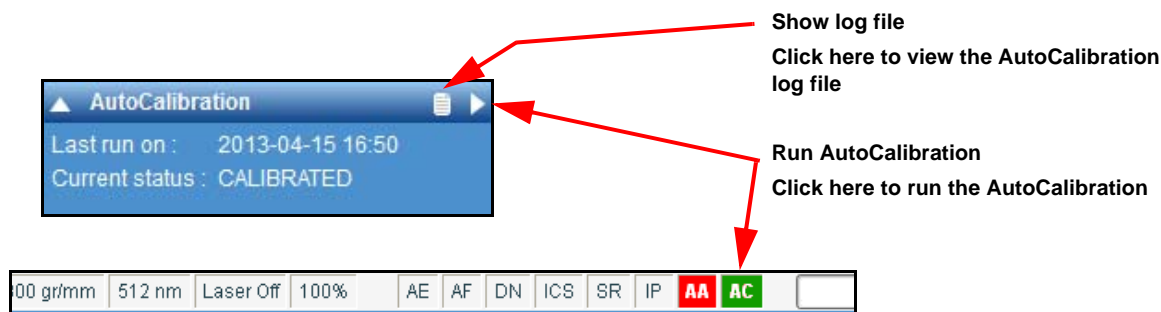


**WARNING:** misuse of this section can result in incorrect results and/or system failure. Modification of these parameters should only be carried out by trained users.

### > AutoCalibration

The AutoCalibration module allows easy automated calibration of the spectrometer. It displays the date when the AutoCalibration was last run, and the current status.

The AutoCalibration routine can be started by clicking on the "Run AutoCalibration" button in the AutoCalibration section; alternatively, it can be started by clicking on the «AC» AutoCalibration indicator button in the Status Bar.



The AutoCalibration log file displays the results from previous AutoCalibration procedures, including the date and time when it was run, the grating/laser which was calibrated, the previous and new calibration values, and the status (PASS/FAIL).

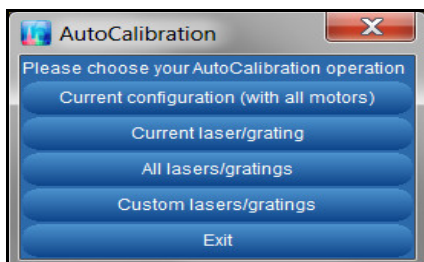
Date_Time	Operator	Methods	Grating	Laser	Peak_Pos(tolerance)	Peak_Intens	New_Offset	Old_Offset	New_Coeff	Old_Coeff	New_Laser_Int	Old_Laser_Int	Status
2013-04-15_16:50:35		AutoCalibration	1800 g/mm	532 nm	136.77 nm (± 0.18 nm - 0.21 nm)	134 units	NA	NA	0.0040208	0.0040212	NA	NA	PASS
2013-04-15_16:36:18		AutoCalibration	1800 g/mm	532 nm	-0.01 nm (± 0.04 - 0.04)	1374 units	-4	0	NA	NA	NA	NA	PASS

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## Choosing the AutoCalibration Mode

There are four modes of operation for AutoCalibration, depending on the specific system and configuration. When AutoCalibration is started you will be prompted to choose the AutoCalibration mode that is to be used.

Note that depending on the system configuration you may not see all four modes available.



### **«Current configuration (with all motors)» AutoCalibration**

This mode will use the exact system configuration and all acquisition parameters set in the Acquisition tab of the Control Panel. Only the acquisition time, number of accumulations and calibration position will be set automatically by the AutoCalibration module. This mode is only available on systems with a large configuration requiring a highly flexible calibration routine.

### **«Current laser/grating» AutoCalibration**

This mode will calibrate just the currently selected laser and grating combination. It is ideally suited for very fast calibration of the current configuration. Note that in this case other lasers and gratings will not be calibrated, so if you switch laser or grating it will be necessary to run a new calibration.

### **«All lasers/gratings» AutoCalibration**

This mode will calibrate the full system, including all lasers and gratings; it is only suitable for fully automated systems. After full calibration it is possible to freely switch to any laser or grating combination and obtain well calibrated data.

### **«Custom lasers/gratings» AutoCalibration**

This mode will calibrate a custom configuration of lasers and gratings. You will be prompted to select which lasers and gratings will be calibrated. Note that in this case other lasers and gratings will not be calibrated, so if you switch to an uncalibrated laser or grating it will be necessary to run a new calibration.

## AutoCalibration Status

Status indicators are used to display the status of the current laser/grating combination. When a laser or grating is switched, the status indicator will automatically update.

The following status indicators are used for AutoCalibration:

### **CALIBRATED**

The current laser/grating is calibrated, and can be used to acquire calibrated data.

### **NOT CALIBRATED**

The current laser/grating is not calibrated and should not be used to acquire calibrated data.



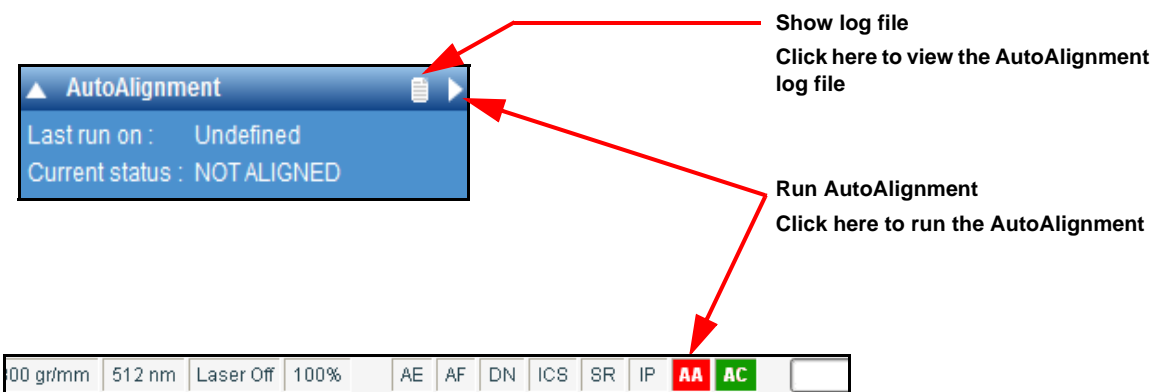
**EXPIRED**

The last run calibration has now expired. The expiry time is set in the configuration of the AutoCalibration. The default setting is 12 hours, but this can be modified by a system Administrator.

> **AutoAlignment**

The AutoAlignment module allows easy automated optical alignment of the spectrometer. It displays the date when the AutoAlignment was last run, and the current status.

The AutoAlignment routine can be started by clicking on the “Run AutoAlignment” button in the AutoAlignment section; alternatively, it can be started by clicking on the «AA» AutoAlignment indicator button in the Status Bar.



The AutoCalibration log file displays the results from previous AutoCalibration procedures, including the date and time when it was run, the grating/laser which was calibrated, the previous and new calibration values, and the status (PASS/FAIL).

Date_Time	Laser	Result
2013-01-04_16:40:15	633nm	Current alignment good. AutoAlignment not required.
2013-01-04_16:40:15	633nm	AutoAlignment complete. System aligned.
2013-01-04_16:40:15	633nm	High-precision AutoAlignment complete. System aligned.
2013-01-04_16:40:15	633nm	System not aligned. Please retry.
2013-01-04_16:35:03	633nm	AutoAlignment canceled. System not aligned.
2013-01-04_16:34:02	633nm	AutoAlignment canceled. System not aligned.
2013-01-04_16:32:21	633nm	AutoAlignment canceled. System not aligned.
2013-01-04_16:28:16	633nm	AutoAlignment canceled. System not aligned.
2013-01-04_16:27:19	633nm	AutoAlignment canceled. System not aligned.

**AutoAlignment Status**

Status indicators are used to display the status of the current alignment.

The follow status indicators are used for AutoAlignment:

**ALIGNED**

The current laser is aligned.

**NOT ALIGNED**

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The current laser is not aligned; although it can be used, care should be taken since signal levels may be very low due to the poor alignment. If in doubt please speak to a system Administrator.

**EXPIRED**

The last run alignment has now expired. The expiry time is set in the configuration of the AutoCalibration, and can be modified by a system Administrator.

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