SPECTRUM

User's Guide

PerkinElmer®

IR Microscopes

Spectrum software allows you to control two different types of infrared microscope that, depending on your system, can be connected to your spectrometer:

The Spotlight 150 microscope, which has a manual stage and allows scanning at a single point only; and

The Spotlight 200 microscope, which has an automated stage and can record images you can use to select points, lines or areas at which to collect spectra.

Click a link to find more information about setting up each of these microscopes.

Setup Microscope tab (Spotlight 150)

NOTE: The Setup Microscope tab is only available when the instrument configuration file includes a Spotlight 150. To use the controls on the tab, the beam path must be directed to the microscope on the Setup Instrument BeamPath tab. The Microscope icon  will then be displayed in the Accessory bar.

Use the Setup Instrument Microscope tab to set up the microscope apertures, the microscope illumination and the cassegrain correction.

1. Select **Instrument** from the Setup menu.

   OR

   Select  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

   NOTE: To see the tabs, you may have to resize the Dialog Pane.

2. Select the Setup Microscope tab.

   The first time you access the Setup Microscope tab within a Spectrum work session, you will be prompted to start the microscope initialization and aperture calibration procedure. During the initialization, a message will be displayed in the Status bar. You can choose not to initialize the microscope at this time, but you will not be able to use the microscope controls until you do so. You can also choose to **Skip** the initialization, which sets up the microscope using the settings from your previous session. You can initialize your microscope at any time in your Spectrum session – see Initialize.

On the left of the Setup Microscope tab the live image from the video camera is displayed. On the right of the Setup Microscope tab the microscope parameters that can be controlled by Spectrum software are displayed. The controls on the Setup Microscope tab can be expanded, or collapsed, independently. When collapsed, the current settings of each parameter are displayed in brackets beside the control name.

Live Image

Displayed on the live image are cross hairs showing the center point of the image. Also marked are the aperture dimensions, superimposed on the image. The aperture can be changed using the mouse controls. See Apertures.

The coordinates of the center of the image are 0, 0. Horizontal and vertical scales give the dimensions of the image.

The live image resizes with the Setup Microscope tab, but the aspect ratio is maintained.

Initialize

If you chose not to initialize your microscope upon selecting the Setup Microscope tab for the first time in your current Spectrum session, the Initialize button will be available.

- Click **Initialize** to start the microscope initialization and aperture calibration procedure.

  You will be prompted to remove any sample from the stage, to ensure that any ATR objective fitted is retracted and to ensure that the lower cassegrain is fitted.

When the microscope has been initialized, the button becomes **Reset Image**.

Reset Image

Reset Image is only available when the microscope has been initialized.

- Click **Reset Image** to restore the illumination, brightness and contrast to the default values.

Sampling Mode

The Spotlight 150 can be used in Transmittance, Reflectance or ATR mode.

- Select **Reflectance**, **Transmittance** or **ATR** in the Sampling mode section.

  The Spotlight 150 changes to the mode you have chosen. During the sampling mode change, a message will be displayed in the Status bar.

  The default is Transmittance.

  You can also switch between Transmittance and Reflectance modes using the button on the accessory bar.

Illumination

The **Illumination** control enables you to change the amount of light illuminating the sample on the sample stage.

To change the illumination:

- Move the slider on the Illumination slider bar left or right.

  Moving the slider to the left decreases the illumination and moving it to the right increases the illumination. The percentage (**%**) illumination is updated. The default is 20%.

The **Auto** button sets the illumination to an appropriate level for your sample.

Image Quality

The **Brightness** control enables you to adjust the brightness of the camera.

- Move the slider on the **Brightness** slider bar left or right.

  Moving the slider to the left decreases the brightness and moving it to the right increases the brightness. The default is 128. The available range is 0–255.

The **Contrast** control enables you to adjust the contrast of the camera.

- Move the slider on the **Contrast** slider bar left or right.

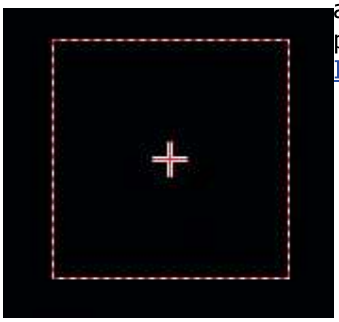
  Moving the slider to the left decreases the contrast and moving it to the right increases the contrast. The default is 32. The available range is 0–64.

The Brightness and Contrast controls are useful when measuring highly absorbing materials (for example, black rubber), where changing the Illumination alone does not provide sufficient control.

As you increase the brightness, the quality of the image decreases. We therefore recommend that before you increase the brightness, you maximize the illumination of the sample using the Illumination control. As you increase the brightness, the contrast of the image decreases; this means that you will probably need to increase the contrast.

When you have finished viewing a very dark or light image, we recommend that you reset the brightness and contrast to their default values using Reset Image.

Apertures



You can change the size and shape of the infrared aperture. The box, which is displayed at the current position, represents the current aperture (see Setting the Infrared Aperture).

The default aperture dimensions are 100 microns by 100 microns with a 0° angle of rotation.

Setting the Infrared Aperture

1. Move the sample stage so that the cross hairs are on the feature you want to set the aperture around.
2. Using the mouse, set the aperture size:

   Move the pointer to one of the sides of the current aperture marker. The cursor changes to a two-headed arrow ⇖, ⇗, ⇔ or ⇕.

   The aperture shape can be sized in the same way as a grow box. Move the edges of the aperture shape so that it fits around the feature – it is best to make the aperture slightly smaller than the feature in order to correct for diffraction of the infrared beam. The numbers in the Apertures section of the Setup Microscope tab are updated as you drag the edges.

   The minimum width and height values are 3 microns and the available range is 3–800 microns.

NOTE: The width and height values are for the full aperture size. The scale displays the dimensions from either side of the 0,0 position. Therefore a 100 micron aperture size will display from +50 to -50 on the axes.

3. Using the mouse, set the aperture rotation:

   The pointer changes into a curved, two-headed arrow when the pointer is moved to the corner of the current aperture marker. The angle can be 0–360°.

   Hold down the left-hand mouse button and move the mouse in a circle.

   The current position marker rotates, and the Angle is updated as you drag the marker.

4. Click **Test** to move the aperture blades to the size and orientation you have set.

   This allows you to check that the settings are correct before collecting data. While the apertures are moving a message will be displayed in the Status bar.

5. Click **Park** to move the blades back to the open position.

NOTE: When you click **Scan** to start data collection, if the apertures are in the open position (Park), they will close to the dimensions set on the Setup Microscope tab for the duration of the scan, and then return to the open position. If the apertures are in the closed position (Test) when a scan starts, they will remain closed.

You can also modify the **Width**, **Height** and **Angle** of the aperture by typing the numbers into the Apertures section of the Setup Microscope tab.

For more information, see Size and Shape of the Infrared Aperture.

Calibrate

- Click **Calibrate** to start the aperture alignment procedure and calibrate the automatic apertures.

  You will be prompted to remove any sample from the sample stage, and to ensure that the lower cassegrain is fitted before continuing. Click **OK**.

  During the calibration, a message will be displayed in the Status bar. The controls on the Setup Microscope tab are disabled until the calibration is completed.

We recommend that the calibration is done regularly for optimal performance of the instrument.

Validation Position

- Click **Validation Position** to move the apertures to the position required to collect reference spectra for Ready Checks or Instrument Verification.

Size and Shape of the Infrared Aperture

The size and shape of the infrared aperture that you use depends on the size and shape of the features from which you want to collect spectra. If you are scanning at individual, isolated points in a sample, you can use an aperture that is similar in size and shape to the smallest feature of interest. If the features you are interested in have different sizes and shapes, resize the aperture for each feature: the variable aperture can be made to fit different size squares or rectangles.

If you change the size of the aperture, the current background spectrum is invalidated and a new background spectrum must be collected before you can scan samples.

Maximum Aperture Size

In general, the larger the aperture setting, the better the spectral quality. The maximum aperture size setting is typically larger than the detector size. However, setting the aperture size to values higher than the detector size may not produce a proportionally larger signal – in fact, it may overload the detector. For example, this can happen at aperture sizes above 100 microns for MCT detector systems or 200 microns for DTGS detector systems.

Correction

When you are using the Spotlight 150 in Transmittance mode, the infrared beam passes through the lower cassegrain. The buttons in the Correction section of the Setup Microscope tab move the lower cassegrain up and down so that you can compensate for sample thickness and thus maximize the energy reaching the detector.

NOTE:	Correction is only required when the Spotlight 150 is in Transmittance mode.

The cassegrain correction controls enable you to move the lower cassegrain up or down, and so correct for the refractive index of the sample. When you are using the Spotlight 150 in Transmittance mode, you can use this control to adjust the energy reaching the detector.

The up  and down  arrows move the lower cassegrain under the sample stage up or down, respectively. The default correction is 0. The correction values are in microns.

Zero moves the lower cassegrain to the point of maximum energy for an open beam. This is suitable for very thin samples. For thicker samples, you may need to increase the correction.

Park moves the lower cassegrain to its lowest position. If the lower cassegrain has been removed, the lower cassegrain bracket can then be moved down to a lower position by pressing **Lower Park**. The sample stage can then be moved down to accommodate very thick samples. See Collecting Spectra from Very Thick Samples.

While the lower cassegrain is moving the Zero, Park or Lower Park positions, a message will be displayed in the Status bar.

You can use Monitor to see the energy reaching the detector while adjusting the cassegrain controls. See Maximizing the Energy.

Save Image

Save Image allows you to save the live image at any time, in Bitmap (*.bmp) or Portable Network Graphic (*.png) format. The scales will be saved with the image. You can browse to select a file location.

If you select **Save apertures on image**, the cross hairs and the aperture box will be overlaid on the image.

Stage Lighting

If your system is equipped with the stage lighting assembly, then **Stage Lighting** will be available. Use the button to turn the lighting on or off. You can also use the button on the accessory bar.

ATR

If your system is equipped with an automated ATR objective, then the ATR option is available for lowering and raising the crystal. You can also use the button on the accessory bar. When you attempt to lower the crystal, a message will be displayed reminding you to lower the stage to a safe distance to prevent a collision when the crystal is lowered.

Additional Information

For information about the configuration of the microscope in Transmittance or Reflectance modes, see *A Guided Tour of the Spotlight 150* in your *Spotlight 150 User's Guide*.

For information on collecting backgrounds with a Spotlight 150, see Collecting Backgrounds with the Spotlight 150.

For information on collecting sample spectra with the Spotlight 150, see Scanning a Sample with the Spotlight 150.

Maximizing the Energy

When you are using the Spotlight 150 in transmittance mode, the infrared beam passes through the lower cassegrain. The buttons in the Correction section of the Setup Microscope tab move the lower cassegrain of the Spotlight 150 up and down so that you can maximize the energy reaching the detector before scanning your sample.

NOTE: If you are working with the Spotlight 150 in reflectance mode, the infrared focus is the same as the visible focus, so provided that the visible image is focused correctly using the Z-control on the microscope, you do not need to maximize the energy.

1. Adjust the visible focus using the Z-axis control on the microscope.
2. Select **Monitor** from the Measurement menu, or click  .

   The Live tab is displayed in the Viewing area for the current Samples View. The apertures are moved into the positions set in the Aperture section of the Setup Microscope tab.

3. Select **Energy** to monitor the level of infrared energy reaching the detector in your instrument or accessory.

   The Monitor Energy dialog is displayed.

4. Use the buttons in the **Correction** section of the Setup Microscope tab to focus the infrared beam onto the sample.

5. When you have maximized the energy reaching the detector, click Halt to exit the Monitor mode.

Collecting Spectra from Very Thick Samples

Removing the lower cassegrain enables you to study very thick samples in reflectance mode.

The **Park** button in the **Correction** section of the Control dialog moves the lower cassegrain down to its lowest position; if the lower cassegrain is removed the sample stage can then be moved down to accommodate very thick samples.

1. Click **Zero**.

2. Remove the lower cassegrain.

   For information on how to do this, see Collecting the Spectrum of a Thick Sample in your Spotlight 150 User's Guide.

3. Click **Park**.

   The lower cassegrain bracket moves to the Park position. The button text then changes to **Lower Park**.

4. Click **Lower Park**.

   You will be prompted to confirm that the lower cassegrain has been removed from the bracket before continuing.

5. Click **OK**.

   The lower cassegrain bracket moves to its lowest position. The sample stage now can be moved down. The text on the button returns to **Park**.

NOTE: From the Lower Park position, you can only use the **Park** or **Zero** buttons to move the lower cassegrain bracket up, and not the up arrow. Before moving the lower cassegrain bracket to either position you must ensure that the sample stage is centered, and move the sample stage up.

Setup Microscope Basic tab (Spotlight 200)

NOTE: The Setup Microscope Basic tab is only available when the instrument configuration file includes a Spotlight 200. To use the controls on the tab, the beam path must be directed to the microscope on the Setup Instrument BeamPath tab. The Microscope icon  will then be displayed in the Accessory bar.

The first time you connect to your Spotlight 200 microscope within a Spectrum work session, you will be prompted to start the microscope initialization and aperture calibration procedure. During the initialization, a message will be displayed in the Status bar. You can choose not to initialize the microscope at this time, but you will not be able to use the microscope controls. You can also choose to **Skip** the initialization, which sets up the microscope using the settings from the previous session.

You can initialize your microscope at any time during your Spectrum session.

To initialize your microscope:

- Click the  button on the Accessory bar.
  OR
  Select **Initialize Microscope** in the Microscope menu.
  You will be prompted to remove any sample from the stage, to ensure that the ATR objective crystal (if fitted) is retracted and to ensure that the lower cassegrain is fitted. Click **Start** when ready.

When the microscope has been initialized, the Setup Microscope tabs are activated.

NOTE: To see the tabs, you may have to resize the Dialog Pane.

Use the Setup Microscope Basic tab to set up the microscope sampling mode, illumination, stage control and automated ATR objective (if fitted).

Sampling Mode

- Select whether to analyze your sample in **Transmittance**, **Reflectance** or **ATR** mode.
  Transmittance mode directs light through the sample from below the stage.
  Reflectance mode directs light on to the sample from above and collects the light that is reflected from the sample's surface.
  ATR mode is designed for use with an ATR objective. The microscope operates in reflectance, but the stage moves downwards before moving horizontally if the ATR crystal is lowered, to protect the crystal from striking the sample. It also restricts the Z-axis adjustment increments that are available, to prevent sudden stage movements from damaging the sample or the ATR crystal.

NOTE: If you select ATR mode but do not have an ATR objective attached to your microscope, Spectrum will display a message and you will need to change the sampling mode to perform a scan.

You can also change the sampling mode using the Transmittance/Reflectance button on the Accessory bar.

Illumination

- Adjust the Illumination of the sample using the slider, and observe the effect in the **Camera View** pane above.
  You can click and drag the sliders with the mouse, or click a slider and use the left and right arrow keys on the keyboard.
  OR
  Click the **Auto** button and the software will automatically set the illumination to the optimum level for your sample.

You can also click the  button in the Camera View toolbar to automatically set the illumination level.

Stage Control

Focussing

When you are focussing your sample, you have to adjust the Z-axis of the stage (move it up and down). You can do this with the joystick provided with your microscope, but for fine focusing, it is easier to use the Stage Control options.

To adjust the focus manually:

1. Twist the joystick to adjust the Z-axis position until the sample is visible in the Camera View image.
2. Select an option for the size of the **Z-Axis Adjustment** increment.
   This is the distance that the stage will move during each step of the focusing. Some of the options are not available if you are using ATR mode to prevent damage to the crystal.
3. Click the  and  buttons to move the stage by the adjustment increment until the image is focused.

   You can also use the **Adjust Up**  or **Adjust Down**  buttons on the Accessory bar.

To adjust the focus automatically:

1. Use the joystick to adjust the Z-axis position until the sample is visible in the Camera View image.
2. Click **Auto-Focus** to run the auto-focusing routine.
   OR
   Click the  button on the Camera View toolbar.

Stage Lighting

Certain Spotlight 200 microscopes have two white LEDs at the rear of the sample stage area. These illuminate the sample stage and assist with positioning samples.

To turn the LEDs on:

- Click the green **Stage Lighting** button on the Setup Instrument Basic tab.
  OR
  Click the  button on the Accessory bar.

To turn the LEDs off:

- Click the red **Stage Lighting** button on the Setup Instrument Basic tab.
  OR
  Click the  button on the Accessory bar.

NOTE:	We recommend that you turn the LEDs off before collecting a spectrum.

ATR

If you have an automated ATR objective fitted to your microscope, there will be additional settings available on this tab to set the pressure applied to your sample.

Setup Microscope Advanced

NOTE: The Setup Microscope Advanced tab is only available when the instrument configuration file includes a Spotlight 200. To use the controls on the tab, the beam path must be directed to the microscope on the Setup Instrument BeamPath tab, and the microscope must be initialized. The Microscope icon  will then be displayed in the Accessory bar.

Use the Setup Microscope Advanced tab to set up the correction, the image quality and the apertures.

Correction

The Correction control moves the lower cassegrain on the microscope up or down to correct for the refractive index of the sample. It is only used in transmittance mode. When you are viewing a sample using the camera, Correction changes the amount of visible light reaching the sample. When you are scanning a sample, it changes the amount of infrared radiation reaching the detector.

To adjust the correction manually:

- Click the ^ and v buttons to move the lower cassegrain until the image in the Camera View is evenly illuminated.
  The distance that the cassegrain has moved from its original position is shown in parentheses above the buttons. Movement downwards is shown as a negative number.

To adjust the correction automatically:

- Click the **Maximize Energy** button.
  OR
  Click the **Maximize Energy**  button on the Camera View toolbar.
  The cassegrain position is adjusted to maximize the infrared radiation reaching the detector.

There are two other buttons in the Correction section of the tab, Zero and Park.

- Click the **Zero** button to move the lower cassegrain to the point of maximum energy for an open beam (that is, with no sample present).

The Park button is used to remove the lower cassegrain. The sample stage can then be moved down to allow you to collect spectra in reflectance mode from very thick samples.

1. Click the **Park** button.
   The lower cassegrain moves to its lowest position, and the button label changes to **Lower Park**.
2. Remove the lower cassegrain.
   For information on how to do this, refer to *Collecting the Spectrum of a Thick Sample* in the *Spotlight 200 User's Guide* supplied with your microscope.
3. Click the **Lower Park** button.
   The lower cassegrain bracket moves to its lowest position and the sample stage can then be moved downwards.

NOTE: Take care not to collide with the cassegrain's connector as the stage is lowered. Refer to the *Spotlight 200 User's Guide* for further information.

Raise the stage and click the **Zero** button before you replace the lower cassegrain.

Image Quality

- Use the sliders to adjust the **Brightness** and **Contrast** of the camera.
  You can click and drag the sliders with the mouse, or click a slider and use the left and right arrow keys on the keyboard.
  The results are shown in the Camera View, and the scale values are shown in parentheses above the sliders.

NOTE: Check the Illumination of your sample after adjusting the brightness and contrast, as the optimum setting may have changed.

Apertures

Use this section of the tab to set up and calibrate the apertures that are used to define the area being scanned.

- Enter the values of **Width**, **Height** and **Rotation Angle** that you want to use for the apertures.
  The Camera View shows the area to be scanned as a red, dashed rectangle at the center of the image.

NOTE: You can choose to apply these apertures to all the markers, lines and maps set up in your image by clicking the **Apply to all** button.

You can also adjust the apertures using the Camera View:

1. To set the aperture size, move the mouse pointer to one of the sides of the aperture indicator (the red, dashed rectangle).
   The mouse pointer changes to a two-headed arrow ⇔, ⇕, ⤢ or ⤡.
2. Drag the edges of the aperture shape so that it fits around the feature you want to scan.
   It is best to make the aperture slightly smaller than the feature to correct for diffraction of the infrared beam.
3. To set the aperture rotation, move the mouse pointer to a corner of the aperture indicator.
   The mouse pointer changes to a curly arrow ↺.
4. Drag the corner of the rectangle left or right to rotate the apertures.

To visually check the aperture calibration:

1. Click the **Test** button.
   You will see the apertures move into position in the Camera View. If the apertures are correctly calibrated, then the edges of the apertures should line up with the sides of the red, dashed shape in the image.
2. To return to the full Camera View, click the **Park** button.

If the aperture edges did not line up with the red, dashed rectangle in the image, then you need to calibrate the apertures.

1. Click **Calibrate**.
   You are requested to remove any samples form the software and ensure that the lower cassegrain is fitted.

2. Click **OK.**
   The aperture calibration routine will be performed. Once this is completed, click the **Test** button again to check the aperture positions.

Validation Position

- Click the **Validation Position** button to set the apertures to the required positions for collecting Instrument Verification and Ready Check reference spectra (600 x 600 µm, 0° rotation).

Setup Microscope Data Collection

NOTE: The Setup Microscope Data Collection tab is only available when the instrument configuration file includes a Spotlight 200. To use the controls on the tab, the beam path must be directed to the microscope on the Setup Instrument BeamPath tab, and the microscope must be initialized. The Microscope icon  will then be displayed in the Accessory bar.

Use the Setup Microscope Data Collection tab to set up the background collection, auto-focusing, markers layout and ATR position (when fitted) that will be used when you are collecting spectra from your sample.

Background Location

- Choose an option for the location from where to collect the background spectrum before scanning your sample.
  Select **Always at Background position** to always collect a background spectrum at the location shown by the crosshairs marked "B" on the Camera View or Stage View.
  Select **Always at current stage position** to always collect a background spectrum at the location shown by the crosshairs marked "C" on the Camera View or Stage View.
  Select **Prompt for position** if you want the software to ask you if the stage should move to the background spectrum collection position ("B") every time a background is collected. If you click **No**, the background scan will be collected from the current stage position ("C") instead.

NOTE: If you are using the automated ATR objective, these settings will be ignored and every background spectrum will be collected with the crystal held in the air above the sample.

Background Options

In normal operation, any difference in the aperture area or rotation of markers will require a corresponding background to be collected. This ensures that the spectrum baseline will be at 100% transmittance, but this can often significantly increase the time taken for the data collection, especially after an image has been analyzed automatically when there may be many markers with unique aperture settings. There are two options available for reducing the number of backgrounds collected to speed up the data acquisition.

Checking the checkbox marked **Minimize backgrounds collected** causes the software to reuse backgrounds for markers with similar aperture areas. By default, two apertures are defined as similar if both the widths and heights are within 5% of each other. The rotation of the apertures is also ignored if this option is selected, that is, the same background will be used for markers with similar aperture areas even if the rotation values are very different. Both these compromises has only a very small effect on the spectral data collected, but may give a significant time saving.

You can also choose to reduce the number of background spectra collected during a line or map scan by checking the checkbox marked **Scan background every [] points**, and using the arrow buttons to select the number of points to scan in between the collection of each background spectrum.

NOTE: Refer to Setting Up Markers for more information on markers, lines and maps.

Auto-Focus Options

To reduce the time needed to collect sample spectra, Spectrum will, by default, focus on the sample at the start of the analysis but then not focus again. This should give good results if your sample is flat, but if you have a sample with an uneven surface, you can choose to auto-focus the microscope more frequently:

- To auto-focus before every marker is scanned, check the checkbox marked **Auto-Focus at each marker**.
- To auto-focus after scanning a fixed number of points in a line or map scan, check the checkbox marked **Auto-Focus every [] points**, and use the arrow buttons to choose the focusing interval.
  This can be important in obtaining good results, especially if the line or map covers a large area, as the focal position of the first point may not apply to all the other points.

NOTE: If you are using the automated ATR objective to scan markers, these settings will be ignored and the image will be focused automatically before every marker is scanned.

Markers Layout

You can set up markers, lines or maps to scan different areas of your sample. The position and aperture settings of these markers can be saved and reused again in future as a markers layout file.

To load a previously saved markers layout:

1. Click the **Load Layout** button.
   A Browse dialog will open.
2. Select the markers layout file you want to load, and click **OK**.
   Markers layout files have a *.slf file extension.

To save the current markers layout:

1. Click the **Save Layout** button.
2. Enter a name for the markers layout file, and click **OK**.
   The saved markers will appear in the Stage View. If they are not visible, select **Microscope** > **Stage View Range** > **Full Range** to show the entire stage in case they are not in the location currently being viewed.

To automatically load the current markers layout when you start up Spectrum:

- Check the checkbox marked **Remember markers layout on startup**.

Save Options

Select whether you want to save the accumulated results from each line or map in a *.lsc or *.fsm file respectively (in addition to the *.sp files for each spectrum.)

*.lsc and *.fsm files can only be viewed using a separate application, such as SpectrumIMAGE Viewer.

ATR

If you have an automated ATR objective fitted to your microscope, there will be additional data collection options available.

Setup Raman Instrument

Use the Setup Instrument tabs to edit the settings used by your instrument, such as the initial values used in the Instrument Settings bar, or to restore the default settings. If your system has a video camera, you can use the Setup Instrument tabs to view the video camera image of your sample, or, if you have a motorized stage, you can set up data collection at multiple locations.

- Select **Raman Instrument** from the Setup menu.

  OR

  Select  ,  or  in the **Setup** section of the Navigation Pane.

  The Setup Instrument tabs are displayed in the Dialog Pane.

NOTE:	To see the tabs, you may have to resize the Dialog Pane.

Setup Instrument - Basic	
Setup Instrument - Advanced	Toolbox
Setup Sample Area XYZ Stage/Microscope XYZ Stage (motorized stages only)	Live Video Visible Image Survey Graphic
Setup Sample Area (RamanStation Sample Area without stage only)	Live Video
Setup Microscope (manual-stage Raman microscope only)	Live Video
Setup Instrument - Data Collection	
Setup Instrument - Auto-Name	

Additional Information

Spectrum remembers the settings last used for a particular type and configuration of the instrument and any accessory fitted. You can also save the current instrument settings, or load a previously saved set of instrument settings. See Load and Save.

Setup Instrument - Basic

Use the Setup Instrument Basic tab to edit the settings used by your instrument, such as some of the values used in the Instrument Settings bar, to save instrument settings for reuse later, or to restore the default settings. You can also select the sampling accessory, such as a fiber optic probe or a Raman microscope.

1. Select **Raman Instrument** from the Setup menu.

   OR

   Select  ,  or  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

NOTE:	To see the tabs, you may have to resize the Dialog Pane.

2. Select the Setup Instrument Basic tab.

Restore Defaults

Spectrum has a separate default instrument setting for each combination of instrument type, configuration and accessory.

For a particular combination of instrument type, configuration and accessory, Spectrum retains the settings used for the most recent scan. If you change instrument settings, but do not perform a scan, those changes are not retained.

To restore the factory default instrument settings for the current combination of instrument type, configuration and accessory, click **Restore Defaults**.

Load and Save

You can save the current instrument settings, or load a previously saved set of instrument settings. To access the Instrument Settings dialog, click **Load and Save**.

The Instrument Settings dialog contains a list of settings that have been saved. To add the current instrument settings to the list, click **Save Settings**. You can select a previously saved set of instrument settings and make these the instrument settings that will be used to perform the next scan, using **Load Settings**.

The instrument settings list is specific to the User currently logged on to Spectrum. You can **Export** your instrument settings to a *.set file to be available to another user, or **Import** an existing *.set file.

You can load settings that were created on a different instrument for the same accessory type. However, if you select an instrument setting that is not allowed, the **Load Settings** button will not be available.

Click **OK** to save changes to the Settings list.

Instrument settings can also be loaded and used as part of a Macro.

In Spectrum ES, you can also add an electronic signature to a set of instrument settings. See Signing for more information. If you attempt to Export or Import an unsigned instrument setup, you may be prompted for a signature.

NOTE:	Instrument Setup files in Common Instrument Setups directory will be visible to all users. The Common Instrument Setups directory is defined at Spectrum installation. The default is

C:\Pel_data\instrumentsetups.

Export

The Export button allows you to save the instrument settings as a *.rex file. A *.rex file is required to set up your Raman instrument for use with the AssureID software application.

Settings

Start (cm-1) **End (cm-1)**	Set the scan range to the region of the spectrum that you are interested in, in Raman shift (cm^{-1}). In this scale, the Start value must be greater than the End value. The effective scan range that your instrument can collect depends on its configuration.
Data Interval (cm-1)	The Data interval specifies the resolution of the spectrum. The options are **1**, **1.5**, **2**, **4**, **8** and **16** cm^{-1}. Typical selections are 1.5 cm^{-1} or 2 cm^{-1}.
Accumulations	Select the number of **Exposures** or enter a specific exposure time in **Minutes** or **Seconds**. If Accumulations is set to an exposure time, Spectrum will calculate the number of exposures required.
Exposure Time (s)	Adjust the exposure time to collect a well-resolved spectrum that maximizes signal quality and minimizes noise. This option is only available if a number of exposures was selected in Accumulations.

Accessory

- Select your Accessory from the drop-down list.

  The options in the drop-down list depend on your instrument configuration.

  They may include **Sample Area** (RamanStation 400 Series without a motorized stage), **Sample Area XYZ Stage** (RamanStation 400 Series with a motorized stage), **Microscope** (Raman microscope with a manual stage), **Microscope XYZ Stage** (Raman microscope with a motorized stage), **Fiber Optic Probe** or **Triggered Fiber Optic Probe**.

  When you select your accessory further options may become available. See Sample Area XYZ Stage, Sample Area, Microscope XYZ Stage or Microscope.

If a Raman microscope or a fiber probe is selected as your accessory, the system is a Class 3B laser system. When changing to a Class 3B system a warning message is displayed. Click **Continue** to direct the beam path to the microscope or probe. The Class 3B Laser Status icon  is displayed on the Measurement bar when the shutter is open.

Sample Area XYZ Stage

This option is available if you have a RamanStation 400 Series instrument with a motorized stage.

When **Sample Area XYZ Stage** is selected as the accessory the following options may be available:

Snout Orientation (Standard Snout)

If you have a RamanStation 400 Series instrument with a standard snout fitted you can select the snout orientation.

The **Snout Orientation** can be either **Horizontal** or **Vertical**.

Select Vertical if the snout is pointing downwards towards the sample stage. Select Horizontal if the snout is rotated horizontally to point at a sample holder (for example, the cuvette in the Versatile Sample Holder).

The Sample Holder options displayed will update for the Snout Orientation selected.

Sample Holder

The options in the Sample Holder drop-down list depend on your instrument configuration and the snout orientation. They may include:

Sample Area XYZ Stage (Snout Orientation/Polarizer Head - Vertical)	96 Well Plate, 384 Well Plate, 1536 Well Plate, 6 x 9 Well Powder Holder Plate, 7 x 11 Well Tablet Holder Plate, Versatile - Microscope Slide, Final Test Plate – NIST, Final Test Plate – Calcite, Final Test Plate – Polystyrene, Final Test Plate – Stray Light, Final Test Plate – Diamond, Final Test Plate – Laser/Webcam alignment and Maldi plate 96 well.
Sample Area XYZ Stage (Snout Orientation/Polarizer Head - Horizontal)	Versatile – Cuvette, Versatile – Capillary Tube and Versatile – 7 mm Sample Bottle

When you change the Sample Holder selection, the stage will move to a safe position for you to unload your current sample holder without damaging the snout. You are prompted to confirm that you have removed the sample holder and fitted the new sample holder. The stage then moves to a suitable position on the new sample holder, for example, the A1 position of a multi-well plate.

NOTE: If you wish to replace a sample holder from the stage, but are not changing the sample holder selection, you should click Remove Sample Holder on the Setup Sample Area XYZ Stage tab. The stage will then move to a safe position for changing the sample holder.

NOTE: If you change Sample Holder any Cell Markers, Markers, Maps or Line Scans will be deleted and the Visible Image Survey will be cleared. The Graphic on the Setup Sample Area XYZ Stage tab will be updated.

Polarizer Options

If you have a RamanStation 400 Series instrument with a polarizer installed (replacing the standard snout), you can select the orientations of the Polarizer Head and of the polarizers in the laser excitation path and the Raman collection path.

- Select the **Polarizer Head** orientation, either **Horizontal** or **Vertical**.

  Select Vertical if the polarizer head is pointing downwards towards the sample stage. Select Horizontal if the polarizer head is rotated horizontally to point at a sample holder (for example, the cuvette in the Versatile Sample Holder).
- Select the appropriate **Laser Polarizer** option, from **None**, **Horizontal** or **Vertical**.
- Select the appropriate **Raman Analyzer** option, from **None**, **Horizontal** or **Vertical**.

For more information see the *Raman Polarization Accessory* leaflet that shipped with the accessory.

Sample Area

This option is available if you have a RamanStation 400 Series instrument with a manual stage.

When Sample Area is selected as the accessory the following options may be available:

Snout Orientation (Standard Snout)

If you have a RamanStation 400 Series instrument with a standard snout fitted you can select the snout orientation.

The **Snout Orientation** can be either **Horizontal** or **Vertical**.

Select Vertical if the snout is pointing downwards towards the sample stage. Select Horizontal if the snout is rotated horizontally to point at a sample holder (for example, the cuvette in the Versatile Sample Holder).

The Sample Holder options displayed will update for the Snout Orientation selected.

Sample Holder

The options in the Sample Holder drop-down list depend on your instrument configuration and the snout orientation. They may include:

Sample Area (Snout Orientation/Polarizer Head - Vertical)	96 Well Plate, 384 Well Plate, 1536 Well Plate, 6 x 9 Well Powder Holder Plate, 7 x 11 Well Tablet Holder Plate, Versatile - Microscope Slide, Final Test Plate – NIST, Final Test Plate – Calcite, Final Test Plate – Polystyrene, Final Test Plate – Stray Light, Final Test Plate – Diamond, Final Test Plate – Laser/Webcam alignment and Maldi plate 96 well.
Sample Area (Snout Orientation/Polarizer Head - Horizontal)	Versatile – Cuvette, Versatile – Capillary Tube and Versatile – 7 mm Sample Bottle

Polarizer Options

If you have a RamanStation 400 Series instrument with a polarizer installed (replacing the standard snout), you can select the orientations of the Polarizer head and of polarizers in the laser excitation path and the Raman collection path.

- Select the **Polarizer Head** orientation, either **Horizontal** or **Vertical**.

  Select Vertical if the polarizer head is pointing downwards towards the sample stage. Select Horizontal if the polarizer head is rotated horizontally to point at a sample holder (for example, the cuvette in the Versatile Sample Holder).

- Select the appropriate **Laser Polarizer** option, from **None**, **Horizontal** or **Vertical**.

- Select the appropriate **Raman Analyzer** option, from **None**, **Horizontal** or **Vertical**.

For more information see the *Raman Polarization Accessory* leaflet that shipped with the accessory.

Microscope XYZ Stage

When Microscope XYZ Stage is selected as the accessory the following options become available:

Microscope Objective

The options available depend on which objective lenses are fitted in the microscope. The default Microscope Objectives are **50x**, **20x** and **5x**. If any additional objective lenses were installed by a PerkinElmer Service Engineer, these will also appear in the drop-down list. For example, **100x**, **100x LWD**, **50x LWD** and **20x LWD**.

Sample Holder

The options in the Sample Holder drop-down list depend on your instrument configuration.

They may include: Microscope Slide, 6 x 9 Well Powder Holder Plate, 7 x 11 Well Tablet Holder Plate, Final Test Plate – NIST, Final Test Plate – Calcite, Final Test Plate – Polystyrene and Final Test Plate – Silicon

When you change the Sample Holder selection, the stage will move to a safe position for you to unload your current sample holder without damaging the microscope objective. You are prompted to confirm that you have removed the sample holder and fitted the new sample holder. The stage then moves to a suitable position on the new sample holder, for example, the A1 position of a multi-well plate.

NOTE: If you wish to replace a sample holder from the stage, but are not changing the sample holder selection, you should click  Remove Sample Holder on the Setup Microscope XYZ Stage tab. The stage will then move to a safe position for changing the sample holder.

NOTE: If you change Sample Holder any Cell Markers, Markers, Maps or Line Scans will be deleted and the Visible Image Survey will be cleared. The Graphic on the Setup Microscope XYZ Stage tab will be updated.

Microscope

When Microscope is selected as the accessory the following options become available:

Microscope Objective

The options available depend on which objective lenses are fitted in the microscope. The default Microscope Objectives are **50x**, **20x** and **5x.** If any additional objective lenses were installed by a PerkinElmer Service Engineer, these will also appear in the drop-down list. For example, **100x**, **100x LWD**, **50x LWD** and **20x LWD**.

Sample Holder

The options in the Sample Holder drop-down list depend on your instrument configuration.

They may include: Microscope Slide, 6 x 9 Well Powder Holder Plate, 7 x 11 Well Tablet Holder Plate, Final Test Plate – NIST, Final Test Plate – Calcite, Final Test Plate – Polystyrene and Final Test Plate – Silicon.

Additional Information

- For more information on the accessories available for your instrument, refer to the appropriate *Getting Started Guide*.
- For more information on the use of the triggered fiber optic probe, see the *Raman Triggered Fiber Optic Probe* leaflet.
- For more information on the use of the polarization accessory, see the *Raman Polarization Accessory* leaflet.

Choosing Data Intervals

When chosing the Data Interval, you need to consider the resolution that you require. For example, typical resolutions for Raman data are 3.2–3.5 cm^{-1}. Data Interval values higher than 2 cm^{-1} will artificially decrease the resolution of the instrument.

Setup Instrument - Advanced

Use the Setup Instrument Advanced tab to adjust the laser power and to edit the less routine settings used by your instrument and to access the instrument Adjustments Toolbox, which enables you to access a selection of calibration and verification tools.

1. Select **Raman Instrument** from the Setup menu.

   OR

   Select  ,  or  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

NOTE: To see the tabs, you may have to resize the Dialog Pane.

2. Select the Setup Instrument Advanced tab.

Toolbox

Click **Toolbox** to access the Adjustments Toolbox, which enables you to access a selection of calibration and verification tools.

Laser

Laser Power

- Enter the **Laser power (%)** for your experiment, or adjust using the slider.

  Occasionally, samples may be damaged by the laser power. This can be overcome by turning the laser power down.

  The laser power should be determined using a meter if an accurate power value is critical to your experiment.

Manual ON

Switches the laser on.

Manual OFF

Switches the laser off.

Auto Laser off after inactivity (min)

If the laser is on, when there is no activity for the specified period of time the laser will automatically turn off. The default time is 60 minutes.

We recommend that you select this option to help extend the life of your laser.

Options

Cosmic ray removal (on single-exposure samples)

Cosmic ray removal applies a median filter on all collections of one exposure.

Halt scan on saturation

If during a scan the detector is saturated (if, for example, the selected accumulation time is too long) and **Halt scan on saturation** is selected, then a message will be displayed and data collection will stop. No data will be saved.

If the option is not enabled and the detector saturates a message will be displayed but the data collection will continue as normal.

Use photo bleach

Enter the **Photo bleach time**. Photo bleaching is used to reduce background fluorescence. In many cases when analyzing solid samples, irradiating the sample with laser light for a period of time can "quench" the fluorescence, and so minimize the magnitude of the fluorescence. The irradiation time required depends on the sample, but would typically be about 60 seconds. After sample irradiation, data collection will begin as normal.

NOTE: To see if photobleaching is likely to be suitable for your sample, preview your sample first using Monitor. If the intensity does not decrease with time, then photobleaching may not be appropriate.

Adjustments Toolbox

Use the Adjustments Toolbox to access a selection of instrument adjustment dialogs.

1. Select **Raman Instrument** from the Setup menu.

   OR

   Select  ,  or  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

NOTE:	To see the tabs, you may have to resize the Dialog Pane.

2. Select the Setup Instrument Advanced tab and then click **Toolbox**.

   The Adjustments Toolbox dialog is displayed:

	Wavelength Calibration	Set the wavelength calibration for your instrument.
	Frequency Calibration	Set the frequency calibration for your instrument.
	Intensity Calibration	Set the intensity calibration for your instrument.
	Wavelength Calibration Verification	Perform a wavelength calibration verification for your instrument.

3. Click on the icon for the calibration procedure you want to run.

   The appropriate calibration dialog is displayed.

4. When you have finished, click **Exit** to close the Adjustments Toolbox.

Additional Information

We recommend that Wavelength Calibration Verification is performed on a regular basis to ensure that the instrument is performing within specification. If the Wavelength Calibration Verification fails, you should perform a Wavelength Calibration.

Calibration

The Wavelength Calibration Verification should be performed on a regular basis to ensure that the instrument is performing within specification. This can be once a day or once a week depending on your requirements and the laboratory environment. The test compares a series of peaks for polystyrene (ASTM E 1840) with values collected using a polystyrene sample.

When the test is complete, the outcome of the calibration verification procedure is displayed. If your instrument passes the Wavelength Calibration Verification procedure, the instrument is optimized for performance and does not require Wavelength or Frequency calibrations.

There are three types of calibration available:

- Wavelength (x-axis) calibration

  This uses the position of precisely known lines in a neon emission spectrum to calibrate the detector.
  Wavelength calibration should only be performed if the instrument has failed a Wavelength Calibration Verification.

- Laser frequency calibration

  This determines the exact laser wavelength.
  Laser Frequency Calibration should only be performed if the instrument has failed a Wavelength Calibration Verification.

- Intensity (y-axis) calibration

  This is performed using special doped glass with a well characterized fluorescence spectrum (NIST standard); this is used to correct for non-linearity in detector response.
  Intensity calibration should typically be performed on an annual basis – that is, during an annual instrument service – or whenever the instrument is moved, or if there have been appreciable temperature variations in the local environment.

NOTE: The calibration procedures describe how to calibrate a RamanStation 400 Series Instrument with the Sample Area XYZ Stage Accessory. For details of the calibration procedure for your instrument or accessory, refer to the appropriate *Getting Started Guide* or leaflet.

Wavelength Calibration

1. Ensure that the instrument is switched on and ready.
2. Attach the external neon calibration accessory (L1320220) to the rear panel of the instrument.

   Push the large connector into the socket labeled **CAL ASSY** on the rear of your instrument, and carefully connect the small cable to the **COL** coupler.
3. Select **Raman Instrument** from the Setup menu.

   OR

   Select  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

   NOTE: To see the tabs, you may have to resize the Dialog Pane.
4. Ensure that the sample compartment lid on the RamanStation is closed.
5. Select the Setup Instrument Advanced tab and then click **Toolbox**.

The Adjustments Toolbox dialog is displayed.

6. Click  .

   The Wavelength Calibration dialog is displayed.

7. Ensure that you have followed the instructions on the dialog, and then click **Next**.

   The instrument will now perform the Wavelength Calibration. This will take some time. A progress bar is displayed.

   When the Wavelength Calibration has finished the result of the calibration is displayed.

   In the unlikely event that the calibration should fail, contact your PerkinElmer Service Representative.

Frequency Calibration

To perform the Laser Frequency calibration:

1. Ensure that the instrument is switched on and ready.
2. Select **Raman Instrument** from the Setup menu.

   OR

   Select  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

   NOTE: To see the tabs, you may have to resize the Dialog Pane.

3. Select the Setup Instrument Advanced tab.
4. Ensure that the **Laser power** is set to 100%.
5. Select the Setup Sample Area XYZ Stage tab.
6. Focus on the polystyrene calibration sample (L118122) using  **Raman AutoFocus**.

   NOTE: The **Focus** limits should be set to **Full Range**.

7. When the polystyrene sample is optimally focused, select the Setup Instrument Advanced tab.
8. Click **Toolbox**.

   The Adjustments Toolbox dialog is displayed.

9. Click  .

   The Laser Frequency calibration dialog is displayed.

10. Ensure that you have followed the instructions on the dialog, and then click **Next**.

    The instrument will now perform the Laser Frequency calibration. This will take a few minutes. A progress bar is displayed

    After completing the Laser Frequency calibration the result of the calibration is displayed.

    In the unlikely event that the Laser Frequency Calibration should fail, please perform a Wavelength Calibration Verification and then repeat the Laser Frequency Calibration.

11. Remove the polystyrene sample and store it safely.

Intensity Calibration

The Intensity Calibration must be performed using the National Institute of Standards and Technology (NIST) Standard Reference Material. NIST SRM 2241 is the Relative Intensity Correction Standard for Raman Spectroscopy when using 785 nm Excitation.

NOTE: As the intensity calibration is not required on a regular basis, NIST Standards are not included in the basic RamanStation package. Standard Reference Materials can be purchased from PerkinElmer (L1321831) or http://www.nist.gov. Your instrument will be intensity calibrated prior to shipping or during installation.

1. Ensure that the instrument is switched on and ready.
2. Select **Raman Instrument** from the Setup menu.

   OR

   Select  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

   NOTE: To see the tabs, you may have to resize the Dialog Pane.

3. Select the Setup Instrument Basic tab, and ensure that the **Data Interval** is set to 1.5 or 2 cm^{-1}.
4. Select the Setup Instrument Advanced tab.
5. Ensure that the **Laser power** is set to 100%.
6. Select the Setup Sample Area XYZ Stage tab.
7. Focus on the NIST sample using  **Raman AutoFocus**.

   NOTE: The **Focus** limits should be set to **Full Range**.

8. When the NIST sample is optimally focused, select the Setup Instrument Advanced tab.
9. Click **Toolbox**.

   The Adjustments Toolbox dialog is displayed.

10. Click  .

    The Intensity calibration (Y-axis) dialog is displayed.

    The instrument will now perform the Intensity Calibration. This will take approximately 30 minutes.

After completing the Intensity Calibration the result of the calibration is displayed.

In the unlikely event that the Intensity Calibration should fail, please ensure the NIST sample is correctly positioned and that the laser key switch is set to on (armed). If this is the case shut down the PC, switch the instrument off and restart the PC, then switch the instrument back on and repeat the calibration.

11. Remove the Intensity Calibration sample and store it safely.

    Any data acquired is now intensity corrected.

Wavelength Calibration Verification

The wavelength calibration verification routine compares a series of peaks for polystyrene (ASTM E 1840) with values collected using a polystyrene sample.

1. Ensure the instrument is switched on and ready.
2. Position the polystyrene calibration sample (L118122) in the beam path.
3. Select **Raman Instrument** from the Setup menu.

   OR

   Select  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

   NOTE: To see the tabs, you may have to resize the Dialog Pane.

4. Select the Setup Instrument Advanced tab.
5. Ensure that the **Laser power** is set to 100%.
6. Select the Setup Instrument XYZ stage tab.
7. Focus on the polystyrene sample using  **Raman Autofocus**.

   NOTE: The **Focus** limits should be set to **Full Range**.

8. When the polystyrene sample is optimally focused, select the Setup Instrument Advanced tab.
9. Select **Toolbox** from the Instrument menu in Spectrum.

   The Adjustments toolbox dialog is displayed.

10. Click  .

11. Ensure that you have followed the instructions in the dialog, and then click **Next**.

    The Wavelength Calibration Verification routine begins. This will take a few minutes. After completing the Wavelength Calibration Verification routine the result is displayed. If the Wavelength Calibration Verification passes, then the instrument is ready for use.

12. Remove the polystyrene sample and store safely.

If the Wavelength Calibration Verification fails, check that calibration sample holder was placed in the sample accessory and correctly positioned, that the sample is optimally focused and that the laser key is switched on. Then repeat the Wavelength Calibration Verification to ensure that instrument has been brought back within specification.

If the instrument fails the Wavelength Calibration Verification again, then wavelength and laser frequency calibrations should be performed. When these calibrations have finished, repeat the wavelength verification to ensure that the instrument has been brought back within specification. If it fails again, please contact your PerkinElmer Service Representative.

If the RamanStation Wavelength Calibration Verification fails for the Sample Area Accessory option, this does not necessarily mean that the performance of Fiber Optic Probe or Triggered Fiber Optic probe accessories would also be affected.

Additional Information

- The spectrometer has no moving parts in its detection system, therefore frequent calibration is not necessary.
- The only calibration procedure that needs to be performed on a regular basis is the Wavelength Calibration Verification.
- The Wavelength and Laser Frequency Calibrations should only be performed if the instrument fails the Wavelength Calibration Verification.
- We recommend that the Intensity Calibration is performed on a yearly basis or if the environment (temperature and/or humidity) changes significantly, or the instrument is moved.

NOTE: If you are using a Triggered Fiber Optic Probe, you should refer to the *Raman Triggered Fiber Optic Probe* leaflet (L1321887) supplied with the probe for configuration and usage details.

Polystyrene Calibration Sample

The polystyrene sample supplied with the instrument is primarily intended for testing that the instrument is performing consistently, rather than for calibrating the Raman shift (abscissa) scale.

The external neon calibration accessory (L1320220) is used for Wavelength Calibration.

Before using the polystyrene sample, ensure that you have removed the protective plastic from either side of the sample. Take care not to touch the polystyrene.

NIST 2241 Relative Intensity Correction Glass

This Standard Reference Material (SRM) is a certified spectroscopic standard for the correction of the relative intensity of Raman spectra obtained with instruments employing 785 nm laser excitation. SRM 2241 consists of an optical glass that emits a broadband fluorescence spectrum when excited with 785 nm laser radiation. The relative spectral intensity of the glass fluorescence has been determined through the use of a white-light, uniform-source, integrating sphere that has been calibrated for its irradiance at NIST. The shape of the fluorescence spectrum of this glass is described by a polynomial expression that relates the relative spectral intensity to the wavenumber (cm^{-1}) expressed as the Raman shift from the excitation wavelength of 785 nm. This polynomial, together with a measurement of the fluorescence spectrum of the standard, can be used to determine the spectral intensity-response correction that is unique to each Raman system. The resulting instrument-intensity-response correction may then be used to obtain Raman spectra that are instrument independent.

This SRM is intended for use in measurements over the range of 20 °C to 25 °C and with Raman systems that employ laser excitation at 785 nm. It may also be used for Raman excitation with lasers that range from 784 nm to 786 nm in excitation wavelength.

If you plan to operate your Raman Instrument outside the 20 °C to 25 °C range, please contact your PerkinElmer Service Engineer.

Setup XYZ Stage

If you have a motorized stage, you can use the Setup Sample Area XYZ Stage tab (RamanStation 400 Series) or Setup Microscope XYZ Stage tab (RamanMicro 200 Series/RamanMicro 300 Accessory) to set up the sample collection parameters.

NOTE: The Setup Sample Area XYZ Stage tab or Setup Microscope XYZ Stage tab is only available when Sample Area XYZ or Microscope XYZ Stage is selected as the Accessory on the Setup Instrument Basic tab.

1. Select **Raman Instrument** from the Setup menu.

   OR

   Select  or  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

   NOTE: To see the tabs, you may have to resize the Dialog Pane.

2. Select the Setup Sample Area XYZ Stage tab or the Setup Microscope XYZ Stage tab, as appropriate.

   On the left of the Setup XYZ tabs is the sample area display. You can toggle between three views in the sample area display: Live Video, Visible Image Survey and Graphic. The sample area display includes a toolbar that allows you to control the stage, and add objects to the display. On the right of the Setup XYZ stage tab, click ⊞ to expand a group of settings or ⊟ to collapse them. You can only expand one group at a time. When collapsed, the currently selected settings are displayed in brackets in the group title.

3. Select **Live Video**, **Visible Image Survey** or **Graphic**.

   Live Video view – shows the live image from the video camera.

   Visible Image Survey view – shows the current Visual Image Survey, which allows you to build an image from several video camera images.

   Graphic view – shows a graphic representation of the whole sampling area, including a schematic of the currently fitted Sample Holder.

Live Video, Visible Image Survey and Graphic views

Click the name of the view to update the sample area display.

The Live Video, Visible Image Survey or Graphic views use the same coordinate system. The 0,0 position of the XYZ Stage is the top left point of the Graphic view. This need not be the top left of the Live Video or Visible Image Survey displays, as only part of the sample stage is displayed. The 0,0,0 position of the stage has the stage at the lowest position in the z-axis.

The views have a scale bar in the bottom-left corner. The dimension displayed on the Visible Image Survey tab will depend on the area chosen.

NOTE: If you have a Raman microscope, the Microscope Objective selected on the Setup Instrument Basic tab is displayed next to the scale bar. For the scale bar dimensions to be correct you must ensure that you have selected the current Microscope Objective for your Accessory on the Setup Instrument Basic tab.

To move the scale bar:

- Click the scale bar with the left mouse button and drag to the new position. When you next click **Scan**, the scale bar will return to the default position.

On the Graphic view, the current Visible Image Survey is shown as a green rectangle, and the current sample stage position is shown by the cursor.

For certain sample holders, the Graphic view will have regions displayed with red hashed lines. These are areas of the sample holder that you cannot move to because it may cause damage to the instrument. You cannot drag a Visible Image Survey or a Marker, Map or Line Scan into the restricted areas.

CAUTION: You can still move the stage into the red hashed area using the joystick if available for your system. This should be avoided to prevent damage to your instrument.

NOTE: If the stage control box is switched off or loses power, you will need to reset the stage before you can use any of the stage controls, or take a measurement. Click the **Reset Stage** button displayed on the Setup XYZ Stage tab.

Sampling Pattern

NOTE: Custom Grid and Custom Line sampling patterns should not be confused with Maps and Line scans.
When Custom Grid or Custom Line is selected, the points in the grid or line are coadded to produce one spectrum. To produce multiple spectra at different points of a sample, Maps or Line scans should be used.

NOTE: If you select a Sampling Pattern on the Setup XYZ Stage tab for your Accessory, this Sampling Pattern option will be applied to all scans, including those samples added to the Sample Table.

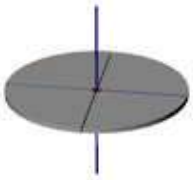
1. Click ⊞ to display the sampling pattern options.
2. Select the sampling pattern type from **Single Point**, **Super Macro Point**, **Custom Grid** and **Custom Line.** The point options displayed depend on the sampling pattern selected.

   For **Custom Line** you can select the **Orientation** (**Left/Right**, **Back/Forward** or **Vertical**) of the line, the **Number** of points in the line and the **Spacing** of the points (mm).

   For **Custom Grid** you can select the **Orientation** (**Horizontal**, **Vertical Left/Right** or **Vertical Back/Forward**) of the grid, the number of points in the grid (in the **Horizontal**, **Vertical Left/Right** or **Vertical Back/Forward** directions, depending on the orientation of the grid) and the **Spacing** of the points (mm).
   The orientation plane is always relative to the Live Video image. So a Horizontal Custom Grid is in the same plane as the Live video image, no matter what snout orientation you have on a RamanStation 400 Series instrument.
3. Click ⊟ to collapse the sampling pattern options.

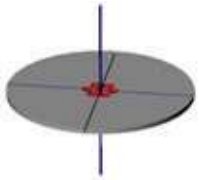
Sampling Pattern Types

- Single Point

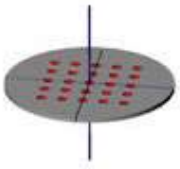
  

  A single spectrum will be collected either at the current position or the center of each selected region.
- SuperMacro Point

  Allows you to analyze a larger sample spot. A SuperMacro spot consists of 7 points arranged as 6 points around 1 point. Spectra from all 7 points are coadded, resulting in a spectrum that is representative of collection from a large spot. The stage position defined will be at the center of the SuperMacro spot.

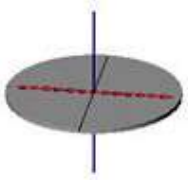
  
- Custom Grid

  Multiple spectra will be collected in a defined grid of points with specified distance between each spectra and then coadded. The stage position defined for the scan will be at the center of the grid.

- Custom Line

  Spectra will be collected along a line in the specified direction and then coadded. The stage position defined will be at the center of the line.



Focus

- Click ⊞ to display the Focus settings and select the type of focus to be performed before running each sample.

None

The sample will be run without focusing.

Raman

This option auto focuses onto the sample by moving the stage through the range set by the Focus Limits and collecting Raman data. The point at which the Raman spectrum is most intense is deemed to be the focal position.

Select the **Use derivative** check box if you want to apply a first derivative. This should improve the quality of the Raman focus mechanism. If Use derivative is selected in the Focus settings, it will also be applied if you click Raman AutoFocus.

Video

This option auto focuses using the image of the sample. The stage moves through the range set by the Focus Limits and measures the sharpness at the center of the image. The point at which the image is sharpest is deemed to be the focal point.

Focus Limits (mm)

The range of the focus sweep can be adjusted using the Focus Limits (mm) drop-down list.

Full Range will sweep between the limits set for the current instrument configuration, while the other options will sweep the specified range (for example, +/- 1mm). If the range falls outside the limit set for the current instrument configuration, the sweep will stop at the limit to avoid damaging the lens snout or objective and/or sample.

Reducing the Focus Limits can significantly decrease the time taken for the auto focus and thus data collection times. However, the focus position may not be found if it falls outside the focus limits.

CAUTION:	Care must be taken when setting auto focus limits for a Raman microscope where long microscope objectives and/or thick samples are used, as a collision could occur.

NOTE: The focus limits selected will be used if you click Video AutoFocus or Raman AutoFocus. However, you do not need to enable Video or Raman focus here to use the auto focus functions.

Additional Information

You need to chose the focus settings appropriate for your analysis. Video focus will scan at or near the surface. Raman focus will focus at the position with the strongest Raman signal. For this reason it is important to chose an appropriate sample holder as, for example, glass has a strong Raman signal, and the Raman focus might measure the sample holder rather than the sample.

Raman focus is particularly useful for samples that are difficult to focus using Video focus, or where reproducibility of focus is important (as this is defined by the CCD, not the operator).

You should chose focus limits appropriate for the profile of your sample. If the sample is reasonably flat, and you are focused at the current position, you can chose a smaller auto focus limit, decreasing the scan time.

Stage Position

Click ⊞ to display the controls for the XYZ stage position.

The details of current stage position are displayed, given as a distance from the top-left corner of the stage.

- Enter a value in the **Horizontal**, **Vertical** or **Focus** fields to move the stage to a new location.

  OR

  Click the arrows below the field to move the stage in predefined steps.

  Click ◀ or ▶ in the **Horizontal** or **Vertical** sections to move the stage 0.01 mm left or right, or forwards or backwards, respectively.
  Click ⏮ or ⏭ in the **Horizontal** or **Vertical** sections to move the stage one Live Video image left or right, or forwards or backwards, respectively.

  Click ◀ or ▶, ⏮ or ⏭, in the **Focus** section to move the stage up or down. The distance moved will depend on your instrument and accessory.

You can select the **Units** for the stage position. The options are: **mm**, **µm** and **nm**. The default units are mm.

If you select **Show Extended Cursor**, the crosshairs will extend to the edges of the image.

The Horizontal, Vertical and Focus directions are always relative to the Live Video image. So the Focus direction will always be closer to or further from the sample, even if you change the snout orientation on a RamanStation 400 Series instrument. When the focus position is 0 mm, the stage is at the point of travel nearest to the sample.

Marker Settings

If you select **Add Marker** from the Setup XYZ Stage toolbar, a marker is added to the display and the Marker Settings are displayed.

If collapsed, click ⊞ to display the Marker Settings.

For each marker, enter the **Sample Name** for the spectrum that will be collected.

The **Horizontal**, **Vertical** and **Focus** positions of the currently selected marker are displayed.

To change the position of a marker, enter the new position in the Marker Settings fields or select the marker on the display with the left mouse button and drag it to a new location.

See Using Markers for more information.

Cell Marker Settings

If you click the right mouse button over a cell on the Graphics view and select **Insert Cell Marker**, a marker is added to the center of the well. The Cell Marker Settings are displayed.

If collapsed, click ⊞ to display the Cell Marker Settings.

Enter the **Base Sample ID** for the spectra that will be collected. The Base Sample ID is the root spectrum file name to which the current cell position will be appended. The default Base Sample ID is Cell Marker. For example, Cell Marker_A2.sp.

The **Cell** position (for example, A1) is displayed. You cannot change the position of a cell marker. Cell markers are always added to the center of the cell.

The **Focus** value is the position of the XYZ stage when the cell marker was added. If required, enter a new position.

See Using Cell Markers for more information.

Line Scan Settings

NOTE: Line scans should not be confused with the Custom Line sampling pattern. When Custom Line is selected, the points in the line are coadded to produce one spectrum. To produce multiple spectra at different points of a sample, Line scans should be used.

If you select **Add Line** from the Setup XYZ Stage toolbar, a line is added to the display and the Line Scan Settings are displayed.

If collapsed, click ⊞ to display the Line Scan Settings.

You can drag-and-drop the line to reposition it or change the horizontal (**H**) and vertical (**V**) Anchor positions. You can also grab one of the ends of the line and drag to resize. New points will be added at the current point spacing.

- Enter the **Base Sample ID** for the spectra that will be collected. The Base Sample ID is the root spectrum file name to which the line position will be appended. The default Base Sample ID is Line Scan, so for position 2 the file name would be Line Scan_(2).sp

- Select the **Orientation** (**Horizontal** or **Vertical**).

  If you change the Orientation of a Line Scan from Horizontal to Vertical, the line will be displayed as a point. The direction of the line scan is effectively through the sample.

  The orientation plane is relative to the Live Video image. So Horizontal is in the same plane as the Live video image.

- Specify the number of **Points** in the line.

  OR

  Set the point **Spacing (mm)** between your points.

- Set the **Rotation** angle of the line.

  You can also select one end of the line and drag to rotate. When a line is selected, the color changes from red to blue. You can only set the rotation if the Orientation is set to Horizontal. If the orientation is set to Vertical, the Rotation angle is fixed at 0°.

If you select **Display in Graph** all the spectra collected will be added to a Samples View.

If you select **Save as LSC** the individual spectra are collated to create an LSC (Line Scan) file that you can view using the SpectrumIMAGE software. SpectrumIMAGE is supplied with your instrument, and can be installed on the PC if required.

See Using Line Scans for more information.

Map Settings

NOTE:	Maps should not be confused with the Custom Grid sampling pattern. When Custom Grid is selected as the Sampling Pattern, the points in the grid are coadded to produce one spectrum. To produce multiple spectra at different points of a sample, Maps should be used.

If you select, **Add Map** from the Setup XYZ Stage toolbar, a map is added to the display and the Map Settings are displayed.

If collapsed, click ⊞ to display the Marker Settings.

Multiple spectra will be collected in a defined grid of points with specified distance between each point.

You can drag-and-drop the map to reposition it or change the horizontal (**V**) Anchor positions. You can also select one of the edges or corners of the map and drag to resize it. New points will be added at the current point spacings.

- Enter the **Base Sample Name** for the spectra that will be collected. The **Base Sample Name** is the file name under which each spectrum in the map will be displayed with a number representing the position in the map appended. The default Base Sample Name is Map Scan, so for row 1 column 2 the file name would be Map Scan_(1)(2).sp

- Specify the number of **Points**, horizontal (**H**) and vertical (**V**), in the map.

  OR

  Set the point **Spacing (mm)**, horizontal (**H**) and vertical (**V**), in the map.

If you select **Display in Graph** all the spectra collected will be added to a Samples View.

If you select the **Save as FSM** check box the individual spectra are collated as a full spectral map that you can view using the SpectrumIMAGE software. SpectrumIMAGE is supplied with your instrument, and can be installed on the PC if required.

See Using Maps for more information.

Additional Information

Setup XYZ Stage Toolbars

Video AutoFocus		This option auto focuses using the video image of the sample. The Live Video view is displayed. To stop the Video AutoFocus, click **Halt**.
Raman AutoFocus		This option auto focuses onto the sample by sweeping the focal range and collecting Raman data to find the most intense Raman signal. To stop the Raman AutoFocus, click **Halt**.
Save Image >	Save image with annotations	Saves the current Live Video or Visible Image Survey image, including the scale bar, cross hairs and any markers, maps or line scans, as a *.bmp or *.jpg. This option is not available on the Graphic view.
	Save image without annotations	Saves the current Live Video or Visible Image Survey image as a *.bmp or *.jpg. Any annotations are not saved to the image. This option is not available on the Graphic view.
Toolbox >	Set First Well	Sets the position as the center-point of the first well (A1) of a multi-well plate (or the only well of a single-well plate).
	Set Last Well	Sets the position as the center-point of the last well of a multi-well plate (or the only well of a single-well plate).

	Reset Stage	Sends the stage to the limit stops to realign the position of the stage. **NOTE:** If the stage control box is switched off or loses power, you will need to reset the stage before you can use any of the stage controls, or take a measurement.
	Align Video with Laser	Allows you to align the Laser and Video camera. This should ideally be performed by a PerkinElmer Service Engineer.
Illumination >	Off Low High	Illumination enables you to control the illumination LEDs on the base of the alignment camera assembly. You have the option to turn them **Off**, switch them to **Low** power, or switch them to **High** power. **NOTE:** Illumination is not available on the Setup Microscope XYZ Stage tab. Illumination only applies to the RamanStation 400 Sample Area.

New Visible Image Survey >	User Drawn Survey	If you have drawn a selection box on the image, User Drawn Survey is available. Creates a Visible Image Survey with the dimensions of the selection box.
	2 x 2 Survey	Creates a 2 x 2 Visible Image Survey.
	3 x 3 Survey	Creates a 3 x 3 Visible Image Survey.
	5 x 5 Survey	Creates a 5 x 5 Visible Image Survey.
	10 x 10 Survey	Creates a 10 x 10 Visible Image Survey.
	20 x 20 Survey	Creates a 20 x 20 Visible Image Survey.
Move to First Well		Moves the sample stage to the first position in the well plate (A1).
Move stage to remove sample holder		Moves the sample stage to a safe position for removing the Sample Holder.

Add Marker	Adds a Marker to the display and displays the Marker Settings.
Add Map	Adds a Map to the display and displays the Map Settings.
Add Line	Adds a Line Scan to the display and displays the Line Scan Settings.
Delete	Deletes the currently selected Marker, Map or Line Scan.
Delete All Shapes	Deletes all the objects in the display. This will delete all objects, including measured shapes.
Delete All Measured Shapes	Deletes all measured shapes from the display.

Scanning Markers, Maps and Line Scans

Any shapes added to the Setup XYZ tab, such as cell markers, markers, maps and lines will be scanned when **Scan** is clicked. These will take priority over any samples added to the Sample Table, which will not be run.

Repeat collections is not available for cell markers, markers, maps and line scans.

Moving the Stage to a Position of Interest

There are a number of ways you can move the stage in Spectrum software.

Moving the Stage using the Mouse

- At the position of interest, click the right mouse button and select **Move Here** from the shortcut menu.

  OR

  Click on the cross hairs with the left mouse button and drag the cross hairs to a new position.

  The cross hairs will move to the new position on the Graphic view and, if applicable, the Visible Image Survey. On the Live Video, the new video image will be displayed. The cross hairs indicate the position of the stage.

Moving the Stage using the Stage Position Controls

To move the stage to a position of interest:

1. Click the plus sign to display the Stage Position controls.
2. Enter **Horizontal**, **Vertical** and **Focus** coordinates of the position you would like to move to.

   OR

   Use the **Horizontal**, **Vertical** and **Focus** scroll arrows to change the coordinates.

   The stage moves to the new position.

Moving the Stage using the Joystick

- If available for your stage, use the joystick to move the cross hairs to the new position.

Additional Information

The cross hairs show the current position of the stage.

NOTE: The step motor on the stage is very powerful, and inertia could cause the sample to shift relative to the sample holder. Therefore, we recommend that you fix the sample to the sample holder.

Visible Image Survey

The Visible Image Survey enables you to view an area of your sample using the video camera. You can use the Visible Image Survey to view a large area of your sample to help to define a region, for example, for a map, or to identify a specific point at which to collect a measurement, for example, by using markers.

NOTE: Visible Image Survey is only available if you have a motorized sample stage.

Performing a User-Defined Visible Image Survey

1. Select **Raman Instrument** from the Setup menu.

   OR

   Select  or  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

   NOTE: To see the tabs, you may have to resize the Dialog Pane.

2. Select the Setup XYZ Stage tab and display the **Live Video** view.
3. Select a region in the area you would like to survey and focus on the sample.

   If your sample is not flat, regions of the Visible Image Survey will not be in focus.
4. Select the **Graphic** view.

   If you have already collected a Visible Image Survey, the current Visible Image Survey is shown as a green rectangle.
5. On the sample holder graphic, click the left mouse button and drag the mouse over the area you want to survey.

   When you release the left mouse button, the selected region is marked by a solid pink line.

   If you wish to clear the selection, you can either click the right mouse button inside the box and select **Clear Selection** from the shortcut menu, click outside the box or simply click and drag the mouse over a new area. Alternatively, you can click the selection box with the left mouse button and drag it to a new position.

   You can also click on the sides to the selection box and drag to increase or decrease the size.

   NOTE: The Visible Image Survey will take longer to collect if you select a larger area. If you have a Raman microscope and are using high-magnification objectives, even a small region on the Graphic view may be a large number of Live Video images and take some time to collect. Therefore, it may be more appropriate to use a pre-defined visible image survey.
6. Click the right mouse button inside the selected area and then select **New Image Survey** from the shortcut menu.

   The Visible Image Survey view is displayed, and the stage moves to the start position for the survey. If at any time you wish to stop the Visible Image Survey, click the **Halt** button displayed on the image.

7. When the survey is complete, if you want to save an image of your survey select **Save Image** and then select **Save Image with Annotations** or **Save Image without Annotations.**

   The Save As dialog is displayed. You can save the image as a *.jpg or *.bmp.

   If you select Save Image with Annotations, the scale bar, cross hairs and any markers, maps or line scans are saved with the image.

8. Click **Save.**

Using Pre-defined Visible Image Surveys

- Click on the toolbar and select from a list of pre-defined areas for your visible image survey.

  The options are **2 x 2 Survey**, **3 x 3 Survey**, **5 x 5 Survey**, **10 x 10 Survey** and **20 x 20 Survey.** The Visible Image Survey window is displayed and the stage moves to the start position for the survey.

Collecting a Sub-set of a Visible Image Survey

After you have performed a Visible Image Survey, you may want to perform another survey that is a subset of the current Visible Image Survey. To do this:

- Click and drag to draw a selection box on the Visible Image Survey window or inside the current survey on the Graphic view.

Halting a Visible Image Survey

- If you want to stop the Visible Image Survey while it is in progress, click **Halt.**

  The Visible Image Survey stops immediately and the stage returns to position it was at before the visible image survey was started. The data collected so far will be displayed in the Visible Image Survey window and can be saved.

Additional Information

On the Graphic view, the current Visible Image Survey is marked by a solid green line.

When you exit the software or if the Sample Holder is changed on the Setup Instrument Basic tab, the Visible Image Survey will be cleared.

If the Sample Holder is changed on the Setup Instrument Basic tab, then the Visible Image Survey will be cleared.

Using Markers

Markers enable you to perform a sequence of scans at different positions of interest on your sample. You can chose to perform a Video or Raman focus at each point, or measure using the focus settings current when the Marker was added. Markers can be useful to, for example, help to define suitable parameters before running a map of a heterogeneous samples.

NOTE: Markers are only available if you have a motorized sample stage, and are defined on the Setup Sample Area XYZ Stage tab or Setup Microscope XYZ Stage tab.

Adding Markers and Collecting Data

1. Select **Raman Instrument** from the Setup menu.

   OR

   Select  or  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

   NOTE: To see the tabs, you may have to resize the Dialog Pane.

2. Select the Setup XYZ Stage tab and then select the appropriate view.

   The options are **Live Video**, **Visible Image Survey** or **Graphic**.

3. Select the region of interest on your sample.

4. Click  to perform a Video AutoFocus.

5. Select the **Sampling Pattern** for your measurements.

   See Sampling Pattern for more information.

6. Select the **Focus** options for your scan.

   You can select to perform a **Video** or **Raman** focus before each sample, and set the **Focus Limits** through which the stage will move when trying to find the focus. See Focus for more information.

7. Click  **Add Marker** on the Setup XYZ Stage toolbar to add a marker, or right click on the display and select **Insert Marker** from the shortcut menu.

   The marker is added to the display. If you use the toolbar button to add the marker it will be added at the center of the display. If you use the shortcut menu, the marker will be added at the point you clicked in the display.

8. Enter a **Sample Name** for the spectrum.

   The default Sample Name is Marker.

9. Repeat steps 9–10 until you have added all the markers you require.

10. Select **Monitor** from the Measurement menu, or click  .

> **NOTE:** We recommend that you preview the spectrum to verify that the instrument parameters are suitable for your sample. If you have several markers over a large area of sample, it is advisable to check the instrument parameters in more than one position.

11. Adjust the **Laser power (%)** and **Exposure time** using the controls on the Live tab.
12. Adjust the instrument parameters to give well-resolved spectrum that maximizes signal quality and minimizes noise and to check for detector saturation.
13. To stop monitoring and scan the sample, select **Scan** from the Measurement menu, or click  .

    The data is collected at each marker position and the spectra are added to a new Samples View named Markers *n*. The progress of the total scan is displayed and the progress of the current measurement is displayed. The color of the marker will change to green to indicate that data has been collected at that position. The Description and the file header of each spectrum will include the stage position.

Moving Markers

- Select the marker you want to move by clicking with the left mouse button, and then drag it to a new position.

  OR

  Select the marker, and then enter the **Horizontal**, **Vertical** or **Focus** (z-axis) values for the new marker position in the Marker Settings section of the Setup Sample Area XYZ Stage tab or Setup Microscope XYZ Stage tab.

  The marker  will change appearance  to show it is selected.

> **NOTE:** Once a marker has been scanned, it cannot be moved.

Deleting Markers

- Select the marker you want to delete by clicking with the left mouse button, and then click the right mouse button and select **Delete Marker** from the shortcut menu.

  OR

  Select the marker you want to delete by clicking with the left mouse button, and then click  **Delete Marker** on the toolbar.

  The marker is deleted.

Deleting All Markers

1. Click the right mouse button and select **Delete All Markers** from the shortcut menu.
2. Click **Yes** to confirm that you want to delete all the markers.
3. All markers on the display are deleted.

Deleting All Markers, Maps and Line Scans

- Click **Delete All Shapes** on the toolbar and then click **Yes** to delete all markers, maps and line scans.

  All objects on the display are deleted.

Deleting All Measured Shapes

- Click **Delete All Measured Shapes** on the toolbar and then click **Yes** to remove all points that have already been scanned.

  Maps and line scans will not be cleared unless all the points have been scanned.

Additional Information

You can add as many markers, maps and line scans as you want to the display, but the more data points you wish to collect, the longer the scan will take.

If you close the software, any markers will be retained when the software is restarted. If you change Sample Holder on the Setup Instrument Basic tab, any markers will be deleted.

Using Cell Markers

Cell markers enable you to enter a marker in the center of a cell, for example on a multi-well sample holder.

NOTE: Cell markers are only available if you have a motorized sample stage, and are defined on the Graphic view of the Setup Sample Area XYZ tab or Setup Microscope XYZ tab.

Adding Cell Markers and Collecting Data

1. Select **Raman Instrument** from the Setup menu.

   OR

   Select  or  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

   NOTE: To see the tabs, you may have to resize the Dialog Pane.

2. Select the Setup XYZ Stage tab and then select the appropriate view.

   The options are **Live Video**, **Visible Image Survey** or **Graphic**.

3. Select the region of interest on your sample.

4. Click  to perform a Video AutoFocus.

5. Select the **Sampling Pattern** for your measurements.

   See Sampling Pattern for more information.

6. Select the **Focus** options for your scan.

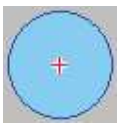
   You can select to perform a **Video** or **Raman** focus before each sample, and set the **Focus Limits** through which the stage will move when trying to find the focus. See Focus for more information.

7. Select the **Graphic**.

8. Right click in the cell where you want to add a cell marker and select **Insert Cell Marker** from the shortcut menu.

   The cell color changes and the marker is added to the center of the cell. The Cell Marker Settings are displayed.

   NOTE: Insert Cell Marker is not available if you are not over a recognized cell position. Only one cell marker can be added to each cell. Once added, a cell marker cannot be moved.



9. If required, modify the **Focus** position of the cell marker.

> **NOTE:** The Focus value is the stage z-axis position at which the spectrum will be collected *unless* you have selected one of the **Focus before each sample options**.

10. If required, modify the **Base Sample ID**.

    Base Sample ID is the root file name under which each spectrum will be saved with the current cell position will be appended. The default Base Sample ID is Cell Marker. The Spectra are named [Base Sample ID]_[Row][Column]_[*nnn*].sp where _*nnn* is a unique identifier added if the Base Sample ID has already been used. For example Cell Marker_(A2)_001.sp.

11. Repeat steps 7–9 until you have added all the cell markers you require.

12. Select **Monitor** from the Measurement menu, or click  .

> **NOTE:** We recommend that you preview the spectrum to verify that the instrument parameters are suitable for your sample. If you have several markers over a large area of sample, it is advisable to check the instrument parameters in more than one position.

13. Adjust the **Laser power (%)** and **Exposure time** using the controls on the Live tab.

    Adjust the instrument parameters to give well-resolved spectrum that maximizes signal quality and minimizes noise and to check for detector saturation.

14. To stop monitoring and scan the sample, select **Scan** from the Measurement menu, or click  .

    The data is collected at each point in cell position order and the spectra are added to a new Samples View named [Base Sample ID]. The color of the cell and cell marker will change to indicate that data has been collected. The progress of the total scan is displayed and the progress of the current measurement is displayed. The Description and the file header of each spectrum will include the cell reference and the stage position.

Adding Multiple Cell Markers

On the Graphics view:

- Hold down the left mouse button and drag diagonally to draw a selection box around the cells you want to add a cell marker to, and then right click inside the box and select **Add Cell Markers**.

  A cell marker will be added to the center of each of the cells selected. If the center of the well is inside the box, a cell marker will be added to the cell – you do not need to select the whole cell.

  OR

  Hold the Ctrl button and then click with the left mouse button in the cells where you would like to add a cell marker.

  A cell marker will be added to the center of each cell.

Deleting Cell Markers

- Select the cell marker you want to delete by clicking with the left mouse button, then click the right mouse button and select **Delete Cell Marker** from the shortcut menu.

  OR

  Select the cell marker you want to delete by clicking with the left mouse button, and then click  **Delete Cell Marker** on the toolbar.

  OR

  Hold the Ctrl button and then click with the left mouse button in the cells containing a cell marker you want to delete.

  The cell marker is deleted.

Deleting All Markers, Maps and line scans

- Click  **Delete All Shapes** on the toolbar and then click **Yes** to delete all markers, maps and line scans.

  All objects on the display are deleted.

Deleting All Measured Shapes

- Click  **Delete All Measured Shapes** on the toolbar and then click **Yes** to remove all points that have already been scanned.

  Maps and line scans will not be cleared unless all the points have been scanned.

Additional Information

You can add as many markers, maps and line scans as you want, but the more data points you wish to collect, the longer the scan will take.

If you close the software, any cell markers will be retained when the software is restarted. If you change Sample Holder on the Setup Instrument Basic tab, any cell markers will be deleted.

Using Maps

Maps enable you to collect multiple spectra from a defined grid of points with specified distance between each spectra. You can also combine these scans into a full spectral map file that can be opened in SpectrumIMAGE software.

Collecting a Visible Image Survey can be useful to view a region of your sample before choosing where to add a map. And using markers to collect data at several points over the region of interest can be useful when defining instrument settings that will produce good-quality spectra over the whole map.

You can chose to perform a Video or Raman focus at each point, or measure using the focus settings current when the Map was added.

NOTE: Maps are only available if you have a motorized sample stage and are defined using the Setup Sample Area XYZ tab or Setup Microscope XYZ tab.

Adding Maps and Collecting Data

1. Select **Raman Instrument** from the Setup menu.

   OR

   Select  or  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

   NOTE: To see the tabs, you may have to resize the Dialog Pane.

2. Select the Setup XYZ Stage tab and then select the appropriate view.

   The options are **Live Video**, **Visible Image Survey** or **Graphic**.

3. Select the region of interest on your sample.

4. Click  to perform a Video AutoFocus.

5. Select the **Sampling Pattern** for your measurements.

   See Sampling Pattern for more information. You must take care that you do not chose sampling pattern options that result in overlap between data points in your map.

6. Select the **Focus** options for your scan.

   You can select to perform a **Video** or **Raman** focus before each point, and set the **Focus Limits** through which the stage will move when trying to find the focus. See Focus for more information.

   Choosing to auto focus before each point in a map can be useful when your sample is very heterogeneous and has a wide variation in focal heights, but will increase the time taken to scan your map. So you should only select this when it is appropriate for your sample.

7. Click **Add Map** on the Setup XYZ Stage toolbar or click the left mouse button and drag diagonally to draw a selection box, and then right click and select **Add Map** from the shortcut menu.

   The map is added to the display and the Map Settings are displayed. The Anchor coordinates define the position of the first point in the map. If you use the toolbar button to add the map it will be added at the center of the display at the default map size of 5 x 5 with 1.0 mm point spacings. If you use the shortcut menu to add the map, it will add a map the size of the selected area with a point in each corner.

   NOTE: If you do not select an auto focus option, the map will be generated at the focus setting (Z position) of the instrument when the map was added.

8. If required, modify the **Base Sample Name**.

   Base Sample Name is the file name under which each spectrum in the map will be displayed with a number representing the position in the map appended. The default Base Sample Name is Map Scan. For example, the file name for position row 1, column 2, using the default Base Sample Name, would be Map Scan_(1)(2)_sp. If the Base Sample Name has already been used, a number *_nnn* is appended to give the spectrum a unique file name. For example, Map Scan_(1)(1)_001.sp.

9. If required, adjust the horizontal (**H**) and vertical (**V**) anchor points or click on the map and drag to move it to the position of interest.

10. Move the pointer to one of the sides or corners of the map.

    The cursor changes to a two-headed arrow.

11. Using the arrows, click and drag to increase or decrease the map size.

    As you change the size of the map, the points keep the same spacing. Points are added or removed from the map.

12. Adjust the point **Spacing**, or change the number of **Points**.

    This will not increase the size of the map.

    The horizontal (**H**) and vertical (**V**) points and spacings can be adjusted independently.

13. Select **Display in Graph** if you want the spectra collected to be added to the Samples View.

    NOTE: You will not be able to select this option if your map exceeds a certain size limit. The individual spectra will still be generated but they will not be displayed in the Graph.

14. Select **Save as FSM** if you would like to generate a full spectral map of your data that you can view using SpectrumIMAGE software.

    The *.fsm file will be generated with the same Base SampleID as the spectra. Base Sample Name as the spectra. For example [Base Sample Name].fsm.

15. Repeat steps 6–13 for any additional maps you would like to add.

16. Select **Monitor** from the Measurement menu, or click  .

> **NOTE:** We recommend that you preview the spectrum to verify that the instrument parameters are suitable for your sample. Depending on how widely spaced the points in the map are, or whether you have a heterogeneous sample, it is advisable to check the instrument parameters in more than one position.

17. Adjust the **Laser power (%)** and **Exposure time** using the controls on the Live tab.

    Adjust the instrument parameters to give a well-resolved spectrum that maximizes signal quality and minimizes noise and to check for detector saturation.

18. To stop monitoring and scan the sample, select **Scan** from the Measurement menu, or click  .

    The data is collected at each point in the map, starting at the top left position, and the color of each measured point changes to green. If **Display in Graph** is selected, the spectra are added to the Samples View. The progress of the total scan is displayed and the progress of the current measurement is displayed. Once the points in the map have been measured you can access the Map Scan Settings, but you cannot modify them. The Description and the file header of each spectrum will include the stage position.

Resizing Maps

- Move the pointer to one of the sides or corners of the map, then click with the left mouse button and drag to resize it.

  New columns or rows will be added to the map at the current point spacings.

Moving Maps

- Select the map you want to move by clicking with the left mouse button, and then drag to a new position.

  OR

  Select the map and then enter the coordinates of the new **Anchor** position in the Map Settings section of the Setup XYZ Stage tab.

Changing the Appearance of Maps

You can change the way that maps are displayed. The default is to show all the points in the map.

To change to just show the outline of the map, rather than the individual data points, click the right mouse button and deselect **Show Map Markers**.

You can change the color of an individual map by clicking the right mouse button and selecting **Choose Color**. The Color dialog is displayed. Select a new color for your map and click **OK**.

> **NOTE:** You can change the color of the map before and after the points have been scanned, but when scanned the color will default to green.

Deleting Maps

- Select the map you want to delete by clicking with the left mouse button, and then click the right mouse button and select **Delete Map** from the context menu.

  OR

  Select the map you want to delete by clicking with the left mouse button, and then click **Delete Map** on the toolbar.

  The marker is deleted.

Deleting All Markers, Maps and line scans

- Click **Delete All Shapes** on the toolbar and then click **Yes** to delete all markers, maps and line scans.

  All objects on the display are deleted.

Deleting All Measured Shapes

- Click **Delete All Measured Shapes** on the toolbar and then click **Yes** to remove all points that have already been scanned.

  Maps and line scans will not be cleared unless all the points have been scanned.

Additional Information

Each data point in a map is saved before the next data point is collected. This ensures that no data is lost. If you exit Spectrum software before a Map is completed, upon reopening Spectrum and clicking Scan the software will continue the map at the next data point.

You can add as many markers, maps and line scans as you want to the display, but the more data points you wish to collect, the longer the scan will take.

If you close the software, any maps will be retained when the software is restarted. If you change Sample Holder on the Setup Instrument Basic tab, any maps will be deleted.

Saturation

If you have **Halt scan on saturation** selected on the Setup Instrument Advanced tab, the scan will halt if saturation is detected at a point.

If you do not have the option selected and saturation is detected at a point, the scan will continue. A message will be displayed indicating that saturation has occurred during the scan, and this information will be saved to the History of the spectrum. However, there will be no indication in the map that the saturation event occurred.

Using Line Scans

Line scans enable you to measure points along a line in the x, y or z axes. Line scans are particularly useful when you want to examine a pattern across a sample, or for depth profiling (z-axis). You can chose to perform a Video or Raman focus at each point, or measure using the focus settings current when the Line Scan was added.

You can collect data in two ways: as separate spectra and as a single Line Scan (LSC) file that you can view using SpectrumIMAGE software.

NOTE: Line scans are only available if you have a motorized sample stage and are defined on the Setup Sample Area XYZ tab or Setup Microscope XYZ tab.

Adding Line Scans and Collecting Data

1. Select **Raman Instrument** from the Setup menu.

   OR

   Select  or  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

   NOTE: To see the tabs, you may have to resize the Dialog Pane.

2. Select the Setup XYZ Stage tab and then select the appropriate view.

   The options are **Live Video**, **Visible Image Survey** or **Graphic**.

3. Select the region of interest on your sample.

4. Click  to perform a Video AutoFocus.

5. Select the **Sampling Pattern** for your measurements.

   See Sampling Pattern for more information.

6. Select the **Focus** options for your scan.

   You can select to perform a **Video** or **Raman** focus before each point, and set the **Focus Limits** through which the stage will move when trying to find the focus. See Focus for more information.

7. Click  **Add Line** on the Setup XYZ Stage toolbar or click the left mouse button and drag diagonally to draw a selection box, and then right click and select **Add Line** from the shortcut menu.

   The line is added to the display and the Line Scan Settings are displayed. The Anchor coordinates define the position of the first point in the line scan. If you use the toolbar button to add the line it will be added at the center of the display. If you use the shortcut menu it will add a horizontal line in the center of the selected area.

8. If required, modify the **Base Sample Name**.

   Base Sample Name is the file name under which each spectrum in the line scan will be saved with a number representing the position in the line appended. The default Base Sample Name is Line Scan. For example, the file name for position 2 would be Line Scan_(2).sp. If the Base Sample Name has already been used, a number *_nnn* is appended to give the spectrum a unique file name. For example Line Scan_(2)_001.sp.

9. Select the Orientation, **Horizontal** or **Vertical**, of the line scan.

The default Orientation is Horizontal. If you change the Orientation of a Line Scan from Horizontal to Vertical, the line will be displayed as a point. The direction of the line scan is effectively through the sample.

10. If required, adjust the horizontal (**H**) and vertical (**V**) anchor points or click on the line and drag to move it to a new position.

11. Modify the number of **Points** or the **Spacing** between the points in the line.

    This will not increase the length of the line. Reducing the point spacing will increase the number of points. Reducing the number of points will increase the point spacing.

12. If the Orientation of the line scan is set to Horizontal, adjust the **Rotation** angle (in degrees) of the line scan.

    If you selected Vertical as the Orientation you cannot set the Rotation angle, which is fixed at 0°.

13. Select **Display in Graph** if you want the spectra collected to be added to the Samples View.

    The Base Sample ID provides the name of the Samples View that the spectra will be added to.

    NOTE: You will not be able to select this option if your line scan exceeds a certain size limit. The individual spectra will still be generated but they will not be displayed in the Graph.

14. Select **Save as LSC** to collate your spectra into a single file that you can view using SpectrumIMAGE software.

    The *.lsc file will be generated with the same Base Sample Name as the spectra. For example [Base Sample Name].lsc.

15. Repeat steps 6–13 until you have added all the lines to the view that you require.

16. Select **Monitor** from the Measurement menu, or click  .

    NOTE: We recommend that you preview the spectrum to verify that the instrument parameters are suitable for your sample. If you have several line scans over a large area of sample, have widely spaced points within a line scan, or have a heterogeneous sample, it is advisable to check the instrument parameters in more than one position.

17. Adjust the **Laser power (%)** and **Exposure time** using the controls on the Live tab.

    Adjust the instrument parameters to give a well-resolved spectrum that maximizes signal quality and minimizes noise and to check for detector saturation.

18. To stop monitoring and scan the sample, select **Scan** from the Measurement menu, or click  .

    The data is collected at each point in the line. If **Display in Graph** is selected, the spectra are added to the Samples View. The color of the measured points of the line changes to green. The progress of the total scan is displayed and the progress of the current measurement is displayed. Once the points in the line have been measured you can access the Line Scan Settings, but you cannot modify them. The Description and the file header of each spectrum will include the stage position.

Resizing Line Scans

- To resize a line, click with the left mouse button and then drag to the required length.

  The current point spacing in the Line Scan Settings is maintained, and number of points in the line will be increased or decreased accordingly.

Rotating Line Scans

- Select the line you want to rotate by clicking with the left mouse button on one end of the line (the cursor changes to ) and dragging to rotate.

  OR

  Select the line and then enter a value for the **Rotation** in the Line Scan Settings section of the Setup XYZ Stage tab.

  You can only select a **Rotation** angle when the line orientation is Horizontal.

Moving Line Scans

- Select the line you want to move by clicking with the left mouse button, and then drag to a new position.

  OR

  Select the line and then enter the coordinates of the new **Anchor** position in the Line Scan Settings section of the Setup XYZ Stage tab.

Changing the Appearance of Line Scans

You can change the color of an individual map by clicking the right mouse button and selecting **Choose Color**. The Color dialog is displayed. Select a new color for your map and click **OK**.

NOTE: You can change the color of the line before and after the points have been scanned, but when scanned the color will default to green.

Deleting Line Scans

- Select the line you want to delete by clicking with the left mouse button, and then click the right mouse button and select **Delete Line** from the context menu.

  OR

  Select the line you want to delete by clicking with the left mouse button, and then click **Delete Line** on the toolbar.

  The line is deleted.

Deleting All Markers, Maps and Line scans

- Click **Delete All Shapes** on the toolbar and then click **Yes** to delete all markers, maps and line scans.

  All objects on the display are deleted.

Deleting All Measured Shapes

- Click **Delete All Measured Shapes** on the toolbar and then click **Yes** to remove all points that have already been scanned.

  Maps and line scans will not be cleared unless all the points have been scanned.

Additional Information

If you wish to halt the scan, the spectra collected so far will be added to the Samples View. If you click Scan again, the remaining points will be measured and the spectra added to the same Samples View.

You can add as many markers, maps and line scans as you want to your but the data points you add, the longer the scan will take.

If you close the software, any lines will be retained when the software is restarted. If you change Sample Holder on the Setup Instrument Basic tab, any line scans defined will be deleted.

Calibrating a Sample Holder

NOTE: This information is relevant only if you have a motorized stage.

Before using a sample holder, you need check that your sample holder is calibrated. This is important to ensure that the actual live video position agrees with the position displayed on the **Graphic** view.

To check that your sample holder is calibrated:

1. Ensure that the appropriate sample holder is selected in the Accessory section of the Setup Instrument Basic tab.
2. Select the Setup Sample Area XYZ tab or Setup Microscope XYZ tab, as applicable, and then click **Graphic**.
3. Ensure that the Graphic view displays the appropriate sample holder type and that all the wells are displayed.

   You will need to calibrate the sample holder if the Graphic image does not display all the wells in your sample holder.
4. Click **Move to first well** on the Setup XYZ Stage toolbar.

   The stage is directed to move to the center of the A1 well on the sample holder. You should see cross hairs positioned over the center of the A1 position. on the Graphic view.
5. Check that the snout or objective is positioned over the center of the A1 position.

   You can see visually if the snout or objective is roughly positioned over the A1 region of your sample holder. Then focus the **Live Video** image and see if you are at the center of the well. You can also use a Visible Image Survey to view a larger area to check that you are in the A1 well. You may find it useful to mark the A1 well on a multi-well plate to make it easy to identify.

NOTE: If you have a Raman microscope and are using standard objectives (as opposed to long working distance objectives) you will not be able to focus at the bottom of a multi-well plate.

If you find there is an offset between the snout of objective position and the position on the Graphic view, then you will need to calibrate your sample holder. It may be advisable to reset your stage first:

1. To reset the stage, remove the sample holder and, if you have a RamanStation 400 Series instrument ensure that the snout or polarizer is pointing vertically downwards.
2. Click **Toolbox** on the Setup XYZ Stage toolbar and then select **Reset Stage**.
3. Ensure that you have followed the instructions on-screen, and then click **OK**.

   The stage will move to the limits of the XYZ stage and then move to position A1.
4. Click **Remove Sample Holder**.

   The stage will then move to a safe position for installing the sample holder.
5. Replace the sample holder.
6. Click **Move to first well**.

   The stage is directed to move to the center of the A1 well on the sample holder.
7. Focus the **Live Video** image to help you find the center.

You can also use a Visible Image Survey to view a larger area to check that you are in the A1 well. You may find it useful to mark the A1 well on a multi-well plate to make it easy to identify.

> **NOTE:** If you have a Raman microscope and are using standard objectives (as opposed to long working distance objectives) you will not be able to focus at the bottom of a multi-well plate.

8. If there is still an offset, move the stage using either the joystick or Stage Position controls to position the cross hairs at the center of the first well (A1).

9. Click **Toolbox** on the Setup XYZ Stage toolbar and then select **Set First Well** to set the position of the center-point.

   If your sample holder has one well only, for example the Versatile - Microscope Slide, then you have completed the calibration. If you have a multi-well plate, continue at Step 10.

10. Move the stage to position the cross hairs at the center of the last well in the multi-well plate.

11. Click **Toolbox** and then select **Set Last Well**.

    The new center-points are used to calculate the center positions of all the wells in the well plate.

    The wells of the multi-well plate should now be displayed correctly and the position on the graphic correspond to the snout location.

Align Video with Laser

The cross hairs indicate the position of the video camera. If there is an offset between the cross hairs and the position of the laser spot, the cross hairs no longer represent the position where data will be collected. You can adjust this offset using the Align Video with Laser option available on the Setup Sample Area XYZ Stage tab, the Setup Microscope XYZ Stage tab or the Setup Microscope tab.

1. Select **Raman Instrument** from the Setup menu.

   OR

   Select  or  in the **Setup** section of the Navigation Pane.

   The Setup Instrument tabs are displayed in the Dialog Pane.

   > **NOTE:** To see the tabs, you may have to resize the Dialog Pane.

2. Select the Setup Sample Area XYZ Stage tab or the Setup Microscope XYZ Stage tab, as appropriate.
3. Select a flat, non-reflective area of sample, and focus the video image.
4. Click  on the Setup XYZ Stage toolbar and then select **Align Video with Laser** from the sub-menu.

   The Align Video with Laser wizard and the Live Video view is displayed on the Setup XYZ Stage tab. The cross hairs on the Live Video view mark the position of the video camera and the laser spot shows the position of the laser.
5. Ensure that you have followed the instructions on the dialog, and then click **Next**.
6. Adjust the laser power to the lowest value that produces a visible spot and then click **Next**.
7. Using the controls on the Align Video with Laser dialog, move the cross hairs until they are in the center of the laser spot.

   To set the position of the cross hairs back to the original position, click **Reset**.
8. Click **Finish** to exit the wizard.

The position on the cross hairs is now updated on the Live Video image to match the laser spot position. The cross hairs, therefore, may not be at the center of the Live Video image. If the cross hairs are far from the center of the image, you can manually adjust the position of the alignment camera. Contact your PerkinElmer Service Representative.