DXRxi Raman Imaging Microscope—Quick Reference

Welcome to the Thermo Scientific™ DXR™xi Raman imaging microscope. The instrument combines rapid chemical characterization with high-end spectral performance. It has interchangeable lasers and their associated filters and gratings, and accommodates polarizers and various optical filters. The instrument offers standard (brightfield) reflected light sample illumination. Optional reflected light brightfield/darkfield and transmission illumination kits are also available.

The system runs with the Thermo Scientific OMNIC™xi Raman imaging software, a game-changing problem solving tool. You can use the software to collect *spectra* at selected sample locations and to acquire a quick preview *chemical image*. Then perform real-time collection of image data in multiple regions. Image data can be acquired in two planes—the sample surface or a cross section.

This document will get you started using the microscope and software and demonstrate how to run experiments using a provided sample and image file. The examples will help you learn to apply the instrument's features to your own analyses. These topics are covered:

Contents

Once you are familiar with the basics, you can explore the more advanced features. Complete instructions for the microscope and software are available in PDF format in the electronic documentation set or by clicking the Help ? button in the OMNICxi software. See "Find Information" in this document for details. A glossary of important terms is provided at the end of this document.

Note The information in this document is intended for typical users of a properly configured DXRxi system—that is, we assume the DXRxi instrument with OMNICxi software has been installed and its performance has been verified by a certified Thermo Scientific representative.

⚠WARNING

Avoid personal injury or equipment damage. Be sure that all persons operating this system read the site and safety manual first.

Safety Precautions

The DXRxi Raman imaging microscope may be configured as a Class 1 laser-safe system or as a Class 3B laser product.When configured as a Class 1 system, safety interlocks protect the operator from exposure to harmful laser radiation during normal operation and when performing configuration and maintenance procedures that are documented in the *DXRxi User Guide* and the OMNICxi Help system.

WARNING Avoid eye injury.

- Do not use magnets near the instrument. Magnets can defeat safety interlocks causing emission of visible and/or invisible laser radiation when sample compartment doors are open.
- The system is a class 3B laser product emitting visible or invisible laser radiation when using the fiber optic accessory or operating with the microscope cover removed. Exposure to laser radiation could cause permanent eye damage.

For complete safety information, refer to the *DXR Site and Safety Guide*. A copy in PDF format is included in the provided electronic documentation set.

Start the System

❖ **To start the DXRxi system**

1. Make sure the desired laser and a compatible filter and grating are installed.

   For information on selecting and changing the laser, filter or grating, refer to the OMNICxi Help system.

2. Make sure the power strip for the microscope and all of its components is plugged into a wall outlet.

3. Power up the instrument.

   Turn on the power button for the instrument power strip.

   Open the sample compartment doors on the microscope and remove any sample or other items from the stage.

NOTICE The microscope stage moves extensively during power up and initialization. Items left on the stage during this process could cause damage. Anything that protrudes above the surface of the stage may interfere.

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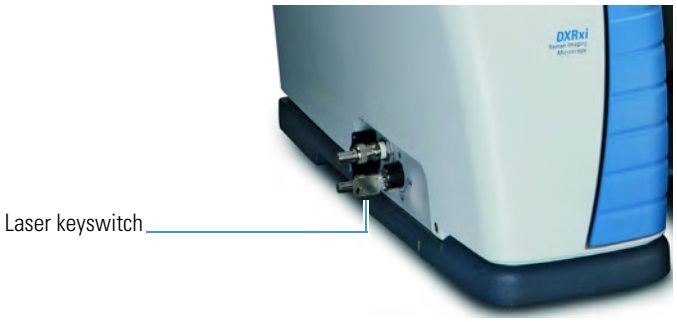
Hold down the instrument power button for 3 seconds. The power indicator LED should light.

Figure 1. DXRxi instrument power button and power indicator LED

Make sure the laser keyswitch is in the unlocked position.

Figure 2. DXRxi microscope laser keyswitch

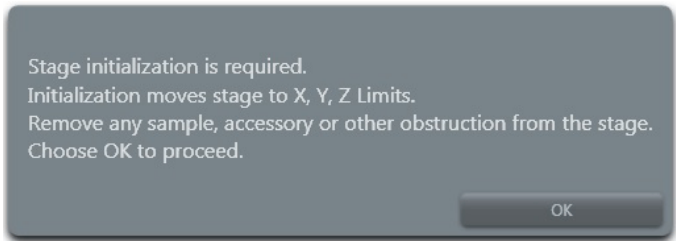


4. Power up the computer and the display monitor.

CAUTION Avoid pinch hazard. Keep hands free of moving parts in the sample compartment after you power up the instrument and start the OMNICxi software.

5. Double-click the OMNICxi program icon on the computer desktop to start the OMNICxi software.

   A message is displayed asking you to wait while the software initializes the instrument's motorized components (this takes about 2 minutes). **Wait until initialization completes!**



When you start the OMNICxi application, two windows are opened:

- the **Acquisition window,** for data collection and on-the-fly analysis. See "Collect and Analyze a Basic Sample."
- the **Analysis window,** for viewing and processing previously acquired image data. See "Open and Reanalyze an Image File."

Raman Sampling

Monochromatic light from the Raman excitation laser is directed to the sample. When the light strikes the sample, the sample emits Raman radiation. This radiation travels through the microscope optics to the detector.

The Raman spectrum shows the inelastically scattered light over the wavelength range defined by the installed grating and thus can be used to identify the sample. Intensity is expressed in detector counts. Frequency is expressed in terms of wavenumber (cm^{-1}). In Raman spectroscopy, the x-axis is converted to Raman shift, which is a measure of the difference between the observed spectral bands and the wavelength of the excitation laser.

A visible light source is directed through the microscope optics allowing you to view the sample and locate areas of interest. To accommodate different sample materials and configurations, the visible light can be reflected off the sample or transmitted through it (requires the optional transmitted light illumination kit). If the system has the optional reflected light brightfield/darkfield (BF/DF) illuminator, observations can be made with brightfield (straight down) or darkfield (edge) illumination. For more information, find "Configuring Sample Illumination" in the OMNICxi Help system.

Collect and Analyze a Basic Sample

This section steps you through a basic analysis using a *silicon test sample* provided with the instrument. You will learn how to operate the microscope and use the data collection features of the software. The steps include:

- installing and focusing the sample
- collecting spectra at selected locations and optimizing settings
- acquiring a preview chemical image and optimizing settings
- selecting analysis regions
- collecting and analyzing the selected regions

Tip If this is the first time you are using the DXRxi system, we recommend that you read these instructions carefully and perform the steps as you go. All other information in the system documentation will assume you have completed this training.

Before you begin, check the microscope configuration. We used these components to perform the analysis:

- 532 nm laser with associated filter and a full range grating
- No optical filters or polarizers
- Standard single slide holder

For information about changing the laser, laser filter or grating or the sample holder for the microscope stage, click ? in the OMNICxi software and search for "Operating the Microscope."

Note If you are using a laser set that has a different excitation wavelength, the images you see in the software may look slightly different than the ones in this document (especially the intensities of spectra shown in the *spectral pane*).

❖ To analyze the silicon test sample

1. Power up the instrument and start the software.

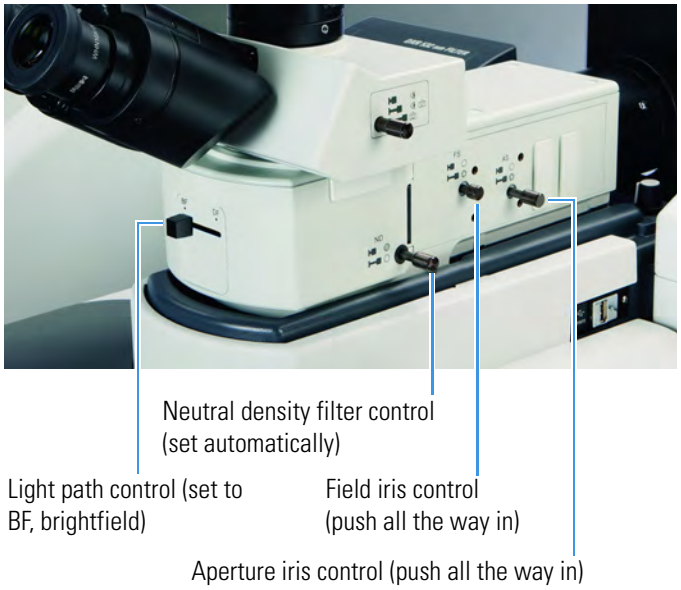
   If the instrument is not powered up, follow the instructions at the beginning of this document to start the instrument and software and initialize the sample stage.

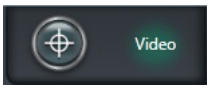
   If the instrument is already powered up, double-click the icon on your computer desktop to start the software.

2. Set up the microscope for reflected light brightfield illumination.

   If you have the optional reflected light brightfield/darkfield (BF/DF) illuminator, set the *light path* control to BF and push the *field iris* (FS) and *aperture iris* (AS) controls all the way in. (The *neutral density filter* control is set automatically.) For more information, find "Configuring Sample Illumination" in the OMNICxi Help system.

Figure 3. Sample illumination controls with optional BF/DF illuminator



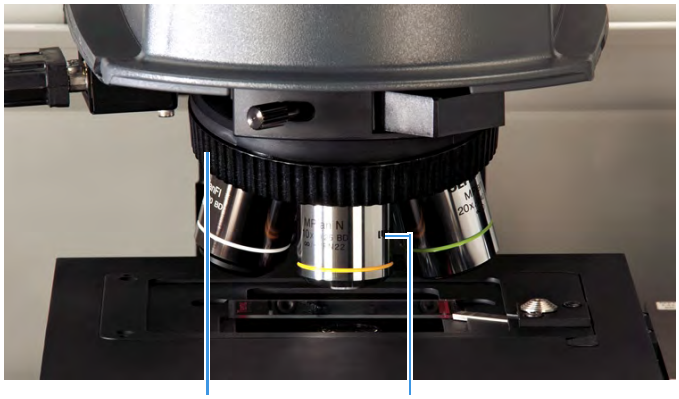
3. Click the **Video** 

button in the software to select *video mode*.

4. Select the 10x objective.

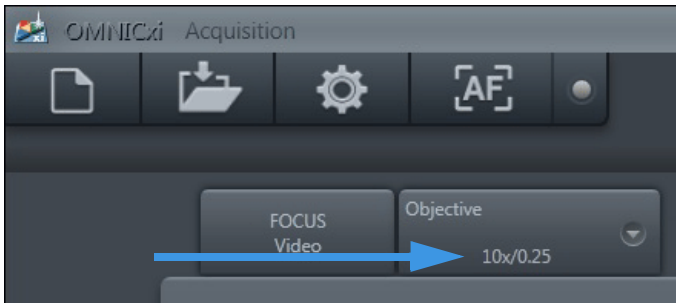
   Open the sample compartment doors on the instrument and rotate the 10x objective to the front objective position.

Figure 4. Rotate knurled ring to select 10x objective



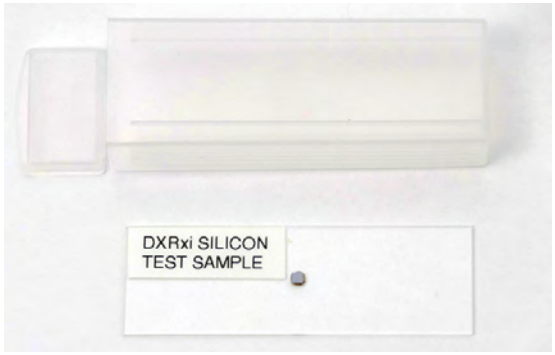
Select the *video calibration* for this 10x objective in the OMNICxi Acquisition window to synchronize the scales.

Figure 5. Select 10x objective video calibration in OMNICxi software



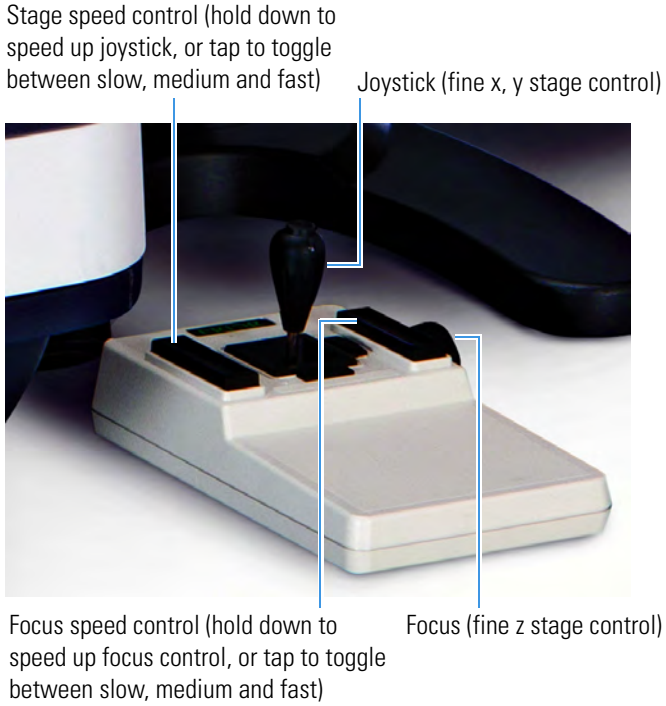
5. Load the silicon test sample that came with the DXRxi system onto the single slide holder on the microscope stage.

Figure 6. DXRxi microscope silicon test sample



NOTICE To avoid dropping the slide into the microscope, before you install the slide, use the focus knob on the stage controller to lower the stage (see figure 7). For help installing the slide, read about the "single sample slide holder" in the OMNICxi Help system.

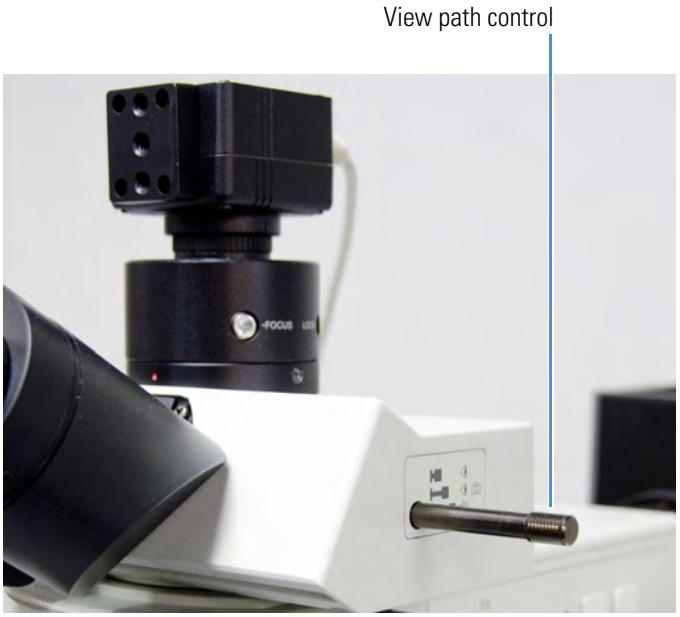
Figure 7. Using the stage controller



6. Set the view path control to its middle position.

   This allows you to see through the eyepiece on the instrument and simultaneously produce a *video image* in the software.

Figure 8. Set view path control to middle position



7. Use the microscope to locate and focus the sample.

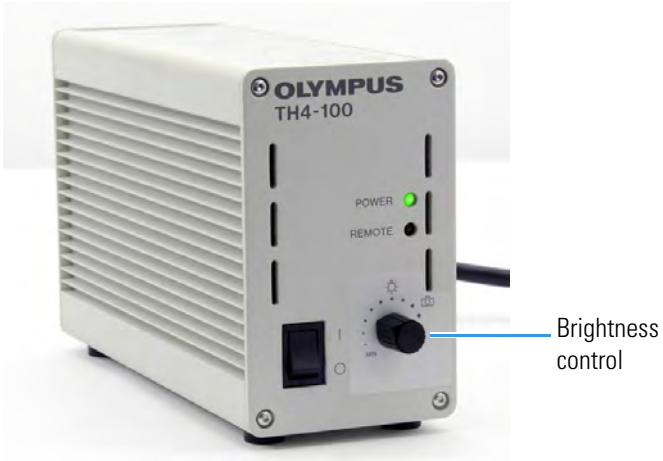
> **NOTICE** Do not allow the sample to touch the objective lens. It may damage the lens or the sample.

Use the joystick to position the sample under the objective and the focus control to focus the image (see Figure 7).

> **Tip** For quicker focusing, first watch the stage directly (not through the eyepiece) while you use the joystick to position the sample under the spot of white light. Then use the focus control to adjust the stage height until the spot appears in focus at the sample surface. Then look through the eyepiece while you center and focus on features of the sample.

You may need to adjust the brightness to bring the sample into view. If you have the optional reflected light BF/DF illuminator, the brightness control is on the illuminator power supply.

Figure 9. Brightness control for reflected light BF/DF illuminator



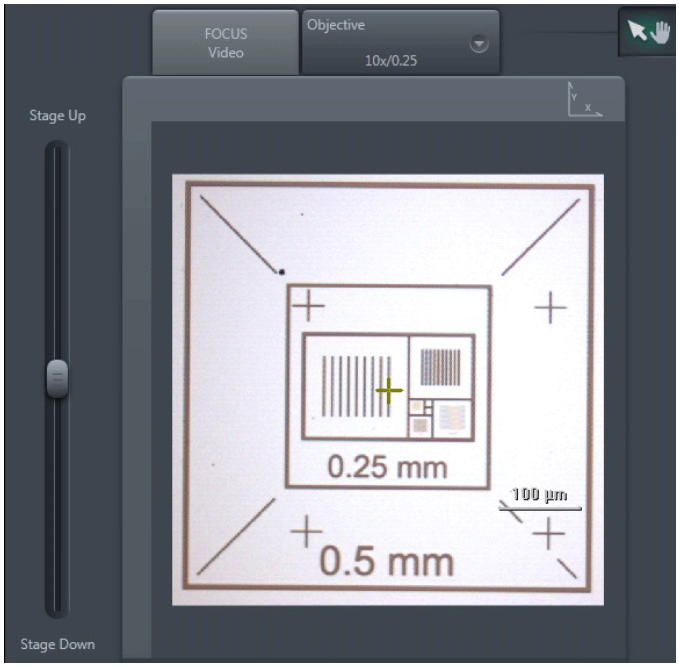
Otherwise, the brightness control is on the microscope. For more information, find "Configuring Sample Illumination" in the OMNICxi Help system.

> **Tip** Make sure a distinct feature is centered under the cross hairs when viewed through the eyepiece before you continue, especially if you plan to switch to a high magnification (100x or higher) objective. This ensures the sample stays in the new objective's field of view.

8. View the focused video image in the software.

   When a sample is positioned under the objective, a live *video image* of the sample surface appears in the left (video) pane in the Acquisition window. (If the image is faint or missing, use the microscope brightness control to bring it into view.) Here is a focused image of the silicon test sample in the video pane.

Figure 10. Focused video image of test sample at 10x magnification



The *video image* shows approximately one field of view using the selected objective.

The test sample is fabricated from a silicon wafer that has been patterned with an array of metallized features. The features are spaced in precise intervals which can be used to verify imaging performance. The sample is ideal for training purposes because it is easy to focus on and reference specific features.

Click **Zoom To Region**  to center the optical image in the right (mosaic) pane. The *optical image* shows what you see when you look through the microscope eyepiece (one field of view).

Figure 11. Optical image of silicon test sample at 10x magnification

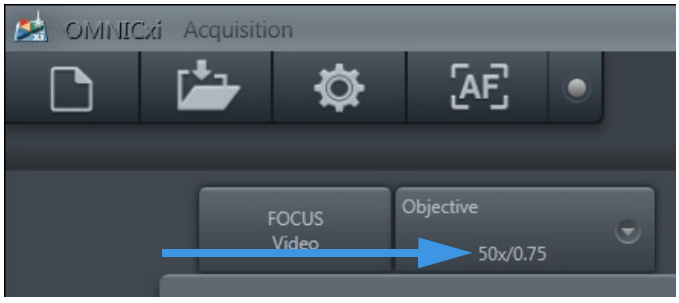
9. Change to a higher magnification objective if available.

   If you have a 20x or higher magnification objective, rotate it to the front of the microscope.

   Select the video calibration for the new objective in the software to synchronize the scales. For this demonstration, we used a 50x objective.

> **Tip** If the objective you selected on the microscope isn't listed under the Objective option in the software, create a *video calibration* for that objective. See "Creating a New Video Calibration" in the OMNICxi Help for instructions.

Figure 12. Select 50x objective in OMNICxi Acquisition window

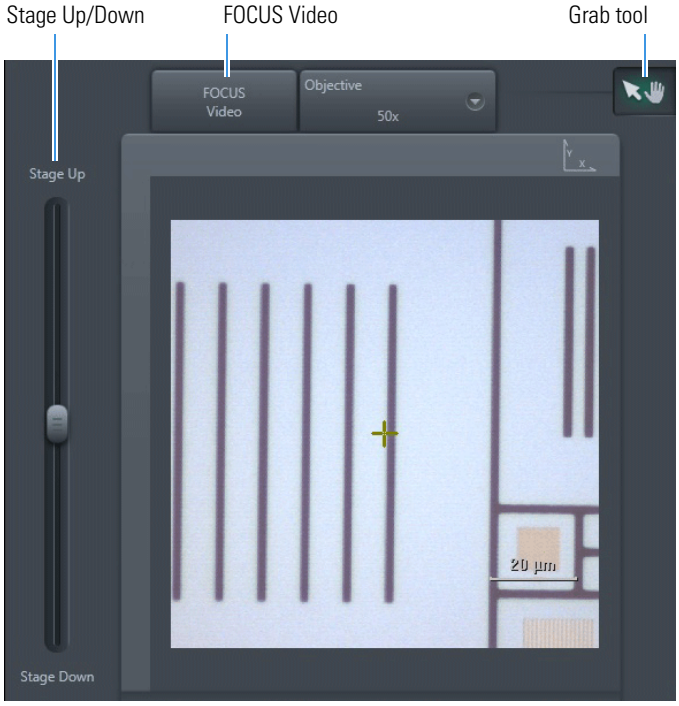


10. Bring the sample into sharp focus (adjust brightness as needed).

    To quickly bring the sample into focus, click **FOCUS Video** in the software. The sample should still be centered under the new objective and focused in the left (video) pane.

Here is the same sample at 50x magnification.

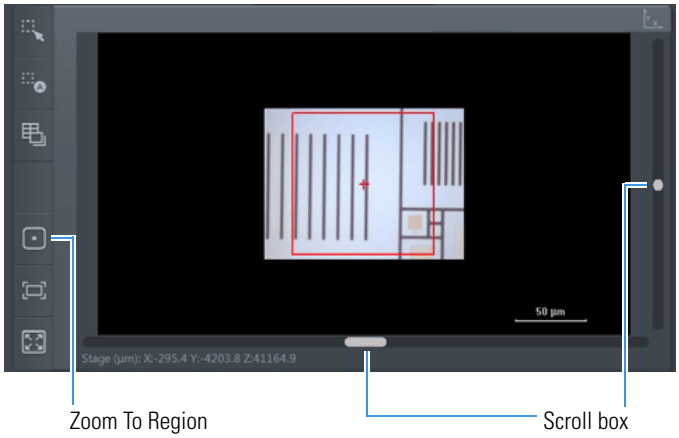
Figure 13. Focused video image of test sample at 50x magnification



> **Tip** There are several ways to bring a sample into focus:
>
> - Using the **Focus control** on the stage controller while viewing through the microscope eyepiece
> - Using the **Stage Up/Stage Down** control in the software (see figure 13). Drag scroll box up/down for coarse focus. Click directly above/below scroll box for fine focus.
> - Using the **scroll wheel** on the mouse when the video pane and Grab  tool are active
> - By clicking the **FOCUS Video** button in the software

11. Click **Zoom To Region**  in the software to center and scale the optical image.

Figure 14. Optical image of test sample at 50x magnification



Tip There are a several ways to zoom the optical image:

- By dragging one end of the **scroll box** at the right edge of the pane (see figure 14)
- Using the **scroll wheel** on the mouse when the pane and Grab tool are active
- By clicking the **Zoom To Region** button in the software

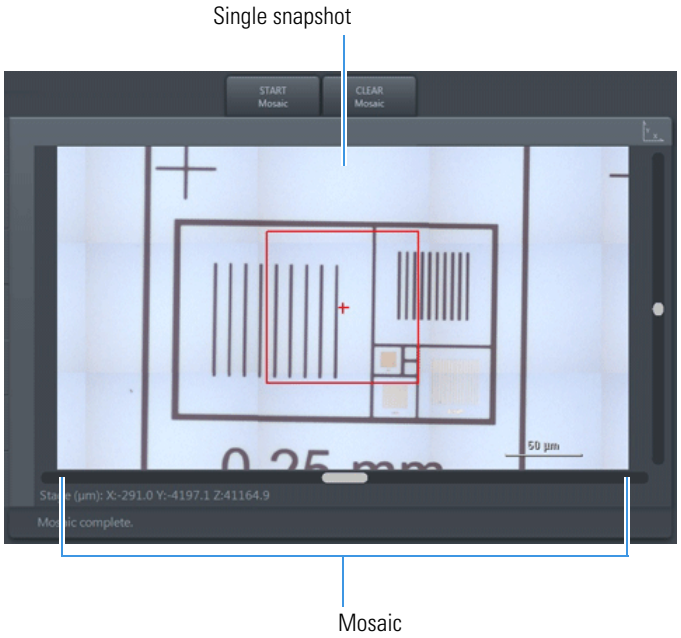
To reposition the optical image in its pane, drag a scroll box.

12. Collect a mosaic.

    Click **START Mosaic** START Mosaic.

    The microscope stage begins moving the test sample in a spiral pattern while the camera takes static pictures of the sample so you can view a larger area in the software. The pictures appear in the right (mosaic) pane.

Figure 15. Mosaic shown in right (mosaic) pane



The pictures are stitched together with the edges slightly overlapped to ensure that all areas are captured. The software continues collecting pictures until the mosaic pane (at the current zoom level) is filled or until you click **STOP Mosaic** in the software. We refer to the combined pictures as a *mosaic*.

Note If your sample is large, you can collect a mosaic before you switch to the higher magnification objective. Collecting the mosaic with a low magnification objective is faster and uses less computer memory. However, the resulting mosaic may not show sufficient detail if the sample or features of interest are very small.

Tip Explore the mosaic features further by using the buttons above the right pane to start, stop, restart and clear the mosaic. Allow the final mosaic to complete before you proceed to the next step.

13. Use **Live Spectrum mode** to determine appropriate acquisition settings for the test sample.

    Close the sample compartment doors on the instrument.

Tip The system can collect Raman data only when the laser is directed onto the sample. For your protection, the laser light is blocked when the sample compartment doors are open.

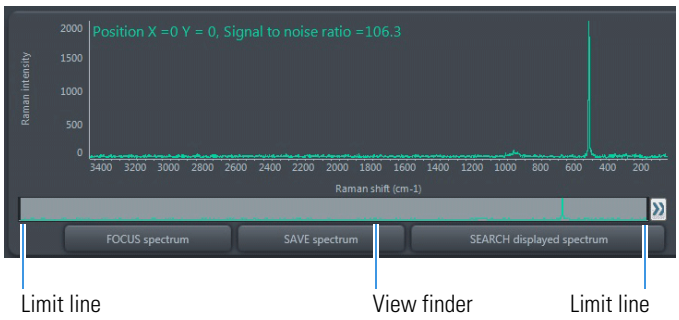
Click Live Spectrum.

Select the **Grab tool**.

Set **Laser power** to high (10 mW for the 532 nm laser set).

Click a point in the silicon (background) portion of the test sample *mosaic*. A spectrum similar to the following should appear in the *spectral pane*.

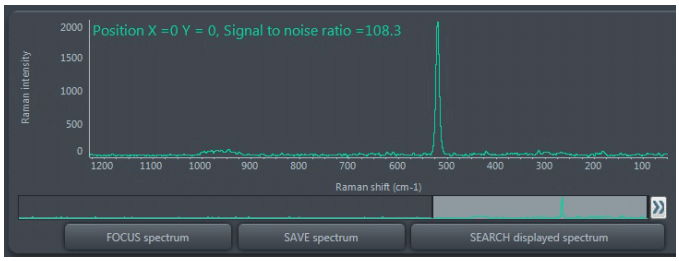
Figure 16. Spectral pane showing silicon spectrum collected in Live Spectrum mode (before optimizing settings)



Tip You can drag the displayed spectrum left and right to pan the x-axis, or up and down to expand or contract the y-axis. To display the original spectrum, **double-click** the spectral pane. To autoscale the vertical axis, **right-click** the spectral pane, choose **Display** and select **Autoscale**. More display options are available in the right-click menus.

Zoom in on the large silicon peak at 521 wavenumbers (cm^{-1}). To zoom in, use the mouse to draw a box around the peak in the *spectral pane* and then click inside the box. You can also zoom by adjusting the vertical limit lines in the view finder (see the previous image). The zoomed in image will look something like this:

Figure 17. Spectral pane showing close up of silicon peak



Adjust the Laser power, Exposure time, Number of scans and Aperture settings to achieve the spectral quality you need. Each time you change a setting, the software immediately refreshes the collected spectrum at the current cursor (and stage) location. The updated spectrum appears in the spectral pane.

We used these settings for the test sample.

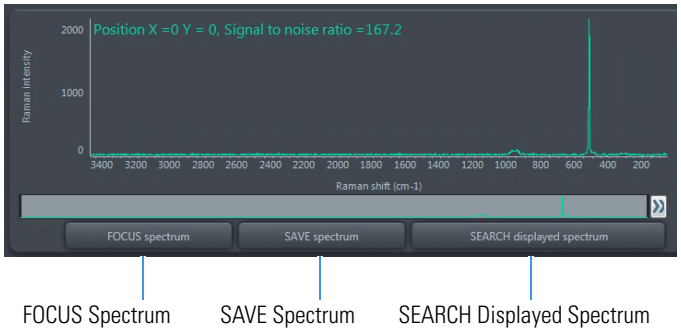
Table 1. Live Spectrum mode acquisition settings for silicon test sample

Feature	Description	Setting
Laser power	Laser energy at the sample (0 blocks the laser)	10.0 mW[a]
Exposure time	Laser exposure of each collected spectrum	.002 sec[b]
Number of scans	Number of scans used to produce each spectrum	4[c]
Aperture	Size of Raman beam entrance slit or pinhole	50 micron slit[d]

[a] Use highest available Laser power setting that doesn't burn the sample.
[b] Lower Exposure time settings may improve spectral quality for some samples.
[c] Multiple scans are averaged.
[d] 50 micron slit allows maximum Raman energy at the detector.

Here is an optimized silicon spectrum. The shape of the peak at 521 cm^{-1} is better and there is less noise in the baseline region.

Figure 18. Spectral pane showing optimized silicon spectrum collected in Live Spectrum mode



Tip

- To bring the sample into sharp focus at each clicked location, click **FOCUS Spectrum**.
- To save the displayed spectrum, click **SAVE Spectrum**.
- To search the displayed spectrum against the available spectral libraries, click **SEARCH Displayed Spectrum**. A search results window appears above the button. The name of the best matched spectrum from the available spectral libraries appears at the top of the search results window and its spectrum is added to the spectral pane. After you close the search results window, the library spectrum disappears from the spectral pane.
- To correct a curved baseline, click **Settings**, select **Correct Baseline** and select a correction strength that matches the degree of correction required.

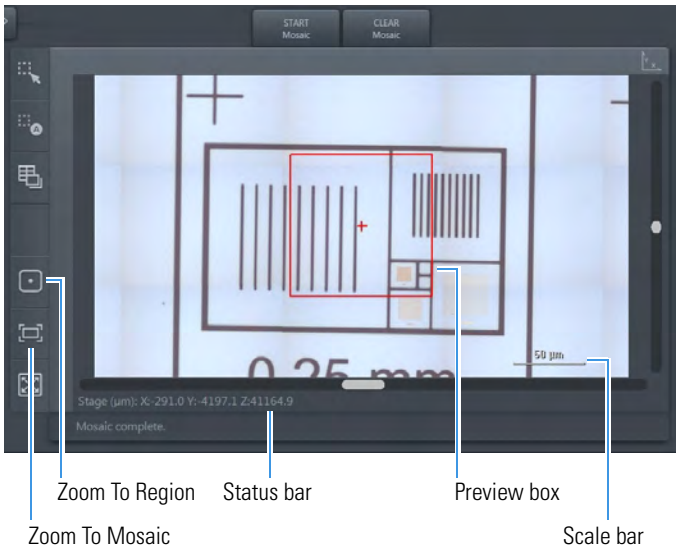
14. Select a region that will be used to scan a preview chemical image.

Click **Video** 

to return to *video mode*.

Click **Zoom To Region**  to center and scale the *mosaic*.

Figure 19. Mosaic pane with mosaic and preview box



The box in the center of the mosaic is called the *preview box*. It defines the region that will be used to scan a quick preview *chemical image* of a representative area of the sample.

The *status bar* shows the X, Y and Z coordinates of the microscope stage and the current location of the cursor.

Center a small region of interest in the preview box. If possible, select a region that is representative of all the regions you plan to analyze.

Use these features to manipulate the preview box.

Tool	Description
Grab 	Moves or resizes the preview box: • To move the preview box, drag the "+" in the center of the box (doing this also moves the microscope stage and updates the video image in the left pane). • To resize the preview box, drag an edge.
Zoom To Region 	Zooms and centers the mosaic and any selected areas (such as the preview box) in the right pane.
Zoom To Mosaic 	Zooms to show the full mosaic in the right pane. If there are multiple mosaics, this tool zooms out so that all mosaics are visible.

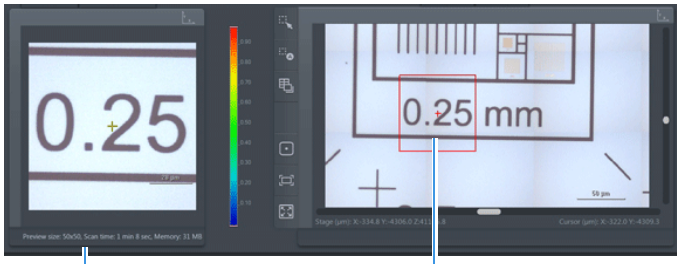
Tip Take a moment to click several locations in the mosaic. Notice that each time you click, the preview box moves to that location and the stage coordinates in the status bar are updated. The video image (left pane) shows the selected area in more detail.

Now drag the image in the left (video) pane and notice that the preview box (right pane) moves to that location and the stage coordinates are updated.

The limits of the video pane are defined by the magnification and aperture opening of the objective. The scale bar is helpful for estimating the actual size of features in the sample.

For this demonstration, we selected the preview region shown below.

Figure 20. Preview region



This region will be scanned in the next step and the results used to optimize the *Image pixel size* setting for the final collected data. Image pixel size specifies the distance between sampling points in the chemical image. It determines the ability to distinguish small details in the sample. It also affects the length of time and amount of computer memory needed to collect the image, and the size of the image file if you save it.

Start with these settings (leave the other settings alone).

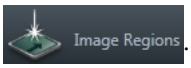
Table 2. Acquisition settings for preview scan

Feature	Description	Setting
Image pixel size	Distance between sampling points	1.6 µm
Profile	Defines the mathematical algorithm used to create the chemical image	Correlation

Tip Before you scan the preview region, the status bar below the video pane tells you the size of the preview region, the estimated time required to scan the data and the amount of computer memory needed to store it. These values update each time you change the boundaries of the *preview box* or the *Image pixel size* setting to help you evaluate the impact on time and available memory.

15. Use Image Regions mode to scan the preview region.

    Make sure the sample compartment doors are closed.

    Click **Image Regions** 

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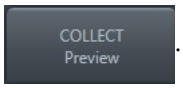
    Click **COLLECT Preview** 

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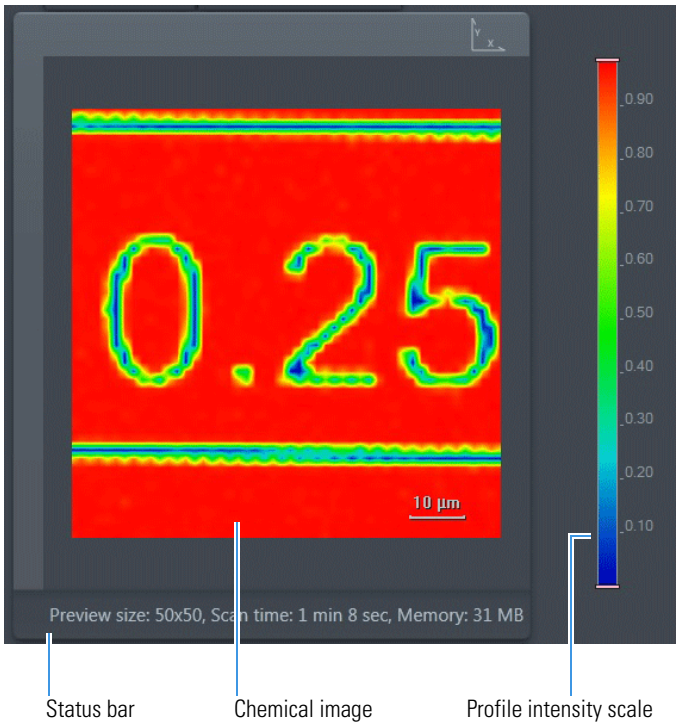
    The software begins creating a *chemical image* of the *preview region*. The image is displayed in the left pane.

    The chemical image contains a single (or average) spectrum collected at each sampling point. The number and locations of the spectra that make up the chemical image are defined by the *Image pixel size* setting. The number of passes is defined by the *Number of scans* setting. A mathematical algorithm (defined by the selected Profile) is applied to each spectrum to create the chemical image.

    During the preview scan, the *status bar* shows the start time, percent completion and total time for each scan and the estimated time to complete all requested scans. Wait until all four scans are completed. Our preview scan is shown below.

Figure 21. Chemical image of preview region

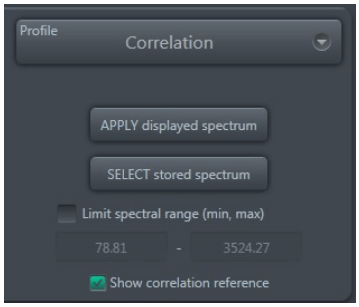


When the Correlation (default) profile is selected, the chemical image shows the correlation between the acquired spectra and the current reference spectrum. This lets you find the locations of the reference compound (or similar compounds) in the sample. The *default reference spectrum* is the first acquired spectrum (taken in the lower left corner of the preview box).

The *profile intensity scale* shows the scale and corresponding colors used by the correlation algorithm (right-click it to customize the colors). Each color represents a degree of correlation with the

reference spectrum. Spectra that are exactly like the correlation reference have a correlation value of 1.00 on the profile intensity scale and are displayed in the color at the top of the scale. The Correlation profile settings are shown below (we used defaults).

Figure 22. Correlation profile settings

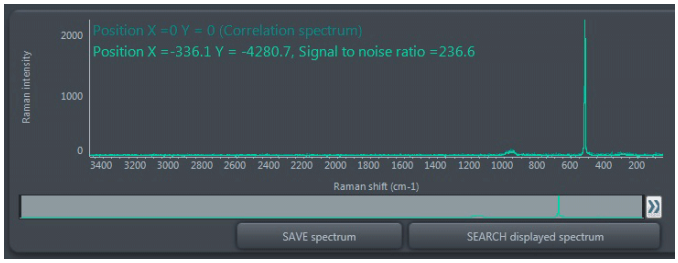


Tip

- Try changing the correlation reference and notice its effect on the chemical image. (Changing the reference won't force the image to redraw.) To change the reference, click the **Spectrum tool**  , then click a point in the preview image and choose **APPLY displayed spectrum** under the Correlation profile settings. If you select a (silicon) background point on the test sample, the correlation image will look similar to the one in Figure 21. If you click a point directly on a metallized (gold or chromium) feature, such as the "2" in figure 21, the colors are reversed. (Keep in mind that metals do not produce a Raman spectrum so ignore the data in the spectral pane.) Make sure a silicon correlation reference is selected before you proceed.
- Drag the lower limit of the profile intensity scale to a correlation value of about 0.5. This changes the image to show only areas of high correlation. (It can improve contrast especially for a sample that has a small number of components like the silicon test sample.)

Click the **Spectrum tool**  and then click a point in a high correlation (red or orange) area of the chemical image. (You don't have to wait until the image is completed to do this.) The spectrum collected at that time and location appears in the spectral pane.

Figure 23. Spectral pane showing preview spectrum from a background (silicon) area of the sample



To search the displayed spectrum against the available spectral libraries, click **SEARCH Displayed Spectrum**. The search results window appears above the button. The name of the best matched spectrum from the available spectral libraries appears at the top of the search results window and its spectrum is added to the spectral pane. Click Search Displayed Spectrum again to close the search results window. The library spectrum disappears from the spectral pane. For more information, see "Run a Spectral Search" in this document.

To see the correlation reference spectrum, make sure **Show correlation reference** is selected in the Correlation profile settings. The reference spectrum is added to the spectral pane (along with the spectrum at the current cursor location).

16. Adjust the settings if needed to optimize the image data.

    Update the data acquisition settings as needed to optimize the image quality, for example to sharpen the image or show more or less detail. For this example, we changed the following settings:

Table 3. Acquisition settings for final chemical image

Feature	Setting
Image pixel size	0.5 µm
Aperture	25 micron confocal pinhole[a]

[a] Maximizes the spatial resolution at this objective magnification.

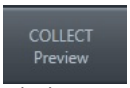
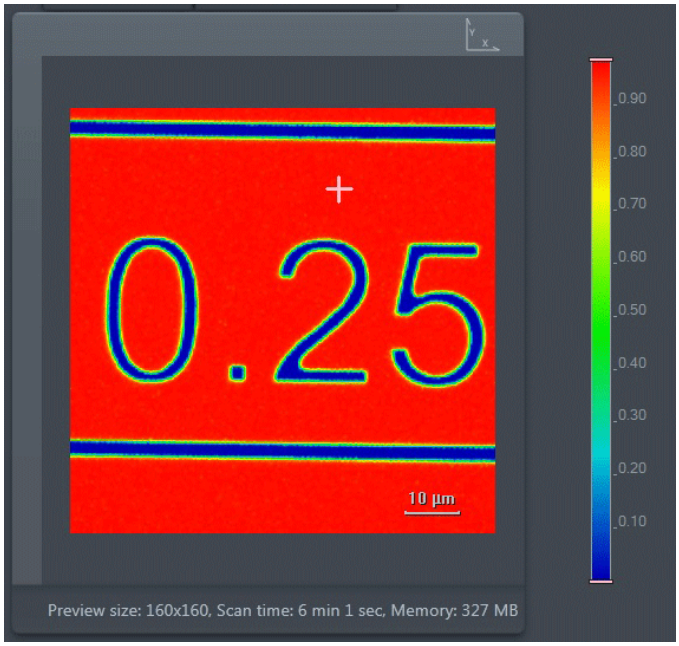
After you change a setting, click **COLLECT Preview**  to redraw the image so you can see any improvements in image quality. Here is our optimized chemical image.

Figure 24. Optimized chemical image



Tip At this point, you can continue optimizing data acquisition settings and redrawing the chemical image as needed until the desired image quality is achieved. Wait until all requested scans are completed before proceeding.

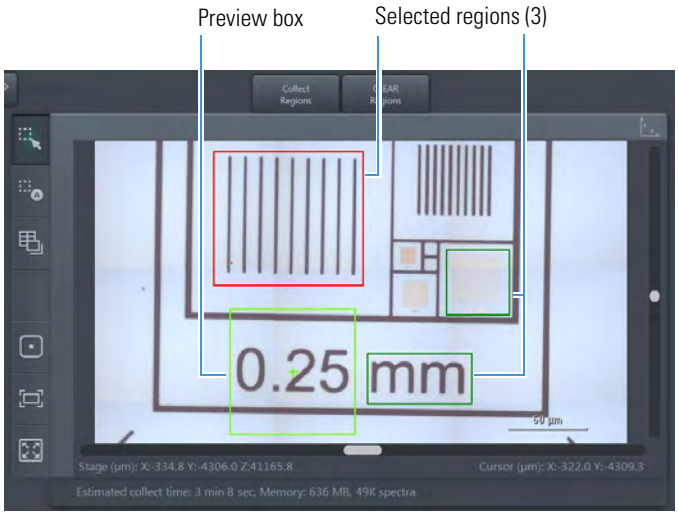
17. Select one or more regions for further analysis.

    Click **Manual Regions** and use the mouse to draw a box around several regions of interest in the *mosaic*.

> **Tip**
>
> - Each region has associated acquisition settings. To specify a setting, such as Number of scans, for a particular region, adjust the setting and then draw the region.
> - To view the settings for a drawn region, select the region. Its settings are shown in the Acquisition Settings panel.
> - Use **CLEAR Regions** to delete all drawn regions.

For this demonstration, we selected the regions shown below. The currently selected region is shown in red. The light green box is the *preview box*.

Figure 25. Mosaic pane showing regions selected for data acquisition



> **Tip** You can also define or edit regions by entering their X, Y, and Z coordinates and measurements. Click **Region Queue** to see the current list of regions.
>
> To add a region, click + and enter its coordinates.
>
> The region list—including all associated collection parameters—can be saved and reloaded for use with subsequent samples. (This is similar to saving an experiment in the OMNIC Workstation for DXRxi Raman Imaging Microscope software.)
>
> To close the Region Queue window, click the Region Queue button again.

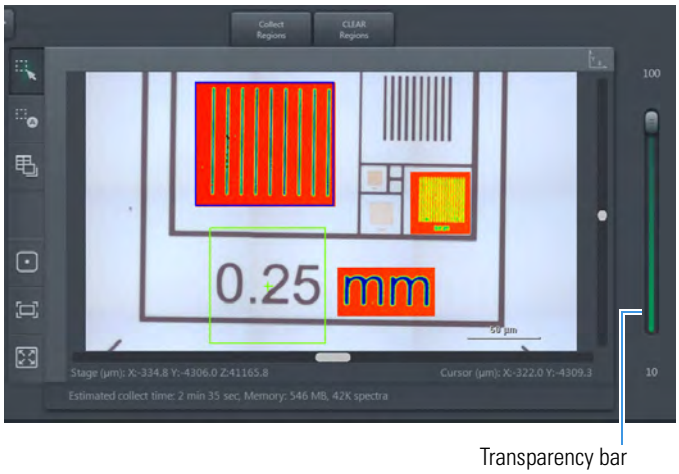
18. Collect a detailed chemical image of each selected region.

    Click **COLLECT Regions** Collect Regions.

    The regions are collected in the order in which they were created (and listed in the region queue). The chemical images appear on top of the mosaic. Wait until all requested scans are completed for all of your selected regions.

> **Tip** Use the *transparency bar* (see the next image) to change the transparency of the chemical images so you can view features in the mosaic.

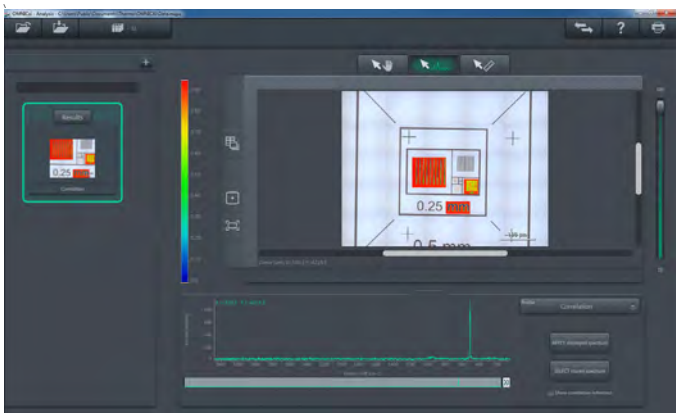
Figure 26. Detailed chemical image of selected regions



The collected data also appear in the *Analysis window*, which can be used to perform other types of analyses with the data or to search sample components against a spectral library.

> **Tip** If the system computer is connected to one display monitor (rather than two), use the button in the upper right corner of either window to switch between the *Acquisition window* and the *Analysis window*.

Figure 27. OMNICxi Analysis window showing final collected regions



19. To save the acquired data, click **Save Current Data**, select a file format (.MAPX or .H5) and click **Save**.

    For more information, see "Save Data" in this document.

> **Tip** To collect data from another sample, click **New Sample** in the Acquisition window. This clears the mosaic and discards all data stored in the computer's internal memory. Then load the new sample onto the microscope stage.

Open and Reanalyze an Image File

When you start the OMNICxi application, two windows are opened, the Acquisition window and the Analysis window. This section explores the purpose and main features of the Analysis window, which can be used for additional processing of acquired data. We will use an example data file that is included with your OMNICxi software.

This section covers these topics:

- Display the Analysis Window
- Open an Image File
- Tools for Working with Displayed Data
- Generate Several Profiles for Visual Comparison
- Generate an MCR Analysis Profile

Display the Analysis Window

When you start the OMNICxi software, it opens the Acquisition and Analysis windows. If your computer has a single display monitor, use the button to switch between the two windows. The button is located in the upper right corner of both windows.

Open an Image File

From the OMNICxi Analysis window, you can open a stored image file, view or reanalyze the data and save the file with additional profile information. The original acquired data remains intact. Stored image files include the mosaic if one was created, the acquired data, the acquisition settings for each region and any applied profiles and associated profile settings.

NOTICE If you attempt to open an image file in the Analysis window when the window contains previously collected but unsaved image data from the Acquisition window, the software will ask if you want to save the acquired data before it opens the selected file. After the file is open, the data collection features in the Acquisition window will be disabled.

To enable the data collection features, click **New Sample** in the Acquisition window. The software will ask if you want to save the data in the Analysis window before it clears both windows for data collection.

❖ **To open an OMNICxi image file**

1. From the Analysis window, click **Open Data File** .
2. Select the format of the image file to be opened.
3. Select the image file.
4. Click **Open**.

Tip To set the default folder and path where OMNICxi opens and saves image files, click **Settings** in the Acquisition window.

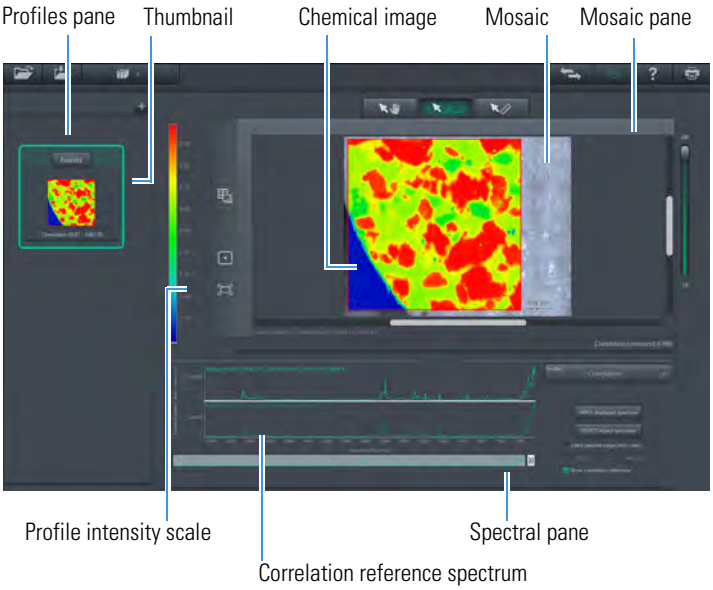
A status bar appears above the mosaic pane. If the file is large, it may take a few seconds to open. If one or more profiles were run on the data, the profile results are recreated and will take additional time.

If the file includes a mosaic, the *mosaic* appears in the *mosaic pane*. If the file contains acquired image data, a *chemical image* of each acquired region also appears in the mosaic pane (on top of the mosaic). Profile results appear in the chemical image and in the corresponding *thumbnail* in the *profiles pane*. All of these items are identified in figure 28.

Try this!

Open the file OMNICxi Tablet Sample.MAPX, which is included with the OMNICxi software (in the default folder for storing image files). The file contains data collected from a common pharmaceutical tablet and correlation profile results. Here is the data from the demonstration file displayed in the Analysis window.

Figure 28. Analysis window with opened image file



The *mosaic* and the *chemical image* for the correlation profile appear in the *mosaic pane*. The *thumbnail* for the correlation profile appears in the *profiles pane*. The *correlation reference spectrum* appears in the *spectral pane*.

Tip To see the settings used to acquire the chemical image, click **Region Queue** .

To close the Region Queue window, click the Region Queue button again.

The chemical image shows the correlation between the collected spectra and the current reference spectrum. This lets you find the locations of the reference compound (or similar compounds) in the sample. Areas of the chemical image that are similar to the correlation reference have a correlation value of 1.00 on the *profile intensity scale* and are displayed in the color at the top of the scale.

Select the **Spectrum tool** and click a point in the chemical image to show the spectrum acquired at that location in the spectral pane.

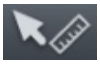
To see search results for the spectrum at the current cursor location, click the **Results** button above the corresponding thumbnail. The search results window appears below the button. The name of the best matched spectrum from the available spectral libraries appears at the top of the search results window and its spectrum is added to the spectral pane. After you close the search results window, the library spectrum disappears from the spectral pane. For more information, see Run a Spectral Search.

Tools for Working with Displayed Data

The Analysis window provides these tools for working with displayed image data.

Try these!

Table 4. Tools for working with a mosaic or chemical image in the mosaic pane

Tool	Can be used to...in the mosaic pane
Grab 	• **Drag the zoomed in mosaic or chemical image** within the pane (use the scroll wheel on the mouse or the scroll bars to zoom the image in or out).
Spectrum 	• Click **points** in the chemical image **to view the associated spectrum**. The spectrum appears in the spectral pane. • Select **areas** in the chemical image **to view the associated average spectrum** in the spectral pane (use the mouse to draw a box around the area to select it).
Ruler 	• Measure the length of features in the chemical image or mosaic. (Click to define the start of the measurement, then drag to the end point. The length in µm (or mm) is displayed in the status bar below the mosaic pane.)

These features are also available to work with displayed spectra.

Table 5. Features for working with displayed spectra[a]

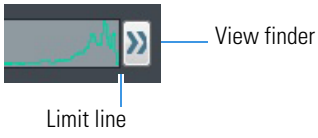
Feature	What to do...
Pan the x-axis	Drag displayed spectra left and right
Expand or contract the y-axis	Drag displayed spectra up and down
Zoom the x-axis to show more detail	• Draw a box around a region of a displayed spectrum and then click inside the box • Drag the vertical limit lines at each end of the view finder 

Table 5. Features for working with displayed spectra[a]

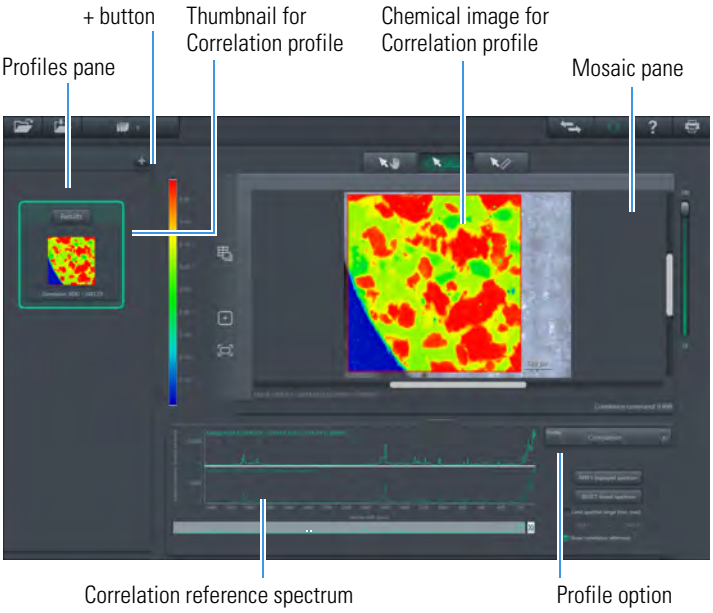
Feature	What to do...
Expand the x-axis to show entire spectra	• Double-click the spectral pane • Double-click between the limit lines in the view finder
Stack multiple spectra to view them individually	Right-click the spectral pane, choose **Display** and make sure **Stack spectra** is selected

[a] Right-click the spectral pane for more display options.

Generate Several Profiles for Visual Comparison

Our example data file includes a correlation analysis. The *chemical image* for the correlation profile appears in the *mosaic pane*. The *thumbnail* for the correlation profile appears in the *profiles pane*. The *correlation reference spectrum* appears in the *spectral pane*.

Figure 29. Analysis window with Correlation analysis profile



Follow these steps to generate another profile in order to visually compare the results.

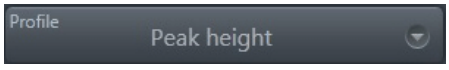
❖ To add a profile analysis to the example data file

1. Add a thumbnail to the profiles pane by clicking the  button above the pane.

   Make sure the new profile is selected before you continue.

2. Select a profile analysis type by clicking the **Profile** option.

   For this demonstration, we selected the Peak Height profile.

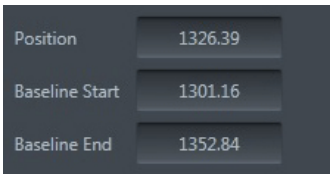
   

3. Select the **Spectrum tool**  , then click a point of interest in the chemical image.

   The spectrum collected at that location is displayed in the spectral pane.

4. Specify a peak for the peak height analysis.

   You can do this by entering values in the boxes, like this:



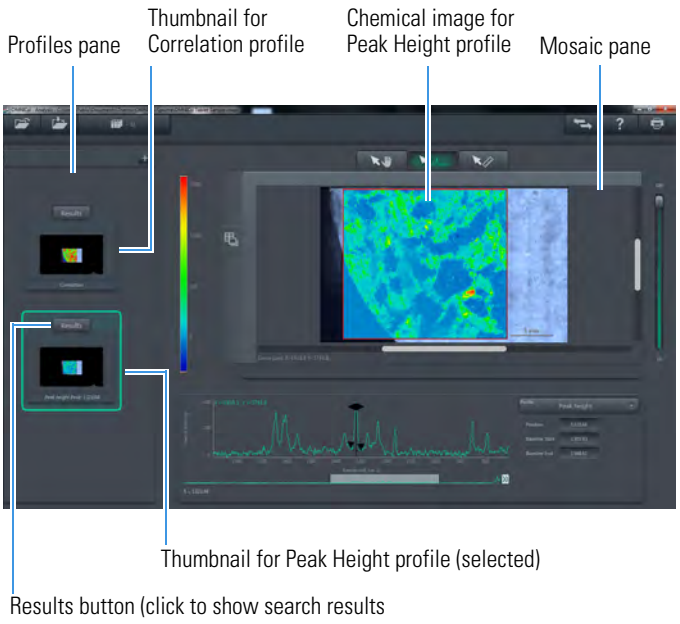
   or by using the tools in the spectral pane to mark the locations on the displayed spectrum:

> **Tip** To zoom the spectrum, use the mouse to draw a box around the spectral region, then click inside the box.

After you enter a value or move one of the selection tools in the spectral pane, the software starts calculating the new profile using the new settings. Each time you adjust a setting, the calculation immediately restarts.

The *chemical image* for the peak height profile appears in the *mosaic pane*. The *thumbnail* for the peak height profile appears in the *profiles pane*, below the Correlation thumbnail.

Figure 30. Analysis window with two profile analysis results



The *peak height profile* shows the corrected height of the specified peak for each sample point in the chemical image. Each color in the chemical image represents the relative intensity of the specified peak. A higher intensity value (closer to the top of the profile intensity scale) indicates a larger peak.

To see search results for the spectrum at the current cursor location, click the **Results** button above the peak height profile thumbnail. The search results window appears below the button. The name of the best matched spectrum from the available spectral libraries appears at the top of the search results window and its spectrum is added to the spectral pane. After you close the search results window, the library spectrum disappears from the spectral pane. For more information, see Run a Spectral Search.

You can add as many profiles as you like (only the profile specifications are saved with the data). The thumbnails let you compare profile results visually. All of the profiles are active and can be tweaked and quickly recalculated. For more information, find "Profile Settings" in the OMNICxi help system.

5. To save the example data, click **Save Current Data**  .

   The data are saved with the new profile information (that is, the number and type of profiles that were run and the settings that were used). The specified profiles are recalculated each time the file is opened.

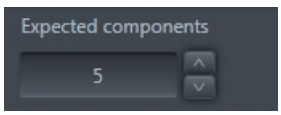
Generate an MCR Analysis Profile

Multivariate Curve Resolution (MCR) is a featured analysis type for image data. It lets you estimate which pure components are present in the image as well as the locations of those components in the sample. The calculated pure component spectra that result from MCR can be searched against spectral libraries for identification. This section provides an overview of the MCR profile analysis using our example data file.

❖ To run an MCR profile analysis on the example data file

1. Select the MCR profile analysis type.

2. Specify one more than the number of different components you expect to find in your sample (up to 32 components total).

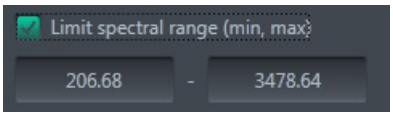


   For example, if you think your sample has four significant components, specify five components in the software. (The last component will show any residual information that is unaccounted for.)

> **Tip** MCR requires a large amount of computer processing power and speed. Depending on its capability, your computer may not be able to calculate more than four or five components. We recommend starting with a small number of components and increasing the number in increments of one.

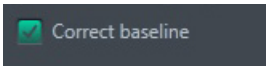
3. If you want to refine the spectral range used for the analysis (for example to ignore a high intensity peak that is not of interest), click **Limit spectral range** and either enter minimum and

maximum x-axis values in the boxes (in the displayed unit) or use the tools in the spectral pane to define the analysis region.



For our example data, we cut off the low end at 206 cm^{-1} to eliminate the totally absorbing region below that point.

4. If you want to apply a baseline correction to the sample data before performing the MCR analysis (and it was not corrected during data acquisition), select **Correct Baseline**.



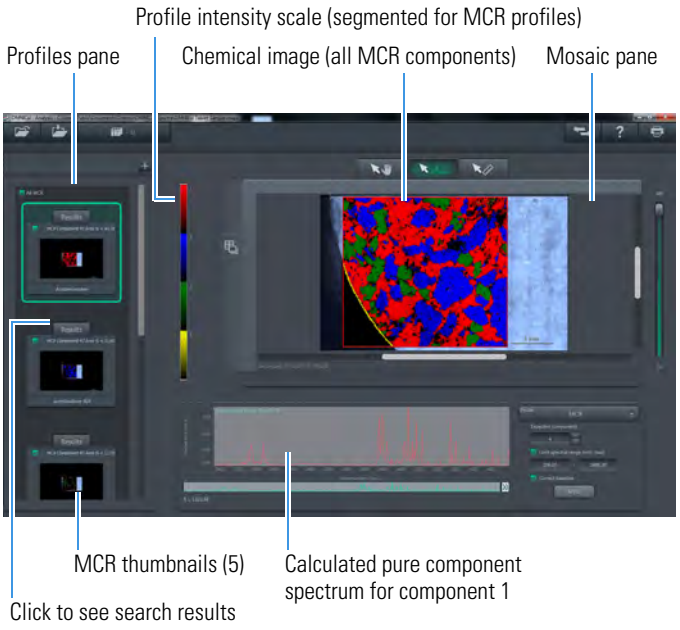
The feature applies a 4th order polynomial baseline correction to each spectrum in the chemical image. It can be useful for correcting any effects on the baseline curvature caused by the sample response. Fluorescence is a good example.

5. To start the MCR analysis, click the **APPLY** button below the MCR profile settings.

The length of time for the analysis depends on the number of specified components and the number of spectra in the chemical image. A status bar shows the progress. Wait until the analysis is completed.

The analysis results appear in the *mosaic pane* and at the top of the *profiles pane* (one MCR *thumbnail* for each found component).

Figure 31. Analysis window with MCR profile results



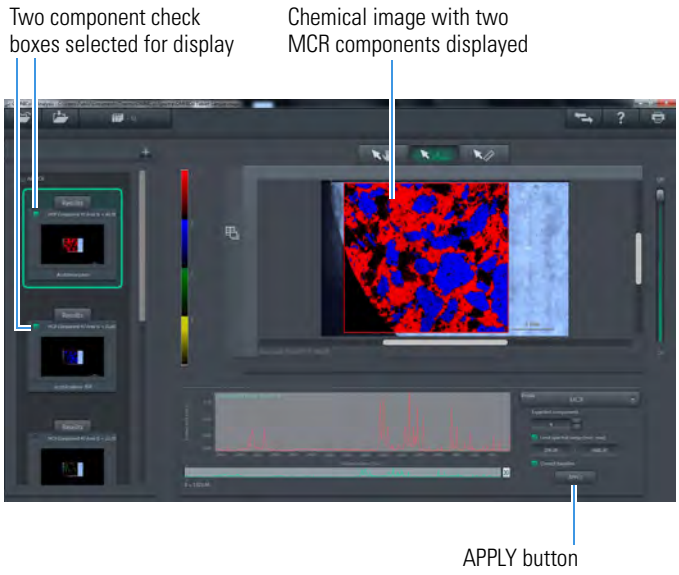
The thumbnails show the locations of the found components in the *chemical image*. To select a component, click its thumbnail in the profiles pane. The selected thumbnail is highlighted and its chemical image appears in the *mosaic pane*. The *profile intensity scale* is segmented to show the color associated with each found component in the chemical image.

The *spectral pane* shows the calculated pure component spectrum for the selected component. Select the **Spectrum tool** and click a point in the chemical image to add the spectrum at that location to the spectral pane.

If OMNICxi has associated spectral libraries, the found MCR components are searched against those libraries automatically. To see the search results for a found component, click the **Results** button in the component thumbnail. The search results window appears below the button. The name of the best matched spectrum from the available spectral libraries appears at the top of the search results window and its spectrum is added to the spectral pane. Click the Results button again to close the search results window; the library spectrum disappears from the spectral pane.

Use the **check boxes** in the thumbnails to add components to the chemical image in the mosaic pane. Here is the same analysis results with two found MCR components selected for display.

Figure 32. Analysis window with two MCR components selected for display



To quickly display all found MCR components in the chemical image (mosaic pane), select All MCR at the top of the profiles pane.

6. To update the current MCR analysis, for example, after you have changed the **Expected components** setting, click **APPLY** (see figure 32).

The updated results replace the previous ones in the Profiles pane and in the mosaic pane.

7. To save the example data, click **Save Current Data**  .

The data are saved with the new profile information (that is, the number and type of profiles that were run and the settings that were used). The specified profiles are recalculated each time the file is opened.

Work with Image Files

This section provides an overview of the features available in both OMNICxi windows (Acquisition and Analysis) for saving, copying, exporting and printing data and searching found components against spectral libraries for component identification.

These topics are covered:

- Run a Spectral Search
- Save Data
- Print Data
- Copy or Export Data

Run a Spectral Search

OMNICxi includes features for searching spectra and analysis results against one or more spectral libraries to identify the associated compound. A spectral library is a database of spectra that you can purchase or create using spectra you collect with the instrument.

The OMNICxi Analysis window provides features for selecting libraries. Your library selections apply to search features in both the Acquisition and Analysis windows.

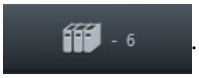
Tip Additional library management features are available in the OMNIC Workstation for DXRxi Raman Imaging Microscope software (included in the OMNCxi suite). These rules apply:

- OMNICxi spectral libraries must contain Raman spectra
- OMNICxi spectral libraries must be identified as Raman libraries when they are created
- OMNICxi spectral libraries must be stored in the following folder in Windows™ Explorer:

  C:\My Documents\OMNIC\Libs

  If you have OMNIC libraries that do not appear in OMNICxi software, copy the associated library files to this folder.
- To create a user library, use **Analyze > Library Manager** in the OMNIC Workstation software.

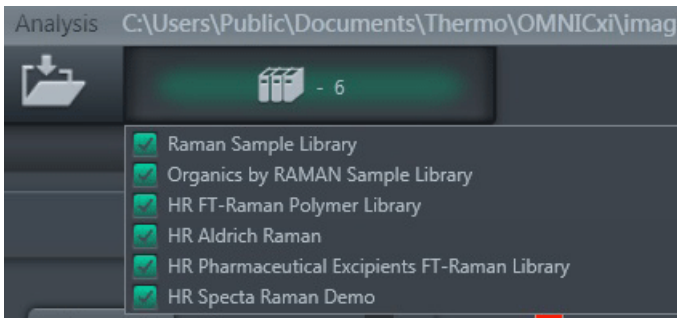
❖ To select the spectral libraries to search

1. From the OMNICxi Analysis window, click 

.

   The software shows a list of the available Raman libraries.

Figure 33. OMNICxi Analysis window showing selected spectral libraries



2. Select or deselect libraries by clicking their check boxes.

   The software searches only libraries that are selected in this list. Depending upon the selected window, display mode or analysis profile, if you change which libraries are selected, the software may automatically initiate a new search.

❖ To Display Search Results

Search results are available for the following display modes:

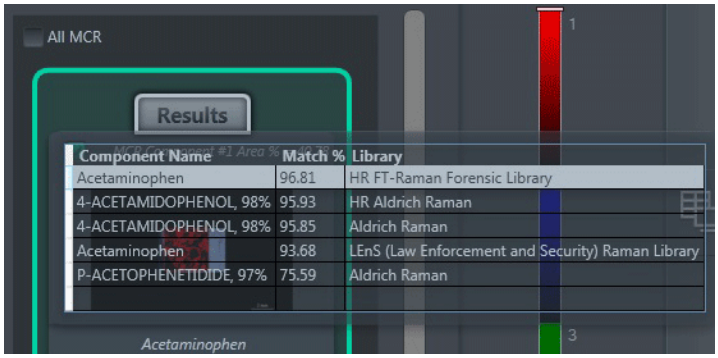
- In the Analysis window, select a profile *thumbnail* (other than MCR), select the **Spectrum tool** and click a location in the chemical image (mosaic pane) to display the associated spectrum in the spectral pane. To see search results for the spectrum, click the **Results** button in the thumbnail. A search results window appears below the button (see Figure 28).
- In the Analysis window, select an MCR profile *thumbnail* to display the associated pure component spectrum in the spectral pane. To see search results for the pure component spectrum, click the **Results** button in the associated thumbnail. A search results window appears below the button (see figure Figure 34).
- In the Acquisition window with a mosaic displayed, choose **Live Spectrum** mode and click a location in the mosaic pane to acquire a spectrum at that location and display it in the spectral pane. To see search results for the spectrum, click **SEARCH displayed spectrum** below the spectral pane (see figure Figure 18). A search results window appears above the button.
- In the Acquisition window with one or more regions of acquired chemical image data displayed, select the **Spectrum tool** and click a location in a chemical image to display the spectrum collected at that location in the spectral pane. To see search results for the spectrum, click **SEARCH displayed spectrum** below the spectral pane (see figure Figure 23). A search results window appears above the button.

In each case, a search results window is displayed. The best matched spectrum from the available spectral libraries appears at the top of the search results window and its spectrum is added to the spectral pane. You can select another library spectrum in the search results window and its spectrum will replace the previous one in the spectral pane. After you close the search results window, the library spectrum disappears from the spectral pane.

About the Search Results Window

The search results window includes a list of the five best matched spectra from the available libraries. Here is an example.

Figure 34. Search results window for MCR profile analysis



The library matches are shown in order of match value, with the best match listed first.

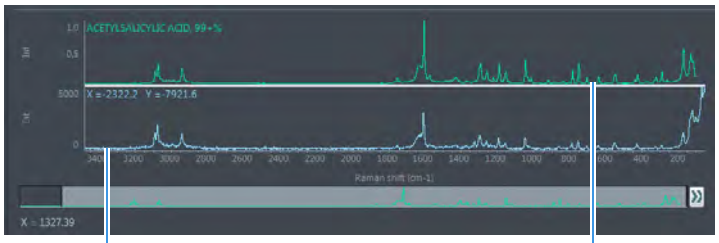
The following information is provided for each found match:

- compound name
- name of the library that contains the spectrum
- match value between 0 and 100

The match value tells you how well the library spectrum matches the unknown. The closer the value is to 100, the better is the match.

Whenever a search results window is open, the selected library spectrum is temporarily added to the spectral pane. Here is the spectral pane showing the spectrum at a selected location in a chemical image and its library match. (For the MCR profile analysis, the spectral pane will show the spectrum at the clicked location, the calculated pure component spectrum and the selected library spectrum.)

Figure 35. Spectral pane showing displayed spectrum and library match



Spectrum at current cursor location

Best matched library spectrum

Save Data

Acquired data can be saved in a variety of formats to reopen in OMNICxi software or similar applications or add to Word processing or presentation software.

Save Image Data

From the OMNICxi Acquisition or Analysis window, you can save newly acquired chemical image data and its associated profile information, or save previously collected image data with new profile information. The original acquired data remain intact.

❖ To save chemical image data as a MAPX or H5 file

1. From either OMNICxi window, click **Save Current Data** .
2. Select a folder.

> **Tip** To set the default folder and path where OMNICxi opens and saves image files, click **Settings** in the Acquisition window and change the Default Directory.

3. Select a file format.

The following file formats are available:

Table 6. OMNICxi image file formats

Format	Description
.MAPX	Can be opened in the OMNICxi application
.H5	HDF (hierarchical data format), version 5. Binary format developed by the US National Center for Supercomputing Applications (NCSA). Used for storage, management and exchange of scientific data. Can be opened in any compatible application such as the OMNICxi or MATLAB™ software.

4. Enter a filename and click **Save**.

   The following information is saved:

   - mosaic, if one was created
   - all acquired regions and their associated acquisition settings
   - all applied profiles and their associated profile settings

❖ To save the preview or chemical image as a TIFF file

1. Right-click the preview image in the video pane or a region of acquired chemical image data in the mosaic pane of the OMNICxi Acquisition or Analysis window and choose **Save preview as TIFF** (or **Save region as TIFF**).
2. Select a folder, enter a filename and click **Save**.

The image is saved as a TIFF file (*.Tagged Image File Format) which is an uncompressed raster-based image format. The TIFF image can be imported to a wide selection of publishing, page layout, scanning, faxing, word processing and other applications.

❖ To save the preview or chemical image data as a MAP file

1. Right-click the preview image in the video pane or a a region of acquired chemical image data in the mosaic pane of the OMNICxi Acquisition or Analysis window and choose **Save preview as OMNIC/Atlμs MAP** (or **Save Region as OMNIC/Atlμs MAP**).
2. Select a folder, enter a filename and click **Save**.

The image is saved as a .MAP file which can be opened with the Thermo Scientific Atlus mapping software. (The computer must also have the OMNIC Workstation for DXRxi Raman Imaging Microscope software installed.)

❖ To send preview or chemical image data to OMNIC/Atlμs

Right-click the preview image in the video pane or a region of acquired chemical image data in the mosaic pane of the OMNICxi Acquisition or Analysis window and choose **Send preview to OMNIC/Atlμs as MAP** (or **Send region to OMNIC/Atlμs as MAP**).

If the computer has the Thermo Scientific Atlus and OMNIC Workstation for DXRxi Raman Imaging Microscope software installed, the Atlμs software opens with the image displayed in a spectral window.

Save Spectral Data

Data displayed in the spectral pane in the OMNICxi Acquisition or Analysis window can be saved as:

- SPA or SPG files, which are proprietary formats for Thermo Scientific general purpose spectroscopy software
- EMF (Enhanced MetaFile), which is a 32-bit version of the Windows metafile image file format

or sent directly to the Thermo Scientific OMNIC Workstation for DXRxi Raman Imaging Microscope or OMNIC Specta software.

❖ To save selected spectra as an OMNIC .SPA or .SPG file

1. Select the spectrum or spectra to be saved (use Shift + click to select additional spectra).
2. Right-click the spectral pane and choose **Save Selected Spectrum As > OMNIC.SPA** (or **OMNIC.SPG** for multiple spectra).
3. Select a folder, enter a filename and click **Save**.

The file can be opened using the Thermo Scientific OMNIC Workstation for DXRxi Raman Imaging Microscope or OMNIC Specta application for further processing.

❖ To send selected spectra to OMNIC or OMNIC Specta

1. Select the spectrum or spectra to be exported (use Shift + click to select additional spectra).
2. Right-click the spectral pane and choose **Send Selected Spectrum To > OMNIC (or OMNIC Specta)**.

If the computer has the Thermo Scientific OMNIC Workstation for DXRxi Raman Imaging Microscope or OMNIC Specta software installed, that software opens with the spectrum displayed in a spectral window (OMNIC) or the data tray (OMNIC Specta).

❖ To save an image of displayed spectra as an EMF file

1. Select the spectrum or spectra to be saved (use Shift + click to select additional spectra).
2. Right-click the spectral pane and choose **Save displayed Spectra As EMF**.
3. Select a folder, enter a filename and click **Save**.

The spectral pane is saved as an image, exactly as it is displayed in the OMNICxi software. An EMF (Enhanced Metafile) is a 32-bit version of the Windows metafile image file format.

Copy or Export Data

Acquired data can be copied to the Windows Clipboard or exported in a wide range of formats. Copied images provide a low-resolution visual snapshot of the data or profile results that can be pasted into reports and presentations. Use the export features if you need to access the raw image or spectral data from other applications.

❖ To copy data to the Windows Clipboard

To copy the contents of the any pane in the OMNICxi Acquisition or Analysis window to the Clipboard, right-click the pane and choose **Copy as picture** (or **Copy ___ as picture**). The copied image can be pasted into any word processing, presentation, or other compatible application.

> **Tip** The resolution of a copied image will be the same as the computer's display monitor (typically 72 dpi or 96 dpi).

❖ To export chemical image data as an ENVI (flat-binary raster) file

1. Right-click the preview image or acquired region and choose **Export preview data as ENVI** or **Export region data as ENVI**.
2. Select a folder, enter a filename and click **Save**.

The image is saved as an .ENVI (flat-binary raster) file, which can be opened with the Thermo Scientific Atlμs software, MATLAB software or other similar applications.

❖ To export spectral data as text

1. In the spectral pane, select the spectrum or spectra to be exported (use Shift + click to select additional spectra).
2. Right-click the spectral pane and choose **Export > Selected spectrum (or spectra) as TXT**.
3. Select a folder, enter a filename and click **Save**.

The data are saved in a tab separated text file, which can be opened with any text processing application such as Notepad. If multiple spectra are selected, they will be exported to multiple text files.

Print Data

Acquired data can be printed in a pre-formatted report. You can select the images and information to include. Images and information are formatted exactly as displayed in the software.

> **Tip** To create a report using a customized format, copy or export the data to a compatible application.

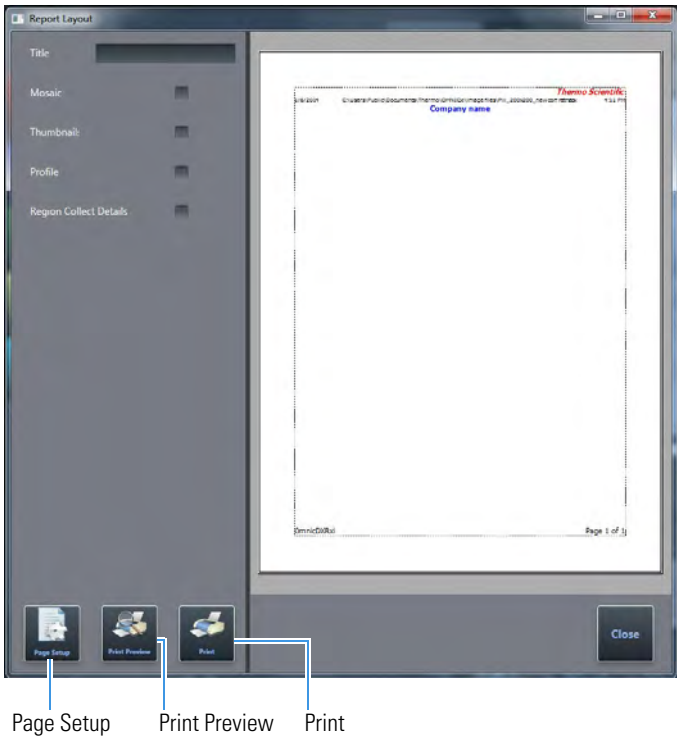
❖ To format and print a data report

1. Collect an image or open an image file.
2. Display the Analysis window.
3. Run any additional analysis profiles on the data.
4. To include profile information, select the profile thumbnail.

   Make sure your selected items are displayed exactly as you want them to look in your printed report.

5. Click  to display the Print Layout window.

Figure 36. Print layout window



6. Click **Page Setup**  to define margins and page orientation.
7. Select the items to include in the report.

Table 7. OMNICxi Report Layout window options

Format	Description
Title	Adds the text you enter to the top of the report. The title is centered.
Mosaic	Adds the contents of the mosaic pane (including any regions of acquired chemical image data) and the profile intensity scale to the report.
Thumbnail	Adds the selected profile thumbnail, the profile analysis results, any search results, and the contents of the spectral pane to the report. Maintains all color assignments.
Profile	Adds the analysis results for the selected profile thumbnail, any search results for the selected profile thumbnail, and the contents of the spectral pane to the report.
Region Collect Details	Adds the acquisition settings for all regions of acquired chemical image data to the report.

8. Click **Print Preview**  to preview the report.
9. To select a printer and print the report, click **Print**  .

Tip Select a PDF print driver to print to a portable document format (PDF) file.

Find Information

This document provides a comprehensive overview of the OMNICxi software and important training for first-time users. Detailed reference information is provided in these locations:

- The OMNICxi software includes a complete help system for the instrument and software. To open the help system, start the OMNICxi software and click the ? button.
- PDF files of all the available system documents, including the *Site and Safety Guide*, are provided in the electronic documentation set, which is installed on the system computer during installation.

Glossary

Acquisition window One of two windows that comprise the OMNICxi software. Used to acquire, analyze and identify *chemical image* data from a sample positioned on the microscope stage.

Analysis window One of two windows that comprise the OMNICxi software. Used to view, analyze and identify previously collected *chemical image* data.

chemical image A scan that contains one *spectrum* collected at each sampling point in the *preview box* or a selected region of the displayed *mosaic*. The number and spacing of the spectra that make up the chemical image are defined by the *Image pixel size* setting in the *Acquisition window*. The number of passes is defined by the *Number of scans* setting. A mathematical algorithm (defined by the selected *profile*) is applied to each spectrum to create the chemical image. The colors in the image are determined by the *profile intensity scale*.

Exposure time An acquisition setting that specifies the amount of time spent acquiring each spectrum. Longer exposure times increase the height of the spectral peaks but also increase scan time.

Grab tool  Can be used to drag the *video image*, *mosaic* or *chemical image* within their respective panes and to move and resize the *preview box*. Also moves the stage to a clicked location in the *video image*, *optical image* or *mosaic*. Also used in *Live Spectrum mode* to select a location in the mosaic pane to acquire a spectrum.

Image Cross Section mode One of four modes of operation available in the *Acquisition window*. Useful for acquiring a *preview image* representing *spectra* acquired at different depths in the sample along a vertical (y-z axis) line.

Image pixel size An acquisition setting that specifies how far the stage moves before it acquires another spectrum. For example, if the pixel size is 5 µm, each spectrum represents 5 µm of sample area averaged together.

Image Regions mode One of four modes of operation available in the *Acquisition window*. Useful for acquiring *preview* or *chemical image* data in a selected region or regions of the sample surface (x-y plane).

Laser power An acquisition setting that specifies the strength of the excitation laser present at the sample. The laser power scale depends on the installed laser. Higher power increases the height of spectral peaks. However, some samples may be burned or damaged when laser power is too high.

Live Spectrum mode One of four modes of operation available in the *Acquisition window*. Useful for quickly surveying the sample surface to locate and identify components. Use the *Grab tool* to click a location in the *mosaic pane*; the software acquires a *spectrum* at that location using the current acquisition settings (*Laser power*, *Exposure time* and *Number of scans*) and displays it in the *spectral pane*.

mosaic An image comprised of static pictures of the sample surface. The pictures are stitched together with the edges slightly overlapped to ensure that all areas are captured. The mosaic is created in the *Acquisition window* and appears in the *mosaic pane*. It also appears in the mosaic pane of the *Analysis window*.

mosaic pane The right display pane of the *Acquisition window* and *Analysis window*. Can contain a *mosaic*, if one was collected, and any regions of acquired *chemical image* data.

Number of scans An acquisition setting that specifies how many times a region is scanned to create a *preview image* or region of acquired *chemical image* data. Multiple scans are used to reduce the noise in the spectra through signal averaging.

optical image An image of the sample as it appears when viewed through the microscope eyepiece (one field of view). The optical image appears in the *mosaic pane* of the *Acquisition window* before a *mosaic* has been collected.

preview box Defines the boundaries of the *preview region* in the *mosaic pane* of the *Acquisition window*.

preview image A *chemical image* of the *preview region*.

preview region The region defined by the *preview box* and used to acquire a preview *chemical image* of the sample.

profile A mathematical algorithm (defined by the current Profile setting) that is applied to each spectrum in the *preview image* or region of acquired *chemical image* data. The algorithm converts each spectrum into a number that is represented by a color in the chemical image. The colors in the chemical image are defined by the *profile intensity scale*.

profile intensity scale

- For all profile types except MCR, shows the scale or range and corresponding colors used by the selected *profile*. Areas of the *chemical image* that correlate with the profile algorithm are displayed in the colors at the top of the profile intensity scale.
- For the MCR profile type, shows the color associated with each found component in the chemical image.

profiles pane The left display pane of the *Analysis window*. Contains *thumbnails* of all *profiles* run on the *chemical image* data displayed in the *mosaic pane*.

Ruler tool  Can be used to measure the length of features in the *optical image* or *mosaic* or a region of acquired *chemical image* data. Click to define the start of the measurement, then drag to the end point. The length in µm (or mm) is displayed in the status bar below the *mosaic pane*.

spectral pane The bottom right display pane in the *Acquisition window* and the *Analysis window*. Can be used to display *spectra* from these windows and display modes:

- In *Image Regions mode* or *Image Cross Section mode* (*Acquisition window*) and in the *Analysis window*, use the *Spectrum tool* to click a location in the *preview image* or a region of acquired *chemical image* data to display the associated spectrum in the spectral pane. Can be used to display individual *spectra* (clicked location with *Number of scans* set to 1), coadded spectra (clicked location with Number of scans greater than 1) or average *spectrum* (selected area).
- In *Live Spectrum mode* (*Acquisition window*), use the *Grab tool* to click a location in the *mosaic pane*; the software acquires a spectrum at that location using the current acquisition settings (*Laser power, Exposure time* and *Number of scans*) and displays it in the *spectral pane.*

spectrum or **spectra** A measure of Raman radiation emitted by a sample. Intensity is expressed in detector counts. Frequency is expressed in terms of wavenumber (cm^{-1}). In Raman spectroscopy, the x-axis is converted to Raman shift, which is a measure of the difference between the observed spectral bands and the wavelength of the excitation laser.

Spectrum tool  Can be used to display the *spectrum* associated with a clicked location in the *preview image* or region of acquired *chemical image* data in the *Acquisition window* or *Analysis window*. The spectrum is displayed in the *spectral pane*. If an area is selected in the preview or chemical image, the associated average spectrum appears in the spectral pane.

thumbnail Contains an image and associated analysis and spectral search information for the selected *profile* in the *Analysis window*.

video image A live (constantly updated) video image of the sample surface at the current location of the microscope stage. Appears only in the *Acquisition window* in *video mode*.

video calibration Defines the size, in micrometers, of the video system's field of view for the currently selected objective so the *chemical image* data accurately describes the sample. Each objective installed on the microscope requires an associated video calibration file.

Video mode One of four modes of operation available in the *Acquisition window*. Shows a *video image* of the sample surface in the *video (left) pane* and an *optical image* or *mosaic* in the *mosaic pane*. Useful for creating a *mosaic* and for viewing, focusing, and zooming images of the sample surface. Can also be used to manipulate the *preview box* (using the *Grab tool*).

video pane The left display pane of the *Acquisition window*. Shows a live image of the sample surface captured by the microscope's video camera. The limits of the video pane are defined by the magnification and aperture opening of the selected objective.

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