thermo scientific

DXRxi Instruments and Software

DXRxi
Raman Imaging Microscope
with OMNICxi Raman Imaging Software
User Guide

269-300706 Revision A • September 2021

ThermoFisher SCIENTIFIC

© 2014-2021 Thermo Fisher Scientific Inc. All rights reserved.

Microsoft, Windows and Excel are either trademarks or registered trademarks of Microsoft Corporation in the United States and/or other countries. MATLAB is either a trademark or a registered trademark of The MathWorks, Inc. in the United States or other countries. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

For technical support, please contact: www.thermofisher.com

Thermo Fisher Scientific Inc. provides this document to its customers with a product purchase to use in the product operation. This document is copyright protected and any reproduction of the whole or any part of this document is strictly prohibited, except with the written authorization of Thermo Fisher Scientific Inc.

The contents of this document are subject to change without notice. All technical information in this document is for reference purposes only. System configurations and specifications in this document supersede all previous information received by the purchaser.

Thermo Fisher Scientific Inc. makes no representations that this document is complete, accurate or error-free and assumes no responsibility and will not be liable for any errors, omissions, damage or loss that might result from any use of this document, even if the information in the document is followed properly.

This document is not part of any sales contract between Thermo Fisher Scientific Inc. and a purchaser. This document shall in no way govern or modify any Terms and Conditions of Sale, which Terms and Conditions of Sale shall govern all conflicting information between the two documents.

For Research Use Only. This instrument or accessory is not a medical device and is not intended to be used for the prevention, diagnosis, treatment or cure of disease.

WARNING Avoid an explosion or fire hazard. This instrument or accessory is not designed for use in an explosive atmosphere.

Contents

Chapter 1 Introducing the DXRxi Raman Imaging System 1

About the System 1
Getting Started 2
Safety and Operating Precautions 2
About this Help System 3
Organization 3
Conventions 4
Contacting Us 4
Trademarks 5

Chapter 2 DXRxi Microscope Operation 7

Microscope Features and Controls 7
Front View 7
Right Side View 8
Left Side View 10
Inside Sample Compartment 11
Stage Controls 12
Operating the Microscope 13
Indicators 13
Powering the System Up or Down 14
Loading a Sample 14
Available Sample Holders 15
Rotating Stage Holder 17
Sample Holder Breadboard Kit With Clips 18
XPS Sample Holder 18
Raman Macro Sampler 19
Leveling the Sample Holder 19
Installing Objectives 23
Configuring Sample Illumination 24
Controlling Illumination with the Optional Reflected Light Brightfield/Darkfield Illuminator 24
Controlling Illumination with the Optional Reflected Light Brightfield Illuminator 26
Controlling Transmitted Light Illumination 27
Using Optical Filters 27

Using Optical Polarizers 29
Positioning the Motorized Stage 37
Using Confocal Operation 38
Using the Raman Polarization Option 38
Changing the Laser, Filter, and Grating 39
Installing a 633 nm Laser 43
Enabling the Laser 43
Optional Class 3B Laser Configuration 43
Using a Class 3B Fiber Optic Launcher Accessory 44
Installing the Microscope Alignment and Calibration Tool 46
Servicing the Microscope 51
Cleaning Your Instrument 51
Replacing the Nosepiece 52
Replacing an Illuminator Bulb 52
Safety Information 55
Laser Precautions 55
Biohazards or Radioactive Materials and Infectious Agents 56
Cooling Vents 56
Ventilation 57
Microscope Safety Labels 58

Chapter 3 OMNICxi Software Operation 63
General Workflow 63
Overview of the Workspace 64
OMNICxi Acquisition Window 64
General Features and Controls 66
Video Mode Features and Controls 77
Image Regions Mode 82
Image Cross Section Mode 93
Live Spectrum Mode 94
OMNICxi Analysis Window 99
3D Analysis and Terrain Windows 110
Instrument Settings 116
Collect a Mosaic 120
Acquire and Analyze Data 125
Analyze Regions 126
Acquire a Preview Chemical Image 126
Settings for Acquiring a Chemical Image 127
Acquire and Analyze a Chemical Image 130
Profile Settings 131
Reanalyze Image Data 143
Identify and Analyze Particles 145
Video Image-Based Particle Analysis 145
Chemical Image-Based Particle Analysis 146
Settings for Particle Analysis 146

View Particle Analysis Results 149
View Spectrum at Specific Location 150
Analyze Sample Cross Sections 153
Measure Change Over Time 159
View Time-Based Data 162
Measure and View 3D Volumes 163
Acquire 3D Image Data 163
View 3D Data 164
Verify Instrument Performance 164
System Tools 155
Aligning and Calibrating Laser Sets 155
Creating a New Video Calibration 160

Chapter 4 Troubleshooting 171
Problems and Solutions 171
Performance Verification fails 171
Sample Bands are too Small 172
Sample Bands Are Too Large or CCD Overflowed 172
No Raman Signal Appears in the Live Display or Spectrum Has No Sample Bands 172
No Visible Image Appears in the Video Pane or Viewer 173
The Spectrum Has a Sloping Background or a Severe Positive Offset 173
The Spectrum Baseline Is Severely Tilted, Curved or Saturating 173
The Video Image is Obscured or Distorted 174
The Computer Cannot Communicate with the Instrument 174
Chemical Image Appears to be Different Than the Video Image 174
Chemical Image Does Not Achieve Required Spatial Resolution 175
Chemical Image Shows Missing Data 175
X-Axis Calibration Fails 175
Error Messages 176
General Errors 176
Instrument off or disconnected 177
Video camera disconnected 177
Stage controller off or disconnected 177
Stage controller failed 178
Unknown stage location 178
Detector too warm 178
Detector disconnected 179
No filter 179
No grating 179
No laser 179
Mismatched grating 179
Mismatched filter 179
Invalid fiber probe configuration 180
Uncalibrated laser, grating, filter set 180

Alignment/calibration out of date. 180
Stage firmware out of date 180
Sample door open 180
Laser off 180
Laser key off 180
Laser warming up 180
Sample signal too high 181
Cannot read instrument EEPROM 181
No communication 181
Align and Calibration Errors 181
Align/calibrate tool not connected 182
Laser output too low 182
No laser at sample 182
Poor quality align/calibrate spectra 183
Spectral response too low 184
Spectral response too high 184
Performance check value(s) out of range. 185
Misaligned filter or grating 185
Unable to read system info 186
Unable to collect spectral data 186
Unable to calibrate laser power 186

1

Introducing the DXRxi Raman Imaging System

Welcome to the Thermo Scientific™ DXR™xi Raman imaging microscope with OMNIC™xi Raman imaging software. This system is intended to be used to analyze the chemical composition of materials using dispersive Raman spectroscopy. This help system will get you started using the microscope and software and demonstrate how to run experiments using a provided sample.

This section covers these topics:

Contents

- About the System
- Getting Started
- Safety and Operating Precautions
- About this Help System
- Contacting Us
- Trademarks

NOTICE Be sure that all persons operating this system read the site and safety manual first.

About the System

The DXRxi Raman Imaging Microscope 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 reflected light brightfield sample illumination. Optional reflected light brightfield/darkfield and transmission illumination kits are also available.

The instrument includes our OMNICxi Raman imaging software for data collection and analysis. The OMNICxi software comprises two main windows:

- the **Acquisition window** for data collection and on-the-fly analysis
- the **Analysis window** for viewing and processing previously collected image data

With automatic feature recognition and real-time optimization of imaging parameters, this imaging system is a powerful tool for component identification that will transform the way you approach materials analysis.

Getting Started

The instrument can be run from a Windows™-compatible computer connected to the instrument. The computer must meet our requirements for processing speed and memory. The system is set up and installed by one of our service representatives. For best performance, leave the power connected and the instrument powered up even when not in use.

The *DXRxi Quick Reference* guide provides a comprehensive overview of the microscope and software. If you are using the instrument the first time, start by reviewing the quick reference guide and completing the steps as you go. A printed copy of the guide is provided with the system. A PDF version is also available in the electronic documentation set.

Safety and Operating Precautions

The instrument contains precise optical components. Handle it carefully. Before using the system, read the site preparation and safety manual. A copy in PDF format is included in the provided documentation set. Always follow the safety and operating precautions in that manual and in this help system when using the instrument.

The DXRxi Raman imaging microscope may be configured as a Class 1 laser-safe system or optionally as a Class 3B laser product.

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

WARNING Avoid eye injury. 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.

- All persons using or in the vicinity of a Class 3B system must wear safety eyewear that exceeds the specifications for the laser in use
- Do not look directly into a laser, even when wearing laser safety eyewear
- Set up a laser safety area in the location where a Class 3B system is being used
- Making adjustments, using controls, or performing procedures that are not specified in the documentation could result in exposure to hazardous visible or invisible laser radiation.

CAUTION 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 compartment doors on the instrument are open.

Follow these additional precautions when using the instrument:

- **Avoid pinch hazard**. Keep hands and fingers clear of the microscope sampling stage when the stage is in motion.
- **Avoid explosion hazard**. Do not use the instrument to analyze flammable or explosive materials.

About this Help System

- Organization
- Conventions

Organization

This help system has the following main sections:

Section	Description
Introduction	Overview of the instrument and this help system plus information about contacting us and the system warranty
DXRxi Raman Imaging Microscope	Complete instructions for operating, maintaining and servicing the microscope

Section	Description
OMNICxi Raman Imaging Software	Descriptions of all software features and controls
Troubleshooting	Troubleshooting information and descriptions of possible error messages

Conventions

This help system uses the following conventions for safety and special notices:

WARNING Indicates a hazardous situation which, if not avoided, could result in death or serious injury.

CAUTION Indicates a hazardous situation which, if not avoided, could result in minor or moderate injury.

NOTICE Follow instructions with this label to avoid damaging the system hardware or losing data.

Note Contains helpful supplementary information.

Tip Provides information that can make a task easier.

Contacting Us

For U.S. Technical Support, please contact:

Unity Lab Services
Part of Thermo Fisher Scientific
5225 Verona Road
Madison WI 53711-4495 U.S.A.
Telephone: 1 800 532 4752
E-mail: us.techsupport.analyze@thermofisher.com

For International Support, please contact:

Thermo Fisher Scientific
Telephone: +1 608 273 5017
E-mail: support.madison@thermofisher.com

Note Please have the instrument serial number available when you contact us.

Trademarks

Microsoft, Windows and Excel are either trademarks or registered trademarks of Microsoft Corporation in the United States and/or other countries. MATLAB is either a trademark or a registered trademark of The MathWorks, Inc. in the United States or other countries. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

2

DXRxi Microscope Operation

The DXRxi Raman Imaging Microscope 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 reflected light brightfield sample illumination. Optional reflected light brightfield/darkfield and transmission illumination kits are also available.

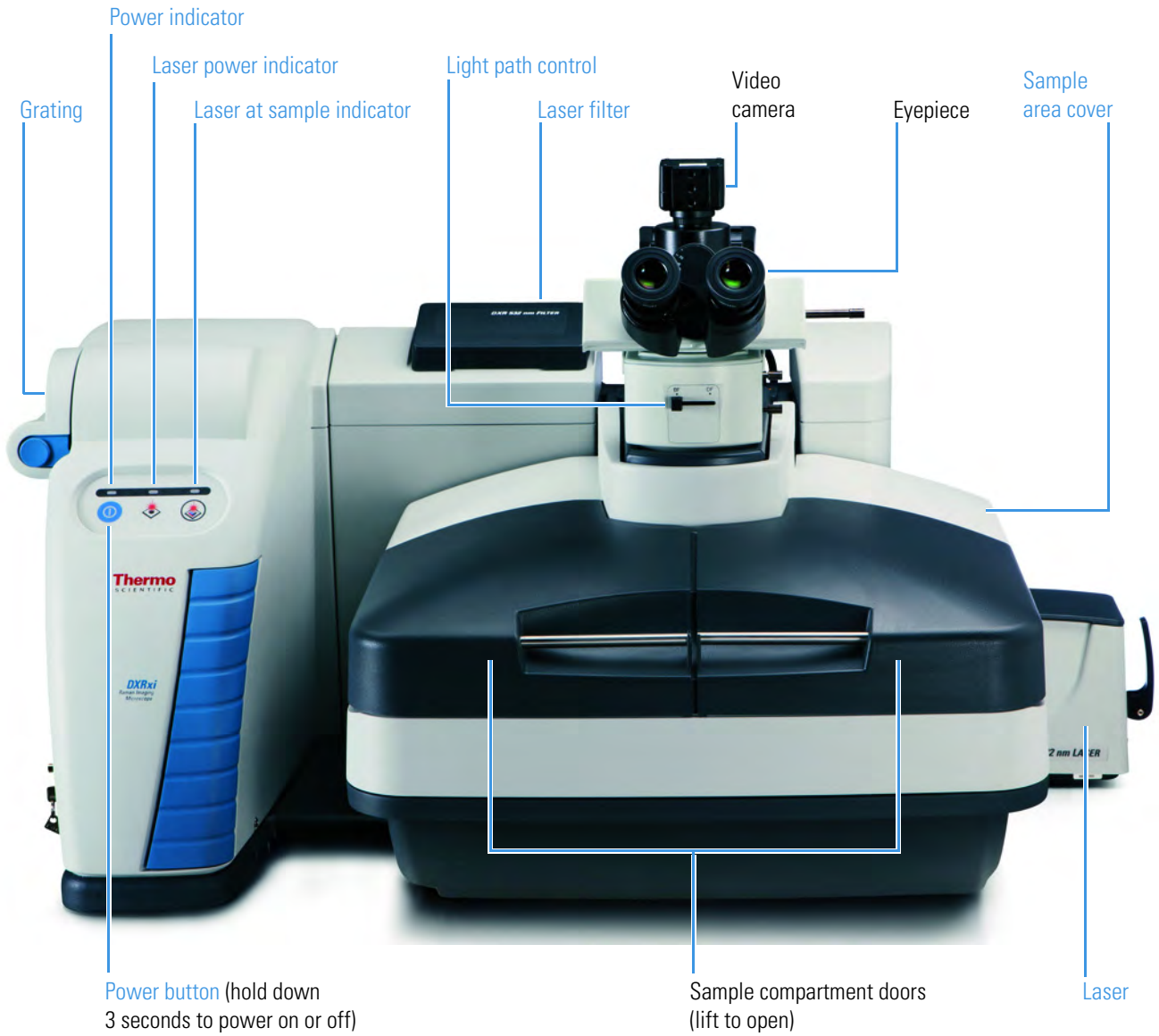
Contents

- Microscope Features and Controls
- Operating the Microscope
- Servicing the Microscope
- Safety Information

Microscope Features and Controls

Front View

Figure 1. Microscope with optional reflected light brightfield/darkfield (BF/DF) illuminator



Right Side View

Figure 2. Microscope with optional reflected light brightfield/darkfield (BF/DF) illuminator

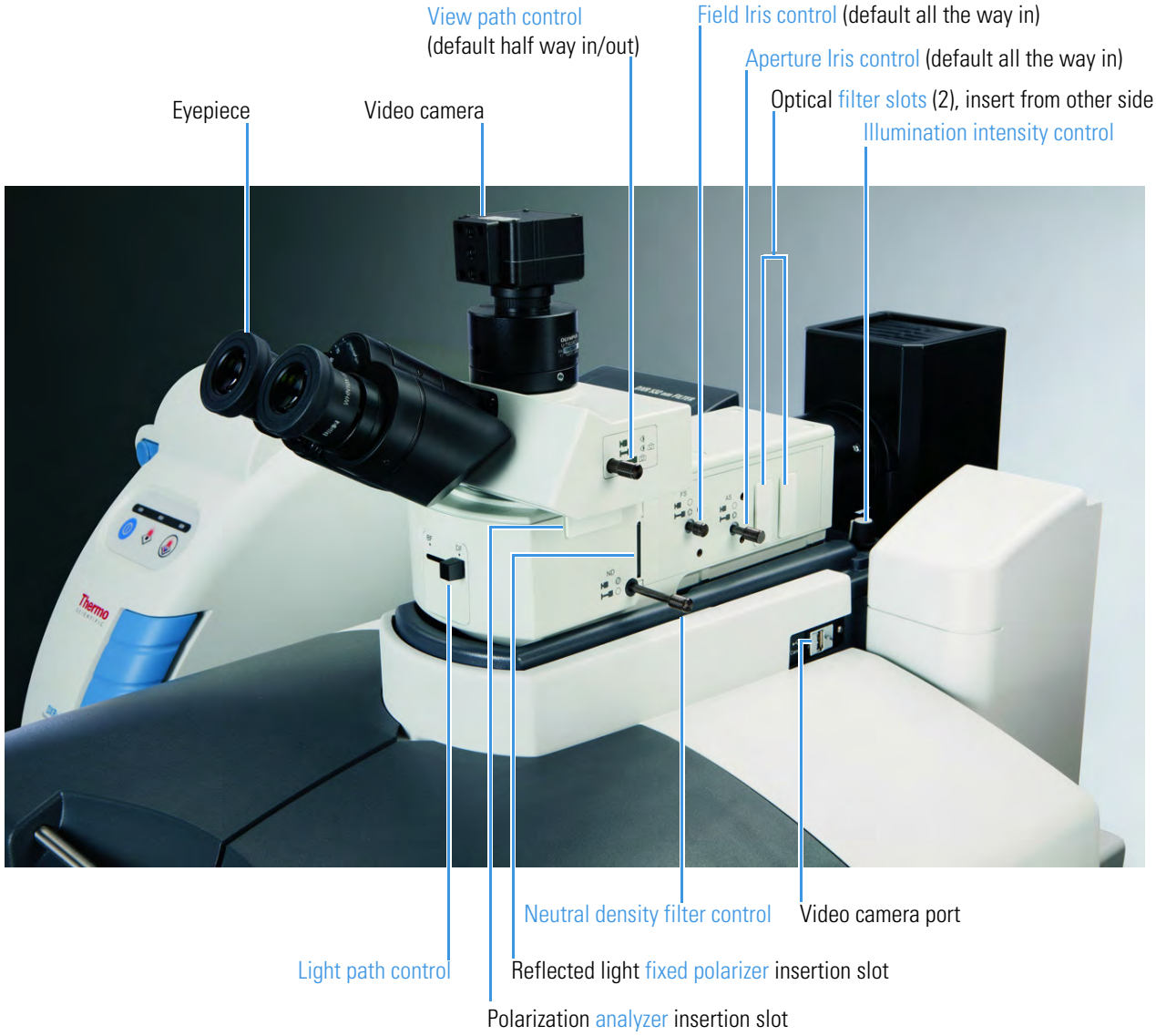
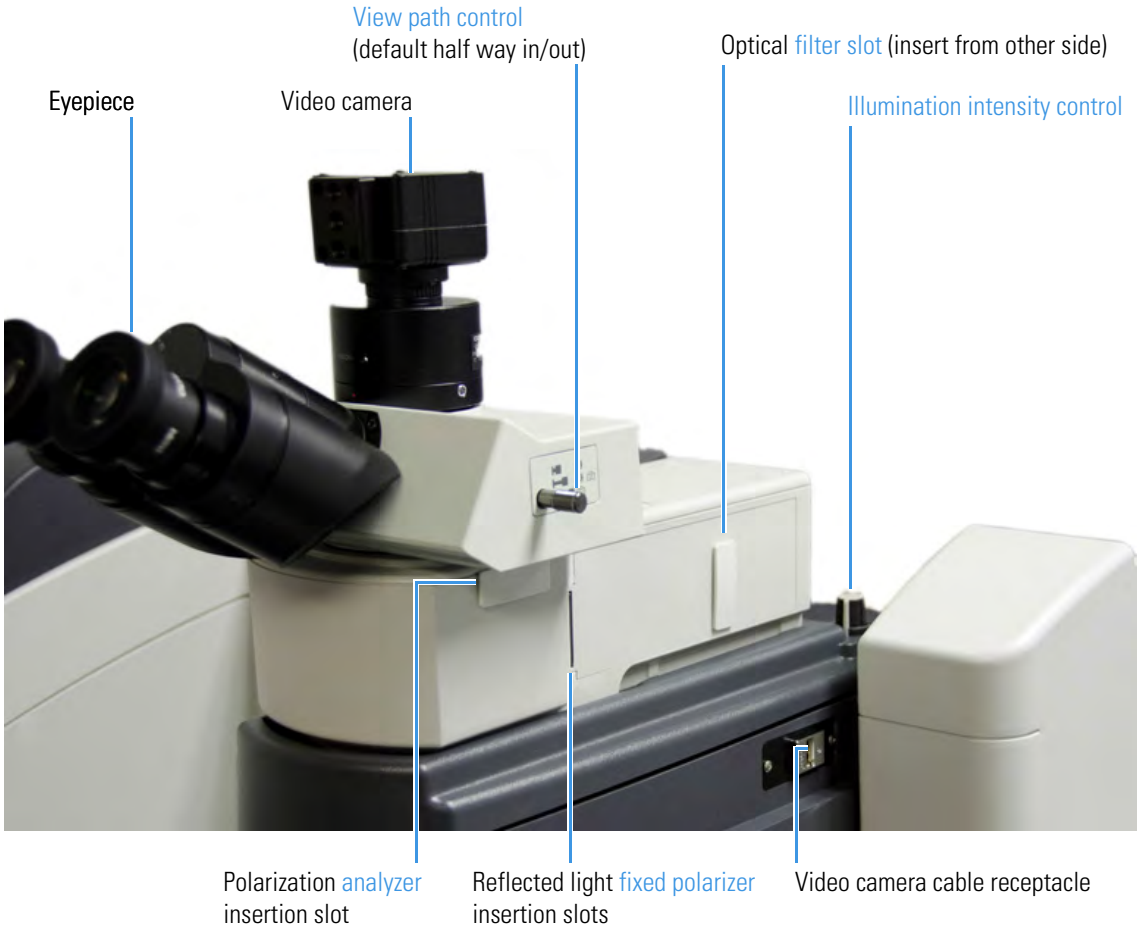


Figure 3. Power supply for optional reflected light brightfield/darkfield (BF/DF) illuminator

Figure 4. Microscope with reflected light brightfield only illuminator



Left Side View

Figure 5. Microscope, left side view with laser keyswitch

Inside Sample Compartment

Figure 6. Microscope, inside sample compartment

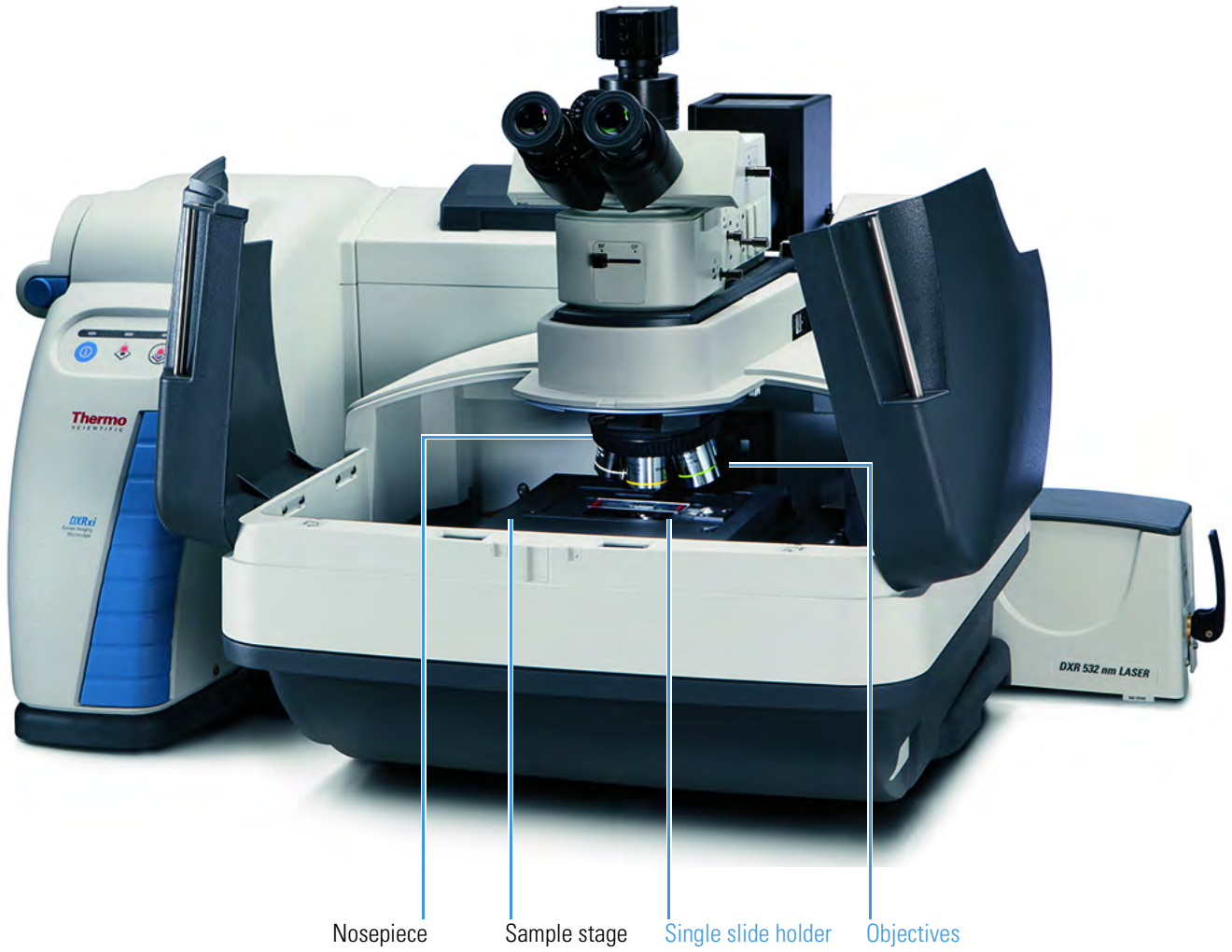
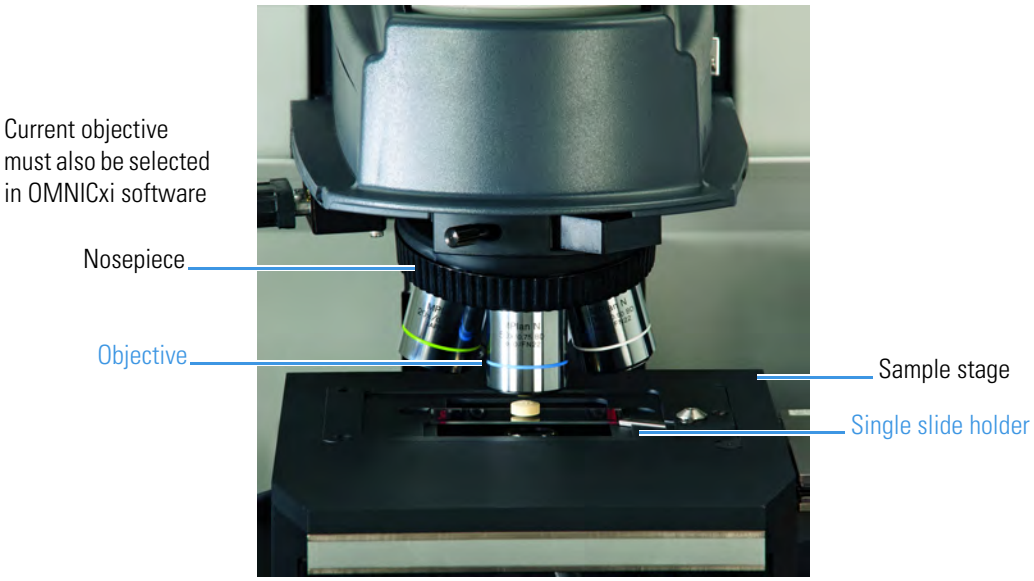
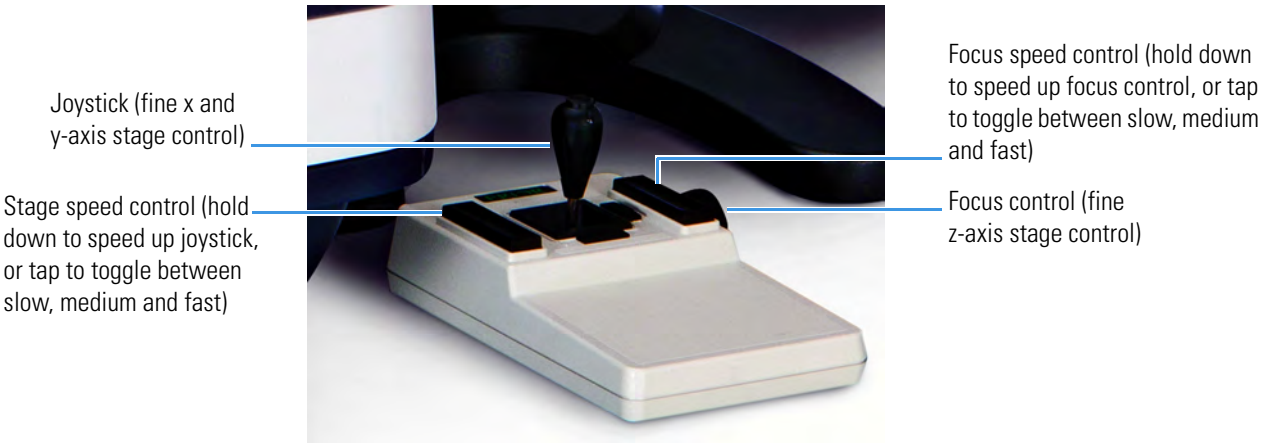


Figure 7. Microscope objectives and sample stage (10x objective is included)



Stage Controls

Figure 8. Microscope sample stage controls



Operating the Microscope

Indicators

Figure 9. Indicators

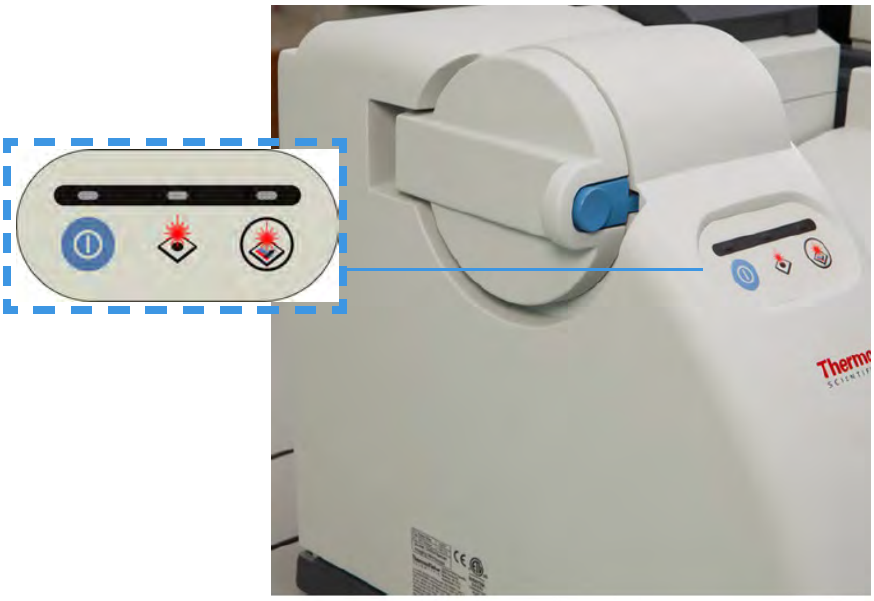


Table 1. Indicator descriptions

Indicator	Description
	Power button and blue indicator LED. Hold down power button 3 seconds to power instrument on or off. LED lights when instrument is powered on.
	Laser icon and green indicator LED. LED lights when laser is powered on.
	Green indicator LED. LED lights when laser is present at sample and data acquisition is in progress.

Powering the System Up or Down

❖ To power up the system

1. Turn on the power strip.
2. Hold down the blue power button on the front of the microscope keypad for three (3) seconds.
3. Ensure stage controller switch is on.
4. Ensure illuminator switch is on (if using the brightfield/darkfield illuminator).
5. Power up the computer and monitor(s).

❖ To power down the system

1. Shut down the computer.
2. Turn off the power strip.

Loading a Sample

The sample must be held securely to prevent movement during image collection. This is commonly done by affixing the sample to a glass slide using double-sided adhesive tape or an epoxy. We offer a wide selection of sample holders to accommodate many different sample types. Contact your sales representative for more information.

When installing a sample holder be sure to install it in the orientation shown. For collections over large areas while maintaining focus the sample holder must be leveled to the stage bearings. This is done by adjusting the 3 set screws in the sample holder. After adjustment is complete, secure the sample holder with the 3 hold-down screws.

Available Sample Holders

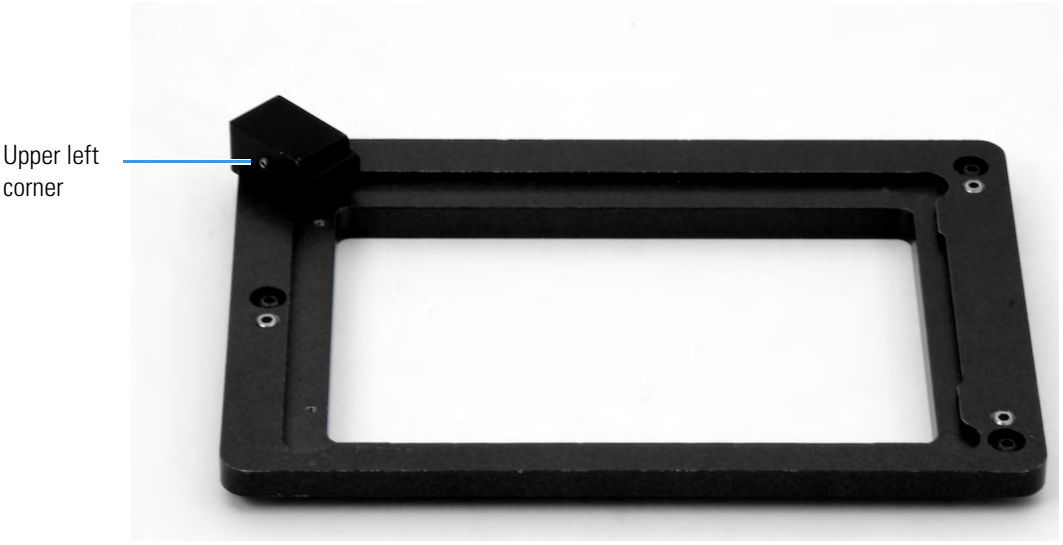
Note This holder is also used to hold the align/calibrate tool for system alignment and calibration.

Single Slide Holder

Figure 10. Single slide holder

Microtiter Well-Plate Holder

Figure 11. Microtiter Well-Plate holder



Dual Slide Holder

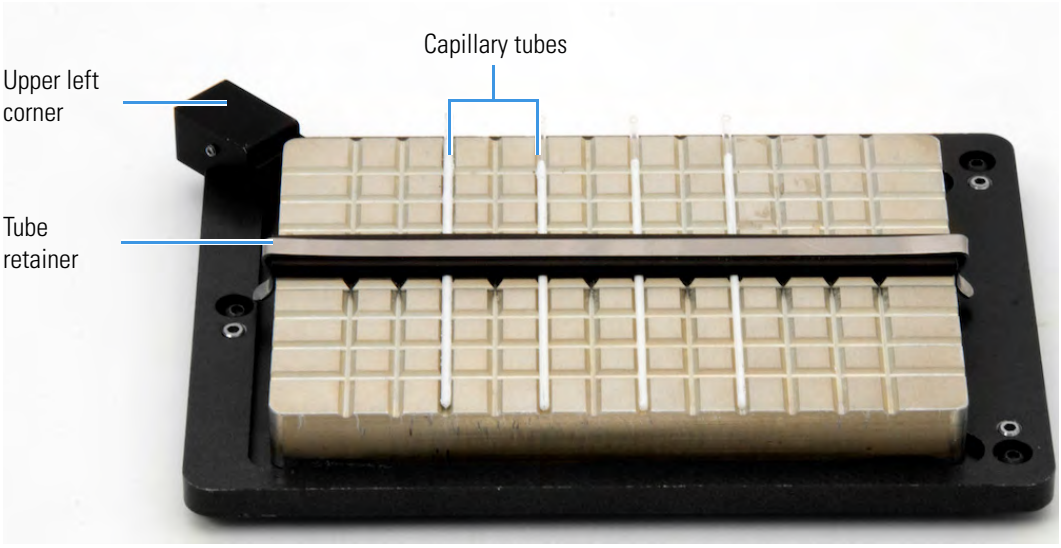
Figure 12. Dual slide holder set inside Well-Plate holder

Acrylic Vacuum Plate

Figure 13. 96-acrylic vacuum plate mounted on Well-Plate holder

Capillary Tube Array Holder

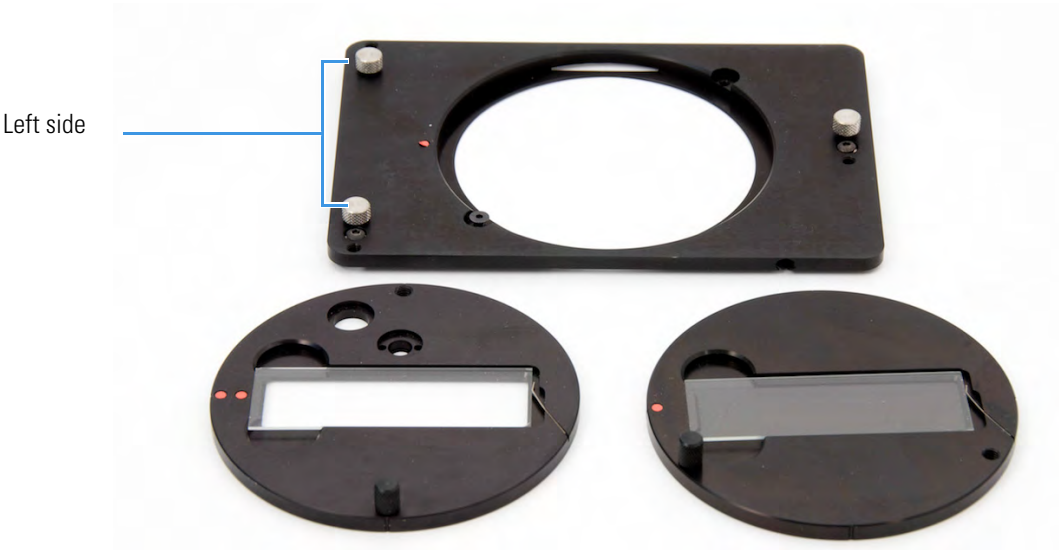
Figure 14. Capillary tube array holder mounted on Well-Plate holder



Rotating Stage Holder

Accepts standard microscope slide, provides manual rotation to any arbitrary position. Holds standard microscope single slide.

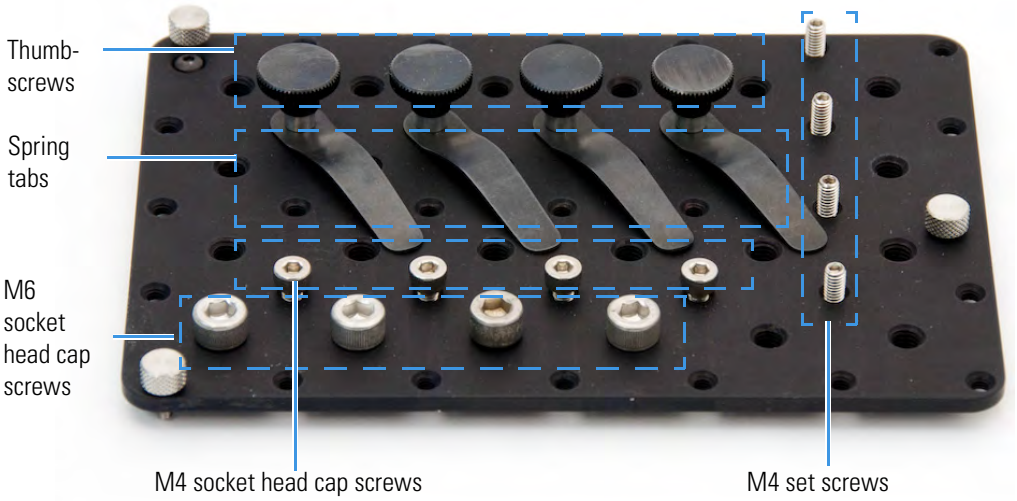
Figure 15. Rotating stage and holders



Sample Holder Breadboard Kit With Clips

This sample holder provides maximum flexibility for holding small and uniquely shaped samples without the risk of contamination with adhesives. It is compatible with Ted Pella sample holders.

Figure 16. Breadboard kit



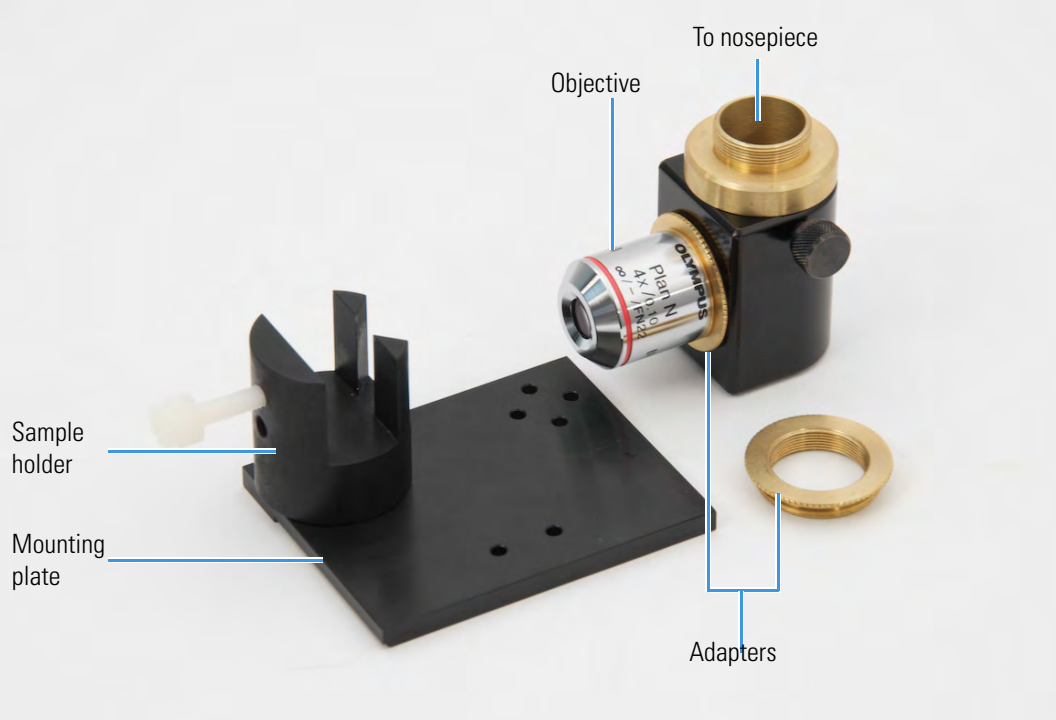
XPS Sample Holder

Permits easy transfer of samples from the Thermo Scientific K-Alpha™ X-ray photoelectron spectrometer.

Figure 17. XPS sample holder

Raman Macro Sampler

Figure 18. Microscope Macro Sampler Adapter



Leveling the Sample Holder

Figure 19. Screw locations

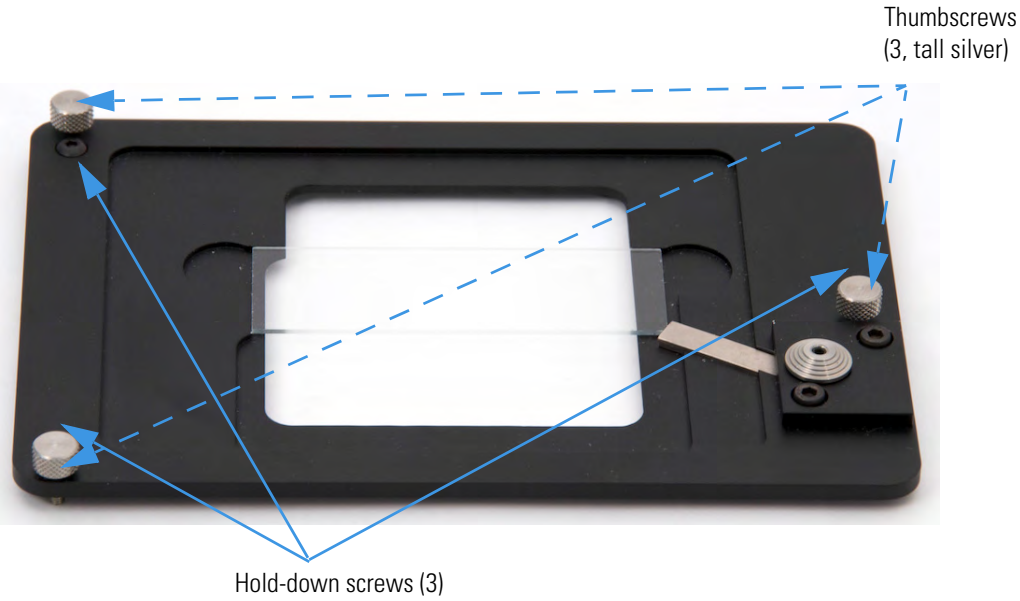
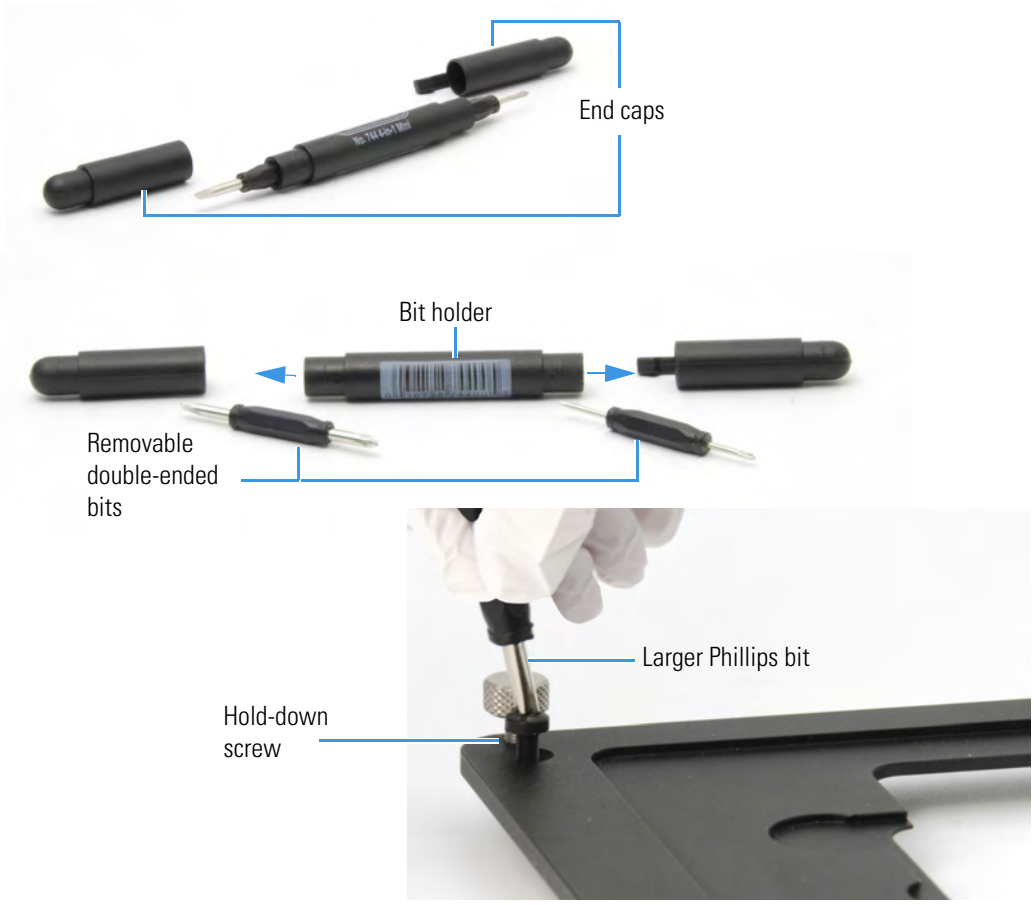


Figure 20. Using the screwdriver



❖ How to level the sample holder

1. Lower stage and bring to front to make it easier to access single slide holder fasteners.

   NOTICE Anytime during leveling when moving the stage, the objectives can potentially collide with objects on the sample holder.

2. Loosen the three hold-down screws so they are barely threaded in their holes.
3. Unthread the three thumbscrews so that the bottom of each thumbscrew is not in contact with the stage.
4. Use your fingers to push down gently on the back-left corner of the slide holder (see Figure 19). Then turn the back-left thumbscrew clockwise until you feel the bottom of the screw touch the stage.
5. Continue turning the back-left thumbscrew clockwise 1.5 turns to raise the slide holder in that corner.
6. Repeat step 4 for the front-left corner of the slide holder and the front-left thumbscrew.

7. Continue turning the front-left thumbscrew clockwise 1 turn to raise the slide holder in that corner.
8. Repeat step 4 for the right side of the slide holder and the middle-right thumbscrew.
9. Continue turning the middle-right thumbscrew clockwise one-half turn to raise the slide holder on that side.

Note

- Any time the thumbscrews are adjusted, it is recommend to gently apply pressure to the slide holder to assure that the adjustment thumbscrews are properly contacting the stage.
- If the slide holder is running into the hold-down screws, back them out further.

10. Select the 10x objective on the microscope.
11. Move the stage all the way to its limits to position the back-left corner of the slide holder under the objective.

NOTICE When focusing on the slide holder, the thumbscrews can potentially collide with the objectives. Objectives to either side of the primary objective may need to be removed in order to focus on the surface. Video calibration will need to be done if any objectives are changed.

12. Use the stage up/down control to bring the top surface of the slide holder into focus.

    Take note of the z position located below the mosaic pane in OMNICxi software for a later calculation.

13. Move the stage straight all the way to the back to position the front-left corner of the slide holder under the objective.
14. Bring the top surface of the slide holder at that location into focus.
15. Take the new z value and subtract the original z position (step 12).

    Z2 (step 15) - Z1 (step 12) = Z for Table 2

    Use this number and find the closest match from Table 2. The table provides an approximate amount to rotate the front-left thumbscrew to level the slide holder from front to back. Rotate the thumbscrew clockwise if the number is positive; counterclockwise if it is negative.

Table 2. Amount to rotate thumbscrew

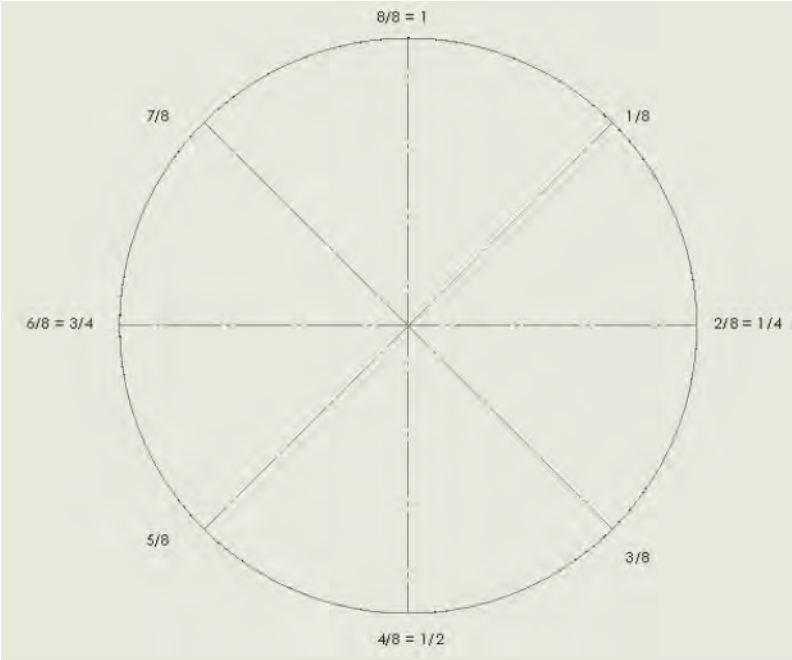
µm	Revolutions
400	1
350	7/8
300	6/8 (3/4)

Table 2. Amount to rotate thumbscrew

µm	Revolutions
250	5/8
200	4/8 (1/2)
150	3/8
100	2/8 (1/4)
50	1/8

Tip Envision pictures of wheels to visualize the rotation amount.

Figure 21. Rotation wheels showing fractions of a full revolution



16. Move the stage to position the middle of the slide holder under the objective.
17. Move the stage all the way to the right and position a front-to-back location that is on the same level as a point on the opposite side of the slide holder under the objective.
18. Bring the top surface of the slide holder at that location into focus.

    Take note of the z position located below mosaic pane for a later calculation.
19. Move the stage all the way to the left to position the same front-to-back location on the right side under the objective.
20. Bring the top surface of the slide holder at that location into focus.
21. If better flatness is required, repeat step 11 through step 20.

Note If it is critical for the top surface of the sample to be flat, the same leveling technique can be applied to the top of a sample, except Table 1 will no longer be valid. If sample is not very parallel, then leveling the top of the sample will result in the sample holder no longer being level. Using a higher powered objective may help.

22. Take the new z value and subtract the original z position (step 16).

    Z2 (step 20) - Z1 (step 16) = Z for Table 2

    Use this number and find the closest match from Table 2. The table provides an approximate amount to rotate the middle-right thumbscrew to level the slide holder from left to right. Rotate the thumbscrew clockwise if the number is positive; counterclockwise if it is negative.

    Note To check the flatness, move the stage to any location on the same surface, verifying that the image is in focus or near focus.

23. Carefully fasten the 3 hold-down screws starting at the back left corner of the slide holder, then the front left, and finish with the middle-right hold-down screw.

Installing Objectives

Each objective is designed to work with a specific illuminator. For example, if your microscope has the reflected light brightfield illuminator, only brightfield objectives will work. If you have the optional reflected light brightfield/darkfield illuminator, then brightfield and darkfield objectives can be used.

Note If you have the reflected light brightfield/darkfield illuminator, you can use objectives designed for the brightfield only illuminator if you use a special adapter. Contact us for more information.

❖ To install an objective

1. If there is no free space on the microscope nosepiece, unscrew one of the installed objectives and gently remove if from the nosepiece.
2. If installing a brightfield only objective into a brightfield/darkfield illuminator, screw the adapter ring into the nosepiece.
3. Gently screw the new objective into the nosepiece (or adapter).
4. If you just installed this objective for the first time, create a new video calibration for the new objective.

   If this objective has an existing video calibration, update the calibration.

Configuring Sample Illumination

This section explains how to set the microscope features that control sample illumination and how to control the brightness for each configuration. It also shows how to install and use the optional filters and polarizers. These topics are covered:

Contents

- Controlling Illumination with the Optional Reflected Light Brightfield/Darkfield Illuminator
- Controlling Illumination with the Optional Reflected Light Brightfield Illuminator
- Controlling Transmitted Light Illumination
- Using Optical Filters
- Using Optical Polarizers

The features and controls available for sample illumination depend on the options installed. Every microscope allows reflected light brightfield (BF) illumination. Reflected light darkfield (DF) illumination is available only if your microscope has the optional reflected light BF/DF illuminator and at least one objective that allows darkfield illumination. Transmitted light illumination is available only if the microscope also has the optional transmitted light illuminator. Polarized illumination is available only if you purchased an optional reflected light or transmitted light polarizer.

Controlling Illumination with the Optional Reflected Light Brightfield/Darkfield Illuminator

The features described below control sample illumination when the optional reflected light brightfield/darkfield illuminator is installed.

Figure 22. Sample illumination controls with the optional BF/DF illuminator

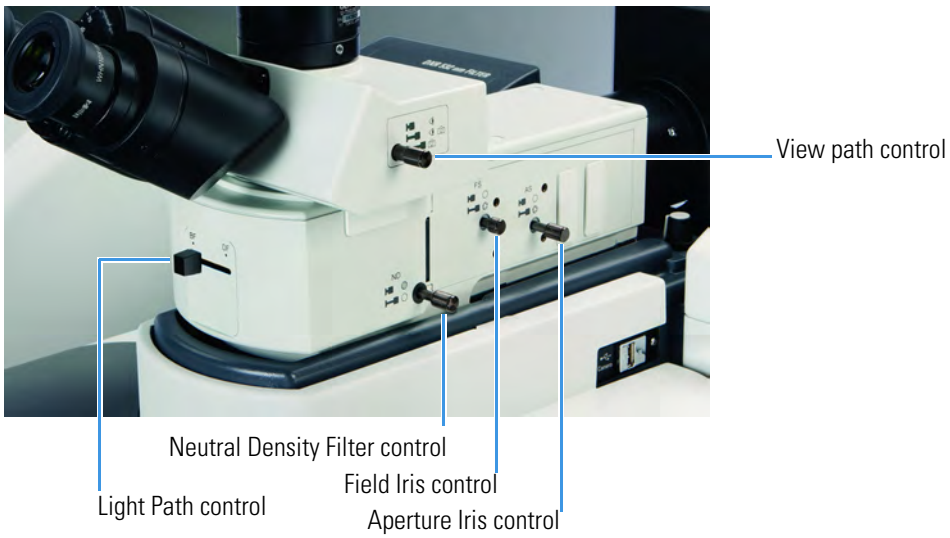


Table 3. Sample illumination controls with the optional BF/DF illuminator*

Feature	Description	Available Settings
View Path	Directs light through the microscope eyepiece or to the video camera (to produce a video image in the software) or both.	• In (eyepiece only) • Middle (eyepiece and video image) • Out (video image only)
Light Path	Directs light straight down onto the sample (BF) or from the edges (DF) to increase the contrast.	• BF (Brightfield) • DF (Darkfield)
Neutral Density Filter**	Reduces glare (protects the viewer from sudden changes in illumination, e.g., when switching the *light path* between darkfield and brightfield).	• In (ND filter in light path) • Out (no ND filter)
Field Iris (also referred to as a "field stop")	Blocks unnecessary light from the field of view (FOV) to optimize contrast.	• In (iris fully open and matched to 10x objective) • Out (iris fully closed)
Aperture Iris (also referred to as an "aperture stop")	Reduces reflected illumination across entire field of view. Typically used with high magnification objectives.	• In (iris fully open) • Out (iris fully closed)

* *Understanding Conjugate Planes and Köhler Illumination*, Davidson, M. and Fellers, T.National High Magnetic Field Laboratory, the Florida State University, 1800 E. Paul Dirac Dr., Tallahassee, FL 32306

** The ND filter setting is determined by the Light Path control. When Light Path is set to BF, the ND filter is in the light path; when Light Path is set to DF, the ND filter is not in the light path.

The information in the next table shows how to configure the illumination controls for reflected light observations (brightfield and darkfield) when the optional reflected light BF/DF illuminator is installed.

Table 4. Configuring sample illumination with the optional BF/DF Illuminator

Illumination Control	Reflected Light Brightfield Observation	Reflected Light Darkfield Observation*
View Path	Middle position	Middle position
Light Path	BF	DF
ND Filter	In (filter engaged)	Out (no filter)
Field Iris (FS)	Adjust so that iris image circumscribes objective FOV	In (open)
Aperture Iris (AS)	In (open) or slightly closed	In (open) or slightly closed

* Requires an objective that allows darkfield illumination.

Use the **Illumination Intensity control** on the illuminator power supply to adjust the brightness.

Figure 23. Illumination intensity control for optional BF/DF Illuminator

Controlling Illumination with the Optional Reflected Light Brightfield Illuminator

If your system has the optional reflected light illuminator, the light path is always configured for reflected light brightfield illumination. Use the **View Path control** to direct light through the microscope eyepiece or to the video camera (to produce a video image in the software) or both. The View Path control is normally set to the middle position which sends light to both locations.

Use the **Illumination Intensity control** on the microscope to adjust the brightness.

Figure 24. Illumination intensity control for the reflected light brightfield (only) illuminator

Controlling Transmitted Light Illumination

If your system has the optional transmitted light illumination kit, you can use transmitted light to observe transparent samples mounted on a transparent slide, or use transmitted and reflected light illumination together to optimize brightness and contrast. Transmitted light observations also require a sample holder that is open in the center.

The transmitted light illumination kit adds an LED light source and condensing optics under the microscope stage. The light source is positioned directly under the objective.

Use the **Illumination Intensity control** on the microscope to control the brightness of the transmitted light source.

Figure 25. Illumination intensity control for optional transmitted light illuminator (requires optional brightfield/darkfield illuminator)

Using Optical Filters

Various optical filters are available for customizing the character or overall brightness of the light from the reflected light illuminators. For more information about the optical filters available and how they affect the light, contact our local representative.

The reflected light brightfield/darkfield illuminator has two filter insertion slots (see the image below); the brightfield only illuminator has one.

NOTICE Insert optical filters only from the left side of the microscope.

Figure 26. Optical filter insertion slots[a] for reflected light BF/DF illuminator

[a] Insert optical filters from left side only.

❖ To install an optical filter

1. Remove the caps from one set of filter openings on the left and right side of the microscope.
2. Hold the filter by the labeled end with the label facing you.
3. Gently push the filter all the way into one of the filter insertion slots and then slowly draw it out until you feel the first click and then a second click.

   The two positions are described below.

   Table 5. Optical filter positions

Position	Used for...
First click	Filter in sample beam
Second click	Filter out of sample beam (beam path is open)

4. When you are finished using filters, replace the caps on the filter openings on both sides of the microscope.

Using Optical Polarizers

Optional polarizers are available for the reflected light illuminators and the transmitted light illuminator. There are two fixed polarizers that send polarized light to the sample (one for each type of illuminator).

Two polarization analyzers are also available (configurable and fixed). The polarization analyzers can be used to capture any change in polarization that is due to the state of the light interacting with the sample. The configurable polarization analyzer can be used with both of the fixed polarizers (reflected light and transmitted light). The fixed polarization analyzer works only with the fixed reflected light polarizer.

NOTICE Store polarizers in shipping cartons when not in use. Polarizer performance may deteriorate with extended exposure to light.

Figure 27. Optional fixed polarizers (send polarized light to the sample)

Figure 28. Optional polarization analyzers (can be used to capture any change in polarization that is due to the state of the light interacting with the sample)

Installing the Reflected Light Fixed Polarizer

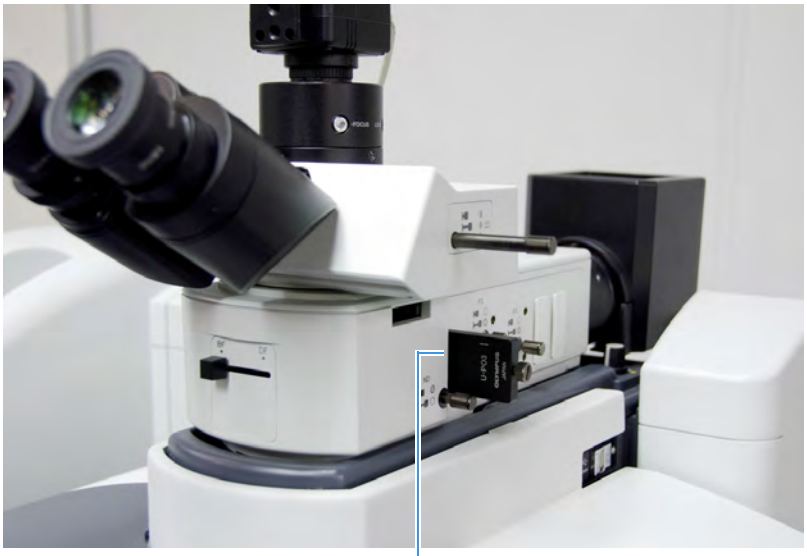
The fixed polarizer for the reflected light illuminators sends polarized light to the sample when reflected light illumination is in use. It can be used with an optional polarization analyzer to capture any change in polarization that is due to the state of the light interacting with the sample.

Figure 29. Reflected light fixed polarizer

The image below shows the location for inserting the reflected light fixed polarizer into the microscope.

NOTICE Insert the reflected light fixed polarizer only from the right side of the microscope.

Figure 30. Reflected light fixed polarizer insertion slot on the microscope



Insertion slot for fixed polarizer for reflected light illuminators

❖ To install the reflected light fixed polarizer

1. Remove the placeholder from the polarizer insertion slot.

   The placeholder prevents light from entering the opening when the polarizer is not in use. The placeholder looks like this:

   Figure 31. Placeholder for fixed reflected light polarizer insertion slot

2. Hold the polarizer by the labeled end with the label facing you.
3. Gently push the polarizer all the way into the insertion slot and then slowly draw it out until you feel the first click and then a second click.

   The two positions are described below.

   Table 6. Fixed reflected light polarizer positions

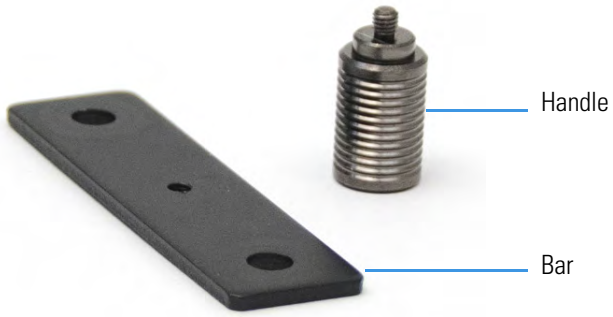
Position	Used for...
First click	Polarizer in sample beam
Second click	Polarizer out of sample beam (beampath is open)

4. When you are finished using the polarizer, remove it from the slot and replace the placeholder.

Reflected Light Fixed Polarizer Attachment

The reflected light fixed polarizer includes a small bar and handle that can be used to connect it to the configurable polarization analyzer if one is used. This allows the two to be clamped together for easier positioning into and out of the sample beam. Click here for more information.

Figure 32. Attachment for reflected light fixed polarizer



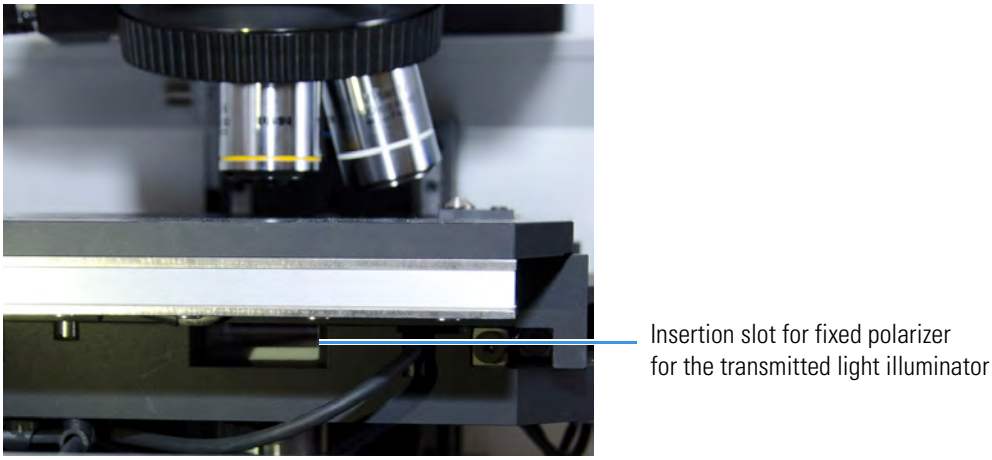
Installing the Transmitted Light Fixed Polarizer

The fixed polarizer for the optional transmitted light illuminator sends polarized light to the sample when the transmitted light illuminator is in use. It can be used with the optional configurable polarization analyzer to capture any change in polarization that is due to the state of the light interacting with the sample.

Figure 33. Transmitted light fixed polarizer

The transmitted light fixed polarizer fits in the transmission illumination assembly below the stage platform.

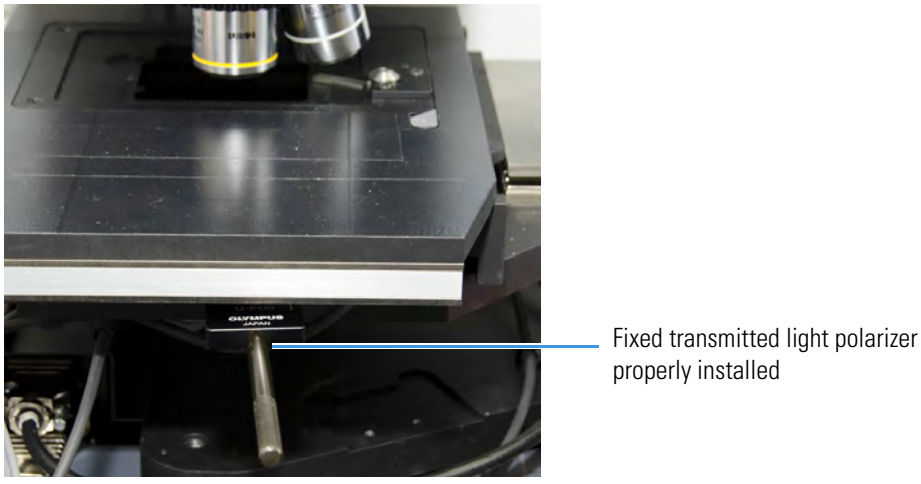
Figure 34. Transmitted light fixed polarizer insertion slot on the microscope



❖ To install the transmitted light fixed polarizer

1. Open the sample compartment doors and remove any sample from the microscope stage.
2. Lower the stage just enough to clear the selected objective and then move the stage toward the back of the microscope as far as it will go.
3. Remove the sample area cover.
4. If the handle is not attached to the fixed transmitted light polarizer, screw the handle into the hole at the end of the polarizer and tighten it finger tight.
5. Hold the polarizer by the handle (label should face up) while you gently slide it into the insertion slot under the stage platform.

   Figure 35. Transmitted light polarizer properly installed



6. Replace the sample area cover.

❖ To use the transmitted light fixed polarizer

Use the handle to gently push the polarizer all the way back and then slowly draw it forward until you feel the first click, second click and third click. The three positions are described below.

Table 7. Polarization analyzer positions

Position	Used for...
First click	Polarizer in sample beam
Second click	does nothing (beam path is blocked)
Third click	Polarizer out of sample beam (beam path is open)

Installing a Polarization Analyzer

Two polarization analyzers are available for the microscope (configurable and fixed). The polarization analyzers can be used along with the fixed polarizers to capture any change in polarization that is due to the sample.

The configurable polarization analyzer can be used with the fixed reflected light polarizer and the fixed transmitted light polarizer.

Figure 36. Configurable polarization analyzer (for use with both types of fixed polarizers)

The fixed polarization analyzer works only with the fixed reflected light polarizer.

Figure 37. Fixed polarization analyzer (for use with fixed reflected light polarizer only)

The image below shows the location for inserting a polarization analyzer into the microscope.

NOTICE Insert the polarization analyzer only from the right side of the microscope.

Figure 38. Polarization analyzer insertion slot on the microscope

❖ To install the configurable polarization analyzer

1. Remove the cap from the polarization analyzer opening on the left and right side of the microscope.
2. Hold the polarization analyzer by the labeled end with the label facing up.
3. Gently push the analyzer all the way into the insertion slot and then slowly draw it out until you feel the first click and then a second click.

   The two positions are described below.

   Table 8. Configurable polarization analyzer positions

Position	Used for...
First click	Polarization analyzer in sample beam*
Second click	Polarization analyzer out of sample beam (beampath is open)

   * Before installing sample, start with polarizer dial positioned so field of view is darkest when viewed through eyepiece.

4. When you are finished using the analyzer, replace the caps on the analyzer openings on both sides of the microscope.

❖ To install the fixed polarization analyzer

1. Remove the cap from the polarization analyzer opening on the left and right side of the microscope.
2. Hold the analyzer by the labeled end with the label facing up.
3. Gently push the analyzer in until you hear the first click and then a second click

   The two positions are described below.

   Table 9. Fixed polarization analyzer positions

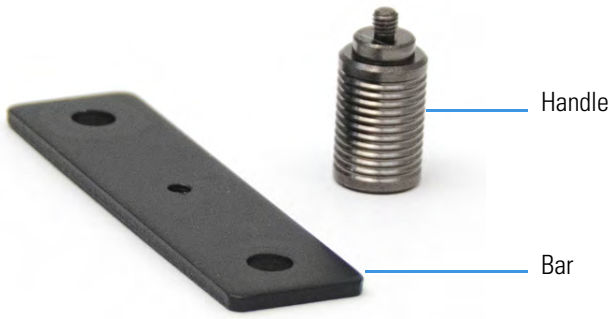
Position	Used for...
First click	Polarization analyzer **out** of sample beam (beampath is open)
Second click	Polarization analyzer **in** sample beam

4. When you are finished using the analyzer, replace the caps on the analyzer openings on both sides of the microscope.

Installing the Polarization Analyzer Attachment

The fixed reflected light polarizer includes a small bar and handle that can be used to connect it to the configurable polarization analyzer if one is used. This allows the two to be clamped together for easier positioning into and out of the sample beam.

Figure 39. Attachment for fixed reflected light polarizer



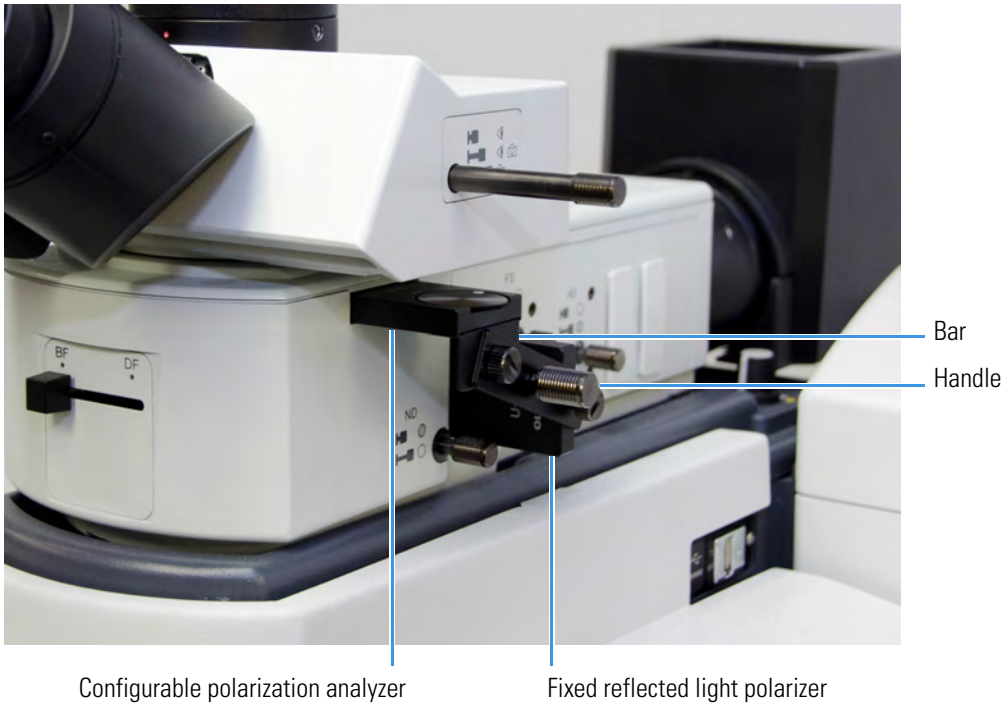
❖ To install the attachment

1. Attach the handle to the center hole in the bar.
2. Install the fixed reflected light polarizer.
3. Install the configurable polarization analyzer.
4. Use the screw at the end of the fixed polarizer to attach it to one end of the bar attachment.

5. Use the screw at the end of the polarization analyzer to attach it to the other end of the bar attachment.

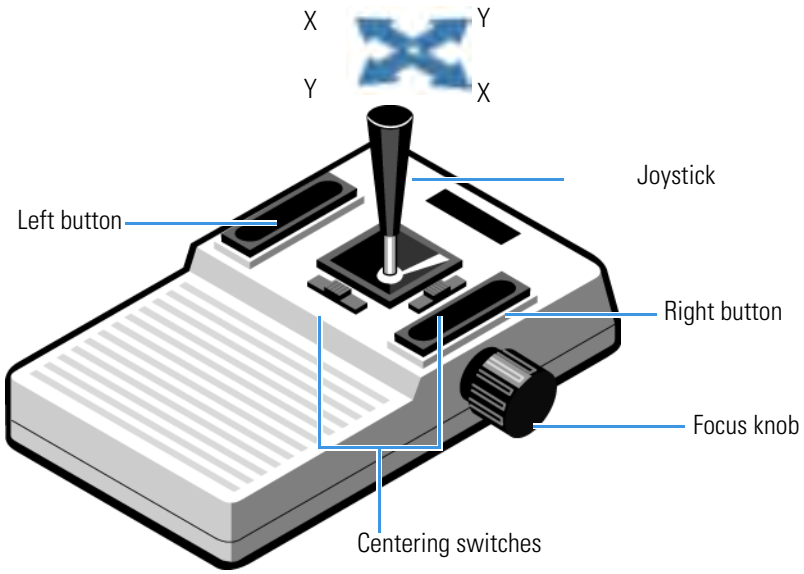
   Here is an image of the polarizer, analyzer and bar properly installed.

Figure 40. Fixed reflected light polarizer and configurable polarization analyzer clamped together for easy operation



Positioning the Motorized Stage

Figure 41. Joystick



Feature	Description
Joystick	Moves the motorized stage left and right along the X-axis or forward and back along the Y-axis
Focus knob	Adjusts the height of the stage along the Z-axis
Left button*	Press to adjust the speed of the XY movement
Right button*	Press to adjust the focus speed
Centering switches	Engage/disengage the centering springs on the joystick. If disengaged, the joystick will not self-center and will cause the stage to move when not expected. It is recommended that centering springs remain engaged.

* Three different settings: press once for slow; press twice for medium; press three times for fast. Press a fourth time to cycle back to slow.

Additional features that occur when the software is running:

- The stage speed will adjust automatically depending on the selected video calibration. This will allow the user to more accurately position a sample depending on the objective magnification (i.e., stage will move faster if 10x video calibration is selected as compared to a 100x video calibration).
- To aid in sample positioning (insert/eject) if the **Left** button is depressed, the XY joystick speed will increase to allow fast positioning. When released, the XY joystick will cycle to the next speed setting.
- When the **Right** button is depressed, the Z axis speed will increase as the "Focus knob" is rotated to allow fast sample positioning. This feature is particularly useful when loading/unloading samples. When the button is released, the previous focus speed is enabled.

Using Confocal Operation

Confocal operation provides a well defined focus that is useful when examining layered materials or very small samples.

To make maximum use of your microscope's confocal capability, use a 100x objective and select the 25 micrometer confocal pinhole aperture in the software.

Using the Raman Polarization Option

The microscope can be purchased with optional optics to collect polarized Raman measurements. If the polarization kit is installed, polarization settings will be available in the Advanced Acquisition settings tab within the OMNICxi software.

The microscope employs a 180° degree back-scattering configuration. The z(Y,Y)z, z(X,X)z, z(X,Y)z, and z(Y,X)z polarization states can be measured by selecting the appropriate laser polarization and analyzer polarization. The z(X,X)z is denoted as Horizontal polarization and the z(Y,Y)z is denoted as Vertical polarization. The horizontal axis is across the stage from left to right and the vertical axis goes from front to back.

There are three laser polarization options (Depolarized, Horizontal (X) and Vertical(Y)). Raman experiments that do not require the use of polarization optics should select **Depolarized** for the Laser Polarization.

The polarization analyzer can be set to Horizontal (X) or Vertical (Y) or any angle from 0 to 180 degrees by specifying a custom angle. See polarization acquisition settings for more information.

Test samples can be used to verify the performance of the polarization accessory. Recommended test samples are lithium niobate crystals (X-cut) or carbon tetrachloride.

Changing the Laser, Filter, and Grating

The primary reason for changing the laser is to avoid a fluorescence response from the sample. When the laser is changed, the filter and grating must also be changed.

❖ To install a laser

1. Slide the laser onto the metal tongue until it seats firmly against the instrument.

Figure 42. System with no laser installed

Figure 43. System with laser being slid into place

Figure 44. System with laser slid firmly into place

2. Rotate the handle and lock the laser into place.

   The handle should be point up as shown.

Figure 45. System with laser in place and locked

❖ To install a grating

1. Open the grating door.
2. Remove any installed grating.

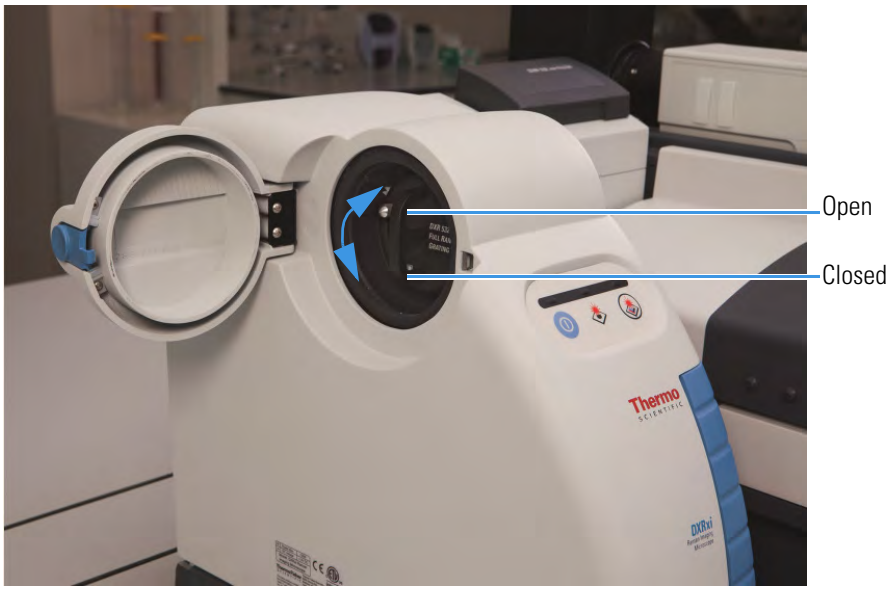
Figure 46. Removing a grating

3. Use a clean, lint-free cotton cloth to wipe the magnets that secure the grating when installed. (Magnets are located on the bottom of the new grating and inside the grating compartment on the instrument).
4. Install the grating.

Figure 47. Installing a grating

5. Open the grating shutter.

Figure 48. Grating shutter button



❖ **To change the filter**

1. Remove the installed filter.

Figure 49. Removing filter

2. Use a clean, lint-free cotton cloth to wipe the magnets that secure the filter when installed. (Magnets are located on the bottom of the filter and inside the filter compartment on the instrument).

3. Install the filter.

Installing a 633 nm Laser

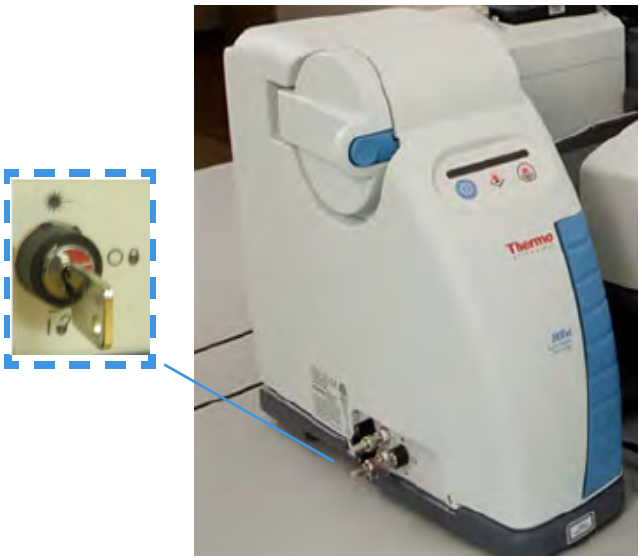
If you specifically ordered this laser wavelength with your system, see “Changing the Laser, Filter and Grating.” If you need to add this capability in the future, a service visit is required to ensure optimum alignment and performance. Contact our sales or service representative in your area.

Enabling the Laser

The laser must be enabled before it can be turned on. To enable the laser, rotate the laser key to the unlocked (vertical) position.

Note The laser key can only be removed when the laser keyswitch is in the locked (horizontal) position. The laser is disabled when the laser keyswitch is locked.

Figure 50. Laser unlocked (enabled)



Optional Class 3B Laser Configuration

The DXRxi microscope can be configured as either a Class 1 or Class 3B laser product. Class 3B laser radiation is hazardous and can cause permanent eye damage. Exposure to laser radiation should be avoided by setting up a laser safe area.

See the DXR Raman Instruments Site and Safety Guide that came with your instrument for a full description of necessary precautions.

WARNING Avoid eye injury. The system is a Class 3B laser product emitting visible or invisible laser radiation. Exposure to laser radiation could cause permanent eye damage.

- All persons using or in the vicinity of a Class 3B system must wear safety eyewear that exceeds the specifications for the laser in use
- Do not look directly into a laser, even when wearing laser safety eyewear
- Set up a laser safety area in the location where a Class 3B system is being used
- Making adjustments, using controls, or performing procedures that are not specified in the documentation could result in exposure to hazardous visible or invisible laser radiation.

You must purchase the Class 3B Conversion Kit to support removing the sample area cover the fiber optic accessory. If you purchase the kit after the base system is already installed, the kit must be installed by a Thermo Scientific Field Service Engineer (FSE). The FSE will apply proper Class 3B rating labels and will provide basic laser safety training.

Using a Class 3B Fiber Optic Launcher Accessory

WARNING Avoid eye injury. The fiber optic accessory is a Class 3B laser product. Exposure to laser radiation could cause severe burns or permanent eye damage.

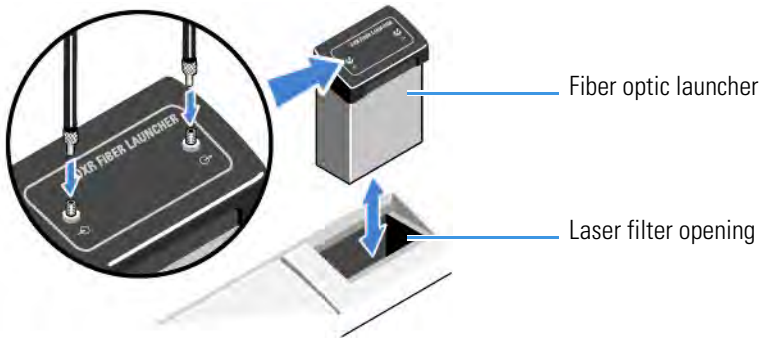
- Do not put anything except samples against the tip of the fiber optic probe.
- Always wear laser safety eyewear that exceed the specifications for the laser in use.
- Do not look directly into the tip of a fiber optic probe or the fiber optic probe connectors, even when wearing laser safety eyewear.
- Set up a laser safety area in the location where the fiber optic accessory is being used.

Make sure to follow all safety precautions before installing the fiber optic accessory. Making adjustments, using controls, or performing procedures not specified in the documentation could result in exposure to hazardous visible or invisible laser radiation.

❖ To install a fiber optic accessory

1. Remove the laser filter from the system and slide the fiber optic launcher into the laser filter opening.

Figure 51. Fiber optic launcher installation



CAUTION To ensure safe operation, always connect the collection probe cable to the fiber optic launcher before you connect the excitation probe cable.

2. Connect the fiber optic cables from the probe to the appropriate ports on the fiber optic launcher (see previous image).
   a. Insert the end of the probe cable labeled "Collection" into the connector and rotate the connector so it slides into the keyhole slot on the launcher. Tighten the knurled nut finger tight.
   b. Insert the end of the probe cable labeled "Excitation" into the connector and rotate the connector and tighten the nut as described above.

CAUTION To ensure safe operation, always replace the safety cap onto the excitation connector on the fiber optic launcher when the probe cable is not connected.

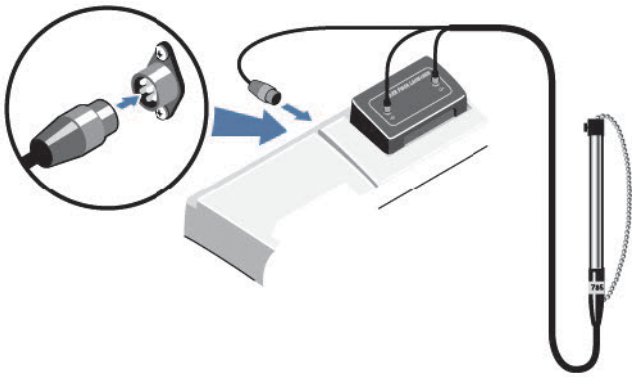
3. Insert the probe into the probe alignment tool to perform alignment and calibration.

   Rotate the probe into the tool so that the ball inside the tool slides into the indent on the side of the probe.

Figure 52. Probe positioned for alignment and calibration

4. Connect the power cable for the probe LED.

Figure 53. Connect power cable for probe LED



5. Attach the strain relief hook (provided) to the microscope by inserting the two screws (provided) into the two holes at the top of the microscope back panel (behind the laser filter compartment). Tighten the two screws.
6. Attach the probe cable to the strain relief hook.

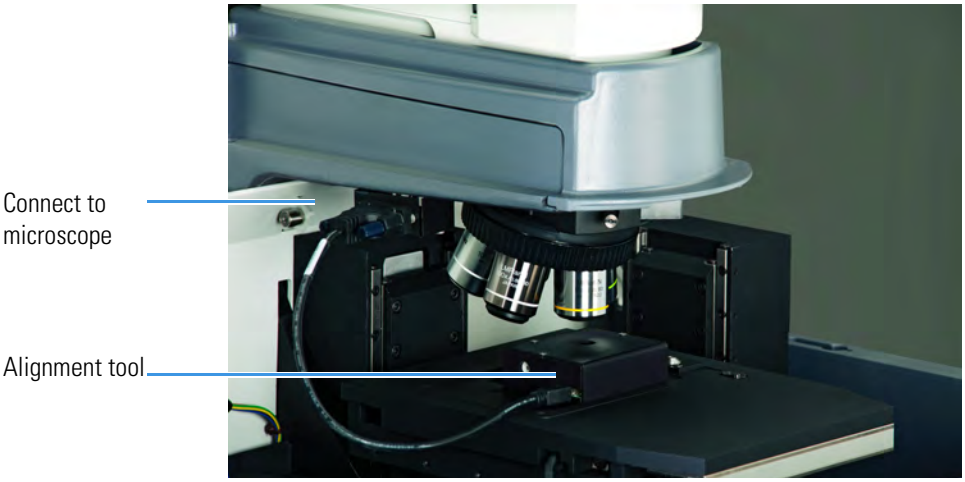
   Loop the nylon strain relief attached to the probe cable (before it splits) over the hook installed in the previous step.
7. Open the **Settings** box in the OMNICxi Acquisition window and click **Align/Calibrate** and choose OK to align and calibrate the instrument with the probe.

The Fiber Launcher includes two caps to cover the ports when the accessory is not attached. Always replace the caps to cover the ports when the accessory is not in connected.

Installing the Microscope Alignment and Calibration Tool

The alignment and calibration tool is connected to the instrument during installation, and only needs to be disconnected if the sample area cover is removed by the user. The tool is designed to fit the standard single slide holder that comes with the microscope.

Figure 54. Connecting alignment and calibration tool

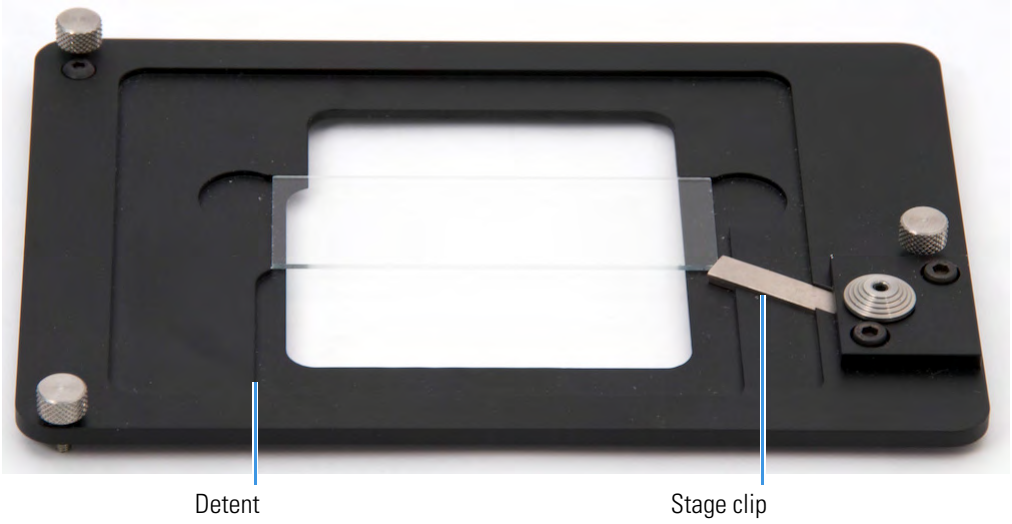


❖ To install the align/calibrate tool

1. Position the tool with the connector extending from the left (see image above).
2. Pull the stage clip toward you while you out slide the tool into the detent at the front of the single slide holder and then release the stage clip.

   Make sure the tool is all the way forward in the detent, level and secured by the stage clip and both connectors are secured.

Figure 55. Single slide holder



Tip For alignment and calibration instructions, see Aligning and Calibrating Laser Sets in the software help system.

Cleaning the Glass Window on the Alignment Tool

❖ To clean the glass window on the alignment tool

1. Use a can of dry compressed air or a dust blower to blow off any particles on the surface of the glass.
2. Tear a lens tissue in half to make bristles or use high purity cotton and dip it in distilled water.
3. Apply low pressure at the center of the glass and make an outward corkscrew motion to clean the glass.

   Do not repeat motion unless step 2 is repeated.
4. If there is still material on the glass that needs to be dissolved, many optics companies sell cleaning supplies to dissolve specific materials.

NOTICE Some cleaning supplies can dissolve materials that are used to hold the glass in place. Using a swab with a stick for cleaning optical components with a minimal amount of cleaning agent is preferred.

Servicing the Microscope

Cleaning Your Instrument

If the outside of the instrument needs cleaning, turn off the power to all components, unplug the power strip from the wall outlet, and wipe the outside of the instrument with a damp (not wet), soft cloth and a mild soap. Do not use harsh detergents, solvents, chemicals or abrasives. Additionally, any spilled sample around the stage can be cleaned by removing the sample holder and using a small vacuum then wiping with a damp cloth.

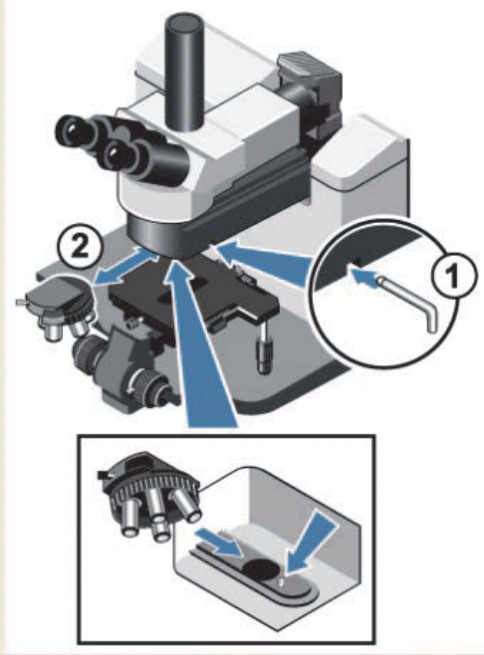
CAUTION Avoid shock hazard or system damage. Do not allow liquid to run into the power supply or the back of the instrument.

NOTICE Avoid permanent damage inside the instrument. The optical components are delicate, so be extremely careful when cleaning the microscope objectives.

- Do not attempt to clean or touch any optical surface inside the instrument
- Do not attempt to clean a filter or grating
- If an objective needs to be cleaned, use pressurized, filtered nitrogen or air to blow off dust and other particles
  - If this is not sufficient, use commercial cleaning solution and lens paper that is specifically for cleaning delicate glass lenses

Replacing the Nosepiece

Figure 56. Replacing the nosepiece



When installing a nosepiece, make sure to slide it in until it stops, and then tighten the screw until it is just tight. Do not over tighten.

When installing a new nosepiece or changing objectives, set the Objective selection in the software to the new objective you will be using.

Tip Do not remove the screw because it is difficult to replace.

Replacing an Illuminator Bulb

Note If you suspect a brightfield or transmission illuminator needs to be replaced, or if there is a problem with the illuminator, contact us.

Time needed: Approximately 10 minutes

Tools needed: A 3.0 mm hex wrench

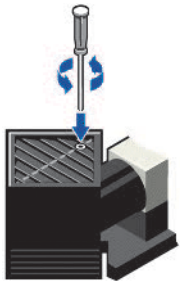
Parts needed: Replacement illuminator bulbs (contact us for ordering information)

CAUTION Avoid fire hazard. Use only illuminator bulbs supplied by us to avoid fire hazard.

❖ To replace a brightfield/darkfield illuminator bulb

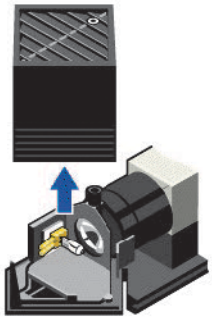
1. Turn off the instrument power and unplug the power cable from the power source.
2. Use a 3.0 mm hex wrench to loosen, but not remove, the screw.

   Figure 57. Loosening the screw



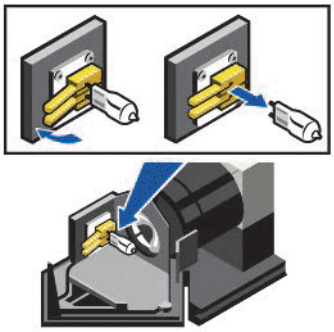
3. Lift the cover off.

   Figure 58. Lifting cover



4. Squeeze the levers to release the bulb.

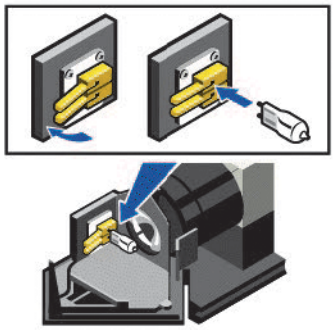
   Figure 59. Releasing the bulb



5. Squeeze the levers, insert the new bulb all the way, and release the levers.

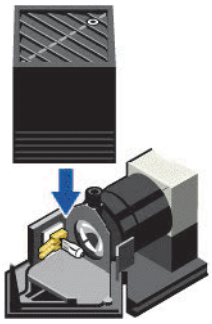
   To extend bulb life, always handle a new bulb with a clean tissue or cloth.

   Figure 60. Inserting the bulb



6. Replace the cover.

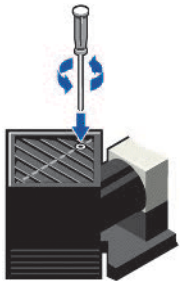
   Figure 61. Replacing the cover



7. Use a 3.0 mm hex wrench to tighten the screw.

   Do not over tighten.

   Figure 62. Tightening the screw



8. Connect the instrument power cable to a power source.

   For example, an electrical outlet or power strip.

9. Turn on the instrument power.

Safety Information

NOTICE **Be sure that all persons operating this system read the site and safety manual first.**

Laser Precautions

WARNING Avoid eye injury. The system may be configured as a Class 1 or Class 3B laser product emitting visible or invisible laser radiation. Exposure to laser radiation could cause permanent eye damage.

- All persons using or in the vicinity of a Class 3B system must wear safety eyewear that exceeds the specifications for the laser in use
- Do not look directly into a laser, even when wearing laser safety eyewear
- Set up a laser safety area in the location where a Class 3B system is being used
- Making adjustments, using controls, or performing procedures that are not specified in the documentation could result in exposure to hazardous visible or invisible laser radiation.

For more detailed information, see the Site and Safety Guide that came with your instrument.

Moving Parts

CAUTION Avoid pinch hazard. To prevent personal injury, keep hands free from moving parts.

The microscope contains an automated stage that is controlled by the software. There are several places on and around the stage where a person or object could be pinched by moving parts. When operating the system, keep hands and fingers away from the stage area except to position the sample or change accessories. Remove your hands from the system when operating the software.

Biohazards or Radioactive Materials and Infectious Agents

Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Wear appropriate protective equipment. Individuals should be trained according to applicable regulatory and organization requirements before working with potentially infectious materials. Follow your organization's Biosafety Program protocols for working with and/or handling potentially infectious materials.

WARNING Reduce the risk associated with potentially infectious samples:

- Do not spill samples inside the sample compartment or any of the instrument components.
- If spill occurs, disinfect the external surfaces immediately.

Instruments, accessories, components or other associated materials should not be disposed of and may not be returned to us or other accessory manufacturers if they are contaminated with biohazard or radioactive materials, infectious agents, or any other materials and/or conditions that could constitute a health or injury hazard to employees. Contact us if you have questions about decontamination requirements.

Cooling Vents

To avoid overheating, do not block the free flow of air around the instrument. There should be at least 2 cm (about 1 in) of clearance behind the instrument and at least 30 cm (about 12 in) of clearance on each side.

Ventilation

There are no special ventilation requirements for your instrument. The types of analysis you plan may require special ventilation (for example, if you will be analyzing highly toxic samples). Chlorinated solvents, perfluorochlorinated solvents, and other solvents containing halogenated hydrocarbons are often used as sample solvents. The pyrolysis of these solvents by laser absorption may produce hydrochloric acid (HCl), hydrofluoric acid (HF), or phosgene ($COCl_2$).

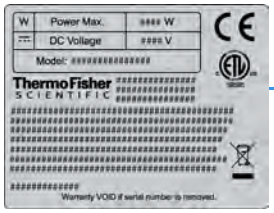
Hydrochloric acid and hydrofluoric acid are highly corrosive and may cause accelerated corrosion of the metallic and optical components in the instrument if seals are not properly maintained or the concentration level of corrosive gases in the air is excessively high due to improper sampling techniques.

WARNING Avoid toxic inhalation. Hydrochloric acid, hydrofluoric acid, and phosgene are highly toxic. If you plan to regularly use solvents containing halogenated hydrocarbons, be sure your work area is properly ventilated.

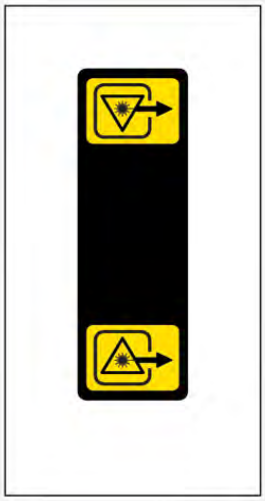
Be sure to provide storage space for solvents containing halogenated hydrocarbons that is away from the instrument, and do not leave these types of solvents in the sample compartment for an extended time.

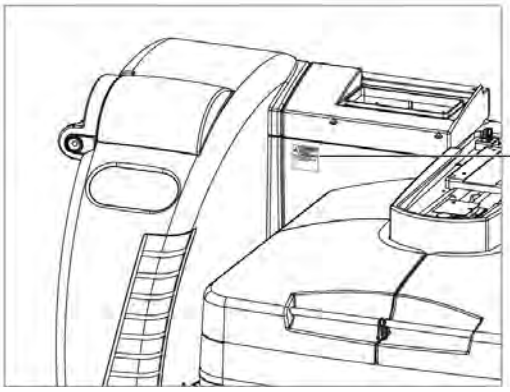
Microscope Safety Labels

CAUTION
AVOID EXPOSURE
VISIBLE AND/OR INVISIBLE LASER RADIATION EMITTED FROM THIS APERTURE.

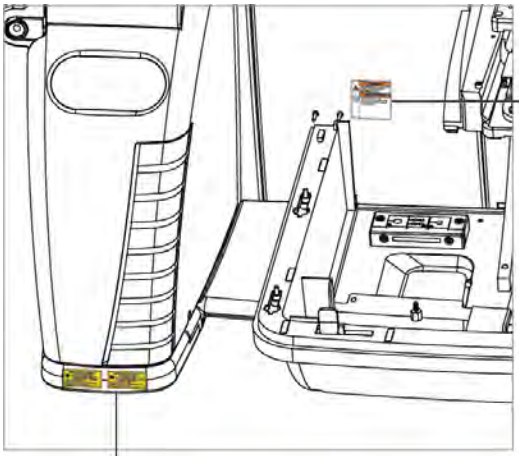

W
Power Max.
DC Voltage
Model:
ThermoFisher
SCIENTIFIC

Emits Class 3B
laser radiation
WARNING
Class 3B visible and/or invisible laser radiation when in use.
AVOID EXPOSURE TO BEAM.
AVERTISSEMENT
Classe 3B visible et / ou un rayonnement laser invisible lors de son utilisation. ÉVITER L'EXPOSITION AU RAYON.
532nm
Collection
Excitation
DXR FIBER LAUNCHER
Emits Class 3B laser r
fiber accessory is not
covers are not installe





⚠ WARNING
Class 3B visible and/or invisible laser radiation when in use. AVOID EXPOSURE TO BEAM.
⚠ AVERTISSEMENT
Classe 3B visible et / ou un rayonnement laser invisible lors de son utilisation. ÉVITER L'EXPOSITION AU RAYON.



⚠ WARNING
Class 3B visible and/or invisible laser radiation when in use. AVOID EXPOSURE TO BEAM.
⚠ AVERTISSEMENT
Classe 3B visible et / ou un rayonnement laser invisible lors de son utilisation. ÉVITER L'EXPOSITION AU RAYON.

VISIBLE AND/OR INVISIBLE LASER RADIATION
CLASS 3B LASER PRODUCT
AVOID EXPOSURE TO BEAM.
Power Max. 350 mW
Wavelength nm: 455, 532, 633, 780, 785
Pulse Duration: Continuous

RADIATION LASER VISIBLE ET/OU INVISIBLE
PRODUIT LASER DE CLASSE 3B ÉVITEZ L'EXPOSITION AU FAISCEAU.
Puissance maximum 350 mW
Longueur d'onde en nm : 455, 532, 633, 780, 785
Durée de l'impulsion : continue

3

OMNICxi Software Operation

Use the OMNICxi Raman imaging software with your DXRxi Raman Imaging Microscope to quickly identify and characterize solids, liquids, or microparticles. Analyze regions of the sample surface, locate and characterize particles, view live spectra, measure how your sample changes over time, or explore 3D visualizations.

General Workflow

The standard workflow for collecting and analyzing data can be described in the following high-level steps:

- **Collect a mosaic:** Before acquiring and analyzing data, collect a mosaic. The mosaic is a collection of static pictures of the sample surface and is used to define regions to analyze, locate particles, view spectra and more.

  Collect a terrain mosaic for samples with rough or uneven surfaces. A terrain mosaic takes longer to collect than a classic mosaic, but results in an image that is completely in focus without special sample preparation.

- **Acquire and analyze data:** Set the parameters for data acquisition and analysis, then collect and analyze data.

- **Create reports or share data:** Create reports or export your raw data for further analysis in other applications.

Collect a Mosaic

Before acquiring new data, first collect a mosaic. A mosaic is a collection of static pictures of the sample surface. Use the mosaic to set the parameters for data acquisition and analysis. For example, draw regions to create a chemical image or locate particles for particle analysis.

A terrain mosaic allows you to create a mosaic in which every part of the sample surface is in focus, even if the sample surface is rough or uneven. After collecting a terrain mosaic, you can view the terrain map, which is a 3D visualization of the surface topography of the sample.

Acquire and Analyze

Once you have collected a mosaic, set the parameters for your data acquisition and then collect and analyze the data. Your settings and techniques depend on the type of analysis you are performing.

Report

After collecting and analyzing your data, generate a customizable report with chemical images, spectra, and information on acquisition settings. You can also export images and raw data for further analysis in other applications.

Overview of the Workspace

In the OMNICxi Raman Imaging software, you will use two primary windows for collecting and analyzing data:

- Use the OMNICxi Acquisition Window to view your sample and collect data.
- Use the OMNICxi Analysis Window to view analysis results and explore the sample in depth.

For the best experience, use two monitors to view both windows at once, allowing you to view a live image of your sample while you perform your analysis.

The secondary windows for 3D analysis and terrain mapping can be used to explore 3D sample data and to view 3D visualizations of the sample's surface topography.

This section describes all of the tools and features in the Acquisition, Analysis, 3D Analysis, and Terrain windows.

OMNICxi Acquisition Window

This section covers all of the features and controls available in the Acquisition window, which can be used to:

- View a large area of the sample surface at any zoom level (see Video mode)
- Quickly collect and view spectra collected at selected locations on a sample (see Live Spectrum Mode)
- Acquire a preview chemical image (see Image Regions Mode or Image Cross Section Mode)
- Perform real-time acquisition of multiple regions on the sample surface (X, Y plane) (see Image Regions Mode)
- Perform real-time collection of a sample cross-section (Y, Z plane) (see Image Regions Mode)

- Create a terrain map to visualize the sample's surface topography
- Locate and characterize particles (see Identify and Analyze Particles)

This section covers these topics:

- General Features and Controls
- Video Mode Features and Controls
- Image Regions Mode
- Image Cross Section Mode
- Live Spectrum Mode

For an overview of the Acquisition window and a tutorial on how to collect and analyze a basic sample, refer to the *DXRxi Quick Reference* guide. A copy of the guide in PDF format is included in the electronic documentation set provided with the instrument.

General Features and Controls

This section explains the features that are visible in the Acquisition window.

To learn about a feature in the OMNICxi Acquisition window, click its name in the image below.

Figure 63. OMNICxi Acquisition window, general features and controls

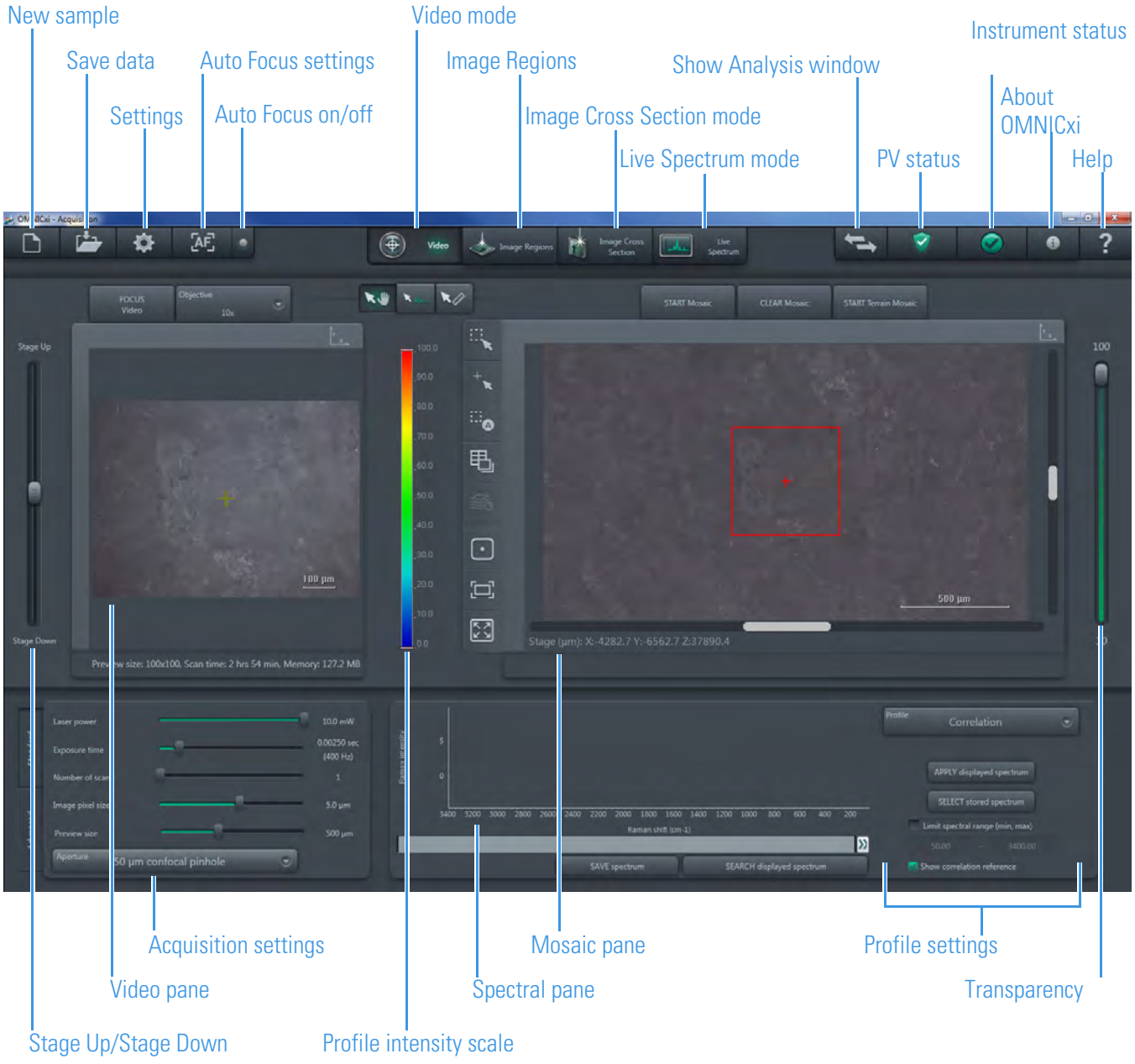


Table 10. Acquisition window, general features and controls

Feature	Description
New sample	Clears all data from the video and mosaic panes in the Acquisition and Analysis windows to prepare the system for collecting data with a new sample. After you confirm this action, the data collection features in the OMNICxi Acquisition window are enabled.
Save current data	Saves any newly acquired or updated data in the Acquisition and Analysis windows. The following information is saved: • mosaic, if one was created • all regions of acquired chemical image data and their associated acquisition settings • all applied profiles and their associated profile settings OMNICxi saves 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. Image data can be saved in these file formats: • **.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. **Tip**: Use Save Current data to set the filename before data collection. Click here to set the default folder and path where OMNICxi saves image files.
Settings	Instrument settings

Table 10. Acquisition window, general features and controls

Feature	Description
Auto Focus Settings	Defines settings for focusing the sample while collecting a mosaic in the Acquisition window, and for applying the focus (Z height) information while collecting data in Live Spectrum mode and Image Regions mode. Settings are used only when the Auto Focus On/Off indicator is selected. These options are available for adjusting the auto focus: • **Video** auto focus mode and settings: – **Each video image**. Brings the sample area into focus before adding each static picture to the mosaic. – **Every __ µm**. Focuses the sample whenever the stage is more than the specified distance from the nearest focus location during collection of a mosaic. • **Spectral** auto focus mode and settings: – **Limit spectral range**. Allows you to limit the frequency range used for spectral auto focus in order to emphasize specific features in the spectral data. To set this feature, collect a mosaic, choose Live Spectrum mode, click a point of interest in the mosaic to acquire a spectrum at that location. Locate a region of interest in the displayed spectrum. Enter the region's minimum and maximum X-axis values in the Spectral auto focus settings. The software will create the mosaic and apply the focus (Z height) information during data collection in order to maximize the features in the selected region. Setting is applied only when Auto Focus On/Off indicator is selected.

Table 10. Acquisition window, general features and controls

Feature	Description
Auto Focus On/Off indicator	Turns the Auto Focus settings on and off. The indicator is on when this option is selected and off when deselected. • **Video**. When a mosaic is collected with this option selected, the software either focuses the sample before collecting each picture in the mosaic or it brings the sample into focus every time the stage moves the specified distance (depending on the specified Auto Focus Video settings). When deselected, the entire mosaic is collected using the current Z-axis position of the sample stage. When collecting data in Live Spectrum mode or Image Regions mode with this option selected, the software moves the stage to the selected X, Y location and then uses the stored Z-axis (focus) position to collect the data. • **Spectral**. When collecting data in Live Spectrum mode with the Spectral auto focus and Limit spectral range options selected, each time you click a location in the mosaic, the software moves the stage to the selected X, Y location and performs a spectral auto focus at that location before collecting the data. When acquiring image data in a selected region with the Spectral auto focus and Limit spectral range options selected, the software performs a spectral auto focus at the center of each region before acquiring the data.
Video mode	Lets you view the sample surface (video pane) and create a mosaic or a terrain mosaic of a larger area of the sample surface (mosaic pane). For more information, see Video Mode Features and Controls.
Image Regions mode	Can be used to: • collect a preview chemical image • select regions for data acquisition and analysis • acquire a chemical image of all selected regions For more information, see Image Regions Mode.
Image Cross Section mode	Can be used to collect a Y-, Z-axis depth profile of spectra along a line for samples that allow you to see spectra at different layers in the material. The sample must be optically transparent to the laser wavelength. For more information, see Image Cross Section Mode.
Live Spectrum mode	Can be used to acquire a spectrum at the current cursor location in the mosaic. The spectrum is displayed in the spectral pane. For more information, see Live Spectrum Mode.

Table 10. Acquisition window, general features and controls

Feature	Description
Show Analysis window	If the system computer is connected to one display monitor (rather than two), use this button to quickly display the OMNICxi Analysis window. A similar button in the Analysis window allows you to quickly switch back to the Acquisition window.
PV status	Shows performance verification (PV) status for the currently installed laser set (laser, filter and grating): PV information for current laser set is valid (all tests passed) and up-to-date PV information for current laser set is invalid (one or more tests failed during last PV run) PV information for current laser set is either out-of-date or this laser set has never been validated No PV tests available for current laser set **Note**: Only full range gratings can be verified. PV is also unavailable for the fiber probe. Also lets you run PV tests and view PV test results. To run PV, click the PV status icon and choose **Run**. To see PV test results for this instrument, click the PV status icon and choose **Results**.
Instrument status	Shows the status of the instrument: Instrument is operational Instrument is waiting for a response. Click the icon to see the status. Click a status condition to display more information. One or more errors occurred. Click the icon to list the errors. Click a listed error to display more information.
About OMNICxi	Provides the instrument serial number and version information about the firmware and software

Table 10. Acquisition window, general features and controls

Feature	Description
Help	Displays Help information for the instrument and software
Stage Up/Stage Down	Brings the sample into focus at the current stage location. Drag the scroll box up or down for coarse focus. Use the up and down arrow buttons for fine focus. **NOTICE** Avoid possible damage to your sample or the objective lens. If you need to move the stage a great distance along the Z-axis, open the sample compartment doors and use the Focus knob on the stage controller to move the stage while you watch the sample to avoid bumping the sample into the lens.
Video pane	In video mode, 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 numerical aperture of the currently selected objective.
Mosaic pane	Before you collect a mosaic in video mode, this pane shows a static picture of the sample as it appears when viewed through the microscope eyepiece (one field of view). After you collect a mosaic or a terrain mosaic, the mosaic appears in the mosaic pane.
Spectral pane	• In Video mode, if a spectrum was displayed in this pane in Image Regions, Image Cross Section, or Live Spectrum mode, the spectrum will remain displayed when you switch to Video mode. • In Image Regions mode and Image Cross Section mode (and in the OMNICxi Analysis window), the spectral pane shows the spectrum collected at the current cursor location in a region of acquired chemical image data. The spectral pane is also used for specifying peak heights and areas for the peak height, peak area, and other profile types. • In Live Spectrum mode, shows the spectrum acquired at the current cursor location. Click here for details about the features available in the spectral pane.
Acquisition settings	Settings available to acquire spectral data in Image Regions mode or Image Cross Section mode. The settings affect the quality and speed of acquired chemical image data. A limited set is available in Live Spectrum mode.
Profile settings	Defines the algorithm used to convert the spectral data into a color map, referred to as a chemical image. The chemical image is created in Image Regions mode or Image Cross Section mode.

Table 10. Acquisition window, general features and controls

Feature	Description
Profile intensity scale	In Image Regions mode and Image Cross Section mode, defines the scale or range and corresponding colors used to create the chemical image.
Transparency	In Image Regions mode and Image Cross Section mode, changes the transparency of any acquired chemical image data so you can view features below them in the mosaic.

Spectral Pane Features and Controls

The spectral pane appears in all of the available display modes in the OMNICxi Acquisition window. A spectral pane with reduced features also appears in the OMNICxi Analysis window. The spectral pane has these general characteristics:

- In Video mode, if a spectrum was displayed in the spectral pane in Image Regions, Image Cross Section, or Live Spectrum mode, the spectrum will remain displayed when you switch to Video mode.
- In Image Regions mode and Image Cross Section mode (and in the OMNICxi Analysis window), the spectral pane shows the spectrum collected at the current cursor location in a region of acquired chemical image data. The average of the selected location and the eight adjacent spectra is shown when "Show Average Spectrum at Location" is selected in the right click menu of the mosaic pane. The spectral pane is also used for specifying peak heights and areas for the peak height, peak area and other profile types.
- Live Spectrum mode shows the spectrum acquired at the current cursor location in the collected mosaic.

If OMNICxi has associated spectral libraries, any displayed spectrum is quickly searched against the selected libraries. To see the search results, click **SEARCH Displayed Spectrum,** if available, below the spectral pane. The results are displayed in a popup window.

Use the Search Setup options in the Analysis window to specify regions to use for the spectral search.

In some instances, other spectra may appear in the spectral pane. Here are some examples:

- Opening a search results window temporarily adds the spectrum of the selected library match 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. The library spectrum disappears after you close the search results window.
- When Correlation profile analysis results are displayed, use **Show correlation reference** to add the correlation reference spectrum to (or remove it from) the spectral pane.
- When MCR profile results are displayed, select an MCR thumbnail to add its associated pure component spectrum to the spectral pane.

To learn about a feature in the spectral pane, click its name in the image below.

Figure 64. Spectral pane features and controls

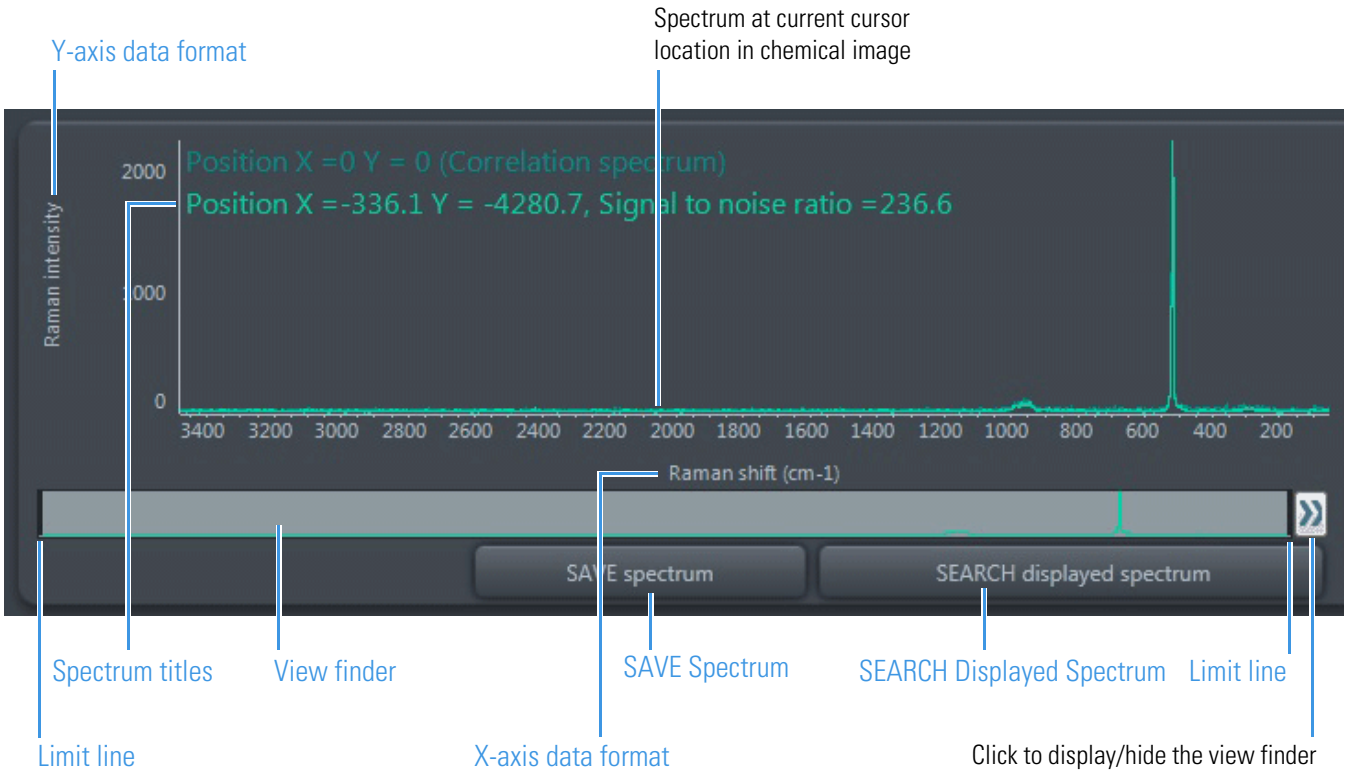


Table 11. Spectral pane features and controls

Feature	Description
Y-axis data format	The Y-axis of displayed spectra is always Raman intensity (detector counts)
X-axis data format	Defined by the Data Format option in the Settings box
Spectrum title	Shows the associated spectrum's location in the acquired chemical image and the signal-to-noise ratio at that location
View finder	Can be used to: • **Change** the display limits of spectra displayed in the spectral pane (drag the vertical limit lines) • **Display** a different region of spectra displayed in the spectral pane (click the region) • **Expand** the X-axis of spectra displayed in the spectral pane to their limits (double-click between the limit lines)

Table 11. Spectral pane features and controls

Feature	Description
Save Spectrum	Saves the selected spectrum (or spectra) as an OMNIC .SPA (or .SPG) file, which can be opened using the Thermo Scientific OMNIC Workstation for DXRxi Raman Imaging Microscope or OMNIC Specta application for further processing. To select a spectrum, click its title in the spectral pane. To select additional spectra, hold down the Shift key while you click another spectrum title.
Search Displayed Spectrum	Searches the displayed spectrum against the available spectral libraries to identify the associated compound. A search results window appears above the button. The name of the best matched library spectrum 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. If no suitable match is found in the library you can select Unknown and no matching spectrum will be displayed. To close the search results window, click Search Displayed Spectrum again. After you close the window, the library spectrum disappears from the spectral pane. 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: • The compound name • The name of the library that contains the spectrum • A 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 the match. Use the Select Libraries button in the OMNICxi Analysis window to select the libraries to search. Use the Search Setup button in the OMNICxi Analysis window to specify one or more spectral regions to use in the search. **NOTE** The Search Displayed Spectrum button is available in the Acquisition window only. To see search results from the OMNICxi Analysis window, click the **Results** buttons in the profile thumbnail.

Table 11. Spectral pane features and controls

Feature	Description
Right-click menu options	• **Select all displayed spectra**. Selects all the spectra that are currently displayed in the spectral pane. • **Copy displayed spectra as picture**. Copies an image of the spectral pane and its contents to the Windows Clipboard. The copied image can be pasted into any word processing, presentation or other compatible application. The resolution of a copied image will be the same as the computer's display monitor (typically 72 dpi or 96 dpi). • **Save displayed spectra as EMF**. Saves an image of the spectral pane as an EMF (Enhanced Metafile), which is a 32-bit version of the Windows Metafile image file format. • **Export > Selected spectrum (or spectra) as TXT**. Exports displayed spectral data to a tab separated text file (one spectrum per file), which can be opened with any text processing application such as Notepad. • **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 Workstation) or the data tray (OMNIC Specta). • **Save selected spectrum (or spectra) as SPA (or SPG)**. If one spectrum is selected, it can be saved in an OMNIC spectral (.SPA) file. If multiple spectra are selected, they can be saved in an OMNIC group (.SPG) file. • **Show background**. Displays the current background spectrum in the spectral pane (replaces the spectrum at the current cursor location if one was displayed). The instrument makes background measurements automatically as needed. The laser is blocked during background collection and no sample information is used. Information from the background is used to separate absorptions that are due to the instrument or molecules in the environment from absorptions that are due solely to the sample.

Table 11. Spectral pane features and controls

Feature	Description
Right-click menu options (*continued*)	• **Display** – **Full Y scale**. Shows all displayed spectra so that they fill the pane vertically. – **Common Y scale**. Shows all displayed spectra using the same Y-axis scale, making it easy to compare their relative peak heights visually. The minimum and maximum Y values from all the spectra become the new Y-axis display limits. Uses the displayed region to calculate the highest and lowest points that define the common scale. – **Full X and Y scale**. Applies Full Y Scale to all displayed spectra and also expands the X-axis to show the entire spectral range. (Double-clicking the spectral pane performs this function as well.) – **Auto scale**. Applies Full Y Scale to all spectra at all times for optimum viewing. – **Stack spectra**. Stacks multiple displayed spectra in the spectral pane so you can view them individually.

These additional features are available for working with displayed spectra:

Table 12. Features for working with displayed spectra

If you want to...	then...
Pan X-axis	Use the Grab tool to drag displayed spectra left and right. To reset the X-axis, double-click the spectral pane.
Expand X-axis to show entire spectra	• Double-click the spectral pane • Double-click between the two limit lines in the view finder
Expand or contract Y-axis	Use the Grab tool to drag displayed spectra up and down
Zoom X-axis to show more detail	• Draw a box around a region of a displayed spectrum, then click inside the box • Drag the vertical limit line at each end of the view finder

Video Mode Features and Controls

Select **Video mode** in the OMNICxi Acquisition window for the following tasks:

- View a live video image of the sample surface. The image is displayed in the video pane.
- View a static picture of the sample surface as seen through the microscope eyepiece (one field of view). The optical image is displayed in the mosaic pane.
- Collect a mosaic (collection of static pictures) of a larger area of the sample surface (use the scroll wheel on the mouse to change the scale in the mosaic pane). The mosaic is displayed in the mosaic pane. (It replaces the optical image.)
- Collect a terrain mosaic of an area of the sample surface. A terrain mosaic creates a mosaic image that is completely in focus, even if the sample surface is rough or uneven. With a terrain mosaic, you can view the terrain map of the surface topography.
- Focus, zoom or pan the video image
- Zoom or pan the optical image or mosaic
- Measure the length of features in the optical image or mosaic
- Select a preview region (for acquiring a preview chemical image)

This section explains the main features available in the Acquisition window when Video mode is selected. To learn about a feature, click its name in the image below.

Figure 65. Video mode features and controls

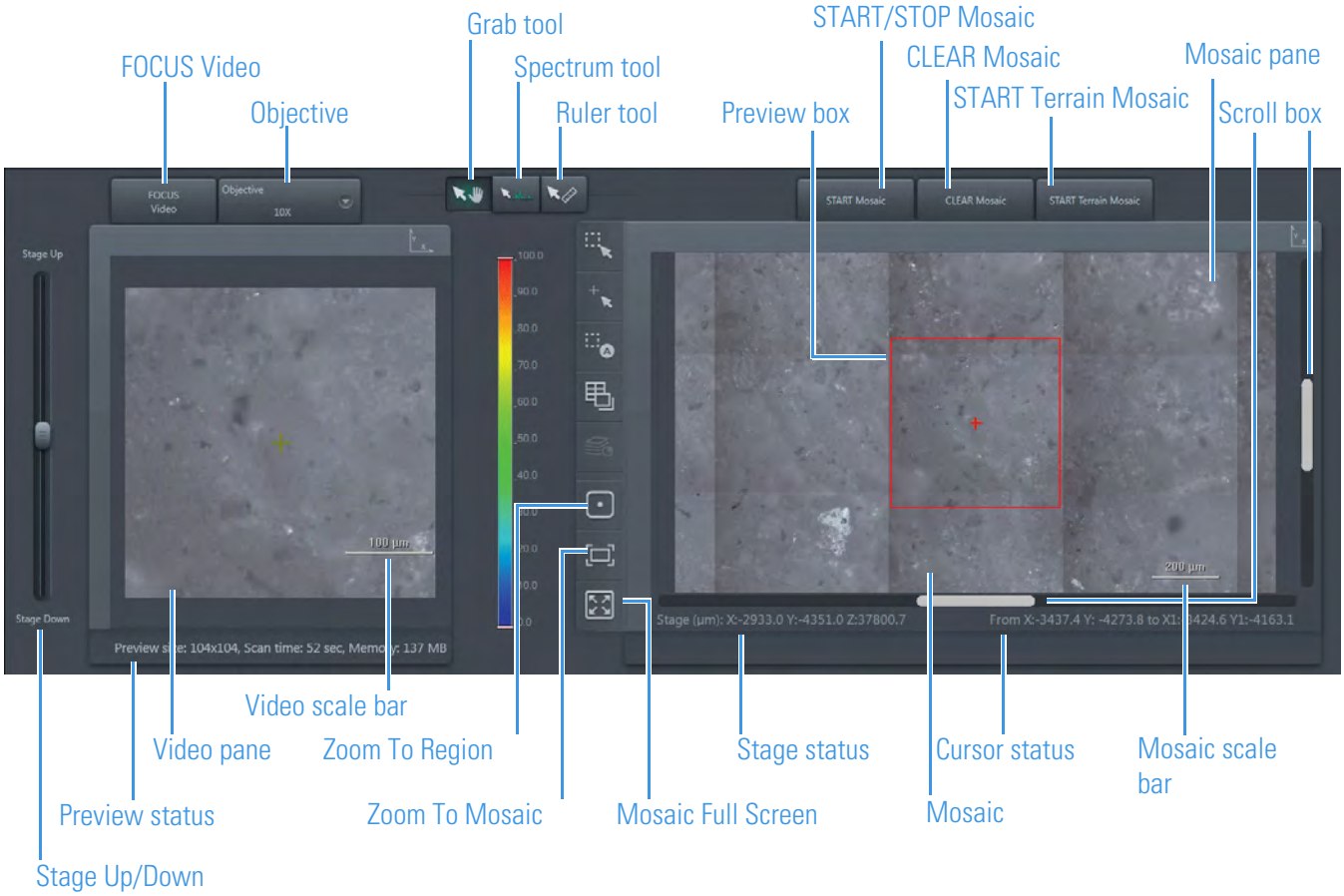


Table 13. Video mode features and controls

Feature	Description
FOCUS Video	Brings the sample into focus at the current stage location.
Objective	Allows you to select a video calibration for the objective that is currently selected on the microscope. The video calibration matches the number of micrometers displayed in the video pane to the number of micrometers in the microscope's field of view. Each microscope objective must have a matching video calibration and video calibrations sometimes require updates. Click here for instructions to create or update video calibrations. **NOTE** Make sure the correct video calibration is selected in the software before you collect a mosaic.

Table 13. Video mode features and controls

Feature	Description
Grab tool	In Video mode, the Grab tool can be used to: • Drag the video image, chemical image or zoomed in mosaic within their respective panes. (Use the scroll wheel on the mouse or the scroll bars to zoom the mosaic in or out.) • Move and resize the preview box in the mosaic pane: – 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. • Move the microscope stage to the current cursor location in the video image or mosaic.
Spectrum tool	In Video mode, if a region of acquired chemical image data is displayed in the mosaic pane (or a preview image is in the left pane), use this tool to click a location (or select an area) in the chemical image to display the associated spectrum (or the average spectrum) in the spectral pane. Hold down the Shift key while selecting spectra to display multiple spectra.
Ruler tool	In Video mode, can be used to measure the length of features in the optical 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.
START Mosaic	Collects a mosaic at the current stage location. To collect a mosaic, the stage moves in a spiral pattern while the video camera takes static pictures of the sample so you can view a larger area of the sample surface in the software. The pictures appear in the 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.
STOP Mosaic	Cancels mosaic collection. Appears only while the software is actively collecting a mosaic.
CLEAR Mosaic	Clears the mosaic pane. Any collected mosaics are deleted.

Table 13. Video mode features and controls

Feature	Description
START Terrain Mosaic	Opens the Terrain Mosaic Setup dialog to configure the terrain mosaic collection settings. With a classic mosaic, if your sample had a very rough or uneven surface, parts of the sample that were closer or farther than the focal plane may have been out of focus. With a terrain mosaic, every part of the sample surface is in focus. A terrain mosaic takes longer to collect but results in more accurate data if your sample is not perfectly flat and level. After collecting a terrain mosaic, you can view the sample's surface topography by viewing the terrain map.
Mosaic	An image comprised of static pictures of the sample surface. The mosaic is created in the OMNICxi Acquisition window and appears in the mosaic pane. It also appears in the mosaic pane of the Analysis window.
Preview box	Defines a region of the optical image or mosaic that will be used to scan a preview chemical image of a representative area of a sample. Use Image Regions mode or Image Cross Section mode to scan the preview image.
Scroll boxes (Mosaic pane)	Can be used to zoom or position the optical image or mosaic within the mosaic pane. • **To zoom** the optical image or mosaic, drag one end of the scroll box at the right or bottom of the mosaic pane • **To reposition** the optical image or mosaic, drag the scroll box at the right or bottom of the mosaic pane
Video pane	Shows a live video image of the sample surface captured by the microscope's video camera. The limits of the video pane are defined by the magnification and numerical aperture of the currently selected objective.
Video scale bar	For a properly calibrated objective, shows the scale of the video pane in micrometers. Helpful for estimating the actual size of features in the sample.
Preview status	In Video mode, this area shows the size of the preview region (inside the preview box) in pixels, the estimated time required to acquire the preview 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.

Table 13. Video mode features and controls

Feature	Description
Mosaic pane	Before you collect a mosaic in video mode, this pane shows a static picture of the sample as it appears when viewed through the microscope eyepiece (one field of view). After you collect a mosaic, the mosaic appears in the mosaic pane. Right-click the mosaic pane for these options for copying and saving a mosaic: • **Copy as picture**. Copies the contents of the mosaic pane to the Windows™ Clipboard. The copied image can be pasted into any word processing, presentation or other compatible application. The resolution of a copied image will be the same as the computer's display monitor (typically 72 dpi or 96 dpi).
Mosaic scale bar	For a properly calibrated objective, shows the scale of the mosaic pane in nanometers, micrometers or millimeters (depending on the zoom level). Helpful for estimating the actual size of features in the sample.
Stage Up/Stage Down	Brings the sample into focus at the current stage location. Drag the scroll box up or down for coarse focus. Click directly above or below the scroll box for fine focus (or use the scroll wheel on the mouse). **NOTICE**: If you need to move the stage a great distance along the Z-axis, we recommend that you open the sample compartment doors and use the Focus knob on the stage controller to move the stage while you watch the sample to avoid bumping the sample into the objective.
Zoom To Region	Zooms and centers the mosaic and any selected areas (such as the preview box) in the mosaic pane
Zoom To Mosaic	Zooms the mosaic pane to show the full mosaic. If there are multiple mosaics, this tool zooms out so that all mosaics are visible.
Mosaic Full Screen	Expands the mosaic pane to the limits of the computer screen, or contracts the mosaic pane back to its original size.
Stage status	Shows the X, Y, and Z coordinates of the microscope stage in micrometers
Cursor status	Shows the X and Y coordinates of the current cursor location in the mosaic pane

Image Regions Mode

Select **Image Regions mode** in the OMNICxi Acquisition window for the following tasks:

- Adjust acquisition and profile settings before acquiring a chemical image
- Adjust the size and location of the preview box
- Acquire a chemical image of the preview region
- Select regions for acquiring chemical image data
- Acquire a chemical image of the selected regions
- Locate and select particles for particle analysis
- Zoom or pan the optical image or mosaic
- Measure the length of features in the optical image, mosaic or chemical image
- View the spectrum associated with the current cursor location in an acquired chemical image
- Search a displayed spectrum against the available spectral libraries

Note When using Image Regions mode to acquire a chemical image, make sure the sample compartment doors are closed and Laser power is greater than 0. 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.

This section explains the main features available in the Acquisition window when Image Regions mode is selected. To learn about a feature, click its name in the images below.

Figure 66. Image Regions mode features and controls

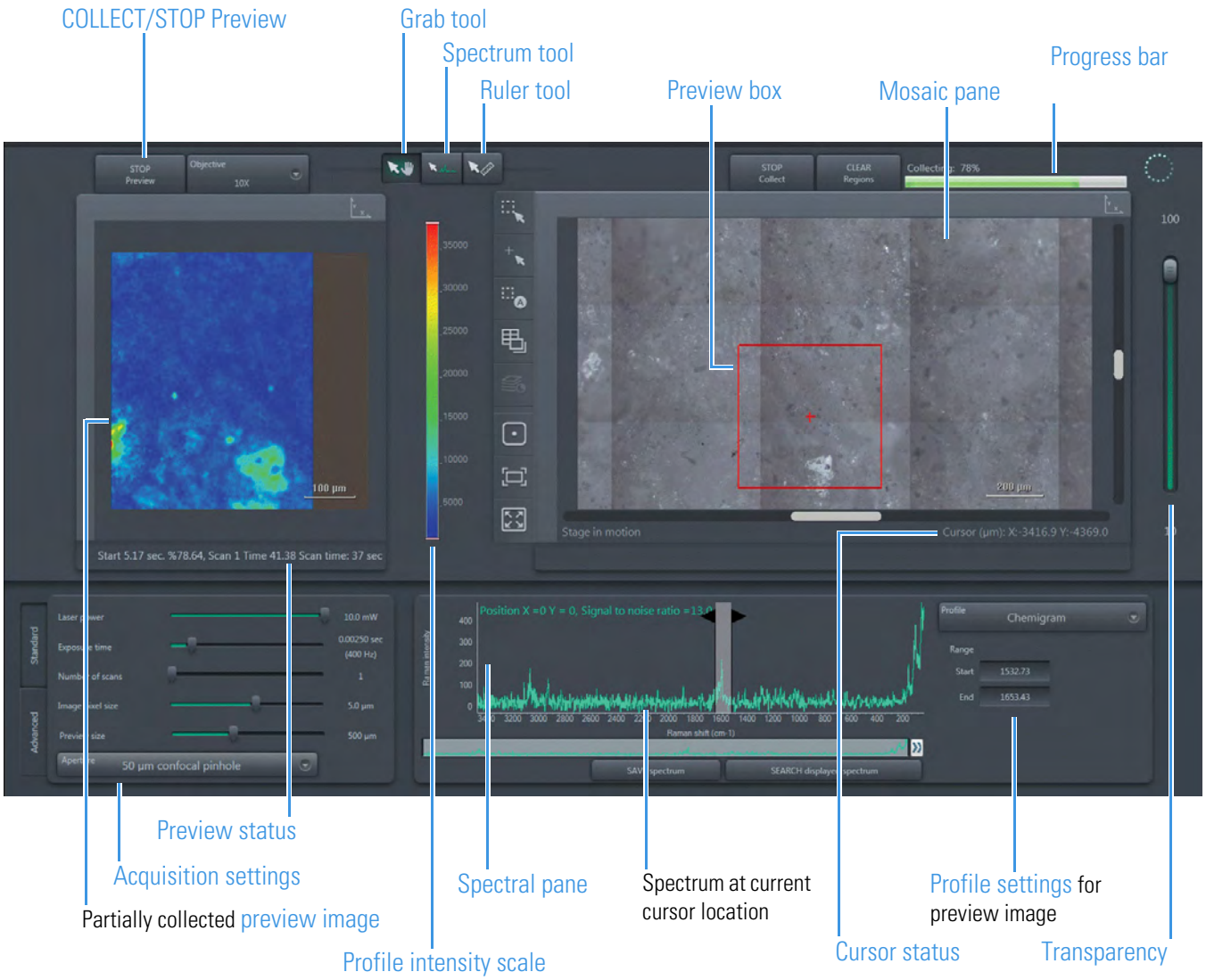


Figure 67. Image Regions mode features and controls

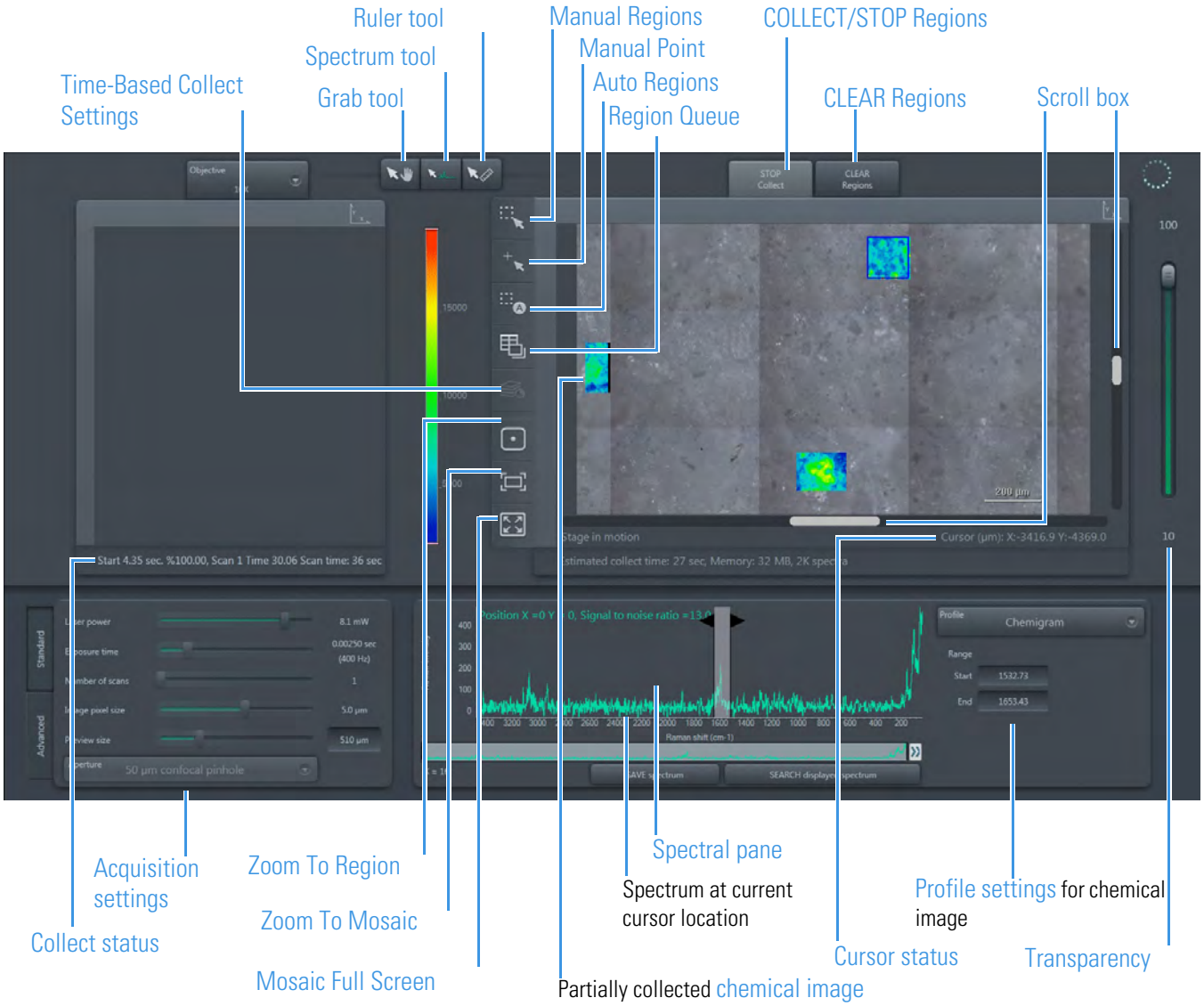


Table 14. Image Regions mode features and controls

Feature	Description
Collect/Stop Preview	Collects a chemical image of the selected preview region using the current acquisition settings. The preview region is the area inside the preview box. The preview image is displayed in the left pane. If you move or resize the preview box during acquisition, the software will restart acquisition of the preview region using the new preview size and location.
Preview box	Defines a region of the optical image or mosaic that will be used to scan a preview image of the sample. Use COLLECT Preview to acquire the preview image. Use the Grab tool to move or resize the preview box.
Preview image	A scan that contains an average spectrum collected at each sampling point in the preview box. The number and locations of the spectra that make up the preview image are defined by the Image pixel size setting. The number of passes is defined by the Number of scans setting. The preview image is useful for optimizing the Image pixel size setting before acquiring chemical image data in the final selected regions.
Preview status	Before collecting the preview data, this area shows the size of the preview region in pixels, the estimated time required to acquire the preview 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. During preview acquisition, this area shows the start time, percent completion and time for each scan and the estimated time to complete all requested scans of the preview region.
Collect/Stop Regions	Acquires a chemical image of each selected region in the mosaic. The acquired images are displayed in the mosaic pane (overlaid on the mosaic). Use Manual Regions, Auto Regions or the Region Queue to select regions.
Clear Regions	Deletes all drawn regions from the mosaic and any acquired regions of chemical image data, as well as the acquired data.
Scroll boxes (mosaic pane)	Can be used to zoom or position the mosaic within the mosaic pane. • **To zoom** the mosaic, drag one end of the scroll box at the right or bottom of the mosaic pane • **To reposition** the mosaic, drag the scroll box at the right or bottom of the mosaic pane

Table 14. Image Regions mode features and controls

Feature	Description
Collect Status	During acquisition of one or more selected regions, this area shows the start time, percent completion and time for each scan and the estimated time to complete all requested scans of each selected region.
Progress bar	Shows the progress of data acquisition (percent completed)
Grab tool	In Image Regions mode, can be used to: • Drag the chemical image or zoomed in mosaic within the mosaic pane. (Use the scroll wheel on the mouse or the scroll bars to zoom the image or mosaic in or out.) • Move the microscope stage to the current cursor location in the mosaic (click the mosaic to change the location). • Move and resize the preview box in the mosaic pane: – To move the preview box, drag its center. (Doing this also moves the microscope stage and updates the video image in the left pane.) – To resize the preview box, drag an edge. • Restart acquisition of the preview region. Click a location in the mosaic during acquisition of the preview image to restart acquisition with the current cursor location in the center of the new preview image.
Spectrum tool	In Image Regions mode, can be used to display the spectrum associated with the current cursor location in the preview image or region of acquired chemical image data. Select an area of the preview image or acquired region to display the associated average spectrum in the spectral pane. Hold down the Shift key while selecting spectra to display multiple spectra.
Ruler tool	In Image Regions mode, can be used to measure the length of features in the mosaic, preview image or region of acquired chemical image data. Click to define the start of the measurement, then drag to the end point. The length in micrometers (or mm) is displayed in the status bar below the mosaic pane.

Table 14. Image Regions mode features and controls

Feature	Description
Manual Regions	Can be used to manually select the regions that will be used to acquire a chemical image. To select a region, click this option and then use the mouse to draw a box around the region in the mosaic pane. The coordinates of the drawn box are automatically added to the region queue. After you have boxed all regions of interest, click Collect Regions to acquire a chemical image of each region. To delete a drawn region, right-click the region and choose **Delete > Selected Region**. To delete all drawn regions, click Clear Regions.
Manual Point	Use to manually add or select a single point. To add a point to your selection, click this option and click in the mosaic pane. The new point is numbered. Click a point to select it. Hold Ctrl and click to select multiple points. To delete a point, select one or more points, right-click in the mosaic pane, and select **Delete > Selected Point** or **Delete > All Points**.

Table 14. Image Regions mode features and controls

Feature	Description
Auto Regions	Automatically selects regions in the optical image or mosaic using either the Auto Region Selection settings or Particle Selection settings. See Settings for Particle Analysis for a description of the Particle Selection settings. The following Auto Region Selection settings are available: • **Auto**. Uses the mosaic at the current zoom level to find suggested regions. To change the zoom level, click Cancel to close the Auto Regions box, zoom the mosaic in or out, then click Auto Regions again. • **Exemplar**. Uses a selected location in the to find similar regions in the displayed optical image or mosaic. Use the mouse to click a representative location in the optical image or mosaic. • **Adjust sensitivity**. Adjusts the sensitivity of the auto regions feature to changes in contrast in the mosaic. • **Sieve regions by size**. Filters regions based on size. • **Dilate and coalesce**. Merges nearby small regions into larger regions. • **Keep Regions**. Saves automatically selected regions in the mosaic pane. • **Cancel**. Cancels auto region selection and deletes the selected regions from the mosaic pane.

Table 14. Image Regions mode features and controls

Feature	Description
Region Queue	Can be used to manually add to or edit the coordinates of currently selected regions in the optical image or mosaic. These options are available: • **Add region (+)**. Adds a row to the bottom of the region queue. (Also adds a corresponding region to the mosaic pane.) The new row is populated with the settings from the previous row. Enter new X, Y, and Z coordinates of the new region in micrometers and define the region width and height. The X and Y coordinates you enter will become the lower left corner of the new region. The Z position is the current focus (Z-axis) value when the region is created. After you enter an X, Y or Z value, the corresponding region is updated in the mosaic pane. • **Delete region (-)**. Deletes the selected row in the region queue. (Also deletes the corresponding region from the mosaic pane.) • **Load Regions**. Loads a previously saved region queue (.REGIONS filename extension) and displays its associated regions in the mosaic pane. • **Save Regions**. Saves the current region queue to a file that can be reloaded later with a different sample. The file is assigned a .REGIONS filename extension and is saved in the default directory for OMNICxi data. • **Duplicate**. Creates a series of regions that are offset in their X, Y and/or Z coordinates. Enter the number of regions to be created with an offset X, Y, and/or Z axis and the offset in micrometers and then click **Duplicate**. The new regions are displayed in the mosaic pane. To close the Region Queue window, click the Region Queue button again.
Time-Based Collect Settings	Can be used to collect a region repeatedly over time at specified intervals or upon external trigger input in order to study how a sample changes over time. **Note**: This feature is available only after you have selected at least one region. Click here for details about the settings available for time-based data acquisition.

Table 14. Image Regions mode features and controls

Feature	Description
Chemical image	A scan that contains one spectrum collected at each sampling point in the preview box or a selected region of the mosaic. 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 Profile settings) is applied to each spectrum to create the chemical image. The colors in the chemical image are defined by the profile intensity scale.
Acquisition settings	Settings used to acquire a chemical image of the selected region or regions of the displayed optical image or mosaic. Use Collect Preview to acquire a chemical image of the preview region. Use Collect Regions to acquire a chemical image of the selected region or regions. Click here for details about the settings available for acquiring a chemical image.
Profile settings	Defines the mathematical algorithm applied to the acquired spectra to create the chemical image. The algorithm converts each spectrum into a number that is assigned a color in the chemical image. The colors used in the chemical image are determined by the profile intensity scale. Click here for details about the profile types available and their associated settings and analysis results.

Table 14. Image Regions mode features and controls

Feature	Description
Profile intensity scale	Shows the scale 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. Right-click the profile intensity scale for these options: • **Copy as picture**. Copies an image of the profile intensity scale to the Windows Clipboard. The copied image can be pasted into any word processing, presentation, or other compatible application. • **Save as EMF**. Saves an image of the profile intensity scale as an EMF (Enhanced Metafile), which is a 32-bit version of the Windows Metafile image file format. • **Select colormap**. Select an available color combination for all profile types except MCR. • **Select MCR profile colormap**. Select an available color combination for the MCR profile type. **Tip** Drag the lower limit of the profile intensity scale to shorten the scale.
Mosaic pane	Shows the optical image of the sample surface (i.e., what you see when you look through the microscope eyepiece) and any collected mosaics. Any regions of acquired chemical image data are overlaid on the mosaic. Right-click the mosaic pane for these options for copying and saving data: • **Select all items.** • **Select all within specified rectangle.** Click and drag to draw a rectangle and select all regions within the boundary. This is especially useful for selecting many particles at once during particle analysis setup. Also selects points. • **Delete all items.** Deletes all regions and points in the mosaic. • **Delete selected items.** Deletes all selected regions and points.

Table 14. Image Regions mode features and controls

Feature	Description
Right-click menu options (*continued*)	• **Copy region as picture**. Copies an image of a region of acquired chemical image data to the Windows Clipboard. The copied image can be pasted into any word processing, presentation or other compatible application. The resolution of a copied image will be the same as the computer's display monitor (typically 72 dpi or 96 dpi). • **Save region as TIFF**. Saves the selected region of acquired chemical image data as a TIFF file (.TIFF filename extension), which can be imported to a wide selection of publishing, page layout, scanning, faxing, word processing and other applications. The resolution of the TIFF image will be the same as the acquired data. For data with multiple frames the TIFF output will have multiple pages showing each individual frame. This file follows the BioFormats format that can be directly imported to image processing applications such as ImageJ and FIJI. • **Export region data as ENVI**. Exports the selected region of acquired chemical image data as an ENVI file (*.ENVI file name extension), which can be opened with the Thermo Scientific OMNIC and Atlμs software, MATLAB software or other similar applications for further processing. • **Send region to OMNIC/Atlμs as .MAP**. Sends the selected region of acquired chemical image data to OMNIC/Atlμs software as a .MAP file. If the computer has the Thermo Scientific Atlus and OMNIC Workstation for DXRxi Raman Imaging Microscope software installed, that software opens with the image displayed in a spectral window. • **Show averaged spectrum at selection**. Displayed spectrum is a 9 point average of all the spectra surrounding the point that was clicked. • **Show terrain map.** Opens the terrain map in the OMNICxi - Terrain window. The terrain map is a 3D visualization of the surface topography of the sample.

Table 14. Image Regions mode features and controls

Feature	Description
Spectral pane	In Image Regions mode, displays the spectrum collected at the current cursor location in a region of acquired chemical image data. The spectral pane is also used for specifying peak heights and areas for the peak height, peak area, and other profile types. Click here for details about the features and tools available for the spectral pane.
Cursor status	Shows the X and Y coordinates of the cursor in the mosaic or acquired chemical image. Click the mosaic or chemical image to move the cursor to that location.
Zoom To Region	Zooms and centers the optical image or mosaic and any selected areas (such as the preview box or a selected region) in the mosaic pane.
Zoom To Mosaic	Zooms the mosaic pane to show the full mosaic. If there are multiple mosaics, this tool zooms out so that all mosaics are visible.
Mosaic Full Screen	Expands the mosaic pane to the limits of the computer screen, or contracts the mosaic pane back to its original size.
Transparency	Changes the transparency of any acquired chemical image data so you can view features below them in the mosaic.

Image Cross Section Mode

Use Image Cross Section mode to collect a Y-, Z-axis depth profile of your sample. This allows you to view and analyze image data from different layers in the material. The spectra are collected along a line starting in the middle of the preview box.

The sample must be optically transparent to the laser wavelength. Generally, if you can see through the material with your eyes it is a good candidate for this application. (Mounting the sample on a slide that has an opening in the middle will eliminate the possibility of including a spectrum of the slide in the analysis results.)

Note When using Image Cross Section mode to acquire a chemical image, make sure the sample compartment doors are closed and Laser power is greater than 0. 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.

Use the preview box in the mosaic pane to specify the sample location for the depth profile. Use the Grab tool to move and resize the preview box.

The features and tools available in Image Cross Section mode are the same as in Image Regions mode with these exceptions:

- COLLECT Regions and CLEAR Regions buttons are unavailable
- Manual Regions, Auto Regions, Region Queue, and the Time-Based Collect Setup are not applicable
- Transparency bar is not applicable

Note When acquiring cross sectional image data, it is helpful to use one of these techniques to estimate the number and thickness of the layers in order to optimize the Y and Z position of the cross section for the final analysis:

- In Image Cross Section mode, acquire a preview chemical image with a large **Image pixel size** setting. This provides a quick view of the entire sample which can be used to estimate the thickness and to view and analyze the spectra in the different layers. Keep in mind that the focus position is the midway point in the Z-axis travel (click a position in the chemical image to adjust it).
- Use Live Spectrum mode to acquire, view and search individual spectra in each focal plane. This provides information about the materials present in each layer and their approximate depths. This information will help you determine the height of the preview box needed to capture the layers of interest in the acquired chemical image. This method is detailed below in the steps to acquire a cross sectional image.

Live Spectrum Mode

In Live Spectrum mode, a spectrum can be acquired at any location in the mosaic pane by clicking the location. Use Live Spectrum mode to view and search spectra acquired at any location on a sample and to optimize acquisition settings for acquired spectra.

The spectrum is acquired at the current focal plane. These additional focusing options are available:

- FOCUS Spectrum button. Performs spectral auto focus to maximize height of displayed spectrum by changing the sample focus.
- Spectral Auto Focus mode. If the Auto Focus On/Off indicator is on and Focus type in Auto Focus Settings is set to **Spectral**, each time you click in the mosaic pane to acquire a spectrum, the software moves the stage to that location and performs a spectral focus before it acquires the spectrum.
- Video Auto Focus mode. If the Auto Focus On/Off indicator is on and focus type in Auto Focus Settings is set to **Video**, each time you click in the mosaic pane to acquire a spectrum, the software moves the stage to that location and uses the stored Z-axis (focus) position to acquire the spectrum.

Note When using Live Spectrum mode to acquire and view spectra, make sure the sample compartment doors are closed and Laser power is greater than 0. 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.

This section explains the main features available in the Acquisition window when Live Spectrum mode is selected. To learn about a feature, click its name in the images below.

Only the acquisition settings that affect acquired spectra (Laser power, Exposure time, and Number of scans) are available in Live Spectrum mode.

Profile settings are not used since the instrument is acquiring spectra but not using them to create a chemical image. However, for Profile types that require peak height, area, region or baseline information, you can use spectra displayed in Live Spectrum mode and the Profile tools to specify these settings.

Figure 68. Live Spectrum mode features and controls

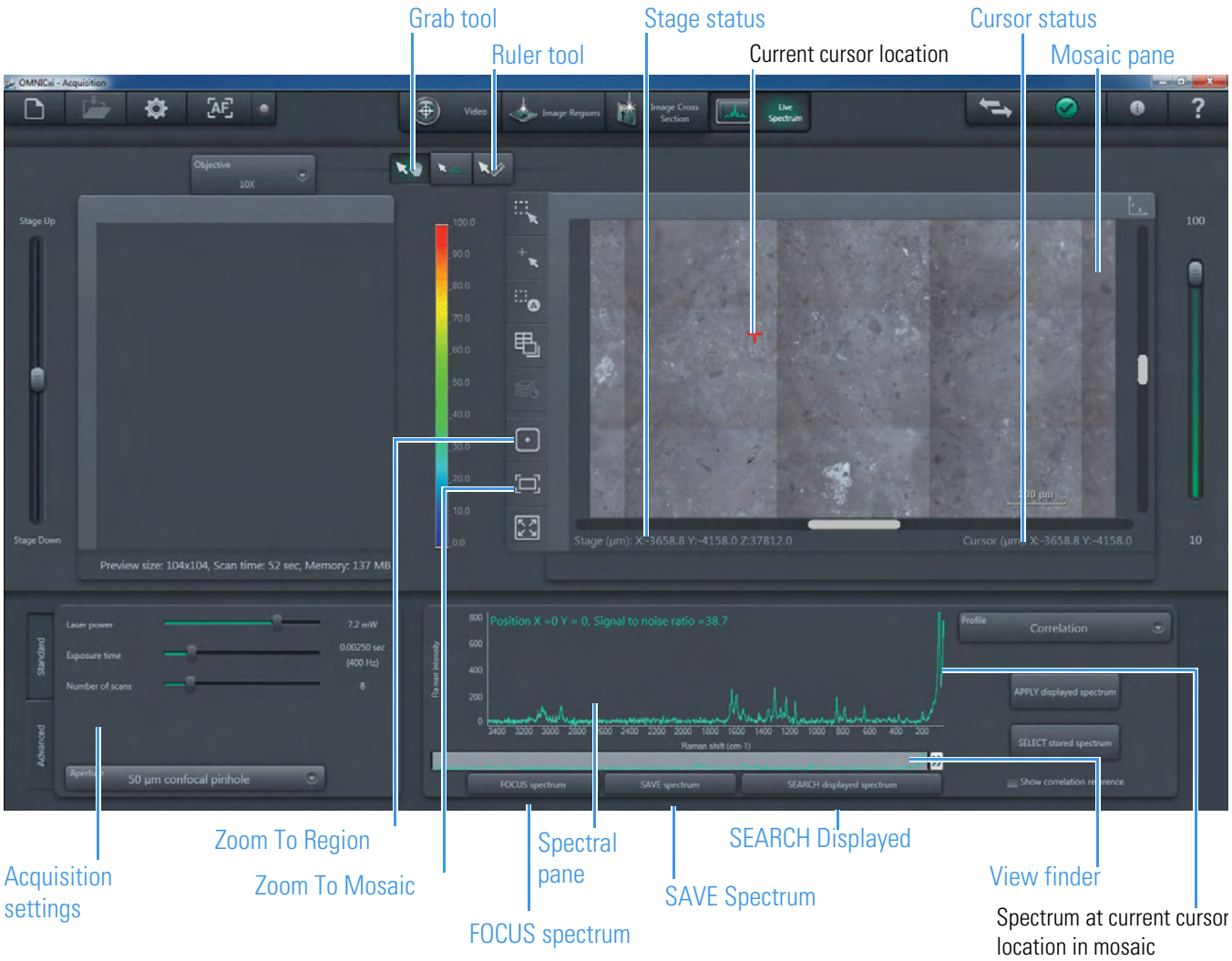


Table 15. Live Spectrum mode features and controls

Feature	Description
Grab tool	In Live spectrum mode, the Grab tool can be used to: • Drag the zoomed in mosaic within its respective pane (use the scroll wheel on the mouse or the scroll bars at the edges of the mosaic pane to zoom the mosaic in or out) • Move the microscope stage to a selected location in the mosaic (click the mosaic pane to specify the location)
Ruler tool	In Live Spectrum mode, can be used to measure the length of features in the 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.
Stage status	Shows the X, Y, and Z coordinates of the microscope stage in micrometers
Current cursor location	Shows the most recently clicked location in the mosaic. The spectrum acquired at this location is displayed in the spectral pane.
Cursor status	Shows the X and Y coordinates of the current cursor location in the mosaic
Mosaic pane	In live spectrum mode, shows any collected mosaics
Zoom To Region	Zooms and centers any collected mosaic in the mosaic pane
Zoom To Mosaic	Zooms the mosaic pane to show the full mosaic. If there are multiple mosaics, this tool zooms out so that all mosaics are visible.
Spectral pane	In Live Spectrum mode, shows the spectrum acquired at the current cursor location in the collected mosaic. The Y-axis format of the displayed spectrum is always Raman intensity (detector counts). The X-axis format is defined by the Data Format option in Instrument Settings. The spectrum title shows the location of the acquired spectrum in the mosaic and the signal-to-noise ratio at that location. To add the background spectrum used for this spectrum to the spectral pane, right-click the spectral pane and choose **Show Background**.

Table 15. Live Spectrum mode features and controls

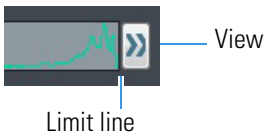
Feature	Description
View finder	Contains an image of the displayed spectrum. Can be used to: • Change the display limits of the spectral pane (drag the vertical limit line at each end of the view finder)  • Show a different region of the displayed spectrum (click the region you want to show in the view finder) • Expand the X-axis to its limits (double-click between the limit lines in the view finder)
FOCUS Spectrum	Performs a spectral auto focus at the current cursor location, which maximizes the height of the displayed spectrum by changing the sample focus.

Table 15. Live Spectrum mode features and controls

Feature	Description
SAVE Spectrum	Saves the selected spectrum as an OMNIC SPA file, which can be opened using the Thermo Scientific OMNIC Workstation for DXRxi Raman Imaging Microscope or OMNIC Specta application for further processing. Right-click the spectral pane for more options for saving and exporting spectra. Click here for more information.
SEARCH Displayed Spectrum	Searches the displayed spectrum against the available spectral libraries to identify the associated compound. A search results window appears above the button. The name of the best matched library spectrum 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. If no suitable match is found in the library you can select Unknown and no matching spectrum will be displayed. To close the search results window, click Search Displayed Spectrum again. After you close the window, the library spectrum disappears from the spectral pane. 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: • The compound name • The name of the library that contains the spectrum • A 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. Use the Select Libraries button in the OMNICxi Analysis window to select the libraries to search. Use the Search Setup button in the OMNICxi Analysis window to specify regions to use tin the spectral search.

These additional features are available for working with displayed spectra:

Table 16. Features for working with displayed spectra

If you want to...	then...
Pan X-axis	Use the Grab tool to drag spectra in the spectral pane left and right.
Expand X-axis to show entire spectra	• Double-click the spectral pane • Double-click between the two limit lines in the view finder
Expand or contract Y-axis	Use the Grab tool to drag spectra in the spectral pane up and down
Zoom X-axis to show more detail	• Draw a box around a region of a spectrum in the spectral pane, then click inside the box • Drag the vertical limit line at each end of the view finder

OMNICxi Analysis Window

This section covers all features and controls available in the Analysis window, which can be used to:

- Reanalyze sample data using a selected profile
- Compare the analysis results from several profiles
- Search image spectra against one or more spectral libraries to identify the associated compound
- Create and print data reports
- Open, view and reanalyze previously acquired sample data
- Save sample data with new profile information
- View terrain map
- View particle analysis results

For an overview of the Analysis window and a tutorial on how to open and reanalyze an image file, refer to the *DXRxi Quick Reference* guide. A copy of the guide in PDF format is included in the electronic documentation set provided with the instrument.

General Features and Controls

All of the features visible in the OMNICxi Analysis window are discussed below. To learn about a feature, click its name in the image below.

Figure 69. OMNICxi Analysis window, general features and controls

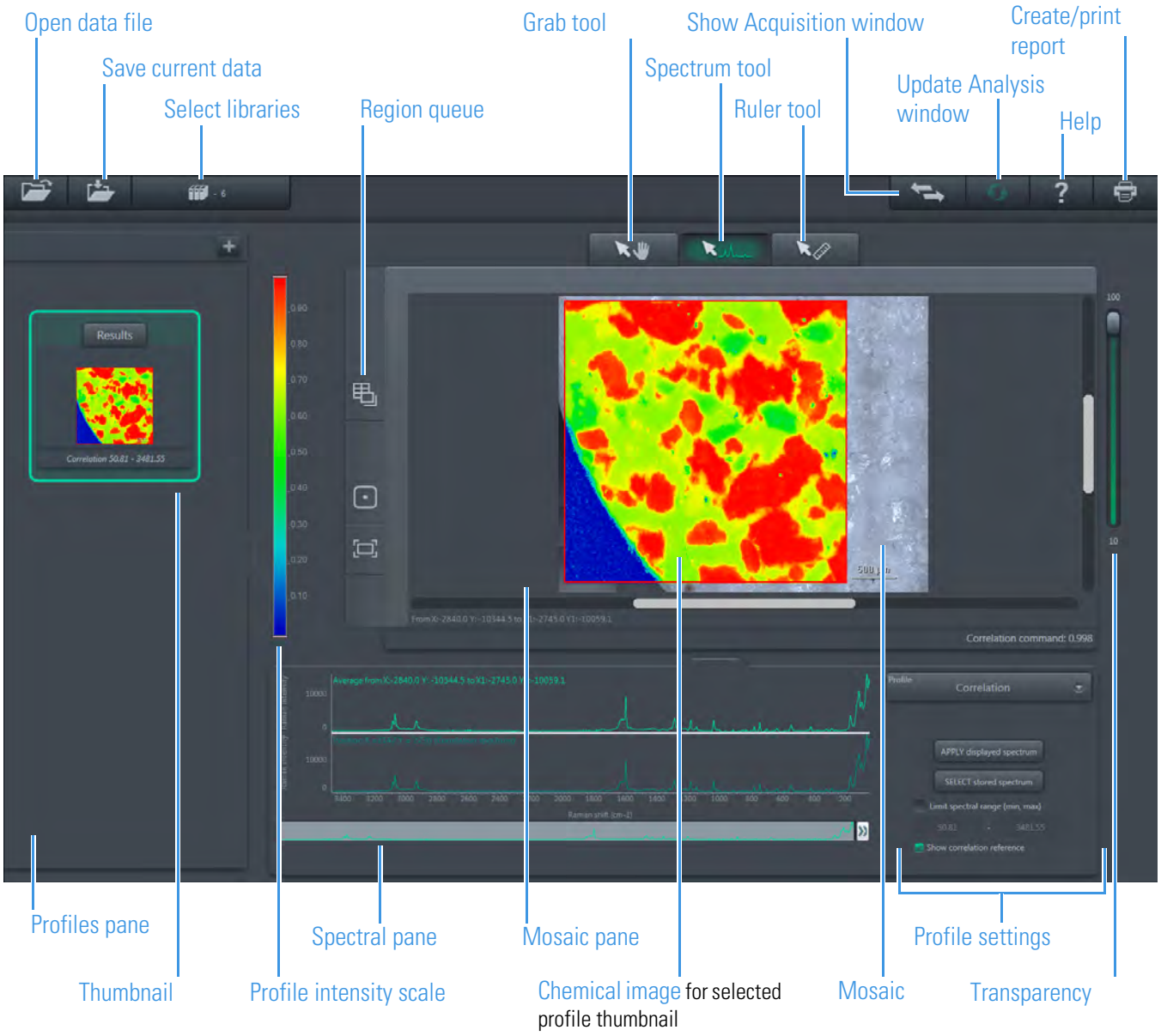


Table 17. Analysis window, general features and controls

Feature	Description
Open data file	Opens an image file that was previously saved. OMNICxi recognizes these file formats: • **.MAPX**. Can be opened only in OMNICxi software • **.H5**, OMNICxi binary format. OMNICxi image data saved in this format can be opened in any compatible application including OMNICxi and MATLAB™ software. **NOTE**: OMNICxi can only open H5 format data files that were created in OMNICxi software. After a file is opened, 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 thumbnails 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). A thumbnail picture of the image data appears at the top of the profiles pane. The thumbnail indicates the type of profile used to create the chemical image. All of these items are called out in Figure 69. If additional profiles were generated for this sample, their thumbnails will also appear in the profiles pane. To select another profile, click its thumbnail. Click here to set the default folder and path where OMNICxi opens image files. **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, use New Sample. The software will ask if you want to save the data in the Analysis window before it clears both windows for data collection.

Table 18. Analysis window, general features and controls

Feature	Description
Save current data	Saves any newly acquired or updated data in the Acquisition and Analysis windows. The following information is saved: • mosaic, if one was created • all regions of acquired chemical image data and their associated acquisition settings • all applied profiles and their associated profile settings OMNICxi saves 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. Image data can be saved in these file formats: • **.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. Click here to set the default folder and path where OMNICxi saves image files.

Table 19. Analysis window, general features and controls

Feature	Description
Select libraries	Click the Select Libraries button to specify regions and to select libraries for the search. **Search Setup** opens the Search Setup dialog. Use this dialog to specify one or more regions to use for the spectral search. Select **Use Regions in Search** to limit the spectral search to the specified regions. If this is not selected, the entire spectrum will be used. Use the Spectrum Tool and select a point on a collected region before opening the Search Setup. The select library button also lists the Raman spectral libraries available on this computer and lets you select which libraries to use for spectral searches performed in the OMNICxi application. A Raman spectral library is a database of spectra that you can purchase or create using spectra you collect with the instrument. To select or deselect a library, click its check box in the library list. OMNICxi includes features for searching spectra and analysis results against one or more spectral libraries to identify the associated compound. Depending on the selected window (Acquisition or Analysis), display mode or analysis profile, if you change which libraries are selected, the software may automatically initiate a new search. Additional library management features are available in the Thermo Scientific OMNIC Workstation for DXRxi Raman Imaging Microscope software, which is 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 spectral libraries that do not appear in OMNICxi software, copy the associated library files to this folder. You must copy both the .lbd and the .lbt file to the Libs folder. • To create a user library, use **Analyze > Library Manager** in the OMNIC Workstation software.

Table 19. Analysis window, general features and controls

Feature	Description
Region queue	Can be used to view the position, dimensions and acquisition settings for all regions of acquired chemical image data. These options are available: • **Delete**. Deletes the selected row in the region queue. (Also deletes the corresponding region from the mosaic pane and any image data it contains.) • **Select All.** Selects all of the regions. This is useful for exporting all of the regions. To close the Region Queue window, click the Region Queue button again.
Grab tool	In the Analysis window, lets you drags the chemical image or zoomed in mosaic within the mosaic pane. (Use the scroll wheel on the mouse or the scroll bars to zoom the chemical image or mosaic in or out.)
Spectrum tool	In the Analysis window, can be used to display the spectrum acquired at the current cursor location in a region of acquired chemical image data. The spectrum appears in the spectral pane. Select an area of the acquired region to display the associated average spectrum in the spectral pane. Hold down the Shift key while selecting spectra to display multiple spectra. When collecting time-based frames, you can extract the spectrum at the selected position from all frames. To do this, right-click and select "Extract spectra from all frames of a region."
Ruler tool	In the Analysis window, can be used to measure the length of features in the mosaic or region of acquired chemical image data. Click to define the start of the measurement, then drag to the end point. The length in micrometers (or mm) is displayed in the status bar below the mosaic pane.
Show Acquisition window	If the system computer is connected to one display monitor (rather than two), use this button to quickly display the OMNICxi Acquisition window. A similar button in the Acquisition window allows you to quickly switch back to the Analysis window.
Update Analysis window	You can begin analyzing data as soon as it appears in the Analysis window. During data collection of multiple regions or time-based frames, the Update Analysis Window button illuminates, indicating new data is available. Click the button to update the Analysis window with the new data.
Help	Displays Help information for the instrument and software

Table 19. Analysis window, general features and controls

Feature	Description
Create/print report	Creates a printable, preformatted report from the data displayed in the Analysis window. When creating a report, the following options are available: • **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 the chemical image) 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 thumbnail, and the contents of the spectral pane to the report. • **Region Collect Details**. Adds the acquisition settings for all acquired regions to the report. • **History details.** Adds information about operations carried out. • **Page Setup**. Defines page margins and orientation. • **Print Preview**. Opens a Print Preview window with additional page layout options. • **Print**. Allows you to select a printer and print the report. 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.
Profiles pane	Contains thumbnail images of all profile analyses run on the chemical image data displayed in the mosaic pane. Allows you to visually compare profile results. All created profiles are active and can be tweaked and quickly recalculated. Click here for steps to update or add a profile analysis. The analysis results appear in the mosaic pane and in the selected thumbnail picture in the profiles pane. The MCR profile analysis creates one thumbnail image for each found component. All the other profiles produce one thumbnail image.

Table 19. Analysis window, general features and controls

Feature	Description
Thumbnail	Contains a small picture of the chemical image and its associated analysis and search information for each generated profile (or for each found component for the MCR profile). All generated profile thumbnails are displayed in the profiles pane. The analysis information includes the profile type and key profile settings. Click the **Results** button in the thumbnail to see search results for the spectrum at the current cursor location in the chemical image (or for the selected pure component spectrum for the MCR profile). Use Select Libraries to select the libraries to search.
Chemical image	A scan that contains one spectrum collected at each sampling point in a selected region of the displayed mosaic. 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 Profile settings) is applied to each spectrum to create the chemical image. The colors in the chemical image are determined by the profile intensity scale.
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 OMNICxi Acquisition window and appears in the mosaic pane. It also appears in the mosaic pane of the Analysis window.
Mosaic pane	Shows any collected mosaics. Any regions of acquired chemical image data are overlaid on top of the mosaic. Right-click the mosaic pane for these options for copying and saving data: • **Extract spectra from all frames of a region**. Displays all the spectra at the clicked point from all the time-based collect frames. For example, if you have five time frames, click once with the spectrum tool to display the five spectra. • **Delete Selected Region** (or **Delete All Regions**). Deletes selected (or all) regions of acquired chemical image data. • **Crop frames from region.** Allows removal of frames from either end of a multiple-frame file. This option is not available on recently collected data. It is only available for data opened from a file. Note that the trim function immediately and irreversibly deletes the trimmed data from the file.

Table 19. Analysis window, general features and controls

Feature	Description
Right-click menu options (*continued*)	• **Copy region as picture**. Copies an image of a region of acquired chemical image data to the Windows Clipboard. The copied image can be pasted into any word processing, presentation or other compatible application. The resolution of a copied image will be the same as the computer's display monitor (typically 72 dpi or 96 dpi). • **Save region as TIFF**. Saves the selected region of acquired chemical image data as a TIFF file (.TIFF filename extension), which can be imported to a wide selection of publishing, page layout, scanning, faxing, word processing and other applications. The resolution of the TIFF image will be the same as the acquired data. For data with multiple frames the TIFF output will have multiple pages showing each individual frame. This file follows the BioFormats format that can be directly imported to image processing applications such as ImageJ and FIJI. • **Export region data as ENVI**. Exports the selected region of acquired chemical image data as an ENVI file (*.ENVI file name extension), which can be opened with the Thermo Scientific OMNIC and Atlμs software, MATLAB software or other similar applications for further processing. • **Send region to OMNIC/Atlμs as .MAP**. Sends the selected region of acquired chemical image data to OMNIC/Atlμs software as a .MAP file. If the computer has the Thermo Scientific Atlus and OMNIC Workstation for DXRxi Raman Imaging Microscope software installed, that software opens with the image displayed in a spectral window. • **Show averaged spectrum at selection**. Displayed spectrum is a 9 point average of all the spectra surrounding the point that was clicked.

Table 19. Analysis window, general features and controls

Feature	Description
Right-click menu options (*continued*)	• **Show region information**. Displays the instrument setup and collection parameters for the selected region. This information can be copied to the clipboard for use in other software. • **Analyze region in 3D.** Opens the region in the 3D Analysis window. • **Show terrain map.** – **Show region terrain map.** Opens the terrain map of only the selected region. – **Show whole terrain map.** Opens the full terrain map of the mosaic, including all regions. • **Analyze particles in selected region.** Opens the particle analysis tools in the Analysis window. • **Show map information**. Displays the history and digital signature information for the selected map file.
Spectral pane	In the Analysis window, displays the spectrum at the current cursor location in a region of acquired chemical image data. To show the average of the selected location and the eight adjacent spectra, right-click in the mosaic pane and choose **Show Averaged Spectrum At Selection**. Click the **Results** button in a profile thumbnail image to temporarily add the spectrum of the selected library match to the spectral pane. The library spectrum disappears after you close the search results window. When Correlation profile results are displayed, use **Show correlation reference** to add the correlation reference spectrum to (or remove it from) the spectral pane. When MCR profile results are displayed, select an MCR thumbnail to add its associated pure components spectrum to the spectral pane. Click here for details about special features and tools available for the spectral pane such as the right-click menu options and view finder.

Table 19. Analysis window, general features and controls

Feature	Description
Profile settings	Define the mathematical algorithm applied to each spectrum in an acquired region of data to create the chemical image. 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. The profile analysis results (i.e., the chemical image) appear in the mosaic pane on top of the mosaic. A thumbnail picture of each profile analysis also appears in the profiles pane. Each profile type has unique settings and results. Click here for details on the available Profile types, and their profile settings and generated results.
Profile intensity scale	For all profile types except MCR, shows the scale or range and corresponding colors used by the selected profile to create the chemical image. 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 in the chemical image that is associated with each found component in the sample. Right-click the profile intensity scale for these options: • **Copy as picture**. Copies an image of the profile intensity scale to the Windows Clipboard. The copied image can be pasted into any word processing, presentation or other compatible application. • **Save as EMF**. Saves an image of the profile intensity scale as an EMF (Enhanced Metafile), which is a 32-bit version of the Windows Metafile image file format. • **Select color map**. Select an available color combination for the chemical image (for all profile types except MCR). • **Select MCR profile color map**. Select an available color combination for the chemical image (for the MCR profile type). **Tip** Drag the lower limit of the profile intensity scale to shorten the scale (can improve contrast in the chemical image).
Transparency	Changes the transparency of any acquired chemical image data so you can view features in the mosaic.

3D Analysis and Terrain Windows

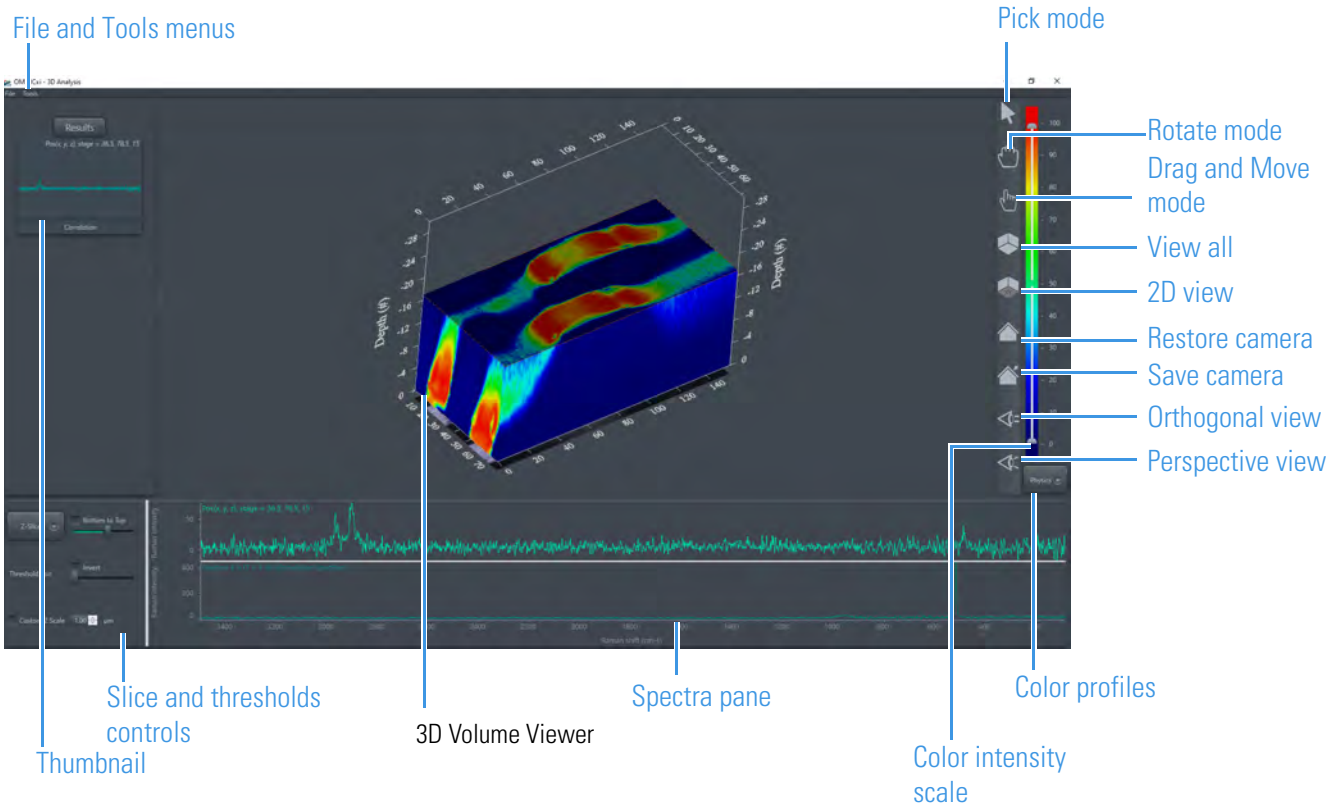
This section covers all features and controls available in the 3D Analysis and Terrain windows.

- **3D Analysis window.** Used to explore 3D data.
- **Terrain window.** Used to view the terrain map, a 3D visualization of the sample surface topography.

3D Analysis Window General Features and Controls

All of the features visible in the OMNICxi 3D Analysis window are discussed below. To learn about a feature, click its name in the image below.

Figure 70. 3D Analysis Window general features and controls



The OMNICxi Analysis window also allows you to view 2D data in the 3D analysis window. If you right-click a ... and select Analyze Region in 3D but the region does not have any z-slice data, the 3D Analysis window opens with a few different tools and features.

Figure 71. Viewing 2D data in the 3D Analysis window

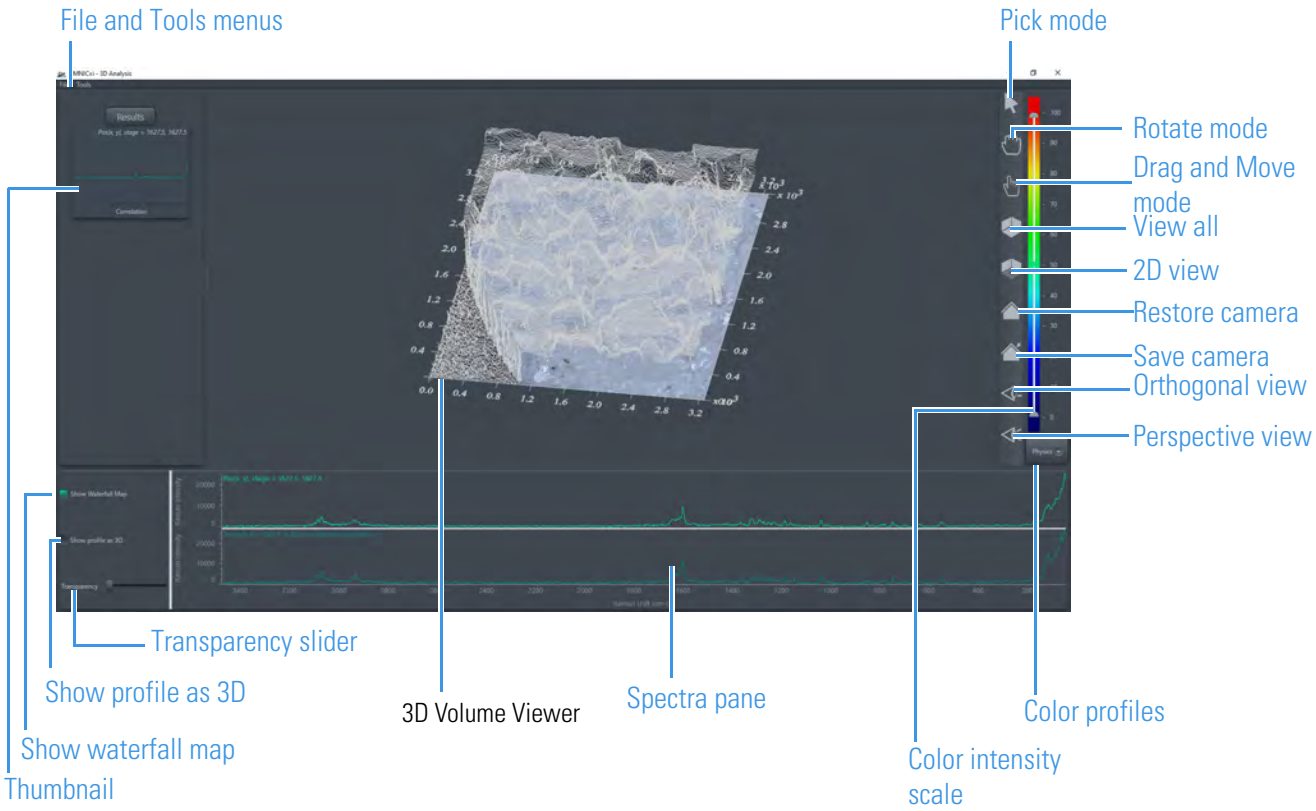


Table 20. 3D Analysis Window features and controls

Feature	Description
File and Tools Menus	Used to copy the image to your clipboard or to change the background color. Select **File > Copy to Clipboard** to copy an image of the 3D volume as it is currently displayed. You can then paste the image in another application. Select **Tools > Set Background Color** to choose a background color to display behind the 3D volume.
Pick mode	Select a point on the 3D volume to view the spectrum at that point. When you pick a point on the 3D volume, the 2D Analysis window will display the corresponding location. With the ruler tool enabled, use Pick mode and click and drag to measure distance on the surface of the volume.
Rotate mode	Click and drag to rotate the 3D volume.
Drag and move mode	Move the 3D volume on the canvas.
View all	Centers the 3D volume on the canvas.
2D view	Snaps to a 2D view along the currently selected slice option. For example, with X-Slice selected, the image will snap to a 2D view along the y- and z-axes.
Restore camera	Returns the view to a position previously set with the Save Camera tool.
Save camera	Set the viewing angle to return to when you click Restore Camera
Switch to orthographic camera	Displays the 3D volume in parallel line orthographic view.
Switch to perspective camera	Displays the 3D volume in perspective view.
Color intensity scale	Defines the scale or range and corresponding colors used in the 3D volume.
Color profiles	Select to change the colors displayed on the 3D volume.
Spectra pane	View spectra at the location indicated with Pick mode.

Table 20. 3D Analysis Window features and controls

Feature	Description
Slice and thresholds	• **X-, Y-, and Z-Slice slider.** Drag slider to view slices along the indicated axis. Select "Bottom to Top" to reverse the direction of slice. • **Threshold min.** Trims the 3D volume to show only colors within the threshold. Select "Invert" to reverse the threshold. • **Custom Z-Scale.** Select to set a custom Z-Scale. This is useful for visually stretching or compressing the 3D volume along the z-axis.
Profile thumbnail	Displays the results of the profile selected in the Analysis window. For example, when using the Correlation profile, use Pick to select a point on the 3D volume, and click Results in the thumbnail to see the correlation search results. When using the MCR profile, the thumbnails can be used to show or hide components or to change the color of components.
Right-Click Menu Options	• **Show Video Image.** Overlays the 2D video image onto the 3D volume. • **Show XYZ Position.** Diplays the XYZ position of the last point selected with the Select tool. • **Show Outline Box.** Displays a light border around the 3D volume. This is useful for seeing the empty space when viewing a slice of the volume. • **Show axes.** Displays axes to show the measurements of the volume. • **Enable Ruler.** You must use the slice tools to measure with the ruler. To use the ruler, slice the 3D volume, select Pick mode, and then click and drag on the sliced side of the 3D volume. • **Show as Isosurface.** Displays the 3D volume as an isosurface.
Show Waterfall Map	• The waterfall map is a 3D representation of the profile results. For example, with a correlation profile, the heights show the relative match value, with greater matches shown as higher points than poor matches.
Show Profile as 3D	Show or hide the profile intensity colors overlaid on the waterfall map.
Show Transparency	• Use the slider to adjust the transparency of the chemical image and to show the video image.

Terrain Window General Features and Controls

All of the features visible in the OMNICxi Terrain window are discussed below. To learn about a feature, click its name in the image below.

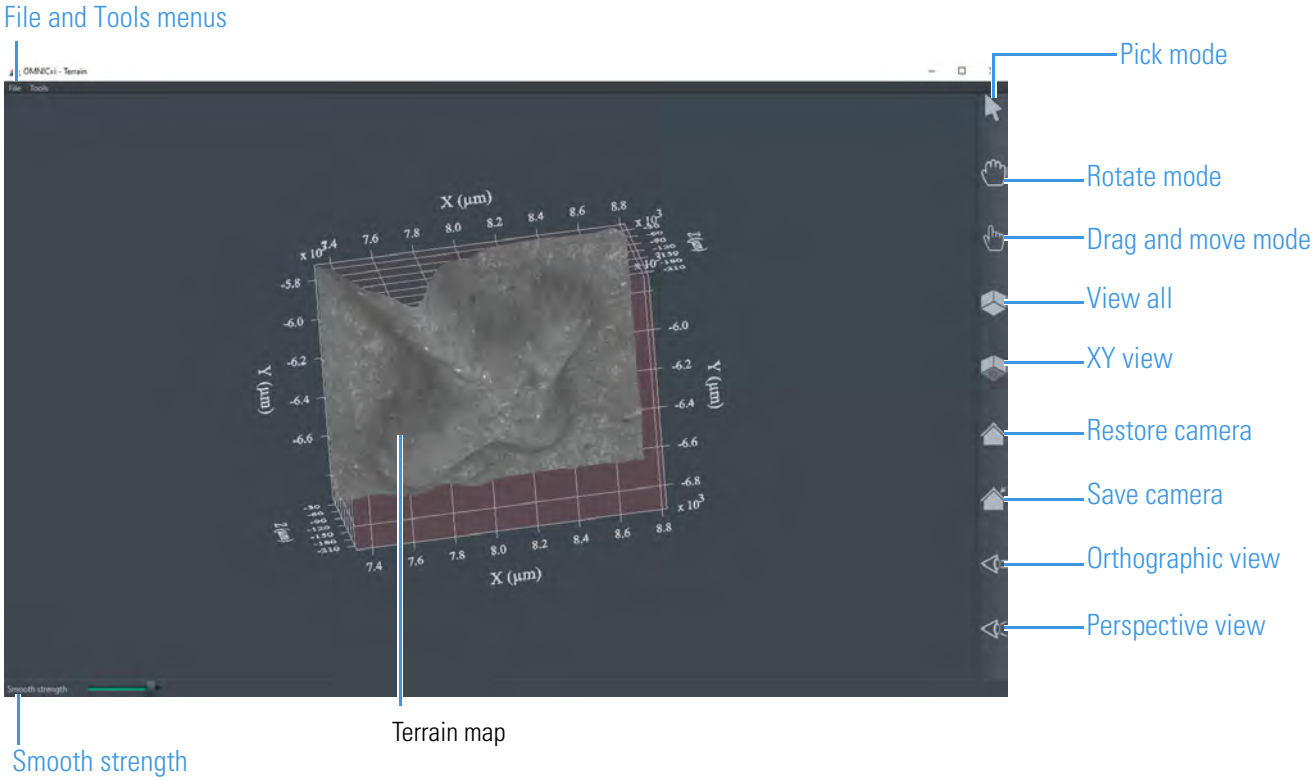


Table 21. Terrain Window features and controls

Feature	Description
File and tools menu	File. Copy to clipboard Tools. Set background color.
Pick mode	Select a point on the terrain map to view the spectrum at that point. When you pick a point on the terrain map, the 2D Analysis window will display the corresponding location.
Rotate mode	Click and drag to rotate the terrain map.
Drag and move mode	Move the terrain map on the canvas.
View all	Centers the terrain map on the canvas.
XY view	Snaps to a flat, XY view of the terrain map.

Table 21. Terrain Window features and controls

Feature	Description
Restore camera	Returns the view to a position previously set with the Save Camera tool.
Save camera	Set the viewing angle to return to when you click Restore Camera
Orthographic view	Displays the 3D volume in parallel line orthographic view.
Perspective view	Displays the 3D volume in perspective view.
Terrain map	Shows the topographic terrain map.
Smooth strength	Set the level of smoothing used to collect data. Adjust smoothing to find an optimal balance that reduces noise while maintaining sufficient definition. Smooth strength is only available if you open the terrain mosaic from the Acquisition window. It is not available when viewing a chemical region from the Analysis window. Once set, the smooth strength value is used to collect the chemical region. This value is also saved with the map file. When you open the saved data in the Analysis window, the image is automatically smoothed using the given value.
Right-click menu	• **Show video image.** Overlays the video image of the sample surface onto the 3D terrain map. If show video image and show regions are both selected, the region is shown on top of the video image. • **Show regions.** Overlays the chemical image on top of the 3D terrain map. • **Show axes.** Show or hide the x, y, and z axes. • **Use real scale.** By default, the terrain map displays an exaggerated z-scale to emphasize variation in the surface topography. Select to view the real z-scale.

Instrument Settings

To view or change these settings, click **Settings** at the top of the Acquisition window in OMNICxi software.

Figure 72. Acquisition window, Settings options

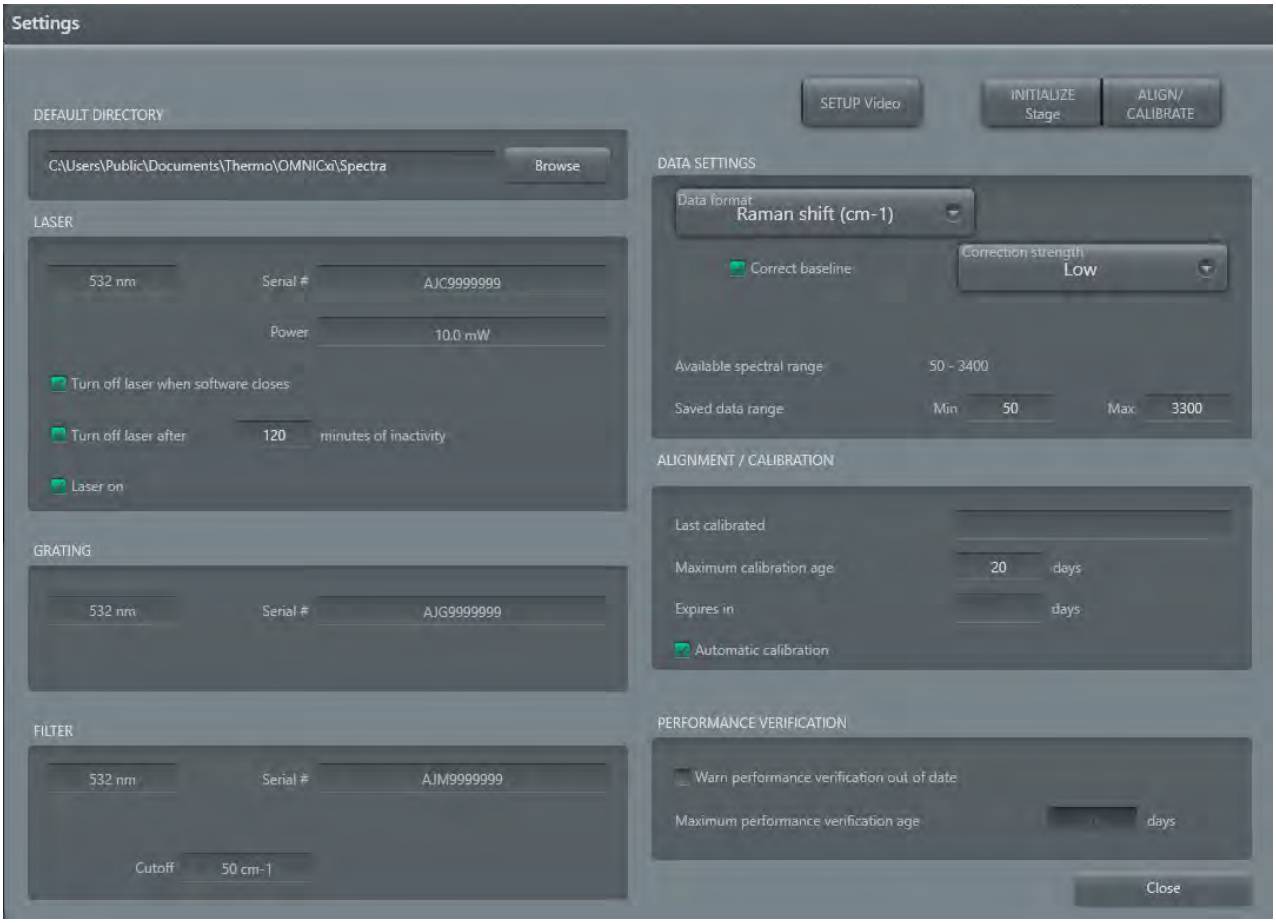


Table 22. Acquisition window, Settings options

Feature	Description
Default Directory	Defines the folder in Windows™ Explorer that OMNICxi software will use to open and save image and spectral data and to load and save the region queue.
Laser	Shows the serial number, maximum power and frequency in nanometers of the installed laser. Also provides these options to control laser operation: • **Turn off laser when software closes**. When selected, automatically turns off the laser when you exit the OMNICxi software. Recommended to preserve laser life. • **Turn off laser after _ minutes inactivity**. When selected and a valid number of minutes (greater than 0) is entered, automatically turns off the laser after the specified time has elapsed with no data collection activity. Recommended to preserve laser life. • **Laser on**. Turns the laser on or off. This setting is selected automatically when you start the OMNICxi software. You can use this option to turn off the laser if you want to leave the instrument running with the software open and a sample in place (prevents the sample from burning). The laser must be on in order to collect data with the instrument.
Grating	Shows the serial number and frequency in nanometers of the installed grating.
Filter	Shows the serial number, frequency in nanometers, and cutoff of the installed laser filter. Cutoff defines the lowest frequency, in wavenumbers, that will be displayed in Raman shifted spectra. See Data Format for more information.

Table 22. Acquisition window, Settings options

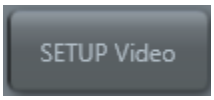
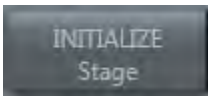
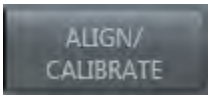
Feature	Description
Setup Video 	Provides tools to: • Calibrate the video image in the OMNICxi software for each installed microscope objective. This lets you retrieve the appropriate video calibration when the objective is changed. To retrieve a video calibration for an objective that was previously calibrated, use the Objective option in the OMNICxi Acquisition window. If you have a new objective that has never been calibrated, follow these steps to create and save a calibration for that objective. You can also update or delete a saved calibration. • Adjust video camera settings (**not** recommended). Use the **Video Settings** button to access settings for the installed video camera. These settings are defined at the factory and adjusted during system installation and do not need further adjustment.
Initialize Stage 	Initializes the sample stage on the microscope. The stage is initialized automatically after the microscope is powered up and OMNICxi software is opened. **We recommend** selecting the 10x objective on the microscope before you power up the instrument or initialize the sample 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.
Align/Calibrate 	Aligns the installed laser and calibrates the laser wavelength. If you have multiple lasers, gratings and filters, each laser, grating, and filter set must have associated alignment and calibration data. Once the alignment and calibration data are created, you can interchange your laser sets without recalibrating them. Click here for more information.

Table 22. Acquisition window, Settings options

Feature	Description
Alignment/Calibration information	Shows the date the current laser set (laser, filter and grating) was last calibrated. These options are available to control the frequency of alignment and calibration: • **Maximum calibration age**. Lets you define a time limit in days before new alignment/calibration data is required for the installed laser set. • **Expires in**. Tells you the number of days remaining before the alignment and calibration information for the current laser set will expire. • **Automatic calibration**. Calibrates the laser automatically every 30 minutes.
Data format	Defines the format of Raman data displayed in the spectral pane. These options are available: • **Raman shift** (cm^{-1}). Converts the X-axis to Raman shift, which is a measure of the difference between the observed spectral bands and the wavelength of the excitation laser. The unit is wavenumbers (cm^{-1}). • **Wavelength** (nm). Converts the X-axis to wavelength in nanometers. • **Pixels**. Raw data collected from the detector (uncalibrated in the X- and Y-axis).
Correct baseline	Select this option to apply a baseline correction to all acquired spectra. Can be used to correct any effects on the baseline curvature caused by the sample response. Fluorescence is a good example. This option is available: • **Correction strength**. Select a correction strength (auto, low, medium or high) that matches the degree of correction required.

Table 22. Acquisition window, Settings options

Feature	Description
Spectral range	Use this feature to specify spectral range limits for the installed laser set. **Available spectral range** shows the full range for the current laser set. To limit the spectral range, for example to eliminate a totally absorbing region at one end, enter **Min** and **Max** values in the selected unit (see Data format).
Performance verification	Use these features to set up a system to warn users when performance verification information is out-of-date for the installed laser set. Click here for more information. • **Warn if performance verification is out-of-date**. Select this option to configure the software to automatically check the current PV information for the installed laser set and warn the user if the information is out-of-date. • **Maximum performance verification age**. Lets you define a time limit in days before new PV data is required for the installed laser set.

Collect a Mosaic

The first step in analyzing your sample is collecting a mosaic image. A mosaic is a collection of visual images of the sample surface. Use the mosaic to visually inspect the sample surface and to set up your analysis. For example, once the mosaic is collected, use the automatic or manual region tools to specify regions to analyze, use Live Spectrum mode to refine your settings, or locate particles in the mosaic for particle analysis.

You can collect a standard mosaic or a terrain mosaic. A terrain mosaic automatically ensures that the entire mosaic is in focus, even if your sample surface is rough or uneven. A terrain mosaic takes longer to collect but results in more accurate data if your sample is not perfectly flat or level. With a terrain mosaic, you can view a terrain map. A terrain map is a 3D visualization of the sample surface topography.

If you are collecting a terrain mosaic, you may want to use a longer working distance lens. A long working distance lens will allow more room between the lens and the sample and can help prevent accidentally bumping the lens into the sample.

To collect a mosaic, you will load a sample and bring it into focus and then collect the mosaic.

❖ To bring a sample into focus and collect a classic mosaic

1. Load the sample onto the microscope stage.
2. Configure sample illumination.
3. Click the **Video** button in the software to select video mode.

4. Open the sample compartment doors on the microscope and rotate the desired objective to the front objective position (for easier initial focusing, start with the 10x objective).
5. Select the video calibration for this objective by setting the **Objective** option in the OMNICxi Acquisition window.

   Note 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.

6. Set the view path control to its middle position.
7. Use the microscope to locate and bring the sample into focus.

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

   Use the joystick on the stage controller to position the sample under the objective and the focus control to focus the image.

   Tip For quicker focusing, first watch the stage directly (not through the eyepiece) while using 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 are using reflected light illumination and have the optional reflected light brightfield/darkfield illuminator, the brightness control is on the illuminator power supply. Otherwise, the brightness control is on the microscope.

   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.) The video image shows approximately one field of view.

   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).

9. Change to a higher magnification objective if desired.

   Rotate the objective to the front objective position on the microscope and select the video calibration for that objective by setting the Objective option in the Acquisition window.

10. Click **FOCUS Video** in the software to bring the sample into sharp focus (adjust brightness as needed).

    The sample should still be centered under the new objective and focused in the left (video) pane.

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

12. Click **START Mosaic** to collect a mosaic.

    The microscope stage begins moving the sample in a spiral pattern while the camera takes static pictures of the sample surface so you can view a larger area in the software. The pictures appear in the right (mosaic) pane (see Figure 65).

    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.

❖ To collect a terrain mosaic

1. Load the sample onto the microscope stage.
2. Configure sample illumination.
3. Click the **Video** button in the software to select video mode.
4. Open the sample compartment doors on the microscope and rotate the desired objective to the front objective position (for easier initial focusing, start with the 10x objective).
5. Select the video calibration for this objective by setting the **Objective** option in the OMNICxi Acquisition window.

> **Note** 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.

6. Set the view path control on the microscope to its middle position to direct light through the microscope eyepiece and to the video camera.
7. Use the microscope to locate and bring the sample into focus.

> **NOTICE** Be careful not to bump the sample into the lens when bringing the sample into focus.

Use the joystick on the stage controller to position the sample under the objective and the focus control to focus the image.

Tip For quicker focusing, first watch the stage directly (not through the eyepiece) while using 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 are using reflected light illumination and have the optional reflected light brightfield/darkfield illuminator, the brightness control is on the illuminator power supply. Otherwise, the brightness control is on the microscope.

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.) The video image shows approximately one field of view.

   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).

9. Change to a higher magnification objective if desired.

   Rotate the objective to the front objective position on the microscope and select the video calibration for that objective by setting the Objective option in the Acquisition window.

10. Click **FOCUS Video** in the software to bring the sample into sharp focus (adjust brightness as needed).

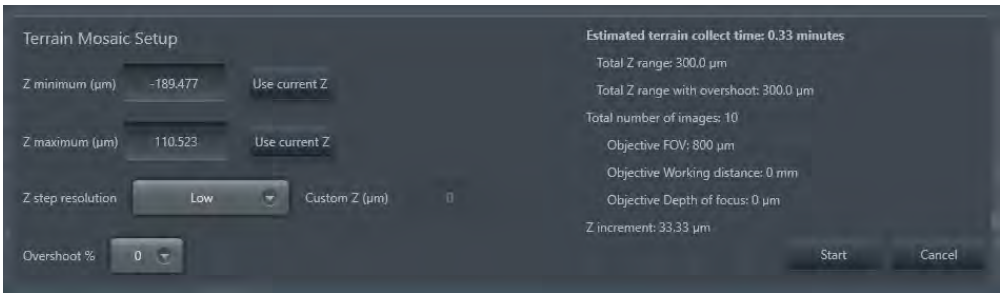
    The sample should still be centered under the new objective and focused in the left (video) pane.

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

12. With the cursor over the mosaic pane, use the scroll wheel on the mouse to adjust the size of the mosaic that will be collected.

13. Click **START Terrain Mosaic.**

Figure 73. The Terrain Mosaic Setup dialog



NOTICE When adjusting the stage position, be careful not to bump the sample into the microscope objective. The sample will be very close to objective at the highest stage position. Use a long working distance lens allow more space between the sample and the lens.

14. With the Terrain Mosaic Setup open, use the preview pane and the stage controls to bring the highest part of the sample into focus. This will be the lowest stage position with the sample farthest from the objective. When you have found a high point on the sample, click **Focus Video** to bring the sample into focus.

15. With the highest part of the sample surface in focus, next to Z minimum, click **Use Current Z** to set the Z minimum. This will be the lowest stage position used to collect the terrain mosaic.

16. Using the preview pane and stage controls, move the stage toward the lens to bring the lowest part of the sample into focus. This will be the highest stage position with the sample nearest to the lens. Click **Focus Video** to bring the sample into focus.

17. With a low point on the sample surface in focus, next to Z maximum, click **Use Current Z** to set the Z maximum. This will be the highest stage position used to collect the terrain mosaic.

18. Select an overshoot percentage. The overshoot adds a percent of the total z range to maximum and minimum z position to ensure that any points just outside the z range are in focus.

    The overshoot percent is added to both maximum and minimum. For example, with a full range of 300 µm, 30 µm will be added to both the max and min, for a total z range of approximately 360 µm. The total z-range is rounded to fit the z increment, so the range may be somewhat larger than the exact percent overshoot.

    An overshoot percent of 5% is good for most applications. You may need a larger range if your sample is very rough or uneven. Use 0% if you are concerned about bumping the sample into the lens.

19. Select high, medium, or low z-step resolution. A high resolution takes longer to collect, but collects more z positions, resulting in more accurate data. The z-step resolution is

determined by depth of focus of the objective. A higher resolution uses a smaller z increment.

The estimated time to collect is displayed in the Terrain Mosaic Setup dialog. The collection time depends on the resolution, total z-range, and collection settings.

20. Click **Start**.

    The instrument begins collecting a terrain mosaic. Click STOP Mosaic to stop collecting the terrain mosaic before the mosaic is complete.

    When the terrain mosaic is collected, the terrain map opens automatically.

21. In the Terrain window, use the slider to adjust the **smooth strength**. The smoothing affects how spectral data is collected. Use the image of the terrain map to find a balance that reduces noise while preserving sufficient surface definition. Noisy data may look like fur, with many high and low points. If smoothing is too strong, you may loose important details in the sample surface.

22. When you are satisfied with the smooth strength, close the window. Smooth strength is saved automatically.

    You can now use the terrain mosaic to collect a chemical region or to analyze particles.

    To use the terrain information when collecting a region or analyzing particles, open the Region Queue tool to ensure that Terrain is selected in the Terrain column. Clear the selection to ignore the terrain mosaic and to instead use a single z position during collection.

Acquire and Analyze Data

Use the OMNICxi software to do the following:

- Analyze Regions: Collect a chemical image of one or more regions of the sample surface. Specify regions automatically or select only specific areas of interest with manual regions.
- Identify and Analyze Particles: Use the particle selection tools to locate and characterize particles in the sample.
- View Spectrum at Specific Location: Select Live Spectrum mode to view the spectrum of a specific location on the sample surface.
- Analyze Sample Cross Sections: With Image Cross Section mode, collect a cross section of transparent sample.
- Measure Change Over Time: Acquire a series of measurements to analyze how your sample changes over time.
- Measure and View 3D Volumes: View 3D data sets in the 3D volume viewer.

Analyze Regions

Create a chemical image of an area of the sample surface by specifying one or more regions to analyze.

To analyze one or more regions, you will select one or more regions to analyze, acquire a preview chemical image, adjust acquisition settings, and collect data.

Acquire a Preview Chemical Image

Acquire a preview chemical image to optimize your collection parameters before investing time in collecting a final chemical image.

Acquiring a preview chemical image requires you to collect a mosaic, adjust the preview box and collection settings, and collect the preview.

❖ To acquire a preview chemical image

1. Load the sample onto the microscope stage.
2. Configure sample illumination.
3. Click the **Video** button in the software to select video mode.
4. Bring the sample into focus and collect a mosaic or collect a terrain mosaic.
5. Use Live spectrum mode to acquire spectra at several locations of the sample in order to optimize the Laser power, Exposure time and Number of scans settings.
6. Click **Image Regions**.
7. Use the preview box in the mosaic pane to select a region for the preview chemical image.

   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 the **Grab tool** (see Figure 66) to position and size the preview box.

   - To move the preview box, drag the "+" in the center of the box.
   - To resize the preview box, drag an edge.

   Note 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.

8. Make sure the sample compartment doors are tightly closed.
9. Click **COLLECT Preview**.

The software begins creating a chemical image of the preview region. The image is displayed in the left pane (see Figure 66).

The chemical image contains an 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. The profile intensity scale shows the scale or range and corresponding colors used by the selected profile.

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. You can interrupt (and restart) the preview scan at any time.

10. Adjust the **Image pixel size** and **Aperture** settings, if needed, to optimize the image data (for example to sharpen the image or show more or less detail).

11. After you change a setting, click **COLLECT Preview** to redraw the preview image so you can see any improvements in image quality. When you are satisfied with the settings, you are ready to select analysis regions and acquire the final chemical image data.

Tip Click the **Spectrum tool** and then click a point in the preview image. The spectrum collected at that time and location appears in the spectral pane.

Settings for Acquiring a Chemical Image

These settings are used to acquire a chemical image of the selected region or regions in the displayed optical image or mosaic.

- In Image Regions or Image Cross Section mode, use Collect Preview to acquire a chemical image of the preview region.
- In Image Regions mode, use Collect Regions to acquire a chemical image of each selected region.

Figure 74. Standard settings for acquiring a chemical image

Table 23. Acquisition window, standard settings for acquiring a chemical image

Feature	Description
Laser power	Laser energy at the sample in milliwatts (0 blocks the laser). Highest available laser power setting 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.
Exposure time	Laser exposure time for each collected spectrum in fractions of a second. Also indicates the speed of acquisition in Hz (i.e., number of spectra acquired per second). Longer exposure times may increase the height of spectral peaks but will also increase scan time.
Number of scans	Number of times a region is scanned during data acquisition. Spectra collected from multiple scans are averaged. Using a higher number of scans setting may reduce noise in the spectral baseline due to signal averaging. However, increasing number of scans also increases the time required to acquire the data.
Image pixel size	Distance the stage moves between sampling points in the chemical image in micrometers. For example, if Image pixel size is 5 µm, each spectrum represents 5 µm of sample area averaged together.

Table 23. Acquisition window, standard settings for acquiring a chemical image

Feature	Description
Preview size	Defines the size of the preview region (defined by the preview box in the mosaic pane) in micrometers.
Aperture	Defines the size of the Raman beam entrance slit or pinhole. The beam passes through the aperture on its way to the instrument detector. Available settings: • **25 µm confocal pinhole**. Maximizes spatial resolution (ability to discriminate very small changes in a sample) and spectral resolution (ability to resolve narrow bands in the spectrum), but allows less Raman energy from the sample which may decrease signal intensity. Recommended for analyzing samples with fine detail, cross sectional analysis, and when using a high resolution grating. • **50 µm confocal pinhole**. Increases Raman energy at the detector but may reduce spatial and spectral resolution. Recommended when using higher image pixel size settings and working with a full range grating. • **25 µm (or 50 µm) slit**. Maximizes Raman energy at the detector which produces higher quality spectra (higher signal-to-noise ratio) but may reduce spatial and spectral resolution. Use the smaller (25 µm) slit to improve spectral resolution. Do not use slit settings for cross sectional analysis.

Figure 75. Advanced settings for acquiring a chemical image

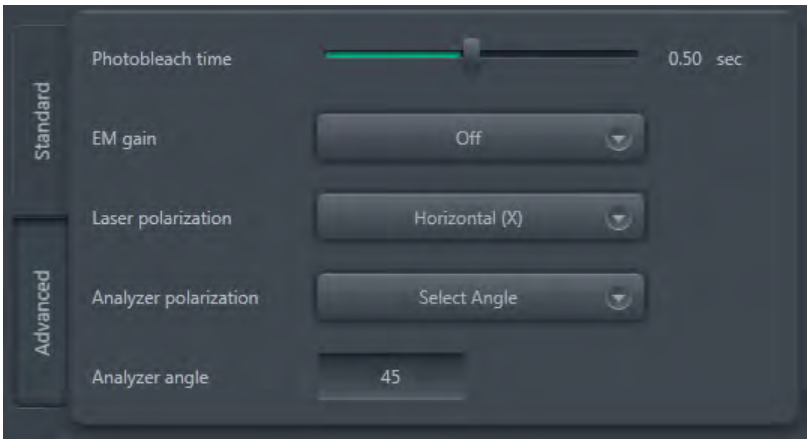


Table 24. Acquisition window, Advanced settings for acquiring a chemical image

Feature	Description
Photobleach time	Time for each sample point to be exposed to the laser for photobleaching before collection begins. Adding photobleach time may reduce sample fluorescence but will also increase scan time.
EM gain	Controls the electron multiplying gain of the detector camera. EM gain increases the signal to overcome the readout noise, but it also increases other sources of noise. The optimal setting for this is highly dependent on the experiment. In general, it is recommended to run with EM gain on for most operation but to turn it off when collecting with a longer exposure time.
Laser polarization (requires polarization option)	Sets the polarization for the laser light incident on the sample
Analyzer polarization (requires polarization option)	Sets the position for the polarization analyzer controlling the Raman emission. This option is only available when Laser polarization is set to "Horizontal(X)" or "Vertical(Y)" to run a polarized experiment.
Analyzer angle (requires polarization option)	Sets an arbitrary angle (0–180 degrees) for the polarization analyzer controlling the Raman emission. This option is only available when Analyzer angle is set to "Select Angle."

Acquire and Analyze a Chemical Image

Create a chemical image to identify components of your sample or to gain understanding of the sample's structure and composition.

❖ To acquire and analyze image data

1. Load the sample onto the microscope stage.
2. Configure sample illumination.
3. Click the **Video** button in the software to select video mode.
4. Bring the sample into focus and collect a mosaic or a terrain mosaic.
5. Use Live spectrum mode to acquire spectra at several locations of the sample in order to optimize the Laser power, Exposure time and Number of scans settings.

6. Acquire a preview chemical image and use it to optimize the Image pixel size and Aperture settings.
7. Use one of the following methods to select one or more regions of the sample for analysis.
   - Click **Manual Regions** and use the mouse to draw a box around several regions of interest in the mosaic.
   - Click **Auto Regions** and adjust any settings for the auto region selection wizard. The auto selected regions appear in the mosaic pane.
   - Click **Region Queue** and use the tools to define or edit regions. Added or edited regions appear in the mosaic pane.

   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 delete a region, right-click the region and choose **Delete > Selected Region**.
8. When you are ready to collect the image data, click **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 selected regions.

   Tip Use the transparency bar to change the transparency of the chemical image data so you can view features in the mosaic.
9. To save the acquired data, click **Save Current Data**, select a file format and click **Save**.
10. To collect data from another sample, click **New Sample**.

Note 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.

After collecting the chemical image, apply another profile to the image to visualize other properties of the sample data.

Profile Settings

Profile Settings appear in the OMNICxi Acquisition window and in the OMNICxi Analysis window. The profile settings define the mathematical algorithm applied to each spectrum in the selected region (or the preview region) to create the chemical image. The algorithm converts each spectrum into a number that is represented by a color in the chemical image.

The available profile types are described below. After you select a profile, the software immediately starts calculating it. Each time you adjust a profile setting, the calculation restarts. For each profile type, the profile intensity scale defines the scale or range and corresponding colors used to create the chemical image.

The Analysis window can be used to analyze the same sample using different profile settings in order to compare the results visually. Click here for more information.

Use the Spectrum tool to click a point in the acquired (or partially acquired) chemical image. The spectrum collected at that time and location appears in the spectral pane. For the MCR profile type, the calculated pure component spectrum associated with the current cursor location is also displayed in the spectral pane.

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. After you close the search results window, the library spectrum disappears from the spectral pane. Click here for details about the search results.

These profile types are available:

- Correlation Profile Settings
- Chemigram Profile Settings
- Peak Area Profile Settings
- Peak Height Profile Settings
- Peak Area Ratio Profile Settings
- Peak Height Ratio Profile Settings
- Peak Width Profile Settings
- Peak Position Profile Settings
- MCR Profile Settings

Correlation Profile Settings

The Correlation profile shows the correlation between each sample point in the chemical image 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 collected spectrum (taken in the lower left corner of the preview box or selected region).

Each color in the profile intensity scale 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.

Figure 76. Correlation profile settings

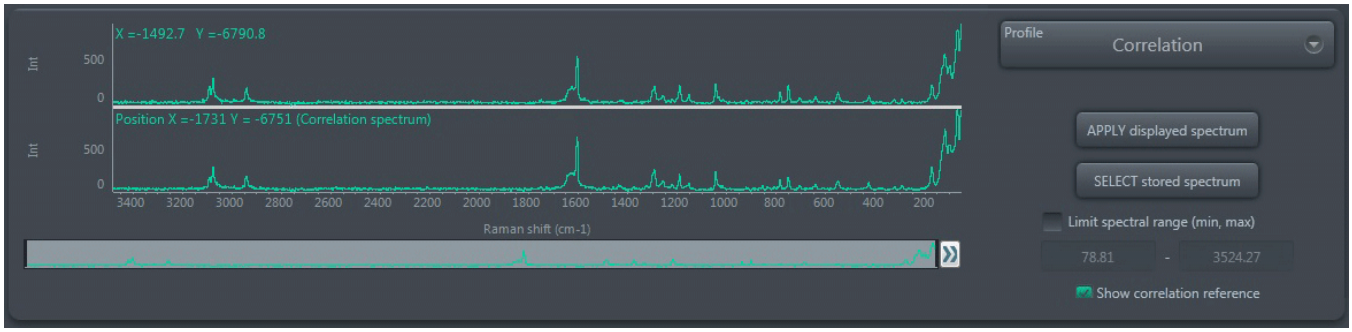


Table 25. Correlation profile settings

Setting	Description
Apply Displayed Spectrum	Lets you select a spectrum for the correlation reference. To select the reference, click the **Spectrum tool** , then click a point in a chemical image (preview image or acquired region) and choose **Apply Displayed Spectrum**.
Select Stored Spectrum	Lets you select a spectrum stored in an OMNIC format spectral data file (.SPA filename extension) for the correlation reference. To save a spectrum for use as a correlation reference, display and select the spectrum in the spectral pane, right-click the spectral pane and choose **Send selected spectrum to OMNIC** (or **OMNIC Specta**). Then use the Thermo Scientific OMNIC Workstation for DXRxi Raman Imaging Microscope or OMNIC Specta software to save the spectrum to an .SPA file.
Limit spectral range (min, max)	Refines the spectral range used for the correlation analysis (for example to ignore a high intensity peak or region that is not of interest). Enter minimum and maximum X-axis values or use the tools in the spectral pane to mark the start and end of the region used for the analysis. **Tip** To zoom the spectrum, use the mouse to draw a box around the region, then click inside the box. Right-click the spectral pane for more display options.
Show correlation reference	Displays the currently selected correlation reference spectrum in the spectral pane (along with the spectrum at the current cursor location)

Chemigram Profile Settings

The Chemigram profile shows the uncorrected area of the specified region for each sample point in the chemical image. The colors in the chemical image represent the relative area under the specified region at each sample point. A higher intensity value (closer to the top of the profile intensity scale) indicates a larger peak area.

Figure 77. Chemigram profile settings

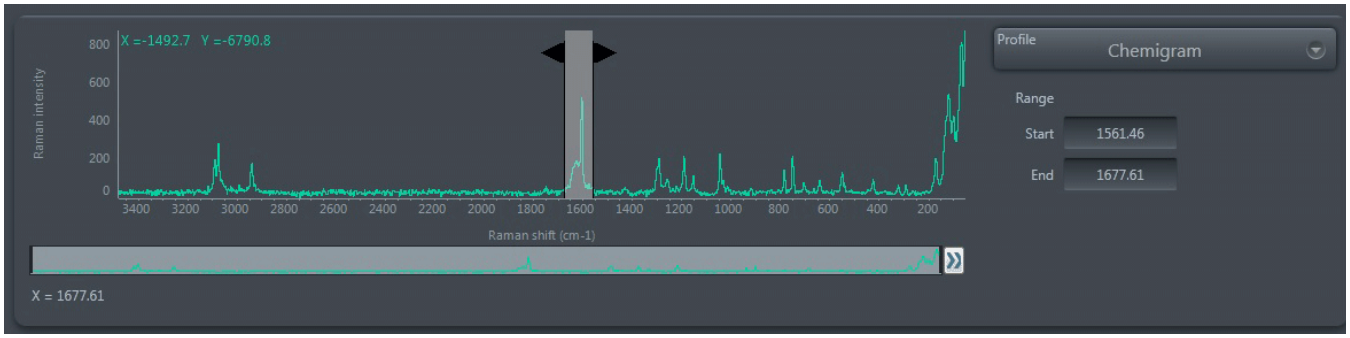
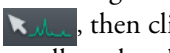


Table 26. Chemigram profile settings

Profile Type	Description
Range start/end	Defines the location of the region of interest. To specify the region, 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. Enter X-axis start and end locations in their respective boxes or drag the left and right facing triangle tools in the spectral pane to mark the region start and end. **Tip** To zoom the spectrum, use the mouse to draw a box around the region, then click inside the box.

Peak Area Profile Settings

The Peak Area profile shows the corrected area of the specified peak for each sample point in the chemical image. The colors in the chemical image represent the relative area of the specified peak at each sample point. A higher intensity value (closer to the top of the profile intensity scale) indicates a larger peak area.

Figure 78. Peak area profile settings

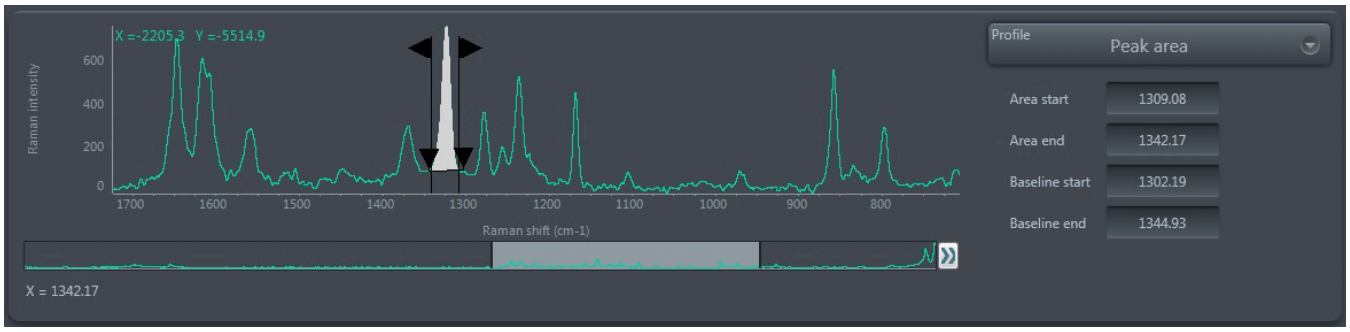


Table 27. Peak area profile settings

Profile Type	Description
Area start/end	Defines the location of the peak of interest. To specify the peak position, 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. Enter X-axis start and end locations in their respective boxes or drag the left and right facing triangle tools in the spectral pane to mark the peak start and end. **Tip**: To zoom the spectrum, use the mouse to draw a box around the region, then click inside the box.
Baseline start/end	Defines baseline values for the specified peak area. Enter X-axis locations in the **Baseline start** and **Baseline end** boxes or drag the two downward facing triangle tools in the spectral pane (see image above) to mark the baseline points.

Peak Height Profile Settings

The Peak Height profile shows the corrected height of the specified peak for each sample point in the chemical image. The colors in the chemical image represent the relative intensity of the specified peak at each sample point. A higher intensity value (closer to the top of the profile intensity scale) indicates a larger peak.

Figure 79. Peak height profile settings

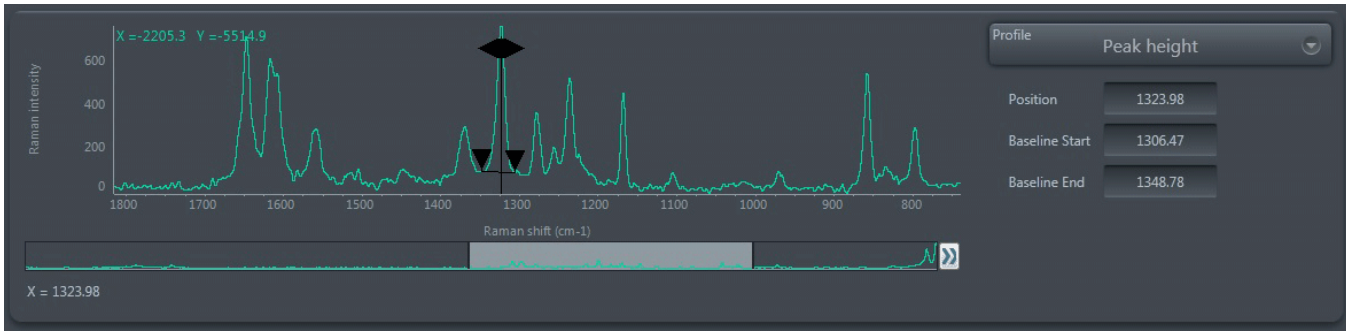


Table 28. Peak height profile settings

Profile Type	Description
Position	Defines the location of the peak of interest. To specify the peak location, 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. Enter an X-axis location in the **Position** box or drag the double triangle tool in the spectral pane to mark the peak location. **Tip** To zoom the spectrum, use the mouse to draw a box around the region, then click inside the box.
Baseline start/end	Defines baseline values for the specified peak. Enter X-axis locations in the **Baseline start** and **Baseline end** boxes or drag the two single triangle tools in the spectral pane (see image above) to mark the baseline points.

Peak Area Ratio Profile Settings

The Peak Area Ratio profile shows the ratio of the corrected areas of two specified peaks for each sample point in the chemical image. Using the ratio of two peaks lets you look at:

- Changes in sample material (some peaks will increase while others may decrease)
- Changes in two sample components
- Changes in peak area normalized against another peak to make sure physical variations (focus for example) are not causing variations in measured peak area
- Information about the number of layers present and the quality of the materials in a layered sample such as graphene

The colors in the chemical image represent the relative ratioed areas of the specified peaks. A higher intensity value (closer to the top of the profile intensity scale) indicates a larger peak area ratio.

Figure 80. Peak area ratio profile settings

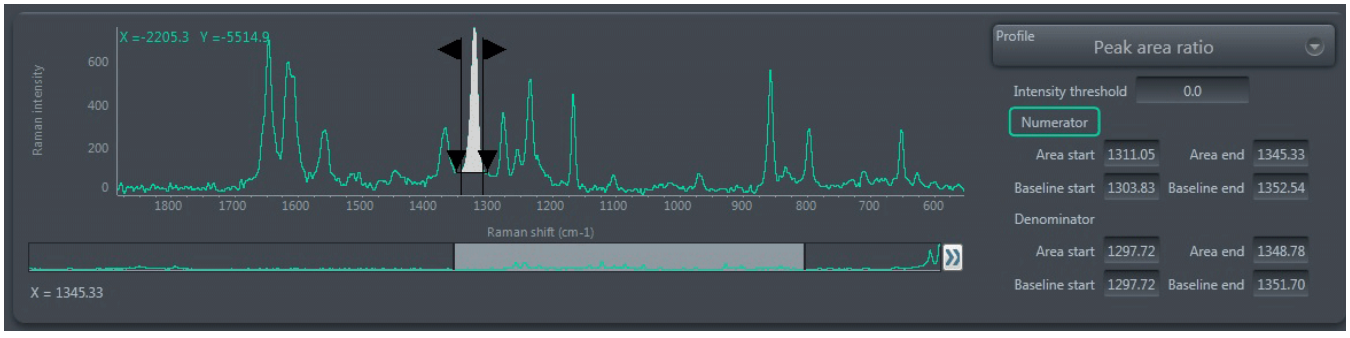


Table 29. Peak area ratio profile settings

Profile Type	Description
Intensity threshold	Specify a peak area ratio value below which will be reported as 0 (to eliminate peaks below a certain size). Prevents dividing a small peak (or noise spike) by an even smaller peak (or noise spike), which generates a high response even when the peak of interest is not present in the spectra.
Numerator/ Denominator	To define the areas of the numerator and denominator peaks, select **Numerator** (or **Denominator**) and then specify the following: • **Area start/end**. Defines the location of the numerator or denominator peak. To specify the peak location, 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. Enter X-axis start and end locations in their respective boxes or drag the left and right facing triangle tools in the spectral pane to mark the peak start and end. • **Baseline start/end**. Defines baseline values for the specified peak. Enter X-axis locations in the **Baseline start** and **Baseline end** boxes or drag the downward facing triangle tools in the spectral pane to mark the baseline points. **Tip** To zoom the spectrum, use the mouse to draw a box around the region, then click inside the box.

Peak Height Ratio Profile Settings

The Peak Height Ratio profile shows the ratio of the corrected heights of two specified peaks for each sample point in the chemical image. The colors in the chemical image represent the relative ratioed heights of the specified peaks. A higher intensity value (closer to the top of the profile intensity scale) indicates a larger peak height ratio.

Figure 81. Peak height ratio profile settings

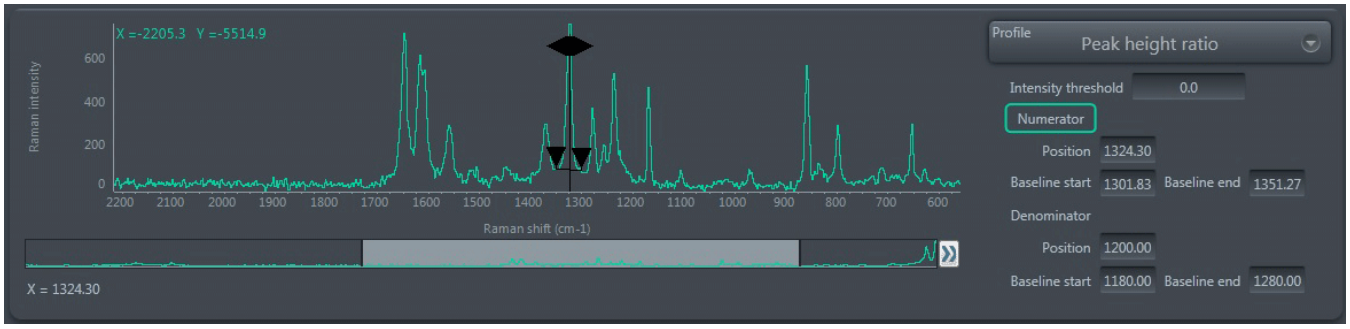


Table 30. Peak height ratio profile settings

Profile Type	Description
Intensity threshold	Specify a peak height ratio value below which will be reported as 0 (to eliminate peaks below a certain size). This prevents dividing a small peak (or noise spike) by an even smaller peak (or noise spike), which generates a high response even when the peak of interest is not present in the spectra.
Numerator/ Denominator	To define the heights of the numerator and denominator peaks, select **Numerator** (or **Denominator**) and then specify the following: • **Position**. Defines the location of the numerator or denominator peak. To specify the peak location, 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. Enter the X-axis location in the Position box or drag the double triangle tool in the spectral pane to mark the peak position. • **Baseline start/end**. Defines baseline values for the specified peak. Enter X-axis locations in the **Baseline start** and **Baseline end** boxes or drag the downward facing triangle tools in the spectral pane to mark the baseline points. **Tip** To zoom the spectrum, use the mouse to draw a box around the region, then click inside the box.

Peak Width Profile Settings

The Peak Width profile shows the width of the specified peak for each sample point in the chemical image. The measurement is based on the full width of the peak at the specified height. The colors in the chemical image represent the relative width of the specified peak at each sample point. A higher intensity value (closer to the top of the profile intensity scale) indicates a wider peak.

Figure 82. Peak width profile settings

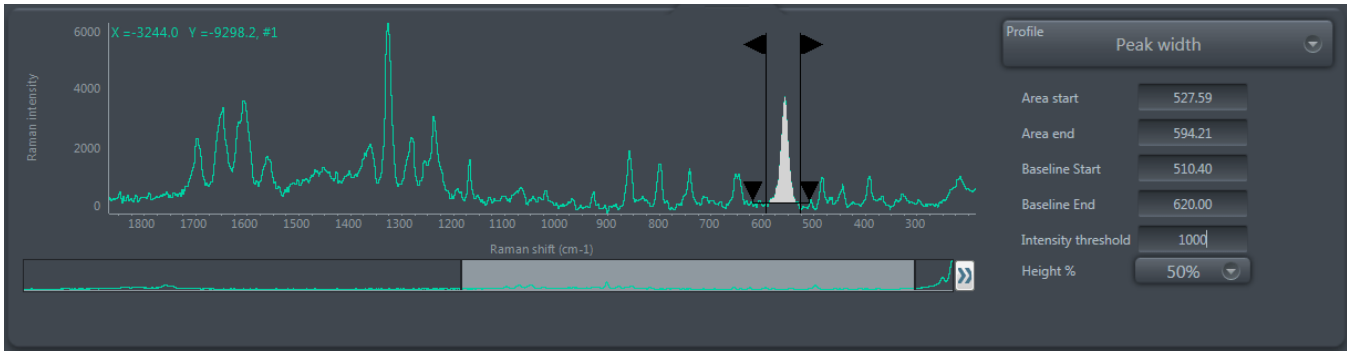


Table 31. Peak width profile settings

Profile Type	Description
Area start/end	Defines the location of the peak of interest. To specify the peak position, 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. Enter X-axis start and end locations in their respective boxes or drag the left and right facing triangle tools in the spectral pane to mark the peak start and end. **Tip:** To zoom the spectrum, use the mouse to draw a box around the region, then click inside the box.
Baseline start/end	Defines baseline values for the specified area. Enter X-axis locations in the **Baseline start** and **Baseline end** boxes or drag the two single triangle tools in the spectral pane (see image above) to mark the baseline points.
Height	Specifies the height of the peak where the width measurement is to be made. For example, selecting 50% will measure the width at one half of the peak height resulting in a full width half max (FWHM) measurement.
Intensity threshold	Specifies a peak height value below which this profile will report 0. This eliminates peaks below a certain size to prevent reporting noise when the peak of interest is not present in the spectra.

Peak Position Profile Settings

The Peak position profile shows the shift in the location of a specified peak along the X-axis. The measurement is based on the peak maximum in a specified region for each sample point in the chemical image. The colors and their associated X-axis values in the profile intensity scale indicate the relative locations of the peak maximum in the specified region.

Figure 83. Peak position profile settings

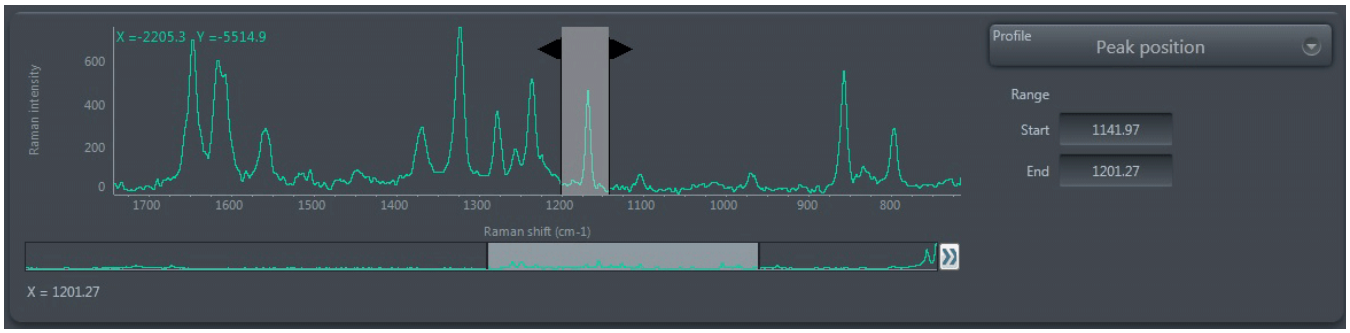


Table 32. Peak position profile settings

Profile Type	Description
Range start/end	Defines the location of the region of interest. To specify the region, 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. Enter X-axis start and end locations in their respective boxes or drag the left and right facing triangle tools in the spectral pane to mark the region start and end. **Tip** To zoom the spectrum, use the mouse to draw a box around the region, then click inside the box.
Intensity Threshold	Specifies a peak height value below which this profile will report 0. This eliminates peaks below a certain size to prevent reporting noise when the peak of interest is not present in the spectra.

MCR Profile Settings

The MCR profile finds components in the sample data based on their (calculated) pure component spectra and shows their locations in the chemical image. Click a point in the chemical image to display the spectrum acquired at that location and its associated pure component spectrum in the spectral pane.

If OMNICxi has associated spectral libraries, the found MCR components are searched against those libraries automatically. To see search results for the displayed pure component spectrum, click Search Displayed Spectrum below the spectral pane. 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. After you close the search results window, the library spectrum disappears from the spectral pane. Click here for details about the search results.

Figure 84. MCR profile settings

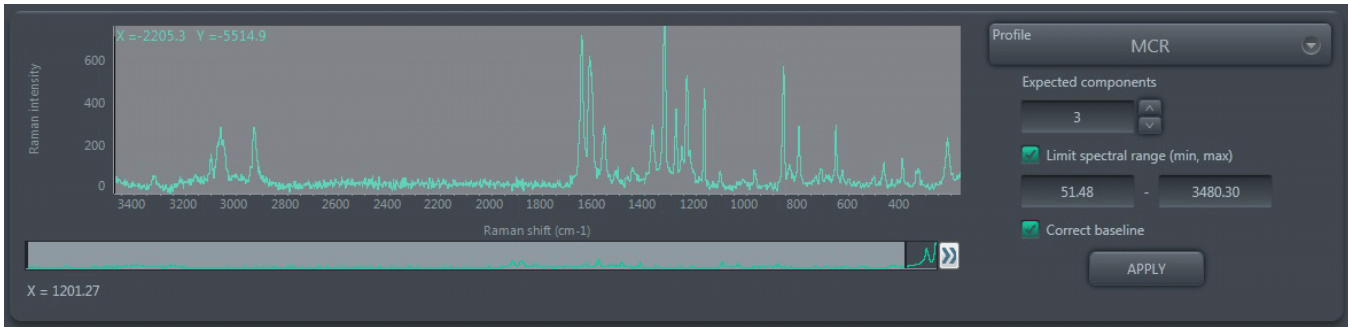


Table 33. MCR profile settings

Profile Type	Description
Expected components	Specifies the number of components that will be reported after the MCR analysis. Enter the number of different components you expect to find in the sample plus one (up to 32 components total). For example, if you think your sample has 4 significant components, specify 5 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.
Limit spectral range (min, max)	Refines the spectral range used for the MCR analysis (for example to ignore a high intensity peak or region that is not of interest). Enter minimum and maximum X-axis values or use the tools in the spectral pane to mark the start and end of the region used for the analysis. **Tip**: To zoom the spectrum, use the mouse to draw a box around the region, then click inside the box. Right-click the spectral pane for more display options.
Correct baseline	Applies a 4th order polynomial baseline correction to each spectrum in the chemical image before performing the MCR analysis. It can be useful for correcting any effects on the baseline curvature caused by the sample response. Fluorescence is a good example. **Tip**: You can also apply a configurable baseline correction to your sample data as it is acquired instead of this pre-processing correction.
APPLY	Starts the MCR analysis. The length of time for the analysis depends on the number of specified components and the number of spectra in the chemical image.

View Search Results for a Generated Profile Analysis

❖ To view search results for a generated profile analysis

1. Use the Select Libraries button at the top of the Analysis window to select the Raman libraries to be searched.
2. Select the profile thumbnail in the profiles pane.

   If you are searching MCR analysis results, select an MCR component thumbnail in the profiles pane.

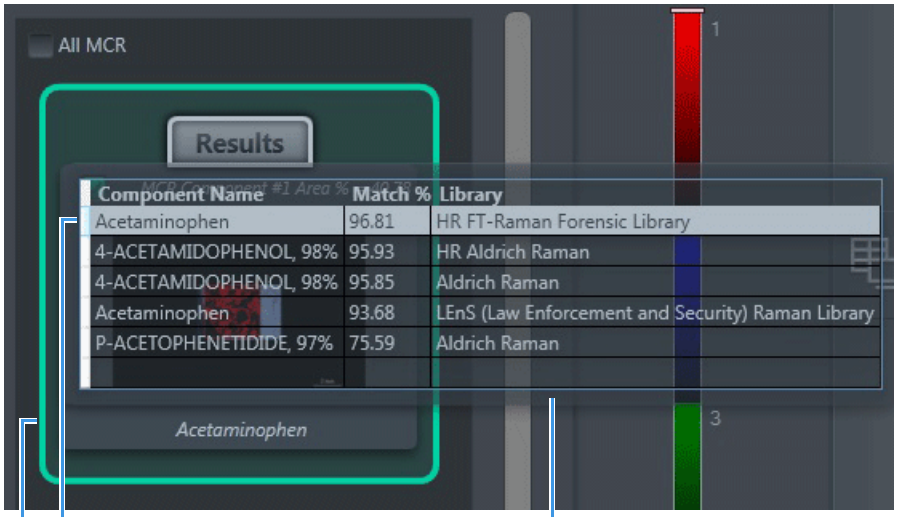
3. Select the Spectrum tool, then click a point of interest in a region of acquired chemical image data.

   The spectrum collected at that location is displayed in the spectral pane. The spectrum is automatically searched against the selected libraries. (For the MCR profile, the selected spectrum and the calculated pure component spectrum are displayed in the spectral pane. The pure component spectrum is searched instead of the spectrum at the current cursor location.)

4. Click the **Results** button in the thumbnail.

   A search results window appears below the button.

Figure 85. Search results window for a found MCR component



The name of the best matched library spectrum 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. If no suitable match is found in the library you can select Unknown and no matching spectrum will be displayed.

To close the search results window, click the **Results** button again. After you close the window, the library spectrum disappears from the spectral pane.

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:

- The compound name
- The name of the library that contains the spectrum
- A 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.

Reanalyze Image Data

Reanalyze previously collected data to add profile information.

❖ To reanalyze image data

1. If you want to reanalyze the image data using different profile settings, select a thumbnail in the profiles pane.

   If you are adding a profile, click the **+** button to add a profile thumbnail to the profiles pane. Make sure the new thumbnail is selected before you continue.

2. Use the Profile option to select a profile analysis type.

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

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

4. Specify any Profile settings.

   Enter values in the boxes or use the tools in the spectral pane to mark the locations on the displayed spectrum. To zoom the spectrum, use the mouse to draw a box around the region, then click inside the box.

   For all profile types except MCR, 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 appears in the mosaic pane. The thumbnail image appears in the profiles pane below any previously generated profile thumbnails.

5. If you are running an MCR analysis, click **APPLY** to start the analysis.

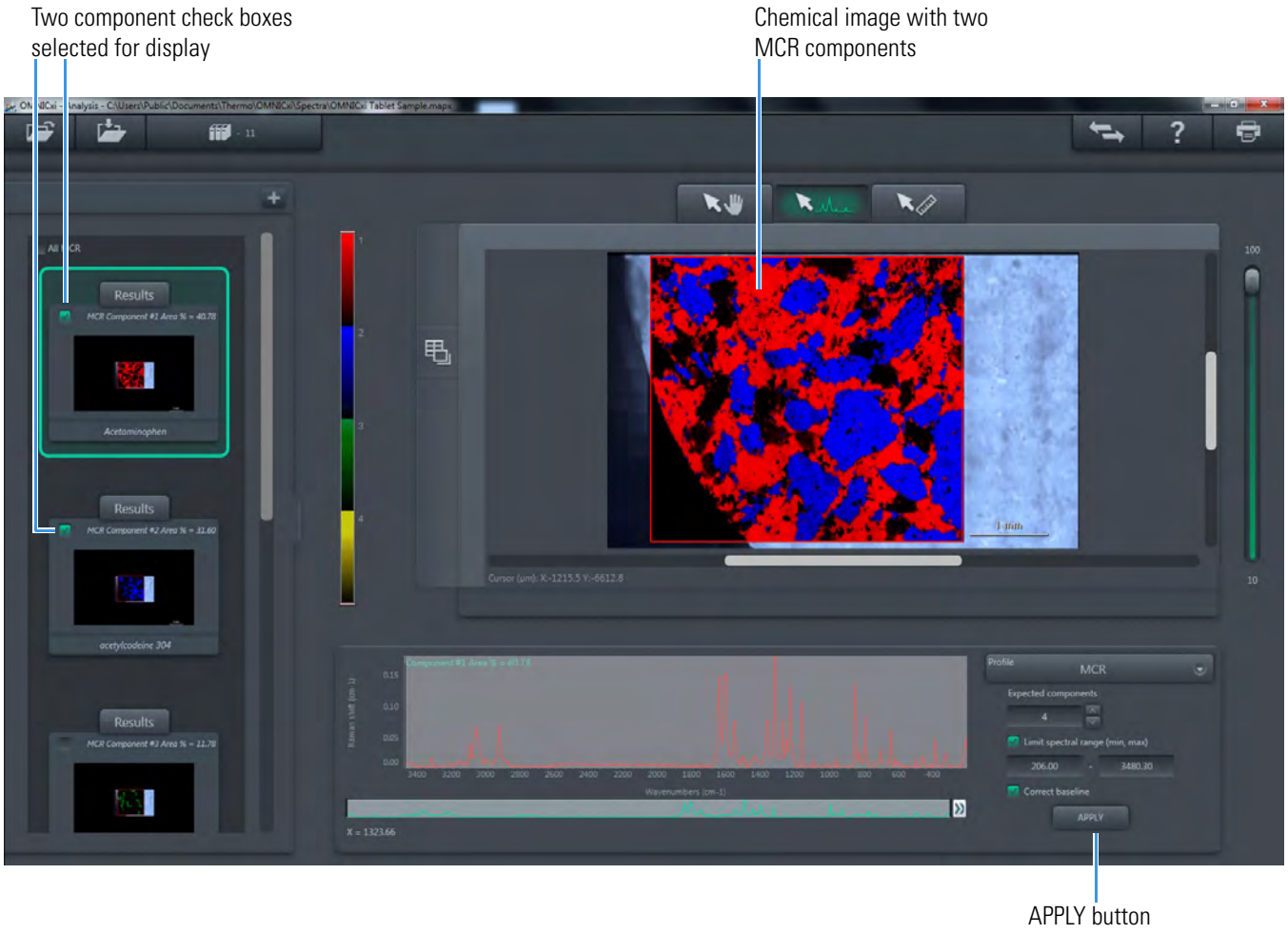
   The length of time for the analysis depends on the number of specified components and the number of spectra in the chemical image. A status bar shows the progress. Wait until the analysis is completed.

   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.

   The MCR analysis produces one thumbnail for each found component. Only one set of MCR thumbnails can be displayed and they always appear at the top of the profiles pane.

   Use the check boxes in the thumbnails to add components to the chemical image in the mosaic pane. Here is an example with two found MCR components selected for display.

Figure 86. Analysis window with two MCR components selected for display



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

To update the current MCR analysis, for example, after you have changed the Expected components setting, click **APPLY**. The updated results replace the previous ones in the profiles pane and in the mosaic pane.

6. To save the analysis results, 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.

Identify and Analyze Particles

Use the advanced particle analysis tools to locate, characterize, and identify particles.

The particle selection tools are available only with the optional Particle Analysis Add-On.

Begin particle analysis with either a video image of your sample or with a chemical image. Collecting a chemical image before performing particle analysis allows you more options to analyze your sample but data collection takes longer.

Video Image-Based Particle Analysis

Use the particle selection tools in the Acquisition window to automatically detect and select particles in your sample. Collect single point spectra or regions for each particle. After measuring the particles, view particle properties such as size, quantity, and type in the results table in the Analysis window.

❖ To detect and measure particles using a video image

1. Load the sample onto the microscope stage.
2. Configure sample illumination.
3. Click the **Video** button in the software to select video mode.
4. Bring the sample into focus and collect a mosaic.
5. Use Live spectrum mode to acquire spectra at several locations of the sample in order to optimize the Laser power, Exposure time and Number of scans settings.
6. Click **Image Regions** to select image regions mode.
7. Select the **Auto Regions** tool ( ) and navigate to the **Particle Selection** tab.

   The currently selected particles are displayed in the mosaic pane. Use the particle selection tools to refine the selection. See Settings for Particle Analysis for details.
8. When you are satisfied with the selection, click **Keep Regions**.

   You can add or remove regions and points manually before collecting data.ffg
9. When you are ready to collect the image data, click **Collect Regions**.

   The collected data appear the Analysis window.

For more on exploring your results, see View Particle Analysis Results. You can refine your results by using the Search Setup tools to specify one or more regions to use in the spectral search.

Chemical Image-Based Particle Analysis

For chemical image-based particle analysis, either open a saved chemical image or collect a region in the Acquisition window and then analyze the chemical image in the Analysis window.

❖ To detect and measure particles using a chemical image

1. Collect a new chemical image for your sample or open the chemical image in the analysis window. For more on collecting a chemical image, see Acquire and Analyze a Chemical Image.
2. In the Analysis window, right-click the image and select Analyze Particles in Selected Region.

   The Particle analysis window opens, showing the particle selection tools and the results tab.
3. In the Setup tab, refine your settings to include particles in the image that you want to analyze and to exclude other areas from the analysis. For details, see Settings for Particle Analysis.
4. When you are satisfied with the selection, click the Results tab to view the results of the particle analysis.

You can use the Search Setup to refine your results. Open the Search Setup and add one or more spectral regions to use in the search. Select Use Region in Search. After you click OK, the particle analysis is re-run using the specified regions.

For more on exploring your results, see View Particle Analysis Results.

Settings for Particle Analysis

Use settings in the Particle Selection tab to refine your selection of particles before measuring. The Particle Selection tab is only available with the optional Particle Analysis Add-On.

There are a few differences between setup options for video- and chemical-image based particle analysis.

❖ To set up particle analysis

1. Select Auto Regions and navigate to the particle analysis tab. The following particle analysis settings are available:

Figure 87. Video-image based particle selection settings

These settings are available:

Table 34. Particle Analysis settings

Setting	Description
Use Defaults	Resets all settings to the default values.
Highlight Regions	Select to highlight particles that have been identified in the sample image.
Save and Load	Save your particle analysis settings as a .particle file. The .particle file saves all of the settings related to particle analysis, including background intensity and number of particle settings. Loading a .particle file automatically updates the particle analysis settings.

Table 34. Particle Analysis settings

Setting	Description
Image Processing	Use the Image Processing group to specify how the selection handles particles with holes, touching particles, and partially visible particles. The following tools are available: • **Fill Holes Whose Size Is.** If detecting particles as a region, data from the hole will be ignored. Use the slider to set a threshold for how large of a hole should be ignored. • **Separate Touching Particles.** Select to treat particles that are touching as if they were separate particles. Clear to treat the group as a single particle. • **Exclude Partially Visible Particles.** Excludes partial or incomplete particles that are cut off on the edge of the mosaic or region.
Background Intensity	Use the background intensity group to limit the selection based on visual contrast. The following tools are available: • **Auto Detect.** Automatically detects what is a background and what is a particle based on visible contrast. The histogram shows what percentage of the image is a given intensity. Intensity is a measurement of how light or dark a pixel is, from completely black to completely white, with white being the most intense. If you have a very light background with only dark particles, the histogram may show that more than 80% of the image is high intensity. Clear to refine the selection with the image intensity histogram. • **Use 2 regions.** Select to manually refine the selection using to regions on the histogram. This is useful for selecting particles that are lighter and darker than a neutral background. • **Image Intensity Histogram.** Manually refine the selection based on visual contrast.

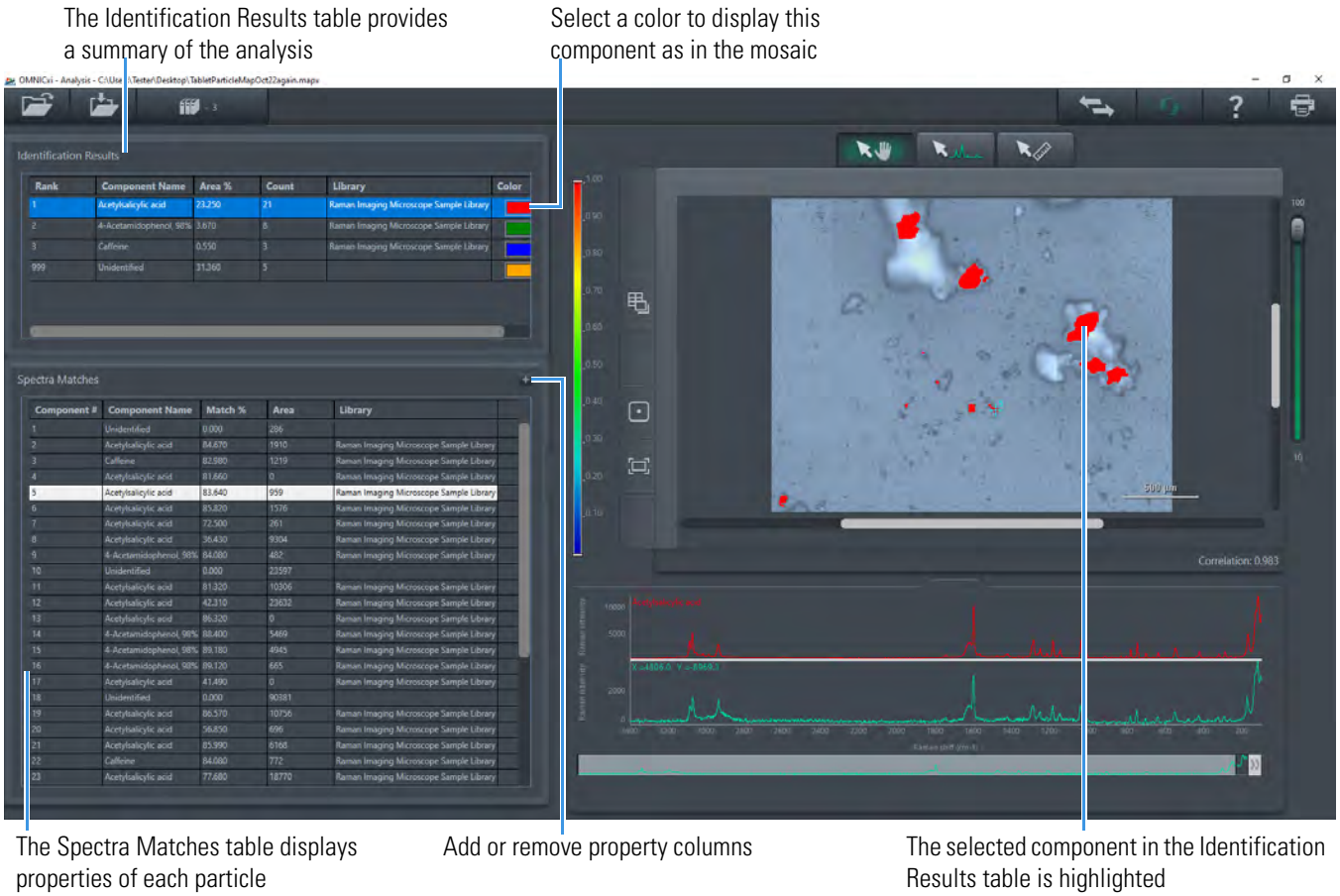
Table 34. Particle Analysis settings

Setting	Description
Number of Particles	Use the Number of Particles group to limit the selection based on a maximum count or based on particle size in micrometers. The following tools are available: • **Max # of Particles.** Limits the number of particles to the value specified. Includes the largest particles and excludes smaller particles. For example, if you limit to 200 particles, the 200 largest particles are selected. Select Limit to specify a maximum value. Selects the largest particles. Clear to select all detected particles. • **Detect as Points.** (Only available for video-image based particle analysis.) When detecting as points, instead of collecting all spectra within the region, the spectrum is measured as the average of 9 spectra at the location marked by the plus sign. Deselect to collect all of the spectra in the region for each particle. • **Particle Size Histogram.** Refine the selection based on the size of particles. This is useful for excluding particles that are too small or too large. The particle size is given by the diameter of the particle in micrometers.
Manual update	Select to update the particle selection manually. If your sample has many particles, it can be time consuming to wait for the calculation each time you edit a setting. Updating manually allows you to edit multiple settings before recalculating. With Manual update selected, click Recalculate to refresh the particle selection.

View Particle Analysis Results

Use the Analysis Window to explore particle data. The Spectra Matches and Identification Results tables display information about the particles in your sample.

When viewing particle analysis data in the Analysis window, the Analysis window will display Particle Analysis mode.



- Click the column headers to sort by that property.
- Customize the Spectra Matches table by adding or removing property columns.
- Drag columns to change their order.
- Select a spectrum in the mosaic to highlight it the Spectra Matches table.
- Click a row in the Spectra Matches table to point to the particle in the mosaic
- Click a row in the Identification Results table to highlight the component in the mosaic

View Spectrum at Specific Location

Select Live Spectrum mode to view a spectrum at a specific location on the sample surface and to compare that spectrum to library spectra.

Acquire Spectra at Selected Sample Locations

To compare the spectrum at a specific location to library spectra, you will collect a mosaic, select a spectrum, and search the displayed spectrum.

❖ To acquire spectra at selected sample locations

1. Select Video mode and use the available tools to locate, zoom and bring the sample into focus.
2. Click **START Mosaic** to collect a mosaic.
3. Close the sample compartment doors.
4. Click **Live Spectrum mode**.
5. Set **Laser power** greater than 0.
6. Use the **Grab tool** to select a location in the mosaic.

   The large red **+** moves to the selected location The spectrum acquired at that location and the current focal plane is displayed in the spectral pane.
7. Adjust the Acquisition settings as needed to optimize the displayed spectrum.

   If the spectrum has a curved baseline, use Correct baseline in the **Settings** box to correct it.
8. Use **SEARCH Displayed Spectrum** below the spectral pane to search the spectrum against the available spectral libraries.

Settings for Acquiring Spectra

These settings are used to acquire a spectrum at the current cursor location in the mosaic pane.

Figure 88. Live Spectrum mode, standard acquisition settings

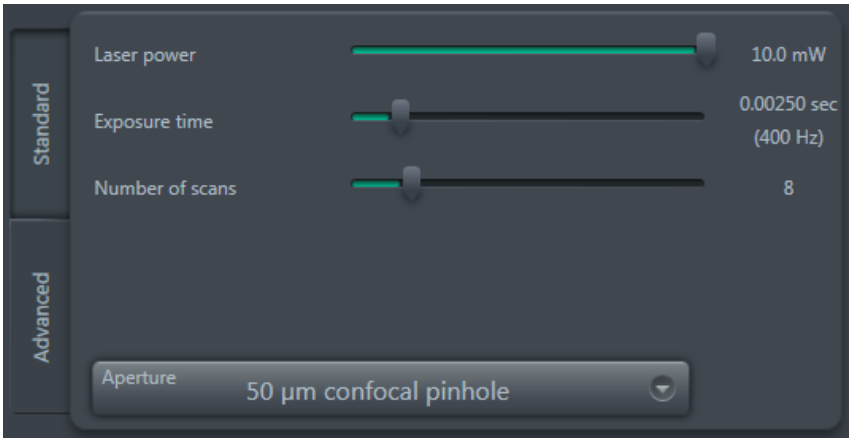


Table 35. Acquisition window, standard acquisition settings in Live Spectrum mode

Feature	Description
Laser power	Laser energy at the sample in milliwatts (0 blocks the laser). Highest available laser power settings 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.
Exposure time	Laser exposure time for each acquired spectrum in fractions of a second. Also indicates the speed of acquisition in Hz (i.e., number of spectra acquired per second). Longer exposure times may increase the height of spectral peaks but will also increase scan time.
Number of scans	Number of scans used to produce the acquired spectrum. Spectra collected from multiple scans are averaged. Using a higher number of scans setting may reduce noise in the spectral baseline due to signal averaging. However, increasing number of scans also increases the time required to acquire the data.
Aperture	Defines the size of the Raman beam entrance slit or pinhole. The beam passes through the aperture on its way to the instrument detector. Available settings: • **25 µm confocal pinhole**. Maximizes spatial resolution (ability to discriminate very small changes in a sample) and spectral resolution (ability to resolve narrow bands in the spectrum), but allows less Raman energy from the sample which may decrease signal intensity. Recommended for analyzing samples with fine detail, cross sectional analysis, and when using a high resolution grating. • **50 µm confocal pinhole**. Increases Raman energy at the detector but may reduce spatial and spectral resolution. Recommended when using higher image pixel size settings and working with a full range grating. • **25 µm (or 50 µm) slit**. Maximizes Raman energy at the detector which produces higher quality spectra (higher signal-to-noise ratio) but may reduce spatial and spectral resolution. Use the smaller (25 µm) slit to improve spectral resolution. Do not use slit settings for cross sectional analysis.

Figure 89. Live Spectrum mode, advanced acquisition settings

Table 36. Acquisition window, advanced acquisition settings in Live Spectrum mode

Feature	Description
EM gain	Controls the electron multiplying gain of the detector camera. EM gain increases the signal to overcome the readout noise, but it also increases other sources of noise. The optimal setting for this is highly dependent on the experiment. In general, it is recommended to run with EM gain on for most operation but to turn it off when collecting with a longer exposure time.
Laser polarization (requires polarization option)	Sets the polarization for the laser light incident on the sample
Analyzer polarization (requires polarization option)	Sets the position for the polarization analyzer controlling the Raman emission. This option is only available when Laser polarization is set to "Horizontal(X)" or "Vertical(Y)" to run a polarized experiment.
Analyzer angle (requires polarization option)	Sets an arbitrary angle (0–180 degrees) for the polarization analyzer controlling the Raman emission. This option is only available when Analyzer angle is set to "Select Angle."

Analyze Sample Cross Sections

Acquire a cross sectional chemical image to view depth information (Y-, Z-axis) rather than the normal surface (X-, Y-axis) information.

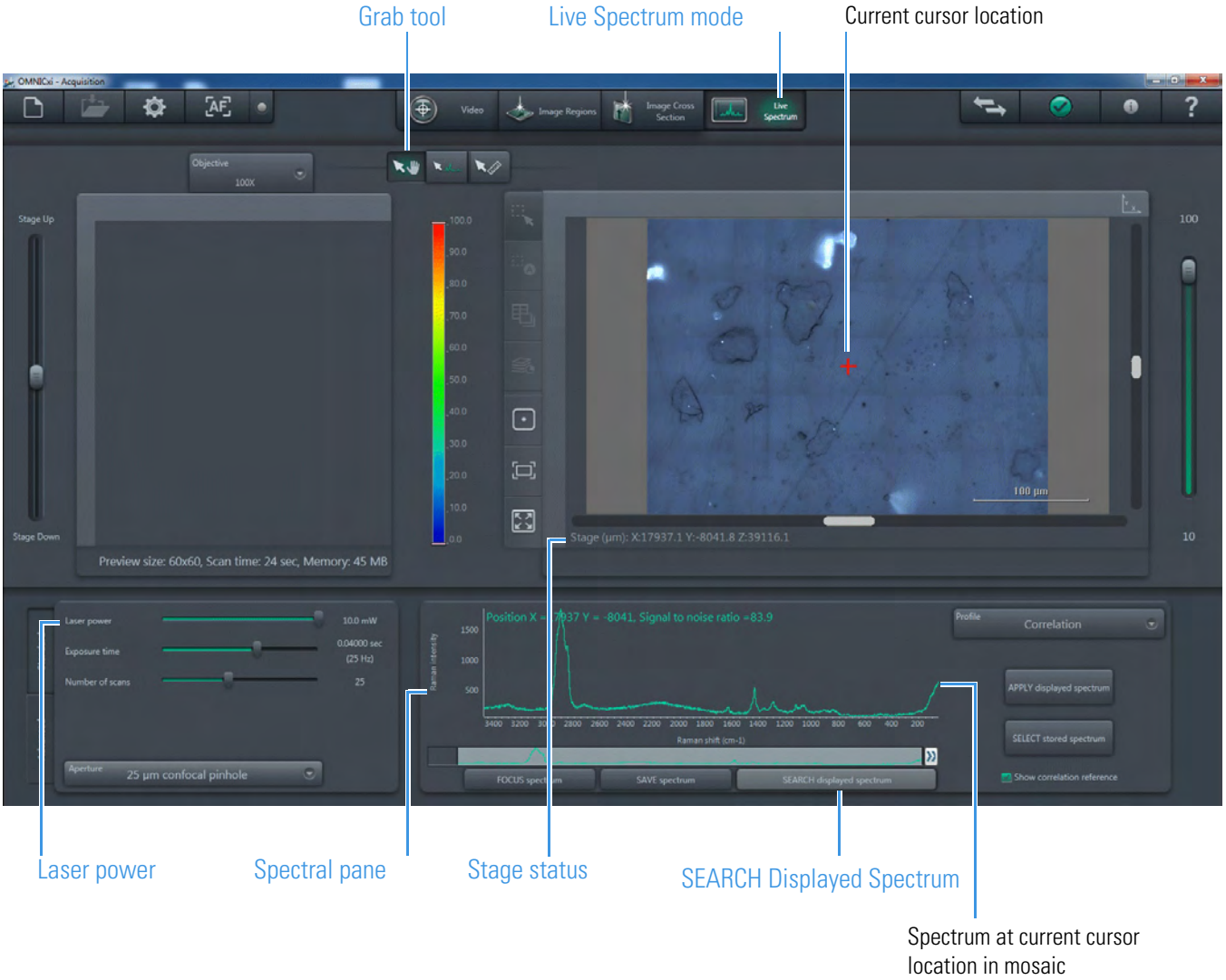
For a cross sectional image, the sample must be optically transparent to the laser wavelength. Generally, if you can see through the material with your eyes it is a good candidate for this application. (Mounting the sample on a slide that has an opening in the middle will eliminate the possibility of including a spectrum of the slide in the analysis results.)

❖ To acquire a cross sectional chemical image of a layered sample:

1. Select Video mode and use the available tools to locate, zoom and bring the surface layer of the sample into sharp focus.
2. Click **START Mosaic** to collect a mosaic.
3. Close the sample compartment doors.
4. Click **Live Spectrum**.
5. Set **Laser power** greater than 0 (use an appropriate setting for your sample material).
6. Use the **Grab tool** to click a location in the mosaic.

The large **+** moves to the selected location. The spectrum acquired at that location is displayed in the **spectral pane**. The Stage status shows the current X, Y, and Z location of the microscope stage.

Figure 90. Live Spectrum mode used to explore the layers in an optically transparent sample



7. Adjust the Acquisition settings (Laser power, Exposure time, Number of Scans, Aperture, Photobleach time, polarization settings, and EM gain) as needed to optimize the spectrum.

   Make sure **Aperture** is set to one of the "confocal pinhole" options.

   If the spectrum has a curved baseline, use Correct baseline in the **Settings** box to correct it.

8. Click SEARCH Displayed Spectrum to search the displayed spectrum against the available spectral libraries.

9. Locate and identify other layers in the sample.

NOTICE Be careful not to bump the sample against the objective during this step or you may damage the objective or the sample.

Use the Focus control on the stage controller to slowly move the sample toward the objective approximately 10 to 20 µm to change the focal (Z) depth. The distance will depend upon the thickness of the layers. When the spectrum changes significantly, click SEARCH Displayed Spectrum to see the search results. Continue moving further into the sample to identify all layers.

10. Select a **Profile** type and adjust the profile settings as needed to create the chemical image.

    For this demonstration, we used the MCR profile type with 5 expected components.

11. Click **Image Cross Section** and use the **Grab tool** to position and size the **preview box** over an area of interest in the sample.

    Note that there are separate Preview Size sliders for Y and Z (depth). The height of the preview box shows the length along the Y-axis of the chemical image data.

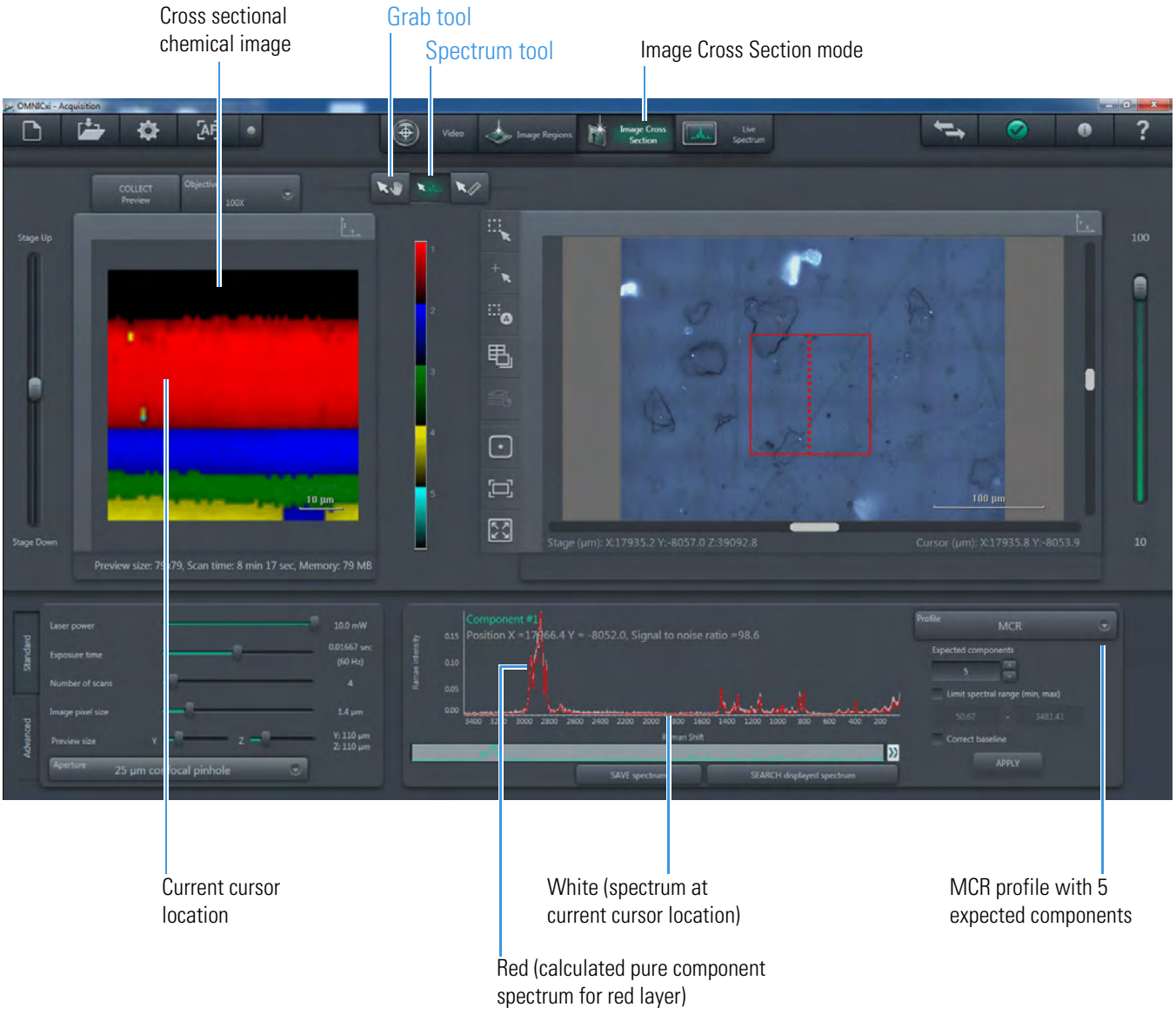
12. Adjust the Image pixel size setting.

13. Adjust other acquisition settings as needed.

14. Click **COLLECT Cross Section** to collect the chemical image.

    The image appears in the left pane. The cross sectional chemical image shows depth (Y-, Z-axis) information rather than the normal surface (X-, Y-axis) information acquired in Image Regions mode. Here is an example of a cross sectional image using the MCR profile. Each color in the chemical image represents a new layer in the sample.

    Figure 91. MCR cross sectional image of an optically transparent sample



15. Adjust the Image pixel size setting if needed to refine the chemical image.

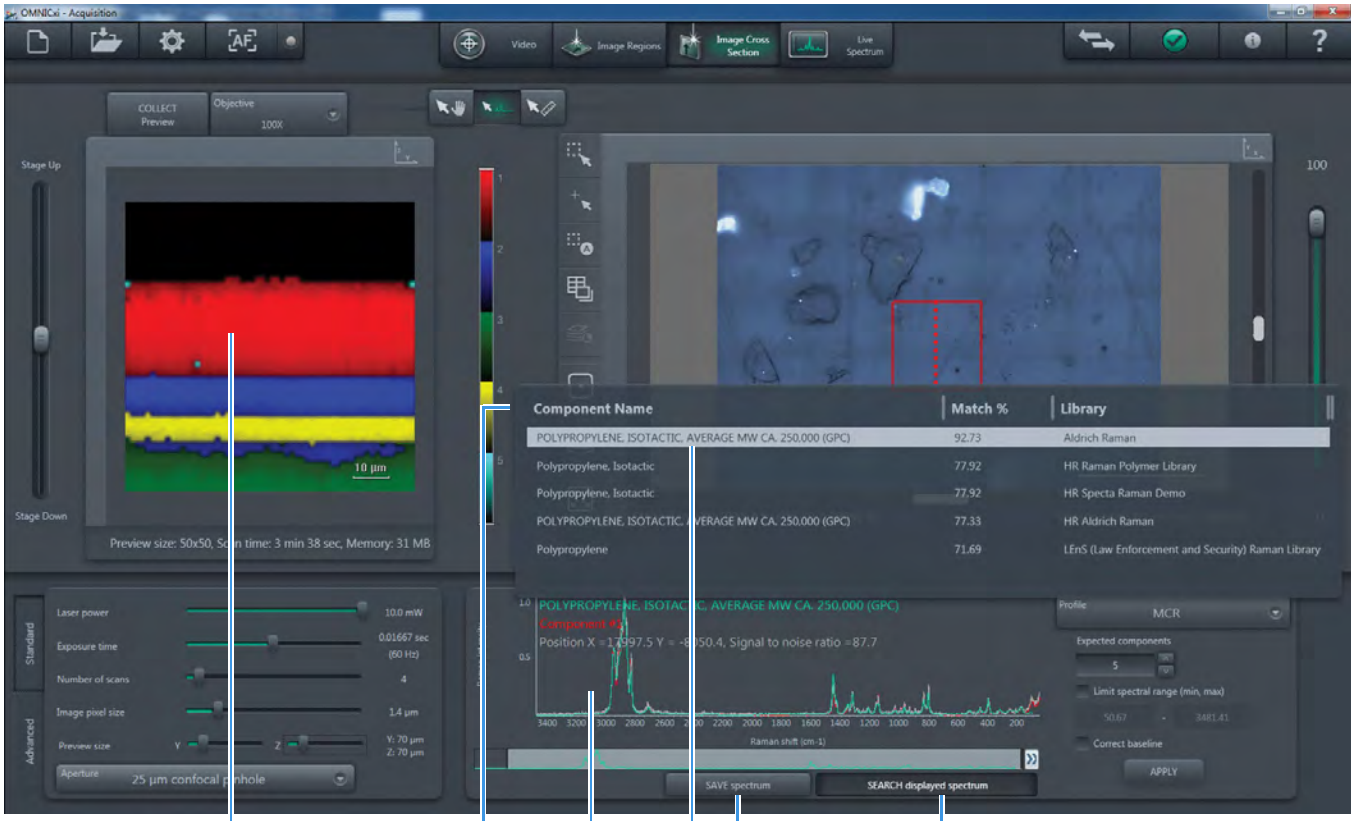
16. If you adjusted a setting, click **COLLECT Cross Section** again to recollect the image data.

17. Select the **Spectrum tool** and then click each colored layer in the chemical image to display the spectrum acquired at that focal plane in the sample and its associated (calculated) pure component spectrum in the spectral pane.

18. To search a displayed pure component spectrum against the available spectral libraries, click SEARCH Displayed Spectrum.

    A search results window appears above the button and the best matched library spectrum is temporarily added to the spectral pane. Here is an example.

Figure 92. Search results for pure component spectrum associated with cross sectional image of an optically transparent sample



Current cursor location

Search results window

SEARCH Displayed Spectrum

SAVE Spectrum

Best library match for red layer

Spectral pane
White (spectrum at current cursor location)
Red (pure component spectrum for red layer)
Green (best library match for red layer)

19. To save the selected spectrum as an OMNIC format .SPA file, click **SAVE Spectrum**.

    To select a spectrum, click its title in the spectral pane. To select additional spectra, hold down the Shift key while you click another spectrum title. Multiple selected spectra are saved as an OMNIC .SPG file. SPA and SPG files can be opened using the Thermo Scientific OMNIC Workstation for DXRxi Raman Imaging Microscope application for further processing.

20. Right-click the acquired chemical image for these options for copying and saving data:

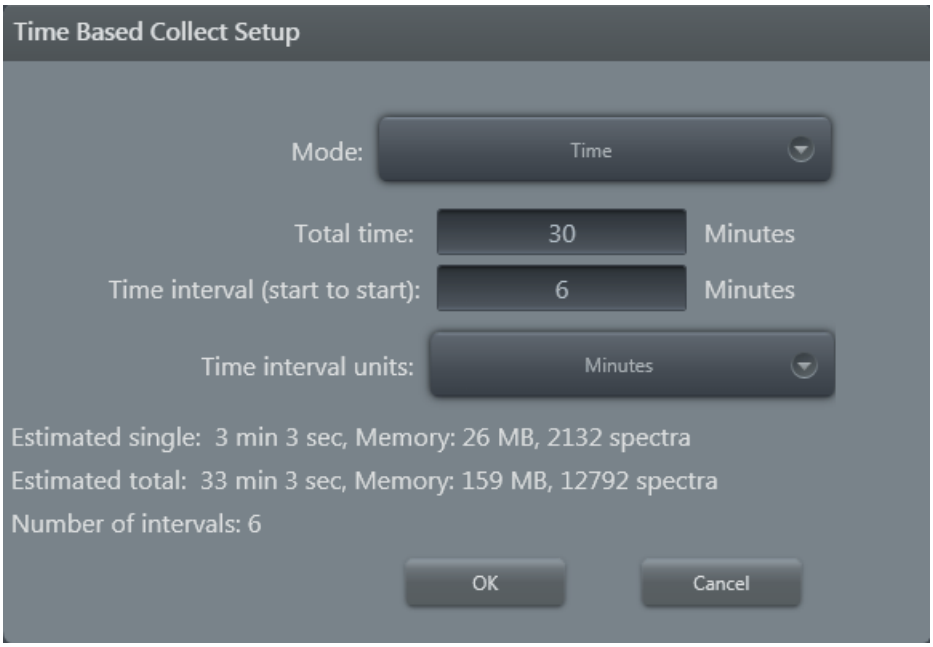
    - **Copy preview as picture**. Copies the chemical image to the Windows Clipboard. The copied image can be pasted into any word processing, presentation or other compatible application. The resolution of a copied image will be the same as the computer's display monitor (typically 72 dpi or 96 dpi).
    - **Save preview as TIFF**. Saves the chemical image as a TIFF file (.TIFF filename extension), which can be imported to a wide selection of publishing, page layout, scanning, faxing, word processing and other applications.
    - **Export preview data as ENVI**. Exports the chemical image as an ENVI file (.ENVI file name extension), which can be opened with the Thermo Scientific Atlμs and OMNIC Workstation for DXRxi Raman Imaging Microscope software, MATLAB software or other similar applications.
    - **Send preview to OMNIC/Atlμs as .MAP**. Sends the selected region to OMNIC/Atlμs software as a .MAP file. If the computer has the Thermo Scientific Atlus and OMNIC Workstation for DXRxi Raman Imaging Microscope software installed, that software opens with the image displayed in a spectral window.
    - **Add preview to analysis window**. Adds the chemical image to the mosaic pane in both the Acquisition and Analysis windows.

Measure Change Over Time

A region can be acquired repeatedly over time building up multiple 'frames' in order to study how the sample changes over time. The collection can be done at specified intervals or on input from an external trigger. The data can be viewed in the Acquisition window or the Analysis window. See Viewing Time-Based Data for details.

❖ To set up time-based acquisition

1. Draw one or more regions in the mosaic pane.
2. Click the Time-Based Collect Setup icon to show the settings.Time-based Collect settings


Time Based Collect Setup
Mode:
Time
Total time:
30
Minutes
Time interval (start to start):
6
Minutes
Time interval units:
Minutes
Estimated single: 3 min 3 sec, Memory: 26 MB, 2132 spectra
Estimated total: 33 min 3 sec, Memory: 159 MB, 12792 spectra
Number of intervals: 6
OK
Cancel

These settings are available:

Table 37. Time-Based Collect settings

Setting	Description
Mode	Available settings: • **None**. Runs standard collection. • **Time**. Runs time-based collection with frames collected at fixed time intervals. – **Total Time**. Defines the total length of the experiment from the start of the first frame to the start of the last frame. – **Time Interval**. Defines the time from the start of one frame to the start of the next frame. – **Time Interval Unit**. Defines whether settings are measured in: – Minutes (for shorter collections) – Hours (for longer collections) • **Trigger**. Runs multiple-frame collection with the start of each frame triggered by an external input. – **Total Intervals**. Defines the number of frames collected using the trigger. This function requires the optional external trigger accessory, available from us. The inputs and outputs are described below. – Each frame waits to begin collection until a trigger is received by a low-to-high transition on the "DI00" input on the external trigger module. – Output "DI02" is asserted high when the software is waiting for a trigger and transitions low once collection of each frame begins. – Output "DI04" is asserted high at the start of the time-based collect (when "Collect Regions" is pressed) and transitions low at the end of the last frame collection. – The inputs and outputs are 5 Volt TTL-level digital signals.

View Time-Based Data

You can view individual frames of time-based data in the Analysis window, or animate a series of frames. Many of the features available in the OMNICxi Analysis window also function with time-based data.

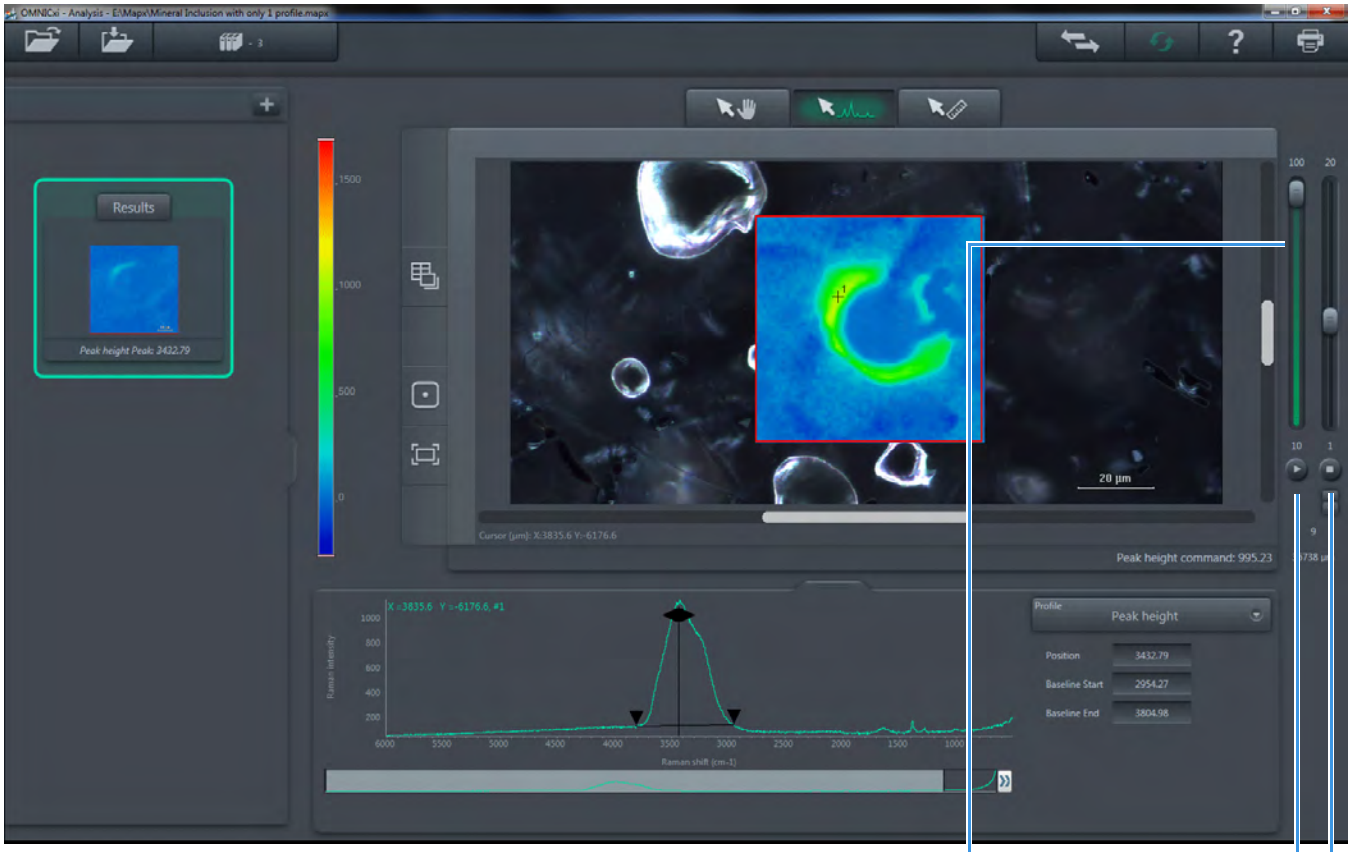
❖ To view selected frames of a time-based collection

- Use the up/down arrows or the Frame Selection bar to select a frame of interest.
- To play through or "animate" a series of frames, select a frame and then use the Play, Fast Forward and Stop buttons to start/stop and control speed.

Table 38. Analysis window showing time-based data and animation controls

Feature	Description
Frame Selection bar	Controls which frame of a time-based collection is being displayed. The up and down arrows can also be used to adjust the slider. **Note:** Single-frame collects will remain static.
Play	Animates subsequent frames of a time-based collection. After you click Play once, the button changes to the Fast Forward button.
Fast Forward	Speeds up the animation
Stop	Stops the animation

Figure 93. Time-based data collection



Measure and View 3D Volumes

Acquire 3D image data to view in the 3D Analysis window. To collect 3D image data, the sample must be optically transparent to the laser wavelength. Generally, if you can see through the material with your eyes, it is a good candidate for this application.

Acquire 3D Image Data

Use the Region Queue to collect data in the Z-Axis and analyze it in the OMNICxi 3D Analysis window.

❖ **To acquire 3D image data**

1. Load the sample onto the microscope stage.
2. Configure sample illumination.

3. Click the **Video** button in the software to select video mode.
4. Bring the sample into focus and collect a mosaic.
5. Use Live spectrum mode to acquire spectra at several locations of the sample in order to optimize the Laser power, Exposure time and Number of scans settings.

   Make sure Aperture is set to one of the "confocal pinhole" options.
6. Click **Image Regions** to select image regions mode.
7. Select the **Manual Regions** tool and draw a region on the mosaic.
8. Click the **Region Queue** tool.
9. In the Region Queue settings, select **Duplicate.**
10. Enter a value for the number of duplicates along the Z-Axis.
11. Enter a value for the spacing in micrometers and click **Duplicate**.
12. Click **Collect Regions.**

When collection is complete, you can view the data in 3D with the OMNICxi 3D Analysis Window. See 3D Analysis Window General Features and Controls for details.

View 3D Data

The 3D Analysis window includes many features and controls for viewing 3D data.

❖ **To view 3D data in the 3D Analysis window**

1. With your 3d data open in the analysis window, right-click the image and select **Analyze Region in 3D.**

   The 3D volume opens in the 3D Analysis window. Changes made in the Analysis window are reflected in the 3D Analysis window, so it is recommended that you view them simultaneously on two separate monitors.

Verify Instrument Performance

Performance Verification (PV) runs a set of predefined tests to verify instrument operation. If you have multiple laser sets—each set includes a matching laser, filter and grating—PV can be repeated to verify operation with each set.

The PV status icon in the Acquisition window shows you the status for the currently installed laser set.

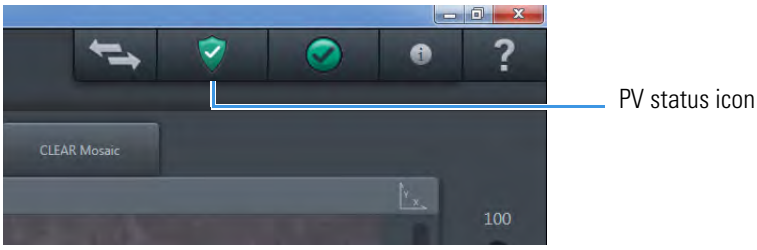


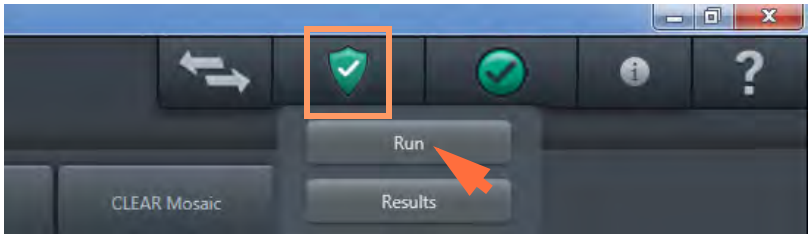
Table 39. PV status, available options

Icon	Meaning
	PV information for current laser set is valid (all tests passed) and up-to-date
	PV information for current laser set is invalid (one or more tests failed during last PV run)
	PV information for current laser set is either out-of-date or this laser set has never been validated
	No PV tests available for current laser set **Note**: Only full range gratings can be verified. PV is also unavailable for the fiber probe.

Click the PV status icon to access features for running PV tests and viewing PV test results.

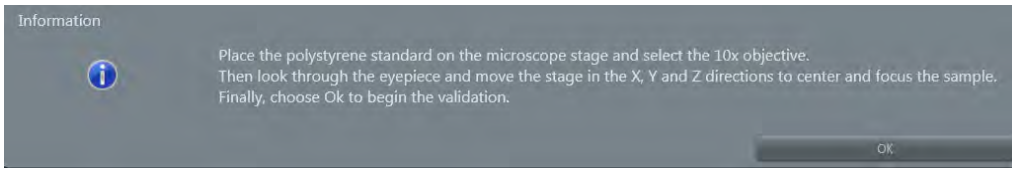
❖ To run performance verification

1. Set up the microscope:
   - Make sure the correct laser, filter and grating are installed in the instrument
   - Set up the microscope for reflected light brightfield illumination (see the *DXRxi Quick Reference Guide* for details)
   - Set the view path control to its middle position
   - Make sure the single slide sample holder is installed on the microscope stage
2. Click the PV status icon and choose **Run**.



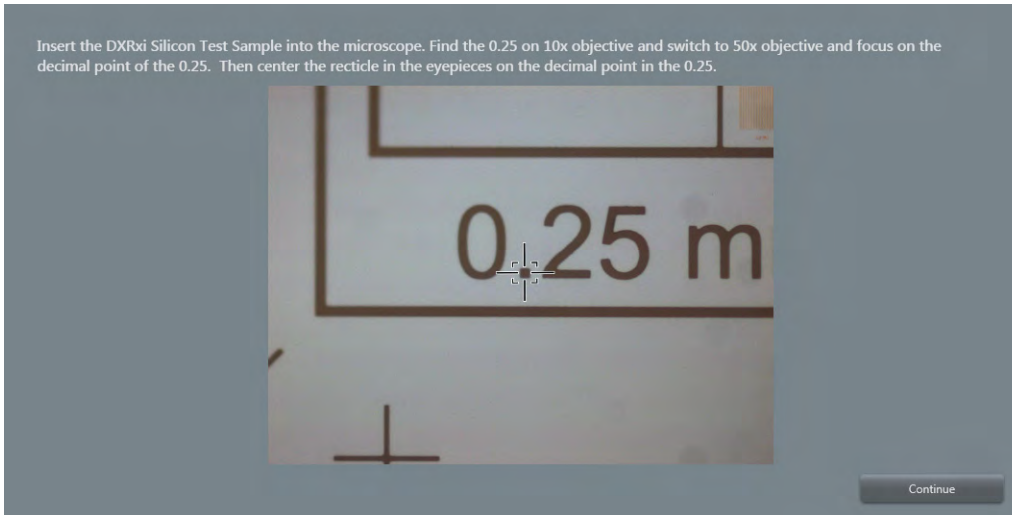
Note The Run button is disabled if no PV tests are available for the current laser set.

A message similar to the following is displayed:



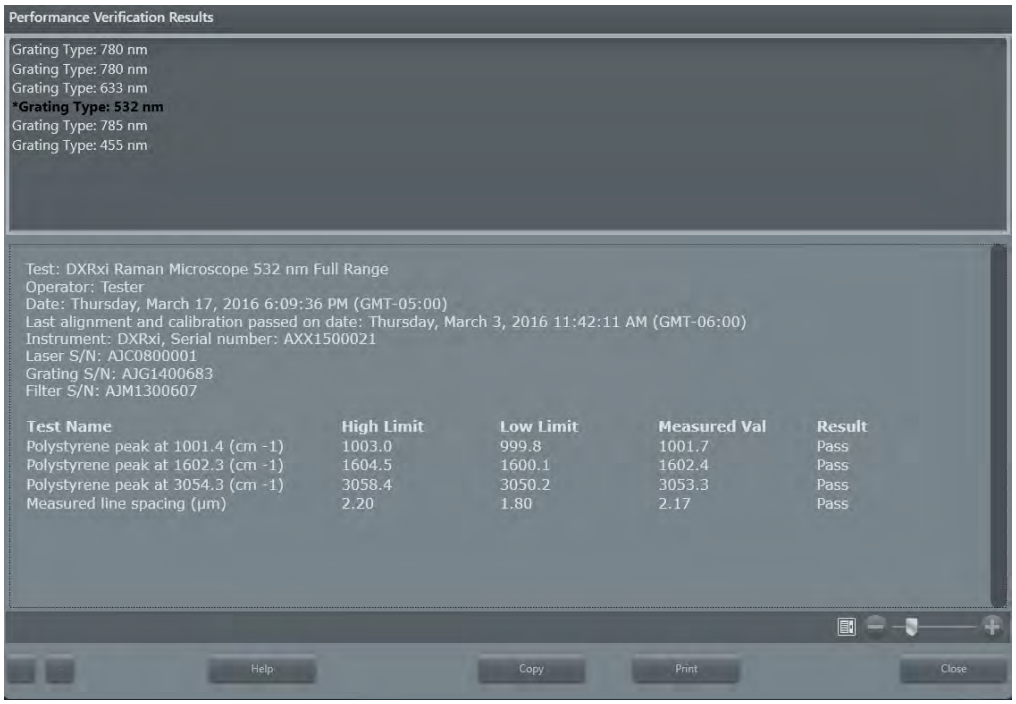
3. Place the polystyrene sample provided with the instrument on the slide holder.
4. Rotate the 10x objective into the front objective position. (The software automatically selects the video calibration for the 10x objective in the Acquisition window.)
5. Use the joystick on the microscope to position the polystyrene sample under the objective, and the focus control to focus the image.
6. Choose **OK** in the software to start the wavelength accuracy test, which collects a spectrum and compares measured peak locations to known values in wavenumbers.

   When the test is completed, a message similar to the following is displayed:



7. Place the DXRxi silicon test sample provided with the instrument on the slide holder.
8. Locate and focus the sample:
   - Use the 10x objective to locate and focus an area of the silicon test sample that matches the image shown above
   - Switch to the 50x objective
   - Focus the image and center the crosshair on the decimal point in the “0.25” text on the test sample
9. Click **Continue** in the software to start the image resolution test, which collects a map (2 scans) of a specific region of the sample and compares measured line spacing values to known values in micrometers.

   When the test is completed, the PV results are displayed. Here is an example:



If all the test results show "Passed" as in the example above, the instrument passed the PV test. If one or more tests show a "fail" result, refer to the PV troubleshooting information for help.

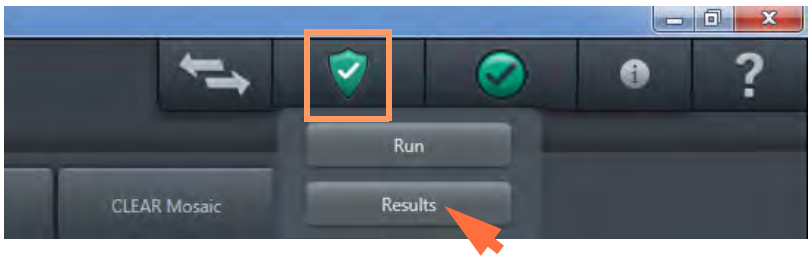
10. When you are finished viewing the results, click **Close** to close the window.

    The software automatically saves the measured PV data (polystyrene spectrum and image map) in this location:

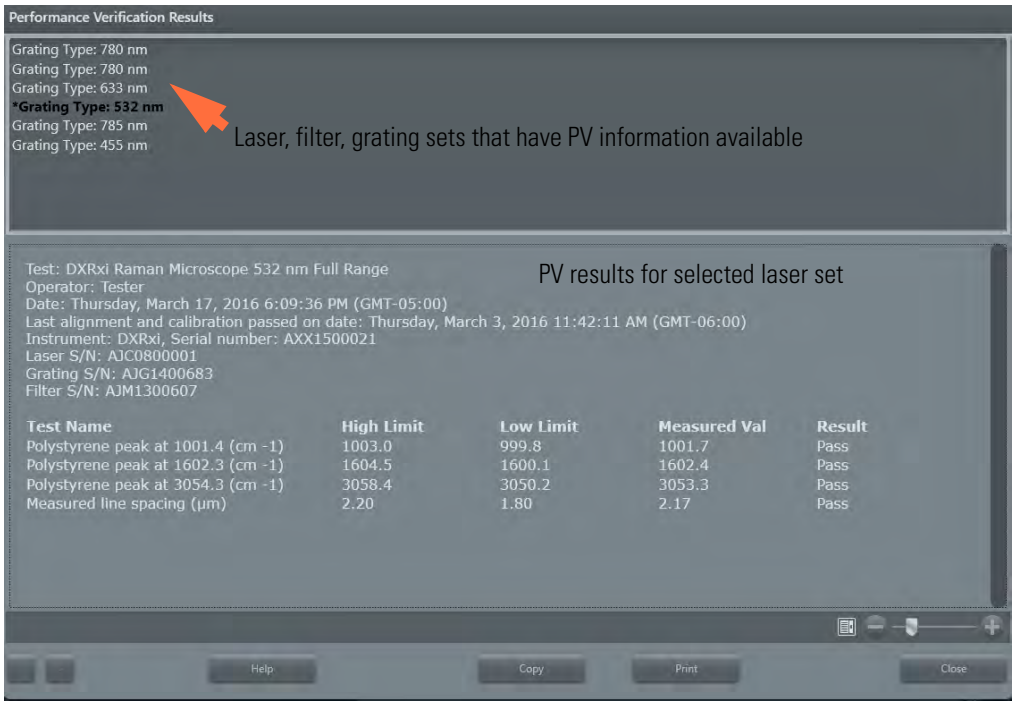
    Thermo/OMNICxi/Spectra/PV Data

❖ To view PV test results

1. Click the PV status icon and choose **Results**.



A window opens with results from the most recent PV test for the installed laser set. Here is an example:



All known laser sets for this instrument are listed in the top pane. An asterisk (*) appears to the left of the installed laser set. The selected laser set appears in bold.

To show the most recent PV test results for a different laser set, select the laser set in the top pane. The results appear in the bottom pane.

The following additional features are available in the PV results window.

Table 40. PV results window, available features

Feature	Description
Print	Prints a report of the displayed PV results, including instrument ID and status, detailed PV test results and a section for signatures (operator and reviewer)
Copy	Copies a report of the displayed PV results to the Windows Clipboard
Help	Displays a Help window with PV information
< >	Displays previous (or next) PV results, if available, for the laser set (laser, filter grating) selected in the top pane

System Tools

Use these tools to align and calibrate a laser set (laser, filter, and grating) or to create or edit the video calibrations for your microscope objectives.

Contents

- Aligning and Calibrating Laser Sets
- Creating a New Video Calibration

Aligning and Calibrating Laser Sets

Use the Align/Calibrate feature in the OMNICxi software to align the installed laser, filter and grating and to calibrate the laser wavelength. If you have multiple lasers, gratings and filters, each laser, grating, and filter set must have associated alignment and calibration data. Once the alignment and calibration data are created, you can interchange your laser sets without recalibrating them.

Recalibrate all of your laser sets in the following situations:

- It has been more than one week since the laser sets were aligned and calibrated. We recommend aligning and calibrating once a week.
- You have just moved the instrument.
- You see an error message related to the detector signal intensity (no signal or signal too low) or spectral quality (peaks too broad, peaks in wrong position, artifacts in the spectrum), your spectral search match numbers are lower than expected or the performance check failed.

To ensure the accuracy of your data, OMNICxi software performs automatic x-axis calibration every 30 minutes to account for any changes in the temperature of the environment around your instrument.

A notification alerts you when the x axis is being calibrated, and calibration typically takes about 1 minute to complete. When calibration is complete, the software resumes any task that was interrupted.

You can turn automatic calibration on or off in the Instrument Settings view.

The instrument should be powered up and running for at least one hour before you align and calibrate a laser set. OMNICxi software should be running and the status indicator in the upper right corner should show a green check mark. Wait at least 5 minutes after changing the installed grating before you align and calibrate a laser set.

Note If the currently installed laser set does not have associated alignment and calibration data, the status indicator will show a red "X." If you see a red X that indicates the current laser set is uncalibrated, follow the steps below to create alignment and calibration data for this laser, grating, and filter set.

Tools needed:

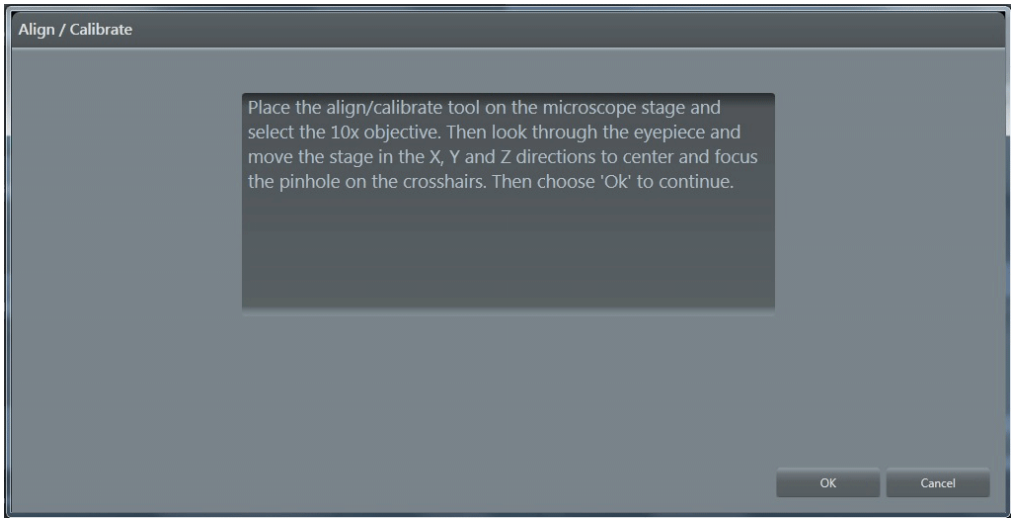
- Align/calibrate tool (normally stored in the holder attached to the left side wall inside the microscope sample compartment and left connected to the instrument)

❖ To align and calibrate the current laser, grating and filter set

1. Open the sample compartment doors.
2. Make sure the standard (single slide) sample holder is installed on the microscope stage.
3. Install the alignment and calibration tool on the single slide sample holder.

   Make sure the tool is in the detent, level and secured by the stage clip and both connectors are secured.
4. Rotate the 10x objective into the front objective position so it engages the detent. (Not necessary to select the objective in OMNICxi software for this procedure.)
5. From the OMNICxi Acquisition window, click **Settings** and then click ALIGN/ CALIBRATE.

   A message appears.



6. Use the stage controller to move the stage so that the alignment tool is under the white light from the illuminator.

Turn up the brightness as far as it will go. When you look at the alignment tool directly (not through the eyepiece), you should see a pool of white light from the illuminator on the surface of the tool.

Continue looking at the alignment tool directly while using the joystick on the stage controller to roughly position the opening in the surface of the alignment tool under the white light.

Tip Hold down the button to the left of the joystick to speed up the X- and Y-axis stage movement.

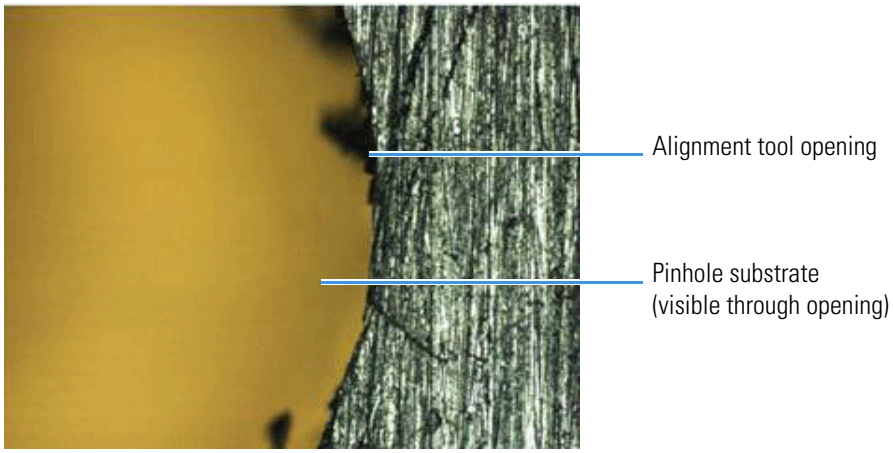
Look through the eyepiece and adjust the brightness to eliminate all glare.

Continue looking through the eyepiece while using the focus knob on the stage controller to focus on the metal surface of the alignment tool (clockwise knob rotation moves the stage up).

Tip Hold down the button to the right of the joystick to speed up the Z-axis stage movement.

You should see the edge of the opening in the alignment tool through the eyepiece. The pinhole is located on a substrate mounted inside the alignment tool. The substrate is visible through the opening as shown below.

Figure 94. Alignment tool opening (pinhole substrate is visible through opening)



If you don't see the opening in the alignment tool through the eyepiece, use the joystick to adjust the X or Y position of the stage until the opening comes into view.

7. Focus on the substrate.

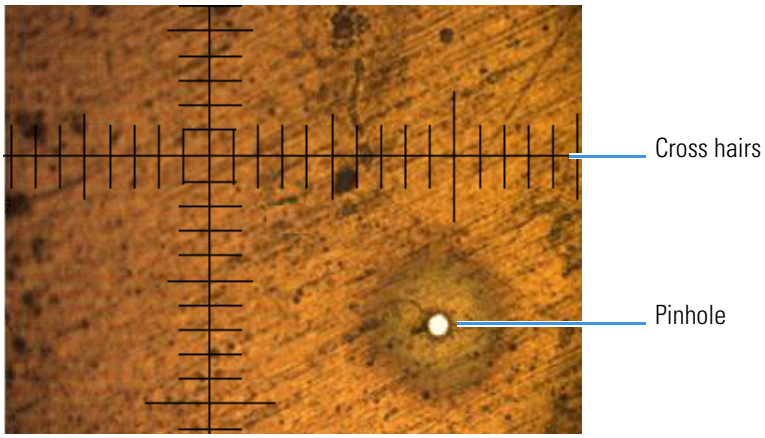
   Use the focus control to bring the substrate into focus, adjusting the brightness to optimize the image contrast. You should see the texture of the substrate; the presence of dark spots and streaks is normal. You may or may not see the pinhole at this point.

8. Locate the pinhole in the substrate.

   Turn the brightness control most of the way down (the pinhole is backlit). Adjust the X and Y position of the stage until the substrate fills the field of view. Then look for the pinhole. (It is not necessarily in the center of the opening in the alignment tool.)

   Use the focus control to bring the pinhole into sharp focus, adjusting the brightness as needed to optimize the image contrast.

Figure 95. Backlit pinhole (in focus but not centered)

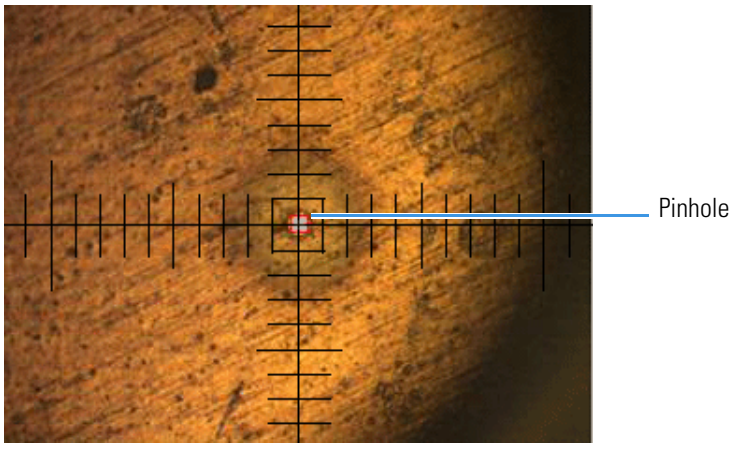


9. Position the focused pinhole exactly in the cross hairs of the microscope eyepiece.

   Look through the eyepiece on the microscope and adjust the diopter ring on the right lens if necessary to straighten the cross hairs.

   Adjust the X and Y position of the stage until the pinhole is **exactly** under the box in the center of the cross hairs.

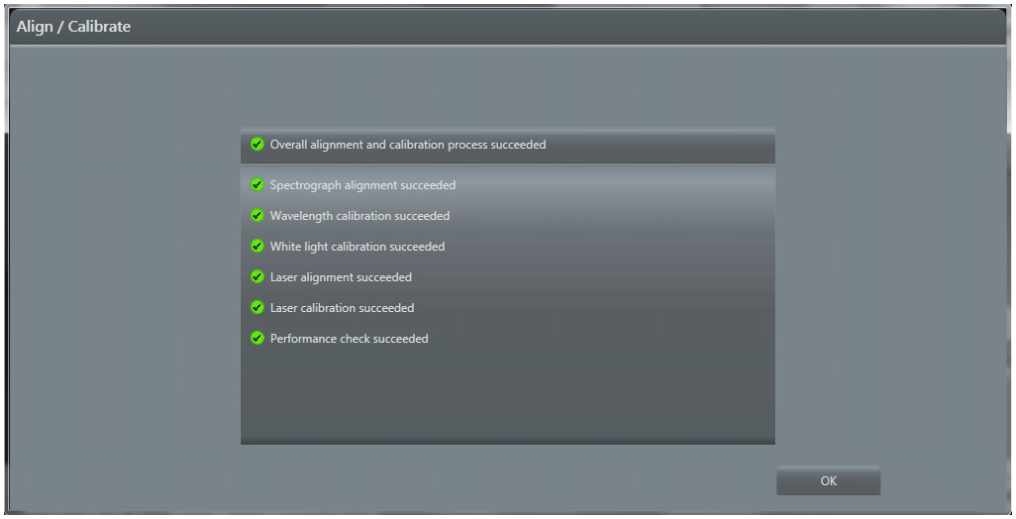
Figure 96. Pinhole in focus and in position



10. When the pinhole is in focus and perfectly centered, close the sample compartment doors and then click **OK** in the software to start the align/calibrate process.

    The software aligns the spectrograph to maximize the signal, calibrates the laser wavelength and then checks system performance. Depending on the type of laser installed, it may take up to 30 minutes to complete. When the process is completed, the align/calibrate results are displayed. Here is an example.

    Figure 97. Align/calibrate results (passed)

    If the performance check passed, a green check mark appears next to each result (see above). If the performance check fails, a yellow triangle or a red "X" appears instead. One of the following conditions may apply:

    - Grating door is open (close the grating door)
    - Grating shutter is closed (open the shutter)
    - Grating is not fully seated (Remove the grating, wipe its bottom surface with a clean, lint-free cloth and then install the grating.)

    Check each of these items and then restart Align/Calibrate. If that doesn't fix the problem, cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software and run Align/Calibrate. If the problem persists, contact technical support.

11. If the performance check passed, gently remove the align/calibrate tool from the microscope stage and place the tool in the holder attached to the left side wall inside the microscope sample compartment.

    You can leave the cable attached.

Creating a New Video Calibration

The video image in OMNICxi software is directly related to the stage position and the magnification of the selected objective. When you change the objective on the microscope, the dimensions of the video system's field of view change. Calibrating the video image defines the new size, in micrometers, of the video system's field of view so the image data accurately describes the sample.

OMNICxi software provides a way to create and save video calibrations for your different objectives. This lets you retrieve the appropriate calibration when you change the objective, rather than having to recalibrate the video image. To retrieve a video calibration for an objective that was previously calibrated, use the **Objective** list box in the OMNICxi Acquisition window. If you have a new objective that has never been calibrated, follow these steps to create and save a calibration for that objective.

After the calibration data has been created for each of your objectives, recalibrate in the following situations:

- After reinstalling a single objective, recalibrate that objective
- After reinstalling the nosepiece on the microscope, recalibrate all the objectives
- After moving the instrument, recalibrate all the objectives

This section explains how to create a new video calibration and work with existing calibrations.

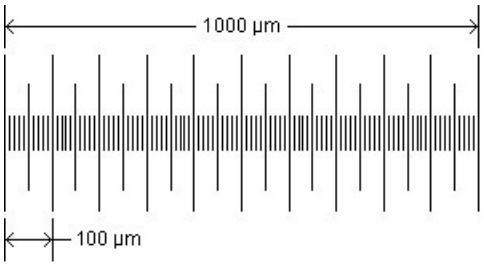
Tools needed:

- Objective that requires a video calibration
- Video calibration slide (provided with the system)

Figure 98. Video calibration slide example

The slide has a scale in the center that can be used to calibrate the video image. The image below shows the distance between major division lines. The distance between the minor division lines is 10 micrometers.

Figure 99. Calibration slide micrometer scale



The instrument should be powered up and running for at least one hour before you create or update a video calibration. OMNICxi software should be running and the status indicator in the upper right corner should show a green check mark.

❖ To create a new video calibration or update an existing one

1. Open the sample compartment doors.
2. Make sure the standard (single slide) sample holder is installed on the microscope stage.
3. Install the new objective on the microscope nosepiece.
4. Rotate the **10x objective** to the front objective position.

   We will use this low magnification objective to bring the scale on the calibration slide into focus.
5. Install the video calibration slide on the single slide holder.

   Make sure the calibration slide is level and secured by the stage clip.

6. From the OMNICxi Acquisition window, click **Settings** and then click SETUP Video.

   The Setup Video window is displayed with an area for the video image and features for calibrating it.

   Figure 100. Setup Video window

7. In the Setup Video window, set **Current calibrations** to the correct calibration for the 10x objective.

   Note If you are calibrating the 10x objective for the first time, skip this step.

8. Position the calibration slide under the white light from the illuminator.

   Turn up the brightness to optimize the contrast. When you look at the calibration slide directly (not through the eyepiece), you should see a pool of white light from the illuminator on the surface of the slide.

   Continue looking at the calibration slide directly while using the joystick and focus control on the stage controller to roughly center the large circle in the middle of the slide under the white light.

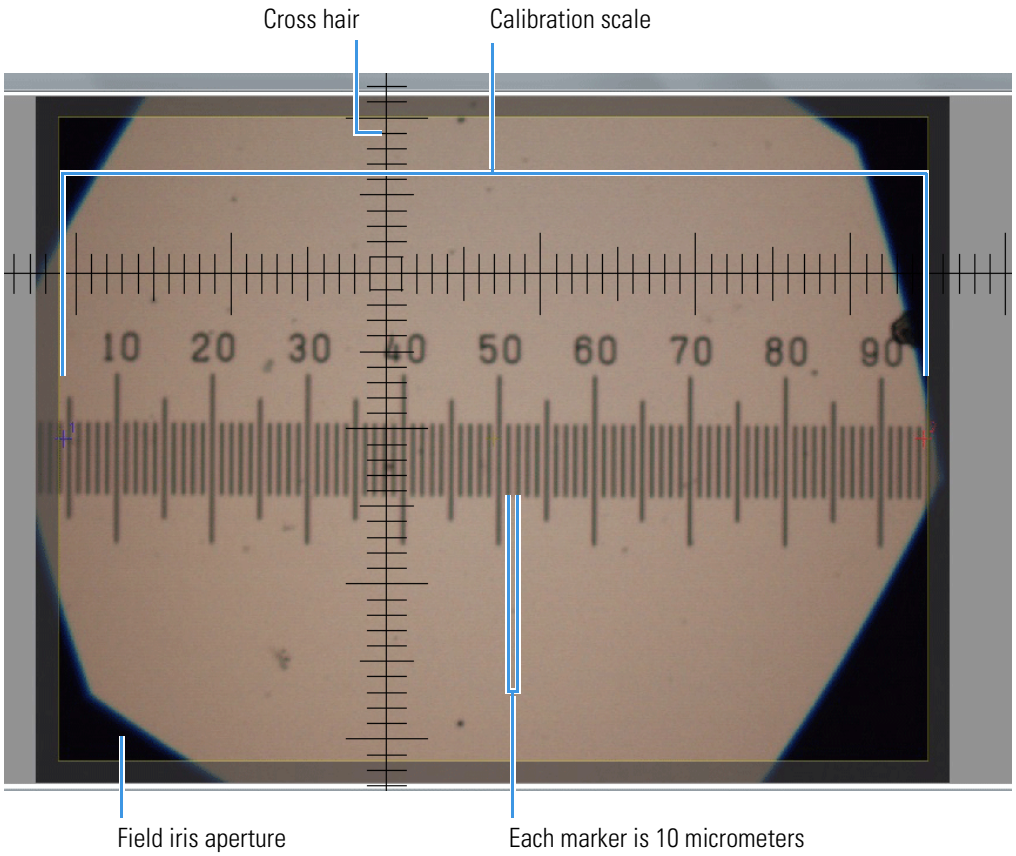
   Tip Hold down the button to the left of the joystick or focus control to speed up the respective control.

9. Bring the scale in the center of the calibration slide into focus.

   Close the sample compartment doors.

   If you have the optional reflected light brightfield/darkfield illuminator, pull out the field iris (FS) control. Then look through the eyepiece while using the focus knob on the stage controller to bring the edge of the field iris aperture into sharp focus (clockwise knob rotation moves the stage up). Bringing the aperture into focus also focuses on the calibration slide.

   Figure 101. Calibration scale in focus at 10x magnification

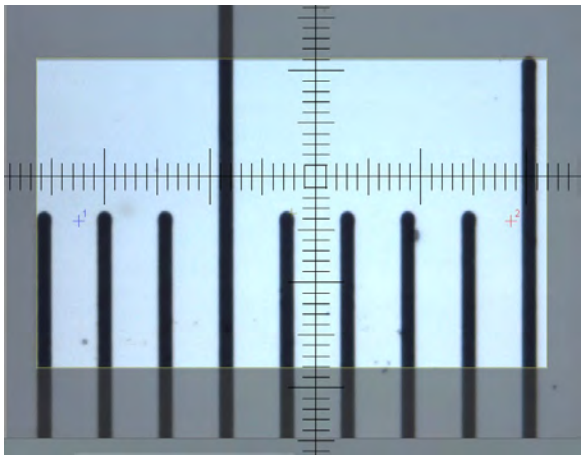


   When the aperture is in focus, push in the field iris control to widen the field of view.

   Note If you have the brightfield only reflected light illuminator (which doesn't have a field iris control), bring the scale in the center of the calibration slide (shown in the image above) into focus instead of the field iris aperture.

10. Rotate the objective you are calibrating (or recalibrating) to the front objective position and then look through the eyepiece while you use the fine focus control to bring the scale (or a portion of it) into sharp focus. Here is the calibration slide in focus using a 100x objective.

Figure 102. Calibration slide in focus using 100x objective



11. If you are creating a new calibration for this objective, click NEW Calibration in the Setup Video window.

Note If you are updating an existing calibration, skip this step and use the **Current calibrations** list box to select the calibration to update.

Enter a name for the new objective in the **Objective name** box. We suggest using the magnification of the objective followed by its numerical aperture (for example, 10x/0.25) or the model name. Objectives are typically labeled with this information. After you complete the calibration, this name will appear under **Current calibrations** in the Setup Video window, and in the **Objective** list box in the OMNICxi Acquisition window.

Enter a more detailed description of the calibration in the **Objective description** box (especially if there are two objectives with the same magnification and numerical aperture).

Set **Magnification** to match the magnification of the objective you are calibrating.

12. Line up the scale on the calibration slide with the cross hairs in the microscope eyepiece.

    Look through the right eyepiece on the microscope and use the joystick to move the stage.

    Line up one of the vertical division lines on the calibration slide with the vertical cross hair in the eyepiece. Use the diopter ring on the right lens if necessary to straighten the cross hairs. The horizontal cross hair should be just above the shortest division lines so you can see it clearly.

    Figure 103. Calibration scale properly aligned with cross hairs for 10x objective

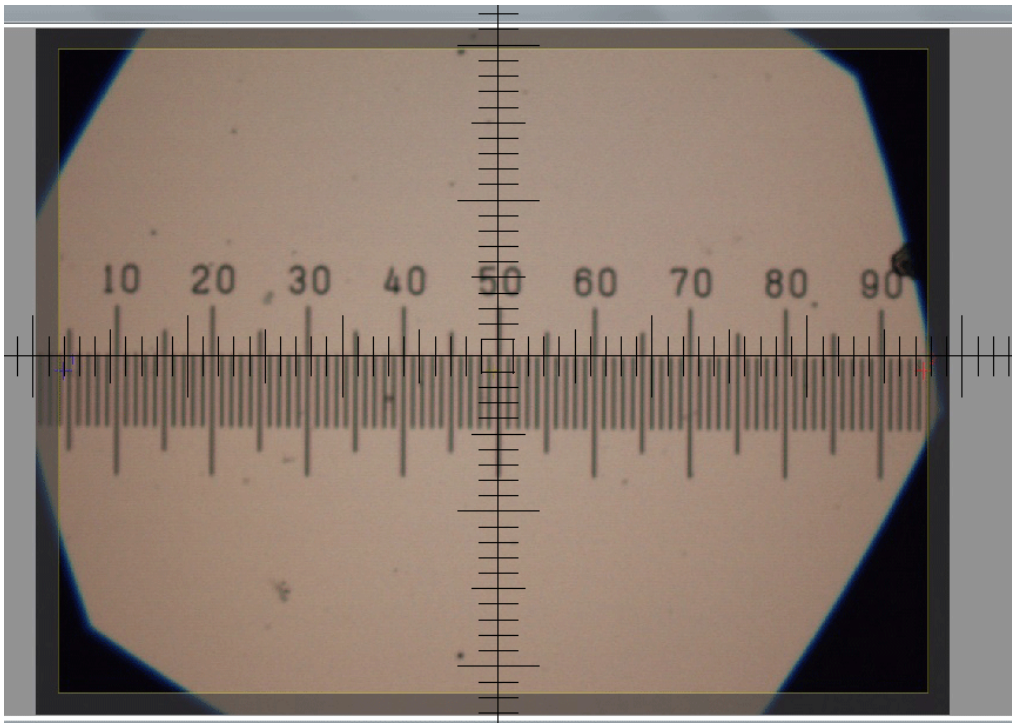
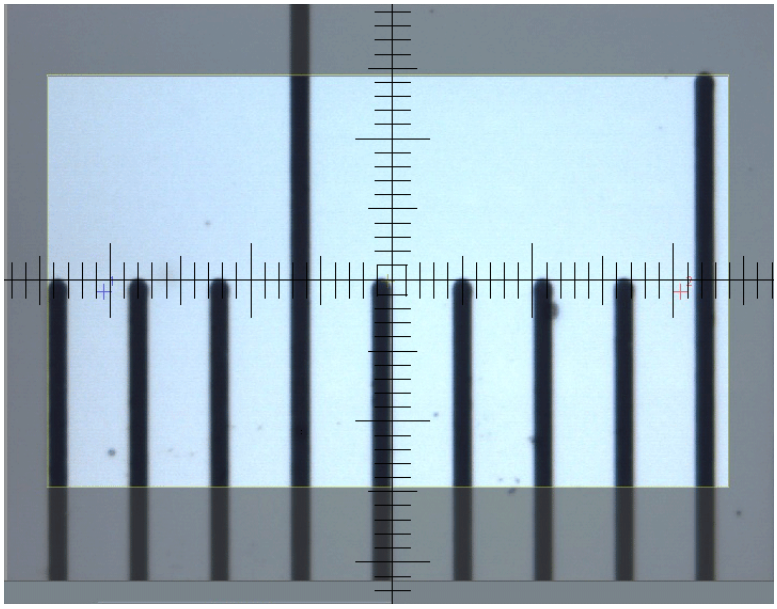


Figure 104. Calibration scale properly aligned with cross hairs for 100x objective



Tip It can be helpful to place the vertical cross hair at the left or right edge of the division line on the calibration slide, especially for high magnification objectives (see the image above).

13. Maximize the Setup Video window to enlarge the video pane.

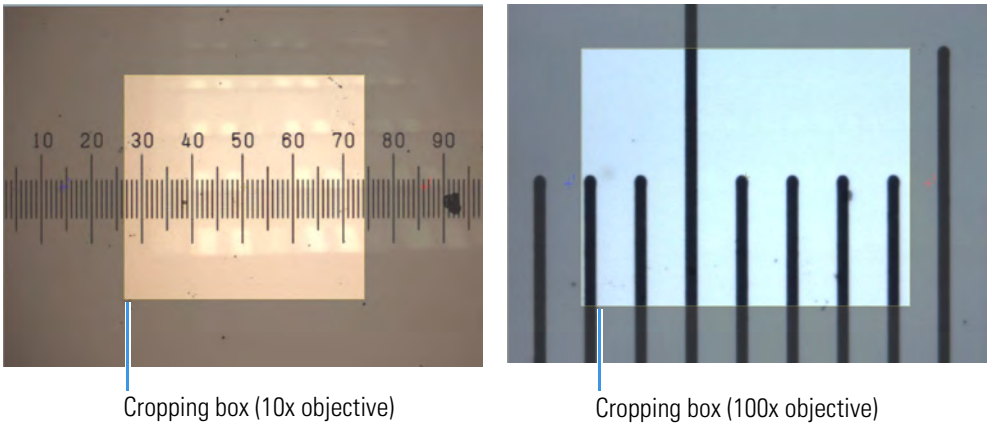
    Turn down the brightness until a video image appears in the video pane.

14. Adjust the edges of the light colored rectangular area in the center of the video image so the area covers about half the screen.

    We will refer to this light colored area as the “cropping” box. It contains a centering marker and two distance markers that are used to calibrate the video image.

    To adjust the cropping box, grab an edge.

    Figure 105. Cropping box adjusted to cover half of available area for two different objectives



15. Use the mouse to place the centering (yellow) marker in the video image at exactly the point where the cross hairs in the right eyepiece meet.

    Figure 106. Centering marker correctly positioned for 10x objective

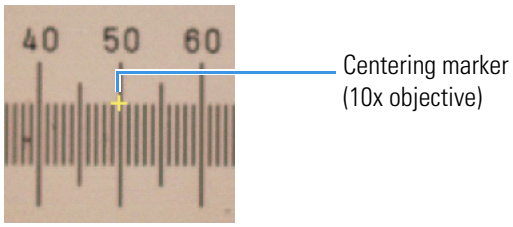
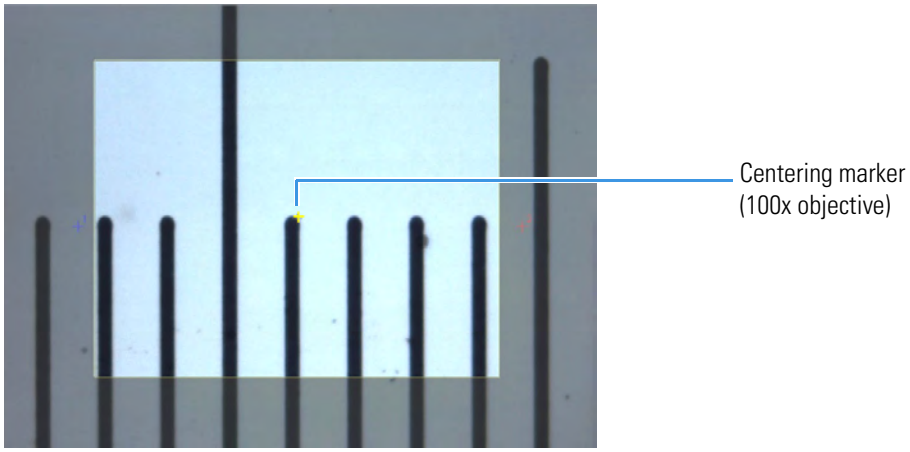


    Figure 107. Centering marker correctly positioned for 100x objective

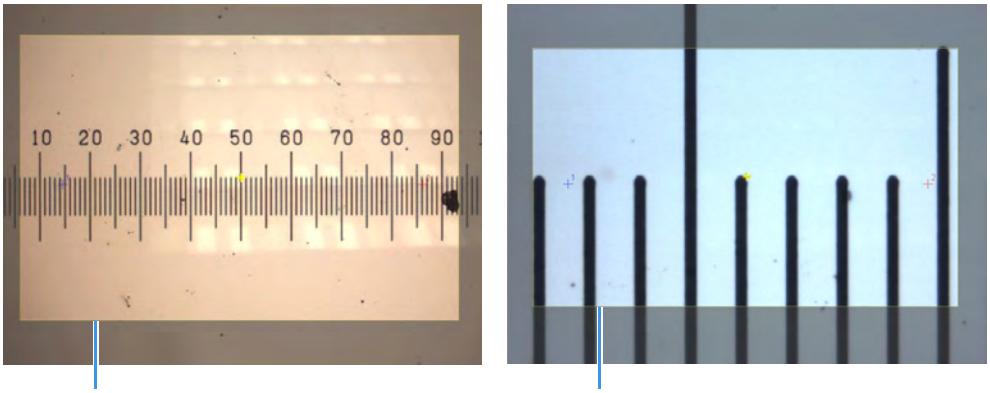


16. Use the cropping box to define the size of the video image in the OMNICxi Acquisition window.

    Expand the size of the cropping box to the full extent of the screen, and then bring the edges in to eliminate areas where the illumination is not ideal. Only this area will appear in the video pane of the OMNICxi Acquisition window and be used for capturing video images. By adjusting the size, shape and location of the cropping box, you can eliminate distortions and uneven illumination that can occur around the perimeter of a video image.

    To adjust the size and shape of the cropping box, drag its border. To move the cropping box, drag its center to the desired location.

    Figure 108. Cropping box correctly sized and positioned for two different objectives



Final cropping box (10x objective)

Final cropping box (100x objective)

17. Drag the distance markers, labeled “1” and “2” in the video image, to equidistant division lines to the right and left of the center marker.

    The distance markers must be on the same horizontal line as the centering marker, and they must be positioned within the cropping box.

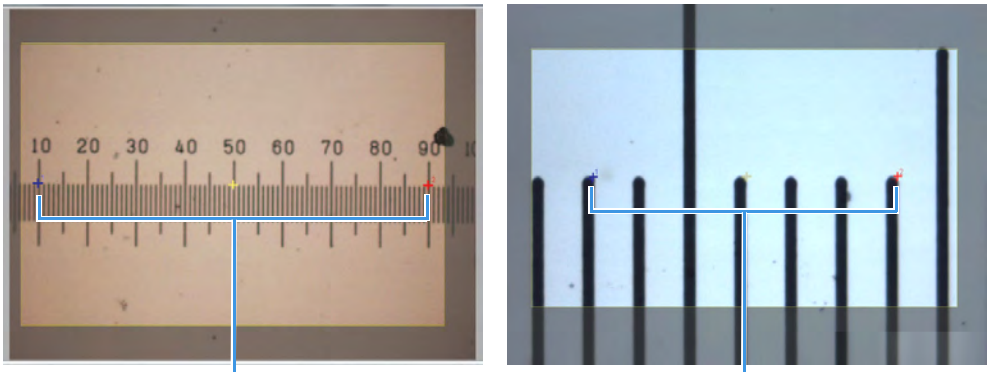
    For best accuracy, use division lines that are far apart.

    You can use the Left and Right buttons and the arrows in the **Adjust marker positions** box to more finely adjust the position of the selected distance marker.

Here are some examples showing the calibration markers correctly placed for two different objectives.

Tip If you lined up the vertical cross hair at the left or right edge of a division line, place the other two markers at the same edge of their respective division lines for greater accuracy. This is especially important for high magnification objectives.

Figure 109. Distance markers correctly placed for two different objectives



18. Set **Distance between markers** in the Setup Video window to the distance between the markers in micrometers. For the examples above, this would be 800 micrometers for the 10x objective and 60 micrometers for the 100x objective.
19. Click **Save** to save the calibration data for this objective.
20. If you have another objective to calibrate, repeat steps 10 through 20.

    If you are finished calibrating objectives, close the Video Setup window and the Settings window and wrap the calibration slide in lens paper and place it in the provided box.

Tip To check a new calibration, select **Video mode** in the OMNICxi Acquisition window and collect a mosaic. Then use the Grab tool to click a location in the video image. If that location moves to the center of the video pane, the video image has been calibrated correctly.

❖ To edit an existing video calibration

1. Rotate the objective you are recalibrating to the front objective position.
2. From the OMNICxi Acquisition window, click **Settings** and then click SETUP Video.
3. In the Setup Video window, set **Current calibration** to the calibration you want to edit.
4. Adjust the calibration as desired.

   If you just want to edit typed information, complete your edits and then proceed to the next step.

If you want to move the markers or adjust the size, shape and location of the cropping box to change the video image in OMNICxi software, follow the steps to reset the box or move markers in Creating a New Video Calibration.

5. Click **Save**.

❖ To delete a saved calibration

1. From the OMNICxi Acquisition window, click **Settings** and then click

.
2. In the Setup Video window, set **Current calibration** to the calibration you want to delete.
3. Click **Delete**.

   A message asks you to confirm the deletion.
4. Click **Yes**.

4

Troubleshooting

- Problems and Solutions
- Error Messages

Problems and Solutions

Performance Verification fails

- If a PV wavelength accuracy test failed (peak location is outside the specified limits), use OMNIC software to open the spectral (.SPA) file from the most recent PV test. The most recent PV test files (polystyrene spectrum and image map) are located here:

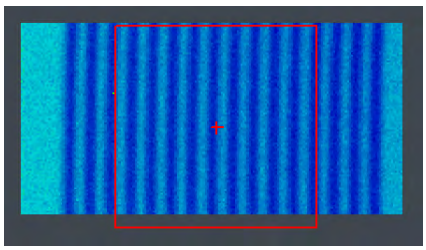
  Thermo/OMNICxi/Spectra/PV Data

  – If the spectrum contains excessive instrument noise (spikes), the polystyrene sample may have been out of focus. Rerun the PV test making sure the focus is correct.

  – If a wavelength accuracy test fails again, align and calibrate the installed laser set and then rerun the PV test

  If a wavelength accuracy test fails again, contact us.

- If the PV image resolution test failed (measured line spacing is outside the specified limits), open the image map from the most recent PV test (see location above). The map should contain vertical lines as in this example:



  If there are no vertical lines in the collected map, check the following and then rerun the PV test:

  – Make sure the silicon test sample is right side up (the wording on the sample should be right side up when you are looking at the sample through the microscope)

- Make sure the 50x objective is fully rotated into the front objective position
- Make sure the correct video calibration for your 50x objective is selected in the OMNICxi Acquisition window so the scales are synchronized.
- Make sure the sample is in sharp focus
- Make sure the crosshair in the microscope eyepiece is centered on the period in the "0.25" feature on the test sample

If the image resolution test fails again, contact us.

Sample Bands are too Small

The Raman signal is too small. You can increase it by doing one or more of the following:

- Increase the setting of exposure time in the software
- Increase the aperture size by setting in the software
- Select a slit instead of a pinhole aperture
- Increase the setting of laser power in the software

If this problem persists, contact our sales or service representative in your area.

Sample Bands Are Too Large or CCD Overflowed

The Raman signal is too large, which saturates the CCD. You can decrease the signal by doing one or more of the following:

- Decrease the setting of exposure time in the software
- Decrease the aperture size by setting in the software
- Select a pinhole aperture instead of a slit aperture
- Decrease the setting of laser power in the software
- Make sure all doors of the system are tightly closed

If this problem persists, contact our sales or service representative in your area.

No Raman Signal Appears in the Live Display or Spectrum Has No Sample Bands

- Make sure all doors of the system are tightly closed
- If the laser power is off, turn on the laser
- Increase the laser power in the software

- Make sure the sample is directly under the objective and in focus
- Make sure the shutter door on the grating is open
- The sample may not be Raman active; test the system by using a known good sample

If this problem persists, contact our sales or service representative in your area.

No Visible Image Appears in the Video Pane or Viewer

- If the illuminator is set to low intensity, set it to higher intensity
- Change the software to video mode
- Make sure the viewer selection control (below the video camera) is in the middle position
- Push the ND, FS and AS controls on the illuminator all the way in, if present

If this problem persists, contact our sales or service representative in your area.

The Spectrum Has a Sloping Background or a Severe Positive Offset

The sample may be heating. To account for this condition try one or more of the following:

- Use a different laser (shorter wavelengths heat samples less)
- If the sample looks burned, replace it or move it so the laser will strike a fresh area
- Reduce the laser power and collect the sample again

  Watch the live display to see if the sample is still heating. (Reducing the laser power reduces the Raman signal generated at the sample and therefore the quality of the data. To compensate, try increasing the number of scans or the exposure time.)
- Reduce the exposure time and collect the sample again
- Turn on baseline correction in the settings panel

If this problem persists, contact our sales or service representative in your area.

The Spectrum Baseline Is Severely Tilted, Curved or Saturating

The sample is fluorescing during data collection. To correct this problem, try one or more of the following:

- Turn on baseline correction in the settings panel
- Use a longer wavelength laser
- Go to live spectrum mode and expose the sample to the laser beam for a period of time before beginning the collection of the sample spectrum.

This is known as “photobleaching.” Photobleaching time can be automatically added to an image collection on the advanced tab of the acquisition settings.

- Make sure the slide, coverslip, or other sample properties are not responsible for the fluorescence

If this problem persists, contact our sales or service representative in your area.

The Video Image is Obscured or Distorted

- Check the selected objective is in the right position (not eight position), by rotating nose piece and make sure the objective click and centered
- Push the ND, FS and AS controls on the illuminator all the way in, if present
- Make sure the viewer selection control (below the video camera) is in the middle position
- If the objective is dirty or contaminated, clean it (see Cleaning Your Instrument)

If this problem persists, contact our sales or service representative in your area.

The Computer Cannot Communicate with the Instrument

- If the front panel indicators are not on, make sure the instrument is plugged in and the power strip is on
- If the instrument is turned off, turn it on by holding down the blue button on the front of the instrument for five (5) seconds
- Check the cables between the computer and the instrument

  If they are disconnected, connect them and restart the software.
- Check the cable between the stage and the computer

  If the stage is not responding, turn the stage power supply off and on again to regain the control.

If this problem persists, contact our sales or service representative in your area.

Chemical Image Appears to be Different Than the Video Image

- Check that the objective selected in the software matches up the actual objective focused on the sample
- Check that the video setup has the correct setting and calibration for that objective
- Switch to video mode, make sure the video image is centered on the area of interest, and collect a mosaic to confirm the proper stitching.

Chemical Image Does Not Achieve Required Spatial Resolution

- Check if the correct objective is selected and in position for the spacial resolution selected
- Switch to video mode and make sure the sample is in focus
- Check the sample slide to make sure it is properly seated
- Check the aperture setting, make sure 25 micro pinhole was selected for high spatial resolution imaging
- Lower the image pixel size setting in the software
- Switch to a shorter wavelength laser

  Spatial resolution depends on the wavelength of the laser.

Chemical Image Shows Missing Data

- Increase the exposure time in the software
- Increase the number of scans in the software
- Reduce vibrations in the environment
- Use a vibration isolation table

  Make sure that the isolation is active (for example, there is air pressure to the table)

X-Axis Calibration Fails

The neon bulb used for automatic x-axis calibration may have failed.

- Turn off automatic x-axis calibration and run manual calibration. Contact our service representative in your area for repair of the automatic x-axis calibration feature.

Error Messages

Contents

- General Errors
- Align and Calibration Errors

General Errors

- Instrument off or disconnected
- Video camera disconnected
- Stage controller off or disconnected
- Stage controller failed
- Unknown stage location
- Detector too warm
- Detector disconnected
- No filter
- No grating
- No laser
- Mismatched grating
- Mismatched filter
- Invalid fiber probe configuration
- Uncalibrated laser, grating, filter set
- Alignment/calibration out of date
- Stage firmware out of date
- Sample door open
- Laser off
- Laser warming up
- Sample signal too high
- Cannot read instrument EEPROM
- No communication
- No communication

Instrument off or disconnected

The instrument is powered off or a cable is disconnected. Check the following:

- Both instrument USB cables are securely connected to the instrument and to USB 2.0 connectors on the system computer
- Instrument power supply is connected to a power source and powered on
- Instrument power strip is connected to a working outlet that meets the instrument's electrical specifications and the strip is powered on (Refer to the instrument site and safety information guide for electrical specifications.)
- Power LED on the instrument front panel is lit (hold down 3 seconds to power up)

If this message appears and the instrument is powered on and the USB cables are connected to the computer, cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software. If that doesn't fix the problem, contact technical support.

Video camera disconnected

A video camera cable is disconnected. Check the following:

- Video camera USB cable is securely fastened to the instrument
- Both instrument USB cables are connected to the instrument and to USB 2.0 connectors on the system computer

If the cables are connected and this message still appears, disconnect the video USB cable, wait 5 seconds and then reconnect it to restart the video camera. If this doesn't fix the problem, contact technical support.

Stage controller off or disconnected

The stage controller is powered off or the cable is disconnected. Check the following:

- USB cable for the stage controller is securely connected to the computer
- Stage controller is plugged into the instrument power strip
- Stage controller is powered on

If the stage controller is powered on and connected to the computer and this message still appears, contact technical support.

Stage controller failed

The OMNICxi software can detect the sample stage but not control its position. Turn off the stage controller, wait 5 seconds and then turn it back on. Then initialize the sample stage.

NOTICE Before you initialize the sample stage, remove any sample or other items from the stage. (Items left on the stage during initialization could cause damage. Anything that protrudes above the stage may interfere.)

To initialize the stage, from the Acquisition window click **Settings > Initialize Stage**.

If this message appears after the stage is initialized, note the status (color) of the LED indicator on the front of the stage controller and then contact technical support.

Unknown stage location

The OMNICxi software cannot determine the location of the microscope stage. Initialize the stage.

NOTICE Before you initialize the stage, remove any sample or other items from the stage. (Items left on the stage during initialization could cause damage. Anything that protrudes above the stage may interfere.)

To initialize the stage, from the Acquisition window click **Settings > Initialize Stage**.

If this message appears after you initialize the stage, cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software. If that doesn't fix the problem, contact technical support.

Detector too warm

The instrument detector has not cooled to the proper operating temperature. Wait 15 minutes for the detector to cool and then restart data collection.

If this message reappears, check the following:

- Make sure nothing is blocking the vents on the bottom of the instrument (left side)
- Check that the temperature of the room is below the upper limit of the recommended range (below 27 °C or 80 °F).

If this message appears after the detector has had sufficient time to cool and the room temperature is within our specifications, contact technical support.

Detector disconnected

The instrument cannot communicate with the detector. Check the following:

- Both instrument USB cables are securely connected to the instrument and to a USB 2.0 connector on the system computer
- USB cable for the stage controller is securely connected to the computer
- Instrument power supply is connected to a power source and powered on
- Illuminator power supply is connected to a power source and powered on
- Stage controller is connected to a power source and powered on

If this message appears and the instrument is powered on and all three USB cables are connected to the computer, cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software. If that doesn't fix the problem, contact technical support.

No filter

The instrument does not have a filter installed. Install a filter with a wavelength range that is compatible with the installed laser or grating.

No grating

The instrument does not have a grating installed. Install a grating with a wavelength range that is compatible with the installed laser and filter or fiber optic launcher.

No laser

The instrument does not have a laser installed. Install a laser that is compatible with the installed grating and filter or fiber optic launcher.

Mismatched grating

The wavelength range of the grating installed in the instrument does not match the wavelength of the installed laser. Install a grating that matches the laser frequency.

Mismatched filter

The wavelength range of the filter installed in the instrument does not match the wavelength of the installed laser. Install a filter that matches the laser frequency.

Invalid fiber probe configuration

The installed fiber probe is not compatible with the current laser set (laser or grating). Install a compatible laser set.

Uncalibrated laser, grating, filter set

The laser, grating, and filter set installed in the instrument has not been aligned and calibrated. The instrument cannot collect data until the installed laser set (laser, filter, grating) has been aligned and calibrated. For instructions, see Aligning and Calibrating Laser Sets.

Alignment/calibration out of date

The alignment and calibration for the laser, grating, and filter installed in the instrument occurred longer ago than the specified interval. The instrument cannot collect data until the installed laser set (laser, filter, grating) has been aligned and calibrated. For instructions, see Aligning and Calibrating Laser Sets.

Stage firmware out of date

A newer version of firmware for the stage controller is required. Contact technical support.

Sample door open

The instrument cannot collect data because a sample compartment door is open or a laser interlock override is missing (Class 3B instrument configurations only). Close both sample compartment doors (or install the override), wait for the laser to warm up and restart data collection.

Laser off

The instrument cannot collect data because the laser setting is off in the software. From the OMNICxi Acquisition window, open the Settings box, select **Laser On**, close the Settings box and restart data collection.

Laser key off

The instrument cannot collect data because the laser keyswitch is off. Set the laser keyswitch to the On position and restart data collection.

Laser warming up

The instrument may not produce stable results right now because the laser is warming up. Depending on the type of laser installed, allow 1 to 15 minutes for the laser to warm up and then restart data collection.

Sample signal too high

The sample signal is saturating the instrument detector. Change one or more of the following settings and measure the sample again:

- Reduce the laser power
- Reduce the exposure time
- Select an aperture that has a smaller opening

If the problem persists, contact technical support.

Cannot read instrument EEPROM

The instrument EEPROM has incorrect contents or is corrupted. Contact technical support.

No communication

The software is unable to communicate with the instrument or open the shutter that allows the laser to enter the sample compartment. Cycle the power on the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software. If that doesn't fix the problem, contact technical support.

Align and Calibration Errors

- Align/calibrate tool not connected
- Laser output too low
- No laser at sample
- Poor quality align/calibrate spectra
- Spectral response too low
- Spectral response too high
- Performance check value(s) out of range
- Misaligned filter or grating
- Unable to read system info
- Unable to collect spectral data
- Unable to calibrate laser power

Align/calibrate tool not connected

The align/calibrate tool is not connected to the instrument. Before you start the align/calibrate process, install the align/calibrate tool on the microscope stage and plug in the cable.

If this message appears when the align/calibrate tool is installed and connected to the instrument, cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software and run Align/Calibrate. If the problem persists, contact technical support.

Laser output too low

The laser output of the instrument is below the minimum specification. Possible causes:

- Laser is not fully seated. (Remove and reinstall the laser making sure the latch is fully vertical.)
- Filter is not fully seated. (Remove the filter and wipe its bottom surface with a clean, lint-free cloth, wipe the magnet inside the filter compartment and then reinstall the filter.)

If the problem persists, contact technical support.

No laser at sample

The system is unable to open the shutter that allows the laser to enter the sample compartment. One of the following conditions applies:

- A sample compartment door is open. (Close the doors.)
- Laser keyswitch is off. (Set the laser keyswitch to the On position.)
- Laser is off. (Select the **Laser on** option in the Settings box).
- Grating is not fully seated. (Remove the grating, wipe its bottom surface with a clean, lint-free cloth, wipe the magnet inside the grating compartment and then install the grating and secure the compartment door.)
- Grating shutter is closed. (Open the shutter.)
- Filter is not fully seated. (Remove the filter and wipe its bottom surface with a clean, lint-free cloth and then install the filter.)
- Laser is not fully seated. (Remove and reinstall the laser making sure the latch is fully vertical.)

Check each of these items and then restart Align/Calibrate. If that doesn't fix the problem, cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software and run Align/Calibrate. If the problem persists, contact technical support (laser shutter may be broken or its cable has failed).

Poor quality align/calibrate spectra

The align/calibrate process is unable to align or calibrate the instrument due to poor quality spectral data. One of the following conditions applies:

- Align/calibrate tool pinhole was not in focus before Align/Calibrate was started. (Focus the pinhole.)
- Align/calibrate tool is dirty. (Clean the glass window on the tool.)
- Align/calibrate tool is misaligned. (Make sure the tool lays flat and is secured by the stage clip.)
- Align/calibrate tool cable is loose. (Check the cable connection at both ends.)
- Objective is misaligned. (Make sure the 10x objective is rotated into position and engaged in the detent.)
- Sample compartment door is ajar. (Make sure both doors are tightly closed.)
- Grating is not fully seated or the shutter is closed. (Remove the grating, wipe its bottom surface with a clean, lint-free cloth, wipe the magnet inside the grating compartment, open the shutter and then install the grating and latch the grating door.)
- Filter is not fully seated. (Remove the filter, wipe its bottom surface with a clean, lint-free cloth, wipe the magnet inside the filter compartment, and then install the filter.)
- Laser is not fully seated. (Remove and reinstall the laser making sure the latch is fully vertical.)
- If using the fiber optic probe, check that the fiber optic connections to the system are fully seated and not reversed. Ensure the probe is fully seated in the probe alignment tool.

Check each of these items and then restart Align/Calibrate. If that doesn't fix the problem, cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software and run Align/Calibrate. If the problem persists, contact technical support.

Spectral response too low

The spectral response is too low for the align/calibrate process to continue. One of the following conditions applies:

- Align/calibrate tool is dirty. (Clean the glass window on the tool.)
- Align/calibrate tool is misaligned. (Make sure the tool lays flat and is secured by the stage clip.)
- Align/calibrate tool cable is loose. (Check the cable connection at both ends.)
- Objective is misaligned. (Make sure the 10x objective is rotated into position and engaged in the detent.)
- Sample compartment door is ajar. (Make sure both doors are tightly closed.)
- Grating is not fully seated or the shutter is closed. (Remove the grating, wipe its bottom surface with a clean, lint-free cloth, wipe the magnet inside the grating compartment, open the shutter and then install the grating and latch the grating door.)
- Filter is not fully seated. (Remove the filter, wipe its bottom surface with a clean, lint-free cloth, wipe the magnet inside the filter compartment, and then install the filter.)
- Laser is not fully seated. (Remove and reinstall the laser making sure the latch is fully vertical.)

Check each of these items and then restart Align/Calibrate. If that doesn't fix the problem, cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software and run Align/Calibrate. If the problem persists, contact technical support.

Spectral response too high

The spectral response is too high for the align/calibrate process to continue. One of the following conditions applies:

- Align/calibrate tool is dirty. (Clean the glass window on the tool.)
- Align/calibrate tool is misaligned. (Make sure the tool lays flat and is secured by the stage clip.)
- Align/calibrate tool cable is loose. (Check the cable connection at both ends.)
- Objective is misaligned. (Make sure the 10x objective is rotated into position and engaged in the detent.)
- Sample compartment door is ajar. (Make sure both doors are tightly closed.)
- Grating is not fully seated or the shutter is closed. (Remove the grating, wipe its bottom surface with a clean, lint-free cloth, wipe the magnet inside the grating compartment, open the shutter and then install the grating and latch the grating door.)

- Filter is not fully seated. (Remove the filter, wipe its bottom surface with a clean, lint-free cloth, wipe the magnet inside the filter compartment, and then install the filter.)
- Laser is not fully seated. (Remove and reinstall the laser making sure the latch is fully vertical.)

Check each of these items and then restart Align/Calibrate. If that doesn't fix the problem, cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software and run Align/Calibrate. If the problem persists, contact technical support.

Performance check value(s) out of range

The performance check step of the align/calibrate process completed but the results indicate the instrument is not performing as expected. Contact technical support.

Misaligned filter or grating

The currently installed grating or filter may be damaged or severely out of alignment. Check the following:

- Align/calibrate tool is dirty. (Clean the glass window on the tool.)
- Align/calibrate tool is misaligned. (Make sure the tool lays flat and is secured by the stage clip.)
- Align/calibrate tool cable is loose. (Check the cable connection at both ends.)
- Objective is misaligned. (Make sure the 10x objective is rotated into position and engaged in the detent.)
- Sample compartment door is ajar. (Make sure both doors are tightly closed.)
- Grating is not fully seated or the shutter is closed. (Remove the grating, wipe its bottom surface with a clean, lint-free cloth, wipe the magnet inside the grating compartment, open the shutter and then reinstall the grating and latch the grating door.)
- Filter is not fully seated. (Remove the filter, wipe its bottom surface with a clean, lint-free cloth, wipe the magnet inside the filter compartment and then reinstall the filter.)

Check each of these items and then restart Align/Calibrate. If that doesn't fix the problem, cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software and run Align/Calibrate. If the problem persists, contact technical support.

Unable to read system info

The software is unable to read the instrument EEPROM, laser, grating or filter. Follow these steps:

- Remove the grating, wipe its bottom surface with a clean, lint-free cloth, wipe the magnet inside the grating compartment, make sure the shutter is open and then reinstall the grating and secure the grating compartment door.
- Remove the filter, wipe its bottom surface with a clean, lint-free cloth, wipe the magnet inside the filter compartment and then reinstall the filter.
- Remove and reinstall the laser making sure the latch is fully vertical.

Check each of these items and then restart Align/Calibrate. If that doesn't fix the problem, cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software and run Align/Calibrate. If the problem persists, contact technical support.

Unable to collect spectral data

The instrument is unable to collect spectral data during the align/calibrate process. Cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software. If the problem persists, contact technical support.

Unable to calibrate laser power

The system is unable to calibrate the laser power during the align/calibrate process. Remove and reinstall the laser making sure the latch is fully vertical and then restart Align/Calibrate. If that doesn't fix the problem, cycle the power on the computer and the instrument (turn the power switch on the power strip off and back on and then hold down the instrument power button for 3 seconds) and then restart the OMNICxi software and run Align/Calibrate. If the problem persists, contact technical support.