

Nicolet RaptIR

FTIR Microscope



USER GUIDE

Contents

1. Introduction	5
1.1 Intended use	6
1.2 Disclaimer	7
1.3 Conventions used	8
1.4 Warranty information	9
1.5 Microscope safety labels	10
2. Overview	11
2.1 Features and controls	12
2.2 Connections and ports	14
2.3 Optional Joystick	15
2.4 Optional trinocular eyepiece	16
2.5 Using OMNIC Paradigm software	17
3. Operation	21
3.1 Prepare the microscope	23
3.2 Analyze samples	26
3.3 ATR measurements	34
3.4 Locate, light, and mask the sample	36
3.5 Verify microscope performance	42
3.6 Using the polarizer	44
3.7 Single-point data measurement	47
3.8 Using the grazing angle objective (optional)	50
3.9 Differential interference contrast (optional)	52
3.10 Fluorescence illuminator (optional)	54
4. Maintenance	59
4.1 Clean the microscope	60
4.2 Maintain the liquid nitrogen dewar	61

5. Troubleshooting	63
---------------------------------	-----------

6. Contacting us	67
-------------------------------	-----------

© 2023 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. Teflon is a trademark of Chemours in the United States and/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.

1. Introduction

1.1 Intended use

The Thermo Scientific Nicolet RaptIR FTIR Microscope is a Fourier-transform (FTIR) microscope designed for use in a controlled laboratory environment, and is intended to be used with Nicolet series spectrometers.

With the RaptIR microscope, you can quickly find your target, collect high-resolution visual images, and generate high spatial resolution IR data for analysis.

OMNIC Paradigm software includes a full suite of analytical tools, customizable workflows to automate your routine tasks, and simple to use tools for microparticle analysis as well as area, point, and line analysis.

With the RaptIR Microscope, you can sample thick (up to 4 cm) and heavy (up to 5 kg) samples, and with multiple objectives and an automated nose piece, the microscope supports a range of options for viewing samples and collecting IR data.

1.2 Disclaimer

Do not use the microscope for anything other than its intended purpose as described in this user guide.

NOTICE

Read the site and safety information for your system before using the microscope.

1.3 Conventions used

Safety precautions and other important information use the following format:

DANGER



Avoid hazard. Indicates a hazardous situation which, if not avoided, will result in serious injury or death.

WARNING



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

CAUTION



Avoid hazard. 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.

1.4 Warranty information

Thermo Fisher Scientific warrants that each product we sell is free from defects in labor and materials and shall conform to its product specifications as defined in the product user documentation. If the product does not function as warranted during the warranty period, we will repair or replace it without charge. If, in our judgment, we are unable to do so, you may return it to us and we will refund your money.

This warranty replaces all other warranties, expressed or implied, including the implied warranties of merchantability and fitness for a particular purpose and any other obligations or liabilities on the part of Thermo Fisher Scientific whether in contract, warranty, negligence, or otherwise. Thermo Fisher Scientific shall not be liable for and disclaims all consequential, incidental, and contingent damages.

1.4.1 Warranty period

The system warranty period is 12 months in the U.S.A. and Canada. The warranty period begins on the date of installation or 30 days from the date of invoice, whichever is sooner.

The system warranty period for products sold outside the U.S.A. and Canada is 12 months from the date of installation or 14 months from the date of shipment, whichever is sooner.

1.4.2 Limit of warranty

Misuse, accident, modification, unsuitable physical or operating environment, improper maintenance, or damage caused by a product for which we are not responsible will void the warranty.

Consumables are not covered under warranty.

We do not warrant uninterrupted or error-free operation of a product. We provide certain non-Thermo Fisher Scientific products on an “as is” basis. Non-Thermo Fisher Scientific manufacturers or suppliers may provide their own warranties. A separate software warranty is provided with the software user documentation.

NOTICE

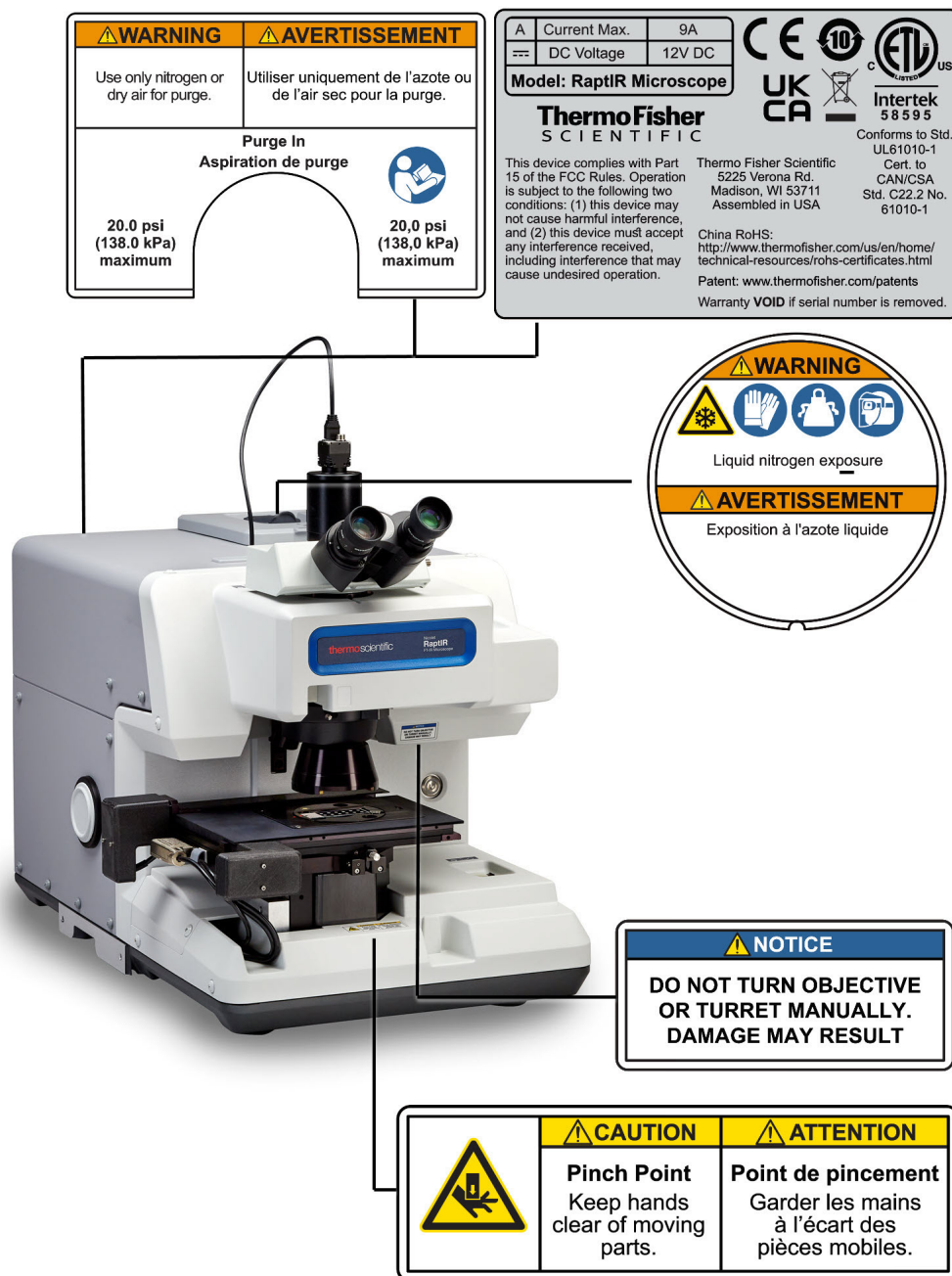
Inside the shipping box, the instrument is sealed in a plastic bag to keep the optical components dry. You must allow 24 hours for the instrument to reach room temperature before opening the bag. If the bag is opened before the instrument reaches room temperature, moisture could condense on the optical components and cause permanent damage.

Your warranty will not cover:

- Damage due to improper moving techniques.
- Missing or damaged parts if the shipping boxes are unpacked before our service engineer installs the system.
- Damage due to removing the sealed plastic bag before the instrument has come to room temperature.

1.5 Microscope safety labels

Your microscope comes with numerous safety labels. Read all cautionary text to prevent damage, hazard, or injury.



2. Overview

2.1 Features and controls

Figure 2-1: The main features of the microscope



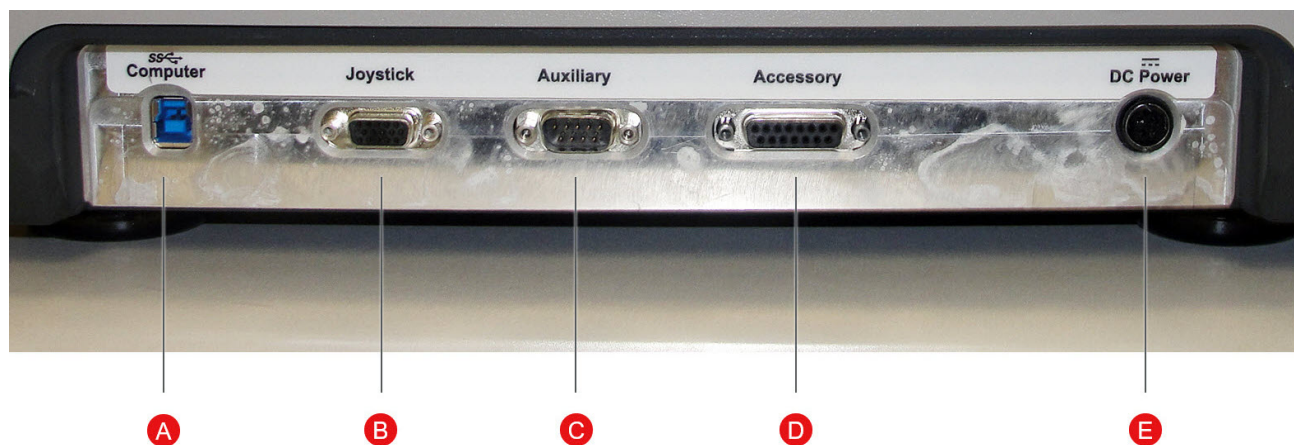
A	Trinocular video eyepiece	The optional trinocular eyepiece provides a visual and a video image in OMNIC Paradigm software. The monocular eyepiece (not shown) provides only a video.
B	Rotating objective turret	The turret supports 1 IR objective and 1 visual objectives. Typically, you will use a 15x IR objective and a 4x visual objective. The microscope also supports optional GAO and 40x visual objectives.
C	Motorized stage	The stage supports a 40 mm working distance and samples up to 5 kg. Control the stage through OMNIC Paradigm software or with the optional joystick. Never attempt to move the stage by hand.
D	Transmission illumination iris	Use the field of view iris to adjust the size of the illuminated field of view. It opens and closes concentrically. Typically, the iris is fully opened so that it is out of the full field of view. If a sample surface is uneven, you may find it easier to focus on the edges of the iris blades to find the best focus.
E	Power indicator and button	Press to turn the microscope on or off. The blue power indicator blinks while the microscope is starting up and stays a steady blue when the microscope is ready for use.

F	Reflection illumination iris	Use the field of view iris to adjust the size of the illuminated field of view. It opens and closes concentrically with respect to the reticle cross hairs. Typically, the iris is fully opened so that it is out of the full field of view. If a sample surface is uneven, you may find it easier to focus on the area of interest if you first partially close the iris and focus on the edges of the iris blades.
G	1-liter liquid nitrogen dewar	The liquid nitrogen dewar holds 1 liter of liquid nitrogen. Once cooled, the detector will remain cool for about 18 hours.

Figure 2-2: Close-up of the stage

A	ATR sensor	The ATR sensor detects whether the slide-in ATR attachment is installed.
B	Sample slide alignment indicator	Align the red indicator dots on the stage and the sample slide.
C	Control stage orientation	Used to rotate the stage during installation. Do not adjust after installation.
D	Slide-in ATR attachment	The optional slide-in ATR attachment is used for ATR measurements.

2.2 Connections and ports



- | | |
|----------|--|
| A | USB 3.0 connection to computer's USB 3.0 port |
| B | Connection to the optional joystick |
| C | Connection to the Auxiliary Signals port on the spectrometer |
| D | Connection to the Accessory port on the spectrometer |
| E | Connection to the power supply cable |

2.3 Optional Joystick

You can use the optional joystick to control the stage position and sample illumination. The stage and illumination can also be controlled in the software.

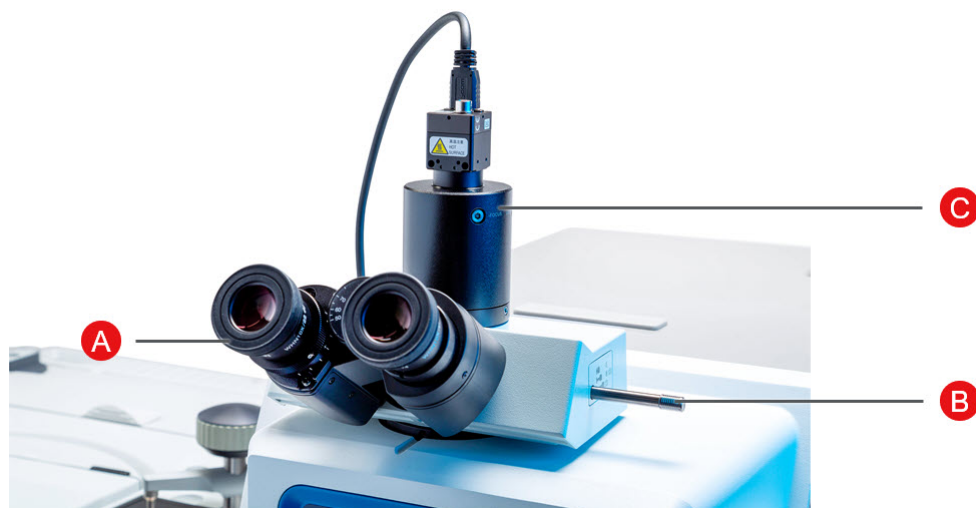
To connect the joystick, plug the data cable into the "Joystick" port on the back of the microscope.



A	Transmittance illumination control	Rotate to manually dim or brighten transmittance illumination.
B	Reflectance illumination control	Rotate to manually dim or brighten reflectance illumination.
C	Joystick for stage control	Push the joystick forward, backward, left, or right to move the stage along the sample plane. Rotate to move the stage up or down.
D	Speed control	Controls the speed of the stage for precise, slow movement, or fast movement.

2.4 Optional trinocular eyepiece

Your microscope may have either a monocular eyepiece with only the camera or it may have the trinocular eyepiece with the camera and visual eyepieces.



A	Visual eyepieces	Adjustable eyepieces for looking at the sample. Best used with the optional joystick.
B	Three-position view selector	Controls the light path for the eyepieces. <ul style="list-style-type: none">• In: Eyepiece only, no camera• Middle: Eyepiece and camera• Out: Camera only, no eyepieces
C	Camera	The USB camera is operated with OMNIC Paradigm software.

2.5 Using OMNIC Paradigm software

Run your microscope and analyze your samples using OMNIC Paradigm software, Thermo Scientific's streamlined material analysis software. The user-friendly dashboard screen helps you view instrument status and recent work, process your spectra, conduct multi-component searching, and create new libraries. Designed with lab managers and science educators in mind, this software helps automate workflows using an intuitive drag-and-drop workflow creator. Work remotely and collaborate with colleagues around the globe when you upload OMNIC Paradigm data to the cloud using the Thermo Scientific OMNIC Anywhere application.

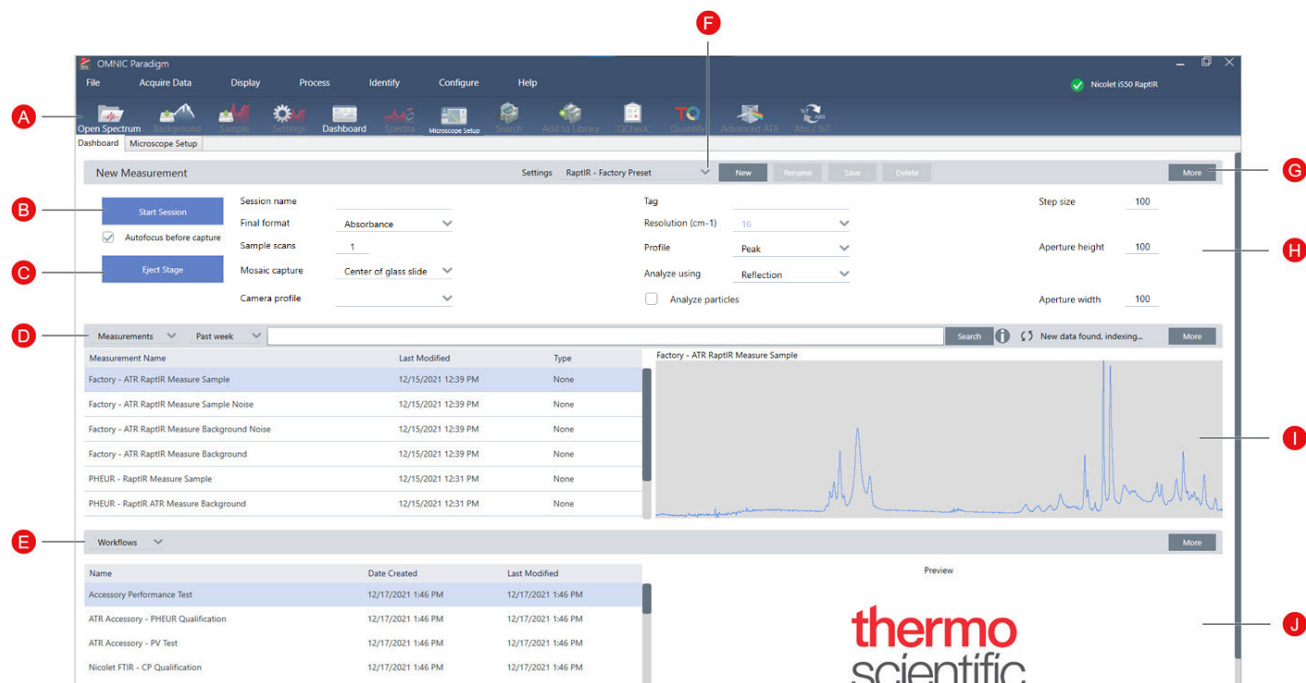
2.5.1 Interface

When working in microscopy, you will primarily work in the dashboard and the session views.

The dashboard

Begin a new session, edit measurement settings, view recent measurements, reports, and sessions, and view workflows on the dashboard.

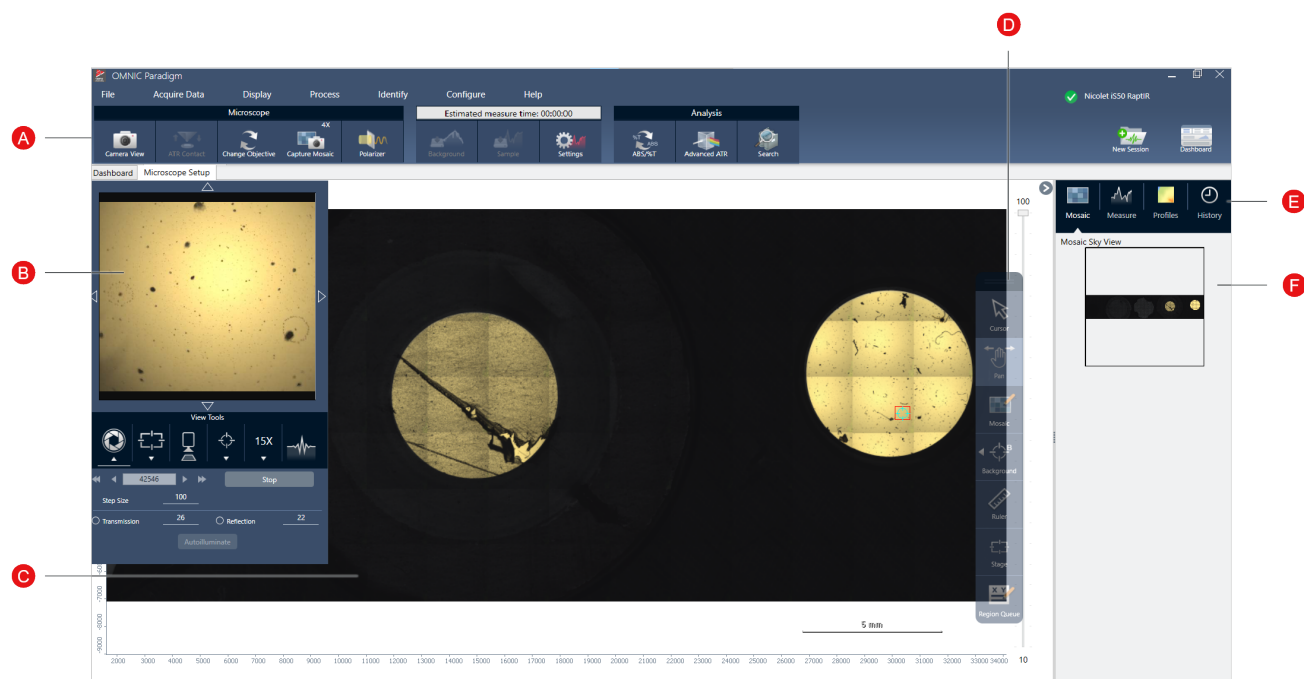
Figure 2-1: The dashboard showing tools for microscopy



A	Toolbar	The toolbar includes buttons for features and tools you will need to use often and is used to navigate between the Dashboard, Map, and Spectral views.
B	Start Session	After you've loaded your sample and selected a mosaic capture location, click Start Session to switch to the Session view and automatically collect a mosaic image of the sample. Select Auto-focus before capture to automatically bring the sample into the best focus.
C	Eject Stage	Ejecting the stage is optional. Ejecting lowers the stage and moves it forward so that you have more room to load your sample. After the sample is loaded, click Start Session to move the stage back into position or move it manually.
D	Measurements, Sessions, and Reports	Displays your Measurements, Sessions, and Reports. Choose a category from the list to switch views.
E	Workflows and Packages	Displays qualification and performance verification workflows as well as your custom workflows. For more on creating and using workflows, see OMNIC Paradigm software guides and tutorials.
F	Settings	Create, select, save, or delete collections of settings.
G	More	Click More to expand any of the main sections of the dashboard and to view additional settings or details.
H	Collection settings	The most commonly used settings are shown here, in the New Measurement pane. Click More to view additional advanced settings, including settings for background measurements.
I	Preview	Shows a preview image of the selected measurement, map, or report.
J	Workflow or package preview	Shows a preview image of the selected workflow or package. Locked workflows display a logo instead of the preview of the workflow.

The microscopy session view

Analyze your sample using the microscopy session view. Here you can view your sample, define areas of interest for your analysis, and measure sample data.

Figure 2-2: The microscopy session view in OMNIC Paradigm software

- | | | |
|----------|--------------------|---|
| A | Toolbar | View additional microscope settings and open the live camera view, capture a new mosaic, measure a spectrum, or navigate back to the dashboard. |
| B | Camera view | Open the camera view to see the live visual image of the sample. Use the View Tools to adjust the illumination, focus, and aperture and to take a quick, snapshot mosaic, change objectives, or view a live interferogram signal.

Use the arrows around the live image to move the stage without a joystick. |
| C | Mosaic view | Shows the mosaic image and is the primary workspace where you define your background point and regions for analysis. |

D	Floating toolbar	<p>Analysis and navigation tools for interacting with the mosaic image.</p> <ul style="list-style-type: none"> • Cursor: select regions, points, and spectra • Pan: Use to move the viewable portion of the mosaic • Mosaic: Use to draw a region for a high-magnification mosaic • Analysis tools <ul style="list-style-type: none"> • Background point: select a point at which you will measure the background spectrum • Area: draw an area for region analysis • Point: select a point to measure a spectrum • Line: measure a line map • Particle analysis: draw a region for particle analysis (this tool is only available when Particle Analysis is selected on the dashboard) • Ruler: use the ruler to measure objects in the mosaic view • Stage: click a point on the mosaic to move the stage to that position • Region queue: view all of the regions and points that are currently selected for your analysis (these are the positions that will be measured when you click Sample)
E	Analysis panel	View your mosaics, background spectra, profiles, and history in the analysis panel.
F	Mosaic sky view	The sky view shows a high-level view of the mosaic. When you zoom in, the sky view shows you what part of the mosaic you are viewing. You can use the sky view to navigate around the whole mosaic.

3. Operation

3. Operation

3.1 Prepare the microscope

To start your analysis, cool the microscope detector (if applicable), turn the system on, and start OMNIC Paradigm software.

3.1.1 Turn the computer on

Before turning on the microscope, turn the computer on and wait for the Windows sign in screen to appear. If you turn on the microscope before turning on the computer, OMNIC Paradigm software may not be able to communicate with the microscope or the camera.

- If this happens, turn the microscope off and back on.
- If the issue persists, turn off the computer and the microscope. Start the computer and wait for the sign-in screen to appear. Then turn on the microscope.

3.1.2 Turn the microscope and spectrometer on

Turn on the spectrometer. See the spectrometer user guide for instructions.

To turn the microscope on, press the power button on the front panel. The blue indicator light blinks during initialization and shows a continuous blue glow when the microscope is ready to use.



3.1.3 Start OMNIC Paradigm software

Use OMNIC Paradigm software to control the microscope and analyze your sample.

When you first start the software and turn on the microscope, the software checks the limits of the stage movement to ensure that everything is operating correctly.

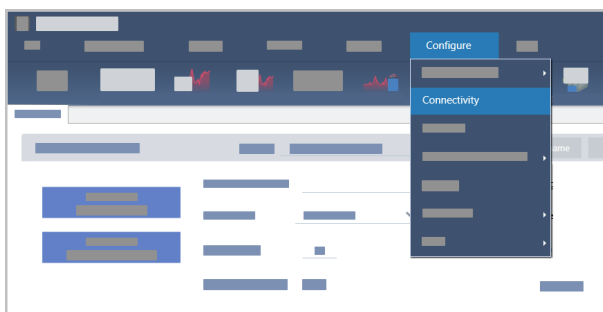
◆ **To start OMNIC Paradigm software and connect to the microscope**

1. Start OMNIC Paradigm software.
2. If the software is connected to the microscope already, the instrument status displays Nicolet iS50 RaptIR and a green checkmark.

Figure 3-1: Green checkmark in OMNIC Paradigm software



3. If the software is not already connected to the instrument, connect now.
 - a. Go to **Configure > Connectivity** and select the spectrometer. Click **Connect**.



- b. To switch to the microscopy view, go to **Configure > Sample Location > RaptIR**. The dashboard changes to show microscopy tools. To switch back to spectroscopy tools, change the sample location back to another accessory or module on your main spectrometer.

3.1.4 Cool your detector

Your microscope uses a liquid nitrogen-cooled detector. Before using the microscope, always ensure that there is enough liquid nitrogen in the dewar.

The liquid nitrogen dewar holds 1 liter of liquid nitrogen. When cooled according to the following procedure, the detector should remain cool for approximately 18 hours.

WARNING



Avoid freeze burns.

Liquid nitrogen is extremely cold and therefore hazardous.

- Wear protective clothing and eyewear and follow standard laboratory safety practices to prevent injury.
- Pour the liquid nitrogen slowly. If you pour too quickly, the nitrogen may splash.

◆ To refill the liquid nitrogen dewar

1. Open the dewar cover and remove the plastic stopper from the dewar.
2. Insert the funnel into the detector dewar and pour the liquid nitrogen slowly into the funnel. (A small amount of liquid nitrogen typically spills out of the funnel. This will not harm your instrument.) Then let the liquid nitrogen drain completely two or three times. Wait until the vapor plume disappears and repeat until the dewar is filled. Continue to slowly fill the funnel until 1 liter of liquid nitrogen is consumed, or until nitrogen bubbles under the funnel. Stop filling at this point.
3. Remove the funnel.
4. Wait until the vapor plume disappears, and wait 5 minutes before closing the dewar cover to allow the gasket to thaw.
5. Wait 20 minutes, then repeat the procedure to make sure the dewar is filled.

3.2 Analyze samples

Use OMNIC Paradigm software to operate the microscope and to analyze samples. Typically, you will analyze your sample by following these steps:

- Prepare and load the sample.
- Capture a visual image of the sample surface. This image is called a mosaic.
- Collect a background spectrum.
- Analyze the sample.

With OMNIC Paradigm software, you can either set up and use the microscope with automation features to help you light the sample and bring it into focus, capture a mosaic, and locate a background point, or you can run the microscope manually, without automation, and visually inspect your sample before capturing any visual images or data.

In either case, the first step is to load your sample.

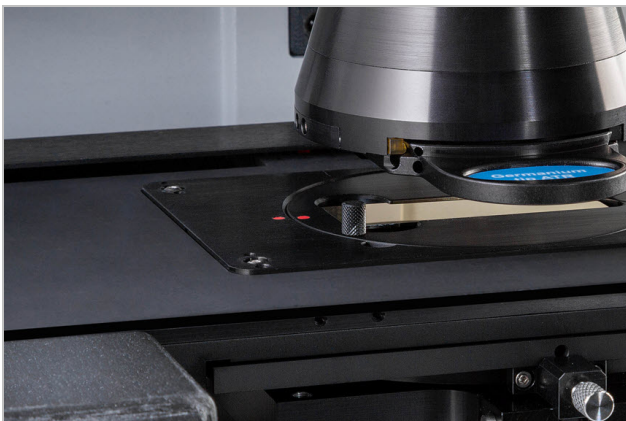
3.2.1 Load a sample

Eject the stage so that you have more access to the stage to position your sample. If your sample is small and fits in position easily, you can position the sample without ejecting the stage.

◆ To insert a sample

1. In the software, click **Eject Stage**. Ejecting the stage lowers the stage and moves it out to make it easier for you to load a sample.
2. Insert the sample slide. The stage fits a universal sample holder. Use the red indicators to properly orient the sample holder.

Figure 3-1: Sample insert



When your sample is installed, you are ready to begin the session and collect a mosaic.

If you ejected the stage, the stage will move back into position automatically when you begin the session. You can leave the stage ejected until you start the session.

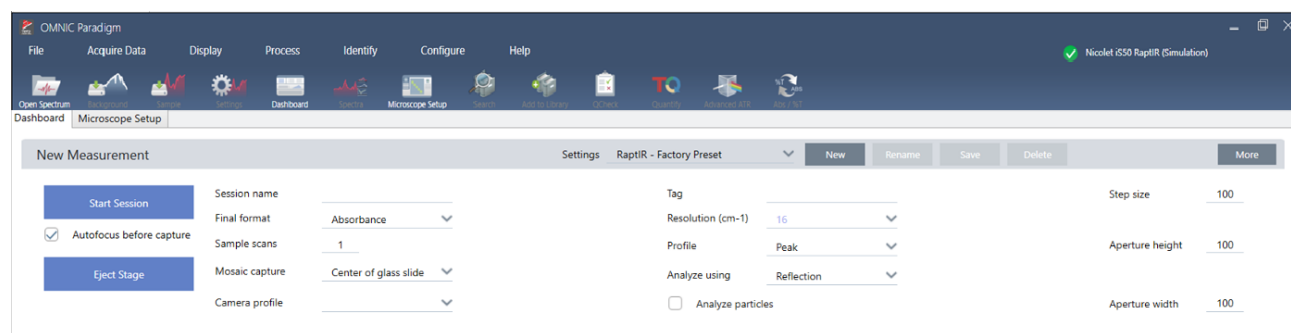
3.2.2 Prepare your measurement settings

When your sample is in place, review your measurement settings on the Dashboard. The most commonly used settings are shown at the top and you can view additional, advanced settings if you click the More button.

For more on each of the measurement settings, see the OMNIC Paradigm software user guide.

- To simplify your microscopy session by using the automation features, select a mosaic type from the Mosaic Capture list and select Autofocus before capture. If you are using the RaptIR sample holder, select **Use the fixed reference location on the RaptIR sample holder** in the background settings.
- To run your session manually, clear the selection for Autofocus before capture and select Custom Mosaic from the Mosaic Capture list.

Figure 3-2: Mosaic capture settings



Note Select **Particle Analysis** to analyze groups of small particles. With this option selected, the particle analysis tools will be present in the session view. Clear this Particle Analysis selection to instead analyze areas, lines, and individual sampling points.

Note If you are using the 40x objective with the manual focus ring, make sure that the focus ring is set to the 0 position. An incorrectly positioned focus ring may interfere with autofocusing.

3.2.3 Review your background settings

Review your settings for background collection before you begin your session.

BACKGROUND	
<input type="checkbox"/> Use the fixed reference location on the RaptIR sample holder	
<input checked="" type="radio"/> Match sample scans	<input checked="" type="radio"/> Measure background before each region
<input type="radio"/> Set background scans <u>16</u>	<input type="radio"/> Measure background once for entire sample

Table 3-1: Background settings for microscopy

Setting	Description
Use the fixed reference location on the RaptIR sample holder	<p>The RaptIR sample holder has built-in reference points for transmission and reflection analyses. Select this option to automatically move the stage to the reference location after capturing your initial mosaic.</p> <p>This option is not available if you selected Custom Mosaic in the Mosaic Capture list.</p> <p>See Measure a background spectrum for details.</p>
Match sample scans	If selected, the number of scans for the background collection is the same as the number of scans used for the sample measurement.
Set background scans	Select to use a different number of scans for the background than for the sample.
Measure background before each region	<p>If selected, the software will measure a new background before each new region of the sample. For example, if you have three area regions in your sample measurement, with this option selected, the software collects a new background before each area.</p> <p>Note that a set of points that use the same measurement settings is considered a single region.</p>
Measure background once for entire sampler	With the option selected, a new background is collected only when the measurement settings change. For example, if you have this option selected, if you measure three areas in one sample collection and they all use the same measurement settings, only one background is collected.

3.2.4 Capture a mosaic

When your sample is in position, capture a mosaic. A mosaic is a visual image of your sample surface. The camera captures a series of small, high-resolution images and stitches them together into a single mosaic, giving you a large image of the sample surface that you can use for your analysis. The mosaic acts as a workspace for your analysis, where you can explore regions of interest and specify areas and points for your IR data collection.

In general, when you are analyzing a sample, you will collect a low-magnification mosaic image with a 4x magnification objective, adjust your settings as needed, then, if needed, capture a high-magnification mosaic of a smaller area using the 15x or 30x objective. When you have captured a mosaic, draw regions or select particles, and begin measuring data.

Capturing a mosaic requires you to review your collection settings, choose a location for the mosaic capture, and click Start Session.

◆ To capture a mosaic

1. On the dashboard, select **Autofocus before capture** and review your session settings. With this option selected, the software automatically illuminates the sample and brings it into focus. Clear the selection to focus manually instead.
2. Select a location from the **Mosaic Capture** list. This tells the software where to find your sample and where to capture the mosaic. To start the session without automatically collecting a mosaic, select **Custom Mosaic**.
3. Select **Start Session**.

The stage moves your sample into position and brings it into focus, and the software collects a low-magnification mosaic and then changes objectives to the high-magnification objective. If you selected **Use the fixed reference location on the RaptIR sample holder**, the software captures a high-magnification snapshot and then the stage automatically moves to the reference location to collect a background measurement.

If you selected "Custom Mosaic" from the Mosaic capture list, the software switches to the Session view without capturing a mosaic.

3.2.5 Measure a background spectrum

Before you collect sample data, measure a background spectrum.

The RaptIR sample holder features built-in reference points to use for your background measurements. If you selected **Use the fixed reference location on the RaptIR sample holder**, the stage automatically moves to the reference location and takes a mosaic snapshot. You can then place a background point as usual.

Figure 3-3: Sample holder



- A** Reference point for reflection analyses
- B** Reference point for transmission analyses

◆ To measure a background spectrum


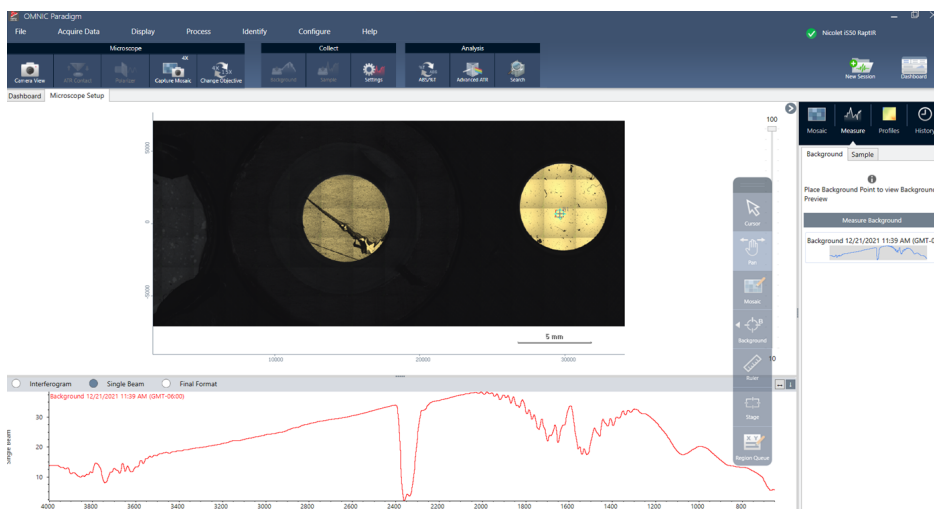
1. From the floating toolbar, select the **Background** tool. 
2. Click the mosaic at the point where you want to measure the background. A live single beam spectrum is displayed in the spectra pane. Use this spectrum to determine if you want to use this point for your background measurement. Click the mosaic again to move the background point.
3. When you are satisfied with the background point, click **Accept Background**. This is the opportunity to select a better background location before measuring the data.
4. Click **Measure Background**. The background spectrum is collected. When it is complete, it is added to the Background tab of the Spectra tab.

Figure 3-4: Mosaic background



If you are measuring multiple areas over a period of time, replace your background measurement periodically. In general, you should always have a recent measurement of the background before your measure the sample.




3.2.6 Analyze areas, lines, and points

Create a chemical image of an area of the sample surface by specifying one or more regions to analyze. You can also measure the sample at individual points using the point tool, or measure along a line with the line tool. You can measure areas, lines, and points together.

Measuring areas, points, and lines requires you to first capture a mosaic and measure the background.

◆ **To analyze areas, lines, and points**

1. See [Capture a mosaic](#).
2. See [Measure a background spectrum](#).
3. Specify areas, lines, and points to analyze. You can add multiple areas and points to a single analysis.

To analyze	Select this tool	Perform these steps
Areas		<ol style="list-style-type: none"> 1. Select the area tool. 2. Click and drag on the mosaic to draw the area.
Lines (Not available when using the polarizer during IR data collection)		<ol style="list-style-type: none"> 1. Select the line tool. 2. Click and drag to draw a line.
Points		<ol style="list-style-type: none"> 1. Select the point tool. 2. Click to add a point.

Use the cursor tool to select or delete areas, lines and points.

4. When you are finished adding regions and points, click **Sample**.
5. Open the Region Queue to view and refine details for all areas, lines, and points in your measurement. You can also select a background measurement to associate with each region.

When the measurement is complete, view the results in the new tab.

See [Next steps](#) for more on analyzing and sharing your results.

3.2.7 Analyze particles

Use the Particle Analysis tools to locate, characterize, and identify particles.

◆ **To analyze particles**

1. Prepare your sample.
2. On the dashboard, select **Analyze particles**.

Figure 3-5: Analyze particles checkbox

New Measurement

Settings RaptIR - Factory Preset

New Rename Save Delete More

Start Session

Session name

Final format Absorbance

Sample scans 1

Mosaic capture Center of glass slide

Camera profile

Tag

Resolution (cm-1) 16

Profile Peak

Analyze using Reflection

☒ Analyze particles

☐ Enable Polarizer acquisition

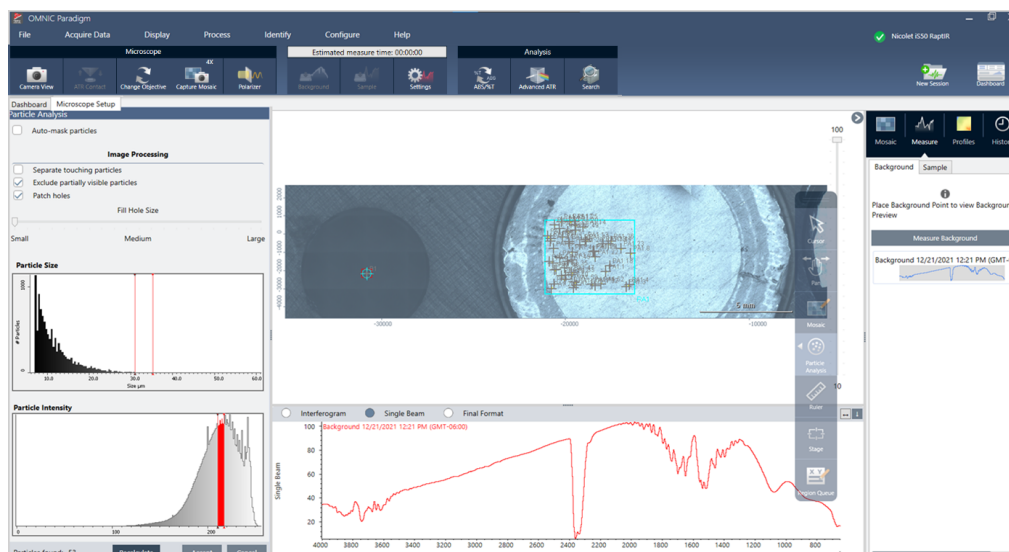
Step size 100

Aperture height 100

Aperture width 100

3. [Capture a mosaic.](#)
4. In the session view, review the mosaic and make any needed changes to focus and illumination. Capture a high-magnification mosaic if needed.
5. [Measure a background spectrum.](#)
6. Analyze particles.
 - a. Select the Particle Analysis tool and click and drag to draw a rectangle on the mosaic. This is the region of interest, where the software will detect particles. When you have drawn a region, the Particle Analysis pane opens.

Figure 3-6: OMNIC Paradigm software particle analysis setup



- b. Refine your selection using the options and selection tools. Select **Recalculate** after updating settings to update the particles.

See the OMNIC Paradigm guides and tutorials for detailed explanations of the particle analysis tools and settings.

- c. When you are satisfied with your selection, click **Accept**. This saves the selection settings but does not yet measure the data.
- d. Click **Sample**.

9. When the measurement is complete, view the results in the new tab. See [Next steps](#) for more on analyzing and sharing your results.

3.2.8 Next steps

- Apply profiles to visualize properties of the sample data
- Apply processing to selected spectra
- Create reports or export data
- Explore spectra further in the Spectra view.

3.3 ATR measurements

With the optional slide-on attenuated total reflection (ATR) attachment, you can analyze highly infrared-absorbant or hard-to-prepare microscopic materials, often with little or no sample preparation. Examples of these materials include polymers, coatings, rubbers, coated papers, and biological materials.

Applications of ATR microscopy include:

- Analyzing the surface of a sample.
- Analyzing highly-absorbing materials and the surfaces of thick samples.
- Analyzing surface coatings.
- Analyzing surface defects, inclusions, or degradation.

3.3.1 Installing a slide-on ATR attachment

The slide-on ATR attachment fits into the 15x objective and has two positions:

- Slide half-way / first stopping point to see through. Use with camera mode to view the sample.
- Slide all the way to second stopping point for ATR.

A sensor on the microscope detects when the ATR attachment is installed, and the software prompts you to install or remove it as needed.

3.3.2 Measure using ATR

To use the ATR crystal attachment for your measurement, you will install the crystal attachment, prepare your measurement settings, and measure the sample.

◆ To measure using ATR

1. On the dashboard, select **ATR** from the Analyze Using list.

Figure 3-1: Analyze using: ATR

The screenshot shows the 'New Measurement' settings window. The 'Analyze using' dropdown menu is open, showing options: 'Analyze particle', 'Reflection', 'Transmission', and 'ATR'. The 'ATR' option is highlighted. Other settings visible include 'Session name', 'Final format' (Absorbance), 'Sample scans' (1), 'Mosaic capture' (Center of glass slide), 'Camera profile', 'Tag', 'Resolution (cm-1)' (16), 'Profile' (Peak), 'Step size' (100), 'Aperture height' (100), and 'Aperture width' (100).

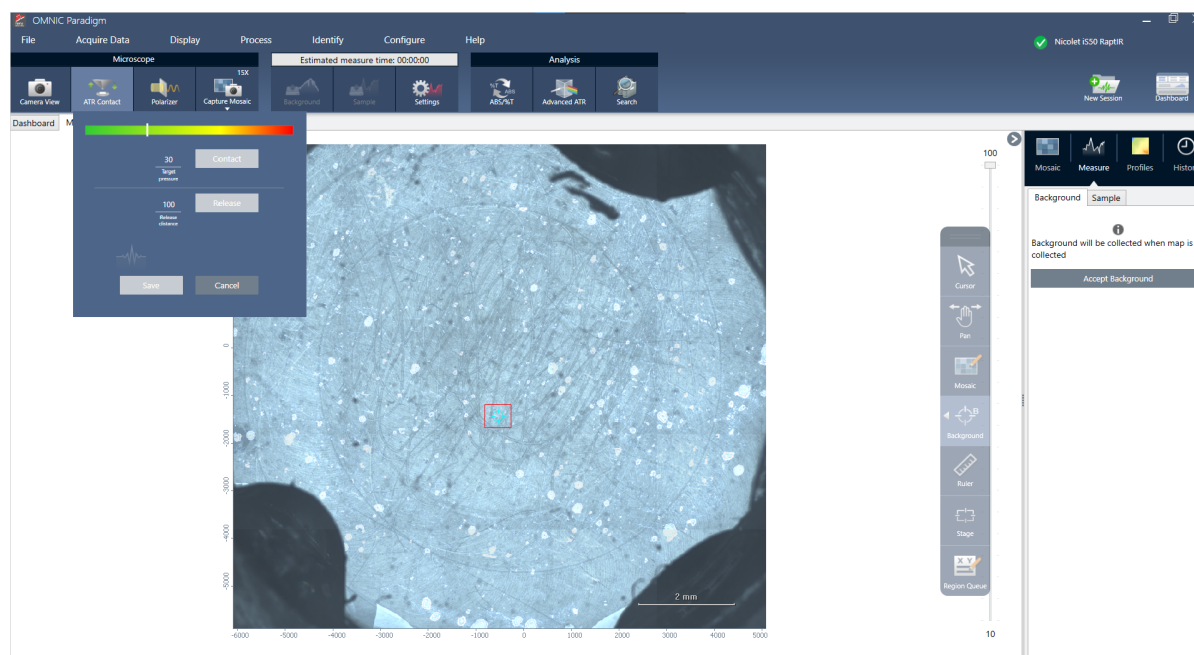
2. [Capture a mosaic.](#)

Once you have captured a mosaic, you can measure the background with the crystal installed and measure your sample using the area or points tools, just like a standard reflection measurement. In general, the default ATR Contact settings are sufficient. However, you want to view or change the contact settings, open the ATR Contact view before measuring the background or sample.

3. Optional: Review and edit the ATR Contact settings.

- a. In the Session view, click **ATR Contact** to view the ATR settings.

Figure 3-2: ATR Contact



Setting	Description
Target pressure	This is the target pressure that will be applied during measurement. Click and drag the slider or enter an exact value.
Release distance	The vertical distance that the stage moves once the ATR contact is released. A larger distance provides more clearance but will cause the ATR measurement to take longer, since the stage will move farther at every point.
Contact	Press to test contact.
Release	Press to release contact.

4. [Analyze areas, lines, and points](#) or [Analyze particles](#). The software prompts you to insert or remove the ATR crystal attachment as needed.

3.4 Locate, light, and mask the sample

To manually optimize your mosaic image and your IR data, use the Camera View to find the region of interest, bring the sample into focus, adjust the illumination, and change the aperture.

3.4.1 Move the stage and bring the sample into focus

The easiest way to bring the sample into focus is to select an approximate location of interest from the Mosaic capture list and to select the Autofocus before capture option. With these options selected, when you start the session, the stage will automatically move to the correct location, bring the sample into focus, and capture a mosaic.

If you want to move to another location and bring a new region into focus, you can move the stage and bring the sample into focus using either the software or the optional joystick.

Move the stage using either OMNIC Paradigm software or the optional joystick. Never attempt to move the stage by hand.

WITH THE SOFTWARE

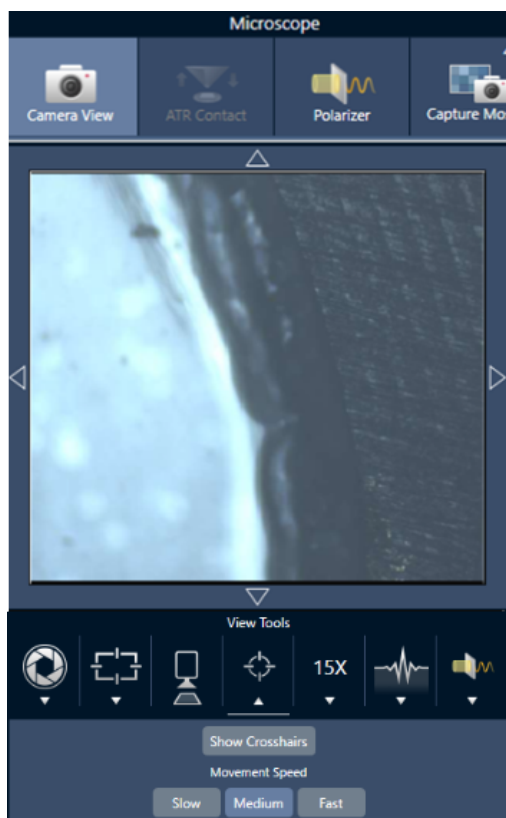
In the session view, open the Camera View to see the sample.

- **To move the stage horizontally**, open the Camera View and open the Stage tools.

Click the arrows to the sides and above and below the sample image to move the stage. Change the movement speed to change how far the stage moves with each click.

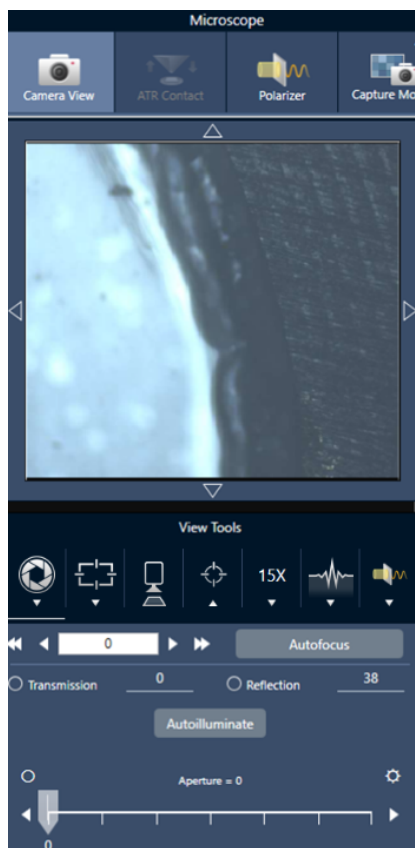
Double-click inside the live video image to center the stage on that position.

Figure 3-1: Camera view stage tools



- **To move the stage up and down**, open the Camera View and open the focus settings. Click the left and right arrows to move the stage up or down.

Figure 3-2: Camera focus



Autofocus

To bring the sample into focus automatically, click Autofocus. The software moves the stage and up down to find the optimal focus. Autofocus works best with areas of high visual contrast. Autofocus may struggle with some low-contrast samples and samples with multiple focal planes.

Tips for autofocus

- Adjust the illumination for optimal viewing. If the illumination is too high or too low, there may not be enough contrast for autofocus to find the proper focus.
- If you are using the 40x objective with the manual focus ring, make sure that the focus ring is set to the 0 position. An incorrectly positioned focus ring may interfere with autofocusing.

WITH THE JOYSTICK

You can move the stage horizontally or vertically with the joystick, and with the movement speed control, you can move quickly or carefully. Use the Camera View or the optional eyepieces to judge your position.

3. Operation

- **To move the stage horizontally**, push or pull the joystick forward, backward, left, and right.
- **To move the stage up or down**, rotate the joystick clockwise to move the stage up or counterclockwise to move the stage down.

Use the speed selector to change movement speeds.

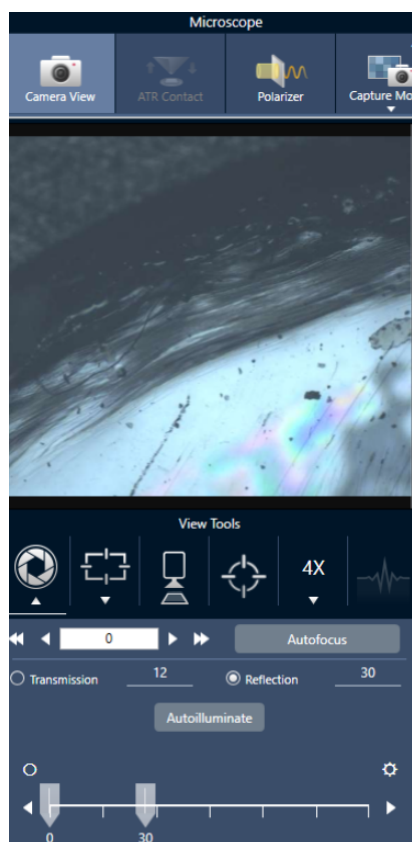
3.4.1 Illuminate the sample

You can control the amount of light that reaches the sample by using either the software or the optional joystick. Use the reflection illumination controls to set the light from above the sample and the transmission illumination controls to set the light from below the sample.

With the software

To control the illumination in the software, open the Camera View. Select either **Transmission** or **Reflection** and drag the slider to the desired light setting. You can also enter an exact value.

Figure 3-1: Camera view (illumination)



Autoilluminate

Click Autoilluminate for the software to automatically optimize the sample illumination.

With the optional joystick

The optional joystick has two control knobs to set the transmission and reflection illumination. Use the Camera View or the optional eyepieces to view the sample illumination. Rotate the knobs to control the light.

3.4.1 Adjust aperture

The adjustable aperture defines the area that the IR beam interacts with the sample. This ensures that IR energy strikes only the area of interest and not the adjacent sample material, and it ensures that the small amount of diffracted radiation that passes around the edge of the area of interest does not reach the detector.

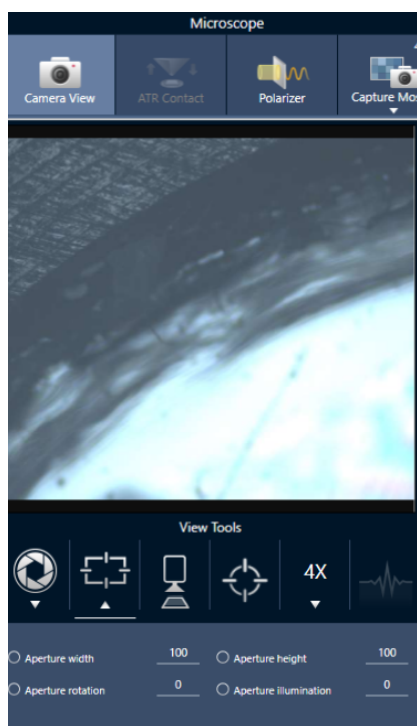
During particle analysis, the software finds a set of ideal apertures for all of the particles, then uses those apertures during the sample measurement.

Set the aperture manually in the Advanced settings area of the dashboard or in the Camera view.

◆ **To adjust the size, shape, and rotation of the aperture**

1. Open the Camera view and select the aperture settings.

Figure 3-1: Camera view (aperture settings)



2. Use the sliders or enter an exact value to adjust the height, width, and rotation of the aperture.

Note To visualize the aperture, adjust the illumination until you can see the bright blue rectangle of light passing through the aperture.

3.5 Verify microscope performance

Ensure that your microscope is working well by running PV workflows and checking the system status.

3.5.1 Performance verification and qualification workflows

Check the performance of your microscope by running the qualification or performance verification (PV) workflows. These workflows use an established standard sample to check performance of the instrument. Each test follows different regulatory standards.

PV and qualification workflows use the polystyrene standards plate to test the microscope's performance.

Table 3-1: Descriptions of qualification and performance verification workflows

Test	Description
Nicolet RaptIR - Factory Qualification	Runs factory recommended tests and all qualification tests.
Nicolet RaptIR - Factory ATR Qualification	Runs factory recommended tests and all qualification tests using the ATR accessory.
Nicolet RaptIR - PV Test	Measures the basic performance of the RaptIR based on factory recommended tests.
Nicolet RaptIR - PV ATR Test	Using ATR, measures the basic performance of the RaptIR based on factory recommended.
Nicolet RaptIR - PHEUR Qualification	Executes the qualification tests for the RaptIR as defined in the European Pharmacopoeia.
Nicolet RaptIR - PHEUR ATR Qualification	Executes the qualification tests for the ATR accessory on the RaptIR as defined in the European Pharmacopoeia.
Nicolet RaptIR - USP Qualification	Executes the qualification tests for the RaptIR as defined in the US Pharmacopoeia.
Nicolet RaptIR - JP Qualification	Executes the qualification tests for the RaptIR as defined in the Japanese Pharmacopoeia.
Nicolet RaptIR - CP Qualification	Executes the qualification tests for the RaptIR as defined in the Chinese Pharmacopoeia.

◆ To run a qualification or performance verification workflow

1. Right-click the workflow and select **Run**.
2. Follow the on-screen prompts.

When the workflow is complete, the final reports are added to the Reports pane in the dashboard and can be printed.

3.5.2 System Status

The system status icon displays information about your instrument and software services.

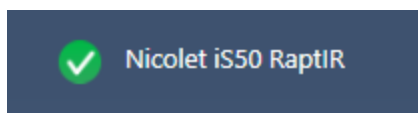








Table 3-2: System status icons

Icon	Icon with Security Suite installed	Description
		The system is connected and all services are running correctly. You are ready to measure and save data. Click the system status icon to view details about the system.
		<p>A yellow icon means there may be a problem with the instrument, such as the following:</p> <ul style="list-style-type: none"> • The detector is warm • The instrument is not scanning • The instrument is not connected <p>Click the system status icon for more details about the problem. Also visually inspect the instrument and connection.</p>
		There is a problem with one or more software services. Click the system status icon for details. If the service does not start automatically after a few minutes, restart the computer.

If you continue to have trouble with system status errors, contact customer support.

3.6 Using the polarizer

Microscopes with the polarizer option include separate polarizers for visible and for infrared light.

For each light source, the microscope includes two polarizing filters called the polarizer and the analyzer.

- **Polarizer:** Positioned between the light source and the sample
- **Analyzer:** Positioned between the sample and the camera or eyepieces and the detector

When using the polarizer, you can insert either the polarizer alone (for plane-polarized light) or the polarizer and the analyzer (for cross-polarized light). The polarizer and analyzer can be rotated together or independently.

3.6.1 Using the polarizer and analyzer

To use the polarizers, start by viewing the sample in the Camera View. Here, you can use the visible light polarizer and preview your polarizer settings for spectral data. The polarizer cannot be used during Particle Analysis or when measuring lines. It can be used to measure areas and points.

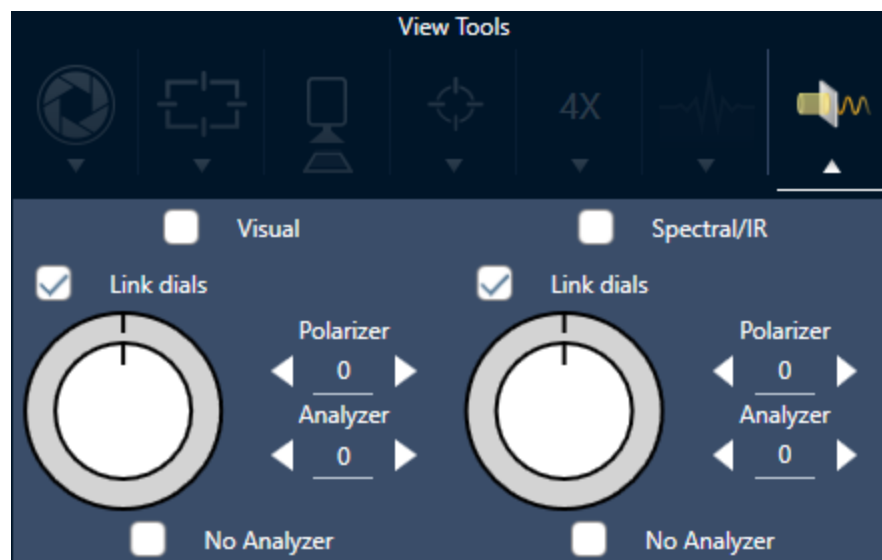
◆ To use the polarizer and analyzer in the Camera View

1. On the dashboard, select **Enable Polarizer acquisition**.

With this option selected, regions are measured using the polarizer. If you do not change any settings, the default polarizer and analyzer settings are used. You can change your mind during the session and enable or disable the polarizer before you collect data.

2. Begin your microscopy session as usual.
3. In the Microscopy Setup view, open the Camera View and go to the Polarizer tab.

Figure 3-1: The polarizer tab in the Camera View



4. Select **Visual** to use the visible light polarizer. Select **Spectral/IR** to use the IR polarizer.
 - View the sample image in the Camera View while you adjust the visible light polarizer settings.
 - To preview the IR polarizer settings, turn on the Live Spectra view and view the spectrum.

Previewing polarized images and IR data in the Camera View

Table 1. Polarizer settings in the Camera view

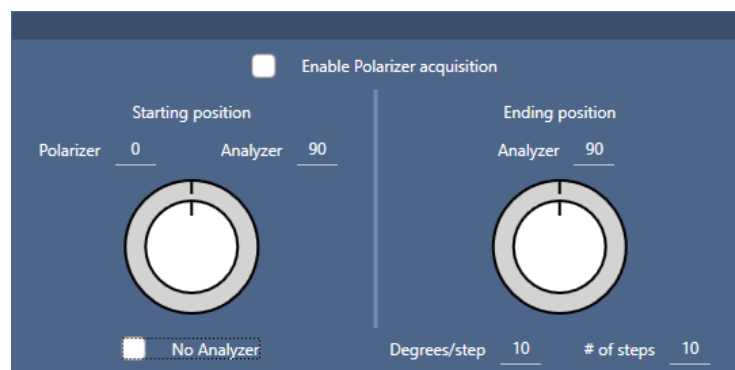
Setting	Description
Visual	Select to enable the visual polarizer.
Spectral/IR	Select to enable the IR polarizer.
Link dials	If selected, the polarizer and analyzer rotate at the same rate. Changing the angle for one also changes the other.
Polarizer angle	Set the angle of the polarizer.
Analyzer angle	Set the angle of the analyzer.
No analyzer	If selected, the analyzer is removed from the beam path and only the polarizer is used.

3.6.2 Collecting IR data with the polarizer

When measuring your sample with the polarizer enabled, you can measure in two ways:

- Measure the entire region with the polarizer (and analyzer, optionally) at a single, fixed angle.
- Automatically rotate the analyzer during the measurement and collect data at a specified interval, such as every 5 degrees of rotation.

Figure 3-2: The Polarizer Setup options



◆ **To collect data with the polarizer and analyzer**

1. In the Microscopy Setup view, click Polarizer Setup in the toolbar to view the polarizer settings.
2. Select **Enable Polarizer acquisition**.
3. Edit and review your polarizer settings.
 - To use a single, fixed angle, set the **# of steps** to 1. Only the starting position of the analyzer is used.
 - For a stepped collection...
 - a. Set the angle of the polarizer.
 - b. Set the starting and ending angles of the analyzers.
 - c. Set either the **# of steps** or the **Degrees/step**. The other setting is updated automatically.
4. Define one or more regions and measure the sample as usual.

The background point is automatically measured at each analyzer angle to correspond with the sample. During sample collection, each region is measured at every specified angle.

Figure 3-3: Polarizer acquisition settings

Setting	Description
Enable Polarizer acquisition	Select to use the polarizer (and, optionally, the analyzer) in the data collection
Starting position	Polarizer: Fixed angle of the polarizer. Analyzer: The starting angle of the analyzer. If the # of steps is set to 1, this is the only angle used.
No analyzer	Select to remove the analyzer from the beam path and use only the polarizer.
Ending position	Analyzer: The analyzer ending angle. Degrees/step (degrees per step): Enter either the degrees per step or the number of steps. The other value is calculated automatically. # of steps (number of steps): Enter either the degrees per step or the number of steps. The other value is calculated automatically.

3.6.3 Explore the polarized data

After collecting data with the polarizer, the results are shown in the Analysis tab. When viewing data collected with a polarizer, you can use the angle slider to view the profile image at each angle that was used in the collection.

3.7 Single-point data measurement

Note Single-point data measurement is not available for ATR mode in OMNIC Paradigm software version 2.3 or lower.

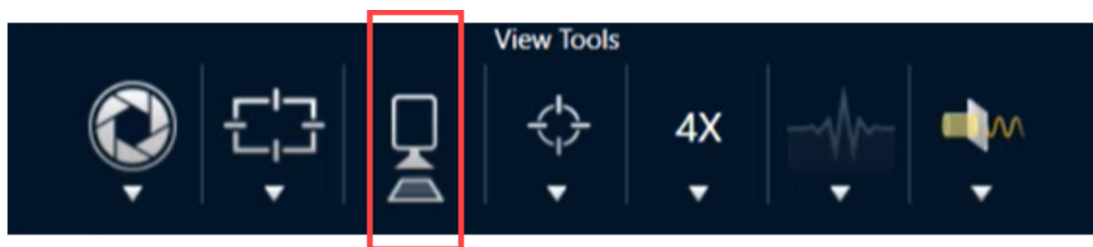
You can use the Measure Now button in OMNIC Paradigm software to measure data at the point currently shown in the live camera view. The new measurement is then automatically added to the measurement list on your dashboard.

There is no need to use the Start Session button to measure single-point data. Instead, set up your session as normal and go to the Microscope Setup tab.

◆ To measure single-point data

1. Select **Camera View** and center your sample, either by using the Joystick or eyepiece or by using a previously captured mosaic.
2. Select the **single image icon** under the **View Tools** menu to collect a single-shot mosaic using a 4x objective.

Figure 3-1: Single image icon.



3. Select **Change Objective** to switch over to the 15x objective.

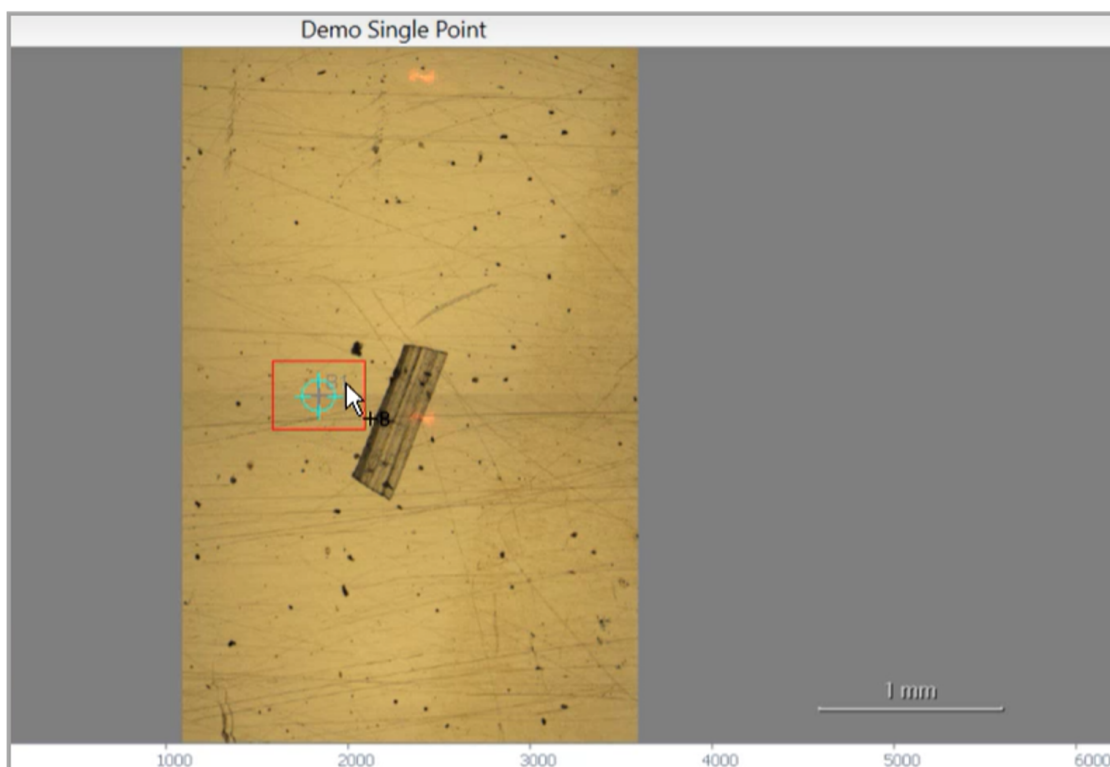
Figure 3-2: Change Objective (15x)



4. Select the **Background** button.

Using single-point measurement, the software will lay down whatever background point is centered on a reticle.

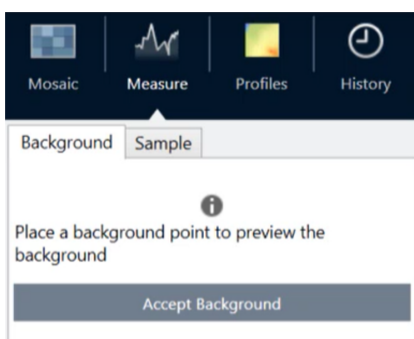
Figure 3-3: Single-point background



You may freely move the camera and click on the screen to place or relocate your desired background point.

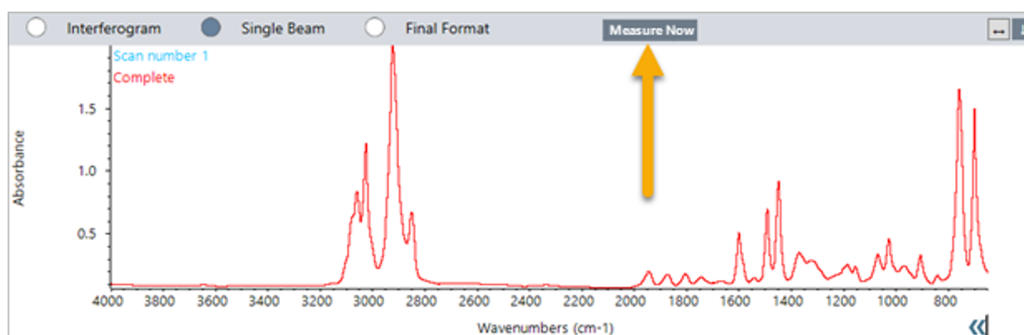
When you are ready, select **Accept Background**.

Figure 3-4: Accept Background button



5. Select **Measure Background** and wait for the process to complete.
6. Move your camera view back onto your sample. You can do this by using the Camera View arrows.
7. Select **Measure Now** to measure your sample data.

Figure 3-5: Measure Now button



Note

OMNIC Paradigm v2.3 known issues:

- The software displays all results for each scan as a stack of 10. The result that is labeled "Complete" is the correct one.
- Data measured is stored under the measurement, not the session. As such, trying to reopen the same session again will yield no data. The data must be accessed directly.

3.8 Using the grazing angle objective (optional)

The grazing angle objective (GAO) is an optional, factory-installed addition for the RaptIR+ microscope that allows for analysis of ultra-thin film samples on reflecting surfaces. Using the GAO is similar to using other IR objectives but differs in several ways due to its specialized design.

NOTICE

Because of its narrower depth of focus, you must always bring the sample into focus manually when using the GAO, and you must never use the **Autofocus** feature.

NOTICE

The grazing angle objective is very tight, so the mirror head needs to be closer to the sample than normal. This lack of space is correct and expected, but brings a risk of scraping the sample while moving the stage. Be careful not to bump the objective onto the sample during collection.

NOTICE

You do not need to use the illuminator when working the GAO. The extra light is not necessary and the cable might get tangled with the objective during rotation.

◆ Collecting data with the GAO

NOTICE

The grazing angle objective will interfere with the insertion of the ATR slide add-on. To operate with the ATR slide, unscrew the GAO objective and ensure that you do not unscrew the upper alignment ring of the GAO assembly, as this is aligned for proper operation of the GAO. To use the GAO again, simply screw the objective back into place.

1. [Load a sample](#). In the Dashboard, set **Analyze Using** to **Reflection**.

The GAO works best with ultra-thin coatings on reflecting surfaces, such as a gold mirror.

2. [Prepare your measurement settings](#).

Before selecting **Start Session** or going into microscope view, start the session as normal but skip the Autofocus step at all points and focus manually. Never use Autofocus while using the GAO.

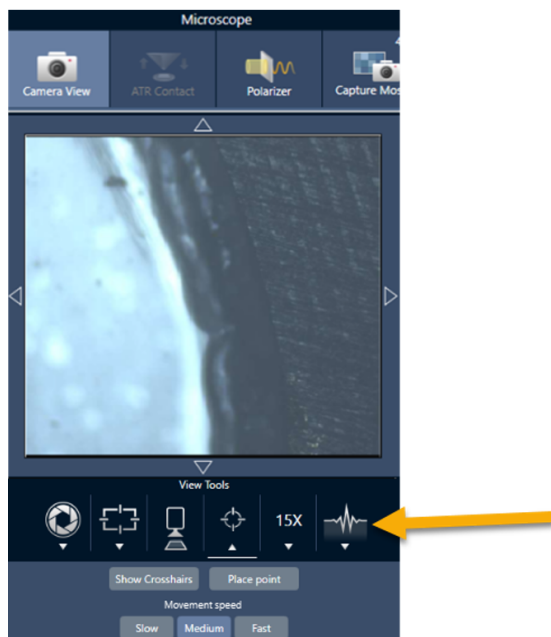
3. [Capture a mosaic](#).

Start your session as normal. Collect a 4x mosaic, then a 15x mosaic if needed.

4. In the toolbar, select **Change objective** to switch over to the grazing angle objective.
5. Change the GAO focus. With the software or the optional Joystick, use fine movements to adjust the stage height.

- Open the **Camera View** and select the Live Spectra tab under View Tools.

Figure 3-1: Camera View live spectra



- Use the IR signal to determine when the sample is in focus.
6. [Measure a background spectrum.](#)
 7. [Analyze areas, lines, and points.](#)

Collect your sample as usual.

NOTICE

Be careful not to bump the objective onto the sample during the measurement. Sampling large areas or point that are particularly far apart increases this risk.

When you finish a session, the objective automatically switches to the 4x objective. To avoid the risk of collision between the objective and the sample during rotation, move the stage down before ending the session.

3.9 Differential interference contrast (optional)

Your microscope can be configured with differential interference contrast (DIC) capabilities.

DIC is an optical technique that uses Wollaston prisms and crossed polarizers to enhance contrast and visibility in otherwise transparent samples, such as layered laminates, unstained biological samples, or certain polymers.

Performing DIC is only possible if your microscope has been specifically configured with that capability by Thermo Fisher Scientific. For more information, [contact us](#).

Note The DIC technique can only be used in Transmission mode. Additionally, the DIC optics will only work with the 15x IR visible objective and condenser.

◆ To perform DIC

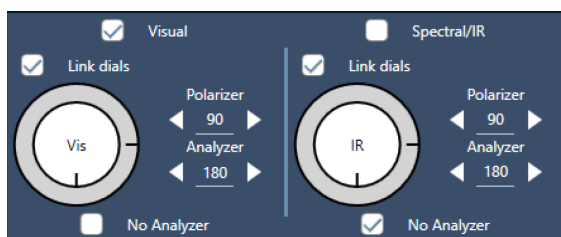
1. Illuminate and bring a sample into focus, then capture a 15x mosaic. For more information, see the [Move the stage and bring the sample into focus](#) and [Capture a mosaic](#) sections. When the reflection and condenser image are properly focused, the view will show a bright light.

Note You can use the manual iris on the lower right side of the microscope; when the condenser is at the proper focus, the edges of the iris will appear sharp.

2. Select the polarizer function on the right end of the Camera View menu. Turn on the visual polarizer and ensure that the **No Analyzer** check box at the bottom is unchecked (you will need the analyzer). Set the polarizer to 90° and the analyzer to 180°.

The camera view should go dark, indicating that the polarizers have been crossed at a 90° angle. Make sure to check the **Link dials** box to ensure they remain linked. For more information, see [Using the polarizer](#).

Figure 3-1: DIC settings for polarizer in Camera View



3. Gently place the first Wollaston prism into the bottom slot near the base of your unit.

The prism may require firm pressure to insert. It should fit firmly and go all the way in until there is resistance.

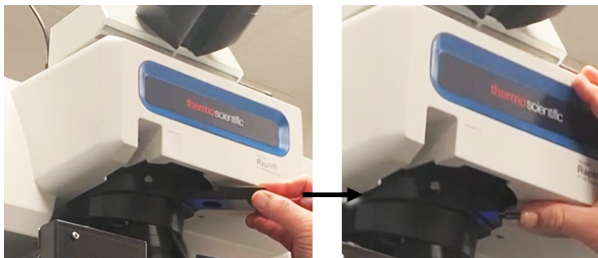
Figure 3-2: Insert Wollaston prism into base slot



3. Operation

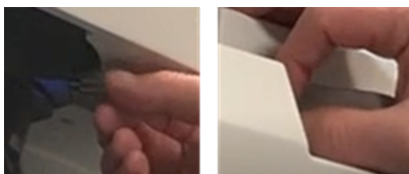
4. Loosen the microscope nose piece thumbscrew. This will allow the second Wollaston prism to slide past the indentations. Slide the prism into the nose piece (indent side up) until it stops, then secure the prism by gently tightening the thumbscrew.

Figure 3-3: Insert Wollaston prism into the nose piece slot



5. Inserting the DIC optics into the microscope will darken your sample image. To remedy this, increase illumination in the software (turning it up to 5 is recommended).
6. Use your fingers to rotate the Wollaston prisms and manipulate the sample image. This will result in visible color changes to give you higher visibility of your sample with a three-dimensional effect. You can also record mosaics or single frame captures of the resulting image.

Figure 3-4: Wollaston prisms rotation



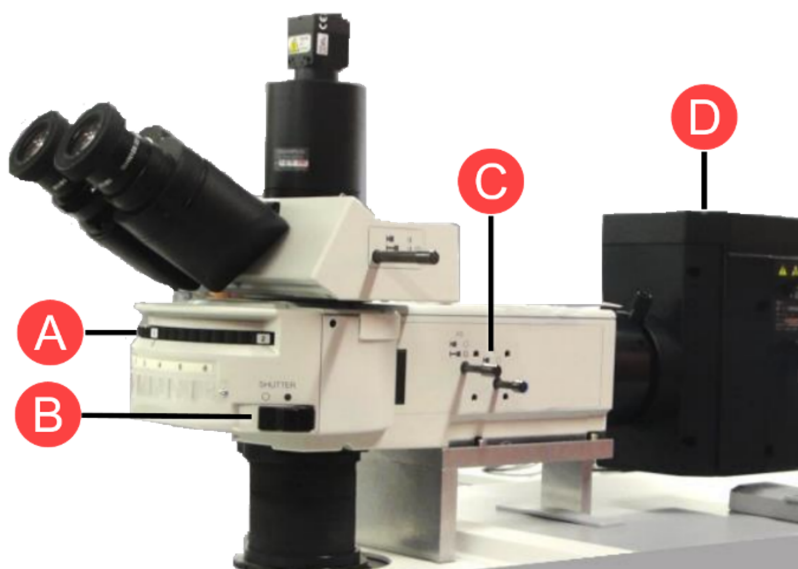
3.10 Fluorescence illuminator (optional)

Your microscope is compatible with a fluorescence illuminator designed to locate and illuminate portions of a fluorescent sample that are of interest.

Note The fluorescence illuminator is to be used to locate points of interest in a sample. It is not, in itself, used to collect or measure data.

The fluorescence illuminator can be installed by a Thermo Fisher representative. For more information, [contact us](#).

Figure 3-1: RaptIR fluorescence illuminator



A	Rotating mount: Used to switch to different illumination frequencies if needed.
B	Shutter: Switch on or off to block the light or allow it through.
C	Optics and apertures: May also be used to manipulate the flow of light.
D	Illuminator lamp: Positioned near the back of the instrument.

◆ To illuminate your sample with the fluorescence illuminator

1. Center your sample using the OMNIC Paradigm software microscopy view.

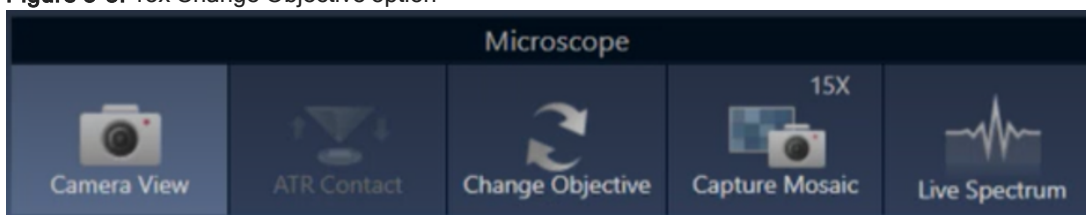
Start with a 4x objective at medium Joystick speed for a wider area view. This allows you to find the sample's fluorescent locations more easily.

Figure 3-2: Centered sample (dyed fluorescent polystyrene beads) using 4x objective and blue excitation. One bead is seen fluorescing.



2. Once you have centered your target, select **Change Objective** to switch to the 15x objective. You will see a much brighter image of your sample and points of interest.

Figure 3-3: 15x Change Objective option



3. Focus on the sample as needed. For more information, see [Move the stage and bring the sample into focus](#).

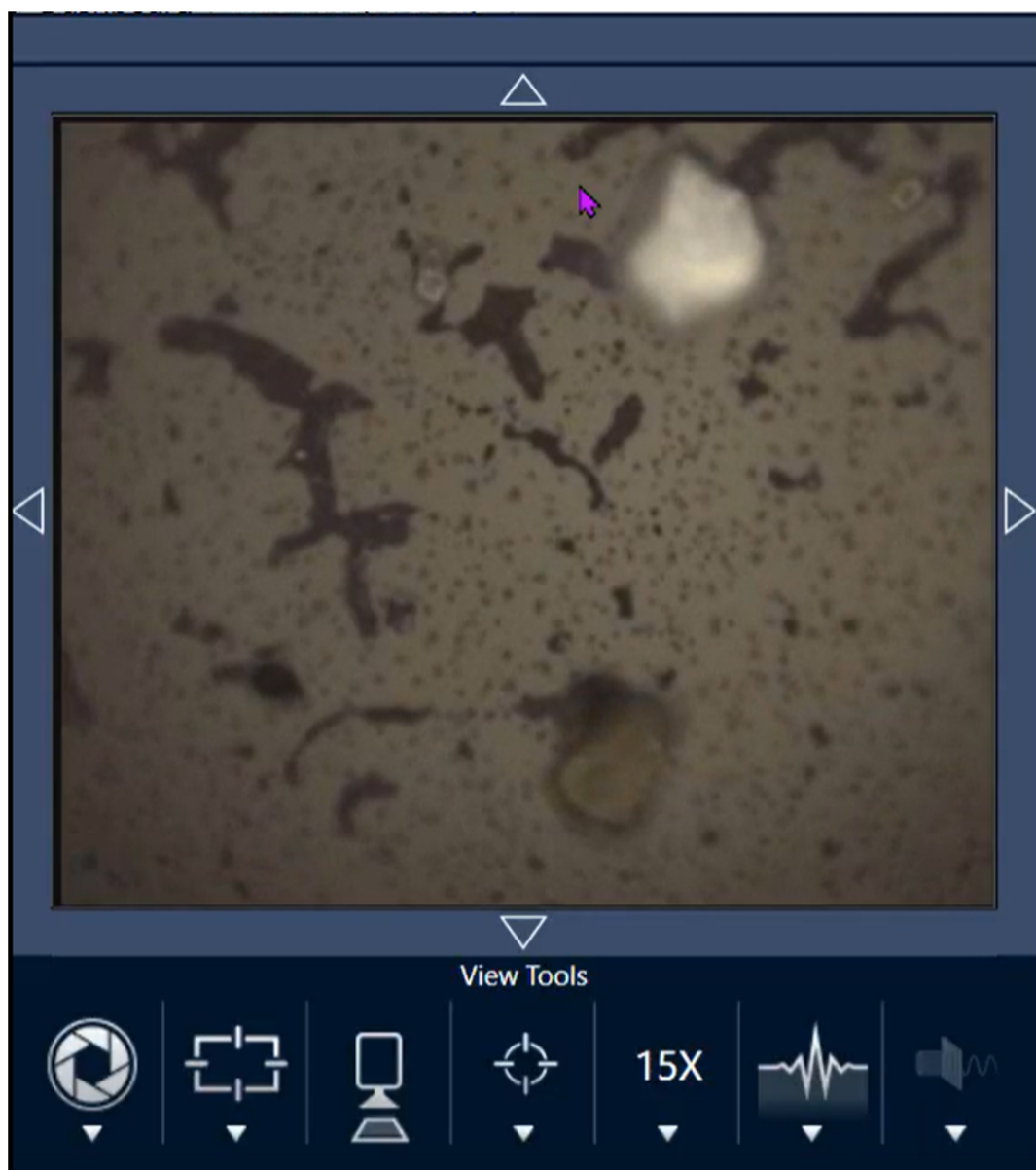
Figure 3-4: Centered sample (dyed polystyrene beads) using 15x objective.



The bead in the example is now much brighter. No change was made to the fluorescence illumination. A second nearby bead is also visible now.

4. Close the shutter (shutting off fluorescence) when you are ready to measure data. You may use the microscope's regular illuminator as needed at this point.

Figure 3-5: Dyed polystyrene beads using 15x objective (closed shutter)



[This page intentionally left blank]

4. Maintenance

4.1 Clean the microscope

If you wish to remove dust from a mirror, window, or optical component, blow it off with the dust blower that was included with the microscope. Do not use canned air or duster, which may damage the instrument. Never allow any liquid to come into contact with a window or optical component.

4.2 Maintain the liquid nitrogen dewar

The detector dewar should maintain its insulating vacuum for several years. If the vacuum is lost, the insulation will lose effectiveness and the following symptoms may occur:

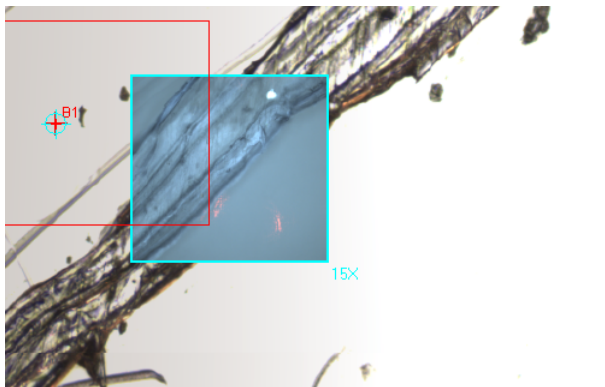
- Shortened hold times and condensation (liquid nitrogen boils off much faster than usual)
- Water and atmospheric contaminants condensing on the detector window will show up in spectra as unwanted peaks
- Note that water condensation may also appear during normal detector cooling in high-humidity environments

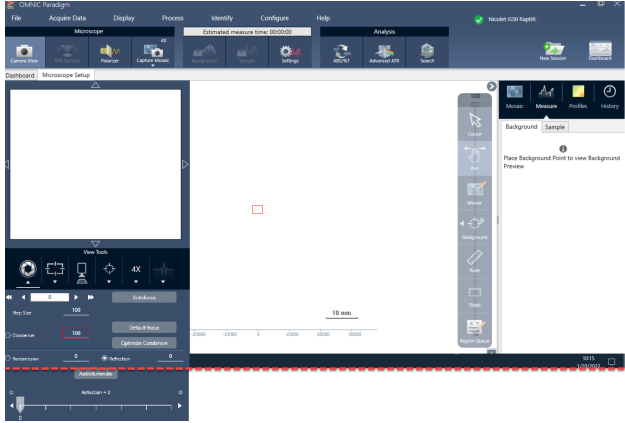
NOTICE

If your instrument shows any of these symptoms, the detector dewar may have a vacuum leak. Contact us immediately for assistance.

[This page intentionally left blank]

5. Troubleshooting

Problem	Possible cause	Solution
<p>15x mosaics do not reproducibly align with the 4x image. For example, in the image below, the 15x image is not aligned with the 4x image.</p> 	<p>The objective is loose.</p>	<p>The objective can sometimes become loose. This usually happens when inserting and removing the ATR attachment.</p> <p>If the objective feels loose, hand-tighten the objective. The slot for the ATR attachment should face directly to the front.</p> <p>Do not overtighten the objective, and do not use the ATR attachment as a lever to tighten the objective. Overtightening the objective will damage it.</p> <p>If the objective feels snug and you are still having alignment issues, contact your service representative.</p>

Problem	Possible cause	Solution
<p>Sections of the software interface do not fit on the screen.</p> 	<p>Your display scaling settings are not compatible with the software.</p>	<p>If parts of the software interface do not fit on the screen, you may need to adjust the display scaling in your device's display settings. For example, on some monitors, the Camera tools may not fit on the screen if the display scaling is set to above 100%.</p> <p>For help changing your display settings, see Windows help information.</p>
<p>The mosaic or camera view are completely black.</p>	<p>The camera is not connected.</p>	<p>Verify that the camera cable is plugged into the nose piece.</p> <p>Verify that the microscope USB cable is plugged into the USB 3.0 connector.</p>
<p>The system status shows a yellow or red icon.</p>	<p>There may be a problem with the instrument or with software services.</p>	<p>See System Status for more information.</p>

[This page intentionally left blank]

6. Contacting us

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

6.0.1 Ordering parts

To order parts, [contact us](#).

If you need to send the instrument or an accessory to us for repair, call or e-mail us first for any shipping requirements or other instructions.

6.0.2 Further information

To find guides and tutorials for how to use your microscope or OMNIC Paradigm software, please consult our online [knowledge base](#).

[This page intentionally left blank]