Nicolet RaptIR+

FTIR Microscope



USER GUIDE



269-3625 02

Revision A

December 2023

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	
2.2 Connections and ports	14
2.3 Optional Joystick	
2.4 Optional trinocular eyepiece	
2.5 Using OMNIC Paradigm software	

3. Operation	
3.1 Prepare the microscope	
3.2 User-swappable detectors	
3.3 Analyze samples	
3.4 ATR measurements	40
3.5 Right side emission	45
3.6 Locate, light, and mask the sample	
3.7 Verify microscope performance	51
3.8 Using the polarizer	
3.9 Single-point data measurement	
3.10 Using the grazing angle objective (optional)	
3.11 Differential interference contrast (optional)	61
3.12 Fluorescence illuminator (optional)	63
3.13 Using the Linkam stage in Transmission mode	67

4.	Maintenance	75
	4.1 Clean the microscope	76

4.2 Maintain the liquid nitrogen dewar 4.3 Storing and shipping your detector	
5. Troubleshooting	80
6. Contacting us	83

© 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 errorfree, 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. 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:



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

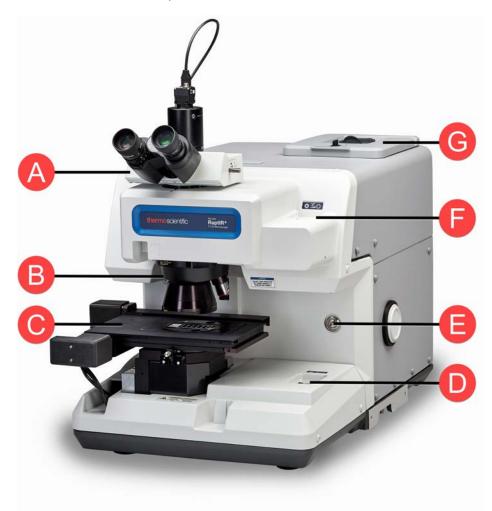
Your microscope comes with numerous safety labels. Read all cautionary text to prevent hazard or injury.



2. Overview

2.1 Features and controls

Figure 2-1: The main features of the microscope



А	Trinocular video eye- piece	The optional trinocular eyepiece provides a visual and a video image in OMNIC Paradigm soft- ware. The monocular eyepiece (not shown) provides only a video.
В	Rotating objective tur- ret	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.
С	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	Swappable detector hatch and 1-liter liquid nitrogen dewar	The detector hatch can open to allow swapping of detectors. 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



Α	ATR sensor	The ATR sensor detects whether the slide-in ATR attachment is installed.
В	Sample slide alignment indicator	Align the red indicator dots on the stage and the sample slide.
С	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



А	USB 3.0 connection to computer's USB 3.0 port
В	Connection to the optional joystick
С	Connection to the Auxiliary Signals port on the spectrometer
D	Connection to the Accessory port on the spectrometer
Е	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.

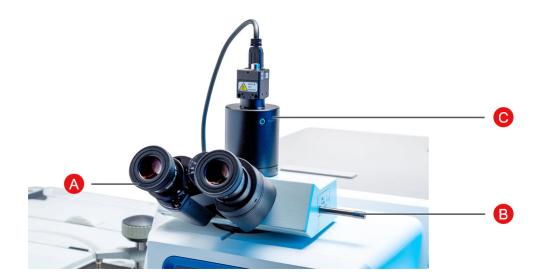
To connect the joystick, plug the data cable into the "Joystick" port on the back of the microscope.



A	Transmittance illumination control	Rotate to manually dim or brighten transmittance illumination.
В	Reflectance illumination control	Rotate to manually dim or brighten reflectance illumination.
С	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.



Α	Visual eyepieces	Adjustable eyepieces for looking at the sample. Best used with the optional joystick.
В	Three-position view selector	Controls the light path for the eyepieces.
		 In: Eyepiece only, no camera Middle: Eyepiece and camera Out: Camera only, no eyepieces
С	Camera	The USB camera is operated with OMNIC Paradigm software.

2.5 Using OMNIC Paradigm software

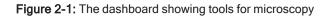
Run your microscope and analyze your samples using OMNIC Paradigm software, Thermo Scientific's streamlined material analysis software. The user-friendly dashboard screen helps you view instrument status and recent work, process your spectra, conduct multi-component searching, and create new libraries. 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.

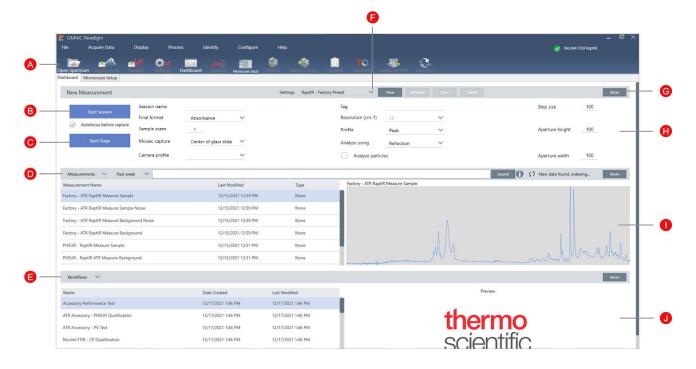
2.5.1 Interface

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.







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.

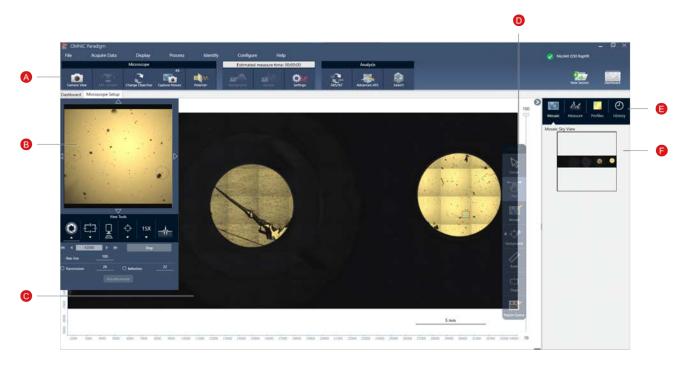
В	Start Session	After you've loaded your sample and selected a mosaic capture location, click Start Session to switch to the Session view and automatically collect a mosaic image of the sample. Select Auto-focus before capture to automatically bring the sample into the best focus.
С	Eject Stage	Ejecting 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.
Н	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 pack- age 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.

2. Overview

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.
В	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.
С	Mosaic view	Shows the mosaic image and is the primary workspace where you define your background point and regions for analysis.

D	Floating toolbar	Analysis and navigation tools for interacting with the mosaic image.
	toonbai	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
		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

3.1 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**.



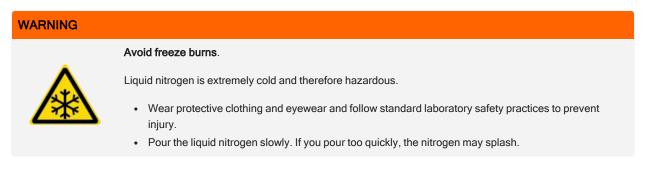
b. To switch to the microscopy view, go to Configure > Sample Location > Right Microscope. The dashboard changes to show microscopy tools. To switch back to spectroscopy tools, change the sample location back to another accessory or module on your main spectrometer.

3.1.4 Cool your detector

Your microscope can use liquid nitrogen-cooled MCT detectors. Before using the microscope with these detectors, always ensure that there is enough liquid nitrogen in the dewar.

For more information on the detectors available to you, see User-swappable detectors.

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.



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 User-swappable detectors

The RaptIR+ microscope is compatible with several easily swappable detectors to help you optimize your data collection. Each available detector is recognized by OMNIC Paradigm software upon installation and loads its own factory preset settings, but you are free to create your own settings based on your analysis needs.

The detectors are universal and will work on any RaptIR+ unit without recalibration. They can also be swapped without shutting the power down.

Always use personal protective equipment (PPE) while handling the detectors.

The available detectors for the microscope are:

Detector	Description	Spectral range
MCT-A	Fast, high-sensitivity detector with limited spectral range. Requires liquid nitrogen.	7, 800 - 650 cm-1
MCT-B	Fast detector with 20-30% less sensitivity compared to MCT-A, but a wider spectral range for low-frequency vibrations, ideal for work with polymers and other inorganic materials. Requires liquid nitrogen.	7, 800 - 450 cm-1
InGaAs	Specialized near-infrared detector with high spectral range, ideal for pharmaceuticals. Does not require liquid nitrogen. Note that the InGaAs detector requires a CaF2 or XT-KBr beamsplitter configuration (preferably the former) in order to function correctly. This is configured at the time of purchase.	11, 700 - 3, 800 cm-1
TEC-MCT	TE-cooled, lower sensitivity detector. Does not require liquid nitrogen. Please allow up to one minute to bring this detector to temperature.	7,800 - 1, 000 cm-1

WARNING



Avoid hazard. Make sure the detector dewars are empty before you move them. Do not attempt to remove a detector with any liquid nitrogen in the dewar.

CAUTION



Avoid hazard. Do not let anything fall into the detector chamber when the lids are open.

3.2.1 To install a RaptIR+ swappable detector

1. Open the top hatch of the RaptIR+ microscope. If it is locked, turn the main latch to the "unlocked" position to open it.

If there is a detector already inside, uninstall it by following the instructions in <u>To uninstall a RaptIR+ swappable</u> <u>detector</u>.

Figure 3-1: Top hatch, closed and open



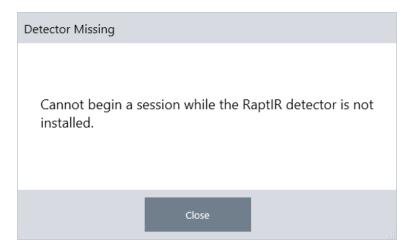
Figure 3-2: Latch: locked position and unlocked position





Note OMNIC Paradigm software checks for a detector in five second intervals. It is not possible to begin a data measurement session without a detector installed. If this occurs, a pop-up will appear as a warning.

Figure 3-3: "Detector Missing" pop-up box in	OMNIC Paradigm software
--	-------------------------



2. Lift your chosen detector by the handles and place it into the receptacle. Guide the detector in gently until you reach the bottom; do not drop it in. Available detectors include MCT-A, MCT-B, InGaAs, and TEC-MCT.

Figure 3-4: Lifting / inserting a detector



3. When the detector is in, turn the two smaller latches to the "locked" position until tight to secure the detector onto the unit.

- 3. Operation
- Figure 3-5: Detector latch



4. Close the top hatch and turn the main latch to the "locked" position.

Figure 3-6: Top latch (locked)



5. (Optional) The top hatch includes a liquid nitrogen fill port for detectors that require it.

To fill the port, see Cool your detector.

Figure 3-7: Dewar fill port (closed, open)



3.2.2 To uninstall a RaptIR+ swappable detector

NOTICE

Do not attempt to remove a detector during a data measurement session in OMNIC Paradigm software. Doing so will automatically close and interrupt your session.

1. Open the top hatch of the RaptIR+ microscope. If it is locked, turn the main latch to the "unlocked" position in order to open it. (Wait until the dewar is empty before removing the detector.)

Figure 3-8: Open latch with detector inside



Figure 3-9: Latch: locked and unlocked positions



2. Inside, turn the two smaller latches on the detector to the "unlocked" position.

Figure 3-10: Unlocked detector latches



3. Lift the detector by its handles to remove it from the receptacle, then place it in a safe location on a flat, even, unmoving

3. Operation

surface.

Figure 3-11: Lifting / inserting a detector



4. Close the top hatch of the RaptIR+ microscope (or insert a new detector), then turn the main latch to the "locked" position.

Note To best protect your detectors, store them in their originally supplied packaging.

3.2.3 Optimizing the swappable detectors

Optimization of your detectors is important for ensuring the quality of your spectral data. OMNIC Paradigm software will alert you with a yellow system status icon when optimization is recommended. Optimization will set the system gain to improve sensitivity while ensuring that the signals do not saturate the detector.

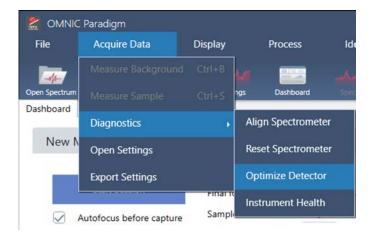
Note If you are using a liquid-nitrogen-cooled detector, wait for it to cool before performing optimization steps. See <u>Cool</u> your detector for further details.

Note Optimization should be done with the correct default source and beamsplitter installed. If the optimization fails because the correct configuration is not available for other reasons, it does not necessarily indicate a problem or prevent the use of your system.

Click the status icon in the upper-right hand corner of the screen to see the yellow status details. If you see a yellow icon next to **Not optimized**, you should optimize your detector.

- To optimize your detector
- 1. Go to Acquire Data > Diagnostics > Optimize Detector.

Figure 3-12: OMNIC Paradigm software Optimize Detector path



2. Follow the on-screen prompt and wait for the process to complete.

Figure 3-13: Detector optimization pop-up message

nformation			
		move the sample holde	r from
the instrument	and keep clea	ar of the stage.	
the instrument	and keep clea	n of the stage.	

If the status turns green, you have successfully optimized your detector.

Figure 3-14: Successful optimization pop-up message

Information	
	Optimization successful.
	Close

3.3 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.
- Measure 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.

3.3.1 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.

3.3.2 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

Acquire Data Dis	play Process	Identify Confi	gure	Help					Nicolet iS50 RaptIR (Simulation	n)
num Alicroscope Setup	Cathboard	Microscope Setup	P	-		70 🚯	2			
w Measurement				Set	tings Raptl	R - Factory Preset	✓ New	Renamo Save Delete		Mot
Start Session	Session name					Tag			Step size	100
	Final format	Absorbance	1			Resolution (cm-1)	16	~		
Autofocus before capture	Sample scans	1				Profile	Peak	~	Aperture height	100
Eject Stage	Mosaic capture	Center of glass slide	/			Analyze using	Reflection	~		
	Camera profile		1			Analyze partic	loc		Aperture width	100

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.

3.3.3 Review your background settings

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



3. Operation

Setting	Description
Use the fixed ref- erence location on the RaptIR sample holder	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. This option is not available if you selected Custom Mosaic in the Mosaic Capture list. See Measure a background spectrum for details.
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 back- ground before each region	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. Note that a set of points that use the same measurement settings is considered a single region.
Measure back- ground 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.

Table 3-1: Background settings for microscopy

3.3.4 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.

3.3.5 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

Reference point for transmission analyses

В

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.

3.3.6 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	Area	 Select the area tool. Click and drag on the mosaic to draw the area.
Lines (Not available when using the polarizer during IR data collection)	Line	 Select the line tool. Click and drag to draw a line.
Points	-< → Point	 Select the point tool. 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 <u>Next steps</u> for more on analyzing and sharing your results.

3.3.7 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.

3. Operation

New Measurement Settings RaptIR - Factory Preset V New More Session name Tag 100 Step size Final format Absorbance Resolution (cm-1) ~ Sample scans 100 Autofocus before capture 1 Profile Peak Aperture height Analyze Mosaic capture Center of glass slide ~ Analyze particles Camera profile 100 Aperture width Enable Polarizer acquisition

Figure 3-5: Analyze particles checkbox

- 3. Capture a mosaic.
- 4. In the session view, review the mosaic and make any needed changes to focus and illumination. Capture a highmagnification 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

File Acquire Data Display Process	Identify Configure Help		😪 Nicolet ISO Reptik
		Analysis Alicent Alix Sector	<u>.</u>
Dashteoand Microscope Setup			2 - 44 - 0
Auto-mask particles			100 - Lan 100 - Lan 100
Image Processing			Mosaic Measure Profiles Histor
Separate touching particles			Background Sample
Exclude partially visible particles Fatch holes	The second se		0
Fill Hole Size	1	ALCOREM TO	Place Background Point to view Backgroun Preview
Small Medium Larg		and the second second	A A A A A A A A A A A A A A A A A A A
		A CONTRACTOR OF A	Background 12/21/2021 12:21 PM (GMT-
Particle Size	1 2 49	The second second	All all man
1 · · · · · · · · · · · · · · · · · · ·		Contraction to	
, k	1 Aller Aller Aller		-100 Mart - 100 Mart -
			• @
			Terrer
a na na sta sta sta			
Particle Intensity			
	Interferogram Single Beam Interferogram Interferogram Single Beam Interferogram Single Beam Interferogram Interferogram Interferogram Single Beam Interferogram Int	Final Format	
	40	16 1.1.	<u>~</u>
W.	6	WIN	"
	3 60 -	- II	
	" " NW		
	20 -	V	~
Particles Found: 53 Recalculate Access Concert	4000 3000 3600 3400 320	20 3000 2800 2600 2400 2200 2000 1800 1600 1	450 1250 1000 800

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 <u>Next steps</u> for more on analyzing and sharing your results.

3.3.8 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.

3.4 ATR measurements

With the optional slide-on attenuated total reflection (ATR) attachment, you can analyze highly infrared-absorbant or hardto-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.

3.4.1 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.

3.4.2 Measure data 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

Vew	Measurement		Settings	RaptiR - Factory Preset	New	Periatria			More
		Session name			Tag			Step size	100
	Start Session	Final format	Absorbance	~	Resolution (cm-1)	16	~		
Ø	Autofocus before capture	Sample scans	1		Profile	Peak	\sim	Aperture height	100
		Mosaic capture	Center of glass slide	~	Analyze using	Reflection	~		
	Eject Stage	Camera profile		~	Analyze particle Enable Polarize	Transmission		Aperture width	100

2. Capture a mosaic.

Once you have captured a mosaic, you can measure the background with the crystal installed and measure you sample using the area or points tools, just like a standard reflection measurement. In general, the default **ATR Contact** settings are sufficient. If 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.	ΔTR	Contact
Figure	J-Z.	AIN	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 dis- tance 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. See <u>Analyze areas, lines, and points</u> or <u>Analyze particles</u>. The software prompts you to insert or remove the ATR crystal attachment as needed.

3.4.3 ATR clean check

Running a manual "clean check" in OMNIC Paradigm software to check the cleanliness of an ATR crystal after completion of an experiment. A comparison is made between a clean sample and a contaminated one, which is captured via peaks in the spectra. As this is a manual process, the ATR clean check involves certain necessary steps that would not be normally required. An eggshell business card would be an adequate sample for this type of check.

The process involves collecting a spectrum of your sample, then releasing the pressure (by raising the crystal off of the sample) and measuring another spectrum. If the crystal has picked up contamination from the sample, such as oil or adhesive residue, the spectrum of the crystal will show residual peaks. This then requires cleaning the crystal.

To run an ATR clean check

- 1. Place your sample and set your parameters (Analyze using should be set to ATR).
- 2. Go to Microscope Setup and select Camera View. You will see a live video of your sample. (Do not start a session yet).
- 3. Select Change Objective to set it to 15x.
- 4. Under View Tools, select the middle icon and choose Show Crosshairs to aid in seeing your target.
- 5. Capture a single-shot mosaic.

Figure 3-3: Add single image to the mosaic



6. Close Camera View and go to ATR Contact.

Note A message appears at this point if the ATR crystal attachment is not installed. If this occurs, simply insert the attachment and select **OK** (the option will be unavailable until the attachment is inserted).

Figure 3-4: ATR attachment pop-up message

ATR Insert			
	quired for the	e next operation, please i	nsert the
	mple position		isert the
			insert the

7. Under the **ATR Contact** menu, set the target pressure and release distance. The suggested settings for the purposes of the check are a target pressure of 50 and a release distance of 3500 microns, but you are free to set different values if preferred. A large release distance ensures that the crystal comes clear of the sample.



Figure 3-5: ATR Contact menu (setting target pressure)



- 8. <u>Measure a background spectrum</u>. You can do this using the **Background** tool in the floating toolbar. Accept and measure the background as normal, selecting **Accept Background** and **Measure Background** from the side menu.
- 9. When the background is complete, select **Contact** under the **ATR Contact** menu to manually bring the sample into contact with the ATR. This does not move the stage; the ATR crystal should not be in contact with the sample while

collecting the background.

10. Select Measure Now to measure the spectrum.

Note The **Measure Now** function stores your data under Measurements in the OMNIC Paradigm software dashboard, where you are able to load and review them.

11. Select **Release** under the **ATR Contact** menu to release the crystal, then select **Measure Now** again to obtain a measurement of the crystal itself.

Examine the spectrum from the released crystal. If there are peaks present, it indicates contamination from the sample. Shown here is a residual oil. If the spectrum is essentially flat, you can continue. Otherwise, you should clean the crystal before proceeding.

Figure 3-6: Residual oil sample



3.5 Right side emission

Coupled with an iS50 spectrometer, your microscope can be configured for right side emission (RSE) operation with a "passport synchro" in order to allow your microscope to analyze emitter samples.

CAUTION Avoid eye hazard. Light emitted by the sample has a path to the eyepiece. If your sample emits hazardous light, please take appropriate precautions to ensure no one uses the eyepiece while the sample is emitting.

Note By this method, right side emission is achieved from the perspective of your spectrometer as opposed to the microscope.

To enable right side emission measurements, the RaptIR microscope must be attached on the right side of a Nicolet iS50 spectrometer, which must be equipped with the passport synchro option. The data will be collected using the detector in the iS50, not the microscope detector. As such, this setup directs the beam from the microscope "backwards" to the spectrometer, where it will be modulated and detected.

To analyze using right side emission

- 1. Open OMNIC Paradigm software and go to **Connectivity > Nicolet iS50** to connect to the spectrometer. Then go to **Configure > Sample Location > Main Compartment**.
- 2. In the dashboard, set the Source setting to **Right collimated**. This will move the spectrometer's internal mirrors to allow the beam to properly travel for right side emission.
- 3. Use the Joystick to focus and center your sample. To change objectives, select **Camera View** on the toolbar and select your desired objective in the virtual controls.

You may now measure your data as needed (see Analyze samples).

3.6 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.

3.6.1 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.

- 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.

3.6.1 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.





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.

3.6.1 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.

Microscope

Camera View

ATR Contact

Polarizer

Camera View

View Tools

View Tools

View Tools

Image: Contact

View Tools

Image: Contact

View Tools

Image: Contact

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.

3.7 Verify microscope performance

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

3.7.1 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 Qual- ification	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.

3.7.2 System Status

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



Table 3-2: System status icons

lcon	Icon with Security Suite installed	Description
v	V	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.
•	Ų	A yellow icon means there may be a problem with the instrument, such as the following: The detector is warm The instrument is not scanning The instrument is not connected Click the system status icon for more details about the problem. Also visually inspect the instrument and connection.
8	8	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.

3.8 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.

3.8.1 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

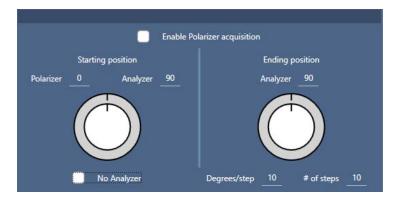
Setting	Description
Visual	Select to enable the visual polarizer.
Spectral/IR	Select to enable the IR polarizer.
Link dials	If selected, the polarizer and analyzer rotate at the same rate. Changing the angle for one also changes the other.
Polarizer angle	Set the angle of the polarizer.
Analyzer angle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

3.8.2 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.					
Starting position	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.					
	Analyzer: The analyzer ending angle.					
Ending position	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.					

3.8.3 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.

3.9 Single-point data measurement

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

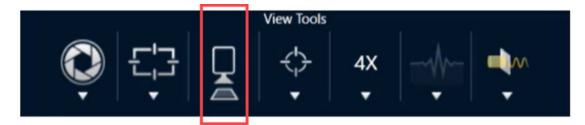
You can use the Measure Now button in OMNIC Paradigm software to measure data at the point currently shown in the live camera view. The new measurement is then automatically added to the measurement list on your dashboard.

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.

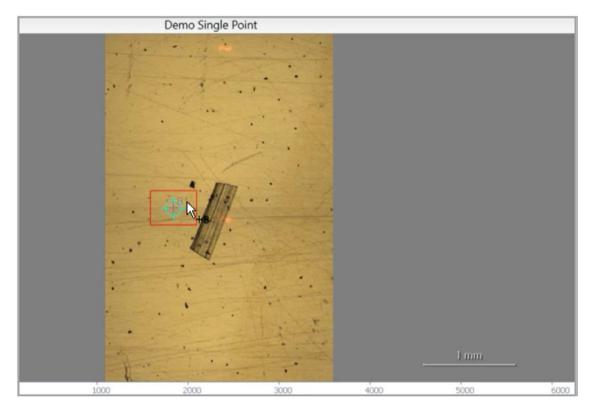
Figure 3-2: Change Objective (15x)



4. Select the Background button.

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

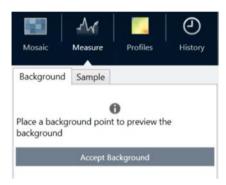
Figure 3-3: Single-point background



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.

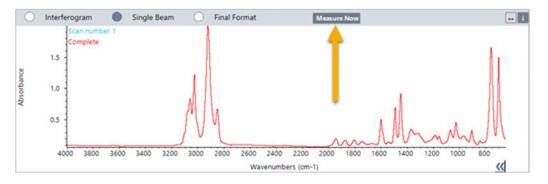




- 5. Select Measure Background and wait for the process to complete.
- 6. Move your camera view back onto your sample. You can do this by using the Camera View arrows.
- 7. Select Measure Now to measure your sample data.

3. Operation

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, nor the session. As such, trying to reopen the same session again will yield no data. The data must be accessed directly.

3.10 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.

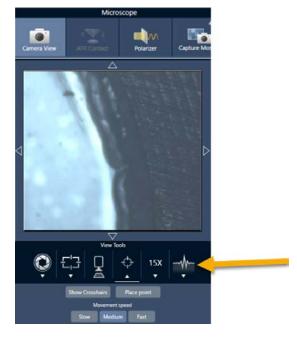


Figure 3-1: Camera View live spectra

- 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.

3.11 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

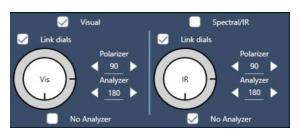
 Illuminate and bring a sample into focus, then capture a 15x mosaic. For more information, see the <u>Move the stage and</u> <u>bring the sample into focus</u> and <u>Capture a mosaic</u> sections. When the reflection and condenser image are properly focused, the view will show a bright light.

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.

 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 <u>Using the polarizer</u>.

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



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



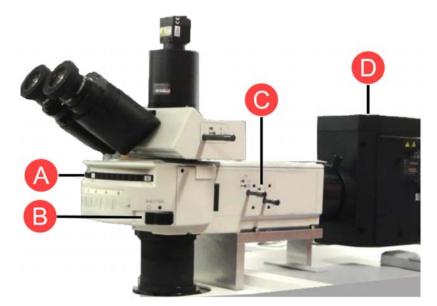
3.12 Fluorescence illuminator (optional)

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.
В	Shutter: Switch on or off to block the light or allow it through.
С	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.

3. Operation



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 Microscope Total Contact Change Objective Capture Mosaic Capture Mosaic Live Spectrum

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.

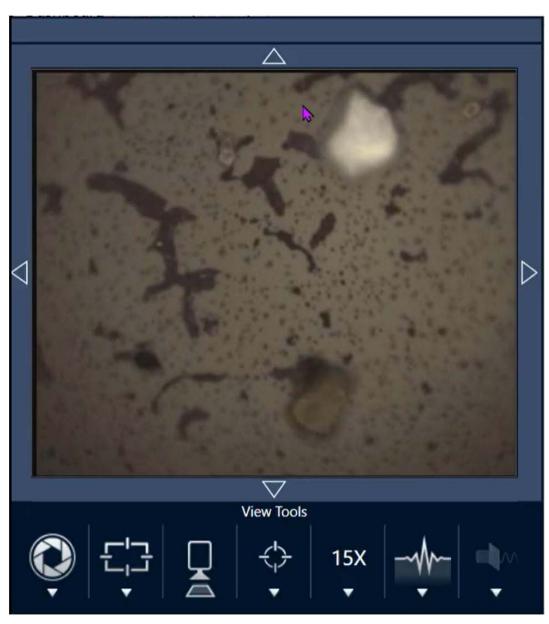


Figure 3-5: Dyed polystyrene beads using 15x objective (closed shutter)

3.13 Using the Linkam stage in Transmission mode

Your microscope supports the use of a Linkam stage to let you analyze samples in an environmental chamber. In this configuration, the standard stage is removed and the Linkam stage is installed directly on the dovetail mount.

NOTICE

Before you begin operations, wait three to five minutes for the LVDT to initialize after you turn on the microscope.

Note This configuration is fixed and does not allow for x-y movement of the Linkam stage.

3.13.1 Inserting and removing the Linkam stage

The Linkam stage configuration is installed and assembled by a Thermo Fisher representative, but you have the ability to remove the stage and reinsert it as needed to swap between stages. If you have any questions not covered by these instructions, contact us.

To insert the Linkam stage

- 1. Remove the standard stage if it is currently mounted. (See Inserting and removing the standard stage.)
- 2. Ensure that the Linkam stage is sitting flat on the dovetail mount and line up the alignment pins on the bottom of the stage to the to the holes in the mount, then push down. If the stage is correctly lined up, it snaps down with minimal force.
- 3. Tighten the front knob with your hand, finger-tight, to secure the stage.

Figure 3-1: Front stage knob



4. Configure the Linkam stage. Go to Configure > Instrument > Microscope and select RaptIR with Manual Stage. Then restart OMNIC Paradigm software.

3. Operation

OMNIC File	Paradigm Acquire Data	Display	Process	Identify	Configure Help								🕺 Nicolet ISSO Left RaptiR	
ashboard	Microscope Setup	Settings	Centered	Monocope Selup	Sample Location Connectivity	•		10	*	3				
-	rement Settings	Session n			Database Database Maintenance	. TEC-)	MCT - Factory P	veset	V New	ktaw.	Sast Dealer	Step size	100	More_
	Start Session utofocus before cap	Final form	uat ,	Absorbance 8	Options Instrument		ce Settings	(cm-1)	16 Peak	× ×		Aperture height	100	
	Eject Stage	Mosaic ca		Center of glass slide	Qualification Standards		ctor Settings ascope	ing yze parti	Transmission	~				
		Camera p	rofile	Auto	~		0.	Enable Polari	zer acquisition			Aperture width	100	

Figure 3-2: OMNIC Paradigm software Microscope option

Figure 3-3: Configure Microscopes - manual stage option

Configu	re Microscopes	×
0	None	
0	iN5	
0	RaptIR	
	RaptIR with Manual Stag	e
	Save Cance	I

To remove the Linkam stage

1. From the OMNIC Paradigm software dashboard, set **Analyze using** to **Reflection** or **ATR**. Doing this drops down the condenser, giving you the space you need to remove the stage.

Figure 3-4: Condenser up



3. Operation

Figure 3-5: Condenser drop



2. Loosen the front knob with your hand (counterclockwise).



Figure 3-6: Front stage knob

3. Remove the Linkam stage. If it is sufficiently loosened, it should snap out of position with an audible pop. If you cannot remove the stage, simply loosen the knob further.

Figure 3-7: Removing the Linkam stage



To best protect your Linkam stage when not in use, store it in a secure location in its original packaging, if available. You may leave the black mounting ring attached.

3.13.2 Inserting and removing the standard stage

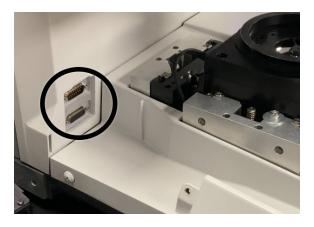
You can reinstall the standard RaptIR+ stage as needed when you do not have use for the Linkam stage.

To insert the standard stage

1. Use a Phillips screwdriver to open the side cover on your microscope.

Figure 3-8: Side cover removal





2. With the cover removed, connect the standard stage cables. The cables and connectors are male and female, so they cannot be installed backwards. Once connected, use a small flathead screwdriver to tighten the cable set screws. Do not over-tighten.



- 3. Take the standard stage and align it onto the dovetail mount (make sure the front stage knob is properly loosened), then press it down. The stage will pop into place.
- 4. Secure the stage by tightening the front knob, finger-tight, until secure.
- 5. To configure the standard stage, go to **Configure > Instrument > Microscope** and select **RaptIR**.

Close and restart OMNIC Paradigm software.

Figure 3-9: OMNIC Paradigm software Microscope option

OMNIC Paradigm ile Acquire D	ata C)isplay I	Process Identify	Configure Help							🤣 Nicolet iSSO Left RaptiR	
hboard Microscope S	etup X	Circle Series	Confloard Microscope Seller	Sample Location Connectivity Database	•	TO	*	3				
Measurement Se	ttings			Database Maintenance	TEC-MCT - Factory Pre	iset	∼ New	Artana 2	an Duite			More_
Start Senie	20	Session name Final format	Absorbance	Options		s(cm-1)	16	~		Step size	100	
Autofocus bef	1	Sample scans	a	Instrument Qualification Standards	Source Settings Detector Settings	(and (Peak	~		Aperture height	100	
Eject Stag	•	Mosaic capture	Center of glass slide	Quanticasion Standards	Microscope	ing yze parti	Transmission	~				
		Camera profile	Auto	~	0.0	-	zer acquisition			Aperture width	100	

Figure 3-10: Configure Microscopes - RaptIR option

Configu	re Microsco	pes	×
0	None		
\bigcirc	iN5		
•	RaptIR		
0	RaptIR with	Manual Stage	
	Save	Cancel	

If the stage is properly installed and configured, the software asks if you want to initialize the stage. Select OK to continue normal operation.

To remove the stage, simply follow the physical steps above in reverse order or see the section below.

To remove the standard stage

- 1. Loosen the set screws on the connector cables.
- 2. Disconnect the connector cables.
- 3. Loosen the front knob on the standard stage.
- 4. Remove the standard stage by pulling it up and out of position. If you cannot remove the stage, simply loosen the knob further.

3.13.3 Using the Linkam stage

This section describes how to set up a measurement in Transmission mode using the Linkam stage and OMNIC Paradigm software.

1. Make sure the stage is configured. If not, go to Configure > Instrument > Microscope and select RaptIR with Manual Stage.

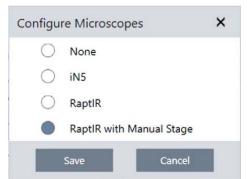


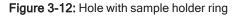
Figure 3-11: Configure Microscopes message

- 2. Use the stage knob to adjust the sample holder ring and bring it closer to the center.
- 3. Set Analyze using to Transmission.

Note If the condenser is physically all the way up, you can temporarily set **Analyze using** to **ATR** in order to drop the condenser down, allowing you to move the stage. Make sure to go back to **Transmission** afterward.

- 4. Go to Microscope Setup and select Camera View.
- 5. Set your objective to 4x.
- 6. Use the Joystick to turn up illumination and raise the stage. (You can also do this using the virtual controls in Microscope View). Do this until you can see the focused image in the Camera View.

You see a clear image of the hole and sample holder ring. Move the sample holder ring out of the way to achieve a clear view of the hole.





3. Operation

Figure 3-13: Clear image of hole



Note At this point, do not use the z-axis controls as doing so will defocus your image.

- 7. Switch to the 15x objective. The screen goes dark.
- 8. Select the Condenser option and set it to about 40, 000. You will see light from the Transmission illuminator.
- 9. Turn the Reflectance illuminator all the way down.
- 10. Using the lower iris control knob to the right side of the stage, close the iris slightly. The iris edges indicate when you are in focus. Be sure to use the lower iris knob and not the one on the top.
- 11. Focus the condenser image until the edges of the iris become clear and sharp.

3. Operation

Figure 3-14: Focused iris condenser image



Note At this point, do not manipulate the objective or condenser

- 12. Select **Show Crosshairs** in the Camera View. This step is optional, but it shows the precise location where the system will perform its analysis.
- 13. Go to Live Spectrum.

You are now ready to insert your sample and measure data as normal.

Note You may move your sample in the x and y directions using the micrometers on the Linkam stage. Do *not* adjust the Z location as this will defocus the system. The x and y Joystick options will have no effect.

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

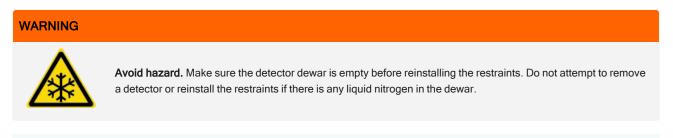
If your instrument shows any of these symptoms, the detector dewar may have a vacuum leak. Contact us immediately for assistance.

4.3 Storing and shipping your detector

Your detector can degrade over time, resulting in performance issues and the need to re-pump the dewar.

This section includes instructions on how to store and ship your detector to Thermo Fisher for re-pumping, service, and maintenance. A general recommendation is to get your detectors re-pumped every seven years, though this can vary with usage.

The detector shipping restraints must be reinstalled prior to storing the detector for shipment.



Note If you are simply storing your detectors on a shelf and do not plan to ship them to Thermo Fisher for service, it is not necessary to reinstall the shipping restraints. Only reinstall the restraints if you plan to ship your detectors for pumping and maintenance.

If you have lost your detector's original shipping restraints, contact us to order a replacement.

To reinstall the detector restraints

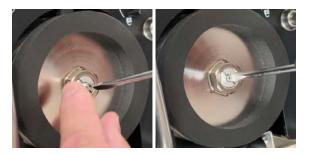
1. Insert the larger restraint piece into the detector's dewar fill port and screw it in until snug.

Figure 4-1: Reinstalling the larger restraint



2. Insert the smaller shipping restraint piece into the hole and screw it in until snug.

Figure 4-2: Reinstalling the smaller restraint



When both pieces are installed and secure, you are ready to store your detector for shipping.

Contact us (or a Thermo Fisher representative in your area) for details on how to ship your detectors to us.

Detector storage

- Store your detectors at room temperature in their originally supplied packaging. Place them on a flat, level surface.
- Make sure the detector dewars are empty before storage.
- If your detector did not come with packaging, or if you have lost your packaging, please contact us to request it.

5. Troubleshooting

5. Troubleshooting

Problem	Possible cause	Solution
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.	The objective is loose.	The objective can sometimes become loose. This usually happens when inserting and removing the ATR attachment. If the objective feels loose, hand-tighten the objective. The slot for the ATR attachment should face directly to the front. Do not overtighten the objective, and do not use the ATR attachment as a lever to tighten the objective. Ovetightening the objective will damage it. If the objective feels snug and you are still having alignment issues, contact your service representative.

Problem	Possible cause	Solution
<text></text>	Your display scaling set- tings are not compatible with the soft- ware.	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%. For help changing your display settings, see Windows help information.
The mosaic or camera view are completely black.	The camera is not connected.	Verify that the camera cable is plugged into the nose piece. Verify that the microscope USB cable is plugged into the USB 3.0 connector.
The system status shows a yellow or red icon.	There may be a problem with the instrument or with soft- ware services.	See <u>System</u> <u>Status</u> for more information.

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 out online knowledge base.