

thermo scientific

# Nicolet iN5 with OMNIC Paradigm software



## User Guide

269-3641 00

Revision A

November 2023

**ThermoFisher**  
SCIENTIFIC

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## WARNING



Avoid an explosion or fire hazard.

This instrument or accessory is not designed for use in an explosive atmosphere.

# Introduction

The Thermo Fisher Scientific™ Nicolet™ iN5 microscope lets you quickly and easily collect spectra of Transmittance (transmission), Reflectance (reflection), and ATR samples, with continuous viewing of the sample during collection.

You should be familiar with the operation of your spectrometer and system software before using the microscope. You can use the microscope with Thermo Fisher Scientific OMNIC Paradigm software.

The Nicolet iN5 microscope must be installed as part of a Thermo Fisher Scientific spectrometer system and can be used only after it is properly connected to the spectrometer.

## NOTICE

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

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## Conventions

### 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.

**Tip** Provides helpful information that can make a task easier.

# Contacting us

For technical support, [contact us](#).

If you have problems with your system, refer to the [Troubleshooting](#) section for help. If you require additional help, contact technical support.

To order parts, or to send an instrument or accessory to us for repair, contact us for shipping requirements and other instructions.

# Microscope basics

This section describes the major components of your microscope as well as how to turn on the microscope power, calibrate the video image, focus a sample, and move the stage horizontally.

## Microscope components

The following illustrations identify some important components of the microscope. Refer to these illustrations when you use other sections of this manual.

Figure 2-1: iN5 components (side view)

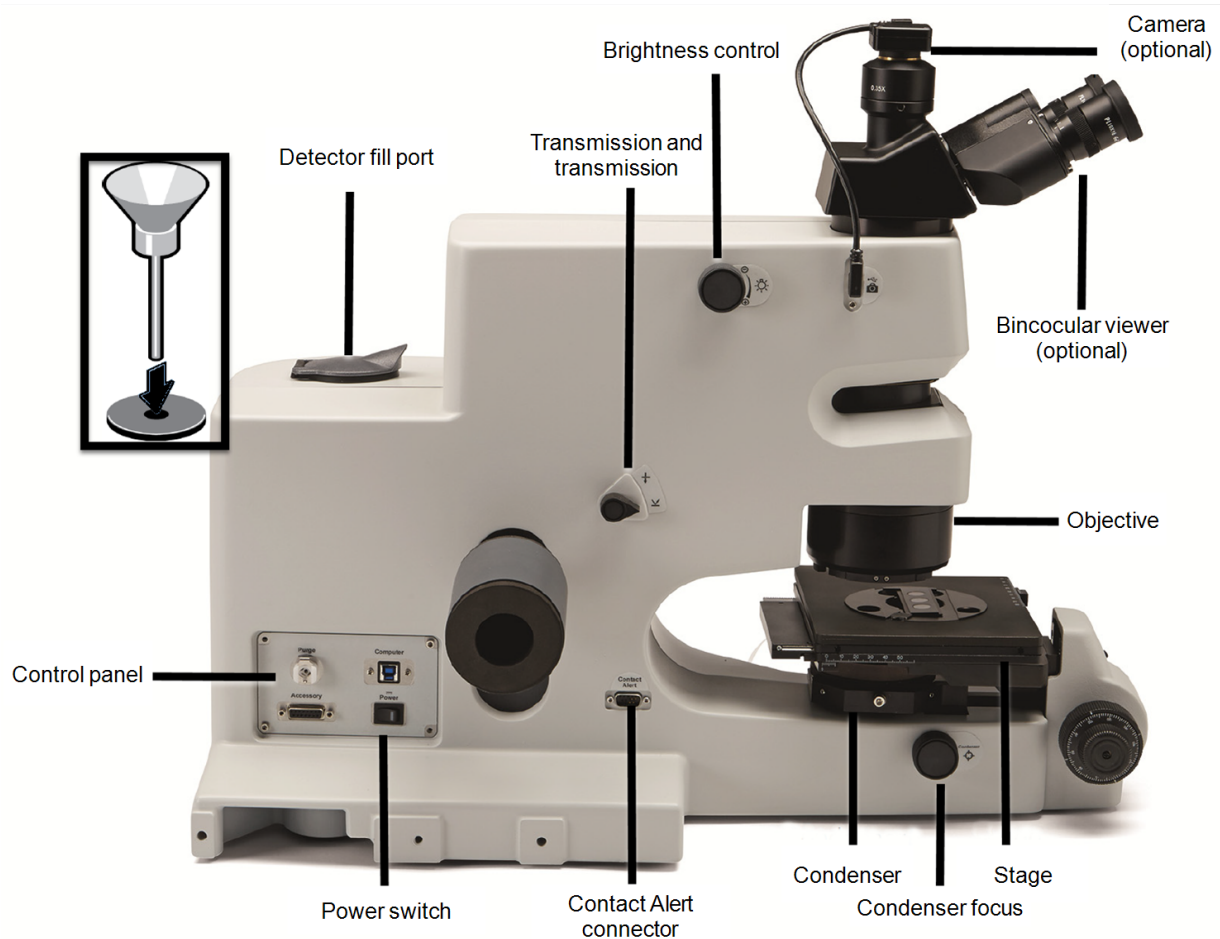
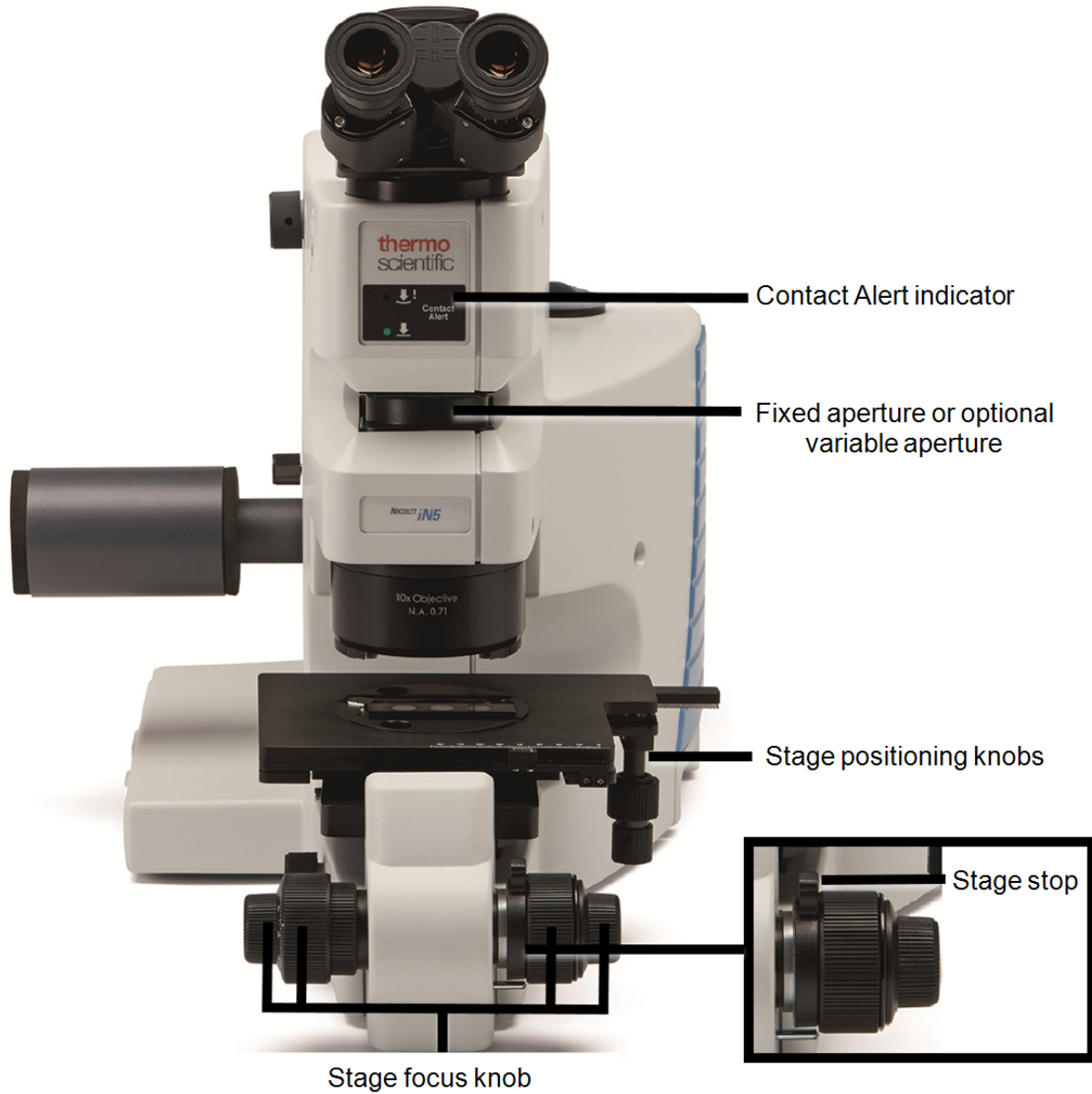


Figure 2-2: iN5 components (front view)

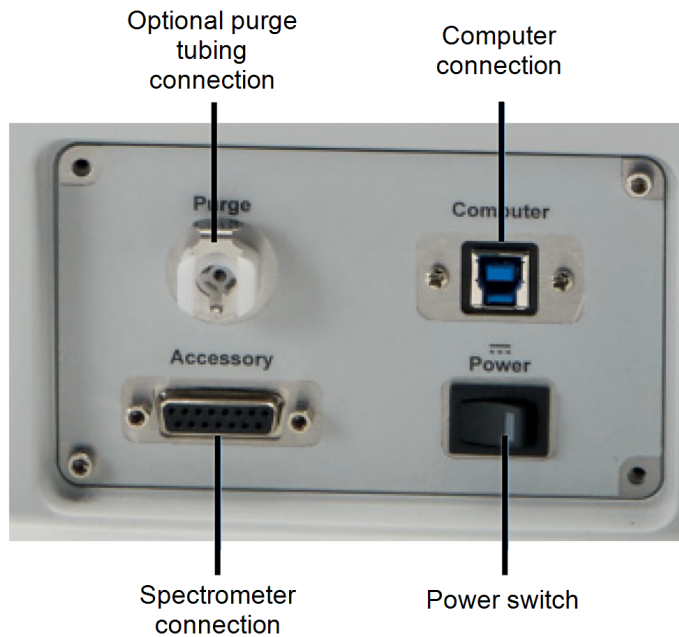


## Control panel

The control panel is on the left side of the microscope.



Figure 2-3: iN5 control panel



## Turning on the microscope

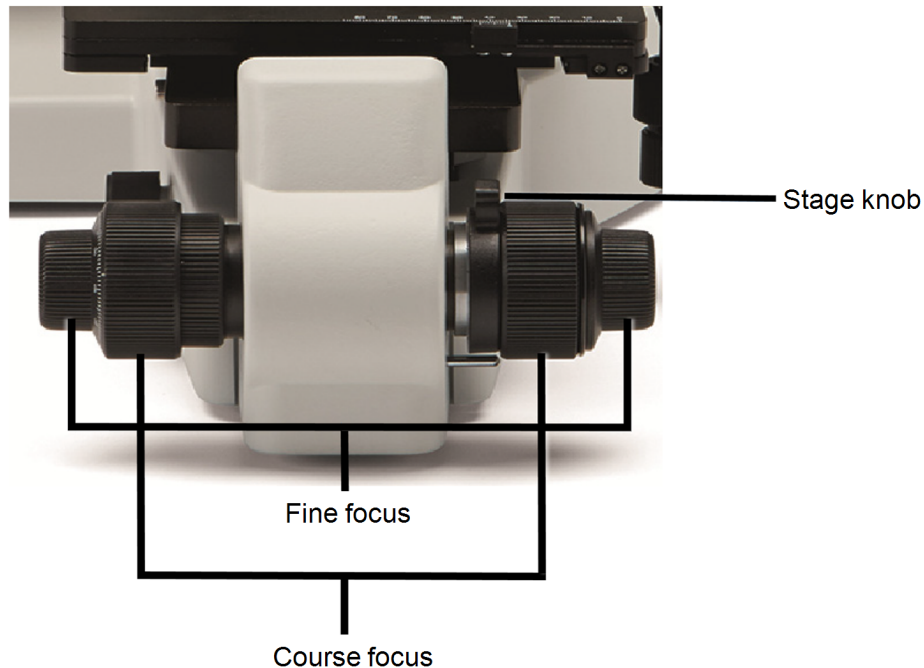
It is advisable to keep the spectrometer power and microscope power on at all times, unless your building is subject to power outages or if you need to perform a service or maintenance procedure. Leaving the system on keeps it equilibrated with the surroundings and gives you the most consistent results.

To turn on the microscope power, set the power switch to **ON**.

## Focusing

The Course focus and Fine focus knobs on the left and right side of the microscope let you raise or lower the stage (in the Z direction). Use these knobs to lower the stage before installing a sample and to bring a sample into focus.

Figure 2-4: iN5 course, stage, and fine focus knobs



- When focusing, first use one of the coarse focus knobs to bring the sample into approximate focus
  - The easiest way to do this is to look directly at the sample rather than through the viewer or at the video image
  - You should be able to see a small, bright spot of light on the sample when it is close to the focal point
  - Look through the viewer or at the video image (if you have a camera option) and use one of the fine focus knobs to focus the sample sharply
  - You can activate your camera by selecting **Live Display** in OMNIC Paradigm software

## Setting the stage stop

Next to the focus knobs on the right side of the microscope is a ring used for setting a vertical stage position. You can use this ring both as a stage stop that lets you quickly return to a stage position and as a safety measure to protect the microscope hardware.

◆ **To set the stop**

1. Use the Stage focus knobs to adjust the vertical position of the stage.

Normally this is the position at which a sample is in sharp focus.

2. Lift the Stage stop handle until it feels tight.

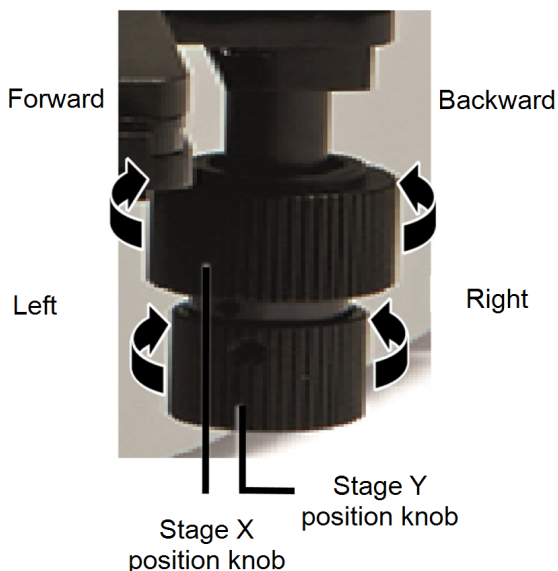
You can now move the stage downward—for example, to change samples—and then quickly return the stage to its former vertical position by turning the Course focus knob until the stage stops.

You can also use this feature to protect the objective and stage. First carefully move the stage upward until it is slightly below the bottom of the objective. Then lift the stop handle until it feels tight. With the stop set, you can begin working with samples without worry of hitting the objective accidentally when you raise the stage.

## Moving the stage horizontally

If you have the standard manual stage, use the stage positioning knobs to move the stage in the X and Y directions, as shown.

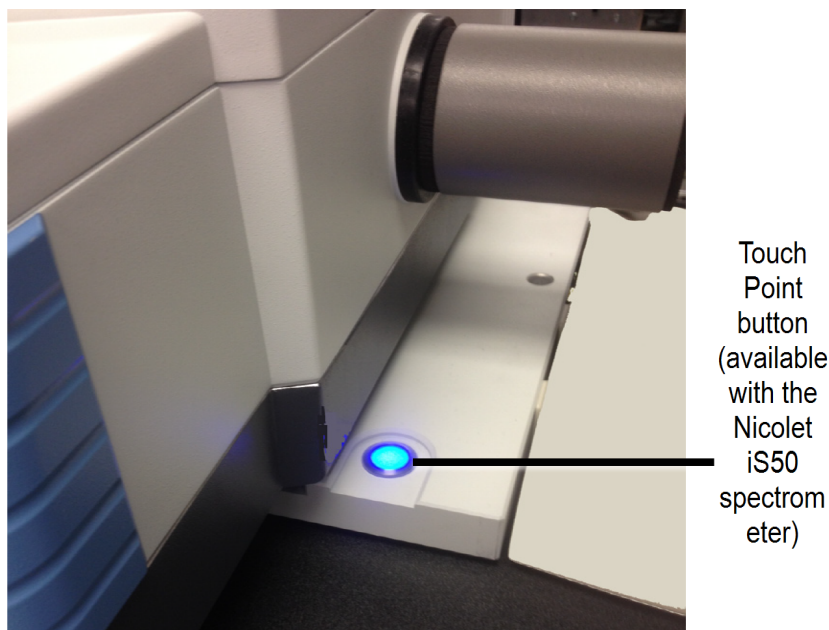
Figure 2-5: Moving the stage



# Touch point

The iN5/iS50 interface bracket contains a Touch Point button that allows the user to easily switch to the microscope beam path. Additionally, the button enables easy integrated sampling while viewing your sample through the eyepieces. This button automatically configures the spectrometer for microscope sampling.

Figure 2-6: Touch Point



Touch Point button (available with the Nicolet iS50 spectrometer)

The Touch Point LED shows the status of the ATR module and has three states:

LED status	Meaning
On	System is ready for use
Off	System is not configured for microscope sampling.
Blinking	Optics are reconfiguring

The microscope spectral range options are the same as the spectrometer's and dependent on spectrometer configuration.

Refer to the spectrometer help topics or iS50 ATR Module user guide for more information.

# Before you measure spectra

This section provides necessary information on what to do before measuring data and collecting spectra.

## Turn on the system components

We recommend that you keep the spectrometer power and microscope power on at all times, unless the building is subject to power outages or you need to perform a service or maintenance procedure. Leaving the system on keeps it equilibrated with the surroundings and gives you the most consistent results.

**Note** Always follow the safety precautions described in this manual and in the spectrometer site and safety guide that came with your system.

Your microscope uses power from your spectrometer.

1. Turn on the microscope power.

See power switch in the section. If the microscope has been off for several hours, allow at least one hour for its temperature to stabilize for best performance.

2. Turn on the computer.

Turn on the printer and computer as explained in the documentation that came with those components.

## Cool the detector

If your microscope is configured with an MCT detector, the detector requires cooling with liquid nitrogen before you can collect infrared spectra.

If your microscope has been configured with a DTGS detector, no cooling is required and you may skip this procedure.

## WARNING



**Avoid freeze burns.** Liquid nitrogen is extremely cold and therefore potentially hazardous.

- Wear protective equipment and follow standard laboratory safety practices.
- To avoid hazardous contact with liquid nitrogen, make sure that any dewar or container used to hold liquid nitrogen can do so safely without breaking.
- When filling the dewar, be careful not to contact the liquid nitrogen with your skin. Fill the dewar slowly. Cooling the detector too quickly may cause the dewar to rapidly boil off liquid nitrogen.

1. Fill a laboratory dewar with approximately 750 mL (26 fluid ounces) of liquid nitrogen.

Less liquid nitrogen is needed if the detector was cooled recently.

2. Open the Detector fill port and insert the stem of the provided laboratory funnel into the hole in the Detector fill port; slowly pour the liquid nitrogen into the lab funnel until the detector dewar is full.

The warm funnel and detector may cause the liquid nitrogen to boil rapidly.

- After you have filled the lab funnel three times, allow the detector dewar to cool for one to two minutes before adding more liquid nitrogen.
- Continue filling the detector dewar by slowly pouring the remaining liquid nitrogen into the funnel.
- The dewar is full when liquid nitrogen no longer drains into it.
- For best results, allow the detector to stabilize for at least 20 minutes before analyzing samples.
- The Thermo Scientific stainless steel dewar detector will remain cold for up to 18 hours.
- You can add liquid nitrogen as needed at any time except when you are collecting infrared spectra.

## Start OMNIC Paradigm software

OMNIC Paradigm software is Thermo Fisher Scientific's streamlined material analysis software. To start the software, double-click the icon on your desktop.

**Note** A Thermo Fisher representative will configure your microscope to work with your spectrometer. *There is no need for a user to change the configuration setting thereafter.*

If your device does become mis-configured, go to **Configure > Instrument > Microscope > iN5** in OMNIC Paradigm software, then reboot your computer.

The preset factory settings and parameters for the iN5 populate automatically, but you may create your own settings based on your specific microscopy needs. For additional information, consult the [OMNIC Paradigm User Guide](#) in the Thermo Fisher Scientific Knowledge Base.

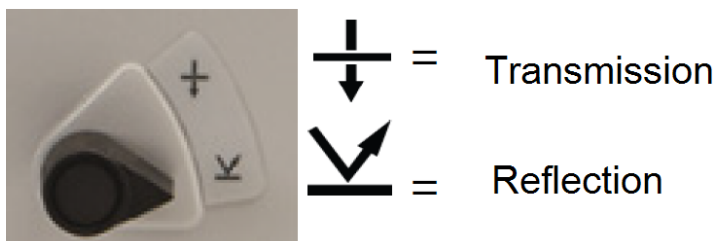
## Set up the binocular viewer

It is important to set up the viewing system for optimal performance before measuring data. This includes selecting the correct final format and manipulating the brightness of the image.

### ❖ To set up the binocular viewer (optional)

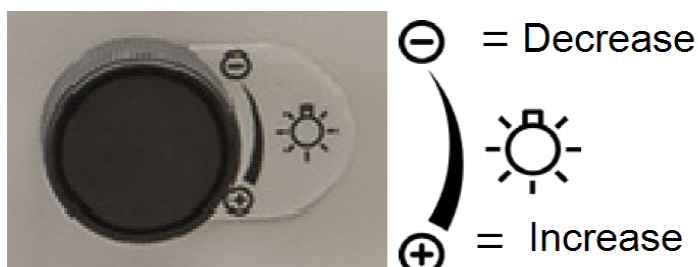
1. Set the microscope for reflection by turning the reflection and transmission knob.

Figure 3-1: iN5 transmission and reflection knob



2. Position a sample under the objective, and rotate the brightness control to adjust the illumination to a comfortable viewing level.

Figure 3-2: iN5 brightness control



Before you measure spectra

3. Use the Stage focus knobs to obtain a sharp image of the sample.
4. Grasp the base of both eyepieces and move them until they are aligned with your eyes as you look through the viewer.

Figure 3-3: Eyepiece adjustment



You should see a single, clear image of the sample through the viewer when the eyepieces are positioned correctly.

When you are finished, look at the reading on the scale between the eyepieces (the white dot indicates the numerical value). In the future you will be able adjust the eyepieces quickly by moving them to this reading.

5. While looking through the right eyepiece with your right eye, use the stage focus knobs to focus the sample image.
6. While looking through the left eyepiece with your left eye, turn the eyepiece until the image is in focus.



Figure 3-4: Turning the left eyepiece



This sets the diopter, ensuring that the sample image will be in focus for both your eyes. The correct diopter setting will vary from person to person because of vision differences.

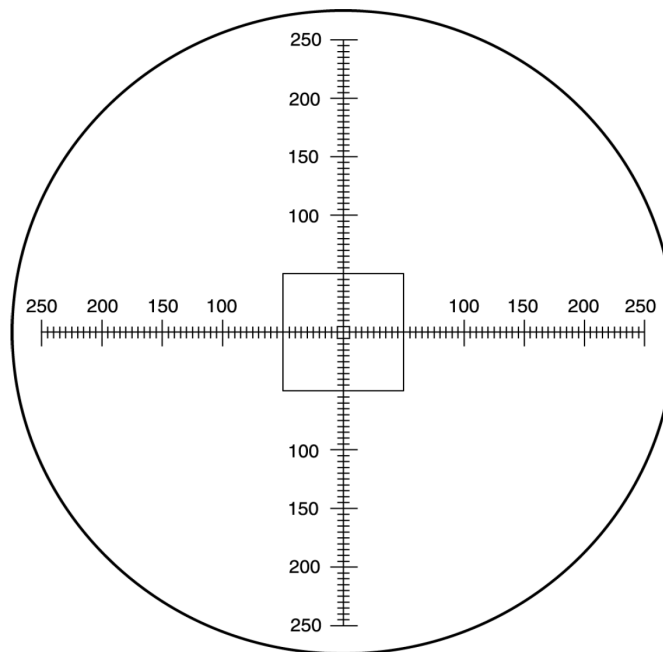
7. While looking through the right eyepiece, focus the reticle by holding the inner ring with one hand and turning the outer ring until the reticle cross hairs are clear.

Figure 3-5: Focusing the eyepiece reticle



You can rotate the entire right eyepiece to orient the cross hairs as desired. Normally the cross hairs are positioned as shown.

Figure 3-6: iN5 crosshair



You can use the reticle to measure distances on sample surfaces; the numerical scale is in micrometers. The square at the center of the reticle is the reticle reference square. You can use it when adjusting the aperture to the size and shape of a square 100  $\mu\text{m}$  on a side.

# Run performance verification workflows

Verify that your microscope is working well by running qualification and performance verification (PV) workflows as well as checking the system status in OMNIC Paradigm software.

Test	Description
<b>Nicolet iN5 - Factory Qualification</b>	Run this workflow to check performance for Transmission and Reflection.
<b>Nicolet iN5 - Factory ATR MCTA Qualification</b>	Run this workflow to check performance for ATR.
<b>Nicolet iN5 - CP Qualification</b>	Run this workflow to execute qualification tests for the microscope as defined in the Japanese Pharmacopoeia.
<b>Nicolet iN5 - JP Qualification</b>	Run this workflow to execute qualification tests for the microscope as defined in the Chinese Pharmacopoeia.
<b>Nicolet iN5 - PHEUR ATR MCTA Qualification</b>	Run this workflow to execute qualification tests for the ATR accessory as defined in the European Pharmacopoeia.
<b>Nicolet iN5 - PHEUR Qualification</b>	Run this workflow to execute qualification tests for the microscope as defined in the European Pharmacopoeia.
<b>Nicolet iN5 - PV Test</b>	Run this workflow to measure the basic performance of the microscope based on factory recommended tests.

Test	Description
Nicolet iN5 - USP Qualification	Run this workflow to execute qualification tests for the microscope as defined in the US Pharmacopoeia.



❖ To run a PV 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.

## Check system status

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

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

Icon	Icon with Security Suite installed	Description
		<p>A yellow icon means there may be a problem with the instrument, such as:</p> <ul style="list-style-type: none"><li>• The detector is warm</li><li>• The instrument is not scanning</li><li>• The instrument is not connected</li></ul>
		<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.</p>

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# Basic measurements

This section will get you started measuring spectra with your Nicolet iN5 microscope. You will learn how to measure spectra of a solid Reflectance sample, a fiber Transmittance sample, and an ATR sample. We recommend performing all three data measurements in order to learn the most about using the system.

## ❖ Before you begin

- Make sure the spectrometer power and microscope power are turned on (see [Turn on the system components](#) for details)
- Make sure the detector is cool, if applicable; the DTGS detector does not require cooling (see [Cool the detector](#) if needed)
- Start OMNIC Paradigm software if it is not already running
- See [Before you measure spectra](#) for details on system components, software, tests, and operation

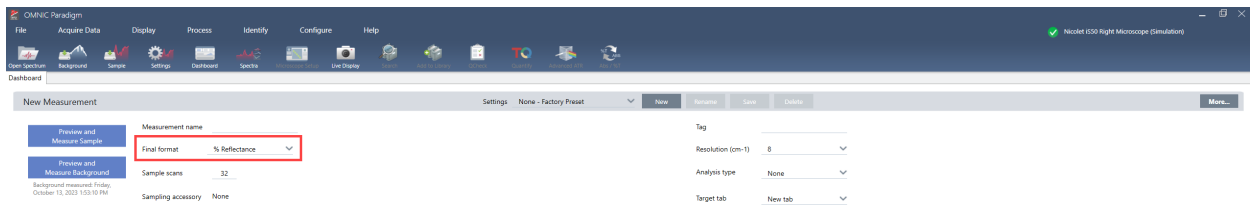
## Reflectance measurement

When the microscope is set for Reflectance analysis, you can use it to analyze samples that reflect infrared light. Typical infrared-reflecting samples include films and coatings on metal substrates. The sample must be on a reflective substrate to allow you to measure high quality Reflectance data.

For this experiment you will need to prepare a sample slide with a thick ink spot from a permanent marker.

1. Open OMNIC Paradigm software. In the dashboard, set **Final format** to **% Reflectance**.

Figure 4-1: Final format - % Reflectance



2. Set the microscope for Reflectance by turning the knob on the left side.

This directs both the visible light and infrared beam downward through the objective onto the stage.

3. Turn the illumination to proper intensity using the brightness control dial.

Proper illumination will make it easier to focus the microscope.

4. Remove the fixed aperture or open the optional variable aperture all the way.

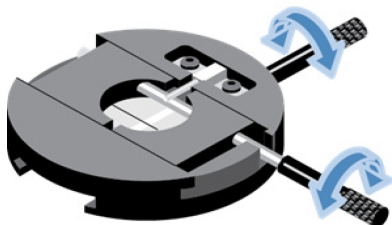
### NOTICE

- Handle the fixed aperture and optional metal variable aperture with care.
  - The blackening used to eliminate scatter can easily be rubbed off.
  - System performance can be negatively affected if the blackening is removed.
- Handle and store the apertures with care.
  - Contamination such as dust and finger oil can negatively affect system performance.
  - Use the air duster provided to gently remove any dust visible in the aperture opening.



- a. Turn the knobs until the blades are fully open as shown.

Figure 4-2: iN5 aperture knobs



- b. Replace the variable aperture in the slot when you are finished.

If you are using the fixed aperture, do not replace in the slot.

Once you are accustomed to working with the optional variable aperture, you will find it easy to adjust without removing it from the slot. You will be able to see the aperture opening change in size and shape in the video or viewer image as you turn the knobs.

5. Prepare a simple sample slide. For this first exercise, create a thick ink dot with a permanent marker on the aluminum mirrored slide provided.
6. Place the prepared sample slide containing the ink spot sample on the stage.

When you analyze your own Reflectance samples, you can simply place the sample on a slide or tape it down if it doesn't lie flat.

Depending on the sample and the background material you want to use, you may need to install the sample on a slide that has a gold or aluminum mirror for measuring the background. In some cases the material to be analyzed is on top of a reflective substrate, and an exposed area of the substrate can be used for the background measurement.

**Note** If you ever need to clean one of the provided sample slides to remove adhesive or other material, wipe the slide with a clean cotton cloth containing a small amount of methanol.

**Note** If there is not enough clearance between the stage and the objective to install a large sample, you can remove the condenser so that the stage can be lowered further (see [Removing the condenser before installing a large sample](#)).

7. Move the stage horizontally to position the sample ink spot under the objective.

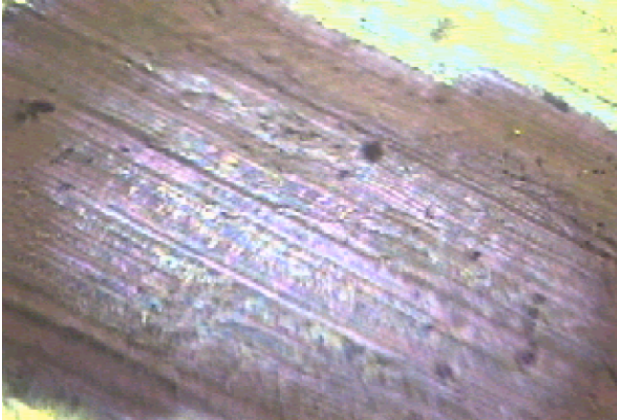
## Basic measurements

8. While looking directly at the sample (not at the video or viewer image), move the stage vertically to focus the spot of light on the sample.

Depending on the current position of the stage, you may have to move it up or down. The spot of light will be in focus when the slide is approximately one centimeter below the objective.

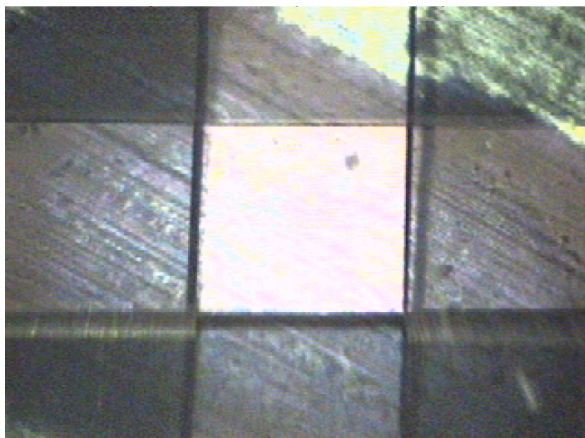
9. Move the stage horizontally to position the spot of ink under the objective.

Figure 4-3: Image of an ink spot



10. While looking at the viewer image, use the fine focus knob to sharply focus the image of the ink spot.
11. If you have a variable aperture option, adjust the aperture so that the opening is entirely within the area of the ink spot.
  - Hold the aperture in place in its slot with one hand while turning the knobs with your other hand
  - Push or pull the knobs slightly to adjust the orientation of the opening
  - You can also rotate the aperture within the slot

Figure 4-4: Image with aperture rotation

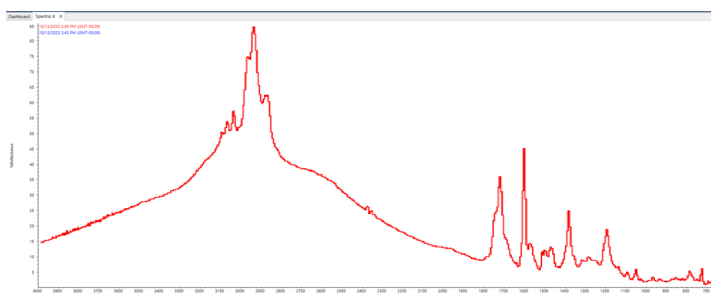


12. Move the stage horizontally so that an unmarked area of the sample is under the objective. You will use this area to measure a background for ratioing your sample spectrum.
13. In the dashboard, select **More** to see a live display of the detector signal.  
If no signal appears, see [Troubleshooting](#).
14. Select **Preview and Measure Background**. This opens a preview Spectra tab.
15. In the new tab, select **Start Background Measurement** and wait for the scans to complete.
16. Move the stage horizontally to position the ink so that it once again fills the aperture opening.  
You are now ready to measure a sample spectrum.
17. Select **Start Sample Measurement** from the Spectra tab and wait for the process to complete.

Your sample spectrum is now saved in OMNIC Paradigm software and can be accessed at any time from the Measurements menu in the dashboard.

If you run into errors or other problems during your measurement, see [Troubleshooting](#) as well as the [latest user guide for OMNIC Paradigm software](#).

Figure 4-5: Ink spectrum measurement



The locations and intensities of the peaks in the final spectrum are unique for a particular sample material.

The sloped baseline of this spectrum is due to the thickness of the ink on the sample. The baseline of a spectrum should normally be consistent and smooth.

The closer the baseline is to 0 log (1/R) units (100% reflectance), the better. This makes the band intensities easier to measure and allows you to compare spectra visually or by searching the spectrum against a spectral library.

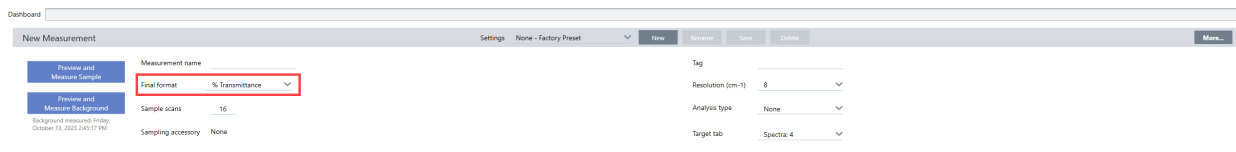
## Transmittance measurement

When the microscope is set for Transmittance analysis, you can use it to analyze samples that transmit infrared light. Typical infrared-transmitting samples include fibers, films and biological cross sections from 5 to 15 micrometers thick, depending on the application.

For this experiment we will use the provided sample slide, on which you will mount a fiber sample.

1. Open OMNIC Paradigm software. In the dashboard, set **Final format** to **% Transmittance**.

Figure 4-6: Final format - % Transmittance



2. Set the microscope for Transmittance by turning the Reflectance and Transmittance knob on the left side.

This directs both the visible light and infrared beam upward from below the stage, through the opening in the stage and to the objective.

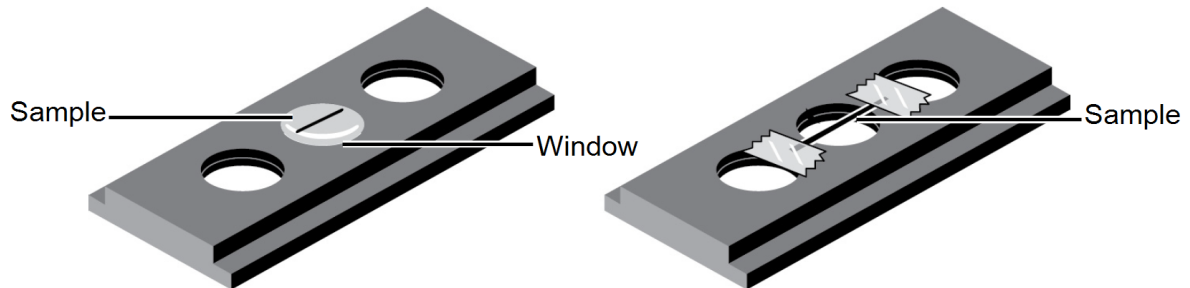
3. Turn the illumination all the way up.
4. Open the microscope aperture all the way.
5. Prepare a fiber sample and install it on the sample slide.

You can use a fiber from the provided fiber swatch or another fiber that is similar.

You can support the sample on an infrared-transparent substrate such as a potassium bromide (KBr) window or lay it across the sample slide opening, securing the ends of the sample with adhesive tape.

The sample should be thin enough to ensure that the sample spectrum will not have any totally absorbing peaks (peaks that are below 0.4 %T or above 2.4 absorbance units).

Figure 4-7: Sample slides



**Note** If you ever need to clean one of the provided sample slides to remove adhesive or other material, wipe the slide with a clean cotton cloth containing a small amount of methanol.

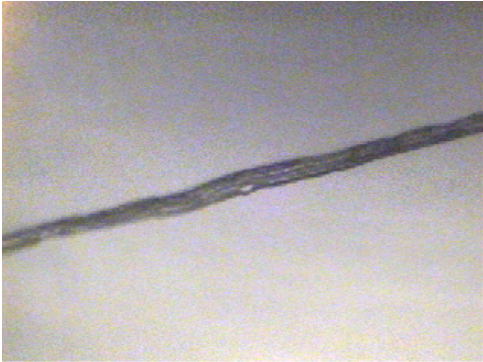
- After taping a sample to the slide as shown above, you can turn the slide over and use the roller tool provided with your microscope to flatten the sample on a hard surface.
- A fiber sample becomes wider and somewhat transparent when flattened.
  - You may need to roll a section of a fiber four or five times to flatten it.
  - If a non-flattened fiber is 50 micrometers or more in diameter, the flattened section should be visible with the naked eye.
  - If the diameter of the fiber is smaller than 50 micrometers, it may help to view the fiber under a conventional light microscope while you roll it.

**Note** If you want to measure a spectrum with the sample between two windows, use the optional compression cell as explained in the Micro Compression Cell and Micro Compression Diamond Cell Kit User Guide.

6. Move the stage horizontally to position the fiber under the objective.
7. While looking directly at the fiber (not at the video or viewer image), move the stage vertically to focus the spot of light on the fiber.

Since the fiber is very thin, you may need to move the stage horizontally as well to position the spot on the fiber.

Figure 4-8: Image of the fiber sample



8. While looking at the image, use the fine knob to sharply focus the image of the fiber.

#### NOTICE

- Handle the fixed aperture and optional metal variable aperture with care.
  - The blackening used to eliminate scatter can easily be rubbed off.
  - System performance can be negatively affected if the blackening is removed.
- Handle and store the apertures with care.
  - Contamination such as dust and finger oil can negatively affect system performance.
  - Use the air duster provided to gently remove any dust visible in the aperture opening.

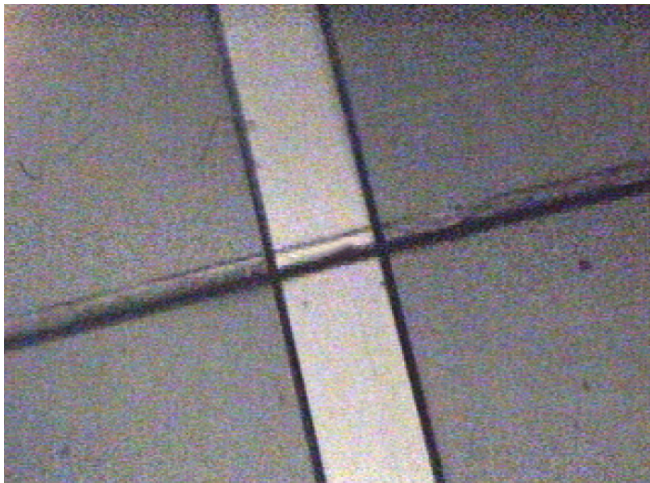
9. If you are using the standard fixed aperture:
  - a. Center the fiber in the field of view.
  - b. Insert the fixed aperture in the slot.
  - c. Move the X-Y knobs on the stage to ensure the fiber is centered in the aperture. the aperture so that the opening is entirely within the area of the fiber.

With the fixed aperture installed, you may need to move the stage from side-to-side to see the edges of the fiber to ensure the desired portion of the sample is centered within the aperture.

10. If you have an optional variable aperture, adjust the aperture so that the opening is entirely within the area of the fiber.
  - Hold the aperture in place in its slot with one hand while turning the knobs with your other hand

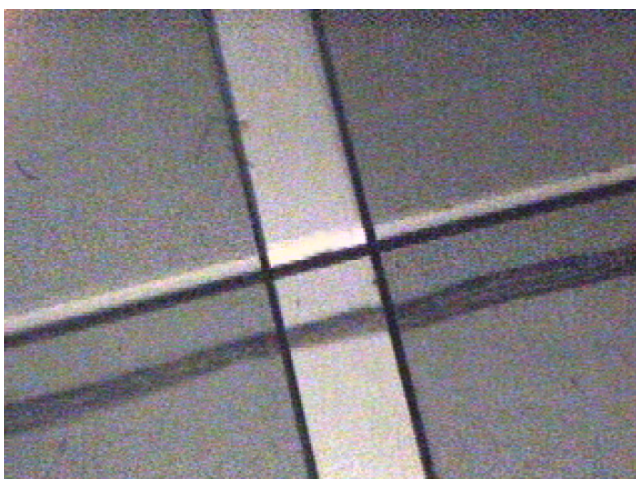
- You can push or pull the knobs slightly to adjust the position of the opening
- You can also rotate the aperture within the slot

Figure 4-9: Aperture opening within area of fiber



11. Move the stage horizontally so that the fiber is completely outside the aperture opening.

Figure 4-10: Fiber completely outside aperture



**Note** When you analyze a sample that is installed on a substrate, the background must be measuring through the substrate. In that case move a clean area of the substrate under the objective.

12. In the dashboard, select **More** to see a live display of the detector signal.

If no signal appears, see [Troubleshooting](#).

13. Maximize the signal intensity by adjusting the condenser focus.

The condenser focuses the infrared beam on the plane of the sample. To adjust the focus, turn the Condenser focus knob until the signal intensity is greatest. You may need to turn the knob in both directions to find the optimum focus.

Generally, the condenser is optimally focused when the video or viewer image is at or near its brightest.

When you analyze your own samples, you don't need to focus the condenser before each sample unless the samples are of different thicknesses. Focusing the condenser before the first sample in a sequence of similar samples is usually adequate.

14. Select **Preview and Measure Background**. This opens a preview Spectra tab.
15. In the new tab, select **Start Background Measurement** and wait for the scans to complete.
16. Move the stage horizontally to position the fiber so that it once again fills the aperture opening.

You are now ready to measure a sample spectrum.

17. Select **Start Sample Measurement** from the Spectra tab and wait for the process to complete.

Your sample spectrum is now saved in OMNIC Paradigm software and can be accessed at any time from the Measurements menu in the dashboard.

18. The locations and intensities of the peaks in the final spectrum are unique for a particular sample material.

The baseline of the spectrum should be consistent and smooth. The closer the baseline is to 0 absorbance units (100% transmittance) the better. This makes the band intensities easier to measure and allows you to compare spectra visually or by searching the spectrum against a spectral library.

## ATR measurement

The optional Slide-On ATR™ system lets you analyze highly infrared-absorbent or hard-to-prepare microscopic materials like polymers, coatings, rubbers, coated papers, and biological materials, often with little or no sample preparation.



Figure 4-11: Ge ATR slider and diamond ATR



Applications of ATR microscopy include these:

- Analyzing surface defects or inclusions
- Analyzing glass-filled fibers
- Analyzing biological materials
- Analyzing surface degradation
- Performing polymer studies
- Performing quantitative analyses

Your iN5 microscope configuration may include one or more optional Slide-On ATR crystal sliders. The iN5 microscope is optionally available with either a germanium (GE) tip ATR or a diamond ATR crystal.

The Contact Alert™ system helps you achieve proper contact pressure with the crystal in an ATR crystal slider. During an ATR experiment, the system monitors the force between the sample and crystal. Indicators illuminate to show when you have achieved initial contact and optimum pressure.

For this experiment, we will use a polycarbonate sample slide that may have been provided with the Slide-On ATR.

If your Slide-On ATR is not provided with a polycarbonate sample slide, prepare a simple sample slide by taping a piece of a plastic bag to a glass slide.

1. Open OMNIC Paradigm software. In the dashboard, set **Final format** to **Absorbance** (or **Log1/Reflection** if desired).
2. Set the microscope for Reflectance by turning the knob on the left side.
3. Turn the illumination all the way up by rotating the dial on the left side of the microscope.
4. Open the microscope aperture all the way.
5. Install the Contact Alert plate on the stage.

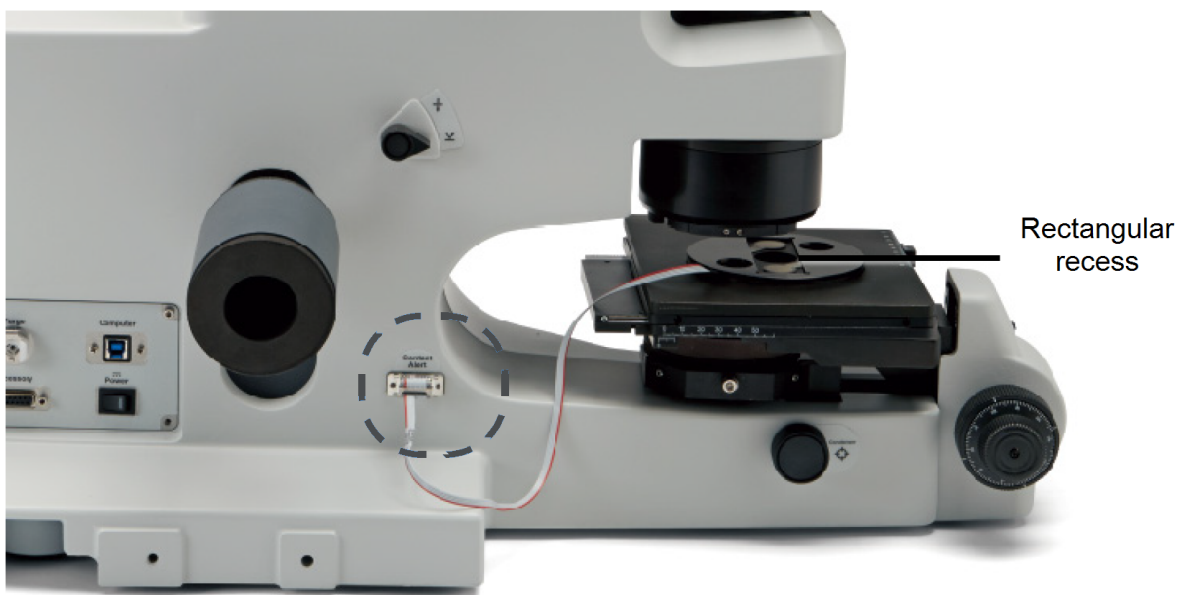
Remove the round insert from the stage and then replace it with the Contact Alert plate.

Figure 4-12: Contact Alert plate



6. Connect the cable from the plate to the Contact Alert connector on the microscope.

Figure 4-13: Contact Alert connector



7. Place the polycarbonate sample slide, or your previously prepared slide, into the rectangular recess on the plate.

#### NOTICE

This will protect the ATR crystal in case too much pressure is applied when sample contact is made. A glass slide will typically break before the ATR crystal cracks. Replacing the glass slide is much less expensive than replacing the ATR crystal.

8. Move the stage horizontally to position the plastic sample under the objective.

9. While looking directly at the plastic sample (not at the video or viewer image), move the stage vertically to focus the spot of light on the plastic.

Since the plastic is very thin, you may need to move the stage horizontally as well to position the spot on the plastic.

10. While looking at the video or viewer image, sharply focus the image of the plastic sample.
11. Remove the plastic cap from the ATR crystal slider and use a laboratory tissue to wipe the crystal clean.

Any residue on the crystal from previous experiments could produce unwanted peaks in your sample spectrum.

12. Insert the slider all the way into the slot at the front of the objective.

Figure 4-14: Insertion of the slider



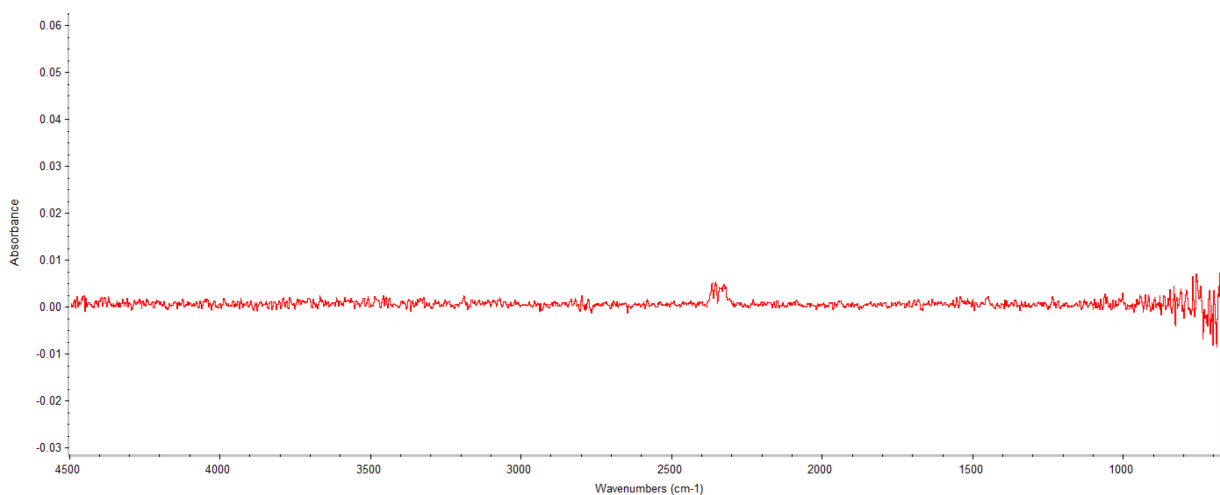
13. Select **Preview and Measure Background**. This opens a preview Spectra tab.
14. In the new tab, select **Start Background Measurement** and wait for the scans to complete.  
You are now ready to preview your sample spectrum.
15. Select **Preview Sample** from the Spectra tab. You will see a live preview of your sample spectra.

**Note** There are no peaks associated with your sample present, as the crystal is not in contact with the sample.

Figure 4-15: Contact Alert panel



Figure 4-16: ATR sample spectrum



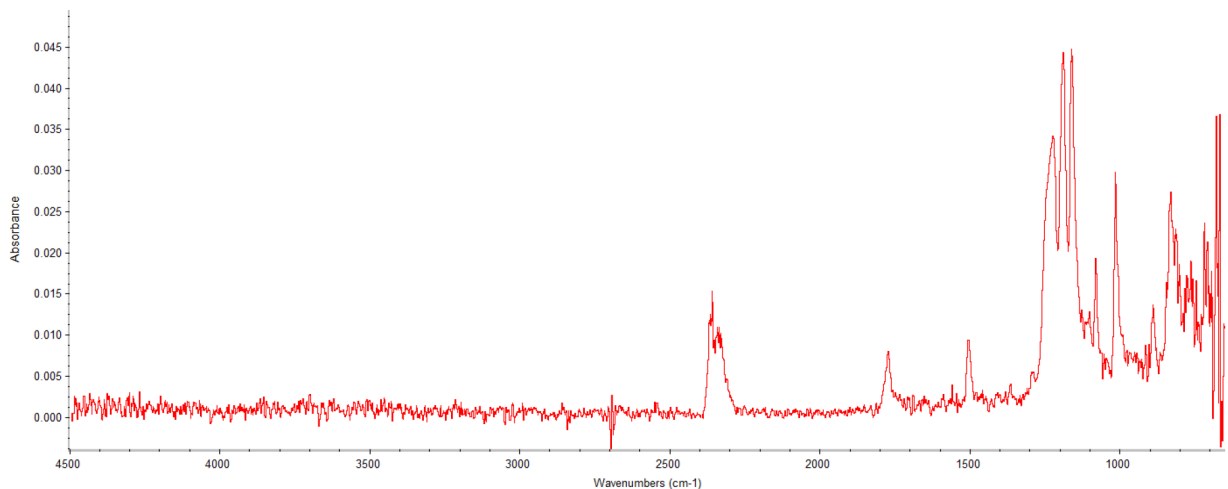
16. Slowly and carefully raise the stage until the green indicator on the front of the microscope illuminates.

The sample has made initial contact with the ATR crystal at this point.

Figure 4-17: Contact Alert green indicator



Figure 4-18: Sample spectrum in contact with the ATR crystal



#### NOTICE

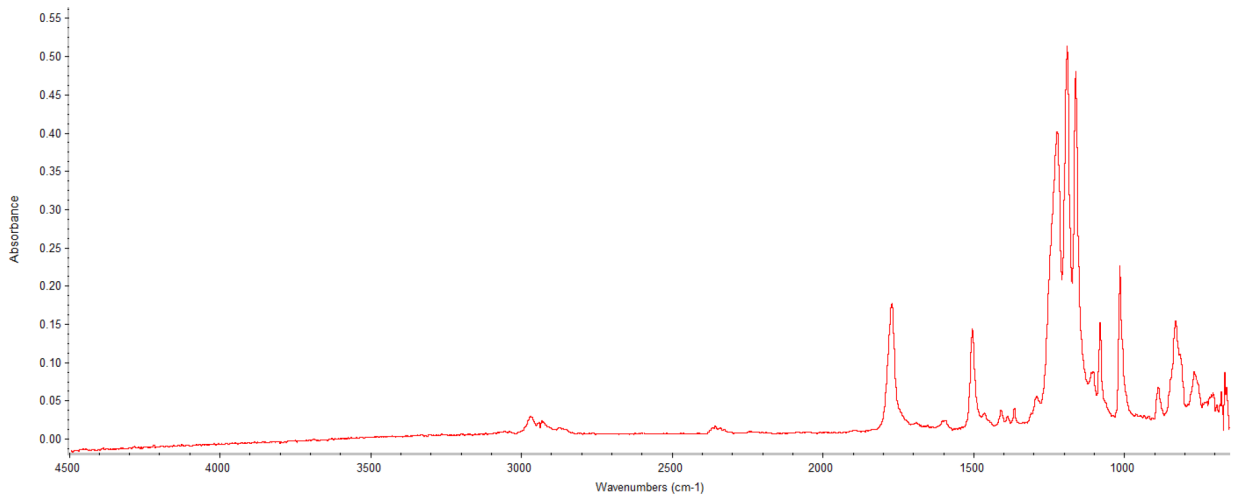
Excessive pressure can damage the ATR crystal or crack the glass slide. Be sure to stop raising the stage in the next step when the red indicator illuminates. Red indication means you are at the maximum pressure for the ATR.

17. Continue to slowly raise the stage until the red indicator illuminates, then **stop immediately**. You will have seen the spectral peak intensity of your sample increase with additional force.

Figure 4-19: Contact Alert red indicator



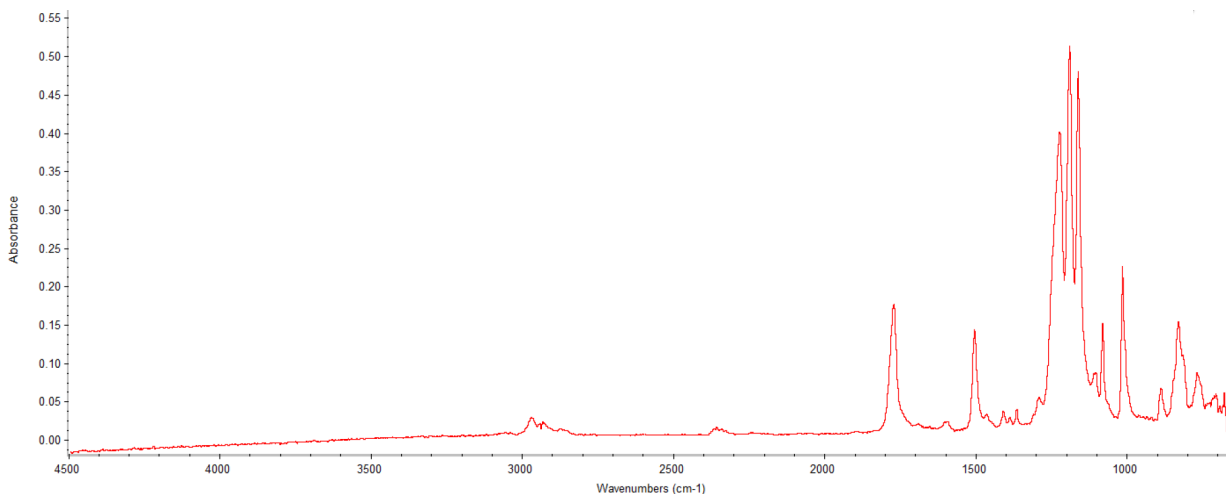
Figure 4-20: Spectral peak intensity



18. Back down the stage slightly until the just the green indicator is illuminated.



Figure 4-21: Optimal signal intensity



The contact pressure and signal intensity is now adequate for measuring data.

19. Select **Start Sample Measurement** from the Spectra tab and wait for the process to complete.

Your sample spectrum is now saved in OMNIC Paradigm software and can be accessed at any time from the Measurements menu in the dashboard.

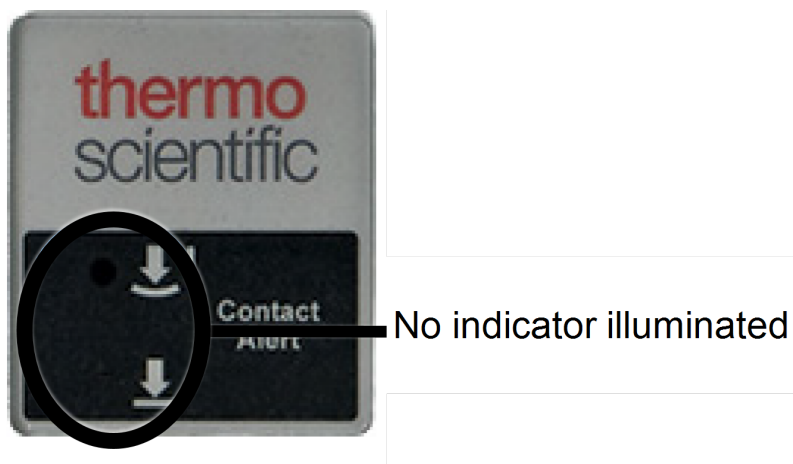
The baseline of the spectrum should be consistent and smooth. The closer the baseline is to 0 log (1/R) units (100% reflectance) the better. This makes the band intensities easier to measure and lets you compare spectra visually or by searching the spectrum against a spectral library.

**Note** ATR spectra show stronger bands at the long wavelength (lower wavenumber value) end of the spectrum.

You may wish to use OMNIC Paradigm software's ATR correction feature to render the spectrum more similar in relative band intensities to a Transmittance spectrum

20. In the OMNIC Paradigm software dashboard, go to **Process** and select **Advanced ATR Correction**.
21. Carefully raise stage. The green indicator will turn off. Continue to lower the stage to the full extent of travel.

Figure 4-22: Contact Alert. no illuminated indicator



It is now safe to remove the slider.

#### NOTICE

- Damage to the crystal can occur while the crystal is in contact with the sample
- The crystal cannot be in contact with sample when moving the stage horizontally or during removal of slider

22. Carefully remove the slider from the objective, use a laboratory tissue to wipe the crystal clean; return slider to the protective packaging.

## Tips for measuring a background

Since your background spectra can have a significant effect on the quality of your ratioed sample spectra, it is important to measure a background using the technique that is most appropriate for your application. Follow the tips below to obtain the best possible backgrounds for your Transmittance, Reflectance, and ATR experiments.

- To minimize the effects of water vapor and carbon dioxide absorptions on your background and sample spectra, make sure the microscope and spectrometer are adequately purged when you measure data.
- Depending on the sample and the background material you want to use for a Reflectance experiment, you may need to install the sample on a slide that has a gold or aluminum mirror for measuring the background. In some cases the material to be analyzed is on top of a reflective substrate, and an exposed area of the substrate can be used for the background measurement.



- Before you measure a background spectrum in an ATR experiment, make sure the stage is lowered so that the sample is not in contact with the ATR crystal. Also make sure the crystal is clean.
- When using a compression cell to measure Transmittance data, place a single crystal of KBr on the cell substrate. The crystal should be about the same thickness as the sample. Measure the background through the crystal, making sure the apertured area does not extend beyond the crystal.
- If you are measuring spectra with the sample installed on a window or between two windows, measure the background through the same window material. This will help prevent offset of the sample spectrum baseline.
- Fringing occurs when the infrared beam encounters a thin region of space defined by two substances that don't have the same refractive index, such as an air gap between two salt windows or a thin film surrounded by air. If you are sampling between two windows, you can eliminate or minimize fringing by placing a single crystal of KBr between the plates and measuring the background through the KBr crystal.

## Removing the condenser before installing a large sample

If you find that there is not enough clearance between the stage and the objective to install a large Reflectance sample, you can remove the condenser so that the stage can be lowered further. (The condenser is not used in a Reflectance experiment.)

Before you start:

- Select **More** in the OMNIC Paradigm software dashboard to see the spectrum preview and maximize the signal by adjusting the condenser focus knob
- Make a record of the detector signal intensity obtained with the microscope set to % **Transmittance** and with no sample on the stage
- After you reinstall the condenser, you will maximize the signal again and compare it with the recorded value to make sure the condenser is installed correctly

❖ **To remove the condenser**

1. Remove any sample from the stage.
2. Use the Course focus knob to raise the condenser stage to its highest position.

**NOTICE**

To prevent damage to the internal mirror, avoid inserting your finger or any other object through the opening on the top of the condenser.

3. Use the Condenser focus to lower the condenser to its lowest position.
4. Loosen the captive thumbscrew all the way.
5. Use the stage positioning knobs to move the stage all the way to the back.

This moves the Stage positioning knobs out of the way so that you can remove the condenser in the next step.

6. Carefully slide the Condenser out through the opening at the right.

You can now lower the stage and install your sample.

## Reinstalling the condenser

❖ **To reinstall the condenser**

1. Remove any sample from the stage.

**NOTICE**

Be careful not to hit the objective with the stage when you raise the stage.

2. Use the Course focus knob to raise the stage to its highest position.
3. Use the Stage positioning knobs to move the stage all the way to the back.
4. Carefully slide the Condenser into position from the right.

The bracket containing the captive thumbscrew should still be in its lowest position. If it is not, use the condenser focus knob to lower it all the way.

5. Secure the condenser with the captive thumbscrew.

- a. Position the condenser so that the screw hole aligns with the thumbscrew.
- b. Hold the condenser with your right hand while tightening the thumbscrew with your left hand.

Do not tighten the thumbscrew more than finger-tight or performance may be compromised.

6. While observing the spectrum preview (click **More** to see it if needed), use the condenser focus knob to maximize the detector signal.
7. Adjust the condenser Setscrews slightly in either direction to maximize the detector signal again.

The signal intensity you achieve should be within plus or minus 20% of the value you recorded before you removed the condenser. If it is not, try removing and reinstalling the condenser as described above.

When you are finished, the microscope is ready for use.

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# Sampling accessories

This section describes the types of sampling accessories offered by Thermo Scientific. Be sure to read the manual that came with the accessory, if one was provided.

## Compression cell accessory

The optional compression cell lets you easily flatten and install infrared-transmitting samples, such as fibers, films, chips and beads up to 15 micrometers thick. The cell reduces the thickness of samples that are too thick and also helps hold small samples in position. You can also use a compression cell to analyze powders and liquids.

For directions on using the Compression Cell, refer to the *Micro Compression Cell and Micro Compression Diamond Cell Kit User Guide*.

## ATR sampling accessories

Germanium (Ge) and diamond ATR crystal sliders are available for performing ATR experiments with your microscope. The crystal material type affects the depth of penetration of the infrared beam into the sample. You can also purchase replacement crystals for the sliders.

For ordering information, [contact us](#).

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# Maintenance and service

For information on maintaining and servicing your microscope, see the [Run performance verification workflows](#) section.

For additional service or ordering information, [contact us](#).

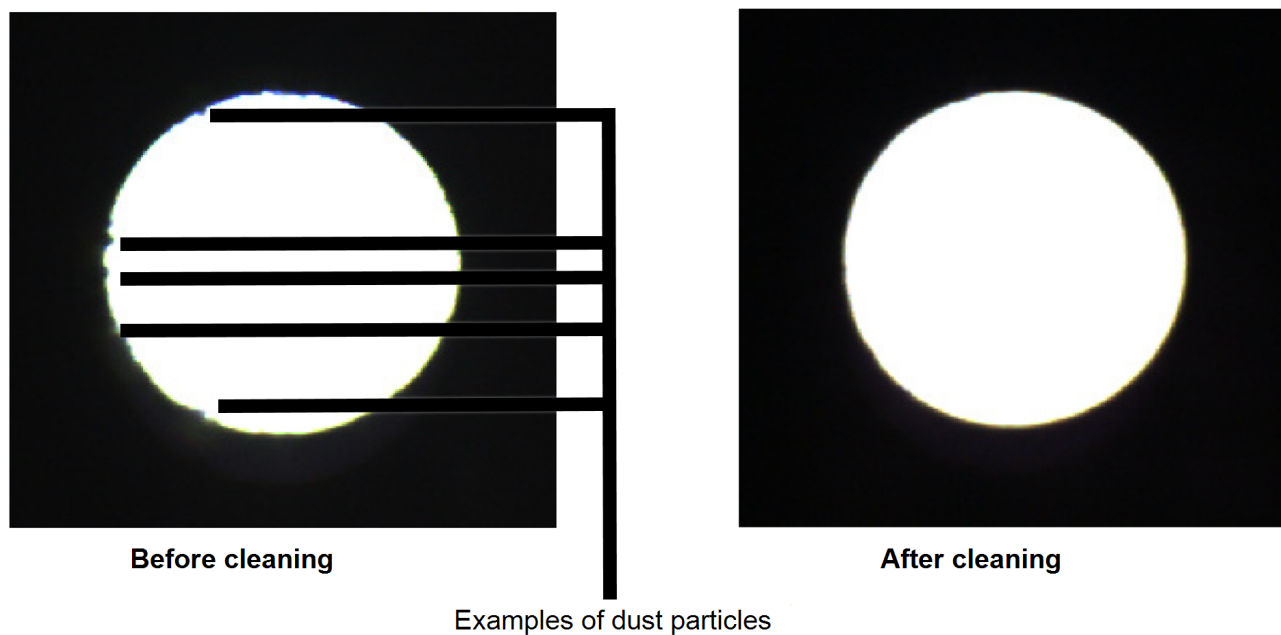
## Cleaning the aperture

### NOTICE

Refurbishment of the blackening on the fixed aperture or optional variable metal aperture can be easily performed using a household candle. The optional variable glass aperture does not require blackening.

- Clean the aperture with isopropyl alcohol
- Hold the aperture briefly over the flame of the candle (repeat until a uniform deposit of soot appears around the hole in the aperture)
- Repeat this process on the opposite side of the aperture
- Inspect under magnification to ensure aperture is clear of soot particles
- Remove soot particles with a household pin or equivalent sharp object

Figure 6-1: Before and after cleaning the aperture





# Troubleshooting

The following table describes some problems that could occur when you use the microscope and explains how to solve them. If you are unable to solve a problem after following the instructions here, contact us.

Problem	Possible Cause	Solution
<b>The noise test failed.</b>	The microscope is not focused.	Focus the microscope. For Transmittance experiments, be sure to check both the sample and condenser focus.
	The spectrometer is not functioning properly.	Check the operation of the spectrometer. See the documentation that came with the spectrometer for details.
<b>No image appears.</b>	The microscope is not focused.	Focus the microscope. For Transmittance experiments, be sure to check both the sample and condenser focus.
	The illumination is too low.	Increase the illumination with the illumination knob.
	The illuminator is not receiving power.	Make sure the microscope power switch is turned on.
<b>The image is blurred</b>	The microscope is not focused.	Focus the microscope. For Transmittance experiments, be sure to check both the sample and condenser focus.
<b>The signal is weak without a sample in the beam path.</b>	The microscope is not in the correct mode.	Set the microscope to Transmittance or Reflectance as needed.

Problem	Possible Cause	Solution
	The microscope is not focused.	Focus the microscope. For Transmittance experiments, be sure to check both the sample and condenser focus.
	Focus the microscope. For Transmittance experiments, be sure to check both the sample and condenser focus.	Focus the microscope. For Transmittance experiments, be sure to check both the sample and condenser focus.
	The experiment parameters are not set correctly.	Make sure the Sample Compartment, Detector, Aperture, Velocity and Gain parameters are set correctly in the Experiment Setup dialog box.
<b>No signal appears in the live display.</b>	The microscope is not in the correct mode	Set the microscope to Transmittance or Reflectance as needed.
	The microscope is not focused.	Focus the microscope. For Transmittance experiments, be sure to check both the sample and condenser focus.
	The detector is not cooled.	<a href="#">Cool the detector</a> . Allow at least 10 minutes for the detector to cool.
	“Cooling the Detector” on page 7. Allow at least 10 minutes for the detector to cool.	Remove any foreign objects from the beam path. Make sure the Sample Compartment parameter in the Experiment Setup dialog box is set for the microscope mode and location.
	The aperture is not adjusted correctly.	Adjust the aperture.

Problem	Possible Cause	Solution
	The external camera cable is disconnected.	Connect the external camera cable between the camera and the microscope body.
	The USB cable is disconnected.	Connect the USB cable between the control panel and the computer.
	The spectrometer cable is not securely attached to the spectrometer connector on the control panel.	Attach the cable.
<b>The signal is weak with a sample in the beampath.</b>	The microscope is not in the correct mode.	Set the microscope to Transmittance or Reflectance as needed.
	The microscope is not focused.	Focus the microscope. For Transmittance experiments, be sure to check both the sample and condenser focus.
	The detector is not cooled.	<a href="#">Cool the detector</a> . Allow at least 10 minutes for the detector to cool.
	The condenser thumbscrew has been overtightened (for Transmittance experiments only).	Make sure the thumbscrew is not overtightened.
	The experiment parameters are not set correctly.	Make sure the Sample Compartment, Detector, Aperture, Velocity and Gain parameters are set correctly in the Experiment Setup dialog box.

Problem	Possible Cause	Solution
	The infrared energy level in the spectrometer is low.	Check the signal intensity. If it is low, make sure the sample compartment is clear and then align the spectrometer. If the problem persists, check the spectrometer source power and consult the documentation for your specific spectrometer.
	The sample was not properly prepared.	Prepare the sample properly. For example, a Transmittance sample should not be too thick.
<b>Sample focus stage drifts out of focus.</b>	Sample stage focus knob tension is not set properly.	Increase sample stage tension.
<b>Live display updates slowly.</b>	Cable isn't USB3-compatible.	Use a USB3-compatible cable and corresponding port.
	The computer's USB port is not USB3.	Try a different USB port (look for blue plastic in the port)

## To set the tension

1. Hold left side course focus knob firmly with your left hand.
2. Simultaneously, grasp the right side course focus knob with your right hand.
3. Turn the right side knob clockwise to increase the tension or counterclockwise to decrease the tension.
4. Test to ensure you have achieved the desired tension.
5. Repeat process, as needed.

### NOTICE

Too little tension will allow the stage to drift downward under its own weight and weight of the sample, causing your sample to go out of focus.