Nicolet iN5 User Guide

The Thermo Scientific™ Nicolet™ iN5 microscope lets you quickly and easily collect spectra of Transmission, Reflection and ATR samples, with continuous viewing of the sample during collection.

Contents

Introduction

You should be familiar with the operation of your spectrometer and system software before using the microscope. You can use the microscope with Thermo Scientific OMNIC™ or µView™ software. For complete information on using these packages, see the Help system or other documentation that came with the software. In this manual, we will refer mostly to OMNIC commands.

The Nicolet iN5 microscope must be installed as part of a Thermo 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.

Conventions

DANGER Indicates a hazardous situation which, if not avoided, will result in death or serious injury.
WARNING Indicates a hazardous situation which, if not avoided, could result in death or serious injury.
CAUTION Indicates a hazardous situation which, if not avoided, could result in minor or moderate injury.

NOTICE Follow instructions with this label to avoid damaging the system hardware or losing data.
Note Contains helpful supplementary information.

Where to Find the Information You Need

Tutorials are included in the OMNIC software Help system.

The tutorials teach you how to do these things:

- Set up your system for a variety of experiments
- Perform mathematical operations on spectra
- Create reports and report notebooks containing the results of your work
- Install optional hardware
- Change replaceable parts
- Get part number and ordering information

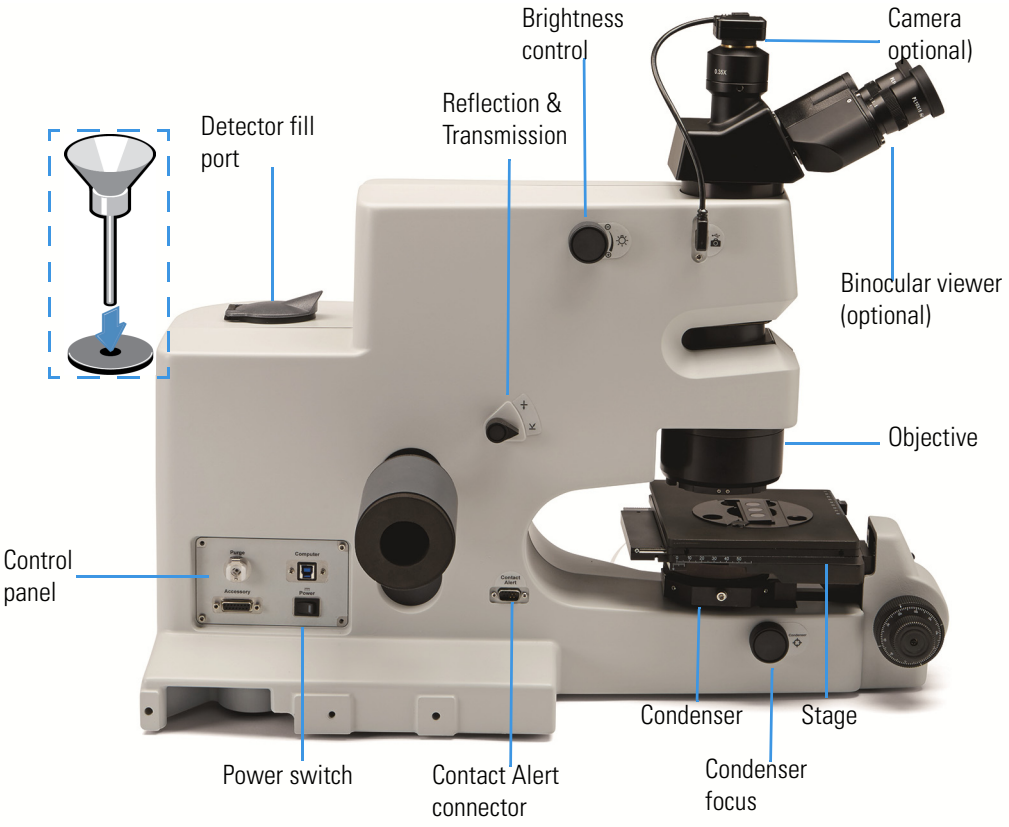
Note You must have the media: Microscope Help and Tutorials media in the ROM drive to view any videotaped parts replacement procedures.

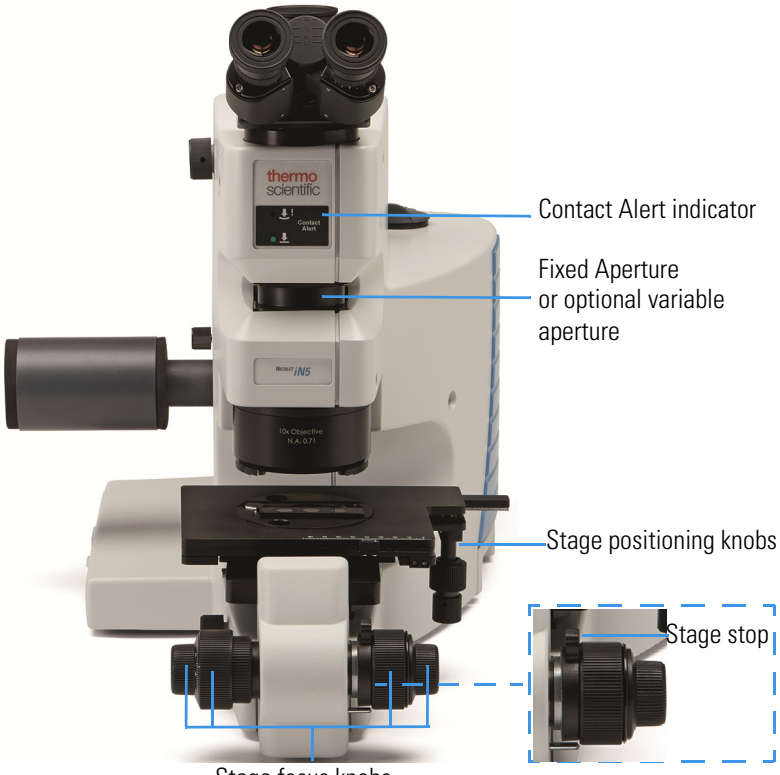
Microscope Basics

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

Microscope Components

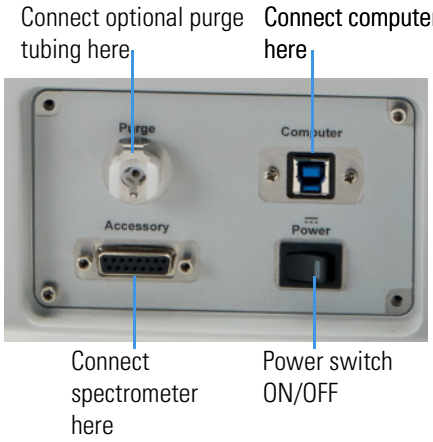
The following illustrations identify some important components of the microscope. Refer to these illustrations when you use other sections of this manual.


Brightness control
Camera optional)
Reflection & Transmission
Detector fill port
Binocular viewer (optional)
Objective
Control panel
Condenser
Stage
Power switch
Contact Alert connector
Condenser focus


thermo scientific
Contact Alert indicator
Fixed Aperture or optional variable aperture
Stage positioning knobs
Stage stop
Stage focus knobs

Control Panel

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



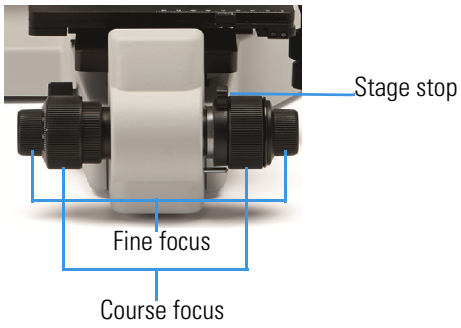
Turning on the Microscope Power

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.

To turn on the microscope power, press Power switch ON/OFF.

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.



- 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

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.

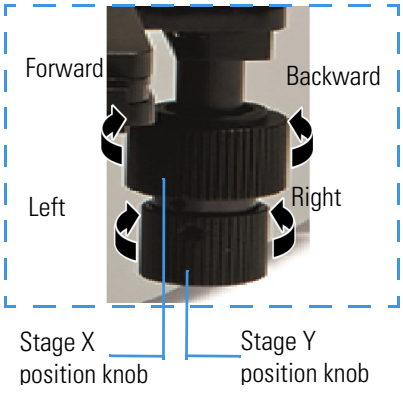
2. Lift the Stage stop handle until it feels tight.

   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.

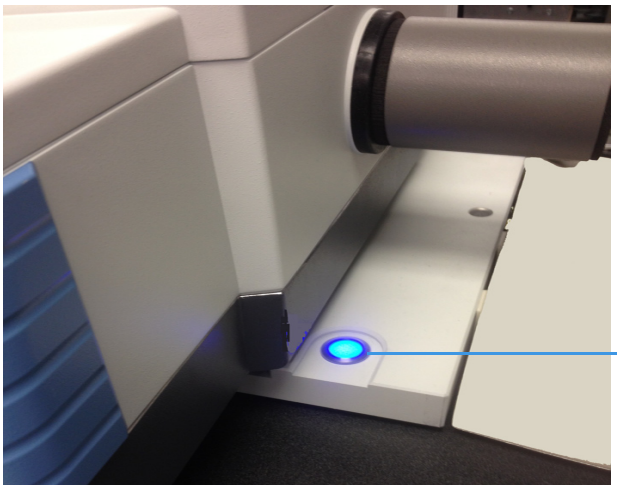
You can also use this feature to protect the objective and stage. First carefully move the stage upward until it is slightly below the bottom of the objective. Then lift the stop handle until it feels tight. With the stop set, you can begin working with samples without worry of hitting the objective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



Touch Point



Touch Point button
(only available with the
Nicolet iS50 spectrometer)

Before You Collect Spectra

Turning 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" on page 3. 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.

Cooling 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 (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 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.

Starting OMNIC Software

Double-click the OMNIC icon on your desktop.

Opening a Configuration

Opening a configuration lets you quickly set a number of software options. You can create and save your own configurations to customize OMNIC software for the way you prefer to use it. In OMNIC Help Topics find "configuration" in the Index and go to "Customizing OMNIC by setting options" for more information.

Note If you are using Thermo Scientific EZ OMNIC™, only the current configuration is available. You cannot open a different configuration.

If you will be checking the performance of the microscope (explained later in this section) or collecting your first sample spectrum (explained in the next section), open the provided configuration file named **DEFAULT.CON**.

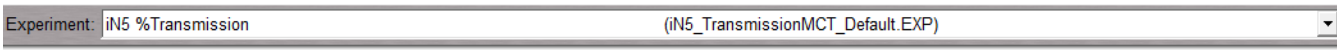
1. Choose **Open Configuration** from the File menu.

   The Open Configuration dialog box lists the available configuration files.

2. Select the file named **DEFAULT.CON**

3. Click **Open**.

Selecting an Experiment



By selecting an experiment from the Experiment drop-down list box below the OMNIC menu bar, you can quickly set the software parameters for the type of data collection you want to perform. The drop-down list contains all of the experiments you have opened, plus the default experiment. You can create and save your own experiments with the parameter settings you need for different applications.

Note Choosing either iN5 %Transmission or iN5 %Reflection through the software does not change the mode, this has to be done at the microscope.

If the desired experiment does not appear in the Experiment drop-down list box, choose **Experiment Setup** from the Collect menu and use the **Open** button in the Experiment Setup dialog box to open the experiment. Once you have opened the experiment, it will appear in the Experiment drop-down list box for future selection. In OMNIC Help Topics find "experiment" in the Index and go to "Using Experiment Setup" for more information on opening experiments.

If you want to check or change the parameters after selecting an experiment, use **Experiment Setup** in the Collect menu.

Later in this section you will select (or open) the experiments needed to collect Transmission or Reflection sample data with the microscope.

Setting up the Viewing System

Before you collect sample data, it is important to set up the viewing system for optimum performance. This involves setting the µView parameters that affect the display of the video image of the sample and calibrating the video image.

Setting the Video Parameters

The µView, the video parameters determine how the video driver displays the video image. You set the parameters by using the **Video Source** command in µView. For details see "Setting the video parameters" in the "Setting Up the Software and Display" section of the *MicroView User Guide*.

Calibrating the Video Image

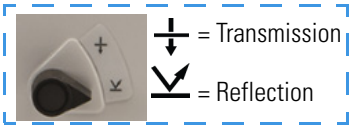
The µView can be used to calibrate the video image to coordinate what is displayed in the video pane with the actual field of view. That is, the number of micrometers represented by the video pane axes must match the number of micrometers in the field of view. The calibration is very important. If you don't calibrate the video image, the axes and any annotation such as the ruler and drawn circles and rectangles will not have correct micrometer values.

For complete instructions see "Calibrating the video image" in the "Setting Up the Software and Display" section of the *MicroView Software User Guide*.

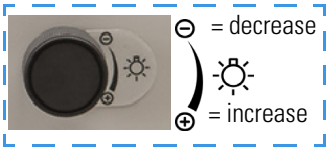
Setting up the Binocular Viewer

❖ **To set up binocular viewer (optional)**

1. Set the microscope for Reflection by turning the Reflection & Transmission knob.



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



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.

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

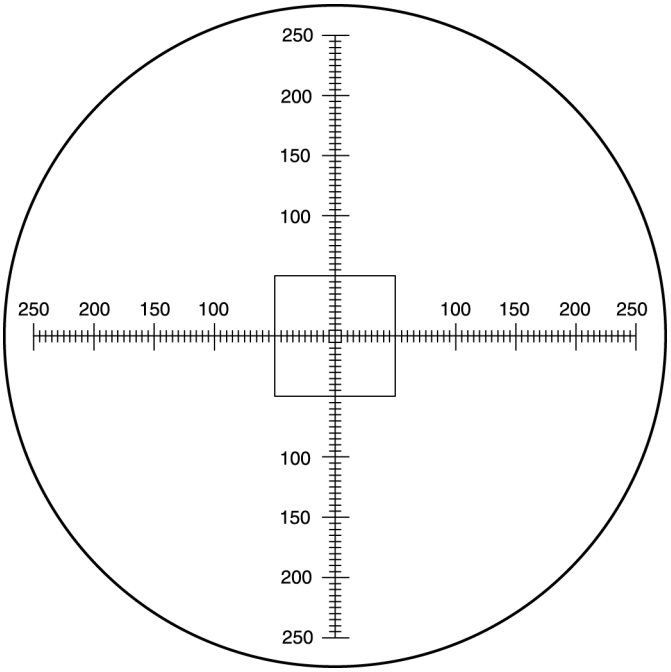
When you are finished, look at the reading on the scale between the eyepieces (the white dot indicates the numerical value). In the future you will be able adjust the eyepieces quickly by moving them to this reading.

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.

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.

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



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 µm on a side.

System Performance Check

To verify that the microscope is performing well, you should measure its performance weekly by checking the signal-to-noise ratio (SNR) from 2600 to 2500 wavenumbers. The procedures in the next sections give the desired noise level limits for both Transmission and Reflection. If you will be performing only Reflection experiments, you do not need to run the Transmission noise test.

Before you begin...

- Make sure the detector is cool. See Cooling the Detector if you need help
- Prepare the optics by following the procedures in the preceding sections
- If a camera is installed, start µView if it is not already running.
  (Refer to the *MicroView Software User Guide* for instructions.)

Running the Noise Test in Transmission

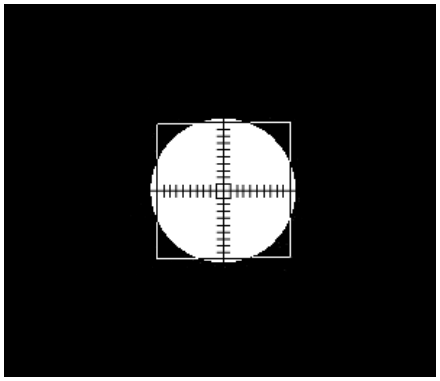
1. Set the microscope for Transmission by turning the Reflection & Transmission knob on the left side.

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

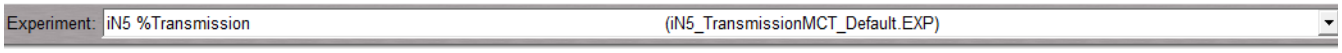
2. Set the illumination properly by rotating the dial on the left side of the microscope.
3. Remove any slide from the stage.
4. Move the stage horizontally to position the large hole in the stage under the objective.

   See "Moving the Stage Horizontally" on page 5.

5. Adjust the Condenser focus and adjust the illumination until the video or viewer image is moderately bright and the Transmission pin hole is in focus.



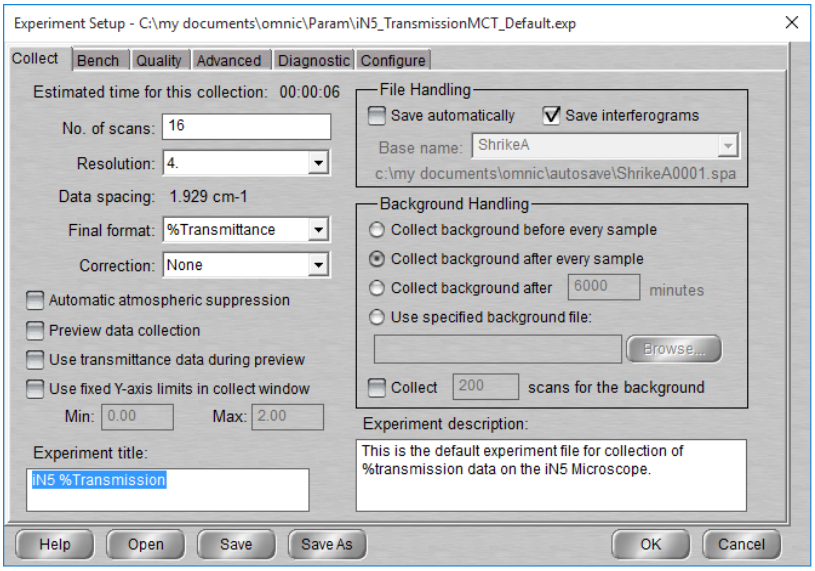
6. Select the **iN5_%Transmission** experiment from the Experiment drop-down list box.



This sets the experiment parameters for collecting Transmission sample data with the microscope. (Later you will set three of the parameters to different settings specifically for the noise test.)

7. If the experiment is not available in the Experiment drop-down list box, use **Experiment Setup** to open it. Its filename is **iN5_Transmission.exp**. For more information, find "experiment" in the OMNIC Help system Index and go to "Selecting an experiment" or "Using Experiment Setup."Choose **Experiment Setup** from the Collect menu.

   The Experiment Setup dialog box appears.

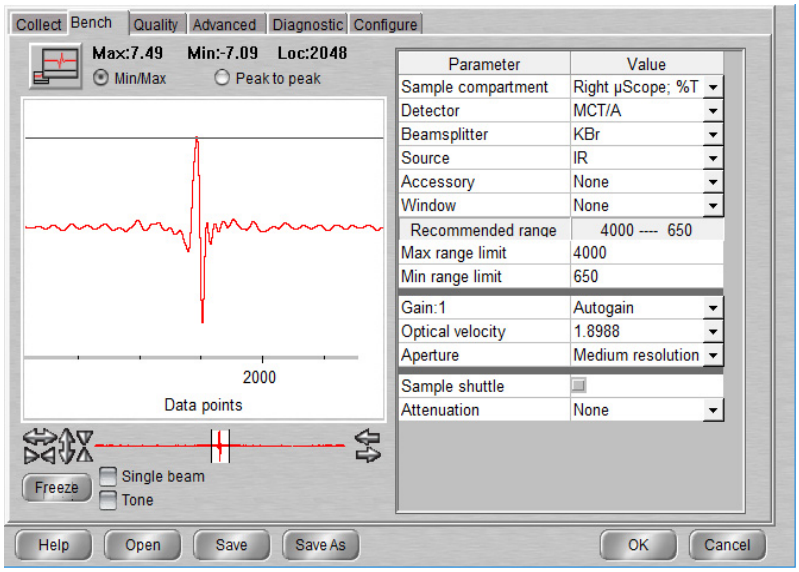


   This dialog box contains the experiment parameters used by the **iN5_Transmission** experiment. For complete information about the parameters, find "parameters" in the OMNIC Help system Index and go to "Using Experiment Setup."

8. Set the parameters for the noise test.
   a. Click the **Collect** tab.
   b. Set Number of Scans to **200.**
   c. Set Final Format to **%Transmittance.**
   d. Click the **Advanced** tab.
   e. Set Apodization to **Triangular**.

9. Click the **Bench** tab.

   The Bench tab contains a live display of the detector signal.



   The intensity of the signal is indicated by the values above the live display. If no signal appears, see the table in Troubleshooting section for instructions.

   For more information about the live display, find “live display” in the OMNIC Help system Index and go to “Using the live display.”

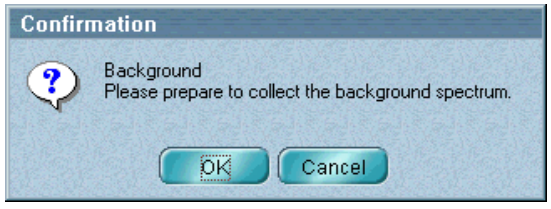
10. Maximize the signal intensity by adjusting the condenser focus and the horizontal stage position.

    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.

    Tip 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.

11. Click **OK** to close the Experiment Setup dialog box.

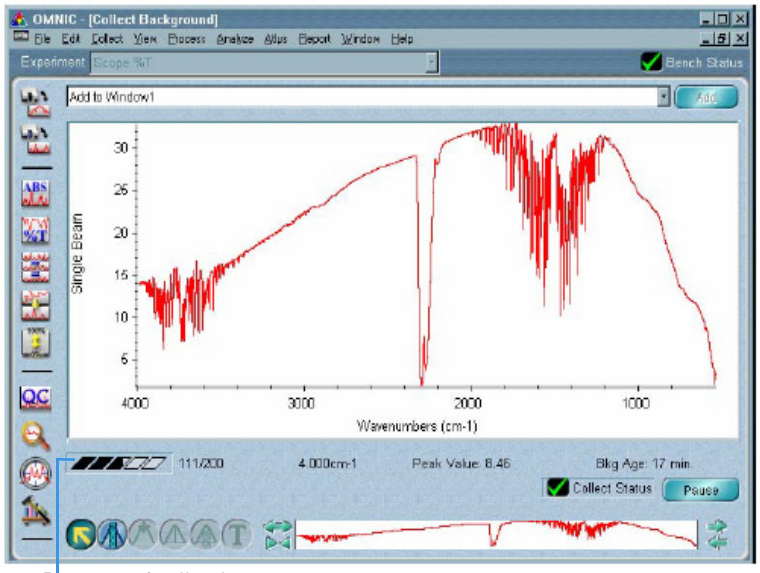
12. Click **Collect Background** on the toolbar.



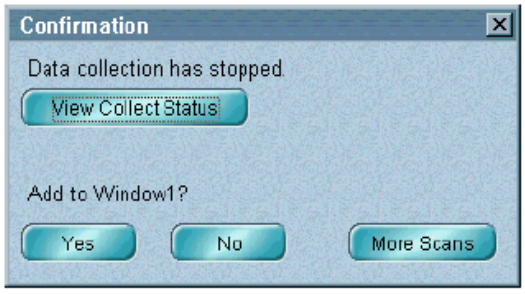
A message asks you to prepare for the background collection.

13. Click **OK.**

    The Collect Background window shows the background spectrum as it is collected.



Progress of collection gauge

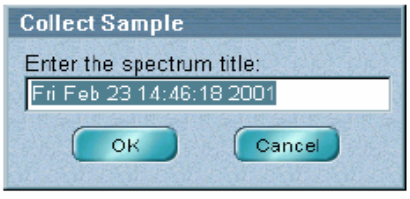


When data collection is finished, a message asks whether to add the spectrum to a spectral window.

14. Click **No**.

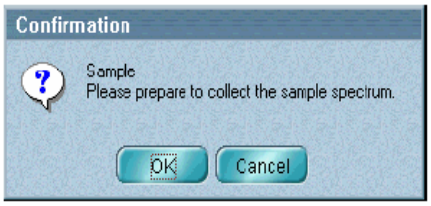
    Even though you are not adding the background to a spectral window, it will remain in memory and will be used for ratioing the sample spectrum you are about collect.

15. Click **Collect Sample** on the toolbar.



The Collect Sample window appears and then a dialog box showing the default title for the sample spectrum.

16. Type a title in the text box or click **OK** to accept the default.



A message asks you to prepare for the sample collection.

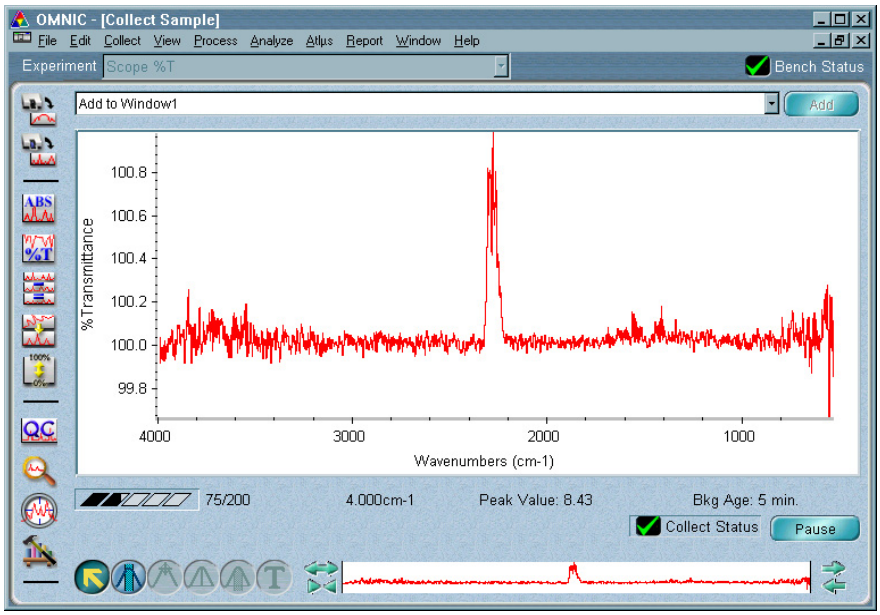
17. Click **OK**.

    Collection of the sample spectrum begins. The progress of the collection is indicated by the gauge near the left side of the window.

    When data collection is finished, a message asks whether to add the spectrum to a spectral window.

18. Click **Yes** to add the spectrum to a spectral window.

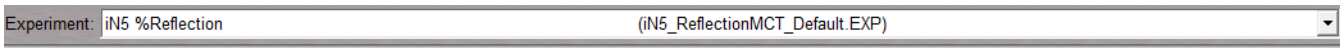
    Example: Sample spectrum displayed in a spectral window:



19. Use the region tool to select the spectral region from approximately 2600 to 2500 wavenumbers.

20. Choose **Noise** from the Analyze menu.

    Example: Typical noise dialog box



    The peak-to-peak noise should be less than 0.10. The spectrometer contributes significantly to the performance of the microscope. (Older spectrometers that do not meet basic mid-IR performance standards may have a higher noise level.) If the noise level exceeds 0.10, make sure the spectrometer is functioning properly and then repeat this procedure. See the documentation that came with your spectrometer for information on checking its performance.

    If you repeat the test, make sure these conditions are met:

    - The detector is cool
    - The knob on the left side of the microscope is set to Transmission
    - The aperture is fully open
    - The Sample Compartment parameter is set for the location of the microscope and for Transmission (%T)
    - The condenser is focused

    For more information about the Noise command, find "noise" in the OMNIC Help system Index and go to "Measuring noise."

21. Click **OK** to close the Noise dialog box.
22. Choose Clear from the Edit menu to delete the spectrum.

Running the Noise Test in Reflection

1. Set the microscope for Reflection by turning the Reflection & Transmission knob on the left side.

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

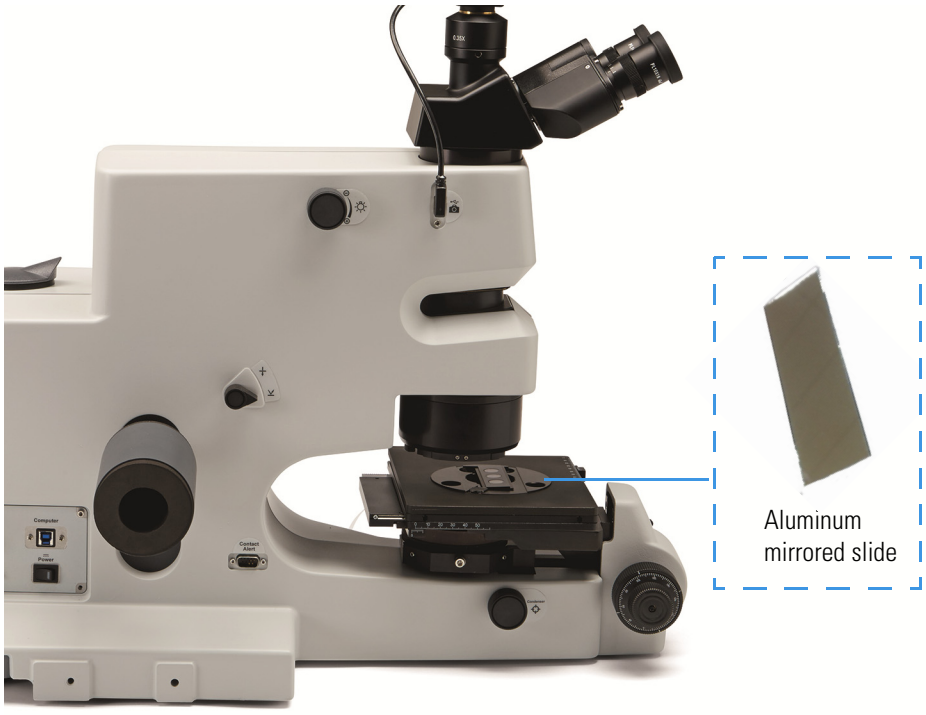
2. Turn the illumination all the way up.

    See step 2 in the preceding procedure if you need help.

3. If you have the optional variable aperture, set the microscope aperture to a 100 µm square.

    See step 8 in the preceding procedure if you need help.

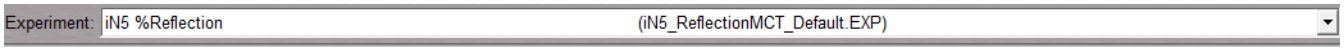
4. Place the provided aluminum mirrored slide on the stage.



5. Move the stage horizontally to position the aluminum mirrored slide under the objective.

   See step 5 in the preceding procedure if you need help.

6. Select the **iN5_%Reflection** experiment from the Experiment drop-down list.



   This sets the experiment parameters for collecting Reflection sample data with the microscope. (Later you will set three of the parameters to different settings specifically for the noise test.)

   If the experiment is not available in the Experiment drop-down list box, use **Experiment Setup** to open it. Its filename is **iN5_%Reflection.exp**. For more information, find "experiment" in the OMNIC Help system Index and go to "Selecting an experiment" or "Using Experiment Setup."While looking directly at the gold mirror (not at the video or viewer image), move the stage vertically to focus the spot of light on the mirror.

7. While looking at the video or viewer image, use the fine focus knob to sharply focus the image of the mirror.

   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.

8. While looking at the video or viewer image, use the fine focus knob to sharply focus the image of the mirror.

   You should be able to see slight surface imperfections that you can bring into focus.

9. Set the parameters for the noise test.

   a. Click the **Collect** tab if it is not already displayed in front.

   b. Set Number Of Scans to **200**

   c. Set Final Format to **%Reflectance**.

   d. Click the **Advanced** tab and set Apodization to **Triangular**.

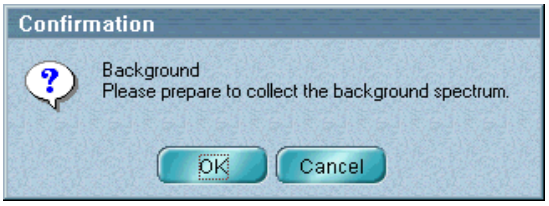
10. Click the **Bench** tab.

    The intensity of the detector signal is indicated by the values above the live display. If no signal appears, see the table in Troubleshooting for instructions.

    For more information about the live display, find "live display" in the OMNIC Help system Index and go to "Using the live display."

11. Click **OK** to close the Experiment Setup dialog box.

12. Click **Collect Background** on the toolbar.



A message asks you to prepare for the background collection.

13. Click **OK**.

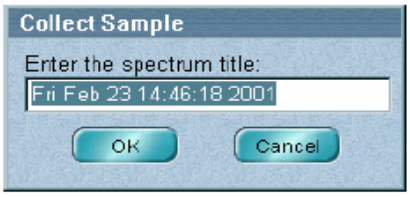
    The Collect Background window shows the background spectrum as it is collected. The progress of the collection is indicated by the gauge near the left side of the window.

    When data collection is finished, a message asks whether to add the spectrum to a spectral window.

14. Click **No**.

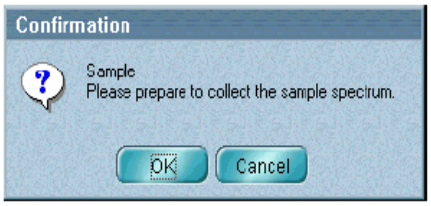
    Even though you are not adding the background to a spectral window, it will remain in memory and will be used for ratioing the sample spectrum you are about collect.

15. Click **Collect Sample** on the toolbar.



The Collect Sample window appears and then a dialog box showing the default title for the sample spectrum.

16. Type a title in the text box or click **OK** to accept the default.



A message asks you to prepare for the sample collection.

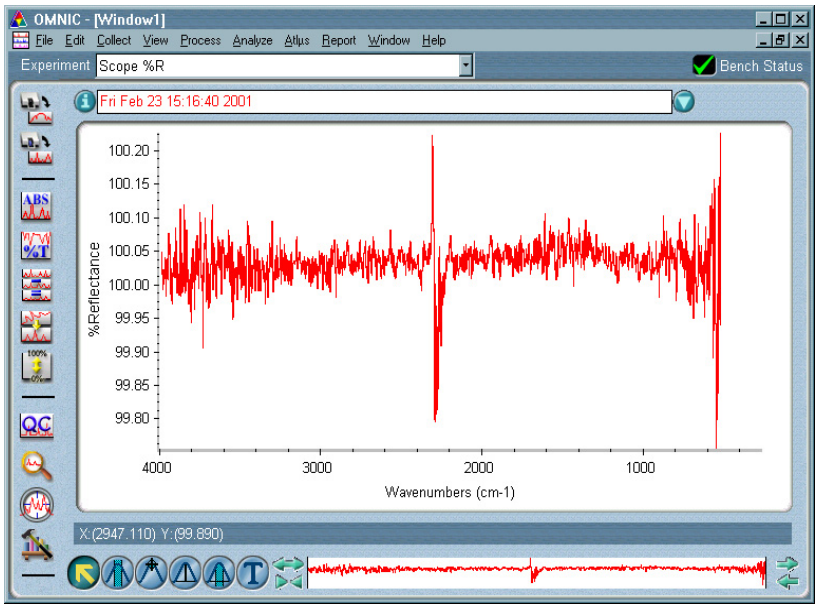
17. Click **OK**.

    Collection of the sample spectrum begins. The progress of the collection is indicated by the gauge near the left side of the window.

    When data collection is finished, a message asks whether to add the spectrum to a spectral window.

18. Click **Yes** to add the spectrum to a spectral window.

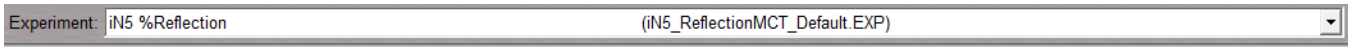
    Example: Sample spectrum displayed in a spectral window:



19. Use the region tool to select the spectral region from approximately 2600 to 2500 wavenumbers.

20. Choose **Noise** from the Analyze menu.

    Example: Typical noise dialog box



    The peak-to-peak noise should be less than 0.10. (Older spectrometers that do not meet basic mid-IR performance standards may have a higher noise level.) If the noise level exceeds 0.10, make sure the spectrometer is functioning properly and then repeat this procedure. See the documentation that came with your spectrometer for information on checking its performance.

    If you repeat the test, make sure these conditions are met:

    - The detector is cool
    - The knob on the left side of the microscope is set to Transmission
    - The aperture is fully open
    - The Sample Compartment parameter is set for the location of the microscope and for Transmission (%T)
    - The condenser is focused

    For more information about the Noise command, find "noise" in the OMNIC Help system Index and go to "Measuring noise."

21. Click **OK** to close the Noise dialog box.
22. Choose **Clear** from the Edit menu to delete the spectrum.

Basic Experiments

This section will get you started collecting spectra with your Nicolet iN5 microscope. You will learn how to collect spectra of a solid Reflection sample, a fiber Transmission sample and an ATR sample. We recommend performing all three data collections, 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 Turning on the System Components in "Before You Collect Spectra" on page 6 for details.

Make sure the detector is cool. See Cooling the Detector in "Before You Collect Spectra" on page 6 if you need help.

Start OMNIC if it is not running.

Start µView if it is not already running. Alternatively, if configured with optional camera, refer to the *MicroView Software User Guide* for instructions.

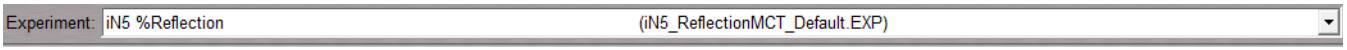
Open the configuration file named **DEFAULT.CON** if you have not already done so. See "Opening a configuration" in "Before You Collect Spectra" on page 6 for details.

Reflection Experiment

When the microscope is set for Reflection 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 collect high quality Reflection data.

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

1. Select the **iN5_%Reflection** experiment from the Experiment drop-down list.



> **Note** If you just performed the Reflection noise test using this experiment (with three parameter settings changed as described in the preceding section), first select the **iN5_%Transmission** experiment and then select the **iN5_%Reflection** experiment. This will reset all the experiment parameters to their original settings in the iN5_%Reflection experiment in one step.

This sets the experiment parameters for collecting Reflection sample data with the microscope.

If the experiment is not available in the Experiment drop-down list box, use **Experiment Setup** to open it. Its filename is **iN5_%Reflection.exp**. For more information, find "experiment" in the OMNIC Help system Index and go to "Selecting an experiment" or "Using Experiment Setup."

2. Set the microscope for Reflection 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.

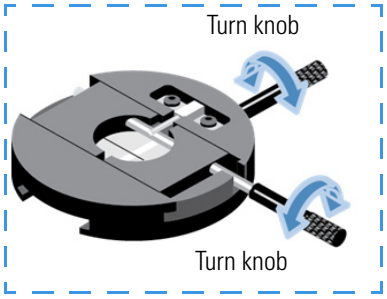
   Using proper illumination will make it easier to focus the microscope.

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.



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 example, 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 Reflection 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 collecting 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.

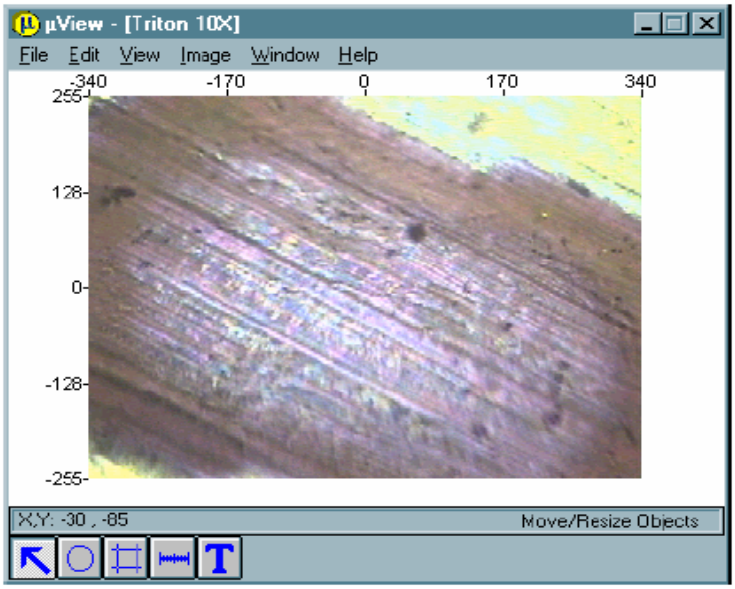
Note

- 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.
- 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 in Microscope Basics for instructions.

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

   Example: Video image of an ink spot

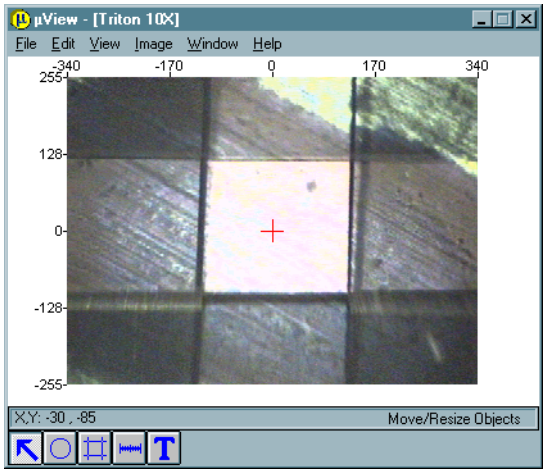


10. While looking at the video or viewer image, use the fine focus knob to sharply focus the image of the ink spot.
11. If you have a Variable Aperture option, adjust the aperture so that the opening is entirely within the area of the ink spot.

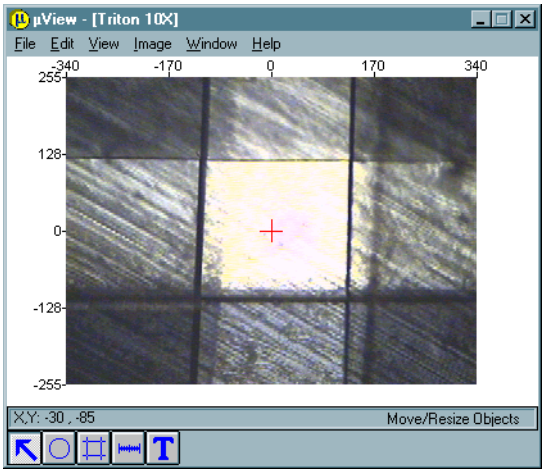
    - Hold the aperture in place in its slot with one hand while turning the knobs with your other hand

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

Example:



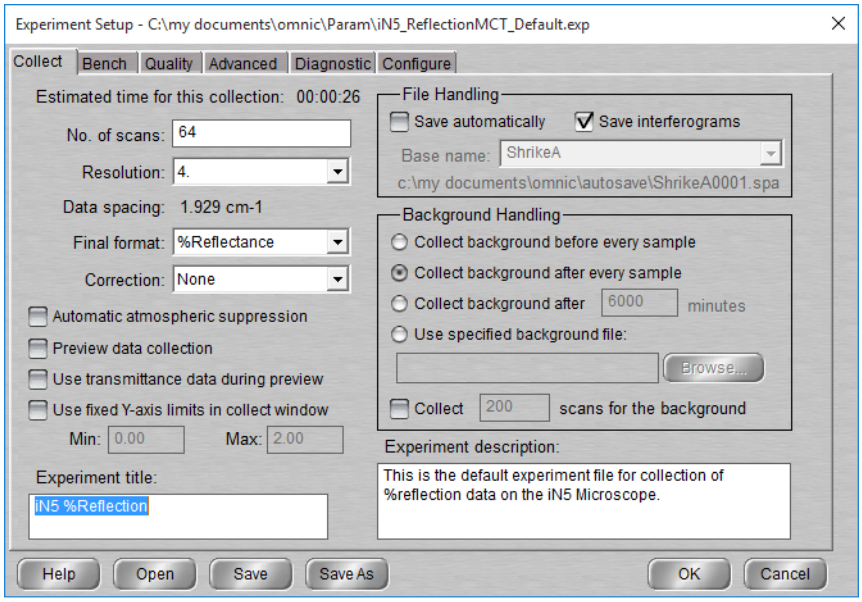
12. Move the stage horizontally so that an unmarked area of the sample is under the objective.



You will use this area to collect a background for ratioing your sample spectrum.

13. Choose **Experiment Setup** from the Collect menu.

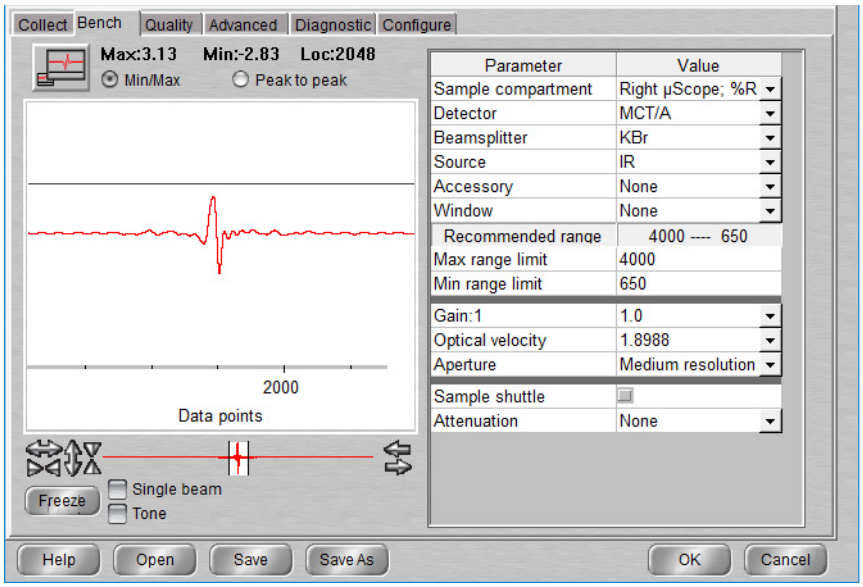
    The Experiment Setup dialog box appears.



    This dialog box contains the experiment parameters used by the iN5_%Reflection experiment. For complete information about the parameters, find "parameters" in the OMNIC Help system Index and go to "Using Experiment Setup."

14. Click the **Bench** tab.

    The Bench tab contains a live display of the detector signal.

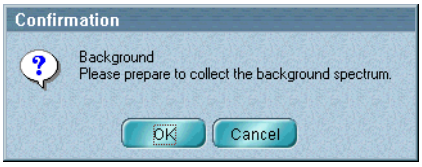


    The intensity of the signal is indicated by the values above the live display. If no signal appears, see "Troubleshooting" on page 50 for instructions.

For more information about the live display, find “live display” in the OMNIC Help system Index and go to “Using the live display.”

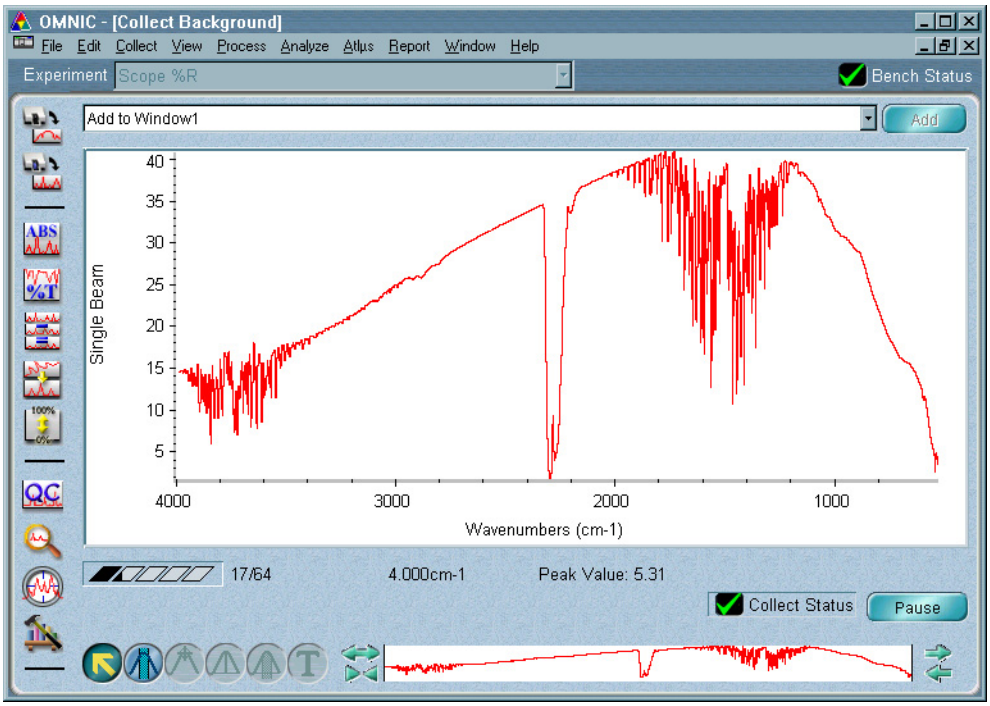
15. Click **OK** to close the Experiment Setup dialog box.
16. Click the **Collect Background** button on the toolbar.

    A message asks you to prepare for the background collection.



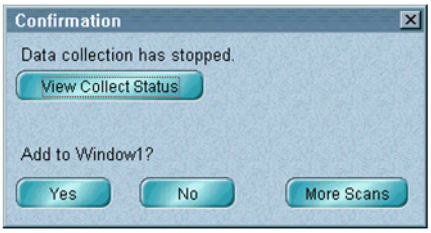
17. Click **OK**.

    The Collect Background window shows the background spectrum as it is collected.



The progress of the collection is indicated by the gauge near the left side of the window.

When data collection is finished, a message asks whether to add the spectrum to a spectral window.



18. Click **No**.

    Even though you are not adding the background to a spectral window, it will remain in memory and will be used for ratioing the sample spectrum you are about collect.

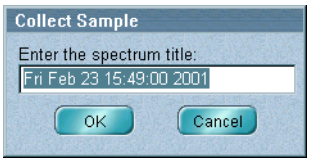
19. Move the stage horizontally to position the ink so that it once again fills the aperture opening.

    You are now ready to collect a sample spectrum.

20. Click the **Collect Sample** button on the toolbar.

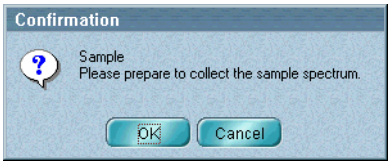
    The Collect Sample window appears and then a dialog box showing the default title for the sample spectrum.

    Example:



21. Type a title in the text box or click **OK** to accept the default.

    A message asks you to prepare for the sample collection.



22. Click **OK**.

    Collection of the sample spectrum begins. The progress of the collection is indicated by the gauge near the left side of the window.

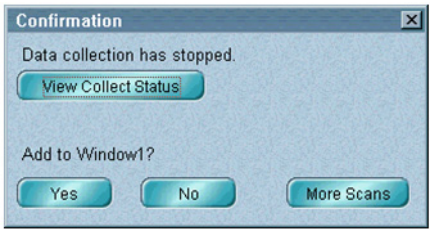
    The Collect Status indicator above the view finder shows the status of the collection during and after collection. When the indicator is a green check mark, it shows that the spectrum has passed all of the selected spectral quality checks made so far. In OMNIC Help Topics find "quality checks" in the Index and go to "Quality checks" for information on specifying the quality checks to perform.

    If you ever have a problem with data collection while one or more quality checks are turned on, the Collect Status indicator may change. If the indicator is a yellow circle, the spectrum has failed a spectral quality check (a measured value was not within the allowed range), but it is not serious enough to stop the collection. If the indicator is a red X, there is a problem with the quality of the spectrum. After correcting the problem, collect the spectrum again.

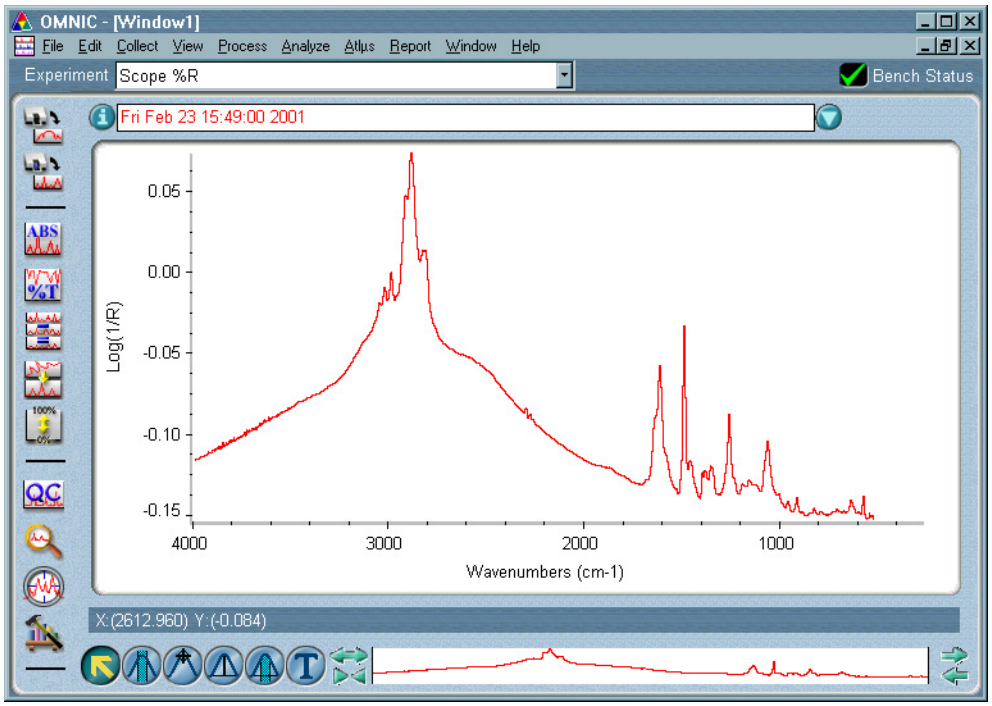
    When data collection is finished, a message asks whether to add the spectrum to a spectral window.

    You can click **View Collect Status** to display the Results window, showing a summary of any problems encountered during data collection and other information about the collection. After you view the information, click **Close**.

23. Click **Yes** to add the spectrum to a spectral window.

    Here is the ink spectrum displayed in a spectral window:



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

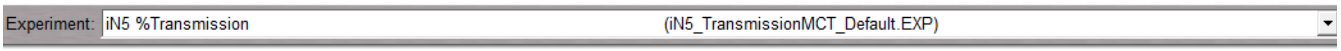
    The sloped baseline of this spectrum is due to the thickness of the ink on the sample. The baseline of a spectrum should normally be consistent and smooth. The closer the baseline is to 0 log (1/R) units (100% reflectance) the better. This makes the band intensities easier to measure and allows you to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

Fiber Transmission Experiment

When the microscope is set for Transmission 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. Select the **iN5_%Transmission** experiment from the Experiment drop-down list box.



NOTICE If you just performed the Transmission noise test using this experiment (with three parameter settings changed as described in the preceding section), first select the **iN5_%Reflection** experiment and then select the **iN5_%Transmission** experiment. This will reset all the experiment parameters to their original settings in the iN5_%Transmission experiment in one step.

This sets the experiment parameters for collecting Transmission data with the microscope.

If the experiment is not available in the Experiment drop-down list box, use **Experiment Setup** to open it. Its filename is **iN5_%Transmission.exp**. For more information, find "experiment" in the OMNIC Help system Index and go to "Selecting an experiment" or "Using Experiment Setup."

2. Set the microscope for Transmission by turning the Reflection & Transmission 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.

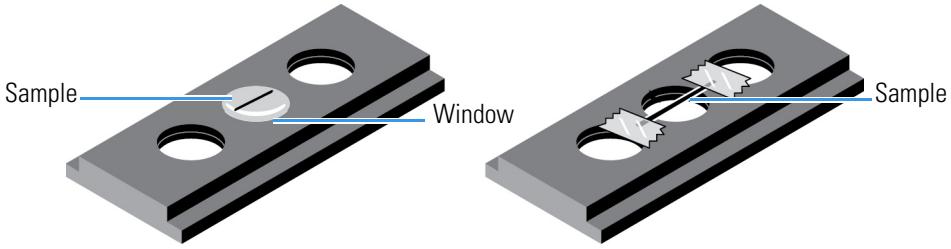
3. Turn the illumination all the way up.

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).



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 an unflattened 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.

If you want to collect a spectrum with the sample between two windows, use the optional compression cell as explained in *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.

   Example: Video image of the fiber

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

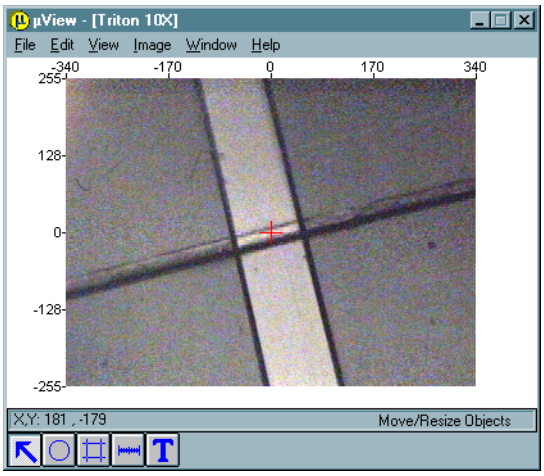
NOTICE

- 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.

Example: Aperture opening entirely within the area of the fiber:



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

    Example:

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

12. Choose **Experiment Setup** from the Collect menu.

    The Experiment Setup dialog box appears.

13. Click the **Bench** tab to view the live display of the detector signal.

14. 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.

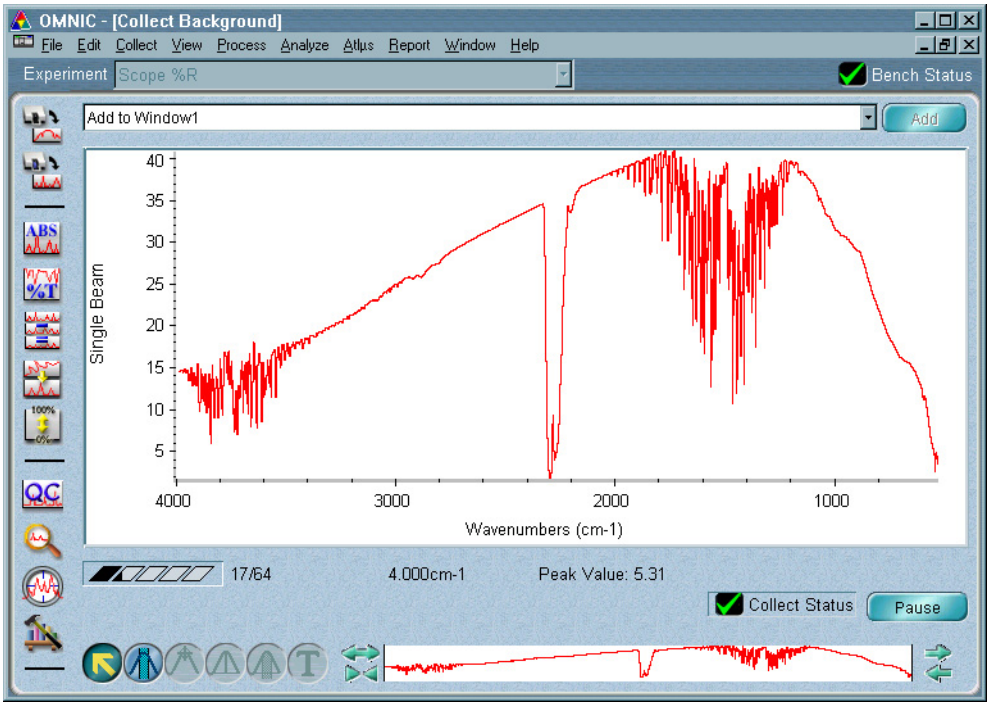
When you analyze your own samples, you don't need to focus the condenser before each sample unless the samples are of different thicknesses. Focusing the condenser before the first sample in a sequence of similar samples is usually adequate.

15. Click **OK** to close the Experiment Setup dialog box.
16. Click **Collect Background** on the toolbar.

    A message asks you to prepare for the background collection.

16. Click **OK**.

    The Collect Background window shows the background spectrum as it is collected.



The progress of the collection is indicated by the gauge near the left side of the window.

When data collection is finished, a message asks whether to add the spectrum to a spectral window.

17. Click **No**.

    The background will remain in memory and will be used for ratioing the sample spectrum you are about collect.

18. Move the stage horizontally to position the fiber so that it once again fills the aperture opening.
19. Click **Collect Sample** on the toolbar.

The Collect Sample window appears and then a dialog box showing the default title for the sample spectrum.

20. Type a title in the text box or click **OK** to accept the default.

    A message asks you to prepare for the sample collection.

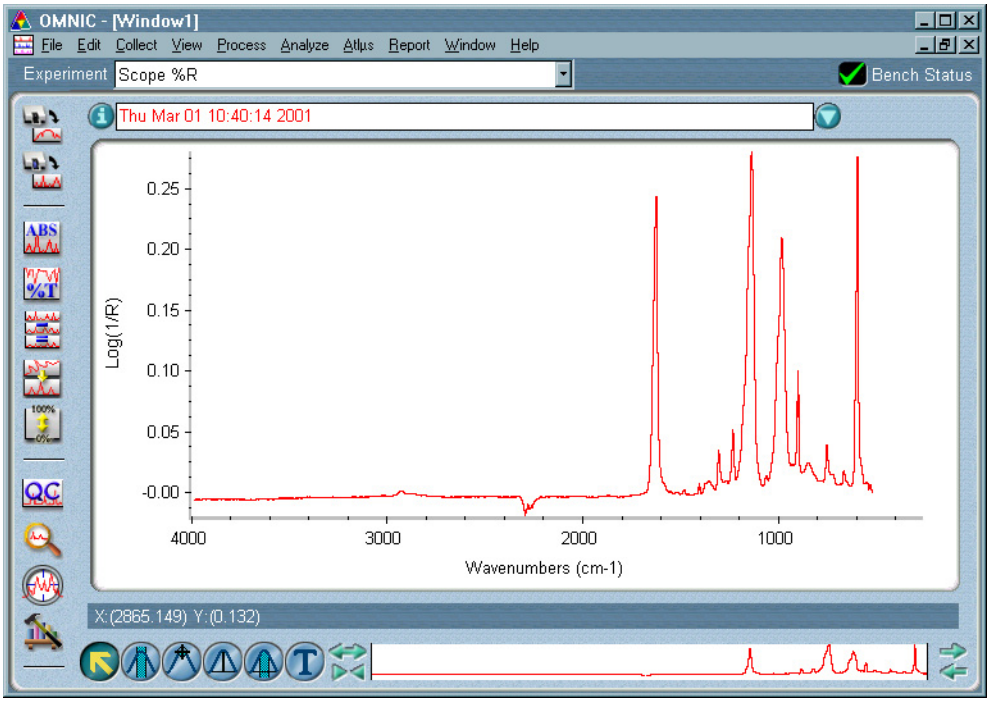
21. Click **OK**.

    Collection of the sample spectrum begins. The progress of the collection is indicated by the gauge near the left side of the window.

    When data collection is finished, a message asks whether to add the spectrum to a spectral window.

22. Click **Yes** to add the spectrum to a spectral window.

    Example: Fiber spectrum displayed in a spectral window:



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 Experiment

Ge ATR slider

Diamond ATR

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

Applications of ATR microscopy include these:

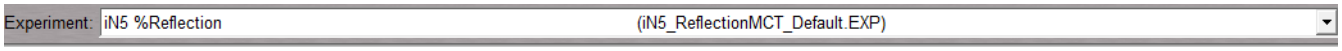
- Analyzing surface defects or inclusions
- Analyzing glass-filled fibers
- Analyzing biological materials
- Analyzing surface degradation
- Performing polymer studies
- Performing quantitative analyses

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 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. Alternatively, a prepared sample is included with the optional Polystyrene Standards Plate used with the ValPro™ System Qualification option.

1. Select the **iN5_Reflection** experiment from the Experiment drop-down list box.



NOTICE If you just performed the Reflection noise test using this experiment (with three parameter settings changed as described in the preceding section), first select the **iN5_%Transmission** experiment and then select the **iN5_%Reflection** experiment. This will reset all the experiment parameters to their original settings in the **iN5_%Reflection** experiment in one step.

This sets the experiment parameters for collecting ATR data.

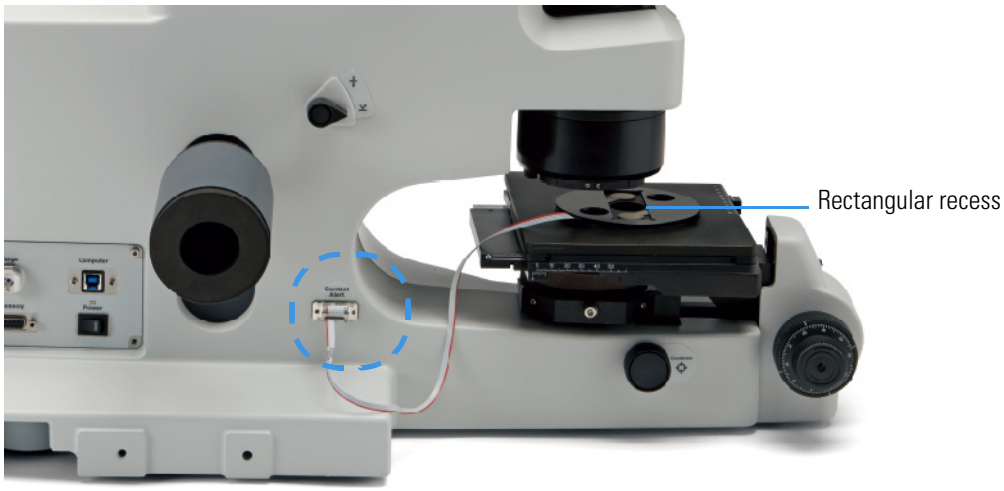
If the experiment is not available in the Experiment drop-down list box, use **Experiment Setup** to open it. Its filename is **iN5_Reflection**. For more information, find "experiment" in the OMNIC Help system Index and go to "Selecting an experiment" or "Using Experiment Setup."

2. Set the microscope for Reflection 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.

Contact Alert plate

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



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 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.

13. Choose **Experiment Setup** from the Collect menu.

    The Experiment Setup dialog box appears.

14. Select the **Preview sample....** option.
15. Click **OK** to close the Experiment Setup dialog box.
16. Click **Collect Background** on the toolbar.

    A message asks you to prepare for the background collection.

17. Click **OK**.

    The Collect Background window shows the background spectrum as it is collected. When data collection is finished, a message asks whether to add the spectrum to a spectral window.

18. Click **No**.

    The background will remain in memory and will be used for ratioing the sample spectrum you are about collect.

19. Click **Collect Sample** on the toolbar.

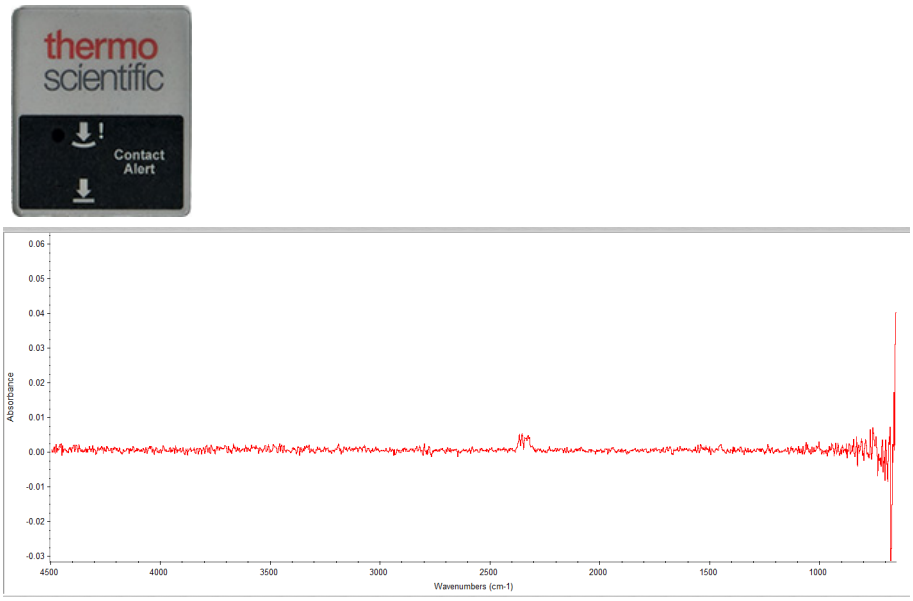
    The Collect Sample window appears and then a dialog box showing the default title for the sample spectrum.

20. Type a title in the text box or click **OK** to accept the default.

    A message asks you to prepare for the sample collection.

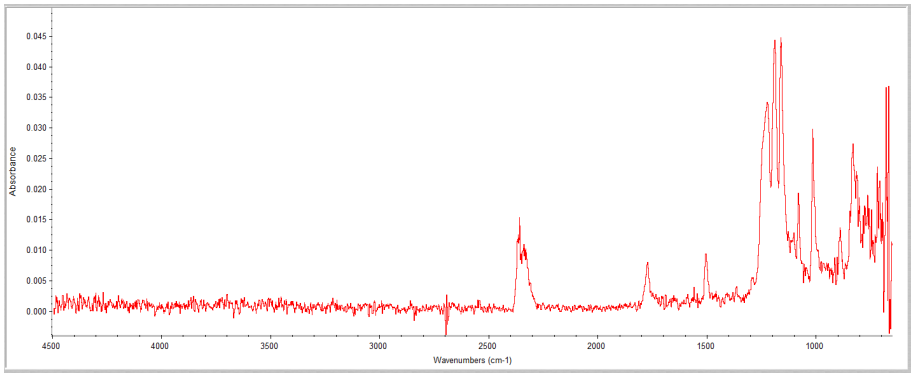
21. A preview of your sample spectra will appear.

    Note, there are no peaks associated with your sample present since the crystal is not in contact with the sample.



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

Green indicator illuminated



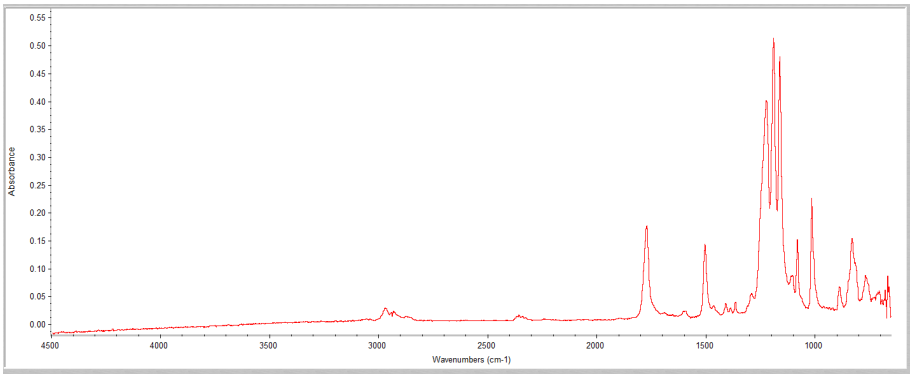
The sample has made initial contact with the ATR crystal at this point.

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.

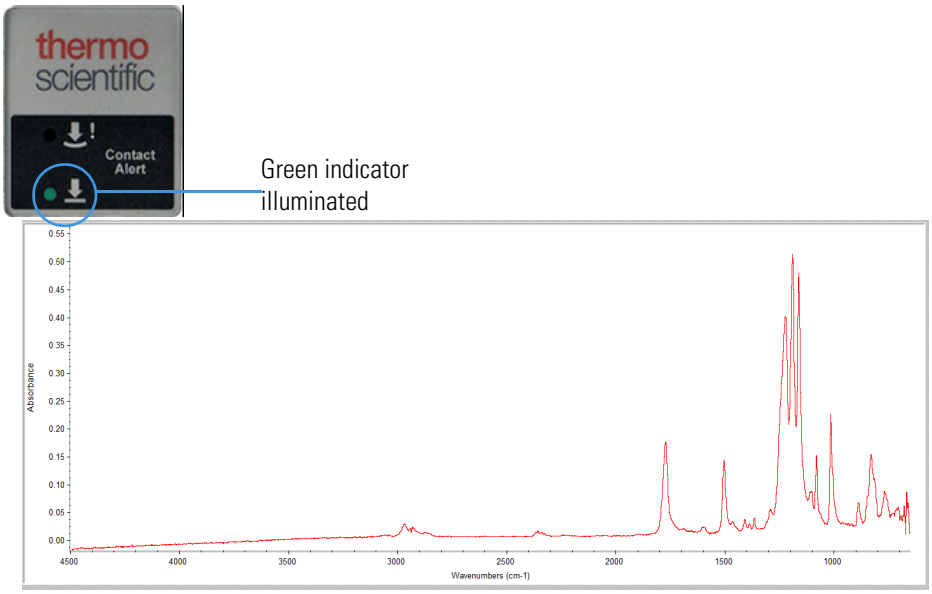
23. Continue to slowly raise the stage until the red indicator illuminates–STOP IMMEDIATELY.

You will have seen the spectral peak intensity of your sample increase with increased force.

Red indicator illuminated



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



The contact pressure and signal intensity is now optimum for collecting data.

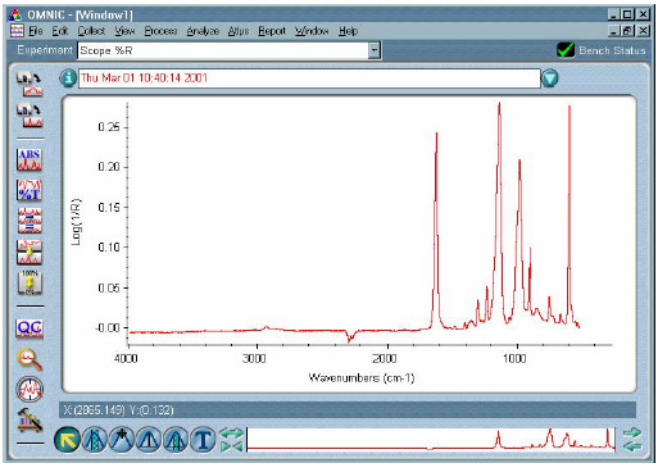
25. Click **Continue Collect** to begin sample data collection.

The progress of the collection is indicated by the gauge near the left side of the window.

When data collection is finished, a message asks whether to add the spectrum to a spectral window.

26. Click **Yes** to add the spectrum to a spectral window.

Example: Sample spectrum displayed in a spectral window



- 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 that ATR spectra show stronger bands at the long wavelength (lower wavenumber value) end of the spectrum
- You may wish to use OMNIC's ATR correction feature to render the spectrum more similar in relative band intensities to a Transmission spectrum

27. Click **Other Corrections** from the **Process** menu and select **ATR**
28. Carefully raise stage.

    The green indicator will turn off. Continue to lower the stage to the full extent of travel.

    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

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

Including a Video Image in a Report

It is often helpful to include a video image of your sample in a report created with OMNIC. You can do this easily if you have µView.

1. Display the desired image of the sample in the video pane.

   Focus the image sharply for best results.

2. Choose **Copy Video Image from the Image menu of µView**.

   This copies the video image to the Windows™ Clipboard.

3. Use Template in the Report menu of OMNIC to create or select a report template that includes a Current Clipboard Picture item or Clipboard Picture item.

   - If a template includes a Current Clipboard Picture item, reports generated using the template will include the contents of the Clipboard at the time the report is generated.
     - This allows you to use the same template to generate reports for a variety of samples. You simply copy the desired sample video image to the Clipboard just before generating the report.
   - If a template includes a Clipboard Picture item, reports generated using the template will include the contents of the Clipboard at the time the template was created.
     - This means that the video image you just copied to the Clipboard will appear in all reports you generate with the template in the future.
     - This item is most useful when you want your reports to include an image that doesn't change; for example, a corporate logo.
     - For video images, you will probably find the Current Clipboard Picture item more useful.

   For complete instructions on using the Template command, find "template" in the OMNIC Help system Index and go to the "Selecting, editing or creating a report template" topic. Click the How To button near the top of the topic to see a step-by-step procedure.

4. Use **Preview/Print Report** in the Report menu of OMNIC to view and print the report.

   The displayed report is generated using the template that you just created or selected.

   - If the template includes a Current Clipboard Picture item, the report contains whatever image you copied to the Clipboard most recently; in this case, the sample video image.
     - After you print the report or close the report preview, you can use µView to copy a different sample video image to the Clipboard and then use Preview/Print Report again to view and print a report containing that video image.
   - If the template includes a Clipboard Picture item, the sample video image you copied in step 2 appears in the report—and will appear in all future reports generated with the template.

   For complete instructions on using the Preview/Print Report command, find "report" in the OMNIC Help system Index and go to the "Previewing or printing a report" topic. Click the How To button near the top of the topic to see a step-by-step procedure.

Tips for Collecting a Background

Since your background spectra can have a significant effect on the quality of your ratioed sample spectra, it is important to collect a background using the technique that is most appropriate for your application. Follow the tips below to obtain the best possible backgrounds for your Transmission, Reflection 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 collect data.
- Depending on the sample and the background material you want to use for a Reflection experiment, you may need to install the sample on a slide that has a gold or aluminum mirror for collecting 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 collect 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 collect Transmission data, place a single crystal of KBr on the cell substrate. The crystal should be about the same thickness as the sample. Collect the background through the crystal, making sure the apertured area does not extend beyond the crystal.
- If you are collecting spectra with the sample installed on a window or between two windows, collect 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.

Touch Point Button

iN5 Microscope

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.

Touch Point button (only available with the Nicolet iS50 spectrometer). This button automatically configures the spectrometer for microscope sampling. The Touch Point LED shows the status of the ATR module and has three states:

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

The microscope spectral range options are the same as for the spectrometer and are dependent upon the spectrometer configuration. Refer to the spectrometer help topics or iS50 ATR Module user guide for more information.

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 Reflection sample, you can remove the condenser so that the stage can be lowered further. (The condenser is not used in a Reflection experiment.)

Before you start:

- Maximize the signal by adjusting the condenser focus knob
- Make a record of the detector signal intensity obtained with the microscope set for Transmission and with no sample on the stage

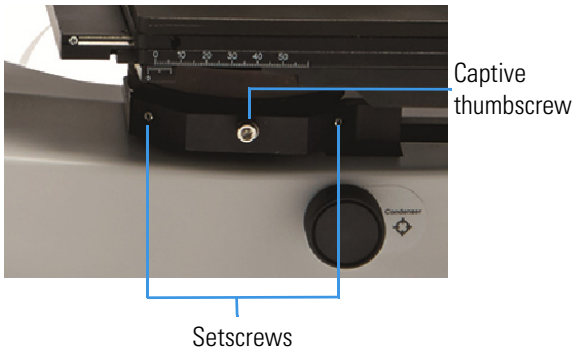
  The signal intensity appears above the live display on the Bench tab of the Experiment Setup dialog box
- Display the intensity as a peak-to-peak value by selecting Peak To Peak to the right of the live display
- After you reinstall the condenser, you will maximize the signal again and compare it with the recorded value to make sure the condenser is installed correctly

❖ To remove the condenser

1. Remove any sample from the stage

   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 condenser stage to its highest position.
3. Use the Condenser focus to lower the condenser to its lowest position.

4. Loosen the captive thumbscrew all the way.



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.

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 watching the live display on the Bench tab of the Experiment Setup dialog box, 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.

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.*"

For ordering information, point to Microscope in the Help menu of OMNIC, choose **Replacing Parts** and then view "Ordering parts" in the "Ordering parts" book.

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.

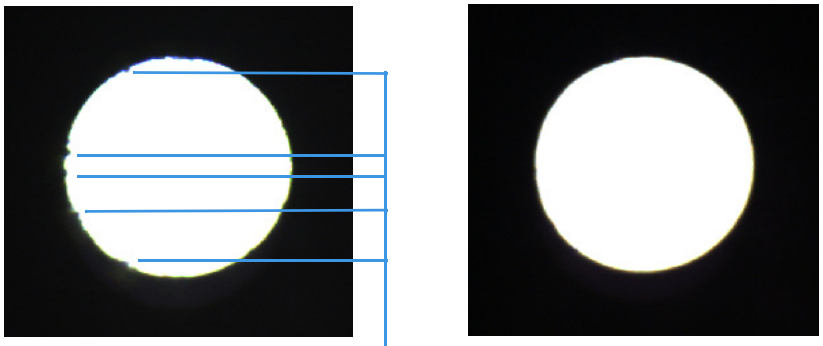
For ordering information, point to Microscope in the Help menu of OMNIC, choose **Replacing Parts** and then view "Ordering parts" in the "Ordering parts" book.

Maintenance and Service

For information on maintaining and servicing your microscope, point to Microscope in the Help menu of OMNIC, choose **Replacing Parts** and then view the topics in the "Service and maintenance" book. Also see "System Performance Check" on page 12 in the Before You Collect Spectra section of this manual.

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.



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
The noise test failed.	The microscope is not focused.	Focus the microscope. For Transmission 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.
No image appears.	The microscope is not focused.	Focus the microscope. For Transmission 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.
The image is blurred	The microscope is not focused.	Focus the microscope. For Transmission experiments, be sure to check both the sample and condenser focus.

Problem	Possible Cause	Solution
The signal is weak without a sample in the beam path.	The microscope is not in the correct mode.	Set the microscope to Transmission or Reflection as needed.
	The microscope is not focused.	Focus the microscope. For Transmission experiments, be sure to check both the sample and condenser focus.
	The detector is not cooled.	"Cooling the Detector" on page 7. Allow at least 10 minutes for the detector to cool.
	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
No signal appears in the live display.	The microscope is not in the correct mode.	Set the microscope to Transmission or Reflection as needed.
	The microscope is not focused.	Focus the microscope. For Transmission experiments, be sure to check both the sample and condenser focus.
	The detector is not cooled.	"Cooling the Detector" on page 7. Allow at least 10 minutes for the detector to cool.
	The beam path is blocked or not set correctly.	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.
	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.

Problem	Possible Cause	Solution
The signal is weak with a sample in the beampath.	The microscope is not in the correct mode.	Set the microscope to Transmission or Reflection as needed.
	The microscope is not focused.	Focus the microscope. For Transmission experiments, be sure to check both the sample and condenser focus.
	The detector is not cooled.	"Cooling the Detector" on page 7. Allow at least 10 minutes for the detector to cool.
	The condenser thumbscrew has been overtightened (for Transmission 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.
	The infrared energy level in the spectrometer is low.	Set Sample Compartment to **Main** and check the signal intensity on the **Bench** tab of the Experiment Setup dialog box. 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. See the documentation that came with your spectrometer for more information.
	The sample was not properly prepared.	Prepare the sample properly. For example, a Transmission sample should not be too thick.
Sample focus stage drifts out of focus.	Sample stage focus knob tension is not set properly.	Increase sample stage tension.

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.

Contact Us

Technical Support: www.thermofisher.com