FT-IR Microscopes

Imaging ATR Accessory

User Guide

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Imaging ATR Accessory

The Thermo Scientific Imaging ATR (Attenuated Total Reflection) accessory for our Nicolet™ iN10, Nicolet iN10MX and Nicolet Continuμm™ FT-IR microscopes supports chemical imaging studies with enhanced spatial resolution from a single, large area contact point. The large contact area allows you to collect area maps and images very quickly. It also makes this accessory ideal for mapping samples that may be disturbed or deformed by using multiple contact points. Examples include paints, cultural artifacts, biological tissues, and materials that are sticky or oily. (The accessory is generally not applicable for liquids and loose powders.)

This document covers:

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Conventions Used in This Manual

This manual uses the following conventions for providing safety and other special information:

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.

Operating Precautions

NOTICE The germanium crystal can be broken if small, hard materials are compressed against it at maximum pressure.

To maximize the life of your Imaging ATR accessory, heed these precautions:

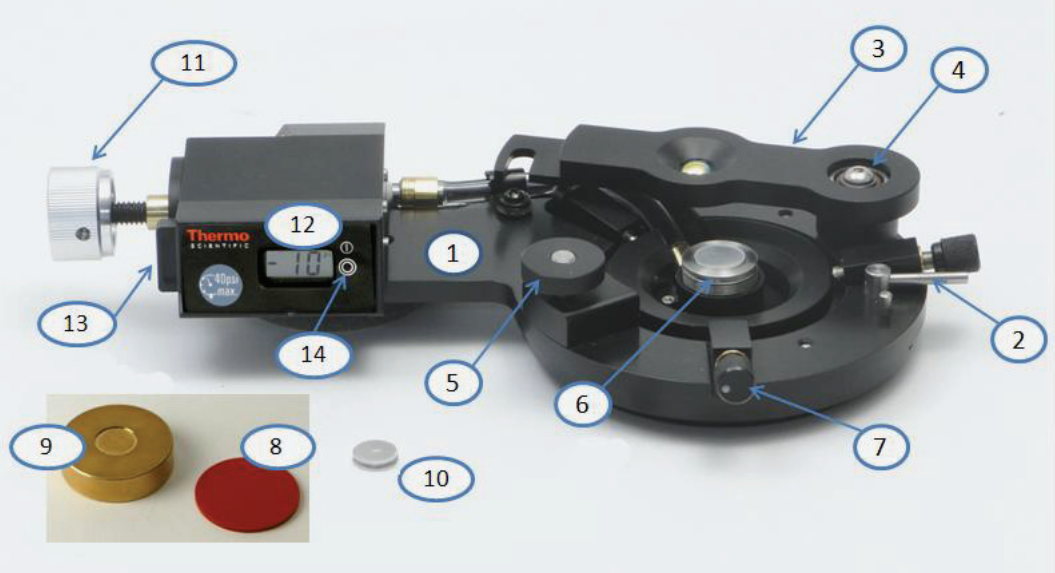
- Do not apply pressure to the crystal without a sample in place.
- Do not use the pressure device to crush a sample.
- Make sure your sample will not react with the germanium crystal.
- Do not rub the crystal against the sample.
- Do not scrape the crystal with any type of metal tool.
- Do not use abrasive cleaning agents, pads or strongly acidic or basic solutions to clean any surface of the accessory.

Features and Operation

The Imaging ATR accessory is designed to support chemical imaging studies with enhanced spatial resolution. Micro-ATR techniques place the sample in direct contact with a germanium ATR crystal. That technique results in an effective improvement in spatial resolution compared to open beam transmission or reflection measurements in air. Single point ATR mapping with our slide-on micro-ATR accessory offers the same benefit and is also more flexible in terms of total image size and dimensions. However, with single point mapping many separate contact points are needed to build up the imaging data set. The Imaging ATR accessory is a faster alternative to slide-on micro-ATR because only a single, large area contact point is required.

Components

Figure 1. Imaging ATR accessory features



The key components are:

1 **Baseplate**. Mates to the standard 3 3/8 in diameter sample holder recess found on all Thermo Scientific infrared microscope stages.

2 **Stage Lock Lever**. Activates a cam to secure the accessory to prevent rotation or shifting that may affect results.

3 **Crystal Arm**. Holds the anti-reflection coated germanium ATR crystal. If the crystal becomes damaged or broken or otherwise needs replacement, you can order a new crystal arm and replace it yourself. Refer to Maintenance for details.

4 **Crystal Pivot Screw**. Hex head machine screw that is removed to change the crystal arm. The screw is covered by a cap. Remove the cap to access the screw.

5 **Crystal Arm Lock**. A detent lets you know the crystal arm is in the proper position for data collection. The crystal arm lock ensures the crystal arm does not move under pressure. Tighten the knob finger tight to lock the arm during operation.

6 **Stainless Steel Pressure Column**. Holds the sample and presses it (upward) against the ATR crystal to achieve optical contact.

7 **Pressure Column Centering Screws** (2). Used to bring the sample directly onto the optical axis. The sample should be placed as close to the center of the pressure column as is practical.

8 **Storage Disk**. Must be installed in the sampling area under positive pressure when the Imaging ATR accessory is not in use. The disk is required to maintain proper operation of the hydraulic assembly. It also protects the germanium crystal from being scratched by metal parts. Refer to Installing the Storage Disk for details.

9 **Pressure Column Cap**. Can be placed on top of the pressure column to provide a larger, more stable sampling surface. Remove the cap to accommodate thick samples.

10 **Sample Holders** (3). Hold the sample in place during the analysis. The recess in the top of the pressure column accepts 13 mm diameter disks. Custom sample holders may also be used. Mount your sample as close to the center of the sample holder as is practical.

11 **Pressure Column Control Knob**. Rotate the knob clockwise to raise the pressure column and counterclockwise to lower it. The control either draws a vacuum or creates pressure inside the fluid-driven assembly to expand or compress the column spring. An internal clutch helps protect the hydraulic assembly from being over pressurized. For details, refer to Using the Pressure Column Controls.

12 **Pressure Indicator**. Monitors the internal pressure of the drive assembly. When the pressure reading is negative, the pressure column is compressed. A positive value indicates the column is expanded.

13 **Pressure Indicator Battery Access**. Provides access to the battery for the pressure indicator. The indicator requires a 3 volt lithium coin size battery. Battery life is estimated at 7 months of continuous use.

14 **Pressure Indicator On/off Switch**. To extend the indicator battery life, use this switch to turn off the display when not in use. Press the button once momentarily to change between the on and off states.

NOTICE If you press and hold the on/off switch for 5 seconds, the pressure indicator records the current pressure as zero and reports changes in pressure relative to this new zero state. **Avoid** operating in this state because the absolute internal pressure is no longer known. To restore the factory settings, remove the battery, wait a few seconds and then reinstall the battery.

In addition to the main accessory, the kit includes sample spacers in several thicknesses, a tweezer for manipulating the spacers or other 13 mm disks, and a set of self adhesive polyimide masking discs that may be used as practice samples or for performance checks.

Using the Pressure Column Controls

NOTICE Handle the pressure column controls carefully and follow the instructions below or you may damage the crystal.

Before you place a sample on the crystal, turn the pressure column control knob counter clockwise to lower the pressure column so that the crystal will not scrape or rub against the sample when you rotate the crystal arm.

When the column is retracted, the pressure reading is negative. The safe value depends on the thickness of your sample. When the column is retracted as far as it will go (to accommodate the thickest sample), the indicator typically reads between -10 and -12. Thin samples may not require full vacuum for clearance. After you establish the safe indicator reading for a particular sample type, you may use the indicator alone without visually checking for clearance.

After you load the sample (see Mounting and Loading a Sample for instructions), turn the pressure column control knob clockwise to raise the pressure column. The indicator becomes less negative and then swings to positive values as contact is made with the crystal. Once contact is made, the pressure column no longer expands so the internal pressure builds rapidly with relatively small turns of the control knob.

Just as sample thickness influences the indicator reading for clearance, sample thickness also influences the absolute reading of the indicator upon contact with the sample. Once this contact point is established for a particular sample type, you can use the indicator reading to quickly re-establish the same contact pressure for similar samples.

The maximum safe internal pressure is 40 psi. **For most samples, good optical contact is achieved at quite low pressures (2 to 15 psi). The pressure control knob contains an internal clutch to prevent gross over-pressure conditions. The clutch slip-point is far above typical sampling pressure. Always use the digital indicator, and not the clutch slip-point, to monitor sample pressure.**

Tip It is important to understand that the pressure indicator reports the internal pressure of the hydraulic fluid. While related, it is **not** the pressure applied to the sample. The purpose of the indicator is to ensure the accessory is operating within safe limits, and to allow reproducible settings for retracted and contact positions for different sample types. Therefore, you should consider the indicator to report arbitrary units.

Specifications

Feature	Specification
Crystal material	Germanium
Refractive index	4.0
Median angle of incidence	32 degrees
Contact area	1 mm diameter
Minimum IR active area	500 x 500 micrometers[a]
Spectral range cutoff	650 cm^{-1}
Maximum sample thickness	8 mm

[a] 400 x 400 micrometers on the Nicolet Continuμm microscope

Installation

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Installing the Accessory

Follow these steps to install the Imaging ATR accessory in your FT-IR microscope.

❖ To install the Imaging ATR accessory

1. Lower the condenser on your microscope all the way, or demount it if applicable (see "Tips for specific Thermo Scientific microscopes" below).
2. Lower the microscope stage far enough to accommodate the accessory (typically near its lower limit).

   It may help to move the stage as far to the left and forward as possible to make more room for the accessory. Refer to the documentation that came with your microscope for help with performing these operations.
3. Place the accessory in the round recess of the stage top plate.

4. Rotate the accessory in the stage until it is square with the front top plate of the stage.

Figure 2. Imaging ATR accessory mounted on a typical motorized microscope stage

5. Activate the stage lock lever to keep the stage firmly in place when in use.

   Move the lever clockwise until it touches the post.

Tips for specific Thermo Scientific microscopes:

- **Nicolet iN10 family microscopes** – The Imaging ATR accessory always operates in reflection mode. When in reflection mode, use the IR Energy tool to control the condenser and stage motors. Use the Park Condenser button to move the condenser to its lower limit. The Eject Stage button automatically lowers the stage and moves it to a convenient location for mounting the accessory. To control the stage height, use the slider for the IR Energy tool, or the Focus control on the virtual joy stick.
- **Nicolet Continuμm microscopes** – The condenser is normally mounted or demounted to accommodate different magnifications. The condenser must be demounted while using the Imaging ATR accessory (be sure to place the condenser in a clean, safe location). To control the stage height, use the motorized Focus control knob on the right side of the microscope, or the optional focus control in the Atlμs window, if Atlμs software is installed.

Installing the Storage Disk

The Imaging ATR accessory includes a red plastic disk that must be installed in the sampling area under positive pressure when the accessory is not in active use. The disk is required to maintain proper operation of the hydraulic assembly. It also protects the germanium crystal from being scratched by metal parts.

Install the disk when you are finished analyzing samples for the day, and leave it in place if you remove the accessory from the microscope.

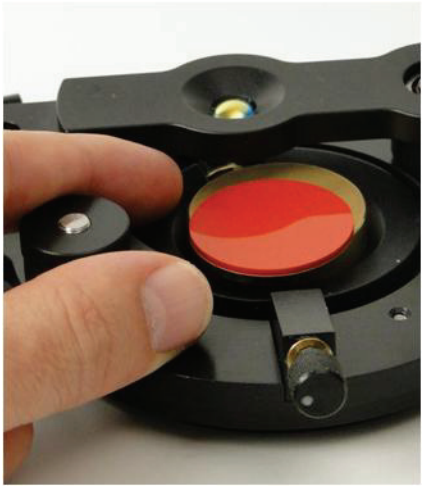
NOTICE Do not store the Imaging ATR accessory at vacuum, that is, with a negative reading on the pressure indicator.

❖ To install the storage disk

1. Lower the pressure column by turning the control knob counter clockwise.
2. Release the crystal arm lock and swing the arm out of the way.
3. Remove any sample or spacer from the pressure column.

   The pressure column cap may remain in place if it is installed.
4. Place the red plastic storage disk on the pressure column.

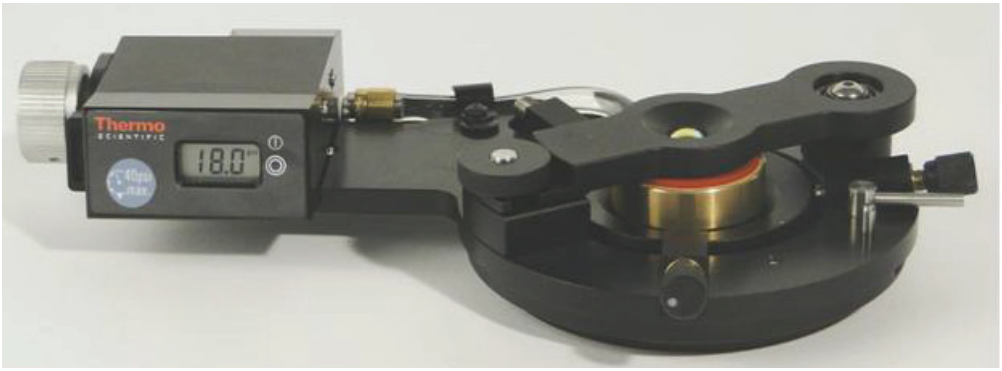
   Figure 3. Inserting the storage disk on the pressure column



5. Close and lock the crystal arm.
6. Rotate the pressure column control knob clockwise until the indicator reads about 20 psi.

   The exact value is not important; however, do not exceed a reading of 40 psi.

   Figure 4. Storage disk properly installed



Operation

Note If the accessory has been stored under negative pressure, the pressure column may no longer retract. To restore normal operation, follow the instructions above to install the storage disk and leave the column under pressure overnight.

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Preparing the Accessory for Operation

Perform these operations before you begin using the imaging ATR accessory:

- Center the accessory and the ATR crystal
- Center the pressure column.

Centering the Accessory and the ATR Crystal

The purpose of this step is to place the apex of the germanium hemisphere crystal on the optical axis of the microscope. One way to find this position is by lowering the stage to its maximum extent, and then moving the stage until the top of the crystal is in the middle of the field of view. Because the crystal surface is curved, this can be a challenge. Also, on some microscopes the stage may not lower far enough to bring the top of the crystal into focus. The following is an alternative, and preferred, method.

❖ **To center the accessory and the ATR crystal**

1. Press the Home button of your stage control software.

   Assuming that your stage is correctly initialized, this places the center of the stage recess, and thus your ATR crystal, very close to the correct x-y location (that is, directly below the center of the microscope objective).

   Note In this document, x refers to the right-left axis, y refers to the front-back axis, and z refers to the up-down axis.

2. Position the stage near its lower limit and then monitor the reflected IR energy as you raise the stage (the energy will increase).

   Adjust the stage height so that the reflected IR energy is at its maximum value. (Watch the intensity of the interferogram in a real-time display, for example on the OMNIC Experiment Setup > Bench tab, or use the IR Energy control in OMNIC Picta software.)

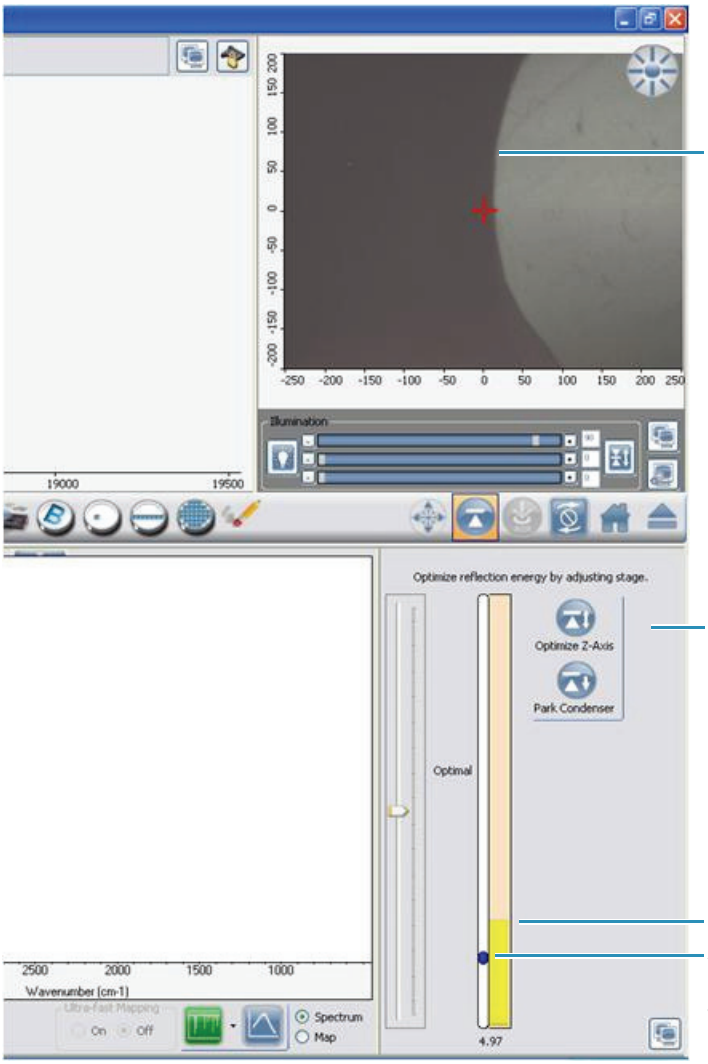
   This will be close to the correct focus (z) position but the crystal is still not centered on the optical (x-y) axis.

3. With the reflected light illuminator set to maximum, move the stage up to 500 micrometers in the x and/or y direction until you see a brightly reflected circle of light on the video display or through the eye pieces.

4. Adjust the stage height to bring the edge of the circle in sharp focus.

   At this point, the image should look similar to Figure 2.

Figure 5. Typical view in OMNIC™ Picta software while adjusting the focus position

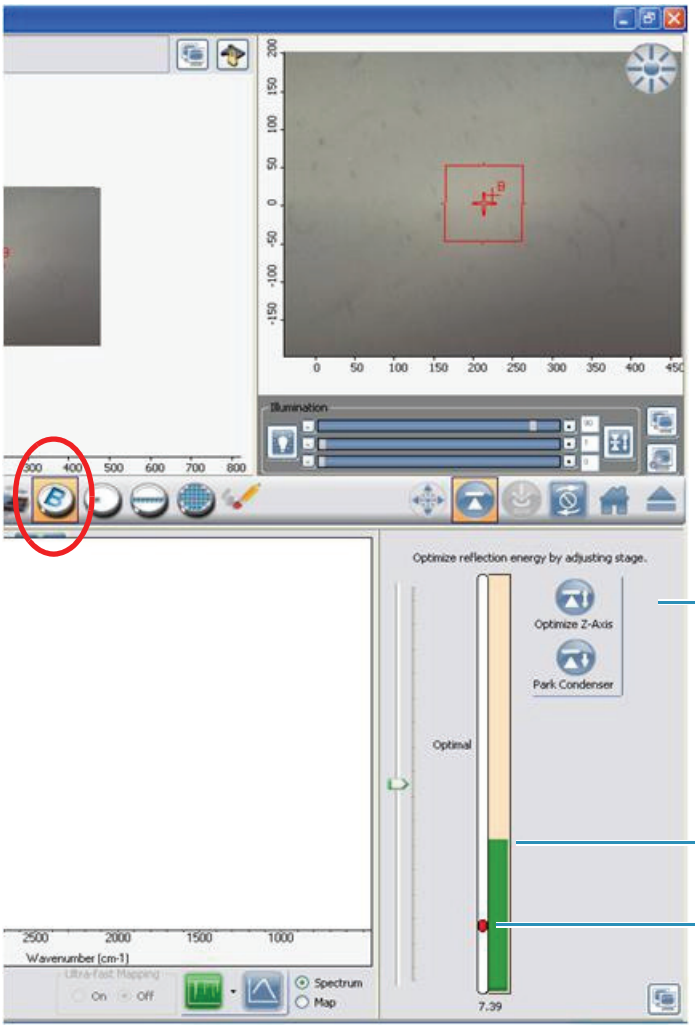


5. Move the center of the field of view to approximately the center of the bright circle.

   You can maximize the focus again by adjusting the stage height and observing the IR energy as described above, but it should be close.

Tip At this point, it is helpful to place a background point in the center to mark the optical axis as shown in Figure 3 (use the Map Background Point tool in OMNIC Picta or Atlμs software). This will allow you to use the Move To Background feature later to quickly return to this position.

Figure 6. Typical setup view with the background location marked



At focus, and close to center, use Map Background Point tool to mark this position. Background point stores current x, y, z coordinates

OMNIC Picta IR Energy control in reflection mode

IR energy bar shows maximum value (standard MCT)

Approximate height indicator (dot) position when in focus

Centering the Pressure Column

After you have centered the crystal on the optical axis of the microscope, you must also center the pressure column.

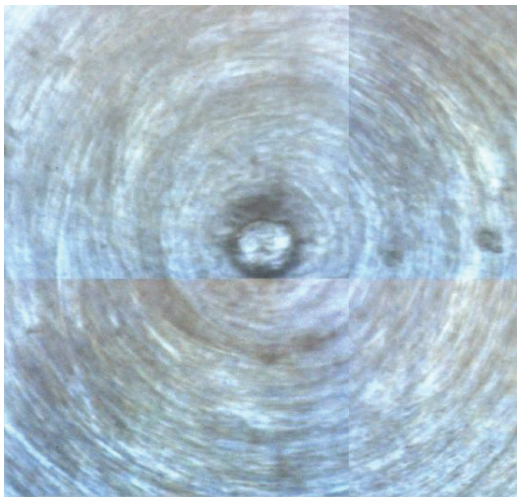
❖ To center the pressure column

1. Release the crystal arm lock and swing the arm out of the way so you can see the top of the column.

   Note Do not move the microscope stage x-y controls; the accessory must remain on the optical axis.

2. Remove any sample and/or spacer from the column.
3. Raise the column and/or the focus so that the top of the column (or column cap) is in view.
4. Adjust the pressure column centering screws so that the obvious concentric machining rings lead you to the center.

   Figure 7. Concentric machining rings seen through the microscope eyepieces

   The crystal and column are now aligned.

Mounting and Loading a Sample

❖ To mount a sample

Samples can be mounted in many different ways. A common method is to place the sample at the center of a sample spacer (included). Spacers are provided in several thicknesses to take up or allow more space for thin or think samples. Use appropriate adhesive, double-sided tape, clay, or “blue-tack” to secure the sample, if necessary, to keep it from moving during the analysis.

The pressure column cap slips over the pressure column and provides a larger, more stable surface for holding most samples. However, the cap reduces the available space between the pressure column and the germanium crystal. If you require more space for your application, remove the column cap.

Tip Place the region of interest as close to the center of the spacer as possible.

❖ To load a sample

1. Use the round-tipped tweezers to lift the spacer and place it into the 13 mm recess on the top of the pressure column or the pressure column cap.
2. If you moved the microscope stage for sample loading, return the stage to the optical axis (using the stored background point mentioned above, for example).
3. View the sample and use the centering screws to make fine adjustments to place the region of interest on axis.

Achieving Optical Contact

Optical contact is the most important consideration for achieving good results with the Imaging ATR accessory. If you want to collect an area map, the entire area of interest must be in optical contact with the crystal surface. Because ATR is a surface sensitive technique, any air gaps, even on the submicron scale, will lead to loss of data at the point of the gap. Therefore, the sample must be very flat (by polishing or cutting with a razor or microtome) or it must conform to the crystal surface under pressure. Particulate contamination on the sample or the crystal surface can also create air gaps. Therefore, it is important to work in a clean area.

The Imaging ATR accessory is capable of generating large pressures on the sample. However, higher pressure does not result in more complete contact. In fact, deformation of the sample by high pressure can result in poorer contact. Well prepared samples of common materials such as polymers, paper, and biological tissues should achieve good contact at pressure indictor readings below 15 psi. Harder samples may require more pressure. In the event good contact (characterized by a strong absorbance signal) is not achieved at a reading of 40 psi, there is very likely something wrong with the sample presentation. Inspect the sample and the crystal surface under a stereoscope for cleanliness or uneven surfaces that are preventing the crystal from making intimate optical contact with the sample.

Collecting Spectra and Maps

This section explains how to use the Imaging ATR accessory to collect a single point or map.

Note To maximize the life of your Imaging ATR accessory, after you are finished using it for the day, be sure to install the storage disk. Please refer to Installing the Storage Disk for instructions.

Single Point Collection

The Imaging ATR accessory is primarily intended for collecting imaging data sets. However, the accessory provides high quality ATR spectra of single contact points and can complement our slide-on micro-ATR tools.

❖ To measure a single point

1. Collect the background before making contact with the sample.
2. Without moving the microscope x-y stage, raise the pressure column until contact is achieved and then collect the sample spectrum.

Collecting Maps

The Imaging ATR accessory is useful for collecting line and area maps using the mapping features of OMNIC Picta and Atlμs software. When collecting imaging data sets with the accessory, consider these two important concepts.

- **First, the actual field of view on the sample is about ¼ the size of what you can see through your camera or eyepieces**, so you are sampling a region that is ¼ the size of what you see in air. (Please refer to Video Calibration with OMNIC Picta Software for more information.)
- **Second, as the stage moves the crystal off the optical axis during the mapping process, the IR light travels at different angles through the crystal**. This makes a single background spectrum inappropriate unless the mapped area is quite small. When the mapped area is large, collecting a separate background for each stage position is ideal. In addition, collecting maps in the Single Beam Spectra final format allows flexibility in applying appropriate background files. It also lets you collect the background data set before or after the sample data set.

Using the Map Math Process Function

The Map Math feature allows you to divide two maps that have the same dimensions and coordinates. For example, you can use Map Math to divide two single beam maps to produce a map of ratioed spectra. Map Math is located in the Atlμs menu in OMNIC software (if Atlμs software is installed) and in the Analyze group in OMNIC Picta software. Please refer to the Atlμs or Picta Help for more information about Map Math.

Video Calibration with OMNIC Picta Software

The video calibration in OMNIC Picta software is fixed and does not account for the effective magnification of the ATR crystal. When viewing the sample directly with the crystal arm open, the software tools operate normally. However, when the crystal arm is closed, the effective magnification of 4x makes the indicated calibration on the video panes 4 time larger at the focus. This affects your interaction with the software in the following ways:

- **Aperture setting is magnified**. For example, a 100 micrometer aperture setting becomes a 25 micrometer image at the sample. This means you can use large apertures and still achieve good spatial resolution.
- **Mapping step size is reduced at the sample**. For example, a 40 micrometer step size becomes a 10 micrometer step size at the sample. This means you can use larger step sizes and achieve the same spatial resolution.
- **Display area is magnified**. For example, drawing a 1000 x 1000 micrometer area map results in a 250 x 250 micrometer area at the sample focus.
- **Stage movement is reduced at the sample**. When using the Move Stage tool, the stage travel is reduced by 4x at the sample. For example, clicking on the video or mosaic video panes physically moves the stage the distance indicated by the calibration, but that is 4 times less at the sample.

Step-By-Step Example Using OMNIC Picta Software

This section takes you through a typical sequence to prepare the Imaging ATR accessory for use and collect background and sample area maps and ratio and save them using OMNIC Picta software.

Note Similar commands and procedures are also available in Atlμs software.

❖ To prepare the accessory and measure a sample using OMNIC Picta software

1. In Experiment Setup, set the following:

   Mode: Reflection

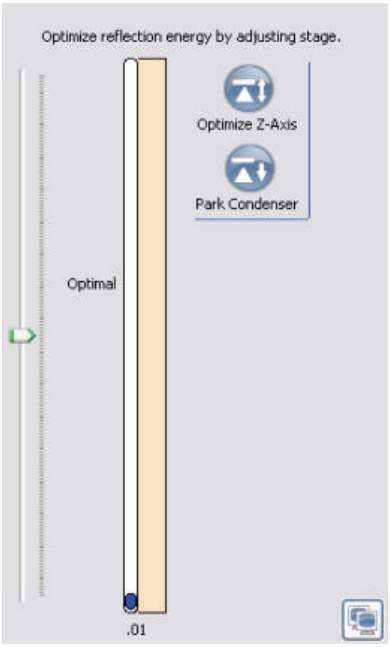
   Final Format: Single Beam

   Collect Background after __ minutes: 0 (manual background control)

   Focus Handling: Do Not Focus During Map Collection

2. Click **Home**.
3. Activate the IR Energy control.
4. Click **Park Condenser**.

5. Manually lower the microscope stage until the indicator is near its lower limit.



6. Click **Eject Stage**.

   This saves the low position as the provisional sampling position.

7. Install the Imaging ATR accessory. Refer to Installation for instructions.

8. Follow the steps in Preparing the Accessory for Operation to center the accessory, crystal, and pressure column.

9. Open the crystal arm and place the sample spacer on the pressure column or pressure column cap.

10. Lower the pressure column so that there is clearance between the sample and crystal when you swing the crystal arm.

11. Close and lock the crystal arm.

12. Click the **Load** Stage button to move the sample stage to the correct position to start your map. (The stage was centered and focused in the previous steps.)

13. Collect a Spectrum background (rather than a Map background). This will allow you to use the %T final format later in preview mode when you monitor the contact. (This step is optional but recommended.)

14. Clear the mosaic to remove any temporary frames stored in the window, and then draw the area map around the current centered position. The magnification of the germanium crystal is not indicated in the coordinate values; they are 4x larger than the mapped area. The drawn map is typically larger than the live video pane. In all cases, the map area should be centered. Use the background point as a reference.

15. Open the crystal arm, and then use the pressure column control knob to elevate the sample until it is visible in the video camera. Use the positioning screws on the accessory (instead of the joy stick) to fine tune the position of the sample. Do not move the motorized stage.
16. Use the pressure column control knob to lower the sample until it is completely out of view. This should allow enough clearance for the crystal arm to close.
17. Close and lock the crystal arm.
18. Collect the background map.
19. Raise the sample and monitor the pressure indicator. The pressure reading will increase rapidly upon contact with the sample.

    Alternatively, you may activate the preview mode and watch the spectrum as you raise the pressure column. When a spectrum appears, stop increasing the pressure. Increasing the pressure further does not usually increase absorbance.
20. Collect the sample map.
21. Use **Process > Map Math** to ratio the sample map to the background map to create a reflectance map result.
22. Use **Save Map As** to store the ratioed map separately from the raw data, or **Save Map** to store the maps and overwrite the raw data.
23. If you are finished running samples for the day, install the storage disk. Please refer to Installing the Storage Disk for instructions.

Maintenance

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Storing the Accessory

Before you store the accessory, make sure the red plastic storage disk is installed in the sampling area under positive pressure. Refer to Installing the Storage Disk for more information.

The disk is required to maintain proper operation of the hydraulic assembly. It also protects the germanium crystal from being scratched by metal parts.

Cleaning the Crystal

Clean the crystal thoroughly before you collect background data and after you measure each sample. Use a clean, soft cloth, lens paper, or a cotton swab.

NOTICE

- Do not use laboratory wipes to clean the crystal (too rough) or paper that is scented or contains lotions (they will show up in your spectra).
- Use only water, alcohol or hexane to clean the crystal. Other solvents may damage the crystal or other components of the accessory.
- The cleaning fluid should be at or close to room temperature (or the temperature of the previous sample). Applying extremely hot or cold liquids may crack the crystal.

❖ To clean the crystal

1. Dab the crystal with a clean cloth dampened with a recommended cleaning fluid from the list above, or place a drop of fluid on the crystal and then wipe it to remove most of the sample.
2. Repeat with a clean cloth.
3. If you used a solvent, allow enough time for it to evaporate completely or dry the crystal with clean, dry air or nitrogen.

Inspecting the Hydraulic Assembly

Keep all sharp sample preparation tools away from the accessory. Periodically inspect the tubing and tubing fittings for signs of abrasion or small cuts. If you see signs of accidental damage, contact our sales or service representative in your area to order a replacement hydraulic assembly. If you see leakage of fluid in any amount, immediately stop using the accessory and remove it from your microscope.

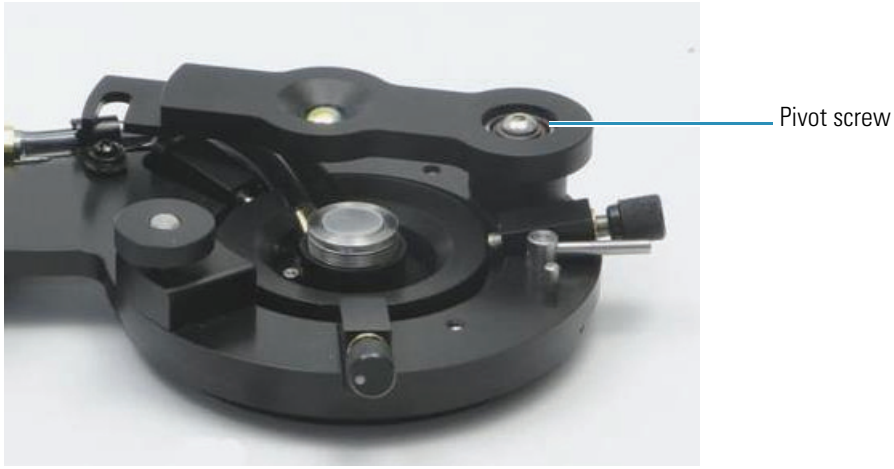
Replacing the Crystal Arm and Crystal

If the crystal becomes damaged or broken or otherwise needs replacement, you can order a new crystal arm and replace it yourself. To order parts, contact our sales or service representative in your area or use the information at the beginning of this document to contact us.

❖ To replace the crystal arm

1. Use a thin spatula or screw driver to remove the cap from the pivot screw.

   Figure 8. Pivot screw and cap

2. Use a 3/32 inch hex driver to gently remove the hex head pivot screw, the crystal arm, and up to four washers above and below the arm.

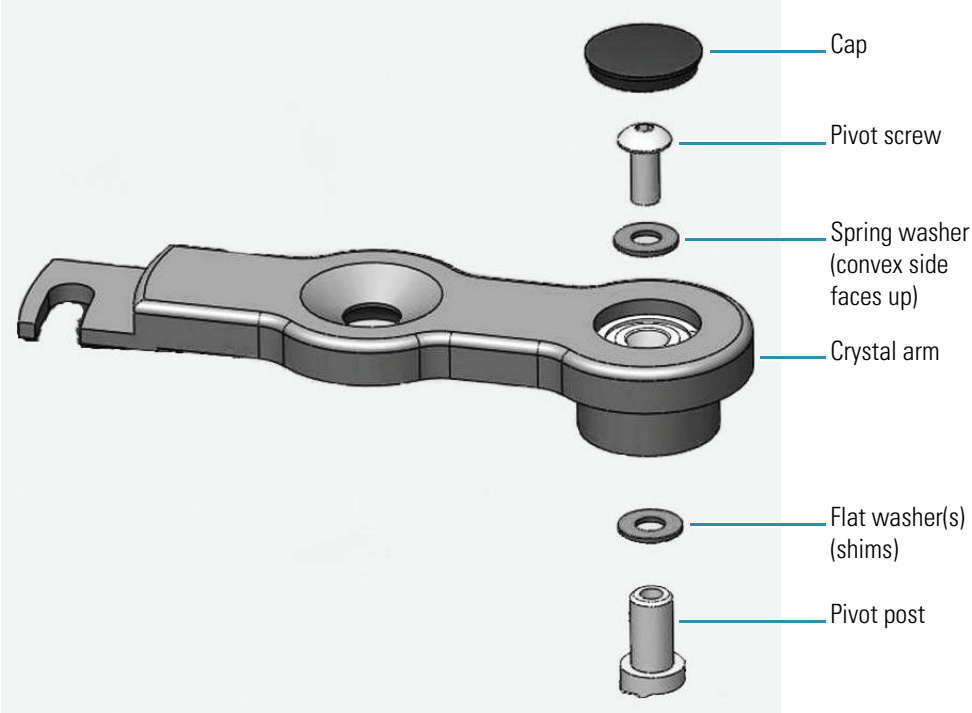
   Your accessory will have one dome shaped spring washer and between zero and three flat shim washers installed above and below the crystal arm. Look for shim washers that may have remained on the receiving post, or are stuck to the bottom of the crystal arm bearing during disassembly.

3. Install the screw, spring washer (convex side up), new crystal arm, shims, if necessary, and cap according to the diagram below.

   Use the same number of shim washers with your new crystal arm as you removed from the old one. The shims allow the arm to engage at the correct height with the arm lock support.

   The upper washer is a cone shaped lock washer. The convex side must face up.

Figure 9. Installing the crystal arm



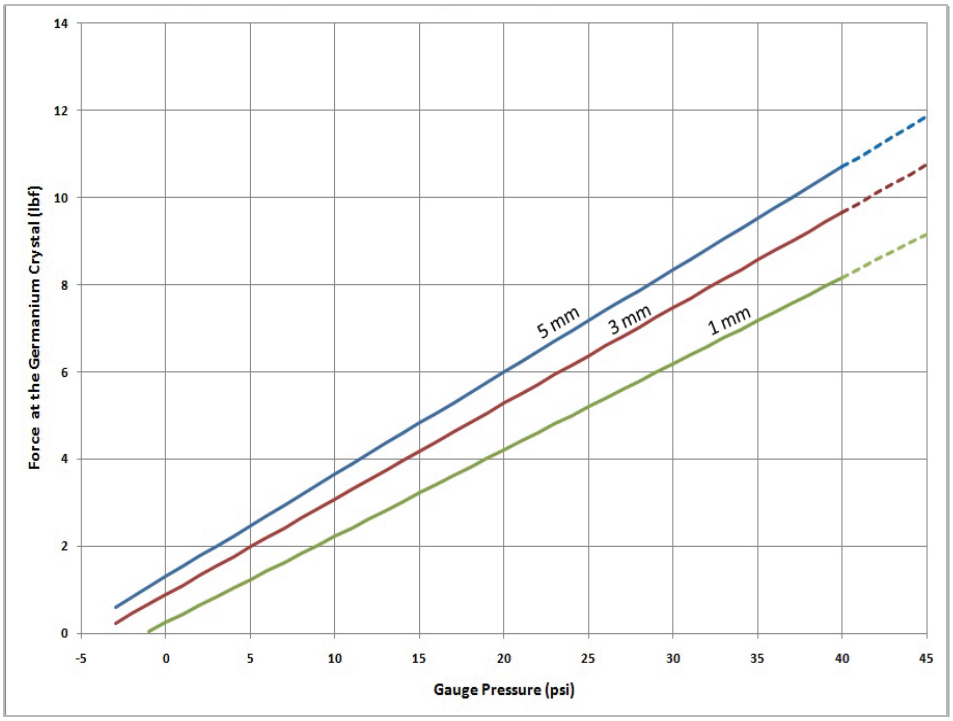
4. Use the hex driver to tighten the pivot screw until the arm swings freely but with some friction.
5. Replace the cap.

Appendix A - Total Force Applied by the Pressure Column

The pressure column has a natural spring rate that is overcome as the column advances. This makes the total force applied to the sample at a given internal pressure (shown on the integrated pressure indicator) dependent upon the actual thickness of the sample (including the spacer) mounted in the accessory. The following plot shows the typical behavior of the Imaging ATR accessory for 1, 3 and 5 mm thick samples at indicated internal pressures between zero and 40 psi.

Note Avoid operating the accessory at internal pressures over 40 psi.

Figure 10. Applied Force vs Gauge Reading for Different Sample Thicknesses[1]



[1] Thicknesses values shown above include sample and spacer.