# **ATR Imaging Accessory**



# **User's Guide**



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# **Introduction**

# About this User's Guide

The accessory enables you to generate Attenuated Total Reflectance (ATR) images using the Spotlight series of imaging systems.

This guide is divided into the following chapters:

### Introduction

This chapter describes the conventions and warnings used in this guide.

### Safety Information

This chapter provides important safety information and warnings.

### **Overview of the ATR Imaging Accessory**

This chapter describes the ATR Imaging Accessory and its theory of operation.

### Unpacking and Installing the Accessory

This chapter describes how to unpack, assemble and install the ATR Imaging Accessory.

### Sample Preparation

This chapter provides advice on preparing samples.

### Collecting ATR Images

This chapter describes collecting the reference data used to correct for system effects and then how to routinely collect ATR images.

### Processing ATR Images

This chapter describes how to enhance your images, specifically by using Principal Component Analysis (PCA) to improve spatial contrast.

### **Optimizing the Accessory**

This chapter describes how to set up the accessory for optimal results.

### Cleaning

This chapter describes how to examine and clean the ATR Imaging Accessory.

# Conventions Used in this User's Guide

Normal text is used to provide information and instructions.

**Bold** text refers to text that is displayed on the screen.

UPPERCASE text, for example ENTER or ALT, refers to keys on the PC keyboard. '+' is used to show that you have to press two keys at the same time, for example, ALT+F.

All eight digit numbers are PerkinElmer part numbers unless stated otherwise.

### Notes, Cautions and Warnings

Three terms, in the following standard formats, are used to highlight special circumstances and warnings.

**NOTE:** A note indicates additional, significant information that is provided with some procedures.

CAUTION	We use the term CAUTION to inform you about situations that could result in <b>serious damage to the instrument</b> or other equipment. Details about these circumstances are in a box like this one.
D	<b>Caution (Achtung)</b> Bedeutet, daß die genannte Anleitung genau befolgt werden muß, um einen <b>Geräteschaden</b> zu vermeiden.
DK	<b>Caution (Bemærk)</b> Dette betyder, at den nævnte vejledning skal overholdes nøje for at undgå en <b>beskadigelse af apparatet</b> .
E	<b>Caution (Advertencia)</b> Utilizamos el término <b>CAUTION</b> (ADVERTENCIA) para advertir sobre situaciones que pueden provocar <b>averías graves en este equipo</b> o en otros. En recuadros éste se proporciona información sobre este tipo de circunstancias.
F	<b>Caution (Attention)</b> Nous utilisons le terme <b>CAUTION</b> (ATTENTION) pour signaler les situations susceptibles de provoquer de <b>graves détériorations de</b> <b>l'instrument</b> ou d'autre matériel. Les détails sur ces circonstances figurent dans un encadré semblable à celui-ci.
	<b>Caution (Attenzione)</b> Con il termine <b>CAUTION</b> (ATTENZIONE) vengono segnalate situazioni che potrebbero arrecare <b>gravi danni allo strumento</b> o ad altra apparecchiatura. Troverete informazioni su tali circostanze in un riquadro come questo.
NL	<b>Caution (Opgelet)</b> Betekent dat de genoemde handleiding nauwkeurig moet worden opgevolgd, om <b>beschadiging van het instrument</b> te voorkomen.
P	<b>Caution (Atenção)</b> Significa que a instrução referida tem de ser respeitada para evitar a <b>danificação do aparelho</b> .
CN	Caution (小心) 我们使用"小心"这一术语来通知您有关可能会对 本仪器或其它设备造成严重损害的情况。 有关这些情况的详细信息可在此类方框中找到。
JP	Caution (注意) 分光器や他の機材等に深刻なダメージを与える恐れがある場合は、 この様なボックスの中に表示しています。



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# **Safety Information**

# Summary

This chapter describes the general safety practices and precautions that you must observe when operating the ATR Imaging Accessory.

This advice is intended to supplement, not supersede, the normal safety codes in the user's country. The information provided does not cover every safety procedure that should be practiced. Ultimately, maintenance of a safe laboratory environment is the responsibility of the analyst and the analyst's organization.

Please consult all manuals and CDs supplied with your Spotlight Imaging System. Carefully read the safety information in this chapter and in the other manuals supplied. When setting up the microscope and accessory, or performing analyses or maintenance procedures, strictly follow the instructions provided.



When using the motorized stage do not place your fingers between the moving and fixed parts of the instrument. The motors driving the stage from side to side, front to back, or up and down are powerful and do not stall easily.

# Warnings and Safety Information

The ATR crystal is made from germanium (Ge). Before handling Ge crystals, please ensure that you have read the appropriate Material Safety Data Sheet (MSDS).

You can search for up-to-date copies of safety data sheets on materials used in PerkinElmer products that are known to have safety issues from the Technical Resources section of the PerkinElmer website. The MSDS information is available in a range of languages, and includes data items required in specific national, supra-national and state jurisdictions.

To obtain a safety data sheet for a particular compound, follow the steps described below.

**NOTE:** To read MSDS .pdf files you will need Adobe Reader 5.0 or later. An installation of this software is available on the *Software Utilities CD*.

- Launch your web browser and navigate to the PerkinElmer web site: www.perkinelmer.com
   If you are not redirected automatically, you may have to select the home page appropriate to your location.
- 2. Search for the term MSDS using the search box located at the top of the home page. The Search for Material Safety Data Sheets (MSDS) page is displayed.
- 3. Enter the key words for the compound in the **Product name** box, and then click **Go**. A full list of all MSDS documents that refer to the compound is displayed.
- 4. Select the MSDS document you want to view.

## Ge (germanium) crystals



### Cleaning Ge crystals

Clean the crystal using an organic solvent; do not use acids or oxidizers.

# **General Operating Conditions**

The ATR Imaging Accessory has been designed and tested in accordance with PerkinElmer specifications and in accordance with the safety requirements of the International Electrotechnical Commission (IEC).

Only use the ATR Imaging Accessory indoors and under the following conditions:

Temperature 15 °C to 35 °C

Relative Humidity 80% maximum (non-condensing)

Do not use the ATR Imaging Accessory if it:

- Shows visible damage;
- Has been subjected to prolonged storage in unfavorable conditions, such as a humid condensing atmosphere;
- Has been subjected to severe transport stresses.

The crystal tip is resistant to solvents such as de-ionized water, ethanol, and iso-propyl alcohol (IPA). However, the coating on the upper surface of the crystal is fragile, and the resin used to bond the crystal is not resistant to solvents such as acetone, chloroform, or dichloromethane. Refer to *Cleaning the Crystal* on page 82.

# <u>Overview of the ATR</u> <u>Imaging Accessory</u>

# Theory of Operation

Traditionally, Attenuated Total Reflectance (ATR) techniques are used to obtain infrared spectra from samples that are too thick or too opaque for transmission measurements, and are too strongly absorbing for good reflectance measurements.

The Spotlight ATR Imaging Accessory employs a germanium crystal with a high refractive index (about 4.0). This crystal is placed in intimate contact with the sample and the sample imaged through the germanium. This arrangement permits a spatial resolution up to four times better than that available in conventional reflection or transmission IR imaging techniques.



### Figure 1 Attenuated total reflectance

The infrared beam is directed through the crystal towards the sample; most of the beam is totally internally reflected and returns to the detector system via the crystal. However, some of the energy penetrates a short distance into the sample where, depending on the properties of the sample, it may be absorbed. When absorption occurs the reflected beam is attenuated and carries a modified form of the sample's absorption spectrum. The energy within the sample is strongest at its surface and decreases exponentially with depth. The effective penetration depth  $d_p$  is given by:

$$d_p = \frac{\lambda}{2\pi n_1 (\sin^2 \theta - n_{21}^2)^{1/2}}$$

Where:

 $n_1$  is the refractive index of the ATR crystal (4.0 for germanium)

 $\lambda$  is the wavelength of the radiation

heta is the angle of incidence of the beam

 $n_{21}$  is the ratio of the refractive indices of the sample and the ATR crystal.

The depth of penetration, and therefore the effective pathlength within the sample, varies with both the wavelength and the refractive index of the sample. It is greatest at long wavelengths. For typical samples viewed through the germanium crystal, the effective penetration depth is no more than a few microns. Close contact between the ATR crystal and sample is essential.

The variability of penetration depth that is characteristic of the ATR technique gives rise to spectral shapes that are 'distorted' in comparison to conventional transmission or reflectance spectra. The most obvious effect is a linear enhancement of absorption features at longer wavelengths due to increased effective pathlength. This effect can be corrected mathematically if direct comparison with conventional reflectance or transmission library spectra is required; refer to *ATR Images and Spectral Libraries* on page 68. Alternatively, comparisons can be made directly with spectral libraries acquired using the ATR technique.

The ATR technique is sensitive only to material directly in contact with, or within a few microns of, the ATR crystal sampling surface. It is well suited to the examination of surfaces, coatings, laminates or paint systems in cross section, and samples that might otherwise scatter light deeply.

Figure 1 shows the ATR crystal and sample in a central position, with the IR beam accessing one point on the sample. To acquire an image, both the sample and the crystal to which it is clamped are moved in the plane of the sample using the motorized X,Y stage. As the crystal and sample move, the infrared beam and the detector array visit different parts of the sample. At each point a complete spectrum is recorded to build up the image, sometimes referred to as a Full Spectral Map.

The ATR crystal is coated to minimize reflections on the curved surface as the infrared beam enters and exits. The ATR reflection at the crystal tip is highly efficient when a sample is not present. The effects of residual reflections and any small absorption by the germanium itself may be compensated in a variety of ways, principally:

- ATR background spectra, which measure and correct for the transmission of the ATR crystal at the center of the image. Refer to *Collecting ATR Imaging Backgrounds* on page 49.
- ATR crystal images, which measure and correct for the transmission of the ATR crystal and the effects of variable illumination as a function of position across the sample area. Refer to *Compensating for Uneven Illumination* on page 51.

# System Description

The ATR Imaging Accessory enables you to collect ATR images from a sample area that you have visually identified using the microscope.



# Figure 2 The ATR imaging accessory, showing the base (top) and the ATR crystal arm (bottom)

The accessory fits to the motorized stage in place of the slide-holder, and utilizes the microscope stage control to place the ATR crystal at precise positions in the beam path. A scribed line on the crystal arm (h) helps you find and focus on a registration mark at the center of the top face of the crystal. This position is set as a nominal origin in the (X,Y) plane. The system is calibrated with the height of the crystal. For data collection, the stage automatically moves focus from the top surface of the ATR crystal along the vertical Z axis to its tip.

The ATR crystal is held in an arm mounted on the accessory using pillars (a, b), which allows the crystal to be swung in and out of the field of view. The crystal arm is secured by a clamping thumbscrew (i) on each side. When the crystal arm is lifted to clear the pillar on the left of the accessory (b), it turns on the taller pillar on the right (a).

	The crystal tip may be damaged by accidental contact with a hard surface, such as the steel anvil (d).
CAUTION	The pillar on the right of the ATR Accessory (a) is fitted with a 3.5 mm O ring (c) to help prevent such contact. Only remove this O ring to examine thin samples.

The accessory includes a mini-stage to aid precise sample positioning. To view a sample on the mini-stage, the crystal arm is lifted and swung out of the beam path. Two mini-stage adjusters (e) enable you to center the area of interest on your sample in the field of view.

To ensure good optical contact during data collection, the accessory includes a mechanism that applies a consistent and controllable compressive force between the sample and the ATR crystal via the anvil (d). Before collecting an ATR image, swing the crystal arm into the field of view, lower it gently on to the sample, and then clamp it. To apply the compressive force, slide the force lever (g) on the accessory from right to left by pinching the force-lever towards the thumb pillar (f) using one hand. This mechanism is adjustable; refer to *Adjusting the Mini-Stage Pre-Load* on page 70.

# ATR Imaging Accessory Specification

ATR crystal material	Germanium (Ge) [refractive index 4.0, critical angle in air 14.4°]
Range of measurement	4500 cm <sup><math>-1</math></sup> to 720 cm <sup><math>-1</math></sup> (ATR Imaging mode)
Area of contact with sample	Nominally, a 600 $\mu$ m diameter circular flat surface for the standard crystal, or a 1200 $\mu$ m diameter circular flat surface for the larger-area crystal.
Spatial Resolution	3.1 $\mu$ m typical at center of field, measured at about 6 $\mu$ m wavelength (about 1667 cm <sup>-1</sup> ).
Angle of Incidence, $\theta$	The beam subtends a range of angles between approximately 18° and 30° at the sample.

# System Requirements

The ATR Imaging Accessory is designed for the Spotlight family of Imaging Systems.

CAUTION	Any upgrades to the Spotlight system must be completed BEFORE you fit the ATR Imaging Accessory to the stage.
CAUTION	These upgrades help protect your system from damage by mechanical interference.

The following systems need attention from a PerkinElmer Service Engineer before being fitted with the ATR Imaging Accessory:

- Spotlight 300 FT-IR and 350 FT-NIR microscopes with a serial number lower than 76366.
- Stage Control Boxes with a serial number lower than 70536.
- Spectrum 100 series FT-IR and FT-NIR spectrometers with a serial number lower than 76000, or any Spectrum One series spectrometer.

## Software Requirements

The imaging system will also require SpectrumImage Software version 1.4.1 or later (if you have a standard crystal) or SpectrumImage Software version 1.6.5 or later (if you have a larger-area crystal).

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# <u>Unpacking and Installing</u> <u>the Accessory</u>

# Preparing the Imaging System

### Using the accessory for the first time

1. Make sure that **ATR Imaging** mode is enabled in your SpectrumImage software. See *Enabling ATR Imaging in the SpectrumImage Software* on page 25.

Enable **ATR Imaging** mode BEFORE you unpack and fit the ATR Imaging Accessory.

**CAUTION** The SpectrumImage software in ATR Imaging mode includes routines that help protect the instrument from damage by mechanical interference.

2. Unpack and assemble the ATR Imaging Accessory.

# Routinely preparing your Spotlight Imaging System for ATR Imaging

- Remove any accessories, such as the single-point ATR objective, and the lower cassegrain (as described on page 31).
   This *essential* step provides working space for the accessory.
- 2. Log in to the SpectrumImage software in **ATR Imaging** mode.

OR

In the SpectrumImage Control window, select **ATR Imaging** from the Operation mode drop-down list.

- If required, adjust the mini-stage pre-load setting.
   You may want to do this when working with particularly hard, or particularly soft, samples. Refer to *Adjusting the Mini-Stage Pre-Load* on page 70.
- 4. Mount the ATR Imaging Accessory on the stage, as described on page 33. The method employed depends on the type of stage.
- Ensure that the ATR Imaging Accessory is level.
   Initially, this is a minor adjustment. However, for optimal measurements refer to Leveling the Accessory on page 71.
- 6. Find and register the ATR crystal position (as described on page 36).

You are now ready to collect the reference data required to optimize the ATR images generated for your samples. Refer to *Collecting ATR Images* on page 45.

# Enabling ATR Imaging in the SpectrumImage Software

When SpectrumImage software version 1.4.1 or later is installed (select **About SpectrumImage** from the **Help** menu), support for the ATR Imaging Accessory is optional. When ATR Imaging is enabled:

• The SpectrumImage Login dialog includes a **Startup Mode** named ATR Imaging.

Login		
Please enter your login details:		
Login Name Instrument Startup Mode	Attached Instrument	▼ ▼
	ОК	Cancel

**NOTE:** If you have purchased both the standard ATR imaging accessory and the larger-area ATR imaging accessory (Spectrum Image version 1.6.5 or later) the options **Standard ATR Imaging** and **Enhanced ATR Imaging** will be available.

• The SpectrumImage Control window includes an Operation mode named ATR Imaging.



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If a SpectrumImage release 1.4 or later is installed, but ATR Imaging is not enabled:

- 1. Exit the SpectrumImage software.
- 2. Browse to and run C:\Program Files\PerkinElmer\SpectrumImage\EnableATR.exe.
- Remove any accessories, such as the single-point ATR objective, and the lower cassegrain (as described on page 31).
   This *essential* step provides working space for the accessory.
- 4. Log in to SpectrumImage in **ATR Imaging** mode.
- 5. Select **Set ATR Crystal Height** from the Options menu in the Stage Control window. The Set ATR Crystal Height dialog is displayed.

Set ATR Crystal Height			×
Current ATD crustel beight.	7000	1150	
New ATR crystal height:	0	μm	
ОК		Cancel	

6. Enter the correct value for the **New ATR crystal height**. The crystal height value is on a label on the crystal arm.

**NOTE:** If the value entered is incorrect, your ATR images will be out of focus or completely dark.

7. Click **OK**.

## Unpacking the Accessory

	Handle the accessory carefully; the crystal can be scratched, or the accessory damaged, by careless handling.
CAUTION	Treat the anti-reflective coating on the top surface of the crystal as fragile; it can be scratched by hard particles. When the accessory is not in use, close the dust cover.
	Do not touch the ATR crystal; ATR is a surface technique so the crystal must be extremely clean.



### Figure 3 The ATR imaging accessory

The accessory is packed in a foam-filled case containing:

- Accessory base assembly;
- Crystal arm;
- Sample carrier;
- Sample carriers (disposable x10);
- Stainless Steel Tweezers (Fine point);
- Magnifier (Eye Glass x7);
- Hex Key (1.5 mm).

The accessory also comes with a pack of 25 lens tissues. Refer to *Checking for Contamination on the ATR Crystal* on page 80.

If the ATR crystal is damaged in service, it can be replaced. Refer to *Appendix 1: Replacing the ATR Crystal* on page 85.

# Assembling the Accessory



### Figure 4 Fitting the ATR crystal arm

- 1. Carefully unpack the accessory base, crystal arm and a sample carrier. Keep the dust cover on the arm closed.
- 2. Place the sample carrier on the mini-stage, rimmed side down.
- 3. Slacken the clamp thumbscrew at each end of the crystal arm.
- 4. Using the forefinger and thumb of each hand, hold the crystal arm at both ends with the crystal dust cover and scribed line uppermost.
- 5. Press the brake button with your right thumb and gently slide the arm on to the right and then the left mounting pillars.



Support the arm by bracing your fingers on the accessory base.

**NOTE:** Hold the arm level and be careful not to apply any tilting or twisting forces, which will make the arm far more difficult to slide on the pillars.

6. Release the brake button and then clamp the arm by tightening the thumbscrew firmly at each end.

**NOTE:** When released, the brake button prevents the arm dropping towards the mini-stage from a mid-position on the pillars, but cannot hold the arm in place for data collection.

## Swinging the Crystal Arm

### CAUTION

Avoid touching the crystal. Use gloved hands to avoid transferring finger oils. Remember that even with gloved hands, it may be possible to transfer traces of lubricants or other materials.

If the crystal arm is clamped to the accessory pillars:

- 1. Make sure that the force lever is to the right, so that no compressive force is being applied by the anvil.
- 2. Slacken the thumbscrew at each end of the crystal arm.
- 3. Using the forefinger and thumb of each hand, hold the crystal arm at *both* ends.
- Press the brake button with your right thumb and *gently* slide the arm up the mounting pillars until the arm clears the left pillar.
   Support the arm by bracing your fingers on the accessory base.

**NOTE:** Hold the arm level and be careful not to apply any tilting or twisting forces, which will make the arm far more difficult to slide on the pillars.

- After the arm clears the short support pillar on the right, release the brake button. The brake button prevents the arm dropping towards the mini-stage from a mid-position on the pillars, which could damage the crystal tip.
- 6. Swing the arm forwards (anti-clockwise) around the right pillar.

To avoid the possibility of mechanical interference with the upper cassegrain, swing the crystal arm until the top of the left clamp screw is facing towards you. Ensure the crystal arm is now at 90° to the stage, and is resting on the support pillar (rather than being higher up the right pillar held by the brake).

CAUTION

Do not move or carry the accessory when the arm is free to swing. Make sure the arm is clamped on the accessory pillars.

If the crystal arm has been swung out of the field of view:

- 1. Ensure that the force lever is to the right.
- 2. Using the forefinger and thumb of each hand, hold the crystal arm at *both* ends.
- 3. Swing the arm around the right pillar over the stage, until it clears the support pillar and aligns with the left mounting pillar.

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4. Press the brake button with your right thumb and *gently* slide the arm on to the left mounting pillar.

Do not allow the crystal tip to drop into contact with either the anvil or a<br/>sample carrier. The crystal tip may be damaged.CAUTIONThe pillar on the right of the ATR Accessory is fitted with a 3.5 mm O ring<br/>to help prevent such contact. Only remove this O ring to examine thin<br/>samples.

Support the arm by bracing your fingers on the accessory base.

**NOTE:** Hold the arm level and be careful not to apply any tilting or twisting forces, which will make the arm far more difficult to slide on the pillars.

5. Release the brake button and then clamp the arm by tightening the thumbscrew at each end.

**NOTE:** When released, the brake button prevents the arm dropping towards the mini-stage from a mid-position on the pillars, but cannot hold the arm in place for data collection.

# Maximizing Stage Movement

The ATR Imaging Accessory utilizes the full range of vertical stage movement. To ensure that your Spotlight Imaging System is not inadvertently damaged by mechanical interference, you *must* remove all accessories that might restrict the full movement of the microscope stage, such as the single-point ATR objective.

In particular, to maximize the range of movement in the Z direction, remove the lower cassegrain, including the purge gaiter (if fitted).

Do not use the SpectrumImage software in ATR Imaging mode when the lower cassegrain (or 'correction mirror') is fitted.

**CAUTION** To accommodate the ATR Imaging Accessory, the stage must be able to move down as far as possible. If the lower cassegrain is fitted, the stage motors may be seriously damaged.

## Removing the Lower Cassegrain

Remove any sample, tools or accessories from the microscope stage, and then:

1. Select the **Control** window and then, in the Correction pane, click **Zero**.

The lower cassegrain moves to the position where the infrared beam is focused when no sample is on the stage.

CAUTION

When moving the stage upwards, do not drive the stage into the upper cassegrain, which may be damaged as a result.

- 2. Using the Z-control on the joystick, move the stage to its highest possible position.
- Release the locking lever.
   This is at the back of the lower cassegrain assembly on the right.
- 4. Gently slide the lower cassegrain assembly forward and out of the dovetail connector.
- In the Control window, click **Park**. The lower cassegrain stub moves down.
- 6. Click **Park** again.

Do not cancel these stub movements or manually drive the stub upwards.

### CAUTION

If you drive the stub upwards, and you then drive the stage to its forward and downward limits, the stage drive mechanism may be damaged.

The lower cassegrain stub moves to its lowest position, which can take about 20 seconds.

7. Lower the stage using the Z-control on the joystick.

## Replacing the Lower Cassegrain

After your ATR imaging session, to replace the lower cassegrain, remove any sample, tools and the ATR imaging accessory from the microscope stage, and then:

1. Using the Z-control on the joystick, move the stage to its highest possible position.

**CAUTION** When moving the stage upwards, do not drive the stage into the upper cassegrain, which may be damaged as a result.

- 2. Select the **Control** window and then, in the Correction pane, click **Zero**. The lower cassegrain stub moves up.
- 3. Make sure that the cassegrain is correctly seated in the lower cassegrain assembly.
- 4. If required, fit the purge gaiter.
- 5. Gently slide the cassegrain assembly as far as it will go on to the dovetail connector on the lower cassegrain stub.

The lower cassegrain is at the position where the infrared beam is focused when there is no sample on the stage.

Tighten the locking lever.
 This is at the back of the lower cassegrain assembly on the right.

# Installing the Accessory on the Stage

1. Log in to the SpectrumImage software in **ATR Imaging** mode.

OR

In the **SpectrumImage Control** window, select **ATR Imaging** from the **Operation mode** drop-down list.

A message is displayed:

ATR Imaging Mode 🛛 🛛 🕅			
If the ATR Imaging accessory is already fitted, click OK to proceed. Otherwise:			
1: Remove the lower cassegrain.			
2: Drive the stage forwards and down to enable easy access.			
3: Remove the sample holder.			
<ol> <li>Fit the ATR Imaging accessory securely, and ensure that the clamping screws for the crystal are tight.</li> </ol>			
5: Click OK to proceed.			
The lower cassegrain motor will move to its lower park position.			
Cancel			

- 2. Make sure that you have removed any accessories that might restrict the full movement of the stage, specifically the lower cassegrain.
- 3. Use the joystick to drive the stage to its travel limit, both towards you and down.
- 4. Remove the sample slide holder.

The sample slide holder is held by between two and six screws (newer stage design), or a pair of spring clips at the forward left corner of the stage aperture (older stage design).

5. Make sure that the thumbscrews that clamp the crystal arm to the ATR Imaging Accessory are not slack and then lift the accessory by its handles (see Figure 4 on page 28) and place it securely on the stage.

6. For the newer stage design, fix the accessory to the stage using the two thumbscrews. These thumbscrews are usually captive. If not, make sure you fit the longer thumbscrew to the left rear of the accessory.



### Figure 5 New stage design - thumbscrews

### OR

For the older stage design, make sure the stage springs engage by gently sliding the accessory base from the right into the forward left corner of the stage aperture.



Figure 6 Old stage design — stage springs

7. For optimum performance, level the stage. Refer to *Leveling the Accessory* on page 71.

OR

Gently rock the accessory. Remove any rocking movement using the leveling screws.



### Figure 7 Level the stage

A 1.5 mm hex key is provided. Only a slight adjustment from flush with the underside of the accessory should be necessary.

8. Click **OK**.

You may be reminded that you must remove the lower cassegrain.

Spotlight	×	
Ple	ving the lower cassegrain drive mechanism to its lowest sition, to maximise available stage Z movement. ase remove the lower cassegrain to continue.	
CAUTION	If the lower cassegrain is fitted, th damaged.	he stage motors may be seriously

9. Click **OK**.

The lower cassegrain mechanism drives to a low park position (20 mm), which can take 20 seconds.

In addition, the Crystal Registration wizard starts automatically. Refer to *Registering the Crystal Position* on page 36.

# **Registering the Crystal Position**

ATR imaging requires that the Z axis of the ATR crystal is carefully and consistently aligned with the optical axis of the microscope, and that the IR focus is on the tip of the crystal.

The ATR crystal has a registration mark at the center of its top surface. When the system is focused on this mark, and the image centered, the ATR crystal is precisely aligned with the optical axis of the microscope. This important reference is registered as the origin in the (X,Y) plane.

Prior to data collection, you are asked to focus on the registration mark, and to center the image. The system can then use the height of the crystal to move the IR focus to the tip of the crystal automatically.

The Register Crystal Position Wizard helps you to find, focus on, and center the registration mark at the center of the top surface of the ATR crystal. It then registers this position as the origin in the (X,Y) plane.

To register the crystal position:

1. While logging in to SpectrumImage, select **ATR Imaging** as the Startup Mode.

### OR

In the Control window, select **ATR Imaging** as the Operation mode. The ATR Register Crystal Position Wizard starts automatically.

ATR Register Crystal Position Wizard	
ATR Register Crystal Position Wizard	Welcome to the ATR Register Crystal Position Wizard For ATR Imaging, the crystal must be aligned with
Register Crystal Position	<text><text><text><text><text></text></text></text></text></text>
	<u> </u>

- 2. Make sure the ATR crystal arm is clamped to the accessory pillars and that its dust cover is open.
- 3. Use the optical microscope to find the line scribed along the upper surface of the arm.
4. Move the field of view along the line, and center at the edge of the crystal well. The Visible window displays an image similar to:



#### 5. Click Move to ATR Crystal Position.

The Visible window displays an image of the flat top surface of the crystal.

6. Center, and focus on, the registration mark at the center of this surface. The Visible window displays an image similar to:



### 7. Click Finish.

The crystal axis is registered as the origin in the (X,Y) plane.

**NOTE:** Alternatively, while the system is in ATR Imaging mode, you can find, focus on, and center the registration mark at the center of the top surface of the ATR crystal manually. Then, to register this position as the origin in the (X,Y) plane, select **Register ATR Crystal Position** from the Options menu in the Stage Control window.

# **Sample Preparation**

### The Need for Sample Preparation

To achieve the best possible ATR imaging results, the surface of the sample must be in contact with the entire area of the ATR crystal tip. Ideally, the sample should be presented level and have a surface finish as flat and smooth as the crystal tip itself.

Slightly compressing the crystal into the sample can help ensure good contact, to a degree that depends on the nature of the sample. However, this compression cannot flatten a rough surface on a fine scale.

CAUTION	For rougher surfaces, or if the sample is not horizontal, minimize the risk of crystal damage by setting the mini-stage pre-load to zero.
	Refer to Adjusting the Mini-Stage Pre-Load on page 70.

In many cases, the sample must be supported, mounted and polished. This chapter provides brief notes on sample preparation techniques that have yielded good ATR images for certain types of sample.

The techniques used to prepare a sample for ATR imaging, such as cold encapsulation in resin followed by polishing, are very similar to the mounting, supporting and finishing techniques used in optical microscopy. These methods are described in detail in the literature on art conservation and forensics. The manufacturers and suppliers of the tools and materials used in light microscopy are also a useful source of hints and tips.

For ATR imaging, you must consider the IR spectrum of the resin or mounting material used and how it permeates the sample. The suppliers of mounting resins are a good source of guidance on the selection of a suitable resin.

## Sample Preparation Criteria

Whatever sample preparation techniques you use, prepare your samples to a standard that meets as many of the following criteria as possible:

- Although acceptable results can be obtained with a poorer surface finish, ideally the prepared sample should have a surface finish similar to that of the tip of the crystal. Specifically, the sample surface should not have scratches wider or deeper than approximately 1 or 2 µm, and should be flat to within a micron or two over any area 600 µm in diameter.
- To ensure uniform contact across the face of crystal tip, the prepared surface must be level and parallel with the (X,Y) plane.
- To avoid distortion, the area of interest on the sample surface must be firmly supported by surrounding material. The load applied by the compression mechanism can be up to 30 N.
- The sample surface must be clean and uncontaminated.
- The total thickness of a sample and its supporting block should ideally be between around 3 mm to 10 mm, and must not exceed 12 mm.



# Sample Preparation Techniques

### **Cold Encapsulation**

Samples can be encapsulated in polyester or epoxy resin. This technique is known as 'potting'. The nature of the sample determines the precise technique and the appropriate resin. If small bubbles are a problem, de-gas the resin before use. The finished sample must sit firmly on the sample stage without rocking and the prepared surface must be horizontal, polished and cleaned such that there is no trace of the potting resin on the sample surface.

While the resin cures, you can hold small samples vertical using the clips sold for the purpose. Hold a bundle of fibers vertical using a small hole drilled in a piece of plastic.

### Sandwiching

Small samples, such as paint flakes can be glued between acrylic blocks, and then polished to expose the sample edge.

### Film Sheets and Laminates

For optical microscopy, sheet samples, such as laminates, can be held in a small vice. Sometimes, thicker plastic sheets are used to support, or 'back', thin samples, or several layers of the sample material are stacked and the stack polished.

### Microtoming

For some samples, it is possible to obtain a reasonable surface by sectioning using a microtome. If small burrs spoil the optical contact, polish the surface using lapping film.

### Polishing

If the surface of the sample is not naturally smooth, it must be polished. This is best achieved by grinding the surface using clean de-ionized water and successively-finer grades of abrasive. Begin with light finger pressure and 1200 grit wet-and-dry paper used wet on a hard, flat surface such as a sheet of glass. For finer polishing, use 'Lapping Film', such as that produced by 3M, finishing with the 1  $\mu$ m grade. The aim is to create a new surface at each stage, where all the defects created by the previous grade of abrasive have been removed. Polish in a circular, or figure-of-eight, motion until no trace of the scratches produced by the previous grade of abrasive can be seen. Then draw the sample repeatedly across the lapping film in one direction so that the final marks made on the surface are parallel; this makes it much easier to see when polishing with a finer grade of abrasive has created a new surface.

**NOTE:** Between each grade of polishing film, remove grit particles by cleaning the sample thoroughly using clean de-ionized water or a suitable solvent, being careful to avoid scratching the surface by wiping it with dirty tissue. To avoid contamination from previous samples, use fresh lapping film for each sample.

Starting from a roughly-cut sample, the entire polishing process can often be completed within an hour. There are a number of commercially available polishing machines to assist with the task.

### Tablets and Powders

Pressed, uncoated, pharmaceutical tablets can be examined by ATR imaging provided you take care to ensure the tablet is brought into intimate and uniform contact with the ATR crystal.

One way to obtain ATR images from pharmaceutical powder blends is to make sample disks using a commercial KBr press with well polished dies. Typically, a KBr press makes 13 mm or 7 mm disks. A micro KBr pellet press, which makes 7 mm, 4 mm or 2 mm diameter disks, is also suitable. Use enough powder to make disks at least 2 mm thick.

Tablets presented directly from the press often contain voids that preclude ATR measurement; these appear as 'holes' in the ATR image, areas showing a flat baseline with no spectra present.

If tablets are cut and milled prior to measurement (which is sometimes the practice for NIR imaging) the milled surface finish may dominate the ATR image. This is unsurprising because ATR imaging is a surface technique with potentially a much higher spatial resolution than NIR diffuse reflectance imaging.

For optimum ATR imaging, pay attention to the morphology of the sample at the crystal tip. Most tablets contain tiny voids, and the appearance of 'holes' in the ATR image shows that the system is reporting the surface of the sample accurately with a shallow depth of field. In conventional reflectance imaging these 'holes' are filled by scattered light.

**NOTE:** Small particles of sample may adhere to the tip of the ATR crystal. Refer to *Checking for Contamination on the ATR Crystal* on page 80 and *Cleaning the Crystal* on page 82.

### Sample Disks

Sample disks are disposable items that need not be cleaned. They are ideal for supporting pressed disks or resin blocks. A sample can also be fixed to a disk with adhesive for permanent mounting or to facilitate microtoming, milling or polishing.

CAUTION	Do not allow the crystal tip to make direct contact with the surface of the sample disk.
	The crystal tip may be damaged.

Part Number	Item
L1860318	ATR IMAGING SAMPLE CARRIER STD PK10

# **Collecting ATR Images**

### Overview

There are some practical ways in which ATR imaging differs from conventional transmission or reflection imaging:

• For optimum performance, images are recorded using the central axis of the ATR crystal as a reference point. Correct alignment is assured by moving the crystal using the (X,Y) stage and focusing on the registration mark at the top centre of the crystal. Once this alignment has been set (see *Registering the Crystal Position* on page 36), the sample is positioned using the mini-stage controls, NOT the motorized stage controls. Follow the software wizards that facilitate image collection with care; they disable the lateral motion of the motorized stage at certain times to prevent unintentional misalignment.

**NOTE:** Any slight misalignment of the registration mark can be carefully corrected using the (X, Y) controls. Before collecting any images, select **Register ATR Crystal Position** from the Options menu in the Stage Control window to re-register the crystal position.

- ATR imaging is a contact technique that applies pressure to the sample. Some samples, if soft, may be slightly indented. It is possible to acquire multiple images of one region of sample before removing the crystal, but it may be difficult to revisit exactly the same site on another occasion without re-preparing the sample surface.
- It is important to keep the sampling tip clean and free of damage. If the crystal tip becomes contaminated, perhaps as a result of contact with a previous sample, the next image may show this contamination in sharp focus superimposed on the sample image.
- ATR imaging offers more options for processing the images and applying corrections for spectral transmission and illumination variations across the image than conventional infrared imaging.

All ATR images are processed using an ATR imaging background, which is a set of spectra taken through the crystal with no sample present, and using the same spectral and spatial resolution as the final sample image. These spectra measure the response of the whole system at the center of the image. When an ATR sample image is recorded, the spectrum at each pixel is divided by the ATR imaging background so that the results may be interpreted as percentage transmission or as true absorbance. If you prefer to work in absorbance, the ATR imaging background is subtracted from the absorbance spectrum at each point in the image.

A separate ATR imaging background must be recorded from a clean crystal for each spectral and spatial resolution that you intend to use. ATR imaging backgrounds must be recorded before any sample images are acquired; the software prevents sample image acquisition if an appropriate ATR imaging background cannot be found. Assuming that the crystal is clean, you can collect a new ATR imaging background at any time; collect new ATR imaging backgrounds whenever the crystal is changed, or the system is serviced or adjusted. Refer to *Collecting ATR Imaging Backgrounds* on page 49.

ATR imaging backgrounds compensate for the system throughput at the center of the image. If a slight drop in illumination at positions away from the center of the image is significant for your application, you can apply a Baseline Offset Correction. This correction makes assumptions about the lack of absorption in certain defined spectral regions and adjusts the spectra accordingly. Refer to *Compensating for Uneven Illumination* on page 51.

You can correct sample images both for illumination and subtle spectral variations using an ATR crystal image, which measures the system's spectral throughput at all points across the crystal tip. ATR crystal images must be recorded from the clean crystal, and use the same spatial and spectral resolutions as the sample image. Refer to *Compensating for Uneven Illumination* on page 51.

This chapter assumes that you have removed the lower cassegrain, selected **ATR Imaging** mode, fitted the ATR Imaging Accessory, and registered the crystal position. If these steps are unfamiliar, refer to Unpacking on page 23.

Once you have prepared the sample (refer to *The Need for Sample Preparation* on page 40), ATR imaging requires you to:

- Collect an ATR Imaging Background for each combination of Resolution and Pixel size that you intend to use.
   Refer to *Collecting ATR Imaging Backgrounds* on page 49.
- Decide whether to apply any automatic processing, either by applying a Baseline Offset Correction or by subtracting an ATR Crystal Image.
   Refer to *Compensating for Uneven Illumination* on page 51.
- Collect ATR images from areas of interest on your sample. Refer to *Collecting an ATR Sample Image* on page 55.

Later, you may want to:

- Process your images to improve contrast, reveal spectral structure, and minimize noise.
- Apply ATR Correction to allow spectra extracted from your images to be directly compared to the normalized transmission spectra held in spectral libraries.

Refer to Processing ATR Images on page 61.

While collecting ATR images, remember that:

- The joystick is used in the (X,Y) plane to place the ATR crystal on the optical axis of the microscope. Center the region of interest on your sample in the visible field using the mini-stage on the accessory.
- When the ATR crystal is in the field of view, focus on the registration mark on the top surface of the crystal. Immediately prior to data collection, the system uses the crystal height parameter to shift the microscope stage automatically and focus on the tip of the crystal.

### ATR Image Parameters and Quality

The size of the ATR image dataset is determined by the size of the image, and its quality.

The image is usually square or rectangular with sides between 25  $\mu$ m and 500  $\mu$ m for the standard crystal (although larger sizes are available for when maximum coverage is paramount). With the larger-area crystal (at least 1200 mm diameter sample area) you can collect data from a rectangular sample area with sides between 25  $\mu$ m and 1000  $\mu$ m, although again a larger area is available.

The quality of an ATR image is defined by:

Resolution	The spectral resolution, which affects the amount of spectral data stored at each pixel of the image. Typical samples are imaged using 8 or $16 \text{ cm}^{-1}$ , but the system offers spectral resolutions ranging from 1 to $128 \text{ cm}^{-1}$ .
Pixel size	The size of the image sampling grid. Image fine spatial features using 1.56 µm pixels. Image coarser features over a wider area using 6.25 µm pixels.
Scans per pixel	The fidelity of the image; the greater the number of Scans per pixel, the higher the signal-to-noise ratio. The best signal quality is obtained using 16 scans per pixel or greater.
	For most samples, an adequate signal-to-noise ratio can be achieved using as few as 1 or 2 Scans per pixel, especially if the image is post-processed using, for example, the <b>Show Structure</b> command (refer to <i>Revealing Spectral</i> Structure on page 65).
	If you consistently acquire ATR images using 64 or 128 Scans per pixel, refer to <i>Optimizing ATR Imaging Backgrounds</i> on page 77.

These factors not only affect the quality and size of the ATR image but also the time taken to acquire it. Before collecting an ATR image (or an ATR crystal image), the system provides an estimate of the elapsed time required.

You can collect multiple ATR images from the same point on the sample, so you can select the various parameters to suit the task in hand. For example, a quick survey to check the quality of the sample might use a low spectral resolution (such as 16 cm<sup>-1</sup>) and only 1 or 2 Scans per pixel over a wide area. Once the sample has been verified and a region of interest selected, a higher quality image can be acquired using a finer spectral resolution and a greater number of scans per pixel.

## **Collecting ATR Imaging Backgrounds**

ATR imaging backgrounds are used to remove the spectral contribution of the system measured at the center of the image. This includes contributions from the crystal and the detector, and also environmental factors such as ambient humidity and carbon dioxide concentration.

All ATR image data requires an appropriate ATR imaging background. The microscope configuration is subtly different at high and low magnifications, so an ATR imaging background is required for each combination of pixel size and spectral resolution.

**NOTE:** You cannot collect an ATR sample image unless a suitable ATR imaging background is available.

ATR imaging backgrounds are collected when the crystal is not in contact with a sample, and with the system focused on the tip of the crystal. It is important that the tip of the crystal is clean. Refer to *Checking for Contamination on the ATR Crystal* on page 80.

An ATR imaging background does not take very long to collect, so you may decide to update them quite frequently.

To collect an ATR Imaging Background:

- 1. If there is a sample on the mini-stage, raise the crystal arm to make sure that the sample is not in contact with the crystal.
- 2. Make sure the crystal arm is clamped, and the crystal dust cover open.
- 3. Select **Start ATR Background** from the Scan menu of the Stage Control window. The ATR Imaging Background Wizard starts.



4. If the microscope is not already focused on the registration mark on the top surface of the crystal, click **Go to Last Known Crystal Position**.

The stage moves to the registered crystal position, that is the origin in the (X,Y) plane.

- 5. Focus on the registration mark on the top surface of the crystal.
- 6. Select the Resolution and Pixel size required, and then click Finish.

The system automatically focuses on the tip of the crystal, and the ATR imaging background is collected and saved.

An ATR background consists of 16 spectra contained in a .fsm file.

By default, ATR background files are stored in the form:

C:\pel data\SpectrumImage\BackgroundATR*nMag*.fsm,

where *n* is the resolution (2, 4, 8, 16, 32, or 64), and *Mag* is the Magnification (High for a pixel size of 1.56  $\mu$ m, or Low for a pixel size of 6.25  $\mu$ m).



- **NOTE:** If the ATR imaging background is noisy, the MCT detector coolant may require refilling. If the ATR imaging background contains unexpected artifacts, the tip of the ATR crystal may be contaminated. Refer to *Checking for Contamination on the ATR Crystal* on page 80.
  - 7. Repeat this procedure for as many combinations of resolution and pixel size as you need, which is likely to be a subset of the 12 combinations available.

**NOTE:** If you routinely collect ATR sample images (and ATR crystal images) using 64 or 128 scans per pixel, and want to achieve the best signal-to-noise ratio, you can improve the signal-to-noise ratio of the ATR imaging backgrounds used to remove the spectral contribution from the system. Refer to *Optimizing ATR Imaging Backgrounds* on page 77.

### **Compensating for Uneven Illumination**

When an ATR sample image is collected, it includes any field effects inherent in the system, such as a variation in IR illumination and spectral shape across the field.

If you wish to compensate for illumination effects quickly and automatically, you can apply a Baseline Offset Correction, which sets the absorption in a designated spectral range to zero in every spectrum in the image. Both the raw and processed images are saved. By default, this process assumes that there is no significant absorption in the region 2100 to 2000 cm<sup>-1</sup>, which is true for most samples. Where this assumption is not valid, you can apply the correction to your ATR sample image using a different spectral region; refer to *Baseline Offset Correction* on page 63.

To compensate for both illumination variations and subtle spectral shape changes, you can divide the sample image by (or subtract in absorbance terms) an ATR crystal image, which is an ATR image collected under identical instrument conditions to the ATR sample images but when the crystal is not in contact with a sample. If you have stored a suitable crystal image, this subtraction can be done automatically when you collect an ATR sample image. Alternatively, you can ratio your sample image with the crystal image later. As previously, both raw and processed images are saved.

ATR crystal images are collected with no sample on the stage, and with the system focused on the tip of the clean crystal. To ensure optimum results, separate crystal images are required for each spectral resolution and each spatial sampling interval ('Pixel size') that will be used for sample images. The crystal image must be at least as large as, or larger than, the largest sample image.

**NOTE:** It is not necessary to collect an ATR crystal image before you collect an ATR sample image. However, to preserve signal quality, the number of scans per pixel in the crystal image must equal or exceed the number of scans per pixel in the sample image. In particular, if the number of scans used for the sample image is greater than 16, the number of scans used for the ATR crystal image must be greater than 16.

To collect an ATR Crystal Image:

**NOTE:** For the best results, it is important to ensure that the crystal is aligned accurately and consistently for both the crystal image and the sample image.

- 1. If there is a sample on the mini-stage, raise the crystal arm to make sure that the sample is not in contact with the crystal.
- 2. Make sure the crystal arm is clamped, and the crystal dust cover is open.

3. Select **Start ATR Crystal Image** from the Scan menu of the Stage Control window. The ATR Crystal Image Wizard starts.

ATR Crystal Image Wizard	
ATR Crystal Image Wizard ▶ File Details	Welcome to the ATR Crystal Image       Image         Wizard       Image         This wizard helps you create a new ATR crystal image.
Focus on Crystal     Image parameters	Enter file details for the ATR crystal image. Experiment name: Default
	File name: C:\pel_data\Spotlight\Default\ATRCrystal.fsm Browse Comment:
	<u>N</u> ext > Cancel

4. If necessary, amend the **File Details** displayed.

If you enter an Experiment name the path for the File name is automatically updated. A new folder will be created if required.

**NOTE:** You may want to save a high quality crystal image from the clean crystal in a reference folder to use for checking for crystal contamination. Refer to *Checking for Contamination on the ATR Crystal* on page 80.

The Focus on the Crystal page is displayed.



6. Focus on the registration mark on the top surface of the ATR crystal, and then click **Next**.

The Image parameters page is displayed.

ATR Crystal Image Wizard		
ब ATR Crystal Image Wizard	Collect ATR Crystal Select the paramete image.	Image rs for the ATR crystal
<ul> <li>File Details</li> <li>Focus on Crystal</li> <li>Image parameters</li> </ul>	Resolution: Scans per pixet: Pixel size: Background size: Estimated file size:	16 v cm <sup>-1</sup> 16 v • 1.56μm 6.25μm 300 v x 300 v μm 57.9 MB
	Estimated duration: Click Finish to raise th and collect the ATR cry	122.9 minutes
	< <u>B</u>	ack <u>F</u> inish Cancel

The joystick is now disabled, so the stage cannot be moved.

Enter Image parameters identical to those of your ATR sample images.
 The Estimated file size and Estimated duration fields are updated automatically.

#### 8. Click Finish.

If the ATR crystal image file already exists you are asked to confirm that you want to replace it.

An estimate of the time needed to create the image, and the approximate size of the file, is displayed.

9. Click OK.

The ATR crystal image is collected and saved to the folder and filename specified at step 4. By default, this is C:\pel\_data\SpectrumImage\default\ATRCrystal.fsm.



**NOTE:** The colored bands in this ATR crystal image are not necessarily representative of an image collected from your system.

### Collecting an ATR Sample Image

 Before you begin, make sure you have stored an ATR imaging background for the Resolution and Pixel Size you want to use for your ATR sample image.

 CAUTION
 If a suitable ATR imaging background is not available, you will not be able to collect the sample image. Refer to Collecting ATR Imaging Backgrounds on page 49.

 NOTE: While collecting a sample image, the stage is used in the (X,Y) plane to align the ATR crystal on the optical axis of the microscope rather than to position the sample.

 The stage control is used in the Z direction to focus the system.

 You position the sample on the accessory stage manually, and use its mini-stage controls to center the region of interest.

The ATR Imaging Wizard guides you through collecting an ATR image from your prepared sample. At certain points, the stage control joystick is disabled for particular directions.

To collect an ATR Image from your sample:

1. Select **Start ATR Image** from the Scan menu of the Stage Control window. The ATR Imaging Wizard starts.

ATR Imaging Wizard		
ATR Imaging Wizard	Welcome to the ATR Imaging Wizard This wizard helps you create a new ATR Sample Image	<u>жү</u> г
Automatic processing options     Prepare accessory to	Enter file details for the ATR sample image. Experiment name:	
mount sample	Default	_
<ul> <li>Mount sample and prepare accessory for imaging</li> </ul>	File name: C:\pel_data\Spotlight\Default\ATRImage.fsm	_
Confirm focus	Browse	
Image parameters	Comment:	
	To continue, click <b>Next</b> .	
	<u>N</u> ext >	Cancel

2. If necessary, amend the File details displayed.

If you enter an Experiment name the path for the File name is automatically updated. A new folder will be created if required.

#### 3. Click Next.

The Automatic processing options page is displayed.

ATR Imaging Wizard			
ATR Imaging Wizard	Automatic Processing Options		
<ul> <li>File details</li> <li>Automatic processing options</li> <li>Prepare accessory to mount sample</li> <li>Mount sample and prepare accessory for imaging</li> <li>Confirm focus</li> <li>Image parameters</li> </ul>	Select a processing option: Current crystal Image Current crystal image: None Browse Collect New Crystal Image Collect New Crystal Image Collect New Crystal Image Satisper Content of the con		
	< <u>B</u> ack <u>Next</u> Cancel		

4. Select Subtract Crystal Image, or Baseline Offset Correction, or No Automatic Processing.

Refer to Compensating for Uneven Illumination on page 51.

5. If you are collecting a second or subsequent image from a sample that is already mounted in the accessory, select **Skip sample mounting**.

If you selected **Skip sample mounting**, the Confirm focus page is displayed. Go to step 16.

OR

The Prepare accessory to mount sample page is displayed.

ATR Imaging Wizard	
ATR Imaging Wizard	Prepare accessory to mount sample
<ul> <li>File details</li> <li>Automatic processing options</li> <li>Prepare accessory to mount sample and prepare accessory for imaging</li> <li>Confirm focus</li> <li>Image parameters</li> </ul>	<text></text>
	< <u>B</u> ack <u>N</u> ext> Cancel

- 7. Before collecting a new ATR image, make sure the pressure applied to the sample by the mini-stage for a previous image has been released by moving the force lever to the right.
- 8. Lift and swing the crystal arm away from the field of view. Refer to *Swinging the Crystal Arm* on page 29.

The Mount sample and prepare the accessory for imaging page is displayed, which includes four tabbed steps.



The joystick is disabled in the (X,Y) plane, so the stage can only be moved in the Z direction.

- 10. Click through **Step 1** to **Step 4** to see a sequence of photographs that illustrate how to place the sample on the mini-stage; center the region of interest; swing the crystal arm over the sample and place the crystal in contact with the sample; and apply a compressive pressure between the sample and crystal.
- 11. Place the sample on the mini-stage.
- 12. Focus on the sample and then gently push the sample with tweezers to find the region of interest.

Use the manual mini-stage adjusters to center the area of interest. The mini-stage adjusters move the sample at  $45^{\circ}$  to the X and Y axes. The range of travel is appropriate for centering the image.

 Swing the crystal arm over the sample, lower the arm on to the accessory pillars until the crystal tip touches the sample, and then clamp the arm at both ends. Refer to Swinging the Crystal Arm on page 29.

**CAUTION** Do not drop the crystal arm on to a sample, lower the arm forcibly, or attempt to tilt the arm by pushing preferentially on one side. The crystal tip may be damaged.

14. Being careful not to move the accessory, slide the force lever from right to left. The accessory base has a pillar to the left of the force lever slide that enables you to do this with the finger and thumb of one hand.

The Confirm focus page is displayed.



- Focus on the registration mark on the top surface of the ATR crystal.
   The joystick allows you to re-center the registration mark in the field of view.
- 17. Click Next.

The Image parameters page is displayed.

ATR Imaging Wizard		<b>X</b>	
ATR Imaging Wizard	ATR Sample Image options Select the parameters for the ATR sample		
<ul> <li>Automatic processing options</li> </ul>	Resolution:	16 💌 cm <sup>-1</sup>	
Prepare accessory to mount sample	Scans per pixel: Scan from:	16 <b>•</b>	
<ul> <li>Mount sample and prepare accessory for imaging</li> </ul>	Scan to:	720 cm <sup>-1</sup>	
Confirm focus	Pixel size:	🖲 1.56µm 🔿 6.25µm	
Image parameters	Sample size: Estimated file size:	150 ¥ x 150 ¥ μm 14.5 MB	
	Estimated duration: Click <b>Finish</b> to raise the collect the ATR sample i	34.8 minutes stage to focus on the sample and nage.	
8	< <u>B</u>	ack <u>F</u> inish Cancel	

The joystick is now disabled so the stage cannot be moved.

18. Enter **Image parameters** appropriate for the images you wish to collect. Refer to ATR Image Parameters and Quality on page 48. Do not enter parameters for which you do not have an ATR imaging background, or an appropriate ATR crystal image (if used). The Estimated file size and Estimated duration fields are updated automatically.

#### 19. Click Finish.

An estimate of the time needed to create the image, and the approximate size of the file, is displayed.

#### 20. Click **OK**.

The ATR sample image is collected and saved to the folder and filename specified at step 2. By default, this is C:\pel\_data\SpectrumImage\default\ATRImage.fsm.

If you chose to automatically apply a **Baseline Offset Correction** at step 4, the unprocessed image is also saved. By default, this is

C:\pel\_data\SpectrumImage\default\ATRImage\_raw.fsm.

# **Processing ATR Images**

### Introduction

Although you can process an ATR sample image automatically during data collection by applying a default Baseline Offset Correction, or by subtracting an ATR crystal image, you may prefer to process your images manually using one or more of the processing options available in your SpectrumImage software.

Only the most significant processing options are described here.

### **Baseline Offset Correction**

By default, the Baseline Offset Correction option in the Process menu of the View window assumes that there is no significant absorption in the region 2100 to 2000 cm<sup>-1</sup>. For each spectrum in the image, the measured absorption averaged over this region is calculated and removed, which compensates for variations in illumination across the field. This is valid for spectra collected from most samples.

If this assumption is not valid, find a region within your spectrum where there is no significant absorption, and then specify its **Start** and **End** points as the **Baseline range** to be used for the Baseline Offset Correction.

Baseline Offset Co	rrection	
Baseline range Start		ОК
2100.00	cm-1	Cancel
End		
2000.00	cm-1	Help

#### **NOTE:** This setting has no effect on the Baseline range used by the Baseline Offset Correction option offered by the ATR Imaging Wizard. To change this default Baseline range, amend the values of the **BaselineRangeStart** and **BaselineRangeEnd** parameters in the [ATR] section of the pelimage.ini file.

## Ratioing ATR Images

One method used to remove artifacts from spectral images is to ratio them against a suitable background image, such as a crystal image. The ATR Imaging Wizard enables you to automate this process during data collection using a suitable ATR crystal image file, but you can also process your image files at a later stage:

> In the **SpectrumImage** window, select **Ratio** from the **Process** menu.

The Ratio dialog is displayed.

Ratio	×
Sample data type Full Spectral Image Line Scan Data Spectrum	
File names	
Sample	
Background	
Result	
Options	
Align Centres	
C Align Bottom Left	
OK Cancel	Help

Compared to the Sample image, the Background image must have been acquired using the same spectral resolution and pixel size, and be the same size (or larger, although this adds no value).

Provided the sample image has been collected using 16 scans per pixel or greater, it can be helpful for the Background image to have a better signal-to-noise ratio. When the Background image is collected, this can be achieved by selecting a number of **Scans per pixel** one or two increments greater.

Use the align Options when the Sample image and Background image are of different sizes. For ATR background images and ATR sample images, select **Align Centers**.

For more detail, refer to the SpectrumImage software Help system.

### **Revealing Spectral Structure**

The aims of image processing include improving contrast, revealing spectral structure, and minimizing noise.

### Principal Component Analysis

Principal Component Analysis (PCA) allows the spectral information in the data set to be analyzed, ordered and therefore displayed by statistical significance. It is a statistical technique that does not use any assumed or previously known information about the sample. PCA first calculates the spectral covariance matrix of a multi-dimensional data set, and then calculates its eigenvectors and eigenvalues. The eigenvector with the highest eigenvalue identifies the first principal component of the data set, or the most significant spectral feature within it.

Subsequent principal components (represented by the eignevectors ranked according to their corresponding eigenvalues) show statistically independent and less significant spectral features. Often, the most interesting spectral information in an image is within the first few principal components; the less significant components are often dominated by noise. The original image can be reconstructed using only the most significant principal components, thus reducing noise.

The SpectrumImage software Help system describes how scoring variance using PCA can relate to absorption bands in the underlying spectra.

When applied to ATR imaging, especially where absorptions are weak, PCA is a convenient method for exposing the spectral structures within the image. Its usefulness is enhanced by the ability to display the principal component images as color composites.

If your data would benefit from more sophisticated and flexible processing, consider using the Hyperview package, which is available via your PerkinElmer technical support representative.

### The Show Structure Command

The Show Structure command is a simple way to enhance independent chemical variation in an ATR sample image relative to variations arising largely from physical effects and random noise.

It applies a number of processes using default parameters that reduce baseline effects and high frequency noise, and then applies PCA to extract the most significant independent spectral features. The results are displayed as score images that can be viewed independently or overlaid.

The Show Structure command is very useful during initial sample examination. It can confirm the presence of useful image structure prior to a more detailed analysis, or prior to collecting an image using a greater number of scans to improve the signal-to-noise ratio.

To use the Show Structure command and Layer Manager dialog:

- 1. Display your ATR sample image in an IR Image window.
- 2. For optimum results, select **Atmospheric Correction** from the Process menu. This step removes a subtle source of variability.

3. Select **Show Structure** from the Process menu.

The Layer Manager window is displayed, and the display format changes to an Overlay image.

Layer Manager - Total Abs.( lamdemo.fsm )		
View C Single © Mix Sh	ow Overlaps Average	
List		
h master	Move Up Insert	
Score 3.imp h Score 4.imp h Score 5.imp	Move Down Delete	
h Score 6.imp h Score 7.imp	Hide	
Layer	Ordinate Scaling	
Name: Score 1.imp	🔽 Autoscale	
Color: Red	Contrast:	
Score map for factor 1 from 2000.00 to	4	
Mask	Brightness:	
Threshold:	<b>₹</b>	
0.2548 🗖 Opaque	Min: Max:	
No Mask	0.1720 1.0000	
OK Cancel	Help	

As with any Overlay Image, the master layer corresponds to the original dataset, and is the source of any spectral information extracted from the image.

The Score layers are image maps corresponding to the principal components. Eight score layers are available in this simplified processing chain.

By default, the image maps corresponding to the three most significant components are displayed using the default colors red, green and blue.

4. Use the **Layer Manager** dialog to manipulate the layers and discover areas of interest in your image.

For more information, refer to the SpectrumImage software Help system.

5. To view a spectrum at any point in the image, click the right mouse button and select **View Spectrum**.

A Spectrum View window is displayed.

The Show Structure command creates a folder named *imagename*.fsm\_PCA at the location of your image file. This folder contains:

Filename	Description
Score 1.imp to Score 8.imp	The score files are image maps of the principal components
Composite.imp	A composite image map that sums the score files
Reconstructed.fsm	An image reconstructed, by default, from the first eight principal components
Factor 1.sp to Factor 8.sp	Factor files describe the contribution of the dataset associated with its principal components
Leverage.imp and Leverage.sp	The leverage.imp file shows the degree of influence each pixel had in the principal component analysis, and often reveals areas of particularly high scores on a principal component. The leverage.sp file shows those spectral regions that had a high influence on the principal component analysis
Outliers 0.imp to Outliers 8.imp and Outliers.imp	Image maps for data whose Cook's distance is > 1, and therefore unlikely to be part of the distribution being characterized

### ATR Images and Spectral Libraries

Ideally, ATR spectra should be compared with ATR libraries containing reference spectra obtained using a germanium ATR system; this allows any spectral matching software to work to its optimum capability.

Apply ATR Correction before comparing ATR spectra to conventional reflectance or transmission library spectra. ATR Correction adjusts the ATR sample image for the increase in penetration depth (and therefore in effective pathlength) that occurs at longer wavelengths. Absorbance values at the shortest wavelength (highest wavenumber value) are unchanged, but as the wavelength increases the measured absorbance is increasingly attenuated.

This correction enables spectra extracted from your image to be better compared to the normalized transmission spectra held in a spectral library, and accessible using your Spectrum software.

To perform an ATR Correction:

- 1. Display your ATR sample image in an IR Image window.
- 2. Select ATR Correction from the Process menu.

The ATR Correction dialog is displayed.

ATR Correction	X
Parameters	ОК
Contact 5.000000	Cancel
	Help

3. If necessary, enter a **Contact** factor.

The Contact factor helps compensate for a less than perfect contact between your sample and the ATR crystal by allowing for a small air gap. It cannot compensate for poor sample preparation.

A value of zero assumes perfect contact. Refer to the SpectrumImage software Help system for more information.

4. Click **OK**.

The image is processed.

# **Optimizing the Accessory**

# Adjusting the Mini-Stage Pre-Load

The mini-stage compression mechanism presses the sample against the crystal tip to improve optical contact between the crystal tip and the sample.

When the force lever is to the right, no force is applied. When the force lever is moved a little to the left a pre-load is applied that is adjustable between 0 and 15 N. When the force lever is moved fully to the left a further 15 N is applied.



#### Figure 8 Mini-stage force lever

### CAUTION

For rougher surfaces, or if the sample is not horizontal, minimize the risk of crystal damage by setting the pre-load to zero.

- For softer samples, you may decide to decrease the pre-load. This can lessen surface distortion or indentation by the crystal tip.
- For a hard, polished sample it may be appropriate to improve crystal contact by increasing the pre-load.
- Set the pre-load by turning the adjustment screw anti-clockwise (to decrease the pre-load) or, if the preload has been reduced, clockwise (to increase the pre-load).
   By default, the adjustment screw is set fully clockwise, which applies the maximum pre-load of 15 N.



#### Figure 9 Mini-stage pre-load adjustment screw

The pre-load is adjustable over one full turn of the adjustment screw.

## Leveling the Accessory

This procedure helps ensure the optimum performance of the accessory. Check the stage level periodically, especially if you remove the accessory from the stage often.



1. Back the four leveling screws to just under flush with the underside of the accessory.



#### Figure 10 Accessory leveling screws

Use the 1.5 mm hex key supplied.

- Fit the ATR Imaging Accessory to the microscope stage. Refer to *Installing the Accessory on the Stage* on page 33.
- 3. For the newer stage design, fix the accessory using the two thumbscrews.



#### Figure 11 Accessory thumbscrews

Use these thumbscrews to position the accessory on the stage, but do not tighten them. Some vertical movement is required to level the accessory.

- 4. Very gently screw in each of the leveling screws until a resistance is felt and then back off by about quarter of a turn.
- 5. Move the crystal arm until the tip is a few mm above the surface of the anvil, and then tighten both clamps.

6. Focus on the top surface of the crystal arm at position A.



#### Figure 12 Crystal arm

A small spot of light is visible on the arm. The Auto functions for both Illumination and Focus in the Control window may be useful.

7. In the Stage Control window, select **Origin** from the Options menu, click **Zero X,Y Z** and then click **OK** to confirm.

The coordinates in the Control window are set to X: 0, Y: 0, Z: 0.

- 8. Use the stage control joystick to move the stage along the Y axis until the spot of light falls on point B, re-focus the microscope, and note the value of the Z coordinate from the Control window.
- 9. If the Z coordinate is within  $\pm 20 \ \mu m$  of zero, the stage is adequately leveled along the Y axis. Go to step 11.

OR

If the Z coordinate is negative by more than 20  $\mu$ m, the rear of the accessory is low. Move the stage back to point A, and then use both of the rear leveling screws to gently and evenly raise the accessory until the visible image is in focus. Next, in the Stage Control window, click **Zero X,Y Z** and then **OK** to confirm.

OR

If the Z coordinate is positive by more than 20  $\mu$ m, the front of the accessory is low. Move the stage vertically until the Z coordinate is zero, and then use both of the front leveling screws to gently and evenly raise the accessory until the visible image is in focus. Next, move the stage back to point A and then, in the Stage Control window, click **Zero X,Y, Z** and then **OK** to confirm.

- 10. If any adjustments were made at step 9, the microscope is looking at point A, is in focus, and the Z coordinate is 0. Move to point B, refocus, and then return to step 8. With practice, it is possible to level the accessory in the Y direction to within  $\pm 10 \ \mu$ m, but  $\pm 20 \ \mu$ m is adequate.
- Focus on the top surface of the crystal arm at position C.
   Points C and D are offset towards the front of the accessory to avoid the scribed line.
- In the Stage Control window, select **Origin** from the Options menu, click **Zero X,Y Z** and then click **OK** to confirm.

The coordinates in the Control window are set to X: 0, Y: 0, Z: 0.

13. Use the stage control joystick to move the stage along the X axis until the spot of light falls on point D, re-focus the microscope, and note the value of the Z coordinate from the **Control** window.

The image must not contain the scribed line.
14. If the Z coordinate is within  $\pm 20 \ \mu m$  of zero, the stage is adequately leveled along the X axis. Move to step 16.

OR

If the Z coordinate is negative by more than  $20 \ \mu\text{m}$ , the left of the accessory is low. Move the stage back to point C, and then use both of the left leveling screws to gently and evenly raise the accessory until the visible image is in focus. Next, in the Stage Control window, click Zero X,Y Z and then OK to confirm.

OR

If the Z coordinate is positive by more than 20  $\mu$ m, the right of the accessory is low. Move the stage until the Z coordinate is zero, and then use both of the front leveling screws to gently and evenly raise the accessory until the visible image is in focus. Next, move the stage back to point C and then, in the **Stage Control** window, click **Zero X,Y Z** and then **OK** to confirm.

- 15. If any adjustments were made at step 14, the microscope is looking at point C, is in focus, and the Z coordinate is 0. Move to point D, refocus, and then return to step 14. With practice, it is possible to level the accessory in the X direction to within  $\pm 10 \ \mu m$ .
- 16. For the newer stage design, gently tighten the two thumbscrews that fix the accessory to the stage.

# Correcting Image Alignment

Provided the sample has not moved, and the sample disk has not moved on the anvil, the visible and ATR sample images can be aligned to around  $\pm 20 \ \mu m$  for low magnification images (pixel size 6.25  $\mu m$ ), and  $\pm 10 \ \mu m$  for high magnification images (pixel size 1.56  $\mu m$ ). You can measure the image misalignment and, if necessary, amend the appropriate ImageCorrection parameters in the pelimage.ini file.

### Making a target sample

To check and correct visible and IR image alignment, you need a sample containing a feature that can be readily identified on the visible and ATR images, and centered to within a few microns. If you do not have a suitable sample:

- Cut a piece of clean, unscratched plastic sheet to a convenient size; say 10 mm x 10 mm. Its thickness should be between 1 mm and 6 mm. A piece of acrylic from a CD case is ideal.
- 2. Apply a piece of clear cellulose sticky tape to one surface, being careful to avoid any bubbles.
- 3. Remove any finger oils from the surface of the tape using a piece of lens tissue dampened with a little isopropyl alcohol (IPA) or ethanol.
- 4. Fix a new blade to a scalpel handle, and then lightly scribe some crosses on the surface of the tape.

Your scribed lines should be barely visible to the naked eye. The aim is produce cuts no wider than 50  $\mu\text{m}.$ 

You will also need a thin piece of rubber to prevent your sample from slipping on the accessory. A piece of rubber cut from the palm of a disposable latex glove is ideal.

# Measuring the misalignment between the visible and ATR images

- 1. Set up your system for ATR Imaging.
- Remove the sample carrier from the mini-stage.
   Slight movement between the sample carrier and the mini-stage can invalidate your measurements.
- 3. Position the crystal arm such that the tip of the crystal is close to the height of your sample above the stage and then clamp it at both ends.
- 4. Register the crystal position.
- 5. Making sure that the microscope stage remains at the origin in the (X,Y) plane, swing the crystal arm out of the field of view.
- Place a thin sheet of rubber on the anvil. This helps prevent your sample from slipping.
- 7. Place your target sample on the rubber sheet, taped surface uppermost.

- 8. Use the Monitor Visible window to find, focus on, and center on a recognizable finely scribed feature.
  Do not use the joystick control to move the microscope stage laterally. Move the sample using tweezers and center the feature using the accessory mini-stage controls.
  The feature is centered at the origin in the (X,Y) plane.
- 9. In the Monitor Visible window, select **Copy Image to New Window** from the File menu.
- 10. Gently lower the crystal tip onto the sample, clamp the crystal arm and apply pressure using the force lever.

To avoid moving the stage in the (X,Y) plane, gently pinch the force lever towards the left thumb pillar.

11. Collect an ATR sample image.

For low magnification, use the following parameters: **Baseline Offset Correction** 

Skip sample mounting Resolution: 16 cm<sup>-1</sup> Scans per pixel: 1 Pixel size: 6.25 µm Image size: 300 µm x 300 µm.

OR

For high magnification, use the following parameters:

Baseline Offset Correction Skip sample mounting Resolution: 16 cm<sup>-1</sup> Scans per pixel: 1 Pixel size: 1.56 µm Image size: 200 µm x 200 µm.

12. When the ATR sample image has been collected, select **Show Structure** from the Process menu.

The system takes around 2 minutes to collect the ATR sample image. Your target feature should be clearly visible when the Show Structure function terminates.

- 13. In the window containing the ATR sample image, click on the cross-hair cursor button. A cross-hair cursor is displayed in the center of the image with its (X,Y) coordinates.
- 14. Drag the cross-hair cursor to the center of the feature and note the sign and value of its coordinates.

If the image offset is greater than  $\pm 20 \ \mu m$  for a low magnification ATR sample image (pixel size 6.25  $\mu m$ ), and  $\pm 10 \ \mu m$  for a high magnification ATR sample image (pixel size 1.56  $\mu m$ ), consider amending the image alignment parameters in the pel\_image.ini file.

OR

If the image offsets are less than those specified above, there is no need to amend the alignment parameters used by the system.

### Amending the Image Alignment Parameters

- 1. Make a backup of the C:\WINDOWS\pelimage.ini file.
- 2. Open the C:\WINDOWS\pelimage.ini file using a text editor such as Notepad, and then find the [ATR] section.
- Note the existing values of the ImageCorrectionLowX and ImageCorrectionLowY parameters, and of the existing ImageCorrectionHighX and ImageCorrectionHighY parameters.

The default setting for these parameters is zero, which signifies that no correction is applied.

- For each parameter, calculate the sum of the existing ImageCorrection value and the corresponding misalignment measured earlier, at step 14.
   The LowX and LowY parameters apply to measurements at low magnification, and the
- 5. Using the text editor, replace each parameter by the corresponding summed value.
- 6. Save the C:\WINDOWS\pelimage.ini file, and exit your text editor.

HighX and HighY parameters are used for high magnification.

### ATR Crystal Images and ATR Imaging Backgrounds

After amending the image alignment parameters:

- If you use crystals images for background compensation, collect new crystal images as described in *Compensating for Uneven Illumination* on page 51.
- > Collect new ATR imaging backgrounds, as described on page 49.

# **Optimizing ATR Imaging Backgrounds**

By default, ATR imaging backgrounds are collected using 60 accumulations, which is more than sufficient for most ATR imaging tasks.

However, if you routinely collect ATR sample images (and ATR crystal images) using 64 or 128 scans per pixel, and want to achieve the best signal-to-noise ratio, you can improve the signal-to-noise ratio of the ATR imaging backgrounds used to remove the spectral contribution from the system.

### Amending the ATR imaging background parameter

- 1. Make a backup of the C:\WINDOWS\pelimage.ini file.
- 2. Open the C:\WINDOWS\pelimage.ini file using a text editor such as Notepad.
- 3. Find the ReferenceSpectraAccumulations parameter in the [ATR] section. The default value for this parameter is 60.
- Set ReferenceSpectraAccumulations=120.
   The value of this parameter must be a multiple of 15.
- 5. Save the C:\WINDOWS\pelimage.ini file, and exit your text editor.
- 6. Collect new ATR imaging backgrounds, as described on page 49.

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# <u>Cleaning</u>

## Checking for Contamination on the ATR Crystal

It is important that the ATR crystal tip be kept clean and its top surface free of dust. Always close the dust shutter on the crystal arm when the accessory is not in use, and avoid contaminating the crystal with finger oils.

You can quickly detect gross contamination adhering to the ATR crystal by visually inspecting the crystal tip and its curved upper surface.

You can detect subtle contamination adhering to the ATR crystal tip by visually inspecting the ATR crystal image. However, an ATR crystal image collected from a clean crystal includes field effects, so any changes may not be immediately apparent.

Alternatively, provided you have previously collected (and stored for reference) a high quality (Resolution, Pixel size, Scans per pixel) ATR crystal image from the clean crystal that is at least as large as your ATR sample image:

1. Collect an ATR crystal image of the same size and using the same parameters as those used to collect your reference ATR crystal image. See *Compensating for Uneven Illumination* on page 51.

**NOTE:** To display the parameters used to acquire a displayed image, select **Status** from the **File** menu.

2. Ratio the two ATR crystal images using the **Ratio** command in the Process menu of the main SpectrumImage window.

Refer to *Ratioing Full Spectral Images* in the onscreen Help.

The resulting image should be uniform, although some noise is to be expected.

- Select Show Structure from the Process menu. Any features indicate contamination adhering to the ATR crystal.
- 4. Display spectra from any features to help confirm the nature of the contaminant. Refer to *Displaying a Spectrum from an Image* in the onscreen Help.

### Visually Examining the Crystal Tip

You can examine the crystal tip for gross contamination using the eyepiece supplied, catching reflections from the room lighting. Alternatively you can mount the crystal arm so that the crystal tip can be examined using the microscope.

1. Invert the crystal arm and fit the larger end on the left post of the accessory base.



#### Figure 13 The crystal arm inverted to examine the crystal tip

The crystal tip is aligned with the registered crystal position, that is the origin in the (X,Y) plane.

- Adjust the height of the motorized stage until the crystal tip comes into focus. If necessary, adjust the visible illumination in the microscope Control window. The crystal tip is too large to be entirely within the field of view.
- 3. In the Stage Control window, select a View area of 1000 x 1000 microns.
- 4. Make sure the crystal tip is approximately central under the microscope. If necessary, drive the stage to view the extreme edges of the tip in the X and Y directions, note the appropriate coordinate from the Control window, and then move to the central point.
- 5. In the Stage Control window, select **Visible Image Survey** from the Survey menu. The survey captures an image of the entire crystal tip.

## Cleaning the Crystal

	The crystal tip is resistant to solvents such as de-ionized water, ethanol, and iso-propyl alcohol (IPA). The resin used to bond the crystal is not resistant to solvents such as acetone, chloroform, or dichloromethane; these solvents could damage the bond between the ATR crystal and its holder.
CAUTION	When cleaning the crystal, use minimal pressure and avoid circular scrubbing movements.
	The germanium crystal tip can be scratched if particles are rubbed across its surface. Regard all particles as potentially abrasive.
	The anti-reflective coating on the top surface of the crystal is fragile.

### **Cleaning Procedure for Slight Soiling**

If the crystal tip is only slightly soiled, blow off any dry particulates using a proprietary dust blower (NOT an air-line, which may contain trace oils and contaminate the accessory) and then:

1. Tear off a fresh 2 cm wide strip of lens tissue.

Wear gloves to avoid contaminating its central area with finger oils.

2. Place a small drop of solvent on one side of the lens tissue and then shake to remove any excess.

Use Analar, or 'optical', grades of either ethanol or IPA. Evaporation of these high purity solvents leaves no deposits on the crystal surface.

If you use de-ionized water as a cleaning agent, follow by cleaning using a drop of ethanol or IPA to remove any residual water from the crystal tip.

3. In one motion, lower the wet area of the tissue onto the crystal tip and then drag the tissue gently towards the dry area.

This draws the solvent from the tip, leaving it dry and clean.

**NOTE:** To ensure that no contaminants are carried to the crystal tip, the tissue must not touch any adjacent areas.

4. Stop when the tissue no longer appears to be wetted by the crystal. It is important not to use too much solvent.

Allow the crystal tip to dry thoroughly before collecting any data.

**CAUTION** Failure to observe this precaution may lead to images and spectra that are contaminated by the solvent used.

### **Cleaning Procedure for Heavier Contamination**

If the crystal tip is more heavily contaminated:

- Tear off a fresh 2 cm wide strip of lens tissue.
   Wear gloves to avoid contaminating the tissue with finger oils.
- 2. Use tweezers to tightly roll the strip of lens tissue into a thin cylindrical shape.
- 3. Grip the lens tissue roll with tweezers or fine artery clamps and then moisten one end with a small amount of solvent.

Use Analar, or 'optical', grades of either ethanol or iso-propyl alcohol (IPA). Evaporation of these high purity solvents leaves no deposits on the crystal surface.

For stubborn contaminants on the crystal tip that are not soluble in water, ethanol or IPA, a very small quantity of a high purity grade of a more aggressive solvent such as acetone, chloroform, or dichloromethane may be used.

CAUTION

Do not allow the solvent to come into contact with the anti-reflection coating or the resin that surrounds the crystal, which may be damaged as a result.

- 4. If you used de-ionized water as a cleaning agent, follow by cleaning using a little ethanol or IPA.
- 5. Taking great care not to exert excessive pressure or allow metal tweezers to contact the crystal, gently use the end of the roll to dislodge contaminants from the crystal tip. The roll of tissue is compliant enough to prevent damage to the crystal provided it is used end on, especially as the edge is torn.
- 6. Repeat steps 1 to 4 until the contaminants have been removed.
- 7. Now follow the *Cleaning Procedure for Slight Soiling* on page 82.

### Cleaning the Accessory Base

CAUTION

Always remove the ATR crystal arm before attempting to clean the accessory base.

Never immerse, or attempt to wash, the accessory.

### **Cleaning Procedure for Slight Soiling**

If the accessory is only slightly soiled, blow off any dry particulates using a proprietary dust blower (NOT an air-line, which may contain trace oils and contaminate the accessory).

### **Cleaning Procedure for Heavier Contamination**

If the accessory is more heavily contaminated:

1. Remove the crystal arm.

To avoid damaging the crystal tip, clean the crystal arm and crystal separately (see *Cleaning the Crystal* on page 80).

 Wipe the accessory (NOT the crystal arm) using a lint-free cloth. If necessary, this cloth may be dampened with a little de-ionized water, the recommended concentration of a standard laboratory detergent, or a little alcohol. Allow the accessory to air dry.

**NOTE:** The self-lubricating bearing in the crystal arm transfers a little lubricant to the right pillar. When you re-assemble the accessory, take care not to contaminate the crystal.

# Appendix 1: Replacing the ATR Crystal

If the ATR crystal is damaged, it can be replaced.

Part Number	Item
L1860317	ATR Crystal Kit

### Fitting a New ATR Crystal

1. Invert the crystal arm and fit the larger end on the left pillar of the accessory.



#### Figure 14 Retaining screws

The crystal holder is held to the underside of the crystal arm using two counter-sunk retaining screws.

- 2. Using a flat-bladed screwdriver, carefully remove the two retaining screws.
- 3. Remove the crystal (in its steel sleeve) and discard it.



CAUTION

These materials are hazardous. See the Materials Safety Data Sheets (MSDS) supplied by your local Safety Officer for details.

They must be disposed of with care, following your laboratory procedures.

- 4. Make sure that the recess in the arm is clean and free of debris.
- 5. Unpack the new crystal.

Take care not to contaminate any part of the germanium crystal with finger oils or any other materials, or to rub or scratch its surface.

Lower the new crystal and its steel sleeve into the recess in the crystal arm.
 Keep the crystal level, with its tip uppermost. Do not force the crystal into the recess.

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- 7. Carefully fit the two new retaining screws provided, and then tighten them evenly. Take great care not to scratch the crystal tip with the screwdriver blade.
- Inspect the crystal tip for contamination.
   Refer to *Visually Examining the Crystal Tip* on page 81.
- 9. Remove the crystal arm, and then replace it on the accessory in its usual orientation.
- Peel the label off the crystal arm and apply the label for the new crystal. The new crystal comes with a self-adhesive label specifying its height.

#### Setting the Crystal Height

1. In the Stage Control window, select **Set ATR Crystal Height** from the Options menu.

Set ATR Crystal Height		X
Current ATR crystal height:	7000	μm
New ATR crystal height:	0	μm
OK		Cancel

The Set ATR Crystal Height dialog is displayed.

2. Enter the **New ATR crystal height** in microns.

If the value entered is incorrect your ATR images will be out of focus or completely dark.

3. Click **OK**.

#### **Optimizing ATR Crystal Registration**

When registering the ATR crystal position (see *Registering the Crystal Position* on page 36), it is important that the registration mark is consistently centered in the red alignment circle. If you want to optimize the diameter of the red alignment circle:

- 1. Make a backup of the C:\WINDOWS\pelimage.ini file.
- 2. Open the C:\WINDOWS\pelimage.ini file using a text editor such as Notepad, and then find the [ATR] section.
- Amend the value of the RegistrationDiameter parameter. A suitable value is likely to be in the range 275 to 325.
- 4. Save the C:\WINDOWS\pelimage.ini file, and exit your text editor.
- 5. Select another Operation mode in the Control window and then re-select **ATR Imaging** mode.

The Stage Control and Monitor Visible windows are refreshed.

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