Sample Preparation

The Spotlight imaging system enables you to examine the sample in the Monitor Visible window and to select the area where you want to collect images or spectra. To make sure that you collect good quality spectra, it is important that you prepare samples properly. Sample preparation is needed when the sample is too thick for transmission work, or the area of interest is on the inside (for example in a laminated sample).

- If you are going to collect transmission spectra, the sample should ideally be thin enough (approximately 5 to 20 µm) to give good detail and undistorted absorption bands.
- The area of the sample must be large enough to give an adequate signal or the scan time must be increased.
- Preparing a sample, therefore, often involves flattening it; this both thins it and increases its area. The sample can be flattened by rolling, squeezing, or pressing.

This chapter tells you how to prepare samples for spectroscopy with the Spotlight imaging system. It includes:

- A list of useful tools;
- A list of window materials commonly used for mounting samples;
- Descriptions of special techniques used to prepare particular types of samples.

If you are not familiar with the techniques described here we recommend that you work through the SpectrumIMAGE on-screen tutorials, which you can access by selecting **Learning** from the Help menu on the main SpectrumIMAGE window.

Tools for Sample Preparation

This section lists the tools you need for preparing samples:

- Tools provided with the imaging system;
- Tools in the microsampling toolkit;
- Materials to have available;
- Specialized accessories you may want to purchase.

Tools Provided with the Imaging System

The following items for use in sampling are provided in the Sampling Accessories Kit that is supplied:

Item	Use
Holder for 13 mm disks	Supporting 13 mm disks on the sample stage.
Slides, glass (box)	Supporting samples for sample preparation.
Rotating 13 mm disk holder	Supporting samples and allows rotation of the sample.
Support for large samples	Clips on to the sample stage. Supporting bulky samples so that the stage clip does not interfere with them.
Gold mirror assembly	Reflection measurements.
KBr windows (2)	Supporting samples.

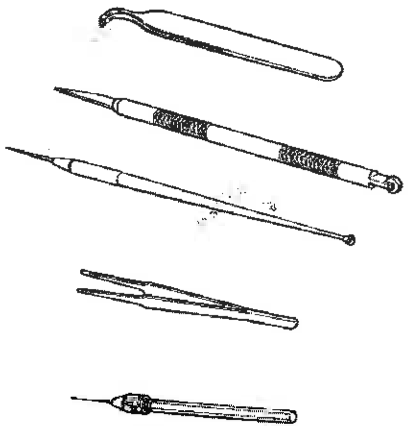

Figure 17 Some of the Tools in the Microsampling Toolkit

The following tools are provided in the Microsampling toolkit (Figure 17):

Tool	Use
Steel tweezers	Picking up extremely small objects
Roller knife	Cutting (knife end) and flattening (roller end)
Steel probe	Pulling samples apart, separating fibers
Forceps, 4½ inch, Cd plated	Picking up small objects
Tungsten alloy needle	Transferring particles
Pin vise	Holds needles for sharpening or for flattening samples
Interchangeable handle for micro tools	Handle for tungsten needle or steel probe

Other Useful Tools

Depending on the type of samples that you usually work with, it may be helpful to have some of the following tools:

Tool	Part Number
Microprobe with right angle bend	N930 2606
Forceps, round tips	N930 2607
Forceps, narrow needle points	N930 2608
Windows (all 13 mm diameter):	
BaF_2 (1 mm thick)	N930 2611
BaF_2 (2 mm thick)	N930 2612
ZnSe (1 mm thick)	N930 2613
NaCl (2 mm thick)	N930 2614
KBr (2 mm thick)	N930 2615
Wide-tipped forceps, hooked	0990 8138
Wide-tipped forceps, flat	0990 8400
1.5 mm microdisk. Fits in 13 mm disk holder to support very small samples.	0186 1043

These items are available from your local PerkinElmer sales office or agent.

Specialized Accessories

The following accessories are extremely useful in preparing certain types of samples (as described in "Techniques for Preparing Samples", starting on page 66):

- Miniature Diamond Anvil Cell (N930 2618);
- Fiber Optic Illuminator (N930 2602);
- Microtome.

Items to Have Available

In addition to the items provided with the Spotlight imaging system, we recommend that you have the following available:

- Tape with adhesive on both sides ("double-sided tape") for holding long or large samples on the sample stage;
- Single-edge razor blades for cutting samples.

Common Window Materials

For work in the mid-infrared, using the Spotlight 400 imaging system, both liquid and solid samples are often mounted on salt windows. Very thin windows, 1 to 2 mm thick, give the best spectra. The following materials are commonly used in windows:

- KBr: Potassium bromide is inexpensive, and it transmits infrared radiation to below 400 cm^{-1}. The major disadvantage of this material is that it is hygroscopic, so that the windows fog easily.
- BaF_2: Barium fluoride is not hygroscopic. Its transmittance cut-off is 750 cm^{-1}. It can break or crack easily.
- NaCl: Sodium chloride transmits infrared down to 600 cm^{-1}. Otherwise, its properties are similar to KBr.
- ZnSe: Zinc selenide is not hygroscopic. Its transmittance cut-off is 650 cm^{-1}. ZnSe is more durable than the other windows but is yellow, so that the field of the Monitor visible window appears yellow.

The Spotlight 400N FT-NIR imaging system has a range of 7800 cm^{-1} to 2400 cm^{-1}. However, for most near-infrared work in the region 7800 – 4000 cm^{-1}, samples can be mounted on standard quartz microscope slides.

Techniques for Preparing Samples

This section describes some useful techniques for preparing various types of samples.

Flattening Solids

Flattening samples by pressing or squeezing often enables you to make thick samples thin enough to give good infrared spectra. Because the samples are usually quite small, only moderate force is necessary.

Rolling with the roller knife

The roller end of the roller knife provided in the microsampling tool is one of the simplest and most effective devices for flattening samples. It is especially useful for flattening fibers or particles.

You can treat different types of sample in different ways:

- If the sample is soft, you can roll it on a small salt window.
- If the sample is hard, you can roll it on a hard surface, such as glass or metal. A flat, black cap from a jar makes a good surface for rolling a light-colored sample.
- If you roll the sample on a small, flat piece of metal you can view it and collect spectra in reflectance mode. Samples rolled on windows transparent to infrared can be examined in transmittance.
- If you flatten fibers on a glass microscope slide, they can then be peeled off and mounted either on a window or over the aperture for the microscope slide.

Squeezing with a pellet press

You can squeeze samples between the polished anvils of a KBr pellet press without KBr. To collect the spectra use one of the following methods:

- Peel the flattened sample off the anvil with a probe or knife and place it on a sample mount. Collect the transmittance spectrum.
- Leave the sample on the polished anvil and collect the reflection spectrum. Use a clean area of the anvil as the reference.

Using a diamond anvil cell

See the procedure in "Polymers" (page 69) for information on flattening samples with a miniature diamond anvil cell.

Compressing between infrared transmitting windows

Pressing two windows together, with the sample between them, compresses the sample. This also provides optical contact between the windows and the sample, reducing surface scattering.

Windows made of NaCl or KBr are relatively soft. If your sample is hard, or if it is wet, use BaF_2 or ZnSe.

Pressing with the heel of a probe

Press on small samples with the flat end of the probe handle. Even moderate pressure usually produces considerable thinning.

Pressing with a needle

Pressing with the point of a needle or probe applies a high force per unit area, because the area of contact is small.

Rolling a hard sample with the side of a sewing needle held in a pin vise presses it into a flake.

Slicing Samples from Solids

Cutting a wedge of sample

Cutting a wedge-shaped piece from its edge enables you to produce a thin sample while destroying very little of the original. This technique can be used with laminates, plastics, films, paint chips and paper.

To cut a wedge-shaped piece from a relatively thick sample:

➢ Hold the sample in tweezers as you slice a thin wedge from it with a razor blade. Taper the wedge to as thin a slice as possible.

To cut a wedge-shaped piece from a relatively thin sample:

1. Place the sample between two offset glass slides. Allow a triangular portion of the sample to protrude as shown on the left in Figure 18.

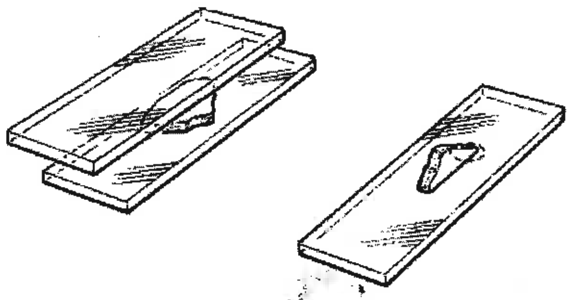

Figure 18 Cutting a Wedge-Shaped Sample

2. Run a razor blade or the roller knife along the edge of the upper slide.
   The triangular piece of the sample is sliced off, giving a wedge-shaped sample.
3. To mount the sample, rotate it so that it is positioned as shown on the right in Figure 18.
4. Position it under the imager so that the infrared beam goes through the thin end of the wedge (circled in Figure 18).

Microtoming

A microtome enables you to slice a sample into thin cross-sections, 0.5 to 20 µm thick. It is commonly used to prepare samples for light microscopy; the same range of thicknesses is also appropriate for infrared microspectroscopy.

If you are trying to identify the individual components of a laminate, microtomed samples give the best results.

Samples are often embedded in a supporting medium before they are microtomed. If you must use an embedding material, choose it carefully, so that it does not alter the sample by reacting with it, dissolving it, or contaminating it. Some commonly used materials are:

- **paraffin wax:** This is the preferred medium for infrared spectroscopy. It produces few spectral interferences, and it can usually be easily removed from the sample with warm xylene.
- **β-pinene wax:** This material is similar to paraffin.
- **plastic embedding materials:** These can be used depending on the size and porosity of the sample.
- **acrylic and epoxy resins:** Although these are commonly used in light microscopy, they are not recommended for infrared, because they are hard to remove and can cause spectral interferences.

Polymers

Pressing or squeezing enables you to reduce the pathlength of polymer samples such as paint chips, thick films, elastomers, or fibers.

Diamond anvil cell

The miniature diamond anvil cell, shown in Figure 19, enables you to press polymers (or other compressible samples). It enables you to both thin the sample and collect its spectrum in the same device; this is an advantage when you have limited material available. It is small enough to be easy to manipulate, and fits in the recessed retainer in the support for large samples. By collecting a background spectrum of an empty area of the cell, you can completely compensate for the absorption bands of the diamonds.

To thin a sample in the miniature diamond anvil cell:

1. Loosen and remove the three screws that hold the cell together.
2. Lift off the top half of the cell and set it aside.
3. Place the sample on the bottom half of the cell. (The sample must be small.)
4. Put the top half back on the cell, lining up the red dots on the top and bottom halves. Do not tighten the screws yet; applying uneven shear forces may damage the diamonds.

CAUTION *One diamond can damage another.*

5. Press down on the cell with your thumb to thin the sample. Replace the three screws and tighten them finger tight.

NOTE: If the spectrum collected with the diamond anvil cell shows interference fringes, place some KBr in the cell and collect a background spectrum through it.

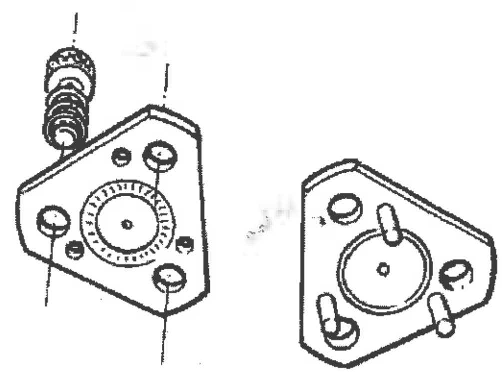

Figure 19 The Miniature Diamond Anvil Cell

Pressing elastomers between windows

If your sample is elastic and you are compressing it between windows, you must apply pressure continuously. Use the following procedure:

1. Press on the windows with a probe, flattening the sample.
2. While maintaining the pressure, apply small amounts of quick-setting nitrocellulose cement to the edges of the salt plates.

   When the cement is dry, the sample remains compressed.

The compression cell (see "Using the Compression Cell" on page 77) enables you to compress this kind of sample.

Filled polymers

When a polymer contains a high concentration of fillers, and you want to analyze the polymer, you have to prepare a sample for analysis that is free of filler.

Often you can obtain a suitable sample by cutting a thin wedge of the material with a sharp blade. If the filler is not uniformly dispersed, you can find clear regions of polymer for analysis.

You can use pyrolysis to remove the fillers. As you heat the sample, the polymer volatilizes, and the fillers are reduced to ash. The sample can be pyrolyzed in the following ways:

Using a disposable pipette:

1. Place the polymer in a disposable pipette and seal the large end.

2. When this end cools, tap the polymer into it, then heat the sample gently.

   The pyrolyzed polymer condenses on the walls of the pipette. The filler is left behind as ash.
3. Score and break the pipette between the ash and the pyrolysate.
4. Add a drop of solvent to the pyrolysate to wash it on to a salt plate.

If the amount of sample is small, use a capillary tube instead of a disposable pipette.

Using a microbrush to pyrolyze micro amounts:

1. Seal the end that is away from the brush fibers and tap the sample particle into this end then heat it gently with a microtorch.
2. After pyrolysis, break off and discard the end of the tube that contains the ash.
3. While holding the fibers of the brush against the salt plate, add a drop of solvent to the broken end.
4. Allow the solvent containing the pyrolysate to flow into the fibers.

   When it evaporates, the pyrolysate remains on the salt plate for analysis.

Particles

Crushing

Crushing enables you to thin samples such as large particles that cannot be sliced. This can be done in various ways:

- If the sample is small, crush it with the roller end of the roller knife.
- If the sample is larger, use a pestle and mortar.

Separating by aperturing

Powders and other particulate solids may contain several different components. Instead of separating them, use the infrared aperture to isolate the component you want to sample:

1. Spread the sample out with a probe so that you can visually distinguish the components.
2. Looking at the Monitor Visible window, find a particle of the component you want to sample.
3. Center this particle in the field of view.

4. Adjust the infrared aperture until only the particle that is of interest is visible.

You can easily pick up extremely small particles and transfer them with a very fine-pointed tungsten needle. Scoring the surface of the salt plate with the needle makes a simple map to help you positively identify the particles under the imager.

Transferring with a tungsten needle

When necessary, sharpen the tungsten needle.

Nujol or fluorolube mulls

Suspending fine particles of a solid sample in nujol or fluorolube reduces or eliminates the surface reflections that can distort absorption measurements. These oils also reduce the amount of radiation lost to reflection or scattering.

If the film is thin enough, you can correct the spectrum for the presence of the oil by subtracting a spectrum of the pure liquid. It is difficult, however, to obtain the correct thickness for a good subtraction.

Fibers

You can roll fibers to flatten them (see "Flattening Solids" on page 66), or they can be pressed in a diamond anvil cell (see "Polymers" on page 66). The following is another approach.

Fibrous Solids

If a fibrous sample, such as paper, is too thick, tear it and examine the torn edges. The edges contain single fibers and thin clumps of fibers.

Coatings on Substrates

If the sample is coated on a substrate, the method for collecting its spectrum depends on the nature of the substrate:

- If the substrate is reflective, you can analyze the sample in reflectance.
- If the substrate is opaque, scrape off a sample of the coating; use the roller knife to scrape a small piece on to a KBr or BaF_2 disk.

Liquids

Solutions of samples

Although liquids are seldom analyzed with the imaging system, sometimes the sample of interest is in solution.

1. Transfer the solution on to a salt plate.
2. Allow the solvent to evaporate, leaving the sample on the plate.

Micropipettes

You can use a micropipette to apply liquid to the surface of a salt plate, or to the edge of the junction between two salt plates. In the latter case, the liquid flows between the plates by capillary action.

Preventing liquids from spreading

If the amount of liquid being transferred to the salt plate is very small, restrict it to a small area of the plate. There are several ways to do this:

- Use a microbrush to transfer solutions. The bristles of the microbrush hold the liquid in a small region of the salt plate until the solvent evaporates.
- Repeatedly jab a small area of the salt plate with a tungsten probe. Leave the resulting small salt particles in the well that is produced.

  The capillary spaces between the salt particles retain the liquid and minimize spreading.
- Place the salt plate on a small metal washer that is being gently heated.

Because there is more heat at the outside of the salt plate than near the center (over the hole in the washer), the droplet of liquid is forced toward the center.