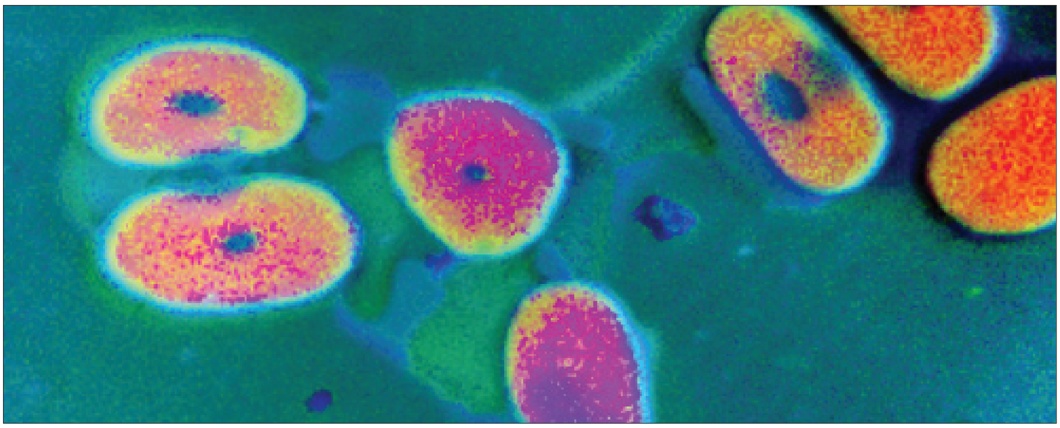
Large Area ATR FT-IR Imaging Using the Spotlight FT-IR Imaging System



Processed ATR image of human hairs encapsulated in resin. Image size is 500 microns wide by 200 microns tall with 1.56 micron pixel spacing.

Introduction

ATR FT-IR imaging offers users easy spectroscopic access to "difficult" samples which are hard to image in transmission or reflection, or which have confusing depth structure. It is a contact technique in which the sample is pressed against an ATR crystal which is illuminated by an infrared beam from a spectrometer. The crystal in the PerkinElmer ATR FT-IR Imaging Accessory is made of germanium with a refractive index of 4.01 which gives excellent spatial resolution and shallow sample penetration, both of which contribute to clear and sharp images.

The ATR FT-IR Imaging Accessory provides a much improved sample area, at least 600 microns in diameter, compared to other ATR systems. This allows users to cope easily with large samples (such as multi-layer paint samples a few hundred microns thick) in a single operation without having to resort to "stitching together" or "mosaicing" several small images. Because ATR imaging involves contact with the sample which may lead to indentation, the acquisition of several adjacent images can be difficult; not having to reposition the sample is therefore a distinct advantage.

The ATR FT-IR Imaging Accessory uses a crystal with an optimized design (patent pending) which seeks to maximize both resolution and uniformity of illumination over the extended sample area. This optimization is described along with the advanced processing techniques offered by the Spotlight™ Imaging Software that provide the ultimate in data quality.

Optimized crystal design

Conventional transmission or reflection microscopic imaging systems operate over relatively small areas of sample at any one time. Sample illumination in this situation is relatively easy to arrange. As sample area is increased however, efficient and uniform illumination becomes progressively more difficult, and must be designed in from the start in order to achieve high-quality results.

The ATR FT-IR Imaging Accessory provides both high spatial resolution and high-quality sample illumination by virtue of a novel (patent pending) design optimization technique. The crystal shape and size are selected with reference to the properties of the Spotlight FT-IR Imaging System optics system to optimize the total signal strength over the sample area and its spatial uniformity.

Figure 1 shows how the optimization is performed: the crystal has several design parameters, the most significant of which is the radius of curvature of the input and output faces (which appears along the horizontal axis). As the radius is increased, the peak signal within the field of view drops slightly, but the illumination becomes more and more uniform. The total amount of signal collected over the whole sample area shows a broad peak suggesting an optimum value for the crystal radius. A larger radius would produce a more uniform sample illumination but at a lower intensity level, while a smaller radius would produce a more peaky illumination which is brighter in the center but dimmer at the edges.

The PerkinElmer ATR Crystal is designed to have the radius that gives best total signal throughput. Having selected the radius, the other optical parameters are optimized to ensure best possible imaging performance.

Having produced a final design, the input and output faces of the ATR crystal are treated with a broad-band, multi-layer, anti-reflection coating to counteract the naturally high reflectivity of germanium (around 36% per surface). This ensures that the optical throughput of the ATR crystal is excellent when no sample is present – equivalent to more than 80% of that from a standard gold mirror as shown in Figure 2. The ATR accessory does not significantly degrade the Spotlight's excellent signal-to-noise ratios. The spectral shape is dominated (especially at the extremes of the range) by the characteristics of the broad-band anti-reflection coating.

Although the ATR crystal has been optimized for large-area working, the Spotlight Imaging Software allows users to collect smaller, square or rectangular images from 500 microns down to 25 microns on a side. Smaller images give even better uniformity of illumination, and there is no need to re-position the sample between image acquisitions. This is an especially important feature since it is common for ATR crystals to indent or damage softer, more delicate samples, making the stitching together of small adjacent images difficult.

Precision mechanics

The ATR crystal is precision laser-aligned and supported by the mechanical fixtures. The crystal is provided with an index mark at its pole which is used as a visible position reference; this ensures that crystal alignment is maintained across imaging operations.

A variable sample pressure mechanism is provided that has been designed to ensure good sample contact on a wide variety of sample types. Both features ensure that the image quality is maintained across the whole sample area.

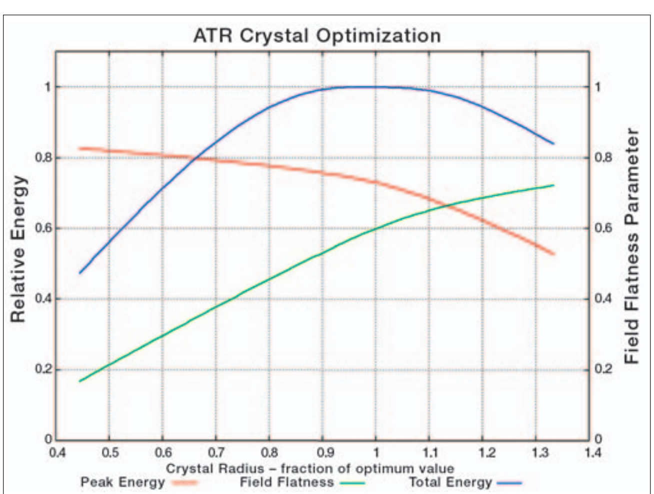


Figure 1. ATR imaging performance in terms of peak signal, field flatness and total signal all as a function of ATR crystal relative radius. The ATR Imaging Crystal is designed to have optimum radius.

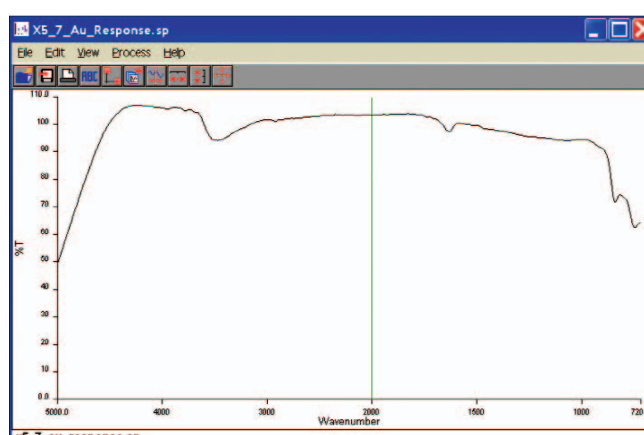


Figure 2. Typical spectral response of the ATR crystal compared to a standard gold mirror.

Optimizing spectral quality

The Spotlight Imaging Software has been enhanced to make the best possible use of the ATR imaging accessory. Three methods are provided for processing spectral data according to the needs of the user: ATR backgrounds, baseline offset correction and crystal images.

ATR backgrounds

In common with conventional transmission and reflection imaging modes, the system is able to record ATR background spectra that characterize the spectral throughput and sensitivity of the entire system from source, via the ATR crystal, through to the detector. A separate ATR background is required for each combination of spectral resolution and magnification that will be required for sample images. These ATR backgrounds are used as references against which to ratio all the spectra obtained from a sample. This enables the sample characteristics to be viewed directly as relative absorbance or transmission.

ATR backgrounds are recorded from a region near the center of the crystal, and offer a rapid means of processing spectra that is suitable for the majority of tasks. There is an implicit assumption that the center of the crystal is representative of the whole sample area; this might not be the case if, for example, the crystal became contaminated with sample residues.

When a large sample is scanned, the illumination varies slightly across the image, and there may be small variations in the spectral throughput of the system caused by the anti-reflection coating on the crystal and variations in beam angles at the sample surface. Typical systems may show illumination at the edges of the sample area that is about 40% to 60% of the central value.

The use of an ATR background is rapid, automatic and convenient: it is ideal when baseline variations across an image are not important. This may be the case if the user routinely caries out their own advanced baseline compensation and spectral processing such as Principal Components Analysis. ATR backgrounds are applied routinely and transparently by the Spotlight Imaging Software to all images.

Baseline offset correction

Baseline offset correction is a simple and efficient technique for compensating illumination variations: it is based upon the assumption that there is a region in the spectral range where the user's sample has no absorption. This region can be pre-selected by editing the appropriate entry in the configuration file – the default region is 2000 cm^{-1} to 2100 cm^{-1}. The method operates by offsetting the measured absorption in each spectrum so that the selected spectral region shows no absorption. This method is very effective at correcting variations in illumination across the whole sample area, but does not compensate the small spectral throughput variations that might occur. The method is very rapid. For maximum flexibility, the Spotlight Imaging Software always saves the "raw" image data as well as the baseline-offset-corrected image.

Baseline offset correction is a good default process, and results in images which are easy to interpret and analyze using simple or advanced processing tools, including the built-in "Show Structure" command that is provided by the Spotlight Imaging Software.

Crystal image

For ultimate spectral quality, where accuracy of shape is critical, the Spotlight Imaging Software offers the crystal image facility. This is useful for example where spectra will be compared directly against library data with little intermediate processing.

A crystal image is a full spectral map or image recorded from a (clean) crystal with no sample present under the same conditions of spectral and spatial resolution that will be used for a sample image. Users may require several crystal images if they routinely use different imaging conditions. The crystal image records the effective spectral throughput of the entire system at each point across the crystal surface when there is no sample present, including all the effects noted above. These data may then be used to process a sample image so that the spectra that it contains are all referenced to a truly "flat" background right across the field of view. The processing may be applied "live" as the image is scanned, or at a later stage. The sample image is simply divided by the crystal image, spectra are ratioed point-by-point, and the result is a processed sample image where the baseline is truly flat across the whole area. The ATR crystal should be aligned identically for both the crystal image and the sample image if the correction is to be fully effective.

Users should record crystal images over the largest area that might be occupied by their samples. The Spotlight Software will automatically adapt if the subsequent sample images are smaller by selecting the appropriate region of the crystal image – there is no loss of flexibility in selecting image sizes. For highest final data quality, it is preferable to record the crystal image with a similar or larger number of accumulations than might be used for a sample image. As for the baseline offset correction, the Spotlight Software always saves the raw image as well as the processed image.

A single crystal image may be applied to multiple sample images, but it is wise to rerecord the crystal image periodically to compensate for any changes in the system, for example after a service visit, or the installation of a new crystal. A crystal image is also a good way for the user to check on the state of his/her ATR crystal. The presence of contamination from samples can be determined by inspecting and processing the crystal image, for example by using the Spotlight Imaging Software's "show structure" command to extract the most significant spectral features. This approach can be used to check the efficacy of cleaning and to check for the presence of scratches. Users who desire ultimate image quality may opt to record a crystal image more frequently, for example just after a crystal has been cleaned and just prior to taking a sample image.

Figure 3 shows a typical crystal image in a false-color representation selected to emphasize the small variations in illumination across the field. This image shows an area 400 microns by 400 microns (the diagonal is about 566 microns across), and has been divided by an ATR background to give results that can be interpreted as absorbance.

Spectra were extracted from points placed in a three by three grid across this image to illustrate the variations that are encountered across the field (Figure 4 shows the results).

The highest and flattest spectrum has been recorded in the center of the crystal, whereas the others come from the middles of the edges and from the corners of the image. The variation of intensity (as measured at 2000 wavenumbers) amounts to a factor of 2.25 across the image: this is a typical value, although some systems show a smaller variation. The spectra are all relatively flat. This indicates that simple baseline offset correction would do a good job of "flattening out" the variations in illumination.

A few small and relatively broad variations from flatness are evident at the extremes of the spectral range;

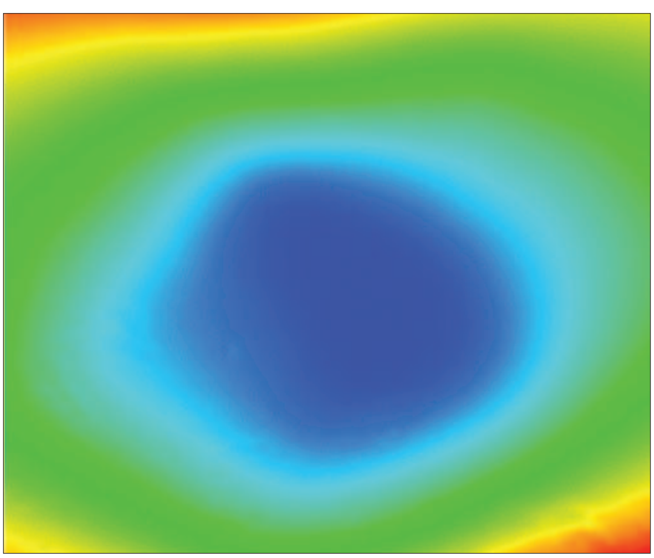


Figure 3. A typical crystal image recorded over a 400 micron by 400 micron patch of the sample surface. The colors represent a total absorbance range of 0.35 from low (deep blue) to high (red).

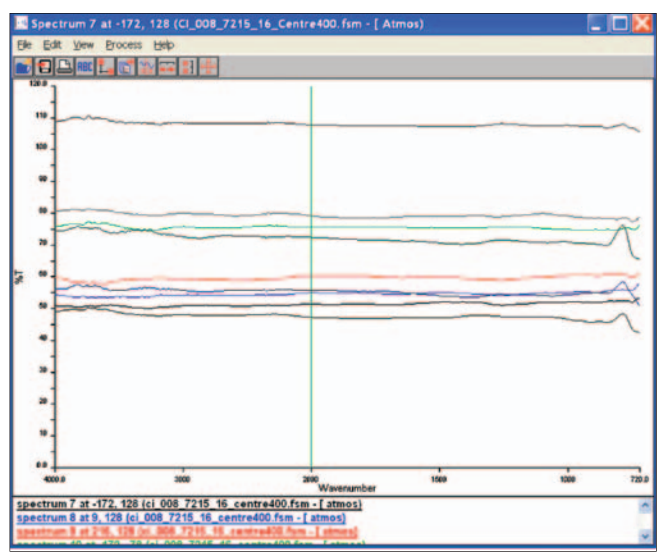


Figure 4. Spectra obtained from the image in Figure 1 at 9 points arranged to cover center, the corners and the middle of each side.

these are caused primarily by the anti-reflection coating and are most noticeable at the long-wave end of the range. It is worth remembering that these variations may not be so obvious in a sample image since all ATR spectra tend to have exaggerated long-wave absorption strengths due to deeper sample penetration. When a crystal image such as that in Figure 3 is used to process sample images, both the intensity variations and the small spectral variations will be completely removed. This processing improves the quality of the spectra; applications involving fine discrimination between spectra or requiring highly accurate matches to spectral libraries will benefit as a result.

Example image

A sample of paint taken from a car was embedded in resin between two plastic (PMMA) blocks and polished in such a way as to reveal the layered structure produced by the different base and top coats. The paint layer is about 200 microns wide, and the large sample capability of the Spotlight ATR Imaging Accessory allows the entire thickness to be imaged in one operation, together with the supporting plastic and resin on either side (this permits the user to check easily for the effects of contamination by the encapsulation materials).

An ATR image of the paint sample appears in Figure 5 where the colors show total absorbance. The layers in the paint sample run top-to-bottom; there is an air gap to the left (deep blue) and a plastic block at the extreme right. The image shows a reducing signal from the center to the corners whose form is similar to that of the crystal image of Figure 3. The total absorbance range is about 0.275 A. The spectra are drawn from three points at the extreme right of the image where the material is nominally uniform: there are obvious differences in offset and some more subtle changes in shape.

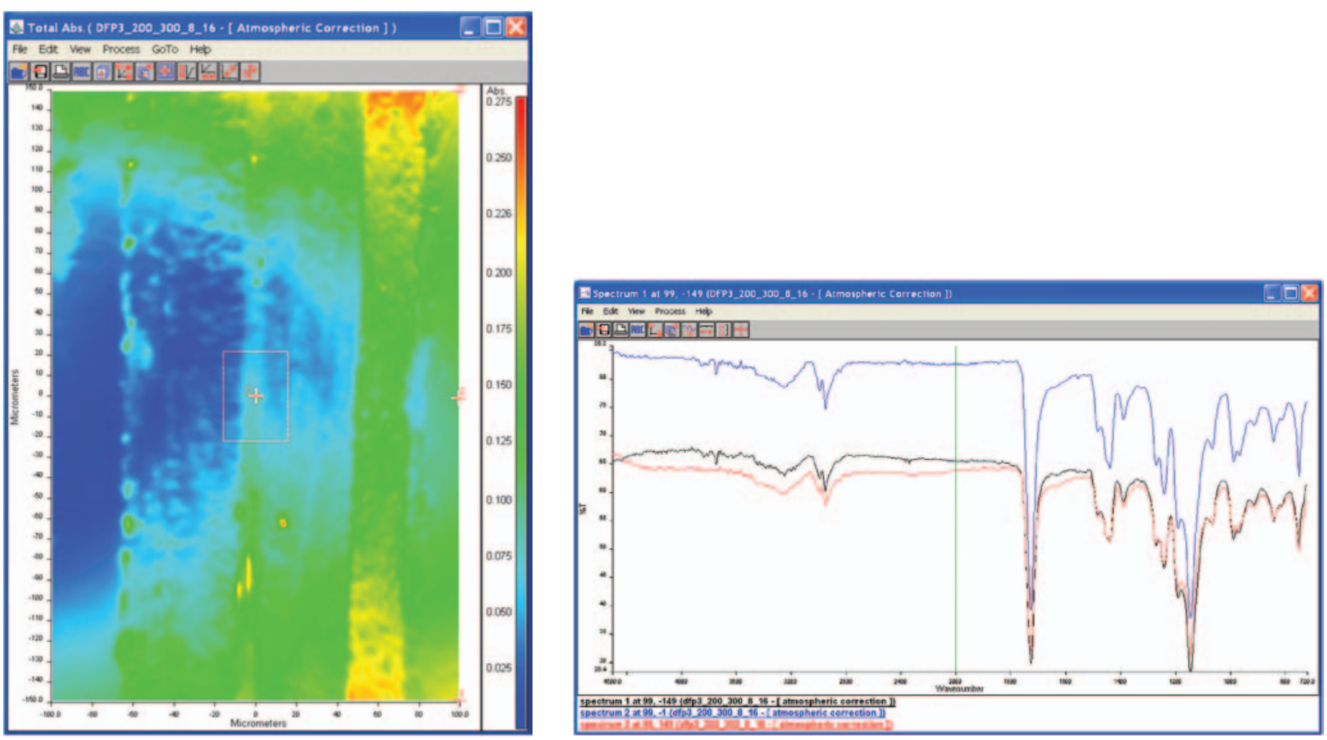


Figure 5. ATR image of a multi-layer paint sample shown as total absorbance. The image was recorded using 8 cm^{-1} spectral resolution and 1.56 µm pixel size over an area 200 µm wide by 300 µm tall. The spectra are taken from three points on the right-hand side where the material is nominally uniform.

The image was processed using baseline offset correction to compensate for the illumination variations, and the result appears in Figure 6. The spectral region from 2000 cm^{-1} to 2100 cm^{-1} was used for the calculations. The gross changes in absorbance have been removed. The overall range of absorbance has reduced from 0.274 to about 0.03, and there is more discrimination within the image. The three sample spectra have been adjusted to have the same 100% transmittance at 2000 cm^{-1}, but some slight shape variations remain.

Figure 7 shows the effect of using a crystal image that was recorded immediately prior to the sample image. The crystal image was in fact 300 µm by 300 µm, and only the central 200 µm wide area was used to perform the processing. Again, the gross illumination effects are removed, but in this case subtle spectral variations are also compensated, and the image appears cleaner. The three spectra are now better matched.

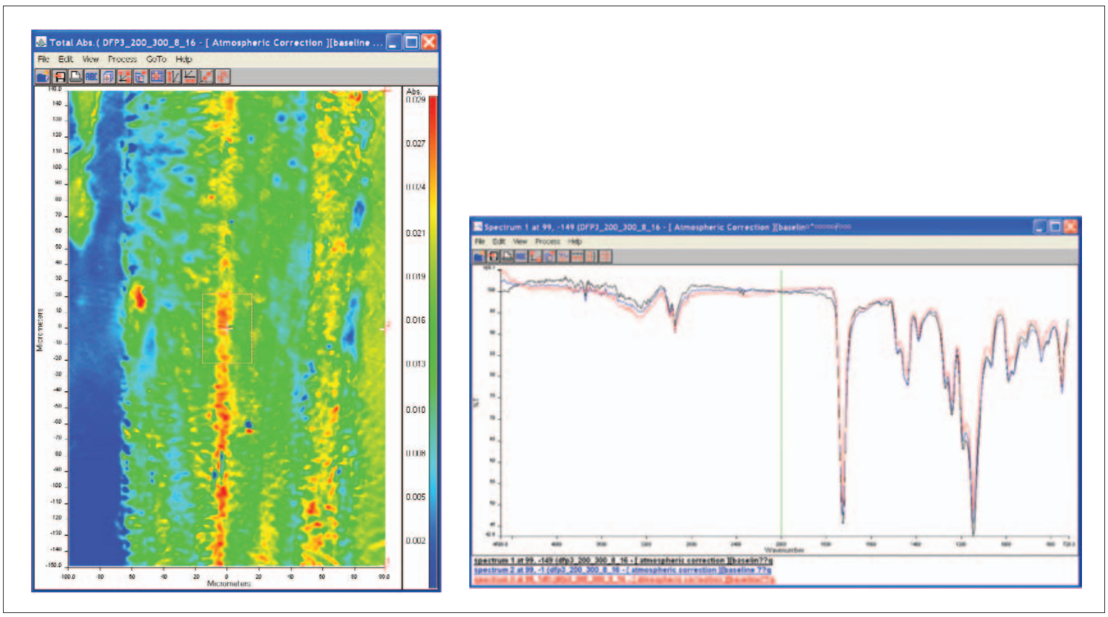


Figure 6. Paint sample ATR image with baseline offset correction and spectra taken from three points on the right-hand edge. Note that all spectra cross between 2000 cm^{-1} and 2100 cm^{-1}.

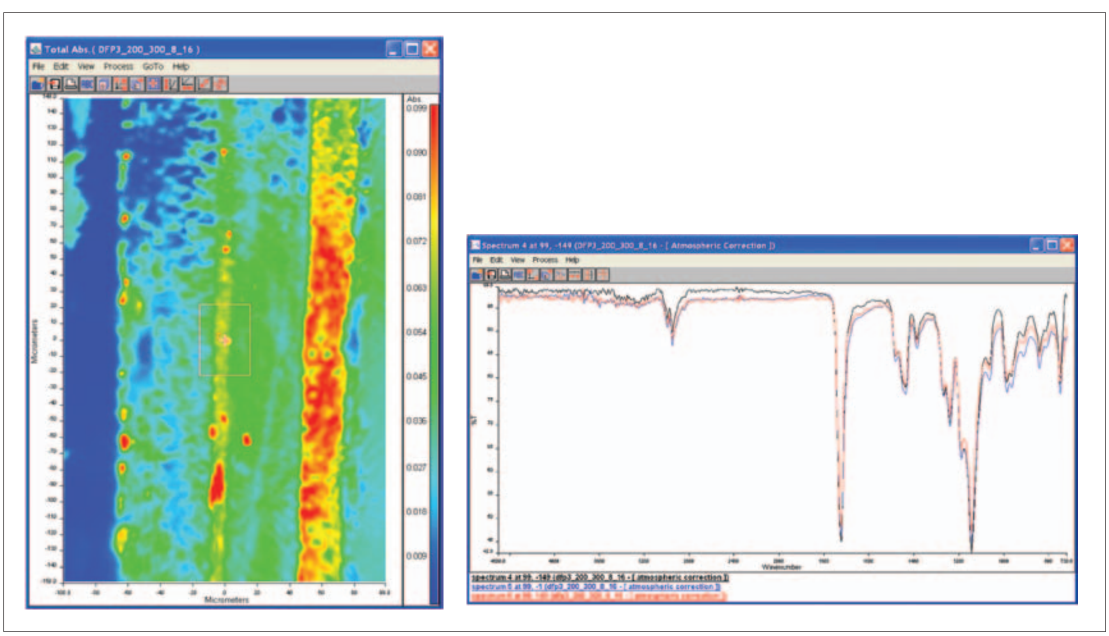


Figure 7. Paint sample ATR image with crystal image compensation.

Figure 8 shows two examples of advanced processing applied to the uncorrected paint chip image in Figure 5 in order to reveal the chemical structure. The raw data have been subjected to principal components analysis to extract the most significant spectral features, and selected components have been combined into color composite pictures. The Spotlight Imaging Software provides this functionality in a basic, automated form via the new "Show Structure" command. For more advanced processing options, the Hyperview package is also available.

Conclusions

- The PerkinElmer ATR FT-IR Imaging Accessory provides an optimized, highly-efficient germanium ATR crystal.
- The sample area is 600 microns in diameter: users can image large samples in one operation without using "stitching" or "mosaicing."
- The Spotlight Imaging Software provides graded levels of processing which can be selected to match the task at hand, including ATR backgrounds, baseline offset correction and, for the most faithful spectra, crystal images.
- A new "Show Structure" command performs automated principal components analysis followed by color compositing to bring out the most significant spectral features in any image and reduce the effects of noise – all at the touch of a button.
- More advanced spectral processing can be performed using the Hyperview package.

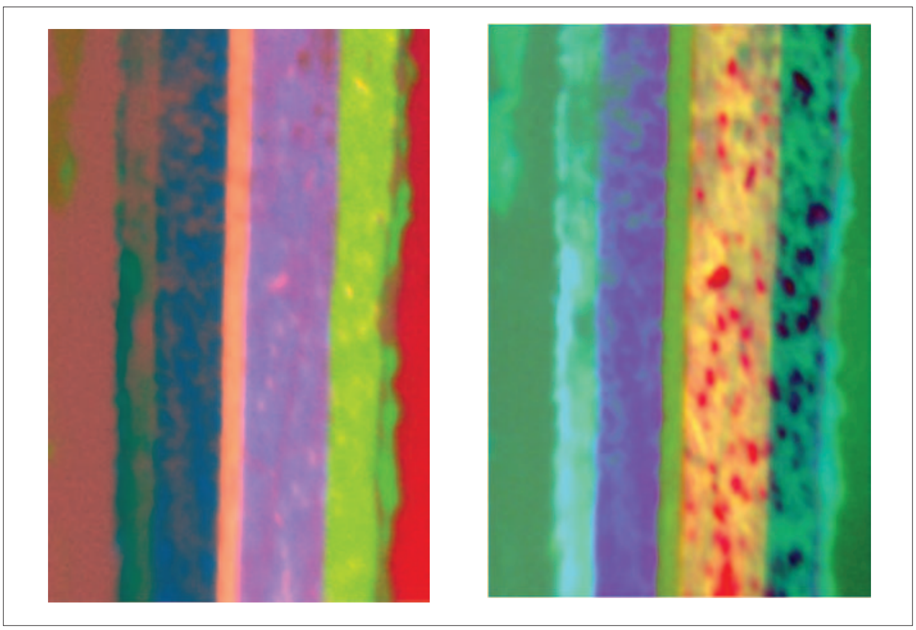


Figure 8. Paint sample ATR image of Figure 5 processed using the new automatic "Show Structure" command. Each image is 200 µm wide and 300 µm high, with 1.56 µm pixel spacing. The layer structure of the paint is clearly visible, including the presence of filler materials within some layers, and the noise has been reduced.

PerkinElmer Life and Analytical Sciences
710 Bridgeport Avenue
Shelton, CT 06484-4794 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/lasoffices

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