

Spatial Resolution in ATR FT-IR Imaging: Measurement and Interpretation



Introduction

ATR FT-IR imaging with germanium crystals offers the potential for acquiring infrared spectral images at up to four times higher spatial resolution than is possible using conventional reflectance or transmission imaging. There is often some confusion over the meaning and inter-relationship of terms such as “spatial resolution,” “pixel size” or “sampling interval,” and it may be hard to understand how any given system’s specifications will affect the imaging of a particular sample. Using the PerkinElmer ATR

FT-IR Imaging Accessory with the Spotlight™ FT-IR Imaging System as an example, this note explains what is meant by spatial resolution and sets out a generally applicable method for its measurement on any imaging system. Guidance is given regarding practical questions such as “can this instrument see a feature in my sample which is only X microns wide?”

Key points which are discussed include:

- The ATR Imaging Accessory offers a resolution of 3.1 microns, expressed as the FWHM of the combined spread function. The resolution is diffraction limited over most of the spectral range.
- The ATR Imaging Accessory provides near-optimum oversampling by utilizing a pixel spacing of 1.56 microns, which is half the resolution.
- Spatial resolution is determined by a combination of diffraction and detector element size

ATR Imaging Accessory

Figure 1 shows the arrangement of the ATR Imaging Accessory, comprising a Cassegrain-style reflective objective and a high-refractive-index germanium ATR “crystal”. The sample is pushed into direct contact with the tip of the crystal which is 600 microns in diameter. Infrared radiation from the Spotlight FT-IR Imaging System enters the crystal via the anti-reflection-coated curved surface and undergoes total internal reflection at the sample surface before exiting the crystal via the curved surface. There is some energy in the space immediately below the crystal tip which can be absorbed by a sample. If this occurs, the reflected beam is slightly attenuated according to the absorption, and carries spectral information about the nature of the sample.

The light reflected from the sample plane is directed by the ATR crystal and the Cassegrain Objective to form an image (somewhere above the top of the system shown in Figure 1). A 16-element linear array detector is placed at the final image plane of the system: both the crystal and

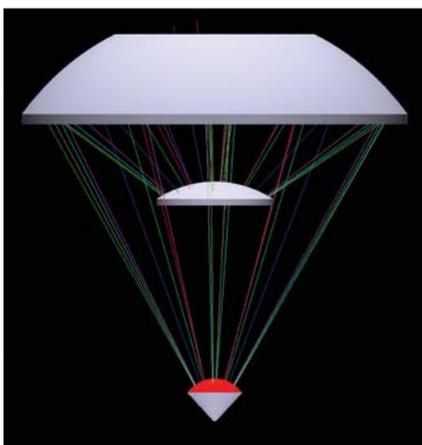


Figure 1. Layout of the ATR crystal below the Spotlight FT-IR Imaging System’s reflective Cassegrain Objective. The sample is pressed against the 600-micron diameter crystal tip at the bottom using a special mechanism. The curved crystal face (colored red) is anti-reflection coated for maximum efficiency.

the attached sample are scanned laterally under the Cassegrain Objective, causing the image to move across the detector until all points on the sample have been “seen” by at least one detector element. This is achieved using a stepper-motor-driven X, Y stage, and a full IR spectrum is recorded for each position of the crystal and sample until a “full spectral map” has been acquired. It is possible to acquire and accumulate several spectra at each position to improve signal quality.

Factors affecting resolution

Diffraction

This is a fundamental limit to the resolution of any instrument: it is simply impossible to confine light into regions smaller than about half the wavelength – the harder one tries, the more the light tries to escape and spread out. Diffraction effects depend upon the convergence angle of the beam (sometimes referred to as the Numerical Aperture of the system), its uniformity or shape and on the wavelength. Longer wavelengths suffer more diffraction in absolute terms than shorter wavelengths, and higher convergence angles give better resolution.

The ATR Imaging Accessory utilizes a high-refractive index material (germanium) to “immerse” the sample: this has the effect of shortening all the wavelengths that reach the sample by a considerable factor – 4.01 in this case. This is highly advantageous since it reduces the effects of diffraction by the same factor. For

example, a 6 micron wavelength in air becomes only 1.5 microns at the sample, and the potential spatial resolution improves in proportion.

The net effect of diffraction is to blur the image slightly as it falls on the detector. The light at any single point in the diffraction limited image is composed of an average over a small region of the sample, the size of the region being set by the diffraction properties of the system and the wavelength.

Detector element size

At any particular instant, each detector element “sees” light from a finite area of the image according to its size. The corresponding area of the sample depends on the optical magnification of the system. It is difficult to distinguish features in the image which are smaller than the detector size. Fortunately, the high refractive index of the ATR crystal increases the usual magnification approximately fourfold, allowing each detector element to look effectively at a smaller portion of the sample and thus to resolve finer details.

Finer resolution can be obtained by using smaller detector elements and a higher optical magnification, but the unfortunate and inescapable side effects are a loss of optical efficiency and therefore signal quality. This can only be compensated by a (much) longer image acquisition time. The system resolution is therefore influenced in practice by a compromise between practical detector element size and tolerable signal-to-noise ratio.

The Combined Spread Function (CSF)

In summary, each detector element can be thought of as summing or averaging the signals over a small area of the image formed by the optical system according to its size and shape. In addition, each point in this image may be thought of as the sum or average of the signals over a small area of the sample according to the diffraction properties of the system. Both effects act so as to blur or smear out fine detail. Rather than accounting for each effect separately (and including the optical magnification), they can be combined into a single, composite blurring effect by means of the Combined Spread Function (CSF).

Each detector element can be thought of as carrying out a weighted spatial average of the signal from different parts of the sample using the CSF as the weighting function. The CSF is a two-dimensional function of position in the sample plane.

A high resolution system has a very sharp or narrow CSF, indicating that the detector element only sees a small region of the sample, and can thus discriminate fine detail. A low-resolution system is characterized by a broad CSF so that the detector sees a large area of the sample and tends to “average out” fine spatial detail. In general the CSF may not be symmetric, and it will vary in size with both wavelength and position across the image.

The resolution of any imaging system is set by the width of its CSF, and this is the quantity which is measured in the sections which follow.

Scatter and Depth Effects

Conventional reflection or transmission imaging may be influenced by scatter within the depth of the sample even though the microscope may be focused on a single plane of the sample. This can be important if the sample is weakly absorbing but strongly scattering. Light from one region or feature may bounce around within a significant volume of the sample, visiting several different materials on the way, before emerging somewhere else and confusing the image. Scattering is really just another form of blurring.

ATR imaging has a significant advantage over conventional (reflection or transmission) imaging techniques in this regard. The physics of internal reflection is such that the depth of the energy field that extends just outside the ATR crystal is extremely limited, only a few microns at most. This is especially true when high-refractive index materials such as Germanium are employed (as in the Spotlight ATR Imaging accessory). This means that an ATR image responds to and shows only those parts of the sample that are essentially in direct contact with the sample face of the crystal. The system is not susceptible to long-range scatter and gives clearer, sharper images as a result.

Image Sampling

The fundamental limit to resolution is set by the CSF; this is distinct from the image sampling interval – the distance over which the sample and ATR crystal is moved between collecting spectra. If the sample is moved by a distance much greater than the size of the CSF between

spectral acquisitions, there will be areas of the sample which will never be “seen” by any detector element, and spatial detail may be lost (“skipped over”). At the other extreme it is possible, although potentially wasteful, to step the sample by an interval which is much smaller than the size of the CSF. This generates a lot more data but does not improve the resolution because each detector element still carries out a spatial average over the area defined by the CSF as described above. This practice is referred to as “over-sampling.”

In fact, the optimum image sampling interval is half the characteristic size of the CSF; this is the scheme used by the ATR Imaging Accessory. This scheme ensures that no detail is missed, and that there are effectively always two samples within each resolution element.

Measuring ATR resolution

A variation on the traditional edge response test has been developed specifically for ATR Imaging systems. The test requires a sample with a sharply defined edge: there must be a transition between contrasting materials that occurs over a distance scale which is short compared to the expected width of the CSF. The test involves acquiring an image of the sample and examining a cross-section taken across the edge – known as the “Edge Response”. A system with high resolution will produce a sharp image with a high slope at the edge, whereas a low-resolution system will produce a blurred edge image with a lower slope. Resolution is therefore related to the slope of the edge response function.

Slope can be measured by computing the first derivative of the edge response. It can be shown mathematically that the differentiated edge response is equivalent to a cross-section through the system's CSF in the direction orthogonal to the edge. The width of the differentiated edge response signal is therefore equivalent to the resolution of the system in that direction.

For conventional imaging systems, the edge may take the form of a blade or a chrome-on-glass mask. An ATR-compatible sample is not so easy to prepare as it must come into intimate contact with the germanium crystal and must present a mid-IR absorption. The ATR Image Accessory resolution is measured using a sample of micro-embossed plastic film whose upper surface takes the form of an array of triangular prisms as shown in Figure 2. The sample is mounted under the ATR crystal and sufficient pressure is applied to distort and flatten the tops of the prisms, leaving a set of parallel rectangular contact

regions with regular pitch as shown in Figure 3.

The plastic material exhibits some strong ATR absorptions which give contrast in the ATR image. The transitions between the deformed plastic regions and the intervening air gaps form the test edges. The edges are made sharper by the fact that the effective penetration of the energy into the sample and the air under the crystal is very shallow indeed (less than a micron).

A single scan is performed across a small area of the target and an image is extracted at a wavelength in the middle of the range where the plastic material absorbs strongly; Figure 4 shows four cross sections extracted from such an image which correspond to the separate elements of the Spotlight Imaging System's MCT Detector Array. The sections are all very similar, showing sharp-edged contact areas a little more than 10 microns wide and separated by 50 microns.

Figure 5 shows a section from a typical edge response (in red): the part at the left corresponds to an air gap where there is high reflection, and the absorption rises towards the right in the plastic contact region. Also plotted is the calculated first derivative signal (which is equivalent to a section through the CSF, in green). The derivative shows a clear peak at the point where the edge is steepest and falls away on either side. The signal appears a little noisy because of the differentiation process; conventional smoothing techniques cannot be applied as they would broaden the peak and corrupt the resolution measurement. Instead, the curve is fitted using a heuristic model – a pseudo Voigt profile – using nonlinear weighted least-squares methods. The final resolution figure may then be derived from the shape of the fitted curve.

In practice, the same measurement is carried out independently for each of the 16 detector elements in the

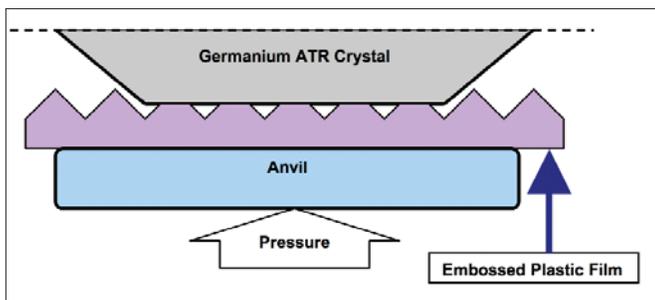


Figure 2. ATR resolution test target formed by pressing micro-embossed plastic film onto the ATR crystal's sample surface.

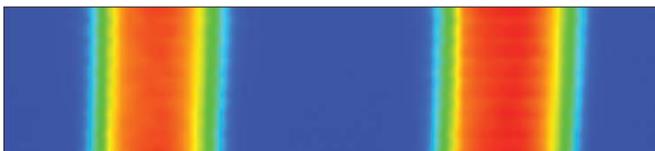


Figure 3. A single un-processed scan across the ATR test target covering a 100 micron width and showing absorption at 1726 cm^{-1} . The air gaps show as deep blue (zero absorption), whereas the plastic regions in contact with the crystal show as red (0.21 absorbance). There are scan lines from each of the 16 detector elements in this image.

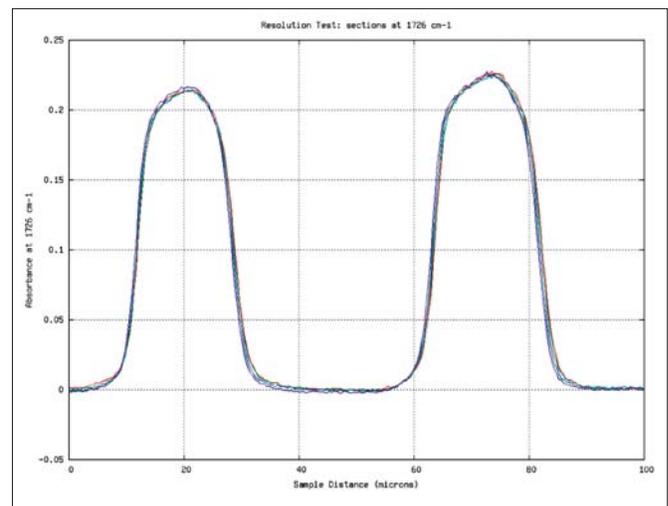


Figure 4. Edge responses recorded by four of the 16 elements in the Spotlight imaging detector array. Note that each element shows a very similar response with good signal-to-noise ratio.

array, and an average figure is calculated together with an estimate of the spread or uncertainty.

Quoting a figure

The CSF measured above does not closely resemble any simple textbook pattern usually calculated for a conventional microscope (an Airy disk): this is to be expected given the nature of the ATR optical system and the finite detector size, and it means that conventional measures of optical resolution such as the “Rayleigh” criterion are perhaps inappropriate. Instead, it is convenient to quote the full-width-at-half-maximum (FWHM) of the fitted profile.

The typical value of the FWHM measured at 1726 wave-numbers is 3.1 microns, with an uncertainty of about ± 0.15 microns.

It was noted above that the resolution is affected by both diffraction and detector size; at shorter wavelengths there is a small improvement in resolution, while at longer wavelengths the resolution falls because of diffraction, for example to about 4 microns FWHM at

1170 cm^{-1} . This indicates that the ATR Imaging Accessory is effectively diffraction limited over most of its spectral range.

Interpretation

There are two main effects of having a finite resolution when imaging fine or sharp features in a sample:

- Spectral mixing or loss of spectral contrast
- Inability to measure the true size of a small feature accurately

Figure 6 shows the effect of scanning at three successive sampling positions using Spotlight’s fine spatial sampling mode. The sample and crystal are moved by 1.56 microns per step, which is approximately half the resolution figure of 3.1 microns quoted above. At each position, the detector element forms a weighted average over an area of the sample, the weighting function being the CSF.

Imagine that the sample is made up of two different materials with a boundary between them at the origin on the X axis. When the detector is at position “A”, it “sees”

mostly the left-hand material, but because the “wing” of the CSF extends to the right past $X=0$, it will also pick up a small amount of the right-hand material. The spectrum will therefore be slightly mixed, by less than about 15%. A similar argument can be made for the situation at position “C”. When the detector is placed at position “B”, it detects an even mixture between the two materials.

Consider next what would happen if the sample were to be a very narrow layer centered at the origin ($X=0$) in Figure 6 and embedded between slabs of a different material (such as an adhesive between polymers). When the detector is placed centrally at “B” it will certainly “see” the narrow layer, but the spectrum will be polluted by mixing from the surroundings to either side. The extent of the mixing will depend upon the width of the narrow layer compared to the width of the CSF. Note also that the detector will still “see” the narrow layer when it is placed at positions “A” or “C”, but to a lesser extent because of increased mixing. In summary, the Spotlight Imaging System will certainly “see” the narrow layer,

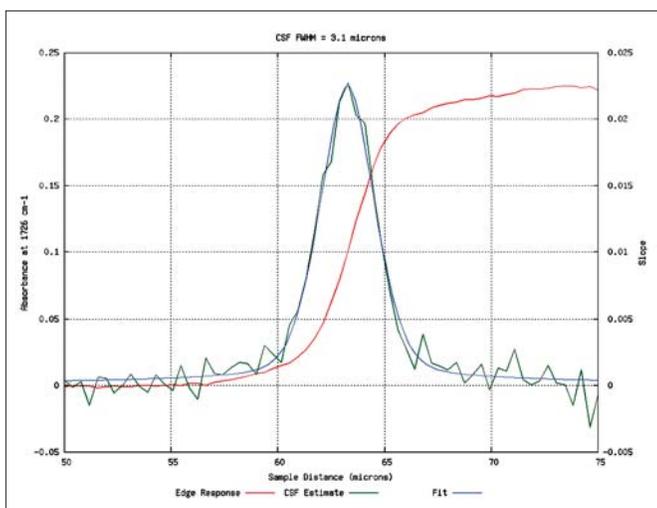


Figure 5. A typical edge response function and its associated derivative. The derivative is well-fitted by a pseudo-Boigt profile and has a FWHM of about 3.1 microns.

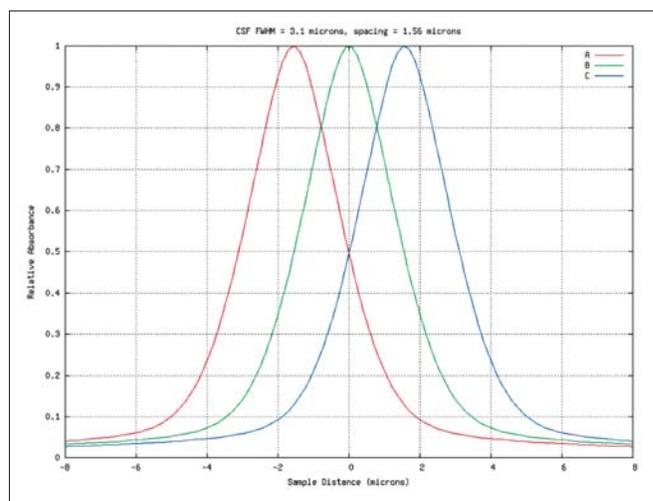


Figure 6. Three combined spread functions separated by the Spotlight fine sampling interval.

but its width will appear increased slightly – by about the width of the CSF – and its contrast will be reduced. It may be possible to recover useable spectral information if the signal-to-noise ratio is high enough.

Spectral mixing only becomes serious when the feature under investigation is of similar size to the CSF or in the region immediately surrounding a transition between two materials in the sample. If a region in the sample is wider than about 3 times the resolution of the system, then at least one pixel will contain a “pure” spectrum.

An Example

Figure 7 shows a processed high-resolution ATR image of a plastic laminate structure containing seven distinct layers made of four chemically distinct materials. The sampling interval was 1.56 microns, which is half the Spotlight FT-IR Imaging System’s resolution as

measured above. The spectral data in the image have been processed using Principal Components Analysis to reveal the spectral differences between the materials most clearly.

The two red bands correspond to distinct, identical layers in the laminate which appear to be 5 pixels wide in the raw image, equivalent to a physical width of just under 7.8 microns. “Pure” (unmixed) spectra can be obtained easily from within each layer and there is evidence of still finer spatial structure in the central (blue-green) layer.

The small white line which has been added to Figure 7 indicates a region where there is a spatially sharp transition from one material to another. Spectra were obtained from *every other pixel* along the white line to show the effects of mixing across the transition; this spacing is equivalent to the FWHM

of the Combined Spread Function (like curves A and C in Figure 6).

Figure 8 shows five spectra obtained along the white line in Figure 7, each one taken from a point spaced two pixels (3.1 microns) apart. The total length of the line is only 12.4 microns. The uppermost spectrum (with an absorption band at about 1470 cm^{-1}) comes from the left-hand end of the sampling line, whereas the lowest spectrum (with strong absorptions at 1510 cm^{-1} and 1230 cm^{-1}) comes from the right-hand end. These may be regarded as the two “pure” spectra. Moving in by one pixel from either end of the line (i.e. to within about 3 or 4 microns of the transition) does give a little mixing, but the effect is very small, even in the strong absorption bands. At the center of the line, which coincides with the transition between materials, the Spotlight measures a truly mixed spectrum (shown in red).

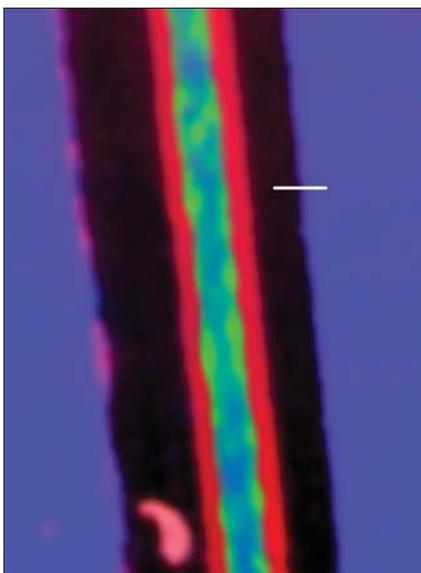


Figure 7. ATR image of a section of a plastic laminate. The image covers 150 microns width by 200 microns height, and has been sampled on a 1.56 micron pitch. The white bar indicates the position where spectra have been extracted. This image is a composite of three principal component images to show the spectral structure to best advantage.

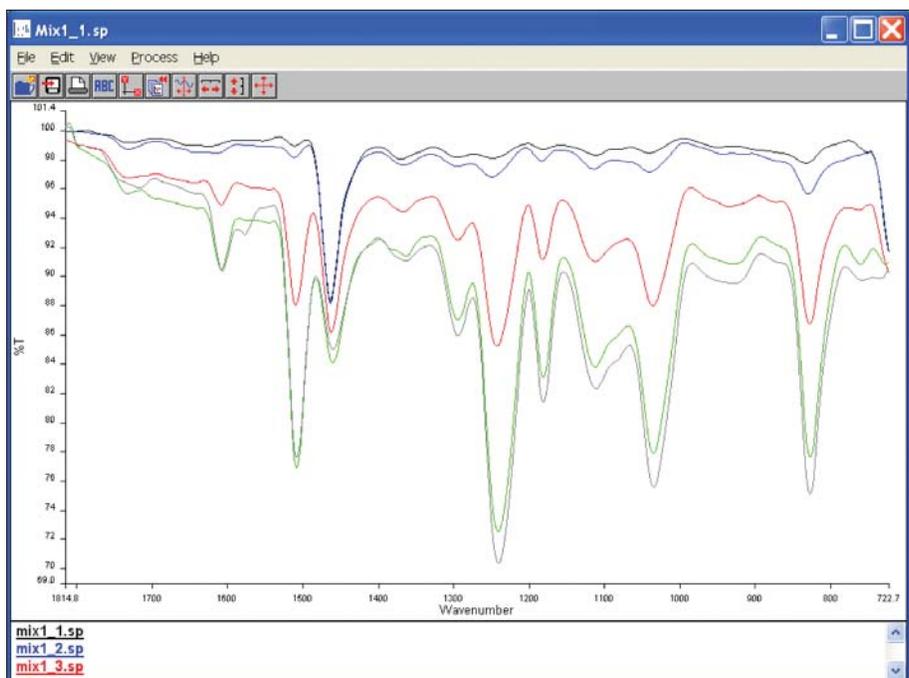


Figure 8. Five spectra obtained from every other pixel along the sampling line in Figure 7. The spatial interval is 3.12 microns.

Conclusions

- Spatial resolution is determined by a combination of diffraction and detector element size
- The Spotlight FT-IR ATR Imaging accessory offers a resolution of 3.1 microns, expressed as the FWHM of the Combined Spread Function. The resolution is diffraction limited over most of the spectral range.
- The Spotlight FT-IR Imaging system with the ATR Imaging Accessory provides near-optimum over-sampling by utilizing a pixel spacing of 1.56 microns, which is half the resolution.
- The practical effects of finite resolution – spectral mixing and a loss of contrast for structures whose scale is similar to the resolution – have been described and illustrated using a real example.
- A generally-applicable method has been presented for measuring the spatial resolution of ATR imaging systems using a micro-embossed polymer target.

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