Technical note

Spatial resolution

Considerations for FTIR microscopy applications

Introduction

Spatial resolution can be defined as “the resolving power to distinguish between closely spaced objects” but expressions for spatial resolution can vary depending on the field, context, and application. Even within FTIR microscopy itself, spatial resolution has been defined in different ways. The details and merits of the various approaches are beyond the scope of this note and will not be further mentioned here. From a practical standpoint the most important consideration concerning spatial resolution is not how to measure it but whether or not it is sufficient for the intended application.

There are some fundamentals that should be considered when thinking about FTIR microscopy and spatial resolution. The first is that the diffraction limit is usually considered a lower limit on what spatial resolution can be achieved.

$$\text{Diffraction limit} = \frac{0.63\ (\text{wavelength})}{(\text{index of refraction})\ (\text{numerical aperture of the objective})}$$

The actual calculation is less important in a practical sense than appreciating the factors that go into it. The consideration of the index of refraction is important when using ATR but not with measurements done in air. The diffraction limit as well as the spatial resolution are both wavelength dependent and vary across an infrared spectrum.

In FTIR microscopy, the beam size is typically large compared to the desired spatial resolution so an aperture or an effective aperture based on the size of pixels in an array detector is used to define the smallest area on the sample represented by a single spectrum. While a smaller aperture (or effective aperture) will provide better spatial resolution, as it gets smaller, it limits the amount of infrared light that reaches the detector. This means weaker signals and a loss of signal-to-noise which is one area that is sometimes neglected in considerations of spatial resolution. The end goal of the analysis depends on the spectral information generated and whether it is a single point measurement where the spectrum might be used for identification or quantification or whether it is for infrared imaging where the images depend on detecting and utilizing meaningful differences in the spectra collected across the sample. The quality of the spectral data really has to be considered as part of the spatial resolution discussion. Diffraction plays another role in spatial resolution. While the previously mentioned diffraction limit is in relation to the sample, diffraction also occurs with optical components in the beampath (apertures, objectives, etc.). This additional diffraction restricts how closely the spatial resolution can approach the limits.

Since there are numerous factors that contribute to the spatial resolution, it is more common to experimentally measure spatial resolution than to calculate it. This is not always as straightforward as it seems due to different methods of measuring spatial resolution and different definitions. Spectral purity is often used as a measure of the spatial resolution. The ideal situation is where the spectrum from the sample is obtained without any features from the surrounding material. That idealized definition is not typically fully achieved with small samples so the question becomes what is the acceptable level of spectral features from the surrounding material that is permissible in the sample spectrum? In infrared imaging this is the contrast of the image, and it is often adjustable. That is why it is important to understand how spatial resolution is being defined to access the correct results. How important this is for a particular application will depend on the goal of the application.

This note will show examples of how spatial resolution affects images collected from a U.S. Air Force (USAF) 1951 resolution target as well as examples representative of actual infrared samples.

Experimental details

The infrared data in this note was collected using a Thermo Scientific™ Nicolet™ RaptIR™ FTIR Microscope. The infrared data presented here was collected in transmission mode. The images from the USAF 1951 target and the cross-section of the polystyrene sphere were collected using a 5 µm x 5 µm aperture and a step size of 1 µm. A 1 µm step and oversampling provides for better image resolution and some additional detail in the images, but the spatial resolution of each spectrum is still ultimately limited by the 5 µm x 5 µm aperture. The data from the cross-section of the multi-layer polymer composite was collected using similar parameters but a 5 µm x 10 µm aperture was used with the longer edge of the aperture in the direction along the layers in the polymer. The Raman images in this note were collected with a Thermo Scientific™ DXR3xi Raman Imaging Microscope using a 532 nm laser and either a 100X or 50X objective.

Results

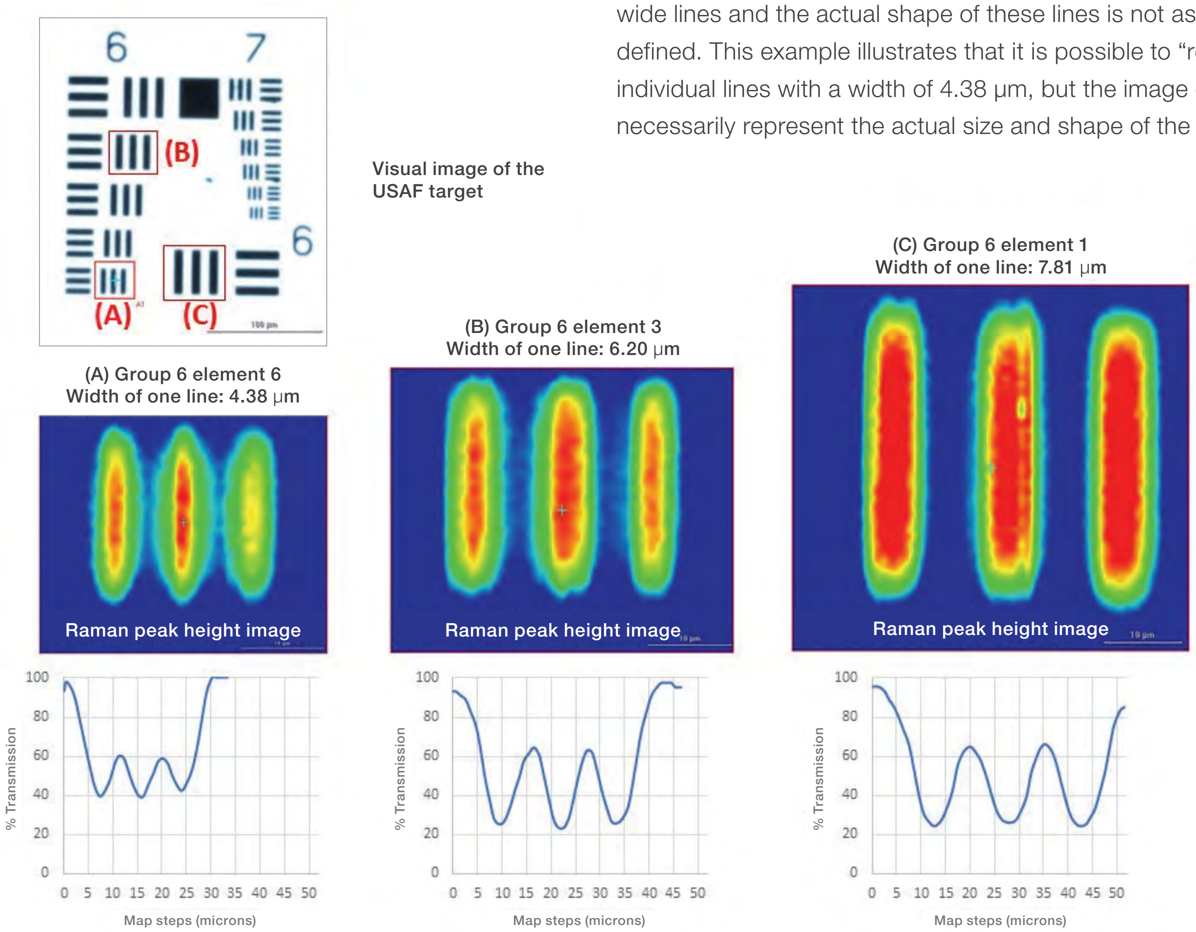
One of the most common tests is done using a USAF 1951 resolution target. It has a spiral arrangement of equally spaced lines of varying size. Each group consists of six elements and the resolution of the line pairs is given by,

$$\text{Resolution (lp/mm)} = 2^{\text{group}+(\text{element}-1)/6}$$

$$\text{Width of 1 line (µm)} = [\tfrac{1}{2}\,_{\text{(line pair resolution (lp/mm))}}] \times 1000$$

While this is a commonly used test sample, it is also important to remember that this target is not representative of most FTIR microscopy samples. The lines don't have a distinct infrared spectrum but just block transmission, so the variation ideally is between the extremes of 100% and 0% transmission. With the smaller lines these ideal limits are not fully achieved, and what defines a significant distinction between peaks and valleys is part of the discussion.

Figure 1 shows infrared images of elements (1, 3, and 6) of group 6 from a USAF target. The infrared images are based on the absorbance at 3990 cm^{-1}. Below each of the images is a plot of the percent transmission measured at points along a vertical line bisecting the middle of each of the series of lines. It can be clearly resolved that there are 3 lines even when the line width is below 5 microns, but the range of values defining the peaks and valleys is significantly smaller with the 4.38 µm wide lines and the actual shape of these lines is not as well defined. This example illustrates that it is possible to "resolve" individual lines with a width of 4.38 µm, but the image does not necessarily represent the actual size and shape of the lines.



(A) Group 6 element 6
Width of one line: 4.38 µm

(B) Group 6 element 3
Width of one line: 6.20 µm

(C) Group 6 element 1
Width of one line: 7.81 µm

Figure 1: Infrared images from (A) Element 6 (line width 4.38 µm), (B) Element 3 (line width 6.20 µm), and (C) Element 1 (line width 7.81 µm) of Group 6 from a USAF 1951 resolution target. The infrared images are based on the absorbance at 3990 cm^{-1}. 5 µm x 5 µm aperture and 1 µm steps.

Another way to assess spatial resolution is to do a knife edge test. In this experiment spectra are collected from an area of 100% transmission to 0% transmission, and the transition from full transmission to fully blocked is accessed to see the width of the transition (spatial resolution). The results of such an experiment can be seen in Figure 2. The percent transmission is plotted as a function of map position. The transition is sharp, and the width is highly dependent on the limits selected. The values at 10% and 90% transmission give a transition width of 10 µm, and with 20% and 80% transmission the width drops to 6 µm. How the test limits are defined affects the results.

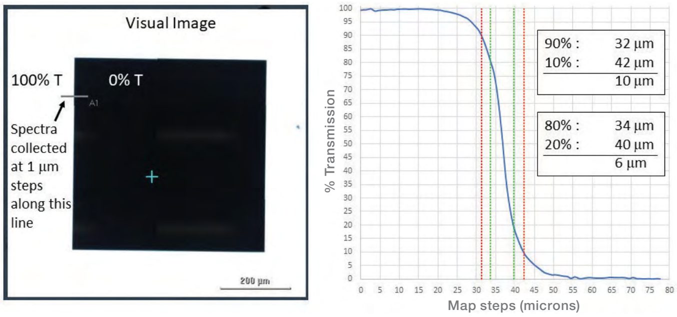


Figure 2: Knife edge measurement using the USAF 1951 resolution target. 5 µm x 5 µm aperture and 1 µm steps. Plot of the percent transmission versus maps steps in microns. Red lines are at 90% and 10% transmission and the green lines are at 20% and 80% transmission.

While these tests can give some idea of expected spatial resolution, they do not really exemplify real samples. To further illustrate spatial resolution, two additional samples were analyzed. In the first case small polystyrene spheres (approximately 10 µm in diameter) were embedded in epoxy resin and then cross-sectioned using a microtome. This represents what might be encountered as a defect or contaminant in a polymer film. The second example is a multi-layer polymer composite, and the analysis focuses on looking at a thin layer in that sample. These samples were also analyzed with a Raman microscope as a secondary check on the results.

Figure 3 shows the visible image and FTIR peak area image obtained from the cross-section of the polystyrene sphere. The FTIR area image is based on the peak area (3013–3034 cm^{-1}) of a polystyrene peak (see Figure 4). The infrared spectra of the epoxy resin and the polystyrene are very similar, and this illustrates why having good quality spectra is important for FTIR imaging. Having access to a wide spectral range and good quality data is important in finding significant differences in the spectra that can be used to generate meaningful infrared images. While an approximate diameter of the sphere cross-section can be measured directly from the image (10 µm), it was also instructive to plot the peak area values (reported as a percent of the maximum value) as a function of map position along a line bisecting the sphere cross-section (Figure 5). The full width at half height (FWHH) is approximately 10 µm. Raman imaging of the same sample at higher spatial resolution using a 100X objective was done as a secondary check, and Figure 6 shows there is excellent agreement with the infrared results.

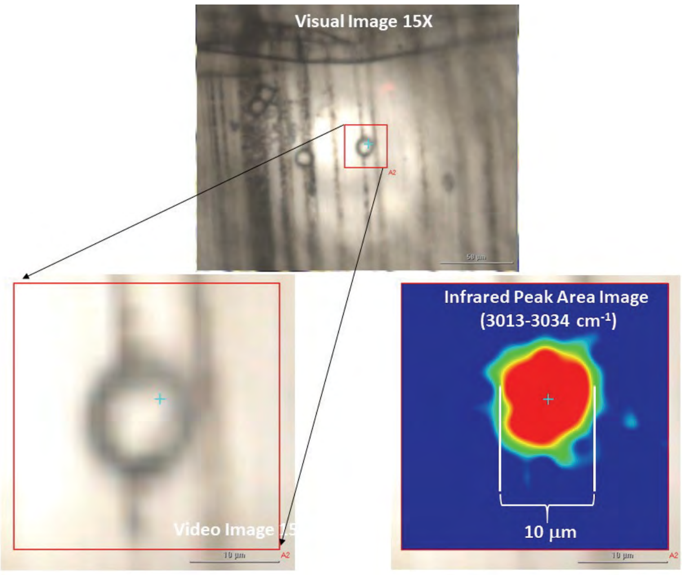


Figure 3: Visual and infrared images of a cross-section of an approximately 10 µm diameter polystyrene sphere embedded in epoxy collected with a 15X infrared objective. The infrared peak area image is based on the area of a polystyrene peak (3013–3034 cm^{-1}). 5 µm x 5 µm aperture and 1 µm steps.

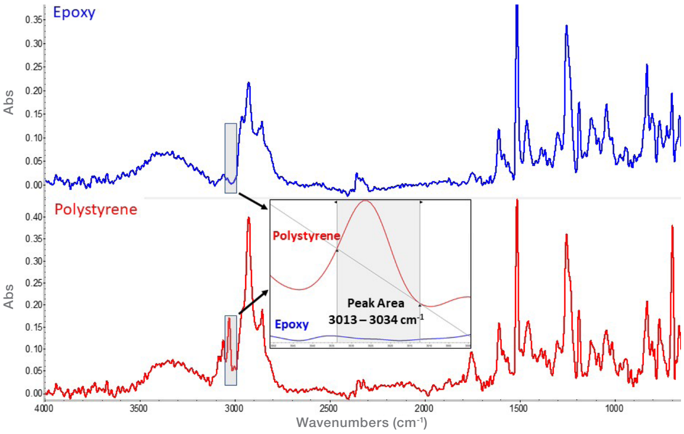


Figure 4: Representative infrared spectra from the polystyrene (red) and epoxy (blue) portions of the sample. The inset shows the peak area (3013 cm^{-1}–3034 cm^{-1}) used to generate the infrared image in Figure 3.

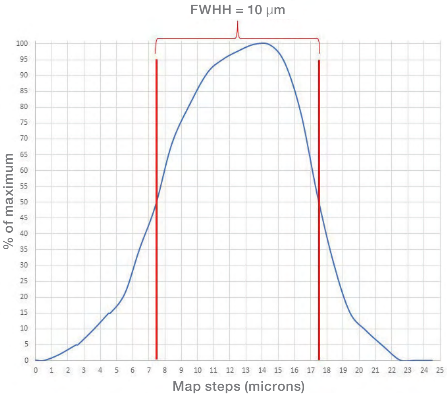


Figure 5: Plot of peak area expressed as the percent of the maximum value versus map step (microns) along a line bisecting the cross-section of the polystyrene sphere. Red lines at half height. FWHH = 10 µm.

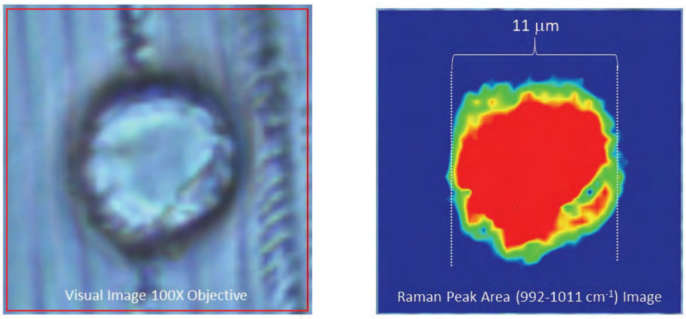


Figure 6: Cross-section of the polystyrene sphere embedded in epoxy. Visual image and Raman peak area image (polystyrene peak at 992–1011 cm^{-1}) collected using a 100x objective, 532 nm laser, 10 mW, 0.5 µm image pixel size, and 25 µm pinhole.

One of the more common applications for FTIR microscopy is the analysis of multi-layer polymer composites. Some of the layers can be quite thin so spatial resolution can be a consideration. To illustrate this, an example with a relatively thin (approximately 6 µm) polyurethane layer between nylon and polyethylene layers was analyzed. Figure 7 shows a visual image of the cross-section of this layered polymer composite as well as FTIR peak area images for the polyurethane layer and the two adjacent layers. The peak area (1302–1330 cm^{-1}) used to generate the polyurethane infrared image is shown in Figure 8. While an approximate thickness could be determined directly from the infrared image, an alternative approach is to use a plot of the peak area values (expressed as a percent of the maximum) versus map position for a line perpendicular to the orientation of the polymer layers (Figure 9). The FWHH provides a measure of the width of the polyurethane peak (8 µm). This is not too far off from the value of 6 µm obtained from the Raman image (Figure 10).

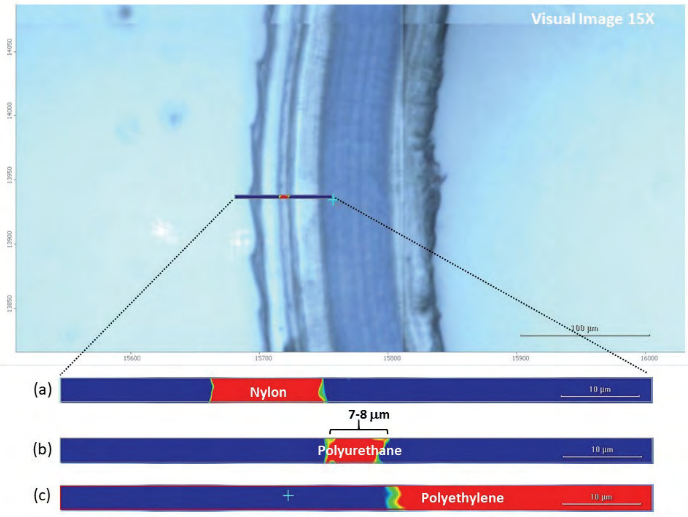


Figure 7: Visual Image of the cross-section of the multi-layered polymer composite. The area indicated was analyzed using a 5 µm x 10 µm aperture and 1 µm steps. Raman peak area images (a–c) show the location of the polyurethane layer (b) and the two adjacent layers (a) nylon, and (c) polyethylene.

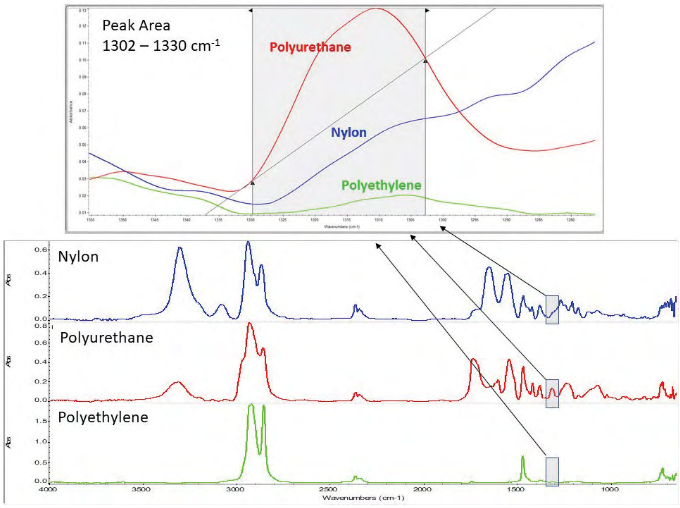


Figure 8: Representative infrared spectra from the three layers (red-polyurethane, green-polyethylene, and blue-nylon. The inset shows the peak area used for generating the infrared peak area image of the polyurethane peak shown in Figure 7.

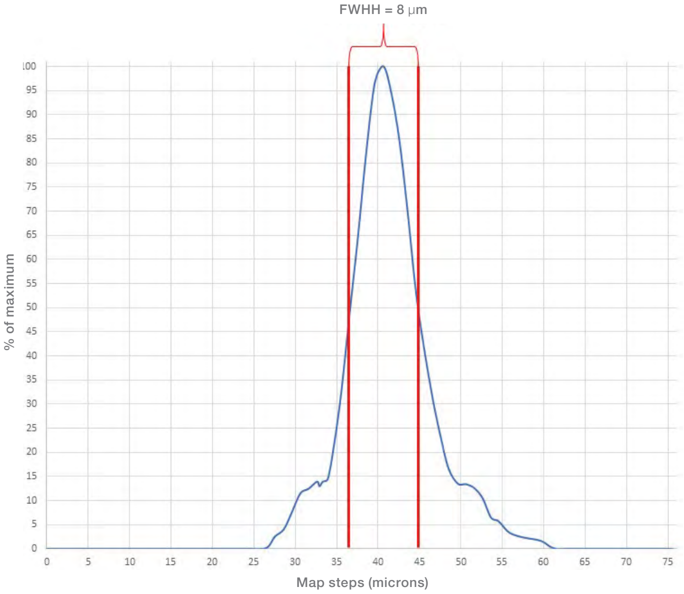


Figure 9: Plot of peak area expressed as the percent of the maximum value versus map step (microns) along a line perpendicular to the direction of the polymer layers. Red lines at half height. FWHH = 8 µm.

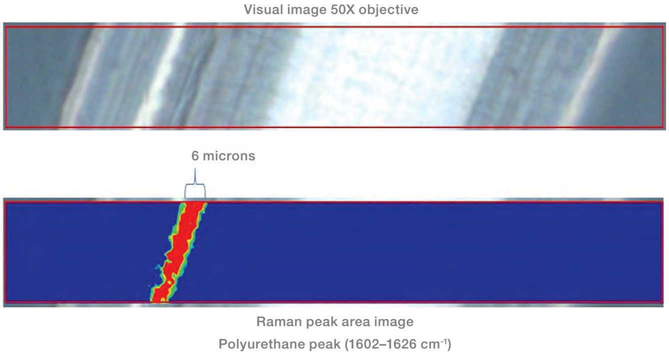


Figure 10: Cross-section of the multi-layer polymer composite. Visual image and Raman peak area image (polyurethane peak at 1602–1626 cm^{-1}) collected using a 50X objective, 532 nm laser, 10 mW, 1 µm image pixel size, and 25 µm pinhole.

Conclusion

Spatial resolution is one of the factors to consider when thinking about FTIR microscopy applications. Appreciating the basics of spatial resolution is useful because it is often defined in different ways and it can vary in importance to the success of a particular application. While some spatial resolution tests have their merits, they seldom represent actual FTIR samples so these should be considered alongside more representative samples as well. Often the success of an FTIR application depends on balancing all the factors to optimize the results. Greater spatial resolution at the expense of spectral quality may not be the best trade-off, particularly in FTIR imaging where the quality of the spectral data is possibly more important than the spatial resolution for generating high-quality and meaningful infrared images.

The results presented here show that the Nicolet RaptIR FTIR Microscope can spatially differentiate between objects spaced closer than 5 microns and provides a good balance between spatial resolution and spectral quality. This makes it an excellent choice for addressing a wide variety of FTIR microscopy applications.

Nicolet RaptIR FTIR Microscope

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