Part I of this article series (Spectroscopy 19[10], 22–28 [2004]) discussed the distortion of the laser focal volume, but what of the confocal aperture? What is the depth resolution of the detected Raman signal? Initial papers on these topics had an over-simplified treatment of the aperture, concluding that the aperture is remarkably inefficient at improving the depth resolution and that the observed Raman signal originates from a very broad region throughout the laser focus (1, 2). Subsequently, Baldwin and Batchelder (3) presented a more rigorous treatment of the aperture, which showed that while the Raman depth resolution remains degraded, it still is significantly better than the axial blurring of the focused laser beam alone would imply. This explains the monotonic decrease in Raman signal that is obtained in practice on focusing deeper into a transparent sample; the laser profile broadens dramatically but only Raman photons originating within a reduced fraction of the excitation volume can pass through the aperture. Baldwin and Batchelder suggested also that the axial resolution might be improved in some circumstances by choosing a lower numerical aperture (NA) objective. Their treatment still neglected the effects of diffraction, however, and only considered on-axis aberrations.

Refined Treatments of the Confocal Aperture and the Effects of Diffraction

A number of authors have produced treatments that accommodate, with varying degrees of rigor, the effects of diffraction (4–6). In general, these treatments all support the fundamental conclusions regarding the distortion of the apparent position and dimensions of buried structures, but generally they conclude that the depth resolution should not be badly degraded. Baia et al. (4, 5) modeled on-axis refraction and diffraction, including a simple treatment of the confocal aperture, and showed that this approach correctly modeled the observed intensity variation from a 20-µm coating on polycarbonate. More recently, Sourisseau and Maraval (6) presented an elegant and rigorous treatment of axial and lateral intensity distributions due to refraction and diffraction, coupled with a detailed analysis of throughput through the confocal aperture. They concluded that diffraction reduces the axial spreading of the laser focus significantly and claimed that the depth resolution should remain fairly constant (at 5–6 µm for the optical system they studied), irrespective of the sampling depth. They, too, correctly computed the intensity profile expected from a 20-µm coating on a thick substrate. Whether these more intensive computations provide a significantly better match to experimental data than the more simplified methods (1, 4) has yet to be determined. They certainly are more demanding: 24 h of computer time was required to calculate a 60-µm depth profile at 1-µm intervals, according to Sourisseau and Maraval (6).

Resolution of Deeply Buried Structures

In practice, one finds that the apparent thickness of a buried layer depends upon its true thickness and its depth below the surface. In other words, the blurring of the confocal intensity profile from a buried layer does degrade significantly with depth, despite suggestions to the contrary (6). Figure 1

Neil Everall

Depth Profiling with Confocal Raman Microscopy, Part II

Part II of this two-part series continues the discussion on the interpretation of confocal Raman data, including how depth resolution is degraded when focusing deep within a sample and how intensity variations can occur when focusing near a sample’s surface.
schematically indicates why this is so. If a thick layer is near the surface, then the laser profile remains relatively narrow compared with the layer thickness irrespective of \( \Delta \) (the axial displacement) (Figure 1a), and the apparent thickness is governed primarily by the rate at which the laser maximum traverses the layer (the breadth of the laser profile contributes only a marginal additional broadening to the apparent layer thickness). If the laser axial intensity profile is much narrower than the layer thickness, then the apparent layer thickness depends mainly upon the rate at which the intensity maximum traverses the layer, because the breadth of the laser intensity distribution contributes negligible additional broadening. If the laser axial intensity profile is much wider than the layer thickness, there will be an additional broadening due to the convolution of the (changing) intensity profile with the material spatial distribution as a function of \( \Delta \). Hence buried layers, which will be illuminated by a broader axial intensity profile, will appear to be thicker than the same layer positioned at the sample surface. For a high NA objective, the laser intensity maximum traverses the sample at approximately twice the rate at which the sample is moved (~2\( \delta \Delta \), where \( \delta \Delta \) is the incremental change in the axial displacement \( \Delta \)), hence the reduction in the apparent thickness by a factor of ~2. However, if the layer is buried deeply, then the laser intensity profile can be much wider than the layer (Figure 1b). The breadth of the laser profile then significantly contributes to broadening of the apparent layer thickness, because even though the laser maximum passes quickly through the sample, Raman signals are generated from regions of the sample that are distant from the laser maximum (including when it lies far outside the layer boundaries). In other words, in one case we...
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convolve a narrow intensity distribution with the layer thickness, and in the other case we convolve a broadened distribution. Of course, this simplified treatment assumes that the confocal aperture is ineffective in rejecting signals generated throughout the broadened laser profile — more on this below.

The idea that the apparent thickness of a structure depends upon its position in the sample is disconcerting to say the least, but it is tested easily using the scheme outlined in Figure 2. We simply take a transparent laminate with a thin coating (A) on a thick substrate (B), and record the confocal Raman profile with layer A uppermost (Figure 2a). We then flip the sample and record another confocal profile of layer A, this time focusing through layer B (Figure 2b). If the resolution of the system is unaffected by the penetration depth, then the width of the intensity profile from layer A should be the same for each configuration.

Figure 3 shows the result of just such an experiment, performed on a laminate consisting of a 10-µm coating of poly(ethylene naphthoate) (PEN) on a ~180-µm substrate of poly(ethylene terephthalate) (PET). Two different objectives (100X, 0.9 NA and 50X, 0.55 NA) were used in this experiment. The intensity profiles of the PEN layer are shown for each configuration (overlapped on the same depth axis for simplicity). This result shows unambiguously that the apparent layer thickness depends strongly upon its distance from the surface; the thickness was much lower using the configuration shown in Figure 3a. It shows also that using a lower-NA objective does not solve the problem; the layer still appears to be much thicker when it is buried deeply.

This result poses a major problem when interpreting confocal intensity profiles because it is possible for a structure to appear to be either too thin, too thick, or presumably even the correct thickness, depending upon where it lies in the sample. This effect was apparent in the data of Reinecke et al. (7), who used confocal Raman measurements on surface-modified polyvinyl chloride (PVC) to determine the thickness of the chemically derivatized surface layer. The

![Figure 3. Results for the experiment defined in Figure 2 using 0.9-NA and 0.55-NA objectives.](image_url)
authors noted that the surface-modified layer appeared much thicker when the laser had to pass through the ~80-µm-thick PVC film before reaching the surface of interest, and correctly ascribed this effect to broadening of the laser focus.

This provides further evidence that the axial resolution degrades with depth.

**Static Versus Dynamic Resolution**

The experiment above is important because it confirms that the confocal aperture is not fully effective in rejecting Raman photons generated away from the center of the laser axial intensity profile. But how should we specify the actual depth resolution? This depends to some extent upon the experiment we are performing. Figure 3 shows that the observed width of the PEN response was ~16 µm (fwhm) with the 0.9-NA objective. Does this mean that if we position the laser focus so that we are at the maximum of the PEN response, we primarily will sample material within a region of ±8 µm (considering only the fwhm of the response)? Not necessarily. We know that the laser focus moves through the sample approximately twice as fast as the sample physically is moved. This results in the scale on the confocal depth axis being compressed, giving an apparent improvement in the depth resolution (as judged by the observed width of the

**Figure 4.** Comparison of the spatial resolution attained for a sharp interface for a sample prepared by (a) microtomy and (b) optical sectioning. The sample was a laminate of PE and PET, which has very little interfacial mixing. The PE Raman signal is plotted in each case as a function of position. Here Δ refers to sample displacement both laterally and axially along the surface normal.

**Figure 5.** Results from experiments testing the surface specificity of confocal Raman microscopy. Spectra of a PEN–PET laminate are obtained as a function of axial displacement to identify the point at which the PEN–PET contrast ratio is maximized.
response from a given layer). Let’s call this the “dynamic” depth resolution, because it entails moving the sample during the experiment. The dynamic depth resolution obviously depends upon both the (evolving) shape of the laser axial profile, and the rate at which it traverses the sample.

However, what if one is trying to record a pure Raman spectrum of a buried feature in a static configuration, that is, simply focused at a fixed depth? In this case the spectral purity depends primarily upon the breadth of the laser intensity profile. We no longer benefit from the depth-scale compression, and in simple terms we expect the “static” depth resolution to be worsened by a factor of ~2. In other words, when nominally focusing ~90 µm below the surface, the Raman spectrum could contain features from a region at least ±16 µm about the response center. This is better than the resolution predicted using the equation shown, (± ~60 µm), but it shows that the confocal aperture still passes photons that originate throughout a significant portion of the broadened laser focus. As a consequence of this effect, it actually is very difficult to obtain pure Raman spectra from buried layers that are tens of micrometers thick; usually there is significant spectral contamination from surrounding material (2).

**Interfacial Broadening**

Confocal Raman spectroscopy, in principle, is an attractive approach for studying mixing and component migration at buried interfaces. However, spherical aberration degrades the resolution that can be attained significantly and means that only quite pronounced interfacial broadening can be observed with the confocal approach. Figure 4 shows this for a polyethylene (PE)–PET laminate examined using microtomy followed by lateral mapping (Figure 4a) and optical sectioning (Figure 4b). The sample in Figure 4a was prepared by cutting a cross section through the sample with a diamond blade. One would expect a very sharp interface for this system. The method shown in Figure 4a yields an apparent interfacial width that is determined mainly by the diameter of the focused laser spot (~1 µm), whereas the method shown in Figure 4b yields a much broader interfacial profile due to spherical aberration. This simple experi-
ment demonstrates unambiguously that interfaces can be studied with better precision if the sample first can be microtomed to yield an undamaged cross section; the resolution, as judged by the apparent sharpness of interfaces, is better and there is no ambiguity about exactly where one is focused within the sample. The interfacial transition was much sharper for lateral scanning across a microtomed cross section. However, if one cannot prepare a cross section, then confocal profiling using an immersion rather than a metallurgical objective helps to minimize the aberrations and yields more accurate raw intensity profiles; this approach is discussed at the end of this article.

**Surface Specificity**

Spherical aberration poses obvious problems when focusing deep into a sample, but when one simply is trying to obtain a pure spectrum at the surface of a material, one might imagine that the depth resolution would approximate to the depth of focus of the laser beam in air — that is, ~1 µm for a high-NA lens. One reasonably might expect to obtain a fairly pure spectrum of, for example, a 5-µm coating on a thick substrate. But what happens in practice? This was investigated using a 5-µm layer of PEN on a thick PET substrate, noting the spectral purity as a function of the position of the laser focus about the upper surface (see Figure 5). Focusing exactly on the top surface ($\Delta = 0$ µm) gave a reasonably pure spectrum of PEN (the main PET peak at ~1612 cm$^{-1}$ was small), while focusing deeper into the sample ($\Delta = +3$ µm) increased the relative (and absolute) PET intensity, as expected. Surprisingly, focusing above the film surface ($\Delta = -3$ µm) also increased the relative PET intensity (although the absolute PEN and PET intensities both decreased, as expected). Figure 6 shows the variation in contrast ratio as a function of $\Delta$ and confirms that if one deviates from the upper surface in either direction, the PEN–PET contrast decreases. The optimum coating contrast (that is, the minimum PET/PEN ratio) is obtained when focused on the air–PEN surface ($\Delta = 0$), and as expected the PET substrate contribution increases as one focuses below the PEN surface. However, perhaps surprisingly, the PET contribution also

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**Figure 7.** Schematic illustrating why the coating/substrate ratio decreases when $\Delta <0$. 
increases on focusing above the PEN surface ($\Delta < 0$). The purest spectrum of a thin coating on a thick substrate therefore will be obtained when focusing very near to the coating–air interface; one cannot improve the surface specificity by focusing above the top surface.

This result seems surprising at first sight, but can be understood by considering the limiting situations sketched in Figure 7. When focusing on the coating (Figure 7a), the coating/substrate signal ratio primarily depends upon the extent to which the two layers are sampled by the laser focal volume. When focusing well above the top surface (Figure 7b), no part of the sample is in focus and the only signals observed by the detector are those due to laser and Raman photons propagating along the principal optical axis. Hence in this case the signal ratio largely depends upon the relative thickness of the different layers of the sample, rather than their proximity to the laser focus. If the laser focus is well above the surface, only Raman rays traveling along the main optical axis of the microscope will reach the detector, and the proportion of such rays originating in the coating or the substrate will vary as the ratio of coating/substrate thickness. Therefore, the thickest layer will dominate the Raman spectrum when the sample is far removed from the laser focus, giving rise to the effects seen in Figures 5 and 6. This serves again to illustrate how careful one must be when interpreting confocal intensity profiles, whether one is focused near the surface or deep within a sample.

Note that in the discussion above we have assumed explicitly that there is no difference in either the Raman scattering cross section or the polymer density when comparing the surface with the bulk. This probably is a safe assumption given the depth (several micrometers) to which Raman microscopy penetrates into the bulk even when one is focused on the surface.

Use of an Immersion Objective

The use of metallurgical objectives produces confocal profiles that are difficult to interpret, to say the least. This suggests the obvious approach of using an objective that is designed for sub-surface focusing, such as an immersion objective. In this manner one avoids the large air–sample index discontinuity that causes refraction. This approach was shown to work well for a polymer laminate, yielding raw intensity profiles that have approximately the correct thickness for each layer, albeit with significant signal attenuation when focusing deep into the sample (2). Figure 8 illustrates the results obtained for a multilayer polypropylene–PET laminate using a 1.25-NA oil immersion objective. It plots the PET signal as a function of nominal focal position in the sample. The immersion objective gives a more accurate representation of the sizes of the individual layers, because the immersion oil minimizes the discontinuity in refractive index that gives rise to refraction at the sample surface. The metallurgical objective,
as expected, gave a foreshortened profile, in good agreement with the theoretical trace. The immersion objective gave a profile in which each layer thickness was in good agreement with the known values, although the signal attenuation for the bottom layer was very pronounced. This could have been due to the poor quality of the objective that was used for this work (only a rather old objective was available). Signal attenuation is not a fundamental limitation, as was shown by Vyorykka et al. (8), who obtained unattenuated confocal data using an immersion objective. More recently, Froud et al. (9) used a metallurgical objective to obtain excellent confocal Raman profiles from a polymer laminate with 11 layers within a 100-µm film; the layer thicknesses were determined accurately with this approach. Unfortunately the authors did not discuss the spectral purity of the data obtained from each layer, and our own work leads us to believe that this still is significantly worse than that obtained by mechanically sectioning a sample. In other words, spectral features from surrounding layers still are manifested, to some extent, in data acquired from the middle of a thick buried layer. Further work is required to establish whether this is in fact a significant problem.

The benefits of using immersion objectives for depth profiling now are becoming fairly widely known. For example, Fleming et al. studied morphological gradients in PET film that was processed with supercritical carbon dioxide (10). They showed that confocal profiles using an immersion objective matched well the profiles generated by sectioning the sample mechanically, but that metallurgical objectives gave incorrect profiles, as expected. Vyorykka et al. (8) showed how immersion objectives are useful for studying porous, turbid coatings. The immersion oil can penetrate the coating and wet the particles, reducing the scatter and hence the turbidity. This allows one to focus through coatings that would otherwise be too opaque to permit transmission. Larsson et al. (11) successfully used a water immersion objective to depth profile the distribution of allyl and sulphopropyl ligands in water-swollen agarose parti-
icles. Pudney et al. (12) studied gellan and carrageenan distributions in carbohydrate polymers in water. These and similar studies show the potential of immersion objectives to yield useful data with a minimum of distortion; although if the best possible spatial resolution is required, then Figure 5 demonstrates that mechanical, rather than optical, sectioning usually will give superior results, provided the samples are amenable to microtomy or polishing.

Finally, it is worth noting that the distortion of the depth scale from a metallurgical objective can, in some circumstances, be treated using a simple correction factor. Reinecke et al. (7) studied surface-modified 80 µm-thick PVC film using confocal Raman depth profiling. They used the known total thickness of the sample to scale the Raman depth correctly so that the distribution of aminothiophenol modifier could be mapped. However, simply scaling the depth axis alone was not sufficient, because this does not account for the progressive blurring of the laser focal volume on focusing deeper into the sample. The key aspect of this work was the use of an internal standard band from the PVC to normalize the intensity response of the aminothiophenol. When this was done, the scaled confocal profile agreed quite well with data obtained by microtomy and lateral scanning. Normalization with the internal standard had the effect of broadening the response due to the top surface layer and narrowing the response of the bottom layer. More work is required to assess whether this is a generally applicable method.

**Conclusions**

I have attempted to highlight in this series of articles some of the effects that must be considered when performing confocal Raman experiments. The metallurgical objectives that usually are supplied as standard with a Raman microscope are not well suited to depth profiling, due to the inevitable effects of refraction and spherical aberration. Raw data must be modeled carefully in order to extract meaningful quantitative results, otherwise the depth scale and the size of buried structures are grossly incorrect. Immersion objectives yield raw intensity profiles that correspond much better to the true structure of the sample, and they are recommended for confocal depth profiling. However, the best spatial resolution usually will be attained by mechanical preparation of a cross section, when this is possible.

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**Neil Everall** is ICI group research associate with the Measurement Science Group at ICI plc (U.K.). E-mail the author at: neil_everall@ici.com.