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Correction of diffraction effects in confocal Raman microspectroscopy

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Abstract: A mathematical approach developed to correct depth profiles of wet-chemically modified polymer films obtained by confocal Raman microscopy is presented which takes into account scattered contributions originated from a diffraction-limited laser focal volume. It is demonstrated that the problem can be described using a linear Fredholm integral equation of the first kind which correlates apparent and true Raman intensities with the depth resolution curve of the instrument.

The calculations of the corrected depth profiles show that considerable differences between apparent and corrected depth profiles exist at the surface, especially when profiles with strong concentration gradients are dealt with or an instrument with poor depth resolution is used. Degrees of modification at the surface obtained by calculation of the corrected depth profiles are compared with those measured by FTIR-ATR and show an excellent concordance.

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1. Introduction

In recent years it has been shown that wet-chemical treatments of polymer films can successfully be used to modify surface specific properties of the material [1, 2]. This type of selective modification reaction entails generally the formation of a gradient in the µm-range of modifying groups across the film. It has been demonstrated [3-6], that confocal Raman microspectroscopy can be used to characterize these films with respect to the number of modified groups as a function of depth. The technique has furthermore been shown to be a powerful tool for studying coatings [7, 8], morphological changes in polymer films [9] and polymer-polymer interphases [10] and to be of interest in industrial research [11].

In the experiment the exciting laser spot is focused first on the film surface and then, in steps of 1 or 2 µm on fictitious planes inside the film recording a spectrum at each step. In this way a characteristic depth profile is obtained.

However, these profiles contain an intrinsic error which is due to the fact that confocal Raman microscopy only in the ideal case of a laser beam with diameter approaching zero gives the Raman scattering of a single plane. In the real case, due to diffraction phenomena of the optical system, the laser beam has significant intensity up to a certain distance from the focal plane x. Consequently, it has to be considered that a measured intensity $I_m(x)$ contains contributions proceeding from regions in the neighborhood of x. These contributions which are given by the depth resolution of the Raman instrument lead to an erroneous depth profile which becomes especially evident at the surfaces of the analyzed films and always when samples with high concentration gradients of a species are measured.

It has to be emphasized that the depth resolution discussed here is only the ideal value measured at the outer surface of a film (at depth x=0) as it is determined in air. It has been demonstrated [12-14] that, for reasons of the refractive index difference between air and the polymer sample, Raman scattering originates from a region deeper below the surface than the nominal point; furthermore the depth resolution curve broadens quickly when penetrating deeper into the film. These refractive index effects which often have a larger influence on the final depth profile than the resolution of the microscope have to be considered when the measured data are obtained on a microscope without oil-immersion objective. In this first paper we present a new mathematical approach which can be used as starting point to develop correction procedures for depth profiles. Here we present a way to obtain corrected depth profiles with intensities which are much closer to those of the true ones by calculation of the contributions of the Raman radiation that originates from a diffraction-limited laser focal volume. In a future paper the approach presented here will be applied to correct depth profiles also with respect to the refractive index effects.

2. Experimental part

Raman spectra were recorded using a Renishaw Ramascope 2000 spectrometer, with the 632.8 nm line of a He-Ne laser for excitation.

FTIR-ATR measurements were performed using a Nicolet 520 spectrometer with a diamond crystal acting as the internal reflection element.

Solutions of Fredholm Integrals were obtained with the help of the software Mathcad 7 Professiona*l*.

3. Results and discussion

3.1 Homogeneous single-layer films

The simplest case to investigate the Raman response in a depth profiling study using the confocal configuration of a Raman microscope is that of a homogeneous single-layer film. In Fig. 1(a) theoretical constant group concentration is represented schematically in scheme (a) while a typical Raman measurement for this type of homogeneous band is depicted in scheme (b).
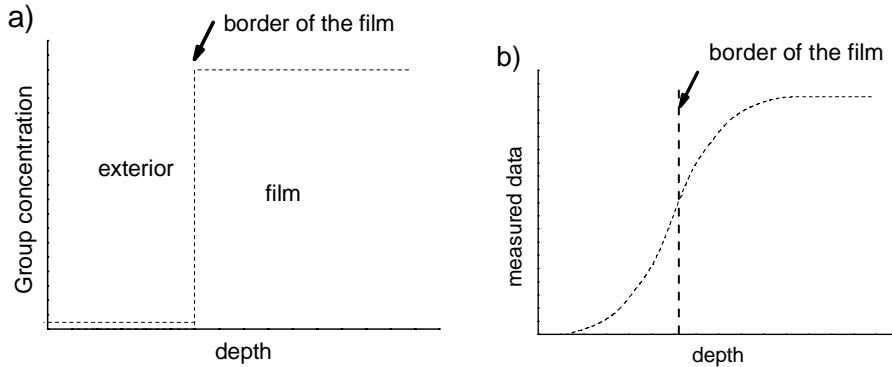

Fig. 1. Schematic presentation of true (a) and experimentally obtained (b) depth profiles of a 40 mm thick transparent polymer film of refractive index n=1.

The main difference one can observe when comparing the measured and the true profiles is that the experimental data show a Raman response outside the borders of the film and that, near the borders inside the film, the scattered intensities are lower than those in the centre of the film. This effect can be explained by the fact that due to diffraction effects of the optical system the Raman scattering measured is that of a small volume element and the measured intensity at a focussed plane at depth x does also contain contributions from the surrounding planes. The extent of the contributions of the surrounding planes to the measured intensity at x is given by the resolution curve for the experiment (i.e. the response curve from a silicon wafer). In order to correct the measured depth profiles and obtain the true ones one has to find a correlation between measured profile, resolution curve and true profile. As this correlation is not obtained in a straightforward manner, one can, in a first step, describe the problem as a system of linear algebraic equations.

(I) System of linear algebraic equations

The film is considered to be composed of a number of finite intervals i and the measured intensity value of each interval must be corrected by substraction of the contributions made by the intervals in its vicinity. These contributions are given by the resolution curve (Fig. 2) of the experiment which has been measured in this case on a thin transparent PVC film. The left part with respect to x=0 (the "negative" distances from the surface) has been obtained focusing the laser spot first 10 μm above the polymer surface and then, in steps of 2 μm toward the surface recording a spectrum at each step. The right part is measured focussing first onto the surface of the opposite side of the film and then, in steps of 2 μm, downwards.

The intensities of the 1425 cm^{-1} band were then normalized with respect to those of the surfaces. The resolution curve obtained is symmetric within the experimental error as it is shown in Fig. 2 and can be interpreted in the following way: if the Raman intensity scattered by the interval around x is I(x), this intensity contributes to those of the intervals in the vicinity of x as follows:

for interval (x+1): $I(x+1) = f_2 * I(x)$, at (x-1): $I(x-1) = g_2 * I(x)$

at (x+2): I(x+2)= f_3*I(x), at x-2: I(x-2)= g_3*I(x)

....

at (x+n): I(x+n)= f_{n+1}*I(x), at (x-n): I(x-n)= g_{n+1}*I(x)

being f(x) and g(x) the correction factors given for each interval by the resolution curve.
The problem can, then, graphically be presented as in Fig. 3: as the measured intensity in the first interval $I_m(1)$ is composed of the true intensity $I_t(1)$ in this interval plus the sum of the
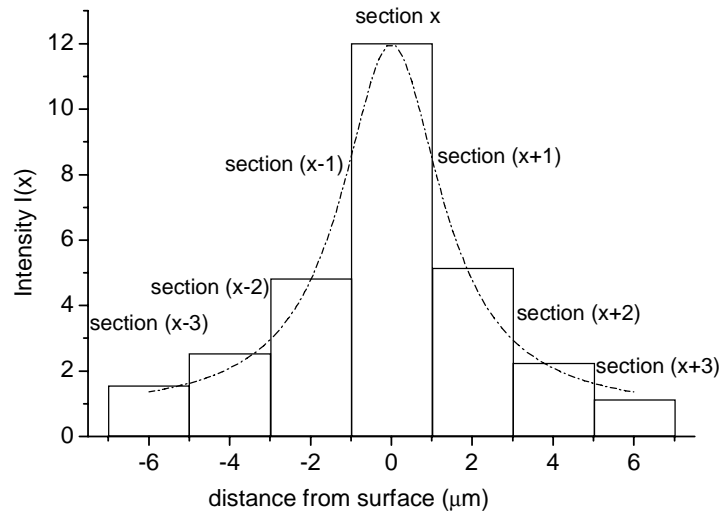

Fig. 2. Resolution curve determined by measuring the Raman response of the signal at 1425 cm^{-1} of a PVC film.

true intensities ($I_t(2)$, $I_t(3)$, $I_t(4)$) proceeding from the neighbourhood of interval (1) contributing each interval according to its distance with a correction factor, the measured intensity I_m of this interval is written

$I_m(1)$= $I_t(1)$+f2*$I_t(2)$+f3*$I_t(3)$+..... From the second interval on one has to consider also the contributions from the neighbourhood on the left side:

$I_m(2)$= g2*$I_t(1)$+f2*$I_t(3)$+f3*$I_t(4)$+.....

$I_m(3)$= g3*$I_t(1)$+g2*$I_t(2)$+f2*$I_t(4)$+f3*$I_t(5)$+.....

$I_m(4)$= g4*$I_t(1)$+g3*$I_t(2)$+g2*$I_t(3)$+f2*$I_t(5)$+f3*$I_t(6)$+....

....

In this way, as the measured intensities $I_m(x)$ and the correction factors f(x) and g(x) are given, one obtains a system of n equations with n unknown parameters $I_t(x)$. The system of equations has been solved using the programme *Mathcad 7 Profesional*. However, the true intensities $I_t(x)$ (which in the simplest case of a reference signal should be a constant) calculated in this way are not physically reasonable. The failure of the method used might be due to the fact that the corresponding matrix is symmetric and might be badly conditioned. In this case, a direct solution of the equations is contaminated by round-off errors and is useless.

II) Integral equations

An alternative methodology to calculate true intensities is to consider the film as continuous instead of a succession of finite intervals. In this case, the Raman intensity measured at each point is the sum of the contributions of the entire film to this point while the extent of the contributions is given by the resolution curve. Mathematically, this correction can be carried out by transformation of the system of linear equations into a linear Fredholm integral equation of the first kind where y is the variable needed to obtain the contribution of the entire film on each point x by integration in the limits of the film thickness (from 0 to b).
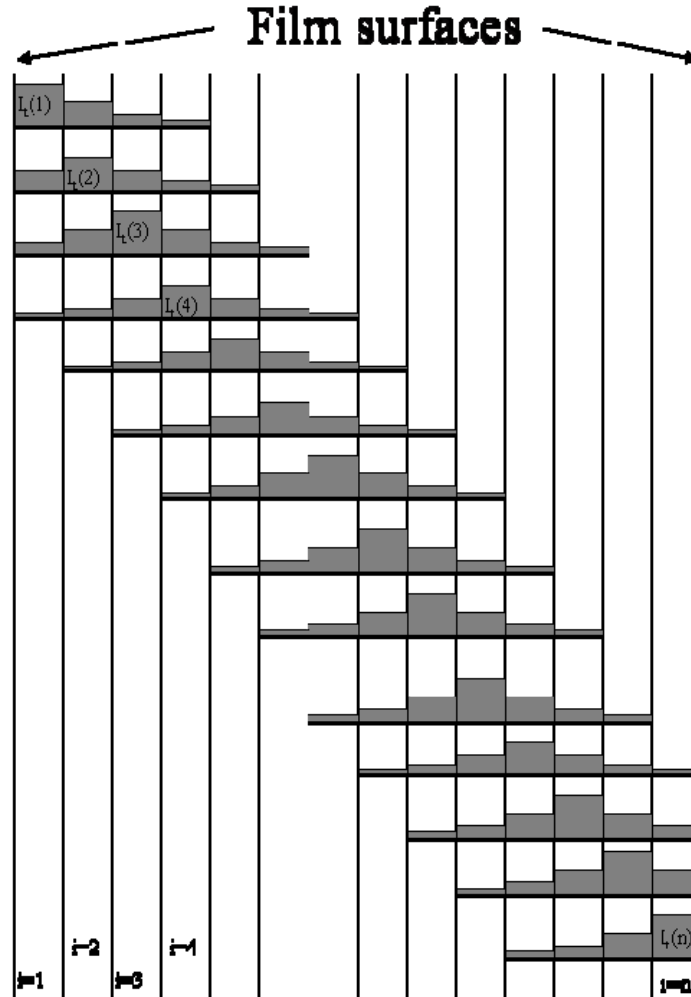

Fig. 3. Sectioning of the film in a number of finite intervals i. The measured intensity $I_m(i)$ of each interval is given by the sum of the true intensity $I_t(i)$ of this interval plus the contributions made by the intervals in its vicinity.

$$f(x) = \int_0^b \left[I(y)H(x, y) \right] dy \tag{1}$$

I(y) is the true intensity at each point y and the kernel *H(x,y)* is a function which describes the contribution of all planes in the neighbourhood of this point. This function H which corresponds to the resolution curve is related to the optical properties of the microscope and the diameter of the laser beam and depends on the wave length of the laser and the numerical aperture and fill factor of the lens.

A typical resolution curve of a confocal experiment can be excellently fitted by a Lorentzian function. Consequently the resolution curve can be expressed mathematically as:

$$H(x,y) = \frac{A^2}{4(x-y)^2 + A^2}$$

(2)

being, in the confocal case studied in this work, *A*= 1.17. Using this expression for *H(x, y)* and knowing that the true depth profile for a homogeneous film is a constant *I(y)*= const= k_0 for *b>=y>=0*, the experimental (apparent) depth profile *f(x)* becomes, for a constant concentration band as the one in Fig. 1:

$$f(x) = \int_0^b \left[k_0 \frac{A^2}{4(x-y)^2 + A^2} \right] dy = \frac{1}{2\sqrt{A^2}} \arctan\left[2\frac{(b-x)}{\sqrt{A^2}} \right] k_0 A^2 + \frac{1}{2\sqrt{A^2}} \arctan\left[2\frac{x}{\sqrt{A^2}} \right] k_0 A^2$$

(3)

Using this function the experimentally obtained data may be fitted, which would allow one to calculate the value of the constant k_0.

3.2 Films with concentration gradient

Confocal Raman spectroscopy has been shown to be an excellent means for analyzing thin transparent films whose surface has been modified by a wet-chemical treatment in solvent/non-solvent mixtures [2, 3, 6]. This kind of treatment leads to a concentration gradient for modifier groups in the micrometer range across the film. When these films are analyzed using confocal Raman spectroscopy a series of spectra recorded at different depths are obtained. In order to determine the degree of modification as a function of depth two signals are usually analysed, one that corresponds to the modifier molecule and a second one that is not affected by the reaction and should be constant. The conventional way to obtain the depth profile for such a system is to normalize the modifier signal of each spectrum by division by the reference band and use a calibration curve (measured on homogeneously modified samples) which correlates the calculated ratio of the two band intensities to the degree of modification. We have shown, however, that this method presents intrinsic errors, which become important at the surface, especially in the case of high concentration gradients [16]. It is therefore necessary to apply the approach presented above and correct both, the profile of the reference and of the modifier bands. The reference signal corresponds to the case of a constant true intensity discussed above. Using the resolution curve of our Raman microscope the true function is *I(y)*= const= k_0= 981.

In order to correct the measured modifier profile we have used again Eq. (1) using the same depth resolution curve, *H(x,y)*, as before. Although the Fredholm Integral equation describes the problem in a correct way (no approximation) and two of three variable are known (*H(x,y)* and the measured modifier profile *f(x)*), for mathematical reasons it is not possible to obtain the desired function *I(y)* in a direct way. Our approach consists therefore in the use of guess functions *I(y)* that are likely to describe best the physical conditions of the experiment. The procedure consists then in finding the optimal parameters of this function *I(y)* which allow to fit the experimental points *f(x_i)* determined by the intensities of the corresponding Raman spectra.

As possible functions we have tried in this work a Lorentzian function which can be supposed to be especially suitable for Fickian mass transport (the gradient formed by wetchemical modification of dense polymer membranes is often related to Fickian mass transport and can be described by a Lorentzian relation). If we use Eq. (1) employing the same

function $H(x,y)$ for the resolution curve as in the case discussed above and for the true profile $I(y)$ a Lorentzian dependence we have to solve the following integrals:

$$f(x) = \int_0^b \left[k_1 \frac{k_2}{4y^2 + (k_2)^2} \frac{A^2}{4(x-y)^2 + A^2} \right] dy \tag{4}$$

The programme *Mathcad 7 Professional* is able to integrate this expression. The solution is given by the following equation:

$$f(x) = \frac{1}{2} k_1 k_2 A^2 \frac{\begin{bmatrix} 2x\ln(4b^2 + k_2^2)\sqrt{k_2^2}\sqrt{A^2} + k_2\arctan\left[2\frac{(b-x)}{\sqrt{A^2}}\right]\sqrt{k_2^2} + A^2\arctan\left[2\frac{b}{\sqrt{k_2^2}}\right]\sqrt{A^2} - \\[2mm] k_2^2\arctan\left[2\frac{b}{\sqrt{k_2^2}}\right] + 4\arctan\left[2\frac{b}{\sqrt{k_2^2}}\right]x^2\sqrt{A^2} - 2x\ln(4x^2 - 8xb + A^2 + 4b^2)\sqrt{k_2^2}\sqrt{A^2} + \\[2mm] 4\arctan\left[2\frac{(b-x)}{\sqrt{A^2}}\right]x^2\sqrt{k_2^2} - A^2\arctan\left[2\frac{(b-x)}{\sqrt{A^2}}\right]x^2\sqrt{k_2^2} \end{bmatrix}}{\left[(8k_2^2 x^2 + A^4 - 2A^2 k_2^2 + 8A^2 x^2 + k_2^4 + 16x^4)\left(\sqrt{k_2^2}\sqrt{A^2}\right)\right]} +$$

$$\frac{1}{2} k_1 k_2 A^2 \frac{\left(2x\ln(k_2^2)\sqrt{A^2} + 2x\ln(4x^2 + A^2)\sqrt{A^2} + 4\arctan\left[2\frac{x}{\sqrt{A^2}}\right]x^2 - k_2^2 rc\tan\left[2\frac{x}{\sqrt{A^2}}\right]\right)}{\left[(8k_2^2 x^2 + A^4 - 2A^2 k_2^2 + 8A^2 x^2 + k_2^4 + 16x^4)\left(\sqrt{A^2}\right)\right]}$$

$$\tag{5}$$

We have applied this new treatment to a real experimental sample of 40 μm thickness. We suppose again that no absorption or scattering effects take place, and for the sake of simplicity we assume here a constant resolution curve across the sample (what is definitively not the
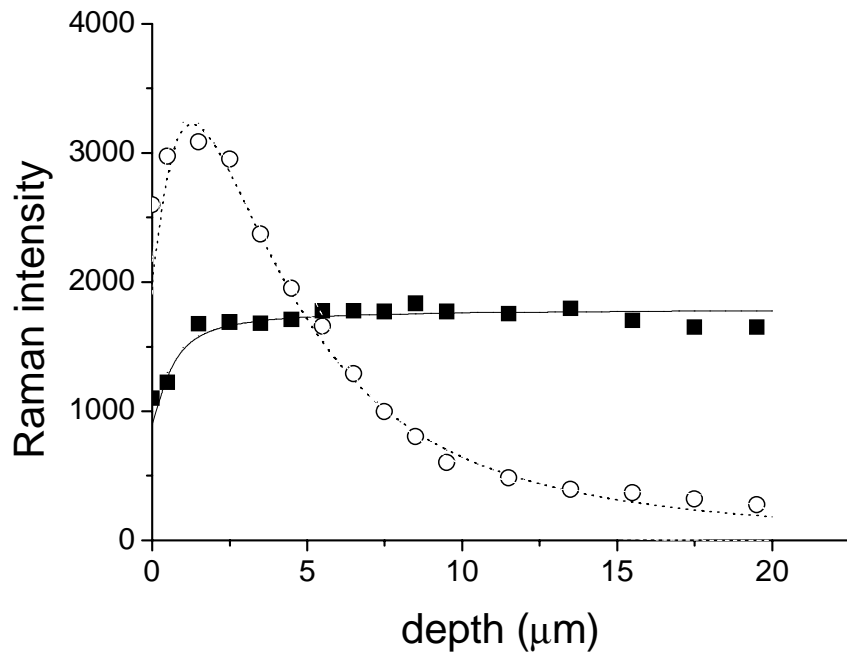
Fig. 4. Raw data with corresponding fits assuming a constant true function (solid line) for the reference signal and a Lorentzian fit (dotted line) for the true modifier profile.

case) and have taken into consideration the refractive index difference between sample and air only for the correction of the depth scale. These solutions have been used to fit the experimental data of the modifier band intensities with a non-linear least square algorithm. In Fig. 4 the fit is presented together with the measured raw data. The Lorentzian function adequately fit the data and has been used in what follows to construct the corrected depth profile. From the fit in Fig. 4 the characteristic parameters k_1 and k_2 of the Lorentzian are obtained which can be used to represent the true modifier intensity as a function of depth. After division by the corrected reference intensity $(I(y)=981)$ and transformation into degrees of modification using a calibration curve one obtains the true depth profile which is compared in Fig. 5 with that of the depth profile obtained in the conventional way by simple division of modifier intensities and reference band intensities. In general, a good agreement of both curves is observed. However, comparing the values at and near the surface an important difference between true and apparent

modifier content can be seen, in agreement with the predictions reported previously [16].
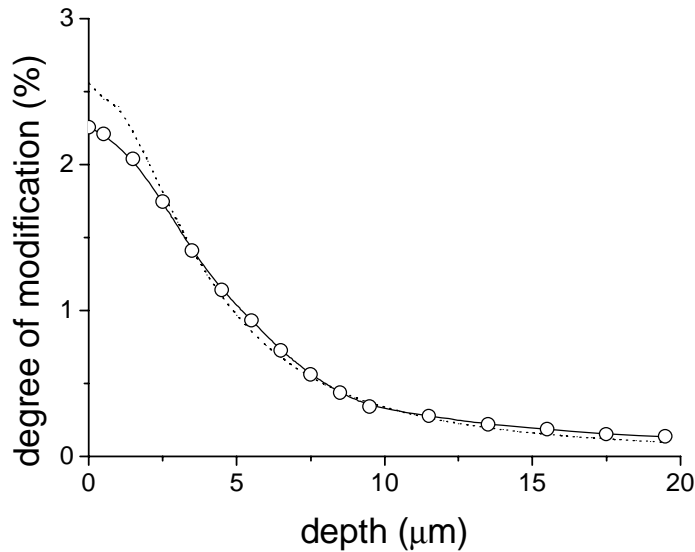

Fig. 5. Comparison of depth profiles obtained by simple normalization (solid line) and normalization after correction of the data (dotted line) using a Lorentzian relation.

In order to study in more detail the extent of the error between corrected and apparent depth profiles, the kinetic of a wet-chemical film modification reaction is recorded. Experimentally, a polymer film is introduced in a reactive solution which contains the modifier. After different time intervals samples are withdrawn, purified by extraction and dried. Then the corresponding depth profiles are determined using confocal Raman microscopy. Three apparent profiles with different gradients obtained on films after 22, 46 and 70 hours of reaction time have been measured and corrected using the approach presented above. Values of the degrees of modification obtained at the surface in apparent and corrected depth profiles have been compared in table 1. Furthermore, we include a column in table 1 which shows the

Table 1: Comparison of degrees of modification at the surface obtained by confocal Raman spectroscopy (without and with correction) and FTIR-ATR measurement

sample	Degree of modification at the surface (apparent depth profile)	Degree of modification at the surface (true depth profile)	Fractional difference between apparent and true degrees of modification at the surface	Degree of modification at the surface obtained by FTIR-ATR
22 hours	5.1 %	6.4 %	20.3%	6.4 %
46 hours	6.9 %	7.6 %	9.2%	7.6 %
70 hours	8.0 %	8.2 %	2.4%	8.1 %

degree of modification of the films measured with a FTIR-ATR spectrometer. The instrument was equipped with a diamond as the internal reflection element as it is known that in these conditions a sample depth of 1-2 μm can be analyzed [15].

Two conclusions can be drawn from table 1. It is shown that the error obtained at the surface when using the conventional way to obtain depth profiles is greater the more pronounced the gradient of the true profile. This means, that flat profiles do not necessarily need a correction while in highly surface selective reactions it is indispensable to perform the fitting treatment shown in this work. The second conclusion from table 1 is the excellent agreement between corrected confocal Raman data and ATR values at the surface what confirms the accuracy of this treatment.

4. Conclusions

In this contribution a mathematical treatment has been presented which allows one to correct depth profiles obtained by confocal Raman microscopy with respect to an intrinsic error of this technique which is due to diffraction effects of the optical system. This treatment allows the calculation of the corrected depth profiles using the data of the corresponding apparent profile, taking in consideration the depth resolution of the Raman instrument. It has been shown that the major difference between apparent and corrected profiles is observed at the film surface and that this difference depends on the slope of the gradient and the depth resolution.

The major advantage of this treatment is the possibility to obtain more reliable depth profiles from experimental data recorded with a poor or even non-confocal optical system. On the one hand this allows one to obtain good results even on inferior Raman instruments; on the other hand, as a consequence of the higher detected count rate in the non-confocal configuration, accumulation times of the spectra can be drastically shortened.

A second paper about a mathematical treatment of data from confocal Raman microscopy that also considers the effect of refraction and that is based on the approach shown in this word is now being prepared.

Acknowledgments