

# Avoiding Coupling Fluid–Sample Interaction in Confocal Raman Depth Profiling with Immersion Objectives

J. PABLO TOMBA\* and JOSÉ M. PASTOR

*Institute of Materials Science and Technology (INTEMA), National Research Council (CONICET), University of Mar del Plata, Juan B. Justo 4302, (7600) Mar del Plata, Argentina (J.P.T.); and Department of Physics of Condensed Matter, University of Valladolid, Paseo del Cauce s/n, (47011) Valladolid, España (J.M.P.)*

Index Headings: **Confocal Raman microspectroscopy; Polymer films; Depth resolution; Immersion objectives.**

## INTRODUCTION

Confocal Raman microspectroscopy (CRM) has become one of the most valuable techniques for probing molecular/physical properties in small sample regions.<sup>1,2</sup> It is possible, for instance, to perform nondestructive in-depth analyses of transparent samples by optical sectioning; in this approach, the laser focus is moved to successively deeper positions into the sample to obtain spectral information as a function of depth. Although theory predicts depth resolutions of a few micrometers, operative values may be far beyond this limit. Several reports have shown the adverse effect of laser refraction on depth discrimination, which is particularly severe when “dry” metallurgical objectives are employed in the optical path.<sup>3–6</sup> When the laser beam is focused through air at a given point below the sample surface, it deviates at the sample entrance in a degree that depends on the sample refractive index ( $n$ ), as predicted by Snell’s law. The effect causes significant spreading of the laser focal volume and a marked loss in spatial discrimination. As a consequence, sharp sample features appear broadened and artificially closer to the microscope objective. In addition, laser refraction perturbs the collection efficiency of the confocal aperture, causing a continuous reduction in Raman intensity with focusing depth.<sup>7,8</sup>

Many experimental strategies have been considered to minimize these undesirable effects. A recent work by Everall and co-workers nicely summarizes most of them, concluding that the most effective way to suppress distortions by laser refraction is the employment of immersion optics with a coupling fluid that matches the refractive index of the sample.<sup>9</sup>

One important issue one has to consider in the implementation of this methodology is the compatibility of the fluid with the sample. There are many cases in which the fluid, typically organic oil, may physically interact with the sample, altering the system under study. One remarkable example can be found in amorphous polymers. These transparent materials, particularly suited to be studied by CRM, are extremely sensitive to the contact with small organic molecules: they can penetrate and swell the sample by diffusion, cause extensive damage (i.e., cracking) by osmotic swelling or dissolve the polymer in case of thermodynamic affinity. A smart alternative, proposed by Vyorykka et al. consists of the use of a double oil configuration, with a thin glass as a separating element between the oils.<sup>10</sup> One might use different oils suitably chosen to be in contact either with the sample or with the objective; although the strategy has shown to be effective in depth profiling of semicrystalline polymers, we may be in some cases constrained by the selection of at least one fluid compatible with the sample. Other promising approaches, for instance the implementation of microscope objectives with cover slip correction proposed by Adar et al., only appear to be effective in improving the intensity of the Raman signal but not the actual depth resolution nor the underestimation of the depth scale.<sup>9,11</sup>

In this Note, we test a strategy based on the use of a protective coating as a separating medium to prevent sample-coupling fluid direct contact when one performs depth profiling by CRM with immersion objectives. A transparent medium, with a refractive index of  $\sim 1.5$ , easily exchangeable, with good ability to adapt to sample surface imperfections and to tolerate direct contact with organic penetrants, were prerequisites in the selection of the coating. Specifically, we show that a thin polyethylene (PE) film 30  $\mu\text{m}$  thick can be reversibly applied onto a polymeric substrate used as test sample, allowing safe depth profiling of the substrate with good spatial resolution.

## EXPERIMENTAL SETUP AND RESULTS

We designed a simple device for reversible application of the protecting coating onto the sample surface, whose main parts are shown in Fig. 1. Scheme 1 shows how the different parts are assembled. An aluminum frame, internally threaded, supports the protective coating, a commercial low-density PE film ( $n = 1.51$ ) with nominal thickness of 30  $\mu\text{m}$ . The film was cut from the original sheet to perfectly fit in the dimensions of the frame. The internal diameter of the frame, 30 mm, allows the housing of the microscope objective (18–24 mm diameter). An externally threaded aluminum ring tightly fixes the edges of the protective film to the frame, maintaining the film in place. A key point for the success of the strategy is to achieve a uniform optical path for the laser beam when passing from coating to substrate. To promote good physical contact between these elements, we evacuated the air between them through a channel formed in the main frame, in turn connected to a standard laboratory vacuum pump. A rubber gasket, with a cut at the point of air evacuation, is placed between frame and

Received 18 March 2008; accepted 22 April 2008.

\* Author to whom correspondence should be sent. E-mail: jptomba@fi.mdp.edu.ar.

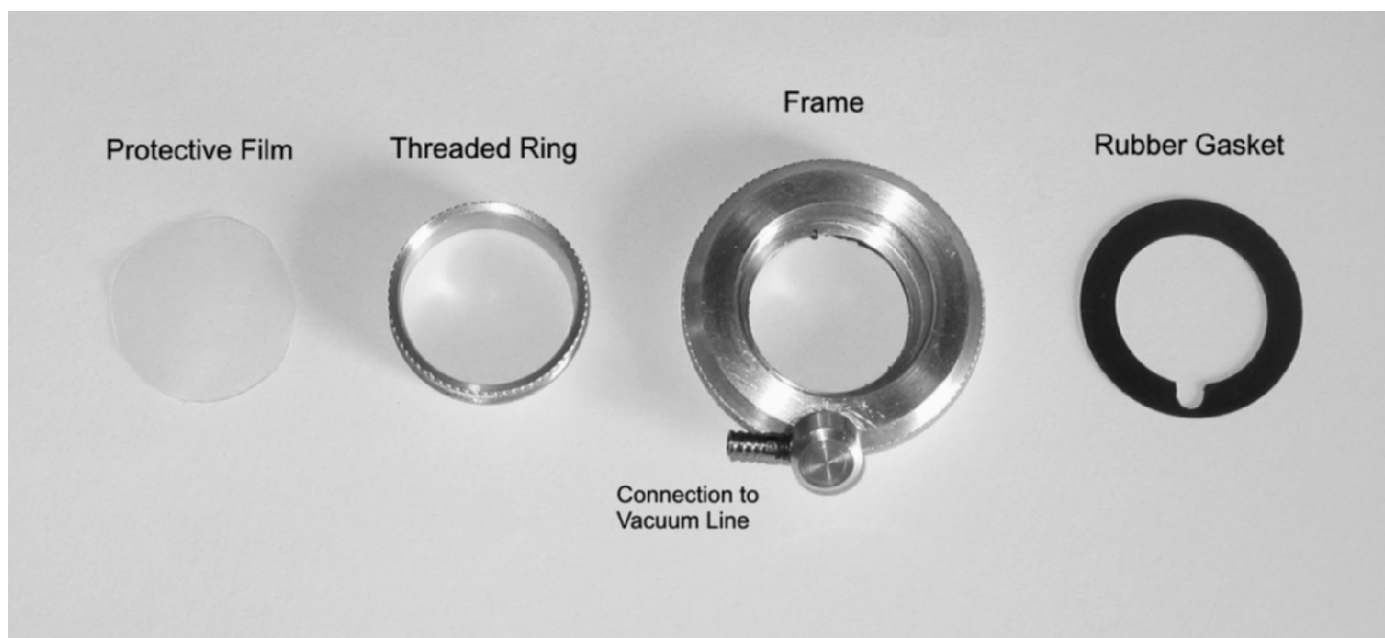


FIG. 1. Main components of the experimental setup used to reversibly apply the protective film.

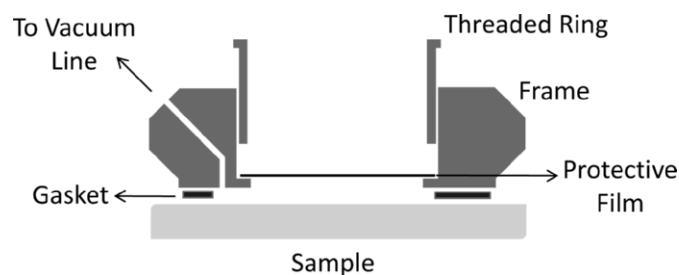
sample to prevent air entrance. Once air is evacuated by the pump, the difference of pressure thus established at both sides of the protective film is largely enough to tightly adhere it to the counterpart. At the same time, the PE film is flexible enough to copy possible surface imperfections of the substrate, helping to achieve good physical contact with most parts of the sample surface. Other films commercially available, i.e., polyethylene terephthalate and polypropylene, are too stiff for this application. The same occurs with thicker films; besides, they reduce the working distance even further.

The strategy was tested by measuring the confocal Raman response of a polymeric substrate with known thickness, a thin polypropylene (PP) film, supported on a glass slide. The PP film ( $n = 1.49$ ) was  $44\ \mu\text{m}$  thick, as measured with a Mitutoyo micrometer (model 395-271), with  $\pm 1\ \mu\text{m}$  precision. Good optical contact between the PP and glass was achieved by placing a drop of coupling oil between them, as suggested in Ref. 9. The coupling oil was purchased from Merck (B446082,  $n = 1.50$ ). The model sample, protected with the PE film on top through the above-described system, was mounted on the stage of a Raman microspectrometer (DILOR LabRam). Air evacuation was maintained during measurements. A drop of coupling oil was applied onto the surface of the protective film, as in conventional depth profiling with immersion optics, to minimize deviations of the laser path at the sample entrance. Due to its semicrystalline nature, PE resists well the contact with oils, although some whitening was observed after prolonged contact; in any case, the film can be easily replaced between measurements.

Intensity confocal profiles were measured by taking Raman spectra from different depths, moving the stage manually in the vertical direction ( $z$ ), through a micrometric screw, in steps of  $2\ \mu\text{m}$ . An immersion Olympus  $100\times$  ( $NA = 1.3$ ,  $210\ \mu\text{m}$  working distance) was the objective primarily used. Samples were excited with a  $16\ \text{mW}$  HeNe laser,  $632.8\ \text{nm}$  wavelength. The pinhole aperture was set to  $200\ \mu\text{m}$  (the maximum aperture is  $1000\ \mu\text{m}$ ). The nominal depth resolution in these instrumental

conditions was  $4.5\ \mu\text{m}$ , as estimated by scanning in the  $z$ -direction a silicon wafer immersed in the coupling oil.<sup>12</sup>

Figures 2A and 2B show as-measured confocal profiles of the model system. The depth scale corresponds to the nominal focusing depth ( $\Delta$ ) as determined from the micrometric screw; the zero corresponds to some arbitrary point within the coupling oil layer where depth profiling was originally started. Figure 2A shows the depth response of each of the components of the test system, through a plot of intensity of their specific Raman bands:  $\sim 1000\ \text{cm}^{-1}$  for the oil,  $\sim 1300\ \text{cm}^{-1}$  for PE, and  $\sim 815\ \text{cm}^{-1}$  for PP; all of them have been normalized with respect to its maximum value. The oil used as coupling fluid appears here in the  $0\text{--}10\ \mu\text{m}$  depth range, followed by the response of the PE protective coating, which extends from  $9.5$  to  $41\ \mu\text{m}$ , as determined from the full width at half-maximum (FWHM) of the profile. The apparent thickness of the PE coating is about  $29.5\ \mu\text{m}$ , in good agreement with the expected  $30\ \mu\text{m}$ ; this is not surprising, given the good optical contact between the coupling oil and the PE film, and with both similar refractive indexes. The response of the PP test film is found at higher depths ( $40\text{--}82\ \mu\text{m}$ ) and yields an apparent thickness of  $42\ \mu\text{m}$ , in good agreement with the nominal value ( $44\ \mu\text{m}$ ). We also see that the broadening of all the planar interfaces found (oil-PE, PE-PP, PP-glass) is rather similar, indicating that depth resolution remains fairly constant with focusing depth.



SCHEME 1. Schematic assembly of the components shown in Fig. 1.

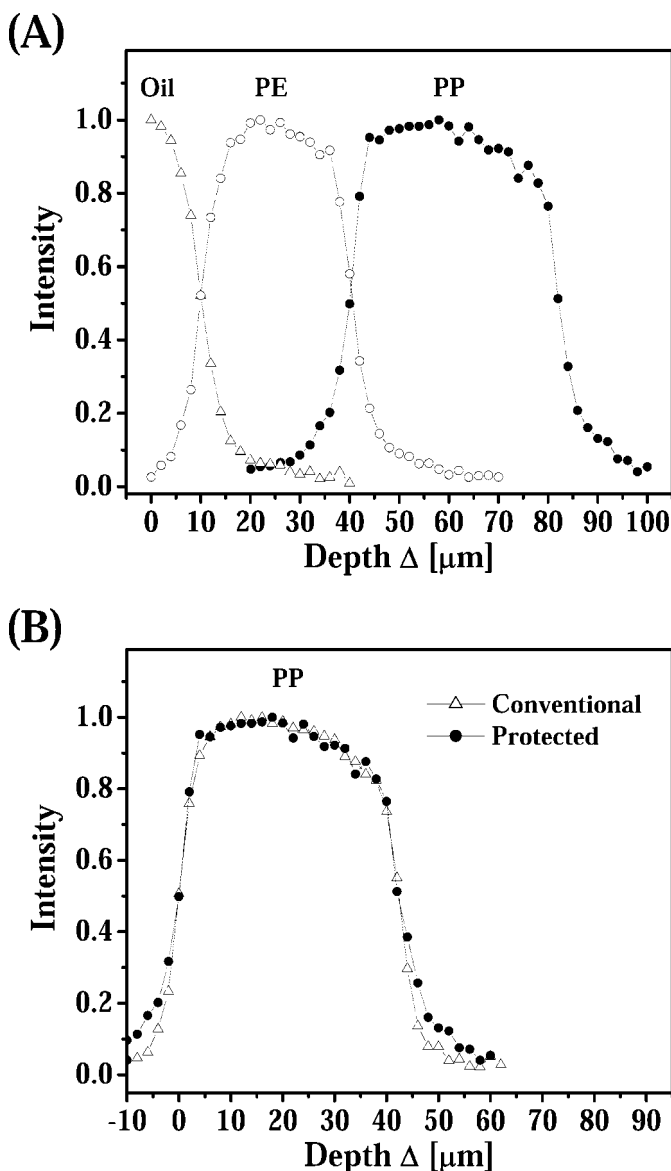


FIG. 2. (A) Confocal profiles of the test system; (B) Comparison of the confocal response of the PP film, as measured through the protective film and in the conventional way. In all the cases, the objective was an oil immersion Olympus 100 $\times$  (NA = 1.3), in conjunction with a confocal aperture of 200  $\mu\text{m}$  and a coupling oil with  $n = 1.5$ .

The FWHM of the corresponding derivative curves is, on average,  $\sim 6 \mu\text{m}$ , somewhat higher than the nominal depth resolution (4.5  $\mu\text{m}$ ), but satisfactory for the purposes of the work.

Figure 2B shows a direct comparison between the results obtained with the proposed approach and measurements carried out in the conventional way, i.e., using immersion objectives with the coupling oil directly applied onto the PP film. In the comparison, the PP responses have been shifted with respect to the maximum of the derivative curve of the respective intensity profile and have been scaled with respect to unity. We see that both strategies yielded practically the same confocal response. In terms of collection efficiency of Raman signal, we observed a minor decrease, about 10%, with respect to the conventional approach. Overall, the same good agreement was found for other PP substrates analyzed (films 25 and 65  $\mu\text{m}$  thick).

We conclude that this simple approach efficiently protects the substrate from potential damage by direct contact with the coupling oil, keeping at the same time the benefits of working with immersion optics: almost invariant depth resolution, close to the diffraction limit, and good optical throughput. There are some obvious limitations in terms of specimen dimensions and surface roughness of the sample, but they are not much more stringent than those required for carrying out conventional depth profiling with immersion objectives.

#### ACKNOWLEDGMENTS

This work is part of a project funded by ANPCYT (PICT06-1359) and AECI (A60606).

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## A Novel Wavelength Calibration for Fiber-Optical Spectrographs Based on the Grating-Diffractive Equation

ZHIMIN PENG, QIANSUO YANG,\*  
CHUN LIU, NAIYI ZHU, and ZON-  
GLIN JIANG

*Laboratory of High Temperature Dynamics, Institute of  
Mechanics, Chinese Academy of Sciences, 100080 Beijing,  
China*

Index Headings: **Fiber-optical spectrograph; Wavelength calibration; Standard line spectra; Charge-coupled device; CCD; Low-pressure mercury discharge lamp.**

#### INTRODUCTION

Fiber-optical spectrographs can be used for spectral measurement in a spectral area from the ultraviolet-visible to the near-infrared regions because generally the response wave range of the charge-coupled device (CCD) is from 200 to 1100

Received 5 December 2007; accepted 15 April 2008.

\* Author to whom correspondence should be sent. E-mail: qsyang@imech.ac.cn.