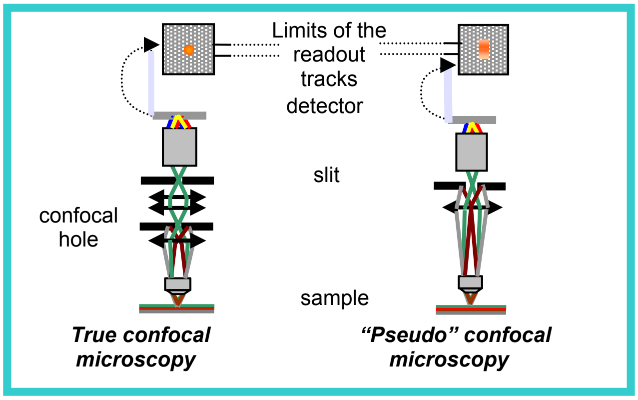
Study of Different Confocal Techniques

A - Confocal microscopy

The controversy about the different confocal techniques existing is still a current topic for Raman spectroscopists. Some consider the "true" confocal approach as too demanding from the alignment point of view. Therefore, other techniques have been suggested, among them, the so-called "pseudo" or "easy" confocal microscopy. Its basic principle consists in using the combination of a one-dimensional slit and CCD aperturing instead of a pinhole as the filtering element. This combination has been termed a "virtual" pinhole. Both techniques are claimed to yield the same results for depth and lateral resolution. The purpose of this paper is to objectively compare these two methods, considering both their advantages and their drawbacks.

B - Principle of confocal microscopy

The confocality principle is based on the selection of a restricted collection volume via various means (spatially with a pinhole or by CCD aperturing). This dramatically improves lateral and depth resolution by filtering out the signal coming from out-of-focus or adjacent regions. In certain cases, this may also lead to a significant reduction of fluorescence background generated by surrounding regions. The two main methods used by Raman manufacturers are illustrated below.



Orange spots show where Raman light is imaged on the CCD. In the method of true confocal microscopy, only light from the focal spot at the sample is passed by the pinhole. In the pseudo-confocal method, Raman light from material above and below the plane of focus is passed and only partially rejected by the entrance slit. Then a careful selection of the readout tracks on the CCD filters the light along the other dimension.

C - True confocal microscopy

This technique is based on the presence of an adjustable confocal hole in the image plane of the sampling point. This element enables a controlled and accurate definition of the probed volume by tuning the hole aperture. This optical scheme has often been criticised due to the purported difficulty in adjustment and sensitivity to mechanical drift. In reality any instability actually depends on the stability of the whole system. To avoid mechanical instabilities, Jobin-Yvon has developed a new generation of compact, and totally integrated systems known as the LabRAM family. In all of these new systems, measurements requiring long acquisition times such as high resolution 2D maps can be performed routinely.
In addition, the hole defines the precise reference axis for the whole system, while preserving the high throughput of the system even when using small apertures. As a result of the selection occurring in the pinhole plane, no stray light reaches the detector and the spot image on the detector is sharp. This eliminates possible crosstalk between adjacent volume elements of the sample. Reducing the number of CCD tracks that are read has the additional advantage of reducing the readout noise and the potential number of cosmic ray events.

Confocal line-scanning as a method for collecting confocal Raman maps

Finally the true confocal approach can also be applied to a line-scanning system that has been implemented in the Dilor XY and Jobin-Yvon's T64000. Because the confocality is achieved in these systems by optical filtering, a diffraction-limited spot can be scanned along the sample surface, an image of this line can be transferred to the entrance slit, and spectra from all points on the line can be collected simultaneously through an aberration-free spectrograph. Thus, a quick acquisition of a 2D image while preserving the high spatial accuracy is possible. Confocality provides optimum spatial resolution and multiplex detection enables minimisation of time required to capture the information.

D . .Pseudo. confocal microscopy

The pseudo confocal technique uses a slit and a computer-controlled detector aperture. The confocal operation is not achieved with hardware alone, but by closing the slit down, and at the very final stage of the optical system by selecting the relevant rows on the CCD. This approach is said to provide a "virtual" pinhole.
The proposed advantage of this technique is the easy adjustment of the "virtual" slit via software control of the CCD image. This method allows for a "correction" of small alignment drifts because the binning area necessarily has to be reset each time a new confocal experiment is set up. However, using control of the CCD to correct for alignment drifts is not a good approach as these drifts eventually result in a shift of the spatial origin of the Raman signal, thus the measured Raman spectrum is not from the area on the sample that the operator believes it to be.
The pseudo confocal method is not continually adjustable due to the finite size of the pixels, which provides less flexibility in the selection of the collection volume. Moreover, there is a risk of crosstalk between different sample regions as the slit is a single dimension spatial filter.

E . True versus pseudo-confocality

The purpose of the following measurements is to compare empirically the performance of these two techniques.

1 - Confocality measurements under different optical schemes

A common and relevant test to estimate the confocality performances of a Raman instrument is to move a flat absorber (such as silicon) in the Z-direction with respect to the focus plane. The resulting curve illustrates the variation in signal intensity versus depth and its FWHM is characteristic of the depth of focus and thereby of the confocality. Such a method was applied to evaluate the confocality under two system configurations, respectively simulating true and pseudo confocal methods.

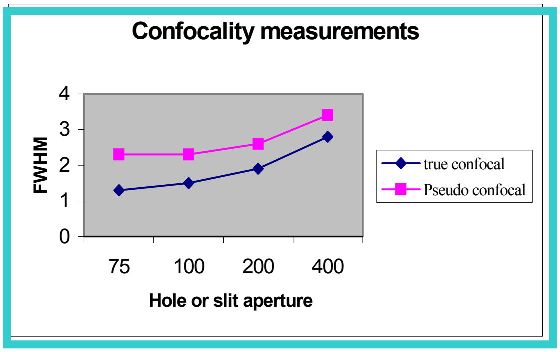


Figure 2 : Diagram showing the full width at half maximum of the curves of signal intensity versus depth for various hole apertures. These highlight the gain in depth discrimination under true confocal conditions.

The following diagram (fig.2) illustrates the resulting values for the depth f focus (i.e. .confocal depth.) in both cases. Obviously, the true confocal approach yields better results, that will automatically affect the application results.

2 . Analysis of a thin film under different optical configurations

<u>a- Principle of the experiment</u>

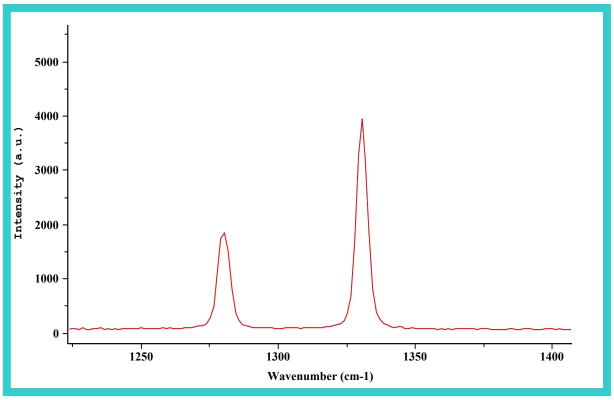


Figure 3:
Raman spectrum showing characteristic peaks of ^{12}C and ^{13}C

The sample consists of a small diamond chip with a 2 mm film deposited on the surface from 13C feeder gases. Using the automated z-piezo device, spectra were collected as a function of depth, using 0.1 mm increments. The diamond film from natural diamond (mostly 12C) is well separated from the 13C film; the respective frequencies are 1332 and 1284 cm-1.

The first figure shows a spectrum taken, under best confocal conditions (LabRAM equipped with the 1800l/mm grating, 514.5 nm laser excitation, 100X objective), where the film peak at 1284 cm-1 shows maximum intensity.

b - Results and discussion

Selecting the Raman band relative to the top layer, depth profiles are generated. The following figure (fig.4) shows typical intensity profiles obtained respectively for the top layer and the substrate, versus depth. Taking into account the magnification factors between the different optical components, the slit and hole apertures are set in order to establish a correspondence between the two methods. To perform the pseudo-confocal measurements, the confocal pinhole was removed from the Raman path. The recording was started from 2 to 5 µm above the surface in order to show the increase in signal as well as decrease below the film.

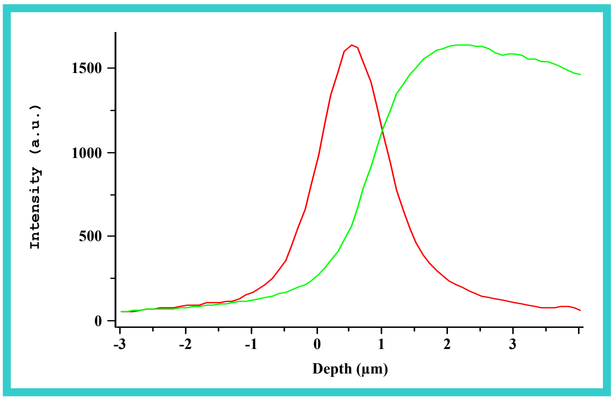


Figure 4: Intensity depth profiles of the ^{13}C film (red) and ^{12}C substrate (green)

True confocal				Pseudo-confocal			
Hole	**Slit**	**Binning**	**FWHM**	**Hole**	**Slit**	**Binning**	**FWHM**
400	160	147-156	2.52	removed	80	153-156	2.87
200	160	147-156	1.51	--	40	154-155	1.78
100	160	147-156	1.15	--	20	155	1.65
50	160	147-156	1.08	--	10	155	1.64

Table 1

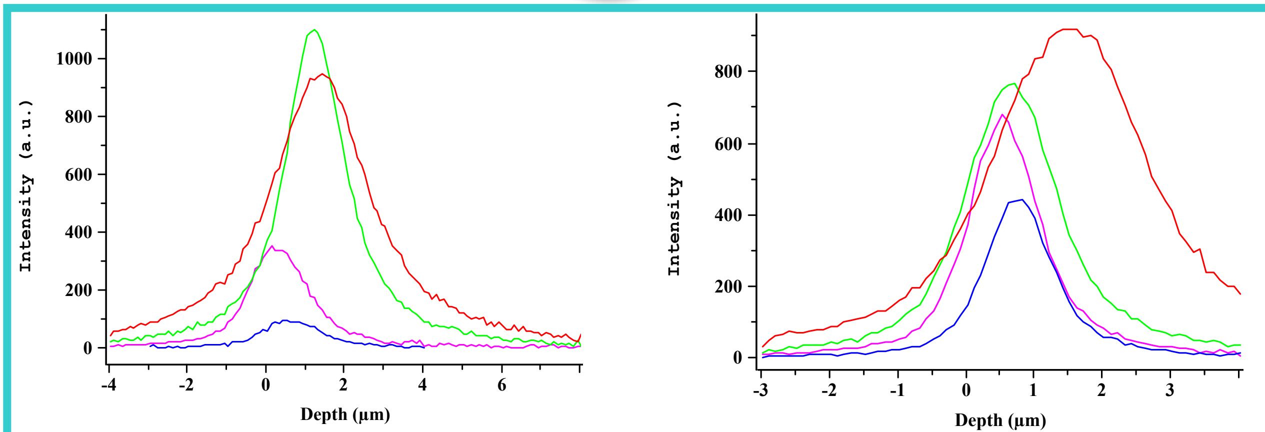
Theoretically, for the system in use, the magnification factors gives :
1 ∝m at sample Æ 140 ∝m at hole Æ 28 ∝m at slit Æ 28 ∝m at CCD
This determines the CCD readout region with respect to the pixel size.

The profiles shown in figure 5 result from depth profiles acquired under various confocal conditions. These are summarised in table 1. The z interval considered for the different measurements varies as, by closing the pinhole for instance, the maximum film signal is achieved at a location closer to the surface. By fitting the resulting profiles, one can derive an approximate value for the film thickness defined as the full width at half maximum.

The calculated values are given in table1. These results clearly show that, even with a rather large binning, the true confocal approach gives better results. The difference gets larger when going to more and more limited apertures (below 100 ∝m in the confocal mode). Contrary to what was expected, the reduction of the readout region in the pseudo-confocal case does not give rise to a large decrease of the signal; this is certainly due to the lack of confocal discrimination of the out-of-focus signal.

The similar values obtained with the slit at 20 and 10 ∝m for the pseudo-confocal arrangement, and 100-50 ∝m for the hole in the confocal set-up, indicate the depth resolution of each method. The values obtained under optimum conditions (1.64 vs. 1.08∝m) in both cases were smaller than the nominal 2 ∝m thickness. However, if one accounts for the optical shift induced by the refractive index of the material, the actual thickness of the film would be 1.17 ∝m. This value is then very close to that obtained under true confocal conditions (1.08-1.15 µm).

For very thin films, thickness measurements therefore require an ultimate depth discrimination that only true-confocal microscopy can offer.



	red	green	magenta	blue
hole (µm)	400	200	100	50
slit (µm)	80	40	20	10

3 - Application example: Depth profiling of a laminar polymer sample

The investigation of multilayer transparent samples is of major concern in the polymer industry. The confocal microscope allows such Raman measurements without any sample preparation, giving access to the interface and layer chemical properties. The following results concern the analysis of a 75µm-laminar film made of 2 layers of polyethylene sandwiching a middle nylon layer. Mapping in the XZ plane was carried out by recording spectra by point imaging along a line in the X direction and repeating that at different depths separated by 5µm. This provides the same type of information as the measurement over a cross section of the sample but does not require any sample preparation; in this case this could be quite important because of the difficulties encountered in microtoming polymers. The results shown in the figure 6 illustrates the confocal approach when high depth spatial resolution is required.

F - The confocal hole : a reliable ally for optimal spatial resolution.

The comparison tests performed using a true confocal and a pseudo-confocal configuration showed that when it comes to very critical issues for which ultimate resolution is required, the true confocal approach yields better results.

This is attributed to the superior spatial filtering of the confocal hole that gives rise to a sharper and non-distorted image on the CCD detector, resulting in higher spatial resolution in chemical maps.

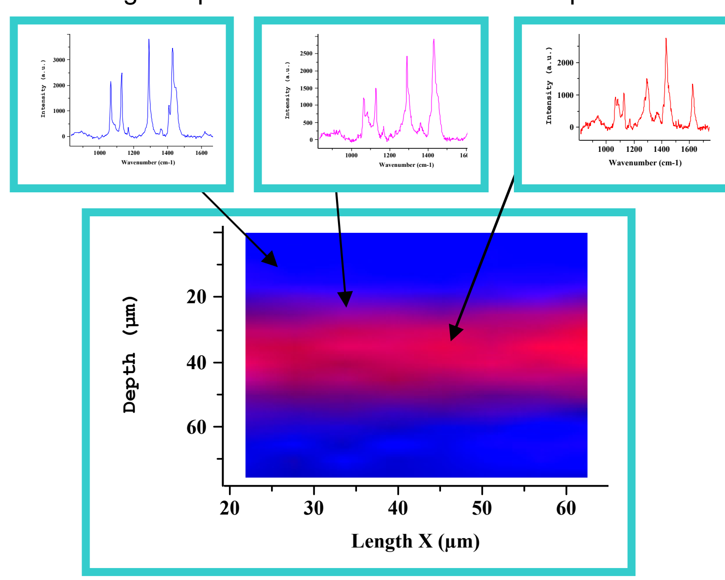


Figure 6. 2D map generated from spectra collected from different depths within the multilayer structure. This image highlights the sharp discrimination between the different polymer layers that true confocal microscopy can achieve.

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