NOTES

Use of a Microscope Objective Corrected for a Cover Glass to Improve Confocal Spatial Resolution inside a Sample with a Finite Index of Refraction

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INTRODUCTION

The Raman microscope has gained wide recognition in the last years for its ability to combine molecular/crystalline spectroscopy with the spatial resolution inherent in optical techniques (better than 1 μm). In particular, it is attractive for identifying and/or characterizing small amounts of material included in matrices and for measuring interfaces in laminated structures. Contaminants in manufactured products[1] and fluid inclusions[2] in minerals are two areas where the technique has shown to be particularly useful. Depth profiling without sample preparation is also an area being pursued. However, it has been shown that the predicted confocal (vertical) spatial resolution is degenerated inside a material with index of refraction greater than 1,[3–9] and the loss increases with the depth of examination.

The source of this error is the optical system itself. The microscope objective performs best when used in a manner consistent with its design. Typical objectives supplied with Raman microscopes are metallographic objectives that are engineered to correct optical aberrations of light passing through air (medium with index equal to 1). Clearly, when probing inside a material with an index different from 1, these corrections will no longer work, which is the source of the degradation in confocal performance.

EXPERIMENTAL

The first proposal for recovering confocality was to use immersion objectives.[10–12] These objectives are meant to be used with a film of water or oil between the sample and the last optic of the objective. Note that the index of water is 1.33 and that of many oils is close to 1.5, which is in the range of many organic materials. This solution has been shown to provide the predicted improvement, but one is left with the issue of chemical compatibility with the sample. For example, if one is trying to measure hydration of skin as a function of depth, introduction of water on the skin surface can result in diffusion of the water into the underlying layers, thus putting the measurement in question. Use of an immersion oil could likewise potentially interact with the skin's structure.

A second solution that has been recently tested and the results of which are shown below is to use a dry objective with cover slip correction. Such an objective is designed to correct for a layer of glass, index about 1.4, above the sample. This actually should be an ideal solution for studies of fluid inclusions where the inclusions are always embedded in a mineral matrix whose index of refraction is at least 1.4 (quartz, for example, has an index of refraction of 1.54).

An Olympus UPLAPO 60× objective (NA = 0.9, working distance = 0.2 mm above cover glass, thickness 0.11–0.23 mm, producing a 100% flat field, corrected from the blue to the red range of the spectrum) was tested on the LabRAM (equipped with a HeNe laser and an 1800 grooves/mm grating). The sample was the CO_2 bubble in an H_2O–CO_2 fluid inclusion (containing 5 mol% CO_2) in a quartz matrix. The UPLAPO 60× objective has an adjustable collar that enables the user to select parameters corresponding to corrections for variations in the ''glass cover slip thickness''. In this case measurements were made over the range of the collar in order to determine the effect of this parameter on the measurement.

All measurements were made on the gas bubble (about 15 μm in diameter, see Fig. 1) in an inclusion that was about 40 μm long and 15–20 μm wide.

The signal with the UPLAPO objective was so intense that it was possible to maximize the signal in real time by adjusting the focus of the stage. The spectrum recorded with the ring set for a 160 μm thick cover glass is shown in Fig. 2. The strong bands at 1285 and 1388 cm^{-1} are assigned to CO_2, as are the weaker side bands at about 1265 and 1409 cm^{-1}. Measurements were made for a series of positions of the ring, tabulated, and then plotted in Fig. 3.
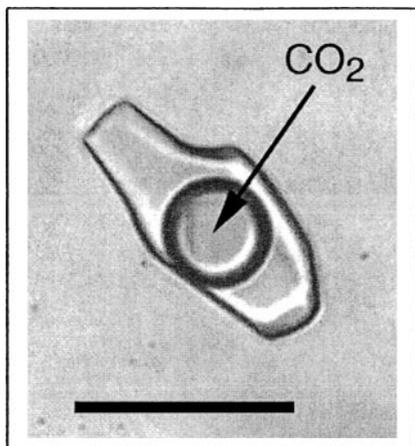
FIG. 1. Optical micrograph of a synthetic fluid inclusion (H_2O–5% CO_2) in a quartz matrix. The dimensional bar is 30 μm long.

FIG. 3. Peak intensity of the Raman signal at 1385 cm^{-1} as a function of position of the adjustable collar on the UPLAPO 60× objective. Also shown are the intensities of this line as measured by the dry 100× (NA = 0.9) and oil immersion (NA = 1.4) objectives.

The signal was also measured with the metallurgical 100× dry and 100× oil immersion objectives, as noted in the figure. What is immediately apparent is that the signal with the objective corrected for glass over the sample is much stronger than that of the dry objective. The fact that the signal from the metallurgical objective could not be maximized in real time because it was too weak does not really affect the results in Fig. 3 because real-time focusing only improved the signal by about 12–15%. The signal for the 130, 140, and 150 μm thicknesses were repeated in random sequence in order to confirm the behavior in that region. The dip at the 150 μm setting was reproducible.

In order to estimate the depth of the inclusion, it was subsequently examined optically with the 100× dry objective. The difference between the mechanical position for focus at the surface of the mineral and at the inclusion was about 100 μm. That makes the depth of the inclusion about 154 μm below the surface (using the simple calculation of real depth = measured depth × n). Curiously, this value is centered at the double peak in Fig. 3. It has been suggested by one of the reviewers that this anomaly results from the strong reflection of the laser at the interface of the inclusion.

CONCLUSION

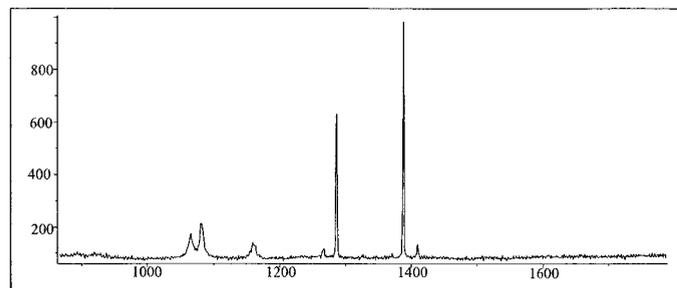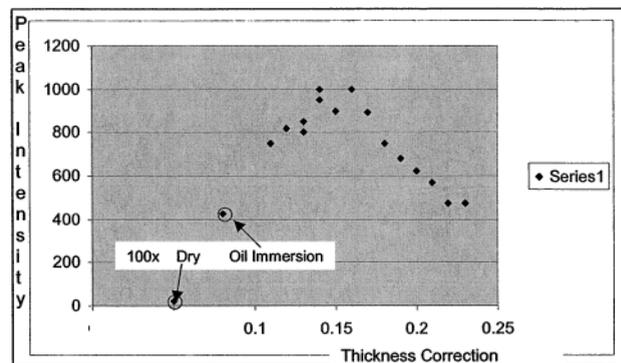This simple study shows that it is possible to improve the confocality of the Raman microprobe when examining materials below their surfaces by selecting an appropriate type of optic. This study shows that the UPLAPO 60× objective with correction collar for variable "cover slip thickness" can be very useful in maximizing the signal intensity by compensating for the non-unity index of refraction of the sample matrix, thereby improving the focus at the spectrograph slit. Equally as important is the improvement in spatial resolution when measuring the depth of an interface, as well as its sharpness.

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FIG. 2. Raman microprobe spectrum of the gas bubble in the inclusion shown in Fig. 1, recorded with the UPLAPO 60× objective, with the collar set for 160 μm nominal matrix thickness.

Surface Preparation and Direction Dependence of Diffuse Reflectance Infrared Fourier Transform Spectra of Wood

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