1764 CD
DOI: 10.1017/S1431927606066943

Microsc Microanal 12(Supp 2), 2006

The Development of a Spatial Resolution Standard for IR Microspectroscopy: A preliminary Investigation

Luis Lavalle, David Oertel and André Sommer.

Molecular Microspectroscopy Laboratory, Department of Chemistry and Biochemistry,
Miami University, Oxford, OH 45056.

Infrared microspectroscopy has experienced tremendous growth over the past two decades in regard to the types and variety of problems this method is being applied to. The method is now very mature and the standard microscope allows one to obtain both one and two dimensional images of the samples being investigated. These images convey to the user molecular information as a function of spatial position in the x-y plane of the sample. However, the reliability of this information is not known unless the microscopes spatial resolution is measured by using a structured sample with known dimensions. This paper will look at preliminary considerations for the design of a standard sample.

The spatial distribution of light at the focus of an infrared microscope, assuming a circular aperture, is given by a first order Bessel function [1, 2]. This distribution, commonly known as an Airy diffraction pattern, is depicted in Figure 1 for an arbitrary wavelength. The distance between the first minima of the large central maximum can be related to experimental parameters via the well known equation:

$$d = 1.22\, \lambda / n_1 \sin \theta \qquad (1)$$

where λ is the wavelength of light, n_1 is the refractive index in which the measurement is made (typically air, n_1=1.0), and θ is the half angle acceptance of the objective or condenser. The large central maximum contains 84% of the intensity of the original light with the remaining 16 % lying at distance greater than the first minima. In practice one can determine "*d*" by stepping a metal edge or an infrared absorbing film through the focused spot and plotting the normalized energy or absorbance as a function of position. In doing so one is basically integrating the function which yields a step function (also shown in Figure 1). The value of "*d*" can then be extracted from the step function by measuring the distance at which the intensity is 0.08 (8%) to that at which it reaches 0.92 (92%). In practice, the 5% and 95% values are usually taken [3].

Initial experiments were conducted using two different test samples. These included a razor blade edge, and a patterned photo-resist on a 2 mm thick barium fluoride substrate. The razor blade was chosen due to the facts that it was readily available and presented a high contrast edge. However, the patterned photo-resist simulates a real world sample but is more difficult to obtain. Figures 2 and 3 show the step functions obtained at a wavelength of 6 micrometers for both samples. The step function of the razor blade is not optimal since the high absorbance side is severely degraded. The extracted value for "*d*" in this case is 202 micrometers when it should be (ca) 12 micrometers. As of this time we suspect that the degradation may be due to diffraction effects or a non-linear detector under low light level conditions.

For the photo-resist sample both of these drawbacks are circumvented. The step function in Figure 3 is better defined, however, there is an anomaly again on the high absorbance side. That is, when the infrared beam begins to enter the photo-resist. This anomaly arises from specular reflection off the edge of the photo-resist and is a consequence of the optical index mismatch between the resist and air. This effect has been used in the past to study the nature of the interface [4]. The extracted value for "*d*" in this case is 167 micrometers when it should be (ca) 18 micrometers.

Figure 4 illustrates the step function obtained on the photo-resist but with a drop of Nujol oil placed on the sample and a 1 mm thick barium fluoride window placed on top of the resist and Nujol. The sample is, in effect, a cross-sectioned bi-layer laminate in which the refractive index disparity has been drastically reduced. One can observe that the step function is relatively clean and agrees with what is expected experimentally. The extracted "*d*" value is 37 micrometers which compares more favorably to the theoretical value of (ca) 18 micrometers.

In conclusion, the results presented in this investigation show that care must be taken in the design of a spatial resolution standard for infrared microspectroscopy. For example, although sin θ is approximately 0.6 for most microscopes and equation 1 reduces to $\sim 2\lambda$, when the sample is mounted on a window, the angle of light impinging on the sample is reduced as a result of the refractive index of the window. In the present example the angle of light is reduced to 25 degrees resulting in a theoretical spatial resolution of 18 micrometers. The data also illustrate that sample structure considerations are also extremely important.

References

[1] W. Driscoll, Handbook of Optics, First edition, McGraw-Hill, 1978.
[2] M. Born and E. Wolf, Principles of Optics, Third edition, Pergamon Press, 1965.
[3] D. J. Gardiner, M. Bowden and P. R. Graves, Phil. Trans. R. Soc. Lond. A 320 (1986) 295.
[4] R. Bhargava, S. Wang and J. Koenig, Appl Spec 52 (3) (1998) 323.

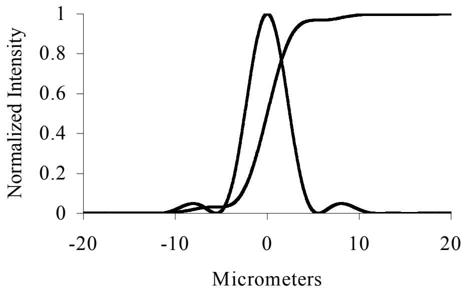

Figure 1: Airy Diffraction Pattern and Step Function

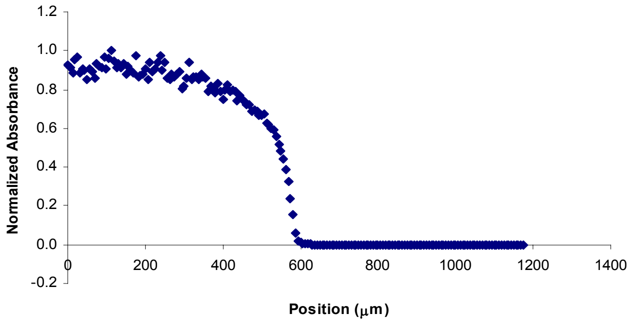

Figure 2: Blade Step Function

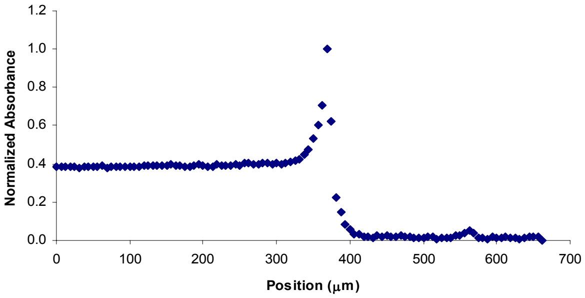

Figure 3: Photoresist Step Function in air

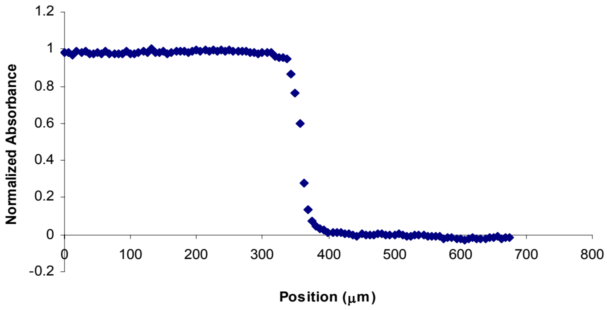

Figure 4: Photoresist Step Function immersed