FTIR Microscopy: How experimental decisions affect the signal-to-noise ratio

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A high-performance FTIR microscope is characterized by its ability to analyze small materials—a combination of high spatial resolution and a good signal-to-noise ratio (SNR). The spatial resolution results from high-quality optics, especially the objective and condenser. The SNR depends upon the design of the optics, including the source, interferometer, beamsplitter, and detector; the efficiency of the microscope optics (internal mirrors, the objective, and condenser); and the data treatments, including averaging, apodization, spectral range, and smoothing. A high spatial resolution and a good SNR are necessary to generate meaningful spectra, especially for samples below 10 microns in size or for monolayers on reflective substrates. Moreover, it is important to recognize the trade-offs between SNR and user-settable parameters, including number of scans, spectral resolution, and aperture size, as demonstrated in this note using a Thermo Scientific™ Nicolet™ RaptIR™+ FTIR microscope.

SNR is customarily measured through open beam experiments, where both a background and sample spectrum are obtained with no sample present in the IR beam. When ratioed, these should theoretically obtain a straight line at 100% transmittance. Any variation around that value is noise, which is quantified as the root-mean-square (RMS) noise. The S/N ratio is therefore defined as $\frac{100}{RMS\ noise}$.

$$\frac{S}{N} = \frac{S_{avg.}}{\sqrt{n\sigma^2/n}} = \sqrt{n}\,\frac{S_{avg.}}{\sigma} = \sqrt{n}\,\frac{100}{RMS\ noise}$$

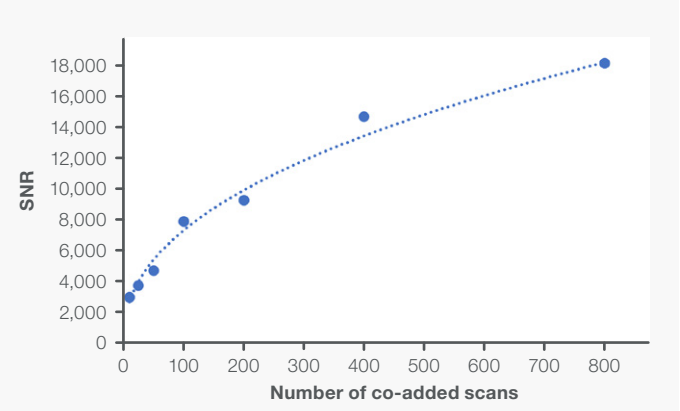


Figure 1. Signal-to-noise ratio as a function of the number of co-added scans.

SNR is often measured between 2550-2500 cm^{-1} where single beam signal intensity is the strongest and there is no water vapor or CO_2 interference. All the results shown in this note were obtained in this spectral range. SNR as a function of the number of co-added scans and spectral resolution are shown in Figures 1 and 2, respectively. SNR increases as the number of scans increases and exhibits a $n^{1/2}$ relationship, but the total analysis time also increases n-fold.

SNR decreases as the spectral resolution increases. In order to achieve a higher resolution, the mirror inside the interferometer must travel a longer distance for a single scan. Therefore, fewer scans can be recorded in the same time period. If fewer scans are recorded, the SNR of the resulting spectra will decrease.

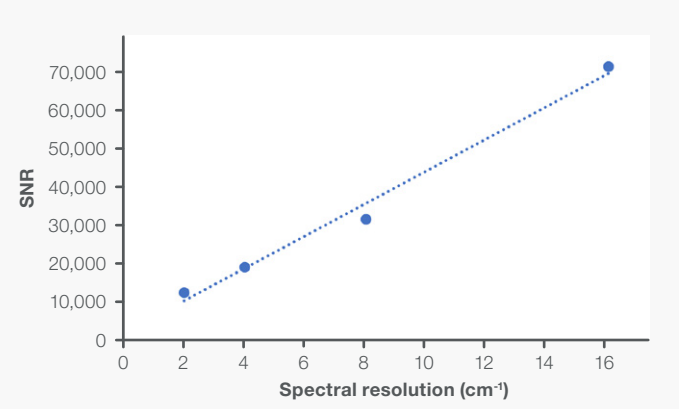


Figure 2. Signal-to-noise ratio as a function of spectral resolution.

FTIR microscopy combines the power of an optical microscope with the analytical capabilities of FTIR spectroscopy and provides chemical annotation to spatially distributed sample composition or small features, usually within 10 to 100 micron range. Spatial resolution is the fundamental characteristic determining why use of a microscope is necessary rather than a spectrometer alone.

Because the beam size is typically larger than desired spatial resolution, an aperture (or an effective aperture in an array detector) is used to define the smallest area on the sample represented by a single spectrum. While a smaller aperture (or effective aperture) provides better spatial resolution, as it gets smaller, it limits the amount of infrared light that reaches the detector, resulting in weaker signals and a loss of SNR, as shown in Figure 3.

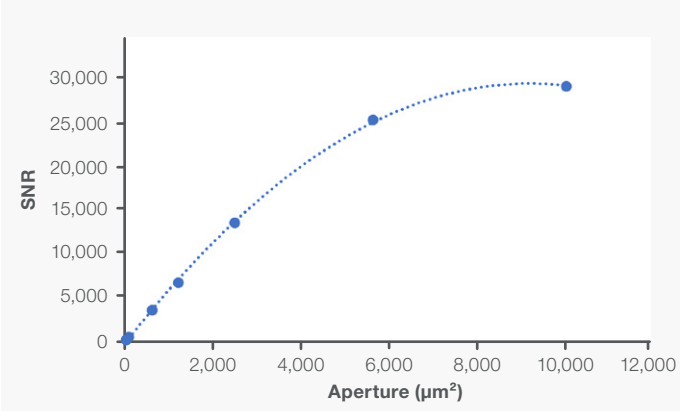
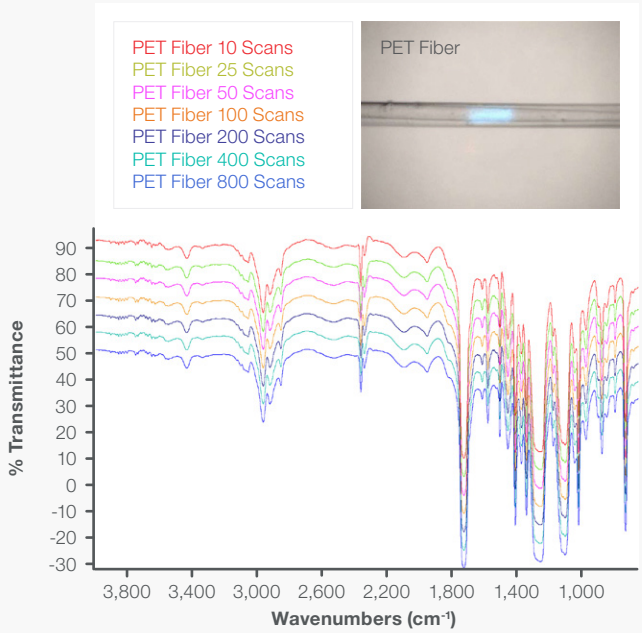


Figure 3. Signal-to-noise ratio as a function of aperture size.

FTIR microscope analysis often involves the identification of chemical species through library matching, whether the analysis is a single point measurement or imaging across a sample. Figure 4 illustrates an example of analyzing a PET fiber. By increasing the number of scans, the SNR increases accordingly. However, there is no discernible difference in the resultant spectra. More importantly, there is no correlation between SNR and Library Match Value. Between 10 scans and 800 scans, the SNR increases ~6× at the expense of 80× longer experiment time (result not shown). The improved SNR has no impact on the identification. Note that the example here is not to underplay the importance of SNR. In the cases where a sample has low IR absorbance or low concentration components, high SNR can be crucial to detect, identify and quantify the sample.

In this note, we have demonstrated the intrinsic trade-offs between SNR and most common user-settable parameters in FTIR microscope experiments, including spatial resolution, spectral resolution, and scan numbers. A monomaniacal pursuit of SNR is not only impractical but also has diminishing returns for library matching. Low-spatial resolution spectra with high SNR can be easily obtained even with simple microscopes. High SNR can also be obtained by increasing the number of co-added scans or reducing spectral resolution, resulting in prohibitively long analysis time or potential loss of valuable spectral information. When considering FTIR microscopes, the critical performance needs for a specific application must be clearly defined. It is only when the combination of high spatial resolution and good SNR is obtained that users can accomplish the ultimate goal of microanalysis of materials.



Number of scans	S/N	Library match value
10	2,989	93.93
25	3,765	93.78
50	4,713	93.81
100	7,914	93.80
200	9,304	93.85
400	14,723	93.80
800	18,222	93.85

Figure 4. FTIR microscope analysis of a PET fiber. Top: spectra obtained with different number of co-added scans. Bottom: signal-to-nose ratio of the spectra and their respective Library Match Value.

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