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Raman Microscopical Analysis Made Easy

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Raman microscopy has historically been difficult to perform due to the high level of instrument complexity. Calibration of the wavelength axis has been probably the most tedious part of operating a Raman spectrometer, where user intervention was required and had to be performed at least daily. The SureCal approach to wavelength calibration conducts the calibration automatically to better than 0.2 cm^{-1} accuracy and precision without the necessity of user intervention. This patented method utilizes a Neon lamp that travels coaxial to the excitation laser and impinges onto the detector and is analogous to the HeNe laser reference used in FTIR spectrometers. Since the calibration is highly effective, single frequency or frequency stable lasers are no longer necessary for careful Raman spectroscopy, thereby significantly reducing the costs without any sacrifice in performance.

Fluorescence can be very problematic, when attempting to conduct Raman measurements. Many dyes and pigments, as well as textiles and active drug ingredients, exhibit fluorescence. On occasion, longer excitation wavelengths may be employed to circumvent fluorescence with limited success. The Raman scattering efficiency drops off as a fourth power as the wavelength is increased, thereby reducing sensitivity. A patented Automatic Fluorescence Rejection (AFR) method[2] has been used for rejecting fluorescence. This novel method drives the excitation laser at two different temperatures or current to generate two spectra. These two spectra are subtracted one from another to generate the Raman difference spectrum, where the fluorescence is significantly rejected. The Raman difference spectrum is then integrated yielding the desired Raman spectrum without fluorescence. As shown in Figure 1, for acetaminophen, a fluorescent background can be rejected without degradation of the Raman spectrum of interest.

Confocal Raman depth profiling has become an important capability for analyzing samples without cross-sectioning or otherwise damaging the sample. A novel optical design (patent pending) allows for depth resolution of better than two microns. A confocal aperture is placed in an image plane to reduce the sampling depth of field or collection volume. Only the in-focus and on-axis light rays are recorded by the spectrograph system as the out-of-focus and off-axis light rays are blocked by the confocal aperture. Confocal optics restrict the sampling depth to a region smaller than for conventional optics. In addition confocal measurements can also improve the rejection of stray light and reduce fluorescence interference. The pinhole that is utilized to achieve the high degree of confocality resides on a slide with slits that when engaged provide high throughput. Switching between confocal and high throughput modes of operation can be accomplished by simply selecting the desired pinhole or slit size in the software, as evidenced in Figure 2.

1 - U.S. Patent 6,141,095, 2 – U.S. Patent 6,281,971, 3 – U.S. Patent Pending

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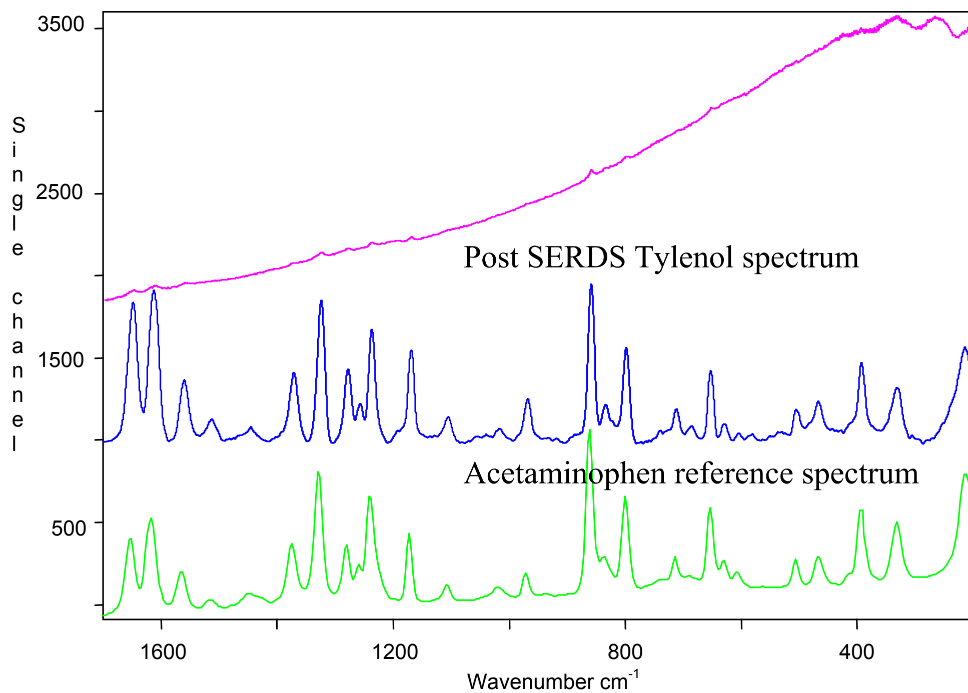

Figure 1 – Tylenol tablet Raman spectrum at 785nm (top), after rejection of fluorescence using SERDS (middle), and acetaminophen reference spectrum (bottom).

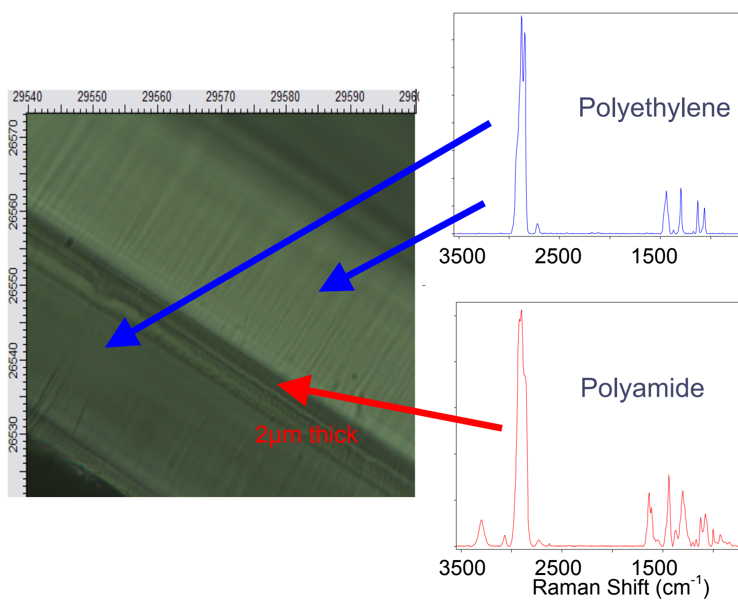

Figure 2 – Confocal depth profile of a multilayer polymer film. The middle polyamide layer was 2 microns thick and could be analyzed without contamination from adjacent layers.

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