A132 Micro-Absorption Spectroscopy as a Non-Destructive Tool for Forensic Analysis

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The goal of this presentation is to illustrate a new method for forensic analysis on the micron scale that combines absorption spectroscopy and confocal microscopy. Results on the characterization of representative samples, including sensitivity and linear dynamic range of micro-absorption measurements will be presented.

This presentation will impact the forensic science community by illustrating a method that uses spatially resolved absorption spectroscopy on a micron scale. It may improve trace analysis of samples that are obtained in micron size with minimal sample preparation.

The ability to investigate samples at a micron level with non destructive probes is a key factor in forensic studies. Fluorescence probes employing confocal or other geometries are available; however they generally require labeling and are limited by photo-bleaching and quenching. On the other hand, micro-spectroscopy based on absorption measurements provides a convenient label free way for characterizing an unknown material. A new experimental approach for micro-absorption spectroscopy is developed and applied to the investigation of fluid and solid samples relevant to forensics. By exploiting the spatial variation of the intensity due to Beer-Lambert's law ground state absorption spectra with a spatial resolution better than 1.4 micron in the lateral and 3.6 micron in the axial direction is measured. A confocal detection system is employed to probe and spectrally resolve the attenuation of a white light beam in the axial direction. It enables the measurement of absorption spectra of biological assemblies at the single cell level and small samples with a thickness of few microns. Confocal absorption microscopy is nondestructive and is capable of collecting both spatial and physical information based on light absorption by microscopic structures.

The quantities of samples often obtained in crime investigations are minute. Micro-absorption spectroscopy enables measurements on smaller sample volumes and with rapid acquisition time on the timescale of seconds. To examine the sensitivity of the technique, absorption spectra of nanoliter solutions in micro-capillaries with a pathlength of 50 microns are investigated. Through measurements of the transmitted intensity in calcein dye solutions at fixed pathlengths, it is established that the absorbance varies linearly with concentration over the range from 0.1 to 2 mM. Preliminary results indicate detection limits of better than 0.1mM in a sample volume of less than a picoliter.

The technology has been used to analyze micro-fibers and can be employed to distinguish the fibers from the known and suspected material at the molecular level. Another important aspect that is addressed is to acquire a spectrum of an individual hair, which is difficult using a Raman spectroscopic probe as it may lead to damage of the sample. As an application to the analysis of biological samples at the single cell level, the visible absorption spectrum of hemoglobin in a single live red blood cell (diameter ~ 7 microns) is measured under physiological conditions. Spectroscopic changes due to heme degradation under pathological conditions are investigated. Variations in the composition of inhomogeneous samples (e.g., thin films) can be determined from spatially resolved absorption spectra. Extensions of the micro-spectroscopic method to the ultraviolet and infrared regions of the spectrum will be discussed.

Micro-Absorption Spectroscopy, Nano-Liter Samples, Non-Destructive

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