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# Experiments in Raman spectroscopy of hair: Exciting light and molecular orientation

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**Synopsis**

Hair fibers have been analyzed by Raman spectroscopy to assess the suitability of a range of excitation wavelengths for data collection over extended periods of time. It is found that the optimum excitation wavelength for spectral detail, of these tested, was 780 nm and that this wavelength resulted in little signal degradation over time. It was found that with higher energy excitation sources that the signal intensity of the spectra degraded significantly in short periods of time. This work suggests that near-IR Raman spectroscopy therefore offers the most suitable conditions to analyze the nature of secondary structural feature in hair fibers.

In addition, a preliminary exploration of the structural orientation of spectral features of the hair was attempted. Through the use of a linearly polarized excitation source the intensity of the Raman spectral features were observed to change as the alignment of the fiber axis with respect to the plane of polarization was changed. It was found that the spectral features associated with the α-helical vibrations decreased in intensity as the fiber axis was rotated from an orientation parallel to the exciting beam.

## INTRODUCTION

Sensorial properties of hair, specifically the tactile and visual attributes, are related primarily to the surface morphology and mechanical properties of single hair fibers. Reliable quantification of fiber properties at various length scales is therefore critical in the prediction of sensorial behavior within the personal care industry. In particular, characterization of fibers at the micron and sub-micron scale—correlating local mechanical properties with chemical composition—will lead to an improved understanding of the behavior of hair, and will assist in the design and development of hair care technologies.

Raman spectroscopy is a technique commonly used to study vibrational, rotational and other frequency dependent structural motion within simple and complex molecular systems. The technique is commonly used in protein chemistry as the vibrational information is specific for different chemical bonds and environments within molecules but also has applications for the exploration of protein films, and as in the experiments detailed below, protein fibers.

A wide range of physical processes and chemical and treatments alter the tactile and visual characteristics of hair and these high order changes have been reported to be accompanied by changes in the frictional properties, chemical composition and mechanical response.

Raman spectroscopy has been used previously to investigate changes to keratin fibers after chemical and physical treatments (1–4) and this paper describes two outcomes from a series of preliminary experiments scoping the suitability of the technique to hair fiber analysis.

Consequently, Raman spectroscopy has been applied to obtain baseline measures with the intent of examining the structural phenomena associated with the chemical and physical changes further in a continuing series of experiments. The first objective of this piece of the work was to optimize experimental parameters for spectra quality and collection times of Raman spectra. It is well known that Raman signal intensity of hair should increase significantly with a shorter excitation wavelength (3) as Raman signal intensity is proportional to the fourth power of the inverse of the incident wavelength. However, the background luminescence would also be expected to increase significantly with shorter excitation wavelengths (5).

Secondly, an investigation has been carried out to determine whether methodologies that have been applied to determine molecular order in commercial polymers can be applied to hair fibers. The Raman spectra, obtained using a polarized light source, of commercial polymers have been shown to exhibit sensitivity to orientation and this methodology has previously been used to estimate molecular orientation of polypropylene in spun fibers (6). Similar methods have been applied here.

## EXPERIMENTAL

Virgin yak hair samples were supplied to Avacta Analytical Ltd. (York, UK) for the collection of Raman spectra. As in previous work, yak hair has been used as a pigment-free representative model of human hair reducing the problems that are encountered from background fluorescence.

To determine the optimum collection protocol laser wavelengths of 780 nm, 633 nm (red) and 514 nm (green) were selected. A Renishw® System 1000 Raman Microspectrometer was used. The detector slit was set at 15 μm and the binning box to 20 pixels wide. A quarter wave plate was used in the excitation path to make the light incident to the sample approximately circularly polarized to minimize variations in band intensities due to polarization/orientation effects. The fiber was orientated at no specific alignment to the light source. The system was calibrated using a set of emission lines from a neon lamp and the system performance verified using a standard silicon sample. The accuracy of the spectra is within approximately ±1 $cm^{-1}$. The samples were secured onto a microscope slide, which was placed onto the Raman microscope and the surface focused using a 50×, 0.75NA microscope objective.

To explore the fiber orientation characteristics the Raman microspectrometer was set up as described above, though a quarter wave-plate was not used; the light incident to the fiber was therefore linearly polarized. The laser wavelength selected was 780 nm. The fiber longitudinal axis was orientated parallel to direction of polarization of the beam and the spectra recorded. The samples were then rotated 90° so that the longitudinal axis was perpendicular to the direction of polarization and further spectra recorded. Though measurements were made at different rotations it was ensured that measurements were made at the same position of the fiber surface. Spectra were recorded using the synchronous scanning mode, covering the spectral range from 200–1700 $cm^{-1}$ with an exposure time of 60 seconds.

## RESULTS AND DISCUSSION

The laser of wavelength 780 nm was considered as the control for this set of experiments and compared to the spectra obtained at 633 nm (red laser) 514 nm (green laser). The time series spectra for each of three excitation wavelengths are shown in Figures 1, 2 and 3.

The analysis performed to determine the most suitable laser wavelength selected for data collection examined a spectral region that has a flat background level: 1550 $cm^{-1}$ +/− 10 $cm^{-1}$. The mean signal intensity at this spectral position was determined and plotted against the integrated exposure time of the fibers to the excitation laser. This is shown on a log-linear plot in Figure 4.

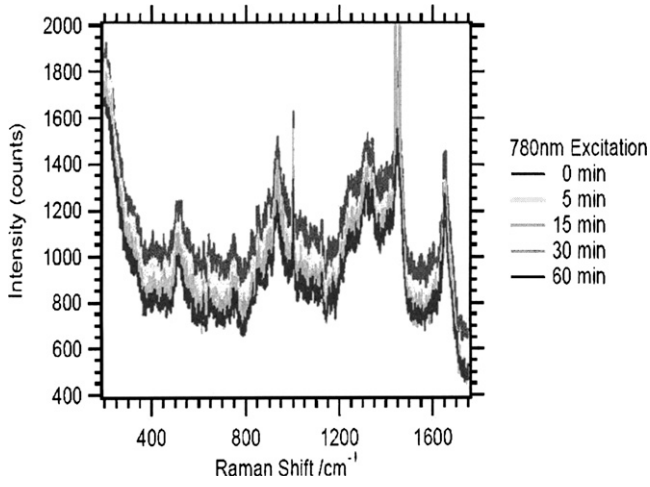


**Figure 1.** Spectra collected at different time points with 780-nm excitation. Spectra decrease in intensity with increasing excitation time.

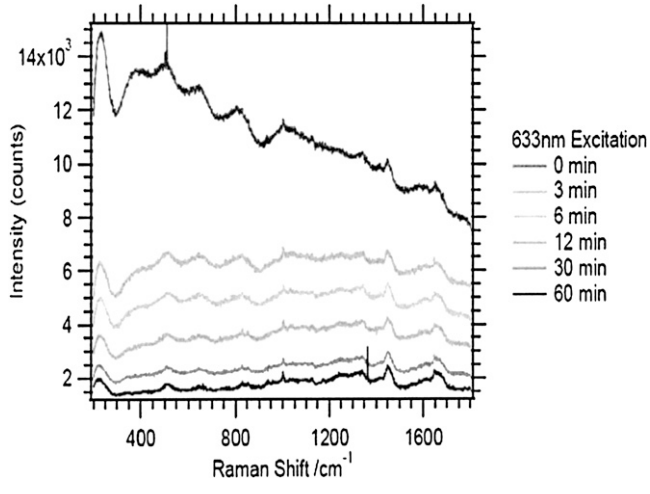


**Figure 2.** Spectra collected at different time points with 633-nm excitation. Spectral intensity decreases with increasing exposure time.

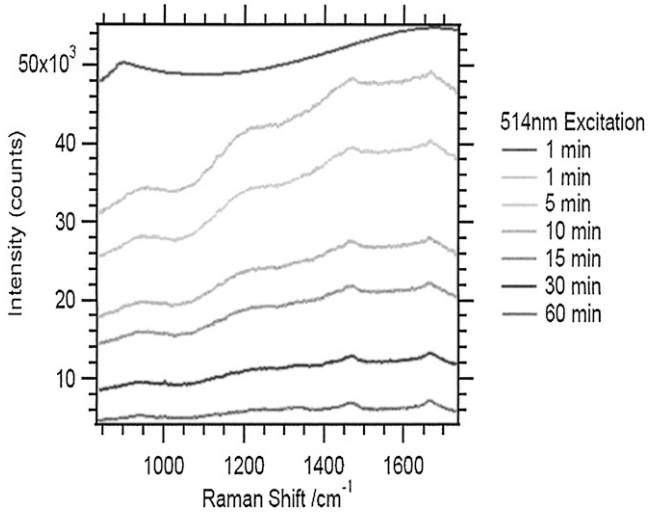


**Figure 3.** Spectra collected at different time points with 514-nm excitation. Spectral intensity decreases with increasing exposure time.

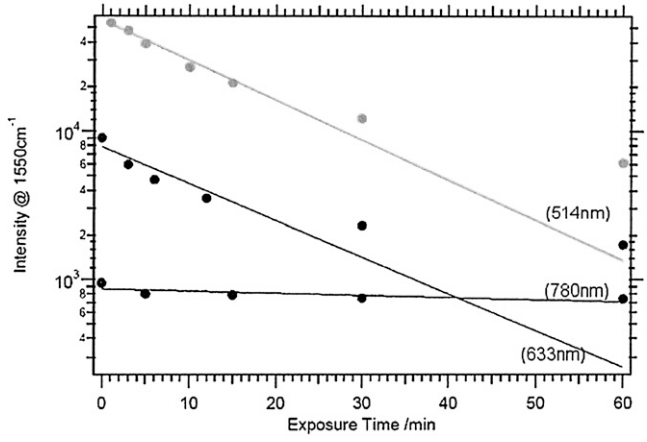


**Figure 4.** Plot of decay in Raman signal intensity (at 1550 $cm^{-1}$) at different excitation wavelengths.

The data contained in Figure 4 show that there is a clear decrease in the overall signal level in this spectral region as a function of time for all the excitation wavelengths. However, this decay is especially apparent for the red and green excitation wavelengths, which exhibit decay times (time for the intensity to fall by a factor of 10) of the order of minutes compared with hours for the 780-nm excitation.

The increase in luminescence background observed when higher energy lasers are used in Raman excitation is mainly due to the natural fluorescence of the hair. The majority of signal decrease over time at shorter wavelengths is likely due to photobleaching effects and photon adsorption leading to localized heating of the sample at the area where the bean is incident, leading to thermal sample degradation.

It is important to note that the decay curves are non-linear, oven on this log-linear plot. This supports the conclusion that the signal decay occurs due to multiple processes.

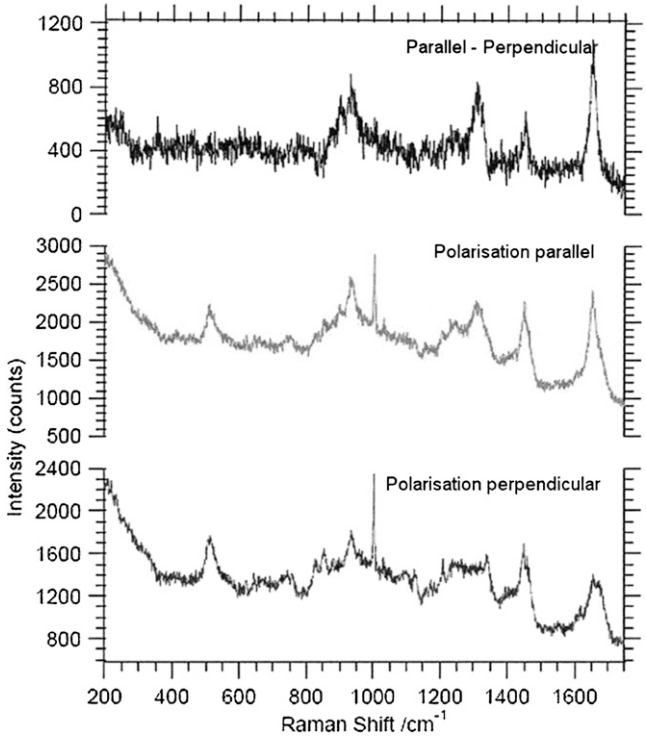


**Figure 5.** Comparison of spectra from sample parallel and perpendicular to plane of polarization. Middle spectrum: long axis of fiber parallel to plane of polarized laser. Bottom spectrum: long axis perpendicular to polarization. Top spectrum: Difference spectrum (parallel – perpendicular).

In summary, this work demonstrates that 780 nm is the most appropriate excitation wavelength to use to investigate hair samples by Raman spectroscopy.

The spectra collected when the fiber is oriented parallel and when oriented perpendicular to the direction of polarization of the excitation laser are shown in Figure 5: the difference spectrum of the two is also shown. The Raman spectra recorded are observed to be sensitive to the orientation of the hair sample with respect to the laser polarization.

The difference spectra features two α-helical at 1655 $cm^{-1}$ (Amide I) and 935 $cm^{-1}$ (C-C stretch), together with the Cα-H bending mode at 1314 $cm^{-1}$ as being the most orientation sensitive bands.

As the anisotropic component of the fiber is comprised mainly of α-helical intermediate filaments, these results suggest that the relative intensities at 1655 $cm^{-1}$ and 935 $cm^{-1}$ could be used as a measure of alignment of the α-helical intermediate filaments with the fiber axis. This could be exploited to explore structural differences between hair samples of different ethnicity or hair subjected to different treatments.

## CONCLUSION

The multiple wavelength experiments show that the 780-nm excitation is the most appropriate wavelength out of the set of visible-to-NIR wavelengths explored. It has been

observed that while shorter wavelength lasers can be used to obtain greater signal intensity, potentially reducing collection times, the shorter wavelength causes a large increase in background intensity, negating this benefit.

Exploratory experiments designed to determine the applicability of polarized Raman show changes in the spectra depending on orientation of the fibers to the direction of the exciting beam. This may therefore offer potential to explore and estimate orientation of the crystalline intermediate filaments in hair fibers.

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