Application note

Forensic analysis of fiber and hair using FTIR microscopy

Introduction

Fibers and hairs are abundant and easily transferred trace evidence that can link individuals or items to a crime scene or contact with a victim. Textiles represent a major source of fibers and are either of natural origin or manmade synthetics. Light microscopy, including the use of polarized light, is commonly used for fiber analysis, and an experienced analyst can categorize fibers based on their physical appearance and perform a direct comparison with fibers from known sources. The success and reliability of this analysis, unfortunately, largely depends on the experience of the analyst. Fourier-transform infrared (FTIR) microscopy provides supporting information for fiber analysis while also being less dependent on user experience. Spectra obtained from FTIR microscopy can be used to quickly identify fiber materials. It is also possible to do a direct comparison between fibers to help verify a suspected source. As FTIR microscopy is a non-destructive technique, it can be followed by other, potentially destructive, analytical methods for further characterization and verification.

Hair is primarily composed of protein, but other components may also be present in smaller quantities, including water, lipids, dyes, cosmetic residues, etc. Infrared spectra of hairs are generally quite similar, but differences caused by chemical treatments (bleaching, straightening, etc.) and/or sun exposure that affects the cuticle (outer protective layer) of the hair can be detected. Infrared spectra of hair can also show differences based on race, gender, age, diet, and health. Classifying an unknown hair sample to this degree, based only on its spectrum, would require chemometric methods and large databases comprised of representative spectra. FTIR microscopy is, therefore, better suited for providing corroborating information on hair samples as it is quick, easy, and non-destructive, allowing it to serve as the first step in an analysis workflow. Direct comparison of spectra from an unknown hair sample to a limited number of known sources is much more manageable.

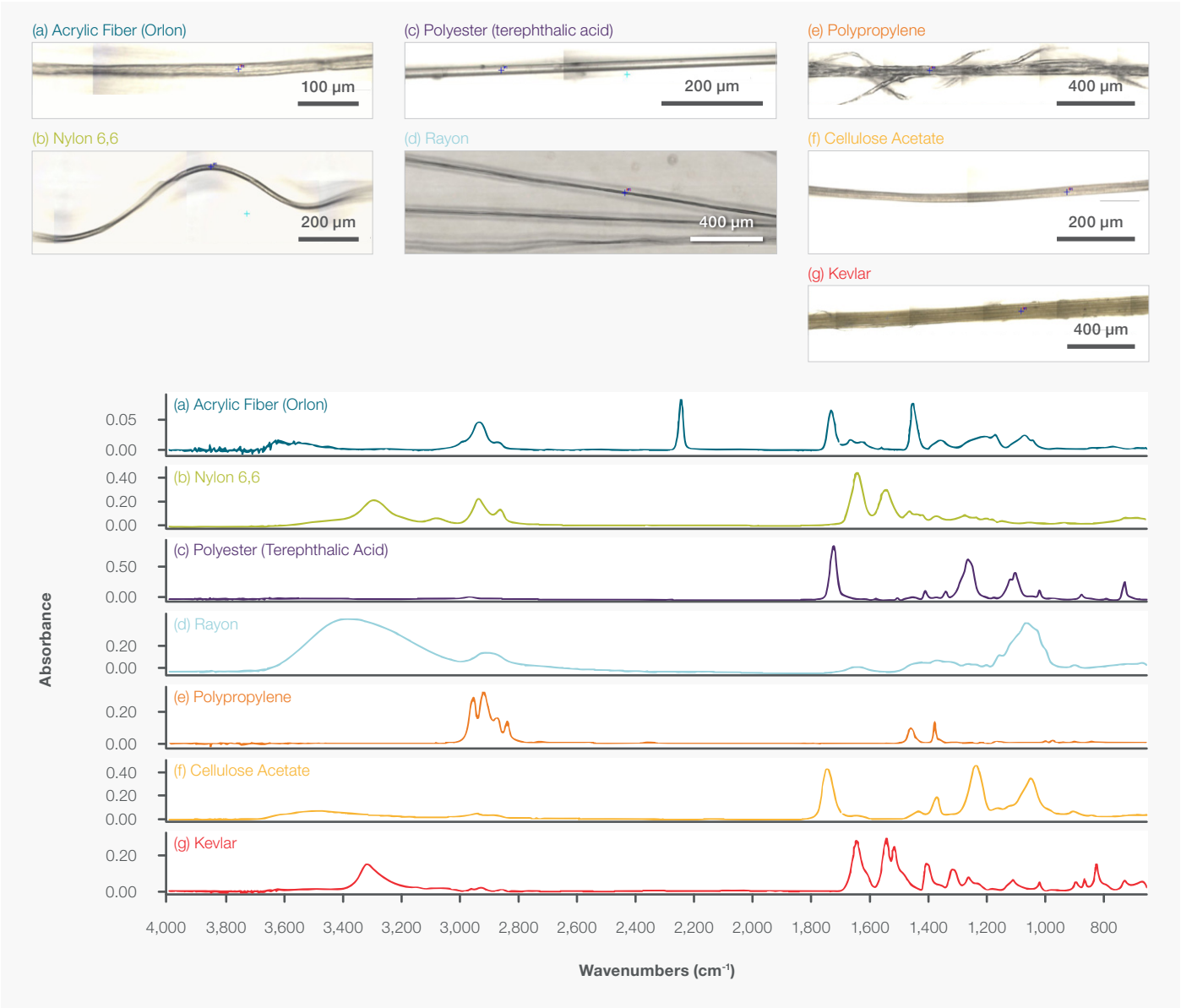

Figure 1. Infrared spectra from a selection of synthetic fibers. Video mosaic images were collected using the 15x objective; spectra were collected using a slide-on germanium ATR accessory.

Experimental details

A Thermo Scientific™ Nicolet™ RaptIR™ FTIR Microscope was used to analyze the fiber and hair samples in this application note. The Nicolet RaptIR FTIR Microscope easily locates regions of interest with a 4x visible objective lens, which is used to quickly collect a large, high-quality visual mosaic. The microscope then automatically switches to a proprietary 15x objective for both excellent higher magnification visual images as well as infrared spectra. A germanium slide-on attenuated total reflection (ATR) accessory allows for the collection of spectra with little to no sample preparation. A built-in pressure sensor can be used to adjust the automatic contact between the sample and the ATR crystal, ensuring the pressure is both repeatable and customizable for different samples. For most of the fiber and hair specimens, several positions along the sample were selected for automatic spectra collection in order to verify homogeneity. The high-precision stage ensures that spectra are collected from the positions chosen in the visual mosaics. For infrared mapping, an area and step size are selected, and ATR spectra are then automatically collected over that area.

Synthetic fibers

A selection of 7 synthetic fibers was analyzed, with visual images and FTIR spectra shown in Figure 1. The differences in the infrared spectra make it relatively straightforward to use commercial libraries for fiber material identification. Distinguishing between different fibers of the same material, meanwhile, is largely sample dependent; for instance, contributions from dyes and other additives might prove to be unique to a particular source. These are, however, relatively minor components, and a direct comparison between an unknown fiber and fibers from possible matching sources will likely prove to be more definitive.

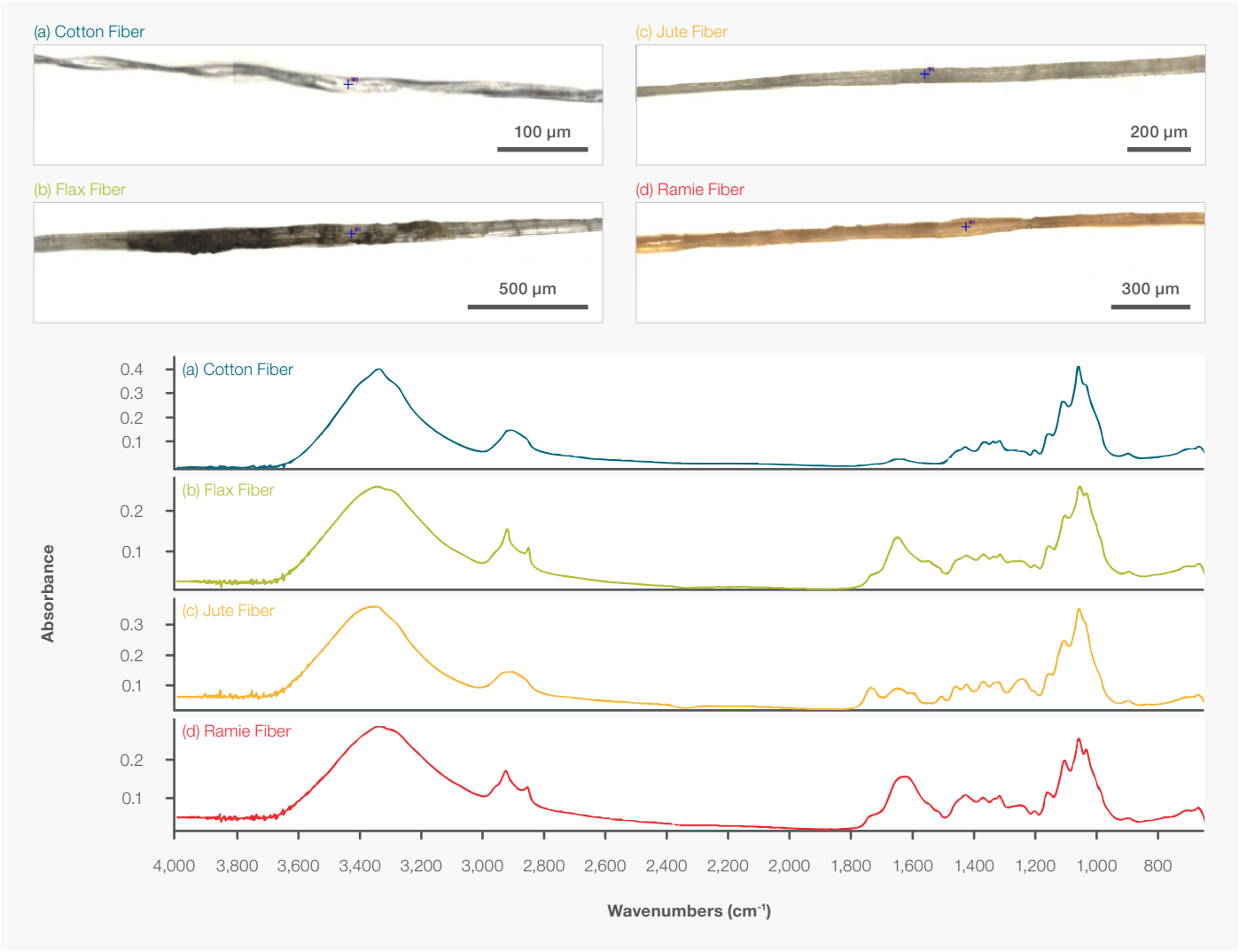

Figure 2. Infrared spectra from cellulose-based fibers. Video mosaic images were collected using the 15x objective; spectra were collected using a slide-on germanium ATR accessory.

Natural fibers

Similar to their synthetic counterparts, natural fibers can readily be categorized based on their general material (cotton, keratin, etc.). However, differences within a class of material are more subtle. Figure 2 shows the analysis of four different cellulose fibers; cotton, flax, jute, and ramie. Their infrared spectra are largely similar but can be differentiated. Within a particular type of cellulose fiber, differences associated with dyes and processing treatments are still best suited for a direct comparison of an unknown fiber to fibers from known sources.

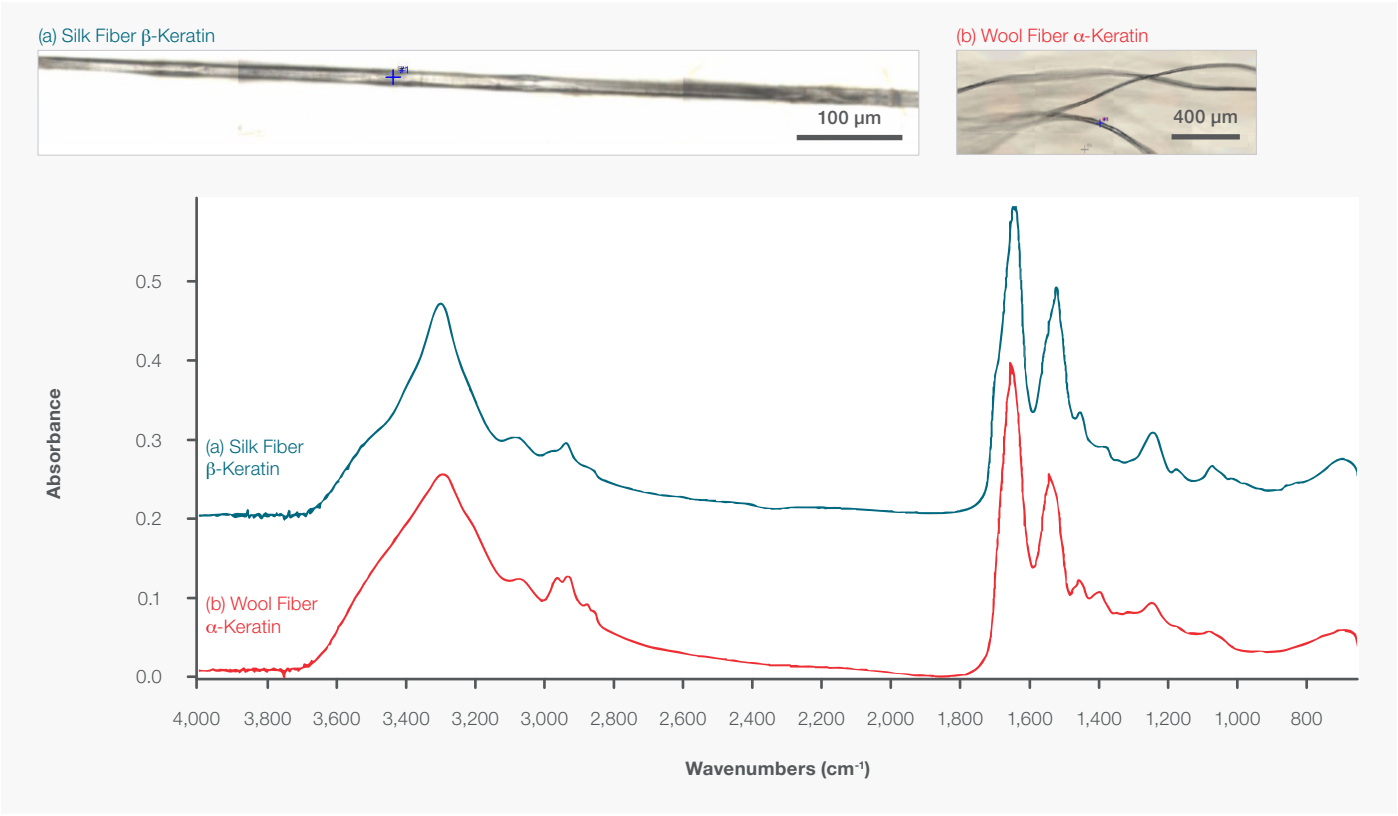

Figure 3. Infrared spectra from keratin-based textile fibers. a) Silk, composed of β-keratin. b) Wool, composed of α-keratin. Video mosaic images were collected using the 15x objective; spectra were collected using a slide-on germanium ATR accessory.

Another distinct group of natural fibers are those made of keratin and other fibrous proteins. This includes wool and silk fibers, which are commonly used in textiles. Wool, like human hair, is mainly α-keratin, whereas silk is composed of β-keratin; a comparison of their infrared spectra can be seen in Figure 3. Since these materials are chemically similar, so are their infrared spectra; nevertheless, there are still enough differences to distinguish between them.

Embedded fibers

Fibers do not always appear as loose strands; they can also be part of a larger sample. Figure 4 shows an example of a nylon fiber embedded in a paper substrate. Since ATR is a surface analysis technique, the fiber can only be observed when it is above the surface of the paper, as is seen in Figure 4. This example shows peaks for both a synthetic fiber and a matrix of natural origin (paper cellulose).

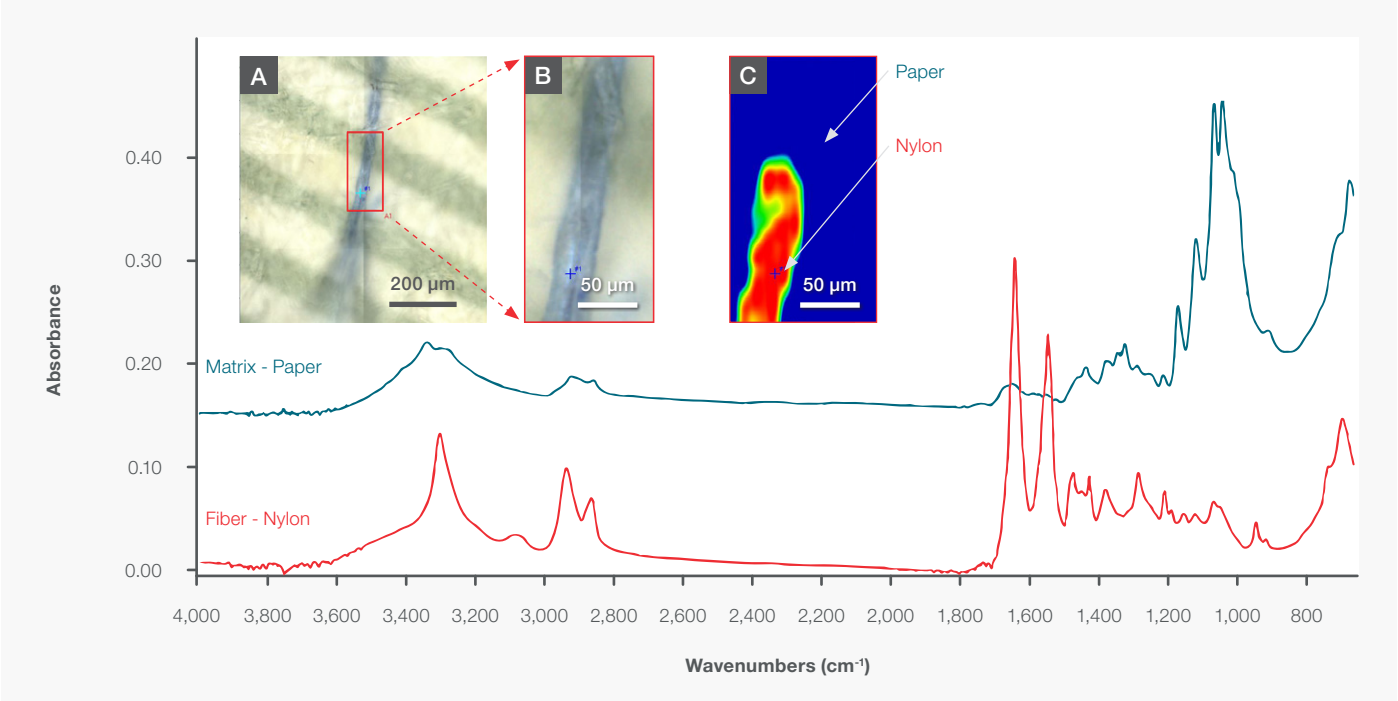

Figure 4. Infrared ATR mapping of a nylon fiber embedded in a paper substrate. a) Video mosaic collected using the 15x objective. b) Video image of the sample area being analyzed. c) Infrared correlation image based on the nylon spectrum from the sample. Area: 110 x 240 µm², effective aperture: 25 x 25 µm², 10 µm steps, 300 spectra total.

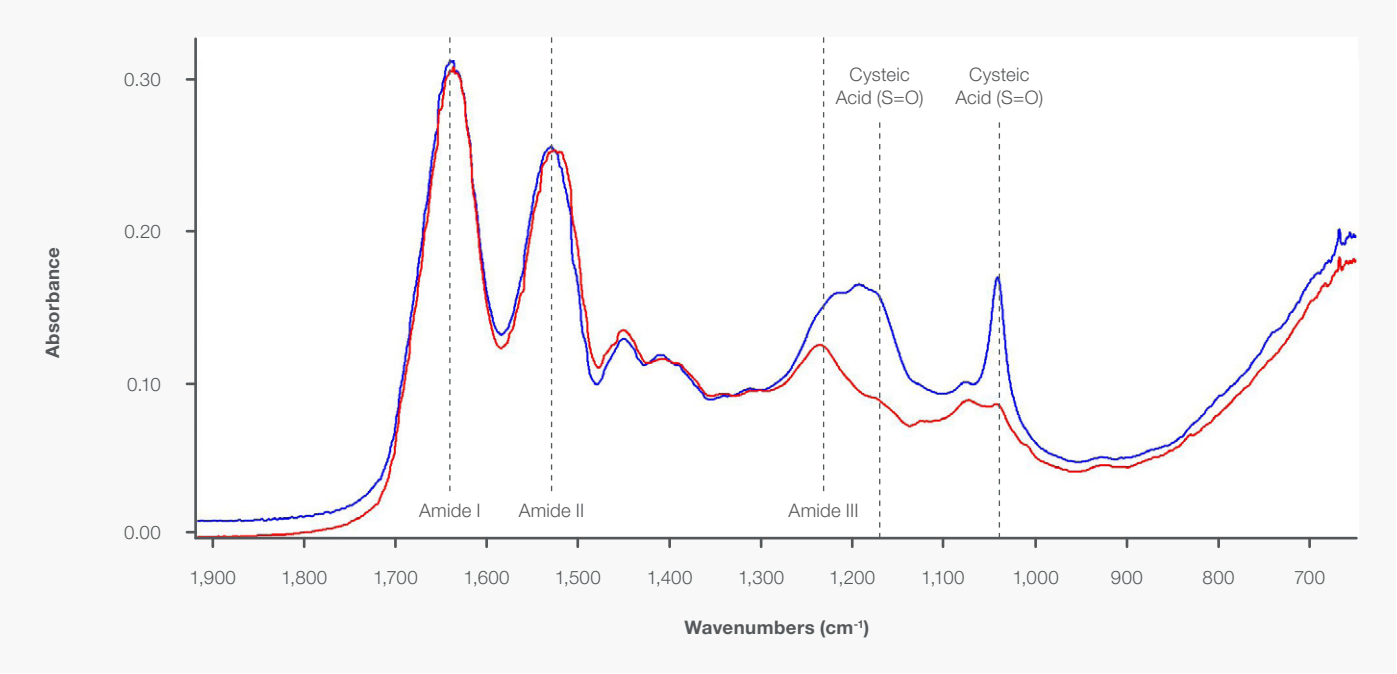

Figure 5. Infrared spectra from an untreated hair sample (red) and a treated hair sample (blue). Stronger peaks associated with cysteic acid are observed in the treated hair sample.

Hair

Like wool, hair is made of α-keratin, however, hair fibers are typically linked to particular people/pets rather than textile sources. While the basic chemical structure of all hair is very much the same, differences can arise from chemical treatments such as bleaching, straighteners, and dyeing. One common effect is the oxidation of sulfur during hair treatments, resulting in the disruption of disulfide bonds in hair. Oxidation of cystines can produce cysteic acid with an enhanced peak at 1042 cm^{-1}. There is also an additional cysteic acid peak at ~1077 cm^{-1} that is often observed as a shoulder on the amide III peak. A comparison of untreated and treated hair can be seen in Figure 5, illustrating the effect that chemical treatments can have on the structure of hair and the corresponding infrared spectra.

Distinguishing hair from different sources can be challenging but is simplified when direct comparisons can be made between unknown and known hair samples. Figure 6 shows such a comparison, with samples from three different individuals living in the same dwelling. A spectrum of cat hair was also included for comparison. While the basic structures are the same, there are still differences in the spectra that could be used to distinguish them.

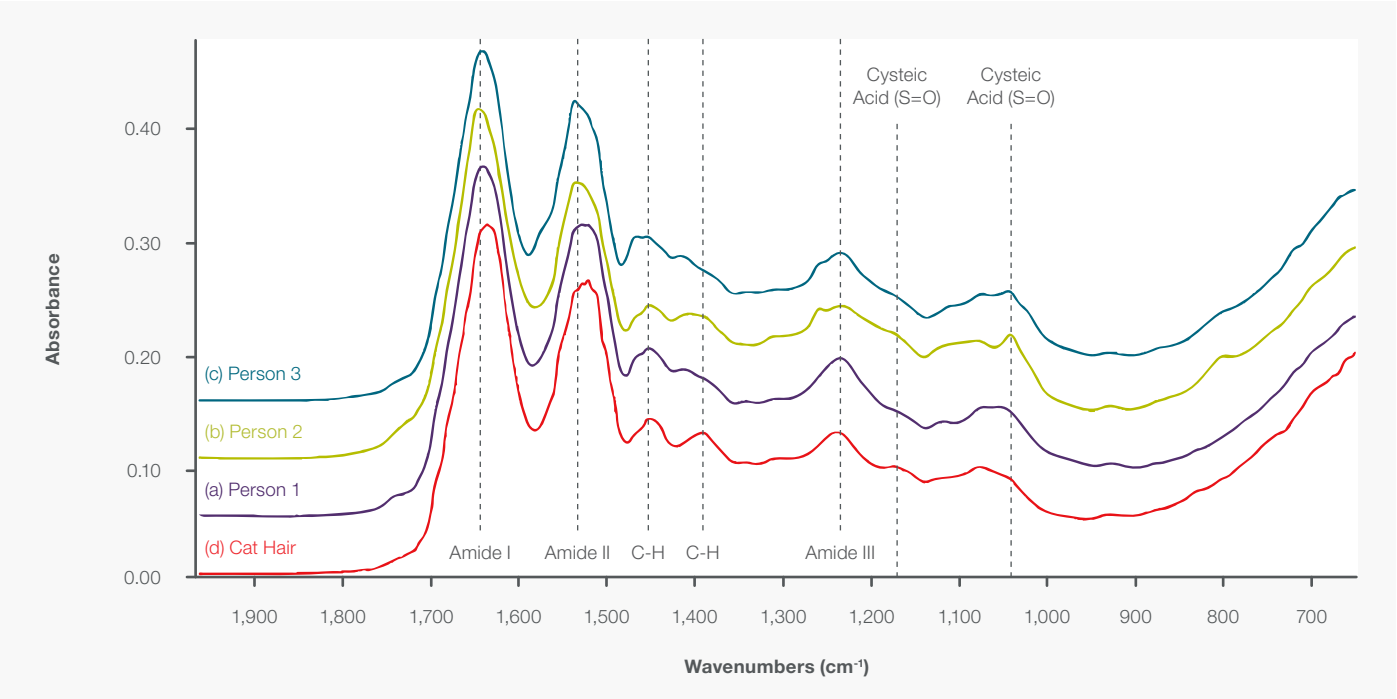

Figure 6. Infrared spectra from three individuals, (a) a male adult, (b) a female child, and (c) a female adult, living in the same dwelling, as well as (d) cat hair. Differences include shifts in the amide I and amide II bands, the C-H bending regions, and the area associate with cysteic acid.

In these situations, it is possible to generate a discriminant analysis method where spectra from multiple sources serve as the basis for classification; it could then be determined which class an unknown sample belongs to. In this application note, the discriminant analysis method was generated using Thermo Scientific™ TQ Analyst™ Software; the method was then validated using the hair spectra from the three individuals. In each case the spectra were classified correctly. This analysis can subsequently be used to determine if an unknown hair sample belongs to one of these known sources. To classify hairs based on general characteristics such as race, gender, and diet would require much more extensive and representative databases and more complex and carefully constructed chemometric methods. While some attempts have been made to achieve this, it is not clear if this is practical for such a wide-ranging classification because of the amount of data involved and all the possible variables that would need to be considered.

Conclusions

FTIR microscopy provides two tiers of fiber analysis. The first is a fast, easy, and effective compositional analysis for a particular fiber. This general classification is quite straightforward and can be effective with a minimal amount of experience. The use of ATR requires very little sample preparation, is non-destructive, and provides a wealth of information on the fibers' chemical structure. This initial classification can be used as the first step in an analysis workflow, directing subsequent tests.

The second tier of fiber analysis involves assessing variations within the same class of material. While this is much more sample dependent, the examples highlighted in this application note show that there can be sufficient differences between fibers of the same material that the spectrum of an unknown sample can be matched to fibers from known sources. Hair in particular can be analyzed in this fashion; hair treatments such as bleaching, dyeing, and straightening can show intense differences. While this type of analysis requires the collection of additional spectral information, it has the potential to link an unknown fiber to a specific source.

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