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Application News

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Spectrophotometric Analysis

Human Hair Cross-Section Analysis Using the AIM-9000 Infrared Microscope

In order to control the permeability of the product components of hair-treatment and hair-coloring products into human hair, measurement methods that allow direct and straightforward analysis of human hair need to be established.[1)]

The AIM-9000 infrared microscope enables visualization of component distributions in minute areas. This article introduces an example of analyzing cross sections of human hair using the AIM-9000. The cross sections of human hair were prepared using a microtome manufactured by Leica Biosystems.

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AIM-9000 Infrared Microscope with Mapping Program

Minute areas of samples can be analyzed in detail by combining the AIM-9000 infrared microscope with the optional mapping program. Fig. 1 shows the instruments used for analysis. The mapping program is capable of both area mapping measurement for analyzing the in-plane distribution of sample components, and line mapping measurement which is effective for analysis at regular intervals along straight lines. In addition to mapping measurement in the standard transmission and reflectance modes, ATR mapping measurement that uses the optional ATR objective mirror and pressure sensor is also available.

Fig. 1 **IRTracer^{TM}-100 Fourier Transform Infrared Spectrophotometer (Left) and AIM-9000 Infrared Microscope (Right)**

Preparation of Human Hair Sections

Untreated black hair and permed/bleached hair were prepared as samples of human hair.

A Leica Biosystems fully automatic rotary microtome was used to prepare the sample sections. Fig. 2 shows the HistoCore NANOCUT R which is the latest model. The cutting method on the HistoCore NANOCUT R can be switched between automatic and manual modes and the cutting thickness can be set from 0.25 to 300 µm. In this example, 3 µm thick sections were created through vitreous ice-embedding using the EF-13 electronic sample freezing device.

Fig. 2 **Leica Biosystems HistoCore NANOCUT R Fully Automatic Rotary Microtome**

Analysis of Human Hair Cross Sections

Mapping measurement was performed using the infrared microscope. The human hair sections were placed on a diamond cell and measured using transmission microspectroscopy. The aperture was set to 10 µm × 10 µm and the measurement interval was set to 5 µm. Table 1 lists the measurement conditions and Fig. 3 shows the representative infrared spectra of untreated black hair and permed/bleached hair.

The spectra show an amide I peak (C=O stretching vibrations) in the vicinity of 1650 cm^{-1} and a peak originating from cysteic acid (S-O stretching vibrations), which is an indicator of hair damage, in the vicinity of 1040 cm^{-1}. The cysteic acid peak only appears for the permed/bleached hair.

Table 1 **Measurement Conditions**

Instrument	: IRTracer-100, AIM-9000
Resolution	: 8 cm^{-1}
Accumulation	: 10
Apodization function	: Sqr-Triangle
Aperture size	: 10 µm × 10 µm
Measurement interval	: 5 µm
Detector	: MCT

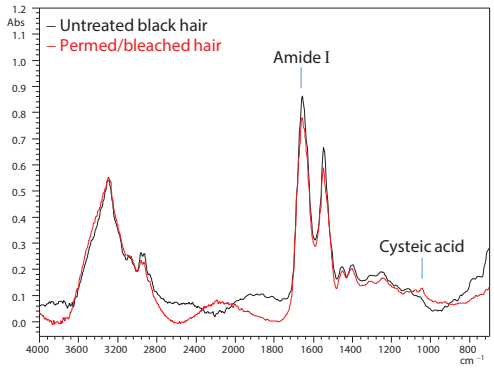

Fig. 3 **Representative Infrared Spectra of Untreated Black Hair and Permed/Bleached Hair**

Chemical Images of Hair Cross Sections

Observation images of untreated black hair and permed/bleached hair were acquired and chemical images were created from the mapping measurement results.
Chemical images were created from the results of mapping measurement using peak height and area, multivariate analysis (PCR/MCR), and the degree of similarity with target spectra. By this, component distributions that cannot be confirmed visually were successfully visualized. Mapping measurement is widely used for defect analyses as well as analysis of industrial materials and biological samples.
Fig. 4 (a) shows chemical images derived from amide I (peak correction area values near 1650 cm^{-1}). Amide I is widely distributed from the surface to the interior of the hair for both the untreated black hair and permed/bleached hair, and both favorably agree with their observation image counterparts.
Fig. 4 (b) shows chemical images derived from cysteic acid (peak correction area values near 1040 cm^{-1}). Compared to the untreated black hair, cysteic acid is distributed throughout the permed/bleached hair, which is assumed to be the result of hair damage.

Conclusion

This article introduced an analysis of human hair cross sections using the AIM-9000 infrared microscope. We were able to demonstrate the changes in composition of internal proteins resulting from hair damage. FTIR is an effective method for observing the components inside human hair and the changes that occur due to hair damage.

Acknowledgments:
We would like to thank Leica Microsystems GmbH for their cooperation in the cutting of samples.

References:
1) Satoshi Inamasu et al., "Analysis of Human Hair Cross Section Using Infrared Microspectroscopy"
J. Soc. Cosmet. Chem. Jpn. Vol.50, No.3 2016 P.209-217

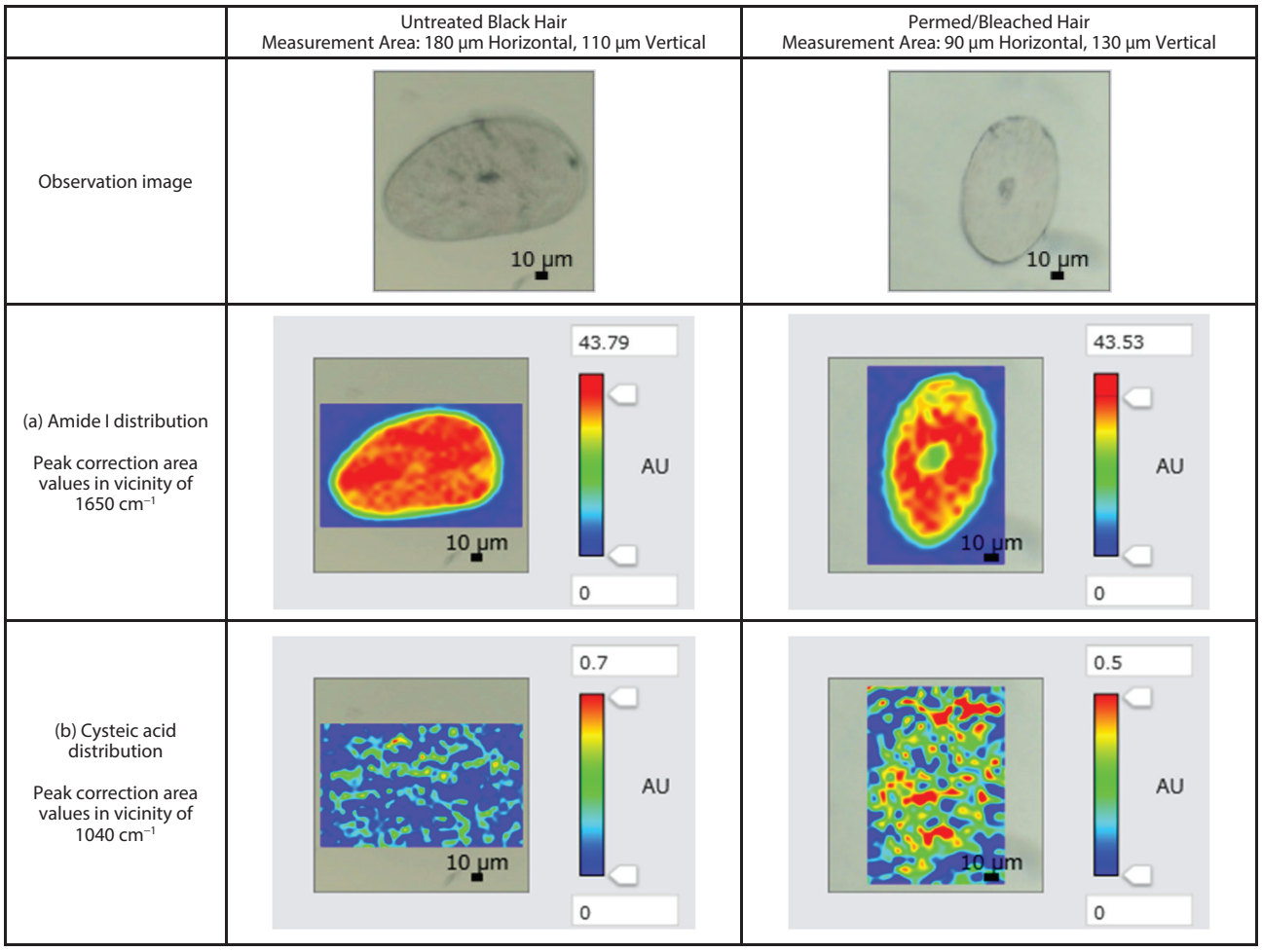

Fig. 4 **Chemical Images of Hair Cross Sections (Untreated Black Hair, Permed/Bleached Hair)**

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