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Ivan M. Kempson,[1] *B.App.Sci. (Hons); William M. Skinner,*[1] *Ph.D.; Paul K. Kirkbride,*[2] *Ph.D.*

A Method for the Longitudinal Sectioning of Single Hair Samples*

ABSTRACT: A simple technique is presented for the longitudinal sectioning of hair samples without the need for any embedding medium. The technique applies to single hair samples that are placed in a groove of a particular depth and can either be scraped or cut to the desired level. Planar sections are obtained that reveal the internal structure of the sample and are ideal for the application of surface analytical techniques for the study of internal transverse and longitudinal distributions of xenobiotics.

KEYWORDS: forensic science, criminalistics, hair, cross-sectioning, hair structure

Hair analysis has developed rapidly over the last few years, however, current methods still do not allow discrimination between endogenous and exogenous sources of particular elemental and molecular xenobiotics (1,2). Analysis of the transverse and longitudinal distributions of these species in the internal structure of a hair sample using surface sensitive techniques may aid to distinguish between these contributions for forensic and environmental analysis. Methods suitable for the sectioning of single hairs have been published previously (3,4), but none enables controlled longitudinal sectioning, and all require the use of an embedding medium. For surface-sensitive analytical techniques it is desirable to avoid the use of a mounting medium in order to reduce the chance of spectral contamination. It is the intention of the authors to utilize Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) to investigate and identify the distributions of various elemental and molecular species as being of ingested or environmental origin.

Two simple techniques are described here to section single hair samples longitudinally to provide a planar surface that exposes the internal structure. The advantages of the methods given here are that they require only single hairs, and do not use any embedding medium. In addition to allowing unique chemical analysis, longitudinal sectioning also reveals interesting microscopical features in hairs.

A steel plate (60 × 110 mm) was machined to contain a series of grooves approximately 5 mm wide of varying depths as shown in Fig. 1. The depths varied from 20 μm to 80 μm in 10 μm increments.

By loosely laying a hair in a groove of appropriate depth, securing one end pressed with a gloved (latex) finger, held by a small clamp or taped down, and carefully manipulating a sharp blade, a section can be produced that exposes the internal structure of the sample. Two methods for achieving a longitudinal section, scraping and cutting, have been investigated. The quality of sectioning was determined using SEM imaging.

Method 1: Scraping

The first method was performed using the edge of a blade (single edged industrial blade) held at slightly less than 90° to the direction of movement to scrape the thickness of the hair down to the desired depth (Fig. 2).

For comparison, SEM images have been given in Figs. 3 and 4 of a hair before sectioning. The overlapping scale nature of the exterior cuticle surface can clearly be seen.

Figures 5 and 6 show SEM images of a hair sectioned using a scraping action against the direction of the cuticle scales, i.e., towards the proximal end of the hair. The cuticle structure can be seen as a narrow layer on the outside of the hair surrounding the bulk cortex region. A reasonably flat surface resulted in the cortex region, which suffered only minor gouging and tearing to its fibrous structure. However, the cuticle structure was significantly damaged.

If this method is used to prepare sections for surface chemical analysis, caution needs to be taken to ensure that detached cuticle scales or other debris does not contaminate exposed surfaces and influence results. This method is perhaps the more damaging of the two described here but is easier to implement for samples that are particularly brittle or short (down to just a couple of millimeters).

Method 2: Cutting

This technique was implemented by using the blade at a very fine angle (approximately 20° from the plane of the plate) to cut along the length of the hair sample as shown in Fig. 7.

This method retained the cuticular structure far better than the scraping method given previously. SEM images for a cut against the cuticle direction are presented in Figs. 8 and 9 where the section passed through the center of the hair and revealed the inner medulla structure. The finish of the cortex surface was considerably rougher than obtained from the first method described, however, the long fibrous composition was more evident. Long, fine, parallel units can be seen to comprise the cortex region. The central medulla core presented a dramatically different structure in appearance. A much more complicated networked structure was ob-

[1] Ian Wark Research Institute, University of South Australia, Mawson Lakes, South Australia, 5095, Australia.
[2] Forensic Science, South Australia
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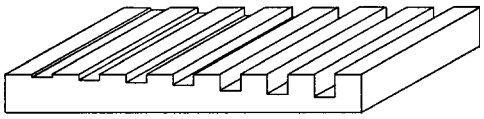

FIG. 1—*A schematic of the plate with grooves 10 to 80 (μm deep and 60 mm long (not to scale).*

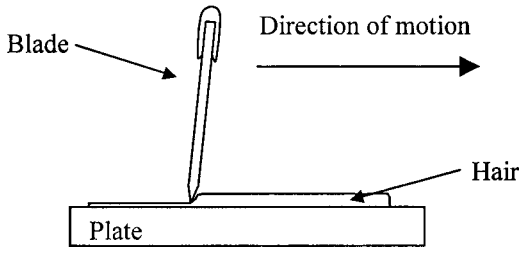

FIG. 2—*Longitudinal section of a hair sample obtained by scraping to the desired depth with a blade.*

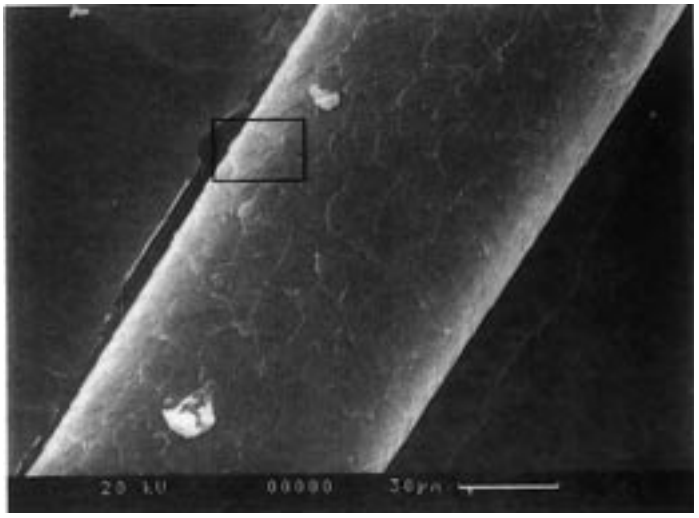

FIG. 3—*A hair sample before sectioning. The boxed region is magnified in Fig. 4.*

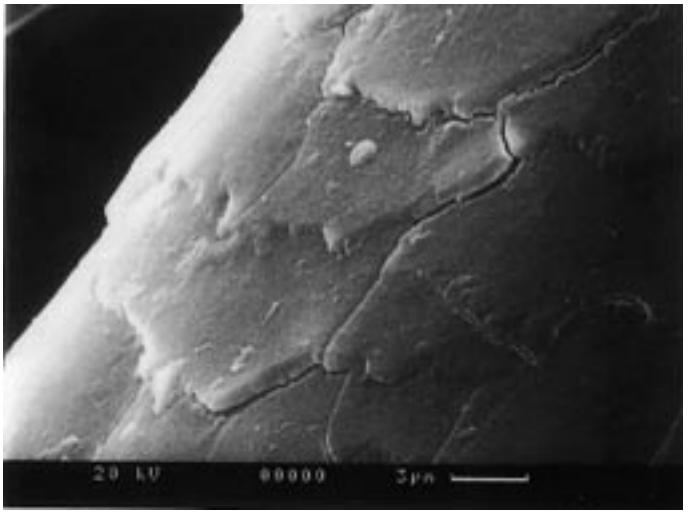

FIG. 4—*A close up of the pre-sectioned hair demonstrating the cylindrical scale covered structure.*

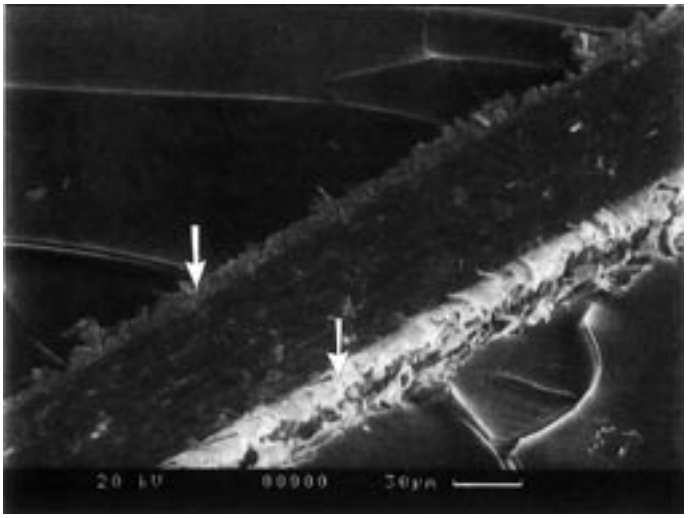

FIG. 5—*A longitudinally scraped hair section. Of note is the damage to the cuticle structure (arrow).*

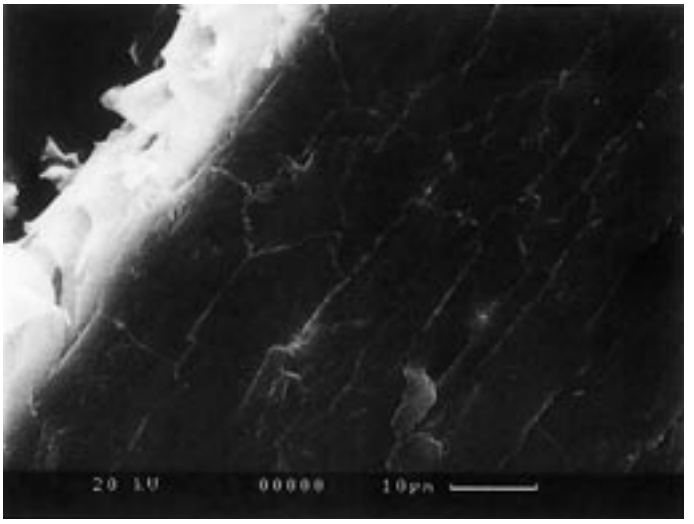

FIG. 6—*Although the cuticle is significantly damaged a reasonably smooth surface in the cortex region is obtained.*

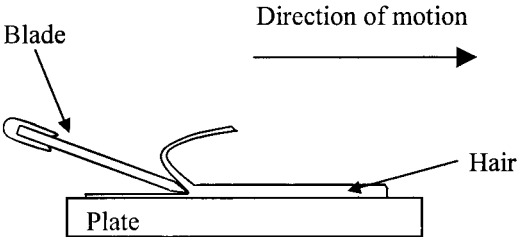

FIG. 7—*Longitudinal section of a hair sample obtained by cutting to the desired depth with a blade.*

served within a relatively hollow core (Fig. 10). Figure 11 has been included to demonstrate the overlapping scale nature of the cuticle.

Preliminary Results

Very little published material exists implementing ToF-SIMS for hair analysis (5,6). Some preliminary results are given to

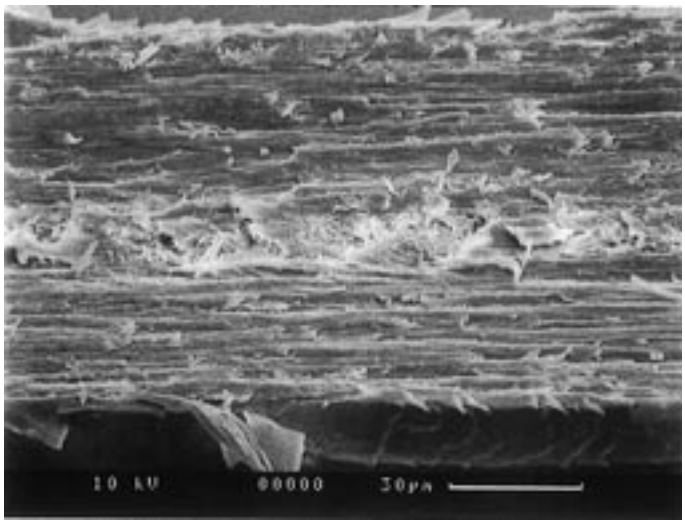

FIG. 8—*The retention of the cuticle scale structure can be seen along the lower edge of the picture along with the fibrous cortex structure.*

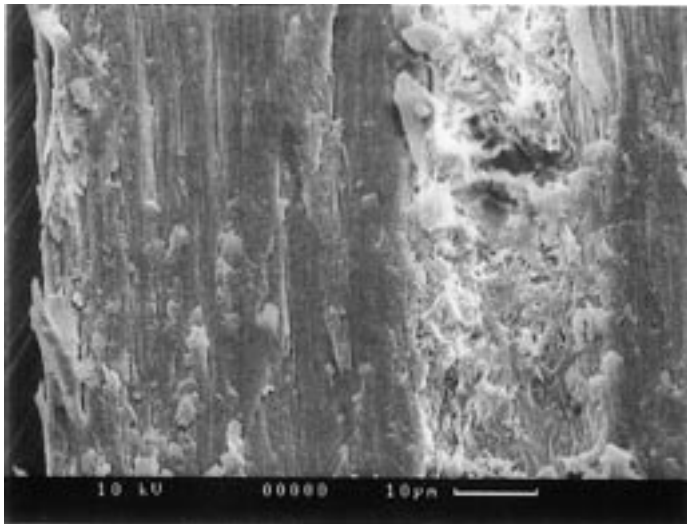

FIG. 9—*A dramatically different structure including hollow regions is observed in the medulla.*

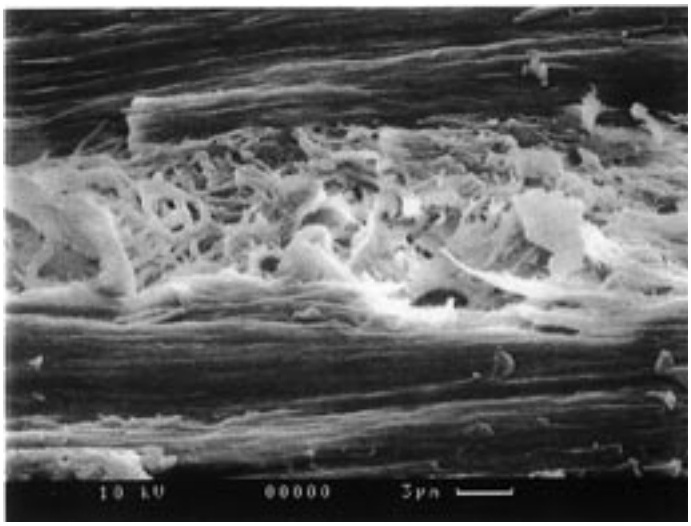

FIG. 10—*Curious structures such as that in the left portion of the figure can be seen in the medulla.*

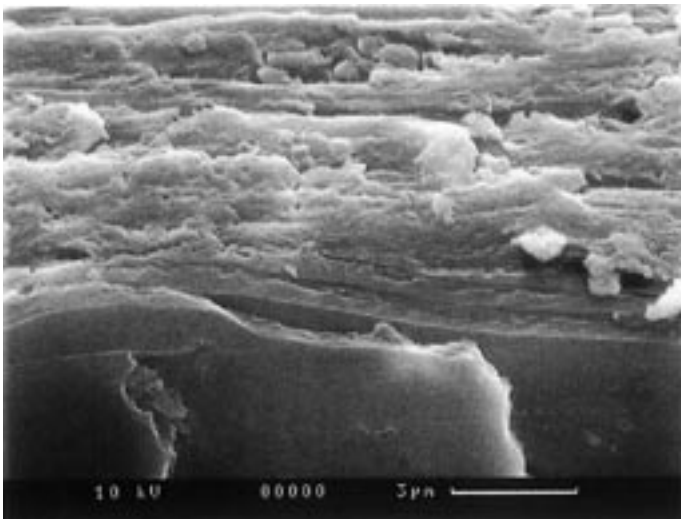

FIG. 11—*The overlapping nature of the cuticle scale structure can be observed.*

demonstrate the potential use of ToF-SIMS analysis combined with the sectioning techniques described here. A Physical Electronics Inc PHI TRIFT II ToF-SIMS with a 25kV liquid metal Ga ion gun was used to image elemental and molecular species in a hair sample sectioned using the cutting method. Figure 12 gives examples of the images produced. The total positive ion image is given along with the corresponding distribution of calcium and the presently unidentified molecular species' with mass to charge ratios of 368.9 amu and 388.3 amu. All images have been normalized with the total ion image to remove topographic and secondary ion yield effects. The total ion image clearly shows that the analyzed section includes the medulla, the bulk cortex region, and a thin cuticle layer. A few cuticle scales can be seen to have lifted from the hair surface during sectioning. The calcium distribution demonstrates a high concentration at the hair surface as well as localised concentrations in the cortex and medulla regions. Further work should distinguish the surface component of the calcium distribution and the molecular species given in Fig. 12(*d*) as being of either endogenous or exogenous origin.

The selection of images shown here is given only as a preliminary demonstration of the potential use of this technique. Numerous other elemental and molecular images can and have been produced but their analysis and interpretation is beyond the scope of this paper, and will be reported in a future publication.

Discussion

Often the portion of hair of most interest for chemical analysis is the first centimeter(s) at the root end as it is in this region that xenobiotics taken within the previous month(s) will be detectable (assuming a growth rate of approximately 1 cm per month) (7–9). Since these methods require one end of the hair to be restrained, cutting or scraping must be performed against the cuticle scale direction to obtain samples at the skin level. This results in substantial loss in the cuticle structure when scraping is used, and minor disturbance from the cutting method. Unfortunately, for proximal analysis this cannot be avoided.

These techniques are very rapid, do not require embedding and provide longitudinal sections up to a few centimeters long. So far, hairs with diameters from 50 to 150 μm have been successfully sectioned. Difficulty may arise, however, sectioning hairs of smaller di-

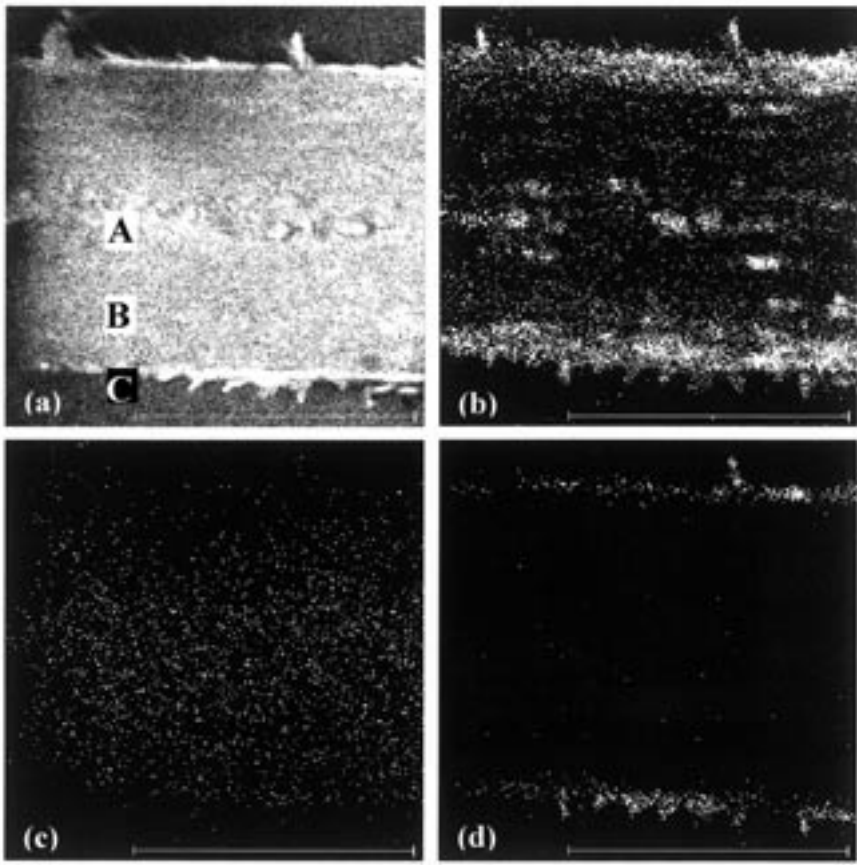

FIG. 12—*ToF-SIMS images of* (a), *the total positive ion yield which clearly shows the medulla (A), cortex (B) and the cuticle (C) regions of the sample*, (b), *the normalized calcium distribution*, (c), *an internal molecular species' distribution, and* (d), *another molecular species predominant in the cuticle. Scale = 100* μ*m.*

ameter. A diameter of approximately 30 μm is expected to be about the limit of this technique due to the physical size of the groove and the blade as well as the reduced strength of the hair strand. Few human hairs have diameters less than this and as such will only result in problems if this technique was to be extended to some animal hairs or furs. Very short hairs (down to a couple of millimeters long) can be sectioned using the scraping technique, the cutting method, however, requires hairs that are at least one centimeter long to allow for restraining the sample and maneuvering the blade. Thick, brittle hairs over 500 years old have been sectioned using the cutting method for short (approximately 5mm) lengths before breakage. The scraping technique would probably provide longer sections but at this point has not been attempted. Curly hair samples have not been a problem since the blade, correctly positioned, holds the hair strand in the groove as it passes along its length. No interracial variability has so far been observed after sectioning of various European, Mediterranean and Australasian samples.

The resulting sections produced by the methods described here provide relatively smooth, planar samples ideal for further analysis with surface sensitive techniques for the investigation of internal distributions of elemental and molecular species.

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Additional information and reprint requests:
Ivan M. Kempson
Ian Wark Research Institute
University of South Australia
Mawson Lakes
S.A. 5095
Australia