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Calculation of Cuticle Step Heights from AFM Images of Outer Surfaces of Human Hair

James R. Smith

1. Introduction

Atomic force microscopy (AFM) is an ideal technique for noninvasive examination of hair surfaces ***(1–11)***, providing a wealth of structural information not always apparent from electron microscopy. The fine cuticular structure of human head hair is of interest to those engaged in the fields of dermatology ***(12–14)***, cosmetics ***(15–17)***, and forensic science ***(18–20***). In the former, the morphology of hair can be affected by an underlying inherited or congenital metabolic disorder, such as maple syrup urine disease ***(21)*** or monilethrix ***(22)***, respectively. The cosmetics industry is interested in the effects of haircare formulations, such as conditioning and bleaching agents, on hair cuticle surfaces ***(23)***. There is now increasing legislation on cosmetic manufacturers to be able to substantiate claims made concerning their products.

Cuticle step height, as shown in **Fig. 1**, is an important parameter for the quantitative assessment of human hair ***(5,24,25)***. Step heights typically range from 300–500 nm ***(5,15)*** but can vary further as a result of swelling or lifting caused by clinical, cosmetic, or environmental effects. This large variation in step height can be attributed to the heterogeneous character of hair cuticular structure.

The wide distribution of step height coupled with the need to obtain many step measurements clearly calls for a computational image processing technique. Such an approach is necessary to perform vast numbers of step height measurements for statistical comparisons. This becomes even more apparent when it is realized that there are a multitude of often-subtle differences in cuticle patterns between hairs from different parts of the head, between hairs from different body sites, and within each hair according to the distance from

From: *Methods in Molecular Biology, vol. 242: Atomic Force Microscopy: Biomedical Methods and Applications*
Edited by: P. C. Braga and D. Ricci © Humana Press Inc., Totowa, NJ

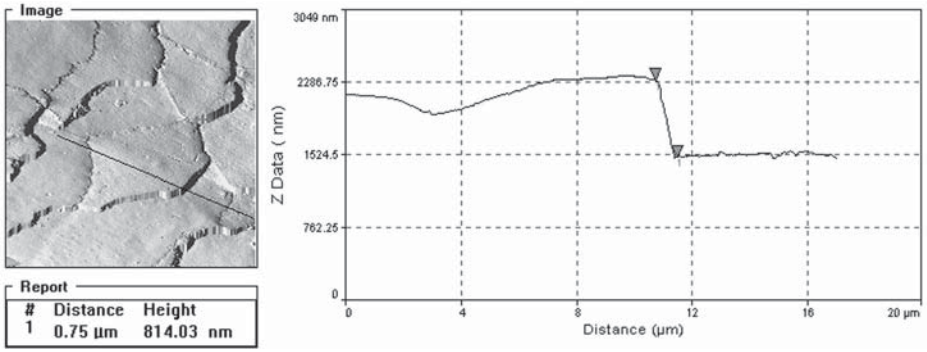

Fig. 1. A transect across an image of a hair cuticle showing a typical scale edge profile. In this case, the cuticle step height is shown to be 814 nm.

the skin surface. Except as a means for illustrating specific surface features, the single atomic force micrograph cannot be said to be representative of that hair and certainly not of a whole head of hair. This therefore focuses our attention on the need for quantitative extraction of surface architectural information, which by adequate sampling will enable systematic statistical comparisons to be made within and between hairs.

In this chapter, the method is demonstrated on a hair sample half of which has been bleached with a cosmetic formulation and the other left untreated. AFM topography imaging (**Fig. 2**) was conducted near the boarder region where the known length variation on surface architecture was considered to play a negligible role ***(8)***.

2. Materials

1. A tress of human hair. In this chapter, a tress of European brown hair, of length 20 cm, was used.
2. Sodium dodecyl sulfate solution (1%).
3. Cosmetic formulation for hair treatment. Here, a commercial bleaching product was used.
4. Double-sided, adhesive carbon tape (*see* **Note 1**).
5. Tweezers for manipulating hair fibers.
6. Atomic force microscope. Here, a TopoMetrix TMX2000 Discoverer Scanning Probe Microscope, operated in contact mode, in air, using 200-µm V-shaped silicon nitride cantilevers (spring constant 0.032 N/m) was used.

3. Methods

3.1. Sample Preparation

1. Place about 20 fibers in a 100-cm^3 beaker half filled with sodium dodecyl sulfate solution (1%), ensuring all the hairs are immersed.

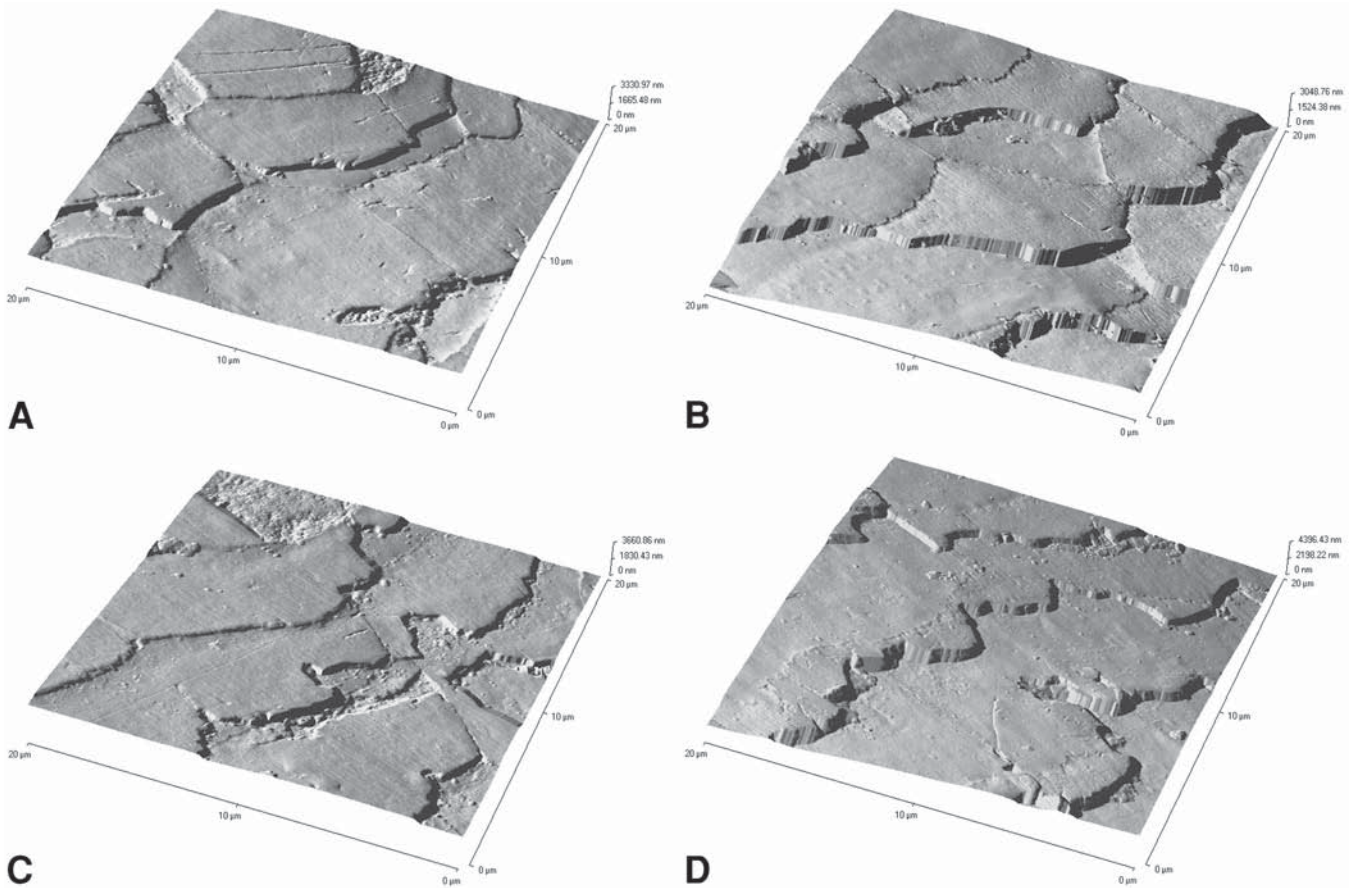

Fig. 2 . Typical AFM topography images of European brown human hairs: two untreated hairs: **(A)** hair 1, and **(B)** hair 2; and two bleached hairs: **(C)** hair 1, and **(D)** hair 2. Examination of 10 images of each hair for both conditions suggests that the bleached hairs appear to be more damaged than the untreated hairs.

2. Place the beaker in an ultrasonic bath and sonicate at room temperature for 30 s.
3. Pour away the detergent solution, rinse the hairs with copious amounts of double-distilled water, and allow to air dry. Fibers can be subsequently stored between filter papers.
4. Hair samples should be fixed to an AFM mounting assembly (nickel stub) using double-sided carbon tape before AFM imaging.

3.2. AFM Imaging

1. The end of the cantilever should be placed over the center of the short axis of the hair specimen.
2. The scan range should ideally be limited to an upper value of 20 µm. This obviates the scanner from exceeding its *z* range while tracking the curvature of hair surfaces. Such artifacts have been reported elsewhere ***(4)***.
3. The resolution should be set to 500, that is, the topography dataset will comprise 500 lines × 500 pixels. A greater resolution can be used if desired.
4. An optimal scan rate of 3 Hz is recommended.
5. Surface architecture information is best revealed using a hypothetical light source positioned to the left of the image.
6. Obtain an image of the hair surface and check the direction in which the cuticular sheets overlap one another.
7. Change the scan direction so that the cuticular sheets overlap from left (root end) to right (tip end; *see* **Note 2**).
8. Obtain 10 images each for treated and untreated regions for two hairs (4 × 10 images). Ideally, more hair fibers (approx 10) should be examined.

3.3. Image Analysis

1. Use the TopoMetrix Image analysis software (TopoMetrix SPM Lab. 1996, Version 3.06.06, TopoMetrix Corporation, Santa Clara, CA) to load the saved topography image and export the dataset as a text file. The images should not be levelled and/or shaded before exporting the data. The cuticle step height program requires the data to be delimited with comma separation and for the file header information (first 18 rows of data) to be retained. The 40 text files should be stored in a directory C:\subdirectory.
2. The text files should have three digit filenames. For example, "1b0.txt" refers to hair 1 (the prefix), bleached (b, or u for untreated), first image (0). Image numbers range from 0 to 9.
3. Use the cuticle step height program, coded in Microsoft MS-DOS QuickBasic, to calculate the cuticle step heights in all the images. A program listing is provided in **Subheading 3.4.** (*see* **Notes 3** and **4**).
4. Four output text files, consisting of one long column of cuticle step heights in nanometres (nm), are produced: hair 1, untreated; hair 1, treated (bleached); hair 2, untreated; and hair 2, treated.
5. The output text files can be read into a spreadsheet, such as Microsoft Excel, and imported into a software package such as Microcal Origin 4.2 to produce line

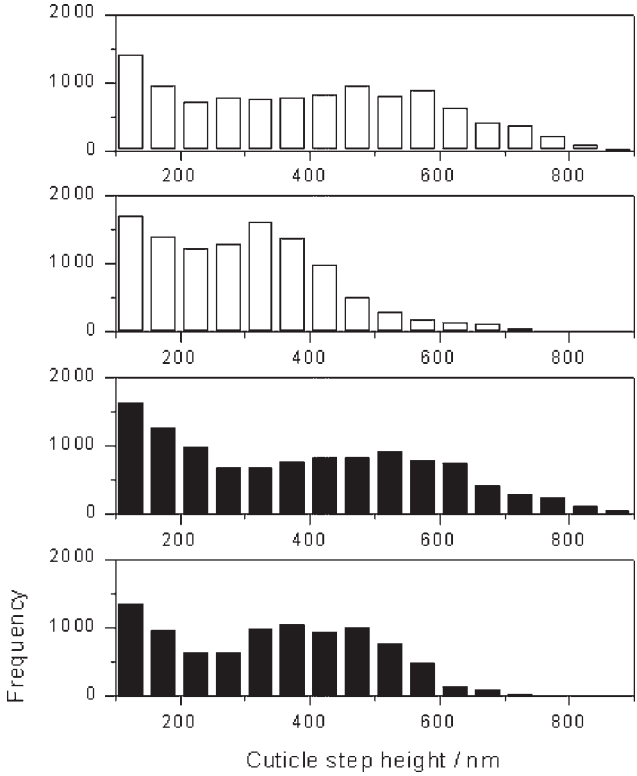

Fig. 3. Frequency histograms of cuticle step heights observed for bleach treated (unshaded) and untreated (shaded) hairs. One-way analysis of variance showed there to be no significant differences in the mean step heights for bleached and untreated hairs (p <0.05, N ≃ 10,000 steps per image).

graphs and histograms and to perform statistical analysis using, for example, one-way analysis of variance (**Fig. 3**).

3.4. Cuticle Step Height Program Listing

```
REM*********************************************************************
REM*** IMAGE ANALYSIS OF ATOMIC FORCE MICROSCOPY IMAGES     ***
REM*** OF HUMAN HAIR                                       ***
REM***                                                    ***
REM*** J.R. SMITH, SPM LABORATORY,                         ***
REM*** UNIVERSITY OF PORTSMOUTH                            ***
REM*********************************************************************

DIM height(1000): REM z-height at position 1 to 500
DIM deriv(1000): REM first derivative of height data
DIM maxgrad(1000): REM x-axis pixel position when deriv() >
              gradient threshold
```

```
DIM newstep(100): REM x-axis pixel position marking start of
                each cuticle step
DIM endstep(100): REM x-axis pixel position marking end of each
                cuticle step
DIM stepedge(100): REM calculated cuticle step height for output

sca = 20: REM scan range = 20 microns
res = 500: REM resolution = 500 pixels, image is 500 lines x 500
                pixels
minh = 100: REM minimum height accepted as cuticle step
maxh = 900: REM maximum height accepted as cuticle step
numhair = 2: REM number of hairs to be examined
numtrt = 2: REM number of treatments to be examined (untreated &
                bleached)
numimag = 10: REM number of AFM images per treatment, per hair

CLS
FOR hair = 1 TO numhair
  FOR treat = 1 TO numtrt
    IF treat = 1 THEN outname$ = RIGHT$(STR$(hair), 1) + "u"
                ELSE outname$ = RIGHT$(STR$(hair), 1) + "b"
     OPEN "C:\subdirectory\" + outname$ + ".txt" FOR OUTPUT AS
                #2

       FOR sample = 0 TO numimag-1
       file$ = outname$ + RIGHT$(STR$(sample), 1)
       OPEN "c:\subdirectory\" + file$ + ".txt" FOR INPUT AS #1

       FOR n = 1 TO 18:  REM reads superfluous header information
            INPUT #1, x$
         NEXT n

       FOR major = 1 TO res: REM for 'res' lines of data

         FOR n = 1 TO res: REM for 'res' columns of data
         INPUT #1, height(n)
         NEXT n

         deriv(1) = 0
         FOR n = 2 TO res
           deriv(n) = height(n) - height(n-1)
         NEXT n

         num = 0: REM number of pixels positions where deriv() >
                threshold, set to 10
         FOR n = 1 TO res
           check = ABS(deriv(n))
         IF check > 10 THEN num = num+1: maxgrad(num) = n
         NEXT n

         count = 1
         newstep(count) = maxgrad(count)
         FOR n = 2 TO num
```

```
            gap = maxgrad(n) - maxgrad(n-1)
              IF gap >= 10 AND n >= 5 THEN count = count+1:
                newstep(count) = maxgrad(n): endstep(count-1)
            maxgrad(n-1)
              NEXT n
            endstep(count) = maxgrad(num)

            FOR n = 1 TO count
              height1 = newstep(n)
              zheight1 = height(height1)
              height2 = endstep(n)
              zheight2 = height(zheight2)
              stepedge(n) = INT(ABS(zheight1-zheight2))
            NEXT n

            FOR n = 1 TO count: REM removes step heights equal to
                zero
                IF stepedge(n) = 0 THEN count = count-1
            NEXT n

            FOR n = 1 TO count
            IF stepedge(n) > minh AND stepedge(n) < maxh THEN
                PRINT #2, stepedge(n): PRINT hair; treat; sample;
                major; n; stepedge(n)
            NEXT n

        NEXT major

        CLOSE #1
      NEXT sample
      CLOSE #2
    NEXT treat
NEXT hair
END
```

4. Notes

1. Self-adhesive carbon discs (Agar Scientific, UK) tend to be more adhesive than carbon tape, and are especially suited for wet-cell work (although the experiments described here were performed in air).
2. The best way of viewing the surface architecture of cuticle scales is with the orientation such that scales overlap from top–left (root end, highest point) to bottom–right (tip end, lowest). With the TopoMetrix Discoverer TMX2000 instrument, this can be obtained by mounting the hair perpendicular to the long axis of the cantilever and performing the scan at a scan rotation of either 90 or 270°, depending on juxtapositions of the root and tip ends. However, the required orientation for the cuticle step height program is for the cuticle scales to overlap from left to right. This can either be achieved by rotating the scan direction before acquisition, or rotating the image, typically by about 20°, during the image-analysis routine (**Fig. 4**). The latter, less favorable method reduces the scan range of the image and so reduces the number of cuticle steps per image.

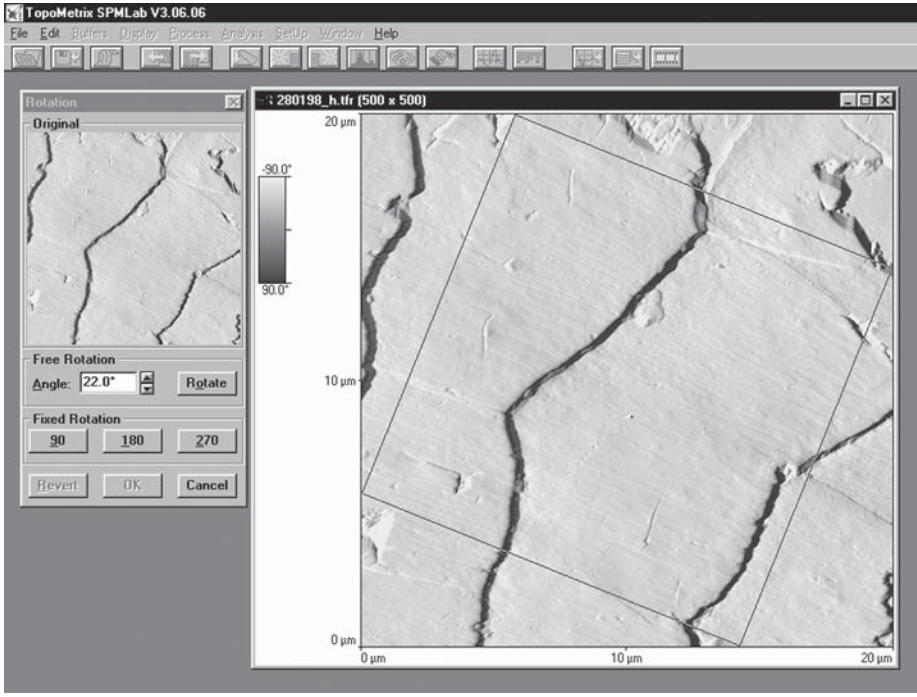

Fig. 4. The cuticle step height program requires the scales to overlap from left (root end) to right (tip end). This can be achieved either by rotating the sample (preferred method), or, as shown here, rotating the image (dataset) through 22°.

3. The program also assumes that the image is free from artefacts caused by the scanner going off-range as a result of the gross curvature of the hair sample (*see* **Subheading 3.2., step 4**). It is also necessary for the hair sample to be free from endocuticular debris, which is sometimes observed at the foot of cuticle steps, otherwise an erroneously low step height may be recorded.
4. The general program methodology is as follows: The program opens the input text file and examines the first line consisting of 500 data points of height values recorded in nanometres. The line of data is then derivatized using a first-order algorithm to locate the positions of the cuticle steps on the *x* axis. Markers are set that tag the start and end of each cuticle step on the line profile. This is achieved by defining a threshold gradient, currently set to –10 nm/pixel. The minus sign indicates that the cuticular sheets step down from left to right across the image; the units are simply a result of *z* data being recorded in nanometers and lateral information being recorded in pixels. The points where the derivative plot falls below the threshold and subsequently rise above it mark the start and end of the cuticle step, respectively. For this, the program considers all the points below the threshold gradient and measures the gap between the current pixel position and

its previous value. If the gap is greater than a given clearance, currently set to 10 pixels (0.4 μm for a resolution of 500 pixels), then a new cuticle has been identified. The routine continues until the end of the line. Cuticle step heights are then calculated by measuring the vertical distance between the start and end markers for each cuticle step. Steps heights less than 100 nm or greater than 900 nm are neglected. The process is repeated for the remaining 499 lines of the input text file. The program can easily be adapted to open further text files corresponding to more hair images to construct a more representative sample set.

Acknowledgments

Thanks are owed to the Royal Society of Chemistry and Royal Society for financial support.

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