

ORIGINAL ARTICLE

Critical study of hair growth analysis with computer-assisted methods

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Introduction

Computer-assisted image analysis has been proposed for human hair growth analysis. Some patents have been filed and publications followed in the 1980s.^{1,2} However, it soon became clear that hair is a tricky material for automated computer-assisted image analysis,³ and that numbers might not all be considered as reflecting hair measurements. Physical properties of hair, that is, the object and the variability of the skin, and their background are very complex. The multilayered fibre is composed of a non-pigmented cuticle, a cortex with presence or absence of pigment granules, and a medulla filled with proteinaceous material or air cavities. On top, its organization and orientation at the exit point from the skin must also be taken into account. A follicular unit comprising a number of hair follicles (occasionally up to

Abstract

Background Computer-assisted image analysis has been proposed for human hair growth studies.

Methods The performances of Trichoscan, a commercially available automated system combining epiluminescence microscopy with digital image analysis, developed for office-based hair growth measurements, have been evaluated comparatively on the same skin sites using standardized photographic equipment and calibrated processing for contrast-enhanced phototrichogram (CE-PTG) analysis. This reference method has been validated with scalp biopsies and histological examination of serial sectioning.

Results Besides edge effects, hair fibres escaped the Trichoscan analysis for various reasons including, but not limited to, thickness, pigmentation, closeness and crossing.

Conclusion Most of these problems have been identified in the late 1980s and remain largely unsolved by the processing software that was evaluated in 2004. Therefore claims promoting the Trichoscan method for accurate hair measurements in clinical trials on scalp and body hair are not supported by the present investigation. The speed at which the analysis is performed is outweighed by the errors in signal detection. Therefore we suggest that improvements must be clearly documented before Trichoscan is established for quantified diagnostic purposes and detailed hair cycle monitoring during hair trials.

5) may exit from a single orifice at the skin surface and it may be difficult to count individual hair fibres. Some attempts have suggested that use of fully automatic systems may be an option⁴ but this has not been made available to the public. A software named Trichoscan⁵ combining epiluminescence microscopy with digital image analysis has recently been proposed for automated image analysis of scalp hair. This method requires the use of hair dyes for improved detection of less pigmented and thinner hair as suggested⁶ and reported⁷ earlier. Using standardized photographic equipment and calibrated processing for contrast-enhanced phototrichogram (CE-PTG) analysis, we established a protocol that was equally sensitive as scalp biopsies⁸ for hair detection and growth staging. Taking this as a reference method, the aim of this study was to evaluate the advantages and limits of Trichoscan for human hair growth analysis.

Materials and methods

Preliminary observations and study rationale

A detailed examination of one image showed that some hair might be undetected,⁹ and this prompted us to evaluate a reproducibility study.

Duplicate images were taken a few seconds apart from a given scalp site of two different patients (Subject 1 TG and Subject 2 UD). The images were subject to the fully automated Trichoscan analysis. The same images were reprocessed according to our method.⁸

Duplicate analysis of high-resolution image

Similarly results of a high-resolution image published by R. Hoffmann using the Trichoscan software in 2003¹⁰ appear to have been subject to a second analysis using the same software. The same image (sufficiently detailed to ascertain identity between images but less than optimal resolution for our image analysis) appeared again in 2004 (June 2004; website: <http://www.Hairlosstalk.com>) together with results but some numbers were not consistent. The high-resolution image (published in 2003) was taken as another source of information. The duplicate source data (numbers dated 2003 and 2004) may be considered as totally independent from the present team of investigators.

The results of variations between the analyses stimulated us to proceed with a new series of images and an extended paired comparison of results using both methods for analysis.

Extended paired comparison

We planned to target a population of 1000 hair fibres for paired analysis. With an average density of 200 per cm², 5 scalp sites would be required. In our laboratory, we had at our disposal an image bank taken of 4 different areas of one subject, that is, containing 192 contrast enhanced phototrichograms (CE-PTG) using a validated and calibrated method (described elsewhere⁸).

We performed CE-PTG on all sites every consecutive month during 4 years (48 months),^{11,12} the density obtained in our preliminary studies ranged between 250 and 305 hair per cm².

Six months after the last session (after the 48th month), we decided to select the same 4 scalp sites for the present comparative study. In addition, because it has been mentioned that Trichoscan could be used on all body sites, we added one site outside the scalp area, that is, on the beard.

Trichoscan method

As detailed in the Trichoscan website (<http://www.Trichoscan.com>) we used the hair clipping method (3 days before imaging) and used hair dyeing as recommended (15–20 min of contact) 3 days after clipping, that is, immediately before imaging. Using a Fotofinder epiluminescence microscope equipped with the Trichoscan software we explored 4 scalp sites. Three were barely affected by hair loss and one in the vertex showing incipient androgenetic alopecia grade III vertex according to the usual classification,¹³ and one spot was located in the beard with a mixture of pigmented and unpigmented hair.

After completing all technical procedures prescribed in the Trichoscan operating instruction manual, analysis was performed.

The computer automatically displays a standard area (0.651 mm²) in which the software performs the analysis.

The data generated display the hair number in the target site, hair fibres in contact with the borders of the target site, anagen and telogen hair counts.

The process of the analysis and the results were captured on a CCD camera. This allowed a hair-by-hair evaluation of which fibres were indeed correctly detected and measured by the software and at the same time we were able to characterize those fibres that were either not detected or erroneously analyzed.

Hair data processing with reference method

The same sites were photographed at the same session within minutes. Images were stored and analyzed later using our standard internal imaging procedures based on the calibrated method published elsewhere.⁸ Accordingly, we updated and calibrated the analytical process, that is, computer-assisted processing for the specific requirements of the present study protocol (internal reference: H.A.I.R. Technology® processing 03P23¹⁴), individual hair fibres were calibrated for length and thickness the first time on images captured immediately after clipping (without contrast enhancement) and a second time at day 3, that is, immediately after the Trichoscan processing. These deviations from our standard protocol, that is, no dye at the baseline photograph and 72 h interval between two photographs instead of 48 h, were required in order to comply with the standard Trichoscan instructions for users. This deviation was mandatory in order to prevent troubleshooting or erroneous analysis due to a hair segment that would have been dyed twice during the procedure, that is, at baseline and at day 3 after clipping.

Results

Initial observations

The duplicate images were subject to Trichoscan analysis and the telogen percentage same site – same day were Subject 1 TG 19 and 24% and Subject 2 UD 27 and 19% (i.e. range of variation 5%–8%). While applying H.A.I.R. Technology® processing 03P23 to the same images, we noticed a drastic reduction in telogen percentage variation in Subject 1 TG 14 and 11% and Subject 2 UD 11 and 16% (i.e. range of variation 3%–5%).

Duplicate analysis of high-resolution image

Data of hair analysis on a high-resolution image published by R. Hoffmann using Trichoscan in 2003 also appeared as a duplicate in 2004 (website: <http://www.Hairlosstalk.com>).

Upon visual inspection the images appeared exactly the same as were the published results of hair counts, that is, 149 hair in both publications. However, the anagen percentage shifted from 69% (2003) to 82% (2004) and, as would be expected, the telogen percentage dropped from 31% (2003) to 18% (2004).

Besides, there was also unnoticed hair in the Trichoscan processing. Indeed, next to the 149 hair fibres, H.A.I.R. Technology® processing 03P23 detected another set of 27 unnoticed hair. The high-resolution images contained 18% undetected hair.

The thickness of these 27 fibres was estimated to range from less than 20 up to 30 μm (< 20 μm : $n = 10$; 20 μm : $n = 14$; 30 μm : $n = 3$).

Apart from thickness, we estimated hair cycle staging on the length of the fibre. We estimated that 8 telogen hair

fibres were missed by the Trichoscan method. Therefore a more investigative study was warranted.

Paired analysis

Imaging and qualitative observations of the analysis procedure

Obvious errors were seen on the computer screen while the Trichoscan analysis process was ongoing (fig. 1). Because a preset area of 0.651 mm^2 was used, over 500 hair fibres were available for a paired analysis. This fits with study plans of probing a 1000-hair population.

Hair counts (Table 1)

As shown in Table 1, the software was clearly unable to count hair in the beard area. Each hair, pigmented as well as unpigmented, was counted twice (> 200% error). When this systematic error was excluded, in the beard as in the almost unaffected scalp areas the error ranged from +18.32 to +25.23%. There is an overestimation in all sites except in the vertex (–17.8%) because of a substantial proportion of thinner hair (32%). We estimate that about

Table 1 Hair counts

Hair number	Reference	Trichoscan	$\partial (T-R)/R$ (%)	%
Beard	19	61	221	
Top left	131	155		18.32
Occipital	107	134		25.23
Top right	150	181		20.67
Vertex	118	97		–17.80

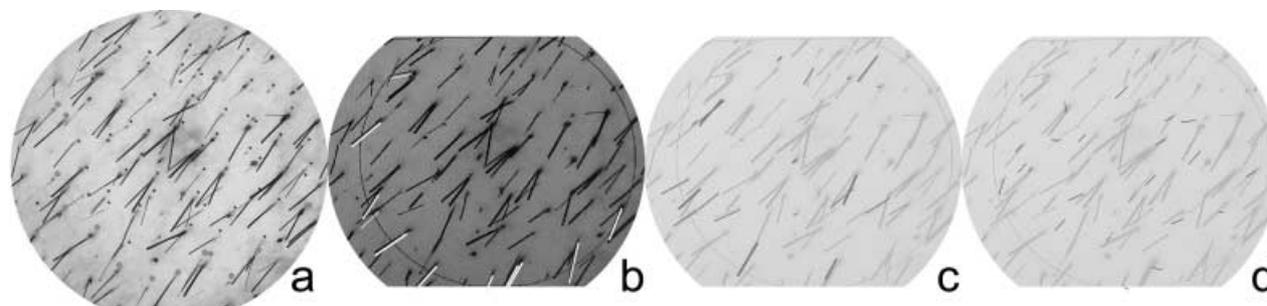


fig. 1 Detailed identification of errors in scalp hair analysis by Trichoscan. The source image from the vertex (a) shows individual hair fibres together with a series of dots indicating the origin of hair fibres belonging to the target area. The bigger dots show hairs that might touch the edge or outline of the target area. In the paired analysis, we identified 55 hairs that were either deleted from the analysis (edge effect; b; $n = 11$ hair) or that were not detected by Trichoscan analysis. The undetected hairs (c, d) were 21 thick

and 23 thin hairs. Using the H.A.I.R. Technology® approach, the missing hair were not only evaluated for thickness but also for growth: the thicker hair (c) were classified as telogen (6 short fibres) or anagen (15 long fibres); the thinner hair (23 fibres; d) were mostly non-growing vellus-like hair.

Of a total of 118 hair that we identified in the field outlined by Trichoscan, there were 55 hair errors, that is, 46% (for a detailed split of errors, see Tables 1–4).

half of the thinner hair remained undetected by the Trichoscan.

Anagen percentage (Table 2)

Anagen percentage was underestimated in the beard area, overestimated in the vertex site and almost correctly evaluated in the other scalp sites (range -6.92 to +1.37%; Table 2).

The influence of inappropriate detection and measurements of the thickest (beard) and thinner (vertex) hair may be responsible for these discrepancies.

Also the length of the fibres immediately after clipping conditions affects the classification into anagen or telogen. Even though a trained technician performed the clipping carefully, the range of clipping extends from less than 300 µm up to more than 1 mm (10th centile: 313.62 µm; 90th centile 705.24 µm; data not shown). This indicates that a fixed cut-off point of 700 µm to discriminate between anagen and telogen would inevitably display a 10% error, that is, including more anagen. On the contrary, too close clipping and slower growth rates might result in underestimation of anagen. In this particular assay growth rates ranged from 166 µm/day (in the thinnest fibres) up to 457 µm/day in the thickest fibres. After three days under the worst conditions (10th centile and slow growth rate) the length of the fibre would reach 313 + 498 = 811 µm. Therefore we presume that error in detection of the fibre is responsible for errors in anagen percentage discrimination.

Surface analysis (Table 3)

Definition of the surface that contains hair fibres remains problematic with the Trichoscan. Indeed a lot of hair fibres that cross the borders of the target area are fragmented so that they may be considered by the software either to belong to the target site while in fact they are not (fragmented anagen) or erroneously excluded from the area (especially the longer anagen hair fibres). The algorithm that excludes all hair touching the borders is not appropriate and leads inevitably to a systematic

Table 2 Anagen percentage

Anagen %	Reference	Trichoscan	∂ (T-R)/R (%)	%
Beard	94.74%	81%	-15	
Top left	87.02%	81%		-6.92
Occipital	88.79%	90%		1.37
Top right	88.00%	82%		-6.82
Vertex	56.78%	75%		32.09

Table 3 Surface analysis

Surface	Reference	Trichoscan	∂ (T-R)/R (%)	%
Beard	0.3859	0.651	69	
Top left	0.5170	0.651		25.92
Occipital	0.4424	0.651		47.15
Top right	0.4988	0.651		30.52
Vertex	0.5374	0.651		21.15

Table 4 Density

Density	Reference	Trichoscan	∂ (T-R)/R (%)	%
Beard	49.23	93.70	90	
Top left	253.39	238.10		-6.03
Occipital	241.87	205.84		-14.90
Top right	300.74	278.03		-7.55
Vertex	219.59	149.00		-32.15

underestimation of the density as illustrated by our experimental results.

Density (Table 4)

Because of all of the other differences (+ or -), one should not be surprised to find differences when scalp counts are to be expressed relative to the area. The latter was indeed systematically underestimated. Finally the major error of beard hair counts accounts for the sign change observed in calculation of hair density.

Discussion

Our study was prompted by a number of variations that were unexpected after considering the original claims for accuracy promoting the Trichoscan method for hair growth measurement.^{5,15} Indeed, as mentioned in such publications, intra-class correlation of approximately 91% within the same operator and an inter-class correlation of approximately 97% for different operators, suggested that the method was very precise and reproducible. Advocates for the method declare that a system must be able to analyze the biological parameters that constitute hair growth, which are: (1) hair density (n/cm²), (2) hair diameter (µm), (3) hair growth rate (mm/day) and (4) anagen/telogen ratio. Our present investigation does not support these claims.

With the available software, numbers were displayed for hair counts (all fibres detected by the software in the target area, and those that touched the border of the target area). This number is also split into resting hair (telogen) and those considered growing, that is, in anagen

phase of the hair growth cycle. The commercially available software provided to dermatologists and hair clinics for office-based use does not display the thickness of hair fibres and the hair growth rate. Although cumulative thickness may be an indirect way to approach the hair thickness measurement, it provides a global measure that depends on hair cycle duration.¹² Also we doubt that Trichoscan measures growth accurately. First, there are no growth rates on the data display. Second, the precision of anagen hair detection is not optimal. Indeed the anagen percentage was underestimated (difference > 5%) in two out of four scalp sites as well as in the beard area but it was overestimated when thinning was more important (overestimation of 32% anagen hair proportion in the vertex).

A number of these errors (especially with thin hair detection) have been described by others using the Trichoscan method in normal scalp sites, especially a density that was underestimated by 22% and the lack of detection of thinner hair (Seung Ho Lee, Oh Sang Kwon, Jun Gyu Oh, Kyu Han Kim, Hee Chul Eun: *Phototrichogram: Evaluation of Modified Methods with Bleaching and Trichoscan*. Seoul, Korea, poster presentation at the European Hair Research Society June 2004, and personal communication). As thinning is a phenomenon associated with male pattern hair loss, published documents⁹ and the present experimental study clearly document that thin, and even thick hair counts, as well as growth staging generated by the Trichoscan method may not be considered as reliable. Therefore, we conclude that Trichoscan in the present form would not qualify as a test method for quantification of hair loss according to our internal and other standards.¹⁶

We agree with other authors that computerized methods still require optimization. Indeed, computerized methods have been quoted as 'less than ideal' in a more academic statement.¹⁷ This view is now supported by experimental evidence from different sources (Seung Ho Lee *et al.* personal communication and the present paper). Our statement⁹ 'Hair evaluation as performed with automated systems does not match the quality standards prevailing in our laboratory. Many problems identified in 1989³ still cause problems for automatic evaluation in 2004!' is confirmed herewith. Ease of use and fast image processing, as pointed out by others^{18,19} (and Seung Ho Lee *et al.* personal communication), are certainly appreciated. Nevertheless, albeit speed is considered smart in our culture, we believe that customers, that is, clinicians, patients, and pharmaceutical or cosmetic companies, deserve the highest standard and a better service than merely a fast one. All should be given the best possible and clinically most relevant information about hair measurements – both qualitatively and quantitatively – that have diagnostic, prognostic and

therapeutic relevance. Therefore we suggest that substantial improvements must be documented before Trichoscan is established for office-based quantified diagnostic purposes and for detailed hair cycle monitoring during hair trials.

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