

## Key concepts

- ✓ There is a need for a validated tool to monitor hair loss and treatment response in clinical practice as well as in experimental trials.
- ✓ Since the introduction of the TrichoScan® as an *in vivo* non-invasive method to evaluate human hair in 2002, several validation- as well as study results have been published.
- ✓ TrichoScan® is capable to measure all important hair growth parameters such as hair density and thickness and with this chapter some practical as well as theoretical aspects will be described.

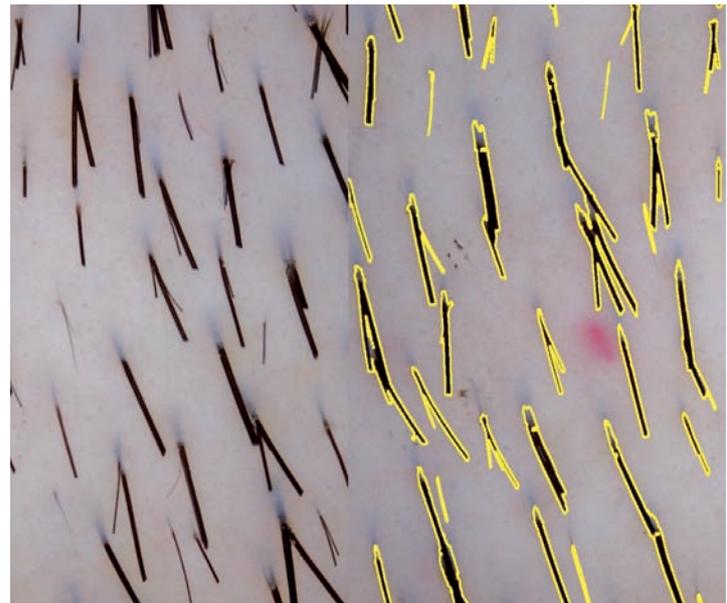
## 1. INTRODUCTION

Hair loss, or thinning hair, is a common complaint in clinical dermatology. However, patients seeking advice for hair loss are not necessarily bald and the effects of treatment attempts can be hard to measure. Consequently, there is a need for a sensitive tool to monitor hair loss and treatment response in clinical practice as well as in experimental trials. Such a tool must be able to analyze at least the most important biological parameters of hair growth, such as hair density ( $n/cm^2$ ), hair diameter ( $\mu m$ ), hair growth rate (mm/day), or anagen/telogen ratio, just to mention a few. In addition, any technique must produce results which are representative for the whole scalp.

Since the introduction of the phototrichogram as an *in vivo* non-invasive method to evaluate the human hair cycle in 1970, variations of the technique have been used as a basis for quantitative analysis of hair growth<sup>1-10</sup>. This chapter describes the TrichoScan® software and provides an insight into its practical, theoretical and statistical analysis background.

## 2. CLIPPING OF HAIRS WITHIN TARGET AREA

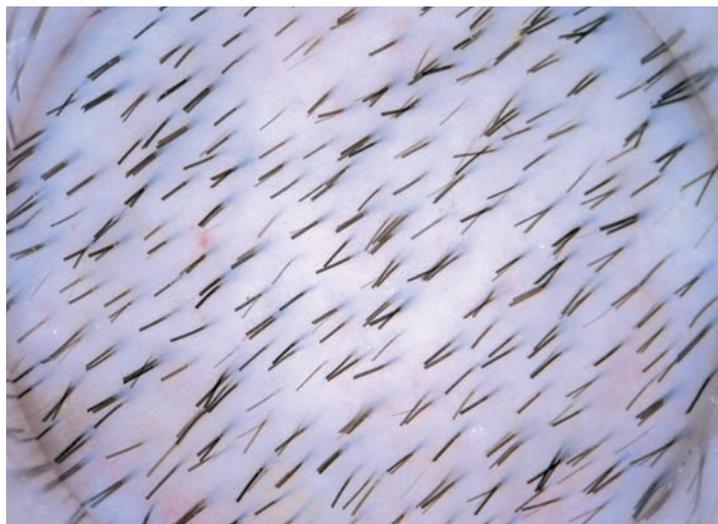
To prepare an area of scalp for TrichoScan® use, it is common practice that the hairs of the subject are clipped with an electric hair clipper. In our experience the need for precise



hair clipping must not be underestimated; the hair clipping procedure is prone to some avoidable errors. Hairs must not be clipped too short, simply because manual and automatic hair counting cannot count hairs which are not visible, and the hair clipping must be even throughout the image (Figs. 1, 2).

## Contents

1. Introduction
2. Clipping of hairs within target area
3. Contact images from the scalp
4. Contrast enhancement of hairs
5. Data base
6. Quality control of images
7. Validation trials using TrichoScan® Research 3.0
8. Discussion and future developments



**Figure 1.** TrichoScan® images directly after even hair clipping without hair dye. We accomplish this with a modified hair clipper with a very narrow clipping knife and a spacer piece that avoids clipping hair too short (Fig. 2).

### 3. CONTACT IMAGES FROM THE SCALP

A prerequisite to measure hair length is that images are taken in 2 dimensions, which means clipped hairs must lay flat onto the scalp. To accomplish this, all systems use a rigid optical contact plate of different size, which is firmly pressed onto the scalp (Fig. 3). A LED ring-light ensure proper lighting for all images.

This also ensures that images are always taken at the same distance from the scalp. To enhance the contact of the optical plate and the scalp we use an alcoholic disinfection spray; after taking the image, the alcohol spray will dry and leave no remnants behind.

Others use a clear gel, however, this is much more difficult to remove and leaves a greasy-looking hair coating behind, which is of some concern for some patients, especially women.



**Figure 3.** TrichoScan®-optics with contact plate.

### 4. CONTRAST ENHANCEMENT OF HAIRS

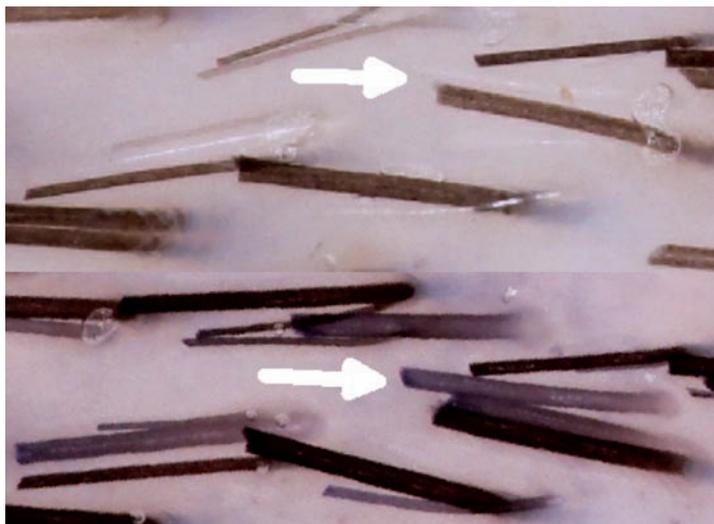
White, grey or fair hairs have only limited contrast in comparison to the scalp skin, which makes it difficult or even impossible for manual and automatic hair counting (Fig. 4). Therefore a hair dye was introduced by Van Neste when he described his contrast-enhanced phototrichogram.

### 5. DATA BASE

All cameras can be manually adjusted to meet personal needs. For a scientific instrument this has to be avoided, as



**Figure 2.** TrichoScan® hair clipper.

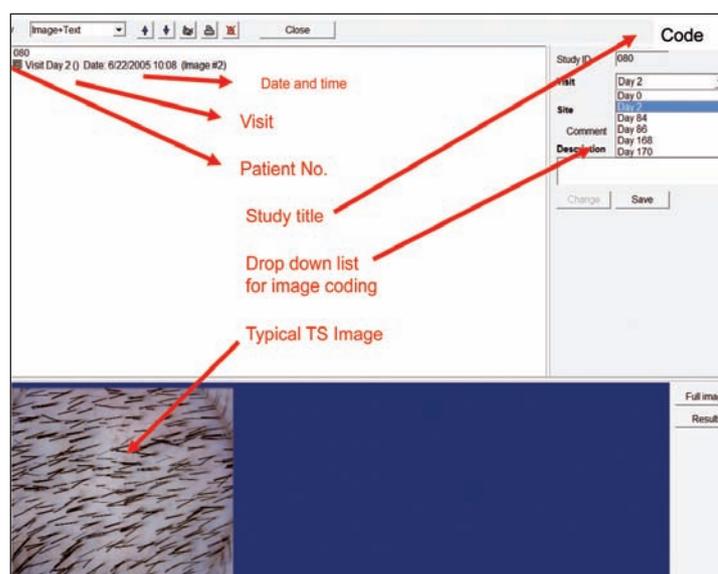


**Figure 4.** TrichoScan® images with and without hair dye. White hairs (white arrow) are virtually invisible without a hair dye.

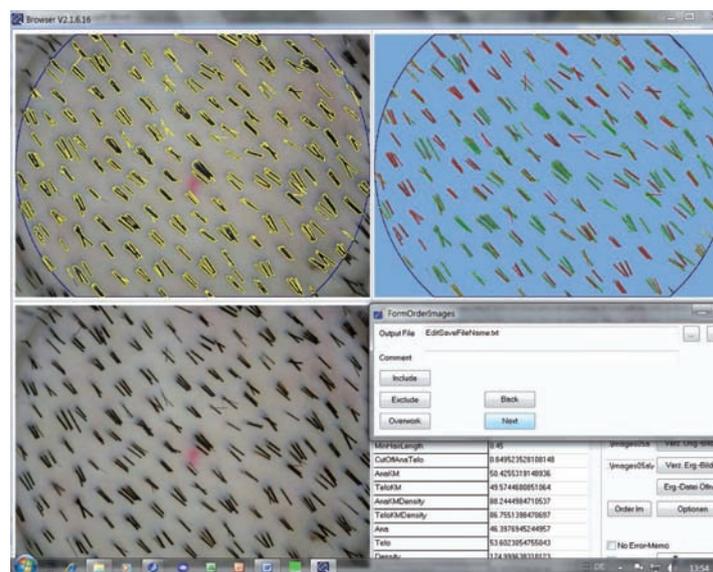
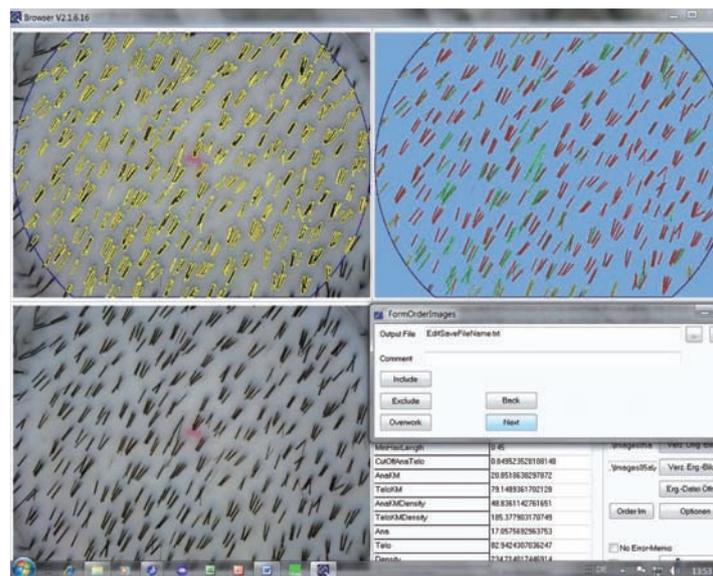
otherwise no reproducible images can be made. In our tool, the camera is totally controlled by the software, what ensures that camera settings such as zoom, contrast, resolution, compression and much more are always the same from image to image. Images are then stored in a data base (Fig. 5).

## 6. QUALITY CONTROL OF IMAGES

In a clinical trial with several study sites and different study personal, all images must be of uniform quality. We usually train all study persons involved in the TrichoScan® procedure and perform quality assurance after taking images.



**Figure 5.** Example of TrichoScan® images as they are stored in the data base. Every image is described by the date and time, as well the study code and image code.



**Figure 6.** Example of TrichoScan® images quality assurance software. All images are checked to meet TrichoScan® technical requirements.

Before images are analyzed, all images will be assessed whether they fit to the technical requirements such as good contrast and lightning, no air bubbles or no hair dye remnants (Fig. 6). Those assessment are done in a blinded fashion where the assessor has no information about the study drug.

## 7. VALIDATION TRIALS USING TRICHOSCAN® RESEARCH 3.0

In 2009 we performed a validation trial of the TrichoScan® method and compared hair growth parameters using TrichoScan® software versus manual identification of hairs<sup>11</sup>. In this trial, digital images for TrichoScan® or conventional visual analysis were taken from 10 patients aged

18 years or older with AGA, Norwood-Hamilton grade III-IV/Ludwig grade I or II. The participants were selected from a volunteer panel at bioskin GmbH, Hamburg. All patients included in this study had also taken part in a previous hair growth study at bioskin and already had a suitable measurement area marked with a tattoo. There were no other pigmented lesions in the treatment area. The recommendations of the Helsinki Declaration and the ICH GCP guidelines were followed. Written informed consent was obtained before inclusion in the study.

On day 1 the measurement area on the anterior border of the vertex balding spot was identified (marked with a central tattoo). The area was clipped evenly (Moser, TrichoScan® Edition) and short clipped hair was removed by pressing an adhesive strip onto the shaved area three times. The quality was checked with a magnifying glass. Afterwards a digital image was taken for documentation of the time. Digital images were stored in an image database (Image DB).

Hair dye and TrichoScan® image on day 3: On day 3 (48 ± 2 hours after hair clipping) the clipped hairs within the target area were dyed (Goldwell topchic, black 2N, Darmstadt, Germany with Rondo 6% Crème-Oxyd, Coiffeur, Cologne, Germany). After 12 minutes the colored area was thoroughly cleaned with an alcoholic solution (Kodan® Spray, Schülke & Mayr, Vienna, Austria) and digital images were taken using a digital ELM system. Three separate images (B1, B2, B3) were taken by three investigators (U1, U2, U3), altogether nine images were taken of each area.

To be admissible the following requirements had to be fulfilled for all images: All hairs were uniformly dyed, all hairs were evenly clipped, no remnants of hair dye were present, no air bubbles were present around the hairs, the image was bright and sharp, no hairs from outside the measurement area crossed the field, and all hairs were straight. None of the images which were taken had to be excluded from analysis. All images were analyzed using TrichoScan® Research Edition 3.0 and results were imported into Excel. Data obtained by manual evaluation were extracted with special software into a tab delimited text file which was also imported into an Excel® data sheet. The statistical analysis was performed at bioskin.

Three CDs of the images were produced for manual analysis by three independent evaluators. These CDs contained the same images which were analyzed by TrichoScan®, but they were additionally embedded in a software program ("hair measure tool" provided by Datinf GmbH, Tübingen). This software contained all 90 images in random order. Randomization was done by Datinf GmbH, Tübingen, Germany. The images were numbered 1, 2, (x), – 90. No information was given about who took the image and from which subject the image was taken. It was not possible to delete images from, or to add images to, this CD. Each evaluator used a computer mouse to outline the perimeters of each hair fiber on each image. He/she had to click on every hair where the hair left the scalp skin, then follow the hair and release the mouse button at the end of the hair tip. The thickness of the

yellow line (the hair) was adjusted to the actual hair thickness with the scroll wheel on the computer mouse. When the yellow line had the same thickness as the actual underlying hair, the correct thickness of this hair was determined. Hair density (number per unit area on the image) and hair thickness (hair diameter) were recorded automatically by the software. Hairs starting from outside the target area which had the hair tips inside the target area were not counted. Hairs which started inside the target area but left it were counted, however, these were not used for the analysis of hair growth as the complete hair shaft was not in the target area. The evaluator did not have access to any of the calculated results such as hair density and thickness, therefore he/she was unable to compare different analyses. After each analysis the manual evaluator had to click "finish" and thereafter this image was no longer available for counting. No information was given about the results of the computerized TrichoScan® images.

In this GCP-conforming study the clinical trial situation was imitated where different investigators use the same equipment on different patients. In this study, there was a highly significant correlation between evaluation of hair parameters using manual identification of hairs and the fully-automated TrichoScan® method. This demonstrates that the TrichoScan® software, although working by statistics and mathematical approximation, counts hairs and not artefacts. Nevertheless, there were some differences between the methods with regards to absolute values. The strongest differences were seen in the mean thickness and total density of the hairs. TrichoScan® values for hair thickness were approximately 10% higher and density values approximately 10% lower than the values obtained by the manual evaluators. The differences in most other parameters were less than 5%. In particular, the critical parameter, cumulative hair thickness, was in good agreement with the manual evaluation. It is not surprising that TrichoScan® underestimates the total hair density: As a digital tool TrichoScan® relies on camera resolution. In this study resolution was 2 Megapixels, a resolution at which very thin (below 7 μm) hairs are not analyzed. In our opinion this is not a disadvantage as these hairs are not clinically relevant. Regarding the overestimation of hair thickness by TrichoScan®, it must be kept in mind that this is a systematic difference and occurs for every hair. Therefore, it has no impact on the TrichoScan® capability to measure changes in hair thickness. If needed, the thickness can be mathematically readjusted.

Not surprisingly, considerable variability was noted for the manually marked images<sup>12</sup>. Manual marking of hairs is tedious and time-consuming, making it near to impossible to repeatedly count hundreds of hairs without variation for density, length and thickness. The mean data variability for one evaluator who marked the same image three times ranged from 2.71-12.95%, depending on the parameter (Table 1). Some evaluators showed more variability than others. The correlation between different evaluators was best for total hair density and parameters related to hair length; and worst for the parameters related to hair thickness. Of course, there

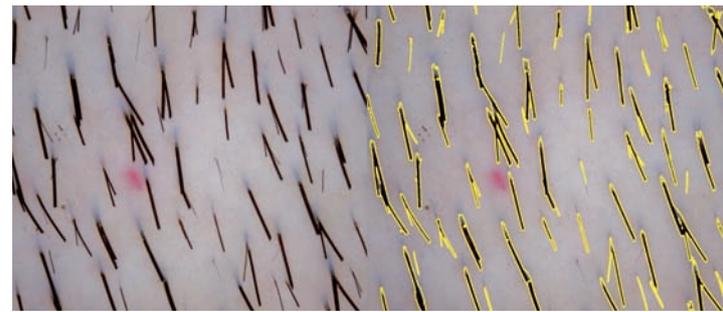
Table 1.

Variable	Hand	TrichoScan®
Hair count total	7.07%	0.00%
Hair count terminal	11.41%	0.00%
Cumulative thickness total	8.09%	0.00%
Cumulative thickness terminal	12.95%	0.00%
Mean thickness	6.53%	0.00%
Mean thickness terminal	3.99%	0.00%
Growth rate total	2.71%	0.00%
Growth rate terminal	3.38%	0.00%
Cumulative growth rate total	8.12%	0.00%

was no variability in repeated measures with TrichoScan®, the software delivered completely reliable results. Keeping in mind the definition of repeatability, the maximum of the difference between two measurements on the same patient, this is a tremendous difference between manual marking of hairs and TrichoScan®.

## 8. DISCUSSION AND FUTURE DEVELOPMENTS

In summary, in our validation study we saw an excellent correlation of hair growth parameters analyzed between the fully-automated TrichoScan® method and manual marking of hairs prior to analysis. Considerable variability was seen in the results from manually identified hairs, compared to no variability in TrichoScan®-analyzed images. In a clinical trial, the wider margin of error and consequent data variability from manually evaluated images would necessitate a larger study sample size to overcome the effect of the variability on the statistical calculations. Therefore, the TrichoScan® research technique is particularly suitable for clinical studies with treatment comparisons. That this is indeed the case can be seen in the results of a clinical trial evaluating changes of hair growth and thickness in 34 Minoxidil-treated men. Using the TrichoScan® method, treatment benefits could be seen 8 weeks after treatment initiation<sup>13</sup>. Moreover, TrichoScan® can be adopted to study the effect of drugs or laser treatment on hypertrichosis or hirsutism<sup>14</sup>. Another very important point is the objectiveness of the fully-automated TrichoScan® method. This implies that even in open trials or trials with apparent effects there is no bias due to the study groups.

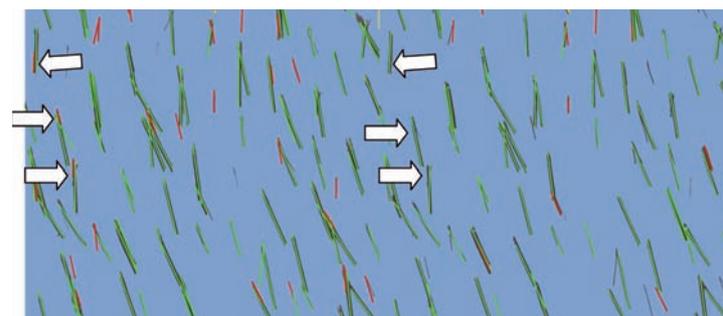


**Figure 7.** Example: Original image (left) with perfect image illumination but, due to the nature of asian scalp, with dark hair ostia. Raw results (right) of original image. The red tattoo is not analysed.

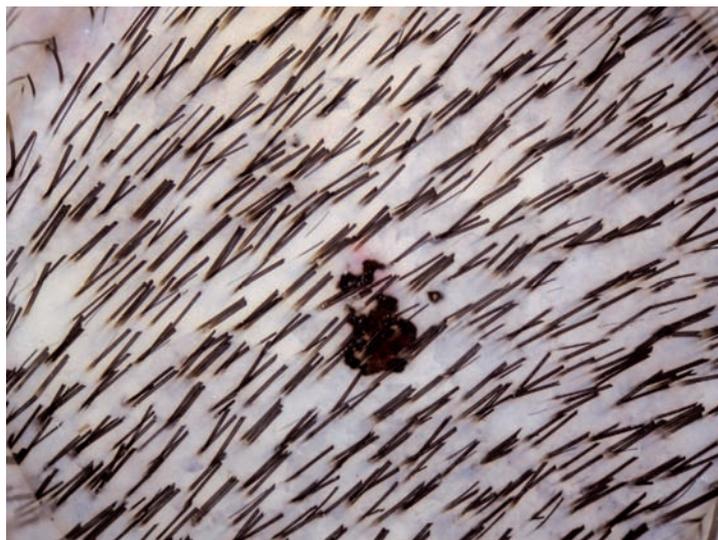
One drawback with TrichoScan® is the management of dark hair ostia, which can be typically seen on asian scalp (Fig. 7). Here Saraogi et al.<sup>15</sup> as well as VanNeste<sup>16</sup> stated that our software detects hairs where no hairs are present. We also subsequently observed similar phenomena in some asian people and therefore adjusted TrichoScan® settings for those cases where such phenomena occur. In contrast to Caucasians, people in asia have much darker hair ostia which results in a larger visible diameter of hair follicle ostia. As the TrichoScan® software works using color contrast, in some cases the diameter of a large and dark ostium is incorrectly considered as a hair. This is of course an artefact and with slight adjustments of the TrichoScan® settings it is very easy for us to solve this problem (Fig. 8).

A second potential drawback with TrichoScan® is that hairs still need to be dyed prior to image collection. Although simple to do, in clinical practice the hair dye is, in our experience, the single most likely procedure which is prone to investigator-incurred . We have been presented with images which were not cleansed enough after the application of the hair dye, which led to potentially avoidable artefacts (Fig 9).

To improve this procedure, in our 4<sup>th</sup> TrichoScan® version we introduced a “short contact hair dye”, where the dye has to be applied only for 5 minutes. In our hands, with our evolved device and software, this is enough to enhance the



**Figure 8.** Example: TrichoScan® settings for caucasian hairs incorrectly count large dark hair ostia as hairs (left) but with proper adjustments (right) to the software settings these artefacts are no longer present (right).



**Figure 9.** Remnants of hair dye in a TrichoScan® image. These images cannot be accurately analysed. To improve this procedure, in our 4th TrichoScan® version we introduced a “short contact hair dye”, where the dye has to be applied only for 5 minutes. In our hands, with our evolved device and software, this is enough to enhance the contrast and to avoid unnecessary dying of the scalp skin.

contrast and to avoid unnecessary dying of the scalp skin. Furthermore, we will be able to increase the detectability of hairs by the use of 18 MP cameras, equipped with our custom made optics (Fig. 10)



**Figure 10.** Canon EOS 600 camera with Canon macro and adjusted TrichoScan-optics for highest resolution images from the scalp.

## \* REFERENCES

- Guarrera M, Ciulla MP. A quantitative evaluation of hair loss: the phototrichogram. *J Appl Cosmetol.* 1986;4: 61-6.
- Bouhanna P. The phototrichogram, a macrophotographic study of the scalp. *Bioengineer Skin.* 1985;3:265.
- Van Neste DJJ, Dumrotier M, De Coster W. Phototrichogram analysis: technical aspects and problems in relation with automated quantitative evaluation of hair growth by computer-assisted image analysis. In: Van Neste DJJ, Lachapelle JM, Antoine JL, eds. *Trends in human hair growth and alopecia research.* Dordrecht: Kluwer; 1989. p. 155-65.
- Van Neste DJJ, de Brouwer B, de Coster W. The phototrichogram: analysis of some technical factors of variation. *Skin Pharmacol.* 1994;7:67-72.
- Rushton DH, de Brouwer B, de Coster W, Van Neste DJ. Comparative evaluation of scalp hair by phototrichogram and unit area trichogram analysis within the same subjects. *Acta Derm Venereol.* 1993;73: 150-3.
- Price VH, Menefee E. Quantitative estimation of hair growth. I. androgenetic alopecia in women: effect of minoxidil. *J Invest Dermatol.* 1990;95:683-7.
- Hoffmann R. TrichoScan: combining epiluminescence microscopy with digital image analysis for the measurement of hair growth in vivo. *Eur J Dermatol.* 2001;11:362-8.
- Rushton H, James KC, Mortimer CH. The unit area trichogram in the assessment of androgen-dependent alopecia. *Br J Dermatol.* 1983; 109:429-37.
- Friedel J, Will F, Grosshans E. Phototrichogram: adaptation, standardization and applications. *Ann Dermatol Venereol.* 1989;116: 629-36.
- Van Neste DJ, Dumrotier M, de Brouwer B, de Coster W. Scalp immersion proxigraphy (SIP): an improved imaging technique for phototrichogram analysis. *J Eur Acad Derm Venereol.* 1992;1: 187-91.
- Gassmueller J, Rowold E, Frase T and Hughes-Formella B. Validation of TrichoScan® technology as a fully-automated tool for evaluation of hair growth parameters. *Eur J Dermatol.* 2009;19:224-31.
- Hayashi S, Miyamoto I, Takeda K. Measurement of human hair growth by optical microscopy and image analysis. *Br J Dermatol.* 1991;125:123-9.
- Gassmueller J, Hoffmann R, Webster A. Treatment of androgenetic alopecia using topical fulvestrant solution in males and postmenopausal females; results from two randomized, proof-of-concept studies. *Br J Dermatol.* 2008;158:109-15.
- Hoffmann R. A 4-month, open-label study evaluating the efficacy of eflornithine 11.5% cream in the treatment of unwanted facial hair in women using TrichoScan. *Eur J Dermatol.* 2008;18:65-70.
- Saraogi PP, Dhurat RS. Automated Digital Image Analysis (TrichoScan®) for Human Hair Growth Analysis: Ease versus Errors. *Int J Trichol.* 2010;2:5-13.
- Van Neste D, Trüeb RM. Critical study of hair growth analysis with computer-assisted methods. *J Eur Acad Dermatol Venereol.* 2006; 20:578-83.