

The Quantification of Terminal Hair by Digital Microscopy: Advancements Towards a More Objective Diagnosis of Hirsutism

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Purpose: The modified Ferriman-Gallwey score, currently used to clinically evaluate and quantify excessive hair growth in women with hirsutism, has limitations because of its subjective nature. Therefore, we aim to investigate whether we could quantify terminal hairs on the output of a digital microscope camera to improve the clinical evaluation of hirsutism.

Patients and Methods: This feasibility cross-sectional study included 20 healthy men and 15 healthy women. Two independent researchers used a digital microscope camera to obtain photos of the upper lip and chin in all participants. The hair thickness (when $\geq 60 \mu\text{m}$), number of terminal hairs and hair color were determined to indicate mean differences between men and women by an independent *t*-test. Additionally, intraclass correlation coefficients were determined to assess the inter-observer variability.

Results: The mean (standard deviation) number of terminal hairs on the upper lip was 27 (15) in men and 0 (1) in women. On the chin, men had a mean (standard deviation) of 29 (18) terminal hairs, compared to 0 (0) in women. This corresponds to mean differences of 27 hairs (range: 19–34) on the upper lip and 28 hairs (range: 19–39) on the chin between men and women. Minimal inter-observer variability was observed, particularly in visible light analyses (intraclass correlation coefficient: 0.998).

Conclusion: Digital microscopy with visible light may contribute to a more objective method for diagnosing hirsutism by the quantification of terminal hair. Future studies should focus on the applicability of this new method in women with hirsutism.

Keywords: digital microscopy, hirsutism, Ferriman-Gallwey score, terminal hair, hyperandrogenism, androgens

Introduction

Hirsutism is defined as the excessive growth of terminal hair in a typical male pattern in a female.^{1,2} Approximately 5% to 10% of premenopausal women in the general population are affected by hirsutism.³ The majority of hirsutism cases are due to an underlying endocrine disorder and show elevated androgen levels,^{4,5} suggesting hyperandrogenism (HA). High levels of androgens are associated with evident health conditions, such as obesity, hypertension, hypercholesterolemia, type 2 diabetes, amenorrhea and ovulatory dysfunction, which can lead to infertility.⁶ Moreover, hirsutism is highly associated with social and psychological difficulties, including anxiety, social avoidance and confusion of one's gender identity.^{7–9}

Out of all sex steroids, androgens are of utmost importance for the regulation of hair growth.¹⁰ Under the influence of androgens, hair follicles that are producing vellus-type hairs can be stimulated to produce terminal hairs, which are larger, thicker and more pigmented. Consequently, elevated levels of androgens lead to stimulation of terminal hair growth, and manifest hirsutism in females.

The modified Ferriman-Gallwey (mFG) score is the current standard scoring system to clinically assess the severity and distribution of excess terminal hair growth.¹¹ This scoring system grades 9 body areas from 0 (no excessive hair growth) to 4 (severe excessive hair growth), including the upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, upper arms and thighs. A score equal to or larger than 8 typically indicates hirsutism. Consequently, hirsutism can be classified into mild (score 8–16), moderate (score 17–24) and severe (score ≥ 25). Unfortunately, the mFG scoring system has its drawbacks. First of all, current assessment using the mFG score requires a full body examination. In clinical practice, the requirement for a comprehensive physical evaluation can pose practical challenges. These include time constraints, patient discomfort, and cultural sensitivities, which may limit the feasibility of applying the full mFG scoring system consistently. Second, the assessment of excessive hair using the mFG score gets complicated by the use of hair removal methods. Ideally, women should discontinue their current method for hair removal for approximately 3 to 4 weeks to properly assess hirsutism. Third, the Androgen Excess-PCOS (AE-PCOS) Society has proposed the need for ethnic-specific mFG cut-off values to define hirsutism.^{12,13} The different cut-off values used to diagnose hirsutism may complicate the use of the mFG score in routine clinical practice and prevent it from serving as a universal measure for identifying hirsutism. Lastly, although the mFG score has been shown to reflect androgen excess, a large variability, intra- and inter-observer variability have been reported.^{14–16} These substantial limitations are possible points of engagement to develop a new method to objectify the quantification of terminal hair. After all, a more objective and patient-friendly clinical diagnosis is valuable for future research, and the treatment, follow-up and monitoring of hirsutism patients.

Various previous studies have focused on the development of more objective methods for the quantification of hair by measuring the diameter of plucked hairs¹⁷ and measuring the rate of hair growth, using trichometry^{17,18} or other photography techniques combined with image analysis.^{19,20} The use of trichoscopic microscope cameras is very useful for diagnoses of scalp and hair disorders and may greatly improve the clinical management of these disorders.²¹ Trichoscopic cameras have previously been used to assess the results of laser treatment in women with hirsutism.²² However, manual image analysis has not yet been used to diagnostically differentiate between vellus and terminal hairs in hirsutism.

The main aim of this study is to investigate whether terminal hairs can be quantified using a digital microscope camera and to whether visible light or ultraviolet (UV) light is preferred. Additionally, this study aims to investigate the inter-observer variability when using this new technique.

Materials and Methods

This feasibility cross-sectional study included a sample of 35 healthy, mainly Caucasian, volunteers. Given the exploratory nature of this study and the absence of prior data, the sample size was determined pragmatically and based on evident physiological differences in hair growth patterns between men and women. All volunteers were enrolled between April 4, 2023, and May 18, 2023. Consequently, all volunteers were divided into three different groups. For group A, 15 healthy adult male volunteers with short hairs were recruited. For group B, 15 healthy adult female volunteers with no signs hirsutism were recruited. For group C, with 5 healthy adult male volunteers with long hairs were recruited. Participants assigned to group A had dry shaved in less than 48 hours prior to the measurements, whereas those in group C had refrained from dry shaving for at least 48 hours prior to the measurements. The exclusion criterion for the adult males was insufficient development of secondary sex characteristics (eg, hypogonadotropic hypogonadism).

Materials

The Dino-Lite Edge AM4115T-FVW digital handheld microscope camera has been used to obtain the photos. This camera has a resolution of 1.3M pixels and enables magnification between 20 and 220 times. Moreover, it is equipped with both visible light as well as UV fluorescence LEDs (400–450nm). The assisting DinoXCope software has been used on a MacBook OS to capture and analyze the photos. A room with dimmable artificial light and the ability to darken the room was employed to assess the output of the camera on different ambient light conditions. Both researchers were trained in the use of the camera.

Procedures

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Participants were recruited in the Amsterdam UMC, location Amsterdam Medical Center. Written and informed consent was obtained from each individual participant before the start of the measurements. A questionnaire was handed to each participant to assess some additional patient characteristics (ie, age, sex, ethnicity). The researcher assessed one's skin color by using the Fitzpatrick skin color scale and one's facial mFG score to assess the severity and distribution of facial terminal hair growth (score 0–8).

Two independent researchers individually took photos of two different androgen-sensitive locations on the face (ie, upper lip and chin), using both visible and UV fluorescence light with a magnification of 31 times. At the time of capturing and analyzing the photos, the researchers were located in separate rooms. In total, 8 photos were taken from each individual participant. The photos from groups A and B were independently assessed by two researchers, with each researcher performing a single, separate quantification. The analysis encompassed three specific hair parameters: hair thickness, the number of terminal hairs per photograph, and hair color. The inter-observer variabilities were assessed by comparing the outcomes of both researchers.

The hair parameters (ie, hair thickness, number of terminal hairs and hair color) were assessed in all participants in group A and B to differentiate between the groups. For each individual photo, the DinoXcope software was used to perform line measurement across individual hair shafts to assess the hair thickness of individual hairs. A cut-off value of 60 μm was used to differentiate between vellus hairs and terminal hairs.²³ If the diameter exceeded 60 μm , the hair was included in the terminal hair count. Hairs with a diameter < 60 μm , short overlapping hairs, ingrown hairs or hairs that barely reached the skin's surface, and photos with insufficient contrast between a hair and the skin (ie, blurry hairs or very light-colored hairs) were excluded from the analysis. The hair color was assessed by comparing the hairs on the photos with a self-composed hair color scale, consisting of the following 7 items: 1) black, 2) brown, 3) ginger, 4) dark blonde, 5) light blonde, 6) very light blonde, and 7) unpigmented. The hair colors were eventually pooled into three categories, suggesting 1) dark-colored hair (black, brown, ginger, dark blonde), 2) light-colored hair (light blonde, very light blonde), and 3) unpigmented hair. These hair parameters have not been assessed in men with long facial hair (group C). However, we did use the photos to visually compare them to those of men in group A.

In order to investigate whether the visible light or UV fluorescence light function of the camera is preferred for image analysis, four visible light and four UV fluorescence light photos were taken under different ambient light conditions, namely 1) 100% daylight, 2) 100% artificial light, 3) 50% artificial light, and 4) no ambient light (darkened room) to visually compare the outcomes of visible light to those of UV fluorescence light.

Statistics

The obtained data was compiled and then exported to data editor SPSS Version 28.0 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp). Continuous variables were expressed as mean and standard deviation, discrete variables were expressed as median and interquartile range and categorical data were summarized in percentages. An independent *T*-test was employed to analyze mean differences and confidence intervals between the groups between continuous variables. One-way random intraclass correlation coefficients were used to analyze the inter-observer variability in obtaining the photos. Two-way mixed intraclass correlation coefficients were used to analyze the inter-observer variability in the analysis of outcomes. Pearson correlation coefficients (*r*) were used to investigate whether there is a positive correlation between hair parameters assessed on visible light and UV fluorescence light photos. A *p*-value < 0.05 was considered statistically significant.

Results

Patient Characteristics

The demographic characteristics of the healthy volunteers are summarized in Table 1. Thirty-five participants could be divided into 15 men with short facial hair (42.9%), 15 women (42.9%) and 5 men with long facial hair (14.2%). The median age was 26 years old (IQR 4) for women, and 32 years old (IQR 22) for men. Most participants were Caucasian

Table 1 Demographic Patient Characteristics

Participants (n)	35
Sex (n, %)	
Men	20 (57.1%)
Women	15 (42.9%)
Age in years (median, range)	
Men	32 (19–63)
Women	26 (20–58)
Ethnicity (n, %)	
Caucasian	31 (88.4%)
Mediterranean	0 (0.0%)
Latin-American	0 (0.0%)
North-African/Middle Eastern	0 (0.0%)
African	0 (0.0%)
Asian	1 (2.9%)
Other	3 (8.6%)
Fitzpatrick skin color (n, %)	
Type 1: Pale white skin	2 (5.7%)
Type 2: White skin	23 (65.7%)
Type 3: Light brown skin	9 (25.7%)
Type 4: Moderate brown skin	0 (0.0%)
Type 5: Dark brown skin	1 (2.9%)
Type 6: Deeply pigmented dark brown to black skin	0 (0.0%)
Facial mFG score (median, IQR)	
Men	7 (1)
Women	0 (0)

(88.6%) and had a low score (1–2) on the Fitzpatrick skin color scale (71.4%). Only one male participant (2.9%) was Afro-Caribbean with Fitzpatrick skin color type 5, indicating a dark brown skin. The median facial mFG score was 0 (IQR 0) in women and 7 (IQR 1) in men.

Visible Light Analyses

Figure 1A–C show the visual differences in hair growth by using the digital microscope camera between group A, B and C. Male participants were distributed into two different groups based on the length of their facial hair. It must be noted that the hair parameters have not been assessed in group C (n=5) since long overlapping hairs impede analyzing the output, leading to, respectively, an under- or overestimation of the actual hair thickness and number of terminal hairs. Figure 1D and E show, respectively, visible light and UV fluorescence photos of a male participant with Fitzpatrick skin color type 5.

In women, the number of hairs with a diameter greater than 60 μm varied between 0 and 3 hairs, whereas in healthy men, it varied between 1 and 71 hairs per view on visible light photos of both the upper lip and chin. The results of the independent *T*-tests are depicted in Table S1. The mean (standard deviation) number of terminal hairs varied between men and women on both the upper lip (0 (1) hairs in women, compared to 27 (15) hairs in men) and the chin (0 (0) hairs in women, compared to 28 (18) hairs in men), with mean differences of, respectively, 27 (95% CI 19–34) and 28 (95% CI 19–39) terminal hairs between men and women. If terminal hairs were counted in women, the mean (standard deviation) hair thickness on the upper lip was lower in women (66.0 (6.2) μm , compared to men (97.4 (14.7) μm). The mean hair thickness on the chin was 97.5 μm (13.6) in men, yet no hair thickness above the cut-off value of 60 μm were found in women.

The most common hair color was 7 (50.0%) in women and 1 (33.3%) in men. This reflects most women had unpigmented to very light blonde facial hair, whereas most men had brown or black colored facial hair.

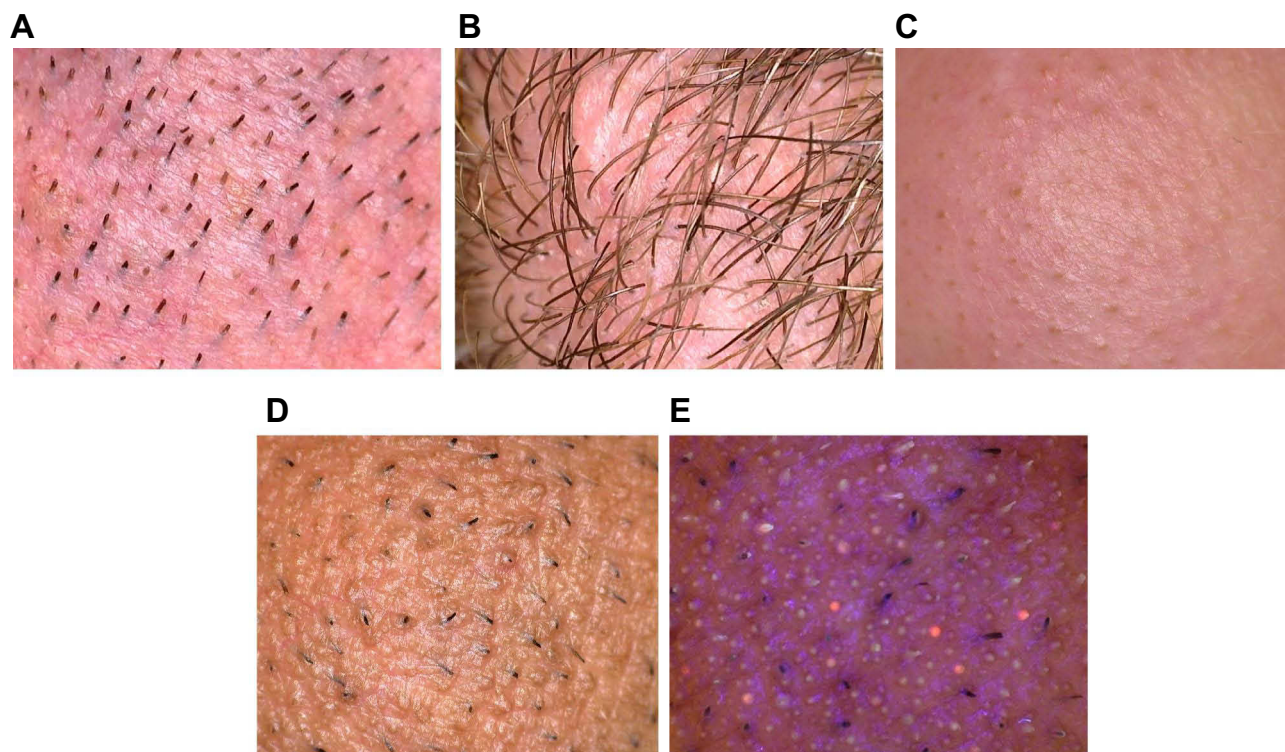


Figure 1 Visual differences in hair growth by digital microscopy. (A) Male participant with short hair; (B) Male participant with long hair; (C) Female participant; (D) Male participant with a dark skin color (visible light); (E) Male participant with a dark skin color (UV fluorescence light).

UV Fluorescence Light Analyses

On UV fluorescence photos, the number of terminal hairs varied between 0 and 65 in men, whereas no terminal hairs were observed in women. The results of the independent *t*-test are depicted in [Table S1](#). Mean (standard deviation) number of terminal hairs varied between the sexes on both the upper lip (0 (0) in women, compared to 21 (15) in men) and the chin (0 (0) in women, compared to 24 (17) in men), with mean differences of, respectively, 21 (13–29) and 24 (15–34) terminal hairs between men and women. In men, the mean hair thickness was 101.7 μm (13.9) on the upper lip and 108.0 μm (14.9) on the chin. Mean hair thicknesses in women could not be assessed since none of the hair thicknesses were above the cut-off value of 60 μm . The most common hair color was 6 (36.7%) in women and 1 (33.3%) in men.

Visible Light Versus UV Fluorescence Light

The outcomes of the visible light photos were correlated to the outcomes of the UV fluorescence photos. There is a statistically significant correlation between visible light and UV light outcomes in number of terminal hairs on the upper lip ($r = 0.897$) and chin ($r = 0.971$). A weaker correlation has been found regarding the hair thickness in both the upper lip ($r = 0.694$) and the chin ($r = 0.639$). The Fisher's exact test showed significant associations between UV fluorescence light pooled hair colors and visible light pooled hair colors on both the upper lip ($p = 0.002$) and chin ($p < 0.001$).

[Figure S1](#) visually shows the influence of four different ambient light conditions, respectively, 100% daylight, 100% artificial light, 50% artificial light and no ambient light (eg, darkened room), on the output of the digital microscope camera. The results indicate that the visible light function of the microscopic camera provides consistent photos under different ambient light conditions, whereas UV fluorescence light photos seem to show more variability in output under variable ambient light conditions, particularly in 100% daylight. This could be explained by the fact that daylight itself contains light in the 400–450nm range, which could not be filtered out.

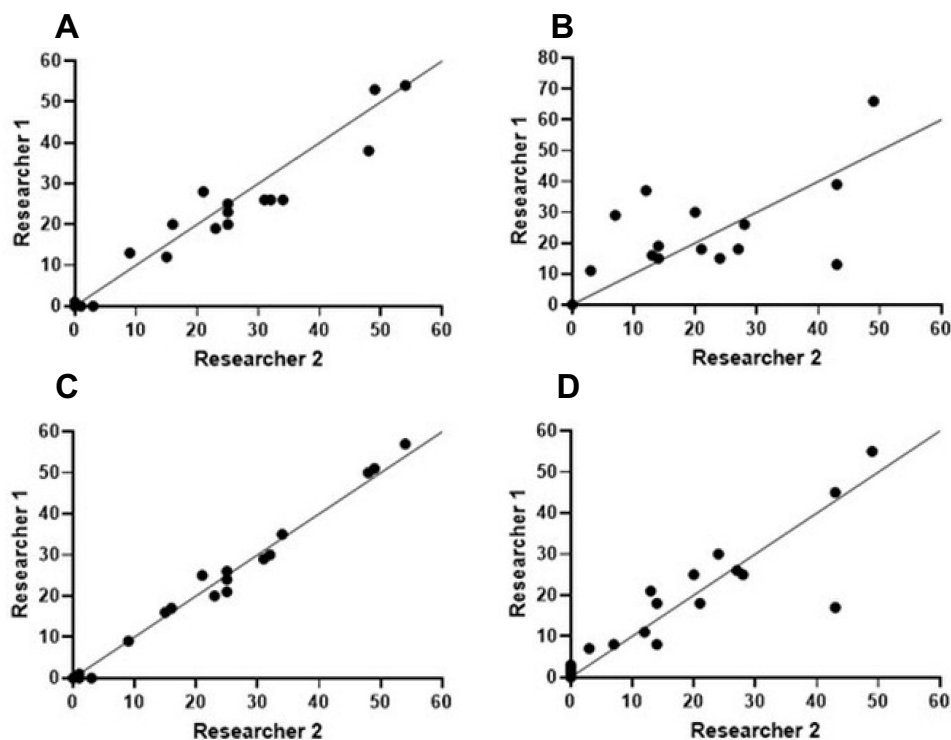


Figure 2 Correlations between two independent researchers in number of terminal hairs on the upper lip in obtaining the photos and the analysis of the outcomes. (A) Correlation of obtaining the photos (visible light); (B) Correlation of obtaining the photos (UV fluorescence light); (C) Correlation of analyzing the number of terminal hairs (visible light); (D) Correlation of analyzing the number of terminal hairs (UV fluorescence light).

Inter-Observer Variability

Figure 2 depicts the correlations between two independent researchers in both obtaining the photos (Figure 2A and B) as well as analyzing the output (Figure 2C and D). The intraclass correlation coefficients were calculated and are depicted in Table S2. There is a strong intraclass correlation between the visible light photos obtained by two independent researchers and which were analyzed by one researcher (0.988 [95% CI 0.976–0.994]). A slightly weaker one-way random intraclass correlation was found between obtaining the UV fluorescence light photos (0.897 [95% CI 0.786–0.951]). In terms of the analysis of the number of terminal hairs by two independent researchers, there is a strong two-way mixed intraclass correlation for both visible light (0.998 [95% CI 0.995–0.999]) and UV fluorescence light (0.961 [95% CI 0.918–0.984]) photos.

Regarding the hair thickness, the one-way random intraclass coefficient showed a strong correlation between the visible light photos obtained by two independent researchers and which were analyzed by one researcher (0.919 [95% CI 0.765–0.972]). Again, a weaker intraclass correlation was found for UV fluorescence light photos (0.835 [95% CI 0.502–0.946]). The two-way mixed intraclass correlations showed a strong correlation between two researchers regarding the analysis of the hair thickness for visible light photos (0.938 [95% CI 0.828–0.977]). However, a weak intraclass correlation was found in terms of UV fluorescence light photos (0.248 [p=0.307, 95% CI –1.342–0.749]). All correlations were statistically significant, except for the analysis of hair thickness in UV fluorescence light photos.

Discussion

The results of this feasibility study indicate that terminal hairs can be quantified by digital microscopy. We showed significant differences between men (terminal hairs) and women (vellus hairs) regarding the hair thickness, number of terminal hairs and hair color, compliant to physiological differences in hair growth patterns between healthy men and women (ie, without signs of HA). To our knowledge, this is the first time that digital microscopy was studied to differentiate between terminal hairs and vellus hairs. For optimal determination of the mFG score, it is essential that women refrain from

using hair removal methods for an average of 3 to 4 weeks prior to an appointment in order for hair to be observable without the help of medical instruments. Interestingly, using digital microscopy, terminal hairs could be observed even after shaving (ie, < 48 hours), indicating that this new method may potentially reduce discomfort compared to current hirsutism assessments in women who prefer to shave their terminal hair. Moreover, in healthy men, long terminal hairs could visually be observed, yet overlapping of long hairs greatly complicated the image analysis.

This study was the first study to quantify terminal hairs by digital microscopy combined with manual image analysis. Overall, the use of digital microscopy demonstrates a strong positive correlation between two independent researchers, meaning an overall low inter-observer variability for, especially, visible light analyses. Previously, the inter-observer variability of the mFG score has been evaluated and showed a kappa value of 0.585 for the upper lip and an overall mean kappa value of 0.744.¹⁶ Digital microscopy and manual image analysis, on the other hand, showed intraclass correlation coefficients of, respectively, 0.998 for visible light and 0.961 for UV fluorescence light for assessing the number of terminal hairs. Thus, digital microscopy shows less variability than the use of the mFG score does.

In our study, visible light analyses seemed to show better correlations between two researchers compared to UV fluorescence light analyses. Moreover, the output of the UV fluorescence light function of the camera is greatly disturbed by environmental daylight containing abundant light with wavelengths of 400–450nm. There seems minimal disturbance by artificial room light. The originally expected higher contrast using UV light illumination was not obtained as shown by the poor correlations between visible light and UV fluorescence light analyses in this study, particularly for the assessment of the hair thickness, and the poor contrast between the skin and hairs on UV fluorescence light photos in a darker skin color. A better result could be expected if the photos were taken with the original 395nm UV light without the fluorescence filter. Consequently, UV light with a wavelength of 395nm will result in higher intensity illumination, less noise in the photos and high contrast for pigment. However, this pigment contrast will only be beneficial for light skin types. For dark skin types, the skin will also darken.

It remains imperative to develop a method applicable to all individuals, irrespective of their skin color. Instead of UV light, the use of near infrared (NIR) light could be considered. Light with a wavelength of 700–900nm could penetrate deeper into the skin and therefore capture the hair root, also in individuals with a darker pigmented skin. However, this will also result in less contrast for the pigmented hair. Polarized light illumination is commonly used for high contrast photos of the skin. Nevertheless, this will also show more pigmented structures and the microvasculature and is not expected to improve the contrast between hair and skin.

In this study, three different hair parameters were determined to differentiate between vellus hair and terminal hair, namely the hair thickness, number of terminal hairs and the hair color. Although terminal hair is defined as dark, course and thick hair, the hair color did not seem to reflect a sufficient parameter since large inter-personal differences regarding this parameter were observed. In general, vellus hairs were very light blonde to unpigmented in this study, however we also observed darker colored vellus hairs in women. On the contrary, there were also men with very light blonde terminal hairs. Therefore, this study suggests that hair thickness is the most distinctive parameter to quantify a terminal hair.

This study has some limitations. First, the volunteers in our study did not have large diversity in Fitzpatrick skin color type, age and ethnicity, which limits its generalizability. Only one Afro-Caribbean man with Fitzpatrick skin color type 5 was included. Second, the arbitrary cut-off value of 60 μ m seemed sufficient to differentiate between vellus hair and terminal hair. Yet, in literature, this cut-off value is not highly unambiguous and could be more accurately determined through subsequent research investigating the optimal cut-off value for hair thickness of vellus hair. Third, although manual image analysis offers a promising new method to quantify terminal hair, the process of manual hair quantification is still time-consuming. Finally, this study is limited by the absence of intra-observer variability and a formal sample size justification. However, given that the inter-observer variability is very low, we also expect the intra-observer variability to be minimal. Due to the exploratory nature of this study, it was not feasible to conduct a formal sample size calculation based on previous studies. However, the sample size was determined based on known physiological differences in hair distribution patterns between males and females, and the observed differences in hair parameters in this study were large enough to confirm the adequacy of the chosen sample size.

Future studies should be performed to find the optimal light band (UV, visual or NIR) in relation to skin color type. Moreover, future studies should focus on validating this method in diverse clinical populations with a broader range in

ethnicity, skin color and age, and to investigate its applicability in the clinical diagnosis and the evaluation of treatment in women with hirsutism. Another next step would be to automatize this new method to improve its user friendliness and ease the potential integration into routine diagnostics for hirsutism.

Conclusion

This study has shown that terminal hairs could be identified and quantified by the use of digital microscopy and manual image analysis, with a minimal inter-observer variability. This method could potentially be superior to the use of the mFG score to clinically diagnose hirsutism and to measure the effects of anti-androgenic medication more objectively. Since the results of this study suggest that terminal hairs could be observed in shaved hairs, digital microscopy could improve the patient-friendliness and reduce patient discomfort compared to current hirsutism assessments where women have to restrain from shaving prior to an appointment. Future studies on this new method should consider a wider range of populations, including those with different skin colors and women with hirsutism. Furthermore, automating the analyses is recommended to optimize clinical applicability.

Ethics Approval and Informed Consent

Ethical review and approval were waived for this study due to the fact that the Medical Ethics Committee of the Amsterdam University Medical Center assessed that the study did not fall within the scope of the Medical Research Involving Human Subjects Act (WMO) since this study does not include scientific research as referred to in section 1(b) of the WMO (W23_085#23.208, 03-02-2023).

Informed consent was obtained from all subjects involved in the study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted and agreed to be accountable for all aspects of the work.

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The authors report no conflicts of interest in this work.

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