IADVL

SIG Trichology and Hair Transplantation (IADVL Academy) Newsletter

July 2019

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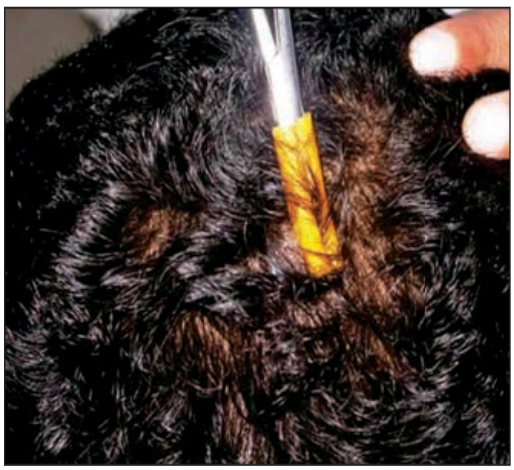

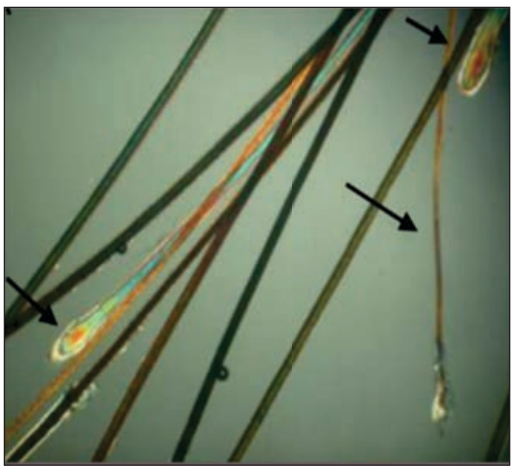

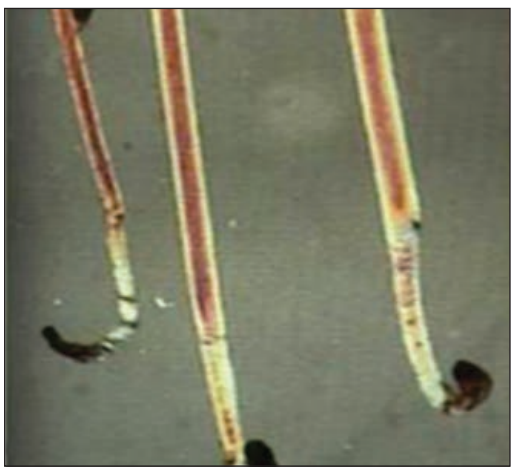

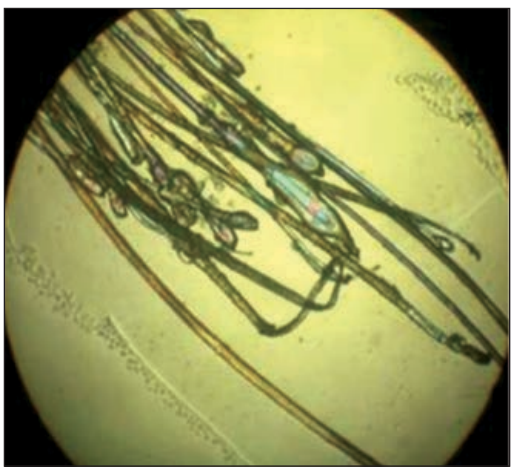

Contents

Photography in trichology

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Clinical photography in the field of trichology could be either to document the condition or as a series of images to document the results post treatment. Unlike the photographic documentation in general dermatology, trichology photography gives unique challenges. The major limitation in trichology imaging is the reflection of the light by the shiny hair shaft and subsequent alteration of the image quality. Courses, CMEs and workshops are devoted to hon the professional skills of the physician, but unfortunately adequate training is not being considered to document the physicians work in the form of photography. By using a digital camera and few accessories the photographic documentation could be made simple, reproducible and standardised. This article gives a glimpse of tips and tricks in trichology imaging.

Tips and tricks for good quality images in Trichology :

1. **Space and equipment :** A corridor of 3 x 8 feet is sufficient for taking good quality images. Simple point and shoot camera would suffice. SLR cameras has the added advantage for modifying the parameters. Camera should be placed at a distance of 4-6 feet from the patient. Ceiling light should be behind the photographer and an appropriate background behind the patient.
2. **Disadvantages of mobile cameras:**
   - lack of macro mode
   - lack of consistent positioning / setting
   - reflection of light from the inbuilt flashes

3. **Background :**
   - white background gives a good visibility for trichology (figure 1)
   - black background for white hairs gives a good contrast for the visibility

4. Lighting : 1

   - Inbuilt flashes and sharp overhead lighting should be avoided to eliminate reflection of light by the hair shaft.

   - While taking images with the available room lighting the lights should be above and in the front of subjects and should never be behind or directly above the subject.

   - Available light settings should be used consistently to get a constant output .

   - Oily hair reflects light more than dry hair. Hence photographs should be taken with dry hair.

   - Ring flash helps in better imaging of scalp lesions in low light condition and to get clear macro mode images.

   - A white cardboard of size 11x14 inches can be held by the patient at 60 to 90 degree angle to the body to reflect any light from overhead lighting and avoid deep shadows on face and neck. (figure 2)

- Reflected light can be utilised instead of direct light than the direct light : ceiling lights projected at 45 degree angle to the patient along with the white cardboard held by the patient gives maximum illumination of the scalp.

5. **Tripod :**

Tripod if available helps in standardising the height and the distance from which the camera is positioned and provides consistent and comparable imaging which is of use in analysing serial photographs for treatment assessment.

6. **Rotating sitting stool:**

A stool allows the patient to maintain an upright posture and position. Simple rotation of the stool positions the patient in appropriate angles for consistent photographs.(figure 3)

7. **Gobal photography for AGA & HT :**

- Hair style should be precisely in the same way during every visit.
- Preferable to have dry and clean hair.
- Same colour of hair for every visit during serial photography is preferable for comparison.
- Standard views are frontal, chin to chest, right oblique, left oblique, vertex and occipital view.

(figure 4a,b,c,d,e)

1.Frontal view - patient looking at the camera directly with the nose perpendicular to the ceiling (figure 4a)

2.Chin to chest view - patient looking downwards with the nose perpendicular to the floor

(figure 4b)

3.Right and left oblique view - patient looking at an angle to the camera with the nose and distal cheek appearing to be just touching (figure 4c,d)

4.Vertex view - patient looking at the ceiling with the nose at 45 degree angle to the ceiling. patient can be asked to look at the junction of the wall and ceiling to get this angle. (figure 4e)

5.Occipital view - patient looking away from the camera with the nose perpendicular to the ceiling. (figure 4e)

8. **Canfield technique : 2,3**

The Canfield technique uses a stereotactic positioning device on which the patient's chin and forehead are fixed, and the device is mounted with a camera and flash device. This fixed position of the device assures that the view, magnification and lighting are the same at consecutive study visits. (figure 5)

9. Minimal blurring of images are not seen during the preview in LCD screen on the camera but gets visible in the monitor. Hence an additional copy of the image becomes handy .

10. For imaging the lesion on scal , it is recommended to take two images. One image should include the entire scalp at a distance of at least 4 feet to denote the location of the lesion and the other with a macromode and without flash if lighting is good or ring flash if the lighting is poor.

11. Informed consent is a must before photography. Images can be shown to the patient for their opinion to make sure they are comfortable with the images.

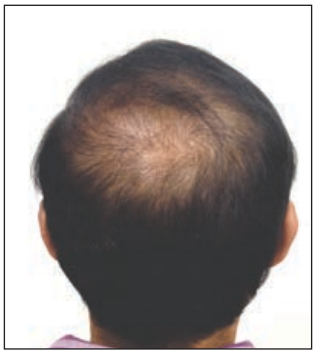
12. One of the images can have the name/ id number for easy recognition .

13. Manual adjustment for the technical parameters can be limited to the professionals and auto focus mode can be utilized always.

14. Accessories on the hair like bands and clips to be removed to avoid distraction in the final image.

15. **Storage of data:**

- JPEG is the preferred format for storage
- Two sources for storage helps in back up for the data
- Google picaso/ iCloud etc., are the cloud storage platforms that can be utilised for safe storage and easy retrieval.



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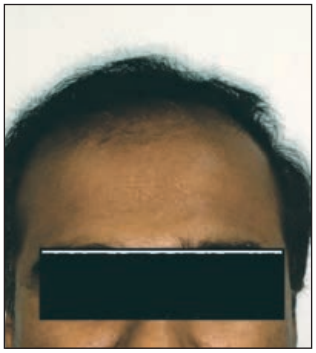

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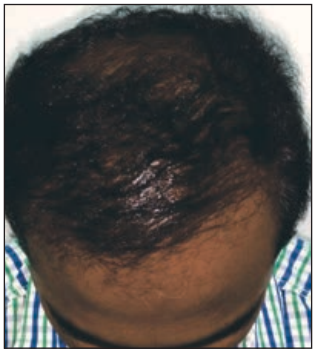

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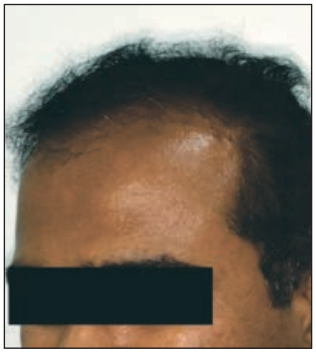

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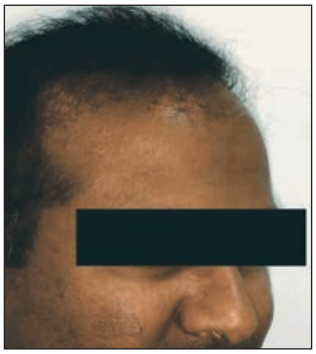

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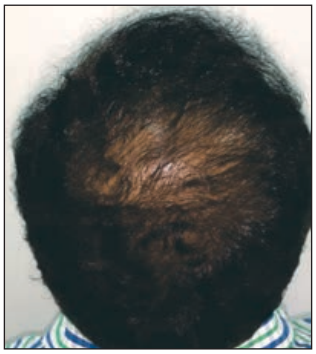

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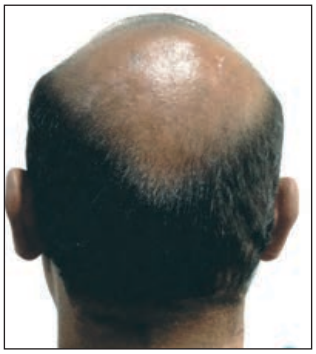

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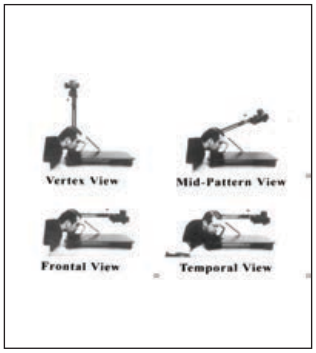

Figure No.5

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Hair Mass and Weight Measurement- Need of The Hour

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Hair loss is a continuously increasing and being attended to symptom by patients as well as dermatologists.[1] At present most of the evaluation methods are scales such as Norwood / Ludwig scale and informal clinical photography.[2] It is usually not possible to see appreciable visible difference until 50% of hair is lost.[3] Therefore, comparing "before and after" global photographs has little value if the loss is less than 50%. The diameter of hair fibers also contributes greatly to the overall volume of hair as well as appearance of hairs apart from hair density.

Miniaturisation is known part of AGA progression but very few methods are available to monitor changes in hair diameter in an office based setting besides global photography and dermatoscopy, both these methods are not quantitative. [4,5,6]

Sophisticated measuring methods availability is limited to research facilities and industry laboratories. These include conventional and contrast-enhanced phototrichogram,[7,8,9,10] measurement of dry hair weight,[11,12] electron microscopy, and confocal laser scanning microscopy.

AGA and other hair loss treatments can very well be treated better if convenient ways of quantification of hair loss measurement are available. Quantification of hair loss measurement should take into account continuous miniaturisation of terminal hair follicles as this influences appearance by decrease in density as well as diameter. So at least in situations such as AGA a meaningful measurement would consider both density and diameter. This was termed as " hair mass" [13] by Arnold. Procedures or devices capable of measuring hair mass accurately, reproducibly and easily will greatly aid to the assessment of alopecia, progression and treatment response. This article describes in brief some methods of estimating the hair mass and weight.

Dry hair weight measurement:

Procedure for measuring the dry weight of hair:

A representative site is selected on the thinning frontal/parietal scalp. Hair in the designated area is carefully hand clipped under magnification on the screening visit (designated as week –6) and at specific weekly intervals thereafter. No treatment is given during the first 6 weeks, so that the sample collected at the end of this interval represents baseline growth (week 0). A template consisting of a plastic sheet with a square hole (1 cm^2) is placed over the selected site. All hairs within the template square are pulled through it, with the help of a magnifying lamp to ensure that only hair originating within the square will be included. The hairs will be grasped and hand clipped to about l mm in length with small straight surgical scissors. Clipped hairs will be collected in a paper envelope and stored for weight measurement. After the templateis removed, the 4 corners are permanently marked using ink for repeating the measurement from the same site during subsequent visits. [12]

Measurement method:

Hair samples collected by clipping are degreased in trichlorotrifluoroethane (Freon TF) and dried. The hair sample are placed in the chamber of an analytical balance having 0.01 mg readability. After conditioning for at least 1 hour in the balance chamber, the ambient relative humidity will be recorded, and the samples weighed. Sample weights are corrected to a standard humidity of 65%.[12]

Interpretation :

Interval weight :

weight of the hair (mg/cm^2) measured during every periodical visit (ex., 6 weekly intervals)

Excess cumulative weight :

It is defined as the difference between the actual cumulative mean weight and the hypothetical cumulative weight that would accrue if growth continued at the same rate as during the baseline pretreatment period (from week –6 to week 0). Thus excess cumulative weight represents the aggregate change in weight, if any, caused by treatment. Expressed differently, the excess cumulative weight reflects the total cumulative hair protein production induced by treatment.

Excess cumulative weight = baseline weight (0-6 weeks) subtracted from interval weight at a particular fixed time period (ex., 6 weekly intervals) .

The dry weight of the hair shaft varies widely among the individuals, hence the percentage change in the value from the baseline

would be a useful criteria to compare rather than the actual weight values for easy interpretation.

Advantages :

- A quantitative estimation of the growth rate best suited for comparing different modalities of treatment accurately.
- Takes into account both the changes in density and the diameter of the hair shaft over fixed period of time.

Disadvantages :

- Does not take into account the changes in the growth rate. Changes in growth rate can significantly alter dry hair weight unless samples are corrected for length.
- Wide variations in the hair caliber and resulting hair weight among different population might alter the interpretation of the results.
- it is practically almost always done in laboratory settings and not used as a part of common clinical evaluation.

Cross section Trichometry (CST) : [14,15]

In 2008, Cohen published a report introducing the cross-section trichometer, a hand-held device for measuring hair mass. This device "grabs" the bundle of hair from a 2 × 2 cm scalp area in a J-slot and measures the cross-sectional area of the hair bundle. It then displays the Trichometric Index (TI) – which equals to bundle cross-sectional area in mm2 per cm2 of scalp surface multiplied by 100. A direct correlation was detected between the observed hair loss severity and the Trichometric Index. For clinical application of this methodology a commercial prototype instrument Hair check TM was introduced to calculate the TI, it was termed as Hair mass index (HMI).

The HairCheck® measuring device :

The HairCheck® measuring device, together with a locating strip, comprised the HairCheck® System (Divi International Co., Miami, FL, USA), which was designed to quantitatively measure scalp hair mass (Image 1). The locating strip in the device enabled the return to the same sample site on the scalp on subsequent visits without the application of tattoos. The measuring device was a hand-held mechanical device with paired levers that transmitted a pre-determined load to a captured bundle of hair. The capture chamber has a slotted hook and anvil integrated into a disposable cartridge. Its size was designed to measure the cross-sectional area of a bundle of hair growing within a 2 × 2 cm (4 cm2) scalp area. The hair needed to be at least 2.5 cm (1 inch) in length for the device to function properly. An LED screen displayed the HMI value, expressed as mm2 of hair per cm2 of scalp × 100, rounded up to the closest integer.

HMI:

HMI is calculated by measuring the cross-sectional area of a bundle of hair (mm2) per cm2 of the sample scalp area multiplied by 100. The numeric value of HMI allows physicians and patients to monitor the hair mass over time, much like the way body mass index (BMI) is used in the management of weight. HMI values are not influenced by hair length, but reflect hair mass as determined by density and diameter alone – the two anatomic hallmarks of hair loss and growth.

Advantages :

1. Quick and convenient way to quantify the hair mass in the same scalp area during different office visits without hair clipping and without the application of tattoos.
2. The locating strip enables the return to the same sample site (2 × 2 cm) on the scalp on subsequent visits without the application of tattoos.
3. This system is highly reproducible .
4. A precise numeric HMI score is easy to document and compare between office visits and between different treatment groups.
5. Sensitive tool to detect small changes in hair number/density and shaft diameter, including hair shafts of mixed diameters, makes it a valuable tool in a wide range of hair loss situations regardless of etiology.
6. Can measure all types of hair, from super fine to very coarse.
7. HMI estimation in the donor area during initial consultation for hair transplantation will help the surgeon and patient develop realistic expectations of the outcome.

8. In addition to measuring hair loss and growth, HMI can be used to measure hair breakage. When breakage is present, the bundle cross-sectional area decreases from the proximal portion toward the tip, and the ratio of the distal HMI to the proximal HMI can be used as an indicator of breakage severity.

Disadvantages :

1.The hair shaft must be at least 2.5 cm in length for accurate measurement

2. Device is expensive, disposable cartridges for every use limits the widespread utilization of this device.

Practical applications of HMI estimation :

- To diagnose early patterned alopecia before visible loss is seen
- Can alert the physician in cases of non response to treatment and thus opting for further addition of minoxidil/ finasteride/ procedures
- Occipital HMI values can serve as baseline to measure the further loss
- It can also be used in other types of hair loss such as post partum effluvium, traction alopecia
- It can also in addition be used to measure hair breakage by carrying out proximal and distal readings on hair shaft.
- In hair transplantation baseline evaluation of both donor and receipient areas can help plan the hair transplant better.
- HMI estimation can be used to manage additional hair loss conditions unique to women, such as post-partum effluvium and traction-induced alopecia, assessing their clinical course and treatment efficacy.
- Similarly, this device can be used to evaluate devices and over-the-counter products (lasers, food supplements, etc.) that promise to grow hair.

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   Ellis JA, Sinclair R, Harrap SB. Androgenetic alopecia: Pathogenesis and potential for therapy. Expert Rev Mol Med. 2002;4:1–11.
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PHOTO feature – trichoscopic findings

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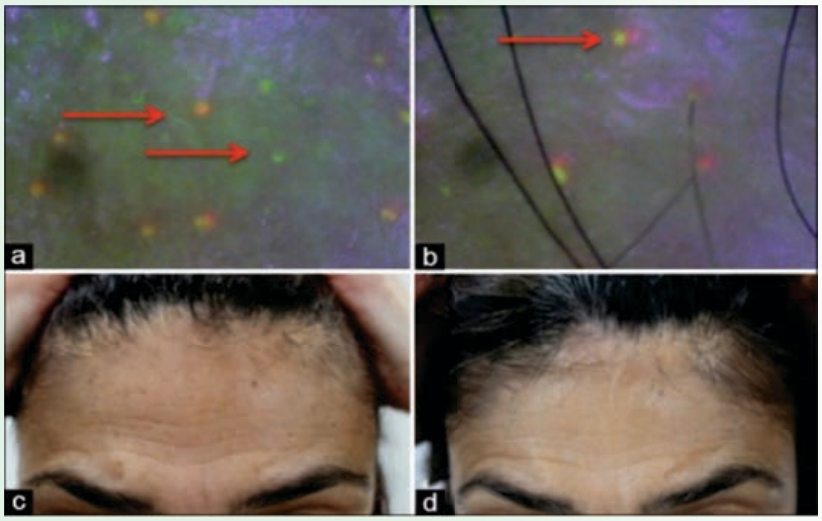
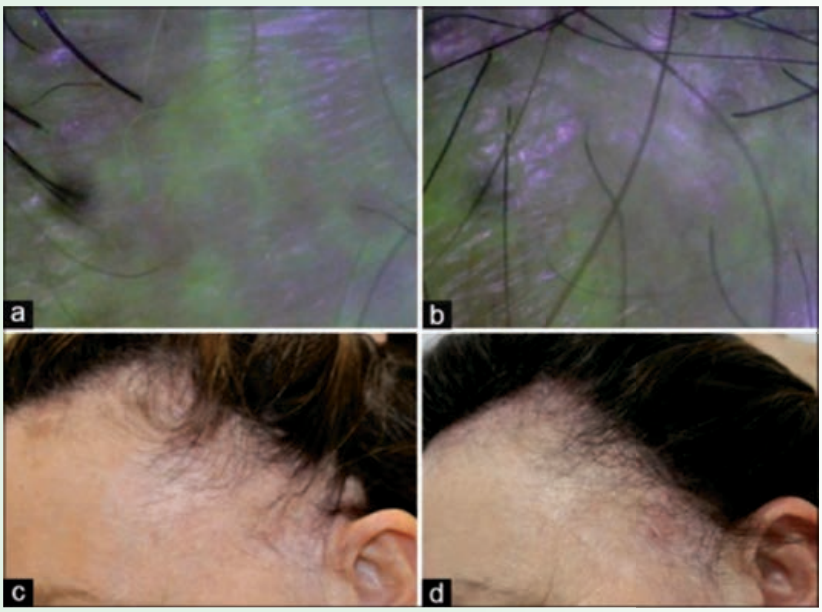


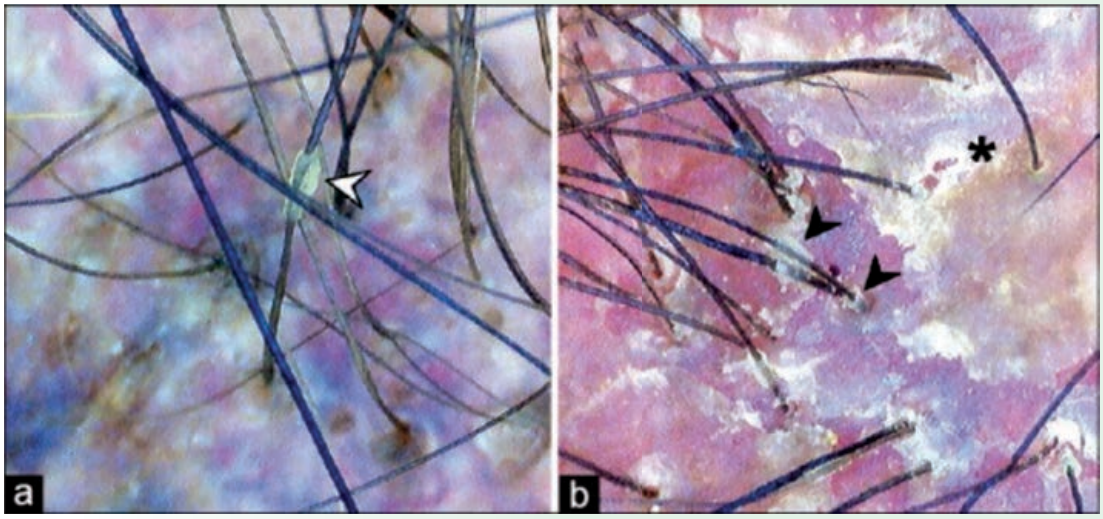
This is a case of Frontal Fibrosing Alopecia.
The pictures show a "Starry night sky sign" which is positive in frontal region (a and b) in a 46-year-old female patient before partial hair regrowth in the frontal hairline was observed. She was on a 6-month treatment with a topical corticosteroid, 5% topical minoxidil, and oral dutasteride (c and d, respectively).
The Red arrows depicts Follicular Propionibacterium, which was observed with ultraviolet-light-enhanced trichoscopy with ×50.

Rodrigues-Barata AR, Moreno-Arrones OM, Corralo DS, Galvan SV. The "Starry Night Sky Sign" Using Ultraviolet-Light-Enhanced Trichoscopy: A New Sign That May Predict Efficacy of Treatment in Frontal Fibrosing Alopecia. Int J Trichology. 2018;10(5):241–243. doi:10.4103/ijt.ijt_17_18



This is another case of Frontal fibrosing alopecia. This is "Starry night sky sign" negative (a and b) case in a 63-year-old female patient with no hair regrowth after treatment with 5% topical minoxidil and oral dutasteride after a 6-month period (c and d, respectively)1

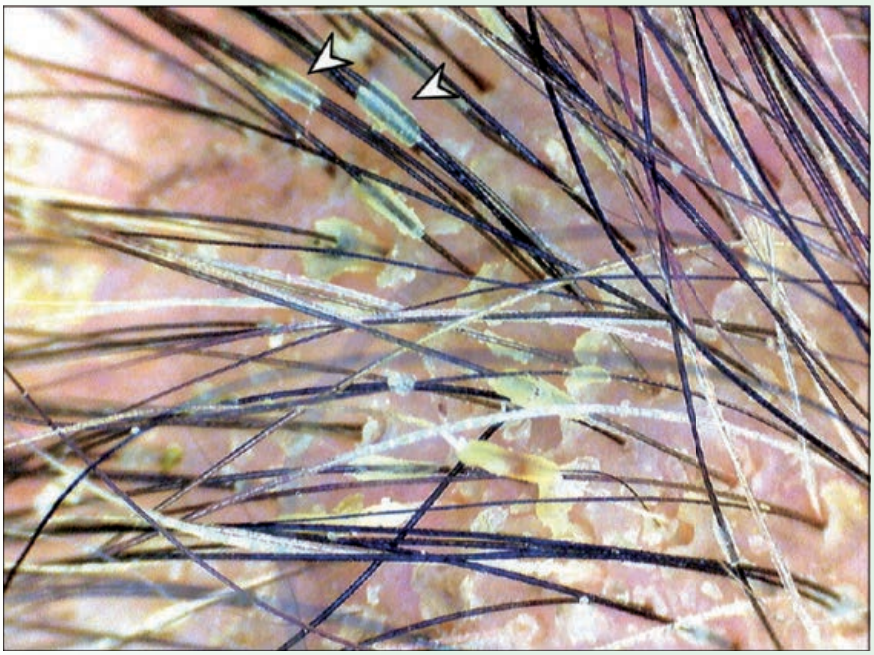
Photo Courtesy
Rodrigues-Barata AR, Moreno-Arrones OM, Corralo DS, Galvan SV. The "Starry Night Sky Sign" Using Ultraviolet-Light-Enhanced Trichoscopy: A New Sign That May Predict Efficacy of Treatment in Frontal Fibrosing Alopecia. Int J Trichology. 2018;10(5):241–243. doi:10.4103/ijt.ijt_17_18



Trichoscopy *(×20) showing (a) Discoid Lupus Erythematosus presenting with a distal tubular hair cast, (b) Discoid Lupus Erythematosus presenting with two casts within a shaft (black arrowheads) and extensive surrounding scales (asterisk)*

Photo Courtesy

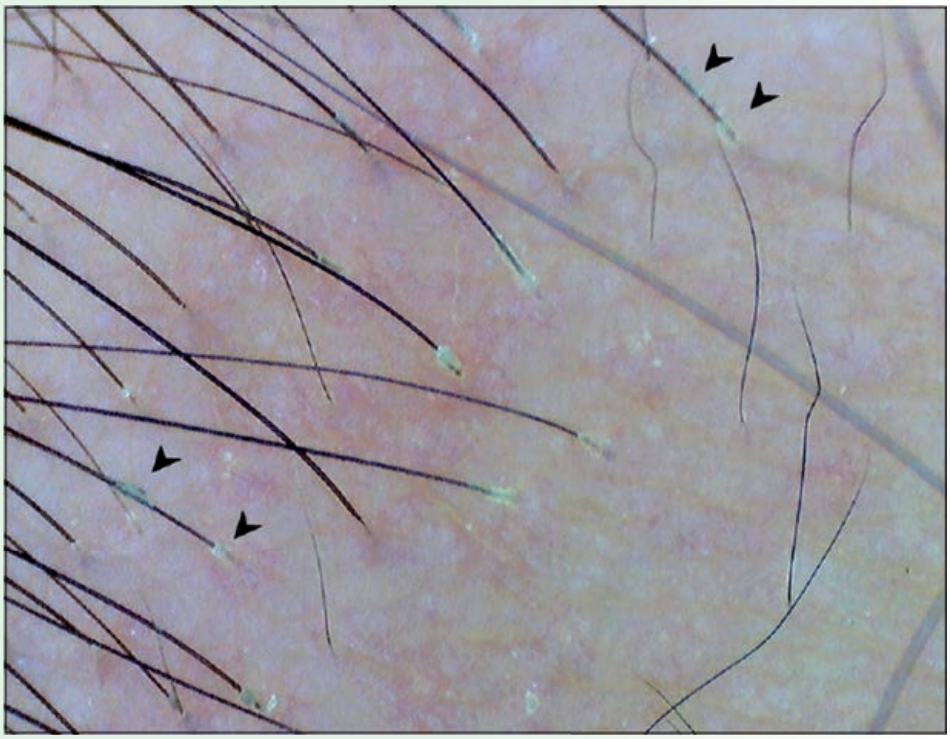
Mathur M, Acharya P, Karki A, Shah J, Kc N. Tubular Hair Casts in Trichoscopy of Hair and Scalp Disorders. Int J Trichology. 2019;11(1):14–19. doi:10.4103/ijt.ijt_77_18



Trichoscopy *(×20) This is a case of Pemphigus Foliaceus with a single cast surrounding two hair shafts (white arrowhead). Extensive surrounding scaling can also be seen.(×20) showing (a) Discoid Lupus Erythematosus presenting with a distal tubular hair cast, (b) Discoid Lupus Erythematosus presenting with two casts within a shaft (black arrowheads) and extensive surrounding scales (asterisk)*

Photo Courtesy

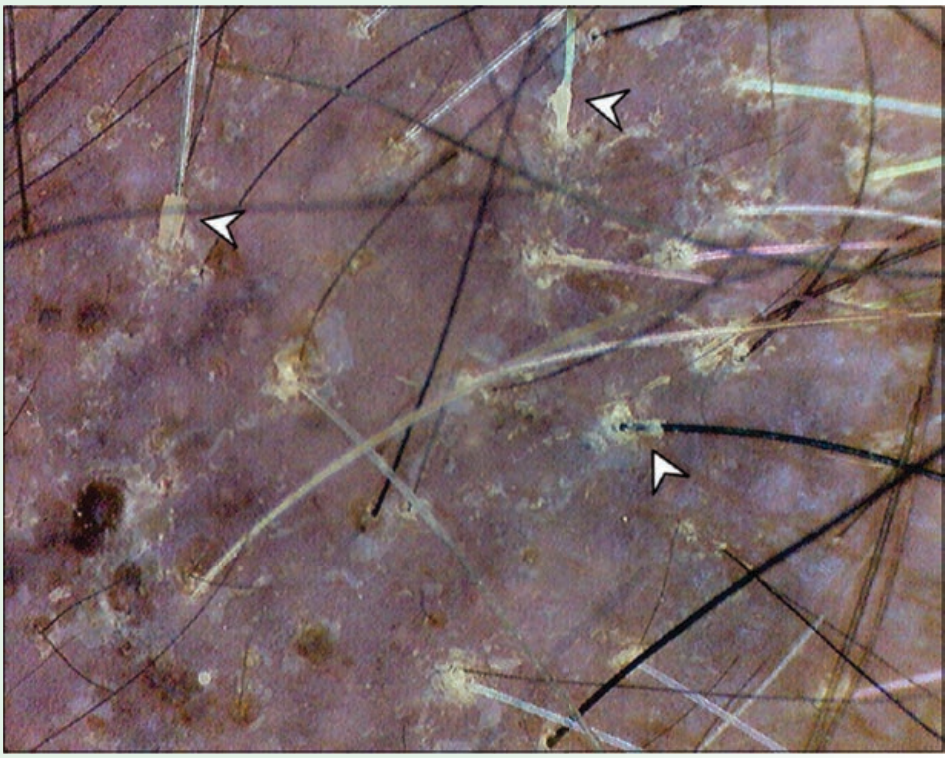
Mathur M, Acharya P, Karki A, Shah J, Kc N. Tubular Hair Casts in Trichoscopy of Hair and Scalp Disorders. Int J Trichology. 2019;11(1):14–19. doi:10.4103/ijt.ijt_77_18



Trichoscopy *(×20) A case of Alopecia Areata where two casts within a shaft (black arrowhead) can be seen.*

Photo Courtesy

Mathur M, Acharya P, Karki A, Shah J, Kc N. Tubular Hair Casts in Trichoscopy of Hair and Scalp Disorders. Int J Trichology. 2019;11(1):14–19. doi:10.4103/ijt.ijt_77_18



Trichoscopy *(×20) here shows a case of Lichen Planopilaris withmultiple proximal hair casts (white arrowheads) and minimum surrounding scales*

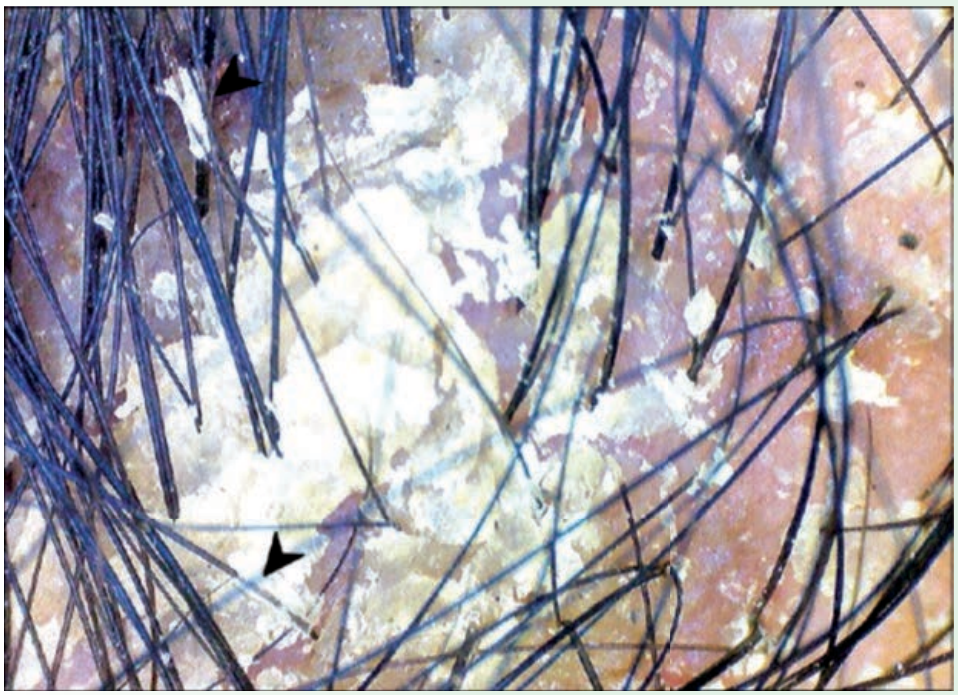
Photo Courtesy

Mathur M, Acharya P, Karki A, Shah J, Kc N. Tubular Hair Casts in Trichoscopy of Hair and Scalp Disorders. Int J Trichology. 2019;11(1):14–19. doi:10.4103/ijt.ijt_77_18

Trichoscopy *(×20) shows a case of Frontal Fibrosing Alopecia. Multiple proximal hair casts (black arrowheads) can be seen with absent/minimum surrounding scales*

Photo Courtesy

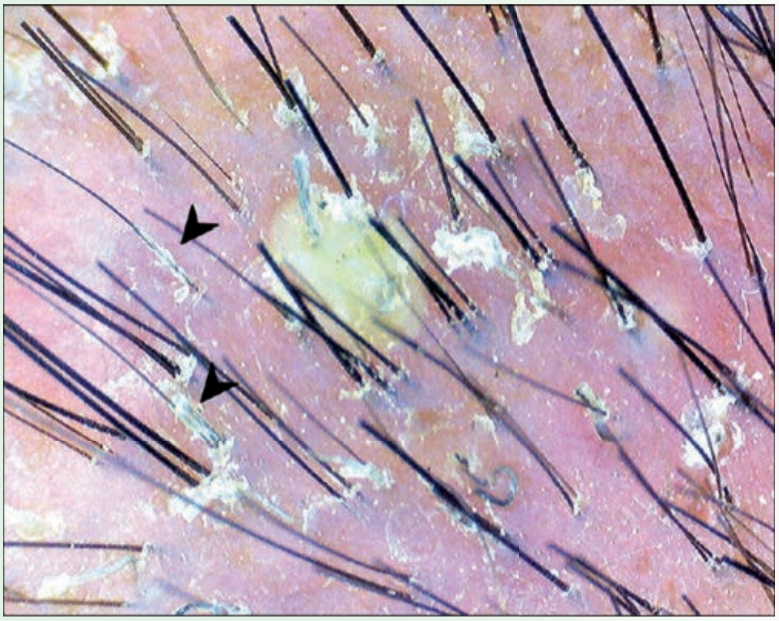
Mathur M, Acharya P, Karki A, Shah J, Kc N. Tubular Hair Casts in Trichoscopy of Hair and Scalp Disorders. Int J Trichology. 2019;11(1):14–19. doi:10.4103/ijt.ijt_77_18



Trichoscopy *(×20) A case of Pemphigus Vulgaris where both proximal and distal hair casts (black arrowheads) and extensive surrounding scales is visible*

Photo Courtesy

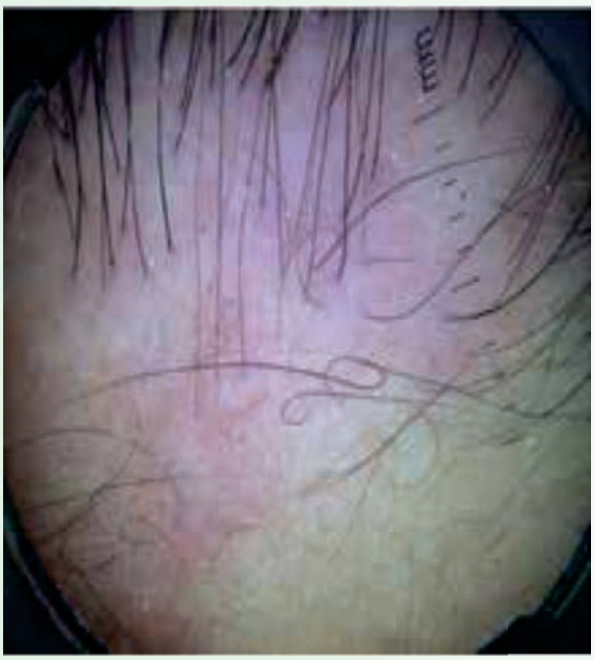
Mathur M, Acharya P, Karki A, Shah J, Kc N. Tubular Hair Casts in Trichoscopy of Hair and Scalp Disorders. Int J Trichology. 2019;11(1):14–19. doi:10.4103/ijt.ijt_77_18

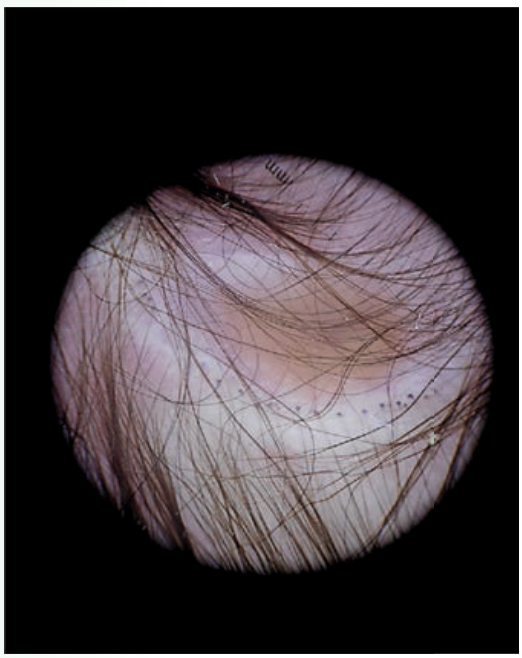


***Trichoscopy** (×20) depicting a case of tinea capitis where irregular tubular hair casts (black arrowheads) and medium surrounding scales are present2*

Photo Courtesy

Mathur M, Acharya P, Karki A, Shah J, Kc N. Tubular Hair Casts in Trichoscopy of Hair and Scalp Disorders. Int J Trichology. 2019;11(1):14–19. doi:10.4103/ijt.ijt_77_18





***Frontal fibrosing alopecia:** Plaques with alopecia in the frontal region sparing the implantation hairline.*
Dermoscopy of the hairline evidencing the presence of vellus hair in the anterior region, absence of hairs with central erythema and posterior terminal hairline with discrete follicular hyperkeratosis4

Photo Courtesy

Contin LA, Rocha VB. Pseudo "fringe sign" in frontal fibrosing alopecia. An Bras Dermatol. 2017;92(6):892–894.

1. Rodrigues-Barata AR, Moreno-Arrones OM, Corralo DS, Galvan SV. The "Starry Night Sky Sign" Using Ultraviolet-Light-Enhanced Trichoscopy: A New Sign That May Predict Efficacy of Treatment in Frontal Fibrosing Alopecia. Int J Trichology. 2018;10(5):241–243.

2. Mathur M, Acharya P, Karki A, Shah J, Kc N. Tubular Hair Casts in Trichoscopy of Hair and Scalp Disorders. Int J Trichology. 2019;11(1):14–19.

3. Vañó-Galván, Sergio et al. Updated diagnostic criteria for Frontal Fibrosing Alopecia. Journal of the American Academy of Dermatology , Volume 78 , Issue 1 , e21 - e22

4. Contin LA, Rocha VB. Pseudo "fringe sign" in frontal fibrosing alopecia. An Bras Dermatol. 2017;92(6):892–894.

Trichogram and Phototrichogram

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Trichogram

- Trichogram is a semi invasive (plucking) microscopic method for hair root and hair cycle evaluation.
- In 1957, Scott et al described morphological examination of hair roots,
- In 1964, a "trichogram" was coined by Pecoraro & also described parameters such as
- Hair shaft diameter
- Hair growth and
- Telogen

Indications

- Androgenetic alopecia
- Alopecia areata
- Anagen dysplastic effluvium
- Telogen effluvium

Methodology :

Pre procedure instructions

1. Hair should not be washed for 3-5 days
2. Application of EMLA / topical anaesthetic spray for >30 mins, will help reducing the pain or discomfort.
3. Marking the point for extracting the hair

   In AGA, diffuse effluvium & loose anagen hair, 1st site is 2 cms behind the frontal line and 2 cms from midline. 2nd site is on occipital area.
   In alopecia areata, 1st site is on the border of the alopecia patch, 2nd site clinically unaffected patch.

   In female-pattern hair loss, samples should be taken from the center and the vertex of the scalp. The sites for telogen hair loss and scarring alopecia are, respectively, the central interparietal area and the advancing border of the alopecic patch.

4. Rubber arms on straight artery forceps (Kochers Foreceps) can be used to collect the hair samples.

Procedure

The hairs in a group of 10-15 shafts are held at above 0.5 to 1 cm from the scalp and grasped tightly by the Kochers forceps (Image 1). A pull has to be given in a firm and quick action in the direction of hair growth .

Mounting the hair samples on the slide :

The collected hair samples are carefully placed on a glass slide, ensuring that they are parallel to each other and that the roots are aligned. Hair shafts should be covered with clear adhesive tape. This is the simplest method but the use of adhesive tape can produce artifacts, such as bubbles and black spots, that distort the image. To avoid these artifacts and obtain a sharper, cleaner image, several drops of balsam (such as that used to mount histological slides) can be placed on top of the mounted hairs and covered with a cover slip. The use of polarized light improves image quality

Hair examination :

The sample are examined using a 4x objective, although a 10x or 40x objective can be used if higher magnification is needed (Images 2-5). A higher-quality image can be obtained by fitting 2 polarizers to the microscope: 1 between the condenser and the sample and the other between the sample and the observer. The different parts of the hair shaft should be analyzed in the trichogram for the following points

Total no. of hairs
Total no. of anagen hairs
Total no. of telogen hairs
Total no. of dystrophic hairs
Total no. of broken hairs

Clinical correlation along with detailed history taking, clinical examination and trichogram findings can help us in diagnosing the condition as suggested in the following table.

Complications
Patient experiences pain or discomfort during this procedure.
This procedure is operator dependent and needs to be done properly or otherwise may result in broken or dystrophic hair

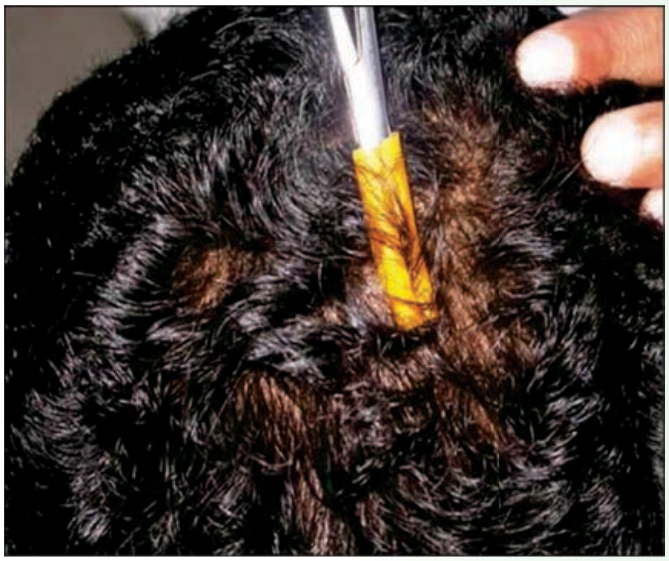


Image 1 : *collecting hair samples using a Kochers foceps*

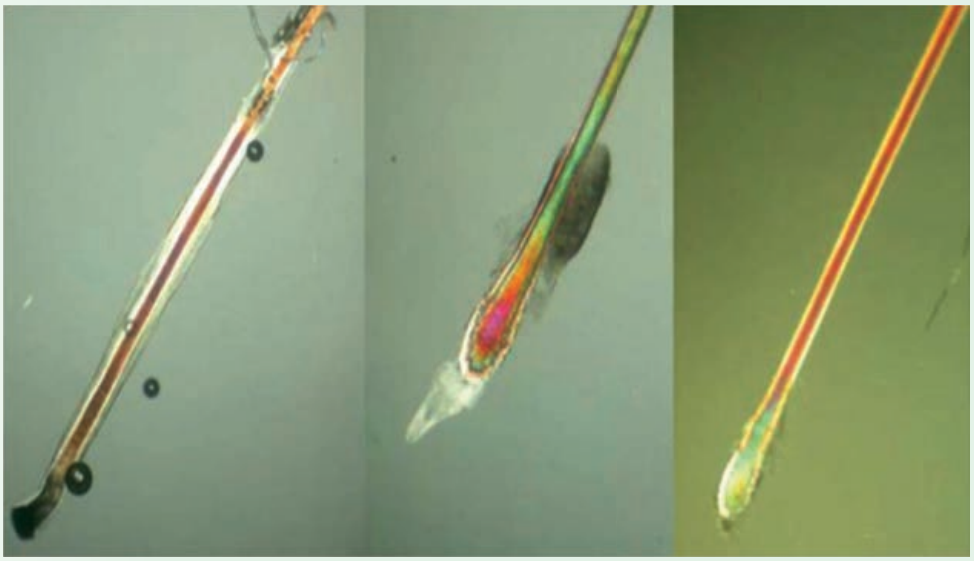


Image 2 : *Anagen catagen and Telogen hair under light microscope*

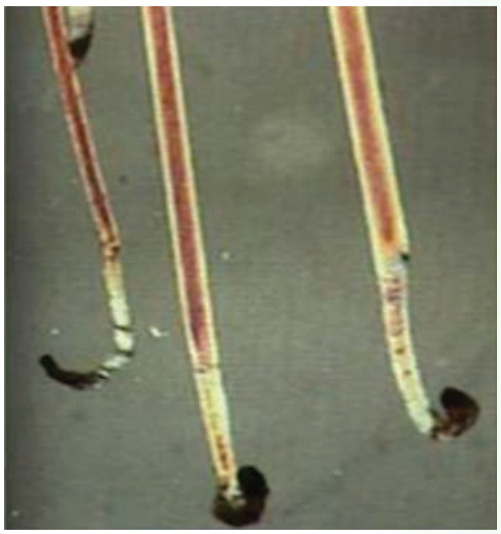


Image 3 : *Dystrophic anagen hair under light microscope*

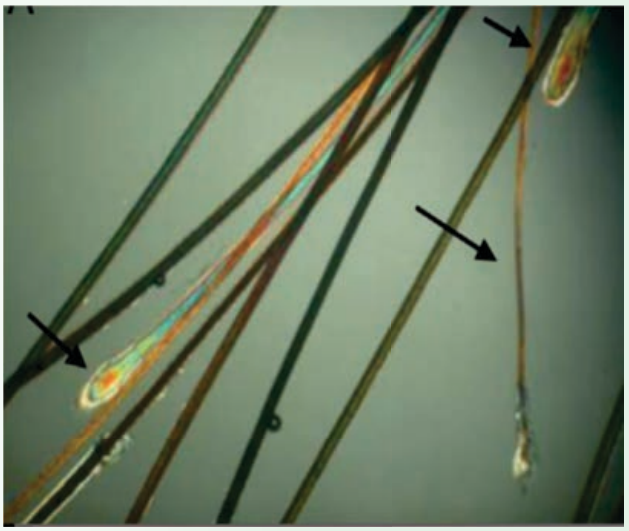


Image 4 : *Androgenetic alopecia with variation in hair shaft diameter*

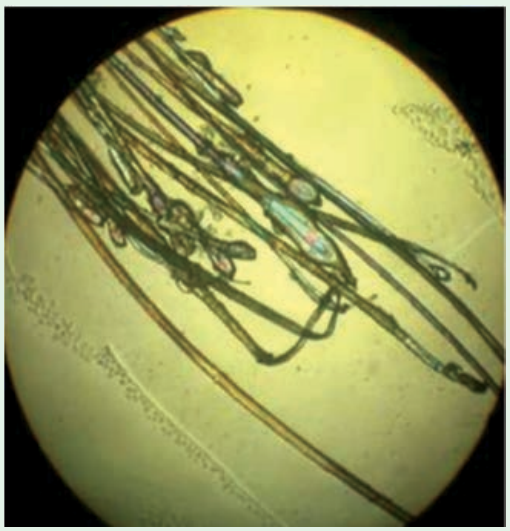


Image 3 : *Telogen effluvium – more number of telogen hairs*

Image courtesy :

Hair and Scalp Evaluation: The Trichogram C. Serrano-Falcón, M.A. Fernández-Pugnaire, S. Serrano-Ortega. Actas Dermosifiliogr. 2013;104(10):867---876

Table 1 : Characteristics of anagen, telogen and dystrophic anagen hair under light microscope

Anagen	Telogen	Dystrophic hair
Longer	Shorter	
Uniform Diameter	Thickened root(club shaped)	Tapering diameter: exclamation mark
Rectangular shape		Irregular contour
Intense pigmentation	Weak pigmentation	
Presence of sheaths	Sheath at root only or absent	No sheaths
Slight distal angle($<20^0$)	No angles	Distal Angle($>20^0$)

Table 2 : Hair loss pattern clinical and trichogram findings correlation

Clinical condition	Clinical examination	Trichogram
Androgenetic alopecia	Less density of hairs	Different diameters, some dystrophic hairs
	Pull test + if active.	
Alopecia areata	Exclamation mark hairs	Paintbrush tip
	Black dots	
	Pull test +++	Predominance of anagen hairs(Golf club shaped distal end)
Anagen effluvium	Immediate cause	
	Pull test ++	>20 % of hairs in telogen phase(club shaped distal end)
Telogen effluvium	Cause 3-4 months earlier	
	History and Clinical examination	Cut hairs(clean cut distal end)
Trichotillomania		

Phototrichogram (PTG)

Phototrichogram is a non–invasive reproducible method with basic principle of taking a close up photograph of a certain defined scalp area

History

1. In 1970, Saitoh introduced phototrichogram.
2. In 1991, Blume et al has used for vellus hair diagnostics.
3. Van Neste used immersion oil (SIPP-scalp immersion proxigraphy photographic method) and Contrast enhancement PTG (CE – PTG).

Types

1) Phototrichogram,
2) Contrast Enhancement Phototrichogram

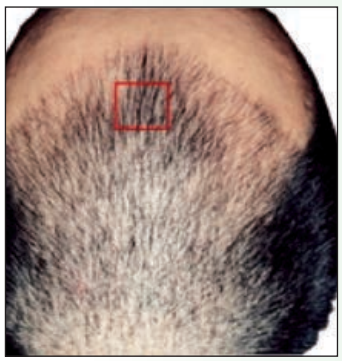
Indications

1) In androgenetic alopecia,
2) Assessment of hair growth and hair loss.
3) Early changes of hair density.
4) Hair miniaturisation at single hair level

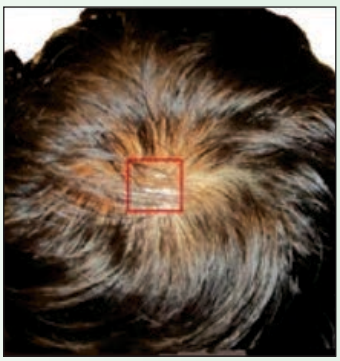
Method

Pre procedure

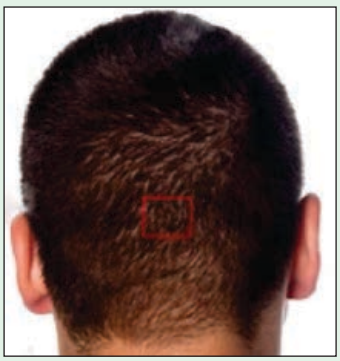
1. Marking the site for measurement
   - i. two progressing areas
     1. 2 cms from frontal hair line
     2. Vertex
   - ii. one control area
   - 1. occipital region.



Frontal area



Vertex area



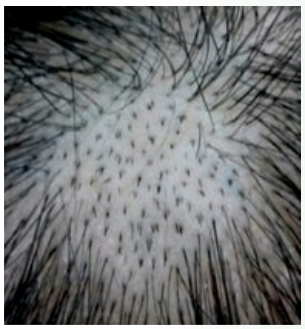
Occipital area

2. Glass slide
3. Immersion oil (SIPP)
4. Hair clipper.
5. Hair dye
6. Camera
7. µm ruler

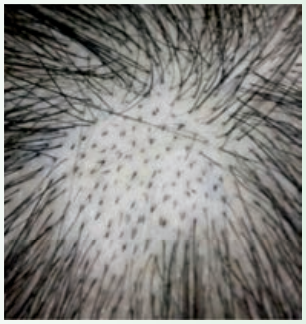
Procedure

1. An area of 1 sq. cm is marked and hairs are trimmed to 1 mm length
2. A drop of immersion oil is placed on glass slide and slide is applied on the scalp,
3. Photograph is taken by a camera, by placing it on the glass slide,
4. In contrast enhanced PTG, hair dye may be used to colour the vellus hair as well as terminal hair,
5. Day 1 photo (no trimming is done),
   Day 2 photo (no trimming is done).

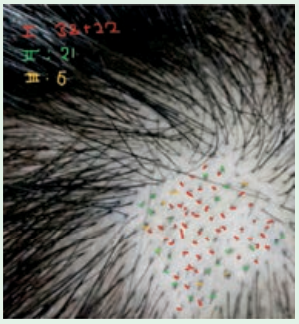
Phototrichogram



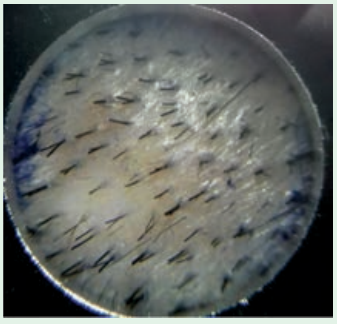
Day 0



Day 2



Color coding for single or double or three or more hairs



Dermoscopic image on clipped area

Points to look for are

1. Total no. of hair in 1 cm2 area
2. Total hair density
3. Total no. of anagen hairs
4. Linear hair growth rate
   Day 1 length
   Day 2 length (measured with m ruler)
5. Hair thickness can be measured with a (m ruler)
   < 40 m –thin hair
   >40 m – thick hair

Points to be noted are

Anagen hair - substantial prolongation of hair
Catagen hairs - moderate elongation of hair
Telogen hairs - no elongation of hair
Hair shedding - missing hair

If more anagen growing hairs are present with more than 8:1 ratio, then a diagnosis of Androgenetic Alopecia can be made.
If more telogen hairs are seen, then acute or chronic telogen effluvium can be diagnosed. This test is mainly indicated to differentiate androgenetic alopecia and telogen effluvium than the other hairloss conditions. It may be a good choice in patients who are reluctant for biopsy.

Complications

- When dye is not applied correctly, dye can get stuck on to open pores and may mimic hair.
- Pt's do not easily accept for 3 areas to be trimmed

In comparison with trichoscan,

With more easier access to dermoscopy, trichoscopy and early diagnosis of AGA, the photo trichogram is gradually losing its place in hair growth assessment techniques.

Practical implications and use in clinical practice

1. Only for AGA,
2. Can be used for PRP patients to assure that the results are good,
3. Early diagnosis of AGA,
4. Clinical therapeutic trials efficacy of drugs,

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Light Microscopy

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Light microscopy of the hair is a important bedside clinical tool for the diagnosis of various disorders affecting the hair and the adjacent scalp. Hair abnormalities also form a part of various genodermatoses and syndromes, and can also be seen in a host of acquired infectious and non-infectious diseases.

Procedures for light microscopy of hair :

- The hair sample are collected by either clipping or plucking.

Clipping -

Hair clipping is performed in suspected hair shaft disorders and in tinea capitis. This involves clipping a few hairs close to the scalp.

Plucking -

Hair plucking is performed when the hair root is required to be examined. This technique is also employed in conventional trichogram. The routine hair pull test also yields the hair shaft with intact root in some cases.

- The site of hair sampling depends on the area affected. In certain conditions, e.g. Netherton's syndrome, changes may not be detected on examining limited areas over the scalp. In such situations, examination of eyebrows may yield the result. In cases of white piedra, genital hair should also be examined.

- Processing hair samples for the light microscopy :

- Dry mount : Hair samples are placed on a glass slide and covered with a cover slip.
- Wet mount : Hair samples are placed on a glass slide, covered with a cover slip and KOH solution is added to the hair samples.

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3. Rudnicka L, Olszewska M, Rakowska A. In vivo reflectance confocal microscopy: usefulness for diagnosing hair diseases. J Dermatol Case Rep. 2008;2(4):55–59.

Image 1 : Scheme of hair microscopy

Image courtesy :
Adya, K. A., Inamadar, A. C., Palit, A., Shivanna, R., & Deshmukh, N. S. Light microscopy of the hair: a simple tool to "untangle" hair disorders. International journal of trichology,2011 3(1), 46–56.

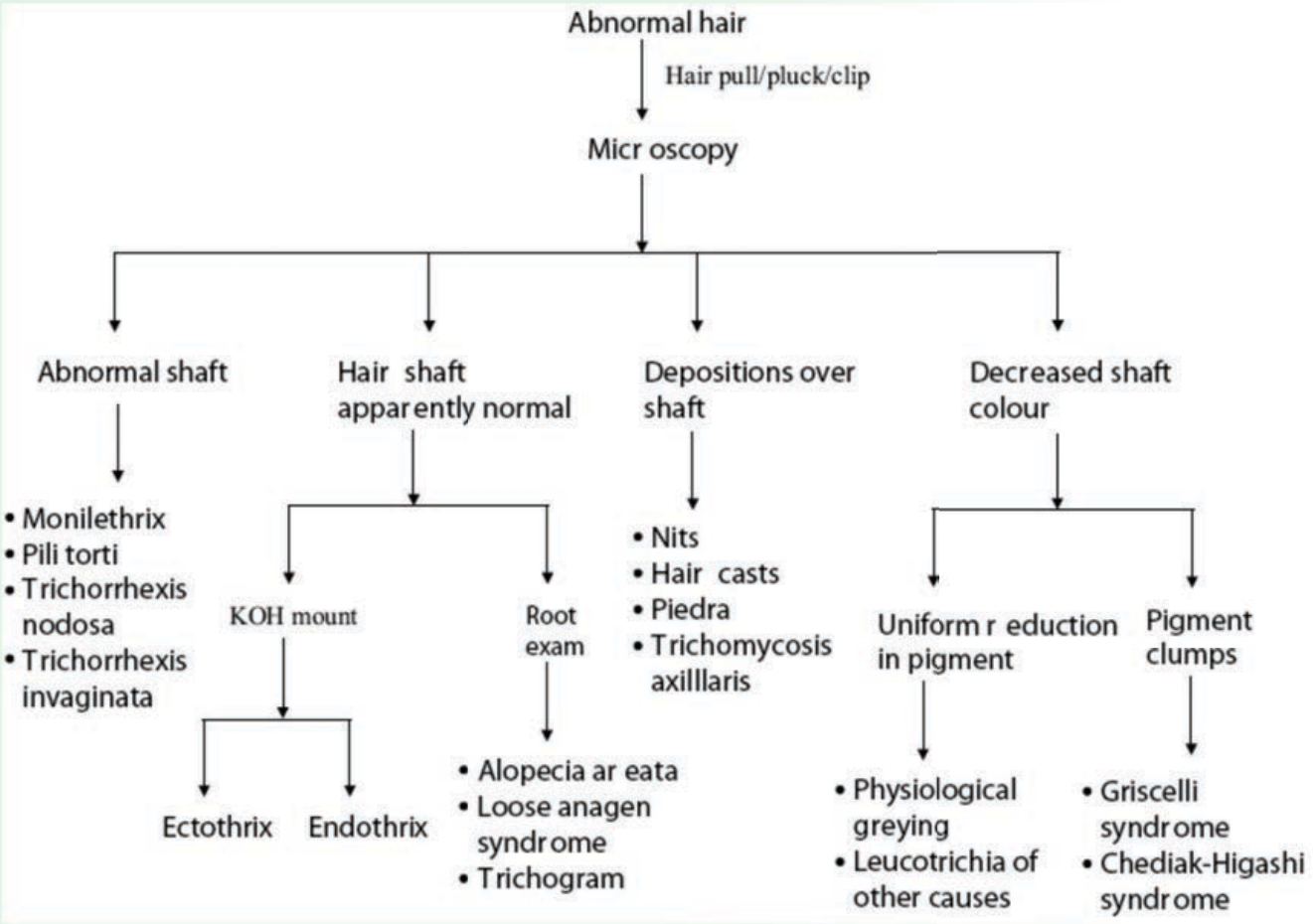

Image 2 : Appearances of hair shaft in specific disorders

Image courtesy :
Adya, K. A., Inamadar, A. C., Palit, A., Shivanna, R., & Deshmukh, N. S. Light microscopy of the hair: a simple tool to "untangle" hair disorders. International journal of trichology,2011 3(1), 46–56.

Hair disorders	Microscopic appearances
Monilethrix	"Beaded" appearance
Pili torti	"Twisted" appearance
Trichorrhexis invaginata	"Bamboo" appearance
Trichorrhexis nodosa	An appearance of "two paint brushes thrust in together"
Alopecia areata	"Exclamation mark" appearance
Loose anagen syndrome	"Rumpled sock" appearance
Trichorrhexis invaginata (with only proximal deformity)	"Golf tee" appearance

Confocal microscopy, Optical coherence tomography, Scanning electron microscopy, Atomic Force Microscopy

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Confocal microscopy

Confocal laser scanning microscopy also called as laser scanning microscopy is a non-invasive method which generates a three-dimensional image of the surface structure of the hair as well as different internal structures of hair (cortex and medulla fibers).The hair can be observed in its natural environment with less damage than produced by other microscopic methods such as scanning electron microscopy where there is unacceptable modification of the sample like coating with an electrically conducting film, drying by exposure to high vacuum, and chemical change wrought by exposure to high-energy electrons.

Confocal microscopy provides rapid, easy, elegant, and nondestructive observations of the hair in its natural environment. The technique analyzes the surface (size of the scales, optical properties of the hair such as opacity or brilliancy), internal structures of the hair (cortex and medulla fibers), and the emission spectrum. The condition of the hair surface can be evaluated according to the chemical or physical injuries sustained. Surface deposits can be observed in terms of thickness, homogeneity and brilliancy as well as their resistance to cosmetic treatments. This is helpful for wide range of applications in cosmetic research.

Confocal microscopy also provides fluorescent images either by exploiting the natural fluorescence of keratin or by adding different fluorescent dyes as markers of various structures. Confocal laser scanning microscopy is useful in obtaining “dynamic studies,” such as the routes of penetration of fluorochromes into the cortex and “optical sections” of the specimen.

Preserving the integrity of the sample, including almost any kind of surface depositsand repeated chemical or physical treatments.

In a study by Rudnicka et al, 2 healthy persons and 6 patients with hair diseases (1 with alopecia areata, 1 with androgenic alopecia and 4 with genetic hair shaft abnormalities) were examined with the use of Vivascope 1500. It gave in all cases high quality images of the hair shaft intersections, at 1µm intervals, which allowed detailed analysis of the hair structure. Also hair follicles could be partly visualized at a depth of up to 200µm, which allowed analysis of only superficial parts of the hair follicles. They concluded that CSM can be valuable tool in evaluation of hair shaft diseases.

Hadjur C in his articles mentions that confocal microscopy is relatively noninvasive, nondestructive technique and used by them routinely to monitor the efficiency of cleansing shampoos, to assess the homogeneity of layering polymers, and to evaluate the changes they induce in the optical properties of the hair surface in terms of opacity, transparency, and brilliancy. It also helps for investigation by using the fluorescence channel which reveals the internal structure of the hair. Fluorescent probes (rhodamine and its derivatives) demonstrate the routes of penetration and outline the geometry of cortical cells and of the medulla according to their lipophilic or hydrophilic properties. A volume rendering of a hair cylinder provides a better understanding of the interrelationships between cuticle cells, cortical cells, and the medullar channel.

Optical coherence tomography

Optical coherence tomography (OCT) as a diagnostic tool in dermatology was introduced in 1995 by Schmitt and very recently introduced for diagnostic trichology.

It provides highly reproducible in vivo and ex vivo measurements of hair shaft thickness, including the inner-hair variation of diameter and shape. Thus it can be used for measuring hair diameter, cross-section surface, and hair shape. It helps to investigate the influence of hair growth promoting agents in clinical studies via the OCT pictures, the cross-sectional surface as well as the longitudinal and transversal diameters, which can be done in vivo.

Scanning electron microscopy

Scanning electron microscopy and transmission electron microscopy (TEM) are used in high-resolution trichological studies. The SEM is a valuable instrument for obtaining detailed architecture of the human hair surface. It is used for obtaining high-resolution images of the hair cuticle surface with illustrations of hair shaft abnormalities. It is also used when longitudinal or transversal images of the inner structures are required.

The pre-treatment of the hair required for electron microscopy which is a very tedious process as compared to confocal or optical microscopy.

Kaliyadan et al have done a cross-sectional, controlled study in a sample of 25 female volunteers (19 study group and 6 controls) in the age group of 18–45 years. The study group was composed of volunteers who regularly used different cosmetic hair treatment procedures such as bleaching, dyeing, and straightening (any one of these or a combination). The control group had never used any specific hair treatment procedure and were regularly using coconut oil for hairs. The hair shaft damage as seen on SEM was assessed using a standardized scoring system and compared among the two groups statistically. The hair shafts were also examined clinically and with light microscopy.

No significant differences were seen between the test and control groups with regard to normal clinical examination and light microscopy findings. However, a higher degree of hair shaft damage was evident under SEM in the study group as compared to the control group. The damaged was seen as irregular overlay of the cuticle without cracks or holes, severe lift up of the cuticle with cracks or holes but without exposure of the cortex, partial exposure of cortex and complete disappearance of cortex.
The study ends with conclusion that regular use of procedures such as bleaching, dyeing, or straightening can lead to subtle changes in the hair shaft which can be detected early by SEM.

Atomic Force Microscopy (AFM)

The atomic force microscope is a combination of the principles of the scanning tunneling microscope and the stylus profilometer. It supplies 3D images (profi- lometry) with high resolution at the nanometer scale, and qualitative and quantitative measurements of the sample, giving a mathematical description of the surface.

It operates without any sample preparation, avoiding contact between the tip probe and the sample surface. It can be useful to investigate the roughness and the weathering of the cuticle, and to measure lifting of the scales. This technology provides complementary information on hair shaft condition.

Atomic force microscopy is not for clinical practice, but in hair cosmetology it can assess the effect of dyeing, bleaching, perm, or conditioners. The limitation of AFM is that it only measures topographic morphology perpendicular to the sample plane, and the re-entrant surfaces (i.e., spaces obscured by the main surface) and subsurface information cannot be detected, in contrast to SEM or confocal microscopy using fluorescence.

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Trichoscopy quiz

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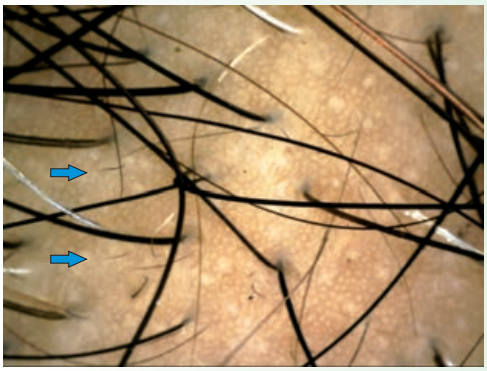
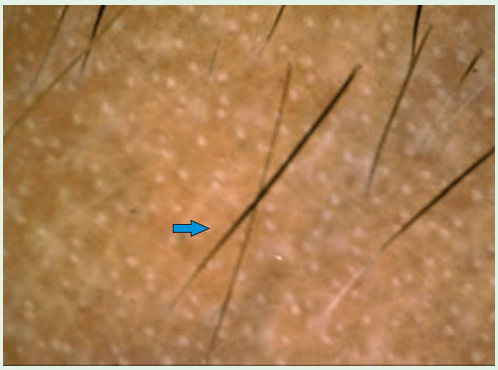
1) What is the hair shaft called in trichoscopy marked with blue arrow ?

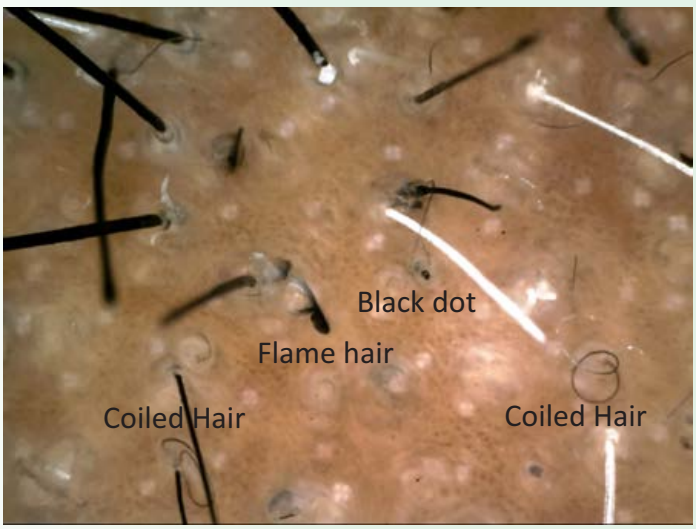
A



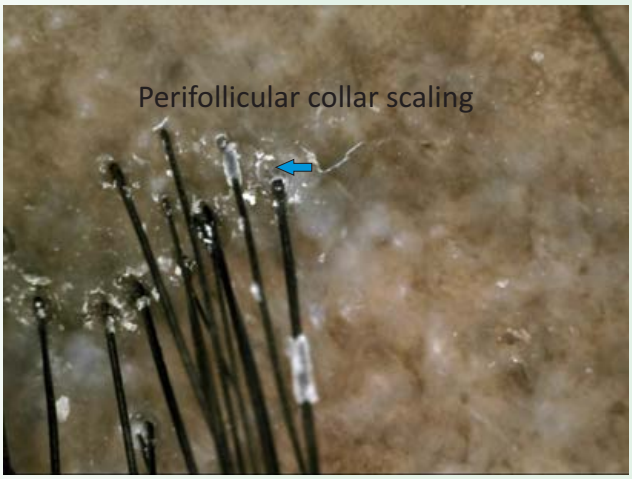
B



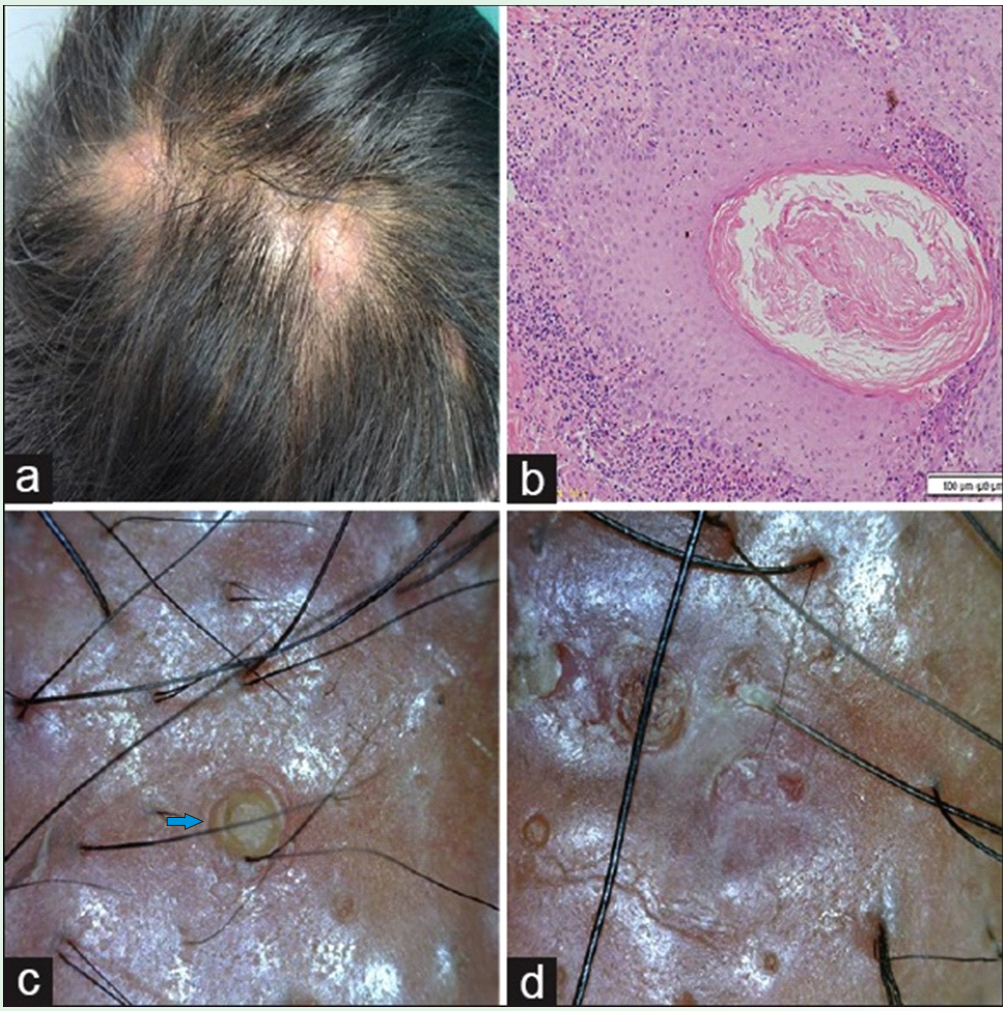
2) What is the diagnosis with these trichoscopy findings?



3) Which type of alopecia shows the perifollicular collar scaling (silver white tubular structure) as hall mark feature?



4) The clinical, histopathology and trichoscopic picture of Dissecting cellulitis of scalp is shown. Name the structure marked with blue arrow?



Answers

1) A-vellus hair, B-Short regrowing hair

- Vellus hairs are hypopigmented, nonmedullated hairs, less than 30 micrometer thick and less than 2-3 mm long.
- In androgenetic alopecia, terminal hairs become vellus hair through miniaturization process
- Regrowing hairs are short, thin, not hypopigmented and has pointed ends.
- Multiple short regrowing hairs is typical finding of telogen effluvium.

2) Trichotillomania

- Presence of multiple broken hairs of different length is the most frequent finding in Trichotillomania
- Trichoscopy shows the evidence of pulling the hair which results in coiled hair, flame hair and broken hair

3) Lichen Planopilaris

- The presence of perifollicular collar scaling in trichoscopy in scarring alopecia patient strongly suggest lichen planopilaris.
- This corresponds to the changes in the outer hair follicle root sheath resulting in excessive perifollicular scaling.

4)3D yellow Dot

- Yellow dots, appearing as large "3D" soap bubbles imposed over dark dystrophic hairs are specific for dissecting cellulitis.

Excerpts From Recent Literature

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1. Hair Growth in Two Alopecia Patients after Fecal Microbiota Transplant.
ACG Case Rep J. 2017;4:e107. Rebello D, Wang E, Yen E, Lio PA, Kelly CR.

Two patients with alopecia universalis had subsequent hair regrowth after fecal microbiota transplant for treatment of recurrent C. difficile infections (CDI). Treatment options for alopecia universalis is currently limited. Gut microbiota may have immunomodulatory effects in alopecia areata, and further study may be required to elucidate mechanisms. This case report highlights suggests not only an intestinal effect but a profound immunological response to FMT in alopecia areata. Though mechanism is still poorly understood, FMT is being investigated as a treatment option for other inflammatory conditions.

2. Sunscreen and facial skincare products in frontal fibrosing alopecia: a case-control study.
Br J Dermatol. 2019 Apr;180:943-944. Cranwell WC, Sinclair R.

Subjects with frontal fibrosing alopecia (FFA) reported more frequent use of sunscreen-containing products, with 88% reporting daily use year-round, compared with 29% in controls.The high frequency of sunscreen use among women with FFA supports the hypothesis thatsunscreen use on the forehead may be involved in the aetiology of FFA.There are a number of hypotheses for the pathogenic role of sunscreen in FFA. One hypothesis, is that sunscreen enters the follicular infundibulum and incites a lichenoid reaction against the vellus and epidermal antigens. The preponderance of FFA in postmenopausal women may be partly explained by reduced sebum production in older women, with retention of pathogenic chemicals in the follicular infundibulum.

3. A Review of the Use of Biotin for Hair Loss.
Skin Appendage Disord. 2017;3:166–169. Patel DP, Swink SM, Castelo-Soccio L.
Though use of biotin as a hair and nail growth supplement is prevalent, research demonstrating its efficacy is limited. Biotin supplementation may be of benefit in cases of acquired and inherited causes of biotin deficiency as well as pathologies, such as brittle nail syndrome or uncombable hair. However, these cases are uncommon and there is lack of sufficient evidence for supplementation in healthy individuals. Despite its popularity, biotin has no proven efficacy in hair and nail growth of healthy individuals and there is no evidence to suggest benefit from biotin supplementation outside of known deficiencies secondary to congenital or acquired causes.

4. Morphological classification system of hair regrowth patterns in alopecia areata patches: DIMT classification.
J Eur Acad Dermatol Venereol. 2019;33:e96-e97. Lee H, Lee S, Lee WS.
The patterns of hair regrowth in alopecia areata (AA) known by the acronym DIMT include: (i) diffuse (regrowth of hair throughout the patch) (ii) marginal (regrowth started from the margins and progressed in) (iii) targetoid (concentric areas of hair regrowth within the patch) (iv) irregular (no pattern). The classification needs further investigation that whether these hair regrowth patterns are associated with clinical outcomes of AA and demographic variations. The pathogenesis of AA related with hair regrowth patterns deserves further study.

5. Tofacitinib for the treatment of alopecia areata in preadolescent children.
J Am Acad Dermatol. 2019;80:568-570. Craiglow BG, King BA.
The treatment options for alopecia areata (AA) have historically been limited. Janus kinase (JAK) inhibitors have recently emerged as a pathogenesis directed therapy. 4 pediatric patients aged 8 to 10 years with alopecia totalis and alopecia universalis were treated with oral tofacitinib. Although this is a small case series, the results are favorable and corroborate those in adolescents and adults. After proper counseling regarding the risks, including severe infection and malignancy, the use of tofacitinibmay be considered for preadolescent children with AA.

6. The Higher Number and Longer Duration of Kenogen Hairs Are the Main Cause of the Hair Rarefaction in Androgenetic Alopecia
Skin Appendage Disord. 2019;5:152-154. Guarrera M, Rebora A.
Theconcept of hair miniaturization as the sole cause of androgenetic alopecia (AGA) is hardly tenable. In fact, againcludes two distinct phenomena: the progressive hair softness and the areas of hair rarefaction. If hair miniaturization explains the first feature, the kenogen hypothesis seems more likely to explain the second. The increased duration and frequency of kenogen are the real mechanism through which the scalp hairs rarefy. Physiologically, kenogen affects about 10% of all hairs and lasts about 2 months. Over time, with progressing AGA,the number of hairs that go into telogen increases, causing a small area of hair rarefaction to develop. Confluence of such areas produces further areas of alopecia.

Advances in scalp biopsy technique

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Alopecia is a very common problem and is often a cause of great concern for cosmetic and psychological reasons. Although it has several causes, it may be an important sign of systemic disease. Alopecia can be either scarring or non-scarring. Scalp biopsy provides worthwhilediagnostic clues when clinical observation and medical historyalone fail to diagnose the non-cicatricial or cicatricialtype of alopecia. Scalp biopsy is considered mandatory in all cases of scarring alopecia while in non-scarring type, it may be required if the cause of hair loss is unclear.

The key to a good scalp biopsy in a patient with alopecia is to take an adequate sample of scalp in both size and degree of involvement. The current gold-standard for a scalp biopsy specimen is the use of a 4-mm punch which must include subcutaneous fat to ensure sampling of the entire follicular unit and any anagenfollicles. The specimen may be sectioned vertically(V) or transversely(T). It is important to select the appropriate site of biopsy to have a correct diagnosis ofalopecia.In a scarring alopecia,the biopsy should be taken from the active border of hair loss where some hairs still remain andare more likely to display diagnostic findings. For non-scarring alopecia, the preferred site ofbiopsy is generally the border of a lesion or fromthe site of a positive pull test in the setting of a diffuse alopecia. In androgenic alopecia, two biopsies, one from the involved scalp (vertex) and one from theuninvolved scalp (occiput) may be beneficial.

In 1984, Headington established the morphology of hair follicles in TS of the scalp. Later various techniques like Frishbergtechnique, Tyler technique and St. John's Protocol were proposed. Recently, “Hovert technique”is gaining popularity,wherein the scalp biopsy is transected approximately 1 mmbelow the epidermal surface to obtain an “epidermal disc (image 1).”This epidermal disc is subjected to vertical sectioning inconventional fashion, whereas the remaining lower portionof the biopsy is sliced for horizontal sections.The main advantage of vertical sections (VS) is that the dermoepidermal junction, papillary dermis, and subcutis are better demonstrated. The major drawback of VS is that the follicular counts andratio cannot be assessed. The advantages of transverse section (TS), in addition to the higher yield of follicles are the rapid, easy, and accurate assessment of follicular density, follicle and shaft diameters, anagen, telogen, terminal, and vellus hairs. The disadvantage of TS is that the grossing is cumbersome and demands some degree of expertise and experience. Since each sections have their own advantages, many authors have clearly demonstrated that the combination of both V and T provides increased diagnostic utility than either section alone.When one skin sample is provided the decision to make V or T can becomeparticularly challenging.The North American Hair Research Society (NAHRS) consensusstated that combining V and T is optimal when there are two biopsiesand it suggested the T when a single biopsy was submitted.

The “figure 8” a new hair biopsy technique which is efficient and confines the area of scarring (image 2).The area is cleaned and injected with a 1-cc bolus of lidocaine and epinephrine. Two 4mm punch biopsy specimens are obtained from adjacent site, leaving a narrow isthmus of skin between them. A small nick with a no.15 scalpel is made in the centre of the isthmus, producing 2 small peninsulas that act as flaps. The 2 holes are crossed as 1 wound because the superior peninsula moves inferiorly and to the right while the inferior peninsula moves superiorly and to the left. Using a nonabsorbablesuture (polypropylene or nylon) a simple interrupted stitch is placed attaching the right inferior border to the left inferior border of the defect. A second stitch attaches the left superior-medial border to the left inferior border of the defect to close the wound. This results in an S-shaped linear scar.

Conclusion: The accurate diagnosis of alopecia requires both vertical and transverse section examination.However, when expertise in such novel techniques are lacking, the higher diagnostic accuracy for Tsection (noncicatricial alopecia) justifies if patient consented for single biopsy.

Image1: Hovert technique for scalp biopsy

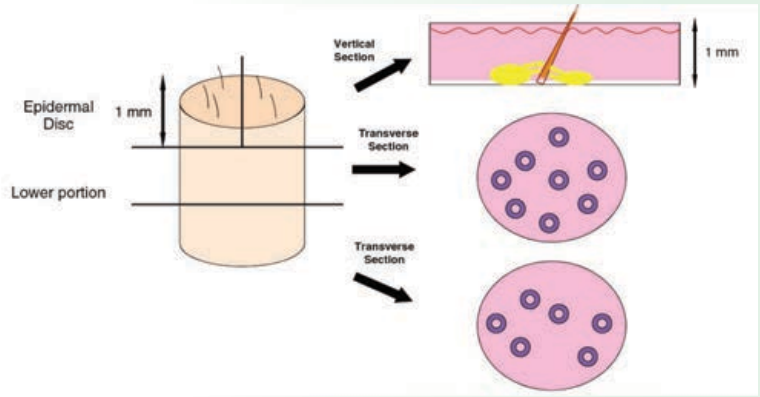
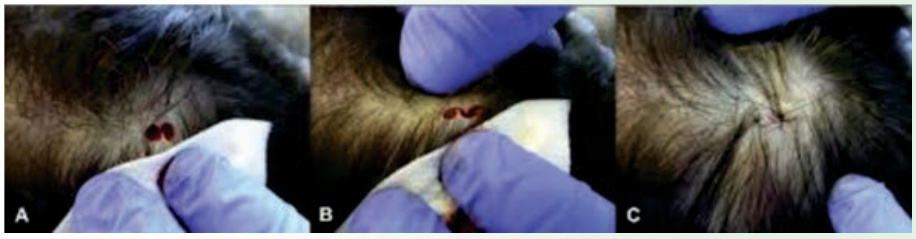


Image 2 : figure 8 scalp biopsy technique

image credit : for image 2

Zaiac M.N, Bloom R, Morrison B.W, Tosti A. The figure 8: A new hair biopsy technique. Journal of the American Academy of Dermatology 2014; 71(5):201



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3) Palo S, Biligi DS. Utility ofhorizontal and vertical sections of scalp biopsies invarious forms of primary alopecias. J Lab Physicians 2018;10:95-100.

4) Knopp E. The scalp biopsy for hair loss and its interpretation. SeminCutan Med Surg 2015;34:57-66.

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