Diagnosis of Hair Disorders

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Hair disorders include hair loss, increased hair growth, and hair structure defects with increased breakage, as well as unacceptable cosmetic appearance, such as reduced shine, strength, curliness, and elasticity. It is the task of the dermatologist to choose the right diagnostic tool depending on the suspected clinical diagnosis. Moreover, certain tools are best suited for diagnosis in private practice, whereas others can only be used to monitor hair growth under treatment in clinical studies. The techniques can be classified as either invasive (eg, biopsies in scarring alopecia), semi-invasive (trichogram, unit area trichogram), or noninvasive (eg, global hair counts, phototrichogram, electron microscopy, laser scanning microscopy) methods. Further, one must differentiate between subjective and objective techniques. For the practicing dermatologist, body and scalp hair distribution by use of different grading systems, the hair pull test, and dermoscopy belong in the category of basic diagnostic tools. Basic techniques may be extended by computer-assisted phototrichogram and, in selected cases, by use of the trichogram and/or scalp biopsies. For research purposes optical coherent tomography, electron microscopy, biochemical methods, atomic force microscopy, and confocal laser scanning microscopy are optional tools. For clinical studies global photographs (global expert panel), hair weighing, phototrichogram, and different clinical scoring systems have proven to be objective tools for documentation and evaluation of hair growth and hair quality.
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The precise and reliable diagnosis of hair growth disorders is mandatory to develop a successful therapeutic and cosmetic management strategy. Although the diagnosis of hair disorders is significantly based on the clinical experience and trichological knowledge of the dermatologist, there is a broad spectrum of diagnostic techniques that are very helpful in diagnostic work-up and for monitoring hair growth cycle disturbances

For the practicing dermatologist, two types of diagnostic tools should be distinguished: obligate and facultative procedures. Obligate or basic diagnostic procedures are composed of a thorough evaluation of the patient's and his/her family's history, supported by a complete and thorough clinical examination. Basic examination techniques include grading of hair loss or excessive hair growth, a pull test, and dermoscopy of the scalp, especially for the examination of hair follicle openings and hair shaft irregularities. Depending on the suspected diagnosis, facultative techniques, such as microscopic examination of hair roots or hair shafts, phototrichogram, or other techniques may be added.

History

An evaluation of the patient's personal history, as well as family and drug history, gynecological problems, and general internal medical disorders, as well as a discussion and patient's expectations and wishes should be performed. The disorder's impact on quality of life as well as any psychological disturbances, also are important and contributing factors for developing a management approach.

Clinical Examination

The clinical examination should begin with complete inspection of scalp and body hair distribution and the whole skin examination. When undertaking an in-depth inspection of the scalp, the physician should look for signs of inflammation, scaling, erythema, and scarring. The presence of follicular openings, exclamation mark hairs, or tufted hairs is important. For further classification, the hair loss pattern and density should be analyzed. In addition, hair shaft quality should be evaluated based on caliber, fragility, length, and elasticity.

Grading systems have been established, particularly for androgenetic alopecia (AGA) and for alopecia areata. For grading male pattern baldness, the Hamilton-Norwood scale is the most-used classification. In women the best-known scale for female-pattern AGA is the Ludwig scale which is graded on a 3-point scale. The Gan-Sinclair scale has 5 grades and the Savin-scale has 8 differentiation classes and are more accurate for specification and have become accepted.[1-3]

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Table 1 Different Types of Effluvium in Trichogram

Suspected Diagnosis	Plucking Sides	Anagen	Catagen	Telogen	Dysplastic	Dystrophic	Broken
Normal	Frontal + occipital	70-90%	<2	<15%	3-5%	<2%	<8%
Pattern hair loss	Frontal	↓	↔ ↑	↑	↔ ↑		
	Occipital	↔	↔	↔	↔		
Diffuse telogen effluvium	Frontal	↓	↔ ↑	↑ ↑	↔ ↑		
	Occipital	↓	↔ ↑	↑ ↑	↔ ↑		
Diffuse alopecia areata	Frontal	↓ ↓	↔ ↑	↑	↑	↑	
	Occipital	↓ ↓	↔ ↑	↑	↑	↑	
Loose anagen hair	Frontal	↓ ↓	↔	↔ ↑	↑ ↑ ↑		
	Occipital	↓	↔	↔ ↑	↑ ↑		
Alopecia areata circumscripta	Border of alopecic patch	↓ ↓	↔ ↑	↑	↑ ↑	↑	
	Contralateral untouched area	↔	↔	↔	↔ ↑	↔ ↑	
Trichotillomania	Border of alopecic patch	↔	↔ ↑	↓	↑		
	Contralateral untouched area	↔	↔	↔	↔		

In women with excessive hair growth, diffuse hair growth (hypertrichosis) must be distinguished from excessive hair growth with a male pattern distribution (hirsutism). The latter can be graded with the use of an evaluation score based on hair distribution and density for example, using the Ferriman-Gallwey score. This score evaluates nine regions of the face and body separately for intensity of hair growth on a scale from 0 to 4, adding all points together; today in daily practice, a score >6 (in certain countries, >8) is considered as hirsutism.[4]

Pull Test

The hair pull test is a simple test for the clinician to determine the ongoing activity and severity of any type of hair loss. The pull test technique shows rather high interexaminer variability; however, each examiner standardizes this procedure for him or herself and is able to compare this proper standard examination in his or her own patients. For comparable results and for quantification, it is necessary that the patient refrain from hair washing for 5 days to obtain at least a certain standardization among subjects.

A bundle of approximately 50-60 hairs is grasped between the thumb, index finger, and middle finger from the base near the scalp. The hair is firmly, but not forcibly, tugged away from the scalp as the fingers slide along the hair shaft. Afterward, the number of extracted hairs is counted and, depending on the diagnosis, sometimes also examined under the microscope, for example, in loose anagen hair. If more than 10% of grasped hairs, or 6 hairs, are pulled away from the scalp, this constitutes a positive pull test and implies active hair shedding. If fewer than 6 hairs can be easily pulled out, this is considered normal physiologic shedding.[5]

Wash Test

The wash test is a noninvasive method to measure hair loss by counting and identifying rinsed out hairs. It was modified by Rebora and colleagues[6] as the so-called AGA/TE wash test to distinguish AGA and telogen effluvium by counting vellus and terminal telogen rinsed-out hairs. After 5 days of abstinence from shampooing, patients wash and rinse their hair in a basin with a gauze-covered hole used to entrap the lost hairs. These hairs are collected and examined for hair count and further analyzes. The hairs are divided into 3 classes of hair length, long hair >5 cm; intermediate hair (>3 to <5 cm); short telogen or vellus hair <3 cm, and counted individually. The final results of the AGA/TE-modified wash test are given as the total number of telogen hairs and the percentage of telogen vellus.

The disadvantages of the hair wash technique are that (1) the method can lead to hair breakage and double counting of broken hairs; (2) the method cannot be used in patients with very short or curly hairs; and (3) the method can be very time-consuming. For a precise diagnosis, the counted hairs should be examined microscopically for hair cycle phases, shaft abnormalities, and morphologic appearance of the distal tip.[7]

Hair Weighing

Hair weighing can be used in clinical studies to evaluate and analyze the effect of topically or systemically applied drugs or cosmetic molecules.[8] A well-defined rectangular site, sized via a plastic template, is clipped 1 mm short, outlined, and later permanently marked by 2 mini tattoos in nonadjacent corners of the square. After a first treatment-free period (eg, 6 weeks, depending on the expected effectiveness of hair growth promotion), hairs are hand clipped short under magnification and collected carefully (baseline growth value over a defined time). In the subsequent treatment period, hairs have the same time (6 weeks) to grow as during the screening period before clipping and collecting. At the end of the study, hairs of all sampling periods are degreased and weighed separately by an experienced technician in order of their being collected.[9]

The method is rather time consuming, but when entirely analyzed by a trained technician, good, reproducible results can be obtained. Hair weighing has its place in hair growth studies because no immediate measurements results are available, as the weighing of all samples is performed at the end of all sampling periods. Combining hair weighing with hair count and microscopic measurement of width and length can be useful for conducting several analysis with collected hairs. Inclusively linking the measurement of hair shaft thickness with optical coherent tomography (OCT).

Trichogram

The trichogram is a good tool to distinguish between the different types of effluvia (Table 1). After the patient abstains from hair washing for 5 days, 60-80 hairs are plucked with a rubber-armed forceps. For optimal evaluation of results, hair bulbs are immediately placed with their roots on a glass slide in an embedding medium, which allows microscopic evaluation later and storage of the slides for teaching (Figure 1).[10] Depending on the hair disorder, the plucking sides vary (Table 2).

Figure 1 Trichogram: telogen hair roots, epilated, and embedded hair roots under magnification.

To obtain optimal and reliable results, experienced technicians and examiners are needed. For the patient, it is an unpleasant procedure, mainly because of the 5 days without hair washing, as well as the discomfort of the hair plucking. The trichogram also has significance when the evaluation of the hair root is necessary, for example, in the diagnosis of loose anagen hair and in anagen-dysplastic effluvium.

Unit Area Trichogram
The unit area trichogram is a semi-invasive quantitative method that uses hair plucking to estimate the following hair growth parameters: hair follicle density, proportion of anagen fibers, and hair shaft diameter. It is based on plucking hairs in a defined area (usually >30 mm^2), which are then counted and measured. Microscopic analysis enables one to differentiate hair growth phases and measurement of hair length.[11] The unit area trichogram can be used for follow-up of scalp hair changes in a study cohort for observing hair growth cycling and for monitoring topical or systemic drug effect; nevertheless, it is rather time-consuming and is unsuitable for large-scale clinical trials.

Phototrichogram
The phototrichogram (PTG) is a noninvasive, reproducible method of taking close-up photographs of certain defined, shaved scalp areas to follow hair growth during a given time period. Several manual, semi-automatic, and automatic PTG methods exist. All have in common the dyeing of hair a black or brown color to enhance hair/skin contrast before taking photos. One automated phototrichogram technique is known as the TrichoScan, a well-established method in many dermatology offices and trichological centers in Europe.[1] The use of PTG technology enables the investigator to objectively quantify hair growth rate, density of hair follicles, and hair shaft thickness, as well as the number of vellus and terminal hairs. For differences in evaluation of hair growth parameters see Table 3.

Contrast-Enhanced PTG (CE-PTG)
The CE-PTG is a potent method for analysis of hair growth and loss. The photographs of the scalp areas are taken twice, at intervals of 2 to 5 days. Depending on the clinical demand and trial protocol, usually 2 to 3 areas are shaved, dyed, and photographed (see Table 2).[12]

At the first visit (day 0), all hairs in the defined areas (1 cm^2) are trimmed 1 mm from the skin surface. At both visits, the hair sites are covered with a transient (brown or black) hair dye for contrast enhancement. Afterward, photographs are taken with a macro camera by the use of a scalp immersion proxigraphy method. In other PTG methods the use of gel-like intermediate-substances is described.

In hair loss studies as well as in the measurement of body hair density, it is fundamental that exactly the same area must be visualized on both photos. This area can be guaranteed by the use of a semipermanent tattoo on the investigational area.

Automated Phototrichogram: TrichoScan
The TrichoScan® is a GCP-validated, investigator-independent, automated software program for the analysis of hair growth. It combines standard epiluminescence microscopy with automatic digital image analysis for the measurement of all important hair parameters.[13,14] The defined scalp areas are trimmed with a standardized hair clipper (Hairliner, Wella, Germany) and exactly 3 days later are dyed with a commercially available solution (e.g., Goldwell top chic, black 2N, Darmstadt, Germany). After the extra color is removed with an alcoholic solution and, the area is still wet, images are obtained with a digital camera with a rigid contact lens, which ensures that the images are always taken at the same distance from the scalp. As an automated image analysis tool, accurate TrichoScan results strongly depend on the image quality; therefore, stray hairs, excessive hair dye, or air bubbles and other confounders have to be eliminated.

The TrichoScan is used to support clinical diagnosis in hair consultation as well as in clinical studies (Figure 2). During follow-up, it is fundamental that exactly the same area is seen on both photos; otherwise, accurate results will not be achieved.

Dermoscopy and Videodermoscopy
Today, hair diagnostic consultation is impossible to imagine without dermoscopy of the scalp. With handheld systems, videodermoscopy has the advantage of storing the hair and skin findings for use as further controls. The magnification enhances the images and detects the hair shaft in the follicle (if present) and its length, diameter, and possible anomalies. It is useful for the differential diagnosis

Table 2 Investigational Sites for Trichogram; Phototrichogram; TrichoScan®

Suspected Diagnosis	Investigational Site A	Investigational Site B
Male pattern hair loss	Frontal regression hair line and/or vertex region	2 cm besides occiput region (Protuberantia occipitalis externa)
Female pattern hair loss, diffuse effluvium, alopecia areata diffusa, loose anagen hair	Approx. 2 cm behind frontal hair line	2 cm besides occiput region (Protuberantia occipitalis externa)
Alopecia areata circumscripta, trichotillomania	Border of the alopecic patch	Contralateral

Table 3 Evaluation Parameters for Phototrichogram Methods

	TrichoScan	CE-PTG
Number of all hairs and hair density (n/cm²)	Note: TrichoScan cannot pick up hairs that are too short or too fine (approx. <6-10 μm) for analysis. +	+
Terminal hair density (n/cm²) and total terminal hair count (n)	Automatic detection of all hairs that are thicker than 40 μm (terminal hairs). The relative number of terminal hairs is also given.	All hairs compared with a calibrated ruler thicker than 40 μm
Vellus hair density (n/cm²) and total vellus hair count (n)	Automatic detection of all hairs that are thinner than 40 μm (terminal hairs); hair diameters <6-10 μm. The relative number of terminal hairs is also given.	All hairs compared with a calibrated ruler thinner than 40 μm
Mean (μm) and cumulative hair thickness (mm)	+	+
Telogen hair count (n/cm²) and telogen-rate (%)	Hair that has not grown in the 3 days after hair clipping in which hair stubble is left behind. In that sense, nongrowing hairs are judged as telogen	No elongation of the hair in comparison of both pictures. A missing hair in the second picture suggests a hair shedding process.
Anagen hair count (n/cm²) and anagen-rate (%)	All hairs that have grown >0.20 mm after 3 days.	The ratio of growing hair to all visible hair per area multiplied by 100
Catagen hair count (n/cm²) and catagen-rate (%)	−	Moderate elongation of the hair in comparisons of both pictures
Linear hair growth rate (LHGR: mm/d)	−	The change in length of the renewable hair (hair length in the second photo minus the hair length on day 0) between the times the two

between cicatricial and noncicatricial alopecia and between alopecia areata and trichotillomania. Follicular atrophy or keratotic plugs can easily be identified in the follicular openings. In alopecia areata and androgenetic alopecia, peripilar dots may be present. In trichotillomania, the hair is broken at different distances from the scalp. In addition, one may study the vascular pattern of the scalp by using the epiluminescent mode. Although it is a technically easy-to-handle method, interpretation of (video) dermoscopic images requires experience.[15]

The dermoscope can be used daily in hair consultations. The videodermoscope should be used for the follow-up of therapeutic results and for teaching purposes.

Global Photos

The increase in hair coverage can be the result of an increase in hair density and hair thickness, as well as a continuous increase in hair weight. Thus, global photographs are an essential tool in evaluating the overall course of hair volume, in objectively monitoring hair growth activity, hair quality, and fullness in clinical trials and for long-term follow-up of patients under long-term treatment. This standardized method uses a stereotactic device with subsequent blinded evaluation by an expert panel comparing the subject's before- and after-treatment photos.

In a stereotactic positioning device, the patient's chin and forehead are fixed on the stationary component, and the camera and flash device are mounted on the flexible arm, enabling photos of the regions of interest with vertex, midpattern, frontal, and temporal views. Assuring a constant view, magnification, and lighting, precise follow-up pictures are created.[16] This technique is used mostly in AGA hair loss studies.

Structural and Microscopic Methods

Light and Polarizing Microscopy

Ten to 20 hairs are clipped close to the scalp surface and afterwards cut in portions of approximately 7 cm, which are embedded in a mounting medium (i.e., Eukitt) from the proximal to the distal part in sections on different glass slides, allowing examination of the entire hair. These hairs are examined using light microscopy for thickness, form, and twists about the entire hair length.

Polarizing microscopy can be performed on the same embedded hair specimen. It helps to detect disorders of protein composition and keratinization or storage, for example, in trichothiodystrophy with the tiger tail pattern or in monilethrix. Light and polarizing microscopy are used in suspected hair shaft anomalies and unknown increased fragility of hairs, in clinical studies, or in hair consultation.

Electron Microscopy

Electron microscope possesses a greater resolving capacity compared with the light microscope. With electron microscopy, one can produce high-resolution images of the hair cuticle surface with illustration of hair shaft abnormalities and longitudinal or transversal images of the inner structures. Electron microscopy is mainly used for research purposes and not for routine diagnostic procedures, except for genotrichoses.
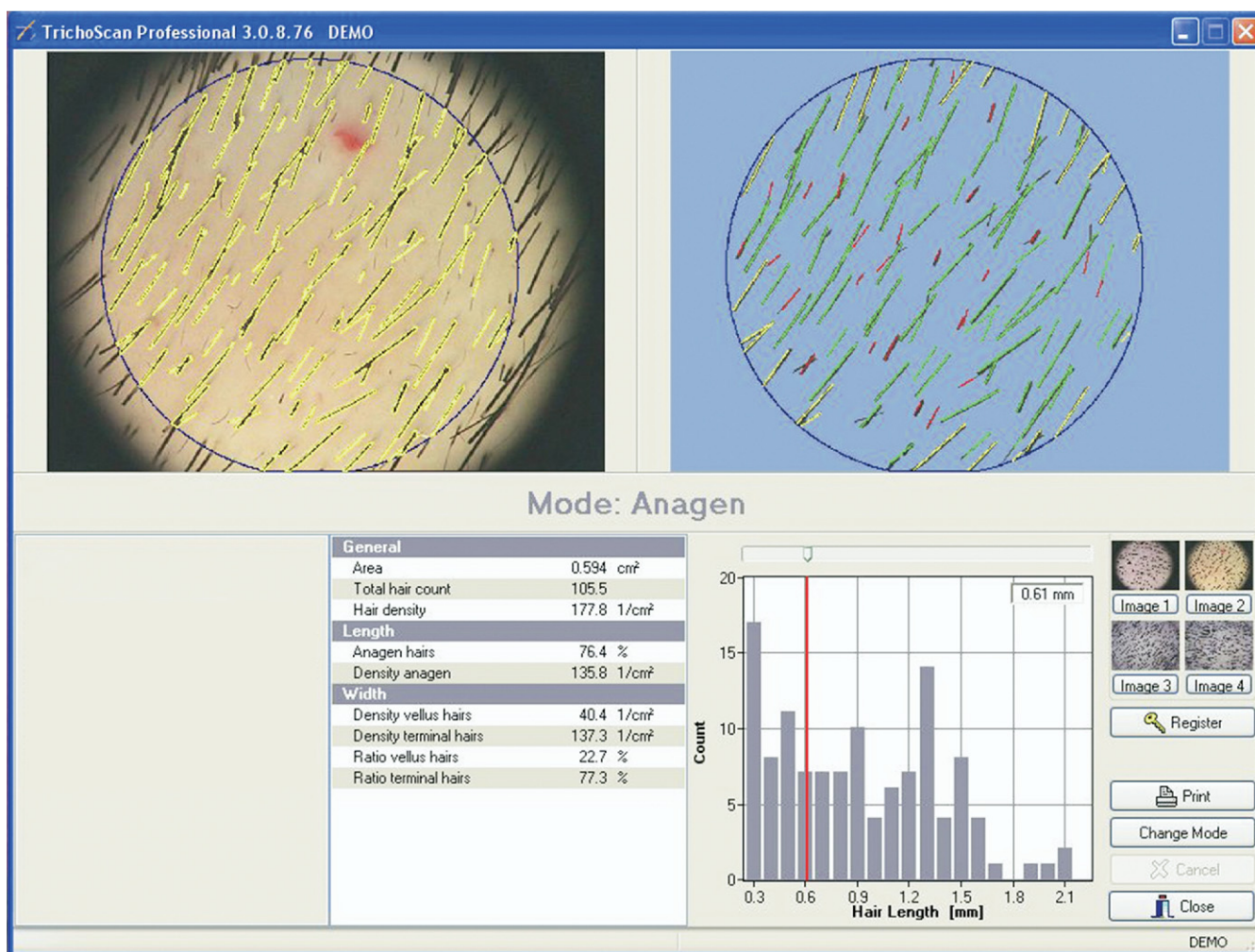
Figure 2 Typical screenshot of the TrichoScan result presentation. With precise clipping, anagen hairs can be analysed. For this purpose the hair in the target area must first be clipped completely to the skin surface with a standard single use hair clipper. After three days the hair in the area is dyed and analysed. As only anagen hairs grow considerably within these three days, by mathematical approximation the TrichoScan counts only anagen hairs.

Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy noninvasively generates a three-dimensional image of the surface structure of a hair as well as different internal structures of hair (cortex and medulla fibers) and the emission spectrum. It is a useful, noninvasive method for examining objects with curved surfaces and does not require any sample preparation; the hair can be observed in its natural environment with less damage than produced by other microscopic methods such as scanning electron microscopy.[17] It 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.[17]

Atomic Force Microscopy

Atomic force microscopy (AFM) supplies three-dimensional images (profilometry) with high resolution at the nanometer scale, producing qualitative and quantitative measurements of the sample. The method requires no preparation of the sample, avoiding contact between the tip probe and the sample surface. It can be used to

investigate the roughness and the weathering of the cuticle and to measure the lifting of the scales. AFM is, however, limited to measurement of the topographic morphology perpendicular to the sample plane, meaning that re-entrant surfaces (i.e., spaces obscured by the main surface) and subsurface information cannot be detected, in contrast to the results available with scanning electron micrsocopy or confocal microscopy using fluorescence.[18]

AFM be used as an imaging technique as well as a tool for quantitative assessment of the effects of human hair treatment.[19] As a noninvasive method, it requires no special or extensive sample preparation, like single-electron microscopy, and provides accurate topographic information.

Optical Coherence Tomography

OCT is able to provide highly reproducible in vivo and ex vivo measurements of hair shaft thickness, including the inner-hair variation of diameter and shape.[20] It produces a two-dimensional image of optical scattering from internal tissue microstructures in a way that is analogous to the pulse-echo image seen with ultrasound.[21]

OCT can be used for research purposes in trichological examination, especially for measuring the hair diameter, cross section sur-

face, and hair shape. In the future, it may be used to help investigate the influence of hair growth promoting agents to follow in vivo hair shaft changes over time.

Scalp Biopsy

The scalp biopsy, mostly performed with a 4-mm cylindrical punch, is an important tool in the diagnosis of cicatricial, but also of certain cases of noncicatricial, alopecia. The selection of the correct biopsy site, depending on the disease, is crucial for successful histologic findings. In nonscarring alopecia (e.g., trichotillomania, AGA) a punch in the center of the lesion is appropriate; in scarring alopecia, the sample has to be taken from the active peripheral margin.

Generally, two biopsies should be carried out, one for transverse and the other for vertical sectioning. For transverse sectioning, the punch is embedded and cut horizontally, allowing a quick overview of the hair follicle quantity, diameter, grouping, and morphometric data.[22] The vertical specimen should be cut in half longitudinally to hair growth direction, one part is for hematoxylin-eosin stain the other for immunofluorescence. The vertical slices show hair follicle histology for the examination of whole hair shafts and their structures as well as potential infiltrates.

Since it is an invasive technique, the indication for a scalp biopsy should be carefully considered but, when necessary, the intervention should not be delayed. This technique is used in atrophic as well as cicatricial alopecia, nonspecific inflammatory scalp diseases, scalp tumors, nonspecific differential diagnosis of noncicatricial alopecia, and in clinical studies.

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