Imaging Tools for Noninvasive Hair Assessment

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PRACTICE **POINTS**

- Reflectance confocal microscopy (RCM) imaging can be taken at levels from the stratum corneum to the papillary dermis and can be used to study scalp discoid lupus, lichen planopilaris, frontal fibrosing alopecia, alopecia areata, and androgenetic alopecia.
- Because of its ability to distinguish different stages of disease, RCM can be recommended as an intermediate step between trichoscopy and histology for the diagnosis and management of hair disease.
- Optical coherence tomography has the potential to monitor early subclinical responses to alopecia therapies while also improving hair transplantation outcomes by allowing for visualization of the subcutaneous angle of hair follicles.
- Software development paired with trichoscopy has the ability to quantify hair growth parameters such as hair count, density, and diameter.

Trichology tools historically have been limited in their ability to provide noninvasive detailed assessments of the hair and scalp. Recent advances in diagnostic and treatment monitoring technologies have begun to fill this gap. Global photography previously relied on a film camera and stereotactic imaging equipment but has been simplified by the advent of cameras that use software analysis and provide adjustable outlines to match facial features for the capture of standardized views. Reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) both enable in vivo visualization of subcutaneous structures and provide new insight into the dynamic subclinical changes of alopecia. Recent efforts focus on training convolutional neural networks to quantify various hair parameters on OCT scans. When scalp biopsy is necessary, trichoscopy, RCM, and OCT can guide in selecting biopsy sites. Because of the growing clinical applications of these technologies, clinicians should be aware of the advantages and limitations of noninvasive hair-imaging tools. *Cutis.* 2023;112:E52-E57.

ew imaging tools along with adaptations to existing technologies have been emerging in recent years, with the potential to improve hair diagnostics and treatment monitoring. We provide an overview of 4 noninvasive hair imaging technologies: global photography, trichoscopy, reflectance confocal microscopy (RCM), and optical coherence tomography (OCT). For each instrument, we discuss current and future applications in clinical practice and research along with advantages and disadvantages.

Global Photography

Global photography allows for the analysis of hair growth, volume, distribution, and density through serial standardized photographs.¹ Global photography was first introduced for hair growth studies in 1987 and soon after was used for hair and scalp assessments in finasteride clinical trials.²

Hair Assessment—Washed, dried, and combed hair, without hair product, are required for accurate imaging; wet conditions increase reflection and promote

From the Center for Dermatology, Department of Pathology and Laboratory Medicine, Rutgers Robert Wood Johnson Medical School, Somerset, New Jersey. Dr. Rao also is from Department of Dermatology, New York-Presbyterian Hospital/Weill Cornell Medical Center, New York, New York. Dr. Rubin, Rohan R. Shah, Samavia Khan, and Dr. Haroon report no conflict of interest. Dr. Rao is a consultant for Caliber Imaging & Diagnostics, Inc. Correspondence: Rohan R. Shah, BA, Center for Dermatology, Department of Pathology and Laboratory Medicine, Rutgers Robert Wood Johnson Medical School, 1 World's Fair Dr, Somerset, NJ 08901 (rs1520@njms.rutgers.edu). doi:10.12788/cutis.0834 hair clumping, thus revealing more scalp and depicting the patient as having less hair.¹ Headshots are taken from short distances and use stereotactic positioning devices to create 4 global views: vertex, midline, frontal, and temporal.³ Stereotactic positioning involves fixing the patient's chin and forehead as well as mounting the camera and flash device to ensure proper magnification. These adjustments ensure lighting remains consistent throughout consecutive study visits.⁴ Various grading scales are available for use in hair growth clinical studies to increase objectivity in the analysis of serial global photographs. A blinded evaluator should assess the before and after photographs to limit experimenter bias. Global photography often is combined with quantitative software analysis for improved detection of hair changes.¹

Advancements—Growing interest in improving global photography has resulted in various application-based, artificial intelligence (AI)–mediated tools to simplify photograph collection and analysis. For instance, new hair analysis software utilizes AI algorithms to account for facial features in determining the optimal angle for capturing global photographs (Figure 1), which simplifies the generation of global photography images through smartphone applications and obviates the need for additional stereotactic positioning equipment.^{5,6}

Limitations—Clinicians should be aware of global photography's requirements for consistency in lighting, camera settings, film, and image processing, which can limit the accuracy of hair assessment over time if not



FIGURE 1. Global photography provides adjustable outlines for consistent head positioning.

replicated correctly.^{7,8} Emerging global photography software has helped to overcome some of these limitations.

Global photography is less precise when a patient's hair loss is less than 50%, as it is difficult to discern subtle hair changes. Thus, global photography provides limited utility in assessing minimal to moderate hair loss.⁹ Currently, global photography largely functions as an adjunct tool for other hair analysis methods rather than as a stand-alone tool.

Trichoscopy

Trichoscopy (also known as dermoscopy of the hair and scalp) may be performed with a manual dermoscope (with $10 \times$ magnification) or a digital videodermatoscope (up to $1000 \times$ magnification).¹⁰⁻¹² Unlike global photography, trichoscopy provides a detailed structural analysis of hair shafts, follicular openings, and perifollicular and interfollicular areas.¹³ Kinoshita-Ise and Sachdeva¹³ provided an in-depth, updated review of trichoscopy terminology with their definitions and associated conditions (with prevalence), which should be referenced when performing trichoscopic examination.

Hair Assessment—Trichoscopic assessment begins with inspection of follicular openings (also referred to as "dots"), which vary in color depending on the material filling them-degrading keratinocytes, keratin, sebaceous debris, melanin, or fractured hairs.¹³ The structure of hair shafts also is examined, showing broken hairs, short vellus hairs, and comma hairs, among others. Perifollicular areas are examined for scale, erythema, blue-gray dots, and whitish halos. Interfollicular areas are examined for pigment pattern as well as vascularization, which often presents in a looping configuration under dermoscopy. A combination of dot colorization, hair shaft structure, and perifollicular and interfollicular findings inform diagnostic algorithms of hair and scalp conditions. For example, central centrifugal cicatricial alopecia, the most common alopecia seen in Black women, has been associated with a combination of honeycomb pigment pattern, perifollicular whitish halo, pinpoint white dots, white patches, and perifollicular erythema.13

Advantages-Perhaps the most useful feature of trichoscopy is its ability to translate visualized features into simple diagnostic algorithms. For instance, if the clinician has diagnosed the patient with noncicatricial alopecia, they would next focus on dot colors. With black dots, the next step would be to determine whether the hairs are tapered or coiled, and so on. This systematic approach enables the clinician to narrow possible diagnoses.² An additional advantage of trichoscopy is that it examines large surface areas noninvasively as compared to hair-pull tests and scalp biopsy.14,15 Trichoscopy allows temporal comparisons of the same area for disease and treatment monitoring with more diagnostic detail than global photography.¹⁶ Trichoscopy also is useful in selecting biopsy locations by discerning and avoiding areas of scar tissue.17

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Limitations—Diagnosis via the trichoscopy algorithm is limiting because it is not comprehensive of all hair and scalp disease.¹⁸ Additionally, many pathologies exhibit overlapping follicular and interfollicular patterning. For example, almost all subtypes of scarring alopecia present with hair loss and scarred follicles once they have progressed to advanced stages. Further studies should identify more specific patterns of hair and scalp pathologies, which could then be incorporated into a diagnostic algorithm.¹³

Advancements-The advent of hair analysis software has expanded the role of videodermoscopy by rapidly quantifying hair growth parameters such as hair count, follicular density, and follicular diameter, as well as interfollicular distances (Figure 2).^{14,17} Vellus and terminal hairs are differentiated according to their thickness and length.¹⁷ Moreover, the software can analyze the same area of the scalp over time by either virtual tattoos, semipermanent markings, or precise location measurements, increasing intra- and interclass correlation. The rate of hair growth, hair shedding, and parameters of anagen and telogen hairs can be studied by a method termed phototrichogram whereby a transitional area of hair loss and normal hair growth is identified and trimmed to less than 1 mm from the skin surface.¹⁹ A baseline photograph is taken using videodermoscopy. After approximately 3 days, the identical region is photographed and compared with the initial image to observe changes in the hair. Software programs can distinguish the growing hair as anagen and nongrowing hair as telogen, calculating the anagen-to-telogen ratio as well as hair growth rate, which are essential measurements in hair research and clinical studies. Software programs have replaced laborious and time-consuming manual hair counts and have rapidly grown in popularity in evaluating patterned hair loss.

Reflectance Confocal Microscopy

Reflectance confocal microscopy is a noninvasive imaging tool that visualizes skin and its appendages at near-histologic resolution (lateral resolution of



FIGURE 2. Hair analysis software accompanying videodermoscopy assists in calculations of hair count, follicular density, follicular diameter, and interfollicular distance.

0.5–1 μ m). It produces grayscale horizontal images that can be taken at levels ranging from the stratum corneum to the superficial papillary dermis, corresponding to a depth of approximately 100 to 150 μ m. Thus, a hair follicle can be imaged starting from the follicular ostia down to the reachable papillary dermis (Figure 3).²⁰ Image contrast is provided by differences in the size and refractive indices of cellular organelles.^{21,22} There are 2 commercially available RCM devices: VivaScope 1500 and VivaScope 3000 (Caliber Imaging & Diagnostics, Inc).

VivaScope 1500, a wide-probe microscope, requires the attachment of a plastic window to the desired imaging area. The plastic window is lined with medical adhesive tape to prevent movement during imaging. The adhesive tape can pull on hair upon removal, which is not ideal for patients with existing hair loss. Additionally, the image quality of VivaSope 1500 is best in flat areas and areas where hair is shaved.^{20,23,24} Despite these disadvantages, VivaScope 1500 has successfully shown utility in research studies, which suggests that these obstacles can be overcome by experienced users. The handheldVivaScope 3000 is ergonomically designed and suitable for curved surfaces such as the scalp, with the advantage of not requiring any adhesive. However, the images acquired from the VivaScope 3000 cover a smaller surface area.

Structures Visualized—Structures distinguished with RCM include keratinocytes, melanocytes, inflammatory cells, hair follicles, hair shafts, adnexal infundibular epithelium, blood vessels, fibroblasts, and collagen.²³ Real-time visualization of blood flow also can be seen.



FIGURE 3. Distinguishable structures on reflectance confocal microscopy (RCM) images include individual keratinocytes, melanocytes, inflammatory cells, hair follicles, blood vessels, fibroblasts, and collagen. Real-time visualization of blood flow also can be seen. Reflectance confocal microscopy can provide detailed information about hair shafts, adnexal infundibular epithelium, and stroma. This RCM image shows multiple hair shafts arising from follicles within the dermoepidermal junction.

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Applications of RCM-Reflectance confocal microscopy has been used to study scalp discoid lupus, lichen planopilaris, frontal fibrosing alopecia, folliculitis decalvans, chemotherapy-induced alopecia (CIA), alopecia areata, and androgenetic alopecia. Diagnostic RCM criteria for such alopecias have been developed based on their correspondence to histopathology. An RCM study of classic lichen planopilaris and frontal fibrosing alopecia identified features of epidermal disarray, infundibular hyperkeratosis, inflammatory cells, pigment incontinence, perifollicular fibrosis, bandlike scarring, melanophages in the dermis, dilated blood vessels, basal layer vacuolar degeneration, and necrotic keratinocytes.²⁵ Pigment incontinence in the superficial epidermis, perifollicular lichenoid inflammation, and hyperkeratosis were characteristic RCM features of early-stage lichen planopilaris, while perifollicular fibrosis and dilated blood vessels were characteristic RCM features of late-stage disease. The ability of RCM features to distinguish different stages of lichen planopilaris shows its potential in treating early disease and preventing irreversible hair loss.

Differentiating between scarring and nonscarring alopecia also is possible through RCM. The presence of periadnexal, epidermal, and dermal inflammatory cells, in addition to periadnexal sclerosis, are defining RCM features of scarring alopecia.26 These features are absent in nonscarring alopecias. Reflectance confocal microscopy additionally has been shown to be useful in the treatment monitoring of lichen planopilaris and discoid lupus erythematosus.²⁰ Independent reviewers, blinded to the patients' identities, were able to characterize and follow features of these scarring alopecias by RCM. The assessed RCM features were comparable to those observed by histopathologic evaluation: epidermal disarray, spongiosis, exocytosis of inflammatory cells in the epidermis, interface dermatitis, peri- and intra-adnexal infiltration of inflammatory cells, dilated vessels in the dermis, dermal infiltration of inflammatory cells and melanophages, and dermal sclerosis. A reduction in inflammatory cells across multiple skin layers and at the level of the adnexal epithelium correlated with clinical response to treatment. Reflectance confocal microscopy also was able to detect recurrence of inflammation in cases where treatment had been interrupted before clinical signs of disease recurrence were evident. The authors thus concluded that RCM's sensitivity can guide timing of treatment and avoid delays in starting or restarting treatment.²⁰

Reflectance confocal microscopy also has served as a learning tool for new subclinical understandings of alopecia. In a study of CIA, the disease was found to be a dynamic process that could be categorized into 4 distinct phases distinguishable by combined confocal and dermoscopic features. This study also identified a new feature observable on RCM images—a CIA dot—defined as a dilated follicular infundibulum containing mashed, malted, nonhomogeneous material and normal or fragmented hair. This dot is thought to represent the initial microscopic sign of direct toxicity of chemotherapy on the hair follicle. Chemotherapy-induced alopecia dots persist throughout chemotherapy and subsequently disappear after chemotherapy ends.²⁷

Limitations and Advantages—Currently, subtypes of cicatricial alopecias cannot be characterized on RCM because inflammatory cell types are not distinguished from each other (eg, eosinophils vs neutrophils). Another limitation of RCM is the loss of resolution below the superficial papillary dermis (a depth of approximately 150 μ m); thus, deeper structures, such as the hair bulb, cannot be visualized.

Unlike global photography and trichoscopy, which are low-cost methods, RCM is much more costly, ranging upwards of several thousand dollars, and it may require additional technical support fees, making it less accessible for clinical practice. However, RCM imaging continues to be recommended as an intermediate step between trichoscopy and histology for the diagnosis and management of hair disease.²⁶ If a biopsy is required, RCM can aid in the selection of a biopsy site, as areas with active inflammation are more informative than atrophic and fibrosed areas.²³ The role of RCM in trichoscopy can be expanded by designing a more cost-effective and ergonomically suited scope for hair and scalp assessment.

Optical Coherence Tomography

Optical coherence tomography is a noninvasive handheld device that emits low-power infrared light to visualize the skin and adnexal structures. Optical coherence tomography relies on the principle of interferometry to detect phase differences in optical backscattering at varying tissue depths.^{28,29} It allows visualization up to 2 mm, which is 2 to 5 times deeper than RCM.³⁶ Unlike RCM, which has cellular resolution, OCT has an axial resolution of 3 to 15 µm, which allows only for the detection of structural boundaries.³⁰ There are various OCT modalities that differ in lateral and axial resolutions and maximum depth. Commercial software is available that measures changes in vascular density by depth, epidermal thickness, skin surface texture, and optical attenuationthe latter being an indirect measurement of collagen density and skin hydration.

Structures Visualized—Hair follicles can be well distinguished on OCT images, and as such, OCT is recognized as a diagnostic tool in trichology (Figure 4).³¹ Follicular openings, interfollicular collagen, and outlines of the hair shafts are visible; however, detailed components of the follicular unit cannot be visualized by OCT. Keratin hyperrefractivity identifies the hair shaft. Additionally, the hair matrix is denoted by a slightly granular texture in the dermis. Dynamic OCT produces colorized images that visualize blood flow within vessels.

Applications of OCT—Optical coherence tomography is utilized in investigative trichology because it provides highly reproducible measurements of hair shaft diameters, cross-sectional surface areas, and form factor, which

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FIGURE 4. A, Optical coherence tomography (OCT) shows outlines of hair shafts above the epidermis (yellow arrow) in addition to the shaft's shadow cast below the skin surface (orange arrow). B, Dynamic OCT imaging of the scalp shows vascular flow below the skin's surface.

is a surrogate parameter for hair shape. The cross-section of hair shafts provides insight into local metabolism and perifollicular inflammation. Cross-sections of hair shafts in areas of alopecia areata were found to be smaller than cross-sections in the unaffected scalp within the same individual.³² Follicular density can be manually quantified on OCT images, but there also is promise for automated quantification. A recent study by Urban et al³³ described training a convolutional neural network to automatically count hair as well as hair-bearing and non-hair-bearing follicles in OCT scans. These investigators also were able to color-code hair according to height, resulting in the creation of a "height" map.

Optical coherence tomography has furthered our understanding of the pathophysiology of cicatricial and nonscarring alopecias. Vazquez-Herrera et al³⁴ assessed the inflammatory and cicatricial stages of frontal fibrosing alopecia by OCT imaging. Inflammatory hairlines, which are seen in the early stages of frontal fibrosing alopecia, exhibited a thickened dermis, irregular distribution of collagen, and increased vascularity in both the superficial and deep dermal layers compared to cicatricial and healthy scalp. Conversely, late-stage cicatricial areas exhibited a thin dermis and collagen that appeared in a hyperreflective, concentric, onion-shaped pattern around remnant follicular openings. Vascular flow was reduced in the superficial dermis of a cicatricial scalp but increased in the deep dermal layers compared with a healthy scalp. The

attenuation coefficients of these disease stages also were assessed. The attenuation coefficient of the inflammatory hairline was higher compared with normal skin, likely as a reflection of inflammatory infiltrate and edema, whereas the attenuation coefficient of cicatricial scalp was lower compared with normal skin, likely reflecting the reduced water content of atrophic skin.34 This differentiation of early- and late-stage cicatricial alopecias has implications for early treatment and improved prognosis. Additionally, there is potential for OCT to assist in the differentiation of alopecia subtypes, as it can measure the epidermal thickness and follicular density and was previously used to compare scarring and nonscarring alopecia.³⁵

Advantages and Limitations—Similar to RCM, OCT may be cost prohibitive for some clinicians. In addition, OCT cannot visualize the follicular unit in cellular detail. However, the extent of OCT's capabilities may not be fully realized. Dynamic OCT is a new angiographic type of OCT that shows potential in monitoring early subclinical responses to novel alopecia therapies, such as platelet-rich plasminogen, which is hypothesized to stimulate hair growth through angiogenesis. Additionally, OCT may improve outcomes of hair transplantation procedures by allowing for visualization of the subcutaneous angle of hair follicles. Blind extraction of hair follicles in follicular unit extraction procedures can result in inadvertent transection and damage to the hair follicle; OCT could help identify good candidates for follicular unit extraction, such as patients with hair follicles in parallel arrangement, who are predicted to have better results.36

Conclusion

The field of trichology will continue to evolve with the emergence of noninvasive imaging technologies that diagnose hair disease in early stages and enable treatment monitoring with quantification of hair parameters. As discussed in this review, global photography, trichoscopy, RCM, and OCT have furthered our understanding of alopecia pathophysiology and provided objective methods of treatment evaluation. The capabilities of these tools will continue to expand with advancements in addon software and AI algorithms.

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