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In Vivo Directional-Hemispherical Reflectance of Vitiligo-Affected and Adjacent Healthy Skin Across the Ultraviolet-to-Infrared Spectrum: A Preliminary Observational Study

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Abstract

Vitiligo is a chronic depigmenting skin disorder. Objective assessment of depigmentation and treatment efficacy remains challenging because many methods rely on subjective clinical evaluation. This study assessed reflectometry as a quantitative method for differentiating vitiligo-affected skin from healthy skin. Six patients with stable acquired vitiligo and 28 paired measurement sites were assessed. Reflectance was measured at 335–2500 nm using an SOC 410 Solar DHR reflectometer and at 1500–21,000 nm using an ET-100 emissometer. Site-level differences were aggregated at the participant level and analyzed using the exact two-sided Wilcoxon signed-rank test with Holm correction. Vitiligo-affected skin showed consistently higher reflectance in the 335–720 nm range, with the largest median difference at 335–380 nm; however, none remained statistically significant after Holm correction. At longer wavelengths, differences were smaller and less consistent. In the infrared ranges, no spectral band showed a statistically significant difference after Holm correction, and direct comparison of contrasts at incidence angles of 20° and 60° showed no statistically significant angle-dependent effect. These preliminary findings indicate that the most pronounced descriptive contrast occurs in the ultraviolet and visible spectral ranges. Larger studies are required to confirm the potential usefulness of reflectometry for quantitative assessment and monitoring of vitiligo.

Keywords: vitiligo; reflectance; infrared reflectance; emissometer

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1. Introduction

Vitiligo is a chronic depigmenting disorder of the skin characterized by the loss of functional melanocytes. This process results in the appearance of sharply demarcated, milk-white macules of variable size and shape [1–4]. The disease may become apparent at any period of life and affects individuals of both sexes and of different ethnic groups, although its onset frequently occurs at a younger age. The prevalence of vitiligo in the world population is estimated at approximately 0.5–2%, which makes it one of the most common pigmentary disorders of the skin [1–4]. Although vitiligo does not pose a direct threat to

life, the visible nature of the disease may exert a substantial influence on self-esteem, social relationships, and patients' quality of life [2].

Two principal clinical forms are distinguished: segmental and non-segmental vitiligo [1,5,6]. The segmental form most often involves lesions distributed unilaterally or in a band-like pattern, which frequently stabilize after a period of rapid progression. Non-segmental vitiligo, which is diagnosed considerably more often, is usually characterized by a bilateral and symmetrical distribution of depigmentation [1,3,5,6]. Lesions are most commonly located on the face, hands, fingers, extensor surfaces of the limbs, and in the vicinity of natural body orifices [1,3,5]. The course of the disease is unpredictable: in some patients the lesions remain stable for many years, whereas in others the changes spread at a variable rate [5,6].

The etiopathogenesis of vitiligo is complex and multifactorial. Its development is considered to involve the interplay of genetic predisposition, oxidative stress, disturbances of innate and adaptive immunity, abnormalities of melanocyte adhesion, environmental factors, and physical and emotional stress [2,3,7,8]. Studies conducted to date have identified numerous genomic regions associated with susceptibility to the disease. These encompass genes related both to melanocyte function and to the regulation of the immune response, as well as to the familial occurrence of vitiligo and its co-occurrence with other autoimmune disorders [7,8]. An important role in the pathogenesis is also attributed to oxidative stress. Melanocytes of patients with vitiligo exhibit increased sensitivity to reactive oxygen species and impairment of antioxidant mechanisms, which leads to cellular damage, inhibition of melanogenesis, and cell death [3,8]. Oxidative stress may also favor the maintenance of an aberrant immune response [9]. In turn, with respect to segmental vitiligo, characterized by a typical unilateral distribution of lesions, the participation of local neurogenic factors influencing the function and survival of melanocytes is considered [3,8].

Current concepts of pathogenesis regard vitiligo as the result of mutually reinforcing genetic, oxidative, and immunological processes, the consequence of which is the loss of melanocytes. A more detailed understanding of these relationships has provided the basis for the development of more targeted therapeutic strategies [3,4,7,9–11]. In clinical practice, combination therapy directed both at suppressing disease activity and at stimulating repigmentation is increasingly employed. Achieving a visible improvement usually requires a long duration of treatment as well as regular and, as far as possible, objective assessment of its efficacy [4,12,13]. The evaluation of treatment effects in vitiligo nevertheless encounters substantial methodological limitations. The absence of international standardization and the use of different assessment tools and scales restrict the possibility of direct and reliable comparison of results obtained by different research teams [6,14–16]. Conventional approaches to assessing the extent of lesions depend to a large degree on the visual interpretation of the investigator [14,15]. Among the most frequently used tools is the Vitiligo Area Scoring Index (VASI), based on the "rule of hand", according to which the patient's palm is assumed to correspond to 1% of the body surface area. This method is time-consuming and subject to the risk of error, because the flat, closed human hand in fact represents only approximately 0.70–0.76% of the body surface area, which may lead to overestimation of the estimated area of depigmentation [6,14,15,17,18]. The limited objectivity and reproducibility of this method hinder accurate assessment of change, particularly in the case of early repigmentation. More advanced graphical assessment tools, such as the Vitiligo Extent Score (VES), constitute an alternative and are characterized by higher reliability and greater ease of application. As a tool based on semi-quantitative analysis, however, VES still remains partly dependent on investigator interpretation [6,18]. For this reason, further methods intended to increase the objectivity of lesion monitoring are being implemented in both research and clinical practice [15,17]. Wood's lamp allows

visualization of the borders of the macules and of the first foci of repigmentation that are not visible in daylight [6]. Digital photography and body mapping enable computer-based calculation of lesion area [6,19]. Dermoscopy, in turn, permits assessment of microscopic patterns of repigmentation, which is of importance in monitoring early treatment effects, for example after UVB phototherapy. Despite their undoubted advantages, these methods also have significant limitations. Wood's lamp, although useful in individuals with fair complexions, still requires interpretation by the investigator. Optical photographic systems require rigorously standardized lighting conditions and appropriate patient positioning, which may be difficult to reproduce and may lead to technical errors. Dermoscopy, in turn, is a point-based and time-consuming method, which limits its usefulness in monitoring extensive areas of skin. In this situation, growing interest is being attracted by digital solutions whose purpose is to limit the contribution of subjective assessment in the analysis of vitiligo lesions [15,17,20]. Computational methods allow quantitative determination of the area of depigmentation and may improve the reproducibility of the measurements performed [17,20]. Their application nevertheless remains limited by the high cost of the equipment and by the fact that many systems still map the three-dimensional body surface onto flat two-dimensional (2D) images. An alternative may be provided by optical techniques based on the measurement of light reflected from the skin surface, which enable objective and quantitative assessment of its optical properties without the need for complex image analysis [21,22]. It therefore appears justified to seek simpler, readily available, and at the same time more objective methods of assessing depigmentation. In this context, the aim of our study was to assess whether reflectometry can quantitatively differentiate vitiligo-affected from adjacent healthy skin across a broad spectral range.

2. Materials and Methods

2.1. Participants

Six patients with a diagnosis of acquired vitiligo were enrolled in the study, including four women and two men, aged 35–65 years (mean age 51.6 ± 13.2 years). The diagnosis of vitiligo was established by a dermatologist. Vitiligo was considered clinically stable if no new depigmented lesions had appeared and no enlargement of existing lesions had been observed for at least six months prior to study enrollment. Clinical characteristics, including skin phototype, vitiligo subtype, disease duration, treatment history, and lesion progression, are presented in Table 1. The inclusion criteria were: age ≥18 years, the presence of stable vitiligo lesions, and the feasibility of performing measurements both within the lesion and on the directly adjacent area of skin unaffected by vitiligo. Individuals with active inflammatory skin lesions, infections, scars, or tattoos at the measurement site were excluded from the study.

Table 1. Clinical characteristics of the study participants with vitiligo.

Patient No.	Skin Phototype	Vitiligo Subtype	Duration of the Disease = Duration of the Lesions	Treatment History	Progression of Lesions
Patient 1	I	acquired non-segmental generalized	7 years	• Tacrolimus—topical—2020 • NB-UVB—2021, 2023	NO—no new changes for 6 months
Patient 2	II	acquired non-segmental generalized	15 years	• PUVA—2013 • NB-UVB—2024	NO—no new changes for 10 months
Patient 3	II	acquired non-segmental generalized	10 years	• small UVA 2024	NO—no new changes for 7 months

Table 1. *Cont.*

Patient No.	Skin Phototype	Vitiligo Subtype	Duration of the Disease = Duration of the Lesions	Treatment History	Progression of Lesions
Patient 4	II	acquired non-segmental generalized	12 years	• NB-UVB—2021	NO—no new changes for 13 months
Patient 5	I	acquired non-segmental generalized	11 years	• NB-UVB—2016, 2018 • Tacrolimus—topical—2023	NO—no new changes for 8 months
Patient 6	II	acquired non-segmental generalized	20 years	No treatment history	NO—no new changes for 12 months

PUVA—psoralen plus ultraviolet A photochemotherapy; UVA—ultraviolet A radiation; NB-UVB—narrowband ultraviolet B phototherapy at 311 nm; NO—no progression of vitiligo lesions during the specified period before study enrollment.

All participants provided written informed consent to participate in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki. Approval for the study was obtained from the Bioethics Committee of the Medical University of Silesia in Katowice (no. BNW/NWN/0052/KB1/52/I/24).

2.2. Measurement Sites

Reflectance measurements were performed in pairs: on skin affected by vitiligo and on a directly adjacent area of normally pigmented skin located within the same anatomical region (Figure 1). This approach made it possible to limit the influence of differences arising from anatomical location, epidermal thickness, vascularization, and exposure to solar radiation. Measurement sites were selected so that the entire 12.7-mm-diameter measurement field was located within the vitiligo-affected or adjacent healthy skin area, respectively.

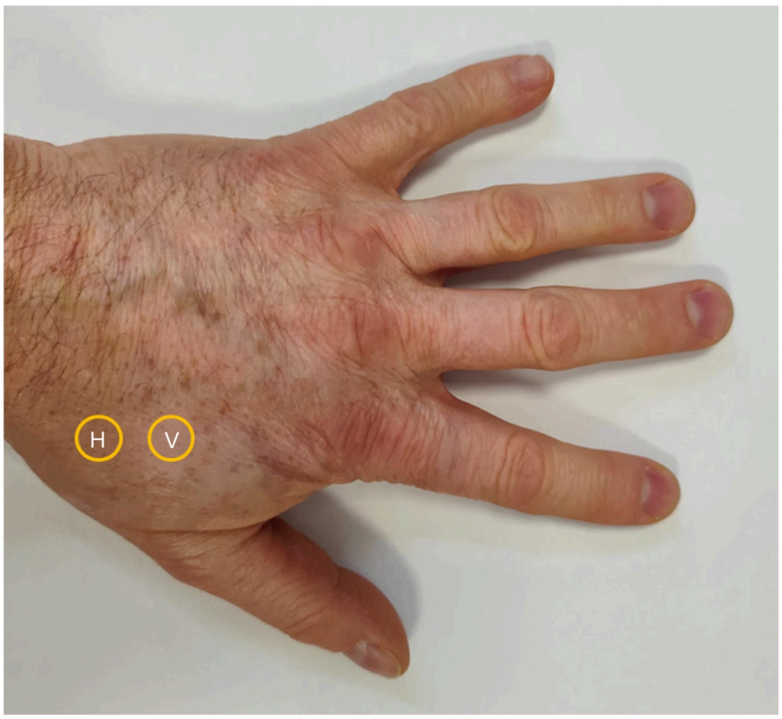


Figure 1. Representative probe-placement sites for reflectance measurements on vitiligo-affected skin (V) and directly adjacent healthy skin (H). The circles indicate the approximate locations of the measurement sites.

A total of 28 paired measurement sites (vitiligo-affected skin vs. adjacent healthy skin) were obtained from six participants. The measurement sites included the axillary region; the upper limb (dorsum of the hand, wrist, elbow region, and palmar aspect of the forearm); the chest region; the inguinal region; the lower leg; the foot; and the back. For each anatomical measurement site, the vitiligo lesion and the directly adjacent area of healthy skin constituted one corresponding V–H pair. The number and anatomical distribution of paired measurement sites for each participant are presented in Table 2.

Table 2. Number and anatomical distribution of paired measurement sites in individual participants.

Patient No.	Number of Measurement Pairs (V-H)	ET-100 Pairs Included in Analysis (V-H)	Anatomical Measurement Sites
Patient 1	6	6	Right axillary region; left axillary region; dorsum of the right hand; dorsum of the left hand; right chest region; left chest region
Patient 2	5	5	Dorsum of the right hand; right elbow region; dorsum of the left hand; left elbow region; back
Patient 3	2	2	Right wrist; left wrist
Patient 4	6	6	Palmar aspect of the right forearm; palmar aspect of the left forearm; right axillary region; left axillary region; right inguinal region; left inguinal region
Patient 5	4	4	Dorsum of the right hand; dorsum of the left hand; right lower leg; left lower leg
Patient 6	5	0 *	Right foot; left foot; right axillary region; left axillary region; dorsum of the left hand
Total	28	23	—

V-H—paired measurement consisting of vitiligo-affected skin (V) and directly adjacent healthy skin (H). Each anatomical measurement site represents one V-H pair obtained within the same anatomical region. * Five ET-100 measurement pairs from Patient 6 were excluded from the final ET-100 analysis because of a calibration error; therefore, the ET-100 analysis included 23 paired sites from five participants.

2.3. Skin Reflectance Measurements in the Ultraviolet, Visible Light, and Near-Infrared Ranges

Directional-hemispherical reflectance of the skin in the ultraviolet, visible light, and near-infrared ranges was assessed using an SOC 410 Solar DHR Reflectometer (Surface Optics Corporation, San Diego, CA, USA). The device enables measurement of total hemispherical reflectance in seven spectral bands: 335–380 nm, 400–540 nm, 480–600 nm, 590–720 nm, 700–1100 nm, 1000–1700 nm, and 1700–2500 nm. The diameter of the measurement field was 12.7 mm. Prior to the measurements, the device was calibrated using a Diffuse Calibration Coupon and a Specular Calibration Coupon, both traceable to NIST standards. All measurements were performed at an angle of incidence of 20°.

2.4. Infrared Reflectance Measurements

Directional-hemispherical reflectance in the infrared range was assessed using an ET-100 Emissometer (Surface Optics Corporation, San Diego, CA, USA). The device enables measurements to be performed at angles of incidence of 20° and 60° in six spectral bands: 1.5–2.0 µm, 2.0–3.5 µm, 3.0–4.0 µm, 4.0–5.0 µm, 5.0–10.5 µm, and 10.5–21.0 µm. The diameter of the measurement field was likewise 12.7 mm. The emissometer was calibrated before measurements were performed on each participant, using the supplied Specular Gold Calibration Coupon. According to the manufacturer's specifications, the measurement accuracy of the ET-100 is ±0.03 reflectance units and its repeatability is ±0.005 units. In the present study, reflectance values obtained at both 20° and 60°angles of incidence were analyzed, comparing skin affected by vitiligo with directly adjacent healthy skin.

2.5. Measurement Conditions

All measurements were carried out under controlled environmental conditions, at a temperature of 21 ± 1 °C and a relative air humidity of approximately 45%. Before the start of the examinations, participants remained in the measurement room for at least 15 min in order to acclimatize. The measurement sites were clean and dry, free from cosmetics or other preparations that might influence the optical properties of the skin. Participants were instructed not to apply cosmetics to the examined skin areas on the day of measurement. No additional skin-cleaning procedure was performed immediately before the measurements. All measurements were performed by the same operator. For each paired measurement site, healthy skin was measured first, followed by the adjacent vitiligo-affected skin. The probe was gently placed against the skin without applying intentional additional pressure, while ensuring complete contact of the measurement aperture with the skin surface to prevent external light from entering the measurement area. No additional device or procedure for quantitative control of contact pressure was used. Three consecutive measurements were obtained at each site, with the probe repositioned between readings. The mean of the three readings was used for further analysis.

2.6. Statistical Analysis

Data were organized and prepared for analysis using Microsoft Excel. Statistical analyses were performed using Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA, USA). The normality of the distributions was assessed using the Shapiro–Wilk test. Because the distributions of the analyzed variables departed from normality and the number of participants was small, non-parametric methods were used for inferential analyses.

To account for the presence of multiple anatomical measurement sites within individual participants, the participant rather than the individual measurement site was treated as the independent unit of analysis. For each spectral band, a site-specific reflectance difference was first calculated for each anatomically corresponding pair as $\Delta = V - H$, where V denotes reflectance of vitiligo-affected skin, and H denotes reflectance of directly adjacent healthy skin. When more than one anatomical site was examined in the same participant, the site-specific values were subsequently aggregated within that participant using the median. Thus, each participant contributed one value per spectral band to the inferential analysis. The SOC 410 analysis included six participants, whereas the ET-100 analysis included five participants after exclusion of the ET-100 measurements from Patient 6 because of calibration error (Table 2).

For descriptive presentation of reflectance values for vitiligo-affected (V) and healthy (H) skin, site-level V and H values were likewise aggregated within each participant using the median. Participant-level reflectance values for vitiligo-affected and healthy skin and participant-level Δ values were summarized using the median, first quartile (Q1), third quartile (Q3), minimum, and maximum. Differences between vitiligo-affected and adjacent healthy skin were evaluated by applying the Wilcoxon signed-rank test to the participant-level Δ values. Zero differences were excluded from the Wilcoxon signed-rank calculation; therefore, the effective sample size (N_W) could differ between spectral bands. Tied non-zero absolute differences were assigned average ranks. Exact two-sided *p*-values were calculated by enumerating all possible sign assignments for the non-zero participant-level differences, conditional on the observed absolute ranks. Effect size was calculated as $r = |Z|/\sqrt{N_W}$, where Z is the standardized Wilcoxon statistic, and N_W is the number of non-zero participant-level differences included in the test. The Z statistic and effect size r were reported as supplementary measures; statistical significance was determined from the exact *p*-values rather than from the normal approximation.

To control for multiple testing, Holm-adjusted p values were calculated separately within prespecified families of related comparisons: seven SOC 410 spectral bands, six ET-100 spectral bands measured at an incidence angle of 20°, and six ET-100 spectral bands measured at an incidence angle of 60°. Both unadjusted exact two-sided p-values and Holm-adjusted p values (pHolm) are reported. Statistical significance was evaluated at $\alpha = 0.05$ on the basis of the Holm-adjusted p values.

The effect of incidence angle on the vitiligo–healthy skin reflectance contrast measured with the ET-100 was assessed directly. For each participant and spectral band, the difference between the participant-level contrasts obtained at the two incidence angles was calculated as $D = \Delta 20° - \Delta 60°$. The resulting participant-level D values were tested against zero using the Wilcoxon signed-rank test. Holm correction was applied across the six ET-100 spectral bands constituting this separate family of angle comparisons.

Short-term within-site repeatability was assessed using the three consecutive readings obtained at each measurement site, with repositioning of the measuring probe between readings. For each site, the relative standard deviation (RSD) was calculated as RSD = (SD/mean) × 100%, where SD and mean refer to the three repeated readings. The distribution of site-specific RSD values was summarized by the median, Q1, Q3, minimum, and maximum.

The within-site standard deviation (Sw) was pooled across measurement sites according to:

$$Sw = \sqrt{[\Sigma(n_i - 1)s_i^2/\Sigma(n_i - 1)]},$$

where s_i is the standard deviation of the repeated readings at measurement site i, and n_i is the number of repeated readings at that site ($n_i = 3$). Sw was calculated separately for healthy and vitiligo-affected skin to characterize repeatability in each type of skin. The repeatability coefficient (RC) was subsequently calculated as: $RC = 1.96 \times \sqrt{2} \times Sw$.

For an additional descriptive assessment of whether the observed local vitiligo–healthy skin contrasts were large relative to short-term measurement variability, Sw was also pooled across healthy and vitiligo-affected measurement sites for each spectral band and, for the ET-100, separately for each incidence angle. A corresponding pooled RC was then calculated using the same formula. For each anatomically paired measurement site, the absolute reflectance contrast $|\Delta|$ was compared with the respective pooled RC. The number and percentage of paired sites for which $|\Delta|$ exceeded RC were reported together with the direction of the contrast ($\Delta > RC$ or $\Delta < -RC$). This comparison was descriptive only and was not treated as a statistical significance test because multiple measurement sites were clustered within individual participants. Importantly, the pooled RC was derived from the variability of individual repeated readings, whereas the reflectance contrasts were calculated from the means of three measurements at each site. Therefore, the pooled RC does not directly represent the repeatability limit of these contrasts, and the comparison should be interpreted solely as a descriptive assessment of their magnitude relative to short-term measurement variability.

Statistica 13.3 was used for the standard statistical procedures, including descriptive statistics, assessment of normality, and the initial Wilcoxon signed-rank analyses. Microsoft Excel was used for data organization and for calculations not provided as direct automated procedures in Statistica 13.3, including participant-level aggregation, exact Wilcoxon p-value calculations by enumeration of possible sign assignments, Holm adjustment where required, calculation of RSD, pooled Sw and RC according to the equations specified above, and descriptive comparisons of $|\Delta|$ with RC. All calculations were performed using unrounded measurement values, with rounding applied only when presenting the results.

3. Results

3.1. SOC 410 Reflectance Measurements

At the participant level, vitiligo-affected skin showed consistently higher reflectance than adjacent healthy skin in the ultraviolet and visible spectral ranges (335–720 nm), with the largest median difference observed in the 335–380 nm band ($\Delta = 0.1781$). The participant-level differences were smaller and less consistent in the near-infrared ranges. In the 1000–1700 nm and 1700–2500 nm bands, median Δ values were slightly negative (-0.0048 and -0.0033, respectively), indicating a tendency toward lower reflectance in vitiligo-affected skin. Although the exact two-sided Wilcoxon signed-rank test yielded $p = 0.03125$ for each of the four bands between 335 and 720 nm, none of the differences remained statistically significant after Holm correction (pHolm = 0.21875). Thus, the observed UV–visible differences were consistent in direction and accompanied by large effect-size estimates, but they should be interpreted as descriptive patterns rather than statistically significant differences after correction for multiple testing (Tables 3 and 4; Figure 2).

Table 3. Participant-level SOC 410 reflectance values for vitiligo-affected and adjacent healthy skin across spectral bands.

Spectral Band (nm)	Skin	Median	Q1	Q3	Min	Max	N Patients
335–380	Vitiligo	0.2785	0.2496	0.3854	0.1445	0.4830	6
	Healthy skin	0.1068	0.0839	0.1848	0.0690	0.2325	6
400–540	Vitiligo	0.4045	0.3828	0.4668	0.3435	0.4920	6
	Healthy skin	0.3223	0.3056	0.3610	0.2540	0.4160	6
480–600	Vitiligo	0.4103	0.3853	0.4653	0.3415	0.4900	6
	Healthy skin	0.3395	0.3174	0.3751	0.2520	0.4250	6
590–720	Vitiligo	0.5850	0.5736	0.6234	0.4950	0.6445	6
	Healthy skin	0.5208	0.4966	0.5606	0.4180	0.5940	6
700–1100	Vitiligo	0.5585	0.5363	0.5658	0.4720	0.5925	6
	Healthy skin	0.5478	0.5224	0.5615	0.4990	0.5975	6
1000–1700	Vitiligo	0.2820	0.2718	0.2851	0.2585	0.2920	6
	Healthy skin	0.2860	0.2798	0.2919	0.2750	0.3055	6
1700–2500	Vitiligo	0.0720	0.0700	0.0740	0.0650	0.0760	6
	Healthy skin	0.0773	0.0743	0.0818	0.0635	0.0980	6

V—vitiligo-affected skin; H—adjacent healthy skin; Me—median; Q1—first quartile; Q3—third quartile; Min—minimum; Max—maximum.

Table 4. Participant-level differences in SOC 410 reflectance between vitiligo-affected and adjacent healthy skin across spectral bands.

Spectral Band (nm)	Median Δ	Q1	Q3	Min	Max	Nw	T	Z	*p* Exact	pHolm	r
335–380	0.1781	0.1689	0.1826	0.066	0.2427	6	0	2.201	0.03125	0.21875	0.899
400–540	0.0835	0.0793	0.0891	0.0393	0.114	6	0	2.201	0.03125	0.21875	0.899
480–600	0.0684	0.0635	0.0901	0.0298	0.118	6	0	2.201	0.03125	0.21875	0.899
590–720	0.0677	0.0545	0.0718	0.005	0.1223	6	0	2.201	0.03125	0.21875	0.899
700–1100	0.0069	−0.0023	0.0137	−0.0427	0.0297	6	8	0.524	0.6875	0.6875	0.214
1000–1700	−0.0048	−0.0147	0.0002	−0.0222	0.0033	6	4.5	1.261	0.25	0.5	0.515
1700–2500	−0.0033	−0.0039	−0.0009	−0.0212	0.0013	6	2	1.782	0.09375	0.28125	0.728

$\Delta = V - H$; N_W—number of non-zero participant-level differences included in the Wilcoxon signed-rank test; T—Wilcoxon T statistic; Z—standardized Z statistic; *p*—exact two-sided *p*-value; pHolm—Holm-adjusted exact *p* value; r—effect size.

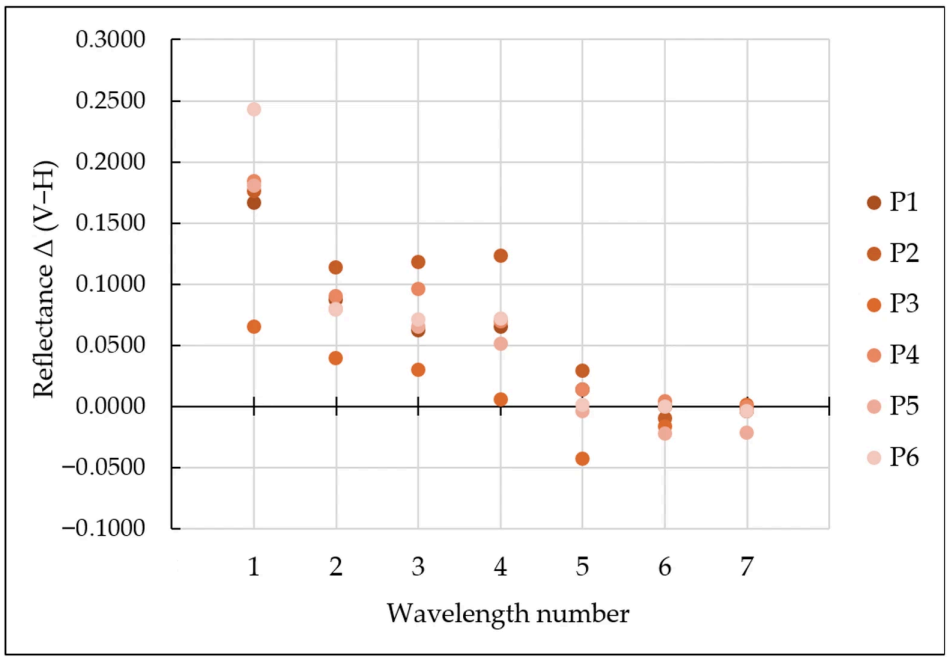


Figure 2. Participant-level SOC 410 reflectance differences (Δ = V − H) between vitiligo-affected and adjacent healthy skin. Spectral bands: 1, 335–380 nm; 2, 400–540 nm; 3, 480–600 nm; 4, 590–720 nm; 5, 700–1100 nm; 6, 1000–1700 nm; 7, 1700–2500 nm.

3.2. ET-100 Reflectance Measurements at an Incidence Angle of 20°

At an incidence angle of 20°, participant-level differences between vitiligo-affected and adjacent healthy skin were small across all ET-100 spectral bands. Median Δ values ranged from −0.0055 to 0.0005, with predominantly negative values in the shorter infrared bands, indicating slightly lower reflectance of vitiligo-affected skin. However, none of the participant-level differences was statistically significant, either before or after correction for multiple testing (all exact two-sided $p \geq 0.31250$; all pHolm = 1.000). Overall, the ET-100 measurements at 20° did not provide evidence of a systematic difference in reflectance between vitiligo-affected and adjacent healthy skin (Tables 5 and 6; Figure 3).

Table 5. Participant-level ET-100 reflectance values for vitiligo-affected and adjacent healthy skin across spectral bands at an incidence angle of 20°.

Spectral Band (µm)	Skin	Median	Q1	Q3	Min	Max	N Patients
1.5–2.0	Vitiligo	0.0810	0.0795	0.0875	0.0755	0.0880	5
	Healthy skin	0.0900	0.0870	0.0940	0.0750	0.0960	5
2.0–3.5	Vitiligo	0.0475	0.0455	0.0510	0.0425	0.0560	5
	Healthy skin	0.0535	0.0455	0.0605	0.0405	0.0610	5
3.0–4.0	Vitiligo	0.0390	0.0365	0.0390	0.0350	0.0415	5
	Healthy skin	0.0370	0.0370	0.0410	0.0335	0.0435	5
4.0–5.0	Vitiligo	0.0375	0.0350	0.0385	0.0345	0.0420	5
	Healthy skin	0.0395	0.0365	0.0455	0.0335	0.0460	5
5.0–10.5	Vitiligo	0.0370	0.0350	0.0375	0.0340	0.0390	5
	Healthy skin	0.0375	0.0350	0.0395	0.0330	0.0435	5
10.5–21.0	Vitiligo	0.0360	0.0350	0.0395	0.0345	0.0400	5
	Healthy skin	0.0380	0.0375	0.0395	0.0335	0.0450	5

V—vitiligo-affected skin; H—adjacent healthy skin; Me—median; Q1—first quartile; Q3—third quartile; Min—minimum; Max—maximum.

Table 6. Participant-level differences in ET-100 reflectance between vitiligo-affected and adjacent healthy skin across spectral bands at an incidence angle of 20°.

Spectral Band (μm)	Median Δ	Q1	Q3	Min	Max	Nw	T	Z	*p* (Exact)	pHolm	r
1.5–2.0	−0.0055	−0.0107	0.0005	−0.0158	0.0015	5	3	1.214	0.3125	1	0.543
2.0–3.5	−0.0033	−0.0050	0.0003	−0.0148	0.0017	5	3	1.214	0.3125	1	0.543
3.0–4.0	−0.0007	−0.0007	0.0005	−0.0012	0.0017	5	6	0.406	0.75	1	0.182
4.0–5.0	0.0005	−0.0010	0.0008	−0.0097	0.0017	5	7	0.135	1	1	0.060
5.0–10.5	−0.0002	−0.0008	0.0000	−0.0062	0.0012	4	3	0.730	0.625	1	0.365
10.5–21.0	−0.0010	−0.0012	−0.0007	−0.0058	0.0012	5	3.5	1.084	0.375	1	0.485

Δ = V − H; N_W—number of non-zero participant-level differences included in the Wilcoxon signed-rank test; T—Wilcoxon T statistic; Z—standardized Z statistic; *p*—exact two-sided *p*-value; pHolm—Holm-adjusted exact *p* value; r—effect size.

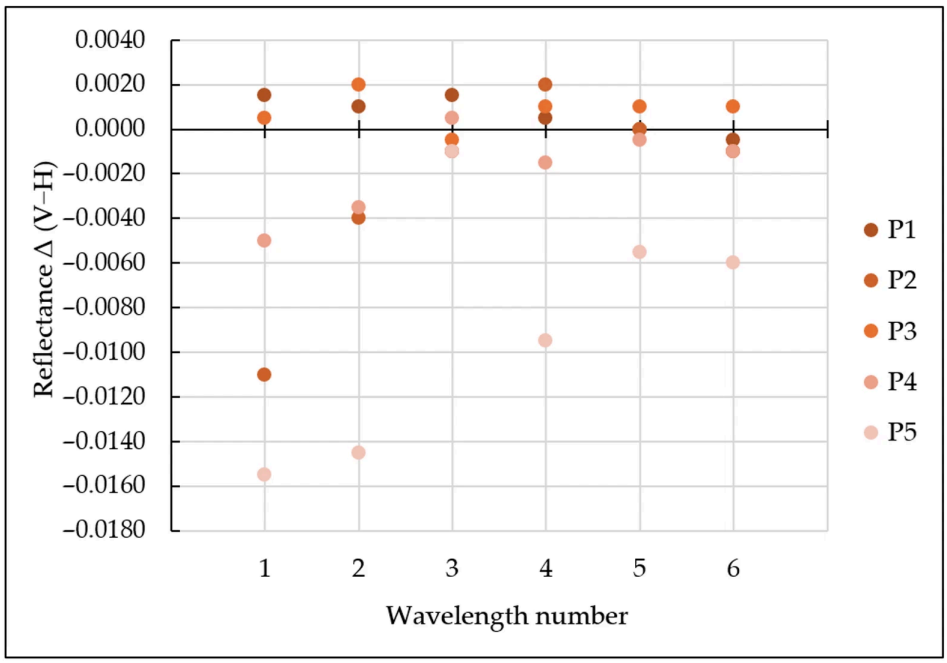


Figure 3. Participant-level ET-100 reflectance differences (Δ = V − H) between vitiligo-affected and adjacent healthy skin at an incidence angle of 20°. Spectral bands: 1, 1.5–2.0 μm; 2, 2.0–3.5 μm; 3, 3.0–4.0 μm; 4, 4.0–5.0 μm; 5, 5.0–10.5 μm; 6, 10.5–21.0 μm.

3.3. ET-100 Reflectance Measurements at an Incidence Angle of 60°

At an incidence angle of 60°, participant-level reflectance differences between vitiligo-affected and adjacent healthy skin were likewise small and varied across spectral bands. The largest median difference was observed in the 1.5–2.0 μm band (Δ = −0.0057), indicating lower reflectance of vitiligo-affected skin. This band showed the lowest exact two-sided *p*-value among the six spectral bands ($p = 0.06250$) and a large effect-size estimate (r = 0.905), but the difference was not statistically significant either before or after Holm correction (pHolm = 0.37500). No other spectral band showed a statistically significant difference. Thus, no ET-100 spectral band measured at 60° demonstrated a statistically significant vitiligo–healthy skin reflectance difference after correction for multiple testing (Tables 7 and 8; Figure 4).

Table 7. Participant-level ET-100 reflectance values for vitiligo-affected and adjacent healthy skin across spectral bands at an incidence angle of 60°.

Spectral Band (µm)	Skin	Median	Q1	Q3	Min	Max	N Patients
1.5–2.0	Vitiligo	0.1170	0.1135	0.1255	0.1115	0.1830	5
	Healthy skin	0.1330	0.1210	0.1395	0.1140	0.1980	5
2.0–3.5	Vitiligo	0.0725	0.0695	0.0790	0.0695	0.1470	5
	Healthy skin	0.0860	0.0750	0.0960	0.0705	0.1540	5
3.0–4.0	Vitiligo	0.0630	0.0585	0.0700	0.0550	0.1090	5
	Healthy skin	0.0620	0.0615	0.0790	0.0605	0.1060	5
4.0–5.0	Vitiligo	0.0635	0.0585	0.0635	0.0575	0.1350	5
	Healthy skin	0.0675	0.0650	0.0800	0.0595	0.1340	5
5.0–10.5	Vitiligo	0.0645	0.0620	0.0660	0.0570	0.1140	5
	Healthy skin	0.0660	0.0650	0.0705	0.0635	0.1100	5
10.5–21.0	Vitiligo	0.0635	0.0630	0.0650	0.0530	0.1270	5
	Healthy skin	0.0640	0.0635	0.0720	0.0620	0.1260	5

V—vitiligo-affected skin; H—adjacent healthy skin; Me—median; Q1—first quartile; Q3—third quartile; Min—minimum; Max—maximum.

Table 8. Participant-level differences in ET-100 reflectance between vitiligo-affected and adjacent healthy skin across spectral bands at an incidence angle of 60°.

Spectral Band [µm]	Median Δ	Q1	Q3	Min	Max	N_W	T	Z	*p*	pHolm	r
1.5–2.0	−0.0057	−0.0060	−0.0032	−0.0250	−0.0007	5	0.000	2.023	0.063	0.375	0.905
2.0–3.5	−0.0010	−0.0080	−0.0005	−0.0272	0.0018	5	3.000	1.214	0.313	1.000	0.543
3.0–4.0	0.0017	−0.0018	0.0028	−0.0087	0.0067	5	7.000	0.135	1.000	1.000	0.060
4.0–5.0	−0.0022	−0.0072	0.0010	−0.0215	0.0037	5	4.000	0.944	0.438	1.000	0.422
5.0–10.5	−0.0023	−0.0052	0.0015	−0.0102	0.0017	5	3.000	1.214	0.313	1.000	0.543
10.5–21.0	−0.0032	−0.0033	−0.0017	−0.0108	0.0013	5	1.000	1.753	0.125	0.625	0.784

Δ = V − H; N_W—number of non-zero participant-level differences included in the Wilcoxon signed-rank test; T—Wilcoxon T statistic; Z—standardized Z statistic; *p*—exact two-sided *p*-value; pHolm—Holm-adjusted exact *p* value; r—effect size.

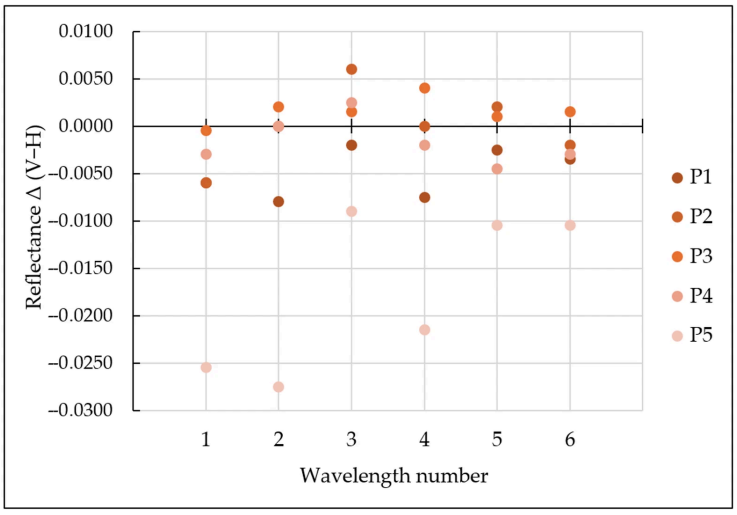


Figure 4. Participant-level ET-100 reflectance differences (Δ = V − H) between vitiligo-affected and adjacent healthy skin at an incidence angle of 60°. Spectral bands: 1, 1.5–2.0 µm; 2, 2.0–3.5 µm; 3, 3.0–4.0 µm; 4, 4.0–5.0 µm; 5, 5.0–10.5 µm; 6, 10.5–21.0 µm.

3.4. Direct Comparison of ET-100 Reflectance Contrasts Between Incidence Angles

Direct within-participant comparison of the vitiligo–healthy skin reflectance contrasts obtained at incidence angles of 20° and 60° showed only small differences between angles across the ET-100 spectral bands. Median D values (Δ20°–Δ60°) ranged from −0.0023 to 0.0022. None of the direct angle comparisons reached statistical significance, either before or after Holm correction (all exact two-sided $p \geq 0.12500$; all pHolm ≥ 0.75000). These findings indicate that the magnitude of the vitiligo–healthy skin reflectance contrast did not differ significantly between the two incidence angles in any of the analyzed spectral bands (Table 9).

Table 9. Direct comparison of participant-level vitiligo–healthy skin reflectance contrasts between incidence angles of 20° and 60° measured with the ET-100.

Spectral Band [µm]	Median D	Q1	Q3	Min	Max	N_W	T	Z	*p*	pHolm	r
1.5–2.0	0.0012	−0.0023	0.0075	−0.0050	0.0092	5	5	0.674	0.625	1	0.302
2.0–3.5	−0.0002	−0.0028	0.0083	−0.0040	0.0123	5	6	0.405	0.8125	1	0.181
3.0–4.0	−0.0023	−0.0023	0.0035	−0.0073	0.0075	5	7	0.135	1	1	0.061
4.0–5.0	0.0012	0.0007	0.0077	−0.0028	0.0118	5	3	1.214	0.3125	1	0.543
5.0–10.5	0.0022	−0.0003	0.004	−0.0017	0.0043	5	3	1.214	0.3125	1	0.543
10.5–21.0	0.002	0.0007	0.0027	−0.0002	0.005	5	1	1.753	0.125	0.75	0.784

D = Δ20°–Δ60°, where Δ = V − H; N_W—number of non-zero participant-level differences included in the Wilcoxon signed-rank test; T—Wilcoxon T statistic; Z—standardized Z statistic; *p*—exact two-sided *p*-value; pHolm—Holm-adjusted exact *p* value; r—effect size.

3.5. Short-Term Within-Site Repeatability

Short-term within-site repeatability was assessed for both instruments using three consecutive measurements with probe repositioning between readings. For the SOC 410, repeatability varied across spectral bands, with the pooled repeatability coefficient (RC) ranging from 0.0081 to 0.0420. The observed site-level vitiligo–healthy skin contrasts exceeded the corresponding RC most consistently in the UV and visible ranges: this occurred for all 28 paired sites in the 335–380 nm band, for 25 of 28 pairs (89.3%) at 400–540 nm, for 20 of 28 pairs (71.4%) at 480–600 nm, and for 23 of 28 pairs (82.1%) at 590–720 nm. In these four bands, all contrasts exceeding RC were positive, indicating higher reflectance of vitiligo-affected skin. In the three longer-wavelength SOC 410 bands, |Δ| exceeded RC in 10 of 28 pairs (35.7%) in each band, and the direction of these contrasts was less consistent (Tables 10 and 11).

Table 10. Short-term within-site repeatability of SOC 410 reflectance measurements with probe repositioning.

Spectral Band [nm]	Skin	RSD Me [%]	RSD Q1 [%]	RSD Q3 [%]	RSD Min [%]	RSD Max [%]	Sw	RC	n Sites
335–380	Healthy skin	4.39	2.61	7.08	0.88	34.59	0.0089	0.0247	28
	Vitiligo	4.16	2.38	5.92	0.55	22.65	0.0171	0.0473	28
400–540	Healthy skin	1.87	1.31	2.50	0.22	5.77	0.0074	0.0204	28
	Vitiligo	2.10	1.15	4.92	0.31	6.57	0.0136	0.0376	28
480–600	Healthy skin	3.04	1.48	3.86	0.21	7.37	0.0121	0.0335	28
	Vitiligo	3.18	1.72	5.01	0.41	9.57	0.0177	0.0490	28

Table 10. *Cont.*

Spectral Band [nm]	Skin	RSD Me [%]	RSD Q1 [%]	RSD Q3 [%]	RSD Min [%]	RSD Max [%]	Sw	RC	n Sites
590–720	Healthy skin	1.26	0.82	1.56	0.17	3.91	0.0078	0.0216	28
	Vitiligo	1.34	0.50	2.20	0.33	6.64	0.0131	0.0362	28
700–1100	Healthy skin	0.71	0.53	1.51	0.20	2.51	0.0063	0.0174	28
	Vitiligo	1.15	0.61	2.13	0.20	3.83	0.0097	0.0269	28
1000–1700	Healthy skin	0.95	0.72	1.58	0.19	5.04	0.0046	0.0127	28
	Vitiligo	1.07	0.59	1.58	0.12	5.95	0.0052	0.0145	28
1700–2500	Healthy skin	1.78	1.24	3.57	0.45	8.91	0.0029	0.0079	28
	Vitiligo	2.68	1.34	4.50	0.50	10.83	0.0030	0.0083	28

RSD—relative standard deviation; Me—median; Q1—first quartile; Q3—third quartile; Sw—pooled within-site standard deviation; RC—repeatability coefficient.

Table 11. Site-level vitiligo–healthy skin reflectance contrasts relative to SOC 410 short-term repeatability.

Spectral Band [nm]	Pooled Sw	Pooled RC	Median Δ	Median |Δ|	n |Δ| > RC	% |Δ| > RC	n Δ > RC	n Δ < −RC	n Paired Sites
335–380	0.0136	0.0378	0.1730	0.1730	28	100.00	28	0	28
400–540	0.0109	0.0303	0.0855	0.0855	25	89.29	25	0	28
480–600	0.0151	0.0420	0.0690	0.0690	20	71.43	20	0	28
590–720	0.0108	0.0298	0.0695	0.0695	23	82.14	23	0	28
700–1100	0.0082	0.0226	0.0085	0.0170	10	35.71	8	2	28
1000–1700	0.0049	0.0136	−0.0025	0.0090	10	35.71	2	8	28
1700–2500	0.0029	0.0081	−0.0040	0.0045	10	35.71	1	9	28

Δ = V − H; Sw—pooled within-site standard deviation; RC—repeatability coefficient. The comparison |Δ| > RC is descriptive and does not represent a statistical significance test.

For the ET-100, site-level vitiligo–healthy skin contrasts were generally small relative to short-term measurement variability at both incidence angles. Depending on the spectral band, |Δ| exceeded RC in 0–43.5% of paired sites at 20° and in 0–47.8% at 60°. Where RC was exceeded, negative contrasts predominated in most bands, indicating lower reflectance of vitiligo-affected skin than of adjacent healthy skin. Descriptively, the comparison of reflectance contrasts with the pooled RC indicated more consistent differences between vitiligo-affected and healthy skin in the UV–visible SOC 410 measurements than in the infrared ET-100 measurements. Comparisons of |Δ| with RC were descriptive and were not interpreted as statistical significance tests because multiple measurement sites were clustered within individual participants (Tables 12–14). Furthermore, because the pooled RC was derived from individual repeated readings, it does not directly represent the repeatability limit of contrasts calculated from triplicate means. Therefore, the proportions of paired sites with |Δ| exceeding RC should be interpreted solely as descriptive indicators of contrast magnitude relative to short-term measurement variability.

Table 12. Short-term within-site repeatability of ET-100 reflectance measurements at an incidence angle of 20°.

Spectral Band [μm]	Skin	RSD Me [%]	RSD Q1 [%]	RSD Q3 [%]	RSD Min [%]	RSD Max [%]	Sw	RC	n Sites
1.5–2.0	Healthy skin	2.90	1.48	3.74	0.64	12.01	0.0039	0.0108	23

Table 12. *Cont.*

Spectral Band [µm]	Skin	RSD Me [%]	RSD Q1 [%]	RSD Q3 [%]	RSD Min [%]	RSD Max [%]	Sw	RC	n Sites
	Vitiligo	1.86	0.94	3.34	0.00	9.75	0.0028	0.0078	23
2.0–3.5	Healthy skin	2.56	1.95	4.98	1.14	11.06	0.0026	0.0073	23
	Vitiligo	2.84	1.31	4.06	0.00	9.12	0.0020	0.0057	23
3.0–4.0	Healthy skin	12.22	7.78	17.67	1.71	33.58	0.0061	0.0168	23
	Vitiligo	13.16	7.36	16.82	2.09	31.24	0.0057	0.0157	23
4.0–5.0	Healthy skin	4.09	2.58	5.67	1.17	9.58	0.0020	0.0056	23
	Vitiligo	3.88	2.22	5.34	0.00	10.15	0.0019	0.0053	23
5.0–10.5	Healthy skin	3.46	1.48	4.72	0.00	6.86	0.0015	0.0041	23
	Vitiligo	4.41	3.03	5.72	0.00	8.49	0.0018	0.0051	23
10.5–21.0	Healthy skin	5.09	2.48	5.56	1.43	9.41	0.0019	0.0053	23
	Vitiligo	4.56	2.94	6.93	0.00	11.62	0.0022	0.0061	23

RSD—relative standard deviation; Me—median; Q1—first quartile; Q3—third quartile; Sw—pooled within-site standard deviation; RC—repeatability coefficient.

Table 13. Short-term within-site repeatability of ET-100 reflectance measurements at an incidence angle of 60°.

Spectral Band [µm]	Skin	RSD Me [%]	RSD Q1 [%]	RSD Q3 [%]	RSD Min [%]	RSD Max [%]	Sw	RC	n Sites
1.5–2.0	Healthy skin	1.56	1.18	3.41	0.34	5.77	0.0037	0.0103	23
	Vitiligo	1.45	0.92	3.32	0.43	9.49	0.0046	0.0128	23
2.0–3.5	Healthy skin	2.74	1.20	5.49	0.44	7.60	0.0036	0.0099	23
	Vitiligo	2.33	1.38	5.28	0.00	12.72	0.0038	0.0104	23
3.0–4.0	Healthy skin	9.43	6.57	13.48	0.87	17.90	0.0075	0.0207	23
	Vitiligo	7.84	5.14	15.36	0.00	26.32	0.0080	0.0221	23
4.0–5.0	Healthy skin	3.18	1.97	5.36	0.43	10.54	0.0035	0.0097	23
	Vitiligo	2.74	1.43	5.21	0.46	13.62	0.0033	0.0090	23
5.0–10.5	Healthy skin	3.78	2.25	4.87	0.77	10.31	0.0034	0.0095	23
	Vitiligo	2.60	1.72	4.32	0.00	10.61	0.0028	0.0078	23
10.5–21.0	Healthy skin	3.83	2.26	6.92	0.49	11.10	0.0038	0.0105	23
	Vitiligo	3.23	1.60	5.07	0.84	11.81	0.0032	0.0089	23

RSD—relative standard deviation; Me—median; Q1—first quartile; Q3—third quartile; Sw—pooled within-site standard deviation; RC—repeatability coefficient.

Table 14. Site-level vitiligo–healthy skin reflectance contrasts relative to ET-100 short-term repeatability at incidence angles of 20° and 60°.

Incidence Angle	Spectral Band [µm]	Pooled RC	Median Δ	Median |Δ|	n |Δ| > RC	% |Δ| > RC	n Δ > RC	n Δ < −RC	n Paired Sites
20°	1.5–2.0	0.0095	−0.0030	0.0040	8	34.78	1	7	23
20°	2.0–3.5	0.0065	−0.0030	0.0050	9	39.13	1	8	23
20°	3.0–4.0	0.0162	0.0000	0.0040	0	0.00	0	0	23
20°	4.0–5.0	0.0054	−0.0010	0.0040	10	43.48	3	7	23
20°	5.0–10.5	0.0046	−0.0010	0.0020	6	26.09	1	5	23
20°	10.5–21.0	0.0057	−0.0010	0.0020	5	21.74	1	4	23
60°	1.5–2.0	0.0116	−0.0080	0.0110	10	43.48	0	10	23
60°	2.0–3.5	0.0102	−0.0020	0.0080	11	47.83	2	9	23
60°	3.0–4.0	0.0214	−0.0010	0.0100	0	0.00	0	0	23

Table 14. *Cont.*

Incidence Angle	Spectral Band [μm]	Pooled RC	Median Δ	Median |Δ|	n |Δ| > RC	% |Δ| > RC	n Δ > RC	n Δ < −RC	n Paired Sites
60°	4.0–5.0	0.0094	−0.0010	0.0090	10	43.48	2	8	23
60°	5.0–10.5	0.0087	−0.0010	0.0060	10	43.48	3	7	23
60°	10.5–21.0	0.0098	−0.0030	0.0060	5	21.74	1	4	23

Δ = V − H; RC—repeatability coefficient. The comparison |Δ| > RC is descriptive and does not represent a statistical significance test.

4. Discussion

The directional-hemispherical reflectance (DHR) method constitutes a valuable tool for assessing the optical properties of skin, enabling quantitative characterization of the manner in which it reflects electromagnetic radiation over a broad spectral range. The value of skin reflectance depends on its structure and on the presence of the principal chromophores, such as melanin, hemoglobin, and water, which determine the way in which electromagnetic radiation interacts with the tissue. In view of the above, it was hypothesized that the DHR method may be a useful tool in assessing vitiligo-related changes in the optical properties of skin.

In the present study, the directional-hemispherical reflectance of vitiligo-affected skin and of directly adjacent skin with normal pigmentation was assessed over a broad spectral range, from 335 nm to 21 μm. The largest differences between the examined areas were found in the ultraviolet (UV) and visible light (VIS) ranges, with the largest median difference and the largest effect size estimate recorded for the 335–380 nm band. Although the exact two-sided Wilcoxon signed-rank tests yielded nominally significant results in the 335–720 nm bands ($p = 0.03125$ for each band), none remained statistically significant after correction for multiple testing (pHolm = 0.21875). In the 700–1700 nm range the differences became attenuated, whereas in the 1700–2500 nm band a small negative median difference was observed. Emissometric measurements demonstrated considerably smaller differences and no statistically significant differences between vitiligo-affected and adjacent healthy skin remained at either incidence angle after correction for multiple testing.

The higher reflectance of vitiligo lesions in the UV and VIS ranges is consistent with the optical properties of melanin, which is one of the principal chromophores of the epidermis. Its absorption encompasses a broad range of wavelengths, with particularly strong interaction in the ultraviolet and visible parts of the spectrum [23–25]. Zonios et al. [25] demonstrated that the in vivo absorption spectrum of melanin declines exponentially with increasing wavelength, which translates into weaker absorption at longer wavelengths. In vitiligo, the absence or substantial reduction in melanin content therefore decreases the absorption of radiation, which may lead to an increase in its reflection. This mechanism may contribute to the direction of the differences observed in the present study and to their greater magnitude in the lower spectral bands.

The observed attenuation of the differences between vitiligo-affected skin and healthy skin with increasing wavelength corresponds to the diminishing contribution of melanin to the optical response of the tissue. Yudovsky and Pilon [26] determined the optical parameters of skin exclusively in the 440–620 nm range, because above 600 nm both melanin and hemoglobin exhibit only weak absorption of radiation, and the reflectance value is determined primarily by light scattering within the tissue. Under such conditions, the measurement result is influenced mainly by the blood volume in the dermis and its oxygenation, the epidermal thickness, and the scattering properties of both skin layers [26,27]. Verdel et al. [27] pointed out that scattering is an individual characteristic: in their study the epidermis scattered light approximately twice as strongly as the dermis, and the adoption

of averaged literature values instead of values determined individually in a given subject led to errors in the estimation of the remaining skin parameters. The absence of significant differences in the 700–1100 nm and 1000–1700 nm bands therefore does not signify the absence of biological differences between the compared areas, but rather indicates that the pigmentary component ceases to predominate over individual variability in scattering, vascularization, and the layered structure of the skin [26–31]. This interpretation is also supported by the observation that melanin content and the structural features of the epidermis remain to a large extent independent. Yudovsky and Pilon [26] found that despite a nearly fourfold difference in melanin concentration between individuals with lightly and heavily pigmented skin (4.41 vs. 17.0 mg/mL on the inner surface of the forearm), the epidermal thickness at the same location remained similar (60.1 vs. 63.8 μm). Verdel et al. [27], in a seasonal observation, demonstrated a marked increase in melanin content during the summer period with practically unchanged epidermal thickness. A change in pigmentation alone may therefore substantially modify reflectance in the UV and VIS ranges without altering the parameters that determine the optical response at longer wavelengths. The pattern of differences observed in the present study, namely a high contrast in the 335–720 nm range and its disappearance in the near-infrared, is consistent with a possible contribution of differences in melanin content. Confirmation of this assumption would, however, require the application of an analysis enabling separation of the contributions of pigmentation, vascularization, and scattering [26,27].

In the 1700–2500 nm band, the direction of the difference was reversed, with slightly lower reflectance values observed for vitiligo-affected skin than for healthy skin. However, this difference was not statistically significant after correction for multiple testing. In this part of the spectrum, absorption associated primarily with water and with the organic components of skin predominates [23,28–31]. This result cannot therefore be interpreted as a simple consequence of pigment loss. It may reflect the contribution of other skin characteristics, such as hydration, the structure of the stratum corneum, the thickness of individual layers, and scattering properties. Since these parameters were not assessed in the present study, the explanation presented should be regarded as a hypothesis requiring verification.

The measurements performed with the emissometer indicate that differences in directional-hemispherical reflectance between vitiligo-affected and adjacent healthy skin were considerably smaller in the infrared than in the UV–VIS range. At an angle of 20°, small negative median differences were observed in the 1.5–2.0 μm and 2.0–3.5 μm bands, indicating a tendency toward lower reflectance of vitiligo-affected skin. However, neither difference was statistically significant, and above 3.0 μm the values for both types of skin were very similar. This is consistent with the high absorption and low reflectance of human skin in the infrared, where the skin behaves as a surface of high emissivity [32–37]. In this range, the radiative response of skin is associated with a greater degree with water, temperature, surface condition, and tissue structure than with pigmentation as such.

At an angle of incidence of 60°, no statistically significant differences between vitiligo-affected and healthy skin were observed in any of the six emissometric bands, either before or after Holm correction for multiple testing. This result is consistent with the observation of Charlton et al. [34], who assessed the influence of constitutive pigmentation on the measured emissivity of skin. That work indicates that pronounced pigmentary differences visible in the optical range need not translate into significant differences in the radiative parameters of skin in the thermal infrared. The high emissivity and correspondingly low reflectance of human skin reported in the literature may contribute to the relatively small differences observed in the present study; however, the respective contributions of melanin and other physical and physiological factors cannot be distinguished from the present broad-band reflectance measurements.

Although reflectance values differed descriptively between measurements obtained at incidence angles of 20° and 60°, the direct within-participant comparison of the vitiligo–healthy skin contrasts did not demonstrate a statistically significant angle-dependent effect in any of the analyzed spectral bands. Nevertheless, measurement geometry remains relevant to the interpretation of infrared reflectance. In an earlier study of healthy skin conducted with the use of the same instruments [38], infrared reflectance values measured at an angle of 60° were systematically higher than those obtained at an angle of 20°, despite the preservation of a similar pattern of differences between anatomical locations. A larger angle of incidence may increase the contribution of surface reflection and the sensitivity of the result to local geometry, roughness, and the positioning of the measuring head relative to the skin. This may partly reduce the relative contribution of subtle tissue differences arising from pigmentation. This interpretation nevertheless requires caution, since the specular and diffuse components were not analyzed separately in the present study.

Reflectance values may also have been influenced by features of the skin surface not directly related to vitiligo. Banyś et al. [39] demonstrated an effect of sebum on the directional reflectance of skin, particularly in selected infrared bands. Other studies employing DHR indicate that results may change under the influence of procedures modifying the surface and structure of the epidermis, such as microdermabrasion [40], UVB therapy of psoriatic lesions [41], and the application of cosmetics or protective preparations [32,42,43]. For this reason, in the present study the vitiligo lesion was compared with directly adjacent healthy skin within the same anatomical region. Such a design limits the influence of differences related to location, environmental exposure, and individual skin characteristics, although it does not eliminate them entirely.

Compared with established reflectance-based approaches to skin pigmentation, such as narrow-band reflectance devices and tristimulus colorimetry, which sample a few wavelengths in the visible range, or diffuse reflectance spectroscopy, which usually covers the visible and near-infrared range and requires model-based decomposition of the spectrum [22,25–27], directional-hemispherical reflectance offers several practical features. First, the hemispherical collection of reflected radiation makes the result largely independent of the angular distribution of the reflected light and therefore less sensitive to local surface geometry than measurements performed with a fixed detection angle. Second, the two instruments jointly cover a broad spectral range from 335 nm to 21 µm, which allows pigment-related contrast in the ultraviolet and visible range to be assessed in the same session as the infrared response relevant to thermography and radiative heat exchange [38–43]. Third, the measurement is a direct, point-based reading traceable to NIST calibration standards, obtained within seconds without image acquisition, controlled lighting, or subsequent image analysis [44,45]. The present results indicate that these advantages are most relevant in the 335–720 nm range, whereas in the infrared the method is unlikely to add diagnostic information on vitiligo beyond that available from the visible range.

The results obtained have potential practical relevance primarily for the development of non-invasive and quantitative methods of assessing vitiligo lesions. The greatest contrast between the depigmented area and normally pigmented skin was found in the 335–720 nm range, and these bands therefore appear potentially useful for quantitative differentiation between vitiligo-affected and normally pigmented skin. Instrumental measurement could complement visual assessment, the outcome of which depends on lighting conditions, skin phototype, and the experience of the examiner. Comparison of the same lesion at successive time points could potentially be useful, but this requires evaluation in longitudinal studies. Repeatable measurements may potentially enable quantitative assessment of the response to phototherapy, topical treatment, or other therapeutic modalities, but this potential application requires prospective longitudinal validation. An analogous approach has already been

applied to monitoring the effects of NB-UVB irradiation in psoriasis [41] and of procedures modifying the epidermis [40].

The data obtained may also support the development of spectral imaging devices and algorithms for automatic segmentation of vitiligo lesions. The bands exhibiting the greatest contrast may be used in selecting wavelengths for multispectral cameras, hyperspectral systems, or simpler portable measuring devices [44,45], which would make it possible to create maps of vitiligo lesions and to assess lesion area and homogeneity more objectively. The results obtained in the infrared, by contrast, indicate that these ranges should not be regarded as a stand-alone method for identifying vitiligo. Their significance appears rather to be complementary and concerns research on the radiative properties of skin, the interpretation of thermography, and the assessment of whether depigmentation influences assumptions regarding skin emissivity (which, for an opaque medium such as skin in this spectral range, is approximated by $\varepsilon \approx 1 - R$).

The results presented are not, however, sufficient to regard DHR as a diagnostic method for vitiligo. The study has several limitations. Above all, it encompassed a small group of six patients, despite the fact that 28 measurement pairs were obtained, and individual measurements performed in the same subject cannot be treated as fully independent biological observations. The small number of participants limits the generalizability of the results and justifies designating the study as preliminary. An additional limitation was the diversity of anatomical locations of the lesions, since skin reflectance also depends on the tissue and surface characteristics mentioned above [26,27,39,46]. The fixed measurement order, with healthy skin always assessed before vitiligo-affected skin, may also have introduced an order-related bias. Although the probe was gently applied by the same operator, contact pressure was not quantitatively controlled, potentially contributing to measurement variability. In addition, all participants had Fitzpatrick skin phototypes I–II, which limits the generalizability of the findings to individuals with darker skin phototypes. Furthermore, although skin phototype and disease duration were recorded (Table 1), the small number of participants precluded their use as stratification variables; the analysis also did not take into account a formal quantitative measure of vitiligo activity beyond the clinical stability criterion used for study inclusion, degree of depigmentation, skin hydration, or epidermal thickness. Prior to any potential clinical application, it is therefore necessary to confirm these observations in a larger and more diverse group of patients. Further studies should confirm short-term repeatability in larger samples and assess intersession and interoperator reproducibility, as well as determine the sensitivity and specificity of the measurements and establish reference values taking into account skin phototype, anatomical location, vitiligo duration and activity, and the stage of treatment. It would also be worthwhile to combine DHR with other non-invasive imaging and spectroscopic methods [27,44,45], which would enable simultaneous assessment of pigmentation, vascularization, and skin structure.

In summary, vitiligo-affected skin showed a consistent descriptive pattern of higher reflectance than adjacent healthy skin in the ultraviolet and visible ranges, with the contrast diminishing with increasing wavelength. At longer wavelengths, the differences became smaller and less consistent, with small negative median differences observed in the 1000–1700 nm and 1700–2500 nm bands. In the infrared ranges measured with the ET-100, differences between vitiligo-affected and adjacent healthy skin were small and no spectral band showed a statistically significant difference either before or after Holm correction for multiple testing. Direct comparison of the reflectance contrasts obtained at incidence angles of 20° and 60° likewise showed no statistically significant angle-dependent effect. Owing to the preliminary character of the study and the small number of participants, these observations require confirmation in a larger population. The descriptive comparison with the repeatability coefficient (RC) indicated that, in the 335–720 nm range, the local

vitiligo–healthy skin contrasts exceeded the pooled RC in the majority of paired sites and were consistently directed toward higher reflectance of vitiligo-affected skin. However, because RC was derived from individual repeated measurements rather than contrasts calculated from triplicate means, these findings should not be interpreted as demonstrating that the observed contrasts exceed their corresponding repeatability limits.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app162311684/s1.

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Abbreviations

The following abbreviations are used in this manuscript:

2D	Two-dimensional
DHR	Directional-hemispherical reflectance
H	Healthy skin
IQR	Interquartile range
Mdn	Median
NB-UVB	Narrowband ultraviolet B
NIR	Near-infrared
NIST	National Institute of Standards and Technology
Q1	First quartile
Q3	Third quartile
SD	Standard deviation
UV	Ultraviolet
UVB	Ultraviolet B
V	Vitiligo-affected skin
VASI	Vitiligo Area Scoring Index
VES	Vitiligo Extent Score
VIS	Visible light

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