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Multispectral short-wave infrared imaging for characterization of skin fluid content independent of skin pigmentation

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ABSTRACT

Clinical assessment of inflammatory skin disease relies heavily on visual cues such as erythema, yet this sign is often less conspicuous in darkly pigmented skin because epidermal melanin attenuates visible reflectance and reduces lesion–background contrast. Short-wave infrared (SWIR, 1000–1700 nm) imaging probes a spectral window in which water absorption is strong and melanin absorption is weak, suggesting a pigment-insensitive approach to visualizing inflammation-associated dermal fluid. Here we develop a portable cross-polarized multispectral visible-to-SWIR imaging system and pseudocolor reconstruction method to enhance fluid contrast. In 24 healthy volunteers spanning a wide range of objectively measured pigmentation (individual typology angle ITA -46° to $+45^{\circ}$), visible reflectance varied strongly with pigmentation, whereas SWIR reflectance was largely independent of skin tone. Following intradermal saline injection as a model of localized inflammation-associated dermal fluid, pseudocolor SWIR imaging markedly increased lesion-to-background contrast compared with co-registered color photography (ΔE 50.2 ± 16.7 vs 2.00 ± 1.35 ; $p = 5.9 \times 10^{-13}$) without correlation between SWIR contrast and pigmentation. In pilot subjects with acne, SWIR imaging enhanced lesion visibility across pigmentation levels and revealed clinically occult inflammation in darkly pigmented skin. These findings establish multispectral SWIR imaging as a quantitative, pigment-insensitive strategy for visualizing cutaneous inflammation.

INTRODUCTION

Inflammatory skin diseases like acne, atopic dermatitis, psoriasis, hidradenitis suppurativa, and urticaria affect up to 30% of the global population and collectively contribute to skin disease being the 4th leading cause of disability worldwide¹⁻⁶. These diseases can significantly impair an affected individual's quality of life and are associated with pain, itching, sleep disturbances, social stigmatization, and increased anxiety, depression, and suicidality^{2,5-8}. Furthermore, because the stigma associated with skin disease leads many affected individuals to avoid seeking care, the overall global burden is likely underestimated⁶. For those who do seek care, accurate diagnosis and assessment of disease severity are critical to ensure appropriate and effective therapy.

Skin inflammation is often characterized pathophysiologically by cytokine-mediated vasodilation, resulting in an increase in tissue water and hemoglobin content⁹⁻¹¹. While the low optical absorption of water renders it effectively transparent in the visible range, hemoglobin's strong optical absorption results in inflamed skin appearing more red, which clinically is described as erythema^{12,13}. Along with changes in skin texture and changes in pigmentation, erythema is a key visual feature of inflammatory skin disease that clinicians rely upon to diagnose skin disease and assess severity. These disease-associated changes in skin appearance are also the basis for standardized visual scoring systems that are often employed in clinical trials to achieve reproducible and semi-quantitative measures of disease.

While these visual approaches are effective diagnostic tools for some patients, they are inherently subjective and fail to meet the needs of diverse patients across the full spectrum of human pigmentation. The principal cause of this failure is that the appearance of skin disease is often visually more subtle in darkly-pigmented skin than in lightly-pigmented skin. Epidermal melanin, which is increased in people with darkly-pigmented skin, has strong absorption in the visible range¹². Thus for people with darkly-pigmented inflamed skin, melanin absorbs a significant amount of light interacting with skin and decreases the contrast between affected skin and healthy unaffected skin. For example, erythema, which is a key marker of numerous inflammatory skin diseases, is more violaceous than red and can be so subtle in pigmented skin that it is overlooked during clinical examination^{7,14-18}. This can result in the misdiagnosis of chronic inflammatory skin

conditions, leading to prolonged patient suffering from a treatable disease^{7,15,16,18,19}. Because erythema is a core component of standardized, semi-quantitative disease severity scoring systems, difficulty assessing erythema in pigmented skin leads to underestimation of skin disease severity in patients with darkly pigmented skin²⁰⁻²³. This underestimation may contribute to undertreatment of disease in a patient population that disproportionately experiences medical marginalization^{14,15,18,24}.

Optical imaging modalities, such as digital photography and multispectral reflectance imaging, have the potential to enable objective and noninvasive assessment of skin disease that overcomes the limitations of subjective visual assessment²⁵⁻²⁸. Multispectral reflectance imaging is a promising technology that detects disease-associated shifts in tissue chromophore content by leveraging wavelength-dependent differences in optical properties of disease-relevant tissue chromophores^{26,29}. However, because both digital photography and multispectral reflectance imaging typically operate in the visible (VIS) and near infrared (NIR) spectral ranges (400 - 1000nm), where melanin's strong optical absorption effectively masks the ability to detect hemoglobin, these optical modalities do not overcome the challenges of visual assessment with respect to accurate disease assessment in pigmented skin. These limitations motivate the use of spectral regions that are sensitive to inflammation-driven changes in tissue composition yet minimally affected by melanin absorption.

The short-wave infrared (SWIR) is an emerging medical imaging spectral range extending from 1000 to 1700 nm where, unlike the visible and near infrared range, water and lipid exhibit strong optical absorption while melanin and hemoglobin exhibit relatively weak optical absorption^{27,30-34}. In addition, the reduced optical scattering afforded by longer wavelengths supports increased depth of imaging in the SWIR compared to visible and near infrared imaging^{35,36}. The strong optical absorption of water and weak optical absorption of melanin in the SWIR suggest that SWIR imaging should be both sensitive to inflammation-associated shifts in tissue fluid content and insensitive to skin pigmentation. Thus, compared to imaging modalities operating below 1000 nm, SWIR imaging may offer an objective and quantitative method for the diagnosis and management of skin disease across the full spectrum of human pigmentation.

Prior studies in animals and humans have evaluated SWIR imaging and spectroscopic systems for both cutaneous and non-cutaneous clinical applications. Non-cutaneous applications include SWIR spectroscopic assessment of lung injury³⁷ endoscopic detection of cerebrospinal fluid leaks³⁸ as well as detecting pupil size through closed eyelids³⁹. Cutaneous applications include face detection⁴⁰, assessment of skin structure and optical properties^{25,41}, skin cancer⁴², scleroderma⁴³, bruises⁴⁴, burns⁴⁵, skin water and lipid content⁴⁵⁻⁴⁷, skin allergy testing⁴⁸, and allergic contact dermatitis⁴⁹. However, these prior SWIR imaging systems have either been limited to single wavelength grayscale images or relied on bulky research-grade multispectral and hyperspectral systems that would be challenging to implement in the clinical setting. Furthermore, previous studies have failed to include a representative number of darkly pigmented skin (in many cases, including none), or have not directly tested the impact of skin pigmentation on SWIR imaging. To overcome these limitations, we developed a novel, portable, clinic-ready multispectral imaging system sensitive to wavelengths ranging from 400-1700 nm that generates both qualitative and quantitative visualization of tissue fluid content. We modeled the inflammation-associated increase in skin fluid content by injecting normal saline intradermally in human subjects with pigmentation ranging from objectively-determined light to dark pigmentation. We hypothesized that, unlike imaging below 1000 nm, multispectral SWIR imaging would enable high-contrast visualization of tissue fluid in subjects across the pigmentation spectrum. We then tested whether this approach could reveal visually subtle acne lesions in darkly pigmented skin.

RESULTS

Multispectral VIS-SWIR Imaging Device Characterization

We constructed a portable cross-polarized multispectral imaging device consisting of an InGaAs camera sensitive from 400 nm – 1700 nm combined with a 25 mm focal length lens, a motorized filter wheel with 6 bandpass filters including visible, near infrared, and SWIR wavelengths, polarization filter in front of the lens, and symmetrically positioned broad spectrum halogen lamps with polarization filters oriented orthogonally to the lens polarization filter (Figure 1a-b). The halogen lamps demonstrated a broad spectrum from VIS to SWIR with peak intensity at 1075 nm (Figure 1c). The polarization filter induced a shift in the illumination spectrum, with the peak intensity shifting to 1095.7 nm and 5-10% increased attenuation centered at 1175 nm and 1400 nm. With the imaging and illumination assembly positioned 20 cm from the imaging plane, peak surface irradiance was 12 $\mu\text{W}/\text{cm}^2$, 210 $\mu\text{W}/\text{cm}^2$, 459 $\mu\text{W}/\text{cm}^2$, 181 $\mu\text{W}/\text{cm}^2$, 249 $\mu\text{W}/\text{cm}^2$,

and $226 \mu\text{W}/\text{cm}^2$ with the 540 nm, 650 nm, 850 nm, 1200 nm, 1300 nm, and 1600 nm bandpass filters respectively, (Supplemental Figure S1). With a >99% reflectance Spectralon target placed at the imaging plane, the peak 12-bit pixel intensity was found to linearly increase with exposure time for each of the 6 bandpass filters (Supplemental Figure S2). Plotting normalized pixel intensity (relative to saturation intensity of 4095) vs exposure time for each bandpass filter reveals filter-specific exposure times that achieve constant pixel intensity across all bandpass filters (Figure 1d). For example, to achieve a relative intensity of 80% of pixel saturation, the exposure times range from 34 ms for the 850 nm filter to 1230 ms for 540 nm filter. At an imaging distance of 0.2 m, the field of view was 6.5 cm x 5.2 cm. The Spectralon target reflectance image at each bandpass filter defined a filter-dependent flat field correction matrix. Applying this flat field correction matrix corrected for spatial inhomogeneities in the illumination source (Figure 1e).

Before testing the multispectral functionality of this system, a proof of principle study was performed at a single SWIR wavelength to confirm that intradermal saline injection could be detected. In a healthy subject with objectively determined light pigmentation, injection of 0.2 mL of normal saline on the ventral forearm (Figure 2A) resulted in an initial visible blanching effect where the area of injection appears less red than surrounding skin. Blanching dissipated within 40 seconds with gentle massage of the injection site. In the SWIR 30 frames per second (fps) camera stream with 1300 ± 12 nm bandpass filter in place, the normal saline injection appears as a darker area compared to surrounding skin (indicating increased 1300 nm absorption), and this contrast difference remained visible even after blanching resolved (Figure 2B, Supplemental Video 1). This initial study confirmed that the device was capable of detecting intradermal fluid directly at a single SWIR wavelength.

With the initial validation study demonstrating the feasibility of detecting intradermally-administered fluid, we next tested the hypothesis that SWIR imaging would provide high contrast visualization in a pigment-insensitive manner by imaging intradermal saline in a group of 24 healthy volunteers with skin pigmentation ranging from light to dark pigmentation. While the initial proof of principle involved a single SWIR wavelength, we hypothesized that tissue fluid contrast could be further enhanced with the inclusion of multiple SWIR wavelengths.

Multispectral VIS-SWIR Imaging of Skin in Subjects with Light to Dark Pigmentation

We first explored how differences in skin pigmentation across the study volunteers impacted the reflected intensity of skin images acquired from visible to SWIR prior to injection of fluid. Pigmentation was measured objectively at the dorsal forearm with the spectrophotometer-derived individual typography angle (ITA), which has been used as a surrogate for melanin content^{50,51}. Subjects' ITA values ranged from -46° to $+45^\circ$. Applying established ITA pigmentation categories from Del Bino et al⁵⁰, half of the subjects ($n=12$) comprised a group of subjects with darker pigmentation (3 'dark', 9 with 'brown' pigmentation) and the remaining half had lighter pigmentation (5 'tan', 4 'intermediate', and 2 with 'light' pigmentation) (Figure 3A). The spectrophotometer-derived melanin index demonstrated an expected monotonic decrease (from approximate 2.2 to 0.6) when sorting subjects from darker to lighter pigmentation by ITA, and the spectrophotometer hemoglobin index, a measure of skin erythema, had similar values between lightly and darkly pigmented subjects though exhibited more variation in subjects with lighter pigmentation (Supplemental Figure 3).

Prior to intradermal saline injection, baseline images were acquired at the injection site with a visible camera under room light illumination and with the multispectral VIS-SWIR camera (Figure 3B provides a study schematic). Figure 3C shows white light (iPhone) images and multispectral images of skin at each imaging wavelength prior to intradermal saline injection from representative subjects with dark ($\text{ITA} = -46^\circ$) and light ($\text{ITA} = 43^\circ$) pigmentation. To facilitate visual comparison of image intensities between subjects, the images at each wavelength in the darkly pigmented subject are scaled by a constant multiplicative factor that is derived by taking a ratio of image intensities at 850 nm, thereby ensuring equal image intensities at 850 nm between the two representative subjects. With this image transform applied, it is visually apparent that while the SWIR images appear to have similar relative intensities between lightly and darkly pigmented subjects, at visible wavelengths relative image intensities are lower in the darkly pigmented subject.

To facilitate quantitative comparison of reflected intensity across subjects, we defined a near-infrared-normalized reflectance fraction (RF_{NIR}). For each subject and wavelength, RF_{NIR} was calculated by dividing the mean intensity of unaffected skin at that wavelength by the mean intensity of unaffected skin in the 850 nm image. Values greater than 1 indicate higher reflectance than at 850 nm. In Figure 3D, RF_{NIR} at each imaging wavelength is averaged across the 12

subjects with darker pigmentation (ITA range of -46° to 10°) and the 12 subjects with lighter pigmentation (ITA range of 10° to 45°).

At the visible wavelengths (540 and 650 nm), RF_{NIR} was significantly lower in the darker-pigmented group than in the lighter-pigmented group (ITA $<10^{\circ}$ vs ITA $>10^{\circ}$ $RF_{NIR,540nm} = 0.83 \pm 0.42$ vs. 1.31 ± 0.49 , $p_{540nm} = 0.008$; $RF_{NIR,650nm} = 1.12 \pm 0.33$ vs. 1.54 ± 0.30 , $p_{650nm} = 0.002$), indicating that visible reflectance is strongly dependent on skin pigmentation. In contrast, at the SWIR wavelengths (1200, 1300, and 1600 nm), RF_{NIR} did not differ between darker- and lighter-pigmented subjects (ITA $<10^{\circ}$ vs ITA $>10^{\circ}$ $RF_{NIR,1200nm} = 0.62 \pm 0.15$ vs. 0.57 ± 0.11 , $p_{1200nm} = 0.82$; $RF_{NIR,1300nm} = 0.69 \pm 0.15$ vs. 0.64 ± 0.11 , $p_{1300nm} = 0.81$; $RF_{NIR,1600nm} = 0.31 \pm 0.21$ vs. 0.22 ± 0.13 , $p_{1600nm} = 0.91$). Taken together, these findings show that in darkly pigmented skin, visible light reflectance is suppressed relative to near-infrared reflectance, whereas in lightly pigmented skin, visible reflectance exceeds near-infrared reflectance. By contrast, SWIR reflectance is essentially independent of baseline pigmentation, highlighting the pigment-insensitive behavior of SWIR wavelengths.

Five of the subjects had visible brown spots on their skin, consistent with the clinical diagnosis of lentigo (a freckle) or melanocytic nevus (a brown mole) within the field of view. Figure 4A shows visible and multispectral images from one of these subjects (ITA = 7°). These lesions appear brown compared to adjacent skin in typical visible photographs because they contain more melanin than adjacent skin. At the 540 nm, 650 nm, and 850 nm multispectral images, these lesions appear as dark spots (higher absorption) but do not appear to be distinguishable from background skin in the SWIR wavelengths, which is consistent with SWIR imaging being insensitive to pigmentation due to lower absorption of light by melanin at longer wavelengths.

Multispectral SWIR Skin Imaging of Tissue Fluid and Acne in Subjects with Light to Dark Pigmentation

Next we sought to assess whether the detection of dermal fluid by SWIR imaging was impacted by the degree of skin pigmentation by imaging the dorsal forearms of all 24 subjects with the multispectral VIS-SWIR system every minute for 10 minutes after intradermal injection of normal saline (study schematic in Figure 3B). Consistent with our proof of principle study (Figure 2), in six subjects (1 of 12 with ITA $<10^{\circ}$ darker pigmentation and 5 of 12 with ITA $>10^{\circ}$ lighter pigmentation) the injection resulted in visible skin blanching due to local pressure-induced occlusion of dermal capillary vessels that dissipated after a few minutes. In these cases, the 650 nm and 540 nm images demonstrated higher intensity at the site of injection (consistent with loss of blood) compared to the surrounding unaffected skin (Figure 4B-C). In 13 subjects there was minimal bleeding at the site of injection, which appeared most prominently as a dark spot in the 540 nm images near the injection site but was less prominent or absent at other wavelengths, consistent with strong oxyhemoglobin absorption at 540 nm and relatively weak absorption at the remaining wavelengths (Figure 4B). Across all subjects (with or without blanching) evidence of increased light absorption by fluid at SWIR wavelengths, consisting of darker circular areas within the SWIR images, was present at all time points after injection (Figure 4C).

Intradermal saline was well visualized at SWIR wavelengths for all subjects ranging from light to dark pigmentation, and Figure 5A-B shows representative post injection, visible and multispectral images for subjects with dark (ITA = -46°) and light (ITA = 43°) pigmentation. Notably, the subject with objectively determined dark pigmentation had a VIS photograph that appeared artificially lighter than what was observed by eye – this is a known limitation⁵² of photography and highlights the need for objective colorimetry. To further enhance the visualization of intradermal saline, SWIR-pseudocolor images were generated where pixel-by-pixel RGB values were set to be equal to the pixel-by-pixel intensities from the spectrally-encoded (relative to 850 nm) and contrast-enhanced 1200, 1300, and 1600 nm images respectively (Figure 5C). Figure 5D-E compares the SWIR-pseudocolor images of intradermal saline with coaligned visible photographs 1 minute after injection for the subjects in Figure 5A-B, and it is evident that while in visible photographs the site of fluid injection and surrounding unaffected skin are difficult to distinguish visually, in the SWIR-pseudocolor images there is an obvious perceptual difference between the intradermal fluid and unaffected skin.

To quantify the extent of the visual contrast in the SWIR-pseudocolor images, the CIELAB color space difference ΔE was calculated between the saline injection site and a ring of surrounding unaffected skin ($\Delta E_{injection_vs_unaffected}$) for both SWIR pseudocolor as co-registered visible color images. Figure 6A shows pre-injection SWIR pseudocolor images ($t=0$) and coregistered SWIR pseudocolor and visible images obtained at $t=5$ minutes after injection for each subject, ordered from lowest to highest ITA. Figure 6B plots $\Delta E_{injection_vs_unaffected}$ at $t=5$ minutes for both modalities against subject ITA. Consistent with the representative subjects in Figure 5, the intradermal fluid injection site was difficult to distinguish from

surrounding skin in visible images but was clearly delineated in SWIR-pseudocolor images (Figure 6A). Accordingly, $\Delta E_{\text{injection_vs_unaffected}}$ was significantly higher in SWIR-pseudocolor images compared to visible images across all subjects (Figure 6C, mean $\pm$ standard deviation of 50.2 ± 16.7 vs. 2.00 ± 1.35 , paired t-test $p = 5.9 \cdot 10^{-13}$). There was no correlation between SWIR-pseudocolor $\Delta E_{\text{injection_vs_unaffected}}$ values and ITA (spearman $\rho = 0.05$, $p = 0.81$), indicating that SWIR detection of dermal fluid is minimally affected by skin pigmentation.

To evaluate the ability of the multispectral SWIR system to detect cutaneous inflammation, two additional volunteers (one with light pigmentation ITA = 53° and one with dark pigmentation ITA = -53°) with facial acne were imaged. On visible imaging, acne lesions appeared as red papules that were faintly distinguishable from surrounding skin ($\Delta E_{\text{acne_vs_background}} = 8.4$) in the lightly pigmented subject and as skin-colored papules that were difficult to distinguish from background skin ($\Delta E_{\text{acne_vs_background}} = 3.9$) in the darkly pigmented subject (Figure 7A-B red arrows). In contrast, for both subjects, acne lesions were clearly delineated in SWIR-pseudocolor images (Figure 7C-D red arrows), with over a 12-fold increase in contrast for the darkly pigmented subject ($\Delta E_{\text{acne_vs_background}} = 47$) and a nearly 6-fold increase for the lightly pigmented subject ($\Delta E_{\text{acne_vs_background}} = 50$). Notably, the darkly pigmented subject reported an area of discomfort and fullness on his cheek that was adjacent to his visible acne papule. While this region visually lacked features of active disease, it was prominently highlighted in the SWIR pseudocolor image as an area of increased absorption, consistent with underlying cutaneous inflammation associated with severe cystic acne that was clinically masked by dark pigmentation. (Figure 7D black arrow).

DISCUSSION

This study demonstrates that cross-polarized multispectral SWIR imaging enables high-contrast, pigmentation-insensitive visualization of dermal fluid and cutaneous inflammation. First, we compared conventional photography and multispectral SWIR imaging for detection of intradermally administered saline across subjects spanning objectively determined skin pigmentation from light to dark. Intradermally administered saline served as a proxy for inflammation-associated dermal fluid. Imaging across visible, NIR, and SWIR wavelengths revealed distinct wavelength-dependent contrast features. Blood and pigmented lesions were distinguishable from background skin at visible and NIR wavelengths but became indistinguishable at SWIR wavelengths. In contrast, fluid was poorly visualized at visible and NIR wavelengths yet readily detectable at SWIR wavelengths. Reflectance intensity at SWIR wavelengths remained relatively constant across pigmentation levels, whereas visible reflectance decreased with increasing pigmentation. We then developed a multispectral algorithm that combined individual SWIR wavelengths to generate pseudocolor images. Contrast between the site of saline injection and adjacent unaffected skin was significantly greater in SWIR pseudocolor images than in visible photographs, and this enhancement was independent of skin pigmentation. Furthermore, in subjects with inflammatory acne, SWIR imaging significantly enhanced lesion visibility in both lightly and darkly pigmented skin and revealed evidence of symptomatic inflammation that was not readily apparent on visible inspection. Together, these findings support our hypothesis that reduced melanin absorption and increased water absorption at longer wavelengths enable SWIR imaging to provide pigmentation-insensitive assessment of cutaneous inflammation beyond the capabilities of conventional visible and NIR imaging.

This work addresses a critical and well-recognized limitation in dermatology: the reduced visibility of inflammation in darkly pigmented skin. Cutaneous inflammation induces vasodilation and increases in tissue water and hemoglobin. Because hemoglobin strongly absorbs blue wavelengths, cutaneous inflammation often appears red (termed erythema in clinical practice). However, melanin's optical absorption overlaps substantially with hemoglobin and thus attenuates the visual appearance of erythema in pigmented skin. As a result, clinicians often under-recognize or underestimate disease severity in patients with dark skin, leading to misdiagnosis, delayed treatment, and inadequate disease monitoring. Imaging technologies operating in the visible or near-infrared ranges, such as standard digital photography or conventional multispectral reflectance imaging, do not overcome this limitation, as melanin remains a dominant absorber within these spectral regions. In contrast, SWIR imaging exploits a spectral window characterized by reduced melanin absorption and increased water absorption, enabling visualization of inflammation-associated tissue fluid independent of baseline pigmentation.

Prior studies have explored reflectance SWIR imaging for assessment of skin disease. For example, pseudocolor images derived from hyperspectral SWIR systems were shown to outperform conventional visible imaging in visualizing and quantifying cutaneous inflammation in allergic contact dermatitis⁴⁹, and in enhancing bruise detection⁴⁴. However, these studies did not include subjects with darkly pigmented skin and did not evaluate the effect of pigmentation on image

contrast. Similarly, a multispectral imaging study spanning visible, NIR, and SWIR wavelengths in skin cancer included only Caucasian subjects³⁵. Several groups have proposed that reduced melanin absorption in the SWIR may enable pigment-insensitive imaging^{32,48,49}, but this hypothesis has not been systematically tested. Tversky and MacGlashan demonstrated that allergen-induced wheals were well visualized by SWIR imaging at a single wavelength (1400 nm) and reported comparable qualitative results between Caucasian and African American subjects⁴⁸. While this study represents an important early demonstration of SWIR imaging in individuals spanning a wide range of skin pigmentation, reliance on race as a surrogate for pigmentation and lack of objective skin color measurement limit interpretation. Steiner et al.⁴⁰ developed a multispectral pulsed LED-based SWIR system capable of distinguishing human faces from silicone masks. Although fewer than 3% of participants had darkly pigmented skin, the authors observed that differences in skin pigmentation that were prominent in visible images were largely negligible in SWIR images. Building upon these prior efforts, our study is, to our knowledge, the first to systematically evaluate the pigmentation dependence of multispectral SWIR imaging with pseudocolor image reconstruction across a broad spectrum of objectively-measured skin pigmentation.

This work has important limitations. With respect to experimental methods, to test the pigmentation-independence of SWIR imaging across subjects with varying pigmentation, we attempted to introduce a consistent amount of fluid at a consistent depth for each subject. However, given variation in skin thickness and depth of injection, some unavoidable variability in fluid injection occurred across subjects. Injection near a vein could mask the signal, and in some cases, the fluid dissipated more rapidly, possibly due to differences in lymphatic drainage. This variability underlies why injection site exhibited higher contrast in some subjects than in others. Importantly, although SWIR contrast varied across individuals, there was no systematic difference between subjects with light and dark pigmentation. Furthermore, our use of saline injection to model inflammation may be viewed as not fully physiologic given the volume of saline introduced. It is possible that in some cases inflammation may be sufficiently mild that local changes in tissue fluid content are not appreciable even with SWIR imaging. However, the primary purpose of the saline injection studies was to demonstrate pigmentation insensitivity rather than to define the lower limits of inflammation detection. Prior work^{48,49} and the present results with acne subjects suggest that SWIR imaging can detect inflammation-associated fluid in clinically relevant settings, though additional subjects must be studied to confirm these findings. With respect to the device itself, our initial prototype was built to support full-body imaging using a vertical scanning stage and incorporates multiple halogen lamps and a motorized filter wheel. This configuration results in a bulky and relatively slow system, with extended acquisition times due to filter switching and prolonged exposure times at wavelengths where the halogen source is relatively weak. To better support clinical implementation, multispectral acquisition must be sufficiently rapid to minimize motion artifact and avoid prolonged subject immobility during imaging. Use of a multispectral rather than hyperspectral system required preselection of specific wavelengths, and alternative wavelength combinations may further improve tissue fluid contrast. Finally, SWIR-sensitive InGaAs cameras introduces a significant cost which limits accessibility. While InGaAs camera prices have decreased in recent years, emerging approaches such as quantum dot upconversion coupled with conventional silicon detectors may offer a path toward more affordable SWIR imaging sensors⁵³.

Future work will address these limitations. Next-generation iterations of the imaging system will focus on reducing system size, acquisition time, and motion sensitivity to better support clinical implementation. We will expand clinical studies to include additional subjects with acne, as well as patients with inflammatory skin diseases such as atopic dermatitis, hidradenitis suppurativa, and psoriasis, where visual assessment of erythema informs diagnosis and treatment. Importantly, not all cutaneous inflammation results in visible erythema. For example, alopecia areata is characterized by a dermal inflammatory infiltrate without clinically apparent redness. Whether SWIR imaging can detect inflammation in such settings, even in the absence of visible erythema, warrants further investigation. In addition, modeling-based approaches will be used to optimize wavelength selection and refine pseudocolor reconstruction algorithms to enhance clinically meaningful visualization of disease severity.

In summary, we developed a cross-polarized multispectral SWIR imaging system capable of generating pseudocolor images that highlight dermal fluid in a pigment-insensitive fashion. By enabling objective assessment of dermal fluid independent of melanin content, this technology may help address longstanding disparities in dermatologic care and support diagnosis and monitoring of inflammatory skin disease in a manner that performs equally well across the full spectrum of skin pigmentation. Medical devices must be designed and validated to work for all people, and this work adds to a growing body of literature suggesting that SWIR imaging is less prone to pigmentation-related bias than existing imaging modalities.

METHODS

2.1 Multispectral Imaging System Assembly and Characterization

The multispectral imaging system consists of a light source, camera, optical components, and motorized filter wheel mounted to a linear scanning system with each component controlled through custom electronics and a computer running a custom MATLAB script (Figure 1A-B).

The linear scanning components were sourced from two XL C-Beam Tension Linear Actuator Bundles (Open Builds), which consist of 1.5 m long C-Beam linear rails, a lead screw, and 12.5 cm square sliding gantry plates. The lead screws were driven by high torque Nema 23 Stepper Motor (maximum 26 lbs of pull and max speed 5m/min) and a 24 V power source (Openbuilds), and the motors were driven by a DM542T stepper motor driver (Amazon.com). Limit switches (Amazon.com) were added to the ends of the linear rail so that they would be triggered when the gantry plates were at their extreme positions. The linear rails were placed next to each other and mounted vertically to a 24" square optical breadboard (MB2424, Thorlabs) with right angle mounts (VB01B, Thorlabs). In addition, optical poles and bases (TR12, PH6, Thorlabs) were used to add a shelf behind the linear rails on which electronic components and a system computer could be placed. Locking wheels (Amazon.com) were attached to the bottom of the base optical breadboard to enable transporting the system. Motor positioning was controlled via Arduino and interfaced with the MATLAB imaging system over a serial port. Push button homing, vertical positioning, and automatic acquisition functions were integrated in the imaging systems MATLAB graphical user interface (GUI). A 90 degree mounting plate (AP90L) was centered and secured to the 2 moving gantry plates, and a 180° degree adjustable mounting plate (AP180, Thorlabs) was secured to the center of AP90L. Finally the camera, light source, and filter wheel were secured to an optical breadboard, which is coupled to the adjustable mounting plate, allowing the camera to be positioned vertically or horizontally (Figure 1B).

The camera is a global shutter 12-bit thermoelectrically cooled (minimum 5° C) InGaAs camera (Goldeye G-130 VSWIR TEC1, Allied Vision) with quantum efficiency exceeding 50-70% from 400-1650 nm, 1280 x 1024 pixels, pixel pitch of 5µm, maximum frame rate of 94 frames per second at full resolution, and dark current of 4.8 ke⁻/s at 20°C. A 25 mm focal length VIS-SWIR lens (M2518-APVSW Hyper Apo, Computar) was used. Resolution, field of view, and chromatic aberration were evaluated by imaging a 1951 USAF Resolution Target (Edmund Optics) positioned on top of a Spectralon 99% Diffuse Reflectance Target (LabSphere) 20 cm away with multiple bandpass filters ranging from VIS to SWIR (540 nm, 650 nm, 850 nm, 1200 nm, 1300 nm, 1600 nm). The field of view of the 1280 x 1024 image was 6.5x5.3 cm (~51µm/pixel), and the Group 2 Element 3 line pairs were visually distinguishable (G2E3 lines are 99.21 µm thick, corresponding to a width of approximately 2 pixels). Translation matrices between images at each wavelength and the image at 850 nm were determined with the MATLAB *imregcorr* function. Compared to the 850 nm image, images acquired at 540 nm demonstrated approximately 1 pixel offset vertically and less than 1 pixel offset horizontally, and the remaining images demonstrated less than 0.5 pixel offset vertically and horizontally (Figure S4).

A six-position motorized filter wheel was appended to the camera lens (FW102C, Thorlabs). Motor positioning was controlled via serial interface through MATLAB code that incorporated a C++ SDK included with the Thorlabs filter wheel. The filter wheel has 6 positions for bandpass filters. For the initial prototype, we selected three SWIR wavelengths (1200 ± 10 nm, 1300 ± 12 nm, and 1600 ± 10 nm) that were found in prior work to highlight skin inflammation⁴⁹, one NIR wavelength (850 ± 40 nm) that has been used minimize imaging artifacts associated with skin contours¹⁰, and two visible wavelengths (540 ± 10 nm and 650 ± 40 nm) that enable direct comparison of skin reflectance between VIS and SWIR wavelengths. Each filter was sourced from Thorlabs (FB540-10, FBH650-40, FBH-850-40, FBH1200-10, FBH1300-12, and FBH1600-12). The optical density (OD) of each filter from 400-1700 nm was characterized in transmission mode with a tungsten halogen source (HL-2000-HP, Ocean Optics), a broadband multimode fiber (0.5 NA Low OH, 1500µm diameter M107L02, Thorlabs) an OceanHDX spectrometer (Ocean Optics) sensitive from 400-1000 nm and a NIRQuest spectrometer (Ocean Optics) sensitive from 900-1700 nm. The 650 nm and 850 nm filters showed low OD above ~1250 nm, thus necessitating the addition of an OD 6 short pass 950 nm filter (FF01-940/SP-25, Semrock) to ensure that light above 1250 nm was sufficiently blocked (Figure S5).

Illumination was achieved with broadband (400-2500 nm) halogen light sources placed symmetrically around the camera. Each light source consisted of a modified Thorlabs QT lamp (QTH10, Thorlabs). The QTH10 includes a housing, heat sinks, and a 50.8 mm aspheric collimating lens. To increase illumination power, we replaced the 10W bulb in the QTH10

lamp with a 35 W Bi-pin G4 halogen bulb (Amazon.com). To increase the field of illumination, a -75 mm plano concave diverging lens (LC1315, Thorlabs) was positioned in front of the QTH10 housing. To minimize specular reflection, the QTH10 were appended with a 65 mm VIS/SWIR self-adhesive wire grid linear polarizer placed on acrylic sheets (PSA1000-R65, MidOpt). An additional polarization filter (PSA1000-S25, MidOpt) was appended to the filter wheel and oriented perpendicularly to the polarizers placed in front of the light sources. The lamps were positioned on either side of the camera in an epi-illumination orientation and aligned concentrically at a working distance of 0.2 m. Each of the halogen lamps was controlled by an Arduino UNO via a relay module, thus enabling toggling of the light source through MATLAB control of Arduino digital output. Spectral output of the halogen lamps with and without the polarizers was determined by measuring the spectrum with both the OceanHDX and NIRQuest spectrometer in relative mode utilizing a broadband multimode fiber (0.5 NA Low OH, 1500µm diameter M107L02, Thorlabs) and a reference high power tungsten halogen source (HL-2000-HP, Ocean Optics). Spectra were stitched together between OceanHDX and NIRQuest measurements by selecting a spectral range of overlap between both spectrometers (950-1000 nm) and applying a constant gain to the NIRQuest signal to align both spectrometers in the overlap region. Relative intensity was normalized relative to the maximum signal obtained from the unpolarized halogen lamps (Figure 1C).

The peak surface irradiance at a working distance of 20 cm was determined by positioning power sensors (coupled to a Thorlabs PM100D power meter) at a fixed position 20 cm from the halogen lamps, where irradiance was maximized, and placing the system bandpass filters directly onto the power meter. For the 540 nm, 650 nm, and 850 nm bandpass filters a S120C (Thorlabs) photodiode power sensor was used, while the remaining filters required a S122C (Thorlabs) photodiode power sensor. Measurements were obtained with and without the polarizer in place and included both 2 and 4 halogen sources (Figure S1).

The halogen source is not a constant wavelength source, and camera pixel quantum efficiency is also wavelength dependent. To account for these variations, we sought to determine wavelength-specific exposure times that would ensure consistent wavelength-independent pixel intensity. Images of a Spectralon 99% diffuse reflectance target (with near constant reflectance from VIS to SWIR) were obtained at each of the 6 system bandpass filters at exposure times varying from 10 ms to 1500 ms. Peak pixel intensity (relative 12-bit maximum 4095) was determined for each non-saturated image (Figure 1D). These filter-specific images also enabled assessment of illumination uniformity, from which wavelength-dependent flat-field correction intensity matrices were derived (Figure 1E).

2.2 Human Studies

Human studies protocols were approved by the Institutional Review Board at Washington University in St. Louis. First, a single subject was recruited to undergo intradermal saline injection and have a video obtained during injection using both a conventional visible camera and a SWIR-sensitive camera at a single wavelength. Then, twenty-four (n=24) healthy volunteers across the full spectrum of skin tone were recruited for intradermal saline injection and multispectral VIS-SWIR imaging. Finally, an additional 2 subjects (one lightly pigmented and one darkly pigmented) with facial acne lesions were recruited for multispectral VIS-SWIR imaging of their acne lesions.

Colorimetry

For each subject undergoing saline injection, skin tone was subjectively assessed on the dorsal forearm in a blemish-free region with a Konica Minolta CM700d Spectrophotometer (Konica Minolta Sensing Americas, NJ). For subjects undergoing acne imaging, the site of skin tone assessment was an acne-free region of the forehead or cheek. Measurements were repeated in triplicate at each assessment site. The CM700d measures a 10 nm resolution reflectance spectrum from 400-700 nm. CM-SA skin analysis software (Konica Minolta Sensing Americas, NJ) was used to extract melanin index (MI), hemoglobin index, and CIE L*a*b* color space coordinates (using D65 illuminant and 10° observer settings). Of note, the derived MI is based on a proprietary method that Konica Minolta does not disclose, and may not be the same MI derived from other colorimeters and melanometers⁵⁴. Given these limitations in MI, the individual typography angle (ITA) was used as an objective surrogate for melanin content. ITA is the angle in the b*L* plane of the line defined between (0,50) and measured (b*, L*) coordinates:

$$ITA^{\circ} = \frac{180^{\circ}}{\pi} \tan^{-1} \frac{L^* - 50}{b^*} \quad (1)$$

ITA has been used as an objective measure of pigmentation for decades and has been shown to be linearly related to skin melanin content^{50,51,54}. Furthermore, specific ranges of ITA values have been used to define 6 pigmentation categories:

very light (I, $ITA > 55^\circ$), light (II, $41^\circ < ITA < 55^\circ$), intermediate (III, $28^\circ < ITA < 41^\circ$), tan (IV, $10^\circ < ITA < 28^\circ$), brown (V, $-30^\circ < ITA < 10^\circ$), and dark (VI, $ITA < -30^\circ$). For each subject, ITA was calculated for each of the measured triplicate L^* , b^* coordinate pairs, and then the median value was used as a measure of central tendency. In total $n=24$ healthy volunteers with a range of pigmentation were recruited for the saline injection studies, and $n=2$ volunteers (one with objectively dark pigmentation and one with objectively light pigmentation) with active acne were imaged for the acne imaging pilot studies.

Intradermal injection protocol

A blemish free region of the dorsal forearm was identified and cleaned with an isopropyl alcohol pad (McKesson 191089). L.S. administered a 0.1-0.2 mL intradermal injection of normal saline (McKesson 1145610) with a 27 gauge tuberculin syringe (McKesson 1031816). This formed a dermal wheal, which simulated dermal edema (Figure 1A).

For the subject undergoing real time video, 30 frames per second (fps) video during intradermal saline injection and several minutes after was captured with both an iPhone 11 Pro camera and the VIS-SWIR multispectral imaging system with the filter wheel locked in the 1300 nm bandpass filter position.

For the remaining subjects, the dermal wheal was imaged both with a conventional color camera (iPhone 11/13) and with the custom six-wavelength VIS-SWIR multispectral imaging system oriented perpendicularly (overhead) at 0.2 m imaging distance (Figure 1B). Image sets were acquired of the forearm immediately following the injection ($t=0$), and longitudinally every minute for 10 mins, resulting in 12 image sets per subject. A Band-Aid was placed on the forearms to serve as a fiducial marker for image coregistration. The conventional color camera images were intended to serve as proxies for visual assessment. Color calibration targets were not incorporated into conventional color photography images, but image color was adjusted manually to ensure consistent color of the fiducial marker (Band-Aid) across subjects.

Acne imaging protocol

Subjects were seated 0.2 m away from the VIS-SWIR multispectral imaging system, with the camera and illumination assembly rotated to face the subjects (as opposed to 90° overhead rotated position for the saline injection studies). Subjects rested their chin on a fixed support and had a 3D printed circular fiducial marker (for image coregistration) placed on their cheek in an acne-free area prior to imaging. Subjects were asked to identify areas of active acne (based on self-perception and symptoms of fullness/pain), and the position of the imaging system was adjusted to ensure that the field of view consisted of the chosen active area of disease. Images were then acquired in rapid succession with both an iPhone 11, for conventional color photography, as well as the VIS-SWIR multispectral imaging system across all system wavelengths.

2.3 Image Processing

Flat-field correction and chromatic aberration correction

At each acquisition time point (twelve time points for the saline-injection study and one for the acne study), we recorded six raw images corresponding to each filter-wheel position and processed them in MATLAB using a custom pipeline. Thus for the saline injection study, for example, we start with 72 raw images spanning 6 wavelengths and 12 time points ($I_{\lambda,(1-6),t(1-12)}^{\text{Raw}}$). Flat-field correction was performed per wavelength by using both the wavelength-specific flat-field correction images described in Figure 1E ($I_{\lambda}^{\text{FlatField}}$) as well as dark frame images ($I_{\lambda}^{\text{Dark}}$, obtained at wavelength-specific exposure times with the lens fully blocked). Subtracting the dark frame from both raw images and the flat-field image produced dark-corrected versions ($I_{\lambda,t}^{\text{Raw-Dark}} = I_{\lambda,t}^{\text{Raw}} - I_{\lambda}^{\text{Dark}}$, $I_{\lambda}^{\text{FlatField-Dark}} = I_{\lambda}^{\text{FlatField}} - I_{\lambda}^{\text{Dark}}$). From the dark-corrected flat field, we computed a per-pixel gain map equal to the inverse of that image scaled by its spatial mean. Multiplying the dark-corrected raw image by this wavelength-specific gain map yielded the flat-field-corrected image:

$$I_{\lambda,t}^{\text{FlatFieldCorrected}} = I_{\lambda,t}^{\text{Raw-Dark}} * \left(\frac{\langle I_{\lambda,t}^{\text{FlatField-Dark}} \rangle}{I_{\lambda,t}^{\text{FlatField-Dark}}} \right) \quad (2)$$

To reduce artifacts, we applied a histogram-based clipping of extremely bright outliers and set any negative values to zero. For the 540 nm band, which was more susceptible to dark current noise, we additionally applied a 2-D median filter prior to flat-fielding. Following flat-field correction, images at each wavelength were transformed using predetermined wavelength-specific translation matrices (Figure S4) to correct for chromatic aberration. This process yields aberration and flat-field corrected images ($I_{\lambda,t}^{\text{FlatFieldAberrationCorrected}}$).

Temporal co-registration

Each of these images underwent an additional stage of co-registration to account for subject movement across time. This was achieved by selecting coordinates of fiducial markers within the field of view across all images (including both visible iPhone images as well as the 6 VIS to SWIR wavelength-specific images) across all time points. In most cases, fiducial markers were determined algorithmically by leveraging MATLAB edge-detection algorithms, otherwise manual selection was performed. Once fiducial markers were set, a similarity transform was derived (using Matlab *fitgeotform2d* function) from the fixed (*fPts*, selected at a specific wavelength and time) and moving (*mPts* selected from all remaining images) fiducial marker coordinates and then applied (using Matlab *imwarp* function) to each flat-field and aberration-corrected image to yield co-registered images across all wavelengths and times:

$$I_{\lambda,t}^{FlatFieldAbberationCorrectedCoregistered} = \text{imwarp}(I_{\lambda,t}^{FlatFieldAbberationCorrected}, \text{fitgeotform2d}(mPts, fPts, 'similarity')) \quad (3)$$

For the conventional visible camera (iPhone) images, transforms were similarly derived based on fiducial markers identified within the visible images that matched the selected *fPts* coordinates and then applied to the color-corrected conventional visible camera images as described above. Finally, each co-registered image (including iPhone images) was cropped to a 3x3 cm region to center the lesion around unaffected skin in the field of view.

SWIR Pseudocolor image creation

Following flat-field correction, chromatic aberration correction, and temporal co-registration, we generated spectrally encoded pseudo-RGB SWIR images to emphasize chromophore-dependent contrast. For each time point, we first constructed spectrally encoded images at 1200, 1300, and 1600 nm by subtracting the corresponding 850 nm image from each longer-wavelength image:

$$I_{\lambda=(1200,1300,1600),t}^{SpectralEncoded} = I_{\lambda=(1200,1300,1600),t}^{FlatFieldAbberationCorrectedCoregistered} - I_{850,t}^{FlatFieldAbberationCorrectedCoregistered} \quad (4)$$

Because the 850 nm and longer-wavelength bands share similar large-scale intensity nonuniformity patterns arising from tissue curvature, this subtraction suppresses these contour- and illumination-related variations so that the residual signal more closely reflects local differences in chromophore concentration. The resulting spectrally encoded images were then normalized to [0,1] and denoised by replacing statistical outliers (beyond four standard deviations from the local median) using a nearest-neighbor, median-based filter. To further enhance tissue contrast while limiting the influence of large low-intensity background regions, we analyzed the per-channel intensity histogram, using changes in bin counts to identify the transition from background to tissue signal and temporarily flattening intensities below this data-driven cutoff before estimating display limits. Final per-channel display ranges were determined using percentile-based contrast stretching (MATLAB *stretchlim* followed by *imadjust*), and the processed 1200, 1300, and 1600 nm images were assigned to the red, green, and blue channels, respectively, to form composite pseudo-RGB SWIR images.

Region of interest definition

Finally, to facilitate image quantification, regions of interest (ROI) corresponding to the saline wheal and surrounding unaffected skin were determined. This was achieved through a custom MATLAB GUI that displays the pseudo-RGB SWIR images after injection and allows the user to select an ellipsoidal polygon surrounding the saline wheal that is applied to each time point of imaging. The region can be further adjusted manually to account for the presence of pinpoint bleeding at the injection site or changes in the size of the saline wheal over time. An annular ring corresponding to background skin is then generated symmetrically around the wheal (with inner radius 10% larger than the outer edge of the wheal to provide a buffer). The inner and outer borders of the annular ring were manually adjusted as needed to remove residual imaging artifacts. For the acne subjects, lesional and background regions of interest were determined by manually defining circular regions around the acne lesions and adjacent healthy skin.

2.4 Image Analysis

Comparing baseline multispectral reflection between lightly and darkly pigmented subjects

Flat-field- and aberration-corrected, co-registered, and cropped pre-injection images of unaffected skin

($I_{\lambda,t=0}^{FlatFieldAbberationCorrectedCoregistered}$) were used to assess baseline reflection intensity across subjects. For each subject and

each of the six wavelengths, we computed the mean pixel intensity over the unaffected skin region. To enable comparison across subjects with different overall brightness, we then calculated a near-infrared-normalized reflectance fraction (RF_{NIR}) by dividing the mean intensity at each wavelength (excluding 850 nm) by the mean intensity of the corresponding 850 nm image:

$$RF_{NIR, \lambda=(540,650,1200,1300,1600)} = \frac{\langle I_{\lambda=(540,650,1200,1300,1600), t=0}^{FlatFieldAbberationCorrectedCoregistered} \rangle}{\langle I_{850\text{ nm}, t=0}^{FlatFieldAbberationCorrectedCoregistered} \rangle} \quad (5)$$

Subjects were classified as being in a lighter-pigmented cohort ($ITA > 10^\circ$, $n=12$) or a darker-pigmented cohort ($ITA < 10^\circ$, $n=12$). For each wavelength, RF_{NIR} values were compared between groups using unpaired two-sample t-tests. Group means and standard deviations of RF_{NIR} for the light and dark cohorts were also computed and used for graphical display (Figure 3D).

Comparing imaging contrast for intradermal fluid/inflammation detection between lightly and darkly pigmented subjects

To quantify lesion visibility in both SWIR-pseudocolor and visible images, we measured the CIELAB color space difference between the region of saline injection and the surrounding ring of unaffected skin. For each saline-injection subject and each time point, the pre-defined saline injection area and annular background regions of interest (ROIs) (see Region of interest definition above) were applied to the co-registered pseudo- RGB SWIR images and the corresponding conventional visible iPhone images. Within each image, RGB pixel values inside each ROI were converted to CIE $L^*a^*b^*$ coordinates (D65 illuminant, 10° observer), and a color difference $\Delta E_{injection_vs_unaffected}$ between the saline injection area and the background was calculated as:

$$\Delta E_{injection_vs_unaffected} = \sqrt{(\langle L^*_{injection} \rangle - \langle L^*_{unaffected} \rangle)^2 + (\langle a^*_{injection} \rangle - \langle a^*_{unaffected} \rangle)^2 + (\langle b^*_{injection} \rangle - \langle b^*_{unaffected} \rangle)^2} \quad (6)$$

This calculation was performed independently for the pseudo- RGB SWIR images and the visible images, yielding $\Delta E_{injection_vs_unaffected}^{SWIR}$ and $\Delta E_{injection_vs_unaffected}^{Visible}$ for each subject at each imaging time point. For the saline injection cohort, quantitative analyses focused on $t=5$ minutes after injection, at which time initial blanching resolved.

For cross-subject analyses, the 5-minute $\Delta E_{injection_vs_unaffected}^{SWIR}$ and $\Delta E_{injection_vs_unaffected}^{Visible}$ values were merged with each subject's individual typology angle (ITA°) obtained from spectrophotometry. Subjects were sorted by ITA° and ΔE values were plotted against ITA to visualize how lesion-background color contrast varied with skin pigmentation for each modality (Figure 6). To summarize modality performance across pigmentation groups, subjects were further stratified into lightly pigmented ($ITA > 10^\circ$) and darkly pigmented ($ITA < 10^\circ$) cohorts, consistent with the baseline reflectance analysis. Within each subject, $\Delta E_{injection_vs_unaffected}^{SWIR}$ and $\Delta E_{injection_vs_unaffected}^{Visible}$ values at 5 minutes were treated as paired measurements, and differences between modalities were assessed using two-sided paired t-tests. Distributions of $\Delta E_{injection_vs_unaffected}^{SWIR}$ and $\Delta E_{injection_vs_unaffected}^{Visible}$ were visualized using box plots (MATLAB *boxchart* function with notches), and the association between continuous ITA° and $\Delta E_{injection_vs_unaffected}^{SWIR}$ was evaluated using Spearman rank correlation.

An analogous ΔE calculation was performed for the acne-imaging pilot subjects. For each acne subject, a single imaging time point was available, and $\Delta E_{acne_vs_background}^{SWIR}$ and $\Delta E_{acne_vs_background}^{Visible}$ values quantifying the color difference between the acne lesion and unaffected background skin was similarly determined using the manually defined acne lesion and background ROIs.

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Author contributions: Conceptualization: LS, QM

Methodology: LS, QM, AJ, DB, MH, YJ, MB

Investigation: LS, QM, AJ, FA

Visualization: LS, QM, AJ, MH, YJ

Writing—original draft: QM, LS; review & editing: LS, QM, AJ, MH, YJ

Conflict of Interest Declaration: No relevant conflicts of interest

Supplementary Materials: Video S1, Figures S1-S5, are attached in a supplementary material document.

FIGURES

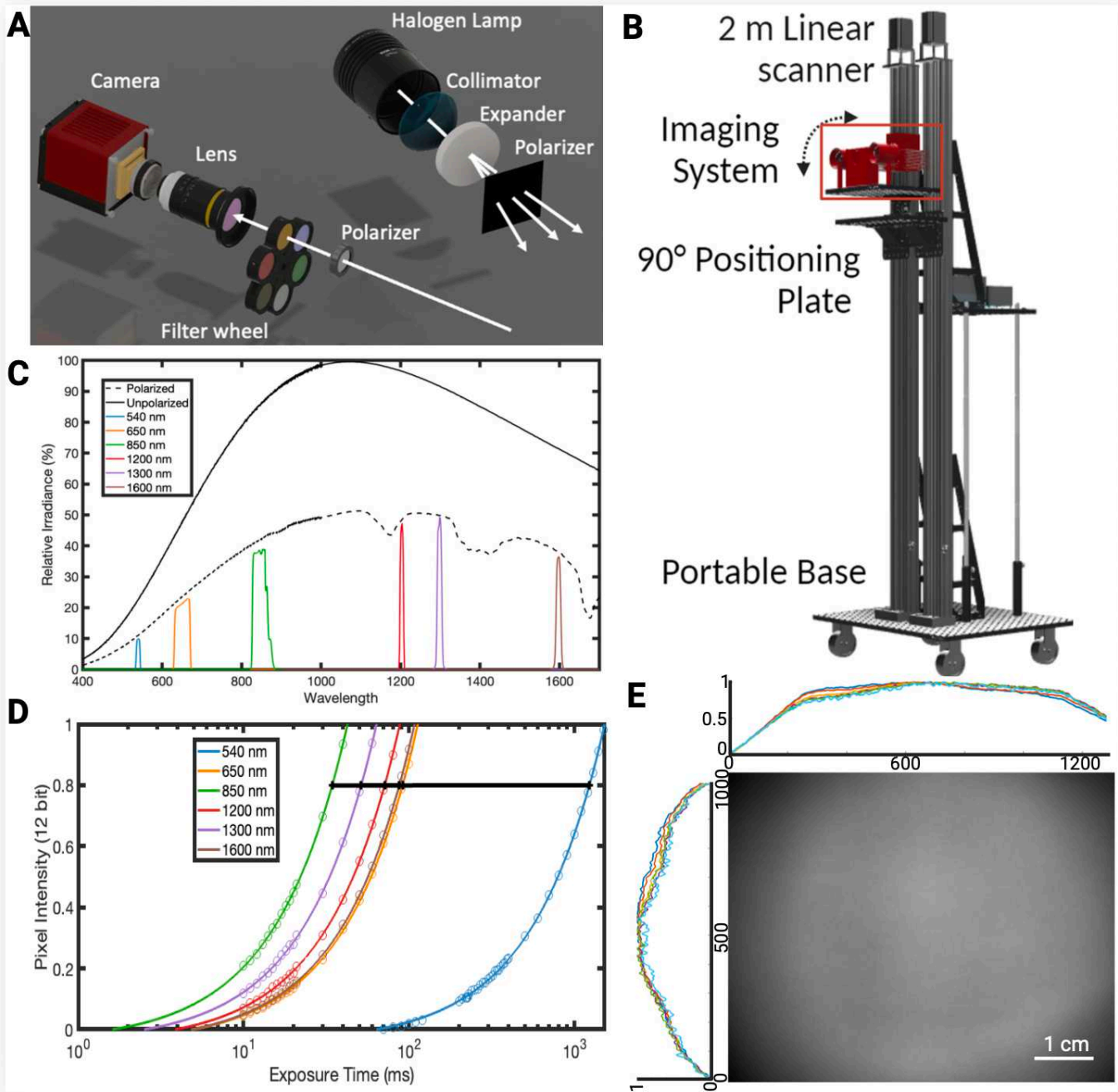


Figure 1. Multispectral VIS-SWIR imaging system design and benchtop characterization. **A.** Optical layout showing the detection path (VIS-SWIR camera, low-chromatic aberration lens, motorized bandpass filter wheel, and imaging polarizer) and the illumination path (broadband halogen source, beam collimator/expander, and illumination polarizer oriented orthogonally to the imaging polarizer for cross-polarized reflectance imaging). **B.** CAD rendering of the integrated system mounted on a portable motorized vertical scanning stage (2 m linear scanner) with a 90° positioning plate. **C.** Relative source irradiance spectrum (400–1700 nm) measured for the halogen lamp alone, with the illumination polarizer, and after insertion of each of the six bandpass filters used for imaging (540, 650, 850, 1200, 1300, and 1600 nm). **D.** Camera intensity response measured by imaging a Spectralon reflectance standard: peak pixel intensity (normalized to the 12-bit maximum, 4095) is plotted versus exposure time (log scale) for each bandpass filter. Differences between curves reflect wavelength-dependent illumination throughput (C) and sensor quantum efficiency; these calibration curves were used to select filter-specific exposure times that yield matched signal levels (example target shown at 0.8, black line). **E.** Example flat-field acquisition on a uniform reflectance standard (850 nm; scale bar, 1 cm) with corresponding horizontal and vertical centerline intensity profiles for each spectral band. These measurements were used to generate wavelength-specific flat-field correction matrices.

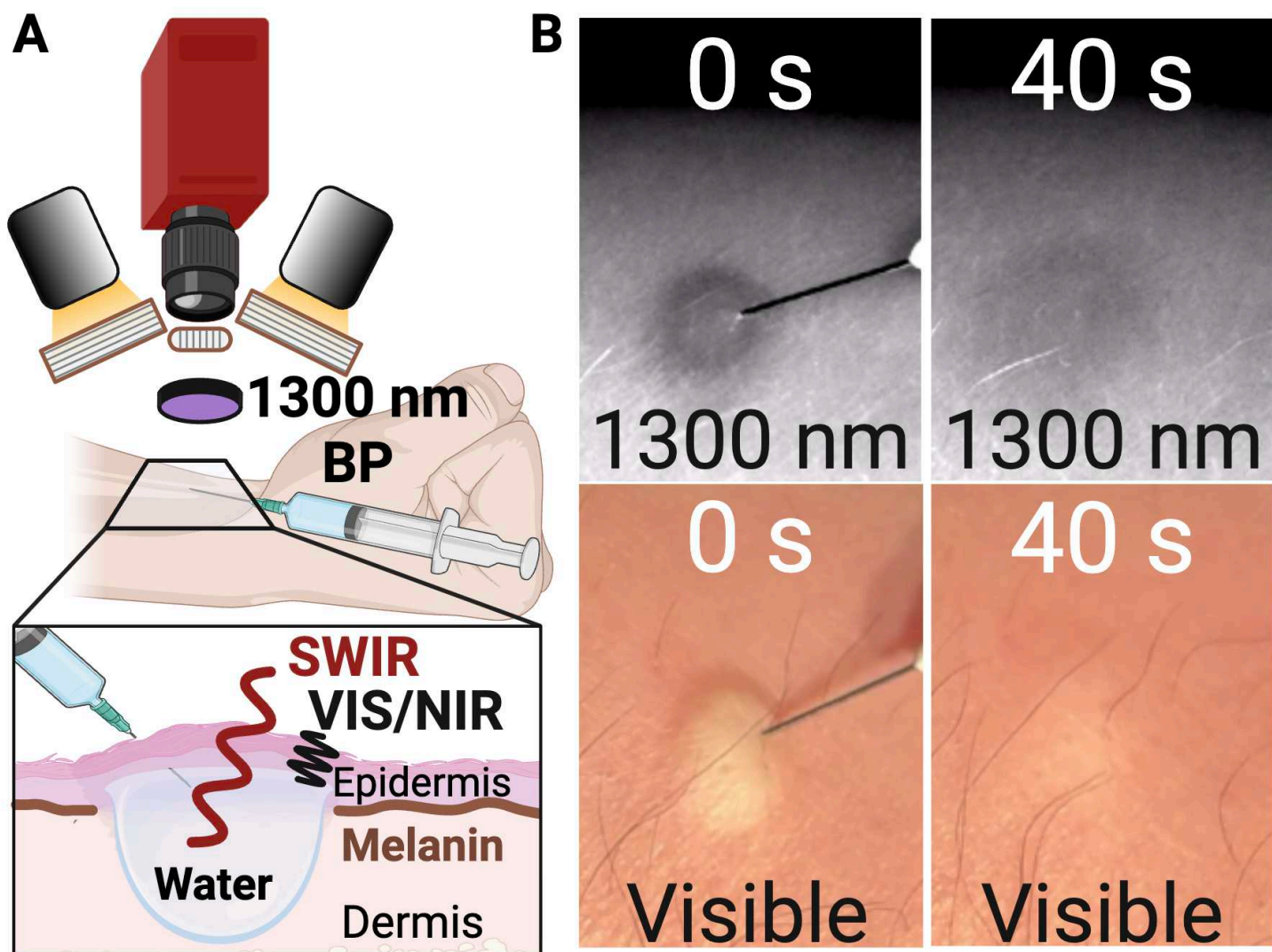


Figure 2. Summary of SWIR single-wavelength real-time detection of intradermal saline. **A.** Schematic of the experimental setup illustrating intradermal saline injection imaged with the VIS–SWIR system using a 1300-nm bandpass filter. Inset depicts the proposed contrast mechanism: SWIR light penetrates to the dermis with reduced sensitivity to epidermal melanin compared with VIS/NIR wavelengths, while water exhibits strong absorption near 1300 nm, producing a localized decrease in reflectance at the injection site. **B)** Representative video frames acquired at the time of injection (0 s) and 40 s post-injection showing SWIR reflectance at 1300 nm (top) and conventional visible imaging (bottom). In SWIR images, the injection site appears as a dark region consistent with increased water content and corresponding reduced reflectance. In visible images, injection-associated blanching (pressure-related) is apparent at 0 s but largely resolves by 40 s, whereas SWIR contrast persists.

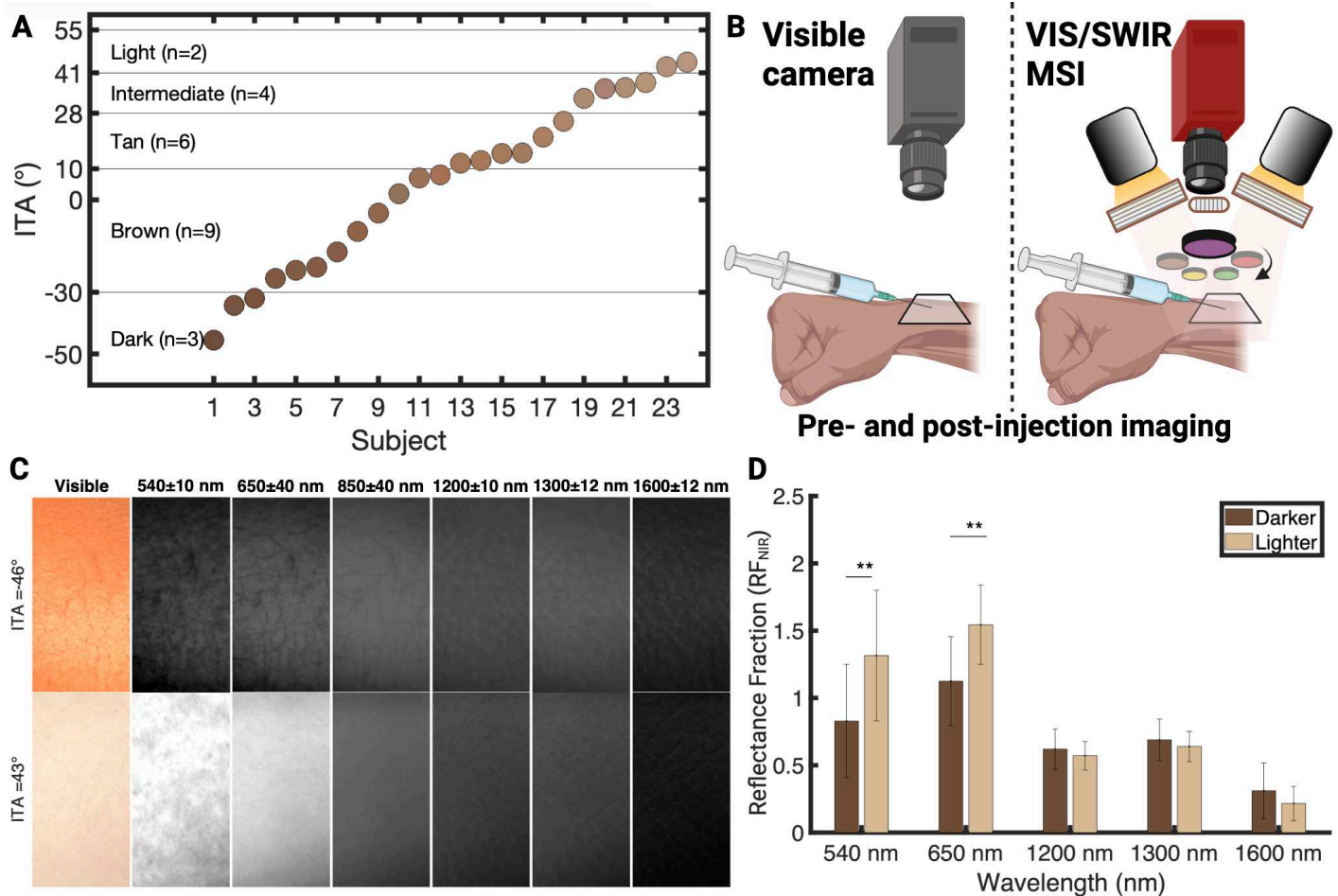


Figure 3. **A.** Spectrophotometer-derived Individual Typology Angle (ITA) for each participant, spanning “Dark” (ITA < -30°) to “Light” (ITA > 31°). Subjects were dichotomized into darker-pigmented (ITA < 10°, n=12) and lighter-pigmented (ITA > 10°, n=12) cohorts. **B.** Experimental schematic illustrating paired pre- and post-injection imaging acquired with a conventional visible camera and the VIS–SWIR multispectral imaging (MSI) system. **C.** Representative visible and multispectral reflectance images (540±10, 650±40, 850±40, 1200±10, 1300±12, and 1600±12 nm bandpass filters) from a lighter-pigmented subject (ITA = 43°) and a darker-pigmented subject (ITA = -46°). For visualization, multispectral image intensities were scaled by a constant factor within each wavelength band to match mean intensity at 850 nm between the two subjects, highlighting wavelength-dependent differences in baseline reflectance; visible-band images show lower relative intensity in darker skin, whereas SWIR-band images appear similar across pigmentation. Because visible images were acquired with automatic exposure/white balance, darker skin tones may appear brighter than expected in the photographs. **D.** Near-infrared–normalized reflectance fraction (RF_{NIR}), defined as the mean pixel intensity at each wavelength divided by the mean intensity of the corresponding 850-nm image from the same subject, plotted as group mean ± SD for darker- versus lighter-pigmented cohorts. RF_{NIR} was significantly lower in the darker-pigmented group at visible wavelengths (540 nm and 650 nm; unpaired two-sample t-tests, **p=0.008 and p=0.002, respectively), while no differences were detected at SWIR wavelengths (1200–1600 nm; p≥0.81), indicating that visible reflectance is strongly pigmentation dependent whereas SWIR reflectance shows reduced dependence on baseline pigmentation in this cohort.

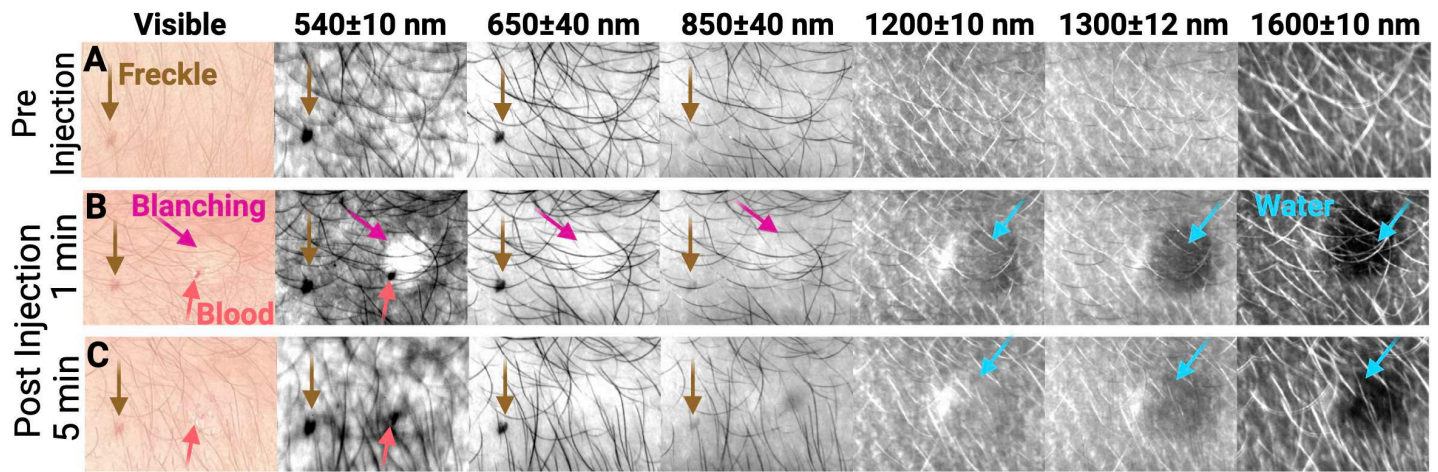


Figure 4. Comparison of skin findings assessed by a conventional visible color camera and narrowband VIS–SWIR imaging. A representative subject with a pigmented lesion (freckle) imaged before and after intradermal saline injection demonstrates the distinct appearance of pigmentation, superficial blood, transient blanching, and injected saline across wavelengths. At each time point (rows) the first column shows the visible color camera image, and the subsequent columns show the same image acquired at increasing narrowband wavelengths. **A) Prior to intradermal saline injection** the pigmented lesion (brown arrows) appears brown on the visible photograph and as a dark, high-absorption focus at 540, and 650 nm, is faded at 850 nm, and becomes difficult to distinguish from surrounding skin at SWIR wavelengths (1200–1600 nm), consistent with reduced melanin contrast at longer wavelengths. **B). At 1 minute after injection**, acute blanching at the injection site (magenta arrows) is visible clinically and manifests as increased intensity at 540/650 nm, consistent with reduced blood volume from pressure-induced capillary occlusion. Minimal superficial bleeding at/near the injection site appears as a focal dark spot most prominent at 540 nm (pink arrow). In contrast, intradermal saline is visualized in the SWIR bands as a circular region of increased absorption (darker signal; cyan arrows), reflecting water-sensitive contrast. **C) At 5 min post-injection**, blanching diminishes while the superficial blood signal remains most apparent at 540 nm. The intradermal saline continues to appear as a darker circular area at SWIR wavelengths (cyan arrows), highlighting the distinct SWIR appearance of dermal fluid compared with pigmentation- and hemoglobin-driven contrast in the visible/NIR bands.

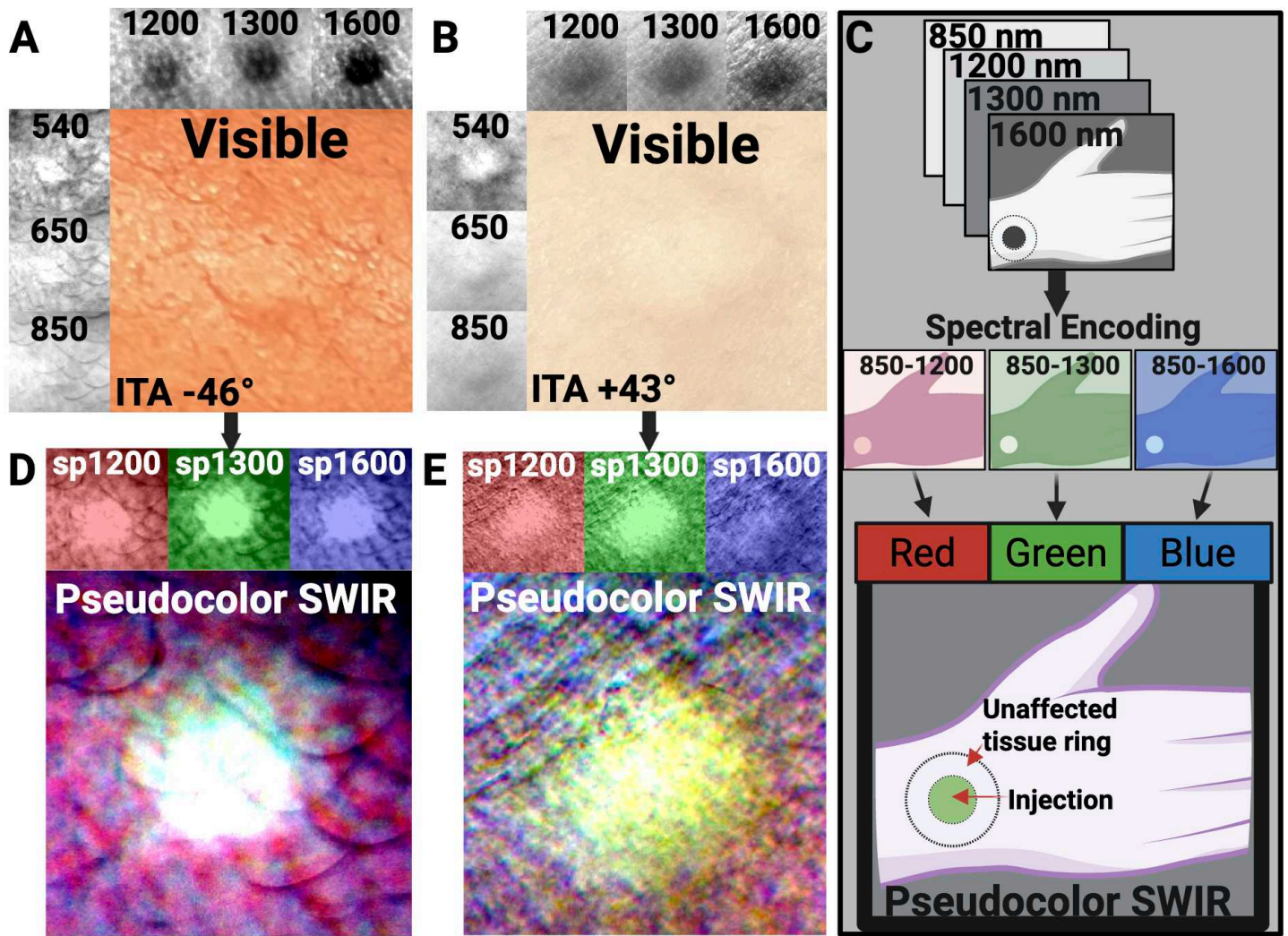


Figure 5. Visible versus multispectral VIS-SWIR appearance of intradermal saline in representative subjects spanning dark and light pigmentation. A-B) images acquired 5 min after intradermal saline injection using a conventional visible camera and a multispectral VIS-SWIR system with six bandpass filters (540, 650, 850, 1200, 1300, and 1600 nm) in a subject with (A) dark pigmentation (ITA = -46°) and (B) light pigmentation (ITA = $+43^\circ$). The intradermal saline is not apparent in the visible color images but appears hypointense (darker) in the SWIR bands. C) Pseudocolor SWIR generation: the 850-nm image is subtracted from the 1200-, 1300-, and 1600-nm images (spectral encoding), and the resulting images are mapped to the red, green, and blue channels, respectively. D-E) The pseudocolor SWIR images highlight intradermal saline with high contrast in both subjects, despite markedly different baseline pigmentation.

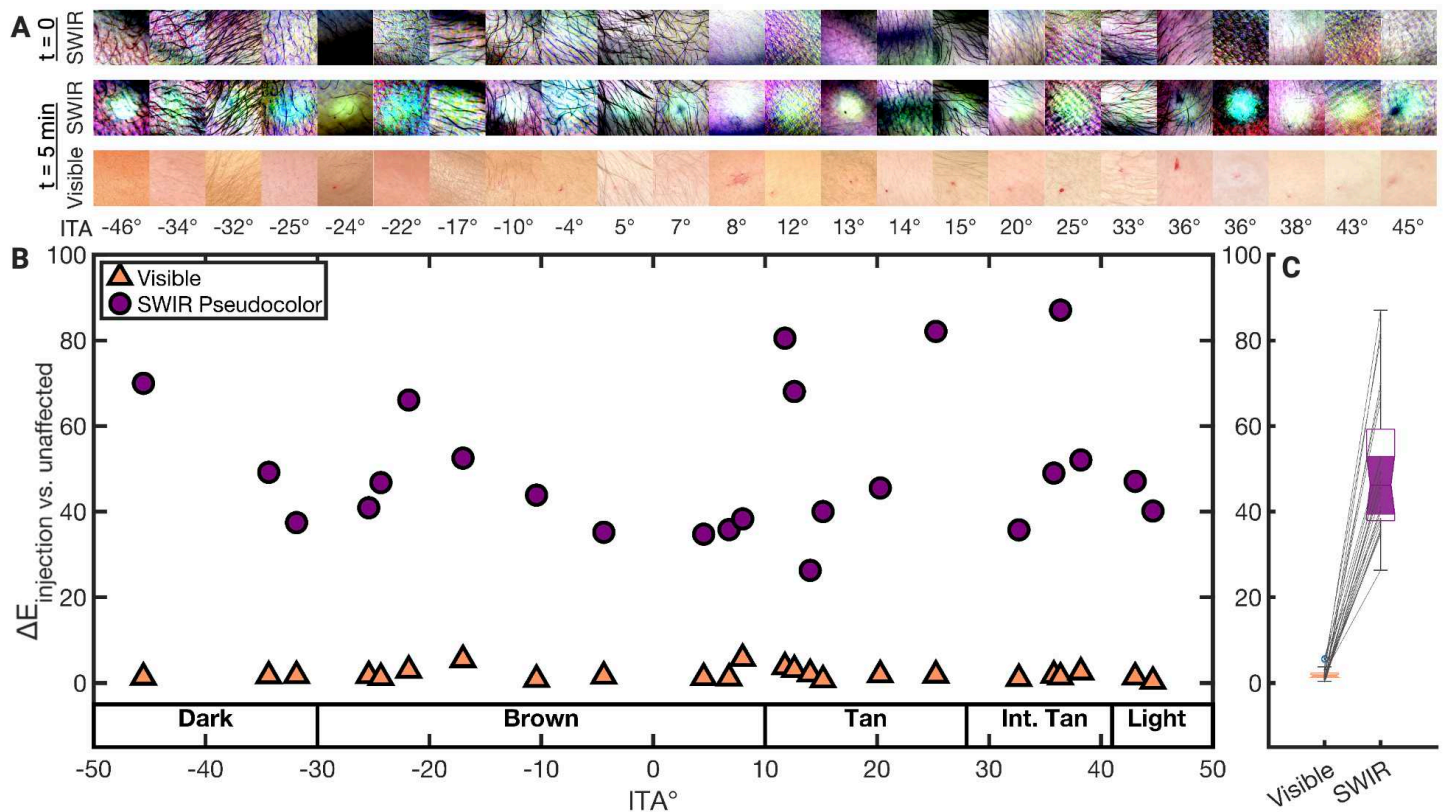


Figure 6. SWIR pseudocolor imaging enhances intradermal saline contrast independent of skin pigmentation. A) Representative image set for all subjects ordered by ITA (dark -46° to light +45°). For each subject SWIR pseudocolor images pre-injection ($t = 0$) and 5 minutes post-injection ($t = 5$ min), as well as co-registered visible color images at $t = 5$ min are shown. The injection site is difficult to distinguish in visible images (aside from occasional pinpoint bleeding/blanching) but is clearly delineated in SWIR pseudocolor images. **B)** The CIELAB color difference between the injection region and surrounding unaffected ring ($\Delta E_{\text{injected vs. unaffected}}$) versus ITA for visible and SWIR pseudocolor images at $t = 5$ min. ITA pigmentation categories are superimposed along the x-axis. SWIR-pseudocolor $\Delta E_{\text{injected vs. unaffected}}$ shows no association with pigmentation (Spearman $\rho = 0.05$, $p = 0.81$). **C)** Paired box plot comparison of $\Delta E_{\text{injected vs. unaffected}}$ across all subjects (visible vs SWIR pseudocolor) demonstrates significantly higher values for SWIR-pseudocolor values (paired t-test $p = 5.9 \cdot 10^{-13}$).

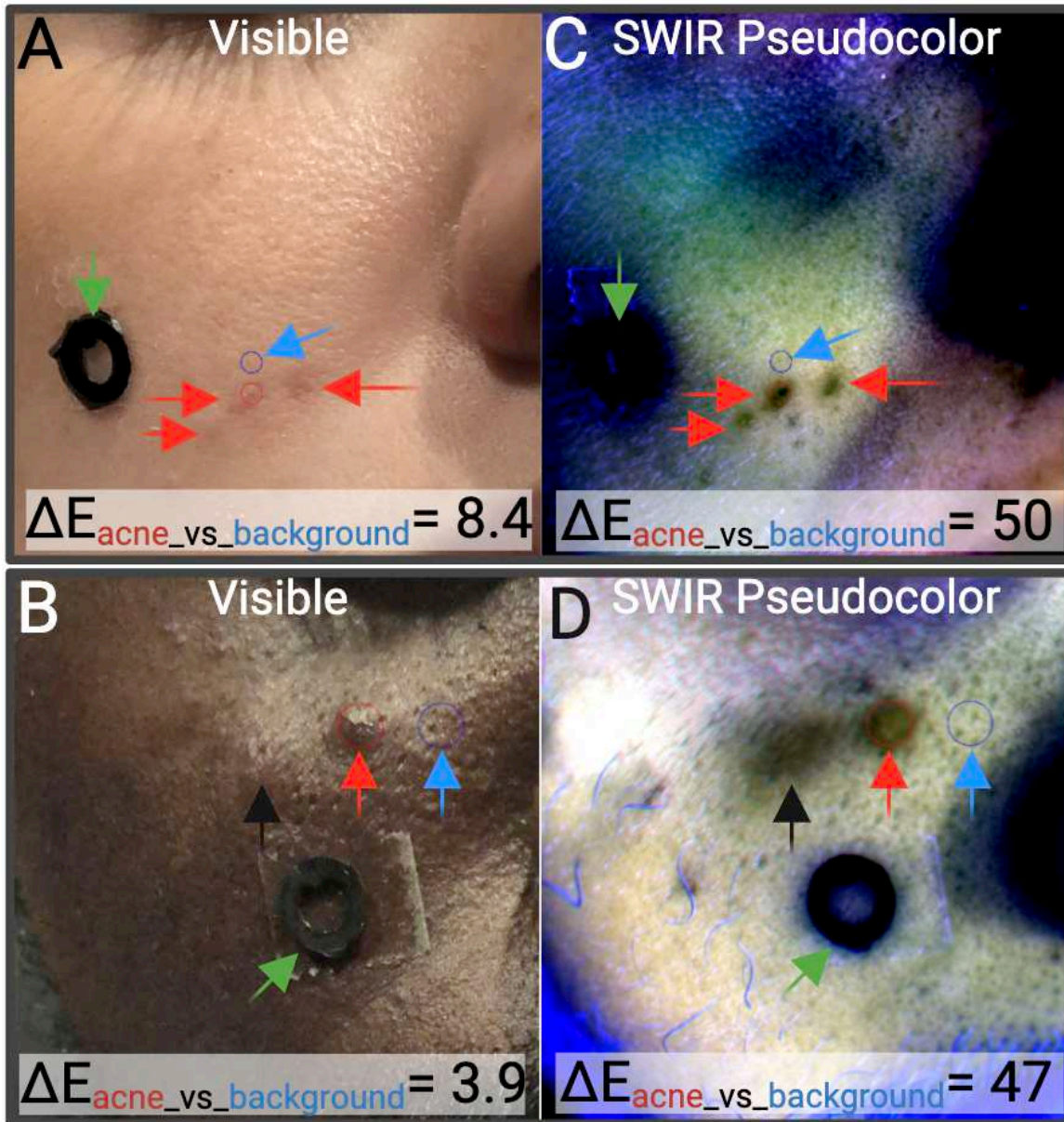


Figure 7. Pseudocolor SWIR images enhance contrast of acne-associated inflammation in lightly and darkly pigmented skin. A,B) Conventional visible camera color images of lightly-pigmented (A) (ITA = 53°) subject and darkly pigmented (B) (ITA = -53°) subjects with facial acne. Acne lesions are indicated (red arrows), and appear as scattered erythematous acne macules in the lightly-pigmented subject and skin-colored papules in the darkly-pigmented subject. Lesion to background contrast was quantified as the CIELAB color difference between an acne region (red circle) and adjacent unaffected skin (blue circle) ($\Delta E_{\text{acne_vs_background}}$). A fiducial marker is affixed to the cheek (green arrow) for image co-registration between visible and SWIR pseudocolor images. C-D) Co-registered SWIR pseudocolor images for the lightly-pigmented (C) and darkly-pigmented (D) subjects show higher lesion to background contrast than the visible images. The black arrow in (B,D) indicates a subject-reported area of discomfort/fullness that is difficult to distinguish in the visible image but appears as a contiguous region of increased absorption in SWIR pseudocolor adjacent to the visible papule, suggestive of deeper inflammation (e.g., severe cystic acne).

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