

Egyptian Dermatology Online Journal Volume 11 Number 1

Subsurface Cutaneous Imaging Techniques

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Egyptian Dermatology Online Journal 11 (1): 2, June 2015

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Abstract

Surface skin imaging has long been the only non-invasive way for skin imaging reflecting only the image provided by the stratum corneum, however in the past few decades, thanks to the technological development, sub-surface skin imaging have been made possible providing non-invasive in depth cutaneous images by means of dermoscopy, optical coherence tomography, cutaneous ultra-sonography and Laser confocal microscopy. This review aims at providing insight into each of these modalities.

Introduction

The attempt to improve the accuracy of the diagnosis of different skin diseases, especially for melanocytic skin lesions, has led to the development of noninvasive imaging tools, such as dermoscopy, optical coherent tomography, in vivo reflectance mode confocal microscopy, spectrophotometric intracutaneous analysis, and high-frequency ultrasound. They use the multiple reflection indices of different chromophores of the skin when exposed to light to provide highly detailed image of skin lesions [1]. Nowadays, dermoscopy is widely used in the routine clinical practice while the other techniques are used mainly for research purposes.

Optical coherence tomography

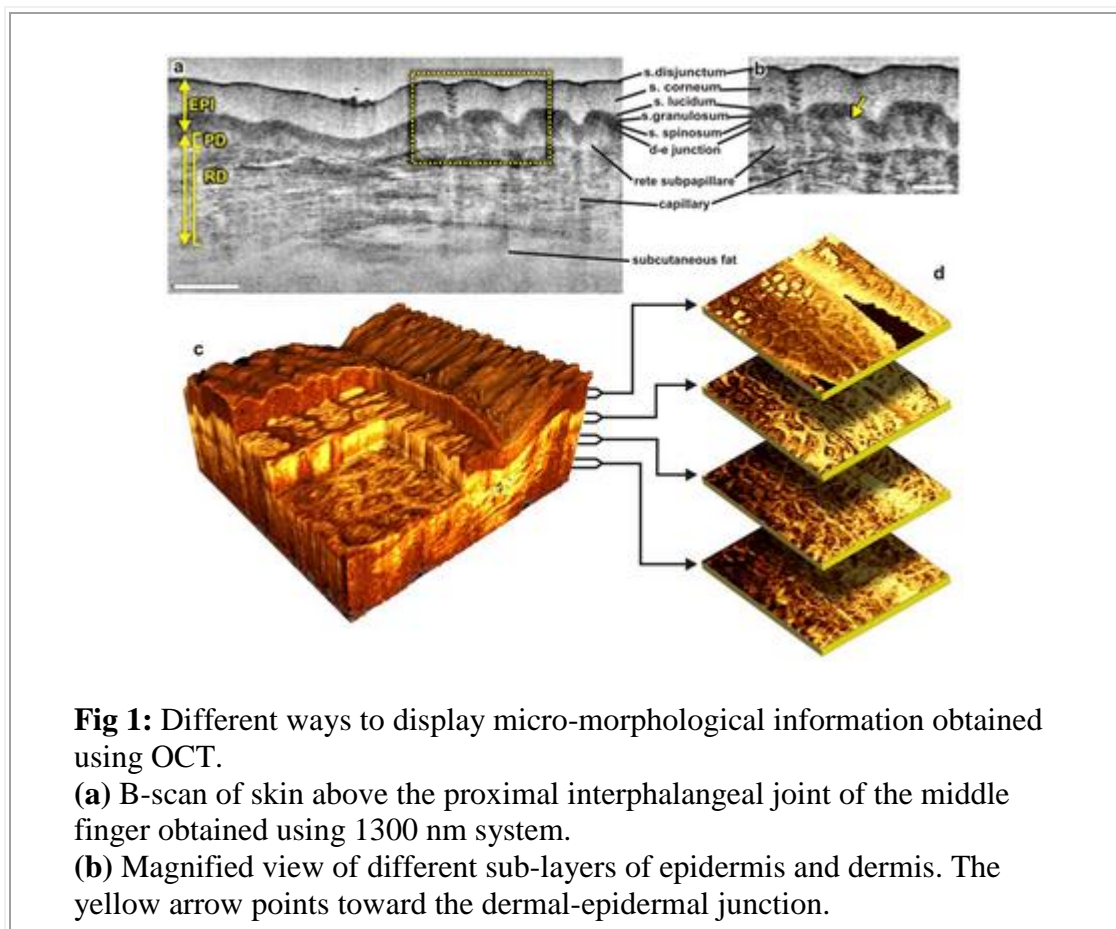
Optical coherence tomography (OCT) is a non-invasive technique for the morphologic investigation of tissues. It is based on the reflection of light waves by biological tissues in the near-infrared region. An optical fiber projects a light beam onto the skin, creating two-dimensional cross sectional images that are comparable with the non-invasive virtual biopsies [2]. The technique provides real-time skin images 2 or 3 dimensional to a depth of 1.8 micro meter with a lateral resolution of 10-20 micro meter [3]. Recently, high-definition OCT (HD-OCT) scanners have been developed to provide significantly higher lateral resolution (1-3 micro meter) [4]

OCT is a promising tool for various dermatologic conditions, allowing for the visualization of the stratum corneum, viable epidermis, papillary dermis, and appendages such as hair follicles and sweat ducts [3]

OCT is an extremely active and dynamic area of research and draws heavily from the rapidly developing technology base in photonics and lasers. Different modified technologies were introduced to improve the image resolution. Polarization-sensitive OCT has the ability to visualize and quantify the birefringence properties of skin especially collagen. Loss of collagen structure and integrity is often associated with abnormalities of the skin, including tumors and connective tissue diseases, suggesting that birefringence assessments may prove valuable as a diagnostic indicator of certain cutaneous pathologies [5].

OCT of normal skin [2]

The appearance of healthy images at different anatomic sites is of importance, in particular when interpreting OCT images of pathological processes. Normally, the stratum corneum is only visible on the palmoplantar skin. At these sites, OCT images display a wavy surface due to the dermatoglyphics. The horny layer of the palmoplantar skin is a well-defined, thick, homogenous, low-scattering band showing some high-scattering sweat gland ducts inside [6]. The border between the cornified and living epidermis is usually distinct, whereas the dermo-epidermal border is frequently blurred (Fig 1).



(c) 3D rendering of the same region reconstructed from 1024 B-scans.
 (d) En face sections of the same region separated in depth by 360 μm .
 The scale bars in (a) denotes 500 μm and the scale bar in (b) denotes 200 μm [6]

Applications in dermatology:

- Skin tumors [7]
 - Basal cell carcinoma: Basal cell nests show a characteristic hypo-reflective border. OCT is used mainly for identification, diagnosis of the type, evaluation of lateral borders, planning extent of excision and follow-up (Fig 2) [8]

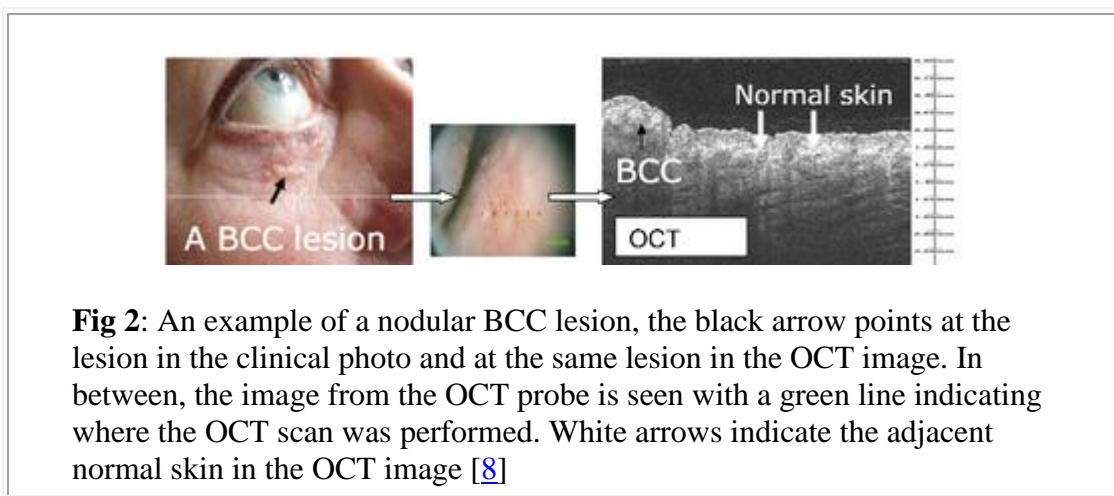


Fig 2: An example of a nodular BCC lesion, the black arrow points at the lesion in the clinical photo and at the same lesion in the OCT image. In between, the image from the OCT probe is seen with a green line indicating where the OCT scan was performed. White arrows indicate the adjacent normal skin in the OCT image [8]

- Squamous cell carcinoma in situ: actinic keratosis, Bowen's disease, and erythroplasia of Queyrat show hyper-reflective keratin depositions, acanthotic epidermis; irregular, but intact DEJ. OCT is specifically important to rule out invasion.
 - Invasive squamous cell carcinoma show hyper-reflective keratin depositions, acanthotic epidermis and indistinguishable DEJ. OCT can be used to evaluate the invasion depth and the efficacy of treatment.
 - Malignant melanoma: show hyper-reflective structures extending into the dermis and indistinguishable DEJ. OCT can be used to evaluate the invasion depth and treatment efficacy.
- Other dermatological uses
 - Hair analysis:
OCT can be used for in-vivo diagnosis of alopecia and follow-up. Results are comparable to histology [9]
 - Nail diagnosis:
Fungal elements are visualized as homogeneous, low-signal nail plate. Currently there is no differentiation of pathogens but it can differentiate it from inflammatory causes of nail changes such as lichen ruber, psoriasis, atopic dermatitis [10]
 - Dermatitis and psoriasis:

Non-invasive, reproducible follow-up, standardized measurement of plaques (hyperkeratosis, acanthosis, papillomatosis). Development of assessment scores is possible [11]

- Autoimmune diseases (e.g., lupus erythematosus): Findings are comparable to histology [12]
- Blistering skin disorders: Differentiation between intraepidermal and subepidermal blistering is possible [13].
- Parasitosis: OCT can be used for the rapid detection of parasitic pathogens such as scabies and larva migrans [8]
- Measuring the skin when using topical treatments: Assessment of skin atrophy (epidermis and dermis) in patients taking corticosteroids [2]

In Vivo reflectance mode confocal microscopy

In vivo reflectance confocal microscopy (RCM) is a non-invasive imaging tool that allows real-time visualization of cells and structures in living skin with near histological resolution [14]. RCM is based on the collection of images from light reflected by living tissue [15]. A confocal microscope consists of a light source, a condenser, an objective lens and a detector, the light source illuminates a small three dimensional spot within a sample, such as skin. This illuminated spot is then imaged onto the detector through a small aperture (pinhole). The small aperture allows only light that originates from the focused illumination spot to be detected, whereas the light that originates away from the spot is reflected [16]. Images are obtained in grey scale in which white represents total light reflection and black represents no reflection at all. More light is reflected when the tissue contains structures of size similar to the wavelength of the light source (**Fig 3**) [17,18]

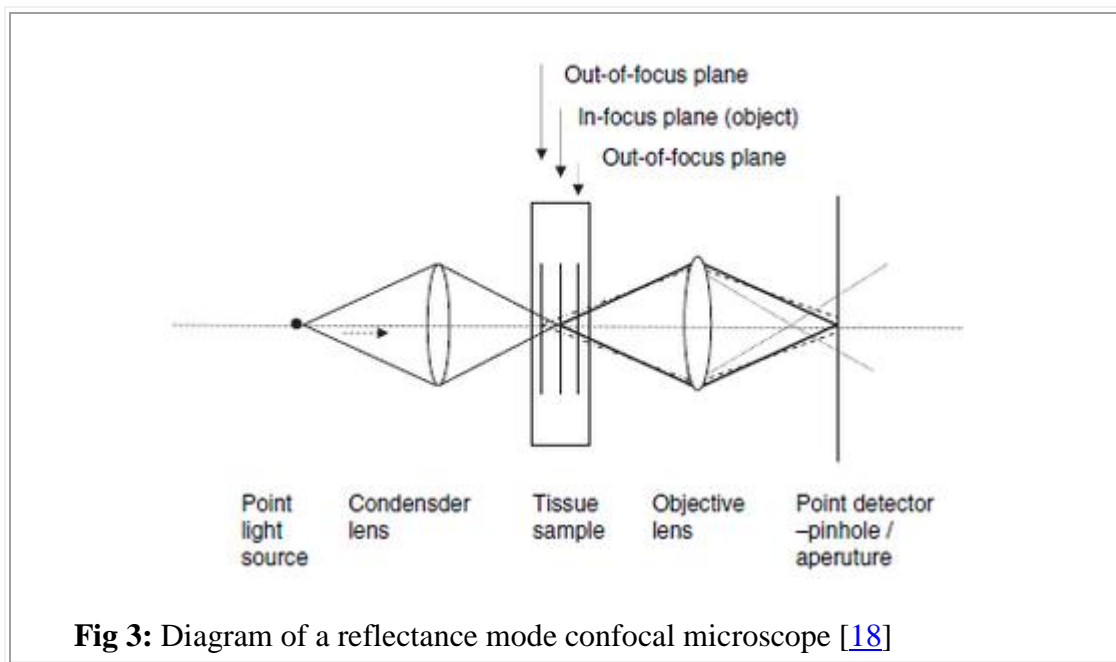


Fig 3: Diagram of a reflectance mode confocal microscope [18]

Evaluation of normal skin

- *Epidermis*

Imaging normal skin in real time usually takes place from the surface and progressing deeper. Most superficial images correspond to the stratum corneum. The stratum corneum produces the first image of the top surface of the skin because of backscattered light at the water-to-stratum corneum interface. Corneocytes are visualized as brilliant polygonal shapes of 10 to 30Mm size, and grouped in 'islands' separated by skin folds, which appear very dark. The next layer is the stratum granulosum keratinocytes which are 20 to 25Mm size and their nuclei show up as dark central oval structures surrounded by a bright grainy cytoplasm. The stratum spinosum is located 20 to 100 micro meter deep. It consists of a tight 'honeycomb pattern' of keratinocytes (10 to 15 micro meter size) with well-demarcated cell borders. Between 50 to 100 micro meter depths we can find the dermo-epidermal junction. Basal keratinocytes are small (7-15 micro meter) and bright [19], due mostly to the presence of melanin inside the cell. The melanin in basal keratinocytes is typically arranged in a supranuclear position.

- *Dermis*

Dermal papillae are observed as dark round areas surrounded by rings of bright circles of basal cells containing highly reflective melanin granules. Capillary loops are located in the centre of dermal papillae as black holes, often showing bright erythrocytes rolling within them. Below the dermo-epidermal junction, a network of collagen fibers and bundles (1Mm and 5 to 25 micro meter diameter, respectively) can be observed within the papillary dermis and superficial reticular dermis. Eccrine ducts appear as bright central hollow structures that spiral through the epidermis and dermis. Hair shafts with pilosebaceous units can be observed as central hollow structures with elliptical elongated cells at the circumference, with a central white structure corresponding to the hair shaft (**Fig 4**) [19]

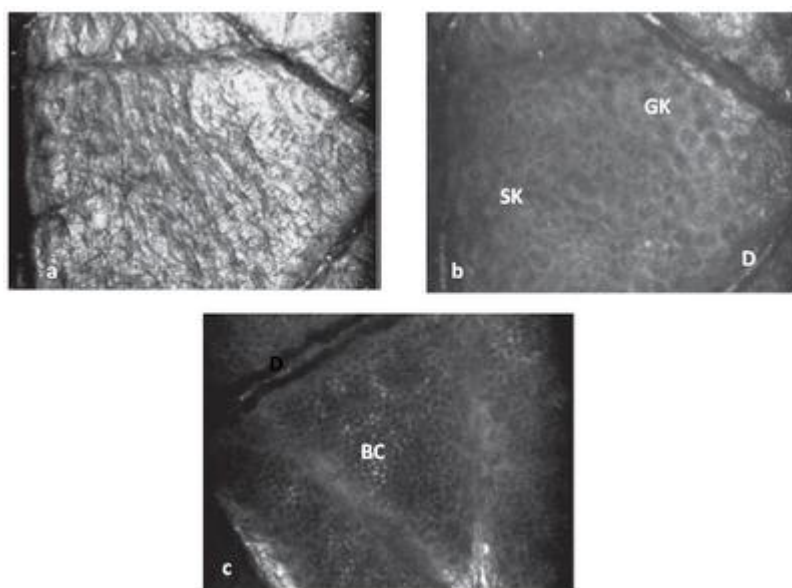


Fig 4: a) Confocal image of stratum corneum level showing high refractivity. b) Slightly oblique image showing the presence of granular keratinocytes (GK) and spinous keratinocytes (SK). c) Confocal image at dermo-epidermal level (D) showing basal cell layer (BC) [18]

Use of RCM in clinical dermatology

- ***RCM findings of inflammatory skin conditions***
 - Contact dermatitis:
Features of contact dermatitis show spongiosis which appears as intercellular brightness. The presence of epidermal inflammatory cellular infiltrate can be visualized as bright round or oval structures 9 to 12 micro meter size interspersed between keratinocytes. Areas of necrotic epidermis, perivascular inflammatory infiltrate, and increased size and brightness of basal keratinocytes are also seen in both types of reaction [20]
 - Psoriasis:
The major features that distinguish the uninvolved skin from the lesional psoriatic skin are increased numbers of dermal papillae with enlarged dermal blood vessels inside them to supply the proliferative lesion with circulating erythrocytes [21]. Other features are parakeratosis, clusters of polymorph-nuclear leucocytes forming the Munro's microabscesses visualized as highly refractile compared with the surrounding keratinized background and thinning of the granular layer [22].
 - Rosacea:
RCM histopathology reveals increased diameters of the pilosebaceous ducts, tortuous capillaries and a characteristic perifollicular and perivascular inflammatory infiltrate [23].
- ***RCM findings of cutaneous infections***

- Fungal infections:
RCM enables rapid real-time identification of branched hyphae, visualized as a network of long, dark, sometimes septated structures [24].
 - Bacterial folliculitis:
Folliculitis imaged with RCM can be diagnosed by direct observation of a hair follicle surrounded by a significant number of small bright granular cells (neutrophils), which can be also found within the subcorneal pustules. In addition, severe spongiotic epidermis and capillary dilatation in the dermal papillae can be observed [23].
 - Viral infections:
Warts imaged with RCM show hyperkeratotic stratum corneum and presence of multiple highly refractile round structures measuring 20 to 40 micro meter size within the lesion.
 - Cutaneous herpes infections:
Their main features are the presence of pleomorphic big round cells with dark cytoplasm, identified as ballooned keratinocytes, and internal round bright structures corresponding to multinucleated giant cells.
- ***RCM findings of skin neoplasms***
- Melanocytic nevi:
RCM of nevi show round to oval bright refractive cells with centrally positioned dark round nuclei [25]. Dermal papillae are uniformly distributed and circumscribed by a rim of refractive monomorphous cells (edge papillae) that correspond to small melanocytes and melanin-rich keratinocytes, without any cytological atypia. In junctional nevi, melanocytes are at the dermo-epidermal junction level [26]. On the contrary, in compound and dermal nevi, they are seen within the papillary and reticular dermis, near the vessels. Sporadically, small brilliant dendrites in the epidermis can be observed [26].
 - Melanoma:
Confocal features suspicious for melanoma include structural changes in the spinous and granular layers, keratinocyte disarrangement, and loss of intercellular demarcation (disruption of the 'honeycomb pattern') [25]. Enlarged atypical cells with pleomorphic morphology, variable refractivity and angular nuclei may be found in several layers of the epidermis (pagetoid dissemination), and in the dermis. A useful advantage of RCM is that it enables identification of abnormal intra-epidermal melanocytic proliferation, granules, and dendritic structures in clinically amelanotic melanomas (**Fig 5**) [27].

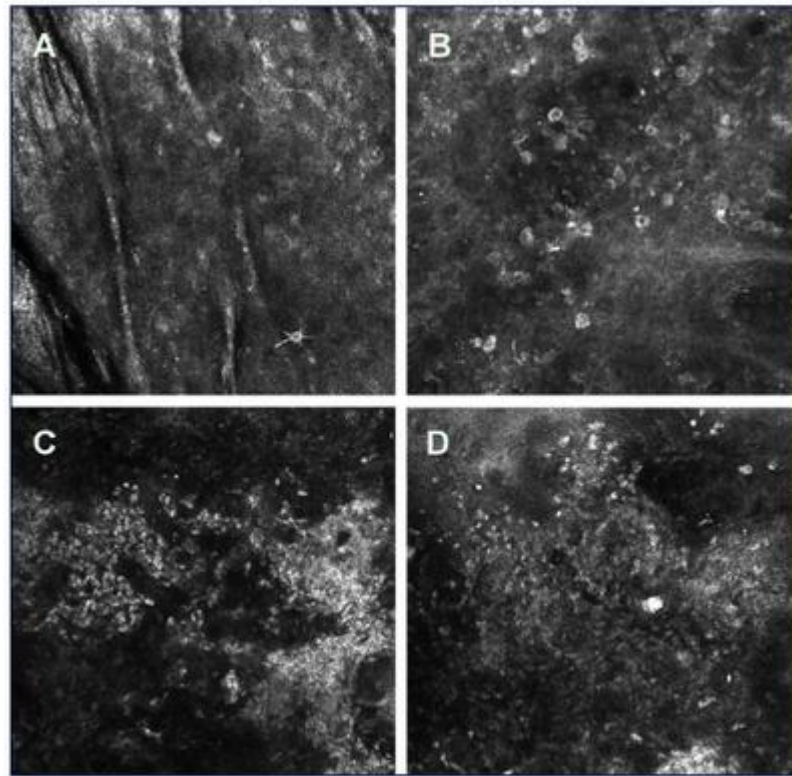


Fig 5: Reflectance confocal microscopy of melanoma. A - melanoma in situ with the presence of a few dendritic cells in superficial layers of the epidermis. B - melanoma in situ with the presence of numerous atypical pigmented cells and disarray of the epidermis. C, D - melanoma 3 micrometer Breslow thickness (C) and 1,3 micrometer Breslow thickness (D) with the total disorganization of the epidermis structure and the presence of polymorphonuclear bright cells [1]

- Actinic keratosis:
The main features of actinic keratosis assessed by in vivo RCM include irregular hyperkeratosis in the stratum corneum. In addition, the stratum granulosum is almost identical to that of normal skin, with dark nuclei, contrasted against the bright refractile cytoplasm of the keratinocytes. Whereas the nuclei in the stratum spinosum and stratum basale vary in shape, size, and haphazard orientation, these findings correspond to nuclear enlargement with pleomorphism in a pattern consistent with architectural disarray, which does not involve the full thickness of the epidermis [28].
- Squamous cell carcinoma:
Confocal features suggestive of SCC are full thickness architectural disarray and nuclear enlargement with pleomorphism observed from the basal layer to the stratum granulosum.
- Basal cell carcinoma:
Reflectance confocal microscopy morphological characteristics of basal cell carcinoma include: the presence of pleomorphism and architectural disorder of the overlying epidermis, indicative of actinic damage or the

presence of the tumour, the presence of islands of refractive tumour cells with elongated monomorphic basaloid nuclei, associated with intervening areas of low refractility, which might correspond to the mucinous stroma, nuclei of tumour cells that are polarized along the same axis of orientation, disrupting the normal honeycomb pattern of the epidermis and the dermal papillae architecture, increased dermal vasculature with prominent dilatation and tortuosity of blood vessels and trafficking of leucocytes is easily identified as bright, highly refractile round cells along the endothelial lining[29]

- **Monitoring of the treatment response:**
RCM is a useful tool in monitoring the response of actinic keratoses [30] and BCC to topical treatment such as imiquimod [31]
- **RCM usefulness in cosmetics:**
Reflectance confocal microscopy is a sensitive tool for the detection of histological changes of the epidermis and papillary dermis because of ageing. Changes in the epidermis and the superficial dermis following the treatment with anti-ageing products, lasers and pulsed light therapies can be monitored by RCM before, during and after application. RCM greatly enhances the assessment of pigmentary changes in human or animal skin over time and in response to specific stimuli such as ultraviolet radiation exposure as well as in pre-evaluation of tattoos before laser removal, helping to predict their clinical outcome [32].

Spectrophotometric intracutaneous analysis

Spectrophotometric intracutaneous analysis (SIA) is a skin-imaging technique that allows the rapid, noninvasive in vivo quantification and assessment of eumelanin, oxyhemoglobin, and dermal collagen within the human skin. SIAscopy produces independent linear measurements of each of these chromophores producing images called SIAscans. The technique uses light reflected from the skin in the visible and infrared spectra to produce images of the epidermal and dermal melanin, the vasculature, and the dermal collagen content within the lesion. The interpretation of image colors allows estimations of the underlying histopathologic features (**Fig 6**). The device is available in contact and non-contact types [33,34].

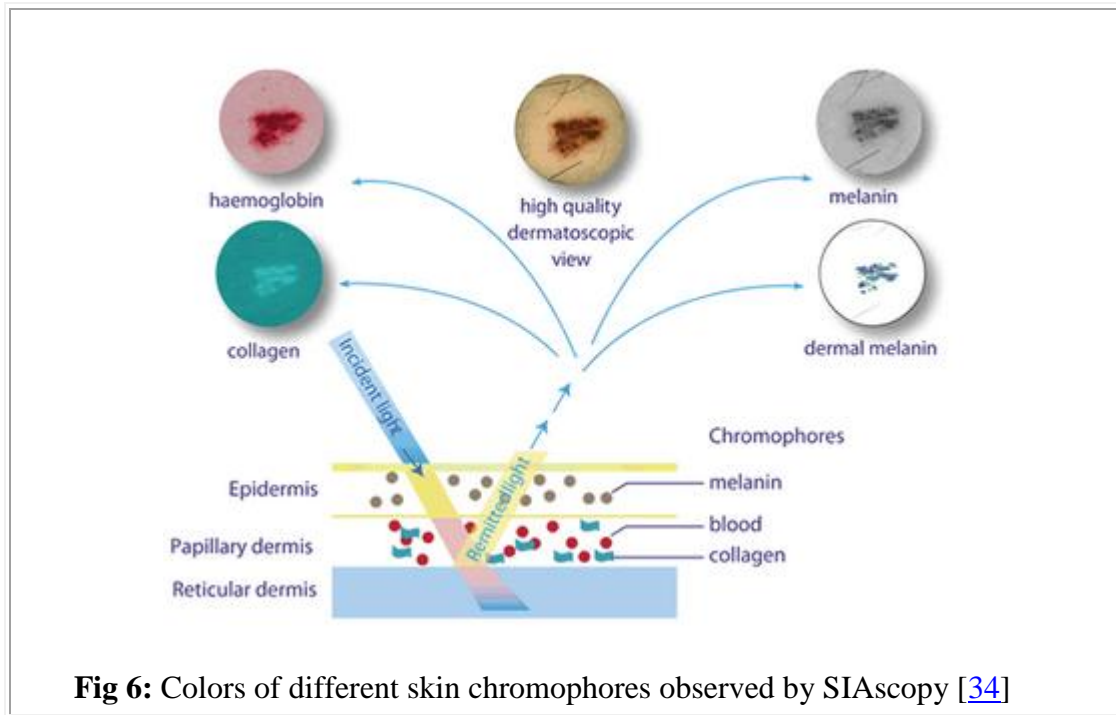


Fig 6: Colors of different skin chromophores observed by SIAscopy [34]

SIAscopy applications in dermatology

- **Pigmented skin lesions**

1. **Malignant melanoma**

Characteristic signs of invasive cutaneous melanoma observed by SIAscopy include; the presence of melanin in the dermis indicating invasion into the papillary dermis, collagen hole, and an area with no papillary collagen present, indicating destruction or replacement of the papillary dermis by melanoma. The blood distribution map shows the increase in blood levels on the lesion periphery ("erythematous blush"), which is indicative of inflammation and vasodilatation, often associated with invasive skin tumors. In addition, there is a total lack of blood in the centre of the lesion in the area which coincides with the dermal melanin, further indicating destruction, or replacement of the papillary dermis. The collagen map also shows fibrosis on the lesion periphery which is associated with early, invasive melanoma (**Fig 7**) [35,36].

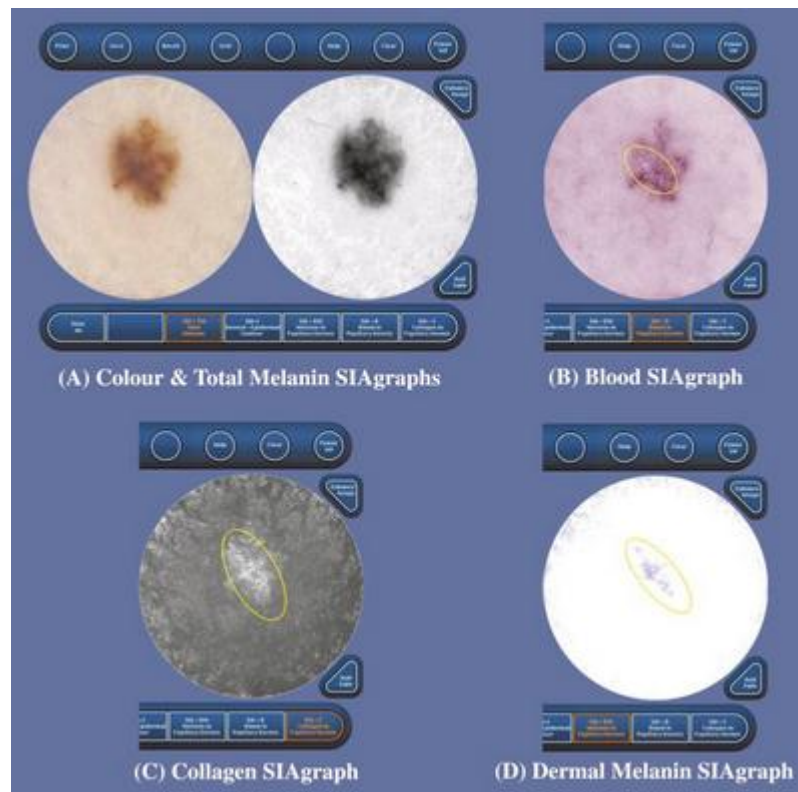


Fig 7: SIAgraphs of a superficial spreading melanoma. The colour and total melanin SIAgraphs (A) are unremarkable. However, the blood SIAgraph (B) shows a subtle blood displacement with erythematous bluish (circled). The collagen SIAgraph (C) shows no holes as this is only a Clark's level II melanoma, although there are large quantities of irregular collagen (circled) consistent with fibrosis. The dermal melanin SIAgraph (D) shows dermal melanin irregularly distributed across a large area of the lesion (circled) [36]

2 - Pigmented basal cell carcinoma

SIAscopy is of little value in differentiating malignant melanoma from pigmented basal cell carcinoma as both show the same features [36]

- ***Psoriasis and eczema***

SIAscopy can be used in diagnosing psoriasis and eczema and assessment of treatment regimen efficacy [37] including phototherapy [38].

- ***Others***

Other possible uses include assessment of aging skin [39], acute burn depth [40] and linear scars hypertrophy [41]

Cutaneous ultrasonography

Used since the 70s in dermatology, ultrasonography is based on the reflection of sound

waves throughout the tissues [42]. According to the anatomical structure, its vascularization, and density, the ultrasound waves are reflected back to the transducer that converts them into a gray scale, observed on the monitor [43]. The higher the frequency of the waves emitted by the transducer, the better the spatial resolution and subsequent visualization of structures near it. The introduction of transducers with frequency higher than 15 MHz produced the high-frequency ultrasound (HFUS). The shortest wavelength obtained by this frequency allowed a better assessment of superficial structures, significantly expanding its use in cutaneous diseases [44].

For dermatologic purposes, the ultrasound scanners using high frequencies, i.e. 15 MHz and more, producing a resolution of at least 50 μm , are essential. For such ultrasound characteristics, the term high-frequency ultrasound or high resolution ultrasound has been introduced [45]. During the propagation in the skin, the ultrasound waves undergo reflection, retraction, scattering, attenuation or absorption by the examined structures, mostly at the border of the adjacent media, generating various amplitudes of echoes influencing the ultrasound image characteristics.

Normal ultrasonographic findings of the skin:

It shows a well-defined hyperechoic band known as epidermal "entry echo" at the interface between the transducer and the skin. Underneath, the epidermis is seen as hyperechoic layer with small hypoechoic areas corresponding to hair follicles, vessels, and sebaceous glands. The next layer, the subcutaneous tissue, is hypoechoic with hyperechoic connective tissue septa separating the adipose lobules, more deeply, the superficial fascia covering the muscular tissue can be seen as hyperechoic regular line (Fig 8) [46]

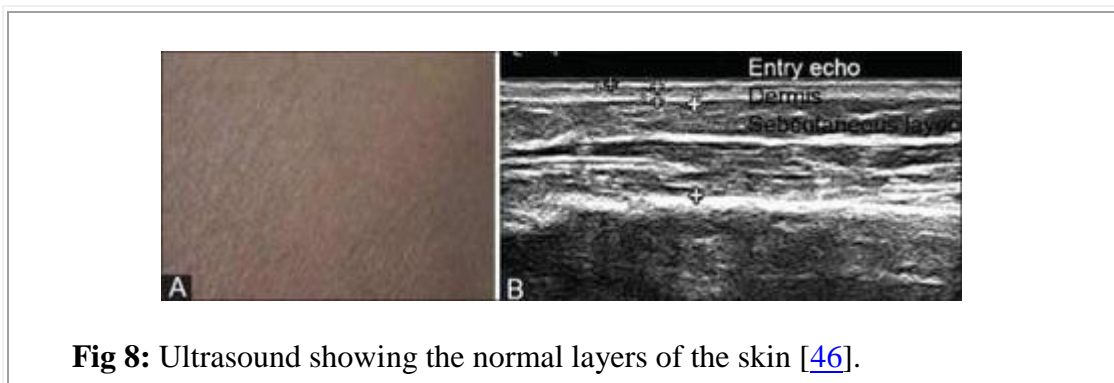


Fig 8: Ultrasound showing the normal layers of the skin [46].

The nail unit structure on HRUS shows superficial bilaminar hyperechoic parallel lines representing dorsal and ventral plates and underlying hypoechoic bed [47].

Cutaneous ultrasonography applications:

- *Skin tumors*

HRUS is used for the measurement of the thickness and the invasion depth, assessment of the borders, and follow-up after surgery, cryotherapy, and laser treatment for different benign and malignant skin tumors such as malignant melanoma, basal cell carcinoma and seborrheic keratosis. Skin tumors present as focal hypoechoic areas

within the hyperechoic epidermis and dermis. Color and power Doppler help to identify the vascularity in the lesions. Presence of abnormal intra or peri-tumoral low resistance pulsatile flow signals suggests the malignant nature of the lesion (**Fig 9**) [48].

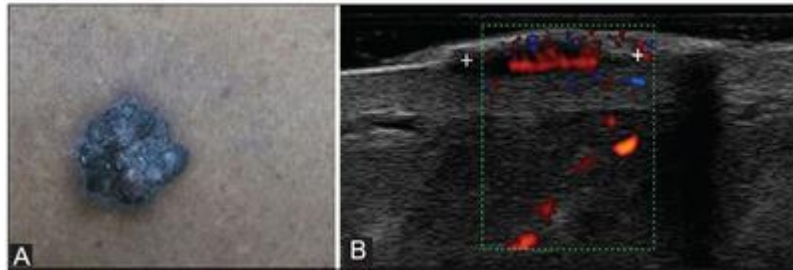


Fig 9: (A) Malignant melanoma seen as an elevated pigmented lesion with irregular shape and borders. (B) HRUS shows well-defined, solid, homogeneously hypoechoic lesion in the dermis with multiple vessels arising from the base, suggestive of high vascular density [46]

- *Sclerodermal changes of skin*

HRUS can be used to monitor the course and therapeutic efficacy of the treatment of diseases e.g. morphea, systemic scleroderma and lipodermatosclerosis [49].

- *Chronic inflammatory dermatoses*

- Psoriasis

HRUS examination shows thickened epidermis with hyperechoic as the superficial scales produce a hyper-reflective epidermal band, and a hypoechoic band of variable thickness may be seen in dermis in the acute phase (**Fig 10**) [50].

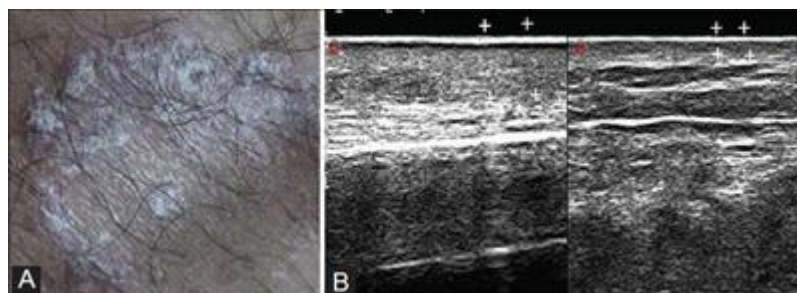


Fig 10: Ultrasonography examination of psoriatic plaque showing thickened hyperechoic epidermis and dermis compared to contralateral skin, as the superficial scales produce a hyper-reflective epidermal band [50]

- Contact dermatitis

HRUS of the affected areas in these cases shows significantly increased

thickness of dermis within a homogeneous echotexture and gross foci of hypoechoic edema [51].

- *Evaluation of exogenous component like foreign bodies and cosmetic fillers in the skin:*

The USG picture of foreign body is typical and consists of small, strong reflector surrounded by hypoechoic tissue. The combination of this appearance and positive clinical history is pathognomonic of the diagnosis [52].

On HRUS, hyaluronic acid and pure silicone are anechoic, while polyethylmethacrylate and silicone oil, calcium hydroxyapatite are hyperechoic with variable posterior acoustic shadowing [53].

- *Evaluation of nail involvement in systemic diseases and nail bed lesions* e.g. glomus tumors, nail bed cysts, and subungual exostosis [42].
- *Evaluation of other dermatologic diseases* [42]
 - Allergic dermatitis
 - Nodular erythema
 - Dermatomyositis
 - Sarcoidosis
 - Lymphedema of the limbs
 - Wound healing
 - Follow-up of localized burn lesions.

Dermatoscopy

Dermoscopy is a non-invasive technique that allows a rapid and magnified in vivo observation of the skin with the visualization of morphologic features invisible to the naked eye. The optical principle involved in dermoscopy is the interactions of light with the skin. The refractive index of the stratum corneum is higher than that of air; much of the incident light is reflected off the surface of the skin overwhelming the retina obscuring the visualization of light that is reflected from the deeper layers of the skin [54]. Two types of dermoscopy are recognized; the non polarised type (NPD) in which a liquid interface is used to eliminate the backscattering of light from the stratum corneum (**Fig 11**) and polarised dermoscope (PD) in which two polarizers are used to achieve cross-polarization allowing the dermoscope to capture the backscattered light from the deeper layers of the skin (**Fig 12**). The main advantages of the cross-polarized system are that it eliminates the necessity of a liquid interface and it does not require direct contact with the skin [55].

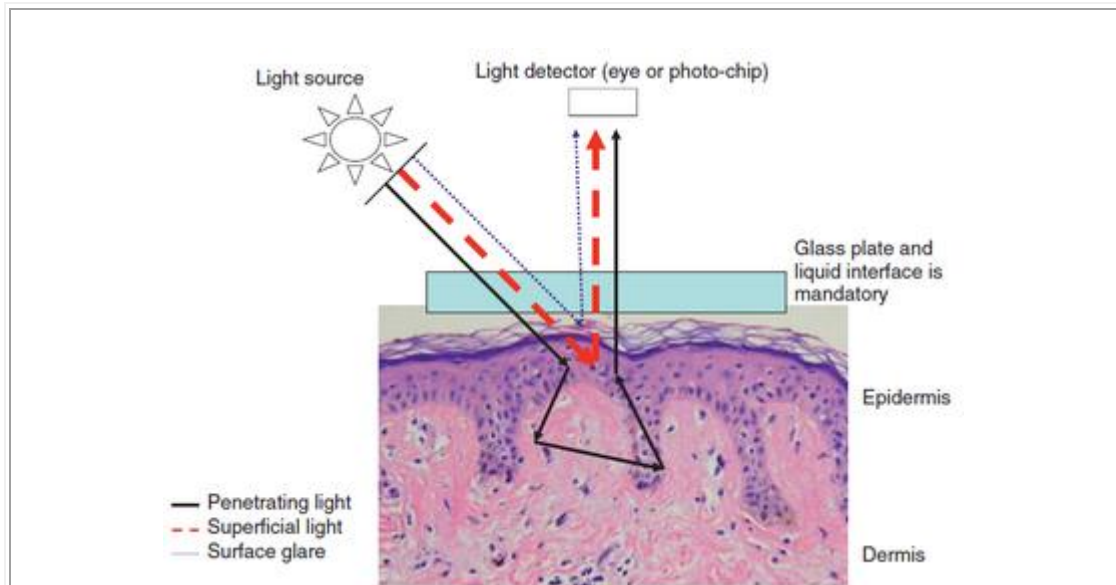


Fig 11: Schematic representation of optical properties of light during the use of contact NPD with a liquid interface [55]

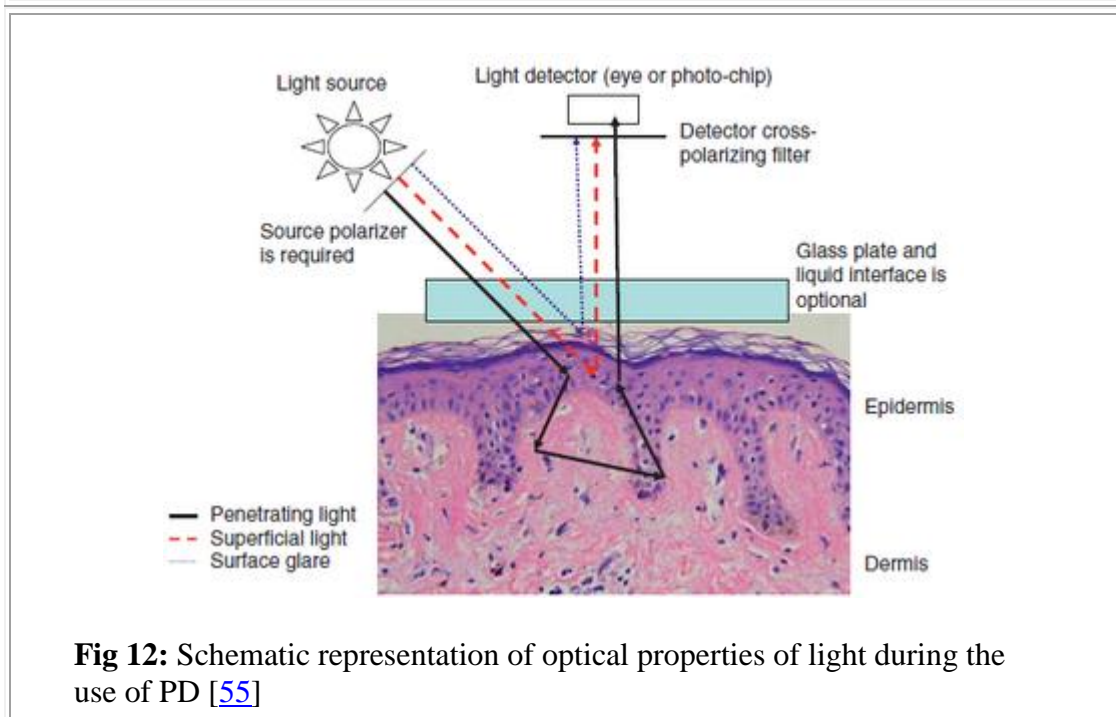


Fig 12: Schematic representation of optical properties of light during the use of PD [55]

Dermoscopy of normal skin

The structures and details of the normal skin vary depending upon the skin site, skin phototype and the degree of photodamage. Once the features of normal skin are recognized, the boundary between normality and pathology can be better recognized (Fig 13) [56].

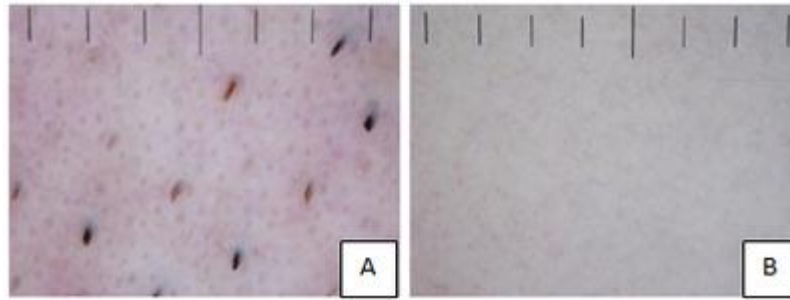


Fig 13:

A) Normal facial skin (male skin, phototype I), showing dense follicular units.

B) Normal truncal skin (male skin, phototype I), showing an absence of detail [56]

Dermoscopy applications [57]

- Classic dermoscopy: for the diagnosis of pigmented and non-pigmented skin tumors including melanocytic and non-melanocytic and benign and malignant skin tumors.
- Entomodermoscopy: for the diagnosis of skin infections and infestations caused by parasites or viral, bacterial, fungal or protozoan infections.
- Inflammoscopy: for the diagnosis of inflammatory skin diseases such as psoriasis, lichen ruber planus, pityriasis rosea and many others.
- Trichoscopy: for diagnosing hair and scalp disorders.
- Capillaroscopy: of the nail fold capillaries for the screening of autoimmune diseases.
- Dermoscopy for treatment decision and monitoring: This application gains importance especially in the light of the steadily increasing availability and use of topical treatment options for non-melanoma skin cancer.

References

1. Kardynal A, Olszewska M. Modern non-invasive diagnostic techniques in the detection of early cutaneous melanoma. *Journal of dermatological case reports*, 2014, 8(1): 1.
2. Welzel J. Optical coherence tomography in dermatology: a review. *Skin Res Technol* 2001; 7: 1-9.
3. Pagnoni A, Knuette A, Welker P, et al. Optical coherence tomography in dermatology. *Skin Res Technol*, 1999; 5: 83-87.
4. Gambichler T, Valavanis K, Plura I, et al. In vivo determination of epidermal thickness using high definition optical coherence tomography. *Br J Dermatol* 2014; 170: 737-739.

5. Applegate BE, Yang C, Rollins AM, Izatt JA. Polarization resolved second-harmonic-generation optical coherence tomography in collagen. *Opt Lett* 2004; 29:2252-4.
6. <http://www.zmpbmt.meduniwien.ac.at>
7. Schmitz L, Reinhold U, Bierhoff E, Dirschka T. Optical coherence tomography: its role in daily dermatological practice. *JDDG: Journal der Deutschen Dermatologischen Gesellschaft*, 2013; 11(6), 499-507.
8. Mogensen M, Thrane L, Jørgensen T M, Andersen P E, Jemec G B E. Optical coherence tomography for imaging of skin and skin diseases. In *Seminars in cutaneous medicine and surgery*, 2009; September, Vol. 28, No. 3, pp. 196-202. WB Saunders.
9. Bartels NG, Stieler K, Richter H, Patzelt A, Lademann J, Blume-Peytavi U. Optical coherent tomography: promising in vivo measurement of hair shaft cross section. *Journal of biomedical optics*, 2011; 16(9), 096003-096003.
10. Hoeller-Obrigkeit D, Abuzahra F, Spoeler F, Foerst M, Brans R, Erdmann S, Merk H F. Optical coherence tomography as a non-invasive diagnostic perspective for real time visualization of onychomycosis-a pilot study. In *Journal Der Deutschen Sermatologischen Gesellschaft* 2009; November (Vol. 7, No. 11, pp. 1014-1014). Commerce Place, 350 Main ST, Malden 02148, MA USA: Wiley-Blackwell Publishing Inc.
11. Welzel J, Bruhns M, Wolff HH. Optical coherence tomography in contact dermatitis and psoriasis. *Archives of dermatological research*, 2003; 295(2), 50-55.
12. Gambichler T, Hyun J, Moussa G, Tomi NS, Boms S, Altmeyer P, Hoffman K, Kreuter A. Optical coherence tomography of cutaneous lupus erythematosus correlates with histopathology. *Lupus*, 2007; 16(1), 35-38.
13. Mogensen M, Morsy HA, Nurnberg BM, Jemec GBE. "Optical coherence tomography imaging of bullous diseases." *Journal of the European Academy of Dermatology and Venereology* 22, no. 12 (2008): 1458-1464.
14. Markisz JA, Aquilia MG. *Technical magnetic resonance imaging*. McGraw Hill Professional 1996.
15. Webb, R. H. *Confocal optical microscopy*. *Reports on Progress in Physics*, 1996; 59(3), 427.
16. Rajadhyaksha M, González S, Zavislan JM. Detectability of contrast agents for confocal reflectance imaging of skin and microcirculation. *Journal of biomedical optics*, 2004; 9(2), 323-331.
17. Rajadhyaksha M, González S, Zavislan JM, Anderson RR, Webb R H. In vivo confocal scanning laser microscopy of human skin II: Advances in instrumentation and comparison with histology1. *Journal of Investigative Dermatology*, 1999; 113(3), 293-303.

18. González S, Gilaberte-Calzada Y. In vivo reflectance-mode confocal microscopy in clinical dermatology and cosmetology. *International journal of cosmetic science*, 2008; 30(1), 1-17.
19. Rajadhyaksha M, Anderson R, Webb RH. Video-rate confocal scanning laser microscope for imaging human tissues in vivo. *Applied optics*, 1999, 38(10), 2105-2115.
20. Swindells K, Burnett N, Rius-Diaz F, González E, Mihm MC, González S. Reflectance confocal microscopy may differentiate acute allergic and irritant contact dermatitis in vivo. *Journal of the American Academy of Dermatology*, 2004, 50(2), 220-228.
21. González S, Rajadhyaksha M, Anderson RR. Non-invasive (real-time) imaging of histologic margin of a proliferative skin lesion in vivo. *Journal of investigative dermatology*, 1998; 111(3), 538-539.
22. González S, Rajadhyaksha M, Rubinstein G, Anderson RR. Characterization of psoriasis in vivo by reflectance confocal microscopy. *Journal of medicine*, 1998; 30(5-6), 337-356.
23. González S, Rajadhyaksha M, González-Serva A, White WM, Anderson R. Confocal reflectance imaging of folliculitis in vivo: correlation with routine histology. *Journal of cutaneous pathology*, 1999; 26(4), 201-205.
24. Markus R, Huzaira M, Anderson RR, González S. A Better Potassium Hydroxide Preparation?: In Vivo Diagnosis of Tinea With Confocal Microscopy. *Archives of dermatology*, 2001; 137(8), 1076-1078.
25. Langley RG, Rajadhyaksha M, Dwyer PJ, Sober A J, Flotte TJ, Anderson RR. Confocal scanning laser microscopy of benign and malignant melanocytic skin lesions in vivo. *Journal of the American Academy of Dermatology*, 2001; 45(3), 365-376.
26. Pellacani G, Cesinaro AM, Seidenari S. In vivo assessment of melanocytic nests in nevi and melanomas by reflectance confocal microscopy. *Modern pathology*, 2005; 18(4), 469-474.
27. Busam K J, Charles C, Lohmann CM, Marghoob A, Goldgeier M, Halpern A C. Detection of intraepidermal malignant melanoma in vivo by confocal scanning laser microscopy. *Melanoma research*, 2002; 12(4), 349-355.
28. Aghassi D, Anderson RR, González S. Confocal laser microscopic imaging of actinic keratoses in vivo: a preliminary report. *Journal of the American Academy of Dermatology*, 2000; 43(1), 42-48.
29. Nori S, Rius-Díaz F, Cuevas J, Goldgeier M, Jaen P, Torres A, González S. Sensitivity and specificity of reflectance-mode confocal microscopy for in vivo diagnosis of basal cell carcinoma: a multicenter study. *Journal of the American Academy of Dermatology*, 2004; 51(6), 923-930.

30. Goldgeier M, Fox CA, Zavislan JM, Harris D, González S. Noninvasive imaging, treatment, and microscopic confirmation of clearance of basal cell carcinoma. *Dermatologic surgery*, 2003; 29(3), 205-210.
31. Torres A, Niemeyer A, Berkes B, Marra D, Schanbacher C, González S, Owens M, Morgan B. 5% Imiquimod cream and reflectance-mode confocal microscopy as adjunct modalities to Mohs micrographic surgery for treatment of basal cell carcinoma. *Dermatologic surgery*, 2004; 30(12p1), 1462-1469.
32. Middelkamp-Hup MA, Park HY, Lee J, Gilchrest BA, González S. Detection of UV-induced pigmentary and epidermal changes over time using in vivo reflectance confocal microscopy. *Journal of investigative dermatology*, 2006; 126(2), 402-407.
33. Cotton SD. A non-invasive imaging system for assisting in the diagnosis of malignant melanoma, 1998. (Doctoral dissertation, The University of Birmingham).
34. www.medxhealth.com
35. Menzies SW, Crotty K A, Ingvar C, McCarthy W H. An atlas of surface microscopy of pigmented skin lesions: dermoscopy 2003. Roseville: McGraw-Hill.
36. Moncrieff M, Cotton S, Claridge E, Hall P. Spectrophotometric intracutaneous analysis: a new technique for imaging pigmented skin lesions. *British Journal of Dermatology*, 2002; 146(3), 448-457.
37. Novaković L, Hawk J. Spectrophotometric intracutaneous analysis: a novel technique in the differential diagnosis of psoriasis and eczema. *BRITISH JOURNAL OF DERMATOLOGY-SUPPLEMENT*, 2002; 147, 104-104.
38. Novaković L, Cotton S, Hawk JL. Spectrophotometric intracutaneous analysis as an early non-invasive predictor of efficacy in the phototherapy of psoriasis. *Photodermatology, photoimmunology & photomedicine*, 2009; 25(2), 81-85.
39. Callaghan TM, Wilhelm KP. A review of ageing and an examination of clinical methods in the assessment of ageing skin. Part 2: Clinical perspectives and clinical methods in the evaluation of ageing skin. *International journal of cosmetic science*, 2008; 30(5), 323-332.
40. Tehrani H, Walls J, Price G, Cotton S, Sassoon EM, Hall P N. A prospective comparison of spectrophotometric intracutaneous analysis to clinical judgment in the diagnosis of nonmelanoma skin cancer. *Annals of plastic surgery*, 2007; 58(2), 209-211.
41. Kaartinen IS, Välisuo PO, Bochko V, Alander JT, Kuokkanen HO. How to assess scar hypertrophy-a comparison of subjective scales and spectrocutometry: a new objective method. *Wound Repair and Regeneration*, 2011; 19(3), 316-323.
42. Kleinerman R, Whang T B, Bard R L, Marmur E S. Ultrasound in dermatology: Principles and applications. *Journal of the American Academy of Dermatology*, 2012,

67(3): 478-487.

43. Wortsman X. Common applications of dermatologic sonography. *Journal of Ultrasound in Medicine*, 2012, 31(1): 97-111.

44. Crisan M, Crisan D, Sannino G, Lupsor M, Badea R, Amzica F. Ultrasonographic staging of cutaneous malignant tumors: an ultrasonographic depth index. *Archives of dermatological research*, 2013, 305(4), 305-313.

45. Zmudzinska M, Czarnecka-Operacz M, Silny W. Principles of dermatologic ultrasound diagnostics. *Acta Dermatovenerologica Croatica*, 2008, 16(3), 0-0.

46. Mandava A., Ravuri P R, Konathan R. High-resolution ultrasound imaging of cutaneous lesions. *The Indian journal of radiology & imaging*, 2013, 23(3), 269.

47. Altmeyer P, Hoffmann K, Stücker M, Goertz S, El-Gammal S. General phenomena of ultrasound in dermatology. *Ultrasound in Dermatology*. Berlin, Springer, 1992: 55-79.

48. Szymańska E, Nowicki A, Mlosek K, Litniewski J, Lewandowski M, Secomski W, Tymkiewicz R. Skin imaging with high frequency ultrasound-preliminary results. *European journal of ultrasound*, 2000, 12(1): 9-16.

49. Hesselstrand R, Scheja A, Wildt M, Åkesson A. High-frequency ultrasound of skin involvement in systemic sclerosis reflects oedema, extension and severity in early disease. *Rheumatology*, 2008, 47(1): 84-87.

50. Cammarota T, Pinto F, Magliaro A, Sarno A. Current uses of diagnostic high-frequency US in dermatology. *European journal of radiology*, 1998, 27: S215-S223.

51. Schmid-Wendtner M H, Burgdorf W. Ultrasound scanning in dermatology. *Archives of dermatology*, 2005, 141(2), 217-224.

52. Soudack M, Nachtigal A, Gaitini D. Clinically unsuspected foreign bodies the importance of sonography. *Journal of ultrasound in medicine*, 2003, 22(12), 1381-1385.

53. Wortsman X, Wortsman J. Sonographic outcomes of cosmetic procedures. *American Journal of Roentgenology*, 2011, 197(5), W910-W918.

54. Anderson R R, Parrish J A. The optics of human skin. *Journal of Investigative Dermatology*, 1981; 77(1), 13-19.

55. Wang S Q, Marghoob A A, Scope A. Principles of dermoscopy and dermoscopic equipment. *An Atlas of Dermoscopy*, 2012.

56. Bowling, J. Normal skin. (2012): *Diagnostic dermoscopy: the illustrated guide*, first edition. Edited by Bowling, J., published by Wiley-Blackwell, London. Chapter 1. Page: 8.

57. Zalaudek I, Lallas A, Moscarella E, Longo C, Soyer H P, Argenziano G. The dermatologist's stethoscope-traditional and new application of dermoscopy. *Dermatol Pract Conc.* 2013; 3 (2): 11.

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