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Abstract. Spectrophotometric intracutaneous analysis (SIAscopy) is an imaging technique developed for diagnostics of pigmented skin lesions. By image analysis, the displayed images indicate the potential distribution and position of melanin, blood, and collagen within the lesion. A topographic comparison was performed between SIAscopic findings and histopathology. In total, 60 patients with suspicious pigmented skin lesions were included. The lesions were SIAscopically imaged and documented before excision and histopathological preparation. Topographical comparisons between SIAscopy findings and histopathology were made. A sensitivity and specificity of 24% and 84%, respectively, were obtained for invasive melanomas. The positive and negative predicted values were 58% and 54%, respectively. The features indicating dermal melanin, blood displacement and collagen holes did only show "no" to "slight" agreement with histopathology, i.e., $\kappa \leq 0.21$. It was concluded that (i) SIAscopy-based diagnosis has low diagnostic accuracy for melanoma, (ii) single SIAscopic features do not provide reliable diagnostic information relating to the lesions internal structure on histopathology examination and (iii) SIAscopy cannot be used as a guide for localizing the maximum tumor thickness when performing the histopathological examination. The importance of validating new optical tools for tumor diagnostics with histopathological findings was demonstrated. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: 10.1117/1.JBO.18.6.061223]

1 Introduction

Clinical diagnosis of melanoma is difficult and there is a need for techniques that allow for noninvasive investigation of tumors, to facilitate early diagnosis. Spectrophotometric intracutaneous analysis (SIAscopy), is a digital computerized colorimetric analysis technique developed for assisting diagnosis of pigmented skin lesions.[1,2] The technique is based on imaging the lesion in the visible and near-infrared wavelength region (400 to 1000 nm). Based on fitting the data into a mathematical model, the technique produces images indicating the distribution, location and quantity of melanin, blood and collagen within the tissue. It is stated that the technique can detect these tissue chromophores as deep as the papillary dermis and the position of melanin relative to the dermoepidermal junction. SIAscopy has been presented as a noninvasive tool to facilitate *in vivo* diagnosis of melanoma and significant findings were the presence of blood displacement with erythematous blush, collagen holes and presence of dermal melanin within the lesion.[2]

One of the most important prognostic factors for primary melanoma without metastases is maximum histological tumor thickness.[3] Despite the importance of accurately measuring invasion depth few studies address the issue of appropriate block sampling.[4–8] If clinical diagnosis, with the aid of *in vivo* diagnostic tools, could lead not only to correct melanoma diagnosis, but also to an estimation of the melanoma invasion depth, the appropriate excision margins, and a possible sentinel node biopsy, could be performed directly. There is also a potential interest of improving the diagnostic accuracy of routine histopathologic investigation of melanoma, since correct measurements of tumor thickness also might result in even better correlation with clinical outcome. In this study the main goal was to investigate if the SIAscopic images, indicating the presence of dermal melanin, blood displacement and collagen holes; topographically correlated with histopathological findings of melanoma. The secondary objective was to investigate if SIAscopy could give a topographic indication of the localization of maximum tumor thickness and by that provide a guide for appropriate sectioning for histopathological evaluation.

2 Materials and Methods

2.1 *Patients*

The study was approved by the local ethics committee and performed in accordance with the Declaration of Helsinki. Initially,

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69 patients were recruited from the University Hospital, Gothenburg, Sweden, of which 60 lesions were included in the study. The inclusion criteria were lesions clinically suspicious for melanoma and showing positive SIAscopic findings. Thirty out of the 60 lesions were processed in total accordance to the protocol, as described in the following paragraphs. Of these 30 lesions, 19 were invasive melanomas (66% of the total number of invasive melanomas), seven melanoma *in situ* (54% of the total number melanoma *in situ*) and four benign nevi (22% of the total number of benign nevi). Nine lesions (2 invasive melanoma, two melanoma *in situ*, and five benign lesions) had to be excluded due to technical problems. Due to stepwise development of the histopathological procedure, precise adherence to all details in the study protocol did not occur for the first 30 lesions. In 25 lesions deviating from the protocol, images or detailed drawings of the sectioned specimens were available, enabling localization of the SIAscopic areas of interest. In five lesions, only schematic drawings of the specimens were available, which could not be used for precise orientation; however, areas of interest indicated by SIAscopy were very large in these lesions and thereby still possible to identify. Remaining uncertainties were eliminated by step-sectioning the tissue slices as described below. There is thus no doubt that the areas of interest were properly scrutinized, and the deviations from protocol deemed not to significantly impair the validity of the observations made.

2.2 SIAscopic Procedure

The lesions were tattoo marked at the periphery (black tattoo ink, Starbrite Colors™, USA), followed by photography, SIAscopic imaging, and, after surgical excision, histopathological investigation. For spectrophotometric intracutaneous analysis a commercial instrument was used (Siascope V, software Dermetrics Version 2.0, Astron Clinica Ltd., Great Britain). The instrument generates four images depicting the concentration of haemoglobin, melanin, collagen and dermal melanin. SIAscopic findings indicating melanoma were applied using the method described by Moncrieff, et al.[2] The obtained SIAgraphs contained signals that varied widely in their distribution within the lesion, as to the proportion of the lesion engaged and the strength of the signals. The areas with maximum signal for dermal melanin sometimes covered such small proportions of the lesions that they would have easily been missed with routine pathologic sectioning.

2.3 Histopathological Investigation

The excised specimens were routinely processed and the histological sections, 4 μm thick, were stained with haematoxylin and eosin. Before cutting the specimen in slices, the lesion was oriented and the positions of the SIAscopic areas of interest were outlined by comparisons with the overview clinical photo of the lesion. The correct localization of the areas of interest was aided by the tattoo markings. Appropriate planes of sectioning of the specimen in relation to the outlined areas were then determined.

A specially developed preparatory and documentary procedure was undertaken to find the areas of interest in the histological sections. Briefly the intended position of each section plane was marked by a shallow incision in the surface. In addition, two straight reference lines perpendicular to the marked section planes were made by a shallow incision in the surface. These incisions, as well as the sides of the specimen
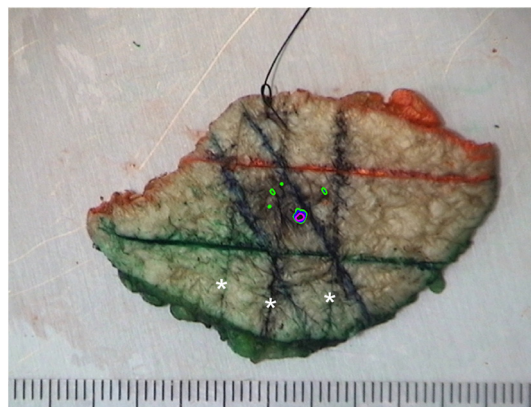
Fig. 1 A specimen of a melanoma *in situ* with the SIAscopically indicated areas of increased dermal melanin content outlined with green and violet dots/lines. The planes of sectioning are indicated with black lines (*). The parallel horizontal reference lines are inked with red and green and the oblique reference line with blue color. Note the small proportions of the lesion with SIAscopic signal displaying dermal melanin.

(corresponding to the ends of the future tissue blocks), were inked with different colors to ensure correct identification in the histological sections. The specimen was photographed with all the incision markings (Fig. 1). The location of every region of interest was identified by its block belonging and within pertinent blocks by the distance of its boundaries to the perpendicularly made reference incisions. The oblique reference lines made it possible to determine the depth of each of the histological step-sections and locate the areas of SIAscopic interest in the histological sections with a high accuracy. The maximal possible error deemed to be within 0.3 to 0.5 mm. To ensure complete cover of areas of interest, these areas were step-sectioned at 100 to 500 μm depending of the size of the area. The remaining parts of the lesions were also step-sectioned at intervals not larger than 500 μm.

2.4 Additional Image Processing

To further improve the method for topographic comparison between the SIAscopic findings and the fixed surgical specimen, additional image processing was implemented in eight cases (four invasive melanomas, three melanomas *in situ* and one dysplastic nevus). This was performed by using an image warping algorithm[9,10] to match the image data from the clinical image, the SIAgraph and the image of the fixed surgical specimen. By selecting characteristic landmarks, including the tattoo markings, in the different images, transformation parameters were determined. The SIAscopic information could then be accurately overlaid the image of the excised fixed lesion with a maximal error rate of about 1 mm.

2.5 Statistical Analysis

Sensitivity and specificity for each feature, as well as for the combined features, were calculated. Positive (PPV) and negative predictive values (NPV) were calculated. 95% confidence intervals (CI) were calculated for sensitivity and specificity and for PPV and NPV.[11] Kappa (κ)-statistics were made to compare the agreement between SIAcopy and histopathology on the features of dermal melanin, blood displacement and collagen holes.

3 Results

Table 1 shows the demography of the lesions. Eight of the 29 identified invasive melanomas in the study had a Breslow thickness less than 0.76 mm (Clark II-III), nine lesions Breslow thickness $0.76 - \leq 1.0$ mm (Clark II-III) and 12 lesions Breslow thickness ≥ 1.1 (Clark III-V). The median and mean Breslow thickness of the invasive melanomas in the study were 1.0 and 1.29 mm, respectively (range 0.4 to 5.5 mm).

Figure 1 illustrates how the SIAscopic data was transferred to the histopathologic specimen. The figure shows a melanoma *in situ* with marked section planes and reference lines. The SIAscopically indicated areas of dermal melanin are outlined together with inked reference incisions on the surface and marked reference lines. Note the small proportions of the lesion with SIAscopic signal displaying dermal melanin.

Detailed analysis of how the separate features topographically correlate with the histopathological findings is summarized in Table 2. Twenty-six of the 29 invasive melanomas showed indications of dermal melanin in the SIAscopic analysis; however, histopathological presence of tumor cells in the dermis in the SIAscopically indicated area, was only histologically confirmed in 18 lesions. On average, the topographic agreement of the SIAscopic finding of dermal melanin and the actual presence of tumor in the dermis was 69%. On the contrary, two of the three invasive melanomas not showing any SIAscopic signal of dermal melanin, revealed ample content of pigmented tumor cells and melanophages in the dermal component of the lesions during histopathologic examination. Thus a topographically distinct SIAscopic signal indicating dermal melanin in these lesions was, contrary to the expectation, missing in the corresponding parts of histological sections.

The SIAscopic finding of blood displacement this was indicated in 22 invasive melanomas, but only histopathogically confirmed in six lesions (Table 2). SIAscopic indications of collagen holes were found in seven invasive melanomas and confirmed in three lesions (Clark II, IV and V, Breslow thickness 1.0, 1.8, and 5.5 mm, respectively). In 12 invasive melanomas, with no SIAscopic indication of collagen holes, the histological findings were such that collagen holes would be expected (eight lesions with Clark level III, Breslow thickness ranging from 0.7 to 1.8 mm, and four lesions with Clark level IV, Breslow thickness ranging from 1.1 to 4.6 mm). The feature of erythematous blush was found in almost all invasive melanomas (28/29), as well as in almost all melanomas *in situ* (12/13) and in many nonmelanoma lesions (11/18). Thus this parameter was found to be unspecific and difficult to verify in histology since it is a variable feature and no effort was put into making further comparisons.

Eight lesions were subjected to complementary digital image processing to improve the matching of SIAscopic data with histopathology in order to seek for improved agreement. Figure 2 illustrates the detailed topographic matching of the SIAscopic image (of dermal melanin) with the image of the lesion. However, a separate analysis of these lesions did not

Table 1 Details of the dataset.

Diagnosis	Number	Breslow thickness (mm)	Clark level
Invasive melanoma	29		
Superficially spreading melanoma	23	0.4–5.5	II–V
Lentigo malignant melanoma	2	1.0–1.2	II–IV
Nodular melanoma	3	1.5–1.7	III
Unclassified melanoma[a]	1	0.7	II
Melanoma *in situ*	13		
Nonmelanoma	18		
Benign melanocytic lesions	4		
Dysplastic melanocytic lesions	10		
Pigmented basal cell carcinoma	2		
Seborrhoeic keratosis	2		

[a]Unclassified because of difficulties in histogenetic typing due to regression.

Table 2 SIAscopic findings and its correlation to histopathological findings in melanoma and nonmelanoma lesions.

	SIAscopic signal								
	Dermal melanin				Blood displacement		Collagen holes		Erythematous blush[a]
Histopathological findings	Present in SIAscopy	Due to pigmented tumor	Due solely to melano-phages	Due to epidermal hyper-pigmentation	Confirmed in histopath.	Not confirmed in histopath.	Confirmed in histopath.	Not confirmed in histopath.	Present in SIAscopy[a]
Invasive melanomas (n = 29)	26	18	25	13	6	16	3	4	28
Melanoma *in situ* (n = 13)	13	0	13	11	0	8	0	4	12
Nonmelanoma lesions (n = 18)	17[k]	6	15	11	2	6	1	1	11

[a]Not all specimens were assessed for this feature, so the feature is not divided into confirmed and not confirmed.
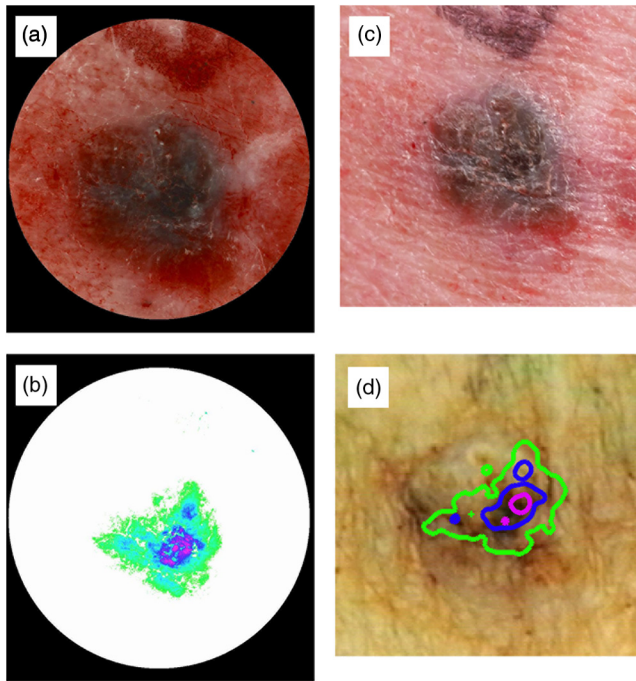
Fig. 2 Images showing equally scaled (a) dermoscopy image, (b) SIAscopy image of dermal melanin, (c) photo of the lesion before surgery and (d) photo of the excised lesion. The lesion is a case of SSM, Breslow thickness 1.7 mm, Clark level III. By performing image warping the SIAscopy image has been mapped onto the excised lesion image so that guided sectioning can be carried out. Only the contours of the SIAscopic signal of dermal melanin are mapped onto the image of the lesion in order to avoid loss of details for orientation.

reveal any improvement in the agreement (data not shown), why the evaluation of these lesions is included in the overall dataset.

To compare the agreement between the separate SIAscopy features and histopatholoic findings κ-statistics was used. For blood displacement κ was found to be 0.21 ($p < 0.01$) indicating a weak agreement. For dermal melanin and collagen holes κ were found to be 0.10 ($p > 0.05$) and 0.03 ($p > 0.05$), respectively, implying no agreement. The sensitivity and specificity for the individual features analyzed are presented in Table 3. The combined features (presence of blood displacement with erythematous blush, collagen holes and presence of dermal melanin) resulted in a sensitivity of 24% (CI: 12% to 42%) and a specificity of 84% (CI: 67% to 93%) for invasive melanoma.

Table 3 SIAscopic features—sensitivity and specificity for invasive melanoma ($n = 29$).

Feature	Sensitivity %(95% CI)	Specificity %(95% CI)
Collagen holes	24 (12–42)	81 (64–91)
Blood displacement	76 (58–88)	48 (32–66)
Erythematous blush	97 (83–99)	26 (14–43)
Blood displacement with erythematous blush	72 (54–85)	61 (44–76)
Dermal melanin	90 (74–96)	3 (1–16)

The reported sensitivity and specificity is exclusively based on the presence of the combination of SIAscopic features and not influenced by clinical examination. To evaluate the proportion of lesions with positive or negative SIAscopy findings that were correctly diagnosed we calculated PPV and NPV. The PPV was found to be 58% (CI: 32% to 81%) and the NPV was 54% (CI: 40% to 67%) for invasive melanomas ($n = 29$) in this study. Taken together, these results indicate poor agreement to histopathology for the identified SIAscopy features.

4 Discussion

This is to our knowledge the first detailed study of the correlation between SIAscopic features and histopathological findings in pigmented skin lesions. The results imply a poor correlation between the SIAscopic information and the histopathologic features of the investigated lesions. Topographical comparison between the individual SIAscopic features and histopathology reveals that the SIAscopic signals of melanin in the dermis, especially as a sign of tumor cells in the dermis, as well as blood displacement and collagen holes, often prove incorrect. Instead, we observed that the SIAscopic signal of dermal melanin very often topographically corresponds to the presence of melanophages and/or epidermal hyperpigmentation, implying that a colorimetric change due to epidermal hyperpigmentation might be interpreted as the presence of dermal melanin.

The SIAscopic findings of blood displacement and collagen holes were verified by histopathology in only a few cases. Instead, we observed a topographical correlation between the histologically confirmed presence of high amounts of melanin, both epidermal and dermal, and SIAscopic indications of blood displacement and collagen holes. Thus it is likely that high amount of melanin disturbs the colorimetric analysis in such a way that it is misinterpreted by the instrument as blood displacement and/or collagen holes.

Since findings of dermal melanin, blood displacement and collagen holes are all findings that are expected to correlate to invasive tumor, our hypothesis was that SIAscopy could help in locating invasive tumor areas. Unfortunately we found that the topographical correlation between SIAscopy and histopathology was poor. Thus we conclude that SIAscopy cannot be used for locating the area with maximal tumor invasion.

The over-all sensitivity of the SIAscopic findings obtained in this study was very low, 24%, but the specificity was high, 84%. Our sensitivity differs substantially from the results obtained by Moncrieff et al, who reported a sensitivity and specificity at around 80%.[2] Our study population differs from Moncrieff et al by numbers (60 versus 348) but the number of included melanomas do not differ substantially (42 versus 52).[2] Thus there are a higher percentage of invasive melanomas in our study population. The levels of invasion probably differ somewhat, since we have the same median Breslow thickness (1.0 mm), but a difference in mean Breslow thickness (1.29 mm in our study population compared to 1.52 mm in the study population of Moncreiff et al). This result was surprising as the enriched population on our study, i.e., incidence of melanoma higher than seen in Moncrieff et al., should have theoretically performed better than was published in the Moncrieff publication.

Taken together, this study demonstrates poor correlation between SIAscopic finding and histopathology. The technique has low diagnostic accuracy for melanoma, and features pointed out by the SIAscope do not provide reliable diagnostic

information relating to the lesions internal structure on histopathology examination. Thus, the methodology cannot be used as a guide for localizing the maximum tumor thickness when performing the histopathological examination. The study also demonstrates the importance of validating new optical tools for tumor diagnostics with histopathological findings.

Acknowledgments

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