

## ORIGINAL ARTICLE

# Optical super-high magnification dermoscopy of benign and malignant melanocytic lesions in correlation with histopathology

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## Summary

**Background and objectives:** Technical advances have allowed for significant improvements in imaging techniques in recent years. Specifically, lesions can now be depicted at a much higher magnification – up to 400 x – using optical super-high magnification dermoscopy (OSHMD).

**Patients and methods:** This is a retrospective, observational study assessing 99 melanocytic lesions in patients from the University Hospital Heidelberg. Dermoscopy (20 x) and OSHMD images (90 x, 120 x, 150 x, 180 x and 270 x) were acquired. OSHMD images were assessed for the presence/absence of pigment network, distribution, size and color of cells, dots and roundish nests (small/large), structureless areas and vessels in nevi versus melanomas. Correlation studies with histopathology were performed.

**Results:** We found that in OSHMD atypical pigment network, irregular dark dots, atypical vessels and irregular grey out-of-focus cells are clues to melanoma. Black dots and small roundish nests in OSHMD images corresponded to nests of atypical melanocytes in melanomas in histopathology. Grey out-of-focus cells in OSHMD images corresponded to melanophages in histopathology and in irregular distribution were found more frequently in melanomas.

**Conclusions:** We conclude that knowing about histopathological correlates OSHMD may support differentiating nevi from melanomas.

## KEYWORDS

dots, melanoma, nevus, network, super-high magnification dermoscopy

## BACKGROUND

The melanoma incidence is further increasing, and the mortality rate remains high.<sup>1</sup> Early diagnosis is of utmost importance for clinical outcomes, as surgical excision of initial melanomas enables curative therapy. In contrast, advanced melanomas are more likely to relapse or metastasize. Dermoscopy is a well-established tool for the clinical

assessment of melanocytic lesions, enhancing melanoma diagnosis for experienced clinicians compared to naked-eye examination.<sup>2</sup>

Technical advances have facilitated significant improvements in imaging techniques in recent years. Specifically, lesions can now be depicted at a much higher magnification of up to 400 x by optical super-high magnification dermoscopy (OSHMD).<sup>3–5</sup> First reports on OSHMD go back to the year 2018, when OSHMD images of a junctional and dermal nevus were presented.<sup>3</sup> OSHMD is performed

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with a capillaroscopy lens arranged to the Medicam1000s (Fotofinder Systems, Bad Birnbach). It is especially allowing sight on pigmented cells and vascular structures. Even single pigmented cells and blood flow in vessels may be assessed by OSHMD.<sup>4</sup> It has already been applied in pigmented lesions, basal cell carcinomas, but also infectious diseases such as scabies and fungal infections.

High hopes were placed on OSHMD to improve the diagnostic accuracy in otherwise clinically unclear lesions including melanocytic lesions.<sup>3,6,7</sup> Cinotti et al. have performed a study classifying 190 clinically atypical melanocytic lesions. Some OSHMD features were found to be particularly more frequent in nevi, others in melanomas.<sup>6,7</sup> Large pigmented cells and cells with irregular shape and size were mainly reported in melanomas.<sup>6</sup> Additionally, polymorphous blue out-of-focus cells were observed more frequently in melanomas in association with regression areas.<sup>6</sup> In melanomas dots were observed in OSHMD and hypothesized to correspond to free melanin.<sup>6</sup> However, in all of the previously published studies correlation with histopathology has not yet been performed.

In our study we aimed to clearly define relevant OSHMD features encountered in nevi and melanomas and correlate these with findings of histopathology. This is the first study to evaluate dermoscopy, OSHMD and histopathology side by side.

## MATERIAL AND METHODS

The study was approved by local ethics committees (approval no. S-836/2020) and performed in accordance with the Declaration of Helsinki principles.

### Study design and setting

This is a retrospective, observational study using descriptive statistics to assess OSHMD in nevi and melanomas. The study was performed at the University Department of Dermatology Heidelberg (Germany).

### Participant selection and imaging procedures

Patients with atypical melanocytic lesions were included. Melanocytic lesions were documented and assessed by dermoscopy at 20-fold magnification and OSHMD by using a digital videodermatoscope (ATBM master, Fotofinder Systems, Bad Birnbach, Germany). For each lesion one dermoscopic image at a 20 x magnification was acquired with polarized light (Medicam 1000s, Fotofinder Systems, Bad Birnbach, Germany) and OSHMD images (90 x, 120 x, 150 x, 180 x, 270 x) with a capillaroscopy lense (D-Scope III, Fotofinder Systems, Bad Birnbach, Germany).

## Variables

Clinical data included patient age and sex as well as lesion localization, procedure (excision versus follow-up/expert opinion) and results of histopathology (nevus, melanoma in situ, invasive melanoma, melanoma metastasis). OSHMD images were retrospectively investigated by J.K.W., H.A.H., and F.T. without blinding to histopathology results for the presence/absence of features previously identified by others<sup>8</sup> including pigment network, distribution, size and color of cells, dots and roundish nests (small/large), structureless and regression areas. Presence of all criteria was assessed in nevi versus melanomas.

## Histopathological correlation

In all excised lesions histopathological characteristics were correlated with OSHMD features. To illustrate correlation with histopathology, images of five exemplary melanomas are depicted for each OSHMD criterion presented (Figures 1–4). Histopathology was performed by using routine hematoxylin and eosin stains at 25 x, 50 x and 100 x magnification. For atypical and/or malignant melanocytic lesions we additionally performed Melan-A immunostains.

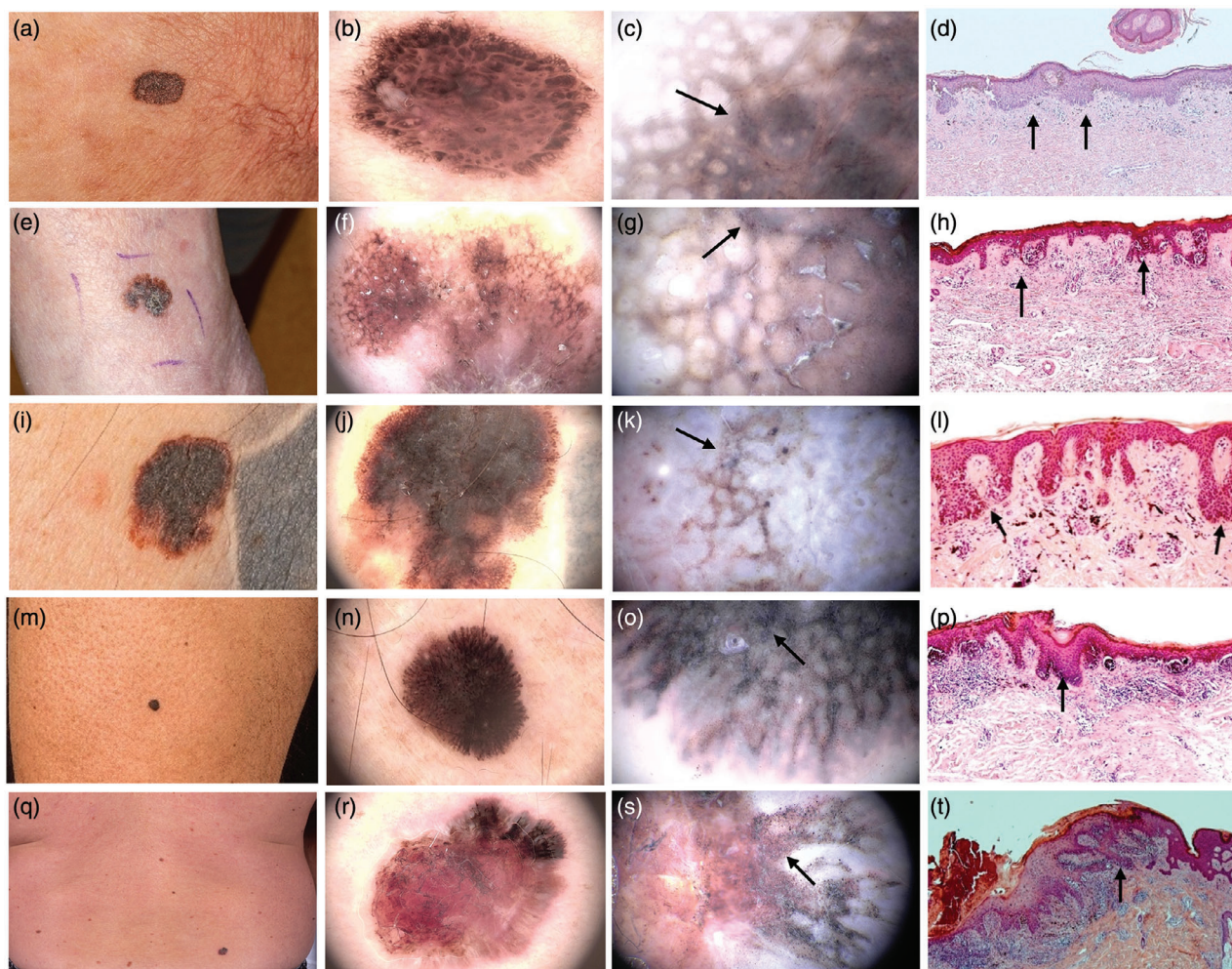
When cutting excised lesions for further histopathological assessment, a printout of the dermoscopic image of each lesion was used to determine the preferred cutting plane. This approach ensured precise correlation between dermoscopic characteristics and the histological plane.

## Statistical analysis

Patient characteristics were analyzed descriptively by tabulation. Means, standard deviations (SD), ranges and percentages are reported. Statistical differences in the distribution of categorical variables were evaluated with a Fisher's exact test. Additionally, a multivariate logistic regression analysis was performed;  $p < 0.05$  was considered statistically significant. Bonferroni corrections for multiple testing were applied whenever applicable. Data analyses was performed with SPSS Version 29 (SPSS, Chicago, IL, U.S.A.).

## RESULTS

In this retrospective observational study, patients from the University Hospital Heidelberg were included, and 99 melanocytic lesions suspicious for malignancy – either primary cutaneous melanoma or cutaneous metastasis of melanoma – were evaluated using OSHMD. Examinations were performed between September 2020 and August 2021.



**FIGURE 1** Atypical network. (a–d) Confluent dermal papillae corresponding to thickened network in melanoma in situ as well as (e–t) atypical network, pseudopods and streaks in invasive melanomas. (a, e, i, m, q) Overview images, (b, f, j, n, r) dermoscopy, (c, g, k, o, s) OSHMD and (d, h, l, p, t) histopathology are provided for each melanoma.

The mean age of patients was 52.3 years (range 8–91 years). Patients included 39 female (39.4%) and 60 male individuals (60.6%) (Table 1). Lesions were mainly localized on the trunk ( $n = 62$ , 62.6%), followed by the upper ( $n = 15$ , 15.2%) and lower extremities ( $n = 13$ , 13.1%), head and neck ( $n = 7$ , 7.1%) and palmoplantar skin ( $n = 2$ , 2%). Investigated lesions included 65 nevi (65.7%), eleven melanomas in situ (11.1%), 22 invasive melanomas (22.2%) and one melanoma metastasis (1.0%). The final diagnosis of all malignant lesions was made by histopathology. Benign lesions were either excised, diagnosed by expert opinion or uneventful follow-up. Encountered OSHMD features were evaluated for all 99 lesions and results are listed in Table 2.

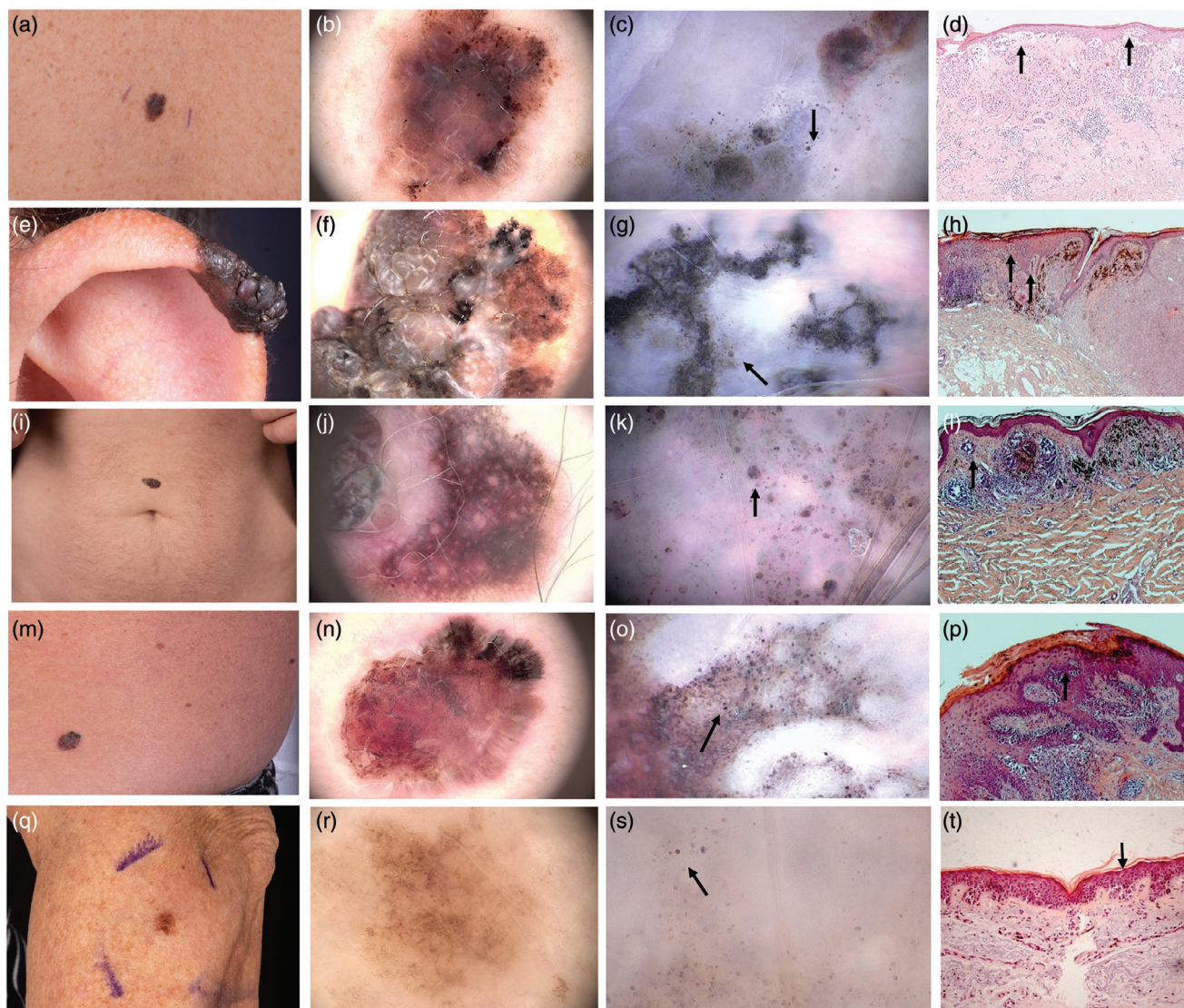
### Assessment of pigment network and cells

Correlation studies revealed that regular pigment network of nevi in dermoscopy corresponded to a delicate network

with regularly distributed cells of homogenous pigmentation in OSHMD (Figure 1). Thickened network found in melanomas in situ in dermoscopy is even better depicted by OSHMD, revealing confluent homogenous pigmentation instead of single cells along a network (Figure 1). Streaks are also a well-known dermoscopic feature in melanomas corresponding to areas of greyish confluent pigmentation with associated black dots in OSHMD (Figure 1). OSHMD confirmed that a regular pigment network was less frequent in melanomas than nevi (11.8% vs. 61.5%,  $p < 0.001$ ). In comparison network only in parts of lesions (52.9% vs. 24.6%,  $p = 0.007$ ), partially thickened network (26.5% vs. 9.2%,  $p = 0.037$ ) or streaks (14.7% vs. 3.1%,  $p = 0.045$ ) were more frequently seen in melanomas versus nevi in OSHMD.

OSHMD, compared to dermoscopy, allows for the examination of single cells, which were assessed in terms of size, distribution, and color. Correlation studies with histopathology allowed to determine polygonal brown cells inside a





**FIGURE 2** Dots and roundish nests. Nests of melanoma cells in epidermis and dermis corresponding to dots/globules in dermoscopy and dots/roundish nests in OSHMD in (a–d, m–p) invasive, (e–l) nodular and (q–t) early melanomas. (a, e, i, m, q) Overview images, (b, f, j, n, r) dermoscopy, (c, g, k, o, s) OSHMD and (d, h, l, p, t) histopathology are provided for each melanoma.

network corresponding to keratinocytes, large roundish cells in irregular distribution to atypical melanocytes and grey out-of-focus cells to melanophages. OSHMD revealed pigmented cells of regular size (72.3%) and light brown color (72.3%) in regular distribution along a pigment network (60%) more often in nevi, while melanomas frequently showed irregularly distributed cells (76.5%) of varying size (79.4%) and black or grey color (52.9%) (Table 1).

### Assessment of dots and roundish nests

Dots and globules are well-defined dermoscopic features to evaluate in melanocytic lesions and may be studied more in detail by OSHMD. Our correlation studies with histopathology indicate that black dots in OSHMD corresponded to

single melanocytes within the epidermis (Figure 2) and greyish pigmentation of bizarre configuration next to dots to confluent tumor nests in the dermis (Figure 2). Small roundish nests in OSHMD images were found to correspond to aggregated tumor cells in melanomas, with the color of nests providing information on localization of tumor cells within the epidermis or dermis (Figure 2). In OSHMD images irregularly distributed dots were more frequent in melanomas; 47.1% of melanomas versus 4.6% of nevi showed black or grey dots ( $p < 0.001$ ). Small roundish nests were also seen more often in melanomas than nevi; black or grey roundish nests were found in 26.5% of melanomas versus 1.5% of nevi ( $p < 0.001$ ) (Table 1). Regarding distribution and size of larger roundish nests, no significant differences were found in nevi versus melanomas. Here, correlation studies indicated that in melanomas larger roundish nests corresponded to an accumulation of tumor





**FIGURE 3** Irregular vessels. Irregular vessels and areas of regression in invasive melanomas depicted by (a, e, i, m, q) overview images, (b, f, j, n, r) dermoscopy, (c, g, k, o, s) OSHMD and (d, h, l, p, t) histopathology. Vessels are indicated by arrows.

cells (Figure 2), whilst they represented nests of pigmented (monomorphic) melanocytes in nevi.

### Assessment of structureless and regression areas

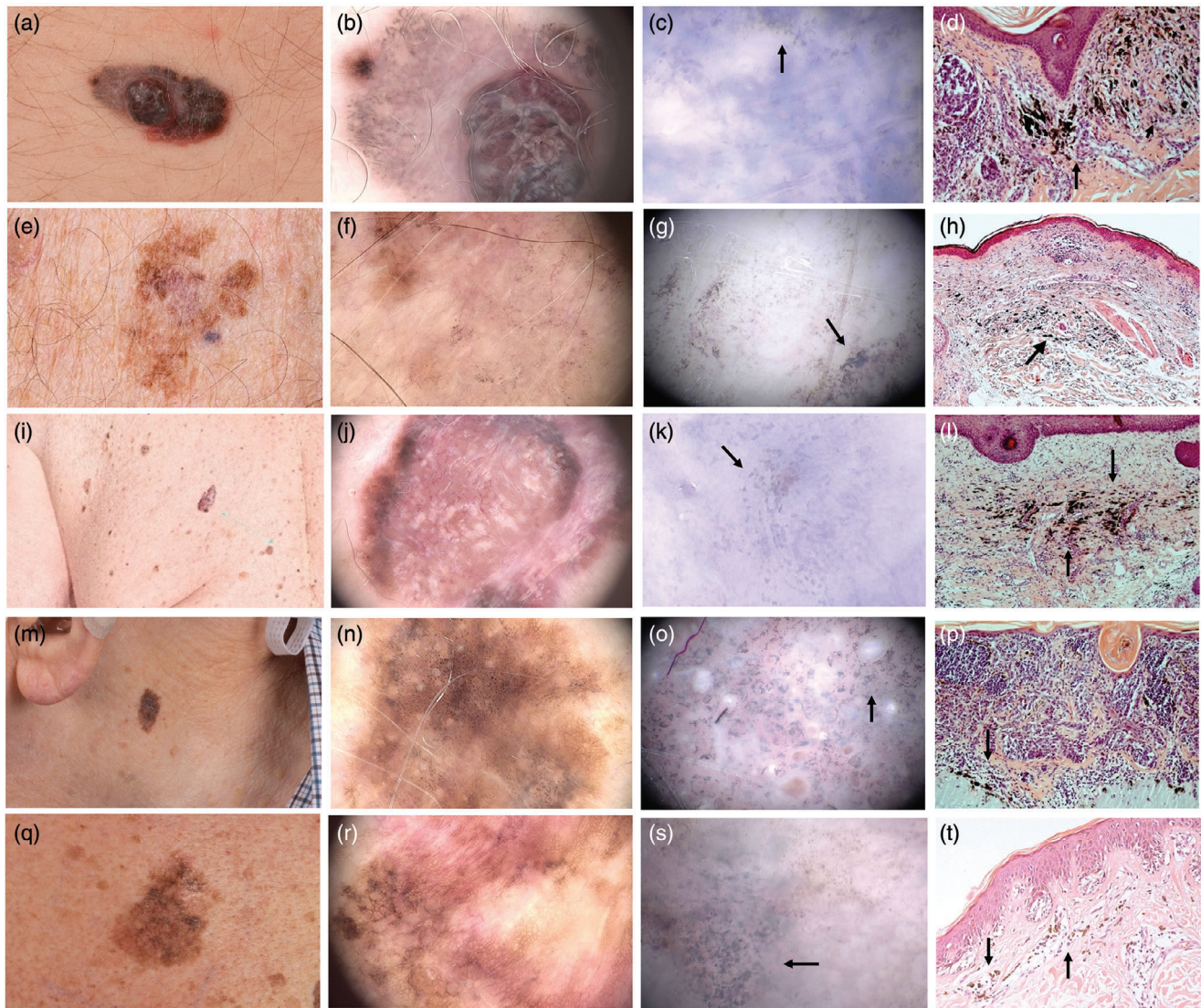
Dermoscopy defines blue-white veil and black, brown and/or grey structureless areas as relevant criteria of malignancy. In OSHMD images red structureless areas (64.7% vs. 12.3%,  $p < 0.001$ ) and blue areas (55.9% vs. 13.8%,  $p < 0.001$ ) were more frequent in melanomas than nevi. Significance was not met for grey areas (41.2% vs. 23.1%,  $p = 0.068$ ). Histopathological correlation confirmed that blue areas corresponded to pigmented cells within the deeper dermis. In OSHMD images, blue areas were observed in blue nevi

with deeply localized pigment but were more frequently found in invasive melanomas, where they corresponded to melanoma cells within the dermis (Figure 2).

Dermoscopy allows to depict vessels especially in areas of regression and atypical vascular pattern is along the criteria of melanoma. OSHMD depicts vessels in more detail; irregular vessels were defined by either asymmetrical distribution and/or polymorphous appearance (Figure 3). In contrast, regular vascular pattern in OSHMD included homogenous vessels in the center of papillae within a network. In OSHMD images irregular vessels were found more frequently in melanomas than nevi (32.4% vs. 4.6%,  $p < 0.001$ ), while regular vessels were less frequent in melanomas (14.7% vs. 40%,  $p = 0.012$ ).

We found that grey out-of-focus cells depicted by OSHMD corresponded to melanophages in histopathology





**FIGURE 4** Melanophages. (d, h, l, p, t) Melanophages corresponding to (c, g, k, o, s) grey out of focus cells in OSHMD and (b, f, j, n, r) peppering in dermoscopy images of (a, e, i, m, q) melanomas depicted.

(Figure 4). Accumulation of such grey out-of-focus cells in OSHMD were found in areas of regression in dermoscopic images. In OSHMD images grey out-of-focus cells were either irregularly distributed without any pattern or regularly distributed within the center of papillae. Irregularly distributed melanophages were mainly found in melanomas (35.2% vs. 9.2%,  $p = 0.002$ ) (Figure 4), while regularly distributed cells were seen in nevi, but also in some melanomas.

### Multivariate analysis

Multivariate logistic regression considered all variables significant according to our previous analyses. Here, the presence of a partially thickened network ( $p = 0.035$ ), cells in irregular distribution ( $p = 0.018$ ) and of different size ( $p = 0.004$ ), black/grey dots ( $p = 0.015$ ), red areas ( $p = 0.011$ )

and blue areas ( $p < 0.001$ ) was more indicative of malignant lesions (Table 3).

### DISCUSSION

Optical super-high magnification dermoscopy is a novel imaging technique depicting lesions at a much higher magnification, thus allowing to detect and define new structural lesion criteria. Until now, reports on OSHMD for the evaluation of melanocytic lesions are still limited, highlighting the need for further investigation. In our study we focused on benign and malignant melanocytic lesions, since differentiating atypical nevi from melanomas is of utmost importance in clinical routine and diagnostic criteria of melanocytic lesions are most complex. Altogether, 99 melanocytic lesions were assessed with one third of lesions being malignant comprising in situ melanomas, invasive

**TABLE 1** Patient characteristics.

	n	Percent
<b>Sex</b>		
Female	39	39.4
Male	60	60.6
<b>Lesion localization</b>		
Trunk	62	62.6
Upper extremities	15	15.2
Lower extremities	13	13.1
Head/neck	7	7.1
Palmoplantar	2	2.0
<b>Lesion excised</b>		
Yes	71	71.7
No	28	28.3
<b>Histopathology</b>		
Nevus	65	65.7
Melanoma in situ	11	11.1
Melanoma invasive	22	22.2
Melanoma metastasis	1	1.0

melanomas and one melanoma metastasis. Lesions were localized on different parts of the body in order to account for possible variations of OSHMD features by anatomic site. All malignant and 56.9% of benign lesions were excised, which allowed correlation studies of OSHMD features with findings of histopathology. Most previous studies have compared OSHMD features to other non-invasive diagnostic techniques as reflectance confocal microscopy (RCM). Here, our study is the first to correlate OSHMD and histopathology.

From our results we conclude that relevant dermoscopic criteria may be visualized by OSHMD and correlated with histopathology.<sup>8</sup> In this context, atypical network of lesions in dermoscopy corresponded to an irregular distribution of cells and confluent pigmentation instead of a delicate pigment network in OSHMD and was found in melanomas more frequently than in nevi. Irregular distribution of cells in OSHMD images has already been reported in melanomas and referred to as scattered cells by Cinotti et al.<sup>6</sup> They additionally reported network with edged papillae in nevi, without edged papillae in melanomas in OSHMD.<sup>6</sup> This criterion was not included in our study, since we could not sufficiently and reproducibly differentiate edged versus unedged papillae in OSHMD images. However, we additionally included streaks as a pattern to evaluate in OSHMD images. As a limitation of OSHMD only small parts of lesions are depicted per image. However, for several features such as streaks taking into consideration the distribution and pattern along the entire lesion is of relevance. Altogether, it is important to consider all prevailing criteria before drawing conclusions from OSHMD images. Providing histopathology allowed to perfectly correlate thickened network in dermoscopy and OSHMD images

with confluent papillae and extensive basal melanocytes in histopathology.

In line with previous studies we found that various cell types are to identify by OSHMD.<sup>6,7,9</sup> Polygonal brown cells inside a network corresponded to keratinocytes, large roundish cells to atypical melanocytes and grey out-of-focus cells to melanophages.<sup>6</sup> This correlation was confirmed by the histopathological analyses we performed. Moreover, our correlation studies corroborated previously published reports demonstrating black dots in OSHMD corresponding to single melanocytes or small groups of melanocytes within the upper epidermis.<sup>10</sup> Cinotti et al. also defined dots as a relevant criterion to assess by OSHMD and reported those more frequently in melanomas than nevi.<sup>9</sup> They additionally evaluated roundish nests and found that brown and black nest were more frequent in nevi, violet nests in melanomas.<sup>9</sup> We also found brown or black small roundish nests more frequently in melanomas, while larger roundish nests were seen in both nevi and melanomas. Differences concerning assessment of differently colored nests by Cinotti et al. versus our study underline that the assessment of criteria in OSHMD images shows a highly subjective character.

Next, we found blue structureless areas in 55.9% of melanomas versus 13.8% of nevi. Correlation studies with histopathology confirmed that blue areas corresponded to pigmented cells within the dermis.<sup>11</sup> In line with our study blue structureless areas in OSHMD images were also reported by Cinotti et al. and more frequent in melanomas.<sup>6,9</sup>

OSHMD reveals a near-perfect sight on vessels not only in areas of regression, but also within network structures. Our OSHMD images revealed irregular vessels in 32.4% and regular vessels in 14.7% of melanomas, which emphasizes that it is not sufficient to just evaluate parts of lesions, but consider presence of criteria throughout the overall lesion. Cinotti et al. also studied shape of vessels and reported irregular and dilated vessels more frequently in melanomas, in contrast to linear vessels in nevi.<sup>6</sup> Further on, examining dynamic changes such as blood flow velocity in OSHMD might be of interest.

Moreover, especially in areas of regression, our correlation studies confirmed that grey out-of-focus cells corresponded to melanophages. Cinotti et al. have previously assumed that those cells corresponded to melanophages, which were more frequent in melanomas.<sup>9</sup> Here, we could further specify that irregularly distributed melanophages were found more frequently in melanomas, while regularly distributed melanophages in the center of papillae were found in both nevi and melanomas. Altogether, symmetry is one of the most relevant criteria regarding dignity of melanocytic lesions. From our study we conclude that for the assessment of lesion symmetry, dermoscopy and histopathology are superior to OSHMD. Single criteria in parts of lesions seem less important with regard to lesion dignity than overall lesion configuration. Even in

**TABLE 2** OSHMD features found in nevi versus melanomas.

			Nevi (n = 65)		Melanomas (n = 34)		p value
			n	%	n	%	
Network							
Regular			40	61.5	4	11.8	< 0.001
In lesion parts			16	24.6	18	52.9	0.007
Partially thickened			6	9.2	9	26.5	0.037
Streaks/ pseudopods			2	3.1	5	14.7	0.045
Cells							
Regular distribution			39	60.0	4	11.8	< 0.001
Irregular distribution			20	30.8	26	76.5	<0.001
Same size			47	72.3	3	8.8	< 0.001
Different size			10	15.4	27	79.4	< 0.001
Brown color			47	72.3	17	50.0	0.014
Grey color			11	16.9	18	52.9	< 0.001
Dots							
Brown color			2	3.1	6	17.6	0.018
Black/grey color			3	4.6	16	47.1	< 0.001
Small roundish nests							
Brown color			1	1.5	5	14.7	0.017
Black/grey color			1	1.5	9	26.5	< 0.001
Larger roundish nests							
Regular distribution			5	7.7	0	0	0.162
Irregular distribution			6	9.2	2	5.9	0.711
Same size			7	10.8	0	0	0.092
Different size			4	6.2	2	5.9	1.00
Color of out-of-focus structureless areas							
Red			8	12.3	22	64.7	< 0.001
Blue			9	13.8	19	55.9	< 0.001
Grey			15	23.1	14	41.2	0.068
Vessels							
Regular			26	40.0	5	14.7	0.012
Irregular			3	4.6	11	32.4	< 0.001
Grey out-of-focus cells							
Regularly distributed grey areas			10	15.4	3	8.8	0.533
Regular			7	10.8	4	11.8	1.0
Irregular			6	9.2	12	35.3	0.002

melanomas regular network was present in parts of lesions in OSHMD images, while partially thickened network and single dots were also found in nevi. Nevertheless, OSHMD may serve as an important add-on tool to closer study lesions.

To best visualize various structures such as cells, network, dots, vessels and melanophages, OSHMD images were taken at different magnifications. Focusing on structures of interest is necessary to acquire excellent images and visualize structural elements within different layers of the epidermis or dermis.

Results from our study illustrate novel insights achieved by OSHMD, allowing a magnification up to single cells. This may be of special interest in the context of research and for more experienced clinicians. How far younger clinicians might benefit from OSHMD remains to be further assessed. Most likely OSHMD might be used for specific tasks such as differentiating lentigo maligna from solar lentigo.<sup>4,9,12,13</sup> Compared with other imaging technologies the fast and easy clinical application of OSHMD is an important advantage.<sup>4</sup> Besides application in melanocytic lesions, OSHMD has been evaluated in non-melanoma



**TABLE 3** Multivariate analysis.

	p value
<b>Network</b>	
Partially thickened	0.035
<b>Cells</b>	
Irregular distribution	0.018
Different size	0.004
<b>Dots</b>	
Black/grey color	0.015
<b>Color of out-of-focus structureless areas</b>	
Red	0.011
Blue	< 0.001

skin cancer and infectious diseases,<sup>14–18</sup> which are further potential application fields of OSHMD. Artificial intelligence (AI) is now integrated in many imaging technologies including dermoscopy and line-field optical coherence tomography (LC-OCT) and might as well be of interest for OSHMD.<sup>19–20</sup>

Limitations of our study include the descriptive and retrospective assessment of lesion criteria. Acquisition and evaluation of OSHMD images considerably depends on the expertise of the investigator. Further studies need to be performed blinded to diagnosis and dermoscopy. A prospective study reflecting clinical workflow is of interest to first perform dermoscopy with recording of the suspected diagnosis, then OSHMD and recording whether the suspected diagnosis changed. Otherwise, OSHMD might be used for unclear lesions only to assess additional benefit. Additionally, with a high frequency of dysplastic nevi included frequencies of specific criteria in nevi versus melanomas were most likely less pronounced compared with including only clear-cut benign versus malignant lesions.<sup>7</sup> Moreover, this was only a pilot study and the number of melanomas for histopathological correlation limited. The limited number of lesions and the many variables included in the multivariate analysis limit the extent of possible conclusions. However, variables significant in our multivariate analysis are among those extensively discussed above. Another limitation is that, similar to dermoscopy, OSHMD provides horizontal images of the skin surface, making an exact correlation with vertical histology sections impossible. Co-localization within a dermoscopic image using OSHMD is problematic. LC-OCT for example enables a direct correlation with histology and dermoscopy, since not only horizontal images corresponding to dermoscopy are provided, but also vertical images corresponding to histology, and ultimately 3D.<sup>21,22</sup>

Altogether, we conclude that OSHMD as an add-on tool may provide relevant clues to diagnose melanocytic lesions. Yet, further studies are necessary to confirm correlations reported within our study and evaluate whether OSHMD conveys a diagnostic benefit in clinical application.

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## CONFLICT OF INTEREST STATEMENT

H.A.H. received honoraria and/or travel expenses from companies involved in the development of devices for skin cancer screening: Scibase AB, FotoFinder Systems GmbH, Heine Optotechnik GmbH, Magnosco GmbH. J.K.W. also received honoraria from FotoFinder Systems GmbH. All other authors state no conflict of interest.

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