Received: 19 May 2022 | Accepted: 1 October 2022

DOI: 10.1111/srt.13215

ORIGINAL ARTICLE

WILEY

Comparison of reflectance confocal microscopy and line-field optical coherence tomography for the identification of keratinocyte skin tumours

Elisa Cinotti[1,2] | Martina Bertello[1] | Alessandra Cartocci[3] | Diletta Fiorani[1] | Linda Tognetti[1] | Valentina Solmi[1] | Simone Cappilli[4,5] | Ketty Peris[4,5] | Jean Luc Perrot[2,6] | Mariano Suppa[2,7,8] | Veronique Del Marmol[7] | Pietro Rubegni[1]

[1]Dermatology Unit, Department of Medical, Surgical and Neurological Sciences, Università degli Studi di Siena, Siena, Italy

[2]Groupe d'Imagerie non invasive de la Société Française de Dermatologie, Paris, France

[3]Department of Medical Biotechnologies, University of Siena, Siena, Italy

[4]Dermatology Unit, Department of Medicine and Traslational Surgery, Catholic University of Sacred Heart, Rome, Italy

[5]Dermatology Unit, Department of Medical and Surgical Sciences, A. Gemelli University Hospital Foundation – IRCCS, Rome, Italy

[6]Department of Dermatology, University Hospital of Saint-Etienne, Saint-Etienne, France

[7]Department of Dermatology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium

[8]Department of Dermatology, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium

Correspondence
Elisa Cinotti and Martina Bertello, Dermatology Unit, Department of Medical, Surgical and Neurological Sciences, University of Siena, Viale Bracci 16, 53100 Siena, Italy.
Email: elisa.cinotti@unisi.it; bertellomartina@hotmail.com

Abstract

Background: Reflectance confocal microscopy (RCM) and line-field confocal optical coherence tomography (LC-OCT) are non-invasive imaging devices that can help in the clinical diagnosis of actinic keratosis (AK) and cutaneous squamous cell carcinoma (SCC). No studies are available on the comparison between these two technologies for the identification of the different features of keratinocyte skin tumours.

Objectives: To compare RCM and LC-OCT findings in AK and SCC.

Methods: A retrospective multicenter study was conducted. Tumours were imaged with RCM and LC-OCT devices before surgery, and the diagnosis was confirmed by histological examinations. LC-OCT and RCM criteria for AK/SCC were identified, and their presence/absence was evaluated in all study lesions. Gwet AC1 concordance index was calculated to compare RCM and LC-OCT.

Results: We included 52 patients with 33 AKs and 19 SCCs. Irregular epidermis was visible in most tumours and with a good degree of agreement between RCM and LC-OCT (Gwet's AC1 0.74). Parakeratosis, dyskeratotic keratinocytes and both linear dilated and glomerular vessels were better visible at LC-OCT than RCM ($p < 0.001$). Erosion/ulceration was identified with both methods in more than half of the cases with a good degree of agreement (Gwet AC1 0.62).

Conclusions: Our results suggest that both LC-OCT and hand-held RCM can help clinicians in the identification of AK and SCC, providing an in vivo and non-invasive identification of an irregular epidermis. LC-OCT proved to be more effective in identifying parakeratosis, dyskeratotic keratinocytes and vessels in this series.

KEYWORDS
actinic keratosis, line-field optical coherence tomography, reflectance confocal microscopy, squamous cell carcinoma

1 | INTRODUCTION

Cutaneous squamous cell carcinoma (SCC) is the second most common cancer in humans.[1] It arises from a malignant proliferation of epidermal keratinocytes usually with benign clinical behavior, despite it could be locally invasive or metastatic.[2] SCC and its premalignant precursor actinic keratosis (AK) represent a significant burden in dermatologic practice because of their rising prevalence as a result of the increasing life expectancy and sun exposure.

Non-invasive imaging tools such as dermoscopy, reflectance confocal microscopy (RCM) and conventional optical coherence tomography (OCT) can help the early diagnosis of keratinocyte skin tumors.[3,4] RCM and OCT allow to appreciate the loss of the normal epidermal layering in keratinocyte tumors, and RCM can detect single-cell proliferation thanks to its higher lateral resolution (1μm vs. 7.5 μm).[5] Recently, line-field confocal OCT (LC-OCT) has been developed to combine the high lateral resolution of RCM (1 μm) and the deep penetration of OCT (500 μm).[6] Our study aimed to describe which features can be observed in AK and SCC using both RCM and LC-OCT and to compare these two imaging techniques for keratinocyte skin tumors.

2 | MATERIAL AND METHODS

SCCs and AKs were retrospectively collected from the database of the Dermatology Departments of the University Hospital of Siena (Italy) and Erasme University Hospital of Brussels (Belgium). All lesions were excised and subjected to confirmatory histopathological examination.

2.1 | LC-OCT device

Vertically-orientated images/videos were acquired with a CE-marked prototype of LC-OCT (DAMAE Medical, Paris, France) by two investigators expert in skin imaging (E.C. and M.S.). The device, composed of a handheld probe connected to a central unit and a display, has 1.2-μm axial resolution, 1.3-μm lateral resolution, 500-μm scanning depth, 1.2-mm lateral field of view, and 10-frames/second acquisition.[6]

2.2 | RCM device

In vivo RCM examination was performed with the hand-held VivaScope 3000 camera (MAVIG GmbH, Munich, Germany), which uses an 830-nm wavelength laser and provides images with a lateral resolution of 1 μm and axial resolution of 3–5 μm that correspond to a horizontal 920 μm × 920 μm section of the skin up to 250 μm of depth.[7]

2.3 | Image acquisition and evaluation

A drop of paraffin oil was placed between the tumour and the glass window at the tip of the LC-OCT or RCM camera to ensure refractive index matching. At least four LC-OCT images and one LC-OCT video and at least one RCM image of different depths (epidermis, DEJ and dermis when allowed by a not excessive thickness of the epidermis) and one RCM stack, considered relevant for the diagnosis, were captured for each lesion (Figure 1).

LC-OCT and RCM criteria were chosen based on previously described histological, RCM, and LC-OCT criteria more predictive of the diagnosis of AK and SCC[5,8–14] (Table 1). Three observers blinded for any clinical, dermoscopic and histopathological data (E.C., L.T. and A.L.) evaluated all study lesions for the presence/absence of each criterion. Any disagreement was solved by consensus among the evaluators.

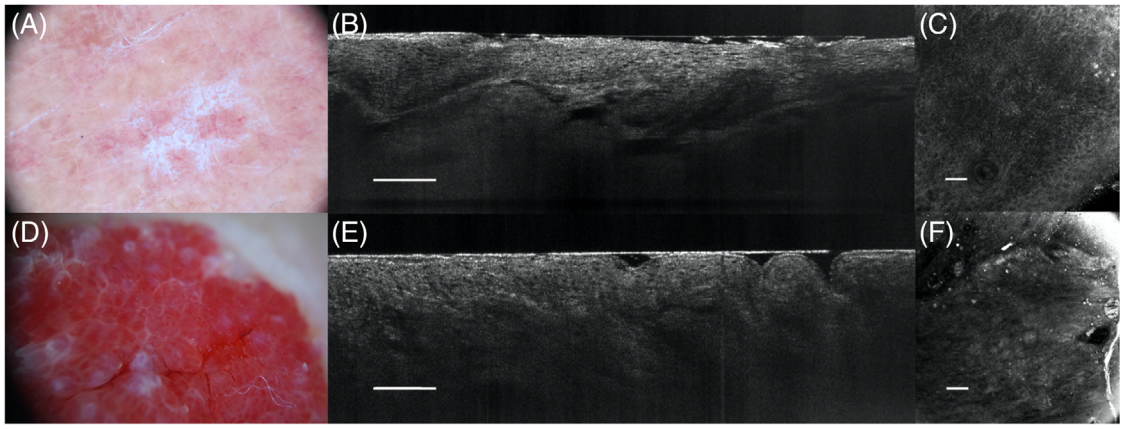

FIGURE 1 (a–c) Actinic keratosis: (A) dermoscopic, (B) line-field confocal optical coherence tomography (LC-OCT) and (C) reflectance confocal microscopy (RCM) images. Scale bar = 100 μm. LC-OCT shows hyperkeratosis, acanthosis and irregular keratinocytes. RCM shows irregular keratinocytes. (D–F) Squamous cell carcinoma: (D) dermoscopic, (E) line-field confocal optical coherence tomography (LC-OCT) and (F) reflectance confocal microscopy (RCM) images. Scale bar = 100 μm. LC-OCT and RCM images reveal the presence of an irregular epidermis. LC-OCT also revealed a not well outlined dermal-epidermal junction.

TABLE 1 Description of the selected criteria for AK and SCC (based on literature review and expert opinion)

RCM/LC-OCT criteria	Definition
Hyperkeratosis	Stratum corneum thicker than 20 μm. It is measured on a stack at RCM and on a vertical section at LC-OCT.
Parakeratosis	Nucleated cells appearing as dark nuclei surrounded by bright outline of corneocytes at RCM. Small dark areas corresponding to cell nuclei inside the stratum corneum at LC-OCT
EROSION/ulceration	Dark areas with sharp borders and irregular contours filled with amorphous material, cellular debris and small particles
Irregular keratinocytes	Variation in shape and size of epidermal keratinocytes (irregular honeycomb pattern) at RCM. Variation in shape and size of epidermal nuclei of keratinocytes and variation in reflectivity of keratinocytes at LC-OCT. Totally disarranged epidermis was also included in this parameter.
Dyskeratotic keratinocytes	Large targetoid cells with either a bright center and dark peripheral halo or with a dark center and bright rim surrounded by a dark halo under RCM. Large hyper-reflective round cells inside the epidermis at LC-OCT.
Pigmented epidermis	Diffuse (cobblestone) or clustered (mottled) hyper-reflective keratinocytes.
Keratin pearls	Whorl-shaped accumulation of keratin, appearing as highly refractive, speckled structure at RCM and as a dark area with hyperreflective and undulated lines under LC-OCT.
Tumour strands	Round, demarcated structures (nest-like structures) in the dermis that are often surrounded by fibrosis at RCM. Broad strands of atypical and dyskeratotic keratinocytes interrupting the dermo-epidermal junction and projecting irregularly at LC-OCT.
Dilated linear vessels	Elongated large hypo-reflective structures with possible visible blood cell flow inside in the dermis.
Glomerular vessels	Coiled canalicular hypo-reflective structures.
Elastosis	Network of thick, highly reflective collagen bundles intermixed with moderately reflective, lace-like elastic fibers under RCM. Hypo-reflective areas in the dermis at LC-OCT.

Abbreviations: AK, actinic keratoses; LC-OCT, line-field confocal optical coherence tomography; RCM, reflectance confocal microscopy; SCC, squamous cell carcinoma.

2.4 | Statistical analysis

Previous descriptive analysis was carried out, absolute frequencies and their percentages were calculated for qualitative variables, mean and standard deviation for quantitative ones. Differences in the proportion of the positive LC-OCT and RCM parameters were evaluated by the proportion test, and the agreement between LC-OCT and RCM parameters was evaluated by the percentage of agreement and the Gwet's AC1 concordance index (moderate 0.4–0.6; good 0.6–0.8; very good 0.8–1). Each statistical test was two-tailed and was considered significant for p-values < 0.05. Statistical analyses were carried out by R software version 3.6.2.

3 | RESULTS

Fifty-two histologically proven tumors, 33 (63.5%) SCCs (23 in situ and 10 invasive) and 19 (26.5%) AKs, were included in the study (Table 2). Study lesions belonged to 26 (50%) females and 26 (50%) males. The mean age was 66.6 ± 13.7 years. Forty-five patients attended the Erasme University Hospital of Brussels and seven attended the University Hospital of Siena. The majority of SCCs were located in the head/neck region (54.5%), followed by upper extremities (24.2%), trunk (12.1%) and lower extremities (9.1%). Similarly, AKs were located on the head/neck region (57.9%), followed by upper extremities (26.3%), trunk (10.5%) and lower extremities (5.3%). No statistically significant differences in gender, age and body locations were found between AK and SCC. Irregular epidermis was found in most AKs (15 [88.2%] RCM and 17 [89.5%] LC-OCT) and SCCs (30 [96.8%] RCM and 28 [93.3%] LC-OCT) under both RCM and LC-OCT (Table 2).

The two devices led to explore the epidermis beneath the stratum corneum in the majority of the cases with a good grade of agreement (Gwet's AC1 0.66) and to a greater extent with LC-OCT ($p = 0.039$). Irregular epidermis and erosion/ulceration were identified with both methods with a good degree of agreement (Gwet's AC1 index 0.74 and 0.62, respectively) (Table 3). Parakeratosis, as well as dyskeratotic keratinocytes, have been found to a greater extent with LC-OCT than with RCM (70.6% vs. 15%, $p < 0.001$ and 82.5% vs. 33.3%, $p < 0.001$, respectively). LC-OCT more frequently identified the presence of vessels, both glomerular and dilatated linear (80 vs. 34.3%, $p < 0.001$ and 68.6% vs. 25,7%, $p < 0.001$, respectively). Invasive tumour strands were seen only in SCC. If we considered invasive SCCs with a visible DEJ, invasive strands were seen in five (62.5%) of eight cases under LC-OCT and in none out of three cases under RCM. Pigmented epidermis and keratin pearls were mainly absent both at RCM and LC-OCT.

4 | DISCUSSION

Comprehensive descriptions of AK/SCC under LC-OCT and RCM have already been performed.[5,15,16] To our better knowledge, this is the first study that compares single criteria of RCM and LC-OCT in AK and SCC. Both technologies have proved to be a valuable aid for the non-invasive diagnosis of these tumors being able to identify an irregular

TABLE 2 Distribution of LC-OCT and RCM criteria across study lesions

	RCM			LC-OCT			*p*-Value
	Total 52	SCC 33	AK 19	Total 52	SCC 33	AK 19	
Hyperkeratosis	22 (48.9)	12 (44.4)	10 (55.6)	32 (61.5)	24 (72.7)	8 (42.1)	0.296
Parakeratosis	6 (15.0)	5 (21.7)	1 (5.9)	24 (70.6)	16 (69.6)	8 (72.7)	<0.001
Erosion/Ulceration	24 (52.2)	16 (57.1)	8 (44.4)	29 (59.2)	19 (63.3)	10 (52.6)	0.631
Irregular keratinocytes	45 (93.8)	30 (96.8)	15 (88.2)	45 (91.8)	28 (93.3)	17 (89.5)	1.000
Dyskeratotic keratinocytes	13 (33.3)	8 (36.4)	5 (29.4)	41 (82.0)	24 (77.4)	17 (89.5)	<0.001
Pigmented epidermis	3 (7.9)	1 (4.8)	2 (11.8)	0 (0.0)	0 (0.0)	0 (0.0)	0.221
Keratin pearls	3 (8.6)	3 (15.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.131
Invasive strands	2 (5.7)	2 (10.5)	0 (0.0)	8 (22.9)	8 (40.0)	0 (0.0)	0.088
Dilatated vessels	9 (25.7)	6 (31.6)	3 (18.8)	35 (68.6)	18 (56.3)	17 (89.5)	<0.001
Glomerular vesselS	12 (34.3)	8 (42.1)	4 (25.0)	40 (80.0)	25 (80.6)	15 (78.9)	<0.001
Elastosis	15 (42.9)	5 (26.3)	10 (62.5)	10 (23.3)	5 (20.0)	5 (27.8)	0.109
Visible epidermidis from GRanular layer	39 (75.0)	22 (66.7)	17 (89.5)	48 (92.3)	29 (87.9)	19 (100.0)	0.039
Visible –DEJ and superficial dermis	35 (67.3)	19 (57.6)	16 (84.2)	28 (62.2)	16 (57.1)	12 (70.6)	0.756

Note: Data are presented as number (%) unless otherwise stated. Percentages are calculated on the total of the evaluable cases: numbers do not always add up to the total of AKs and SCCs due to missing values (criteria were not evaluated in case of poor image quality and/or focal visualization of the corresponding skin layer). *p*-values refer to the comparison between RCM and LC-OCT on both SCC and AK.
Abbreviations: AK, actinic keratoses; DEJ, dermo epidermal junction; LC-OCT, line-field confocal optical coherence tomography; RCM, reflectance confocal microscopy; SCC, squamous cell carcinoma.

TABLE 3 Agreement between LC-OCT and RCM parameters

	Total concordance	SCC concordance	AK concordance	Agreement
Hyperkeratosis	0.20	0.20	0.22	42.3%
Parakeratosis	−0.09	−0.04	−0.15	26.9%
Erosion/Ulceration	0.62	0.49	0.86	73.1%
Irregular keratinocytes	0.74	0.80	0.63	76.9%
Dyskeratotic keratinocytes	−0.09	−0.06	−0.14	23.1%
Pigmented epidermis	0.51	0.53	0.49	61.5%
Keratin pearls	0.54	0.34	0.83	61.5%
Invasive strands	0.26	−0.01	0.62	46.1%
Dilatated vessels	−0.03	0.01	−0.09	28.8%
Glomerular vessels	0.03	0.07	−0.03	32.7%
Elastosis	0.22	0.26	0.18	46.1%
Visible epidermidis from granular layer	0.66	0.49	0.88	75.0%
Visible DEJ and superficial dermis	0.35	0.29	0.47	51.9%

Concordance was calculated by Gwet's AC1 index (moderate 0.4–0.6; good 0.6–0.8; very good 0.8–1). Total Gwet's AC1 refers for both SCC and AK.

epidermis in most cases and with a good agreement both in AK and SCC (total concordance 0.74; SCC concordance 0.80; AK concordance 0.63; agreement 76.9%). The irregular epidermis can be clearly seen as a variation in shape and size of the epidermal keratinocytes under RCM and as a variation in shape and size of epidermal nuclei of keratinocytes at LC-OCT. The few cases that did not show an irregular epidermis were mainly AKs where keratinocytes irregularity can be more subtle and few SCCs with images that were probably collected in not significant areas.

Hyperkeratosis and ulceration were observed in nearly half of the cases, whereas parakeratosis and dyskeratotic keratinocytes were visible in a great number of cases at LC-OCT and in a less extent at RCM (parakeratosis was found in 70.6% at LC-OCT and 15.0% at RCM $p < 0.001$; dyskeratotic keratinocytes were seen in 82.0% at LC-OCT and 33.3% at RCM $p < 0.001$). It should be considered that LC-OCT allows to evaluate a large area of the skin in a single image, whereas the hand-held RCM used in our study has a smaller field of view that can hamper the identification of parakeratosis and dyskeratotic

keratinocytes that can be focally visible. Moreover, identification of parakeratosis is probably easier with LC-OCT than with RCM, since cell nuclei within the stratum corneum are easily visible as hyporeflective areas with LC-OCT, and the definition of parakeratosis is more controversial under RCM.[13] Hyperkeratosis showed a poor agreement between RCM and LC-OCT, probably because of the different fields of view and measurement methods of the skin layer thicknesses.

As expected, vessels were better visible under LC OCT than RCM, probably in relation to the wider and deeper field of view. Also, the epidermis beneath the stratum corneum was visible in a higher number of cases with LC-OCT than RCM, probably due to the deeper penetration of LC-OCT, which can overcome the presence of hyperkeratosis. However, the DEJ was visible in a similar proportion of cases, indicating that excessive hyperkeratosis and acanthosis can prevent the visualization of the DEJ in AKs and SCCs with both devices.

As awaited, tumour strands were seen in SCC and not in AKs. When the analysis was conducted on the ten invasive SCC, LC-OCT and not RCM could detect tumor strands, probably because only three invasive SCCs were visible beneath the DEJ in RCM.

The main limitation of this study was the small sample size due to the difficulty of finding histologically proven keratinocyte tumors that were both imaged at RCM and LC-OCT. In addition, the RCM and LC-OCT imaging parameters could have slightly different definitions related to the peculiarities of the two techniques and lacked histopathological comparison.

It is possible that a poor agreement between RCM and LC-OCT for some parameters could depend on a different imaging site, besides of the different technical features of the two devices.

Our pilot study showed that irregular epidermis can be detected in the majority of AK and SCC by both hand-held RCM and LC-OCT and pointed out that these two techniques could show various aspects of the same diseases because of the different plans of section (horizontal and vertical respectively) and the different fields of view. Notably, parakeratosis, dyskeratotic keratinocytes and dilated vessels seem to be better visible at LC-OCT than RCM. Our study can be a model for further investigations on larger series with histopathologic correlation.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

FUNDING INFORMATION

The authors received no specific funding for this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. cd_value_code=text

ORCID

Martina Bertello https://orcid.org/0000-0002-0676-1519
Diletta Fiorani https://orcid.org/0000-0001-7017-5939
Simone Cappilli https://orcid.org/0000-0001-9813-2391

REFERENCES

1. Neidecker MV, Davis-Ajami ML, Balkrishnan R, Feldman SR. Pharmacoeconomic considerations in treating actinic keratosis. *Pharmacoeconomics* 2009; 27:451-464.
2. Corchado-Cobos R, García-Sancha N, González-Sarmiento R, *et al.* Cutaneous squamous cell carcinoma: from biology to therapy. *Int J Mol Sci* 2020; 21:2956.
3. Zalaudek I, Argenziano G. Dermoscopy of actinic keratosis, intraepidermal carcinoma and squamous cell carcinoma. *Curr Probl Dermatol* 2015; 46:70-76.
4. Valdés-Morales KL, Peralta-Pedrero ML, Cruz FJ-S, Morales-Sánchez MA. Diagnostic accuracy of dermoscopy of actinic keratosis: a systematic review. *Dermatol Pract Concept* 2020; 10:e2020121.
5. Cinotti E, Tognetti L, Cartocci A, *et al.* Line-field confocal optical coherence tomography for actinic keratosis and squamous cell carcinoma: a descriptive study. *Clin Exp Dermatol* 2021;46(8):1530-1541. https://doi.org/10.1111/ced.14801
6. Dubois A, Levecq O, Azimani H, *et al.* Line-field confocal optical coherence tomography for high-resolution noninvasive imaging of skin tumors. *J Biomed Opt* 2018; 23:1-9.
7. Cinotti E, Labeille B, Debarbieux S, *et al.* Dermoscopy vs. reflectance confocal microscopy for the diagnosis of lentigo maligna. *J Eur Acad Dermatol Venereol* 2018; 32:1284-1291.
8. Manfredini M, Longo C, Ferrari B, *et al.* Dermoscopic and reflectance confocal microscopy features of cutaneous squamous cell carcinoma. *J Eur Acad Dermatol Venereol* 2017; 31:1828-1833.
9. Ishioka P, Maia M, Rodrigues SB, Lellis RF, Hirata SH. In vivo confocal laser microscopy for monitoring of actinic keratosis treatment: a comparison with histopathologic assessment after treatment with topical 5% 5-fluorouracil. *J Eur Acad Dermatol Venereol* 2018; 32:1155-1163.
10. Mota ANCM, Carvalho ND, Pellacani G, *et al.* Reflectance confocal microscopy in actinic keratosis—comparison of efficacy between cryotherapy protocols. *Skin Res Technol* 2020; 26:876-882.
11. Peppelman M, Nguyen KP, Hoogedoorn L, van Erp PE, Gerritsen MJ. Reflectance confocal microscopy: non-invasive distinction between actinic keratosis and squamous cell carcinoma. *J Eur Acad Dermatol Venereol* 2015; 29:1302-1309.
12. Teoh YL, Kuan LY, Chong W-S, Chia HY, Thng TGS, Chuah SY. The role of reflectance confocal microscopy in the diagnosis and management of squamous cell carcinoma in situ treated with photodynamic therapy. *Int J Dermatol* 2019; 58:1382-1387.
13. Navarrete-Dechent C, DeRosa AP, Longo C, *et al.* Reflectance confocal microscopy terminology glossary for nonmelanocytic skin lesions: a systematic review. *J Am Acad Dermatol* 2019; 80:1414-1427.e3.
14. Lenoir C, Cinotti E, Tognetti L, *et al.* Line-field confocal optical coherence tomography of actinic keratosis: a case series. *J Eur Acad Dermatol Venereol* 2021; 35:e900-e902.
15. Ulrich M, Maltusch A, Rius-Diaz F, *et al.* Clinical applicability of in vivo reflectance confocal microscopy for the diagnosis of actinic keratoses. *Dermatol Surg Off Publ Am Soc Dermatol Surg Al* 2008; 34:610-619.
16. Ruini C, Schuh S, Gust C, *et al.* Line-field confocal optical coherence tomography for the in vivo real-time diagnosis of different stages of keratinocyte skin cancer: a preliminary study. *J Eur Acad Dermatol Venereol* 2021; 35:2388-2397.

How to cite this article: Cinotti E, Bertello M, Cartocci A, et al. Comparison of reflectance confocal microscopy and line-field optical coherence tomography for the identification of keratinocyte skin tumours. *Skin Res Technol*. 2023;29:1–5. https://doi.org/10.1111/srt.13215