



In vivo reflectance confocal microscopy for evaluating melanoma of the lip and its differential diagnoses

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Objective. To improve prebiopsy diagnostic accuracy and surgical management of pigmented appearing lesions on the lips, particularly melanoma, using in vivo reflectance confocal microscopy (RCM).

Study Design. Prospective case series over a 12-month period between 2015 and 2016. The setting was two specialist dermatology referral centers with expertise in confocal microscopy. The study population was a consecutive sample of patients with pigmentation of the lip for which the cause was uncertain clinically, whose differential diagnosis included melanoma, and who had undergone both in vivo RCM and subsequent biopsy. The outcome measures were RCM features, dermoscopy features, and histopathological diagnosis. Results were reported by descriptive analysis and correlations made between RCM features and histopathology.

Results. Eight patients were recruited for the study. In vivo RCM facilitated the targeting of small biopsies to identify two in situ oral melanoma recurrences and successfully mapped an in situ oral melanoma before wide excision. Suprabasal dendritic pagetoid cells and epidermal disarray on RCM were useful indicators for in situ melanoma of the lip. Previously described dermoscopy features for mucosal melanoma were not very helpful in diagnosing melanoma in our series. Challenges included evaluating inflamed lesions with pigment incontinence.

Conclusions. RCM can assist in the diagnosis and management of pigmented lip lesions, but additional studies are required to further evaluate these initial observations. (Oral Surg Oral Med Oral Pathol Oral Radiol 2017;123:84-94)

Oral melanoma is a rare malignancy, with a poor 5-year survival rate of approximately 15-40%.¹ As the oral cavity is significant for both form (cosmesis) and function, tissue preservation is essential for the optimal management of oral lesions when it is safe to do so. For patients who either have a history of oral melanoma or present with a pigmented-appearing oral lesion, the ideal scenario is to be able to identify which lesions need biopsy, without oversampling, and those that can be safely and noninvasively monitored. Furthermore, if a biopsy or excision is required in larger pigmented-appearing oral lesions, there is a need to determine the best site to biopsy and the optimal width of surgical margins.

In recent years, in vivo reflectance confocal microscopy (RCM) has demonstrated utility as a clinical adjunct for the diagnosis of equivocal cutaneous pigmented lesions, with good evidence and acceptable sensitivity and specificity for the diagnosis of cutaneous melanoma.² For pigmented lesions of the oral cavity that are difficult to clinically diagnose, in vivo RCM could

have similar potential benefit. RCM directs visible or infrared light toward a focal spot in the tissue and collects the back-scattered light via a detector, after it passes through a pinhole to filter out undesired light from out-of-focus regions. This back-scattered light is converted into pixels, and as the tissue in the focal plane is scanned, a series of pixels is collected to form a two-dimensional en-face image. The microscope can be translated toward or away from the tissue along its optical axis to collect a stack of images in depth. Commercial RCM machines can capture images to a depth of 200-300 μm , with lateral resolutions of 0.1-1 μm , optical sections of 1-5 μm , and a field of view of at least 400 \times 400 μm .³ The contrast in the image relies on differing reflectivity, which is due to differing refractive indexes of the tissues and molecules within them. Molecules or structures with a relatively higher refractive index, such as melanin, appear white on RCM images due to their strong back-scatter signal.^{3,4}

In contrast to cutaneous epidermis, normal oral cavity lining mucosa has no surface stratum corneum. This causes significant back-scattering of light and detracts from RCM image clarity. As far as we are aware,

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Statement of Clinical Relevance

In vivo reflectance confocal microscopy (RCM) can assist in the diagnosis of lip melanoma. Inflammation may confound in vivo RCM evaluation of pigmented lip lesions.

there have only been a few brief descriptive studies published to date regarding use of *in vivo* RCM for evaluation of pigmented oral cavity lesions.^{3,5-7} While oral mucosa melanoma is rare, it must be differentiated from other more common or benign differential diagnoses, such as melanotic macules, nevi, melanoacanthomas, and amalgam tattoos.⁸

The purpose of this study was to describe and illustrate the potential uses and pitfalls of *in vivo* RCM as a clinical adjunctive tool for diagnosing and evaluating melanoma of the lip and pigmented and pigmented-like lesions of the lip that are difficult to clinically diagnose. The authors sought to determine whether there were RCM features that could help distinguish melanoma of the lip from other similarly appearing lesions. The hypothesis was that RCM could assist in the diagnosis of lip melanoma.

METHODS

This was a case series of consecutively presenting patients undergoing *in vivo* RCM for atypical pigmented lesions of the lip, including lesions suspicious for melanoma or melanoma recurrence, between February 2015 and January 2016. Patients were recruited from two dermatology specialist referral centers in Sydney, Australia: Melanoma Institute Australia and the Sydney Melanoma Diagnostic Centre, Royal Prince Alfred Hospital. Inclusion criteria were: (1) pigmented lesions of the lip for which the cause was uncertain clinically and a differential diagnosis of melanoma (lesions suspicious for melanoma recurrence were also included), and (2) biopsy of the lesion with histopathological evaluation (as the clinical reference standard). Exclusion criteria included patients for whom the clinical history and clinicodermoscopic examination of a pigmented-appearing lesion of the lip suggested a benign diagnosis (e.g., longstanding history of an isolated, nonchanging pigmented macule and benign appearance on dermoscopy).

A clinical case history, clinical photos (using Canon 750D and Nikon D3300 digital cameras), and RCM images (Vivascope 3000 or Vivascope 1500, Caliber Imaging and Diagnostics Inc., Rochester, NY, USA; wavelength 830 nm, lateral resolution 0.5-1 μm , ≥ 6 frames per second, field of view at least $500 \times 500 \mu\text{m}$) were taken for all cases and, when required, polarized or nonpolarized dermoscopy (Heine Delta 20 T, Heine Optotechnik, Herrsching, Germany, and Solarscan, Polartechnics Ltd., Sydney, Australia) was performed. Vertical stacks (VivaStack) of 32 RCM images, recording the epithelium through to the level of connective tissue, were taken for each lesion. Optical sections were taken every 3.25 μm . A biopsy was performed when clinical suspicion, dermoscopy, or RCM suggested at least a moderate risk of melanoma or

when there was diagnostic uncertainty. An incisional or excisional biopsy was performed according to the clinical indication and size of the lesion. For lesions undergoing excisional biopsy, multiple RCM images at different sites from within the lesion were recorded. RCM images were recorded at the sites of incisional biopsies, which were punch biopsies 3 or 4 mm in diameter. The punch biopsy site was relocated after RCM images were recorded, either manually given the small probe size of the Vivascope 3000, or with dermoscopy when necessary (after using the Vivascope 1500, which has a dermoscopic image-capture feature to guide RCM imaging). The area was marked with a pen to denote the site for biopsy, which was selected as the area having the most concerning RCM features for melanoma based on the authors' experience with RCM. No aceto-whitening was conducted during this study. Where it was clinically appropriate to monitor, patients were requested to return for evaluation at 3 months.

The study adhered to the principles outlined in the Declaration of Helsinki with respect to human patients in biomedical research. The study was part of a project with local ethics committee approval (protocol no. X-11-0090). All study participants gave written consent for the RCM, biopsies, and clinical images obtained in this study, which were done as part of their regular care.

RCM and dermoscopy images were reviewed by two observers (P.G., N.G.M.). Dermoscopy patterns were analyzed according to the multicenter International Dermoscopy Society study on mucosal and mucocutaneous pigmented lesions outlined by Blum et al.⁹ The indication for RCM was recorded, and RCM images were analyzed according to depth (suprabasal epithelium, epithelial-connective tissue junction [ECTJ], and connective tissue), using the standard terminology defined by Pellacani et al.¹⁰ Cell sizes were described relative to the surrounding keratinocyte size. Small size refers to cells smaller than the adjacent keratinocytes, moderate size to larger than the adjacent keratinocytes, and large size to more than twice the size of the adjacent keratinocytes.

RESULTS

Eight patients met the study criteria and are described in [Table I](#). There were 6 female and 2 male patients, and the locations of the lesions involved the vermillion (6), vermillion and lining mucosa (1), and wet lining mucosa of the lip (1). The clinical appearances, along with some selected representative RCM and histopathology images, are illustrated for all cases in [Figures 1 to 5](#) for cases 1 to 5, and in online [Supplemental Figures](#) for cases 6 to 8.

RCM was used in the diagnosis and management of three *in situ* lip melanoma cases (cases 1-3). Two cases ([Figures 1 and 2](#)) involved use of RCM to target a

Table I. Clinical, dermoscopy, RCM, and histopathology findings from the included cases

<i>Case no., age (years), sex</i>	<i>Clinical notes</i>	<i>Oral cavity subsite</i>	<i>Indications</i>	<i>Dermoscopy features</i>	<i>RCM features</i>	<i>Type of biopsy</i>	<i>Histopathology</i>
1. 74, F	Multiple previous surgeries for recurrent LM, most recently 2.5 years prior, with clear margins. Phototype I/II	Upper vermillion	F/U OM	Light brown parallel lines with central scar. No clear change from appearance of 6 months earlier.	SB epithelium: Typical (predominant) and atypical honeycomb patterns. Some small bright round cells. ECTJ: Focus of small bright round cells. Occasional dendritic cells. Mixed edged and nonedged papillae. CT: Broadened reticulated fibers.	3 mm punch	LM; referred for radiotherapy as definitive treatment.
2. 69, M	LM excised 1 year earlier, with close margins (0.5 mm). New brown pigmentation developed since surgery. Phototype II/III	Upper vermillion and lining mucosa	F/u OM	Brown parallel lines, globules, and structureless areas. Telangectasias.	SB epithelium: Focus of moderate-sized dendritic pagetoid cells. Atypical and typical honeycomb patterns, with occasional cobblestone pattern. ECTJ: Scattered dendritic cells. Nonedged papillae. CT: Broadened reticulated fibers.	3 mm punch	LM
3. 73, M	Recent biopsy showing LM. Phototype II	Lower vermillion	Map OM	N/A	SB epithelium: Atypical cobblestone and honeycomb patterns. Epidermal disarray. ECTJ: Large dendritic and round cells. Junctional nests. Edged, nonedged, and polycyclic papillae.	WLE with margins defined by RCM	Atypical melanocytic proliferation with features of LM. Three margins clear, scant scattered, nonconfluent atypical lentiginous melanocytes seen toward the periphery of one margin.

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Table I. Continued

<i>Case no., age (years), sex</i>	<i>Clinical notes</i>	<i>Oral cavity subsite</i>	<i>Indications</i>	<i>Dermoscopy features</i>	<i>RCM features</i>	<i>Type of biopsy</i>	<i>Histopathology</i>
4. 50, F	Three-year history of pigmentation, darkening in the last 2 months. Phototype IV	Upper lip lining mucosa	Diagnosis uncertain	Brown dots and black and brown globules.	CT: Plump irregular bright cells. Thick cordons. SB epithelium: Regular honeycomb pattern. ECTJ: Moderate to large dendritic cells surrounding papillae and forming a sheet of cells at the basal layer. Mostly nonedged papillae.	Excisional	Lentigo simplex (oral melanotic macule).
5. 72, F	Minimal clinical change on F/U over the preceding 12 months. Phototype II	Lower vermillion	Diagnosis uncertain	Nonhomogenous structure. Brown or black globules, parallel lines, structureless light brown area.	CT: Some plump bright cells within papillae. Thick cordons. SB epithelium: Atypical honeycomb pattern. Occasional moderate dendritic pagetoid cells. ECTJ: Moderate to large scattered dendritic cells. Nonedged papillae.	4 mm punch	Suggestive of actinic cheilitis. Pigment incontinence.
6. 54, F	Five-year history. Possibly getting bigger. History of cutaneous melanoma. Phototype III/IV	Lower vermillion	Diagnosis uncertain	Predominantly brown or black globules, some parallel lines and telangectasias. White structureless area.	CT: Large number of plump bright cells. Some nucleated cells. SB epithelium: Atypical honeycomb pattern. Small focus of grainy particles. ECTJ: Diffuse small round and moderate-sized dendritic cells. Some large	Ellipse excisional	Lichenoid reaction pattern. Pigment incontinence.

(continued on next page)

Table I. Continued

<i>Case no., age (years), sex</i>	<i>Clinical notes</i>	<i>Oral cavity subsite</i>	<i>Indications</i>	<i>Dermoscopy features</i>	<i>RCM features</i>	<i>Type of biopsy</i>	<i>Histopathology</i>
7. 72, F	Nine-year history. Biopsy 4 years earlier showed hemangioma. Changing on dermoscopy/RCM monitoring. Phototype II/III	Lower vermillion	Diagnosis uncertain	Brown dots as predominant feature, some red lines, blue-gray globule, and telangectasias.	dendritic pagetoid cells. Nonedged papillae. CT: Small and plump bright cells, some nucleated cells within papillae. Reticulated fibers and thick cordons. SB epithelium: Atypical honeycomb pattern. ECTJ: Small to moderate-sized bright round cells. Scattered large dendritic cells. Nonedged papillae. CT: Large numbers of small and plump bright cells. Broadened reticulated fibers. Horizontal large-diameter vessels.	3 mm punch	Nonspecific changes. Lichenoid inflammation, pigment incontinence, ectatic vessels, acanthosis, and compact parakeratosis.
8. 28, F	2.5 year history. Growing on dermoscopy monitoring. Phototype II/III	Lower vermillion	Diagnosis uncertain	Light to dark brown structureless macule. Changing on monitoring.	SB epithelium: Honeycomb pattern. Scattered grainy particles ECTJ: Numerous vertical vessels within the papillae. Large number of moderate and large dendritic cells. CT: Small and plump bright cells. Reticulated fibers.	Ellipse excisional	Nonspecific changes. Lichenoid and perivascular inflammation, focal areas of basal pigmentation, minor acanthosis, and focal compact hyperkeratosis.

RCM, reflectance confocal microscopy; *F/U*, follow-up; *OM*, oral melanoma; *SB*, suprabasal; *ECTJ*, epithelial–connective tissue junction; *CT*, connective tissue; *LM*, lentigo maligna; *N/A*, not applicable; *WLE*, wide local excision.

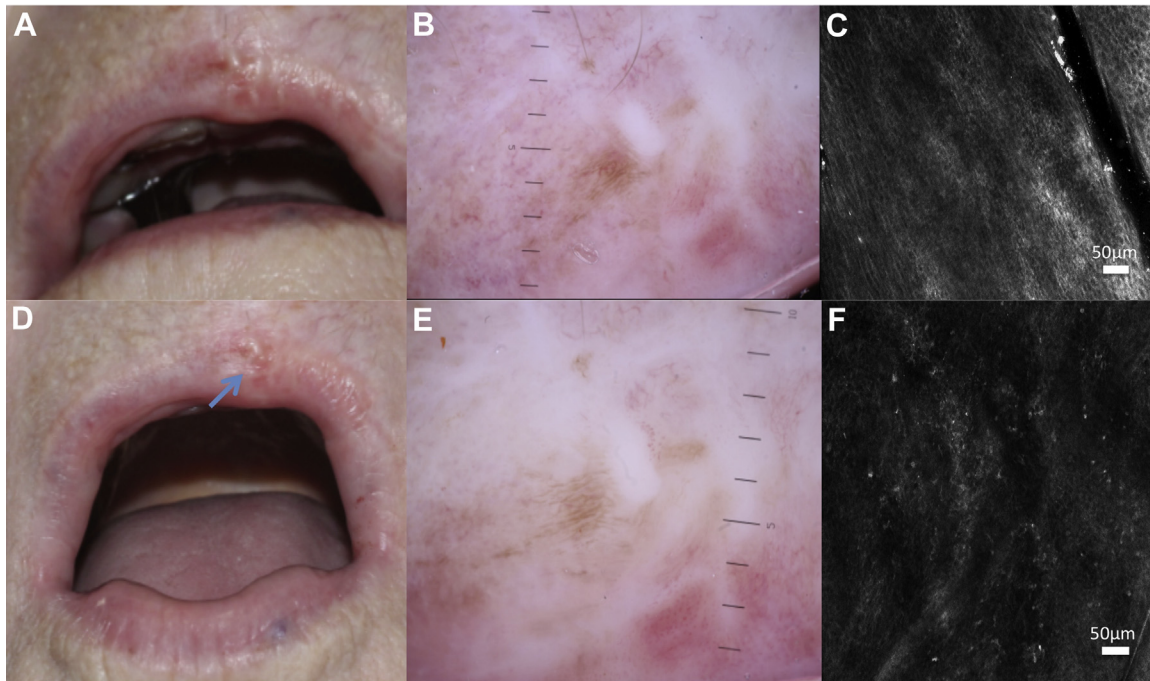


Fig. 1. Case 1: No clear changes between clinical and dermoscopy images taken previously (A, B) and 6 months later (D, E) for previously surgically treated lentigo maligna (LM). Subtle reflectance confocal microscopy (RCM) changes from the earlier visit (C) were found, with small to moderate-sized bright round cells in the suprabasal epithelium at 6-month follow-up (F). A 3-mm punch biopsy targeted by RCM (blue arrow in D) revealed LM recurrence.

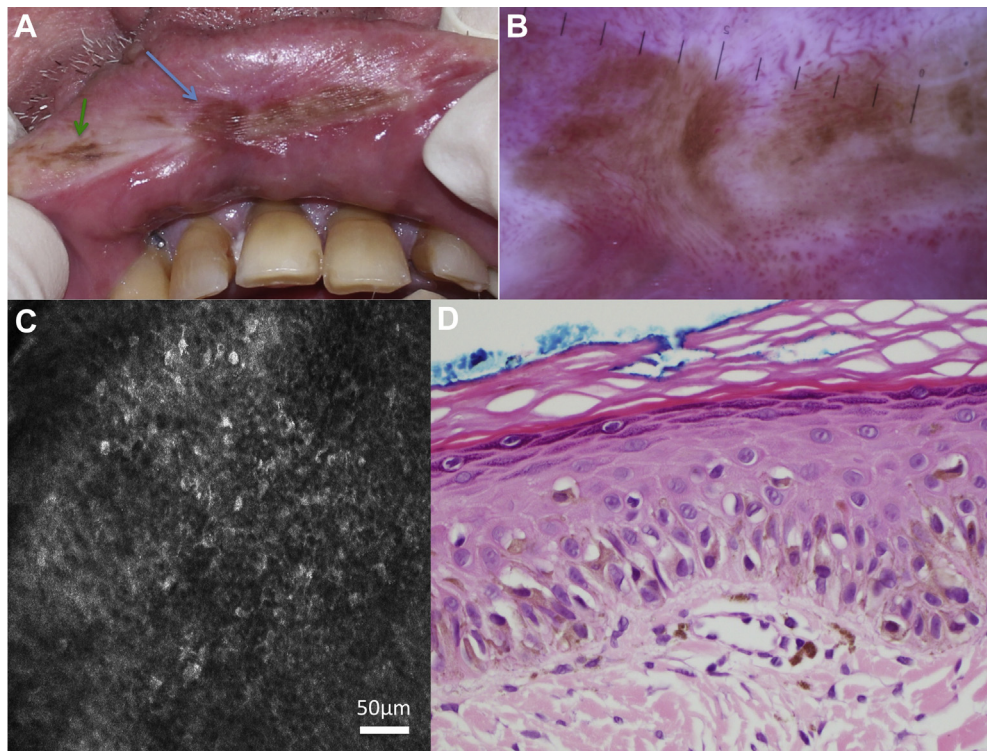


Fig. 2. Case 2: Clinical and dermoscopy images (A, B; blue arrow) reveal new brown pigmentation during follow-up for previously surgically treated lentigo maligna (LM). Reflectance confocal microscopy (RCM) revealed a focal region of moderate-sized, bright, round cells with dendritic processes in the mid and lower epidermis (C) within the brown pigment in the middle of the upper lip (blue arrow), which enabled a targeted biopsy to be performed. Histopathology of the later revealed LM recurrence (D, hematoxylin-eosin stain; original magnification 400×). The brown pigment on the right lateral lip (green arrow) revealed an enlarged and irregularly shaped papillae pattern with small, bright cells, consistent with solar lentigo on RCM (not shown).

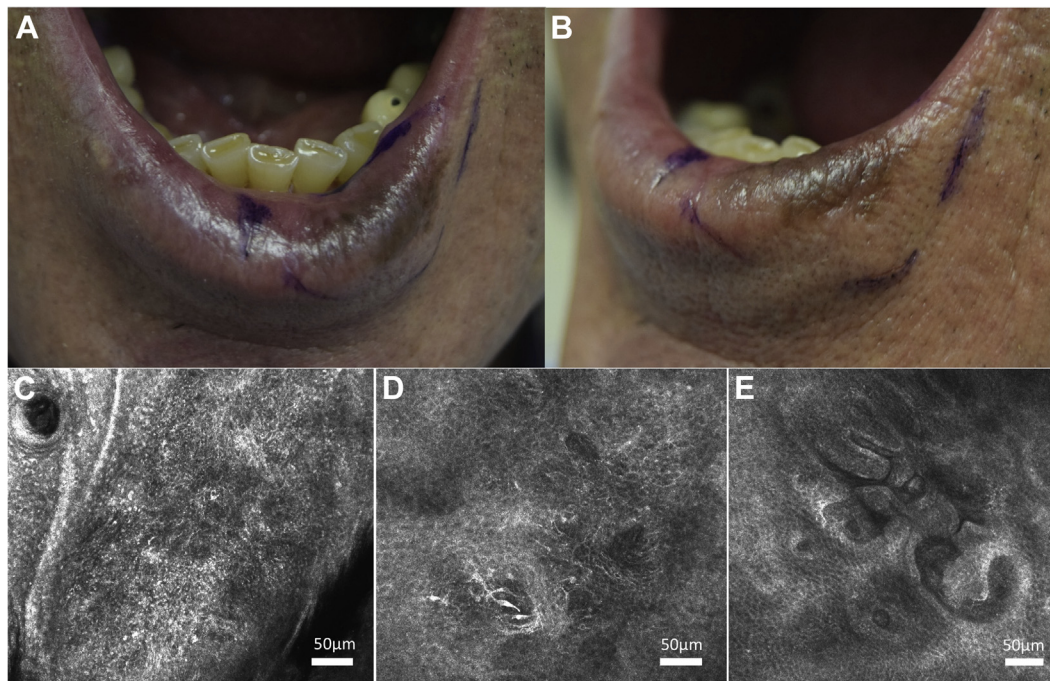


Fig. 3. Case 3: Reflectance confocal microscopy (RCM) guided mapping of margins before wide excision of a biopsy-proven lentigo maligna (LM) of the lower vermillion (A, B); RCM images demonstrating the mapping process. From the left, epidermal disarray, plentiful small and bright cells, and scattered larger dendritic cells are apparent in the suprabasal epithelium, indicating likely LM (C), then a few large dendritic cells, indicating the possibility for LM (D), and then normal vermillion at the medial margin with a polycyclic papillae pattern seen at the level of the epithelial–connective tissue junction (ECTJ) (E).

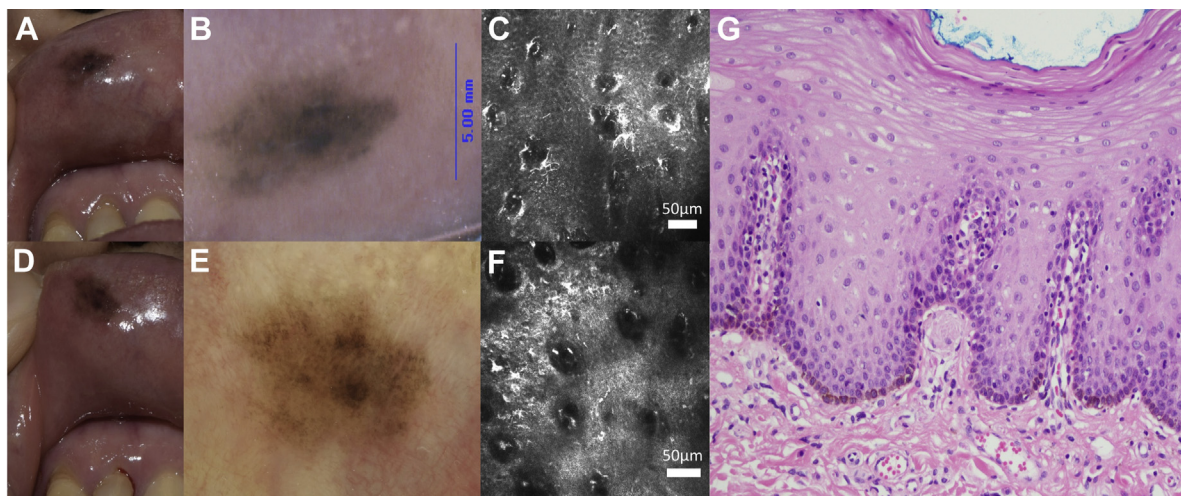


Fig. 4. Case 4: Pigmented macule of the upper lip lining mucosa at initial evaluation with dermoscopy and reflectance confocal microscopy RCM (A-C), and on follow-up 3 months later (D-F). The dendritic cells are around the connective tissue papillae, which is consistent with a melanotic macule; however, interpapillary moderate-sized, bright cells close to the epithelial–connective tissue junction were noted at follow-up with RCM (F). Histopathology taken after the second visit showed broad rete ridges with increased basal pigmentation consistent with an oral melanotic macule (G, hematoxylin-eosin stain; original magnification 200×).

biopsy in the region most suspicious for melanoma, which was confirmed by histopathology. Mapping of margins to assist definitive surgical management was conducted for one case of previously biopsy-proven in situ lip melanoma (Figure 3). RCM could not

reliably exclude melanoma for five of the eight cases (cases 4-8). Table II highlights the relevant RCM features that were helpful in distinguishing the melanomas in this series from the benign or reactive lesions with increased melanin deposition.

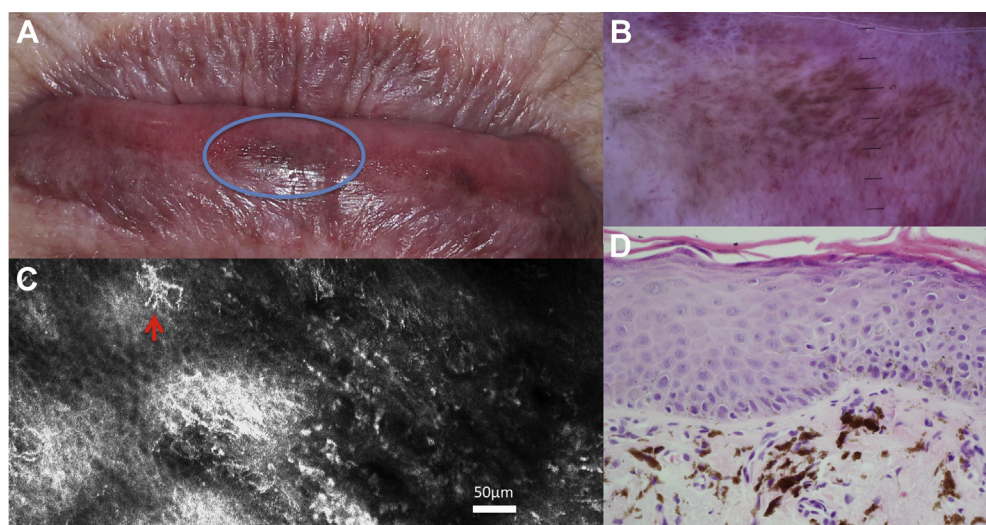


Fig. 5. Case 5: Clinical image shows two pigmented-appearing areas on the lower vermilion (A), with reflectance confocal microscopy (RCM), biopsy, and dermoscopy taken from the blue-circled region (B). RCM demonstrated occasional large dendritic cells (red arrow); scattered small, bright cells (C); and loss of the regular honeycomb pattern in the suprabasal epithelium. The features correlated on histopathology to, respectively, the presence of melanophages and scattered inflammatory infiltrate in the superficial connective tissue, and mild epidermal atypia (D, hematoxylin-eosin stain; original magnification: 400×).

Table II. Selected reflectance confocal microscopy (RCM) features from this series and the authors’ experience that may help distinguish between (in situ) melanoma and benign tumors or reactive lesions with increased melanin deposition from the lip*

RCM feature	Histologic (potential) correlate	Melanoma	Reactive lesion (increased melanin deposition) or benign tumor
Suprabasal dendritic pagetoid cells	Atypical melanocytes Langerhans cells	Highly suspicious	Possible
Suprabasal large, [†] round, or irregularly shaped pagetoid cells	Atypical melanocytes	Highly suspicious	Rare
ECTJ dendritic cells	Melanocytes Langerhans cells	Highly suspicious if interpapillary distribution and large number or focal collection	Highly suspicious for melanotic macule if restricted to only around connective tissue papillae
Epidermal disarray (in superficial epithelium)	Aggregation of atypical melanocytes and inflammatory cells	Highly suspicious	Unlikely
Plump, bright cells in the connective tissue without identifiable nucleus	Melanophages	Possible	Highly suspicious if in large numbers
Small, bright cells (compared to surrounding keratinocytes)	Inflammatory cells	Possible	Highly suspicious if in large numbers

ECTJ, epithelial–connective tissue junction.

*RCM features must be considered together with clinical and dermoscopic findings to formulate the clinical or differential diagnosis.

[†]Twice the size of the surrounding keratinocytes.

In cases 7 and 8, the histologic features were nonspecific, with lichenoid inflammation and acanthosis and either ectatic blood vessels and pigment incontinence (case 7) or a focal area of basal pigmentation and perivascular inflammation (case 8).

DISCUSSION

Diagnostic evaluation of pigmented lesions occurring on mucosal sites can be extremely difficult. Use of dermoscopy has been demonstrated to improve the diagnostic accuracy of clinical diagnosis of these

lesions. Blum et al.⁹ studied 140 pigmented lesions of the oral and anogenital mucosa with dermoscopy and showed that a blue, gray, or white color combined with a structureless pattern had 100% sensitivity for melanoma and 82.3% specificity among benign lesions. One of our lesions had a white structureless area (case 6) and showed lichenoid inflammation on biopsy, with no histologic evidence of malignancy on biopsy. Alternatively, if a blue, gray, or white color was individually identified on dermoscopy from the Blum et al.⁹ study, this provided their series with a sensitivity of 100% and a specificity of 64.3% for the diagnosis of melanoma. Case 7 had a small area of blue-gray color, revealing nonspecific features, which included pigment incontinence on biopsy. Thus, the use of dermoscopy features that were proposed as strong indicators of mucosal melanoma in a prior study was not very helpful in this series.

The literature pertaining to pigmented lesions of the oral cavity studied with *in vivo* RCM has, to date, been limited to descriptive studies of melanotic macules and melanomas.⁵⁻⁷ At this stage, there is no published algorithm using *in vivo* RCM features to predict oral melanoma diagnosis. As such, we used *in vivo* RCM features that have been discussed in the literature for oral melanoma and oral melanotic macules⁶ along with our own clinical experience in cutaneous and mucosal RCM use to guide decision-making. To our knowledge, the RCM features of only two oral melanoma cases have been previously described in the literature. It was reported that these cases showed roundish and fusiform basal and intraepithelial dendritic cells, and in one of the cases, nests and numerous bright roundish cells around the papillae, termed a “pearl necklace,” were noted.⁶

This study sought to investigate the applicability of *in vivo* RCM in real clinical scenarios for patients presenting with pigmented-appearing lesions on the lip with clinically uncertain cause, including lesions suspicious for oral melanoma recurrence. As such, RCM information was evaluated along with clinical information and dermoscopy features in making a decision to monitor or proceed to biopsy or excision.

Our results showed that *in vivo* RCM could be useful in certain clinical scenarios in this context. Cases 1 and 2 demonstrated the value of using *in vivo* RCM to aid in the diagnosis of recurrent oral melanoma during routine surveillance follow-up, when dermoscopy indicates that it may be safe to continue observing (Figures 1 and 2). These cases used *in vivo* RCM to identify the most appropriate site to target a biopsy, each of which showed a lentigo maligna (LM).

Second, *in vivo* RCM may facilitate mapping of oral melanoma margins to assist in the planning of definitive surgical treatment (case 3, Figure 3). This is not

unexpected, since there is good evidence that RCM mapping of cutaneous LM can optimize surgical treatment.¹¹ Mapping was conducted in this case by incrementally moving the probe outward from the center of the lesion and was performed at multiple orientations. The changes in the RCM images were noted as this occurred, and when normally appearing oral mucosa, vermillion, or cutaneous epithelium was first reached, a marker was used to record the site. While this approach is feasible for the lip region, at other sites deeper in the oral cavity this technique is limited by probe design and access and made more difficult by the elasticity of the oral mucosa during the imaging process.

While our series did identify some potentially helpful RCM features for identifying *in situ* lip melanoma (Table II), it did not include any invasive lip melanomas; hence RCM features that were indicative of invasive melanoma were not identified in this research. Cutaneous RCM research has established that nucleated cells in the dermis are commonly found in invasive melanomas.^{12,13}

In our experience, dendritic cells at the ECTJ on RCM may be a confounder for lip melanoma diagnosis. When these cells are only around the connective tissue papillae in small numbers, this is less of a concern, as has been reported by others.⁶ However, in the case of oral melanotic macules, there may be broad-based rete ridges with considerable melanin content in the basal layer and sometimes increased numbers of melanocytes; thus, on RCM, bright dendritic cells may be seen between the papillae in sheet form at the basal epithelial layer. This interpapillary distribution of dendritic cells may raise suspicion for oral melanoma. It was for this reason that a biopsy was performed in case 4 (Figure 4). This case demonstrated that melanotic macules (lentigo simplex) may be difficult to monitor with RCM. In such situations, as with all cases, other RCM features, together with the clinical and dermoscopic information, must be taken into account to establish a diagnosis.

There were other drawbacks as well. For four cases (cases 5-8), despite evaluation of *in vivo* RCM images aided by clinical records, the clinical diagnosis remained uncertain. In each of these cases, there was inflammation in the superficial connective tissue, which appeared as bright, small, round cells on RCM. The specific types of inflammatory cells could not be determined with RCM, which could have been useful in narrowing the differential diagnoses. Another complicating factor was the presence of pigment incontinence with melanophages in the superficial stroma, as seen in cases 5-7 (Figure 5 and Supplemental Figures S1 and S2). These cells may appear as bright, large dendritic cells on RCM and can be difficult to differentiate

from (atypical) melanocytes or Langerhans cells. The shapes of dendritic cells have been proposed by Debarbieux et al.⁶ as a clue in recognizing melanoma, although this observation needs prospective validation in independent studies.

As highlighted by case 5, and from what is known from the literature,^{6,14} pigmented actinic cheilitis can be difficult to distinguish from melanoma of the lip on RCM. This is because both can share RCM features such as loss of the regular honeycomb epithelial pattern; multiple small, bright cells in the upper connective tissue and ECTJ; and large, bright dendritic cells. Similarly, in RCM studies of cutaneous epithelium, pigmented actinic keratoses can be difficult to distinguish from melanoma.^{2,13}

A further limitation of our study was the difficulty in precisely correlating the location of the RCM image to the same area on histopathology. For punch biopsies, this was more precise, as they were small punch biopsies and the RCM images and stacks were analyzed from those particular sites. For excisional biopsies, precise correlation was less reliable. Notwithstanding this, in the cutaneous RCM literature for melanocytic lesions, correlation with histology has been widely accepted, evaluated, and described.¹⁵ Furthermore, the lesions evaluated in this study were not collision lesions (i.e., separate lesions), so that any RCM features found, even if only for a portion of that lesion, were still diagnostically relevant.

Establishing an accepted algorithm for oral melanoma diagnosis using RCM features will be an important next step in defining the role of RCM in diagnosing lip lesions. This requires larger study numbers in order to generate and validate such an algorithm. Recruiting these cases and defining their differential diagnoses for RCM imaging remains challenging due to their rarity, probe access to the oral cavity, and availability of the equipment. The probes that are commercially available with the Vivascope 1500 or 3000 machines that were used in this study can only image anterior sites of the oral cavity. Future work will hopefully also utilize technological advances in RCM equipment and smaller, narrower probe design^{3,16-18} to enable access to other (deeper) oral cavity subsites. This should boost recruitment potential. Furthermore, evaluation of other commonly occurring pigmented-appearing oral lesions, such as amalgam tattoos, should be performed with RCM, as the authors hypothesize that such lesions would be readily identified on RCM due to the strong back-scatter of light from any residual amalgam particles.

In summary, this preliminary investigation showed that in vivo RCM could help in clinically recognizing in situ lip melanoma, mapping oral melanoma for definitive surgery, and targeting oral biopsies of pigmented

lesions. RCM difficulties included evaluation of the inflamed oral mucosa, particularly with pigment incontinence. Larger studies are needed to validate these initial observations. In the future, with greater understanding of oral RCM features and correlation with histopathology, in vivo RCM may become a useful adjunct in deciding which clinically uncertain pigmented-appearing lesions could be safely and non-invasively monitored.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.oooo.2016.08.011>.