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## Application of 3-dimensional reflectance confocal microscopy: Melanocytic proliferations as three-dimensional models

*To the Editor:* Melanoma on chronically sun-damaged skin (MSDS) is challenging to detect.[1] Reflectance confocal microscopy (RCM) increases diagnostic accuracy but relies upon interpretation of 2-dimensional (2D) horizontal sections. Our objective was to determine whether existing technology could be used to create 3-dimensional (3D) models, which would expand on data available for discriminating skin biology.

Melanocytic cells have different morphologic appearances dependent on developmental stage, activation status, and pathogenic genetic and epigenetic alterations. Cell shape, therefore, can provide clues to a cell's behavior. We reconstructed individual melanoma cells from stacks of RCM images (Fig 1; Supplemental Fig 1, available via Mendeley at https://data.mendeley.com/datasets/7kx38d9h2v/3). Distinct morphologies could be identified, including spindle, spheroid, and heavily dendritic cell forms. These 3D reconstructions also allowed for modeling of the nuclear shapes (Fig 1, *insets*). Because image sizes are known, it is possible to calculate nuclear and cellular volumes, which may also increase diagnostic accuracy.[2]

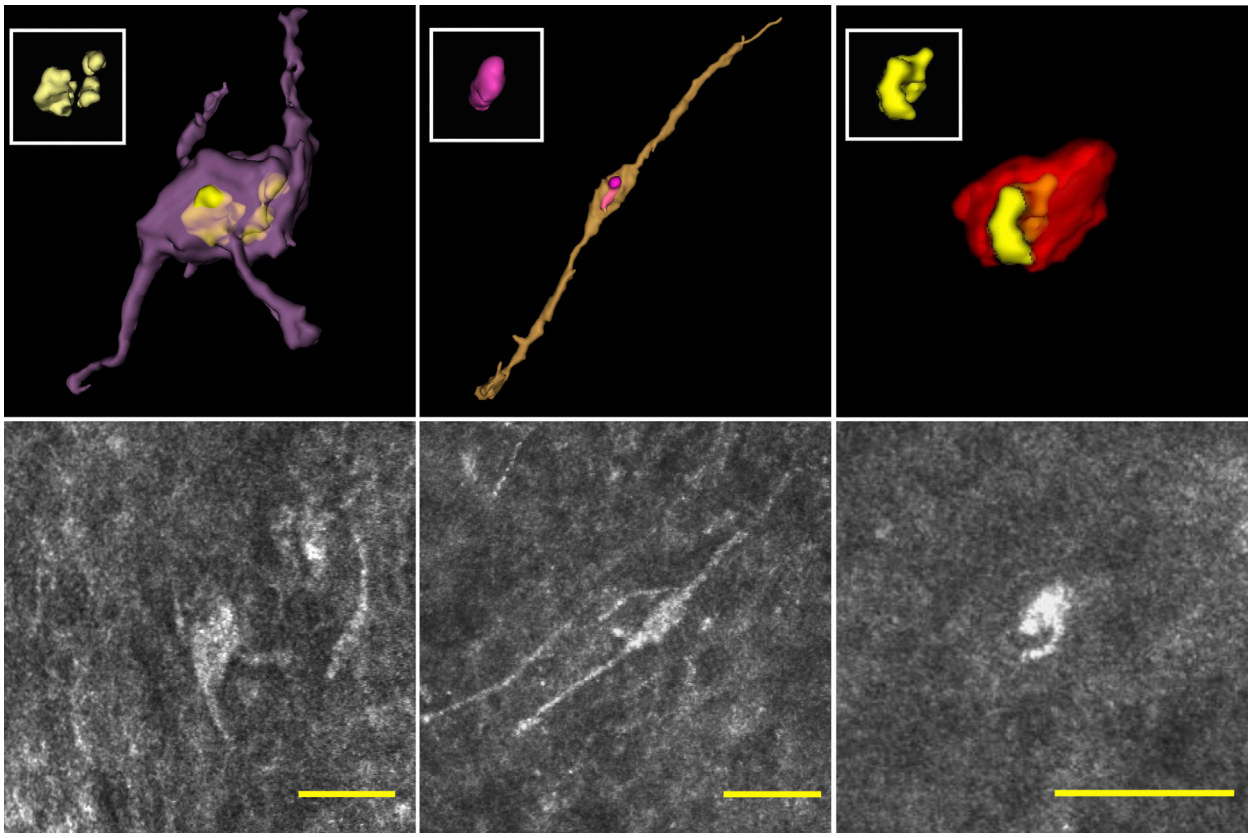

**Fig 1.** Three-dimensional reconstructions of atypical melanocytic cells revealing different morphologies. The *insets* illustrate nuclear morphology. A single slice from the confocal stack is shown below each cell. The reconstructions are enlarged to show features. Scale bar is 50 $\mu$m. A 3-dimensional model can be downloaded and rotated freely to examine from any angle, see Supplemental Fig 1 (.OBJ and associated .MTL)

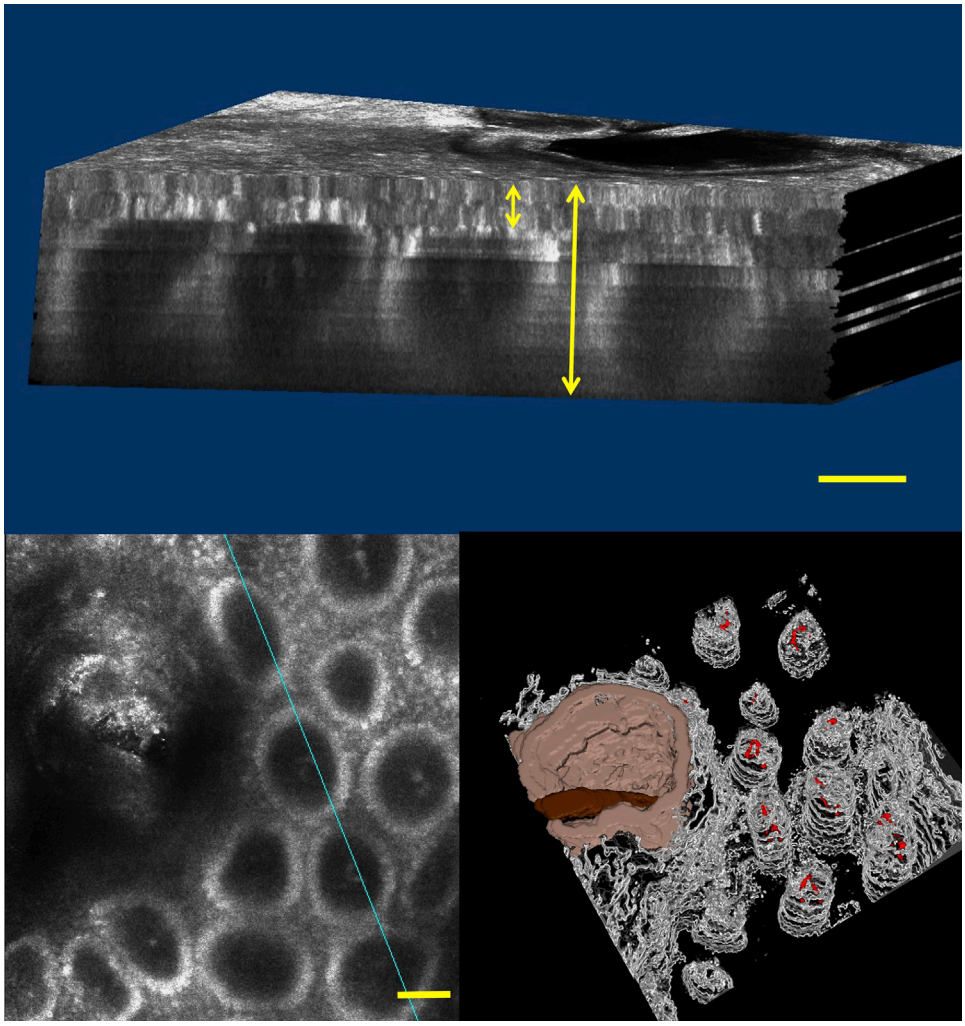

**Fig 2.** Three-dimensional rendering and reconstruction of normal pigmented human skin revealing dermoepidermal structure. A single slice with transection line shown in *blue*. The maximum and minimum thickness of epidermis over the epidermal ridges and dermal papillae can also be calculated (*yellow arrows*). Blood vessels within the papillae were reconstructed. Scale bars are 50 $\mu$m. A 360° rotating video can be viewed of the reconstructed dermoepidermal junction (see Supplemental Fig 4).

Monomorphism or pleomorphism of melanocytes may also provide clues to diagnosis. In some tumors, cells are relatively uniform, presenting as sheets of spindle-shaped cells, monomorphic spheroidal-shaped cells, or heavy mats of dendrites. However, other tumors display more variation with epithelioid, spindle-shaped, and dendritic morphologies all appearing in a 500-$\mu$m field (Supplemental Fig 2).

Location is also important in diagnosis. The presence of melanocytic cells in the middle to upper epidermis (pagetoid spread) may suggest malignancy, whereas cells in the superficial dermis suggest invasion. Within the context of the 3D volume, we used renderings to show upper (epidermal) and lower (dermal) regions simultaneously (Supplemental Fig 3). This can be used not only for identification of pagetoid and invasive cellular locations but also for examining the extent of melanization of cells in different locations that may imply differences in maturation.

When visualizing normal pigmented skin and some benign pigmented neoplasms with RCM, bright rims (edging) around the dermal papillae are visible as rings when viewed in the horizontal plane, primarily due to melanin-containing basal keratinocytes. By rendering the stack on the vertical axis, the normal arrangement of refractile cells along the papillae is apparent (Fig 2, Supplemental Fig 4). Epidermal thickness can be calculated, as well as the length of

epidermal ridge and volume of dermal papillae in 3D. When MSDS is present, asymmetric pigmentation (partially edged or nonedged dermal papillae) may be noted around dermal papillae.[3] This phenomenon may be due to nonuniform melanin transfer or melanoma cell population density differences. The dermoepidermal junction also frequently becomes flattened in malignant tumors. These phenomena may be best evaluated in 3D.

The 3D modeling process currently has limitations. When manual segmentation techniques are used, the more complex models take hours to produce, limiting bedside use. The imaging area is limited to the stack dimensions (500 $\mu$m $\times$ 500 $\mu$m). RCM image stacks are anisotropic, thus the noncubic voxels require interpolation and resampling to render accurately in 3D space.[4]

In summary, 3D modeling of confocal images can provide supplementary information, beyond what is appreciable in 2D images, including melanocyte morphology, volume, pleomorphism, and location. 3D modeling also allows for detailed evaluation of the dermoepidermal contour, which may reflect the summation of keratinocytic, melanocytic, immunologic, and microenvironmental events.

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## Risk factors affecting the first metastasis of acral melanoma: Low-pigmentation independently predicts a first lung metastasis

*To the Editor:* In cutaneous melanomas, the coloration or degree of pigmentation of the melanoma is variable and may be associated with histologic features and prognosis.[1-3] The effect of the degree of pigmentation on the occurrence and site of first metastasis is unknown. We investigated the prognostic factors for acral melanomas and whether the degree of pigmentation of acral melanoma was correlated with the first metastasis site.

The study enrolled 178 patients with acral melanoma who were diagnosed at the Chonnam National University Hospital from January 1992 to September 2019. Patients were divided into subgroups according to the metastatic patterns of their melanoma: melanoma in situ (group 1), invasive melanoma without metastasis (group 2), invasive melanoma with a first lymph node metastasis (group 3), and invasive melanoma with a first distant metastasis (group 4). The degree of pigmentation was evaluated by 2 dermatologists blinded as to outcome, using clinical and dermoscopic photographs. Pigmentation was divided into amelanotic and mild, moderate, severe, and heavy pigmentation; we made a representative typical clinical photo of each category (Fig 1). In addition, low-pigmentation (amelanotic and mild) and high-pigmentation (moderate, severe, and heavy) groups were established to minimize subjective bias.

Patients in the low-pigmentation group had a mean age of 68.3 years, and those in the high-pigmentation group had a mean age of 61.9 years; the difference was statistically significant ($P = .003$) (Table I). In the low-pigmentation group, the tendencies toward a deeper T stage, the presence of ulceration, higher mitotic rate ($P < .001$), and