Ex vivo confocal microscopy of basal cell carcinoma on a 3-color scale

To the Editor: Ex vivo confocal microscopy (CM) is capable of visualizing freshly excised tissue in real-time with cellular resolution without routine processing.[1] Depending on the laser wavelength, either reflectance CM or fluorescence CM (FCM) is utilized. Ex vivo CM is useful for the rapid evaluation of tumor margins during Mohs micrographic surgery.[2]

Initially, ex vivo reflectance CM studies used acetic acid as a contrast agent to enhance basal cell carcinoma (BCC) cell nuclei. However, thin strands of BCC were frequently missed.[3]

The use of fluorophores improves contrast, so that even small strands of BCC can be spotted in FCM mosaics.[4] Acridine orange (AO) is the dye that is most widely used. AO binds specifically to DNA and emits fluorescence, so images of living cell nuclei can be enhanced and displayed as bright structures in FCM mosaics.[1]

Even though good resolution and morphologic correlation are achieved with this standard technique, nowadays confocal mosaics are displayed in a gray scale format.[5] However, dermatopathologists are often neither familiar with nor comfortable assessing these black-and-white images.

We herein report a new technique for obtaining 3-color scale confocal mosaics (FCM on a 3-color scale) with the simultaneous use of AO and ethidium bromide (EB) as fluorescent dyes.

In this technique, the excised skin sample is first soaked with liquid nitrogen. The sample is then sectioned into 20- to 30-μm-thick slices by using a cryostat, after which it is stained with the dye mixture (0.1 mM AO + 0.25 mM EB) for about 1 minute. The sample is then placed in the confocal microscope plate for imaging (Nikon A1R$^+$, Nikon Corporation, Tokyo, Japan). The tissue is scanned simultaneously with lasers at 2 different wavelengths (405 and 488 nm), and the collected fluorescence displayed on the screen as a 3-color scale mosaic. Around 10 to 15 minutes is required for completion of the tissue processing and for the final mosaics to be developed.

Unlike AO, EB binds specifically to the DNA of BCC cells that are damaged on account of freezing. As a result, BCC nests are stained by EB and emit red fluorescence after laser stimulation; in contrast, the epidermis and dermis are stained by AO and emit green fluorescence. Blue color corresponds to the background tissue autofluorescence. All fluorescence is collected by the microscope, displaying the final images in a 3-color scale format. AO and EB staining do not affect additional fixation or staining of the sample.[3]

Fig 1 shows completed BCC mosaics displayed with this new technique. Each color represents a different skin structure, making the mosaics easier to read. In this way, 3-color scale confocal mosaics are more user-friendly and can be interpreted by health care professionals without previous experience with FCM. Moreover, with frozen sample processing, the tissue is completely flattened and the entire sample can be displayed on the screen. These developments represent important advantages over previously described images obtained with CM.[4]

In conclusion, FCM on a 3-color scale is an innovative technique that provides color images, significantly expanding the applicability of FCM. Larger studies are nevertheless required to validate the technique for Mohs micrographic surgery and other applications.

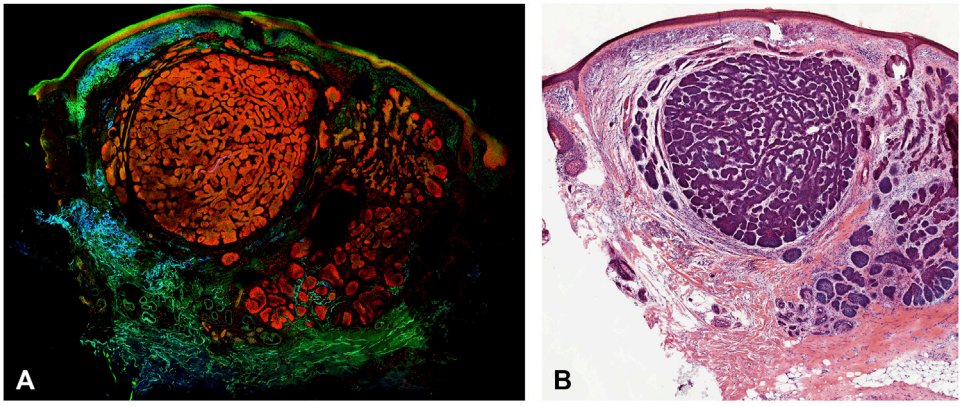

Fig 1. A, Ex vivo confocal microscopy of a nodular basal cell carcinoma based on a 3-color scale. **B**, A section of the same sample stained with hematoxylin and eosin.

Mercedes Sendín-Martín, MD,[a] *Antoni Bennàssar, MD, PhD,*[b] *Konstantin L. Levitsky, PhD,*[c] *and Javier J. Domínguez-Cruz, MD, PhD*[a]

From the Dermatology Department[a] *and Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío, Sevilla, Spain,*[c] *and Clínica Rotger, Palma de Mallorca, Spain*[b]

Funding sources: None.

Conflicts of interest: None disclosed.

Reprints not available from the authors.

Correspondence to: Mercedes Sendín-Martín, MD, Dermatology Department, Hospital Universitario Virgen del Rocío, Sevilla, Av Manuel Siurot, s/n 41013, Sevilla, Spain

E-mail: mercedessendin@gmail.com

REFERENCES

1. Ragazzi M, Longo C, Piana S. Ex vivo (fluorescence) confocal microscopy in surgical pathology: state of the art. *Adv Anat Pathol*. 2016;23(3):159-169.
2. Bennàssar A, Carrera C, Puig S, Vilalta A, Malvehy J. Fast evaluation of 69 basal cell carcinomas with ex vivo fluorescence confocal microscopy. *JAMA Dermatol*. 2013;149(7):839.
3. Keena S, Que T. Research techniques made simple: noninvasive imaging technologies for the delineation of basal cell carcinomas. *J Invest Dermatol*. 2016;136:e33-e38.
4. Bennàssar A, Vilata A, Puig S, Malvehy J. Ex vivo fluorescence confocal microscopy for fast evaluation of tumour margins during Mohs surgery. *Br J Dermatol*. 2014; 170(2):360-365.
5. Villarreal-Martinez A, Bennàssar A, Gonzalez S, Malvehy J, Puig S. Application of in vivo reflectance confocal microscopy and ex vivo fluorescence confocal microscopy in most common subtypes of basal cell carcinoma and correlation with histopathology. *Br J Dermatol*. 2018;178:1215-1217.

https://doi.org/10.1016/j.jaad.2018.12.022