Fast Evaluation of 69 Basal Cell Carcinomas With Ex Vivo Fluorescence Confocal Microscopy
Criteria Description, Histopathological Correlation, and Interobserver Agreement

Antoni Bennàssar, MD; Cristina Carrera, MD; Susana Puig, MD, PhD; Antoni Vilalta, MD; Josep Malvehy, MD, PhD

IMPORTANCE Fluorescence confocal microscopy (FCM) represents a first step toward a rapid “bedside pathology” in the Mohs surgery setting and in other fields of general pathology.

OBJECTIVE To describe and validate FCM criteria for the main basal cell carcinoma (BCC) subtypes and to demonstrate the overall agreement with classic pathologic analysis of hematoxylin-eosin–stained samples.

DESIGN A total of 69 BCCs from 66 patients were prospectively imaged using ex vivo FCM. Confocal mosaics were evaluated in real time and compared with classic pathologic analysis.

SETTING Department of Dermatology, Hospital Clinic of Barcelona, Barcelona, Spain, between November 2010 and July 2011.

PARTICIPANTS Patients with BCC attending the Mohs Surgery Unit.

MAIN OUTCOMES AND MEASURES Presence or absence of BCC and histological subtype (superficial, nodular, and infiltrating) in the confocal mosaics. Eight criteria for BCC were described, evaluated, and validated.

RESULTS Although there were minor differences among BCC subtypes, the most BCC-defining criteria were peripheral palisading, clefting, nuclear pleomorphism, and presence of stroma. These criteria were validated with independent observers (κ values >0.7 for most criteria).

CONCLUSIONS AND RELEVANCE We herein propose, describe, and validate FCM criteria for BCC diagnosis. Fluorescence confocal microscopy is an attractive alternative to histopathologic analysis of frozen sections during Mohs surgery because large areas of freshly excised tissue can be assessed in real time without the need for tissue processing while minimizing labor and costs.

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Real-time high-resolution imaging of human skin is possible in vivo but can also be performed ex vivo with a confocal microscope.\(^1\) Confocal mosaicing microscopy offers an attractive alternative to histopathologic analysis of frozen sections during Mohs surgery because cellular morphology can be observed, in real time, in thin optical sections and directly in freshly excised tissue.\(^2,3\)

Acetic acid was first used as a contrast agent to brighten nuclei in reflectance-mode confocal microscopy (RCM). In RCM, large, densely nucleated basal cell carcinomas (BCCs) are easily detected in mosaics. However, tiny strands of micronodular-infiltrating BCCs remain hidden in RCM mosaics because of the bright scattering interference of the surrounding normal dermis (strong reflectance).\(^4,5\) In the fluorescence mode, a contrast agent that specifically stains nuclei (acridine orange) is used. With fluorescence confocal microscopy (FCM), acridine orange increases the contrast between the nuclei and dermis of BCC cells 1000-fold, and only weak fluorescence is collected from the surrounding dermis. Thus, both large and small BCCs are detected in mosaics.\(^6\)

Fluorescence confocal microscopy devices display large areas of tissue with high resolution and low magnification in a manner that is analogous to that seen in thin histopathologic sections. Confocal images (750 × 750 μm) are stitched together to create mosaics to display large areas of tissue, as required for surgical pathologic analysis. Modern FCM devices are able to display wide field of view mosaics of up to 12 × 12 mm; this corresponds to a view with 2× magnification—a view that is routinely used by Mohs surgeons when reading frozen pathologic sections with a standard light microscope.\(^4,5\) Mosaics are created in less than 3 minutes, whereas histopathologic analysis of frozen sections is time consuming, requiring 15 to 30 minutes per stage.\(^7\)

However, this new imaging technique is entirely different and therefore must be validated. Recent studies have demonstrated an excellent correlation between ex vivo FCM images and histopathologic findings for nonmelanoma skin cancer.\(^8\) In a blind evaluation, residual BCC was detected in freshly excised Mohs tissue with an overall high sensitivity and specificity when compared with the gold standard of frozen section stained with hematoxylin-eosin (H&E).\(^9,10\)

Fluorescence confocal microscopy has the potential to improve diagnostic accuracy. Moreover, large-area mosaicing may offer a means for rapid examination of BCC directly in fresh excisions while minimizing labor and costs. Ex vivo FCM is the biggest advance in Mohs surgery for decades and may evolve into an entirely new alternative to histopathologic analysis of frozen sections. The technology may enable the implementation of rapid surgical bedside pathologic analysis to guide Mohs surgery.

The feasibility of FCM for rapid bedside pathologic analysis has been widely described with good sensitivity and specificity\(^5,9,10\) and may represent a time-saving alternative to the use of frozen sections during classic Mohs surgery or microscopically controlled surgery.\(^4,5,11,12\) However, to our knowledge, no diagnostic BCC criteria have been reported to date.

The goals of this study are 2-fold. First, we aim to describe and validate FCM criteria for BCC because they are lacking in the medical literature. Second, we also want to describe FCM characteristics and criteria for the main BCC subtypes and validate them, demonstrating the overall agreement with classic pathologic analysis.

### Methods

#### Study Sample and Tissue Collection

Eighty consecutive patients from our Mohs Surgery Unit (Hospital Clinic, Barcelona, Spain) with 84 surgically removed BCCs were prospectively enrolled in the study. All patients underwent classic Mohs surgery (45° incision), and all lesions were at least 1 cm in their maximum diameter. The study was approved by the ethics committee of the Hospital Clinic and the University of Barcelona, Spain (registry No. 2010/5492).

Details of tumor location, status (primary or recurrence), and histopathologic subtype, as well as patient demographic data, are listed in Table 1. A 1-mm vertical central strip of tissue from the first Mohs stage center was taken without disruption of the tissue margins for ulceration frozen section assessment. All 84 lesion centers were imaged under FCM, creating a 12 × 12 mm VivaScope mosaic for each one. For technical reasons, 15 panels were not optimal for evaluation and were discarded. Finally, 69 tumor specimens from 66 patients were evaluated. Both surgery and FCM mosaicing imaging were performed prospectively by the first author (A.B.) during Mohs surgery.

#### Fluorescence Confocal Microscopy

Confocal mosaics were acquired using a modified version of a commercially available ex vivo laser scanning FCM (VivaScope 2500; Lucid Inc); this FCM version is specially de-
signed for ex vivo imaging of freshly excised tissue samples. All samples were directly immersed in a 1mM solution of acridine orange for 20 to 30 seconds depending on sample thickness and finally rinsed in isotonic saline solution in preparation for confocal imaging.

Acridine orange provides a strong contrast between nucleus and dermis because it specifically stains the DNA (and also RNA) of nucleated cells. With FCM, only weak fluorescence is collected from the dermis and subcutaneous fat, therefore increasing the contrast of epithelial cells 1000-fold, including epidermis, adnexal structures, and BCC cells. Equally important is the fact that acridine orange immersion affects neither subsequent frozen sections nor the quality of formalin-fixed histopathologic sections.6 To detect orange fluorescence, the wavelength used was 488 nm from the diode laser; illumination laser power was automatically set, but the depth had to be manually adjusted.

For each skin specimen a 12 × 12 mm mosaic was created, such that the magnification is equivalent to that of a standard low-power 2× view of optical microscopes. It took 150 seconds to display each 12 × 12 mm panel and 20 additional seconds for the subsequent image stitching. The instrumentation, imaging, and technical details have been described by Gareau et al.8

**Mosaic Evaluation**

Immediately after surgery, all displayed FCM mosaics from freshly excised samples were evaluated by the attending Mohs surgeon (A.B.) in real time in the operating room. He is experienced in analysis of both frozen Mohs slides and FCM images because he has been formally trained in both fields. Moreover, he has experience in RCM technology, evaluating benign and malignant lesions. For comparison with classic pathologic analysis (gold standard), the corresponding formalin-fixed slides stained with H&E or toluidine blue were processed and obtained for all samples. Permanent formalin-fixed pathologic slides were blindly read by our dermatopathologists.

Fluorescence confocal microscopy panels were visualized at a 2× magnification at first glance and then at 4× (panzoom tool), mimicking the usual frozen section slide reading process during Mohs surgery. “Zooming” mosaics at higher magnifications of 10× to 30× were performed on the samples from only a few patients when necessary.

To our knowledge, BCC characteristics under FCM have not been described as yet. Fluorescence confocal microscopy displays mosaics that reproduce the architecture of normal skin, as well as tumor shape, in real time with cellular resolution. However, when visualizing FCM mosaics we can use neither color terminology as in H&E classic pathologic analysis nor structure refractility as in RCM. In FCM we must describe structures in terms of their fluorescence emission. Thus, nucleated skin structures, such as the epidermis, adnexal structures, and BCC, are described as fluorescent. In contrast, the dermis has only a few cellular structures; thus, fluorescence emission is expected to be absent or weak.

The first step in the evaluation of mosaics consisted in the identification of BCC presence or absence and histological subtype, including superficial BCC (sBCC), nodular BCC (nBCC), and micronodular-infiltrative BCC (iBCC). Each FCM panel was classified into 1 of these 3 subtypes on the basis of general tumor shape and distribution of fluorescent nests and strands of BCC.

**FCM Criteria for BCC**

Classic histopathological BCC features were adapted on FCM images. Eight criteria, including presence of fluorescence, tu-
mor demarcation, nuclear crowding, peripheral palisading, clefting, nuclear pleomorphism, increased nuclear to cytoplasm (N/C) ratio, and stroma, were described and evaluated (Figure 1 and Figure 2).

Fluorescence. Presence of fluorescence was determined when bright-white images were seen on the screen. Fluorescence corresponds to nucleated cells stained with acridine orange. Absence of fluorescence is seen as a black background.

Tumor demarcation. Tumor shape was divided into 2 categories, ill defined when a line could not be clearly drawn to separate the tumor from the surrounding tissue and well demarcated when one could be.

Nuclear crowding. Nuclear crowding was determined when the nuclear density was higher than that of the surrounding epidermis and adnexal structures.

Peripheral palisading. Palisading is described as peripheral polarized and aligned fluorescent ellipses, being the counterpart of the so-called criteria in formalin-fixed H&E-stained slides; it corresponds to the prominent tendency of the outermost row of basal cells to be arranged in a parallel-polarized way.

Clefting. Clefting is a black fluorescence-free half-moon attached to the tumor mass.

Nuclear pleomorphism. Nuclear pleomorphism is a deviation from the normal round or oval nuclear outline present in normal keratinocytes.

Enlarged N/C ratio. As tumoral cells, BCC cells are supposed to have an enlarged N/C ratio when compared with normal keratinocytes. Thus, under FCM, BCC nests are seen as crowded masses of elongated heterogeneous spots of fluorescence (prominent nuclei) with poor or absent cytoplasm.

Stroma. Tumoral stroma is the modified dermis surrounding the BCC mass. When viewed in FCM mosaics, the stroma is seen as a more densely nucleated dermis, as fluorescent dots within a black background. This image is characteristic and is reminiscent of a “starry sky” in which the “stars” correspond to inflammatory cells and activated fibroblast nuclei. The amount of stroma within the tumor was evaluated in a semiquantitative manner.

Results

A total of 69 surgically removed BCCs from 66 patients were imaged. Forty-four of the tumors (64%) came from male patients and 25 from female patients (36%), with a mean (range) patient age of 71.9 years (27-96 years). The nose was the most frequent location (43%), followed by the forehead (23%), trunk (10%), ear (9%), neck (4%), cheek (4%), chin (3%), and eyelid (3%). Most of the BCCs were primary (93%), and the main histological subtype was micronodular-infiltrating, representing approximately three-quarters of the tumors. These results are summarized in Table 1.

FCM Criteria for BCCs

Basal cell carcinoma consists of a malignant proliferation of basaloid cells with prominent nuclei; thus, BCC nests and strands were hyperfluorescent when viewed under FCM. Fluorescence from dermal cells (lymphocytes and fibroblasts), hair follicle (HF) keratinocytes, and eccrine gland (EG) cells was also observed.

Approximately two-thirds of the BCCs were considered well demarcated. The surrounding dermis was obviously ill defined, but 91% of the HF s and all EG cells were considered well demarcated. These differences were statistically significant.

The proposed FCM criteria for BCCs of nuclear crowding (91%), palisading (96%), and nuclear pleomorphism (100%) were present in almost all BCCs. Nuclear crowding was also present in 94% of HFs, especially in the bulb, but less frequently in the surrounding dermis and was almost absent in EG cells. Palisading was one of the most characteristic FCM criteria for BCC. It was present in 96% of BCCs but almost absent in the surrounding dermis and adnexal structures. Thus, this criterion has a high sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).
Eventhoughcleftingwasobservedinonlyroughlyhalfof the BCCs, this criterion was 100% specific and the PPV was 100%inasfarasinnothingoftheremainingskin structures.

NuclearpleomorphismwasobservedinallBCCsbutonly inapproximatelyhalfofthesurroundingdermisandin6% ofHFs.NoEGcellspresentednuclearpleomorphism.Thus,sen- sitivityandNPVforthiscriterionwere100%.

EnlargementoftheN/CratiorawasthepresentinallBCCcells, surrounding dermis cells (lymphocytes and fibroblasts), and EG cells. In HFs, a few bulb cells showed an increased N/C ratio, but most of them and all HF shaft keratinocytes had a low N/C ratio similar to that of normal epidermis.

Bascallcarcinomastromawaspresentin94%ofBCCsbut wasnotobservedinanyHFsorEGcells.Thus,whencompared with adenaxial structures, the presence of stroma has a 100% speci- ficity and PPV. These results are summarized in Table 2.

### FCM Differences Among BCC Subtypes

As Table 3 shows, there were no statistically significant differences between BCC subtypes when comparing fluore- cence, palisading, nuclear pleomorphismsm, and N/C ratio because all these criteria were present in most BCCs.

AllnBCCswereconsideredwelldemarcated.However, the tumor limits were clearly defined in only 31% and 14% of iBCCs and sBCCs, respectively. All iBCCs and nBCCs presented nuclear crowding, but just 1 sBCC (14%) had an increased nuclear density. All sBCCs and most nBCCs showed clefting images, yet just 38% of iBCCs presented clefting.

Inreferenceertosumoralex,29%ofsBCCshadnostromaand57%hadpoorstroma.Incontrast,ins90%and98% ofnBCCsandiBCCs,respectively,thumoraleximageswere observed. These differences were statistically significant.

### Correlation Between FCM and H&E Differentiating BCC Subtypes

WhenviewedunderFCM,allBCCmosaicswereclassifiedby the Mohs surgeon into 1 of the 3 main BCC subtypes, sBCC, nBCC, or iBCC (Figures 3, 4, and 5, respectively). Independently, our dermatopathologist performed the same procedure with H&E slides. The overall agreement between both techniques and observers was high (k = 0.9).

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### Table 2. Fluorescence Confocal Microscopy (FCM) Criteria for BCC and Correlation With Other Skin Structures

<table>
<thead>
<tr>
<th>FCM Criteriaa</th>
<th>No. (%)</th>
<th>BCC (n = 69)</th>
<th>Surrounding Dermis (n = 138)</th>
<th>P Value</th>
<th>Se</th>
<th>Sp</th>
<th>PPV</th>
<th>NPV</th>
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<td>100</td>
<td>100</td>
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<tr>
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<th>No. (%)</th>
<th>BCC (n = 69)</th>
<th>Hair Follicles (n = 138)</th>
<th>P Value</th>
<th>Se</th>
<th>Sp</th>
<th>PPV</th>
<th>NPV</th>
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<td>26</td>
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<td>130 (94)</td>
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<td>61</td>
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<td>100</td>
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<td>90</td>
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<th>Eccrine Gland Cells (n = 138)</th>
<th>P Value</th>
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<th>Sp</th>
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<th>NPV</th>
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<tr>
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<td>62</td>
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<td>24</td>
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<td>&lt;.005</td>
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<td>94</td>
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<td>100</td>
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Abbreviations: BCC, basal cell carcinoma; N/C ratio, nuclear to cytoplasm ratio; NPV, negative predictive value; PPV, predictive positive value; Se, sensitivity; Sp, specificity.

* Eight FCM criteria for BCC were described on the basis of fluorescence images and compared with normal skin structures.
Interobserver Correlation
Criteria validation was carried out by means of cross tables between the main author and 2 independent observers (C.C. and J.M.), evaluating each FCM criterion for BCC. These criteria were validated with $\kappa$ values greater than 0.7 for most criteria in both independent observers as given in Table 4.

Discussion

Ex vivo FCM is a new imaging tool capable of displaying large areas of tissue with a high resolution in a manner that is analogous to that seen in thin sections of histopathologic samples.4,5 Ex vivo FCM may represent an alternative to histopathologic analysis of frozen samples during Mohs surgery because surgical margins can be assessed in real time directly in freshly excised tissue,9,10,12 thus saving time and resources.

However, this new imaging method is entirely different from existing techniques and therefore must be validated. There are many reports demonstrating an excellent correlation between ex vivo FCM images and histopathologic analysis for nonmelanoma skin cancer.3,8-10 However, none have reported a structured description of FCM features for BCCs nor validated truthful criteria to differentiate it from the surrounding dermis and adnexal structures.

FCM Criteria for BCCs
Fluorescence confocal microscopy collects fluorescence from nucleated cells such as BBC nests and strands, basal layers of the epidermis, and adnexal structures including HFs and eccrine sweat glands. Dermal fluorescence is weak, and only within the tumoral stroma can nucleated cells be observed, corresponding to inflammatory lymphocytes and activated fibroblasts.
Fluorescence confocal microscopy displays mosaic images where the more fluorescence one structure collects and subsequently delivers, the brighter it appears on the screen. When a structure does not contain nuclei, no acridine orange is fixed. The structure therefore lacks fluorescence and appears gray or black on the screen. Thus, when visualizing FCM images we should describe the structure first of all by its fluorescence and forget about refractile structures such as in RCM or colors as in classic histopathologic analysis with H&E. This concept seems obvious, but it is the mainstay in FCM. Thus, under FCM, tumoral cells, HFs, EGs, and reactive cells within the tumoral stroma appear hyperfluorescent. However, we should point out that normal dermis does not have any fluorescence and therefore is usually seen as black or gray-black.

In addition, most BCCs and adnexal structures were determined as hyperfluorescent, well-defined bright spots as far as one can precisely demarcate the limits. Fluorescence in the surrounding dermis was diffuse and ill defined.

On the basis of classic histopathologic analysis, 5 morphological-fluorescent criteria for BCC were defined, including nuclear crowding, palisading, clefting, nuclear pleomorphism, and increased N/C ratio. Nuclear crowding was present in most BCCs, but it was also observed in some adnexal structures and in high-density inflammation surrounding the tumor. Therefore, although it is not a very specific criterion, it has a high NPV.

Peripheral palisading and clefting were the best BCC-defining FCM criteria. Palisading was present in most BCCs, and even though clefting was observed in roughly half the BCCs, these criteria were almost pathognomonic for BCC and...
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The BCC stroma FCM image is highly specific and is reminiscent of a starry sky. It was present in 94% of BCCs and their surrounding dermis, but it was never observed in any HF s or EG cells. Thus, when compared with adnexal structures, the presence of stroma is very specific and has a PPV of 100%. We would like to highlight and stress the importance of this criterion. According to our experience, in any of the remaining skin structures.

Nuclear pleomorphism was observed in all BCCs but only in approximately half of the surrounding dermis and in 6% of HFs, but no EG cells showed nuclear pleomorphism. We suggest that this criterion describes a deviation from the round or elliptical nuclear shape and is the counterpart of the so-called nuclear atypia in classic BCC pathologic analysis with H&E. It could be observed in some activated fibroblasts in the surrounding dermis and in some HF bulbs, but the differences are significant.

Enlargement of the N/C ratio was present in all BCC cells, surrounding dermis cells, and EG cells as far as activated fibroblasts, inflammatory lymphocytes, and ductal cells have prominent nuclei and barely show cytoplasm. In HFs, some of the bulb cells showed increased N/C ratio, but most of them and all HF shaft keratinocytes had a low N/C ratio similar to that of normal epidermis.

In summary, when analyzing an FCM mosaic, if we have a well-circumscribed mass or lobule of pleomorphic hyperfluorescent bright dots with a striking tendency to arrange themselves with peripheral palisading next to the clefting, the likelihood of it being BCC is high.

The BCC stroma FCM image is highly specific and is reminiscent of a starry sky. It was present in 94% of BCCs and their surrounding dermis, but it was never observed in any HFs or EG cells. Thus, when compared with adnexal structures, the presence of stroma is very specific and has a PPV of 100%. We would like to highlight and stress the importance of this criterion. According to our experience, in some cases we observed the starry sky image within the freshly excised tumor true margin but we were unable to spot a single BCC nest. Afterward, the Mohs histotechnician went deeper with the cryostat and then the frozen sections stained with H&E showed the presence of BCC. So, as in classic Mohs frozen section reading, all inflammatory foci should be thoroughly investigated for tumor presence (A.B. 2011, unpublished data).

FCM Differences Among BCC Subtypes

We can conclude that BCC under FCM is seen as hyperfluorescent aggregates of pleomorphic nuclei with peripheral palisading arrangement and enlargement of the N/C ratio.

In sBCCs, fluorescent dots corresponding to tumoral nuclei were sparsely distributed along the basal layer of the epidermis without crowding images; here most sBCCs were described as ill defined. Clefting was present in all sBCCs. Moreover, sBCCs had less stromal reaction and inflammation than nBCCs and iBCCs.

Nodular BCCs are seen as a well-demarcated nodular crowding of hyperfluorescent pleomorphic nuclei with high N/C ratio. Fluorescent dots corresponding to tumoral nuclei were arranged in a parallel way in the periphery, and in most mosaics the starry sky image corresponding to stroma was also observed. Tumoral dermis was separated from the main BCC islands by black fluorescence-free areas corresponding to clefting.

Under FCM, iBCCs can be described as nests and strands composed of fluorescent pleomorphic dots infiltrating the surrounding dermis. The thinner the strands are, the less nuclear crowding, palisading, and clefting are present and the more ill defined the tumor is. Furthermore, iBCCs are accompanied by a strong stromal and inflammatory reaction.

Correlation Between FCM and H&E in Differentiation of BCC Subtypes

There was a high overall agreement between the FCM mosaics viewed by the Mohs surgeon and the H&E slides evaluated by a dermatopathologist (κ = 0.9) classifying BCC subtypes. This good agreement was possible because FCM images have cellular resolution and an excellent morphologic correlation with classic H&E histopathological slides.

Conclusion

Ex vivo FCM is a newly developed technology that may be an attractive alternative to frozen or paraffin-fixed, H&E-stained histologic sections during Mohs surgery because freshly excised tissue can be imaged without the need for tissue processing. Furthermore, it takes only a few minutes to obtain a 12 × 12-mm FCM mosaic, compared with 15 to 30 minutes with classic pathologic analysis techniques. This represents a first step toward a rapid “bedside pathology.”

There are a few articles analyzing the sensitivity, specificity, PPV, and NPV of FCM in evaluating residual BCC in Mohs surgery margins. However, to our knowledge, the FCM fluorescent and morphologic characteristics for BCC have not been described thus far.

We herein propose, describe, and validate the criteria for BCC diagnosis under FCM. Moreover, we demonstrate that there is a high correlation with the findings of the dermatopathologist when specimens are classified into the main BCC subtypes and an excellent correlation with frozen sections. However, more studies are required to validate this technique such that it can be incorporated into daily practice in a Mohs surgery setting.

Table 4. Interobserver Agreement

<table>
<thead>
<tr>
<th>Observation</th>
<th>Observer 1</th>
<th>Observer 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence presence</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Well demarcated</td>
<td>0.78</td>
<td>0.68</td>
</tr>
<tr>
<td>Nuclear pleomorphism</td>
<td>0.78</td>
<td>0.68</td>
</tr>
<tr>
<td>Palisading</td>
<td>0.90</td>
<td>0.76</td>
</tr>
<tr>
<td>Clefting</td>
<td>0.90</td>
<td>0.70</td>
</tr>
<tr>
<td>Nuclear pleomorphism</td>
<td>0.86</td>
<td>0.86</td>
</tr>
<tr>
<td>Increased enlargement of the N/C ratio</td>
<td>0.70</td>
<td>0.74</td>
</tr>
<tr>
<td>Stroma presence</td>
<td>0.87</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Abbreviation: N/C ratio, nuclear to cytoplasm ratio.

* The κ value was calculated for each observer compared with the first author.
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Study concept and design: Bennàssar, Puig, Vilalta, and Malvehy.
Acquisition of data: Bennàssar.
Analysis and interpretation of data: Bennàssar, Carrerra, Puig, and Malvehy.
Drafting of the manuscript: Bennàssar.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Puig.
Administrative, technical, and material support: Bennàssar.
Study supervision: Bennàssar, Puig, Vilalta, and Malvehy.

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REFERENCES

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