In this article we discuss the development of noninvasive imaging modalities to help delineate tumor margins of basal cell carcinomas in the setting of Mohs micrographic surgery. A review of the available literature reveals that dermoscopy can help delineate basal cell carcinomas before surgical removal but that it has no benefit over clinical inspection in reducing the number of Mohs stages. In contrast, fluorescence confocal microscopy has a sensitivity of 88–96% and specificity of 89–99% for the detection of basal cell carcinomas and can potentially serve as a rapid means for tumor evaluation on ex vivo specimens. Optical coherence tomography has shown some success in the presurgical evaluation of tumor margins in vivo, before surgical excision. With ongoing developments in device portability, speed of image retrieval, and image resolution, these technologies are likely to gain traction in cutaneous oncology research and practice. It is therefore important for dermatology clinicians and researchers to understand the mechanisms, principal uses, advantages, and limitations of each device.

INTRODUCTION
Basal cell carcinoma (BCC) is the most common type of skin cancer. The incidence of BCC is currently estimated to be 2.8 million and continues to increase. There are multiple treatment annually in the United States modalities currently available for BCC: shave excision, standard surgical excision, Mohs micrographic surgery, curettage alone, curettage followed by electrodessication, radiation therapy, cryotherapy, topical medications, and photodynamic therapy. The highest cure rates for BCC are associated with surgical approaches.

Mohs micrographic surgery (MMS), developed in the 1930s by Frederic Mohs, involves the precise removal of skin cancers with complete margin assessment. Although MMS offers a high cure rate for skin cancers, it can be a time-consuming and labor-intensive process consisting of tumor debulking, preparation of frozen histologic sections, staining with hematoxylin and eosin (H&E), and removal of residual tumor after histologic examination.

This article examines the development of noninvasive imaging devices as an adjunct to the surgical management of BCC, particularly for tumors with subclinical spread. We discuss the use of dermoscopy, confocal microscopy (CM), and optical coherence tomography (OCT) for the perioperative evaluation of BCCs. This paper focuses on how these technologies work, the current uses and limitations of each device, and future directions for improvement.

DERMOSCOPY
Basic principles
Dermoscopy, otherwise known as epiluminescence microscopy, provides a magnified view of subsurface components of the epidermis and papillary dermis (Figure 1). The original dermatoscopes were bulky instruments consisting of a magnifying eyepiece and light source. Immersion oil was

SUMMARY
Non-invasive imaging technologies for tumor margin delineation of BCC
- Dermoscopy can help identify a BCC or scar from a previous biopsy. It can help detect tumor outside the visually apparent margin.
- Fluorescence CM has a sensitivity of 88–96% and a specificity of 89–99% for the ex vivo detection of BCCs and can serve as a rapid means of tumor evaluation, potentially in place of standard histopathology.
- OCT has shown some success for the presurgical evaluation of tumor margins in vivo. OCT is capable of detecting subclinical tumor that crosses the visually apparent tumor margins.

LIMITATIONS
- The use of dermoscopy has not been shown to decrease the number of stages obtained during MMS.
- High costs limit the widespread availability of OCT and CM. Additional training is also needed to adequately interpret the resulting images.
applied to the lens, and direct contact with the skin was necessary. Newer dermatoscopes are hand-held devices with both polarized and nonpolarized lenses, do not require an immersion oil, and do not necessarily require direct contact with the skin.

**Characterization of BCCs on dermoscopy**

The classic BCC patterns observable with dermoscopy include arborizing vessels, large blue-gray ovoid nests, ulceration, maple-leaf and spoke-wheel areas, and multiple blue-gray globules. Other less obvious clues include short, fine, superficial telangiectasias, multiple small erosions, concentric structures, and multiple in-focus blue-gray dots (Altamura et al., 2010).

**Dermoscopy for the delineation of BCCs**

The borders of micronodular and infiltrative BCCs can be difficult to define. For these tumors, dermoscopy may provide some utility in detecting tumor outside the visually apparent margins. However, the use of dermoscopy has not been shown to decrease the number of stages obtained during MMS (Asilian and Momeni, 2013; Gurgen and Gatti, 2012; Suzuki et al., 2014).

**CONFOCAL MICROSCOPY**

**Basic principles**

CM, also known as confocal laser scanning microscopy, is routinely used in ophthalmology for the evaluation of retinal disease and has been more recently adapted for use in other medical specialties, including dermatology. The utility of CM for dermatology applications is currently being investigated. Current studies focus on the implementation of CM for tumor margin delineation and additionally as an adjunct tool for diagnosing melanomas and nonmelanoma skin cancers. CM has also been used for the evaluation of inflammatory diseases and nail conditions.

CM uses near-infrared light at 830 nm to provide imaging of high enough resolution to distinguish subcellular structures. The microscope allows only light back-reflected from a desired focal point within the skin to pass back through a gating pinhole and enter the detector. By blocking light outside the desired focal plane, the microscope is able to attain a lateral resolution of 1 μm (Figure 2) (Nwaneshiudu et al., 2012). One limitation of CM is its limited depth of penetration (200 μm), which enables visualization of only the epidermis and superficial papillary dermis.

**Characterization of BCCs using CM**

Using CM, features suggestive of BCC include polarized, elongated aggregates in the superficial layer, linear telangiectasia-like horizontal vessels, basaloid cords and nodules, and an epidermal shadow corresponding to horizontal clefting. Papillae are often not visible because the tumor alters the normal architecture (Guitera et al., 2012) (Figure 3).

**Role of CM for rapid histopathologic diagnosis**

A recent study investigated the role of reflectance CM for presurgical margin assessment of ill-defined BCCs in vivo (Venturini et al., 2016). Reflectance CM evaluation showed foci of BCCs outside the dermoscopic margins in 3 out of 10 lesions. The accuracy of reflectance CM for the in vivo assessment of BCC was confirmed by histopathology.

Most other published studies have focused on the role of CM as a rapid means of evaluating excised tumor tissue.
ex vivo, between MMS stages (Bennàssar et al., 2014; Chung et al., 2004; Gareau et al., 2009; Kaeb et al., 2009; Longo et al., 2014). Early studies used acetic or citric acid as contrast agents for ex vivo reflectance mode CM, with poor results. These served as weak contrast agents for nuclei, making it difficult to detect micronodular and infiltrative BCCs against the bright background reflectance of collagen. Recent studies have investigated the use of fluorescence CM, which is more effective (Bennàssar et al., 2014; Gareau et al., 2009). Fluorescence CM uses dyes, such as acridine orange, that specifically target subcellular structures. Acridine orange stains nuclear DNA in epidermal keratinocytes and enhances the tumor contrast against the background dermis. Acridine orange staining and CM use do not affect subsequent tissue processing and H&E staining.

The overall sensitivity and specificity of fluorescence-mode CM for detecting BCC with narrow or incomplete margins are 88–96% and 89–99%, respectively (Bennàssar et al., 2014; Gareau et al., 2009). The total time required for CM use is 5–7 minutes with the newer devices, with only 10–20 seconds required for immersion in the acridine orange dye. Increased adoption of this method in clinical settings would

Figure 3. Side-by-side comparison of basal cell carcinoma images. (a) Imaging with reflectance confocal microscopy. On reflectance confocal microscopy, the basal cell carcinoma appears as dark gray circular or oval structures (tumor islands) surrounded by black shadows (clefting). Collagen appears bright white. (b) Imaging with hematoxylin and eosin staining histopathology. Oval structures on reflectance confocal microscopy closely resemble the basaloïd islands seen on histopathology. Images courtesy of Dr. Harold S. Rabinovitz. A high-resolution image of the reflectance confocal microscopy image for use with the Virtual Microscope is available as an eSlide: VM02456.

Figure 4. Schematic representation of optical coherence tomography. Light is split into two directions by a beam splitter, with one part directed toward a reference mirror and the other directed toward biological tissue, reaching up to 1.5 mm beneath the tissue’s surface. The reflected light from each path is recombined, and if coherence is maintained, reflected light interferes at the detector. Basal cell carcinomas present as dark silhouettes that interrupt the normal architecture of the skin, enabling the clinician to scan the skin and delineate the tumor. BCC, basal cell carcinoma.
save time and increase surgical efficiency, considering the 20–45 minutes currently required to process fresh frozen sections for MMS without the use of CM. Current limitations of CM include the high cost of the devices and the need for significant training in image interpretation.

OPTICAL COHERENCE TOMOGRAPHY

Basic principles
OCT works in a manner analogous to ultrasonography, but it uses a 1310-nm light instead of sound waves, resulting in a higher resolution than ultrasonography. Similar to an ultrasound device, an OCT device can be used in vivo on intact tissue, with no adverse risks or tissue damage. A light source is split in two directions by a beam splitter, with one half directed toward a reference mirror and the other directed at the lesion of interest, reaching up to 1.5 mm beneath the tissue’s surface. The reflected light from each path is recombined, and if coherence is maintained, reflected light interferes at the detector (Figure 4).

The image quality of conventional OCT (lateral resolution of 3–15 μm) is of lower resolution than that of CM, making it difficult to visualize cellular details and to distinguish between various types of neoplasms. The advantage of using this device is its higher depth of penetration, which reaches up to 1.5 mm and enables the clinician to view deeper tumor aggregates. Imaging of a lesion also takes less time than with reflectance CM, with conventional OCT devices taking 1 minute or less to obtain an image. Conventional OCT can rapidly define borders of a tumor already diagnosed by traditional histology.

Research is underway investigating the benefits of high-definition OCT in cutaneous oncology. High-definition OCT permits a higher resolution (3 μm in the lateral and vertical directions) than conventional OCT, enabling greater visualization of cellular detail. It achieves this resolution without wholly sacrificing the depth of detection. High-resolution OCT can recognize tumors that are 570 μm deep, in contrast to CM, which has an imaging depth of 200 μm (Boone et al., 2013).

Characterization of BCCs on OCT
With conventional OCT, islands of BCC appear as dark oval silhouettes surrounded by a darker border, with occasional thinning of the epidermis (Figure 5) (Hussain et al., 2015).

OCT for the delineation of BCCs
Given the speed at which images are obtained using OCT, it is possible to manually scan the entire periphery of a tumor or to measure specific points around the boundary. Alawi et al. (2013) studied tumor margin delineation in 19 lesions including 12 BCCs, three squamous cell carcinomas, and four other types of skin tumors. In 84% of cases, lateral margins defined using OCT correctly indicated complete removal of the tumor (Alawi et al., 2013). OCT can also define tumor boundaries with more accuracy than a Mohs surgeon. In one study, lesions that required one MMS stage were predicted by OCT to be 1.4 ± 1.3 mm smaller than the Mohs excision. For lesions that required more than one MMS stage, OCT always predicted that tumor tissue lay outside the planned MMS margin (Wang et al., 2013).

Although the in vivo use of OCT can potentially reduce the number of stages in MMS, ex vivo use is not recommended because excised specimens show reduced contrast and increased optical scatter on OCT. Reported sensitivity and specificity for ex vivo OCT use is only 19% and 56%, respectively (Cunha et al., 2011).

Future developments might include refining the balance between image resolution and maximum imaging depth. High-resolution OCT offers image resolution greater than conventional OCT while maintaining an imaging depth that exceeds that of reflectance CM. Ongoing improvements in image resolution, depth, and processing speed will allow development of imaging techniques like OCT so that they become practical for clinical use.

CONCLUSION
This article highlights the utility, advantages, and limitations of several imaging techniques for delineating BCC margins. Dermoscopy can help delineate BCCs before excision but has no benefit compared with clinical inspection in reducing the number of MMS stages. Noninvasive imaging devices such as CM and OCT hold some promise for improving the evaluation of BCC excision perioroperatively during MMS. To date, no studies have directly compared the sensitivity and specificity of CM versus high-definition OCT for delineating tumor margins and reducing the number of MMS stages. Future projects might include a prospective study comparing the two imaging modalities in the surgical setting. Additionally,
research on optical imaging for the diagnosis of skin cancer, identification of biopsy sites, and monitoring of nonsurgical treatment outcomes will help establish the overall utility of CM and OCT in daily clinical practice.

CONFLICT OF INTEREST
The author states no conflict of interest.

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MULTIPLE CHOICE QUESTIONS

1. Which of these technologies is otherwise known as epiluminescence microscopy?
   A. Dermoscopy
   B. Confocal microscopy (CM)
   C. Optical coherence tomography (OCT)
   D. All of the above

2. When used to enhance tumor contrast in confocal microscopy, what subcellular structure does acridine orange highlight?
   A. Keratin
   B. Cytoplasm
   C. Nuclei
   D. Cell membrane

3. Which device has an excellent lateral resolution of 0.5-1 μm (micrometer) but a limited depth of penetration of 200 μm (micrometer)?
   A. Dermoscopy
   B. Confocal microscopy (CM)
   C. Optical coherence tomography (OCT)
   D. All of the above

4. Which of these technologies works in a manner analogous to ultrasound but uses light instead of sound waves?
   A. Dermoscopy
   B. Confocal microscopy (CM)
   C. Optical coherence tomography (OCT)
   D. All of the above

5. Features of confocal microscopy include which of the following?
   A. Provides 10x magnification and sometimes uses a polarized light source
   B. Utilizes 830 nm light focused through a narrow pinhole
   C. Relies upon low-coherence interferometry
   D. Emits ultraviolet light, which reflects off the object being viewed

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to this paper. Powerpoint teaching slides are available as supplementary material.

REFERENCES


