The assessment of cutaneous melanoma in the clinical setting is often difficult, and important features such as depth and width remain unknown until the pathology report is received. Access to prognostic features such as vertical height before excisional biopsy would offer a basis for guidance in defining surgical margins and early planning of treatment options. Recently developed high-frequency ultrasound imaging in the 40- to 60-MHz range is a noninvasive method that provides in vivo information about cutaneous lesions. Imaging at these frequencies provides high-resolution data within the range of the epidermis and dermis (3–4 mm in depth). Ten cutaneous melanomas and seven pigmented lesions were assessed in this fashion. Vertical height was documented and compared to histopathological findings. High-frequency ultrasound imaging determination of vertical height correlated well with the standard measurement of Breslow's thickness on histological sections only in midrange (1.0–3.0 mm) lesions. Inflammatory cells at the base of three melanomas provoked an overestimation of the depth measurement with ultrasonography. Thick keratin layers such as those found on the feet acted as a virtual block to the high-frequency scanner. The application of this new advance in noninvasive imaging technology to the clinical assessment of cutaneous melanoma provides interesting in vivo data but in its present state does not replace the need for the biopsy of pigmented lesions and histopathological diagnosis.


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The clinical assessment of cutaneous melanoma is often difficult even for the most experienced clinician. Documentation of important features such as depth and width remains speculative until the definitive pathological report arrives. Immediate access to prognostic features such as vertical height and satellitosis before excisional biopsy would offer a basis for early guidance in defining surgical margins and appropriate planning of treatment options.

Although standard ultrasound scanners operating at 3 to 7 MHz have been used for many clinical applications in radiology, cardiology, and obstetrics, higher frequency (20–100 MHz) ultrasound imaging has been used in ophthalmology [1] and for imaging through endoscopic and catheter-based systems [2]. In dermatology, 20-MHz ultrasonography has been used to image benign and malignant lesions and dermatoses such as psoriasis and morphea [3, 4]. There have also been reports of 20-MHz ultrasound technology being applied to measure the dimensions of melanotic lesions [5, 6]. The drawback of these low-frequency systems is their limited resolution. More recently, a few experimental 50-MHz ultrasound imaging systems have been developed for skin imaging [7–9], but no studies of melanoma have been performed at the higher frequency.

In this study, a high-frequency 40- to 60-MHz B-scan ultrasound system was used to assess 10 cutaneous melanomas and 7 benign pigmented lesions in a clinical setting. Higher frequency scanning provides a high degree of axial and lateral resolution, enabling a more precise delineation of superficial lesions confined to the epidermis and dermis [9–11]. Vertical height was documented using the high-frequency scanning
system and then compared with the standard histopathological reporting techniques after excision of the lesion.

Patients and Methods

A real-time B-scan ultrasound backscatter microscope (UBM) operating in the 40- to 100-MHz range has been developed for skin imaging [9]. In this study, the UBM was operated between 40 and 60 MHz, providing axial (depth) resolution between 20 and 30 μm and lateral (horizontal) resolution between 33 and 94 μm. The depth of penetration in skin is 3 to 4 mm at 50 MHz. The scanner uses a focused copolymer (polyvinylidene difluoride and trifluoroethylene) transducer, which is excited by a 400-V monocycle pulse generator. The received ultrasound signal is logarithmically amplified, demodulated, and passed through a custom scan converter, which outputs video images. Each image is produced by scanning the transducer over an 8-mm linear path at a frame rate of 5 to 10 images per second. Sequential 8-mm sections of a lesion can be imaged over a distance up to 25 mm, and the distance between sections can be specified. These features enable the depth of the lesion to be imaged with confidence. The images are digitally stored and can be subsequently retrieved for review.

Patients for the study were recruited from those attending the Pigmented Lesion Clinic at the Toronto-Bayview Cancer Center, Sunnybrook Health Science Center, University of Toronto. The protocol was approved by Sunnybrook Health Science Center, University of Toronto, and written informed consent was obtained from each patient. Each patient was positioned so that the lesion to be imaged was in a horizontal plane. Coupling between the skin and the transducer was provided by placing a 30-mm diameter acrylic cup over the lesion to be imaged, pressing the cup gently against the skin and filling it with saline or a viscous methyl cellulose solution (Fig 1). Alternatively, a viscous gel can be applied over the lesion, taking care to exclude trapped air and then lowering the transducer into this gel. The transducer has been mounted on an articulated arm for stability and maneuverability.

The in vivo depth of 10 melanomas and 7 pigmented lesions was measured using high-frequency ultrasound. For each lesion the depth was measured from the initial entry echo to the bottom of the lesion using electronic calipers. This measurement assumes a speed of sound in skin of 1,600 m/sec, which is expected to be within ±5% of the actual value [12]. The pigmented lesions were echo poor (hypoechoic), and the surrounding tissue was generally more echo rich (echogenic).

The lesions were subsequently excised. The specimens were fixed in buffered formalin. Cryostat cut sections were stained with hematoxylin and eosin. The sections were examined by an experienced dermatopathologist and the clinical diagnosis confirmed. For melanomas, the Breslow depth was measured in the standard way from the bottom of the granular layer to the deepest portion of the lesion. The depth of each lesion was ascertained by two separate methods: from histological sections and using high-frequency ultrasonography by two independent investigators who were unaware of the depth measurement derived using the other methodology. The depth of the histological sections can be measured by microscopy to the nearest 10 μm. Using ultrasonography, the accuracy of the depth measurement is limited by the axial resolution of the system, which has been measured to be between 20 and 30 μm, and by the image contrast.
### Table 1. Comparison of Depth of Melanomas Obtained Histopathologically and by Using High-Frequency Ultrasonography

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Pathology</th>
<th>Vertical Height (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SSM, CL3</td>
<td>0.92</td>
<td>0.95</td>
</tr>
<tr>
<td>2</td>
<td>SSM, CL3</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>3</td>
<td>SSM, CL2</td>
<td>0.42</td>
<td>0.37</td>
</tr>
<tr>
<td>4</td>
<td>SSM, CL3</td>
<td>0.86</td>
<td>1.16</td>
</tr>
<tr>
<td>5</td>
<td>NM, CL4</td>
<td>1.65</td>
<td>1.37</td>
</tr>
<tr>
<td>6</td>
<td>NM, CL4</td>
<td>4.00</td>
<td>≥3.7 (ultrasound beam did not penetrate beyond 3.7 mm)</td>
</tr>
<tr>
<td>7</td>
<td>Melanoma in dysplastic nevus</td>
<td>0.24 (melanoma and adjacent inflammatory infiltrate = 0.50 mm)</td>
<td>0.48</td>
</tr>
<tr>
<td>8</td>
<td>SSM, CL2</td>
<td>0.40 (melanoma and adjacent inflammatory infiltrate = 0.84 mm)</td>
<td>0.88</td>
</tr>
<tr>
<td>9</td>
<td>SSM, CL2</td>
<td>0.54 (melanoma and adjacent lymphocytic infiltrate [regression] = 1.03 mm)</td>
<td>1.16</td>
</tr>
<tr>
<td>10</td>
<td>SSM, CL3</td>
<td>0.83 (melanoma and adjacent scar = 1.75 mm)</td>
<td>2.30 (section adjacent to scar gives depth = 0.85 mm)</td>
</tr>
</tbody>
</table>

SSM = superficial spreading melanoma; NM = nodular melanoma; CL = Clark level of invasion of melanoma.

between the lesion and normal skin. This contrast is a function of the frequency-dependent reflectivity or backscattering properties of the tissues for which there are very few data available. It should also be noted that the ultrasound measurements are of an in vivo nature, whereas the depth is obtained from histopathological specimens that are fixed in formalin and may undergo some degree of distortion.

The generation of ultrasound images is, to some extent, operator dependent. Therefore, the same operator imaged all the lesions. It is important to apply minimal pressure against the acrylic cup when imaging because variations in pressure may alter the thickness measurement of the lesion.

**Results**

The results of the comparison between vertical height estimation with the electronic calipers of the high-frequency ultrasound scanner and standard pathological measurement of Breslow's thickness are listed in Table 1. Ultrasound measurement in Patients 1 to 4 (lesions <1 mm in thickness) showed good correlation with the pathological description of vertical height (Fig 2A–C). In Patients 5 and 6, with lesions greater than 1.5 mm in depth, the ultrasound measurement appeared less accurate. Attenuation of the ultrasound beam below the detection level of the scanner was seen after 3 to 4 mm of depth penetration.

The presence of inflammatory cells at the base of the lesion in Patients 7 and 8 provoked overestimation of vertical height using the electronic calipers of the ultrasound scanner (Fig 3A–C). The scanner accurately marked the extent of the inflammatory response but did not distinguish between melanoma and inflammatory cells. In Patient 9 adjacent lymphocytic infiltrate in an area of regression provoked a similar overestimation.

A partially healed biopsy scar within the melanoma (Patient 10) caused disruption of the architecture and difficulty in assessing vertical height except for the adjacent segments. Ultrasound imaging produced similar-appearing echo patterns for both melanoma and scar [9].

Two patients presented with melanomas on the soles of their feet. The ultrasound imaging system used in this study was unable to penetrate the thick keratin layer overlying the melanoma.

Two patients with in situ melanomas and two with dysplastic nevi had shallow lesions confined entirely to the epidermis. At the 40- to 60-MHz frequencies used in this study, it was not possible to clearly identify these very thin lesions.

Patients with nonmelanoma lesions (Patients 11–17 in Table 2) included those with dysplastic nevus (n = 1), blue nevus (n = 1), compound
nevus (n = 1), congenital nevi (n = 2), pigmented basal cell carcinoma (n = 1), and a pigmented seborrheic keratosis (n = 1). There was an accurate comparison between the vertical heights measured histopathologically and ultrasonographically (see Table 2). Congenital nevi often demonstrate extension of small numbers of nevus cells along hair follicles, sweat ducts and glands, and sebaceous glands around vessel walls, between collagen bundles, and in the perineurium of the nevus. The resolution of the ultrasound scanning system used in this study was not detailed enough to detect such small collections of nevus cells.

Discussion

The single most reliable prognostic indicator of malignant melanoma is the vertical height measured as Breslow’s thickness or Clark’s level of invasion [13]. Immediate access to these data within the clinical setting would provide a basis for defining surgical margins and early planning of management options. The development of 50-MHz high-frequency ultrasound scanners suitable for skin imaging [7–10] and their subsequent application for assessment of skin lesions has provided technology that may be useful in obtaining in vivo clinical data from cutaneous melanoma.

In this study of 10 cutaneous melanomas, the vertical height measurements by high-frequency ultrasound compared well with the Breslow thickness derived from stained fixed sections only in the thin to midthickness lesions (see Table 1). The presence of inflammatory cells at the base of the melanoma or lymphocytic infiltrate, as is present in areas of regression, results in an overestimation of melanoma invasion using the electronic calipers with high-frequency ultra-
sonography (Patients 7–9). The resolution of this scanning system did not enable us to differentiate between the melanocytic lesion and a contiguous cellular infiltrate. This has also been the experience of other investigators [6]. It is interesting, however, to consider the accuracy of this in vivo ultrasound measurement as it correlates with traditional histopathology with its distorting potential of excision, fixatives, and slide preparation. Benign lesions compared similarly with both methods of measurement (see Table 2). In situ lesions, however, were difficult to assess accurately because of the poor definition at shallow levels.
Fig 4. Patient 15 (see Table 2) presented with a suspicious pigmented lesion. Ultrasound evaluation (A) delineated a classic seborrheic keratosis appearance, which correlated with histological evidence (B). The superficial nature of this benign lesion is also easy to recognize. Approximate 1:1 matching of image size is shown between ultrasonography and histopathology.

Fig 5. Patient 6 (see Table 1) presented with a deep melanoma that had a similar clinical appearance to that presented in Figure 4. In this case ultrasonography (A) showed a homogeneous structure, disappearing into the depth of the dermis beyond the range of penetration of the high-frequency ultrasound beam. The histopathological section of this tumor is shown for comparison (B).

The high-frequency ultrasound images also delineate structure, which can provide prebiopsy data in otherwise suspicious lesions. Figure 4A and B shows a pigmented seborrheic keratosis with typical keratin whorls and vacuoles on both ultrasonography and histopathology. The superficial nature of the lesion is also well demarcated. In contrast, a deep nodular melanoma in Figure 5A and B has a homogeneous appearance with severe attenuation of the signal at depths greater than 3 mm, indicating a deeper melanotic lesion.

Thick keratin layers can act as a virtual block to the ultrasound beam; therefore, melanomas occurring on the plantar surface of the foot were not included in this study. Ultrasound penetration of the palmar surface of the hand was variable depending on the development of the keratin layers. In this study, all lesions were from the trunk or limbs. Ultrasound assessment of lesions on the face, on the scalp, or in specialized areas such as the perineum may provide difficult access with poor scanning by the machine. The
scanning probe moves back and forth over an 8-mm linear path and ideally is maintained in a cellulose bath within an acrylic collar as described in the Patients and Methods section. This limitation could be easily addressed by redesigning the probe to allow flexible coupling to irregular patient contours.

To image the deeper lesions within the lower dermis and subcutaneous tissue, it will be necessary to use a lower frequency transducer. Shallow lesions such as those present entirely within the epidermis were also not visualized with adequate clarity using the 40- to 60-MHz frequency system. A higher frequency transducer might enable such thin lesions to be better delineated. In order for high-frequency ultrasound imaging systems to become more effective and for practical assessment of cutaneous melanomas, we envisage a machine that will have the capability of providing imaging over a wide frequency range, from 20 to 100 MHz. A practical design might incorporate multiple transducers similar to the objectives on a microscope. Each of these would have an optimum frequency range and thus could be adjusted for the appropriate depth penetration.

**Summary**

In this study, new advances in high-frequency ultrasound imaging have been applied to the assessment of cutaneous melanoma. The 40- to 60-MHz B-scan ultrasound technology in its present form would not replace the need to perform a biopsy of pigmented lesions and obtain histopathological analysis. It is, however, a noninvasive, reproducible method providing interesting in vivo data regarding the depth of cutaneous melanoma. Its accuracy in this preliminary study was maximum in melanomas ranging in size from thin to midthickness.

This article is not a formal assessment of diagnostic test validity. The sensitivity, specificity, and predictive value would have to be calculated before a true evaluation of its diagnostic ability could be determined. In these times of cost containment in health care, the addition of another diagnostic test must be shown to be cost effective.

If ultrasound imaging technology progresses to a point at which a diagnosis of melanoma and its depth can be accurately made on a consistent basis, this method may provide guidance in defining surgical margins and in early planning of treatment options.

**References**

Invited Discussion

Alexander Zemtsov, MD, MS

The first successful ultrasonographic examination of the skin was performed by United States bioengineers, Alexander and Miller, in 1979 [1]. Over the past 15 years over 100 articles were published in peer reviewed literature dealing with this subject; and most recently, a handbook entitled Ultrasound in Dermatology was printed by Springer-Verlag Publishing House [2]. For reasons beyond the scope of this discussion this research was conducted almost exclusively outside of the United States where it's gaining widespread acceptance; in Germany a physician cannot become board certified in dermatology unless he personally performs 200 ultrasound scans of skin lesions [3]. Furthermore, two of the original three companies selling skin ultrasound equipment are still in business and appear to be prospering (T.P.M., represented in the U.S. by G.W.B. Ltd., and Cortex, represented in the U.S. by Brymill Corporation). Prior to 1992 all skin ultrasonography research was conducted on 20-MHz units. In this issue of Annals, Semple and his Canadian coworker measured the depth of cutaneous melanoma and other skin tumors on the machine operating at 40 to 60-MHz frequency [4]. Before addressing their work directly, I would like to summarize other investigators' observations on the use of 20-MHz skin ultrasonography in clinical management of cutaneous melanoma.

The first definitive studies comparing in vivo ultrasound-measured melanoma depth to histological depth of the tumor (Breslow's depth) were conducted by a group of Israeli plastic surgeons [5, 6]. In all their cases the in vivo ultrasound-measured melanoma depth was slightly, i.e., a few tenths of a millimeter, higher in comparison to Breslow's depth. They hypothesized that this slight discrepancy was due to tissue shrinkage during fixation in formalin, a phenomenon that is used to explain the different thickness of melanomas on frozen section in comparison to paraffin section. Zemtsov and colleagues, utilizing MRI, also discovered that in vivo MRI-derived tumor depths were a few tenths of a millimeter higher than Breslow's depth [7]. Finally, Austrian researchers recently completed a large study where they measured melanoma depth in vivo by 20-MHz ultrasound, then measured the tumor depth by ultrasound in vitro immediately after the tumor's elliptical excision, and compared these in vivo and in vitro ultrasound measurements to histologically-derived Breslow's depth (author personal communications). The results of this study seemed to indicate that immediately after excision due to tissue recoil the ultrasound measured tumor depth is actually higher than in vivo, but after formalin fixation the tissue shrinks to a few tenths of a millimeter below in vivo depth.

In Semple's study published in this issue of the journal, authors utilizing 40 to 60-MHz frequency in some cases reached similar conclusions (Patients 1 and 2) [4]. However, these Canadian investigators were unable to accurately measure melanoma thicker than 1.5 mm and melanomas on the palms and soles. This is most likely due to the fact that their unit in comparison to 20-MHz units has higher resolution in upper 1–2 mm of the skin but does not penetrate and provide good resolution in reticular dermis. El-Gammal and colleagues believe that 40 to 60-MHz units should be primarily used to study epidermal pathology such as lichen planus and psoriasis rather than skin tumors [8]. In conclusion, I believe that as technology continues to improve, skin ultrasonography will find numerous applications in both dermatology and plastic surgery.

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References