Imaging the mechanical stiffness of skin lesions
by in vivo acousto-optical elastography

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Abstract: Optical elastography is an imaging modality that relies on variations in the local mechanical properties of biological tissues as the contrast mechanism for image formation. Skin lesions, such as melanomas and other invasive conditions, are known to alter the arrangement of collagen fibers in the skin and thus should lead to alterations in local skin mechanical properties. We report on an acousto-optical elastography (AOE) imaging modality for quantifying the mechanical behavior of skin lesions. The method relies upon stimulating the tissue with a low frequency acoustic force and imaging the resulting strains in the tissue by means of quantifying the magnitude of the dynamic shift in a back-reflected laser speckle pattern from the skin. The magnitude of the shift reflects the local stiffness of the tissue. We demonstrate AOE on a tissue-mimicking phantom, an in vivo mouse melanoma lesion and two types of in vivo human melanocytic nevi. The skin lesions we examined were found to have distinct mechanical properties that appear to correlate with the varying degrees of dermal involvement of the lesions.

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1. Introduction

The determination of the elastic and viscoelastic properties of soft biological tissues is of fundamental interest in the detection and diagnosis of many diseases, since these properties depend, in a very significant manner, on the state (healthy or pathological) of the tissue. Analogous to manual palpation, pathological tissue regions will have a different strain response (it will “feel” stiffer or softer) to an imposed stress than will surrounding healthy tissue of the same type. The magnitude of the strain response may be greater or less than the surrounding normal tissue depending upon the nature of the pathology. This change in mechanical behavior with pathologies is the reason why manual palpation is successful at assessing the relative health of tissues and organs. For example, the manual location of mechanically distinct nodules is one of the fundamental tools used in the detection of breast tumors. Skin lesions are also manually palpable, presenting as tissue regions with increased surface roughness as well as a different stiffness compared to the surrounding tissue. By imaging the mechanical behavior of skin, not only can diseased tissue regions be detected based upon variations in mechanical behavior, but the margins of these lesions can also be identified with good fidelity. AOE is completely non-invasive and may lead to a reduction in the number of painful, time-consuming, expensive, and invasive biopsy procedures performed each year.

All imaging modalities rely upon some form of contrast mechanism for image formation, for example, acoustic impedance mismatches for ultrasonic imaging, density changes for X-ray tomography, discontinuities in refractive index for optical coherence tomography, and fluorophore concentration for standard fluorescence imaging. Optical elastography relies upon differences in local mechanical properties as a contrast agent. Thus, while AOE depends upon light scattering, the differences in local tissue reflectance due to photon scattering and
absorption do not influence the images. Indeed, the requirements of the imaging system are modest; the only requirement being that the camera resolves the backscattered laser speckle.

Elastography in general is a relatively new method for quantitative imaging of strain and elastic modulus distributions in tissue [1]. The modality is based on the estimation of strain due to tissue compression or expansion [2] and traditionally employs either ultrasonic (US) or magnetic resonance (MR) signals to carry the information and form the images (elastograms). Both US and MR elastography have been used to quantify the elastic behavior of breast tissue and breast lesions [3-9] as well as prostate tissue for the detection of prostatic cancer [10, 11]. In these studies, it was found that lesions were detectable by the elastographic methods based on differences in their elastic modulus from the surrounding tissues. Ultrasound elastography has also been applied to the assessment of vascular conditions such as atherosclerotic lesions [12] and MR elastography has seen applications in studying the contractile properties of skeletal muscle [13]. Elastography has not been applied to any great extent for examining skin, however, a few reports have indicated that ultrasonic imaging may be useful in the evaluation of skin diseases [14, 15].

In our AOE imaging system, a small, low frequency dynamic force is applied acoustically to the skin while the lesion of interest is illuminated with a collimated, coherent light source. At very low frequencies the acoustic wave couples into the tissue primarily as a surface Rayleigh wave [16, 17, 18]. Rayleigh waves propagate along the surface of the tissue with a counter-rotating elliptical particle motion that induces a plane-stress to the surface of the skin [18]. The local strain response of the skin to this stress depends entirely upon the local mechanical properties of the skin, which in turn are dependent in a very significant manner on the health of the tissue. The local strain response is quantified and encoded into a 2-dimensional surface map by tracking the shift in the speckle pattern with sub-pixel resolution [19, 20]. The resulting “speckle shift” image is further processed using a convolution filter to identify regions in the image that have different strain responses to the applied dynamic force. To demonstrate AOE imaging, we elastically imaged a tissue mimicking phantom that had a 3 mm diameter, less stiff inclusion inserted into it, a 4 mm diameter melanoma in an in vivo melanoma mouse model (MM), and 2 different types of in vivo human skin lesions with different degrees of dermal involvement including a 3 mm diameter compound nevus (CN) and a 3 mm junctional nevus (JN).

Human melanocytic nevi (common moles) are of importance because they can develop into melanomas, although at low incidence. Melanocytic nevi are derived from a proliferation of melanocytes which are the pigment producing cells that reside in the basal layer of the epidermis. Nevi come in different varieties based on the location of these nests of melanocytes: along the epidermal-dermal junction (junctional nevi), within the epidermal-dermal junction and dermis (compound nevi), or entirely within the dermis (intradermal nevi). Distinguishing them from melanomas by their clinical appearance can be difficult especially in the earlier stages when the cure rate is high and can only be done reliably by histopathologic evaluation.

2. Methods

2.1 AOE imaging system design

For all of the studies, the procedures for AOE imaging were identical (Fig. 1). A small speaker (8Ω, 0.1 W) was placed approximately 1 cm from the lesions and was driven with a 10 V peak-to-peak sine wave directly from a function generator (Stanford Research Systems, DS345, Sunnyvale, CA, USA) at either 5 Hz (human and phantom studies) or 1 Hz (mouse study). The lesions and surrounding skin were illuminated from an off-axis angle of 40° with an expanded and collimated 5 mW green diode-pumped solid state (DPSS) laser emitting at 543 nm. The diameter of the illuminated spot on the skin was on the order of 12 mm yielding a local power density of 1.13e-4 mW/cm² which is well below the safety threshold as...
specified by the FDA for illumination of skin. The backscattered laser speckle pattern was observed in a direction normal to the skin surface by a 1394b CCD camera running at 200 Hz full frame (Dragonfly Express, Point Grey Research, Vancouver, BC, Canada). The camera had a 640 x 480 CCD chip with a 7.6 μm pitch and was mounted with an f/2.8 telecentric lens. The lens was misfocused by a small amount (~ 0.5 cm) to induce a lateral motion of the speckle pattern in response to the passing acoustic waves. If the system were perfectly focused, the speckle pattern would simply appear to “boil” instead of translate in space and time. Two hundred images were taken for each experiment, so that the total measurement time was 1.0 s.

The magnitude of the shift in the speckle pattern was determined on a pixel-by-pixel basis using a 2-dimensional maximum-likelihood shift estimator [19, 20]. These shifts were then encoded as a 2-D image of speckle shift. To improve the contrast of the shift images, a convolution operator was applied to the images. A rectangular region of the shift image was used as the convolution kernel. In this manner, the magnitudes of the shift would be matched by the operator, and thereby discriminate the speckle shift from the lesion from the speckle shift seen in the surrounding skin.

2.2 Tissue phantom experiments

The tissue phantom was made from 99% hydrolyzed polyvinyl alcohol (PVA; Sigma Aldrich Inc., St. Louis, MO, USA) made at a concentration of 20% w/w with DI water. The PVA was dissolved in the water in a 95°C water bath for 3 hours under constant agitation to ensure thorough mixing. The liquefied PVA material was de-gassed and poured into a 21 cm X 14 cm rectangular mold. The phantom was 3.5 cm thick. The phantom was allowed to cool, placed overnight into a -20°C freezer and subsequently subjected to a series of freezing and thawing cycles by placing it in the freezer for approximately 12 hrs., then removing it from the freezer and allowing it to sit at room temperature for the next 12 hrs. Repeated freezing and thawing of PVA is known to increase the mechanical stiffness of the material in a reproducible manner [21]. The bulk phantom was subjected to a total of 7 freezing and thawing cycles. However after 2 cycles a small 3 mm diameter, 1 cm deep hole was drilled into the phantom and filled with new, liquefied PVA of the same concentration as the bulk.
phantom before the phantom was re-frozen. This 3 mm spot, then, was ultimately subjected to
5 freezing and thawing cycles creating a small ‘lesion’ that was less stiff than the surrounding
bulk PVA phantom. From previous (unpublished) measurements in our laboratory, the
stiffness of the ‘lesion’ was on the order of 80% of that of the bulk phantom. AOE images
were generated subsequently in the manner described above.

2.3 In vivo murine melanoma measurements

Using AOE, we also investigated the elastic imaging of a malignant melanoma in the HGF/B6
transgenic murine melanoma model [22]. These genetically engineered mice over-express
hepatocyte growth factor/scatter factor. Like human melanoma, the HGF/B6 mouse develops
melanoma through progressive stages of increased size and invasiveness after exposure to UV
irradiation. The mouse strain used in this study has a pigmented C57BL/6 genetic background.
The adult mouse (37 g) was anesthetized with a cocktail of 50% sterile H2O, 30 % of 100
mg/ml ketamine, 15 % of 20 mg/ml xylazine and 5 % of 10 mg/ml acepromazine. AOE
imaging with a 1 Hz acoustic stimulation was performed while the mouse was still alive, but
at a reduced respiration rate to eliminate breathing motion artifact. Prior to imaging, the skin
in the region of the melanoma lesion was shaved with an electric shaver and the remaining
hair removed with a commercial human depilatory lotion (Veet, Reckitt Benckiser, Inc.).
Tissue samples for histology were harvested after administering a lethal dose of 250 cc of 1:1
dilution with saline of the cocktail. While there are many similarities between mouse and
human skin, some vital differences relating to melanoma exist. For example, whereas the
melanocytes in humans are mostly located at the epidermal-dermal junction, in mice, the
melanocytes are mostly found in the hair follicles and in the inter-follicular dermis or dermal-
epidermal junction. Furthermore, the early stages of human melanoma is typified by a spread
towards the surface of the skin of atypical melanocytes, this spreading is less characteristic of
murine melanomas. However, in spite of these differences, the actual physical depth of the
early lesions is similar between the two. Thus, murine models of melanoma offer
reproducible means for the investigation of developing lesions in real time and for
optimization of new melanoma imaging modalities that can be applied to human melanotic
lesions.

A real time OCT system was used in this study to provide non-invasive 3-D imaging of the
mouse skin to support our findings in the AOE elastography imaging. OCT utilizes low-
coherence interferometry to perform optical ranging within a biological tissue that enables
visualization of microstructures in the biological tissue based on its optical scattering and
absorption properties. The OCT system used in this study was operated with a partially
coherent light source emitting at a central wavelength at 840nm and implemented with the
spectral domain configuration wherein an ultra-fast linear detector array was used to detect
spectroscopically the light reflected from the skin. The system had a measured imaging
resolution at 20 x 20 x 9 μm in the x-y-z directions, and was capable of providing cross-
sectional images at 58 frames per second or one volume-metric 3-D structural image every 8
seconds covering an area of skin of 3 x 3 x 1.5mm [23]. The focused OCT light source was
delivered to the lesion and surrounding skin of the animal, with an incident energy deposition
on the order of 25 mJ/cm². The light reflected by the skin was coupled back into the OCT
system where the cross-sectional images were obtained. Because of the increased angiogenesis
around the skin lesions, the reflectance was reduced due to the blood absorption of the
incident 840 nm light. This is believed to give the imaging contrast in the OCT images to
differentiate the lesion from the surrounding tissues.

The tissue sample containing the melanoma lesion was fixed and dehydrated by sequential
immersions in formalin and isopropyl alcohol, respectively. Histological sections were
stained with hematoxylin-and-eosin and prepared by standard histological procedures.
2.4 In vivo human melanocytic nevi measurements

We elastically imaged 2 different types of in vivo human skin lesions with expected different degrees of dermal involvement including a 3 mm diameter compound nevus (CN) and a 3 mm diameter junctional nevus (JN) following the procedures outlined above. Because of the predicted differential involvements of the dermis we predicted that the JN would present as a stiffer region in an elastogram and the CN would present as a less stiff region. A deeper lesion would likely alter the collagen structure over a larger mass of tissue than would a shallow lesion.

2.5 Ethical review of procedures

All experimental animal and human procedures were carried out in conformity with the guidelines of the US National Institutes of Health and were approved by the Institutional Review Board and the Institutional Animal Care and Use Committee of the Oregon Health & Science University.

3. Results

3.1 Tissue phantom experiments

The elastogram (x-direction) of the artificial lesion is shown in Fig. 2. The edge of the speaker was located 2 cm from the lesions (to the right in this image). Earlier work has shown that the magnitude of the Rayleigh wave deceases in an exponential fashion from the edge of the speaker [18]. The artificial lesion in the phantom is clearly revealed as an area of higher strain response than the surrounding more stiff phantom material. The strain response of the bulk phantom to the acoustic stimulus was on the order of 80% of that of the lesion, as predicted from our earlier mechanical testing.

![Fig. 2. Strain-encoded elastogram of the artificial lesion in the tissue phantom. The lesion appears as a region of higher strain response than the surrounding phantom material (red region in the center of the elastogram).](image)

3.2 In vivo murine melanoma measurements

The AOE system successfully imaged the murine melanoma lesion. Figure 4 is a photograph of the shaved mouse, illustrating the large size of the lesion we imaged (the lesion of interest is circumscribed by the black rectangle in the image). We chose this large, highly developed lesion on purpose to graphically demonstrate the changes in the mechanical behavior of melanoma lesions versus the surrounding skin. The lesion was on the order of 6 times stiffer than the surrounding tissue as is shown in the elastograms [Figs. 5(a), 5(b) and 5(c)]. Figures 5(a), 5(b), and 5(c) are elastograms of the lesion and are color-coded to show the relative strains in the x- and y-directions, and for the vector sum of the two orthogonal directions, respectively. From the color bars, it can be seen that the lesion displays substantially less
(approximately 6-fold less) strain that does the surrounding skin, implying that the lesion is much stiffer than the surrounding, non-involved skin.

Fig. 4. Photograph of the mouse used in this study. The melanoma lesion of interest is circumscribed by the black rectangle. The arrow points to the location where the speaker was placed during the actual AOE imaging.

Fig. 5. Elastograms of the murine melanoma lesion in the (a) $x$-, (b) $y$-, and (c) resultant-directions, respectively. The width of each elastogram is approximately 8 mm. The dark blue, low strain region in the center of each elastogram reveals the location of the melanoma lesion.

The dimensions of the elastograms are approximately the same as the dimensions of the black rectangle in Fig. 4, with the $x$-direction being about 8 mm long. The dark blue region, indicating an area of low strain, in the center of each elastogram is the melanoma lesion.

To demonstrate the large amount of tissue involvement of the melanoma lesion, we imaged the lesion in vivo with a spectral domain OCT system, and subsequently processed the lesion for histopathology. The OCT images clearly reveal the magnitude of the involved tissue. Figure 6 is an OCT image in the $x$-$z$ plane through the center of the melanoma lesion. As expected for murine melanomas, the top of the lesion lies just below the epidermal layer (~50 μm deep). The large dark region in the center of the image is a portion of the lesion. Because of the increased vasculature and corresponding optical absorbance associated with the lesion, the tumor appears black. The bulk of the lesion is in the dermis.
Figs. 6 (left) & 7 (right). Spectral-domain OCT image (Fig. 6) and histopathology section (Fig. 7) showing the extent of the murine melanoma. The dark region indicated by the arrow is a portion of the lesion in Fig. 6. The top of the lesion is approximately 50 $\mu$m below the skin surface. The black-stained (H & E staining) regions in Fig. 7 are portions of the lesion.

The histopathology image shown in Fig. 7 confirms the extent of the lesion. This histopathology section was taken near, but not necessarily exactly from the same location as the OCT image in Fig. 6. The black stained regions indicate the location of the melanoma. The video sequence shown in Fig. 8 is a 3-dimensional spectral domain OCT image sequence in the $yz$ plane and moving in the $x$-direction (cranio-caudal direction) showing the lateral expanse of the mouse tumor. The highly absorbing regions (dark) reveal the regions of increased angiogenesis associated with the tumor. The total height of the moving block is 1.2 mm. Lateral dimensions of the images are 2.5 X 2.5 mm.

Fig. 8. (2.7 MB). 3-Dimensional spectral-OCT video sequence through the murine melanoma lesion. The dark regions reaching into the upper dermis are all portions of the melanoma lesion.

3.2 In vivo human melanocytic nevi measurements

Because of the assumed differential involvements of the dermis we predicted that the JN would present as a stiffer region in an elastogram and the CN would present as a less stiff region. The junctional nevus was dark and completely flat whereas the compound nevus was lighter in color and very slightly raised. Using our AOE system, we evaluated both nevi with a 5 Hz acoustic stimulation and a probing optical wavelength of 543 nm. The results are shown in Figs. 9(a), 9(b), and Figs. 10(a), and 10(b). Our predictions were confirmed, with the JN being on the order of 44% stiffer than the surrounding skin and the CN being nearly...
31% less stiff than the surrounding skin, as shown by the lower and higher strain responses, respectively.

Fig. 9. White light photograph (a) and elastogram (b) of the JN. The JN clearly appears as a region of lower strain response than the surrounding skin (green region with yellow border), indicating that it is stiffer than the surrounding tissue. The box in Fig. 9(a) shows the region over which the speckle pattern was tracked and the elastogram was generated.

Fig. 10. White light photograph (a) and elastogram (b) of the CN. In this case the nevus presented as a region of higher strain response (deep red oval) than the surrounding tissue, indicating that it is less stiff than the surrounding tissue. The box in Fig. 10(a) shows the approximate region over which the speckle pattern was tracked and the elastogram was generated.

It is interesting to note that the shapes of lesions in the photographs and the elastograms are not quite identical. One explanation is that the collagen orientation is not sufficiently altered over the entire visual area of the lesion to cause a significant change in the local mechanical behavior over the entire lesion.

4. Discussion
The results of the elastic imaging indicate that AOE can image the mechanical behavior of skin lesions and can potentially identify lesions with a greater degree of dermal involvement based on their increased relative stiffness. One very attractive feature of AOE may be the ability to locate and image the circumferential margins of the lesions in a quantifiable manner. However, the ability of AOE to do this efficaciously needs to be further evaluated. This ability
is potentially important in clinical applications of AOE and may aid in the dermatological surgical treatment of skin cancers. Basal cell carcinoma is the most common cancer in the United States. Along with squamous cell carcinoma and melanoma, there are more than 1 million cases per year. Surgical excision of these skin cancers is the standard treatment. In current medical practice, clear histological margins are documented either with frozen section or permanent paraffin sections. Identifying the cancer margins in a real time or near real time manner prior to the surgery can be of tremendous benefit in many ways. The amount of normal skin “margin” can be reduced thereby minimizing the final scar. There would also be a cost savings from avoiding future repeat procedures for positive margins. Of particular note is that no exogenous contrast agents or fluorescent markers need to be employed in order to visualize the lesions. Furthermore, the acoustic and laser powers that are used present no risk to the patient nor the operator. Other prospective applications for AOE include basic cancer research into the development and progression of skin cancers, and clinical utilization of AOE to quantify the degree of damage done to superficial tissues due to injuries or burn. Furthermore, when fully integrated with clinical OCT, AOE may find sub-surface applications, such as identifying and mapping the deep margins of skin cancers or other pathological conditions. This method can be adapted, in principle, to the imaging of any pathological tissue that can be imaged optically while being mechanically stimulated.

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