Intraoperative imaging of surgical margins of canine soft tissue sarcoma using optical coherence tomography

Running title: Intraoperative surgical margin imaging

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Abstract:

Optical coherence tomography (OCT) is a rapid non-invasive imaging technique that has shown high sensitivity for intraoperative surgical margin assessment in human breast cancer clinical trials. This promising technology has not been evaluated in veterinary medicine. The objective of this study was to correlate normal and abnormal histological features with OCT images for surgical margins from excised canine soft tissue sarcoma (STS) and to establish image evaluation criteria for identifying positive surgical margins. Fourteen client-owned dogs underwent surgical resection of a STS and OCT imaging of 2-4 areas of interest on the resected specimen were performed. Following imaging these areas were marked with surgical ink and trimmed for histopathology evaluation. Results showed that different tissue types had distinct characteristic appearances on OCT imaging. Adipose tissue exhibited a relatively low scattering and a honey-comb texture pattern. Skeletal muscle and sarcoma tissue were both dense and highly scattering. While sarcoma tissue was highly scattering, it did not have organized
recognizable structure in contrast to muscle which showed clear fiber alignment patterns. In this investigation, we showed different tissue types had different and characteristic scattering and image texture appearances on OCT, which closely correlate with low power histology images. Given the differentiation between tissue types the results support that OCT could be used to identify positive surgical margins immediately following resection of STS. Further research is needed to assess the diagnostic accuracy of this method for surgical margin assessment.

**Key words:**

Dogs
Margins of Excision
Surgical Oncology
Introduction:

In veterinary medicine, the current standard-of-care for surgical margin evaluation is histologic assessment of surgical margins following formalin fixation. The results of this evaluation often takes days to obtain. Typically, the histopathologic margins for small- and moderate-sized tumors are assessed by cross-sectioning (or radial method) of tissue trimming. In this method, the specimen is sectioned through the center of the tumor on the short axis and then each of these sections is cut again (perpendicular to the original cut) along the long axis.\(^1\) Utilizing this method only a small proportion of the surgical margins is assessed (\(<1\%\) ), which can lead to sampling error due to the limited tissue assessment and the possibility of asymmetric tumor growth.\(^1-3\) This (under-) sampling error can lead to lack of detection of cancer cells extending to the surgical margins (constituting incomplete resection), which is especially possible in the case of invasive tumors that exhibit asymmetric growth like soft tissue sarcomas (STS).\(^1-3\) Failure to recognize incomplete margins can result in inappropriate treatment recommendations and missed opportunities for local disease control.

Soft tissue sarcomas comprise 15\% of all skin tumors in dogs.\(^4\) Surgical removal is commonly the treatment of choice for local control of these tumors; the success of which is often based on histological margin assessment.\(^5-10\) While histopathology is currently the gold standard for surgical margin evaluation in veterinary medicine, reliable intraoperative assessment of surgical specimens is not possible with current methods. Novel imaging techniques that allow intraoperative margin assessment are critically needed to advance the current standard-of-care for the successful surgical treatment of tumors in companion animals. To date, there have been a
few reports of the use of fluorescence imaging techniques in canine patients to assist in identification of residual tumor following resection. \textsuperscript{11-15}

Optical coherence tomography (OCT) is an emerging diagnostic oncologic imaging modality that uses light waves to generate real-time, high-resolution images of tissue at a microscopic level. It has shown promise for the real-time evaluation of human breast cancer margins studies reporting 91.7-100.0\% sensitivity (and 82.0-92.0\% specificity) for identifying residual tumor cells along the entire surgical margin.\textsuperscript{16,17} Preliminary clinical studies in humans and data in rodents and other tumor models strongly support that this imaging modality will be successful in canine patients.\textsuperscript{16-19} The ability to intraoperatively visualize residual microscopic disease in real-time could optimize surgical interventions to allow removal of residual tumor in an attempt to decrease the necessity of second surgeries and potentially other adjunctive therapies. To the authors knowledge, this is the first description of OCT being used for imaging features of normal and abnormal canine tissues at surgical margins, and is a crucial step in the assessment and validation of OCT for the real-time imaging of surgical margins of STS in dogs.

The study aim was to correlate normal and abnormal histological features of surgical margins from excised canine STS with OCT images. Furthermore, this study sought to establish image evaluation criteria for identifying positive surgical margins. The hypothesis was that OCT images would have a high correlation with histological features of surgical margins of excised canine STS.
Materials and Methods:

Fourteen client-owned dogs underwent resection of one or two STS arising from the skin or subcutaneous tissues as part of clinical study protocols approved by the Institutional Animal Care and Use Committee at the XXXX. Prior to enrollment in the study informed owner consent was obtained, the dogs needed a cytologic or histopathologic diagnosis of a STS or mesenchymal tumor. The tumor may have arisen in the skin or subcutaneous tissues for the first time, or be a recurrent tumor. The tumors were excised by an American College of Veterinary Surgeons boarded small animal surgeon either primarily or supervising a resident in training. The planned surgical margins were categorized as ‘marginal’ if the tumor was excised narrowly with excision of gross disease, ‘wide’ if lateral margins of 3 cm from palpable tumor and one fascial plane deep was used, and ‘radical’ if a body part was removed to excise the tumor within a body compartment.

Immediately following surgical excision, the resected specimen was imaged in an adjacent laboratory. The resected specimen was placed on a Petri dish or flattened board to allow positioning on the mounted micrometer stage of a commercial spectral-domain OCT system. Areas of interest were identified to be: a possible area where STS might have been present at the surgical margin (based on palpation or visual assessment) or areas containing different tissue types. Generally, 2-4 areas of interest were imaged on each specimen depending on the size and availability of different tissue types. The sample beam arm was placed directly over the area and 3-D volumetric scans were acquired at each of the 2 to 4 areas of interest per resected tumor sample. The OCT system had a central wavelength of 1310 nm and an incident illumination power of ~ 5 mW. The image resolution was ~ 8 µm axially and ~ 10 µm laterally, with an
imaging depth of ~ 1-2 mm depending on the optical properties of the tissues. In the sample arm of the OCT system, lateral beam scanning was performed with mirrors mounted on computer-controlled galvanometers, passing the OCT beam through a fixed focus objective lens to volumetrically scan the area of interest (5 X 5 X 2 mm$^3$) at 7.4 frames/sec (6 kHz A-scan rate) via raster collection of B-scans along the x-y plane. The imaged area corresponded to 600 sequentially-acquired and adjacent OCT images, taking about 1 minute to collect the volumetric image data at each site. OCT images were evaluated in real-time to ensure the images acquired were of diagnostic quality.

Following imaging of each area of interest, the boundary of the imaged area was marked in a U shape with surgical ink$^b$ (Figure 1) to identify the area for later histologic sectioning, and to correlate the OCT and histologic images. The surgical ink was applied with a toothpick and allowed to dry for 5 minutes. Specimens were then placed in 10% neutral-buffered formalin. The inked areas were paraffin embedded, sectioned, and stained with hematoxylin and eosin (H&E). Prepared slides were digitized with a slide scanner$^c$. The images were orientated based on inked areas for correlation with OCT images. The standard tumor histopathology and assessment of the inked sections was performed by an American College of Veterinary Pathology (ACVP) board certified pathologist (E.D.) or pathology resident (J.S.) blinded to the results of the OCT imaging, thus providing an unbiased assessment of the histology of sections for correlation to OCT images.

The OCT images were acquired with proprietary software$^d$. Processing of the OCT images involved exporting the raw data in another type of propriety software$^d$ to generate 600 OCT
frames. Each B-scan had physical dimensions of 5 mm (lateral) x 2 mm (depth), represented by 600 x 257 pixels, respectively. As described in Mesa et al., a mask was generated for every OCT frame using an active contour segmentation method.\textsuperscript{7} Masks were created by choosing areas of interest, avoiding areas of saturation and background noise, thus rendering a cleaner version of each OCT frame. Correlation of OCT images with histopathology sections of the same areas was performed by an observer experienced in the interpretation of OCT images of surgical margins (K.J.M) with known histopathologic interpretation of tissue type and results of surgical margin interpretation. Tissues were evaluated for two characteristics, which were assessment of light scattering characteristics and evidence of a tissue organized microstructure or texture of tissues. The combination of characteristics that were identified and indicative of incomplete surgical margins or different tissue types were defined as image evaluation criteria.

**Results:**

Fourteen dogs with 15 STS enrolled in this clinical study: one dog had two STS concurrently, which were both surgically excised. The demographics of the study patient population and the location of the tumors are summarized in Table 1. The tumors were resected with different planned surgical margins depending on the area of the body and the size of margins chosen by the primary surgeon. The margins planned at surgery and the number of areas of interest imaged are also summarized in Table 1.

The imaged areas of interest consisted of different tissue types on histologic evaluation. Four tissue types were predominantly present in the evaluated samples: skin, adipose, muscle, and sarcoma. These results are detailed in Table 2. The tissue characteristics based on OCT imaging
were determined for the different tissue types represented in the imaged areas. Depending on the tissue type and the optical properties of the tissue, the OCT imaging depth within the tissue varied. Low backscattering adipose tissue was imaged up to 2 mm in depth, however, denser highly-scattering cellular tissues, like sarcoma, was imaged to shallower depths.

The microscopic histologic and OCT appearance of adipose, skin, muscle, and sarcoma tissues are shown in figure 2. Adipose tissue exhibited relatively lower scattering as well as a hole-filled (honey-comb) appearance and texture pattern. Skin appeared to have intermediate scattering often irregular in contour, and to contain adnexal structures which were seen clearly on OCT images. On the other hand, muscle and sarcoma tissue were both dense and highly scattering; thus, more challenging to differentiate with OCT. However, sarcoma tissue did not have a defined structure, while alignment patterns typically present in muscle fibers could be observed. Three examples of where sarcoma was present at surgical margins (incomplete margins) are shown in figure 3. Incomplete surgical margins were characterized by dense, highly scattering tissues with no evident alignment patterns at the edge of the specimen. Of the three areas that are seen, two are from the same dog and illustrate differences in the outer-most part of the sarcoma. In dog 8 area 1, there are some air bubbles underneath the initial layers of tissue/pseudocapsule giving the appearance of black linear structures. Looking at another area in this dog, area 4, there is the same distinct pseudocapsule but more strongly scattering and deeper tissue imaging is possible with the lack of air.
Discussion

This study represents the first descriptive study of the use of OCT for imaging of surgical margins in dogs. We showed it was possible to image and characterize different tissue types that occur at surgical margins of canine STS including skin, adipose, muscle, and sarcoma based on their light scattering properties and their texture appearance on OCT. The greatest contrast between tissues was between fat and skin or muscle or STS. The proposed reason why adipose tissue is more distinct when comparing to the other tissue types is likely due to large size and regular arrangement pattern of mature adipose cells that create the honey-comb appearance. Although muscle and sarcoma were both high scattering, there were other visual characteristics that allowed differentiation of these two tissues and recognition of STS at surgical margins, representing incomplete excision of the tumor. We found that if muscle tissue is imaged in a plane perpendicular to the direction of the muscle fibers, then the bundled fiber structure is most clearly observed. Given the similarity of appearance of these tissues, there has been limited investigation into methods to objectively differentiate between these tissues. Mesa et al. found that a novel image texture based algorithm could be used to objectively differentiate between OCT images of sarcoma and muscle.\textsuperscript{20} Wang et al. reported on the use of 3-D computational methods of assessment of STS from normal tissue types.\textsuperscript{21} Polarization-sensitive OCT, a variant of OCT that is sensitive to the birefringent optical properties of tissues, such as highly ordered collagen or muscle fibers, has been shown to differentiate between human breast tumors and normal fibrous breast stroma.\textsuperscript{22} Polarization-Sensitive OCT similarly may be helpful to differentiate between muscle tissue with highly ordered fibers and STS that lacks such aligned and organized microstructure. In the future, these and other techniques may be further developed.
to automate objective differentiation of the tissue types to aid in observer interpretation of the
OCT images, even during surgery, and in real time.

Characteristics seen between different tissue types varied but there was also variation in
appearance of sarcoma between different dogs and different areas in the same dog. Sarcoma
tissue was always highly light scattering on evaluation, but the texture of the tumor in the OCT
images was often different between tumors. This represents differences in sarcoma tissue
microstructure, particularly the pseudocapsule which is very superficial, which varies between
tumor types. There was a predominance of low grade tumors in this study, which could have led
to the structure having a prominent pseudocapsule and being less irregular as the sarcomas were
less invasive and heterogenous.

In this study, different characteristics of tissues and tumor at surgical margins following STS
removal in dogs have been identified. Differentiation between normal and cancerous tissues is
key for utilization of intraoperative imaging for assessment of surgical margins of the excised
sample or more ideally the in vivo tumor bed to assess whether there is residual microscopic STS
at the surgical site. There is some evidence that clean resection in canine STS may be associated
with prolonged tumor-free intervals and decreased risk of tumor recurrence.\textsuperscript{5,7,9,23} It remains
underdetermined but possible that removal of residual microscopic disease may be associated
with decreased recurrence similar to scar re-excision when primary resection is incomplete.\textsuperscript{24}
Alternatively, that if the areas of residual microscopic disease could be identified that these cases
could have targeted treatment of this residual microscopic disease with radiation therapy or
topical chemotherapy or similar.
The population of dogs in this study consisted of middle-aged to older, pure and mixed bred dogs, which reflects the population of dogs developing STS as reported in other studies.\textsuperscript{24-26} Many dogs had tumors arising on limbs, which may reflect our referral population as referring veterinarians may be removing truncal STSs and referring distal limb STSs to specialists in oncology and surgery. Most tumors were grade I or II, which is typical of most STSs reported in the veterinary literature.\textsuperscript{24-26} Given the high proportion of limb tumors and the high number of cases where planned marginal excision was performed, it was not surprising there were several cases where the histologic margins were incomplete.

Good correlations were found between OCT and histological images of different normal and abnormal tissue types at the surgical margins of excised canine STSs. This early promise would suggest continued research is warranted to assess this technique for diagnostic accuracy of detection of incomplete margins during STS resection. The future goals of our group are to develop an accurate tool for assessment of surgical margins and detection of incomplete margins that could improve the efficacy of cancer surgery and reduce patient morbidity and mortality.

**Conflict of interest:** Stephen Boppart is co-founder and Chief Medical Officer for Diagnostic Photonics, Inc., which is developing Interferometric Synthetic Aperture (ISAM) and optical coherence tomography (OCT) for real-time tissue assessment during surgery. He also receives royalties from patents licensed by the Massachusetts Institute of Technology related to OCT.
References:


**Tables:**

**Table 1:** Demographics, description of tumor characteristics, and surgical approach.

<table>
<thead>
<tr>
<th>Dog number</th>
<th>Breed</th>
<th>Age at surgery (years)</th>
<th>Tumor location</th>
<th>Longest tumor diameter (cm)</th>
<th>Surgical resection (marginal/wide/radical)</th>
<th>Number of imaged areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vizsla</td>
<td>9.1</td>
<td>Right elbow</td>
<td>1.0</td>
<td>Marginal</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Boxer</td>
<td>8.1</td>
<td>Ribs</td>
<td>4.5</td>
<td>Marginal</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Rat terrier</td>
<td>10.1</td>
<td>Right hock</td>
<td>3.5</td>
<td>Marginal</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Mixed breed</td>
<td>13.6</td>
<td>Left hip</td>
<td>6.2</td>
<td>Wide</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Siberian Husky</td>
<td>11.1</td>
<td>Left hip</td>
<td>5.0</td>
<td>Wide</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Labrador retriever</td>
<td>13.7</td>
<td>Left lateral thorax</td>
<td>24.0</td>
<td>Marginal</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Labrador retriever</td>
<td>4.8</td>
<td>Right carpus</td>
<td>5.0</td>
<td>Marginal</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>Bulldog</td>
<td>9.3</td>
<td>Interscapular</td>
<td>7.0</td>
<td>Wide</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>Mixed breed</td>
<td>6.3</td>
<td>Left metacarpus</td>
<td>2.0</td>
<td>Radical</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>Labrador retriever</td>
<td>6.8</td>
<td>Right elbow</td>
<td>2.0</td>
<td>Marginal</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>Mixed breed</td>
<td>10.8</td>
<td>Left flank</td>
<td>12.0</td>
<td>Marginal</td>
<td>4</td>
</tr>
<tr>
<td>13</td>
<td>Labrador retriever</td>
<td>8.5</td>
<td>Left metacarpus</td>
<td>2.0</td>
<td>Marginal</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>Mixed breed</td>
<td>16.1</td>
<td>Right metacarpus</td>
<td>7.0</td>
<td>Marginal</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 2: Description of tissue types present in histologic sections.

<table>
<thead>
<tr>
<th>Dog #</th>
<th>Surgical resection (marginal/wide/radical)</th>
<th>Histopathologic diagnosis</th>
<th>Area 1</th>
<th>Area 2</th>
<th>Area 3</th>
<th>Area 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Marginal</td>
<td>Grade I STS, margins less than 0.1cm.</td>
<td>75% STS 1mm from margins, other tissue types skin and subcutis</td>
<td>75% STS &lt;1mm from margins, remaining skin and subcutis</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Marginal</td>
<td>Grade I STS, complete, deep margin 1mm, lateral margins 1.5-3cm.</td>
<td>90% STS, &lt;1mm from deep margin. Other tissues adipose and skeletal muscle</td>
<td>Adipose tissue and skeletal muscle</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Marginal</td>
<td>Grade I STS, complete margins.</td>
<td>70-75% STS, tumor extends to deep margin. Other tissue skin.</td>
<td>75-90% STS, &lt;1mm from deep margins. Remaining tissue is skin and fibrous connective tissue.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Wide</td>
<td>Grade II STS, complete, lateral margins &gt;0.7cm and deep margin 1cm.</td>
<td>Adipose tissue with small amount of fibrous connective tissue</td>
<td>&lt;15% STS, 6mm from deep margins, other tissue skin and subcutis</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Wide</td>
<td>Grade II STS, complete excision, &gt;0.3cm lateral and 0.1cm deep.</td>
<td>85% STS, tumor is 1mm from deep margin. Other tissues subcutis.</td>
<td>90% STS, &lt;1mm from deep margin. Other tissues subcutis.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Mass 1: Wide</td>
<td>Mass 1: Grade II STS, complete excision, deep margin 0.1cm and lateral &gt;1.2cm. Mass 2: Grade III STS, complete excision, &gt;0.5cm lateral and 0.1cm deep.</td>
<td>Mass 1: 5-20% STS, 5-7.5mm from margin. Other tissues skin and subcutis Mass 2: 5-60% STS, 7mm from margin. Other tissues skeletal muscle and subcutis</td>
<td>Mass 1: Skeletal muscle and adipose tissue, other tissues haired skin and subcutis Mass 2: 35-70% STS, extends to deep margin</td>
<td>Mass 1: Skeletal muscle and adipose tissue, other tissues haired skin and subcutis Mass 2: 60-75% STS, extends to deep margin. Other tissues skin, subcutis and skeletal muscle.</td>
<td>Mass 1: Skeletal muscle and adipose tissue, other tissues haired skin and subcutis. Mass 2: 80-85% STS, extends to deep margin. Other tissues subcutis and skeletal muscle.</td>
</tr>
<tr>
<td>7</td>
<td>Marginal</td>
<td>Grade II STS, incomplete excision, neoplastic</td>
<td>&gt;90% STS, extends to margin. Other tissue</td>
<td>&gt;95% STS, extends to margin. Other tissue</td>
<td>&gt;95% STS, extends to margin. Other tissue</td>
<td>&gt;95% STS, extends to margin. Other tissue</td>
</tr>
<tr>
<td></td>
<td>Grade</td>
<td>Complete Excision</td>
<td>Subcutis</td>
<td>Subcutis</td>
<td>Subcutis</td>
<td>Subcutis</td>
</tr>
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<td>---</td>
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</tr>
<tr>
<td>8</td>
<td>Marginal</td>
<td>Grade I STS, incomplete excision, neoplastic cells extend to multiple margins.</td>
<td>&gt;90% STS, &lt;1mm from margin. Other tissues fibrous connective tissue and adipose tissue.</td>
<td>85% STS, extends to margin. Other tissue fibrous connective tissue and adipose tissue.</td>
<td>&gt;95% STS, extends to margin. Other tissues fibrous connective tissue and adipose tissue.</td>
<td>&gt;85% STS, extends to margin. Other tissues fibrous connective tissue and adipose tissue.</td>
</tr>
<tr>
<td>9</td>
<td>Wide</td>
<td>Grade II STS, complete excision, &gt;1cm lateral margins and 1cm deep margins.</td>
<td>Skeletal muscle, fibrous connective tissue and adipose tissue.</td>
<td>Adipose tissue and connective tissue.</td>
<td>Subcutis and skin.</td>
<td>Adipose tissue and connective tissue.</td>
</tr>
<tr>
<td>10</td>
<td>Radical</td>
<td>Grade II STS, complete excision, &gt;30cm.</td>
<td>Skin, subcutis and connective tissue.</td>
<td>Skin and subcutis.</td>
<td>Skeletal muscle and adipose tissue.</td>
<td>Skeletal muscle and adipose tissue.</td>
</tr>
<tr>
<td>11</td>
<td>Marginal</td>
<td>Grade I STS, complete excision, &gt;0.1mm lateral margins and &lt;0.1mm deep margins.</td>
<td>75% STS, &lt;1mm from margins. Other tissues skin and subcutis.</td>
<td>35% STS, &lt;1mm from margin. Other tissue skin and subcutis.</td>
<td>25% STS, &lt;1mm from margin. Other tissues skin and subcutis.</td>
<td>75% STS, &lt;1mm from margins. Other tissues skin and subcutis.</td>
</tr>
<tr>
<td>12</td>
<td>Marginal</td>
<td>Grade II STS, complete excision, lateral margins &gt;1.2cm and deep margin 0.4cm.</td>
<td>Skin and subcutis.</td>
<td>80% STS, 0.3cm from margins. Other tissues skin and subcutis.</td>
<td>80% STS, 0.5cm from margin. Other tissues skin and subcutis.</td>
<td>Skin and subcutis.</td>
</tr>
<tr>
<td>13</td>
<td>Marginal</td>
<td>Grade I STS, complete excision, &lt;0.1cm from margins.</td>
<td>80-85% STS, &lt;1mm from margins. Other tissues skin.</td>
<td>80-85% STS, &lt;1mm from margins. Other tissues skin.</td>
<td>80-85% STS, &lt;1mm from margins. Other tissues skin.</td>
<td>80-85% STS, &lt;1mm from margins. Other tissues skin.</td>
</tr>
<tr>
<td>14</td>
<td>Marginal</td>
<td>Grade III STS, incomplete excision, extends to 3 out of 4 submitted margins.</td>
<td>&gt;98% STS, &lt;1mm from margins. Other tissue connective tissue.</td>
<td>95% STS, &lt;1mm from margins. Other tissue connective tissue.</td>
<td>90% STS, &lt;1mm from margins. Other tissue connective tissue.</td>
<td>95% STS, extends to margin. Other tissue connective tissue.</td>
</tr>
</tbody>
</table>

Footnote: STS: Soft tissue sarcoma;
Figures:

Figure 1: Photograph illustrating resected soft tissue sarcoma prepared for the OCT imaging with the U-shaped marking of an area of interest (black and yellow colored).
**Figure 2:** Correlation of histology properties of (A) adipose, (B) skeletal muscle, (C) skin and (D) soft tissue sarcoma with corresponding OCT images (E–H, respectively). The left sided row (A–D) shows the haemotoxylin and eosin stained digitized histological images of the resected tissue and the right sided row (E–H) shows the corresponding OCT images. The black and white scale bars across the bottom of images D and H represent 500 µm to show the scale of all histologic and OCT images shown.
Figure 3: (A)-(C) Examples of surgical specimens and corresponding OCT and histology images with sarcoma at surgical margins. On the left side, is a photograph of gross surgical specimen with incomplete boxes marked with surgical inks highlighting imaged areas. On the right side, the bottom row shows haemotoxylin and eosin stained digitized histological images, and the corresponding OCT images are shown in the top row. The white or black scale bars at the bottom of each OCT or histologic image represent 500 µm.

(A) Images for case 2, area 1 sample

The OCT and histological sections were taken from the area bounded by the U-shaped yellow surgical ink. The OCT images show layer of skeletal muscle with deeper high scattering area consistent with underlying sarcoma (white arrows).
(B) Images for case 8, area 1

The OCT and histological sections were taken from the area bounded by the U-shaped orange surgical ink. The OCT image shows a thin layer of tissue with low scattering areas consistent with the pseudocapsule with air bubbles (black linear structures, yellow arrow) overlying a high scattering tissue with no alignment patterns underlying consistent with sarcoma (white arrowheads). The imaging of the deeper tissues is attenuated by the overlying air bubbles. This result is consistent with sarcoma < 1mm from the deep surgical margin.

(C) Images for case 8, area 4

The OCT and histological sections were taken from the area bounded by the U-shaped yellow surgical ink. The OCT image shows a distinct and condense pseudocapsule immediately under the probe (red arrow) but with high scattering tissue consistent with sarcoma underlying. This result is consistent with sarcoma < 1mm from the deep tissue
margins. Deeper tissue imaging of the tumor is seen in comparison to that seen in Figure 3B due to the lack of air attenuating light penetration.
Footnotes:

a Envisu C2300, Bioptigen Inc., Durham, NC.

b The Davidson Marking System, Bradley Products, Bloomington, MN.

c NanoZoomer 2.0 RS, Hamamatsu C10730, Bridgewater, NJ.

d InVivoVue 1.7, Bioptigen Inc., Durham, NC.

e MatLab, Mathworks®, Natick, MA.