Clinical need: Local control

Post surgical pathology

Optical technologies offer advantages in that they can quickly and non-destructively cover an entire margin, which can range from 5 to 40 cm².

High resolution vital fluorescence imaging

Advantages:
- View microanatomy in live tissue – "virtual pathology"
- In vivo
- Intraoperative
- No tissue processing

Through leveraging the coverage of the 49-channel spectral system and the resolution of the high resolution microscope, we hope to improve the overall performance of our approach for intraoperative margin assessment.

Experimental methodology

Specimen resected and prepared

AcridineRed

AcriflavineRed

Images acquired with high resolution microscope

Sites inked for touch-1

Image registration

Pathology obtained for each site

Microendoscopy

High resolution imaging

Histopathology

Contrast Agent – AcridineRed

- Non-toxic
- Topical antisepcific
- Reversibly associates with nucleic acids
- Stains in seconds

49-channel spectral system

High resolution microscope

Resolution: 6 mm

Coverage: 17 cm²

0.5 mm²

Intraoperative Diffuse Reflectance Spectroscopic Imaging

Monte Carlo Model

Conditional Inference Model

Path Negative

Path Positive

Probe Negative

Probe Positive

Area Examined

Optical

Frozen Section

Depth

Surface

> 2 mm

1-2 mm

Pathologist

Yes

Yes

No

Time

30 min

30 min

A few minutes

Figure 1: Photomicrographs of H&E-stained breast tumor margin sections, representative of (A) invasive ductal carcinoma and (B) greater than 2 mm from the inked surface (negative).

Representative in vivo images captured in a murine sarcoma mouse model compared directly to pathology. High resolution microscope clearly recapitulates tissue structures seen in H&E. Scale bar 1000 µm.

Qualitative image diagnosis

Table 1. Training and test set of images shown to reviewers

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Training set</th>
<th>Test set</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant</td>
<td>5</td>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>Tumor</td>
<td>4</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Tumor + Muscle</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Benign</td>
<td>7</td>
<td>22</td>
<td>29</td>
</tr>
<tr>
<td>Muscle</td>
<td>6</td>
<td>20</td>
<td>26</td>
</tr>
<tr>
<td>Adipose</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Muscle + Adipose</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>43</td>
<td>55</td>
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</tbody>
</table>

Table 2. Diagnostic accuracy of 4 reviewers

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Reviewer Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>Malignant</td>
<td>90%</td>
<td>73%</td>
</tr>
<tr>
<td>Tumor</td>
<td>95%</td>
<td>73%</td>
</tr>
<tr>
<td>Tumor + Muscle</td>
<td>95%</td>
<td>77%</td>
</tr>
<tr>
<td>Benign</td>
<td>90%</td>
<td>59%</td>
</tr>
<tr>
<td>Muscle</td>
<td>93%</td>
<td>71%</td>
</tr>
<tr>
<td>Adipose</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>90%</td>
<td>81%</td>
</tr>
</tbody>
</table>

Table 3. Diagnostic accuracy stratified by tissue type

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Agreement</th>
<th>Disagreement</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant</td>
<td>19</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Tumor</td>
<td>16</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Tumor + Muscle</td>
<td>3</td>
<td>2</td>
<td>60%</td>
</tr>
<tr>
<td>Benign</td>
<td>16</td>
<td>6</td>
<td>73%</td>
</tr>
<tr>
<td>Muscle</td>
<td>14</td>
<td>6</td>
<td>70%</td>
</tr>
<tr>
<td>Adipose</td>
<td>1</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>8</td>
<td>81%</td>
</tr>
</tbody>
</table>

Figure 3. Representative ex vivo images captured from clinical breast specimens. Images are shown both in gray-scale (row 1) and color (row 2) to enable better visualization. H&E images are shown in row 3. IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; mucinous = mucinous carcinoma. Scale bar 100 µm.

Discussion

- High resolution imaging enables compelling visualization of tumor microanatomic instantly with no tissue processing.
- Qualitative analysis by trained reviewers yielded good performance, achieving a sensitivity and specificity of 93% and 71%, respectively.
- Trained reviewers performed most poorly on mixed images; however, quantitative image processing could be used to improve analysis.
- By combining high resolution fluorescence imaging with our group’s wide-field spectral platform, we hope to improve the overall performance of our approach for intraoperative margin assessment.

References