Multimodal imaging microscope for intraoperative detection of breast tumor positive margins


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Outline

• Background & Introduction
  – Breast cancer incidence and state of the art
• Description of the Instrument and Methods
• Brief Results
  – Contrast agent and image analysis
• Conclusion
• Future Work
According to the American Cancer Society, over 200,000 cases of breast cancer are diagnosed annually and over 150,000 of the patients diagnosed with early stage breast cancer choose to undergo Breast Conserving Surgery (BCS) [1].

Unfortunately, intraoperative assessment of breast cancer margins using current strategies is inadequate, and thus rarely performed.

Positive margins rate requiring a second operative procedure remains high, at about 20-40% nationwide [2].

What is a safe surgical margin?

The consensus among most of the surgeons and radiation oncologists is that there should be no tumor left within at least 1-2 mm distance from the surface of the surgical specimen [1].


Source: Wikimedia Commons

Background • Instrument & Methods • Results • Conclusion & Future Work
Current Approaches

• Current techniques for intraoperative pathologic assessment of surgical margins involve **touch prep** and **frozen section analysis**

• Touch prep analysis has a poor sensitivity and specificity, and therefore is not often used

• Frozen section analysis- is very difficult: breast specimens have a high percentage of fat tissue-very difficult to freeze and cut in thin slices for histopathological analysis during the surgery

• Surgical specimens are sent to the pathology lab, fixed, sectioned, stained, and read for results days later, after the patient has gone home

• Published reports indicate a 20-70% rate of positive margins left after surgery [1]

• If positive margins are found, surgery is repeated

Alternative Experimental Approaches

- **Surgical bed analysis**
  - NIR Fluorescence imaging: Frangiony’s group at. BID [1] - sensitivity/specificity issues
  - Cancer targeting contrast agent imaging: U. of Washington, Dartmouth, etc.- Long road to get FDA approval

- **Surgical specimen analysis**
  - Micro CT- limited resolution, low sensitivity/specificity
  - High resolution optical imaging (FCM, OCT, FFOCT, 2PM, etc.) – time consuming – not very suitable for real-time feedback
  - Fluorescence guided multimodal imaging – fast, reduced rates of FPs/FNs -

Proposed Approach

A. Incubation & FI Imaging

B. RCM/OCT Imaging

C. Confocal Imaging

C'. OCT Imaging

D. Automated Image Analysis

E. Tumor

F. Adipose Tumor
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- High sensitivity – minimal false negatives
- Short incubation time for activation – not extend the duration of the procedure with more than few minutes
- No impact on histological analysis – histology will still be the ultimate test

**Contrast Agent Requirements**

**Phase I Agent**

  - uPA specific sequence
  - uPA cleavage

**Phase II Agent**

  - Cancer Tissue Targeting Moiety (Peptide)
  - Peptide Probe Quenched
  - Peptide Probe Cleavage Products
  - Fluorescent
  - Cancer Tissue Targeting Moiety (Peptide)

**Near IR Agent** – $\lambda_{\text{max}}$ 675 nm (abs.)

**Similar kinetics of enzyme activation (minimal effect of the tissue target moiety)**

Background • Instrument & Methods • Results • Conclusion & Future Work
Contrast Agent Characterization

- Absorption and Emission spectra obtained in 10X PBS Buffer
- Red-shift in UV spectrum maximum upon cleavage (635 nm → 675 nm)
- 18.7X increase in fluorescence upon full cleavage (690 nm max)

Absorption Spectra

Emission Spectra (ex: 675 nm)

Background • Instrument & Methods • Results • Conclusion & Future Work
Goals and Design Parameters of the System

- Demonstrate an inverted combined fluorescence imaging-microscopy instrument which can be used in the surgical suite.

<table>
<thead>
<tr>
<th>Design Parameters</th>
<th>RCM</th>
<th>OCT</th>
<th>FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>830 nm</td>
<td>1310 nm</td>
<td>675nm Ex/700 nm Em</td>
</tr>
<tr>
<td>Imaging speed</td>
<td>&gt;10fps</td>
<td>&gt;50fps</td>
<td>20 fps</td>
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<tr>
<td>Imaging range-axial</td>
<td>0.25 mm</td>
<td>2 mm</td>
<td>N/A</td>
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<tr>
<td>Axial resolution</td>
<td>2 µm</td>
<td>7 µm</td>
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<tr>
<td>Lateral resolution</td>
<td>1 µm</td>
<td>10 µm</td>
<td>100 µm</td>
</tr>
<tr>
<td>Field of view</td>
<td>600 µm</td>
<td>2 mm</td>
<td>25 mm</td>
</tr>
</tbody>
</table>

- Demonstrate 90% specificity in determining positive margins in a study at MDACC – over 50 specimens.
Instrument Schematic

Background • Instrument & Methods • Results • Conclusion & Future Work
Instrument Overview

Fluorescence Module  RCM – OCT Modules

Background • Instrument & Methods • Results • Conclusion & Future Work
Instrument Overview

Side view + LabVIEW software

View from above

Background • Instrument & Methods • Results • Conclusion & Future Work
RCM-OCT: Registering

Background • Instrument & Methods • Results • Conclusion & Future Work
Trimodal

Background • Instrument & Methods • Results • Conclusion & Future Work
Investigating Boundaries: ROI Registering

Depth ~ 50µm

Depth ~ 100 µm

Background • Instrument & Methods • Results • Conclusion & Future Work
Histological Comparisons

Red: Normal lobule
Conclusions

• Highly specific and reactive contrast agent
  • Very stable, low staining concentration [200nM]

• Enhanced contrast fluorescence imaging was very useful in highlighting suspicious cancer presence - reduces the amount of time needed for analyzing the specimen with higher resolution microscopy, which can be applied only on the highlighted areas

• Histological comparison = proper algorithmic training based off ground truth
  • Measures of specificity and sensitivity can be computed
Future Work

- Validate image quality and predictive capability against histological ground-truth
- RCM acquisition speed improvements
- Data saving speed and handling improvements
  - Adding flexibility (these files can quickly become prohibitively large)
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