Histological Analysis of Biological Tissues using High-Frequency Ultrasound

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Objective

• Previous findings
  • High-frequency (HF) ultrasound can differentiate between a range of breast pathologies in surgical specimens
  • Two parameters are sensitive to histopathology

• Direction
  • Determine the mechanism linking HF ultrasound to histology

• Approach
  • Review breast specimen results
  • Test range of bovine tissue from heart, kidney, and liver

• Hypothesis
  • Ultrasound sensitivity is a function of the microscopic heterogeneity—and therefore histology—of the tissue
Measurements

(a) Set screw
- Plastic bag
- Transmit-receive transducer
- Tissue specimen
- Receive transducer
- Cable to pulser-receiver

(b) Through-transmission
- Plastic bag
- Tissue
- Receiver
- Transmitter

(c) Pulse-echo
- Plastic bag
- Transmitter + Receiver
- Reflection Surface
- Tissue
Waveform Analysis

Through-Transmission

First-Order Spectra

FA
Normal
LCIS

Normalized Amplitude
Time (µs)

Normalized Amplitude
Time (µs)

Pulse Echo

Second-Order Spectra

FA
Normal
LCIS

Log Amplitude
Frequency (MHz)

Log Amplitude
Quefrency (µs)
# Surgical Specimens

<table>
<thead>
<tr>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph nodes</td>
<td>Ductal Carcinomas (DC):</td>
</tr>
<tr>
<td></td>
<td>- Ductal carcinoma in situ (DCIS)</td>
</tr>
<tr>
<td></td>
<td>- DCIS, solid &amp; cribriform</td>
</tr>
<tr>
<td></td>
<td>- Invasive ductal carcinoma (IDC)</td>
</tr>
<tr>
<td></td>
<td>- DCIS + IDC</td>
</tr>
<tr>
<td>Normal breast</td>
<td><strong>Atypical:</strong></td>
</tr>
<tr>
<td></td>
<td>- Benign with calcifications</td>
</tr>
<tr>
<td></td>
<td>- Atypical ductal hyperplasia</td>
</tr>
<tr>
<td></td>
<td>- Fibrocystic change</td>
</tr>
<tr>
<td></td>
<td>- Papilloma</td>
</tr>
<tr>
<td>FN-FA-TA:</td>
<td>Lobular Carcinomas (LC):</td>
</tr>
<tr>
<td></td>
<td>- Lobular carcinoma in situ (LCIS)</td>
</tr>
<tr>
<td></td>
<td>- Invasive lobular carcinoma (ILC)</td>
</tr>
<tr>
<td>Total measurement positions = 29</td>
<td>Total measurement positions = 26</td>
</tr>
</tbody>
</table>
Breast Histology Correlations

- Tissue heterogeneity increases from fibroadenoma (mostly stroma) to LCIS (tumor proliferation in lobules)
- Heterogeneity correlates with peak density, inversely with slope
Surgical Margin Results

- Peak density (number of peaks and valleys) from first-order spectra
- Normalized Slope from second-order spectra
Multivariate Analysis

- Attenuation vs. peak density
- Rotated and translated plot
## Statistical Significance

**t-test results:** Differentiation from normal tissue

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Peak density</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobular carcinomas</td>
<td>$p &lt; 0.02$</td>
<td>$p &gt; 0.20$</td>
</tr>
<tr>
<td>Ductal carcinomas</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.20$</td>
</tr>
<tr>
<td>FN-FA-TA</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>Benign pathologies</td>
<td>$p &lt; 0.10$</td>
<td>$p &lt; 0.01$</td>
</tr>
</tbody>
</table>

**One-way ANOVA test:** 5% significance level for peak density, close to 1%
Heart Histology Correlations

- Cardiac tissue displays uniform structure
- Vascular structure is more complex with 3 layers (tunica intima, tunica media, and tunica adventitia), smooth muscle, collagen, etc.
Bovine Heart Results

- Vascular tissue (aorta, vena cava, etc.) shows significantly higher peak densities
- No significant variations observed for normalized slope
Kidney Histology Correlations

- Tissue uniformity decreases from cortex to ureter
- Medulla has uniaxial collecting ducts similar to normal breast glands
- Size of heterogeneities increase from cortex to ureter
Bovine Kidney Results

- Ureter and surrounding fat tissue show significantly higher peak densities
- Renal medulla shows significantly lower normalized slope values
Discussion

- Repeatable correlations shown between tissue heterogeneity and peak density of 1\textsuperscript{st}-order spectra
  - Human breast: Ductal and lobular carcinomas
  - Bovine heart: Vascular tissue
  - Bovine kidney: Ureter and surrounding stroma

- Correlations less repeatable for slope of 2\textsuperscript{nd}-order spectra
  - Slopes are lowest for normal ductal structures
  - Normal breast tissue and medullary collecting ducts

- No correlations seen for liver tissue or between organs
  - Tissue uniformity (liver)
  - Averaging effects (between organs)
Conclusions

- Tissues with greater peak densities in 1st-order spectra exhibit:
  - More complex, less uniform histology
  - Larger or more widespread heterogeneities
- Tissues with lower slopes in 2nd-order spectra exhibit:
  - Normal microscopic ductal structures
  - Examples: Normal breast glands and renal medulla
- Results support hypothesis that HF ultrasound is sensitive to microscopic heterogeneity—and thus histology—in tissues
- Applications
  - Intraoperative evaluation of margins during breast cancer surgery
  - Real-time pathology for other cancers and procedures
Acknowledgments

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