Physiologic, Metabolic, and Structural Alterations in Breast Cancer: Assessment via Optical Technologies

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Breast Cancer – Facts and Figures

- Approx. 213,000 women in the US develop invasive breast cancer each year; 62,000 are diagnosed with \textit{in situ} carcinomas

- The NCI estimates that 1 in 8 women will be diagnosed with breast cancer at some time in their lives

- 41,000 will die from breast cancer each year

- \textit{Early detection and treatment is the key to surviving breast cancer}

Sources: American Cancer Society, National Cancer Institute
Breast Cancer Detection and Treatment

Unmet clinical need: Reduce number of unnecessary biopsies, and reduce surgical re-excision rates

Screening

www.cancer.gov

Diagnosis

www4.umndj.edu/~koniges/stereo.html

Treatment

www.cooperhealth.org
Optical signals can probe cancer hallmarks

- Hemoglobin saturation, vascularity
- Water content
- Lipid content
- Cellular metabolism
- Structural protein content
- Amino acids
- Size and density of scattering centers
Optical Spectroscopy: A Useful Method for Cancer Detection

• **Hypothesis**: Optical spectroscopy can exploit differences between malignant and non-malignant tissues

• Allows assessment of tissue structure and function prior to histopathologic observation

• Inexpensive, may result in real-time diagnosis
Optical Spectroscopy in Breast Cancer Detection and Treatment

Screening → Diagnosis → Treatment
Impacting Patient Care on Multiple Levels

Pre-interventional diagnosis

Core-needle biopsy

Intraoperative tumor margin assessment

Intervention guidance / assessment

Therapeutic monitoring

Local recurrence detection

Post-interventional assessment
**Our approach**: Systematic investigation of underlying features of breast disease using optical spectroscopy and development of appropriate optical tools and spectral analysis algorithms.

**Methodology**

- **Instrument / probe design**
- **Toolbox**
- **Modeling / simulation**
- **Phantom models**
- **Validation**
- **Animal models**
- **Diagnostic potential**
- **Ex vivo clinical studies**
- **In vivo clinical studies**
- **Feasibility**
Roadmap

I. Toolbox development

II. Validation

III. Ex vivo assessment

IV. Intraoperative in vivo assessment
Fast EEM Spectrometer

- System components:
  - 450W Xe arc lamp
  - Double excitation monochromator
  - Illumination / collection fiber couplers
  - Filter wheel
  - Triple-grating imaging spectrograph
  - 16-bit Peltier-cooled 1024x256 CCD array
  - Laptop PC
Optimized Fiber Probe Design

Monte Carlo simulations used in the design of optimized application-specific fiber-optic probes

Different tissue types require different probe geometries to optimally interrogate regions of interest

Considerations for probe design:
- Signal / throughput
- Penetration depth
- Forward-firing / side-firing

- For multiseparation probe: 90% reflectance sensing depth = 0.5 – 2 mm

Physically-Based Spectral Analysis

- **Forward model**
  - Fast, scalable inverse MC model; outputs absorption and scattering properties of measured samples
  - Extendable to any probe geometry; valid over wavelength ranges and sampling volumes used in our studies
  - Requires a single phantom calibration measurement

- **Inverse model**

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Inverse Monte Carlo Model

Convergence ~0.5-2 min
Roadmap

I. Toolbox development

II. Validation

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IV. Intraoperative in vivo assessment
Phantom validation

• Ability to extract absorption and scattering properties from optical spectra tested on liquid phantoms

• Variable concentration of scatterer (polystyrene spheres) and absorber (human Hb, nigrosin ink)

• Free parameters: Oxy-Hb or nigrosin concentration, scatter size and density

• Inverse MC model used to extract optical properties, compared to known values for phantoms

## Phantom validation

<table>
<thead>
<tr>
<th>Optical Property</th>
<th>λ Range (nm)</th>
<th>RMS% Extraction Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_a$</td>
<td>350-800</td>
<td>3.1 ± 1.1</td>
</tr>
<tr>
<td>$\mu_s'$</td>
<td>350-800</td>
<td>2.5 ± 0.7</td>
</tr>
</tbody>
</table>

![Graphs showing extraction of optical properties](image.png)
Validation in Animal Models

- Validate optical spectroscopy methods in murine model

- Monitor tumor physiology non-invasively in response to an externally applied perturbation

- Establish ability of optical spectroscopy to track tumor physiology changes in response to various treatments

- Demonstrate superior performance over existing technologies
Monitoring tumor oxygenation

• 4T1 tumors grown in flank of nude mice

• Oxylite pO₂ sensors inserted into tumor (current technology)

• Optical probe placed in contact with tumor to collect diffuse reflectance and fluorescence

• Carbogen atmosphere introduced at designated time point

• Inverse MC model used to extract Hb content, Hb saturation
Perturbation validation

- Hb sat. (from diffuse reflectance) tracks changes in tissue oxygenation
- Note decreased response time for optical spectroscopy
- Samples entire tumor volume non-invasively
Perturbation validation

**Optical spectroscopy**

- Optical spectroscopy consistently tracks Hb saturation before and after perturbation
Roadmap

I. Toolbox development

II. Validation

III. *Ex vivo* assessment

IV. Intraoperative *in vivo* assessment
**Ex vivo Study**

Breast Cancer Or Reduction Surgery → *Ex vivo* Samples → Spectroscopic Measurements

Spectroscopic Measurements → Histopathology

Histopathology → Analysis
## Ex vivo Sample Breakdown

<table>
<thead>
<tr>
<th>Type</th>
<th>Sub-type</th>
<th>Number of samples</th>
<th>Sub total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant</td>
<td>Invasive ductal carcinoma</td>
<td>28</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Invasive lobule carcinoma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ductal carcinoma in situ</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lubular carcinoma in situ</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infiltrating tubulolobular</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Fibrous</td>
<td>Benign</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Dense fibrous</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loose connective</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glandular</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Adipose</td>
<td>Adipose</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>85</td>
</tr>
</tbody>
</table>
Spectral Data Analysis

**MOTIVATION**

**METHODOLOGY / TOOLS**

**VALIDATION**

**EX VIVO ANALYSIS**

**IN VIVO ANALYSIS**

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Raw spectral data → Calibration → Calibrated spectral data → MC inversion → Parameter extraction → Key variables → Classification

Calibrated Reflectance Intensity vs. Wavelength (nm)

[\text{HbO}_2] → [\text{HbCO}_2] → \text{HbSat} → \text{Beta carotene} → Mean \mu_s'

Classification

Classification accuracy → Cross-validation

Wilcoxon rank-sum → Linear or non-linear support vector machine
Representative spectra

Adipose

Malignant (IDC)

Extracted Optical Properties

**Extracted $\mu_a$**

**Extracted $\mu_s'$**

Variables Display Differences

Variables Display Differences

$\text{Zhu et al., Lasers Surg Med, 38, 714-724. 2006.}$
# Model Based Analysis Results

## Extracted Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta carotene concentration</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean reduced scattering coefficient ($\mu_s$)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Hemoglobin saturation</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total hemoglobin concentration</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

## Reflectance Only

<table>
<thead>
<tr>
<th></th>
<th>(a) 2 features (p &lt; 0.0005)</th>
<th>(b) 3 features (p &lt; 0.001)</th>
<th>(c) all features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification Rate (%)</td>
<td>80</td>
<td>82</td>
<td>81</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>83</td>
<td>86</td>
<td>83</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>78</td>
<td>80</td>
<td>80</td>
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</tbody>
</table>
Conclusions - Ex Vivo Study

• There exist significant differences in the optical spectra of malignant and non-malignant breast tissues

• Our method gives us insight into physiological basis for differences

• These spectroscopic differences can be exploited for breast cancer diagnosis

Can optical spectroscopy be used to diagnose tissues in vivo?
Roadmap

I. Toolbox development

II. Validation

III. Ex vivo assessment

IV. Intraoperative in vivo assessment
Intraoperative Breast Tumor Assessment

• Extend prior *ex vivo* studies to *in vivo* optical assessment of breast tissue

• Determine differences in optical spectra of breast tissues of varying physiology *in vivo*

• Assess tissues in “natural environment” – vasculature and local environment intact

• Establish feasibility of optical spectroscopy as a useful clinical diagnostic adjunct
Intraoperative Breast Tumor Assessment

**Motivation**

Mastectomy or lumpectomy surgery

**Methodology / Tools**

1cm or larger lesions targeted

**Validation**

Spectroscopic Measurements

Histopathology

**Ex Vivo Analysis**

Analysis

**In Vivo Analysis**
Procedure:

1. Incision made in skin
2. Ultrasound used to guide needle-biopsy cannula to site of interest
3. Needle is retracted, and probe inserted through cannula to interface with tissue
4. Optical measurement made
5. Probe retracted, and biopsy of interrogated tissue made through cannula
Histopathologic correlation

- Region of interest
  - 3 mm section at proximal end of core
  - Distal end inked to preserve orientation

- Pathologic diagnosis in gross percentage
  - % Adipose
  - % Fibrous / stromal
  - % Epithelial
  - % Invasive (malignant only)
  - % In situ (malignant only)
  - % Hemorrhage
Sample spectra from a single patient

**Adipose**

**Invasive Ductal Carcinoma**
Optical Property / Tissue Parameter Extraction

### Extracted $\mu_a$

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Adipose</th>
<th>IDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>450</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>550</td>
<td></td>
<td></td>
</tr>
<tr>
<td>600</td>
<td></td>
<td></td>
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</tbody>
</table>

### Extracted $\mu_s'$

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Adipose</th>
<th>IDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>450</td>
<td></td>
<td></td>
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<tr>
<td>500</td>
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<tr>
<td>550</td>
<td></td>
<td></td>
</tr>
<tr>
<td>600</td>
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</tr>
</tbody>
</table>

### Table: Beta Carotene Content and Hb Saturation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adipose</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean $\mu_s'$</td>
<td>8.4 cm$^{-1}$</td>
<td>44.7 cm$^{-1}$</td>
</tr>
<tr>
<td>Hb Content</td>
<td>14.2 μM</td>
<td>17.7 μM</td>
</tr>
<tr>
<td>Hb Saturation</td>
<td>88%</td>
<td>41%</td>
</tr>
<tr>
<td>Beta Carotene Content</td>
<td>16.0 μM</td>
<td>5.1 μM</td>
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</table>
Intraoperative Sample Breakdown

<table>
<thead>
<tr>
<th>Tissue type</th>
<th># of Samples, as of 10/1/2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>29</td>
</tr>
<tr>
<td>Benign</td>
<td>12</td>
</tr>
<tr>
<td>Malignant</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients Enrolled</th>
<th>As of 10/1/2006</th>
<th>Expected Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17</td>
<td>75</td>
</tr>
</tbody>
</table>
Intraoperative assessment - Summary

• Feasibility of optical spectroscopy for intraoperative assessment of intact breast tissues demonstrated

• Diffuse reflectance spectroscopy can discriminate between malignant and non-malignant tissues

• Analysis of fluorescence spectra from *in vivo* study in progress

• Future work: Correlation of optical measurements with immunohistological markers (hypoxia, angiogenesis, etc.)
Current *In Vivo* Breast Cancer Studies

Intraoperative assessment

- Core-needle biopsy
- Therapeutic monitoring
Summary

• Optical spectroscopy is a useful tool for detection of cancer and assessment of tissue composition

• Clinical significance: Potential to improve patient care at multiple levels

• Cancer discovery significance: Aid in development of effective cancer therapies
Acknowledgments

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  Lee Wilke, M.D.
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  Tara Breslin, M.D.
  Josephine Harter, M.D.

- Lab members

- Study patients

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  Department of Defense