Multimodal Optical Imaging for Rapid and Accurate Delineation of Cancer Margins

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Ideal Technology for Intraoperative Tumor Margin Delineation

• Assess large fields rapidly and with adequate resolution
• High contrast of pathology / tissue structures
• Robust
• Simple
• Inexpensive
• Resolve signal from single cells (melanoma, glioma)
Wide-Field Polarization Optical Imaging

Subtraction of cross-polarized from co-polarized components of scattered light allows for imaging of the superficial tissue layers.

Imaging cross-polarized scattered component allows for glare rejection and imaging deeper tissue layers.
Dependence of the scattering coefficient and imaging depth on the wavelength of imaging light

Imaging Depth: \( D = \frac{1}{\mu'_s} \)

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>( \mu'_s ) (1/mm)</th>
<th>Image below</th>
</tr>
</thead>
<tbody>
<tr>
<td>410 nm</td>
<td>10.3</td>
<td>100 (±10) µm</td>
</tr>
<tr>
<td>440 nm</td>
<td>8.70</td>
<td>115 (±11) µm</td>
</tr>
<tr>
<td>570 nm</td>
<td>4.52</td>
<td>220 (±25) µm</td>
</tr>
<tr>
<td>650 nm</td>
<td>3.76</td>
<td>270 (±31) µm</td>
</tr>
</tbody>
</table>

Contrast Agents – Methylene Blue

- Positively charged nuclear stain
- Shown effective in grossly demarcating different types of cancer
- Fluorescence and reflectance images of MB stained tissue provide complimentary information
- Concern over toxicity effects in CNS

Gill et al., Cancer 1984
Kaisary et al., Urology 1986
Fukui et al., J Urol 1983
Fedorak et al., Surgery 1983
Yaroslavsky et al., JID 2003
Yaroslavsky et al., Opt.Let. 2004
Al-Arashi et al., Las.Surg.Med. 2007
Contrast Agent - Demeclocycline

- FDA approved antibiotic
- Demeclocycline is capable of distinguishing healthy tissue from cancer in human skin

Rall et al., Journal of National Institute (1957)
Holman et al., Radiology. (1974)
Davis et al., Cancer Res. (1977).
Superficial Reflectance Images of Squamous Cell Carcinoma Stained in Aqueous Methylene Blue

Superficial reflectance image

\( \lambda = 670 \text{nm} \)

frozen histological section
Fluorescence Polarization Images of Squamous Cell Carcinoma Stained in Aqueous Methylene Blue
LumaMed’s Platform Technology: LumaScan™ I and LumaScan™ II
NCI sponsored LumaScan™ clinical study at the University of Wisconsin – Madison
Breast Cancer

- Breast cancer is the leading cause of cancer death in women

- Each year it is estimated that over 290,000 women in the US will be diagnosed with breast cancer and more than 40,000 will die

- 1% of all breast cancers develop in males

- Breast conserving surgery (BCS) is the preferred treatment for early stage breast cancer but re-excision is required in up to 40% cases, as most are performed without intraoperative margin control
Motivation

- Wide field fluorescence polarization imaging can provide rapid en face imaging of breast tissue *Patel et al.*, *J. Biomed. Opt.*, 2012

- A commonly accepted definition of adequate margins requires a 2-mm distance between excision margin and tumor

- Optical coherence tomography (OCT) can provide rapid cross-sectional imaging at depths of a 1-3 mm

- Combining these wide-field fluorescence polarization and OCT can potentially offer a complete assessment of the extent of the tumor laterally and axially *Patel et al.*, *Cancer Res.*, 2014
Tissue Handling and Imaging

- Samples are obtained from UMass Memorial following surgeries.

- Tissue stained in 0.05 - 0.2 mg/ml DPBS solution Methylene Blue. OCT imaging (1310 nm) is not affected by MB.

- The stained tissue is imaged with wide-field polarization imaging system followed by imaging with the OCT system (Thorlabs).

- After imaging, H&E sections were processed from the same tissue block. *En face* sections are initially processed to correlate with wide-field images. The tissue block is then melted and the tissue is re-embedded to process vertical sections for correlation to OCT images.
Invasive Ductal Carcinoma:
Wide-Field Fluorescence Polarization

- Outline tumor and normal areas in histopathology
- Examine fluorescence polarization image of the specimen
- Outline tumor and normal areas in corresponding fluorescence polarization image
- Measure mean fluorescence polarization for both areas

**Mean Fluorescence Polarization**

Tumor = 6.5
Normal = 2.5
Fluorescence Polarization in Breast Cancer

P<0.001

Summary

- Reflectance and OCT images emphasized the structure of connective, fibrous and adipose tissues
  - did not delineate cancer margins reliably, most probably due to high scattering exhibited by breast tumors

- Wide-field fluorescence polarization accurately revealed the location, shape, size and morphology of tumor

- Fluorescence polarization of cancer, was reproducibly higher as compared to benign breast tissues

- Standard OCT delineates breast cancer margins only when cancer resides in adipose tissue. Delineation of tumours on the fibrous background is challenging

- Polarization-sensitive OCT is required for the reliable discrimination of tumor from fibrous background

Patel et al., Cancer Research, 2014
Brain Cancers

Currently the 7th most common human cancer

70,000 new cases and 14,000 deaths in adults in the US in 2013*

*American Cancer Society

Distribution of brain tumor diagnoses in the US in 2013

- Adults (20+):
  - Malignant 23,130
  - Non-Malignant 46,870

- Children (0-19):
  - Malignant 4,500
  - Non-Malignant 2,600
Motivation

- Gliomas, which account for 80% of primary brain malignancies, are among the most challenging to treat and have the worst prognosis, due to their diffuse nature.

- Standard of care for diagnosing brain cancer is three stage examination:
  - intraoperative analysis of fresh tissue
  - preliminary microscopic H&E examination at high magnification
  - IHC examination or genetic analysis

- Technology that can efficiently image a wide field and inspect suspicious areas with single cell resolution would be valuable.
Wide-Field High-Resolution Imager

- Fluorophore: Methylene Blue
- Excited at 402 nm and 532 nm fluorescence measured between 430 nm and 520 nm
- MATLAB/ImageJ used to process polarization images

Simultaneous reflectance, fluorescence, fluorescence polarization imaging

Yaroslavsky et al., Patent # US 20120071764
## Wide-Field High-Resolution Imaging System

<table>
<thead>
<tr>
<th></th>
<th>Field of View</th>
<th>Lateral Resolution</th>
<th>Axial Resolution</th>
<th>G factor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wide-Field CCD camera</strong></td>
<td>43 mm x 32 mm</td>
<td>~13 μm</td>
<td>70 - 200 μm (skin)</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>High-Resolution Confocal</strong></td>
<td>250 - 800 μm</td>
<td>Better than 0.8 μm</td>
<td>3 – 5 μm</td>
<td>1.3</td>
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</table>
Materials and Methods: Tissue Handling

Fresh excess human brain samples were obtained from University of Massachusetts Memorial Medical Center immediately after the surgeries.

Tissues were soaked in 0.75 mg/mL DPBS solution of demeclocycline.

The stained samples were rinsed to remove excess dye and imaged.

After imaging, H&E paraffin embedded sections were processed from the same tissue block.
Optical Pathology – Contrast Enhanced Confocal Imaging

- Optical images were evaluated by pathologists
- NP trained pathologists were able to diagnose brain tumors in MB enhanced optical images
- Optical images can provide results rapidly and effectively, improving time necessary for processing histology

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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<tbody>
<tr>
<td>Normal vs. abnormal brain tissue</td>
<td>95%</td>
<td>100%</td>
<td>100%</td>
<td>98%</td>
</tr>
<tr>
<td>Glial vs. non-glial tumors</td>
<td>83%</td>
<td>90%</td>
<td>91%</td>
<td>80%</td>
</tr>
<tr>
<td>Grade &amp; type of the tumor: all pathologists</td>
<td>63%</td>
<td>46%</td>
<td>76%</td>
<td>32%</td>
</tr>
<tr>
<td>NP trained</td>
<td>88%</td>
<td>100%</td>
<td>100%</td>
<td>86%</td>
</tr>
</tbody>
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Multimodal confocal imaging compared to H&E diagnosis as a standard of care; PPV: positive predictive value, NPV: negative predictive value, NP: Neuropathology

Snuderl et al., Brain Pathology 2012
Summary

- The multimodal system can rapidly image wide field areas and automatically focus into multiple regions of interest to be inspected at high resolution.

- Automated location of multiple selected areas eliminates manual registration.

- Rapid acquisition of wide-field images, followed by high resolution inspection of regions of interest minimizes data processing and image storage requirements.

- Optical images acquired of tissue stained in MB and DMN correlate well with H&E histopathology.

- Pilot trial with brain cancer specimens demonstrated the ability of the imager to grossly delineate tumor regions and detect cell morphology, using wide-field and high-resolution components of the imager, respectively.

- The wide-field high-resolution imaging technology shows promise for the intraoperative delineation of brain pathology.
Nonmelanoma Skin Cancers (NMSC)

- More than 3.5 million new cases of nonmelanoma skin cancer are diagnosed each year
  - 3000 deaths per year
  - Cost of treatments exceeds $600 million per year

- Treatment usually involves surgery

- Micrographic Surgery (MMS) is the most effective form of treatment, as it enables intraoperative tumor margin control using frozen H&E histopathology
Motivation

• Even though Mohs Micrographic Surgery (MMS) is the most effective form of treatment it involves
  - 2 - 20 stages
  - Time and cost ineffective
  - Labor intensive

• More than 80% of lesions occur on the face
  - Tissue preservation
  - Intrinsic contrast
Approach: Multimodal Imaging
Terahertz and Optical Imaging

- Both wavelength ranges are nonionizing
- Terahertz radiation is sensitive to intrinsic contrast between normal and cancerous skin, however, the relatively long wavelength ($\approx 500 \mu m$) limits the resolution of terahertz imagery and thus margin delineation is difficult
- Optical polarized light imaging has the resolution to provide morphological information but lacks chemical sensitivity
- Thus combining the two modalities yields complementary information that can be processed to determine tumor margins \textit{in vivo}
Experimental Methodology

• Fresh skin cancer specimens were obtained from Mohs surgeries at Massachusetts General Hospital under an IRB approved protocol.

• The samples were delivered to UMASS Lowell within an hour after surgery, placed on a gauze moistened with saline solution, covered with a z-cut quartz cover glass and imaged using optical (at 410 nm) and CW terahertz (at 513 μm) systems.

• Hematoxylin & Eosin (H&E) frozen histology was processed from the imaged tissue.

• Optical and Terahertz images were compared to histopathology.
Multimodal Polarization Sensitive Terahertz and Optical Imaging

Squamous Cell Carcinoma (SCC), FOV: ~ 2.6 cm X 1.6 cm

C. Joseph et al., *J. Biophotonics*, 2012
Terahertz and Optical Imaging

SCC Cross-Polarized Optical

SCC Histology

Joseph et al., *J. Biophotonics*, 2012
Joseph et al., *J. Biophotonics*, 2012

Cross-polarized

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Histology

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Tumor → Collagen → Fat → Epidermis → Pilo-sebaceous complex → Hair Follicle
# Statistics

<table>
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<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>THz</td>
<td>79.4%</td>
<td>74.1%</td>
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<tr>
<td>THz+Optical</td>
<td>97.3%</td>
<td>95.7%</td>
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Summary

• Terahertz imaging was capable of identifying the gross location of cancer, but also highlighted non-cancerous areas with similar responses.

• Pixel-by-pixel analysis revealed that quantitative terahertz imaging alone exhibited the sensitivity of 79.4% and specificity of 74.1%.

• Polarization optical imaging was able to discern tissue morphology and discriminate benign areas within terahertz responses that were close to tumor.

• Combined cross-polarized CW terahertz and polarization sensitive optical imaging delivered the sensitivity and specificity of 97.3% and 95.7%, respectively.
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