PhotoOncology

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Parenchymal tissue density is a breast cancer risk indicator. 


Asymmetry in optical spectra indicative for presence of cancer. 


Optical Transillumination Breast Spectroscopy (TIBS) may be able to quantify breast cancer risk.

Prototype engineering
Patient recruitment for cross section proof of principle study.
Understand spectra
Establish predictive value for different applications.
Research tool: Evaluate lifestyle and environmental effects in young women.
Clinical tool: Identify women at risk early in life and permit feedback during Risk reduction intervention.

Longitudinal monitoring study
Diet study in adolescent girls
TIBS correlation to cancer risk.
OUTLINE

• Photodynamic Therapy
  - specific clinical example: prostate cancer
  - other applications/concepts

• Fluorescence Image-Guided Surgery
  - Brain, prostate,...

• GI Endoscopy
  - Fluorescence
  - Raman
  - DOCT
  - future trends

Focus: clinical realities/challenges in photo-oncology

PDT is the use of drugs (photosensitizers) activated by light to produce specific biological effects, usually mediated by oxygen

- cellular (necrosis, apoptosis)
- vascular
- immunologic
**Current Clinical Status**

- *Photofrin*
- *BPD*
- *ALA*

**Approved indications**

- Skin-BCC*
- Lung*
- Esophagus*
- Bladder*
- Head & Neck
- Brain
- Ocular melanoma
- Ovarian
- Prostate
- Renal Cell
- Cervix*
- Pancreas
- Bone

**Current projects**

PDT for (recurrent) prostate cancer using vascular-targeted PS
**Studies in Dog Normal Prostate**

![Image of prostate study](image1)
![Image of prostate study](image2)

**Minimal damage to:**
- urethra
- rectum, bladder
- nerve bundle
- ± radiation

**SKIN PHOTOSENSITIZATION STUDIES**

*pre-clinical and clinical*

![Image of skin response study](image3)

Treatment Planning

'Moderate' plan.
79.0% peripheral prostate treated

'Conservative' plan.
47.2% peripheral prostate treated

'Aggressive' plan.
91.3% peripheral prostate treated

Light Delivery

Prostate
Urethra
Rectum
Hydrodissection

Light Dosimetry Unit
Laptop for Display and Collection

0 5 10 15
Treatment Time (min)

Fluence rate (W/cm²)

Prostate d=5mm
Urethra d=10mm
Hydrodissection
Rectum

10⁻¹
10⁻²
10⁻³
10⁻⁴

0 5 10 15
Treatment Time (min)

Fluence rate (W/cm²)

Prostate d=5mm
Urethra d=10mm
Hydrodissection
Rectum

Monitoring

Tissue optical properties

Tissue optical properties

Measured Fluence Rate (mW/cm²)

Calculated Fluence Rate (mW/cm²)

Measured Fluence Rate (mW/cm²)

Tissue optical properties

Tissue optical properties

Tissue optical properties

Tissue optical properties

0.0 0.2 0.4 0.6 0.8 1.0

µ_s' (cm⁻¹)

0 1 2 3 4 5

µ_a (cm⁻¹)
8/14 biopsy-proven complete responses in Rx-recurrence pts. receiving the highest light doses
Clinical reality: intra- and inter-pt. heterogeneity

- Inadequate light delivery?
  - inaccurate treatment planning
  - errors in fiber placement/delivered energy density
- Non-uniform PS, O$_2$?
- Variable tissue sensitivity?

Dosimetry: light

Treatment reconstruction

1. Target tracing on T2 MRI
2. Necrosis tracing
3. Placement of source fibres
4. Calculation of delivered dose
Light Dose Accuracy

Avg. prediction error: ±44%,
⇒ 1.4/-2.3 mm error in predicting the light dose contour position.

How to cover complex volume of prostate with uniform cylindrically-diffusing fibers?
Inverse treatment plan to calculate output power profile along the length of an intra-urethral fiber to give fixed light fluence at the prostate boundary (radial symmetry)
→ design Bragg grating, build fiber
Dosimetry: photosensitizer

Whole Blood Absorption Spectroscopy

Absorbance

Time (mins)

Relative Concentration

Monomer

Aggregate

Diffuse Transmittance Spectroscopy

Source (Halogen lamp)

Detector (Spectrometer)

Prostate

start of treatment

Option?

biological effect

NIR phosphorescence spectroscopy in situ

Dosimetry: tissue oxygenation

Source (Halogen lamp)

Detector (Spectrometer)

Prostate

Optical Density

Measured

Fit

**Planned Studies**

- Retreatment in Rx recurrences
- Phase IIb/III in Rx recurrences: ‘aggressive’ TPs
- Phase I/II in Primary: ?tissue sensitivity altered
- MRI-guided focal irradiation
- **pre-clinical studies**: ± hyper/hypo-thermia
- Water-soluble PS
- Effect of hormone treatment

**PDT - other planned/ongoing work**

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronomic PDT</td>
<td>Very low drug and light dose rates over extended times (brain, skin)</td>
</tr>
<tr>
<td>New applications</td>
<td>Epilepsy, bone growth, infections</td>
</tr>
<tr>
<td>Therapeutic ‘beacons’</td>
<td>Enzyme and/or mRNA hybridization activated</td>
</tr>
<tr>
<td>2-photon PDT</td>
<td>For age-related macular degeneration</td>
</tr>
<tr>
<td>Nanoparticle-based</td>
<td>Qdot-based 2-photon</td>
</tr>
</tbody>
</table>
**mPDT-brain tumors**

Acute-dose PDT

![Image of brain tumor treatment](image1)

**Morbidity score**

- Pre-ALA: slight health risk
- Post-ALA: pronounced risk
- Post-ALA: severe health risk

ANTECEDENT ALA DRINKING (DAYS)

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**PDT of spinal metastases**

![Image of spinal metastasis treatment](image2)

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Cone Beam CT-guided Photodynamic Therapy

for treatment of spine metastasis
using verteporfin in a pig model

S. Burch, A. Bogaards, S. Bistand, D. Moseley, J. Siewerdsen
A. Yee, J. Finkelstein, D. Jaffray and B.C. Wilson

August 2003

Princess Margaret Hospital

PDT of osteomyelitis

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Focus: clinical realities/challenges in photo-oncology
Can fluorescence imaging provide clearer visualization of residual tumor?

- autofluorescence
- drug-induced (e.g. PDT sensitizer)

Fluorescence-Guided Resection: brain tumor
‘Point & Shoot’ fluorescence camera/spectometer

[Diagram showing multiple spectral windows: 400nm: blue, 500nm: green, 550nm: yellow, 630nm: red, 720nm: near IR]


Red fluorescence at brain surface
Completeness of tumor resection:

WLR 68% ± 38%
WLR+FGR 98% ± 3.5%

Clinical trial (multi-center)

- Pre-op CT scan
- ALA administration

- 50% tumor resection
  Time point A: Bulk tumor measurements
  1. White light images
  2. Fluorescence images
  3. Fluorescent Point Spectroscopy
  4. Tissue Biopsy
  - White light normal areas
  - White light tumor areas
  - Fluorescence – ve areas
  - Fluorescence + ve areas

- Maximum tumor ressection [white light]
  Time point B: Tumor bed measurements
  1. White light images
  2. Fluorescence images
  3. Fluorescent Point Spectroscopy
  4. Tissue Biopsy
  5. OTS localization
  - White light normal areas
  - White light tumor areas
  - Fluorescence – ve areas
  - Fluorescence + ve areas

Procedure End
Fluorescence Image-Guidance at Prostatectomy
FGR- future trends

Time-resolved imaging/spectroscopy

Targeted fluorescent contrast, including NP

Multispectral imaging

Combination with, e.g. MRI

NP
Intraoperative Sentinel Lymph Node Mapping of the Lung Using Near-Infrared Fluorescent Quantum Dots

Fig 1. Intraoperative near-infrared (NIR) fluorescence imaging system. View of the NIR-fluorescence imaging system deployed in the operating room.

Rat 9L glioma model

Qdot-MAb for fluorescence-guided tumor resection

WL Qdot580 + CD44 GFP filter set Background Autofluorescence subtracted
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Focus: clinical realities/challenges in photo-oncology

• most solid tumors (excl. skin) arise as thin lesions on the lining of hollow organs (lung, GI tract, cervix, bladder,…)
Conventional White Light Endoscopy

Detect lesions early

Clinical Challenges
- Detect lesions that are occult to white light endoscopy
- Differentiate benign and (pre)malignant lesions

Fluorescence endoscopy

Autofluorescence imaging (bronchoscopy)

Dr. S. Lam, BCCA
OncoLIFE system

White-light mode         autofluorescence mode ($F_{\text{green}}/R_{\text{red}}$)

Colon
to distinguish hyperplastic (benign) and adenomatous polyps
&
to detect flat adenomas
<table>
<thead>
<tr>
<th>TYPE OF LESION</th>
<th>WL</th>
<th>WL+FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenomatous Polyp</td>
<td>69/120 (57.5%)</td>
<td>97/120 (80.8%)</td>
</tr>
<tr>
<td>Hyperplastic Polyp</td>
<td>34/55 (61.8%)</td>
<td>40/55 (72.7%)</td>
</tr>
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</table>

per-lesion sensitivity

Zanati et al, in prep
**In vivo autofluorescence emission spectra-colon**

Fluorescence microscopy to investigate underlying tissue changes that cause the fluorescence

- **Normal colon**
- **Hyperplastic polyp**
- **Dysplastic polyp**
- **Adenocarcinoma**

**Tytgat et al., Gastrointest. Endosc. 53: 642-650, 2001**

**Fluorescence microscopy to investigate underlying tissue changes that cause the fluorescence**

- **Normal Crypt**
- **Adenomatous crypt**

**DaCosta et al., J. Clin. Path. 58: 766-774, 2005**
Autofluorescence endoscopy: colon

- improved sensitivity and specificity for hyperplastic vs adenomatous polyps (provisional)

- ability to detect flat adenomas (sensitivity)

- understanding of tissue changes in polyps

- definite sensitivity/specificity trial in progress

main GI endoscopic challenges:

Esophagus
to detect dysplasia in Barrett’s Esophagus
Auto-fluorescence spectra in esophagus (blue excitation)

Average spectra

Individual spectra

Statistically-significant differences on average but huge pt-to-pt variations

→ no quantitative differences in the intrinsic autofluorescence between BE and dysplasia

Differences seen in vivo likely due to altered morphology/attenuation

Kara M et al., Am. J. Gastroenter., in press
**Problem: high red autofluorescence in non-dysplastic BE**

White light endoscopy  
LIFE

Alternatives:  
- find optimal LIFE parameters ($\lambda_{ex}, \lambda_{em}$)  
- use targeted fluorescence contrast agent  
- Raman spectroscopy  
- Optical Coherence Tomography  
- Confocal endoscopy, ...

![Diagram showing ALA metabolism and fluorescence in skin and Barrett's Esophagus](image)

**ALA**  
↓  
**PpIX**  
↓  
**Fe**  
↓  
**heme**

**ALA in Barrett's Esophagus - 2mg/kg at 6 hours**

**also highly variable**  
use targeted “contrast agent”
Challenges:

• Identify effective Ab* (high target specificity and affinity, available, human-compatible,...)

  *or peptides, antisense...

• Optimum fluorophore(s): especially for multiplexed imaging

• In vivo imaging instrumentation with correct spectral-spatial-temporal response
Potential advantages:

- Broad excitation spectra
- Narrow, size-dependent emission spectra
- Low photobleaching
- High brightness

Challenges:

- cost
- toxicity
- bioconjugation
- biodistribution

• QD670 + Anti-Villin Mab: topical application

- Multiplexed QDots-Fluorescence (simulated multiplexing)
- Mouse model for colorectal Ca
- Cell line – LS174T (human adenoCa)
- Exc 470 nm, Em LP500 nm
- Multiplexed QDots-Fluorescence
- 0.5 cm
- (simulated multiplexing)
**In Vivo 3QDot-Monoclonal Antibody “Cocktail” Targeted Imaging in an LS174T Colon Adenocarcinoma Mouse Xenograft Model (QD560+CEA, QD580+EGFr, QD700+CC49)**

<table>
<thead>
<tr>
<th>QD560</th>
<th>QD580</th>
<th>QD700</th>
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</thead>
<tbody>
<tr>
<td>CEA</td>
<td>EGFr</td>
<td>CC49</td>
</tr>
</tbody>
</table>

**LS174T Xenograft mouse**
- White light

**3QD+MAbs mouse 2**
- GFP filter 72 h 3 vials
- Pre-injection

**3QD+MAbs mouse 2**
- Texas Red filter 72 h 3 vials
- Pre-injection

**No tumor localization!**

**Non-Specific Accumulation**

<table>
<thead>
<tr>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
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</table>

**Need to get surface chemistry right**
- for adequate tumor uptake and
- to minimize toxicity

**-major challenge**
HOW TO ENHANCE DYSPLASIA DETECTION?

WHITE LIGHT ENDOSCOPY with Random Biopsies

- CHROMOENDOSCOPY
  - REGULAR
  - MAGNIFICATION

- FLUORESCENCE
  - POINT SPECTROSCOPY
  - IMAGING
  - AUTO OR WITH FLUOROPHORE(S)

- NARROW BAND IMAGING

- RAMAN SPECTROSCOPY
  - CONFOCAL
  - ENDOCYTOSCOPY
  - (Doppler) OPTICAL COHERENCE TOMOGRAPHY

Optical biopsy
- many clinical studies of different optical modalities (fluorescence, Raman, elastic scattering,....)
Clinical Trial: Barrett’s esophagus

- 65 patients (59 males)
- Mean age = 65 (range 35-88)
- Mean Barrett’s length = 6 cm (range 3-15)
- # of spectra/biopsies = 233
  - Excluded = 41
    - Gastric-type mucosa
    - Significant reactive changes
  - Analyzed = 192
    - 112 IM
    - 54 LGD
    - 26 HGD/early ACA
Linear Discriminant Analysis on Principal Component Scores
(with leave-one-out cross-validation)

Differentiating ‘high-risk’ from ‘low-risk’ Barrett’s

<table>
<thead>
<tr>
<th>Spectral Diagnosis</th>
<th>Histological Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>IM/LGD</td>
<td>147</td>
</tr>
<tr>
<td>HGD/ACA</td>
<td>19</td>
</tr>
</tbody>
</table>

Sensitivity = 88%
Specificity = 89%
Accuracy = 89%

WongKeeSong et al, Proc. SPIE 5692: 2005
optical analogue of high-frequency ultrasound
...cross-sectional subsurface imaging using interferometry.

e.g. esophagus

Rat esophagus mucosa
Conventional OCT

Measures interference in the time domain
- One point at a time

Sensitivity $\Delta z \cdot$ Power
Imaging Speed

Frequency Domain

Fourier Transform

High Speed Esophageal Imaging

Brouma & Tearney, MGH
FUTURE?

Multimodal Optical Diagnosis
Accuracy of Spectroscopic Classification

<table>
<thead>
<tr>
<th>Method</th>
<th>Sens (HGD vs NDB/LGD)</th>
<th>Spec (HGD vs NDB/LGD)</th>
<th>Sens (LGD/HGD vs NDB)</th>
<th>Spec (LGD/HGD vs NDB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence</td>
<td>100</td>
<td>97</td>
<td>79</td>
<td>88</td>
</tr>
<tr>
<td>Reflectance</td>
<td>86</td>
<td>100</td>
<td>79</td>
<td>88</td>
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<tr>
<td>LSS</td>
<td>100</td>
<td>91</td>
<td>90</td>
<td>96</td>
</tr>
<tr>
<td>Combination</td>
<td>100</td>
<td>100</td>
<td>93</td>
<td>100</td>
</tr>
</tbody>
</table>

Tools or Toys?

Functional (e.g. DOCT)

Gene expression (e.g. Ab-F*)

(ultra) structural (e.g. OCT Confocal, LSS)

Biochemical (e.g. Raman)
National Cancer Institute of Canada
National Cancer Institute (US)
Canadian Institutes of Health Research
Ontario Research & Development Challenge Fund
Photonics Research Ontario
Canadian Institute for Photonic Innovations
Canadian Foundation for Innovation

Princess Margaret Hospital Foundation
St Michael’s Hospital Foundation

DUSA Pharmaceuticals, NY
Axcan, Quebec
Xillix Technologies Corp, BC
Negma-Lerads, France
QLT Inc, BC

Collaborators
UHN Biophotonics Faculty and Students