HANDHELD CONFOCAL MICROSCOPES FOR FLUORESCENCE IN VIVO ENDOMICROSCOPY

Optiscan Imaging Limited (ASX:OIL)
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Endomicroscopy in Breast Cancer Surgery

- What is Endomicroscopy?
- Its use in breast cancer
- Other applications
Medical Imaging Technologies

- **MRI, CT, Ultrasound, X-Ray**
  Real time imaging of organs and body structures in living tissue

- **Pathology**
  Subcellular resolution, high complexity, fixed tissue, slow

- **Endomicroscopy**
  Real-time, cellular detail, minimally invasive, live or fixed tissue
Endomicroscopy

- Miniature Confocal Laser Scanning Microscope
- Real Time Virtual Biopsy
- Requires Fluorescent Contrast Agent

Histology: en face view

Conventional Histology of Colonic Crypts

Confocal Endomicroscopy
Confocal Microscopy

- Optical imaging technique
- Increases resolution and contrast by spatially rejecting out of focus light
Confocal Fluorescence Images....

- Laser focused to a point in the sample, exciting fluorescence.
- A detector measures the intensity of fluorescent light from that point.
- The point is scanned through the specimen.
- Image is an optical “slice” of point-intensity measurements.
- Maps local fluorophore concentration.
Endomicroscopy - In Vivo Virtual Histology

- < 1mm Field of View
- ~1000X magnification
- Micron (µm) scale lateral and axial resolution
- Shows cellular and subcellular detail
- Images surface and subsurface features
Endomicroscope Systems

Commercially Available Imaging Systems for *In Vivo* Confocal Imaging

Point Scanning Endomicroscopes
- Optiscan (FIVE2)
- Optiscan - Carl Zeiss (Convivo)

Fibre Bundle Endomicroscopes
Point Scanner Vs Scanned Bundle

**Point Scanning**
- Scanned single fibre is used for excitation and detection
- Scanner contained within distal probe tip
- Real time optical sectioning in Z axis
- Resolution limited by scanner lens optics (Megapixel images)

**Fibre Bundle**
- Processor end of a fibre bundle is sequentially scanned
- Image is an array of spots
- Fixed z-depth
- Resolution limited by number of fibres in bundle (~30K pixel images)
In Vivo Endomicroscopy Sample Images

Point Scanning
- Mouse ilium
- Human lung
- Barrett’s esophagus
- Adenocarcinoma

Bundle Fibre
- Mouse ilium
- Human lung bronchitis
- Barrett’s esophagus
- Adenocarcinoma
Intraoperative Assessment of Breast Cancer Margin with Confocal Laser Endomicroscopy (CLE)
Breast Cancer - Most Common Cancer in Women

- 2.1 Million
- 15% of cases
- 556 centers
- 145 hospitals

2.1 million new cases of breast cancer predicted in 2018 (globally)¹
15% of all new cancer cases in the United States are breast cancer²
There are 556 Breast Centers in the USA accredited by the US Commission on Cancer³
There are 145 hospitals in Australia performing breast cancer surgeries⁴

The number of breast cancer cases and defined cancer centres represent a large target market. 20-30% of lumpectomy patients currently require repeat surgery with current practice (histopathology analysis) often taking up to 3-4 days post initial surgery.

Lumpectomy/Breast Conserving Surgery (BCS)

- 60% of breast cancer surgery is now breast conservation surgery with advent of effective adjuvant therapy

- Often treatment of choice is complete tumour excision with margin while still maintaining acceptable cosmetic outcome

- Gold standard of surgical tumour margin is histopathological analysis performed days after surgery
What Is The Clinical Problem?

- Positive surgical margins are associated with a significantly higher risk of developing local recurrence
- Can be as high as 30% in ductal carcinoma in situ (DCIS) resulting in re-excisions
- Negative consequences – emotional trauma to patient, post-operative infections, poor cosmesis, prolonged hospital stay, delayed adjuvant therapy and higher costs
- No reliable intra-operative imaging tool for margin assessments
What are the Economics of BCS – Cost of Reop?

First operation:
* Surgeon $650
* Anaesthetist $300
#Hospital (Theatre & Day Surgery) $3570
* Pathology $467

Reoperation: Occurring in 25-30% of cases ($4987)
* Surgeon $650
* Anaesthetist $300
#Hospital (Theatre & Day Surgery) $3570
* Pathology $467

* Medicare Fees Only    # Private Hospital Charges
Standard Imaging Protocol During BCS

- X-Ray or US excised tissue
- Review images to assess margins
- Perform histopathology on excised tissue confirm margins
Margins were clear on pathology.

Small area of calcifications appears clear of margins. Pathology showed invasive cancer was clear but the margins were involved with DCIS. Subsequent further surgery showed more extensive radiologically occult DCIS.
Ultrasound of Breast Cancer Lump During Surgery
Confocal Laser Endomicroscopy (CLE)

- Bridge the gap between macroscopic and microscopic imaging
- Real-time imaging using optical digital biopsy
- Miniaturized microscope for ex-vivo and in-vivo tissue imaging using flexible fibre-optics

Advantages
- Non-invasive
- Real-time high resolution histology of infinite sites
- Reduced sampling errors
- Digital permitting telemedicine and AI application
Endomicroscopy in Breast Cancer Surgery

- **Intraoperative Assessment of Breast Cancer Surgical Margin with CLE**

- **Goal:** Assist breast surgeons and pathologists to provide real-time cellular assessment of surgical margin.

- **Benefits:** Reduce risk of residual tumour, need for repeat surgery, patient emotional distress, costs for patients, hospitals, insurers and the taxpayer by reducing the number of repeat surgeries.
**Progress to date on Breast Cancer Trial**

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**Stage 1**  
October 2018  
Examination of ex vivo excised breast tissue specimens by CLE and Histopathology  
16 patients

**Stage 2**  
March 2019  
Examination of ex vivo fresh breast tissue specimens in conjunction with PARPi-FL imaging agent in pathology lab  
14 patients

**Stage 3**  
TBC  
Examination of ex vivo fresh breast tissue specimens in the operating theatre during operating procedure  
Correlation with X-ray, ultrasound and histopathology

**Stage 4**  
TBC  
Examination of margins in breast wound bed during operation  
Utilising the Optiscan Clinical FIVE2 system Potential IV PARPi-FL

**Today**

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**Trial:** Breast Cancer Surgical Margin Assessment Trial conducted at Hollywood Private Hospital and Western Diagnostic Pathology. In conjunction with Dr Peter Willsher (Breast Surgeon) and Dr Jespal Gill (Pathologist)
Breast Cancer Trial (Stage 1)

Ex vivo CLE images show clear distinction between normal, fibrous and tumour, and excellent correlation with H&E histopathology.

Contrast agent used is 0.1% Acriflavine.

Courtesy of Dr Philip Currie
Breast Cancer (Stage 1)

Contrast agent Acriflavine 1mg/ml.

Images ex-vivo from mastectomy tissue, courtesy of Dr Philip Currie.
Tumour Labelling with PARPi-FL

PARPi-FL: A PARP1 inhibitor (olaparib) with fluorescent tag

Interested NOT in therapeutic
But in the expression for imaging

DNA Repair Pathway

DNA damage

NAD+

NAD

PARP1

Apoptosis

DNA Repair

CELL SURVIVAL

DNA ligase III
DNA polymerase
XRCC1

Courtesy Thomas Reiner Lab
Memorial Sloan Kettering Cancer Center
Summit Biomedical Imaging
Breast Cancer Trial (Stage 2)

- Surgical Margin Assessment Trial conducted at Hollywood Private Hospital (W.A. largest private hospital).

- Underway with multiple specimens currently from 14 mastectomy patients with PARPi-FL matching histopathology.

Matching CLE and H&E – Cancer cells throughout

Matching CLE and H&E – Cluster of cancer cells (Arrows)
Breast Cancer – Invasive ductal carcinoma

Contrast agent PARPi-FL. Labels PARP1 single break DNA repair enzyme.

Images ex-vivo from mastectomy tissue, courtesy of Dr Philip Currie.
Breast Cancer Trial (Stage 3 is next)

- Intraoperative
- Ex-vivo CLE imaging of the excised breast lump
- Correlation with operative X-ray, ultrasound and histopathology
- Macro and micro imaging of optical fluorescent probe
- Clinical decision - to increase surgical resection
- Endpoint – reduction of reoperation
Endomicroscopy: A Platform Technology Applicable to Many Fields of Research
Clinical Applications of Fluorescence In-Vivo Endomicroscopy

Optiscan Endomicroscope Clinical Devices
- Neurosurgery (Zeiss Convivo endomicroscope – 2nd generation scanner)
- GI (Pentax ISC-1000 gastroscopy/colonoscopy – 1st generation scanner)

Other Clinical Research Projects
- Cancer detection and margin identification in mouth, cervix, oesophagus,
Optiscan Endomicroscopes are integrated into Zeiss Convivo for use in tumour margin identification during neurosurgery.
Rat Brain – Glioblastoma

**Tumour islands surrounded by leaky blood vessels in live rat brain**

The tumour is a Glioblastoma (Causes hemorrhage, highly infiltrative). The contrast agent here is IV fluorescein.

Courtesy of researchers in Barrow Neurological Institute, Phoenix, Arizona, USA.
Tumour: Glioblastoma. Montage of images as the surgeon moves the Optiscan probe over a small region of brain and tumour. The Grey tissue at lower left is normal brain and regular fine microvessels (capillaries) can be seen as clear white lines throughout.

However, at upper right, a clear island of large round tumour cells is seen, surrounded by a characteristic region of oedema and some blood leakage.

Between these two areas lies a “rift” between the tumour margin and normal tissue. However, characteristic larger round tumour cells are also seen infiltrating the border of the normal brain tissue.
Pentax ISC-1000 Endomicroscopy system

- Biopsy channel Ø 2.8 mm
- Auxiliary water jet channel
- Confocal imaging window
- Air and water jets
- Light jets
- Light guide
- CCD
- Light guide
Human Colon

Images courtesy of Dr Ralf Kiesslich, University Hospital, Mainz, Germany and Professor Adrian Polglase, Cabrini Hospital, Melbourne, Australia
Barrett’s Oesophagus and Barrett’s Cancer

Normal Barrett’s Oesophagus

Barrett’s carcinoma (early cancer)

Images courtesy of Dr Ralf Kiesslich, Mainz University Hospital, Germany
Barrett’s Oesophagus and Barrett’s Cancer

A blinded, multi-center, randomized, controlled trial comparing traditional endoscopy and confocal laser endoscopy (CLE) concludes that CLE improves diagnostic accuracy (P<.0001) for neoplasia and allows for real-time decision making.

White squamous islands spotted by white light endoscopy (A). CLE shows intestinal metaplastic glands with loss of normal mucosal pattern, darkening of epithelial cells, lack of goblet cells, glandular distortion, and a cribriform pattern, consistent with high-grade dysplasia (C,D).

Canto et al., Gastrointestinal Endoscopy (2014)

Institutions

1. Johns Hopkins Medical Institutions, Maryland, USA
2. Mount Sinai Medical Center, New York, New York, USA
3. Harvard Medical School, Boston, Massachusetts, USA
4. University of Pennsylvania, Pennsylvania, USA
5. Dallas Veterans Affairs Medical Center, Texas, USA
6. Emory University Hospital, Atlanta, Georgia, USA
7. Johannes Guttenberg University, Mainz, Germany
8. Department of Anatomic Pathology, Ohio, USA
Barrier Dysfunction Predicts Relapse in Inflammatory Bowel Disease

Loss of barrier function during mucosal cell shedding in human small intestine visualised by confocal endomicroscopy.


Institutions

1. Medical Department, Johannes Gutenberg University of Mainz, Germany
2. Department of Gastroenterology, University of Liverpool
3. Department of Gastroenterology, Lyon Sud Hospital, France
4. Department of Gastroenterology and Hepatology, National University of Singapore
5. Department of Medicine I, University of Erlangen-Nuremberg, Germany
6. Faculty of Health, Norwich Medical School, University East Anglia, Norwich Research Park, UK
Confocal Imaging in the Oral Cavity Using Acriflavine and Fluorescein

Images of confocal endomicroscopy

After topical application of acriflavine hydrochloride in ex vivo specimens.

(a) Normal mucosa with regular configuration of cell nuclei.
(b) Invasive carcinoma of the floor of the mouth with different sizes of nuclei.

After intravenous fluorescein sodium.
(c) Normal mucosa (imaging plane depth 50um).
(d) An invasive carcinoma of the floor of the mouth with irregular cell patterns and leaking of contrast agent.

Normal Squamous Epithelium & CIN

Contrast agent: Topical Acriflavine solution (0.05%); FOV = 400 um

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