

HANDHELD CONFOCAL MICROSCOPES FOR FLUORESCENCE IN VIVO ENDOMICROSCOPY

Optiscan Imaging Limited
(ASX:OIL)



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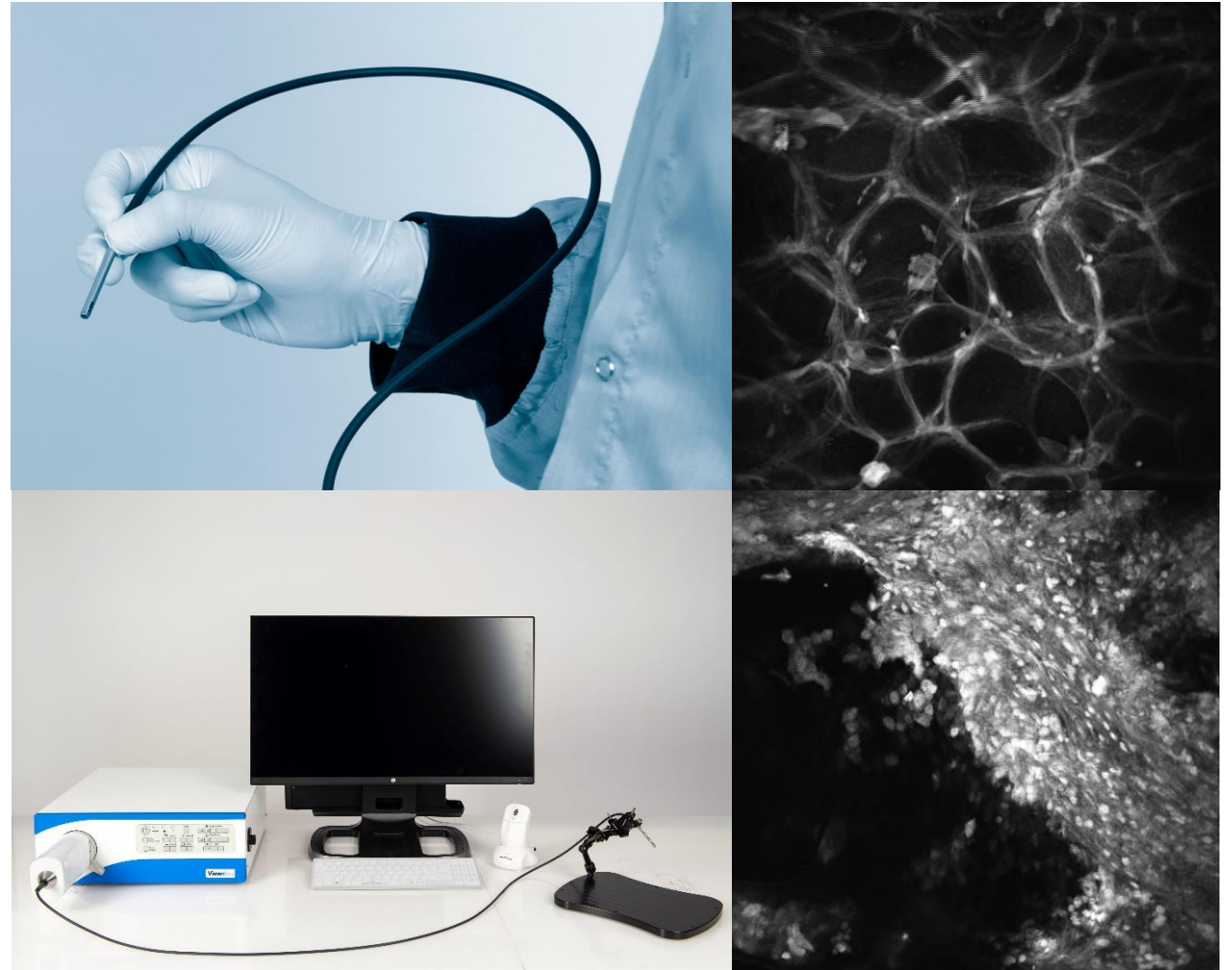
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Endomicroscopy in Breast Cancer Surgery

- **What is Endomicroscopy?**
- **Its use in breast cancer**
- **Other applications**



Medical Imaging Technologies

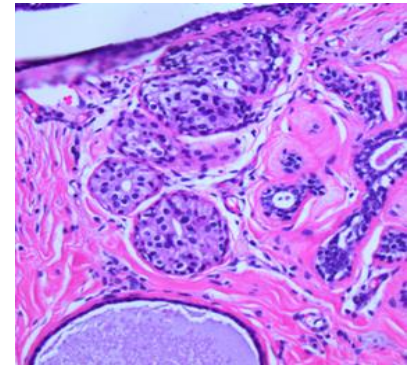
- **MRI, CT, Ultrasound, X-Ray**

Real time imaging of organs and body structures in living tissue



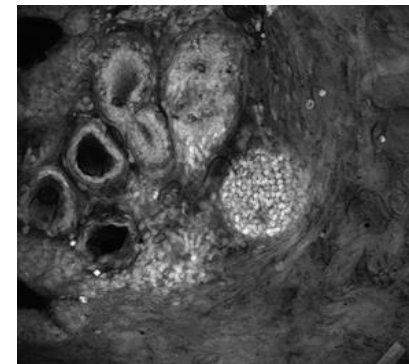
- **Pathology**

Subcellular resolution, high complexity, fixed tissue, slow



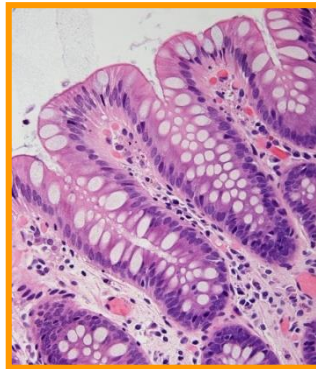
- **Endomicroscopy**

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

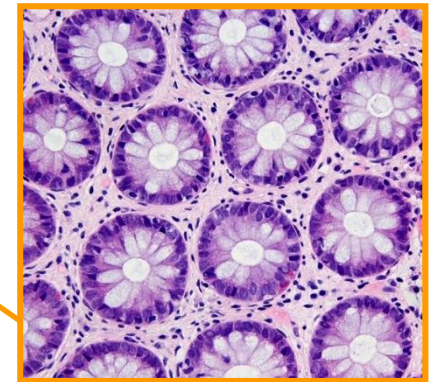
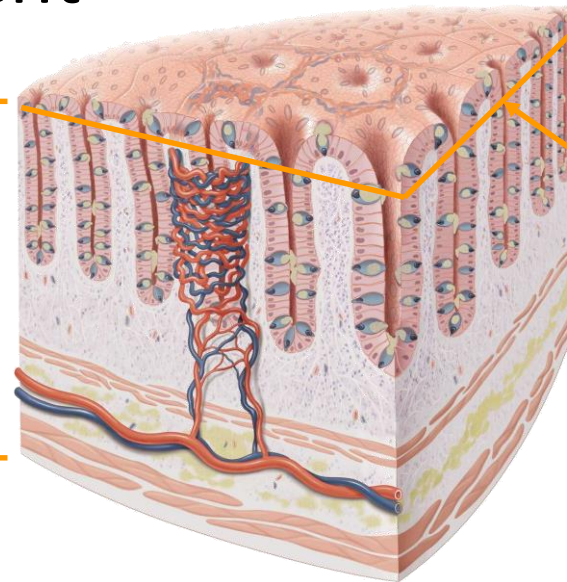


Endomicroscopy

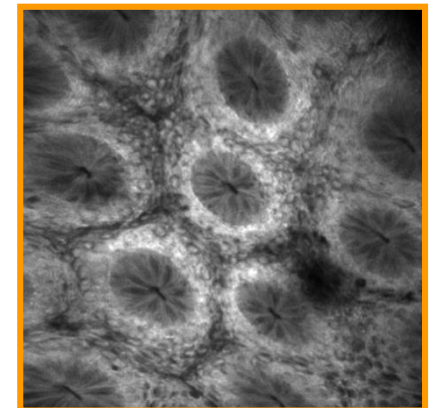
- Miniature Confocal Laser Scanning Microscope
- Real Time Virtual Biopsy
- Requires Fluorescent Contrast Agent



Conventional
Histology of
Colonic Crypts



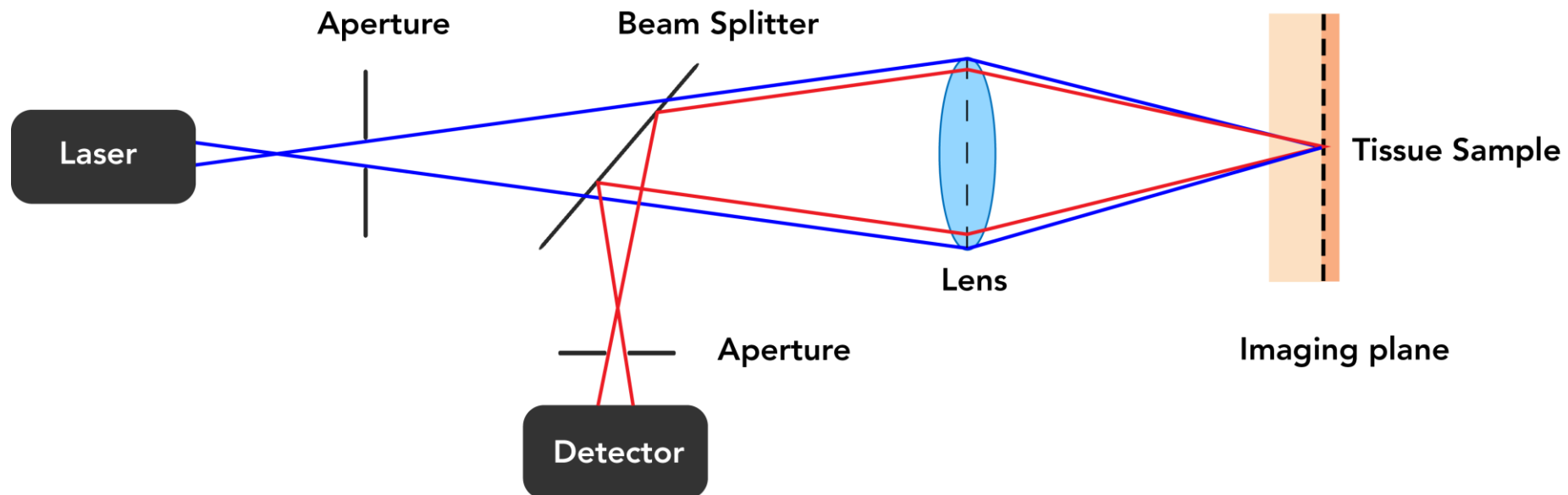
Histology: en face view



Confocal
Endomicroscopy

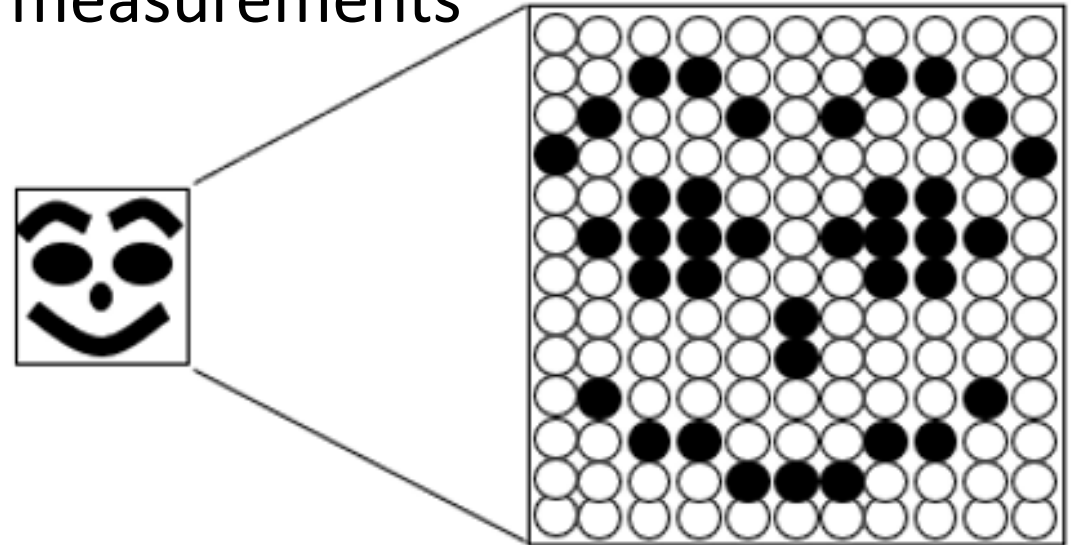
Confocal Microscopy

- Optical imaging technique
- Increases resolution and contrast by spatially rejecting out of focus light



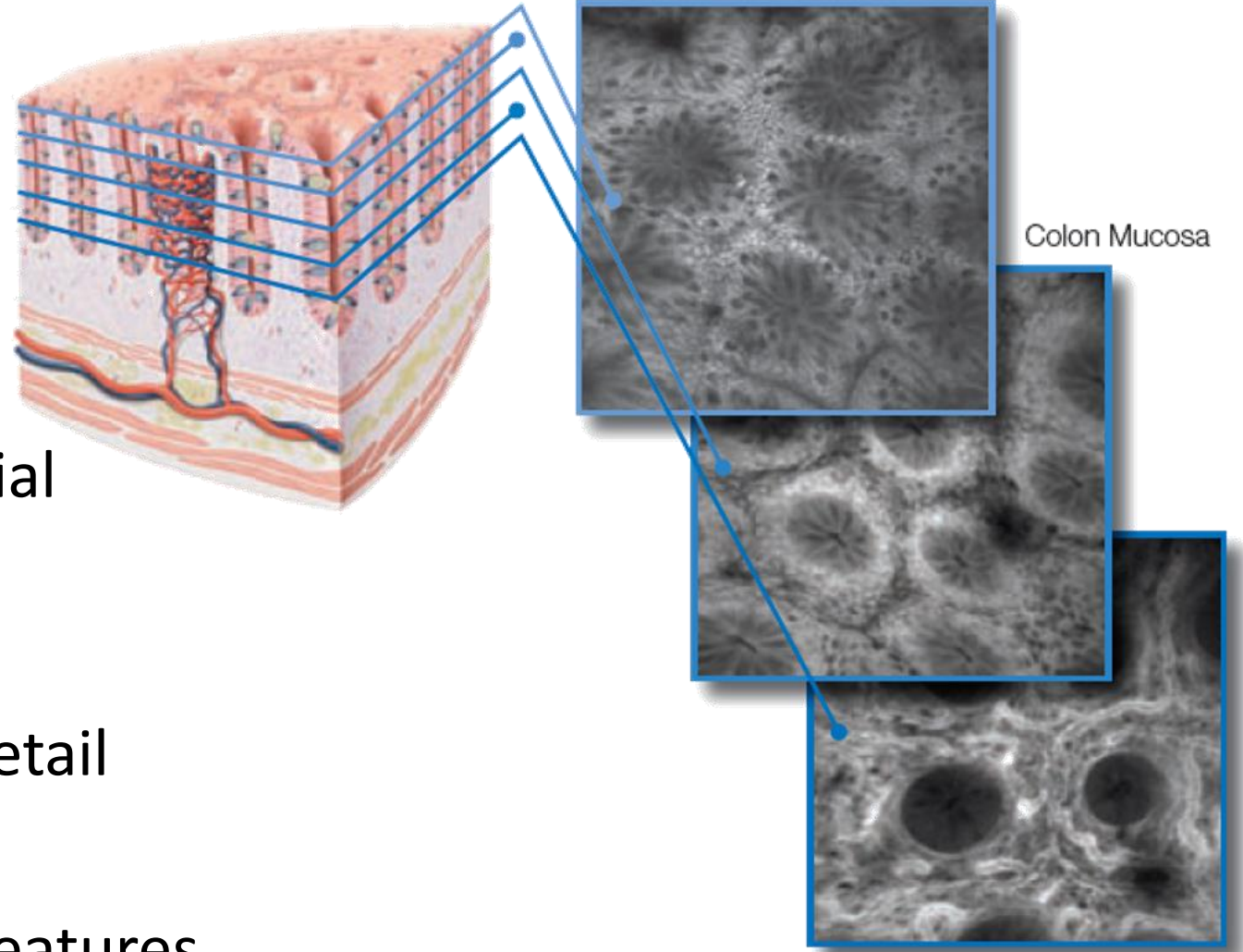
Confocal Fluorescence Images....

- Laser focussed 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

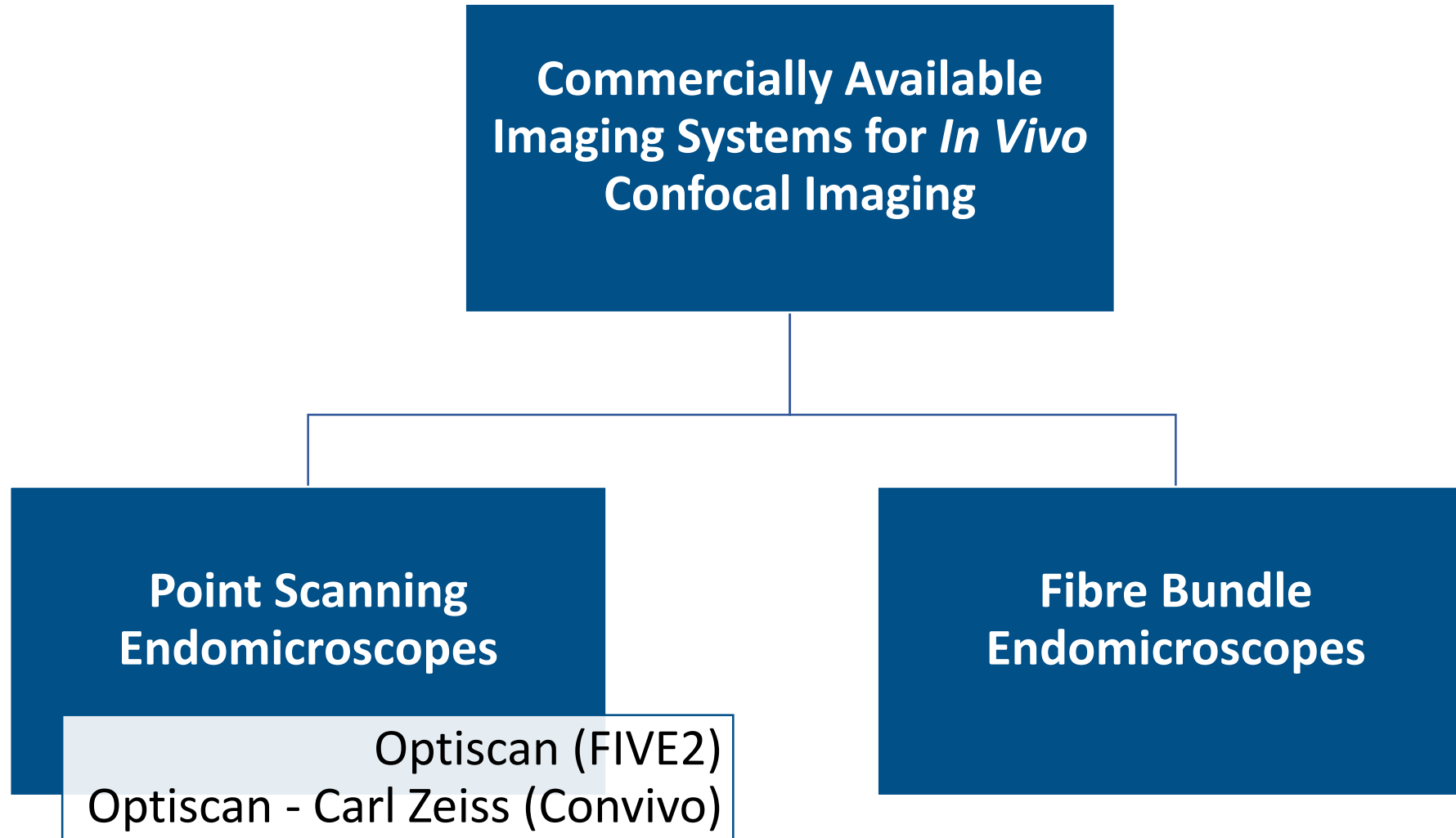


Endomicroscopy - In Vivo Virtual Histology

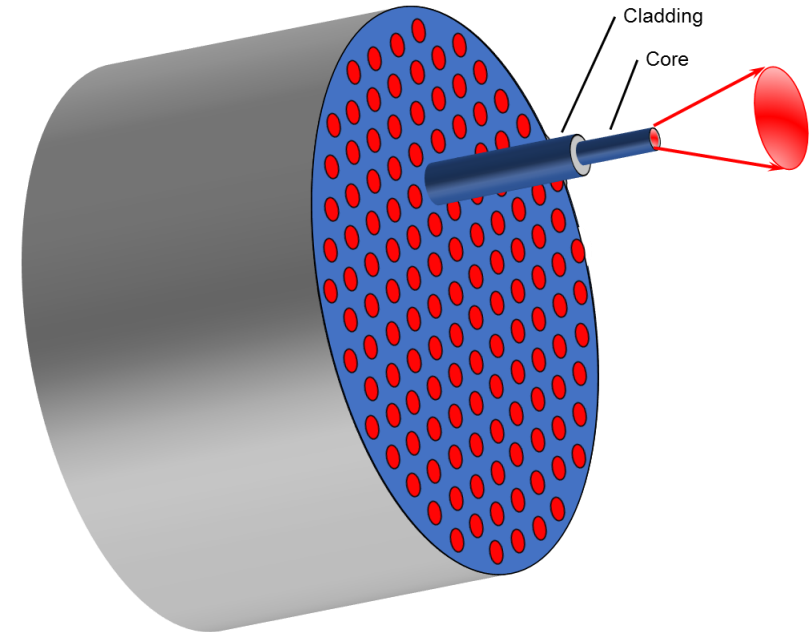
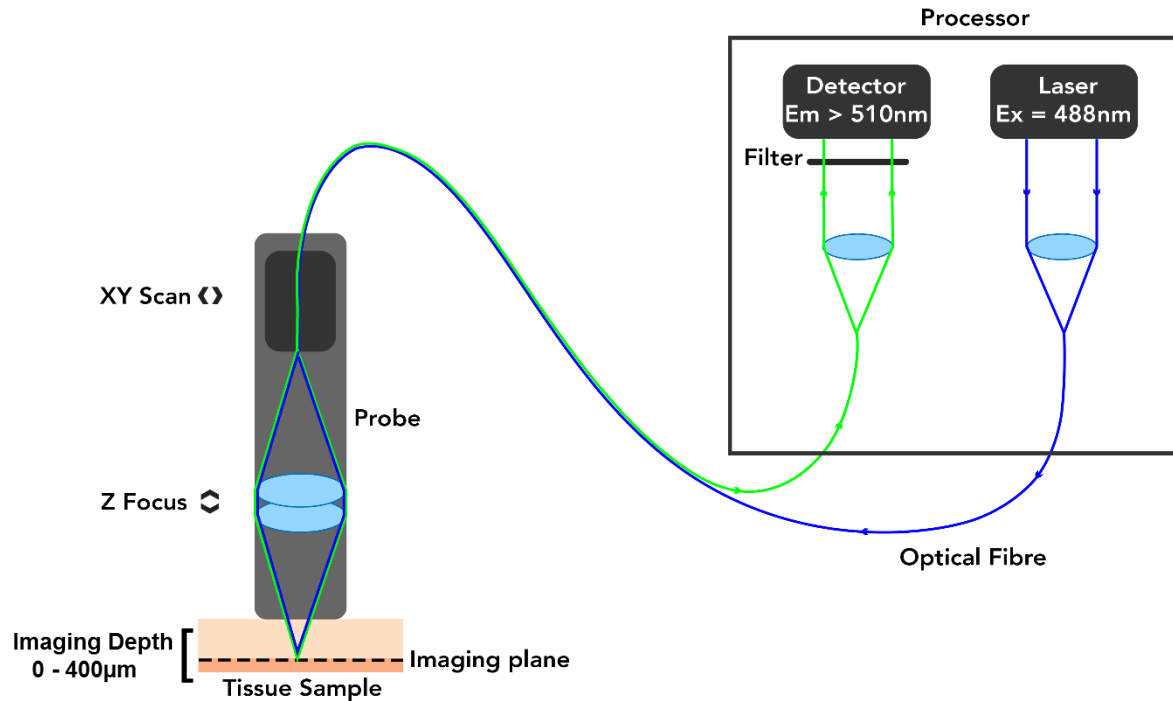
- < 1mm Field of View
- ~1000X magnification
- Micron (μm) scale lateral and axial resolution
- Shows cellular and subcellular detail
- Images surface and subsurface features



Endomicroscope Systems



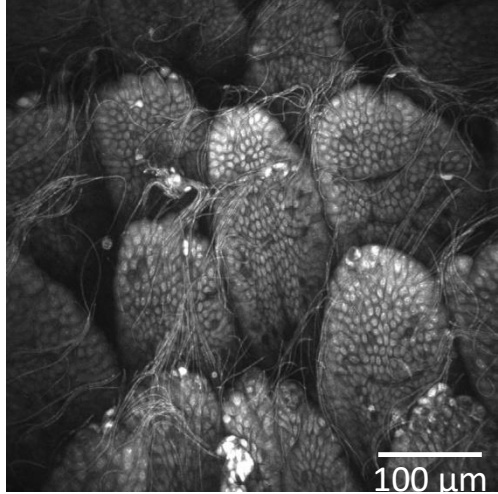
Point Scanner Vs Scanned Bundle



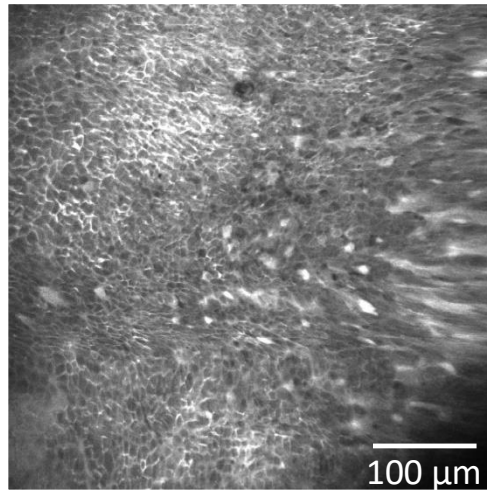
Point Scanning	Fibre Bundle
Scanned single fibre is used for excitation and detection	Processor end of a fibre bundle is sequentially scanned
Scanner contained within distal probe tip	Image is an array of spots
Real time optical sectioning in Z axis	Fixed z-depth
Resolution limited by scanner lens optics (Megapixel images)	Resolution limited by number of fibres in bundle (~30K pixel images)

In Vivo Endomicroscopy Sample Images

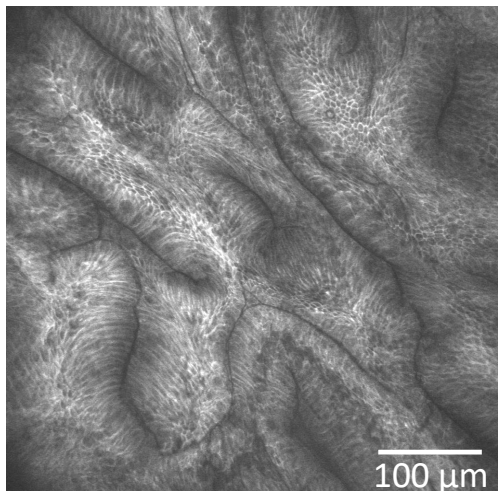
Point Scanning



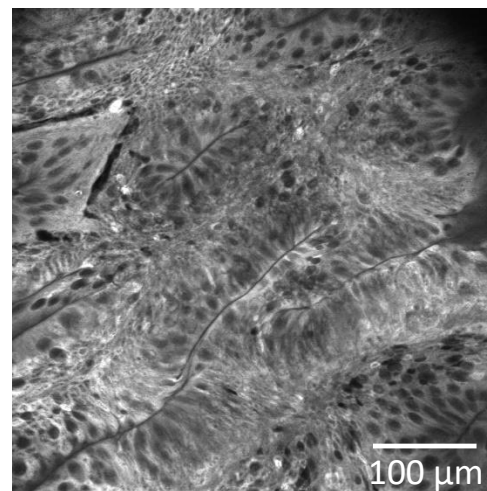
Mouse ileum



Human lung

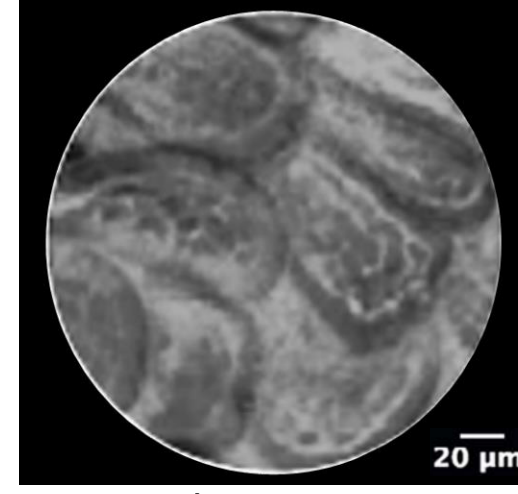


Barrett's esophagus

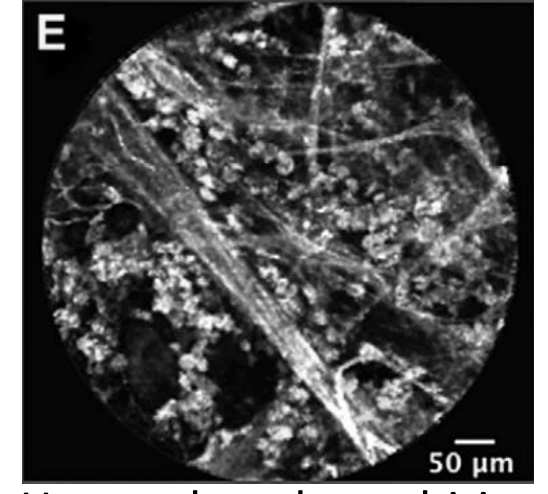


Adenocarcinoma

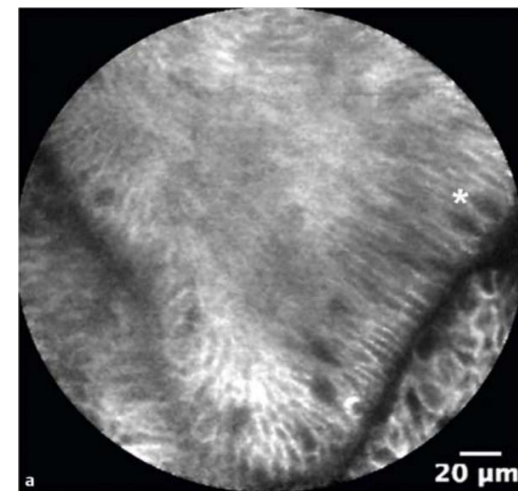
Bundle Fibre



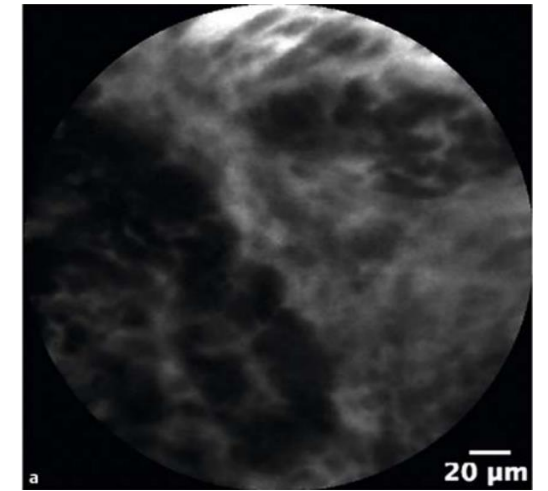
Mouse ileum



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

2.1 million new cases of breast cancer predicted in 2018 (globally)¹

15%
of cases

15% of all new cancer cases in the United States are breast cancer²

556
centers

There are 556 Breast Centers in the USA accredited by the US Commission on Cancer³

145
hospitals

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.

1. GLOBOCAN 2018 estimates; uses Bray et al 2018 paper in CA: A Cancer Journal for Clinicians, page 398 2. American Cancer Society Estimated 2019 statistics 3. <https://www.facs.org/search/cancer-programs>

4. <https://www.myhospitals.gov.au/compare-hospitals/cancer-surgery-waiting-times/breast-cancer>

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

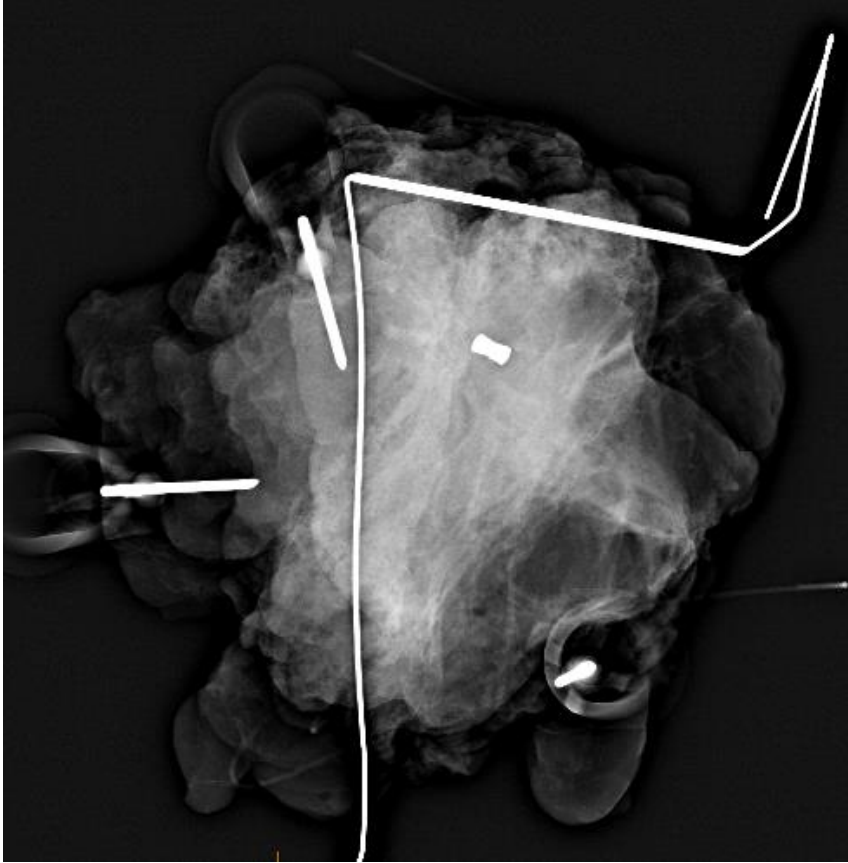
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graph LR; A[X-Ray or US excised tissue] --> B[Review images to assess margins]; B --> C[Perform histopathology on excised tissue confirm margins];
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X-Ray or US
excised tissue

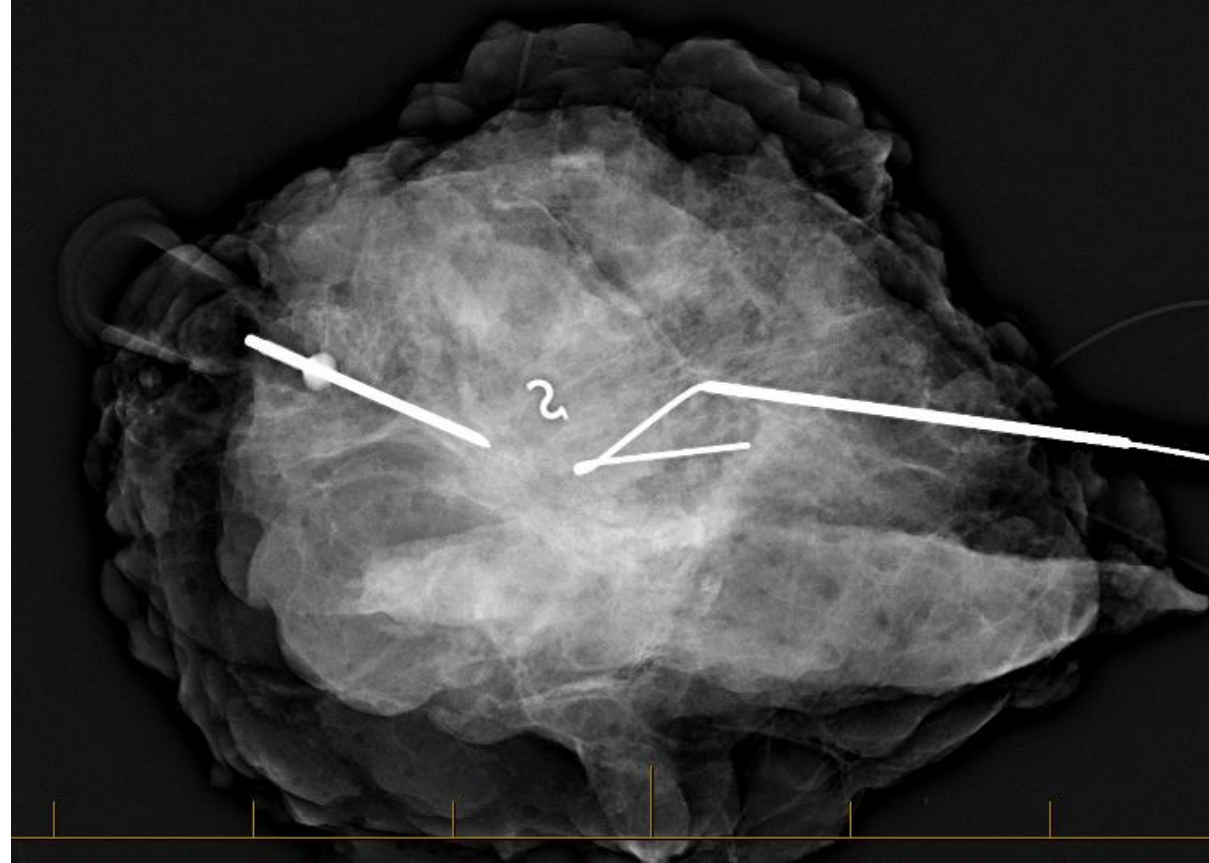
Review images to
assess margins

Perform
histopathology on
excised tissue
confirm margins

X-Ray of Breast Cancer Lump During Surgery

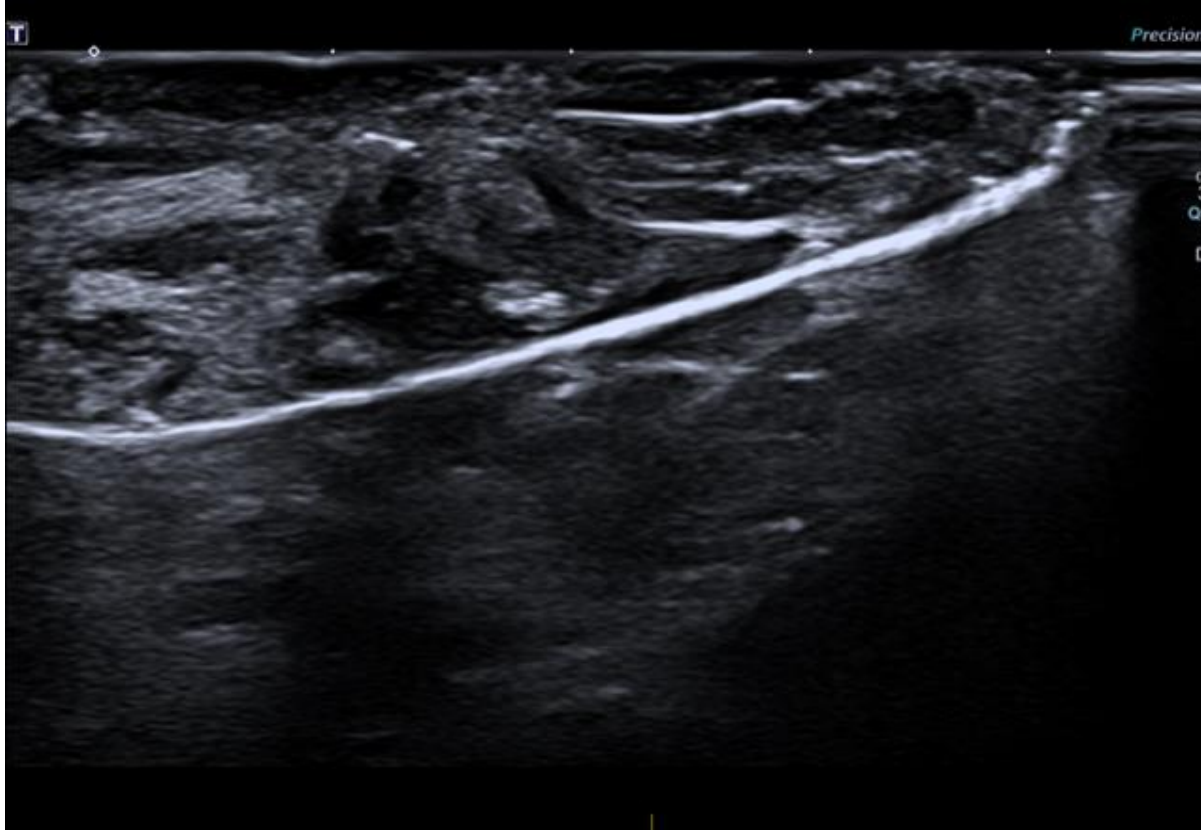


Margins were clear on pathology



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

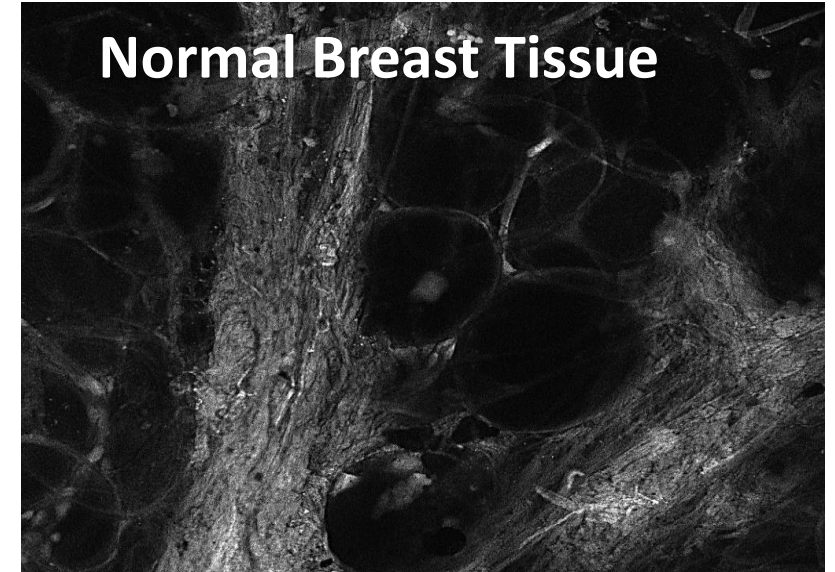


■ Confocal Laser Endomicroscopy (CLE)

- 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

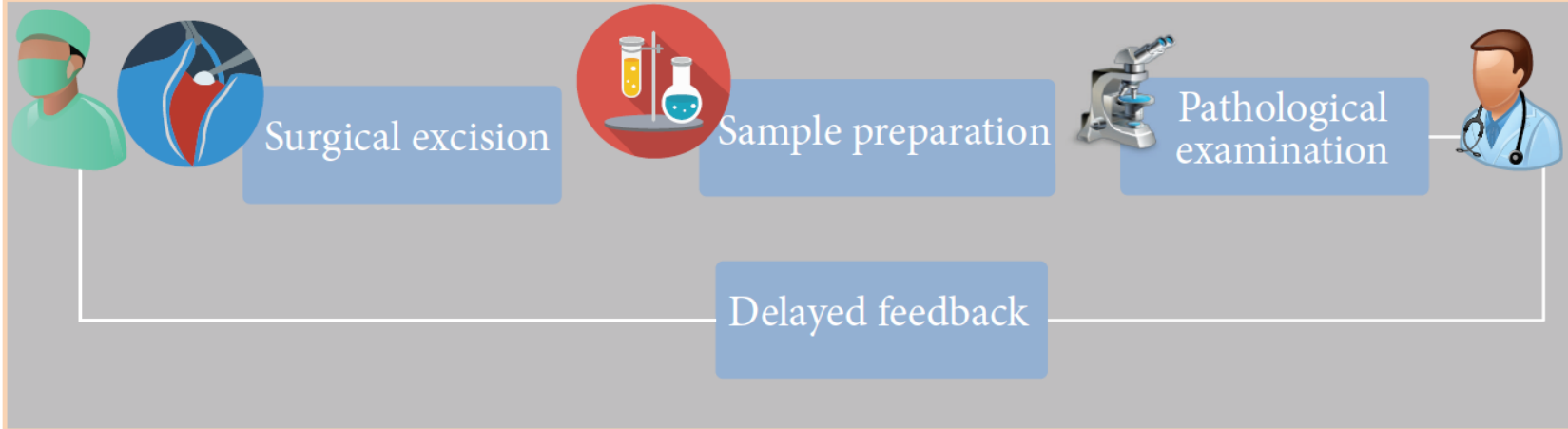
Endomicroscopy in Breast Cancer Surgery

- **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

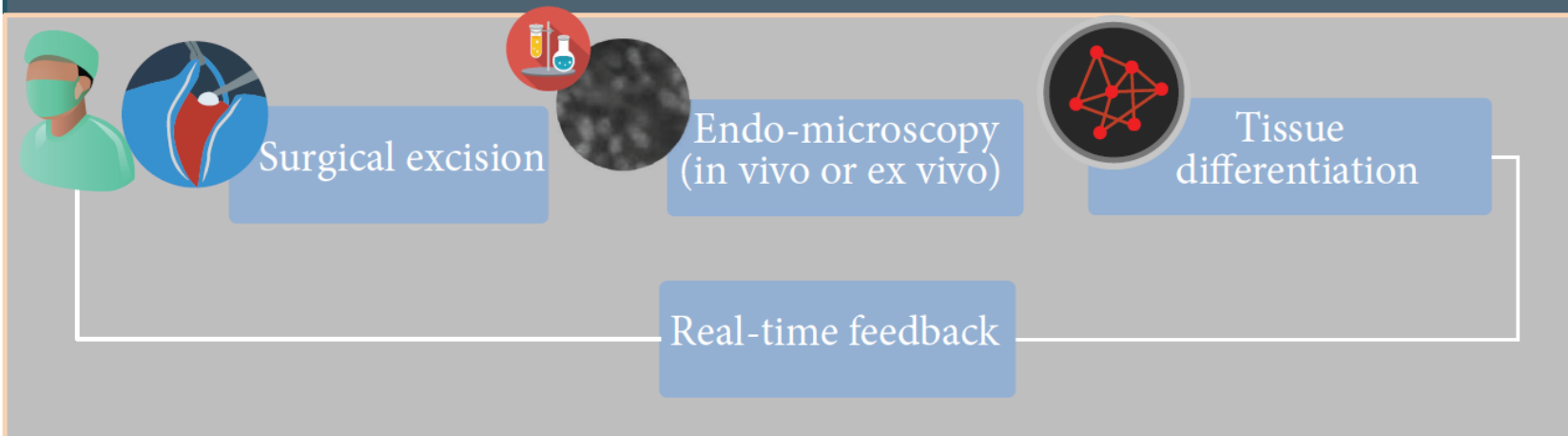


Conventional Intraop Pathology vs Proposed Intraop Pathology

Conventional surgical pathology

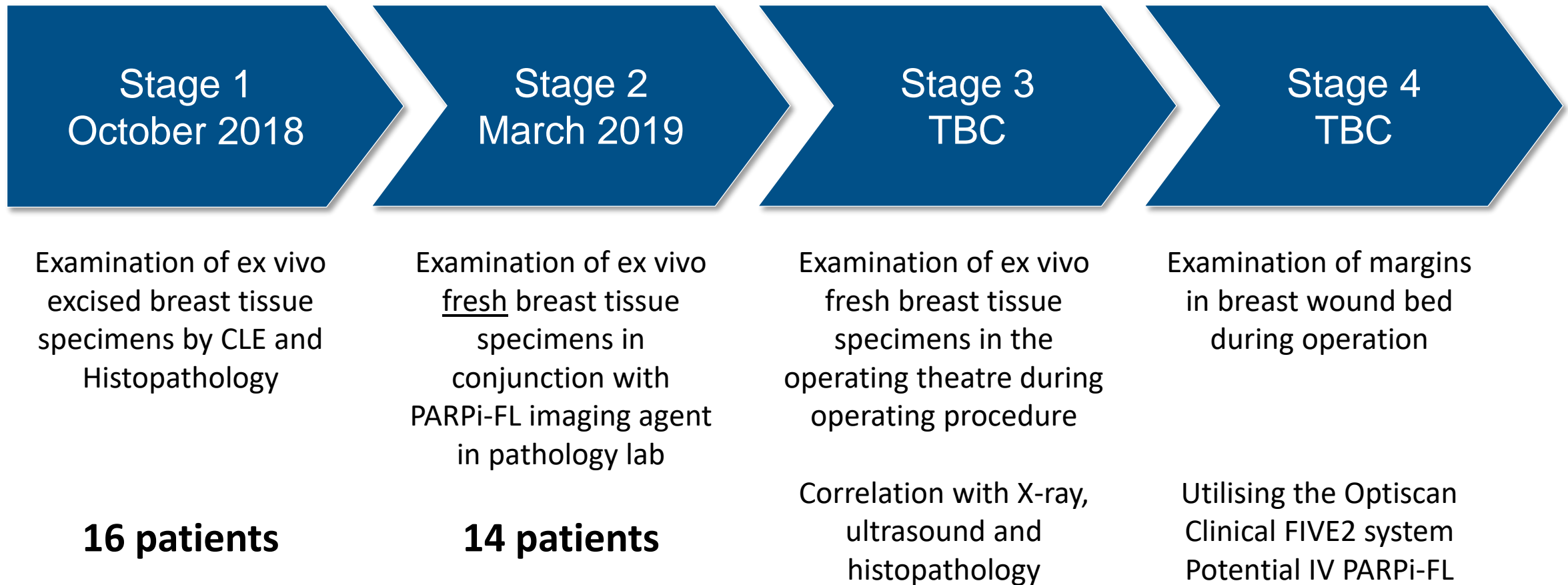


Intraoperative pathology

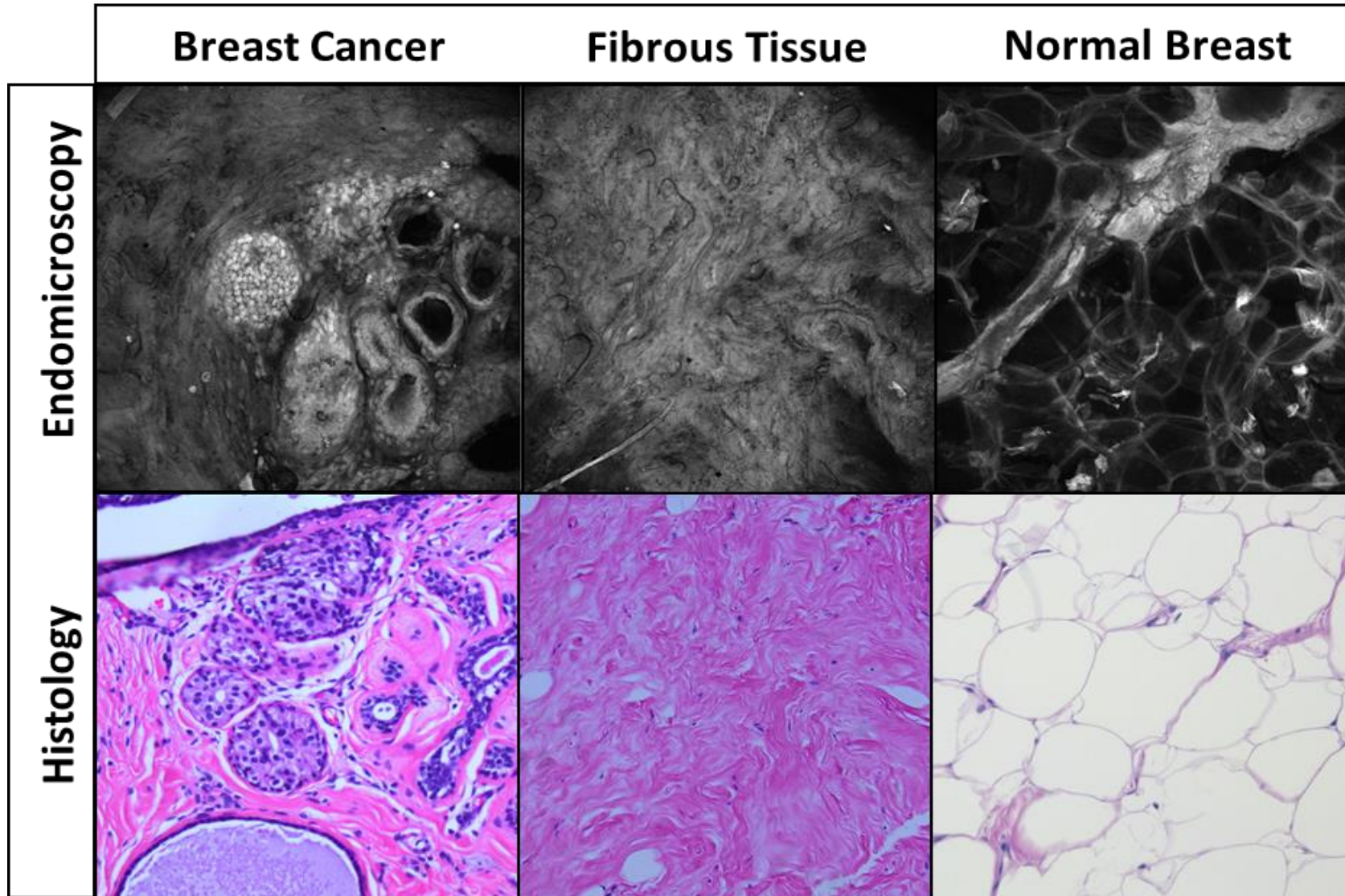


Progress to date on Breast Cancer Trial

Today



Breast Cancer Trial (Stage 1)

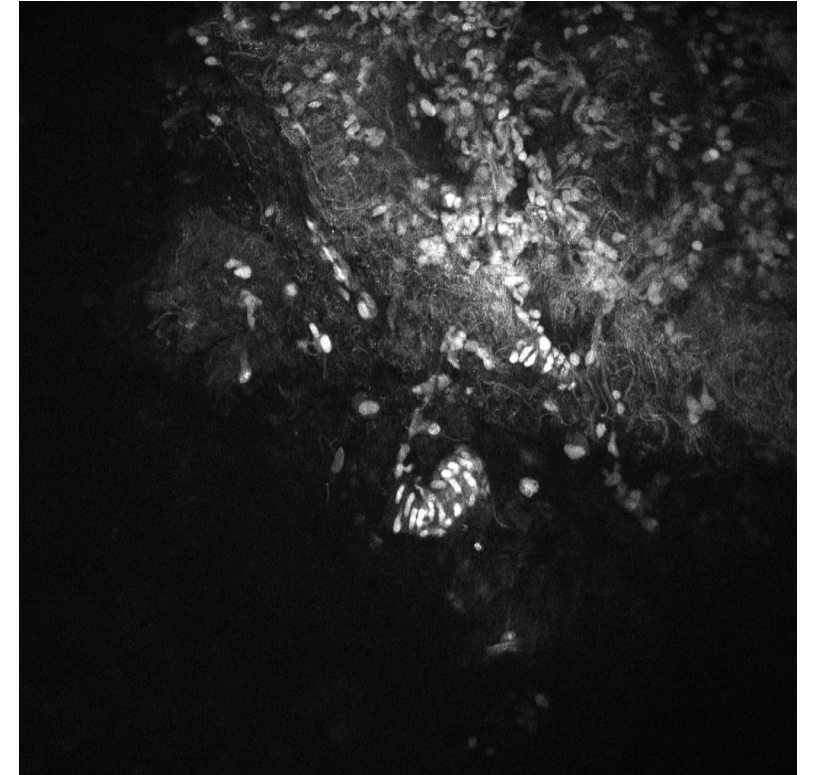
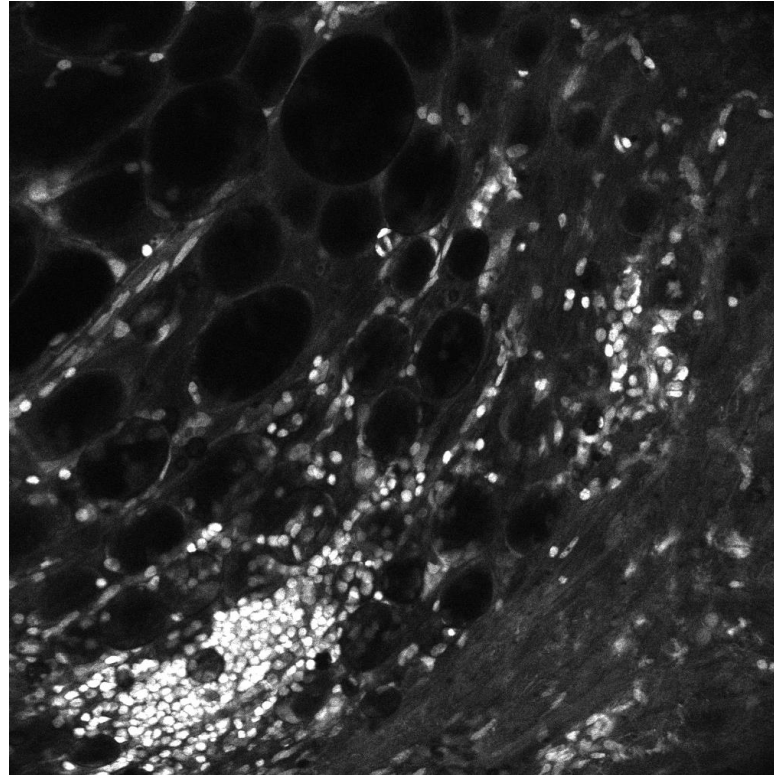
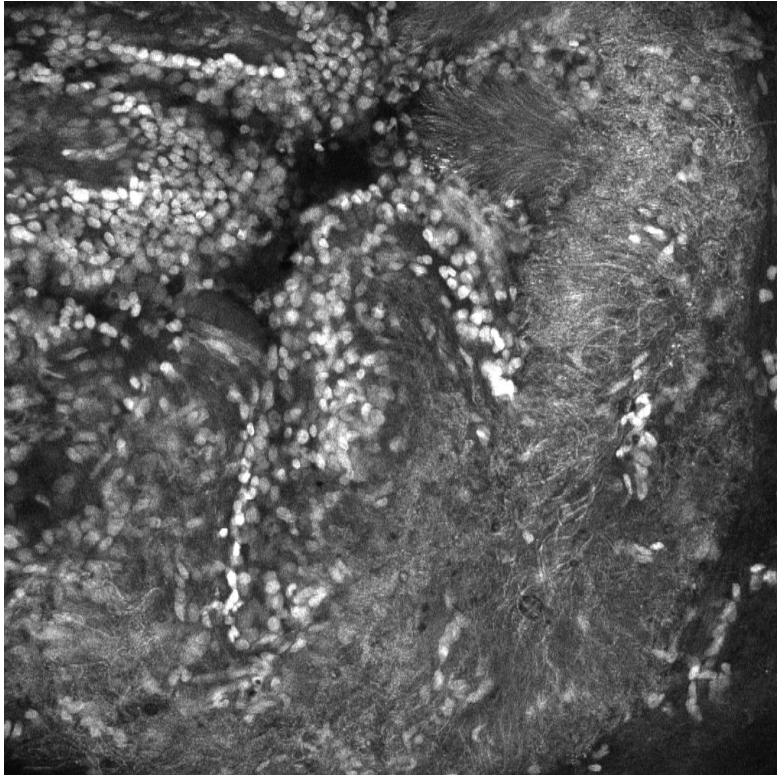


Ex vivo CLE images show clear distinction between normal, fibrous and tumour, and excellent correlation with H&E histopathology.

Contrast agent used is 0.1% Acriflavine.

Courtesy of Dr Philip Currie

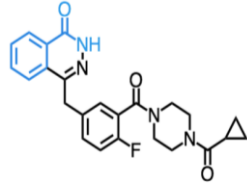
Breast Cancer (Stage 1)



Contrast agent Acriflavine 1mg/ml.

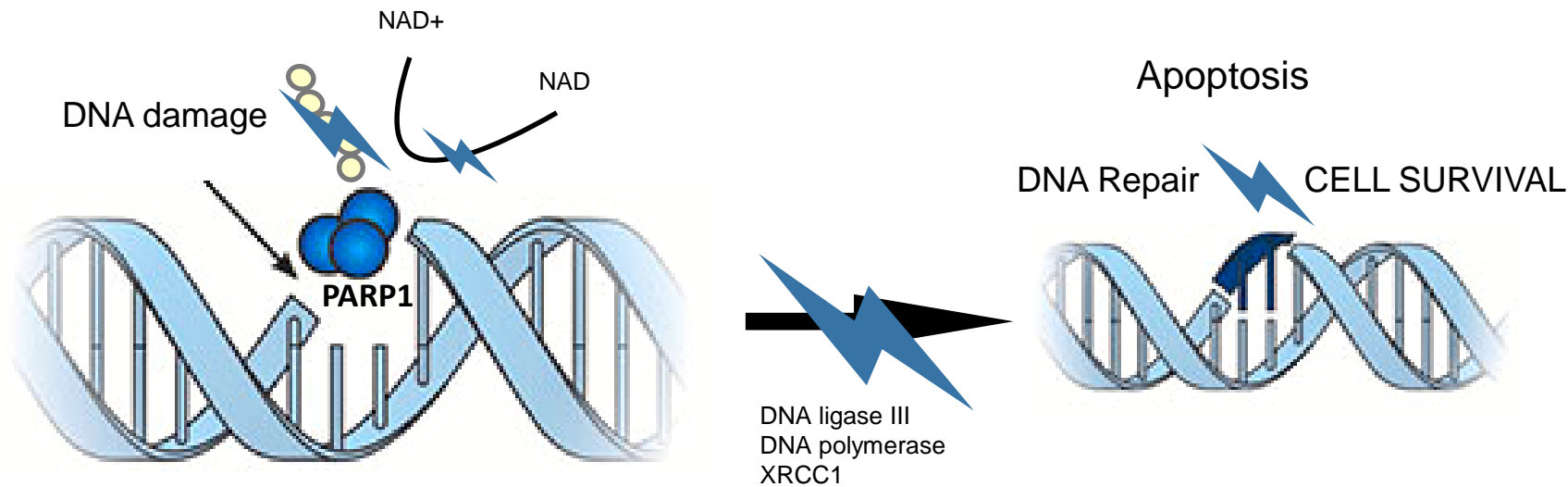
Images ex-vivo from mastectomy tissue, courtesy of Dr Philip Currie.

Tumour Labelling with PARPi-FL



Interested NOT in therapeutic
But in the expression for imaging

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

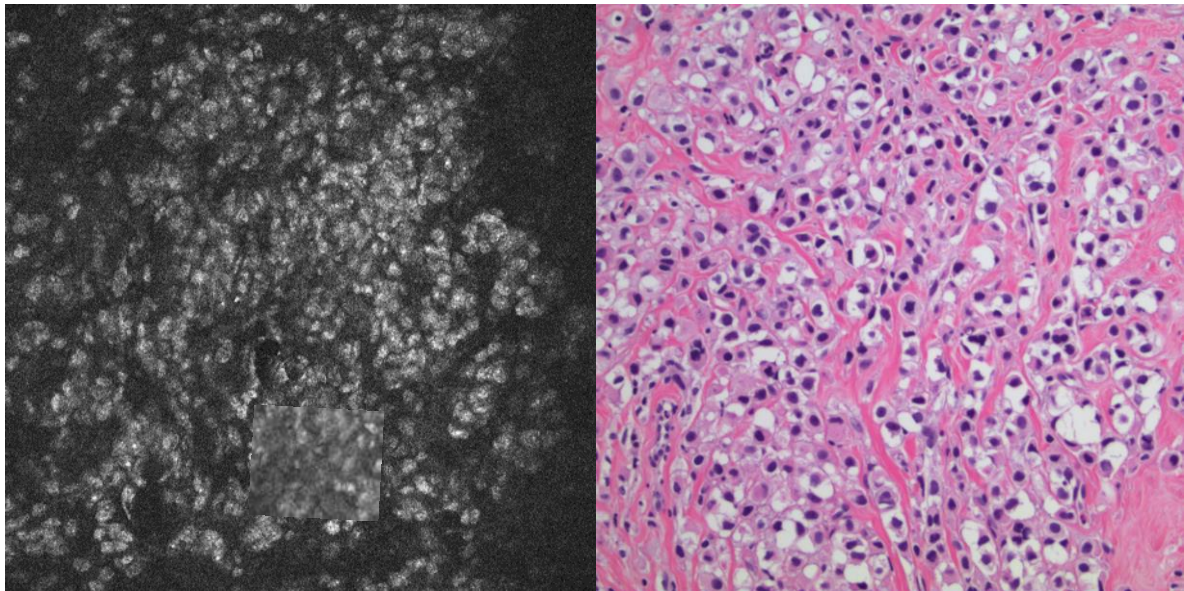


DNA Repair Pathway

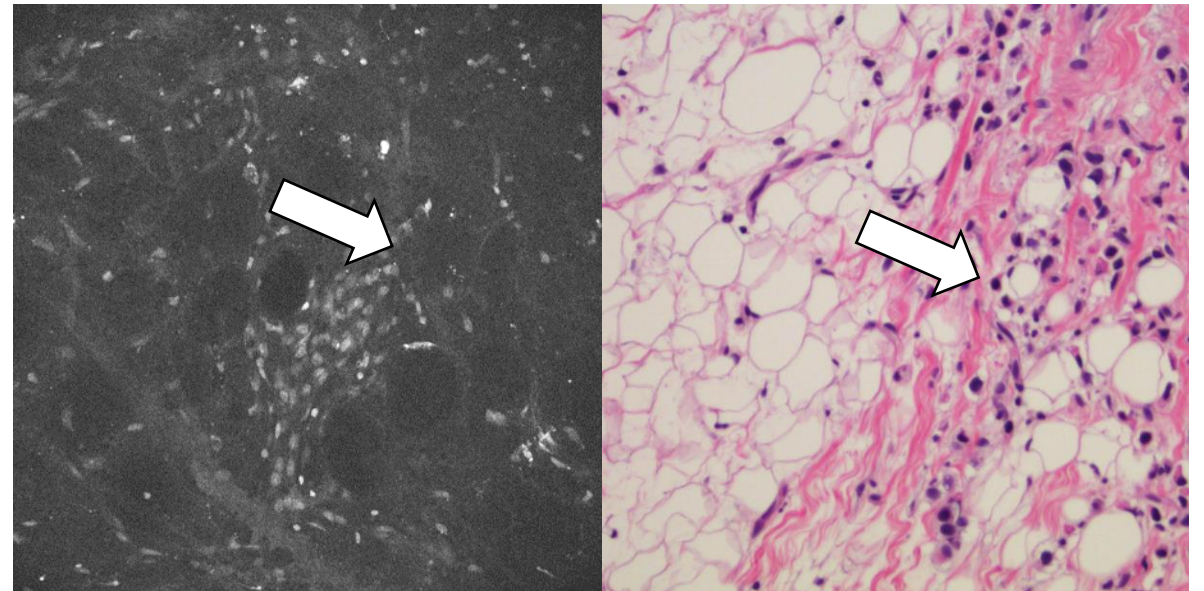
Courtesy Thomas Reiner Lab
Memorial Sloan Kettering Cancer Center
Summit Biomedical Imaging

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

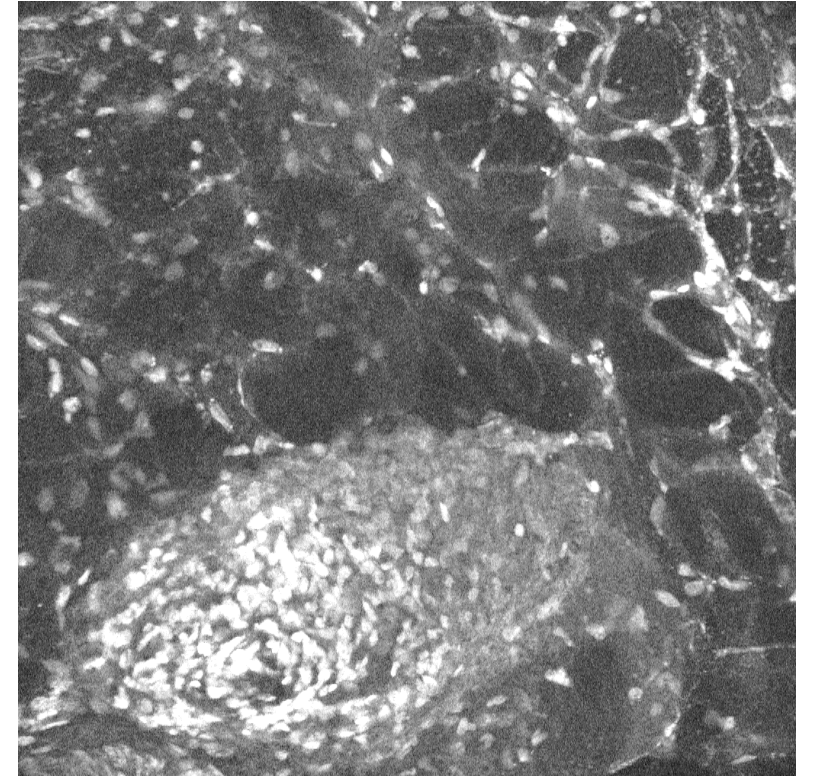
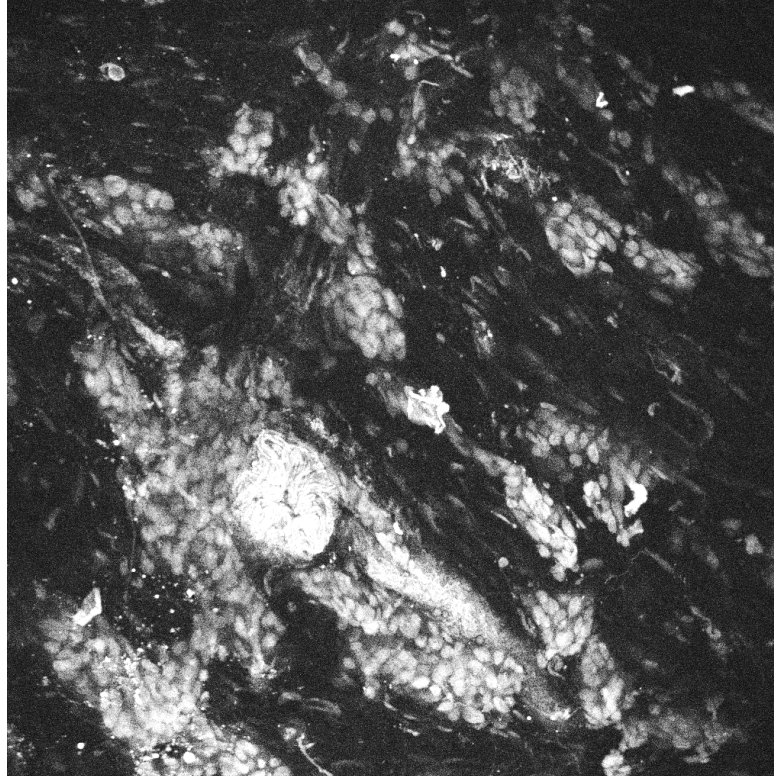
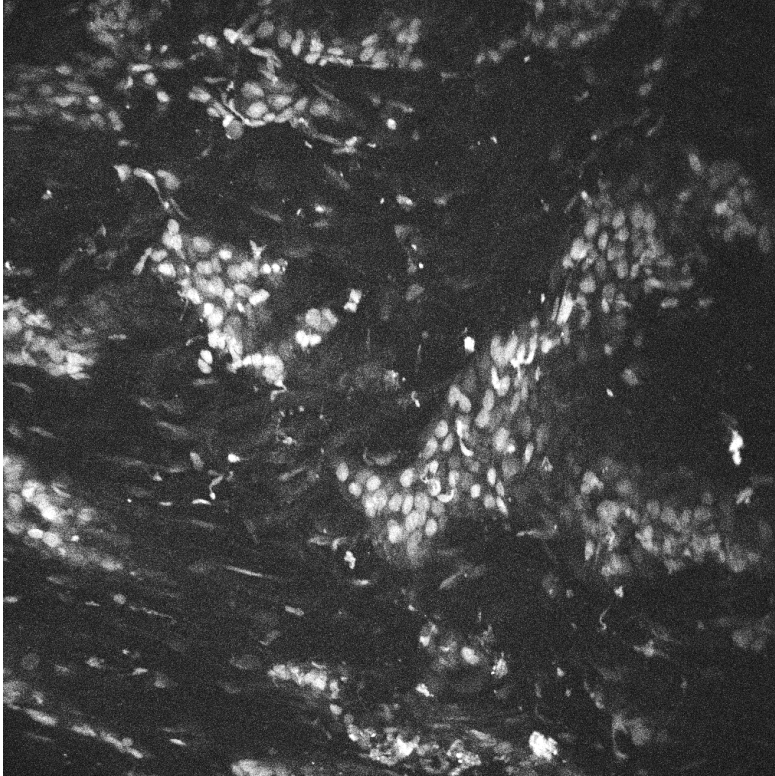


Matching CLE and H&E – Cancer cells throughout



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.

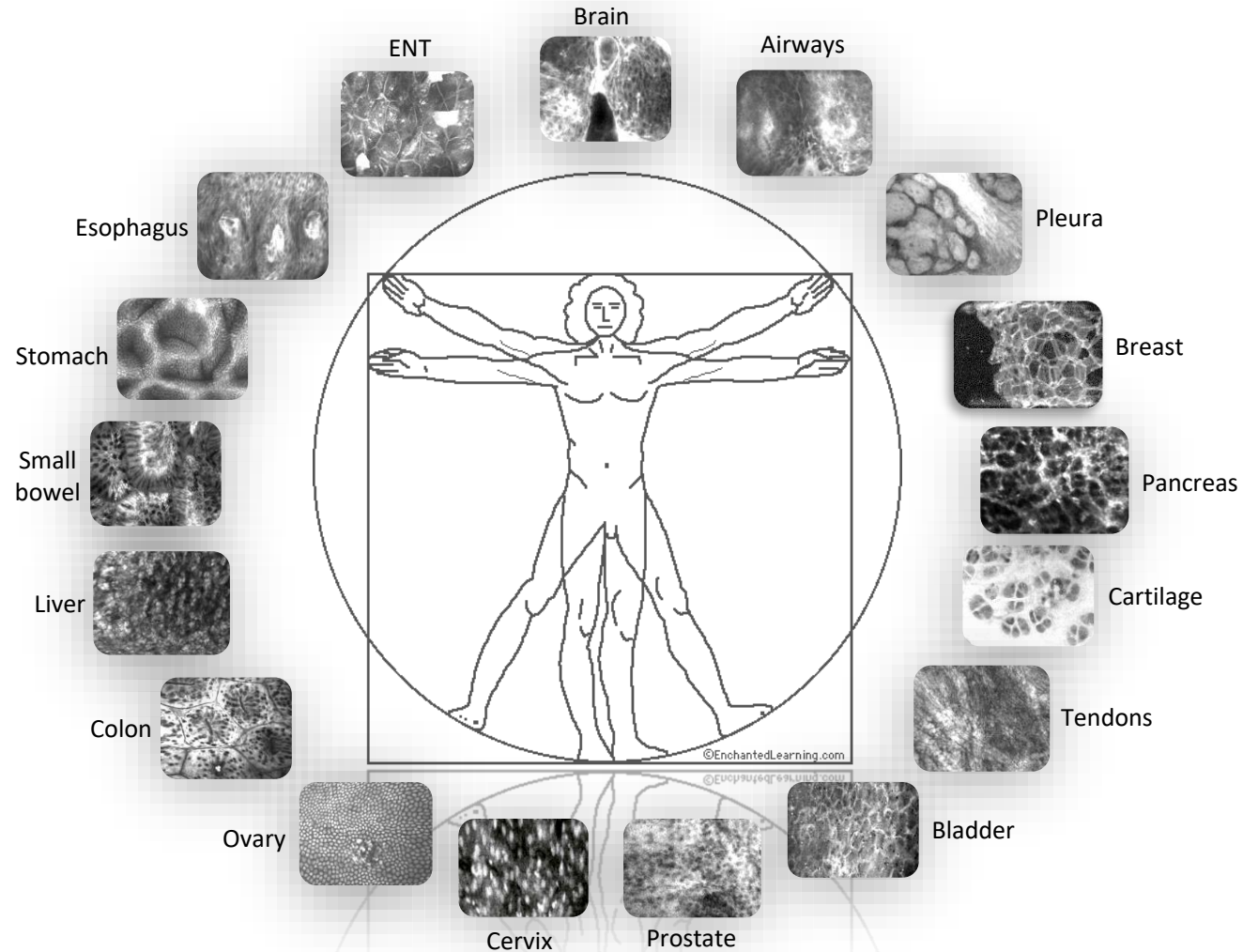
Images ex-vivo from mastectomy tissue, courtesy of Dr Philip Currie.

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

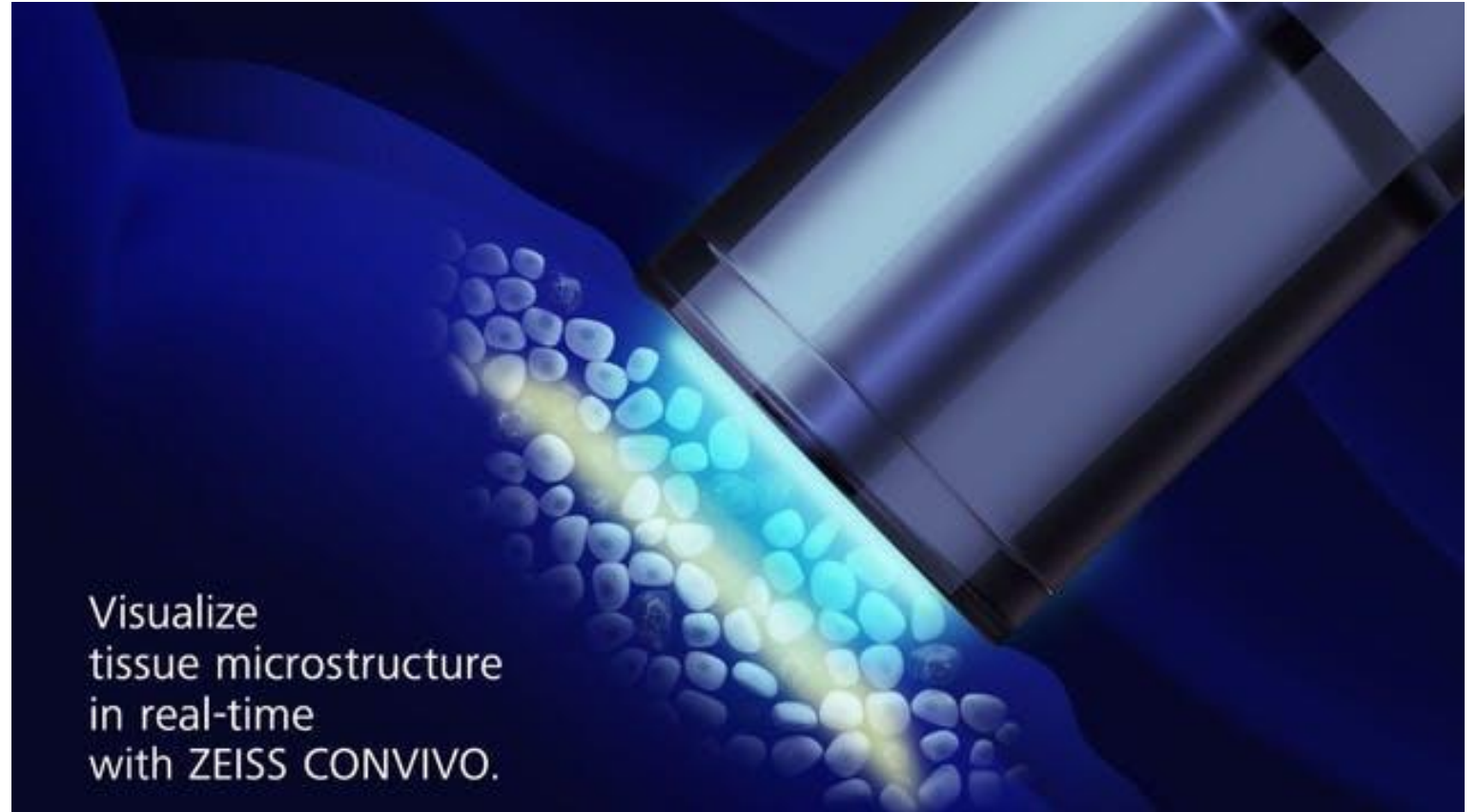
Optiscan Endomicroscope Clinical Devices

- Neurosurgery (Zeiss Convivo endomicroscope – (2nd generation scanner)
- GI (Pentax ISC-1000 gastroscope/colonoscope – (1st generation scanner)

Other Clinical Research Projects

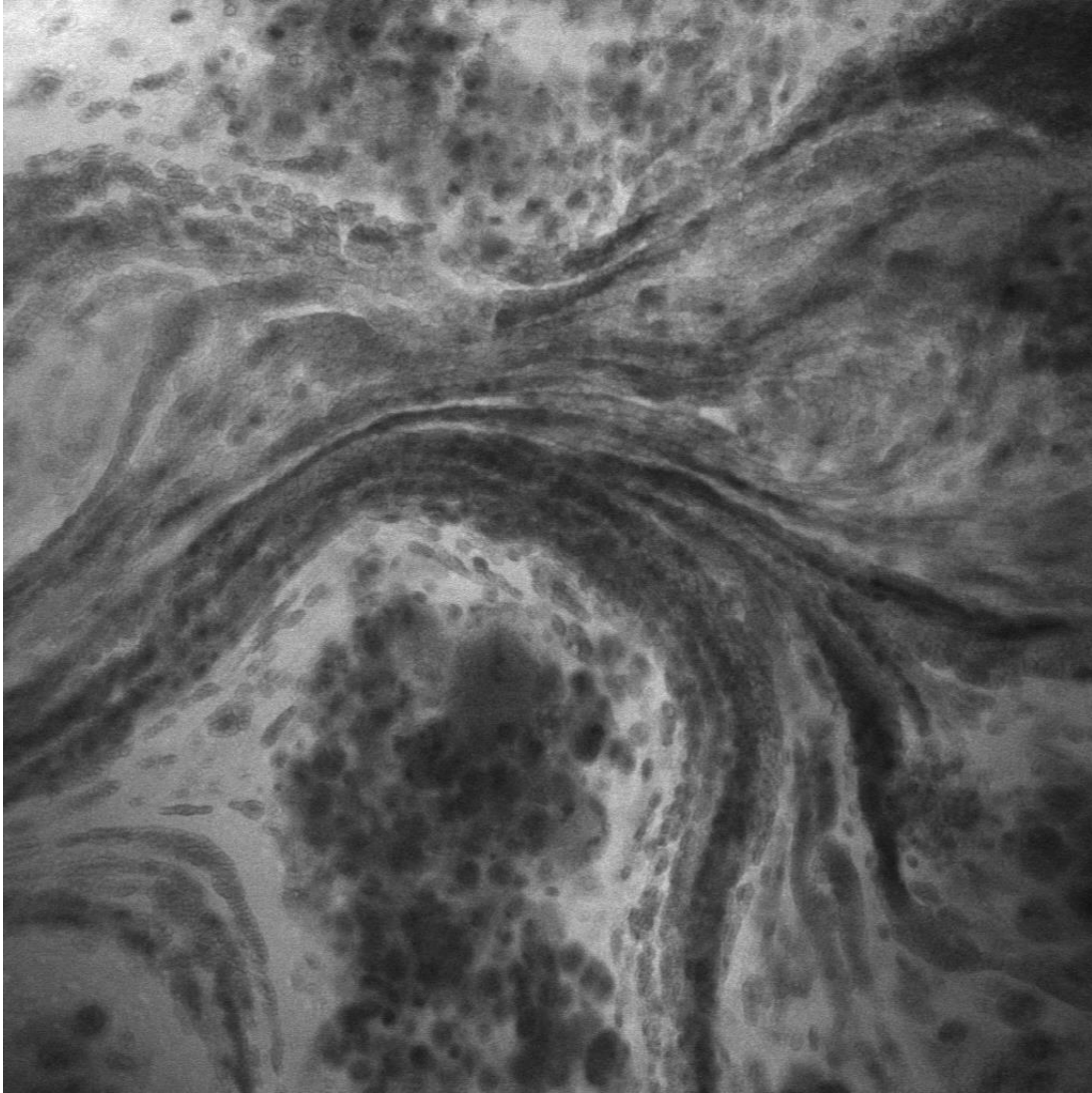
- Cancer detection and margin identification in mouth, cervix, oesophagus,

Carl Zeiss Meditec Collaboration



Optiscan Endomicroscopes are integrated into Zeiss Convivo for use in tumour margin identification during neurosurgery.

Rat Brain – Glioblastoma

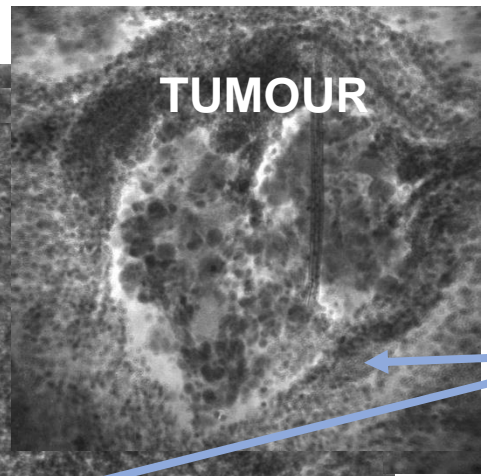


Tumour islands surrounded by leaky blood vessels in live rat brain

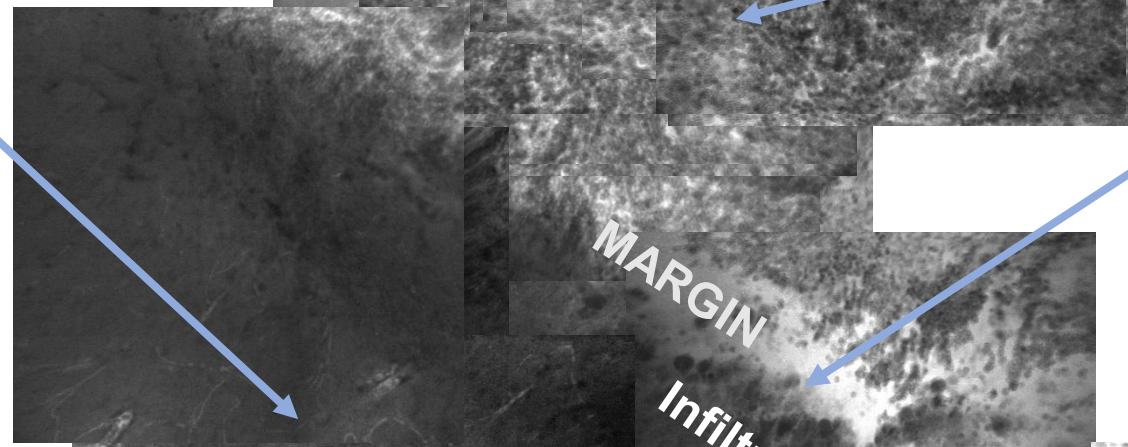
The tumour is a Glioblastoma (Causes hemorrhage, highly infiltrative). The contrast agent here is IV fluorescein

Courtesy of researchers in Barrow
Neurological Institute, Phoenix, Arizona,
USA

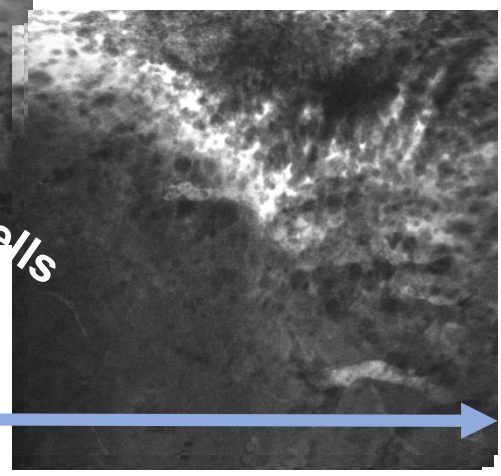
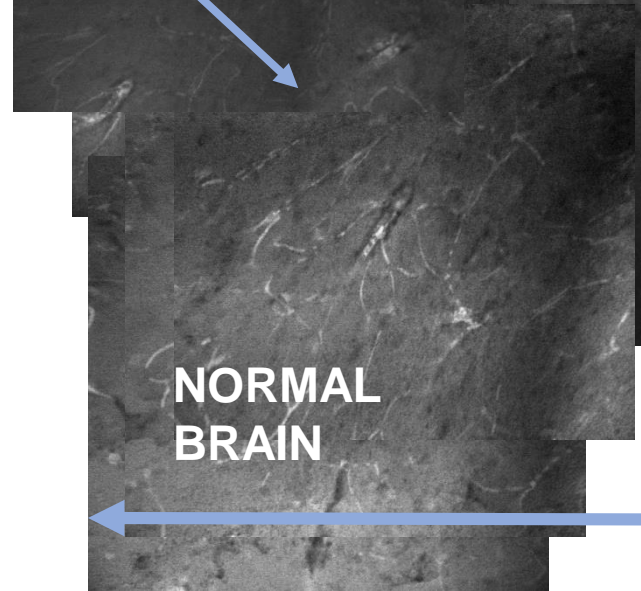
Tumour: Glioblastoma.
Montage of images as the surgeon moves the Optiscan probe over a small region of brain and tumour. The Grey tissue at lower left is normal brain and regular fine microvessels (capillaries) can be seen as clear white lines throughout.



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.



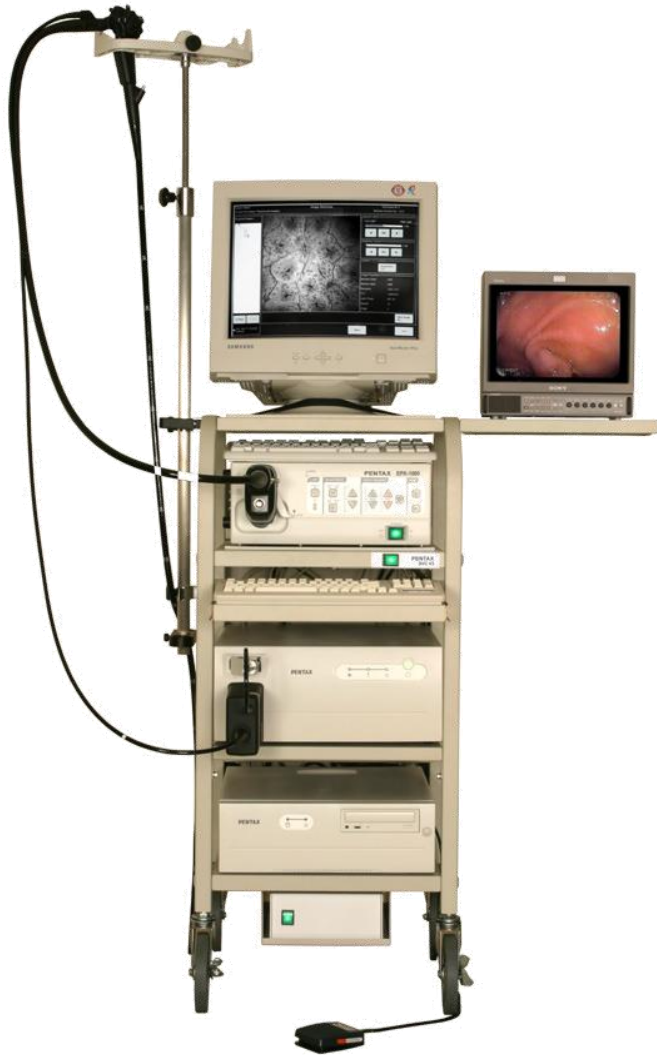
Between these two areas lies a "rift" between the tumour margin and normal tissue. However, characteristic larger round tumour cells are also seen infiltrating the border of the normal brain tissue.



1.5mm



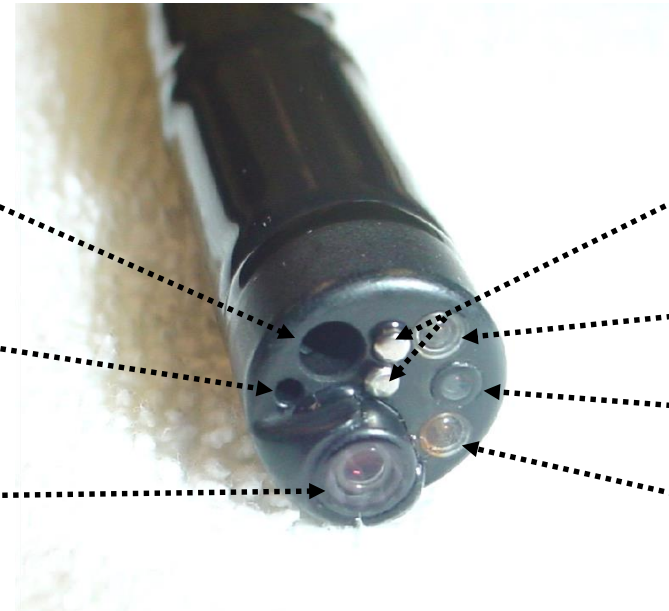
Pentax ISC-1000 Endomicroscopy system



Biopsy channel
Ø 2.8 mm

Auxiliary
water jet
channel

Confocal
imaging
window



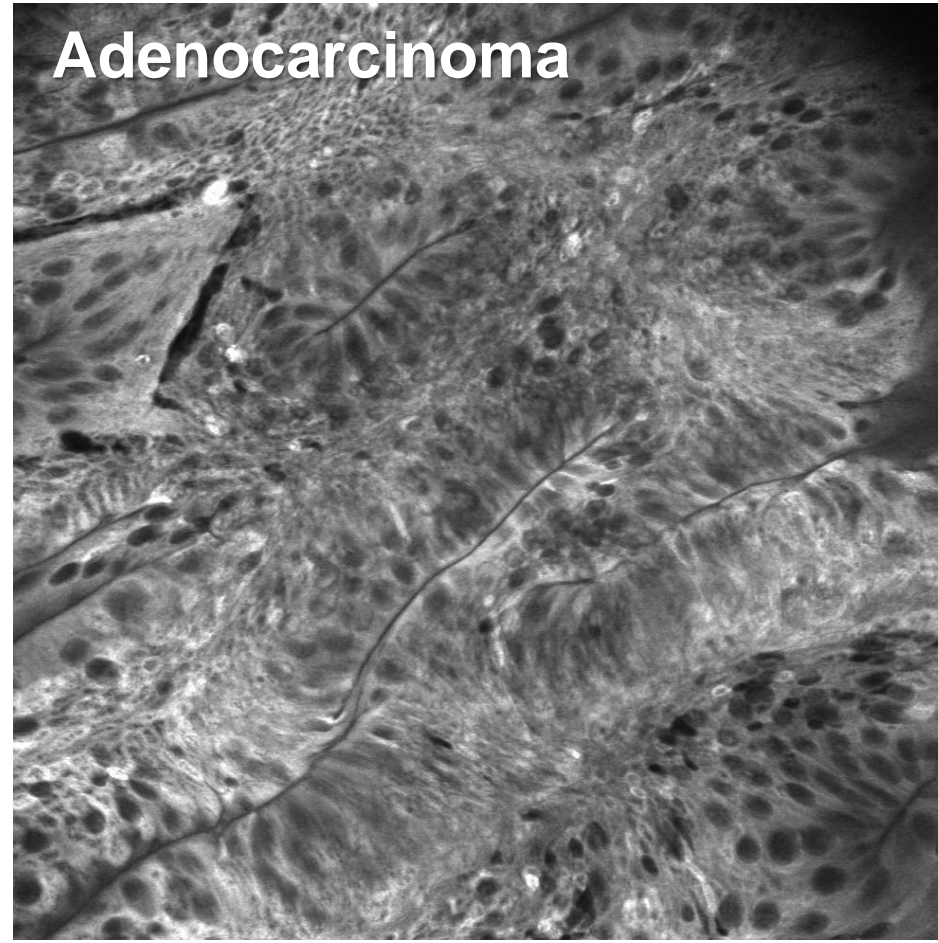
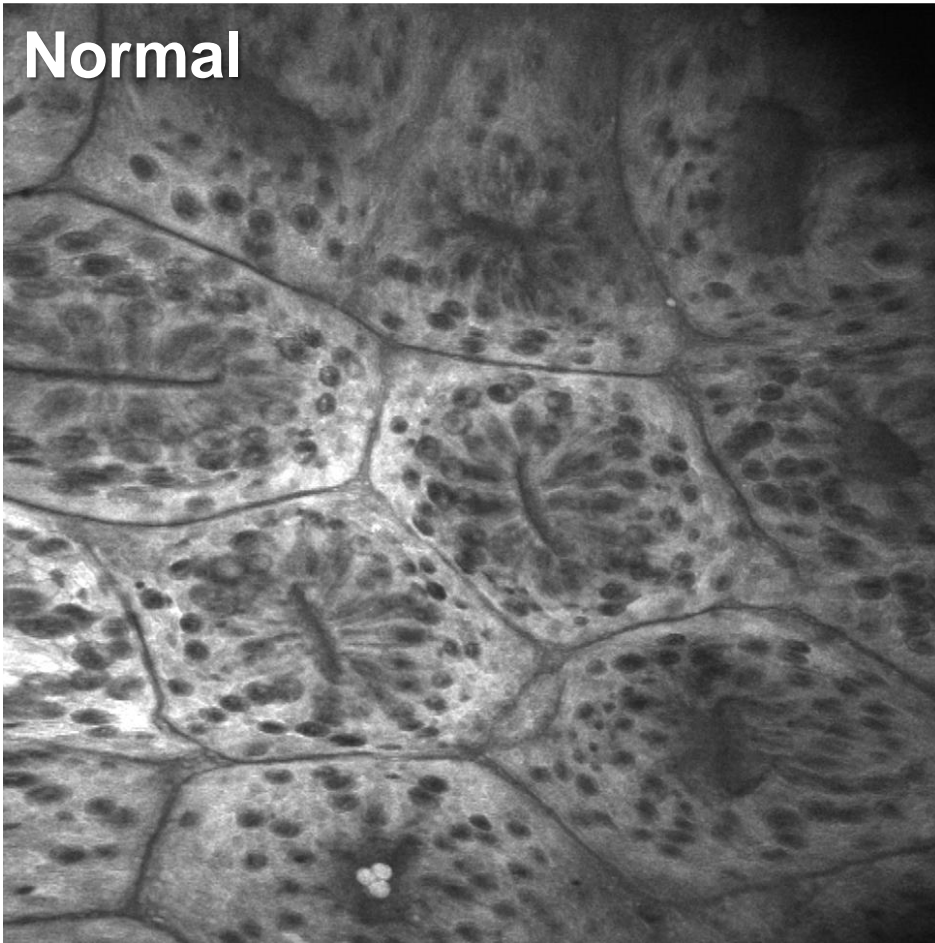
Air and
water jets

Light
guide

CCD

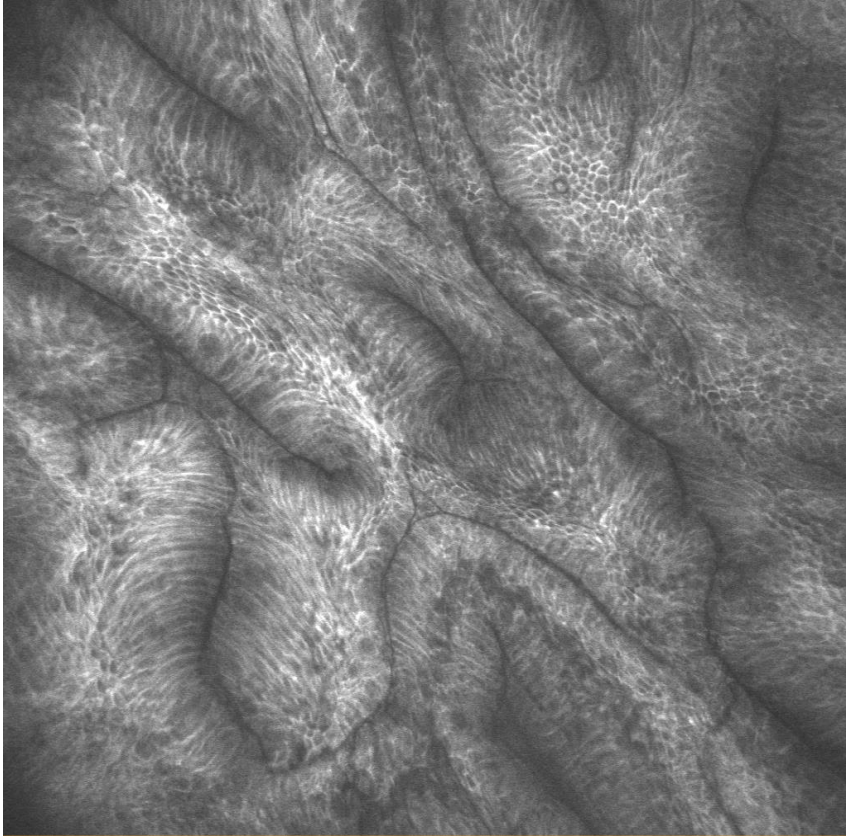
Light guide

Human Colon

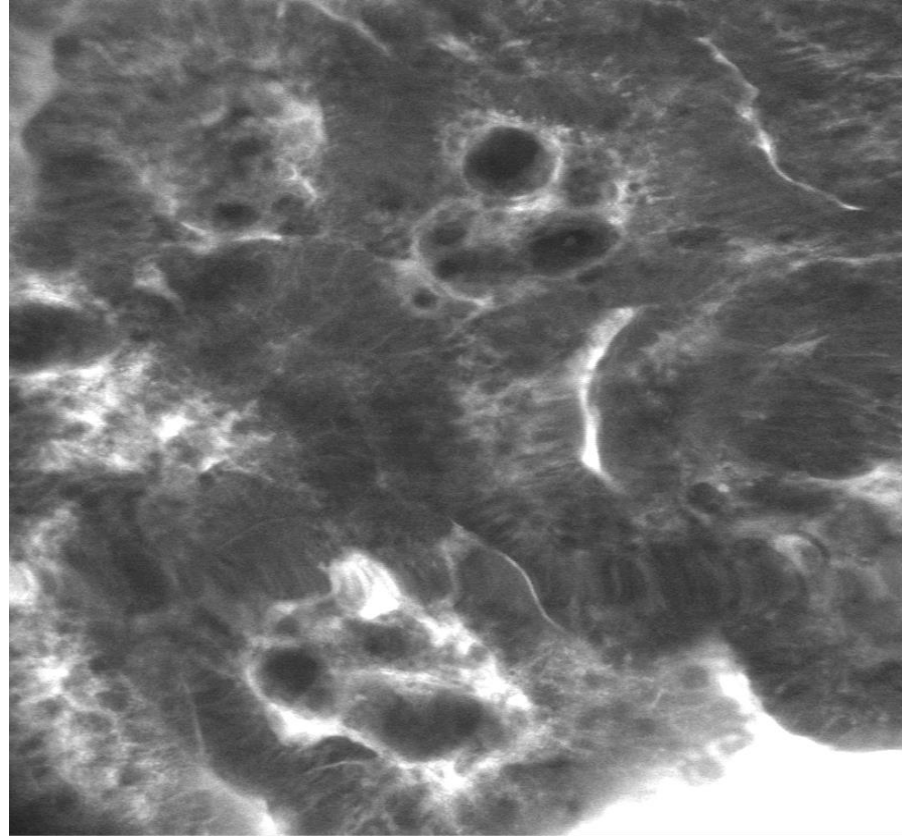


Images courtesy of Dr Ralf Kiesslich, University Hospital, Mainz, Germany and Professor Adrian Polglase, Cabrini Hospital, Melbourne, Australia

Barrett's Oesophagus and Barrett's Cancer



Normal Barrett's Oesophagus

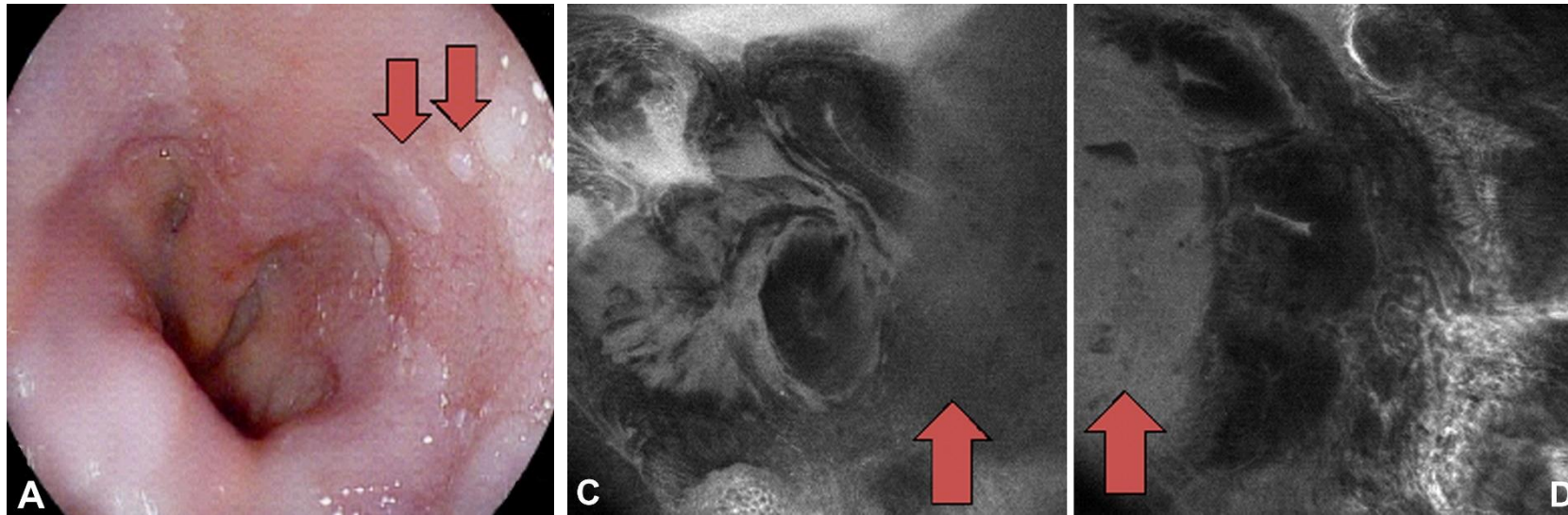


Barrett's carcinoma (early cancer)

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.



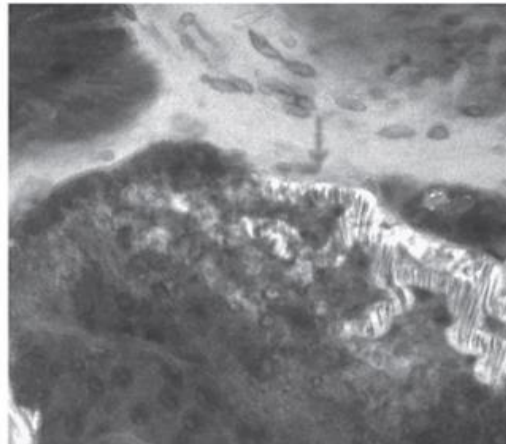
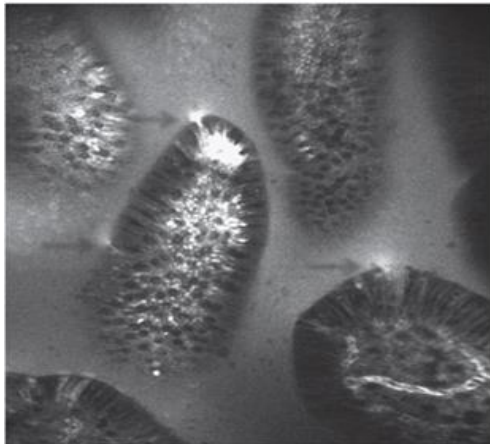
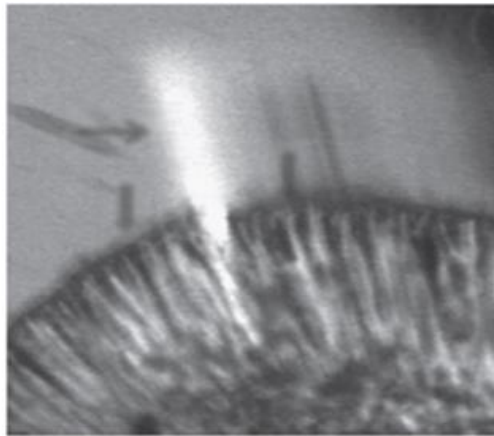
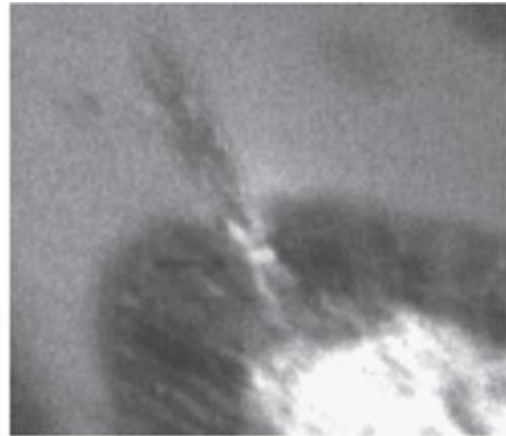
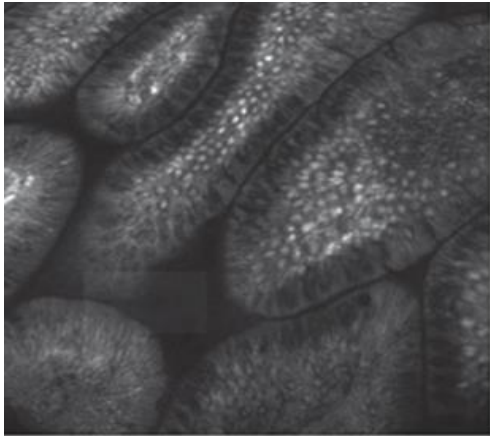
White squamous islands spotted by white light endoscopy (A). CLE shows intestinal metaplastic glands with loss of normal mucosal pattern, darkening of epithelial cells, lack of goblet cells, glandular distortion, and a cribriform pattern, consistent with high-grade dysplasia (C,D).

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



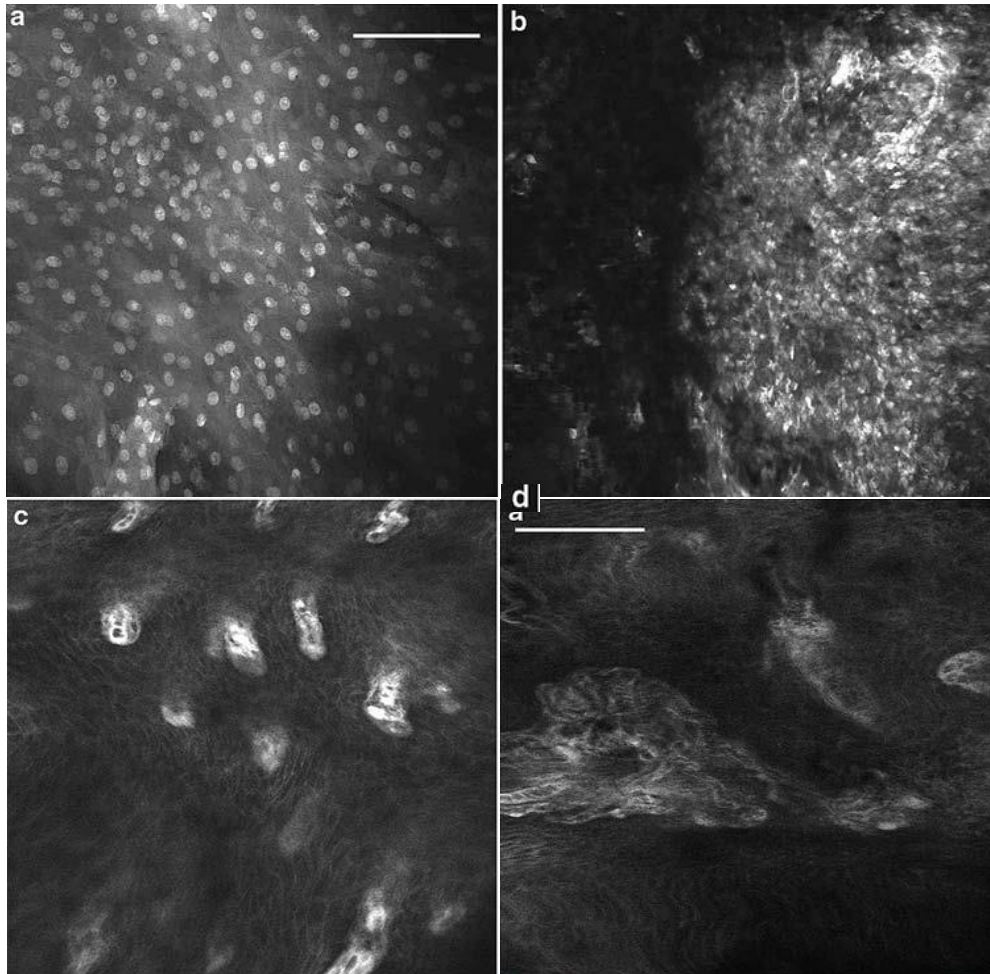
Loss of barrier function during mucosal cell shedding in human small intestine visualised by confocal endomicroscopy.

Kiesslich et.al. Gut. (2012)

Institutions

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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
different sizes of nuclei

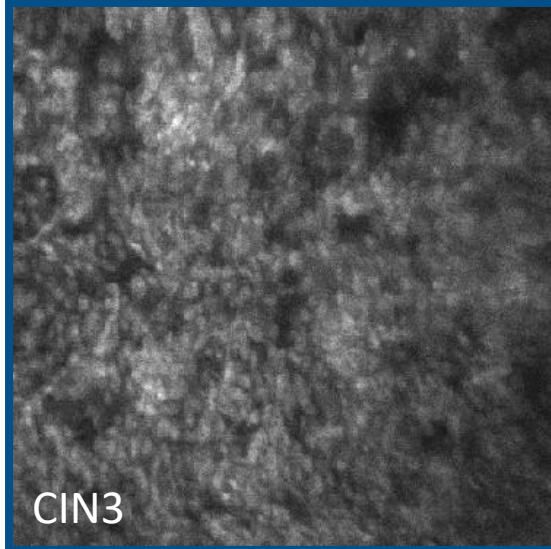
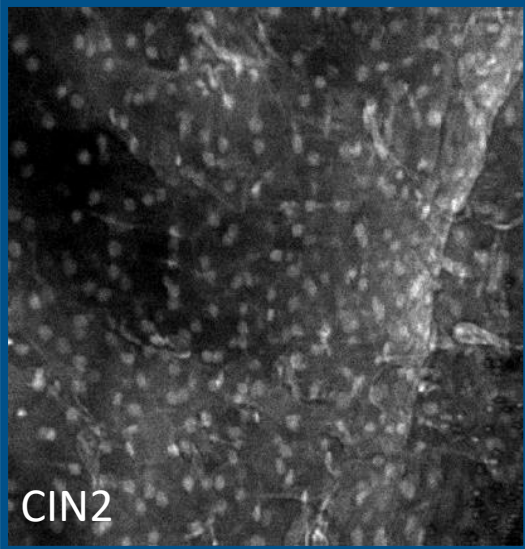
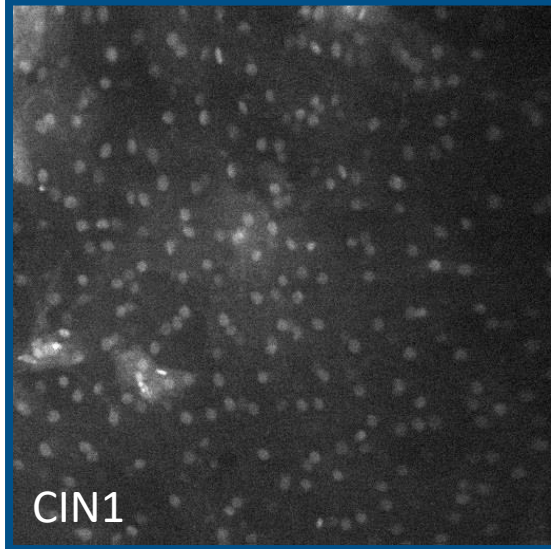
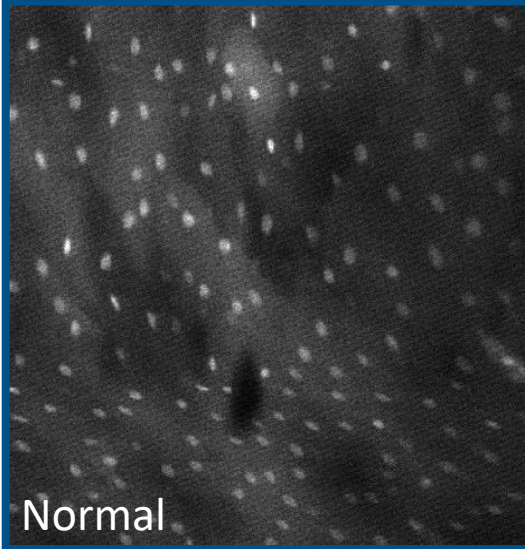
After intravenous fluorescein sodium

(c) Normal mucosa (imaging plane depth 50um.

(d) an invasive carcinoma of the floor of the mouth
irregular cell patterns
leaking of contrast agent

Haxel, B. R., et al., *European Archives of Oto-Rhino-Laryngology*, 2010; 267(3), 443-448.

Normal Squamous Epithelium & CIN

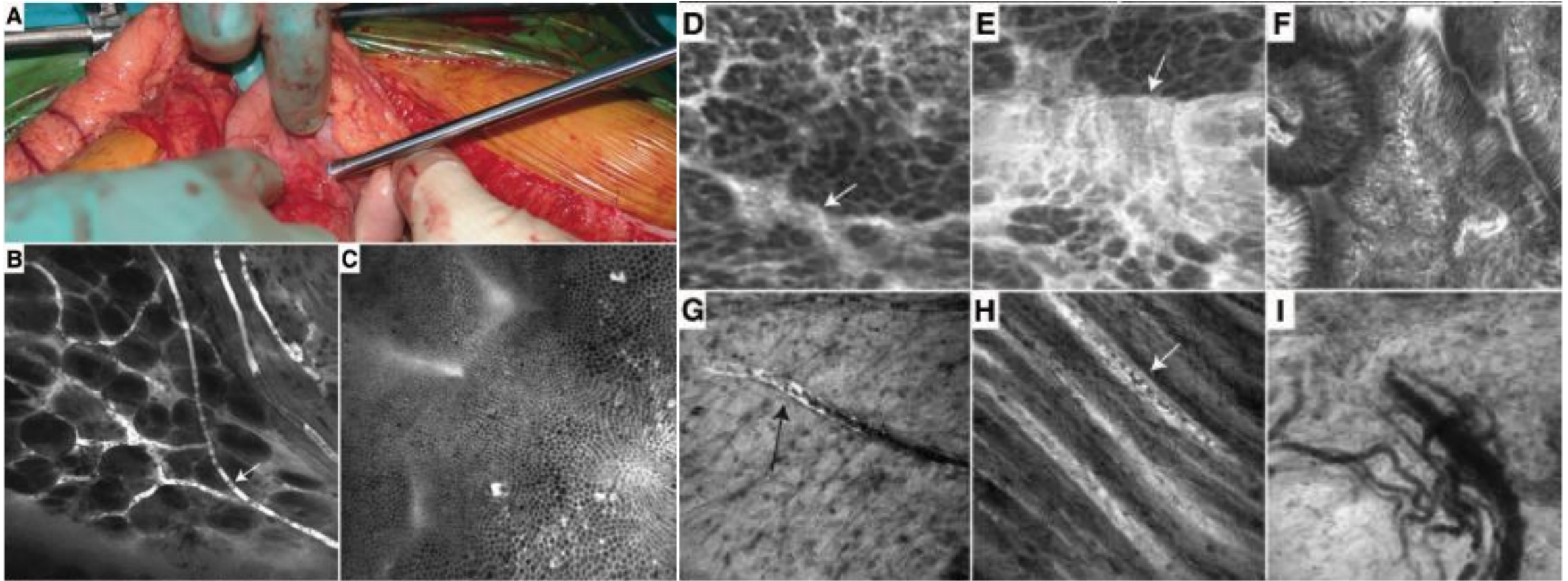


Contrast agent: Topical Acriflavine solution (0.05%);

FOV = 400 μm

Detection of cervical intraepithelial neoplasia in vivo using confocal endomicroscopy. Tan *et al*, BJOG 2009;116:1663–1670.

Real Time Intraoperative Confocal Laser Microscopy-Guided Surgery



Assessment of the potential utility of in vivo histologic surface and subsurface imaging in real-time using the Optiscan confocal laser microscope to detect diseased tissue at the time of surgery. A) intraoperative confocal microscopy B) Omentum C) bile duct epithelium D) normal pancreas E) Pancreatitis F) Small intestine mucosa G) small intestine serosa surface H) deeper blood vessels in serosa I) tortuous subserosal vessels associated with small intestinal lymphoma. [Nguyen et al., Ann Surg 2009;249: 735–737](#)

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