Using Histoindex Genesis® 200 imaging technology to analyse the Pattern of ECM Deposition in Human Breast Cancer Tissue
Introduction to technology

**High Resolution**
Detects fine collagen fibers up to 0.1 µm

**2D & 3D Scanning**
Realistic and 3D scanning visualisation of cellular and ECM architecture

**Stain-Free**
Eliminates staining artefacts & shortened sample preparation time

**Reproducible Staging**
Eliminates inter- and intra-observer discrepancy

**Quantifiable Data**
Provides fine measurements of disease progression and regression
Background

• The ECM consists of structural proteins, such as collagens, elastins, laminins, and fibronectins which transduce important signals to direct growth, survival, migration and differentiation of cells (Frantz, Stewart and Weaver, 2010). The hallmarks of cancer, initially described in 2000 (Hanahan and Weinberg, 2000), result from alterations to these processes.

• Mammographic density, which is characterised by increased collagen type I, is associated with higher risk of breast cancer (Boyd et al, 2013). However, the underlying mechanisms are still unclear.

• A malignant tumour usually presents as a hard lump which is thought to be related to remodelling and stiffening of the ECM. It is possible that these alterations contribute to enhanced cell growth and survival as well as increase cell tension which alters morphogenesis of tissue and promotes metastasis (Butcher et al., 2009).

• Despite identification of biomarkers (Her2/ER) and screening efforts, there is still a number of breast cancers that are not easily detected using such methods. Therefore, The development of novel diagnostic approaches, such as imaging methods to improve detection of primary tumours and their characteristics is imperative.

Hypothesis

There would be measurable differences in collagen type I and III of the ECM in normal and tumour associated stroma.
Method

3 Regions of Interest (ROI) selected

Slides Scanned using Genesis® 200

Digital Image of ROI produced

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<th>B</th>
<th>C</th>
<th>D intensity-based Area</th>
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Comparison of SHG/TPE and H&E Images

BB167933 Normal

BB167933 Tumour

BB167932 Normal

BB167932 Tumour
Quantification of Collagen in Tissue (FibroIndex™)

Percent of Tissue Occupied by Collagen (Average of 3 ROI)

Average Amount of Collagen in Tissue (%)

Sample ID

Normal Tissue
Malignant Tissue
Analysis of Collagen Fibers (FibroIndex™)

**Thickness of Collagen Fibers (Average of 3 ROI)**

- Normal Tissue
- Malignant Tissue

**Density of Collagen Fibers (Average of 3 ROI)**

- Normal Tissue
- Malignant Tissue

**Collagen Cross-linking Ratio (Average of 3 ROI)**

- Normal Tissue
- Malignant Tissue

**Collagen Reticulation Index**
Overall Conclusions

• The images produced using multiphoton imaging highlight a reduced signal from collagen in the tumour tissue compared to the normal tissue. This could be due to a possible alteration of collagen structure, as SHG is sensitive to the crystalline triple-helix structure of collagen for it to auto-fluoresce.

• Quantification of the amount of collagen within the tissue showed that the collagen in ECM of normal tissue is comparable to and possibly more than the tumour associated ECM. Quantification of the structure of collagen fibers (Density, thickness and cross-linking status) did not yield significantly different results between all of the samples.

• The direct measurement of collagen Type I and III deposition in the ECM of normal and tumour associated stroma is too simple to characterise the differences of stromal composition in normal and malignant breast tissue.

• It is expected that future research using multiphoton imaging will further contribute to the understanding of the pattern of ECM deposition in malignant tissue and assist in the discovery of other important characteristics of the stroma in normal and malignant tissue.
To Further this Research

• Compare intra- and inter-lobular collagen deposition as these results illustrate an apparent inter-lobular stroma which is collagen rich whereas the intra-lobular stroma is collagen poor.

• Categorise into subtypes of breast cancer (10 slides per subtype) to generate more reliable results.

• More work focusing on other stromal components would be beneficial, such as other types of collagen and elastins or integrins could possibly better characterise the differences in normal and tumour associated stroma.
Thank you
References


