Novel IR spectroscopic imaging for the elucidation of disease specific changes in breast calcifications

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Introduction

In 2014, breast cancer was the second most common cause of death from cancer in the UK. Microcalcifications are the unique early marker of breast cancer. However, the relationship between their occurrence and malignancy is still unknown. Vibrational spectroscopy techniques are label-free and chemically specific. They have been used to characterize these calcifications based on their composition, e.g. content of phosphates, proteins and carbonates. IR imaging provides detail information about their distribution and amount in breast tissue.

Aims of the PhD project

1- Characterise standard minerals and their spectral signatures in breast calcifications (hydroxyapatite, calcium oxalate) by Attenuated Total Reflection (ATR) FTIR spectroscopy and Raman micro-spectroscopy.

2- Determine the carbonate content and the carbonated calcium hydroxyapatite amount in breast tissue by using micro-FTIR imaging also in combination with novel technologies (upconversion and Quantum Cascade Laser (QCL)).

3- Investigate in vitro breast cancer cell lines developing calcifications using micro-FTIR imaging and Raman microscopy.

Materials and Methods

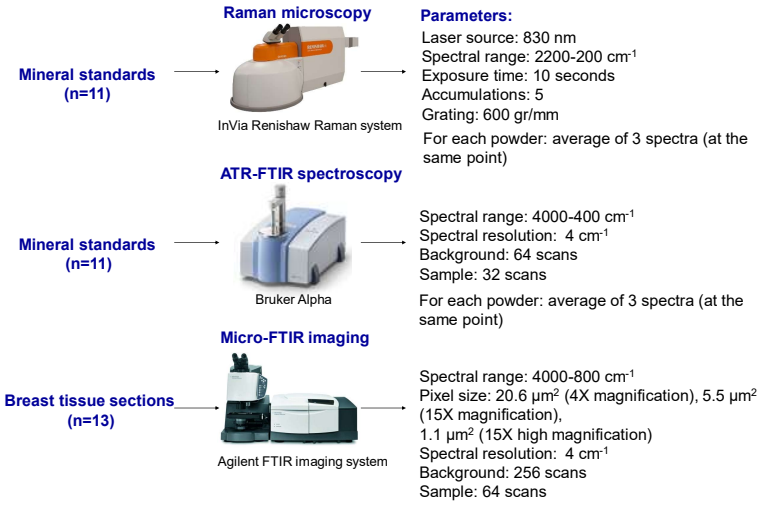

Figure 1: Details of the experimental protocol for the analysis of standards minerals and breast tissue biopsies by ATR-FTIR spectroscopy, micro-FTIR imaging and Raman micro-spectroscopy.

Results

I. Characterisation of standards minerals found in human body by Raman and ATR-FTIR spectroscopy

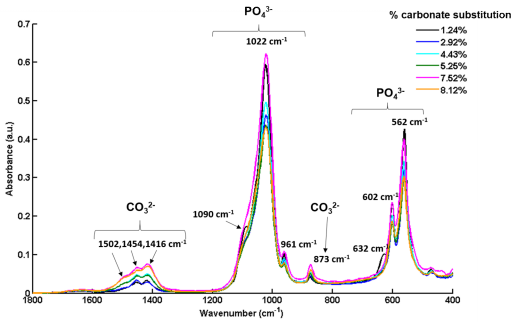

Figure 2: Representative ATR-FTIR spectra of Hydroxyapatite (Hap) with different percentage of carbonate substitution.

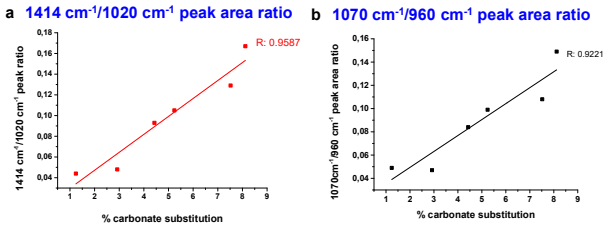

Figure 3: Intensity ratio between carbonate and phosphate bands for different carbonate substitution obtained from (a) ATR-FTIR spectra and (b) micro-Raman spectra.

Fig. 2 shows ATR-FTIR spectral signatures of carbonated Hap with carbonate bands (around 1514, 1454 and 1020 cm^{-1}) and phosphate bands (960 cm^{-1}). In Raman spectra, the carbonate and phosphate bands are found at 1070 and 960 cm^{-1}, respectively (data not shown). Fig. 3 shows a increase of the ratio in peak intensity (derived from fitting) between carbonate and phosphate bands when the carbonate percentage increases. The same values are found between ATR-FTIR and Raman data and a linear correlation is derived.

II. Study of breast biopsies with microcalcifications by micro-FTIR imaging

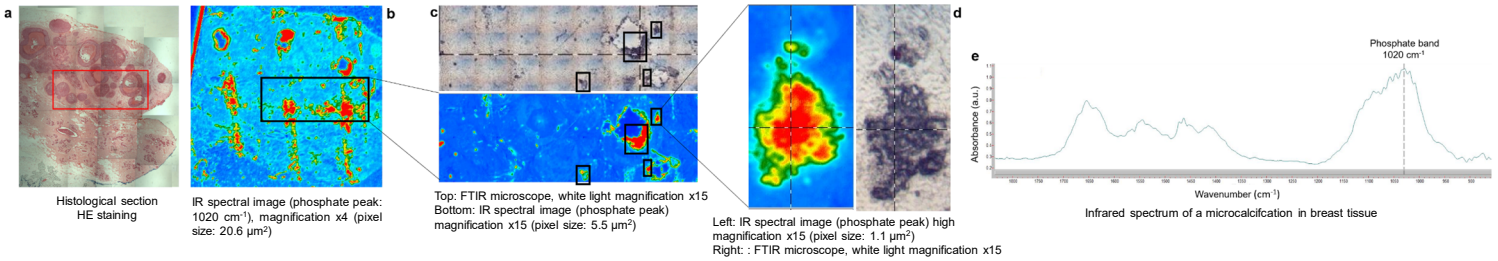

Figure 4 : Visible and micro-transmission FTIR images of a breast cancer biopsy. (a) Histological section stained with Hematoxyline Eosin. The FTIR images refer to the distribution of the phosphate peak (1020 cm^{-1}) obtained using (b) 4X magnification, (c) 15X magnification and (d) 15X high magnification. (e) FTIR spectrum extracted from a microcalcification in breast tissue (crossbar in figure 4d).

Fig. 4 shows the same morphological features between histological section with H&E staining (Fig.4a) and FTIR spectral images (Fig.4b, c, d). High magnification allows to focus on microcalcifications and to obtain their specific signatures, for instance the phosphate band at 1020 cm^{-1}(Fig.4e).

Conclusion and perspectives

- We obtained the same intensity ratio between carbonate and phosphate bands in ATR-FTIR spectra and Raman spectra of mineral standard.
- We found a linear correlation between intensity ratios and percentage of carbonate substitution.
- Future: - Determine the level of carbonate substitution in each breast biopsy microcalcifications and any correlation with pathology.
  - Explore novel IR technologies to provide more rapid analysis of calcifications in tissue.
  - Collaboration with the Royal College of Surgeon in Dublin (Dr. Maria Morgan)

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