Mid-IR Spectroscopy and UV/VIS Fluorescence Imaging of Breast Tissue

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Abstract

We studied instrinsic contrast agents for the differentiation of cancer and normal breast tissues; the methods used are Fourier Transform Infrared spectrometry (FTIR) and fluorescence imaging. FTIR, a standard transmission configuration was used to study the spectra of cancerous and normal tissue samples obtained in the mid-IR range of 815cm^{-1}-1715 cm^{-1}. We cut the tissues to 10µm in thickness using a cryostat and placed the slices on ZnSe before taking the spectra. We compared the spectra of both normal and cancerous tissues looking for variations in the intensity. This data will serve as an initial study for a Quantum Cascade Laser (QCL) imaging device for breast tissues. Fluorescence imaging is a technique that relies on the emission of light from a molecule after absorption of light with a shorter wavelength. The fluorescence images of the breast tissues were analyzed together with their corresponding FTIR spectra. The FTIR method provided us with contrast agents in the mid-IR, whilst the fluorescence method gave us instrinsic contrast agents in the visible wavelength range.

Introduction

Breast cancer is the second leading cause of death for women and the leading overall cause of cancer death in women between the ages of 20 and 59. In the United States, breast cancer is expected to be newly diagnosed every three minutes, and a woman will die from breast cancer every 13 minutes. Inflammatory breast cancer(IBC) is an aggressive type of breast cancer that has a 25% to 50% lower five year survival rate than normal breast cancer. IBC occurs in the superficial skin, thus is an ideal target disease for optical technologies that do not provide significant depth penetration. There is a immediate need for healthcare professionals and scientists to develop early detection sources for breast cancer. The purpose of our research is to lay the foundation towards developing a non-invasive imaging device that could be used to categorize breast cancer. We start by looking at the spectra of cancerous and non cancerous tissues.

Specific Aims

- To study FTIR spectra of formalin-fixed breast tissues and analyse the differences between spectra belonging to the normal and cancer histopathological groups.
- Study and deduce from the FTIR spectra and fluorescence images the biological components that can act as intrinsic contrast agents for the differentiation of cancer and normal tissues.

Spectra Collection and Analysis

Methodology:

- Thaw frozen tissue and embed in OCT media
- Cut sections in cryostat at ~-20c
- 10um microtome sections
- Air dry our samples for about 5 minutes
- Place tissue in ZnSe crystal and mount in sample holder
- Purge sample chamber with nitrogen
- Collect 128 scans at a resolution ~4 cm-1 using a DTGS detector

Data set:

Cancer: 17 sites

Normal: 14 sites

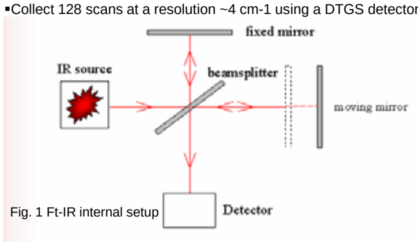

Fig. 1 Ft-IR internal setup

Fig.2 Fluorescent imaging device setup

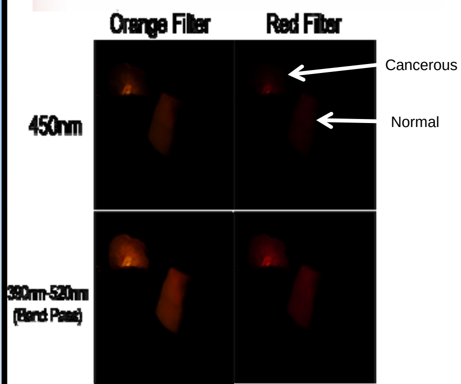

Fig. 3 Fluorescence images of cancerous and normal tissues.Top: image at excittion of 450nm. Bottom: Bandpass excitation

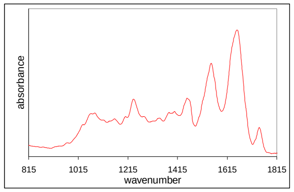

Fig.4 FT-IR Spectra of Normal Tissue

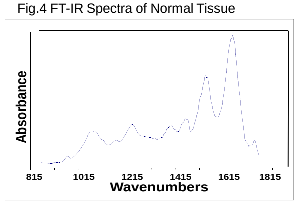

Fig. 5 FT-IR Spectra of Cancerous Tissue

Fig.6. Mean intensity ratios of some different bands throughout the fingerprint region in the mid-IR are compared for cancer and normal groups.

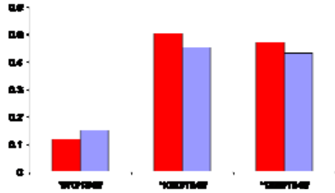

Table. 1 Table of Absorption band with corresponding biomolecules

Absorption band (cm-1)	Corresponding biomolecules and their functional groups
1650	Amide I band arises from in plane C=O stretching vibration weakly coupled with C-N stretching and in plane N-H bending of the amide
1548	Amide II band which arises from the N-H bending vibration strongly coupled to the C-N stretching vibration of protein
1400	Symmetric and asymmetric CH_3 bending modes respectively of the methyl groups of proteins
1338	Vibrations of collagen
1240	Asymmetric phosphate (PO^-_2) stretching modes (νas PO^-_2) of nucleic acids and the amide III/CH_2 wagging vibrations of collagen
1080	Symmetric phosphate PO^-_2 stretching modes (νsPO^-_2) of nucleic acid and the vibrational modes of collagen carbohydrate residues
970	O-P–O antisymmetric stretching mode of DNA or to a phosphate monoester band of phosphorylated proteins and nucleic acids

Conclusion

We have examined the FTIR spectra of formalin-fixed frozen tissue sections from the breast, and shown that there is significant variation between the spectra of normal and cancerous tissues throughout the fingerprint region. We have presented an initial study of the differences between the spectra of normal and cancerous tissues of the breast. We have shown that some major biological components intrinsically present in tissues can be assessed by IR spectroscopy. There are a lot more of these components in the fingerprint region of the mid-IR, but as a complementary procedure we also studied additional components which are also accessible but via fluorescence imaging.

Acknowledgements and References

The authors gratefully acknowledge the support of the Mid-Infrared Technologies for Health and the Environment Center (MIRTHE)

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