Clinical Measurements of Refractive Index of Biological and Human Breast Tissue

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Abstract

Refractive index of biological tissue has been known to be a very good indication of tissue state. It can vary according to tissue hydration, calcification and age. This project aims to determine whether the refractive index of human breast tissue can be used as a diagnosing factor of cancer. Optical Coherence Tomography was used in an imaging technique to measure the refractive index of homogeneous and heterogeneous biological tissue and non-biological media using the 'displaced reflector' method. A key aspect of the process of measuring involved evaluating the accuracy of the testing system before measuring breast tissue. Results thus far have indicated that tissue handling procedures may need to be altered before breast tissue is measured

1.0 Introduction

The incidence of breast cancer is a source of concern for many women and their families. Worldwide, one in eight women will develop breast cancer at some point in their lives.

For women who undergo diagnostic testing following the discovery of a suspicious area in their breast, the conventional ensuing procedures are often painful and arduous. A mammogram that identifies the area of risk is often followed by a core needle biopsy. This biopsy effectively excises a portion of tissue from the breast that is suspicious. Histological tests are then carried out on excised tissue to assess its pathology.

At present there are several studies that have been conducted that highlight the need for high-resolution imaging of breast cancer sites. The need has arisen as results from studies of mastectomies have indicated that as many as 41% of patients who undergo excisions had additional areas of diseased tissue within 2cm of the tumour sites, areas which may have been able to be detected had there been better *in vivo* imaging and tissue differentiating capabilities. Approximately 40-50% of all solid tumours exhibit recurrence following surgical or radiation therapy. The reasons for the recurrence stem from the lack of adequate technology that can differentiate between normal and abnormal (containing nests of occult cancer) tissue surrounding the area of the tumour.

This project aims to evaluate the indicating strength of the refractive index of tissue as a diagnosing factor in differentiating between healthy and cancerous breast tissue.

2.0 Background

2.1 Optical Coherence Tomography

Optical coherence tomography (OCT) is a relatively new imaging technology that is able to image biological systems non-invasively, utilising the coherent properties of light. Somewhat analagous to conventional ultrasound imaging, OCT uses low coherence interferometry to acutely image the morphology of structures of size in the order of micrometers.

Having a penetration depth of only 2-3 mm in highly scattering structures, OCT is limited to superficial imaging of biological tissue such as skin, muscle or breast tissue. However, with a penetration depth extending to approximately 2-3 cm in translucent surfaces such as the eye, the use of optical coherence tomography has become commonplace in areas like opthalmia.

One of the main advantages of OCT is that the periphary of the device consists only of a dual optical fibre, as opposed to a large transducer such as in ultrasound equipment. This enables OCT technology to be incorporated in small devices such as catheters, endoscopes and/or potentially 'optical' biopsy needles to image tissue where access by conventional imaging methods is difficult.

2.2 Refractive Index Measurement of Biological Media

The refractive index (RI) of a medium refers to an index number given to the medium (usually larger than 1) commensurate with the optical delay that the medium imposes upon light that travels through it. Several reasons exist as to why refractive index is measured in biological tissue, the main one being that it has been suspected to vary with tumour malignancy. This is the application of RI wich is of paramount interest to this project.

Different methods have been used in the past to measure the refractive index of biological tissue. These methods include the focus tracking method as demonstrated by Tearney et al. (1995), the total internal reflection method, and the use of bifocal optical coherence refractometry. Although the measurement of refractive index of certain substances is a controversial science, the accuracies of these aforementioned methods have been accepted, moreso than that of the displaced reflector method. However the accuracy of displaced reflector method must also be evaluated because it has several advantages of being able to potentially measure refractive index of tissue *in vivo*, and hence has more applications in healthcare.

3.0 Methodology

A very recent previous study conducted with the aim of measuring refractive index differences between cancerous and healthy breast tissue was deemed inconclusive. Reasons for this result include the fact that the sample tissue was held between two glass coverslips that may have led to squashing of the tissue and hence innacuracies in measured values. The displaced reflector method, is hypothesised to preserve the optical characteristics of the tissue better as it doesn't squash the tissue. A large portion of this study involves the evaluation of accuracy and repeatability of the 'displaced reflector' method of RI measurement, so that breast tissue measurements have a solid foundation for comparing.

3.1 The Displaced Reflector Method

The diplaced reflector method utilises the reference slide upon which the sample is placed to measure the physical axial distance of the OCT beam. In the OCT image, the slide looks "deeper" as the optical delay is imposed upon the light as it travels through the sample. The ratio of the optical distance to the physical distance gives the refractive index of the medium. This can be seen in the diagram below as

$$n = \frac{z+z'}{z} \qquad (1)$$

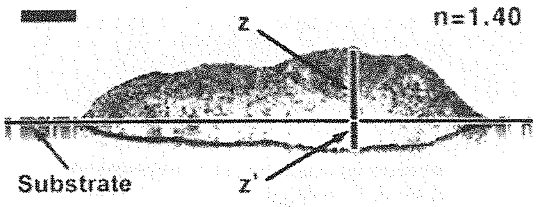

Figure 1. a displaced reflector OCT image

3.2 Top-imaging Sample Arm

Because of the need to image from above the sample stage a new OCT sample arm was built to accommodate the requirement of the 'bird's eye view' imaging requirements of the displaced reflector method.

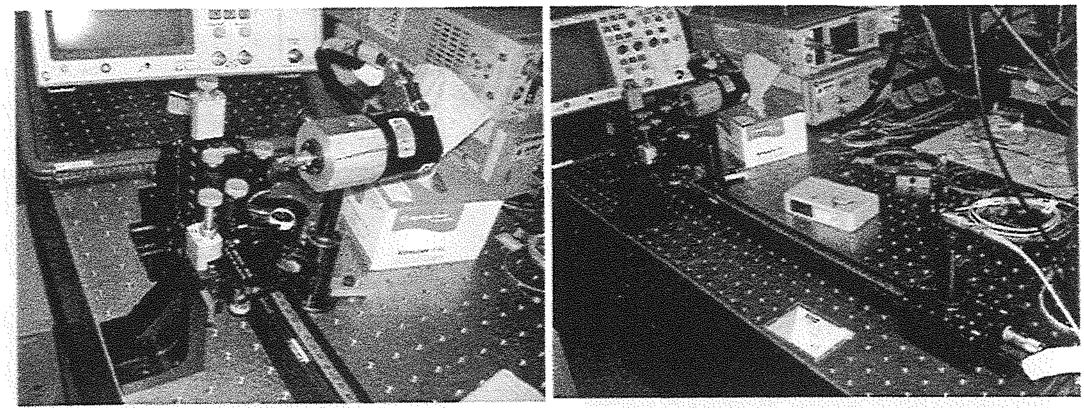

Figure 2. the OCT sample arm that images from above

3.4 Scattering and Refraction in Tissue

Whether or not a highly scattering media such as biological tissue will induce a beam of light (such as that from a broadband source in a OCT system) to refract upon passing through an air/tissue interface is a point of controversy. This is particularly applicable to the the displaced reflector method of refractive index measurement because slight refraction may cause significant changes in the measured RI. To overcome this problem, the RI calculation algorithm was run on settings to take account for refraction correction, as well as for no refraction correction.

3.5 Manual vs Automated Analysis

Automated analysis (algorithm based) provides large numbers of data points, and theretically, more accurate results. Increased accuracy is only theoretical, because significant noise content that is inherent in OCT images may dictate that the edge detection algorithms employed may not be a true surface as seen by a human, interpreting the image. This can be seen in Figure 4a below.

Manual analysis, on the other hand, is time consuming, and hence may not be able to provide as many data points, and also may not be as scientifically credible as automated analysis because it incorporates human measurement error. However, both methods will be utilised here to evaluate which is more suited for this study.

4.0 Preliminary Results

Initial procedures to assess the accuracy of measuring system yielded results of interest. At least 20 samples of each tissue/media type were imaged and analysed using the displaced reflector method. The accuracy of the system was tested over several parameters. These included the image acquisiton, the state of the tissue (drying or thawing) and the algorithm by which the refractive index is calculated.

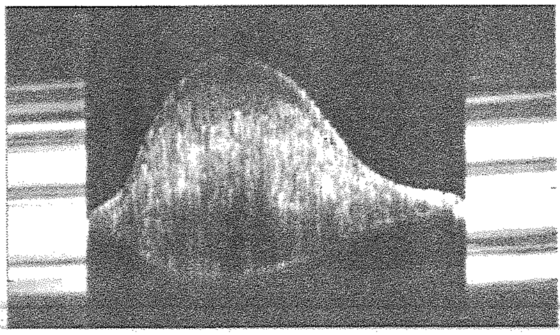

Figure 3. A typical OCT image of a chicken liver sample

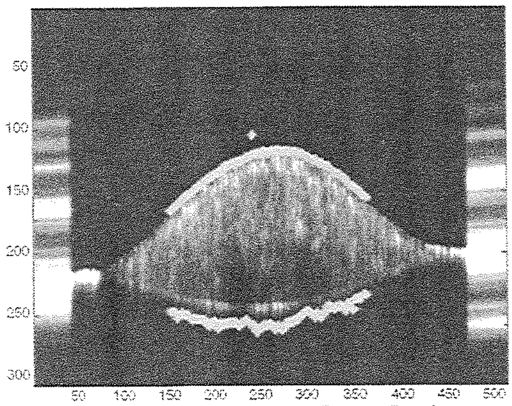

Fig 4a. – Automated analysis

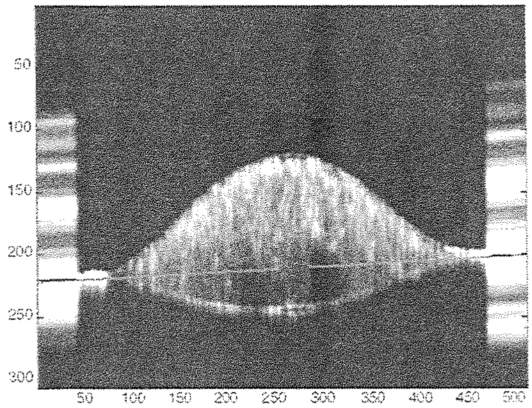

Fig 4b. – Manual analysis

Table 1. Measured and published RI's of bio and non-bio media, manual analysis

Media	Measured RI @ 1310nm	Published RI @ 623nm
Chicken Liver	1.3740	1.3950
Porcine Adipose	1.4244	1.4699
Epoxy	1.5004	1.55
Agarose Gel	1.2890	1.331

The results for the chicken liver and porcine adipose demonstrated the repeatability of the measurement process used in this study. Consecutive experiments spaced across months yielded only a 0.002 difference across all biological tissues. The data sets for the above samples exhibited refractive indices of slightly below, but relatively close to published values. These, however may still be valid, after taking into account the difference in wavelength at which the RI is published (623nm) and measured (1310nm). The manual analysis as seen in Figure 4b provided much more accurate results, and are presented in Table 1.

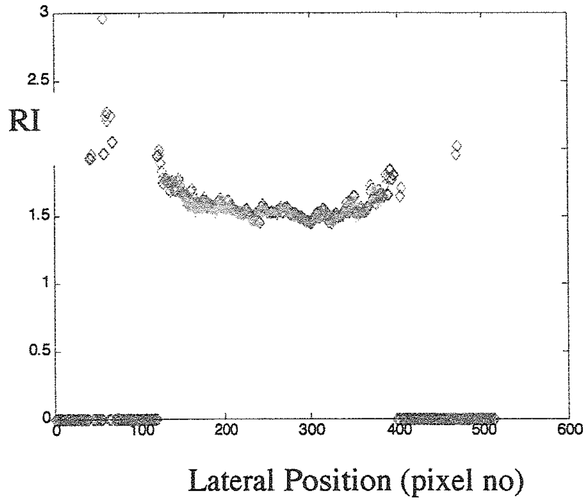

Figure 4. The distribution of corrected (green stars) and uncorrected RI's (red diamonds)

The above figure is the refractive index calculated over the surface of the sample in figure 3, for chicken liver. The uncorrected RI yielded a value of 1.4489 while the corrected value yielded 1.3870, which agreed better with the published values. On each of the other samples, the uncorrected RI gave higher and more innacurate measures of RI, and as the distribution in Figure 4 suggests, has a 'well'-shaped trend, which is undesirable.

5.0 Conclusions

From these initial results, several conclusions can be made. After testing with both interface refraction correction and with no correction, it was clear that the refraction correction algorithm was much more accurate in assessing the RI needed. Although refractive indices were slightly lower than published values, account must be taken of the fact that the much higher wavelength used in experimental OCT setup result in lower RI values. These differences will be quantified in future work. Most importantly, the repeatability of these tests have been demonstrated, and hence, even if the quantitative value of the RI for a particular tissue differs slightly from published values, the ability of RI to distinguish between tissue types is established, and this bodes well for testing on cancerous and healthy breast tissue.

6.0 Further Work

Careful revision of tissue handling procedure for breast tissue must be undertaken before final measurements of breast tissue are taken. After imaging and analysing the breast tissue, statistical analysis of correlation and confidence intervals in relation to cancerous and healthy breast tissue will be completed.

Upon completion of this project, if the refractive index of breast tissue does change with tumour malignancy, the next step is towards clinical application of this finding is design of a system so that the OCT peripherals can fit inside a biopsy needle.

7.0 References

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