The Effects of Formalin Fixing on Terahertz Properties of Biological Samples

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***Abstract*- Terahertz radiation is sensitive to covalently cross-linked proteins and can be used to probe unique spectroscopic signatures. In this paper we demonstrate how the terahertz properties of tissue are affected by formalin fixing. We study the detailed changes arising from different fixation times and see that formalin fixing reduces the refractive index and the absorption coefficient of our samples in the terahertz regime.**

I. Introduction

The terahertz regime, which lies between the millimetre and infrared regions of the electromagnetic (EM) spectrum [1], is typically defined as 0.1–10 THz. The vibrational modes corresponding to protein tertiary structural motion lie towards the far-infrared end of the terahertz range: the molecular properties that can be probed at these frequencies sensitively include bulk dielectric relaxation modes, phonon modes and intermolecular vibrations. Formalin fixed tissues are commonly used to preserve tissues for routine histopathological diagnosis [2], but few data are available on the optical properties of fixed tissue by formalin at terahertz frequencies. In this paper we demonstrate the application of THz reflection spectroscopy for extracting the optical properties of the tissue which was fixed by formalin solution.

II. Experimental Methods

The terahertz Pulsed Imaging (TPI^{TM}) system used in this study was the TPI Imaga 1000^{TM} (TeraView Limited, Cambridge, UK). In this system optical excitation is achieved by a Vitesse femtosecond pulsed laser. The relaxation of the excited carriers produces broadband electromagnetic pulses typically with a FWHM of 0.3ps. The optics are purged using nitrogen gas to remove the water vapour from the air. The usable frequency range is from about 0.1 THz to 3 THz with an average power of approximately 1uW. The system has a reflection geometry, such that the terahertz pulses are focused using off axis parabolic mirrors onto the top surface of a z-cut quartz window with an angle of incidence of 30º. The sample is placed on the quartz window and the reflected terahertz pulse from the quartz/sample interface is detected coherently. It is also possible to raster scan the terahertz optics to build up a terahertz map of the sample. A more detailed description of the system can be found in reference [3].

We chose to use pig muscle as the sample. Terahertz radiation can penetrate several millimeters of dehydrated tissue [4], therefore, to avoid etaloning within the sample, the sample was cut such that the thinnest dimension is no less than 1cm in thickness. After imaging the fresh sample it was placed in formalin. The volume of formalin used was at least 20 times the volume of the tissue. The fixative and the sample were stored in the refrigerator at a constant temperature since the penetration of formalin is related to the temperature of the solution. In order to monitor the changes caused by fixing the protein structure, the sample was measured after being fixed for 24 hours, 48 hours and 72 hours. A photograph of the fixed tissue is shown in Figures 1a).

III. Results And Discussion

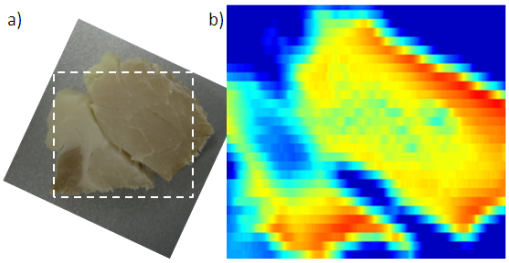

Fig. 1 a) The photograph of the fixed tissue. b) Terahertz image corresponding to the area in the square dashed in the fig 1a.

The calculated refractive index and absorption coefficient from the sample measurements are plotted in Fig. 2. The pink line with circles symbol represents the fresh tissue and the red dash line, the blue real line and the black line with triangles symbol represent the tissue which was fixed for 24 hours, 48 hours and 72 hours respectively. The preliminary results demonstrate that the refractive index of the tissue reduced as the fixation time increased and seem to plateau after 48hours. Comparing the fresh and fixed sample, the refractive index changed obviously at the low frequency in the terahertz range, from 3.2 reduced to 2.3 at 0.1THz. The gradient of the curves tend to be flat after fixing in the formalin solution especially in the high frequency range from 0.9-2THz, the value of refractive index is approximate 2 (Fig. 2a). Since water is being removed from the tissue, it is logical that the absorption coeffi-

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cient should be reduced by the fixing process, as shown in Fig 2b). Before fixing, the absorption coefficient of the tissue is about 100cm^{-1} at 0.1THz, it reduced after 24hours and the value down to 50cm^{-1} approximately. Also, during this phase the percentage of moisture being replaced decreases, so the change in optical properties becomes negligible as time increases.

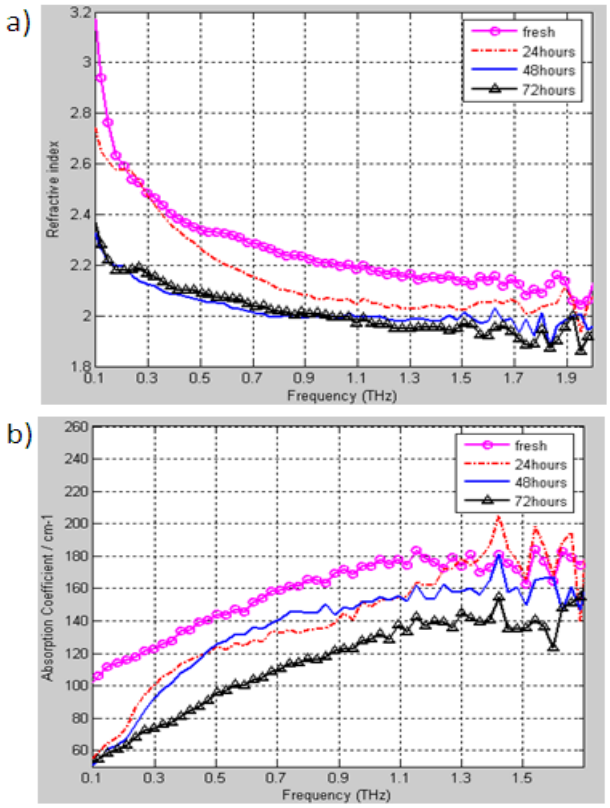

Fig. 2 The refractive index and the absorption coefficient of the tissue sample before and after formalin fixing for 24, 48 and 72 hours.

Formalin is a saturated solution of formaldehyde (HCHO), water, and methanol. Unlike most anti-bacterial and germicidal agents which poison the bacteria and germ cells, formaldehyde kills cell tissue by dehydrating the tissue and bacteria cells and replacing the normal fluid in the cells with a gel-like rigid compound. Tissue and bacterium cells are made of protoplasm and as such, contain large amounts of moisture. The introduction of formaldehyde into the tissue dries out the protoplasm and destroys the cell. Additionally, the structure of the protein in the "new" cell will resist further bacterial attacks.

IV. Conclusion

In summary, we have used terahertz pulse imaging and spectroscopic technology to determine the frequency dependent optical properties of tissue undergoing formalin fixation. The spectral information is sensitive to the conformational changes of the protein molecules due to the formalin. Therefore we predict that terahertz pulsed imaging and spectroscopy could potentially be used for evaluating the quality of biological samples. In this work we will also investigate different types of tissue and calculate the absorption coefficient, so as to understand and relate the measurements to the molecular changes occurring.

V. Acknowledgment

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