Terahertz spectroscopy of snap-frozen human brain tissue: an initial study

G.M. Png, R. Flook, B.W.-H. Ng and D. Abbott

A report is presented of a novel study on the use of terahertz (THz) spectroscopy to distinguish between healthy and diseased snap-frozen tissue samples obtained from three regions of the human brain. The diseased tissue samples are neuropathologically diagnosed as containing abnormally high numbers of protein plaques consistent with Alzheimer's disease. As protein structures have been successfully probed elsewhere using THz radiation, it is expected that the collective vibrational modes of protein plaques can be detected in the THz frequency range. Furthermore, measurements of frozen samples assist in removing uncertainties caused by the presence of water in the sample. Results show some distinction in the THz absorption spectra, which could be attributed to pathological changes in the diseased tissue.

Introduction: Terahertz (THz or T-ray) spectroscopy and imaging have been successfully applied to a wide range of medically-inspired applications. Among these applications, THz spectroscopy has been shown to be potentially useful as a diagnostic tool for protein sensing and histomorphology studies of healthy and diseased excised tissue [1, 2], where fresh and fixed tissue samples are used respectively. Snap-frozen and lyophilised tissue samples from various organs have also been explored [3].

Terahertz protein sensing of tissue is particularly applicable in diseases where an abnormal accumulation of protein occurs, such as the accumulation of protein plaques ('senile plaques') in the cerebral cortex of the brain due to Alzheimer's disease (AD). Diagnosis of AD is currently made on the post-mortem identification of high numbers of lesions (plaques and tangles) in the grey matter of cortical brain regions [4, 5]. The development of a non-invasive diagnostic tool for identifying the accumulation of protein plaques during the early stages of AD would greatly improve medical treatment of the disease, hence improving the quality of a patient's remaining lifetime. Mathematical models have shown that it is plausible to do *in vivo* THz spectroscopy of the cerebral cortex [6, 7], but at higher power levels than presently available. The most readily available techniques at present are therefore *ex vivo* THz studies of protein plaques in excised tissue, and the constituents of plaques. Results from these studies will contribute towards expanding our limited understanding of AD pathogenesis.

This novel investigation uses THz spectroscopic examination of healthy and diseased snap-frozen human brain tissue samples taken from three regions of the cerebral cortex. Frozen tissue is, in theory, ideal because the strong THz resonant activity of liquid water is suspended when frozen [8], hence improving confidence that any resonant activity detected with THz radiation is caused solely by characteristics of the tissue. Snap-frozen tissue has the added advantage of containing smaller ice crystals than those in slow-frozen tissue (frozen in a domestic freezer), so snap-frozen tissue samples are expected to suffer from less THz scattering. Our results do indeed show differences between healthy and diseased tissue. However many challenges exist and will be highlighted in this Letter.

Experiment: Human ethics clearance from the University of Adelaide has been obtained. Excised brain cores (cylinders) of approximately 15 mm in length and <7 mm in diameter are extracted from the cerebral cortices of a normal brain, and one that has been neuropathologically diagnosed as containing abnormally high numbers of protein plaques (indicative of AD). The brains are seated on a dry ice bed to maintain the tissue in optimal condition, while the cores are removed using a slow-speed trepanning drill with a 7 mm diameter hollow drill bit. The cores are taken from three regions in the cerebral cortex of one hemisphere in each brain: superior frontal gyrus (SFG), inferior frontal gyrus (IFG) and cingulate gyrus (CG). These regions are chosen because Bielschowsky silver stains of the exact regions on the contralateral hemisphere, which is fixed in formalin, has revealed numerous protein plaques ($\simeq$102 plaques per $\times$250 microscope magnification field) for the diseased tissue, and no plaques for the healthy tissue.

Measurements are made with a Picometrix transmission mode THz time domain system (TDS) in conjunction with a closed-cycle cryostat (Janis CCS-450) enclosed inside a nitrogen-purged chamber. Samples are mounted as shown in Fig. 1 and are measured at -28°C (245 K) in air, hence the reference signal is the THz signal through the empty cryostat.

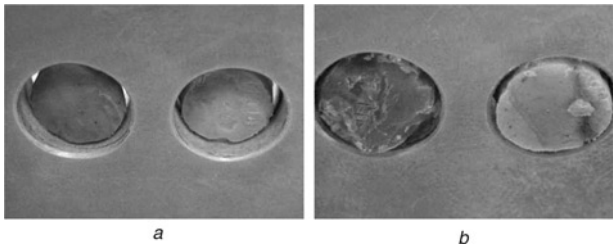

Fig. 1 *Diseased (left) and normal (right) brain cores*
a Cingulate gyri (CG)
b Superior frontal gyri (SFG)
Different colour of samples shown in Figure not indicative of health of the samples, but rather because the samples are from brains of two different donors. Surface of diseased SFG sample is uneven

Results: Fig. 2 shows the THz absorption coefficients of the three tissue types. The diseased CG and IFG tissue samples appear to have lower THz absorption than healthy tissue, however the opposite is observed in SFG tissue. The abnormal behaviour of SFG may be attributed to scattering from the uneven surface of the diseased sample as shown in Fig. 1*b*. The trend exhibited by CG and IFG tissue may indicate that the extraneous protein plaques absorb less THz. It is not possible to conclude that any distinction between the healthy and diseased tissue samples is due to a specific type of protein (e.g. β-amyloid). It is possible that the difference is due to the collective response of a variety of known abnormal proteins that accumulate in diseased brain tissue, which for AD include β-amyloid, lipofuscin, tau, and glial fibrillary acidic protein (GFA) [9]. Tissue atrophy is also a common occurrence in AD, thus atrophy could have also contributed to the observed differences.

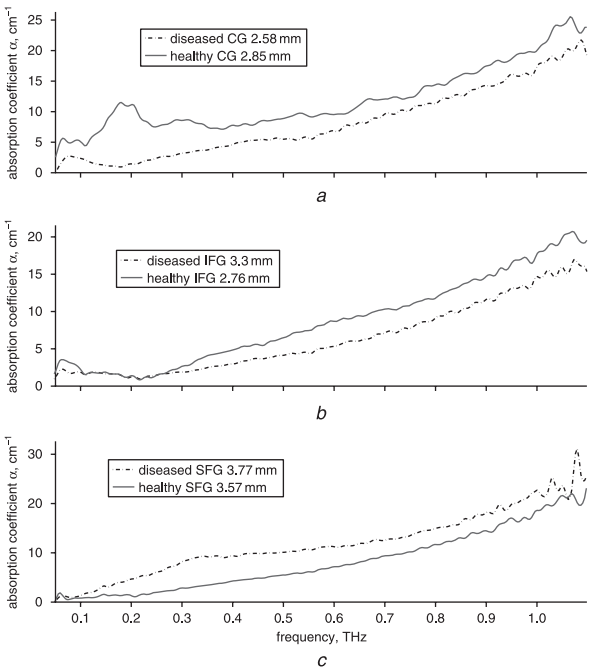

Fig. 2 *Clear distinction between absorption coefficients of diseased and healthy CG is evident (Fig. 2a); similar trend to that of CG is observed in IFG (Fig. 2b); although absorption coefficients of diseased and healthy SFG are distinctly different, diseased SFG absorbs more THz, which is unlike CG and IFG (Fig. 2c)*
a THz absorption coefficients of CG
b THz absorption coefficients of IFG
c THz absorption coefficients of SFG

The temperature at which water activity is completely suspended is commonly accepted to be -80°C but this temperature is unattainable in our cryostat without applying a vacuum. In a vacuum at -80°C, the tissue is expected to become irreversibly lyophilised – this effect is unwanted in this study of snap-frozen tissue, hence vacuum use is

avoided. It has been reported that the THz absorption coefficient of ice at temperatures between −180 to −3°C (93–270 K) does not vary significantly in the frequency range of ≃0.1 to 0.8 THz [8], thus water activity at −28°C is not expected to interfere with the results reported in our study for this particular range of frequencies.

Challenges: Given the small diameter of the cores (<7 mm), minimal handling is paramount in order to reduce thawing caused by heat from contact with fingers/gloves, forceps, dishes, etc. This is a particularly difficult endeavour when cutting the cores to ensure flat incident surfaces to the THz signal. Anatomically matching healthy and diseased tissue samples is also a challenge owing to the poor physical condition of many diseased brains. Moreover, the thickness of grey matter can vary on a case by case basis, and may be reduced in AD brains, resulting in a mixture of white and grey matter in a fixed length of core sample.

Conclusion: These early results are encouraging but many challenges, both experimental and histological, will need to be overcome before THz spectroscopy can be confidently used to analyse frozen brain tissue. Diagnosis of AD currently involves a series of tests and evaluations, generally with the intention of eliminating other possible conditions. Brain imaging scans (computed tomography and magnetic resonance imaging) are used to rule out brain tumours or blood clots. Given the paucity of knowledge on the etiology of AD, any discovery will contribute to the small but growing pool of scientific knowledge to facilitate advances in diagnosis and treatment of AD.

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References

1 Wallace, V.P., Fitzgerald, A.J., Pickwell, E., Pye, R.J., Taday, P.F., Flanagan, N., and Ha, T.: 'Terahertz pulsed spectroscopy of human basal cell carcinoma,', *Appl. Spectrosc.*, 2006, **60**, (10), pp. 1127–1133
2 Knobloch, P., Schildknecht, C., Kleine-Ostmann, T., Koch, M., Hoffmann, S., Hofmann, M., Rehberg, E., Sperling, M., Donhuijsen, K., Hein, G., and Pierz, K.: 'Medical THz imaging: an investigation of histo-pathological samples', *Phys. Med. Biol.*, 2002, **47**, (21), pp. 3875–3884
3 Png, G.M., Choi, J.-W., Ng, B.W.-H., Mickan, S.P., Abbott, D., and Zhang, X.-C.: 'The impact of hydration changes in fresh bio-tissue on THz spectroscopic measurements', *Phys. Med. Biol.*, 2008, **53**, (13), pp. 3501–3517
4 Braak, H., and Braak, E.: 'Neurofibrillary changes confined to the entorhinal region and an abundance of cortical amyloid in cases of presenile and senile dementia', *Acta Neuropathologica.*, 1990, **80**, (5), pp. 479–486
5 Morris, J.C., Storandt, M., McKeel, D.W., Rubin, E.H., Price, J.L., Grant, E.A., and Berg, L.: 'Cerebral amyloid deposition and diffuse plaques in "normal" aging: evidence for presymptomatic and very mild Alzheimer's disease', *Neurology.*, 1996, **46**, (3), pp. 707–719
6 Png, G.M., Mickan, S.P., and Abbott, D.: 'Simulation of terahertz radiation in stratified media'. Proc. SPIE Photonics: Design Technology, and Packaging II, Brisbane, Australia, 2005, Vol. 6038, pp. 116–126
7 Withayachumnankul, W., Png, G.M., Yin, X.-X., Atakaramians, S., Jones, I., Lin, H., Ung, B., Balakrishnan, J., Ng, B.W.-H., Ferguson, B., Mickan, S.P., Fischer, B.M., and Abbott, D.: 'T-ray sensing and imaging', *Proc. IEEE.*, 2007, **95**, (8), pp. 1528–1558
8 Ashworth, P.C., Zeitler, J.A., Pepper, M., and Wallace, V.P.: 'Terahertz spectroscopy of biologically relevant liquids at low temperatures'. Joint 31st Int. Conf. on Infrared and Millimeter Waves and 14th Int. Conf. on Terahertz Electronics (IRMMW-THz), Shanghai, China, 2006, p. 184
9 Selkoe, D.J.: 'Alzheimers disease: genes, proteins, and therapy', *Physiol. Rev.*, 2001, **81**, (2), pp. 741–766