Rapid screening of brain tissue with a high spatial resolution over large analysis areas using Agilent's 670 and 620 FTIR imaging analysis

Application note

Biomedical

Authors

Kathleen M. Gough*, Alexandra Kuzyk*, Jonah Kirkwood†

* Department of Chemistry
University of Manitoba
Winnipeg, MB, Canada

† Agilent Technologies
Mississauga, ON, Canada

Introduction

Infrared imaging analysis is a valuable tool that is widely used in the study of diseases that affect tissue structure. Alzheimer's is a progressive and fatal brain disease that is characterized by the deterioration of mental functions. There is no known cure for Alzheimer's although medication can slow its progress. Two abnormal structures called plaques and tangles are prime suspects in damaging and killing neurons in the brain. Plaques build up between nerve cells and contain deposits of a protein fragment called beta-amyloid. Tangles are twisted fibers of another protein called tau. This study focused on the rapid screening of an entire hippocampus section of mouse brain for the study of Alzheimer's disease. The aim was to investigate whether there was a correlation between the tissue's chemical composition and protein secondary structure with tissue morphology, in order to provide insight into the disease process.

A chemical imaging microscope, featuring a wide range of spatial resolution options was used in combination with an FTIR spectrometer to investigate tissue heterogeneity across large areas, providing a comprehensive understanding of sample chemistry on the micron scale.

Experimental

Instrumentation

To perform the large area tissue analysis, all measurements were performed with an Agilent Cary 670 FTIR spectrometer interfaced to an Agilent Cary 620 FTIR spectrochemical microscope with a 64 × 64 focal plane array (FPA) detector at 5.5 micron pixel resolution. All data was collected using reflection mode mosaic imaging analysis and processed using Agilent's Resolutions Pro software. Mosaic imaging is ideal for chemical imaging experiments as it extends the field of view dramatically and allows for the collection of larger images.

The Resolutions Pro software also allows the automated, unattended collection of spectral data at a high spatial resolution across a large area of analysis. In contrast, the alternate FTIR approaches would involve IR mapping, the use of a linear array, or synchrotron analysis. All of these approaches would require several hours to days to collect comparable data that the Agilent system can acquire in minutes.

Instrument operating parameters are given in Table 1.

Table 1. Agilent Cary 670 FTIR and 620 FTIR microscope collection parameters

Parameter	Value
Speed	5 kHz
Spectral resolution	4 cm^{-1}
Scans	128
Spatial resolution	5.5 µm
Mosaic	4 x 6

Sample analysis

Figure 1 shows the size of a typical mouse hippocampus. It measures about 1.4 mm x 2.2 mm and each cell is only a few microns in size.

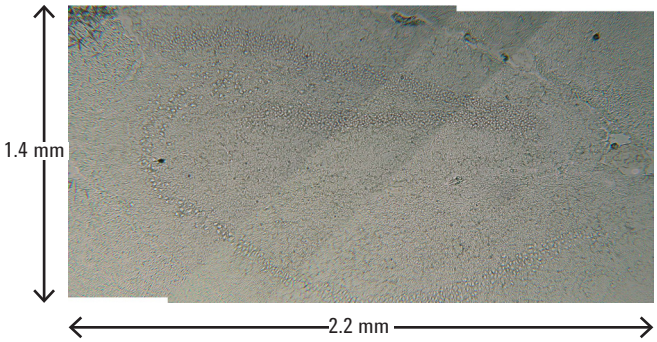

Figure 1. The hippocampus section of the brain of a normal 5 month old mouse[1]

Results and discussion

Approximately 100,000 spectra were acquired at high spatial resolution (5.5 µm) from the brain tissue section in ~30 minutes. The results can then be visualized in various ways using the Resolutions Pro software to provide a better understanding of the sample's chemical composition. For example, 2-D and 3-D chemical images are shown in Figures 2A and B respectively, while representative spectra from the white matter, neuropil and neuron are shown in Figure 2C. White matter consists mostly of myelinated axons, that is, lipid tissue veined with capillaries. Gray matter is made up of neural cell bodies, neuropil, glial cells, and capillaries and so it contains very little lipid. Labeling specific peaks of interest is a useful way to highlight that there are some significant spectral differences in the CH region of the spectrum at ~2730 cm^{-1}. The highlighted changes in CH_2 and CH_3 peaks illustrate the differences in lipid content between white matter and gray matter. In addition, morphological features such as plaques and tangles can be resolved and investigated in order to understand the mechanism of a pathology.

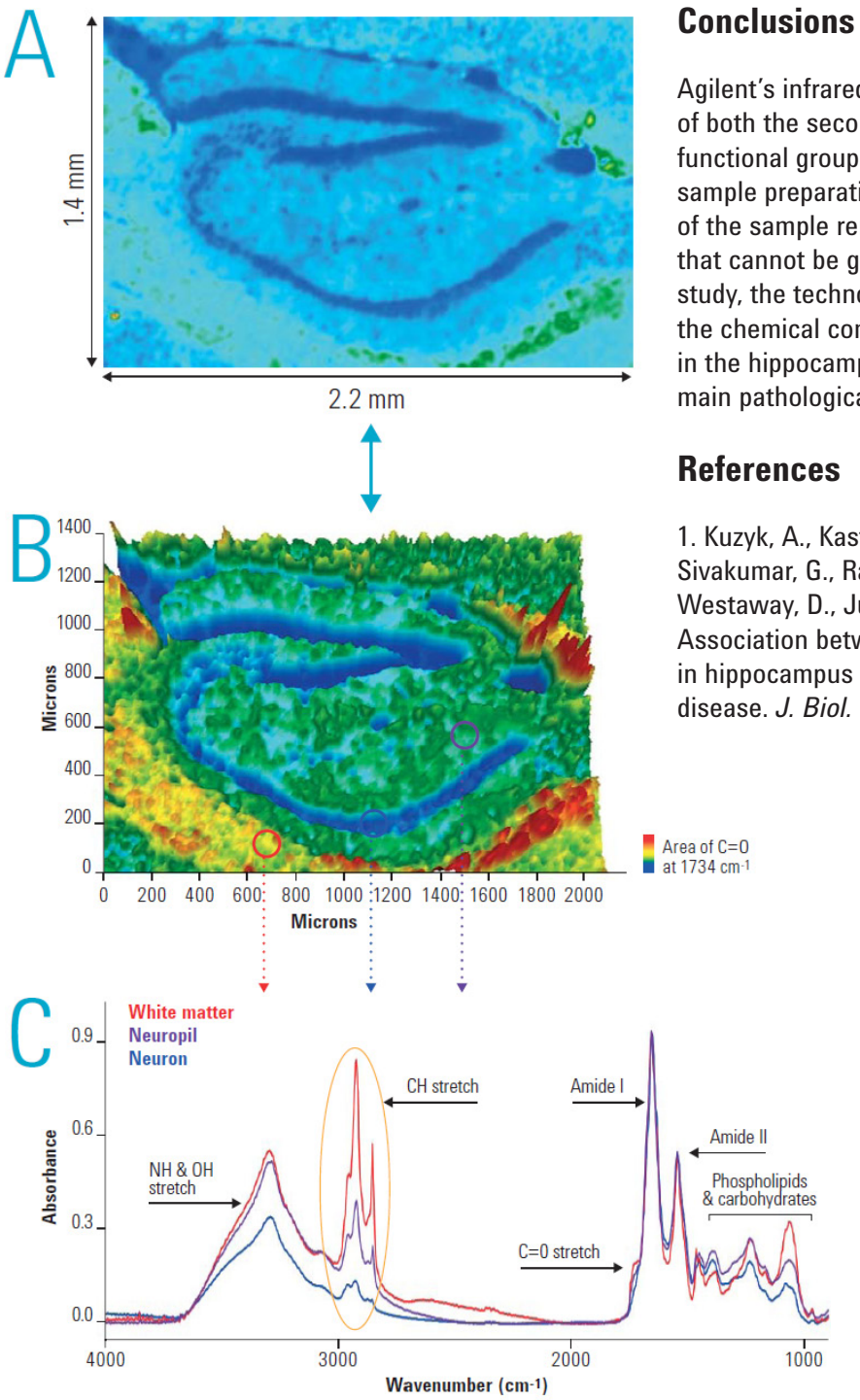

Figure 2. 2-D and 3-D chemical images are shown in A and B respectively. Representative spectra from the white matter, neuropil and neuron are shown in C.

Conclusions

Agilent's infrared imaging system allows for the study of both the secondary structure of proteins and the functional group content of tissue sections with minimal sample preparation, at the cellular level. The content of the sample remains unaltered, providing information that cannot be gained by any other method. In this study, the technology enabled scientists to rapidly study the chemical composition and development of plaques in the hippocampus section of a mouse's brain — the main pathological feature of Alzheimer's disease.

References

1. Kuzyk, A., Kastyak, M., Agrawal, V., Gallant, M., Sivakumar, G., Rak, M., Del Bigio, M., Mai, S., Westaway, D., Julian, R. & Gough, K. M. (2010). Association between amyloid plaque, lipid, and creatine in hippocampus of TgCRND8 mouse model for Alzheimer disease. *J. Biol. Chem.*, *285*, 31202–31207.

www.agilent.com/chem

Published April 2, 2012
Publication number: 5991-0018EN

Agilent Technologies