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Analysis of the Bronze Age Heslington Brain by FTIR Imaging and Hierarchical Cluster Analysis

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The Archaeological Find

In the summer of 2008 the York Archeological Trust (YAT) discovered an intact human skull (Figure 1) during excavations on the site of the new University of York campus at Heslington in York. The skull was found with its mandible (lower jaw) and the two first neck vertebrae *in situ* and a single finger bone. During the careful removal of the soil it became apparent that the skull contained what appeared to be the preserved brain. This was confirmed by Dr. Sonia O'Connor by endoscopy and radiography of the skull (Figure 2). CT and MRI were also used to explore details of the brain masses prior to removal from the skull.

Figure 1: Skull found at Heslington. © YAT

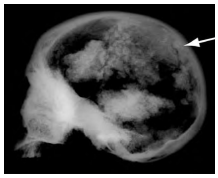

Figure 2: Radiograph of the Heslington skull. © S. O'Connor

Figure 3: Brain recovered from skull. © YAT

Carbon dating suggests that the individual, might have lived in the VI century BC, i.e. *ca.* 2500 years ago at the very end of the Bronze Age or the very beginning of the Iron Age. This is believed to be the oldest preserved brain (Figure 3) found in Britain and might even be the earliest known example of preserved brain material from Europe, and one of the earliest in the world.

The Role of FTIR

FTIR analysis of Heslington brain tissue samples was carried out to add information to that obtained by other researchers attached to the Heslington Brain Project, to determine the extent of preservation and, if possible, to determine the mechanism by which the brain was preserved. The aim is to improve understanding of how brains survive and what that could indicate about funerary practices or the individuals to whom they belonged. This could allow more accurate prediction of the burial conditions in which preserved brains might be found, improving the rate of recovery on future excavations.

FTIR Analysis: 5 µm thick slices of the brain sample were analysed in reflection mode using a Varian 670-IR FTIR system (Varian Inc., Palo Alto, CA, USA) equipped with a liquid nitrogen-cooled 128 x 128 focal plane array (FPA) detector that images a 700 x 700 µm^2 area (pixel resolution is 5.5 x 5.5 µm^2, diffraction limited spatial resolution is 10 µm at 1000 cm^{-1} and 2.5 µm at 4000 cm^{-1}) and coupled to a Varian 620-IR microscope.

Data Processing: The first derivatives of the spectra were calculated and vector normalised before Hierarchical Cluster Analysis was performed for up to seven clusters. The average spectra for each cluster were corrected with the RMieS-EMSC (Resonant Mie scattering EMSC) algorithm.

HCA Separates Tissue into 4 Clusters

The results of the Hierarchical Cluster Analysis into seven clusters of the spectral image obtained for the tissue shown in Figure 4 can be seen in the cluster map in Figure 5. Comparison of the two images shows that the areas of no tissue were clustered into three different clusters (brown, turquoise and lime), while the tissue areas were clustered into 4 clusters (red, orange, light blue and dark blue). Whether these areas correspond to different brain features is not know yet.

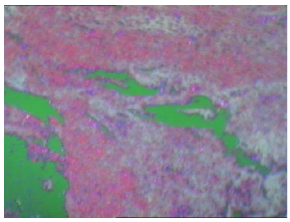

Figure 4: Optical image of the brain tissue analysed - 5 µm thick slice on MirrIR slide. Optical image is slightly smaller than the FTIR image.

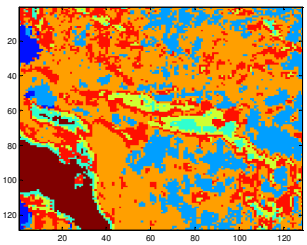

Figure 5: Seven-cluster map corresponding to the HCA analysis of the area of tissue shown in Figure 1. The area is 700 x 700 µm^2.

Edges of Tissue are Very Different from Bulk of Tissue

Comparison of the mean spectra obtained for the different clusters shows that the spectra corresponding to the orange, red and light blue clusters do no differ greatly. The greatest variation is observed in the spectrum corresponding to the small areas of dark blue clusters. The spectrum shows a decrease in absorbance in the amide bands as well as in the 1140 to 1430 cm^{-1} region followed by an increase in the 1000 to 1140 cm^{-1} region exhibiting maxima at 1120 and 1038 cm^{-1}. Absorption bands around 1120 cm^{-1} have been associated with RNA[1] and bands around 1044 cm^{-1} have been attributed to the phosphate monoester asymmetric P-O stretch.[2] The region between 1000 and 1140 cm^{-1} is also associated with glycogen and collagen. However, it is not possible to say which type of molecules these observed bands can be attributed to without further analysis. The IR pseudo-colour image of IR absorbance intensity distribution at 1029 cm^{-1} shown in Figure 7 confirmed that high intensity for this band is limited to the areas identified by the dark blue clusters. The IR pseudo-colour image of a 3500 x 3500 µm^2 area of the brain (Figure 8) shows that these features seem to only be present in the edges of tissue. Further analysis is required to identify what they correspond to.

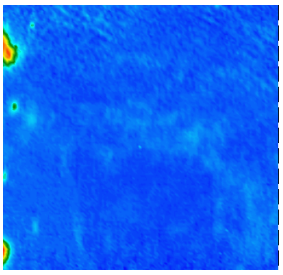

Figure 7: IR pseudo-colour image of absorbance intensity at 1029 cm^{-1} of the tissue shown in Figure 4. The area is 700 x 700 µm^2. High intensity areas correspond to dark blue clusters in Figure 5.

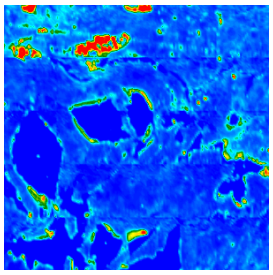

Figure 8: IR pseudo-colour image of a 3500 x 3500 µm^2 area of the brain.

Brain Tissue Still Contains Proteins and Lipids

The average spectra in the 1000-4000 cm^{-1} region for each set of clusters, shown in Figure 6, provide evidence that proteins and lipids are still present in the tissue. Evidence of proteins comes form the presence of characteristic bands in the spectra, such as:

Amide I band, which shows the characteristic absorption maximum at 1657 cm^{-1} attributed to the α-helix and a shoulder at 1628 cm^{-1} which is usually attributed to anti-parallel β-sheets;
Amide II band at 1543 cm^{-1};
N-H stretch 3283 cm^{-1};
The band at 1251 cm^{-1}, might correspond to the amide III band.
The band at 1394 cm^{-1}, which is associated with the -COO symmetric stretch for fatty acids and methyl groups in proteins.

The bands visible between 2800 and 3000 cm^{-1} are characteristic of lipids.

Nothing can be said about the presence of DNA as the characteristic bands usually observed in the 1000-1100 cm^{-1} region cannot be distinguished from contributions from other entities in this region.

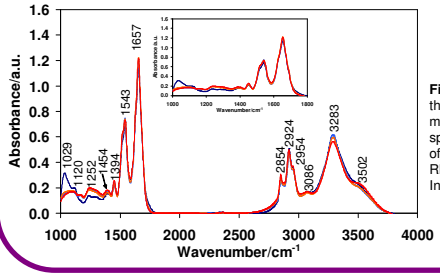

Figure 6: The mean FTIR spectra obtained for the clusters that represent tissue. The colour matches the colour of the clusters. The mean spectra were obtained by averaging the spectra of each cluster and then correcting them with RMieS-EMSC (Resonant Mie scattering EMSC). Inset: fingerprint region of the spectra.

Comparison with Fresh Brain

Comparison between the spectra obtained for the Heslington brain with spectra obtained by Kraft *et al.*[3] (Figure 9) for normal brain tissue that had been snap frozen in liquid nitrogen immediately after resection shows that on the whole the spectra are similar. However, some differences are visible. These are:

The band at 1454 cm^{-1}, which is associated to CH_3 groups in proteins visible for both tissues, might also have contributions from the lipid band associated with the CH_2 scissoring vibration, which in the spectra of the "normal" brain is visible at 1466 cm^{-1}. It seems that this band associated with lipids is shifted for the Heslington brain.

The band around 1735 cm^{-1} that corresponds to the C=O stretching in lipids, which is present in the "normal" brain spectra, is missing from the Heslington brain spectra.

A large spread of absorbance in the region between 1000 and 1350 cm-1 is visible in the Heslington brain spectra, which could be a consequence of the degradation of the brain tissue.

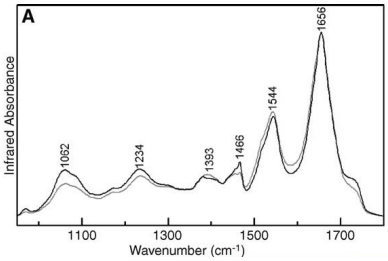

Figure 9: Average IR spectra of normal brain tissue obtained by Kraft *et al.*[3]

References

1 E. Gazi, J. Dwyer, P. Gardner, A. Ghanbari-Siahkali, A. P. Wade, J. Miyan, N. P. Lockyer, J. C. Vickerman, N. W. Clarke, J. H. Shanks, L. J. Scott, C. A. Hart and M. Brown, *Journal of Pathology*, 2003, **201,** 99-108.
2 P. S. Bromberg, K. M. Gough, M. Ogg, M. R. Del Bigio and R. Julian (1999) in *Proceedings of SPIE - The International Society for Optical Engineering*, Vol. 3775, pp. 118-126.
3 C. Krafft, L. Shapoval, S. B. Sobottka, K. D. Geiger, G. Schackert and R. Salzer, *Biochimica et Biophysica Acta - Biomembranes*, 2006, **1758,** 883-891.

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