Micro-elemental mapping of trace elements in breast tissue and their interactions with pathological markers.

M.J.Farquharson[1], A. Al-Ebraheem[2]

[1] Department of Medical Physics and Applied Radiation Sciences, McMaster University, 1280 Main St. W, L8S 4L8, Ontario, Canada.
[2] Department of Radiography, City Community and Health Sciences, City University, London, EC1V 0HB, UK.

Introduction:

Breast cancer accounts for the majority of new cancer cases and the second most common cause of cancer death in women. However, breast cancer survival rates have been improving for thirty years, with survival rates being better the earlier the cancer is diagnosed. This work is a continuation of our investigation into trace element involvement with cancer and part of our long term award at the Hasylab, beamline L. Micro SRXRF has been used for further evaluation of cellular localization of these metals in breast tumour and normal surrounding tissue and the findings were correlated with the histological information given for these samples (Farquharson et al 2008, 2009). This work has lead to two specific cancer related projects in collaboration with Prof. Adrian Harris, Director of the Cancer Research UK Medical Oncology Unit at the Institute of Molecular Medicine in Oxford. The first project with our clinical collaborators was to identify which cell types contain copper at the highest level which will be helpful in future development of copper chelation therapy. The second project focused on analysis of Zn and oestrogen receptor expression as a predictor of tamoxifen failure in a series of tamoxifen treated breast carcinomas. In tamoxifen resistant tumours there is upregulation of alternative non-estrogen receptor growth pathways such as HERII. HERII is an important negative prognostic indicator in breast cancer and is part of the EGFR receptor family. The aim of this experiment is to map the distribution of Zn, Fe, Cu, S, P and Ca in breast cancer tissue and to compare their levels with surrounding normal tissue in a HERII positive clinical breast cancer samples.

Method:

The data was collected at Hasylab, beamline L (Hamburg, Germany). At the energy of 10.5 keV used in this study an on sample spot size of approximately 15μm x 15μm was obtained. A stepwise scan was used with 5-seconds measurement time at each point. The fluorescence signal is recorded using two Peltier cooled energy dispersive Silicon drift detectors (Radiant, Vortex). The samples are formalin fixed paraffin embedded tissue of human primary invasive breast cancer. The samples were in the form of tissue micro arrays consisting of 1.0 mm diameter sections of tissue. Two slices were cut from the paraffin block, one being 10μm thick the other being 5μm thick and cut adjacent to the 10μm slice. The 10μm thick slice was mounted on a 4μm ultralene XRF film. The 5μm thick slice was mounted on a standard glass slide and then stained using Hematoxylin and Eosin (H & E) stain. This slide was then optically imaged to produce high resolution images which clearly identify tumour regions in the samples and can be used as a reference slide for the elemental distribution maps produced from the experimental slide.

Results

Approximately 22 samples were scanned, each region being around 800 x 800 microns with 15 micron steps. The results investigated the Zn, Fe, Cu, S, P and Ca, levels in human primary tumour tissue compared to surrounding normal tissue in samples of both oestrogen receptor positive (ER+ve) and ER−ve in a HERII positive breast cancer samples. The results have shown that this type of samples, HERII +ve invasive ductal carcinoma of breast, seem to have increased levels of metal content with almost three times higher levels of Cu compared to the our previous results. We are currently still working on this data and planning further studies of this work. . Figure (1) below shows a reference slide and elemental map of Zn, Fe, Cu, P, S and Ca distributions in an HERII +ve sample. The dark areas are the tumour cell nuclei and in this example the clusters of cancer cells are well defined. The pink areas are normal tissue. An overview of the statistical analysis and the percentage

difference of elemental mean and medians levels in the tumour breast tissue compared to the normal tissues are listed in table (1).

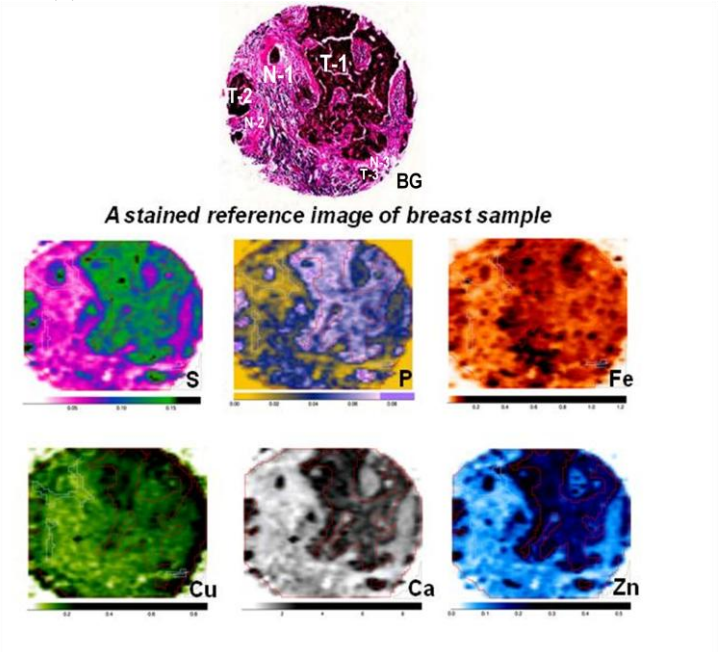

Figure 1: A stained reference image of breast sample. The dark areas are the cancer cell regions. The corresponding S, P, Zn, Fe, Cu and Ca distribution maps in the tumour and the normal regions.

	P		S		Ca		Fe		Cu		Zn	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Tumour-Roi 1	0.06195	0.01241	0.12529	0.02341	2.38621	0.45663	0.0754	0.01952	0.16753	0.02787	0.21996	0.04397
Tumour-Roi 2	0.05858	0.00781	0.12672	0.01284	2.32481	0.29188	0.06847	0.01896	0.19293	0.04759	0.20629	0.03148
Tumour-Roi 3	0.07095	0.01297	0.1406	0.0168	2.88433	0.39217	0.06989	0.00784	0.22748	0.02513	0.24	0.04135
mean:	0.06383		0.13087		2.53178		0.07125		0.19598		0.22208	
median:	0.06195		0.12672		2.38621		0.06989		0.19293		0.21996	
Normal-Roi 1	0.0168	0.00379	0.04891	0.00928	0.82183	0.14037	0.04796	0.02251	0.13193	0.02876	0.0753	0.04825
Normal-Roi 2	0.02177	0.00373	0.0585	0.00939	0.95878	0.13108	0.04276	0.01043	0.13168	0.02059	0.06243	0.01048
Normal-Roi 3	0.01618	0.00484	0.05322	0.01369	0.9041	0.30135	0.09414	0.0417	0.21124	0.06233	0.05358	0.01287
mean:	0.01825		0.05354		0.8949		0.06162		0.15828		0.06377	
median:	0.0168		0.05322		0.9041		0.04796		0.13193		0.06243	
Background	0.00074	0.00092	0.00287	0.00206	0.0289	0.03166	0.00508	0.00773	0.02514	0.00966	0.00223	0.00292
% diff of the means	249.712		144.438		182.912		15.6371		23.8155		248.254	
% diff of the medians	268.67		138.121		163.932		45.7295		46.2318		252.349	

Table 1: Overview of statistical analysis and percentage difference between the mean/medians levels of elements.

Conclusion:

This work will help us to correlate the distribution of Fe, Cu, Zn, P, S and Ca levels with other markers particularly the HERII status of the patient. The results obtained will enable us to further understand the correlation between the metal distribution and the biological markers. Additionally, the results obtained would be helpful for other research groups who are interested in developing therapeutic strategies for breast cancer.

Acknowledgements:

This work was performed using Beamline L, Hasylab, DESY, Hamburg, Germany. We would like to thank Dr. Karen Appel for her assistance.

References

[1] M.J. Farquharson, A. Al-Ebraheem, G. Falkenberg, R. Leek, A.L. Harris, and D.A. Bradley, Phys. Med Biol. **53,** 3023 (2008)

[2] M.J. Farquharson, A. Al-Ebraheem, K. Geraki,R. Leek, A. Jubb, and A.L Harris, Phys. Med Biol. **54** ,4213 (2009).