**Introduction:** Tissue biopsies often require application of a local anaesthetic, producing additional peaks in $^1$H MR spectra. These may be problematic when pattern recognition techniques are used for data analysis. In addition the anaesthetic may lead to metabolic changes in the cells. In this study, we investigated whether the anaesthetic, lignocaine, had any metabolic effect on the $^1$H-HRMAS spectra of cervical tissue samples. Although lignocaine is understood to be toxic to neural cells [1] and preservative for ischemic tissue [2] the “mechanism of action of lignocaine still remains, to a large extent, a mystery” [3]. Statistical Total Correlation Spectroscopy (STOCSY) [4] has previously [5] been used to aid structural assignment of unknown resonances and also to quickly identify xenometabolites in urine [6]. Here it was also used to look for correlations between lignocaine and other metabolites.

**Theory:** Statistical Total Correlation Spectroscopy (STOCSY) is a post-processing technique used to highlight correlations between peaks/variables for the purpose of structural assignment of resonances [4]. This involves the creation of a correlation matrix, C, obtained by normalising (multiply by $1/n-1$) the covariance matrix $X^T X$ where $X$ is an autoscaled, $m \times n$, data matrix, $m$ is the number of spectral points and $n$ the number of spectra. If one peak in particular is to be investigated, the values of the corresponding column in C represent the correlation coefficients of that peak with all other peaks. These coefficients can be used to colour-code a reference spectrum to easily visualise this relationship.

**Data Collection:** Cervical punch biopsies of twenty-seven women with abnormal smears were acquired at colposcopic examination. Lignocaine was injected locally prior to biopsy. Tissue samples were frozen within 5 min of excision and stored at -80°C before MR analysis.

**Data Acquisition:** Rotor synchronised HR-MAS spectra were recorded using an 11.74 T spectrometer (Bruker Avance, Ettlingen, Germany) equipped with a $^1$H/$^13$C/$^31$P HR-MAS probe. Tissues were thawed, washed in phosphate buffered saline made with D$_2$O, loaded into a Kel-F insert, and placed in a cylindrical 4mm ZrO$_2$ rotor. The samples were tuned, shimmed, and spun at 3 kHz at a temperature of 4°C to minimise metabolic activity. $^1$H HR-MAS spectra were acquired with water presaturation and a Carr–Purcell–Meiboom–Gill (CPMG) sequence (TE 135 ms; time between each 180° 333.33 ms). 512 scans were collected using 16k data points, a repetition time of 4.8 s, and a total acquisition time of 41 min.

**Data Analysis:** Each spectrum was apodised with a line broadening factor of 0.9 Hz. Residual water was modelled and removed. Spectra were manually phased and then aligned to lactate (1.32 ppm) and alanine (1.47 ppm). The region between 0.5 and 4.5 ppm was chosen for study, with the region 1.85 to 2.05 ppm removed due to contamination with acetate. The spectra were normalised using probabilistic quotient normalisation [7] prior to auto-scaling (mean centring and dividing each variable by its standard deviation). 2D STOCSY was performed first, to visualise correlations across the whole dataset and then the coefficients of the contaminant triplet at 1.37 ppm were chosen to colour code the mean spectrum.

**Results:** The 2D correlation matrix showed the 4 largest contaminant peaks at 1.37, 2.20, 3.37 and 4.34 ppm to be highly ($R^2 > 0.9$) correlated with each other (fig 1). This was enough to assign the peaks to a single source and aided in identifying the contaminant as lignocaine. No correlation was detected with any other metabolites, even weakly, suggesting that there was no metabolic effect to be accounted for in future analysis. After thresholding, there were few other observable correlations. A region of noise that correlated with itself and a small block of peaks between 3.4 and 4 ppm was the only other regions of note. The 1D plot (fig 2) showed the lignocaine peaks very clearly compared with the 2D version. The quartet at 3.37 ppm was less highly correlated probably as a result of its overlap with taurine and specifically myo-inositol.

**Conclusions:** Comparison of the results with a reference spectrum of pure lignocaine demonstrated that STOCSY is able to identify linked contaminant peaks successfully. More importantly, it shows that there is no correlation between the presence of lignocaine and other visible metabolites. This was an important piece of information to be taken into consideration prior to choosing a modelling/ removal method for the lignocaine peaks and then the multivariate analysis of the dataset.

**Acknowledgements:** We acknowledge the support received from the CRUK and EPSRC Cancer Imaging Centre in association with the MRC and Department of Health (England) grant C1060/A10334, also NHS funding to the NIHR Biomedical Research Centre.