Improvements on histogram analysis for statistical testing of spatially heterogeneous changes within ROI, applied to K^{trans} maps from DCE-MRI assessment of acute treatment with ZD6474

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Introduction

Currently, DCE-MRI tumour perfusion studies are usually analysed voxelwise, but the rich patterns of spatial and functional heterogeneity are lost when the data are summarised for significance testing. Statistical analyses are often performed using a single (or small number of) measures summarising the distribution of a parameter over the ROI, such as mean or median K^{trans}. The substantial limitations of these histogram-based methods include:–

- the median could be zero pre & post if there is a large necrotic "core";
- the mean is dominated by outliers from highly vascular voxels (with high Ktrans);
- there are clear "rim" & "core" effects, but rim and core are functionally defined, creating an element of circularity;
- spatial heterogeneity;
- multiple comparisons issues of multi-parameter "histogram" approaches.

Here we extend the methods to characterise and assess all of the changes in the distribution to provide additional insight and improved interpretation over and above the possibilities provided by crude histogram analysis in the situation where we cannot register images for a complete voxelwise "mapping" approach.

Figure 1: ***Maps of K^{trans} before and after treatment***

Data

The data to be analysed arise from experiments (Checkley 2003) investigating the efficacy of using DCE-MRI with gadopentate dimeglumine to monitor acute effects on tumour vascular permeability, following inhibition of vascular endothelial growth factor. Mice bearing PC-3 human prostate adenocarcinoma xenografts, were treated with ZD6474, a VEGF receptor-2 (KDR) tyrosine kinase inhibitor. There were three experiments, comprising four doses 12.5, 25, 50, 100 and a control

DCE-MRI was performed twice, immediately before and 24 hours after treatment with ZD6474. The pharmacokinetic parameter $\mathbf{K^{trans}}$ was obtained for each voxel within the tumour region of interest, which reflects vascular permeability and perfusion. Figure 1 displays an example of corresponding maps of K^{trans} before and after treatment.

Objective: To explore avenues for statistical analysis of experiments when the responses are images.

Exploratory Analyses

Figure 2 compares the histograms and kernel density estimates of the distributions of K^{trans} before and after treatment. Simple summary statistics from the histograms capture changes in location and scale only and it is clear that treatment generally reduces the values of K^{trans} but more complex changes in distribution are apparent in the kernel density estimates in Figure 3. Comparison between average densities reveals two features common to all treated groups. The first being a higher primary peak post treatment and the second, the absence of a secondary bump post treatment.

Figure 2: ***Kernel Density Estimates for one subject***

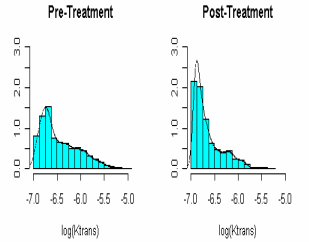
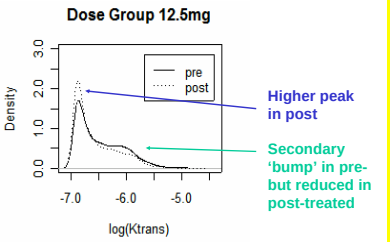


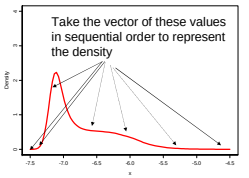
Figure 3: ***Average densities pre and post treatment for 12.5mg dose group***



Main Analyses

The main analysis is based upon *functional principal component analysis* (FPCA). This is a multivariate technique that identifies components of variability and relates them to experimental structure to provide some insight into what causes the variation and whether this can be related to a separation between pre/post treatment groups and/or control and treated samples for example.

Figure 4: ***Discretization Illustration***



Method: The first step is to summarize the functions in a small number of parameters and this is achieved by discretization which is explained in Figure 4. Each distribution can now be represented by a vector of 100 values enabling standard principal component analysis of the data. Sensitivity analysis revealed that using 50 or 200 values did not alter the results. The results however, need to be interpreted in terms of density functions. This method is based on ideas from Ramsay and Silverman (1997). The first few principal components account for most of the variation of the data and will therefore be the most interesting. Later principal components explain decreasing amounts of variation and can be regarded as 'noise' and so discarded.

Summary of process:

Measured response: **image** ⇒ Sample distribution of Ktrans ⇒ Histogram ⇒ Kernel density estimate ⇒ Vector of density values

Results

Figures 5a and 5b display the effect of adding and subtracting a multiple of the first two principal components to the mean density of the entire data set. This allows interpretation of the PCs and aids understanding of the modes of variability between the images.

The first principal component can be identified as reflecting differences between a diffuse distribution and a less variable and more peaked one.

Low PC1 Score ⇔ High Primary Peak

High PC1 Score ⇔ Low Primary Peak

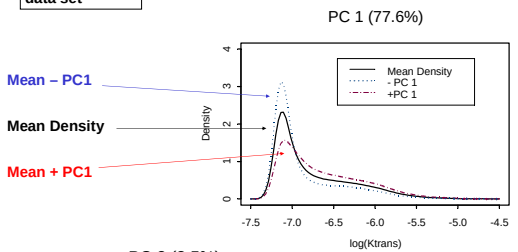
The second component reflects the presence or absence of a secondary mode of higher values of Ktrans.

Low PC2 Score ⇔ No Bump

High PC2 Score ⇔ Bump

FPCA on entire data set

Figure 5a: ***Functional Principal Component One***



PC 2 (8.5%)

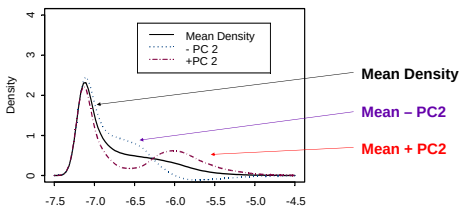


Figure 5b: ***Functional Principal Component Two***

Results (Cont)

Scores for each probability density on the PCs can also be calculated to give principle component score plots of the samples such in Figure 6, which contains the score plot for Experiment 13. Individual pairings reveal a pre to post shift to the bottom left corner, low PC1 and PC2, corresponding to a post-treatment distribution with high primary peak (see Fig. 5a) and no secondary peak (see Fig. 5b). These features indicate a distribution with overall lower values of K^{trans} and no secondary subset of high values post-treatment: a positive treatment effect.

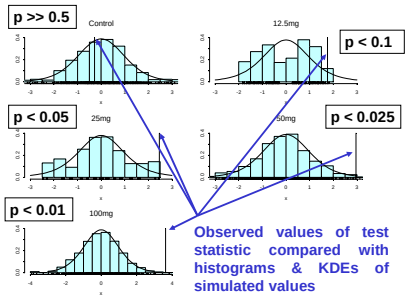
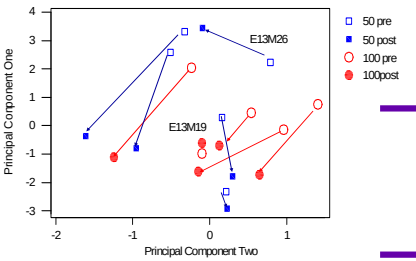


Figure 7: ***Histograms of simulated values***

Figure 6: ***Score Plot and Individual Pairings for one experiment containing two dose groups***



Formal statistical testing of a difference between pre- and post-treatment densities is achieved by a randomisation test based on random relabelling of pre- and post-treated values. The test statistic is based on the sum of the differences in scores on PC1 and PC2 *(a city bock difference).*

Figure 7 shows the histograms of simulated values for each group. The p-values indicate that all four dose groups (but not controls) show clear evidence of effect of treatment. This agrees with a similar test based on the group median but this statistic together with the score plots gives greater insight into where the changes are occurring.

Conclusions

The analysis using FPCA has provided additional insight beyond the simple changes in mean values of K^{trans}. These results could not be obtained from commonly used 'histogram analysis'. Our method could be used to locate where these changes occur within the tumour as the two different PC's are likely to represent different biology such as a well-perfused "rim" and poorly perfused "core", although these areas are not anatomically defined.

These methods are not only limited to tumour vitality studies but could be applied to other areas of imaging where we need to assess heterogeneous voxel values on an ROI basis .

Further Directions

This technique is currently being validated using data from other research areas, where the responses are curves before refining the methodology.

References:

Checkley D, Tessier J, Kendrew J, Waterton J & Wedge S. (2003). *Use of dynamic contrast-enhanced MRI to evaluate acute treatment with ZD6474, a VEGF signalling inhibitor, in PC-3 prostate tumour. Br J Cancer. Nov 17;89(10):1889-95*

Ramsey J. & Silverman B. (1997). *Functional Data Analysis*. Springer-Verlag, London.