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Using Medical Images as Primary Outcome Measures

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Outline

- Introduction
- Exploratory analysis
  - ♦ histograms & kernel densities
- Main analysis
  - ♦ functional principal components & randomization tests
- Extensions to bivariate images
- Parametric models
- Loose ends & refinements

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Introduction

- Medical Images
  - NMR, X-Ray, Microscopy, …
  - ♦ key research tool in drug development
  - quickly reveal changes & nature of effects to scientists but not widely used as outcome measures in clinical trials
  - ♦ Some simple [simplistic?] exceptions
  - x-rays of broken legs
  - brain scans
  - … … …

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Examples

- NMR images of tumours
  - ♦ 3D images of complete tumour can reveal blood flow
    - changes 'obvious' 24 hours after treatment
- NMR images of lungs
  - [hyperpolarised helium-3]
  - ♦ reveals capacity & oxygen take-up
    - differences in CPD, smokers & normals
- High-throughput imaging of cell-based assays
  - *in vivo* dose-response assessment

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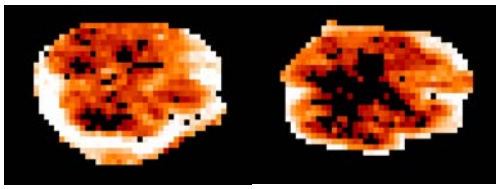
Pre Treatment Post Treatment



E14M34: 50mg dose

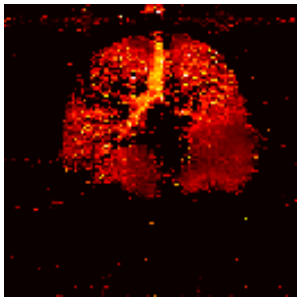
Maps of K^{trans} before and after treatment

Lighter colour indicates higher permeability :–

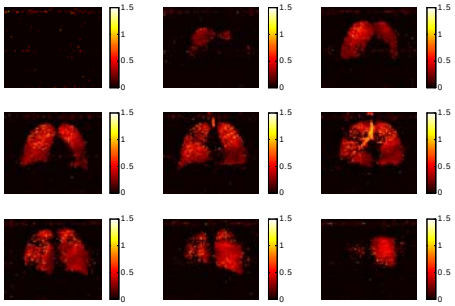
note visible decrease in permeability post treatment

(Similar pairs of images for each of the 44 subjects)

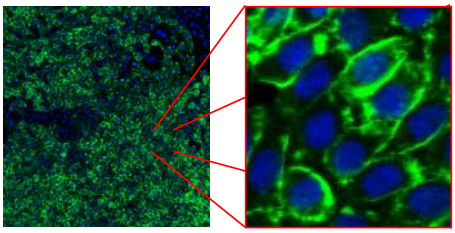
MRI using hyperpolarised helium-3 gas inhaled in the lungs – slice 6



MRI using hyperpolarised helium-3 gas inhaled in the lungs:- 9 slices from one scan



High throughput imaging of cell-based assays



- These images are quick, cheap, non-invasive, clear & so are used in

  drug development

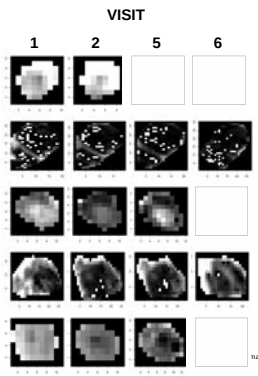
  - does it work?
  - how does it work?
  - can it be made better?
- But for formal statistical assessment some other measure is used
  - tumour weight, survival time, FEPV, … …

WHY?

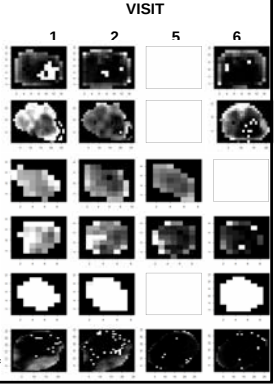
- need statistical techniques for describing & assessing ***variability*** between images
  - before & after treatment
  - ***between*** treatment groups
  - ***within*** treatment groups

(statistics is about describing & assessing variability in the context of uncertainty)

Individual **IAUC60** Maps – Dose 20



Individual **IAUC60** Maps – Dose 30



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Example on Tumours

- 3 experiments:-
  change in vascular permeability in tumours after drug designed to inhibit angiogenesis
- Four doses
  - ♦ 12.5mg, 25mg, 50mg, 100mg + control
- 3D images of tumours by MRI
  before & 24 hrs **after** treatment
- Data provided:-

voxel values of K^{trans}

(& V_e & other parameters, see later)

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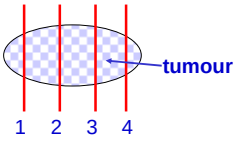
'Raw Data'



NMR scan focuses separately on four near-adjacent 'slices' measuring parameters in each voxel

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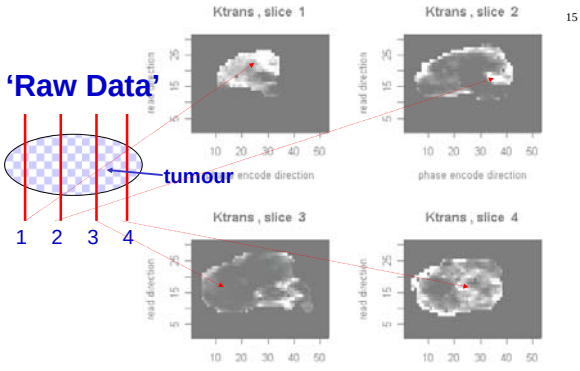
'Raw Data'



K^{trans} Maps of all four slices of *E12M48pre*

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- **Technical Notes:**
  - ♦ Lack of registration between images
    - images 'before' & 'after' treatment are not aligned or 'registered' so cannot look at changes at voxel level
      - – tumours are non-rigid unlike images of the brain
  - ♦ Lack of registration between slices
    - Images provided as four 2D slices that are not perfectly registered so at best only partial information on proximity of voxels in different slices

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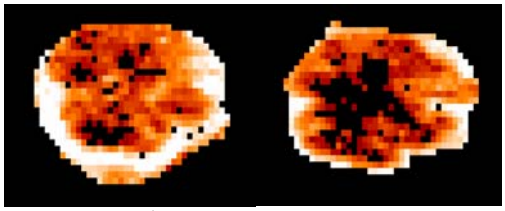

Maps of K^{trans} before and after treatment

E14M34: 50mg dose

Images of tumour before and after treatment

Lighter colour indicates higher permeability :–

note visible decrease in permeability post treatment

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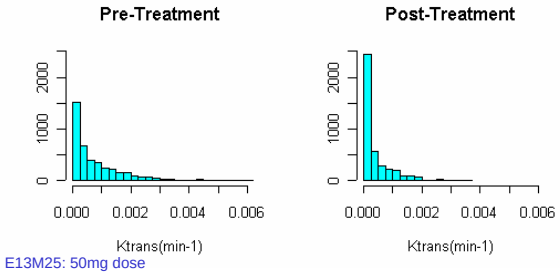
Exploratory analysis

- 3D images provided as four 2D slices
  - ♦ Many zero values of K^{trans}
    - (internal to image – ROI excludes 'external zeros')
  - ♦ Histograms of voxel values have very high positive skewness
  - ♦ ⇒ work with log(K^{trans})
    - or log(1+ K^{trans}) or **log(ε+ K^{trans})** to avoid log(0)



E13M25: 50mg dose

Histograms of pixel values in slices 2 & 3 pooled

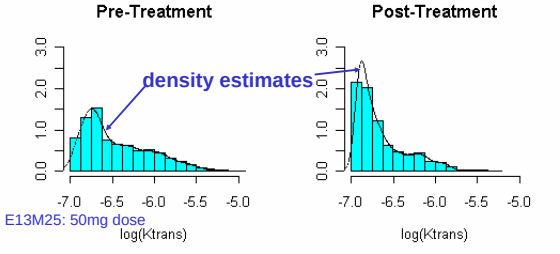
(note overall decrease in values post-treatment)

- **Notes:**
  - Measured response:– Image
  - Working unit:– sample distribution of K^{trans}
  - Losses
    - spatial information
      - (but see later loose end)
  - Gains
    - more familiar statistical object
    - allows calculation of 'mean responses' & assess variability relative to mean
      - (by pooling sample distributions)

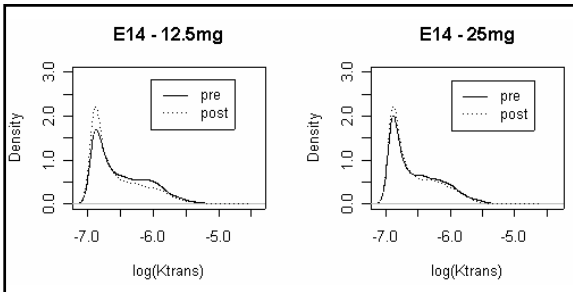
- **Notes(ct$^{d.}$)**
  - Ignoring spatial information masks ***dependence*** of voxel values
    - K^{trans}–values are not independent
    - Beware of statistical techniques which assume independence
    - 'sample size' is ***NOT*** #voxels
    - true sample size is #images, i.e. 88 in total from 44 individuals ~ 6 in each dose group

- **Notes(ct$^{d.}$)**
  - Working with distribution looses information on ***size*** of tumour
    - size change in 24 hours implausible
      - but could be an issue over weeks/months
  - Beware of effects of zero values of K^{trans}
    - (trace zeroes ←→ structural zeroes)
    - reliant on accuracy of segmentation techniques to determine ROI

- *kernel density estimates* better than histograms
  - gives a 'smooth' version of the histogram



E13M25: 50mg dose



Average densities for 12.5mg and 25mg dose groups

Note visible overall decrease in values post-treatment

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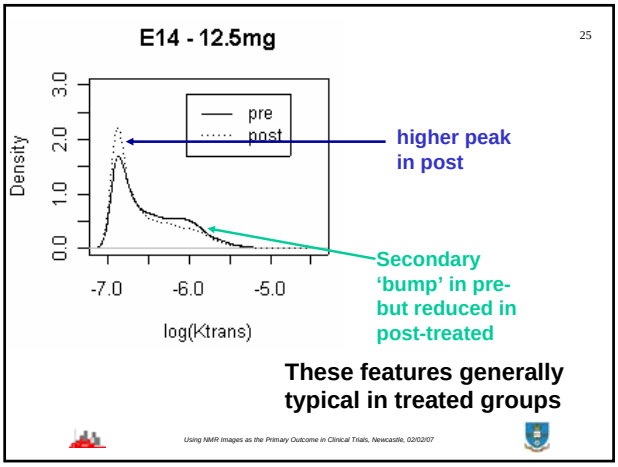

higher peak in post

Secondary 'bump' in pre- but reduced in post-treated

These features generally typical in treated groups

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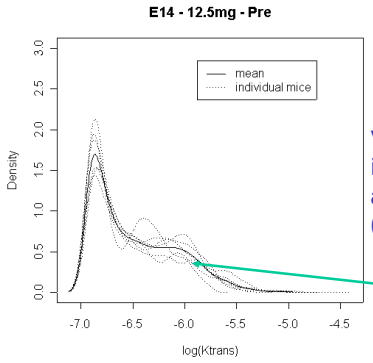

Variability of individuals about mean (pre-treatment)

Secondary bump

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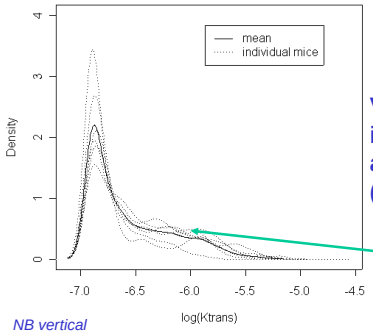

Variability of individuals about mean (post-treatment)

Secondary bump reduced / absent

NB vertical scale changed

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Main Analyses

- Starting point:
  - ♦ measured response is a probability density
    - image ⇒ distribution ⇒ histogram ⇒ **density**
  - ♦ probability densities are **functions**
  - ♦ Key references:

Jim Ramsay & Bernard Silverman:

Functional Data Analysis

Applied Functional Data Analysis

(Springer, 1997 & 2002)

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- **Key ideas:**
  - ♦ investigate components of **variability** between the functions
    - use a type of principal component analysis
  - ♦ relate these components to differences such as pre- / post- treatment or control / treated subjects or different dose levels

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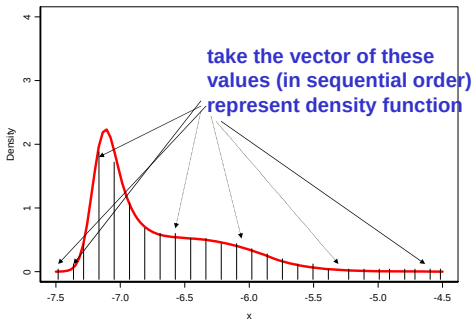

take the vector of these values (in sequential order) to represent density function

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- measured response is a **vector**
  - ♦ image ⇒ distribution ⇒ histogram ⇒ density **⇒ vector of 100 values**
  - ♦ **Losses:–** details of function by discretization
    - sensitivity analysis using grid of 50 or 200 values shewed little difference in results
  - ♦ **Gains :–** all standard multivariate techniques such as PCA & LDA &c available
  - ♦ **But:–** need to interpret results of PCA in terms of density functions

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- Functional principal component analysis
  - ♦ PCA on vector of 100 discrete values
  - ♦ Obtain first few (2 or 3) PCs
    - these are also vectors of 100 values
  - ♦ To interpret a PC we need to examine 'typical' densities that are varied from the overall mean in the direction of that PC
    - can do this by constructing a density function as overall mean density +/– PC
    - i.e. add / subtract PC vector from mean vector and then plot values as a density function

PC 1 (77.6%)

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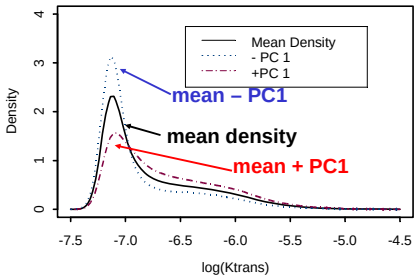

FPCA on complete data set: 77.6% of variation in PC1

PC1 reflects change in height of primary mode

low scores on **PC1** ⇔ high peak, high score ⇔ low peak

PC 2 (8.5%)

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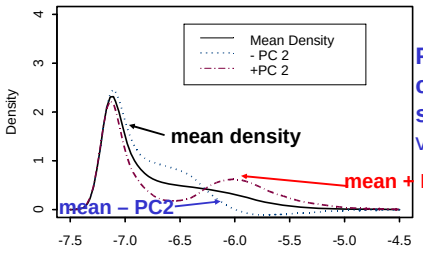

FPCA on complete data set: 8.5% of variation in PC2

PC2 reflects presence of secondary 'bump'

low scores on **PC2** ⇔ no bump, high score ⇔ bump

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- FPCA (continued)
  - ♦ Signs of PCs are arbitrary:–
  - ♦ we have chosen signs so low values are **'desirable'** in current context
  - ♦ low PC1 ⇔ high peak of low values
  - ♦ low PC2 ⇔ no second 'bump' of high values
  - ♦ low scores on first 2 PCs ⇔ generally lower permeability & especially fewer fairly high values

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- FPCA (continued)
  - ♦ Can calculate scores for each probability density on the PCs
  - ♦ image ⇒ distribution ⇒ histogram ⇒ density function ⇒ vector of 100 values **⇒ scores on first two PCs**
  - ♦ **Gains:–** can plot samples in a scatter plot
    - points towards bottom left corner are 'good'
      - (since they have low scores on PCs)
    - Can compare 'pre-' and 'post-' treated

      Discarding low order PCs reduces random noise

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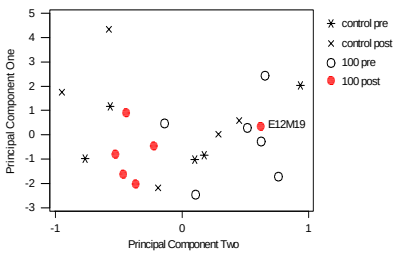

Score plot on first 2 PCs for Expt 12

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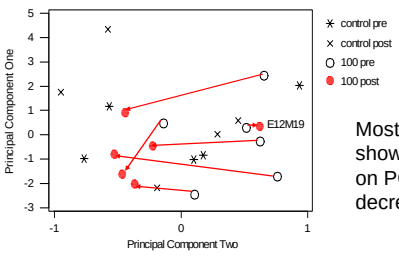

Most treated subjects show decrease in score on PC2 and overall decrease in PC1 score

Score plot on first 2 PCs for Expt 12

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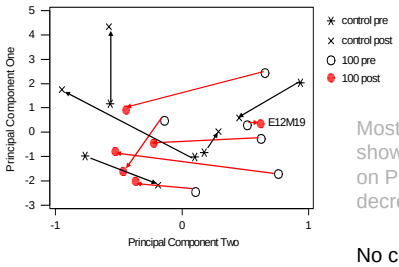

Most treated subjects show decrease in score on PC2 and overall decrease in PC1 score

No consistency in direction of change in controls

Score plot on first 2 PCs for Expt 12

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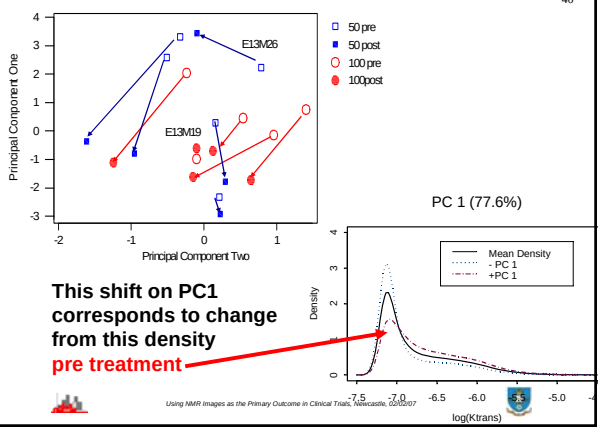

This shift on PC1 corresponds to change from this density pre treatment

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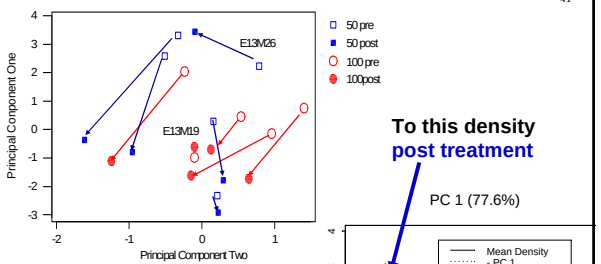
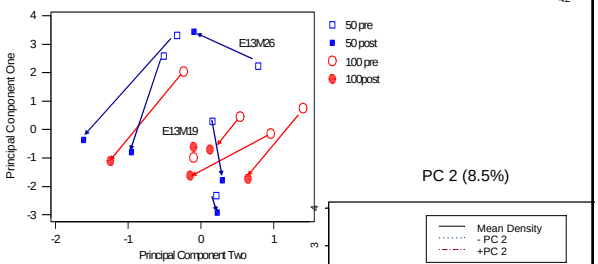


This shift on PC1 corresponds to change from this density pre treatment

42



Likewise on PC2, the shift corresponds to change from this density pre treatment

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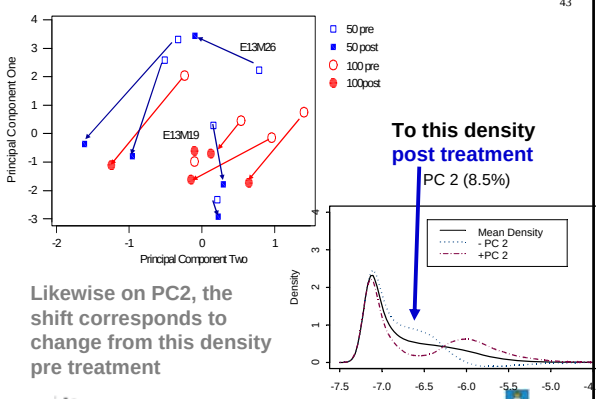

Likewise on PC2, the shift corresponds to change from this density pre treatment

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- **Interim conclusions**
  - overall picture is that treated subjects shew a reduction in permeability both in overall level and in distribution of levels
    - higher proportion of low values
    - lower proportion of fairly high values
  - Control subjects shew no consistent change over 24 hours

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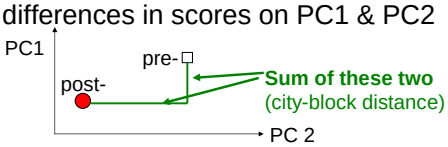
- Formal statistical testing:–
  - Calculate test statistic
  - Assess significance by a randomization test based on random relabelling of pre- & post-treated values
- Test statistic based on sum of differences in scores on PC1 & PC2



Sum of these two (city-block distance)

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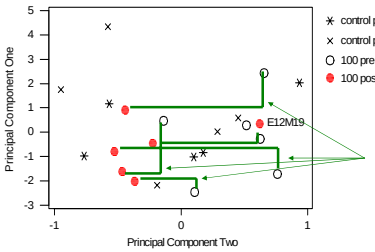

test statistic is sum of all these distances

Compare observed value with values from randomly interchanging pre- & post- labels
(near equivalent of paired t-test)

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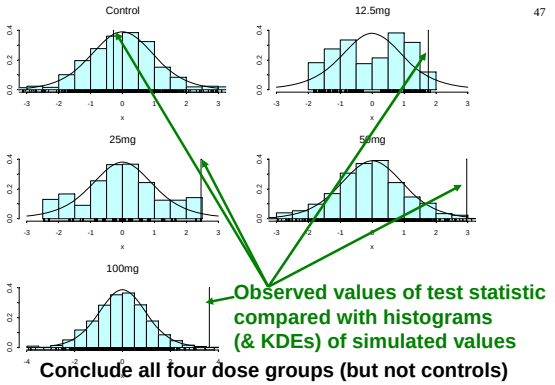

Observed values of test statistic compared with histograms (& KDEs) of simulated values

Conclude all four dose groups (but not controls) shew clear evidence of effect of treatment

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Next step: Multi-dimensional Analysis

- Theory extends to higher dimensions
  - Analyse K^{trans} and V_e simultaneously
- Method in brief...
  - Perform 2D kernel density estimation and discretize distributions over a 2D grid
  - Perform FPCA and interpret results similar to the 1D case, using CONTOUR plot

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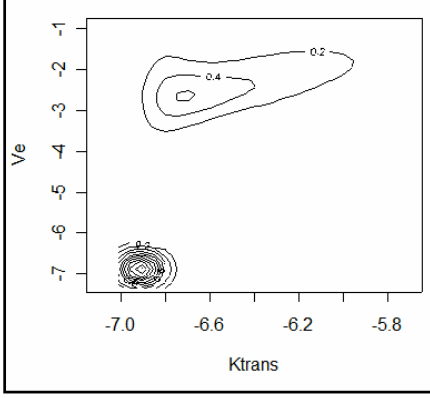
E12 - mean



Contour plot of the average density

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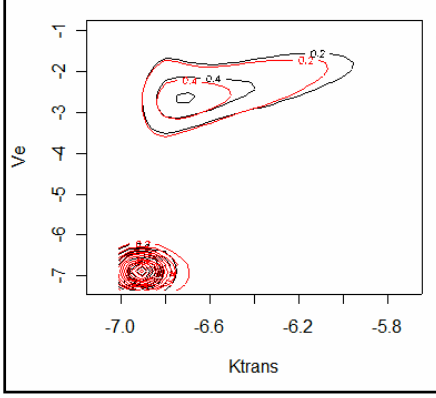
E12: mean - PC1



Contour plot of the average density

FPCA…

Average density minus PC1

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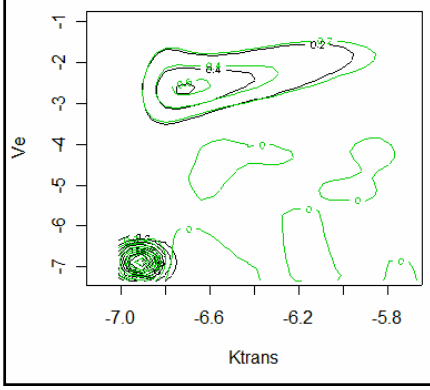
E12: mean + PC1



Contour plot of the average density

FPCA…

Average density plus PC1

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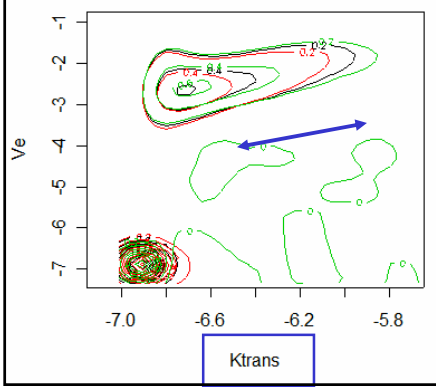
E12: mean +/- PC1



PC 1 shows change in K^{trans}

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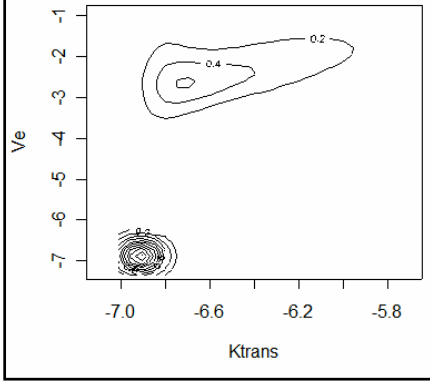
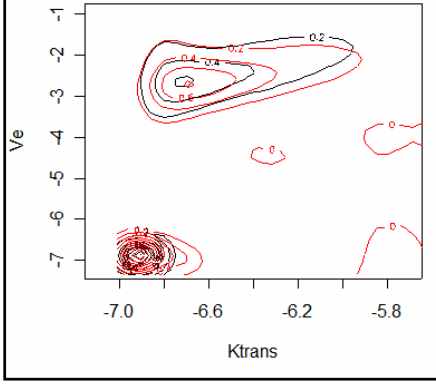
E12 - mean



Contour plot of the average density

54

E12: mean - PC2



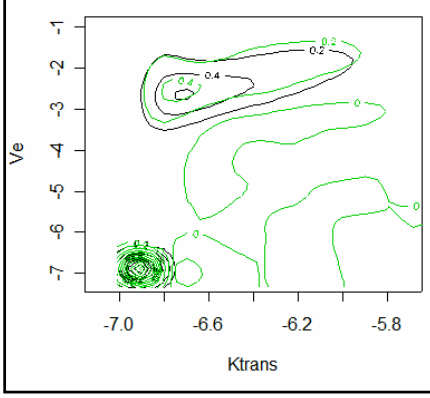
Contour plot of the average density

FPCA…

Average density minus PC2

55

E12: mean + PC2



Contour plot of the average density

FPCA…

Average density plus PC2

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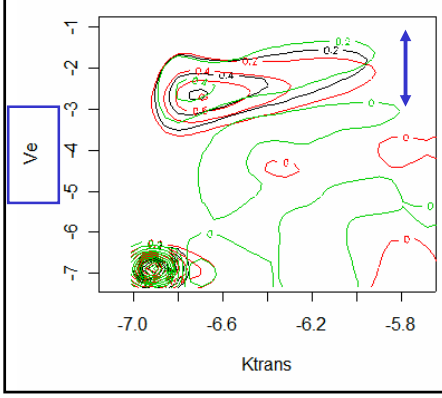
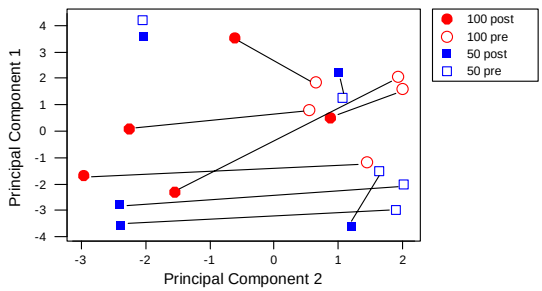
E12: mean +/- PC2



PC 2 shows change in V_e

Note change in V_e only occurs in voxels with high K^{trans} values

Experiment 13: Bivariate density: PC1 vs PC2



Most subjects show decrease in scores on both PC1 & PC2 – a decrease in K^{trans} and Ve post-treatment

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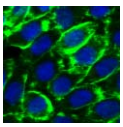
Example of dose-response study: HTS with cell-based assay

- Replicated data
  - 12 dose levels (including controls)
  - Two parameters of interest
    - **Ipeak** and **Ixpr**



- Histograms of both variables show:
  - Clear change in location with dose
  - Systematic changes in skewness and spread

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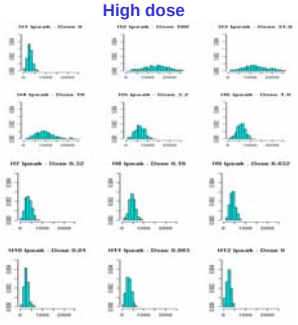
Distribution Histograms: Ipeak: Well H

High dose



Decreasing mean with decreasing dose

Decrease in variance with decreasing dose

Not much visible change in skewness with decreasing dose

low dose

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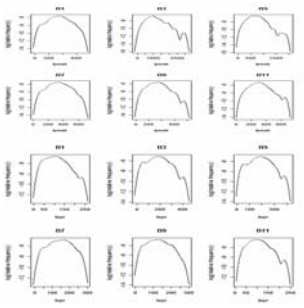
- **Two** analyses presented:
  - Non-parametric using FPCA
  - Parametric using log-hyperbolic distributions
    - Four parameters, allows skewness and 'heavy tails'
- Results consistent (happily!)
  - Both the non-parametric FPCA and the parametric log-hyperbolic pick up consistent changes in mean & in skewness with changing dose (i.e. dose-response)

Log Histograms: Ipeak: Well B

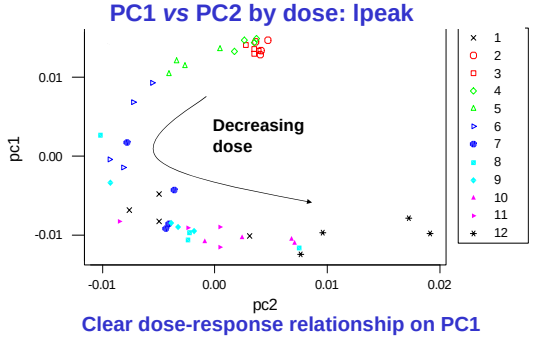


Very distinctive hyperbolae: some suggestion of secondary peak but this is exaggerated by vertical log scale

Suggests fitting log hyperbolic distributions and looking at parameter estimates: we plot
- μ (location) and
- π (skewness)

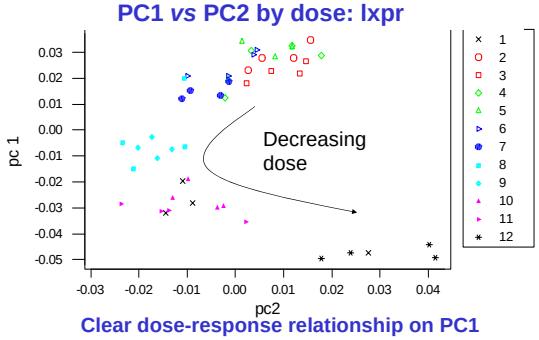
N.B. differing horizontal axes

non-parametric FPCA analysis
PC1 *vs* PC2 by dose: Ipeak

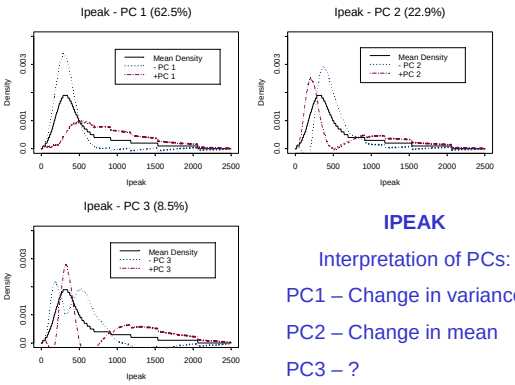


Clear dose-response relationship on PC1
with extra information on PC2 (interpretation of PCs later)

non-parametric FPCA analysis
PC1 *vs* PC2 by dose: lxpr



Clear dose-response relationship on PC1
with extra information on PC2 (interpretation of PCs later)



IPEAK

Interpretation of PCs:

PC1 – Change in variance

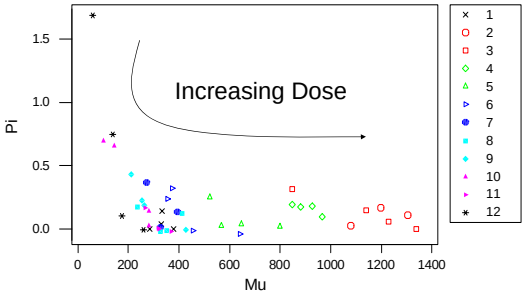
PC2 – Change in mean

PC3 – ?

FPCA Interpretation

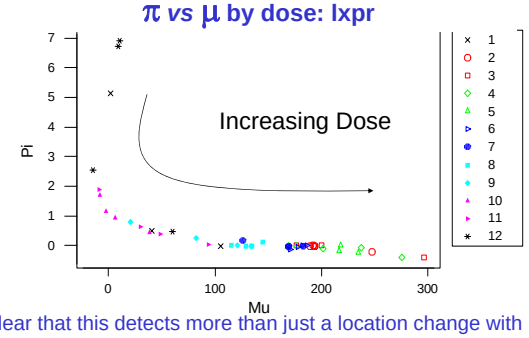
- PC1 separates the middle range doses
- PC2 separates the low doses
- PC3 separates the high doses

parametric analysis with log-hyperbolic
π *vs* μ by dose: Ipeak



parametric analysis with log-hyperbolic

π *vs* μ by dose: lxpr



Clear that this detects more than just a location change with μ: also systematic change in skewness (π)
Yet to know whether this is biologically of interest

Some differences between analyses:

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- Parametric analysis takes **each** distribution separately to estimate the hyperbolic parameters & we can assess how well the model fits each sample
  We then observe the relationship with dose
- Non-parameteric FPCA takes **groups** of distributions & characterizes the modes of variation between those in that particular group

Loose ends & refinements

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- Density estimation
  - needed? why not use histograms?
    - yes unless very large number of observations
      - e.g. brain scans, 20,000 pixels
    - 'smoothing' of histogram important
      - not sensitive to particular choices
- Alternatives to kernel densities?
  - doesn't matter
    - wavelets, splines give similar analyses
      - might be easier to extend into > 2 dimensions?

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- Issues on inference
  - especially with FPCA
    - analysis may be dependent on ***groups*** of observations
      - e.g. should PCA be including/excluding controls??
  - especially with 'testing'
    - prefer just to describe but need a more formal test to show efficacy in a formal clinical trial

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