Data integration of highly dimensional biological data sets with multivariate analysis

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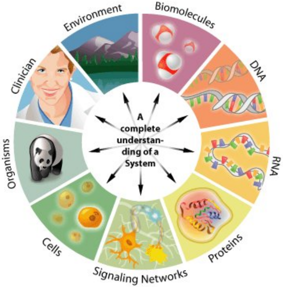
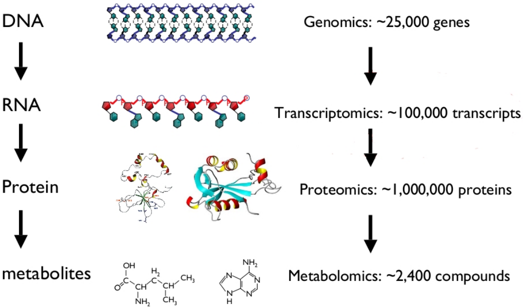
- Study of complex interactions in biological systems
- Holism vs. reductionism

  'Systems biology [...] requires that we develop ways of thinking about integration that are as rigorous as our reductionist programmes, but different [...].It means changing our philosophy, in the full sense of the term', Denis Noble
- Biology-based inter-disciplinary study field



→ Understand better the entirety of processes that happen in a biological system
→ Model and discover emergent properties, properties of cells, tissues and organisms functioning as a system

The biological dogma and the ‘omics’ cascade

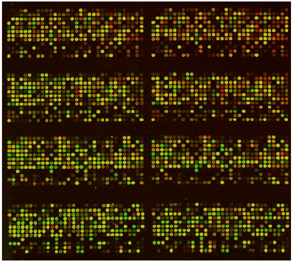


DNA → RNA → Protein → metabolites

Genomics: ~25,000 genes → Transcriptomics: ~100,000 transcripts → Proteomics: ~1,000,000 proteins → Metabolomics: ~2,400 compounds

→ Integrative systems biology: understand the relationships between these functional levels

Transcriptomics: DNA microarray technology



- Measures the expression of thousands of genes on a single individual
- 1 spot = 1 gene
- Gene expression measure = signal intensity
- Spots are ‘on’ (activated) or ‘off’ (silent) across biological conditions

→ **Identify biomarkers or regulated genes to understand the processes of cellular differentiation or carcinogenesis (Supervised analysis)**

High-throughput sequencing

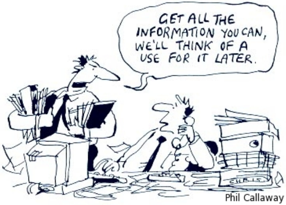
DNA sequencing: methods for determining the order of the nucleotide bases A-C-G-T in a molecule of DNA.

High-throughput sequencing: parallelize the sequencing process, produces thousands or millions of sequences at once

- inexpensive genome-wide sequence and fast
- provides insights into genome variation and evolution
  - genotyping, genome resequencing, de novo genome assembly projects and metagenomics
  - need of efficient methodologies to process and analyse the data

Challenges

Close interaction between statisticians, bioinformaticians and molecular biologists


GET ALL THE INFORMATION YOU CAN, WE'LL THINK OF A USE FOR IT LATER.
Phil Callaway

- Understand the biological problem
- Irrelevant (noisy) variables
- $n << p$ and n very small
  → **limited statistical validation**
- Is the statistical approach is biologically relevant?
- Keep up with new technologies
- Anticipate computational issues

Some research questions

Now, consider the framework of longitudinal 'omics' studies ...

1. Do we observe a 'natural' separation between the different groups of patients across time?
2. Cluster the times profiles for:
   -same type of biological features
   -different type of biological features
   → Identify subsets of correlated features across time
3. Do several assays performed on the same samples contain the same information?

Linear Multivariate approaches

- Dimension reduction
  → project the data in a smaller subspace
- To handle multicollinear, irrelevant, missing variables
- To capture experimental and biological variation

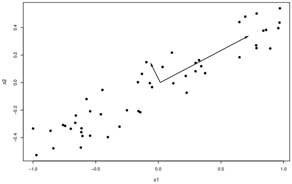
In the R package `mixOmics`, focus is on:

- Data integration
- Variable selection
- Computationally efficient methodologies for large biological data sets
- Interpretable graphical outputs

Principal Component Analysis: PCA

Seek the best directions in the data that account for most of the variability

→ principal components: artificial variables that are linear combinations of the original variables:



$$c_1 = w_1 x_1 + w_2 x_2 + \cdots + w_p x_p$$

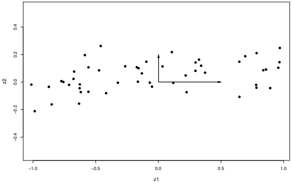
where

- where c_1 is the **first** principal component with max. variance
- $\{w_1, \ldots, w_p\}$ are the weights in the linear combination
- $\{x_1, \ldots, x_p\}$ are the gene expression profiles.

All PCs are mutually orthogonal. $(c_1, c_2, \ldots)$

The new PCs form a a smaller subspace of dimension $< p$

Project the data on these new axes to summarize the information related to the variance.



→ approximate representation of the data points in a lower dimensional space

PCA is an (almost) compulsory first step in exploratory data analysis to:

- Have a first understanding of the underlying data structure
- Identify bias, experimental errors, batch effects

Problem with PCA: interpretation can be difficult with very large number of (possibly) irrelevant variables.

Remember that the principal components are linear combinations of the original variables:

$$\boldsymbol{c} = w_1 \boldsymbol{x}_1 + w_2 \boldsymbol{x}_2 + \cdots + w_p \boldsymbol{x}_p$$

A clearer signal could be observed if some of the variable weights $\{w_1, \dots, w_p\}$ could be set to 0 for the irrelevant variables:

$$\boldsymbol{c} = 0 * \boldsymbol{x}_1 + w_2 \boldsymbol{x}_2 + \cdots + 0 * \boldsymbol{x}_p$$

These variables weights are defined in the loading vectors.
Important weights = important contribution to the PC.
Similar weights = correlated variables.

sparse Principal Component Analysis: sPCA

sparse PCA: sparse loading vectors to remove noisy or irrelevant variables which determine the principal components.

→ Solving PCA through least squares problem (SVD) allows to include regularization parameters

$$\min_{\mathbf{v_h}} ||X_h - \mathbf{u}_h \mathbf{v}_h^T||_F^2 + P_\lambda(\mathbf{u}_h)$$

P_λ is a penalty function with tuning regularization parameter λ

→ use Lasso penalization, or soft-thresholding
→ obtain sparse loading vectors, with very few non-zero elements

Shen, H., Huang, J.Z. 2008. Sparse principal component analysis via regularized low rank matrix approximation, *J. Multivariate Analysis*.

Why PCA can ‘fail’ to summarize the data?

- In some time course experiments, the subject variation can be larger than the time variation
- PCA makes the assumption that samples are independent of each other
- In univariate analysis we use a paired t-test instead of a t-test
- In multivariate analysis we use a multilevel approach:
  - different sources of variation can be separated (treatment effect within subjects and differences between subjects)
  - gain in power

Multilevel approach

- The variation in the data is separated: within matrix and between matrix
- Multivariate tools can then be applied on the within matrix (Westerhuis, 2008)
- We can take into account the repeated measures design of the experiment

VEGFC Study: Human lymphatic endothelial cells were treated in vitro with recombinant VEGF-C for 16 time points: 0min, 15min, 30min, 45min, 60min, 80min, 100min, 2h, 2.5h, 3h, 3.5h, 4h, 5h, 6h, 7h, or 8h) in triplicates, CAGE data (FANTOM5, Riken Institute).

Liquet*, B., Lê Cao*, K-A., Hocini, H., Thiébaut, R. A novel approach for biomarker selection and the integration of repeated measures experiments from two platforms, *BMC Bioinformatics*, accepted (25/09/2012).

VEGFC study: high individual effect

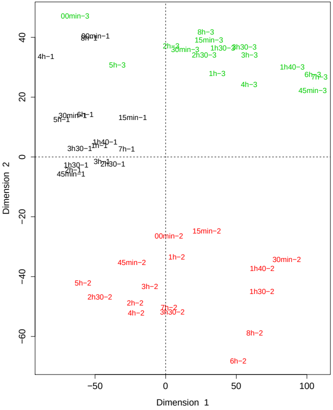


Figure: PCA on original data, color = patient

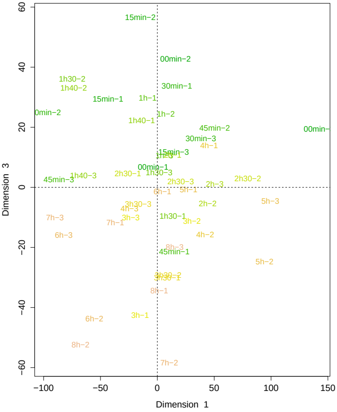
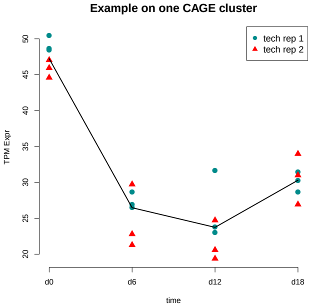


Figure: PCA within matrix, color = time

Modelling trajectories: cubic smoothing splines

Aim: summarize the trajectory of each variable

- Use cubic smoothing splines to summarize each profile
- The derivative between each time point can be estimated
- Fit a non-supervised algorithm to cluster the profiles based on the derivative (k-means, SOM)



Déjean et al. (2008), Clustering Time-Series Gene Expression Data Using Smoothing Spline Derivatives *Eurasip J.*

Example with K-means

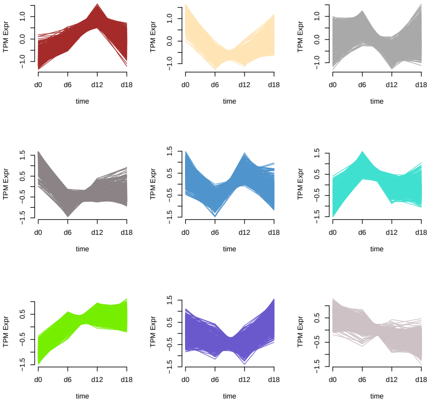


Figure: K-means on derivatives

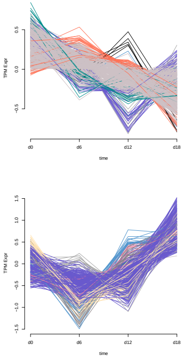


Figure: K-means on original data

How about with Self Organising Maps (SOM)?

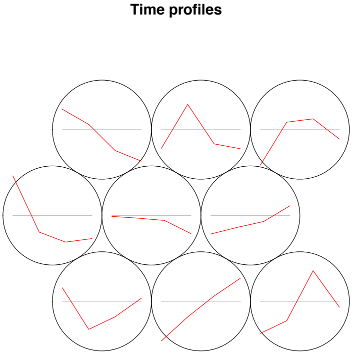


Figure: SOM summary

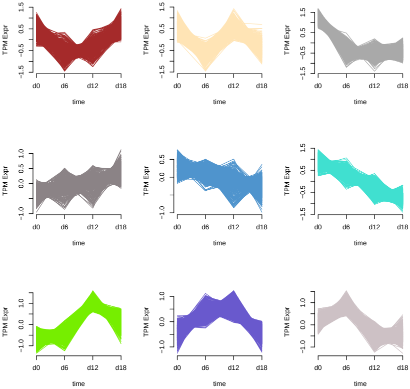


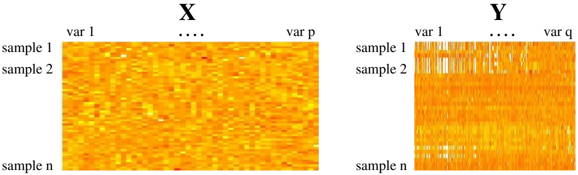
Figure: SOM on original data

Link between smoothing splines and LMM

- Be able to assess the variability for each feature:
  - technical variability
  - biological variability
- Well fitted for correlated repeated measures
- Fits into a linear mixed model framework (not parameters to tune, Verbyla et al. 1999)
- Can take into account random intercepts, bio reps as random effects ...
- Enables interpolation of missing values
- Enables to model the shape of the trajectories
- Variance components and estimates of fixed and random effects can be obtained

Integrating two large data sets

Aim: integrate two data sets and select the relevant features simultaneously:



- Two large data sets X and Y
- Measurements of two types of *variables* on the same samples

- Partial Least Squares regression maximises the covariance between each linear combination (components) associated to each data set

$$\max_{||u_h||=1, ||v_h||=1} cov(X_h\mathbf{u}_h, Y_h\mathbf{v}_h), \qquad h = 1 \dots H$$

where X (n x p) is the transcriptomics data set and Y (n x q) is the proteomics data set

- Similarly to PCA, the PLS components indicate the similarities between samples (useful plots!)
- The loading vectors indicate the contribution of the variables of the same type to the PLS component (useful for variable selection)

sparse PLS-SVD

Use the PLS-SVD variant that directly gives the latent variables and loading vectors and low rank rank approximation.

Let $M_h = X_h^T Y_h$, sparse PLS solves the optimization problem:

$$\min_{\mathbf{u_h},\mathbf{v_h}} ||M_h - \mathbf{u}_h \mathbf{v}'_h||_F^2 + P_{\lambda_1}(\mathbf{u}_h) + P_{\lambda_2}(\mathbf{v}_h)$$

where P_λ is a penalty function

→ obtain simultaneously sparse loadings $\mathbf{u}_h$ and $\mathbf{v}_h$
→ simultaneous select variables from both data sets which are correlated across samples

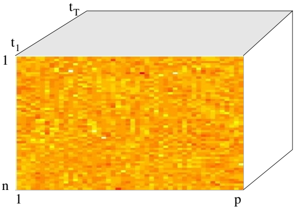
Lê Cao K-A., Rossouw D., Robert-Granié C. and Besse P. 2008. A Sparse PLS for Variable Selection when Integrating Omics data. *SAGMB* **7**(1).

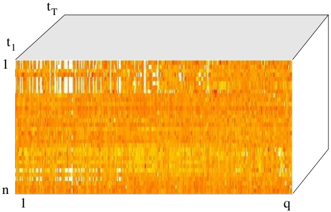
Parameters to tune

- Number of PLS components:
  -Q_h^2 index
  -graphical outputs
- Lasso penalizations λ_1^h, λ_2^h $(h = 1, \dots, H)$:
  -error prediction with cross-validation
  -maximisation of the covariance, stability analysis, permutations(?)

→ the biologist will also help choosing these parameters!

Integration of two longitudinal studies





- Select correlated profiles across time, between and within each data set.
- But difficult to deal with 3D data sets!
- PLS can integrate 2 data sets of 2 dimensions.

Step 1: use cubic smoothing splines to reduce one dimension (samples dimension)

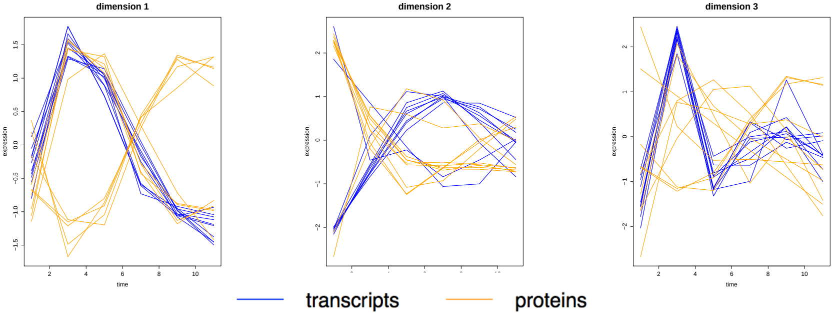
Step 2: apply sPLS on the estimated splines to identify correlated profiles both within and between the two data sets

Two illustrative studies:

Kidney transplant study: Transcriptomics and proteomics study of 40 patients with kidney transplant, rejecting ($n_1 = 20$) or not ($n_2 = 20$) the transplant. Follow up on 5 time points (weeks), PROOF Centre, UBC.

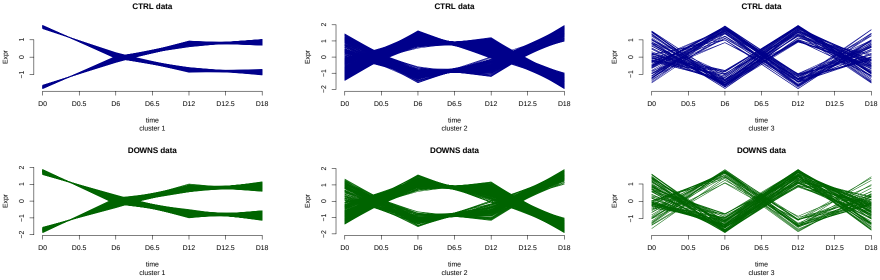
Neuronal study: Human induced pluripotent stem cells from Downs syndrome patients and controls differentiated to neurons (CAGE data). 2 bio reps and 3 tech reps per genotype (control, down syndrome), 4 time points (days), FANTOM5, Riken Institute .

Profile clusters on kidney transplant study



- sPLS selects both transcripts and proteins which are positively or negatively correlated across time
- Quality of clusters decreases with the number of PLS components (dimensions) as obvious patterns cannot be extracted anymore

Concordant profile clusters on Neuronal study

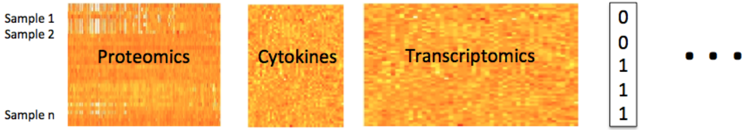

CTRL data

DOWNS data

- Each cluster of profiles corresponds to a PLS component.
- Selection of different features per condition and per component.

Integration of multiple data sets

Integrate heterogeneous data sets



- Need to define the relationships between the different data sets
- Select relevant biological entities which are correlated across the different data sets

Regularized CCA

Classical Canonical Correlation Analysis solves the problem

$$\max cor_{\mathbf{a}_h,\mathbf{b}_h}(\boldsymbol{X}\mathbf{a}_h, \boldsymbol{Y}\mathbf{b}_h) \text{ s.t. } \quad var(\boldsymbol{X}\mathbf{a}_h) = var(\boldsymbol{Y}\mathbf{b}_h) = 1$$

For $n << p + q$, the empirical covariance matrices are ill-conditionned → canonical correlations close to 1.

In regularized CCA the covariance matrices are replaced by:

$$Cov(\boldsymbol{X}) + \lambda_1\mathbf{Id} \text{ and } Cov(\boldsymbol{Y}) + \lambda_2\mathbf{Id}$$

González I., Déjean S., Martin P.G.P., Goncalves O., Besse P. and Baccini A. 2009 Highlighting Relationships Between Heteregeneous Biological Data Through Graphical Displays Based On Regularized Canonical Correlation Analysis, *Journal of Biological Systems*, 17 (2).

Multi-block analysis: Regularized Generalised CCA

- RGCCA generalizes rCCA to more than 2 data sets
- Constitutes a general framework for many multi-block data analysis methods
- Objective: seeks linear combinations of block variables:
  (i) block components explain their own block well and/or
  (ii) block components that are assumed to be connected are highly correlated.

Tenenhaus, A., Tenenhaus, M (2011) Regularized Generalised Canonical Correlation Analysis, *Psychometrika*, 76 (2).

RGCCA

For J blocks of variables $\boldsymbol{X}_1, \ldots, \boldsymbol{X}_j$, the design matrix $\boldsymbol{C} = \{c_{j,k}\}$, the function g and the shrinkage constants $\tau_1, \ldots, \tau_J$,

RGCCA optimizes the problem:

$$\max_{\boldsymbol{a}_1, \ldots, \boldsymbol{a}_J} \sum_{j,k=1, j \neq k}^{J} c_{kj} g(Cov(\boldsymbol{X}_j \boldsymbol{a}_j, \boldsymbol{X}_k \boldsymbol{a}_k))$$

subject to the constraints $\tau_j ||a_j||^2 + (1 - \tau_j) Var(\boldsymbol{X}_j a_j) \quad j = 1, \ldots, J$, where the $\boldsymbol{a}_j$ are the loading vectors associated to each block j.

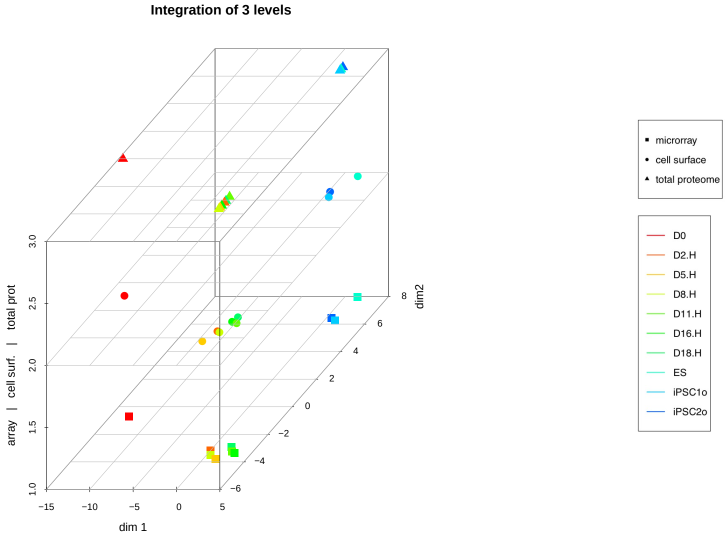
sGCCA

- Similar to the sPLS, L_1 penalizations can be applied to the loading vectors to obtain a sparse version of RGCCA to select different types of biological entities across different functional levels

Grandiose project: Longitudinal study of cell reprogramming. In this study: 8 time points are considered. Multi platform study involving: 6 platforms: microarray, cell surface proteome, total proteome, RA-seq isoform, RNA-seq genes, miRNA.

Tenehaus, A., et al. Variable Selection For Generalized Canonical Correlation Analysis, *submitted.*

Results on Grandiose project



Conclusions

- Statistical exploratory and integrative tools to extract patterns in time course data
- Can be applied to a variety of problems
- Does not provide p-values but can help generating new hypotheses, further statistical tests can then be applied
- Future directions: biological interpretation of the gene lists, time delay, generalised multi-way analysis, identifying discordant clusters across data sets for the same genes ...

Un GRAND MERCI à ...

Neuronal time course

Christine Wells	UQ
Ernst Wolvetang	UQ

Kidney transplant study

Oliver Günther	UBC
Scott Tebutt	UBC

Grandiose proj. and Nagy group

Andras Nagy	Univ. Toronto

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VAC18 Project and multilevel

Benoît Liquet	Univ. Bdx2

Multiple data integration

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Time course developments

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Christèle Robert	INRA Toulouse

FANTOM consortium

sample providers	RIKEN, Japan