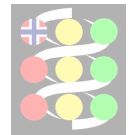


Time series experiments

microarray.no



Time series experiments

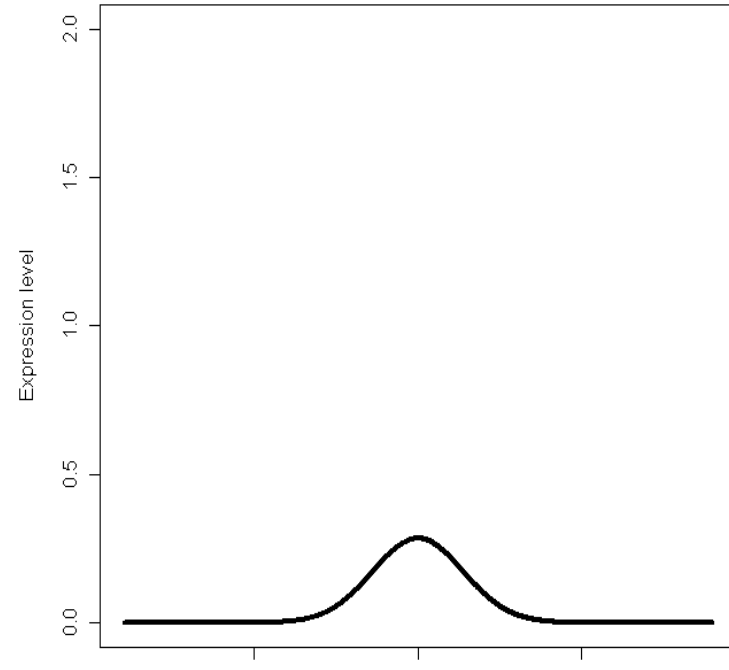
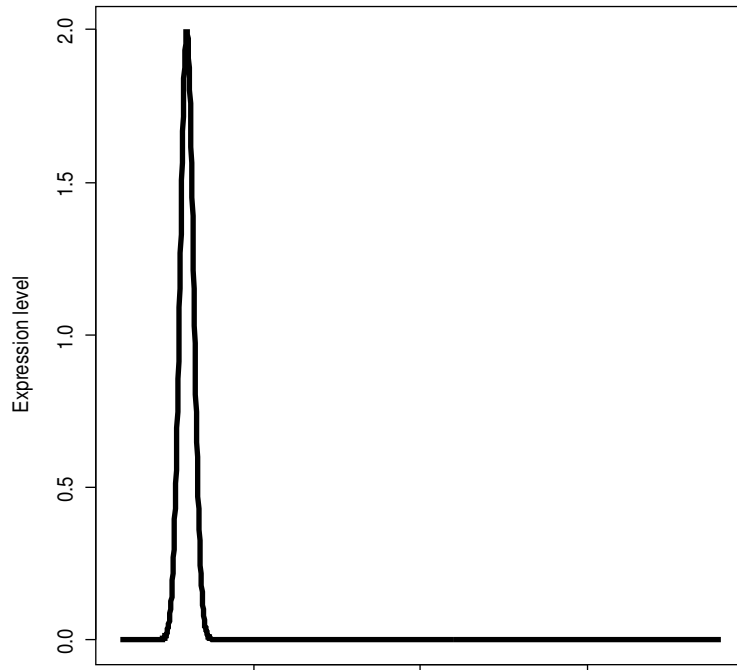
- Why is this a separate lecture:
 - The price of microarrays are decreasing – more time series experiments are coming
 - Often a more complex experimental design
 - Many time points makes this a large experiment
 - The analysis method is often specified by what you are looking for

Time series experiments

- Why consider doing a time series experiment:
 - Biology is dynamic
 - The genes can change their expression in different ways during time
 - Observe cascades and secondary effects

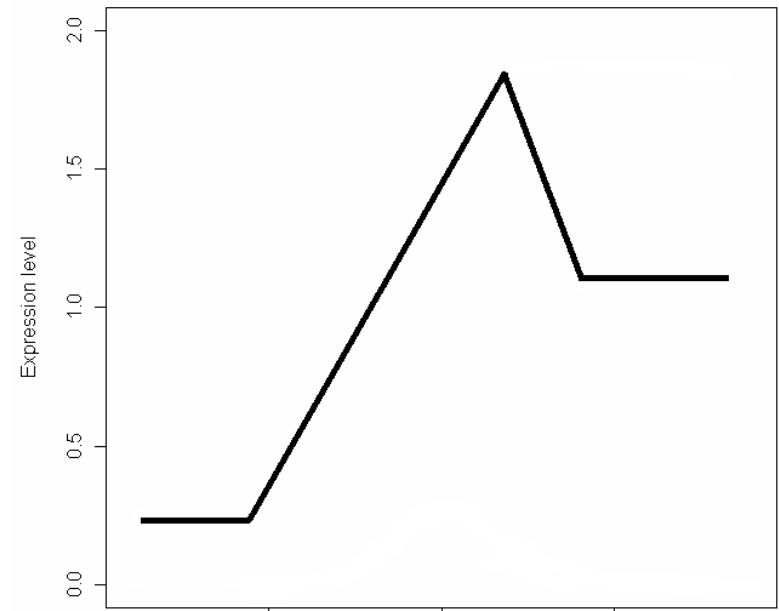
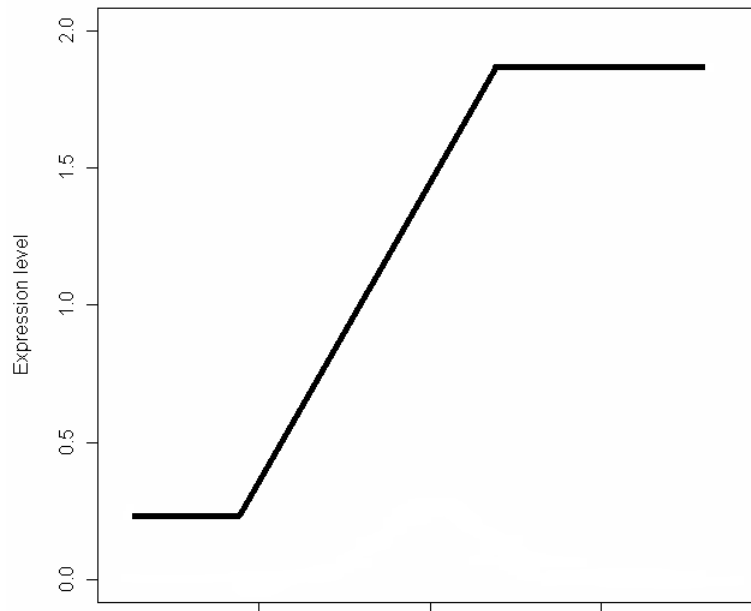
Time series experiments

- Peaks



Time series experiments

- Level change



Experimental design (Ex.d)

- Often larger and complex
- What to consider in an experimental design of a time series study:
 - Time points
 - Distance between time points
 - Replicates
 - Control
 - Technology

Ex.d.: Time points and the distance between them

- How to choose:
 - What is already known about the system/genes
 - Theoretical and experimental knowledge

Early /late response

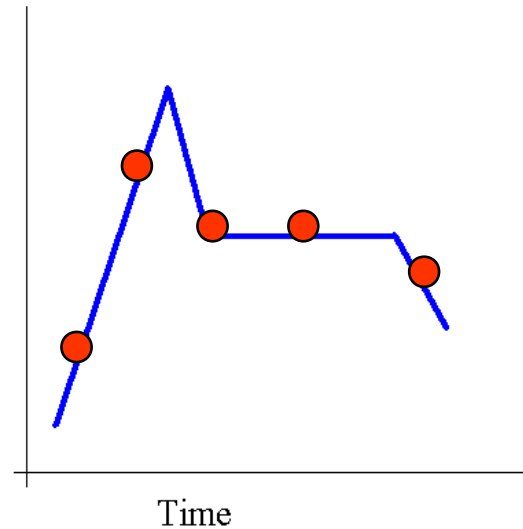
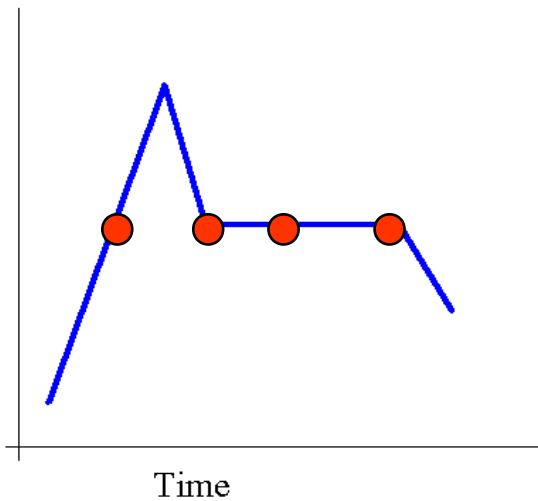
The direction of the response

Will changes in some genes influence others

- What is your field of interest

Ex.d.: Replicates

- How many replicates to use:
 - Many time points but few replicates



- Should be enough to do the analysis you want!

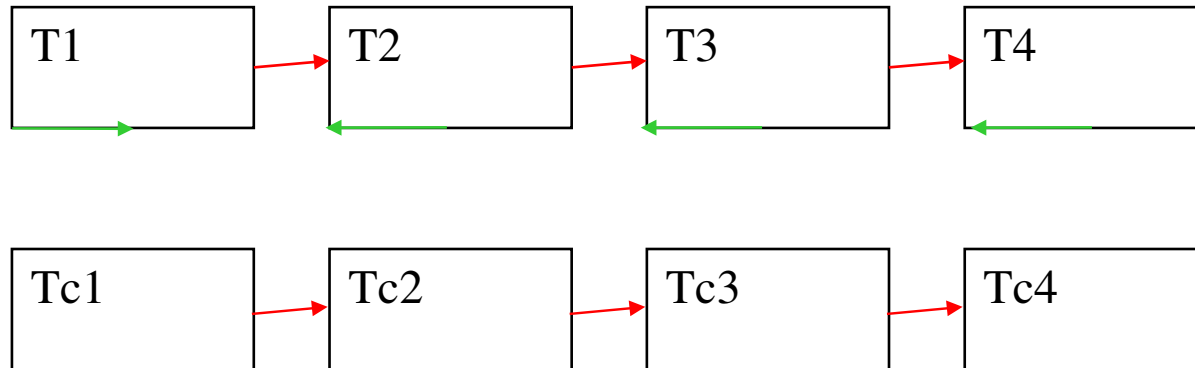
Ex.d.: Control

- What to use as a control and how many controls?
 - One control point or one time series control
 - Differences between treatments
 - The other time points as a control

Ex.d.: Control

- One control
 - Use one time point or a untreated sample as control
 - Ok if no other time effects are expected, such as growth, phase of expression
- A untreated time series as control
 - Same time points, technology, sample source and same handling except the treatment
 - Find genes that change due to treatment over time, filter out some of the effects due to handling and time

Ex.d.: Control



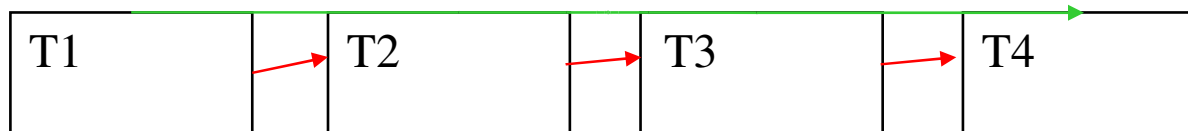
- **Vertically**: Differences due treatment, but you reduce the effect of the handling
- **Horizontally**: Differences due to treatment, time and some handling effect

Ex.d.: Control

- Differences between treatments - compare two or more time series
- Have the same:
 - Time points
 - Conditions
 - Sample source
 - Technology
 - Handling

Ex.d.: Control

- The other time points as a control:
 - Changes due to treatment, time but it does not exclude the effect of the handling
 - Less arrays used
 - See **short** time effects and **long** time effects'



- Ok if no other time effects are expected, such as growth, phase of expression

Ex.d.: Technology

- What technology to choose
 - Homemade/commercial
 - One channel
 - Two channel: Reference design is recommended
- What comparisons are to be done
- Budget

Experimental design

- Often larger and complex
- What to consider in an experimental design of a time series study:
 - Time points
 - Distance between time points
 - Replicates
 - Control
 - Technology

Pre-processing

- The same steps as for non time series microarray experiments, but not always the same algorithm or the same use of the algorithms:
 - QC
 - Filtering
 - Normalization
 -

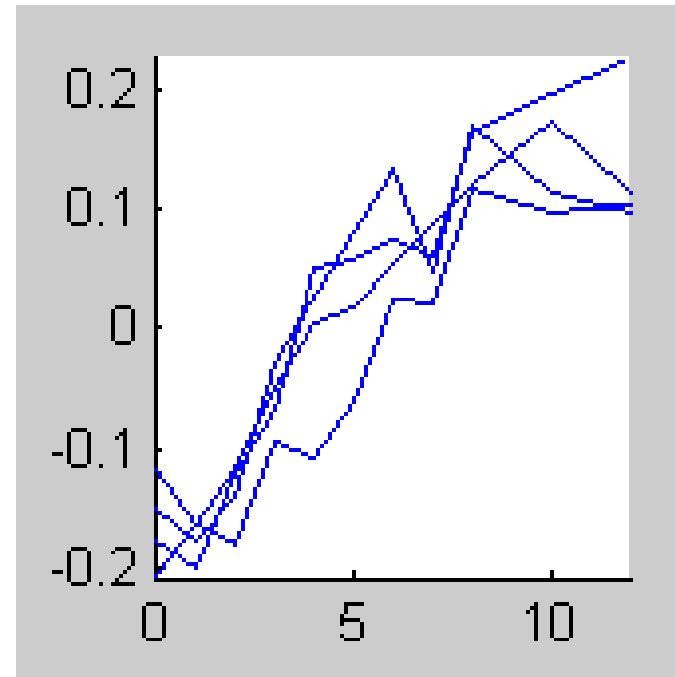
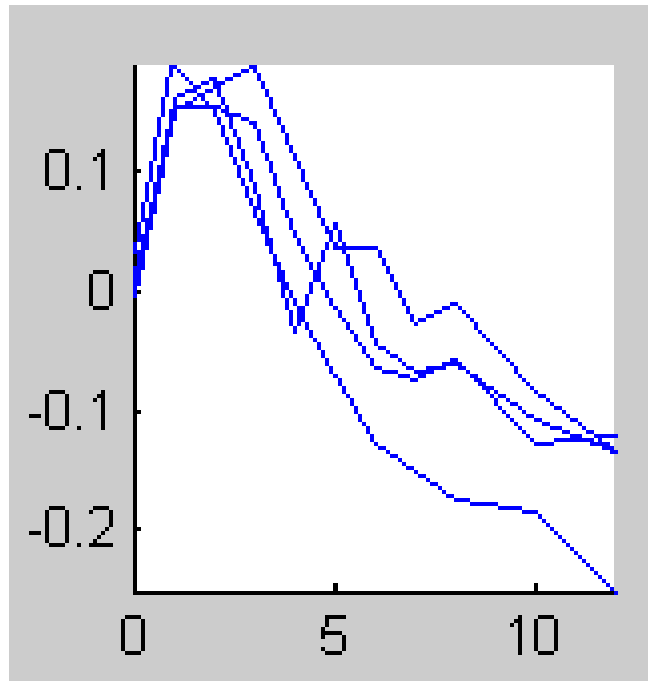
Analysis of time series data

- Clustering
- T-test between time points
- PCA
- PLS
- Cyclic
- Profile
-

Analysis: Clustering

- Filter the data set before clustering
- Cluster the genes
- Use correlation as a distance measure (as a starting point)
- There exist no right number of clusters – *a priori* knowledge is needed

Analysis: Clustering



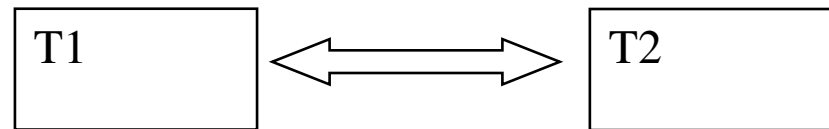
microarray.no

Analysis: T-test

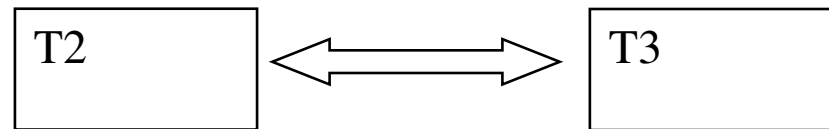
- One time point vs. another time point
- You have 3 time points: T1, T2 and T3
- What should you compare?

Analysis: T-test

- Early response:

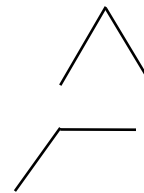


- Late response:



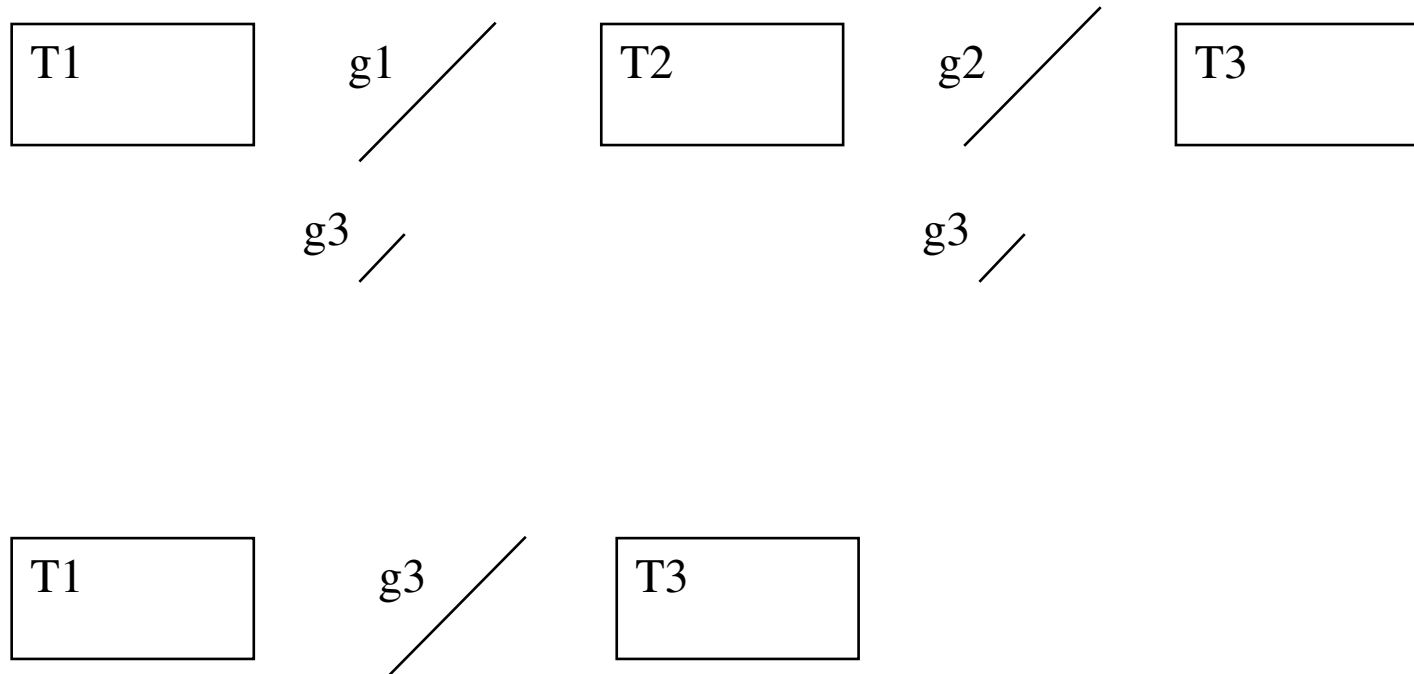
- (T1-T2)(T2-T3)

- In common
- Not in common



Analysis: T-test

- The smaller changes: T1 vs. T3

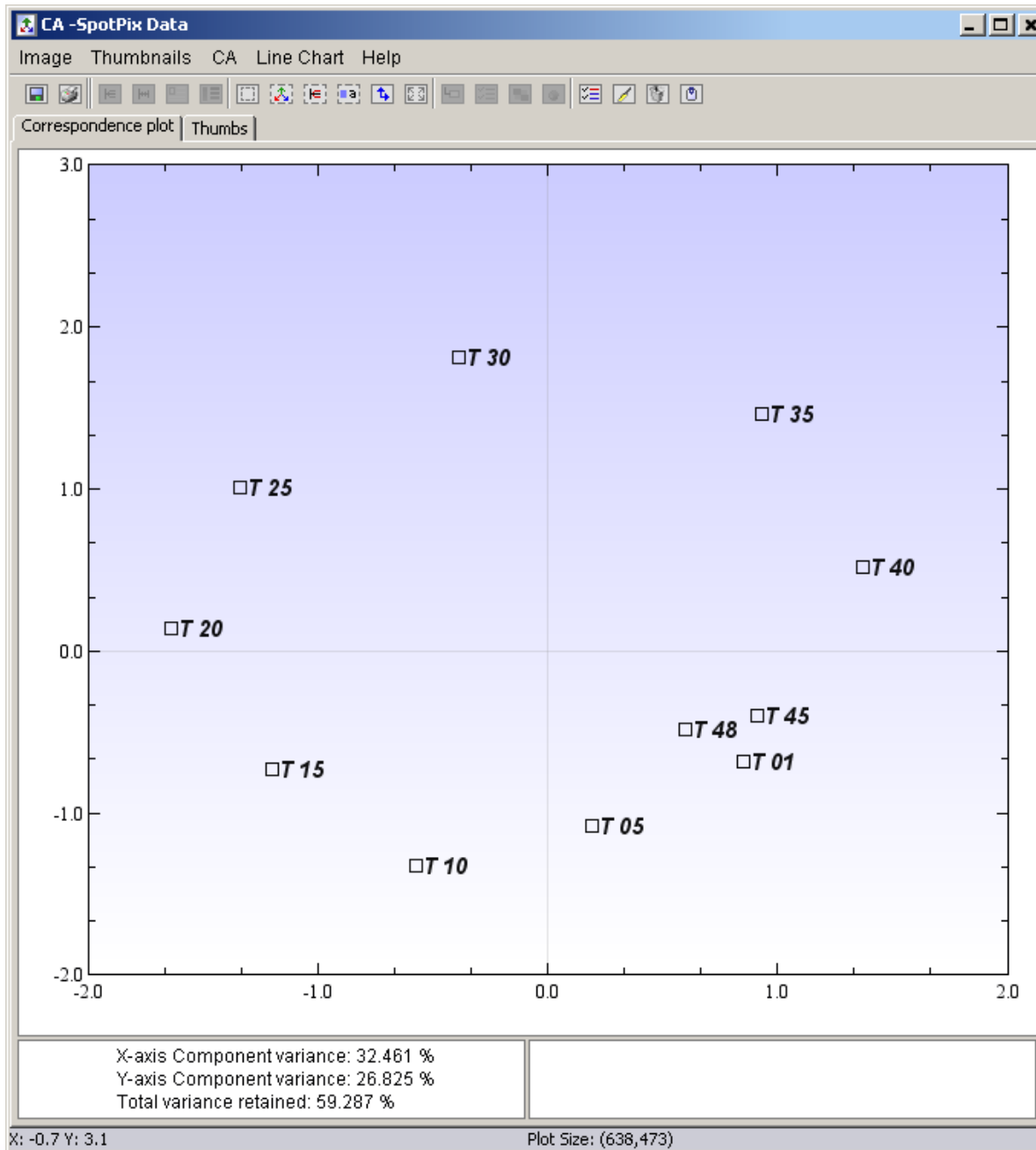


Analysis: T-test

- If you have a large time series, it can be time consuming to do this
- Hard to select the ones to compare
- Solution:
 - Compare all consecutive pairs of time points and then make a profile based on this
 - Other types of analysis: PCA, PLS, ...

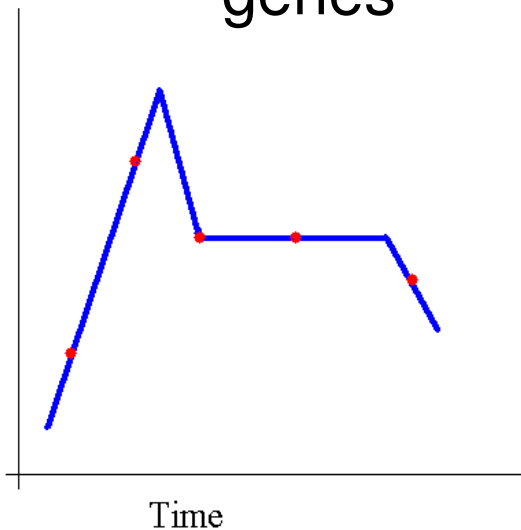
PCA

- Projecting the genes or samples into (a low dimensionality) space that displays most of the variance in the (high dimensionality) data
 - i.e. PC1 and PC2



Partial Least Square (PLS)

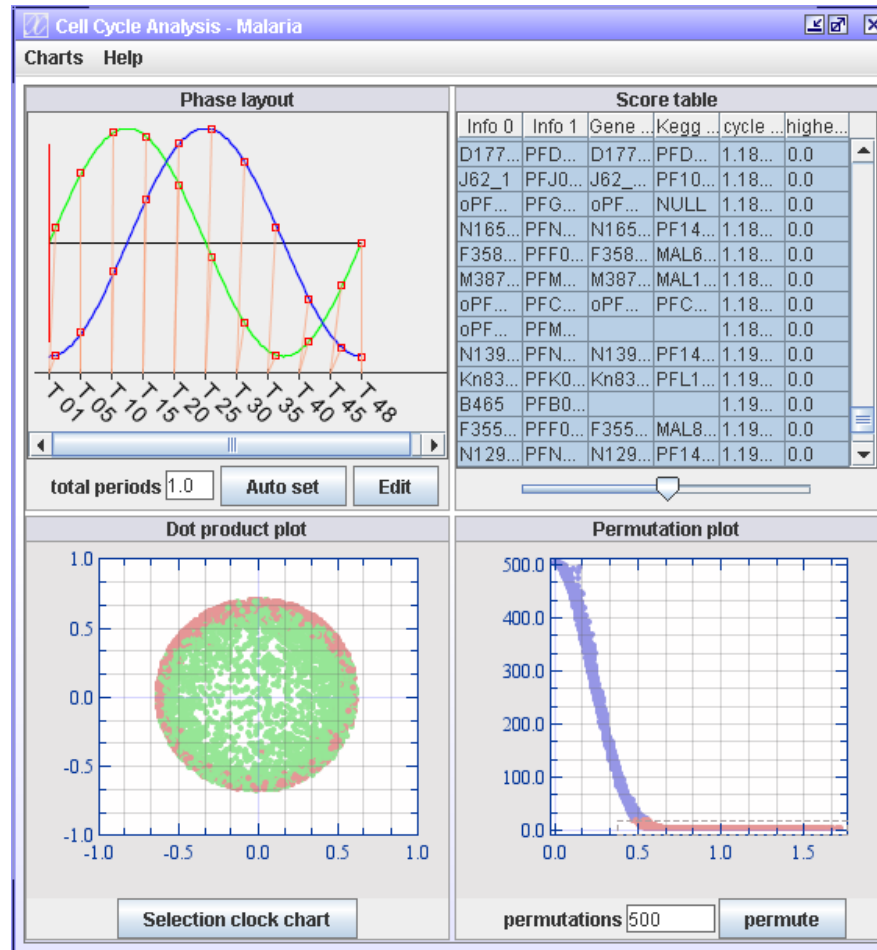
- Using external information to guide the projection
 - Instead of projecting the genes into a space that shows greatest variance, the genes are projected into a space that will help identify genes with particular characteristics, e.g. as below or cyclic genes



Example: Cycling genes

- Expectation of cyclic genes
 - One top and one bottom within one cycle
 - Time between top and bottom is half a cycle
- Sine and cosine are known shapes that have these characteristics
- Use sine and cosine to search for genes that are correlated with these shapes
- The combination of the two allows us to identify all phase shifts of these shapes

J-Express: Cellcycle



microarray.no

GSEA with continuous profiles

- The genes are ranked based on their correlation to an interesting (continuous) profile
- After ranking the genes, looking for genesets that are overrepresented towards the top of the ranked list is the same as for differential expression

GSEA with continuous profiles

- Use a gene profile from the dataset as the search profile
- Create a profile
 - Ascending or decending profile
 - Peak profile

Summary

- Time series experiments are now affordable and within reach for more groups.
- Time series experiments are more complex
- Time series experiments demand more planning

Acknowledgement

- Presentation made in collaboration with Ingrid Østensen (former NMC Oslo).