More on Preprocessing Microarray Data:

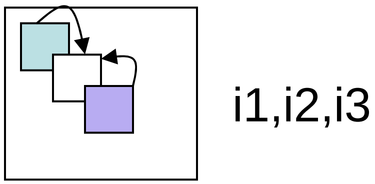
Missing Value Imputation, Preliminary transformations, Filtering.

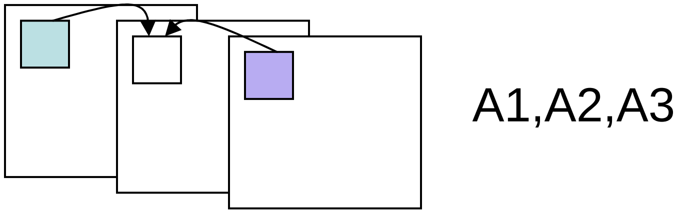
Missing Values:

Deleted rows (genes) with missing entries from the analysis?
… if the number of missing entries in a row is not too high we can retain the row, filling in the missing values according to some rationale.

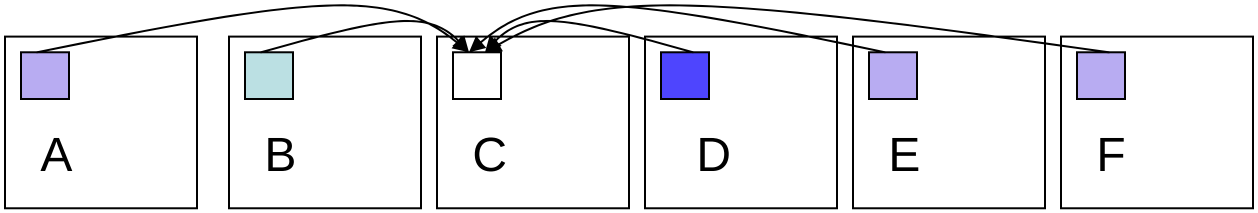
“Unsophisticated” solutions:

- Average on gene (spot;prb cell) and/or condition (chip) replicates – if available

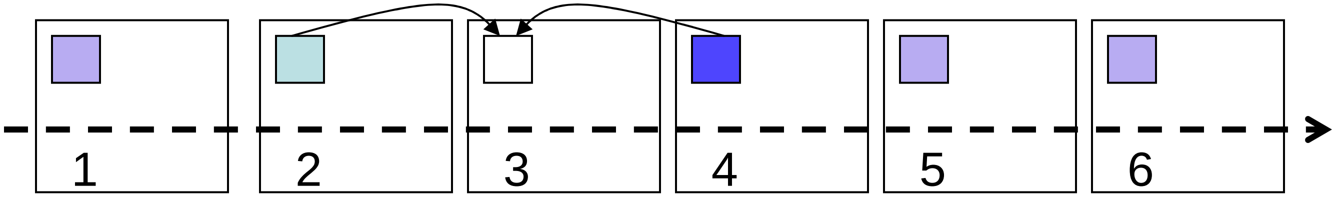




- Average on conditions (ave expression of gene whose profile has missing entries).



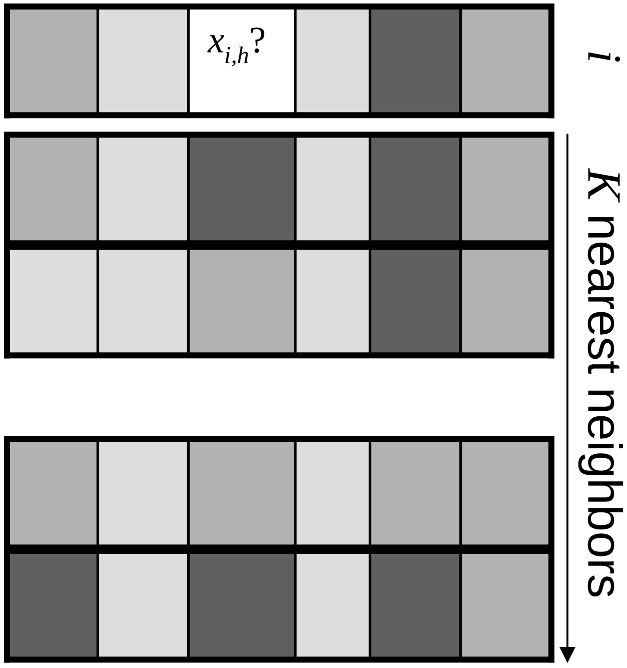
- For time courses (order), interpolation of nearby values in the profile.



None of these solutions exploits relationships among expression of different genes!

More “sophisticated” solutions (Troyanskaya *et al*. 2001)

K-nearest neighbors (KNN) imputation. Identify a set of genes whose expression profile is similar to the one of the gene with missing entries and take averages over these genes, with weights proportional to the similarity. Need to:



$$d_{(-h)}(x_i; x_{\tilde{i}}) \quad N_K(x_i) = \{K \text{ closest } x_{\tilde{i}}\}$$

$$w(x_{\tilde{i}}) = \phi(d_{(-h)}(x_i; x_{\tilde{i}})) \,,\, x_{\tilde{i}} \in N_K(x_i) \quad \text{(non-neg, sum=1)}$$

$$\hat{x}_{i,h} = \sum_{x_{\tilde{i}} \in N_K(x_i)} x_{\tilde{i}} w(x_{\tilde{i}})$$

- choose a metric for profile similarity (Euclidean, correlation, etc.)
- choose size of the neighbors set K (too small miss info, too large “dilute” pattern, but often robust in a range of sizes)

Works very well if gene profiles are clustered; it exploits co-expression relations among genes.

Also in this paper, SVD (i.e. principal components)-based imputation; exploit a few basic expression patterns characterizing the variability in gene profiles.

Missing value imputation: big research area in statistics.

If a data set contains a large share of missing entries, imputation can affect the analysis substantially (inducing spurious features).

In evaluating an imputation procedure, what assumptions can be made on the nature of the process that produces missing entries? Let

X = data = $(X(obs), X(miss))$
R = indicators of whether elements of X are obs or miss

- Missing completely at random: $Pr(R| X(obs), X(miss)) = Pr(R)$ does not depend on the values in X .
- Missing at random: $Pr(R| X(obs), X(miss)) = Pr(R| X(obs))$ depends on the values in X only through the ones we get to observe.
- Missing NOT at random: $Pr(R| X(obs), X(miss))$ depends also on the values we do not get to observe – most complicated situation.

Some analyses (e.g. mixture-based clustering) employ Expectation-Maximization algorithms that can perform imputation.

Multiple Imputation Methods; drawing repeatedly from imputation distributions (any imputation based on averaging will artificially reduce variability).

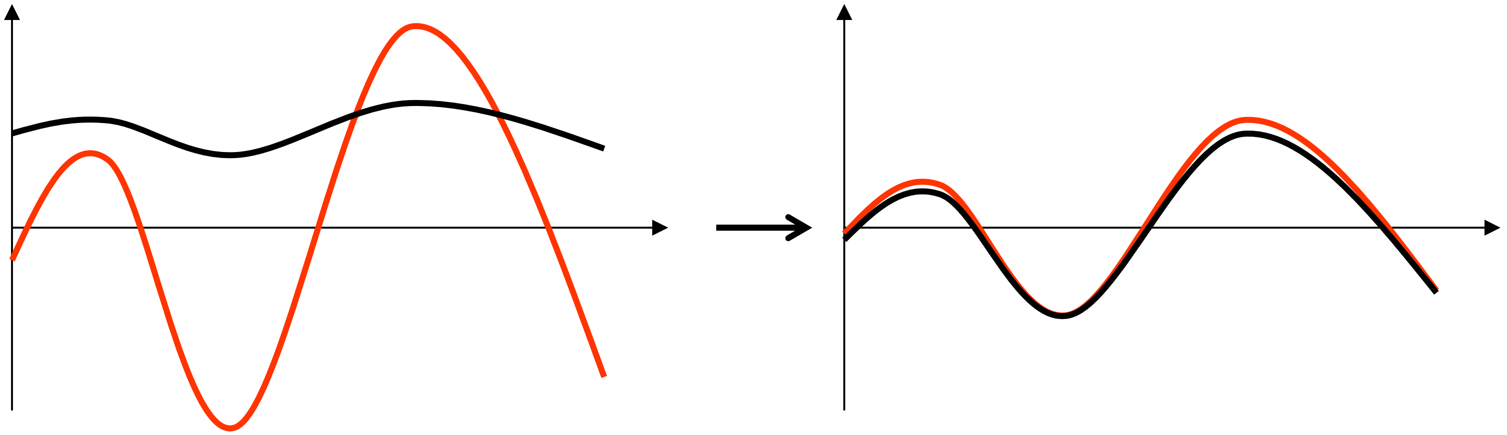
Other preliminary transformations and filtering:

1. Further improve comparability of measurements across experimental conditions and/or across genes with **centering and standardization**:

- by column of the data matrix (i.e. chip, experimental condition or replicate)
- by row in the data matrix (i.e. gene)

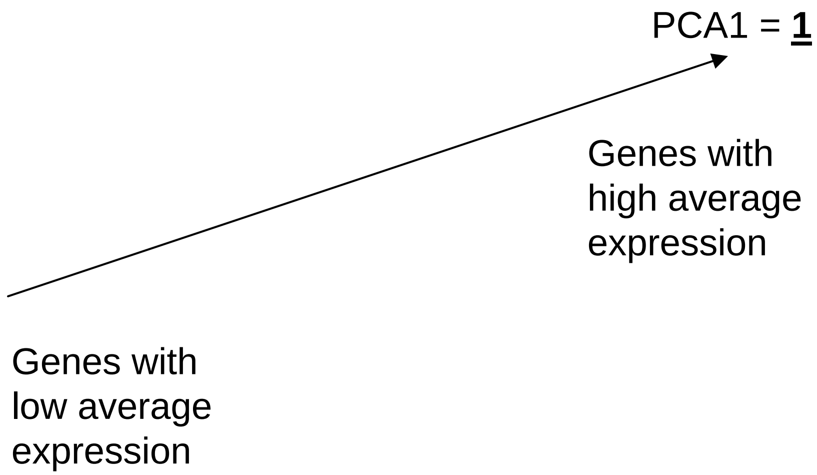
If both, need iterative procedures. Eliminate location and variation magnitude effects: restrict analysis to “pure shapes” (very common). Used in a number of applications.

By column: eliminate location or scale effects that “survived” normalization.
By row: especially when using differential expression and co-expression of genes as means to investigate regulation and co-regulation, often what matters is not the average level of expression or the variation magnitude in absolute terms… but the shape of a gene’s expression profile



Think of the gene profiles as a cloud of N points (genes) $X_1\ X_2 \ldots X_N$ in T dimensions (conditions).

If we do not center and standardize the data matrix by row (gene), many analyses will be dominated by a “small vs large transcription” signal – very strong **<u>1</u>** first PCA.



PCA1 = **<u>1</u>**

Genes with high average expression

Genes with low average expression

BUT: centering and standardizing the data matrix by row (gene) “creates” geometrical structure:

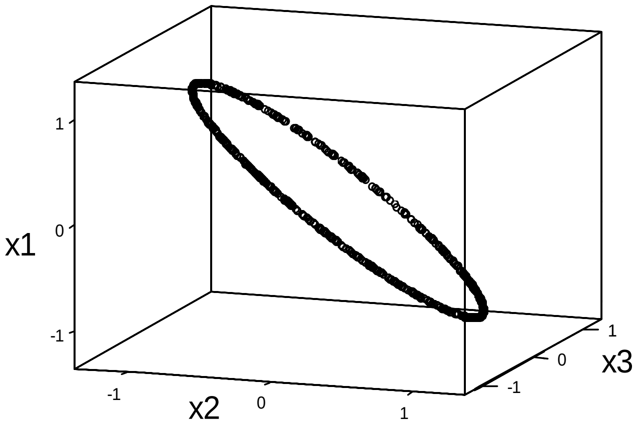
- Centering creates a linear constraint; points live on a hyperplane (dim T-1)

$$X_i{}'\ \underline{\mathbf{1}} = 0$$

- Standardizing puts points on a hypersphere

$$\|\, X_i \,\|^2 = 1$$

(with both, points will live on the intersection)

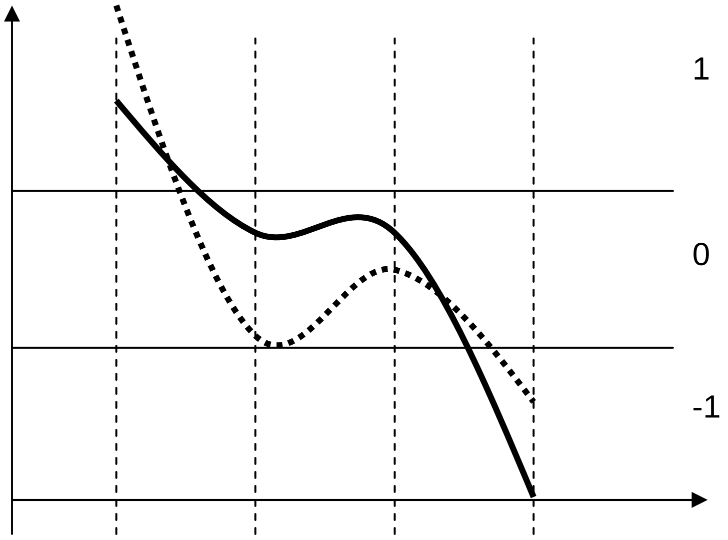


2. Further decrease the effect of unwanted sources of variation by eliminating “detail”, and systematic errors with it:

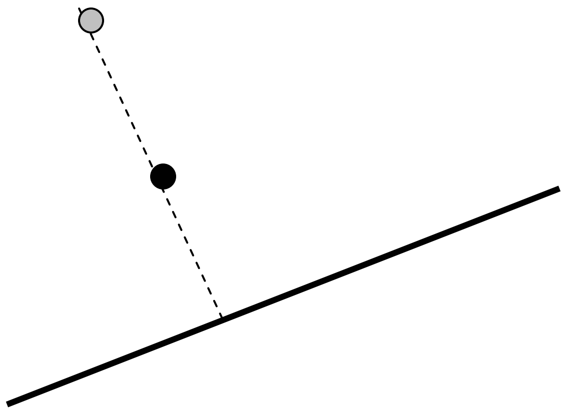
- **Quantization**; discretizing continuous data into (ordered) classes

- **Low-dimensional reconstructions**; approximating expression profiles through a small number of characteristic patterns.

BUT: an arbitrary quantization or low-dimensional reconstruction may induce misleading similarities in gene profiles.



Two profiles are discretized to 1, 0, –1 . Are they similar?



Two profiles share a 1-dimensional reconstruction. Are they similar?

3. Get rid of “inert bulk” that can affect detection of interesting signals by standard methods; **filtering** out genes that

- have very low expression in all conditions of interest (e.g. absent calls in affy, or small normalized affy signals by some other evaluation),
- have very small change wrt a “baseline” in all conditions of interest (e.g. small normalized log-ratios in spotted arrays),
- show very little variation in expression or log-ratios across conditions of interest.

IMPORTANT: NEED TO DO THIS BEFORE ROW (GENE) STANDARDIZATION

Preprocessing step: reduce the number of genes considered in further analyses, eliminate completely uninteresting genes – not an aim in itself (identifying genes presenting significant differential expression). Criteria and methods can be less stringent and/or rigorous, one will tend to retain a larger portion of the genes.

Retaining false positives: in preprocessing, it is better to err towards retaining false positives… just avoid that their number is so large as to obscure patterns of, and relationships among, true positives. However, when identifying differentially expressed genes (as main aim), false positives can be as bad as false negatives:

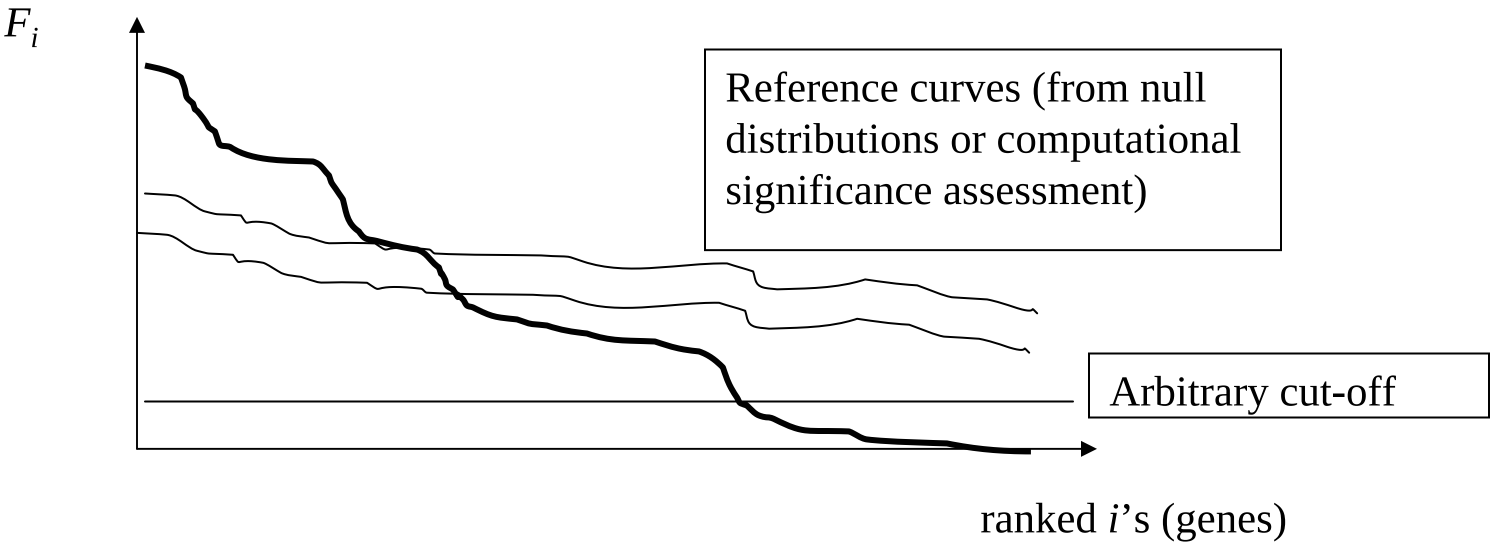
- wrong conclusions
- expenditure for further experimental validation.

(Note: false positive and false negative rates are in trade-off)

General idea: **use a statistic to create a ranking** (partial ordering of the genes)

For preprocessing, we create some arbitrary cut-off along the ranking in terms of
- a value for the statistic
- a number of genes
- a quantile (a percentage of the genes)

But for identifying differentially expressed genes we need to employ a testing mechanism: how many top-ranking genes have a “significant” value of the statistic? VERY multiple testing problem



Issues to remember:

- What is the appropriate "scale" to look at our measurements, given the questions we want to address, and the data analysis methods we want to employ?

- Do we introduce "structure" in the data by imputing missing values, or applying transformations (e.g. row centering and standardization)?

- What is the definition of unnecessary "detail"?

- Many data preprocessing steps (including some normalization and missing value imputation techniques; centering & standardizing by row; low-dimensional reconstructions; some definitions of "bulk" for filtering) move across the columns of the data matrix. Preprocessing of the numbers for an individual chip (condition) is "context dependent"; relative to:
  - the set of chips it will be analyzed with.
  - the data analysis methods and models we want to employ.

Do not perform these steps prior to data-basing of microarray information!