

Sample classification based on high-dimensional data

Hubert Rehrauer



University of
Zurich UZH

ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Example: Classifying tumors

- Tumor diagnosis: is a tumor benign or malignant?
- Classification according to physiological characteristics (appearance, size, shape, ...) not reliable
- Assumption: Malignancy is defined at the molecular level
- Approach: Make a molecular profile and predict malignancy
- More general idea: Use gene expression profiles to identify disease characteristics and disease subtypes

Example: Classifying samples for personalized medicine

- One aspect of pharmacogenomics is to find molecular markers that do predict whether a therapy will work or not
- Example:
 - Measure estrogen receptor status for tamoxifen (antihormone) therapy
 - Measure HER2/NEU status for herceptin therapy in breast cancer
- From the outcome of the measurement one can predict the final endpoint (which may be years away)
- In the above two examples the classification task is simple because the molecular markers that do predict the treatment outcome are known
- If the markers are not known the challenging part is to find which of the 25000 genes are predictive

Classification

A classifier

- is an algorithm or rule that can predict the class of a measured sample
- is always trained using data from a set of training samples with known class

Classification is also known as

- Machine learning
- Supervised classification

Mathematical formulation of the problem:

- Let \mathbf{x} be the vector of feature values for a sample
- x_i with $i=1,\dots,p$ are the measurements for the individual features (for RNA-seq these are the expression values of the p genes)
- Assume y is the class label of the sample
- We are looking for a function that predicts the class label given the feature vector:

$$\hat{y} = C(\mathbf{x})$$

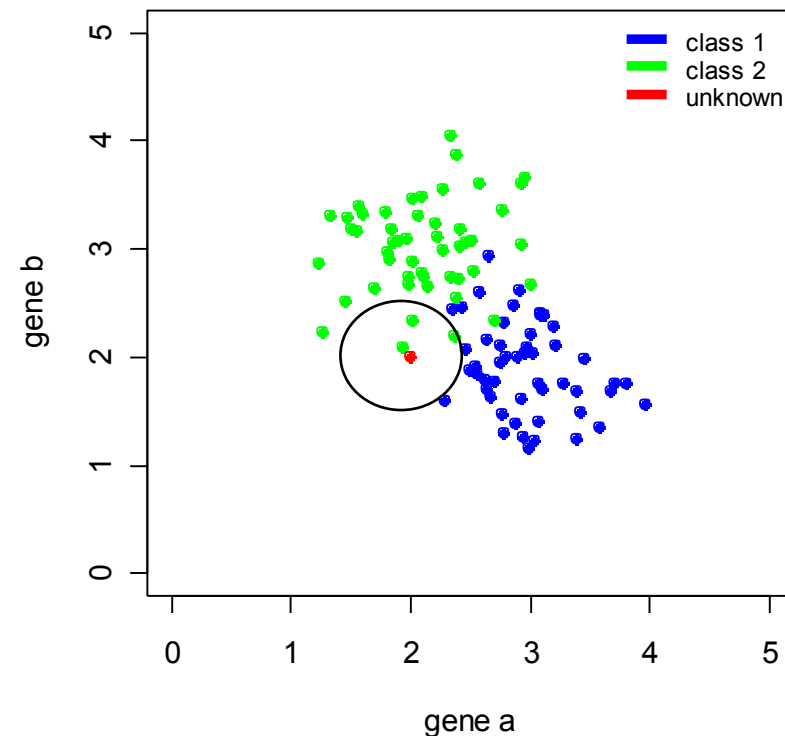
k-nearest neighbor (KNN) classification

Example:

- Sample classification using the measurements of 2 genes
- In the plot (right) every dot is a sample
- KNN rule with $k=3$: Given the unknown sample (red dot), find the 3 closest neighbors
- Assign the sample per majority vote of the k neighbors to one of the classes (in our example the predicted class is “green”)

Parameters

- k : number of nearest neighbors
- Distance measure: Euclidean



Classification using 20000 genes works accordingly

KNN properties

- very simple algorithm
- works in general as well as more complex algorithms
- naïve implementation is computationally very intensive, ($\sim N \log N$ where N is the number of data points)
- strictly consistent estimator (the error rate is at most twice the Bayes error rate if the number of training sample approaches infinity)
- gets worse if
 - noisy or non-relevant features are present
 - if the features are not scaled proportionally to their importance

Problems in classification

- Evaluating classifier performance
- Feature selection
- Optimizing classification
 - optimal method
 - optimal parameters

Classifier performance

- Accuracy: compute the overall rate of correctly assigned samples
- Problematic
 - if performance is different for the different classes
 - if classes have very different sizes

occurrence

Class A	Class B
500	50

correct classifications

Class A	Class B
400/500	0/50

Overall accuracy: $400 / 550 = 72.7\%$, but the error rate for class B is 100%

Balanced accuracy: compute for each class the rate of correctly assigned samples and average the rate across all classes

- $(400 / 500 + 0 / 50) / 2 = 80\% + 0\% = 40\%$

https://en.wikipedia.org/wiki/Accuracy_paradox

Precision and Recall

- If only one class is relevant we can interpret the outcomes as **positive** or **negative** and can define the error measures

- precision (positive predictive value)

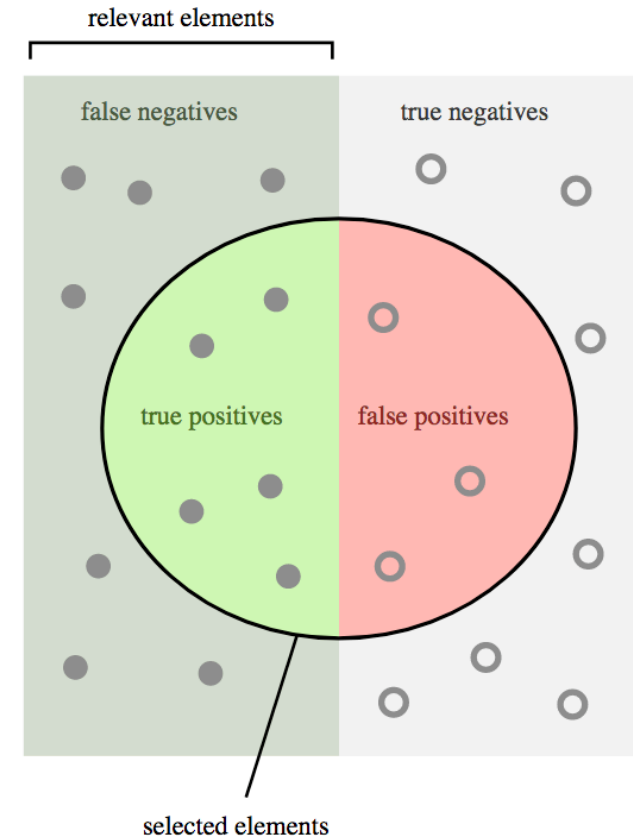
$$\frac{TP}{TP + FP}$$

- recall


$$\frac{TP}{TP + FN}$$

- F-measure – harmonic mean of precision and recall


$$F = 2 \cdot \frac{\text{precision} \cdot \text{recall}}{\text{precision} + \text{recall}}$$



How many selected items are relevant?

Precision = 

How many relevant items are selected?

Recall = 

Determining the error rate

- Cross-validation
- Subdivide the existing samples where the class is known in
 - Learning set
 - Test set
- Use the samples of the training set and define the classifier
- Classify the samples of the test set and compute the error rate
- Can be used to
- optimize parameters of the classifier
- select features to be used for classification
- select the distance measure
-

Cross-validation

- K-fold cross-validation
 - Subdivide the N samples in K groups T_1 to T_K
 - For $k=1, \dots, K$
 - use the k-th group as test set
 - use all other groups as learning set to build the classifier
 - classify the members of the k-th group and determine the error rate
 - repeat with different subdivisions
 - compute the average error rate
- Variants:
 - stratified cross-validation
 - reduces the variance of the error estimate
 - leave-one-out cross-validation:
 - extreme case $K=N$

Empirical and true error

- We are looking for a classifier with minimal error

$$E(C) = \sum_y \int \text{error}(C(\mathbf{x}), y) p(\mathbf{x}, y) d\mathbf{x}$$

- However we can only measure the empirical error based on our N samples and use this as estimator of the true error

$$\hat{E}(C) = \frac{1}{N} \sum_{n=1}^N \text{error}(C(\mathbf{x}_n), y_n)$$

- The empirical error is a biased estimator if several classifiers are used and one reports the empirical error of the best performing classifier.

Precision of the empirical error

- Only probabilistic statements can be made
- Chernoff bound for fixed classifier:

$$\Pr\left(\left|\hat{E}(C) - E(C)\right| < \varepsilon\right) = 2e^{-2n\varepsilon^2}$$

Precision of error estimate depends on samples size

In order to have:

$$\Pr\left(\left|\hat{E}(C) - E(C)\right| \geq 0.1\right) = 0.05$$

one needs N=184 samples

$$\Pr\left(\left|\hat{E}(C) - E(C)\right| \geq 0.05\right) = 0.05$$

one needs N=738 samples

Many samples needed to get a precise estimate of the error

Reported error rates are too optimistic (I)

- Cross-validation is used to find optimal classifier with optimal parameters
- Classifier has parameter \mathbf{a} :
- Based on many cross-validation runs the optimal parameter $\hat{\mathbf{a}}$ that minimizes the error is found:

$$\hat{y} = C(\mathbf{x}; \mathbf{a})$$

$$\hat{\mathbf{a}} = \arg \min_{\mathbf{a}} \text{Err}(C(\mathbf{x}; \mathbf{a}))$$

- The computed error is biased
- Since we chose the classifier with the smallest error, it is too optimistic
- When applying to real-world unknown samples the observed error will be larger

Reported error rates are too optimistic (II)

- Used set of samples is not representative
- Example: Quantitative omics data
 - RNA-seq experiments may have a batch effect
 - but usually all sample come from one batch (e.g. 6-month study in a single hospital)
- Classifier is optimized to classify samples of this batch correctly
- Error rates when classifying samples in a different study from a different hospital will be higher than predicted

Optimal classifier selection and negative bias in error rate estimation: an empirical study on high-dimensional prediction

Anne-Laure Boulesteix  and Carolin Strobl

BMC Medical Research Methodology 2009 9:85

<https://doi.org/10.1186/1471-2288-9-85> | © Boulesteix and Strobl; licensee BioMed Central Ltd. 2009

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Gene expression

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Over-optimism in bioinformatics: an illustration

Monika Jelizarow¹, Vincent Guillemot^{1,2}, Arthur Tenenhaus², Korbinian Strimmer³ and Anne-Laure Boulesteix^{1,*}

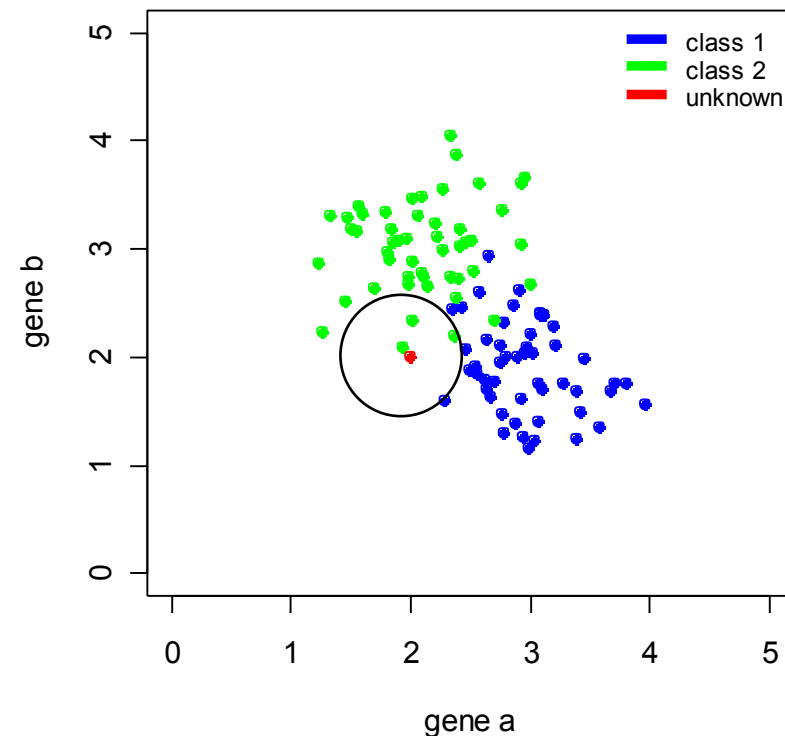
¹Department of Medical Informatics, Biometry and Epidemiology, University of Munich, Marchioninistr. 15, 81377 Munich, Germany, ²SUPELEC Sciences des Systèmes (E3S)-Department of Signal Processing and Electronics Systems - 3, rue Joliot Curie, Plateau de Moulon, 91192 Gif-sur-Yvette Cedex, France and ³Department of Medical Informatics, Statistics and Epidemiology, University of Leipzig, Härtelstr. 16-18, 04107 Leipzig, Germany

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Feature selection

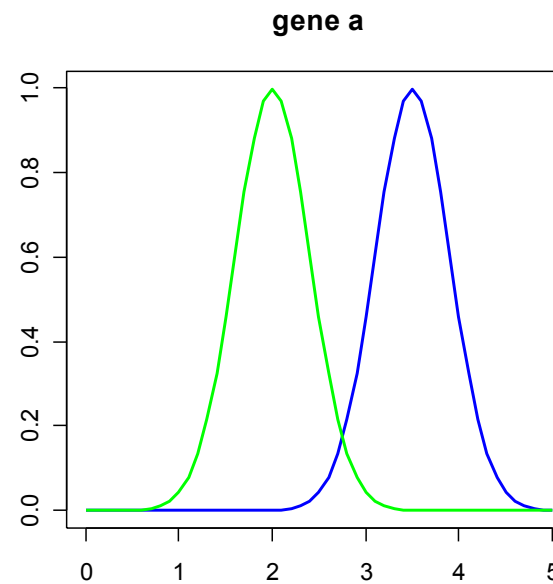
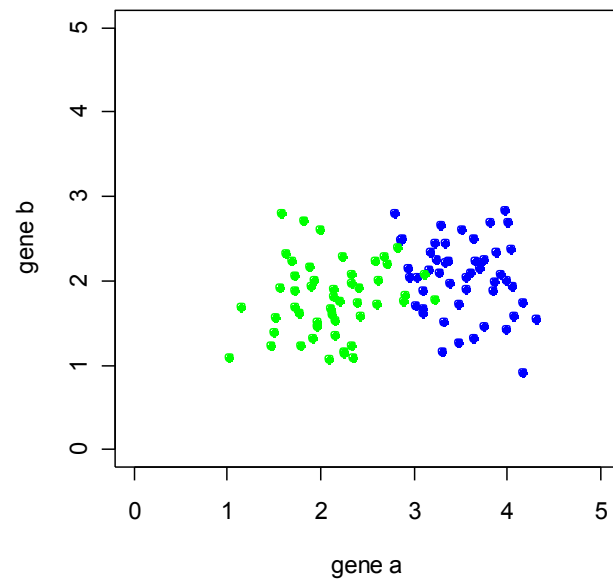
Example:

- Classification with 2 features (2 genes)
- 2-dimensional feature space
- RNA-seq measures 25 000 features
- More genes
 - more information on the samples
 - better classification?
- Not really
- Additional features improve the classification if and only if they provide additional information on class membership

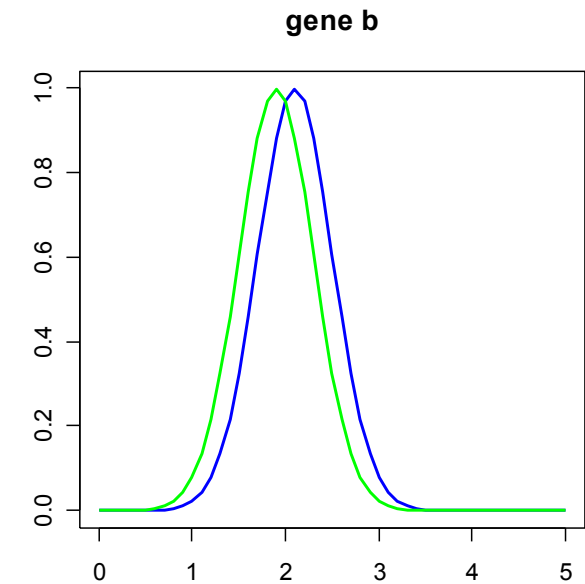


Feature selection

Example:

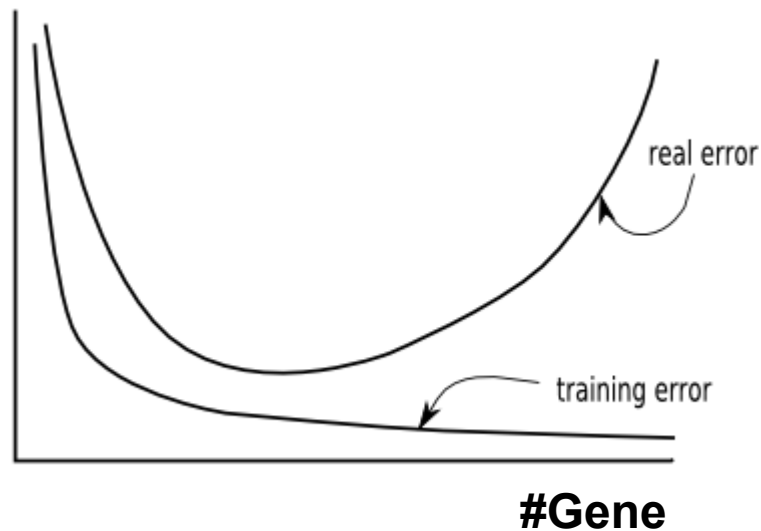


informative



non-informative

Feature selection



The training error decreases with increasing number of genes but the real error (when classifying new samples) does increase
 → Overfitting

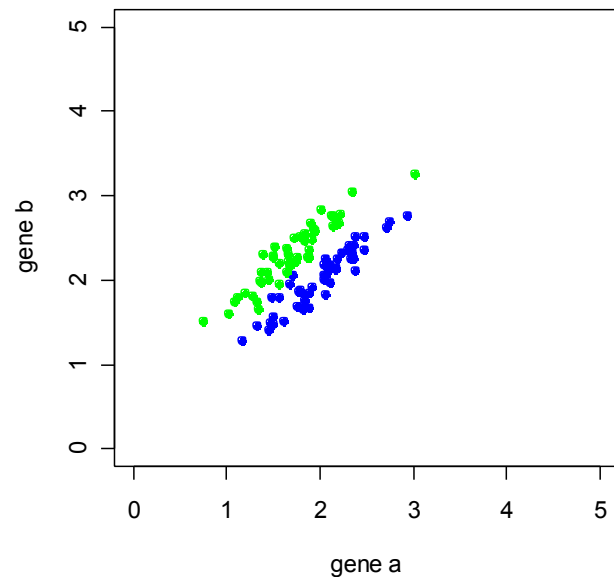
- Why is it not better to have more genes?
- Curse of dimensionality (Richard Bellman)
- Further disadvantages of many genes:
- Learning is slower
- Model is not interpretable

Feature selection: Methods

- Discriminant analysis:
- later ...
- Filtering based on information content of genes
- run t-test, ANOVA, ... and choose the m best genes
- Problems
 - filtering methods work on individual genes
 - significance does not imply predictive power
 - synthetically informative genes are discarded
 - correlated informative genes are kept
- Alternative Methods
- Nearest Shrunken Centroids (Tibshirani et al. 2002)
- Gene shaving (Hastie et al. 2000)
- Elimination of correlated genes (Yeung and Bumgarner 2003)

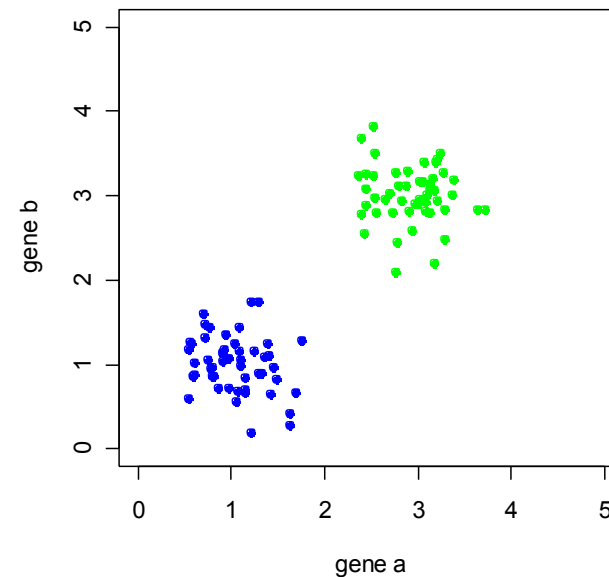
Example: Gene pairs

synthetically informative



Each gene alone cannot discriminate between the two classes
Both genes together allow class discrimination

correlated informative



Each gene alone does already discriminate the classes
Using both genes is no advantage

Distance measure

- Choosing an appropriate distance measure is key to successful classification
- Distance between two samples may be:
 - correlation of gene expression vectors
 - Euclidean distance of gene expression vectors
- Expression values have to be transformed, e.g. log-transformation, such that
 - high expressed genes (signal 20 000 – 40 000)
 - low expressed genes (signal 200 – 400)
 - contribute with similar weights to the classification

Classification algorithms

- K-NN: introduced earlier
- Discriminant analysis
Model probability densities within the classes
 - Linear discriminant analysis
 - Quadratic discriminant analysis
 - Fisher's discriminant analysis
 - Bayesian discriminant analysis
- Support Vector Machines:
Model class boundaries
 - linear kernel functions
 - quadratic kernel functions
- Top-scoring Pairs:
Find pairs of genes whose ordering is inversed in two classes

Discriminant analysis

- Starting point:
The p-dimensional feature vectors \mathbf{x} are multivariate normally distributed given the class k :

$$\Pr[\mathbf{x} | k] = \frac{1}{\sqrt{(2\pi)^p |\boldsymbol{\Sigma}_k|}} e^{-\frac{1}{2}(\mathbf{x} - \boldsymbol{\mu}_k)^T \boldsymbol{\Sigma}_k^{-1} (\mathbf{x} - \boldsymbol{\mu}_k)}$$

Linear Discriminant Analysis

- Assume each class has the same covariance matrix:

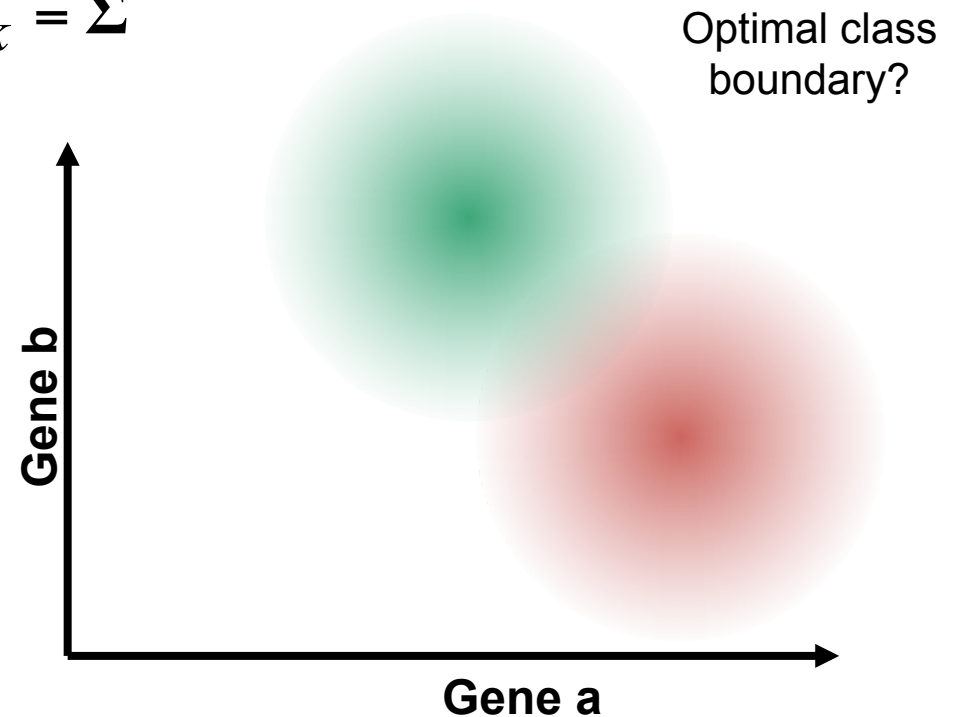
- Example

- 2 classes: $k=1,2$
- 2 genes: a, b

- Classification rule:

$$\hat{k} = \arg \max_k \Pr[\mathbf{x} | k]$$

$$\Sigma_k = \Sigma$$

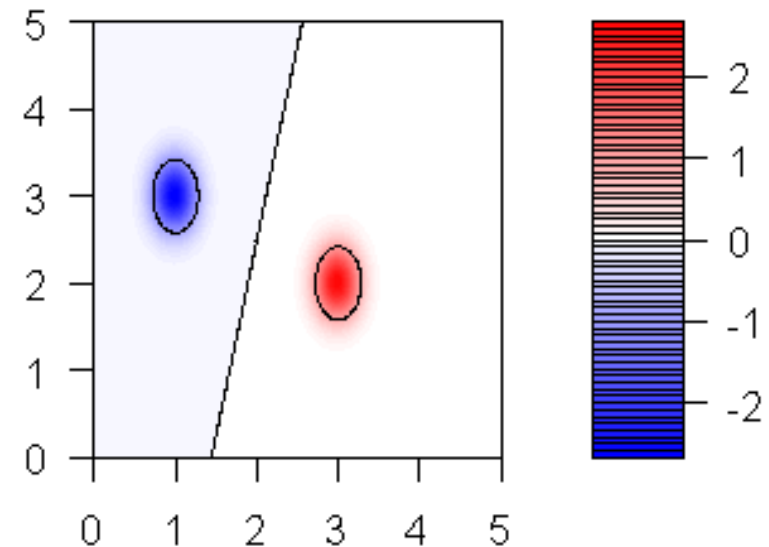


Linear Discriminant Analysis

- Visualize:

$$\Pr[\mathbf{x} \mid k = 1] - \Pr[\mathbf{x} \mid k = 2]$$

- Class boundary where the probabilities are equal
- In higher dimensions (more genes) this boundary is a hyperplane
- In order to classify an unknown sample, we look at which side of the boundary it is located



Linear Discriminant Analysis

- Compute the likelihood ratio:

$$\begin{aligned} \log \frac{\Pr[\mathbf{x} | k = 1]}{\Pr[\mathbf{x} | k = 2]} &= \frac{1}{2} (\mathbf{x} - \mu_2)^T \Sigma^{-1} (\mathbf{x} - \mu_2) - \frac{1}{2} (\mathbf{x} - \mu_1)^T \Sigma^{-1} (\mathbf{x} - \mu_1) \\ &= \mathbf{x}^T \Sigma^{-1} (\mu_1 - \mu_2) + C \\ &= \mathbf{x}^T \mathbf{w} + C \end{aligned}$$

$$\mathbf{w} = \Sigma^{-1} (\mu_1 - \mu_2)$$

Classification only depends on a scalar product
Linear in \mathbf{x}

$$\mathbf{x}^T \mathbf{w}$$

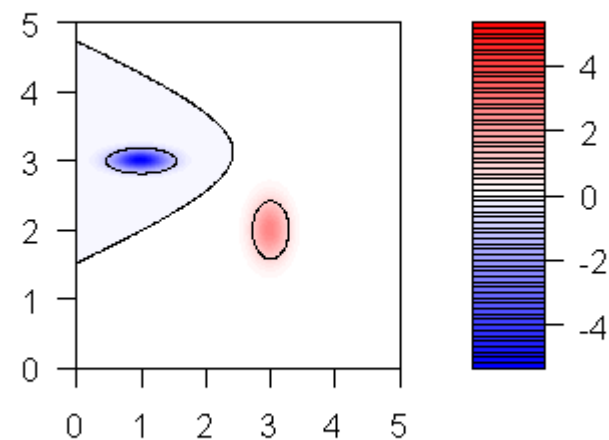
Quadratic Discriminant Analysis

- Assume different covariance matrices

$$\Sigma_1^{-1} \neq \Sigma_2^{-1}$$

$$\log \frac{\Pr[\mathbf{x} | 1]}{\Pr[\mathbf{x} | 2]} = \frac{1}{2} (\mathbf{x} - \boldsymbol{\mu}_2)^T \Sigma_1^{-1} (\mathbf{x} - \boldsymbol{\mu}_2) - \frac{1}{2} (\mathbf{x} - \boldsymbol{\mu}_1)^T \Sigma_2^{-1} (\mathbf{x} - \boldsymbol{\mu}_1)$$

- No simplification possible
- Class boundary is quadratic



Fisher's Discriminant Analysis

- Similar to linear discriminant analysis, but different approach
- Consider linear combination of features (scalar product) :

$$z = \mathbf{x}^T \mathbf{w}$$

- Average in class k:

$$E[z | k] = \boldsymbol{\mu}_k^T \mathbf{w}$$

- Variance in class k:

$$\text{Var}[z | k] = \mathbf{w}^T \boldsymbol{\Sigma}_k \mathbf{w}$$

- Empirical definition of separability of two classes

$$S = \frac{\sigma_{\text{between}}^2}{\sigma_{\text{within}}^2} = \frac{(\mathbf{w}^T \boldsymbol{\mu}_2 - \mathbf{w}^T \boldsymbol{\mu}_1)^2}{\mathbf{w}^T \boldsymbol{\Sigma}_1 \mathbf{w} + \mathbf{w}^T \boldsymbol{\Sigma}_2 \mathbf{w}}$$

- Is maximized by

$$\mathbf{w} = (\boldsymbol{\Sigma}_1 + \boldsymbol{\Sigma}_2)^{-1} (\boldsymbol{\mu}_2 - \boldsymbol{\mu}_1)$$

Fisher's Discriminant Analysis

- Is equivalent to linear discriminant analysis, if covariance matrices are identical
- Separability
 - is similar to F-statistic of the ANOVA
 - can be interpreted as signal-to-noise ratio for the class labels
- can be directly extended to more than two classes
- Finds a linear combination of features that provide good separability
 - Can be used for feature selection

Bayesian Discriminant Analysis

- As an extension if the classes have different sizes (different prior probabilities)
- Maximization of the a posteriori probability:

$$\begin{aligned}\hat{k} &= \arg \max_{k'} \Pr[k' | \mathbf{x}] \\ &= \arg \max_{k'} \frac{\Pr[\mathbf{x} | k'] \pi(k')}{\sum_g \Pr[\mathbf{x} | g] \pi(g)}\end{aligned}$$

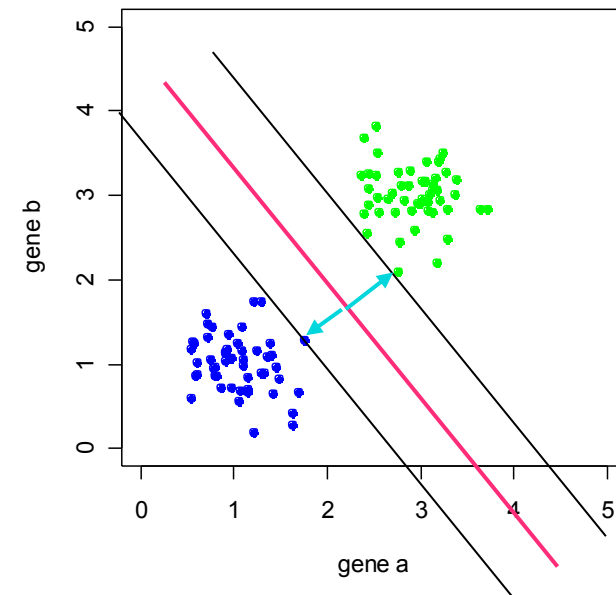
Additional consideration of the costs of misclassifications:

$$\hat{k} = \arg \min_{k'} \sum_g c_{k'g} \Pr[g | \mathbf{x}]$$

$c_{k'g}$ cost if a member of class g is wrongly assigned to class k'

Support Vector Machines

- Consider the boundary of two classes
- Find the **optimal boundary** (in the p-dimensional feature space: Hyperplane)
- A plane is optimal if it maximizes the **margins**
- The boundary is only defined by the points at the border line (support vectors)



Support Vector Machines

- Classification function:
classes: $y_i = \pm 1$

$$y_i = \text{sgn}(\mathbf{w}\mathbf{x}_i + b)$$

- For linear separable data:
Optimization with boundary conditions
using the Lagrange function:

Lagrange multiplier α_i

$$\frac{1}{2} \|\mathbf{w}\|^2 - \sum_i \alpha_i (y_i (\mathbf{w}\mathbf{x}_i + b) - 1)$$

- Classification function given by:

$$y = \text{sgn}\left(\sum_i \alpha_i y_i \mathbf{x}_i \mathbf{x} + b\right)$$

Support Vector Machines

Extension:

- Not linearly separable samples
Introduction of a slack variable that allows misclassifications at additional costs
- Kernel-Trick:
Map the data in to a space with higher dimensionality with the hope that the data are linearly separable in that space

SVM: Kernel Trick

Example:

Function h maps from the 2- to the 3-dim. space:

Classification requires the computation of the scalar product. But in order to compute the scalar product in the new space, the data do not need to be transformed.

Scalar products can be computed using a kernel function:

Computing the scalar product is efficient

SVM classification only needs scalar products

$$h(\mathbf{x}) = h\begin{pmatrix} x_1 \\ x_2 \end{pmatrix} = \begin{pmatrix} z_1 \\ z_2 \\ z_3 \end{pmatrix} = \begin{pmatrix} x_1^2 \\ \sqrt{2}x_1x_2 \\ x_2^2 \end{pmatrix}$$

$$\begin{aligned} h(\mathbf{x})h(\mathbf{x}') &= (\mathbf{xx}')^2 \\ &= k(\mathbf{x}, \mathbf{x}') \end{aligned}$$

$$y = \text{sgn}\left(\sum_i \alpha_i y_i k(\mathbf{x}_i, \mathbf{x}) + b\right)$$

SVM: Typical Kernel-Functions

- linear kernel

$$k(\mathbf{x}, \mathbf{x}') = \mathbf{x}\mathbf{x}'$$

- polynomial kernel

$$k(\mathbf{x}, \mathbf{x}') = (\mathbf{x}\mathbf{x}')^d$$

- Radial-Basis-Function kernel

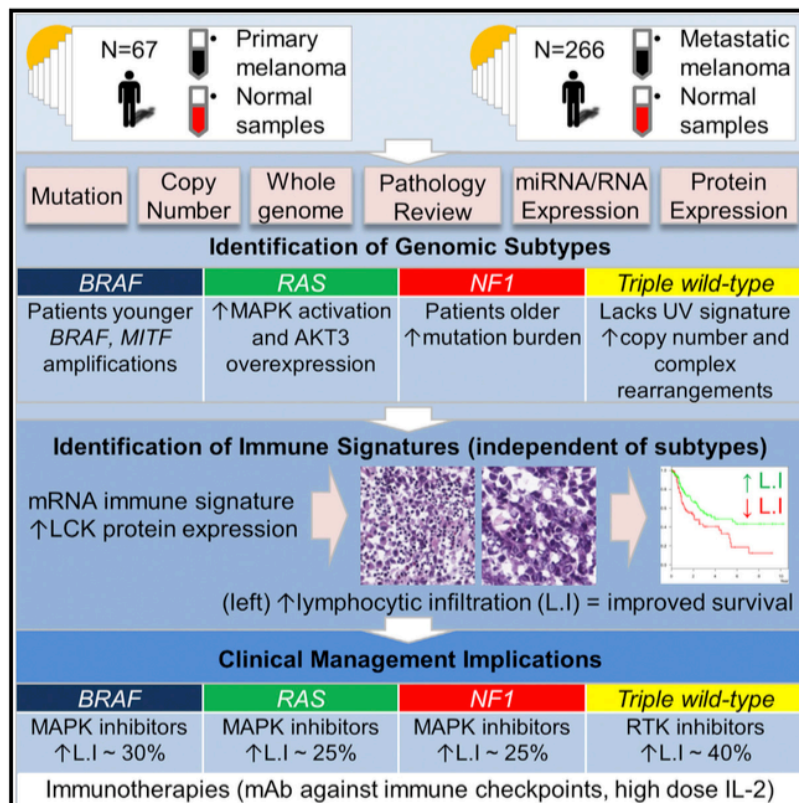
$$k(\mathbf{x}, \mathbf{x}') = \exp\left(-\frac{\|\mathbf{x} - \mathbf{x}'\|^2}{2\sigma^2}\right)$$

More classification methods

- LASSO
- Classification and Regression Trees (CART)
- Bagging: Aggregation of Classifiers by majority vote
- Boosting: Linear Combinations of Classifier outcomes
- Example
 - Random Forests -- Aggregation of Decision Trees (Breiman, 1996, 1998)
- Not considered:
 - What if sample represents a new unknown class?
 - What if sample belongs to more than one class?

Genomic Classification of Cutaneous Melanoma

Graphical Abstract



Authors

The Cancer Genome Atlas Network

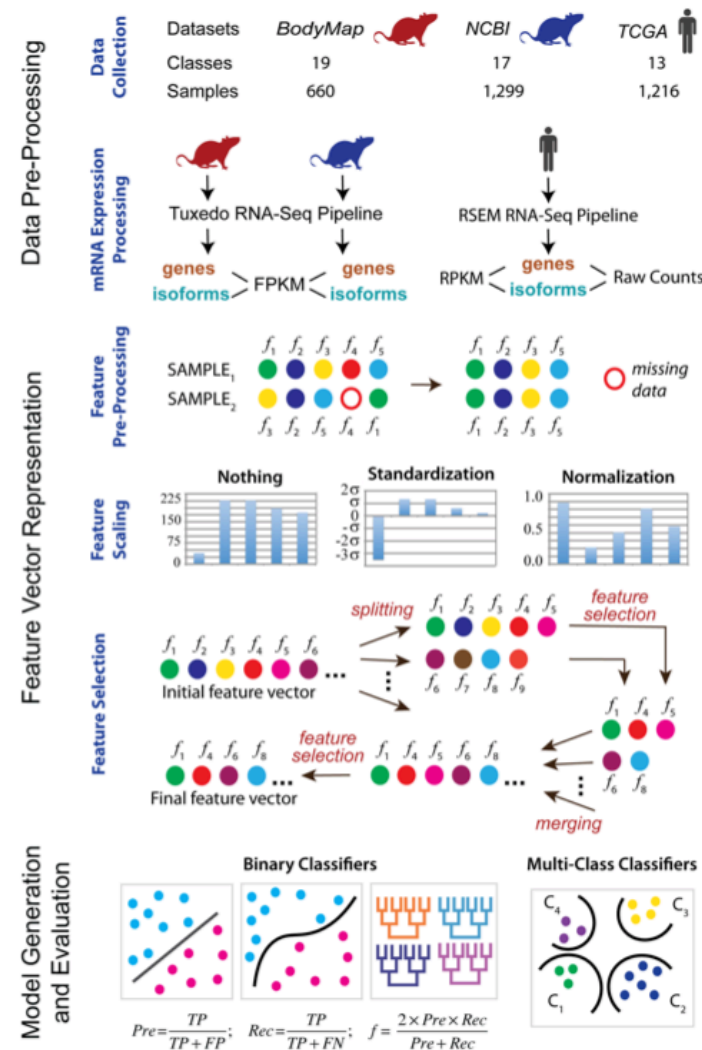
Correspondence

irwatson@mdanderson.org (I.R.W.),
jgershen@mdanderson.org (J.E.G.),
lchin@mdanderson.org (L.C.)

In Brief

An integrative analysis of cutaneous melanomas establishes a framework for genomic classification into four subtypes that can guide clinical decision-making for targeted therapies. A subset of each of the genomic classes expresses considerable immune infiltration markers that are associated with improved survival, with potential implications for immunotherapy.

Johnson NT, Dhroso A, Hughes KJ, Korkin D. Biological classification with RNA-Seq data: Can alternative splicing enhance machine learning classifier?
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iCOBRA: visualizing binary classification performance



Soneson C, Robinson MD. iCOBRA: open, reproducible, standardized and live method benchmarking. Nat Methods. 2016;13:283.

Classification in R

- Existing R packages
 - e1071: SVM
 - MASS: Linear und quadratic discriminant analysis
 - sma: Diagonal LDA
 - class: k-nearest neighbor
 - tspair: top-scoring pairs classifier
 - rpart: classification and regression trees (CART)
 - ipred: bagging
 - LogitBoost: boosting
 - MLSeq: Machine Learning for NGS data
 - MLInterfaces: defines consistent interfaces to many of the above classifiers
 -
- See also:
 - <http://cran.r-project.org/web/views/MachineLearning.html>

Summary

- Molecular profiling data provides insights into intracellular mechanisms that can be used for classification
- Classification with many features and few samples is problematic
- If classifier function is optimized this leads to over-optimistic error rates
- In many cases the simple classifiers, e.g. KNN, provide good performance

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