Supervised learning and analysis of microarray data

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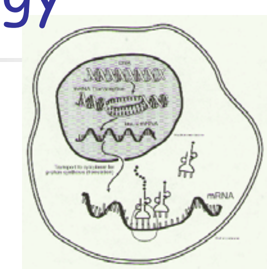
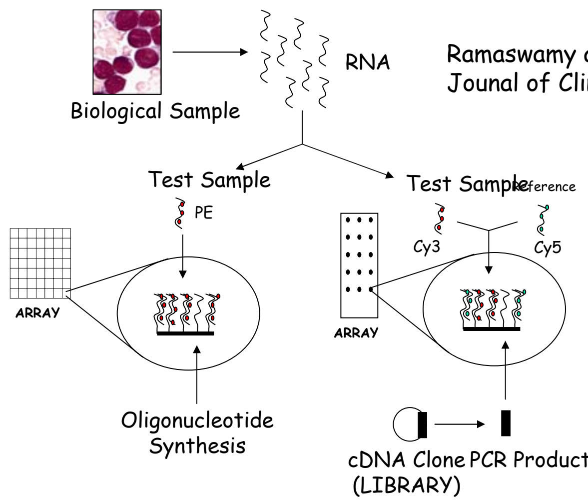
Comp 470

Microarray technology



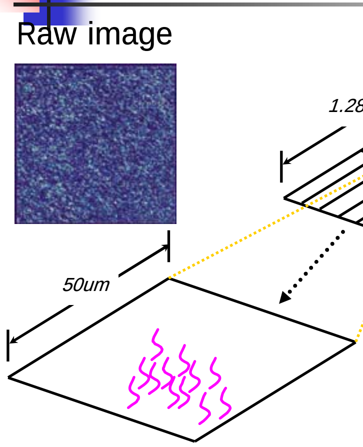
- Quick recap
  - Proteins: state of cell
  - Gene: codes for a protein
  - mRNA: helps assemble a protein
  - mRNA levels ~ gene exp. level ~ protein levels
- Microarrays measure the expression levels of thousands of genes at a time.
- Typical experiment: Measure expression of genes under different conditions and ask what is different at a molecular level and why.

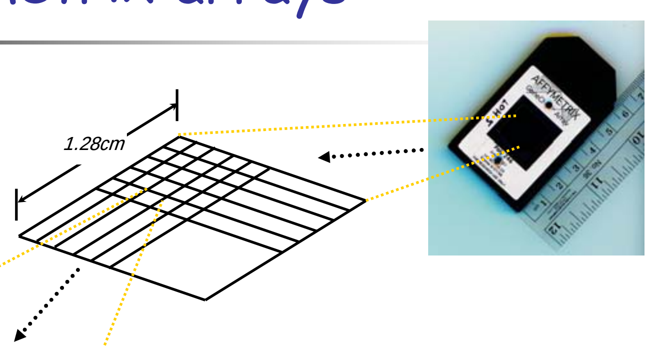
Microarrays



Ramaswamy and Golub,
Jounal of Clinical Oncology

Affymetrix arrays





~10^7 oligonucleotides,
half Perfectly Match mRNA (PM),
half have one Mismatch (MM)
Raw gene expression is intensity
difference: PM - MM

Microarray applications

- Biological discovery
  - new and better molecular diagnostics
  - new molecular targets for therapy
  - finding and refining biological pathways
- Recent examples
  - molecular diagnosis of leukemia, breast cancer.
  - appropriate treatment for genetic signature
  - potential new drug targets

Two computational tasks

- Classifying gene expressions: this week
  - What can be learnt about a cell from the set of all mRNA expressed in a cell? Classifying diseases: does a patient have benign prostate cancer or metastatic prostate cancer?
- Inferring regulatory networks: next week
  - What is the "circuitry" of the cell? What are the genetic pathways of cancer?

Common Approaches

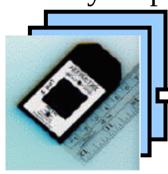
- Comparing two measurements at a time
  - Person 1, gene G: 1000
  - Person 2, gene G: 3200
  - Greater than 3-fold change: flag this gene
- Comparing one measurement with a population of measurements… is it unlikely that the new measurement was drawn from same distribution?

Classification

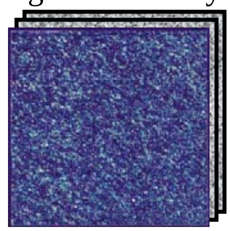
- Use our knowledge of class values, e.g., myeloma vs. normal etc., to gain added insight.
- Find genes that are best predictors of class.
  - Can provide useful tests, e.g. for choosing treatment.
  - If predictor is comprehensible, may provide novel insight, e.g., point to a new therapeutic target.

Classifying gene exp data

Microarray chips



Images scanned by laser



Gene	Value
D26528_at	193
D26561_cds1_at	-70
D26561_cds2_at	144
D26561_cds3_at	33
D26579_at	318
D26598_at	1764
D26599_at	1537
D26600_at	1204
D28114_at	707

Datasets

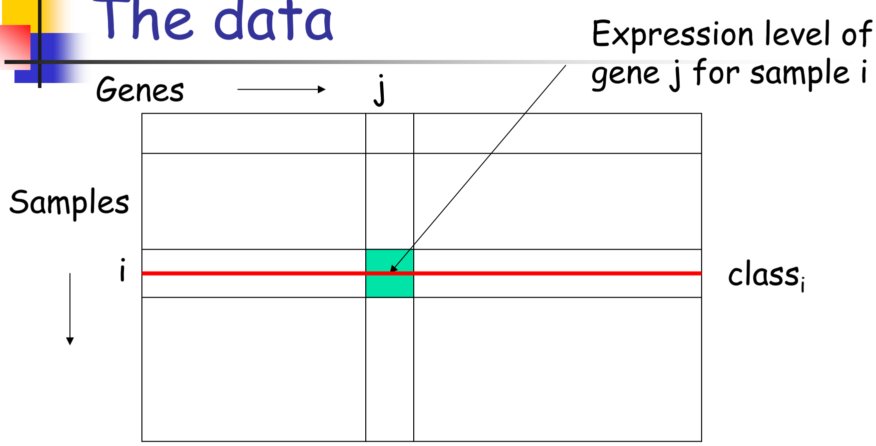
Class	Sno	D26528	D63874	D63880	...
ALL	2	193	4157	556	
ALL	3	129	11557	476	
ALL	4	44	12125	498	
ALL	5	218	8484	1211	
AML	51	109	3537	131	
AML	52	106	4578	94	
AML	53	211	2431	209	
...					

New sample

Prediction function

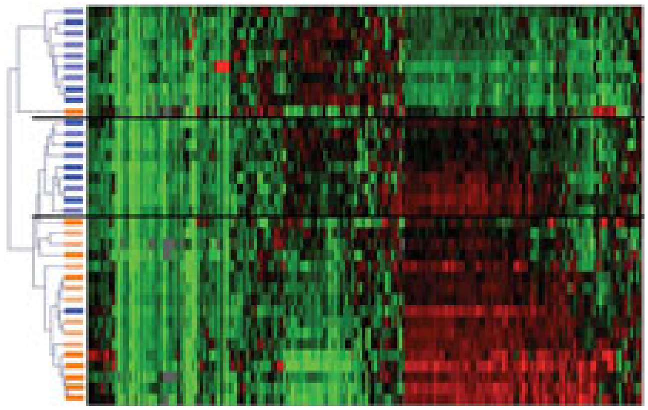
Prediction: AML or ALL

The data



Samples are labeled.
Red line denotes vector of exp levels for sample i

Heat maps



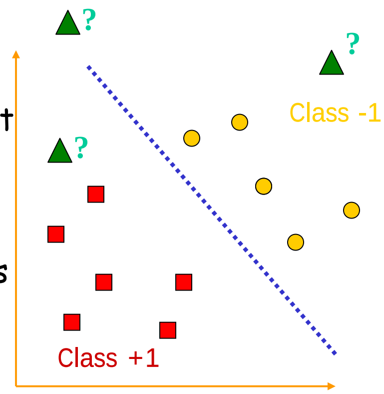
Challenges

- Microarray data inherit large experimental and biological variances
  - experimental bias + tissue heterogeneity
  - cross-hybridisation
  - 'bad design': confounding effects
- Microarray data are sparse
  - high-dimensionality of genes
  - low number of samples/arrays
  - *Curse of dimensionality*
- Microarray data are highly redundant
  - Many genes are co-expressed, thus their expression is strongly correlated.

Classification

Given examples drawn from two classes, learn to classify new examples into the correct class.

Each point represents a vector of gene expression levels

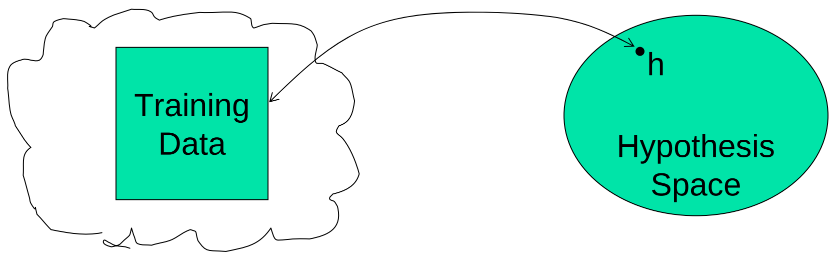


The classification problem

- Given training data $\{(x_1,y_1),\ldots,(x_m,y_m)\}$, x_i in R^n, y_i in $\{+1,-1\}$.
- Estimate function $h:R^n \rightarrow \{+1,-1\}$ such that h will correctly classify new unseen examples from the same underlying probability distribution as the training data.

Classification as optimization

- Set S of training data points
- Class H of hypotheses/models
- Optimization problem: Find the hypothesis/model h in H that best fits all data.



Objective function

- Minimizing training set error does not imply minimizing true error!

$$R_{train}[h] = \frac{1}{m}\sum_{i=1}^{m} \tfrac{1}{2}\left[h(x_i) - y_i\right]^2$$ Empirical risk

$$R[h] = \int \tfrac{1}{2}\left[h(x_i) - y_i\right]^2 dP(x,y)$$ True error

Statistical machine learning theory

- Non-asymptotic theory, based on finite samples which bounds true error in terms of training set error.
- Gives tradeoff between complexity of model and amount of data needed to learn it.

A bound on true error

- VC dimension theory allows us to relate train and test error for particular function classes. The key intuition is that the error of a function is not an absolute, but relative to the class of functions it is drawn from.

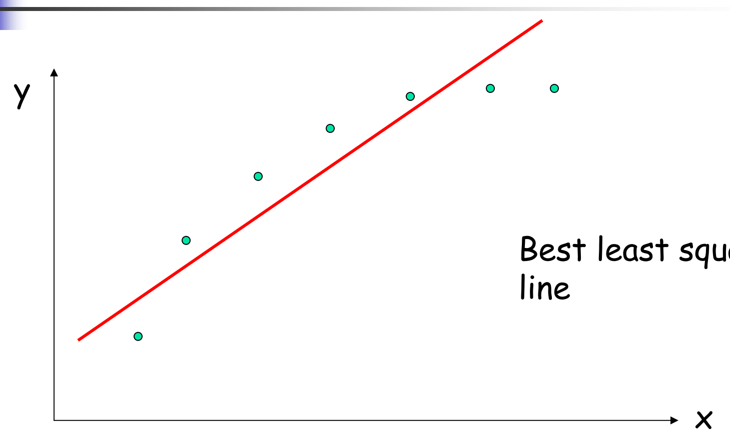
$$R[h] \le R_{train}[h] + \sqrt{\frac{VC(h)(\log 2m / VC(h) + 1) - \log(\delta/4)}{m}}$$

VC(h) is the VC dimension of the class from which h is drawn and delta is the probability bound, m is the size of the training set (Vapnik, 1995).

Tradeoffs

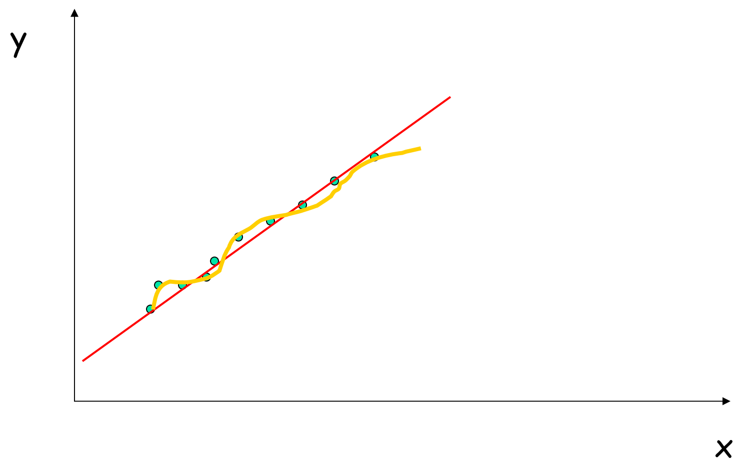
- With only a small amount of data, we can only discriminate between a small number of different hypotheses.
- As we get more data, we have more evidence, so we can consider more alternative hypotheses.
- Complex hypotheses give better fit to the data.

Simple hypothesis will under-fit



Cannot take advantage of more data!

Complex hypotheses will overfit



Adaptive hypothesis space selection

- Find hypothesis h to minimize
  error(h) + λ complexity(h)

Regularization

Support vector machines

- A new generation of learning algorithms based on
  - Non-linear optimization
  - Statistics
  - Functional analysis
- Come with theoretical guarantees on performance, because the learning problem can be reduced to convex optimization.

Applications

- SVMs have been used in a wide variety of tasks and are reputed to be the best for
  - Text categorization
  - Handwriting recognition
  - Classification of gene expression data

History

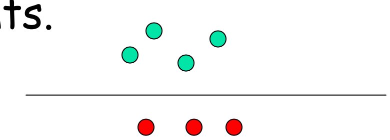
- Introduced in 1992 by Boser, Guyon and Vapnik (COLT 1992).
- Very rapid growth since then. 2 excellent textbooks and lots of new work both in theory and applications.
- www.kernel-machines.org is a great resource for learning about SVMs.

The Problem

- Given training data $\{(x_1,y_1),\ldots,(x_m,y_m)\}$, x_i in R^n, y_i in $\{+1,-1\}$.
- Estimate function $h:R^n \rightarrow \{+1,-1\}$ such that h will correctly classify new unseen examples from the same underlying probability distribution as the training data.

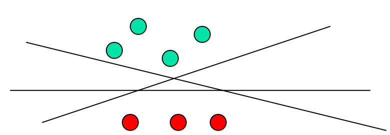
Linear support vector machines

- Consider the class of oriented hyperplanes in R^n.
  - $h(x) = \text{sign}(w.x + b)$
- If data is linearly separable, then there is a function from this class that separates the +1 points from the –1 points.

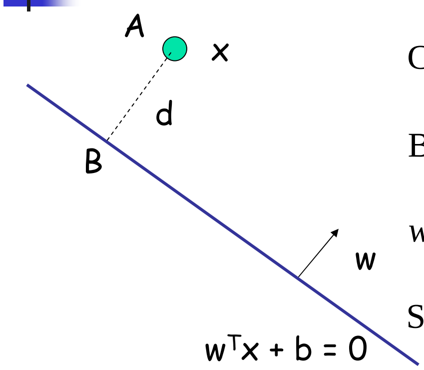


Linear separating hyperplanes

- Unfortunately, there are an infinite number of linear hyperplanes that separate the data!



Geometric Margin



Coordinates of $B = x - d\frac{w}{\|w\|}$

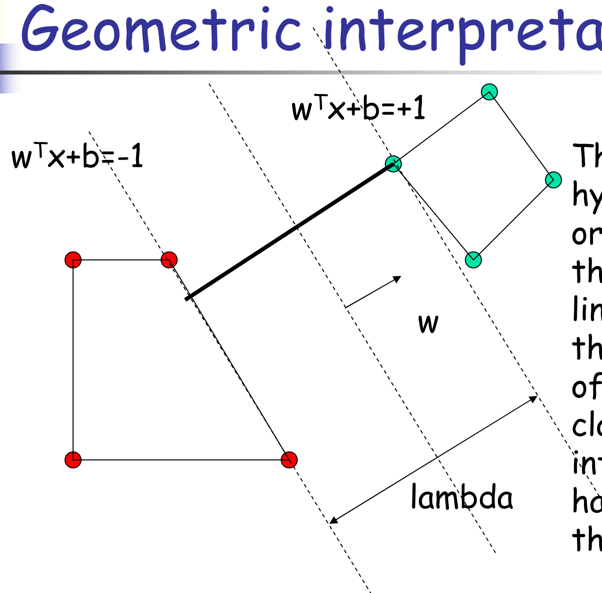
B lies on line defined by $w^T x + b = 0$

$$w^T\left(x - d\frac{w}{\|w\|}\right) + b = 0$$

Solving for d,

$$d = \frac{w^T x + b}{\|w\|}$$

Geometric interpretation



The optimal hyperplane is orthogonal to the shortest line connecting the convex hulls of the two classes and intersects it halfway between them.

Margin maximization

- Let x^+ and x^- be the two points on the convex hulls of the positive and negative data which are closest to the maximal margin hyperplane.

1. $w^T x^+ + b = +1$
2. $w^T x^- + b = -1$
3. $x^+ = x^- + \lambda \frac{w}{\|w\|}$

$w^T(x^+ - x^-) = 2$, from 1. and 2.

$\lambda = \frac{2}{\| \mathrm{w} \|}$, from 3 and above.

Lambda is the margin width, It is inversely proportional to w.w. So to maximize margin, we minimize w.

Optimal separating hyperplane

- Among all separating hyperplanes, there is one with the maximum margin.
- A hyperplane separating data $(x_1,y_1),\dots,(x_m,y_m)$ satisfies
  - $(w.x_i) + b >= 1$ if $y_i = +1$
  - $(w.x_i) + b <= -1$ if $y_i = -1$
- Or in short…
  - $y_i[(w.x_i)+b] >= 1$, for $i = 1..m$
- The optimal hyperplane satisfies the above conditions and has the minimal norm $||w||^2=w.w$

Learning the maximum margin classifier

Find w and b that minimize

$$\tau(\mathrm{w}) = \tfrac{1}{2}\|w\|^2$$

subject to

$$y_i(w^T x_i + b) \geq 1, \text{ for } i = 1..m$$

Quadratic programming!

Solving the quadratic program

$$L(w,b,\alpha) = \tfrac{1}{2}\|w\|^2 - \sum_{i=1}^{m} \alpha_i (y_i (w^T x_i + b) - 1)$$

L must be minimized with respect to w and b and maximized with respect to the Lagrange multipliers alpha_i

The first derivative with respect to w and b must vanish at the saddle point.

Solving the quadratic program

$$\frac{\partial L(w,b,\alpha)}{\partial w} = 0 \text{ which yields } \sum_{i=1}^{m} \alpha_i y_i x_i = w$$

This means w has an expansion in terms of a subset of the training data, namely those (x_i,y_i) for which alpha$_i$ > 0. These data points are called support vectors. None of the other data points matter. The maximal margin hyperplane is completely determined by the support vectors.

Solving the quadratic program

$$\frac{\partial L(w,b,\alpha)}{\partial b} = 0 \text{ which yields } \sum_{i=1}^{m} \alpha_i y_i = 0$$

$$\alpha_i \geq 0, i = 1..m$$

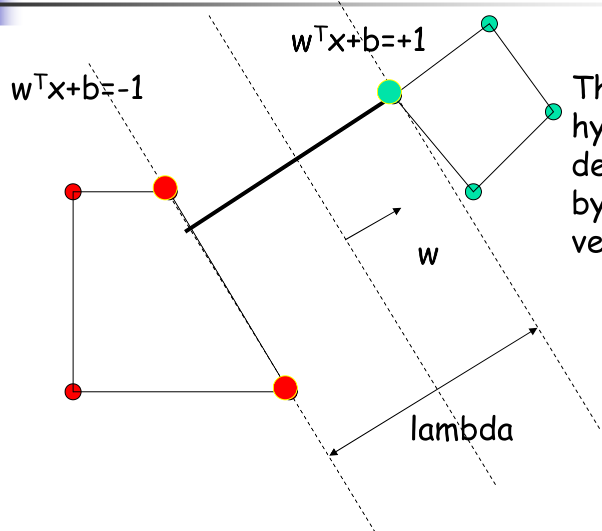
$$y_i(w^T x_i + b) - 1 \geq 0, i = 1..m$$

By the KKT complementarity condition,

$$\alpha_i(y_i(w^T x_i + b) - 1) = 0, i = 1..m$$

Support vectors lie on the margin, because when alpha$_i$ > 0, then y_i((w.x_i + b) –1) = 0.

Geometric interpretation



The optimal hyperplane is determined by the 3 support vectors.

Solution

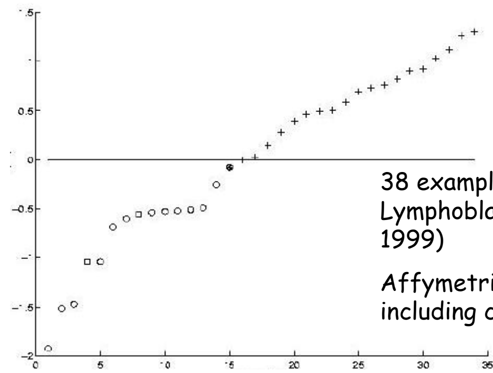
$$h(x) = sign\left(w^T x + b\right)$$
$$= sign\left(\sum_{i=1}^{m} (y_i \alpha_i (x^T x_i) + b)\right)$$

The hyperplane decision function uses the support vectors alone, and takes the dot product of the support vectors with x.

Note: b is calculated from the KKT comp. condn.

Cancer classification

d: distance from hyperplane



Test data

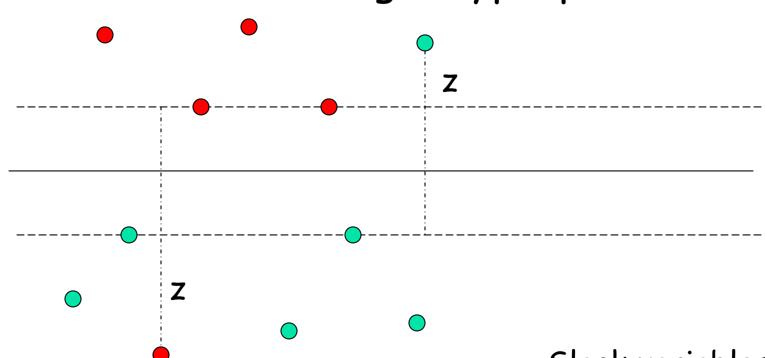
38 examples of Myeloid and Lymphoblastic leukemias (Golub et al, 1999)

Affymetrix human 6800, (7128 genes including control genes)

34 examples to test classifier

Extension to non-separable data

- Idea #1: soft margin hyperplane



Slack variables

Soft margin hyperplanes

$$\text{Minimize } \tfrac{1}{2}\|w\|^2 + c\sum_i \xi_i^\delta, \delta \geq 0$$

subject to

$$y_i(w^T x_i + b) \geq 1 - \xi_i, \xi_i \geq 0$$

For delta = 1, this is a convex optimization problem. We can set up the Lagragian and solve for w, b and zsi using the KKT conditions.

Solving the opt. problem

$$L(w,b,\alpha,\xi) = \tfrac{1}{2}\|w\|^2 + c\sum_{i=1}^{m}\xi_i - \sum_{i=1}^{m}\alpha_i(y_i(w.x_i + b) - 1 + \xi_i) - \sum_{i=1}^{m}\mu_i\xi_i$$

The KKT conditions

$$\frac{\partial L(w,b,\alpha,\xi)}{\partial w} = 0 \text{ which yields } w = \sum_{i=1}^{m} \alpha_i y_i x_i$$

$$\frac{\partial L(w,b,\alpha,\xi)}{\partial b} = 0 \text{ which yields } \sum_{i=1}^{m} \alpha_i y_i = 0$$

$$\frac{\partial L(w,b,\alpha,\xi)}{\partial \xi_i} = 0 \text{ which yields } c - \alpha_i - \mu_i = 0$$

$$\frac{\partial L(w,b,\alpha,\xi)}{\partial \alpha_i} = 0 \text{ which yields } y_i(w^T x_i + b) - 1 + \xi_i \geq 0$$

$$\text{KKT comp. condn. } \alpha_i(y_i(w^T x_i + b) - 1 + \xi_i) = 0$$

The solution

$$w = \sum_{i=1}^{m} \alpha_i y_i x_i$$

From the KKT complementarity condition, we get support vectors are the training data points for which

$$y_i(w.x_i + b) - 1 + \xi_i = 0$$

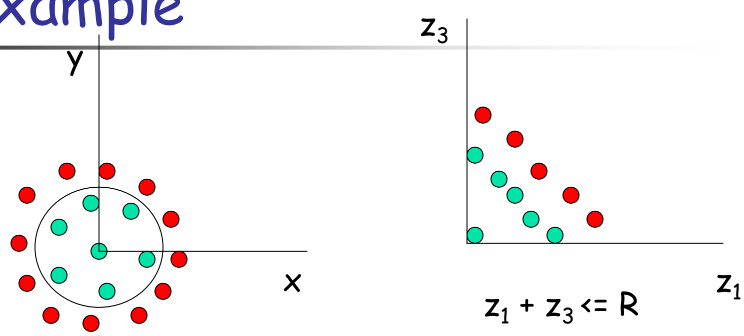
$$y_i(w.x_i + b) = 1 - \xi_i$$

That is, support vectors lie on the margin!

Non-linear support vector machines

- A generalization to handle the case when the decision function f is known to be not a linear function of the input x.
- Central idea: feature spaces. Map the x onto a higher dimensional feature space phi(x). Then, use linear support vector machines to obtain the optimal separating hyperplane in this high dimensional feature space.

Example



$$\varphi : \Re^2 \rightarrow \Re^3$$

$$\varphi((x, y)) = (x^2, \sqrt{2}xy, y^2)$$

Direct mapping

- Direct mapping to a high dimensional space suffers from the curse of dimensionality. To consider all d^{th} order products of an n-dimensional vector, we have to consider
  - (n+d-1)!/(d!(n-1)!) terms
- For n = 16x16, d = 5, we have a 10^{10} dimensional feature space.

A closer look at decision fn

- Note that decision function is of the form

$$h(x) = sign(w^T x + b)$$
$$= sign\left(\sum_i \alpha_i y_i (x^T x_i) + b\right)$$

- We only use dot products of the input vectors for determining the optimal separating hyperplane.

Kernels to the rescue

- If we want to find a separating hyperplane in the feature space, we need to compute the dot product of phi(x) and phi(x_i).
- Define a kernel function K which returns the dot product of the images of its two arguments

$$K(x_1, x_2) = \varphi(x_1)^T \varphi(x_2)$$

Non-linear support vector machines

- The decision function is of the form

$$h(x) = sign(w^T \phi(x) + b)$$

$$= sign\left(\sum_i \alpha_i y_i (K(x, x_i)) + b\right)$$

- We only use dot products of the input vectors for determining the optimal separating hyperplane.

Examples of kernels

- Polynomial kernel

$$K(x,y) = (x^T y)^d$$

- Second degree polynomial kernel

$$\phi((x_1,x_2)) = (x_1^2, \sqrt{2}x_1x_2, x_2^2)$$
$$\phi((y_1,y_2)) = (y_1^2, \sqrt{2}y_1y_2, y_2^2)$$
$$K(x,y) = \phi(x)^T \phi(y) = (x_1^2 y_1^2, 2x_1x_2y_1y_2, x_2^2 y_2^2)$$
$$= (x_1y_1 + x_2y_2)^2 = ((x_1,x_2)^T (y_1,y_2))^2 = (x^T y)^2$$

- Generalized polynomial kernel

$$K(x,y) = (x^T y + c)^d$$

More kernels

- Exponential kernel (Gaussian RBF)

$$K(x,y) = e^{\frac{-\|x-y\|^2}{2\sigma^2}}$$

- Tanh kernel

$$K(x,y) = \tanh(kx^T y - \delta)$$

Wolfe dual form

$$\text{Maximize}\, W(\alpha) = \sum_i \alpha_i - \frac{1}{2}\sum_{i,j} \alpha_i \alpha_j y_i y_j (x_i^T x_j)$$

$$\text{subject to}\; \alpha_i \geq 0; i = 1..m$$

$$\sum_{\mathrm{i}} \alpha_{\mathrm{i}} y_i = 0$$

Derived by substituting for w and b into L(w,b,alpha).

Advantage: maximization expressed in terms of dot products of the x's. Used for learning non-linear SVMs

Mercer condition

- Identifies the class of functions for which K(x,y) is the dot product of phi(x) and phi(y).
- See the excellent tutorial by C. Burges (available from www.kernel-machines.org) for a discussion of this condition.

General support vector machines

- We will substitute phi(x) for x in our previous formulation.
- Solutions are of the form:

$$
\begin{aligned}
h(x) &= sign(w^T x + b) \\
&= sign\left(\sum_{i=1}^{m} \alpha_i y_i (\varphi(x_i)^T \varphi(x) + b)\right) \\
&= sign\left(\sum_{i=1}^{m} \alpha_i y_i K(x_i, x) + b)\right)
\end{aligned}
$$

SVM demo

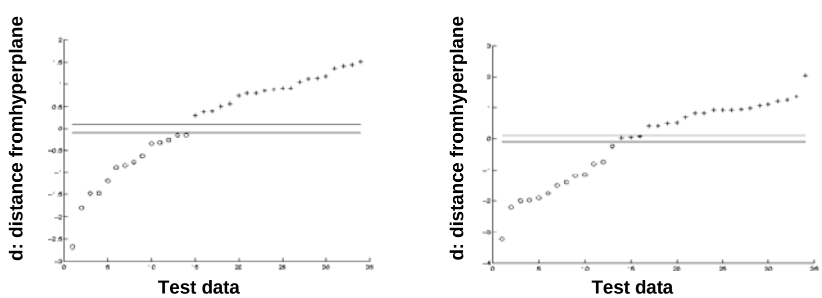
Click here

Feature selection

- SVMs as stated use all genes/features.
- Molecular biologists/oncologists seem to be convinced that only a small subset of genes are responsible for particular biological properties, so they want the "relevant" genes.

Results with feature selection

AML vs ALL: 40 genes 34/34 correct, 0 rejects.
5 genes 31/31 correct, 3 rejects of which 1 is an error.



M
u Mukherjee et. al. 2005

Two feature selection techniques

- Recursive feature elimination (RFE):
  based upon perturbation analysis, eliminate genes that perturb the margin the least.

- Optimize leave one out (LOO): based on the optimized leave-one-out error of an SVM.

Recursive feature elimination

1. Solve the SVM problem for vector $\boldsymbol{w}$

2. Rank order elements of vector $\boldsymbol{w}$ by absolute value

3. Discard input features/genes corresponding to those vector elements with small absolute magnitude (for smallest 10%)

4. Retrain SVM on reduced gene set and goto step (2)

Leave one out estimator

- Leave one point out, train on the others, test on the left out point.
- Repeat this for every point in the training data.
- Leave-one-out estimate is almost unbiased.

Leave-one-out feature selection

- Use the LOO estimator as an objective function in the search for subsets of features.