GS01 0163
Analysis of Microarray Data

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Lecture 22: Classification with Microarrays I

- Clustering and Classification
- Classification Methods
- LDA, DLDA, QDA
- K Nearest Neighbors
- Validation
- Classification and Regression Trees

Discovery and Prediction

Class Discovery is most closely associated with clustering; we are using structure inherent in the data to suggest groupings of interest.

Class Prediction, by contrast, is most closely associated with classification. Here, we start with a few samples from each of a few classes known to be of interest *a priori*, and try to allocate new observations to these classes.

How Classification Works

In general, the first step in classification with microarrays is to select a subset of genes to work with, since the entire set can be problematic.

Given a set of features, various methods are then used to divide up the space of possible values into regions that are “class 1”, “class 2” and so on. Typically, these regions will be divided by smooth curves defining a “decision boundary”.

Smooth boundaries have the advantage that they can suggest some biology.

Some Classification Methods

Linear Discriminant Analysis (LDA, aka Fisher's LDA)

Quadratic Discriminant Analysis (QDA)

Diagonal Linear Discriminant Analysis (DLDA)

Classification and Regression Trees (CART)

k Nearest Neighbors (KNN)

Support Vector Machines (SVM)

Classification and Regression Trees (CART)

Genetic Algorithms

A Data Set

Assessing the importance of BRCA1 and BRCA2 mutations in breast cancer. Initially introduced in Hedenfalk et al (2001), and filtered a bit in Simon et al (2003):

`http://linus.nci.nih.gov/BRB-ArrayTools.html`

under “Book” and “BRCA”.

Log ratio measurements on 3226 genes for 22 breast tumors, 7 with BRCA mutations and 8 with BRCA2 mutations.

Focus on dividing BRCA2 status groups.

Choosing a Subset of Genes

Most commonly, we pick those that show good univariate performance at separating the groups of interest, either by t-tests or Wilcoxon tests (2 groups) or ANOVA or Kruskal-Wallis tests (3 or more groups).

This makes a pretty strong assumption that there will not be any really useful interaction effects in the absence of important information from the individual components.

What we got Here

Here, we used pooled two-sample t-tests to contrast the 8 BRCA2 samples with the 14 others, and chose to focus on just the ones that had a p-value < 0.001 (there are 49 of these).

Telling Groups Apart

In telling two groups apart, a natural starting point is to use as simple a rule as possible – drawing a straight line, mapping every observation down onto that line, and cutting the line at some central point.

Conversely, we can think of this as taking the space and using a sheet to cut it in half – everything on the left side of the sheet will be classed in group 1, and everything on the right will be classed in group 2. This is a simple decision boundary.

So, how do we choose the best line to use?

Fisher's Linear Discriminant Analysis

In 1d, the problem is pretty straightforward – we find the two group means and place our cut precisely at the midpoint.

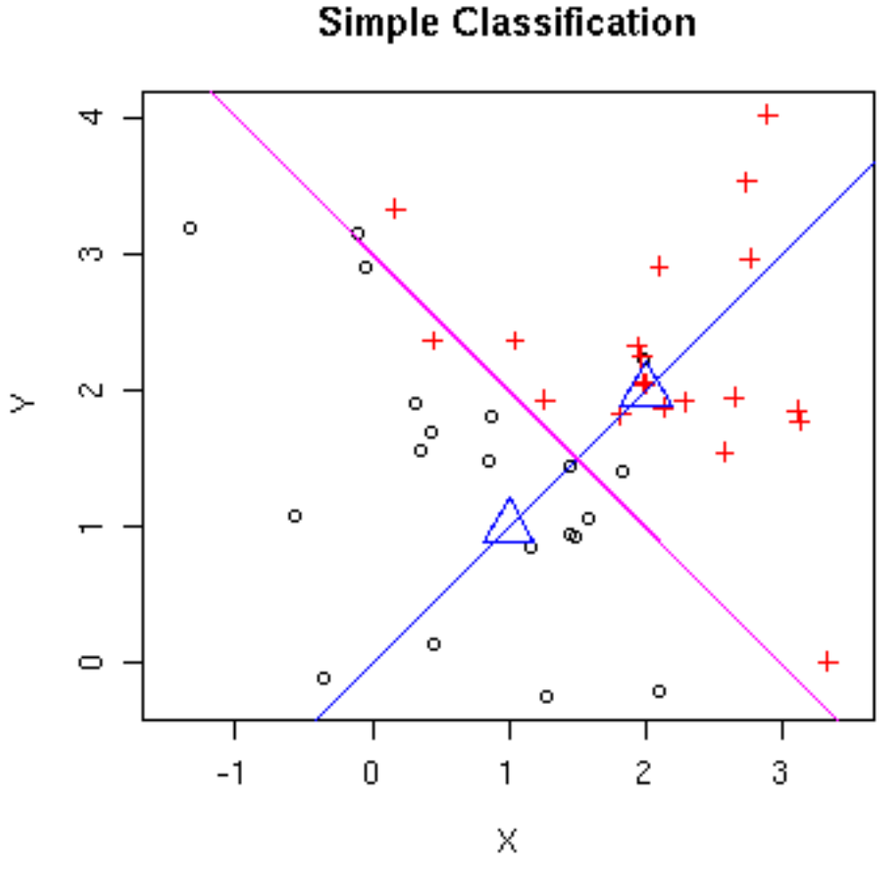
In 2d, this is still partially true – the optimal line to use is the one that connects the two group means, and the cut point is still at the middle. The question is one of how to map points in the plane down onto this line.

A Simple Example

Say that the two groups have centers at (1,1) and (2,2), and that the variances are the same on the two axes. Then

- the line joining the two is $y = x$,
- the center point is (1.5,1.5),
- and the best separating plane is orthogonal to the connecting line and passes through the center – $y = 3 - x$.

Simple Classification 1



Looks pretty easy...

Fisher's Linear Discriminant Analysis

Now say that something got screwed up, and the measurements on the x axis were sent in mm as opposed to m.

The centers are now (1000,1) and (2000,2), the central cut point is now (1500,1.5), and the connecting line is $y = x/1000$.

The optimal separating plane, however, is *not* orthogonal to this line.

If it were, the line would be $y = 1500001.5 - 1000x$.

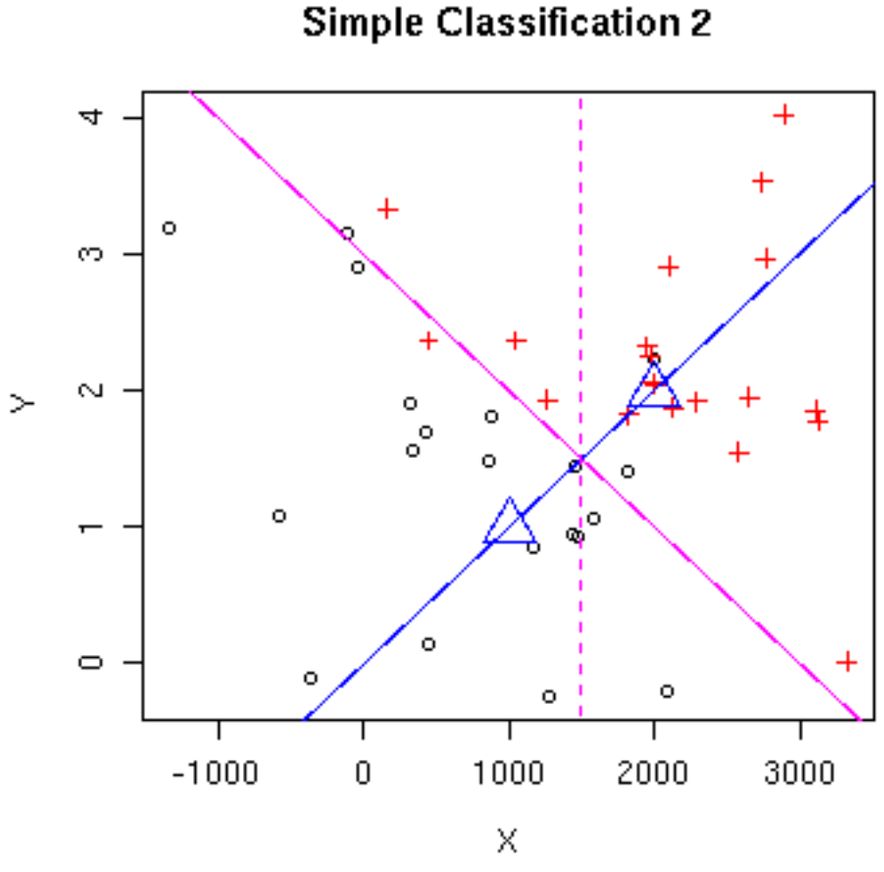
Fisher's Linear Discriminant Analysis

But all I did was stretch the x-axis from what it was before; the optimal separator should also stretch.

Since this separator hit the y axis at 3 before the stretch, it should still do so after. Thus, the new "best line" is $y = 3 - x/1000$.

The optimal separating line should not change if we simply change our measuring units, so we need a method that is scale-invariant.

Simple Classification 2



Hmm.

Extending the t test

The t test is scale-invariant:

$$\frac{\bar{x}_1 - \bar{x}_2}{s_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

as is its square

$$(\bar{x}_1 - \bar{x}_2) \left\{ s_p^2 (\frac{1}{n_1} + \frac{1}{n_2}) \right\}^{-1} (\bar{x}_1 - \bar{x}_2).$$

So we need to extend this by standardizing the data in general.

The Extension

Let

$$W = (\bar{x}_1 - \bar{x}_2)S^{-1}\left\{x - \frac{1}{2}(\bar{x}_1 + \bar{x}_2)\right\}.$$

Here S is the covariance matrix.

If $W > 0$, then x belongs to group 1, else it belongs to group 2.

We have the dividing line, a squashing of the space to standardize things, and a measurement along this line.

Some R Code

```
> library('MASS');
> ds1 <- matrix(rnorm(40), 2, 20) + 1;
> ds2 <- matrix(rnorm(40), 2, 20) + 2;
> data.set <- cbind(ds1, ds2)
> classes <- factor(c(rep('I', 20), rep('II', 20)))
>
> my.lda <- lda(t(data.set),grouping=classes)
> my.predictions <- predict(my.lda, t(data.set))
>
> table(my.predictions$class, classes)
    classes
      I II
  I  14  5
  II  6 15
```

Does this Work?

Well, it certainly did for the problems Fisher used it for back in 1936. For microarray data, however, there can be some problems.

Specifically, in order to use linear discriminant analysis, we need to compute the inverse of the sample covariance matrix, and if we have k variables that means that we are estimating $k(k+1)/2$ different variances, in addition to k means. That's a lot, and can get unstable if k^2 is an appreciable fraction of the total number of samples.

Does it Work Here?

Here, when we began, $n = 22$ and $k = 49$. Whoops. Inverting the matrix will not work at all; we have to restrict our attention to a smaller number of features (say 5).

Fixum Addum Hoccum

What if we don't use the entire covariance matrix, but rather just the main diagonal?

This is much better behaved for microarray data.

This approach is known as Diagonal Linear Discriminant Analysis (DLDA).

More R Code

```
> d1bar <- apply(ds1, 1, mean);
> d2bar <- apply(ds2, 1, mean);
> d1var <- apply(ds1, 1, var);
> d2var <- apply(ds2, 1, var);
> n1 <- ncol(ds1)
> n2 <- ncol(ds2)
> dvar <- ((n1-1)*d1var + (n2-1)*d2var)/(n1+n2-2);
> W <- (d1bar - d2bar) %*% diag(dvar) %*%
+       t(t(data.set) - (d1bar + d2bar)/2);
> table(classes, W>0)

classes FALSE TRUE
      I       7   13
      II     15    5
```

Further Extensions

So far, we've used means and covariance matrices, but we've assumed that the covariances are the same for the two groups.

If we assume that the two groups are both normally distributed, but that the two covariance matrices can be different, then the contours that get drawn result from drawing concentric ellipsoids about the two group centers, and the decision boundary can be curved. This is known as Quadratic Discriminant Analysis (QDA).

And More R Code

```
> library('MASS')
> my.qda <- qda(t(data.set),grouping=classes)
>
> qda.predictions <- predict(my.qda, t(data.set))
> table(qda.predictions$class, classes)
    classes
      I II
  I  15  6
  II  5 14
```

Back away from the Covariance Matrix

and nobody will get hurt. Easy now...

The discriminant methods that we have described so far are focused on mean or central behavior.

We can also carry over some ideas from the clustering/linkage realm, and decide to classify a new sample on the basis of the classification that holds for the known samples closest to it. If we look at the k nearest neighbors (KNN), then the sample is classified according to majority vote among the k.

Something Odd about the Neighbors..

KNN is most explicitly defined in terms of both training and test sets. It is very rarely discussed solely in terms of the training sets which allow us to partition the space into “group 1 regions” and “group 2 regions”.

This can be more flexible, and can produce some odd decision boundaries (a mixed blessing).

Even More R Code

```
library('class');

> my.knn <- knn(
+   train = t(data.set[,c(1:10, 21:30)]),
+   test  = t(data.set[,c(11:20, 31:40)]),
+   k = 1,
+   cl = classes[c(1:10, 21:30)])
> table(my.knn, classes[c(11:20, 31:40)])

my.knn I II
    I  4  8
    II 6  2
```

Relying on more neighbors

```
> my.knn <- knn(
+   train = t(data.set[,c(1:10, 21:30)]),
+   test  = t(data.set[,c(11:20, 31:40)]),
+   k = 3,
+   cl = classes[c(1:10, 21:30)])
> table(my.knn, classes[c(11:20, 31:40)])

my.knn  I II
    I   5 10
    II  5  0
```

How Good is the Rule?

How well does it classify the data?

Is this a valid test of the classifier?

Probably not. If the data to be predicted is used to train the classifier in the first place, then our results will look better than they should. This is the problem of “overfitting” the data.

Our Task

We need to think of a way to assess the prediction accuracy of the rule using samples that the rule hasn't seen.

So, where will these samples come from?

Cross-Validation

We can make use of the samples we have by using only some of them to fit the model, and then predicting the status of the one(s) we haven't seen.

We're going to do this in stages, working first in a context where there should be nothing going on to try to highlight the important issues a bit more clearly.

Working with Noise

```
# start with the null matrix
nothing.here <- matrix(rnorm(20000),
                       1000,20);

# Pick the best 5 t-test "genes"
ds1 <- nothing.here[,1:10];
ds2 <- nothing.here[,11:20];
mu1 <- apply(ds1,1,mean);
mu2 <- apply(ds2,1,mean);
var1 <- apply(ds1,1,var);
var2 <- apply(ds2,1,var);
varpool <- ((10-1)*var1+(10-1)*var2)/18;
```

Fitting the Best

```
nothing.t <- (mu1 - mu2) /
        sqrt(varpool * (1/10 + 1/10));
nothing.ranks <- rank(abs(nothing.t));
nothing.best5 <-
   nothing.here[nothing.ranks > 995,];

# Use LDA to separate the data and to
# predict the status of the 20 samples.
nothing.lda1 <- lda(t(nothing.best5),
  grouping=as.factor(
    c(rep(1,10),rep(2,10))));
```

Counting Successes

```
nothing.predictions1 <- predict(
  nothing.lda1,t(nothing.best5));
nothing.right1 <- sum(
  nothing.predictions1$class[1:10] == 1) +
                    sum(
  nothing.predictions1$class[11:20] == 2);
```

Here, we got 20 right. That looks a bit too good. How are we overfitting?

Leaving one Out

```
# Next, use cross-validation with the 5
# genes chosen above to fit the divider
# on 19, and to predict the status of
# the last one.

groupvec <- as.factor(c(rep(1,10),
                        rep(2,10)));
predvec <- rep(0,20);
for(i1 in 1:20){
  nothing.lda2 <- lda(
    t(nothing.best5[,-i1]),
    grouping=groupvec[-i1]);
```

Making the Predictions

```
  nothing.predictions2 <- predict(
    nothing.lda2,t(nothing.best5[,i1]));
    predvec[i1] <-
      nothing.predictions2$class;
}
nothing.right2 <-
  sum(predvec[1:10] == 1) +
  sum(predvec[11:20] == 2);
```

Doing this, we get 17 right. Still a bit high. How are we overfitting?

Refitting Everything I

```
# Next, use cross-validation by using
# 19 samples, defining the 5 best on
# the basis of those 19, and then
# predicting the status of the last case.

predvec3 <- rep(0,20);
for(i1 in 1:10){
  mu1 <- apply(ds1[,-i1],1,mean);
  mu2 <- apply(ds2,1,mean);
```

Refit the selection of the 5 genes to be used!

Refitting Everything II

```
var1 <- apply(ds1[,-i1],1,var);
var2 <- apply(ds2,1,var);
varpool <- ((9-1)*var1 + (10-1)*var2)/17;

nothing.t <- (mu1 - mu2) /
  sqrt(varpool * (1/9 + 1/10));
nothing.ranks <- rank(abs(nothing.t));
nothing.best5 <- nothing.here[
  nothing.ranks > 995,];
```

Refitting Everything III

```
  nothing.lda3 <- lda(
    t(nothing.best5[,-i1]),
    grouping=groupvec[-i1]);
  nothing.predictions3 <- predict(
    nothing.lda3,t(nothing.best5[,i1]));
  predvec3[i1] <-
    nothing.predictions3$class;
}
```

Here, we get 6 right.

Is This Consistent?

nothing.right1: 20,20,20,etc

nothing.right2: 17,18,20,etc

nothing.right3: 6,12,8,etc

Classification and Regression Trees (CART)

How does CART work?

CART assumes that the axes (genes) have inherent meaning, and tries to work with them directly as opposed to forming linear combinations.

This has some potential advantages in terms of interpretation, and in terms of specifying a rule.

CART splits the data using a series of binary decisions.

CART Questions

How should a split be chosen?

When should we stop splitting?

When we reach a terminal node (a leaf), what class should we say we've found?

Choosing a Split

Before we've done any splitting of the data, we have a mixture of cases and controls. We can view this as the root node, and initially we would say that this node has a certain amount of "impurity" – a node is said to be pure if all of the samples at that node are of the same class.

We want to

define a measure of impurity

find splits that reduce this measure

Defining Impurity

There are a few different mathematical ways of defining the impurity of a node; the two most common are

The entropy or information impurity:

$$-\sum_{classes} P(class) * \log_2(P(class))$$

The Gini index impurity:

$$1 - \sum_{classes} P(class)^2$$

Properties of Impurity

Both of these are peaked in the center – nodes that are split half and half are highly impure.

Similarly, both of these are 0 at the ends – nodes that are all of one class have no impurity.

Working with Gini

In the BRCA example, we compute the overall impurity by computing the impurity of the root node and multiplying it by the number of samples at that node. Here, this becomes

$$22 * \left\{ 1 - \left(\frac{8}{22} \right)^2 - \left(\frac{14}{22} \right)^2 \right\} = 10.18182.$$

Working with Gini

Now let's say that we can split this node according to the values of variable x_1. There are 13 samples with $x_1 < 0$, and all 13 of these have no BRCA2 mutations. There are 9 samples with $x_1 >= 0$, and 8 of these have BRCA2 mutations.

What is the impurity after this split?

Working with Gini Nodes

Here, we have to compute the values at the two nodes and combine them:

$$13 * \left\{ 1 - \left(\frac{13}{13} \right)^2 - \left(\frac{0}{13} \right)^2 \right\} +$$
$$9 * \left\{ 1 - \left(\frac{8}{9} \right)^2 - \left(\frac{1}{9} \right)^2 \right\} = 1.77778.$$

The reduction in impurity that we get by making this split is $10.18182 - 1.77778 = 8.40404$.

Observations about Splitting

In general, we choose the split giving the biggest overall reduction in impurity.

It's easier to split large nodes, as even small reductions in impurity are magnified by the number of samples involved.

At some point, however, it's not worth it anymore.

If we keep splitting the data until every node is completely pure, then in general we will have overfit the data. We want our splits to correspond to things we think are most likely to persist.

Ways to Stop Splitting

We can choose to stop if

The best reduction in impurity that we can get is below a certain threshold value.

The number of samples at a node gets below a specified threshold value.

Specifying these thresholds is something of a black art.

Some R Code

CART can also be viewed as *recursive partitioning*, and R uses the function `rpart`.

```
brcaSampleInfo$BRCA2
> brcaSampleInfo$BRCA2
 [1] - - - - - - + + + + -
[12] - - - - - - - + + + +
Levels: + -
brcaNumbersShort <- brcaNumbers[
   pvalsBRCA2 < 0.001,];

library('rpart');
```

Output I

```
treefit1 <- rpart(brcaSampleInfo$BRCA2 ~ .,$
      data.frame(t(brcaNumbersShort)))
> treefit1
n= 22
node), split, n, loss, yval, (yprob)
      * denotes terminal node

1) root 22 8 - (0.3636364 0.6363636)
  2) X914< -0.2607988 9 1 +
      (0.8888889 0.1111111) *
  3) X914>=-0.2607988 13 0 -
      (0.0000000 1.0000000) *
```

Output II

```
> summary(treefit1)
  n= 22

     CP nsplit rel error xerror      xstd
1 0.875      0     1.000   1.00 0.2820380
2 0.010      1     0.125   1.25 0.2919371

Node number 1: 22 observations,    complexity param=0
  predicted class=-  expected loss=0.3636364
    class counts:     8    14
   probabilities: 0.364 0.636
```

Output III (Split Not Unique?)

```
left son=2 (9 obs) right son=3 (13 obs)
Primary splits:
    X914  < -0.2607988 to the left,
      improve=8.404040, (0 missing)
    X2456 < 0.1496223  to the right,
      improve=8.404040, (0 missing)
    X2804 < 0.02940068 to the left,
      improve=8.404040, (0 missing)
    X35   < 0.5606404  to the right,
      improve=8.315152, (0 missing)
    X501  < 1.533854   to the right,
      improve=8.315152, (0 missing)
```

Output IV

```
Surrogate splits:
    X2977 < 0.1242674  to the left,
     agree=0.955, adj=0.889, (0 split)
    X35   < 0.5606404  to the right,
     agree=0.909, adj=0.778, (0 split)
    X501  < 1.533854   to the right,
     agree=0.909, adj=0.778, (0 split)
    X952  < -0.2179817 to the left,
     agree=0.909, adj=0.778, (0 split)
    X1656 < 1.052772   to the left,
     agree=0.909, adj=0.778, (0 split)
```

Output V

```
Node number 2: 9 observations
  predicted class=+  expected loss=0.1111111
    class counts:     8     1
   probabilities: 0.889 0.111

Node number 3: 13 observations
  predicted class=-  expected loss=0
    class counts:     0    13
   probabilities: 0.000 1.000
```

So, does CART work?

Unfortunately, it doesn't work all that well for most microarray data experiments.

The problem is simply that by focusing so intently on a small number of variables (dealing with ties?) that CART can get misled by random chance splits. This is less of a problem if the number of arrays in the experiment is large (50 or more) such that we are unlikely to see very large reductions in impurity even when we start with 1000s of genes.

Cross-validation shows problems.

Can we fix CART?

By averaging the predictions from several models, we may come up with more robust algorithms. The problem is that by averaging, we are combining the results from many different genes, and the simplicity of interpretation is somewhat lost.

Useful if we start with a small number of variables.