GS01 0163
Analysis of Microarray Data

Keith Baggerly and Kevin Coombes
Section of Bioinformatics
Department of Biostatistics and Applied Mathematics
UT M. D. Anderson Cancer Center
kabagg@mdanderson.org
kcoombes@mdanderson.org

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Lecture 18: Clusters, Partitions, and Silhouettes

- K-Means Clustering
- Partitioning Around Medoids
- Silhouette Widths
- Principal Components
- Principal Coordinates

Review of hierarchical clustering

Last time: we looked at hierarchical clustering of the samples.

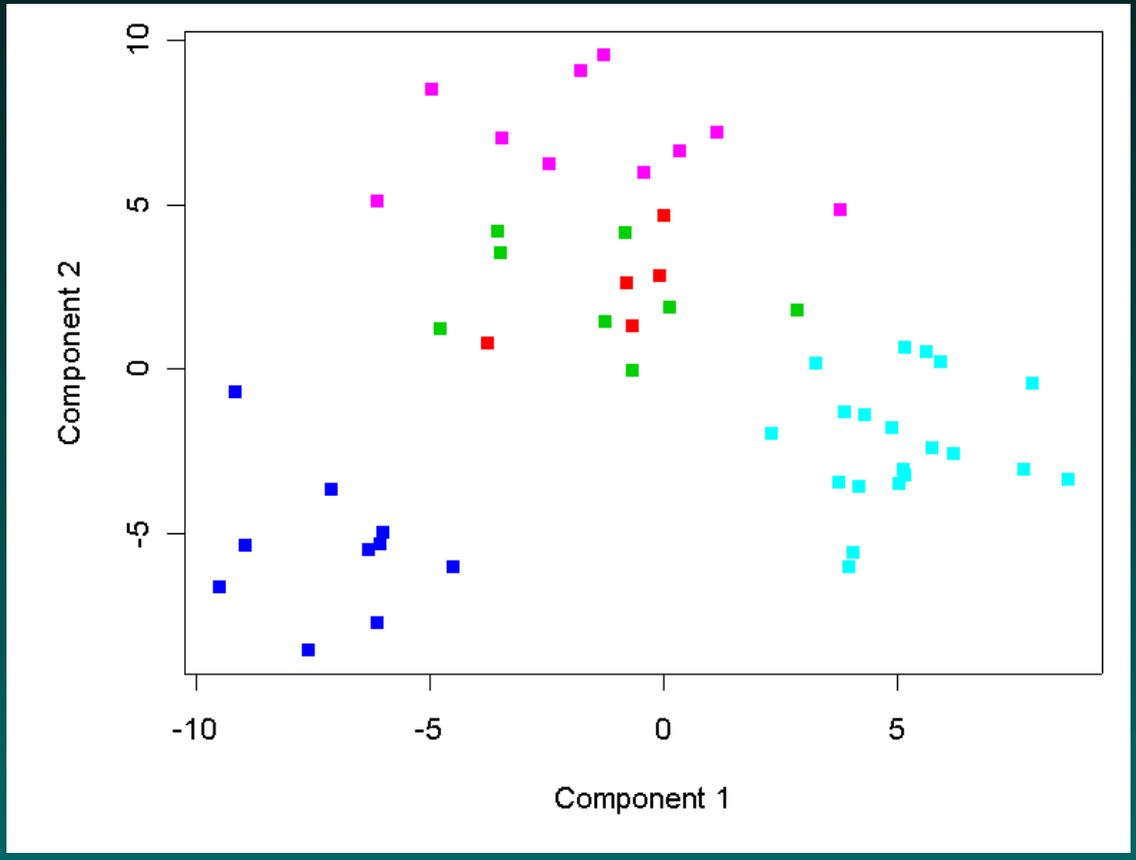
Key issues:

- What distance metric (euclidean, correlation, manhattan, canberra, minkowski) should we use?
- What linkage rule (average, complete, single, ward) should we use?
- Which clusters should we believe? (bootstrap resampling)
- How many clusters should we believe? (bootstrap)

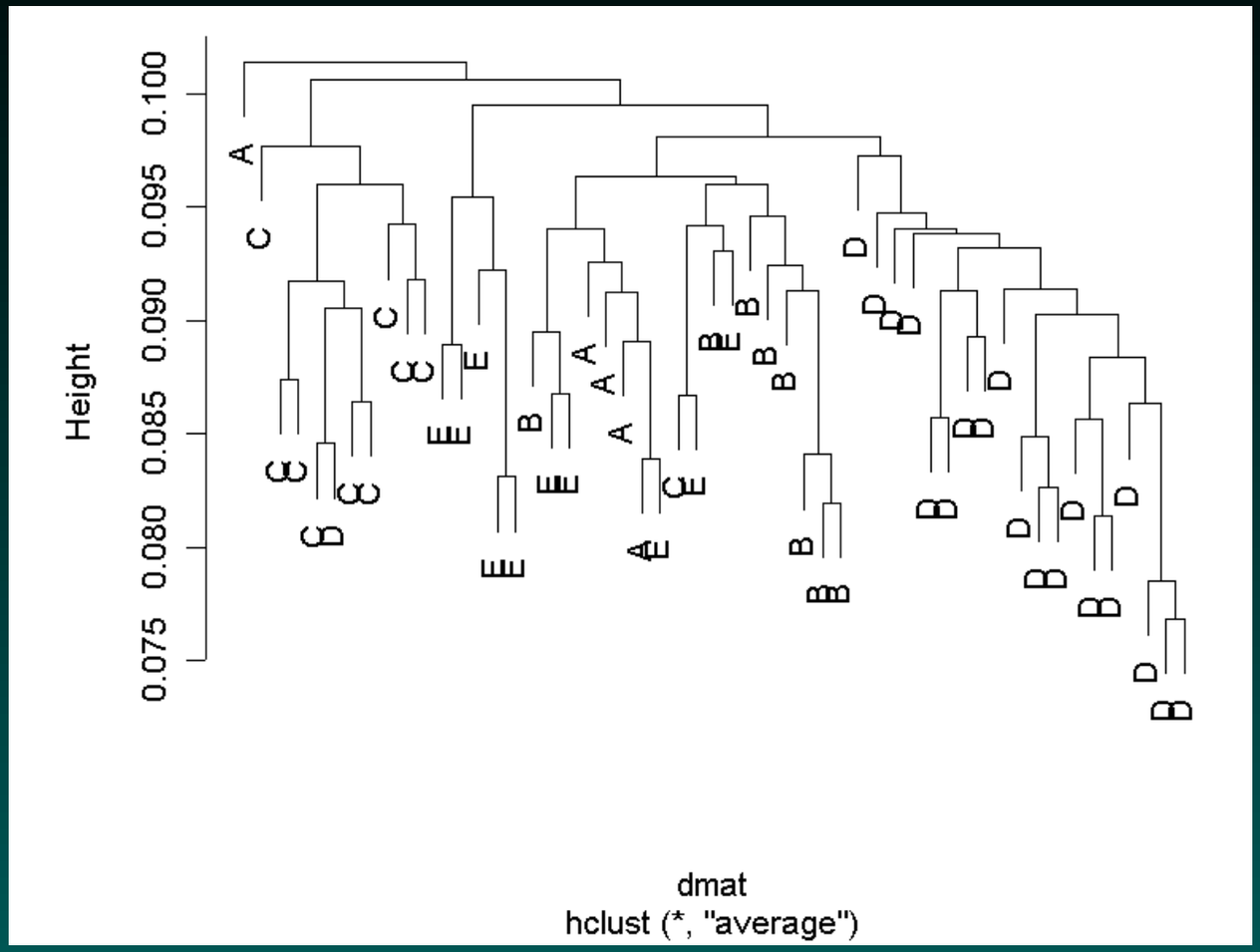
Is there any reason to believe that a hierarchical structure makes sense? Today, we'll look at some other clustering techniques.

Simulated data

To test some algorithms, we simulated data with 1000 genes and 5 different sample classes containing different numbers of samples. Here's a two-dimensional picture of the truth:



Hierarchical clusters (correlation; average)



Three of the classes (B, C, D) are mostly correct. The other two classes are less concentrated.

K-Means Clustering

Input: A data matrix, X, and the desired number of clusters, K.

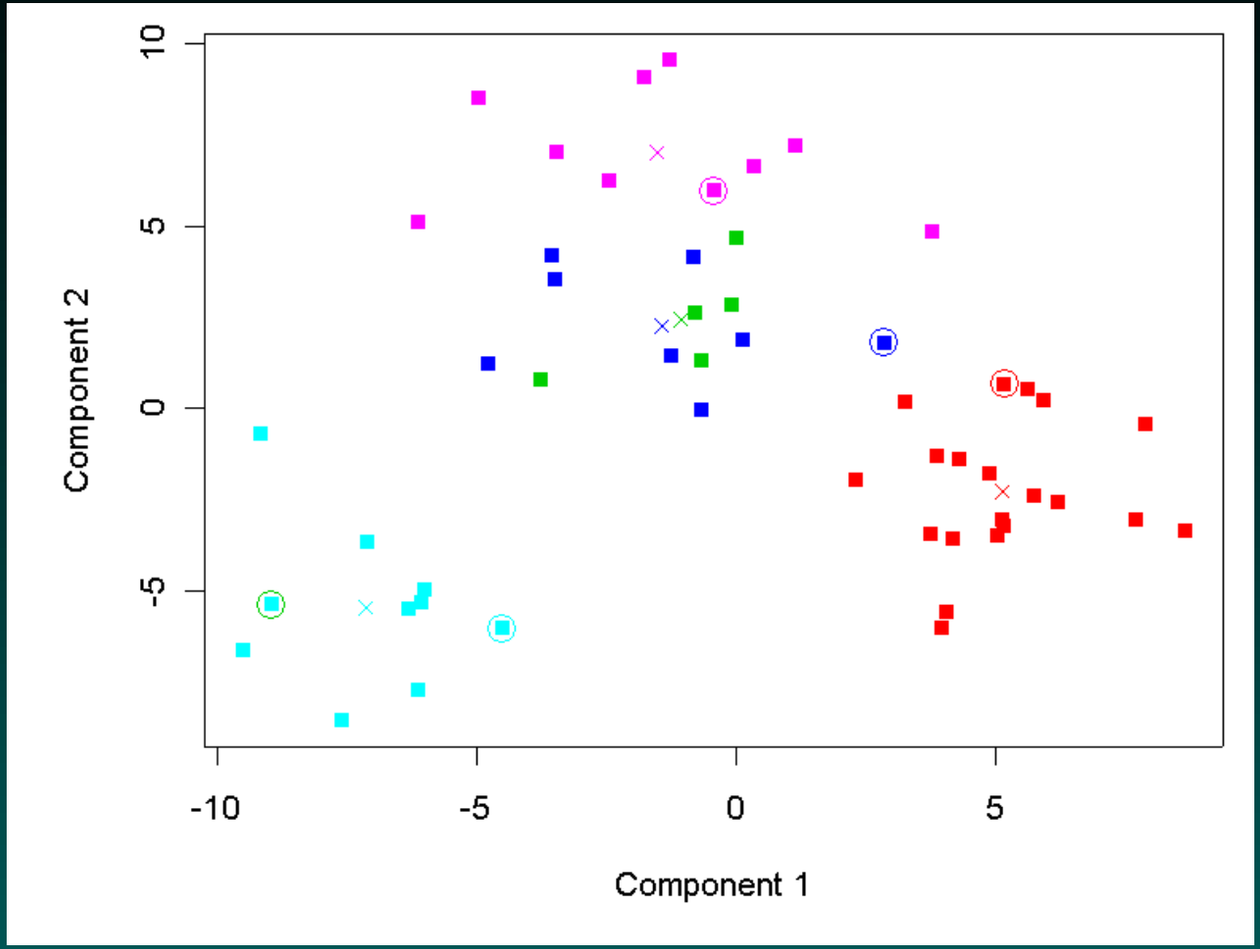
Output: For each sample i, a cluster assignment $C(i) \leq K$.

Idea: Minimize the within-cluster sum of squares

$$\sum_{c=1}^{K} \sum_{C(i)=c, C(j)=c} \sum_{\ell=1}^{N} (x_{i\ell} - x_{j\ell})^2$$

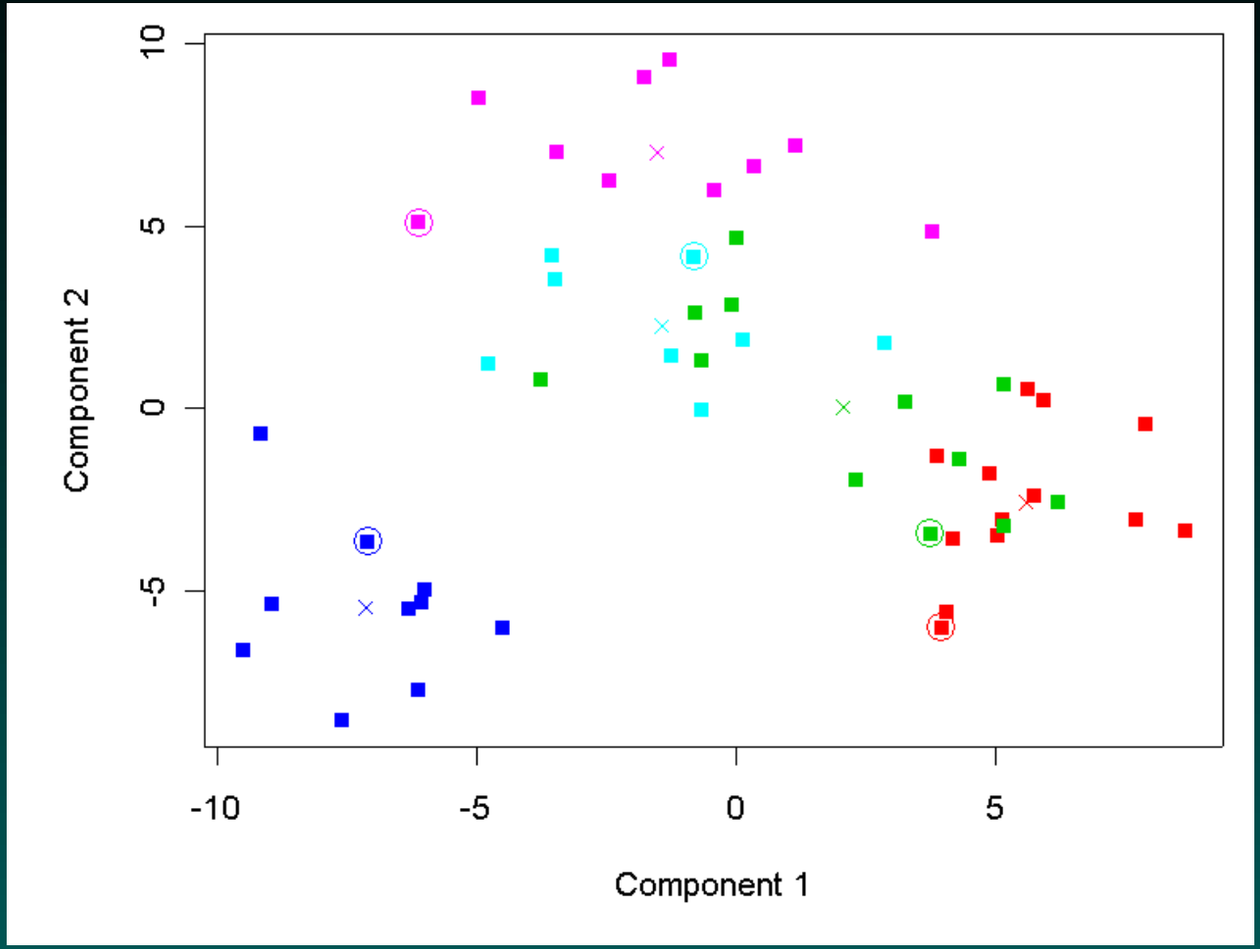
- Algorithm:
  1. Make an initial guess at the centers of the clusters.
  2. For each data point, find the closest cluster (Euclidean).
  3. Replace each cluster center by averaging data points that are closest to it.
  4. Repeat until the assignments stop changing.

K-Means, Take 1



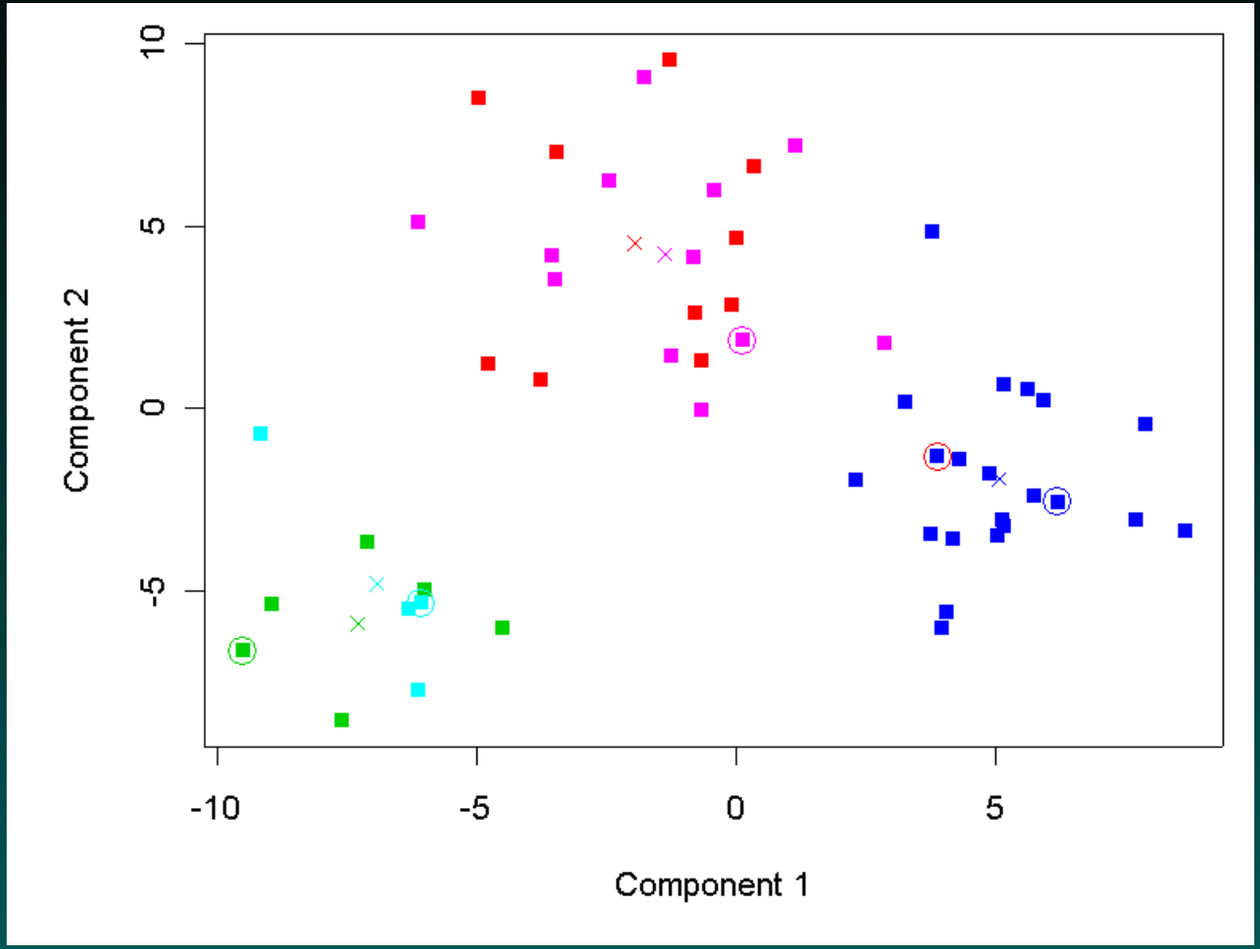
Perfect clustering! (Circles = starting group centers, X = final group centers)

K-Means, Take 2



Oops: bad starting points may mean bad clusters!

K-Means, Take 3



Local minima may not be global

K-means can be very sensitive to the choice of centers used as seeds for the algorithm. The problem is that the algorithm only converges to a local minimum for the within-cluster sum of squares, and different runs with randomly chosen centers (which is the default in the `kmeans` function in R) can converge to different local optima. You can see which of these three runs is better:

```
> sum(kres1$withinss)
[1] 25706.57
> sum(kres2$withinss)
[1] 25736.84
> sum(kres3$withinss)
[1] 25926.12
```

Local minima may not be global

There are two ways around the fact that local minima need not be global.

One is to find better starting seeds for the algorithm. For example, start with hierarchical clustering. Then cut the tree into five branches, and use the average of each branch as the starting points.

Alternatively, you can run the algorithm with many random seeds, keeping track of the within-cluster sum of squares.

Multiple runs of the K-means algorithm

```
kcent <- sample(n.samples, 5)
kres <- kmeans(t(ldata), t(ldata[,kcent])
withinss <- sum(kres$withinss)
for (i in 1:100) {
  tcent <- sample(n.samples, 5)
  tres <- kmeans(t(ldata), t(ldata[,tcent]))
  print(sum(tres$withinss))
  if (sum(tres$withinss) < withinss) {
    kres <- tres
    kcent <- tcent
    withinss <- sum(kres$withinss)
  }
}
```

Can we use other measures of distance?

The K-means clustering algorithm has another limitation. (This is not the last one we will consider).

As described, it always uses Euclidean distance as the measure of dissimilarities between sample vectors. As we saw last time with hierarchical clustering, there are a large number of possible distances that we might want to use. Fortunately, a simple adjustment to the algorithm lets us work with any distance measure.

Partitioning Around Medoids (PAM)

Input: Data matrix, X, distance d, number of clusters, K.

Output: For each sample i, a cluster assignment $C(i) \leq K$.

Idea: Minimize the within-cluster distance

$$\sum_{c=1}^{K} \sum_{C(i)=c, C(j)=c} d(x_i, x_j)$$

- Algorithm:
  1. Make an initial guess at the centers of the clusters.
  2. For each data point, find the closest cluster.
  3. Replace each cluster center by the data point minimizing the total distance to other members in its cluster.
  4. Repeat until the assignments stop changing.

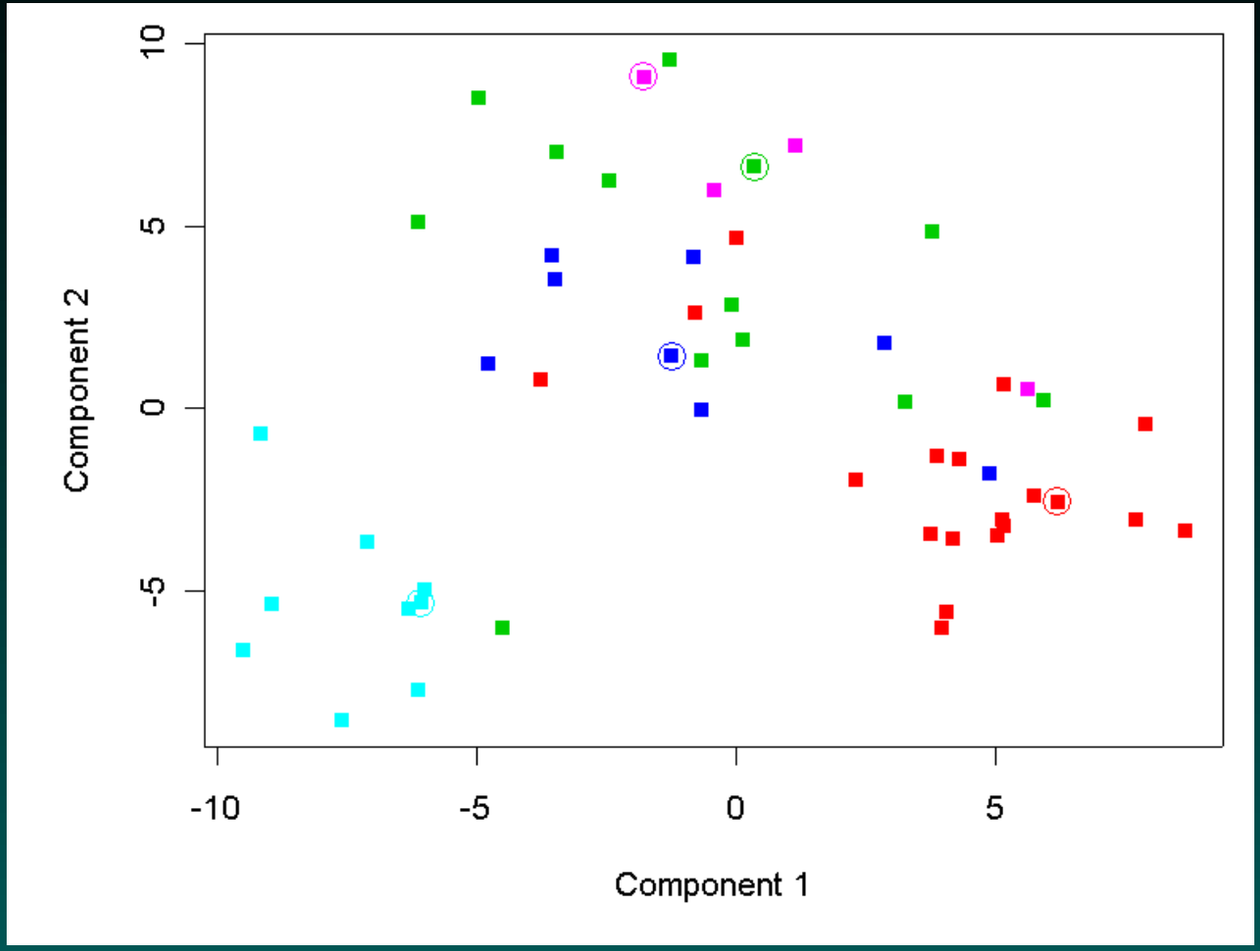
PAM in R

To use PAM in R, you must load another package:

```
> require(cluster)
> dist.matrix <- as.dist(1-cor(ldata)/2)
> pamres <- pam(dist.matrix, 5)
```

Unlike `kmeans`, the implementation of `pam` only lets you specify the number of clusters you want, not the starting point. It also apparently always uses the same method to choose the starting point, so it does not help to run the algorithm multiple times. If their heuristic chooses a poor starting configuration, there is no way to fix it.

PAM results



Not very good on our example data...

How many clusters are there?

Both `kmeans` and `pam` require you to specify the number of clusters before running the algorithm. In our example, we knew before we stated that there were five clusters. In real life, we rarely (if ever) know the number of real clusters before we start. How do we figure out the correct number of clusters?

One way is to run the algorithm with different values of K, and then try to decide which methods gives the best results. The problem that remains is how we measure “best”.

Silhouette Widths

Kaufman and Rousseeuw (who wrote a book on clustering that describes pam along with quite a few other methods) recommend using the silhouette width as a measure of how much individual elements belong to the cluster where they are assigned. To compute the silhouette width of the i^{th} object, define

$$a(i) = \text{average distance to other elements in the cluster}$$

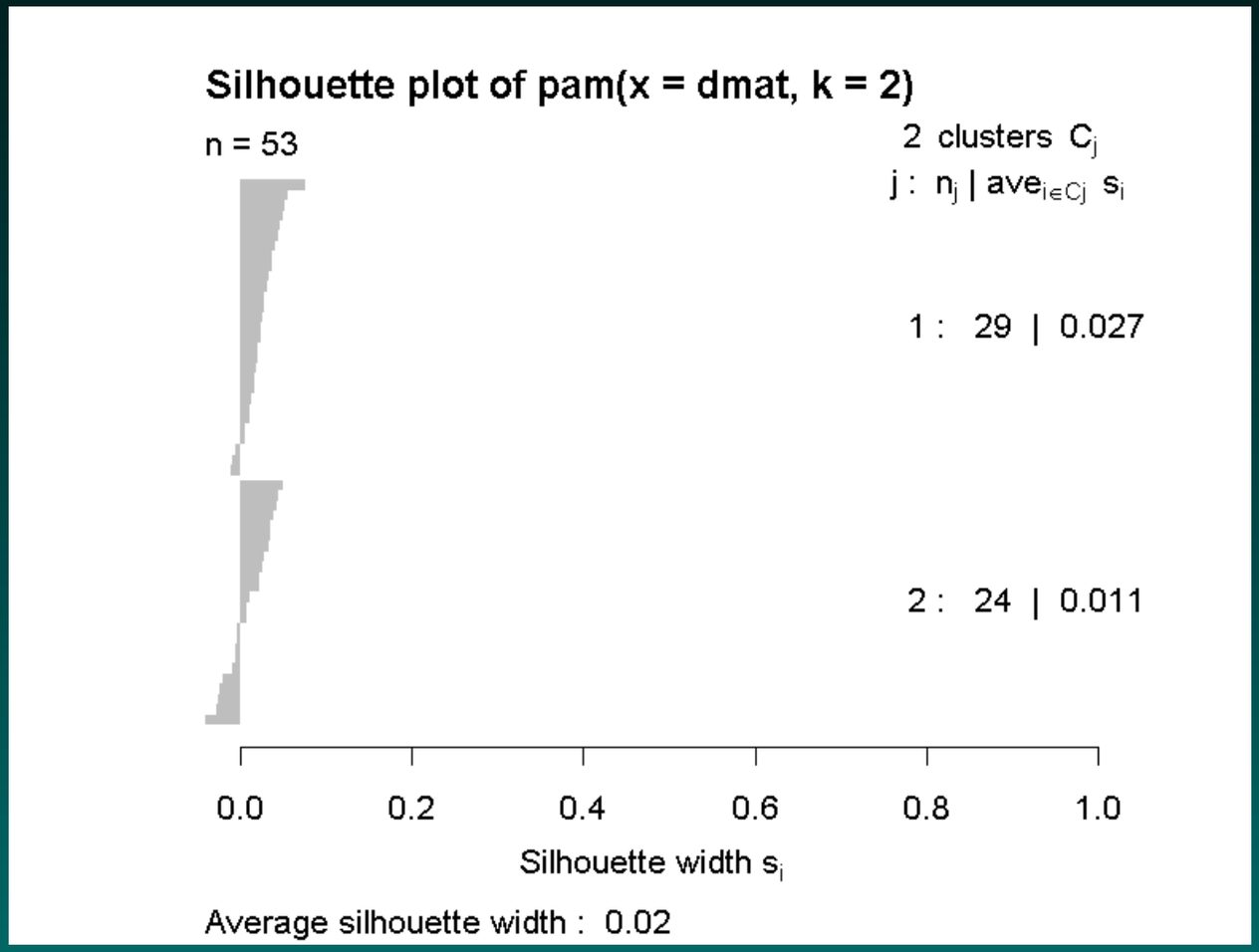
$$b(i) = \text{smallest average distance to other clusters}$$

$$sil(i) = (b(i) - a(i))/max(a(i), b(i)).$$

Interpretation: If $sil(i)$ is near 1, then the object is well clustered. If $sil(i) < 0$, then the object is probably in the wrong cluster. If $sil(i)$ is near 0, then it's on the border between two clusters.

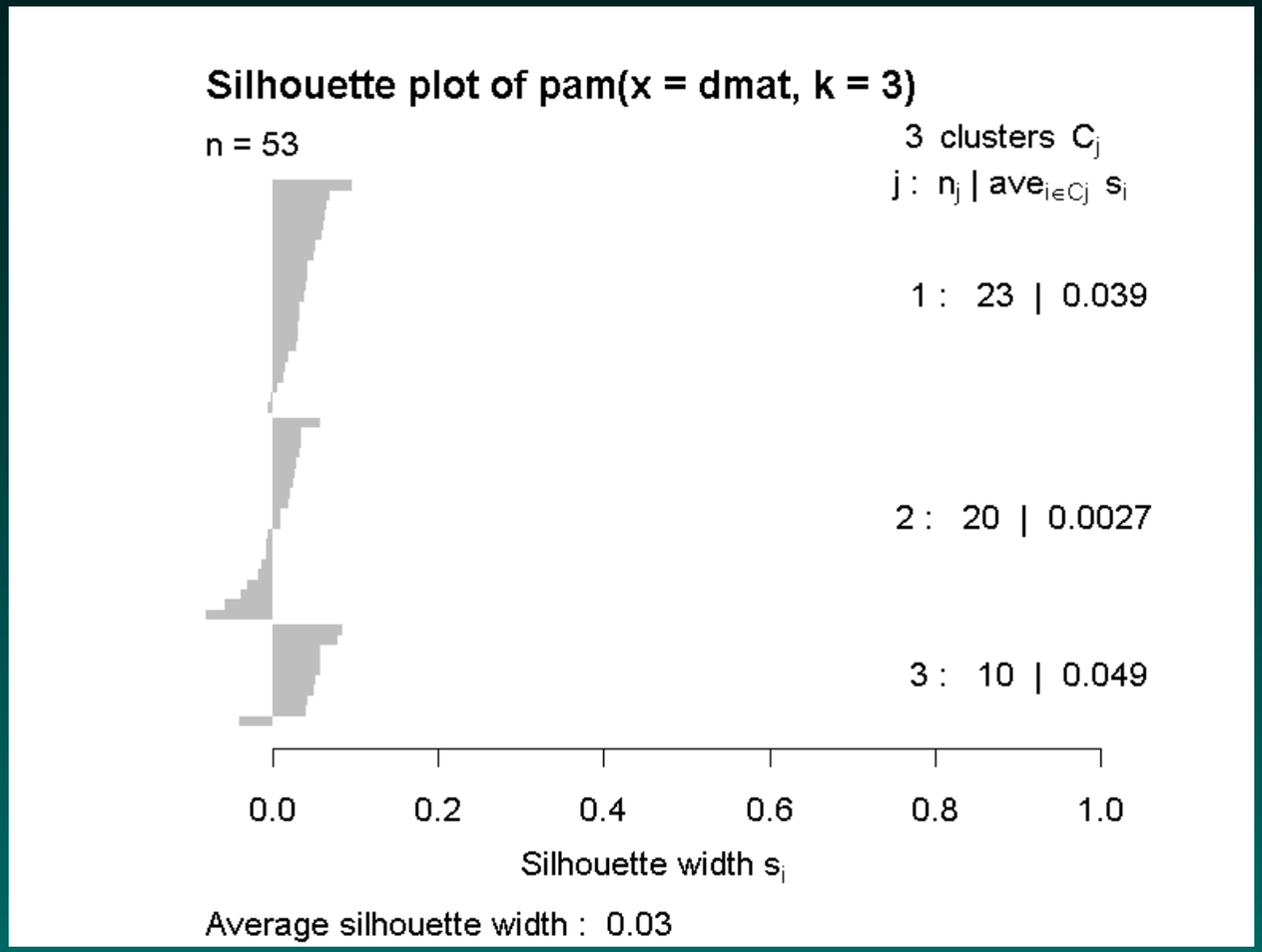
PAM : two clusters

```
> pam2 <- pam(dmat, 2)
> plot(pam2)
```



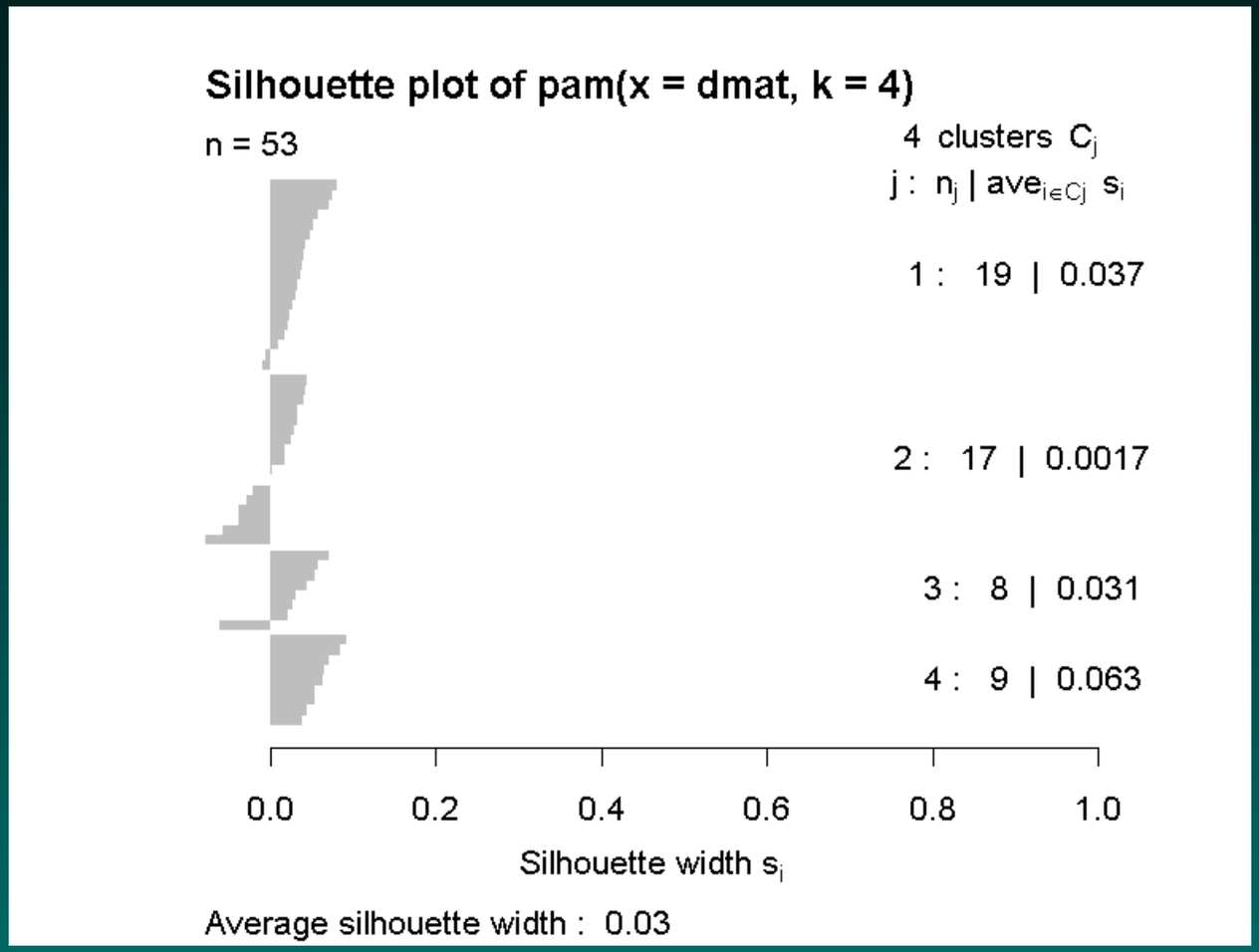
PAM : three clusters

```
> pam3 <- pam(dmat, 3)
> plot(pam3)
```



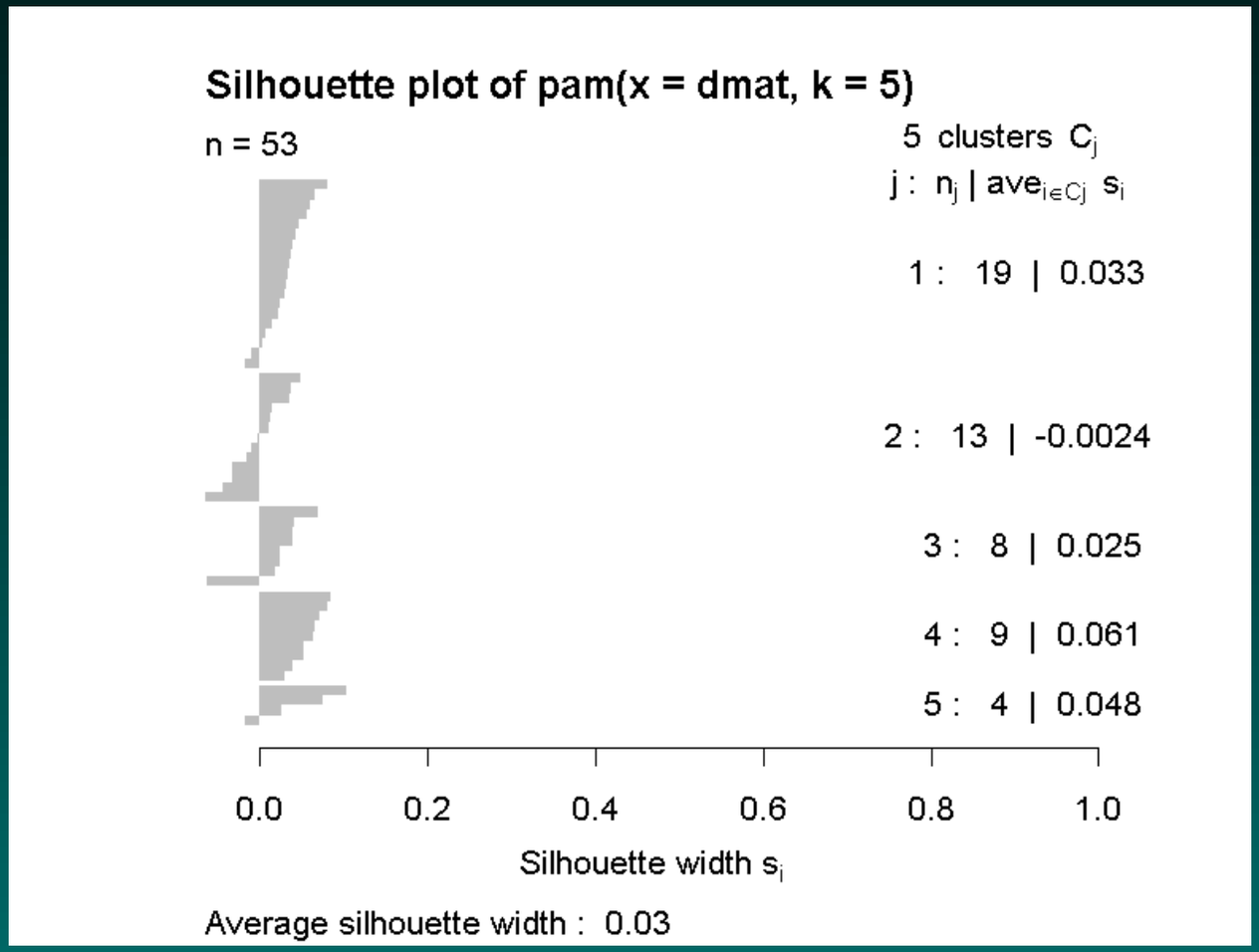
PAM : four clusters

```
> pam4 <- pam(dmat, 4)
> plot(pam4)
```



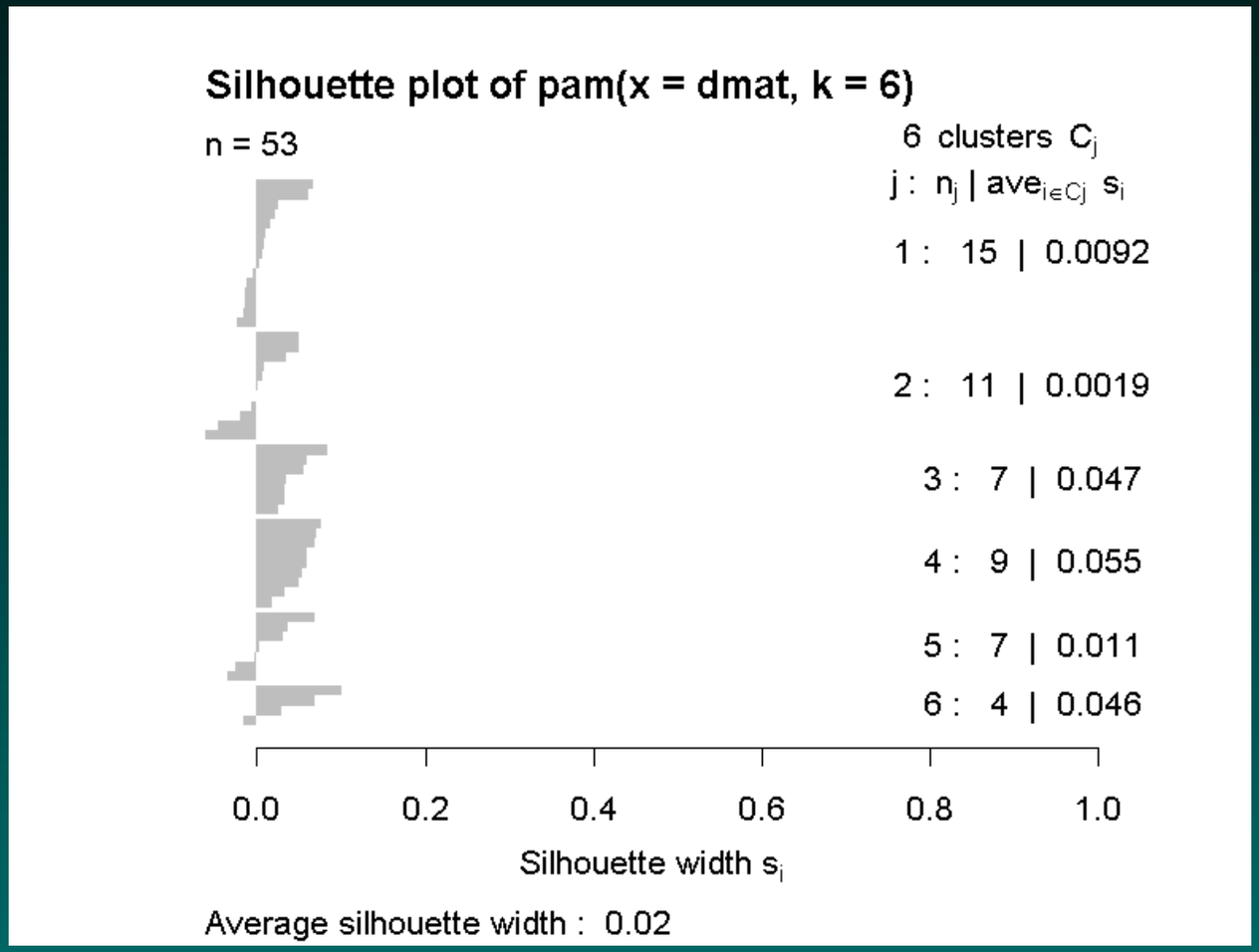
PAM : five clusters

```
> pam5 <- pam(dmat, 5)
> plot(pam5)
```



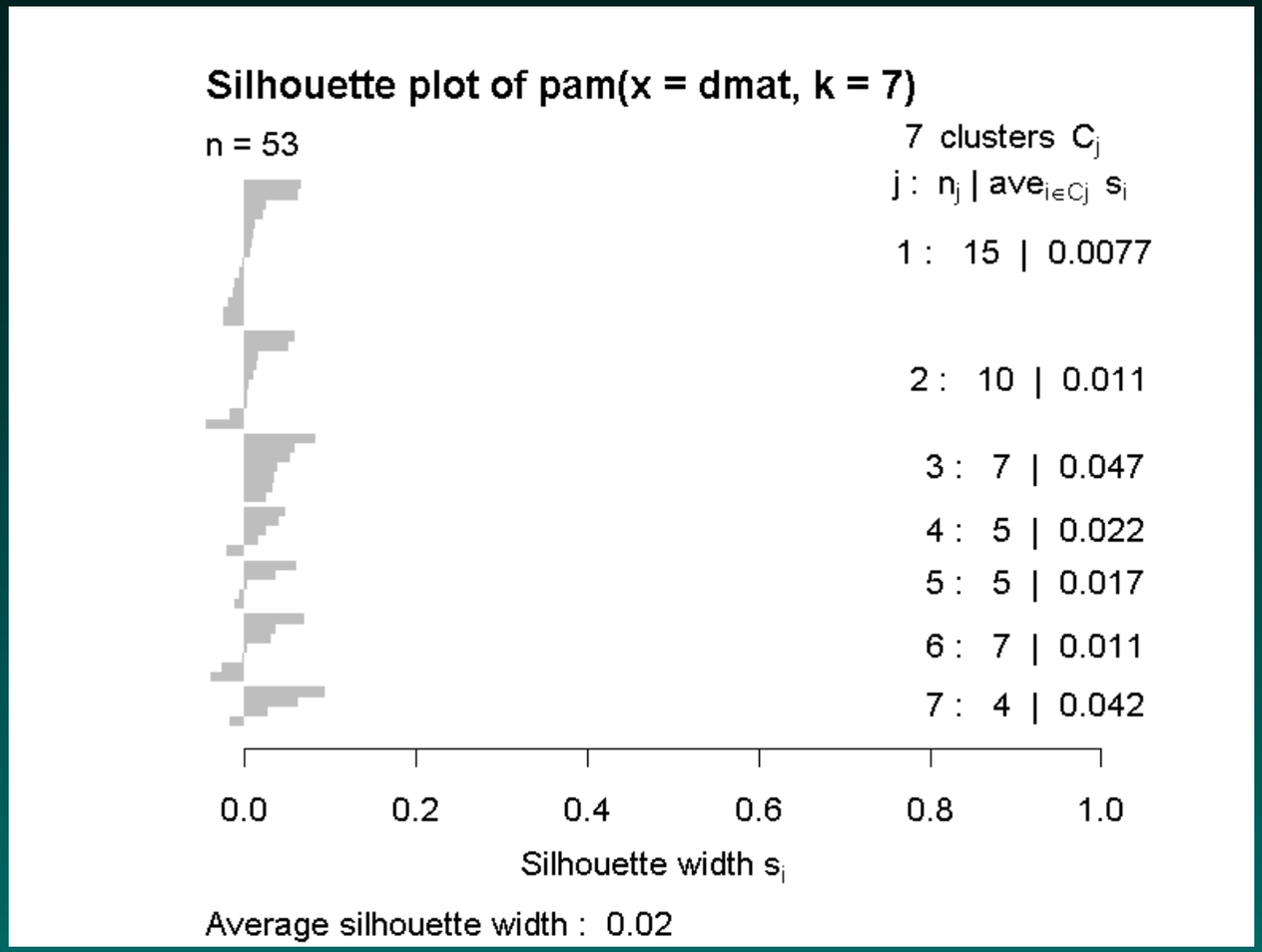
PAM : six clusters

```
> pam6 <- pam(dmat, 6)
> plot(pam6)
```



PAM : seven clusters

```
> pam7 <- pam(dmat, 7)
> plot(pam7)
```



```
> summary(silhouette(pam2))$avg.width
[1] 0.01938651
> summary(silhouette(pam3))$avg.width
[1] 0.02713564
> summary(silhouette(pam4))$avg.width
[1] 0.02904244
> summary(silhouette(pam5))$avg.width
[1] 0.02875473
> summary(silhouette(pam6))$avg.width
[1] 0.02342055
> summary(silhouette(pam7))$avg.width
[1] 0.01862886
```

In general, we want to choose the number of clusters that maximizes the average silhouette width. In this case, that means 4 or 5 clusters.

Using silhouettes with K-means

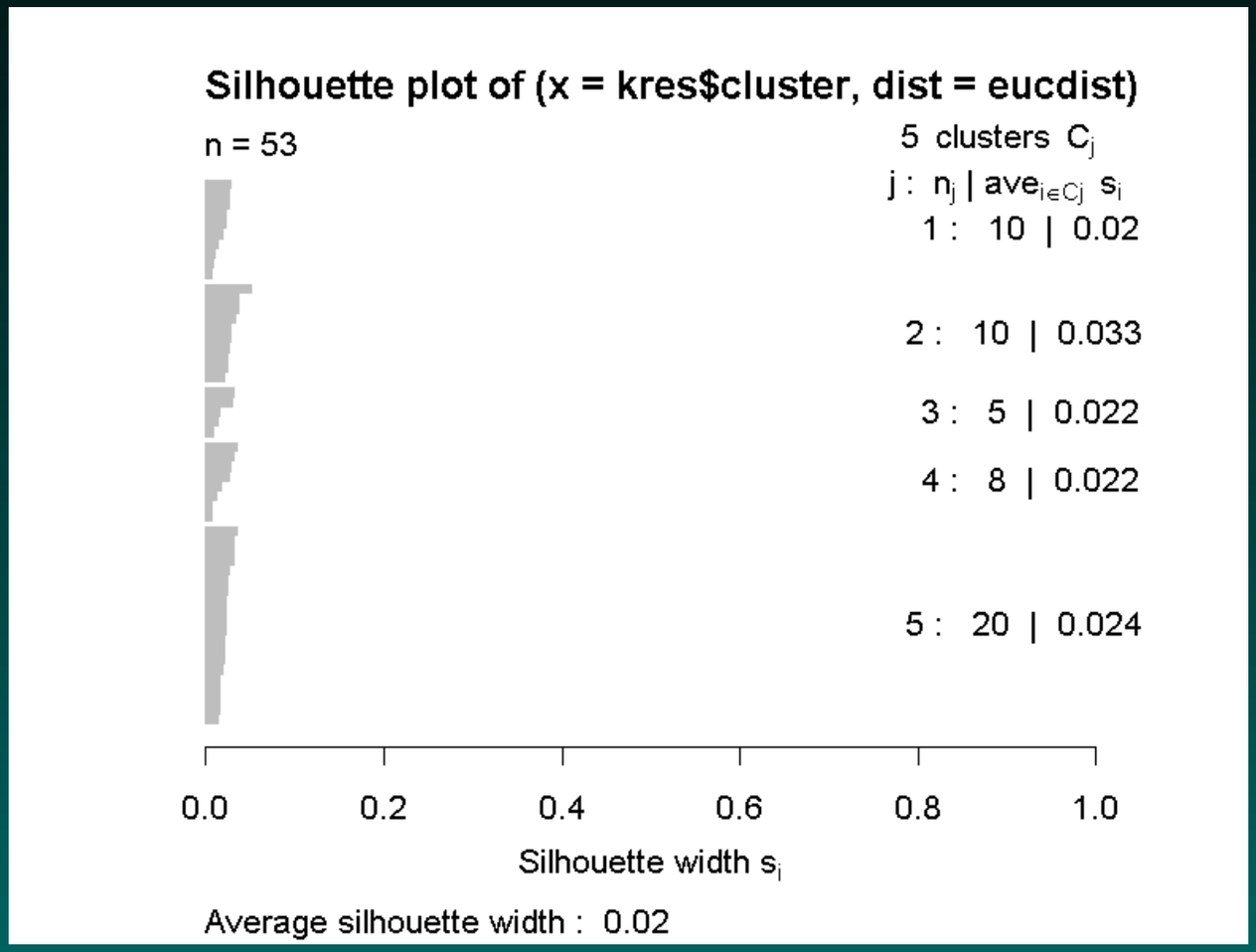
The `silhouette` function knows about `pam` objects, making it relatively easy to use. You can use it with other clustering routines, but you have to supply the clustering vector and the distance matrix. For example, here are the results for the best K-means clustering that we found (as measured by the within-cluster sum of squares).

```
> euc.distance <- dist(t(ldata))
> ksil <- silhouette(kres$cluster, euc.distance)
> summary(ksil)$avg.width
[1] 0.02453796
```

Note that the silhouette width is smaller than the one from PAM using correlation. However, the silhouette plot suggests that everything is classified correctly:

K-means: five cluster silhouette

```
> plot(ksil)
```



Silhouettes with hclust

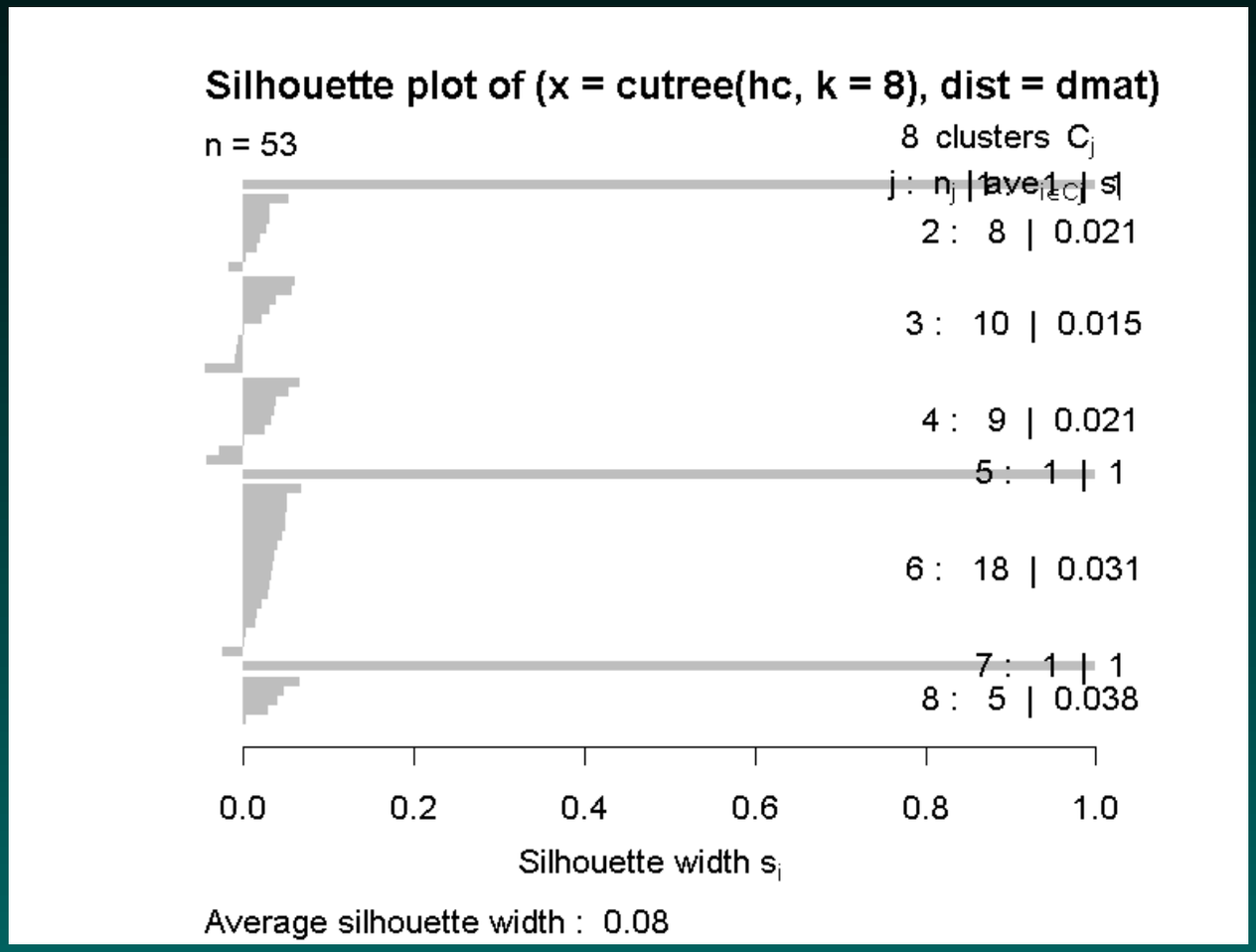
Looking back at the hierachical clustering, we ned to cut eight branches off (including some singletons) to get down to the clusters that look real.

```
> dmat <- as.dist((1-cor(ldata))/2)
> hc <- hclust(dmat, 'average')
> hsil <- silhouette(cutree(hc, k=8), dmat)
> summary(hsil)$avg.width
[1] 0.08024319
```

Why does the average silhouette width look so much better for this method?

Hierarchical clustering: silhouette with singletons

```
> plot(hsil)
```



The silhouette width

General conclusions: The average silhouette width is only a crude guide to the number of clusters present in the data. The silhouette plot seems to be more useful, but requires human intervention (in the form of “cortical filtering”).

The Gap statistic

An alternative method for determining the number of clusters relies on the gap statistic. The idea is to run a clustering algorithm for different valus of the number K of clusters. Let $W(K)$ be the within-cluster error. In the case of Euclidean distance, $W(K)$ is just the within-cluster sum of squares. For other distance measures, it is the term we minimized in the description of PAM. Because adding more clusters will always reduce this error term, $W(K)$ is a decreasing function of K. However, it should decrease faster when K is less than the true number and slower when K is greater than the true number.

The gap statistic measures the difference (on the log scale, for each K) between the observed $W(K)$ and the expected value if the data were uniformly distributed. One then selects the K with the largest gap between the observed and expected values.

Principal Components

You may have wondered how I produced two-dimensional plots of the simulated data that involved 1000 genes and 53 samples. The short answer is: I used principal components analysis (PCA).

As we have been doing throughout our discussion of clustering, we view each sample as a vector $x = (x_1, \ldots, x_G)$ in G-dimensional "gene space". The idea behind PCA is to look for a direction (represented as a linear combination $u_1 = \sum_{i=1}^{G} w_i x_i$) that maximizes the variability across the samples. Next, we find a second direction u_2 at right angles to the first that maximizes what remains of the variability. We keep repeating this process. The u_i vectors are the principal components, and we can rewrite each sample vector as a sum of principal components instead of as a sum of separate gene expression values.

Data reduction

PCA can be used as a data reduction method. Changing from the original x-coordinates to the new u-coordinate system doesn't change the underlying structure of the sample vectors. However, it does let us focus on the directions where the data changes most rapidly. If we just use the first two or three principal components, we can produce plots that show us as much of the intrinsic variability in the data as possible.

Singular value decomposition

The result from linear algebra that allows us to compute principal components efficiently is called the singular value decomposition (SVD). This result (whose proof is based on Gram-Schmidt orthogonalization) tells us that any matrix X with n rows and m columns can be decomposed as a product

$$X = UDV^T$$

where U is an $n \times m$ matrix with

$$U^T U = I_m,$$

D is a diagonal matrix with m nonnegative entries, and V is an $m \times m$ matrix with

$$V^T V = I_m.$$

SVD for PCA

The singular value decomposition of X is, by convention, organized so that the diagonal elements of D are in non-increasing order:

$$d_1 \geq d_2 \geq \ldots \geq d_m.$$

Then the columns of U are the principal components and the product DV^T gives the coefficients that write the columns of X as a sum of columns of U.

If we just have the matrix U, we can recover these coefficients by a simple matrix multiplication:

$$U^T X = U^T(UDV^T) = (U^T U)(DV^T) = I_m DV^T = DV^T.$$

Projection into PCA space

The last computation actually tells us how to take any vector in gene space and rewrite it in terms of the principal components: just multiply by U^T.

Warning: evn though there is a function in R called `princomp` that is supposed to compute principal components, it will not work in the contect of microarrays. The problem is that `princomp` wants to decompose the covariance matrix $\Sigma = XX^T$, which is a square matrix with size given by the number of genes. That's simply too big to manipulate. The linear algebra that lets us avoid this huge matrix reduces to

$$\begin{aligned} XX^T &= (UDV^T)(UDV^T)^T = (UDV^T)(VDU^T) \\ &= (UD)(V^TV)(DU^T) = UD^2U^T. \end{aligned}$$

Sample PCA

We have written a version of PCA in R using SVD. The first polot of our simulated data was produced using the following commands:

```
> spca <- sample.pc(ldata)
> plot(spca, split=group.factor)
```

The plots that added the X's to mark the K-means centers were produced with:

```
> plot(spca, split=factor(kres$cluster))
> x1 <- spca@scores[kcent,1] # start circles
> x2 <- spca@scores[kcent,2]
> points(x1, x2, col=2:6, pch=1, cex=2)
> pcak <- predict(spca, t(kres$centers)) # finish X
> points(pcak[,1], pcak[,2], col=2:6, pch=4)
```

Principal Coordinates

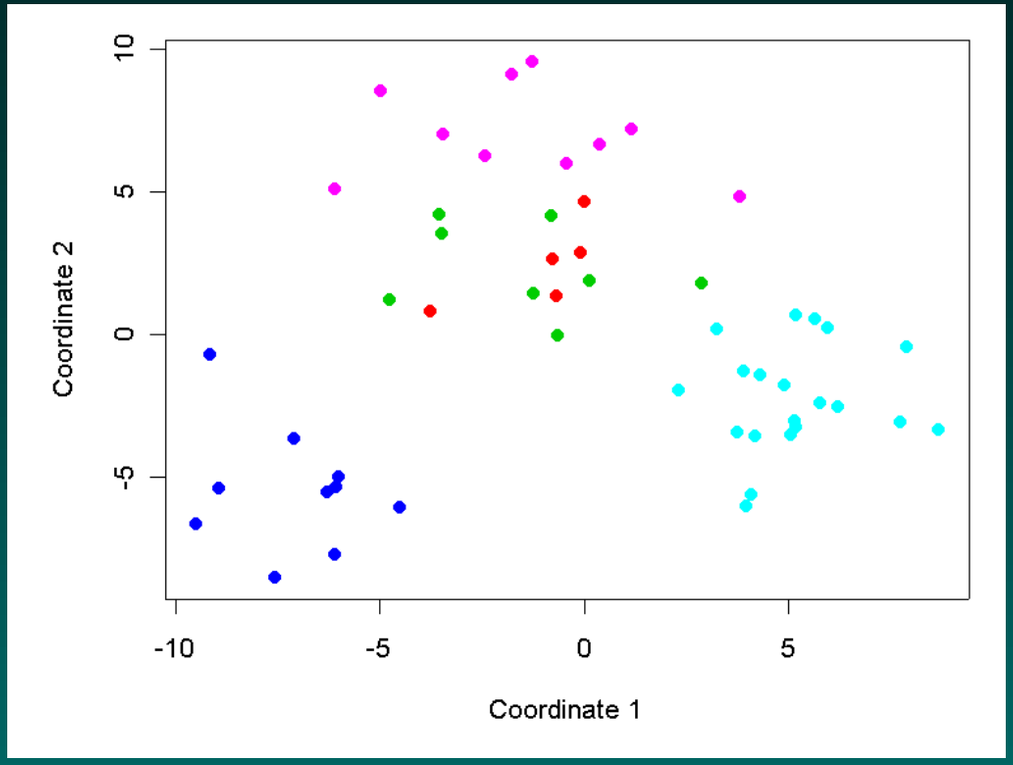
Using the first few principal components provides a view of the data that allows us to see as much of the variability as possible. Sometimes we have a different goal: we'd like to be able to visualize the samples in a way that does as good a job as possible of preserving the distances between samples. In general, this method is called multidimensional scaling (MDS).

The classical form of MDS is also known as principal coordinate analysis, and is implemented in R by the function `cmdscale`.

If you look at the resulting graph carefully, you'll discover that it is identical to the principal components plot: classical MDS using Euclidean distance is equivalent to plotting the first few principal components.

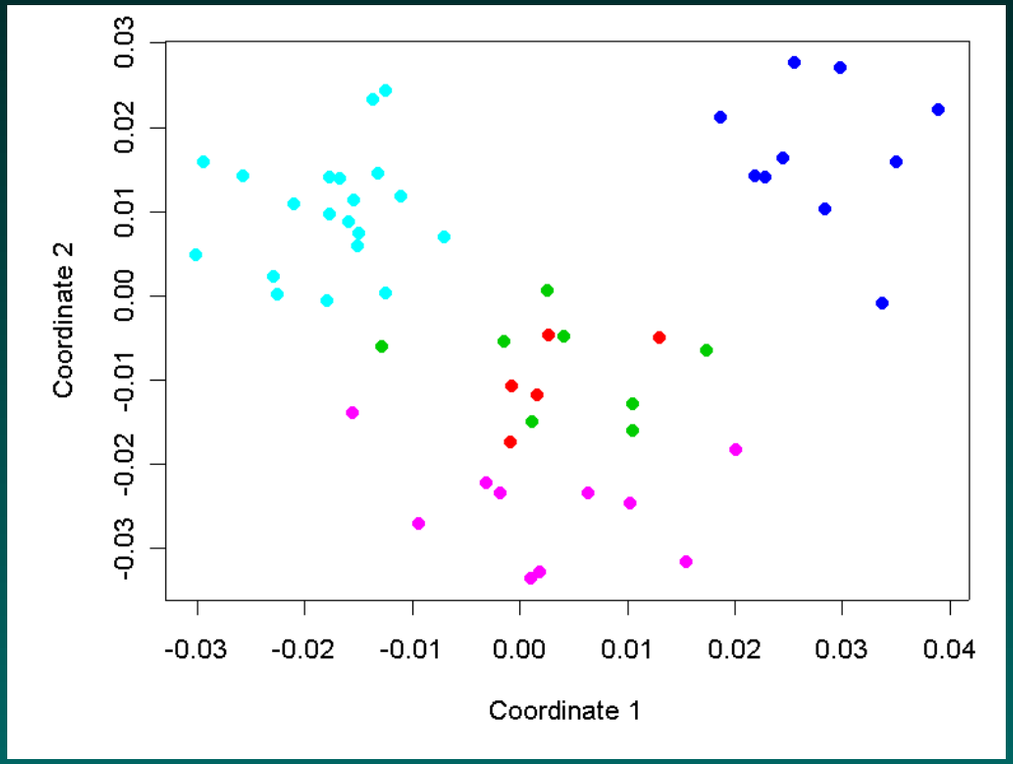
Euclidean classical MDS = PCA

```
> euloc <- cmdscale(euc.distance)
> plot(euloc[,1], euloc[,2], pch=16,
+      col=as.numeric(group.factor),
+      xlab='Coordinate 1', ylab='Coordinate 2')
```



Classical MDS with correlation

```
> loc <- cmdscale(dmat)
> plot(loc[,1], loc[,2], pch=16,
+      col=1+as.numeric(group.factor),
+      xlab='Coordinate 1', ylab='Coordinate 2')
```



Classical MDS with correlation

Usaing the `cloud` function in the `lattice` package, we can also plot the first three principal coordinates:

```
> require(lattice)
> loc3d <- cmdscale(dmat, k=3)
> pc1 <- loc3d[,1]
> pc2 <- loc3d[,2]
>  pc3 <- loc3d[,3]
> cloud(pc3 ~ pc1*pc2, pch=16, cex=1.2,
+       col=1+as.numeric(group.factor),
+        screen=list(z=55, x=-70),
+       perspective=FALSE)
```

Three-D