Introduction to Statistical Methods

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Topics to be Covered

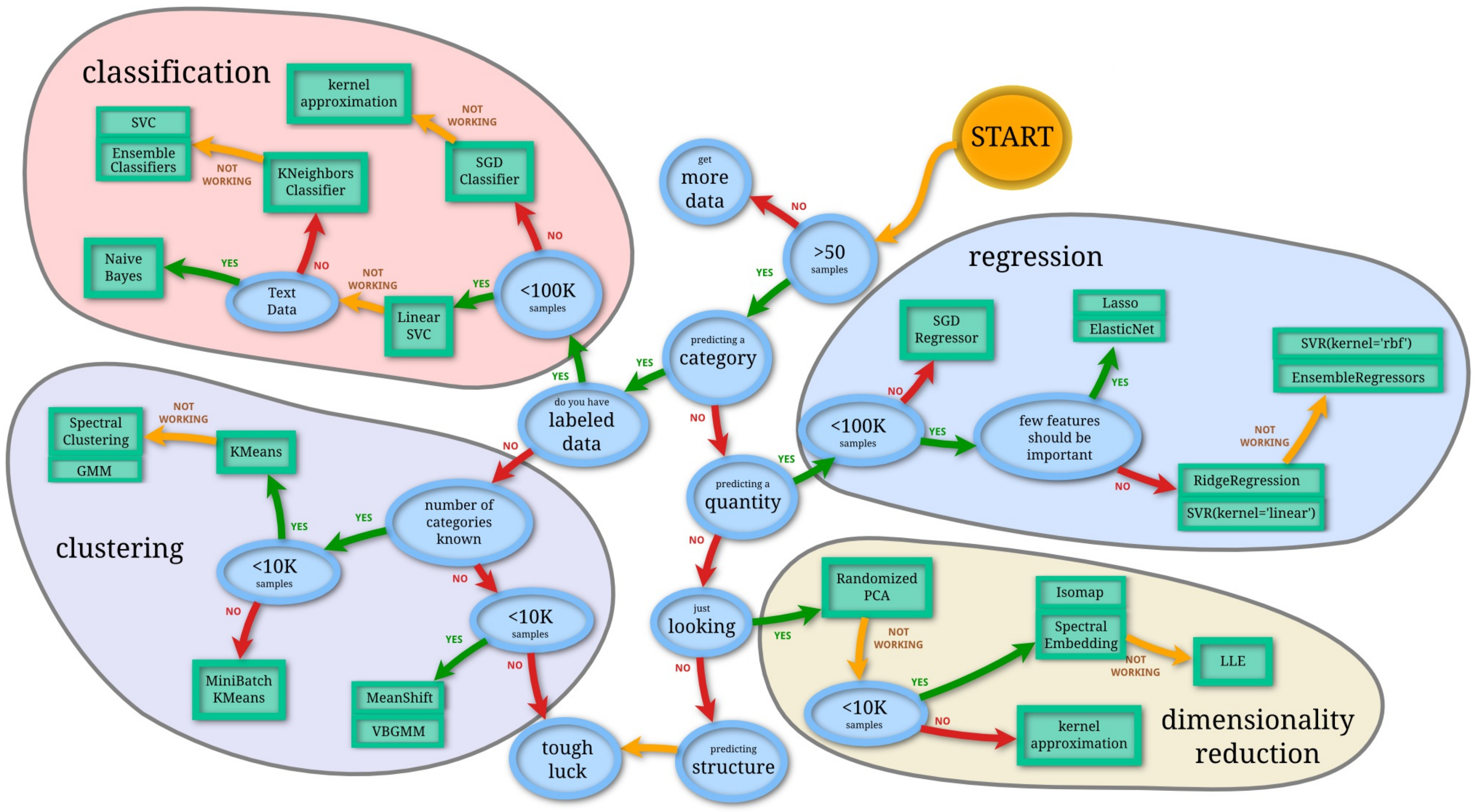
- Correlations
- Enrichment Analyses
- Multiple Testing Correction
- Regression
- Clustering
- Dimensionality Reduction

Introduction

- Data analyses are the product of many tasks
- Statistical Methods
  - Build predictive mathematical models
- Data preparation
  - Extracting structured data from unstructured data sources
  - Merging data sources
  - Ensuring consistency of datasets
- Dataset interpretation
  - Create visualizations to present and communicate findings
- Methods are common in the areas of informatics, data

Statistical Methods Flowchart

- The flowchart below helps find the right method for a given problem
  - http://scikit-learn.org/stable/tutorial/machine_learning_map/



Methods to be Covered

- Basic method will be covered to build confidence with R and the general concepts
  - Dimensionality Reduction: Principal Component Analysis (PCA)
  - Regression: Linear regression
  - Clustering: Hierarchical and K-means clustering
  - Classification will not be covered

Correlating Two Vectors

```r
# Make sure the random numbers are always the same
set.seed(1)

# Generate two sets of 20 random numbers
a <- runif(20); b <- runif(20)

# Calulate the correlation of the two sets
cor.test(a, b)
```

```

	Pearson's product-moment correlation

data:  a and b
t = -1.0368, df = 18, p-value = 0.3136
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.6152730  0.2292138
sample estimates:
       cor 
-0.2373854 
```

Extracting Values From Results

- Values in results are described in the help `?cor.test`
- A p-value is the probability of seeing results as extreme as the ones produced in an analysis.

```
set.seed(1)

a <- runif(20)
b <- runif(20)

results <- cor.test(a, b, method="pearson")
names(results)
```

```
[1] "statistic"   "parameter"   "p.value"     "estimate"
"null.value"
[6] "alternative" "method"      "data.name"   "conf.int"
```

```
results$p.value
```

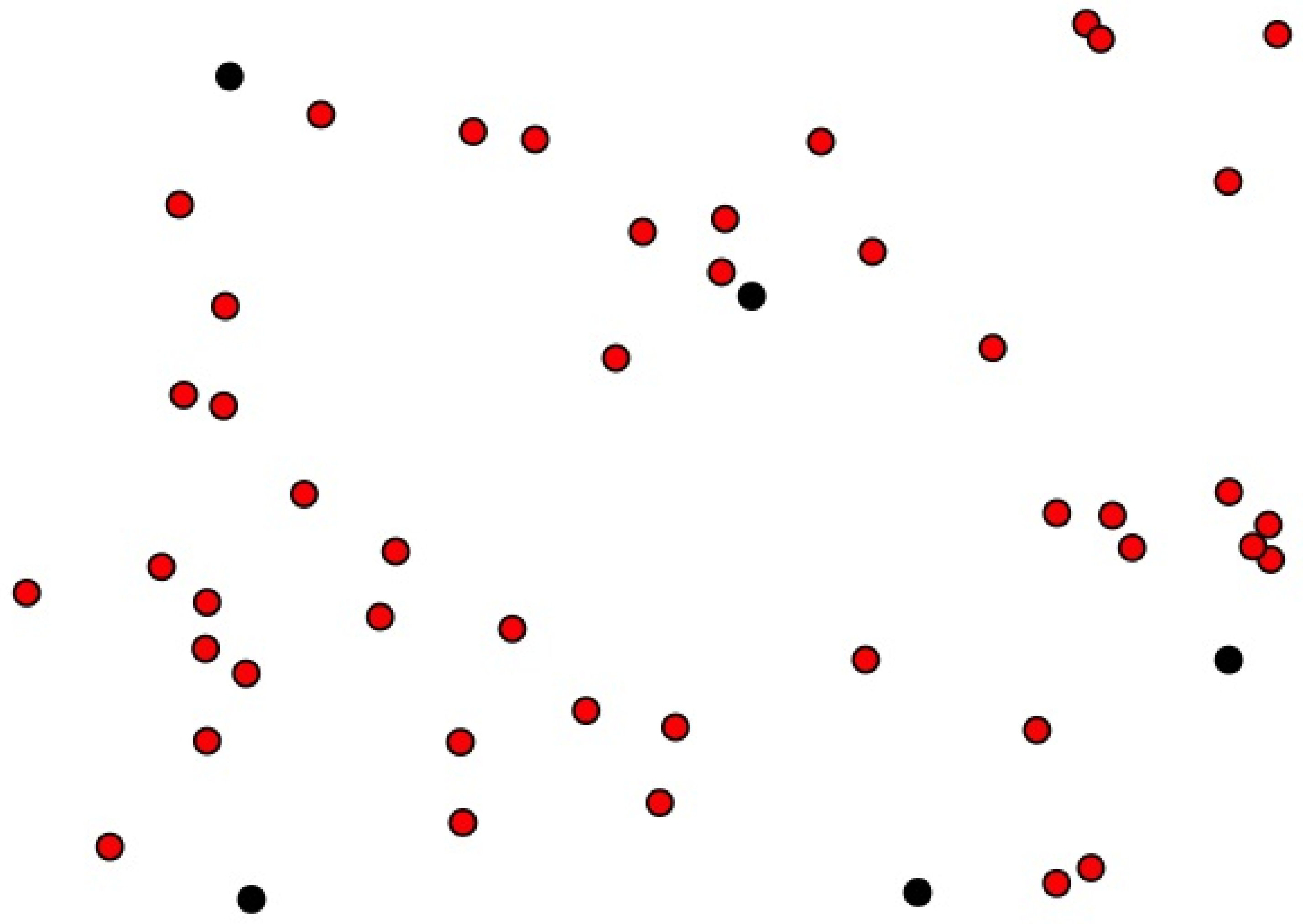
```
[1] 0.3135682
```

Over-Representation (ORA) and Enrichment Analyses

- Enrichment tests are widely used in biology to determine if the genes contain a trait more frequently than a random sampling of genes
  - Gene Ontology (GO) term (e.g. biological process, molecular function, or cellular component) and pathways are the most common comparisons made
- Several tools exist for doing enrichment analyses
  - The tests are either Fisher's exact test or hypergeometric test; these tests produce the same results
  - These calculations can be done in R using `fisher.test` and `phyper`

Enrichment Analysis

- What is the probability of randomly drawing at least 4 black points in a random sample of 10 points?
  - The concept of “black” could be replaced by “genes from a given pathway” or “genes with a common function”



Calculating an ORA (Enrichment) P-Value

The significance (i.e. p-value $P(X \geq k)$) of an over-representation (enrichment analysis) is calculated using a hypergeometric test:

$$P(X \geq k) = 1 - \sum_{i=0}^{k-1} \frac{\binom{K}{i}\binom{N-K}{n-i}}{\binom{N}{n}}$$

where

- N: number of studied genes,
- n: total number of genes identified by a previous analysis
- K: total number of genes in with an annotation
- k: number genes previously identified genes with the annotation
- $\binom{n}{k}$: the number of of ways of choosing k elements from a set of n elements, disregarding order

The p-value of this test indicates the probability that a random selection of genes of the same size as the input gene set from a population would produce the same number of observed annotations (e.g. for a specific GO term or pathway) or more in the gene set

Choose k from N Without Order

```
N <- 5
k <- 3
factorial(N) / (factorial(k)*factorial(N-
k))
```

```
[1] 10
```

```
N <- 5
k <- 3
choose(N, k)
```

```
[1] 10
```

Contingency Table for Enrichment Analysis

	Drawn	**Not Drawn**	**Total**
Black	k=4	K-k=1	K=5
Red	n-k=6	N+k-n-K=39	N-K=45
Total	n=10	N-n=40	N=50

phyper or fisher.test Example

- NOTE: `hitInSample-1` is nescessary in `phyper` beacuse if `lower.tail` is FALSE, probabilities returned are P(X > k). Subtract k by 1 to get P(X ≥ k) (k equal to or greater than).

```
sampleSize <- 10 # size drawn
hitInSample <- 4 # black drawn
hitInPop <- 5 # all black
failInPop <- 50-hitInPop # number of red

phyper(hitInSample-1, hitInPop, failInPop, sampleSize,
lower.tail= FALSE);
```

```
[1] 0.004083521
```

```
fisher.test(matrix(c(hitInSample, hitInPop-hitInSample,
sampleSize-hitInSample, failInPop-sampleSize+hitInSample),
2, 2), alternative='greater')$p.value;
```

```
[1] 0.004083521
```

Multiple Testing Correction for Enrichment Analyses

- Enrichment analyses often do analyses over a large number of molecular functions or pathways
- If we conduct many such tests, we are likely to see false positives
  - A p-value significance cutoff of 0.05 means that we expect 1 test out of 20 to appear significant by random chance (i.e. a false positive)

Multiple Test Corrections Types

- Family-Wise Error Rate (FWER): Controls the probability that any test is a false positive
  - Bonferroni Correction: Very stringent correction; the significance cutoff (i.e. α) is adjusted by the number of tests conducted

$$\alpha_{\mathrm{new}} = \frac{\alpha}{n}$$

  - $\alpha = 0.05$ and 10 tests is adjusted to $\alpha = 0.005$
- False Discovery Rate (FDR): Controls the proportion of tests that are false positives
  - Widely used alternative to FWER (e.g. Bonferroni correction)
  - Example (next slide)

Benjamini-Hochberg (BH) FDR Procedure

- Goal: Calculate the new p-value cutoff for a given set of p-values
- Assume $n = 100, \alpha = 0.05$
  - α denotes the desired false positive rate,

1. Rank n p-values (large to small)
2. Calculate q-values

$$q = \alpha \times \frac{n - \text{rank} + 1}{n}$$

3. Select the lowest ranked p-value that is lower than α

Benjamini-Hochberg Example

p-value	**Rank**	**q-value**	**p < q**
0.9	1	0.05*(100-1+1)/100=0.05	FALSE
0.7	2	0.05*(100-2+1)/100=0.0495	FALSE
0.5	3	0.05*(100-3+1)/100=0.049	FALSE
0.04	4	0.05*(100-4+1)/100=0.0485	TRUE
…	…	…	…
0.005	n	0.05*(100-n+1)/100=5E-4	FALSE

Bonferroni and Benjamini-Hochberg Corrections in R

```r
pVals <- read.table("files/pvalsExample.txt")
head(pVals$V1, 5)
```

```
[1] 0.0001264 0.0001150 0.0000113 0.0000882 0.0000190
```

```r
pValsAdjusted <- p.adjust(pVals$V1, method="bonferroni")
head(pValsAdjusted, 5)
```

```
[1] 0.0039184 0.0035650 0.0003503 0.0027342 0.0005890
```

```r
# 'fdr' or 'BH' for Benjamini-Hochberg method
pValsAdjusted <- p.adjust(pVals$V1, method="fdr")
head(pValsAdjusted, 5)
```

```
[1] 0.00013061333 0.00012293103 0.00002097059
0.00010126667 0.00002804762
```

Additional Enrichment Analyses

- Gene Set Enrichment Analysis (GSEA): GSEA is one of the best known enrichment analyses
  - This method additionally takes into account numeric values associated with the genes (e.g. gene expression levels)
  - They provide many collections of "gene sets" that can be used with GSEA or related methods
  - http://software.broadinstitute.org/gsea/msigdb

Regression

- Goal: Find the relationship between an independent variable and a set of dependent variables (also known as predictor or features)
  - Example: The relationship between drug response (dependent variable) and the expression of some genes (independent variables).

Given n observations each with a response variable y and p predictors (or features)

$$\begin{aligned} Y &= (y_1, \ldots, y_n)^T, \quad n \times 1 \\ X &= (X_1, \ldots, X_p), \quad n \times p \end{aligned}$$

Goal: We want to find a set of regression coefficients β for $x = (x_1, \ldots, x_p)$ to describe the relationship between y and $x_1, \ldots, x_p$

$$\hat{y} = \hat{\beta}_0 + \hat{\beta}_1 x_1 + \hat{\beta}_2 x_2 + \ldots$$

- $\hat{y}$ is the predicted value
- $\hat{\beta}$ are the estimated regression coefficients (as opposed to the true coefficients)

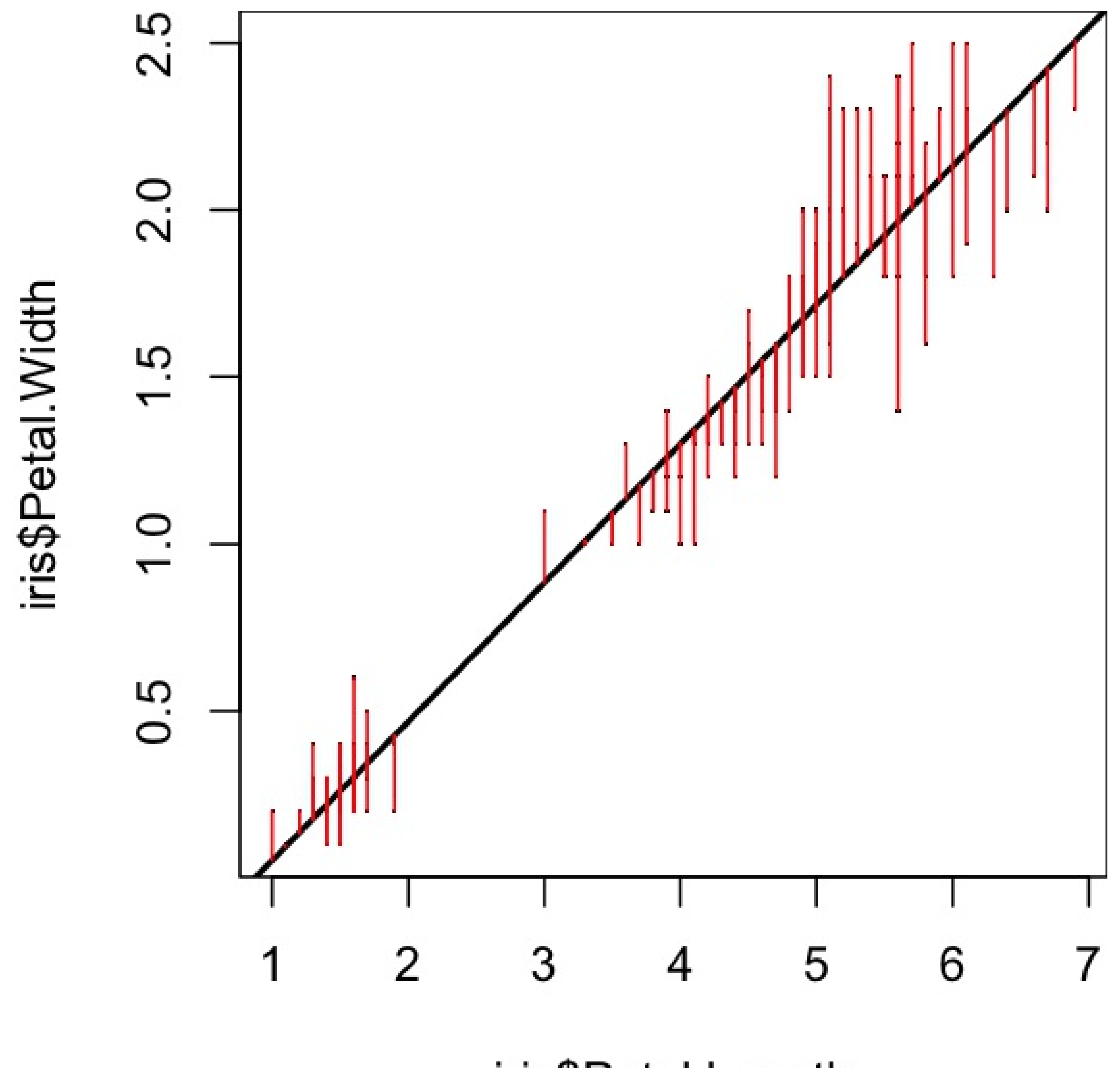
Example Regression

```r
results <- lm(Petal.Width ~ Petal.Length, data=iris)
summary(results)
```

```

Call:
lm(formula = Petal.Width ~ Petal.Length, data = iris)

Residuals:
     Min       1Q   Median       3Q      Max 
-0.56515 -0.12358 -0.01898  0.13288  0.64272 

Coefficients:
              Estimate Std. Error t value Pr(>|t|)    
(Intercept)  -0.363076   0.039762  -9.131  4.7e-16 ***
Petal.Length  0.415755   0.009582  43.387  < 2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '
' 1

Residual standard error: 0.2065 on 148 degrees of freedom
Multiple R-squared:  0.9271,	Adjusted R-squared:  
0.9266 
F-statistic:  1882 on 1 and 148 DF,  p-value: < 2.2e-16
```

Plotting Regression Results

```
par(mai=c(1,1,0,0))
plot(iris$Petal.Length, iris$Petal.Width, pch=".")
abline(results, lwd=2)

# Plot distances between points and the regression line (i.e. residuals)
predictedY <- predict(results)
segments(iris$Petal.Length, iris$Petal.Width, iris$Petal.Length,
predictedY, col="red")
```



Interpreting lm() Results Summary

- Residuals: The difference between the actual and predicted values
- Estimate: Regression coefficient estimates
- Std. Error: Measurement of the variability of the coefficient estimate. Lower is better
- t value: Coefficient score to describe the importance of predictor; used to calculate the p-value
- Pr(>|t|): Coefficient p-value. Probability the predictor is **NOT** relevant
- R-square: Score for evaluating how well the model fits the data. Higher is better. This can be adjusted for the number of predictors used in the model.
    - Values ~0.7 are of more interest, but there is no standard rule
- More information: http://blog.yhat.com/posts/r-lm-summary.html

Predict Values for New Inputs to Regression Model

```
new <- data.frame(Petal.Length=seq(-3, 3, 0.5))
predict(results, new)
```

```
         1          2          3          4          5 
6 
-1.6103418 -1.4024641 -1.1945864 -0.9867086 -0.7788309 
-0.5709532 
         7          8          9         10         11 
12 
-0.3630755 -0.1551978  0.0526799  0.2605576  0.4684353 
0.6763130 
        13 
 0.8841907 
```

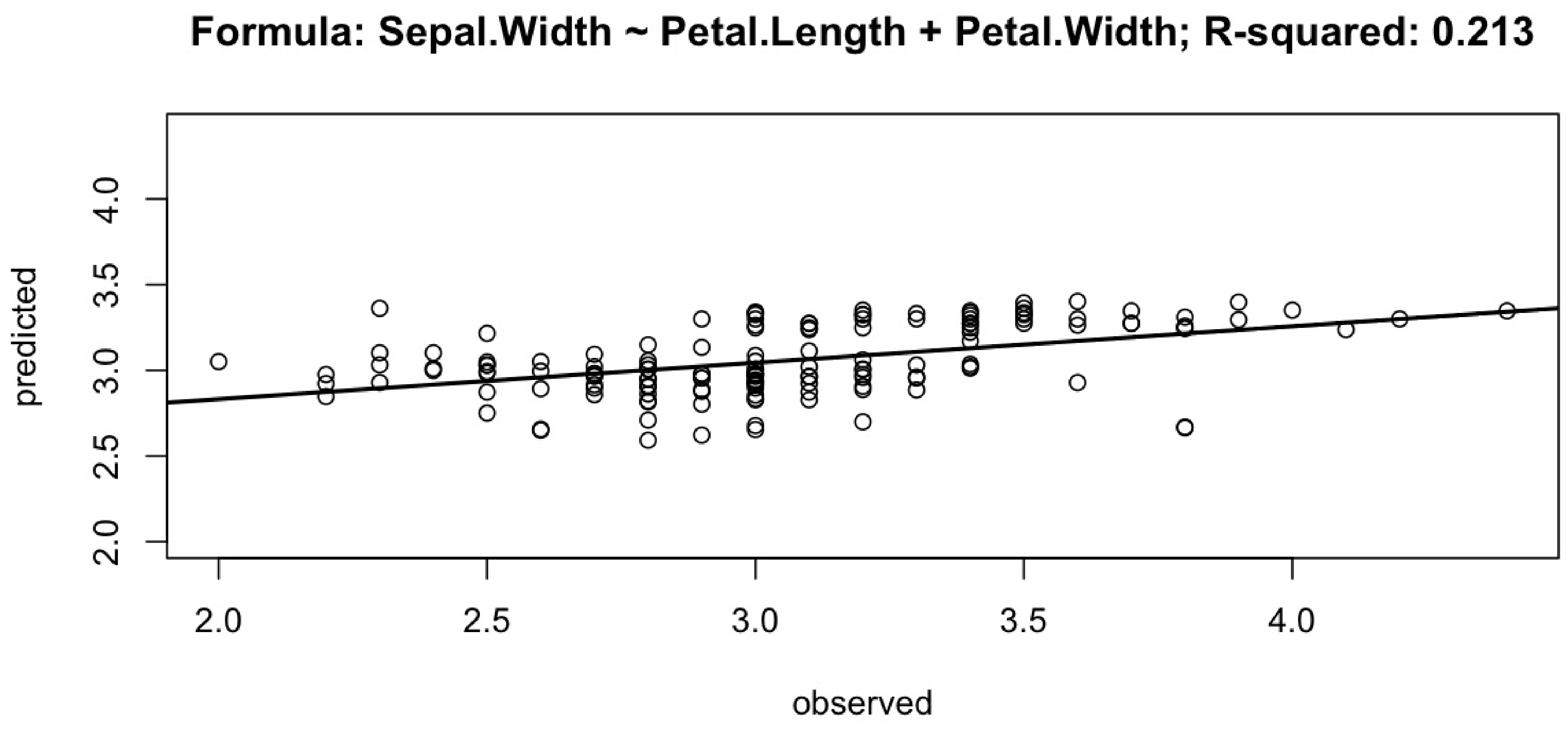
Multiple Regression

```r
formula <- "Sepal.Width ~ Petal.Length + Petal.Width"

y <- iris$Sepal.Width
fit <- lm(as.formula(formula), data=iris[,1:4])
summary(fit)
```

```

Call:
lm(formula = as.formula(formula), data = iris[, 1:4])

Residuals:
     Min       1Q   Median       3Q      Max 
-1.06198 -0.23389  0.01982  0.20580  1.13488 

Coefficients:
             Estimate Std. Error t value Pr(>|t|)    
(Intercept)   3.58705    0.09373  38.272  < 2e-16 ***
Petal.Length -0.25714    0.06691  -3.843  0.00018 ***
Petal.Width   0.36404    0.15496   2.349  0.02014 *  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.3893 on 147 degrees of freedom
Multiple R-squared:  0.2131,	Adjusted R-squared:  0.2024 
F-statistic:  19.9 on 2 and 147 DF,  p-value: 2.238e-08
```

Plotting Multiple Regression

```
pred <- predict(fit)

# Add regression between predicted and observed
fit2 <- lm(pred ~ y)

# Plot predicted versus observed
title <- paste0("Formula: ", formula, "; R-squared: ", round(summary(fit2)$r.squared,
3))

plot(y, pred, xlim=range(c(y, pred)), ylim=range(c(y, pred)), xlab="observed",
ylab="predicted", main=title)

# Add regression line
abline(fit2, lwd=2)
```



Some Issues with Regression

- Missing Data: What if your data has missing values?
  - Imputation can be used to fill in missing values using other data points
- Too many predictors versus the number of samples
  - The “Curse of Dimensionality” (later slide)
  - Regularized regression methods can be used to select features to be included in the model
- Overfitting: Will your model work on other datasets?
  - Excessively complex models (e.g. having too many predictors) can have poor predictive performance when tested on new data
  - Regularized regression methods have properties to avoid overfitting

Missing Data

- By default, `lm()` removes rows that contain missing values
- An alternative is imputation to fill in missing values
  - `impute` imputes using K-Nearest Neighbors (KNN)
  - Step 1: Identify K number of neighbors based on Euclidean distance
  - Step 2: Average the values of the neighbors and replace the missing value

Basic Rules for Imputation

- Should be done when the number of missing values is small
  - A safe maximum threshold is 5% of the total for large datasets
- Should be done when the imputed values are plausible for the missing values
- Should be done when it is assumed that the missing values occur at random
  - If missing values do not occur at random, the data collection should be investigated and/or the values should be dropped

The "Curse of Dimensionality"

- If the number of predictors is greater than the number of samples, it will not be possible to estimate relevant regression parameters in the full model.
  - This is due to the degrees of freedom in the system.
  - There are n observations and $p + 1$ parameters (one regression coefficient for each predictor plus the intercept) leaving $n - p - 1$ degrees of freedom.
  - Increasing the sample size provides more information about the population test.
  - Increasing the number of predictors in the resulting model lowers the degrees of freedom available to estimate the variability of the predictors; this increases the variance of the regression coefficient estimates and reduces confidence in the model.

LASSO, Ridge, and Elastic Net Regularized Regression

- Least Absolute Shrinkage and Selection Operator (LASSO): Tends to produce sparse (i.e. few predictors) whereby the algorithm selects an arbitrary predictor among a set of correlated ones
- Ridge: Tends to select all correlated predictors with their coefficient values equal to each other
- Elastic Net (EN): Blends the concepts of LASSO and Ridge regression to attempt to create a model that is both sparse, but also includes correlated predictors
  - EN parameter $\alpha = 1$ represents LASSO regression, while $\alpha = 0$ approaches Ridge regression
- LASSO, Ridge, and Elastic Net regression are available from the `glmnet` R package
  - Sousa FG et al. DNA damage response alterations and its relation with drug activity across the NCI-60. DNA Repair (2015) for example usage of `glmnet` and Elastic Net

Clustering

- Goal: Divide data into groups (clusters), so that group members are more “similar” to each other than to members outside the group
    - Example: Cluster a set of drugs. For drugs without a known mechanism of action (MOA), predict a potential MOA based on how the unknown MOA drugs cluster with known MOA drugs
- Clustering differs from classification in that in classification we have known groups

Hierarchical Clustering

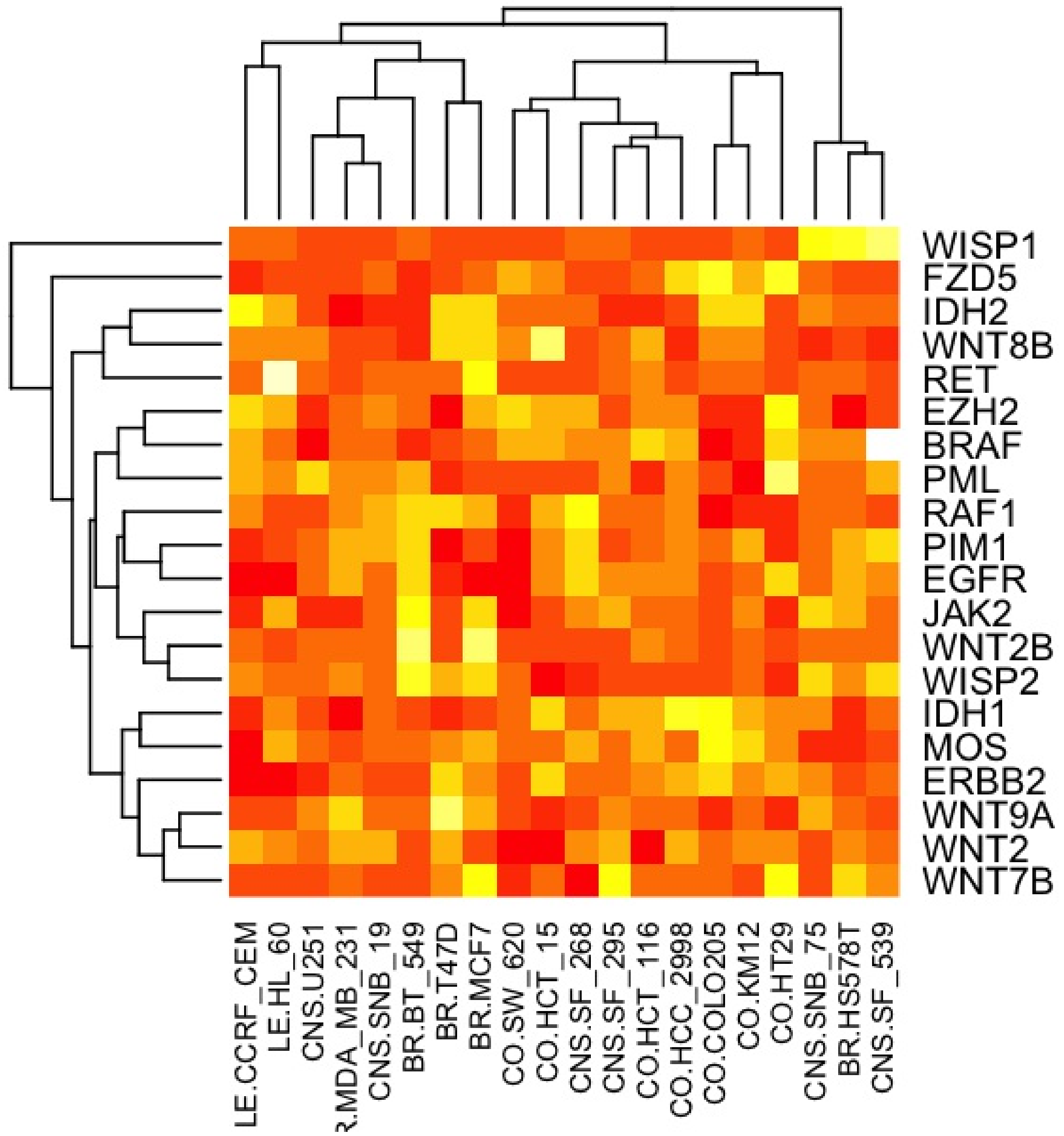
- R uses an agglomerative (bottom-up) clustering approach
  - Alternative: Divise (top-down) is similiar to agglomerative, but in reverse
- Algorithm

1. All points start in their own clusters
2. At each iteration merge the 2 most similar structures
3. Stop if there is a single cluster containing all points, else go to Step 2

Hierarchical Clustering Example

- Heatmap shows the expression of 20 oncogenes from 20 NCI-60 celllines

```
dat <- read.table("files/heatmapExample.txt", sep="\t", header=TRUE)
mat <- as.matrix(dat); heatmap(mat, cexCol=0.75)
```

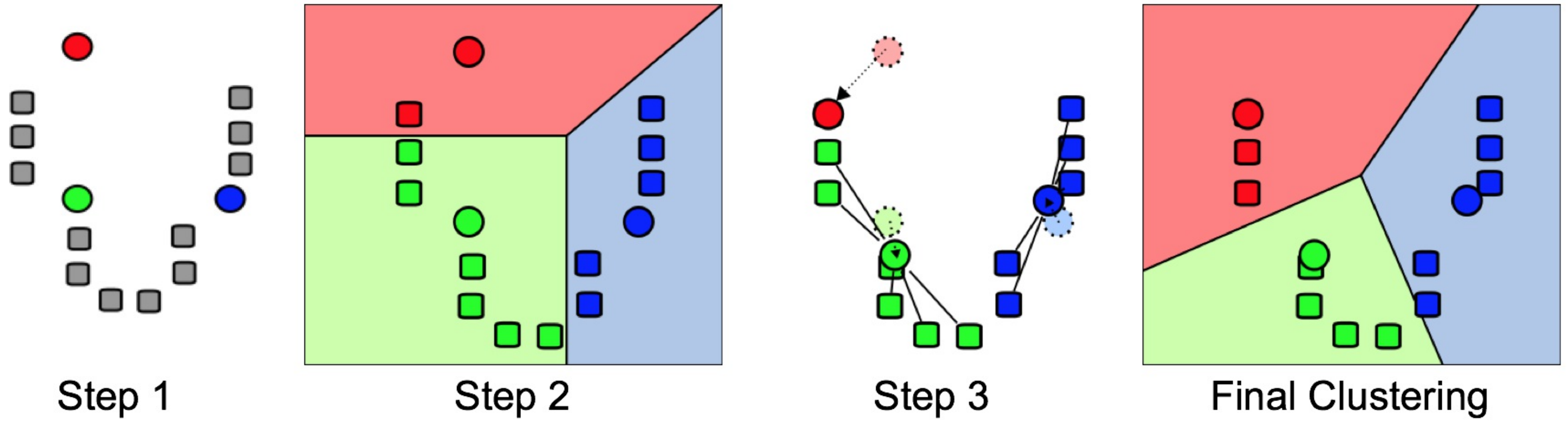


Cluster Similarity (Linkage)

- Distances between clusters are calculated to determine cluster similarity
- Options of Cluster Linkage Distances
  - Single: Distance between two clusters is defined by the distance between the two closest points.
  - Average: Average of all pairwise distances between the points in two clusters
  - Complete: Distance between two clusters is defined by the distance between the two farthest points.
- `hclust()` used by `heatmap()` in R uses the “complete” method by default

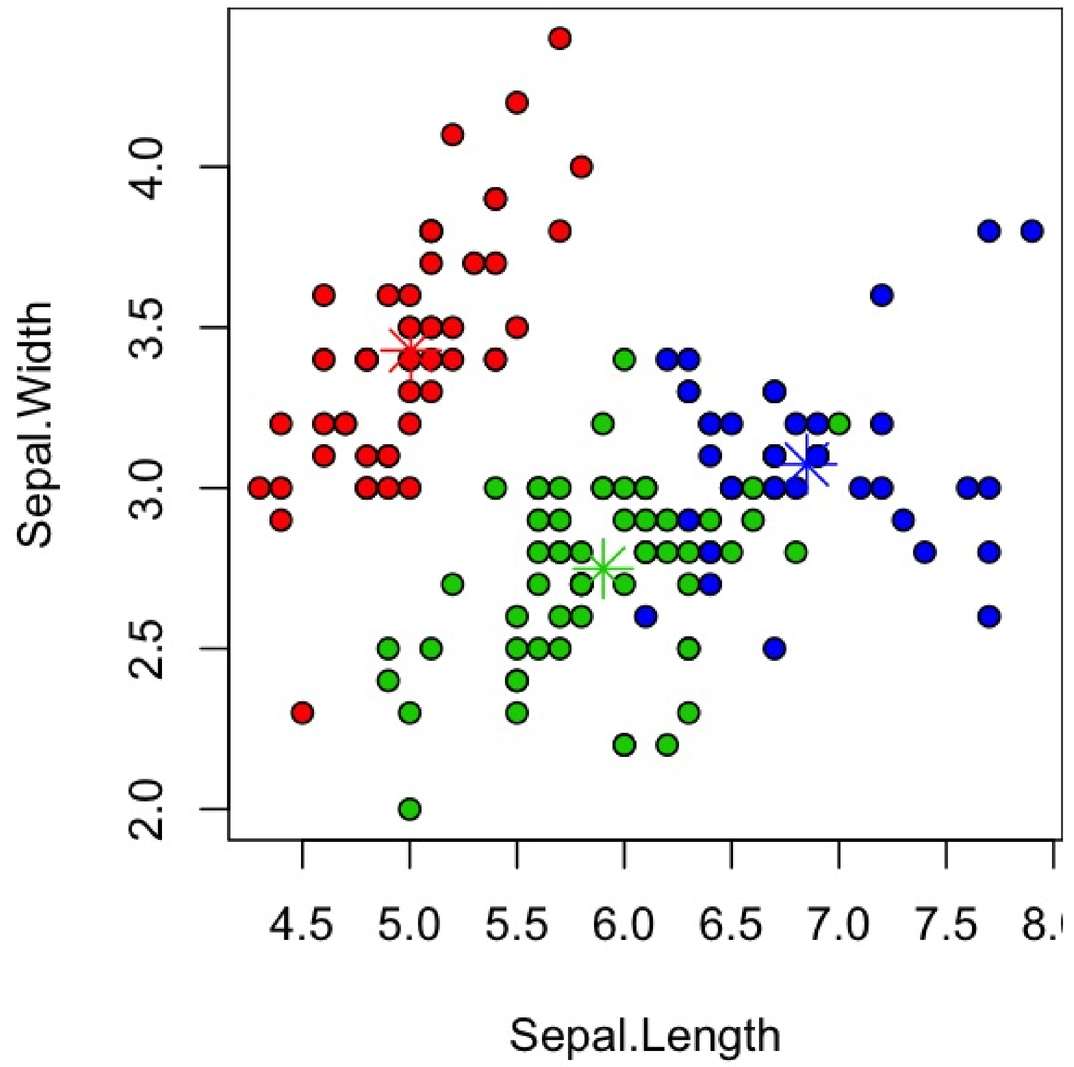
K-Means Clustering

- Algorithm
  1. A user-selected (k) number of means are randomly generated from the data
  2. k clusters are created by grouping data points to the nearest mean.
  3. The centroid of the clusters becomes the new mean.
  4. Steps 2 and 3 are repeated until the clusters do not change anymore



K-Means Clustering Example

```r
# Retain only the numeric data in the iris dataset
iris_data <- iris[, 1:4]

# nstart: try multiple initial configurations and report the best one
kc <- kmeans(iris_data, 3, nstart=25)

par(mai=c(1,1,0,0))
plot(iris[c("Sepal.Length", "Sepal.Width")], bg=c("red","green3","blue")[kc$cluster],
pch=21)
points(kc$centers[,c("Sepal.Length", "Sepal.Width")], col=c("red","green3","blue"),
pch=8, cex=2)
```

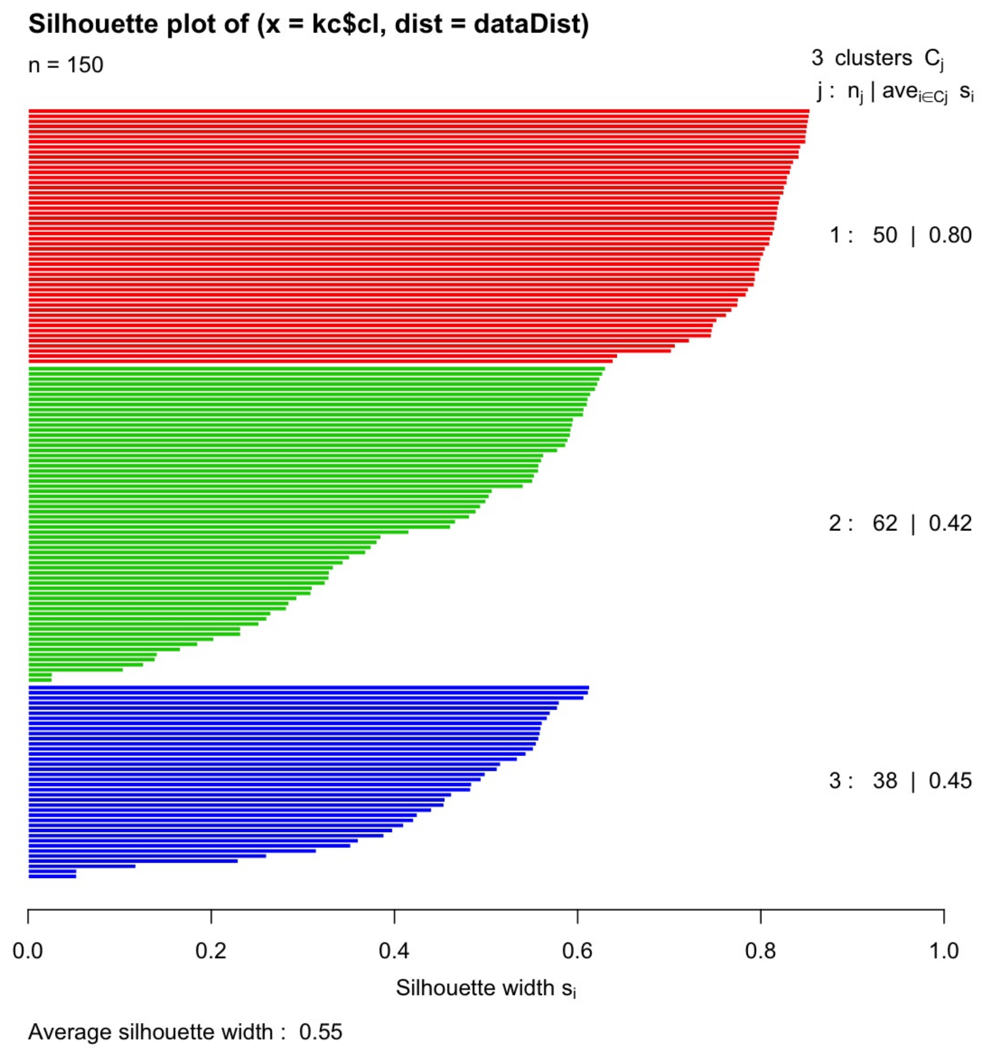


Determine K-Means Cluster Quality

```
library(cluster)
dataDist <- dist(iris_data)
si <- silhouette(kc$cl,
dataDist)
```

```
plot(si, col = c("red",
"green3", "blue"))
```

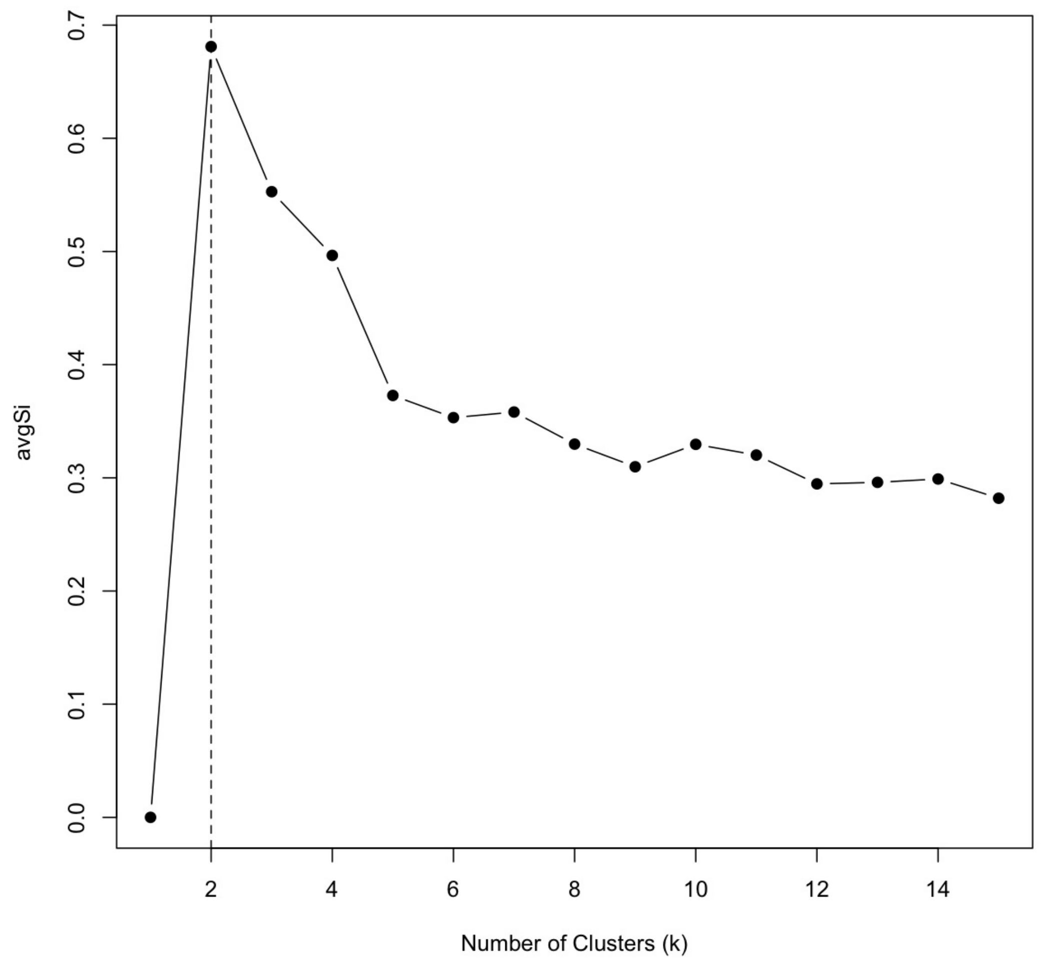
- Silhouette Plot
  - Horizontal barplot is the goodness of fit of sample within the cluster
  - Longer is better
  - Rightmost number, S_i, is average length
- Average Silhouette Guidelines
  - 0.71-1.0: Strong clustering
  - 0.51-0.70: Reasonable clustering
  - < 0.50: Weak clustering



Selecting k with Average Silhouette

```
library(cluster)
kMax <- 15
avgSi <- rep(0, kMax)

# Average silhouette width
# k: 2 to 15
for(i in 2:kMax){
  results <-
kmeans(iris_data,
centers=i)
  si <-
silhouette(results$cluster,
dist(iris_data))
  avgSi[i] <- mean(si[,
"sil_width"])
}
```

```
plot(1:kMax, avgSi,
type="b", pch=19,
xlab="Number of Clusters
(k)")
abline(v=which.max(avgSi),
lty=2)
```



Compare Known Classes with Clusters

```
table(iris$Species, kc$cluster)
```

```
              1  2  3
  setosa     50  0  0
  versicolor  0 48  2
  virginica   0 14 36
```

Differences between Hierarchical and K-Means Clustering

- Clusters
  - K-means produces a single set of clusters
  - Hierarchical produces different clusters depending on where the tree is cut
- Cluster Number
  - K-means requires the number clusters to be set
  - Hierarchical clustering does not require the number of clusters to be set
- Speed
  - K-means is faster than hierarchical clustering

Dimensionality Reduction

- Goal: Seeks to reduce the dimensions of the data without losing (much) information.
  - This is possible if many predictors are correlated with one another, and therefore redundant.
- Principal Component Analysis is a method for dimension reduction

What is Principal Component Analysis (PCA)

- Goal: PCA seeks to simplify a multi-dimensional (e.g. one with 3+ predictors (features)) dataset
  - Used for feature extraction
  - May reveal clusters and help validate clustering results
- Example: We have a dataset with 20 dimensions and it may be interesting to plot the data in two dimensions
- Results:
  - Loadings: Weights for the original values to get the component scores
  - Component Scores: Transformed values for a given point
- `prcomp` and `princomp` can do PCA in R; `prcomp` is the advised function

What are Principal Components?

- Each “principal component” (PC) is an axis that captures the most variance
  - Variance is a measure of the spread of data points; standard deviation is the square root of variance
  - Each PC is a combination of the original variables scaled by a coefficient
  - Every PC explains some variance
  - Each additional PC explains less variance than the previous one
- New coordinate axes are constrained to be perpendicular, so the data are de-correlated

PCA Example

- Using scale=TRUE is advisable
- `prcomp` first transforms the data by centering and scaling
  - Centering is done by subtracting the column means
  - Scaling is done by dividing the (centered) columns of X by their standard deviations

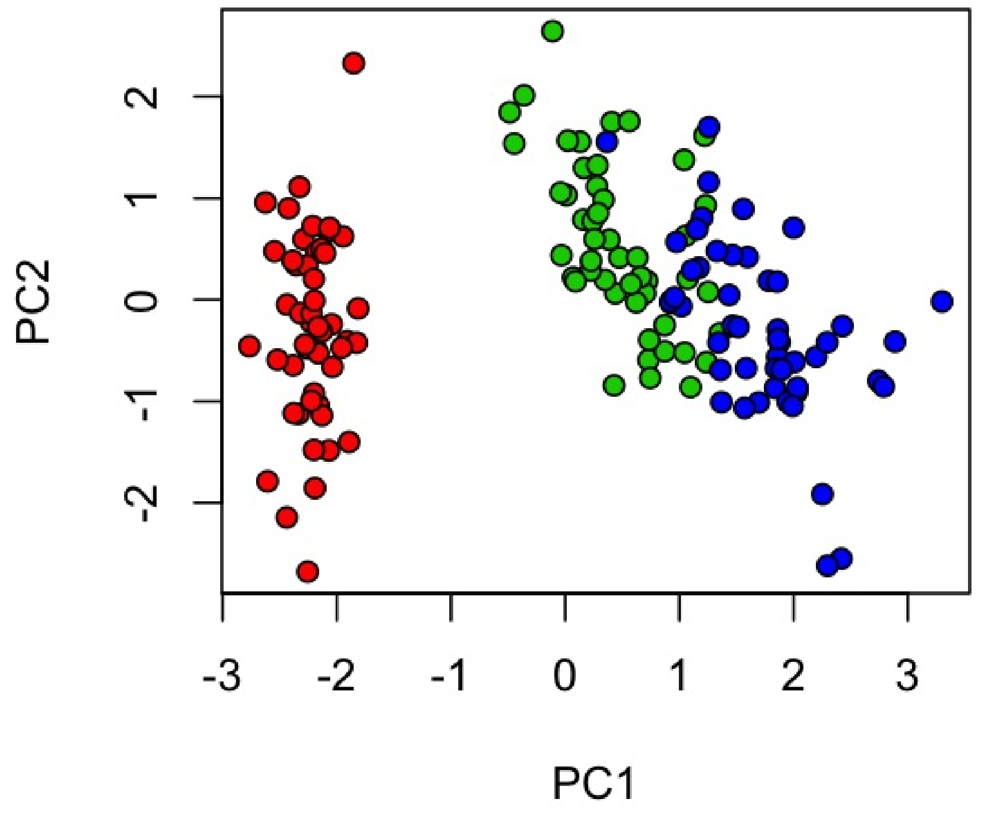
```
iris_data <- iris[, 1:4]

pcaResult <- prcomp(iris_data, scale=TRUE)
summary(pcaResult)
```

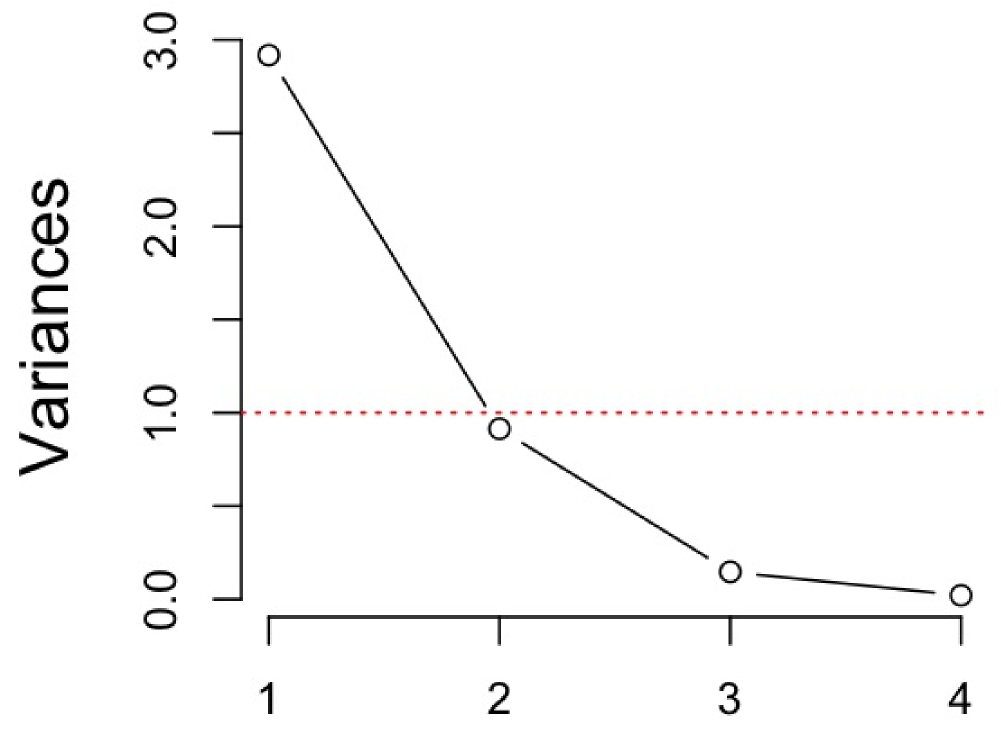
```
Importance of components:
                          PC1    PC2     PC3     PC4
Standard deviation     1.7084 0.9560 0.38309 0.14393
Proportion of Variance 0.7296 0.2285 0.03669 0.00518
Cumulative Proportion  0.7296 0.9581 0.99482 1.00000
```

PCA Example Plots

```
# First 2 principal
components (PC)
plot(pcaResult$x, pch=21,
bg=c("red","green3","blue")
[unclass(iris$Species)])
```

```
# PC variances
plot(pcaResult,
type="line", cex.lab=1.5,
cex.main=1.5, main="")
abline(h=1, lty=3,
col="red")
```





Recovering the Original Data

```r
# Weights (known as loadings)
pcaResult$rotation
```

```
                    PC1         PC2        PC3        PC4
Sepal.Length  0.5210659 -0.37741762  0.7195664  0.2612863
Sepal.Width  -0.2693474 -0.92329566 -0.2443818 -0.1235096
Petal.Length  0.5804131 -0.02449161 -0.1421264 -0.8014492
Petal.Width   0.5648565 -0.06694199 -0.6342727  0.5235971
```

```r
# Original
iris_data[1,]
```

```
  Sepal.Length Sepal.Width Petal.Length Petal.Width
1          5.1         3.5          1.4         0.2
```

```r
# Transformed
pcaResult$x[1,]
```

```
        PC1         PC2         PC3         PC4 
-2.25714118 -0.47842383  0.12727962  0.02408751 
```

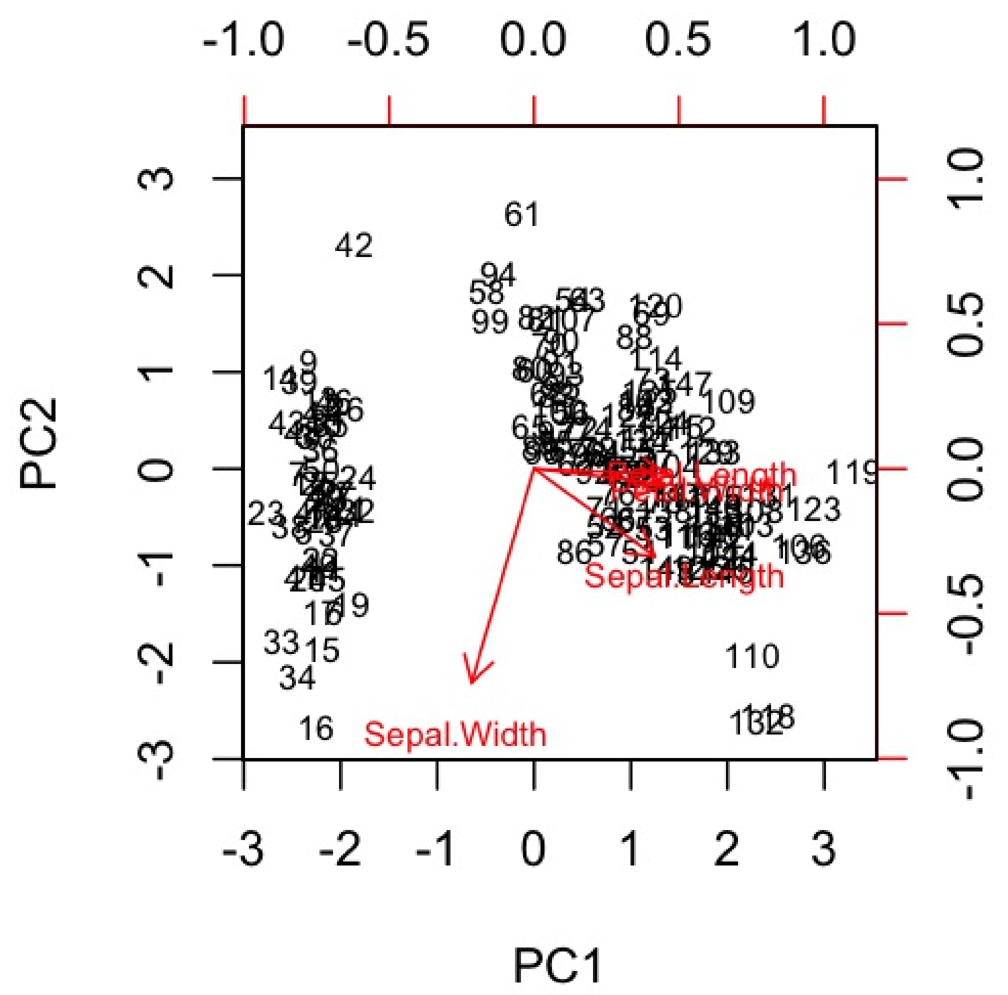
```r
# Recovered
tmp <- t(t(pcaResult$x %*% t(pcaResult$rotation)) * pcaResult$scale + pcaResult$center)
tmp[1,]
```

```
Sepal.Length  Sepal.Width Petal.Length  Petal.Width 
         5.1          3.5          1.4          0.2 
```

Visualizing PCA Results with Biplots

- Visualizes the magnitude and sign of each feature's contribution to a PC
- Visualizes each observation in terms of PCs
- Closeness equals similarity for points and vectors

```
biplot(pcaResult, scale=0, cex=.7)
```



Correlations between Vectors

Feature to Principal Component Correlations

```
cor(iris_data, pcaResult$x)
```

```
                    PC1         PC2         PC3
PC4
Sepal.Length  0.8901688 -0.36082989  0.27565767
0.03760602
Sepal.Width  -0.4601427 -0.88271627 -0.09361987
-0.01777631
Petal.Length  0.9915552 -0.02341519 -0.05444699
-0.11534978
Petal.Width   0.9649790 -0.06399985 -0.24298265
0.07535950
```

Feature to Principal Component Contributions

```
tmp <- abs(pcaResult$rotation)
sweep(tmp, 2, colSums(tmp), "/")
```

```
                   PC1        PC2        PC3        PC4
Sepal.Length 0.2691897 0.27110474 0.41346137 0.15281309
Sepal.Width  0.1391485 0.66321714 0.14042128 0.07223451
```

How Many Principal Components Should be Kept?

- Kaiser criterion
  - Retain only principal components that with a variance greater than 1
  - The variance of every input variable is 1 (because the scaling/centering), therefore only retain PCs with “stronger” variances than individual variables.
  - Simple, but less advisable
- Scree Test
  - Find the place where the smooth decrease in the variances levels off
  - Multiple users may interpret the data different, unless trained the same

Getting Help

- Cross-Validated Stats Exchange
  - Part of Stack Overflow
  - http://stats.stackexchange.com/
- Biostars
  - https://www.biostars.org