Explore the data

Anja Bråthen Kristoffersen

Biomedical Research Group

UNIVERSITY OF OSLO

- This talk is a minor modification of the one given by Raphael Gottardo as part of the Canadian Bioinformatics Workshops course on ”Essential Statistics: Getting the numbers right”. The original material is available from http://bioinformatics.ca/workshops/2009/course-content

bioinformatics.ca

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Workshops

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This talk is mostly about

Looking at your data

Outline

- Basics:
  - Boxplots
  - Histograms
  - Scatter plots
  - Transformations
  - QQ-plot
- Applications to microarray data

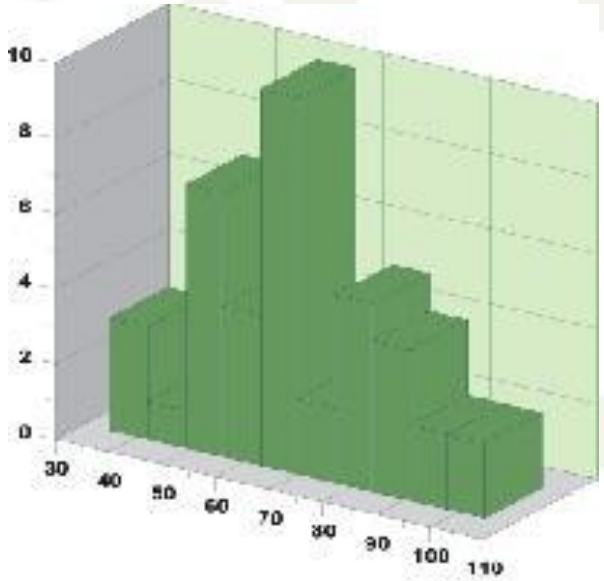
Outline

- Mostly graphical
- Plotting the raw data (histograms, scatterplots, etc.)
- Plotting simple statistics such as means, standard deviations, medians, box plots, etc
- Positioning such plots so as to maximize our natural pattern-recognition abilities
- A **clear** picture is worth a thousand words!

A few tips

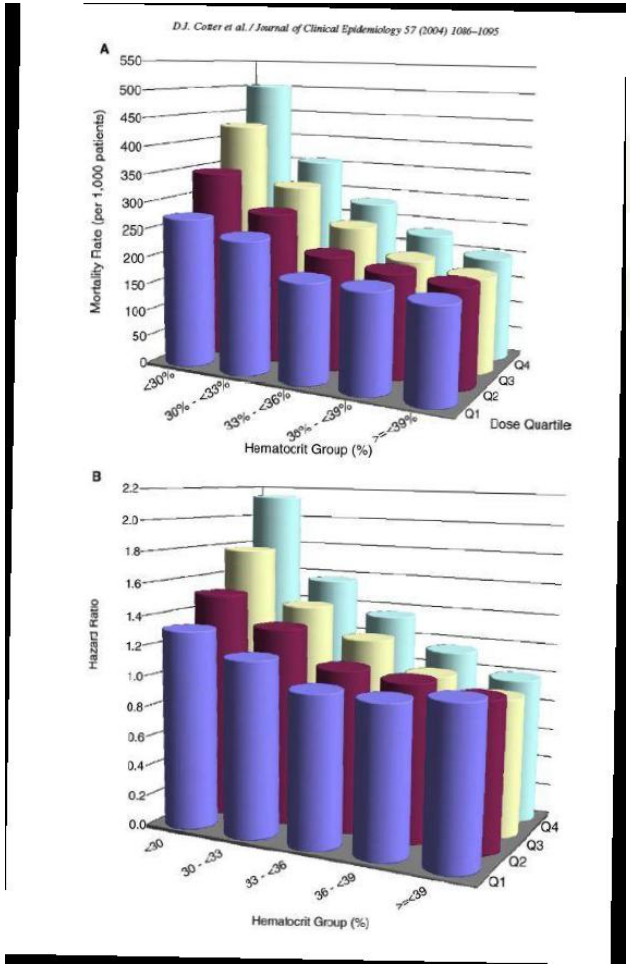
- Aviod 3-D graphics
- Don’t show too much information on the same graph (color, pattern, etc)
- R provides a great environment for Exploratory Data Analysis (EDA) with good graphic capabilities.

A few bad plots



Unnecessary third dimension

A few bad plots



A 2D plot with four lines would be clearer

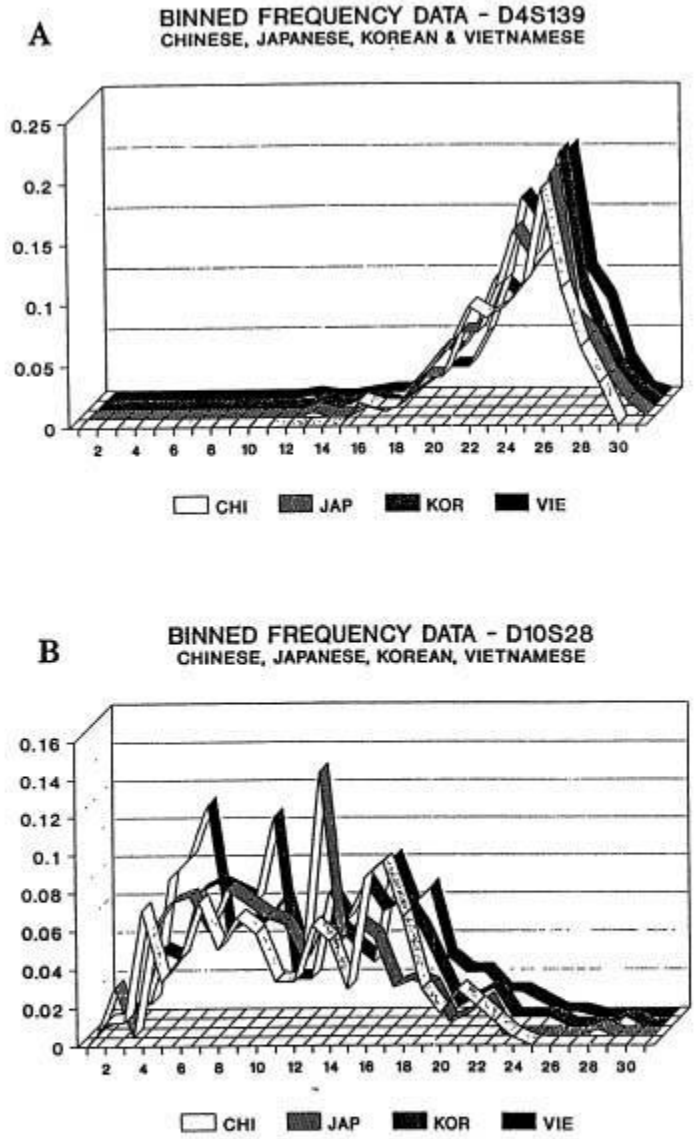


FIG. 4. *Fixed bin distribution (histogram) for two loci and four Asian subpopulations (used with permission from John Hartmann): the boundaries of the* 30 *bins (vertical axis) are determined by the FBI; these bins are not of equal length. Sample sizes (numbers of individuals) for Chinese, Japanese, Korean and Vietnamese are* 103, 125, 93 *and* 215 *for* D4S139 *and* 120, 137, 100 *and* 193 *for* D10S28. *The horizontal axis is the bin number; bins are not of equal length.*

A few bad plots

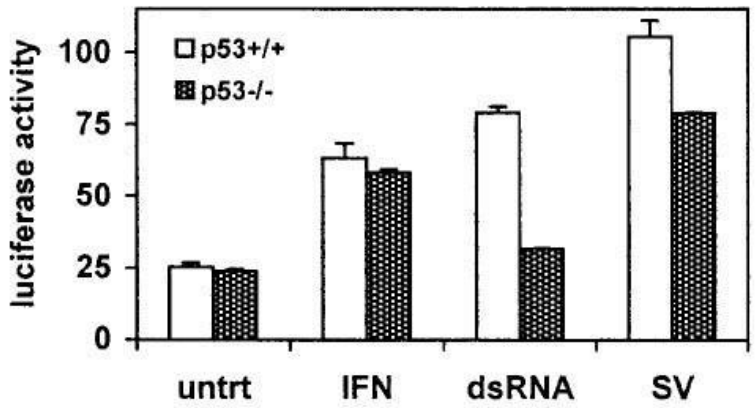


FIG. 4. ISG15 promoter activity mimics endogenous ISG15 mRNA regulation by p53, dsRNA, and virus. Cells (6×10^5 HCT 116) were seeded in 32-mm plates and allowed to attach overnight. Cells were transfected with 500 ng of pGL3/ISG15-Luc, 50 ng of pRL null (Promega), and 450 ng of pcDNA3 for carrier DNA by using Lipofectamine Plus (Life Technologies) following the manufacturer's instructions. Twenty-four hours posttransfection, the medium was aspirated and replaced with medium containing either 1,000 U of IFN-α/ml, 50 μg of dsRNA/ml, or Sendai virus (multiplicity of infection, 10). Cells were incubated for 12 h and then lysed, and luciferase assays were performed. Luciferase activity was assessed on 20 μl of each lysate as directed by the supplier (Dual Luciferase Kit, Promega) using a TD 20/20 luminometer (Turner Designs). Luciferase activity is presented as the ratio of firefly activity to renilla activity to control for differences in transfection efficiency. Each data point is the mean of triplicate samples ± the standard error; the data presented are representative of four independent experiments.

Only three replicates – just showing the numbers would be clearer and more accurate

Show error bars above and below

For more examples

http://www.biostat.wisc.edu/~kbroman/topten_worstgraphs/

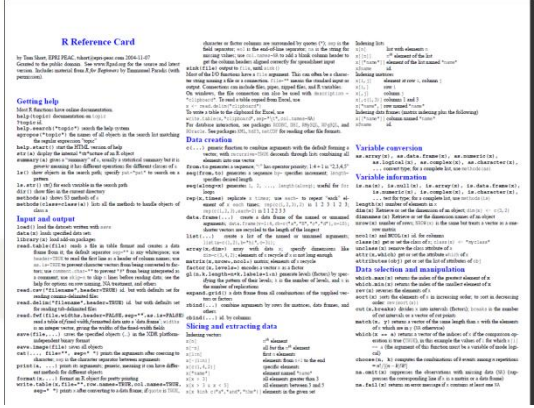
Why you should not use MS Excel for statistics

- Read http://www.practicalstats.com/xlsstats/excelstats.html
- Limited statistical functions
- Misleading/wrong procedures
- Precision errors
- Do not scale well for larger processing
- Excel is not evil, but know when not to use it and
- Don’t box yourself into knowing only Excel

What you should learn

- Learn to use Excel well and appropriately (optional)
- Learn one other package (more statistically)
- R is optimal because you are likely to see it again
- There are a lot of other packages
  - consider using what people around you use.

R



- r-project.org and bioconductor.org
- R reference card http://cran.r-project.org/doc/contrib/Short-refcard.pdf
- *Bioinformatics and Computational Biology Solutions Using R and Bioconductor* by Gentleman et al.

What is R?

- R (http://www.r-project.org). R is a language and environment for statistical computing and graphics
- Provide many statistical techniques
- R provides a great environment for plotting with great graphics capabilities
- Open source
- Highly extensible (e.g. CRAN, Bioconductor)

Probability distributions

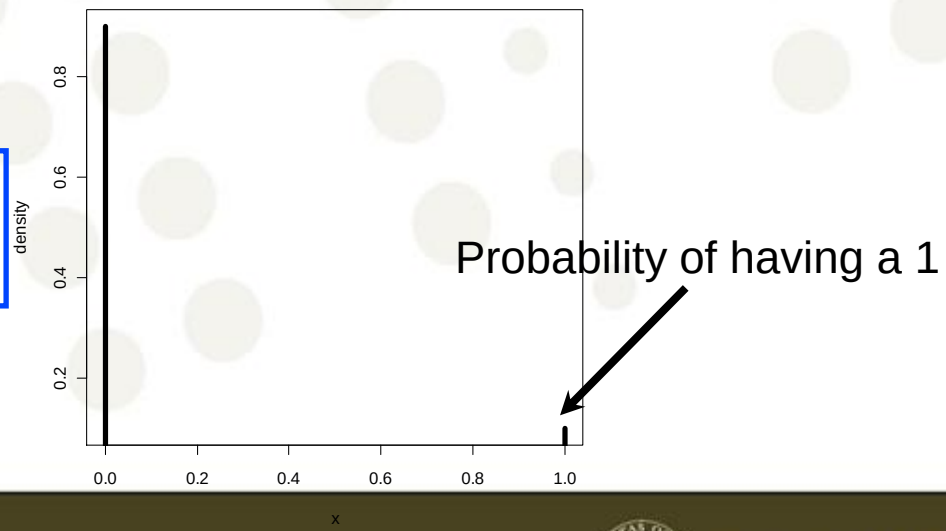
Can be either discrete or continuous (uniform, bernoulli, normal, etc)

Defined by a density function, *p*(x) or *f*(x)

<u>Bernoulli distribution Be(p)</u>

Flip a coin (T=0, H=1). Probability of H is 0.1.

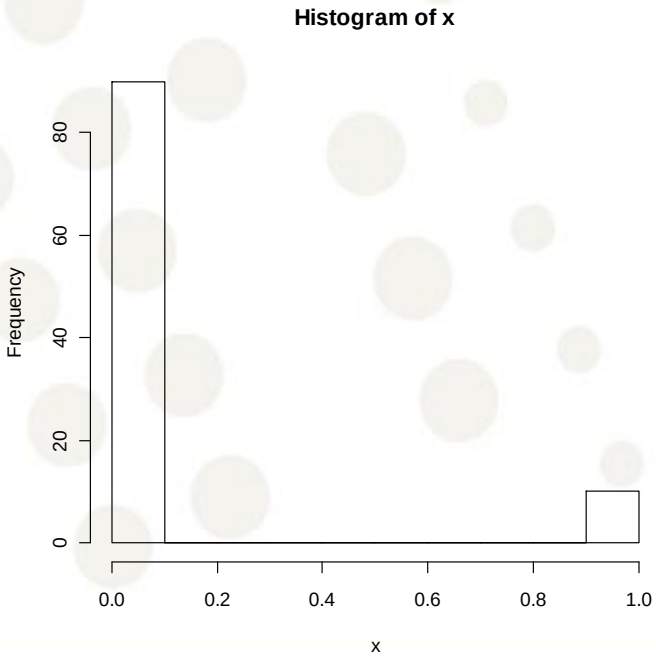
```
x<-0:1
f<-dbinom(x, size=1, prob=0.1)
plot(x,f,xlab="x",ylab="density",type="h",lwd=5)
```



Probability distributions

Random sampling
Generate 100 observations from a Be(0.1)

```
x<-rbinom(100, size=1, prob=0.1)
hist(x)
```

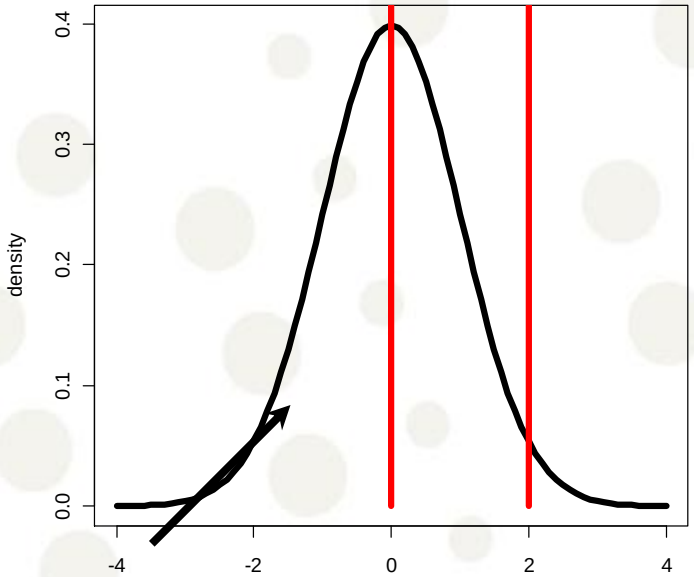


Probability distributions

<u>Normal distribution N(μ,σ²)</u>

μ is the mean and σ² is the variance

```
x<-seq(-4,4,0.1)
f<-dnorm(x, mean=0, sd=1)
plot(x,f,xlab="x",ylab="density",lwd=5,type="l")
lines(c(0,0),c(0,0.5), col=2, lwd=5)
lines(c(2,2),c(0,0.5), col=2, lwd=5)
```

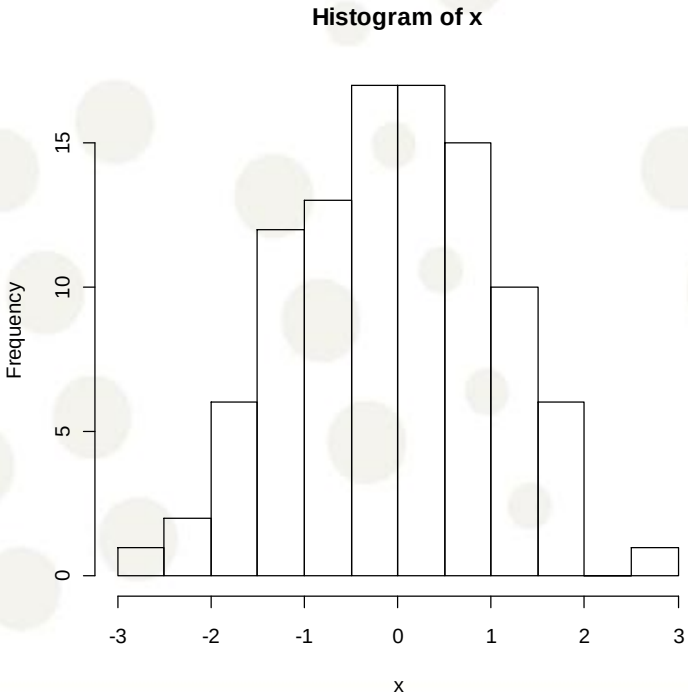


Area under the curve is the probability of having an observation between 0 and 2.

Probability distributions

Random sampling
Generate 100 observations from a N(0,1)

```
x<-rnorm(100, mean=0, sd=1)
hist(x)
```



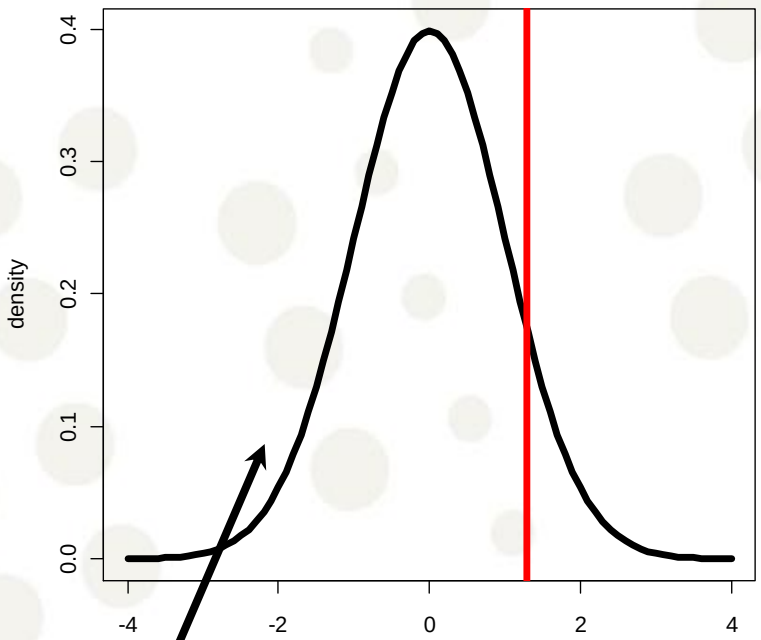
Histograms can be used to estimate densities!

Quantiles

<u>(Theoritical) Quantiles</u>: The *p*-quantile is the value with the property that there is a probability *p* of getting a value less than or equal to it.

```
q90<-qnorm(0.90, mean = 0, sd = 1)
x<-seq(-4,4,.1)
f<-dnorm(x, mean=0, sd=1)
plot(x,f,xlab="x",ylab="density",type="l",lwd=5)
abline(v=q90,col=2,lwd=5)
```

The 50% quantile is called the median



90% of the prob. (area under the curve) is on the left of red vertical line.

Descriptive Statistics

<u>Empirical Quantiles</u>: The *p*-quantile is the value with the property that *p*% of the observations are less than or equal to it.

Empirical quantiles can easily be obtained in R.

```
x<-rnorm(100, mean=0, sd=1)
quantile(x)
```

0%	25%	50%	75%	100%
-2.2719255	-0.6088466	-0.0594199	0.6558911	2.5819589

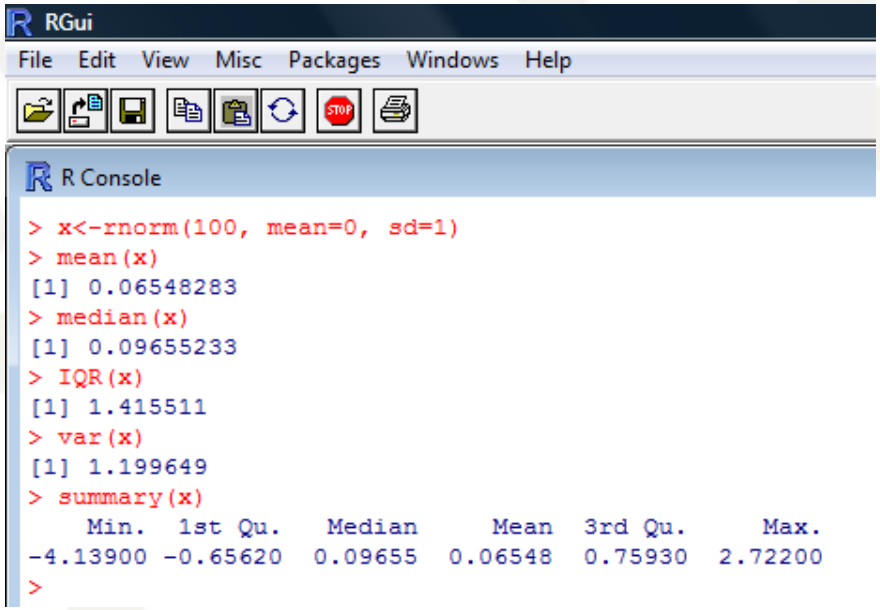
```
quantile(x,probs=c(0.1,0.2,0.9))
```

10%	20%	90%
-1.1744996	-0.8267067	1.3834892

Descriptive Statistics

We often need to quickly ‘quantify’ a data set. This can be done using a set of summary statistics (mean, median, variance, standard deviation)

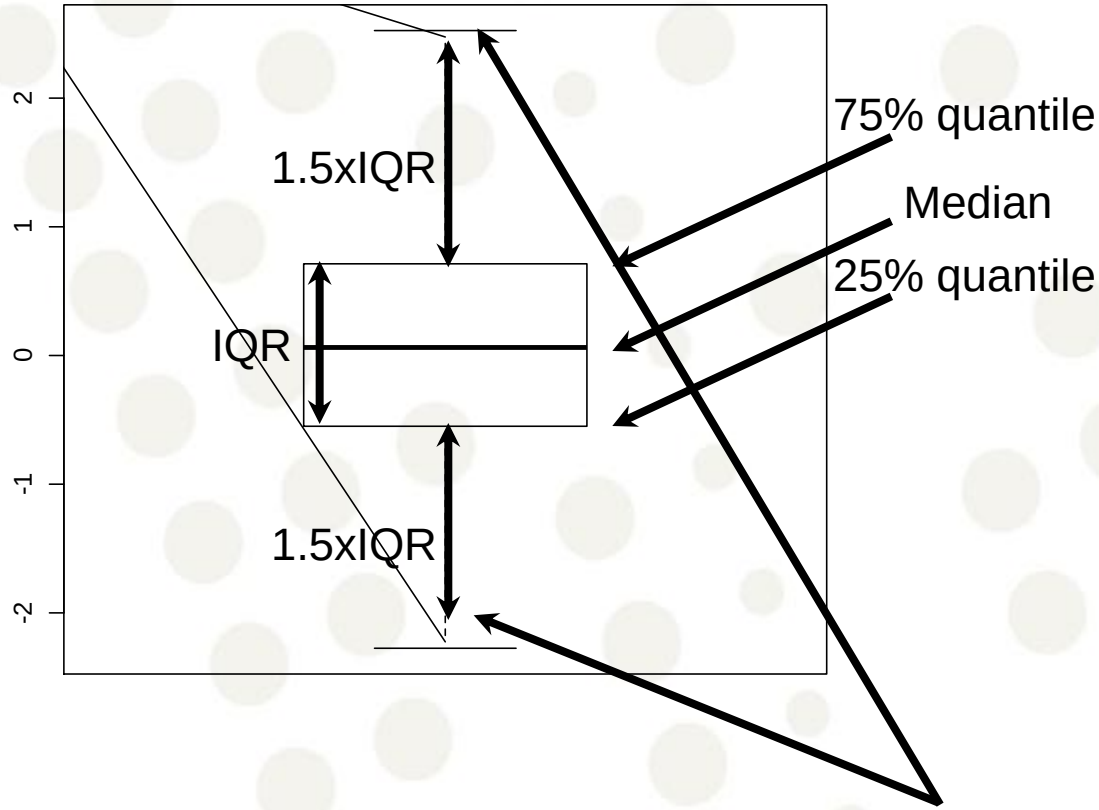
```
x<-rnorm(100, mean=0, sd=1)
mean(x)
median(x)
IQR(x)
var(x)
sd(x)
summary(x)
```



‘summary’ can be used for almost any R object!
R is object oriented (methods/classes).

Descriptive Statistics - Box-plot

```
x<-rnorm(100, mean=0, sd=1)
boxplot(x)
```



IQR= 75% quantile -25% quantile= Inter Quantile Range

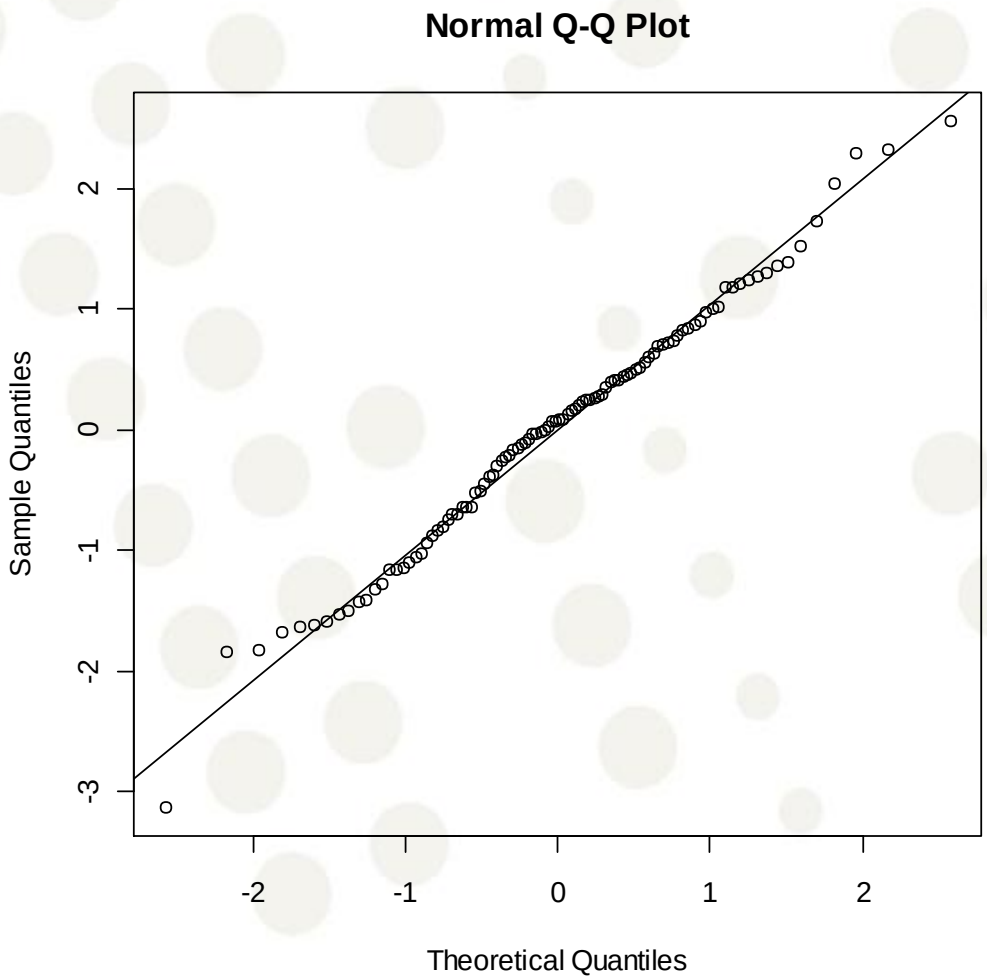
Everything above or below are considered outliers

QQ-plot

- Many statistical methods make some assumption about the distribution of the data (e.g. Normal).
- The quantile-quantile plot provides a way to visually verify such assumptions.
- The QQ-plot shows the theoretical quantiles versus the empirical quantiles. If the distribution assumed (theoretical one) is indeed the correct one, we should observe a straight line.

QQ-plot

```
x<-rnorm(100, mean=0, sd=1)
qqnorm(x)
qqline(x)
```

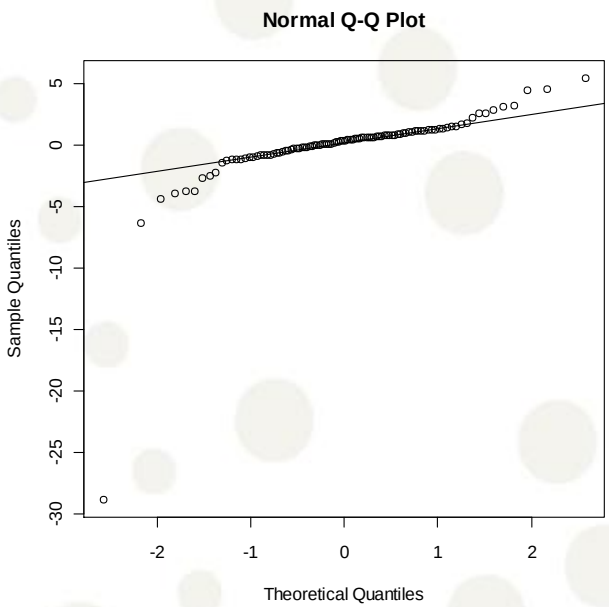


Only valid for the normal distribution!

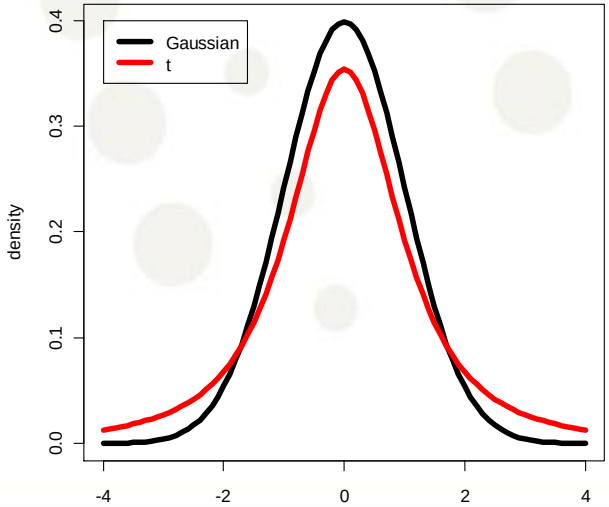
QQ-plot

```
x<-rt(100,df=2)
qqnorm(x)
qqline(x)
```

Clearly the *t* distribution with two degrees of freedom is different from the Normal!



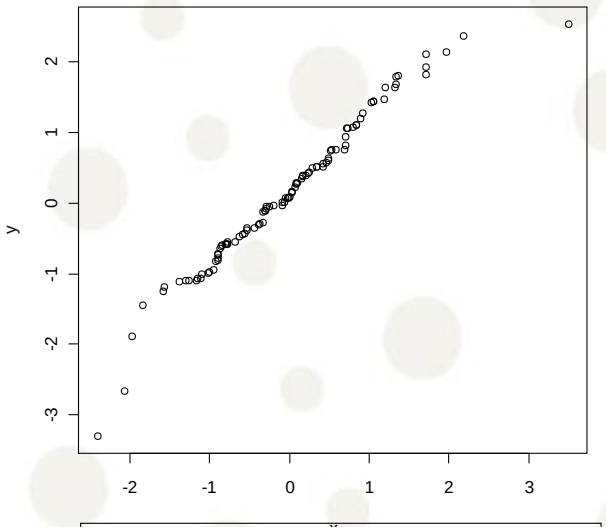

```
x<-seq(-4,4,.1)
f1<-dnorm(x, mean=0, sd=1)
f2<-dt(x, df=2)
plot(x,f1,xlab="x",ylab="density",lwd=5,type="l")
lines(x,f2,xlab="x",ylab="density",lwd=5,col=2)
legend(-4,0.4,c("Gaussian", "t"),col=c(1,2),lty=c(1,1),lwd=5)
```



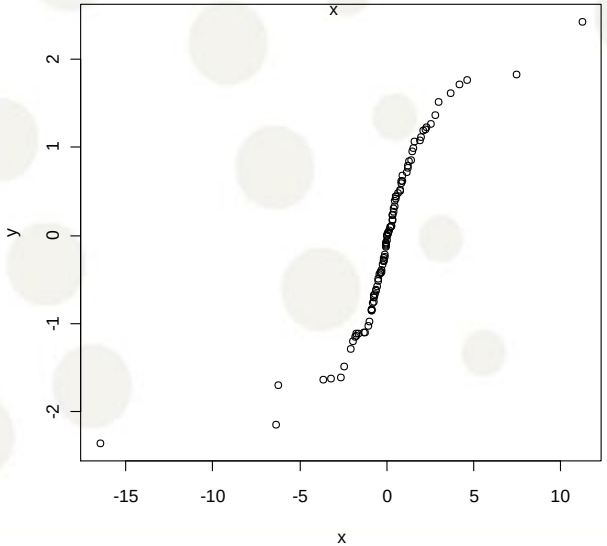
QQ-plot

Comparing two samples

```
x<-rnorm(100, mean=0, sd=1)
y<-rnorm(100, mean=0, sd=1)
qqplot(x,y)
```




```
x<-rt(100, df=2)
y<-rnorm(100, mean=0, sd=1)
qqplot(x,y)
```



Ex: Try with different values of df.

Main idea behind

quantile normalization

http://llmpp.nih.gov/DLBCL/

The New England Journal of Medicine

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THE USE OF MOLECULAR PROFILING TO PREDICT SURVIVAL AFTER CHEMOTHERAPY FOR DIFFUSE LARGE-B-CELL LYMPHOMA

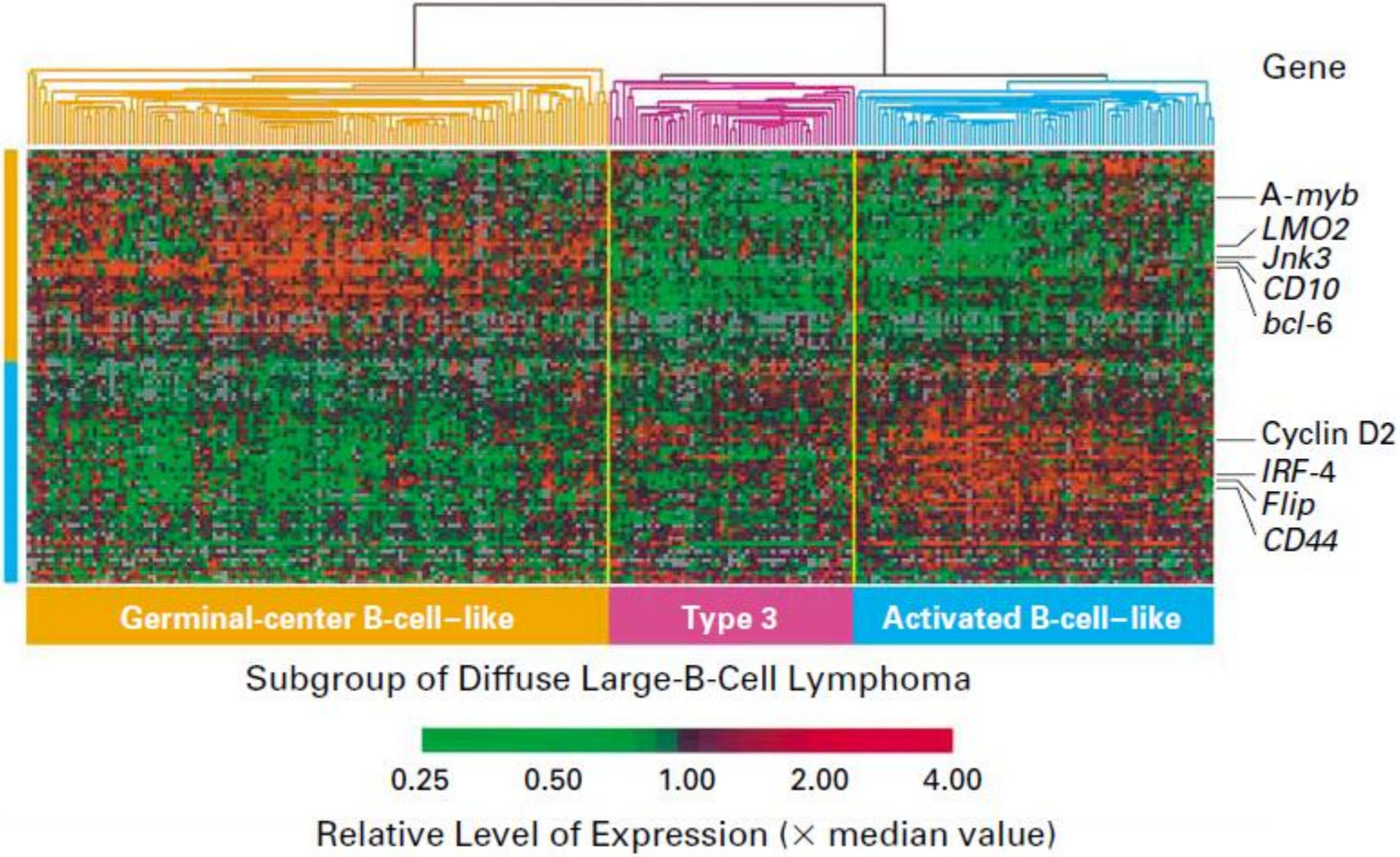
ANDREAS ROSENWALD, M.D., GEORGE WRIGHT, PH.D., WING C. CHAN, M.D., JOSEPH M. CONNORS, M.D., ELIAS CAMPO, M.D., RICHARD I. FISHER, M.D., RANDY D. GASCOYNE, M.D., H. KONRAD MULLER-HERMELINK, M.D., ERLEND B. SMELAND, M.D., PH.D., AND LOUIS M. STAUDT, M.D., PH.D., FOR THE LYMPHOMA/LEUKEMIA MOLECULAR PROFILING PROJECT

ABSTRACT

Background The survival of patients with diffuse large-B-cell lymphoma after chemotherapy is influenced by molecular features of the tumors. We used the gene-expression profiles of these lymphomas to develop a molecular predictor of survival.

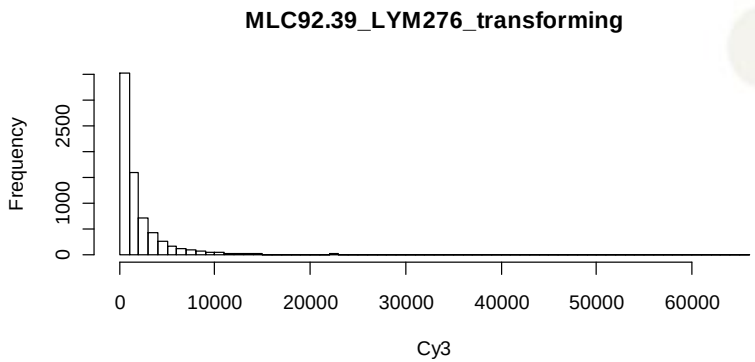
DIFFUSE large-B-cell lymphoma, the most common type of lymphoma in adults, can be cured by anthracycline-based chemotherapy in only 35 to 40 percent of patients.[1] The multiple unsuccessful attempts to increase this rate[2] suggest that diffuse large-B-cell lymphoma

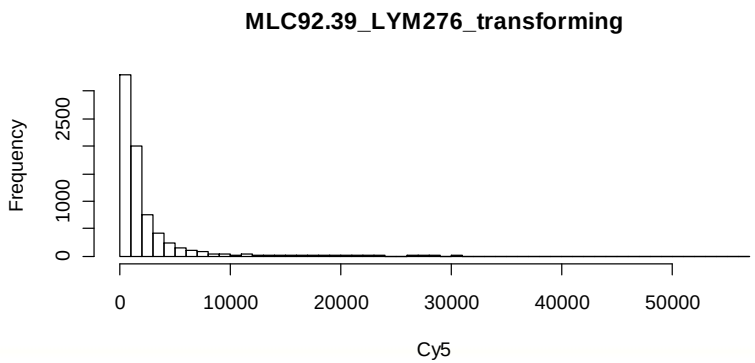
Microarray data



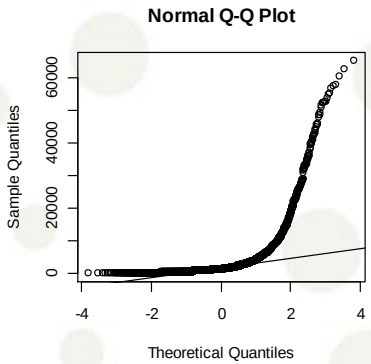
Microarray raw data

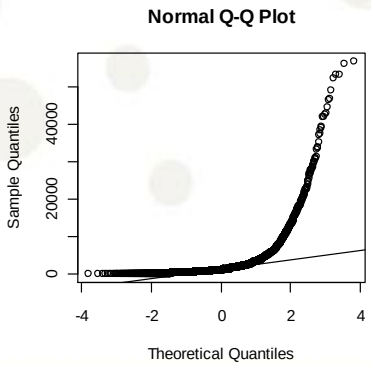
```
Cy3 <- read.table(file="NEJM_Web_Fig1data_CY3.txt", header=TRUE, sep="\t", dec=",")
Cy5 <- read.table(file="NEJM_Web_Fig1data_CY5.txt", header=TRUE, sep="\t", dec=",")

par(mfrow=c(2,1))
hist(Cy3[,55], 50,main=names(Cy3)[55], xlab="Cy3")
hist(Cy5[,55], 50, main=names(Cy3)[55], xlab="Cy5")
```



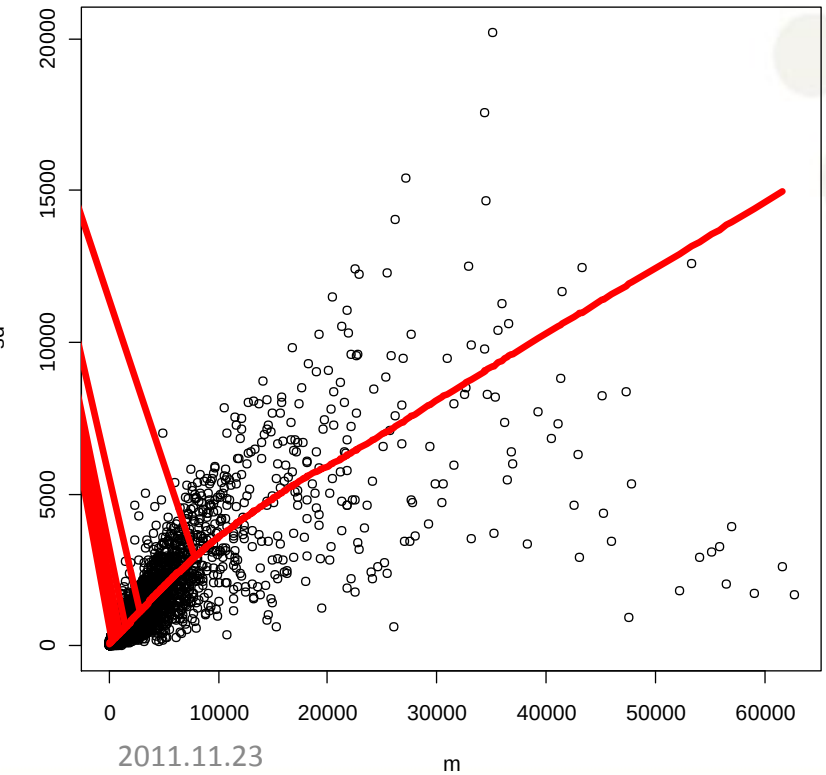



```
par(mfrow=c(2,1))
qqnorm(Cy3[,55])
qqline(Cy3[,55]
qqnorm(Cy5[,55])
qqline(Cy5[,55])
```





Standard deviation depends on signal




```
# 'apply' will apply the function to all rows
# of the data matrix
m <- apply(Cy3[,55:58],1,mean,na.rm=TRUE)
sd <- apply(Cy3[,55:58],1,sd,na.rm=TRUE)
plot(m,sd)
trend<-lowess(m,sd)
lines(trend,col=2,lwd=5)
```

lowess fit

LOcally WEighted Scatter plot Smoother used to estimate the trend in a scatter plot

Non parametric!

Transformations

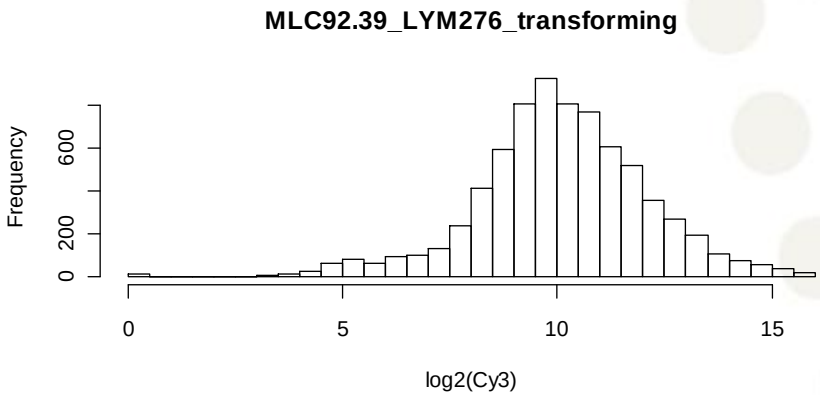
<u>Observations:</u>
The data are highly skewed.
The standard deviation is not constant as it increases with the mean.

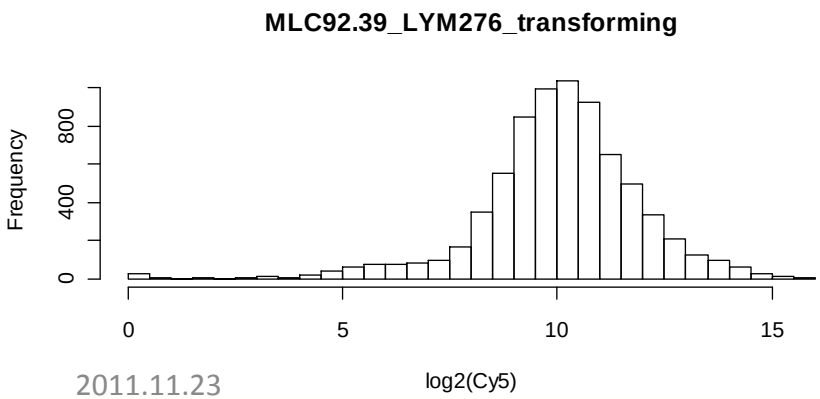
<u>Solution:</u>
Look for a transformation that will make the data more symmetric and the variance more constant.

With positive data the log transformation is often appropriate.

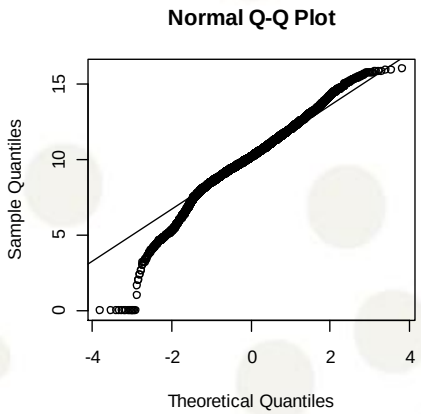
Transformation

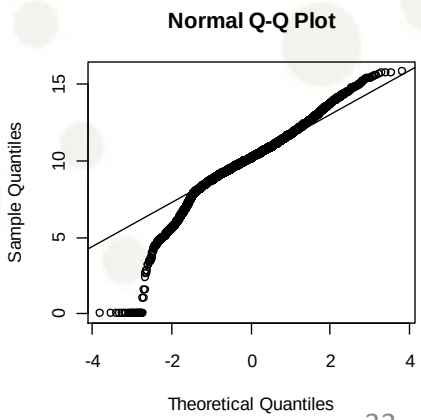
```
hist(log2(Cy3[,55]), 50,main=names(Cy3)[55], xlab="log2(Cy3)")
hist(log2(Cy5[,55]), 50, main=names(Cy3)[55], xlab="log2(Cy5)")
```





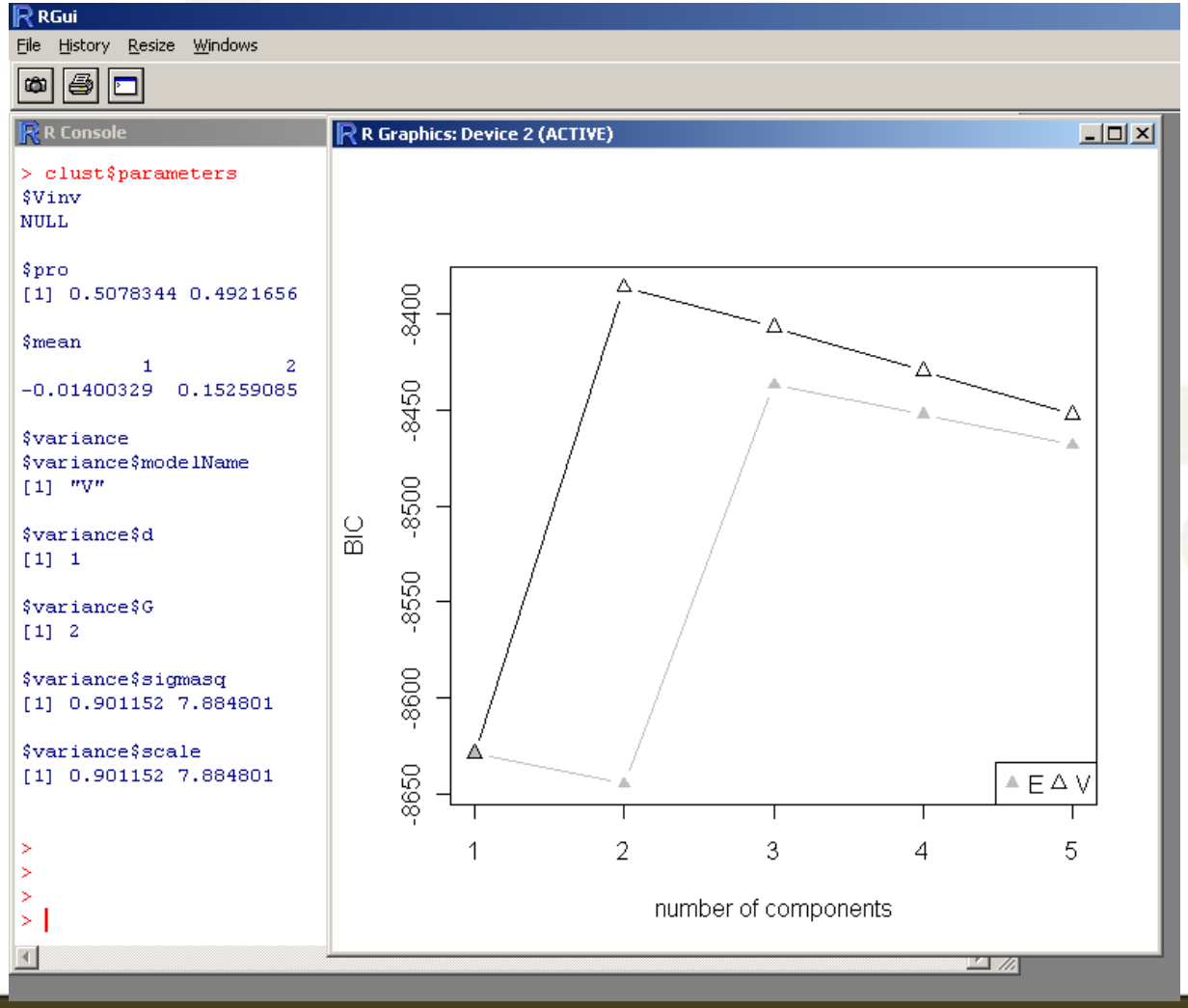

```
par(mfrow=c(2,1))
qqnorm(log2(Cy3[,55]))
qqline(log2(Cy3[,55]))
qqnorm(log2(Cy5[,55]))
qqline(log2Cy5[,55]))
```





One Gaussian distribution?

```
library(mclust)
y<-rnorm(1000,0,1)
x<-rnorm(1000,0,3)
clust <- Mclust(c(x,y), G=1:5)
plot(clust)
clust$parameters
```

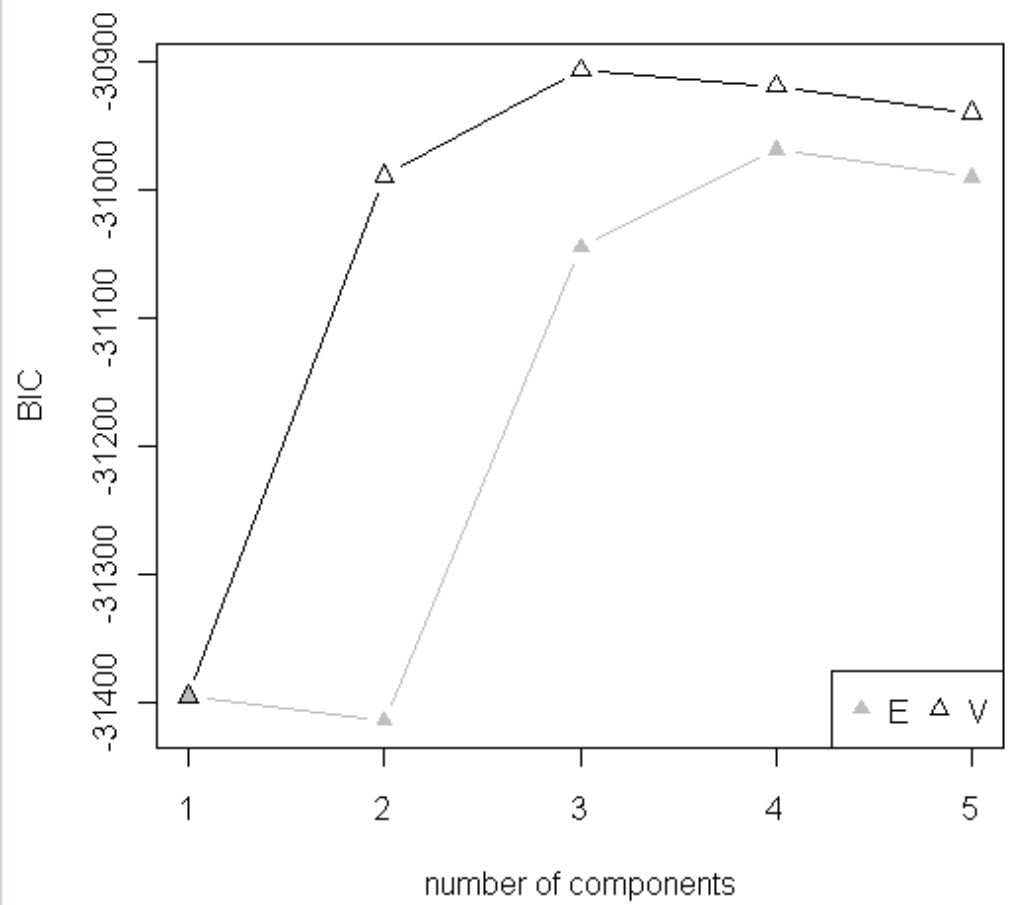


tory Resize Windows

R Console

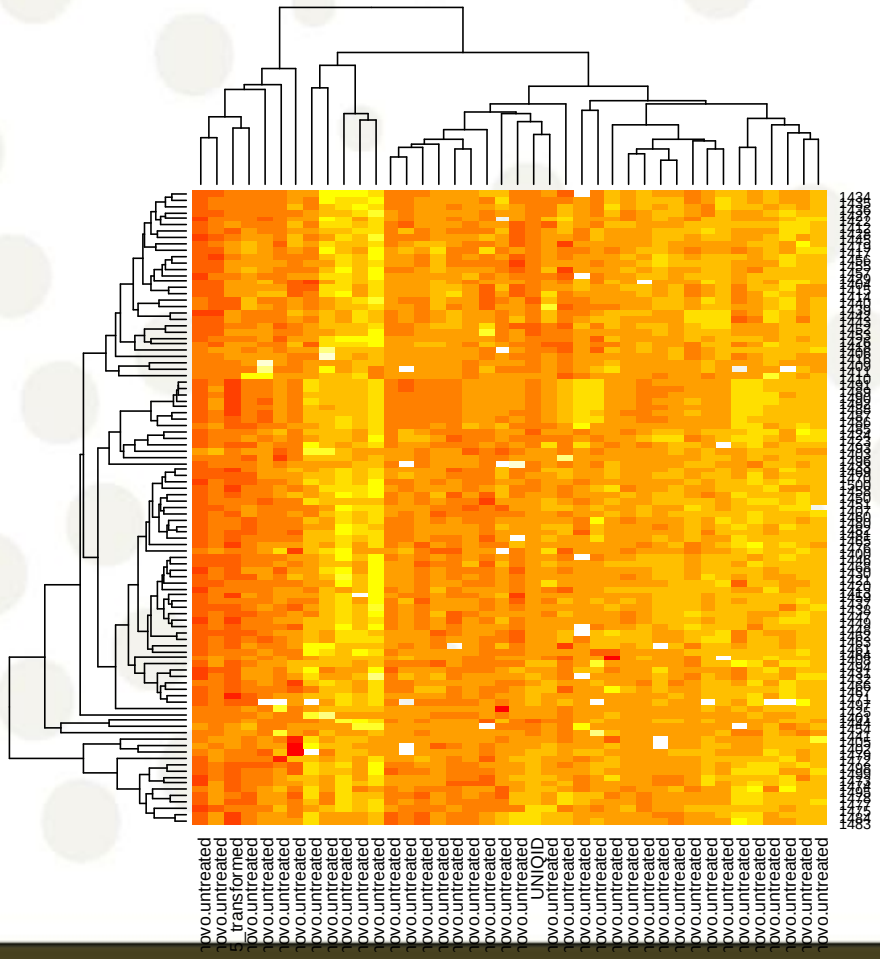
```
> z<-which(is.na(Cy3[,55]))
> clust <- Mclust(log2(Cy3[-z,55]),G=1:5,na.rm=TRUE)
> clust$parameters
$Vinv
NULL

$pro
[1] 0.2828964 0.3778001 0.3393036

$mean
        1         2         3
 9.102280  9.670364 11.328825

$variance
$variance$modelName
[1] "V"

$variance$d
[1] 1

$variance$G
[1] 3

$variance$sigmasq
[1] 7.4456537 0.9734005 2.2411960

$variance$scale
[1] 7.4456537 0.9734005 2.2411960


> plot(clust)
Waiting to confirm page change...
Warning message:
In plot.Mclust(clust) : data not supplied
>
```

R Graphics: Device 2 (ACTIVE)

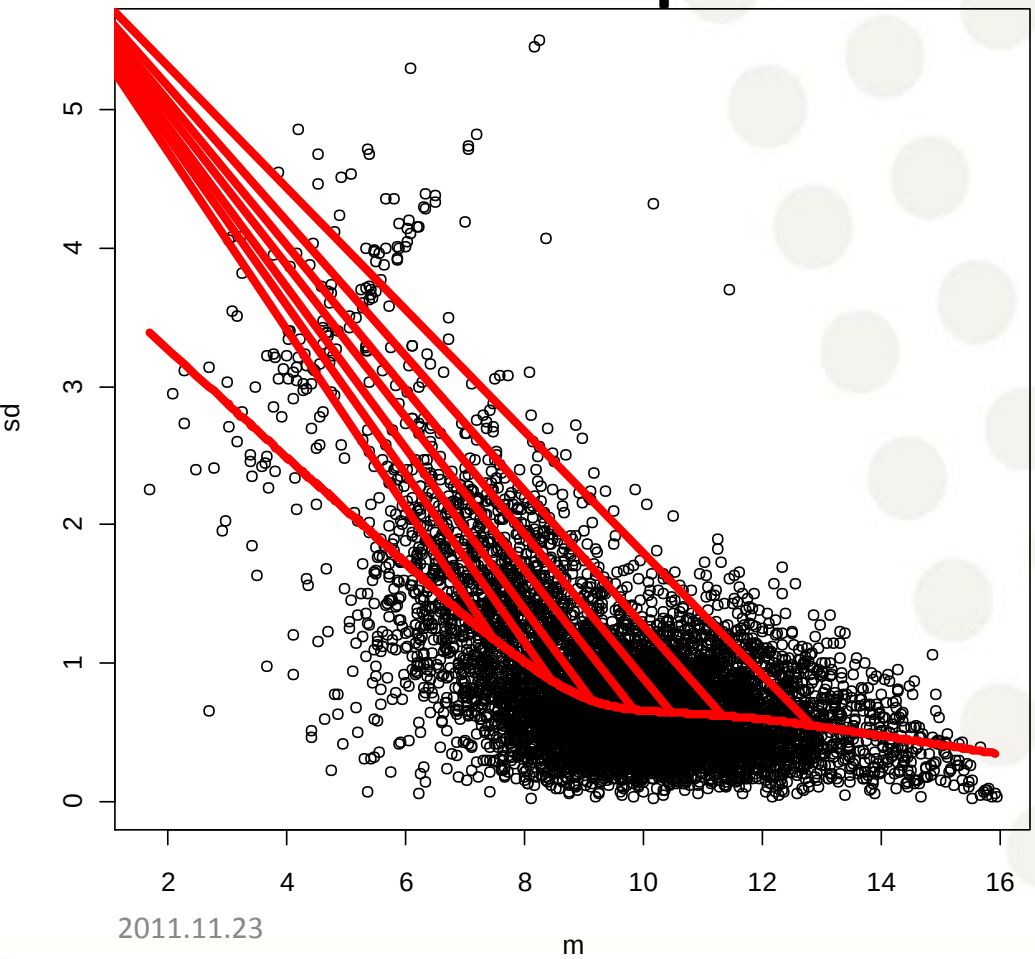


Heatmap

```
z<-as.matrix(log2(Cy3[1400:1500,1:40])-log2(Cy5[1400:1500,1:40]))
heatmap(z)
```



Standard deviation depends on signal




```
# 'apply' will apply the function to all rows
# of the data matrix
m <- apply(log2(Cy3[,55:58]),1,mean,na.rm=T)
sd <- apply(log2(Cy3[,55:58]),1,sd,na.rm=T)
plot(m,sd)
trend<-lowess(m,sd)
lines(trend,col=2,lwd=5)
```

But the dependency is weaker
Especially where most of the data are located.

microarray: Always log

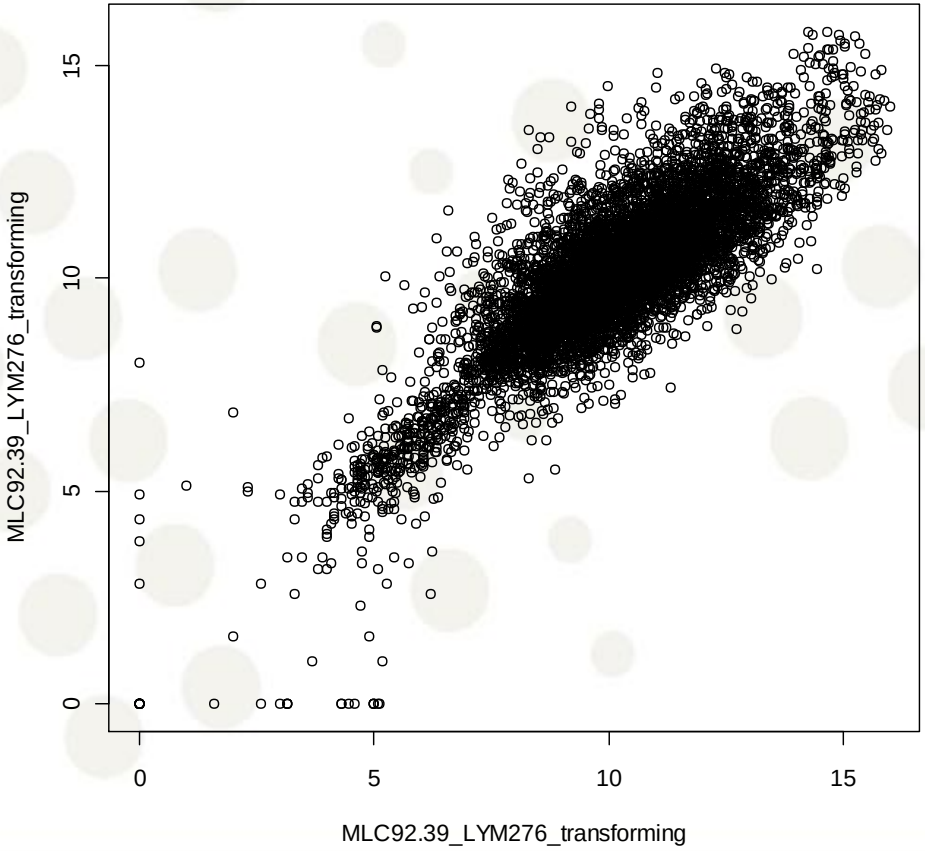
- Makes the data more symmetric, large observations are not as influential
- The variance is (more) constant
- Turns multiplication into addition (log(ab)=log(a)+log(b))
- In practice use log base 2, log2(x)=log(x)/log(2)

gene expression

```
plot(Cy3[,55],Cy5[,55], xlab=names(Cy3)[55], ylab=names(Cy5)[55])
```

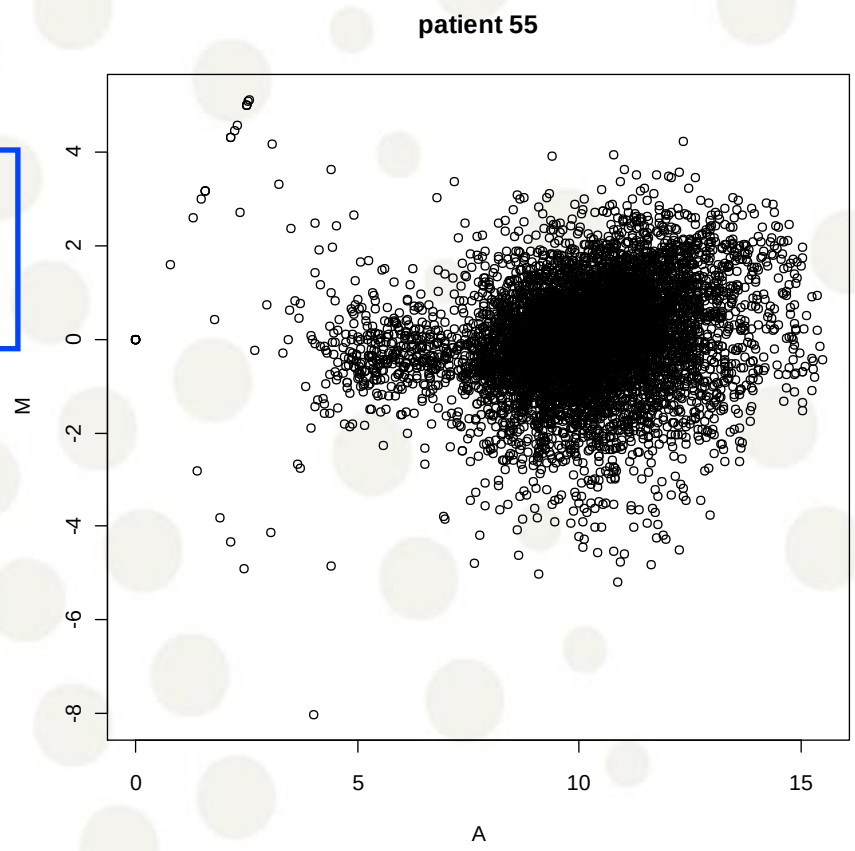
What can you say?

Is this the best way to look at the data?



MA plots

```
# MA plots per replicate
A<-(log2(Cy3[,55])+log2(Cy5[,55]))/2
M<-(log2(Cy3[,55])-log2(Cy5[,55]))
plot(A,M,xlab="A",ylab="M",main="patient 55")
```

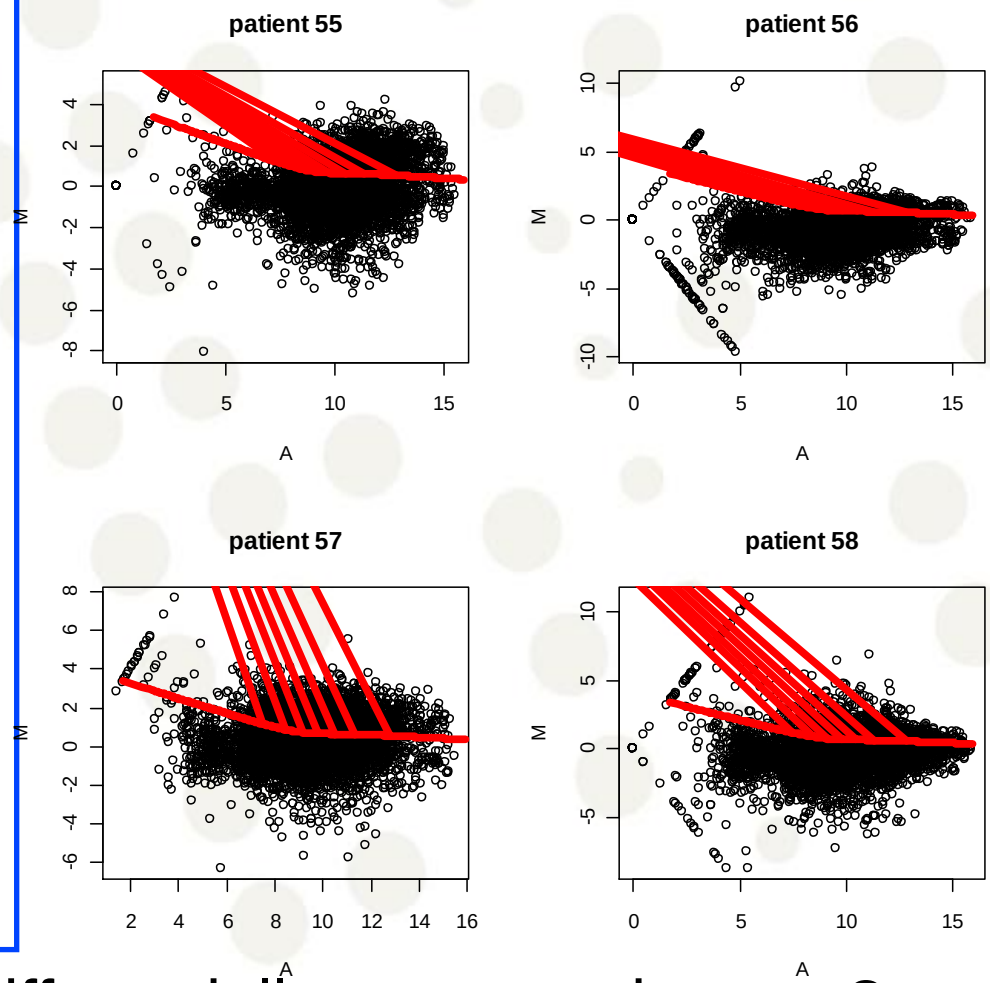


M (minus) is the log ratio

A (average) is overall intensity

MA plots

```
par(mfrow=c(2,2))
A<-(log2(Cy3[,55])+log2(Cy5[,55]))/2
M<-(log2(Cy3[,55])-log2(Cy5[,55]))
plot(A,M,xlab="A",ylab="M",main="patient 55")
trend<-lowess(A,M)
lines(trend,col=2,lwd=5)

A<-(log2(Cy3[,56])+log2(Cy5[,56]))/2
M<-(log2(Cy3[,56])-log2(Cy5[,56]))
plot(A,M,xlab="A",ylab="M",main="patient 56")
trend<-lowess(A,M)
lines(trend,col=2,lwd=5)

A<-(log2(Cy3[,57])+log2(Cy5[,57]))/2
M<-(log2(Cy3[,57])-log2(Cy5[,57]))
plot(A,M,xlab="A",ylab="M",main="patient 57")
trend<-lowess(A,M)
lines(trend,col=2,lwd=5)

A<-(log2(Cy3[,58])+log2(Cy5[,58]))/2
M<-(log2(Cy3[,58])-log2(Cy5[,58]))
plot(A,M,xlab="A",ylab="M",main="patient 58")
trend<-lowess(A,M)
lines(trend,col=2,lwd=5)
```



How do we find differentially expressed genes?

Combining micro-array and survival data

- For each patient five signature are calculated from the micro-array as the mean of the signal from each of the group of genes:
  - Germinal.center.B.cell.signature
  - Lymph.node.signature
  - Proliferation.signature
  - BMP6
  - MHC.class.II.signature

head(dat)

```
> dat <- read.table(file = "M:/Undervisning/Statistikk/DLBCLpatientDataNEW.txt", header =TRUE, sep="\t")
> head(dat)
  DLBCL.sample..LYM.number. Analysis.Set Follow.up..years. Status.at.follow.up Subgroup IPI.Group
1                         2     Training               4.0               Alive      GCB       Low
2                         4     Training               4.9               Alive      GCB    Medium
3                         6     Training               5.6               Alive      GCB       Low
4                         7     Training              12.1               Alive      GCB    Medium
5                         8     Training               0.6                Dead      ABC    Medium
6                        11     Training               0.3                Dead      GCB      High
  Germinal.center.B.cell.signature Lymph.node.signature Proliferation.signature  BMP6 MHC.class.II.signature
1                             0.28                -0.07                   -0.56  0.46                   0.57
2                             1.01                -1.15                   -1.04  0.23                   0.63
3                             0.83                -2.11                    0.52 -0.28                   0.38
4                             0.89                -1.33                    0.01 -0.64                   0.93
5                             0.27                -1.56                    1.56 -0.67                  -2.50
6                            -0.05                 0.06                   -0.68 -0.38                  -2.32
  Outcome.predictor.score
1                   -0.23
2                   -0.38
3                    0.20
4                   -0.41
5                    1.25
6                    0.44
```

summary(dat)

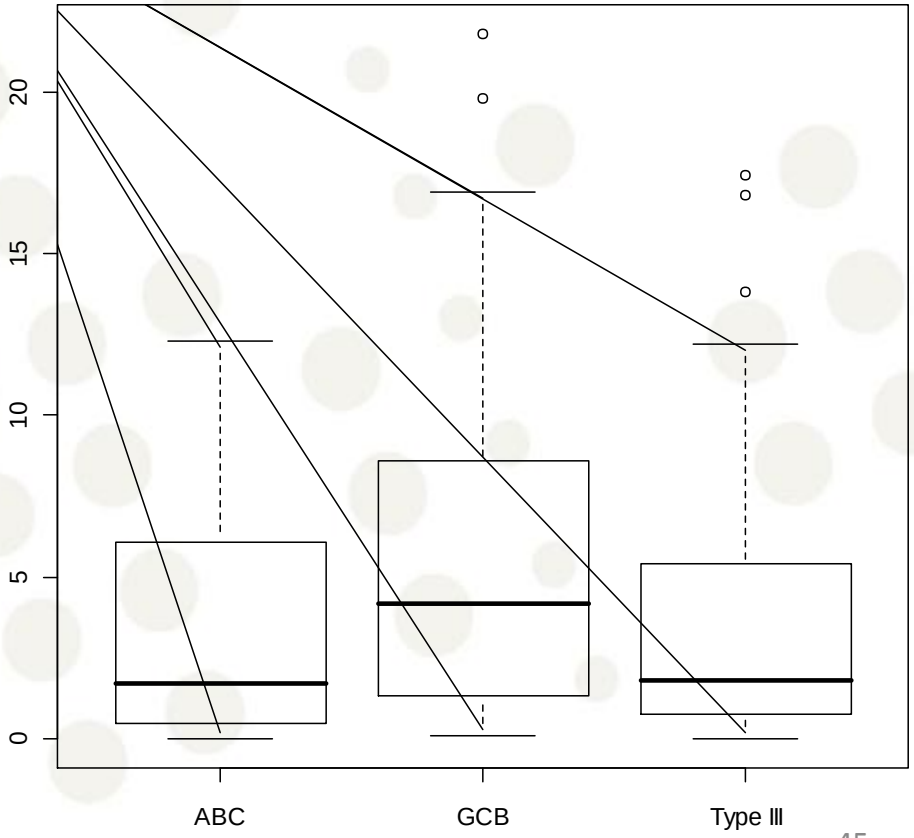
```
> summary(dat)
 DLBCL.sample..LYM.number.     Analysis.Set Follow.up..years.
 Min.   :  1.00            Training  :160   Min.   : 0.000
 1st Qu.: 91.75            Validation: 80   1st Qu.: 0.900
 Median :177.50                             Median : 2.800
 Mean   :190.29                             Mean   : 4.411
 3rd Qu.:284.25                             3rd Qu.: 7.100
 Max.   :439.00                             Max.   :21.800
 Status.at.follow.up     Subgroup     IPI.Group
 Alive:102           ABC      : 73   High   : 32
 Dead :138           GCB      :115   Low    : 82
                     Type III: 52   Medium :108
                                    missing:  1
                                    NA's   : 17

 Germinal.center.B.cell.signature Lymph.node.signature Proliferation.signature
 Min.   :-2.61000                 Min.   :-2.6500      Min.   :-1.700000
 1st Qu.:-0.91000                 1st Qu.:-0.8675      1st Qu.:-0.410000
 Median :-0.16000                 Median : 0.0600      Median :-0.010000
 Mean   :-0.03062                 Mean   : 0.0065      Mean   : 0.005958
 3rd Qu.: 0.86000                 3rd Qu.: 0.8675      3rd Qu.: 0.412500
 Max.   : 2.48000                 Max.   : 2.9800      Max.   : 2.180000
      BMP6          MHC.class.II.signature Outcome.predictor.score
 Min.   :-1.87000   Min.   :-3.020000      Min.   :-1.700000
 1st Qu.:-0.65250   1st Qu.:-0.537500      1st Qu.:-0.537500
 Median :-0.13500   Median : 0.125000      Median :-0.085000
 Mean   :-0.04362   Mean   :-0.006083      Mean   :-0.003208
 3rd Qu.: 0.49250   3rd Qu.: 0.680000      3rd Qu.: 0.522500
 Max.   : 2.69000   Max.   : 1.890000      Max.   : 2.360000
>
```

Boxplot: follow up time for each subgroup

```
boxplot(Follow.up..years.~Subgroup, data = dat)
```

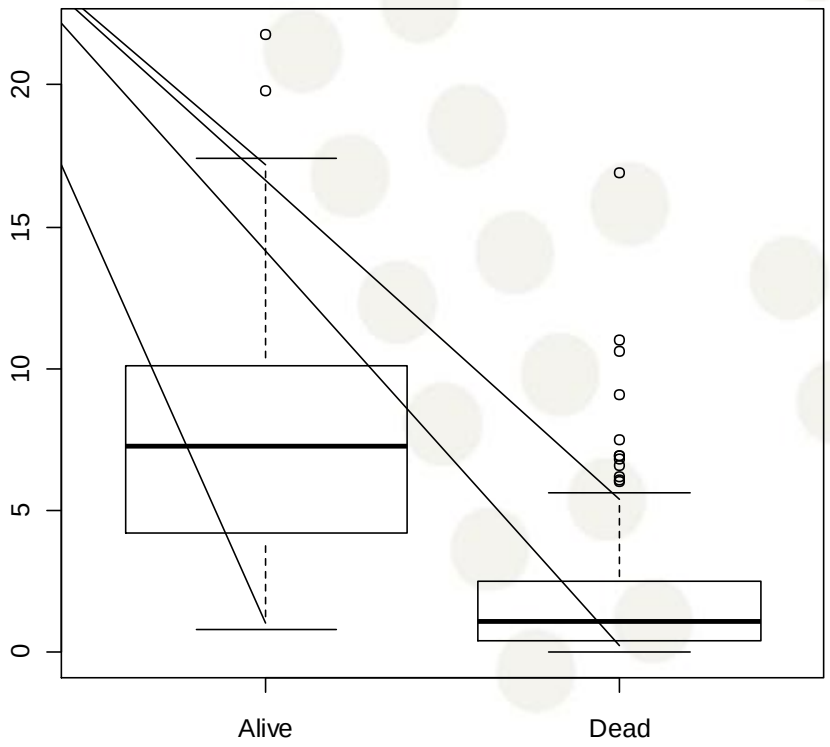
The boxplot function can be used to display several variables at a time!

What can you say here?



Boxplot: follow up time for each subgroup

```
boxplot(Follow.up..years.~Status.at.follow.up, data = dat)
```



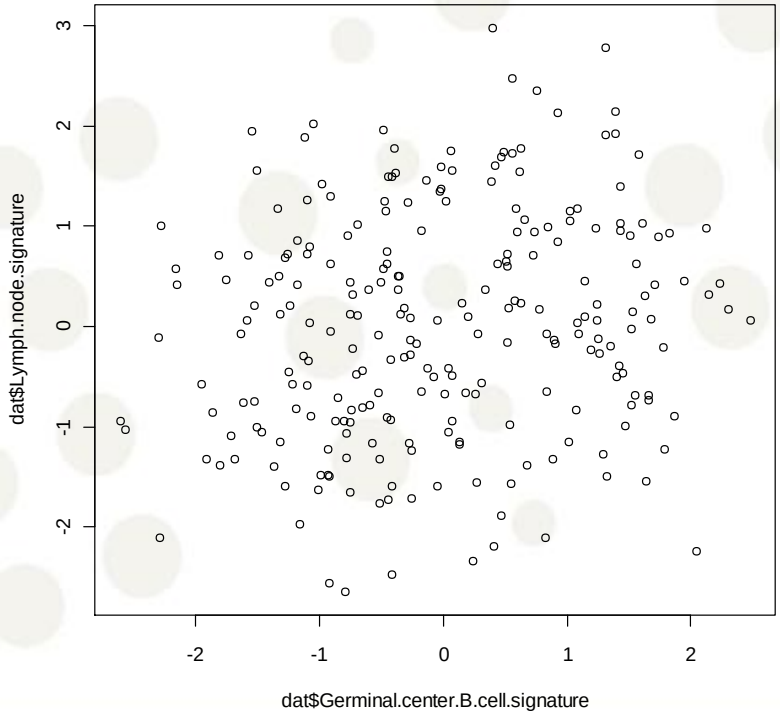
Scatter plots

Biological data sets often contain several variables
So they are multivariate.

Scatter plots allow us to look at two variables at a time.

```
plot(dat$Germinal.center.B.cell.signature,
            dat$Lymph.node.signature)
cor(dat$Germinal.center.B.cell.signature,
            dat$Lymph.node.signature)
#[1] 0.1633608
```

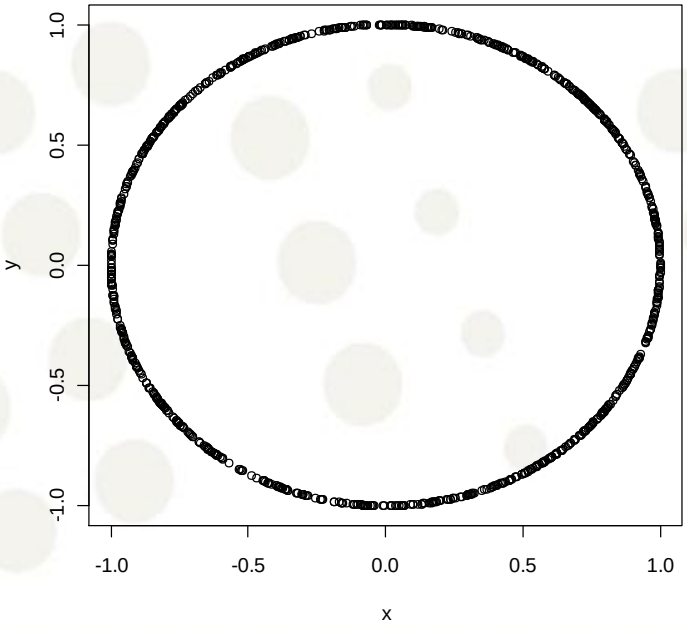
This can be used
to assess independence!



Scatter plots vs. correlations

Correlation is only good for linear dependence.

```
# Quick comment on correlation
theta<-runif(1000,0,2*pi)
x<-cos(theta)
y<-sin(theta)
plot(x,y)
cor(x,y)
```



What is the correlation?

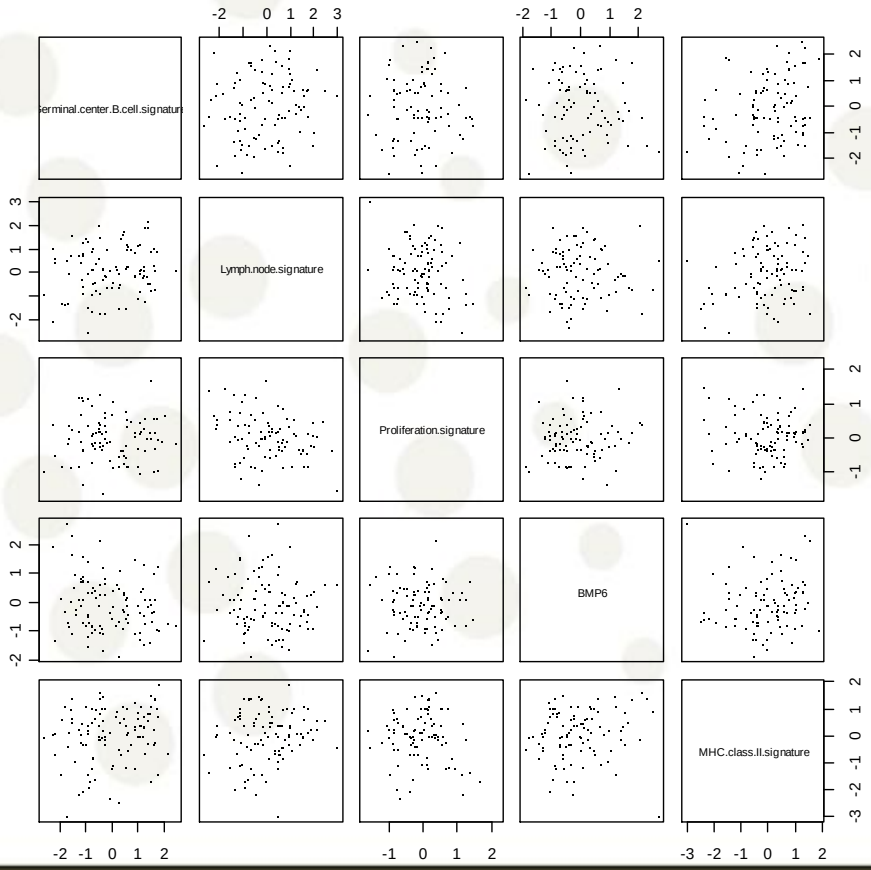
Trellis graphics

Trellis Graphics is a family of techniques for viewing complex, multi-variable data sets.

```
plot(dat[,7:11] pch=".")
```

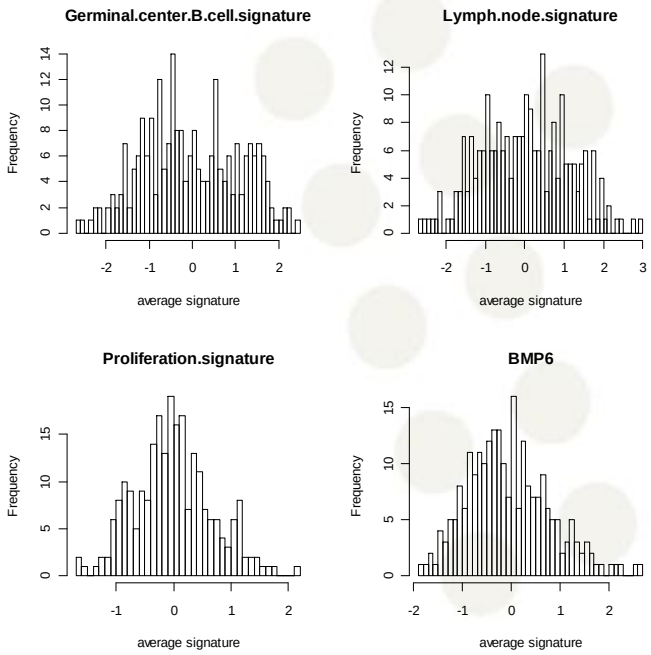
Note that the plotting symbol is changed.

Many more possibilities in the 'lattice' package!



Histogram

```
par(mfrow=c(2,2))
hist(dat[,7], 50, main = names(dat)[7], xlab="average signature")
hist(dat[,8], 50, main = names(dat)[8], xlab="average signature")
hist(dat[,9], 50, main = names(dat)[9], xlab="average signature")
hist(dat[,10], 50, main = names(dat)[10], xlab="average signature")
```



Summary

- Plotting should be the first step in any statistical analysis!
- Extremely Important
- Good modeling starts and ends with plotting
- R provides a great framework for plotting