Introduction to biostatistics

and FSharp.Stats

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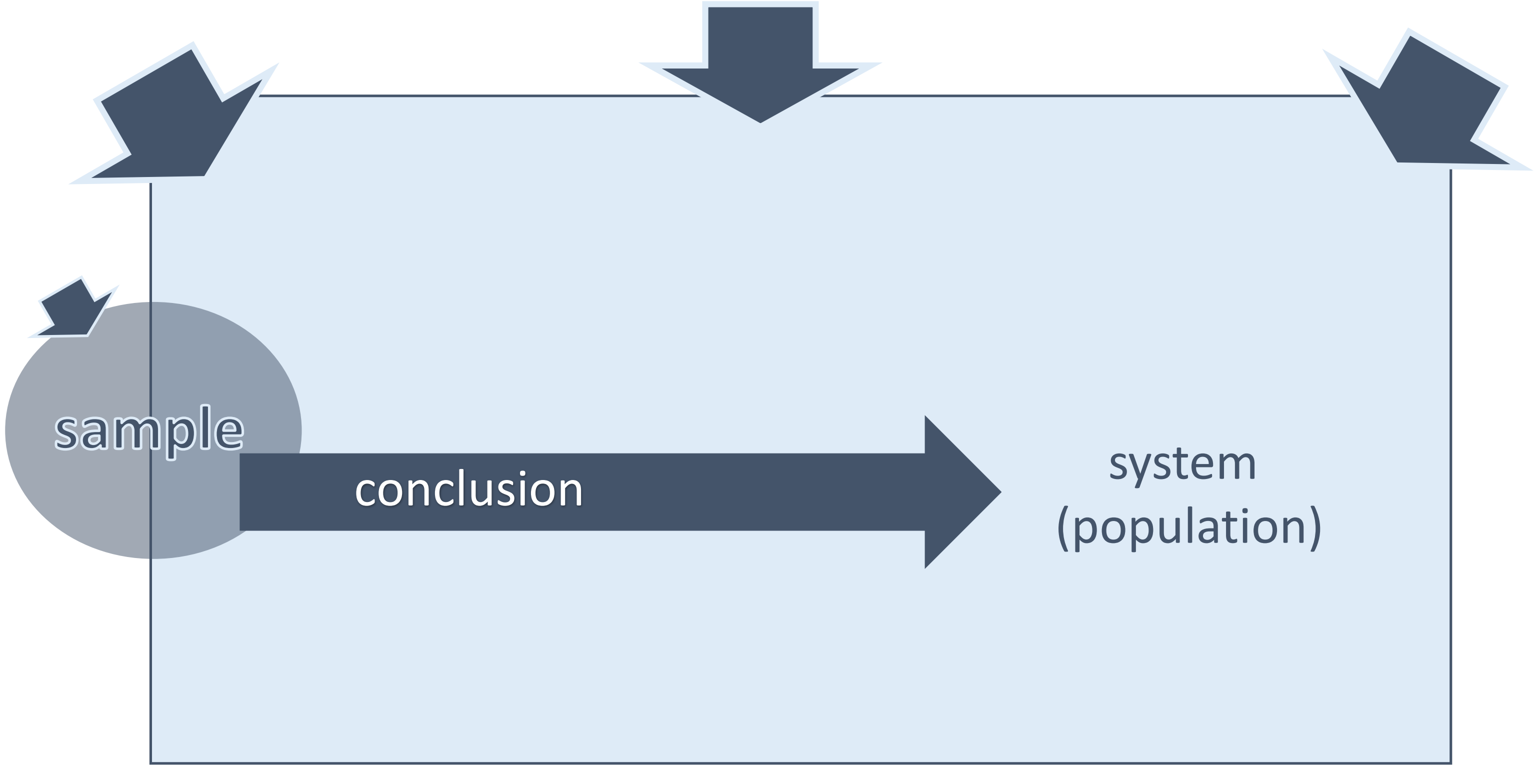
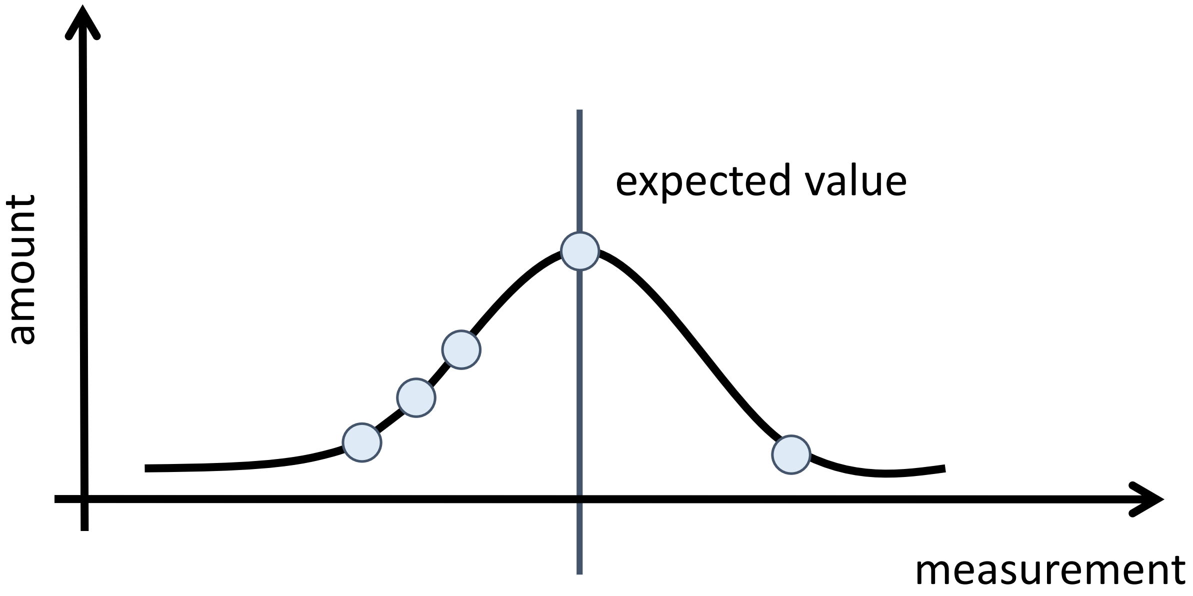
General goal in statistics

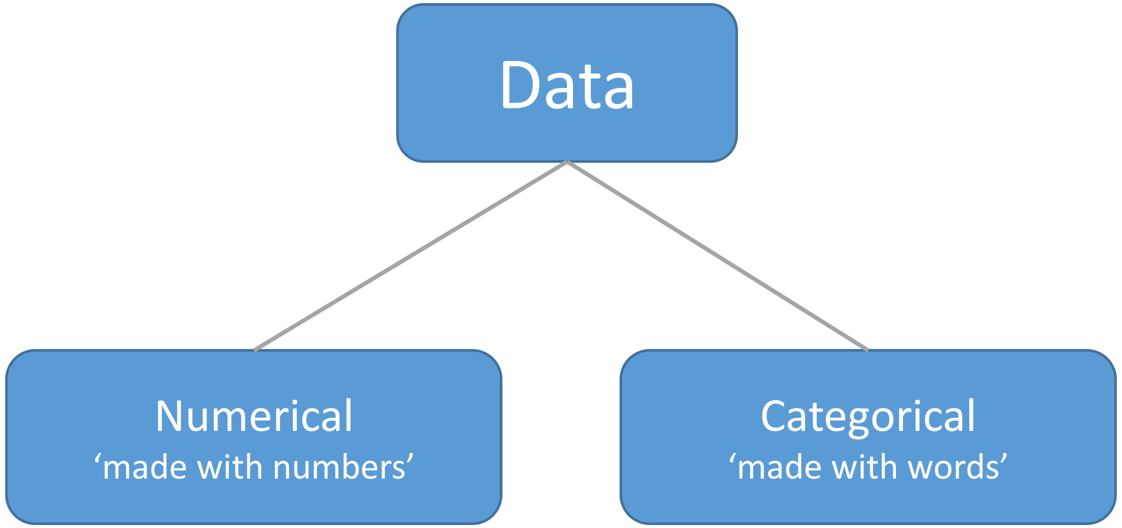


- Drawing conclusions from sample to population

Central tendency



- Finding the expected value by measures of the central tendency using (type L) point estimators

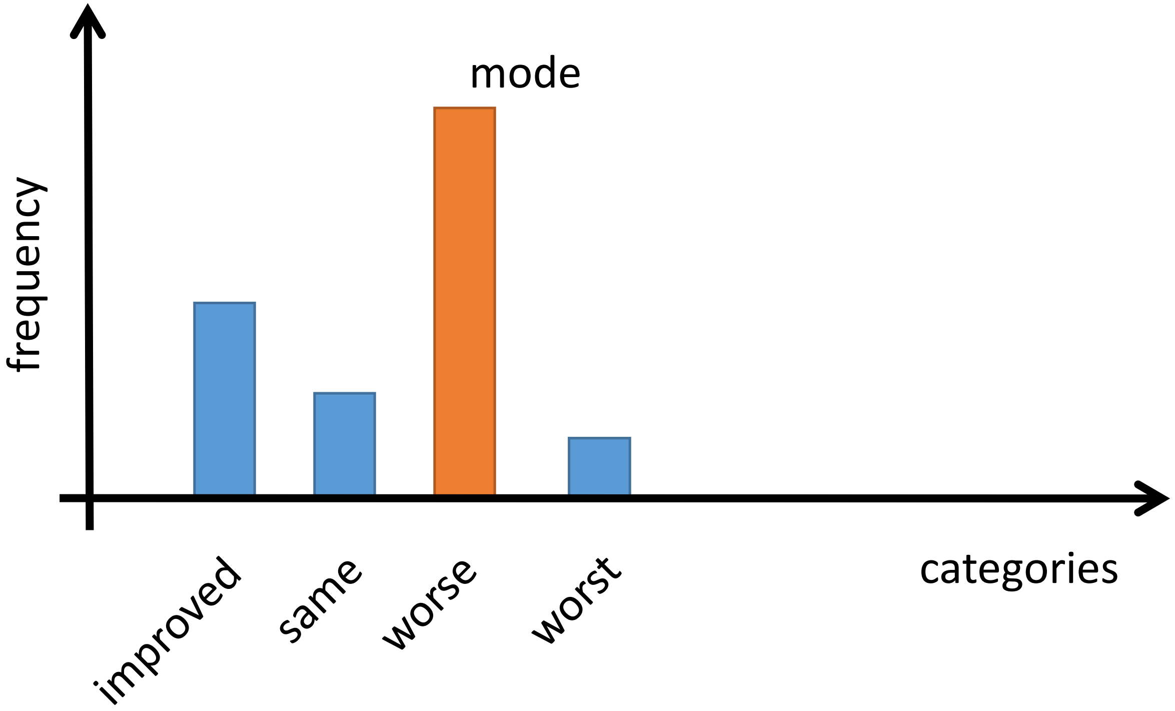


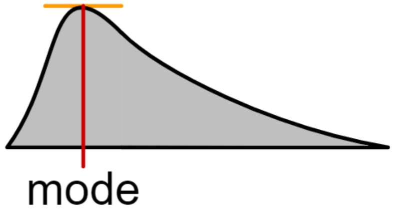
Data

Numerical
‘made with numbers’

Categorical
‘made with words’

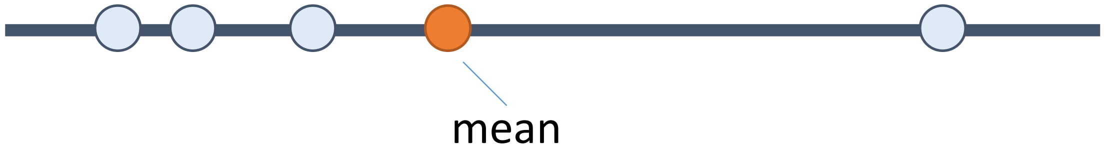
Measures of central tendency: mode



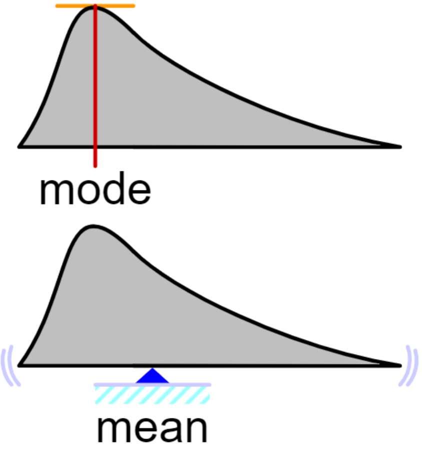


- The mode is the most frequently occurring category

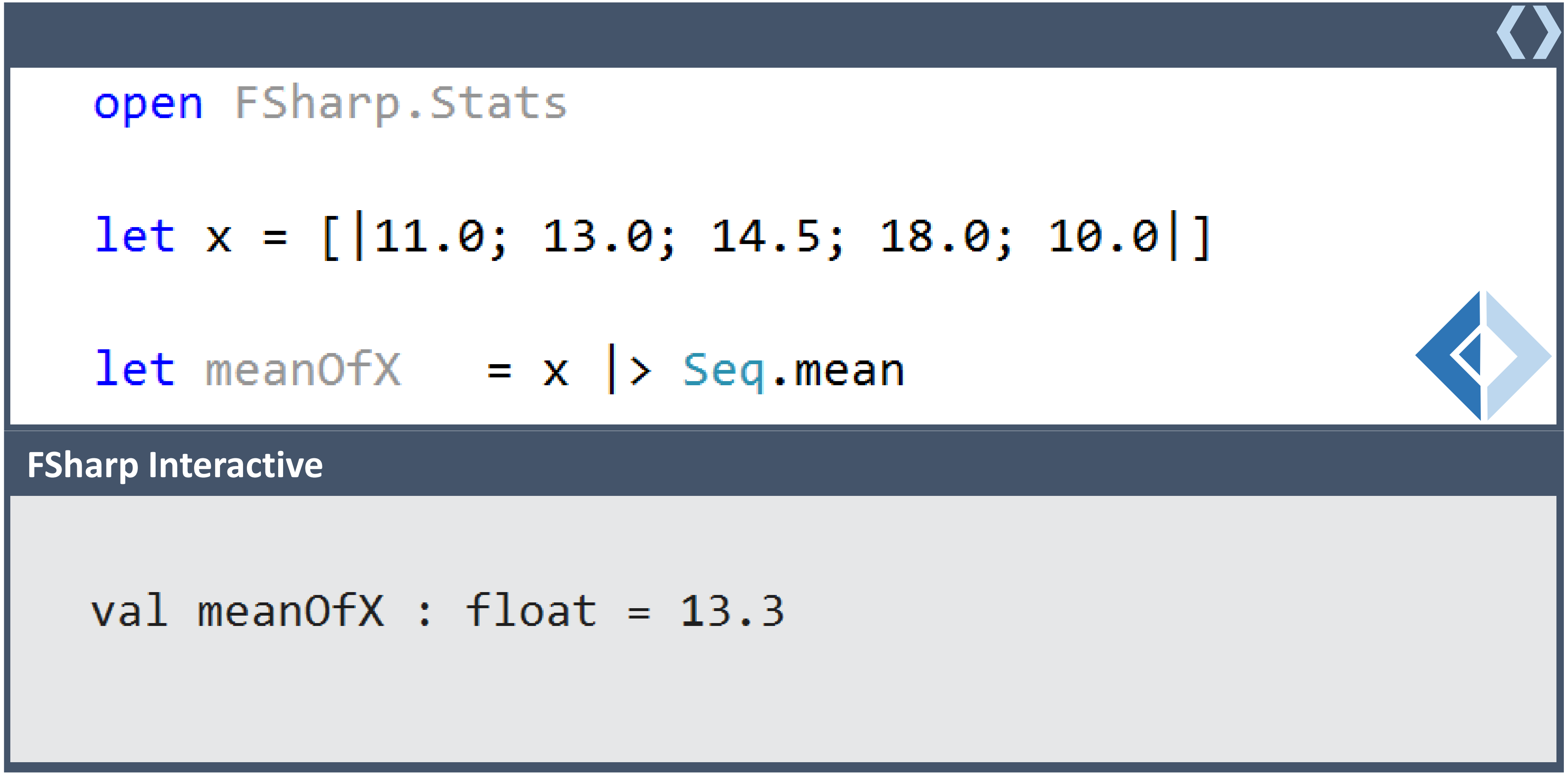
Measures of central tendency: mean



$$\bar{x} = \frac{1}{N}\sum_{i=1}^{n} x_i$$



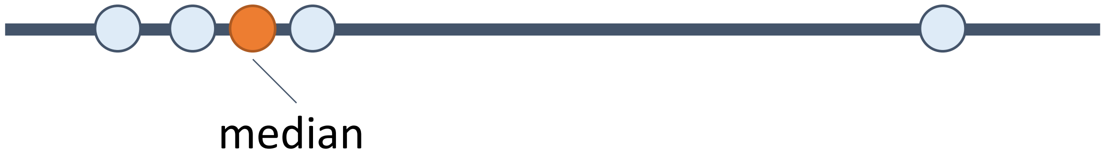
- The mean is not robust against outliers (equally influenced by all values)



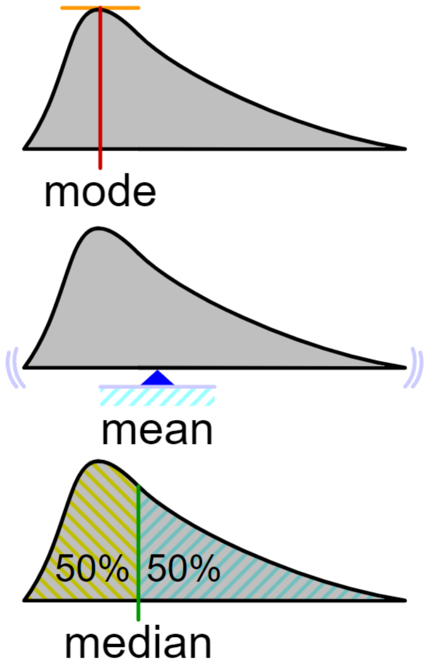
```fsharp
open FSharp.Stats

let x = [|11.0; 13.0; 14.5; 18.0; 10.0|]

let meanOfX    = x |> Seq.mean
```

```
val meanOfX : float = 13.3
```

Measures of central tendency: median



$$P(X \leq m) = P(X \geq m) = \int_{-\infty}^{m} f(x)\,dx = \frac{1}{2}.$$



- The median is that value such that half of data points fall above it an half below it
  => more robust against outliers

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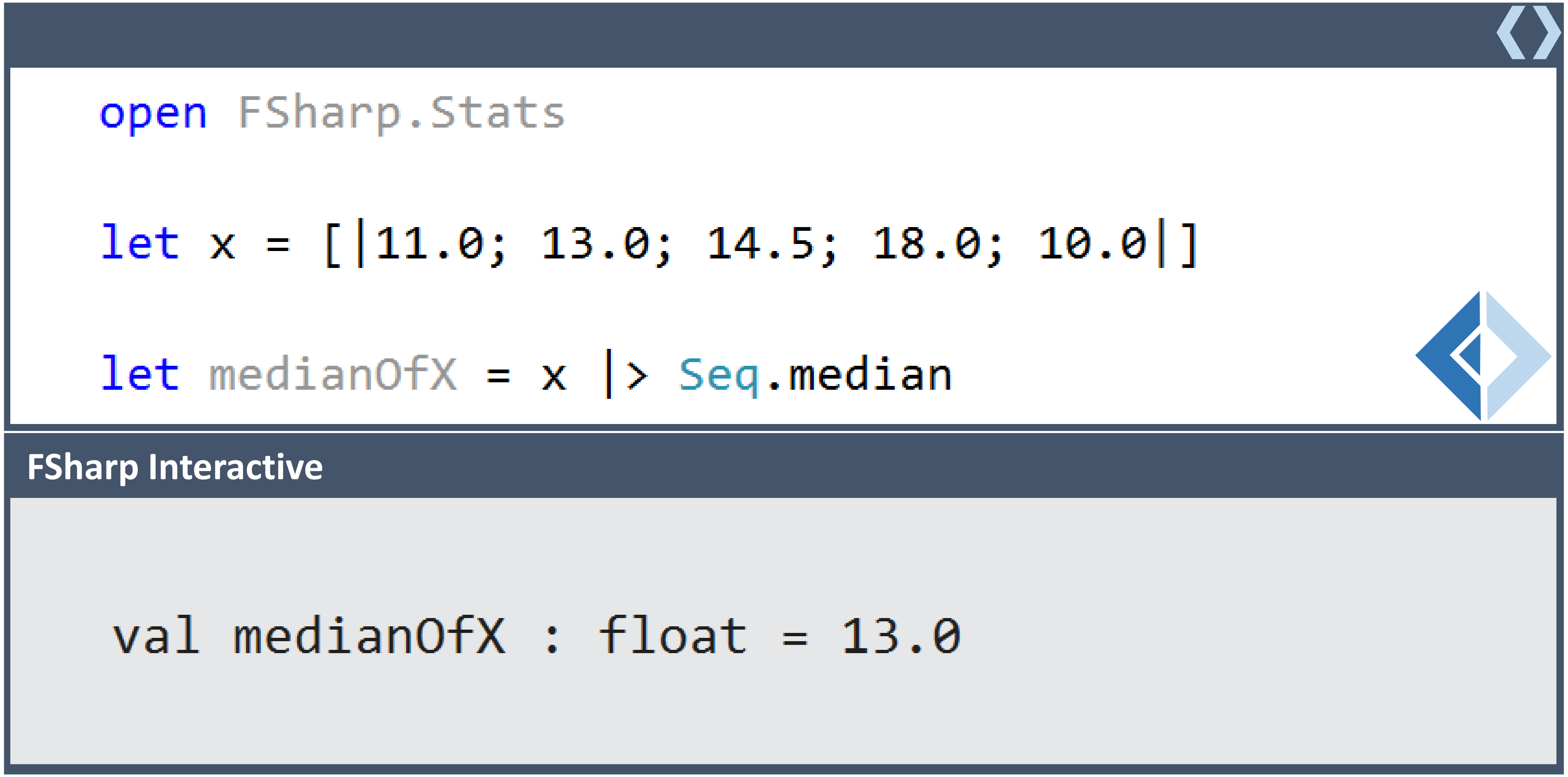
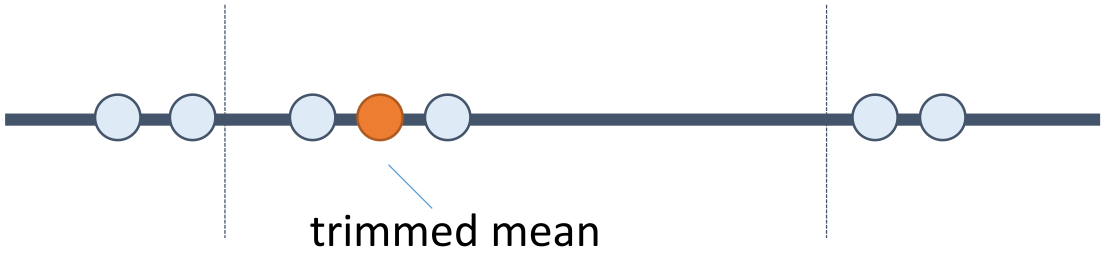


```
open FSharp.Stats

let x = [|11.0; 13.0; 14.5; 18.0; 10.0|]

let medianOfX = x |> Seq.median
```

FSharp Interactive

```
val medianOfX : float = 13.0
```

Trimmed mean



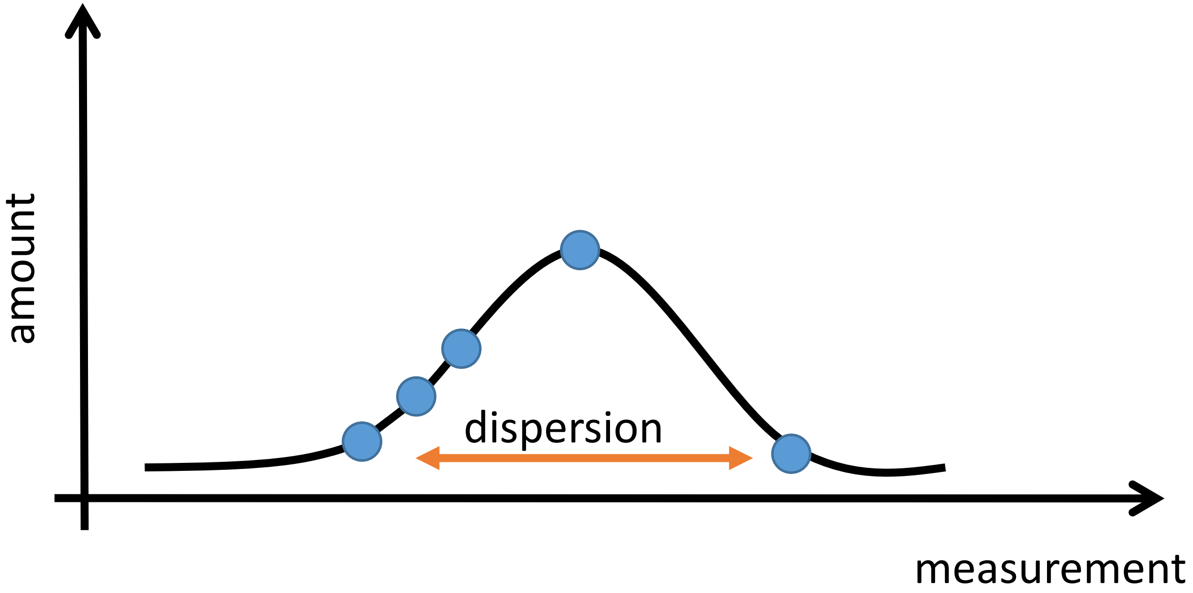
- A trimmed mean involves the calculation of the mean after discarding given parts of a sample at the high and low end
- Typically 5% to 25% of the values are discarded at both ends

Describing distributions

- Central tendency
  - mode
  - mean
  - median
  - trimmed mean

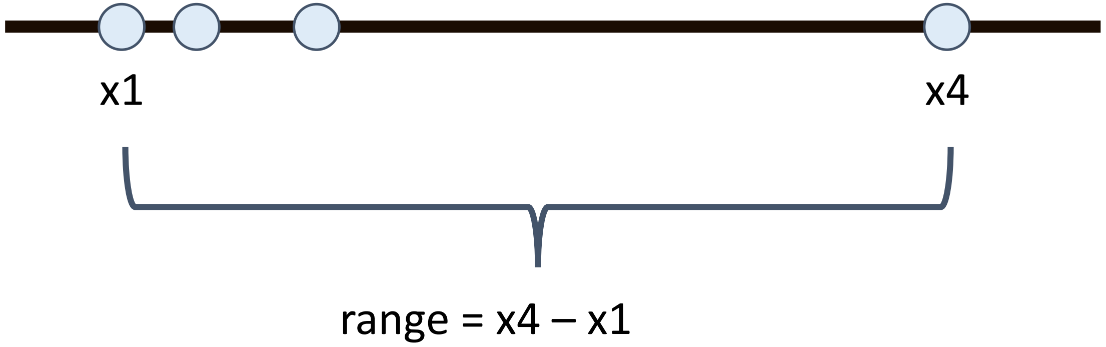
- Dispersion
  - range
  - mean (absolute) deviation
  - variance & standard deviation
  - coefficient of variation

Estimating dispersion



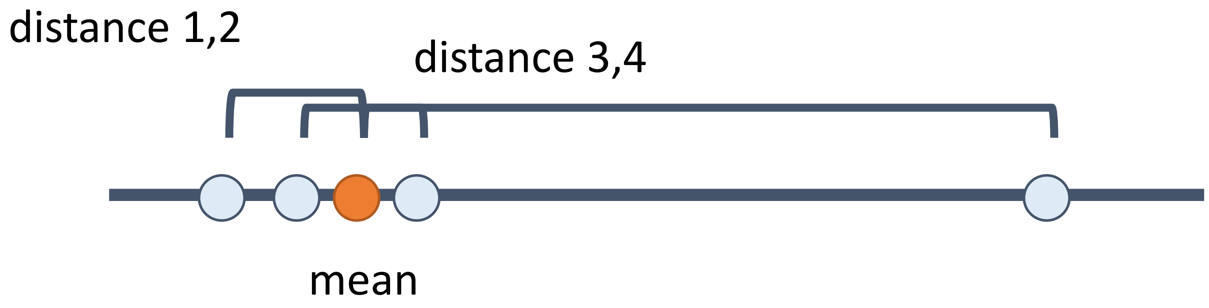
- Estimating the spread/dispersion of the data distribution

The range



- The range is the difference between the highest and lowest value
=> not robust against extrema

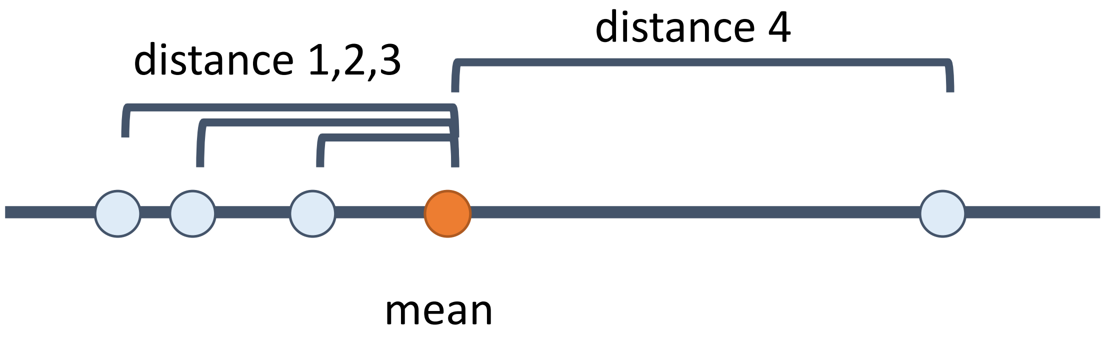
Mean deviation of a sample



$$MD = \frac{1}{N}\sum_{i=1}^{N}|x_i - \bar{x}|$$

- The sum of the absolute amount of deviations from the mean divided by their number

Variance and Standard Deviation of a sample



- Variance: Sum of all squared distances divided by their number

$$\sigma^2 = \frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2$$

- Standard Deviation is the square root of the variance to get back to the original units

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2}$$

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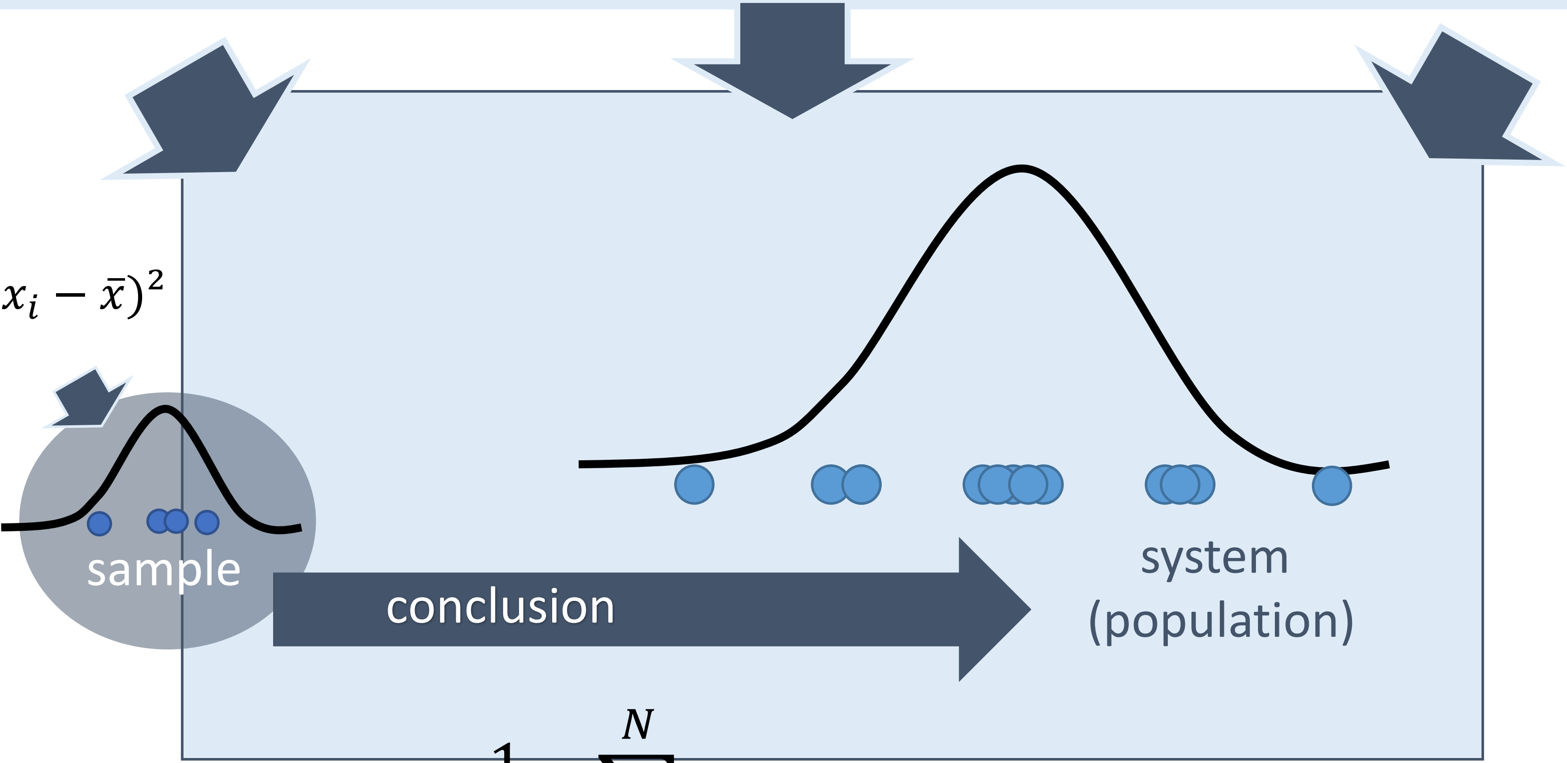
The Variance and Standard Deviation of a population



$$\sigma^2 = \frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2$$

$$\sigma^2 = \frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2$$

- Variance: Sum of all distance quadrates divided by the degrees of freedom (N-1)

The Variance and Standard Deviation of a population
- Bessel's correction -

sample variance

$$\sigma^2 = \frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2 \longrightarrow$$

population variance

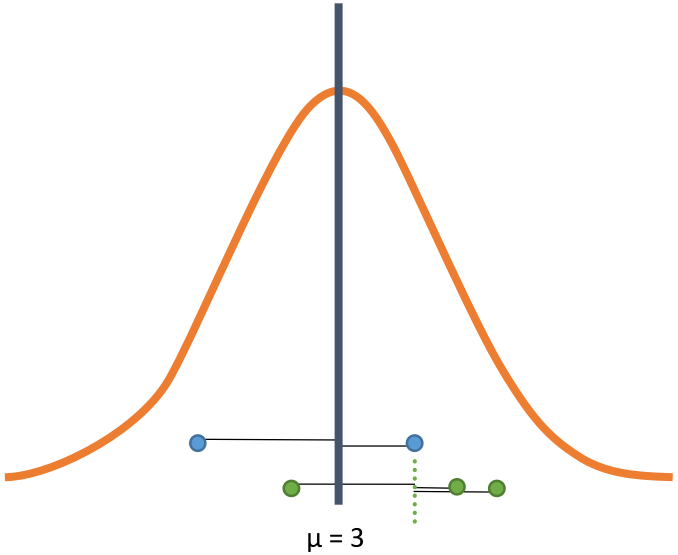
$$\sigma^2 = \frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2$$

3 independent observations from population (μ = 3)

i	x_i	$x_i - \mu$
1	5	5 - 3 = 2
2	0	0 - 3 = -3
3	?	?

3 independent observations from population ($\bar{x}$ = 5)

i	x_i	$x_i - \bar{x}$
1	7	7 - 5 = 2
2	6	6 - 5 = 1
3		



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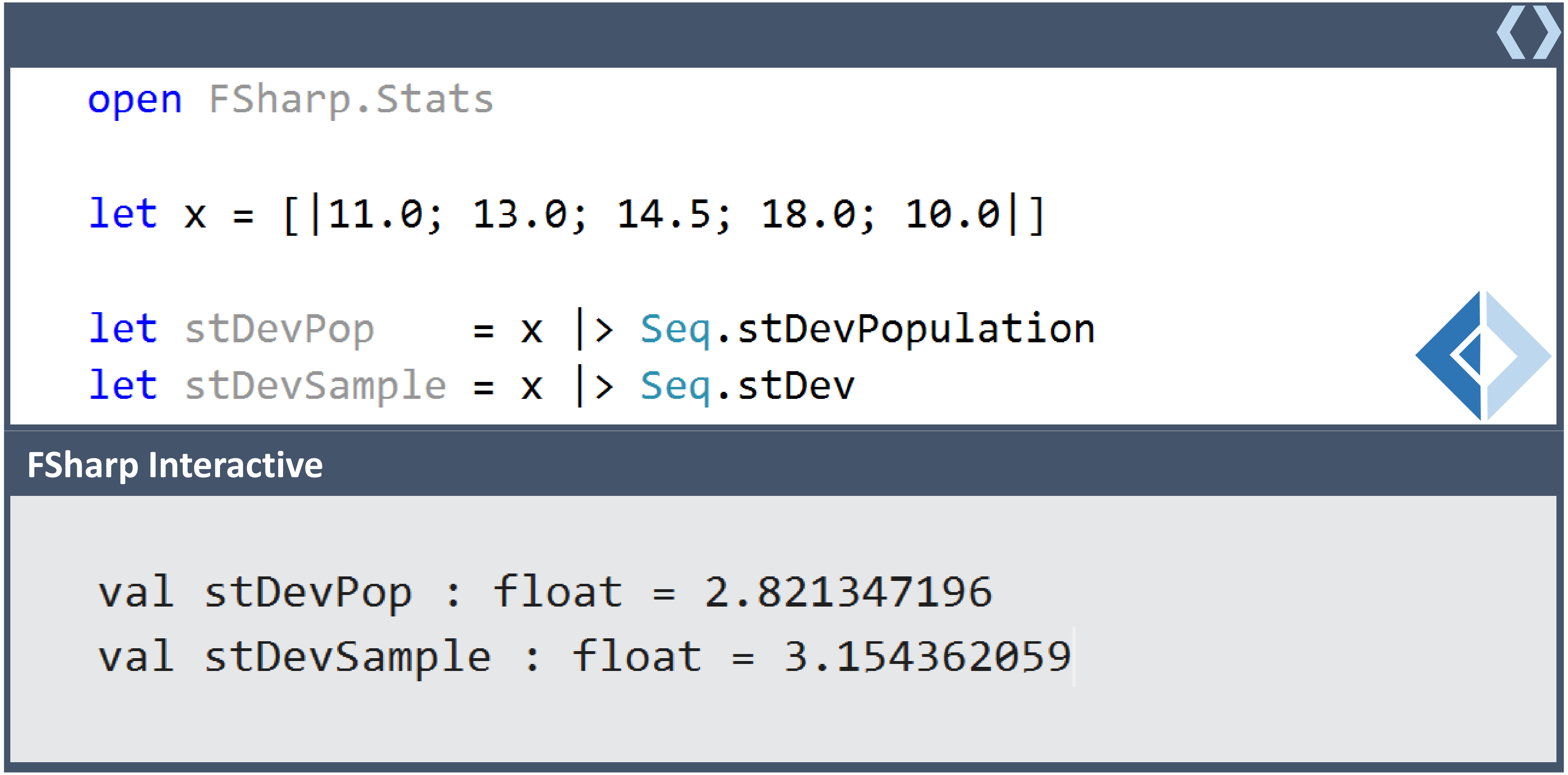


```
open FSharp.Stats

let x = [|11.0; 13.0; 14.5; 18.0; 10.0|]

let stDevPop    = x |> Seq.stDevPopulation
let stDevSample = x |> Seq.stDev
```

FSharp Interactive

```
val stDevPop : float = 2.821347196
val stDevSample : float = 3.154362059
```

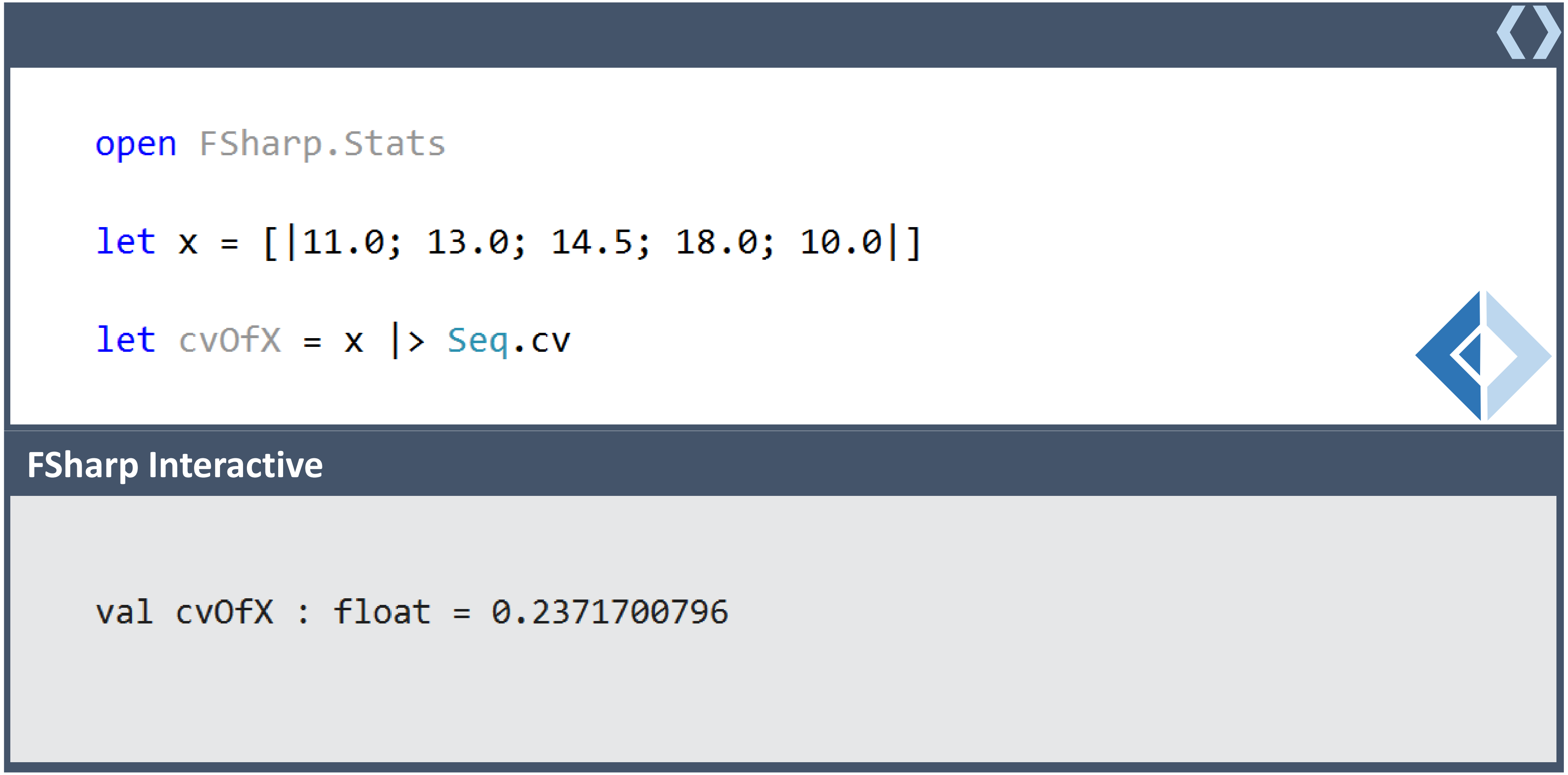
Coefficient of variation

$$c_v = \frac{\sigma}{\mu}$$

σ = standard deviation

μ = mean

- The coefficient of variation represents the ratio of the standard deviation to the mean.
  It is a useful statistic for comparing the degree of variation from one data series to another, even if the means are drastically different from each other



```
open FSharp.Stats

let x = [|11.0; 13.0; 14.5; 18.0; 10.0|]

let cvOfX = x |> Seq.cv
```

FSharp Interactive

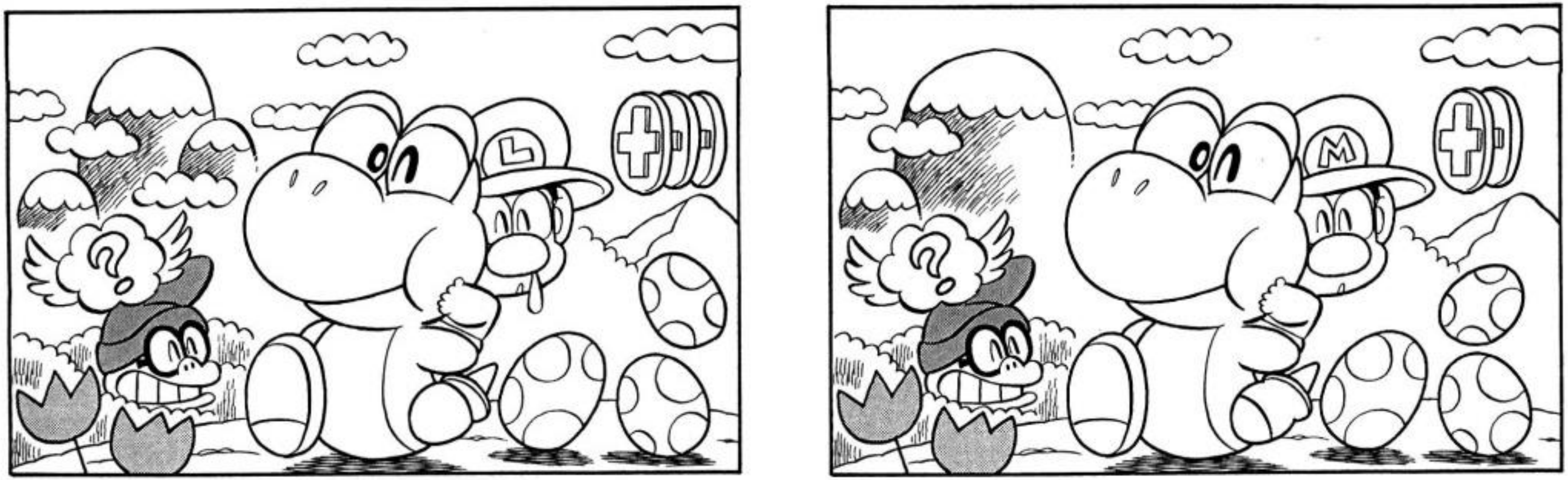
```
val cvOfX : float = 0.2371700796
```

Describing distributions

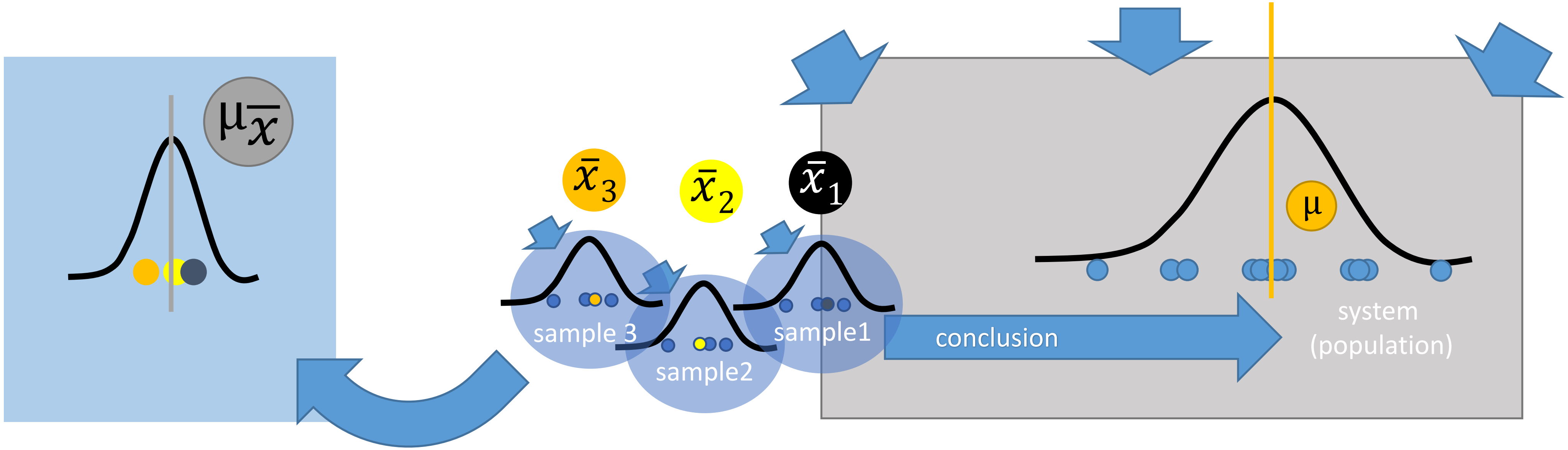
- Central tendency
  - mode
  - mean
  - median
  - trimmed mean

- Dispersion
  - range
  - mean (absolute) deviation
  - variance & standard deviation
  - coefficient of variation

Hypothesis testing: A framework for finding the differences

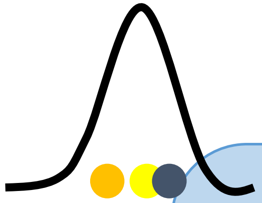


Sampling | sample | population distribution



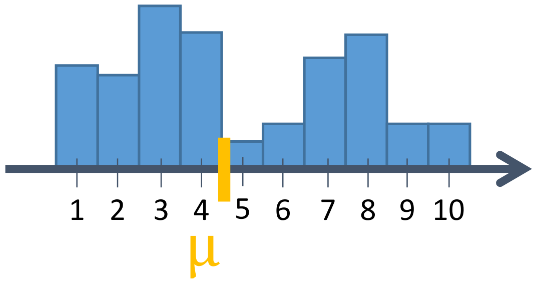
- The *sampling distribution* is the distribution of the estimated parameter values (here: expected value) of the population taken from the sample distribution

Central limit theorem



No matter how the population is distributed: the population of sample means will approximate a Gaussian distribution if the sample size is large enough

Central limit theorem (“simulation”)



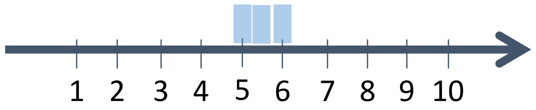
s_1=[3; 4; 7; 8] $\bar{x}_1$= 5.5

s_2=[1; 5; 8; 10] $\bar{x}_2$= 6.0

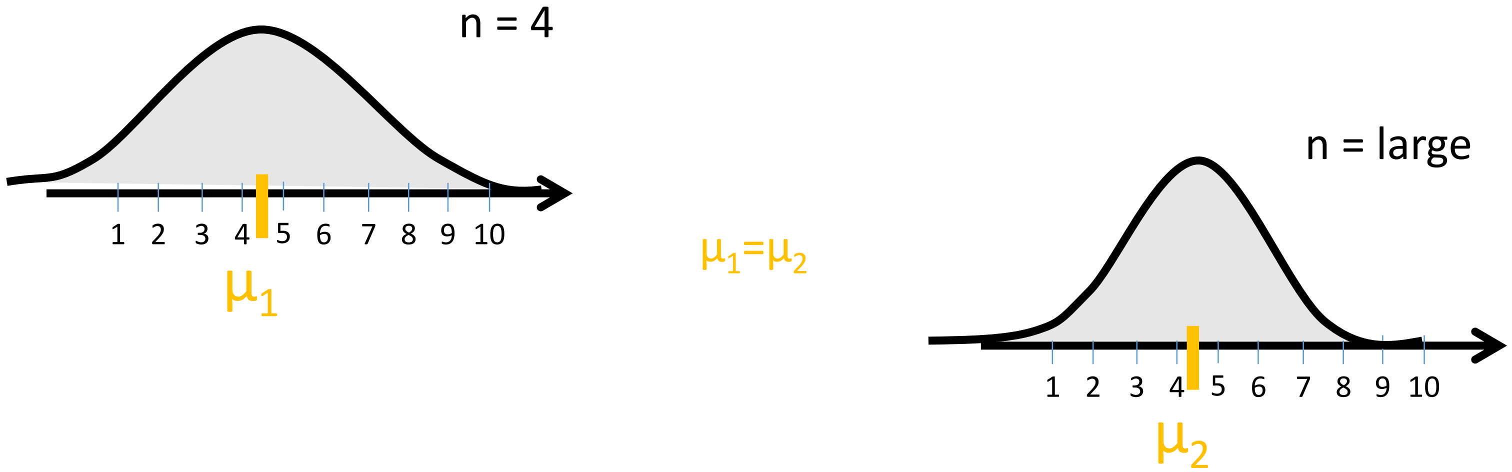
s_3=[2; 3; 6; 9] $\bar{x}_3$= 5.0

…

s_n=[…]



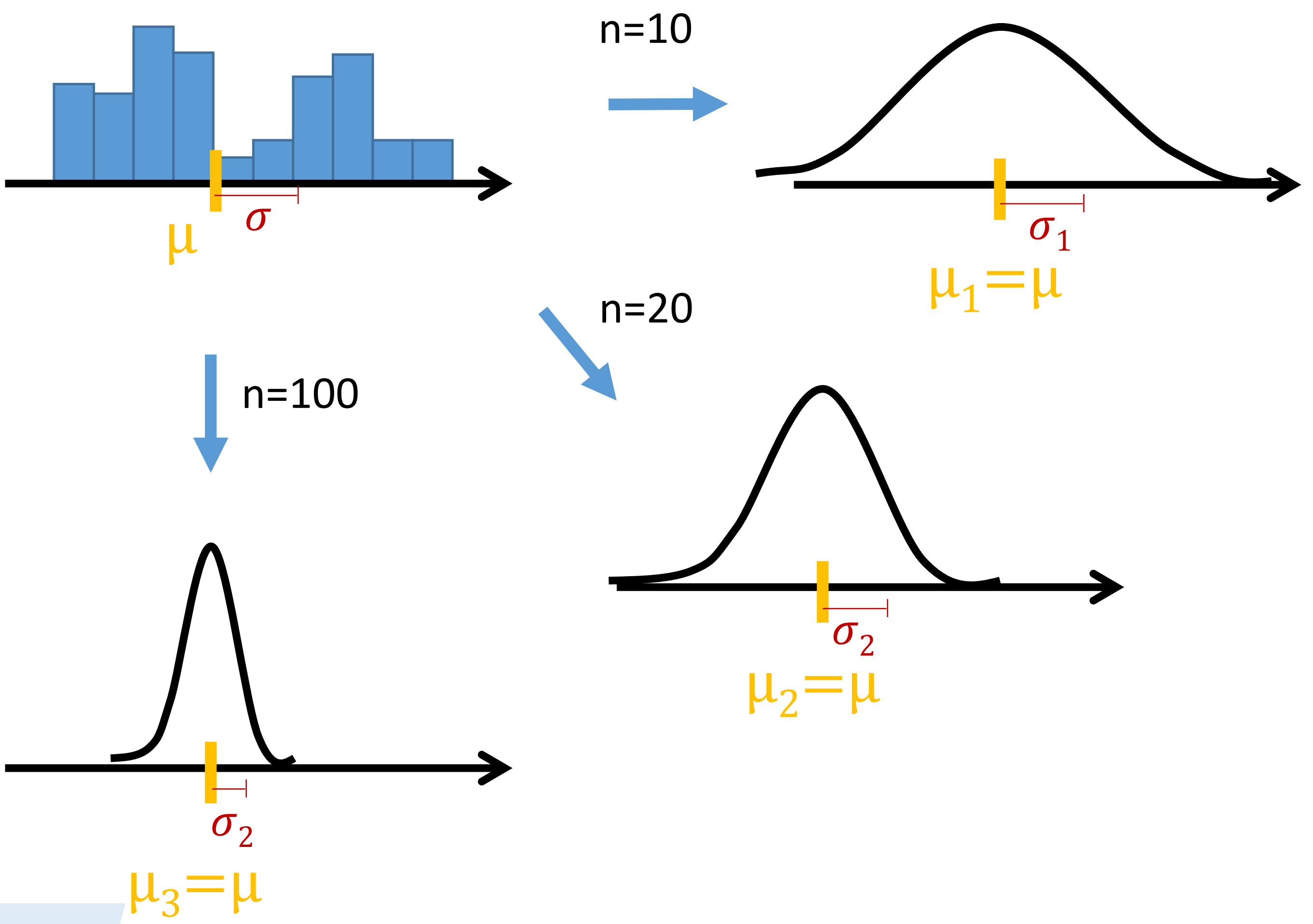
Central limit theorem ("simulation")



- Sample size ---> ∞
- Sampling distribution ---> normal distribution

Standard error of the mean

aka: the standard deviation of the sampling distribution of the sample means



$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

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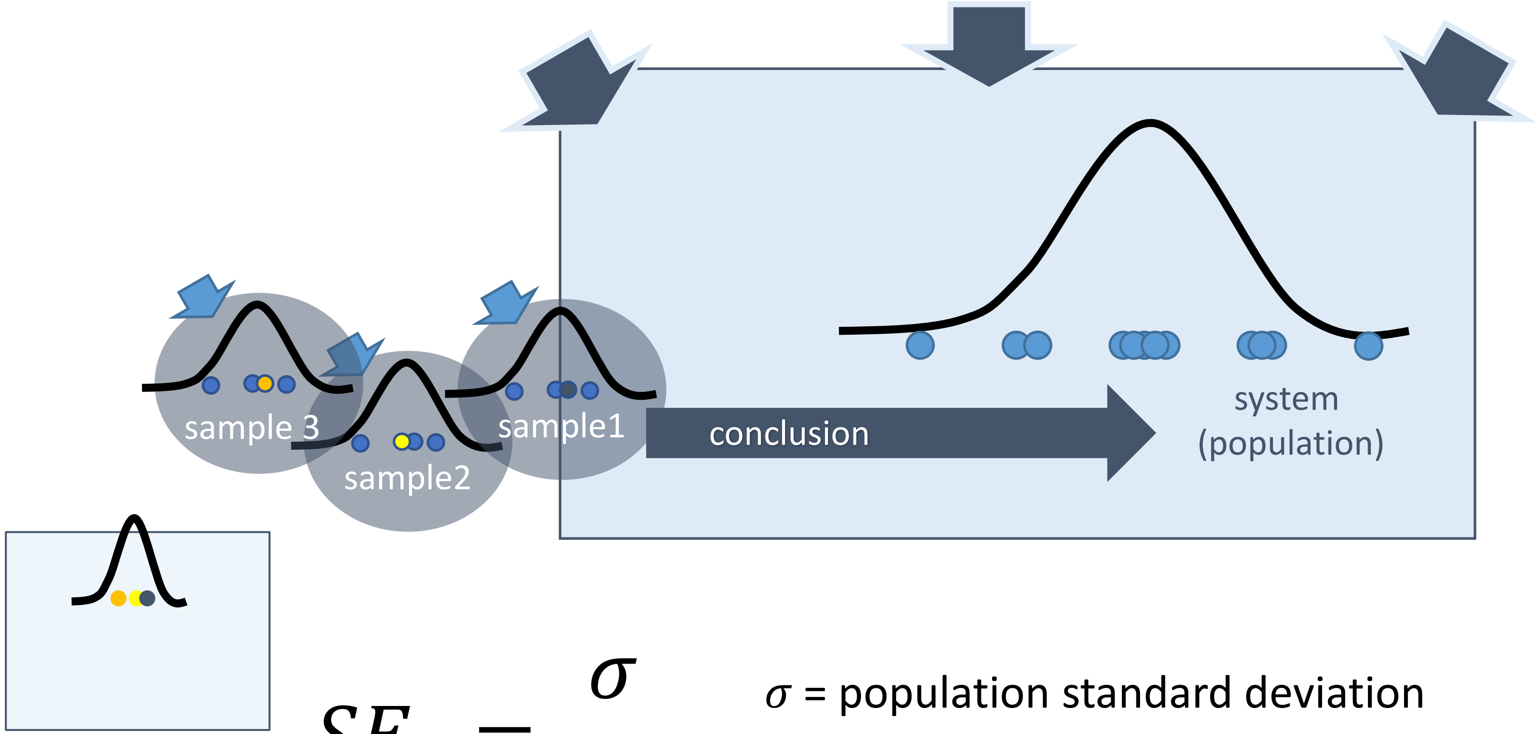
Remark: Standard error of the mean



$$SE_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

σ = population standard deviation

n = sample size

- It defines the standard deviation of different samples means taken from the same population

Hypothesis testing

- Question: Is the effect I observe true/real or occurred by chance?

- Proof by contradiction:

  To prove A, you temporarily assume that A is false. If the assumption leads to a contradiction, you conclude that A must actually be true.

Establish two hypothesis

- Null hypothesis (H_0)

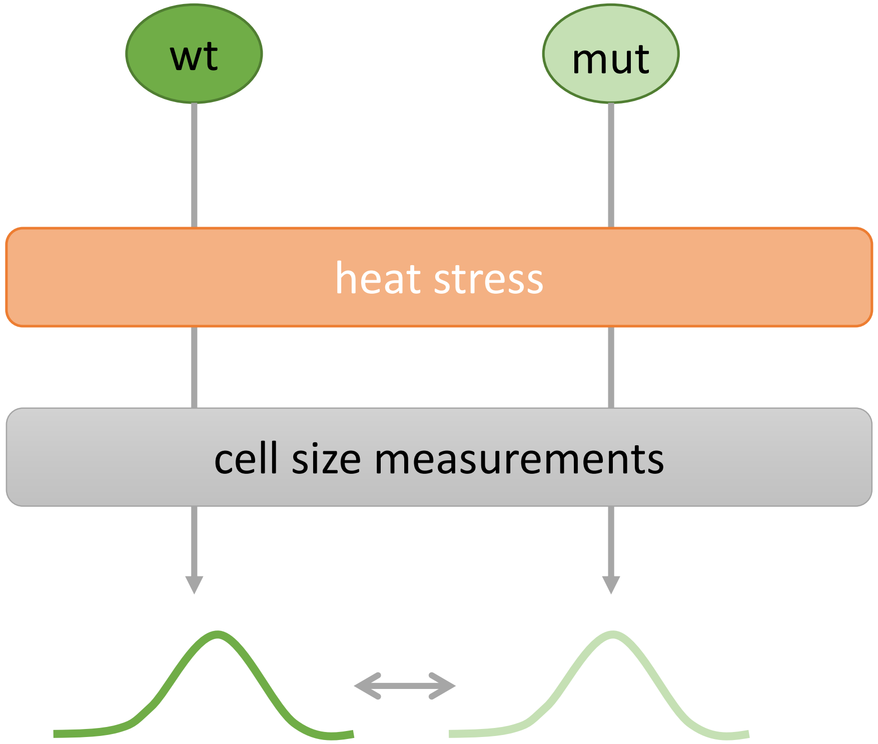
no effect

$\mu 1 = \mu 2$

- Alternative hypothesis (H_1)

effect

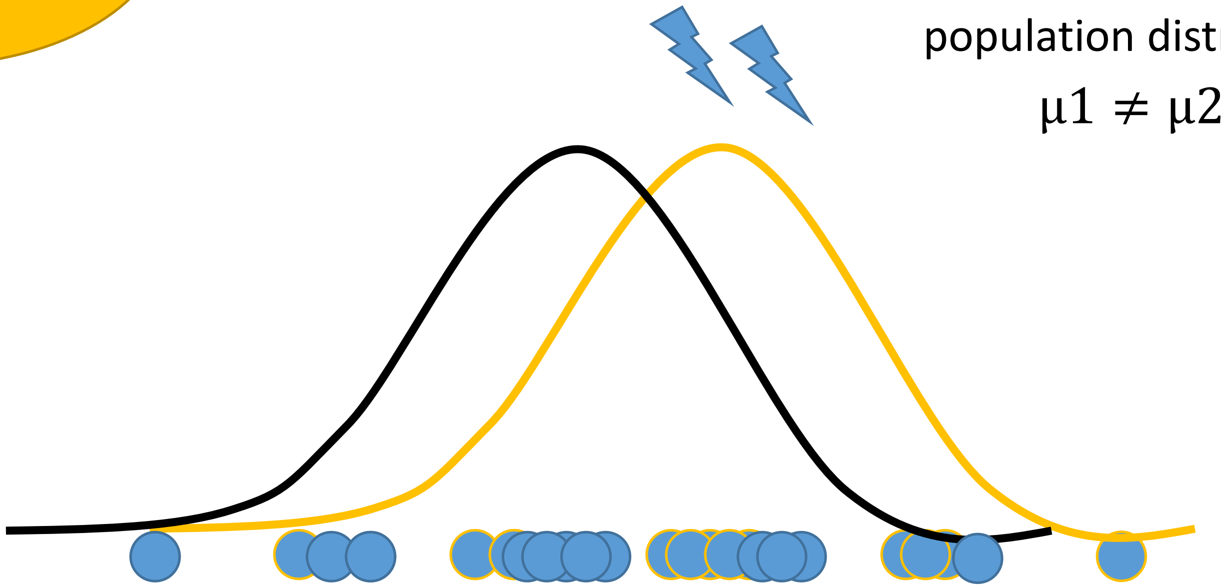
$\mu 1 \neq \mu 2$



Is the effect I observe true ?

H_1= true

effect



population distribution

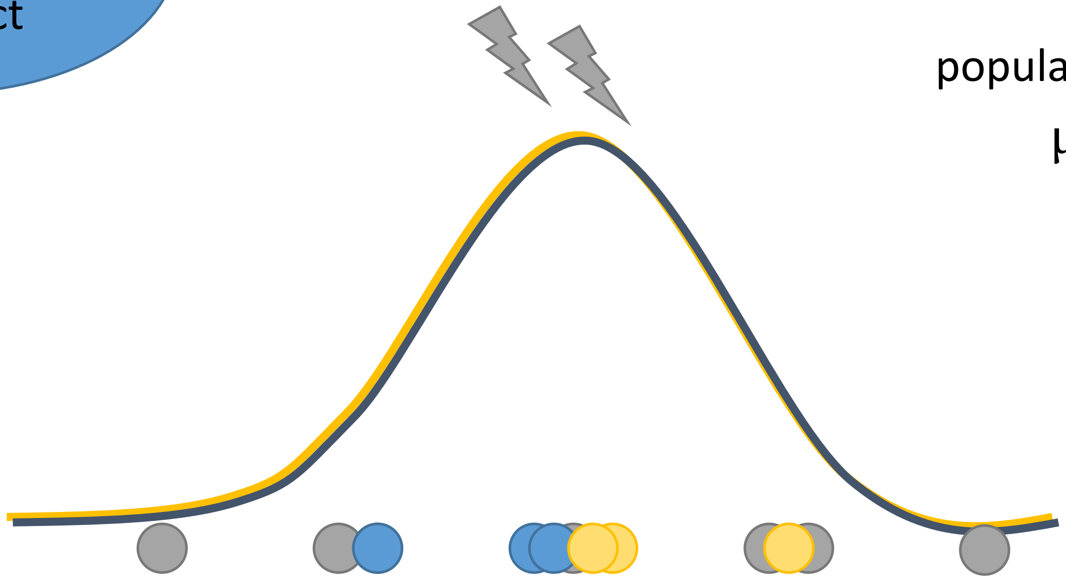
$\mu 1 \neq \mu 2$

- Alternative hypothesis states that the populations are different

Is the effect I observe true ?

H_0= true

no effect

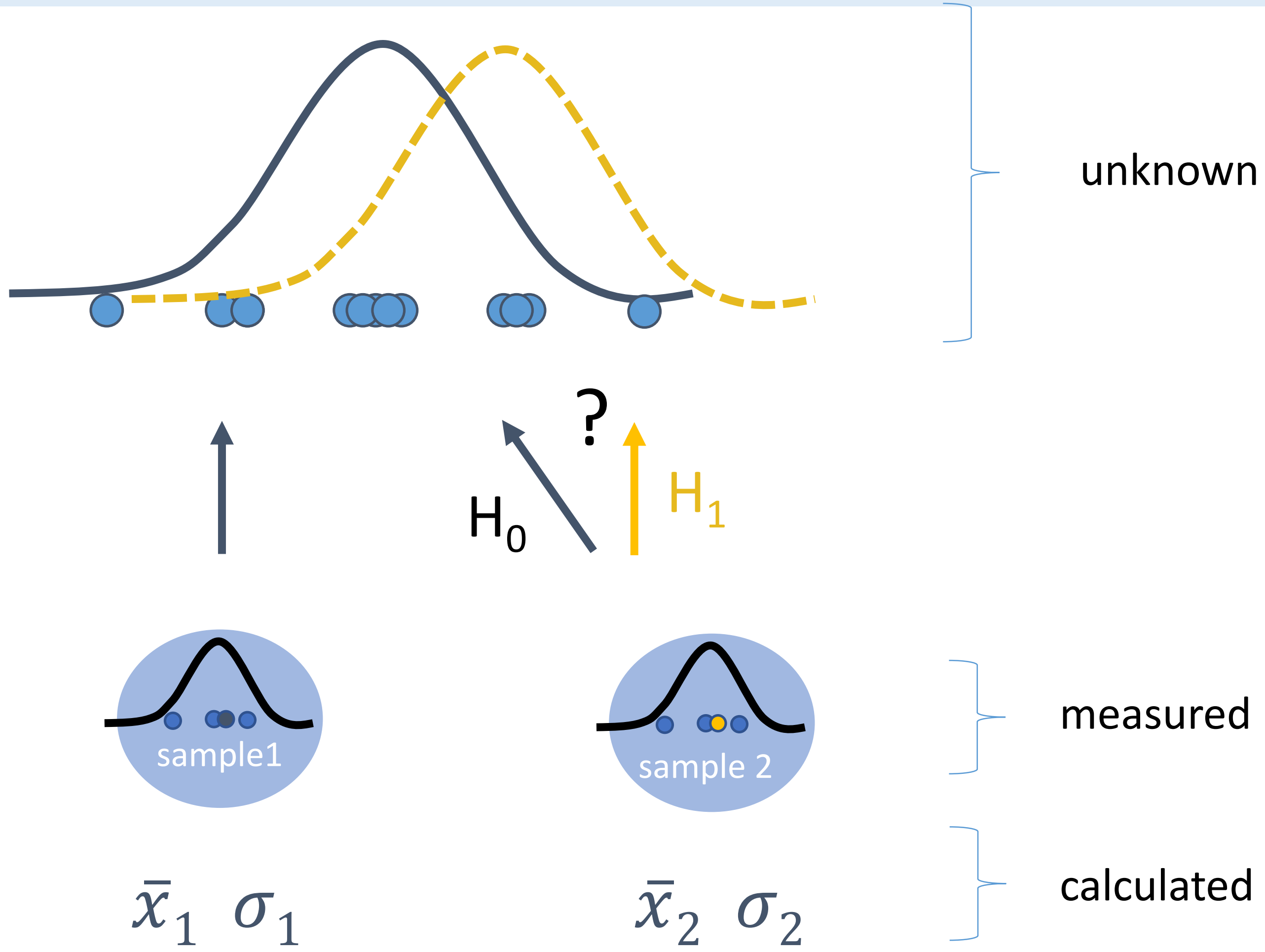


population distribution

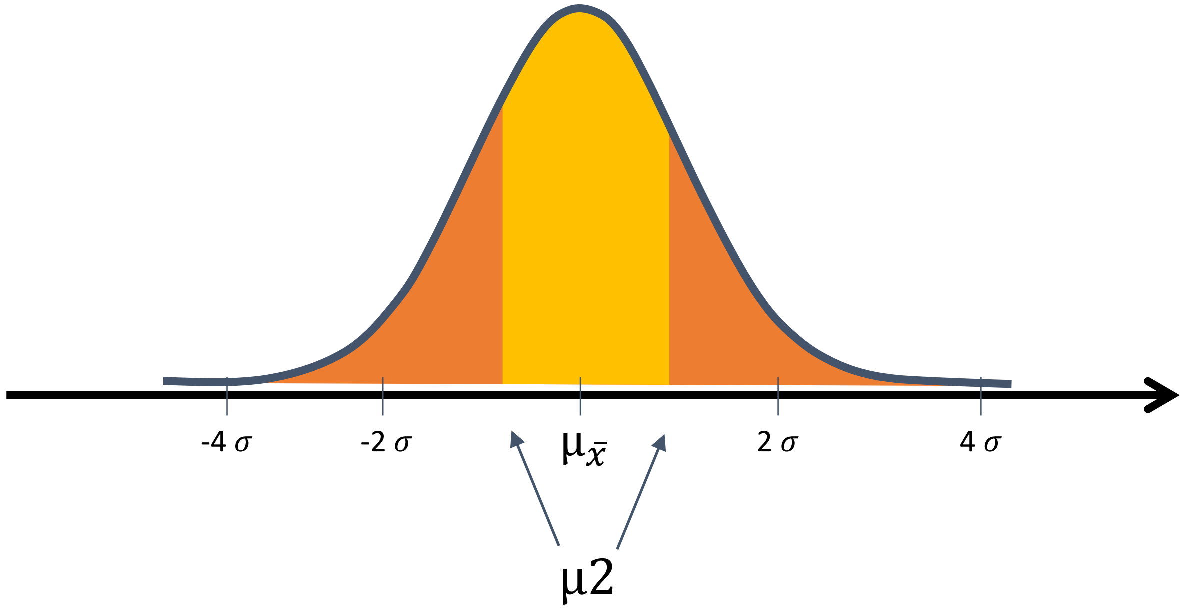
$\mu 1 = \mu 2$

- Null hypothesis states that the populations are equal

Is the effect I observe true ?



What is the probability of obtaining a value at least as extreme as the one that was observed ?

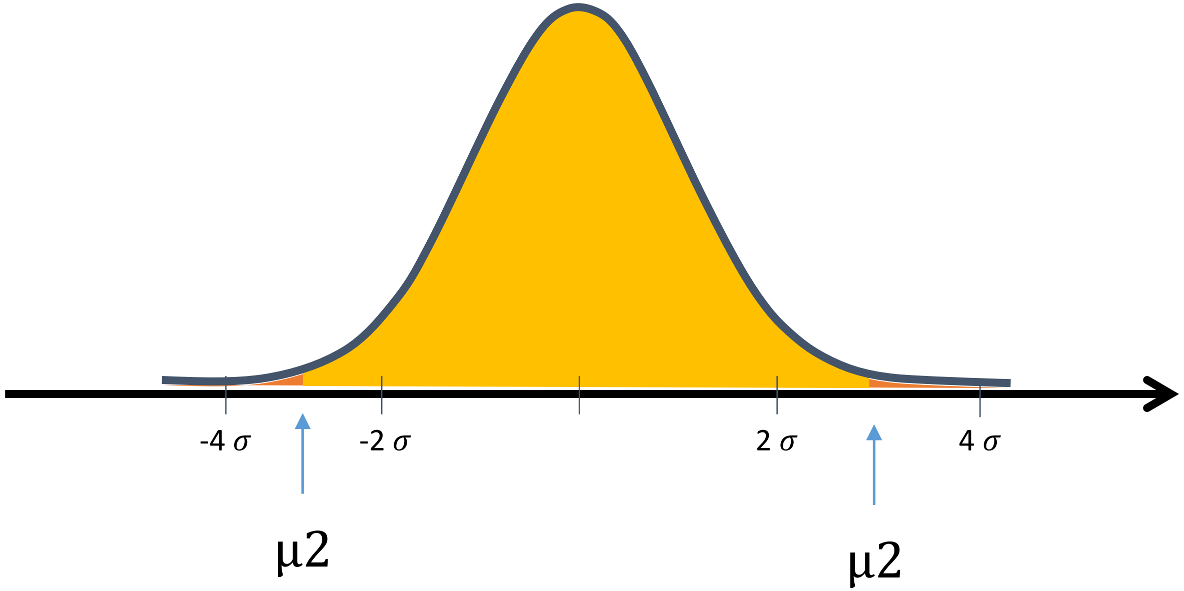


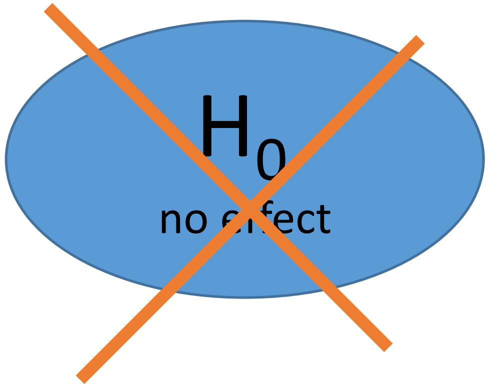
H_0

no effect

- The difference between μ1 and μ2 was most probably by chance: We take H_0 as true -> no effect

What is the probability of obtaining a value at least as extreme as the one that was observed ?





rejected

- Proof by contradiction:
  If we can reject H_0 than we assume H_1 to be true

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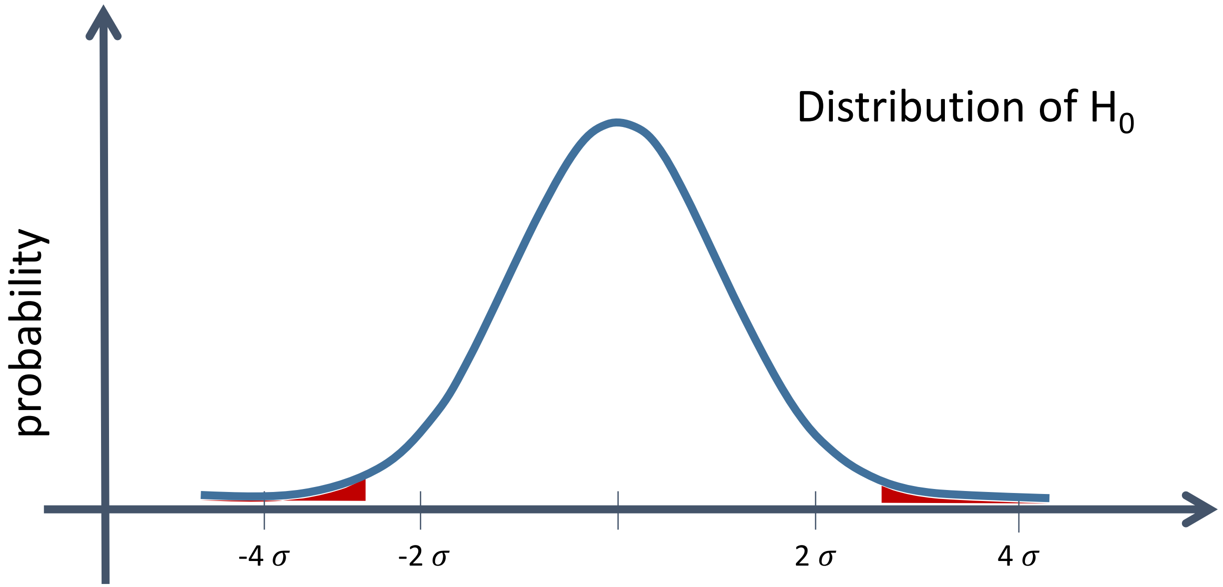
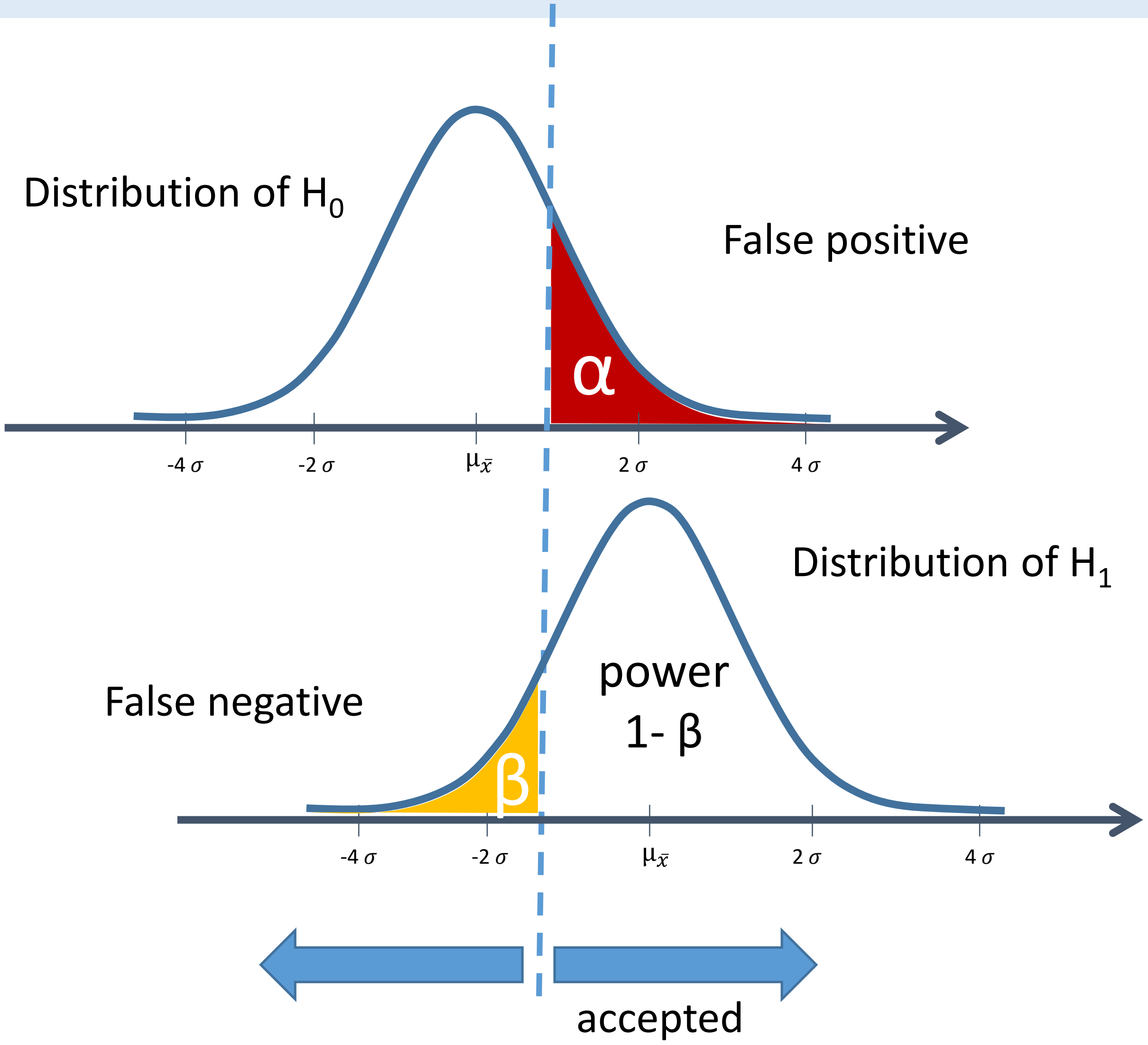
P-Value



- A p-value is the probability of obtaining a value at least as extreme as the one that was observed

Power of a Test



Distribution of H_0

False positive

α

Distribution of H_1

power

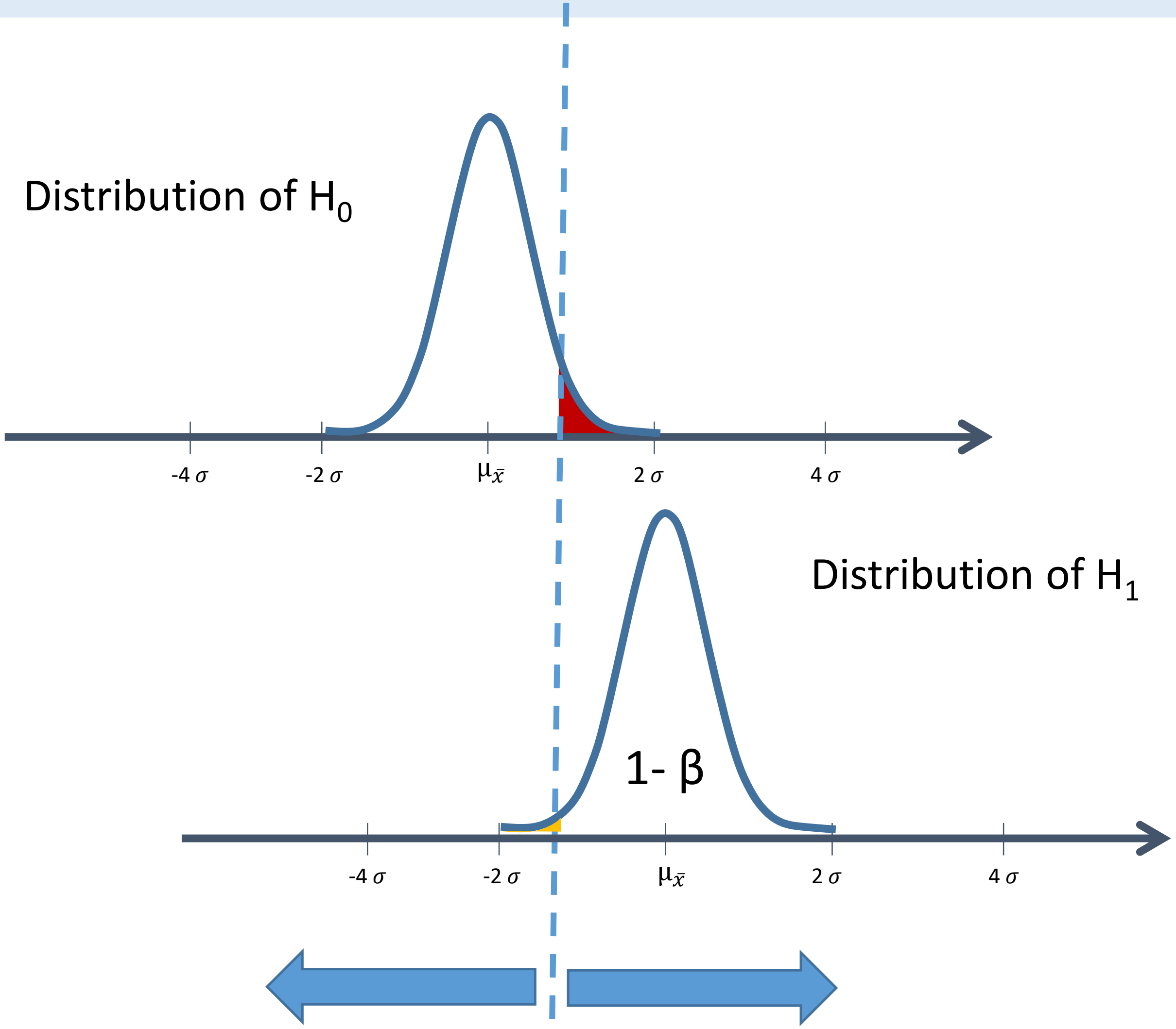
1- β

False negative

β

accepted

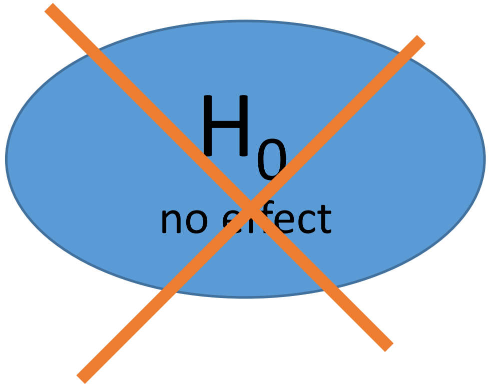
Increase sample size



Significance criterion (when to reject H_0)

- The most common approach to hypothesis testing is to choose a threshold α for the p-value and to accept as significant any effect with a p-value ≤ α

P-value	**Interpretation**
$P < 0.01$	very strong evidence against H_0
$0.01 \leq P < 0.05$	moderate evidence against H_0
$0.05 \leq P < 0.10$	suggestive evidence against H_0
$0.10 \leq P$	little or no real evidences against H_0



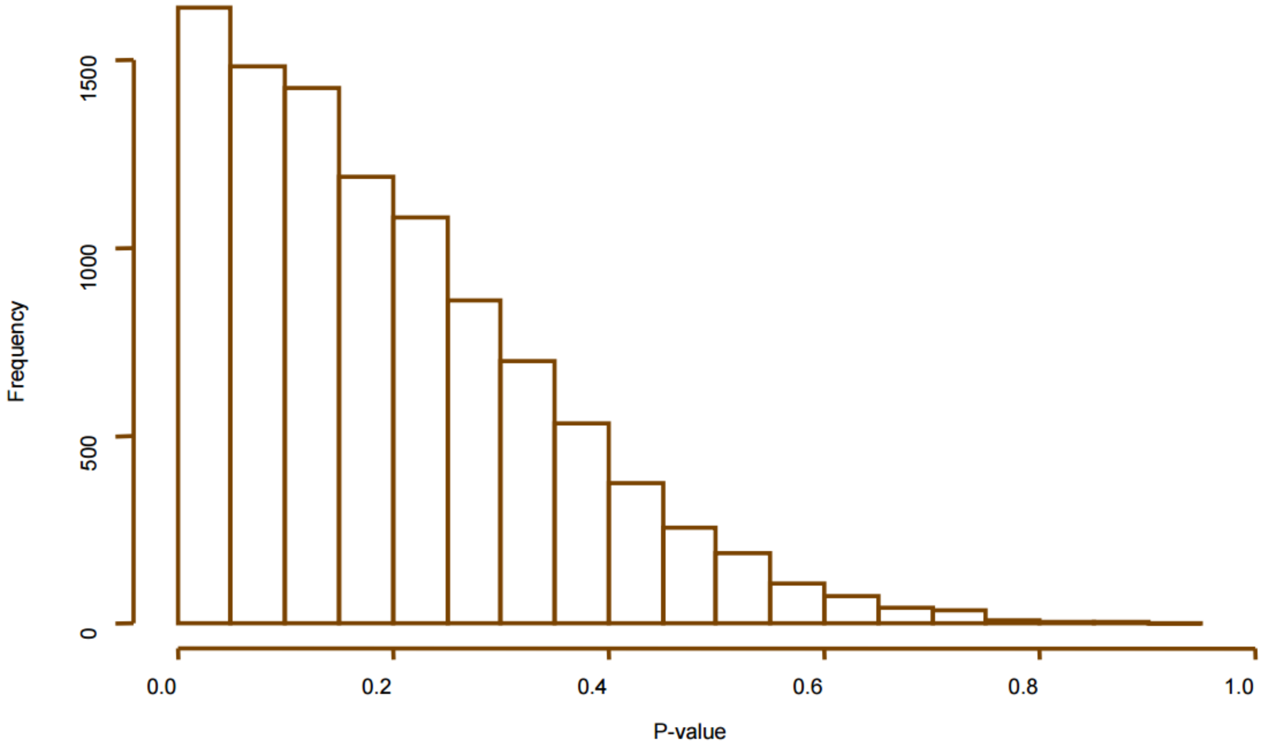
rejected

Multiple testing remarks

- The hypothesis test framework was built to perform one test only.
- What about testing multiple times?
- What does that mean for the p-value?

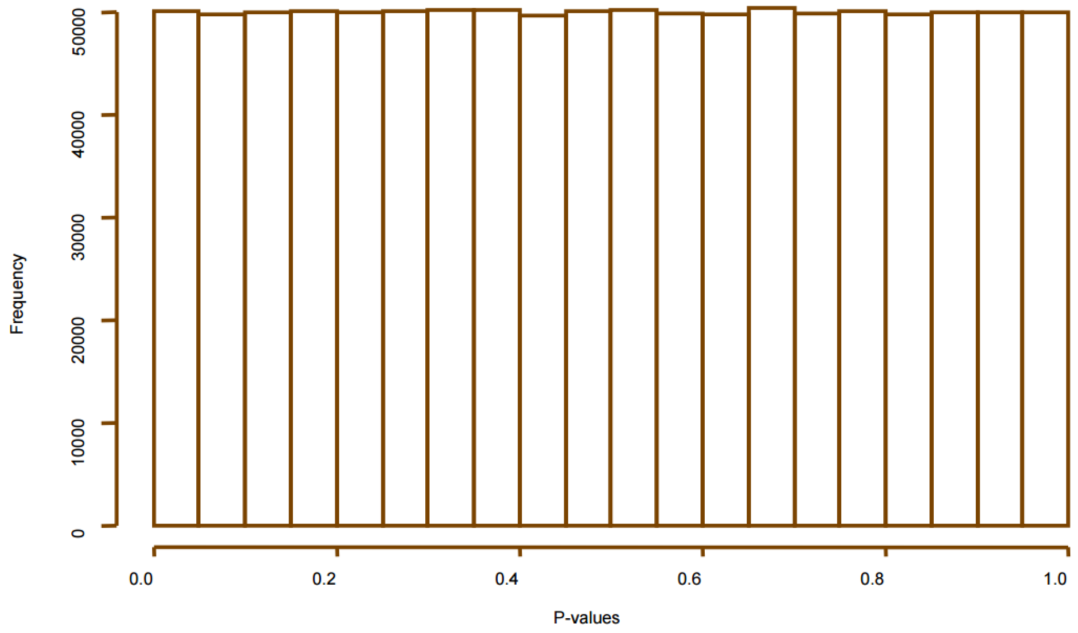
Estimating the proportion of truly Null Tests

- Under the alternative hypothesis p-values are skewed towards 0



Estimating the proportion of truly Null Tests

- Under the null hypothesis p-values are expected to be uniformly distributed between 0 and 1



Adaptation to multiple testing

- Family wise error rates:

$$P(\#false\ positives \quad \geq 1)$$

- False discovery rate:

$$E\left[\frac{\#false\ positives}{\#\ total\ discoveries}\right]$$

Example:

Given: 550 out of 10 000 genes are significant at 0.05 level

- P-value < 0.05
  Expect 0.05 * 10 000 = 500 false positives

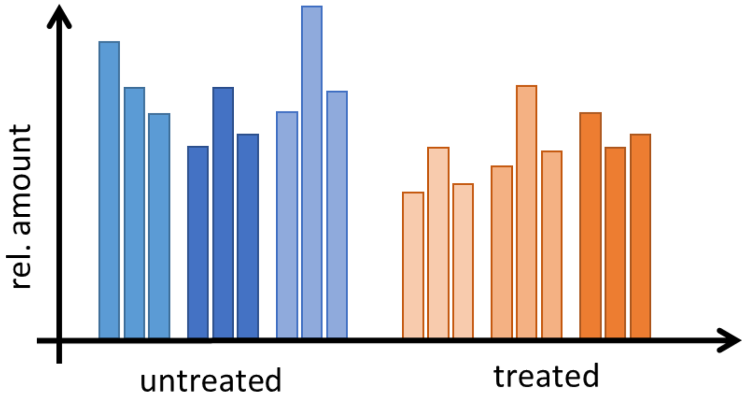
- False discovery rate < 0.05
  Expect 0.05 * 550 = 27.5 false positives

- Family wise error rate < 0.05
  The probability of at least 1 false positive ≤ 0.05

Be aware…

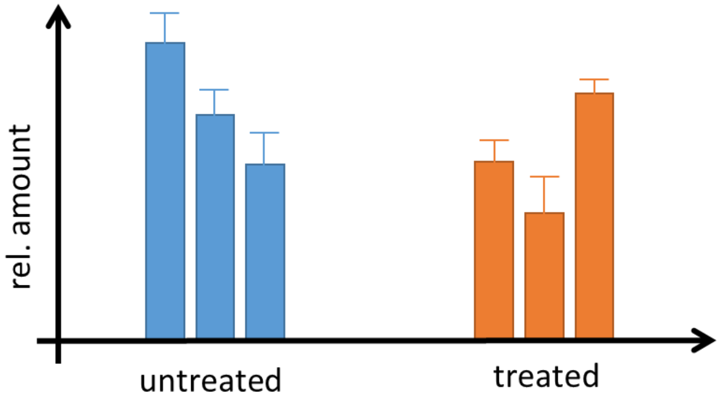
- Statistical significance can mean totally different thing depending on how it is used!

Aggregation and error propagation



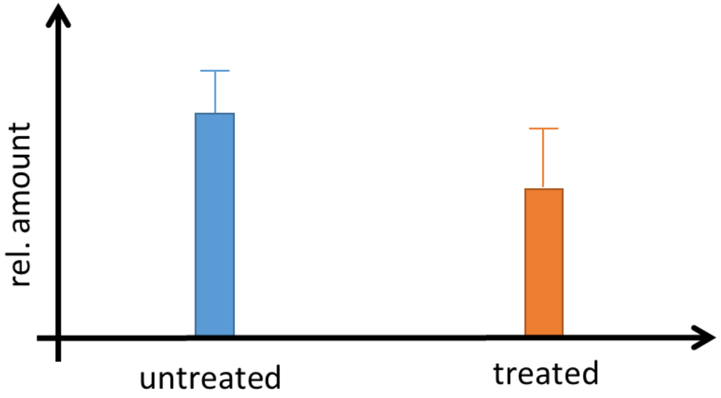
mean

stDev$_{n-1}$

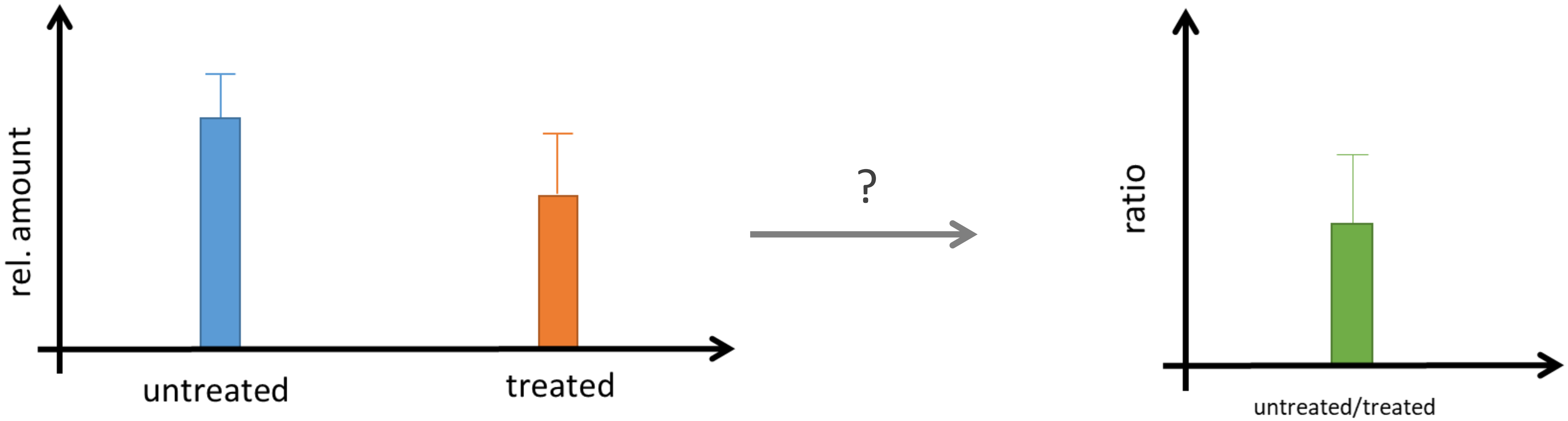


$$X_c = \frac{n_1\overline{X}_1 + n_2\overline{X}_2}{n_1 + n_2}$$

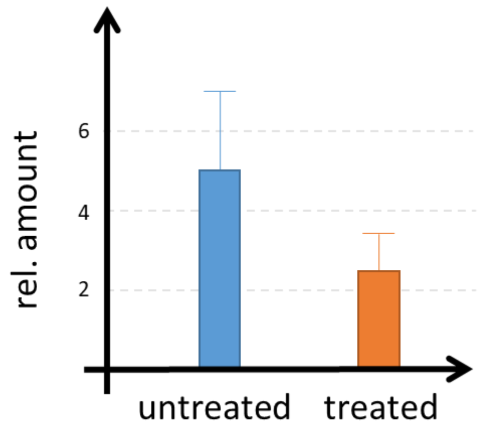
$$S_c{}^2 = \frac{n_1\left[S_1{}^2 + \left(\overline{X}_1 - \overline{X}_c\right)^2\right] + n_2\left[S_2{}^2 + \left(\overline{X}_2 - \overline{X}_c\right)^2\right]}{n_1 + n_2}$$

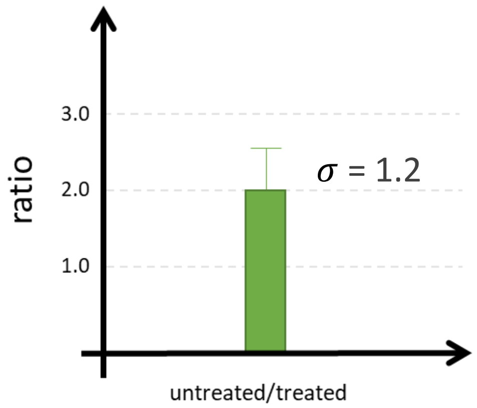


Aggregation and error propagation



Aggregation and error propagation





ratio

$$x_1 = 5.0 \qquad \delta x_1 = 2.0$$

$$x_2 = 2.5 \qquad \delta x_2 = 1.0$$

$$f_{(x1,x2)} = \frac{x_1}{x_2} = 2.0$$

$$\frac{\partial f}{\partial x_1} = \frac{1}{x_2}$$

$$\frac{\partial f}{\partial x_2} = \frac{x_1}{{x_2}^2}$$

error propagation

$$\sigma = \sqrt{\sum_{j=1}^{m} \left(\frac{\partial f}{\partial x_j}\right)^2 \cdot \sigma_{x_j}^2}$$

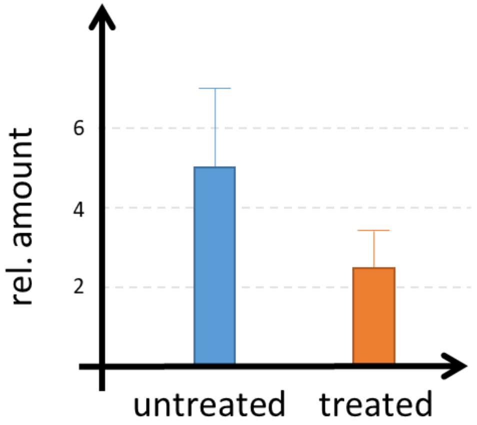
$$\sigma = \sqrt{\left(\frac{\partial f}{\partial x_1}\right)^2 \cdot \sigma_{x_1}^2 + \left(\frac{\partial f}{\partial x_2}\right)^2 \cdot \sigma_{x_2}^2}$$

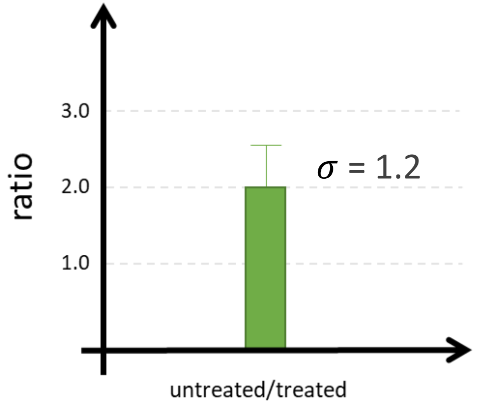
$$\sigma = \sqrt{\left(\frac{1}{x_2}\right)^2 \cdot {\delta x_1}^2 + \left(\frac{x_1}{x_2^2}\right)^2 \cdot {\delta x_2}^2}$$

$$\sigma = \sqrt{\left(\frac{1}{2.5}\right)^2 \cdot 2^2 + \left(\frac{5}{6.25}\right)^2 \cdot 1^2}$$

$$\sigma = \sqrt{1.28} = 1.1314 = 1.2$$

Aggregation and error propagation





ratio

$$x_1 = 5.0 \qquad \delta x_1 = 2.0$$

$$x_2 = 2.5 \qquad \delta x_2 = 1.0$$

$$f_{(x1,x2)} = \frac{x_1}{x_2} = 2.0$$

$$\frac{\partial f}{\partial x_1} = \frac{1}{x_2}$$

$$\frac{\partial f}{\partial x_2} = \frac{x_1}{{x_2}^2}$$

error propagation

addition or subtraction

$$Q = x_1 + x_2 + \cdots$$

$$\delta Q = \sqrt{(\delta x_1)^2 + (\delta x_2)^2 + \cdots}$$

multiplication or division

$$Q = \frac{x_1 \cdot x_3 \ldots}{x_2 \cdot x_4 \ldots}$$

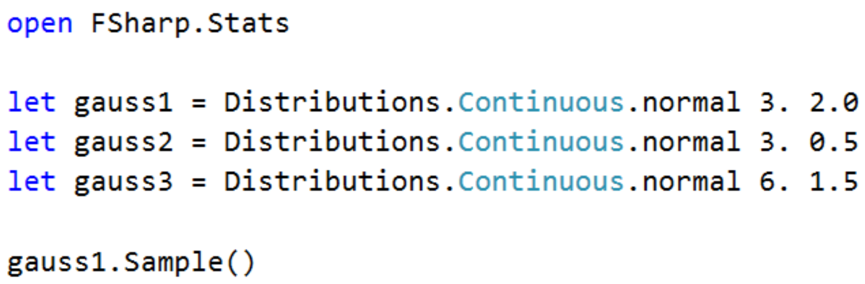
$$\frac{\delta Q}{|Q|} = \sqrt{\left(\frac{\delta x_1}{x_1}\right)^2 + \left(\frac{\delta x_2}{x_2}\right)^2 + \cdots}$$

$$\frac{\delta Q}{2} = \sqrt{\left(\frac{2}{5}\right)^2 + \left(\frac{1}{2.5}\right)^2}$$

$$\frac{\delta Q}{2} = 0.56569$$

$$\delta Q = 1.1318 = 1.2$$

Coding 1: sampling

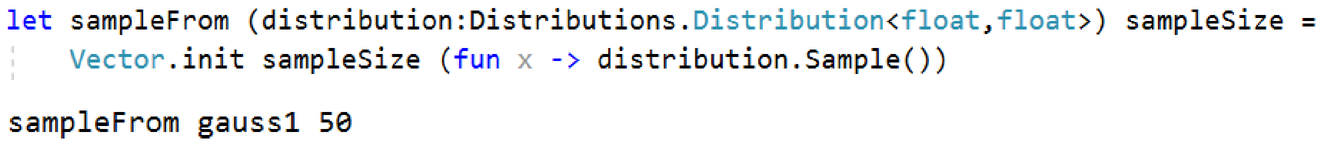


```
open FSharp.Stats

let gauss1 = Distributions.Continuous.normal 3. 2.0
let gauss2 = Distributions.Continuous.normal 3. 0.5
let gauss3 = Distributions.Continuous.normal 6. 1.5

gauss1.Sample()
```

include FSharp.Stats

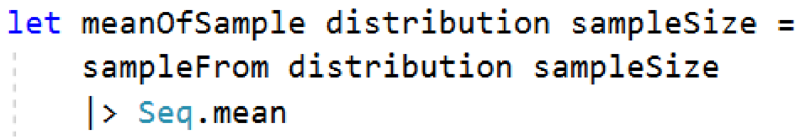
instantiation of normal distributions

get a sample of gauss1 (n=1)



```
let sampleFrom (distribution:Distributions.Distribution<float,float>) sampleSize =
    Vector.init sampleSize (fun x -> distribution.Sample())

sampleFrom gauss1 50
```

function to generate samples

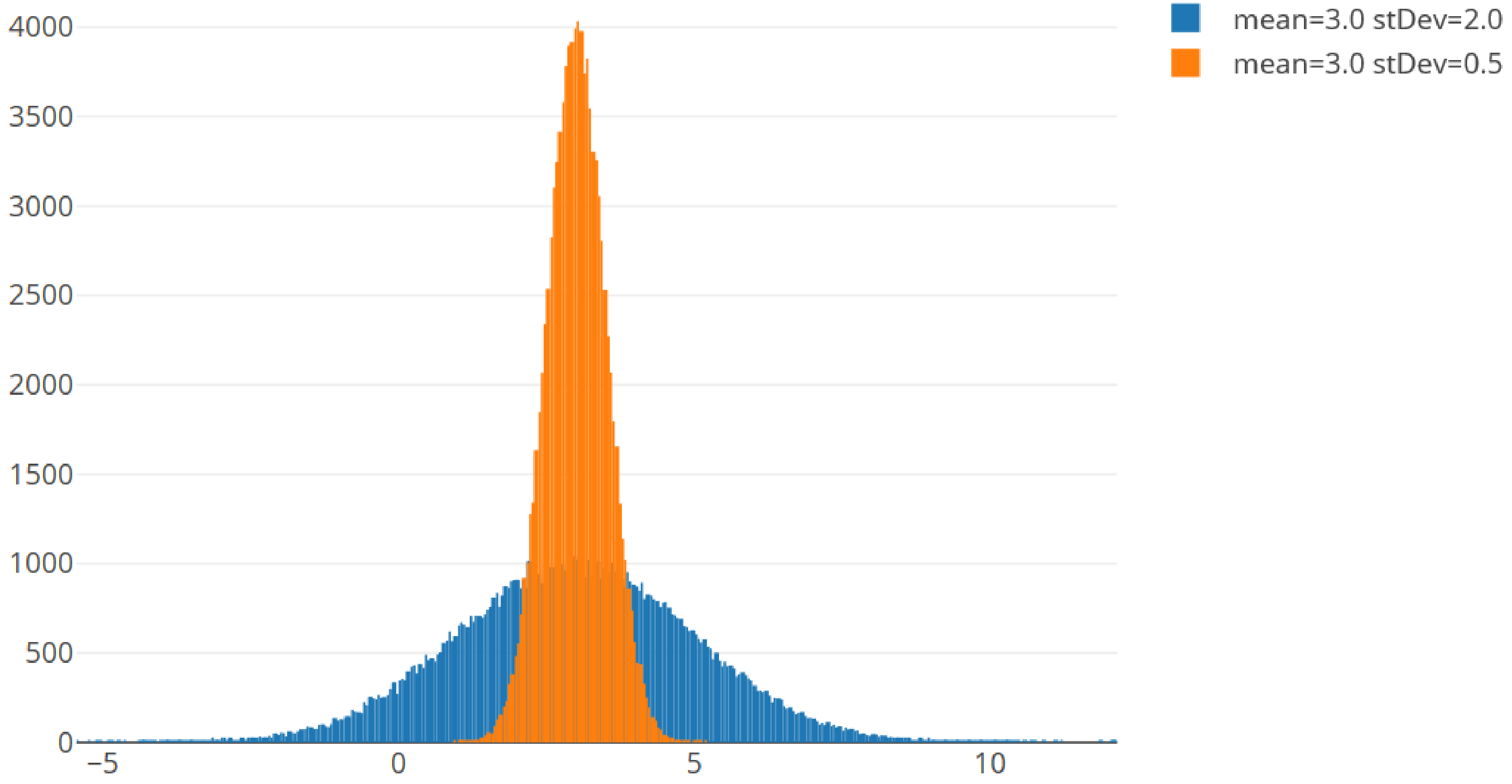


```
let meanOfSample distribution sampleSize =
    sampleFrom distribution sampleSize
    |> Seq.mean
```

function to calculate mean of sample

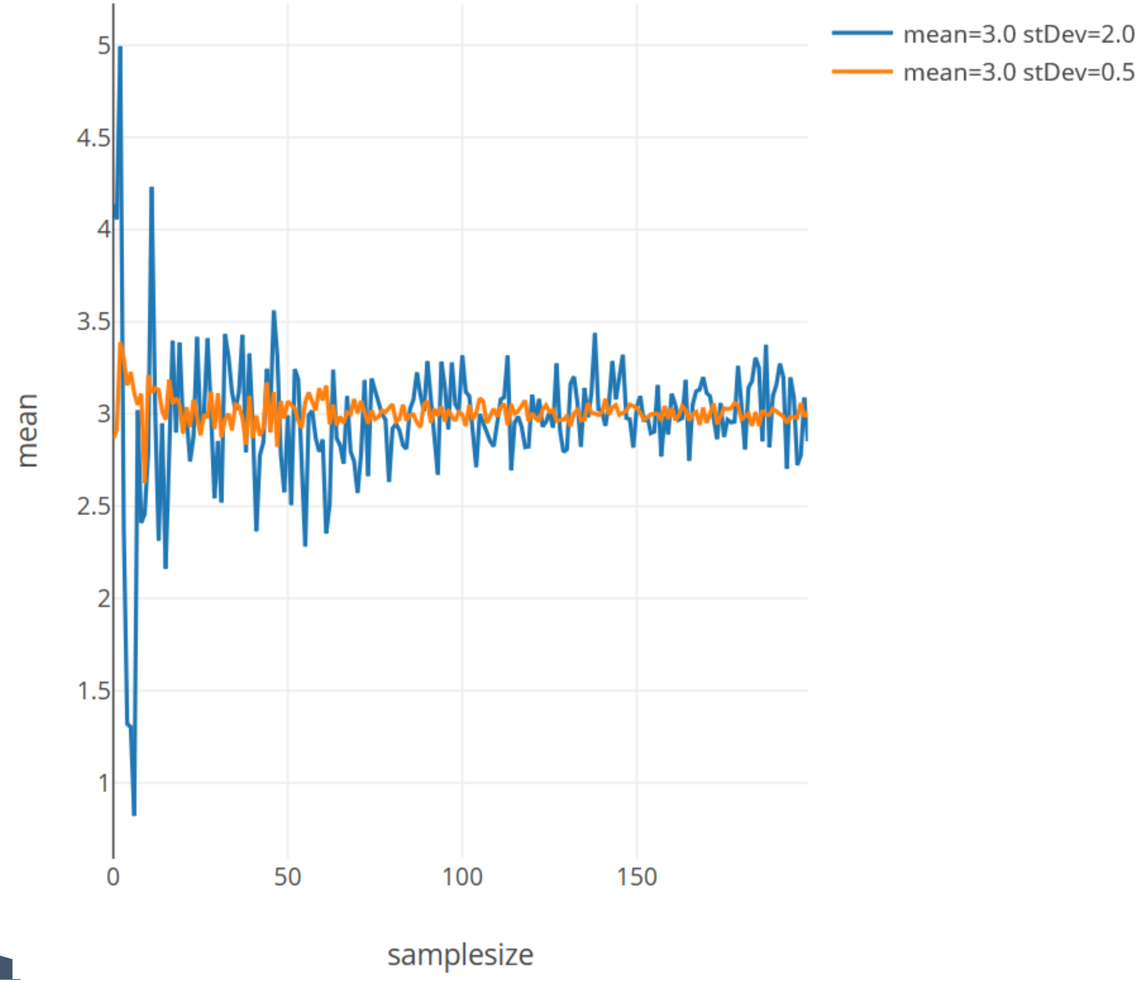
1. write a function that takes a distribution and a sample size and gives the standard deviation
2. calculate means of gauss1 and gaus2-samples of different sample sizes and compare them

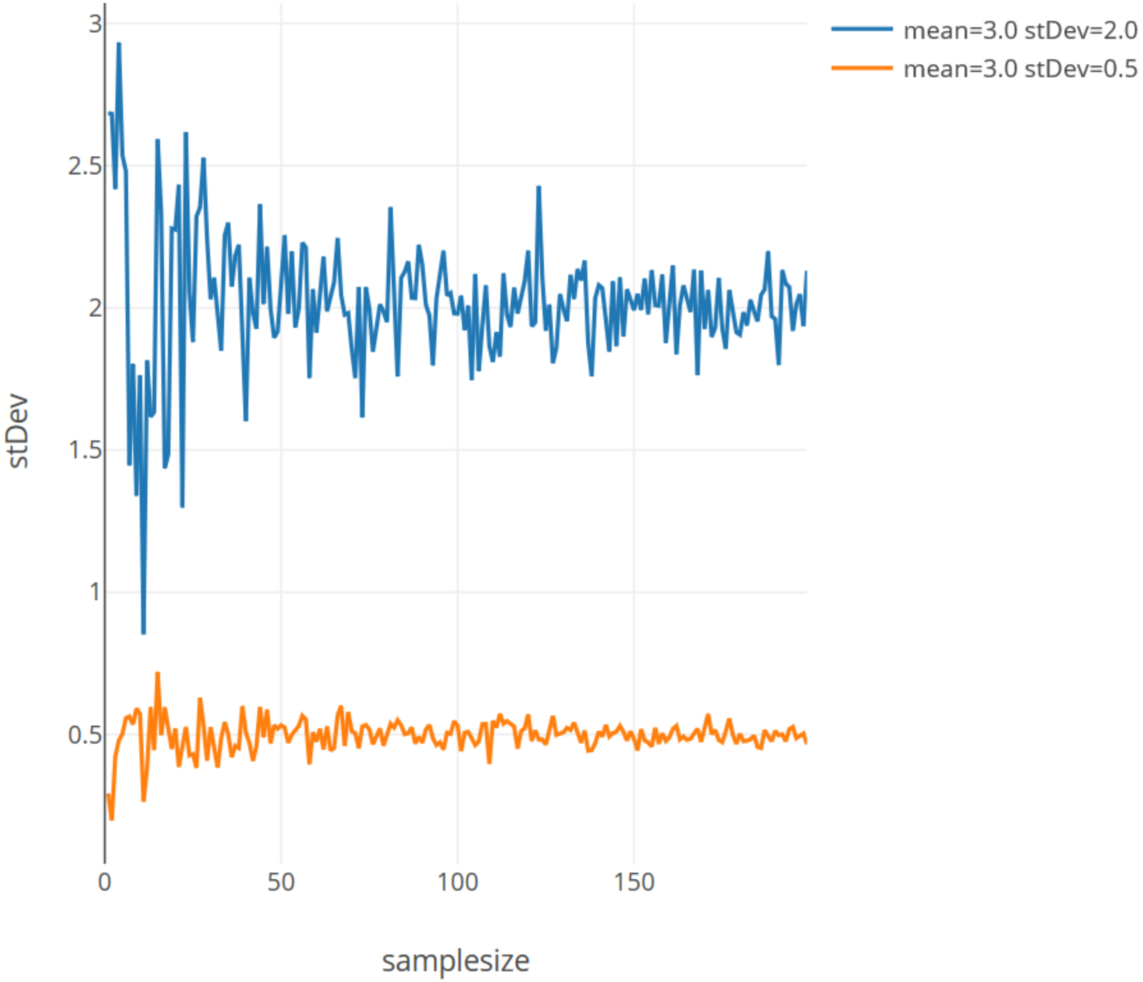
Normal distribution with different σ



N = 100,000

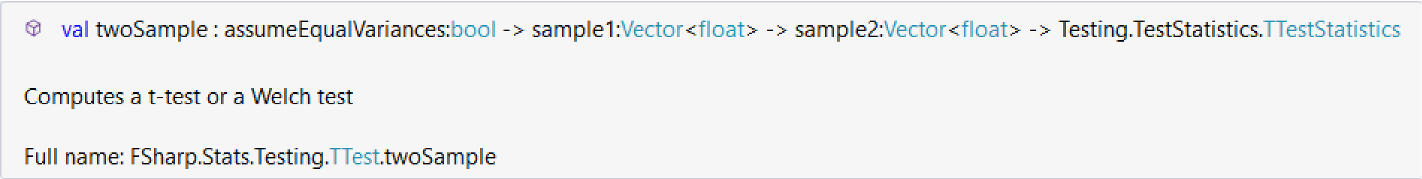
n vs. σ (sample size vs. stDev)





Coding 2: testing

```
Testing.TTest.twoSample
```

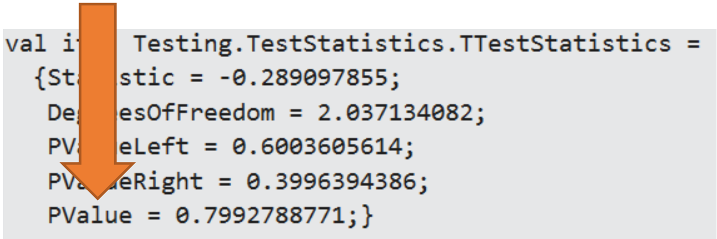


val twoSample : assumeEqualVariances:bool -> sample1:Vector<float> -> sample2:Vector<float> -> Testing.TestStatistics.TTestStatistics

Computes a t-test or a Welch test

Full name: FSharp.Stats.Testing.TTest.twoSample

testing function



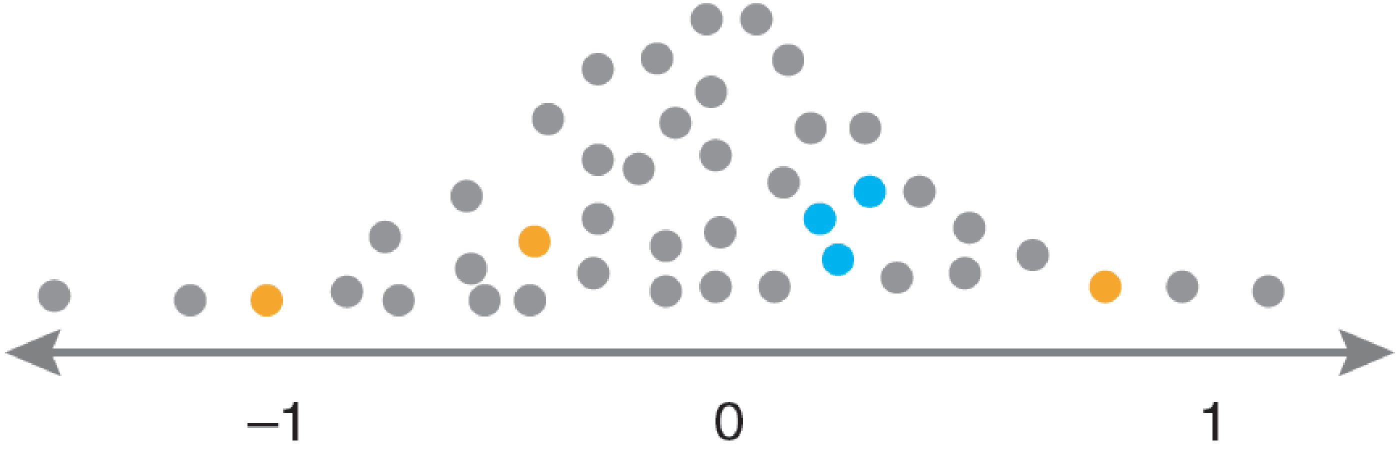
```
val i     Testing.TestStatistics.TTestStatistics =
  {St    stic = -0.289097855;
   De    esOfFreedom = 2.037134082;
   PV    eLeft = 0.6003605614;
   PV    eRight = 0.3996394386;
   PValue = 0.7992788771;}
```

testing result

1. test samples of different distributions (regarding to p value) and mediate the sample size

Pitfall: Small sample sizes

- Small sample sizes (n < 10) can have a strong effect on the estimation of the central tendency and data dispersion of a population

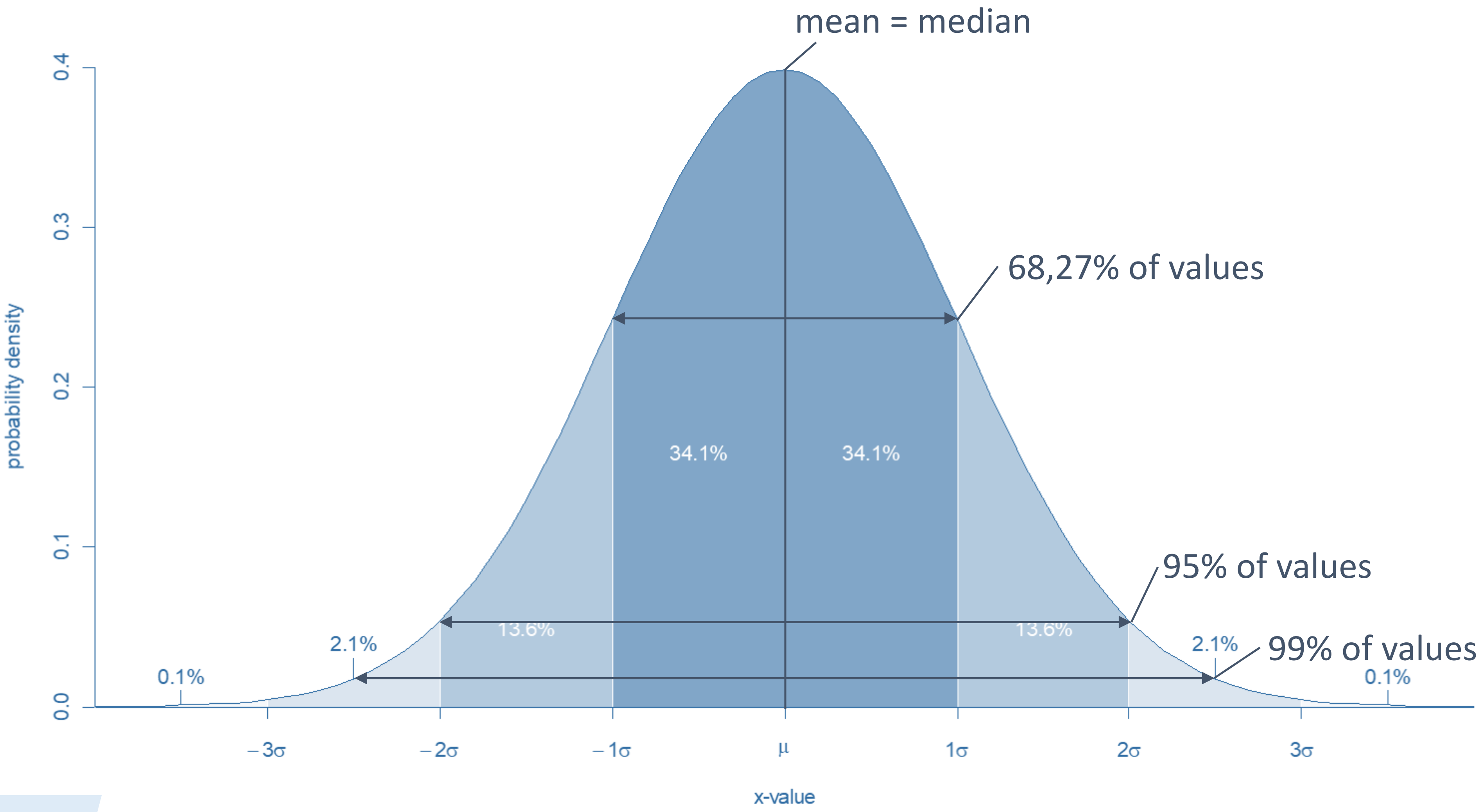


Central limit theorem

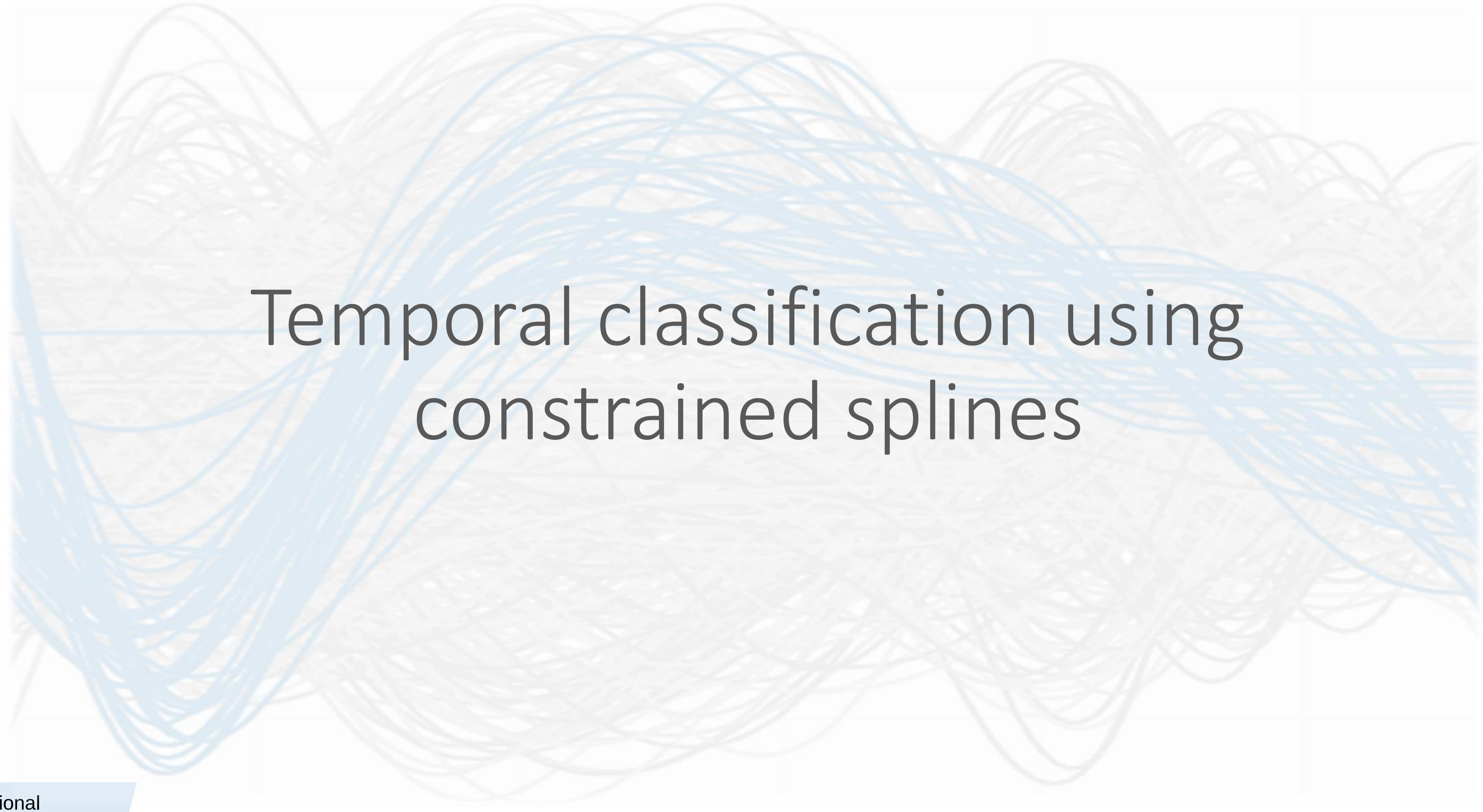
No matter how the population is distributed: the population of sample means will approximate a Gaussian distribution if the sample size is large enough

- “Large” depends on the real population distribution
  - Less normal population distribution => more sample (N >= 100)
  - More normal population distribution => N >= 10)

The Gaussian „Normal Distribution“

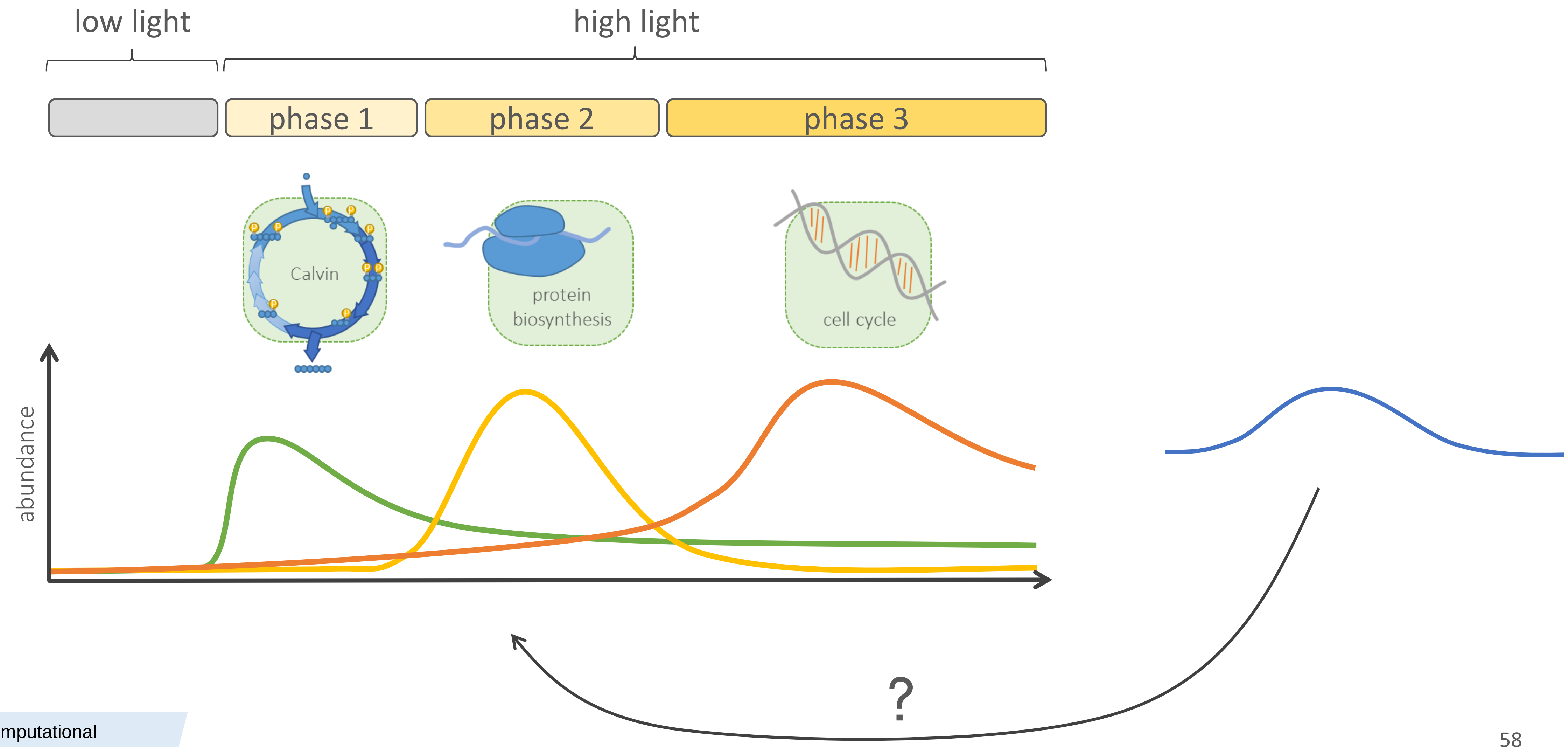


Symmetric around the mean



Temporal classification using constrained splines

Motivation



low light

high light

phase 1

phase 2

phase 3

Calvin

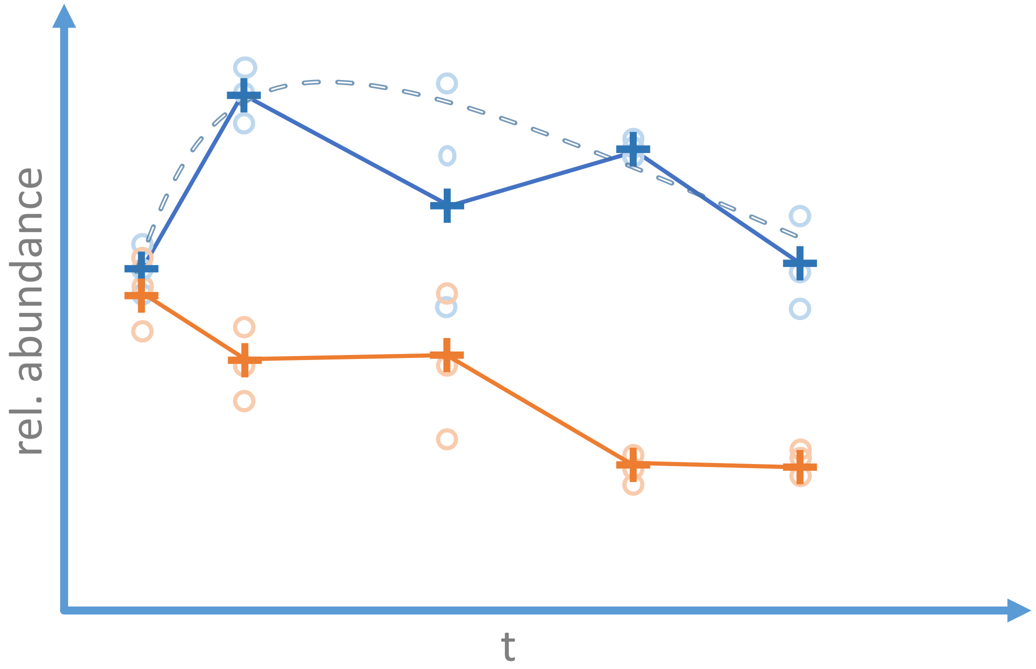
protein biosynthesis

cell cycle

abundance

?

How to choose a model?

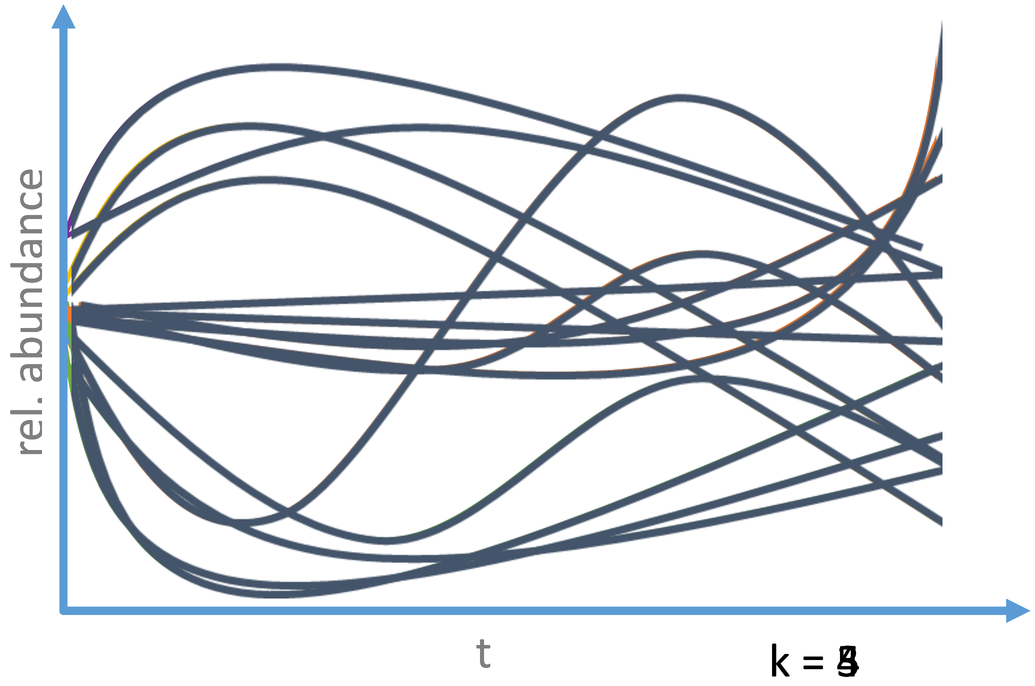


Modelling time courses:

- model has to be tailored to the process being investigated
  - teach the computer to interpret the data
  - measurement variance has to be considered
    → transfer of information
  - dynamics of proteins are known
    → shape assumptions

Computational
Systems Biology

Partition based clustering – kMeans

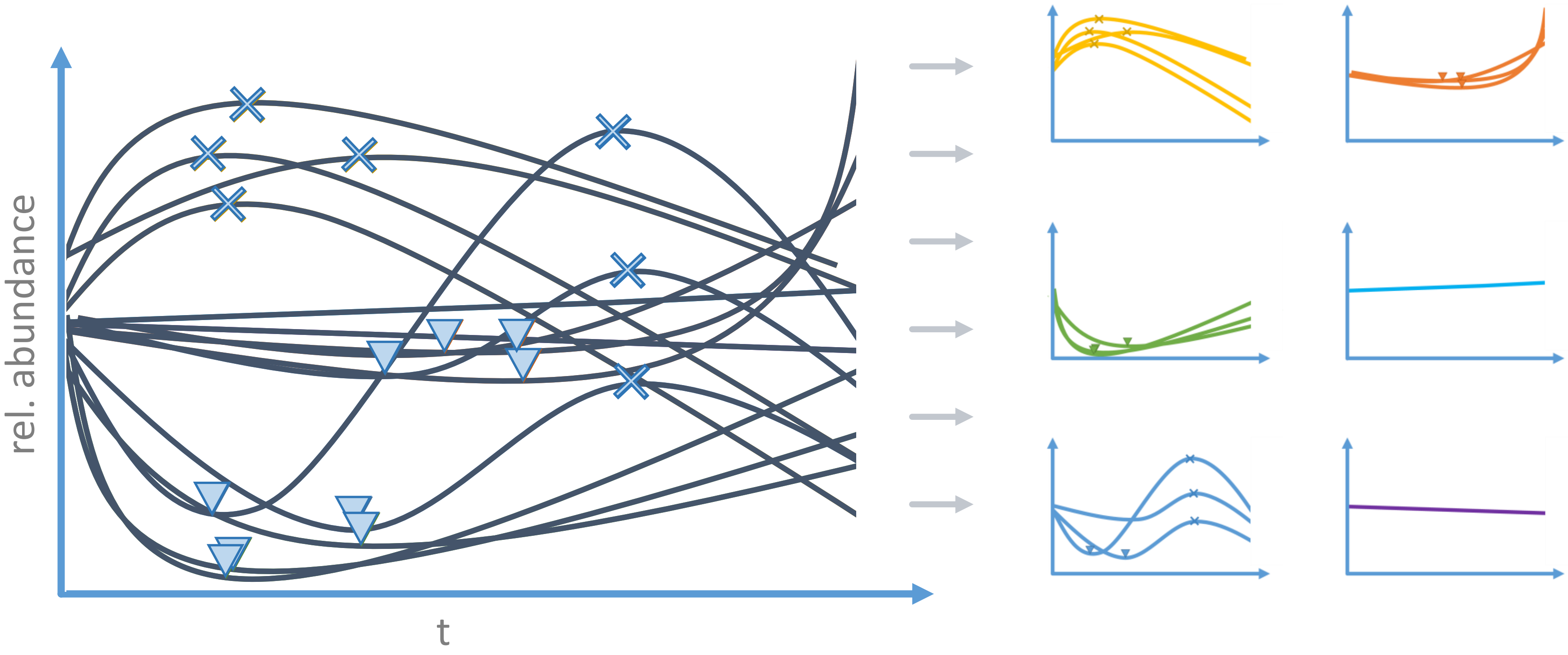


Time series processing – Clustering:

- kMeans clustering
  - set number of clusters beforehand (k)
  - similarity measurement based on object distances

Basic distance measurements are not suited for clustering time series data

Time series analysis - extrema



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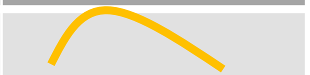
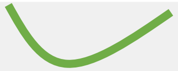
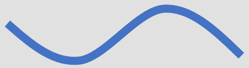
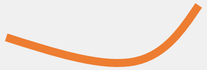
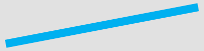
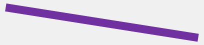
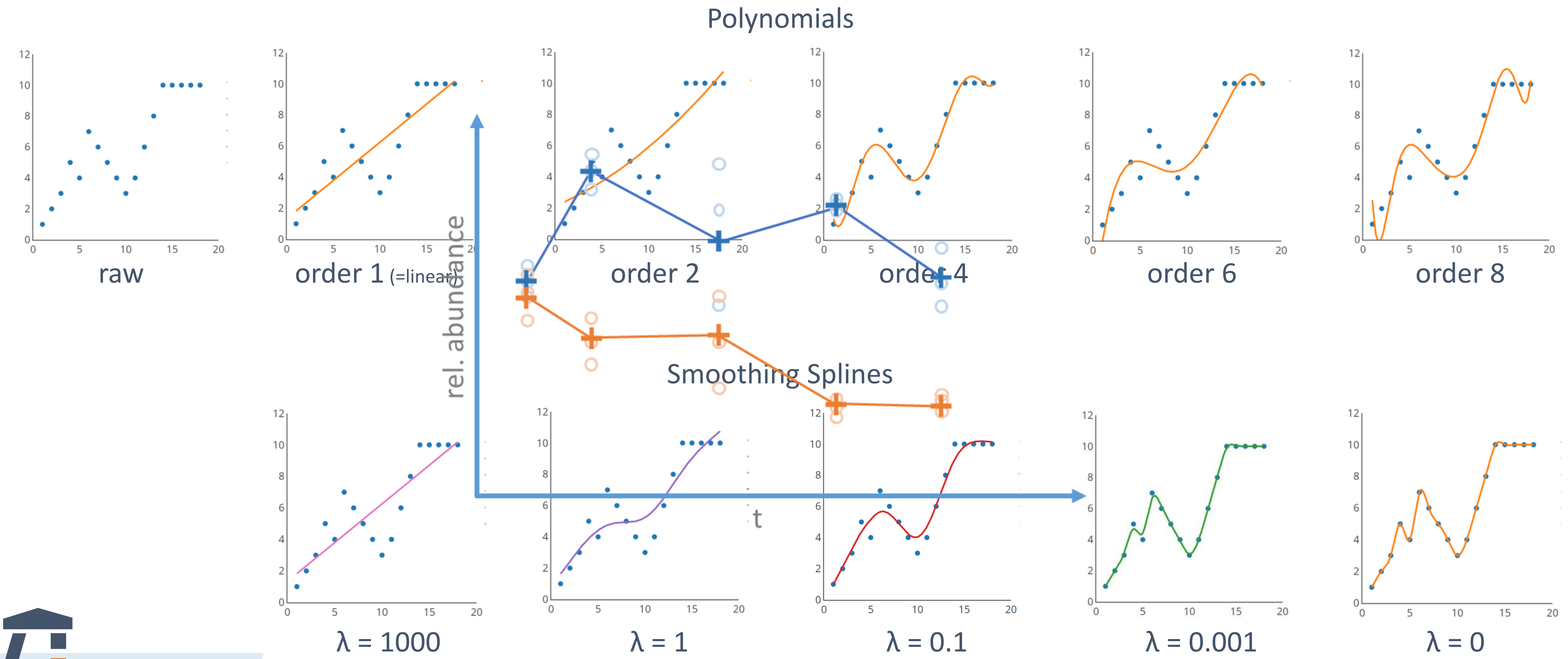
1	
2	
3	
4	
5	
6	

The most interesting features of time series are their extrema

Curve fitting possibilities



Polynomials

raw

order 1 (=linear)

order 2

order 4

order 6

order 8

Smoothing Splines

λ = 1000

λ = 1

λ = 0.1

λ = 0.001

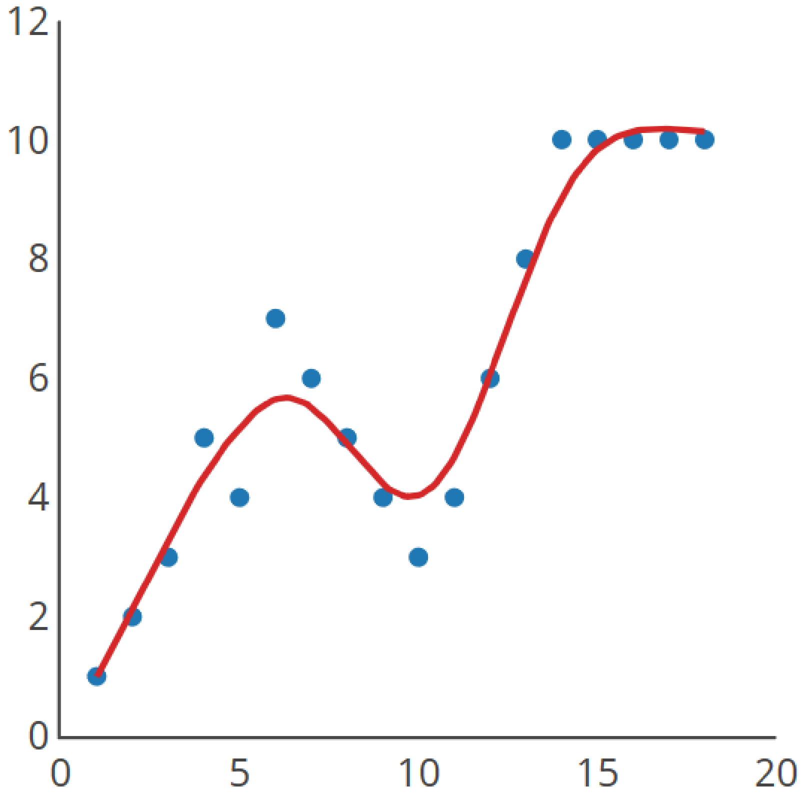
λ = 0

Smoothing splines

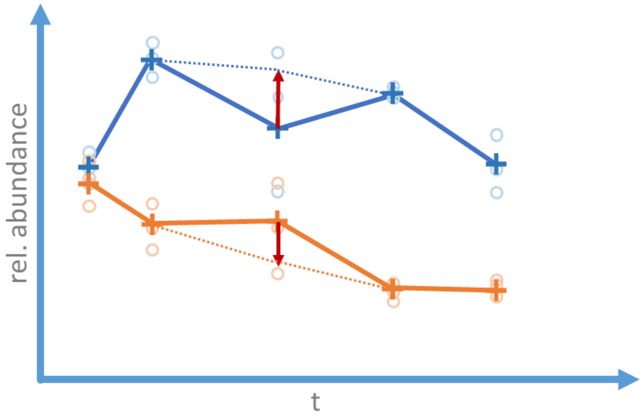
Smoothing splines:

- function formed by **connecting polynomial segments** of degree d
- function is continuous
- incorporates surrounding information

$$min \sum_{i=1}^{n} [y_i - f(x_i)]^2 + \lambda \int_0^{\infty} [f''(x)]^2 dx$$

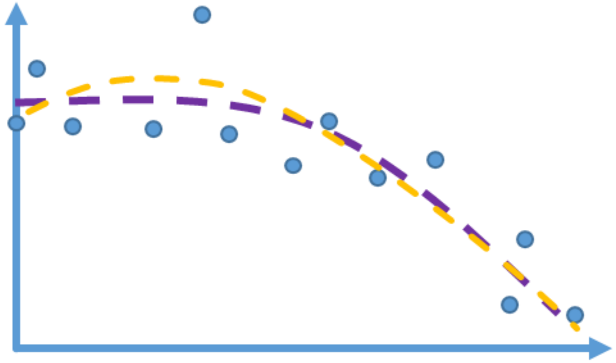


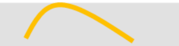
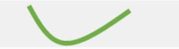
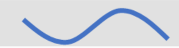
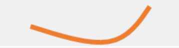
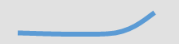
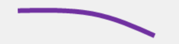
Constrained smoothing splines



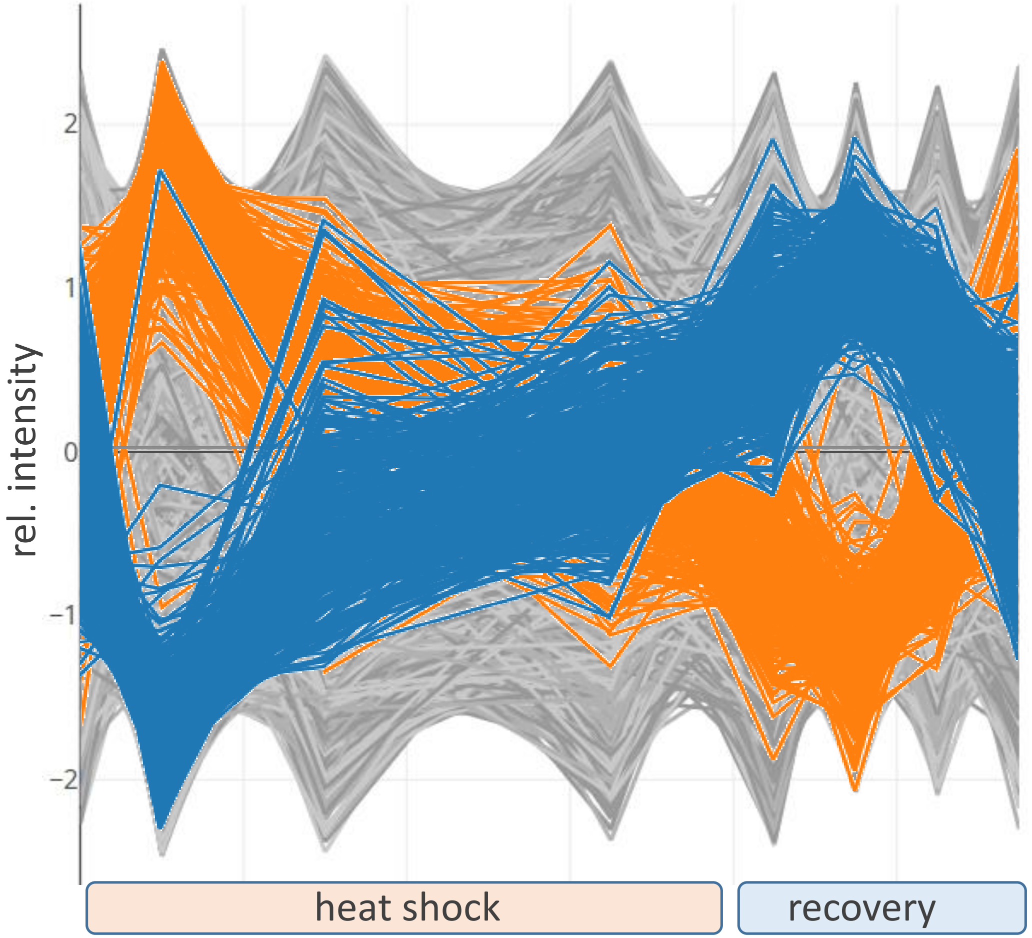
Constraints and weighting matrix:

- correct for noise derived extrema
- limit the degrees of freedom according to the system level looked at (mRNA, Proteins, …)
- choose the best fit for temporal classification



#Class	Shape
1	
2	
3	
4	
5	
6	

Experiment



- *Chlamydomonas reinhardtii*

- Heat shock experiment

  24 h heat shock + 8 h recovery

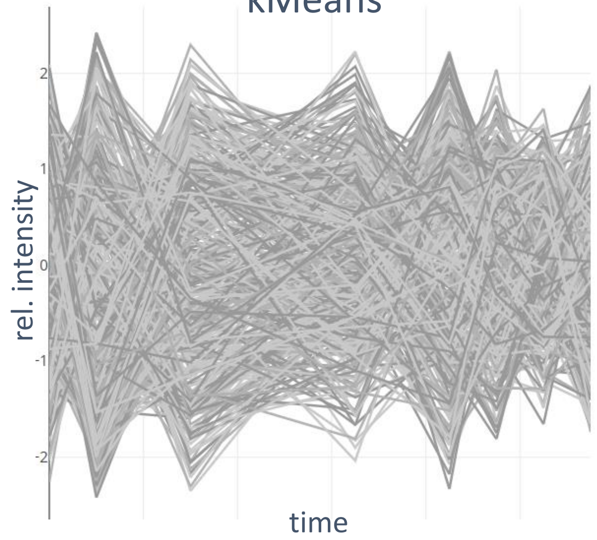
- Temporal classification method yields 45 shape classes

Clusters consist of several shapes

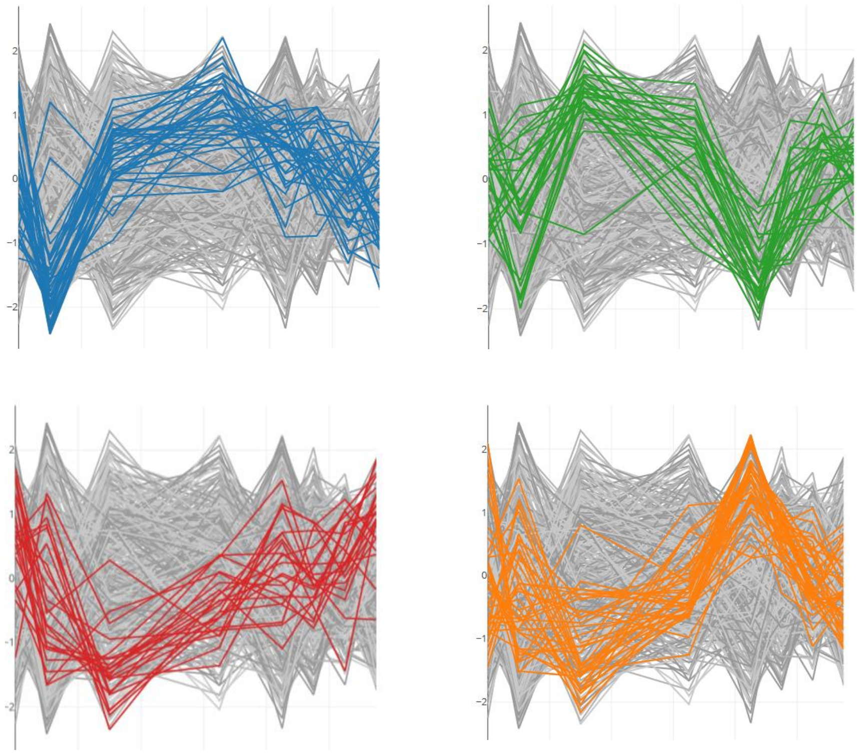
- kMeans clustering exclusively relies on distance measurements
- with temporal classification several subclasses are found within a kMeans cluster

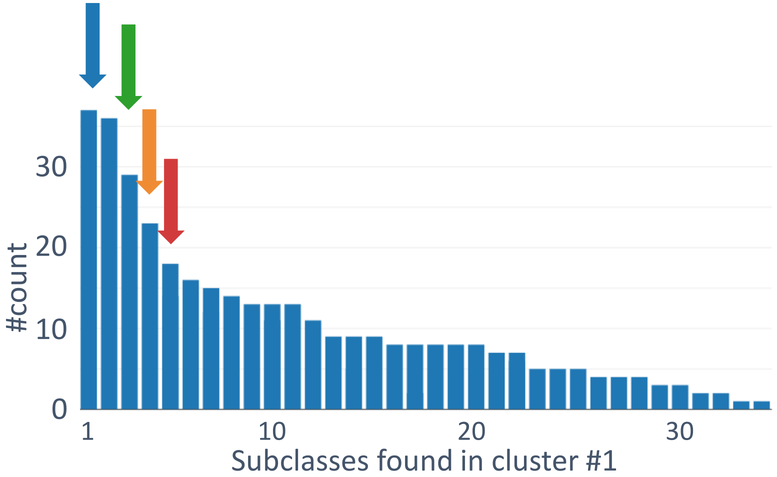
Cluster #1

kMeans

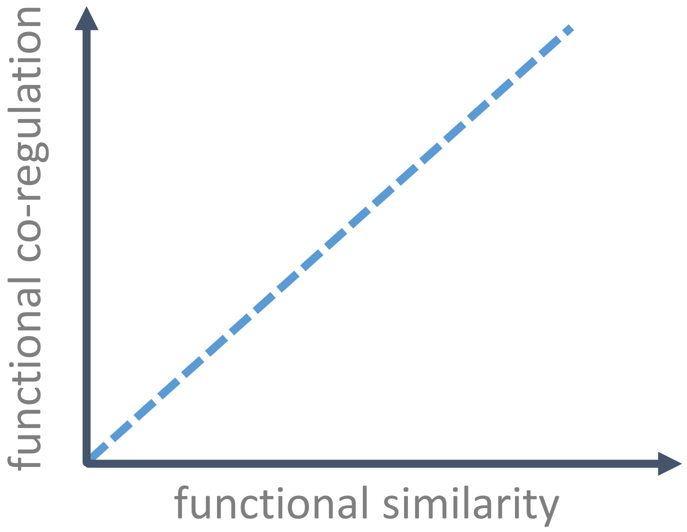


sub classes





Determination of cluster 'pureness'



Assumptions:

- co-regulation encoded in clusters
- functional similarity based on gene ontology terms

Shannon entropy:

- the probability of term *i* appearing in the stream of terms in a cluster

→ **entropy decreases with cluster pureness**

$$\mathrm{H} = -\sum_{i} p_i \cdot log_b\,(p_i) \qquad p_i = \frac{Count(Terms\ in\ cluster)}{Count(Terms\ in\ experiment)}$$

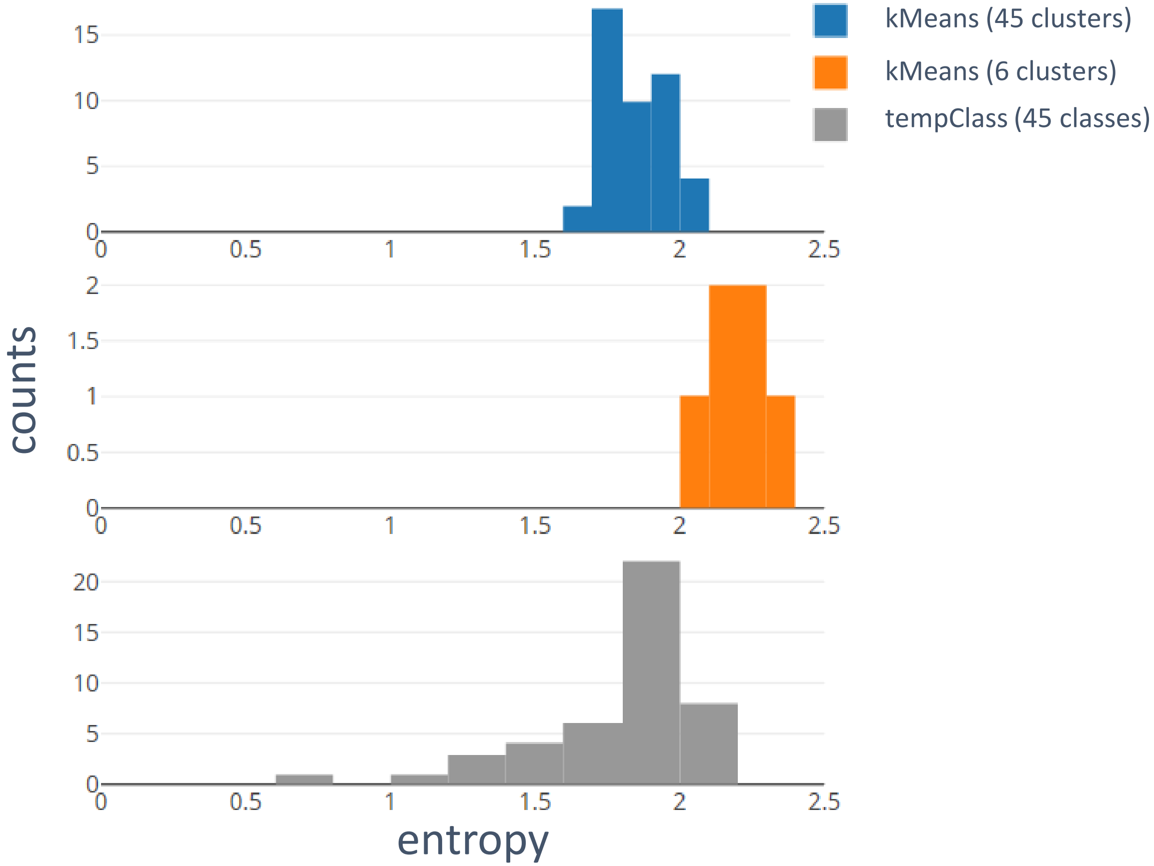
Entropy distribution of clusters/classes

- entropy decreases with cluster pureness -

kMeans vs. temporal classification entropy

- optimal cluster number was determined to be 6
  - entropy is high because of cluster heterogeneity

- 45 classes perform better than 45 kMeans clusters

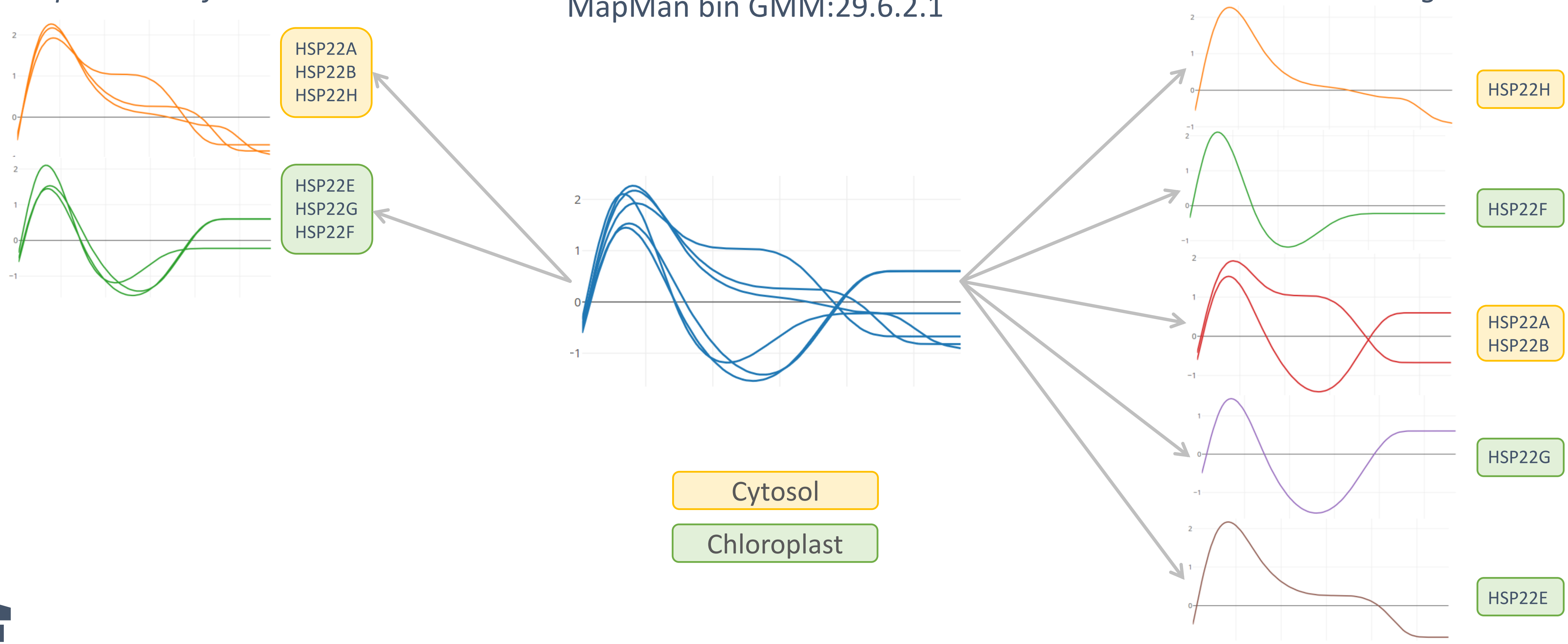


Chaperone HSP22 family

temporal classification

MapMan bin GMM:29.6.2.1

kMeans clustering



Cytosol

Chloroplast

Thank you for your attention!

Timo Mühlhaus

PhD students:

Nathan Mikhaylenko

David Zimmer

Benedikt Venn

Sabrina Gödel

master students:

Lukas Weil

Kevin Schneider

Patrick Blume

Lukas Schuck

bachelor student:

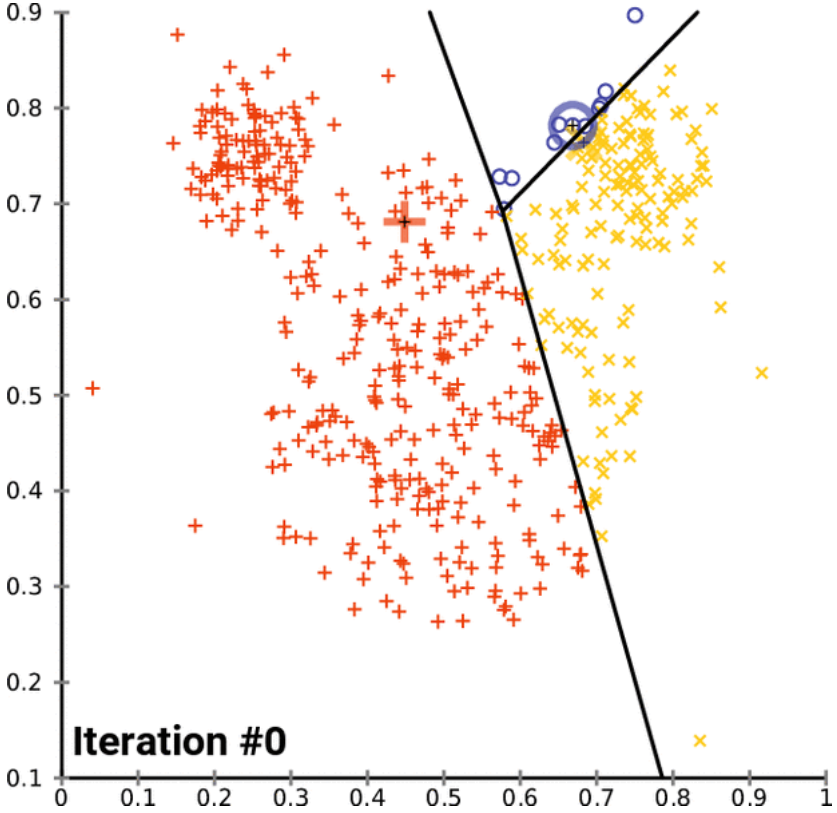
Mark Gottlieb

Thomas Leifeld (EIT, Zhang)

bioComp

TRR
175

kMeans clustering algorithm



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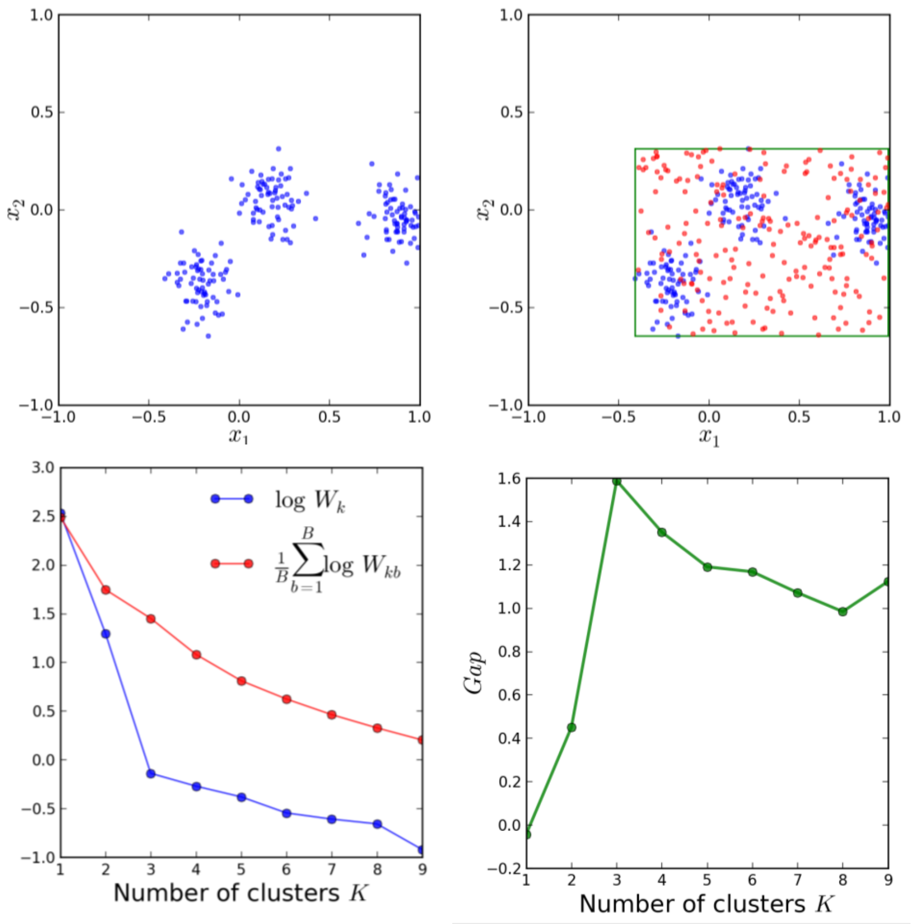
https://commons.wikimedia.org/wiki/User:Chire

1. initiate k random means
2. k clusters are created by sorting to nearest mean
3. means are shifted to new cluster centroids
4. repeat 2 and 3 until convergence has been reached

Clustering – determining optimal k



datasciencelab.wordpress.com/tag/gap-statistic/

- W_k = intra-cluster sums of squares
- Compare dispersion decline of data and random data
- Highest ‘gap’ indicates correct number of k