Biostatistics
Week 5

- Binomial test for proportion
- Terms in hypothesis tests
  - Significance level, error type I and II, rejection zone, power
- sample size calculation / power analysis
- multiple testing

  - Bonferroni correction (for << 100 tests)

  - False discovery rate, p-value histogram (for >100 tests)

Example: Does new treatment lower the relaps risk?

- **Question:** Does a new treatment lower the proportion of 35% relapses that is typical for the state-of-the-art treatment.
  Data: we observe 26 relapses among 100 treated patients

- H_0: „nothing changed“, proportion of relapses is $\pi_0 = 0.35$

- How is the number of relapses among 100 treated patients distributed und H_0?

X : Number of relapses in 100 treated patients
X~ Bin(π=0.35, n=100)

How to assess if the observation is consistent with H_0?

- Probability to observe x = 26 relapses under 100 treated patients under H_0:
- n=100, x=26, π=0.35

$$\binom{n}{x} \cdot \pi^{x} \cdot (1-\pi)^{n-x}$$

In R:

```
> dbinom(26, 100, 0.35)
[1] 0.01400205
```

- What about, if we would have observed x=25 relapses under 100 patients? That would indicate even stronger that H_0 might be wrong.
- **Question (in 2 phrasings)**
  - **What is the rejection zone for H_0?**
  - **How large is the probability to observe 26 or less relapses among 100 patients, if H_0 would be correct?**

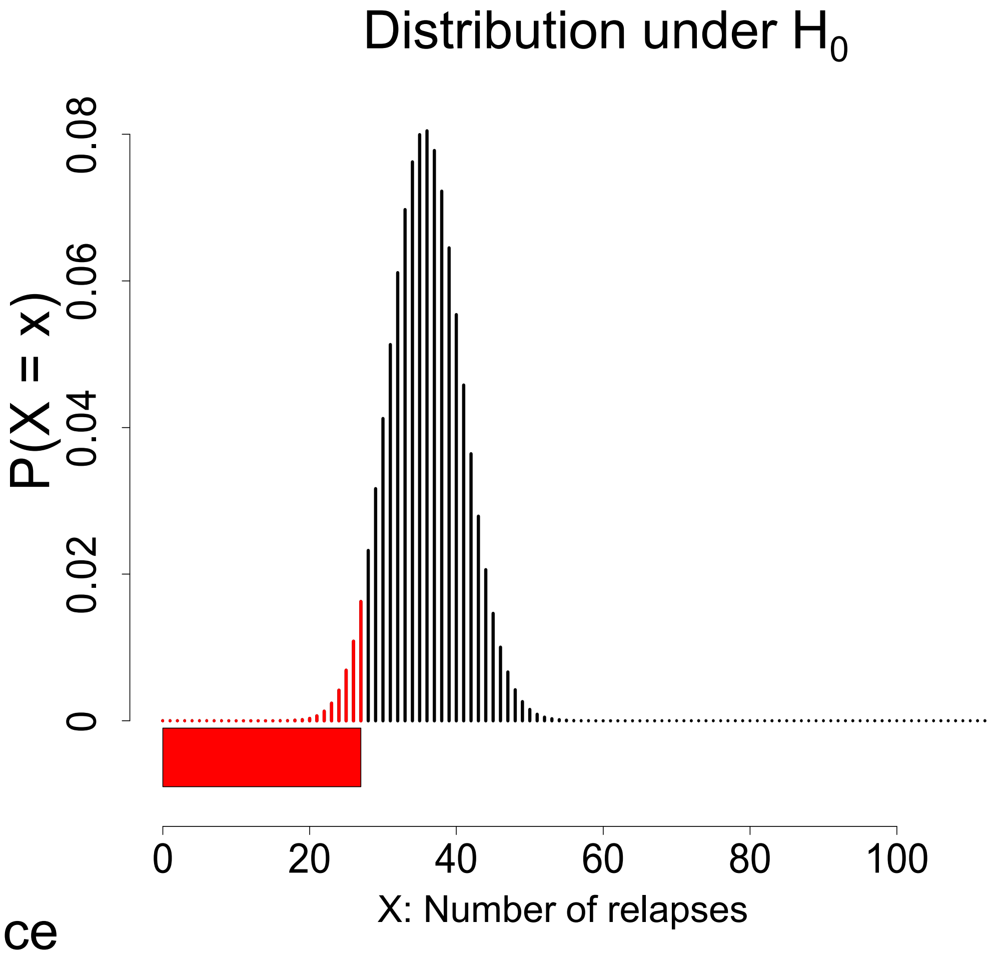
Rejection zone

Look for the maximal range from 0 to k relapses among 100 patients, until the probability of 5% (significance level α) is taken.

```
> pbinom(25:27,100,p=0.35)
[1] 0.021 0.035 0.055
```

Probabilities:
0<X≤25 0.021 # below 5%
0<X≤26 0.035 # below 5%
0<X≤27 0.055 # too large

If the observed number x is between 0 and 26, then we have enogh evidence to reject H_0.
→ Accept H_A



Note: in case of a Binomial distributed variable X, we know the distribution of X under H_0, proposing a certain π_0.
Opposed to that, if X is Normal distributed and H_0 proposed a certain μ_0, we don't know how X is distributed under H_0 because of the unknown σ which needs to be estimated; we just know that the test statistic $T = \frac{\bar{x}-\mu_0}{s/\sqrt{n}}$ is $t_{df=n-1}$ - distributed which we can use to construct from the CI for T a CI for μ.

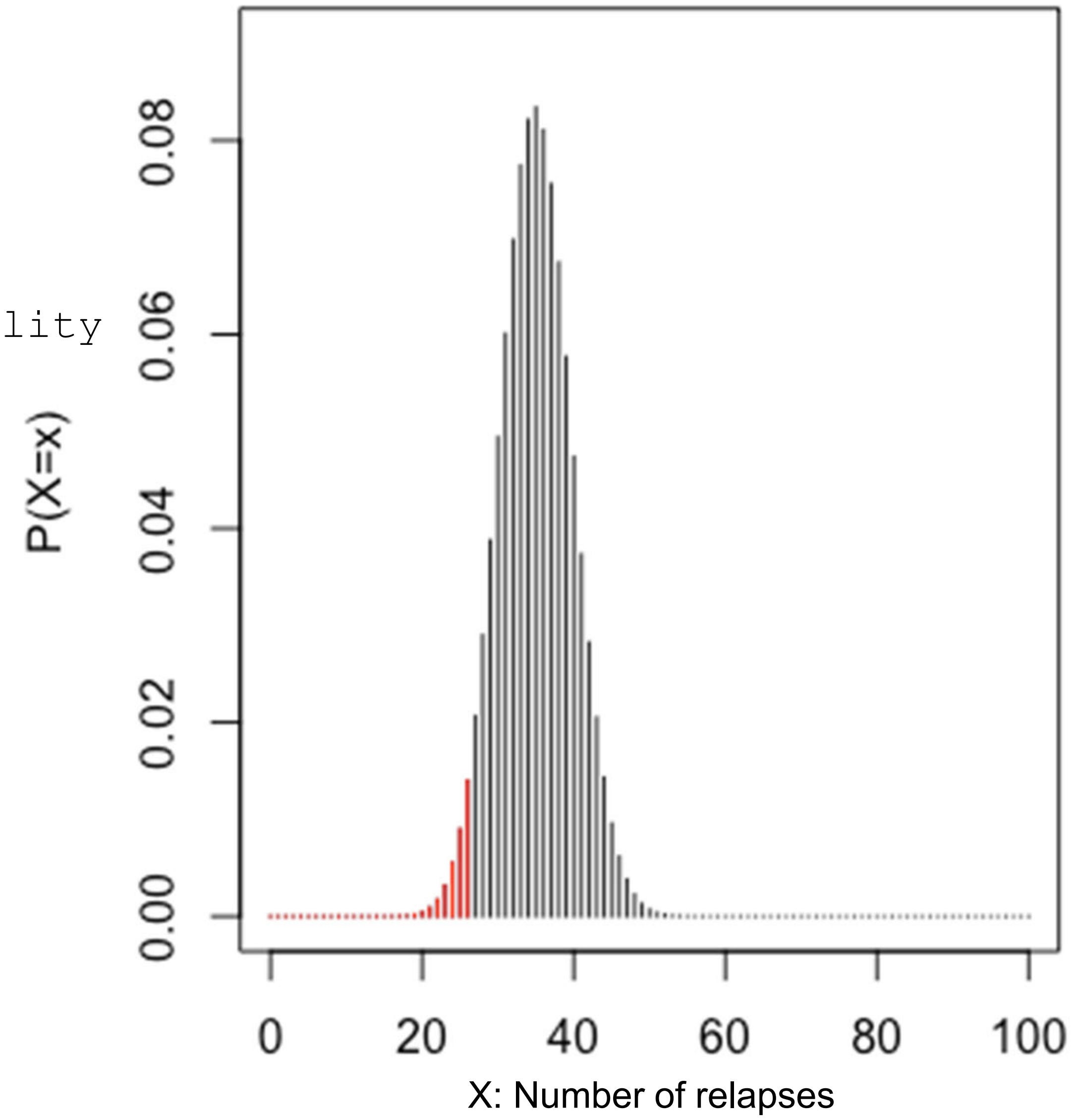
Binomial test in R

# Perform a Binomial test R:

one-sided test
(not recommended in clinical trials)

```
> binom.test(x=26,n=100,p=0.35,
                alternative="less")

          Exact binomial test

data:  26 and 100
number of successes = 26, number of
trials = 100, p-value = 0.03514
alternative hypothesis: true probability
of success is less than 0.35
95 percent confidence interval:
 0.0000000 0.3419918
sample estimates:
probability of success
                  0.26

# compute the p-valu yourself:
> pbinom(26, 100, 0.35)
> 0.0351 # „p-Wert“

# or calculate p-value via
> sum(dbinom(0:26, 100, 0.35))
> 0.0351
```

Distribution of X under H_0



Recall: Construct CI for μ from CI for T

In case of a 2-sided t-test

$$X_i \; i.d.d. \;\; \sim N(\mu,\sigma^2) \;, E(X)=\mu_x \;, \; Var(X)=\sigma_x^2$$

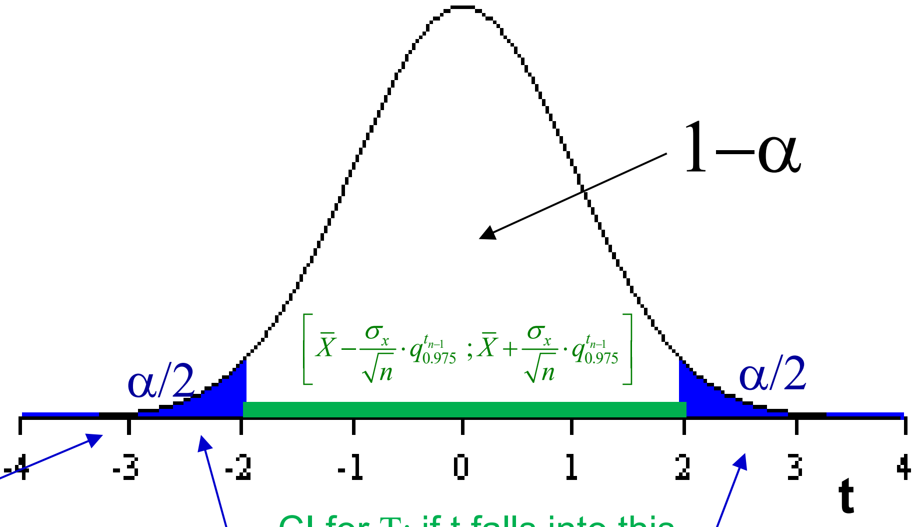
$$\Rightarrow T = \frac{\bar{X}-\mu_x}{s_x / \sqrt{n}} \sim t_{df=n-1}$$

$$P(q^t_{\frac{\alpha}{2}} \le \frac{\bar{X}-\mu_x}{\sigma_x / \sqrt{n}} \le q^t_{1-\frac{\alpha}{2}}) = 1-\alpha$$

$$P(-\frac{\sigma_x}{\sqrt{n}} \cdot q^t_{1-\frac{\alpha}{2}} \le \bar{X}-\mu_x \le \frac{\sigma_x}{\sqrt{n}} \cdot q^t_{1-\frac{\alpha}{2}}) = 1-\alpha$$

$$P(\bar{X} - \frac{\sigma_x}{\sqrt{n}} \cdot q^t_{1-\frac{\alpha}{2}} \le \mu_x \le \bar{X} + \frac{\sigma_x}{\sqrt{n}} \cdot q^t_{1-\frac{\alpha}{2}}) = 1-\alpha$$

Distribution of T (not X!) under H_0



```
> sample = rnorm(100,mean=-0.3,sd=1.2)
> t.test(sample, alternative = "two.sided",
          mu=0, conf.level = 0.95)

        One Sample t-test

data:  sample
t = -3.1524, df = 99, p-value = 0.002144
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
 -0.6206213 -0.1411401
sample estimates:
 mean of x
-0.3808807
```

$0 \notin \text{CI} \Longrightarrow \text{reject } H_0$

CI for μ

CI for T: if t falls into this region, don't reject H_0

Note: in the test output we get the CI for μ, not for T

Rejection zone: if t falls into this region, then reject H_0

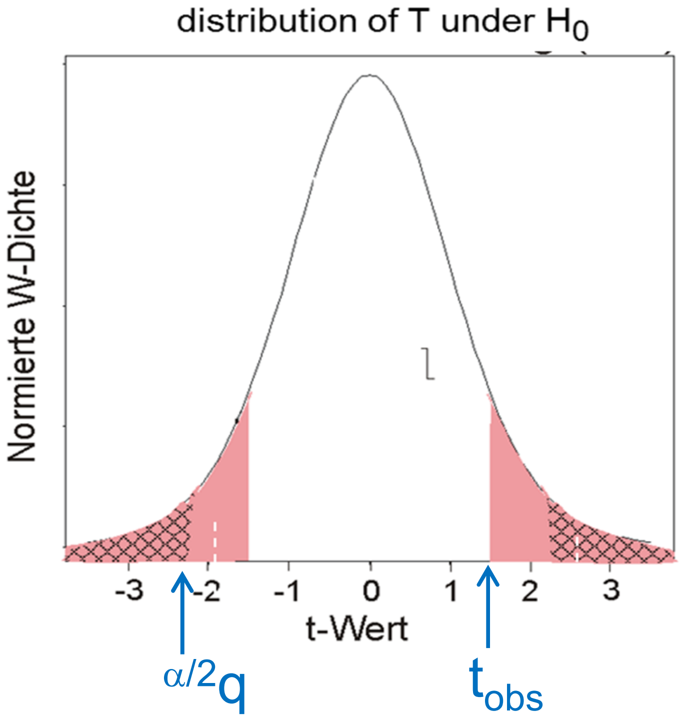
Note: this corresponds to the case where the postulated parameter μ_0 is not covered by the CI for μ,

Recall: Interpretation of the p-value

The p-value corresponds to the probability to get an at least such extreme result as the seen one if we assume that the Null-Hypothesis is valid. (Therefore we reject H_0 if this probability is small)

Graphically: the p-value corresponds to the area in the extreme tails (from the observed t-value outwards) under the density of the test-statistic distribution which is taken for a true H0.

$$T = \frac{\bar{x} - \mu}{s/\sqrt{n}} \sim t_{n-1}$$



$$p = P\left(|t| \geq |t_c| \;\middle|\; H_0 \text{ is true}\right)$$
$$= P\left(p_{new} \leq p \;\middle|\; H_0 \text{ is true}\right)$$

p-value > 0.1 : no evidence for H_A
p-value < 0.1 : weak evidence for H_A
p-value < 0.05 : evidence for H_A
p-value < 0.01 : clear evidence for H_A
p-value < 0.001 : strong evidence for H_A

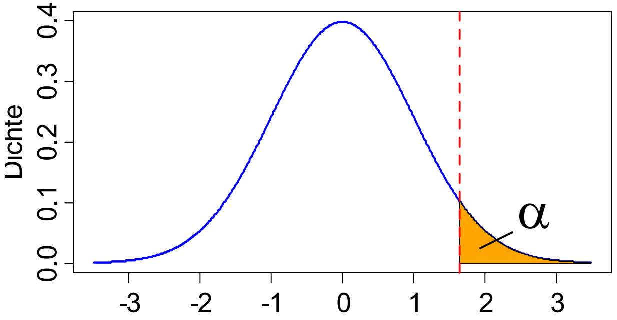
Right-, left- and two-sided Test

z.B.: $$T = \frac{\overline{x} - \mu_0}{s / \sqrt{n}} \overset{a}{\sim} N(0,1)$$

Distribution of T under H_0

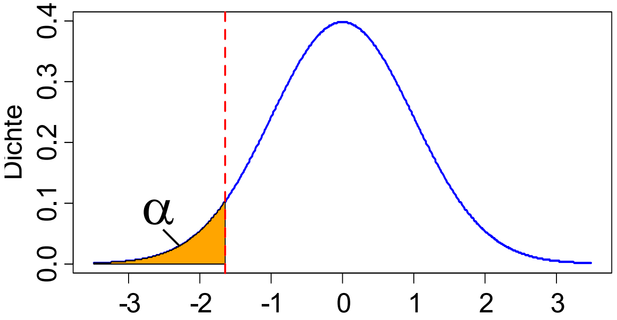
(1-α)-CI for μ

Right-sided Test:
H_A: $\mu > \mu_o$



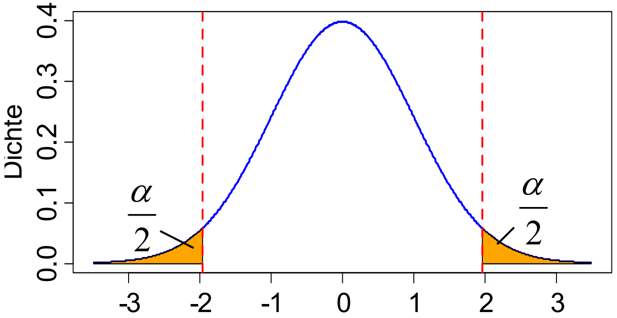
$$[\ -\infty\ ,\ q_{1-\alpha}\ ]$$

Left-sided Test:
H_A: $\mu < \mu_o$



$$[\ q_\alpha\ ,\ \infty\ ]$$

Two-sided Test:
H_A: $\mu \neq \mu_o$



$$[\ q_{\alpha/2}\ ,\ q_{(1-\alpha/2)}\ ]$$

Decision errors revisited

	negative test accepting H_0	positive test rejecting H_0
H_0 is true	**True Negative** (the probability for this correct test decision is (1-α))	**False Positive** (the probability for a type-I error is α)
H_0 is false	**False Negative** (the probability for a type-II error is β)	**True Positive** (the probability for this correct test decision is (1-β))

$P(reject\ H_0 \mid H_0\ true) = \alpha$ probability for type I error

$P(\text{accept}\ H_0 \mid H_0\ false) = \beta$ probability for type II error

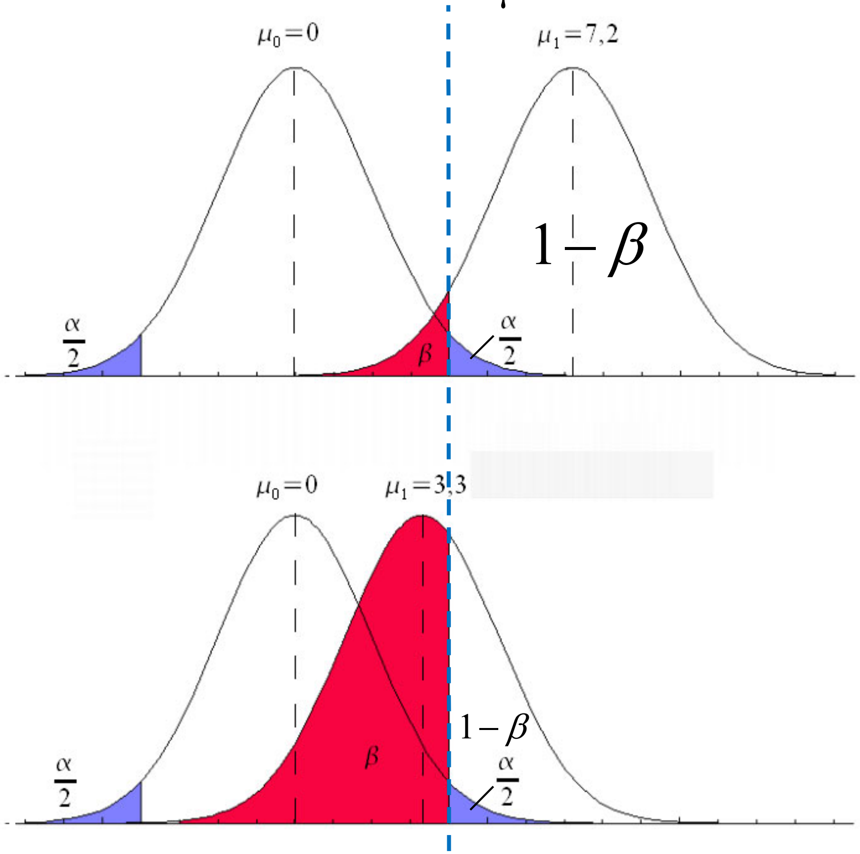
power = $1\text{-}\beta$

Known old reality

H_0: μ_0=0

H_A: μ_A≠0

Unknown new reality: μ=7.2



H_0: μ_0=0

H_A: μ_A≠0

new reality μ=3.3

Effect size = difference between H_0 and unknown new reality

Power Analysis
&
Sample Size Calculation

What is the power of a test

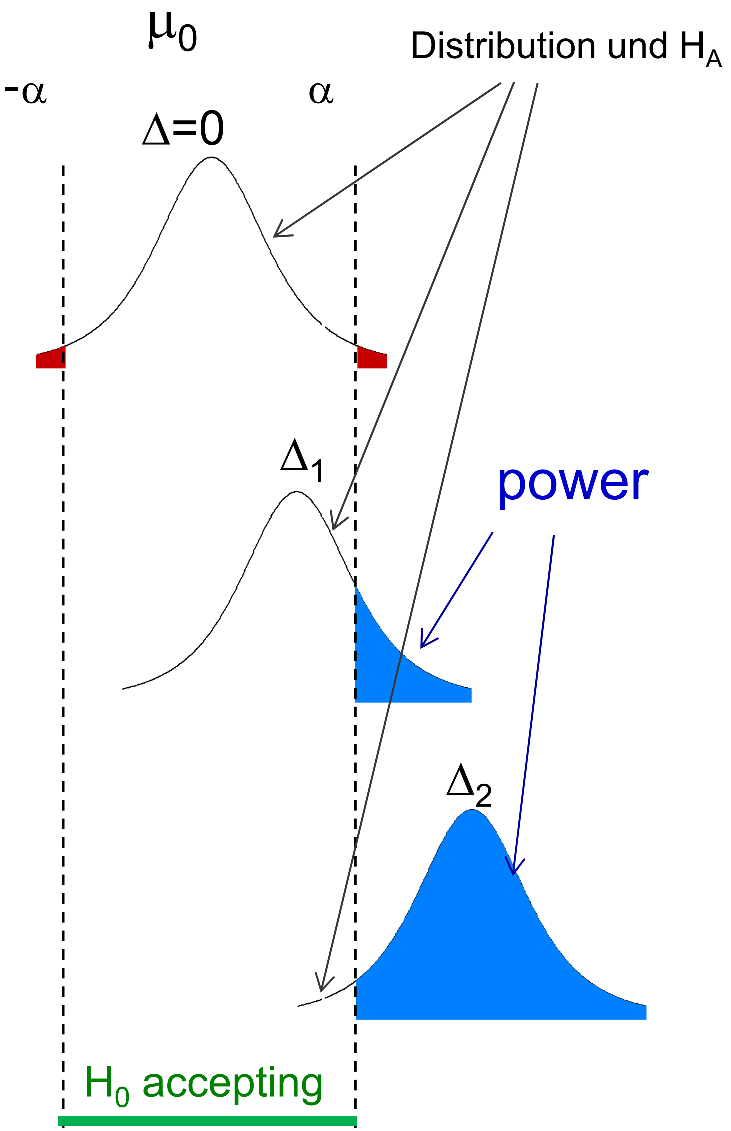
The power $(1–\beta)$, of a test is the probability to reject H_0, if H_A is true.

The power is given by the blue area.

The larger the difference D between H_0 and reality is, the larger gets the power.
However, “reality” is not known -> it is hard to estimate the power.

For a given difference D between H_0 and reality the power gets bigger if the width of the distribution of the Test-Statistic, e.g. mean, gets smaller which can be achieved by increasing the sample size.

Since the reality can not be changed,
in praxis the only way to increase the power is to increase the sample size.



Sample size calculation

Situation:

Your group has developed a new drug for sleeping time elongation.

The new drug is only interesting if its sleeping time elongation surpasses the one from the «golden standard» by at leas 1h (relevant effect).

From a pilot study we know the standard deviation of the sleeping time in individual patients is: sd=1 (1h).

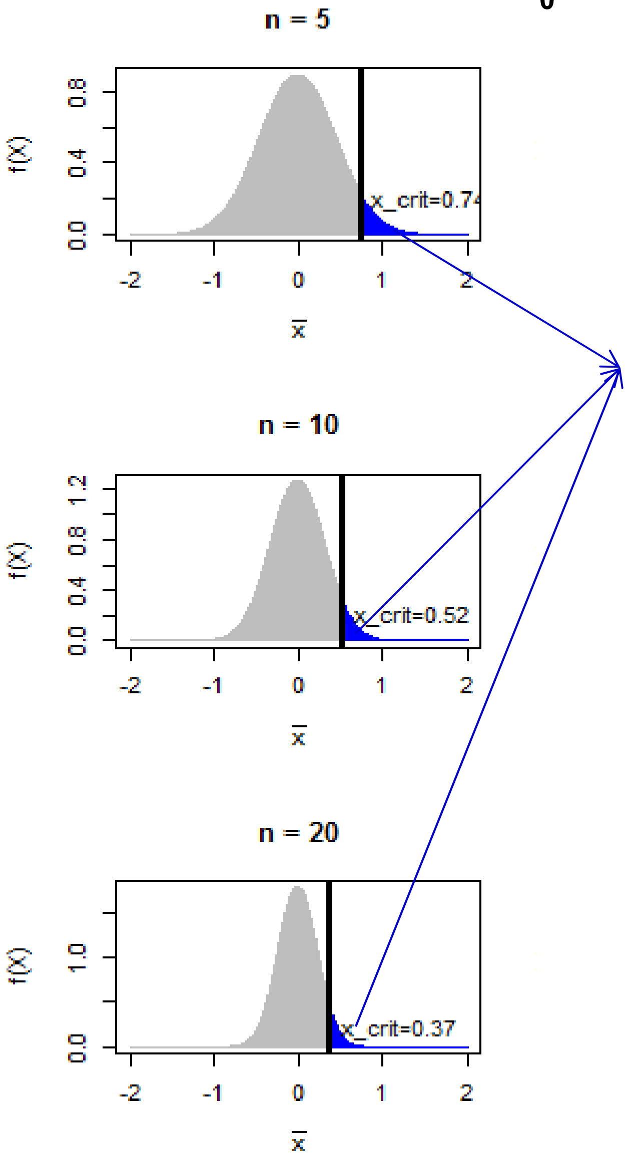
Given your drug surpasses the old drug by a mean sleeping time extension of 1h - how big should the sample size be chosen, so that you have a power of 80% and simultaneously an α=5% that your test rejects the H_0 and proves the superiority of the new drug?

Simulation with n=5,10,20

X: Sleep elongation compared the golden standard drug

H_0: $E(X)_0=\mu_{\Delta 0}=0$

Distribution of T under H_0



$\alpha = 5\%$ is fixed

With a sample of size 5 we would reject H_0, if
$\bar{x} > 0.74$

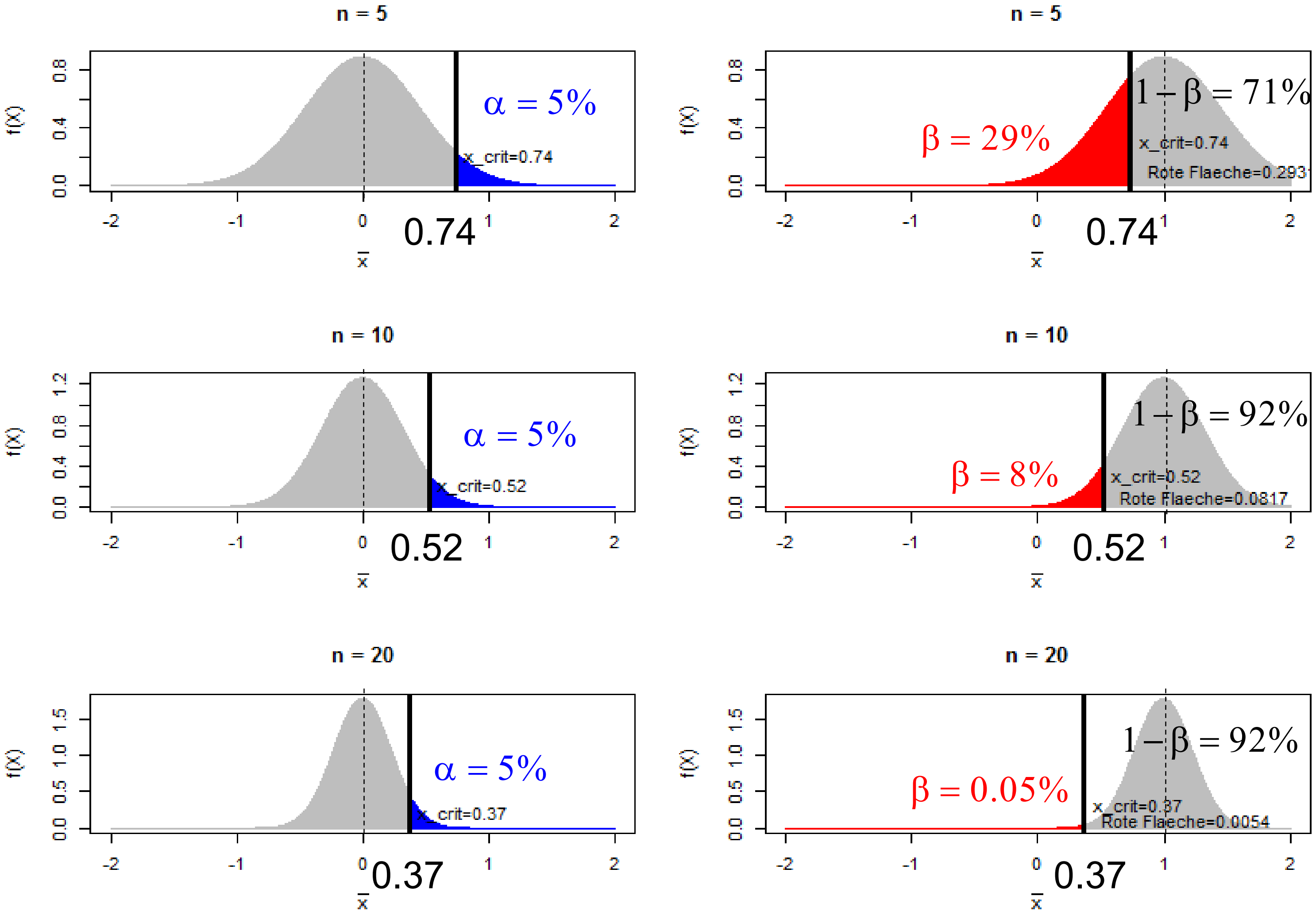
With a sample of size 10 we would reject H_0,
if
$\bar{x} > 0.52$

With a sample of size 20 we would reject H_0,
if
$\bar{x} > 0.37$

Simulation with n=5,10,20

X: Sleep elongation compared the golden standard drug
H_0: $E(X)_0=\mu_{\Delta 0}=0$
H_A: $\mu_\Delta=1$

Distribution of T under H_0 | **Distribution of T given $\mu_\Delta=1$**

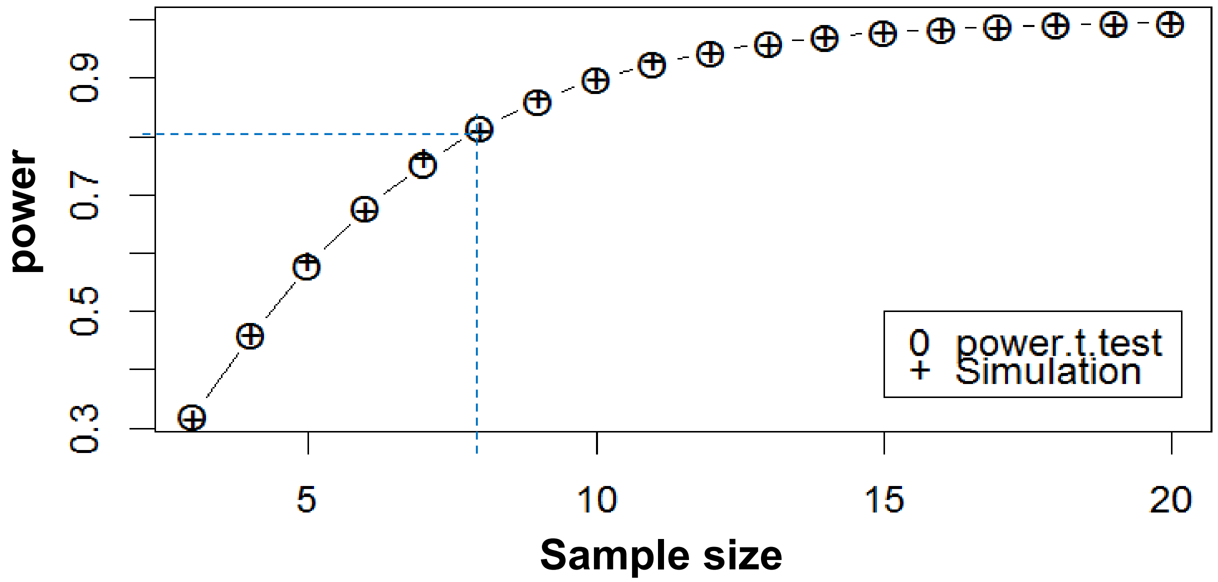


n=5: Reject H_0 in 71% of all simulation runs
Power=1-β=71%

n=10: Reject H_0 in 92% of all simulation runs
Power=1-β=92%

n=20: Reject H_0 in 99.5% of all simulation runs
Power=1-β=99.5%

Results



```
 n       power.simu
 3           0.3171
 4           0.4631
 5           0.5880
 6           0.6737
 7           0.7652
 8           0.8118
 9           0.8685
10           0.9001
11           0.9308
12           0.9452
13           0.9579
14           0.9712
15           0.9803
16           0.9862
17           0.9893
18           0.9926
19           0.9939
20           0.9960
```

```
> power.t.test(power=0.8, delta=1, sd=1, sig.level=0.05,
               alternative="one.sided",type="one.sample")
```

```
     One-sample t test power calculation 

              n = 7.727622            NOTE: n is number in *each* group
          delta = 1
             sd = 1
      sig.level = 0.05
          power = 0.8
    alternative = one.sided
```

How to plan the size of a study?

- Choose the test you want to use in your analysis
- Determine/Estimate the variation of the observations
- fix significance level α (the accepted risk for a type-1-error, typically 5%)
- Fix relevant effect size (the minimal effect which is still relevant)
- Fix the power which gives the probability to detect an relevant effect (typically 80%)
  - Choose 1-β

Perform a sample-size calculation to derive the needed sample size at which the required power is given.

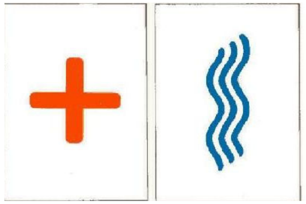
Good webpage for sample size calculations – menu based, but shows corresponding R-code:

http://powerandsamplesize.com/Calculators/

Multiple testing: Rhine Paradox

- The parapsychologist **Joseph Rhine** hypothesized in the 1950’s that some people had *Extra-Sensory Perception* (*ESP*).
- He tested for ESP by an experiment where people were asked to guess the color of 10 hidden cards:

red or blue.



- He discovered that almost 1 in 1000 had ESP – they were able to get all 10 right. **Surprised???**

$$P(10 \text{ correct answers} \mid \text{just guessing}) = \frac{1}{2}\cdot\frac{1}{2}\cdot\frac{1}{2}\cdot\frac{1}{2}\cdot\frac{1}{2}\cdot\frac{1}{2}\cdot\frac{1}{2}\cdot\frac{1}{2}\cdot\frac{1}{2}\cdot\frac{1}{2} = \left(\frac{1}{2}\right)^{10} = \frac{1}{2^{10}} = \frac{1}{1024}$$

No, 1 in 1024 is what we would expect to get by chance if everybody is just guessing

Multiple testing: Rhine Paradox

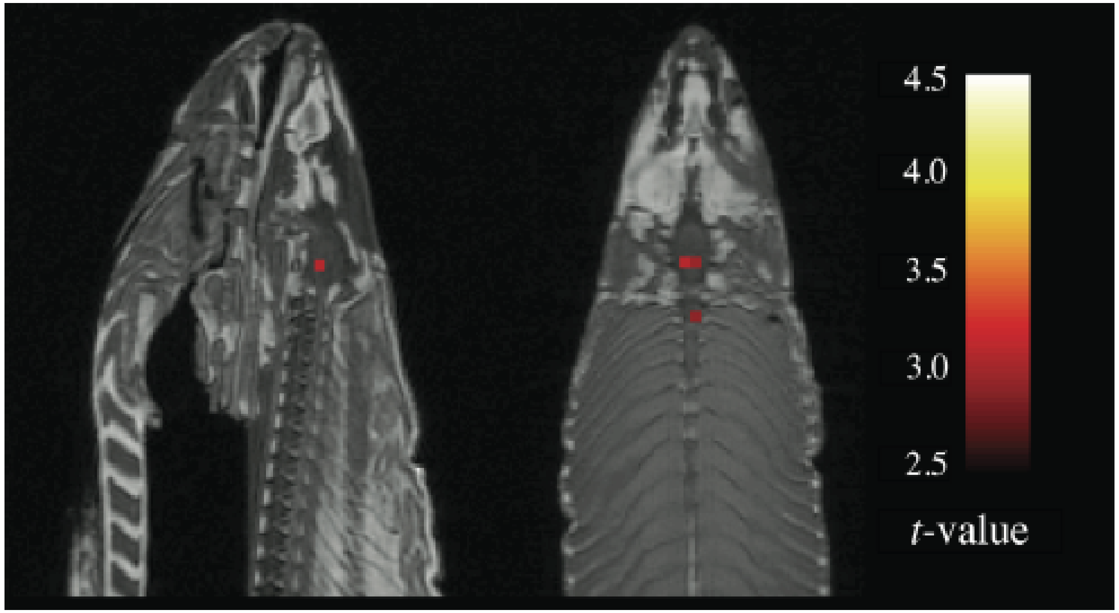
- He told these people they had ESP and called them in for another test of the same type.
- He discovered that all of them had lost their ESP.
- **What did he conclude???**

Multiple testing: Rhine Paradox

He concluded that you shouldn’t tell people that they have ESP, because it causes them to loose it.

☺

fMRI revealed brain response to trans-species emotional stimuli in a dead salmon



A dead salmon was repeatedly confronted with 2 different human emotional stimuli.

Out of 8064 brain voxels in 16 voxels a significant different activity ($p \leq 0.001$!) was observed

This study received 2012 the IG nobel price (for *ignoble,* improbable research).

Source: http://www.guardian.co.uk/science/2012/sep/21/ig-nobel-awards-dead-salmon

The probability to get by chance a significant test result

The risk to get in **one test** an false positive result (that is $p<\alpha$ under H0) is only

$$P(reject\ H_0 \mid H_0\ true) = \alpha$$

$$P(accept\ H_0 \mid H_0\ true) = 1-\alpha$$

Assume n independent test‘s (with n independent samples) where the null-hypothesis H_0 is always valid (no effect nowhere)

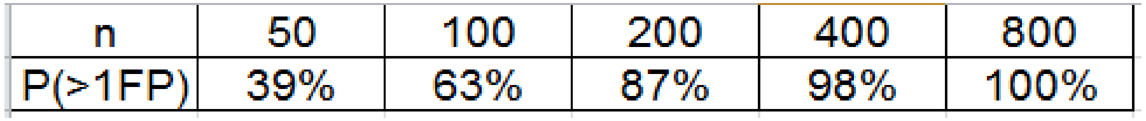
– the probability draw always the right test decision is:

$$P(accept\ n-times\ H_0 \mid H_0\ true) = (1-\alpha)^n$$

– the probability of coming up with at least 1 false positive effect is:

$$P(\geq 1\ rejecting\ H_0 \mid H_0\ true) = 1-(1-\alpha)^n$$

the probability of making at least 1 type one error at **n trials**, when α = 0.01%



n	50	100	200	400	800
P(>1FP)	39%	63%	87%	98%	100%

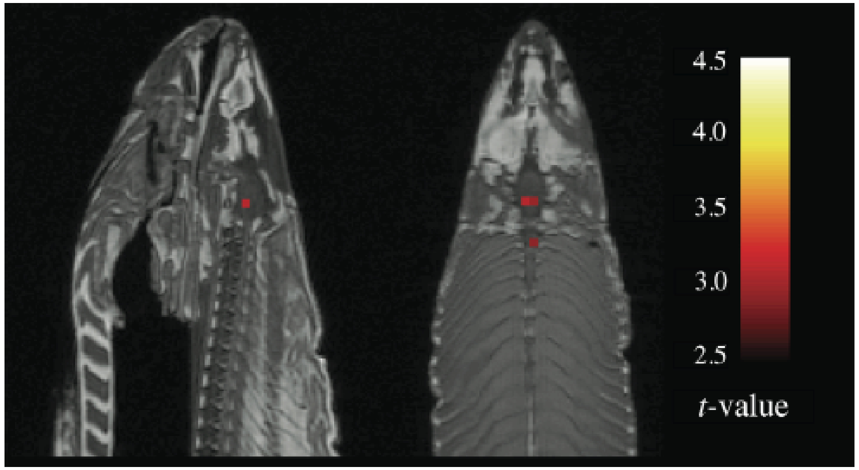
Bonferroni correction for multiple testing and its effect on the dead salmons reaction

Bonferroni: when performing n independentl tests, conduct each test at significance level $\frac{\alpha}{n}$!

When applying Bonferronis rule, we only have a risk of α, to come up with ≥ 1 false positive effects (that is a significant test result although H_0 is true).

No brain region of the dead salmon showed a significant reaction to smiling people after Bonferroni correction.

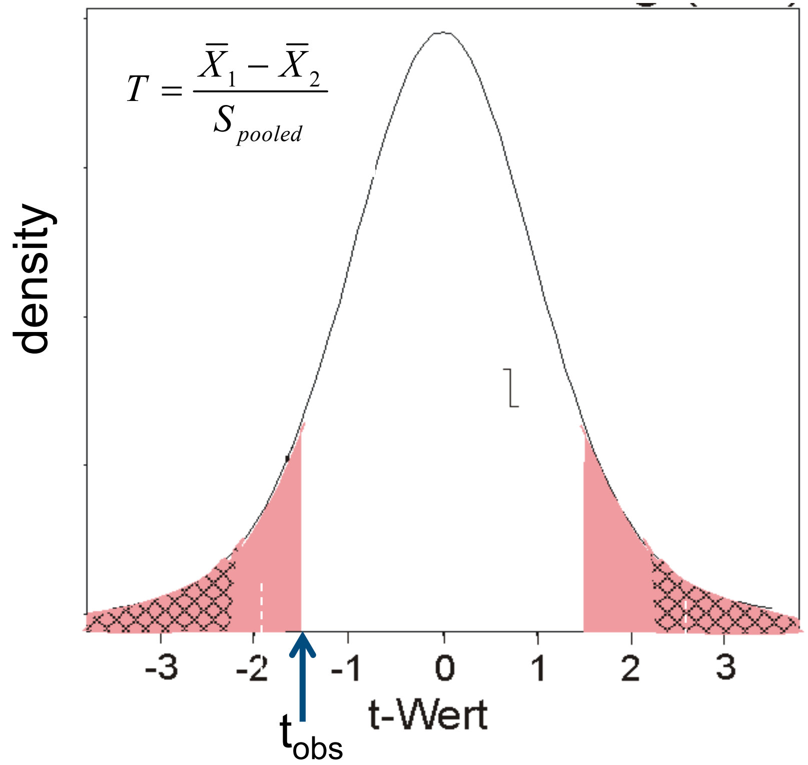
☺



The p-value is uniformly distrubuted under H_0

The p-value corresponds to the probability to get an at least such extreme result as the observed result assuming that the Null-Hypothesis is valid
-> the p-value corresponds to the area in the extreme tails

distribution of T **under H_0**



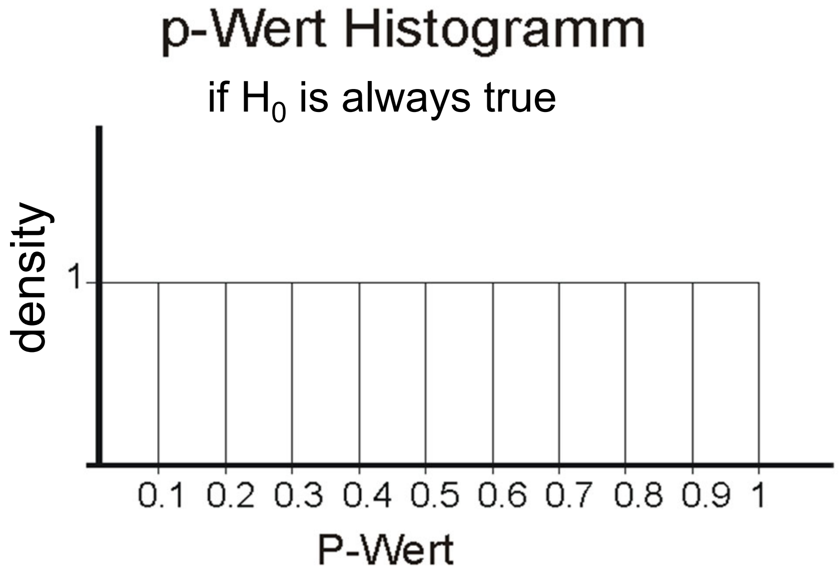
$$p = Prob(\,|t| > |t_{obs}|\;|\;H_0\ true\,)$$
$$= Prob(\,|p| \le |p_{obs}|\;|\;H_0\ true\,)$$

Given H_0 is true in all tests:

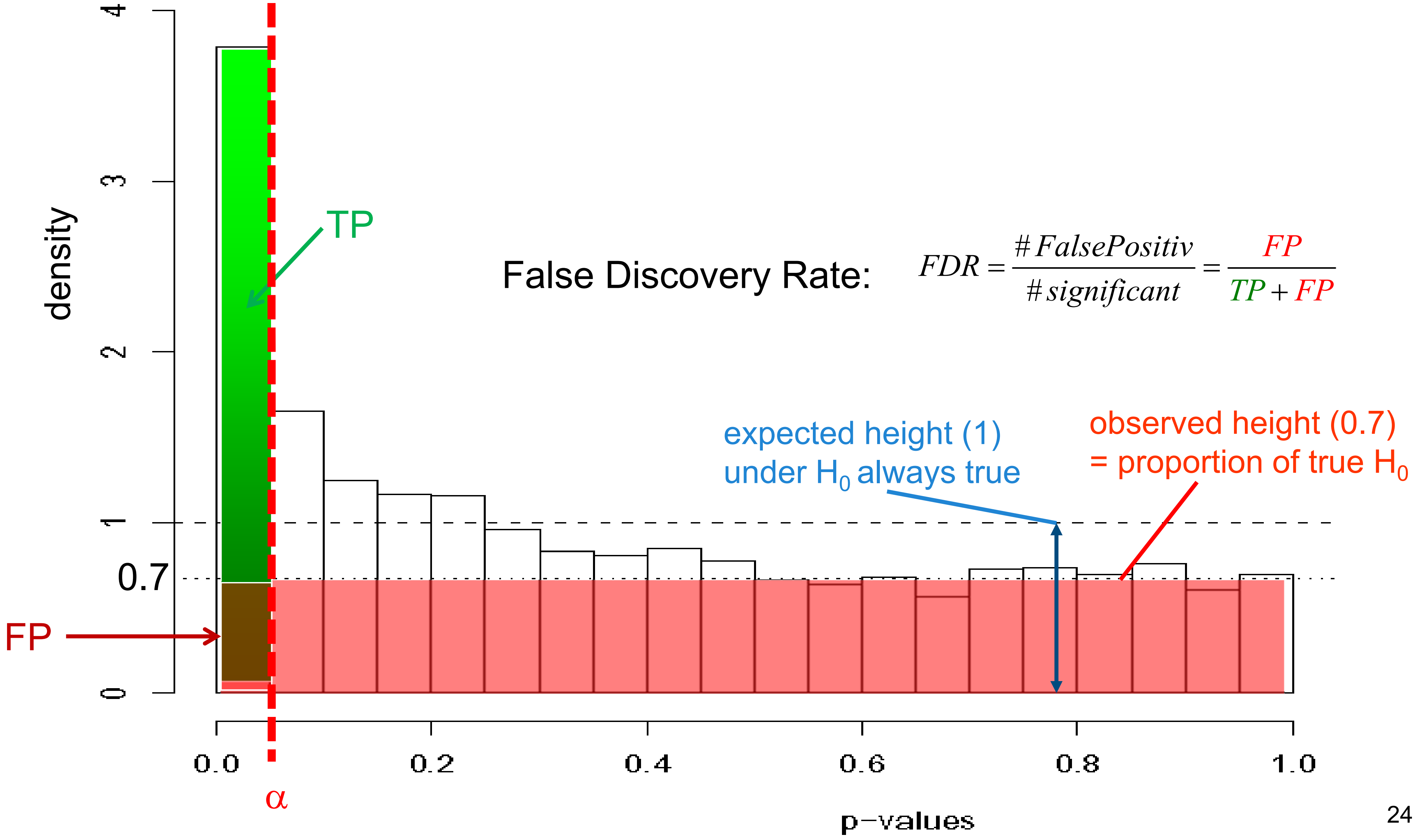
p=0.1: 10% of all tests get a p-value≤0.1 if H_0 is always true

p=0.2: 20% of all tests get a p-value≤0.2 if H_0 is always true

…



How to estimate the ratio p_0 of truly null voxels? How to estimate the false discovery rate?



False Discovery Rate:

$$FDR = \frac{\# FalsePositiv}{\# significant} = \frac{FP}{TP + FP}$$

expected height (1) under H_0 always true

observed height (0.7) = proportion of true H_0

Judging a p-value histogram

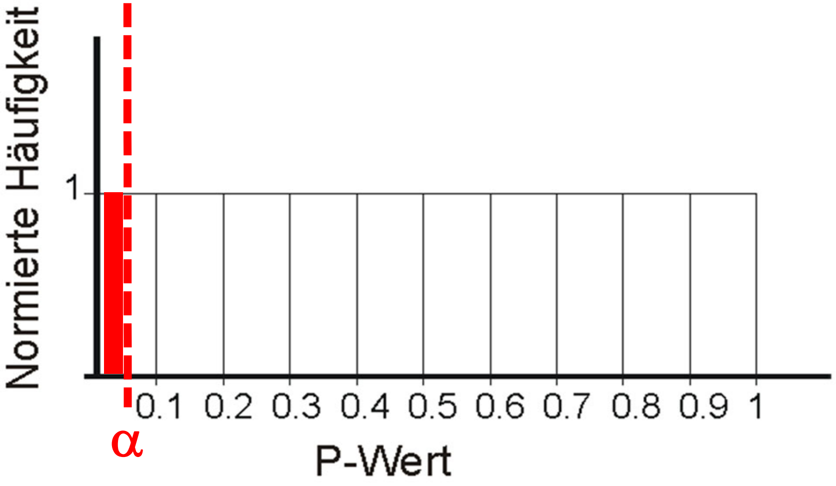
The p-value histogram helps to judge the results from many *independent* tests

Flat is Bad!

For all tests n independent H_0 is true

~ 100% of all significant findings are false positive.

(see proportion of red area in left bar).

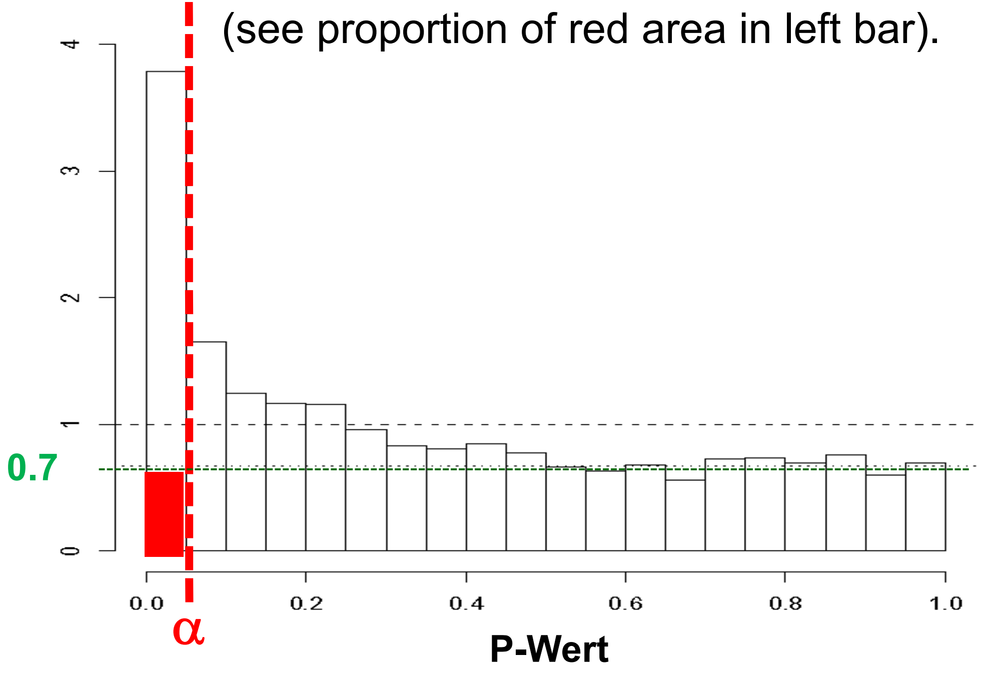


The peak we seak!

For 70% of all tests H_0 is true.

Only ~20% of all significant findings are false positive

(see proportion of red area in left bar).



This method was proposed from John Storey – see http://www.pnas.org/content/100/16/9440.full

Take home message
Multiple Testing

It is tempting, but not o.k. to forget about all non-significant tests and just publish the significant effects.

You **need to take account for the multiple testing**
- by p-value correction such as Bonferroni correction or
- using other measures like FDR or
- confirm the “found effect” in a new control experiment.