Introduction into Biostatistics

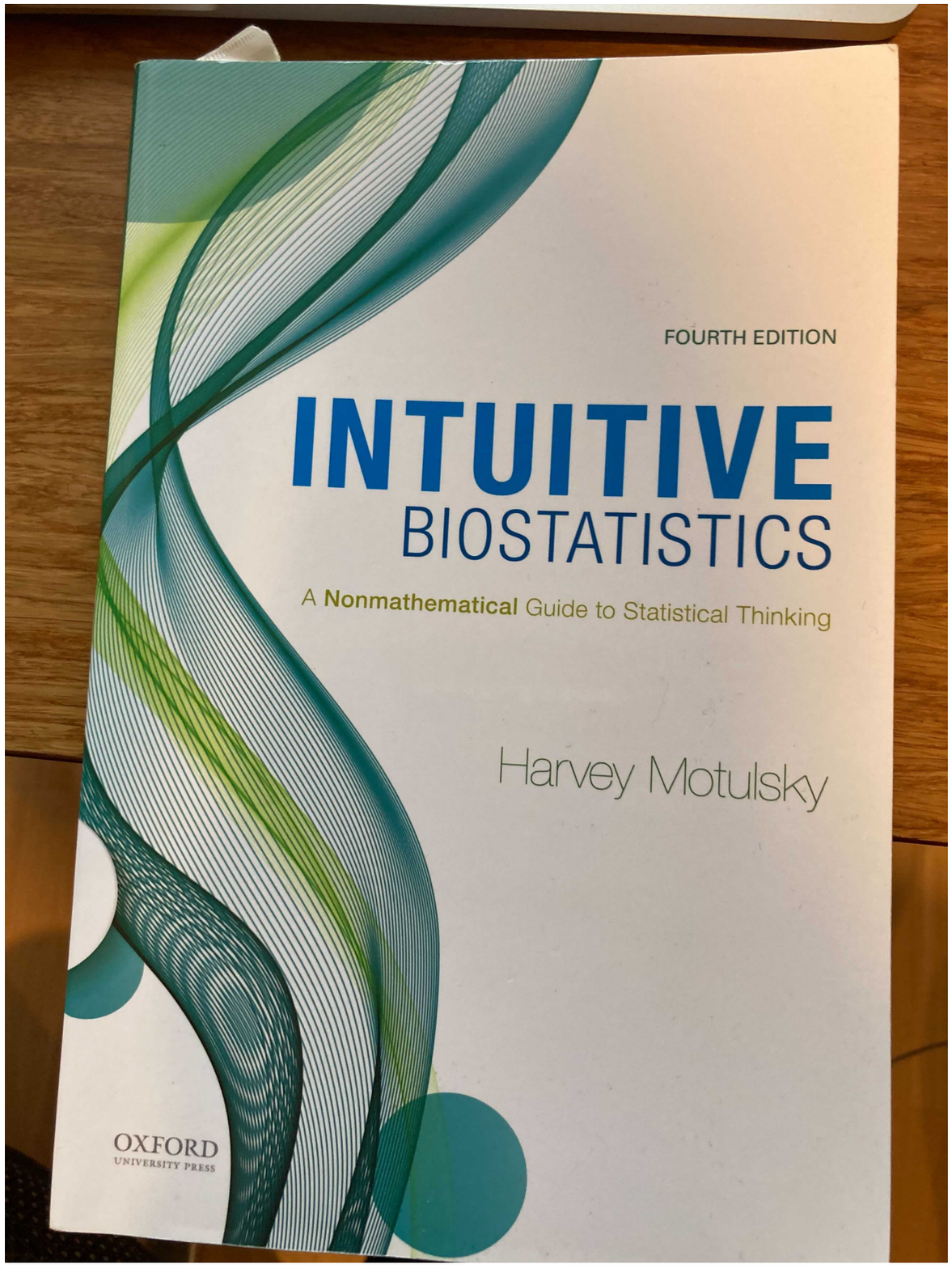
Anna Poetsch, Biotechnology Center, TU Dresden

Organisation

- 16.5. Introduction to biostatistics
- **14.6. Hypothesis testing**
- 21.6. Multiple comparisons and correlations
- 28.6. Big data, clustering, dimensionality reduction

by Melissa Sanabria

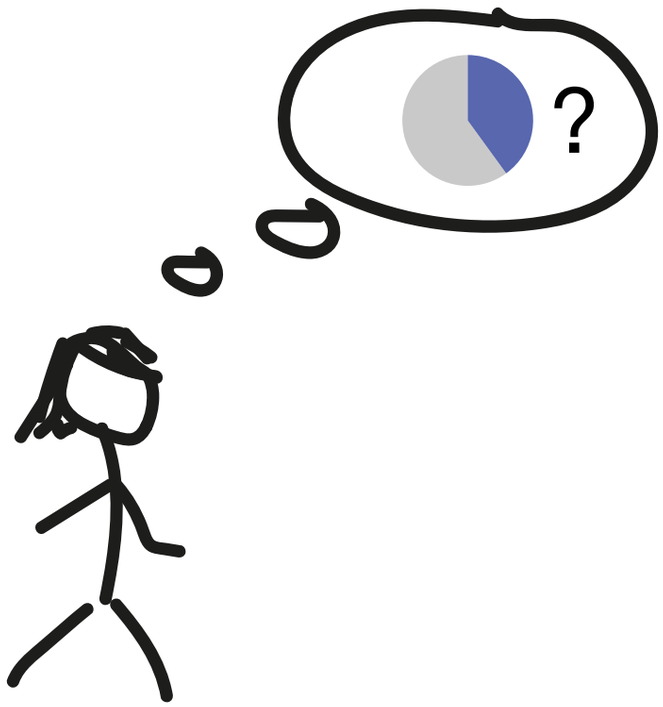
Sources

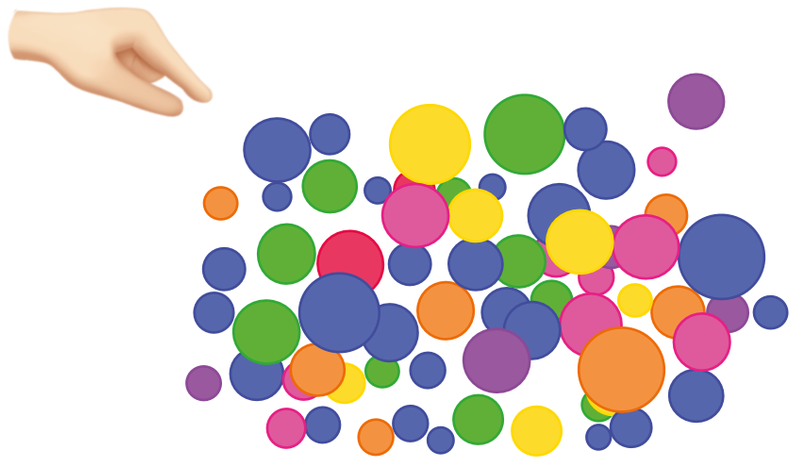


& “the internet”

Recap on confidence of one probability measurement

- A random (or representative) sample!
- They are independent observations!
- The data are accurate!





Recap on confidence of one probability measurement

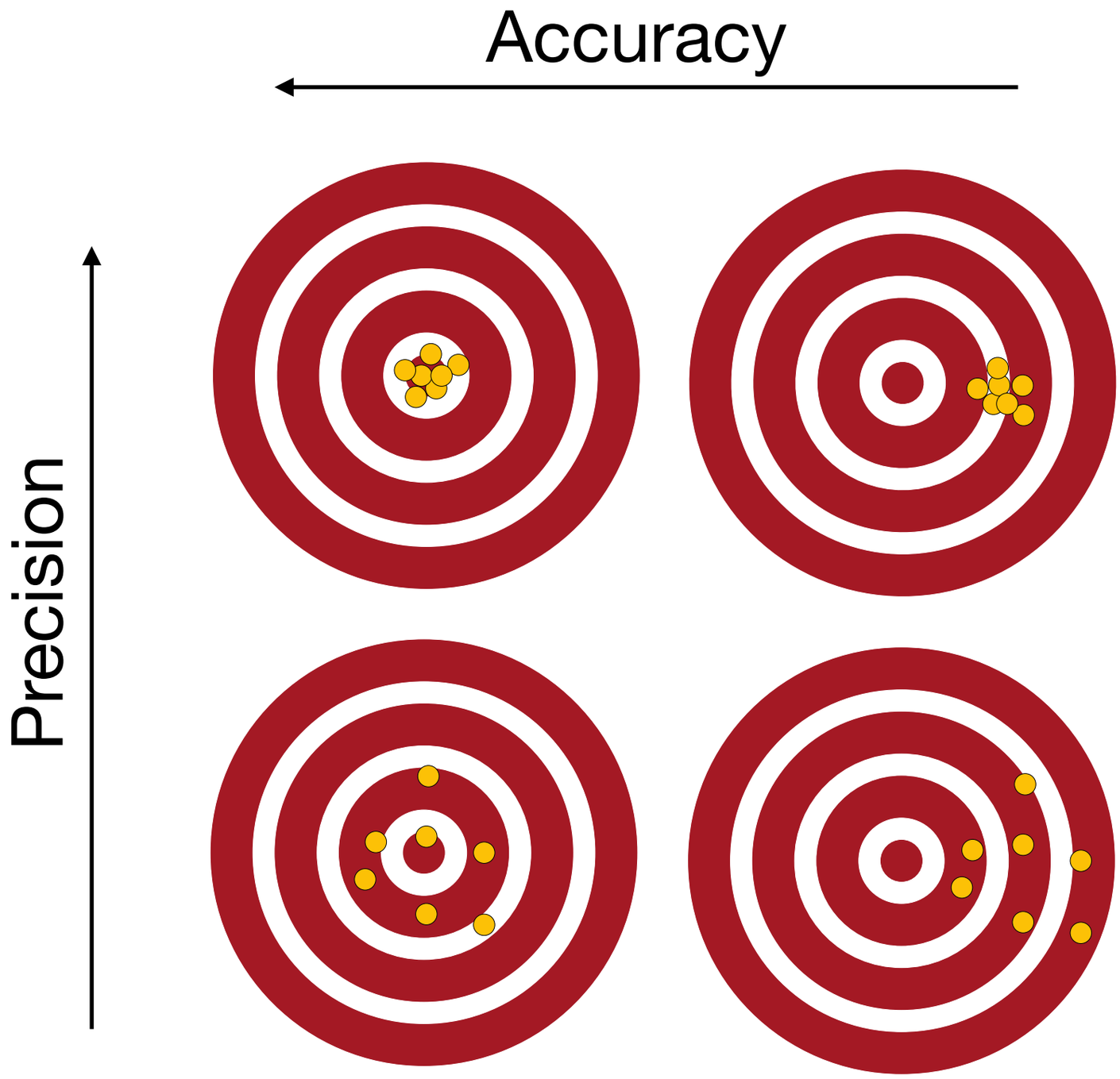
Please answer in the chat:

Does the confidence interval get bigger, if you…

… increase n?

… increase the confidence level, e.g. from 95% to 99%?

Recap on accuracy and precision

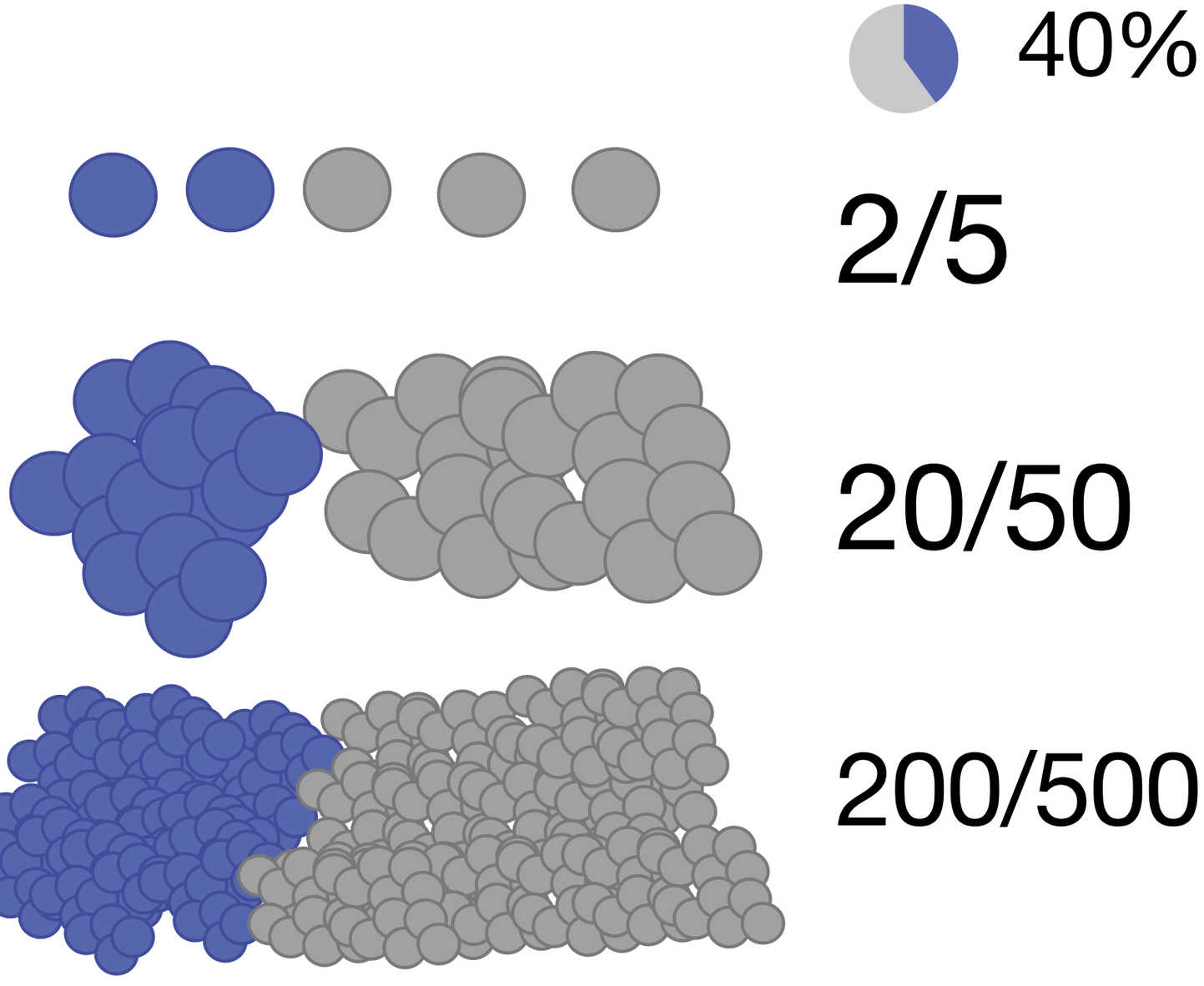


How do these relate to confidence intervals?

Does the confidence interval get bigger, if you increase n?

Know your problem, know your distribution!

Binomial



40%

2/5

20/50

200/500

Confidence increases with n

Normal

Confidence does not increase with n

Descriptive statistics

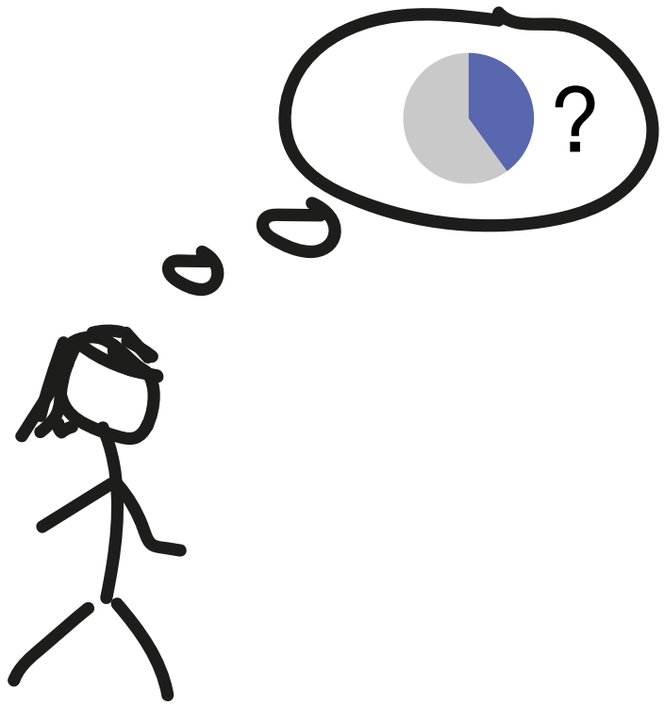
Data types and distributions

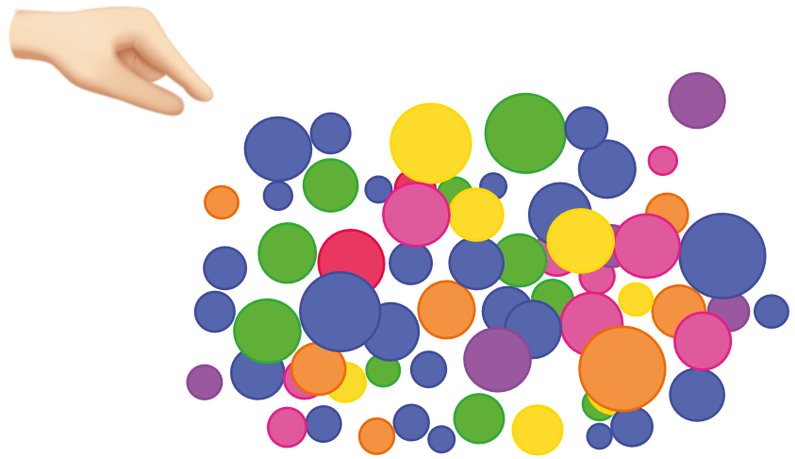
- Probability data (binomial distribution)
- Counted data (Poisson distribution)
- Normal distribution

Summary statistics

- Min, max, mean
- Mode
- Median and quartiles
- Confidence intervals

Probability data/ Binominal distribution





-> Jupyter Notebook

Counted data/ Poisson distribution

- Radioactive decay
- Raisins in a Dresdner Stollen
- Mutations in a genome

-> Jupyter Notebook

Discrete variables

Ordinal variables

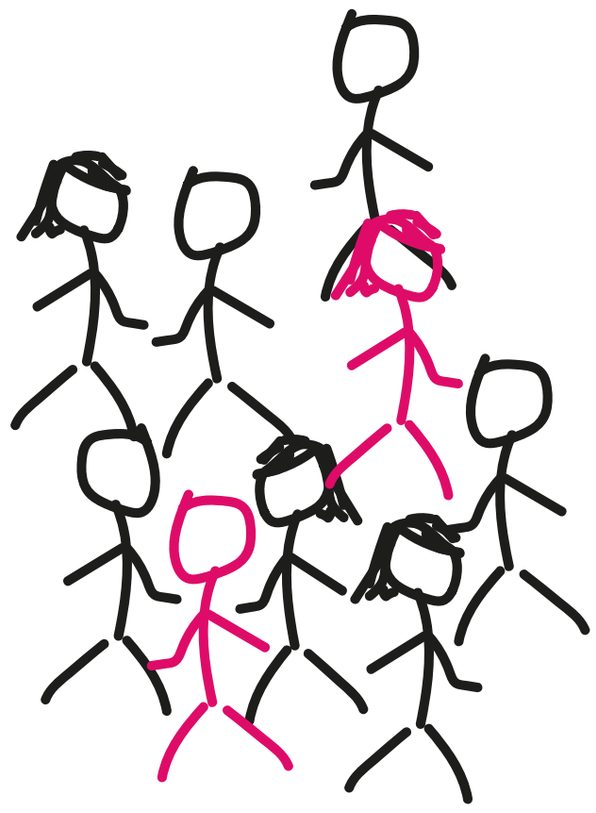
- limited set of discrete values with order

e.g. scale from 1-10

Nominal, binomial variables

- limited set of discrete values without order

e.g. responder <-> non responder



Continuous variables

Interval variables

- continuous value, for which intervals make sense, but no ratios

e.g. °C

Ratio variables

- continuous value, for which ratios make sense

e.g. height, weight, enzyme activity, Kelvin

Summary parameters

1 2 2 5 5 5 10 30

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Min value: 1

Max value: 30

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Parametric measures

Mean: (1+2+2+5+5+5+10+30)/8 = 7.5

Variance: (1+4+4+25+25+25+100+900)/8 = 90.57143

SD: square_root (variance) = 9.516902

SD = standard deviation = sigma

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non-parametric measures:

1 2 2 5 5 5 10 30

Ranks: 1 2 2 4 4 4 7 8

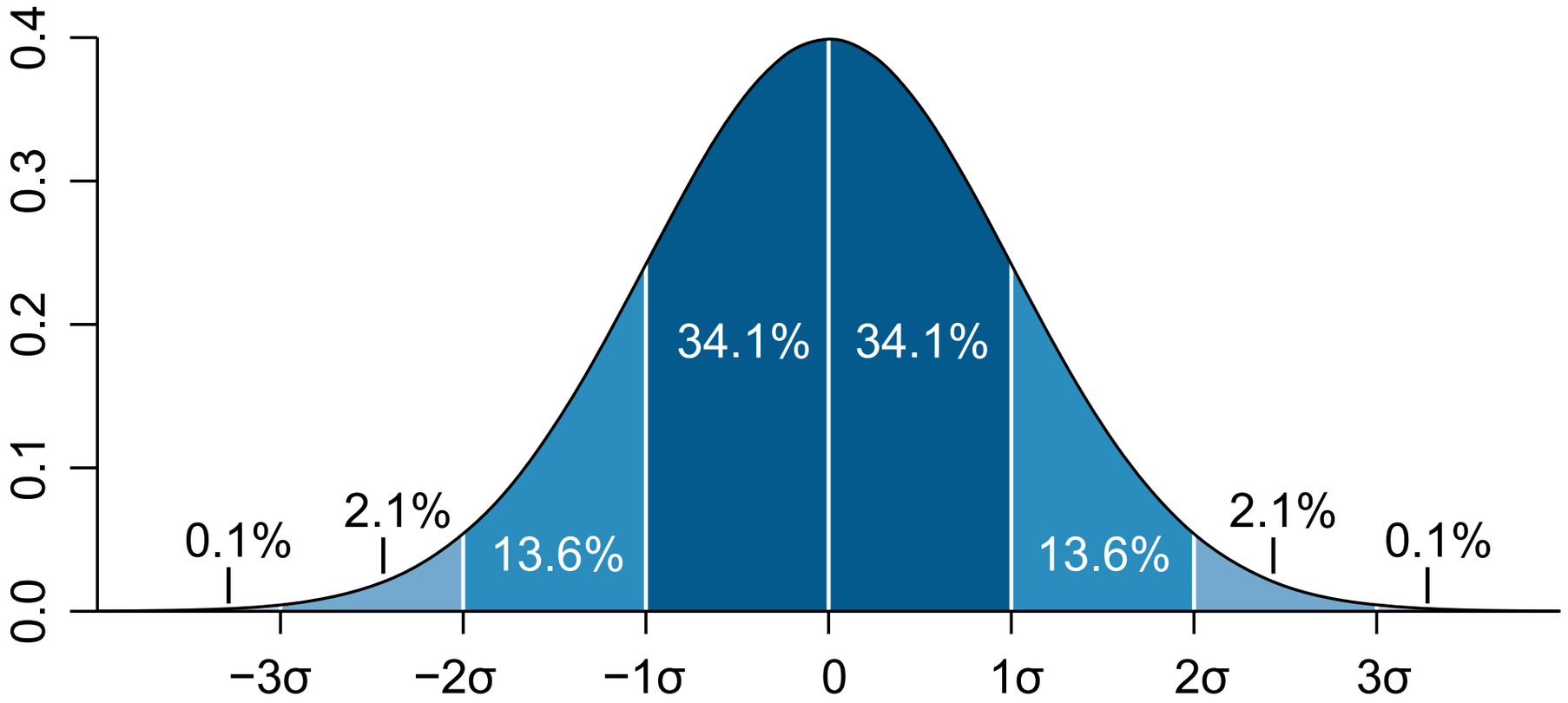
Median: the central value: 5

Quartiles: the value of the lower and upper quarter: 2, 6.25

Inter quartile range (IQR): 6.25-2

Normal distribution

Gaussian distribution, bell-shaped distribution



The result of general imprecision: weighing, pipetting, randomness

Therefore also: height, weight

Density defined by mean and standard deviation

Summary

- Probability data (Binomial distribution)
- Count data (Poisson distribution)
- Categorical and continuous data types
- Normal distributions
- Describing a distribution (mean, median, standard deviation, mode, error)

Hypotheses in the statistical sense

Innocent until proven guilty!

-> at first sight counterintuitive…

0-Hypothesis:

“The Astra Zeneca vaccine does not protect from COVID-19 in > 65 yo”

Test: Can we reject it?

A few months ago: No

Does it mean that it is not protective? No - we just don’t know!

A few months later, H_0 can be rejected

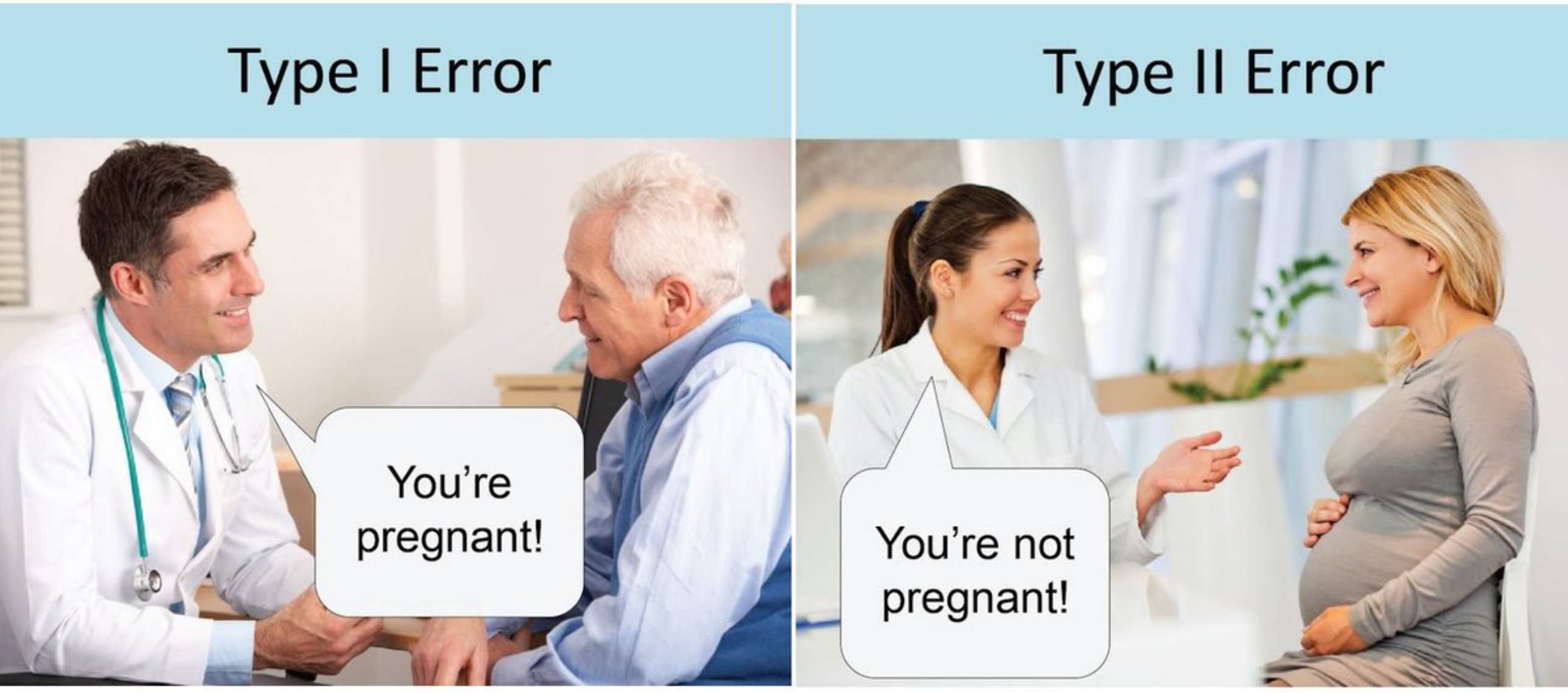
How to reject H_0

How much probability do you allow yourself to be wrong?

- last line chemotherapy treatment: Every bit of hope counts

- vaccination side-effects: Even rare events can be too much

What can go wrong?



False positive

False negative

P-values

The probability that you reject H_0 by chance.

Other ways to phrase it:

The probability that two samples are declared different although they belong to the same population.

The probability of observing a difference as large as you see it (or larger), if the samples are indeed from the same population.

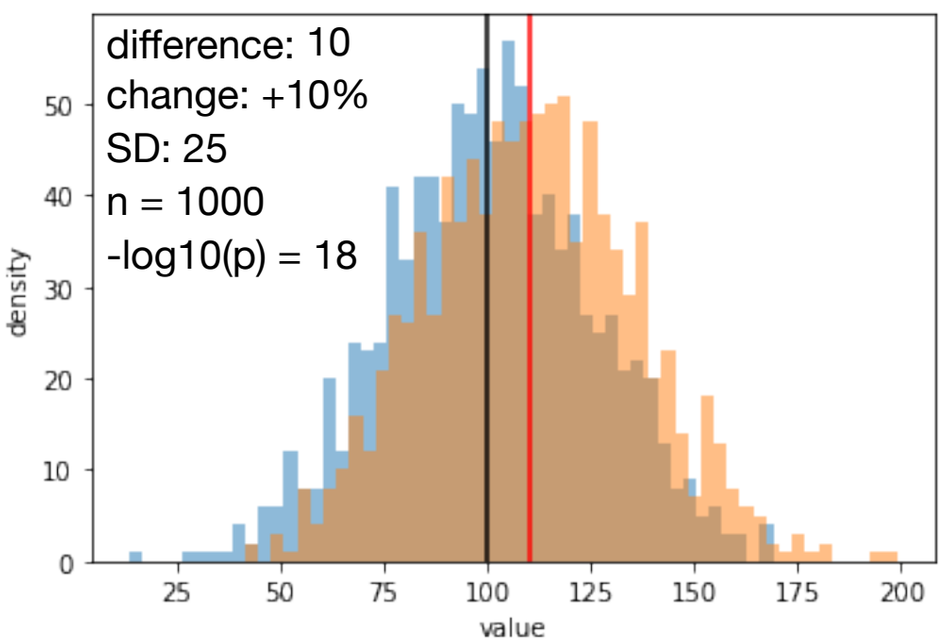
Misconceptions about P-values

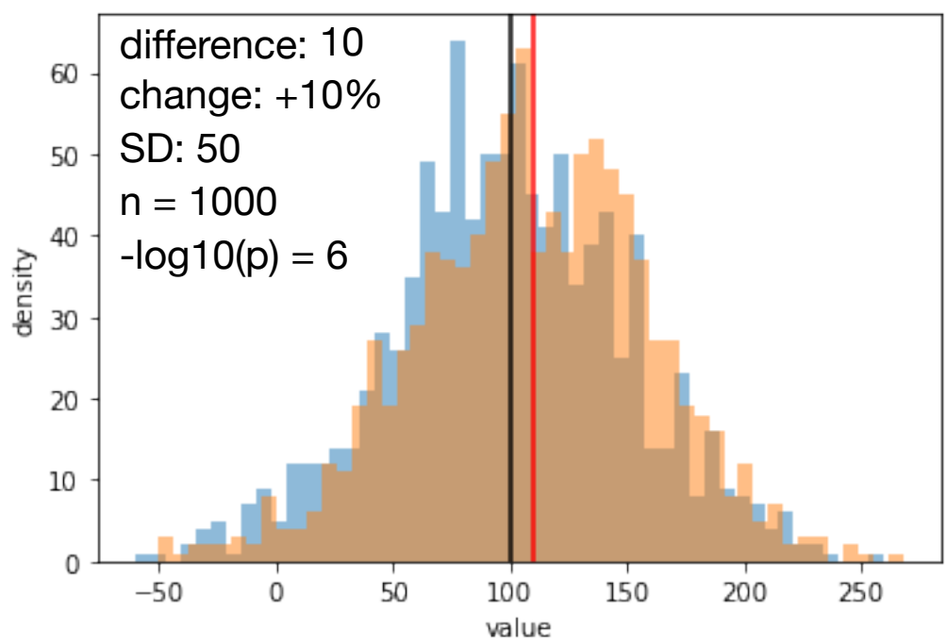
The logic is not reversible:

A p-value of 0.05 means a 5% chance concluding on a difference by chance. Don’t try to interpret the 95%!

You cannot determine whether H_0 is true.

A p-value is not appropriate to conclude about the magnitude of a difference





Misconceptions about P-values

Reproducibility of p-values is inherently very poor.

For measures of reproducibility of an effect the appropriate measure is the effect size, e.g. the actual difference or ratio.

How to put a threshold alpha for P-values

1. Not at all

2. At the next “pleasant number” (0.05, 0.001….)

3. At a threshold that is custom in the field (0.05, 5 sigma,…)

Whatever seems appropriate for your specific setup*

*also consider multiple testing correction

How to perform the actual hypothesis testing

Do we know the distribution?

-> Parametric testing

-> Fit a distribution to our data and compare whether

the two groups are sufficiently different

Do we not know the distribution?

-> Non-parametric testing

-> Determine the ranks of our datapoint and look

whether the ranks are sufficiently unbalanced

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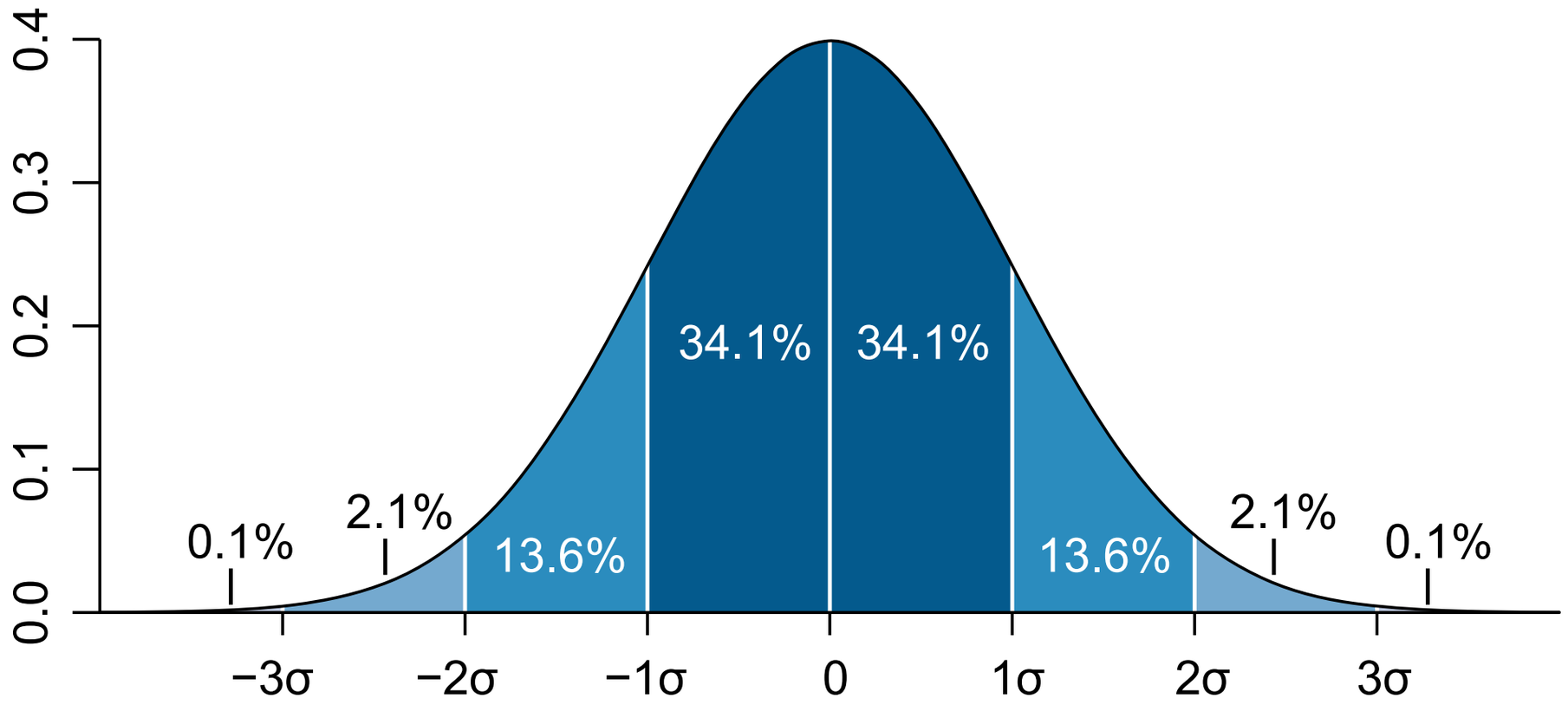

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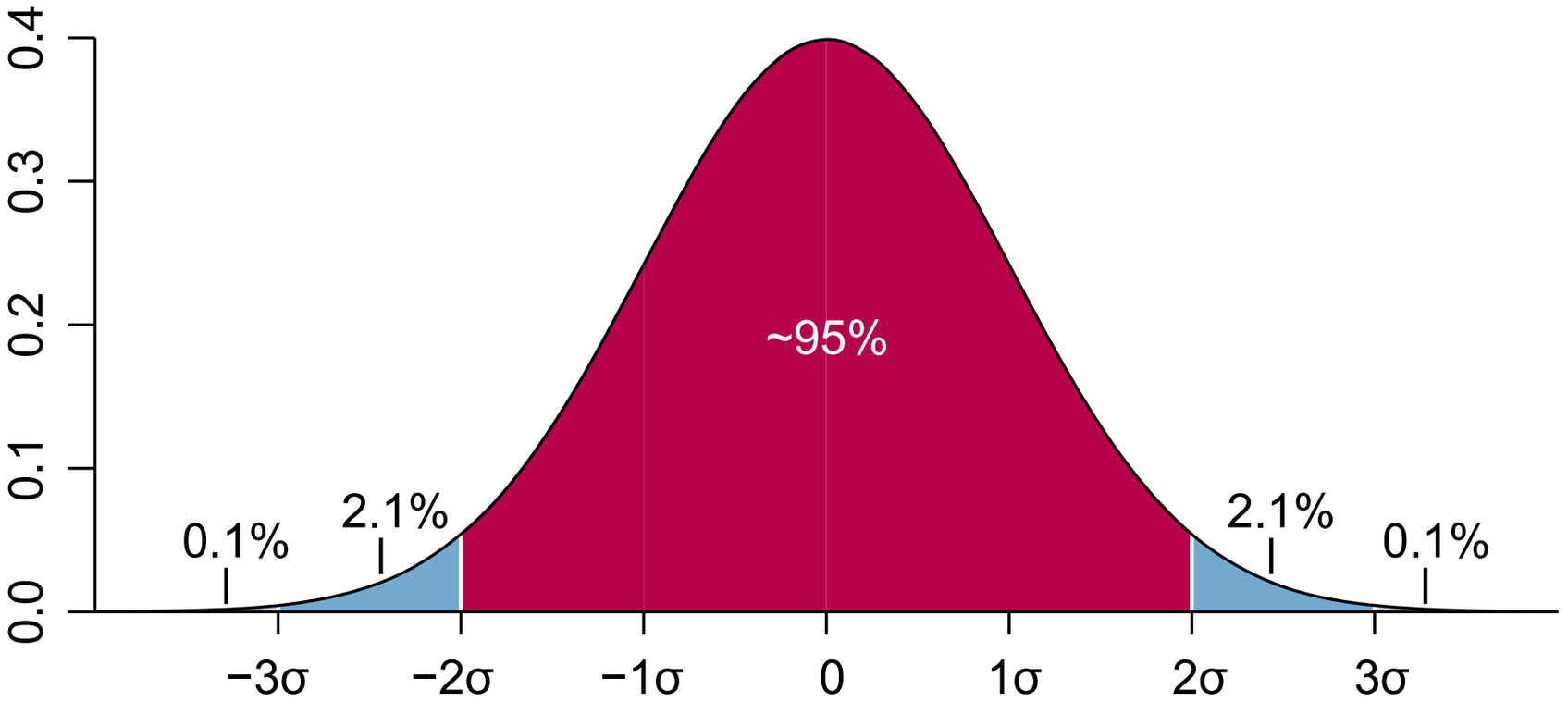
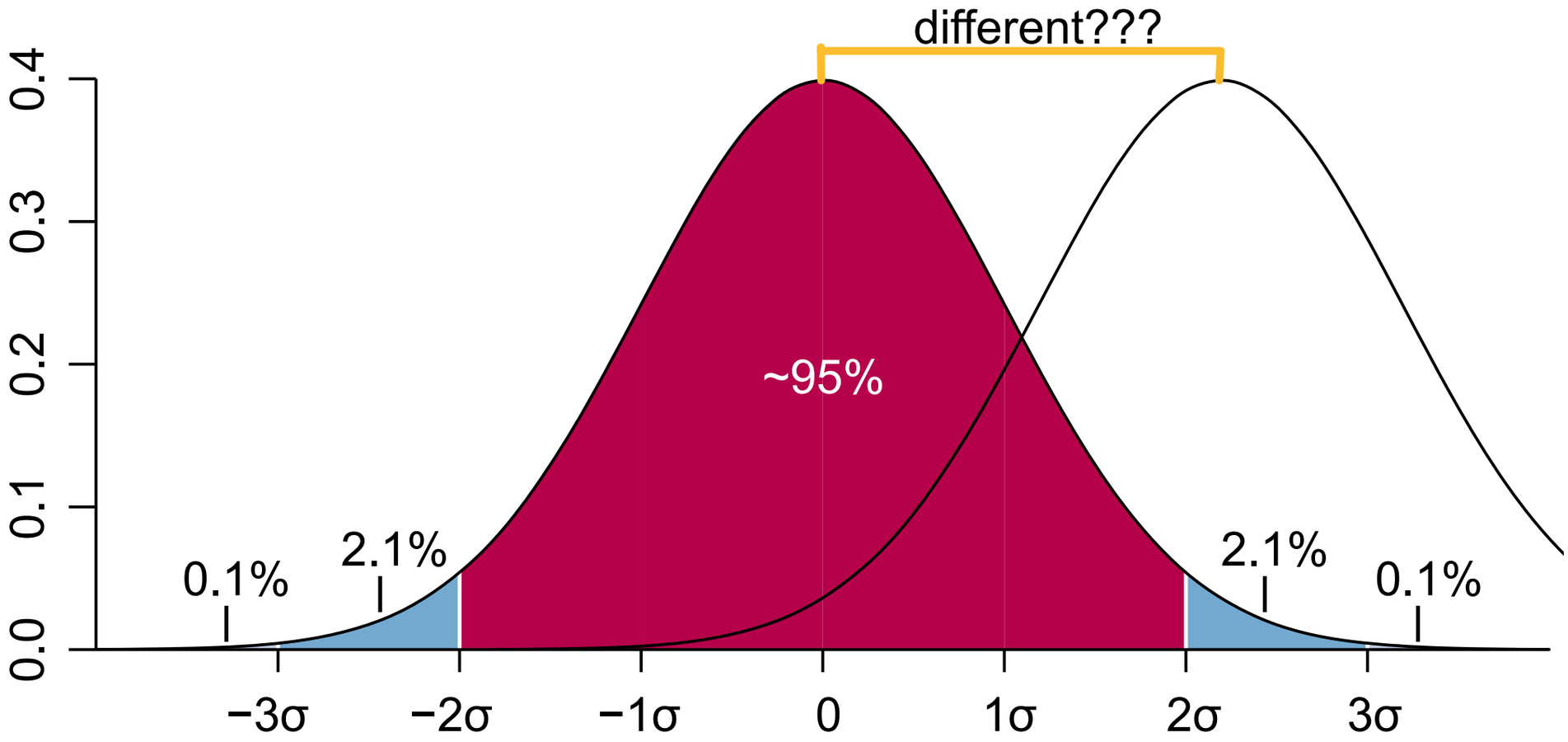


The graph is adapted from: M. W. Toews - Own work, based (in concept) on figure by Jeremy Kemp, on 2005-02-09, CC BY 2.5, https://commons.wikimedia.org/w/index.php?curid=1903871

Assumptions for unpaired parametric statistical testing



Assumptions:

- Our data follow a certain distribution
- They are representative samples
- Independent observations
- Accurate data

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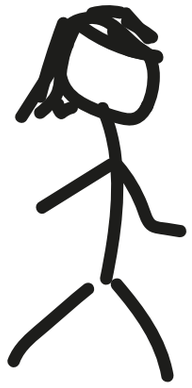
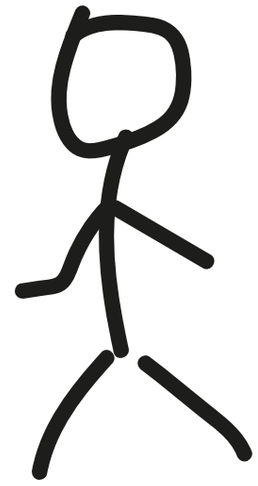
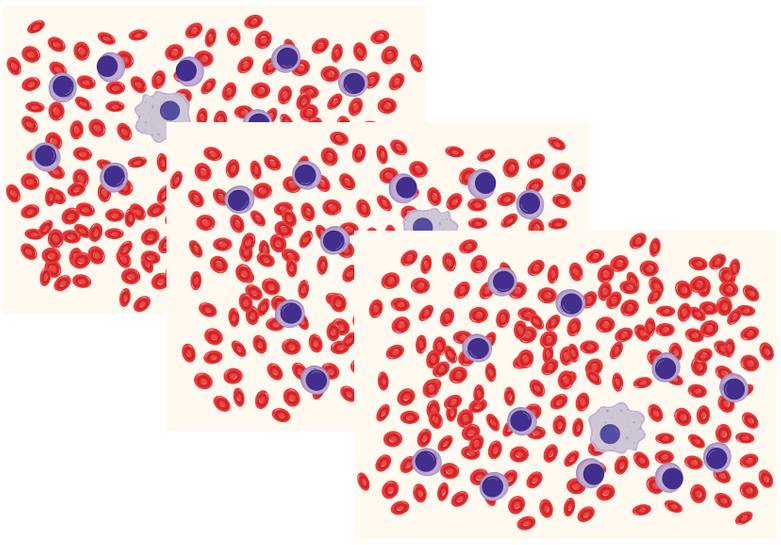
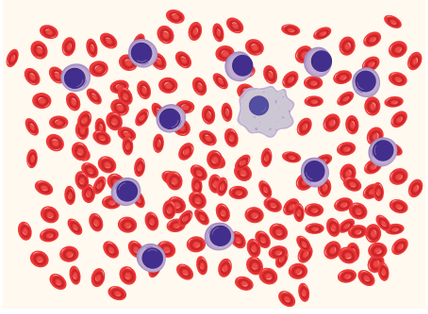


Abnormal white

blood cell count?



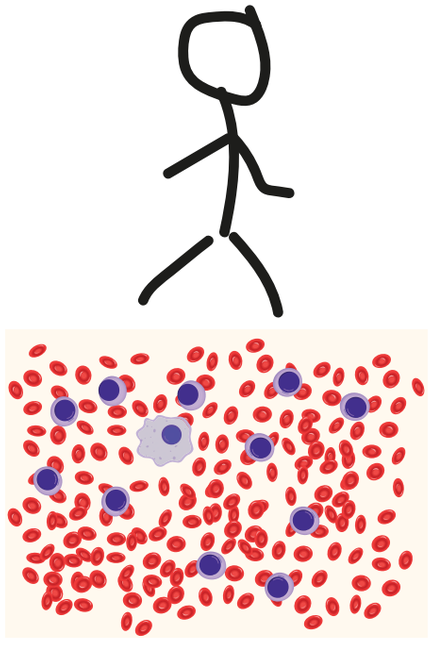


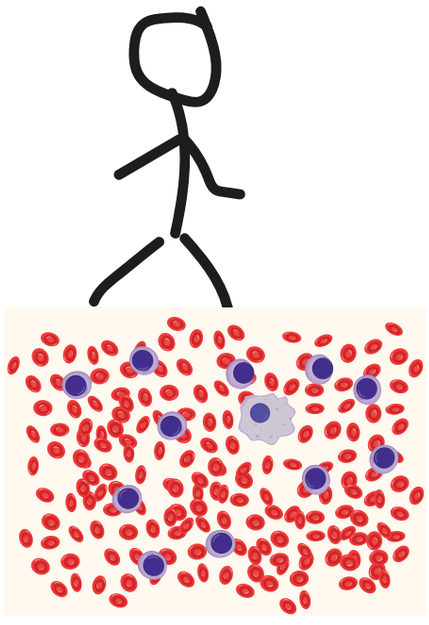


If all control cell counts are from one individual,

they are not independent!!!

Assumptions for statistical testing





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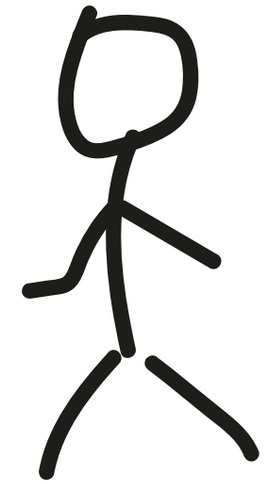
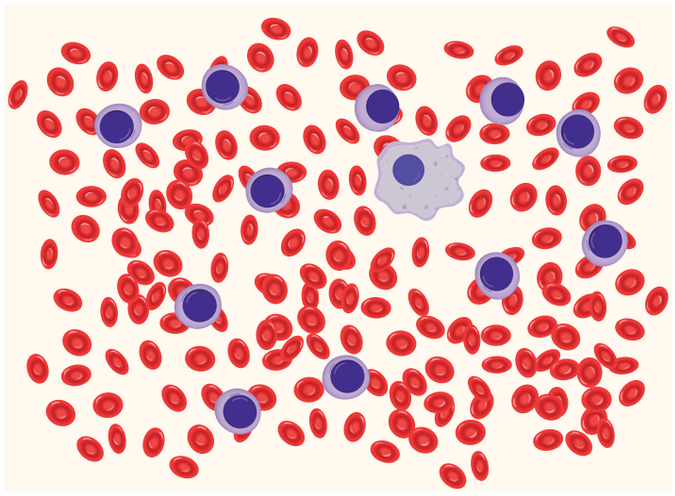
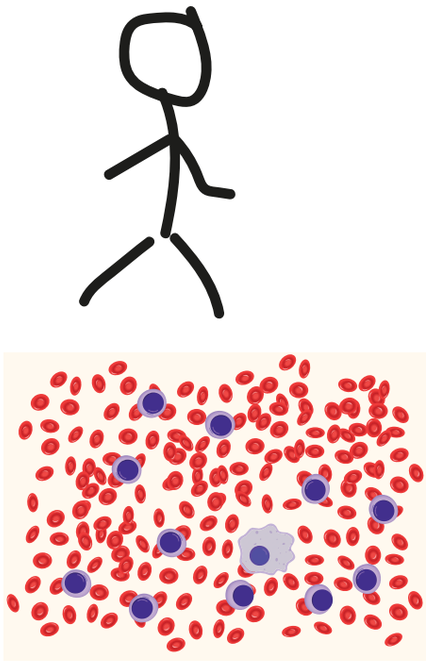


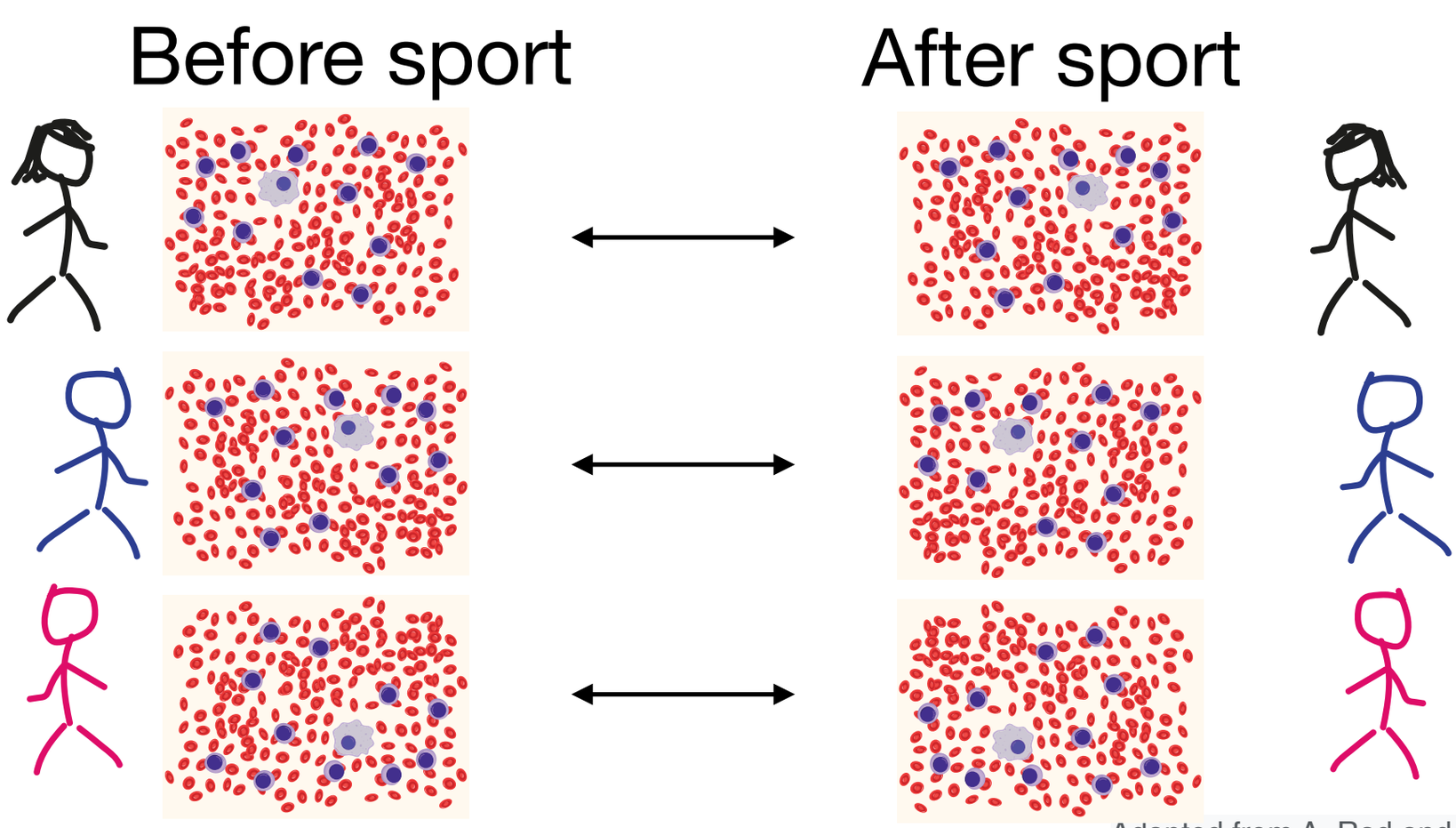




Special considerations

Does our hypothesis have a clear direction?
-> consider a **one-sided** test
i.e. we don't state in H0: There no difference
But: There no increase (or decrease)

Is our data paired?



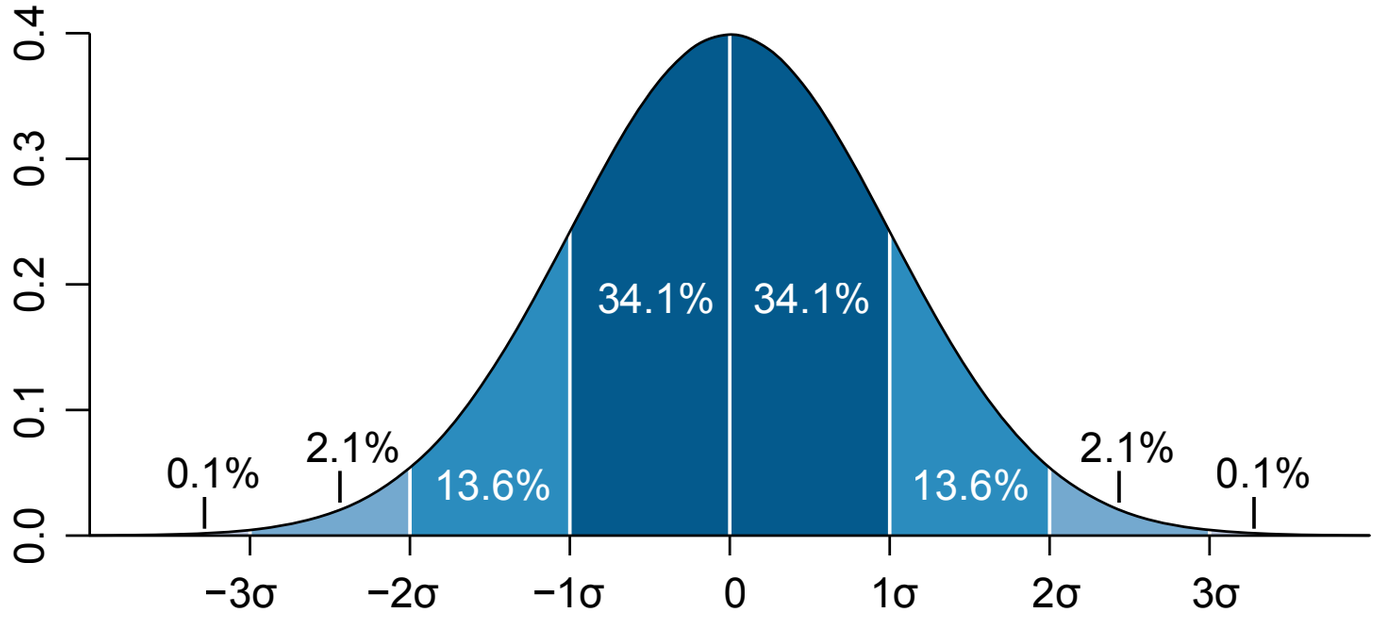
Comparing Two Means

Or: The t-test

Assumptions:

- Our data follow a distribution that can be approximated by the mean
- Equal standard deviation between samples
- They are representative samples
- Independent observations
- Accurate data

The Standard Error of the Mean (SEM)



SEM = SD/square_root(n)

The t-test calculates the standard error of the difference between two means

From this, the t-ratio is generated, the difference of the means divided by the standard error of that difference

The p-value is computed from this t-ratio and total sample size.

What does the p-value from the t-test tell us?

The probability that we are wrong, if we consider the two distributions to be different.

What is different in a paired test?

The difference of each pair is used to compute the standard error and thus the p-value.

When is a t-test inappropriate?

When any of the assumptions is violated, especially the assumption about that the mean needs to be a good approximation of the distribution.

Why?

When using t-tests inappropriately, outliers become very powerful and misleading!!!!

What are the alternatives?

- If data are supposed to meet the criteria theoretically, find the source of your issues

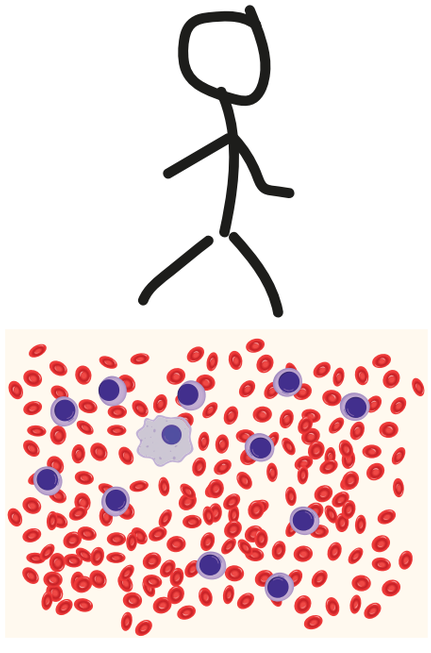
- Assume a different distribution

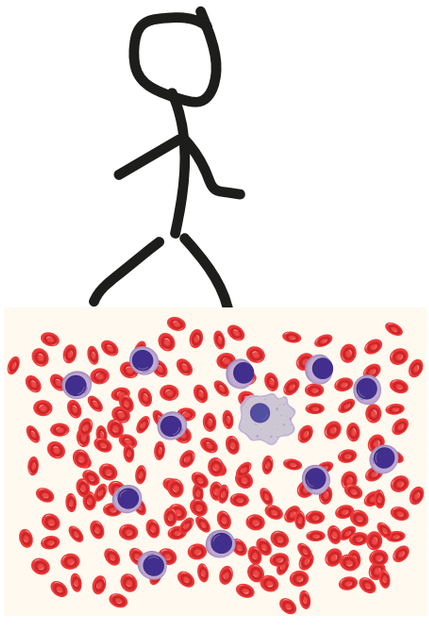
- Change to non-parametric testing

What we have covered

- Raising a hypothesis
- Types of errors
- P-values
- Assumptions for testing
- Comparing two means

Assumptions for statistical testing





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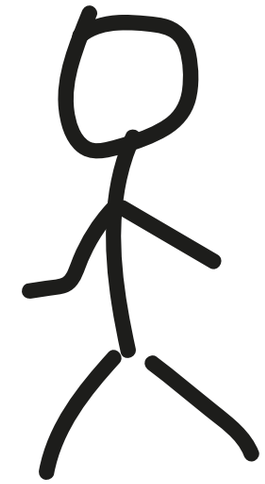
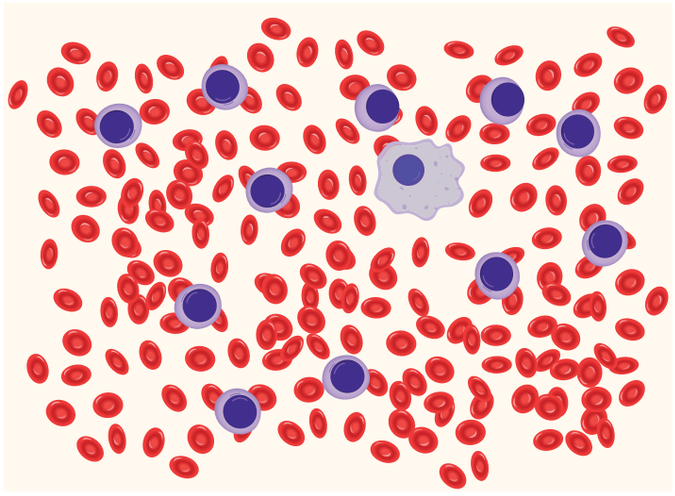
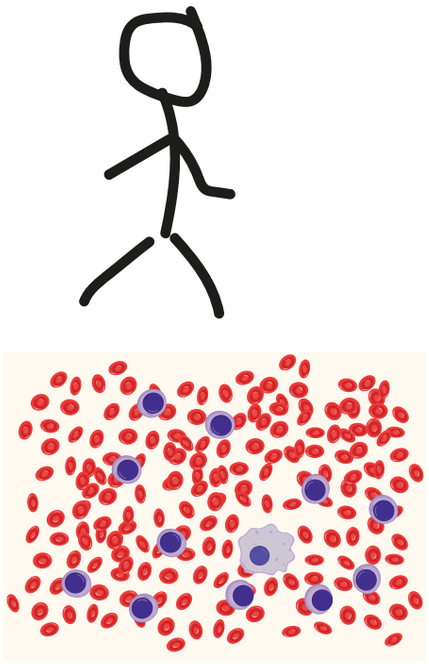
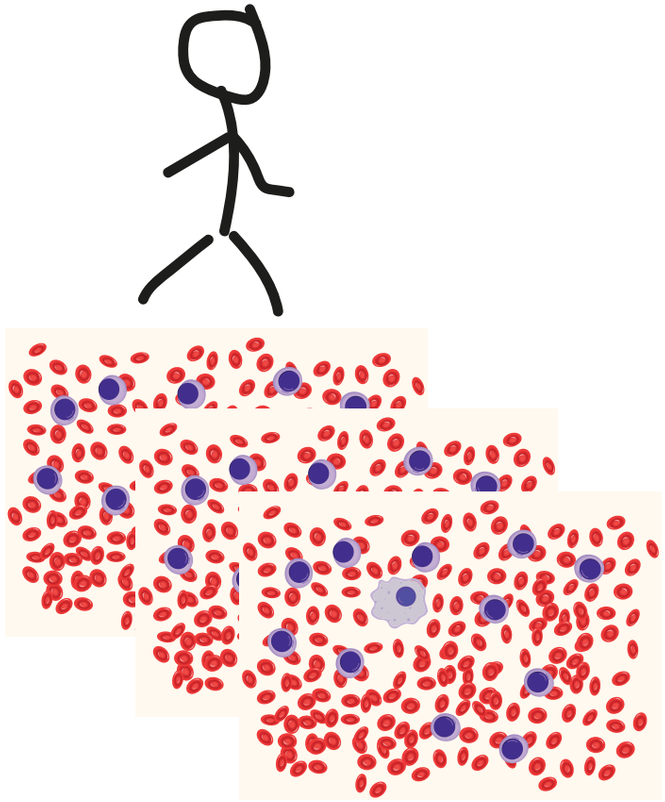
blood cell count?







Technical and biological replicates



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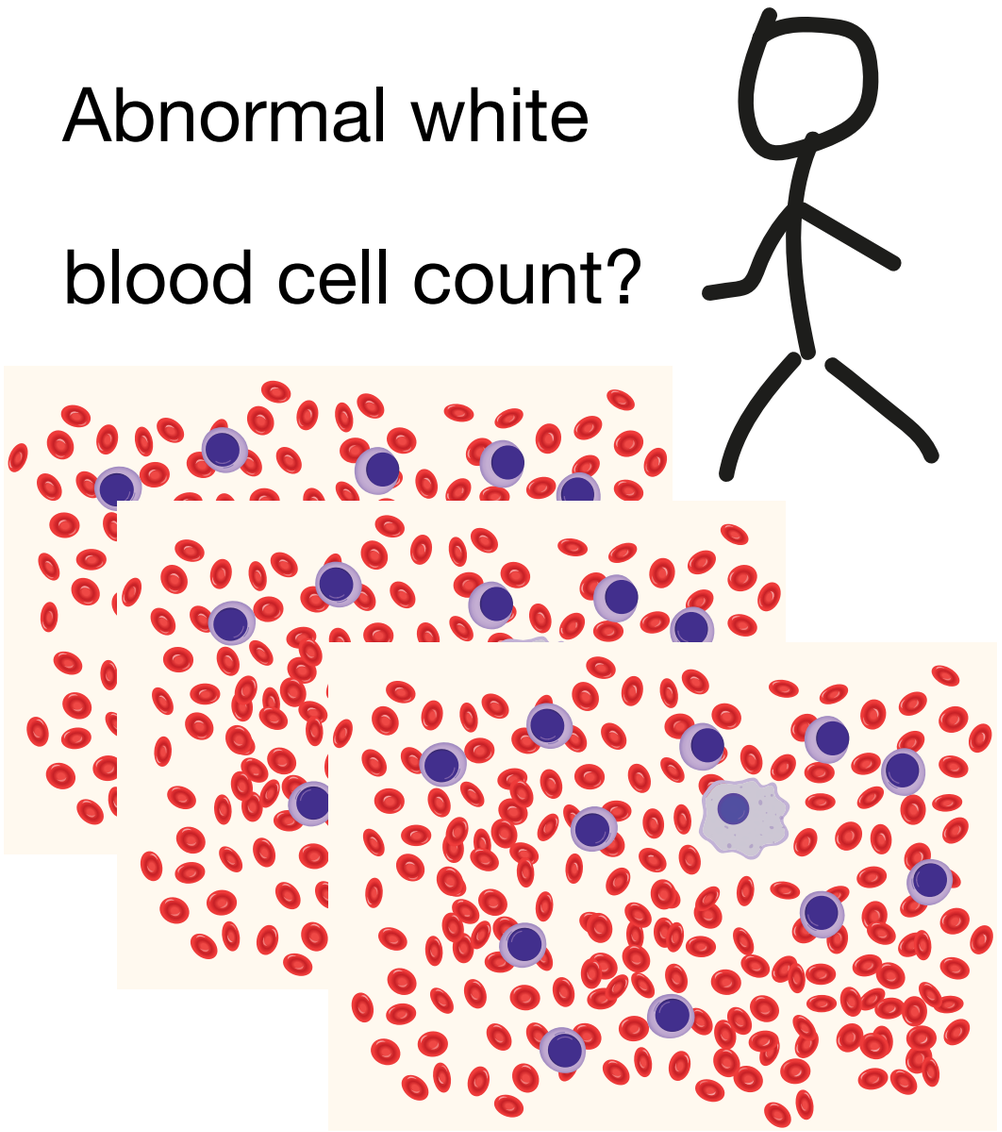

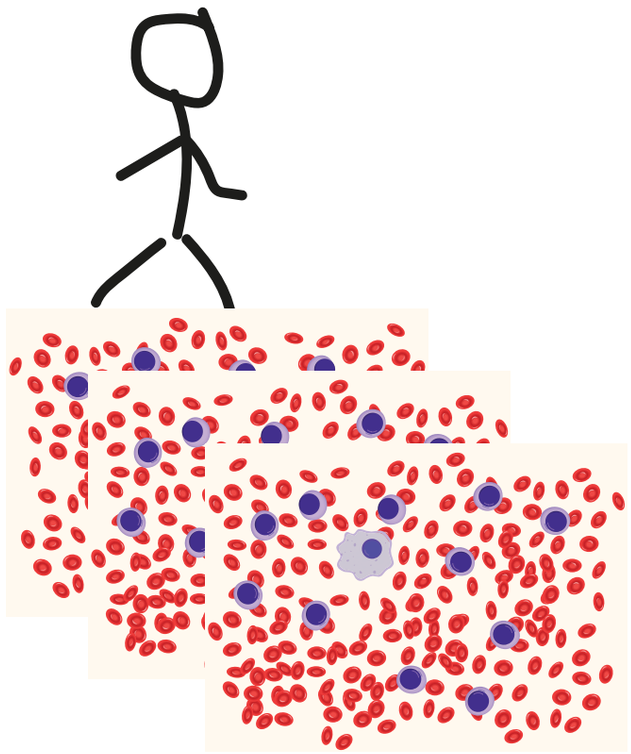

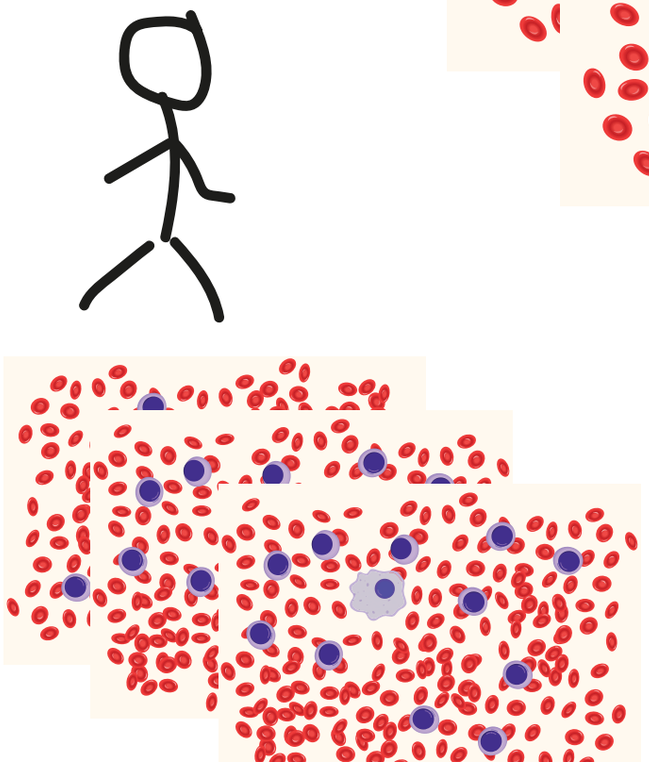

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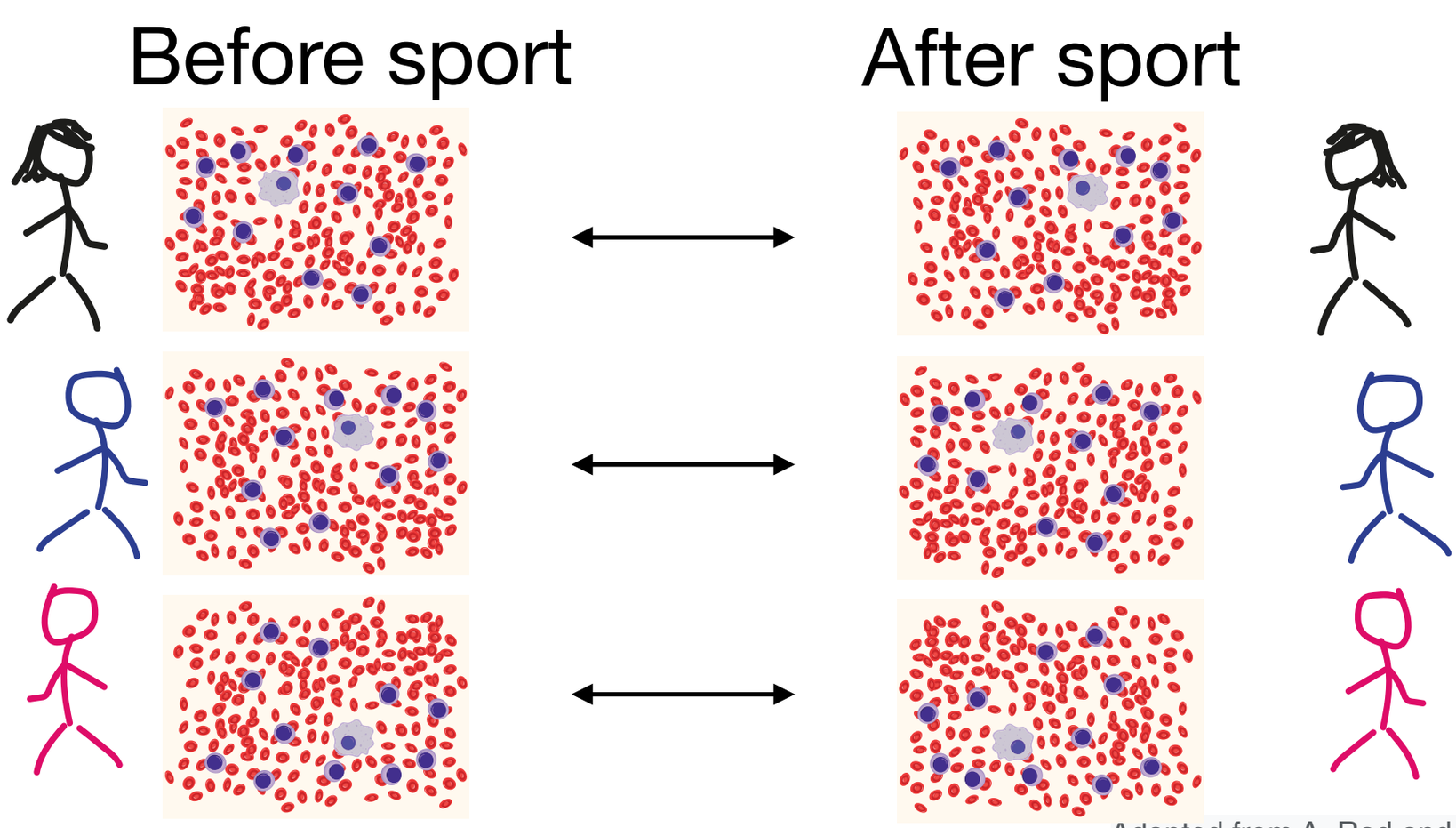

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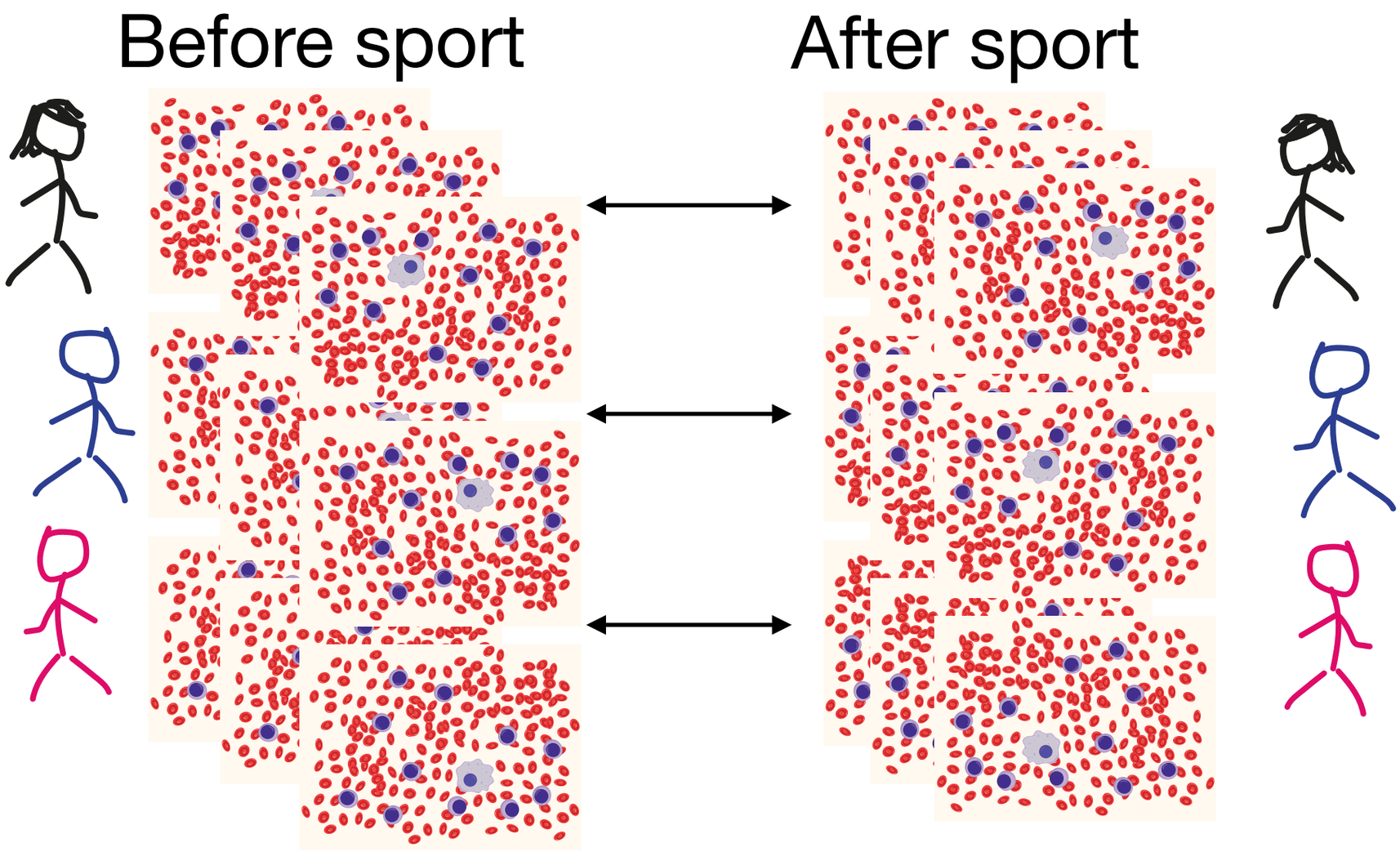

Next week

- Non-parametric testing
- Multiple comparisons
- Correlations
- A glimpse into big data and visualisation

-> Jupyter Notebook