

Lecture on Null Hypothesis Testing & Temporal Correlation

CS – 590.21 Analysis and Modeling of Brain Networks

Department of Computer Science University of Crete





Acknowledgement – Resources used in the slides

On statistical hypothesis test

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- William Morgan (Stanford University)

Proving Your Hypothesis

Mathematics

- 1. We already know a set of axioms & theorems, say K
- We want to show the theorem (hypothesis) H
- We show: K, ¬H ⇒ False (contradiction)
- 4. Thus, if we trust that K holds indeed,
 ¬H cannot hold, and
 H must hold

Real World

- 1. We already "know" K
- 2. We want to show a hypothesis H, e.g., "H: medicine A reduces the mortality of disease B"
- We gather data from the real world.
 We show that K, ¬H makes it very unlikely to observe our data
- 4. We conclude that
 - \neg H is very unlikely

We **reject** ¬**H**, and **accept H**

Notation for the following slides

- Random variables are denoted with a *capital* letter, e.g., X
- Observed quantities of random variables are denoted with their corresponding *small* letter *x*

Example:

- G is the expression level of a specific gene in a patient
- g is the measured expression level of the game in a *specific* patient

The Null Hypothesis

- The hypothesis we hope to accept is called the Alternative Hypothesis
 Sometimes denoted as H₁
- The hypothesis we hope to reject, the **negation of the Alternative Hypothesis**, is called the *Null Hypothesis*

Usually denoted by *H*_o

Think of the H_o as the "status quo"

Standard Single Hypothesis Testing

- 1. Form the Null & Alternative Hypothesis
- 2. Obtain related data
- 3. Find a suitable test statistic T
- 4. Find the distribution of *T given the null*
- 5. Depending on the distribution of T & the <u>observed</u> $t_o = T(x)$ decide to reject or not H_o

Test Statistics

- Test statistic is a function of our data X: T(X) (X: random variable)
 e.g., if X contains a single quantity (variable) T(X) the mean value of X
- T is a random variable (since it depends on X, our data which is random variable)
- Denote with t_o = T(x) the <u>observed</u> value of T in our data
- Instead of calculating P (obtaining data similar to X | H₀) Calculate P (T similar to t_o | H₀)
- If $P(T \text{ similar to } t_o \mid H_0)$ is very low, reject H_0

Statistical significance tests

- Let's just think about a two-tailed test: "difference" or "no difference"
- Null hypothesis: there is no difference between A vs. B
- Assume that o_A & o_B are "sampled" independently from a "**population**"
- Test statistic: a function of the sample data on which the decision is to be based
 t (o₁, o₂) = |e (o₁) e (o₂)|
 e: evaluation metric
- Find the distribution of t under the null hypothesis Assume that the null hypothesis is true
- Where does the t (o_A , o_B) lie in this distribution?

If it's somewhere unlikely, that's evidence that the null hypothesis is false



"Welcome to Lake Wobegon, where all the women are strong, all the men are good-looking, and all the children are above average."

- Garrison Keillor, A Prairie Home Companion

The Lake Wobegon Example: "Where all the children are above average!"

- Let X represent Weschler Adult Intelligence scores (WAIS)
- Typically, X ~ N(100, 15) (μ₀ = 100, σ = 15)

Does sample mean provide strong evidence that population mean $\mu > 100$?

One-Sample z Test

- 1. Hypothesis statements $H_0: \mu = \mu_0$ $H_a: \mu \neq \mu_0$ (two-sided) or $H_a: \mu < \mu_0$ (left-sided) or $H_a: \mu > \mu_0$ (right-sided)
- 3. Test statistic $z_{\text{stat}} = \frac{\overline{x} \mu_0}{SE_{\overline{x}}}$ where $SE_{\overline{x}} = \frac{\sigma}{\sqrt{n}}$
- 4. P-value: convert z_{stat} to P value
- A. Significance statement (usually not necessary)

Example: Two-Sided Hypothesis Test "Lake Wobegon"

1. Formulation of the Hypotheses:

 $H_0: \mu = 100$ $H_a: \mu > 100$ (one-sided)

 $H_a: \mu \neq 100$ (two-sided)

2. Obtain data ...

Obtain data: 9 children from Lake Wobegon population

Their scores: {116, 128, 125, 119, 89, 99, 105, 116, 118} Average of the observations = 112.8

Example: Two-Sided Hypothesis Test "Lake Wobegon"

3. Test statistic

$$SE_{\bar{x}} = \frac{\sigma}{\sqrt{n}} = \frac{15}{\sqrt{9}} = 5$$
$$z_{\text{stat}} = \frac{\bar{x} - \mu_0}{SE_{\bar{x}}} = \frac{112.8 - 100}{5} = 2.56$$

Classical CLT [edit]

Let $\{X_1, ..., X_n\}$ be a random sample of size n — that is, a sequence of independent and identically distributed random variables drawn from distributions of expected values given by μ and finite variances given by σ^2 . Suppose we are interested in the sample average



of these random variables. By the law of large numbers, the sample averages converge in probability and almost surely to the expected value μ as $n \to \infty$. The classical central limit theorem describes the size and the distributional form of the stochastic fluctuations around the deterministic number μ during this convergence. More precisely, it states that as n gets larger, the distribution of the difference between the sample average S_n and its limit μ , when multiplied by the factor \sqrt{n} (that is $\sqrt{n}(S_n - \mu)$), approximates the normal distribution with mean 0 and variance σ^2 . For large enough n, the distribution of S_n is close to the normal distribution with mean μ and variance $\frac{\sigma^2}{n}$. The usefulness of the theorem is that the distribution of $\sqrt{n}(S_n - \mu)$ approaches normality regardless of the snape of the distribution of the individual X_i . Formally, the theorem can be stated as follows:

Central Limit Theory

Establishes that, in most situations, when **independent random variables are added**, their **properly normalized sum tends toward** <u>a normal distribution</u> even if the **original variables themselves are** <u>not normally</u> distributed.

- 1. A sample is obtained containing a large number of observations, each observation being randomly generated in a way that does not depend on the values of the other observations.
- 2. If step 1 is performed many times, the computed values of the average will be distributed according to a normal distribution.

Example: Flip a coin many times. The probability of getting a given number of heads in a series of **K** flips will approach the normal distr. with mean =K/2

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$$S_n:=rac{X_1+\dots+X_n}{n}$$

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 $P = .0052 \Rightarrow$ it is unlikely the sample came from this null distribution \Rightarrow strong evidence against H_0



Conditions for z Test

- 1. Population approximately Normal or large sample (central limit theorem)
- 2. The **population variance** is known!

If the **population variance is unknown** (and therefore has to be estimated from the sample itself) & the **sample size is not large** (n < 30), the **Student's** *t*-test may be more appropriate.

Another Example

- Background knowledge: Breast Cancer is related to mutations in genes BRCA1 & BRCA2
- Hypothesis: Gene G is expressed differently in breast cancer patients with mutation in BRCA1 than BRCA2
- Data: Obtained 7 patients with BRCA1 mutation & 8 with BRCA2 mutation

Patient number	Expression Level of Gene G X_i^1	Have mutation in BRCA1 or not x_i^2
1 (x ₁)	98.2244	1
2	69.6810	1
3	118.4339	1
4	115.2322	1
5	150.7729	1
6	117.7385	1
7	80.6921	1
8	142.8455	2
9	156.8692	2
10	151.9287	2
11	147.3357	2
12	131.2094	2
13	150.3127	2
14	147.0670	2
15 (x ₁₅)	122.3306	2

Hedenfalk et al. N Engl J Med. 2001 Feb 22;344(8):539-48.

1. Form the Null Hypothesis

• Gene G is expressed differently in breast cancer patients with mutation in BRCA1 than BRCA2

Mathematically

- μ_1 : be the mean expression level of gene G in patients with BRCA1 mutation
- μ_2 : be the mean expression level of gene G in patients with BRCA2 mutation

 $H_0: \mu_1 = \mu_2$ $H_1: \mu_1 \neq \mu_2$

2. Obtain data....

DATA: BY THE NUMBERS



www.phdcomics.com

3. Find a suitable test statistic T (Example)



- The larger the difference of the two means, the larger the statistic
- The larger our sample, the larger the statistic
- The smaller the sample variance, the larger the statistic

So T will be quite large (in absolute value), when we can confidently say H_o does not hold

3. Find a suitable test statistic T (cont'd)



3. Find the distribution of T (cont'd)

For the test of this specific example, we will make the following assumptions:

- a) The data in both groups are distributed normally around a mean value μ_1 , μ_2 respectively
- b) Their variance is the same in both groups
- c) Each patient was **sampled independently**

and most importantly that THE NULL HYPOTHESIS HOLDS

This is an assumption for ALL tests!

Then

T(X) has a probability density function of:

$$p(t \mid H_0) = \frac{\Gamma(\frac{\nu+1}{2})}{\sqrt{\nu\pi}\Gamma(\frac{\nu}{2})} \left(1 + \frac{t^2}{\nu}\right)^{-\left(\frac{\nu+1}{2}\right)}$$

where the degrees of freedom of the test V is 15 - 2 = 13 (number of patients - 2)

The *t*-statistic was introduced in 1908 by William Sealy Gosset, a chemist working for the Guinness brewery in Dublin. "Student" was his pen name.



Sampling distribution [edit]

One sample T-distribution

Let $x_1, ..., x_n$ be the numbers observed in a sample from a continuously distributed population with expected value μ

$$ar{x} = rac{x_1 + \dots + x_n}{n},
onumber \ s^2 = rac{1}{n-1} \sum_{i=1}^n (x_i - ar{x})^2.$$

The resulting *t-value* is $t=rac{ar{x}-\mu}{s/\sqrt{n}}.$

The *t*-distribution with n - 1 degrees of freedom is the sampling distribution of the *t*-value when the samples consist distributed population. Thus for inference purposes *t* is a useful "pivotal quantity" in the case when the mean and value $r = \frac{1}{2}$

Student t-distribution (basics)

Probability density function [edit]

Student's *t*-distribution has the probability density function given by

$$f(t)=rac{\Gamma(rac{
u+1}{2})}{\sqrt{
u\pi}\,\Gamma(rac{
u}{2})}\left(1+rac{t^2}{
u}
ight)^{-rac{
u+1}{2}},$$

where u is the number of *degrees of freedom* and Γ is the gamma function. This may also be written as

$$f(t) = rac{1}{\sqrt{
u}\,\mathrm{B}(rac{1}{2},rac{
u}{2})} igg(1+rac{t^2}{
u}igg)^{-rac{
u+1}{2}},$$

t-distribution (basics)



(blue). Previous plots shown in green.



4. Decide on a Rejection Region

- Decide on a rejection region Γ in the range of our statistic
- If $t_o \in \Gamma$, then reject H_0
- If $t_o \not\in \Gamma$, then **do not** reject H_0 accept H_1 ?

Since the pdf of *T* when the null hypothesis holds is known, P($T \in \Gamma \mid H_0$) can be calculated

4. Decide on a Rejection Region

• If $P(T \in \Gamma \mid H_0)$ is too low, we know we are safely rejecting H_0

• What should be our rejection region in our example?

4. Decide on a Rejection Region



Rejection Procedure

- Pre-select a probability threshold *a*
- Find a rejection region $\Gamma = \{ t: |t| > c \}$, such that $P(T \in \Gamma | H_0) = a$
- Decide
 - Reject H_o , if $t_o \in \Gamma$ (recall: t_o is the observed T in our data)
 - Accept H_o, otherwise

What values do we usually use for *a* in science?

0.05 is typical Smaller ones are also used: 0.01, 0.001

When $t_o \in \Gamma$ we say the finding is statistically significant at significance level a

Issues to be Considered

• When there exist two or more tests that are appropriate in a given situation, how can the tests be compared to decide which should be used?

If a test is derived under specific assumptions about the distribution of the population being sampled,

how well will the test procedure work when the assumptions are violated?

Parametric versus non-Parametric Tests

• Parametric test

Makes the assumption that the **data are sampled** from a **particular class of distributions**

It then becomes easier to derive the distribution of the test statistic

Non-Parametric test

No assumption about a particular class of distributions

Permutation Testing

- Often in biological data, we do not know much about the data distribution
- How do we obtain the distribution of our test statistic?
- Great idea in statistics: permutation testing
- Recently practical because it requires computing power (or a lot of patience)

Permutation Testing

- 1. In our first example, we want to calculate $p(t | H_0)$
- 2. If H_0 , then it does **not** matter which group each value x_i^1 comes from
- 3. Then, if we **permute the group labels**, we would get a value for our test statistic **given the null hypothesis holds**
- 4. If we get a lot of such values, we can estimate (approximate) $p(t | H_0)$

Permutation Testing Revisited

- Decide what can be permuted, if the null hypothesis is true
- For all (as many as possible) permutations of the data, calculate the **test statistic** on the permuted data: t_B
- Estimated p-value = # { $|t_B| \ge |t_o|$ } / #B



Does It Really Work?



True distribution calculated theoretically

Estimated distribution from our data: **1,000** permutations

Does It Really Work?



True distribution calculated theoretically

Estimated distribution from our data: **10,000 permutations**



p-value is defined as the probability of obtaining a result equal to or more extreme than what was actually observed

- The area to the right of t(o_A, o_B) is the "significance level"—the probability that some t^{*} ≥ t(o_A, o_B) would be generated if the null hypothesis were true.
 - Also called the p-value.
- The Significance Level
 - Small values suggest the null hypothesis is false, given the observation of $t(o_A, o_B)$.
 - Corollary: all else being equal, a large difference between *e*(*o_A*) and *e*(*o_B*) yields a smaller significance level (as one would hope!).
 - Values below 0.05 are typically considered "good enough."

So all we have to do is calculate the distribution of t.

The classical approach:

- Keep adding assumptions until we arrive at a known distribution which we can calculate analytically.
- E.g.: Student's t-test.

Calculating the Distribution

- Assume that e(o_A) and e(o_B) are sample means from a bivariate Normal distribution with zero covariance. Then we know t is distributed according to Student's t-distribution if the null hypothesis is true.
- Back in the stone age, computing with rocks and twigs, making those assumptions made the problem tractable.
- But the problem with this approach is that you may falsely reject the null hypothesis if one of the additional assumptions is violated. (Type I error.)



- Simulate the distribution using a <u>randomization test</u>.
- It's just as good as analytical approaches, even when the analytical assumptions are met! (Hoeffding 1952)
- And it's better when they're not. (Noreen 1989) What you
- Best of all: dirt simple. SHOULD
- do Intuition:

- Erase the labels "output of A" or "output of B" from all of the observations.
- Now consider the population of every possible labeling. (Order relevant.)
- If the systems are really different, the observed labeling should be unlikely under this distribution.

Statistical Errors

• Type 1 Errors

-Rejecting H₀ when it is actually true
 -Concluding a difference when one does *not actually exist*

• Type 2 Errors

-Accepting H_0 when it is actually false (e.g. previous slide) -Concluding no difference when **one does exist**

Errors can occur due to **biased/inadequate sampling**, **poor experimental design** or the use of **inappropriate/non-parametric** tests.

Regarding the Choice of a Test

When we cannot reject H_{0_i} it does <u>not</u> mean H_1 holds!

- It could be that we do **not have enough power**, i.e.,
 - H_1 is not that "different enough" from H_0 to distinguish it with the given sample size

of all possible tests for a hypothesis choose the one with the maximum power

Power analysis methods need to be employed.

T_A: the proportion of total recording time which lies within $\pm\Delta t$ of any spike from A. **T_B** calculated similarly.



 T_A is given by the fraction of the total recording time (black) which is covered (tiled) by blue bars. Here T_A is 1/3.

P_A: the proportion of spikes from A which lie within $\pm\Delta t$ of any spike from B. **P**_B calculated similarly.



Directional STTC Temporal Correlation Metric

Extended STTC metric to take into consideration the order of the correlation of the spike trains of two neurons

Directional STTC_{AB} represents a measure of the chance that firing events of A will precede firing events of B



 $P_A^{B^-}$: fraction of firing events of A that occur within an interval Δt prior to firing events of B T_{B^-} : fraction of total recording time covered by the intervals Δt prior to each spike of B Δt : specific lag (input in directional STTC)

Advantages of Directional STTC vs. other correlation metrics

$$STTC_{AB} = \frac{1}{2} \left(\frac{P_A^{B^-} - T_B^-}{1 - P_A^{B^-} T_B^-} + \frac{P_B^{A^+} - T_{A^+}}{1 - P_B^{A^+} T_{A^+}} \right)$$

- Relative spike-time shifts (lag parameter)
- Local fluctuations of neural activity or noise
 - accounting the amount of correlation expected by chance $(T_{A^+} \& T_{B^-})$
- The presence of periods without firing events
 - only the firing events contributes on the correlation metric of directional STTC ($P_A^{B-} \& P_B^{A+}$)

Example – Degree of connectivity considering the significant directional STTC edges 1



Conditional STTC (A->B | C) $STTC_{AB}^{C}$

$$STTC_{AB}^{C} = \frac{1}{2} \left(\frac{\frac{N_{B^-A}^{CA}}{N_A} - T_{B^-}}{1 - \frac{N_{B^-A}^{CA}}{N_A} T_{B^-}} + \frac{\frac{N_{A^+B}^{CA}}{N_B} - T_{A^+}}{1 - \frac{N_{A^+B}^{CA}}{N_B} T_{A^+}} \right)$$

 N_A is the number of firing event in A & N_B is the number of firing event in B.

 T_{A^+} is the fraction of the total recording time which is covered by the tiles + Δ t after each spike of A, that fall within the tiles Δ t after each spike of C.

 T_B - is the fraction of the total recording time which is covered by the tiles Δt before each spike of B.



Directional edge "A \rightarrow B" indicate that firing events of A proceed firing events of B by a specific lag Circular shuffling by random delays of the neural traces is used to assess **significance** of directional edges

Null distribution: STTC values for the circular shifted neurons

Significant edge: real STTC value higher than 3 standard deviation of null distribution

Example: Null distribution test for directional STTC

For a given pair (A,B)

- 1. Circular shift the spike train of the neuron A, A^1
- 2. Estimate the directional STTC(A¹,B)
- 3. Repeat the above steps 100 times
- 4. Estimate the mean & standard deviation of the obtained STTC values
- 5. The statistical significant threshold (thr) = mean + 3 std dev

If the directional STTC (A, B) > thr , the edge (A,B) is statistically significant.

The criterion can be strengthen with more repetitions (e.g., **1000**), a larger number of std dev (e.g., **5**).

Strengthen the Criterion of Significant Edge

- Reject the null hypothesis test
- The total number of spikes of A within a STTC lag of spikes of B is above 3.
- The total number of spikes of B within a STTC lag of spikes of A is above 3.



- 1. Control group: each neuron trace is circular shifted by random delay
- 2. For each pair of 'shifted' neurons estimate the directional STTC & null distribution
- 3. Identify the significant edges of the control group

The real neuron traces appear higher values of directional STTC & percentage of significant edges

- Non-parametric test of the equality of continuous 1D probability distributions
- Quantifies a distance between two distribution functions
- Can serve as a goodness of fit test
- Null hypothesis
 - H₀: Two samples drawn from **populations** with same distribution
- The maximum absolute difference between the two CDFs



- Non-parametric test of the equality of continuous 1D probability distributions
- Quantifies a distance between two distribution functions
- Can serve as a goodness of fit test

$$D_{n,m} = \sup_x |F_{1,n}(x) - F_{2,m}(x)|,$$

where $F_{1,n}$ and $F_{2,m}$ are the empirical distribution functions

The null hypothesis is rejected at level lpha if

$$m_m > c(\alpha) \sqrt{\frac{n+m}{nn}}$$
 n & m: size of the sample datasets
 $\alpha = 0.10 \ 0.05 \ 0.025 \ 0.01 \ 0.005 \ 0.001 \ c(\alpha) \ 1.22 \ 1.36 \ 1.48 \ 1.63 \ 1.73 \ 1.95$

and in general by

$$c\left(lpha
ight)=\sqrt{-rac{1}{2}\ln\!\left(rac{lpha}{2}
ight)}.$$



• Kolmogorov computed the expected distribution of the distance of the two CDFs when the null hypothesis is true.

Example: Kolmogorov-Smirnov Test

	Decision		p-value		Distance	
Lag	True Null	Null Null	True Null	Null Null	True Null	Null Null
1	1	0	0	0.5427	0.79	0.0076
2	1	0	0	0.2126	0.78	0.0100
3	1	0	0	0.98485	0.75	0.0043
4	1	0	0	0.9937	0.72	0.0040
5	1	0	0	0.9769	0.68	0.00453

Distance of the two distributions In Sup norm

For **all neuron pairs (**A, B), populate the following distributions with Population 1: real STTC of the pair (A,B) Population 2: random circular shift in one of the two spike trains of (A,B) Population 3: random circular shift in one of the two spike trains of (A,B)

True Null: Population 1 vs. Population 2 Null Null: Population 2 vs. Polulation 3

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