POWER ANALYSIS

Bioinformatics Awareness Days @ TIGEM
July 10th, 2023

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BIOINFORMATICS CORE
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Bioinformatics Core: Tasks

- **STATISTICAL DATA ANALYSIS**
  Experimental Design, Hypothesis Testing, Power Analysis Differential Expression Analysis, Cluster Analysis, Time Series Data Analysis, Survival Analysis, Correlation Analysis
- **OMICS**
  Microarray Analysis, Gene Networks, Pathway Analysis, TFBS Identification, Gene Annotation, Integration, Protein Analysis, Drug Networks
- **NEXT GENERATION SEQUENCING**
  Whole Exome, Targeted Gene, RNA, miRNA, ChIP, Visualization, Interpretation
- **DATABASE AND SOFTWARE**
  DB Creation, DB Maintenance, Web Sites Creation, Web Service Support
- **BIOINFORMATICS AND (BIO)STATISTICS TRAINING**

Bioinformatics Core: People

DIEGO DI BERNARDO

https://www.tigem.it/research/facilities/core-facilities/bioinformatics

https://bioinformatics.tigem.it/

DIEGO CARRELLA

ROSSELLA DE CEGLI

XAVIER BUJANDA CUNDIN

EUGENIO DEL PRETE

Bioinformatics Core: Something about Me

- **TLC ENGINEER** @ UNIVERSITY OF ROME ‘SAPIENZA’
  MAIN TOPICS: Signal Processing, Remote Sensing, Bioinformatics
  THESIS: miRNA Analysis, Genomic Data Mining, Consensus Analysis, PSSM Creation
- **BIOINFORMATICS RESEARCH FELLOW** @ INSTITUTE OF FOOD SCIENCES (CNR)
  Protein Prediction and Classification, Protein Analysis, Proteomic Mass Spectra Analysis, Sequence Alignment and Phylogenetic Tree, Docking
- **PHD IN APPLIED BIOLOGY** @ UNIVERSITY OF BASILICATA
  Celiac Disease and Comorbities, Microarray Data Analysis, Ontologies, Gene Set Enrichment Analysis, Semantic Similarity, Proteomic Mass Spectra Analysis
- **BIOINFORMATICS RESEARCH FELLOW** @ INSTITUTE OF APPLIED MATHEMATICS (CNR)
  Proteomic Mass Spectra Analysis, Metabolomic (Lipidomic) Data Analysis, Web Tools Developer, Hypothesis Tests, Omics Data Integration
- **BIOSTATISTICIAN AND DATA SCIENTIST** @ TIGEM

Outline

DEFINITIONS
- Sample Size
- Replicates
- Power
- Significance Level
- Effect Size
- Power Analysis

G*POWER
- Tool
- Graphical User Interface

EXAMPLES
- One Way ANOVA
- Repeated Measure ANOVA
- Multi Way ANOVA

CONCLUSION
- Take Home Message
- Final Remarks

Sample Size

Question

- **How many samples do I need for my experiment?**

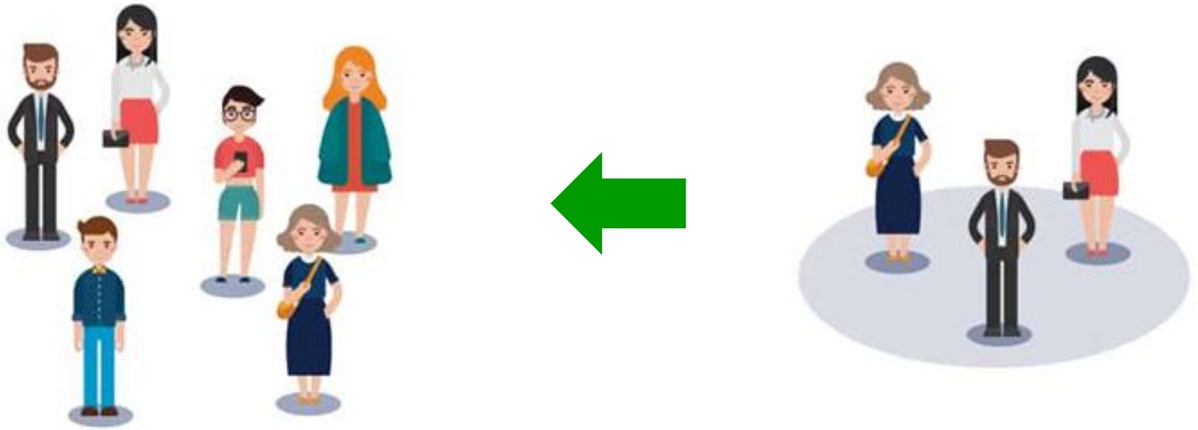
Problems

- **Impossibility** to collect data from an entire population of interest
- **Too small**: under-detection of effect of interest
- **Too large**: unnecessary wasting of resources and/or animals

Goal

- Enough samples to **reasonably detect an effect** (if it really exists) **without wasting limited resources** on too many samples

POPULATION SAMPLE



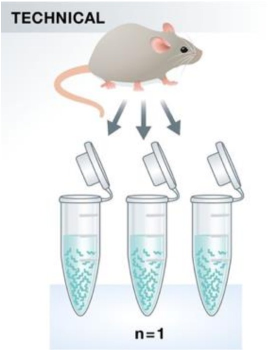
REALITY ≈ MODEL

a) PROBABILITY OF ERROR
b) MARGIN OF ERROR

Replicates

Technical Replicates

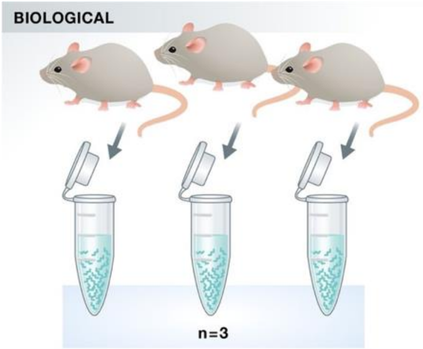
- Use the same biological sample to repeat the technical or experimental steps in order to accurately measure technical variation and remove it during analysis



WITHIN VARIABILITY

Biological Replicates

- Use different biological samples of the same condition to measure the biological variation between samples



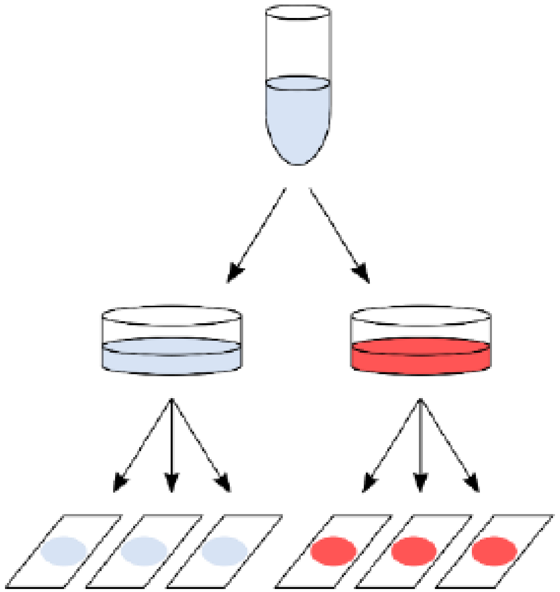
BETWEEN VARIABILITY

WHY THREE (3) IS THE ‘MAGIC’ NUMBER?

Focus on: Experimental Design and Sample Size

Design 1

- Single vial, two culture dishes
- Same time for cellular growth
- Cells counting for the glass slides
- **Which is the sample size?**



ANSWER: N = 1

There is not independence between the three glass slides within the two conditions

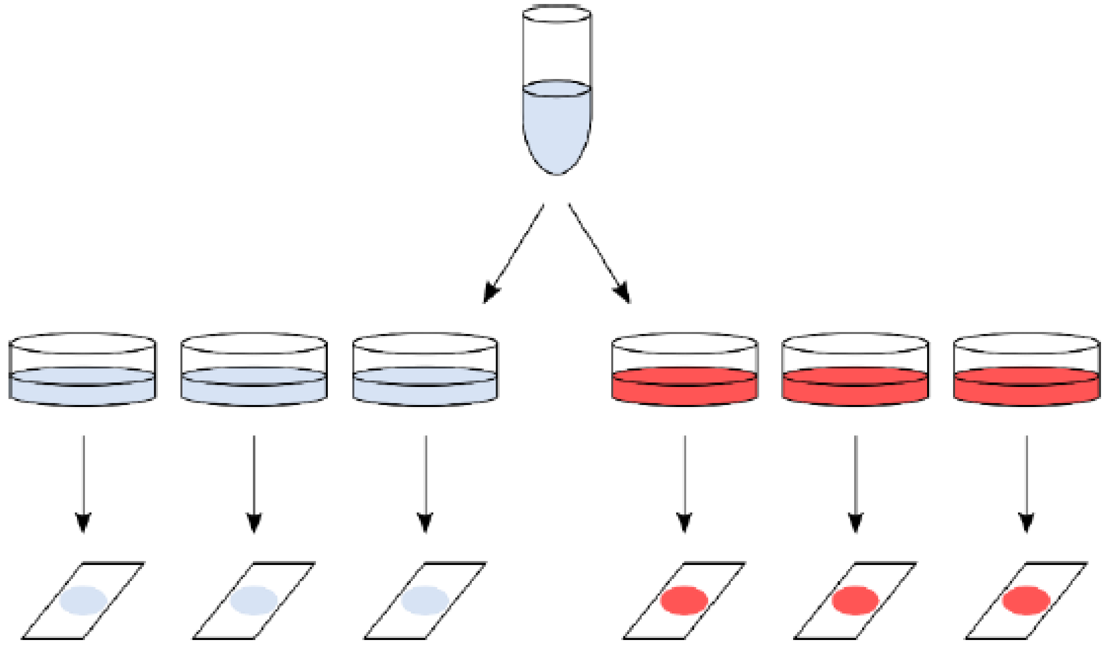
AND

The variability among the glass slides is only due to pipetting error.

Focus on: Experimental Design and Sample Size

Design 2

- Single vial, six culture dishes
- Same time for cellular growth
- Cells counting for the glass slides
- **Which is the sample size?**



ANSWER: N = 1

The glass slides are processed in the same day, same medium, same incubator, and same time.

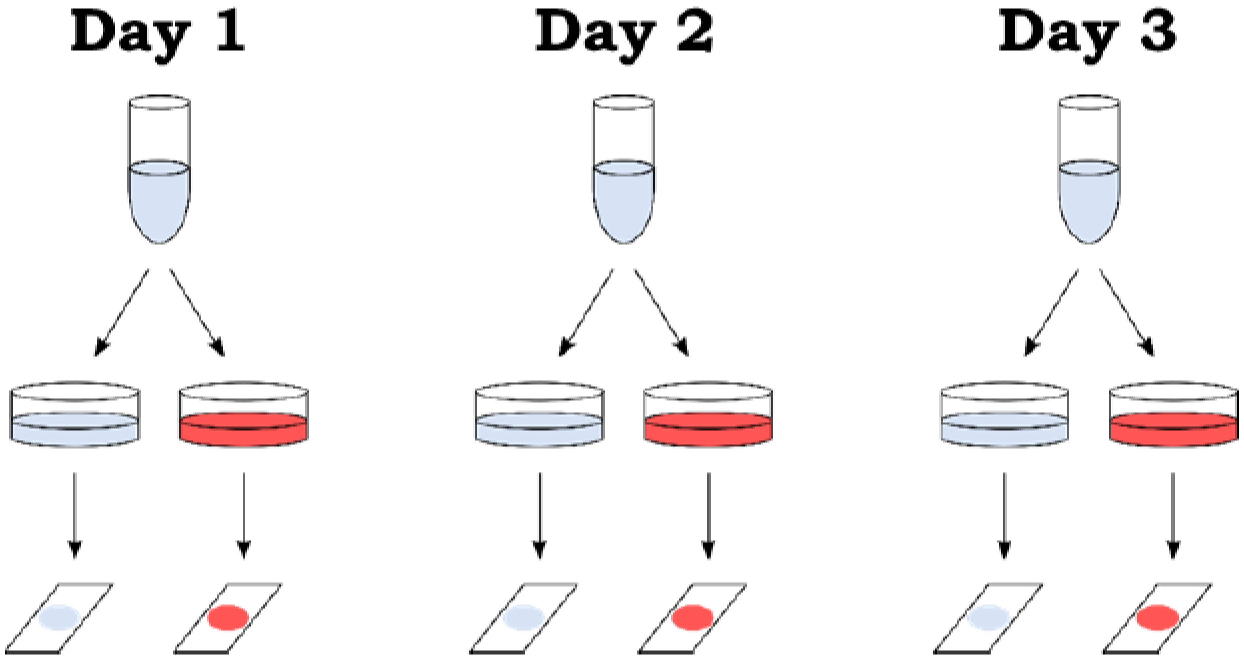
AND

Different plastic does not provide independence.

Focus on: Experimental Design and Sample Size

Design 3

- Three vials, one sample, six culture dishes
- Three time for cellular growth
- Cells counting for the glass slides
- **Which is the sample size?**



ANSWER: N = 3

Three different times introduce the independence, even if the variability is still more technical than biological

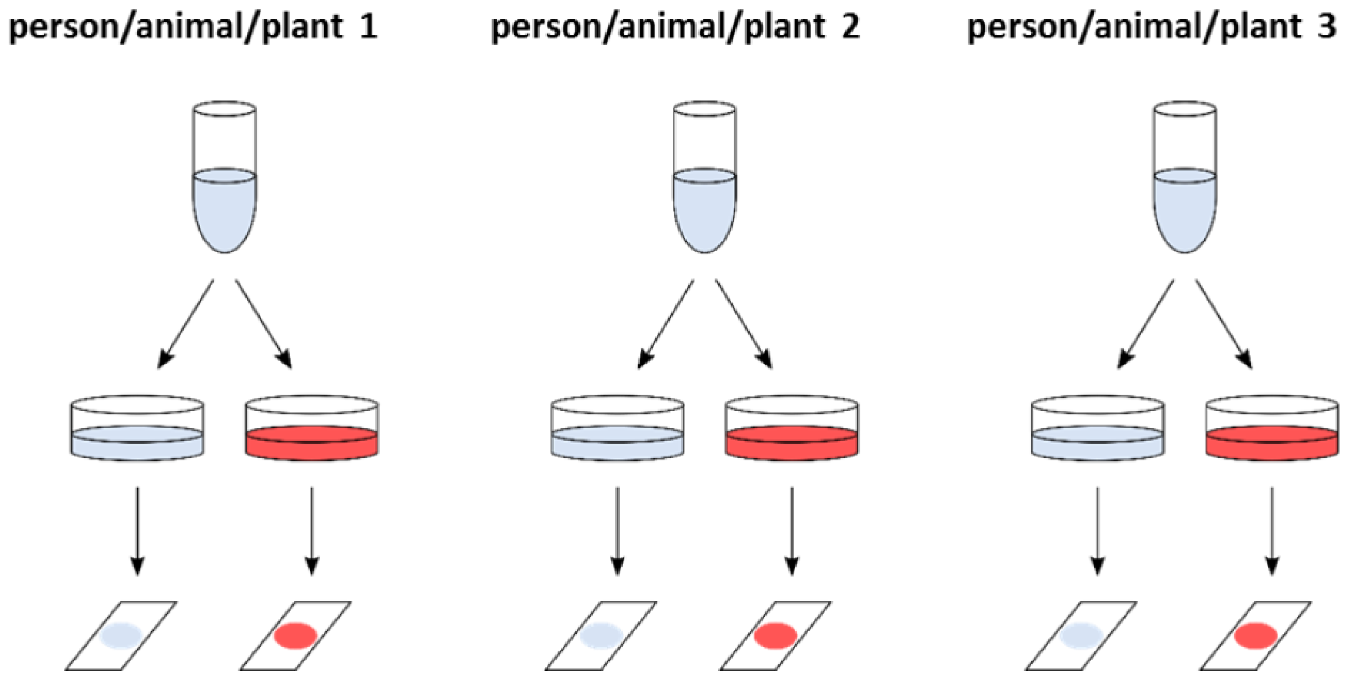
AND

The glass slides for each time are paired

Focus on: Experimental Design and Sample Size

Design 4

- Three vials, three samples, six culture dishes
- Same time for cellular growth
- Cells counting for the glass slides
- **Which is the sample size?**



<u>ANSWER: N = 3</u>

Three independent samples provides biological replicates

AND

The glass slides for each sample are paired

AND

Pay attention to batch effects (e.g., litter)

Power and Significance Level

- H0: LEMUR (NOT SLOTH)

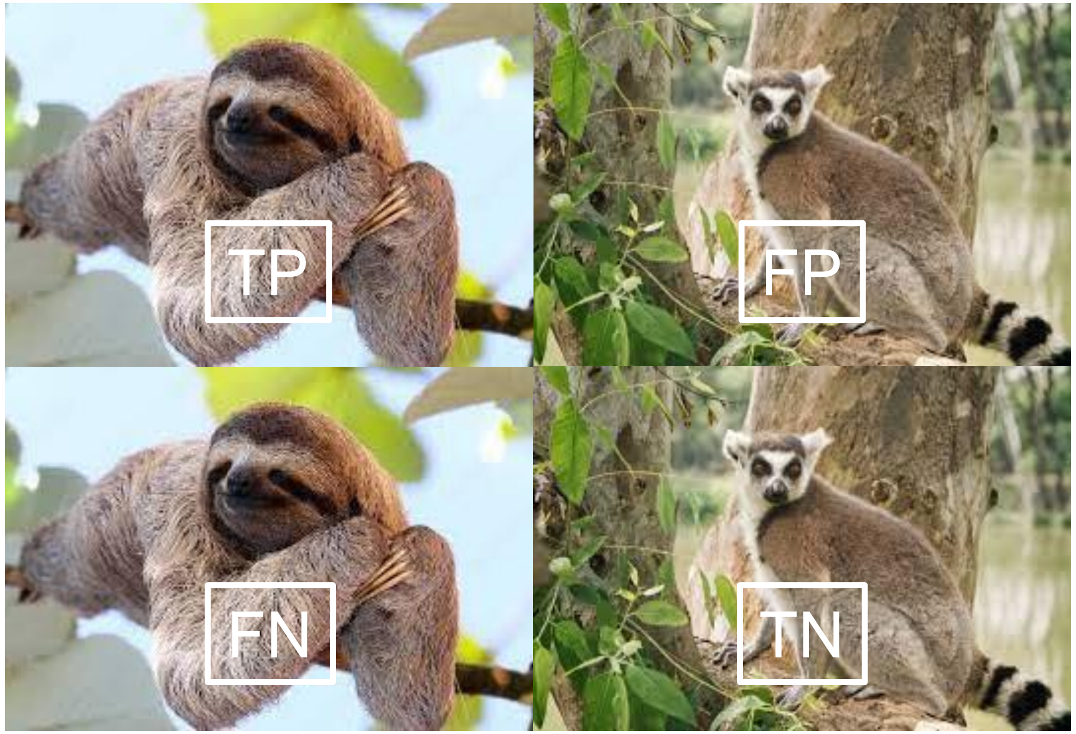
REALITY

H0 FALSE | H0 TRUE

STUDY FINDINGS

REJECT H0

ACCEPT H0



TRUE POSITIVE (TP) - POWER (1-β)
Probability to REJECT H0 when H0 is FALSE

FALSE POSITIVE (FP) - TYPE I ERROR, α
Probability to REJECT H0 when H0 is TRUE

FALSE NEGATIVE (FN) - TYPE II ERROR, β
Probability to ACCEPT H0 when H0 is FALSE

TRUE NEGATIVE (TN)
Probability to ACCEPT H0 when H0 is TRUE

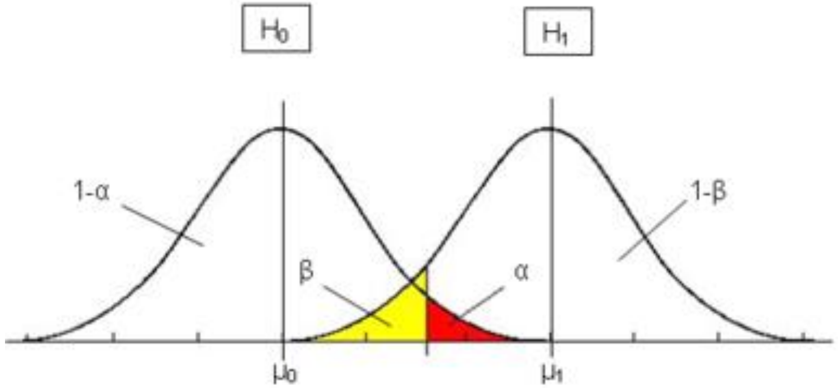
Power and Significance Level

Power (1-β)

- **Probability** to reject a **false** null hypothesis (H0) when the alternative hypothesis (H1) is **true**
- **Probability** of detecting a **true difference** (there is a difference to detect)
- Direct relation with the **Type II Error**

Significance Level (α)

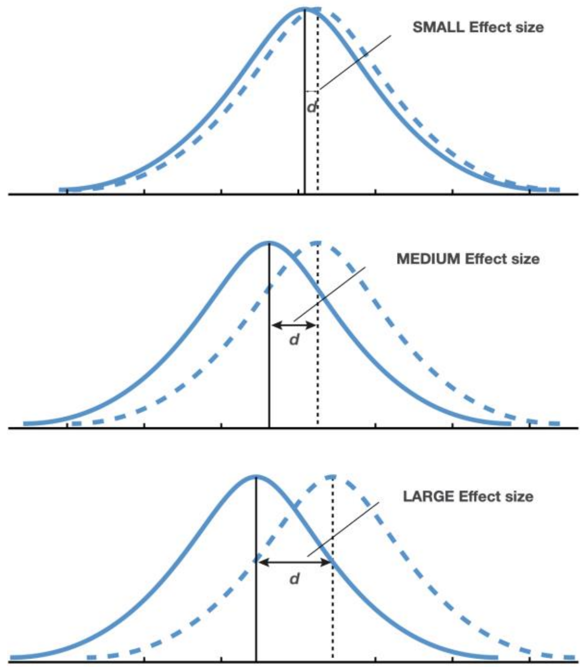
- **Probability** to reject a **false** null hypothesis (H0) when the null hypothesis (H0) is **true**
- **Probability** of detecting a **false difference** (there is not a difference to detect)
- It is the **Type I Error**



Effect Size

Considerations

- **Property** of the sample data (unlike power and significance level)
- To be **estimated**
- **Different statistical** tests have **different values for each category**



Estimation

- Background information from **preliminary data** or **trial data** or similar study
- **Convention** (forced or not)

Formula (Generic)

$$Effect\ Size \approx \frac{Mean\ of\ H1 - Mean\ of\ H0}{Standard\ Deviation}$$

Test	Relevant effect size	Effect Size Threshold		
		Small	Medium	Large
t-test for means	d	0.2	0.5	0.8
F-test for ANOVA	f	0.1	0.25	0.4
t-test for correlation	r	0.1	0.3	0.5
Chi-square	w	0.1	0.3	0.5
2 proportions	h	0.2	0.5	0.8

Power Analysis

Hypothesis
- Biological question
- **One Tail** or **Two Tails** test

One Tail: $\begin{cases} H_0: \mu_0 > \mu_1 \text{ or } H_0: \mu_0 < \mu_1 \\ H_1: \mu_0 < \mu_1 \text{ or } H_1: \mu_0 > \mu_1 \end{cases}$

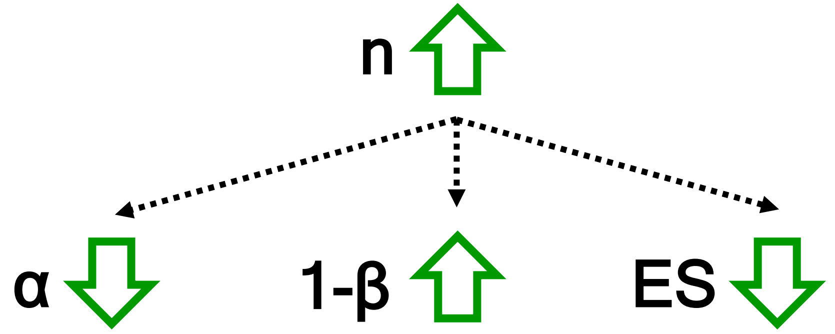
Two Tails: $\begin{cases} H_0: \mu_0 = \mu_1 \\ H_1: \mu_0 \neq \mu_1 \end{cases}$

Experimental Design
- **Variables** (Treatment, Timepoint, Dose, Sex, …)

Statistical Test
- **Comparison** of interest
- **Type of variables**

Parameters
- **Power (1-β)**
- **Significance Level (α)**
- **Effect Size (ES)**
- **Sample Size (n)**

WHICH IS THE LINK WITH n AMONG THE PARAMETERS?



Focus on: Type of Power Analysis

A Priori Power Analysis

- Determine **how many samples** you will need to have a good chance of detecting an effect of a specified size with the desired amount of power

⇩

GIVEN α, 1-β, ES
THEN COMPUTE n

A priori analyses are performed as **part of the research planning process**. They allow you to determine the sample size you need in order to reach a desired level of power.

A Posteriori Power Analysis

- Allow to find **how much power** you would have if you had a specified and sufficient number of samples when you had already conducted a research

⇩

GIVEN α, n, ES
THEN COMPUTE 1-β

Post hoc analyses are performed after your study has been conducted and can be used to assist in **explaining any potential non-significant results**.

G*Power

Heinrich Heine Universität Düsseldorf
Cognitive and Industrial Psychology

Availability

- **WINDOWS:** 3.1.9.7 (March 2020)
- **MAC**: 3.1.9.6 (February 2020)

Statement

- **Free** software for everyone
- **Downloadable** from the webpage
- **Screenshot** without asking permissions

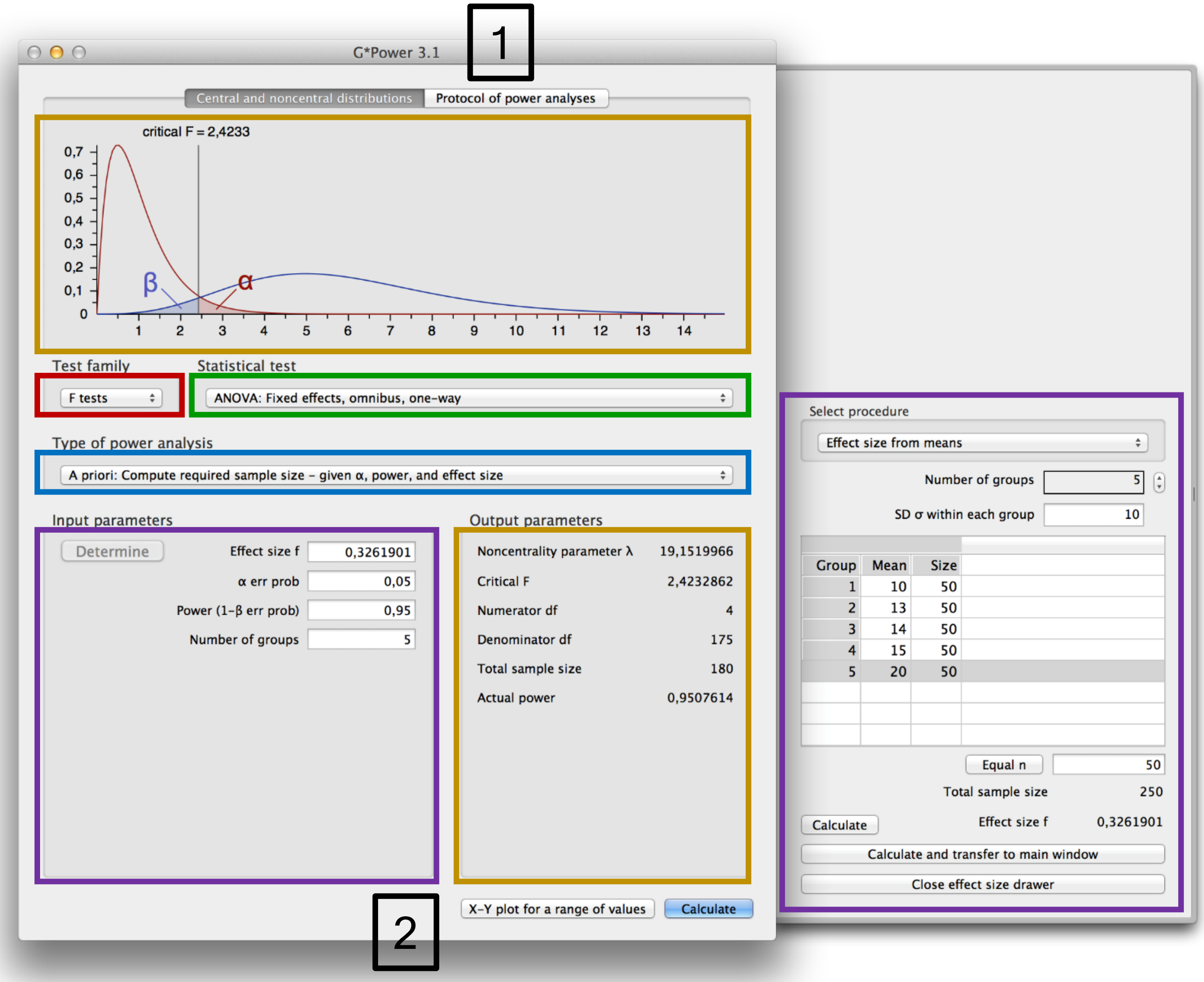
Analysis Type

- Selection for the **parameter of interest**
- Selection for the **test of interest**

Analysis Type	Calculated Value
A priori	Required sample size
Compromise	Implied power and alpha values
Criterion	Required alpha value
Post hoc	Achieved power
Sensitivity	Required effect size

EXACT: 8 specific + 1 general
F-TEST: 16 specific + 1 general
T TEST: 11 specific + 1 general
χ^2 TEST: 2 specific + 1 general
Z TEST: 7 specific + 1 general

G*Power GUI



a) Type of power analysis

b) Test family

c) Statistical test

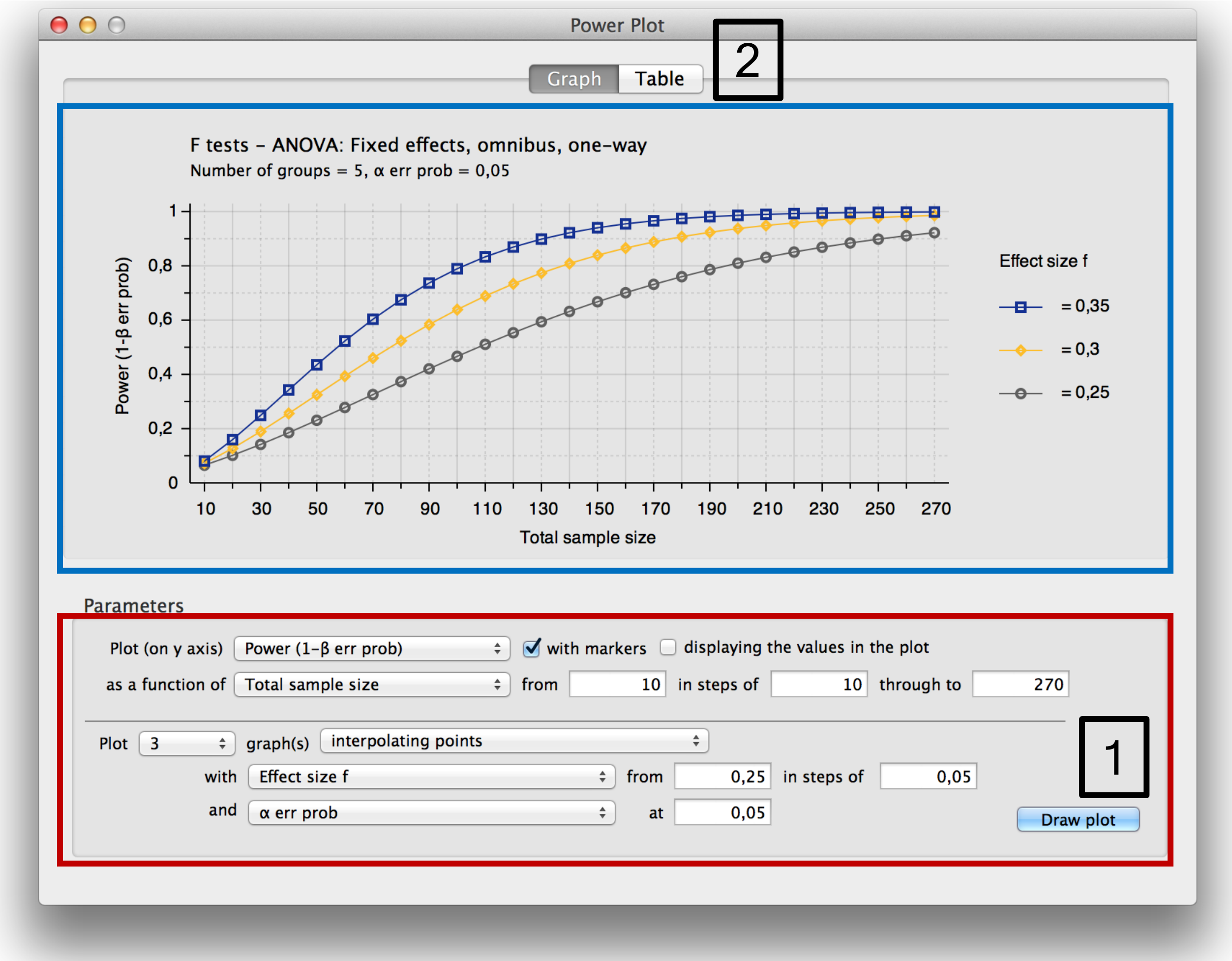
d) Input parameters (with values)

e) Output parameters with density plots

If you click on:

1) **Protocol of power analysis**: Table with I/O parameters
2) **X-Y plot for a range of values:** Plot for parameters

G*Power GUI



a) Parameters and attributes

b) Power Plot

If you click on:

1) **Draw plot**: Power Plot
2) **Table**: X/Y values in tabular format

One Way ANOVA

Description

- Check if **at least one mean** is different among groups
- **Normally distributed (numeric) variables**
- Extension of unpaired t-test for more than two groups

Conditions

- **Numeric variables**: one — MEASUREMENTS
- **Categorical variables**: one — E.G. TREATMENT, TIMEPOINT, DOSE
- **Number of group per categorical variable**: more than two
- **Details**: parametric, unpaired — NORMAL DISTRIBUTION AND DIFFERENT SAMPLES

Parameters

- Significance level: 0.05
- Power: 0.80

One Way ANOVA

Example

- Five different medication regimes on patients after surgery
- Differences in white cell counts
- H0: no difference among medication regimes; H1: difference among medication regimes

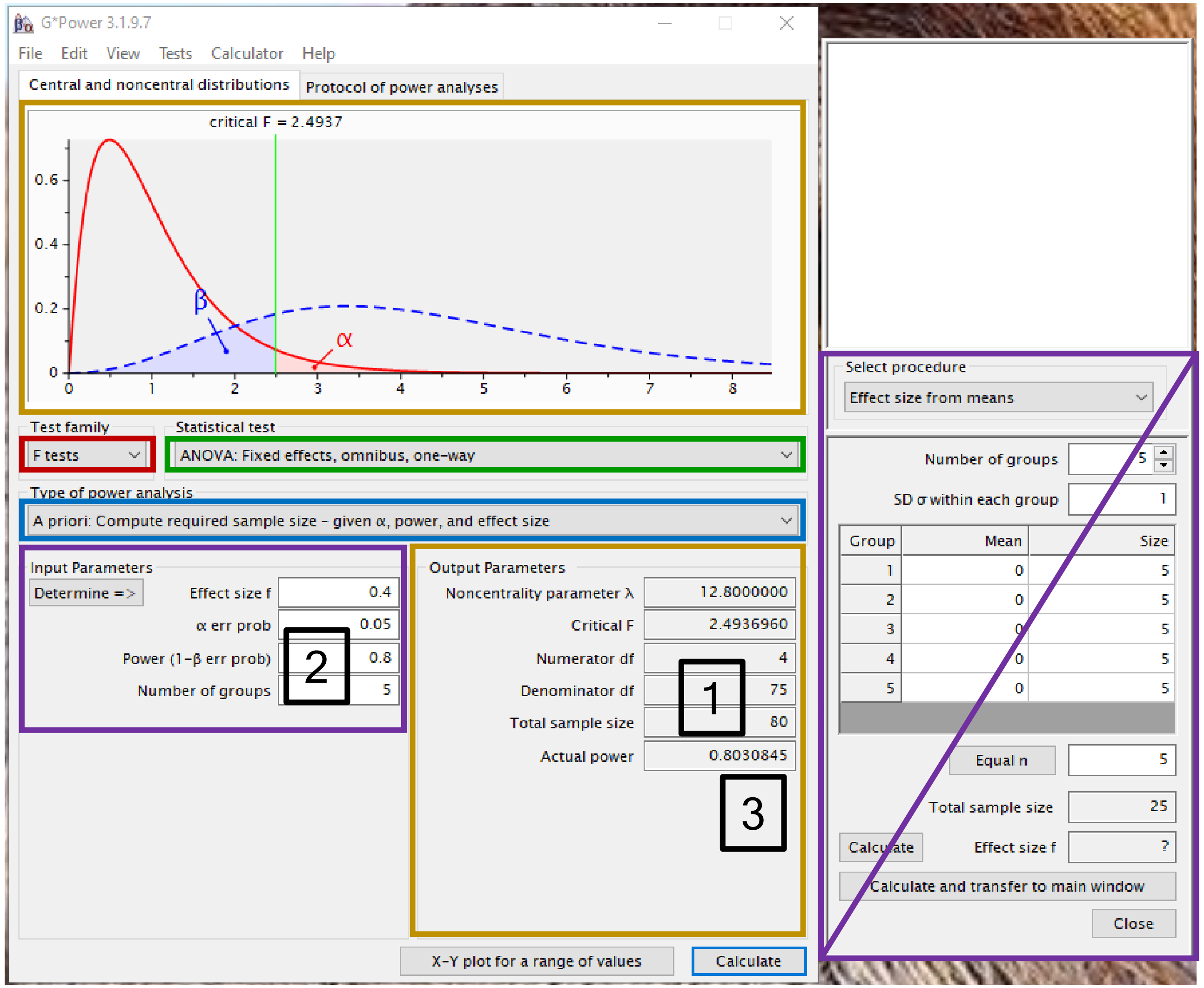
What we know

- **Number of groups**: five
- **Number of measurements**: one per sample
- **Significance level**: 0.05
- **Power**: 0.80

What we guess

- **Effect Size f**: 0.4 (large)

One Way ANOVA



a) A priori: compute required sample size

b) F tests

c) ANOVA: Fixed effects, omnibus, one-way

d) Input parameters (without values)

e) Output parameters with density plots

RESULTS:

1) **Total sample size** (Tss): 80
2) **Sample size per group**: Tss / n_g = 16
3) **Actual power:** 0.801 (check)

Repeated Measure ANOVA

Description

- Check if **at least one mean** is different among groups
- **Normally distributed (numeric) variables**
- Extension of paired t-test for more than two groups

Conditions

- **Numeric variables**: one — MEASUREMENTS
- **Categorical variables**: one — E.G. TREATMENT, TIMEPOINT, DOSE
- **Number of group per categorical variable**: more than two
- **Details**: parametric, paired — NORMAL DISTRIBUTION AND SAME SAMPLES

Parameters

- Significance level: 0.05
- Power: 0.80

Repeated Measure ANOVA

Example

- One treatment on patients
- Four measurements of blood pressure after treatment (1, 2, 3, 4 months)
- H0: no difference during time; H1: difference during time

What we know

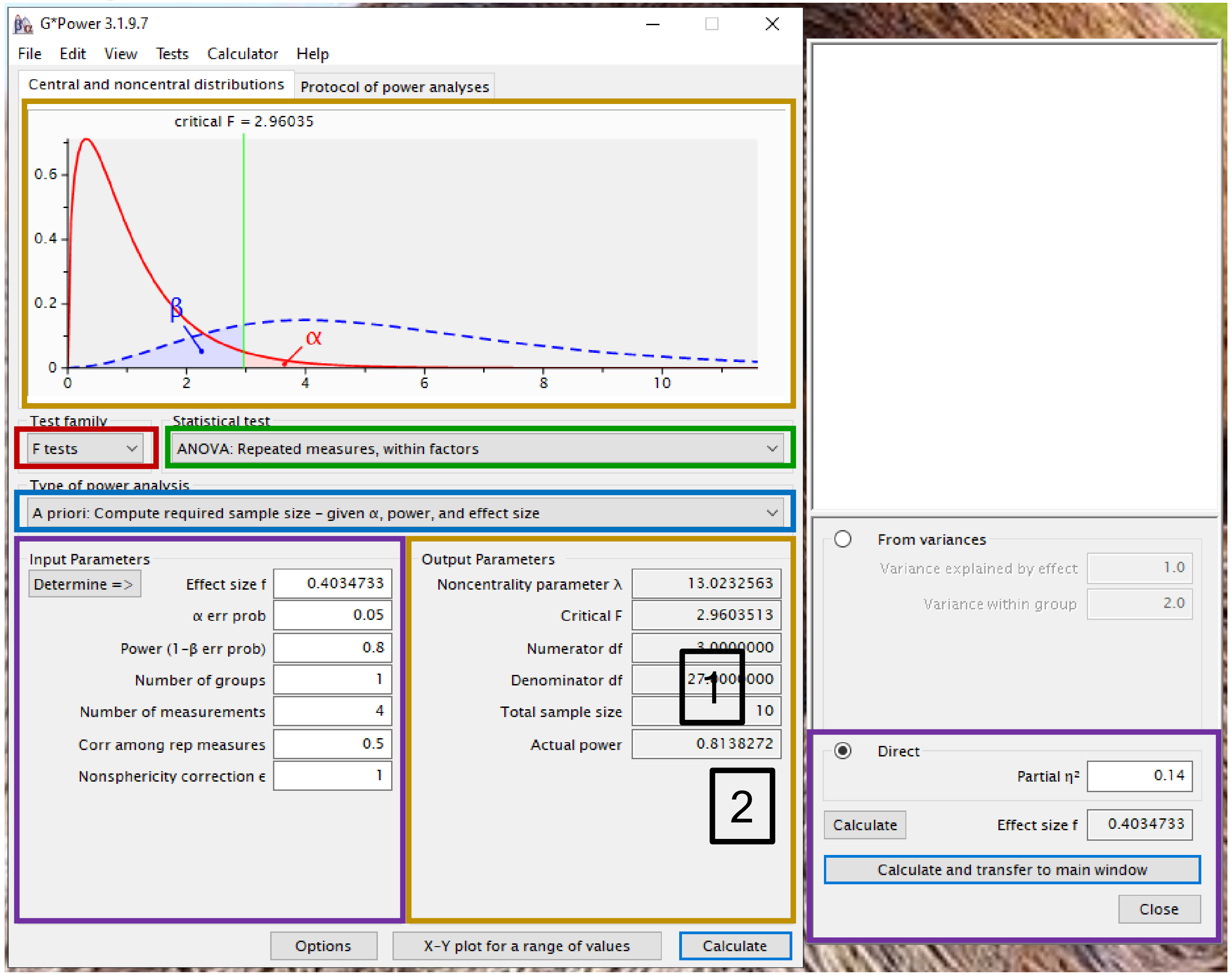
- **Number of groups**: one
- **Number of measurements**: four per sample
- **Significance level**: 0.05
- **Power**: 0.80

What we guess

- **Correlation**: 0.5
- **Non-sphericity correction**: 1
- **Partial** η^2: 0.14 (large)

CORRELATION: relationships among measurements
SPHERICITY: same differences of variances
PARTIAL η^2: ratio between variances (EFFECT SIZE)

Repeated Measure ANOVA



a) A priori: compute required sample size

b) F tests

c) ANOVA: Repeated measures, within factors

d) Input parameters (with Partial η^2)

e) Output parameters with density plots

RESULTS:

1) **Total sample size**: 10
2) **Actual power:** 0.814 (check)

Multi Way ANOVA

Description

- Define the number of **categories of interest** (one or more than one)
- **Normally distributed (numeric) variables**
- Extension of one way ANOVA for more than one category

Conditions

- **Numeric variables**: one — MEASUREMENTS
- **Categorical variables**: more than one — E.G. TREATMENT, TIMEPOINT, DOSE
- **Number of group per categorical variable**: more than one
- **Number of categories of interest**: one or more than one
- **Details**: parametric, unpaired — NORMAL DISTRIBUTION AND DIFFERENT SAMPLES

Parameters

- Significance level: 0.05
- Power: 0.80

Multi Way ANOVA

Example

- Different treatments on patients (a, b, c)
- Different ages (child, adult, elder) and different cancer stages (I, II, III, IV, V)
- H0: no difference in treatment across ages/stages;
  H1: difference in treatment across ages/stages

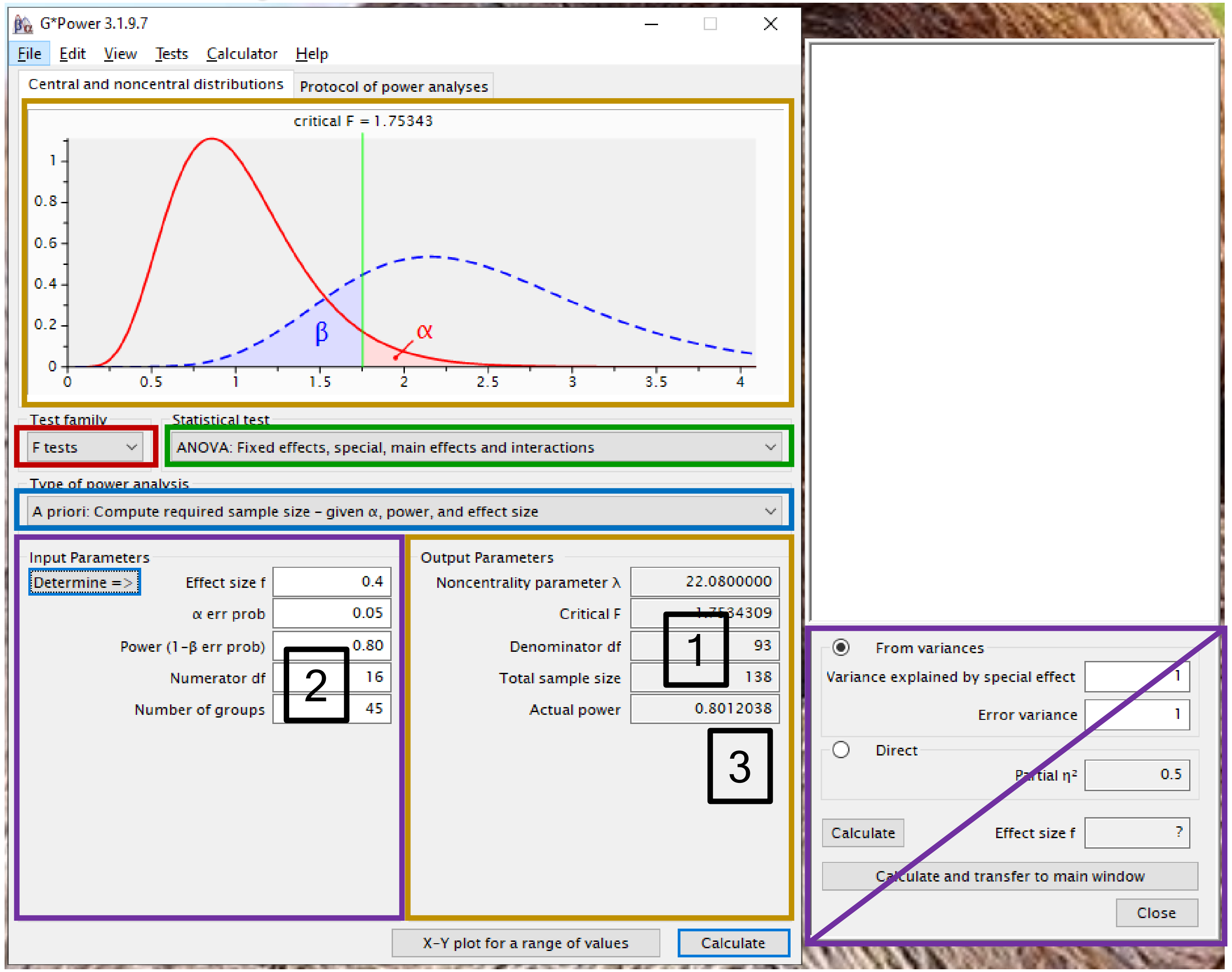
What we know

- **Number of groups**: three treatments (T), three ages (A), five stages (S), $n_g = TAS = 45$
- **Number of degrees of freedom**: $df = (T-1)(A-1)(S-1) = 16$
- **Significance level**: 0.05
- **Power**: 0.80

What we guess

- **Effect Size f**: 0.4 (large)

Multi Way ANOVA



a) A priori: compute required sample size

b) F tests

c) ANOVA: Fixed effects, special, main effects and interactions

d) Input parameters (without values)

e) Output parameters with density plots

RESULTS:

1) **Total sample size** (Tss): 138
2) **Sample size per group**: $Tss/n_g \approx 4$
3) **Actual power:** 0.801 (check)

Focus on: Non-Parametric Tests

Not normally distributed (numeric) variables

- Peculiar distribution and/or low number of samples
- Information obtained from a preliminary/previous/similar experiment
- No relaxation of the hypothesis

Rule of Thumb

- Compute the **parametric test and add 15%** to total sample size
- **Formula**: $Tss_{NP} = Tss + 0.15Tss = 1.15Tss$

Previous Examples

- **One Way ANOVA:** 1.15 x 80 = 92
- **Repeated Measure ANOVA**: 1.15 x 10 ≈ 12
- **Multi Way ANOVA**: 1.15 x 138 ≈ 159

GENERALIZED LINEAR MIXED MODELS

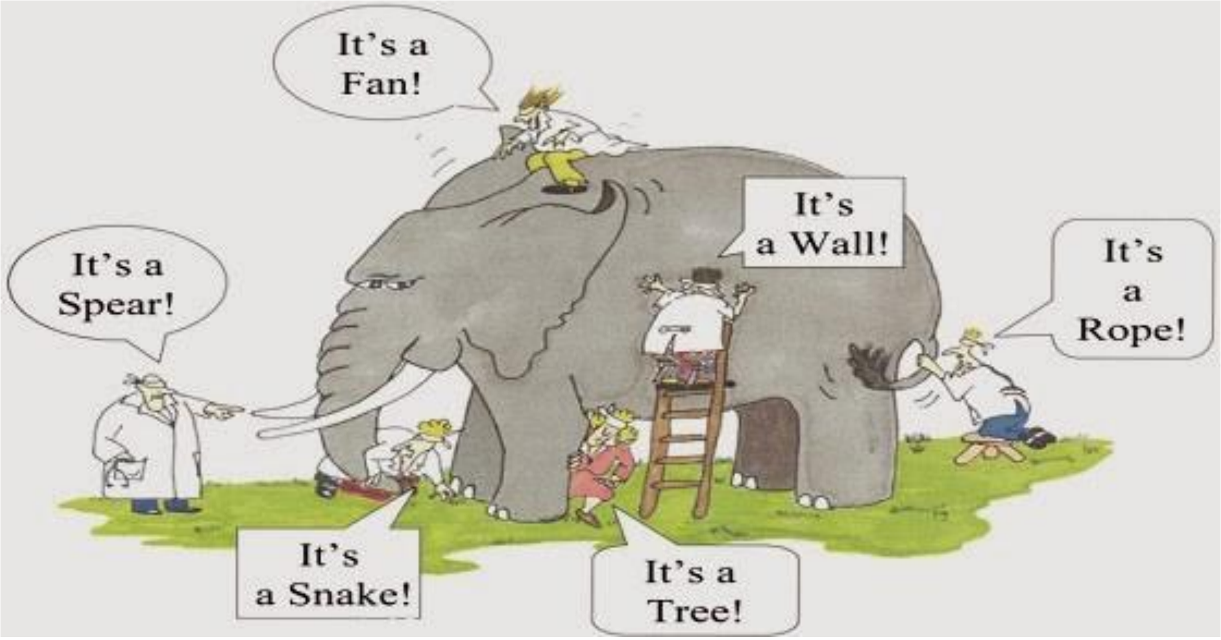
Take Home Message

- **Sample size** is the most important parameter to detect a possible effect among different conditions
- **Sample size**, **significance level**, **power**, and **effect size** are strictly connected each other (considering the distribution of variables)
- **A priori power analysis** provides the minimum number of the samples to detect a possible effect, **with fixed values for the parameters**
- **Different types** of a priori power analysis exist, based on the **parameter of interest** and on the **statistical test** to perform
- **G*Power** is a free, downloadable, and user-friendly tools to perform a priori and a posteriori power analysis

Final Remarks

To consult the statistician after an experiment is finished is often merely to ask him to conduct a postmortem examination. He can perhaps say what the experiment died of.

Sir R. A. Fisher
First Session of the Indian Statistical Conference, Calcutta, 1938



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References

[1] Faul, F. **Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses,** Behavior Research Methods (2009).
[2] Uttley, J. **Power Analysis, Sample Size, and Assessment of Statistical Assumptions - Improving the Evidential Value of Lighting Research**, LEUKOS (2019).
[3] Kang, H. **Sample size determination and power analysis using G*Power software**, Journal of Educational Evaluation of Health Professions (2021).

[h1] *https://www.bioinformatics.babraham.ac.uk/training/*
[h2] *https://med.und.edu/research/daccota/berdc-resources.html*
[h3] *https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower*