R Fundamentals and Best Practices for Mass Spectrometry Data Analysis

Saturday, November 14 (12:00-3:15pm Eastern)

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Module #5: Basic statistics in R

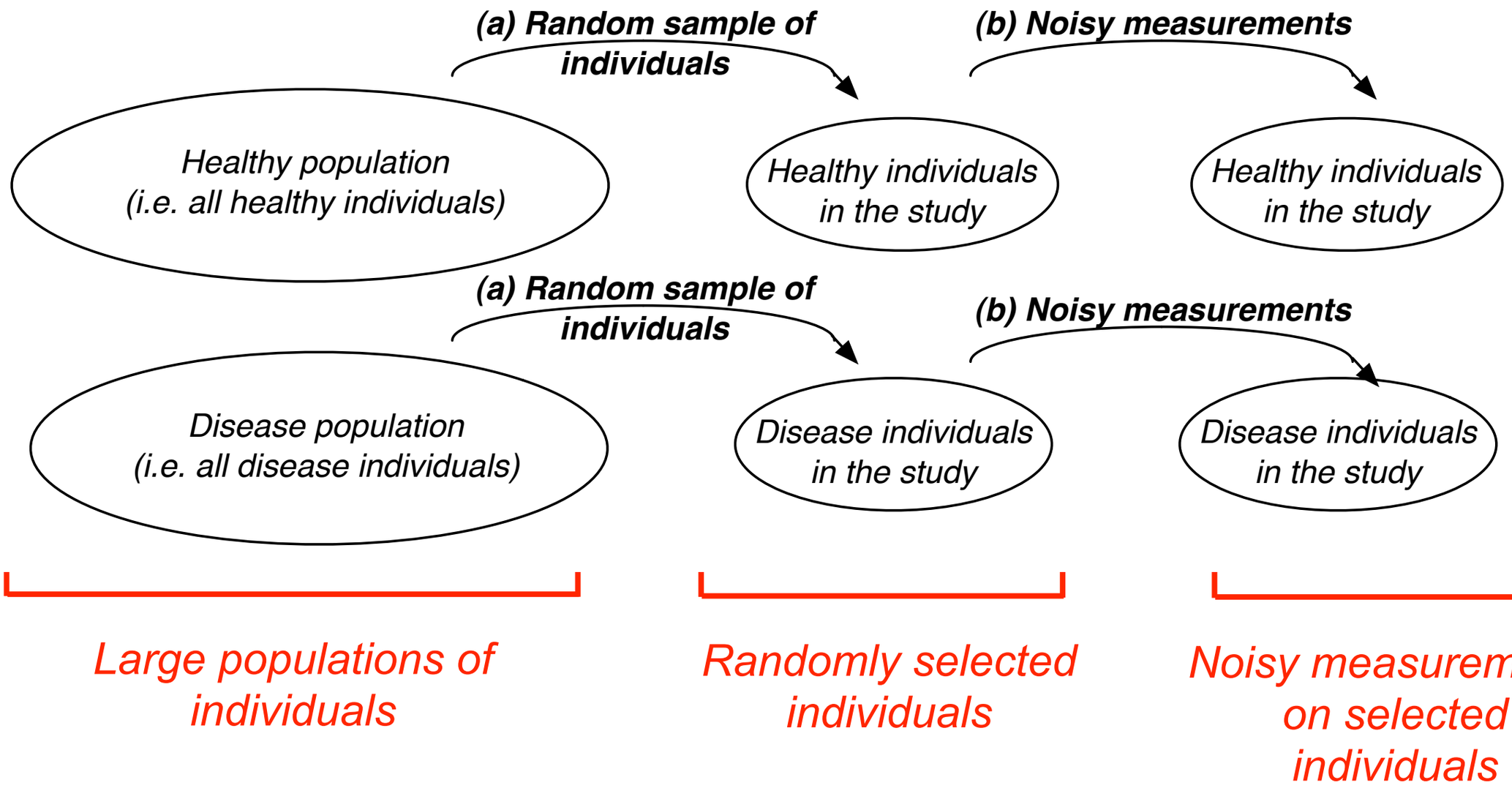
Module #6: Reproducible Data Analysis with RMarkdown

STATISTICAL DESIGN AND ANALYSIS OF EXPERIMENTS: A CRASH COURSE

Beware: this is not enough info!

- Fundamental principles of experimental design
  - Replication, randomization, blocking
- Basics of statistical inference
  - T-test, p-values and error bars
- Adjustments for multiple testing
  - False discovery rate

A STATISTICIAN'S VIEW OF THE EXPERIMENT



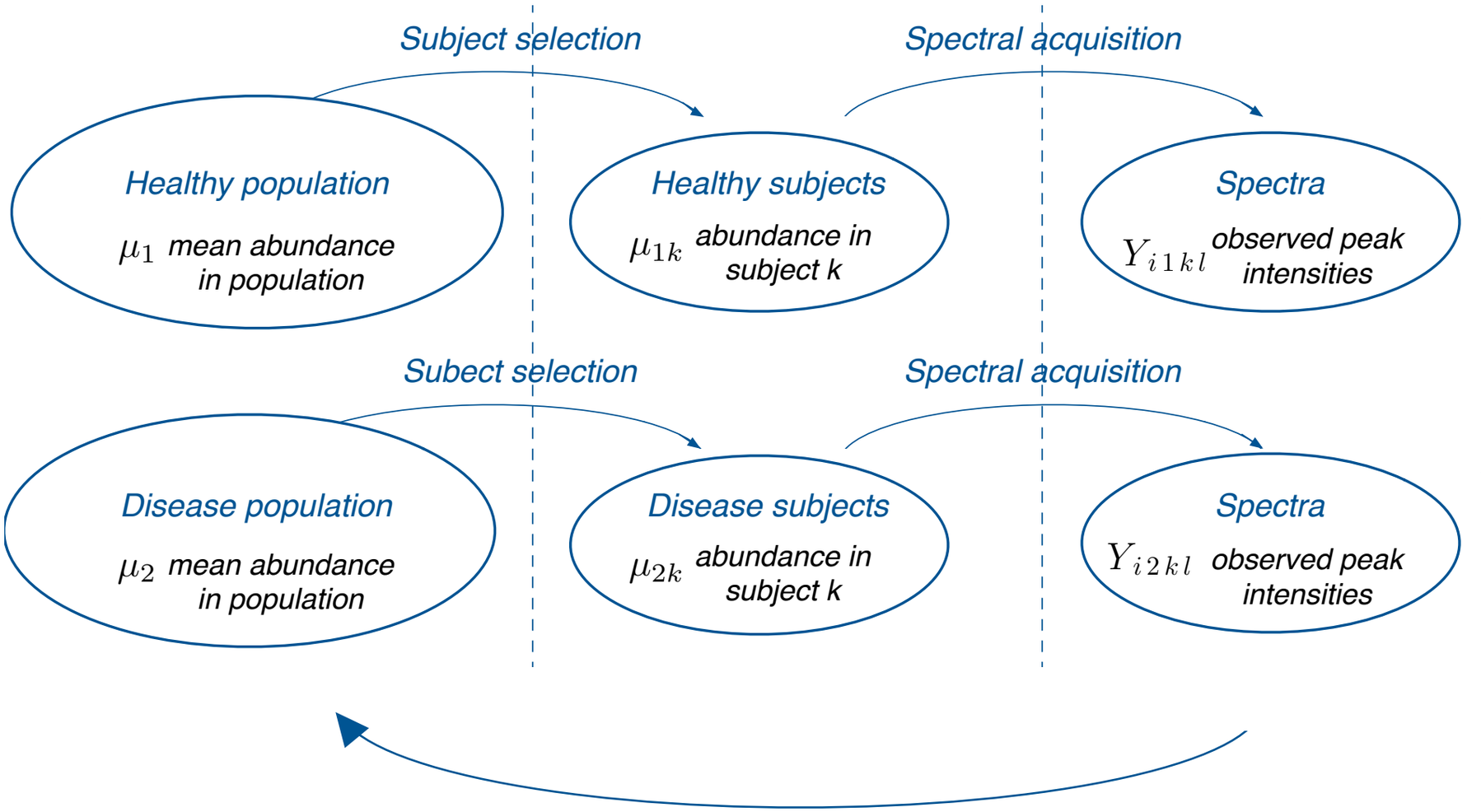
Dangers:

Bias: conclusions systematically differ from truth

Inefficiency: unnecessary variation in the data

COMPARE DESIGNS

In terms of bias and (in)-efficiency



Statistical model: properties of $\bar{Y}_{.1..} - \bar{Y}_{.2..}$

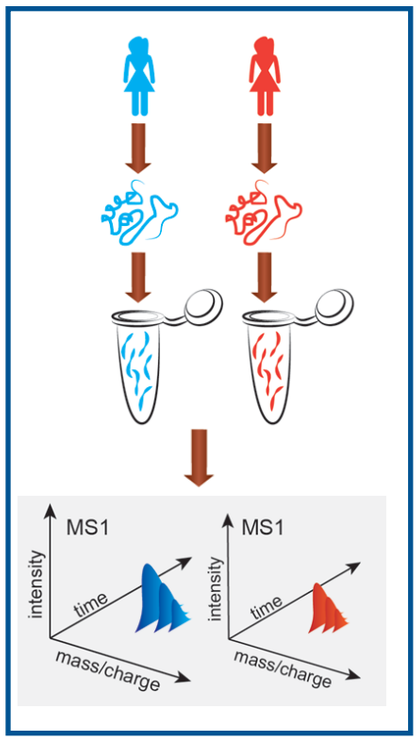
Statistical inference: conclusions on $\mu_1 - \mu_2$

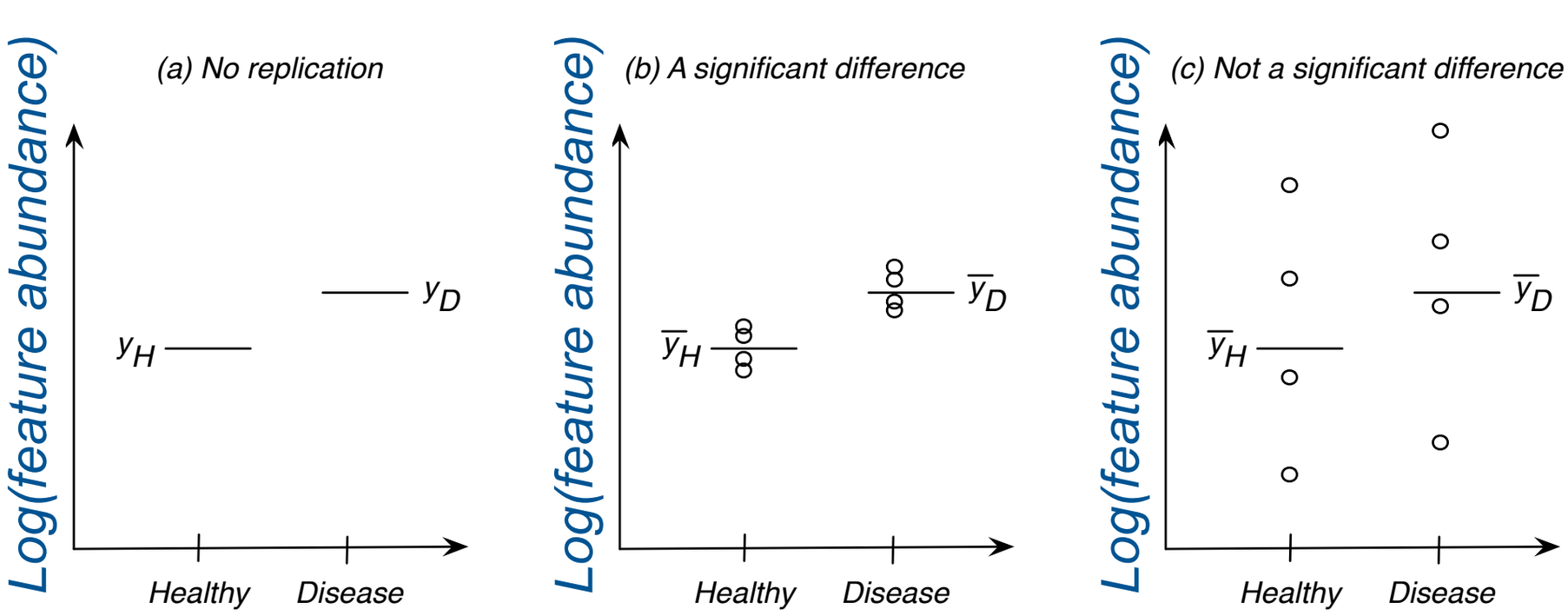
Bias: $\bar{Y}_{.1..} - \bar{Y}_{.2..}$ *systematically different from* $\mu_{1k} - \mu_{2k}$

Inefficiency: *Large* $Var(\bar{Y}_{.1..} - \bar{Y}_{.2..})$

PRINCIPLE 1: REPLICATION

(1) carries out the inference and (2) minimizes inefficiencies





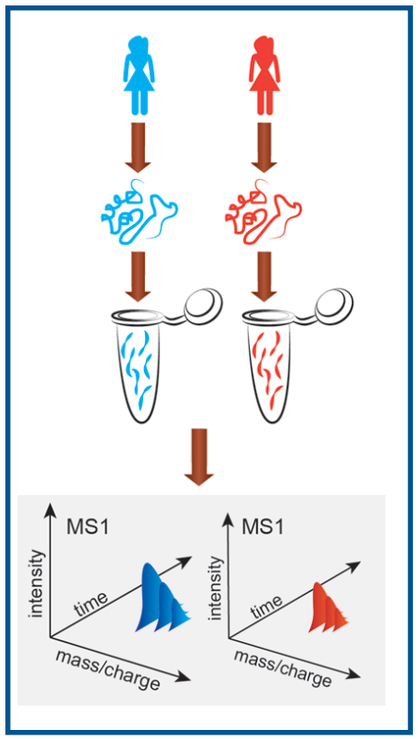
Two levels of randomness imply two types of replication:

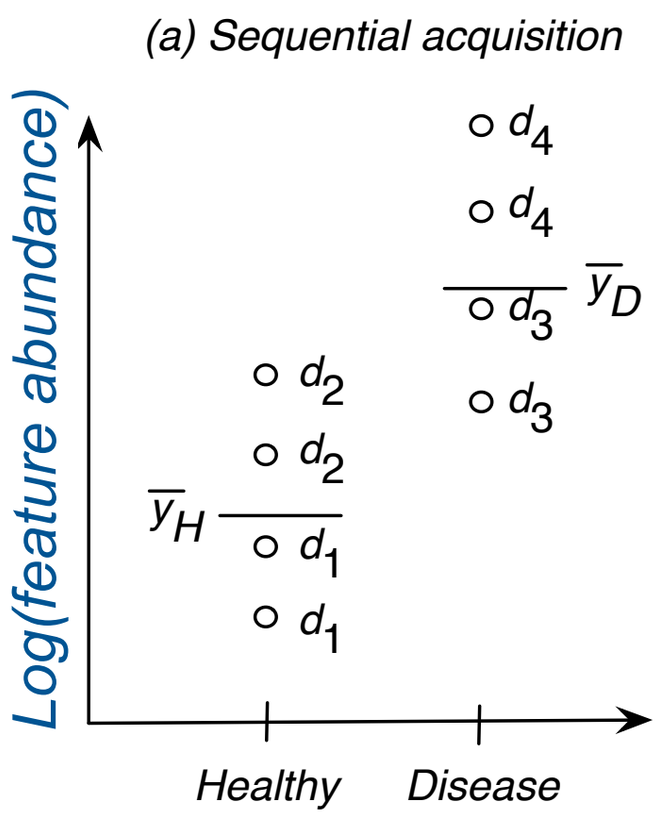
- *Biological replicates:* selecting multiple subjects from the population
- *Technical replicates:* multiple runs per subject

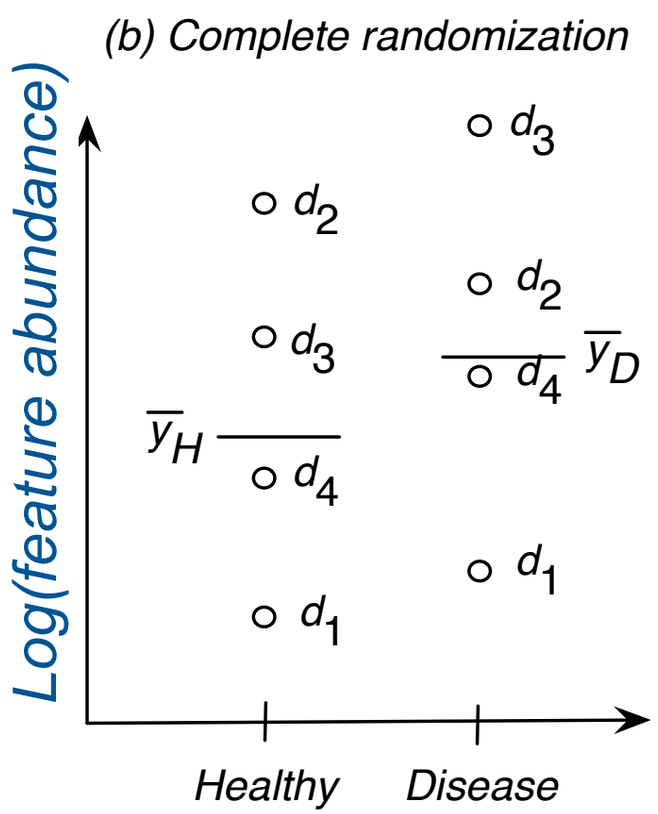
Oberg and Vitek, *J. Proteome Research*, 8, 2009

PRINCIPLE 2: RANDOMIZATION

Prevents bias







No randomization
= confounding
= bias

Complete randomization
= no bias

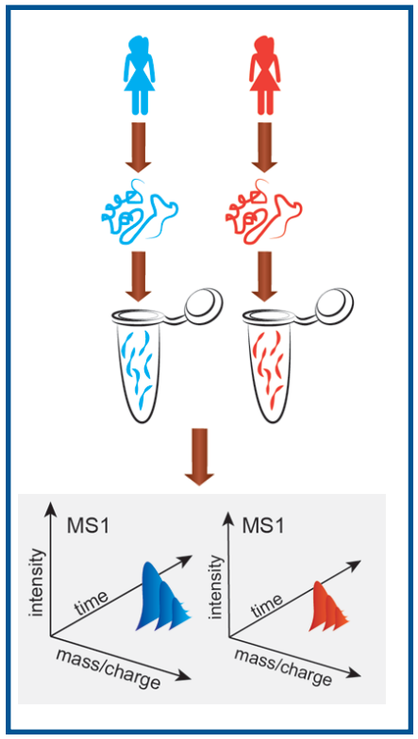
Two levels of randomness imply two types of randomization:

- *Biological replicates:* random selection of subjects from the population
- *Technical replicates:* random allocation of samples to all processing steps

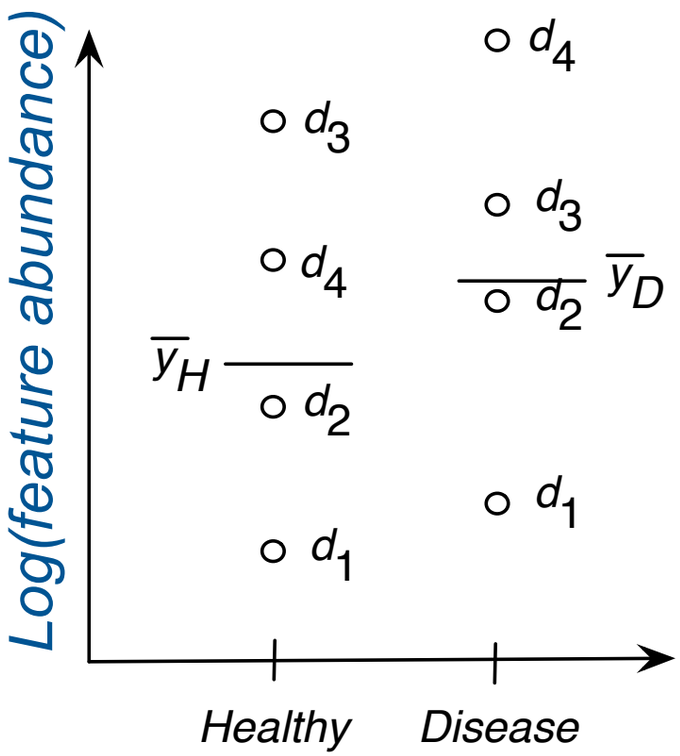
Oberg and Vitek, *J. Proteome Research*, 8, 2009

PRINCIPLE 3: BLOCKING

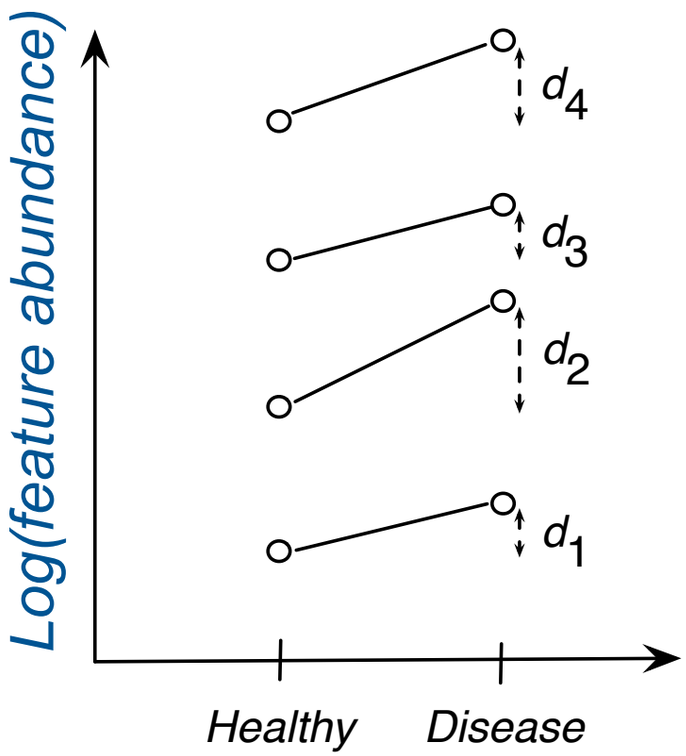
Helps reduce both bias and inefficiency



(b) Complete randomization



(c) Day = block



Complete randomization
= inflated variance

Block-randomization
= restriction on randomization
= systematic allocation

Two levels of randomness imply two types of blocks:

- *Biological replicates:* subjects having similar characteristics (e.g. age)
- *Technical replicates:* samples processed together (e.g. in a same day)

Oberg and Vitek, *J. Proteome Research*, 8, 2009

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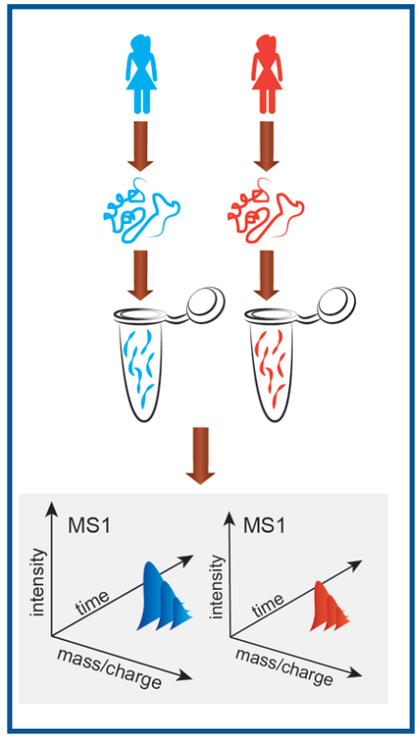
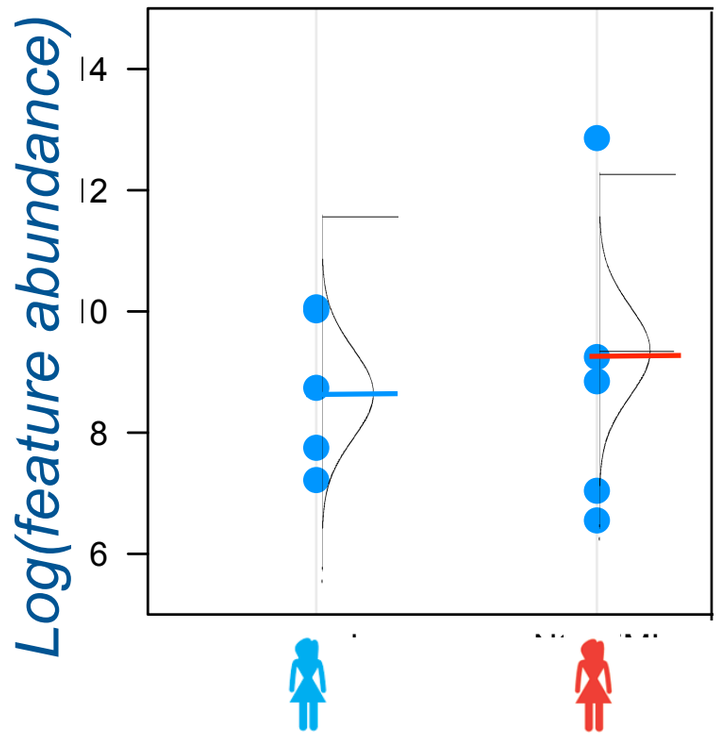
TWO-SAMPLE T-TEST

Simple example: label-free experiment, one feature/protein





Sample means in each group

H0: 'status quo', no change in abundance, $\mu_1 - \mu_2 = 0$

Ha: change in abundance, $\mu_1 - \mu_2 \neq 0$

$$\textbf{observed t} = \frac{\textbf{difference of group means}}{\textbf{estimate of variation}} = \frac{\bar{Y}_{1\cdot} - \bar{Y}_{2\cdot}}{\sqrt{s_1^2/n_1 + s_2^2/n_2}}$$

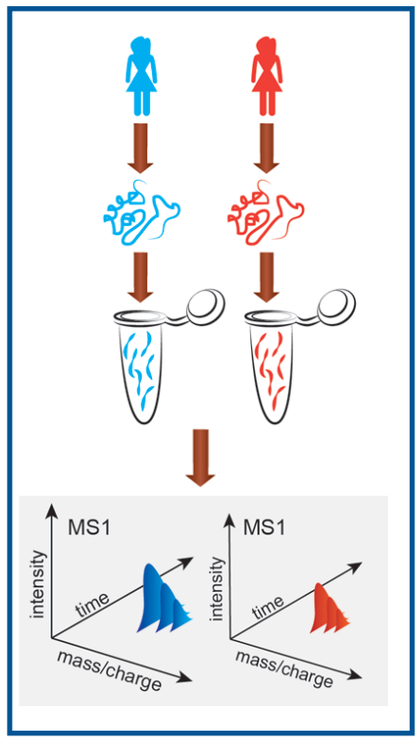
Number of replicates

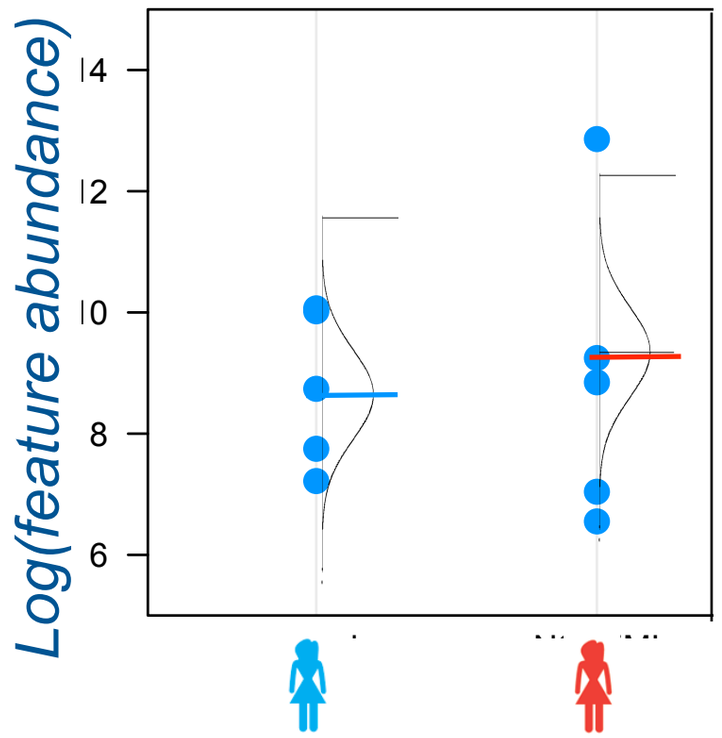
$$s_1^2 = \frac{1}{n_1 - 1} \sum_{i=1}^{n_1} (Y_{1i} - \bar{Y}_{1\cdot})^2$$

Sample variance

TWO-SAMPLE T-TEST

Simple example: label-free experiment, one feature/protein





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$$s_1^2 = \frac{1}{n_1 - 1} \sum_{i=1}^{n_1} (Y_{1i} - \bar{Y}_{1\cdot})^2$$

Properties of the means

$\frac{s_1^2}{n_1}$

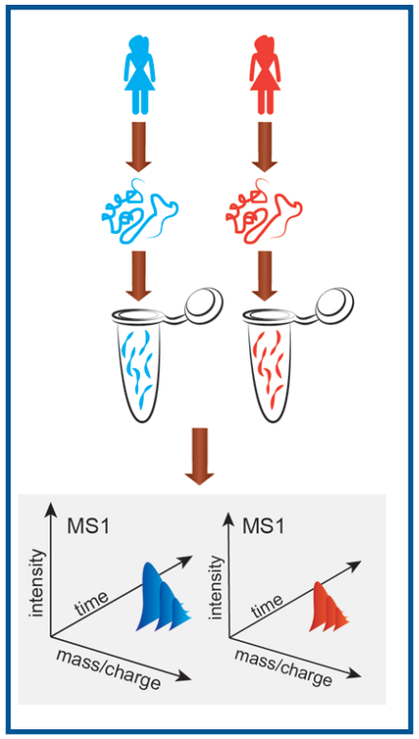
Variance of the sampling distribution of first mean

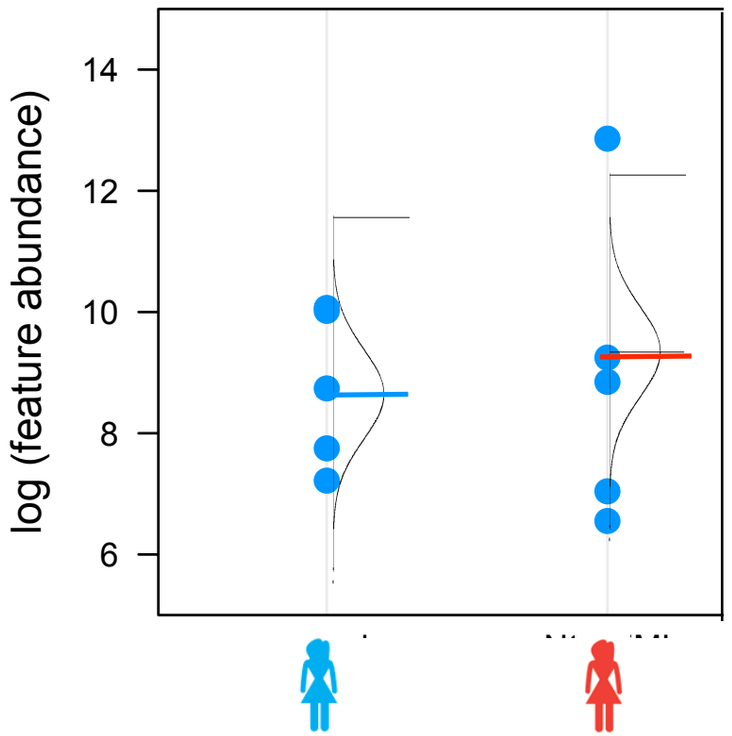
$\sqrt{\frac{s_1^2}{n_1}}$

Standard error of the first mean

FINDING DIFFERENTIALLY ABUNDANT PROTEINS

P-value





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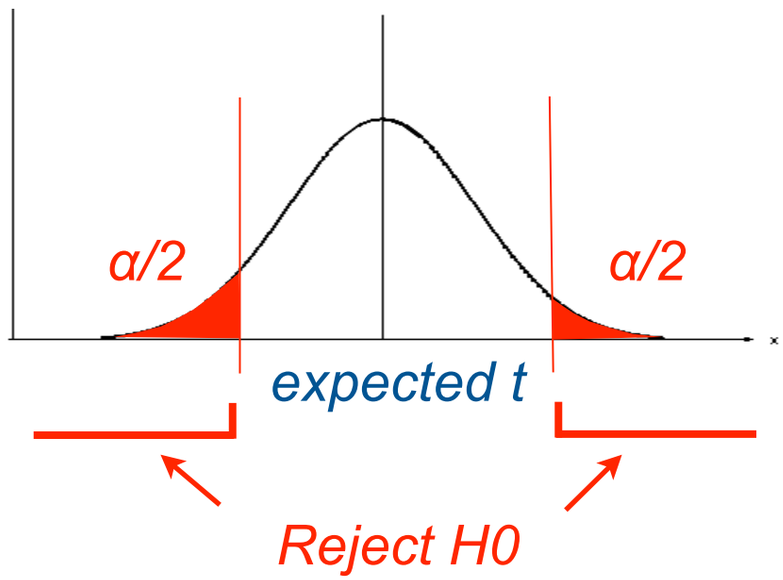
$$\overset{\text{no difference}}{\sim} \textbf{Student distribution}$$

Distribution of the score if H0 is true

α = False Positive Rate



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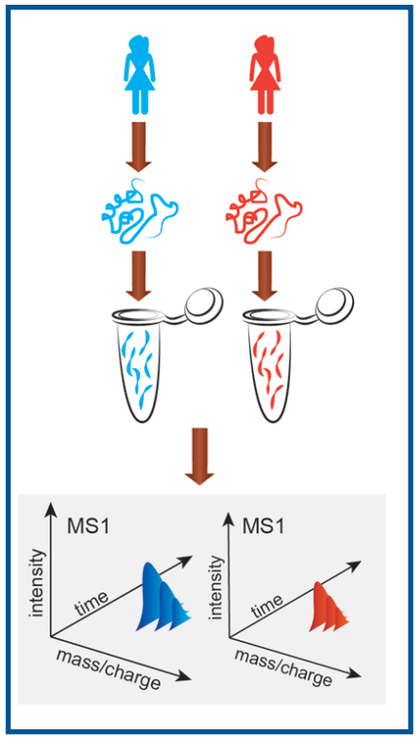

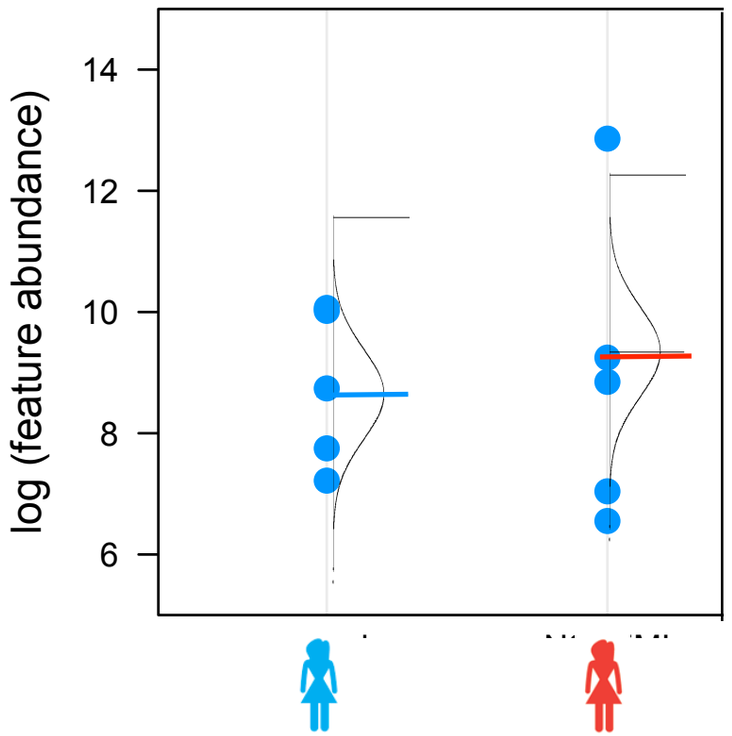

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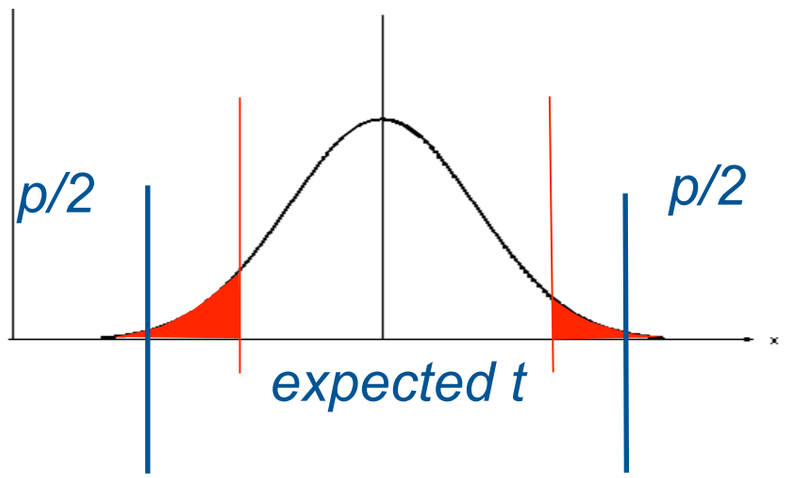
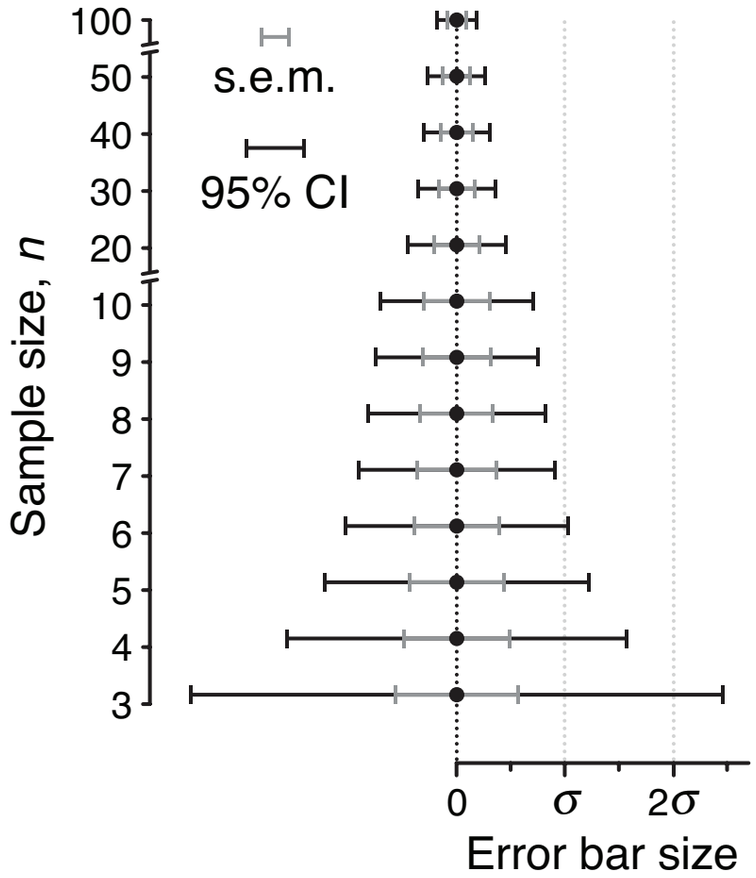
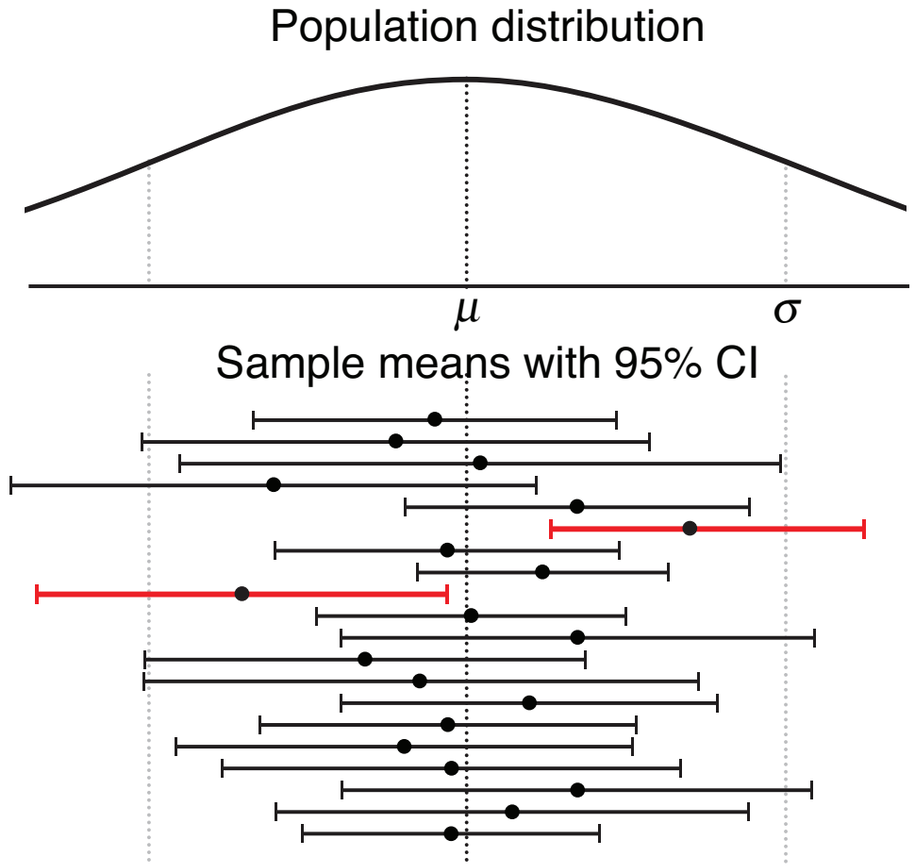


ALTERNATIVE TO TESTING: CONFIDENCE INTERVALS

Not all error bars are made equal





Note on sample size:

- ↑ biological replicates
- ↓ interval width

A 95% CI: if we repeatedly collect data and draw intervals, then 95% of them will contain the true mean

$$\left[(\bar{Y}_{1\cdot} - \bar{Y}_{2\cdot}) - t_{\alpha/2}\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}} \;,\; (\bar{Y}_{1\cdot} - \bar{Y}_{2\cdot}) + t_{\alpha/2}\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}} \right]$$

Krzywinski and Altman, Points of Significance Collection, *Nature Methods*

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MULTIPLE TESTING

Control False Positive Rate for two proteins

For each protein:

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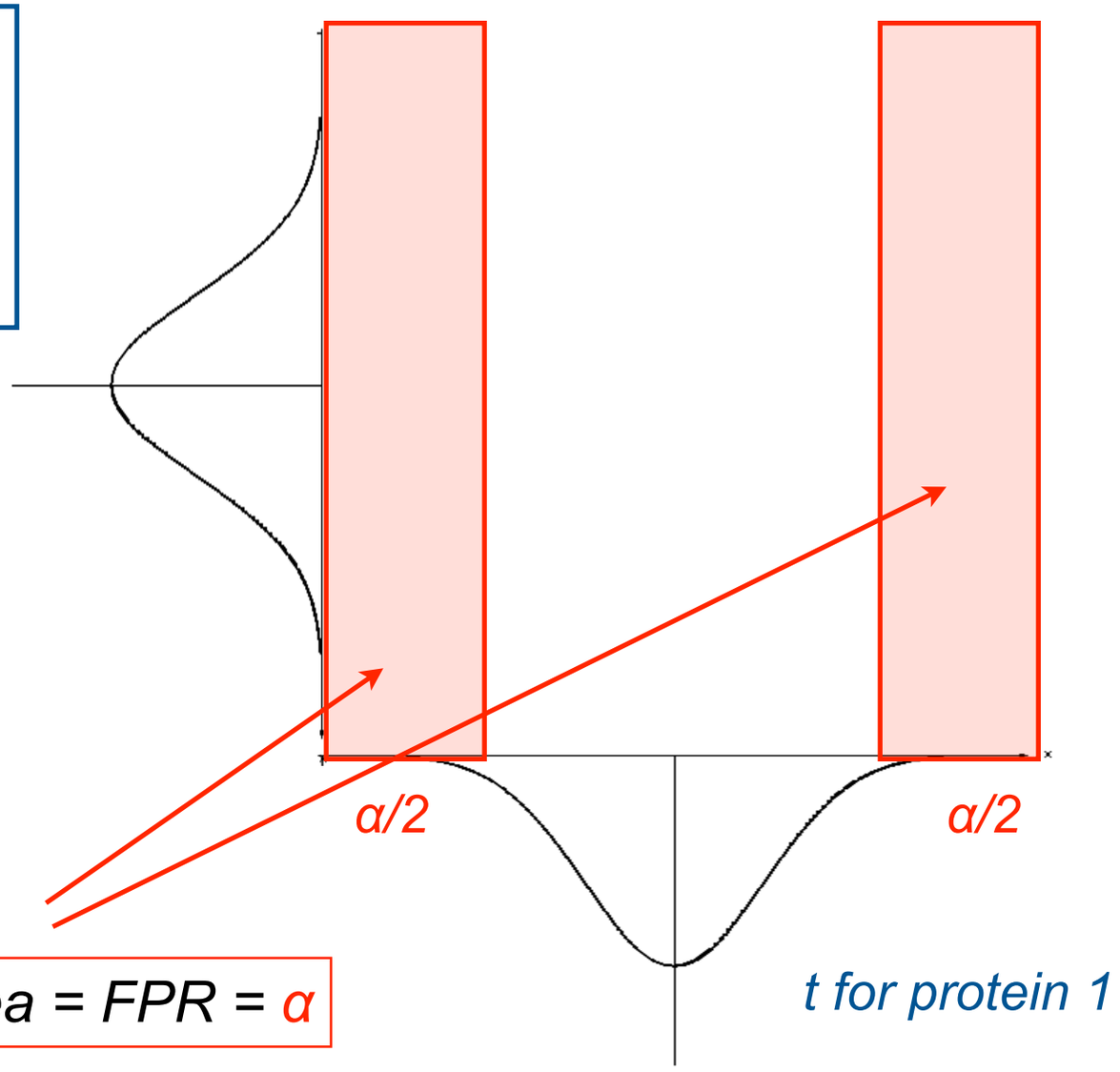

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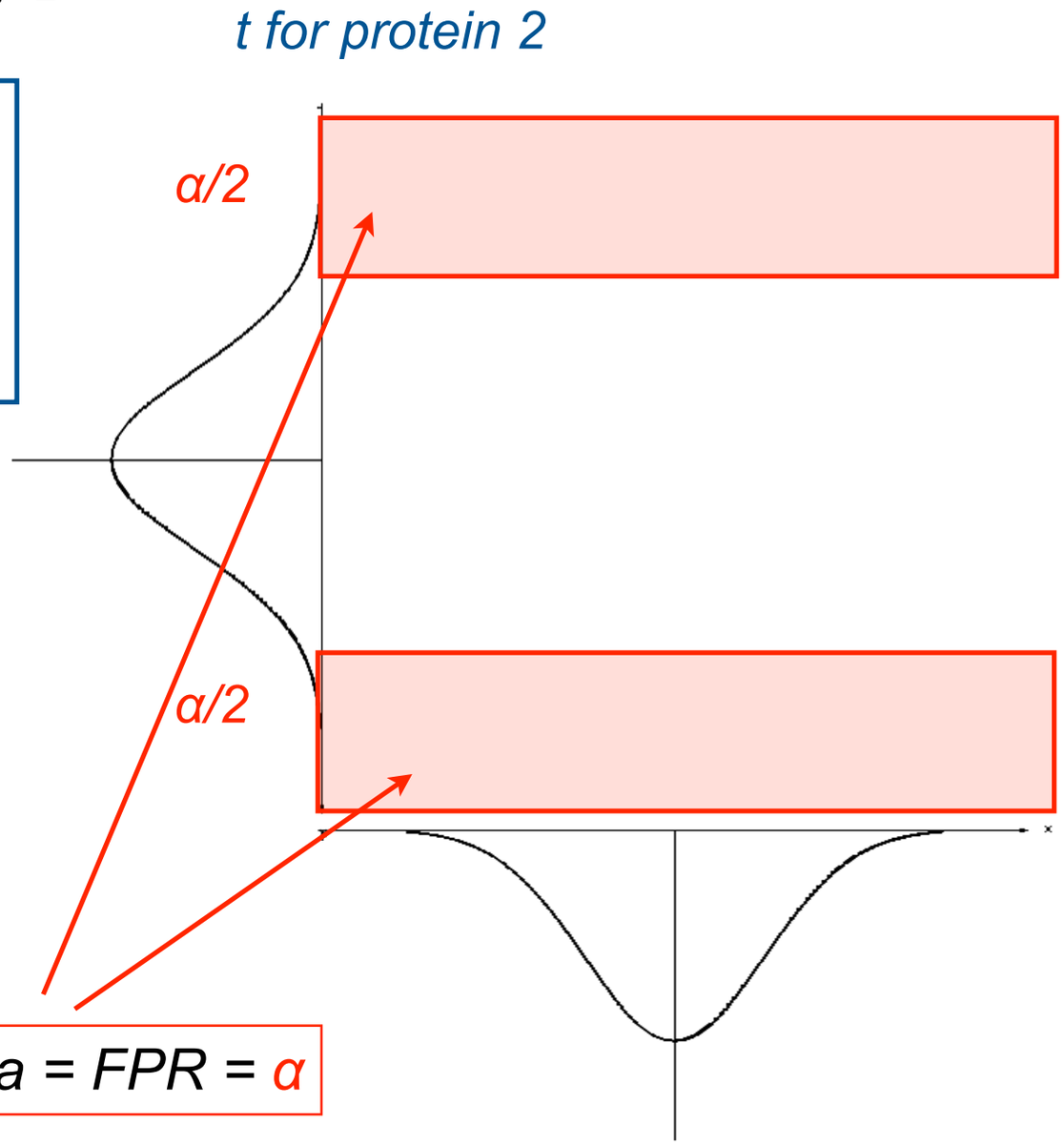

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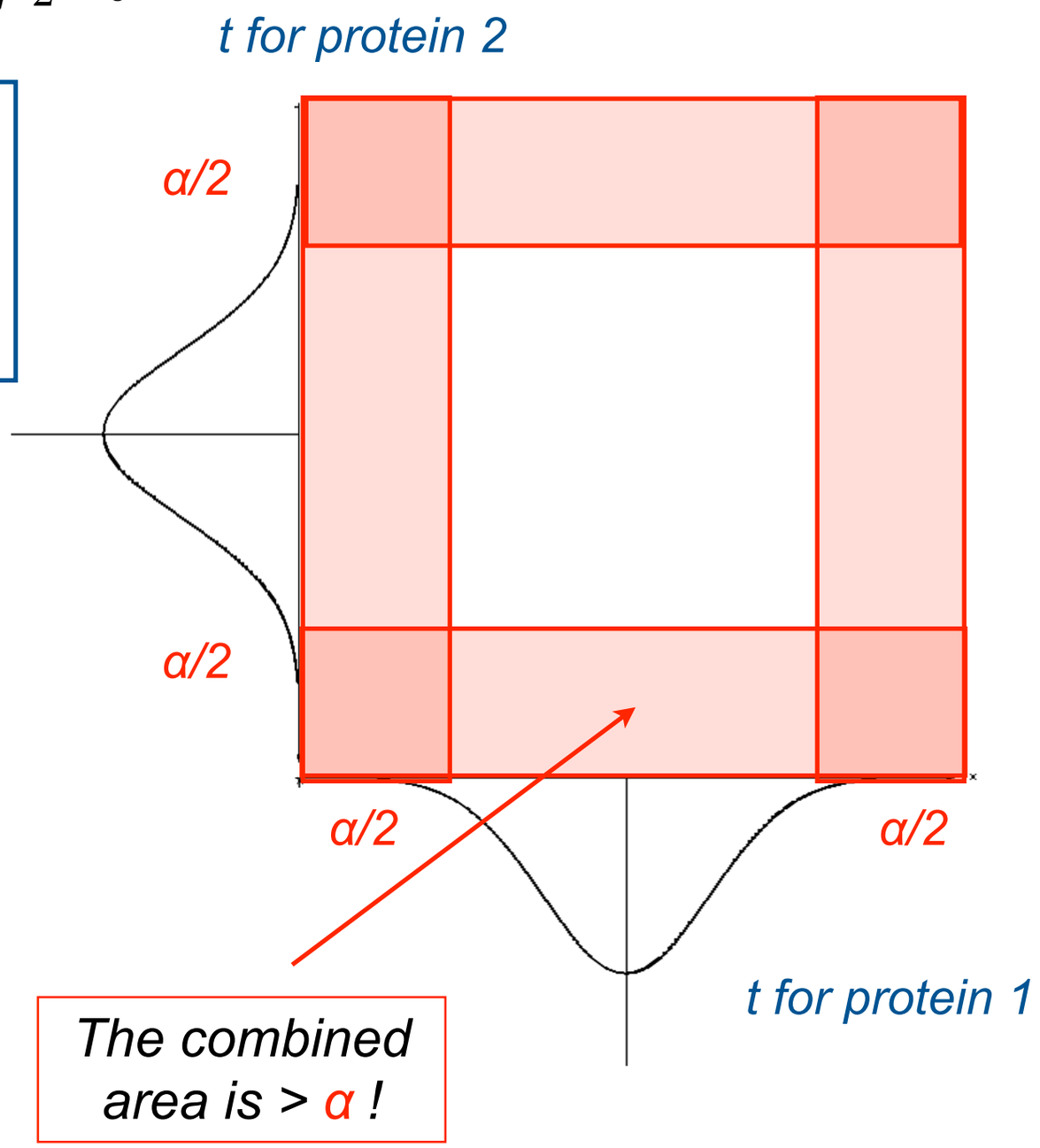
- P(at least one incorrect decision) > α
- The univariate FPR does not hold for the list
- Need to define a *multivariate* error rate



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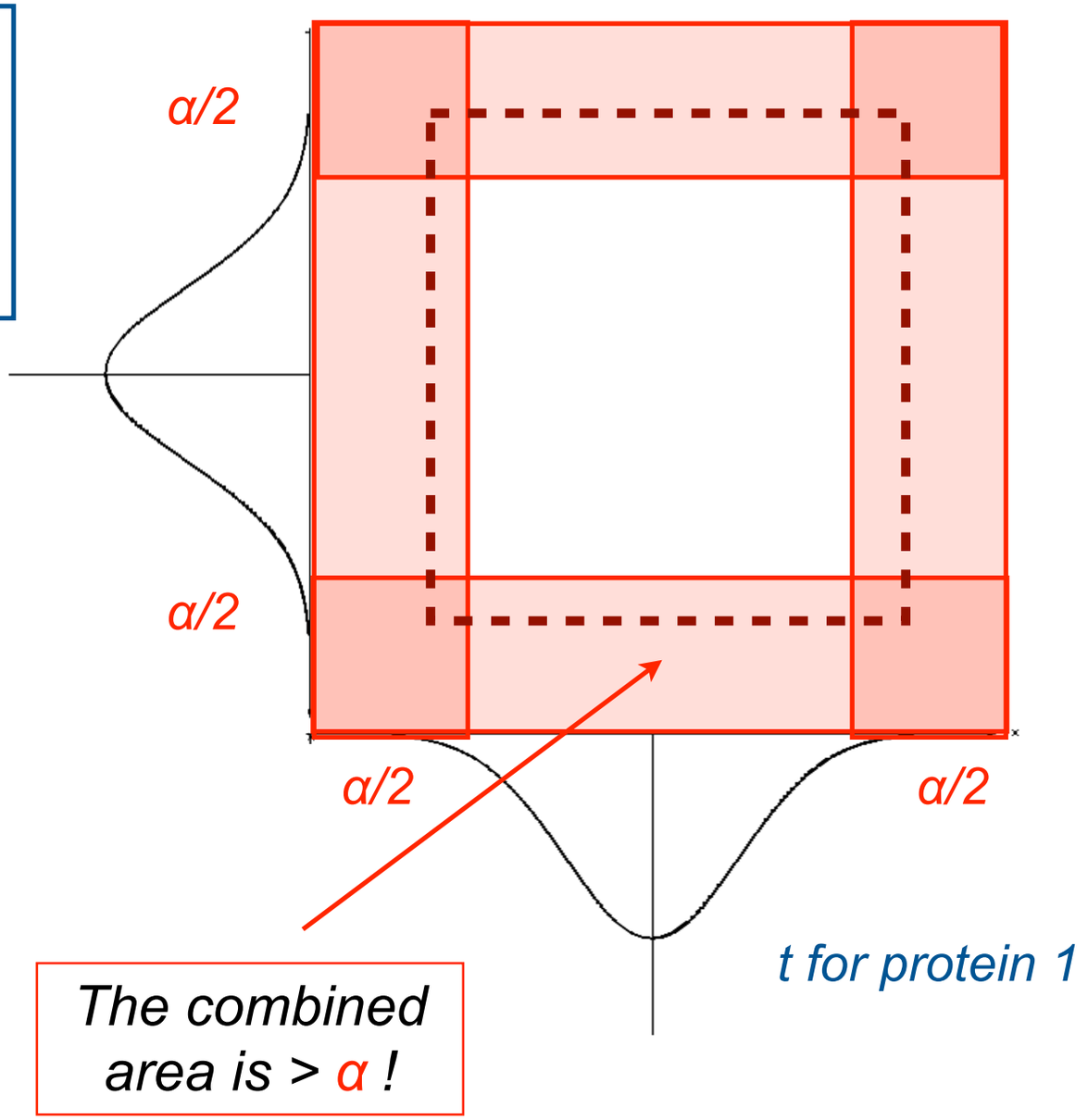


TESTING M PROTEINS

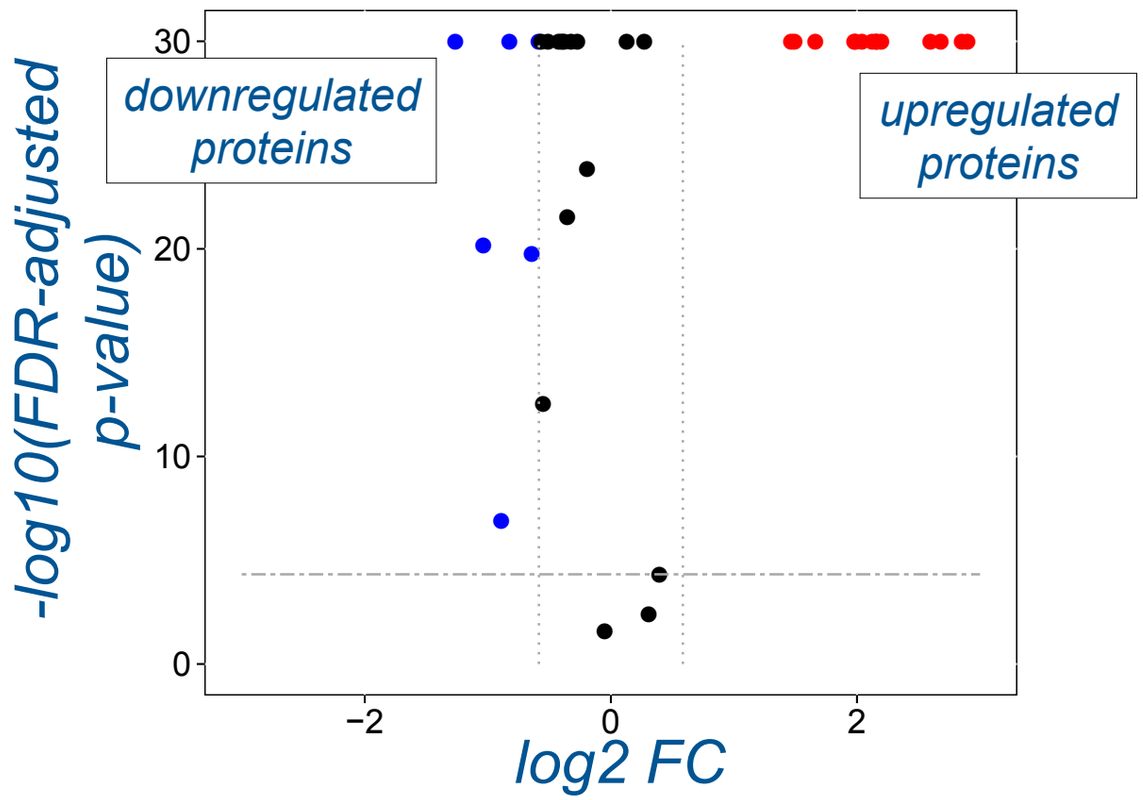
Change criteria from False Positive Rate to False Discovery Rate

	# of proteins with no detected difference	# of proteins with detected difference	Total
# true non-diff. proteins	U	V	m_0
# true diff. proteins	T	S	$m_1 = m - m_0$
Total	$m - R$	R	m

- False discovery rate (FDR)
  - An infinite number of measurements on same proteins
  - FDR: the *average* proportion of false discoveries

$$\mathbf{FDR} = \mathbf{E}\left[\frac{\mathbf{V}}{\max(\mathbf{R}, 1)}\right]$$

Bonferroni approach controls family-wise error rate = $P(V > 0)$



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Devon Kohler
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