Scale Uncertainty in ALDEx2

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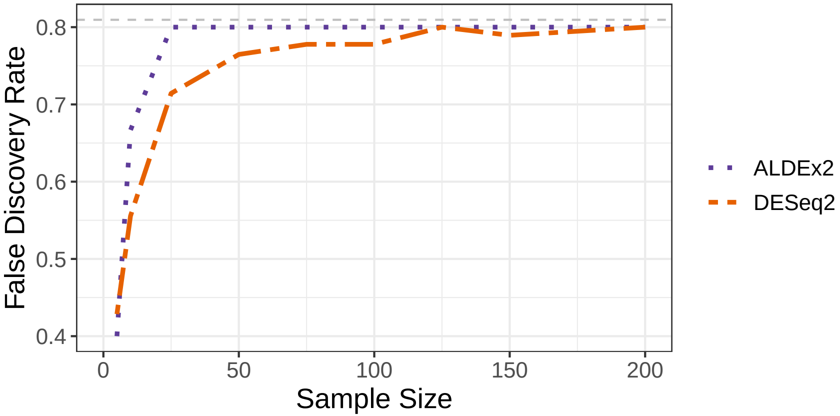
Recap: Sequencing depth can confound conclusions.

Observed data (Y)	Sample 1	Sample 2	Sample 3	
Condition	Health	Health	Disease	Conclusion
Entity 1	5	10	100	Increase
Entity 2	10	25	3	Decrease
Entity 3	0	1	8	Increase
Entity 4	0	0	19	Increase
Sampling Depth	15	36	130	

This can mislead analyses.

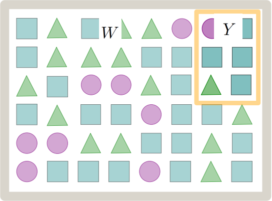
System data (W)	Sample 1	Sample 2	Sample 3	
Condition	Health	Health	Disease	Conclusion
Entity 1	227	351	154	Decrease
Entity 2	684	891	3	Decrease
Entity 3	48	32	15	Decrease
Entity 4	43	39	27	Decrease
Scale ($W^{\perp}$)	1,002	1,313	200	

. . . and lead to unacknowledged bias.



Problem Set-Up

Observed Data as a Sample from the System



■, ●, ▲ = 500 microbes

	Observed (Y)	Proportion ($Y^{\parallel}$)	Truth (W)	Proportion ($W^{\parallel}$)
●	500	0.17	4,500	0.19
■	2,000	0.66	12,000	0.50
▲	500	0.17	7,500	0.31
	3,000		24,000	

↑ Sampling Depth ($Y^{\perp}$)

↑ Scale ($W^{\perp}$)

Notation

- Y is a measurement of the underlying system W.
- W depends on both the composition ($W^{\parallel}_{dn}$) and system scale ($W^{\perp}_{n}$):

$$W_{dn} = W^{\parallel}_{dn} W^{\perp}_{n}$$

$$W^{\perp}_{n} = \sum_{d=1}^{D} W_{dn}$$

- θ is what we want to estimate.

Differential Abundance/Expression Analysis

- Question: How do entities (e.g., taxa or genes) change between conditions?
- In this case, θ is the log-fold change (LFC):

$$\theta_d = \text{mean}_{\text{case}}(\log W_{dn}) - \text{mean}_{\text{control}}(\log W_{dn})$$

The Original ALDEx2 Model

Step 1: Model Sampling Uncertainty

$$Y_{\cdot n} \sim \text{Multinomial}(W^{\parallel}_{\cdot n})$$
$$W^{\parallel}_{\cdot n} \sim \text{Dirichlet}(\alpha)$$

Step 2: Centered Log-Ratio Transformation

$$\log W_{\cdot n} = \left[\log W^{\parallel}_{1n} - \text{mean}(\log W^{\parallel}_{\cdot n}), \ldots, \log W^{\parallel}_{Dn} - \text{mean}(\log W^{\parallel}_{\cdot n})\right]$$

Step 3: Calculate LFCs and Test if Different from Zero.

$$\theta_d = \text{mean}_{\text{case}}(\log W_{dn}) - \text{mean}_{\text{control}}(\log W_{dn})$$

Implied Assumptions about Scale

Step 1: Model Sampling Uncertainty

$$\begin{aligned} Y_{\cdot n} &\sim \text{Multinomial}(W^{\parallel}_{\cdot n}) \\ W^{\parallel}_{\cdot n} &\sim \text{Dirichlet}(\alpha) \end{aligned}$$

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Step 3: Calculate LFCs and Test if Different from Zero.

$$\theta_d = \text{mean}_{\text{case}}(\log W_{dn}) - \text{mean}_{\text{control}}(\log W_{dn})$$

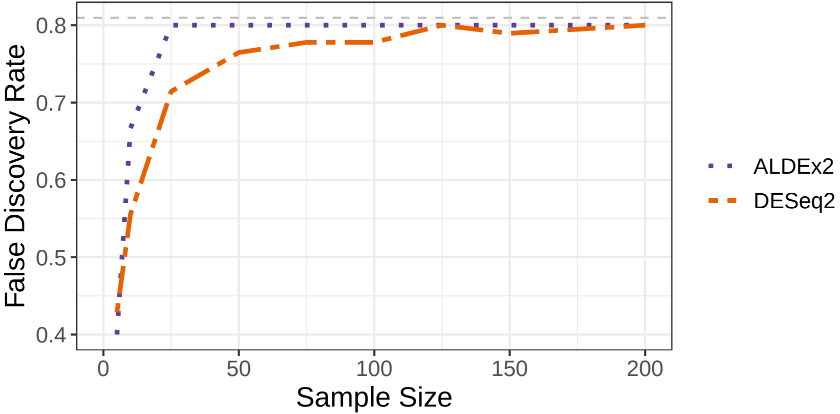
Implied Assumptions about Scale, cont.

Using the relationship $W_{dn} = W^{\parallel}_{dn} W^{\perp}_{n}$ and some math, the CLR normalization implies:

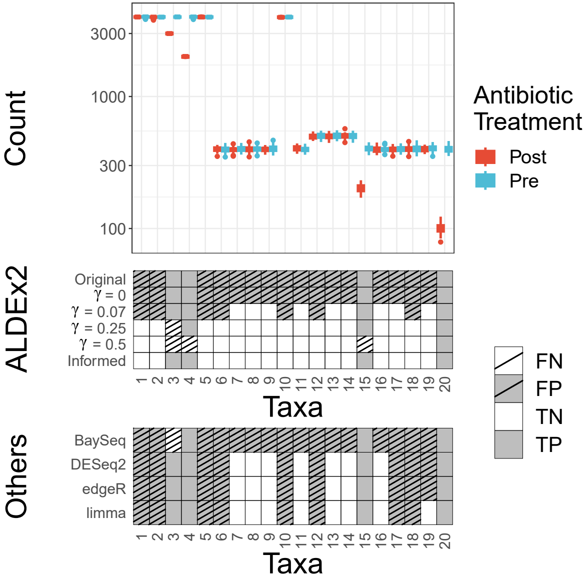
$$\log W^{\perp}_{n} = \text{mean}(\log W^{\parallel}_{\cdot n}).$$

What does this mean? What does this imply for analyses?

Unacknowledged bias!



Adding Uncertainty in Scale can Help.



Scale Reliant Inference (Informal)

Scale Reliant Inference: The Basics

- **The CoDA perspective:** Research questions that depend on scale are not possible.
- **The Normalization perspective:** Research questions that depend on scale can be answered after normalization.
- Who is right?

Scale Reliant Inference: The Basics

- **The CoDA perspective:** Research questions that depend on scale are not possible.
- **The Normalization perspective:** Research questions that depend on scale can be answered after normalization.
- Who is right?
- **The CoDA perspective:** Technically yes, but limiting.
- **The Normalization perspective:** Technically no, but attempting to answer relevant questions.

Scale Reliant Inference: The Basics

- What happens if θ depends on $W^{\perp}$?
- Consider LFCs: how are taxa changing between two conditions?

$$\begin{aligned}
\theta_d &= \text{mean}_{\text{case}}(\log W_{dn}) - \text{mean}_{\text{control}}(\log W_{dn}) \\
&= \ldots \\
&= (\text{mean}_{\text{case}}(\log W^{\parallel}_{dn}) - \text{mean}_{\text{control}}(\log W^{\parallel}_{dn})) \\
&- (\text{mean}_{\text{case}}(\log W^{\perp}_{n}) - \text{mean}_{\text{control}}(\log W^{\perp}_{n})) \\
&= \theta^{\parallel} + \theta^{\perp}
\end{aligned}$$

Don't we need $\theta^{\perp}$?

Scale Reliant Inference: Theory Intro

Recall for LFCs:

$$\begin{aligned}\theta_d &= \text{mean}_{\text{case}}(\log W_{dn}) - \text{mean}_{\text{control}}(\log W_{dn}) \\ &= \theta^{\parallel} + \theta^{\perp}\end{aligned}$$

- What can we say about θ from $\theta^{\parallel}$ alone?
- E.g. If $\theta^{\parallel} = 20$, what does that say about θ? If there are no restrictions, nothing!
- Statistical perspective: θ is not identifiable without $\theta^{\perp}$.
- Practical issues: unbiased estimators, calibrated confidence sets, and type-I error control *NOT* possible!

Scale Simulation Random Variables

Goal: Estimate $\theta = f(W^{\parallel}, W^{\perp})$.

1. Draw samples of $W^{\parallel}$ from a measurement model (can depend on Y).
2. Draw samples of $W^{\perp}$ from a scale model (can depend on $W^{\parallel}$).
3. Estimate samples of $\theta = f(W^{\parallel}, W^{\perp})$.

The Updated ALDEx2 Software

ALDEx2 as an SSRV

Step 1: Model Sampling Uncertainty

$$\begin{aligned} Y_{\cdot n} &\sim \text{Multinomial}(W^{\parallel}_{\cdot n}) \\ W^{\parallel}_{\cdot n} &\sim \text{Dirichlet}(\alpha) \end{aligned}$$

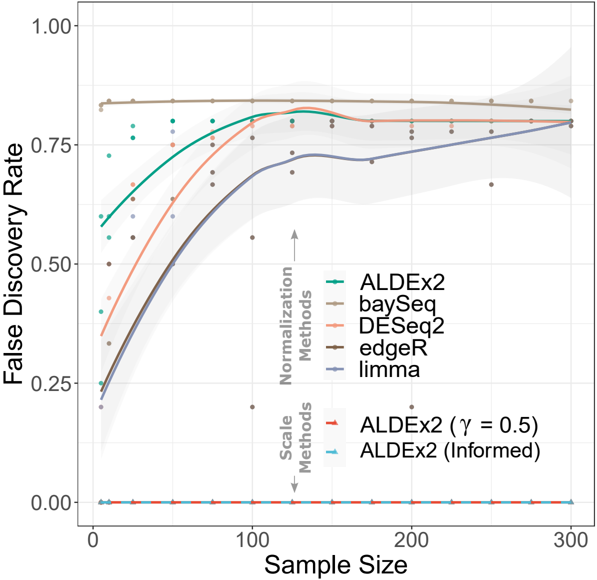
Step 2: Draw Samples from a Scale Model

$$\begin{aligned} \log W^{\perp}_{n} &\sim Q \\ \log W_{\cdot n} &= \log W^{\parallel}_{\cdot n} + \log W^{\perp}_{n} \end{aligned}$$

Step 3: Calculate LFCs and Test if Different from Zero.

$$\theta_d = \text{mean}_{\text{case}}(\log W_{dn}) - \text{mean}_{\text{control}}(\log W_{dn})$$

Benefits of Moving Past Normalizations to Scale



Intro to Scale Models

Normalizations are replaced by a scale model:

$$\log W_n^{\perp} \sim Q$$

There are no restrictions on Q, although there are some helpful options:

1. Based on normalizations.
2. Based on biological knowledge.
3. Based on outside measurements.

Coding Changes to ALDEx2

Including scale

The new ALDEx2 model removes normalizations in lieu of scale models.

Major updates:

1. A new argument `gamma` which inc

The gamma argument

- Added as argument to the `aldex` and `aldex.clr` function.
- `gamma` can either be a single numeric or a matrix.
  1. Single numeric: controls the noise on the default scale model.
  2. Matrix: A $N \times S$ matrix of samples of W.
- `gamma = NULL` returns the default behavior of ALDEx2.

Option 1: Default Scale Model

The default scale model is based on errors in the CLR normalization.

$$\log \hat{W}_{n}^{\perp(s)} = -\mathrm{mean}\left(\log \hat{W}_{\cdot n}^{\parallel(s)}\right) + \Lambda^{\perp} x_{n}$$

$$\Lambda^{\perp} \sim \; N(0, \gamma^{2}).$$

Advantages of the Default Scale Model

1. It is built off the status quo for ALDEx2.
2. Any value of $\gamma > 0$ will reduce false positives compared to the CLR normalization.
3. It has a concrete interpretation to contextualize scale assumptions.

Interpreting the Default Scale Model

Option 2: More Complex Scale Models

Alternatively, can pass a matrix of scale samples to `gamma` so long as:

1. The dimension is $N \times S$.
2. They are samples of $W^{\perp}$ not $\log W^{\perp}$.

Reasons to do this:

1. **Biological beliefs:** Scale is guided by the biological system or the researcher's prior beliefs.
2. **Outside Measurements:** These can be used in building a scale model *if* they are informative on the scale of interest (e.g., qPCR, flow cytometry).

Sensitivity Analyses

Real Data Examples

Real Example: SELEX

Real Example: Vandputte