Empirical Bayes Method for scRNA-Seq Batch Effects

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AACR American Association for Cancer Research

Batch effect correction adapted to the unique distributions of single-cell transcriptomes

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The authors have no conflict of interest to declare.

Research question

Technical variability across datasets

- Datasets from different sources suffer from **technical effects**, caused by differences during sequencing such as protocol, or equipment.

Impact on single-cell data

- Single-cell RNA-Seq data are particularly sensitive to batch effects due to their high sparsity, lower RNA content, and cell-to-cell variability.

Consequences for analysis

- Without correction, batch effects can obscure true biological signals, misleading downstream analysis.

Cell type
- B cell
- Dentritic cell
- Epithelial cell
- Fibroblast
- Innate lymphoid cell
- Malignant cell
- Muscle cell
- Myeloid cell
- Pericyte
- Plasma cell
- T cell

Raw data with uncorrected batch effects

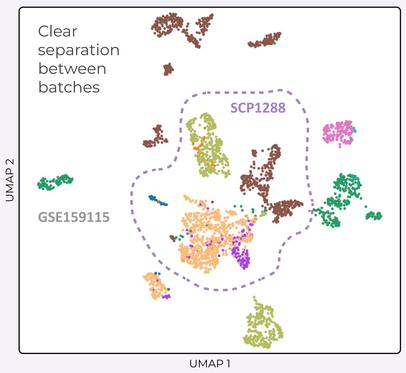
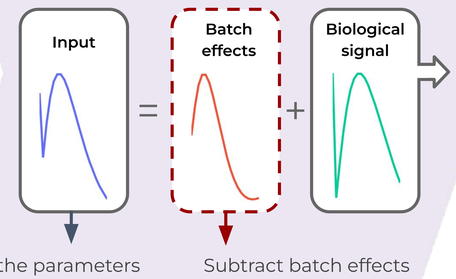


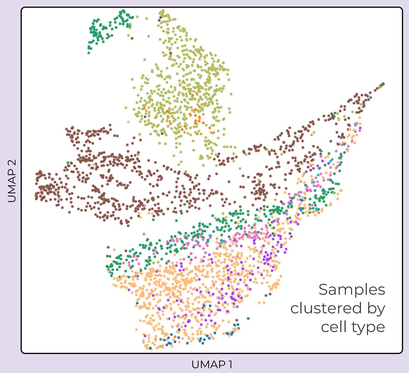
Figure 1: UMAP before batch effect correction.
2 Kidney datasets GSE159115, SCP1288 (See Results part for cohort details)

Batch effect correction

- Reduces the technical effects and makes sure the biological signal dominates.
  - Decompose the input data in 2 components



Corrected data showing biological structure



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References

1. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics. 2007 Jan 1;8(1):118–27.
2. Zhang Y, Parmigiani G, Johnson WE. ComBat-seq: batch effect adjustment for RNA-seq count data. NAR Genomics Bioinforma. 2020 Sep;2(3):lqaa078.
3. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, Zheng S, Butler A, et al. Integrated analysis of multimodal single-cell data. Cell. 2021 Jun 24;184(13):3573-3587.e29.

Results

Cohorts creation

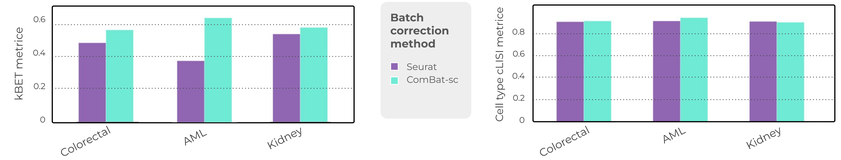
Colorectal cohort	AML cohort	Kidney cohort
3 datasets	2 datasets	2 datasets
14 cell types	7 cell types	11 cell types

For each dataset, **3,000 cells were selected to preserve the original cell type composition**. In each cohort, **4,000 highly variable genes were used**, capturing varying levels of integration complexity (i.e., number of datasets and cell types).

Comparison with Seurat

pyComBat-sc improves batch mixing by **31,11%** compared to Seurat

pyComBat-sc improves cell type clustering by **1,04%** compared to Seurat



kBET measures the bias of a batch variable in the kNN graph.

Cell-type LISI (cLISI) measures the quality of the clustering of cells by cell type.

All quality metrics are scaled between 0 and 1, with higher values indicating better performance.

Approach

Limitations of Existing Methods

- Methods developed for bulk RNA-Seq often fail to account for the sparsity and variability of single-cell data.
- Many single-cell batch correction tools return only low-dimensional embeddings, without gene-level corrected matrices.
  → limiting downstream analyses and reducing data interpretability.

We adapt the **Empirical Bayes framework to better fit the distributional complexity of single-cell data**, enabling effective batch correction while preserving biological signal.

Specificity of the batch effect correction tools

Empirical Bayes methods are state-of-the-art for microarray and bulk RNA-Seq data. → **Apply the methods to scRNA-Seq** Use a formal model and leverage observed data to infer parameters.

Microarray (ComBat, 2007)	RNA-Seq (ComBat-Seq, 2020)	scRNA-Seq (ComBat-sc, 2025)
$y \sim Normal(\mu ; \varphi)$	$y \sim NegBinom(\mu ; \varphi)$	$y \sim \pi\, \delta_0 + (1-\pi)\, NegBinom(\mu ; \varphi)$
ComBat uses a Generalized Linear Model (GLM) to correct batch effects in microarray data.	ComBat-Seq tweaks the distribution of the GLM, and adapts the model to RNA-Seq data.	Our approach further tweaks the distribution to **account for dropouts**, and better fit scRNA-Seq data.

Unique features

- handles **arbitrary experimental designs**, accounts for **covariates** to better preserve the biological signal
- supports selecting a batch as **reference**
- works in **full dimension**, raw count matrices in input and output
  - more **precise**, more **versatile** for downstream analyses: differential expression, signature for deconvolution...