# pyComBat, a Python tool for batch effects correction in high-throughput molecular data using empirical Bayes methods

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## Abstract

**Background:** Variability in datasets is not only the product of biological processes: they are also the product of technical biases. ComBat and ComBat-Seq are among the most widely used tools for correcting those technical biases, called batch effects, in, respectively, microarray and RNA-Seq expression data.

**Results:** In this technical note, we present a new Python implementation of ComBat and ComBat-Seq. While the mathematical framework is strictly the same, we show here that our implementations: (*i*) have similar results in terms of batch effects correction; (*ii*) are as fast or faster than the original implementations in R and; (*iii*) offer new tools for the bioinformatics community to participate in its development. pyComBat is implemented in the Python language and is distributed under GPL-3.0 (https://www.gnu.org/licenses/gpl-3.0.en.html) license as a module of the inmoose package. Source code is available at https://github.com/epigenelabs/inmoose and Python package at https://pypi.org/project/inmoose.

**Conclusions:** We present a new Python implementation of state-of-the-art tools ComBat and ComBat-Seq for the correction of batch effects in microarray and RNA-Seq data. This new implementation, based on the same mathematical frameworks as ComBat and ComBat-Seq, offers similar power for batch effect correction, at reduced computational cost.

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## Keywords

batch effects, transcriptomics, Bayesian statistics, open source

## Availability and requirements

**Project name:** InMoose
**Project home page:** https://github.com/epigenelabs/inmoose
**Operating system:** Platform independent
**Programming language:** Python

**Other requirements:** Numpy, Scipy
**License:** GNU GPL3
**Any restrictions to use by non-academics:** None

## Declarations

**Ethics approval and consent to participate:** Not Applicable
**Consent for publication:** Not Applicable
**Availability of data and materials:** The datasets analyzed during the current study are available in the GEO and dbGAP repositories:
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE18520
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE66957
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE69428
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE9891
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE26712
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE38666
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5900
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE66291
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE68891
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE122231
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE83083
- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59765
- https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000892.v6.p1
- https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000178.v11.p8

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**Authors' contributions:** AB implemented pycombat_norm and compared it with ComBat and Scanpy's implementation of ComBat. MC implemented pycombat_seq and compared it with ComBat-Seq. AB and MC wrote this manuscript. JH, AG, GA, CAA and AN reviewed code and provided scientific guidance. All authors read and approved the final manuscript.
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## 1. Background

Batch effects are the product of technical biases, such as variations in the experimental design or even atmospheric conditions (Lander, 1999; Fare *et al.*, 2003). They particularly reveal themselves when merging different datasets, which have likely been built under different conditions. If not corrected, these batch effects may lead to incorrect biological insight, since the variability can be wrongly interpreted as the product of a biological process.
Multiple methods exist that address this problem. They include approaches related to frequentist statistics, such as simple normalization (Yang *et al.*, 2002; Tai and Speed, 2012) or principal component analysis (Nielsen *et al.*, 2002); and machine learning, such as

support-vector machines (Benito *et al.*, 2004). One of their main flaws is, however, their incapacity to handle low sample sizes or more than two batches at the same time (Chen *et al.*, 2011).
ComBat, originally implemented in the R library sva (Leek *et al.*, 2012), is based on the mathematical framework defined in (Johnson *et al.*, 2007). This tool leverages a parametric and non-parametric empirical Bayes approach for correcting the batch effect in microarray datasets that works for small sample sizes or in the presence of outliers. Note that the parametric method requires strong assumptions but is largely faster than the non-parametric approach.
ComBat-Seq, also implemented in the R library sva, is based on a similar mathematical framework, where normal distributions are replaced by negative binomial distributions, to better reflect the statistical behavior of RNA-Seq data (Zhang *et al.*, 2020).
We introduce in this article pyComBat, a new Python tool implementing ComBat (function pycombat_norm) and ComBat-Seq (function pycombat_seq), following the same mathematical frameworks. Note that the term "pyComBat" implicitly refers to the function pycombat_norm (resp. pycombat_seq) when compared to ComBat (resp. ComBat-Seq). In comparison to both the R implementation and the existing Python implementation of ComBat in the single-cell analysis library Scanpy (Wolf *et al.*, 2018), we show that pyComBat yields similar results for adjusting for batch effects in microarray data, but is generally faster, in particular for the usually slow, but more loose, non-parametric method. Similarly, in comparison to the R implementation, we show that pyComBat yields similar results for adjusting for batch effects in RNA-Seq data, but is generally faster. To our knowledge, it is the sole Python implementation of ComBat-Seq.

## 2. Implementation of pyComBat

pyComBat is a Python 3 implementation of ComBat and ComBat-Seq. It mostly uses generic libraries like Pandas (McKinney, 2010) or NumPy (Van Der Walt *et al.*, 2011) to mimic ComBat and ComBat-Seq, following the exact same mathematical framework.
Two important features are not directly related to the performance of the software but are of utmost importance. First, pyComBat is available as an open-source software under a GPL-3.0 license, which means anyone can use, modify, distribute and share it. Opening pyComBat to the bioinformatics Python community is the best way for maintaining and improving it, while increasing its robustness. Second, the reliability of pyComBat has been thoroughly checked, using a bench of unit tests (code coverage measured at 88% with Python module "coverage") serving both as functional tests (to ensure the proper functioning of each submodule) and as non-regression tests (to ease maintenance).

## 3. Results: Comparing pyComBat with ComBat and ComBat-Seq

### a. Datasets used

For software validation, we created two microarray and two RNA-Seq meta-datasets from public data: one on Ovarian Cancer (6 microarray datasets), one on Multiple Myeloma (4 microarray datasets), one on Breast Cancer (originally used for the validation of ComBat-Seq (Zhang *et al.*, 2020), 2 RNA-Seq datasets) and one on Colon Cancer (2 RNA-Seq datasets) All meta-datasets are described in more detail in Table 1. We then compared

- ComBat, Scanpy's implementation of ComBat and pyComBat on the microarray datasets on one hand,
- ComBat-Seq and pyComBat on the RNA-Seq datasets.

for (*i*) efficacy for batch effect correction and (*ii*) computation time.

### b. Batch effect correction

As an implementation of the ComBat and ComBat-Seq algorithms, pyComBat is expected to have similar, if not identical, power in terms of batch effects correction. This is confirmed in Fig.1A, which shows the distribution of differences between the outputs of ComBat and pyComBat, on the Ovarian Cancer dataset (mean = $-1.06 \cdot 10^{-7}$, 95% CI = $[-1.28 \cdot 10^{-3}, 1.32 \cdot 10^{-4}]$), and on Fig. 2A, which shows the distribution of relative differences between the outputs of ComBat-Seq and pyComBat, on the Colon Cancer dataset (mean = $5.4 \cdot 10^{-6}$, first non-zero quantile 0.9995). As expected, the differences are distributed closely around zero. The slight variability can be explained by the difference between optimization routines in R and Python (Numpy): while small differences in the fitted distribution parameters have little to no impact for the vast majority of data points, they may be amplified by data adjustment for data points located far away in the distribution tails.

Additionally, both ComBat-Seq and pyComBat produce the exact same output on the Breast Cancer dataset.

### c. Computation time

Computation time is evaluated by running pyComBat (resp. Scanpy's implementation of ComBat and ComBat itself) respectively 100 times on both microarray datasets presented in section 3a, with the parametric approach. As Scanpy doesn't handle the non-parametric approach, only ComBat and pycombat_norm have been tested with it, on the Ovarian Cancer dataset. As for pycombat_seq and ComBat-Seq, they have been run respectively 50 times on the Breast Cancer dataset and 20 times on the Colon Cancer dataset. The reduced number of runs on RNA-Seq datasets is due to the longer, and less variable, computation times.

Owing to Python (Numpy) efficiency in handling matrix operations and matrix manipulations as well as thorough optimization of our code, pyComBat is as fast or even faster than ComBat. The parametric version of the pyComBat performs 4 to 5 times as fast as ComBat, and around 1.5 times as fast as the Scanpy implementation of ComBat, in terms of computation time (fig.1B, fig.1C), on both datasets.
Similar results are observed with the non-parametric version (fig.1D), which is inherently more time consuming, but also less dependent on the distribution of the data. In this case, pyComBat is also approximately 4 to 5 times faster than ComBat, going from more than an hour to around 15 minutes.
Finally, pyComBat appears to be 4 to 5 times faster than ComBat-Seq on both RNA-Seq datasets (Fig. 2B, Fig. 2C).

## 4. Discussion and conclusion

We present pyComBat, a new Python implementation of ComBat and ComBat-Seq, the most commonly used software for batch effects correction on high-throughput molecular data. Our implementation offers the same correcting power, with shorter computation time for the parametric method compared to other implementations, and significantly shorter time for the time-consuming non-parametric version compared to the original R implementations. This reduced computing time opens perspectives for a more generic use of the non-parametric approach to a larger range of datasets.

We have attached importance to making the software open source, coupled with comprehensive documentation. We built a robust set of test cases, in an effort to encourage larger participation from the community. We believe that this will be benefiting the Python

bioinformatics community and opening the way towards the translation of other widely used software from R to Python.

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## Figures

Fig. 1

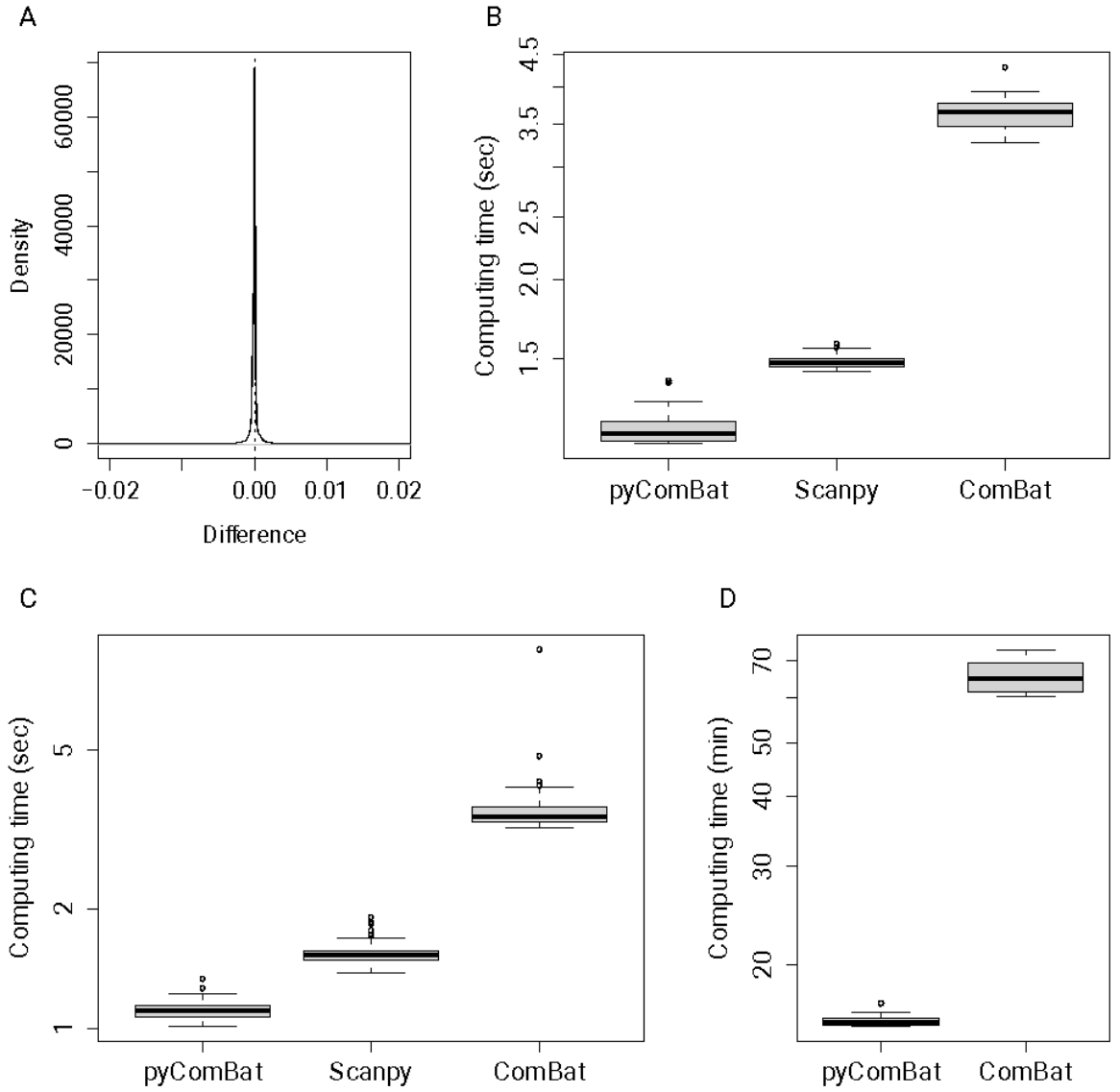
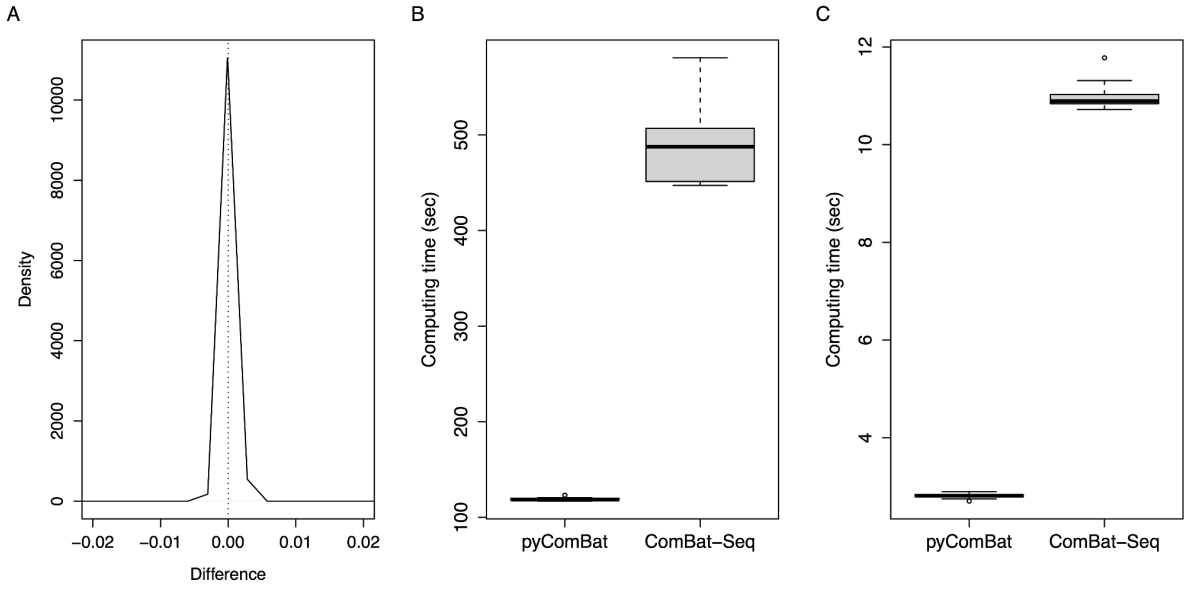


Fig. 2




## Legend of figures

Fig. 1

Performance of pyComBat *vs*. Combat *vs.* Scanpy's implementation of ComBat. **A** Distribution of the differences between the expression matrices corrected for batch effects, respectively by ComBat and pyComBat (parametric version), on the Ovarian Cancer dataset. The vertical dotted line corresponds to zero. **B** Computation time in seconds for pyComBat, Scanpy and ComBat for the parametric method, on the Multiple Myeloma dataset. The y-axis is in a log scale. **C** Computation time in seconds for pyComBat, Scanpy and ComBat for the parametric method, on the Ovarian Cancer dataset. The y-axis is in a log scale. **D** Computation time in minutes for pyComBat (left) and ComBat (right) for the non-parametric method, on the Ovarian Cancer dataset. The y-axis is in a log scale.

Fig. 2

Performance of pyComBat *vs.* ComBat-Seq. **A** Distribution of the relative differences between the expression matrices corrected for batch effects, respectively by ComBat-Seq and pyComBat, on the Colon Cancer dataset. The vertical dotted line corresponds to zero. **B** Computation time in seconds for pyComBat and ComBat-Seq, on the Colon Cancer dataset. **C** Computation time in seconds for pyComBat and ComBat-Seq, on the Breast Cancer dataset.

## Tables

Table 1

| Dataset | Reference(s) |
|---|---|
| | **Ovarian Cancer (microarray)** |
| GSE18520 | (Mok *et al.*, 2009) |
| GSE66957 | |
| GSE69428 | (Yamamoto *et al.*, 2016) |
| GSE9891 | (Tothill *et al.*, 2008) |
| GSE26712 | (Bonome *et al.*, 2008; Vathipadiekal *et al.*, 2015) |
| GSE38666 | (Lili *et al.*, 2013; Huang *et al.*, 2018) |
| | **Multiple Myeloma (microarray)** |
| GSE5900 | (Zhan *et al.*, 2007; Driscoll *et al.*, 2010; Li *et al.*, 2021) |
| GSE66291 | (Lionetti, Barbieri, Todoerti, Agnelli, Marzorati, *et al.*, 2015; Lionetti, Barbieri, Todoerti, Agnelli, Fabris, *et al.*, 2015) |
| GSE68891 | |
| GSE122231 | (Dhodapkar *et al.*, 2014; Khan *et al.*, 2015) |
| | **Breast Cancer (RNA-Seq)** |
| GSE83083 | (Rahman et al., 2017) |
| GSE59765 | (McQuerry et al., 2019) |
| | **Colon Cancer (RNA-Seq)** |
| phs000892.v6.p1 | (Vasaikar et al., 2019) |
| phs000178.v11.p8 | (Cancer Genome Atlas Network, 2012) |

## Legend of tables

Table 1

Composition of each meta-dataset used for benchmarking pyComBat, Scanpy's implementation of ComBat, ComBat and ComBat-Seq