**Systems biology**

**STACAS: Sub-Type Anchor Correction for Alignment in Seurat to integrate single-cell RNA-seq data**

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**Abstract**

**Summary:** STACAS is a computational method for the identification of integration anchors in the Seurat environment, optimized for the integration of single-cell (sc) RNA-seq datasets that share only a subset of cell types. We demonstrate that by (i) correcting batch effects while preserving relevant biological variability across datasets, (ii) filtering aberrant integration anchors with a quantitative distance measure and (iii) constructing optimal guide trees for integration, STACAS can accurately align scRNA-seq datasets composed of only partially overlapping cell populations.

**Availability and implementation:** Source code and R package available at https://github.com/carmonalab/STACAS; Docker image available at https://hub.docker.com/repository/docker/mandrea1/stacas_demo.

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**1 Introduction**

Massively parallel single-cell transcriptomics (scRNA-seq) has emerged as a transformative technology that enables measuring molecular profiles at single-cell resolution. However, despite the highly multiplexed technologies, single-cell data are produced separately for different tissues and organs and are affected by multiple batch effects, such as different sample processing and scRNA-seq protocols. As such, integration of single-cell data might be the ultimate challenge in the field towards the generation of single-cell atlases (Eisenstein, 2020; Lähnemann et al., 2020; Regew et al., 2017).

Seurat (Stuart et al., 2019) is currently one of the most popular and best performing algorithms for single-cell data integration, and can be effortlessly integrated into complex analysis pipelines (Tran et al., 2020). At the core of the Seurat integration algorithm is the identification of mutual nearest neighbors (MNN) across single-cell datasets, named ‘anchors’, in a reduced space obtained from canonical correlation analysis (CCA). These anchors and their scores are used to compute correction vectors for each query cell, transforming (i.e. batch-correcting) its expression profile (Haighverdi et al., 2018). Transformed cell profiles can then be jointly analyzed as part of an integrated space. To handle more than two datasets, a guide tree based on pairwise batch similarities is used to dictate the batch integration order. While Seurat has proven very powerful for the removal of technical artifacts between replicated experiments or even different sequencing technologies (Tran et al., 2020), it tends to overcorrect batch effects and performs poorly when integrating heterogeneous datasets (Luecken et al., 2020), where only a fraction of cell types are shared between individual samples. This is crucial for the creation of reference cell type-specific single-cell atlases where the datasets to integrate were obtained from different tissues or experimental conditions (e.g. T cells from blood versus tumor-infiltrating T cells), and as a consequence are composed of different, partially overlapping cell states or sub-types.

**2 Results**

STACAS is a package for determining integration anchors between heterogeneous datasets, and it is designed to be easily incorporated into Seurat dataset integration pipelines. STACAS uses a reciprocal principal component analysis (PCA) procedure to calculate anchors, where each dataset in a pair is projected onto the reduced PCA space of the other dataset; mutual nearest neighbors are then calculated in these reduced spaces. Crucially, and in contrast to the CCA reduction used by Seurat, the expression values of genes used in generating the PCA spaces are not rescaled to have zero mean and unit variance. When integrating heterogeneous datasets, for instance composed only of CD4⁺ or CD8⁺ T cells, such rescaling can cancel out important biological differences between the datasets (Fig. 1A).

A second innovation introduced in STACAS is the filtering of anchors based on anchor pairwise distance, which is calculated on the reduced PCA spaces used to determine the anchors. We observed that the distribution of anchor distances between datasets with shared cell subtypes (i.e. a sample containing both CD4⁺ and CD8⁺ T cells, compared to a sample of CD8⁺ T cells only) is centered on lower pairwise anchor distances compared with dataset pairs with limited or no overlap (e.g. a CD4⁺ sample and a CD8⁺ sample)
Anchor distance can therefore be used as a quantitative measure to filter spurious anchors and improve dataset integration. In STACAS, the anchor filtering threshold defaults to the 80th percentile of the distance distribution between the two most similar datasets included in the integration task.

Finally, the anchors determined by STACAS can be used directly for dataset integration using the IntegrateData function in Seurat 3. STACAS suggests a guide tree to determine the order in which datasets are to be integrated. In contrast to the Seurat default guide tree, which favors datasets with the highest total number of cells in any given pair, STACAS prioritizes samples with the highest total number of anchors; the rationale being that datasets with many anchors are likely to contain more cell types and represent the ‘centroid’ of the integrated map.

In the example in Figure 1, we integrated four scRNA-seq datasets of mouse T cells from public repositories, composed of (i) CD8+ tumor-infiltrating lymphocytes (TILs) (Carmona et al., 2020); (ii) CD4+ and CD8+ TILs (Xiong et al., 2019); (iii) CD4+ T cells from tumors (Magen et al., 2019) and (iv) CD4+ T cells from tumor-draining lymph nodes (dLN) (Magen et al., 2019). There is an evident batch effect between the samples, with the cells of each sample clustering together regardless of their type (Fig. 1C and F). Consistently with a recent benchmark (Luecken et al., 2020), dataset alignment using Seurat 3 appears to overcorrect these batch effects, overlaying samples with little in common such as CD4+ dLN and CD8+ TILs (Fig. 1D and G). In contrast, STACAS only aligns cells with similar states across samples, limiting the superposition of CD4+ with CD8+ cells (Fig. 1E). Supervised cell state classification using TILPRED (Carmona et al., 2020) confirms that in most cases STACAS was able to cluster cell types across different, heterogeneous datasets (Fig. 1H).

An interactive TIL reference atlas constructed using STACAS can be explored at: [http://tilatlas.unil.ch](http://tilatlas.unil.ch)

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**Conflict of Interest:** none declared.

**Data availability**

The data analyzed in this article are publicly available from NCBI Gene Expression Omnibus (GEO) at [https://www.ncbi.nlm.nih.gov/geo/](https://www.ncbi.nlm.nih.gov/geo/) under the identifiers GSE124691 and GSE116390, and from EMBL-EBI ArrayExpress at [https://www.ebi.ac.uk/arrayexpress/](https://www.ebi.ac.uk/arrayexpress/) under entry E-MTAB-7919.
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