Effect of imputation on gene network reconstruction from single-cell RNA-seq data

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Abstract

Despite the advances in single-cell transcriptomics the reconstruction of gene regulatory networks remains challenging. Both the large amount of zero counts in experimental data and the lack of a consensus preprocessing pipeline for single-cell RNA-seq data make it hard to infer networks from transcriptome data. Data imputation can be applied in order to enhance gene-gene correlations and facilitate downstream data analysis. However, it is unclear what consequences imputation methods have on the reconstruction of gene regulatory networks.

To study this question, we evaluate the effect of imputation methods on the performance and structure of the reconstructed networks in different experimental single-cell RNA-seq data sets. We use state-of-the-art algorithms for both imputation and network reconstruction and evaluate the difference in results before and after imputation. We observe an inflation of gene-gene correlations that affects the predicted network structures and may decrease the performance of network reconstruction in general. Yet, within the modest limits of achievable results, we also make a recommendation as to an advisable combination of algorithms, while warning against the indiscriminate use of imputation before network reconstruction in general.

23 1 Introduction

24 Single-cell transcriptomics has revolutionized genomics. In particular, this new type of data is
 25 widely assumed to advance the unraveling of regulatory interactions in the cell. Thus, there
 26 is great interest in the computational reconstruction of gene regulatory networks (GRNs)
 27 from single-cell transcriptome data.

28 Available methods for GRN reconstruction from single-cell RNA-seq (scRNAseq) data draw
 29 on a plethora of statistical approaches (1–6)). Pratapa et. al. (6) provide an extensive
 30 benchmark study evaluating the performance of various methods. However, for GRN
 31 reconstruction several authors have remarked that preprocessing the data is important,
 32 mostly due to the sparse nature of the data (7,8). Several computational analysis pipelines
 33 have been suggested and are in wide use (9,10). Typically, as one of the early steps, such a
 34 pipeline will include a data normalization and/or imputation step, which statistically estimates
 35 unobserved read counts in cases where the method deems that experimental or technical
 36 noise has led to the absence of a count, i.e., a so-called dropout. While normalization
 37 attempts to correct for different read depths between cells (11,12), imputation attempts to
 38 recover gene counts by predicting missing data and eventually smoothen gene expression
 39 values (13–22). In some tools a prior normalization step is not required but integrated within
 40 the imputation method (20,21). Hou et. al (23) extensively evaluated the impact of imputation
 41 on clustering, differential expression analysis and pseudotime inference and invoked
 42 cautious interpretations of the results.

43 It still remains unclear though how imputation methods affect network structures (24). On the
 44 one hand, it is recommended to use imputation to enhance gene regulatory correlations prior
 45 to network inference (18,20). But on the other hand, results based on imputed data should

46 be interpreted with care (10,23,25). Thus, imputation meets conflicting attitudes within the
47 community.

48 Here, we systematically study the question whether data imputation as a preprocessing step
49 affects results obtained using reconstructed GRNs. We build on previously published
50 benchmark studies and consider the best-performing scRNAseq-based tools for both
51 imputation and network reconstruction in our analysis. We measure the performance of
52 different combinations of imputation method and GRN reconstruction method using multiple
53 experimental datasets and a ground truth network that has been used in other benchmark
54 studies. We compare the performance and network structures obtained using unimputed
55 data and imputed data, respectively, and show that in most cases GRN reconstruction does
56 not profit from imputation. In order to explain the observed results we analyze the effect of
57 imputation on predicted gene interactions. Ultimately, we present a recommendation, how to
58 proceed in a data analysis project.

59 2 Results

60 2.1 Systematic evaluation of network models

61 Evaluating the combination between imputation and network inference on different datasets
62 results in a cubic matrix. To manage this we restrict our selection to state-of-the-art
63 computational tools, both for imputation and network inference, that perform most accurately
64 and have been recommended in recent benchmark studies (6,23). Consequently, we
65 developed a computational pipeline to study seven cell types that were obtained from
66 different scRNAseq experiments, using four state-of-the-art imputation methods combined
67 with three top performing GRN methods as depicted in Figure 1.

68 Information on the seven cell types was derived from five experimental scRNAseq datasets:
69 human embryonic stem cell (hESC) (14), human hepatocytes (hHep) (26), mouse embryonic
70 stem cell (mESC) (27), mouse dendritic cells (mDC) (28) and mouse hematopoietic stem
71 cells (mHSC) (29) that were further separated into the following subtypes: erythrocytes
72 (mHSC-E), granulo monocytes (mHSC-GM) and lymphocytes (mHSC-L).
73 For the four imputation methods, we chose the following methods: two smoothing-based
74 tools *magic* (18) and *knn-smoothing* (22); a model-based tool *saver* (17) and a deep-learning
75 based tool *dca* (20). We included *dca* because the authors specifically expect to improve
76 network reconstruction. A baseline model was established using normalized but unimputed
77 data.
78 As for GRN reconstruction, we selected three tools: an information-based tool PIDC (4), and
79 two tree-based tools GENIE3 (30) and GRNBoost2 (31). In the remainder of this paper we
80 use the term “model” to refer to the combination of a GRN reconstruction algorithm with an
81 imputation method or no imputation, respectively. We obtain the ground truth network from
82 the STRING database — a functional protein-protein interaction network (32) and use the
83 evaluation framework BEELINE (6) for measuring the performance of each network model
84 (see Methods). Furthermore, we inspect the reconstructed network and compare the results
85 with one another.

Figure 1 | Systematic evaluation of network reconstruction from imputed and unimputed data.

Cubic evaluation matrix consists of seven cell types from experimental scRNAseq data, four imputation methods (see text) and three network reconstruction algorithms. Imputed and unimputed (“noimp” in the Figure) scRNAseq data provide input expression matrices which are used by the gene regulatory network (GRN) reconstruction algorithms using the BEELINE framework (6). We evaluate the performances using the early precision ratios (EPR) and compare network results across different models. Additionally, we inspect the effect of gene-gene correlation on prediction classes (true positives (TP), false positives (FP), false negatives (FN)) before and after imputation, and we search for common motifs within the reconstructed networks. hESC: human embryonic stem cells, hHep: human hepatocytes, mDC: mouse dendritic cells, mESC: mouse embryonic stem cells, mHSC-E, mHSC-GM, mHSC-L: mouse hematopoietic stem cells - erythrocytes, granulo monocytes, lymphocytes.

2.2 Imputation does not improve the performance of network reconstruction in general

A compact overview of the results obtained under all the models is provided in Figure 2, where each box summarizes results for one GRN reconstruction method. The performance measurements achieved by the respective model on the seven data sets are arranged on a

vertical axis. Two performance measures have been computed: the early precision ratios (EPR) (6) which are shown in the three boxes of Fig. 2A, and the $\log_2$ -ratios between epr_{imputed} and $epr_{\text{unimputed}}$ which are shown in the three boxes of Fig. 2B. EPR refers to the number of true positive interactions within the top-k network normalized by the network density. Here, k refers to the number of positive interactions found in the ground-truth network (see Methods). An EPR of 1 indicates a random predictor. The second performance measure compares the performance of an imputation method relative to the performance of not using imputation. Here, a value of zero means no change, while a negative value indicates a detrimental effect of the imputation.

The EPR scores for unimputed data that were reported by Pratapa et. al. (6) could be reproduced in our analysis and are illustrated as a dashed line in Figure 2A. Results vary strongly with the datasets; the scores range from approximately 2 (for the mDC dataset) to 8 (for mHSC-GM), with less variation across GRN reconstruction algorithms. Applying imputation with either *dca*, *knnsmooth* or *magic*, does not improve the performance in any of the GRN reconstruction methods. While in mDC data the performance scores in each model scatter around the unimputed model, in mHSC-GM data the performance scores vary strongly, dropping from 8 to just below 5 for the *magic*/GENIE3 model.

Focussing on the change of performance due to imputation as measured using the $\log_2$ -ratios between imputed and unimputed EPR scores, we observe that only *saver* is able to improve the performance (Fig. 2B). The *saver*/PIDC model achieves log-fold-ratios up to +0.5 in 5 out of 7 datasets and 2 out of 7 datasets combined with GENIE3 or GRNBoost2. All other imputation methods worsen the performance with log-fold-ratios down to -1 which represents a performance decline of 100% in comparison to the unimputed model.

126 We further asked the question whether data quality as given by sequencing depth is a
 127 determinant of the success of imputation prior to GRN reconstruction. To answer this, we
 128 simulated cells *in silico* by downsampling the gene counts of the given experiments to 60%
 129 of their sequencing depth, thereby lowering the detection rate (Supp. Fig. 1). The hope
 130 would be that imputation has a more beneficial effect in these simulated data sets as
 131 compared to the original, higher quality data. However, similar results as above were
 132 obtained when we subjected the lower quality *in silico* data to our analysis pipeline (Supp.
 133 Fig. 2). Like with the original datasets, *saver*/PIDC obtain the highest improvements
 134 compared to the downsampled unimputed datasets. Nonetheless on downsampled data,
 135 *dca*, *knnsmooth* and *magic* are able to improve performance in some of the tested datasets,
 136 although not consistently.

137 Overall, our results demonstrate that our model performances are highly dataset-dependent.
 138 Applying imputation on the original data resulted mostly in a drop of performance of GRN
 139 reconstruction compared to the unimputed model, although potentially improving
 140 performance on low-quality data tested *in silico*.

Figure 2 | Impact of imputation on network reconstruction performances. (A) Absolute EPR scores across imputation methods (x axis label) and GRN inference algorithms (box) on seven different cell types (coded by shape and color). Dashed lines represent EPR scores obtained without imputation. EPR = 1 corresponds to a random predictor. (B) log2-ratios between EPR scores obtained using imputed and unimputed data. Log2-ratio = 0 represents no change in performance (grey dashed line) after imputation.

2.3 Imputation method rather than GRN method determines

results

The analysis presented in the preceding Section raises the question how strongly either the choice of imputation method or of network reconstruction algorithm affects the results. To answer this question we first address the variability in results when varying either the one or the other, and then study similarity among computed networks across the models.

With regard to the performance variability, we compare the variance of EPR log-fold-ratios under a fixed GRN reconstruction algorithm while varying across imputation methods, and, vice versa, varying the GRN algorithm while keeping the imputation method fixed. As Figure

155 3A shows EPR log-fold-ratios vary much more strongly when the GRN reconstruction
156 algorithm is fixed than than the other way round (wilcoxon-test p-value $\sim 7.86 \times 10^{-6}$). This
157 implies that the choice of imputation method determines the quality of results to a larger
158 degree than the choice of GRN reconstruction algorithm.

159 A direct consequence of this observation is the suspicion that the topology of the predicted
160 networks may also be largely determined by the imputation method and to a lesser degree
161 by the GRN reconstruction method. To test this, we inspect the overlap among the 500 most
162 important gene-gene interactions of the computed networks. Here, we calculate pairwise
163 similarity scores using the Jaccard index and use it to hierarchically cluster the networks. We
164 found that networks tend to cluster with respect to imputation methods but not GRN methods
165 (Fig. 3B, Supp. Fig. 4). To make this more precise, we use as a measure of cluster purity the
166 adjusted rand index (ARI) (33,34). ARI coefficients calculated across the seven different cell
167 types show higher cluster purity when labelled with imputation method as opposed to
168 network reconstruction algorithms (Fig. 3C).

169 We conclude that the imputation method largely determines model performance, leaving little
170 influence to the subsequent GRN reconstruction algorithm. The choice of imputation method
171 further biases the outcoming network leading to little consensus across the most important
172 recovered gene-gene interactions as computed based on different imputation methods.

Figure 3 | Variability in network results largely stems from imputation methods. (A) Variance distribution of EPR scores across imputation methods. Left violin plot keeps the GRN algorithm fixed and depicts the variances in EPR log-fold-ratios for each dataset across the imputation methods. Right violin plot shows the variances for fixed imputation methods. **** corresponds to $p\text{-value} \leq 0.0001$ by wilcoxon rank sum test. (B) Clustered heatmap of network similarities measured by Jaccard index within top 500 reported interactions. Columns are color-coded by imputation methods. Rows are color-coded by network inference algorithms. (C) Adjusted rand index (ARI) obtained for clustering results in each cell type by annotation label “algorithm” (pink) and “imputation” (blue), respectively.

2.4 Inflation of gene-gene correlations and its impact on the network topology

Based on the reported results, we examine how imputation generally affects gene-gene correlation coefficients. Although not all network reconstruction algorithms use correlation-based measures to recover interactions, we still use Pearson's correlation coefficient as a proxy for the association between two genes. Subsequently, we will investigate whether the interactions within the reconstructed networks affect the global network structure.

189 Exploring the overall distributions of gene-gene correlations after imputation on scRNAseq
 190 data we observe a strong increase in gene-gene correlations (Fig. 4A). Generally,
 191 gene-gene correlations go from almost no correlation when computed using unimputed data
 192 to very good anti- and positive correlations due to imputation. Here, *magic* leads to the most
 193 extreme enhancement. More specifically, Figure 4B exemplifies the association between
 194 three genes before and after imputation, transforming very weak correlations to almost
 195 perfect (anti-)correlations. These associations were only reported after imputation using *dca*
 196 among the top-k network using GRNBoost2 in hESC data. Indeed, we commonly find such
 197 associations across different datasets and imputation methods.

198 In order to see what impact this enhancement of correlation has on the network structure we
 199 next investigated the network density after imputation in relation to the unimputed data using
 200 log-ratios (Supp. Fig. 3A). Here, we looked at the top-k networks according to the EPR
 201 score. Imputation methods alter the network densities with log-ratios ranging from -0.5 and
 202 +0.5 in hESC, hHep, mDC and mESC data, except for *saver* and PIDC in hESC data with a
 203 slightly higher value of 0.59. For the three subtypes of mHSC data we observe larger
 204 changes in network density reaching log-ratios beyond ± 1 . Especially here, imputations
 205 combined with GENIE3 and GRNBoost2 lead to a sparser network whereas all combinations
 206 of imputation methods with PIDC lead to a denser network structure. This is not surprising as
 207 GENIE3 and GRNboost2 are network reconstruction algorithms that provide a directed
 208 network, whereas PIDC results in undirected interactions providing a backward and forward
 209 edge with the same ranks. As we select top-k ranked interactions we take interactions
 210 sharing the same ranks simultaneously, consequently leading to a denser network with
 211 PIDC.

Besides network density, the network topology is also determined by the node degree distribution. Before imputation we observe a heavy tail node degree distribution predominantly in GENIE3 and GRNBoost2 indicating the presence of many hub nodes (Supp. Fig. 3B). After imputation the heavy tail disappears when using *dca*, *magic* and *knnsmooth* while it still exists when using *saver*. Generally, PIDC does not lead to this structural change in node degree distribution.

As a conclusion, the enhancement of gene-gene correlations due to imputation appears to lead to notable changes in the topology of the predicted gene networks.

2.5 Increased correlation values in false positive interactions inflate network results

Since we have observed that imputation may decrease the performance of GRN network reconstruction, we attempt to understand how the altered correlations in imputed data affect network reconstruction. To this end, we explore the change of edge ranks and correlation values of the reported (i.e., positively predicted) and missed (i.e., negatively predicted) interactions.

Overall, the ranks of true positive (TP) interactions reported in the unimputed data change significantly after imputation (Fig. 4C, Supp. Tab. 1, Supp. Fig. 5). Some of the previously reported TP interactions could be recovered after imputation. Nevertheless, the majority of previously reported TP interactions shift after imputation towards the end of the gene interaction ranking list, and are considered less important. As a consequence, other interactions become more important.

Therefore, we look at the change of correlation of positively predicted interactions before and after imputation. Figure 4D (and Supp. Fig. 6) show scatter plots of gene-gene interactions

235 with the absolute values of correlation coefficients before imputation on the horizontal axis
 236 and the correlation coefficient after imputation on the vertical axis. For each model, red dots
 237 are the true positive interactions, yellow are the false positives, and blue are the false
 238 negatives. The general shape of the scatter plot reiterates the observation that correlation
 239 coefficients tend to get enhanced by imputation. For each class we computed regression
 240 lines. For better recognition of true positives after imputation, one would hope for the TP
 241 regression line (shown in red in Fig. 4D) to lie well above the others - which is not really the
 242 case. We generally observe a strong enhancement of correlations as indicated by the height
 243 of the intercept of the regression lines. In 11 out of 12 cases the regression lines for both
 244 true and false positive predictions are almost congruent with each other. Note that the red
 245 color dominates the other ones and the dots below a red one are not visible.

246 Interestingly, we see remarkably different regression lines if we take the false negative (FN,
 247 blue) interactions into account. The majority of FN correlations remain low after imputation,
 248 as indicated by the height of the intercept in Fig. 4D. Presumably, the FN correlation values
 249 that actually get enhanced get lost in the background due to the inflation of FP correlations in
 250 the inferred top-k network. Thus, the boost of correlation values makes it harder for GRN
 251 reconstruction methods to separate the actual signal from the background.

252 Many GRN reconstruction methods have the goal of distinguishing direct interactions from
 253 transitively inferred ones (35). Therefore, we tested whether the GRN reconstruction
 254 algorithms analyzed in this study are able to make the necessary distinction. Given three
 255 genes X, Y, and Z where X is correlated with Y, and Y is correlated with Z, these genes form
 256 a network chain. However, oftentimes by transitivity these associations seem to imply a
 257 correlation between X and Z, thus forming a network loop. Generally, in network theory it is
 258 challenging to distinguish chains from loops. In this context, we analyze how the models deal

259 with the identification of network chains from imputed data. Errors are counted if a
 260 supposedly false loop is detected or a chain is detected instead of a loop (Fig. 4E).

261 In general, PIDC identifies the highest number of network motifs independent of the
 262 imputation used. Using *saver* in combination with PIDC one is able to find the highest
 263 number of TP chains. However, *saver*/PIDC mistakenly identifies network motifs at the same
 264 time. In order to measure the performance between true and false predictions we calculate
 265 the true positive rates (TPR) and false discovery rates (FDR) for each network inference and
 266 imputation method applied to each dataset (Fig. 4F).

267 The performance of network motif search among the top-k networks does not seem to be
 268 affected by imputation. Hence, either imputation methods do not necessarily induce
 269 transitive correlations or the network reconstruction methods deal well with transitively
 270 induced correlations.

Figure 4 | Gene-gene correlation before and after imputation and its impact on the predicted interactions. (A) Gene-gene correlation distributions obtained in each cell type color-coded by imputation method among top 500 most variable genes and significantly varying TFs. (B) Paired density scatter plots before and after imputation with *dca*. GRNBoost2 reported the pairwise interactions between ASXL1, SNAPC3 and ZNF488 among the top-k network after imputation in hESC data. (C) Change of edge ranks in true positive (TP) interactions identified by unimputed model after imputation in hESC data. Dashed line indicates the rank threshold corresponding to the top-k network. Interactions below the dashed line represent TP within the respective model. Low edge ranks represent highly important interactions. (D) Change of correlation values for TP (red crosses), FP (yellow dots) and FN (blue dots) classified by each model in hESC data. Positively predicted interactions differ clearly from FN interactions. (E) Counts of positively and negatively predicted network chain motifs in hESC data for each model. TP network chains agree both in prediction and ground truth. FP network chains are falsely positively predicted chains being actual feed-forward loops in the ground truth. FN network chains are falsely predicted as being feed-forward loops when they are actually network chains in the ground-truth network. (F) TPR and FDR scores for network chain motifs obtained by statistics in E). Ideally, TPR values should be close to 1 whereas FDR values should be close to 0.

3 Discussion

The advent of single-cell transcriptomics has rekindled the interest in reconstructing gene regulatory networks from transcriptomics data, primarily for two reasons. Firstly, it is of great interest to study regulation in individual cells in the hope to eventually uncover how, e.g., differentiation processes proceed. Secondly, the main obstacle in gene network reconstruction from bulk transcriptome data appears to be the low number of available samples in comparison to the large numbers of genes. For example, simulations have demonstrated that high quality reconstruction of gene networks requires a much larger

297 number of samples than the number of genes (35). Seeing each single cell as a sample, the
 298 expectation arose that single-cell transcriptomics would solve this conundrum by providing a
 299 sufficiently large number of samples, thus putting high quality network reconstruction within
 300 reach.

301 It was sobering for us to see that due to the sparse nature of single-cell RNA-seq data,
 302 individual cells cannot contribute as much information to network reconstruction as bulk
 303 samples. Indeed, preprocessing of single-cell data for data analysis is crucial (9), and is
 304 implemented in many computational pipelines. Imputation has become a possible element of
 305 this preprocessing in the hope it would supplement the missing information. In this study we
 306 have however demonstrated that the choice of imputation prior to GRN reconstruction
 307 influences the results in a two-fold manner: First, it affects the performance of network
 308 reconstruction leading to highly variable accuracies and, secondly, the reconstructed
 309 network differs significantly between imputation methods.

310 We have systematically evaluated the effect of imputation on GRN reconstruction using
 311 experimental scRNAseq data on seven cell types. In this context, we have demonstrated
 312 that overall, imputation does not lead to an improvement of GRN reconstruction. However,
 313 *saver* in combination with PIDC may lead in some datasets to an increase in performance.
 314 We have shown and thereby agree with previous studies that imputation may boost
 315 gene-gene correlations in a dubious way, thereby introducing false positives (25). In turn,
 316 these false positives predispose network structures toward forming circular dependencies. In
 317 fact, if network reconstruction methods rely on associations that use correlation to some
 318 extent (for example regression-based methods) the circularity is highly redundant. Andrews
 319 et. al. have warned of this circularity before, albeit in the context of differential expression
 320 analysis (36). Consistent with our findings Andrews et. al. showed that *saver* introduces the

321 smallest number of spurious gene-gene correlations. We speculate that the combination of
322 *saver*/PIDC works well because *saver* is a model-based imputation method and PIDC is a
323 mutual-information based algorithm; the two approaches follow independent assumptions
324 complementing one another, thus avoiding the use of redundant information.

325 In this study we have tested our hypothesis on experimental datasets with fairly large library
326 sizes and gene detection rates (Supp. Fig. 1.). In order to test our hypothesis on more
327 shallowly sequenced single-cell experiments we lowered the detection rate introducing more
328 zero counts *in silico*. Our results have shown that using *saver* with PIDC improves results in
329 most cases. However, generally we discourage the indiscriminate usage of imputation prior
330 to GRN reconstruction because imputation tends to introduce a bias into the derived
331 networks. If need be we recommend the use of *saver* and PIDC. It should be noted that we
332 are not discouraging imputation in general. There may be many other applications that are
333 not studied here, where imputation can be useful, depending on the type of analysis that is
334 subsequently performed.

335 4 Methods

336 4.1 Data collection and preprocessing of scRNAseq data:

337 We collected preprocessed and normalized experimental scRNAseq count data provided in
338 the BEELINE paper (6). Here, the authors also provide the corresponding pseudotime for
339 each dataset / cell type. Please refer to the BEELINE paper for information about
340 preprocessing, normalization, and pseudotime inference.

341 However, *dca* needs unnormalized raw count data. Therefore, we downloaded the fastq files
342 using the corresponding accession numbers: GSE75748 (hESC) (14), GSE81252 (hHEP)

(26), GSE98664 (mESC) (27), GSE48968 (mDC) (28) and GSE81682 (mHSC) (29). For human and mouse we aligned the fastq files to hg19 (GENCODE release 29) or mm10 (GENCODE release M19), respectively and counted the reads per gene using STAR (version 2.7.4a) (37).

Following the BEELINE approach, using normalized count data we select the top 500 most variable genes across pseudotime using a general additive model ('gam' R package). In addition to these genes we also include significantly varying TFs (Bonferroni corrected p-value < 0.01).

We filter both imputed and unimputed scRNAseq data using the same set of (i) top 500 most variable genes and (ii) all significantly varying TFs, in order to make a fair comparison between networks inferred using imputed and unimputed data.

4.2 Code availability

All relevant scripts and R notebooks for reproducing the results are available at Github (https://github.com/lylamha/imputation_GRN_inference). The release includes tutorials from data imputation to the evaluation of the reconstructed networks. It covers the evaluation pipeline with the corresponding analyses and plotting results.

4.3 Imputation

To impute scRNAseq data we use *dca* (version 0.2.3), *knnsmooth* (version 2.1), *magic* ('Rmagic' R package version 2.0.3) and *saver* ('SAVER' R package version 1.1.2). Our rationale for selecting *knnsmooth*, *magic* and *saver* is based on a previous comprehensive benchmark evaluation of various imputation methods (23). Additionally, we also include *dca* as it has been explicitly recommended as improving GRN reconstruction.

365 We apply each imputation method to normalized count data except for dca where we use the
366 raw counts:

367 `dca </path/to/ExpressionData_raw.csv> </path/to/dca_result_folder>`

368 `python3 knn_smooth.py -k 15 -d 10 \`
369 `-f <path/to/ExpressionData.csv> \`
370 `-o <path/to/ExpressionData_knnsmooth_imputed.csv> --sep ,`

371 `magic Rsnippet:`
372 `# so_dat (seurat object using library(Seurat))`
373 `so_dat <- magic(so_dat, assay="RNA", genes="all_genes")`
374 `Dat.magic <- as.data.frame(so_dat@assays$MAGIC_RNA@data)`

375 `saver Rsnippet:`
376 `saver_res <- saver(input_expr_matrix, size.factor = 1, ncores = 20,`
377 `estimates.only = F)`
378 `dat.saver <- as.data.frame(saver_res$estimate)`

379 4.4 Network reconstruction via BEELINE:

380 Several tools have been developed to infer GRNs from scRNAseq data differing in their
381 algorithmic approach. They can be categorized into four main classes: correlation-,
382 regression-, mutual information- or modelling-based approaches (6). In this study we
383 evaluated PIDC, GENIE3 and GRNBoost2 which have been previously recommended by
384 Pratapa et. al. (6) . We use the imputed and unimputed scRNAseq data as input matrices
385 for network reconstruction with PIDC, GENIE3 and GRNBoost2 using default parameters. To
386 this end, we use the evaluation framework BEELINE (version 1.0).

387 As part of the BEELINE pipeline we first run 'BLRunner.py' to reconstruct the networks.
388 Then, we filter the reconstructed networks in order to only include interactions from TFs to
389 genes.

390 Finally, we use 'BLevaluater.py' to compute early precision scores evaluating the
391 performance of each network by comparing it to a ground truth network. Here, we choose
392 the functional protein-protein interaction database STRING and filter for genes that only
393 occur in the input expression matrix.

394 By using early precision scores we only analyze the top-k networks.

395 4.5 Characterizing the reconstructed networks

396 4.5.1 Top-k network

397 For comparability reasons we focus our analyses on the top-k networks. The top-k network
 398 of a reconstructed network includes the first k interactions selected by their ranks which were
 399 assigned by descendingly ordered edge weights. Here, k represents the number of positive
 400 interactions in the ground truth network. Interactions can share the same ranks, e.g., the
 401 forward and backward interactions in an undirected graph. So with k interactions reported in
 402 the ground truth network we select all interactions which ranks are lower than or equal to k
 403 obtaining the top-k network. Note, that the number of reported interactions can be higher
 404 than k .

405 4.5.2 Network density and node degree

406 Taking into account the interaction between transcription factors and genes only the network
 407 density is calculated by $\text{numEdges} / ((\text{numGenes} * \text{numTFs}) - \text{numTFs})$.
 408 In order to calculate the node degree we consider all out- and incoming edges for a given
 409 node.

410 4.6 Methodology of evaluation

411 4.6.1 Early Precision Ratios (EPR)

412 We evaluate the performance of each inferred network based on using early precision scores
 413 (EP) which is given by the number of TP divided by the number of positively predicted
 414 observations within the top-k network. EP scores were calculated using BEELINE. Each

dataset has a different underlying ground truth subnetwork, hence different evaluations regarding the random predictor. To account for these differences and in order to maintain comparability across datasets we divide the EP scores by the network density (see formula above) of each ground truth subnetwork obtaining EP ratios (EPR). Thus, EPR of 1 is indicative of a random predictor in all experimental datasets. To compare the performance of network inference in each imputation method with the corresponding unimputed data, we calculate log2-ratios between EPR_{imputed} and $EPR_{\text{unimputed}}$.

4.6.2 Network similarities

In order to compare similarities across the reconstructed networks we select the top 500 interactions reported in each model. Given two networks, similarity scores are obtained by the Jaccard index which is defined as the number of overlapping interactions divided by the number of unified reported interactions. Repeating this in a pairwise iterative manner we obtain a similarity matrix which we use as an input for a heatmap that is clustered row- and column-wise ('pheatmap' R Package).

We calculate adjusted rand index (ARI) scores ('mclust' R package) in order to evaluate the clustering results based on an annotation label (34). As annotation labels we use the network reconstruction algorithm as well as the imputation method. We compare ARI scores across datasets obtained by the two labels using the pairwise wilcoxon rank sum test.

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441 Contributions

442 L.H.L. and M.V. designed the study. L.H.L. processed and analyzed the data. L.H.L. and M.V.
443 wrote the manuscript.

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446 Competing interests

447 The authors declare no competing interests.

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