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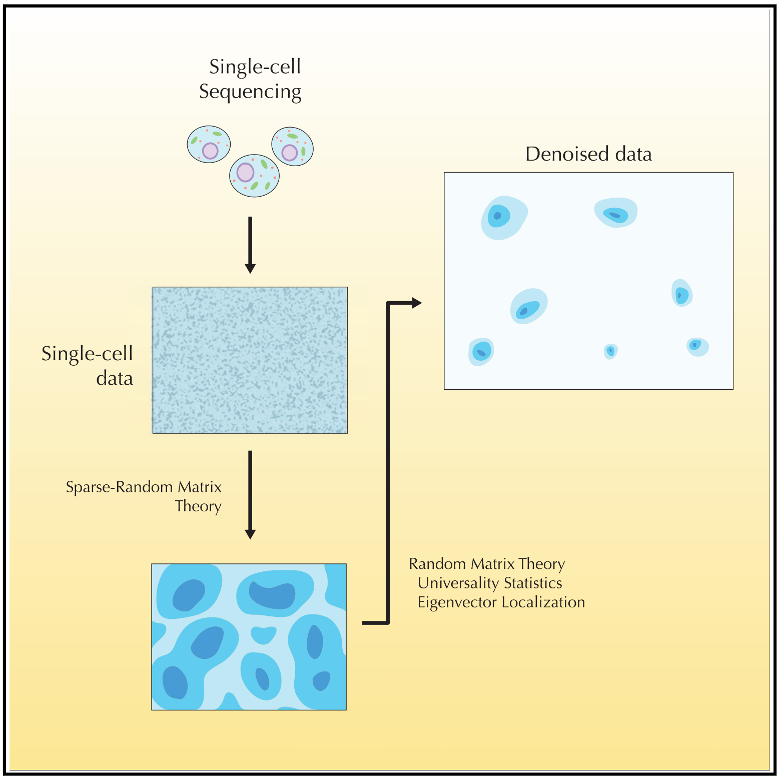
A Random Matrix Theory Approach to Denoise Single-Cell Data

Graphical Abstract



Highlights

- Sparse random matrix theory provides a suitable framework to study single-cell biology
- Eigenvector localization disentangles sparsity-induced signals from biological signals
- 95% of the information is a random matrix, 3% sparsity-induced signal, and 2% true signal
- The method improves clustering and identification of cell populations

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In Brief

We demonstrate the effectiveness of (sparse) random matrix theory for studying the spectrum of the covariance matrix of single-cell genomic data. We show that single-cell data have a 3-fold structure: a random matrix, a sparsity-induced signal, and a biological signal. Most of the spectrum follows the expectations from random matrix theory (95%), but there exist deviations due to artifacts generated by a sparsity-induced signal (~3%) and a biological signal (~2%).

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A Random Matrix Theory Approach to Denoise Single-Cell Data

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THE BIGGER PICTURE Single-cell technologies are able to capture information of a biological system cell by cell. Such a level of precision is changing the way we understand complex systems such as cancer or the immune system. However, a major challenge in studying single-cell systems and their underlying biological phenomena is their inherently noisy nature due to their complexity. Random matrix theory is a field with many applications in different branches of mathematics and physics. In the words of one of its developers, the theoretical physicist Freeman Dyson, it describes a "black box in which a large number of particles are interacting according to unknown laws." A complex system with a large number of components (such as genes, biomolecules, or cells) interacting according to unknown laws is the epitome of systems biology. Therefore, random matrix theory looks like a suitable framework to mathematically describe the noise and complexity of gene-cell expression data coming from single-cell biology.

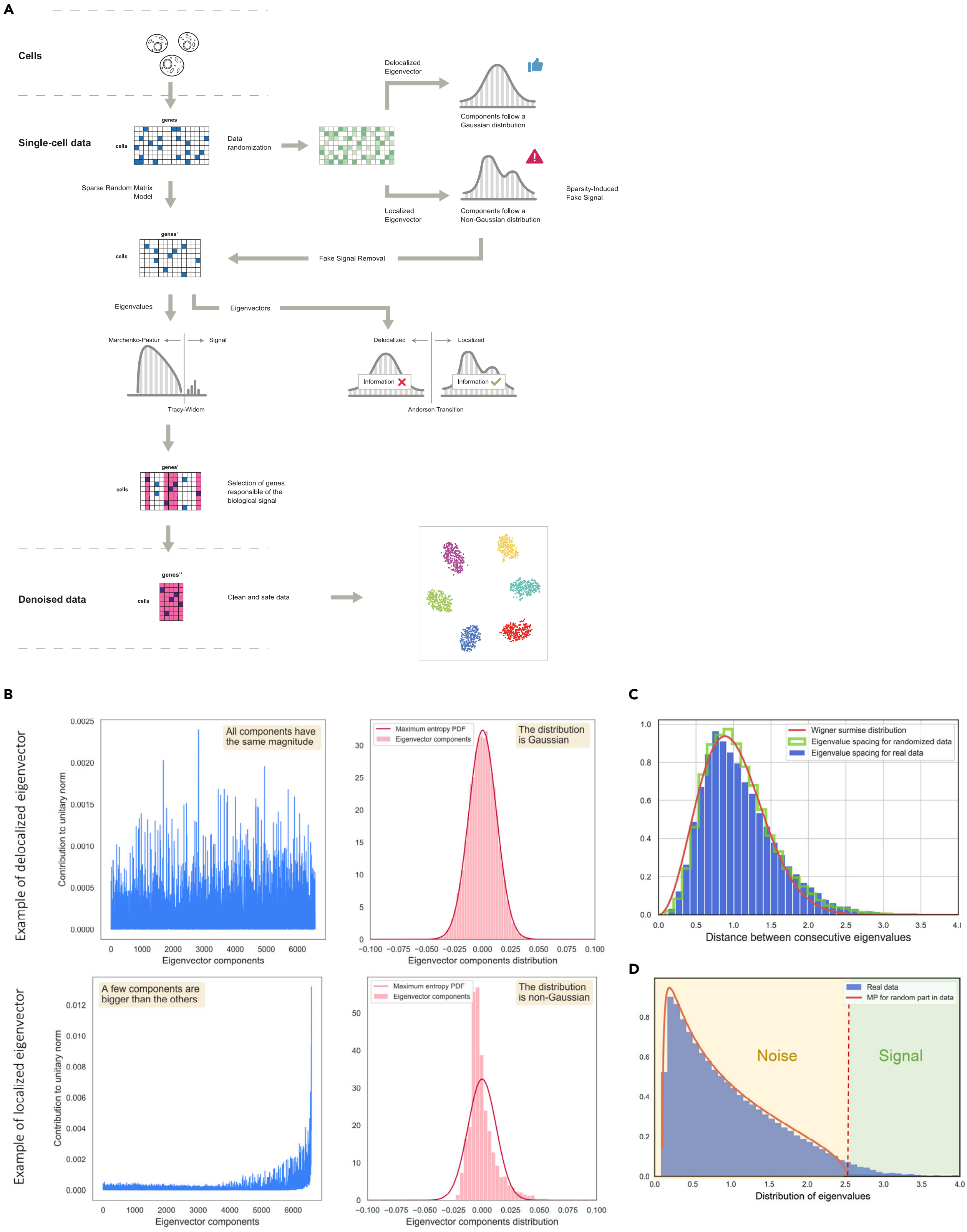
Proof-of-Concept: Data science output has been formulated, implemented, and tested for one domain/problem

SUMMARY

Single-cell technologies provide the opportunity to identify new cellular states. However, a major obstacle to the identification of biological signals is noise in single-cell data. In addition, single-cell data are very sparse. We propose a new method based on random matrix theory to analyze and denoise single-cell sequencing data. The method uses the universal distributions predicted by random matrix theory for the eigenvalues and eigenvectors of random covariance/Wishart matrices to distinguish noise from signal. In addition, we explain how sparsity can cause spurious eigenvector localization, falsely identifying meaningful directions in the data. We show that roughly 95% of the information in single-cell data is compatible with the predictions of random matrix theory, about 3% is spurious signal induced by sparsity, and only the last 2% reflects true biological signal. We demonstrate the effectiveness of our approach by comparing with alternative techniques in a variety of examples with marked cell populations.

INTRODUCTION

Characterizing different cellular subtypes in heterogeneous populations and describing their evolution plays a central role in understanding complex systems such as cancer or the immune system. Single-cell technologies offer the opportunity to identify previously unreported cell types and cellular states and explore the relationship between new and known cell states.[1–7] However, there exist several significant biological and technical challenges that complicate the analysis. The first challenge is the lack of a complete quantitative understanding of the different sorts of noise that arise in single-cell measurements, such as intrinsic cell-to-cell variability and spatial and temporal fluctuations within a cell. Moreover, different technologies show biases arising from the process of detecting, amplifying, and sequencing genomic material that significantly vary across different genomic loci. Correctly estimating noise and distinguishing between biological and technical sources of signal is



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essential for any further analysis, otherwise it is difficult to reliably distinguish states or identify potential variations of a single state. A second complicating factor for single-cell analysis is the sparsity of data (i.e., the large fraction of zero values in the original data matrix), typically caused by the very small amounts of genomic material being amplified.

A number of computational and statistical approaches have been designed to address these challenges.[4,8–13] Imputation methods try to infer the "true" expression levels of missing values from the sample data by empirically modeling the underlying distributions; for instance, using negative binomial plus zero inflation (dropout) for single-cell data. These techniques usually assume that all values are generated by the same distribution (i.e., they assume independent and identically distributed random variables, or i.i.d.). Although there have been efforts to understand the intrinsic stochastic nature of gene expression,[14,15] we currently do not have predictive quantitative models of gene expression. Therefore, it is not clear what the correct distribution is, or whether it is reasonable to make the i.i.d. assumption. Given the lack of a quantitative microscopic description of cell transcription, we would ideally like to have a statistical description of the noise in single-cell data that does not rely on specific details of the underlying distributions of expression.

Universality and Random Matrix Theory in Single-Cell Biology

Historically a similar problem arose in the 1950s in nuclear physics, when the lack of quantitative models of complex nuclei precluded accurate predictions of their energy levels. However, simple theoretical models based on experimental data showed that some observables, such as the spacing between two consecutive energy levels, followed distributions that could be derived from random matrices, i.e., matrices whose entries are independently sampled from a given probability distribution.[16–18] The same distributions were subsequently identified in a variety of complex systems including quantum versions of chaotic systems[19] and patterns of zeros of the Riemann zeta function.[20,21] In this paper, we show that these distributions also appear in the context of single-cell biology and that their properties can be used to denoise single-cell data (Figure 1A).

Random matrix theory (RMT) studies the statistical properties of the eigenvalues and eigenvectors of an ensemble of random matrices. These statistical properties exhibit a phenomenon known as universality, where under mild hypotheses the specific details of the underlying probability distribution generating the entries of the matrix become irrelevant (akin to the central limit theorem).[22,23] Specifically, the observed distributions depend only on the finiteness of the first few moments of the distribution generating the matrix entries.[24–26] RMT universality implies that the density of eigenvalues of covariance matrices obtained from a random matrix follows the Marchenko-Pastur (MP) distribution.[22,27] It also implies that the eigenvectors of a random matrix are delocalized, i.e., their norm is equally distributed across all their components (see Figure 1B and Supplemental Experimental Procedures for an extensive discussion).

We propose here to apply this universality phenomenon to identify statistical features of noise present in single-cell biology (Figure 1). In particular, we claim that any single-cell dataset can be modeled as a random matrix (that encodes the noise) plus a low-rank perturbation (which is the signal). As a consequence, we expect the noise of the system follows the distributions predicted by RMT universality. Large deviations from these distributions indicate the presence of a signal that can be further analyzed. At the level of eigenvalues, random deviations from the MP distribution are described by the Tracy-Widom (TW) distribution, which is the probability distribution for fluctuations on the value of the largest eigenvalue of a random matrix (Figure 1C). Similar strategies using TW and MP distributions have been already discussed in previous works.[28–30]

Eigenvector Localization in Single-Cell Biology

One of the main novelties of this work is the application of the eigenvector statistics predicted by RMT to single-cell sequencing. For the eigenvectors, the transition between noise and signal is described by a phase transition: the delocalized eigenvectors give way to localized eigenvectors, i.e., eigenvectors characterized by having their norm concentrated in a few components (Figure 1B and Supplemental Experimental Procedures). In condensed matter physics this phenomenon is known as Anderson localization.[31] In the single-cell context, localization can be interpreted as groups of cells whose gene expression is correlated. An essential feature of the situation is that the distribution of components for delocalized eigenvectors approximates a Gaussian distribution, whereas the localized eigenvector components have a non-Gaussian distribution (Figure 1B and Supplemental Experimental Procedures). Eigenvalues that lie outside of the MP distribution are associated with localized eigenvectors (Figures 4 and 5).

As noted above, single-cell data are often very sparse. Sparsity introduces a subtlety in the analysis because sparse random matrices can present deviations from the eigenvalue distributions predicted by RMT universality and can have localized eigenvectors (Figure 2A). As a consequence, in a sparse dataset the deviations from the MP distribution and the localized eigenvectors will be partially induced by the sparsity. A way to identify

Figure 1. Random Matrix Theory Applications to Single-Cell Sequencing Data

(A) Schematic of the analysis based on random matrix theory (RMT). Single-cell data can be modeled using sparse random matrix theory (sRMT), showing a 3-fold structure: a random matrix, a sparsity-induced signal, and a biological signal. The strategy proposed here is to identify the biological signal using the predictions from sRMT applied to the covariance matrix of the data.

(B) Deviations from the Tracy-Widom (TW) distribution have been associated to the phenomenon of eigenvector localization. Delocalized eigenvectors are randomly distributed in an N sphere, whereas localized eigenvectors are localized along some directions in the N sphere. Localization can be identified as deviations in components of the eigenvectors from the expected distribution, which is approximately Gaussian in high dimensions. If we think of the components of the eigenvector as a random variable, its probability density function (PDF) (the Gaussian) corresponds to a maximum entropy PDF.

(C) The Wigner surmise distribution captures the spacing between eigenvalues of Wishart matrix across single-cell RNA-sequencing experiments.

(D) Departures from universal distributions predicted by RMT indicate interesting potential biological signals. In red is the non-parametric Marchenko-Pastur (MP) distribution. Deviations from universality can be found by analyzing the larger eigenvalues in relation to the expected TW distribution.

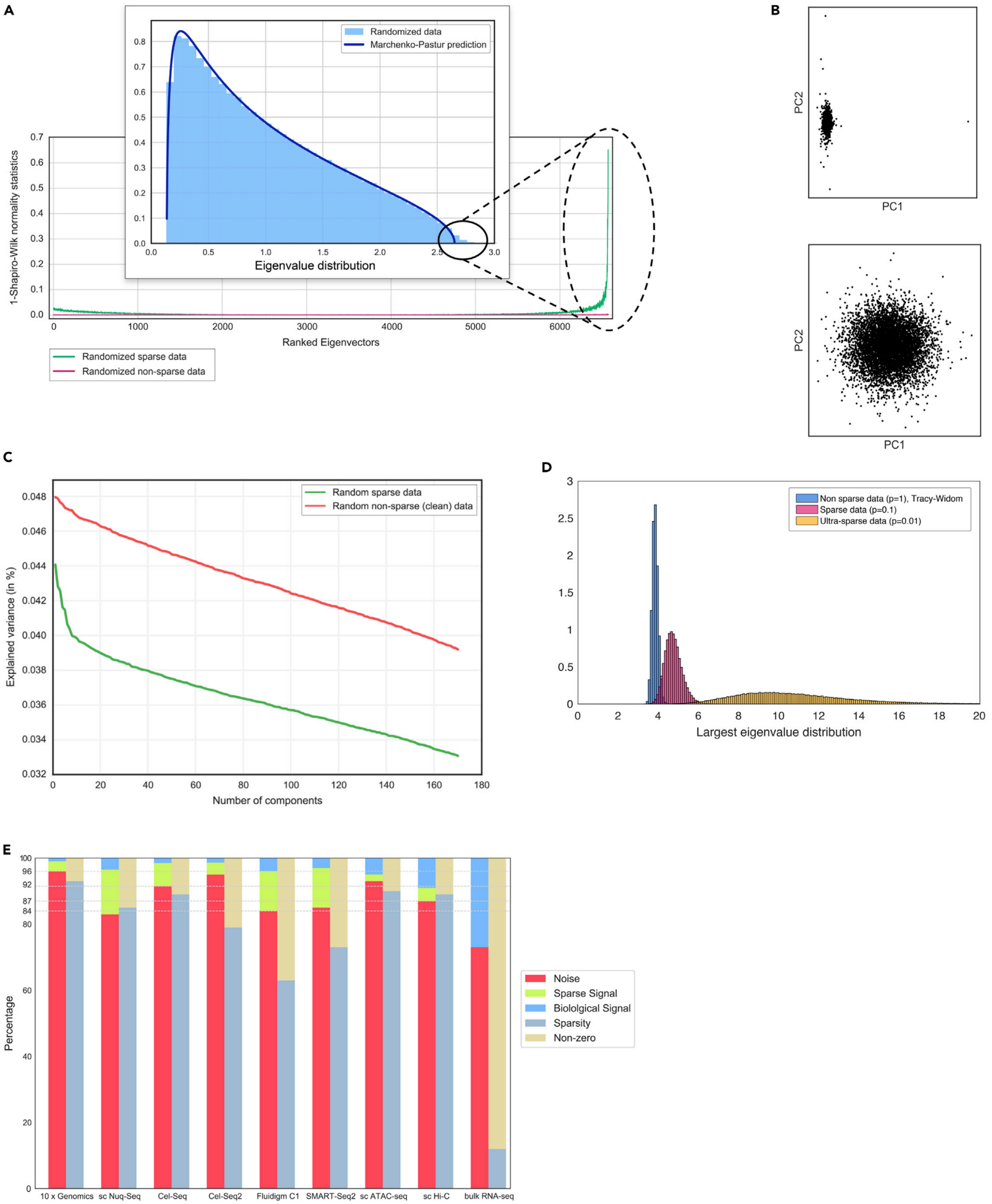

Figure 2. Sparse Random Matrices and Sparsity-Induced Eigenvector Localization

(A) Randomized sparse dataset, corresponding to PBMCs in Kang et al.,[32] where there exist deviations from MP distribution at the eigenvalue level, and presence of localized eigenvectors.

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the effects of sparsity is to randomize the dataset (permuting the cell labels for each gene independently) and observe that, although the entire dataset is now uncorrelated, it still might show localized eigenvectors and potentially also significant deviations in the eigenvalues from the MP prediction (Figure 2A) due to sparsity. We can therefore conclude that single-cell data can be thought of as decomposing into three parts: a random matrix, a sparsity-induced non-biological signal, and a biological signal. To distinguish the biological signal from the sparsity-induced eigenvectors, we propose a feature selection method that discards the features (genes) that are responsible for the localized eigenvectors in the randomized case. This method increases the power for identifying potentially interesting biological signals (Figures 3, 4, and 5). Our approach leads directly to an estimate of the latent space. We show that this procedure is better able than alternative techniques to capture marked single-cell clusters across a variety of datasets (Figure 6).

RESULTS

Quasi-Universality of Single-Cell Sequencing Data

We observed that the distribution of spacing between two consecutive eigenvalues of the sample covariance matrix in different single-cell RNA-sequencing experiments[35–40] resembles the Wigner surmise distribution conjectured by Wigner in 1955[18] in the study of the difference between resonant peaks in slow neutron scattering (Figure 1D). This observation prompted us to investigate the connection between RMT and the spectra of single-cell data, guided by the hypothesis that departures from RMT universality distributions indicate potential biological signals (Figures 1A–1C). We observed that across single-cell datasets these deviations amount to 5% of eigenvalues (Figure 2E). We also demonstrate (Supplemental Experimental Procedures) that the level of localization can be identified as deviations from normality in the distribution of eigenvector components (Figure 2A). Alternatively, localization can be detected using Shannon entropy (Figures S3A–S3C) or by the inverse participation ratio (IPR) (Figures S3D–S3F).

Sparsity-Induced Eigenvector Localization

Single-cell data are usually sparse. Thus, we investigated how sparsity could induce deviations from RMT universality (Figure 2A). By introducing zeros in a random matrix with entries generated with Gaussian or Poisson distributions, we observed deviations in the fluctuations of the eigenvalues from the TW distribution (Figure 2D). A similar phenomenon has been reported in the context of sparse random matrix ensembles, a generalization of RMT to the setting of random matrices with a significant fraction of zero entries. It has been shown[24,41–43] that for the case of sparse Wishart random matrices, the density distribution of eigenvalues deviates from MP and some eigenvectors become localized. We show that this phenomenon can be observed in sparse single-cell data. To this end, we randomized a 95% sparse cell-gene expression matrix corresponding to 6,573 human peripheral blood mononuclear cells (PBMCs) from Kang et al.[32] and analyzed the statistics of its eigenvalues and eigenvectors. Although the bulk of the eigenvalue density seems to follow an MP distribution, it is easily seen that deviations on the upper edge appear. Using a normality test we detected localization in the corresponding eigenvectors (Figures 2A, 4A, and 5A). Eigenvector localization due to sparsity generates artifacts that could potentially be interpreted as true signal in standard application of principal component analysis (PCA). For instance, the highest components of sparse random data show a bias toward the first component (Figure 2B). Another effect of sparsity is the generation of an artifactual "elbow" in randomized sparse data (Figure 2C). Therefore, the first step in our algorithm is to suppress these effects by removing genes that introduce spurious effects due to sparsity. We identify such genes in terms of deviation from normality after random projection (Supplemental Experimental Procedures).

Feature Selection and Application to Single-Cell Transcriptomic Datasets

In this section we explain the application of the RMT analysis to two marked single-cell datasets: 6,573 human PBMCs from Kang et al.[32] (Figure 4) and 3,005 mouse cortex cells from Zeisel et al.[34] (Figure 5). The first step is to remove the sparsity-induced signal. Figures 4A and 5A show the normality test for the eigenvectors before (blue line) and after (red line) removing the sparsity-induced signal. There is a substantial number of eigenvectors that become delocalized once the genes responsible for the sparsity are trimmed out (Supplemental Experimental Procedures). Once the sparsity-induced signal has been removed, the second step in the algorithm is to detect the part of the dataset that corresponds to a random matrix. We first compute the Wishart matrix and then use gradient descent to find the MP distribution in the eigenvalue distribution (Figures 4B and 5B; Supplemental Experimental Procedures). At the same time, the analysis of the normality of the eigenvectors (red line in Figures 4A and 5A) provides an estimate of the amount of information contained in each eigenvector. As mentioned before, the components of delocalized eigenvectors follow a Gaussian distribution; Figures 4A and 5A show the Gaussian profile of each eigenvector through a normality test (Shapiro-Wilk). Interestingly, even some of the eigenvectors corresponding to eigenvalues outside the MP distribution are delocalized and hence do not carry information. A similar argument can be made in terms of other eigenvector features, such as Shannon entropy or IPR (Figure S3).

(B) The localization phenomenon due to sparsity can bias the lower-dimensional representations (*up*). Eliminating the genes that cause eigenvector localization in the randomized dataset generates a more homogeneous distribution in the lower-dimensional representation (*down*), reflecting the random nature of the data.

(C) The effects of sparsity can also be appreciated in the classical elbow plots: sparsity can introduce an artifactual elbow in randomized data.

(D) Deviations from TW distributions can be easily seen in sparse matrices. In this case, 100-by-100 random matrices are drawn a mixture of a normal and a Dirac-delta at zero. Similar results are obtained with other sparse distributions.

(E) Departures from universality amount to near 5% of eigenvalues. However, most of these can be explained by the sparsity of data, suggesting that Sparse Random Matric Theory can provide a better model to understand single-cell sequencing data. Truly potential biological signal amounts to only ~2% of eigenvalues.

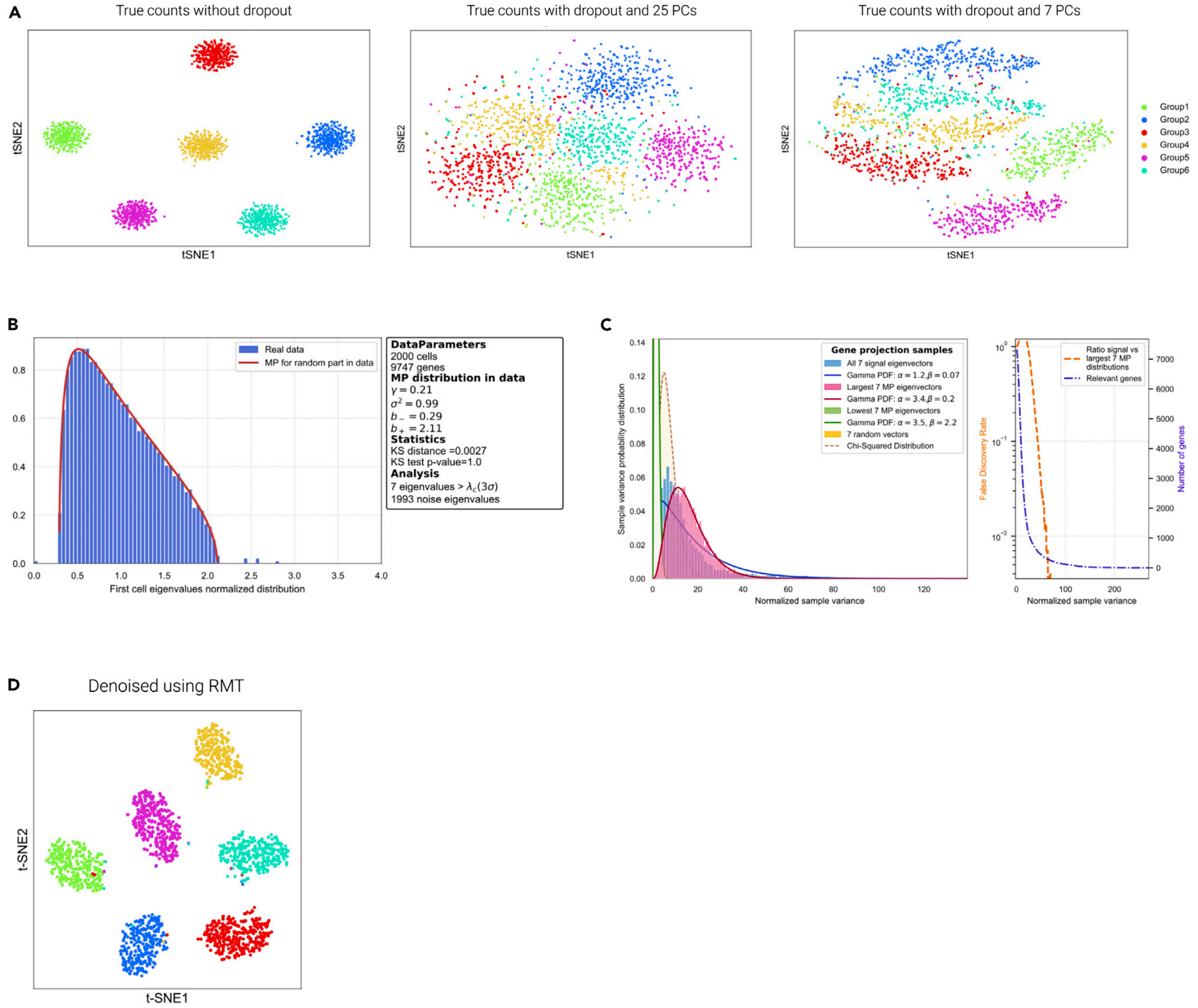


Figure 3. Application to Simulations of Single-Cell and Comparison with Standard PCA

(A) t-SNE representation of a six-cell population single-cell simulation using Splatter[33] for the cases with and without noise associated with dropout effects, and for different selection of principal components after applying a standard PCA technique. The colors correspond to the label of each group of cells simulated, and no clustering has been performed.

(B) MP prediction and identification of the relevant components.

(C) Selection of features (genes) responsible for signal.

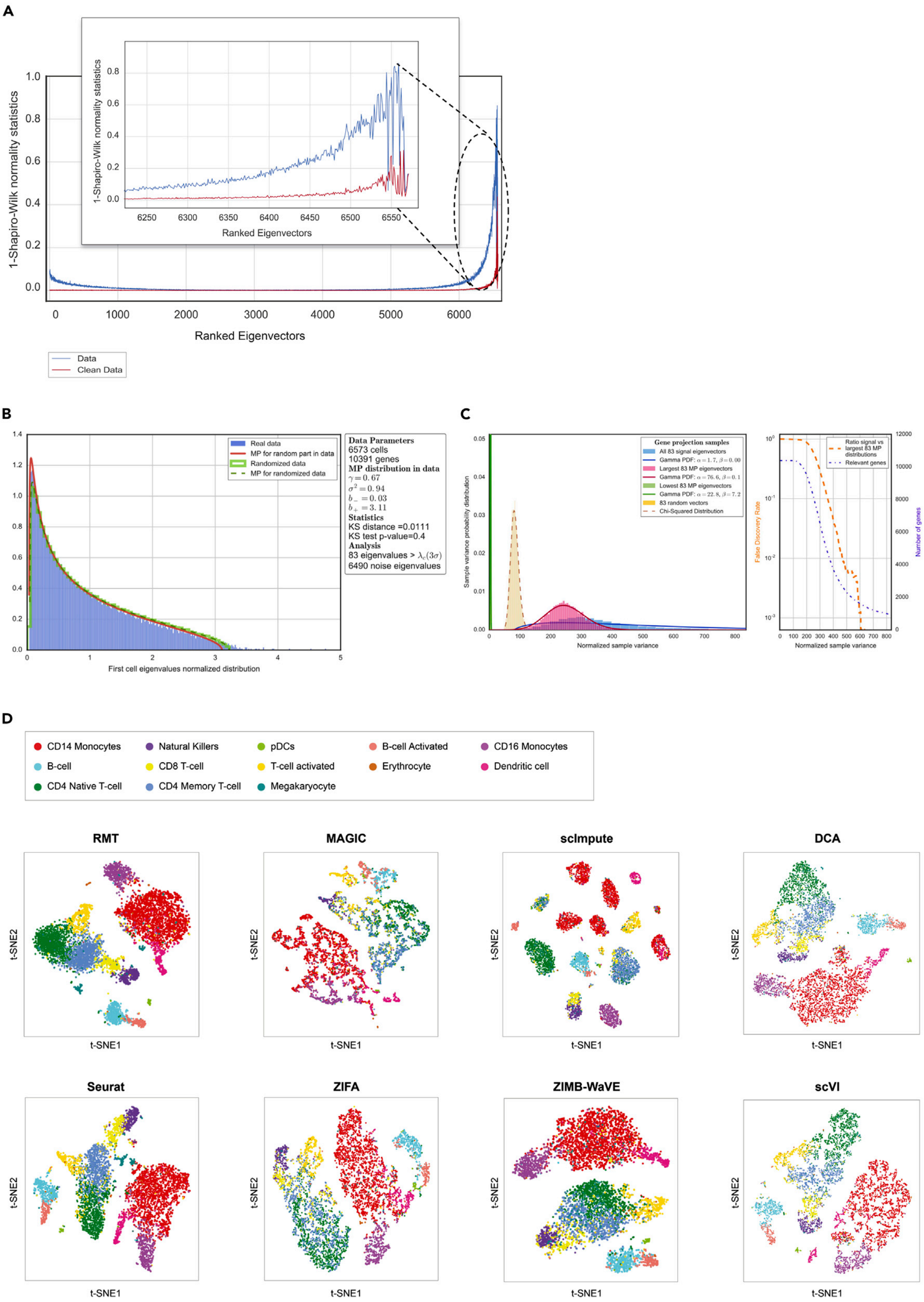
(D) t-SNE representation after results after processing through the RMT.

The strategy of our analysis is to detect and remove these delocalized (non-informative) eigenvectors. Filtering the eigenvectors complements and improves the analysis based only on the eigenvalues.

The third step consists in projecting the dataset onto the eigenvectors that carry signal and also onto different subsets of the eigenvectors that correspond to eigenvalues in the MP distribution (Supplemental Experimental Procedures). Using a chi-squared test for the variance of each gene projected onto the signal and noise eigenvectors, we use a false discovery rate to evaluate which genes are responsible for signal or noise (Figures 4C and 5C). The end result is a selection of features (genes) and a projection of the dataset onto the signal directions. Finally, Figures 4D and 5D (see also Figure S4) show t-distributed stochastic neighbor embedding (t-SNE) representations to visualize in two dimensions the latent space after denoising using our approach. The colors represent the cell populations described in Kang et al.[32] and Butler et al.[11] for human PBMCs, and for marked mouse cortex cell populations described in Zeisel et al.[34] In the same figures we also show a comparison with other methods used to denoise single-cell datasets based on imputation and zero-inflated dimensionality reduction.

Biological Interpretation

We have performed a gene set enrichment analysis on the genes that the algorithm selects as responsible for the biological signal.



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Using a hypergeometric test on reported biological processes in our mouse brain dataset, the top pathways in the signal gene list correspond to specific brain functions (transmission across chemical synapses, q value = 1.4×10^{-23}, Neural system q value = 2.8×10^{-23}) while in the PBMC dataset the top pathways correspond to immune-system-related processes (immune system q value = 1.9×10^{-32}, cytokine signaling in immune system q value = 2.0×10^{-21}). On the other hand, taking the genes that were not selected by our algorithm, the most significant pathways are associated with generic biological processes (S-phase q value = 4.1×10^{-12}; cell-cycle q value = 2.0×10^{-11}). These results support the contention that eigenvector localization can be used to identify biological processes that are specific to independent cell populations within each experiment.

Simulations and Comparison of Alternative Approaches

We now proceed to evaluate the performance of the algorithm for the identification of potential relevant biological signals. We first perform a single-cell RNA-sequencing simulation of six cell populations using Splatter[33] (Supplemental Experimental Procedures). Figure 3A shows a t-SNE representation of a simulation without and with noise associated with dropout effects. Here, 25 and 7 principal components have been selected. The colors correspond to each cell group simulated (no clustering has been performed). Figure 3D shows the result after our algorithm, and Figures 3B and 3C the associated MP statistics. The first example illustrates the challenge of identifying structures based on t-SNE plots before performing the algorithm (Figure 3A); in contrast, after the algorithm has been applied, we see clearly separated clusters (Figure 3D).

We now perform a comparison with some published algorithms in terms of cell-phenotype cluster resolution. We again use the datasets from Kang et al.[32] (human PBMCs) and Zeisel et al.[34] (mouse cortex) described in the previous section. As explained in the previous sections, these references together with Butler et al.[11] have cells already labeled by phenotype. We claimed in previous sections that our method is able to remove system noise such that the cell-phenotype clusters are better resolved. This noise is partially generated due to the missing values in single-cell experiments. For this reason, we compare the two main approaches in the field that address this: imputation (MAGIC[8] and scImpute[10]) and zero-inflated dimensionality reduction (ZIFA[13] and ZIMB-WaVE[9]). We also perform a comparison with non-linear neural networks methods: scVI[44] and DCA.[45] For completeness, we also compare the raw data with a selection of genes based on higher variance (top 300 genes) and with Seurat.[46] The comparison is performed using the knowledge of cell phenotypes in the studies by Butler et al., Kang et al., and Zeisel et al.[11,32,34] and by computing the mean silhouette score in the reduced space, whereby higher values would indicate a better (less noisy) cell-phenotype cluster resolution. In Figures 6A–6D we represent the mean silhouette score as a function of the latent space number of dimensions for 13 PBMC phenotypes described in Butler et al.[11](Figure 6A) and for 7 (Figure 6B), 15 (Figure 6C), and 26 (Figure 6D) marked mouse cortex cell populations described in Zeisel et al.[34] We have selected the 1,500 most signal-like genes using RMT and we can observe how RMT outperforms other methods in the identification of known marked populations. Notice also how this becomes more dramatic as we increase the number of populations. Although this exercise is done with known populations in order to give a comparative quantitative measure, from Figures 6A–6D we can also conclude that RMT method is a suitable one to better disentangle cell populations by noise removal and hence to find new potential cell populations. Moreover, the performance advantage of the RMT method increases with the dimension of the latent space. This last feature is particularly interesting since in the future, the number of required dimensions in the latent space for an accurate analysis is expected to grow due to continuing improvements in resolution and the number of cells that can be measured.

DISCUSSION

In this paper, we demonstrate the effectiveness of (sparse) RMT for studying the spectrum of the covariance matrix of single-cell genomic data. We have shown that single-cell data shows a 3-fold structure: a random matrix, a sparsity-induced signal, and a biological signal. We also show that while most of the spectrum follows the expectations from RMT (95%), there exist deviations due to artifacts generated by a sparsity-induced signal ($\sim$3%) and due to a biological signal ($\sim$2%). The large contribution of the random component to the spectral properties of the covariance matrix of single-cell expression data could be due to the stochastic nature of gene expression at single-cell level, as has been studied in a variety of biological contexts.[14,47]

We have introduced a method to denoise single-cell sequencing data studying eigenvalue and eigenvector properties based on RMT. This method uses RMT universality properties of eigenvalue distributions, e.g., the TW and MP distributions, and extends it to the study of the eigenvector properties, based on the localization/delocalization phase transition. This method is also able to select genes responsible for potentially interesting biological signals. The algorithm provides a powerful

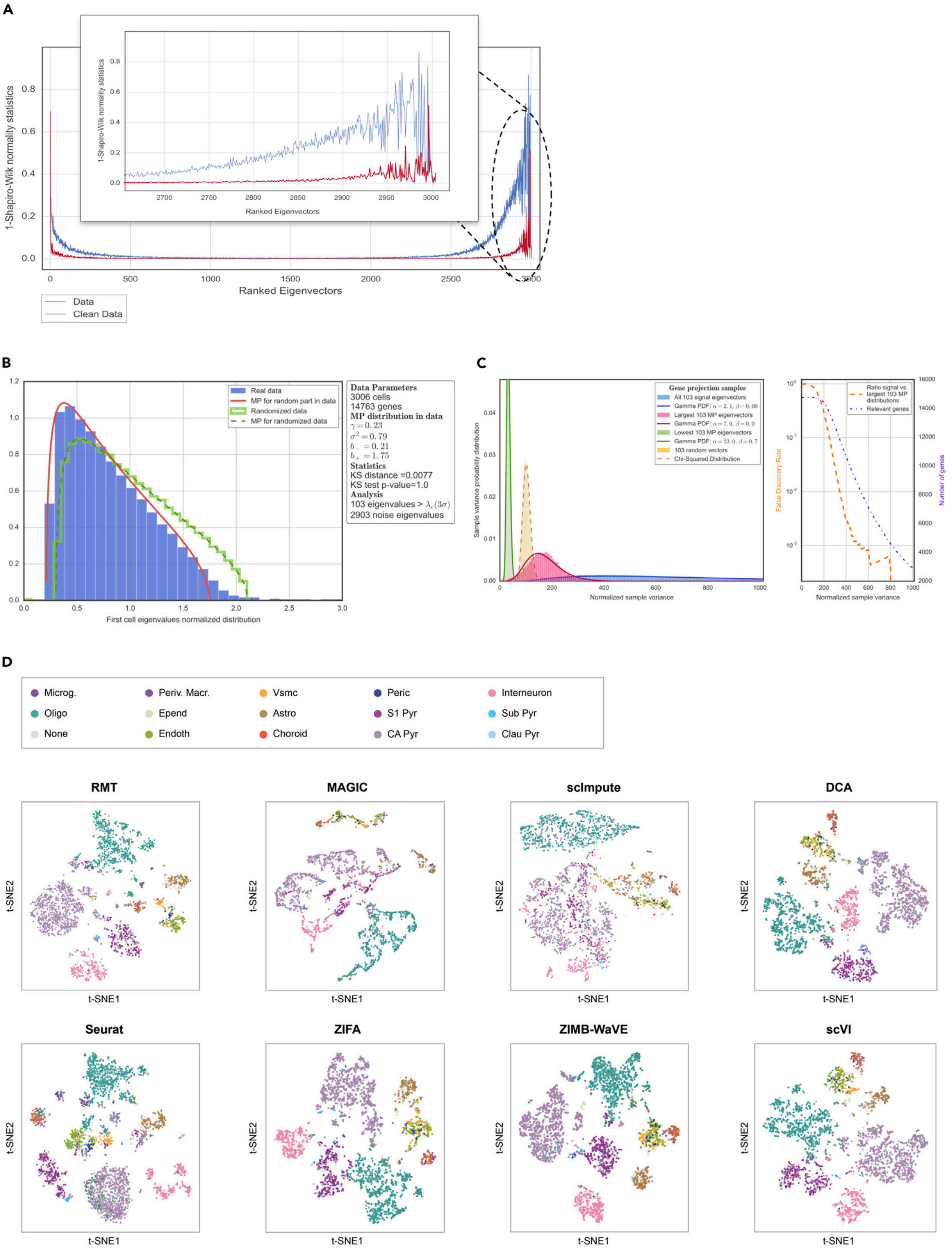
Figure 4. Application to PBMC Single-Cell Expression

(A) Localization properties of the eigenvectors in a single-cell dataset of PBMCs.[32] The blue line represents the system dominated by sparsity and the red line corresponds to the system after removing sparsity. This figure also shows how some eigenvectors corresponding to eigenvalues out of MP distribution are delocalized (red line) and therefore do not carry any information.

(B) MP prediction and identification of relevant components.

(C) Study of the chi-squared test for the variance (normalized sample variance) in signal and noise gene projections. In the left panel, the distributions correspond to a projection of genes into the 83 signal eigenvectors (corresponding to the 83 eigenvalues of A) and the projection into the 83 lowest and 83 largest MP eigenvectors. There is also a projection into 83 random vectors. Finally, the lines show how gamma functions can fit the distributions discussed. The right panel shows the number of relevant genes in terms of the test discussed above, together with a false discovery rate. Higher values for the chi-squared test for variance indicate that the genes are less responsible for the signal.

(D) Comparison of the t-SNE representation for different public algorithms. This case corresponds to 13 different PBMC phenotypes sequenced in Kang et al.[32] and described in Butler et al.[11]



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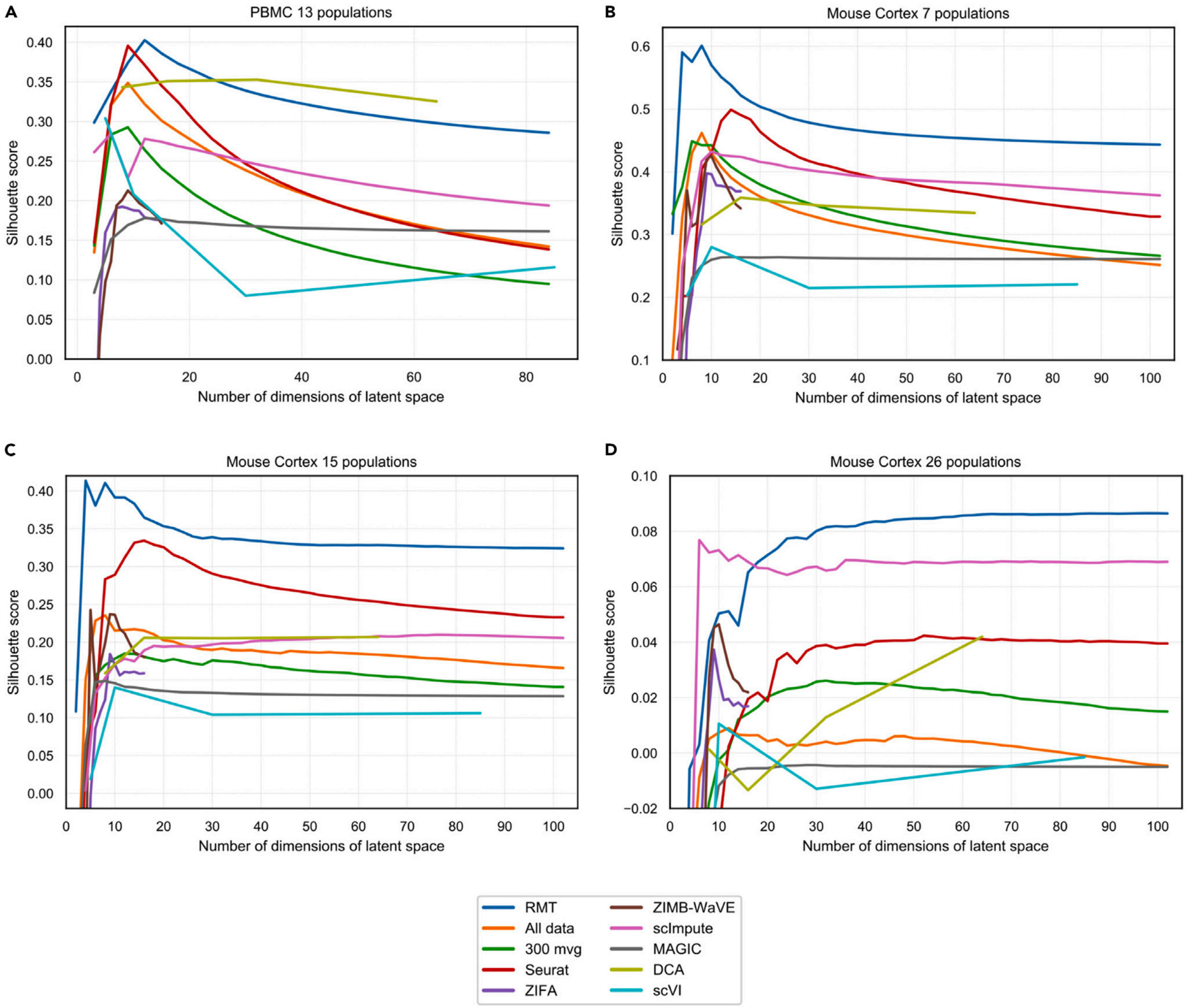

Figure 6. Comparison of Alternative Approaches for Single-Cell Analysis

(A) Mean silhouette score for different methods as a function of the number of dimensions of the latent space for the case of 13 PBMC cell phenotypes described in Butler et al.[11]

(B–D) Mean silhouette score for different methods as a function of the reduced space number of dimensions for the case of 7 (B), 15 (C), and 26 (D) mouse cortex cell phenotypes described in Zeisel et al.[34]

tool to identify this signal and produce a low-rank representation of single-cell data that may be used for further interpretation. Additionally, we should point out that the universality we observed in Wishart/covariance matrices is also observable in the spectra of graph Laplacians (including sparse graphs[48]) and kernel random matrices,[49] which are used in other single-

Figure 5. Application to Mouse Cortex Single-Cell Expression

(A) Localization properties of the eigenvectors in a single-cell dataset of PBMCs.[32] The blue line represents the system dominated by sparsity and the red line corresponds to the system after removing sparsity. This figure also shows how some eigenvectors corresponding to eigenvalues out of MP distribution are delocalized (red line) and therefore do not carry any information.

(B) MP prediction and identification of relevant components.

(C) Study of the chi-squared test for the variance (normalized sample variance) in signal and noise gene projections. In the left panel, the distributions correspond to a projection of genes into the 103 signal eigenvectors (corresponding to the 103 eigenvalues of A) and the projection into the 103 lowest and 103 largest MP eigenvectors. There is also a projection into 103 random vectors. Finally, the lines show how gamma functions can fit the distributions discussed. The right panel shows the number of relevant genes in terms of the test discussed above together with a false discovery rate. Higher values for the chi-squared test for variance indicate that the genes are less responsible for the signal.

(D) Comparison of the t-SNE representation for different methods and algorithms. This case corresponds to 15 different mouse cortex cell phenotypes described in Zeisel et al.[34]

cell analytic techniques, suggesting that the approach followed here could be applied more broadly. The code for the algorithm is publicly available on https://rabadan.c2b2.columbia.edu/html/randomly/.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.patter.2020.100035.

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AUTHOR CONTRIBUTIONS

L.A., M.B., and R.R. developed the application of localization and sparse RMT concepts into single-cell biology. L.A. and M.B. have developed the RMT-based algorithm and applied it to the single-cell datasets described in the main text and methods under the supervision of R.R. L.A., M.B., and R.R. wrote the manuscript. A.J.B. provided valuable mathematical insights and strategies and helped during the writing of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing financial interests.

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