PERSPECTIVE
https://doi.org/10.1038/s41588-021-00873-4

nature genetics

Separating measurement and expression models clarifies confusion in single-cell RNA sequencing analysis

Abhishek Sarkar[1]✉ and Matthew Stephens[1,2]✉

The high proportion of zeros in typical single-cell RNA sequencing datasets has led to widespread but inconsistent use of terminology such as dropout and missing data. Here, we argue that much of this terminology is unhelpful and confusing, and outline simple ideas to help to reduce confusion. These include: (1) observed single-cell RNA sequencing counts reflect both true gene expression levels and measurement error, and carefully distinguishing between these contributions helps to clarify thinking; and (2) method development should start with a Poisson measurement model, rather than more complex models, because it is simple and generally consistent with existing data. We outline how several existing methods can be viewed within this framework and highlight how these methods differ in their assumptions about expression variation. We also illustrate how our perspective helps to address questions of biological interest, such as whether messenger RNA expression levels are multimodal among cells.

Single-cell RNA sequencing (scRNA-seq) has facilitated the investigation of important biological questions that were previously difficult or impossible to study, such as the nature of heterogeneity within classical cell types, the dynamics of cellular processes and the biological pathways underlying cellular differentiation. However, how to model scRNA-seq data has been the subject of considerable confusion and debate. In particular, the high proportion of zeros in typical datasets has garnered special attention and has led to widespread but inconsistent use of terminology such as dropout and missing data. In this Perspective, we argue that much of this terminology is confusing and unnecessary, and we outline simple ways of thinking and talking about scRNA-seq data that can help to reduce confusion.

The first key idea is that observed scRNA-seq counts reflect two distinct factors: the variation in actual expression levels among cells and the imperfect measurement process. Therefore, models for observed scRNA-seq counts, which we will call observation models, are obtained by specifying: (1) an expression model that describes how the true expression levels vary among cells/genes; and (2) a measurement model that describes how observed counts deviate from the true expression levels. Distinguishing among observation, expression and measurement models is important both for avoiding confusion and for performing useful analyses. Indeed, the goal of most RNA-seq analyses is to draw inferences about true expression levels from observed counts, and this is impossible without explicit consideration of how the observed counts are related to the expression levels through a measurement process. Moreover, making measurement and expression models explicit can help to clarify the underlying assumptions and aid interpretation of results such as parameter estimates.

The second key idea is that a Poisson model is a reasonable starting point for modeling scRNA-seq measurement. We summarize theoretical arguments for this model and explain how it can capture the abundance of zeros in scRNA-seq data without special terminology or special treatment. This measurement model is a simplification (Box 1), but we argue that it is a useful simplification that will often suffice in practice.

Both ideas are simple, and neither is new. Modeling the measurement process has a long history[1], as does the use of Poisson measurement models for RNA-seq[2,3]. However, many papers on scRNA-seq analysis do not incorporate these ideas, focusing exclusively on observation models and leaving measurement and expression models implicit (refs. [4,5] are notable exceptions). This ambiguity is especially problematic when the models include components said to capture zero inflation without clearly indicating whether these are part of the measurement model, the expression model or both. Here, we show how many scRNA-seq observation models can be interpreted as combining a Poisson measurement model with different expression models, clarifying their underlying assumptions about expression variation.

These simple ideas can also help to address questions of biological interest. For example, the question of whether gene expression patterns are multimodal among cells is about the expression model, not the observation model. We investigate this question empirically in diverse datasets and find that data are often consistent with surprisingly simple expression models. Specifically, a Gamma distribution often suffices to capture variation in expression levels among cells.

A call to simplify the terminology

One major source of confusion in scRNA-seq analysis is the widespread but inconsistent use of terminology, especially dropout, missing data, imputation and zero inflation. The choice of terminology has many important consequences: it affects the way that researchers think, develop and apply methods and interpret results. We therefore begin by reviewing these terms and explain why, in many cases, we view them as unhelpful.

The term dropout has become commonly used in connection with the zeros in scRNA-seq data[6–10]. Historically, dropout referred to allelic dropout—a failure of PCR in which specific primers would fail

[1]Department of Human Genetics, University of Chicago, Chicago, IL, USA. [2]Department of Statistics, University of Chicago, Chicago, IL, USA.
✉e-mail: aksarkar@uchicago.edu; mstephens@uchicago.edu

Box 1 | Assumptions and limitations of the Poisson measurement model

The Poisson measurement model (equation (1)) is based on the following assumptions (Supplementary Note 1): (1) in each cell, each molecule is equally likely to be observed; (2) each molecule is observed independently of whether or not each other molecule is observed; and (3) only a small proportion of all molecules present are observed. Assumptions (2) and (3) are plausible because the measurement process operates at the molecular level, and only 10–20% of molecules present are estimated to be observed in typical scRNA-seq experiments[8,61]. However, assumption (1) may plausibly be violated. There are many reasons a given mRNA molecule may fail to be observed; for example, it can be lost to diffusion during sample collection and preparation or damaged by cell dissociation or lysis, or it may fail to be amplified or sequenced. Different mRNA molecules will have different chances of surviving these processes due, for example, to differences in RNA stability, their location in the cell (for example, the nucleus versus the cytoplasm) or sequence content. Such factors could make the observed molecules a biased sample of all molecules.

Biased sampling of molecules can be incorporated into the Poisson measurement model by including bias terms (Supplementary Note 1). If the biases are systematic and associated with specific technical covariates (for example, batch), one could estimate their effects within the Poisson model. However, some biases may vary from cell to cell in unknown ways (for example, due to differences in the conditions under which each cell is processed). Such random biases effectively add additional noise to the measurement process and could be dealt with by replacing the Poisson measurement model (equation (1)) with a negative binomial measurement model that allows overdispersion (additional variance) compared with Poisson. However, the negative binomial measurement model raises additional difficulties, not least the question of how much overdispersion to allow for. Our perspective, for which we present empirical evidence, is that for many datasets the measurement overdispersion compared with Poisson will be small, especially compared with the variation in actual expression levels among cells; therefore, the Poisson measurement model (equation (1)) will often suffice.

Interestingly, as technologies improve to measure more molecules per cell—potentially violating assumption (3)—the variance of the measurement process will be reduced relative to a Poisson (Supplementary Note 1). This could conceivably counteract, or even overshadow, some of the overdispersion mentioned above.

to amplify sequences containing a specific allele, leading to genotyping errors for heterozygous individuals[11,12]. In scRNA-seq, the term dropout was introduced to describe a supposed failure that might cause a gene to appear highly expressed in one cell but not expressed in another[6]. Although the source was not specified, this usage seems to refer to some aspect of the measurement process. The term has since spread widely, but its meaning varies among papers and presentations (and sometimes even within papers and presentations). For example, it is used to refer sometimes to observed zeros, sometimes to the unknown subset of zeros at genes that are expressed but undetected and sometimes to the fact that not all molecules present in the original sample are observed (which affects all observations, not just the zeros). Such variation in usage naturally leads to confusion and disagreement about how these phenomena should be modeled. For this reason, we argue the term dropout should be avoided in the context of scRNA-seq data. Instead, zero observations should simply be referred to as zeros, and measurement models should focus on the fact that not all molecules in the original sample are observed, with zeros being just one byproduct.

The term 'missing data' is also commonly used in connection with zeros in scRNA-seq[9,13]. This terminology is misleading because the zeros are not missing data as understood in statistics. To illustrate, consider a survey where a researcher counts the number of cars arriving at an intersection each minute. If no cars arrive in a particular minute, the observation would be recorded as zero; however, this observation is not missing. In contrast, if the researcher takes a lunch break and does not record arrivals for 1 h, this would lead to 60 truly missing observations. The zeros in an scRNA-seq data experiment are more like the former than the latter: there is no analog of a lunch break in the scRNA-seq measurement process. Of course, in RNA-seq data, the observed zeros are noisy and do not necessarily imply that there were zero molecules present in the original cell; however, the same is true of all observations.

Inappropriate use of the term missing data has led to inappropriate application of methods for dealing with missing data to scRNA-seq data. For example, in other applications it is common to impute (fill in) missing values, so many scRNA-seq papers have described methods to impute zeros[9,14–16]. However, since zeros in scRNA-seq are not actually missing data, the meaning of these imputed values is unclear. Thus, the term missing data, and methods that impute (only) zeros, should be avoided. Instead, every observation in scRNA-seq should be treated as a noisy observation of an underlying true expression level, and inference should focus on clearly defined tasks, such as estimating the true expression levels.

Finally, the term zero inflated is another common source of confusion and debate. In statistics, zero inflated describes a model for count data that is obtained from a simpler model by increasing the proportion of zeros. For example, zero-inflated Poisson refers to a distribution obtained by taking a Poisson distribution and then increasing the proportion of zeros. In scRNA-seq applications, the use of zero-inflated models is an understandable reaction to the high proportion of observed zeros. However, recent work suggests that zero-inflated models may be unnecessary; indeed, the title of ref. [17] is 'Droplet scRNA-seq is not zero-inflated'. Here, we take a slightly different perspective: we argue that there is no convincing evidence supporting zero-inflated measurement models; however, zero-inflated observation models could be appropriate, depending on actual expression variation. This perspective makes clear that the need for zero-inflated models may vary among datasets and among genes.

These issues all stem from one major misconception in scRNA-seq: the idea that the measurement process involves some distinct zero-producing technical mechanism. Indeed, some published observation models include a component that randomly creates zero observations irrespective of the true expression level (for example, ref. [15]). Such a mechanism would require a systematic effect that somehow misses all molecules from a particular gene in a particular cell, and there are no convincing theoretical arguments or empirical examples of evidence supporting this idea. Thus, we argue that measurement models for scRNA-seq should begin from a simple assumption: that the measurement process operates independently on each molecule in the cell. This assumption is a simplification (Box 1), but is not strongly contradicted by existing data, and leads to simple observation models and explanations for the large proportion of zeros in scRNA-seq data.

Modeling scRNA-seq data

Observed scRNA-seq counts reflect both the true expression levels of each gene in each cell and the measurement process. We first describe measurement and expression models and how they combine to yield observation models. We focus on data generated using unique molecular identifiers, which substantially reduce unwanted variation, including differences in gene lengths and PCR

amplification efficiencies[18]. It is possible that zero-inflated observation models were initially motivated by a need to account for variation in read counts introduced by PCR. We ignore these sources of variation, so our arguments may not apply to data generated without unique molecular identifiers.

We now introduce some notation. Consider using scRNA-seq to measure gene expression in n single cells. Thinking of each cell as a pool of messenger RNA (mRNA) molecules, let m_{ij} denote the true (but unknown and unobserved) number of molecules present in cell i from gene j ($i=1,\ldots,n$; $j=1,\ldots,p$) and let m_{i+} denote the total number of molecules in cell i. We refer to m_{ij} as the absolute expression level of gene j in cell i and $\lambda_{ij} \triangleq m_{ij}/m_{i+}$ as the relative expression level of gene j in cell i. In words, the absolute expression level of a gene is the number of RNA molecules present from that gene, whereas the relative expression level is the proportion of RNA molecules from that gene. Here, we focus on relative expression levels, since estimating absolute expression levels from scRNA-seq data is more difficult, and use $\Lambda=[\lambda_{ij}]$ to denote the matrix of relative expression levels. Let $X=[x_{ij}]$ denote the observed count matrix, where x_{ij} denotes the number of distinct molecules from gene j observed in cell i, and let x_{i+} denote the total number of molecules observed in cell i.

In this notation, a measurement model is a model that connects the observed counts X to the expression levels Λ by specifying the conditional distribution $p(X \mid \Lambda)$. An expression model is a model for the expression levels $p(\Lambda)$. Together, these two models determine the observation model, which is a model for the observed counts $p(X)$.

Modeling scRNA-seq measurement. We believe that methods should generally start with simple models, adding additional complications only when warranted. In this spirit, we suggest that methods for scRNA-seq should start with a simple Poisson measurement model:

$$x_{ij} \mid x_{i+},\ \lambda_{ij} \sim \text{Poisson}\left(x_{i+}\lambda_{ij}\right). \tag{1}$$

Although simple, this measurement model is supported by theoretical arguments[3,19] (Supplementary Note 1), early empirical analyses of bulk RNA-seq data[2], more recent analyses of control scRNA-seq data[4,17,19] and our own empirical analyses.

Although the Poisson measurement model is essentially the same as that used for bulk RNA-seq data, it can nonetheless account for the fact that there are many more zeros in scRNA-seq data than in bulk RNA-seq data. First, the total number of molecules observed x_{i+} is typically much smaller for single cells than for bulk samples because single cells have less starting material and are typically sequenced to a lower average depth. Second, it is more common that λ_{ij} will be small (or even zero) for a single cell than for a bulk sample. This is because expression levels in bulk samples are averages of expression levels in many single cells, and averaging reduces the frequency of both small and large values. These two facts imply that the rate parameter $x_{i+}\lambda_{ij}$ is often smaller in scRNA-seq data than in bulk RNA-seq data, explaining the higher proportion of zero observations.

The Poisson measurement model also captures aspects of scRNA-seq data that have previously been referred to using terms such as dropout, missing data and technical zeros. It captures the fact that not every molecule that was present in every cell was observed; indeed, this fact is a fundamental assumption. It also captures the fact that x_{ij} may be observed to be zero even when m_{ij} (and hence λ_{ij}) is nonzero. However, it captures these features without introducing a distinct zero-generating mechanism. The zeros, like other observations, are simply imperfect measurements with no need for special terminology or special treatment.

Under the Poisson measurement model, observing $x_{ij}=0$ is different from x_{ij} being missing. If x_{ij} were missing, it would provide no information about the expression level λ_{ij}. However, observing $x_{ij}=0$ provides such information; namely, that λ_{ij} is unlikely to be large. In other words, it correctly reflects that counts x_{ij} are noisy observations of the true expression levels (see also refs. [20–22], for example).

Alternative measurement models. Previous papers have considered zero-inflated measurement models for scRNA-seq data[6,9]. However, there are no convincing empirical analyses supporting such models, nor are there any convincing arguments for why such zero inflation should be expected. Furthermore, two recent analyses of control datasets, in which synthetic mRNA molecules were directly added (spiked in) to droplets at known concentrations, captured and then sequenced, found no evidence supporting a zero-inflated measurement model[4,17]. Our empirical analysis below further supports this position. Including an unnecessary zero-generating component in the measurement process has the cost of increasing the model complexity and introduces the danger that true expression variation may be wrongly attributed to the measurement process.

That said, the Poisson measurement model (equation (1)) is necessarily a simplification. In particular, it ignores biases that may cause some molecules to be more likely to be observed than others (Box 1 and Supplementary Note 1). When such biases are consistent across cells, inferences that involve comparisons among cells will be robust to ignoring them; however, some biases may vary from cell to cell, producing measurements that are overdispersed (more variable) relative to a Poisson distribution. Given the difficulty of precisely modeling all aspects of the measurement process, and given that available data do not strongly contradict a Poisson measurement model, our perspective is that the development of methods should start with the Poisson measurement model and focus more attention on modeling expression variation (as described below). The main downside of this approach is that it risks overstating expression variation if measurement overdispersion is high. However, we view this as a risk worth paying for the benefits of simplicity.

Modeling gene expression. We emphasize that our suggestion to use a Poisson model applies specifically to the measurement model, not the observation model. Indeed, many papers have demonstrated that a Poisson observation model does not capture all variation in observed RNA-seq data; thus, it is common to use a more flexible observation model that can capture additional variation, such as negative binomial or zero-inflated negative binomial (ZINB) observation models. These observation models are not inconsistent with a Poisson measurement model; indeed, in this section, we explain how negative binomial and ZINB observation models, as well as many other existing methods, naturally arise by combining the Poisson measurement model with certain expression models.

To make this idea precise, first consider modeling expression at a single gene j. An expression model for a single gene involves specifying a distribution g_j for the expression levels $\lambda_{1j}, \ldots, \lambda_{nj}$,

$$\lambda_{ij} \sim g_j(\cdot). \tag{2}$$

One simple choice is to assume that g_j is a Gamma distribution; combining this with equation (1) yields the negative binomial observation model[23]. Similarly, combining a point-Gamma expression model (wherein some proportion of the expression levels are exactly zero while the other (nonzero) levels follow a Gamma distribution) with equation (1) yields the ZINB observation model. It is also possible to use nonparametric expression models[4,24]. A list of single-gene expression models is given in Table 1 along with some published methods implementing statistical inference for the corresponding observation models (see Supplementary Note 2 for details).

Similar ideas apply to expression models for multiple genes, although things inevitably become more complex. A multigene

Table 1 | Single-gene models for scRNA-seq data

Expression model	Observation model	Method[a]
Point mass (no variation)	Poisson	Analytic
Gamma	Negative binomial	MASS[41], edgeR[42], DESeq2 (ref. [43]), SAVER[20], BASICS[44]
Point-Gamma	ZINB	PSCL[45]
Unimodal (nonparametric)	Unimodal	ashr[24,46]
Point-exponential family	Flexible	DESCEND[4]
Fully nonparametric[47]	Flexible	ashr

Different expression models, when combined with the Poisson measurement model, yield different observation models. [a]Previously published methods and software packages that use the corresponding observation model to analyze data.

Table 2 | Multigene models for scRNA-seq data

Link function	Noise distribution	Method[a]
Identity	None	NMF[48], scHPF[49]
Identity	Gamma	NBMF[50]
log	None	GLM-PCA[19]
log	Gamma	scNBMF[51], GLM-PCA[19]
log	Point-Gamma	ZINB-WaVE[52]
Neural network	Point-Gamma	scVI[29], DCA[21]

Multigene models partition variation in true expression into structured and stochastic components. The link function describes a transformation and the noise distribution indicates an assumption about the stochastic component. [a]Previously published methods and software packages that use the corresponding observation model to analyze data.

expression model simultaneously describes correlations among expression levels at different genes across cells and different cells across genes. A common and powerful approach to describe these correlations is to use low-rank models, which intuitively assume that the correlations can be captured by a relatively small number of patterns (much smaller than the number of cells or genes). More precisely, these models can be written as:

$$\begin{aligned} \lambda_{ij} &= \mu_{ij} u_{ij} \\ \mu_{ij} &= h^{-1}\left((LF')_{ij}\right) \\ u_{ij} &\sim p\left(u_{ij}\right), \end{aligned} \tag{3}$$

where h denotes a link function, or transformation, and the loadings matrix L and factors matrix F are low rank. This form has an appealing biological interpretation: the matrix LF' represents the structure of expression variation among cells or genes and u_{ij} represents stochastic deviations from this structure. Thus, one might think of LF' as representing different cell types or states and u_{ij} as stochastic expression noise[25]. A list of multigene expression models is given in Table 2 along with some published methods implementing statistical inference for the corresponding observation models (see Supplementary Note 3 for details).

Interestingly, methods that combine expression models of the form from equation (3) with the Poisson measurement model from equation (1) may be robust to misspecification of the measurement model. Specifically, if measurements are overdispersed relative to Poisson, the model fit will tend to include this additional variation in $p(u_{ij})$ (provided this distribution is sufficiently flexible), while leaving estimates of the structured variation LF' unchanged. Intuitively, overdispersered measurement error affects the variance of the observations, but not the mean. Therefore, while estimates of the stochastic noise may be sensitive to assumptions on the measurement process, estimates of the structured expression variation will be more robust.

Summary. In summary, many existing observation models for scRNA-seq data can be derived by combining the Poisson measurement model (equation (1)) with expression models of the form in equation (2) or (3). This framework clarifies several sources of confusion in scRNA-seq analysis. First, it emphasizes that the observation models that are most appropriate for scRNA-seq data may vary among datasets and genes because expression variation will vary among datasets and genes. For example, if data are collected on a set of homogeneous cells, most genes might show relatively little expression variation and may be adequately described by simple expression models. In contrast, if the data contain many cell types, more complex expression models could be required. We study this question empirically below.

Second, this framework provides a different interpretation of a finding that a ZINB observation model fits observations at some gene in some dataset better than a negative binomial model: this would imply that the expression levels at the gene are better modeled by a point-Gamma distribution than a simple Gamma distribution. Importantly, this is a conclusion about the true expression levels, not a conclusion about the measurement process. This interpretation contrasts with the usual way that the ZINB observation model is interpreted, in which zero inflation captures some supposed technical mechanism (see Supplementary Note 4 for details).

Finally, this framework provides a rigorous approach to infer, for example, the mean or variance of true gene expression levels, as well as the true expression levels themselves, from the observed counts. However, these estimates are generally neither simple functions of the observed counts nor functions of simple transformations (for example, log) of the observed counts. We illustrate this procedure for a single-gene point-Gamma expression model (ZINB observation model) in Box 2.

Empirical examples

Single-gene models. There is considerable debate about whether scRNA-seq data are adequately modeled by a negative binomial observation model, or if it is necessary to use a ZINB observation model. Some papers have concluded that observed scRNA-seq data exhibit multimodal expression variation, suggesting that an even more complex observation model may be necessary[26–28]. Under the framework outlined above, these questions translate into questions about the expression model: is a Gamma expression model adequate or is it necessary to use a more complex, even multimodal, expression model?

Since expression variation may vary among genes and datasets, we analyzed datasets from a range of settings, including homogeneous collections of sorted cells, a priori homogeneous cell lines and heterogeneous tissues. We also created in silico mixtures of sorted cells as positive controls for highly heterogeneous expression patterns (Table 3).

For each gene in each dataset, we compared several expression models: a Gamma distribution, a point-Gamma distribution, a nonparametric unimodal distribution and a fully nonparametric distribution (Fig. 1a and Supplementary Note 2). Because these comparisons involve nonparametric families, obtaining P values is not straightforward and is perhaps inappropriate, since specifying any of these models as a null expression model is also questionable. Therefore, we instead compared the support for each model by comparing the likelihood of the data under each model. As a simple heuristic, we considered a likelihood ratio of 100 or more as strong evidence for one model over another.

First, we assessed whether genes show evidence against a Gamma expression model due to excess zeros, by comparing a Gamma expression model with a point-Gamma model. In all of the biological

Box 2 | Inference in the ZINB observation model

In our framework, the ZINB model for observations $x_{1j}, \ldots, x_{nj}$ is written

$$x_{ij} \mid x_{i+}, \lambda_{ij} \sim \text{Poisson}(x_{i+}\lambda_{ij})$$

$$\lambda_{ij} \sim g_j(\cdot) = \pi_j \delta_0(\cdot) + (1-\pi_j)\,\text{Gamma}\left(\phi_j^{-1}, \mu_j^{-1}\phi_j^{-1}\right),$$

where g_j is a point-Gamma distribution, which is a mixture of a point mass on zero (denoted δ_0) and a Gamma distribution (parametrized by shape and rate). Here, we consider two analysis tasks: (1) estimating g_j (that is, estimating π_j, μ_j, ϕ_j) and (2) estimating λ_{ij}. Task (1) can be accomplished by maximizing the marginal likelihood, which has an analytic form but requires numerical optimization. From an estimate $\hat{g}_j$, one can already estimate any useful quantities such as the mean and variance of gene expression[54]:

$$E\left[\lambda_{ij} \mid \hat{g}_j\right] = (1-\hat{\pi}_j)\,\hat{\mu}_j$$

$$V\left[\lambda_{ij} \mid \hat{g}_j\right] = (1-\hat{\pi}_j)\,\hat{\mu}_j^2\hat{\phi}_j + \hat{\pi}_j(1-\hat{\pi}_j)\,\hat{\mu}_j^2.$$

Task (2) can be accomplished by estimating the conditional distribution of λ_{ij} given the observed data and $\hat{g}_j$,

$$\lambda_{ij} \mid x_{ij}, x_{i+}, \hat{g}_j = \hat{\pi}_j\delta_0(\cdot) + (1-\hat{\pi}_j)\,\text{Gamma}\left(x_{ij}+\hat{\phi}_j^{-1}, x_{i+}+\hat{\mu}_j^{-1}\hat{\phi}_j^{-1}\right).$$

If one interprets g_j as a prior, this procedure is empirical Bayes and the mean of the conditional distribution above is the posterior mean estimate of λ_{ij} given the observed data,

$$E\left[\lambda_{ij} \mid x_{ij}, x_{i+}, \hat{g}_j\right] = (1-\hat{\pi}_j)\,\frac{x_{ij}+\hat{\phi}_j^{-1}}{x_{i+}+\hat{\mu}_j^{-1}\hat{\phi}_j^{-1}}.$$

datasets we examined, only a fraction of genes (0.6–8.6%) showed strong evidence in favor of the point-Gamma model (Fig. 1b). The genes showing strong evidence in favor of the point-Gamma expression model included known marker genes in synthetic mixtures of sorted B and T cells (Fig. 1c), providing a positive control that this approach can find such patterns.

Next, we assessed whether genes show evidence for other types of departures from a Gamma expression model, by comparing it to a nonparametric unimodal expression model. In this comparison, many more genes (20–69%) showed strong evidence in favor of the nonparametric unimodal expression model (Fig. 1d). These results suggest that expression variation at many genes might not be captured by either a negative binomial or a ZINB observation model. As an example, in peripheral blood mononuclear cells (PBMCs), the gene *PPBP* exhibits not only many observed zeros, but also many small nonzero observations (for example, ones and twos) and a long tail of large observations (Fig. 1e). Neither the Gamma nor the point-Gamma distribution has sufficient flexibility to simultaneously describe both of these features, explaining the better fit of the nonparametric unimodal model.

Finally, we assessed whether the data show evidence of multimodal expression variation, by comparing a unimodal expression model against a fully nonparametric expression model. In this comparison, few genes (0.4–3.5%) showed strong evidence for the fully nonparametric expression model (Fig. 1f), suggesting that multimodal expression variation may be rarer than was previously suggested[28]. As a positive control, *RPS4Y1* is a Y-chromosome-linked gene showing overwhelming evidence for the nonparametric expression model over the unimodal model (likelihood ratio $> 10^{65}$), due to distinct distributions of gene expression in induced pluripotent stem cells (iPSCs) derived from male and female donors (Fig. 1g). One possible reason that cells from female donors are estimated to have nonzero expression of *RPS4Y1* is that the relevant part of the coding sequence is identical between *RPS4Y1* and its homolog *RPS4X*, and some reads are erroneously mapped.

We emphasize that a lack of multimodal expression variation does not imply a lack of heterogeneity. To illustrate, *SKP1* is a gene at which expression in iPSCs is linked to the genotype of a nearby SNP, meaning that both the mean and mode of expression levels vary across donors depending on the genotype (Supplementary Fig. 1). However, when the data are pooled across all samples, they show only modest evidence for multimodal expression variation (likelihood ratio = 3.1 for the fully nonparametric expression model over the unimodal model). This is partly due to the substantial heterogeneity within each genotype class, which bridges the heterogeneity between genotype classes.

In summary, although some genes show departures from a simple Gamma model of expression variation, in most cases the data are consistent with unimodal expression variation and relatively few genes show strong evidence for zero-inflated (point-Gamma) or multimodal expression variation.

Alternative measurement models. Our analysis above assumed a Poisson measurement model because it is both simple and has strong theoretical foundations. Here, we discuss the empirical support for the Poisson measurement model, as well as alternatives.

Our results on biological datasets above provide substantial evidence that the scRNA-seq measurement process is not zero inflated. If the measurement process involved a distinct zero-generating component, the observed data should have shown many genes supporting a point-Gamma expression model over a Gamma expression model; however, we did not find this. Indeed, only a fraction of genes (2–16%) showed even suggestive evidence supporting a point-Gamma expression model (likelihood ratio > 10; Supplementary Fig. 2). Therefore, we conclude that current data do not support the use of zero-inflated measurement models.

However, our results do not imply that the measurement model is necessarily Poisson. There are plausible reasons why the measurement process could be overdispersed relative to Poisson; however, empirically assessing the extent of measurement overdispersion is difficult. In principle, one could analyze control data containing a known number of molecules of various synthetic genes. However, detecting subtle overdispersion would require very precise control of the number of molecules of each gene, which is difficult[4]. Without additional assumptions, it is not possible to say to what extent overdispersion observed in control data is due to uncontrolled expression variation in the control genes versus measurement overdispersion.

To make progress on this question, we made an additional assumption that the measurement overdispersion is equal across genes. Under this assumption, it is possible to estimate the measurement dispersion by fitting a negative binomial observation model with separate dispersion parameters to reflect measurement dispersion and uncontrolled expression variation (Supplementary Note 5).

We used this approach to bound the measurement dispersion in five control datasets (Table 3) that had previously been pre-processed and analyzed[4,17]. In all five datasets, we found that the profile log likelihood for measurement dispersion dropped off quickly beyond some point, bounding the level that is consistent with the data (Supplementary Fig. 3). Although the bounds vary among protocols, they suggest that measurement dispersion is no

Table 3 | Datasets analyzed

Dataset	Protocol	Number of samples	Number of genes	Source
(Sorted) T cells	GemCode	10,209	6,530	Ref. [53]
(Sorted) B cells	GemCode	10,085	6,417	Ref. [53]
iPSCs	Fluidigm C1	5,597	9,957	Ref. [54]
T cell/B cell mix[a]	GemCode	20,294	6,647	Ref. [53]
Cytotoxic T cell/naive T cell mix[a]	GemCode	20,688	6,246	Ref. [53]
Brain	DroNc-Seq	14,963	11,744	Ref. [55]
Kidney	10x Chromium v2	11,233	15,496	Ref. [56]
PBMCs	10x Chromium v3	11,769	12,144	10x Genomics[b]
Retina	10x Chromium v2	21,285	10,047	Ref. [57]
Control 1	10x Chromium v2	2,000	88	Ref. [58]
Control 2	10x Chromium v2	2,000	88	Ref. [58]
Control	Drop-Seq	84	81	Ref. [59]
Control	GemCode	1,015	91	Ref. [53]
Control	InDrops	953	103	Ref. [60]

This table presents the number of samples passing the previously reported quality control filters and the number of genes with nonzero observations in at least 1% of samples, or passing the previously reported quality control filters[54]. [a]Mixture datasets were generated in silico by concatenating the data and then applying quality control filters. [b]Data downloaded from https://10xgenomics.com/data.

larger than 5×10^{-3} (likelihood ratio <0.1 against the maximum likelihood estimate). This level of measurement dispersion is small (for example, for a gene with mean of ten observed molecules, it increases the variance of the observations by 5% relative to a Poisson, and for genes with a smaller mean the increase in variance is smaller). Although this analysis does not rule out that measurement overdispersion could vary among genes and could be large for some genes, we did not find empirical evidence for this.

Multigene models. In multigene models, a common goal is to estimate the underlying low-rank structure LF', which could describe differences in the cell type or state of different samples, for example. Assessing the effectiveness of different methods for providing such biological insights is important, but also difficult to do objectively. For example, comparing clustering performance[29–31] requires gold-standard labeled data, which are arguably unavailable. Therefore, as a proxy, we instead attempted to assess multigene models in their ability to accurately estimate the underlying expression matrix $\Lambda = LF'$. Surprisingly, we found that expression models making linear versus nonlinear assumptions had largely similar accuracy on this problem (Supplementary Fig. 4 and Supplementary Note 3). The results suggest that practical issues (such as convergence behavior and computational cost) or subjective measures (such as ease of interpretation) may determine which methods are most useful in practice. Assessing expression models on these other metrics will be an important direction for future work.

Conclusion

Here, we describe how models for observed scRNA-seq counts can be helpfully separated into two parts: measurement models (which describe variation introduced by the measurement process) and expression models (which describe variation in true expression levels). We argue that a simple Poisson model is a reasonable starting point for the measurement model and that many existing methods can be interpreted as combining a Poisson measurement model with different expression models. We explain how these simple ideas help clarify confusion about the source and interpretation of zeros in scRNA-seq data and give rigorous procedures to interrogate variation in gene expression among cells.

How should one use these ideas in scRNA-seq analysis? We emphasize that clearly distinguishing among measurement, expression and observation models can help to reduce confusion and misinterpretation. In particular, both individuals who develop statistical methods and individuals who analyze data should make explicit the assumptions made about measurement error and about expression variation when analyzing scRNA-seq data. One important area for future work will be developing fast and accurate diagnostics to assess whether these assumptions are violated by observed data, and whether analysis results are sensitive to these assumptions.

Interestingly, our empirical results suggest that simple expression models should suffice for common analysis tasks such as differential expression, dimension reduction and clustering. Although we found that many genes showed support for a nonparametric unimodal expression model over the Gamma model, the Gamma model is considerably easier to fit and therefore may be preferred. Previous results have suggested that the impact of underestimating expression variation on estimates of mean gene expression could be minimal[24]. Nonetheless, care may be necessary when dealing with long-tailed expression distributions, such as those exhibited by *PPBP* (Fig. 1e).

Our empirical analyses of measurement error have two notable limitations. First, samples in spike-in experiments do not undergo the entire experimental protocol that biological samples do (dissociation, lysis and so on), limiting their use in assessing appropriate measurement models. This limitation is also shared by much previous work in this area[4,17,18,32,33]. Second, our approach to bound measurement dispersion made the strong assumption that measurement dispersion was equal across cells and genes. Therefore, the results may understate the potential for higher overdispersion at some genes. Despite these limitations, our empirical results clearly indicate that the use of zero-inflated measurement models is not supported.

There are commonly used methods that our framework does not encompass. These include methods that first transform the count data (for example, $y_{ij}=\log[x_{ij}/x_{i+}+\epsilon]$) and then apply Gaussian methods such as principal components analysis[34], factor analysis[35,36] or latent variable models[37,38] to the transformed data Y. However, even for these methods, the key idea that observations reflect both expression variation and measurement error may still be useful to keep in mind. One potential way to formalize this is via Taylor series approximations[39,40] (for example, $\mathrm{E}[y_{ij}]\approx\log[\lambda_{ij}+\epsilon]$), which suggest that Gaussian low-rank models of the form $\mathrm{E}[Y]=LF'$ can be interpreted as assuming that $[\log[\lambda_{ij}+\epsilon]]$ is low rank. A key problem moving forward will be to make such connections rigorous and assess their accuracy.

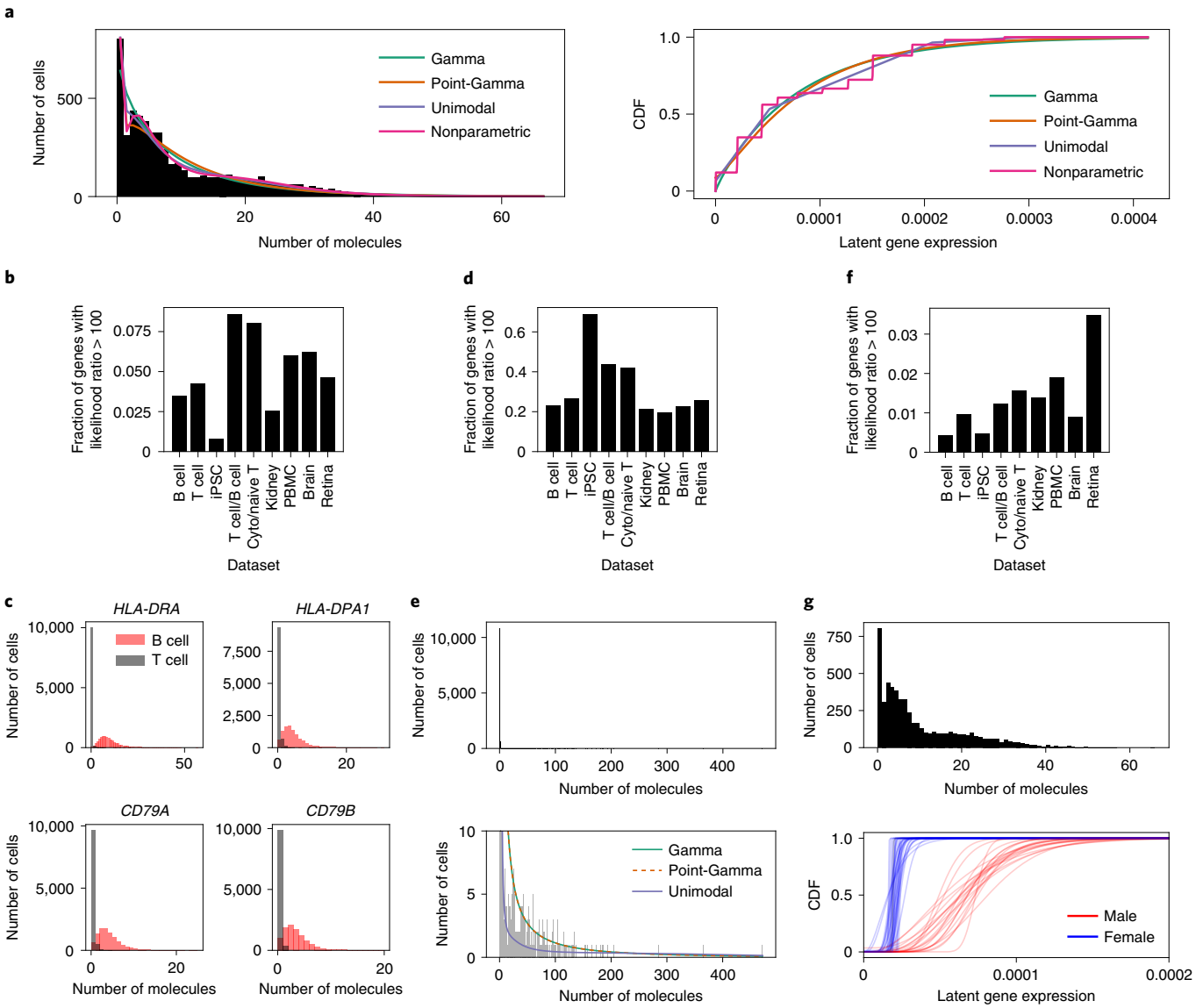

Fig. 1 | Comparing single-gene expression models on scRNA-seq data. a, Example of different expression models (right; CDF: cumulative distribution function), corresponding to different observation models (left), fit to observed data at a single gene (*RPS4Y1*). **b**, Fraction of genes in biological datasets with strong evidence for a point-Gamma expression model over a Gamma model. **c**, Examples of four genes showing strong evidence for a point-Gamma expression model over a Gamma model in an in silico mixture of T cells and B cells. **d**, Fraction of genes with strong evidence in favor of a unimodal expression model over a Gamma model. **e**, Example of a gene showing strong evidence for a unimodal expression model over a Gamma model (*PPBP* in PBMCs). The top panel shows the full range of the observed data, and the bottom panel focuses on a truncated range of the data and shows the fitted observation models. **f**, Fraction of genes with strong evidence in favor of a fully nonparametric expression model over a unimodal nonparametric model. **g**, An example of a gene showing strong evidence for a fully nonparametric expression model over a unimodal nonparametric model (*RPS4Y1* in iPSCs). The top panel shows the observed data, and the bottom panel shows the fitted expression model for each donor, colored by sex.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Sorted immune cell and PBMC data were downloaded from https://10xgenomics.com/data. iPSC data were downloaded from the Gene Expression Omnibus (accession number GSE118723). Brain data were downloaded from the Genotype-Tissue Expression portal (https://www.gtexportal.org/home/datasets). Kidney and retina data were downloaded from the Human Cell Atlas Data Portal (https://data.humancellatlas.org/). Control data were downloaded from https://figshare.com/projects/Zero_inflation_in_negative_control_data/61292. All of the results generated in this study are available at https://zenodo.org/record/4543923 and all analysis notebooks have been published at https://aksarkar.github.io/singlecell-modes/.

Code availability

All of the code used to perform the analysis is available at https://zenodo.org/record/4543921 and https://zenodo.org/record/4543923.

Received: 24 April 2020; Accepted: 22 April 2021;
Published online: 24 May 2021

References

1. Fuller, W. A. *Measurement Error Models* (John Wiley & Sons, 1986).
2. Marioni, J. C., Mason, C. E., Mane, S. M., Stephens, M. & Gilad, Y. RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.* **18**, 1509–1517 (2008).

3. Pachter, L. Models for transcript quantification from RNA-seq. Preprint at https://arxiv.org/abs/1104.3889 (2011).
4. Wang, J. et al. Gene expression distribution deconvolution in single-cell RNA sequencing. *Proc. Natl Acad. Sci. USA* **115**, E6437–E6446 (2018).
5. Zhang, M. J., Ntranos, V. & Tse, D. Determining sequencing depth in a single-cell RNA-seq experiment. *Nat. Commun.* **11**, 774 (2020).
6. Kharchenko, P. V., Silberstein, L. & Scadden, D. T. Bayesian approach to single-cell differential expression analysis. *Nat. Methods* **11**, 740–742 (2014).
7. Stegle, O., Teichmann, S. A. & Marioni, J. C. Computational and analytical challenges in single-cell transcriptomics. *Nat. Rev. Genet.* **16**, 133–145 (2015).
8. Haque, A., Engel, J., Teichmann, S. A. & Lönnberg, T. A practical guide to single-cell RNA-sequencing for biomedical research and clinical applications. *Genome Med.* **9**, 75 (2017).
9. Zhu, L., Lei, J., Devlin, B. & Roeder, K. A unified statistical framework for single cell and bulk RNA sequencing data. *Ann. Appl. Stat.* **12**, 609–632 (2018).
10. Qiu, P. Embracing the dropouts in single-cell RNA-seq analysis. *Nat. Commun.* **11**, 1169 (2020).
11. Fujimura, F. K., Northrup, H., Beaudet, A. L. & O'Brien, W. E. Genotyping errors with the polymerase chain reaction. *N. Engl. J. Med.* **322**, 61 (1990).
12. Whale, A. S., Cowen, S., Foy, C. A. & Huggett, J. F. Methods for applying accurate digital PCR analysis on low copy DNA samples. *PLoS ONE* **8**, e58177 (2013).
13. Hicks, S. C., Townes, F. W., Teng, M. & Irizarry, R. A. Missing data and technical variability in single-cell RNA-sequencing experiments. *Biostatistics* **19**, 562–578 (2018).
14. Li, W. V. & Li, J. J. An accurate and robust imputation method scImpute for single-cell RNA-seq data. *Nat. Commun.* **9**, 997 (2018).
15. Chen, M. & Zhou, X. VIPER: variability-preserving imputation for accurate gene expression recovery in single-cell RNA sequencing studies. *Genome Biol.* **19**, 196 (2018).
16. Talwar, D., Mongia, A., Sengupta, D. & Majumdar, A. AutoImpute: autoencoder based imputation of single-cell RNA-seq data. *Sci. Rep.* **8**, 16329 (2018).
17. Svensson, V. Droplet scRNA-seq is not zero-inflated. *Nat. Biotechnol.* **38**, 147–150 (2020).
18. Islam, S. et al. Quantitative single-cell RNA-seq with unique molecular identifiers. *Nat. Methods* **11**, 163–166 (2013).
19. Townes, F. W., Hicks, S. C., Aryee, M. J. & Irizarry, R. A. Feature selection and dimension reduction for single-cell RNA-seq based on a multinomial model. *Genome Biol.* **20**, 295 (2019).
20. Huang, M. et al. SAVER: gene expression recovery for single-cell RNA sequencing. *Nat. Methods* **15**, 539–542 (2018).
21. Eraslan, G., Simon, L. M., Mircea, M., Mueller, N. S. & Theis, F. J. Single-cell RNA-seq denoising using a deep count autoencoder. *Nat. Commun.* **10**, 390 (2019).
22. Tang, W. et al. bayNorm: Bayesian gene expression recovery, imputation and normalization for single-cell RNA-sequencing data. *Bioinformatics* **36**, 1174–1181 (2019).
23. Hilbe, J. M. *Modeling Count Data* (Cambridge Univ. Press, 2014).
24. Lu, M. *Generalized Adaptive Shrinkage Methods and Applications in Genomics Studies.* PhD thesis, Univ. Chicago (2018).
25. Raj, A. & van Oudenaarden, A. Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell* **135**, 216–226 (2008).
26. Shalek, A. K. et al. Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. *Nature* **498**, 236–240 (2013).
27. Shalek, A. K. et al. Single-cell RNA-seq reveals dynamic paracrine control of cellular variation. *Nature* **510**, 363–369 (2014).
28. Bacher, R. & Kendziorski, C. Design and computational analysis of single-cell RNA-sequencing experiments. *Genome Biol.* **17**, 63 (2016).
29. Lopez, R., Regier, J., Cole, M. B., Jordan, M. I. & Yosef, N. Deep generative modeling for single-cell transcriptomics. *Nat. Methods* **15**, 1053–1058 (2018).
30. Hu, Q. & Greene, C. S. Parameter tuning is a key part of dimensionality reduction via deep variational autoencoders for single cell RNA transcriptomics. *Pac. Symp. Biocomput.* **24**, 362–373 (2019).
31. Sun, S., Zhu, J., Ma, Y. & Zhou, X. Accuracy, robustness and scalability of dimensionality reduction methods for single-cell RNA-seq analysis. *Genome Biol.* **20**, 269 (2019).
32. Brennecke, P. et al. Accounting for technical noise in single-cell RNA-seq experiments. *Nat. Methods* **10**, 1093–1095 (2013).
33. Kim, J. K., Kolodziejczyk, A. A., Ilicic, T., Teichmann, S. A. & Marioni, J. C. Characterizing noise structure in single-cell RNA-seq distinguishes genuine from technical stochastic allelic expression. *Nat. Commun.* **6**, 8687 (2015).
34. Tipping, M. E. & Bishop, C. M. Probabilistic principal component analysis. *J. R. Stat. Soc. B Stat. Methodol.* **61**, 611–622 (1999).
35. Wang, W. & Stephens, M. Empirical Bayes matrix factorization. *J. Mach. Learn. Res.* (in the press).
36. Pierson, E. & Yau, C. ZIFA: dimensionality reduction for zero-inflated single-cell gene expression analysis. *Genome Biol.* **16**, 241 (2015).
37. Buettner, F. et al. Computational analysis of cell-to-cell heterogeneity in single-cell RNA-sequencing data reveals hidden subpopulations of cells. *Nat. Biotechnol.* **33**, 155–160 (2015).
38. Verma, A. & Engelhardt, B. E. A robust nonlinear low-dimensional manifold for single cell RNA-seq data. *BMC Bioinformatics* **21**, 324 (2020).
39. Law, C. W., Chen, Y., Shi, W. & Smyth, G. K. voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol.* **15**, R29 (2014).
40. Lun, A. Overcoming systematic errors caused by log-transformation of normalized single-cell RNA sequencing data. Preprint at *bioRxiv* https://doi.org/10.1101/404962 (2018).
41. Venables, W. N. & Ripley, B. D. *Modern Applied Statistics with S* 4th edn (Springer, 2002).
42. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139–140 (2009).
43. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
44. Vallejos, C. A., Marioni, J. C. & Richardson, S. BASiCS: Bayesian analysis of single-cell sequencing data. *PLoS Comp. Biol.* **11**, e1004333 (2015).
45. Zeileis, A., Kleiber, C. & Jackman, S. Regression models for count data in R. *J. Stat. Softw.* **27**, 1–25 (2008).
46. Stephens, M. False discovery rates: a new deal. *Biostatistics* **18**, 275–294 (2017).
47. Kiefer, J. & Wolfowitz, J. Consistency of the maximum likelihood estimator in the presence of infinitely many incidental parameters. *Ann. Math. Stat.* **27**, 887–906 (1956).
48. Lee, D. D. & Seung, H. S. in *Advances in Neural Information Processing Systems 13: Proceedings of the 2000 Conference* (eds Leen, T. K. et al.) 556–562 (MIT Press, 2000).
49. Levitin, H. M. et al. De novo gene signature identification from single-cell RNA-seq with hierarchical Poisson factorization. *Mol. Syst. Biol.* **15**, e8557 (2019).
50. Gouvert, O., Oberlin, T. & Févotte, C. Negative binomial matrix factorization for recommender systems. *IEEE Signal Process. Lett.* **27**, 815–819 (2020).
51. Sun, S., Chen, Y., Liu, Y. & Shang, X. A fast and efficient count-based matrix factorization method for detecting cell types from single-cell RNA-seq data. *BMC Syst. Biol.* **13**, 28 (2019).
52. Risso, D., Perraudeau, F., Gribkova, S., Dudoit, S. & Vert, J.-P. A general and flexible method for signal extraction from single-cell RNA-seq data. *Nat. Commun.* **9**, 284 (2018).
53. Zheng, G. X. Y. et al. Massively parallel digital transcriptional profiling of single cells. *Nat. Commun.* **8**, 14049 (2017).
54. Sarkar, A. K. et al. Discovery and characterization of variance QTLs in human induced pluripotent stem cells. *PLoS Genet.* **15**, e1008045 (2019).
55. Habib, N. et al. Massively parallel single-nucleus RNA-seq with DroNc-seq. *Nat. Methods* **14**, 955–958 (2017).
56. Stewart, B. J. et al. Spatiotemporal immune zonation of the human kidney. *Science* **365**, 1461–1466 (2019).
57. Lukowski, S. W. et al. A single-cell transcriptome atlas of the adult human retina. *EMBO J.* **38**, e100811 (2019).
58. Svensson, V. et al. Power analysis of single-cell RNA-sequencing experiments. *Nat. Methods* **14**, 381–387 (2017).
59. Macosko, E. Z. et al. Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell* **161**, 1202–1214 (2015).
60. Klein, A. M. et al. Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. *Cell* **161**, 1187–1201 (2015).
61. Islam, S. et al. Characterization of the single-cell transcriptional landscape by highly multiplex RNA-seq. *Genome Res.* **21**, 1160–1167 (2011).

Acknowledgements

We thank members of the M.S. and Y. Gilad laboratories for helpful comments. This work was supported by NIH grant HG002585 and a Gut Cell Atlas grant from The Leona M. and Harry B. Helmsley Charitable Trust (both to M.S.).

Author contributions

A.S. and M.S. developed the theory. A.S. performed the analysis. A.S. and M.S. wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41588-021-00873-4.

Correspondence should be addressed to A.S. or M.S.

Peer review information *Nature Genetics* thanks the anonymous reviewers for their contribution to the peer review of this work.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

nature research

Corresponding author(s): Abhishek Sarkar, Matthew Stephens

Last updated by author(s): Mar 18, 2021

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	N/A
Data analysis	All code used to perform the analysis is available at https://zenodo.org/record/4543921 and https://zenodo.org/record/4543923.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Sorted immune cell and PBMC data were downloaded from https://10xgenomics.com/data. iPSC data were downloaded from Gene Expression Omnibus, accession number GSE118723. Brain data were downloaded from the GTEx portal https://www.gtexportal.org/home/datasets. Kidney and retina data were downloaded from the Human Cell Atlas Data Portal https://data.humancellatlas.org/. Control data were downloaded from https://figshare.com/projects/Zero_inflation_in_negative_control_data/61292. Analysis results generated in this study are available at https://zenodo.org/record/4543923 and https://aksarkar.github.io/singlecell-modes/.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	N/A
Data exclusions	N/A
Replication	N/A
Randomization	N/A
Blinding	N/A

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging