Single-cell generalized trend model (scGTM): a flexible and interpretable model of gene expression trend along cell pseudotime

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ABSTRACT

Modeling single-cell gene expression trends along cell pseudotime is a crucial analysis for exploring biological processes. Most existing methods rely on nonparametric regression models for their flexibility; however, nonparametric models often provide trends too complex to interpret. Other existing methods use interpretable but restrictive models. Since model interpretability and flexibility are both indispensable for understanding biological processes, the single-cell field needs a model that improves the interpretability and largely maintains the flexibility of nonparametric regression models. Here we propose the single-cell generalized trend model (scGTM) for capturing a gene's expression trend, which may be monotone, hill-shaped, or valley-shaped, along cell pseudotime. The scGTM has three advantages: (1) it can capture non-monotonic trends that are still easy to interpret, (2) its parameters are biologically interpretable and trend informative, and (3) it can flexibly accommodate common distributions for modeling gene expression counts. To tackle the complex optimization problems, we use the particle swarm optimization algorithm to find the constrained maximum likelihood estimates for the scGTM parameters. As an application, we analyze several single-cell gene expression data sets using the scGTM and show that it can capture interpretable gene expression trends along cell pseudotime and reveal molecular insights underlying the biological processes. We also provide an open-access Python package for fitting a scGTM at <https://github.com/ElvisCuiHan/scGTM>.

1. Introduction

Pseudotime analysis is one of the most important topics in single-cell transcriptomics. There has been fruitful work on inferring cell pseudotime (1, 2, 3, 4, 5, 6, 7, 8, 9) and constructing statistical models for gene expression along the inferred cell pseudotime (10, 11, 12, 13, 14). Informative trends of gene expression along cell pseudotime may reflect molecular signatures in the biological processes. For instance, a gene may over time exhibit a *hill-shaped* trend (i.e. first-upward-then-downward) (Fig. 1b) or a *valley-shaped* trend (i.e. first-downward-then-upward) (Fig. 1c) trend and either of the trend may indicate the occurrence of some biological event. Hence, it is of great interest to have a statistical model that can capture the different gene expression trends accurately along cell pseudotime.

There are two types of statistical methods that have been developed to model the relationship between a gene's expression in a cell (or a sample) and the cell pseudotime. The latter is the sample's physical time and so modeling issues encountered here are similar to those commonly seen in fitting a regression model to a data set in statistics. Methods of the first type are based on statistical regression models, such as generalized linear models (GLM) and generalized additive models (GAM), where frequently parameters in these models do not have direct relevance to gene expression dynamics. Specifically, the GLM used in the Monocle3 method (8) assumes that a gene's log-transformed expected expression in a cell is a linear function of the cell pseudotime, making it unable to capture hill- and valley-shaped trends that linear trends cannot approximate well. Consequently, most methods use nonparametric regression models, such as the GAM and piece-wise linear models, to capture complex trends. For example, Storey et al. (15) applied basis regression; Trapnell et al. (3) considered the GAM with the Tobit likelihood; Ren and Kuan (13) applied the GAM with Bayesian shrinkage dispersion estimates; Van den Berge et al. (12)

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proposed tradeSeq using the spline-based GAM. More recently, Song and Li (14) proposed the PseudotimeDE method, which fixes the p-value calibration issue in tradeSeq and also uses the spline-based GAM with spline functions. Additionally, Bacher et al. (11) used a piecewise linear model, which is more restrictive than the GAM. Although these nonparametric regression methods can fit complex gene expression trends, they are prone to over-fitting without proper hyper-parameter tuning (as we will show in Section 3) and their parameters either do not directly inform the shape of a trend (e.g., hill-shaped) or carry biological meanings.

Unlike the first type, methods of the second type use models with direct relevance to gene expression dynamics, and notable methods include ImpulseDE/ImpulseDE2 (16, 17, 18) and switchDE (10). Specifically, ImpulseDE2 estimates a gene expression trend using a double-logistic curve to capture the non-monotone trends; however, even though the parameters have biological interpretations, they do not intuitively inform the shape of a trend. In contrast, switchDE uses a restrictive model with parameters that directly inform the shape of a trend (e.g., a gene's activation time) but is unable to detect non-monotonic trends.

The above review suggests that there is no current model that can capture monotone, hill-shaped, and valley-shaped trends with biologically interpretable and trend-informative parameters. To this end, we propose in this paper, the single-cell generalized trend model (scGTM) with three advantages over existing models that (i) can capture both hill- and valley-shaped trends and monotone trends, (ii) has interpretable and trend-informative parameters, and (iii) can also model different types of count data.

To estimate the scGTM parameters, we apply particle swarm optimization (PSO) to find the constrained maximum likelihood estimates (MLE) of the model parameters. PSO has several advantages that make it suitable for our optimization problem: (i) it does not require the objective function to be convex or differentiable; (ii) it can handle boundary constraints and discrete parameters without having to re-formulate the objective function, and (iii) unlike the Newton-type algorithms used in (3, 10, 19), PSO is gradient-free. In addition, PSO codes are widely and freely available, easy to implement and its successes in tackling complex optimization problems are already well documented across disciplines, that includes computer science and engineering.

The rest of the paper is organized as follows. In Section 2, we introduce the scGTM and briefly review the PSO algorithm. In Section 3, we compare the scGTM with the GLM, GAM, ImpulseDE2, and switchDE and show its advantages in capturing informative, interpretable gene expression trends in two real data sets. Section 4 contains a discussion and future work.

2. MATERIALS AND METHODS

2.1. The scGTM formulation

Let $\mathbf{Y} = (y_{gc})$ be an observed $G \times C$ gene expression count matrix, where G is the number of genes, C is the number of cells (i.e., the number of pseudotime values), and y_{gc} is the

(g, c) -th element indicating the observed expression count of gene $g = 1, \dots, G$ in cell $c = 1, \dots, C$. We consider gene expression counts as random variables whose randomness comes from experimental measurement uncertainty, so y_{gc} is a realization of the random count variable Y_{gc} . Given a particular gene g , for notation simplicity, we drop the subscript g and denote Y_{gc} as Y_c and y_{gc} as y_c . We denote by t_c the inferred pseudotime of cell c . In the scGTM, $t_1, \dots, t_C$ are treated as fixed values of pseudotime and serve as the covariate vector of interest.

Given t_c , the scGTM can model the count variable Y_c using four count distributions commonly used for gene expression data: the Poisson, negative binomial (NB), zero-inflated Poisson (ZIP), and zero-inflated negative binomial (ZINB) distributions.

For a hill-shaped gene, the scGTM is

$$Y_c \stackrel{\text{ind}}{\sim} F(\tau_c, \phi, p_c), \quad c = 1, \dots, C, \quad (2.1)$$

$$\log(\tau_c + 1) = \begin{cases} \mu_{\text{mag}} \exp(-k_1(t_c - t_0)^2) & \text{if } t_c \leq t_0 \\ \mu_{\text{mag}} \exp(-k_2(t_c - t_0)^2) & \text{if } t_c > t_0 \end{cases}, \quad (2.2)$$

$$\log\left(\frac{p_c}{1 - p_c}\right) = \alpha \log(\tau_c + 1) + \beta, \quad (2.3)$$

where $F(\tau_c, \phi, p_c)$ in (2.1) represents one of the four common count distributions. The most general case is when $F(\tau_c, \phi, p_c) = \text{ZINB}(\tau_c, \phi, p_c)$ with mean parameter $\tau_c \geq 0$, dispersion parameter $\phi \in \mathbb{Z}_+ := \{1, 2, 3, \dots\}$ and zero-inflated parameter $p_c \in [0, 1]$. As special cases, $F(\tau_c, \phi, 0) = \text{NB}(\tau_c, \phi)$, $F(\tau_c, \infty, p_c) = \text{ZIP}(\tau_c, p_c)$, and $F(\tau_c, \infty, 0) = \text{Poisson}(\tau_c)$.

Fig. 1a displays and shows the roles of the 4 parameters in 2.2 for modelling a hill-shaped trend. For a valley-shaped trend, there are four similar parameters and we note that a monotone increasing trend is a special case of a hill-shaped trend with the increasing part only. The four parameters in the Fig. 1a are the maximum log expected expression μ_{mag} , the activation strength k_1 , the repression strength k_2 , and the change time t_0 where the expected expression stops increasing. Fig. 1b–c show the scGTM fits to the gene (*Tmsb10* in the GYRUS data set and another gene *NFKBIA* in the LPS data set from the Supplementary Table S1). Their trends reveal a hill- and valley-shaped trend, respectively.

In this hill-shaped scGTM, we assume that the gene's expression count Y_c in cell c has mean parameter τ_c and zero-inflation parameter p_c , and both depend on the pseudotime t_c of cell c . In (2.2), we link τ_c to t_c by assuming that $\log(\tau_c + 1)$ is a non-negative transformation that compresses extremely large values of τ_c using a two-part Gaussian function corresponding to $t_c \leq t_0$ and $t_c > t_0$; we choose the Gaussian function for its good mathematical properties and interpretability. We link p_c to t_c in (2.3) using a logistic regression, with predictor $\log(\tau_c + 1)$, i.e., the logistic transformation of p_c is a linear function of $\log(\tau_c + 1)$ (with slope α and intercept β) and thus a function of t_c .

Figure 1. Illustration of the scGTM. (a) Four parameters of the scGTM in Equation (2.2) for a hill-shaped trend: the maximum log expected expression μ_{mag} (horizontal blue line), the activation strength k_1 (absolute value of the left tangent line's slope), the repression strength k_2 (absolute value of the right tangent line's slope), and the change time t_0 (vertical blue line). (b) A hill-shaped trend of gene *Tmsb10* (in the GYRUS dataset) fitted by the scGTM with counts modeled by the Poisson distribution. (c) A valley-shaped trend of gene *NFKBIA* (in the LPS dataset) fitted by the scGTM with counts modeled by the Poisson distribution. In b-c, the scatter points indicate gene expression levels, and the curves are the trends fit by the scGTM.

Besides $\phi \in \mathbb{Z}_+$ and $\alpha, \beta \in \mathbb{R}$, the following parameters of the hill-shaped scGTM shown in Fig. 1a need to be estimated for biological interpretations:

- $\mu_{\text{mag}} \geq 0$: magnitude of the hill, i.e., $\mu_{\text{mag}} = \max_{c \in \{1, \dots, C\}} \log(\tau_c + 1)$;
- $k_1 \geq 0$: activation strength (how fast the gene is up-regulated);
- $k_2 \geq 0$: repression strength (how fast the gene is down-regulated);
- $t_0 \in [0, 1]$: change time (where the gene reaches the maximum expected expression).

For a valley-shaped gene, the scGTM is the same except that we replace (2.2) by

$$\log(\tau_c + 1) = \begin{cases} b - \mu_{\text{mag}} \exp(-k_2(t_c - t_0)^2) & \text{if } t_c \leq t_0 \\ b - \mu_{\text{mag}} \exp(-k_1(t_c - t_0)^2) & \text{if } t_c > t_0 \end{cases}, \quad (2.4)$$

where b indicates the baseline (maximum) log-transformed (expected expression + 1) of the valley-shaped gene. The interpretation of the four key parameters of the valley-shaped scGTM becomes

- $\mu_{\text{mag}} \in [0, b]$: magnitude of the valley, i.e., $b - \mu_{\text{mag}} = \min_{c \in \{1, \dots, C\}} \log(\tau_c + 1)$;
- $k_1 \geq 0$: activation strength (how fast the gene is up-regulated);
- $k_2 \geq 0$: repression strength (how fast the gene is down-regulated);
- $t_0 \in [0, 1]$: change time (where the gene reaches the minimum expected expression).

Compared to the hill-shaped scGTM, the valley-shaped scGTM has an additional baseline parameter b that needs to be estimated. For simplicity, we estimate b by a plug-in estimator $\hat{b} = \max_{c \in \{1, \dots, C\}} \log(y_c + 1)$, where $y_1, \dots, y_C$ are the observed counts of a valley-shaped gene. For the common parameters of the hill- and valley-shaped scGTMs in Section 2.2, we next discuss how PSO can provide constrained likelihood estimates for these parameters.

2.2. Constrained MLE and the PSO algorithm

To fit the scGTM to a gene, we first need to ascertain whether the gene is hill- or valley-shaped: we call the gene valley-shaped only if its expression count y_c is negatively correlated with $t_c \in [0, 0.5]$ and positively correlated with $t_c \in [0.5, 1]$; otherwise, we consider the gene hill-shaped. Next, based on the trend shape, we estimate the scGTM parameters. For a hill-shaped gene, we estimate the scGTM parameters $\Theta = (\mu_{\text{mag}}, k_1, k_2, t_0, \phi, \alpha, \beta)^T$ from the observed expression counts $\mathbf{y} = (y_1, \dots, y_C)^T$ and cell pseudotimes $\mathbf{t} = (t_1, \dots, t_C)^T$ using the constrained maximum likelihood method, which respects each parameter's range and ensures the estimation stability. Let $\log L(\Theta | \mathbf{y}, \mathbf{t})$ be the log likelihood function and the optimization problem is:

$$\max_{\Theta} \log L(\Theta | \mathbf{y}, \mathbf{t}) \quad (2.5)$$

$$\begin{aligned} \text{such that } & \min_{c \in \{1, \dots, C\}} \log(y_c + 1) \leq \mu_{\text{mag}} \\ & \leq \max_{c \in \{1, \dots, C\}} \log(y_c + 1), \\ & k_1, k_2 \geq 0, \\ & \min_{c \in \{1, \dots, C\}} t_c \leq t_0 \leq \max_{c \in \{1, \dots, C\}} t_c, \\ & \phi \in \mathbb{Z}_+, \end{aligned} \quad (2.6)$$

where

$$\begin{aligned} \log L(\Theta | \mathbf{y}, \mathbf{t}) &= \log \left[\prod_{c=1}^C \mathbb{P}(Y_c = y_c | t_c) \right] \\ &= \sum_{c=1}^C \log \left[(1 - p_c) f(y_c | t_c) + p_c \mathbb{I}(y_c = 0) \right] \end{aligned} \quad (2.7)$$

and

$$f(y_c|t_c) = \frac{\tau_c^{y_c}}{y_c!} \frac{\Gamma(\phi + y_c)}{\Gamma(\phi)(\phi + \tau_c)^{y_c}} \frac{1}{\left(1 + \frac{\tau_c}{\phi}\right)^\phi},$$

which can be further specified as a function of Θ based on (2.2) and (2.3).

For a valley-shaped gene, the constrained MLE problem for estimating parameters in the scGTM is similar and we omit the discussion for space consideration.

There are two difficulties in the optimization problem (2.5). First, the likelihood function (2.7) is neither convex nor concave. Second, the constraint is linear in μ_{mag} , k_1 , k_2 , and t_0 but ϕ is a positive integer-valued variable. Hence, conventional optimization algorithms such as P-IRLS in GAM (19, 20) and L-BFGS in switchDE (10, 21) are difficult to apply in this case. Metaheuristics is a class of assumptions-free general purpose optimization algorithms that is widely and increasingly used to tackle challenging and high-dimensional optimization problems in the quantitative sciences (22, 23, 24). PSO is an exemplary and popular member of this class and has been shown to be effective to solve various types of optimization problems and (25) provides a recent review of such algorithms and applications across various disciplines.

PSO first generates a swarm of candidate solutions (known as particles) to the optimization problem (2.5). At each iteration, particles change their positions within the constraints, and the algorithm finds the best solution among all particle trajectories. We summarize the vanilla PSO algorithm (26) for the constrained MLE of the scGTM in Algorithm 1, and provide further details of PSO in the Supplementary Information. The below algorithm applies PSO to solve our optimization problem (2.5).

2.3. Approximate confidence intervals of the four key parameters in the scGTM

The estimated parameters $\hat{\Theta} = (\hat{\mu}_{\text{mag}}, \hat{k}_1, \hat{k}_2, \hat{t}_0, \hat{\phi}, \hat{\alpha}, \hat{\beta})^T$ are next used to construct approximate confidence intervals for μ_{mag} , k_1 , k_2 , and t_0 using the maximum likelihood theory. Specifically, we calculate the plug-in asymptotic covariance matrix of $(\hat{\mu}_{\text{mag}}, \hat{k}_1, \hat{k}_2, \hat{t}_0)^T$ as the inverse of the partial Fisher information matrix of the four parameters evaluated at $(\hat{\mu}_{\text{mag}}, \hat{k}_1, \hat{k}_2, \hat{t}_0)^T$ (detailed derivation in the Supplementary Information). Then we use the diagonal elements of this matrix as the plug-in asymptotic variances of $\hat{\mu}_{\text{mag}}$, $\hat{k}_1$, $\hat{k}_2$, and $\hat{t}_0$, and denote them by $\widehat{\text{Var}}(\hat{\mu}_{\text{mag}})$, $\widehat{\text{Var}}(\hat{k}_1)$, $\widehat{\text{Var}}(\hat{k}_2)$, and $\widehat{\text{Var}}(\hat{t}_0)$, respectively. We then obtain a 95% approximate confidence interval for each of the parameters: $[\hat{\mu}_{\text{mag}}^{\text{lb}}, \hat{\mu}_{\text{mag}}^{\text{ub}}]$, $[\hat{k}_1^{\text{lb}}, \hat{k}_1^{\text{ub}}]$, $[\hat{k}_2^{\text{lb}}, \hat{k}_2^{\text{ub}}]$, and $[\hat{t}_0^{\text{lb}}, \hat{t}_0^{\text{ub}}]$.

Algorithm 1 PSO for the constrained MLE for the scGTM

Input data: a gene's expression counts and cell pseudotime values

$\mathbf{y}$: a $C \times 1$ gene expression count vector;

$\mathbf{t}$: a $C \times 1$ cell pseudotime vector;

Input parameters:

F : count distribution: Poisson, NB, ZIP, or ZINB;

H : number of iterations in PSO; set to $H=100$ by default;

w , c_1 , and c_2 : hyperparameters of PSO; set to $w=0.9$, $c_1=1.2$, and $c_2=0.3$ by default;

Algorithm:

1. Randomly initialize Θ with B particles: $\Theta_1^0, \Theta_2^0, \dots, \Theta_B^0$;

2. Randomly initialize velocity vectors for the B particles: $\mathbf{v}_1^0, \mathbf{v}_2^0, \dots, \mathbf{v}_B^0$;

3. For $h=0$ to H :

(i) Update the best solution of each particle i

$$\hat{\Theta}_i^h = \underset{\Theta \in \mathcal{A}_i^h}{\text{argmax}} \log L(\Theta | \mathbf{y}, \mathbf{t}),$$

$$\text{where } \mathcal{A}_i^h = \{\Theta_i^k : k=0, \dots, h\}, i=1, \dots, B;$$

(ii) Update the global best solution

$$\hat{\Theta}^h = \underset{\Theta \in \bigcup_{i=1}^B \mathcal{A}_i^h}{\text{argmax}} \log L(\Theta | \mathbf{y}, \mathbf{t});$$

(iii) Update velocity of each particle i

$$\mathbf{v}_i^{h+1} = w\mathbf{v}_i^h + c_1 r_{i1}^h (\hat{\Theta}_i^h - \Theta_i^h) + c_2 r_{i2}^h (\hat{\Theta}^h - \Theta_i^h),$$

where r_{i1}^h and r_{i2}^h are independently generated from $\text{Unif}(0,1)$, $i=1, \dots, B$;

(iv) Update each particle i

$$\Theta_i^{h+1} = \Theta_i^h + \mathbf{v}_i^h, \quad i=1, \dots, B;$$

4. Set $\hat{\Theta} = \hat{\Theta}^H$;

5. Calculate 95% approximate confidence intervals of key parameters based on $\hat{\Theta}$ (Section 2.3).

Output:

$-\log L(\hat{\Theta} | \mathbf{y}, \mathbf{t})$: fitted negative log likelihood value;

$\hat{\Theta} = (\hat{\mu}_{\text{mag}}, \hat{k}_1, \hat{k}_2, \hat{t}_0, \hat{\phi}, \hat{\alpha}, \hat{\beta})^T$: estimated parameters;

$[\hat{\mu}_{\text{mag}}^{\text{lb}}, \hat{\mu}_{\text{mag}}^{\text{ub}}]$, $[\hat{k}_1^{\text{lb}}, \hat{k}_1^{\text{ub}}]$, $[\hat{k}_2^{\text{lb}}, \hat{k}_2^{\text{ub}}]$, and $[\hat{t}_0^{\text{lb}}, \hat{t}_0^{\text{ub}}]$: 95% approximate confidence intervals.

where

$$\hat{\mu}_{\text{mag}}^{\text{lb}} = \max\left(0, \hat{\mu}_{\text{mag}} - 1.96\sqrt{\widehat{\text{Var}}(\hat{\mu}_{\text{mag}})}\right),$$

$$\hat{\mu}_{\text{mag}}^{\text{ub}} = \hat{\mu}_{\text{mag}} + 1.96\sqrt{\widehat{\text{Var}}(\hat{\mu}_{\text{mag}})},$$

$$\hat{k}_1^{\text{lb}} = \max\left(0, \hat{k}_1 - 1.96\sqrt{\widehat{\text{Var}}(\hat{k}_1)}\right),$$

$$\hat{k}_1^{\text{ub}} = \hat{k}_1 + 1.96\sqrt{\widehat{\text{Var}}(\hat{k}_1)},$$

$$\hat{k}_2^{\text{lb}} = \max\left(0, \hat{k}_2 - 1.96\sqrt{\widehat{\text{Var}}(\hat{k}_2)}\right),$$

$$\hat{k}_2^{\text{ub}} = \hat{k}_2 + 1.96\sqrt{\widehat{\text{Var}}(\hat{k}_2)},$$

$$\hat{t}_0^{\text{lb}} = \max\left(0, \hat{t}_0 - 1.96\sqrt{\widehat{\text{Var}}(\hat{t}_0)}\right),$$

$$\hat{t}_0^{\text{ub}} = \min\left(\hat{t}_0 + 1.96\sqrt{\widehat{\text{Var}}(\hat{t}_0)}, 1\right).$$

3. RESULTS

3.1. scGTM outperforms GAM, GLM, switchDE, and ImpulseDE2 in capturing informative and interpretable trends

As an example, we use the *MAOA* gene in the WANG dataset (27) (Supplementary Table S1) to compare the fitted trends of the scGTM, GAM, GLM, switchDE, and ImpulseDE2. In the original study, the gene was reported to have a hill-shaped trend. Our comparison results have several interesting observations. First, we show that the scGTM can provide more informative and interpretable gene expression trends than the GAM and GLM when the count outcome comes from the Poisson, ZIP, NB or ZINB distributions. Fig. 2a shows that the scGTM robustly captures the hill-shaped trends for the four distributions and consistently estimates the change time around 0.75, which is where the *MAOA* gene reaches its expected maximum expression. While the GAM also estimates the maximum expression around 0.75, its estimated trends are much more complex. This is likely due to possible overfitting (despite the use of penalization) and consequently, more difficult to interpret them than the scGTM trends (Fig. 2b). Unlike the scGTM and GAM, the GLM only allows for capturing monotone trends, making it unable to detect the possible existence of expression change time (Fig. 2c). Second, we compare the scGTM with the two existing methods, switchDE and ImpulseDE2, that use models with direct relevance to gene expression dynamics. Although switchDE estimates the activation time around 0.75, similar to the scGTM's estimate change time, it cannot capture the downward expression trend as the cell pseudotime approaches 1.00 due to its monotone nature (Fig. 2d). ImpulseDE2 can theoretically capture a hill-shaped trend, but it only fits a monotone increasing trend for the *MAOA* gene (Fig. 2e). A likely reason is that the method

was designed for time-course bulk RNA-seq data. To summarize, the scGTM fits outperform those from GAM, GLM, switchDE and ImpulseDE2 by providing a more informative and interpretable trend with less concern on model overfitting.

In addition to the *MAOA* gene, Wang et al. (27) reported 19 other exemplary genes that define menstrual cycle phases and exhibit hill-shaped expression trends along the cell pseudotime. Supplementary Figs. S1-S19 shows a similar conclusion for these 19 genes when we compare their fits, where we observe that the scGTM consistently provides more informative, interpretable trends than the other models.

Besides visually inspecting the fitted expression trends, we compare the Akaike information criterion (AIC) values of the scGTM, GAM, and GLM used with the four count distributions fitted to the aforementioned 20 genes. Note that a lower AIC value indicates a model's better goodness-of-fit with the model complexity penalized. Supplementary Fig. S20 shows that the scGTM has comparable or even lower AIC values than the GAM's AIC values, confirming that the scGTM fits well to data despite its much simpler model than GAM's. Based on Fig. 2 and Supplementary Figs. S1-S20, we use the scGTM with the Poisson distribution in the following applications for its goodness-of-fit and model simplicity. This choice is consistent with previous research on modeling sequencing data (28) and other count data (29, 30).

3.2. scGTM recapitulates gene expression trends of endometrial transformation in the human menstrual cycle

The WANG dataset contains 20 exemplar genes that exhibit temporal expression trends in unciliated epithelia cells in the human menstrual cycle (27). The original study also ordered the 20 genes by the estimated pseudotime at which they achieved the maximum expression (Fig. 3a; genes ordered from top to bottom), and it was found that the ordering agreed well with the menstrual cycle phases (Fig. 3a; the top bar indicates the phases). Comparing the fitted expression trends of the 20 genes by the scGTM, switchDE, and ImpulseDE2, we observe that only the scGTM trends agree well with the data (Fig. 3). Additionally, we evaluate the 20 genes' estimated change times (i.e., t_0) by the scGTM and their estimated activation times by the switchDE. Although the change times and estimation times are both expected to correlate well with the gene ordering in the original study, only the change times estimated by the scGTM fulfills this expectation (Fig. 3b-c). Compared with the scGTM, switchDE miscalculates the activation times for many hill-shaped genes whose maximum expression occurs in the middle of the cycle; this is likely due to the fact that switchDE can only capture monotone trends (Fig. 3c). Similarly, ImpulseDE2 cannot well capture the trends of those hill-shaped genes (Fig. 3d). Unlike switchDE and ImpulseDE2, the scGTM estimates the change times reasonably for almost all genes. For instance, the *GPX3* gene has an estimated change time at 0.88, consistent with its role as a secretory middle/late phase marker gene (27). In

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Figure 2. Comparison of scGTM with GAM, GLM, switchDE, and ImpulseDE2 for fitting the expression trend of gene *MAOA* in the WANG dataset (27) (Supplementary Table S1). In the first four columns, the three rows correspond to (a) scGTM, (b) GAM, and (c) GLM. From left to right, the first four columns correspond to Poisson, ZIP, NB, and ZINB as the count distribution used in scGTM, GAM, and GLM. The fifth column corresponds to (d) switchDE and (e) ImpulseDE2. Each panel shows the same scatterplot of gene *MAOA*'s log-transformed expression counts vs. cell pseudotime values, as well as a model's fitted trend. With all four count distributions, scGTM robustly captures the gene expression trend and estimates the change time around 0.75. In contrast, GLM, switchDE and ImpulseDE2 only describe the trend as increasing, while GAM overfits the data and does not output trends as interpretable as scGTM does.

Figure 3. Fitted expression trends by scGTM, switchDE, and ImpulseDE2 for 20 exemplar genes in the WANG dataset (27) (Supplementary Table S1). All panels are ordered by cell pseudotime values from 0 (left) to 1 (right). The top color bars show the endometrial phases defined in the original study. (a) The original expression values along pseudotime. (b) The fitted trends of scGTM, with the red segments highlighting the estimated change times t_0 . (c) The fitted trends of switchDE, with the red segments highlighting the estimated activation times. (d) The fitted trends of ImpulseDE2.

summary, the scGTM provides useful summaries for gene expression trends in the human menstrual cycle.

3.3. scGTM identifies informative gene expression trends after immune cell stimulation

As the second real data application, we use the scGTM to categorize gene expression trends in mouse dendritic cells

(DCs) after stimulation with lipopolysaccharide (LPS, a component of gram-negative bacteria) (31). First, we apply the likelihood ratio tests to screen the genes that the scGTM fits significantly better than the null Poisson model (in which τ_c and p_c in (2.1) do not depend on cell pseudotime t_c). Assuming that the likelihood ratio statistic of every gene follows χ^2_3 as the null distribution, we retain 2405 genes whose Benjamini-Hochberg (BH) adjusted p -values ≤ 0.01 .

Second, we use the scGTM's confidence levels of the three parameters t_0 , k_1 , and k_2 to categorize the 2405 genes into three types: (1) *hill-shaped & mostly increasing genes*: $t_0^{\text{lb}} > 0.5 + 0.1$ (change time occurs at late pseudotime) and $k_1^{\text{lb}} > 1$ (strong activation strength), (2) *hill-shaped & mostly decreasing genes*: $t_0^{\text{ub}} < 0.5 - 0.1$ (change time occurs at early pseudotime) and $k_2^{\text{lb}} > 1$ (strong repression strength), and (3) *valley-shaped genes*. To demonstrate that this categorization is biologically meaningful, we perform gene ontology (GO) analysis on the three gene types and compare the enriched GO terms. Fig. 4a shows that the three gene types are enriched with largely unique GO terms, verifying their functional differences. Notably, the hill-shaped & mostly increasing genes are related to immune response processes, showing consistency between their expression trends (activation after the LPS stimulation) and functions (immune response). Further, we visualize 5 illustrative genes from each gene type (Fig. 4b) and observe that the scGTM's fitted trends agree well with the data. In conclusion, the scGTM can help users discern genes with specific trends by its trend-informative parameters.

4. CONCLUSION

We propose the scGTM as a flexible and interpretable statistical model for studying single-cell gene expression trend along cell pseudotime. Using four count distributions and two real data sets, we demonstrated that the scGTM has interpretable parameters that can directly inform a trend for gene expression counts. The scGTM parameters are estimated by the constrained maximum likelihood estimation via PSO, which is one of the most popular metaheuristic algorithms for function optimization. We showed that scGTM has distinct advantages over the classic models GLM and GAM and the two recent methods switchDE and ImpulseDE2 in that it can uniquely capture robust, informative, and interpretable trends. In contrast, the GLM and switchDE can only estimate monotonic trends; the GAM often provides trends that are too complex to interpret, and ImpulseDE2 (a method designed for bulk RNA-seq data) does not have stable performance on single-cell data. The estimated parameters and confidence intervals from the scGTM are then used to characterize the expression trends of the genes.

Strictly speaking, the inference of the scGTM has two caveats. First, the parameter estimation includes a double-dipping procedure: the same data are first used to decide whether a trend is hill- or valley-shaped and it is used to estimate the parameters. Second, since only the key parameters μ_{mag} , k_1 , k_2 , and t_0 are inferential targets, the other parameters ϕ , α and β should be

regarded “nuisance” parameters. However, the construction of confidence intervals of the key parameters does not account for these two caveats and would thus result in overly optimistic confidence intervals. We will investigate how to obtain better-calibrated confidence intervals in future research.

The current implementation of the scGTM is only applicable to a single pseudotime trajectory (i.e., cell lineage). A natural extension is to split a multiple-lineage cell trajectory into single lineages and fit the scGTM to each lineage separately. In addition, the vanilla PSO algorithm in this paper handles each parameter's constraint separately. Hence, if we need a constraint on more than one parameter, e.g., k_1/k_2 should be within a user-specified range, then we have to develop a variant algorithm of PSO or use other metaheuristics algorithms.

5. DATA AVAILABILITY

The code and data for generating the results are available at zenodo (doi: 10.5281/zenodo.5728342). The scGTM Python package is available at <https://github.com/ElvisCuiHan/scGTM>.

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Figure 4. Three types of gene expression trends characterized by the scGTM parameters in the LPS dataset (Supplementary Table S1). (a) GO enrichment analysis of the three gene types. The top enriched GO terms are different among the three gene types. Notably, the hill-shaped & mostly increasing genes (1st column) are functionally related to immune responses. (b) Visualization of example genes in the three types. The scatter plots show gene expression data; the trends estimated by the scGTM (blue curves) well match the data.

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