

A Monte Carlo method to estimate cell population heterogeneity

Ben Lambert^{1,2*}, David J. Gavaghan³, Simon Tavener⁴.

1 Department of Zoology, University of Oxford, Oxford, Oxfordshire, U.K.

2 MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College London, London W2 1PG, UK.

3 Department of Computer Science, University of Oxford, Oxford, U.K.

4 Department of Mathematics, Colorado State University, Fort Collins, Colorado, U.S.A.

*ben.c.lambert@gmail.com.

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1 Abstract

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Variation is characteristic of all living systems. Laboratory techniques such as flow cytometry can probe individual cells and, after decades of experimentation, it is clear that even members of seemingly homogeneous cell populations can exhibit differences. To understand whether this variation is biologically meaningful, it is essential to discern its source. Mathematical models of biological systems are tools that can be used to investigate causes of cell-to-cell variation. From mathematical analysis and simulation of these models, biological hypotheses can be posed and investigated, then parameter inference can determine which of these is most compatible with experimental data. Data from laboratory experiments often takes the form of “snapshots” representing distributions of cellular properties at different points in time, rather than individual cell trajectories. This data is not straightforward to fit using hierarchical Bayesian methods since these approaches require inferring the identities of the groups to which individual cells belong. Here, we introduce a computational sampling method we call “Contour Monte Carlo” for estimating mathematical model parameters from snapshot distributions which is straightforward to implement and does not require assigning cells to a predefined number of categories. Our method is most applicable to systems where the dominant source of uncertainty is heterogeneity in cellular processes rather than experimental measurement error which, due to the increasingly fine scale resolution of laboratory techniques, may be the case for a wide class of systems. In this paper, we illustrate the use of our method by quantifying cellular variation for three biological systems of interest and provide code in the form of a Julia notebook which allows others to apply this approach to their problem.

2 Introduction

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Variation, as opposed to homogeneity, is the rule rather than exception in biology. Indeed, without variation, biology as a discipline would not exist, since as evolutionary biologist JBS Haldane wrote, variation is the “raw material” of evolution. The Red Queen Hypothesis asserts that organisms must continually evolve in order to survive when pitted against other - also evolving - organisms [1]. A corollary of this hypothesis is that multicellular organisms should evolve cellular phenotypic heterogeneity to allow faster adaptation to changing environments, which may explain the observed variation in a range of biological systems [2]. Whilst cell population variation can confer evolutionary advantages, it can also be costly in other circumstances. In biotechnological processes, heterogeneity in cellular function can lead to reduced yields of biochemical products [3]. In human biology, variation across cells can enable pathologies to develop and also prevents effective medical treatment, since medical interventions typically aim to steer modal cellular properties and hence fail to influence key subpopulations. For example, cellular heterogeneity likely assists some cancerous tumours to persist [4] and facilitates evolution of resistance to chemotherapies [5]. Identifying and quantifying sources of variation in populations of cells is important for wide ranging applications to discern whether the variability is benign or alternatively requires remedy.

Mathematical models are essential tools for understanding cellular systems, whose emergent properties are the result of complex interactions between various actors. Perhaps the simplest flavour of mathematical

model used in biological systems is an ordinary differential equation (ODE) that lumps individual actors into partitions according to structure or function, and seeks to model the mean behaviour of each partition. Data from population-averaged experimental assays can be a powerful resource to understand whether such models faithfully reproduce system behaviours and can allow quantification of the interactions of various cellular components of complex metabolic, signalling and transcriptional networks. The worth of such models however is determined by whether averages mask differences in behaviour of individual cells that result in functional consequences [6]. In some cases, differences in cellular protein abundances due to biochemical “noise” may not be meaningful biologically [7] and mean cell behaviour suffices as a description of the system, whereas in others there are functional consequences. For example, a recent study demonstrated that subpopulations of clonally derived hematopoietic progenitor cells with low or high expression of a particular stem cell marker produced different blood lineages [8].

To accommodate cell population heterogeneity in mathematical models, a variety of modelling choices are available, each posing different challenges for parameter inference. A recent review is presented in [9]. These approaches include modelling biochemical processes stochastically, with properties of ensembles of cells represented by probability distributions evolving according to chemical master equations (see [10] for a tutorial on stochastic reaction-diffusion processes; RDEs). Alternatively, population balance equations (PBEs) can be used to dictate the evolution of the “number density” of differing cell types, whose properties are represented as points in \mathbb{R}^n which, in turn, affect their function, including their rate of death and cell division (see [11] for an introduction to PBEs). In a PBE approach, variation in measured quantities results primarily from differing functional properties of heterogeneous cell types and variable initial densities of each type.

The approach we follow here is similar to that of [12], wherein dynamic cellular variation is generated by describing the evolution of each cell’s state using an ODE, but with individual cell differences in the rate parameters of the process. To our knowledge, this flavour of model is unnamed and so, for sake of reference, we term them “heterogenous ODE” models (HODEs). In HODEs, the aim of inference is to estimate the distributions of parameter values across cells that are consistent with observed distributions of measurements at various timepoints. A benefit of using HODEs to model cell heterogeneity is that these models are computationally straightforward to simulate and, arguably, simpler to parameterise than PBEs. An implicit assumption in this modeling approach is that the predominant source of variation is due to differences in biological processes across cells, not inherent stochasticity in biochemical reactions within cells, as in stochastic RDEs.

The difficulty of parameter inference for HODEs is partly due to experimental hurdles in generating data of sufficient quality to allow identification. Unlike models which represent a population by a single scalar ODE, since HODEs are individual-based, they ideally require individual cell data for estimation. A widely-used method for generating data for individual cells is flow cytometry, where a large number of cells are streamed individually through a laser beam and, for example, abundance measurements are made of proteins labelled with fluorescent markers [13]. Alternatively, experimental techniques such as Western blotting and cytometric fluorescence

microscopy can generate single cell measurements [14, 15]. A property of these experimental methods is that they are destructive, meaning that individual cells are sacrificed as part of the measurement process. This means that the measurements of cell properties obtained at a certain point in time represent what are termed “snapshots” of the underlying population [15]. These snapshots are often described by histograms [12] or density functions [9] fit to the underlying data at different points in time. Since HODEs represent the underlying state of individual cells as evolving continuously with time, experimental data tracing individual cell trajectories through time would constitute a richer data resource. The demands of obtaining this data are higher however and typically involve either tracking individual cells through imaging methods [16] or trapping cells in a spatial position where their individual dynamics can be readily monitored [17]. These techniques impose restrictions on experimental practices meaning they cannot be realised in all circumstances, including for online monitoring of biotechnological processes or analysis of *in vivo* studies. For this reason, “snapshot” data continues to play an important role for determining cell level variability in many applications.

A variety of approaches have been proposed to estimate cell-to-cell variability by fitting HODE models to snapshot data. In HODEs, parameter values vary across cells according to a to-be-determined probability distribution, meaning that in order to solve the exact inverse problem, the underlying ODE system needs to be simulated for each individual. Since the numbers of cells in these experiments are typically $>\sim 10^4$ [15], this usually precludes exact inference due to its computational burden and instead the raw snapshot data is approximated by probability densities [12, 15, 18, 19]. Hasenauer et al. (2011) presents a Bayesian approach to inference for HODEs, which models the input parameter space using mixtures of ansatz densities. The authors then use their method to reproduce population substructure on synthetic data generated from a model of tumour necrosis factor stimulus. Hasenauer et al. (2014) uses mixture models to model subpopulation structure in snapshot data with multiple-start local optimisation employed to maximise the non-convex likelihood, which they then apply to synthetic and real data from signalling pathway models. Loos et al. (2018) also uses mixture models to represent subpopulation structure and a maximum likelihood approach that allows for estimation of within- and between-subpopulation variability which permits fitting to multivariate output distributions with complex correlation structures. Dixit et al. (2018) discretises cell abundances into bins, then uses a maximum entropy approach as part of a Bayesian framework to fit the distribution representing cell-to-cell variability.

The framework we present here is Bayesian although is distinct from the traditional Bayesian inferential paradigm used to fit many dynamic models, since the source of stochasticity arises solely due to cell-to-cell parameter variation and not due to measurement noise. Our approach is hence most suitable when measurement error is a minor contributor to observed experimental variability. Our computational method is a two-step Monte Carlo approach which, for reasons described in §3, we term “Contour Monte Carlo” (CMC). Unlike many of the existing methods, CMC is relatively computationally straightforward to implement and does not require extensive computation time. CMC uses MCMC in its second step to sample from the posterior distribution over parameter values and hence does not require specification of ansatz densities. It also does not require *a priori*

representation of subpopulation structure using mixture components, rather, 157
 subpopulations emerge as modes in the posterior parameter distributions. 158
 Like [19], CMC can fit multivariate snapshot data and unlike [12], does 159
 not require this data to be discretised into bins. As more experimental 160
 techniques are developed which elucidate single cell behaviour, there is 161
 likely to be more interest in methods which can be used to recapitulate 162
 the observed snapshots. We argue that due to its simplicity and generality, 163
 CMC is a useful addition to the modeller’s toolkit and can be used to 164
 perform inference on the proliferation of rich single cell data. 165

Outline of the paper: In §3, we present the details of our methodological 166
 framework and detail the CMC algorithm used to generate samples from 167
 the posterior parameter distribution. In §4, we use CMC to estimate cell 168
 population heterogeneity in three systems of biological interest. 169

3 Method

In this section, we first describe the probabilistic framework 171
 that underlies the CMC algorithm, before introducing CMC in pseudocode 172
 (Algorithm 1). We also detail the workflow we have found useful in applying 173
 this approach to analyse cell *snapshot data* and suggest practical remedies 174
 to issues we have encountered in using CMC (Figure 4). A glossary of all 175
 the variables used in this paper is included as Table 1. 176

Experimental methods such as flow cytometry can measure single cell 177
 characteristics at a given point in time. Cells are typically destroyed by 178
 the measurement process and so rather than providing time series for each 179
 individual cell, the data consists of cross-sections or “snapshots” of sampled 180
 individuals from the population (Figure 1). 181

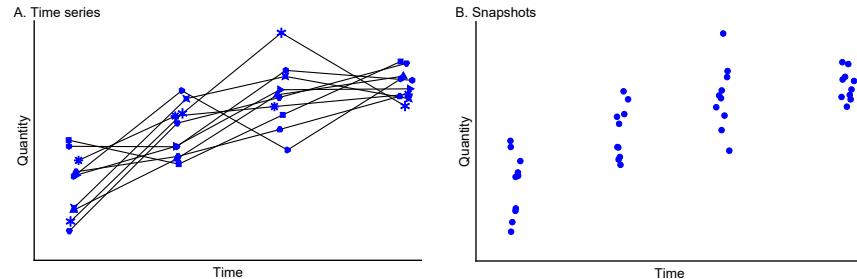


Figure 1: **Time series data (A) versus snapshot data (B) typical of single cell experiments.** In A, note the cell identities are retained at each measurement point (indicated by given plot markers) whereas in the snapshot data in B, either this information is lost or, more often, cells are destroyed by the measurement process and so each observation corresponds to a distinct cell.

We model the processes of an individual cell using a system of ordinary 182
 differential equations (ODEs), 183

$$\begin{aligned} \frac{dx}{dt} &= f(x(t); \theta), & f : \mathbb{R}^k \times \mathbb{R}^p \mapsto \mathbb{R}^k, \\ x(0) &= x_0. \end{aligned} \tag{1}$$

Note that in most circumstances, the initial state of the system, $x(0)$, 184

is unknown and it is convenient to include these as elements of $\boldsymbol{\theta}$ to be estimated. 185
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3.1 Snapshot data 187

In this paper, we assume variation characterised by snapshot data arises due to between-cell heterogeneity in the underlying parameters $\boldsymbol{\theta}$. Therefore, the evolution of the underlying state of cell i is described by an idiosyncratic ODE, 188
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$$\begin{aligned} \frac{d\mathbf{x}^{\{i\}}}{dt} &= \mathbf{f}\left(\mathbf{x}^{\{i\}}(t); \boldsymbol{\theta}^{\{i\}}\right), \quad \mathbf{f}: \mathbb{R}^k \times \mathbb{R}^p \mapsto \mathbb{R}^k, \\ \mathbf{x}^{\{i\}}(0) &= \mathbf{x}_0 \end{aligned} \quad (2)$$

where $\{i\}$ indicates the i th cell. The traditional (non-hierarchical) state-space approach to modelling dynamic systems supposes that measurement randomness generates output variation (Figure 2A). Our approach, by contrast, relies on the assumption that stochasticity in outputs is solely the result of variability in parameter values between cells (Figure 2B). Whether the assumption of “perfect” measurements is reasonable depends on the experimental details of the system under investigation but we argue that our method nevertheless provides a useful approximation in many cases where the signal to noise ratio is high. 192
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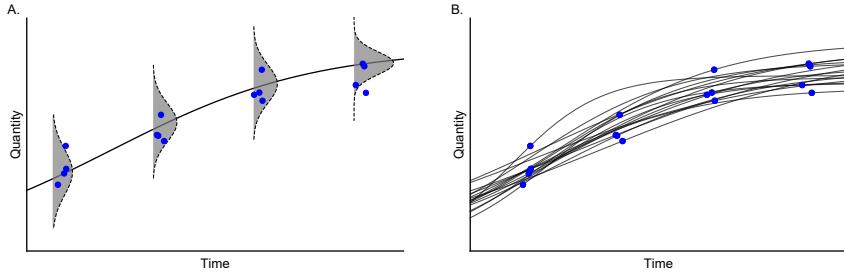


Figure 2: Two ways to generate randomness in measured outputs: the state-space model (A) versus the parameter heterogeneity model (B). For non-hierarchical state-space models (A), there is assumed to be a single “true” latent state where observations result from a noisy measurement process (grey histograms). For models with parameter heterogeneity (B), the uncertainty is generated by differences in cellular processes (black lines) between cells. Note that in both cases, individual cells are measured only once in their lifetime. 201
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We consider the situation in which we measure m quantities of interest (QOIs), 203
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$$\mathbf{q} = (q_1, q_2, \dots, q_m) \in \mathbb{R}^m, \quad (3)$$

where we have n_j observations of each quantity, q_j . Each of these quantities of interest may be different functionals of the solution at the same time or the same functional at different times or a mixture of both. The observed data for QOI j at time t_j then consists of the n_j cellular measurements, 205
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$$\mathbf{y}(t_j)^\top = \left(q_j(x^{\{1\}}(t_j)), q_j(x^{\{2\}}(t_j)), \dots, q_j(x^{\{n_j\}}(t_j)) \right) \in \mathbb{R}^{n_j}. \quad (4)$$

The raw snapshot data \mathbf{X} then consists of the collection of all measured QOIs,

$$\mathbf{X} = (\mathbf{y}(t_1), \mathbf{y}(t_2), \dots, \mathbf{y}(t_m)) \in \mathbb{R}^{n_1} \times \mathbb{R}^{n_2} \times \dots \times \mathbb{R}^{n_m}. \quad (5)$$

Raw snapshot data thus consists of measurements of individual cells with exact inference requiring simulations of the underlying ODE system for each individual cell for each quantity of interest. This is cumbersome and impractical for the numbers of cells sampled in typical experimental setups and so, instead, we follow previous work and represent snapshot data \mathbf{X} using probability distributions [12, 15, 18, 19]. Typically, these distributions are estimated with a kernel density estimator (KDE), where their support is the space of the vector of QOIs $\mathbf{q} \in \mathbb{R}^m$. We denote $\hat{\Phi}$ as the parameter estimates of the corresponding density $p(\mathbf{q}|\Phi)$ fitted to the raw snapshot data.

The goal of inference is to characterise the probability distribution $p(\boldsymbol{\theta}|\mathbf{X})$ representing heterogeneity in cellular processes. The first step in our inference workflow is to fit the output distributions using probability distributions (Figure 4(i)). We assume that the volume of observational data means the estimated probability distributions are approximate sufficient statistics of the outputs, meaning $p(\boldsymbol{\theta}|\hat{\Phi}) \approx p(\boldsymbol{\theta}|\mathbf{X})$.

Variable	Definition	Dimension
$\mathbf{x}(t)$	ODE solution	\mathbb{R}^k
$\boldsymbol{\theta}$	ODE parameters	\mathbb{R}^p
$\mathbf{f}(\mathbf{x}(t); \boldsymbol{\theta})$	ODE RHS	\mathbb{R}^k
$\mathbf{x}^{\{i\}}(t)$	ODE solution for cell i	\mathbb{R}^k
$q_j = q_j(\mathbf{x}(t_j))$	quantity of interest (QOI) j	\mathbb{R}^1
$\mathbf{q} = (q_1, \dots, q_m)$	m distinct QOIs	\mathbb{R}^m
$q_j^{\{i\}} = q_j(\mathbf{x}^{\{i\}}(t_j))$	QOI j for cell i	\mathbb{R}^1
$\mathbf{y}_j^\top = (q_j^{\{1\}}, \dots, q_j^{\{n_j\}})$	QOI j for cells $1, \dots, n_j$	\mathbb{R}^{n_j}
$\mathbf{X} = (\mathbf{y}_1, \dots, \mathbf{y}_m)$	“snapshot” of all QOI	$\mathbb{R}^{n_1} \times \mathbb{R}^{n_2} \times \dots \times \mathbb{R}^{n_m}$
Φ	output target distribution $p(\mathbf{q} \Phi)$	\mathbb{R}^m
Ξ	prior parameter distribution $p(\boldsymbol{\theta} \Xi)$	\mathbb{R}^p
Ψ	prior output distribution $p(\mathbf{q} \Psi)$	\mathbb{R}^p
\hat{a}	estimates of any quantity a	-
$\Omega(\mathbf{z})$	parameter space mapping to $\mathbf{q} = \mathbf{z}$	\mathbb{R}^p
$\mathcal{V}(\mathbf{z})$	volume of $\Omega(\mathbf{z})$	\mathbb{R}^+
V	volume of parameter space	\mathbb{R}^+

Table 1: Glossary of variable names used in this paper.

3.2 Theoretical development of CMC

We consider the underdetermined case where $m < p$, so that each quantity of interest, $\tilde{\mathbf{q}}$, can be generated from a non-singular set of parameter values, which we term iso-output contour regions: $\Omega(\tilde{\mathbf{q}}) = \{\boldsymbol{\theta} : \mathbf{q}(\boldsymbol{\theta}) = \tilde{\mathbf{q}}\}$. In general, these contours have “volumes”, $\mathcal{V}(\tilde{\mathbf{q}})$, which depend on the chosen output value $\tilde{\mathbf{q}}$ (Figure 3). This means that any algorithm which samples parameter values in order to generate a given output target must account for the differential volumes of these sets, otherwise sampling will be biased

towards iso-output contours with larger volumes [32]. The problem in most applied problems however is that we do not know *a priori* the volumes of iso-output contours and so they must be estimated. The following analysis provides a brief introduction to a probabilistic formulation of underdetermined inverse problems (see our companion paper [32] for a more comprehensive discussion) and, in doing so, suggests a sampling approach for estimating the volumes of parameter space mapping to each output value, which forms the foundation of CMC.

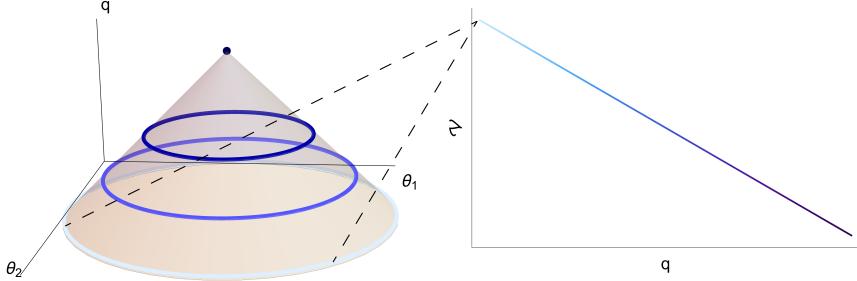


Figure 3: Left: An example output function $q(\theta_1, \theta_2)$ along with iso-output contours indicated (coloured lines) . **Right:** The “volume” of output contours as a function of output value. Note that here, since the input space is two dimensional, the “volume” of each output value corresponds to a length of an iso-output contour.

The aim of solving our inverse problem is to derive the posterior distribution of parameter values, $p(\boldsymbol{\theta}|\hat{\Phi})$, which, when inputted to the forward map, results in the target distribution $p(\mathbf{q}|\hat{\Phi})$. To derive the posterior parameter distribution, we consider the joint density of parameters and outputs $p(\boldsymbol{\theta}, \mathbf{q}|\hat{\Phi})$. This can be decomposed in two ways,

$$p(\boldsymbol{\theta}, \mathbf{q}|\hat{\Phi}) = p(\boldsymbol{\theta}|\mathbf{q}, \hat{\Phi}) \times p(\mathbf{q}|\hat{\Phi}) = p(\mathbf{q}|\boldsymbol{\theta}, \hat{\Phi}) \times p(\boldsymbol{\theta}|\hat{\Phi}). \quad (6)$$

Rearranging to obtain the posterior parameter distribution,

$$p(\boldsymbol{\theta}|\hat{\Phi}) = \frac{p(\boldsymbol{\theta}|\mathbf{q}, \hat{\Phi}) \times p(\mathbf{q}|\hat{\Phi})}{p(\mathbf{q}|\boldsymbol{\theta}, \hat{\Phi})}. \quad (7)$$

Given parameters $\boldsymbol{\theta}$, the mapping from parameters to outputs is deterministic meaning $p(\mathbf{q}|\boldsymbol{\theta}, \hat{\Phi}) = \delta(\mathbf{q}(\boldsymbol{\theta}), \boldsymbol{\theta})$ is the Dirac delta function centred at $\mathbf{q} = \mathbf{q}(\boldsymbol{\theta})$.

In what follows, we assume that the conditional distribution $p(\boldsymbol{\theta}|\mathbf{q}, \hat{\Phi})$ is independent of the data, meaning it represents a conditional “prior”, which can be manipulated by Bayes’ rule,

$$p(\boldsymbol{\theta}|\mathbf{q}(\boldsymbol{\theta})) = \frac{p(\boldsymbol{\theta})}{p(\mathbf{q}(\boldsymbol{\theta}))}, \quad (8)$$

where we have used the Dirac delta function for $p(\mathbf{q}|\boldsymbol{\theta})$. In the same way that a single output value can be caused from many different parameter values, a target output distribution $p(\tilde{\mathbf{q}}|\hat{\Phi})$ does not correspond to a unique parameter distribution. This means that in order to ensure uniqueness of the “posterior” parameter distributions, our probabilistic framework requires that we specify “prior” distributions for the parameters, as in more

traditional Bayesian inference. This results in the form of the posterior parameter distribution targeted by our sampling algorithm, 259
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$$p(\boldsymbol{\theta}|\hat{\Phi}) = \frac{p(\boldsymbol{\theta})}{p(\mathbf{q}(\boldsymbol{\theta}))} p(\mathbf{q}(\boldsymbol{\theta})|\hat{\Phi}). \quad (9)$$

Again, we refer to our companion piece [32] for detailed explanation of eqs. (8) & (9) and instead here provide brief interpretation when considering a uniform prior on parameter space. In this case, $p(\boldsymbol{\theta}) = \frac{1}{V}$, where V is the total volume of parameter space. The denominator term of eq. (8) is the prior induced on output space by the prior over parameter space. For a uniform prior on parameter values, this is 261
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$$p(\boldsymbol{\theta}|\mathbf{q}(\boldsymbol{\theta})) = \frac{1}{\mathcal{V}(\mathbf{q}(\boldsymbol{\theta}))}, \quad (10)$$

where $\mathcal{V}(\mathbf{q}(\boldsymbol{\theta}))$ is the volume of parameter space occupied by the iso-output contour $\Omega(\mathbf{q}(\boldsymbol{\theta}))$. Therefore a uniform prior over parameter space implies a prior structure where all parameter values resulting in the same output $\tilde{\mathbf{q}}$ are given equal weighting. 267
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3.3 Implementation of CMC

Except for some toy examples, the denominator of eq. (8) cannot be calculated, and exact sampling from the posterior parameter distribution of eq. (9) is not, in general, possible. We propose instead a computationally efficient sampling method to estimate $p(\mathbf{q}(\boldsymbol{\theta}))$, which forms the first step of our so-called ‘‘Contour Monte Carlo’’ (CMC) algorithm (Algorithm 1; Figure 4(ii)), where we estimate the volume of iso-output contours with output value $\mathbf{q}(\boldsymbol{\theta})$. This step involves repeated independent sampling from the prior distribution of parameters $\boldsymbol{\theta}^{\{i\}} \sim p(\boldsymbol{\theta}|\Xi)$, where, for completeness, we have conditioned on Ξ parameterising the prior probability density. Each parameter sample is then converted into an output value $\mathbf{q}^{\{i\}} = \mathbf{q}(\boldsymbol{\theta}^{\{i\}})$. The collection of output samples is then fitted using a vine copula kernel density estimator (KDE) [20], $(\mathbf{q}^{\{1\}}, \dots, \mathbf{q}^{\{N_1\}}) \sim p(\mathbf{q}|\hat{\Psi})$. Throughout the course of development of CMC, we have tested many KDE methods and have found vine copula KDE is best suited to approximating the higher dimensional probability distributions required in practice. 271
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The second step in our algorithm then uses Markov chain Monte Carlo (MCMC) to sample from an approximate version of eq. (9) with the estimated density $p(\mathbf{q}(\boldsymbol{\theta})|\hat{\Psi})$ replacing its corresponding estimand (Algorithm 1; Figure 4(iii)),

$$p(\boldsymbol{\theta}|\hat{\Phi}, \Xi, \hat{\Psi}) = \frac{p(\boldsymbol{\theta}|\Xi)}{p(\mathbf{q}(\boldsymbol{\theta})|\hat{\Psi})} p(\mathbf{q}(\boldsymbol{\theta})|\hat{\Phi}). \quad (11)$$

The final step in CMC is to compare output samples generated from the result of the MCMC calculation with the target distribution (Figure 4(iv)). Asymptotically (in terms of the sample size of both sampling steps), CMC produces a sample of parameter values $(\boldsymbol{\theta}^{\{1\}}, \boldsymbol{\theta}^{\{2\}}, \dots)$ which, when mapped to the output space, corresponds to the target distribution $p(\mathbf{q}|\hat{\Psi})$. In developing CMC, we have found that a finite sample of modest size for both steps of CMC results in parameter samples that, when transformed, often represent reasonable approximations of the target. There are however 291
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occasions when this is not the case and we have found this final confirmatory step indispensable since it frequently highlights inadequacies in the contour volume estimation or the MCMC, meaning more samples from either or both of these steps are required. It may also be necessary to tweak hyperparameters of the KDE to ensure reasonable approximation in the contour volume estimation step. If the target distribution is sensitive to the contour volume estimates, this may also indicate that the target snapshot distribution is incompatible with the model: here, we make no claims on existence of a solution to the inverse problem, only that, if one should exist, Contour Monte Carlo is a pragmatic approach to approximate it by sampling. A useful way to diagnose whether the target distribution can be produced from the model and specified priors is to examine the output values from the contour volume estimation step of CMC. If the majority of probability mass of the target lies outside the bounds of the bulk simulated output values obtained by independent sampling from the prior, then the model and/or chosen prior is unlikely to be invertible to this particular target.

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3.4 Workflow and CMC algorithm

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A graphical illustration of the complete CMC workflow is provided in Figure 4. All variables are defined in Table 1.

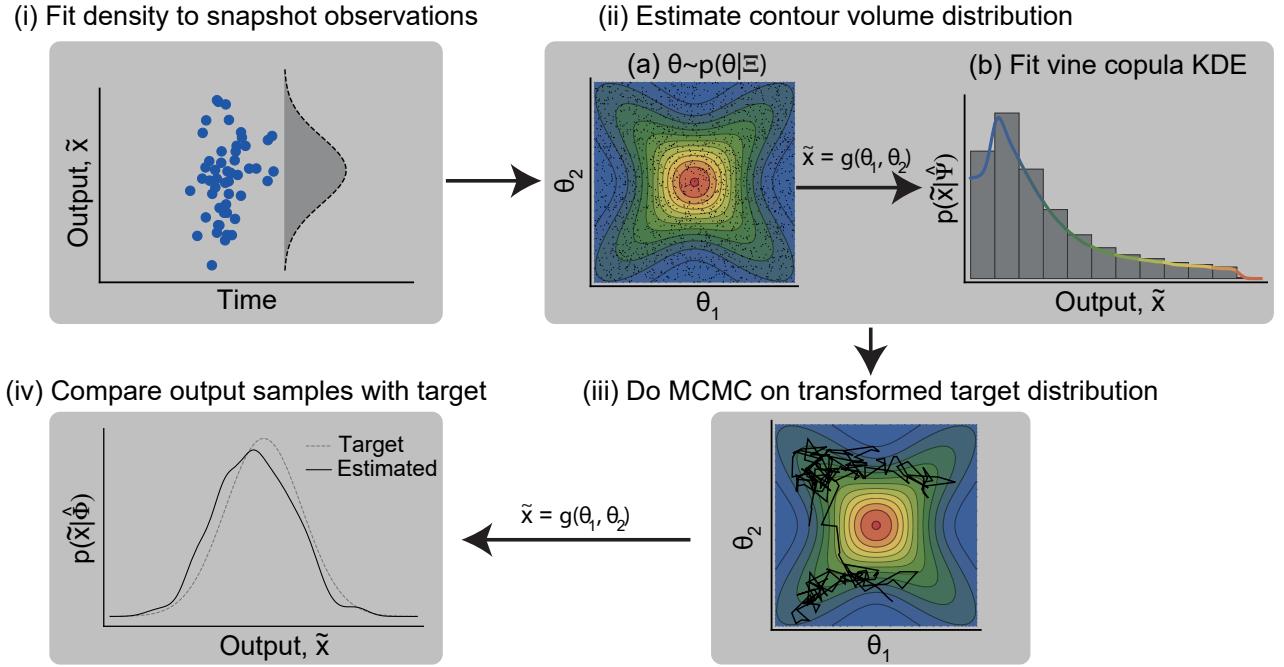


Figure 4: **The workflow for Contour Monte Carlo to estimate cell population heterogeneity.** The distribution targeted in (iii) is given by eq. (11).

The CMC algorithm is provided in Algorithm 1. A definition of all variables is provided in Table 1. For simplicity, in this implementation MCMC sampling is performed via the Random Walk Metropolis algorithm, but for the examples in §4, we use an adaptive MCMC algorithm [21].

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Algorithm 1 Pseudocode for the Contour Monte Carlo algorithm for sampling from the posterior parameter distribution of eq. (11).

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procedure CMC( $\mathbf{X}, \Xi, N_1, N_2$ )       $\triangleright$  Sample from posterior parameter distribution
     $\hat{\Phi} = \text{SNAPSHOTESTIMATOR}(\mathbf{X})$ 
     $\hat{\Psi} = \text{CONTOURVOLUMEESTIMATOR}(\Xi, N_1)$ 
     $(\boldsymbol{\theta}^{\{1\}}, \dots, \boldsymbol{\theta}^{\{N_2\}}) = \text{MCMC}(\hat{\Phi}, \Xi, \hat{\Psi}, N_2)$ 
    converged = COMPAREOUTPUTTOTARGET(( $\boldsymbol{\theta}_1, \dots, \boldsymbol{\theta}_{N_2}$ ),  $\hat{\Phi}$ )
    while converged ≠ 1 do           $\triangleright$  Rerun contour volume estimation and/or MCMC
         $\hat{\Psi} = \text{CONTOURVOLUMEESTIMATOR}(\Xi, N'_1), N'_1 > N_1$ 
         $(\boldsymbol{\theta}^{\{1\}}, \dots, \boldsymbol{\theta}^{\{N'_2\}}) = \text{MCMC}(\hat{\Phi}, \Xi, \hat{\Psi}, N'_2), N'_2 > N_2$ 
        converged = COMPAREOUTPUTTOTARGET(( $\boldsymbol{\theta}_1, \dots, \boldsymbol{\theta}_{N_2}$ ),  $\hat{\Phi}$ )
         $N_1 \leftarrow N'_1, N_2 \leftarrow N'_2$ 
    end while
    return  $(\boldsymbol{\theta}^{\{1\}}, \dots, \boldsymbol{\theta}^{\{N_2\}})$ 
end procedure

procedure SNAPSHOTESTIMATOR( $\mathbf{X}$ )            $\triangleright$  Fit density to snapshot observations
     $\mathbf{X} \sim p(\mathbf{q}|\hat{\Phi})$ 
    return  $\hat{\Phi}$ 
end procedure

procedure CONTOURVOLUMEESTIMATOR( $\Xi, N_1$ )      $\triangleright$  Estimate volume of contours
    for  $i$  in  $1 : N_1$  do
         $\boldsymbol{\theta}^{\{i\}} \sim p(\boldsymbol{\theta}|\Xi)$            $\triangleright$  Sample from prior density
         $\mathbf{q}^{\{i\}} = \mathbf{q}(\boldsymbol{\theta}^{\{i\}})$            $\triangleright$  Calculate corresponding output value
    end for
     $(\mathbf{q}^{\{1\}}, \dots, \mathbf{q}^{\{N_1\}}) \sim p(\mathbf{q}|\hat{\Psi})$        $\triangleright$  Fit vine copula kernel density estimator
    return  $\hat{\Psi}$ 
end procedure

procedure MCMC( $\hat{\Phi}, \Xi, \hat{\Psi}, N_2$ )       $\triangleright$  Random Walk Metropolis algorithm targeting
posterior parameter distribution.
     $\boldsymbol{\theta}^{\{0\}} \sim \pi(\cdot)$            $\triangleright$  Sample from arbitrary initialisation distribution
    for  $i$  in  $1 : N_2$  do
         $\boldsymbol{\theta}^{\{i\}'} \sim \mathcal{N}(\boldsymbol{\theta}^{\{i-1\}}, \Sigma)$        $\triangleright$  Propose new parameter values for parameters
         $r = [p(\boldsymbol{\theta}^i|\Xi) p(\mathbf{q}(\boldsymbol{\theta}^{\{i\}})|\hat{\Psi}) p(\mathbf{q}(\boldsymbol{\theta}^{\{i\}'}|\hat{\Phi})] / [p(\boldsymbol{\theta}^i|\Xi) p(\mathbf{q}(\boldsymbol{\theta}^{\{i\}'}|\hat{\Psi}) p(\mathbf{q}(\boldsymbol{\theta}^i|\hat{\Phi})]$ 
     $\triangleright$  Metropolis acceptance ratio.
         $u \sim U(0, 1)$            $\triangleright$  Sample from uniform distribution
        if  $r > u$  then
             $\boldsymbol{\theta}^{\{i\}} = \boldsymbol{\theta}^{\{i\}'}$            $\triangleright$  Accept proposal
        else
             $\boldsymbol{\theta}^{\{i\}} = \boldsymbol{\theta}^{\{i-1\}}$            $\triangleright$  Reject proposal
        end if
    end for
    return  $(\boldsymbol{\theta}^{\{1\}}, \dots, \boldsymbol{\theta}^{\{N_2\}})$ 
end procedure

procedure COMPAREOUTPUTTOTARGET(( $\boldsymbol{\theta}_1, \dots, \boldsymbol{\theta}_{N_2}$ ),  $\hat{\Phi}$ )  $\triangleright$  Check output distribution
close to target
    for  $i$  in  $1 : N_2$  do
         $\tilde{\mathbf{q}}_i = \mathbf{q}(\boldsymbol{\theta}_i)$            $\triangleright$  Compute output for each parameter sample
    end for
    if  $(\tilde{\mathbf{q}}_1, \dots, \tilde{\mathbf{q}}_{N_2}) \sim p(\tilde{\mathbf{q}}|\hat{\Phi})$ ? then
        return 1           $\triangleright$  Compare outputs with target
    else
        return 0
    end if
end procedure

```

In generating our results in §4, for the contour volume estimation step, we assumed sample sizes were sufficient if the output samples from the MCMC provided a reasonable approximation to the target, although we recognise that future work should refine this process further. For the MCMC step, we use adaptive covariance MCMC (see SOM of [21]) to sample from the target distribution, as we have found that it provides a considerable speed-up over Random Walk Metropolis [22, 23]. We also use the Gelman-Rubin convergence statistic \hat{R} which provides a heuristic measurement of convergence [23, 24], and use a threshold of $\hat{R} \leq \sim 1.1$ to diagnose convergence.

To solve the forward model of each differential equation, we used Julia’s inbuilt “solve” method for ODE models, which automatically chooses an efficient default inbuilt solver [25].

4 Results

In this section, we use CMC to estimate the posterior parameter distribution for three biological systems. In all but one of the examples, we assume that the first step of CMC (“SnapshotEstimator” within Algorithm 1) has already been undertaken and we are faced with inferring a parameter distribution which, when mapped to outputs, recapitulates the target density. To accompany the text, we provide the Julia notebook used to generate the results. A table of priors used for each example is provided in Table 3.

4.1 Growth factor model

We first consider the “growth factor model” introduced by [12], which concerns the dynamics of inactive ligand-free cell surface receptors R and active ligand-bound cell surface receptors P , modulated by an exogenous ligand L . The governing dynamics are determined by the following system,

$$\frac{dR}{dt} = R_T k_{deg} + k_1 L R(t) + k_{-1} P(t) - k_{deg} R(t) \quad (12)$$

$$\frac{dP}{dt} = k_1 L R(t) - k_{-1} P(t) - k_{deg}^* P(t), \quad (13)$$

with initial conditions,

$$R(0) = 0.0, \quad P(0) = 0.0,$$

where $\boldsymbol{\theta} = (R_T, k_1, k_{-1}, k_{deg}, k_{deg}^*)$ are parameters to be determined. In this example, we use measurements of the active ligand-bound receptors P to estimate cellular heterogeneity in processes. We denote the solution of eq. (13) as $P(t; \boldsymbol{\theta}, L)$ and seek to determine the parameter distribution consistent with an output distribution,

$$\mathbf{q} = \begin{pmatrix} q_1 \\ q_2 \end{pmatrix} = \begin{pmatrix} P(10; \boldsymbol{\theta}, 2) \\ P(10; \boldsymbol{\theta}, 10) \end{pmatrix} \sim \mathcal{N} \left[\begin{pmatrix} 2 \times 10^4 \\ 3 \times 10^4 \end{pmatrix}, \begin{pmatrix} 1 \times 10^5 & 0 \\ 0 & 1 \times 10^5 \end{pmatrix} \right]. \quad (14)$$

4.1.1 Uniform prior

To start, we specify a uniform prior for each of the five parameters, with bounds given in Table 3. To estimate the posterior parameter distribution, we use CMC, with adaptive covariance MCMC [21] for the second step.

In Figure 5A, we show the sampled outputs (blue points) versus the contours of the target distribution (black solid closed curves), illustrating a good correspondence between the sampled and target densities. Above and to the right of the main panel, we also display the marginal target densities (solid black lines) versus kernel density estimator reconstructions of the output marginals from the CMC samples (dashed blue lines), which again highlights the fidelity of the CMC sampled density to the target.

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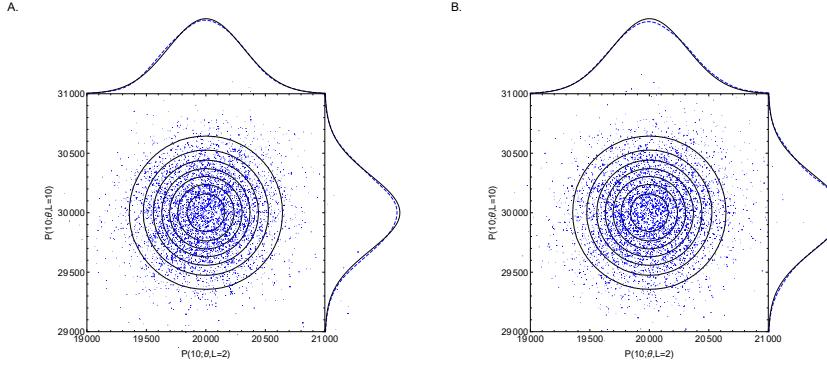


Figure 5: The target joint output distribution (solid contour lines) and target marginal distributions (solid lines; above and to side of figure) versus outputs sampled by CMC (blue points) and reconstructed marginals (dashed lines) for (A) uniform and (B) normal parameter priors. In CMC, 100,000 independent samples were used in the “ContourVolumeEstimator” step and 10,000 MCMC samples across each of 4 Markov chains were used in the second step, with the first half of the chains discarded as “warm-up” [23]. For the reconstructed marginal densities in the plots, we use Mathematica’s “SmoothKernelDistribution” function specifying bandwidths of 100 with Gaussian kernels [26].

In Figure 6A, we plot the joint posterior parameter distribution for k_1 , the rate of ligand binding to inactive receptors, and k_{-1} , which dictates the rate of the reverse reaction, where the ligands unbind. The output measurements we used to fit the model correspond to levels of the bound ligands, which can be generated whenever the ratio of k_1 to k_{-1} is approximately given by the corresponding steady state ratio. Because of this, the distribution representing cell process heterogeneity contains linear positive correlations between these parameters. In Figure 6B, we show the posterior parameter distribution for k_{deg} , the rate of degradation of ligand-free cell surface receptors and R_T , which dictates the rate of introduction of ligand-free cell surface receptors, which shows a concentrated region of posterior probability mass. Why can we better resolve (k_{deg}, R_T) compared to (k_1, k_{-1}) from our measurements? To answer this, it is useful to calculate the sensitivity of $P(t; \theta, L)$ to changes in each of the parameters. To account for the differing magnitudes of each parameter, we calculate elasticities, the proportional changes in measured output for a proportional change in parameter values, using the forward sensitivities method described in [27], which are shown in Figure 7. When the exogenous ligand is set $L = 2$, these indicate the active ligand-bound receptor concentration is most elastic

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to changes in R_T and k_{deg} , meaning that their range is more restricted by the output measurement than for k_1 and k_{-1} , which have elasticities at $t = 10$ closer to 0. In Table 2, we show the posterior quantiles for the estimated parameters and, in the last column, indicate the ratio of the 25%-75% posterior interval widths to the uniform prior range for each parameter. These were strongly negatively correlated with the magnitude of the elasticities for each parameter ($\rho = 0.95$, $t = -5.22$, $df = 3$, $p = 0.01$ Pearson's product-moment correlation), indicating the utility of sensitivity analyses for optimal experimental design. We would suggest however that CMC can also be used for this purpose, using synthetic data in place of real measurements.

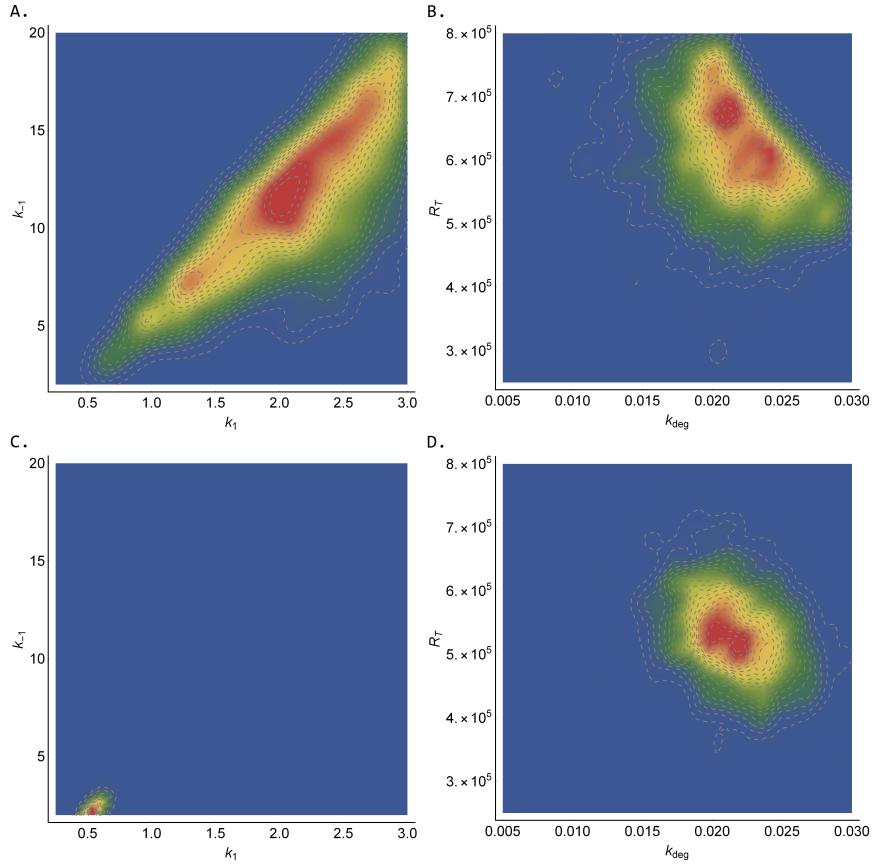


Figure 6: The joint posterior distributions of (k_1, k_{-1}) (left-column) and (k_{deg}, R_T) for the growth factor model estimated by CMC sampling using uniform priors (top row) and normal priors (bottom row). See Figure 5 caption for CMC details and Table 3 for the priors used. Red (blue) indicates areas of relatively high (low) probability mass.

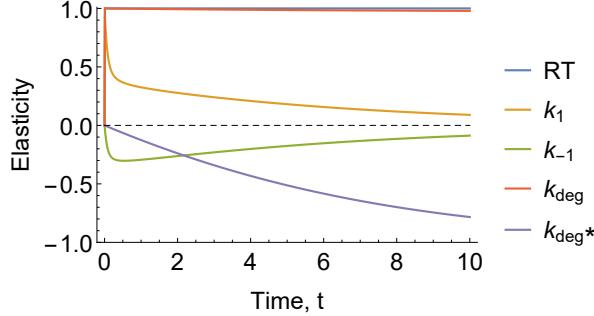


Figure 7: **The elasticities of the measured concentration of active ligand-bound receptors P versus time when $L = 2$.** When calculating the elasticities of each parameter, the other parameters were set to their posterior medians given in Table 2.

4.1.2 Normal prior

For an unidentified model, there is typically a multitude of possible probability distributions over parameter values which map to the same target output distribution. To reduce the space of posterior parameter distributions to one, it is therefore necessary to specify a prior parameter distribution. It is also preferable to allow priors to influence estimates in studies of cellular heterogeneity, since this allows incorporation of pre-existing biological knowledge with compensatory reductions in estimator variance. CMC accommodates different prior choices, with both the “ContourVolumeEstimation” step and the acceptance ratio in the “MCMC” step (Algorithm 1) being affected in such a way that posterior parameter distribution maps to the same output target. We now use CMC to estimate the posterior parameter distribution when changing from uniform priors to more concentrated normal priors (prior hyperparameters shown in Table 3). As desired, the target output distribution appears invariant (Figure 5B) although with substantial changes in the posterior parameter distributions (Figure 6C&D). In particular, the posterior distributions obtained from shifting to the normal prior are more concentrated in parameter space compared to the uniform case (rightmost column of Table 2). The differences in posterior distribution resultant from changes to priors are likely to be more marked the less definitive a guide the data provides on the underlying process and, hence, can be used to stabilise the resultant inferences according to external knowledge about the system.

4.2 Michaelis-Menten kinetics

In this section, we use CMC to invert output measurements from the Michaelis-Menten model of enzyme kinetics (see, for example, [28]); illustrating the capability of CMC to resolve population substructure from multimodality of the output distribution. The Michaelis-Menten model of enzyme kinetics describes the dynamics of concentrations of an enzyme (E), a substrate (S), an enzyme-substrate complex (ES), and a product (P).

Parameter	Quantiles					Posterior 25%-75% conc.
	2.5%	25%	50%	75%	97.5%	
Uniform prior						
R_T	441,006	548,275	606,439	677,055	772,484	23%
k_1	0.90	1.69	2.17	2.56	2.95	32%
k_{-1}	4.35	8.35	11.23	14.23	18.71	33%
k_{deg}	0.013	0.019	0.021	0.024	0.029	20%
k_{deg}^*	0.20	0.34	0.40	0.44	0.49	27%
Normal prior						
R_T	408,396	487,372	529,558	577,970	678,632	16%
k_1	0.39	0.49	0.54	0.60	0.70	4%
k_{-1}	1.39	1.92	2.26	2.63	3.35	4%
k_{deg}	0.016	0.020	0.022	0.024	0.027	16%
k_{deg}^*	0.22	0.29	0.33	0.38	0.46	21%

Table 2: **Estimated quantiles from CMC samples for the growth factor model with uniform and normal priors.** The last column indicates the proportion of the uniform prior bounds occupied by the 25%-75% posterior interval in each case. The particular priors used in each case are given in Table 3.

Specifically,

$$\begin{aligned}\frac{dE}{dt} &= -k_f E(t)S(t) + k_r C(t) + k_{cat}C(t), \\ \frac{dS}{dt} &= -k_f E(t)S(t) + k_r C(t), \\ \frac{dC}{dt} &= k_f E(t)S(t) - k_r C(t) - k_{cat}C(t), \\ \frac{dP}{dt} &= k_{cat}C(t),\end{aligned}\tag{15}$$

with initial conditions,

$$E(0) = E_0, S(0) = S_0, C(0) = C_0, P(0) = P_0,\tag{16}$$

where k_f is the rate constant for the forward reaction $E + S \rightarrow C$, k_r is the rate of the reverse reaction $C \rightarrow E + S$, and k_{cat} is the catalytic rate at which the product is formed by the reaction $C \rightarrow E + P$.

4.2.1 Bimodal output distribution

When subpopulations of cells, each with distinct dynamics, are thought to exist, determining their characteristics - proportions of overall cell number, likely parameter values, and so on - is often of key interest [15, 19]. Before formal inference occurs, multi-modality of the output distribution may signal the existence of fragmented subpopulations of cells. Here we target the following bimodal bivariate normal distribution,

$$\mathbf{q} = \begin{pmatrix} q_1 \\ q_2 \end{pmatrix} = \begin{pmatrix} E(2; \boldsymbol{\theta}) \\ S(1; \boldsymbol{\theta}) \end{pmatrix} \sim p(\mathbf{q}; \boldsymbol{\mu}_1, \Sigma_1, \boldsymbol{\mu}_2, \Sigma_2)\tag{17}$$

$$= \frac{1}{2} (\mathcal{N}(\mathbf{q}; \boldsymbol{\mu}_1, \Sigma_1) + \mathcal{N}(\mathbf{q}; \boldsymbol{\mu}_2, \Sigma_2)),\tag{18}$$

where $\boldsymbol{\theta} = (k_f, k_r, k_{cat})$. The parameters of the mixture normal output distribution we target are

$$\boldsymbol{\mu}_1 = [2.2, 1.6]', \quad \Sigma_1 = \begin{pmatrix} 0.018 & -0.013 \\ -0.013 & 0.010 \end{pmatrix},$$

$$\boldsymbol{\mu}_2 = [2.8, 1.0]', \quad \Sigma_2 = \begin{pmatrix} 0.020 & -0.010 \\ -0.010 & 0.020 \end{pmatrix}.$$

In what follows, we specify uniform priors on each of the elements of $\boldsymbol{\theta}$ (see Table 3).

Using a modest number of samples in each step, CMC was able to recapitulate the output target distribution (Figure 8A). Without specifying *a priori* information on the subpopulations of cells, two distinct clusters of cells emerged from application of CMC (orange and blue points in Figure 8B), each corresponding to distinct modes of the output distribution (corresponding coloured points in Figure 8A). It is worth noting however that the issues inherent with MCMC sampling of multimodal distributions similarly apply here and so, whilst here adaptive MCMC [21] sufficed to explore the posterior surface, it may be necessary to use MCMC methods known to be robust to such geometries (for example, population MCMC [29]).

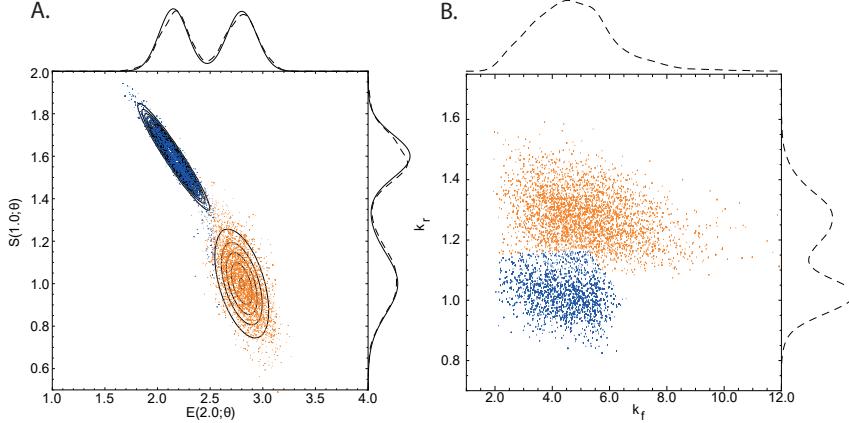


Figure 8: Michaelis-Menten model. (A) Bimodal target distribution q (solid contour lines) versus output samples (points). (B) Posterior parameter samples (points). The solid and dashed lines above and to the side of panel A indicate the target and estimated marginal output distributions, respectively. The orange (blue) points in A were generated by the orange (blue) parameter samples in B. See Figure 5 caption for CMC details. Mathematica’s “SmoothKernelDistribution” function [26] with Gaussian kernels was used to construct marginal densities with: (A) default bandwidths, and (B) bandwidths of 0.3 (horizontal axis) and 0.03 (vertical axis). Mathematica’s “ClusteringComponents” function [26] was used to identify clusters in B.

4.2.2 Four-dimensional output distribution

Loos et al. (2018) consider a multidimensional output distribution, with correlations between system characteristics that evolve over time. Our approach allows arbitrary covariance structure between measurements, and

to exemplify this, we now target a four-dimensional output distribution,
 with paired measurements of enzyme and substrate at $t = 1$ and $t = 2$,

$$\begin{aligned} \mathbf{q} &= \begin{pmatrix} q_1 \\ q_2 \\ q_3 \\ q_4 \end{pmatrix} = \begin{pmatrix} E(1.0; \boldsymbol{\theta}) \\ S(1.0; \boldsymbol{\theta}) \\ E(2.0; \boldsymbol{\theta}) \\ S(2.0; \boldsymbol{\theta}) \end{pmatrix} \\ &\sim \mathcal{N} \left[\begin{pmatrix} 0.5 \\ 2.8 \\ 0.9 \\ 1.4 \end{pmatrix}, \begin{pmatrix} 0.02 & -0.05 & 0.04 & -0.05 \\ -0.05 & 0.30 & -0.15 & 0.20 \\ 0.04 & -0.15 & 0.12 & -0.17 \\ -0.05 & 0.20 & -0.17 & 0.30 \end{pmatrix} \right]. \end{aligned} \quad (19)$$

Since this system has four output measurements, and the Michaelis-Menten model has three rate parameters (k_f, k_r, k_{cat}), the system is over-identified and so CMC cannot be straightforwardly applied. Instead, we allow the four initial states (E_0, S_0, ES_0, P_0) to be uncertain quantities, bringing the total number of parameters to 7, and ensuring that the system is in the unidentified regime where CMC applies. We set uniform priors on all parameters (see Table 3) and to check that the model and priors were consistent with the output distribution given by eq. (19), we plotted the output measurements used to estimate contour volumes (in the first step of the “ContourVolumeEstimator” method in Algorithm 1) against the target (Figure 9). Since the main support of the densities (black contours) lies within a region of output space reached by independent sampling of the priors (blue points), this indicated that the distribution given by eq. (19) could feasibly be generated from this model and priors, and we proceeded to estimation by CMC.

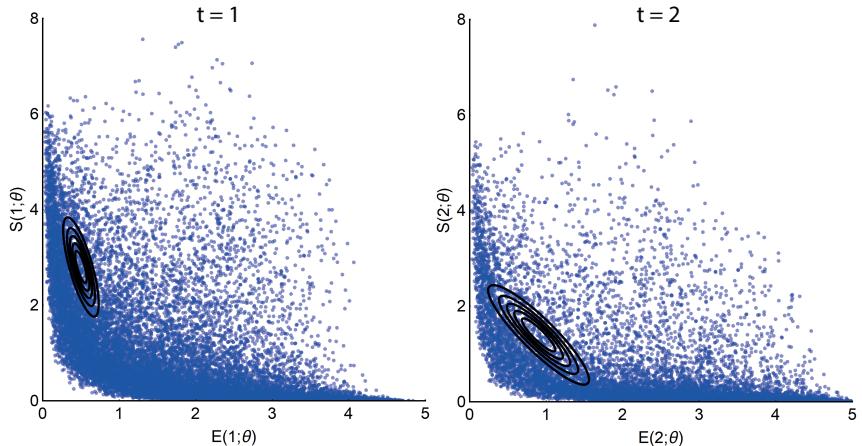


Figure 9: **Output functionals (blue points) (q_1, q_2) (left panel) and (q_3, q_4) (right panel)** obtained by independently sampling the priors $p(\boldsymbol{\theta}|\Xi)$ of the 7 parameter Michaelis-Menten model versus the target distribution (black solid contours). We show 20,000 output samples, where each set of four measurements was obtained from a single sample of the 7 parameters. The output target distribution shown by the contours corresponds to the marginal densities of each pair of enzyme-substrate measurements given by eq. (19).

Figure 10 plots the output samples of enzyme and substrate from

the last step of CMC for $t = 1$ (blue points) and $t = 2$ (orange points) versus the contours (black lines) of the joint marginal distributions of eq. (19). The distribution of paired enzyme-substrate samples illustrates that the CMC output samples approximated the target density, itself representing dynamic evolution of the covariance between enzyme and substrate measurements. The target marginal distributions (solid lines) along with their approximations from kernel density estimation (dashed lines) are also shown above and beside the main panel of Figure 10, and largely indicate correspondence.

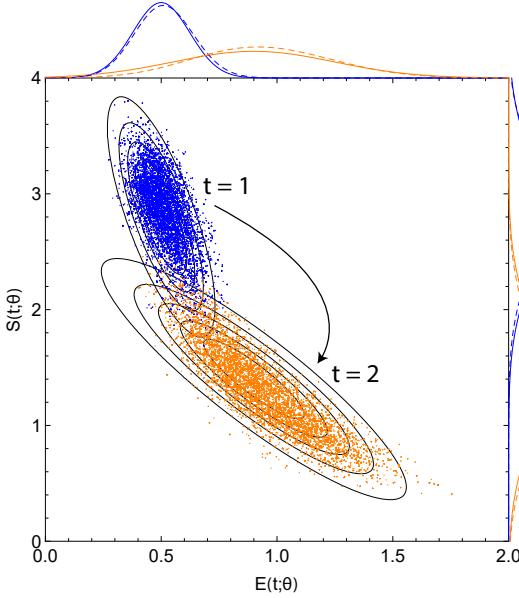


Figure 10: **Posterior output samples from CMC (coloured points) versus the contour plots of the joint marginal distributions of eq. (19) (black solid lines).** Output functionals for (q_1, q_2) and (q_3, q_4) are given by blue and orange points, respectively. Enzyme and substrate measurements are given by the horizontal and vertical axes, respectively. The solid and dashed coloured lines outside of the panels indicate the true target marginals of eq. (19) and those estimated from CMC, respectively. In the “ContourVolumeEstimator” step 200,000 independent samples were used and 10,000 samples across each of 4 Markov chains were used in the MCMC step, with the first half of the chains discarded as “warm-up” [23]. Mathematica’s “SmoothKernelDistribution” function with Gaussian kernels [26] of bandwidths varying from 0.1 to 0.4 for the reconstructed marginal densities.

4.3 TNF signalling pathway

We now illustrate how CMC can be applied to an ODE system of larger size, the tumour necrosis factor (TNF) signalling pathway model introduced in [30] and used by [15] to illustrate a Bayesian approach to cell population variability estimation. The model incorporates known activating and inhibitory interactions between four key species within the TNF pathway: active caspase 8 (x_1) and active caspase 3 (x_2), a nuclear transcription

factor (x_3) and its inhibitor (x_4),

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$$\begin{aligned}\frac{dx_1}{dt} &= -x_1(t) + \frac{1}{2} (\beta_4(x_3(t))\alpha_1(u(t)) + \alpha_3(x_2(t))) \\ \frac{dx_2}{dt} &= -x_2(t) + \alpha_2(x_1(t))\beta_3(x_3(t)) \\ \frac{dx_3}{dt} &= -x_3(t) + \beta_2(x_2(t))\beta_5(x_4(t)) \\ \frac{dx_4}{dt} &= -x_4(t) + \frac{1}{2} (\beta_1(u(t)) + \alpha_4(x_3(t))),\end{aligned}\tag{20}$$

with initial conditions,

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$$x_1(0) = 0.0, \quad x_2(0) = 0.0, \quad x_3(0) = 0.29, \quad x_4(0) = 0.625,\tag{21}$$

which correspond to the steady state of the system when $x_2 = 0$. The functions α_i and β_j represent activating and inhibitory interactions respectively,

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$$\begin{aligned}\alpha_i(z) &= \frac{z^2}{a_i^2 + z^2}, \quad i = 1, \dots, 4, \\ \beta_j(z) &= \frac{b_j^2}{b_j^2 + z^2}, \quad j = 1, \dots, 5,\end{aligned}\tag{22}$$

and the parameters a_i for $i \in (1, 2, 3, 4)$ and b_j for $j \in (1, 2, 3, 4, 5)$ represent activation and inhibition thresholds. The function $u(t)$ represents a TNF stimulus represented by a top hat function,

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$$u(t) = \begin{cases} 1, & \text{if } t \in [0, 2], \\ 0, & \text{otherwise.} \end{cases}\tag{23}$$

When there are fewer output measurements than parameters, models tend to be underdetermined meaning that many combinations of parameters can lead to the same combination of output values. A consequence of this unidentifiability is that we cannot perform “full circle” inference: that is, using a known parameter distribution to generate an output distribution does not result in that parameter distribution being recapitulated through inference. We illustrate this idea by generating an output distribution by varying a single parameter value between runs of the forward model corresponding to the solution of eq. (20) and performing inference on all nine system parameters, whilst collecting only two output measurements. Specifically, we vary $a_1 \sim \mathcal{N}(0.6, 0.05)$, whilst holding the other parameters constant,

$$(a_2, a_3, a_4, b_1, b_2, b_3, b_4, b_5) = (0.2, 0.2, 0.5, 0.4, 0.7, 0.3, 0.5, 0.4)$$

and measure $q_1 = x_1(2.0)$ and $q_2 = x_2(1.0)$ for each forward model simulation. In doing so, we obtain an output distribution well-approximated by the bivariate normal distribution,

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$$\begin{aligned}\mathbf{q} &= \begin{pmatrix} q_1 \\ q_2 \end{pmatrix} = \begin{pmatrix} x_1(2.0) \\ x_2(1.0) \end{pmatrix} \\ &\sim \mathcal{N} \left[\begin{pmatrix} 0.26 \\ 0.07 \end{pmatrix}, \begin{pmatrix} 2.1 \times 10^{-4} & 5.9 \times 10^{-5} \\ 5.9 \times 10^{-5} & 1.8 \times 10^{-5} \end{pmatrix} \right].\end{aligned}\tag{24}$$

We now apply CMC to the target output distribution given by eq. (24) to estimate a posterior distribution over all nine parameters of eq. (20).

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Apart than for a few cases, the priors for each parameter were chosen to *exclude* the values that were used to generate the output distribution (see Table 3), to illustrate the non-equivalence between the recovered posterior distribution and the data generating process. In Figure 11A, we plot the actual parameter values (horizontal axis) used in the true data generating process versus the inferred values (vertical axis). This illustrates that apart from a_1 , where the estimated parameter values correspond well with the range of values used to generate the data, due to the choice of priors there is a disjunction between the actual and estimated values. Despite these differences, due to the model being underdetermined, it is nonetheless possible to use CMC to sample from an output distribution that well approximates the target (Figure 11B).

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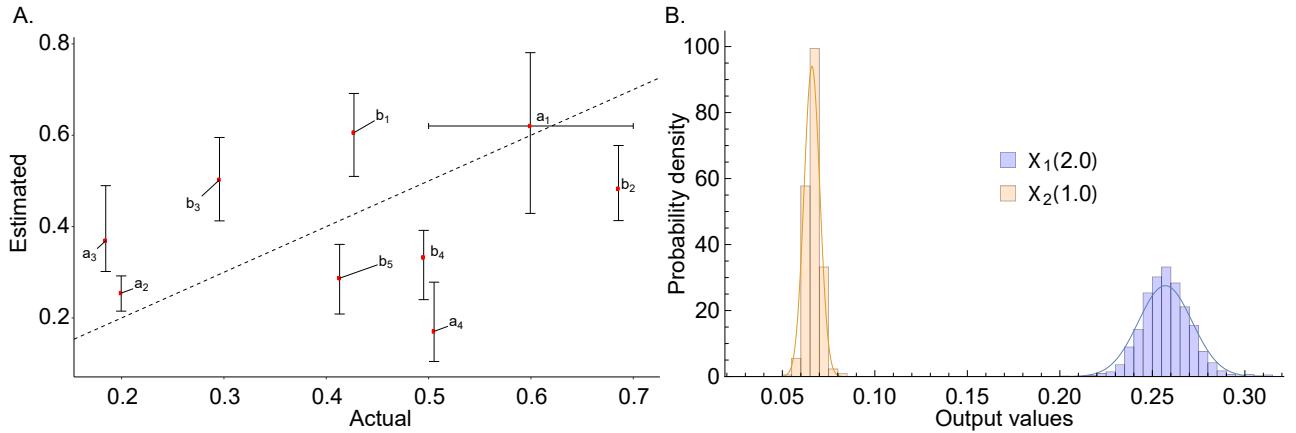


Figure 11: (A) actual parameter values versus estimated quantiles for the output distribution for the TNF signalling pathway model with output distribution given and (B) the marginal output target (solid lines) given by eq. (24) and sampled output distribution (histograms). In A, in the vertical direction, red points indicate 50% posterior quantiles and upper and lower whiskers indicate 97.5% and 2.5% quantiles, respectively; in the horizontal direction, with the exception of a_1 , red points indicate the parameter values used to generate the data; for a_1 the red point indicates the mean of the normal distribution used to generate the data and the whiskers indicate its 95% quantiles. In CMC, 10,000 independent samples were used in the ‘ContourVolumeEstimator’ step and 5,000 MCMC samples across each of 4 Markov chains were used in the second step, with the first half of the chains discarded as ‘warm-up’ [23].

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Cell populations may be well described by subpopulations which each evolve along characteristic trajectories over time. We now apply CMC to investigate a bimodal output distribution for the TNF signalling pathway model similar to that investigated by [15]. In particular, we aim to find a distribution over parameter values which, when used as inputs to the solution to the ODE system, results in the following output distribution,

$$\mathbf{q} = \begin{pmatrix} q_1 \\ q_2 \\ q_3 \end{pmatrix}, \quad (25)$$

where,

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$$\begin{aligned} q_1 &= \mathbf{x}_2(1.0) \sim \mathcal{N}(0.06, 0.01) \\ q_2 &= \mathbf{x}_2(2.0) \sim \frac{1}{2} (\mathcal{N}(0.1, 0.01) + \mathcal{N}(0.14, 0.01)) \\ q_3 &= \mathbf{x}_2(4.0) \sim \frac{1}{2} (\mathcal{N}(0.1, 0.01) + \mathcal{N}(0.20, 0.01)), \end{aligned} \quad (26)$$

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where the target distributions for $q_2(2.0)$ and $q_2(4.0)$ indicate mixtures of univariate normals, and the priors used are described in Table 3. This target distribution, along with the unique trajectories obtained by applying the CMC algorithm for 5,000 MCMC steps, are shown in Figure 12. This figure illustrates that given by bimodality of the output distribution, CMC estimates a corresponding subpopulation structure in the parameter distribution without *a priori* specification of the number of clusters.

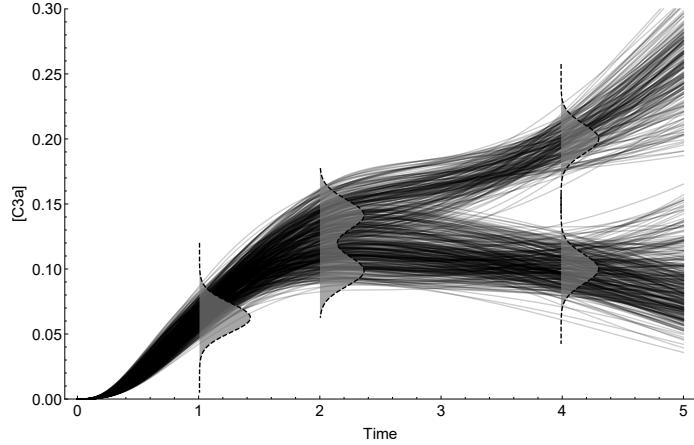


Figure 12: **The target output distribution (dashed plots with grey filling) and unique trajectories (black solid lines) obtained from the posterior parameter distribution.** In CMC, 10,000 independent samples were used in the “ContourVolumeEstimator” step and 5,000 MCMC samples across each of 4 Markov chains were used in the second step, with the first half of the chains discarded as “warm-up” [23].

Model	Target density	Parameter	Prior density	Prior p_1	Prior p_2
Growth factor	2D normal	R_T	uniform	2.5×10^5	8×10^5
		k_1	uniform	0.25	3.0
		k_{-1}	uniform	2.0	20.0
		k_{deg}	uniform	0.005	0.03
		k_{deg}^*	uniform	0.1	0.5
Growth factor	2D normal	R_T	normal	5×10^5	1×10^5
		k_1	normal	0.5	0.1
		k_{-1}	normal	3.0	1.0
		k_{deg}	normal	0.02	0.005
		k_{deg}^*	normal	0.3	0.1
Michaelis-Menten	bimodal normal	k_f	uniform	0.2	15
		k_r	uniform	0.2	2.0
		k_{cat}	uniform	0.5	3.0
Michaelis-Menten	4D normal	k_f	uniform	0.2	15
		k_r	uniform	0.2	2.0
		k_{cat}	uniform	0.2	3.0
		E_0	uniform	3.0	5.0
		S_0	uniform	5.0	10.0
		C_0	uniform	0.0	0.2
TNF signalling	bivariate normal	P_0	uniform	0.0	0.2
		a_1	uniform	0.4	0.8
		a_2	uniform	0.1	0.7
		a_3	uniform	0.3	0.7
		a_4	uniform	0.1	0.3
		b_1	uniform	0.5	0.7
		b_2	uniform	0.4	0.6
		b_3	uniform	0.4	0.6
		b_4	uniform	0.2	0.4
		b_5	uniform	0.2	0.4
TNF signalling	bimodal normal	a_1	uniform	0.5	0.7
		a_2	uniform	0.1	0.3
		a_3	uniform	0.1	0.3
		a_4	uniform	0.4	0.6
		b_1	uniform	0.3	0.5
		b_2	uniform	0.6	0.8
		b_3	uniform	0.2	0.4
		b_4	uniform	0.4	0.6
		b_5	uniform	0.3	0.5

Table 3: **The priors used for each problem in §4.** The parameters p_1 and p_2 indicate the prior hyperparameters: for uniform priors, these correspond to the lower and upper limits; for normal priors, they correspond to the mean and standard deviation.

5 Discussion

Determining the cause of variability in cellular processes is crucial in many applications, ranging from bioengineering to drug development. In this

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paper, we introduce a Bayesian method for estimating cellular heterogeneity from “snapshot” measurements of cellular properties, taken at discrete intervals throughout the experimental course. Our approach assumes what we term a “heterogeneous ordinary differential equation” (HODE) framework, in which biochemical processes in individual cells are assumed to follow dynamics governed by a common ODE, although with idiosyncratic differences in parameter values. In this framework, estimating heterogeneity in cellular processes amounts to determining the probability distributions over parameter values of the governing ODE. Our method of estimation is a two-step Monte Carlo sampling process we term “Contour Monte Carlo” (CMC) which does not require *a priori* specification of cell population substructure unlike other approaches. CMC can be used to process high volumes of individual cellular measurements since the framework involves fitting a kernel density estimator to raw experimental data and using these distributions rather than data as the target outcome. CMC also allows for arbitrary multivariate structure in the measurement space, meaning it can capture correlations that occur between the same cellular species at different timepoints or, for example, contemporaneous correlations between different cellular compartments. Being a Bayesian approach, CMC uses prior distributions over parameter values to ensure uniqueness of the posterior distribution, allowing pre-experimental knowledge to be used to improve estimation robustness. The flexible and robust framework that CMC provides means it can be used to do automatic inference for wide-ranging systems of practical interest.

As well as providing a framework for estimating cellular variation, our approach also provides a natural way to test that it is working as desired. By feeding the posterior parameter samples obtained by CMC into forward model simulations, this results in a distribution over output values that can be compared to the target. Indeed, we have found this comparison indispensable in applying CMC in practice and include it as the last step in the CMC algorithm (Algorithm 1). Discrepancies between the target output distribution and samples from it by CMC can occur either as a result of poor estimates of the “contour volume distribution” in the first stage of the algorithm or due to insufficient MCMC samples in the second. Either of these issues can often be easily addressed and although kernel density estimation in high dimensional spaces remains an open research problem, we have found vine copula kernel density estimation works well for the dimensionality of output measurements we investigate here [20].

Failure to reproduce a given output distribution can also indicate that the generating model (the priors and the forward model) are incongruent with experimental results. This may either be due to misspecification of the ODE system or the inadequacy of the assumed deterministic framework. Our approach currently assumes that output stochasticity is dominated by cellular variation in the parameter values of the underlying ODE, with measurement noise making a minor contribution. Whether this is a reasonable assumption depends on the system under investigation and, more importantly, on experimental details. We recognise that neglecting measurement noise when it is an important determinant of the observed data is likely to mean we overstate the degree of cellular variation. It may also mean that some output distributions cannot be obtained through our assumed model system. Future work allowing inclusion of a stochastic noise process or, more generally, including stochastic cellular mechanisms is thus likely to be worthwhile.

Whilst we have labelled the approach we follow here as Bayesian, since it involves explicit estimation of probability distributions and involves priors over parameter values, we recognise that it is not in the form typically utilised by exponents of this framework. This is because rather than aiming to formulate a model that describes output observations, instead, our approach aims to recapitulate output distributions. Others [31], (including us [32]), have considered this problem before; perhaps most notably by Albert Tarantola in his landmark work on inverse problem theory (see, for example, [33]). In Tarantola's framework, a joint input parameter & output space is considered, where prior knowledge and experimental theory combine elegantly to produce a posterior distribution whose marginal output distribution matches the experimentally obtained one. This work has seen considerable interest in areas such as the geosciences [34,35], and we propose that these methods may prove useful for the biosciences. In particular, we posit that this approach provides a single framework for generalised Bayesian inference that encompasses either output data or output distributions as outcome measures.

The natural world is rife with variation. Mathematical models represent frameworks for understanding the causes of such variation. Typically, the state of biological knowledge is such that one effect, a given pattern of variation, has many possible causes, and observational or experimental data are necessary to apportion weight to each of them, in a process which amounts to solving inverse an problem. The approach we describe here follows the Bayesian paradigm of inverse problem solving whereby uncertainty in potential causes is reflected by probability distributions. Here, we illustrate the utility of our method by applying it to estimate cellular heterogeneity in biochemical processes however, it could equally well be used to understand the inversion of deterministic systems more generally. Whilst describing the inversion process of deterministic models using probability distributions may sound contradictory, it is worth acknowledging that many ODE systems are structurally unidentified meaning there is irreducible uncertainty over some regions of parameter space. Contour Monte Carlo provides an automatic framework for performing inference on these deterministic systems and the use of priors allows for robust and precise parameter estimation unattainable through the data alone.

6 Author contributions

BL, DJG and SJT conceived the study. BL carried out the analysis. All authors helped to write and edit the manuscript.

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