Accounting for randomness in measurement and sampling in studying cancer cell population dynamics

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Abstract: Knowing the expected temporal evolution of the proportion of different cell types in sample tissues gives an indication about the progression of the disease and its possible response to drugs. Such systems have been modelled using Markov processes. We here consider an experimentally realistic scenario in which transition probabilities are estimated from noisy cell population size measurements. Using aggregated data of FACS measurements, we develop MMSE and ML estimators and formulate two problems to find the minimum number of required samples and measurements to guarantee the accuracy of predicted population sizes. Our numerical results show that the convergence mechanism of transition probabilities and steady states differ widely from the real values if one uses the standard deterministic approach for noisy measurements. This provides support for our argument that for the analysis of FACS data one should consider the observed state as a random variable. The second problem we address is about the consequences of estimating the probability of a cell being in a particular state from measurements of small population of cells. We show how the uncertainty arising from small sample sizes can be captured by a distribution for the state probability.

1 Introduction

The development of cancer or cancerous tumours is studied by observing the proportions of different cell types in tissue samples, changing over time as a consequence of cell proliferation. The notion of cancer stem cells (CSCs) exists for over a century [1–5], but only appeared at the front of cancer research with the identification of molecular markers that allowed the isolation of leukaemic CSCs [1, 6]. Recent reviews have discussed important topics in CSC biology, such as the tumour cell of origin [7], therapy resistance [8] and the role of the immune system [1, 9]. A central characteristic of CSCs is their potential for self-renewal and multi-lineage differentiation [1, 2, 8]. For example, using the CSC model, minimal residual disease and tumour recurrence after treatment would result from a remaining, therapy-resistant CSC fraction, whereas metastatic potential would be a CSC-specific property [1, 3, 5]. The hypothesis of CSCs leading to tumours is conceptually attractive but requires further research to be confirmed. The CSCs form a (very) small proportion of the tumour and the theory suggests that conventional chemotherapies kill differentiated or differentiating cells, which however form the bulk of the tumour, but are unable to generate new cells. To develop optimal therapies it is thus essential to know and predict the temporal evolution of proportions of different cell types in tissue samples.

Modelling proliferation in populations of cells is challenging because the intrinsic biological randomness [10] is combined with extrinsic randomness arising from experimental measurements using markers to detect cell states [11]. Although many processes in biology exhibit the Markov property remarkably well [12], if the number of measurements (samples) is limited and the measurements of the counts of cells are noisy, the estimation of transition probabilities for a Markov process is challenging. For populations of cells, the Markov property implies that an initial inhomogeneous population of cells converges to a steady state or oscillatory configuration. We show that conventional Markov models and deterministic approaches to estimate state transition probabilities fail to provide accurate predictions if state measurements are noisy. We here refer to the ‘deterministic approach’ as the scenario without measurement or sampling.
noise, which allows for optimal solution to the estimation of elements of the transition probability matrix through solving a system of linear equations. As demonstrated in the present paper, a solution is to consider a hidden Markov model in which two types of randomness, because of measurement and sampling, are taken into account.

In [13–17], the influence of intrinsic and extrinsic noise on processes occurring at the intracellular and cell population levels is investigated. A linear-noise approximation was used to obtain intrinsic noise statistics of biochemical networks in [13, 14]. In [15, 16], the statistical properties of molecular movement is analysed in crowded intracellular compartments, where the crowding originates from various inert macromolecules in the path of mobile reactant molecules. In [17], it was found that cell–cell variability stemming from both intrinsic and extrinsic sources of noise influences pattern formation at the cell population level, thus showing the importance of noise in developmental biology. Furthermore, in [18, 19], the effects of background light and electronic noises on the overall signals the fluorescence-activated cell sorting (FACS) instrument obtains for each cell is considered.

In [20, 21], the transition probability matrix of a Markov chain is estimated from aggregated data. When the data are noise free, estimating the transition probability matrix of a Markov process with \( M \) states requires only \( M^2 \) measurements. In fact, by solving a system of linear equations, the transition probability matrix of the underlying Markov process can be calculated. Such a deterministic approach was used in [10] for calculating the transition probability matrix of a Markov process describing the proliferation of breast cancer cells. What we would like to point out in the present paper is that when the aggregated data are noisy, the calculation of transition probabilities using the deterministic approach dramatically degrades. Moreover, here, using standard algorithms for estimation of the transition probability matrix in a hidden Markov model such as the Baum–Welch algorithm [22] is not possible because of the fact that as we shall see, only noisy versions of state probabilities can be observed.

Fig. 1 shows the normalised histogram of stem-like cell populations, which is obtained from experimental data of two different samples. The experiment setup is presented in Section A of Supplementary Materials. The number of stem-like cells in the steady-state regime is a random variable. One may argue that this approximately follows a Gaussian distribution. For our study, we will also consider other noise types to compare estimators.

Here, we consider the effect of measurement and sampling randomness on the prediction of the cell population sizes using an estimated transition probability matrix. The transition probability denoted by \( p_{ij} \), is defined between the cell type (state) \( i \), \( i \in \{1, \ldots, M\} \), and the cell type (state) \( j \), \( j \in \{1, \ldots, M\} \). The observation we wish to make from each cell type is their population size or alternatively their proportion among all cell types (state probability) in a given time instant. One of randomness sources is noisy measurements of cell population sizes by FACS techniques. Imperfect cell counting in FACS adds error in estimating the number of cells during proliferation. Statistically, one of the measurement noise sources in cell counting is shot noise [24], which typically amounts to a Poisson distribution [25]. Moreover, our experimental measurements show that for FACS analyses the noise distribution is fitted to Gaussian distribution if the number of cells is reasonably large. The number of cells in a population is always positive, which is why the Gaussian assumption would have to be replaced by the Poisson distribution for small numbers of cells. The randomness in sampling is due to estimation errors for the probability of states, linked to the law of large numbers (LLN) used for the estimation of state probabilities of processes with small population sizes.

For improved accuracy of an estimated transition probability matrix, we use minimum mean square error (MMSE) and maximum likelihood (ML) estimators. Our simulation results show that the error in counting the population size of each cell type dramatically degrades the accuracy of the estimated transition probability matrix when deterministic estimation approaches are used. The principal goal of this work is to determine the minimum number of measurements that is required in order to guarantee the accuracy of population sizes predicted using a transition probability matrix, estimated based on noisy data. The main challenge for this problem is the small number of samples that is typically available from FACS analyses in systems biology. We consider the error in prediction of the size of each cell type population and the average error in prediction of all population sizes as two possible measures of accuracy, which we aim to limit in two design problems we formulate. Table 1 summarises different types of uncertainty in FACS data analysis for the prediction of cell population sizes including (i) imperfect markers [11], (ii) imperfect sorting [11], (iii) imperfect counting of cell population sizes and (iv) imperfect state probability estimation. The types and sources of uncertainty are described and it is also noted how each uncertainty type is dealt within the sequel.

The remainder of this paper is organised as follows. In Section 2, the system model and problem statements are presented. Section 3 reports the stochastic analyses. Simulation results are presented in Section 4. Finally, conclusions are drawn in Section 5.
2 System model and problem statement

2.1 System model

To design novel therapies for cancer treatment, detailed knowledge about the dynamics of cancer cell growth is necessary. It is demonstrated in [1, 10] that cell growth dynamics can be explained by a first-order Markov model in which cells transit stochastically between different states. A second prediction in [10] is that breast cancer stem-like cells arise de novo from non-stem-like cells. These findings contribute to our understanding of cancer heterogeneity and reveal how stochasticity in single-cell behaviours promotes phenotypic equilibrium in populations of cancer cells. Hence, estimating the transition probability matrix of this Markov process has a fundamental role in describing the dynamic process of stem-like and non-stem-like cell growth. The cell population counting is done by using FACS. Imperfect counting in FACS imposes randomness on the measured number of initial cells [26–30]. We will refer to this randomness as measurement noise in the remainder of this paper. Fig. 2 shows the effect of sorting and counting on cell population sizes of parental SUM159 of breast cancer consisting of stem-like, basal and luminal cells [10]. The basal cells and luminal cells form, respectively, the outer and the inner layers of the glandular tissue of the breast [31]. Specific molecular markers are used to identify different cell types. Cells are sorted according to different populations and the cell populations are counted. Ideally, this should be performed after each doubling time with perfect (without noise of counting) and imperfect counting (with noise of counting). The cell population size using imperfect counting is used for state probability estimation and consequently for transition probability estimation, which can be modelled with hidden Markov model. Fig. 2 schematically demonstrates the effect of imperfect counting after one cell doubling. This is one source of error in estimation of the transition probability matrix.

In this manuscript, it is assumed that the probability density function (PDF) of measurement noise is known, which is obtained from our experimental measurements (reported in Fig. 1) and PDF of shot noise. If the number of cells is small, shot noise in particular can add significant uncertainty to the exact values of cell numbers [32, 33]. The statistical characteristics of this noise are described by a Poisson distribution [25]. Moreover, the normalised histogram plot of cell types in experimental tissue samples of two different patients is reported in Fig. 1. It is shown that the noise in cell counting, with a rough degree of approximation, may be described by a Gaussian distribution. When performing such FACS analyses, one thus tries to measure a random process (with unknown statistical moments) with a noisy measurement approach. Moreover, it is assumed that, the initial number of different cancer cell types differ in different tissues or cell samples at the start of each experiment. Also, no information is available about the statistics of the initial population of each cell type in the analysis. The transition probability matrix between different cell types is then given by

\[
P = \begin{bmatrix}
p_{11} & \cdots & p_{1M} \\
\vdots & \ddots & \vdots \\
p_{M1} & \cdots & p_{MM}
\end{bmatrix}
\]

where \(M\) is the number of states, which corresponds to the number of recognisable cell types in the experiment. Increasing the number of samples analysed in the experiment can reduce the effect of noisy measurements, but it cannot directly control the randomness of the initial cell population. Hence, as a first step, we try to minimise the cost of experiments by determining the number of samples and measurements to be analysed, given that a specified level of accuracy of cell population is needed.

After estimating the transition probability matrix using experimental data, we use that matrix for computer
simulations of cell proliferation dynamics to support therapeutic decisions. Hence, in this stage another source of uncertainty in predicting the number of cells using the estimated transition probability matrix, is due to errors in estimating the state probability \( q_j^{(k)} \), which is the probability of a cell being of type \( j \in [1, M] \) at time \( k \). The estimated state probability using LNN is obtained as

\[
q_j^{(k)} = \frac{\tilde{q}_j^{(k)}}{\sum_{j=1}^{M} \tilde{q}_j^{(k)}}
\]  

where \( \tilde{q}_j^{(k)} \) is the population size of cell type \( j \) at time \( k \). The estimated state probability \( q_j^{(k)} \) approaches \( \tilde{q}_j^{(k)} \) for large \( \tilde{q}_j^{(k)} \). Since only a noisy version (approximation) of population size of cell type \( j \) at time \( k \), \( \tilde{y}_j^{(k)} \), is available, the estimated value for probability of cell type \( j \in [1, M] \) at time \( k \) is given by

\[
\tilde{q}_j^{(k)} = \frac{\tilde{y}_j^{(k)}}{\sum_{j=1}^{M} \tilde{y}_j^{(k)}}
\]  

In this model, the state probabilities are the alphabet of the hidden Markov model, which are continuous variables. In this model, the state probability is considered a random variable with Gaussian or Poisson distribution.

2.2 Problem statement

Here, we formulate the effect of randomness in measurement and sampling on cell population counting. When the distribution of measurement noise is known, we use an MMSE or ML estimator to derive the transition probability matrix. For a formal definition of design optimisation problems, we first define the related parameters in Table 2.

In FACS experiment analysis, the maximum number of measurements for cell population is limited. Since the cells die after a few time steps, and the dynamics of cell population growth change. Therefore, in our analysis, we try to minimise the number of measurement in each sample. Moreover, the number of available samples is limited because of the cost of experiments; hence, we consider a limit on the maximum number of samples. Under these limitations, we estimate transition probability matrix defined in (1) from different samples. Our objective function is to minimise the number of measurement in each sample while ensuring that the transition probabilities are estimated to within a specified level of accuracy. Here, two performance measures are defined to compare these two cell populations in the proposed optimisation problems. First performance measure compares the cell populations of each cell types using M error functions for each cell type. In the second problem, we average the error function of cell populations over different subpopulation sizes. This leads us to the following two design optimisation problems. Note that to assess the performance of the proposed solutions for estimation of transition probability of the cell proliferation Markov process, we resort to simulations in Section 4. In this case, a true transition probability matrix is assumed and observations are obtained based on the model described.

Problem 1: The minimum number of measurements required to limit the normalised error of predicted population size for each type of cell using the MMSE or ML estimated \( P \) and
limited NS, is computed in the following optimisation problem

$$\min NMS$$

s.t.

$$\text{PE}^{(k)}_j \leq \varepsilon_j, \quad j \in \{1, \ldots, M\}$$

$$NS \leq NS_{\text{max}}$$

(4)

where NS_{\text{max}} and \(\varepsilon_j\) denote the maximum possible number of samples and the required accuracy in the estimation of cell type \(j\), respectively. Also, the percentage of error for subpopulation \(j\) at time step \(k\), \(\text{PE}^{(k)}_j\) is given by

$$\text{PE}^{(k)}_j = \left| q^{(k)}_j - \hat{q}^{(k)}_j \right| \times 100$$

(5)

In (4), \(k\) indicates the (steady state) time instant in multiples of \(T\) (the time step over which the number for cells is expected to double) at which the error is computed.

**Problem 2:** The minimum number of measurements required to limit the mean of normalised error of a predicted population size using the MMSE or ML estimated \(\hat{P}\) with limited NS, is computed in the following optimisation problem

$$\min NMS$$

s.t.

$$\text{MPE}^{(k)} \leq \varepsilon$$

$$NS \leq NS_{\text{max}}$$

(6)

in which

$$\text{MPE}^{(k)} = \frac{1}{M} \sum_{j=1}^{M} \text{PE}^{(k)}_j$$

(7)

and \(\varepsilon\) denotes the average required accuracy in the estimation of cell types. It can be verified that these problems are convex.
In the next section, we derive the MMSE estimator for \( P \) when the cell population sizes are observed through additive Gaussian noise, which provides the same results as the ML estimation (see Supplementary Materials for details). Then, two ML estimators approximated based on one sample estimation and sample mean approximation are derived in the case of Poisson observations.

### 3 Stochastic analysis

In the present section, we provide results for the proposed MMSE and approximate ML estimators. We consider the case of noisy measurements, assuming a Gaussian distribution, which we found to fit with our experimental data. For small numbers of cells, we assume a Poisson distribution, fitted to PDF of shot noise, to avoid consequences of the Gaussian distribution going into a range of negative values. The Gaussian distribution will allow us to derive a closed-form solution. That is, for the Poisson distribution an approximated solution for problems described in Propositions 1 and 2 and further discussed in the Supplementary Materials.

#### 3.1 Gaussian distribution

It is assumed that we have \( N \) samples, where the initial cell population size of each sample is unknown, and for sample \( i \), the measurements of subpopulation sizes have a Gaussian distribution with mean \( \begin{bmatrix} \tilde{v}_{1,i}^{(0)} & \tilde{v}_{2,i}^{(0)} & \ldots & \tilde{v}_{M,i}^{(0)} \end{bmatrix} \). The MMSE and ML estimators in a general form for \( \hat{P} \) are given by

\[
\hat{P}_{\text{MMSE}} = E(\hat{P}|v_{1,i}^{(0)}, v_{2,i}^{(0)}, \ldots, v_{M,i}^{(NMS-1)}) \quad (8)
\]

\[
\hat{P}_{\text{ML}} = \max_P P(v_{1,i}^{(0)}, v_{2,i}^{(0)}, \ldots, v_{M,i}^{(NMS-1)}|P) \quad (9)
\]

We assume for simplicity that the observed noise for different measurements is independent events. In general, the measurement noise may be because of physical and performance characteristics of the measurement equipment (here the FACS machine) and may be correlated. The exact study and characterisation of such a possible correlated noise model is beyond the scope of the current work. Here, we derive the MMSE estimator when measurements of population sizes are corrupted with additive white Gaussian noise. Hence, because of Markov property for cell proliferation, the observation model is given by

\[
\begin{bmatrix}
\tilde{v}_{1,i}^{(NMS-2)} \\
\tilde{v}_{2,i}^{(NMS-2)} \\
\vdots \\
\tilde{v}_{M,i}^{(NMS-2)} \\
\end{bmatrix} = \begin{bmatrix}
\eta_{1,i}^{(NMS-1)} \\
\eta_{2,i}^{(NMS-1)} \\
\vdots \\
\eta_{M,i}^{(NMS-1)} \\
\end{bmatrix}
\]

where \( \eta_{l,i} \) denotes exact values of cell population sizes. Also, \( \tilde{v}_{l,i}^{(k)} \) and \( \eta_{l,i}^{(k)} \) denote, respectively, the measured values of cell population sizes and the noise term for cell type \( l \), sample \( i \) at time \( k \). The noise term \( \eta_{l,i}^{(k)} \) has a Gaussian distribution with zero mean and standard deviation \( \sigma \). The factor 2 is due to the fact that the measurements take place at intervals when the cell population size is expected to double. In the following Theorem, the transition probability matrix of \( P \) is derived using an MMSE estimator.

**Theorem 1**: The transition probability matrix, \( P \), of the cell proliferation Markov process, when the measurements of population sizes is observed in Gaussian noise, is obtained using an MMSE estimator as follows (see (11))

\[
V_{1:N}^{(NMS-2)} = \begin{bmatrix}
\tilde{v}_{1,1}^{(NMS-2)} & \tilde{v}_{1,2}^{(NMS-2)} & \ldots & \tilde{v}_{1,M}^{(NMS-2)} \\
\vdots & \vdots & \ddots & \vdots \\
\tilde{v}_{N,1}^{(NMS-2)} & \tilde{v}_{N,2}^{(NMS-2)} & \ldots & \tilde{v}_{N,M}^{(NMS-2)} \\
\end{bmatrix}
\]

also, \( V_{1:N}^{(1:M)} \), \( R \), is the covariance matrix of noise and \( A_i \) is the
Lagrange multiplier, and they are given by
\[
P^{(1:NMS-1)} = \begin{bmatrix} \eta_{11} & \cdots & \eta_{1N} \\ \cdots & \cdots & \cdots \\ \eta_{N1} & \cdots & \eta_{NN} \end{bmatrix}^T
\]  
(13)

\[
R_n = \begin{bmatrix} \sigma_1^2 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & \sigma_M^2 \end{bmatrix}
\]  
(14)

\[
\Lambda_i = \begin{bmatrix} \lambda_i \quad \cdots \quad \lambda_i \end{bmatrix}^T
\]  
(15)

where \( \sigma_l^2 \) is noise variance of cell type \( l \), \( \lambda_l > 0 \) is obtained by replacing \( p_l \) from (11) in the following equation for each value of \( l \)
\[
\sum_{k=1}^{M} p_{kl} = 1
\]  
(16)

The above solution is obtained when the constraints \( 0 \leq p_l \leq 1 \) is assumed satisfied. However, if the obtained results violate these constraints, one should enforce them and solve the problem again. The proof and further details are provided in Section B of the Supplementary Material.

This solution is also a solution of the ML estimator for \( NMS - 1 \geq M \) as elaborated in Section C of the Supplementary Material.

### 3.2 Poisson distribution

Based on the distribution of shot noise, which is reported in [26–30], we here assume a Poisson distribution for cell population sizes measured in each sample \( i \in \{1, \ldots, N\} \)
\[
\begin{bmatrix} \eta_{i1}^{(0)} & \cdots & \eta_{i1}^{(0)} \\ \vdots & \ddots & \vdots \\ \eta_{iN}^{(NMS-1)} & \cdots & \eta_{iN}^{(NMS-1)} \end{bmatrix} \sim \begin{bmatrix} P(\tilde{\eta}_{i1}^{(0)}) & \cdots & P(\tilde{\eta}_{i1}^{(0)}) \\ \vdots & \ddots & \vdots \\ P(\tilde{\eta}_{iN}^{(NMS-1)}) & \cdots & P(\tilde{\eta}_{iN}^{(NMS-1)}) \end{bmatrix}
\]  
(17)

Here, the observation model, which can be obtained from the ML estimator (see Section D of Supplementary Materials), is given by
\[
\begin{bmatrix} \eta_{i1}^{(0)} & \cdots & \eta_{i1}^{(0)} \\ \vdots & \ddots & \vdots \\ \eta_{iN}^{(NMS-2)} & \cdots & \eta_{iN}^{(NMS-2)} \end{bmatrix} = \begin{bmatrix} \eta_{i1}^{(1)} \\ \vdots \\ \eta_{iN}^{(NMS-1)} \end{bmatrix}
\]  
(18)

In this setting, the observation noise is signal dependent and not additive. As a result, obtaining a closed-form MMSE solution is challenging even in the case of independent Poisson observations. We can however compute two approximate ML solutions for the problem with Poisson observations in the next following Propositions.

**Proposition 1:** The transition probability matrix, \( P \), for the cell proliferation Markov process, when the measurements of population sizes are Poisson distributed, is obtained using an approximate ML one sample estimator as follows
\[
P_{ML}^T = 0.5 \left( \begin{bmatrix} \tilde{\eta}_{(0:M-1)}^{ \top} \\ \cdots \\ \tilde{\eta}_{(M-1)}^{ \top} \end{bmatrix} - \Lambda_1 \right)^{-1}
\]  
(19)

where \( \Lambda_1 \) is Lagrange multiplier and is given by
\[
\Lambda_1 = \begin{bmatrix} \lambda_1 \quad \cdots \quad \lambda_M \end{bmatrix}^T
\]  
(20)

and \( \lambda_l > 0 \) is obtained by solving the following equation for each value of \( l \)
\[
\sum_{j=1}^{M} p_{jl} = 1
\]  
(21)

The above solution is obtained when the constraints \( 0 \leq p_l \leq 1 \) is assumed satisfied. However, if the obtained results violate these constraints, one should enforce them and solve the problem again (see Section E of the Supplementary Materials for details and proof).

**Proposition 2:** The transition probability matrix, \( P \), for the cell proliferation Markov process, when the measurements of population sizes are Poisson distributed, is obtained using an ML estimator based on approximated sample mean as follows
\[
\tilde{P}_{ML} = 0.5 \left( \begin{bmatrix} \Sigma_{\hat{\epsilon}} & \Sigma_{\hat{\eta}} \end{bmatrix}^{-1} \Sigma_{\hat{\epsilon}}^{\top} \varphi_1 - \Lambda_1 \right)
\]  
(22)

where \( \Lambda_1 \) is given by
\[
\Lambda_1 = \begin{bmatrix} \lambda_1 \quad \cdots \quad \lambda_M \end{bmatrix}^T
\]  
(23)

and \( \lambda_l > 0 \) is obtained from the following equation for each
value of $l$

$$\sum_{j=1}^{M} p_{jl} = 1 \quad (24)$$

and $\Sigma_v$ is given by

$$\Sigma_v = \begin{bmatrix}
\sum_{i=1}^{N} v_{i,j}^{(0)} & \cdots & \sum_{i=1}^{N} v_{i,j}^{(M-2)} \\
\vdots & \ddots & \vdots \\
\sum_{i=1}^{N} v_{i,j}^{(N-3)} & \cdots & \sum_{i=1}^{N} v_{i,j}^{(N-2)}
\end{bmatrix} \quad (25)$$

Also, $\Psi_l$ is given by

$$\Psi_l = \begin{bmatrix}
\sum_{i=1}^{N} v_{i,j}^{(1)} & \cdots & \sum_{i=1}^{N} v_{i,j}^{(N-1)}
\end{bmatrix} \quad (26)$$

The above solution is obtained when the constraints ($0 \leq p_{ij} \leq 1$) is assumed satisfied. However, if the obtained results violate these constraints, one should enforce them and solve the problem again (see Section F of the Supplementary Materials for details and proof).

### 4 Simulation results

In this section, we provide simulation and numerical results for the optimisation Problems 1 and 2. Moreover, the dynamics of the convergence of cell population sizes are studied using a Monte Carlo simulation.

#### 4.1 Simulation scenario

For simulations, the number of cells in each sample is considered a random variable with uniform distribution between 3000 and 6000 cells. The number of measurements in each sample, $NMS$, is selected a multiple of $M$, where in line with [10], the number of states, $M$, is set to three, which corresponds to the three cell types of stem-like cells, basal cells and luminal cells. As stated, two scenarios with either additive white Gaussian observation noise or with Poisson observations are considered. The transition probability matrix for the cell proliferation Markov process simulated in this section is selected as in [10]. This is also reported in the first row of Table 3.

#### 4.2 Numerical results

In this section, first, the MMSE estimator of Theorem 1 is used for the Gaussian scenario and the approximate ML estimator of Proposition 1 for the Poisson scenario.

![Fig. 4 PE(k) in presence of Gaussian measurement noise of CV = 0.2236, as a function of k for different values of NS and with](image-url)

- a. $NMS = 3$
- b. $NMS = 6$
- c. $NMS = 9$
- d. $NMS = 12$

This figure shows the performance of the PE(k) estimator in the presence of Gaussian measurement noise for the cases of $NMS = 3, 6, 9, 12$.
experiments reveal similar performance for the approximate ML estimators in Propositions 1 and 2. The performance of the proposed estimators is quantified by comparing the obtained results with the true (postulated) transition probability in the simulations. Second, using Monte Carlo computer simulations for each cell, we assess the cell proliferation by obtaining the PDF of \( q_j(k) \), \( j \in \{1, 2, 3\} \), over different time steps \( k \in \{T, 2T, ..., 10T\} \).

Fig. 4a shows the percentage of error in the estimation of stem-like cell population, \( \text{PE}^{(1)} \), in terms of the time step index, \( k \), for different number of samples, \( NS \), and the number of measurements in each sample, \( NMS \), set to 3. The measurement noise is Gaussian with coefficient of variation (CV) of 0.2236, or alternatively the power of signal to power of noise ratio (SNR) of 13 dB. Based on the experimental data reported in part in Fig. 1, a SNR between 10 and 15 dB is considered typical. Figs. 4b–d depict the results of similar experiments, but with \( NMS \) set to 6, 9 and 12, respectively. The results demonstrate that as expected \( \text{PE}^{(1)} \) reduces as the number of samples, \( NS \), or the number of measurements in each sample, \( NMS \), increases.

Fig. 5a shows the percentage of error for estimation of stem-like cell population in steady state (\( \text{PE}^{(1)} \) for \( k = 20 \), in terms of \( NS \) and \( NMS \) for additive white Gaussian measurement noise. Figs. 5b and c shows the results of similar experiments for the case of basal and luminal cell subpopulations, respectively. The mean percentage of error in steady state (\( k = 20 \)) estimation of cell population sizes, MPE, is depicted in Fig. 5d. Fig. 5 shows that if the

| Table 3 Estimated transition probability matrix obtained with different approaches with Gaussian noise (CV = 0.2236) |
|---------------------------------------------------------------|
| exact transition probability matrix                           |
| \( P = \begin{bmatrix} 0.58 & 0.35 & 0.07 \\ 0.01 & 0.99 & 0 \\ 0.04 & 0.49 & 0.47 \end{bmatrix} \) |
| estimated transition probability matrix without measurement and sampling randomness |
| \( \hat{P} = \begin{bmatrix} 0.0743 & 0.9257 & 0 \\ 0 & 0.0167 & 0.9833 \\ 0.0659 & 0 & 0.9341 \end{bmatrix} \) |
| estimated transition probability matrix in the presence of noise but ignoring it in the estimation |
| \( \tilde{P} = \begin{bmatrix} 0.5614 & 0.3590 & 0.0795 \\ 0.0203 & 0.9764 & 0.0033 \\ 0.0293 & 0.5149 & 0.4558 \end{bmatrix} \) |
| estimated transition probability matrix based on MMSE in the presence of noise; \( NMS = 12 \) and \( NS = 6 \) |
| \( \hat{P} = \begin{bmatrix} 0.5614 & 0.3590 & 0.0795 \\ 0.0203 & 0.9764 & 0.0033 \\ 0.0293 & 0.5149 & 0.4558 \end{bmatrix} \) |

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**Fig. 5** PE for the proposed MMSE estimator in the presence of Gaussian measurement noise of CV = 0.2236, as a function of NS for different values of NMS

a Stem-like cell  
b Basal cell  
c Luminal cell  
d MPE
number of measurements in each sample is small, increasing the number of samples, \( NS \), generally improves the accuracy of estimation, but it also shows that there are fluctuations in the accuracy of estimation. By increasing the number of measurements in each sample, \( NMS \), the fluctuations disappear. This means that for given \( NS \), we have a lower bound on \( NMS \). For example, if we have ten samples, \( NS = 10 \) and in each sample we only have one measurement, the accuracy of estimation is therefore not guaranteed. A tradeoff exists then between \( NS \) and \( NMS \). Increasing \( NS \) increases the costs for experiments, this means that we need more samples for measurements. Moreover, increasing the number of measurements for cell populations is usually limited for practical purposes because after some steps the cells die and the dynamics of cell population growth change. As a result, Figs. 5 provide a numerical solution for the optimisation problem 1. For example for \( j = 1 \), setting \( NS = NMS = 6 \), limits the PE under 5%. A conclusion is thus that one should select the best values for \( NS \) and \( NMS \) to satisfy the related accuracy and practical constraints.

Figs. 6a–c show the percentage of estimation error, PE, for the proposed approximate ML estimator in Proposition 1, in terms of \( NS \) and \( NMS \) with measurement shot noise for, respectively, the stem-like, the basal and the luminal cells, respectively. Fig. 6d shows the mean percentage of error for the same experiment. As evident, increasing \( NS \) and \( NMS \), reduces PE and for \( NS = 4 \) and \( NMS = 3 \), it is <1% for all cell subpopulations in cell proliferation process. Figs. 6a–c thus provides numerical solutions for the optimisation Problem 1.

Figs. 5d and 6d provide numerical solutions for optimisation Problem 2 and measurement Gaussian and shot noises, respectively. It is evident in Fig. 5d that MPE is bound to 5% for \( NS = NMS = 6 \). Fig. 6d shows MPE is <1% for \( NS = 5 \) and \( NMS = 3 \).

Fig. 7a shows the PDF of \( \tilde{q}_S^{(k)} \), that is, the state probability of stem-like cell, which is denoted by \( P_{1}^{(k)} \) for different time steps of \( k \), when the transition probability matrix is derived using MMSE estimator from noisy data with Gaussian distribution, and with the transition probability matrix shown in Table 3. This PDF is observed by Markov chain Monte Carlo simulation. It can be seen that by increasing \( k \), the variance of \( \tilde{q}_S^{(k)} \) is reduced and converges to a steady-state value. Fig. 7b shows \( P_{1}^{(k)} \) for different values of \( k \), when the transition probability matrix is derived using the deterministic approach (see Table 3). It can be clearly seen, that the dynamics of convergence for the Markov process and the steady-state value of \( \tilde{q}_S^{(k)} \) are distinct.
Figs. 7c and d show the CV of $P^{(k)}_{ij}$ in terms of $k$, which is defined by the ratio of standard deviation to mean of $\tilde{q}^{(k)}_i$, when transition probability matrix is obtained based on the MMSE estimator and the deterministic approach. In both cases, the CV is constant after seven doubling steps, which shows that dispersion of $P^{(k)}_{ij}$ during time is independent of estimation method of transition probability matrix.

5 Conclusions

Predicting the temporal evolution of cell population sizes using FACS is an important task for a variety of biological and biomedical applications. A specific example, which has motivated our research, comes from cancer research where the dynamics of population sizes for normal and CSCs can provide clues for therapeutic decisions. Studying proliferating cell populations and their proportions in tissue samples, Markov processes provide a suitable conceptual framework to model and simulate such systems. A Markov process is characterised by a transition matrix that assembles the probabilities for transitions between states (here cell types). In the present paper, the transition matrix of the Markov process is estimated from the aggregated data of FACS measurements. In this context, noisy measurements are used for the estimation of the transition probability matrix. Sampling randomness, on the other hand, is here related to the error in estimating the state probability from small cell populations. If noiseless observations were available, only $M^2$ measurements would be required for estimating the transition probability matrix; however, this situation does not reflect the situation in experimental laboratories. Assuming the realistic scenario of noisy data, an exact prediction of population sizes directly depends on the number of samples analysed. In our first example, the sample and $M^2$ measurements for population sizes during cell doubling were enough to estimate the transition probability matrix perfectly, allowing exact prediction of subpopulation sizes. We then showed that if measurements are noisy, the number of samples to be analysed and the number of measurement for each sample must be carefully considered. We subsequently developed a MMSE estimator for calculating the transition probability matrix when counts of the cell population are corrupted by Gaussian noise. Moreover, in the case of Poisson noise, we derive two approximate ML solution using one sample estimator and sample mean approximation. Our numerical results show that if the deterministic approach is used for estimating the transition probability matrix, the prediction of subpopulation sizes can easily be erroneous. We
demonstrate that the prediction of the convergence for the proliferation process and the resulting steady states can substantially differ, which would have obvious consequences for the design of therapies that rely on this information.

An interesting point, arising from our simulation study, is that the CV of the PDF for the cell population probability is not changed during proliferation as a consequence of noise in measurements. Numerical results show that when there is shot noise and with the number of samples NS = 5 and the number of measurements per sample, NMS = 3, the mean of error in predicting the population size (MPE) is <1%. Moreover, for noise with a Gaussian distribution and CV of 0.2236, the mean of error in predicting population size MPE was <5%, when NS = NMS = 6 is considered. Our results show that increasing NMS is more effective than increasing NS in improving the accuracy of transition probability matrix estimation. Our study also showed the consequences of estimating the probability of a cell being in a particular state from measurements across a population of cells. For small population sizes, the LLN will not be satisfied, leading to errors. We showed that the uncertainty arising from small sample sizes can be levied by using a distribution for the state probability rather than a single value. Our work thus contributes to a better understanding of randomness and noise when studying stochastic phenomena inevitably linked to FACS experiments.

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7 References

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Supplementary Materials: Accounting for Randomness in Measurement and Sampling in Studying of Cancer Cell Population Dynamics

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Section A: Flow cytometric analysis of HROC87

Flow cytometric analysis of HROC87, a cell line recently established from a primary colorectal cancer [1]. Exemplary data from 1 of 50 (S1) measurements and from 1 of 100 (S2) measurements are given. Similar data have also been generated from another cell line: HROC113 (data not shown). Of note, the analysis was performed in very low passages of the cell line.

The cells were harvested from cell culture in the exponential growth phase (approximately 80% density), washed with phosphate-buffered saline and incubated with 5µM Vybrant®Dye Cycle™ Violet Stain (VDC; Life Technologies, Frankfurt, Germany) in hanks-balanced salt solution for 30min at 37°C in the dark. In the control measurements (S1), 50µM Verapamil (Sigma-Aldrich, Hamburg, Germany) was added before the addition of VDC to block the dye-efflux by membrane-bound pumps. Cells were kept at 37°C until analysis for a maximum of 3 hours. Propidium iodide (1µg/ml; Life Technologies) was added shortly before measurement to allow for life/dead cell discrimination.

Samples were analyzed on a FACS ARIA II cell sorter equipped with standard lasers using the Diva software package (both from Becton Dickinson, Heidelberg, Germany). 100.000 events (all dots in S1(a) and S2(a) were counted per measurement.

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Fig. S1. Exemplary data from 1 of 50 measurements a) Cell size and granularity b) Exclusion of doublets  c) Exclusion of dead cells P3 d) SP Determination.

The gating strategy was as follows. S1(a) and S2(a): gate P1 was set in the forward scatter (FSC; cell size;X-axis) / sideward scatter (SSC; cell granularity;Y-axis) blot on the main cell population (blue dots). S1(b) and S2(b): In the SSC-area (X-axis) versus SSC-height blot (Y-axis), gate P2 was set to exclude doublets (red dots). S1(c) and S2(c): Dead cells were excluded by gating on the PI negative cells measured in the 695 channel (Y-axis; P3; green dots). For a better display, the blots additionally give the empty Fluorescein isothiocyanate (FITC) channel (X-axis). S1(d) and S2(d). Finally, the events were displayed in the 530 nm channel (X-axis) versus the V450 channel (Y-axis). The side population (SP) cells are those able to pump the VDC stain out of their cytoplasm (pink dots). They are not
positively stained for VDC and lie within gate P4. Percentages of cells within P4 are given for the control cells (incubated with Verapamil and VDC; S1) and for the SP cell analysis (incubated with VDC but without inhibitor; S2.)

Fig. S2. Exemplary data from 1 of 100 measurements: a) Cell size and granularity, b) Exclusion of doublets, c) Exclusion of dead cells P3, d) SP Determination.

Section B. Proof of Theorem 1
The observation model for cell division process in each sample is given by
where, $i \in \{1, N\}$. The equation (S1) is satisfied for each sample, $i$, and cell type, $l$. The goal is to find $p_{hl}$, $h, l \in [1, M]$, which fit the equations best in the minimum mean squared sense. We have

$$\hat{P} = \arg \min_P S(P)$$

s.t.

for $\forall 1 \leq h \leq M, 1 \leq l \leq M$,

$$0 \leq p_{hl} \leq 1,$$

$$\sum_{l=1}^{M} p_{hl} = 1,$$

where the objective function $S$ is defined as

$$S(P) = \sum_{l=1}^{M} \sum_{i=1}^{N} \sum_{j=1}^{N_{MS-1}} \left(v_{l,i}^{(j)} - 2 \sum_{h=1}^{M} v_{h,i}^{(j-1)} P_{hl} \right)^2,$$

(S3)

The optimization problem in (S2) is convex, (cost function is quadratic and constraints are linear [2]). Using KKT (Karush–Kuhn–Tucker), the unconstrained solution for this problem is given by

$$S_u(P) = \sum_{l=1}^{M} \left( S(P) + \lambda_i \left( \sum_{h=1}^{M} p_{hl} - 1 \right) + \sum_{h=1}^{M} \left( \lambda_{hl}' - \lambda_{hl}^* \right) P_{hl}, \right)$$

(S4)

where $\lambda_{hl}'$, $\lambda_{hl}^*$ and $\lambda_i$ are Lagrange multipliers and $P_i = [p_{ui} \ldots p_{uml}]$. The value of $S(P)$ can be simplified as
If either of the constraints in (S2-b) is inactive (is not satisfied with equality), the corresponding Lagrange multiplier is set to zero. And if either of them is active the value of \( p_{jl} \) is obtained directly from (S2-b). Hence, here we solve the problem when the set of constraints in (S2-b) is not active, and compute the derivative of (S5) with respect to \( p_{ql} \) and \( \lambda_q, q, l \in [1, M] \). We have

\[
\frac{\partial S_0(P)}{\partial p_{ql}} = -4 \sum_{i=1}^{N} \sum_{j=1}^{N_{M-1}} (v_{i,j}^{(j)})^2 + 4 \sum_{i=1}^{N} \sum_{j=1}^{N_{M-1}} \left( \sum_{h=1}^{M} p_{hh}^{(j-1)} \right)^2 - 4 \sum_{i=1}^{N} \sum_{j=1}^{N_{M-1}} \sum_{h=1}^{M} v_{i,j}^{(j)} p_{hh}^{(j-1)} - 4 \sum_{i=1}^{N} \sum_{j=1}^{N_{M-1}} \sum_{h=1}^{M} v_{i,j}^{(j-1)} p_{hh}^{(j)}
\]

\[
\frac{\partial S_0(P)}{\partial \lambda_q} = \left( \sum_{j=1}^{M} p_{ql} - 1 \right),
\]

By dividing both sides of (S6-a) by \( N \) and because the noise of different samples are centered processes, we have
\[
\frac{1}{N} \frac{\partial S(P_i)}{\partial p_{ij}} = -\frac{4}{N} \left( \sum_{i=1}^{N} \sum_{j=1}^{N_{M.S}} q_{ij}^{(j-1)} \left( v_{ij}^{(j)} - 2 \sum_{h=1}^{M} q_{h,i}^{(j-1)} p_{hl} \right) - 2 \sum_{j=1}^{N} \sum_{h=1}^{M} p_{M} \sum_{i=1}^{N} \eta_{h,i}^{(j-1)} \eta_{h,i}^{(j-1)} + \lambda_{ij}^{(j-1)} / 4 \right)
\]

(S7)

By writing (S7) in matrix form, we have

\[
\begin{bmatrix}
\sum_{j=1}^{N_{M.S}} v_{1,i}^{(j-1)} q_{1,i}^{(j-1)} + \eta_{1,i}^{(j-1)} \eta_{1,i}^{(j-1)} & \cdots & \sum_{j=1}^{N_{M.S}} v_{M,i}^{(j-1)} q_{M,i}^{(j-1)} + \eta_{M,i}^{(j-1)} \eta_{M,i}^{(j-1)} \\
\vdots & \ddots & \vdots \\
\sum_{j=1}^{N_{M.S}} v_{1,i}^{(j-1)} q_{1,i}^{(j-1)} + \eta_{1,i}^{(j-1)} \eta_{1,i}^{(j-1)} & \cdots & \sum_{j=1}^{N_{M.S}} v_{M,i}^{(j-1)} q_{M,i}^{(j-1)} + \eta_{M,i}^{(j-1)} \eta_{M,i}^{(j-1)}
\end{bmatrix}
\begin{bmatrix}
0.5 \sum_{j=1}^{N_{M.S}} q_{1,i}^{(j-1)} v_{1,i}^{(j-1)} - \lambda_{1,i} / 4 \\
\vdots \\
0.5 \sum_{j=1}^{N_{M.S}} q_{M,i}^{(j-1)} v_{M,i}^{(j-1)} - \lambda_{M,i} / 4
\end{bmatrix}
\]

(S8)

If we write the above equation for all values of \( q \), we have following equations

\[
\begin{bmatrix}
\sum_{j=1}^{N_{M.S}} v_{1,i}^{(j-1)} q_{1,i}^{(j-1)} + \eta_{1,i}^{(j-1)} \eta_{1,i}^{(j-1)} & \cdots & \sum_{j=1}^{N_{M.S}} v_{M,i}^{(j-1)} q_{M,i}^{(j-1)} + \eta_{M,i}^{(j-1)} \eta_{M,i}^{(j-1)} \\
\vdots & \ddots & \vdots \\
\sum_{j=1}^{N_{M.S}} v_{1,i}^{(j-1)} q_{1,i}^{(j-1)} + \eta_{1,i}^{(j-1)} \eta_{1,i}^{(j-1)} & \cdots & \sum_{j=1}^{N_{M.S}} v_{M,i}^{(j-1)} q_{M,i}^{(j-1)} + \eta_{M,i}^{(j-1)} \eta_{M,i}^{(j-1)}
\end{bmatrix}
\begin{bmatrix}
0.5 \sum_{j=1}^{N_{M.S}} q_{1,i}^{(j-1)} v_{1,i}^{(j-1)} - \lambda_{1,i} / 4 \\
\vdots \\
0.5 \sum_{j=1}^{N_{M.S}} q_{M,i}^{(j-1)} v_{M,i}^{(j-1)} - \lambda_{M,i} / 4
\end{bmatrix}
\]

(S9)

Hence, \( P_i \) is given by

\[
\begin{bmatrix}
0.5 \\
\vdots \\
0.5
\end{bmatrix}
= \begin{bmatrix}
\sum_{j=1}^{N_{M.S}} v_{1,i}^{(j-1)} q_{1,i}^{(j-1)} \\
\vdots \\
\sum_{j=1}^{N_{M.S}} v_{M,i}^{(j-1)} q_{M,i}^{(j-1)}
\end{bmatrix}
- \begin{bmatrix}
\sum_{j=1}^{N_{M.S}} \eta_{1,i}^{(j-1)} \eta_{1,i}^{(j-1)} \\
\vdots \\
\sum_{j=1}^{N_{M.S}} \eta_{M,i}^{(j-1)} \eta_{M,i}^{(j-1)}
\end{bmatrix}

(S10)

If the variance of noise for each type of cell in all samples is considered equal, i.e., \( \sigma_j^2 = \sigma_j^2 \), we have
\[
\begin{pmatrix}
p_{H} \\
p_{M}
\end{pmatrix} = 0.5 \begin{pmatrix}
\sum_{j=1}^{NMS-1} \sum_{i=1}^{N} v_{i,j}^{(j-1)} v_{i,j}^{(j-1)} & \ldots & \sum_{j=1}^{NMS-1} \sum_{i=1}^{N} v_{i,j}^{(j-1)} v_{M,i}^{(j-1)} \\
\sum_{j=1}^{NMS-1} \sum_{i=1}^{N} v_{M,i}^{(j-1)} v_{i,j}^{(j-1)} & \ldots & \sum_{j=1}^{NMS-1} \sum_{i=1}^{N} v_{M,i}^{(j-1)} v_{M,i}^{(j-1)}
\end{pmatrix}^{-1} + N(NMS-1) \begin{pmatrix}
\sigma_i^2 & \ldots & 0 \\
\vdots & \ddots & \vdots \\
0 & \ldots & \sigma_M^2
\end{pmatrix}
\]

which can be simplified to

\[
\hat{P}_j = 0.5 \left( \left( V_{(0:NMS-2)} \right)^T \left( V_{(0:NMS-2)} \right) + N(NMS-1) R_n \right)^{-1} \left( V_{(0:NMS-1)} \right)^T V_{(l(LN))} \right) - \Lambda_i,
\]

where,

\[
V_{(0:NMS-2)} = \begin{bmatrix}
v_{1,1}^{(0)} & \ldots & v_{M,1}^{(0)} \\
\vdots & \ddots & \vdots \\
v_{l,1}^{(NMS-2)} & \ldots & v_{M,1}^{(NMS-2)} \\
\vdots & \ddots & \vdots \\
v_{1,N}^{(0)} & \ldots & v_{M,N}^{(0)} \\
\vdots & \ddots & \vdots \\
v_{l,N}^{(NMS-2)} & \ldots & v_{M,N}^{(NMS-2)}
\end{bmatrix},
\]

and

\[
V_{(LMS-1)} = \begin{bmatrix}
v_{l,1}^{(1)} & \ldots & v_{l,1}^{(M)} \\
v_{l,1}^{(2)} & \ldots & v_{l,1}^{(2)} \\
\vdots & \ddots & \vdots \\
v_{l,N}^{(LMS-2)} & \ldots & v_{l,N}^{(LMS-2)}
\end{bmatrix}^T
\]

and

\[
R_n = \begin{bmatrix}
\sigma_i^2 & \ldots & 0 \\
\vdots & \ddots & \vdots \\
0 & \ldots & \sigma_M^2
\end{bmatrix}
\]

and

\[
\Lambda_i = \begin{bmatrix}
\frac{\lambda_i}{4} & \ldots & \frac{\lambda_i}{4}
\end{bmatrix}^T
\]

where \( \lambda_i \) is obtained from the following equation for each value of \( l \)
As stated, in the above derivations we did not explicitly incorporated the constraints in (S2-b) and assumed they are inactive. If the assumption is not valid, we will arrive at values of $p_{hl}$ possibly greater than one or negative. In this case, we enforce the violated constraint with equality and solve the optimization problem again. In case, there are multiple such violated constraints, this process is repeated for different combinations of enforced constraints (see [3] pp.314-357 for details).

Section C

In this Section, we derive the ML estimator for the transition probability of the cell proliferation Markov chain with observations made in the presence of additive white Gaussian noise. In the first step, using a joint probability formula for $\hat{P}_{ML}$, we have

$$\hat{P}_{ML} = \max_{\mathbf{p}} P\left(v_{1,1}^{(0)}, \ldots, v_{1,N}^{(0)}, \ldots, v_{M,1}^{(0)}, \ldots, v_{M,N}^{(0)} \mid \mathbf{p}\right)$$

(a) is obtained using the chain rule and (b) is due to first order Markov property of population size. Moreover, it is assumed that the observations are independent, hence, in (S18) we have

$$\sum_{h=1}^{M} p_{hl} = 1$$  \hspace{1cm} (S17)
\[ P\left( v_{1,1}^{(0)}, ..., v_{i,j}^{(0)}, ..., v_{M,1}^{(0)}, ..., v_{M,N}^{(0)} | P \right) = \prod_{i=1}^{N} \prod_{j=1}^{M} P\left( v_{j,i}^{(0)} | P \right) \]

\[ = \prod_{i=1}^{N} \prod_{j=1}^{M} \frac{1}{\sqrt{2\pi \sigma_{ji}}} \exp \left( \frac{-(v_{j,i}^{(0)} - \tilde{v}_{j,i}^{(0)})^2}{2\sigma_{ji}^2} \right) \]

\[ = \frac{1}{(2\pi)^{MN/2}} \prod_{i=1}^{N} \prod_{j=1}^{M} \sigma_{ji} \exp \left( -\sum_{i=1}^{N} \sum_{j=1}^{M} \frac{(v_{j,i}^{(0)} - \tilde{v}_{j,i}^{(0)})^2}{2\sigma_{ji}^2} \right) \tag{S19} \]

\[ P\left( v_{1,1}^{(1)}, ..., v_{i,j}^{(1)}, ..., v_{M,1}^{(1)}, ..., v_{M,N}^{(1)} | P, v_{1,1}^{(0)}, ..., v_{i,j}^{(0)}, ..., v_{M,1}^{(0)}, ..., v_{M,N}^{(0)} \right) = \prod_{i=1}^{N} \prod_{j=1}^{M} P\left( v_{j,i}^{(1)} | P, v_{1,1}^{(0)}, ..., v_{i,j}^{(0)}, ..., v_{M,1}^{(0)}, ..., v_{M,N}^{(0)} \right) \]

\[ = \prod_{i=1}^{N} \prod_{j=1}^{M} \frac{1}{\sqrt{2\pi \sigma_{ji}}} \exp \left( \frac{-(v_{j,i}^{(1)} - 2\sum_{h=1}^{M} \tilde{v}_{h,i}^{(0)} p_{hj})^2}{2\sigma_{ji}^2} \right) \]

\[ = \frac{1}{(2\pi)^{MN/2}} \prod_{i=1}^{N} \prod_{j=1}^{M} \sigma_{ji} \exp \left( -\sum_{i=1}^{N} \sum_{j=1}^{M} \frac{(v_{j,i}^{(1)} - 2\sum_{h=1}^{M} \tilde{v}_{h,i}^{(0)} p_{hj})^2}{2\sigma_{ji}^2} \right) \tag{S20} \]

\[ P\left( v_{1,1}^{(NMS-1)}, ..., v_{i,j}^{(NMS-1)}, ..., v_{M,1}^{(NMS-1)}, ..., v_{M,N}^{(NMS-1)} | P, v_{1,1}^{(NMS-2)}, ..., v_{i,j}^{(NMS-2)}, ..., v_{M,1}^{(NMS-2)}, ..., v_{M,N}^{(NMS-2)} \right) = \prod_{i=1}^{N} \prod_{j=1}^{M} P\left( v_{j,i}^{(NMS-1)} | P, v_{1,1}^{(NMS-2)}, ..., v_{i,j}^{(NMS-2)}, ..., v_{M,1}^{(NMS-2)}, ..., v_{M,N}^{(NMS-2)} \right) = \]

\[ \prod_{i=1}^{N} \prod_{j=1}^{M} \frac{1}{\sqrt{2\pi \sigma_{ji}}} \exp \left( \frac{-(v_{j,i}^{(NMS-1)} - 2\sum_{h=1}^{M} \tilde{v}_{h,i}^{(NMS-2)} p_{hj})^2}{2\sigma_{ji}^2} \right) = \]

\[ = \frac{1}{(2\pi)^{MN/2}} \prod_{i=1}^{N} \prod_{j=1}^{M} \sigma_{ji} \exp \left( -\sum_{i=1}^{N} \sum_{j=1}^{M} \frac{(v_{j,i}^{(NMS-1)} - 2\sum_{h=1}^{M} \tilde{v}_{h,i}^{(NMS-2)} p_{hj})^2}{2\sigma_{ji}^2} \right). \tag{S21} \]
where, $\sigma_{ji}^2$, $j \in [1, M]$ denotes the standard deviation of Gaussian noise for cell type $j$ in sample $i$. Replacing (S19)-(S21) in (S18), we have

$$P\left(v_{i,1}^{(0)}, \ldots, v_{l,N}^{(0)}, \ldots, v_{M,1}^{(0)}, \ldots, v_{l,N}^{(NMS-1)}, \ldots, v_{M,N}^{(NMS-1)}, \ldots, v_{M,N}^{(NMS-1)} \middle| P\right) =$$

$$\frac{1}{(2\pi)^{MN/2} \prod_{i=1}^{N} \prod_{j=1}^{M} \sigma_{ji}^{NMS}}$$

$$\exp \left\{ -\sum_{i=1}^{N} \sum_{j=1}^{M} \frac{v_{j,i}^{(0)} - \tilde{v}_{j,i}^{(0)}}{2\sigma_{ji}^{2}} - \sum_{i=1}^{N} \sum_{j=1}^{M} \frac{v_{j,i}^{(1)} - \tilde{v}_{j,i}^{(0)}}{2\sigma_{ji}^{2}} - \sum_{i=1}^{N} \sum_{j=1}^{M} \frac{v_{j,i}^{(NMS-1)} - \tilde{v}_{j,i}^{(NMS-2)}}{2\sigma_{ji}^{2}} \right\}$$

(S22)

Taking the Logarithm of the above equation and ignoring the constant terms, we have

$$\log P\left(v_{i,1}^{(0)}, \ldots, v_{l,N}^{(0)}, \ldots, v_{M,1}^{(0)}, \ldots, v_{l,N}^{(NMS-1)}, \ldots, v_{M,N}^{(NMS-1)}, \ldots, v_{M,N}^{(NMS-1)} \middle| P\right) =$$

$$-NMS \log \left( \frac{1}{\sqrt{2\pi}} \prod_{i=1}^{N} \prod_{j=1}^{M} \sigma_{ji}^{NMS} \right) - \sum_{i=1}^{N} \sum_{j=1}^{M} \frac{v_{j,i}^{(0)} - \tilde{v}_{j,i}^{(0)}}{2\sigma_{ji}^{2}} - \sum_{i=1}^{N} \sum_{j=1}^{M} \frac{v_{j,i}^{(1)} - \tilde{v}_{j,i}^{(0)}}{2\sigma_{ji}^{2}} - \sum_{i=1}^{N} \sum_{j=1}^{M} \frac{v_{j,i}^{(NMS-1)} - \tilde{v}_{j,i}^{(NMS-2)}}{2\sigma_{ji}^{2}} \right)$$

(S23)

Taking the derivative with respect to $p_{kl}$ and setting it to zero, we have $M$ equations as follows

$$\sum_{j=1}^{M} \left( \frac{\tilde{v}_{k,i}^{(0)} - \tilde{v}_{k,i}^{(1)} - 2\sum_{h=1}^{M} \tilde{v}_{h,i}^{(0)} p_{hl}}{2\sigma_{k}^{2}} \right) + \cdots + \left( \frac{\tilde{v}_{k,i}^{(NMS-2)} - 2\sum_{h=1}^{M} \tilde{v}_{h,i}^{(NMS-2)} p_{hl}}{2\sigma_{k}^{2}} \right) = 0,$$

$$2\sum_{i=1}^{N} \sum_{j=1}^{M} \sum_{h=1}^{M} \frac{\tilde{v}_{k,i}^{(j-1)} - \tilde{v}_{k,i}^{(j)}}{2\sigma_{k}^{2}} = 2\sum_{i=1}^{N} \sum_{j=1}^{M} \frac{\tilde{v}_{h,i}^{(j-1)} - \tilde{v}_{h,i}^{(j)}}{2\sigma_{h}^{2}},$$

$$\sum_{i=1}^{N} \left[ \sum_{j=1}^{M} \tilde{v}_{k,i}^{(0)} \sum_{h=1}^{M} \tilde{v}_{h,i}^{(0)} p_{hl} \right] + \cdots + \left[ \sum_{i=1}^{N} \sum_{j=1}^{M} \tilde{v}_{h,i}^{(NMS-2)} \sum_{h=1}^{M} \tilde{v}_{h,i}^{(NMS-2)} p_{hl} \right] = \sum_{i=1}^{N} \left[ \sum_{j=1}^{M} \tilde{v}_{k,i}^{(0)} \sum_{h=1}^{M} \tilde{v}_{h,i}^{(0)} p_{hl} \right] + \cdots + \left[ \sum_{i=1}^{N} \sum_{j=1}^{M} \tilde{v}_{h,i}^{(NMS-2)} \sum_{h=1}^{M} \tilde{v}_{h,i}^{(NMS-2)} p_{hl} \right],$$

(S24)
In the setting under consideration, we make separate observations based on each sample, and attempt to obtain a common optimized transition probability for the underlying Markov process. As a result, we satisfy the above equation by enforcing the constraint for each sample. We have

\[
\begin{bmatrix}
\tilde{v}_{k,i}^{(0)} & \tilde{v}_{k,i}^{(1)} & \ldots & \tilde{v}_{k,i}^{(NMS-2)} \\
\vdots & \vdots & \ddots & \vdots \\
\tilde{v}_{M,i}^{(0)} & \tilde{v}_{M,i}^{(1)} & \ldots & \tilde{v}_{M,i}^{(NMS-2)} \\
\end{bmatrix}
\begin{bmatrix}
2\sigma_u^{-2} \sum_{h=1}^{M} \tilde{v}_{h,i}^{(0)} p_{hl} \\
2\sigma_u^{-2} \sum_{h=1}^{M} \tilde{v}_{h,i}^{(1)} p_{hl} \\
\vdots \\
2\sigma_u^{-2} \sum_{h=1}^{M} \tilde{v}_{h,i}^{(NMS-2)} p_{hl} \\
\end{bmatrix}
= 
\begin{bmatrix}
\tilde{v}_{k,i}^{(0)} & \tilde{v}_{k,i}^{(1)} & \ldots & \tilde{v}_{k,i}^{(NMS-2)} \\
\vdots & \vdots & \ddots & \vdots \\
\tilde{v}_{M,i}^{(0)} & \tilde{v}_{M,i}^{(1)} & \ldots & \tilde{v}_{M,i}^{(NMS-2)} \\
\end{bmatrix}
\begin{bmatrix}
\sigma_u^{-2} v_{l,i}^{(1)} \\
\sigma_u^{-2} v_{l,i}^{(2)} \\
\vdots \\
\sigma_u^{-2} v_{l,i}^{(NMS-1)} \\
\end{bmatrix}
\]

(S25)

Hence for all values of \( k \), we have following equations,

\[
\begin{bmatrix}
\tilde{v}_{1,i}^{(0)} & \tilde{v}_{1,i}^{(1)} & \ldots & \tilde{v}_{1,i}^{(NMS-2)} \\
\vdots & \vdots & \ddots & \vdots \\
\tilde{v}_{M,i}^{(0)} & \tilde{v}_{M,i}^{(1)} & \ldots & \tilde{v}_{M,i}^{(NMS-2)} \\
\end{bmatrix}
\begin{bmatrix}
2\sigma_u^{-2} \sum_{h=1}^{M} \tilde{v}_{h,i}^{(0)} p_{dl} \\
2\sigma_u^{-2} \sum_{h=1}^{M} \tilde{v}_{h,i}^{(1)} p_{dl} \\
\vdots \\
2\sigma_u^{-2} \sum_{h=1}^{M} \tilde{v}_{h,i}^{(NMS-2)} p_{dl} \\
\end{bmatrix}
= 
\begin{bmatrix}
\tilde{v}_{1,i}^{(0)} & \tilde{v}_{1,i}^{(1)} & \ldots & \tilde{v}_{1,i}^{(NMS-2)} \\
\vdots & \vdots & \ddots & \vdots \\
\tilde{v}_{M,i}^{(0)} & \tilde{v}_{M,i}^{(1)} & \ldots & \tilde{v}_{M,i}^{(NMS-2)} \\
\end{bmatrix}
\begin{bmatrix}
\sigma_u^{-2} v_{l,i}^{(1)} \\
\sigma_u^{-2} v_{l,i}^{(2)} \\
\vdots \\
\sigma_u^{-2} v_{l,i}^{(NMS-1)} \\
\end{bmatrix}
\]

(S26)

If matrix \( V_i \) is full column rank, we simply have

\[
\begin{bmatrix}
2\sum_{h=1}^{M} \tilde{v}_{h,i}^{(0)} p_{dl} \\
2\sum_{h=1}^{M} \tilde{v}_{h,i}^{(1)} p_{dl} \\
\vdots \\
2\sum_{h=1}^{M} \tilde{v}_{h,i}^{(NMS-2)} p_{dl} \\
\end{bmatrix}
= 
\begin{bmatrix}
\tilde{v}_{1,i}^{(0)} & \tilde{v}_{1,i}^{(1)} & \ldots & \tilde{v}_{1,i}^{(NMS-2)} \\
\vdots & \vdots & \ddots & \vdots \\
\tilde{v}_{M,i}^{(0)} & \tilde{v}_{M,i}^{(1)} & \ldots & \tilde{v}_{M,i}^{(NMS-2)} \\
\end{bmatrix}
\begin{bmatrix}
\sigma_u^{-2} v_{l,i}^{(1)} \\
\sigma_u^{-2} v_{l,i}^{(2)} \\
\vdots \\
\sigma_u^{-2} v_{l,i}^{(NMS-1)} \\
\end{bmatrix}
\]

(S27)

For each value of \( i \in [1, N] \), if \( NMS - 1 < M \), the above system of linear equations for \( p_{dl} \) has infinitely many solutions, and is an underdetermined system. If \( NMS - 1 = M \), the above system of linear equations has a single unique solution. Due to independency of noise in different samples, joint probability of observations in (18), can be
factorized to multiplication of joint probability for \( N \) samples. Hence, the solution of (S27) maximizes each component of joint probability in (S18). If \( NMS - 1 > M \), such a system has no solution, and is an over determined system. In this case, if we solve (S27) with \( \ell_2 \) norm, we obtain the same results of Theorem 1.

Section D

In a Poisson process, the number of observed occurrences fluctuates about its mean \( \tilde{v}_{j,i}^{(k)} \) with a standard deviation of \( \sigma_{j,i} = \tilde{v}_{j,i}^{(k)} \). These fluctuations are due to what is known as shot noise and are signal dependent. In this Section, the system of equations for cell proliferation when observations are corrupted by shot noise is derived using an ML estimator. In shot noise, the observations are Poisson distributed, and the terms in (S18) may be computed as follows

\[
P\left(v_{i,1}^{(0)}, \ldots, v_{i,N}^{(0)}, v_{M,1}^{(0)}, \ldots, v_{M,N}^{(0)} \middle| \mathbf{P} \right) = \prod_{i=1}^{N} \prod_{j=1}^{M} P\left(v_{j,i}^{(0)} \middle| \mathbf{P} \right)
\]

\[
= \prod_{i=1}^{N} \prod_{j=1}^{M} \frac{\left(\sum_{h=1}^{M} v_{h,i}^{(0)} P_{j} \right)^{v_{j,i}^{(0)}}}{v_{j,i}^{(0)}!} \exp(-2 \sum_{h=1}^{M} v_{h,i}^{(0)} P_{j})
\]

(S28)

\[
P\left(v_{1,1}^{(NMS-1)}, \ldots, v_{1,N}^{(NMS-1)}, \ldots, v_{M,1}^{(NMS-1)}, \ldots, v_{M,N}^{(NMS-1)} \middle| \mathbf{P}, v_{1,1}^{(NMS-2)}, \ldots, v_{1,N}^{(NMS-2)}, \ldots, v_{M,1}^{(NMS-2)}, \ldots, v_{M,N}^{(NMS-2)} \right)
\]

\[
= \prod_{i=1}^{N} \prod_{j=1}^{M} \frac{\left(\sum_{h=1}^{M} v_{h,i}^{(NMS-2)} P_{j} \right)^{v_{j,i}^{(NMS-1)}}}{v_{j,i}^{(NMS-1)}!} \exp(-2 \sum_{h=1}^{M} v_{h,i}^{(NMS-2)} P_{j})
\]

(S30)

in which \( \tilde{v}_{j,i}^{(0)} \) is the true initial population size of cell type \( j \) in sample \( i \). Hence, using (S28) – (S30) in (S18), we have

...
\[
P(v^{(0)}_{1,1}, ..., v^{(0)}_{1,N}, ..., v^{(NMS-1)}_{M,N}, ..., v^{(NMS-1)}_{M,N} \mid P) = \prod_{i=1}^{N} \prod_{j=1}^{M} \left( \frac{\bar{v}^{(0)}_{j,i}}{v^{(0)}_{j,i}} \right) \prod_{i=1}^{N} \prod_{j=1}^{M} \left( 2 \sum_{h=1}^{M} \bar{v}^{(0)}_{h,i} p_{h,j} \right) \left( \frac{v^{(0)}_{j,i}}{v^{(0)}_{j,i}} \right) ...
\]

\[
\prod_{i=1}^{N} \prod_{j=1}^{M} \left( 2 \sum_{h=1}^{M} \bar{v}^{(NMS-2)}_{h,i} p_{h,j} \right) \left( \frac{v^{(NMS-1)}_{j,i}}{v^{(NMS-1)}_{j,i}} \right)
\]

Taking the Logarithm of the above equation and ignoring the constant terms, we have

\[
\log P(v^{(0)}_{1,1}, ..., v^{(0)}_{1,N}, ..., v^{(NMS-1)}_{M,N}, ..., v^{(NMS-1)}_{M,N} \mid P) = \sum_{i=1}^{N} \sum_{j=1}^{M} \left( v^{(0)}_{j,i} \log \left( \frac{v^{(0)}_{j,i}}{\bar{v}^{(0)}_{j,i}} \right) + v^{(1)}_{j,i} \log \left( \frac{v^{(1)}_{j,i}}{\bar{v}^{(1)}_{j,i}} \right) + 2 \sum_{h=1}^{M} \bar{v}^{(NMS-2)}_{h,i} p_{h,j} - \log \left( v^{(NMS-1)}_{j,i} \right) \right)
\]

\[
= -\sum_{i=1}^{N} \sum_{j=1}^{M} \left( \bar{v}^{(0)}_{j,i} - 2 \sum_{h=1}^{M} \bar{v}^{(0)}_{h,i} p_{h,j} - ... - 2 \sum_{h=1}^{M} \bar{v}^{(NMS-2)}_{h,i} p_{h,j} \right)
\]

Computing the derivative with respect to \( p_{kl} \) and setting the result to zero, we have \( M \) equations

\[
\sum_{i=1}^{N} \sum_{j=1}^{M} \frac{v^{(1)}_{j,i} v^{(0)}_{k,i}}{\bar{v}^{(0)}_{j,i} v^{(NMS-2)}_{k,i}} + ... + \frac{v^{(1)}_{j,i} v^{(NMS-1)}_{k,i}}{\bar{v}^{(NMS-2)}_{k,i}} - 2 \left( \bar{v}^{(0)}_{k,i} + ... + \bar{v}^{(NMS-2)}_{k,i} \right) = 0 \Rightarrow
\]

\[
\sum_{i=1}^{N} \sum_{j=1}^{M} \frac{v^{(1)}_{j,i} v^{(0)}_{k,i}}{\bar{v}^{(0)}_{j,i} v^{(NMS-2)}_{k,i}} + ... + \frac{v^{(1)}_{j,i} v^{(NMS-1)}_{k,i}}{\bar{v}^{(NMS-2)}_{k,i}} = \sum_{i=1}^{N} \sum_{j=1}^{M} \left( \bar{v}^{(0)}_{k,i} + ... + \bar{v}^{(NMS-2)}_{k,i} \right) \Rightarrow
\]

\[
\sum_{i=1}^{N} \sum_{j=1}^{M} \frac{v^{(1)}_{j,i} v^{(0)}_{k,i}}{p_{h,i}} + ... + \frac{v^{(1)}_{j,i} v^{(NMS-1)}_{k,i}}{p_{h,i}} = \sum_{i=1}^{N} \sum_{j=1}^{M} \bar{v}^{(0)}_{h,i} p_{h,i} + ... + \bar{v}^{(NMS-2)}_{h,i} p_{h,i}
\]

\[
\sum_{i=1}^{N} \sum_{j=1}^{M} \left( \frac{v^{(0)}_{h,i} p_{h,i}}{p_{h,i}} \prod_{q=1}^{NMS-2} \prod_{h=1}^{M} v^{(j)}_{h,i} p_{h,i} \right) = 0
\]

\[
= \sum_{i=1}^{N} \sum_{j=1}^{M} \left( \frac{v^{(0)}_{h,i} p_{h,i}}{p_{h,i}} \prod_{q=1}^{NMS-2} \prod_{h=1}^{M} v^{(j)}_{h,i} p_{h,i} \right) \Rightarrow
\]

\[
= \sum_{i=1}^{N} \sum_{j=1}^{M} \left( \frac{v^{(0)}_{h,i} p_{h,i}}{p_{h,i}} \prod_{q=1}^{NMS-2} \prod_{h=1}^{M} v^{(j)}_{h,i} p_{h,i} \right) \Rightarrow
\]

\[
\sum_{i=1}^{N} \sum_{j=1}^{M} \frac{v^{(r-1)}_{h,i} v^{(r-1)}_{h,i}}{p_{h,i}} + ... + \frac{v^{(NMS-2)}_{h,i} v^{(NMS-2)}_{h,i}}{p_{h,i}} = 2 \sum_{i=1}^{N} \sum_{j=1}^{M} \bar{v}^{(r-1)}_{h,i} p_{h,i}
\]

\[
\sum_{i=1}^{N} \sum_{j=1}^{M} \bar{v}^{(r-1)}_{h,i} p_{h,i} = \sum_{i=1}^{N} \sum_{j=1}^{M} \bar{v}^{(r-1)}_{h,i} p_{h,i} = \sum_{i=1}^{N} \sum_{j=1}^{M} \bar{v}^{(r-1)}_{h,i} p_{h,i}
\]

Eq. (S33) in matrix form can be written as
Hence, for all values of $k$, we have the following equations,
If the matrix $\tilde{V}_i$ given by

$$
\tilde{V}_i = \begin{bmatrix}
\tilde{v}_{1,i}^{(0)} & \tilde{v}_{1,i}^{(1)} & \cdots & \tilde{v}_{1,i}^{(NMS-2)} \\
\tilde{v}_{2,i}^{(0)} & \tilde{v}_{2,i}^{(1)} & \cdots & \tilde{v}_{2,i}^{(NMS-2)} \\
\vdots & \vdots & \ddots & \vdots \\
\tilde{v}_{M,i}^{(0)} & \tilde{v}_{M,i}^{(1)} & \cdots & \tilde{v}_{M,i}^{(NMS-2)}
\end{bmatrix}
$$

(S37)

is full column rank, its null space is reduced to the singleton $\{0\}$. This matrix is column full rank when the number of column is less than the number of rows, i.e., $NMS - 1 < M$, which means $V_i^T V_i$ is invertible. Hence, by this assumption we have

$$
\begin{bmatrix}
\prod_{q=1}^{NMS-1} \prod_{(q,j)\neq(i)} v_{i,j}^{(i)} \\
\prod_{q=1}^{NMS-1} \sum_{h=1}^{N} v_{h,q}^{(i)} P_{hl} \\
\vdots \\
\prod_{q=1}^{NMS-1} \sum_{h=1}^{NMS-2} v_{h,q}^{(i)} P_{hl}
\end{bmatrix} - \begin{bmatrix}
2\prod_{q=1}^{N} \prod_{j=0}^{M} v_{h,q}^{(i)} P_{hl} \\
2\prod_{q=1}^{N} \prod_{j=0}^{M} v_{h,q}^{(i)} P_{hl} \\
\vdots \\
2\prod_{q=1}^{N} \prod_{j=0}^{M} v_{h,q}^{(i)} P_{hl}
\end{bmatrix} = 0_{M \times 1}
$$

(S38)

Simplifying (S38), we have

$$
\begin{bmatrix}
\tilde{v}_{1,i}^{(1)} \\
\tilde{v}_{1,i}^{(2)} \\
\vdots \\
\tilde{v}_{1,i}^{(NMS-1)}
\end{bmatrix} = 2 \begin{bmatrix}
\sum_{h=1}^{M} v_{h,i}^{(0)} P_{hl} \\
\sum_{h=1}^{M} \tilde{v}_{h,i}^{(1)} P_{hl} \\
\sum_{h=1}^{NMS-2} \tilde{v}_{h,i}^{(NMS-2)} P_{hl}
\end{bmatrix} 
$$

(S39)

This is the model we use for the Poisson observations of the cell proliferation Markov process.

Section E. Proof of Proposition 1
Starting from (S39), since a direction solution for the transition probabilities is not accessible in this case, we resort to an approximation. To this end, we use an one sample estimator for $v_{1,i}^{(0)}$ and replace it by $v_{1,i}^{(0)}$. We have

$$
\begin{bmatrix}
v_{1,i}^{(0)} & v_{2,i}^{(0)} & \cdots & v_{M,i}^{(0)} \\
v_{1,i}^{(0)} & v_{2,i}^{(0)} & \cdots & v_{M,i}^{(0)} \\
\vdots & \vdots & \ddots & \vdots \\
v_{1,i}^{(NMS-2)} & v_{2,i}^{(NMS-2)} & \cdots & v_{M,i}^{(NMS-2)}
\end{bmatrix}
\begin{bmatrix}
p_{il} \\
p_{2l} \\
\vdots \\
p_{Ml}
\end{bmatrix}
= 
\begin{bmatrix}
v_{1,i}^{(1)} \\
v_{1,i}^{(1)} \\
\vdots \\
v_{1,i}^{(NMS-1)}
\end{bmatrix}.
$$

(S40)

where, $i \in \{1, N\}$. The equation (S40) is satisfied for each sample, $i$, and cell type, $l$. The goal is set to find $p_{hl}$, $h,l \in \{1, M\}$, which fit the equations best in the minimum mean squared sense. We have

$$\hat{\mathbf{P}} = \arg \min_{\mathbf{P}} S(\mathbf{P})$$

s.t.

$$\forall \ 1 \leq h \leq M, \ 1 \leq l \leq M,$$

$$0 \leq p_{hl} \leq 1,$$

$$\sum_{l=1}^{M} p_{hl} = 1,$$

where the objective function $S$ is defined as

$$S(\mathbf{P}) = \sum_{l=1}^{M} \left( \sum_{i=1}^{N} \sum_{j=1}^{NMS-1} \left( v_{ij}^{(j)} - 2 \sum_{h=1}^{M} v_{ih,j}^{(j-1)} p_{hl} \right)^2 \right).$$

(S42)

The optimization problem in (S41) is convex, (cost function is quadratic and constraints are linear [2]). Using KKT (Karush–Kuhn–Tucker), the unconstrained solution for this problem is given by

$$S_{U}(\mathbf{P}) = \sum_{i=1}^{M} \left( S(\mathbf{P}_i) + \lambda_i \left( \sum_{j=1}^{M} p_{j} - 1 \right) + \sum_{j=1}^{M} \left( \lambda'_j - \lambda''_j \right) p_{jl} \right),$$

(S43)

where $\lambda'_j$, $\lambda''_j$ and $\lambda_i$ are Lagrange multipliers and $\mathbf{P}_i = \begin{bmatrix} p_{i1} & \cdots & p_{im} \end{bmatrix}$. Computing the derivative of the cost function, with respect to $p_{hl}$ and following some mathematical manipulations (similar to the Gaussian scenario), we have

$$\hat{\mathbf{P}}_i = 0.5 \left( \mathbf{V}_{l,l}^{(0:NMS-2)} \right)^\dagger \left( \mathbf{V}_{l,l}^{(0:NMS-2)} \right)^{-1} \left( \mathbf{V}_{l,l}^{(0:NMS-2)} \right)^\dagger \mathbf{V}_{l,t(NM-1)}^{(l:NMS-1)} - \Lambda_i,$$

(S44)

where $\mathbf{V}_{l,l}^{(0:NMS-2)}$, $\mathbf{V}_{l,l}^{(l:NMS-1)}$ and $\Lambda_i$ are defined in (S-13), (S-14) and (S-16). The above solution is obtained when the constraints $0 \leq p_{ij} \leq 1$ is assumed satisfied. However, if the obtained results violate these constraints, one should enforce them and solve the problem again (see Section B for details on handling this issue).
Section F. Proof of Proposition 2

Starting from (S39), since a direction solution for the transition probabilities is not accessible in this case, we resort to an alternative approximation in this Section. To this end, we consider the summation of (S39) over \(i\) and obtain

\[
\begin{bmatrix}
\sum_{i=1}^{N} \hat{v}_{i,i}^{(0)} & \cdots & \sum_{i=1}^{N} \hat{v}_{i,i}^{(M,0)} \\
\vdots & \ddots & \vdots \\
\sum_{i=1}^{N} \hat{v}_{i,i}^{(NMS-2)} & \cdots & \sum_{i=1}^{N} \hat{v}_{i,i}^{(NMS-2)}
\end{bmatrix}
\begin{bmatrix}
\hat{p}_{ll} \\
\vdots \\
\hat{p}_{ll}
\end{bmatrix}
= 
\begin{bmatrix}
\sum_{i=1}^{N} v_{i,i}^{(1)} \\
\vdots \\
\sum_{i=1}^{N} v_{i,i}^{(NMS-1)}
\end{bmatrix}
\tag{S45}
\]

Using the definition of a sample mean, and assuming \(\frac{1}{N} \sum_{i=1}^{N} v_{i,i}^{(k)} = \frac{1}{N} \sum_{i=1}^{N} v_{i,i}^{(k)}\), we have

\[
\begin{bmatrix}
\sum_{i=1}^{N} v_{i,i}^{(0)} & \cdots & \sum_{i=1}^{N} v_{i,i}^{(M,0)} \\
\vdots & \ddots & \vdots \\
\sum_{i=1}^{N} v_{i,i}^{(NMS-2)} & \cdots & \sum_{i=1}^{N} v_{i,i}^{(NMS-2)}
\end{bmatrix}
\begin{bmatrix}
\hat{p}_{ll} \\
\vdots \\
\hat{p}_{ll}
\end{bmatrix}
= 
\begin{bmatrix}
\sum_{i=1}^{N} v_{i,i}^{(1)} \\
\vdots \\
\sum_{i=1}^{N} v_{i,i}^{(NMS-1)}
\end{bmatrix}
\tag{S46}
\]

If \(NMS - 1 < M\), this linear algebraic system has an infinite number of solutions. If \(NMS - 1 = M\), the above system of linear equations for \(\hat{p}_{ll}\) has a single solution

\[
\begin{bmatrix}
\hat{p}_{ll} \\
\vdots \\
\hat{p}_{ll}
\end{bmatrix}
= 0.5
\begin{bmatrix}
\sum_{i=1}^{N} v_{i,i}^{(0)} & \cdots & \sum_{i=1}^{N} v_{i,i}^{(0)} \\
\vdots & \ddots & \vdots \\
\sum_{i=1}^{N} v_{i,i}^{(NMS-2)} & \cdots & \sum_{i=1}^{N} v_{i,i}^{(NMS-2)}
\end{bmatrix}^{-1}
\begin{bmatrix}
\sum_{i=1}^{N} v_{i,i}^{(1)} \\
\vdots \\
\sum_{i=1}^{N} v_{i,i}^{(NMS-1)}
\end{bmatrix}
\tag{S47}
\]

If \(NMS - 1 > M\), the solution in MSE sense is obtained with the same approach for the Gaussian distribution by replacing \(\hat{V}_{i,N}^{(0:MS-2)}\) and \(\hat{V}_{i,N}^{(1:MS-1)}\) in (S13) and (S14) by \(\hat{\Sigma}_{w}\) and \(\hat{\Psi}_{l}\), which are described as follows

\[
\hat{\Sigma}_{w} = 
\begin{bmatrix}
\sum_{i=1}^{N} v_{i,i}^{(0)} & \cdots & \sum_{i=1}^{N} v_{i,i}^{(0)} \\
\vdots & \ddots & \vdots \\
\sum_{i=1}^{N} v_{i,i}^{(NMS-2)} & \cdots & \sum_{i=1}^{N} v_{i,i}^{(NMS-2)}
\end{bmatrix}
\tag{S48}
\]

\[
\hat{\Psi}_{l} = 
\begin{bmatrix}
\sum_{i=1}^{N} v_{i,i}^{(1)} & \cdots & \sum_{i=1}^{N} v_{i,i}^{(NMS-1)}
\end{bmatrix}
\tag{S49}
\]
The above solution is obtained when the constraints \((0 \leq p_{ij} \leq 1)\) is assumed satisfied. However, if the obtained results violate these constraints, one should enforce them and solve the problem again (see Section B for details on handling this issue).
References

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