Clustering of time-course gene expression profiles using normal mixture models with AR(1) random effects

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ABSTRACT

Motivation: Time-course gene expression data such as yeast cell cycle data may be periodically expressed. To cluster such data, currently used Fourier series approximations of periodic gene expressions have been found not to be sufficiently adequate to model the complexity of the time-course data, partly due to their ignoring the dependence between the expression measurements over time and the correlation among gene expression profiles. We further investigate the advantages and limitations of available models in the literature and propose a new mixture model with AR(1) random effects for the clustering of time-course gene-expression profiles. Some simulations and real examples are given to demonstrate the usefulness of the proposed models.

Results: We illustrate the applicability of our new model using synthetic and real-time-course datasets. We show that our model outperforms existing models to provide more reliable and robust clustering of time-course data. Our model provides superior results when genetic profiles are correlated. It also gives comparable results when the correlation between the gene profiles is weak. In the applications to real time-course data, relevant clusters of co-regulated genes are obtained, which are supported by gene-function annotation databases.

Availability: An R-program is available on request from the corresponding author.

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Supplementary Information: http://www.maths.uq.edu.au/~gjm/bioinf_2011_supp.pdf.

1 INTRODUCTION

DNA microarray analysis has emerged as a leading technology to enhance our understanding of gene regulation and function in cellular mechanism controls on a genomic scale. This technology has advanced to unravel the genetic machinery of biological rhythms by collecting massive gene-expression data in a time course. Time-course gene expression data such as yeast cell cycle data \cite{Wichert2001} appear to be periodically expressed. To associate the profile of gene expression with a physiological function of interest, it is crucial to cluster the types of gene expression on the basis of their periodic patterns. The identification of co-expressed genes also facilitates the prediction of response to treatment or toxic compounds \cite{Hafemeister2011}. Statistical modelling and algorithms play a central role in cataloguing dynamic gene-expression profiles.

Various computational models have been developed for gene clustering based on cross-sectional microarray data \cite{McLachlan2002}. \textsuperscript{1002} Ramirez et al. \cite{2002}, Fan and Reis \cite{2006}. Also, considerable attention has been paid to methodological derivations for detecting temporal patterns of gene expression in a time course based on functional principal component analysis or mixture model analysis \cite{Qin2006, Xu2003, Luan2003, Luan2004, Storey2005, Hong2006, Ma2006, Ng2006, Kim2008, Booth2008}, including the applications to identify differentially expressed genes over time \cite{Park2003, Sun2011}.

Finite mixture models \cite{McLachlan2000a} have been widely used to model the distributions of a variety of random phenomena. Multivariate normality is generally assumed for multivariate data of a continuous nature. The multivariate normal mixture model is employed to detect different patterns in gene-expression profiles. However, when the two assumptions that are commonly adopted in practice, namely,

(1) there are no replications on any particular entity specifically identified as such and
(2) all the observations on the entities are independent of one another,

are violated, multivariate normal mixture models may not be adequate. For example, condition (2) will not hold for the clustering of gene profiles, since not all the genes are independently distributed, and condition (1) will generally not hold either as the gene profiles may be measured over time or on technical replicates. While this correlated structure can be incorporated into the normal mixture model by appropriate specification of the component-covariance matrices, it is difficult to fit the model under such specifications. For example, the M-step may not exist in closed form \cite{McLachlan2004}.

Accordingly, \cite{Ng2006} have developed the procedure called EMMIX-WIRE (EM-based MIXtire analysis With Random Effects) to handle the clustering of correlated data that may be replicated. They adopted a mixture of linear mixed models to specify the correlation structure between the variables and to allow for correlations among the observations. It also enables covariate information to be incorporated into the clustering process \cite{Ng2006}. Proceeding conditionally on the tissue-specific random effects as formulated in \cite{Ng2006}, the E- and M-steps can be implemented in closed form. In particular, an approximation to the E-step by carrying out time-consuming Monte Carlo methods is not required. A probabilistic or an outright clustering of the genes into g components can be obtained, based on the estimated posterior

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probabilities of component membership given the profile vectors and the estimated tissue-specific random effects; see Ng et al. (2006).

Fourier series approximations have been used to model periodic gene expression, leading to the detection of periodic signals in various organisms including yeast and human cells (Spellman et al., 1998; Wichert et al., 2004; Kim et al., 2004). If the genes studied are periodically regulated, their time-dependent expression can be accurately approximated by a Fourier series approximation (Spellman et al., 1998). A general form of the kth order Fourier series expansion is given as

\[ g_k(t) = a_0 + \sum_{j=1}^{k} a_j \cos(2\pi j t/\omega) + b_j \sin(2\pi j t/\omega), \]  

where \( a_0 \) is the average value of \( g_k(t) \). The other coefficients \( a_k \) and \( b_k \) are the amplitude coefficients that determine the times at which the gene achieves peak and trough expression levels, respectively, and \( \omega \) is the period of the signal of gene expression. While the time-dependent expression value of a gene can be adequately modelled by a Fourier series approximation of the first three orders (Kim et al., 2008), recent results (Kim et al., 2008; Ng et al., 2006) demonstrate that the first-order Fourier series approximation is sufficient to provide good results in terms of clustering the time-course data into meaningful functional groups. Alternatively, the likelihood ratio test may be used to determine the order of the Fourier series approximation within the nested regression models.

The EMMIX-WIRE model of Ng et al. (2006) is developed primarily for clustering genes from general microarray experimental designs. On the other hand, Kim et al. (2008) focus specifically on clustering periodic gene profiles and propose a special covariance structure to incorporate the correlation between observations at different time points. They also review current methods and compare their method with that of Ng et al. (2006). More recently, Schäli et al. (2010) use integrated autoregressive (AR) models to create cluster centers in their simulation study of mixtures of regression models for time-course gene expression data through the new version of software FlexMix of Leisch (2004). Wang and Fan (2010) propose mixtures of multivariate linear mixed models with autoregressive errors to analyse longitudinal data. In this paper, we propose a new EMMIX-WIRE normal mixture regression model with AR(1) random effects for the clustering of time-course data. In particular, the model accounts for the correlation among gene profiles and models the dependence between expressions over time via AR(1) random effects.

The paper is organized as follow: Section 2 presents the development of the extension of the EMMIX-WIRE model to incorporate AR(1) random effects which are fitted under the EM framework. We conduct a simulation study and the data analysis with two real yeast cell data in Section 3. In the last section, some discussion is provided. The technical details of the derivations are provided in the Supplementary Information.

2 EMMIX-WIRE MODEL WITH AR(1) RANDOM EFFECTS

We let \( X \) denote the design matrix and \( \beta \) the associated vector of regression coefficients for the fixed effects. In the specification of the mixture of mixed linear components as adopted by Ng et al. (2006), the vector \( y_j \) for the \( j \)th gene conditional on its membership of the \( k \)th component of the mixture is expressed as

\[ y_j = X \beta_k + Z_1 u_{jh} + Z_2 v_h + \epsilon_{jh} \quad (j = 1, \ldots, n), \]  

where \( \beta_k \) is a \((2k + 1)\) vector containing unknown parameters \( a_0, a_1, \ldots, a_k, b_1, \ldots, b_k \); see (1). \( u_{jh} = (u_{j1h}, \ldots, u_{jmh})^T \) and \( v_h = (v_{h1}, \ldots, v_{hm})^T \) are the random effects, where \( m \) is the number of time points. In (2), \( Z_1 \) and \( Z_2 \) are \( m \times m \) identity matrices. Without loss of generality, we assume \( \epsilon_{jh} \) and \( v_h \) to be independent and normally distributed, \( N(0, \Omega) \) and \( N(0, D) \), independent of \( u_{jh} \). To further account for the time dependent random gene effects, a first-order autoregressive correlation structure is adopted for the gene profiles, so that \( u_{jh} \) follows a \( N(0, \theta^2 A(\rho)) \) distribution, where

\[ A(\rho) = \frac{1}{1 - \rho^2} \begin{pmatrix} 1 & \rho & \cdots & \rho^{m-1} \\ \rho & 1 & \cdots & \rho^{m-2} \\ \vdots & \vdots & \ddots & \vdots \\ \rho^{m-1} & \rho^{m-2} & \cdots & 1 \end{pmatrix}. \]  

The inverse of \( A(\rho) \) can be expressed as

\[ A(\rho)^{-1} = (1 + \rho^2) I - \rho J - \rho^2 K, \]  

and

\[ \text{trace} \left( \frac{\partial A(\rho)^{-1}}{\partial \rho} A(\rho) \right) = -2\rho/(1 - \rho^2), \]  

where \( I, J \) and \( K \) are all \( m \times m \) matrices. Specifically, \( I \) is the identity matrix; \( J \) has its sub-diagonal entries ones and zeros elsewhere, and \( K \) takes on the value 1 at the first and last element of its principal diagonal and zeros elsewhere. The expressions (4) and (5) are needed in the derivation of the maximum likelihood estimates of the parameters.

The assumptions (3) and (4) imply that our new model assumes an auto-correlation covariance structure under which measurements at each time point have a larger variance compared to the model of Kim et al. (2008) under an AR(1) auto-correlation residual structure.

In the context of mixture models, we consider the \( g \)-component mixture with probability density function (pdf) as

\[ f(y \mid \Psi) = \sum_{h=1}^g \frac{p_h f_h(y_j \mid \beta_h, \Omega_h, \theta_h^2, A_h, D_h)}{f_h}, \]  

where \( f_h \) is the component-pdf of the multivariate normal distribution with mean vector \( X_0 \beta_h \) and covariance matrix

\[ \theta_h^2 Z_1 A_h Z_1^T + Z_2 D_h Z_2^T + \Omega_h. \]  

The vector of unknown parameters is denoted by \( \Psi \) and can be estimated by maximum likelihood via the EM algorithm.

In the EM framework adopted here, the observed data vector \( y = (y_1, y_2, \ldots, y_n)^T \) is augmented by the unobservable component labels, \( z_1, z_2, \ldots, z_n \) of \( y_1, y_2, \ldots, y_n \), where \( z_j \) is the \( g \)-dimensional vector with \( h \)th element \( z_{jh} \), which is equal to 1 if \( y_j \) comes from the \( h \)th component of the mixture, and is zero otherwise. These unobservable values are considered to be missing data and are included in the so-called complete-data vector. Finally, we take the random effect vectors \( u_{jh} \) and \( v_h \) as

\( j = 1, \ldots, n; h = 1, \ldots, g \).
1. . . . q), to be missing and include them too in the complete-data vector. Now the so called complete-data log-likelihood \( l_c \) is the sum of four terms \( l_c = l_1 + l_2 + l_3 + l_4 \), where

\[
l_1 = \sum_{h=1}^{q} \sum_{j=1}^{n} z_{jh} \log(p_{hj})
\]

is the logarithm of the probability of the component labels \( z_{jh} \), and where \( l_2 \) is the logarithm of the density function of \( y \) conditional on \( u_j, v_h \), and \( z_{jh}=1 \), and \( l_3 \) and \( l_4 \) is the logarithm of the density function of \( u \) and \( v \), respectively, given \( z_{jh}=1 \),

\[
l_2 = -\frac{1}{2} \sum_{h=1}^{q} \sum_{j=1}^{n} z_{jh} \left( \text{mlog}(2\pi) + \log|\Omega_h| + \epsilon_{jh} \Omega_h^{-1} \epsilon_{jh} \right),
\]

\[
l_3 = -\frac{1}{2} \sum_{h=1}^{q} \sum_{j=1}^{n} z_{jh} \left( \text{mlog}(2\pi \theta_h^2) + \log|\Lambda_h| + \theta_h^{-2} u_j^T A_h^{-1} u_j \right),
\]

\[
l_4 = -\frac{1}{2} \sum_{h=1}^{q} \sum_{j=1}^{n} z_{jh} \left( \text{mlog}(2\pi) + \log|D_h| + v_j^T D_h^{-1} v_h \right),
\]

where

\[
\epsilon_{jh} = y_j - X_j \beta_h - Z_1 u_j - Z_2 v_h.
\]

To maximize the complete-data log-likelihood \( l_c \), the above decomposition implies that each of \( l_1, l_2, l_3, \) and \( l_4 \) can be maximized separately. The EM algorithm proceeds iteratively until the difference between successive values of the log likelihood is less than some specified threshold. All major derivatives are given in the Supplementary Information.

### 3 SIMULATIONS AND APPLICATIONS

#### 3.1 Simulation study

To illustrate the performance of the proposed model, we present a simulation study based on synthetic time-course data. In the following simulation, we consider an autocorrelation dependence for the periodic expressions and compare our model to that of Kim et al. (2008). Synthetic time-course data from three different parametric models (the full model under our new extended \( EMMIX^{*} \)-WIRE approach (denoted by EM-W in the tables), the extended model of Qin and Self (2006), and the model of Kim et al. (2008)), assuming a first-order Fourier series of periodicity, are considered in the simulation study. Within each model, we consider two different settings of \( \theta^2 \) corresponding to low and high auto-correlation among the periodic gene expressions. We also assume that \( \Omega \) and \( D \) are diagonal matrices, where the common diagonal elements are represented by \( \sigma^2 \) and \( \theta^2 \), respectively.

There are three classes of genes. The periods for each class are 6, 10 and 16, respectively. There are 24 measurements at time points 0, 1, ..., 23, and the first order Fourier expansion is adopted in the simulation models. Parameters and simulation results are listed in Tables 1 to 6. In each table, we summarize the results from 1000 simulated sets of data. The true values of the parameters and the means of their estimates are given in these tables, along with the standard errors in parentheses. We terminated the EM algorithm iterations when the absolute values of the relative changes in all estimates between consecutive iterations were smaller than 0.01.

#### Table 1. Bias and standard deviation in brackets from 1000 simulated data points (generated from new \( EMMIX^{*} \)-WIRE (EM-W) model with \( \theta^2_2 \) equal to 0.5

| Parameters | First component | Second component | Third component |
|------------|-----------------|-----------------|----------------|
| \( \rho(0.585, 0.045, 0.017) \) | (0.025, 0.019, 0.001) | (0.000, 0.000, 0.000) | (0.000, 0.000, 0.000) |
| \( \rho(0.585, 0.045, 0.017) \) | (0.025, 0.019, 0.001) | (0.000, 0.000, 0.000) | (0.000, 0.000, 0.000) |

#### Table 2. Bias and standard deviation in brackets from 1000 simulated data points (generated from new \( EMMIX^{*} \)-WIRE (EM-W) model with \( \theta^2_2 \) equal to 1.3

| Parameters | First component | Second component | Third component |
|------------|-----------------|-----------------|----------------|
| \( \rho(0.585, 0.045, 0.017) \) | (0.025, 0.019, 0.001) | (0.000, 0.000, 0.000) | (0.000, 0.000, 0.000) |
| \( \rho(0.585, 0.045, 0.017) \) | (0.025, 0.019, 0.001) | (0.000, 0.000, 0.000) | (0.000, 0.000, 0.000) |

To illustrate the performance of the proposed model, we present a simulation study based on synthetic time-course data. In the following simulation, we consider an autocorrelation dependence for the periodic expressions and compare our model to that of Kim et al. (2008). Synthetic time-course data from three different parametric models (the full model under our new extended \( EMMIX^{*} \)-WIRE approach (denoted by EM-W in the tables), the extended model of Qin and Self (2006), and the model of Kim et al. (2008)), assuming a first-order Fourier series of periodicity, are considered in the simulation study. Within each model, we consider two different settings of \( \theta^2 \) corresponding to low and high auto-correlation among the periodic gene expressions. We also assume that \( \Omega \) and \( D \) are diagonal matrices, where the common diagonal elements are represented by \( \sigma^2 \) and \( \theta^2 \), respectively.

There are three classes of genes. The periods for each class are 6, 10 and 16, respectively. There are 24 measurements at time points 0, 1, ..., 23, and the first order Fourier expansion is adopted in the simulation models. Parameters and simulation results are listed in Tables 1 to 6. In each table, we summarize the results from 1000 simulated sets of data. The true values of the parameters and the means of their estimates are given in these tables, along with the standard errors in parentheses. We terminated the EM algorithm iterations when the absolute values of the relative changes in all estimates between consecutive iterations were smaller than 0.01.
Table 3. Bias and standard deviation in brackets from 1000 simulated data points (generated from new EMMIX-WIRE (EM-W) model with $\theta_R^2$ equal to 0.5 and $d^2$ equal to 0)

| Parameters | First component | Second component | Third component |
|------------|----------------|-----------------|----------------|
|             | EM-W           | Km              | EM-W           | Km              | EM-W           | Km              |
| p(0.585)   | 0.001          | 0.008           | -0.001         | -0.003          | -0.001         | -0.005          |
| 0.1,0.315  | (0.009)        | (0.012)         | (0.008)        | (0.008)         | (0.010)        | (0.011)         |
| a0(0.3)    | 0.001          | 0.008           | -0.001         | -0.018          | 0.003          | -0.014          |
| 1.0,2      | (0.017)        | (0.019)         | (0.018)        | (0.026)         | (0.016)        | (0.016)         |
| a1(0.03)   | -0.002         | -0.023          | -0.001         | -0.005          | 0.003          | -0.006          |
| 1.0,02     | (0.049)        | (0.060)         | (0.059)        | (0.062)         | (0.049)        | (0.049)         |
| b1(0.06)   | -0.001         | -0.014          | 0.016          | 0.019           | 0.002          | 0.004           |
| 0.9,0.01   | (0.026)        | (0.031)         | (0.033)        | (0.038)         | (0.032)        | (0.033)         |
| $\sigma^2$(1,0) | 0.071 | 1.162       | 0.081          | 1.158           | 0.078          | 1.159           |
| 0.5,0.5    | (0.081)        | (1.162)         | (1.19)         | (1.160)         | (0.090)        | (1.159)         |
| $\rho$    | 0.032          | -0.337          | -0.037         | 0.339           | -0.036         | -0.339          |
| 0.6,0.6    | (0.038)        | (0.337)         | (0.062)        | (0.340)         | (0.045)        | (0.340)         |

Table 4. Bias and standard deviation in brackets from 1000 simulated data points (generated from new EMMIX-WIRE (EM-W) model with $\theta_R^2$ equal to 1.3 and $d^2$ equal to 0)

| Parameters | First component | Second component | Third component |
|------------|----------------|-----------------|----------------|
|             | EM-W           | Km              | EM-W           | Km              | EM-W           | Km              |
| p(0.585)   | -0.001         | 0.024           | 0.002          | -0.005          | -0.001         | -0.019          |
| 0.1,0.315  | (0.014)        | (0.029)         | (0.016)        | (0.017)         | (0.017)        | (0.026)         |
| a0(0.3)    | -0.001         | 0.018           | 0.003          | -0.046          | 0.000          | -0.005          |
| 1.0,2      | (0.027)        | (0.035)         | (0.026)        | (0.053)         | (0.021)        | (0.021)         |
| a1(0.03)   | 0.001          | -0.068          | 0.005          | -0.041          | 0.001          | 0.008           |
| 1.0,02     | (0.085)        | (0.146)         | (0.108)        | (0.127)         | (0.086)        | (0.085)         |
| b1(0.06)   | 0.003          | -0.031          | 0.005          | 0.047           | 0.002          | 0.004           |
| 0.9,0.01   | (0.042)        | (0.063)         | (0.054)        | (0.072)         | (0.030)        | (0.054)         |
| $\sigma^2$(1,1.3) | -0.059        | 1.254          | -0.076         | 1.251           | -0.052         | 1.242           |
| 1.3,1.3    | (0.087)        | (1.254)         | (1.178)        | (1.257)         | (1.014)        | (1.243)         |
| $\rho$    | 0.012          | -0.198          | -0.013         | -0.201          | 0.009          | -0.203          |
| 0.6,0.6    | (0.019)        | (0.199)         | (0.039)        | (0.206)         | (0.023)        | (0.204)         |

0.00001, with the maximum iteration of 1000. For our model, we started from the true partition: for [Kim et al., 2008], we started from the true values of parameters. Alternatively, initialization procedures have been considered for mixtures of regression models with and without random effects [Scharl et al., 2010]. For the comparison, we consider the misclassified error rate, the Rand Index, and the adjusted Rand Index [Hubert and Arabie, 1985], where the latter two assess the degree of agreement between the partition and the true clusters of genes. A larger (adjusted) Rand Index indicates a higher level of agreement.

Specifically, we first investigate the performance of our new extended EMMIX-WIRE model and that of [Kim et al., 2008] when the data are generated from the extended EMMIX-WIRE model, in which gene expressions within a cluster are correlated. As listed in Tables 1 and 2, the estimates of the parameters $p$, $a_0$, $a_1$, $b_1$, $\theta_2$, $\rho$, and $\sigma^2$ in the proposed model are approximately unbiased, except for $\sigma^2$, which is slightly underestimated. In contrast, the method of [Kim et al., 2008] fails to capture the contributions from gene-specific and tissue-specific effects on the auto-correlation among periodic gene expressions at each time point, and thus overestimates the correlation between different time points for each gene. Their method therefore leads to an inferior clustering performance in terms of higher error rates and smaller Rand Indices.

We now compare our model with the extended EMMIX-WIRE (EM-W) model of [Kim et al., 2008], which is a special case of our EMMIX-WIRE model (with $d^2 = 0$), where gene expressions are independent. The results are presented in Tables 3 and 4. As we explained in the last paragraph, the system errors are removed in this situation. And our model has unbiased estimation for all parameters. On the other hand, the model of [Kim et al., 2008] still overestimates the residual variance at different time points and underestimates the correlation between different time points for each gene, as it fails to capture the contribution from gene-specific effects to the auto-correlation among periodic gene expressions at each time point. Their method again produces larger error rates and slightly smaller Rand Indices.

Lastly, we generate the data from the model of [Kim et al., 2008] and provide comparative results in Tables 5 and 6. It is observed from Tables 5 and 6 that the clustering performances are comparable between the two models. Our model again provides unbiased estimates for all parameters. In contrast to the model of [Kim et al., 2008], our model accounts for the correlation among gene profiles via the linear effects modelling. As presented in Tables 1 to 6, our model outperforms the model of [Kim et al., 2008] when the genetic profiles are correlated. When the genetic profiles are generated independently, our model has better performance in cases where the variability in gene expressions at each time point is large. In cases where the residual covariance structure follows an AR(1) model ([Kim et al., 2008]), our model still provides comparative results and unbiased estimates as the model of [Kim et al., 2008]. The advantage of our model is to provide more reliable and robust clustering of time-course data is apparent. With microarray experiments including those time-course studies, gene expression levels measured from the same tissue sample (or time point) are correlated [Mclachlan et al., 2004]. Clustering methods which assume independently distributed gene profiles, such as the model of [Kim et al., 2008], may overlook important sources of
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Table 5. Bias and standard deviation in brackets from 1000 simulated data points (generated from Kim et al. [2008] with \( \theta^2 \) equal to 0.5)

| Parameters | First component | Second component | Third component |
|------------|----------------|-----------------|----------------|
| \( p(0.585, 0) \) | 0.003 0.000 | 0.008 0.001 | 0.010 -0.000 |
| 0.1,0.315 | 0.004 0.003 | 0.023 0.003 | 0.024 0.004 |
| \( a_0(0.3, 1.0, 0.2) \) | 0.013 0.010 | 0.010 0.010 | 0.010 0.010 |
| \( a_1(0.03, 1.0, 0.02) \) | 0.015 0.001 | -0.236 -0.002 | 0.047 0.003 |
| \( b_1(0.06, 0.9, 0.01) \) | 0.014 -0.000 | -0.308 -0.001 | 0.058 0.001 |
| \( 0.0, 0.0, 0.6, 0.6, 0.6, 0.0, 0.6, 0.6 \) | 0.026 0.021 | 0.345 0.023 | 0.067 0.025 |
| Error rate | 0.026 | 0.045 | 0.042 |
| Rand | 0.978 | 0.980 |
| Adjusted Rand | 0.955 | 0.960 |

Table 6. Bias and standard deviation in brackets from 1000 simulated data points (generated from Kim et al. [2008] with \( \theta^2 \) equal to 1.3)

| Parameters | First component | Second component | Third component |
|------------|----------------|-----------------|----------------|
| \( p(0.585, 0) \) | 0.009 0.001 | -0.007 0.005 | 0.016 -0.001 |
| 0.1,0.315 | 0.013 0.010 | 0.012 0.011 | 0.020 0.013 |
| \( a_0(0.3, 1.0, 0.2) \) | 0.023 0.023 | 0.024 0.019 | 0.016 0.016 |
| \( a_1(0.03, 1.0, 0.02) \) | -0.005 -0.001 | 0.054 -0.000 | 0.003 0.000 |
| \( b_1(0.06, 0.9, 0.01) \) | 0.015 -0.000 | -0.131 0.001 | 0.020 0.000 |
| \( 0.0, 0.0, 0.6, 0.6, 0.6, 0.0, 0.6, 0.6 \) | 0.036 0.036 | 0.135 0.045 | 0.041 0.043 |
| Error rate | 0.015 | 0.185 | -0.003 -0.186 |
| Rand | 0.978 | 0.980 |
| Adjusted Rand | 0.955 | 0.960 |

Fig. 1. Clustering of gene expression profiles into four groups for the yeast dataset 1.

3.2 Applications: Yeast cell cycle datasets

3.2.1 Yeast cell cycle dataset 1 The first data is the yeast cell cycle data with MIPS criterion from Wong et al. [2001]. This data set is extracted from Cho et al. [2001] and made available by Yeung et al. [2000]. The yeast cell cycle dataset contains 237 genes and 17 samples. These genes corresponding to four categories in the MIPS database (DNA synthesis and replication, organization of centrosome, nitrogen, and sulphur metabolism, and ribosomal proteins); these are assumed to be the true clusters. In this illustration, we fit our new extended EMMIX-WIRE model and the model of Kim et al. [2008] to the yeast cell cycle data, with the period of 85 in the Fourier extension (Luan and Li, 2004).

In the Table 2 of Wong et al. [2007], it shows that the Rand and adjusted Rand Indices for their two-stage method are 0.7087 and 0.3697, respectively, and these indices are higher than other methods considered in their paper. Using the model of Kim et al. [2008], the Rand indices are 0.7330 and 0.4721, respectively. With the model of EMMIX-WIRE (Ng et al. [2006]), we have the Rand and adjusted Rand Indices 0.7799 and 0.5568, respectively. Using the proposed new model, the Rand and adjusted Rand Indices are 0.8123 and 0.6189, respectively, and are the best matches (the largest index) compared with the aforementioned models. The four clusters of genes time-course profiles are presented in Figure 1. It can be seen that the genes have very similar expression patterns within each cluster, except in cluster 2, where there is greater individual variation by some of the genes. The estimation using the proposed model is listed in Table 7. It can be seen that the correlations in the first three components are from 0.27 to 0.72, indicating a significant correlation among gene expressions at different time points. Ignoring this correlation may therefore lead to a lower Rand Index, that is, a worse clustering. We can see the estimates of \( d^2 \) in clusters 1 and 4 are large and are greater than the corresponding estimates of \( \theta^2 \), indicating co-regulation in these two clusters. If we ignore such within-cluster co-regulation, we will have Rand Indices similar to those of Kim et al. [2008]. Our model considers both autocorrelation and co-regulation, and thus obtain the best clustering performance.

variability in the experiments, resulting in the consequent possibility of misleading inferences being made (Ng et al. [2006]).
Table 7. Estimations of parameters for the yeast cell cycle dataset 1 (237 genes)

|               | first cluster | second cluster | third cluster | fourth cluster |
|---------------|---------------|----------------|--------------|----------------|
| $p$           | 0.104         | 0.054          | 0.118        | 0.724          |
| $a_1$         | -0.107        | 0.400          | -0.807       | 0.298          |
| $b_1$         | 1.009         | -0.119         | -0.053       | 0.079          |
| $\sigma^2$    | 0.027         | 0.011          | 0.025        | 0.278          |
| $\theta^2$    | 0.174         | 0.417          | 0.443        | 0.307          |
| $\rho$        | 0.278         | 0.717          | 0.435        | 0.053          |
| $d^2$         | 0.191         | 0.001          | 0.031        | 0.310          |
| $\omega$      | 85            | 85             | 85           | 85             |

Table 8. Estimations of parameters for the yeast cell cycle dataset 2 (384 genes)

|               | first cluster | second cluster | third cluster | fourth cluster | fifth cluster |
|---------------|---------------|----------------|--------------|----------------|--------------|
| $p$           | 0.238         | 0.290          | 0.151        | 0.165          | 0.157        |
| $a_1$         | -0.107        | 0.400          | -0.807       | 0.298          | -0.810       |
| $b_1$         | 1.009         | -0.119         | -0.053       | 0.079          | 0.054        |
| $\sigma^2$    | 0.027         | 0.011          | 0.025        | 0.278          | 0.011        |
| $\theta^2$    | 0.174         | 0.417          | 0.443        | 0.307          | 0.4592       |
| $\rho$        | 0.278         | 0.717          | 0.435        | 0.053          | 0.4484       |
| $d^2$         | 0.191         | 0.001          | 0.031        | 0.310          | 0.300        |
| $\omega$      | 85            | 85             | 85           | 85             | 85           |

3.2.2 Yeast cell cycle dataset 2. The second example is the subset of 384 genes from the yeast cell cycle data [Cho et al. 2001], while the full data set can be found from the Stanford yeast cell cycle website (http://171.65.26.52/yeast_cell_cycle/cellcycle.html).

Each of gene is assigned a "phase". We call each "phase" a "Main Group". There are five "Main Groups" in this dataset, namely, early G1, late G1, S, G2 and M. We now compare and assess the cluster quality with the external criterion (the 5 phases). The raw data is log transformed and normalized by columns and rows. Figure 2 presents the five clusters of genes profiles obtained using the proposed model. It can be seen that the genes have very similar expression patterns within each cluster. The estimations are listed in Table 8. The Rand and adjusted Rand Indices are 0.8102 and 0.4484, respectively. They are 0.8108 and 0.4592 for the model of Kim et al. [2008]. The error rates are the same (0.2813) for the two models. The performances of the two models are very similar because the correlation among gene profiles is weak in this dataset. As indicated in Table 8, the estimates of $d^2$ are all very small compared to the estimates of $\theta^2$.

4 DISCUSSION

We have presented a new mixture model with AR(1) random effects for the clustering of time-course gene expression profiles. Our new model involves three elements taking important role in modelling time-course periodic expression data, namely, (a) Fourier expansion which models the periodic patterns; (b) auto-correlation variance structure that accounts for the auto-correlation among the observations at different time points; and (c) the cluster-specific random effects which incorporate the co-regulation within the clusters. In particular, the latter two elements corresponding to the correlations between time-points and between genes are crucial for reliable and accurate clustering of time-course data. We have demonstrated in the simulation and real examples that the accuracy of clustering is improved if the auto-correlation among the time dependent gene expression profiles has been accounted for along the time points; this is also demonstrated in Kim et al. [2008]. Furthermore, better results are obtained if the co-regulation within the clusters is modelled appropriately. When the correlation between genetic profiles is not small, which is the case for typical time-course data, ignorance of this dependency may lead to less accurate clustering results.

For the purpose of comparison, the periods of the signal of gene expression are assumed to be known in the simulation study and applications to real data. In practice, there are several ways to estimate the periods for each cluster. Kim et al. [2008], Luan and Li, [2004], Spellman et al. [1998], Ng et al. [2006]. For example, in Kim et al. [2008], the periods are estimated using simplex algorithm at the M-step during the EM algorithm. However, when the periods are estimated during the EM iterations, we find that the periods depend also on other parameters. In addition, when we start from an initial period and get the design matrix $X$, then with higher possibility the best period will be the initial periods. So we change the strategy to a slow one, and we call it global grid search method, which guarantees the highest maximum log likelihood at the best periods. It performs as follow, let $S$ is a set with its element as (period $\omega_1$, period $\omega_2$, ..., period $\omega_h$), where $\omega_h$ can take all possible values (grid points). For example, for the yeast cell cycle data, the possible periods are 60, 61, ..., 90. Then for each fixed
(ωg, v, u, . . . , ωg, v, u), we estimate the parameters as if the periods for each component are known. Finally we compare the log likelihood and choose the one with the highest log likelihood as the final result. Since it is very slow if there are too many elements in set S when we have no prior information about periods, we recommend use other method to get the periods first [Booth et al., 2008]. In all the calculation in this paper, we assume the period is fixed, that is, there is only one element in the set S.

The proposed model is very flexible through the different specifications of design matrices or model options as originally available in [Ng et al., 2003]. For example, besides the full model, it enables us to incorporate the model of Qin and Self [2004] as a special case. Specifically, we can obtain their model by assuming zero cluster effects (v = 0) and that random effects u be auto correlated for each gene. Furthermore, when both random effects u and v are assumed to be zero, then we have normal mixture of regression models. In the program we have developed, there are many options and parameters for users to specify the models they want to use in addition to the models we list in our paper. The program is written in R package and is available from the corresponding author.

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