Model-Based Clustering of Microarray Expression Data via Latent Gaussian Mixture Models

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ABSTRACT

Motivation: In recent years, work has been carried out on clustering gene expression microarray data. Some approaches are developed from an algorithmic viewpoint whereas others are developed via the application of mixture models. In this paper, a family of eight mixture models which utilizes the factor analysis covariance structure is extended to twelve models and applied to gene expression microarray data. This modelling approach builds on previous work by introducing a modified factor analysis covariance structure, leading to a family of twelve mixture models, including parsimonious models. This family of models allows for the modelling of the correlation between gene expression levels even when the number of samples is small. Parameter estimation is carried out using a variant of the EM algorithm and model selection is achieved using the Bayesian information criterion. This expanded family of Gaussian mixture models, known as the EPGMM family, is then applied to two well-known gene expression data sets.

Results: The performance of the EPGMM family of models is quantified using the adjusted Rand index. This family of models gives very good performance, relative to existing popular clustering techniques, when applied to real gene expression microarray data.

Availability: The reduced, preprocessed data that were analyzed are available at www.paulmcnicholas.info

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1 INTRODUCTION

1.1 Model-Based Clustering

Cluster analysis methods are used to find subgroups in a population. Clustering is of particular interest when analyzing gene expression data because it can be used to find subgroups that are well distinguished by their expression profiles. A number of clustering techniques are commonly used including agglomerative hierarchical, divisive hierarchical, \(k\)-means, \(k\)-medoids, and model-based clustering. Model-based clustering is a technique for estimating group membership based on parametric finite mixture models. The density of a parametric finite mixture model can be written

\[
  f(x | \pi_1, \ldots, \pi_G, \theta_1, \ldots, \theta_G) = \sum_{g=1}^{G} \pi_g f(x | \theta_g),
\]

where \(\pi_g \in [0, 1]\), such that \(\sum_{g=1}^{G} \pi_g = 1\), is the probability of membership of sub-population \(g\), and \(f(x | \theta_g)\) is the density of a multivariate random variable \(X\) with parameters \(\theta_g\). Overviews of finite mixture models are given by McLachlan and Peel (2000a) and Frühwirth-Schnatter (2006).

In the model-based clustering literature, the finite Gaussian mixture model is most commonly used (examples include Fraley and Raftery, 2002; McLachlan et al., 2002; McNicholas and Murphy, 2008, 2010). The density of a finite Gaussian mixture model is given by,

\[
  f(x | \vartheta) = \sum_{g=1}^{G} \pi_g \phi(x | \mu_g, \Sigma_g),
\]

where \(\phi(x | \mu_g, \Sigma_g)\) is the density of a multivariate Gaussian random variable \(X\) with mean \(\mu_g\) and covariance matrix \(\Sigma_g\), and \(\vartheta = (\pi_1, \ldots, \pi_G, \mu_1, \ldots, \mu_G, \Sigma_1, \ldots, \Sigma_G)\). Note that the Gaussian mixture model has been used within the bioinformatics literature for purposes other than clustering; for example, McLachlan et al. (2006) apply a two-component mixture model to detect differential gene expression.

Gaussian mixture models offer an advantage over other commonly used approaches because the covariance structure can potentially account for correlation between expression levels within an expression profile. Consequently, these models are more flexible than \(k\)-means or hierarchical clustering which commonly use Euclidean distance when clustering. However, due to the high dimensional nature of expression data, additional structure needs to be assumed for the covariance matrices, so that the model can be fitted in high dimensional settings. The MCLUST (Fraley and Raftery, 2002) approach to model-based clustering, which utilizes eigen-decomposed covariance matrices, can only be applied to clustering expression profiles if a diagonal covariance structure is assumed; Yeung et al. (2001) were able to cluster genes using MCLUST but not expression profiles. By assuming a highly parsimonious but non-diagonal covariance structure, it is possible to cluster expression profiles whilst allowing for correlation between gene expressions.

In general, a structure like that given in Equation 1 can be used to model such data. Then the parameters, and hence group memberships, can be estimated using some variant of the expectation-maximization (EM) algorithm (Dempster et al., 1977). The covariance matrices \(\Sigma_g\) can be decomposed to allow the construction of more parsimonious models.
1.2 Parsimonious Gaussian Mixture Models

The factor analysis model (Spearman, 1904) assumes that a p-dimensional random vector \( \mathbf{X}_i \) can be modeled using a q-dimensional vector of latent factors \( \mathbf{U}_i \), where \( q \ll p \). The model can be written \( \mathbf{X}_i = \mu + \mathbf{A} \mathbf{U}_i + \epsilon_i \), where \( \mathbf{A} \) is a \( p \times q \) matrix of factor weights, the latent variables \( \mathbf{U}_i \sim N(0, \mathbf{I}_q) \) and \( \epsilon_i \sim N(0, \mathbf{\Psi}) \), where \( \mathbf{\Psi} \) is a \( q \times q \) diagonal matrix. Therefore, the marginal distribution of \( \mathbf{X}_i \) is \( N(\mu, \mathbf{A}^\top \mathbf{\Psi} \mathbf{A}) \).

To illustrate the implications of the covariance matrix attached to this marginal distribution, \( \mathbf{\Sigma} = \mathbf{A} \mathbf{\Lambda}^\top + \mathbf{\Psi} \), suppose that \( X_{ij} \) and \( X_{ik} \) are expression levels from a sample \( X_i \). Then, \( \text{Cov}(X_{ij}, X_{ik}) = \sigma_{jk} = \sum_{s=1}^q \lambda_{js} \lambda_{ks} \) for \( j \neq k \), and \( \text{Var}(X_{ij}) = \sigma_{jj} = \sum_{s=1}^q \lambda_{js}^2 + \psi_{qq} \). Hence, the matrix \( \mathbf{\Lambda} \) models the covariance between expression levels, and a combination of the \( \mathbf{\Lambda} \) and \( \mathbf{\Psi} \) matrices models the variance of expression levels. The factor analysis model allows for the modelling of a high-dimensional non-diagonal covariance matrix with a low number of parameters.

Ghahramani and Hinton (1997) proposed a mixture of factor analyzers model given by the finite Gaussian mixture model in Equation 1, with \( \mathbf{\Sigma}_q = \mathbf{A}_q \mathbf{\Lambda}_q^\top + \mathbf{\Psi} \), McLachlan and Peel (2000b) used the more general covariance structure \( \mathbf{\Sigma}_q = \mathbf{A}_q \mathbf{\Lambda}_q^\top + \mathbf{\Psi} \). Tipping and Bishop (1999) proposed the mixtures of probabilistic principal component analyzers model, for which the component covariance matrix is \( \mathbf{\Sigma}_q = \mathbf{A}_q \mathbf{\Lambda}_q^\top + \mathbf{\Psi} \).

McNicholas and Murphy (2008) further generalized the factor analysis covariance structure by including the possibility of imposing the constraints: \( \mathbf{\Lambda}_q = \mathbf{\Lambda} \), \( \mathbf{\Psi}_q = \mathbf{\Psi} \) and \( \mathbf{\Psi}_q = \psi_q \mathbf{I}_q \).

The resulting covariance structure \( \mathbf{\Sigma}_q = \mathbf{A}_q \mathbf{\Lambda}_q^\top + \mathbf{\Psi} \) is a \( q \times q \) matrix with a low number of parameters, allowing for the modelling of high-dimensional data in a more stable manner.

### Table 1. The covariance structure of each parsimonious Gaussian mixture model

<table>
<thead>
<tr>
<th>( \mathbf{\Lambda}_q )</th>
<th>( \mathbf{\Psi}_q )</th>
<th>Isotropic Covariance Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>C</td>
<td>( \mathbf{\Sigma}_q = \mathbf{\Lambda}^\top \mathbf{\Lambda} + \mathbf{\Psi} )</td>
</tr>
<tr>
<td>C</td>
<td>U</td>
<td>( \mathbf{\Sigma}_q = \mathbf{\Lambda}^\top \mathbf{\Lambda} + \mathbf{\Psi} )</td>
</tr>
<tr>
<td>C</td>
<td>U</td>
<td>( \mathbf{\Sigma}_q = \mathbf{\Lambda}^\top \mathbf{\Lambda} + \mathbf{\Psi} )</td>
</tr>
<tr>
<td>U</td>
<td>C</td>
<td>( \mathbf{\Sigma}_q = \mathbf{\Lambda}^\top \mathbf{\Lambda} + \mathbf{\Psi} )</td>
</tr>
<tr>
<td>U</td>
<td>C</td>
<td>( \mathbf{\Sigma}_q = \mathbf{\Lambda}^\top \mathbf{\Lambda} + \mathbf{\Psi} )</td>
</tr>
<tr>
<td>U</td>
<td>U</td>
<td>( \mathbf{\Sigma}_q = \mathbf{\Lambda}^\top \mathbf{\Lambda} + \mathbf{\Psi} )</td>
</tr>
</tbody>
</table>

C = constrained, U = unconstrained.

The PGMM family has another significant advantage that is particularly important in applications involving high-dimensional data. When running the alternating expectation-conditional maximization (AECM) algorithm (Meng and van Dyk, 1997) for these models, it is advantageous to make use of the Woodbury identity (Woodbury, 1950) to avoid inverting any non-diagonal \( p \times p \) matrices. Given an \( n \times n \) matrix \( \mathbf{A} \), an \( n \times k \) matrix \( \mathbf{H} \), a \( k \times k \) matrix \( \mathbf{C} \) and a \( k \times n \) matrix \( \mathbf{V} \), the Woodbury identity states that

\[
(\mathbf{A} + \mathbf{H} \mathbf{C} \mathbf{V} \mathbf{C}^\top)^{-1} = \mathbf{A}^{-1} - \mathbf{A}^{-1} \mathbf{H} (\mathbf{C}^\top \mathbf{V} \mathbf{A}^{-1} \mathbf{C} \mathbf{V} \mathbf{H})^{-1} \mathbf{V} \mathbf{A}^{-1}.
\]

### Equations

1. \( (\mathbf{A} + \mathbf{H} \mathbf{C} \mathbf{V} \mathbf{C}^\top)^{-1} = \mathbf{A}^{-1} - \mathbf{A}^{-1} \mathbf{H} (\mathbf{C}^\top \mathbf{V} \mathbf{A}^{-1} \mathbf{C} \mathbf{V} \mathbf{H})^{-1} \mathbf{V} \mathbf{A}^{-1} \).
2. Setting \( \mathbf{H} = \mathbf{A}, \mathbf{V} = \mathbf{A}^\top, \mathbf{A} = \mathbf{\Psi} \) and \( \mathbf{C} = \mathbf{I}_q \) in Equation 2 gives

\[
(\mathbf{\Psi} + \mathbf{\Lambda} \mathbf{\Lambda}^\top)^{-1} = \mathbf{\Psi}^{-1} - \mathbf{\Psi}^{-1} \mathbf{A} (\mathbf{I}_q + \mathbf{A} \mathbf{\Psi}^{-1} \mathbf{A})^{-1} \mathbf{\Lambda} \mathbf{\Psi}^{-1}.
\]

2.2 Parameter Estimation for the PGMM Family

2.2.1 Introduction

Estimation of the model parameters, via the AECM algorithm, is analogous to that of the PAR model parameter estimation procedure described by McNicholas and Murphy (2008). The estimates for the eight pre-existing models are obtained from the PGMM estimates by setting \( \mathbf{\Psi}_g = |\mathbf{\Psi}_g|^{1/2} |\mathbf{\Psi}_g|^1/2 \mathbf{\Psi}_g \) and then setting \( \omega_g = \mathbf{\Psi}_g^{-1} \mathbf{\Psi}_g \). However, the derivation of the maximum likelihood estimates of the model parameters for the new models, requires the method of Lagrange multipliers (Lagrange, 1788). Parameter estimates for the CCU model are derived in Section 2.2.2 and derivations for the other three new models are given at the end of said section.

2.2.2 AECM Algorithm

The EM algorithm is an iterative technique for finding maximum likelihood estimates when data are
Table 2. The covariance structure, number of covariance parameters and nomenclature for each member of the EPGM family, along with the name of the equivalent member of the PGMM family where applicable.

<table>
<thead>
<tr>
<th>EPGM Nomenclature</th>
<th>Covariance Structure</th>
<th>Number of Covariance Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Lambda_y = \Lambda$</td>
<td>$\Sigma_y = \Lambda \Lambda' + \omega I_p$</td>
<td>$p g - q(q - 1)/2 + 1$</td>
</tr>
<tr>
<td>$\Delta_y = \Delta$</td>
<td>$\Sigma_y = \Lambda \Lambda' + \omega I_p$</td>
<td>$p g - q(q - 1)/2 + G$</td>
</tr>
<tr>
<td>$\omega_y = \omega$</td>
<td>$\Sigma_y = \Lambda \Lambda' + \omega I_p$</td>
<td>$G(pq - q(q - 1)/2 + 1$</td>
</tr>
<tr>
<td>$\Delta_y \neq I_p$</td>
<td>$\Sigma_y = \Lambda \Lambda' + \omega I_p$</td>
<td>$G(pq - q(q - 1)/2 + G$</td>
</tr>
</tbody>
</table>

C = constrained, U = unconstrained.

The expected complete-data log-likelihood $Q_2(\Lambda, \omega, \Delta)$ can be written

$$C + \frac{1}{2} \sum_{g=1}^{G} n_g \left[ \rho \log \omega^{-1} + \log |\Delta^{-1}| - \omega^{-1} \text{tr} \{\Delta^{-1} S_g\} + 2 \omega^{-1} \text{tr} \{\Delta^{-1} \Lambda' \Theta_s \Delta \} \right],$$

where $C$ is constant with respect to $\Lambda, \omega$ and $\Delta$, and $\Theta_s = I_q - \beta \Delta \Lambda + \beta_s \Theta_s \beta'$.

To maximize $Q_2$ with respect to $\Lambda, \omega$ and $\Delta$, it is necessary to use the method of Lagrange multipliers. First, form the Lagrange function $L(\Lambda, \omega, \Delta, \kappa) = Q(\Lambda, \omega, \Delta) - \kappa |\Delta - 1|$. Note that we use $\kappa$ to denote the Lagrange multiplier to avoid confusion with the elements of the matrix $\Lambda$. Differentiating $L$ with respect to $\Lambda, \omega$, $\Delta^{-1}$ and $\kappa$, respectively, gives the following score functions.

$$S_1(\Lambda, \omega, \Delta, \kappa) = \frac{\partial L}{\partial \Lambda} = \sum_{g=1}^{G} n_g \left[ \Delta^{-1} S_g \beta_g - \Delta^{-1} \Lambda \Theta_s \right],$$

$$S_2(\Lambda, \omega, \Delta, \kappa) = \frac{\partial L}{\partial \omega} = \frac{n_g}{2} \left[ \omega^{-1} \text{tr} \{\Delta^{-1} S_g\} + 2 \text{tr} \{\Delta^{-1} \Lambda' \beta S_g \} - \text{tr} \{\Delta^{-1} \Theta_s \Delta \} \right],$$

$$S_3(\Lambda, \omega, \Delta, \kappa) = \frac{\partial L}{\partial \Delta} = \sum_{g=1}^{G} n_g \left[ \Delta - \omega^{-1} S_g \right] + 2 \omega^{-1} \text{tr} \{\Delta^{-1} \Theta_s \Delta \} + \kappa |\Delta - 1|,$$

$$S_4(\Lambda, \omega, \Delta, \kappa) = \frac{\partial L}{\partial \kappa} = |\Delta - 1|.$$

Note that $S_4$ is included for completeness only and solving $S_4(\Lambda, \omega, \Delta, \kappa) = 0$ just returns the constraint $|\Delta| = 1$. Now, solving $S_1(\Lambda, \omega, \Delta, \kappa) = 0$ gives

$\Lambda^{\text{new}} = \left[ \sum_{g=1}^{G} n_g S_{g, \beta} \right]^{-1} \left[ \sum_{g=1}^{G} n_g \omega^{-1} \Theta_s \right]^{-1},$
and solving $S_2(\tilde{\Lambda}^{new}, (\hat{\omega}_g)^{new}, \tilde{\Delta}, \kappa) = 0$ gives

$$(\hat{\omega}_g)^{new} = \frac{1}{p} \text{tr} \left\{ \Delta^{-1} \left[ S_g - 2\Lambda^{new} \hat{\beta}_g S_g + \Lambda^{new} \Theta_g (\Lambda^{new})' \right] \right\}.$$ 

Solving $\text{diag}\{S_3(\Lambda^{new}, (\hat{\omega}_g)^{new}, \Delta^{new}, \kappa)\} = 0$ leads to

$$\Delta^{new} = \frac{1}{n + 2\kappa} \text{diag}\left\{ \sum_{g=1}^{G} \frac{n_g}{(\hat{\omega}_g)^{new}} \left[ S_g - 2\Lambda^{new} \hat{\beta}_g S_g + \Lambda^{new} \Theta_g (\Lambda^{new})' \right] \right\}.$$ 

But $\Delta^{new}$ is a diagonal matrix with $|\Delta^{new}| = 1$, therefore

$$n + 2\kappa = \left( \prod_{j=1}^{p} \xi_j \right)^{\frac{1}{p}},$$ 

where $\xi_j$ is the $j$th element along the diagonal of the matrix

$$\sum_{g=1}^{G} \frac{n_g}{(\hat{\omega}_g)^{new}} \left[ S_g - 2\Lambda^{new} \hat{\beta}_g S_g + \Lambda^{new} \Theta_g (\Lambda^{new})' \right].$$ 

Therefore, it follows that

$$\kappa = \frac{1}{2} \left[ \left( \prod_{j=1}^{p} \xi_j \right)^{\frac{1}{p}} - n \right].$$

The derivations for the other three new models are similar. The estimates in the UCUU case are

$$\tilde{\Lambda}^{new} = \hat{S}_g \hat{\beta}_g \Theta_g^{-1}.$$ 

$$(\hat{\omega}_g)^{new} = \frac{1}{p} \text{tr} \left\{ \Delta^{-1} S_g - \Delta^{-1} \tilde{\Lambda}^{new} \hat{\beta}_g S_g \right\},$$ 

$$\Delta^{new} = \frac{1}{n + 2\kappa} \text{diag} \left\{ \sum_{g=1}^{G} \frac{n_g}{(\hat{\omega}_g)^{new}} \left[ S_g - \tilde{\Lambda}^{new} \hat{\beta}_g S_g \right] \right\},$$

where $\kappa$ is as defined in Equation 5 but, in this case, $\xi_j$ is the $j$th element along the diagonal of the matrix

$$\sum_{g=1}^{G} \frac{n_g}{(\hat{\omega}_g)^{new}} \left[ S_g - \tilde{\Lambda}^{new} \hat{\beta}_g S_g \right].$$

In the CUCU case, the estimate for $\Lambda$ is derived in a row-by-row fashion as

$$\Lambda^{new} = r_i \left( \sum_{g=1}^{G} \frac{n_g}{\hat{\delta}_{g(i)}} \Theta_g \right)^{-1},$$

for $i = 1, \ldots, p$ where $r_i$ is the $i$th row of the matrix $\sum_{g=1}^{G} (n_g/\hat{\delta}_{g(i)}) S_g \hat{\beta}_g$, and $\hat{\delta}_{g(i)}$ is the $i$th element along the diagonal of the matrix $\Delta_g$. The other estimates are

$$(\hat{\omega}_g)^{new} = \frac{1}{p} \sum_{g=1}^{G} \hat{\pi}_g \text{tr} \left\{ \Delta^{-1} \left[ S_g - 2\Lambda^{new} \hat{\beta}_g S_g + \Lambda^{new} \Theta_g (\Lambda^{new})' \right] \right\},$$

$$\Delta^{new} = \frac{1}{n + 2\kappa} \text{diag} \left\{ S_g - 2\Lambda^{new} \hat{\beta}_g S_g + \Lambda^{new} \Theta_g (\Lambda^{new})' \right\},$$

$$\kappa = \frac{n_g}{2} \left[ \frac{1}{(\hat{\omega}_g)^{new}} \left( \prod_{j=1}^{p} \xi_j \right)^{\frac{1}{p}} - 1 \right],$$

where $\xi_j$ is the $j$th element along the diagonal of the matrix $S_g - 2\Lambda^{new} \hat{\beta}_g S_g + \Lambda^{new} \Theta_g (\Lambda^{new})'$. In the CCUU case, the parameter estimates are given by

$$\tilde{\Lambda}^{new} = \hat{S}_g \hat{\beta}_g \Theta_g^{-1},$$

$$(\hat{\omega}_g)^{new} = \frac{1}{p} \sum_{g=1}^{G} \hat{\pi}_g \text{tr} \left\{ \Delta^{-1} \left[ S_g - \tilde{\Lambda}^{new} \hat{\beta}_g S_g \right] \right\},$$

$$\Delta^{new} = \frac{1}{(\hat{\omega}_g)^{new}} \left( 1 + 2\kappa / n_g \right) \text{diag} \left\{ S'_g - \Lambda^{new} \hat{\beta}_g S_g \right\},$$

and $\kappa_g$ is as in the CUCC case but with $\xi_{gj}$ given by the $j$th element along the diagonal of the matrix $S'_g - \Lambda^{new} \hat{\beta}_g S_g$.

Note that the predicted clustering for each member of the EPGMF family is given by the maximum a posteriori (MAP) classification. That is, the posterior predicted component membership of tissue $i$ is the value of $g$ for which $\hat{\xi}_{ig}$ is greatest.

### 2.3 Convergence & Model Selection

#### 2.3.1 Convergence Criterion

Aitken’s acceleration (Aitken, 1926) is used in the analyses herein to estimate the asymptotic maximum of the log-likelihood at each iteration. This allows a decision about whether or not a given AECD algorithm has converged. Aitken’s acceleration at iteration $t$ is given by

$$a^{(t)} = \frac{l^{(t+1)} - l^{(t)}}{l^{(t)} - l^{(t-1)},}$$

where $l^{(t+1)}$, $l^{(t)}$ and $l^{(t-1)}$ are the log-likelihood values from iterations $t + 1$, $t$, and $t - 1$, respectively. The asymptotic estimate of the log-likelihood at iteration $t + 1$ is given by

$$l^{(t+1)} = l^{(t)} + \frac{1}{1 - a^{(t)}(l^{(t+1)} - l^{(t)})}.$$ 

(Böhning et al., 1994). Herein, the stopping criterion proposed by McNicholas et al. (2010) is used, so that the algorithm can be stopped when $l^{(t+1)} - l^{(t)} < \varepsilon$. More specifically, $\varepsilon = 0.1$ is used. Note that this criterion is very similar to that proposed by Lindsay (1995), who suggested stopping when $l^{(t+1)} - l^{(t+1)} < \varepsilon$.

#### 2.3.2 Model Selection

The Bayesian information criterion (BIC) is used to select the best member of the EPGMF family, in terms of both model and number of factors. Note that the BIC can also be used to select the number of mixture components (cf. Fraley and Raferty, 1999; McNicholas and Murphy, 2008) but this is not necessary for the analyses herein since we fix $G = 2$. For a model with parameters $\theta$, the Bayesian information criterion (BIC) is given by $\text{BIC} = 2l(x, \hat{\theta}) - m \log n$, where $l(x, \hat{\theta})$ is the maximized log-likelihood, $\hat{\theta}$ is the maximum likelihood estimate of $\theta$, $m$ is the number of free parameters in the model and $n$ is the number of observations. The effectiveness of the BIC for choosing the number of factors in a factor analysis model has been established by Lopes and West (2004), while McNicholas et al. (2010) provide practical evidence that the BIC performs well in choosing the number of factors for the PGMF family of models.

A number of other model selection criteria could be used including the Akaike information criterion (AIC Akaike, 1974), the integrated completed likelihood (ICL Biernacki et al., 2000) and clustering stability (cf. von Luxburg, 2009). However, we found that the BIC gave a quick solution and generally good clustering results.
3 ANALYSES

3.1 Dimensionality Reduction

McLachlan et al. (2002) analyzed two microarray gene expression data sets — one on leukemia data and another on colon tissue samples — using the EMMIX-GENE approach. The first stage of this approach focuses on data reduction where, initially, one and two-component mixtures of t-distributions are fitted to the data. Then a gene is retained only if two conditions are satisfied.

One of these conditions is that the minimum cluster size exceeds some pre-specified threshold $a_1$. The other condition concerns the result of a likelihood ratio test, or tests. First, the hypothesis $H_0 : G = 1$ is tested against $H_1 : G = 2$ and the gene is retained if

$$-2 \log \lambda > a_2,$$

where $\lambda$ is the likelihood ratio statistic. However, if the condition in Equation 6 is not met then the hypothesis $H_0 : G = 2$ is tested against $H_1 : G = 3$ and the gene is retained if the same condition is satisfied, with the same $a_2$, for this test statistic $\lambda$ and at least two of the three components contain at least $a_1$ tissues.

When fitting the two and three-component mixture models for this purpose, starting values for the component memberships are defined randomly or by using starting values based on $k$-means clustering results. This whole process represents the first stage of the EMMIX-GENE approach and can be carried out using the select-genes software that accompanies McLachlan et al. (2004). For the analyses herein, the select-genes software is used with thresholds $a_1 = a_2 = 8$, as in McLachlan et al. (2002), and 50 random and 50 $k$-means starts.

3.2 Leukaemia Data

3.2.1 The Data

Golub et al. (1999) presented data on two forms of acute leukemia: acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Affymetrix arrays were used to collect measurements for 7,129 genes on 72 tissues. There were a total of 47 ALL tissues and 25 with AML. The data were sourced from the website accompanying McLachlan et al. (2004, www.maths.uq.edu.au/~gjm/EMMIX-gene/) and so they had been preprocessed (Dudoit et al., 2002; McLachlan et al., 2002) as follows.

1. Genes with expression less than 100 or greater than 16,000 were removed.
2. Genes with expressions satisfying $\max - \min \leq 5$ and $\max - \min \leq 500$ were removed.
3. The natural logarithm was taken.

Following this preprocessing, a total of 3,731 genes remained. This number was further reduced to 2,030 following application of the select-genes software (cf. Section 3.1).

3.2.2 The EPGMM Approach

Treating this as a clustering problem where the form of leukemia is unknown, all twelve members of the EPGMM family (Table 2) were fitted to these data for $G = 2, q = 1, \ldots, 6$ and ten different random starting values for the $\hat{\xi}_i$. The BIC for the best $q$ for each of the 12 members of the EPGMM family is given in Table 3.

Table 3. The BIC for the best $q$ for each of the 12 members of the EPGMM family for the leukemia data.

<table>
<thead>
<tr>
<th>Model</th>
<th>$q$</th>
<th>BIC</th>
<th>Model</th>
<th>$q$</th>
<th>BIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCCC</td>
<td>3</td>
<td>-411,646.50</td>
<td>CCUC</td>
<td>3</td>
<td>-411,566.29</td>
</tr>
<tr>
<td>UCCC</td>
<td>1</td>
<td>-416,954.56</td>
<td>UCUC</td>
<td>1</td>
<td>-416,803.57</td>
</tr>
<tr>
<td>CCCC</td>
<td>4</td>
<td>-414,615.22</td>
<td>CCUU*</td>
<td>5</td>
<td>-413,207.29</td>
</tr>
<tr>
<td>UCCU</td>
<td>1</td>
<td>-423,354.79</td>
<td>UCUU*</td>
<td>1</td>
<td>-422,089.38</td>
</tr>
<tr>
<td>CUCU*</td>
<td>4</td>
<td>-413,966.90</td>
<td>CUUU</td>
<td>5</td>
<td>-413,978.04</td>
</tr>
<tr>
<td>UUCU*</td>
<td>1</td>
<td>-423,933.46</td>
<td>UUUU</td>
<td>1</td>
<td>-423,532.04</td>
</tr>
</tbody>
</table>

* denotes one of the four new models.

The best of these models, in terms of BIC, was a CCUC model with $q = 3$ latent factors. The chosen model has a non-diagonal covariance structure where the covariance between pairs of genes is equal across different clusters but the variance of each gene is unequal across different clusters (see Section 1.2). The MAP classifications arising from the parameter estimates associated with this model are given in Table 4; only five tissue samples were misclassified.

Table 4. Estimated group membership for the best EPGMM model for the leukemia data.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lymphoblastic leukemia (ALL)</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>Acute myeloid leukemia (AML)</td>
<td>5</td>
<td>25</td>
</tr>
</tbody>
</table>

3.2.3 Hierarchical Clustering, $k$-means, $k$-medoids & MCLUST

In addition to the EPGMM technique, several other techniques were applied to these data using the R software (R Development Core Team, 2010). Agglomerative hierarchical clustering was used, with Euclidean distance and three different linkage methods: complete, average, and single. The $k$-means (cf. Hartigan and Wong, 1979) and $k$-medoids techniques were also used. In the latter case, the partitioning around medoids (PAM; cf. Kaufman and Rousseeuw, 1990, Chapter 2) algorithm was used. Finally, in order to compare our model-based clustering approach to the well-established MCLUST approach, we used the mclust package (Fraley and Raftery, 1999) for the R software.

Table 5. Summary results for all of the clustering techniques that were applied to the leukemia data.

<table>
<thead>
<tr>
<th></th>
<th>BIC</th>
<th>Rand Index</th>
<th>Adjusted Rand Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hierarchical (Complete)</td>
<td>–</td>
<td>0.532</td>
<td>0.058</td>
</tr>
<tr>
<td>Hierarchical (Average)</td>
<td>–</td>
<td>0.525</td>
<td>-0.024</td>
</tr>
<tr>
<td>Hierarchical (Single)</td>
<td>–</td>
<td>0.532</td>
<td>-0.013</td>
</tr>
<tr>
<td>$k$-means</td>
<td>–</td>
<td>0.593</td>
<td>0.187</td>
</tr>
<tr>
<td>PAM</td>
<td>–</td>
<td>0.518</td>
<td>0.023</td>
</tr>
<tr>
<td>MCLUST (VII)</td>
<td>-416,293.2</td>
<td>0.593</td>
<td>0.186</td>
</tr>
<tr>
<td>EPGMM (CCUC, $q = 3$)</td>
<td>-411,566.3</td>
<td>0.869</td>
<td>0.738</td>
</tr>
</tbody>
</table>

The results, which are summarized in Table 5, give the Rand and adjusted Rand indices as measures of class agreement.
The Rand index (Rand, 1971) is based on pairwise agreements and disagreements, and the adjusted Rand index (Hubert and Arabie, 1985) is effectively the Rand index corrected for random chance. These indices reveal that the best of the non-model-based approaches was \( k \)-means clustering, with an adjusted Rand index of 0.187. In fact, \( k \)-means clustering outperformed \texttt{mclust} on these data, but the EPGMM model with the greatest BIC (\( CCUC, q = 4 \)) was the best model overall.

### 3.2.4 The EMMIX-GENE Approach

McLachlan et al. (2002) analyzed the same data using the EMMIX-GENE approach with four random and four \( k \)-means starts in the first stage, which reduced the number of genes to 2,015. In the second stage, a mixture of 40 normal distributions with isotropic covariance structure was fitted to the 2,015 genes. Two of these groups (Groups 1 and 3) provided clusterings that were most similar to the type of leukaemia — of course, in a real clustering scenario this could not be established. A two-component mixture of factor analyzers, with \( q = 6 \) factors, was fitted to the data using the genes from groups 1 and 3, respectively. Using the genes from Group 1 led to the misclassification of 13 tissues and using those from Group 3 led to the misclassification of 6 tissues. Note that McLachlan et al. (2002) did not specify how many different random starts were used but, based on other analyses, it seems likely that 50 random and 50 \( k \)-means starts were used.

### 3.2.5 Two Other Approaches

In addition to the EMMIX-GENE approach, McLachlan et al. (2002) used two other approaches to cluster the leukaemia tissues. In both cases, the first stage was identical to that described in Section 3.2.4. The first alternative approach was to cluster the tissues based on the 40 fitted group means and the top 50 of the 2,015 genes. Fitting a two-component mixture of factor analyzers with \( q = 8 \) factors to these data, using 50 random and 50 \( k \)-means starts, led to the misclassification of just one tissue. The second alternative approach was to base the analysis on the top fifty genes. Fitting a two-component mixture of factor analyzers, with \( q = 8 \) factors to these data, using 50 random and 50 \( k \)-means starts, led to the misclassification of ten tissues.

### 3.2.6 Comments

The EPGMM approach gave very good clustering performance when applied to the leukaemia data. This approach used ten random starts and led to the misclassification of just five tissues. This performance far exceeds that of agglomerative hierarchical clustering, \( k \)-means clustering, PAM, and \texttt{MCLUST}. In fact, the best of all of these techniques had an adjusted Rand index of 0.189, while the best EPGMM model had an adjusted Rand index of 0.738. Although, in one instance, one of the approaches of McLachlan et al. (2002) returned a better predicted classification, it is difficult to make a direct comparison to the EPGMM approach. This difficulty arises because the EPGMM approach is a genuine clustering approach, while the methods described in sections 3.2.4 and 3.2.5 assumed, to some extent, knowledge of the truth. This knowledge was clearly used in the analyses described in Section 3.2.4 but was used in a less obvious fashion in the analyses given in Section 3.2.5. In this latter case, the choice of the number of clusters (40) was validated in some sense by the fact that two of the groups give classifications that were similar to the true leukaemia type. In fact, as mentioned by McLachlan et al. (2002), an objective technique for choosing this number is not possible since genes cannot be assumed to be independently distributed within a tissue sample. Furthermore, it is quite likely that the number of factors \( q \) was selected, in each case, to give the best classification. This could be done objectively, as in Section 3.2.2, using the BIC. Finally, any comparison between the EPGMM approach and the approaches of McLachlan et al. (2002) would have to be taken in context with the fact that different subsets of the 3,731 genes are used in each case.

### 3.3 Colon Data

#### 3.3.1 The Data

Alon et al. (1999) presented gene expression data on 62 colon tissue samples, of which 40 were tumours and the remaining 22 were normal. Affymetrix arrays were used to collect measurements for 6,500 gene expressions on all 62 tissues. Following Alon et al. (1999) and McLachlan et al. (2002), only the 2,000 genes with the highest minimal intensity are focused upon. The data were again sourced from the website mentioned in Section 3.2.1, and this time, the only preprocessing was the taking of natural logarithms, followed by normalization. Application of the \texttt{select-genes} software, with the settings specified in Section 3.1, led to the reduction of the number of genes from 2,000 to just 461.

#### 3.3.2 The EPGMM Approach

Treating this as a clustering problem where the type of tissue is unknown, all twelve members of the EPGMM family (Table 2) were fitted to these data for \( C = 2, q = 1, \ldots, 10 \) and ten different random starting values for the \( z_{1g} \). The BIC for the best \( q \) for each of the 12 members of the EPGMM family is given in Table 6.

<table>
<thead>
<tr>
<th>Model</th>
<th>( q )</th>
<th>BIC</th>
<th>Model</th>
<th>( q )</th>
<th>BIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCUC</td>
<td>4</td>
<td>-79,085.73</td>
<td>CCUC</td>
<td>6</td>
<td>-70,937.72</td>
</tr>
<tr>
<td>UCCC</td>
<td>3</td>
<td>-77,267.65</td>
<td>UCCC</td>
<td>3</td>
<td>-77,268.16</td>
</tr>
<tr>
<td>CCCU</td>
<td>8</td>
<td>-71,064.11</td>
<td>CCCU</td>
<td>7</td>
<td>-71,063.71</td>
</tr>
<tr>
<td>UCCU</td>
<td>3</td>
<td>-77,310.19</td>
<td>UCCU</td>
<td>4</td>
<td>-77,532.97</td>
</tr>
<tr>
<td>CUCU*</td>
<td>8</td>
<td>-71,609.10</td>
<td>CUCU</td>
<td>8</td>
<td>-71,631.35</td>
</tr>
<tr>
<td>UUCU*</td>
<td>2</td>
<td>-78,485.83</td>
<td>UUCU</td>
<td>2</td>
<td>-78,506.33</td>
</tr>
</tbody>
</table>

* denotes one of the four new models.

The best of these models, again in terms of BIC, was a CCUC model with \( q = 6 \) latent factors; the covariance structure in this model is the same as that chosen for the leukaemia data (see Section 3.2.2). The MAP classifications given by the parameter estimates associated with this model are given in Table 7; only five tissue samples were misclassified.

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td>Normal</td>
<td>2</td>
<td>20</td>
</tr>
</tbody>
</table>

#### 3.3.3 Hierarchical Clustering, \( k \)-means, \( k \)-medoids & \texttt{MCLUST}

In addition to the EPGMM technique, the methods used in
Section 3.2.3 were run on these colon data using the \textit{R} software. The results, which are summarized in Table 8, suggest that the best of the non-model-based approaches was PAM, with an adjusted Rand index of 0.218. This time, \textit{mclust} outperformed \textit{k}-means clustering but PAM outperformed \textit{mclust}. The EPGMM model with the greatest BIC (CCUU, \( q = 6 \)) was the best model, misclassifying just five tissues based on ten random starts.

<table>
<thead>
<tr>
<th>Method</th>
<th>BIC</th>
<th>Rand Index</th>
<th>Adjusted Rand Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hierarchical (Complete)</td>
<td>-0.497</td>
<td>-0.018</td>
<td></td>
</tr>
<tr>
<td>Hierarchical (Average)</td>
<td>0.526</td>
<td>-0.008</td>
<td></td>
</tr>
<tr>
<td>Hierarchical (Single)</td>
<td>0.526</td>
<td>-0.014</td>
<td></td>
</tr>
<tr>
<td>k-means</td>
<td>0.494</td>
<td>-0.016</td>
<td></td>
</tr>
<tr>
<td>PAM</td>
<td>0.611</td>
<td>0.218</td>
<td></td>
</tr>
<tr>
<td>MCLUST (VI)</td>
<td>-81,124.36</td>
<td>0.500</td>
<td>-0.006</td>
</tr>
<tr>
<td>EPGMM (CCUU, ( q = 6 ))</td>
<td>-70,937.72</td>
<td>0.849</td>
<td>0.697</td>
</tr>
</tbody>
</table>

### 3.3.4 Correspondence with McLachlan et al. (2002)
Using various techniques, McLachlan \textit{et al.} (2002) found five different clusterings of these data. However, none of these clusterings corresponded to the tissue type. While, once again, the EPGMM results are not directly comparable to those of McLachlan \textit{et al.} (2002), it is interesting to look at the second best of the EPGMM models. The second best of the EPGMM models, in terms of BIC, was a CCUU model with \( q = 7 \) latent factors. Note that this is one of the four new models that were introduced herein and, again, this model has equal covariance between pairs of genes, however the variance structure is more complex than for the CUCUC model. The MAP classification given by the parameter estimates associated with this CCUU model do not separate tumour from normal tissue. However, they are similar to what McLachlan \textit{et al.} (2002) call \( C_1 \), in that they seem sensible when one considers that there was a change of protocol during the experiment (Getz \textit{et al.}, 2000; McLachlan \textit{et al.}, 2002). Specifically, tissues 1–11 and 41–51 were all extracted from the first 11 patients using a poly detector, while the remaining samples were taken from the other patients using total extraction of RNA. Looking at the tissues by extraction method, rather than by tissue type, leads to the estimated classifications given in Table 9; only eight of the tissues were misclassified by this CCUU model when the data are considered by extraction method.

### 3.3.5 Comments
The EPGMM approach gave very good clustering performance when applied to the colon data. Our approach led to the misclassification of just five tissues, when these data were viewed by tissue type. This performance far exceeded that of all of the other techniques that were used — in fact, the performance of these other approaches was surprisingly poor, with only PAM giving better than random classifications (cf. Table 8). This phenomenon is partly explained when one looks at the classifications by extraction method, rather than by tissue type (cf. Table 10). In this case, only one method performed worse than random, which might suggest that techniques like \textit{k}-means clustering and \textit{mclust} were picking up extraction method more-so than tissue type. That said, the performance of these methods was only slightly better than random which suggests that the restrictive cluster shapes imposed by \textit{k}-means clustering and \textit{mclust} were not at all suited to the data. On the other hand, the best of the new EPGMM models gave the based clustering performance, misclassifying just eight samples.

### 4 DISCUSSION
The EPGMM family of models has been shown to give good clustering performance when applied to gene expression microarray data. These applications, concerning leukaemia and colon tissue data, respectively, were conducted as genuine clustering examples. That is, no information on the true tissue classification was used for parameter estimation or model selection. In fact, this information was only used to assess the performance of the selected model. In this context, the clustering performance of the EPGMM family can be looked upon favourably. Moreover, the performance of the EPGMM family on both data sets far exceeded that of a number of popular clustering techniques, including agglomerative hierarchical clustering and \textit{k}-means clustering.

However, like the techniques of McLachlan \textit{et al.} (2002), the EPGMM family relies on multiple random starts. In addition to the obvious drawback of the sensitivity of results to the starting values, there is the computation time that is required. Furthermore, there is no guarantee that increasing the number of random starts will lead to better clustering results. This is due, in the main, to the fact that models with greater BIC do not necessarily give better clustering performance. This phenomenon has been observed previously and
work into finding better model selection techniques is ongoing. That said, the EPGM family did perform well in the analyses in Section 3, based on random starting values and using the BIC.

5 CONCLUSION
The EPGM family of mixture models, for the model-based clustering of gene expression microarray data, has been introduced. This family of models is an extension of the PGMM family of models which, in turn, is an extension of the mixtures of factor analyzers model. The EPGMM family of models are very well suited to the analysis of high dimensional data. The reason for this suitability is three-fold. First, each member of the EPGMM family has a number of covariance parameters that is linear in the data-dimensionality. Second, as shown herein, the Woodbury identity can be used to avoid the inversion of any non-diagonal \( p \times p \) matrices, leading to efficient computation. Thirdly, as shown by McNicholas et al. (2010) in the context of the PGMM family, these models are 'trivially parallelizable', opening up the possibility of even more efficient parameter estimation using parallel computing. The EPGMM family was applied to two well known gene expression microarray data sets. In both cases, the EPGMM family performed well and gave much better clusterings than several popular clustering techniques. Herein, we took \( G = 2 \) for all of the analysis but future work will focus on the selection of \( G \).

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REFERENCES