Model selection for robust learning of mutational signatures using Negative Binomial non-negative matrix factorization

Marta Pelizzola¹, Ragnhild Laursen¹ and Asger Hobolth¹

¹Department of Mathematics, Aarhus University. Emails: {marta, ragnhild, asger}@math.au.dk

June 8, 2022

Abstract

The spectrum of mutations in a collection of cancer genomes can be described by a mixture of a few mutational signatures. The mutational signatures can be found using non-negative matrix factorization (NMF). To extract the mutational signatures we have to assume a distribution for the observed mutational counts and a number of mutational signatures. In most applications, the mutational counts are assumed to be Poisson distributed, but they are often overdispersed, and thus the Negative Binomial distribution is more appropriate. We demonstrate using a simulation study that Negative Binomial NMF requires fewer signatures than Poisson NMF to fit the data and we propose a Negative Binomial NMF with a patient specific overdispersion parameter to capture the variation across patients. We also introduce a robust model selection procedure inspired by cross-validation to determine the number of signatures. Furthermore we study the influence of the distributional assumption in relation to two classical model selection procedures: the Akaike information criterion (AIC) and the Bayesian information criterion (BIC). In the presence of overdispersion we show that our model selection procedure is more robust at determining the correct number of signatures than state-of-the-art methods, which are overestimating the number of signatures. We apply our proposed analysis on a wide range of simulated data and on a data set from breast cancer patients. The code for our algorithms and analysis is available in the R package SigMoS and can be found at https://github.com/MartaPelizzola/SigMoS.

Keywords: cancer genomics, cross-validation, model checking, model selection, mutational signatures, Negative Binomial, non-negative matrix factorization, Poisson.

AMS classification: 92-08, 92-10, 62-08

1 Introduction

Somatic mutations occur relatively often in the human genome and are mostly neutral. However, the accumulation of some mutation types in a genome can have harmful consequences. More specifically, the accumulation of somatic mutations observed in a tumor is called a mutational profile and can often be associated with factors such as aging (Risques and Kennedy, 2018), UV light (Shibai et al., 2017) or tobacco smoking (Alexandrov et al., 2016). A mutational profile is thus a mixture of mutational processes that are represented through mutational signatures. Several signatures have been identified from the mutational profiles and associated with different cancer types (Alexandrov et al., 2020; Tate et al., 2019).
A common strategy to derive the mutational signatures is non-negative matrix factorization (Alexandrov et al., 2013; Nik-Zainal et al., 2012; Lal et al., 2021). Non-negative matrix factorization (NMF) is a factorization of a given data matrix \( V \in \mathbb{R}^{N \times M}_0 \) into the product of two non-negative matrices \( W \in \mathbb{R}^{N \times K}_+ \) and \( H \in \mathbb{R}^{K \times M}_+ \) such that

\[
V \approx WH.
\]

The rank \( K \) of the lower-dimensional matrices \( W \) and \( H \) is much smaller than \( N \) and \( M \).

In cancer genomics, the data matrix \( V \) contains the mutational counts for different patients, also referred to as mutational profiles. The number of rows \( N \) is the number of patients and the number of columns \( M \) is the number of different mutation types. In this paper, \( M = 96 \) corresponding to the 6 base mutations, when assuming strand symmetry times the 4 flanking nucleotides on each side (i.e. \( 4 \cdot 6 \cdot 4 = 96 \)). The matrix \( H \) consists of \( K \) mutational signatures defined by probability vectors over the different mutation types. In the matrix \( W \), each row contains the weights of the signatures for the corresponding patient. In this context, the weights are usually referred to as the exposures of the different signatures. To estimate \( W \) and \( H \) we need to choose a model for the data \( V \). For mutational counts the usual assumption is the Poisson distribution (Alexandrov et al., 2013)

\[
V_{nm} \sim \text{Po}((WH)_{nm}),
\]

where \( W \) and \( H \) are estimated using the algorithm from Lee and Seung (1999) that minimizes the generalized Kullback-Leibler divergence. The algorithm is equivalent to maximum likelihood estimation, as the negative log-likelihood function for the Poisson model is equal to the generalized Kullback-Leibler up to an additive constant.

We suggest using a model where the mutational counts follow a Negative Binomial distribution that has an additional parameter that allows for overdispersion in the data. The Negative Binomial NMF (NB-NMF) is discussed in Gouvert et al. (2020), where it is applied to recommender systems, and it has recently been used in the context of cancer mutations in Lyu et al. (2020). They apply a supervised NB-NMF model to mutational counts from different cancers which uses cancer types as metadata. Their aim is to obtain signatures with a clear etiology, which could be used to classify different cancer types.

We investigate when and why NB-NMF is more suitable for mutational counts than the usual Poisson NMF (Po-NMF). In particular we suggest using a residual-based approach to investigate goodness of fit for a given data set. We consider mutational count data and we extend the NB-NMF model by including patient specific overdispersion. The extended model is referred to as NB\(_N\)-NMF, where \( N \) is the number of overdispersion parameters. As they also mention in Févotte et al. (2009), we believe that a great amount of research has been focusing on improving the performance of NMF algorithms given an underlying model and less attention has been directed to the choice of the underlying model given the data and application.

Additionally, we propose a novel model selection framework to choose the number of signatures. We use both simulated and real data to validate our proposed model selection procedure against other methods. We show that our model selection procedure is more robust against inappropriate model assumptions and that the Negative Binomial model is more suited for mutational count data than the usual Po-NMF.

We have implemented our methods in the R package SigMoS that includes NB-NMF, NB\(_N\)-NMF and the model selection procedure. The R package is available at https://github.com/MartaPelizzola/SigMoS. The package also contains the simulated and real data used in this paper.
2 Negative Binomial non-negative matrix factorization

In this section we first argue why the Negative Binomial model in Gouvert et al. (2020) is a natural model for the number of somatic mutations in a cancer patient. Then we describe our patient specific Negative Binomial non-negative matrix factorization NBN\_N\_NMF and the corresponding estimation procedure.

2.1 Negative Binomial model for mutational counts

We start by illustrating the equivalence of the Negative Binomial to the more natural Beta-Binomial model as a motivation for such model choice. Assume a certain mutation type can occur in \( \tau \) triplets along the genome with a probability \( p \). Then it is natural to model the mutational counts with a binomial distribution (Weinhold et al., 2014; Lochovsky et al., 2015)

\[
V_{nm} \sim \text{Bin}(\tau, p). \tag{2}
\]

However, Lawrence et al. (2013) observed that the probability of a mutation varies along the genome and is correlated with both expression levels and DNA replication timing. We therefore introduce the Beta-Binomial model

\[
V_{nm}|p ~ \text{Bin}(\tau, p) \\
p ~ \text{Beta}(\alpha, \beta), \tag{3}
\]

where the beta prior on \( p \) models the heterogeneity of the probability of a mutation for the different mutation types due to the high variance along the genome. As \( p \) follows a Beta distribution, its expected value is \( E[p] = \alpha/\alpha + \beta \). For mutational counts, the number of triplets \( \tau \) is extremely large and the probability of mutation \( p \) is very small. In the data described in Lawrence et al. (2013) there are typically between 1 and 10 mutations per megabase with an average of 4 mutations per megabase (\( \tau \approx 10^6 \)). This means \( E[p] = \alpha/\alpha + \beta \approx 4 \cdot 10^{-6} \) and thus, \( \beta \gg \alpha \). As \( \tau \) is large and \( p \) is small, the Binomial model is very well approximated by the Poisson model \( \text{Bin}(\tau, p) \approx \text{Pois}(\tau p) \). This distributional equivalence of Poisson and Binomial when \( \tau \) is large and \( p \) is small is well known. This also means that the models (1) and (2) are approximately equivalent to \( \tau p = (WH)_{nm} \).

The Beta and Gamma distributions are also approximately equivalent in our setting. Indeed, as \( \beta \gg \alpha \), the Beta density can be approximated by the Gamma density in the following way

\[
\frac{p^{\alpha-1}(1-p)^{\beta-1}}{B(\alpha, \beta)} = \frac{p^{\alpha-1}}{\Gamma(\alpha)}(\beta - 1 + \alpha)(\beta - 1 + (\alpha - 1)) \cdots (\beta - 1)(1-p)^{\beta-1} \approx \frac{p^{\alpha-1}}{\Gamma(\alpha)} \beta^\alpha(e^{-p})^\beta.
\]

Therefore, for mutational counts the model in (3) is equivalent to

\[
V_{nm}|p ~ \text{Pois}(\tau p) \\
p ~ \text{Gamma}(\alpha, \beta). \tag{4}
\]

Since the Negative Binomial model is a Gamma-Poisson model we can also write the model as

\[
V_{nm} \sim \text{NB}(\alpha, \frac{\tau}{\beta + \tau}) \approx \text{NB}(\alpha, \frac{\tau E[p]}{\alpha + \tau E[p]}) \approx \text{NB}(\alpha, \frac{(WH)_{nm}}{\alpha + (WH)_{nm}}),
\]

where the last parametrization is equivalent to the one in Gouvert et al. (2020). In the first distributional equivalence we use \( E[p] \approx \frac{\alpha}{\beta} \) and in the second we use \( \tau E[p] = (WH)_{nm} \). Compared to the Beta-Binomial model, the Negative Binomial model has one fewer parameter and is analytically more tractable. The mean and variance of this model are given by

\[
E[V_{nm}] = (WH)_{nm} \quad \text{and} \quad \text{Var}(V_{nm}) = (WH)_{nm} \left( 1 + \frac{(WH)_{nm}}{\alpha} \right). \tag{5}
\]
In recent years, this model has become more popular to model the dispersion in mutational
counts (Martincorena et al., 2017; Zhang et al., 2020). When $\alpha \to \infty$ above, the Negative
Binomial model converges to the more commonly used Poisson model as $\text{Var}(V_{nm}) \downarrow (WH)_{nm}$.
As shown in this section, the Negative Binomial model can be seen both as an extension of the
Poisson model and as equivalent to the Beta-Binomial model. Thus, we opted to implement a
NB-NMF model for mutational count data. More details on the approximation of the Negative
Binomial to the Beta-Binomial distribution can also be found in Teerapabolarn (2015).

2.2 Patient specific Negative Binomial NMF: NB\textsubscript{N}-NMF

Gouvert et al. (2020) and Lyu et al. (2020) present a Negative Binomial model where $\alpha$ is
shared across all observations. We extend the model by allowing patient specific dispersion. We
assume

$$V_{nm} \sim \text{NB}\left(\alpha_n, \frac{(WH)_{nm}}{\alpha_n + (WH)_{nm}}\right)$$

where $n \in 1, \ldots, N$ correspond to the different patients. As for the estimation of $W$ and $H$ in
Gouvert et al. (2020), we define the following divergence measure:

$$d_N(V||WH) = \sum_{n=1}^{N} \sum_{m=1}^{M} \left\{V_{nm} \log \left(\frac{V_{nm}}{(WH)_{nm}}\right) - (\alpha_n + V_{nm}) \log \left(\frac{\alpha_n + V_{nm}}{\alpha_n + (WH)_{nm}}\right)\right\}. \quad (6)$$

In Section 6 we show that the negative of the log-likelihood function is equal to this divergence up
to an additive constant. If $V = WH$, it is straightforward to see that $d_N(V||WH) = 0$ and when
$V \neq WH$ we can show $d_N(V||WH) > 0$ by defining $g(t) = (V_{nm} + t) \log \left((V_{nm} + t)/(WH)_{nm+t}\right)$
and showing $g'(t) < 0$. In our application, we find maximum likelihood estimates (MLEs) of
$\alpha_1, \ldots, \alpha_N$ based on the Negative Binomial likelihood using Newton-Raphson together with
the estimate of $WH$ from Po-NMF. We opted for this more precise estimation procedure for
$\alpha_1, \ldots, \alpha_N$ instead of the grid search approach used in Gouvert et al. (2020). Final estimates
of $W$ and $H$ are then found by minimizing the divergence in (6) by the iterative majorize-
minimization procedure (see the derivation in Section 6). The NB\textsubscript{N}-NMF procedure is described
in Algorithm 1 and further details can be found in Section 6.1. The NB-NMF method is similar
except $\alpha_1 = \cdots = \alpha_N = \alpha$.

Algorithm 1 NB\textsubscript{N}-NMF: Estimation of $W$, $H$ and $\{\alpha_1, \ldots, \alpha_N\}$

Input: $V, K, \epsilon$

Output: $W$, $H$, $\{\alpha_1, \ldots, \alpha_N\}$

1. $\hat{W}, \hat{H} \leftarrow$ apply Po-NMF to $V$ with $K$ signatures
2. $\alpha_1, \ldots, \alpha_N \leftarrow$ Negative Binomial MLE using $\hat{W}, \hat{H}$ and $V$
3. Initialize $W^1, H^1$ from a random uniform distribution
4. for $i = 1, 2, \ldots$
5. \hspace{2em} $W_{nk}^{i+1} \leftarrow W_{nk}^{i} \frac{V_{nm}^{i} \sum_{m=1}^{M} \frac{H_{km}^{i}}{(WH)_{nm}^{i}}} {\sum_{m=1}^{M} \frac{V_{nm}^{i} \alpha_n^{i} H_{km}^{i}}{(WH)_{nm}^{i}}}$
6. \hspace{2em} $H_{km}^{i+1} \leftarrow H_{km}^{i} \frac{V_{nm}^{i} \sum_{n=1}^{N} \frac{W_{nk}^{i+1}}{(WH)_{nm}^{i+1} + \alpha_n}} {\sum_{n=1}^{N} \frac{V_{nm}^{i} \alpha_n^{i} W_{nk}^{i+1}}{(WH)_{nm}^{i+1} + \alpha_n}}$
7. \hspace{2em} if $|d_N(V||W_{i+1}^{i}H_{i+1}^{i}) - d_N(V||W_{i}^{i}H_{i}^{i})| < \epsilon$ then
8. \hspace{3em} return $W_{i+1}, H_{i+1}$
9. \hspace{2em} end if
10. end for

4
Estimating the number of signatures is a difficult problem when using NMF. More generally, estimating the number of components for mixture models or the number of clusters is a well-known challenge in applied statistics.

Examples of the complexity of this problem can be found in the $K$-means clustering algorithm and in Gaussian mixture models where the number of clusters $K$ has to be provided for the methods. The silhouette and the elbow method are among the most common techniques to estimate $K$ for $K$-means clustering, however it is often unclear how to find an exact estimate of $K$. A detailed description of these challenges can be found in Gupta et al. (2018). Here the authors also propose a new way of estimating the number of clusters that follows the same rationale as the elbow method, but it combines the detection of optimal well-separated clusters and clusters with equal number of elements. The discrepancy between these two solutions is then used to determine $K$.

Estimating the number of components is also a critical issue for mixed membership models. One example can be found in the estimation of the number of subpopulations in population genetics. Population structure is indeed modeled as a mixture model of $K$ subpopulations and the inference of $K$ is challenging. In Pritchard et al. (2000) an ad hoc solution is proposed under the assumption that the posterior distribution follows a normal distribution, which is often violated in practice. Verity and Nichols (2016) take a different approach and derive a new estimator using thermodynamic integration based on the ”power posterior” distribution. This is nothing more than the ordinary posterior distribution, but with the likelihood raised to a power to ensure that the distribution integrates to 1. This procedure seems to be very accurate, however it is computationally intense and thus can only be used on small data sets.

Classical procedures to perform model selection are the Akaike Information Criterion (AIC)

$$AIC = -2 \ln L + 2n_{prm}$$  \hspace{1cm} (7)$$
and the Bayesian Information Criterion (BIC)

$$BIC = -2 \ln L + \ln(n_{obs})n_{prm}$$  \hspace{1cm} (8)$$

where $\ln L$ is the estimated log-likelihood value, $n_{obs}$ is the number of observations and $n_{prm}$ the number of parameters to be estimated. The two criteria attempt to balance the fit to the data (measured by $-2 \ln L$) and the complexity of the model (measured by the scaled number of free parameters). We have $n_{obs} = N$ where $N$ is the number of patients, so $\ln(n_{obs}) > 2$ if $N \geq 8$, which means that in our context the number of parameters has a higher influence for BIC compared to AIC because real data sets always have at least tens of patients. Additionally, the structure of the data matrix $V$ can lead to two different strategies for choosing $n_{obs}$ when BIC is used. Indeed, the number of observations in this context can be set as the total number of counts (i.e. $N \cdot M$) or as the number of patients $N$, leading to an ambiguity in the definition of this criterion. Verity and Nichols (2016) also presents results on the performance of AIC and BIC, where the power is especially low for BIC. AIC provides higher stability in the scenario from Verity and Nichols (2016), however it does not seem suitable in our situation due to a small penalty term.

A very popular model selection procedure is cross-validation. In Gelman et al. (2013) they compare various model selection methods including AIC and cross-validation. Here, the authors recommend to use cross-validation as they demonstrate that the other methods fail in some circumstances. In Luo et al. (2017) they also show that cross-validation has better performance than the other considered methods, including AIC and BIC.
3.1 Model selection for NMF

For NMF we propose an approach for estimating the rank which is highly inspired by cross-validation. As for classical cross-validation we split the patients in \( V \) in a training and a test set multiple times. However, opposite to cross-validation, we are using information from the full data to validate the fit of the test set. As all the parameters in the model are free parameters, we have chosen to fix the exposures to the ones estimated from the full data. This means our evaluation on the test set is a combination of estimated signatures from the training set and exposures from the full data. The idea is to exploit the fact that the signature matrix should be robust to changes in the patients included in the training set. If the estimated signatures are truly explaining the main patterns in the data, then we expect the signatures obtained from the training set to be equivalent to the ones from the full data. Therefore the product of the exposures from the full data and the signatures from the training set should give a good approximation of the test set, if \( K \) is appropriate.

Inputs for the method are the data \( V \), an NMF procedure, the number of signatures to be considered \( K \), the number of splits into training and test \( J \) and the cost function. We evaluate the model for a range of values of \( K \) and then select the model with the lowest cost. The NMF procedures we are using here are either Po-NMF from Lee and Seung (1999), NB-NMF or NB\(\nu\)-NMF in Algorithm 1, but any NMF procedure could be applied.

A visualization of our model selection algorithm can be found in Figure 1. First, we consider the full data matrix \( V \) and we apply the chosen NMF algorithm to obtain an estimate for both \( W \) and \( H \). Afterwards, for each iteration we sample 90% of the patients randomly to create the training set and determine the remaining 10% as our test set. We then apply the chosen NMF to the mutational counts of the training set obtaining an estimate \( W_{\text{train}} \) and \( H_{\text{train}} \).

\[
\text{Assessment of fit:} \\
\text{Full data: } V \approx W \hat{H} \\
j = 1 \\
V_{\text{train}}1 \approx W_{\text{train}}1 \hat{H}_{\text{train}}1 \\
V_{\text{test}}1 \approx W_{\text{test}}1 \hat{H}_{\text{train}}1 \\
c1 = \text{cost}(V_{\text{test}}1, W_{\text{test}}1 \hat{H}_{\text{train}}1) \\
\vdots \\
j = J \\
V_{\text{train}}J \approx W_{\text{train}}J \hat{H}_{\text{train}}J \\
V_{\text{test}}J \approx W_{\text{test}}J \hat{H}_{\text{train}}J \\
cJ = \text{cost}(V_{\text{test}}J, W_{\text{test}}J \hat{H}_{\text{train}}J) \\
\text{median} = \text{median}(c1, \ldots, cJ)
\]

Figure 1: Model selection procedure for a given number of signatures \( K \) and a count matrix \( V \). Pseudocode can be found in Algorithm 2.

Now, as for classical cross-validation, we want to evaluate our model on the test set. To evaluate the model here, we use the full data: indeed, we multiply the exposures relative to the patients in the test set estimated on the full data times the corresponding signatures estimated from the training set. We use the prediction of the test data to evaluate the model computing the distance between the true data and their prediction with a suitable cost function. This procedure is iterated \( J \) times leading to \( J \) cost values \( c_j, j = 1, \ldots, J \). The median of these values is calculated for each number of signatures \( K \). We call this procedure SigMoS and summarize it in Algorithm 2. The optimal \( K \) is the one with the lowest cost. We use the generalized Kullback-Leibler divergence as a cost function and discuss the choice of cost function in Section 5. We compare the influence of the model choice for our procedure to AIC.
and BIC. We also compare to other recently introduced methods in the literature and present the results from this comparison in Section 4.1.

Algorithm 2  SigMoS: Cost for a given number of signatures $K$ for the count matrix $V$
Input: $V,K,J,cost$, NMF-distribution
Output: $c_{\text{median}}$
1: $\hat{W},\hat{H} \leftarrow$ apply NMF with the chosen distribution to $V$ with $K$ signatures
2: for $j = 1$ to $J$ do
3: $V_{\text{train}} \leftarrow$ mutational counts for the patients in the $j^{th}$ training set
4: $V_{\text{test}} \leftarrow V \setminus V_{\text{train}}$
5: $\hat{W}_{\text{test}} \leftarrow$ exposures from $\hat{W}$ for the patients in the test set
6: $\hat{W}_{\text{train}},\hat{H}_{\text{train}} \leftarrow$ apply NMF with the chosen distribution to $V_{\text{train}}$ with $K$ signatures
7: $c_j \leftarrow cost(V_{\text{test}},\hat{W}_{\text{test}}\hat{H}_{\text{train}})$
8: end for
9: return $c_{\text{median}} \leftarrow \text{median}(c_1,\ldots,c_J)$

4 Results
In this section we describe our results on both simulated and real data. For simulated data we present a study on Negative Binomial simulated data with different levels of dispersion where results from AIC, BIC, SparseSignatures \cite{Lal2021}, SigneR \cite{Rosales2017}, and SignatureAnalyzer \cite{Tan2013} are compared with our proposed model selection procedure. These results are discussed in Section 4.1. We show that our method performs well and is robust to model misspecification. We use a residual analysis to evaluate the goodness of fit and a likelihood ratio test to choose between NB-NMF and NB$_N$-NMF. Furthermore, we apply this analysis to a data set with 21 breast cancer patients from \cite{Alexandrov2013} in Section 4.2.

4.1 Simulation results
We simulate our data following the procedure of \cite{Lal2021}. We consider the set of signatures from \cite{Tate2019} and, for each simulation run, we use signature 1 and 5 from \cite{Tate2019} as they have been shown to be shared across all cancer types, and we sample at random three additional signatures from this set. We simulate the exposures from a Negative Binomial model with mean 6000 and dispersion parameter 1.5 \cite{Lal2021}. The mutational count data is then generated as the product of the exposures and signature matrix. Lastly, Poisson noise, Negative Binomial noise with dispersion parameter $\alpha \in \{10,200\}$ or patient specific dispersion parameters $\{\alpha_1,\ldots,\alpha_N\}$ are added to the mutational counts. The values of the patient specific dispersion are inspired from the data set in Section 4.2. A lower $\alpha$ is associated with higher dispersion, however the actual level of dispersion associated to a given $\alpha$ value depends on the absolute mutational counts as can be seen from the variance in Equation (5). Therefore it is not possible to directly compare these values with the ones estimated for the real data. Using this setup, we simulated 100 data sets with five signatures and 100 patients each.

Figure 2 shows the effect of the model assumption on the estimated number of signatures using AIC, BIC (recall Equations (7) and (8)) and SigMoS as model selection procedures. Using Po-NMF, when the data is overdispersed, leads to an overestimation of the number of signatures as the model explains the overdispersion with additional signatures. The Negative Binomial model allows for more variability and thus a lower number of signatures can explain the data.
Figure 2: Results from AIC, BIC, and SigMoS based on Po-NMF (in the top), NB-NMF (in the middle) and NB$_N$-NMF (in the bottom) using simulated data. Each method is applied on 100 simulated data sets for each value of $\alpha$ and four different scenarios are simulated: Poisson and Negative Binomial dispersion with $\alpha = 10, 200$ and $\alpha \sim U(10, 150)$. The true number of signatures in the simulated data is five and marked in bold. For each data set, the value of the number of signatures is estimated with AIC, BIC, and SigMoS using Po-NMF, NB-NMF and NB$_N$-NMF. The $\alpha$ values are estimated by maximum likelihood estimation.

Correctly specifying the model proves to be essential for determining the optimal number of signatures for AIC and BIC especially when the overdispersion is high ($\alpha = 10$). Nonetheless, SigMoS is robust even when the wrong model is chosen (i.e. when using Po-NMF). These results also illustrate that our model selection method is accurate for detecting the correct number of signatures in situations with some overdispersion i.e. $\alpha = 200$ when assuming the Poisson model. When the true distributional model is unknown, another possibility would be to use the NB$_N$-NMF on the data. Indeed, the patient specific NB$_N$-NMF is a generalization of both Po-NMF and NB-NMF and thus it is robust under any of these distributional assumptions (see bottom row in Figure 2).

In this simulation study we also consider the accuracy of the MLE for the $\alpha$ value in the three scenarios. Our approach estimates the true $\alpha$ with high accuracy when the overdispersion is high i.e. $\hat{\alpha} \in [9.21, 11.78]$ for $\alpha = 10$, $\alpha$ is slightly overestimated when the overdispersion is low: for $\alpha = 200$ we find $\hat{\alpha} \in [225.8, 292.7]$. However according to Figure 2, this does not affect the performance of our model selection procedure.

We considered also an identical set of simulations with 10 signatures: here, we observed that our proposed approach is again estimating the number of signatures accurately and it is still robust to model misspecifications when comparing the results assuming the Poisson and the Negative Binomial model.

Several methods have been proposed in the literature for estimating the number of signatures in cancer data. In the following we present the results of a comparison between our method and three commonly used methods in the literature: SparseSignatures, SignatureAnalyzer, and SigneR. SparseSignatures (Lal et al., 2021) provides an alternative cross-validation approach
where the test set is defined by setting 1% of the entries in the count matrix to 0. Then NMF is iteratively applied to the modified count matrix and the entries are updated at each iteration. The resulting signature and exposure matrices are used to predict the entries of the matrix corresponding to the test set. **SignatureAnalyzer** (Tan and Févotte, 2013), on the other hand, proposes a procedure where a Bayesian model is used and maximum a posteriori estimates are found with a majorize-minimization algorithm. Lastly, with **signeR** (Rosales et al., 2017) an empirical Bayesian approach based on BIC is used to estimate the mutational signatures.

For our method comparison, we run all methods on the simulated data from Figure 2. For each method and simulation setup we only allow the number of signatures to vary from two to eight due to the long running time of some of these methods.

Figure 3: Method comparison using simulated data. Each method is applied on 100 simulated data sets and, for each data set, the value of the estimated number of signatures is kept. We test values for the number of signatures from two to eight and we simulate under four different scenarios: Poisson and Negative Binomial with $\alpha = 10, 200$, and a patient specific dispersion parameter.

Figure 3 shows that, when Poisson data are simulated all methods have a very good performance and can recover the true number of signatures in most of the simulations. The **SparseSignatures** method also has a good performance in this case as it includes a background signature. We would therefore expect it to always estimate one signature more than the true number of signatures present in the data. When Negative Binomial noise is added to the simulated data with a moderate dispersion ($\alpha = 200$), however, both **SignatureAnalyzer** and **signeR** have low power emphasizing the importance of correctly specifying the distribution for these methods, whereas our proposed approach (regardless of the distributional assumption) and **SparseSignatures** maintain good power. For patient specific overdispersion also the power of **SparseSignatures** decreases. Good performance is also achieved with our proposed approach under high overdispersion ($\alpha = 10$) if the correct distribution is assumed. These results demonstrate that SigMoS is accurate for detecting the correct number of signatures and it performs well also in situations with overdispersion.

4.2 Results on Breast Cancer Data

Here, we apply the workflow for mutational count analysis to a data set with 21 breast cancer patients (Alexandrov et al., 2013). In Figure 4, we present the results of this analysis.

We have applied SigMoS to choose the number of signatures for the NMF-methods: Po-NMF, NB-NMF and NB_N-NMF. SigMoS indicates to use three signatures with all methods. This is in line with the results in our simulation study, where we show that our model selection is robust to model misspecification. In Figure 4 we also present results from BIC. According to BIC, six signatures are needed for Po-NMF whereas only three signatures should be used with NB_N-NMF which emphasizes the importance of a correct model choice when using BIC. The confidence intervals for SigMoS also demonstrate that the results are more robust around the optimal number of signatures. When the number of signatures is too high we see a high variability in the model selection which explains the fluctuations in the median.
Figure 4: SigMoS results for Po-NMF, NB-NMF, and NB\textsubscript{N}-NMF applied to a data set with 21 breast cancer patients. The upper panel of the figure shows the median of 100 iterations of our proposed model selection approach and the confidence interval between the 25\% and 75\% quantiles. The two lower panels of the figure show the residuals plots for the optimal number of signatures from SigMoS. The lines in the first plot correspond to two times the expected variance under the chosen distributional assumption. As the NB\textsubscript{N}-NMF holds 21 different expected variances, we have chosen to plot the median, minimum and maximum variance among the 21. The second plots show the normalised residuals. The vertical grey lines depict the theoretical quantiles.

After finding the number of signatures we report the corresponding residuals $R_{nm} = V_{nm} - (WH)_{nm}$. The residuals are plotted against the expected mean $(WH)_{nm}$, as the variance in both the Poisson and Negative Binomial model depends on this value. The colored lines in the residual plots correspond to $\pm 2\sigma$ for the Poisson and the Negative Binomial distribution respectively. The variance $\sigma^2$ can be derived from Equation (5) for the Negative Binomial model and is equal to the mean for the Poisson model.

Starting from the results of Po-NMF, we observe a clear overdispersion in the residuals, which suggest to use a Negative Binomial model. In the first residual plot for the Negative Binomial model we see that the residuals have a much better fit to the variance structure, which is indicated by the colored lines. The quantile lines in the lower panel with normalised residuals show that the quantiles from the NB-NMF and the NB\textsubscript{N}-NMF are much closer to the theoretical ones, suggesting again that the Negative Binomial model is better suited in this case.

We apply a likelihood ratio test to choose between NB-NMF ($\alpha = 65$) and NB\textsubscript{N}-NMF.
\( \alpha_n \in [16, 26083] \) as follows:

\[
LRT = -2(\log L_{\text{NB-NMF}} - \log L_{\text{NB-NMF}}) = 166.68 \sim \chi^2(20)
\] (9)

and we obtain that NB-NMF has a better fit in this case \( p \approx 0 \). We would therefore recommend to use NB-NMF when analysing the 21 breast cancer patients. For this data set indeed there is a high difference in the dispersion parameters of the different patients with one patient with a much lower dispersion \( \alpha = 26083 \) compared to the others \( \alpha_n \in [16, 550] \) and thus NB-NMF is preferable.

We applied the same workflow removing the patient with \( \alpha = 26083 \) from the data, indeed this patient can also be viewed as an outlier in this context [Fischer et al., 2013]. In this case, we find that NB-NMF would give a better fit to the data \( p \approx 1 \), suggesting that the patient specific component in this case would be mainly driven by this one outlier. The usage of NB-NMF instead of NB-NMF would thus depend on the treatment of outliers in downstream analyses in this case.

5 Discussion

Mutational profiles from cancer patients are a widely used source of information and NMF is often applied to these data in order to identify signatures associated with cancer types. We propose a new approach to perform the analysis and signature extraction from mutational count data where we emphasize the importance of validating the model using residual analysis, and we propose a robust model selection procedure.

We use the Negative Binomial model as an alternative to the commonly used Poisson model as the Negative Binomial can account for the high dispersion in the data. As a further extension of this model, we allow the Negative Binomial to have a patient specific variability component to account for heterogeneous variance across patients.

We propose a model selection approach for choosing the number of signatures. As we show in Section 4.1 this method works well with both Negative Binomial and Poisson data and it is a robust procedure for choosing the number of signatures. We note that the choice of the divergence measure for the cost function in Algorithm 2 is not trivial and may favor one or the other model and thus a comparison of the costs between different NMF methods is not possible. For example, in our framework, we use the Kullback-Leibler divergence which would favor the Poisson model. This means that a direct comparison between the cost values for Po-NMF and NB-NMF or NB-NMF is not feasible.

To check the goodness of fit and choose between the Poisson model and the Negative Binomial model we propose to use the residuals and to choose between the classical Negative Binomial (NB-NMF) and the Negative Binomial with patient specific variability (NB-NMF) we use a likelihood ratio test.

We investigated the role of the cost function in our model selection by including the Frobenius norm and the Beta and Itakura-Saito (IS) [Févotte and Idier, 2011] divergence measures from Li et al. (2012) where the authors propose a fast implementation of the NMF algorithm with general Bregman divergence. In this investigation the cost function did not influence the optimal number of signatures. The only difference was how the cost values differed among the NMF methods, as each cost function favored the models differently. Therefore we chose to use the Kullback-Leibler divergence and compared the methods with the residual analysis.

Less signatures are found when accounting for overdispersion with the Negative Binomial model. Indeed, there is no need to have additional signatures explaining noise, which we assume is the case for the Poisson model. We show the Negative Binomial model is more suitable and therefore believe the corresponding signatures are more accurate. This can be helpful when working with mutational profiles for being able to better associate signatures with cancer types and for a clearer interpretation of the signatures when analysing mutational count data. The
6 Methods

In Section 2 we describe the NB-NMF model applied to mutational count data and we propose an extension where a patient specific dispersion coefficient is used. The majorization-minimization (MM) procedure for patient specific overdispersion \( \{\alpha_1, \ldots, \alpha_N\} \) can be found in Section 2.2. In our application, we propose to use negative binomial maximum likelihood estimation for \( \alpha \) (see Section 2.1) and \( \{\alpha_n : 1 \leq n \leq N\} \) (see Section 2.2) instead of the grid search approach adopted in Gouvert et al. (2020). The pseudocode shown in the initial steps of Algorithm 1 describes this approach for patient specific overdispersion. For shared overdispersion among all patients and mutation types we simply set \( \alpha = \alpha_1 = \cdots = \alpha_N \) in Algorithm 1.

6.1 Patient specific NB-NMF

As we discuss in Section 4.2 the variability in mutational counts among different patients can be really high. Thus we extend the NB-NMF model from Gouvert et al. (2020) (see Section 2.1) by including a patient specific component (see Section 2.2). We noticed that the variability among different patients is usually much higher than the one among different mutation types, thus we decided to focus on a patient specific NB-NMF here.

The entries in \( V \) are modeled as

\[
V_{nm} \sim \text{NB}\left(\alpha_n, \frac{(WH^T)_{nm}}{\alpha_n + (WH^T)_{nm}}\right),
\]

where \( \alpha_n \) is the dispersion coefficient of each patient, and the corresponding Gamma-Poisson hierarchical model can be rewritten as:

\[
V_{nm}|a_{nm} \sim \text{Po}(a_{nm}(WH)_{nm}) \\
a_{nm} \sim \text{Gamma}(\alpha_n, \alpha_n).
\]

Here \( a_{nm} \) is the parameter responsible for the variability in the Negative Binomial model.

Now we can write the Negative Binomial log-likelihood function with patient specific \( \alpha_n \)

\[
\ell(W, H; V) = \sum_{n=1}^{N} \sum_{m=1}^{M} \log \left( \frac{\alpha_n + V_{nm} - 1}{\alpha_n} \right) + V_{nm} \log \left( \frac{(WH)_{nm}}{\alpha_n + (WH)_{nm}} \right) + \alpha_n \log \left( 1 - \frac{(WH)_{nm}}{\alpha_n + (WH)_{nm}} \right)
\]

and recognize the negative of the log-likelihood function as proportional to the following divergence:

\[
d_N(V||WH) = \sum_{n=1}^{N} \left\{ \sum_{m=1}^{M} V_{nm} \log \left( \frac{V_{nm}}{(WH)_{nm}} \right) - (\alpha_n + V_{nm}) \log \left( \frac{\alpha_n + V_{nm}}{\alpha_n + (WH)_{nm}} \right) \right\}. \quad (12)
\]

The term \( \log \left( \frac{\alpha_n + V_{nm} - 1}{\alpha_n} \right) \) in the likelihood is a constant we can remove and then we have added the constants \( V_{nm} \log(V_{nm}), \alpha_n \log(\alpha_n) \) and \( (V_{nm} + \alpha_n) \log(V_{nm} + \alpha_n) \).

Following the steps in Gouvert et al. (2020), we will update \( W \) and \( H \) one at a time, while the other is assumed fixed. We will show the procedure for updating \( H \) using a fixed \( W \) and its previous value \( H^t \). First we construct a majorizing function \( G(H, H^t) \) for \( d_N(V||WH) \) with...
the constraint that \( G(H, H) = d_N(V||WH) \). The first term in Equation (12) can be majorized using Jensen’s inequality leading to

\[
d_N(V||WH) = \sum_{n=1}^{N} \sum_{m=1}^{M} V_{nm} \log \left( \frac{V_{nm}}{\sum_{k=1}^{K} W_{nk} H_{km}} \right) - (\alpha_n + V_{nm}) \log \left( \frac{\alpha_n + V_{nm}}{\alpha_n + \sum_{k=1}^{K} W_{nk} H_{km}} \right)
\]

where \( \beta_k = \frac{W_{nk} H_{km}}{\sum_{k=1}^{K} W_{nk} H_{km}} \). The second term can be majorized with the tangent line using the concavity property of the logarithm:

\[
d_N(V||WH) \leq \sum_{n=1}^{N} \sum_{m=1}^{M} V_{nm} \log V_{nm} - V_{nm} \sum_{k=1}^{K} \beta_k \log \frac{W_{nk} H_{km}}{\beta_k}
\]

\[
+ (\alpha_n + V_{nm}) \log \left( \frac{\alpha_n + \sum_{k=1}^{K} W_{nk} H_{km}}{\alpha_n + V_{nm}} \right)
\]

Lastly, we need to show that \( G(H, H) = d_N(V||WH) \). This follows from

\[
G(H, H) = \sum_{n=1}^{N} \sum_{m=1}^{M} V_{nm} \log V_{nm} - V_{nm} \sum_{k=1}^{K} \beta_k \log \frac{W_{nk} H_{km}}{\beta_k}
\]

\[
+ (\alpha_n + V_{nm}) \log \left( \frac{\alpha_n + \sum_{k=1}^{K} W_{nk} H_{km}}{\alpha_n + V_{nm}} \right) + \sum_{n=1}^{N} \sum_{m=1}^{M} V_{nm} \log V_{nm} - V_{nm} \sum_{k=1}^{K} \beta_k \log \frac{W_{nk} H_{km}}{\beta_k}
\]

\[
+ (\alpha_n + V_{nm}) \log \left( \frac{\alpha_n + \sum_{k=1}^{K} W_{nk} H_{km}}{\alpha_n + V_{nm}} \right)
\]

\[
= \sum_{n=1}^{N} \sum_{m=1}^{M} V_{nm} \log V_{nm} - V_{nm} \sum_{k=1}^{K} \log \left( \frac{W_{nk} H_{km}}{\sum_{k=1}^{K} W_{nk} H_{km}} \right)
\]

\[
- (\alpha_n + V_{nm}) \log \left( \frac{\alpha_n + \sum_{k=1}^{K} W_{nk} H_{km}}{\alpha_n + V_{nm}} \right)
\]

\[
= d_N(V||WH)
\]

Having defined the majorizing function \( G(H, H^t) \) in (15), we can derive the following mul-
The multiplicative update for $H$:

$$H_{km}^{t+1} = H_{km}^{t} \frac{\sum_{n=1}^{N} \frac{V_{nm}}{(W'H)_{nm}} W_{nk}^{t}}{\sum_{n=1}^{N} \frac{V_{nm} + \alpha_n}{(W'H)_{nm} + \alpha_n} W_{nk}^{t}}.$$  \hspace{1cm} (16)

Similar calculations can be carried out for $W$ to obtain the following update:

$$W_{nk}^{t+1} = W_{nk}^{t} \frac{\sum_{m=1}^{M} \frac{V_{nm}}{(W'H)_{nm}} H_{km}^{t}}{\sum_{m=1}^{M} \frac{V_{nm} + \alpha_n}{(W'H)_{nm} + \alpha_n} H_{km}^{t}}.$$  \hspace{1cm} (17)

It is straightforward to see that when $\alpha_n = \alpha$ for all $n = 1, \ldots, N$ then the updates for $W$ and $H$ equal those in [Gouvert et al. (2020)](https://doi.org/10.1038/s41467-020-19942-6). Additionally, as shown in [Gouvert et al. (2020)](https://doi.org/10.1038/s41467-020-19942-6) when $\alpha \to \infty$ the updates of the Po-NMF [Lee and Seung (1999)](https://doi.org/10.1162/089976699300017469) are recovered. The pseudo code in Algorithm 1 summarizes the NB$_N$-NMF model discussed in this section.

### 6.2 Code for method comparison

For **SparseSignatures** we use the function `nmflassoCV` with `normalize_counts` being set to `FALSE` and `lambda_values_alpha` and `lambda_values_beta` to zero. All the other parameters are set to their default values. When applying **SignatureAnalyzer** we used the following command:

```
python SignatureAnalyzer-GPU.py --data f --prior on W L1 --prior on H L2 --output_dir d --max_iter 1000000 --tolerance 1e-7 --K0 8
```

For **SigneR** we used the default options.

### Acknowledgement

We would like to thank Simon Opstrup Drue for helpful comments and suggestions on an earlier version of this manuscript. MP acknowledges funding of the Austrian Science Fund (FWF Doctoral Program Vienna Graduate School of Population Genetics”, DK W1225-B20).

### References

Alexandrov, L. B., Ju, Y. S., Haase, K., Van Loo, P., Martincorena, I., Nik-Zainal, S., Totoki, Y., Fujimoto, A., Nakagawa, H., Shibata, T., Campbell, P. J., Vineis, P., Phillips, D. H., and Stratton, M. R. (2016). Mutational signatures associated with tobacco smoking in human cancer. *Science*, 354(6312):618–622.

Alexandrov, L. B., Kim, J., Haradhvala, N. J., Huang, M. N., Tian Ng, A. W., Wu, Y., Boot, A., Covington, K. R., Gordenin, D. A., Bergstrom, E. N., Islam, S. M. A., Lopez-Bigas, N., Klimczak, L. J., McPherson, J. R., Morganella, S., Sabarinathan, R., Wheeler, D. A., Mustonen, V., Getz, G., Rozen, S. G., and Stratton, M. R. (2020). The repertoire of mutational signatures in human cancer. *Nature*, 578(7793):94–101.

Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Campbell, P. J., and Stratton, M. R. (2013). Deciphering signatures of mutational processes operative in human cancer. *Cell reports*, 3(1):264–259.

Févotte, C., Bertin, N., and Durrieu, J. (2009). Nonnegative matrix factorization with the Itakura-Saito divergence: with application to music analysis. *Neural computation*, 21(3):793–830.

Févotte, C. and Idier, J. (2011). Algorithms for nonnegative matrix factorization with the $\beta$-divergence. *Neural Computation*, 23(9):2421–2456.
Fischer, A., Illingworth, C. J., Campbell, P. J., and Mustonen, V. (2013). EMu: Probabilistic inference of mutational processes and their localization in the cancer genome. *Genome Biology*, 14(4):1–10.

Gelman, A., Hwang, J., and Vehtari, A. (2013). Understanding predictive information criteria for Bayesian models. *Statistics and Computing*, 24(6):997–1016.

Gouvert, O., Oberlin, T., and Fevotte, C. (2020). Negative Binomial Matrix Factorization. *IEEE Signal Processing Letters*, 27:815–819.

Gupta, A., Datta, S., and Das, S. (2018). Fast automatic estimation of the number of clusters from the minimum inter-center distance for k-means clustering. *Pattern Recognition Letters*, 116:72–79.

Lal, A., Liu, K., Tibshirani, R., Sidow, A., and Ramazzotti, D. (2021). De novo mutational signature discovery in tumor genomes using SparseSignatures. *PLOS Computational Biology*, 17(6):e1009119.

Lawrence, M. S., Stojanov, P., Polak, P., Kryukov, G. V., Cibulskis, K., Sivachenko, A., Carter, S. L., Stewart, C., Mermel, C. H., Roberts, S. A., Kiezun, A., Hammerman, P. S., McKenna, A., Drier, Y., Zou, L., Ramos, A. H., Pugh, T. J., Stransky, N., Helman, E., Kim, J., Sougnez, C., Ambrogio, L., Nickerson, E., Shefler, E., Cortés, M. L., Auclair, D., Sakserna, G., Voet, D., Noble, M., Dicara, D., Lin, P., Lichtenstein, L., Heiman, D. I., Fennell, T., Imielinski, M., Hernandez, B., Hodis, E., Baca, S., Dulak, A. M., Lohr, J., Landau, D. A., Wu, C. J., Menezes-Zajgla, J., Hidalgo-Miranda, A., Koren, A., McCarroll, S. A., Mora, J., Lee, R. S., Crompton, B., Onofrio, R., Parkin, M., Winckler, W., Ardlie, K., Gabriel, S. B., Roberts, C. W., Biegel, J. A., Stegmaier, K., Bass, A. J., Garraway, L. A., Meyerson, M., Golub, T. R., Gordenin, D. A., Sunyaev, S., Lander, E. S., and Getz, G. (2013). Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature*, 499(7457):214–218.

Lee, D. D. and Seung, H. S. (1999). Learning the parts of objects by non-negative matrix factorization. *Nature*, 401(6755):788–791.

Li, L., Lebanon, G., and Park, H. (2012). Fast Bregman divergence NMF using taylor expansion and coordinate descent. *Proceedings of the 18th ACM SIGKDD international conference on Knowledge discovery and data mining*.

Lochovsky, L., Zhang, J., Fu, Y., Khurana, E., and Gerstein, M. (2015). LARVA: an integrative framework for large-scale analysis of recurrent variants in noncoding annotations. *Nucleic acids research*, 43(17):8123–8134.

Luo, Y., Al-Harbi, K., Luo, Y., and Al-Harbi, K. (2017). Performances of LOO and WAIC as IRT model selection methods. *Psychological Test and Assessment Modeling*, 59(2):183–205.

Lyu, X., Garret, J., Rätsch, G., and Lehmann, K. V. (2020). Mutational signature learning with supervised negative binomial non-negative matrix factorization. *Bioinformatics*, 36(Suppl_1):i154–i160.

Martincorena, I., Raine, K., Gerstung, M., Dawson, K., Haase, K., Van Loo, P., Davies, H., Stratton, M., and Campbell, P. (2017). Universal Patterns of Selection in Cancer and Somatic Tissues. *Cell*, 171(5):1029–1041.e21.

Nik-Zainal, S., Alexandrov, L. B., Wedge, D. C., Van Loo, P., Greenman, C. D., Raine, K., Jones, D., Hinton, J., Marshall, J., Stebbings, L. A., Menzies, A., Martin, S., Leung, K., Chen, L., Leroy, C., Ramakrishna, M., Rance, R., Lau, K. W., Mudie, L. J., Varela, I., McBride, D. J., Bignell, G. R., Cooke, S. L., Shlien, A., Gamble, J., Whitmore, I., Maddison,
M., Tarpey, P. S., Davies, H. R., Papaemmanuil, E., Stephens, P. J., McLaren, S., Butler, A. P., Teague, J. W., Jönsson, G., Garber, J. E., Silver, D., Miron, P., Fatima, A., Boyault, S., Langerod, A., Tutt, A., Martens, J. W., Aparicio, S. A., Borg, A., Salomon, A. V., Thomas, G., Borresen-Dale, A. L., Richardson, A. L., Neuberger, M. S., Futreal, P. A., Campbell, P. J., and Stratton, M. R. (2012). Mutational processes molding the genomes of 21 breast cancers. *Cell*, 149(5):979–993.

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, 155(2):945–959.

Risques, R. A. and Kennedy, S. R. (2018). Aging and the rise of somatic cancer-associated mutations in normal tissues. *PLoS Genetics*, 14(1).

Rosales, R. A., Drummond, R. D., Valieris, R., Dias-Neto, E., and Da Silva, I. T. (2017). signeR: An empirical Bayesian approach to mutational signature discovery. *Bioinformatics*, 33(1):8–16.

Shibai, A., Takahashi, Y., Ishizawa, Y., Motooka, D., Nakamura, S., Ying, B.-W., and Tsuru, S. (2017). Mutation accumulation under UV radiation in Escherichia coli. *Scientific Reports*, 7(1):1–12.

Tan, V. and Févotte, C. (2013). Automatic relevance determination in nonnegative matrix factorization with the β-divergence. *IEEE transactions on pattern analysis and machine intelligence*, 35(7):1592–1605.

Tate, J. G., Bamford, S., Jubb, H. C., Sondka, Z., Beare, D. M., Bindal, N., Boutselakis, H., Cole, C. G., Creatore, C., Dawson, E., Fish, P., Harsha, B., Hathaway, C., Jupe, S. C., Kok, C. Y., Noble, K., Ponting, L., Ramshaw, C. C., Rye, C. E., Speedy, H. E., Stefancsik, R., Thompson, S. L., Wang, S., Ward, S., Campbell, P. J., and Forbes, S. A. (2019). COSMIC: the Catalogue Of Somatic Mutations In Cancer. *Nucleic Acids Research*, 47(D1):D941–D947.

Teerapabolarn, K. (2015). Negative Binomial approximation to the Beta Binomial distribution. *International Journal of Pure and Applied Mathematics*, 98(1).

Verity, R. and Nichols, R. A. (2016). Estimating the Number of Subpopulations (K) in Structured Populations. *Genetics*, 203(4):1827–39.

Weinhold, N., Jacobsen, A., Schultz, N., Sander, C., and Lee, W. (2014). Genome-wide analysis of noncoding regulatory mutations in cancer. *Nature genetics*, 46(11):1160–1165.

Zhang, J., Liu, J., McGillivray, P., Yi, C., Lochovsky, L., Lee, D., and Gerstein, M. (2020). NIM-Bus: a negative binomial regression based integrative method for mutation burden analysis. *BMC Bioinformatics* 2020 21:1, 21(1):1–25.