COMPUTATIONAL TOOL FOR APPLICATIONS OF SPARSE CANONICAL CORRELATION ANALYSIS ON BIOLOGICAL DATA

A Thesis

by

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ABSTRACT

Sparse canonical correlation analysis (sparse CCA) is a method for identifying sparse linear combinations of the two sets of variables that are highly correlated with each other, given that those two sets of measurements are available on the same set of observations. Recently, sparse CCA has become a popular method for analyzing genomic data, where the number of features is large compared to that of observations. Analyzing a set of data using sparse CCA requires multiple steps, including data cleaning, normalizing, and using the right programming packages.

To make sparse CCA accessible for all researchers regardless of their statistical background, a user-friendly computational tool should be created to assist them in walking through the analysis. After the tool is successfully implemented, a few sets of data will be used as case studies for testing efficiency of the sparse CCA computational tool. Eventually, the tool will be added to the computational website hosted by the Center for Translational Environmental Health Research, which currently hosts services for sequencing classification and differential expression analysis.

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All work for the thesis was completed independently by the student.

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1. INTRODUCTION

Canonical correlation analysis (CCA) is a statistical method for exploring the relationships between two multivariate sets of variables that are measured on the same samples. The canonical correlation coefficient measures the strength of association between two canonical variates. CCA has a wide range of application in many research areas including genomics and bioinformatics. Researchers at the Chapkin Lab from Texas A&M University are among those that utilize sparse CCA. One of the papers from this lab, "A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in immune response" by Schwartz et al. (2012) [1], describes how CCA can be used to reveal the correlation structure between a microarray host gene expression and microbial sequencing data.

In order to apply CCA to genomic data where the number of features generally exceeds the number of observations, a penalized version of CCA called sparse CCA must be utilized instead. To complete the whole process of sparse CCA, multiple data-preprocessing tasks are required. Experts in statistical analysis are able to perform those necessary tasks step-by-step to achieve the final results, but those with limited statistical experience such as biologists might find them too complicated. Thus, it is significant to provide an end-to-end computational tool that assists all types of researchers in using sparse CCA.

The Center for Translational Environmental Health Research (CTEHR) is a research center whose goal is to improve human environment health by integrating advances in biomedical and engineering research across translational boundaries. They address the problem of difficulty that a lot of researchers without a rigorous statistics background are facing by launching the Analysis and Predictive Integrative Modeling of Omics Data (APIMOD) computational website . Currently, its beta version is able to provide services for sequencing classification and differential expression.

The core of this project is the implementation of the sparse CCA data processing pipeline that can eventually be added to the APIMOD computational website. The tool should be able to accept two sets of biological data as input and handle the necessary steps from the beginning until the end. Its internal structure will be implemented in a way that any further code modification or addition can be made without much complication.

The report will demonstrate the theoretical idea and purpose behind the implementation. Both synthetic and real-world datasets will be used as case studies to validate the application of the sparse CCA computational tool.

2. BACKGROUND

2.1 Canonical correlation analysis (CCA)

2.1.1 CCA

Due to Hotelling(1936) [2], Canonical correlation analysis (CCA) is a classical method for determining the relationship between two sets of variables. Given two data sets $\boldsymbol{X_1}$ and $\boldsymbol{X_2}$ of dimensions $n \times p_1$ and $n \times p_2$ on the same set of n observations, CCA seeks linear combinations of the variables in $\boldsymbol{X_1}$ and the variables in $\boldsymbol{X_2}$ that are maximally correlated with each other. That is, $\boldsymbol{w_1} \in R^{p_1}$ and $\boldsymbol{w_2} \in R^{p_2}$ maximize the CCA criterion, given by

$$maximize_{w_1,w_2} \boldsymbol{w_1}^T \boldsymbol{X_1}^T \boldsymbol{X_2} \boldsymbol{w_2} \text{ subject to } \boldsymbol{w_1}^T \boldsymbol{X_1}^T \boldsymbol{X_1} \boldsymbol{w_1} = \boldsymbol{w_2}^T \boldsymbol{X_2}^T \boldsymbol{X_2} \boldsymbol{w_2} = 1$$

where it is assumed that the columns of $\boldsymbol{X_1}$ and $\boldsymbol{X_2}$ have been standardized to have mean zero and standard deviation one. In this paper, they refer to $\boldsymbol{w_1}$ and $\boldsymbol{w_2}$ as the canonical vectors (or weights), and they refer to $\boldsymbol{X_1 w_1}$ and $\boldsymbol{X_2 w_2}$ as the canonical variables.

2.1.2 Sparse CCA

Methods for penalized CCA was proposed by Witten et al. (2009) [3]. The criterion for sparse CCA has the following form,

$$maximize_{w_1,w_2} \boldsymbol{w_1}^T \boldsymbol{X_1}^T \boldsymbol{X_2} \boldsymbol{w_2} \text{ subject to } ||\boldsymbol{w_1}||^2 \leq 1,\ ||\boldsymbol{w_2}||^2 \leq 1,\ P_1(\boldsymbol{w_1}) \leq c_1,\ P_2(\boldsymbol{w_2}) \leq c_2$$

for general penalty functions P_1 and P_2 . They are interested in two specific forms of these penalty functions:

• P_1 is an L_1 (or *lasso*) penalty; that is, $P_1(\boldsymbol{w_1}) = ||\boldsymbol{w_1}||_1$. This penalty

will result in $\boldsymbol{w_1}$ sparse for c_1 chosen appropriately. It is assumed that $1 \le c_1 \le \sqrt{p_1}$

• P_1 is a *fused lasso* penalty of the form $P_1(\boldsymbol{w_1}) = \sum_j |w_{1j}| + \sum_j |w_{1j} - w_{1(j-1)}|$. This penalty will result in $\boldsymbol{w_1}$ sparse and smooth, and is intended for cases in which the features in $\boldsymbol{X_1}$ have a natural ordering along which smoothness is expected.

The two forms mentioned above only include the the penalty function P_1, but the penalty function P_2 also follows the same forms.

2.1.3 Important terms

To fully understand the results of CCA and sparse CCA, the understanding of the following terms is required.

1. Canonical variates

They are the linear combinations that represent the weighted sum of two or more variables and can be defined for either dependent or independent variables. Canonical variates can also be referred to as linear composites, linear compounds, and linear combinations.

2. Canonical correlation

It is the measure of the strength of the overall relationships between the linear composites (canonical variates) for the independent and dependent variables. In effect, it represents the bivariate correlation between the two canonical variates.

3. Canonical loadings

They are the measure of the simple linear correlation between the original variables and their respective canonical variates. These can be interpreted like factor loadings, and are also known as canonical structure correlations.

4. Canonical cross-loadings

They are the correlations between the variate of one set of variables and each variable of the other set. They can be interpreted in similar way to that of the canonical loadings.

2.2 Comparison with other types of analysis

2.2.1 Principal component analysis (PCA)

According to Hervé Abdi and Lynne J. Williams [4], PCA is a multivariate technique that analyzes a data table in which observations are described by several inter-correlated quantitative dependent variables. Its goal is to extract a set of uncorrelated features from the table, to represent it as a set of new orthogonal variables called principal components, and to display the pattern of similarity of the observations. Table 1 compares the input and output of PCA and CCA

	PCA	**CCA**
Input	$X = (x_1, x_2, \ldots, x_N)$	$X = (x_1, x_2, \ldots, x_N)$ $Z = (z_1, z_2, \ldots, z_N)$
Output	$X_{out} = W^T X$ where W^T is a projection matrix	$X_{out} = w^T{}_x X$ $Z_{out} = w^T{}_y Z$ where $w^T{}_x$ and $w^T{}_Z$ are canonical loadings

Table 1 A comparison between PCA and CCA

2.2.2 Pearson's correlation coefficient

Pearson's correlation coefficient (r-value) is a measure of the strength of the association between the two continuous variables. The r-value for continuous data ranges from -1 to +1. When $r = -1$, a perfect negative linear correlation is indicated. When $r = 1$, a perfect positive linear correlation is indicated. When $r = 0$, there is no relationship between the variables.

The formula for Pearson's correlation coefficient is:

$$\rho_{X,Z} = \frac{cov(X,Z)}{\sigma_X \sigma_Z}$$

Where X and Z are data vectors.

‘cov’ is the covariance.

‘σ_X’ and ‘σ_Z’ are the standard deviations of X and Z respectively.

The interpretation of an r-value is comparable to the interpretation of a canonical correlation in that they both measure how strong two sets of data are correlated. The main difference is that the analysis of Pearson's correlation coefficient can take into account only one feature at a time for each set of data.

2.3 Probability distribution models for generating synthetic biological data

2.3.1 Multivariate Gaussian distribution

Multivariate Gaussian Distribution (or Multivariate Normal Distribution) is a generalization of the one-dimensional normal distribution to higher dimensions.

For a random-valued random variable **x** = $[X_1, X_2, \ldots, X_n]^T$

If μ is a n-dimensional mean vector and ∑ is the respective $n \times n$ covariance matrix

$$p(x;\mu,\Sigma) = \frac{1}{(2\pi)^{n/2}|\Sigma|^{1/2}} \exp\left(-\frac{1}{2}(x-\mu)^T \Sigma^{-1}(x-\mu)\right)$$

This can be written as

$$\mathbf{x} \sim \mathcal{N}(\boldsymbol{\mu}, \boldsymbol{\Sigma}),$$

The gene-expression levels are modeled as a multivariate Gaussian distribution that statistically captures the real mRNA levels within the cells.

Σ has the following block structure:

$$\boldsymbol{\Sigma} = \begin{bmatrix} \Sigma_\rho & 0 & 0 & \dots & 0 \\ 0 & \Sigma_\rho & 0 & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \dots & 0 & \Sigma_\rho \end{bmatrix}$$

Σ_ρ is a square matrix, with 1 on the diagonal and ρ off the diagonal. All features in the same block are correlated to one another with the correlation coefficient ρ (which ranges from -1 to 1), while features of different blocks are uncorrelated.

2.3.2 Poisson distribution

The National Institute for Biological Standards and Control [5] defines Next Generation Sequencing (NGS) as technologies that offer high-throughput, rapid and accurate methods of determining the precise order of nucleotides within DNA/RNA molecules.

The specific application of NGS for RNA sequencing is called RNA-Seq, which is a high-throughput measurement of gene-expression levels of thousands of genes simultaneously as represented by discrete expression values for regions of interest on the genome (e.g. genes).

According to Ghaffari et al. (2013) [6], two popular models for statistical representation of the discrete NGS data are the negative binomial and Poisson. The negative binomial model is more general because it can mitigate over-dispersion issues associated with the Poisson model; however, with the relatively small number of samples available in most current NGS experiments, it is difficult to accurately estimate the dispersion parameter of the negative binomial model. Therefore, in this study, the RNA-seq model is a combination of a multivariate Gaussian (representing the genes' concentrations) followed by a Poisson process. This method overcomes the dispersion problems associated to the case where data is simply drawn from a Poisson distribution.

The expected number of reads (mean of the Poisson distribution) are calculated from the generalized linear model.

$$E[\, X_{i,j} \mid s_i \,] = s_i \exp(\, \lambda_{i,j} + \theta_{i,j} \,)$$

where s_i is referred to as ‘sequencing depth’

$X_{i,j}$ is the read count for gene j for sample point i

$\lambda_{i,j}$ is the jth gene-expression level in lane i

$\theta_{i,j}$ represents technical effects that might be associated with an experiment. we assume that it follows a Gaussian distribution with zero mean and a variance set by the coefficient of variation (COV). That is,

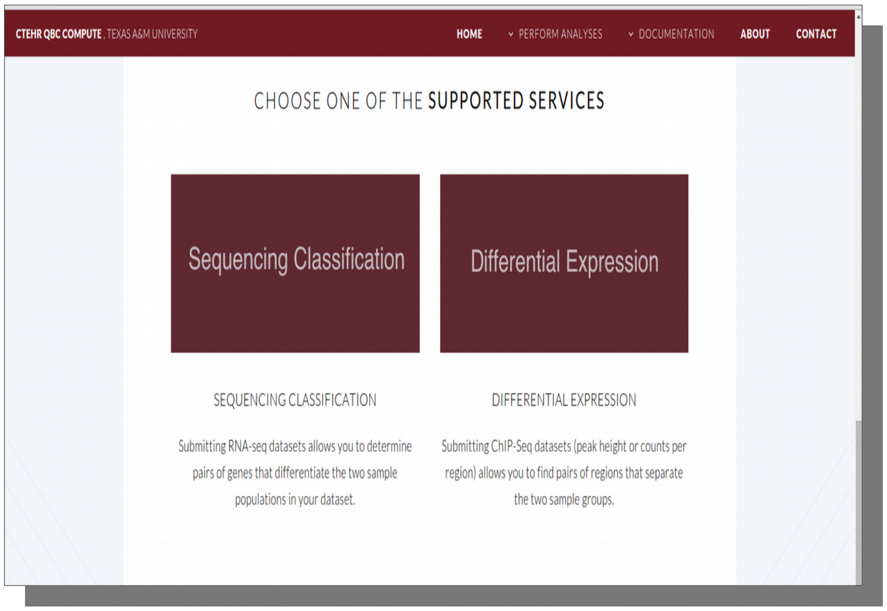
$$\theta_{i,j} \sim N(0, |m_1 - m_0|\text{COV}),$$

2.4 Current state of the computational website

The testing version of the APIMOD computational website is able to host two types of biological data analysis service, a sequencing classification and a differential gene expression. For the sequencing classification, users would submit RNAseq datasets and the tool would determine a pair of genes that differentiate the two sample populations in the dataset. For the differential gene expression, the analysis is done for RNAseq data where the quantitative-biology-core computational pipeline is used to determine differential expression genes. Specifically, mapped reads are normalized and then the result together with the experiment key file are fed to a statistical package called EdgeR to determine differentially expressed genes.

To use the computation site, the users start by navigating on the website and selecting the analysis service of their choice. Figure 1 is the screen-shot of the interface for users to choose between the two choices of analysis. Then they fill in a form describing their information, including name, e-mail, project name, and project descriptions. An example of the form is shown in Figure 2. Depending on the type of service chosen, different types of files are needed to be uploaded. A read file is required for the sequencing classification, and a key file and a count file are required for the differential expression. After filling out those required fields and submitting the service request, a confirmation email is sent to the users. The confirmation email would include a link that redirect the users to a status page, where their service detail as well as the current status are displayed. Once the status is ‘complete’, users are able to view the analysis results on the website as well as download them.

The internal structure of the website is constructed using Python (with Flask framework) and SQLite database. When a service request is submitted, a random identification number is generated and sent to the user, while the user’s information is stored in the computer that hosts the computational website. An SQLite database is used to keep track of the jobs submitted from the users. There are two groups in the database. One is for the analyses that are already completed and the other one is for the analyses waiting to be processed. Once each incomplete task is processed, it is transferred to the completed group.



CHOOSE ONE OF THE SUPPORTED SERVICES

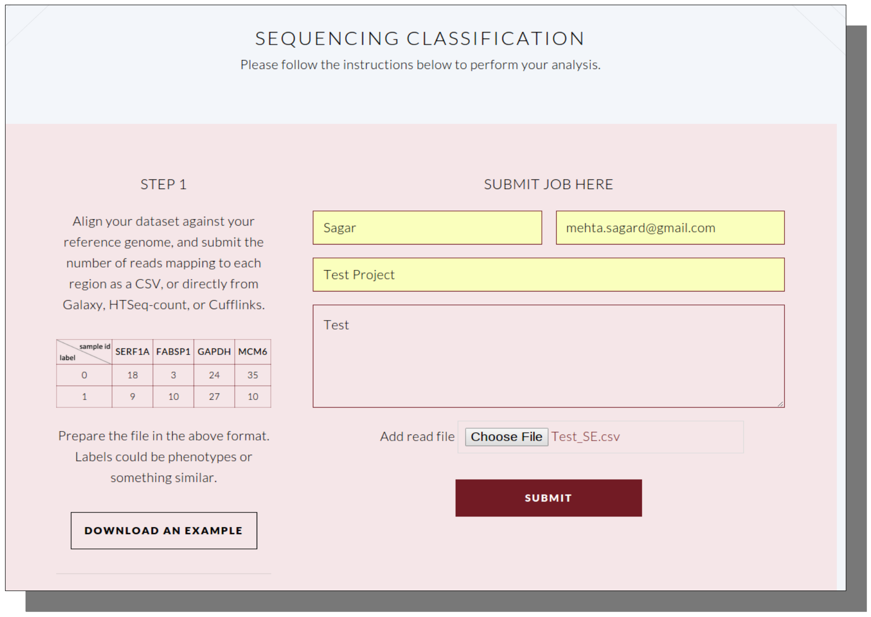
SEQUENCING CLASSIFICATION

Submitting RNA-seq datasets allows you to determine pairs of genes that differentiate the two sample populations in your dataset.

DIFFERENTIAL EXPRESSION

Submitting ChIP-Seq datasets (peak height or counts per region) allows you to find pairs of regions that separate the two sample groups.

Figure 1 The interface when users navigate the website. Users can click either on 'Sequencing Classification' or 'Differential Expression' to proceed to the next step.



SEQUENCING CLASSIFICATION

Please follow the instructions below to perform your analysis.

STEP 1

Align your dataset against your reference genome, and submit the number of reads mapping to each region as a CSV, or directly from Galaxy, HTSeq-count, or Cufflinks.

sample id / label	SERF1A	FABSP1	GAPDH	MCM6
0	18	3	24	35
1	9	10	27	10

Prepare the file in the above format. Labels could be phenotypes or something similar.

SUBMIT JOB HERE

Sagar	mehta.sagard@gmail.com
Test Project	
Test	

Add read file Test_SE.csv

Figure 2 The form for users to upload necessary input

3. RESEARCH DESCRIPTION

3.1 Problem definition

In recent years, an increasing amount of data has become more available in various fields, causing an upward trend in using statistical analysis to solve complicated problems. Researchers in the biology-related fields are among those that can utilize the great quantity of data. Research related to complex system requires a collection of various data types and the subsequent integrative analysis. Despite the meaningful insights statistical tools are able to provide, biologists often lack the adequate background in statistical computation and hesitate to use them.

The Chapkin Lab from the Nutrition department at Texas A&M University is one of the groups that use statistical tools in their research, which eventually leads to the motivation to host the APIMOD computational website. Other than the sequencing classification and differential expression, which are currently available for them, sparse CCA will also need to be added to the website.

Sparse CCA has been growing in popularity as a method for analyzing genomic data, where the number of features is far greater than the number of observations. To complete the whole process of canonical correlation analysis, multiple data-preprocessing steps are required and some parameters have to be determined. People with expertise in statistical analysis might be able to perform those necessary steps until the final results are achieved, but researchers with limited experience such as biologists, these steps might be too complicated. This is why it is highly significant to have an end-to-end computational tool that serves as a helper and makes sparse CCA approachable for all types of researchers.

3.2 Research goals

There are four main goals for the implementation of this sparse CCA computational tool.

1. Tool's validation

The tool must be able to detect the linear relationship manually implemented in the synthetic data and to replicate results for real data

2. User-friendly implementation

The tool should be implemented in a very user-friendly way. Users should be guided to use the tool to achieve their research objectives without any need to consult an expert.

3. Code modularity

Sufficient code modularity should allow any adjustments or incoming data analysis methods to be implemented without complication.

4. Well-written documentation

Tool specification that allow for its seamless integration with the current APIMOD site must be provided.

4. PROJECT APPROACH

The sparse CCA computational tool is implemented in R and Python. To accomplish the research goal, the process as a whole is divided into several steps.

4.1 Framework design

The web-interface portion is implemented using a combination of both Python (Flask) and SQLite, a software library that implements a self-contained, server-less SQL database engine. Initially, users have to submit two files to the system as inputs, each as a CSV or TXT file containing a data table. The two tables must have the same number of samples (rows) but can have different number of features (columns). After submission, the inputs are saved into a specific folder set up in back-end system. Those inputs are then called when the computational code is executed. After the whole process is complete, the results are stored in another folder.

Ideally, the web-based tool will be able to accept many jobs and keep a record of the order of their submission to the system. A database will be set up for that purpose. Once each job is submitted by a user, the database will record its status as ‘incomplete’. Then the R code will process the incomplete job, and once the computational process is done, the database status will be changed to ‘complete’.

Each input’s expected format is a table with the same numbers of samples (rows). The preprocessing step will confirm that the format is acceptable before proceeding to the next step. Also, due to the computational requirement of the sparse CCA, any columns of data with standard deviation equal to zero must be removed.

After preprocessing, the two input tables are now in the form of numerical matrices and ready to be analyzed. Those matrices will be used as input parameters for the sparse CCA functions from the R package ‘PMA’.The generated results are a score table and four graphs showing the component scores of the two inputs. The R package ‘ggplot2’[7] is used for generating the plots. Figure 3 is the diagram showing the tool’s workflow.

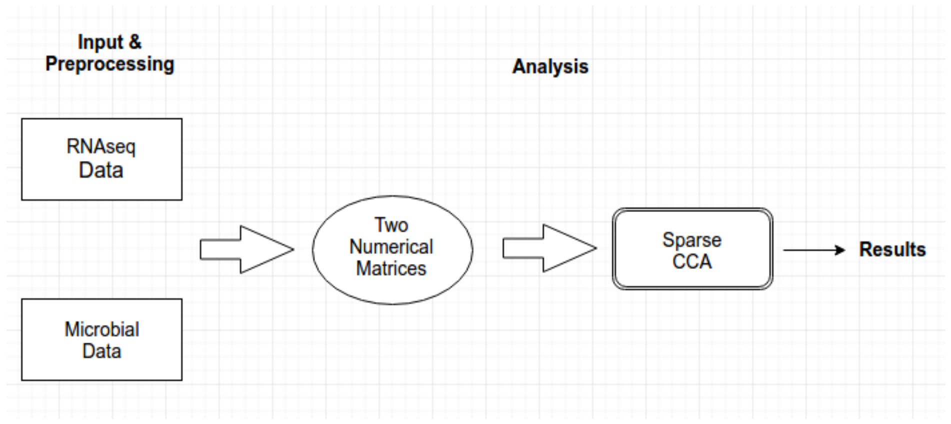


Figure 3 The diagram depicting the overall working structure of the tool

4.2 Implementing a sparse CCA method

'PMA' is an R package that performs penalized multivariate analysis. It offers a wide range of tools including the sparse CCA. The two functions related to the analysis that will be used are 'CCA' and 'CCA.permute'. Below are the detailed descriptions referenced from the official manual of the package 'PMA' [8] on how these two functions can be used.

4.2.1 'CCA.permute' function

'CCA.permute' automatically selects tuning parameters for sparse CCA using the penalized matrix decomposition, as described in Witten et al. (2009) [9]. For each data set X and Z, two types are possible: (1) type 'standard', which does not assume any ordering of the columns of the data set, and (2) type 'ordered', which assumes that columns of the data set are ordered and thus that corresponding canonical vector should be both sparse and smooth (e.g. CGH data). For X and Z, the samples are on the rows and the features are on the columns. The tuning parameters are selected using a permutation scheme. For each candidate tuning parameter value, the following is performed:

1. The samples in X are randomly permuted nperms times, to obtain matrices X*1,X*2,. …

2. Sparse CCA is run on each permuted data set (X^*_i, Z) to obtain factors (u^*_i, v^*_i), which are the canonical coefficients for each pair of (X^*_i, Z).

3. Sparse CCA is run on the original data (X, Z) to obtain factors u and v, which are the resulting canonical coefficients of X and Z respectively..

4. Compute $c^*_i = cor(X^*_i\ u^*_i , Z\ v^*_i)$ and $c = cor(Xu , Zv)$.

Note: cor(x,y) refers to the correlation of x and y.

5. Use Fisher's transformation to convert these correlations into random variables that are approximately normally distributed. Let Fisher(c) denote the Fisher transformation of c.

6. Compute a z-statistic for Fisher(c), using

$$\frac{Fisher(c) - mean(Fisher(c^*))}{sd(Fisher(c^*))}$$

The larger the z-statistic, the 'better' the corresponding tuning parameter value.

As a result, the x penalty and z penalty which result in the highest z-statistic are chosen to be used as part of the input arguments of the 'CCA' function.

Usage:

```
CCA.permute(x,z,typex=c("standard", "ordered"),typez=c("standard","ordered"),
penaltyxs=NULL, penaltyzs=NULL, niter=3,v=NULL,trace=TRUE,nperms=25,
```

standardize=TRUE, chromx=NULL, chromz=NULL,upos=FALSE, uneg=FALSE, vpos=FALSE, vneg=FALSE,outcome=NULL, y=NULL, cens=NULL)

4.2.2 'CCA' function

The function 'CCA' performs sparse canonical correlation analysis using the penalized matrix decomposition. The main parameters to be taken in are the data matrices and their matrix penalty details.

Given matrices X and Z, which represent two sets of features on the same set of samples, the function finds sparse u and v such that $u^T X^T Z v$ is large. For X and Z, the samples are on the rows and the features are on the columns. X and Z must have same number of rows, but may (and usually will) have different numbers of columns. The columns of X and/or Z can be unordered or ordered. If unordered, then a lasso penalty will be used to obtain the corresponding canonical vector. If ordered, then a fused lasso penalty will be used; this will result in smoothness.

Usage:

CCA(x, z, typex=c("standard", "ordered"),typez=c("standard","ordered"), penaltyx=NULL, penaltyz=NULL, K=1, niter=15, v=NULL, trace=TRUE, standardize=TRUE, xnames=NULL, znames=NULL, chromx=NULL, chromz=NULL, upos=FALSE, uneg=FALSE, vpos=FALSE, vneg=FALSE, outcome=NULL, y=NULL, cens=NULL)

4.3 Performance evaluation

To test efficiency of the tool, multiple data sets are used in various scenarios. We first tested the sparse CCA tool on synthetic data sets generated according to the algorithms and their implementations in Ghaffari et al. (2013) [6], Yousefi et al. (2011) [10], and Bahadorinejad, A. (2017) [11]. Once being successful with the synthetic datasets, real datasets are next considered.

For synthetic datasets, there are three types of data being generated, which are the multivariate Gaussian Data, the RNAseq data, and the microbial sequencing data. The multivariate Gaussian data model is based on the multivariate Gaussian distribution. The model has a blocked covariance structure that conforms to various observations made in microarrray expression-based studies [10]. For the RNAseq data, the model used is a combination between the general model based on multivariate Gaussian distribution followed by a transformation based on Poisson distribution model. A series of distribution models can be constructed by changing model parameters to generate different synthetic data samples. For the microbial sequencing data, a python script is used to simulate Operational Taxonomic Units (OTU) frequency vectors that take into account phylogenetic relatedness of OTUs.

We also tested the sparse CCA tool on a real data set generated during the NIH-funded project "Gut Mibrobiota And Colonic Gene Expression: A Lingan Trial In Humans", co-conducted by the Nutrition Department at Texas A&M University.

4.3.1 Applying sparse CCA to synthetic data

There are three sets of data being randomly generated: microbial sequencing, multivariate Gaussian, and RNA sequencing. The RNA sequencing data is generated by processing the multivariate Gaussian data through a Poisson process.

First, two canonical correlations are computed, one is between the microbial sequencing and the multivariate Gaussian data and the other one is between the microbial sequencing and RNA sequencing data. Then, we implement a linear relationship between each pair of data. After the linear relationships are constructed, the two canonical correlations are computed again on the same variables, then the two sets of values are compared.

4.3.1.1 Methods for generating synthetic data

There are three types of synthetic data generated: Microbial OTUs, Multivariate Gaussian, and RNA sequencing (RNAseq).

The code for generating microbial sequencing data is written in Python by Arghavan Bahadorinejad [11]. It basically simulates OTU frequency vectors that take into account phylogenetic relatedness of OTUs.

For generating multivariate Gaussian data, the code was written in C++ by Jianping Hua, as described in Yousefi et al. (2011) [10]. The simulation design uses a general model based on multivariate Gaussian distributions with a blocked covariance structure that conforms to various observations made in microarray expression-based studies. A battery of distribution models can be constructed by changing model parameters to generate different synthetic data samples. The Gaussian distribution model simulates RNA concentrations in real samples.

RNAseq data is generated by the code that was an extension of the one that generates the multivariate Gaussian data, as described in Ghaffari et al. (2013) [6]. After the multivariate Gaussian data is generated, it is pushed through a Poisson process. The Poisson transformation simulates using the sequencing machine in the real experiment. Figure 4 is the flow diagram for generating both the Multivariate Gaussian and RNAseq data.

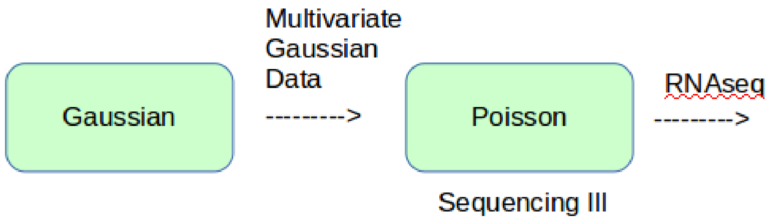


Figure 4 The flow diagram for generating the synthetic data

4.3.1.2 Linear relationship implementation

Since sparse CCA is an analysis that detects linear relationships between two sets of data and the synthetic data are randomly generated, it is necessary that relationships are manually implemented so that the sparse CCA can be tested. The built-in R function 'lm' is used to fit the randomly generated data. The fitted data will be in the form

$$\sum y_k = \sum a_i x_i + \text{intercept}$$

where 'k' and 'i' are the indexes related to OTUs and genes used to be included in the linear implementation respectively. Different ranges of 'k' and 'i' are used so that the results in different scenarios can be compared against one another. Only genes from multivariate Gaussian are used in the implementation in order to observe how the Poisson process affects the linear relationships.

4.3.2 Applying sparse CCA to real data

To further validate the sparse CCA tool, we attempt to replicate the unpublished study "An application of sCCA for integrative analysis of diet-dependent interaction between gut microbiota and host in neonates" [12]. Additional results not included in the original study are also supplied in order to strengthen the validation. The study applies sparse CCA on a pair of host microarray data set and microbial data set, which was aligned using Rapid Annotation using Subsystems Technology (MG-RASTv2) against the SEED subsystem database.

The input for the tool's validation is already in the acceptable form of two tables, resulting from the preprocessing step that was done separately. Quantile normalization was used for RNAseq data while an R package 'metagenomeSeq' [13] was used for the microbial sequencing data. It is possible to add these normalization methods to the tool in the future using steps similar to the explanation made in APPENDIX C.

5. RESULTS

5.1 Tool framework

The sparse CCA computational tool is implemented using the structure similar to that of the sequencing classification and differential expression analysis, so that they can be easily integrated on the website. Specifically, the same tools and folders organization are utilized when building the sparse CCA application. An important feature introduced in the implementation is the modularity of the tool, which allow ease of addition of any new tools in the future. The code component comprised of two major parts. The first part is the one that handles all relations between a user's input, database, and the analysis portion. The other part is the data analysis part.

For the first part, the code initially checks if there is any file in the database. If there is no file, then the process stops. If a file exists, the database is connected using an R package "RSQLite" [14]. Then the database checks for a job with an 'incomplete' status, which will eventually be processed. If none of the jobs has the status of 'incomplete', the process also ends.

The second part of the code component handles the computational portion. Particularly, it is the part where the sparse CCA is implemented. The code is placed within the error handling function called tryCatch, which is inside the first part. This way of implementation allows the modularity feature to be possible. That is the code inside the tryCatch function can be replaced with other types of computational methods in order to add more functionality to the tool.

5.2 Synthetic data

After the sparse CCA is implemented, it is then applied to multiple datasets. Many scenarios for each dataset are considered.

5.2.1 Small number of features

The key property to be considered is the canonical cross loadings, which is the correlation of each variable with the opposite canonical variate.

The data consists of 2 OTUs and 3 genes. We use U1 as the canonical variate for the microbial data, V1 as the canonical variate for the multivariate Gaussian data, and W1 as the canonical variate for the RNAseq data. Table 2 through Table 13 show the canonical cross loadings for each variable in each dataset both before and after the linear relationship is implemented.

In the linear implementation, microbial data is used as a dependent variable while the multivariate Gaussian or RNA sequencing data is used as independent variables. There are three cases in this numerical experiment. Each case has the form of $y_1 \sim \sum x_k$ where 'k' ranges from 1 to 3. The notation '~' indicates a linear relationship. For example, $y \sim x_1+x_2$ is equivalent to the form $y = m_1x_1+m_2x_2+ c$ where m_1 and m_2 are slopes and c is a constant.

<u>Case 1</u>: OTU1 ~ Gene1

V1 vs. OTUs

		OTU 1	**OTU 2**
Dataset 1	Before	-0.3907183	-0.1186813
	After	-1	-0.2260459
Dataset 2	Before	-0.1391491	0.0585454
	After	-1	0.06826512
Dataset 3	Before	-0.4217921	0.0407734
	After	-1	0.1365645
Dataset 4	Before	-0.195657	-0.302892
	After	-1	-0.302892
Dataset 5	Before	-0.4110796	-0.3802128
	After	-1	-0.1696654
Dataset 6	Before	0.0009457134	0.36187219
	After	-1	-0.1616121
Dataset 7	Before	-0.0350365	-0.3819603
	After	-1	0.0793762
Dataset 8	Before	-0.2686624	-0.0550798
	After	-1	-0.2188731
Dataset 9	Before	-0.074364	0.369191
	After	-1	-0.0083163
Dataset 10	Before	-0.32068989	-0.06316337
	After	-1	-0.1439532
Dataset 11	Before	-0.42211989	-0.0194381
	After	-1	0.0958877

Table 2 Canonical cross-loadings between the canonical variate for the multivariate Gaussian data and each of the OTUs, with OTU1 and Gene1 being used in the linear implementation

U1 vs. Genes

		Gene 1	Gene 2	Gene 3
Dataset 1	Before	0.01870357	0.35116653	0.14620568
	After	1	0.2294226	-0.2968851
Dataset 2	Before	0.06022922	-0.12502358	-0.03120631
	After	1	0.1613756	-0.1149653
Dataset 3	Before	-0.3498769	-0.2936249	0.1429806
	After	-1	-0.0481509	0.0795961
Dataset 4	Before	-0.3028919	-0.0242343	-0.2212093
	After	-1	-0.1386472	-0.1513016
Dataset 5	Before	0.3590944	-0.2340555	-0.362238
	After	1	-0.208301	-0.1264478
Dataset 6	Before	0.1616121	-0.3113451	0.1293518
	After	-1	-0.30304246	0.04346897
Dataset 7	Before	-0.0793762	-0.3666335	0.1342536
	After	1	0.3011729	0.0042999
Dataset 8	Before	-0.0912606	0.1299878	0.2418749
	After	-1	-0.3104978	-0.1376715
Dataset 9	Before	0.008316298	0.312365	-0.20366
	After	-1	-0.0589381	0.0059786
Dataset 10	Before	-0.1364311	-0.2840887	0.1965004
	After	-1	-0.1550587	0.1237826
Dataset 11	Before	-0.040835	0.4221199	-0.0133788
	After	-1	0.2846204	0.3264974

Table 3 Canonical cross-loadings between the canonical variate for the microbial data and each of the genes in the multivariate Gaussian data, with OTU1 and Gene1 being used in the linear implementation

W1 vs. OTUs

		OTU 1	OTU 2
Dataset 1	Before	-0.2755202	0.1602694
	After	-0.561442	-0.178859
Dataset 2	Before	-0.2580476	0.041712
	After	-0.32686587	-0.04228278
Dataset 3	Before	-0.3736285	-0.2301289
	After	-0.6839862	0.2431373
Dataset 4	Before	-0.152795	-0.468153
	After	-0.647861	-0.0837184
Dataset 5	Before	-0.2783777	-0.1021217
	After	-0.3591292	0.271894
Dataset 6	Before	-0.13348706	-0.02069273
	After	-0.69750546	-0.05144567
Dataset 7	Before	-0.3150119	-0.2352629
	After	-0.6618332	0.133462
Dataset 8	Before	-0.1401131	0.2371155
	After	-0.6821678	-0.1963306
Dataset 9	Before	-0.2194645	-0.2871148
	After	-0.5676777	0.060138
Dataset 10	Before	-0.1860355	0.1104051
	After	-0.28440878	-0.09500744
Dataset 11	Before	-0.3346095	-0.0444541
	After	-0.4056705	-0.1017371

Table 4 Canonical cross-loadings between the canonical variate for the RNAseq data and each of the OTUs, with OTU1 and Gene1 being used in the linear implementation

U1 vs. Genes

		Gene 1	Gene 2	Gene 3
Dataset 1	Before	-0.22579	0.2202608	0.11878
	After	0.56144203	0.12086873	-0.08553118
Dataset 2	Before	0.01129986	-0.13498591	0.25804763
	After	0.2696895	-0.250798	0.1232999
Dataset 3	Before	-0.1414355	-0.1797505	0.2830388
	After	-0.683986	0.0137319	0.1107876
Dataset 4	Before	-0.1315147	0.4681526	0.0846587
	After	-0.6150942	-0.1289938	0.0955491
Dataset 5	Before	-0.2103968	-0.1799772	-0.24017
	After	0.3477704	-0.1767702	-0.1149791
Dataset 6	Before	0.03006138	0.10799401	0.11165529
	After	-0.69750546	-0.22322208	-0.02736362
Dataset 7	Before	-0.0195874	-0.1594113	0.2648633
	After	0.6628332	0.00229019	0.0071701
Dataset 8	Before	0.1963306	0.2091746	-0.0955025
	After	-0.6821678	-0.226299	0.00122507
Dataset 9	Before	0.1141338	-0.2871148	-0.1946725
	After	-0.503361	-0.0709896	-0.231004
Dataset 10	Before	-0.1475136	-0.0626963	0.1860355
	After	-0.243881766	-0.009474329	0.224025031
Dataset 11	Before	0.274431	0.2645438	0.2794695
	After	-0.2513729	0.3635195	0.211355

Table 5 Canonical cross-loadings between the canonical variate for the microbial data and each of the genes in the RNAseq data, with OTU1 and Gene1 being used in the linear implementation

<u>Case 2</u>: OTU1 ~ Gene1 + Gene2

V1 vs. OTUs

		OTU 1	**OTU 2**
Dataset 1	Before	-0.3907183	-0.1186813
	After	-0.9935857	-0.2103694
Dataset 2	Before	-0.1391491	0.0585454
	After	-0.97641949	0.04373211
Dataset 3	Before	-0.4217921	0.0407734
	After	-0.8327455	-0.2304964
Dataset 4	Before	-0.195657	-0.302892
	After	-0.9391243	-0.087974
Dataset 5	Before	-0.4110796	-0.3802128
	After	-0.9993169	-0.1745127
Dataset 6	Before	0.000945713	0.36187219
	After	-0.959892	-0.2243991
Dataset 7	Before	-0.0350365	-0.3819603
	After	-0.9995626	0.3666335
Dataset 8	Before	-0.2686624	-0.0550798
	After	-0.82040111	0.0685745
Dataset 9	Before	-0.074364	0.369191
	After	-0.986999	0.2802154
Dataset 10	Before	-0.32068989	-0.06316337
	After	-0.99707494	-0.05591419
Dataset 11	Before	-0.42211989	-0.0194381
	After	-0.9682923	-0.0194381

Table 6 Canonical cross-loadings between the canonical variate for the multivariate Gaussian data and each of the OTUs, with OTU1, Gene1, and Gene2 being used in the linear implementation

U1 vs. Genes

		Gene 1	Gene 2	Gene 3
Dataset 1	Before	0.01870357	0.35116653	0.14620568
	After	0.1178854	0.9935857	-0.2103694
Dataset 2	Before	0.06022922	-0.12502358	-0.03120631
	After	0.05548205	-0.97641949	0.18670218
Dataset 3	Before	-0.3498769	-0.2936249	0.1429806
	After	-0.7217637	-0.7260903	-0.028467
Dataset 4	Before	-0.3028919	-0.0242343	-0.2212093
	After	-0.6472933	-0.8446246	-0.1210972
Dataset 5	Before	0.3590944	-0.2340555	-0.362238
	After	0.9882616	-0.3552762	-0.1189485
Dataset 6	Before	0.1616121	-0.3113451	0.1293518
	After	-0.89625939	0.15210658	0.00403712
Dataset 7	Before	-0.0793762	-0.3666335	0.1342536
	After	0.3292405	0.9995626	0.1159866
Dataset 8	Before	-0.0912606	0.1299878	0.2418749
	After	-0.5681438	0.6058479	-0.1373393
Dataset 9	Before	0.008316298	0.312365	-0.20366
	After	-0.1663004	0.9745595	0.1615355
Dataset 10	Before	-0.1364311	-0.2840887	0.1965004
	After	-0.320008	-0.9855761	0.1159428
Dataset 11	Before	-0.040835	0.4221199	-0.0133788
	After	-0.0361081	0.9682923	0.0746215

Table 7 Canonical cross-loadings between the canonical variate for the microbial data and each of the genes in the multivariate Gaussian data, with OTU1, Gene1, and Gene2 being used in the linear implementation

W1 vs. OTUs

		OTU 1	OTU 2
Dataset 1	Before	-0.2755202	0.1602694
	After	-0.65946354	-0.03749887
Dataset 2	Before	-0.2580476	0.041712
	After	-0.4881717	0.2433998
Dataset 3	Before	-0.3736285	-0.2301289
	After	-0.6839862	0.2431373
Dataset 4	Before	-0.152795	-0.468153
	After	-0.4781066	-0.0340261
Dataset 5	Before	-0.2783777	-0.1021217
	After	-0.3674784	0.2625524
Dataset 6	Before	-0.13348706	-0.02069273
	After	-0.63450827	-0.05144567
Dataset 7	Before	-0.3150119	-0.2352629
	After	-0.5576955	0.1228815
Dataset 8	Before	-0.1401131	0.2371155
	After	-0.4225213	-0.2096492
Dataset 9	Before	-0.2194645	-0.2871148
	After	-0.452435	0.233542
Dataset 10	Before	-0.1860355	0.1104051
	After	-0.2708888	0.1158262
Dataset 11	Before	-0.3346095	-0.0444541
	After	-0.4958251	-0.027695

Table 8 Canonical cross-loadings between the canonical variate for the RNAseq data and each of the OTUs, with OTU1, Gene1 and Gene2 being used in the linear implementation

U1 vs. Genes

		Gene 1	Gene 2	Gene 3
Dataset 1	Before	-0.22579	0.2202608	0.11878
	After	-0.05933418	0.65946354	-0.12170565
Dataset 2	Before	0.01129986	-0.13498591	0.25804763
	After	-0.00458042	-0.48817166	0.06294449
Dataset 3	Before	-0.1414355	-0.1797505	0.2830388
	After	-0.6839861	0.0137319	0.1107876
Dataset 4	Before	-0.1315147	0.4681526	0.0846587
	After	-0.4087011	-0.3111239	0.0080849
Dataset 5	Before	-0.2103968	-0.1799772	-0.24017
	After	0.32836222	-0.25266345	-0.07886958
Dataset 6	Before	0.03006138	0.10799401	0.11165529
	After	-0.63450827	0.006577268	-0.055726411
Dataset 7	Before	-0.0195874	-0.1594113	0.2648633
	After	0.3406209	0.5073102	0.3048115
Dataset 8	Before	0.1963306	0.2091746	-0.0955025
	After	-0.375256	0.194175	0.3010886
Dataset 9	Before	0.1141338	-0.2871148	-0.1946725
	After	-0.3266413	0.3441688	0.40991
Dataset 10	Before	-0.1475136	-0.0626963	0.1860355
	After	-0.1378012	-0.1999964	0.233296
Dataset 11	Before	0.274431	0.2645438	0.2794695
	After	-0.168379	0.4073901	0.4161749

Table 9 Canonical cross-loadings between the canonical variate for the microbial data and each of the genes in the RNAseq data, with OTU1, Gene1 and Gene2 being used in the linear implementation

<u>Case 3</u>: OTU1 ~ Gene1 + Gene2 + Gene3

V1 vs. OTUs

		OTU 1	**OTU 2**
Dataset 1	Before	-0.3907183	-0.1186813
	After	-0.9145	-0.1186813
Dataset 2	Before	-0.1391491	0.0585454
	After	-0.95860375	0.05627703
Dataset 3	Before	-0.4217921	0.0407734
	After	-0.8138075	0.0407735
Dataset 4	Before	-0.195657	-0.302892
	After	-0.9387378	-0.087974
Dataset 5	Before	-0.4110796	-0.3802128
	After	-0.9261903	-0.2693177
Dataset 6	Before	0.000945713	0.36187219
	After	-0.77511871	0.09652387
Dataset 7	Before	-0.0350365	-0.3819603
	After	-0.8708296	0.3188008
Dataset 8	Before	-0.2686624	-0.0550798
	After	-0.6899724	-0.2006818
Dataset 9	Before	-0.074364	0.369191
	After	-0.979797	0.240481
Dataset 10	Before	-0.32068989	-0.06316337
	After	-0.95469881	-0.06316337
Dataset 11	Before	-0.42211989	-0.0194381
	After	-0.9625267	-0.0318368

Table 10 Canonical cross-loadings between the canonical variate for the multivariate Gaussian data and each of the OTUs, with OTU1, Gene1, Gene2, and Gene3 being used in the linear implementation

U1 vs. Genes

		Gene 1	Gene 2	Gene 3
Dataset 1	Before	0.01870357	0.35116653	0.14620568
	After	0.0294174	0.8181304	0.3616538
Dataset 2	Before	0.06022922	-0.12502358	-0.03120631
	After	0.01572789	-0.88187657	0.60357339
Dataset 3	Before	-0.3498769	-0.2936249	0.1429806
	After	-0.6575814	-0.615524	0.4498756
Dataset 4	Before	-0.3028919	-0.0242343	-0.2212093
	After	-0.6473556	-0.8442025	-0.0972547
Dataset 5	Before	0.3590944	-0.2340555	-0.362238
	After	0.7781259	-0.3269968	-0.6914781
Dataset 6	Before	0.1616121	-0.3113451	0.1293518
	After	-0.6650068	0.1141568	0.6699263
Dataset 7	Before	-0.0793762	-0.3666335	0.1342536
	After	0.3398491	0.7469429	0.7325247
Dataset 8	Before	-0.0912606	0.1299878	0.2418749
	After	-0.551744	0.5320788	0.2509534
Dataset 9	Before	0.0083163	0.312365	-0.20366
	After	-0.142493	0.9383129	0.4348558
Dataset 10	Before	-0.1364311	-0.2840887	0.1965004
	After	-0.2900495	-0.834644	0.6207757
Dataset 11	Before	-0.040835	0.4221199	-0.0133788
	After	-0.021954	0.953491	-0.0742191

Table 11 Canonical cross-loadings between the canonical variate for the microbial data and each of the genes in the multivariate Gaussian data, with OTU1, Gene1, Gene2, and Gene3 being used in the linear implementation

W1 vs. OTUs

		OTU 1	OTU 2
Dataset 1	Before	-0.2755202	0.1602694
	After	-0.53361876	0.001315121
Dataset 2	Before	-0.2580476	0.041712
	After	-0.4736886	0.2433998
Dataset 3	Before	-0.3736285	-0.2301289
	After	-0.5196779	0.2040253
Dataset 4	Before	-0.152795	-0.468153
	After	-0.4740843	-0.0340261
Dataset 5	Before	-0.2783777	-0.1021217
	After	-0.37496202	-0.03444462
Dataset 6	Before	-0.13348706	-0.02069273
	After	-0.52145469	-0.04873572
Dataset 7	Before	-0.3150119	-0.2352629
	After	-0.5172044	0.153402
Dataset 8	Before	-0.1401131	0.2371155
	After	-0.5053266	-0.097944
Dataset 9	Before	-0.2194645	-0.2871148
	After	-0.5037548	0.2257296
Dataset 10	Before	-0.1860355	0.1104051
	After	-0.5052626	0.1104051
Dataset 11	Before	-0.3346095	-0.0444541
	After	-0.4759663	-0.1445503

Table 12 Canonical cross-loadings between the canonical variate for the RNAseq data and each of the OTUs, with OTU1, Gene1,Gene2, and Gene3 being used in the linear implementation

U1 vs. Genes

		Gene 1	Gene 2	Gene 3
Dataset 1	Before	-0.22579	0.2202608	0.11878
	After	-0.24565406	0.49620364	0.06590824
Dataset 2	Before	0.01129986	-0.13498591	0.25804763
	After	0.06893597	-0.47368864	0.27986026
Dataset 3	Before	-0.1414355	-0.1797505	0.2830388
	After	-0.4653387	-0.1154333	0.4155736
Dataset 4	Before	-0.1315147	0.4681526	0.0846587
	After	0.4037761	-0.3156223	0.0202176
Dataset 5	Before	-0.2103968	-0.1799772	-0.24017
	After	0.1566423	-0.2504334	-0.3273299
Dataset 6	Before	0.03006138	0.10799401	0.11165529
	After	-0.45793806	-0.08802108	0.28627804
Dataset 7	Before	-0.0195874	-0.1594113	0.2648633
	After	0.2949942	0.4179895	0.4339231
Dataset 8	Before	0.1963306	0.2091746	-0.0955025
	After	-0.2600955	0.2516163	0.4542582
Dataset 9	Before	0.1141338	-0.2871148	-0.1946725
	After	-0.3073681	0.3183115	0.4647377
Dataset 10	Before	-0.1475136	-0.0626963	0.1860355
	After	-0.07878463	-0.064644	0.50526263
Dataset 11	Before	0.274431	0.2645438	0.2794695
	After	-0.1379086	0.4176939	0.3195154

Table 13 Canonical cross-loadings between the canonical variate for the microbial data and each of the genes in the RNAseq data, with OTU1, Gene1, Gene2, and Gene3 being used in the linear implementation

5.2.2 Large number of features

For cases where the number of features greatly exceeds the number of samples (with ratio of approximately 10:1 in this experiment), only the canonical correlations are considered instead of the cross-loadings as in the case of small features. The graphs (Figure 5 to Figure 12) show the canonical correlations for each case, where the number of genes ranges from 1 to 10.

2 OTUs, 1-10 genes

The plots show the canonical correlations after the linear relationship implementation for both the multivariate Gaussian and RNAseq data. The y-axis represents the canonical correlations while the x-axis represents the number of genes used in the implementation. The canonical correlations before the linear relationship implementation are 0.6966727 and 0.7593742 for multivariate Gaussian and RNA sequencing respectively.

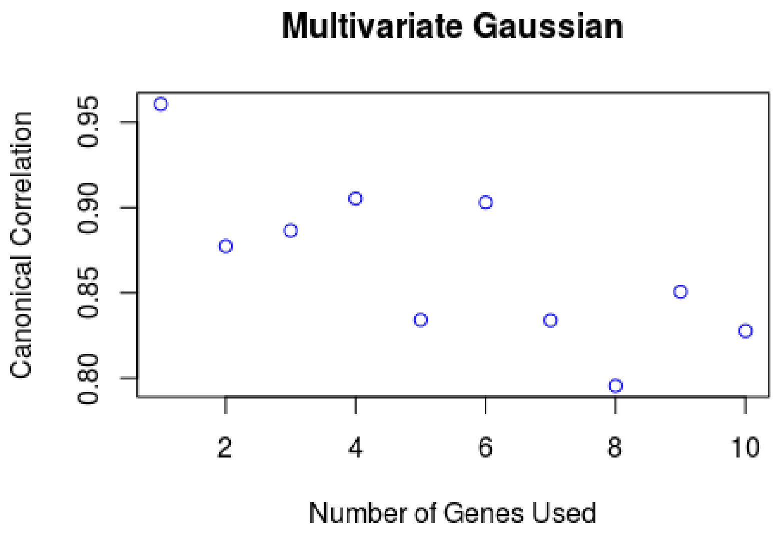


Figure 5 The canonical correlation of the Multivariate Gaussian data after the linear implementation using 2 OTUs

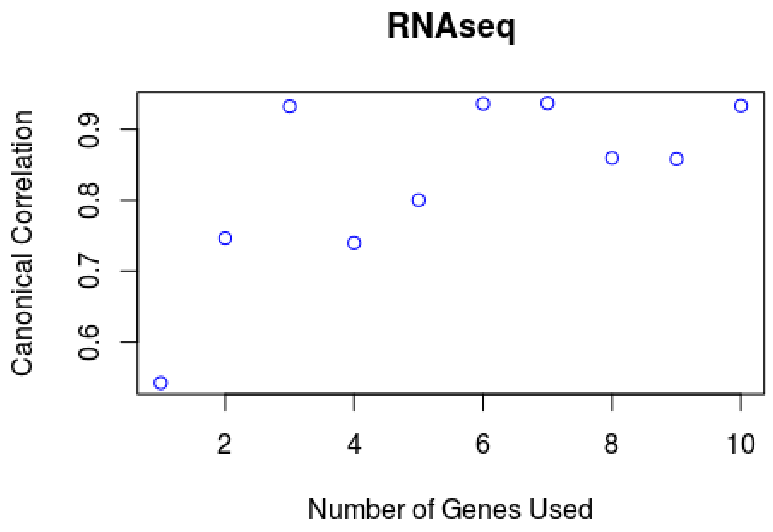


Figure 6 The canonical correlation of the RNAseq data after the linear implementation, using 2 OTUs

<u>3 OTUs, 1-10 genes</u>

The plots show the canonical correlations after the linear relationship implementation for both the multivariate Gaussian and RNAseq data. The y-axis represents the canonical correlations while the x-axis represents the number of genes used in the implementation. The canonical correlations before the linear relationship implementation are 0.6966727 and 0.7593742 for multivariate Gaussian and RNA sequencing respectively.

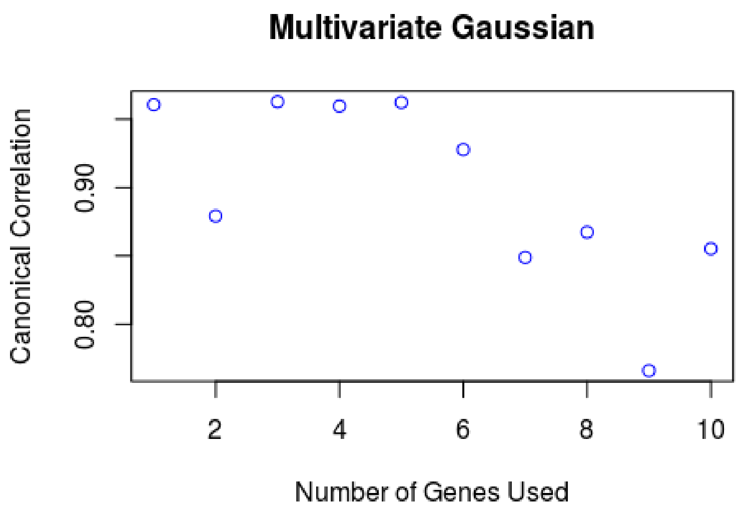


Figure 7 The canonical correlation of the Multivariate Gaussian data after the linear implementation using 3 OTUs

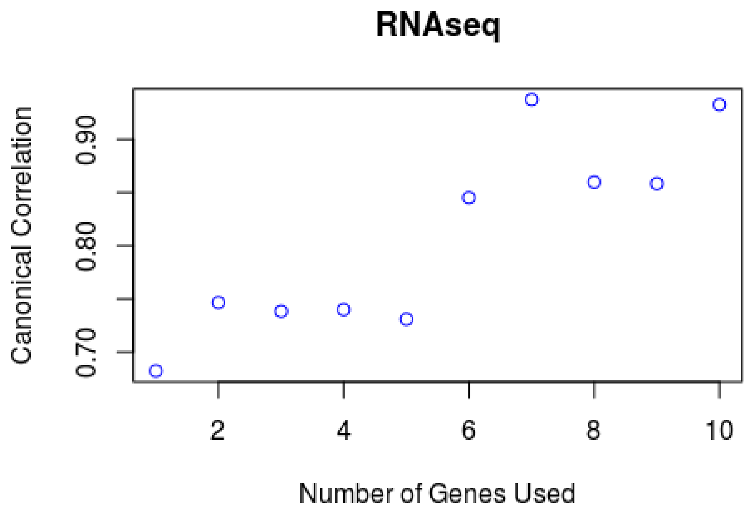


Figure 8 The canonical correlation of the RNAseq data after the linear implementation, using 3 OTUs

4 OTUs, 1-10 genes

The plots show the canonical correlations after the linear relationship implementation for both the multivariate Gaussian and RNAseq data. The y-axis represents the canonical correlations while the x-axis represents the number of genes used in the implementation. The canonical correlations before the linear relationship implementation are 0.6966727 and 0.7593742 for multivariate Gaussian and RNA sequencing respectively.

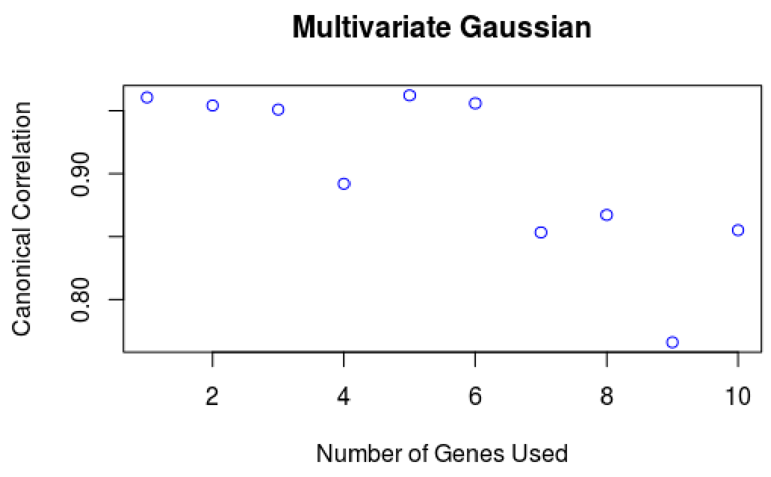


Figure 9 The canonical correlation of the Multivariate Gaussian data after the linear implementation using 4 OTUs

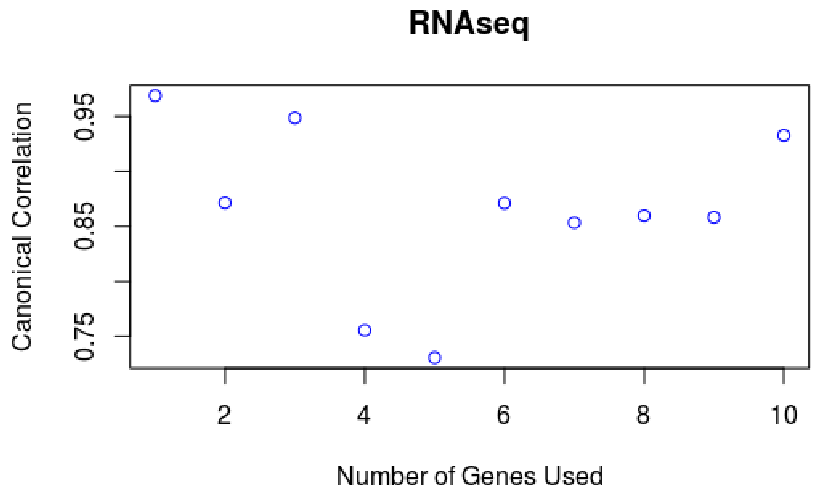


Figure 10 The canonical correlation of the RNAseq data after the linear implementation, using 4 OTUs

<u>5 OTUs, 1-10 genes</u>

The plots show the canonical correlations after the linear relationship implementation for both the multivariate Gaussian and RNAseq data. The y-axis represents the canonical correlations while the x-axis represents the number of genes used in the implementation. The canonical correlations before the linear relationship implementation are 0.6966727 and 0.7593742 for multivariate Gaussian and RNA sequencing respectively.

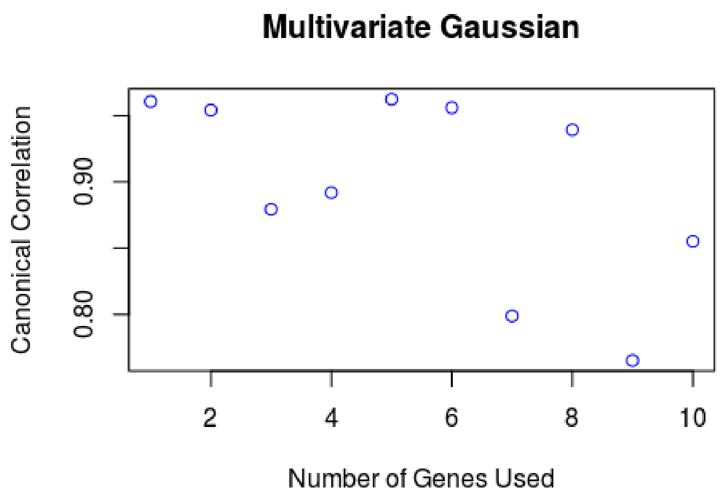


Figure 11 The canonical correlation of the Multivariate Gaussian data after the linear implementation using 5 OTUs

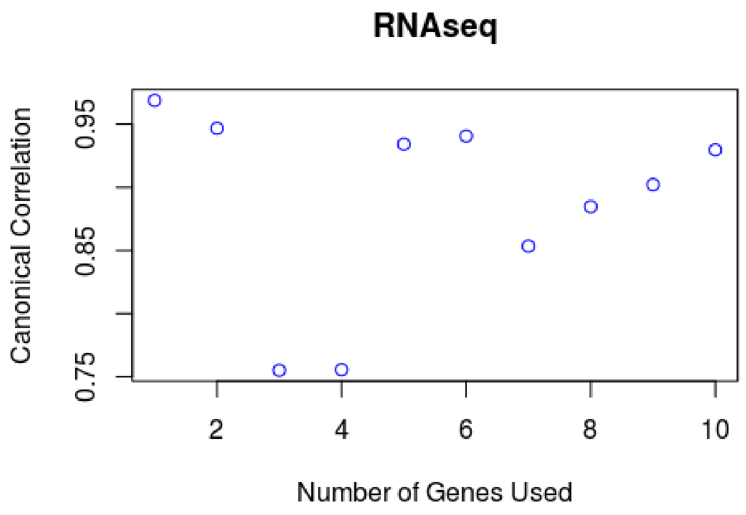


Figure 12 The canonical correlation of the RNAseq data after the linear implementation, using 5 OTUs

5.3 Real data

The real data consists of a host microarray data set and a microbial data set. The objective of this unpublished study we try to replicate is to apply the sparse CCA on this pair of data sets. In that study, both of the input data were not in the right format (as described in APPENDIX A) for the sparse CCA tool, and thus required some manual preprocessing steps before applying sparse CCA. In our replication, however, we start with two data files that are already in the correct format so that they are suitable for the tool. For the following plots, ‘Immunology’ refers to the microarray data set and ‘SeedLevel2’ refers to the microbial dataset. The elliptic shapes in each plot group the similar samples together.

5.3.1 SeedLevel2 vs. Immunology

Both Figure 13 and Figure 14 display the component scores of ‘SeedLevel2’ versus the component scores of ‘Immunology’. Figure 13 represents the first component scores while Figure 14 represents the second component scores. The canonical correlation for the first component scores is 0.964 and the canonical correlation for the second component scores is 0.938.

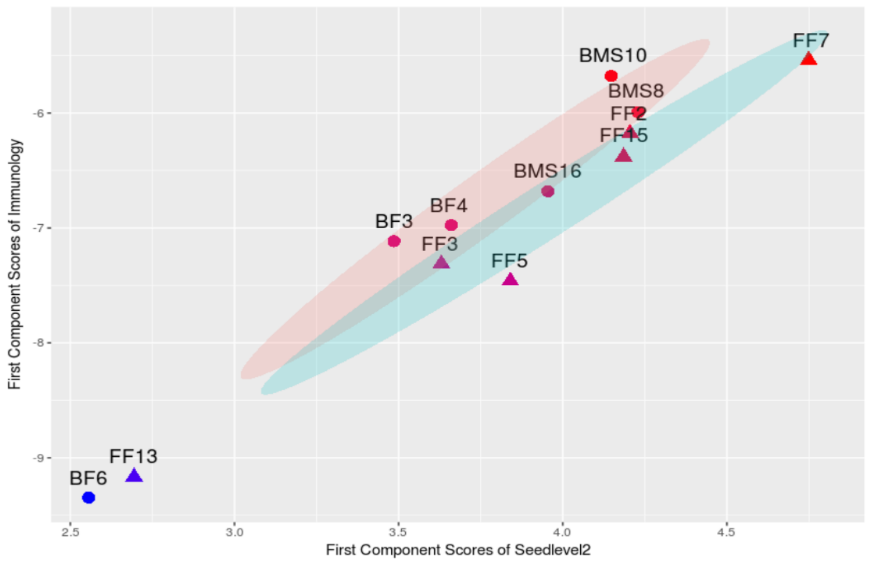


Figure 13 The first component score between Immunology and SeedLevel2

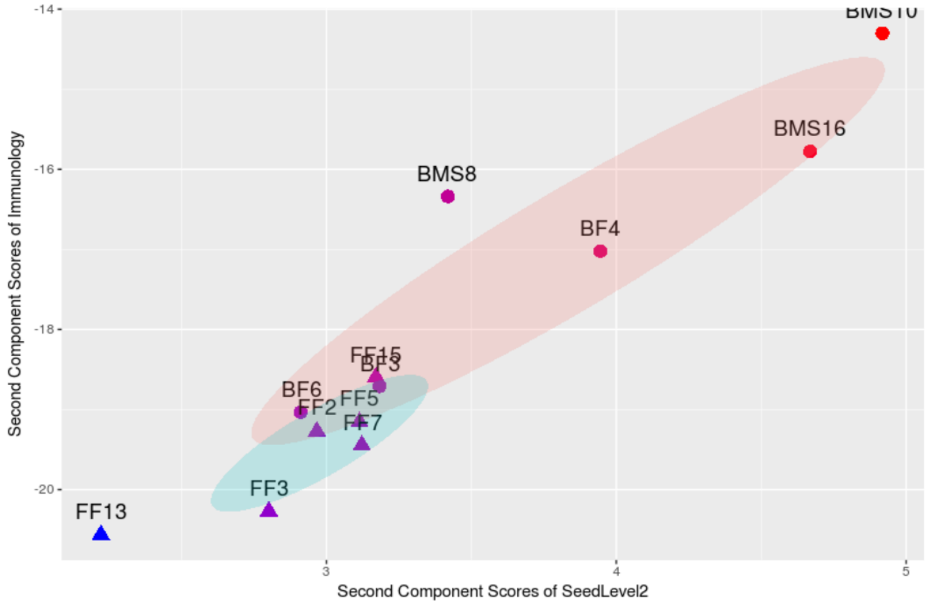


Figure 14 The second component score between Immunology and SeedLevel2

5.3.2 First component scores vs. second component scores

Figure 15 shows the first component scores versus the second component scores of ‘SeedLevel2’. In order to display more than two dimensions, the first component scores of ‘Immunology’ is also shown using the color spectrum. Different feeding types for each sample is also represented by the circular and triangular shapes. Similarly, Figure 16 shows the first component scores versus the second component scores of ‘Immunology’. The first component scores of ‘SeedLevel2’ is also shown via the color spectrum.

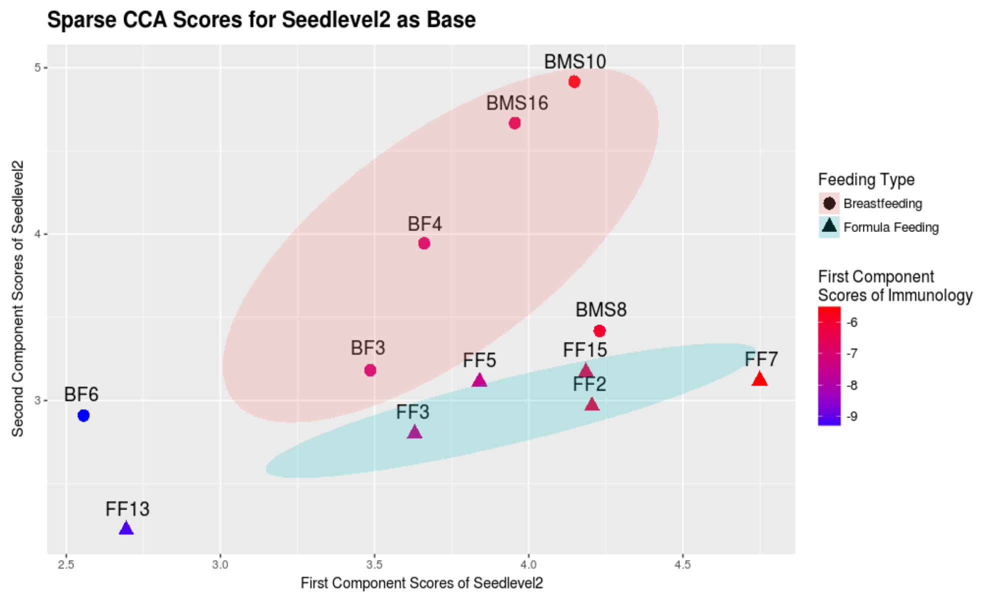


Figure 15 The first component scores vs. the second component scores of SeedLevel2

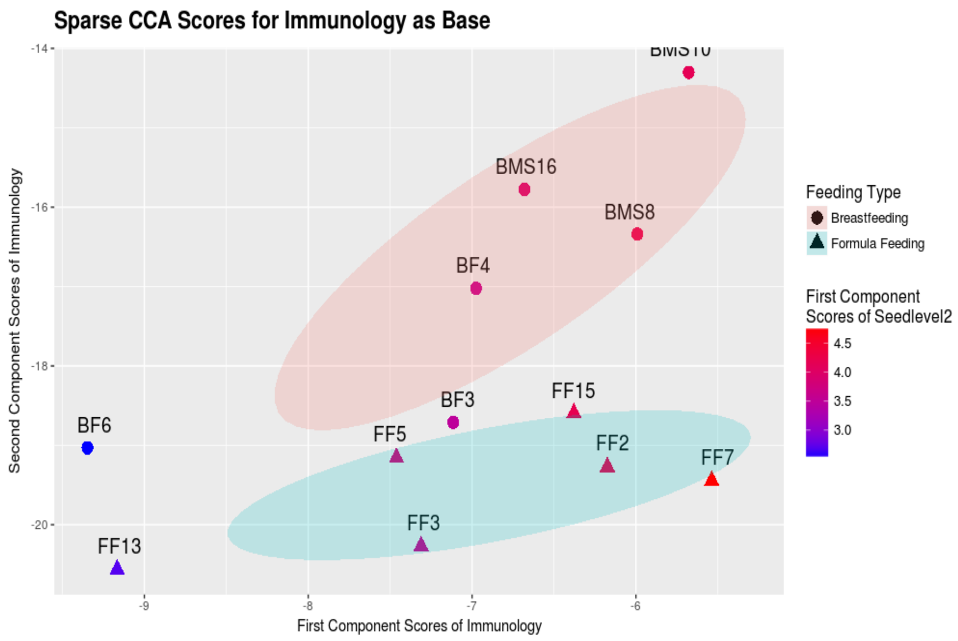


Figure 16 The first component scores vs. the second component scores of Immunology

5.3.3 Sparse PCA

To illustrate that sparse CCA can separate two feeding types better than sparse PCA, the sparse PCA plots are provided as a comparison. Figure 17 represents the PCA scores for ‘SeedLevel2’ while Figure 18 represents the PCA scores for ‘Immunology’. The x-axis represents the first principle component scores while the y-axis represents the second principle component scores. The plots are generated from the Sparse PCA code that are manually implemented using the R package ‘PMA’, which is the same package used in the sparse CCA tool’s back-end.

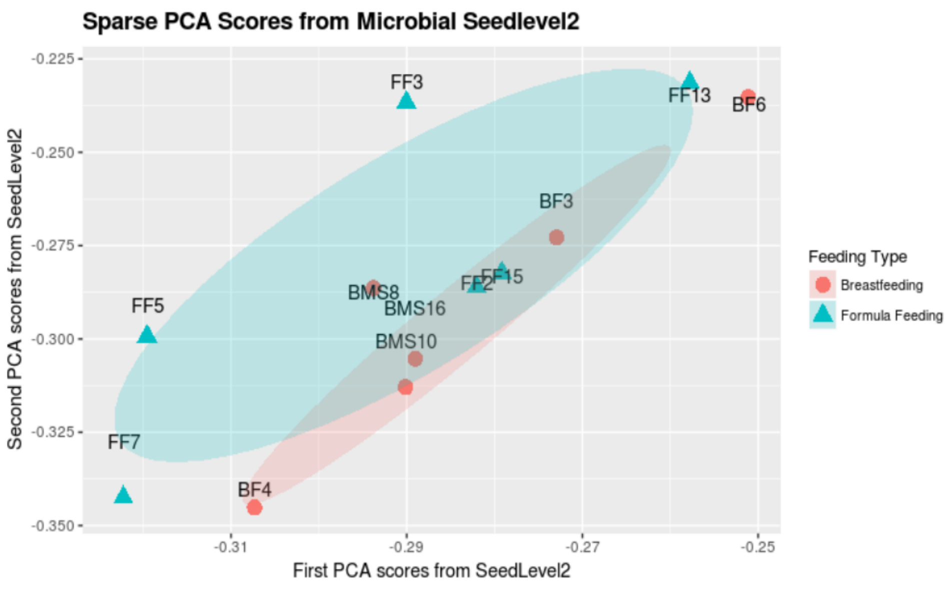


Figure 17 The first vs. second PCA scores of SeedLevel2

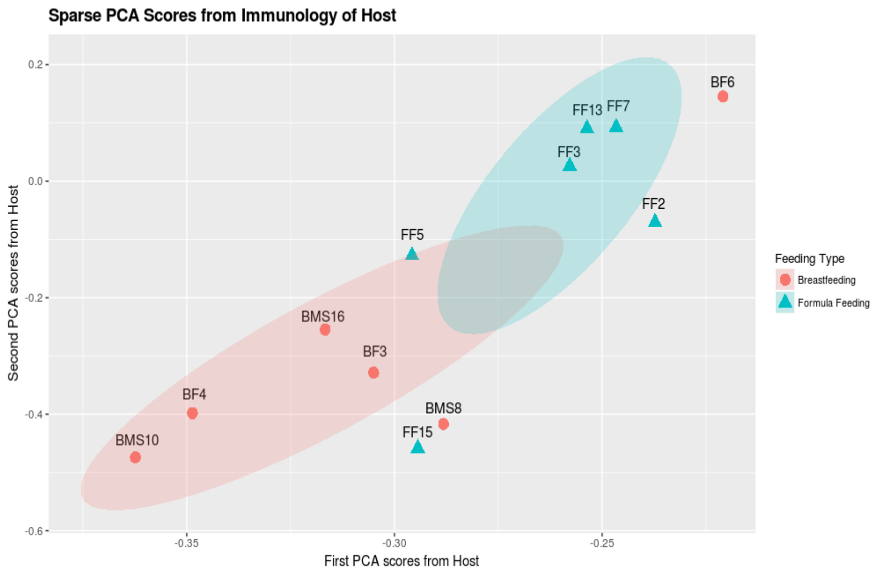


Figure 18 The first vs. second PCA scores of Immunology

6. DISCUSSION

6.1 Synthetic data

6.1.1 Small numbers of features

In this particular part of our validation studies, the data consists of 2 OTUs and 3 genes as features. We consider three separate cases. To solidify the results, each case is repeated with 12 different datasets. The reason for using small numbers of features is that it would be easier to detect the implemented linear relationship. Given that the numbers of features are small, the canonical cross loadings for each feature can easily be tracked. Each case of the numerical experiments results in four tables that display canonical cross-loadings (two for multivariate Gaussian data and two for RNA sequencing data).

The linear relationship is introduced between the variables from the multivariate Gaussian and the OTUs because the multivariate Gaussian models gene RNA concentrations before the library preparation and the subsequent sequencing of those libraries. The first case to consider is when one OTU and one gene are used to construct the linear relationship. Table 1 shows the canonical cross-loadings between each of the OTUs and the canonical variate of the multivariate Gaussian data. Similarly, Table 2 shows the canonical cross-loadings between each of the genes and the canonical variate of the microbial data. After the relationship is introduced, the canonical cross-loadings for the features used in the implementation increase as expected. Table 3 shows the canonical cross-loadings between each of the OTUs and the canonical variate of RNA sequencing data. Table 4 shows the canonical cross-loadings between each of the genes and the canonical variate of the microbial data. Unlike the multivariate Gaussian, the canonical cross-loadings do not display stable results, meaning that the cross-loadings do not always increase. Although most of the numbers do increase, the level of increments are lower than that of the multivariate Gaussian data.

For the second and third cases, where two and three genes are used in the linear relationship implementation respectively, the correlation can also be detected by the sparse CCA tool. For the multivariate Gaussian data, the absolute values of canonical cross loadings increase greatly. With the RNAseq data, however, the linear relationship can still be traced but it is not as obvious as the multivariate Gaussian cases. These results agree with the first case mentioned earlier. Nonetheless, there is a subtle difference when using more than one features in the linear relationship implementation. The canonical cross-loadings for all features do not always increase altogether since the weight of each feature on the linear relationship is not the same.

The results from all three cases indicate that the transformation of the multivariate Gaussian by the Poisson negatively affects the ability of the sparse CCA to detect the linear relationships introduced between the multivariate Gaussian and the OTUs. This suggests that one should use caution when interpreting the sparse CCA results where only sequencing data is used.

6.1.2 Large numbers of features

The numerical experiment for large numbers of features are performed in five scenarios. 789 OTUs are used for microbial data and 300 genes are used for both multivariate Gaussian data and RNA-sequencing data. Similar to the small cases, the linear relationship is introduced between the variables from the multivariate Gaussian and the OTUs. Between two to five OTUs are selected to have a linear relationship with one to ten genes. The chosen OTUs are those that have the minimum number of zero count among the samples. The genes, on the other hand, can be randomly selected.

Each pair of plots display the canonical correlations after implementing the relationship. The five cases presented are based on the number of OTUs in the linear relationship implementation. There are two plots in each case, one for multivariate Gaussian and one for RNAseq. The vertical axis is the canonical correlation for each number of genes used in the implementation. Since the number of features is large, we consider a canonical correlation instead of the canonical cross-loading for each feature used in the previous section.

For 2 OTUs, the original values of the canonical correlation are 0.6966727 and 0.7593742 for multivariate Gaussian data and RNA-sequencing data respectively. In fact, these numbers are the same when different numbers of OTUs are used, since they are the numbers before introducing the linear relationship. Figure 5 shows that after the implementation of linear relationship, the canonical correlations between the microbial data and multivariate Gaussian data significantly increase. For the RNAseq data, the difference is that after the linear relationship is implemented, the canonical correlations between the microbial data and RNAseq data do not increase as much as those for the multivariate Gaussian data, and some do not increase at all, as shown in Figure 6. Following the same trend of thought as in Ghaffari et al. (2013) [6], we speculated that the Poisson process, which is part of the RNAseq generating process, has contributed to the distortion of the linear relationship implemented at the level of the multivariate Gaussian and OTUs level. For the case where three, four , and five OTUs are used, the plots are comparable with those from two OTUs and thus solidify the results obtained in the cases of small number of features.

Similar to the conclusion for the small case, the sparse CCA results should be interpreted with caution in the case where only sequencing data is used because the results indicate that the transformation of the multivariate Gaussian by the Poisson negatively affects the ability of the sparse CCA to detect the linear relationships introduced between the multivariate Gaussian and the OTUs.

6.2 Real data

6.2.1 SeedLevel2 vs. Immunology

Figure 13 and 14 were not included in the original results of the study but are included here in order to aid the sparse CCA interpretation. They are the scatter plots of the first and second canonical variate pair between the two datasets respectively. The canonical correlations between the datasets are also displayed at the top of each graph. The values of canonical correlation reflect how strong the two sets of features are correlated. Higher number indicates stronger correlation which results in a less scattered and more linear positioning of the samples on the graph. Thus, one way of interpretation is to see how much the graph fits to a linear line. The more it fits, the more correlation there is. For 'SeedLevel2' and 'Immunology', the canonical variate plots for both the first and second variate pairs have an obvious linear fit, indicating that the sparse CCA performs as expected since it could find the linear combinations of the original variables such that those linear combinations are strongly correlated.

6.2.2 First component scores vs. second component scores

Figure 15 and 16 are the plots of the first versus the second canonical variate. Figure 15 is for 'SeedLevel2' while Figure 16 is for 'Immunology'. The tool is able to replicate the results made in the paper. For these two plots, the horizontal axis corresponds to the first canonical variate and the vertical axis corresponds to the second canonical variate. In order to add a third dimension to the graph and see the relationship with another variable a color bar on the right is attached. When plotting the result this way, the samples could be separated into groups, based on their canonical variates. This suggest the potential use of the sparse CCA as not only a tool to detect correlations between two sets of variables but also the ability to detect different phenotypes/classes presented by the samples.

6.2.3 Sparse PCA

The sparse PCA plots are used as a comparison in order to show the advantage of using sparse CCA. Spare PCA method was applied on both the host genes expression levels and gut microbiota data. For Figure 17, the x axis and y axis represent the first and second PCA components of the normalized second level of SEED subsystem respectively. Similarly, for Figure 18, the x axis and y axis represent the first and second components of immunology related genes. It is noticeable that the sparse PCA method cannot separate the two groups of samples as well as the sparse CCA.

7. CONCLUSIONS

The sparse CCA tool is built based on the motivation that researchers without expertise in computational fields need to use statistical methods to solve their research related to integrative data analysis. A tool that is easy to use would allow them to perform analyses without having to consult a statistical expert. The R package ‘PMA’ is the primary choice in the implementation because of its capability and efficiency in the sparse CCA computation. Moreover, this package also provides functions for computing a sparse PCA, so the future improvement of the tool will be relatively straightforward if such expansion is necessary.

The four significant objectives to be achieved are the tool’s validation, user-friendly implementation, code modularity, and a well-written documentation. For validation purposes, the sparse CCA tool is applied on synthetic datasets with manual implementation of linear relationship and is also applied on the real data to replicate the result from the unpublished study “An application of sCCA for integrative analysis of diet-dependent interaction between gut microbiota and host in neonates”. For synthetic data, both small and large numbers of features are used in order to confirm the validation results from synthetic data. For real data, supplementary plots that are not in the original study are also included to solidify the validation. The tool’s interface can guide users to complete their analyses, starting from the inputs preparation to the input submission. To test the applicability of the tool, especially its user-interface portion, the tool was tried out by two of the lab members from the Chapkin lab. More details can be found in APPENDIX D. Internally, each part of the framework is designed to be modular so that additional tools can be implemented without complication. The documentation on the tool's usage and specific methods on working with the framework are provided in APPENDIX A and APPENDIX C respectively.

The validation with synthetic data sets confirms the efficiency of the tool when being applied with multivariate Gaussian data. The validation experiments also show that one must be careful when applying the sparse CCA with RNAseq data because, unlike the multivariate Gaussian, the correlation cannot be easily detected due to the effect of the Poisson process transformation during the data generation. For the real data set, the tool is able to successfully replicate the results provided in the study, and thus prove its applicability to the real world problems.

The next step for the tool is to integrate it with the APIMOD website. The necessary steps are covered in the guideline provided in APPENDIX B. There are also a number of possible future extensions of the tool's capability. One is to take advantage of the framework's modularity feature and introduce more options to the tool such as normalization methods or another type of analysis such as the PCA. Another one is to validate more data types for sparse CCA. Although the tool currently is able to accept any type of data as inputs, as long as they have the same number of samples, there is no guarantee that the correlation will always be detected. Because multiple factors must be taken into account such as the process during the generation of data, it is crucial to validate more data types, e.g. ChIP-sequencing data.

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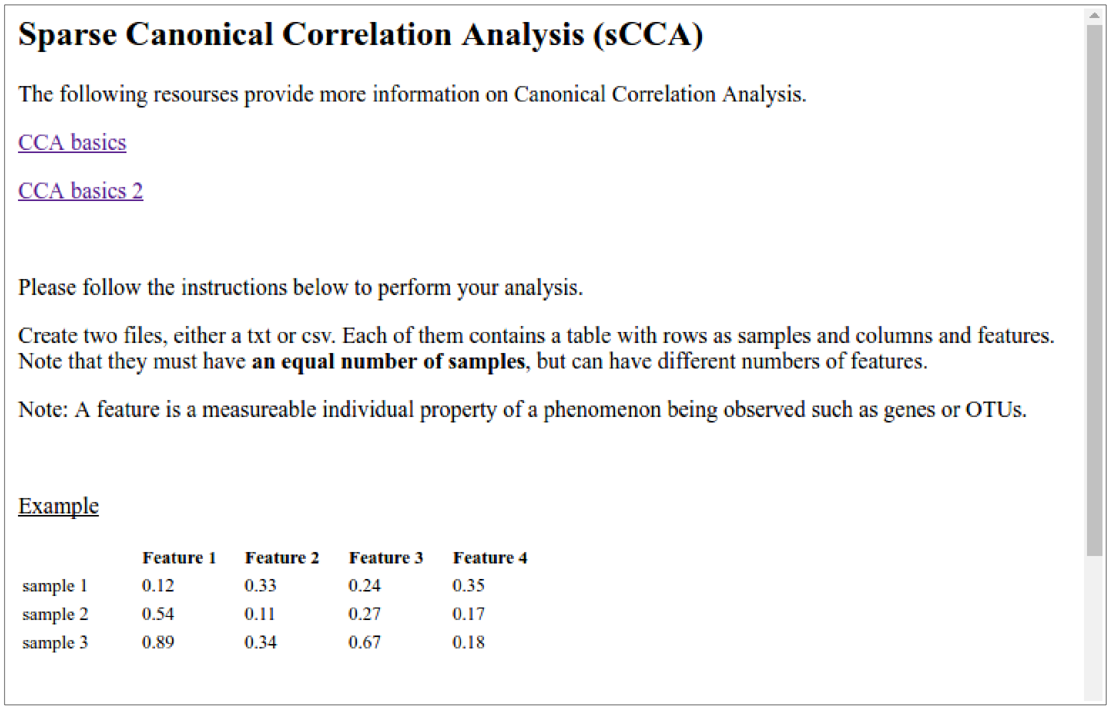
APPENDIX A

TOOL'S DESCRIPTION

Once the users navigate to the sparse CCA page, they will be guided by the instructions to perform their analysis. Some tutorial resources on canonical correlation analysis are provided for those who are interested. An example of the input data format is also given, including the explanation on arranging rows and columns.



Sparse Canonical Correlation Analysis (sCCA)

The following resourses provide more information on Canonical Correlation Analysis.

CCA basics

CCA basics 2

Please follow the instructions below to perform your analysis.

Create two files, either a txt or csv. Each of them contains a table with rows as samples and columns and features. Note that they must have **an equal number of samples**, but can have different numbers of features.

Note: A feature is a measureable individual property of a phenomenon being observed such as genes or OTUs.

Example

	Feature 1	**Feature 2**	**Feature 3**	**Feature 4**
sample 1	0.12	0.33	0.24	0.35
sample 2	0.54	0.11	0.27	0.17
sample 3	0.89	0.34	0.67	0.18

Figure A-1 The instruction for using the tool

On the submission form, they are asked to submit the name, email, project name, and project description. This information is then stored in the database. Then they submit the required input files, each being either a txt or csv file.

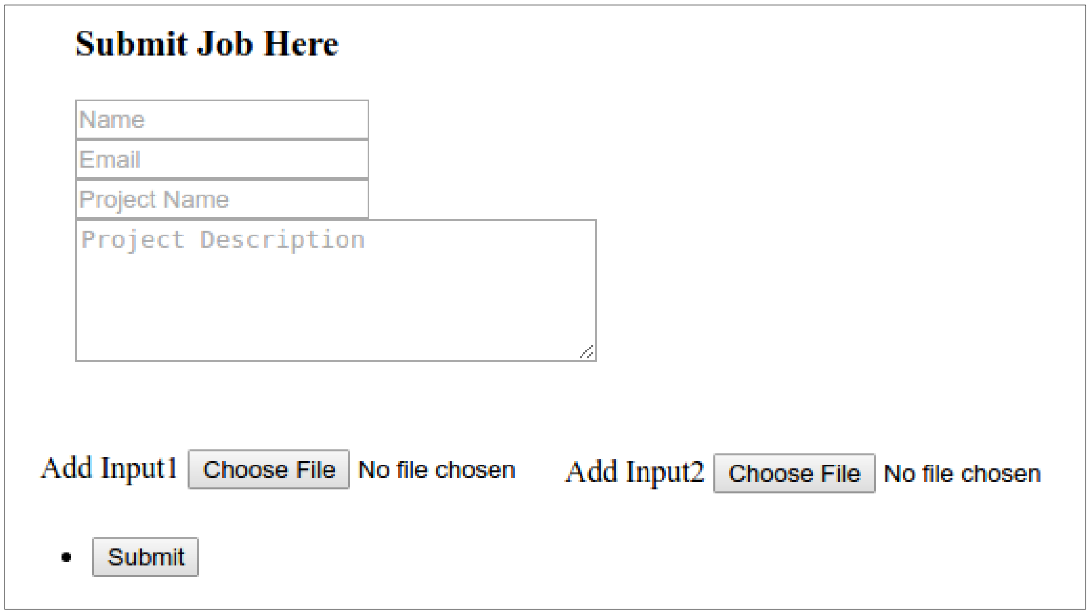


Figure A-2 The form for users to submit input files

APPENDIX B

INTEGRATION WITH THE APIMOD WEBSITE

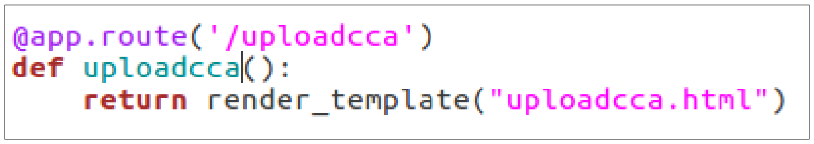
Multiple steps are required in order to integrate the sparse CCA tool with the APIMOD website. An example below will serve as a guideline to complete the integration process.

Example

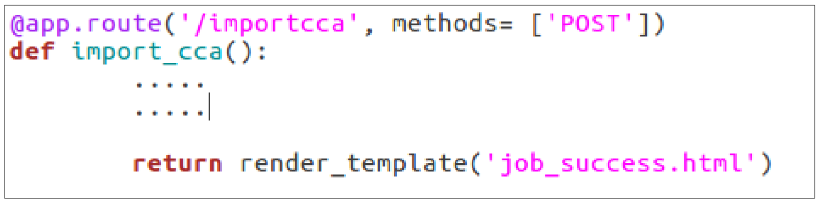
There are two main steps required to integrate the tool to the APIMOD website.

1.) Add and adjust respective portions of the website's back-end code, including the respective HTML templates, to enable the website to offer sparse CCA service. Those parts handle the interactions between the user interface and the system's back-end. The following functions must be adjusted or added to the current code.

The functions 'import_cca' and 'uploadcca' work together to allow users to submit input files to the system. Specifically, 'import_cca' store the users' information into the sqlite database while 'uploadcca' accepts the input files and stores them into the respective folders. The template 'job_success.html' and 'uploadcca.html' are the required templates for these two functions.



```
@app.route('/uploadcca')
def uploadcca():
    return render_template("uploadcca.html")
```

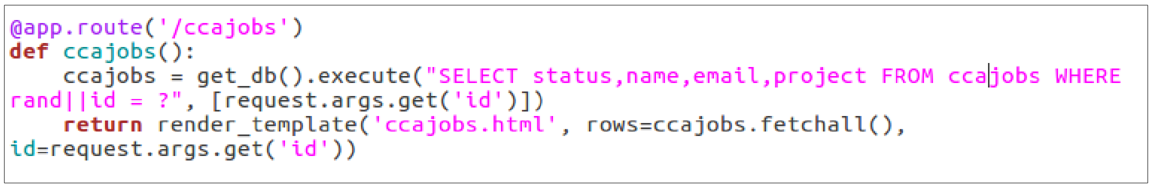


```
@app.route('/importcca', methods= ['POST'])
def import_cca():
        .....
        .....

        return render_template('job_success.html')
```

Figure B-1 Basic structure of the functions ‘uploadcca’ and ‘import_cca’

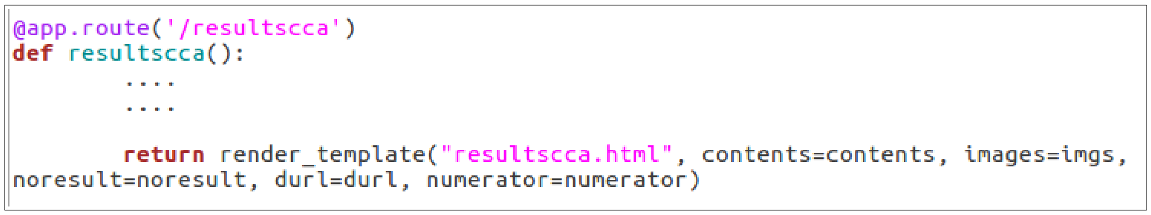
The function ‘ccajobs’ allows the users to check their sparse CCA job status through email. A template ‘ccajobs.html’ must be created to work with this function.



```
@app.route('/ccajobs')
def ccajobs():
    ccajobs = get_db().execute("SELECT status,name,email,project FROM ccajobs WHERE
rand||id = ?", [request.args.get('id')])
    return render_template('ccajobs.html', rows=ccajobs.fetchall(),
id=request.args.get('id'))
```

Figure B-2 The basic structure of the function ‘ccajobs’

The function ‘resultscca’ displays analysis results to the users. A template ‘resultscca.html’ must be created to work with this function.

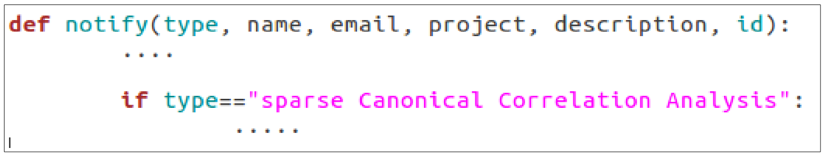


```
@app.route('/resultscca')
def resultscca():
        ....
        ....

        return render_template("resultscca.html", contents=contents, images=imgs,
noresult=noresult, durl=durl, numerator=numerator)
```

Figure B-3 The basic structure of the function ‘resultscca’

The function ‘notify’ handles users’ job notifications for all types of analysis. The necessary step to be added is to have the ‘if’ statement that ensures the specific elements for only the sparse CCA are handled correctly.

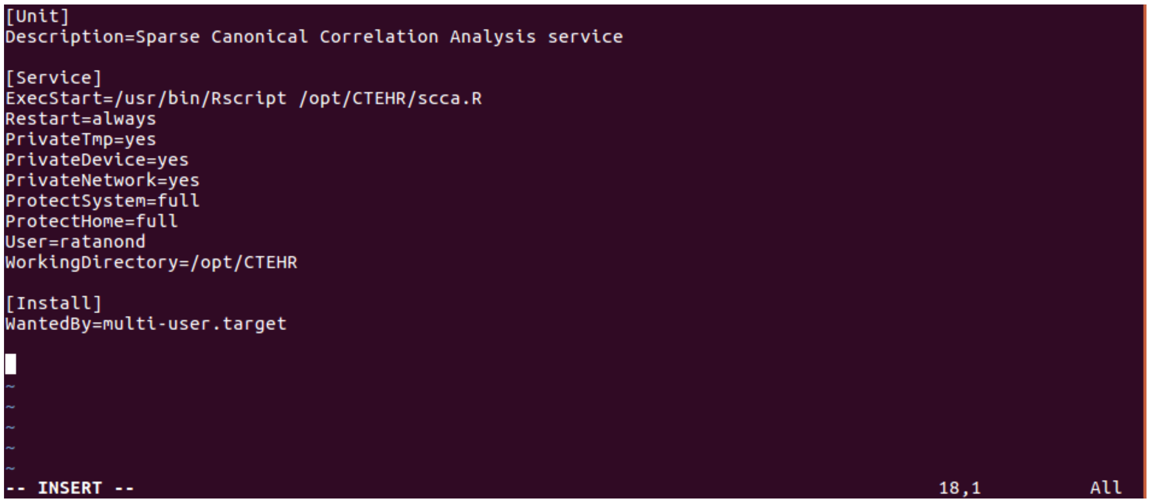


```
def notify(type, name, email, project, description, id):
        ....

        if type=="sparse Canonical Correlation Analysis":
                .....
```

Figure B-4 The basic structure of the function ‘notify’

2.) Create a service file in order to run the sparse CCA code in the background of the computer that hosts the website.



```
[Unit]
Description=Sparse Canonical Correlation Analysis service

[Service]
ExecStart=/usr/bin/Rscript /opt/CTEHR/scca.R
Restart=always
PrivateTmp=yes
PrivateDevice=yes
PrivateNetwork=yes
ProtectSystem=full
ProtectHome=full
User=ratanond
WorkingDirectory=/opt/CTEHR

[Install]
WantedBy=multi-user.target

~
~
~
~
~
-- INSERT --                                                                          18,1          All
```

Figure B-5 The screen-shot of the service file for sparse CCA

APPENDIX C

MODULARITY FEATURE

Once users submit the requests through the user-interface, the relevant information is then stored in the system database waiting to be processed. Thus, a dedicated portion of the framework that connects the database to the analysis code is a necessity. Figure C-1 depicts a brief workflow in the back end, showing that the analysis code must interact with the database in order keep track of the submitted requests. In the current implementation of the APIMOD, each analysis option (sequencing classification or differential expression) has its own way of interacting with the database. However, the downside of this approach is that every time a new tool is added, the code that allow the interactions must always be rewritten. The better approach is to have just one reusable framework to handle the connection.

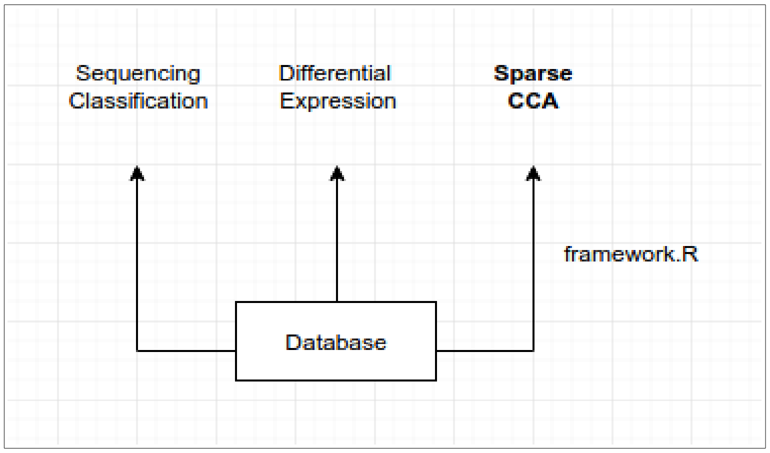


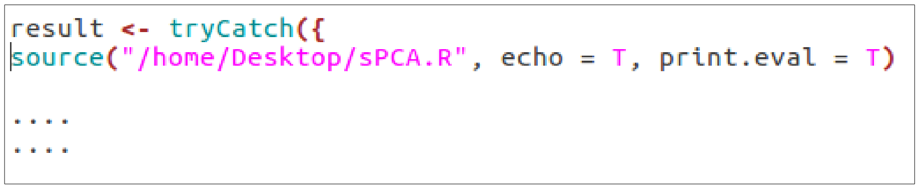
Figure C-1 Back-end workflow

As indicated in the diagram above, 'framework.R' is an R code that manages the connection between the database and the code that performs sparse CCA. 'framework.R' will also be able to accept additional analysis code as well (such as sparse PCA), with a strict requirement being that code used to do the analysis must be in R.

Example

To go through the detailed example, let's assume that a sparse principal component analysis (sparse PCA) is to be implemented and its analysis code 'sPCA.R' is already available.

First , the sparse CCA code in framework.R must be replaced with a directory linking with another analysis code. Given that sPCA.R is at the directory /home/Desktop/, the directory /home/Desktop/sPCA.R must be placed inside the source function, which redirects the code to that directory.

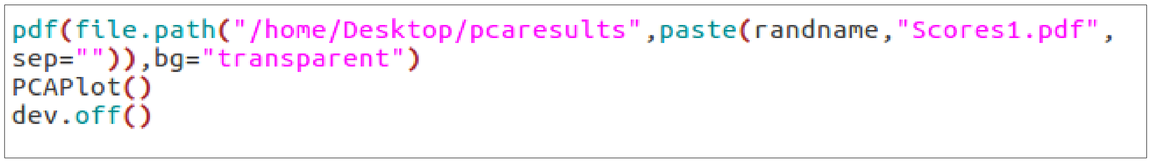


```
result <- tryCatch({
source("/home/Desktop/sPCA.R", echo = T, print.eval = T)

....
....
```

Figure C-2 The 'source' function inside tryCatch

The output has to also be specified inside the analysis code. Suppose that we want to write a plot to a pdf file and place it into the directory /home/Desktop/pcaresults, the code below has to be placed inside sPCA.R.



```
pdf(file.path("/home/Desktop/pcaresults",paste(randname,"Scores1.pdf",
sep="")),bg="transparent")
PCAPlot()
dev.off()
```

Figure C-3 The output destinations

Finally, the names of the associated folders and database in framework.R also have to be changed to correspond with sPCA.R. That is, the database must refer to the users' information who submit requests for sparse PCA and the folders must be those that handle the inputs and outputs of sparse PCA.

APPENDIX D

LAB MEMBERS' RESPONSES

The tool was tested by two members from the Chapkin Lab. A few issues were discovered following their experiment. First, despite having the user-friendly interface and a lot of instructions provided, using sparse CCA without consulting someone with expertise is not quite pragmatic for researchers with less quantitative background. There are a few potential solutions to this issue. One option is to provide tutorial videos that explain the utility and the meaning on the sparse CCA from a biological perspective. Another choice is to design a help menu that is updated according to the problems encountered by the users.

Second, the tool currently accepts inputs where rows represent samples and columns represent features. However, many researchers use Microsoft Excel as their primary data manipulator instead of R. The problem is that while the number of rows Microsoft Excel can handle is around a million, the maximum number of columns it can handle is no more than 20,000. In practical use, it would be common for researchers to have data with more than 30,000 features. Thus it is likely that they would prefer to have rows as features and columns as samples in stead of the other way around, so that there is no trouble opening the file in Microsoft Excel. Because of this, the format of the inputs should be altered to correspond to their need, that is to have columns representing features and rows representing samples. Additionally, regarding the input format, it is strongly suggested by the testers that a screen-shot showing the proper format in Microsoft Excel should be displayed as well.