Reconstructing phenotype specific multi-omics networks with SmCCNet

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Abstract

Sparse multiple canonical correlation network analysis (SmCCNet) is a machine learning technique for integrating multiple omics data on the same subjects, along with a quantitative phenotype of interest, and reconstructing multi-omics networks that are specific to the phenotype. While the current version integrates two omics data types in addition to a phenotype, the framework can be easily generalized to more than two omics data types and multiple quantitative phenotypes. In this document, we illustrate a standard workflow of SmCCNet with a synthetic miRNA, mRNA expression dataset.

Contents

1 SmCCNet overview

Note: if you use SmCCNet in published research, please cite:

> Shi, W.J., Y. Zhuang, P.H. Russell, B.D. Hobbs, M.M. Parker,P.J. Castaldi, P. Rudra, B. Vestal, C.P. Hersh, L.M. Saba, and K. Kechris, "Unsupervised Discovery of Phenotype Specific Multi-Omics Networks." Bioinformatics. 2019 Nov 1;35(21):4336-4343. doi: 10.1093/bioinformatics/btz226.

1.1 Workflow

SmCCNet is a canonical correlation based integration method that reconstructs phenotype-specific multi-omics networks (Shi et al., 2019). The algorithm is based on sparse multiple canonical analysis (SmCCA) for multiple omics data $X_1, X_2, ...X_T$ and a quantitative phenotype Y measured on the same subjects. SmCCA finds the canonical weights $w_1, w_2, ..., w_T$ that maximize the (weighted or unweighted) sum of pairwise canonical correlations between $X_1, X_2, ..., X_k$ and Y, under some constraints (Equation 1). In SmCCNet, the sparsity constraint functions $P_t(\cdot), t = 1, 2, ..., T$, are the least absolute shrinkage and selection operators (LASSO). The weighted version corresponds to a, b, c not all equal; the unweighted version corresponds to $a_s = b_t = 1$ for all $s = 1, 2, ..., \binom{t}{2}$ and $t = 1, 2, ..., T$.

$$(w_1, w_2, ..., w_T) = \arg \max_{\tilde{w}_1, \tilde{w}_2, ..., \tilde{w}_T} \left(\sum_{i<j} a_{i,j} \tilde{w}_i^T X_i^T X_j \tilde{w}_j + \sum_{i=1}^{\binom{t}{2}} b_i \tilde{w}_i^T X_i^T Y \right), \quad (1)$$

$$\text{subject to } \|\tilde{w}_t\|^2 = 1, P_t(\tilde{w}_t) \leq c_t, t = 1, 2, ..., T.$$

The sparsity penalties c_t influence how many features will be included in each subnetwork. With pre-selected sparsity penalties, the SmCCNet algorithm creates a network similarity matrix based on SmCCA canonical weights from repeated subsampled omics data and the phenotype, and then finds multi-omics modules that are relevant to the phenotype. The subsampling scheme improves network robustness by analyzing a subset of omics features multiple times and forms a final similarity matrix by aggregating results from each subsampling step. The general workflow (Figure 1) involves three steps:

- Step I: Determine SmCCA sparsity penalties c_t. The user can select the penalties for omics feature selection based on the study purpose and/or prior knowledge. Alternatively, one can pick sparsity penalties based on a K-fold cross validation (CV) procedure that minimizes the total prediction error (Figure 2). The K-fold CV procedure ensures selected penalties to be generalizable to similar independent data sets and prevents over-fitting.
- Step II: Randomly subsample omics features without replacement, apply SmCCA with chosen penalties, and compute a feature relationship matrix for each subset. Repeat the process many times and define the similarity matrix to be the average of all feature relationship matrices.
- Step III: Apply hierarchical tree cutting to the similarity matrix to find the multi-omics networks. This step simultaneously identifies multiple subnetworks.

1.2 SmCCNet package

The SmCCNet package has the following dependencies:

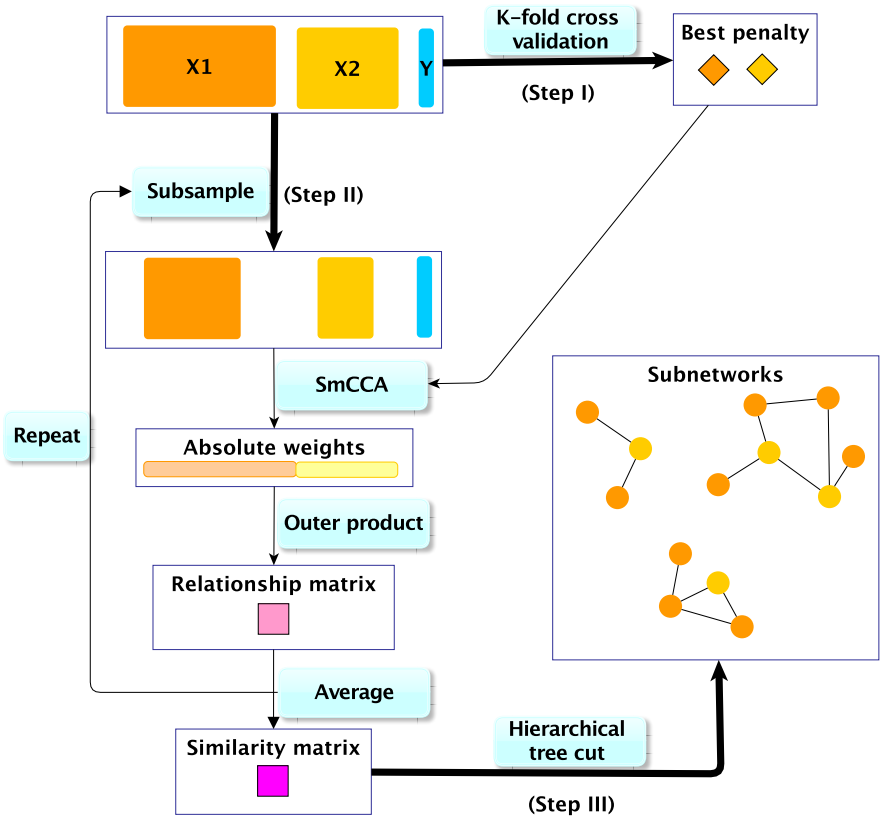

Figure 1: SmCCNet workflow overview. X1 and X2 are two omics data types for the same set of n subjects. Y indicates a quantitative phenotype measure for those n subjects. Note that the flowchart demonstrate workflow for two omics data, it is also compatible with more than two omics data.

```
library(PMA)
library(pbapply)
library(Matrix)
library(igraph)
library(SmCCNet)
library(furrr)
library(future)
```

The older version of the SmCCNet package includes four (external) functions:

- **getRobustPseudoWeights()**: Compute aggregated (SmCCA) canonical weights.
- **getAbar()**: Calculate similarity matrix based on canonical weights.
- **getMultiOmicsModules()**: Perform hierarchical tree cutting on the similarity matrix and extract clades with multi-omics features.
- **plotMultiOmicsNetwork()**: Plot (pruned or full) multi-omics subnetworks.

In the updated package, all functions except for **getAbar** are retired from the package, additional functions have been added to the package to perform single-/multi-omics SmCCNet with quantitative/binary phenotype, and their use is illustrated in this vignette:

- **aggregateCVSingle()**: Saving cross-validation result as the cross-validation table into the working directory and provide recommendation on the penalty term selection.
- **classifierEval()**: Evaluate binary classifier's performance with respect to user-selected metric (accuracy, auc score, precision, recall, f1).
- **dataPreprocess()**: A simple pipeline to preprocess the data before running SmCCNet (center, scale, coefficient of variation filtering and regressing out covariates).
- **fastAutoSmCCNet()**: Automated SmCCNet that automatically identify project problem (single-omics vs multi-omics), and analysis method (CCA vs. PLS) based on the input data that is provided. This method automatically preprocess data, choose scaling factors, subsampling percentage, and optimal penalty terms, then run through the complete SmCCNet pipeline without the requirement for users to provide any information. This function will store all the subnetwork information to local directory user is providing, as well as return all the global network and evaluation information.
- **getCanWeightsMulti()**: Run Sparse Multiple Canonical Correlation Analysis (SmCCA) and return canonical weight.
- **getCanCorMulti()**: Get canonical correlation value for SmCCA given canonical weight vectors and scaling factors.
- **getRobustWeightsMulti()**: Calculate the canonical weights for SmCCA.
- **getRobustWeightsMultiBinary()**: SmCCNet algorithm with multi-omics data and binary phenotype. This is a stepwise approach that (1) Use SmCCA to identify relationship between omics (exlude phenotype), (2) within highly connected omics features selected in step 1, identify relationship between these selected omics features and phenotype of interest with sparse PLS. Sparse PLS algorithm for binary outcome first compute PLS by assuming outcome is continuous, and extract multiple latent factors, then use latent factors to fit logistic regression, and weight latent factor by regression parameters.
- **getRobustPseudoWeights_single()**: Compute aggregated (SmCCA) canonical weights for single omics data with quantitative phenotype.
- **getRobustPseudoWeights_binary()**: Compute aggregated (SmCCA) canonical weights for single omics data with binary phenotype.
- **getOmicsModules()**: Perform hierarchical tree cutting on the similarity matrix and extract clades with omics features.
- **networkPruning()**: Extract summarization scores (the first 3 NetSHy/regular principal components) for specified network module with given network size. The proteins will be ranked based on PageRank algorithm, then the top k proteins (where k is the specified subnetwork size) will be included into the final subnetwork to generate the summarization score. For the PC score, the correlation with respect to the phenotype of interest will be calculated and stored. In addition, the correlation between individual proteins and phenotype of interest will also be recorded. The final subnetwork adjacency matrix will be stored into the user-specified working directory of interest.
- **scalingFactorInput()**: Input the vector of the type of dataset, and return prompts that ask the user to supply the scaling factor intended for SmCCNet algorithm to prioritize the correlation structure of interest. All scaling factor values supplied should be numeric and nonnegative.
- **summarizeNetSHy()**: Implement NetSHy network summarization via a hybrid approach to summarize network by considering the network topology with laplacian matrix (and TOM matrix).

More details on above functions can be found in the package manual.

2 SmCCNet workflow with a synthetic dataset

2.1 Synthetic dataset

As an example, we consider a synthetic data set with 500 genes (X_1) and 100 miRNAs (X_2) expression levels measured for 358 subjects, along with a quantitative phenotype (Y).

```
data(ExampleData)
head(X1[ , 1:6])
##          Gene_1   Gene_2   Gene_3   Gene_4   Gene_5   Gene_6
## Samp_1 22.48570 40.35372 31.02575 20.84721 26.69729 30.20545
## Samp_2 37.05885 34.05223 33.48702 23.53146 26.75463 31.73594
## Samp_3 20.53077 31.66962 35.18957 20.95254 25.01883 32.15723
## Samp_4 33.18689 38.48088 18.89710 31.82330 34.04938 38.79989
## Samp_5 28.96198 41.06049 28.49496 18.37449 30.81524 24.00454
## Samp_6 18.05983 29.55471 32.54002 29.68452 26.19996 26.76684
head(X2[ , 1:6])
##           Mir_1    Mir_2    Mir_3    Mir_4    Mir_5     Mir_6
## Samp_1 15.22391 17.54583 15.78472 14.89198 10.34821  9.689755
## Samp_2 16.30697 16.67283 13.36153 14.48855 12.66090 11.333613
## Samp_3 16.54512 16.73501 14.61747 17.84527 13.82279 11.329333
## Samp_4 13.98690 16.20743 16.29308 17.72529 12.30056  9.844108
## Samp_5 16.33833 17.39387 16.39792 15.85373 13.38767 10.599414
## Samp_6 14.54110 16.51999 14.73958 15.87504 13.21359 10.922393
head(Y)
##           Pheno
## Samp_1 235.0674
## Samp_2 253.5450
## Samp_3 234.2050
## Samp_4 281.0354
## Samp_5 245.4478
## Samp_6 189.6231
```

Denote the number of features in $X_1 \& X_2$ as $p_1 \& p_2$ respectively, and the number of subjects as n.

```
p1 <- ncol(X1)
p2 <- ncol(X2)
n <- nrow(X1)
AbarLabel <- c(colnames(cbind(X1, X2)))
```

Although SmCCNet does not require normality, it calculates the Pearson correlation between linear combinations of omics features and the phenotype, which assumes finite variances and finite covariance. It is necessary to include a transformation if the data are skewed. The algorithm also requires the data to be standardizable (i.e. none of the data matrices include a column with zero variance.)

2.2 Step I: Determine optimal sparsity penalties through CV

For two omics data, to find the optimal sparsity penalties c_1, c_2, we apply a K-fold CV on the synthetic data (Figure 2). Note that under LASSO constraints, $1 \leq c_1 \leq \sqrt{p_1 s_1}, 1 \leq c_2 \leq \sqrt{p_2 s_2}$, where p_1, p_2 denote the number of features in omics data X_1, X_2 respectively, and

s_1, s_2 are the proportions of X_1, X_2 features to be sampled every time. The sparse penalties c_1, c_2 can be re-parametrized as $0 < l_1, l_2 \leq 1$, such that $c_1 = \max\{1, l_1\sqrt{p_1 s_1}\}, c_2 = \max\{1, l_2\sqrt{p_2 s_2}\}$. Large penalty values correspond to more features in each subnetwork, while small penalties correspond to fewer features. Here is the list of parameters that need to be specified:

- K: Number of folds in CV. Typically a 5-fold CV is sufficient. If the training set contains too few (e.g. < 30) samples, or the test or training set becomes unscalable, then choose a smaller K.
- $CCcoef$: Coefficients, (a, b) in Equation 1, for the weighted SmCCA. It can be either supplied manually or interatively (see code below).
- $s = (s_1, s_2)$: Proportions of feature subsampling from X_1, X_2. Default values are $s_1 = 0.7, s_2 = 0.9$.
- $SubsamplingNum$: Number of subsamples.
- $PenExpand$: A penalty option matrix for X_1, X_2. Each row of $PenExpand$ is a pair of penalty options $l = (l_1, l_2)$, where $0 < l_1, l_2 < 1$. Larger penalties correspond to more features to be included in each subnetwork. Typically, it is not necessary to search the entire range (0, 1), and a smaller grid (e.g. $0 < l_1, l_2 < .5$) may be sufficient.
- X A list of omics data, where each omics data should contain same set and order of subjects.
- num_omics The total number of omics data in X.
- $tuneLength$ The total number of candidate penalty terms for each penalty parameter.
- $minTune$ The minimumally possible tuning parameter.
- $maxTune$ The maximally possible tuning parameter.

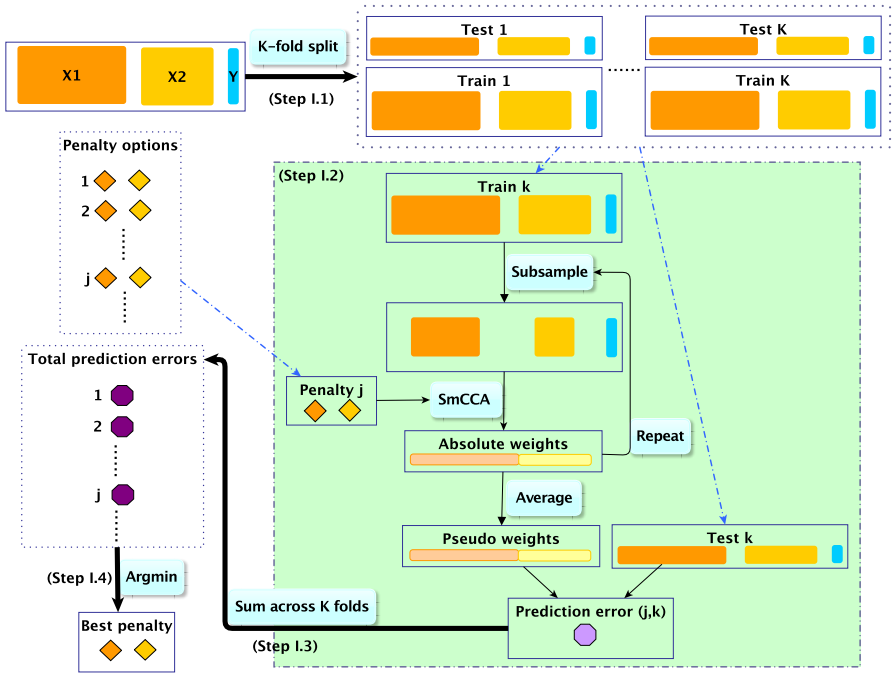

Figure 2: SmCCNet K-fold CV. The best penalty pairs are chosen based on the smallest total prediction error.

In all the code chunks below, if user plans to use more than 2 omics data, please change the code accordingly where a $**$ is marked on the comment line:

```
# number of folds in K-fold CV.
K <- 3
N <- nrow(X1)
# create a list of omics data **
X <- list(X1, X2)
# feature sampling proportions, 0.9 for miRNA since it has less features. **
s <- c(0.7,0.9)
# number of subsamples.
SubsamplingNum <- 50
# number of omics dataset **
num_omics <- 2
# tuning parameter candidate length for each omics data
tuneLength <- 5
# tuning parameter candadate range for each omics data
minTune <- 0.1
maxTune <- 0.5
# create empty matrix to store all possible penalty parameters
penSelect <- matrix(0, nrow = tuneLength, ncol = num_omics)
# create sparsity penalty options.
for (Idx in 1:ncol(penSelect))
{
  penSelect[,Idx] <- seq(from = minTune,
                         to = maxTune,
                         length.out = tuneLength)
}
# expand grid
# convert matrix to list of columns
list_cols <- as.list(as.data.frame(penSelect))
# generate all possible combinations
PenExpand <- do.call(expand.grid, list_cols)

# set a CV directory.
CVDir <- "Example3foldCV/"
dir.create(CVDir)
```

2.2.1 Create test and training data sets.

First, we need to split the data (X_1, X_2, Y) into test and training sets (Figure 2, Step I.1). All CCA methods require data sets to be standardized (centered and scaled) by columns (e.g. features). We have included the standardization step within the SmCCNet algorithm. However, for the CV procedure, we recommend to standardize the training and test sets upfront, since this helps to choose the number of CV folds K. If any data set can not be standardized, we recommend to reduce K. In the code below, we show how to create CV data sets. The standardized training and test data sets will be saved under the "Example3foldCV/" directory.

```
set.seed(12345) # set random seed.

# split data into folds
foldIdx <- suppressWarnings(split(1:nrow(X[[1]]), sample(1:nrow(X[[1]]), K)))
folddata <- purrr::map(1:length(foldIdx), function(x){
```

```
    Y <- as.matrix(Y)
    X_train <- list()
    X_test <- list()
    Y_train <- list()
    Y_test <- list()
    for (i in 1:length(X))
    {
      X_train[[i]] <- scale(X[[i]][-foldIdx[[x]],])
      X_test[[i]] <- scale(X[[i]][foldIdx[[x]],])
    }
    Y_train <- scale(Y[-foldIdx[[x]],])
    Y_test <- scale(Y[foldIdx[[x]],])
    return(list(X_train = X_train, X_test = X_test,Y_train = Y_train,
                Y_test = Y_test))
})
# name each fold of data
names(folddata) <- paste0('Fold_', 1:K)
# saving all preliminary data into local directory for reproducibility purpose
save(folddata, s, PenExpand, SubsamplingNum,
       file = paste0(CVDir, "CVData.RData"))
```

2.2.2 Set Scaling Factors

It is important to set scaling factors for each pairwise correlation to prioritize correlation structure(s) of interest. Below is the function that use prompt to help user define the scaling factor intended for the analysis: default is (1,1,1) for two omics if the first line of code below is run. If user prefer to set up scaling factors in different ways such as (1,10,10) where 10 is set for omics-phenotype correlation to emphasize on omics-phenotype correlation. user can use the interactive function below. This is especially effective when more than two omics data are used since the order of pairwise combinations can be extremely complicated. Note that the argument (DataType) for interactive function below should strictly follow the order of the data list used for SmCCA computation, followed by 'phenotype'. For instance, in the example data, X_1 stands for mRNA, X_2 stands for miRNAs, then the order of the argument is set to ('mRNA', 'miRNA', 'phenotype'). In the example below, we use $(1, 1, 1)$ as the scaling factor to demonstrate the result.

```
# default
scalingFactor <- rep(1,ncol(combn(num_omics + 1,2)))
# interactive **
scalingFactor <- scalingFactorInput(DataType = c('mRNA', 'miRNA', 'phenotype'))
```

2.2.3 Run K-fold CV

For each of the K-fold we compute the prediction error for each penalty pair option (Figure 2, Step I.2). Since there is no subsampling step for cross-validation, we run through cross-validation with nested for loop. However, if the omics data are extremely high-dimensional, we recommend using the R package **parallel** to parallelize the for loop, or use **fastAutoSmCCNet()** directly. **fastAutoSmCCNet()** is the package built-in function that streamline the pipeline with single line of code, and the cross-validation step is parallelized with **future_map()** in **furrr** package.

```
# load cross-validation data
load(paste0(CVDir, "CVData.RData"))
# create an empty list for storing CV result for each fold
CVResult <- list()
for (CVidx in 1:K)
{
    # set scaling factor
    CCcoef <- scalingFactor
    # create empty vector for storing cross-validation result
    RhoTrain <- RhoTest <- DeltaCor <- rep(0, nrow(PenExpand))
    for(idx in 1:nrow(PenExpand))
    {
      # consider one pair of sparsity penalties at a time.
      l <- PenExpand[idx, ]
      # run SmCCA on the subsamples (Figure 1, Step II)
      Ws <- getCanWeightsMulti(folddata[[CVidx]][["X_train"]],
                           Trait = as.matrix(folddata[[CVidx]][["Y_train"]]),
                           Lambda = as.numeric(l), NoTrait = FALSE,
                           CCcoef = CCcoef)
      # evaluate the canonical correlation for training and testing data
      rho.train <-  getCanCorMulti(X = folddata[[CVidx]][["X_train"]],
                                Y = as.matrix(folddata[[CVidx]][["Y_train"]]),
                                CCWeight = Ws,
                                CCcoef = CCcoef)
      rho.test <-  getCanCorMulti(X = folddata[[CVidx]][["X_test"]],
                                Y = as.matrix(folddata[[CVidx]][["Y_test"]]),
                                CCWeight = Ws,
                                CCcoef = CCcoef)

      # store cv result
      RhoTrain[idx] <- round(rho.train, digits = 5)
      RhoTest[idx] <- round(rho.test, digits = 5)
      DeltaCor[idx] <- abs(rho.train - rho.test)

    }

    # record prediction errors for given CV fold and all sparsity penalty
    # options.
    CVResult[[CVidx]] <- cbind(RhoTrain, RhoTest, DeltaCor)
}
```

2.2.4 Extract penalty pair with the smallest total prediction error

Finally, we extract the total prediction errors (Figure 2, Step I.3) and conclude the best penalty pair as the pair with the smallest error (Figure 2, Step I.4). In this step, we want to minimized the scaled prediction error, which is defined as:

$$scaledPredErr = \frac{|trainCC - testCC|}{|testCC|} \quad (2)$$

,where $trainCC$ and $testCC$ is defined as the training canonical correlation and testing canonical correlation respectively. Below is the aggregated cross-validation evaluation result:

```
# aggregate CV result and select the best penalty term
AggregatedCVResult <- Reduce("+", CVResult) / length(CVResult)
# calculate the evaluation metric of interest
EvalMetric <- apply(AggregatedCVResult, 1, function(x) {x[3]/abs(x[2])})
# determine the best CV result
optIdx <- which.min(EvalMetric)
```

We can visualize the scaled prediction errors with a contour plot (Figure 3), note that this method only works for two omics data, if more than two omics data are used, please consider constrcut contour plot for each pair of omics data respectively.

```
library(plotly)
library(reshape2)
f1 <- list(
  family = "Arial, sans-serif",
  size = 20,
  color = "black"
)
f2 <- list(
  family = "Old Standard TT, serif",
  size = 20,
  color = "black"
)
a <- list(
  title = "l1",
  titlefont = f1,
  showticklabels = TRUE,
  tickfont = f2
)
b <- list(
  title = "l2",
  titlefont = f1,
  showticklabels = TRUE,
  tickfont = f2
)
# create melt data
PenExpandMelt <- cbind(PenExpand[,c(1,2)], EvalMetric)
colnames(PenExpandMelt)[c(1,2)] <- c('l1', 'l2')
hmelt <- melt(PenExpandMelt, id.vars = c("l1", "l2"))
contourPlot <- plot_ly(hmelt, x = ~l1, y = ~l2, z = ~value,
                       type = "contour") %>%
  layout(xaxis = a, yaxis = b, showlegend = TRUE, legend = f1)
# orca preinstalltion is required for next step:
# https://github.com/plotly/orca#installation
contourPlot
```

For the synthetic data set, the optimal penalty pair that gives the smallest prediction error is $(l_1, l_2) = (0.1, 0.1)$.

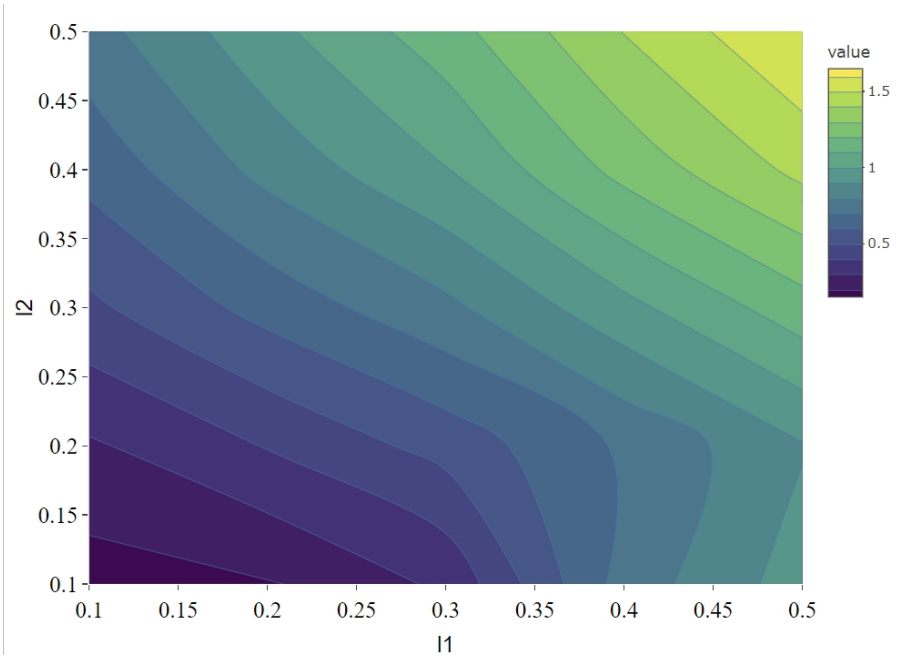

Figure 3: Total scaled prediction error contour plot for evaluation metric (defined as prediction error/test cc). The x- and y-axes indicate LASSO penalties considered for mRNA and miRNA, respectively. Blue to yellow scale indicates increasing total scaled prediction error.

```
# combine CV evaluation result with penalty candidates
overallCVInfo <- cbind(PenExpand, AggregatedCVResult, scaledPredError = EvalMetric)
# set column names for penalty terms
colnames(overallCVInfo)[1:num_omics] <- paste0('l',1:num_omics)
# save overall CV result
write.csv(overallCVInfo, file = paste0(CVDir, 'overallCVInfo.csv'),
          row.names = FALSE)
# print out the best CV penalty pair and associated result
print(overallCVInfo[optIdx,])
```

Table 1 shows the total prediction error (scaledPredError) for all penalty options. Note that in this example, we are only including 25 optional penalty pairs. The fourth column (RhoTest) records the aggregated canonical correlations for the test data set.

2.3 Step II: Integrate two omics data types and a quantitative phenotype

With a pre-selected penalty pair, we apply SmCCA to subsampled features of X_1, X_2 and Y, and repeat the process to generate a robust similarity matrix (Figure 1, Step II). As for the number of subsamples, a larger number of subsamples leads to more accurate results, while a smaller number of subsamples is faster computationally. We use 50 in this example. In general, we recommend to subsample 500-1000 times or more.

```
# run SmCCA on the subsamples (Figure 1, Step II)
Ws <- getRobustWeightsMulti(X,
```

Table 1: Total Prediction Error from a 3-fold CV for the synthetic dataset

l1	l2	RhoTrain	RhoTest	DeltaCor	scaledPredError
0.1	0.1	2.143180	2.021583	0.3073917	0.1520549
0.2	0.1	2.298900	1.937143	0.3617541	0.1867462
0.3	0.1	2.371103	1.802180	0.5689212	0.3156850
0.4	0.1	2.226837	1.281533	0.9453013	0.7376331
0.5	0.1	2.257677	1.161217	1.0964606	0.9442343
0.1	0.2	2.439183	1.903217	0.5359673	0.2816113
0.2	0.2	2.553920	1.827470	0.7264469	0.3975151
0.3	0.2	2.606787	1.708473	0.8983147	0.5257997
0.4	0.2	2.641467	1.553353	1.0881148	0.7004941
0.5	0.2	2.662887	1.420033	1.2428491	0.8752253
0.1	0.3	2.603863	1.770733	0.8331298	0.4704999
0.2	0.3	2.699880	1.663637	1.0362454	0.6228796
0.3	0.3	2.738233	1.560693	1.1775418	0.7544992
0.4	0.3	2.760453	1.420607	1.3398453	0.9431501
0.5	0.3	2.773497	1.303357	1.4701370	1.1279621
0.1	0.4	2.703797	1.665173	1.0386261	0.6237345
0.2	0.4	2.787583	1.537180	1.2504062	0.8134416
0.3	0.4	2.813273	1.427817	1.3854583	0.9703334
0.4	0.4	2.827043	1.284917	1.5421291	1.2001783
0.5	0.4	2.831297	1.185093	1.6461995	1.3890885
0.1	0.5	2.763300	1.581297	1.1820000	0.7474878
0.2	0.5	2.845553	1.459457	1.3860930	0.9497322
0.3	0.5	2.866427	1.346777	1.5196518	1.1283621
0.4	0.5	2.872273	1.213000	1.6592727	1.3679082
0.5	0.5	2.870893	1.121857	1.7490378	1.5590564

```
                    Trait = as.matrix(Y),
                    NoTrait = FALSE,CCcoef = scalingFactor,
                    Lambda = as.numeric(overallCVInfo[optIdx,1:num_omics]),
                    s = s,
                    SubsamplingNum = SubsamplingNum)
```

2.4 Step III: Obtain multi-omics modules and plot subnetworks

From the similarity matrix obtained in the last step, we can get multi-omics modules by applying hierarchical tree cutting and plotting the reconstructed networks (Figure 1). The edge signs are recovered from pairwise feature correlations.

```
# construct adjacency matrix
Abar <- getAbar(Ws, FeatureLabel = AbarLabel)
# perform clustering based on the adjacency matrix Abar
OmicsModule <- getOmicsModules(Abar, PlotTree = FALSE)
save(Ws, Abar, OmicsModule, file = paste0(CVDir, "SmCCNetWeights.RData"))
```

In the following example, the summarization used is the first 3 NetSHy principal component. After clustering, there are 6 network modules, with only 1 network module have more than 10 nodes. The optimal network size after network pruning is 18. The output from this steps contains a network adjacency matrix, summarization scores (first 3 NetSHy PCs), PC loadings and more, which are stored in a .Rdata file in the user specified location. The details about network pruning algorithm can be found in single-omics SmCCNet vignette.

```
# make sure there are no duplicated labels
AbarLabel <- make.unique(AbarLabel)

# create concatenate omics data for network pruning
X_big <- cbind(X1,X2)

# calculate feature correlation matrix
bigCor2 <- cor(X_big)

# data type
types <- c(rep('gene', ncol(X1)), rep('mirna', ncol(X2)))


# filter out network modules with insufficient number of nodes
module_length <- unlist(lapply(OmicsModule, length))
network_modules <- OmicsModule[module_length > 10]
# extract pruned network modules
for(i in 1:length(network_modules))
{
  cat(paste0('For network module: ', i, '\n'))
  # define subnetwork
  abar_sub <- Abar[network_modules[[i]],network_modules[[i]]]
  cor_sub <- bigCor2[network_modules[[i]],network_modules[[i]]]
  # prune network module
  networkPruning(Abar = abar_sub,CorrMatrix = cor_sub,
                           type = types[network_modules[[i]]],
                 data = X_big[,network_modules[[i]]],
               Pheno = Y, ModuleIdx = i, min_mod_size = 10,
                           max_mod_size = 100, method = 'NetSHy',
                           saving_dir = CVDir)
}
```

3 Results

We present the single-omics network result based on the synthetic data. The first table below contains the individual molecular features correlation with respect to phenotype, and their associated p-value (from correlation testing).

Below is the visualization of the PC loadings based on the first NetSHy PC, which represents the contribution of each molecular features to the first NetSHy PC.

In addition, there are two network heatmaps based on (1) correlation matrix, and (2) adjacency matrix. Based on the summarization table, genes 1,2,6,7, and miRNA 2 have relatively high correlation with respect to phenotype. The PC loadings also confirm that genes 6,7, and miRNA 2 and generally have higher correlation with respect to phenotype. From the

Table 2: Individual molecular features correlation table with respect to phenotype (correlation and p-value).

Molecular Feature	Correlation to Phenotype	P-value
Gene_1	0.3828730	0.0000000
Gene_2	0.3411378	0.0000000
Gene_5	0.1310036	0.0131111
Gene_6	0.6284530	0.0000000
Gene_7	0.6531262	0.0000000
Gene_9	0.0774726	0.1434883
Gene_10	0.1201825	0.0229506
Gene_74	-0.1401523	0.0079155
Gene_88	0.1234406	0.0194714
Gene_90	-0.0752956	0.1551147
Gene_123	-0.0703372	0.1842334
Gene_170	0.1007365	0.0568835
Gene_216	-0.0946349	0.0737229
Gene_219	0.1158386	0.0284166
Gene_367	0.1499230	0.0044714
Gene_391	-0.0570075	0.2820460
Gene_485	0.1176494	0.0260153
Mir_2	-0.3474835	0.0000000

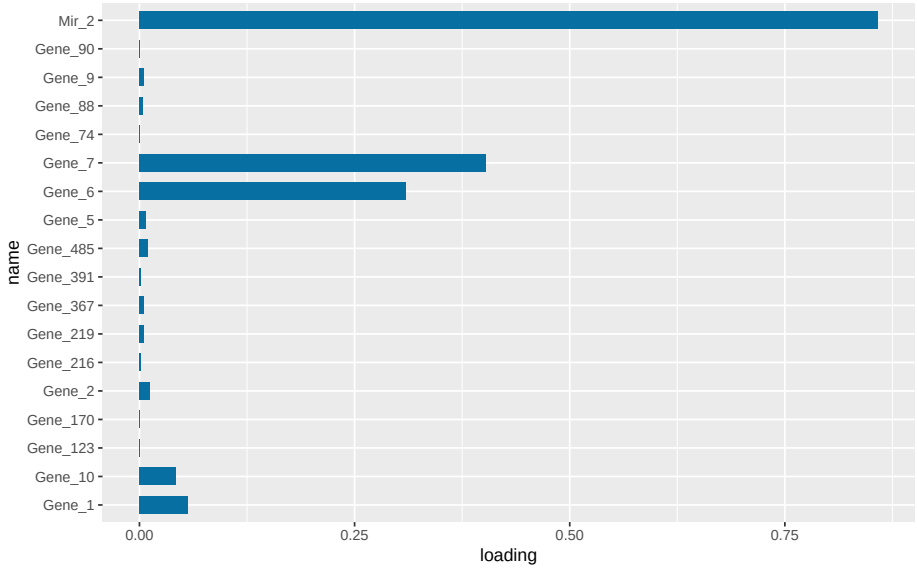

Figure 4: PC1 loading for each subnetwork feature.

correlation heatmap, we do not observe associations between molecular features, but for the adjacency matrix heatmap, we observe the higher connections between genes 367,219 and miRNA 2.

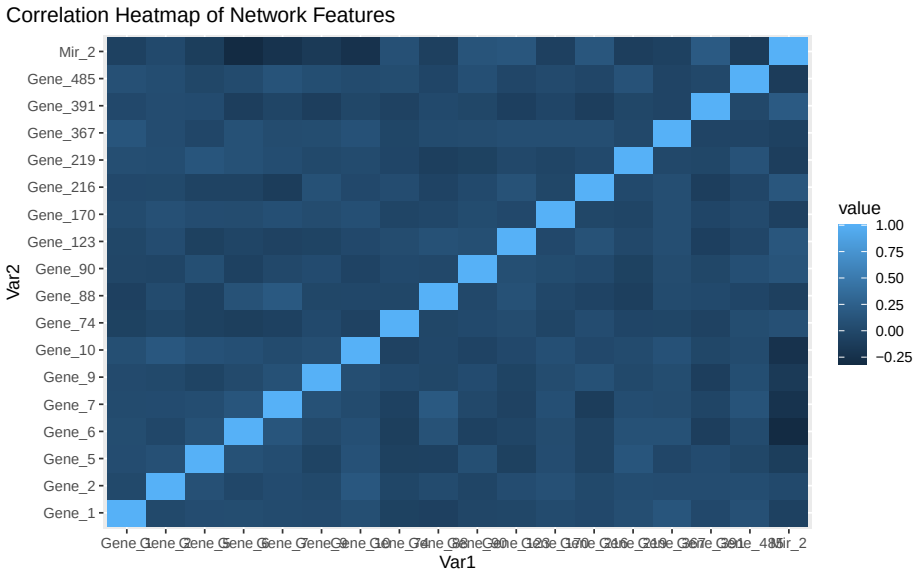

Figure 5: Correlation heatmap for subnetwork features.

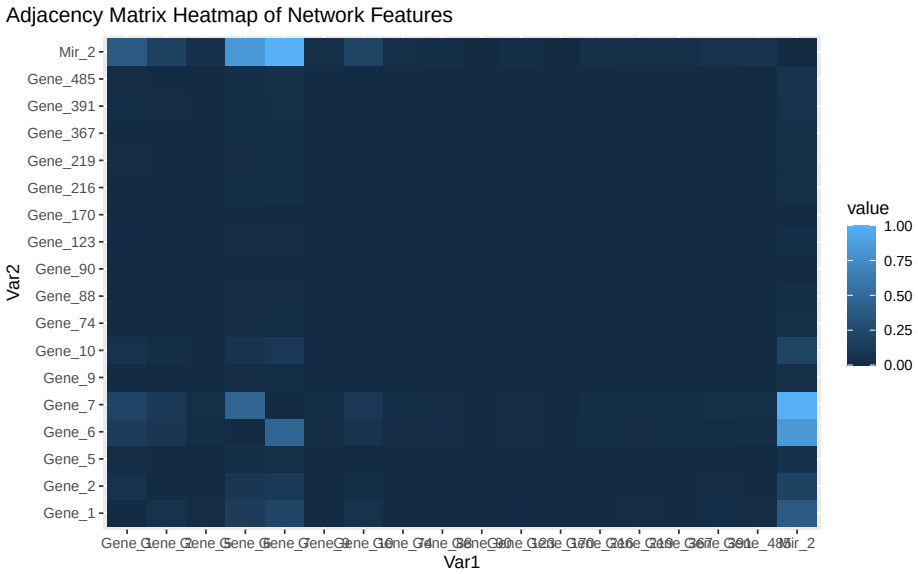

Figure 6: Adjacency matrix heatmap for subnetwork features.

3.1 Step VI: Visualize network module

To visualize the final network module, we need to download the Cytoscape software (Shannon et al., 2003), and use the package RCy3 to visualize the subnetwork generated from the network pruning step. In general, since the network obtained through the PageRank pruning algorithm is densely connected, and some of the edges may be false positive (meaning that two nodes are not associated, but with higher edge values in the adjacency matrix). Therefore, we use the correlation matrix to filter out those weak network edges.

In the network visualization, different colored edges denote different directions of the association between two nodes, where red or blue denotes a positive or negative association respectively. The width of the edge represents the connection strength between two nodes.

```
library(RCy3)
library(igraph)
M <- as.matrix(M)
correlation_filter <- 0.05
# correlation matrix filtering for the subnetwork edge-cut
filter_index <- which(abs(correlation_sub) < correlation_filter)
M_ind <- ifelse(correlation_sub > 0, 1, -1)
M_adj <- M * M_ind
M_adj[filter_index] <- 0
diag(M_adj) <- 0

# network visualization through cytoscape
graph <- igraph::graph_from_adjacency_matrix(M_adj, mode = 'undirected',
         weighted = TRUE, diag = TRUE, add.colnames = NULL, add.rownames = NA)

# define network node type and connectivity and use them in cytoscape
V(graph)$type <- sub_type
V(graph)$type
V(graph)$connectivity <- rowSums(abs(M))
V(graph)$connectivity
# export subnetwork to Cytoscape
createNetworkFromIgraph(graph,"multi_omics_network")
```

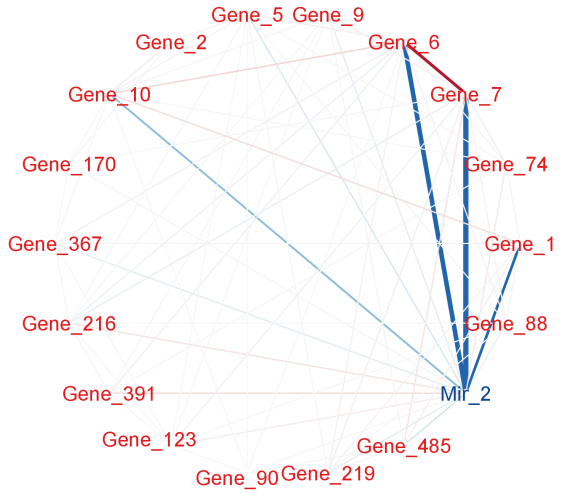

Figure 7: Pruned module 1. The strength of the node connections is indicated by the thickness of edges. Red edges and blue edges are for negative and positive connections respectively. Red node represents genes, and blue node represent miRNAs.

4 Methods for Optimal Scaling Factors Selection

As shown above, the scaling factors must be supplied to prioritize correlation structure of interest. However, it is not always straightforward to determine the scaling factors. In the section below, we provide 3 different methods to select the scaling factors of interest.

4.1 Method 1: Prioiritize Omics-Phenotype Correlation

A common choice for the scaling factors is to prioritize the omics-phenotype correlation. For instance, in multi-omics quantitative phenotype analysis with 2 omics data, let a be the scaling factor for omics-omics correlation, b and c be the scaling factors for omics-phenotype correlation, then a common choice of the scaling factor (a, b, c) is $(1, 10, 10)$. This can be done through manual entry with interactive function 'scalingFactorInput()', and the example is given in section 2.2.2.

4.2 Method 2: Automated SmCCNet Selection Based on Pairwise Correlation.

Another method is to perform the pairwise correlation check between each omics pair. We ran sparse caonical correlation analysis with the most stringent penalty pair $(0.1, 0.1)$, and calculate the canonical correlation. The canonical correlation calculated will be treated as the between-omics scaling factor, while scaling factor of 1 will be used for omics-phenotype relationship. In addition, we introduced another parameter called shrinkage factor to prioritize either omics-omics relationship or omics-phenotype relationship. For instance, in a multi-omics analysis with two omics data, if omics-omics correlation is 0.8, and the shrinkage parameter is 2, then the final scaling factors are set to $(a, b_1, b_2) = c(0.4, 1, 1)$. This method is currently only implemented in **fastAutoSmCCNet()**. If user want to use this method, please refer to the automated SmCCNet vignette.

4.3 Method 3: Scaled SmCCNet analysis

Let's assume that there are two omics data mRNA and miRNA with a quantitative phenotype. We want to systematically evaluate the performance of each pair of scaling factors. The best way is to create a candidate scaling factors set by setting all omics-omics scaling factor to 1, and vary omics-phenotype scaling factor. To scaling factors performance comparable, we scale each set of scaling factors so that they sum up to 1. For instance, with the two omics example, the scaling factor (a, b_1, b_2) should satisfy both $a = 1$ and $a + b_1 + b_2 = 1$. For each set of scaling factors, the sparse penalty parameters (l1, l2) were chosen through a K-fold cross validation to find the penalty pair that minimized the scaled prediction error. All penalty pairs from the set were also tested in a grid search to find the optimal pair (l1, l2). The penalty parameters determine how many miRNA (l1) and mRNA (l2) are in the final results. Scaling factors in the grid search were evaluated to identify which value yielded the best network results with a predefined criterion. Then in the two omics example, the criterion is set to be:

$$(a, b_1, b_2) = \arg\max_{\tilde{a},\tilde{b}_1,\tilde{c}_2} \frac{|trainCC - testCC|}{|testCC|} \quad (3)$$

4.3.1 Cross validation with each set of scaling factors

Below is the parameter setup for the SmCCNet algorithm. Note that it is the same step as the section 2.2:

```
# number of folds in K-fold CV.
K <- 3
N <- nrow(X1)
# create a list of omics data
X <- list(X1, X2)
# feature sampling proportions, 0.9 for miRNA since it has less features.
s <- c(0.7,0.9)
# number of subsamples.
SubsamplingNum <- 50
# number of omics dataset
num_omics <- 2
# tuning parameter candidate length for each omics data
tuneLength <- 5
# tuning parameter candadate range for each omics data
minTune <- 0.1
maxTune <- 0.5
# create empty matrix to store all possible penalty parameters
penSelect <- matrix(0, nrow = tuneLength, ncol = num_omics)
# create sparsity penalty options.
for (Idx in 1:ncol(penSelect))
{
  penSelect[,Idx] <- seq(from = minTune,
                         to = maxTune,
                         length.out = tuneLength)
}
# expand grid
# convert matrix to list of columns
list_cols <- as.list(as.data.frame(penSelect))
# generate all possible combinations
PenExpand <- do.call(expand.grid, list_cols)

# set a CV directory.
CVDir <- "Example3foldCVTune/"
dir.create(CVDir)
```

Same as above, we split the original data into K different folds for evaluation:

```
set.seed(12345) # set random seed.

# split data into folds
X <- lapply(X, scale)
foldIdx <- suppressWarnings(split(1:nrow(X[[1]]), sample(1:nrow(X[[1]]), K)))
folddata <- purrr::map(1:length(foldIdx), function(x){
    Y <- as.matrix(Y)
    X_train <- list()
    X_test <- list()
    Y_train <- list()
    Y_test <- list()
```

```
    for (i in 1:length(X))
    {
      X_train[[i]] <- X[[i]][-foldIdx[[x]],]
      X_test[[i]] <- X[[i]][foldIdx[[x]],]
    }
    Y_train <- Y[-foldIdx[[x]],]
    Y_test <- Y[foldIdx[[x]],]
    return(list(X_train = X_train, X_test = X_test,Y_train = Y_train,
                Y_test = Y_test))
})
# name each fold of data
names(folddata) <- paste0('Fold_', 1:K)
# saving all preliminary data into local directory for reproducibility purpose
save(folddata, s, PenExpand, SubsamplingNum,
       file = paste0(CVDir, "CVData.RData"))
```

Below is the function to set up the tuning grid for scaling factors. There are 3 different arguments in this function:

- $DataType$: The vector with each omics type annotated (in same order as X), followed by 'phenotype'.
- $tuneLength$: The length of candidate scaling factor for each pairwise combination.
- $tuneRangePheno$: A vector of length 2 with the first argument being the minimally possible scaling factor value, and the second argument being the maximally possible scaling factor value.

```
# create a function that set up the scaling factor candidate grids
gridCCcoef <- function(DataType, tuneLength = 5, tuneRangePheno = c(1,10))
{
  # store the length of the data
  datalength <- length(DataType)
  phenotunelength <- datalength - 1
  # create empty matrix for storing the candidate scaling factors
  candCoef <- matrix(1, nrow = tuneLength^phenotunelength,
                     ncol = ncol(utils::combn(datalength, 2)))
  # create storage empty grid
  phenoGrids <- matrix(0, nrow = tuneLength,
                       ncol = phenotunelength)
  # create grids
  for (phenoIdx in 1:phenotunelength)
  {
    phenoGrids[,phenoIdx] <- seq(from = tuneRangePheno[1],
                                 to = tuneRangePheno[2],
                                 length.out = tuneLength)
  }
  # expand grid
  # convert matrix to list of columns
  list_cols <- as.list(as.data.frame(phenoGrids))
  # generate combinations
  phenoGridsExpand <- do.call(expand.grid, list_cols)
  candCoef[,which(utils::combn(datalength,2)[2,] == datalength)] <- as.matrix(
    phenoGridsExpand)
```

```
  # provide column names
  colnames(candCoef) <- apply(utils::combn(datalength,2),2, function(x){
      paste0(DataType[x[1]],'_', DataType[x[2]])
    })
  # scale scaling factors so that each set of scaling factors sum up to 1
  candCoef <- t(apply(candCoef, 1, function(x) {x/sum(x)}))
  return(candCoef)

}

# scaling factor grids
CCcoefMatrix <- gridCCcoef(c('mRNA','miRNA', 'pheno'),
                           tuneLength = 3, tuneRangePheno = c(5,10))


# create data matrix to store the cross-validation result
CVEval <- matrix(0, nrow = nrow(CCcoefMatrix), ncol = num_omics +
                   ncol(utils::combn(num_omics + 1, 2)) + 3)
CVEval[,1:ncol(utils::combn(num_omics + 1, 2))] <- CCcoefMatrix
colnames(CVEval)<- c( paste0('CCcoef:',  colnames(CCcoefMatrix)),
                      paste0('l',1:num_omics), "TrainingCC", "TestCC",
                             "CCPredError")
```

In the example below, with two omics and a quantitative phenotype, we set the $tuneLength$ to 3, with the range of scaling factor to be between 5 and 10. Since there are a large number of candidate scaling factor pairs, to speed up the cross-validation step, the parallel computation can be implemented.

```
for (CCIdx in 1:nrow(CCcoefMatrix))
{
  coef <- CCcoefMatrix[CCIdx,]
  cat(paste0('Now running SmCCA for the scaling factor candidate ',
             CCIdx, '\n'))

  future::plan(future::multisession, workers = K)
  CVResult <- furrr::future_map(1:K, function(CVidx) {
    # set scaling factor
    CCcoef <- coef
    # create empty vector for storing cross-validation result
    RhoTrain <- RhoTest <- DeltaCor <- rep(0, nrow(PenExpand))
    for(idx in 1:nrow(PenExpand))
    {
      # consider one pair of sparsity penalties at a time.
      l <- PenExpand[idx, ]
      # run SmCCA on the subsamples (Figure 1, Step II)
      Ws <- getCanWeightsMulti(folddata[[CVidx]][["X_train"]],
                           Trait = as.matrix(folddata[[CVidx]][["Y_train"]]),
                           Lambda = as.numeric(l), NoTrait = FALSE,
                           CCcoef = CCcoef)
      # evaluate the canonical correlation for training and testing data
      rho.train <-  getCanCorMulti(X = folddata[[CVidx]][["X_train"]],
```

```
                                    Y = as.matrix(folddata[[CVidx]][["Y_train"]]),
                                    CCWeight = Ws,
                                    CCcoef = CCcoef)
          rho.test <-  getCanCorMulti(X = folddata[[CVidx]][["X_test"]],
                                    Y = as.matrix(folddata[[CVidx]][["Y_test"]]),
                                    CCWeight = Ws,
                                    CCcoef = CCcoef)

          # store cv result
          RhoTrain[idx] <- round(rho.train, digits = 5)
          RhoTest[idx] <- round(rho.test, digits = 5)
          DeltaCor[idx] <- abs(rho.train - rho.test)

        }

        # record prediction errors for given CV fold and all sparsity penalty
        # options.
        DeltaCor.all <- cbind(RhoTrain, RhoTest, DeltaCor)
        return(DeltaCor.all)
      },.progress = TRUE,.options = furrr::furrr_options(seed = TRUE))
      cat('\n')
      # aggregate CV result and select the best penalty term
      AggregatedCVResult <- Reduce("+", CVResult) / length(CVResult)
      EvalMetric <- apply(AggregatedCVResult, 1, function(x) {x[3]/abs(x[2])})
      # determine the best CV result
      optIdx <- which.min(EvalMetric)
      # fill in the optimal penalty pair for current scaling scaling
      # factor selection as well as the evaluation result
      CVEval[CCIdx,(ncol(utils::combn(num_omics + 1, 2))+
                  1):ncol(CVEval)] <- c(as.numeric(PenExpand[optIdx,]),
                         as.numeric(AggregatedCVResult[optIdx,]))

      # write out the cross-validation result
      write.csv(CVEval,
                file = paste0(CVDir, "/PredictionError.csv"), row.names = FALSE)
    }
```

4.3.2 Select an optimal scaling factors with its associated penalty parameters

Scaling constants in the grid search were evaluated to identify which value yielded the best network results. How to define a evaluation criterion depends on the interest of study. One simple criterion is to maximize (mean of canonical correlation of test - mean of error of prediction). We might also consider standard deviations of canonical correlations and prediction errors as well.

```
    # read in the overall cv result
    evalResult <- read.csv(paste0(CVDir, "/PredictionError.csv"))
    # find the optn
    evalOptIdx <- which.min(evalResult$CCPredError/abs(evalResult$TestCC))
    # print the optimal result
    evalResult[evalOptIdx,]
```

With the selected parameters of scalineg factors (CCcoef), l1, and l2, we run SmCCNet on the entire dataset as Session 2.3 and identify the networks related to phenotype of interest as session 2.4.

```
# run SmCCA on the subsamples (Figure 1, Step II)
Ws <- getRobustWeightsMulti(X,
                            Trait = as.matrix(Y),
                            NoTrait = FALSE,
                            CCcoef = as.numeric(evalResult[evalOptIdx,
                                     1:ncol(utils::combn(num_omics + 1,2))]),
                            Lambda = as.numeric(evalResult[evalOptIdx,
                                    (ncol(utils::combn(num_omics + 1, 2))
                                     +1):(ncol(utils::combn(num_omics +
                                     1, 2))+num_omics)]), s = s,
                            SubsamplingNum = SubsamplingNum)
```

5 Multi-Omics SmCCNet with Binary Phenotype

Consider $X_1, X_2, ..., X_T$ as T omics datasets, and Y as the phenotype data. Let α and β be two scalars representing the strength of omics-omics and omics-phenotype connections, respectively. The general workflow for multi-omics SmCCNet with binary phenotype is as follows:

1. **Run Weighted/Unweighted Sparse Multiple Canonical Correlation Analysis (SmCCA)**: This is done on $X_1, X_2, ..., X_t$ (excluding phenotype data). The output is canonical weight vectors (with nonzero entries, zero entries are filtered) $\tilde{W}_t \in \mathbb{R}^{p_t^{(sub)} \times 1}, t = 1, 2, ..., T$, which represent the omics-omics connections. In this step, we filter out features that have no connections with other features, which helps reduce dimensionality. Note that we tend to set relaxed penalty terms for this step to include as many omics features as possible to increase the performance of the classifier in the next step.

2. **Subset Omics Data**: Each dataset $X_1, X_2, ..., X_T$ is subsetted to include only omics features selected in Step 1, call subsetted data $X_t(sub) \in \mathbb{R}^{n \times p_t^{(sub)}}$.

3. **Concatenate and Run Sparse PLSDA (Chung and Keles, 2010)**: The subsetted datasets $X_1(sub), X_2(sub), ..., X_T(sub)$ are concatenated into $X(sub) = [X_1(sub), X_2(sub), ..., X_T(sub)] \in \mathbb{R}^{n \times p}, p = \sum_{i=1}^{T} p_i$. The Sparse PLSDA algorithm is then run to extract R latent factors and projection matrix, by default, R is set to 3. Projection matrix is defined as $P \in \mathbb{R}^{p^{(sub)} \times R}$. Latent factors are defined as $r = [r_1, r_2, ..., r_R] = X(sub) \cdot P \in \mathbb{R}^{n \times R}$.

4. **Aggregate Latent Factors**: The R latent factors are aggregated into one using logistic regression, defined by $\text{logit}(Y) = \alpha_1 r_1 + \alpha_2 r_2 + ... + \alpha_R r_R$. Estimate α's and report performance (for instance, accuracy/AUC score). Feature weights is given by aggregation of the projection matrix from Sparse PLSDA $W_t^* = P_t \cdot \alpha \in \mathbb{R}^{p_t^{(sub)} \times 1}, t = 1, 2, ..., T, \alpha = [\alpha_1, \alpha_2, ..., \alpha_r] \in \mathbb{R}^{R \times 1}$, where P_t is the subset of projection matrix P such that it only includes features from the tth omics data.

5. **Normalize and Calculate Final Canonical Weight**: The feature weights $W_1^*, W_2^*, ..., W_T^*$ based on sparse PLSDA are normalized to have an L2 norm of 1. The final canonical weight is obtained by weighted combining the canonical weight from step 1 and the feature weight from classifier $W_t = \frac{a}{a+b}\tilde{W}_t + \frac{b}{a+b}W_t^*, t = 1, 2, ..., T$.
6. **Construct Multi-Omics Network and Perform Network Pruning**: A multi-omics network is constructed and pruned (same as multi-omics SmCCNet with quantitative phenotype).

Note that Steps 1-3 are repeated multiple times with different penalty pairs to select the best pair. The evaluation metric is maximizing the classification evaluation metric such as testing prediction accuracy or testing AUC score. Currently there are 5 different evaluation metrics to choose from: accuracy, AUC score, precision, recall, and F1 score. For instance, if the phenotype is patient's smoking status, then below is the guidence on how to choose the best evaluation metric in this context:

- **Prediction Accuracy:** Use accuracy when both smokers and non-smokers are almost even in number and equally important. It is a method that gives the percentage of all correct predictions out of all patients.
- **AUC Score:** The AUC evaluates the model's ability in distinguishing smokers from non-smokers across varying thresholds when the predicted probability of patient's smoking status is given. It demonstrate the effectiveness in detecting smokers versus non-smokers.
- **Precision:** Precision is used when misclassifying a non-smoker as a smoker has high consequences. It indicates the percentage of true smokers among those predicted as smokers.
- **Recall:** Recall is used when it's important to correctly classify as many smokers as possible, even at the risk of misclassifying some non-smokers. It tells the percentage of actual smokers the model correctly spotted.
- **F1 Score:** The F1 Score is the harmonic mean of precision and recall, ideal for uneven class distributions. We can think of it as the perfect blend of precision and recall in one number.

5.1 Synthetic dataset

As an example, we consider a synthetic data set with 500 genes (X_1) and 100 miRNAs (X_2) expression levels measured for 358 subjects, along with a binary phenotype created by binarizing quantitative phenotype (Y) based on median.

```
data(ExampleData)
head(X1[ , 1:6])
##          Gene_1   Gene_2   Gene_3   Gene_4   Gene_5   Gene_6
## Samp_1 22.48570 40.35372 31.02575 20.84721 26.69729 30.20545
## Samp_2 37.05885 34.05223 33.48702 23.53146 26.75463 31.73594
## Samp_3 20.53077 31.66962 35.18957 20.95254 25.01883 32.15723
## Samp_4 33.18689 38.48088 18.89710 31.82330 34.04938 38.79989
## Samp_5 28.96198 41.06049 28.49496 18.37449 30.81524 24.00454
## Samp_6 18.05983 29.55471 32.54002 29.68452 26.19996 26.76684
head(X2[ , 1:6])
##           Mir_1    Mir_2    Mir_3    Mir_4    Mir_5     Mir_6
## Samp_1 15.22391 17.54583 15.78472 14.89198 10.34821  9.689755
```

```
## Samp_2 16.30697 16.67283 13.36153 14.48855 12.66090 11.333613
## Samp_3 16.54512 16.73501 14.61747 17.84527 13.82279 11.329333
## Samp_4 13.98690 16.20743 16.29308 17.72529 12.30056  9.844108
## Samp_5 16.33833 17.39387 16.39792 15.85373 13.38767 10.599414
## Samp_6 14.54110 16.51999 14.73958 15.87504 13.21359 10.922393
# binarize phenotype variable
Y <- ifelse(Y > median(Y), 1, 0)
head(Y)
##        Pheno
## Samp_1     0
## Samp_2     1
## Samp_3     0
## Samp_4     1
## Samp_5     1
## Samp_6     0
```

Denote the number of features in $X_1 \& X_2$ as $p_1 \& p_2$ respectively, and the number of subjects as n.

```
p1 <- ncol(X1)
p2 <- ncol(X2)
n <- nrow(X1)
AbarLabel <- c(colnames(cbind(X1, X2)))
```

Although SmCCNet does not require normality, it calculates the Pearson correlation between linear combinations of omics features and the phenotype, which assumes finite variances and finite covariance. It is necessary to include a transformation if the data are skewed. The algorithm also requires the data to be standardizable (i.e. none of the data matrices include a column with zero variance.)

5.2 Step I: Determine optimal sparsity penalties through CV

All the parameters set up are the same as multi-omics with quantitative phenotype except that there is an extra penalty term to evaluate: lasso penalty for SPLSDA classifier.

```
# number of folds in K-fold CV.
K <- 3
N <- nrow(X1)
# create a list of omics data
X <- list(X1, X2)
# feature sampling proportions, 0.9 for miRNA since it has less features.
s <- c(0.7,0.9)
# number of component for PLS
ncomp <- 3
# number of subsamples.
SubsamplingNum <- 50
# number of omics dataset
num_omics <- 2
# tuning parameter candidate length for each omics data
tuneLength <- 5
# tuning parameter candadate range for each omics data
```

```
minTune <- 0.1
maxTune <- 0.5
# create empty matrix to store all possible penalty parameters
penSelect <- matrix(0, nrow = tuneLength, ncol = num_omics)
# set up the evaluation metric (choose between 'accuracy', 'auc', 'precision',
# 'recall', 'f1')
metric <- 'auc'
# create sparsity penalty options.
for (Idx in 1:ncol(penSelect))
{
  penSelect[,Idx] <- seq(from = minTune,
                         to = maxTune,
                         length.out = tuneLength)
}
# combine with penalty term for classifier
penSelect <- cbind(penSelect, seq(from = 0.5,
                         to = 0.9,
                         length.out = tuneLength))
# expand grid
# convert matrix to list of columns
list_cols <- as.list(as.data.frame(penSelect))
# generate all possible combinations
PenExpand <- do.call(expand.grid, list_cols)

# set a CV directory.
CVDir <- "Example3foldCVBinary/"
dir.create(CVDir)
```

5.2.1 Create test and training data sets.

Same as before, we need to split the data (X_1, X_2, Y) into test and training sets (Figure 2, Step I.1). Here we scale the complete dataset rather than each fold to increase the performance in predicting the testing phenotype:

```
set.seed(12345) # set random seed.

# split data into folds
X <- lapply(X, scale)
foldIdx <- suppressWarnings(split(1:nrow(X[[1]]), sample(1:nrow(X[[1]]), K)))
folddata <- purrr::map(1:length(foldIdx), function(x){
    Y <- as.matrix(Y)
    X_train <- list()
    X_test <- list()
    Y_train <- list()
    Y_test <- list()
    for (i in 1:length(X))
    {
      X_train[[i]] <- X[[i]][-foldIdx[[x]],]
      X_test[[i]] <- X[[i]][foldIdx[[x]],]
    }
    Y_train <- Y[-foldIdx[[x]],]
```

```
    Y_test <- Y[foldIdx[[x]],]
    return(list(X_train = X_train, X_test = X_test,Y_train = Y_train,
                Y_test = Y_test))
})
# name each fold of data
names(folddata) <- paste0('Fold_', 1:K)
# saving all preliminary data into local directory for reproducibility purpose
save(folddata, s, PenExpand, SubsamplingNum,
       file = paste0(CVDir, "CVData.RData"))
```

5.2.2 Set Scaling Factors

It is important to set scaling factors for each pairwise correlation to prioritize correlation structure(s) of interest. If two omics data are used, there is only one scaling factor for between-omics canonical correlation analysis, then it is natural to set the scaling factor to 1:

```
scalingFactor <- 1
```

If more than two omics data are used, then Below is the function that use prompt to help user define the scaling factor intended for the analysis, note that different from quantitative phenotype, phenotype should be excluded from the scaling factor input:

```
scalingFactor <- scalingFactorInput(c('mRNA', 'miRNA'))
```

5.2.3 Run K-fold CV

Same as multi-omics with quantitative phenotype, cross-validation should be run to determine the best penalty terms combinations. The difference here is that since this is a predictive modeling problem, the evaluation metric used should be based on the testing data, not the comparison between training and testing. In the example here, we use the testing AUC score to evaluate the model performance.

```
# create an empty list to store the cv result
CVResult <- list()
# load cross-validation data
load(paste0(CVDir, "CVData.RData"))
for (CVidx in 1:K)
{
  CCcoef <- scalingFactor
  TrainScore <- TestScore <- rep(0, nrow(PenExpand))
  for(idx in 1:nrow(PenExpand)){
    # consider one pair of sparsity penalties at a time.
    l <- PenExpand[idx, ]
    # run multi-block PLS
    CCcoef <- scalingFactor
    # run multi-block PLS
    suppressMessages(projection <- getRobustWeightsMultiBinary(
                                    folddata[[CVidx]][["X_train"]],
                                    as.numeric(folddata[[CVidx]][["Y_train"]]),
                                    SubsamplingPercent=c(1,1),
                                    Between_Discriminate_Ratio = c(1,1),
```

```
                                    LambdaBetween = l[1,1:num_omics],
                                    LambdaPheno = l[1,(num_omics + 1)],
                                    SubsamplingNum = 1,
                                    CCcoef = CCcoef,
                                    ncomp_pls = ncomp, EvalClassifier = TRUE,
                                    testData = folddata[[CVidx]][["X_test"]]))



        # create training and testing data, and fit logistic regression model
        train_data <- data.frame(x = projection[[1]],
                                 y = as.factor(folddata[[CVidx]][["Y_train"]]))
        test_data <- data.frame(x =  projection[[2]])

        # catching error when performing the logistic regression
        has_error <- FALSE
        suppressWarnings(
        tryCatch({
            # fit logistic regression model
            logisticFit <- stats::glm(y ~ ., family = 'binomial', data = train_data)
            # make prediction for train/test set
            train_pred <- stats::predict(logisticFit, train_data, type = 'response')
            test_pred <- stats::predict(logisticFit, test_data, type = 'response')
            train_score <- classifierEval(obs = folddata[[CVidx]][["Y_train"]],
                                          pred = train_pred,
                                          EvalMethod = metric, print_score = FALSE)
            test_score <- classifierEval(obs = folddata[[CVidx]][["Y_test"]],
                                          pred = test_pred,
                                          EvalMethod = metric, print_score = FALSE)
              },
            error = function(e) {
              cat("Caught an error:", e$message, "\n")
              has_error <- TRUE
            })
            )

            TrainScore[idx] <- round(train_score, digits = 5)
            TestScore[idx] <- round(test_score, digits = 5)


        }

        # record prediction errors for given CV fold and all sparsity penalty
        # options.
        CVResult[[CVidx]] <- cbind(TrainScore, TestScore)
    }
```

5.2.4 Extract penalty terms with the highest testing evaluation score

Finally, we extract the total prediction score and conclude the best penalty pair as the pair with the highest prediction score (in the example's context, AUC score).

```
# aggregate CV result and select the best penalty term
AggregatedCVResult <- Reduce("+", CVResult) / length(CVResult)
# determine the best CV result
optIdx <- which.max(AggregatedCVResult[,2])
```

In the last step, we store the aggregated cross-validation result into the local directory:

```
# combine CV evaluation result with penalty candidates
overallCVInfo <- cbind(PenExpand, AggregatedCVResult)
# set column names for penalty terms for omics
colnames(overallCVInfo)[1:num_omics] <- paste0('l',1:num_omics)
# set column names for penalty terms for classifier
colnames(overallCVInfo)[num_omics+1] <- paste0('lpheno')
# save overall CV result
write.csv(overallCVInfo, file = paste0(CVDir, 'overallCVInfo.csv'),
          row.names = FALSE)
# print out the best CV penalty pair and associated result
print(overallCVInfo[optIdx,])
```

5.3 Step II: Integrate two omics data types and a quantitative phenotype

Same as above, with a pre-selected penalty pair, we apply our method to subsampled features of X_1, X_2 and Y, and repeat the process to generate a robust similarity matrix. Note that in addition to the subsampling, we need to add a between discriminant ratio to control for the trade-off between omics-omics relationship and omics-phenotype relationship presented in the subsequent adjacency matrix. The larger the second number is, the more emphasize is on the omics-phenotype correlation, and vice versa.

```
# run SPLSDA on the subsamples
Ws <- getRobustWeightsMultiBinary(X,
            as.numeric(Y),
            SubsamplingPercent=s,
            Between_Discriminate_Ratio = c(1,1),
            LambdaBetween = as.numeric(overallCVInfo[optIdx,1:num_omics]),
            LambdaPheno = as.numeric(overallCVInfo[optIdx,num_omics + 1]),
            SubsamplingNum = SubsamplingNum,
            CCcoef = scalingFactor,
            ncomp_pls = ncomp, EvalClassifier = FALSE)
```

The rest of the network analysis step is the same as the multi-omics SmCCNet with quantitative phenotype, please refer to section 2.3 and 2.4 for further information. Note that since the cross-validtion penalty terms evaluation with binary phenotype doesn't involve the the participation of canonical weight from step 1, there is no need to tune scaling factors with the method 3 above in section 4.3. However, method 1 and 2 (section 4.1 and 4.2) can be used to determine the scaling factors for between-omics relationship if there are more than 2 omics data.

6 Session info

```
sessionInfo()
## R version 4.2.2 (2022-10-31 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 22621)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_United States.utf8
## [2] LC_CTYPE=English_United States.utf8
## [3] LC_MONETARY=English_United States.utf8
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.utf8
##
## attached base packages:
## [1] grid      stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] reshape2_1.4.4   shadowtext_0.1.2 forcats_0.5.2    stringr_1.4.1
##  [5] dplyr_1.0.10     purrr_0.3.5      readr_2.1.3      tidyr_1.2.1
##  [9] tibble_3.1.8     ggplot2_3.4.0    tidyverse_1.3.2  SmCCNet_1.1.0
## [13] furrr_0.3.1      future_1.33.0    igraph_1.3.5     Matrix_1.5-1
## [17] pbapply_1.7-0    PMA_1.2.1        BiocStyle_2.26.0
##
## loaded via a namespace (and not attached):
##  [1] httr_1.4.4          jsonlite_1.8.4      modelr_0.1.10
##  [4] assertthat_0.2.1    BiocManager_1.30.19 googlesheets4_1.0.1
##  [7] cellranger_1.1.0    yaml_2.3.6          globals_0.16.1
## [10] pillar_1.8.1        backports_1.4.1     lattice_0.20-45
## [13] glue_1.6.2          digest_0.6.30       rvest_1.0.3
## [16] colorspace_2.0-3    plyr_1.8.7          htmltools_0.5.4
## [19] pkgconfig_2.0.3     broom_1.0.1         listenv_0.8.0
## [22] haven_2.5.1         bookdown_0.33       scales_1.2.1
## [25] tzdb_0.3.0          timechange_0.1.1    googledrive_2.0.0
## [28] farver_2.1.1        generics_0.1.3      ellipsis_0.3.2
## [31] withr_2.5.0         cli_3.4.1           crayon_1.5.2
## [34] magrittr_2.0.3      readxl_1.4.1        evaluate_0.18
## [37] fs_1.5.2            fansi_1.0.3         parallelly_1.36.0
## [40] xml2_1.3.3          tools_4.2.2         hms_1.1.2
## [43] gargle_1.2.1        lifecycle_1.0.3     munsell_0.5.0
## [46] reprex_2.0.2        compiler_4.2.2      tinytex_0.42
## [49] rlang_1.0.6         rstudioapi_0.14     labeling_0.4.2
## [52] rmarkdown_2.18      gtable_0.3.1        codetools_0.2-18
## [55] DBI_1.1.3           R6_2.5.1            lubridate_1.9.0
## [58] knitr_1.40          fastmap_1.1.0       utf8_1.2.2
## [61] stringi_1.7.8       Rcpp_1.0.9          parallel_4.2.2
## [64] vctrs_0.5.0         dbplyr_2.2.1        tidyselect_1.2.0
```

```
## [67] xfun_0.34
warnings()
```

7 References

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