Strategies to Analyze Artificial Neural Network Models of Metabolomics Data

Danhui Wang[1], Peyton Greenwood[1], Matthias S. Klein[1]* (klein.663@osu.edu)

[1]Department of Food Science and Technology, The Ohio State University, Columbus, Ohio, USA

[2]Department of Food Science, University of Massachusetts, Amherst, MA, USA

INTRODUCTION

Artificial neural networks (ANN) are emerging as powerful new tools in metabolomics. ANN can be used to predict group membership of unknown samples by applying a set of mathematical functions (neurons) to the metabolomics feature intensities (Fig. 1).

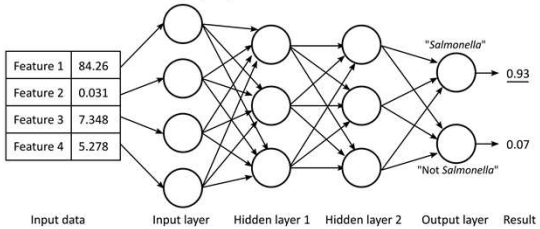

Fig. 1. ANN network model, using 4 features as input, 2 hidden layers with 3 neurons each for calculations, and 2 possible outcome neurons ("*Salmonella*" or "Not *Salmonella*"). In this example, "Salmonella" is predicted as it has the highest value.

However, interpretation of ANN is challenging since model parameters are hard to visualize, effectively turning ANN models into "black boxes". In metabolomics, it is of high interest to identify features of importance for the observed group differences. Also, translation into clinical practice requires targeting a small number of metabolites rather than relying on expensive metabolomics platforms to measure hundreds or thousands of features. The lack of methods to identify features of interest is hampering the use of powerful ANN approaches in metabolomics studies.

AIM

This study aims at creating methods to identify features of importance in ANN classifiers of metabolomics data sets.

METHODS

Dataset. 1D ^{1}H NMR spectra were acquired for various microbial strains after growth in medium, binned[1], and then used to train a predictive ANN model of 2x800 neurons using R packages keras and tensorflow (Fig. 2).[2]

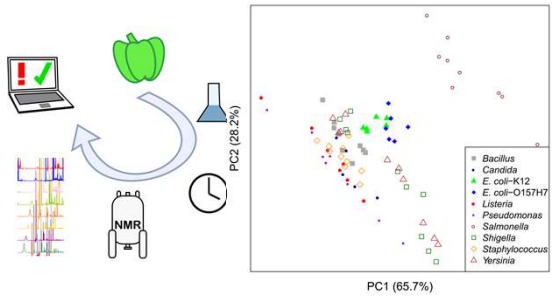

Fig. 2. Left: Workflow of microbe detection, including food sampling, growth in medium, NMR analysis, and ANN prediction. Right: PCA of 1D ^{1}H spectra of microbial medium

For each microbial strain, NMR spectra were screened for signals significantly changing during microbial growth using two-tailed unpaired one-sample t-tests. p-values were corrected using false discovery rate controlling (FDR) at the 20% level.

Algorithm development. We developed a new metric score of feature importance in ANN predictions named the **Feature Impact Assessment (FIA)** score. Fig. 3 shows the workflow of calculating FIA scores from an ANN model and a corresponding real-life dataset. In a sample that is correctly predicted by the ANN, one feature is first replaced by its 1% percentile and then by its 99% percentile. If prediction outcomes change, this feature gets a "raw" score of 1. This is repeated for all features. Next, combinations of two features are checked, and features changing the prediction are assigned a raw score of 2. This is repeated for combinations of three, four, etc. features. FIA scores are then calculated as the sum of the lowest raw score of the respective metabolite and a value between 0 ("in all samples") and 0.999 ("in 0.1% of samples") so that lower FIA scores indicate higher importance. FIA scores can take values between 1 and the number of features of the respective data; e.g., FIA=1.0 or FIA=4.33.

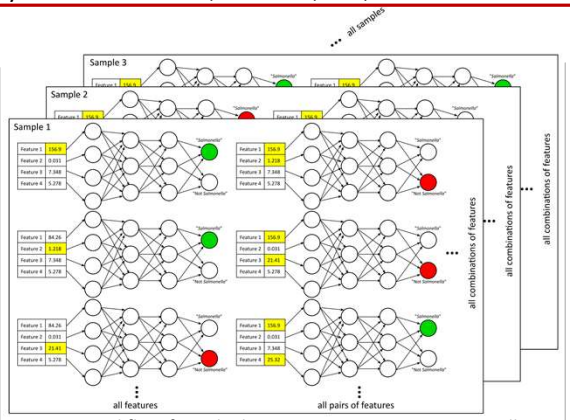

Fig. 3. Workflow for calculating FIA scores. Features in yellow were replaced by their 99% percentile, green indicates correct prediction, red indicates incorrect prediction. In this example, Feature 3 has FIA=1.0 (can change prediction on its own in all samples), while Feature 2 has FIA=2.8 (can change prediction in combinations of 2 features in 20% of samples).

RESULTS AND DISCUSSION

FIA scores were calculated for an ANN model of microbial metabolism. We hypothesized that features with low FIA scores should also be of higher statistical significance. Fig. 4A compares p-values of features with FIA<4 to all other features. FIA<4 was connected to lower p-vales in all groups, highlighting that the FIA algorithm indeed identified features of high significance. Modeling the number of scores <4 vs. number of significant features showed an inverse correlation (Fig. 4B). Groups with few significant p-values are poorly defined and it makes sense that many different features can disrupt predictions in such cases. Strong positive correlations between the mean of the top 100 FIA scores and the number of significant p-values (Fig. 2C) indicates that lower averages of the FIA top 100 are found in less well-defined groups. In this way, analysis of FIA scores may provide additional information about how well-defined a group is regarding metabolite signatures.

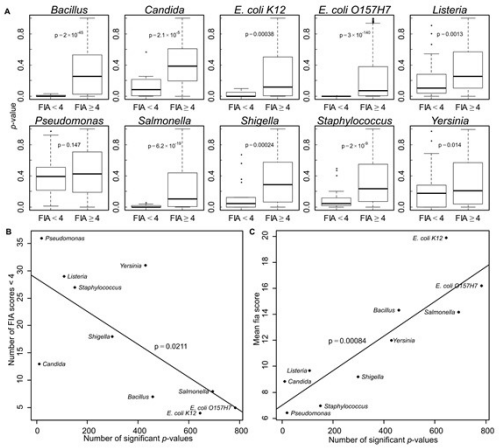

Fig. 4. Relationship of FIA scores to statistical scores

CONCLUSIONS

FIA scores are a new numeric score to assess feature importance in ANN. Analyses of microbial metabolism data showed that low FIA scores are connected to high statistical significance. FIA scores enable better interpretations and, thus, may support the use of ANN in metabolomics. An optimized algorithm for calculating FIA scores was implemented in the R package mrbin, available at CRAN.

BIBLIOGRAPHY

[1]Klein MS (2021): Affine Transformation of Negative Values for NMR Metabolomics Using the mrbin R Package. Journal of Proteome Research 20:1397-1404

[2]Wang D, Greenwood P & Klein MS (2021): Deep Learning for Rapid Identification of Microbes Using Metabolomics Profiles. Metabolites, 11, 863

ACKNOWLEDGEMENTS

This research was supported by the *Foods for Health* Discovery Theme and by the USDA National Institute of Food and Agriculture, Hatch project 1018603.