On the feasibility of deep learning applications using raw mass spectrometry data

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Abstract

Summary: In recent years, SWATH-MS has become the proteomic method of choice for data-independent-acquisition, as it enables high proteome coverage, accuracy and reproducibility. However, data analysis is convoluted and requires prior information and expert curation. Furthermore, as quantification is limited to a small set of peptides, potentially important biological information may be discarded. Here we demonstrate that deep learning can be used to learn discriminative features directly from raw MS data, eliminating hence the need of elaborate data processing pipelines. Using transfer learning to overcome sample sparsity, we exploit a collection of publicly available deep learning models already trained for the task of natural image classification. These models are used to produce feature vectors from each mass spectrometry (MS) raw image, which are later used as input for a classifier trained to distinguish tumor from normal prostate biopsies. Although the deep learning models were originally trained for a completely different classification task and no additional fine-tuning is performed on them, we achieve a highly remarkable classification performance of 0.876 AUC. We investigate different types of image pre-processing and encoding. We also investigate whether the inclusion of the secondary MS2 spectra improves the classification performance. Throughout all tested models, we use standard protein expression vectors as gold standards. Even with our naı¨ve implementation, our results suggest that the application of deep learning and transfer learning techniques might pave the way to the broader usage of raw mass spectrometry data in real-time diagnosis.

Availability and implementation: The open source code used to generate the results from MS images is available on GitHub: https://ibm.biz/mstransc. The raw MS data underlying this article cannot be shared publicly for the privacy of individuals that participated in the study. Processed data including the MS images, their encodings, classification labels and results can be accessed at the following link: https://ibm.box.com/v/mst-supplementary.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Proteins participate in virtually every process in the cell, and are directly responsible for its observed phenotype. Their accurate identification and quantification can therefore enable the precise characterization of phenotypes. Proteins are most commonly analyzed by mass spectrometry (MS). Among the available mass spectrometry approaches, SWATH-MS (Sequential Window Acquisition of all THeoretical fragment ion spectra) has emerged as a technology that combines deep proteome coverage, high reproducibility and quantitative consistency and accuracy (Gillett et al., 2012a). In a SWATH-MS measurement, all ionized peptides falling within a specified mass range are fragmented in a systematic and unbiased fashion using large precursor isolation windows (Ludwig et al., 2018). Spectral profiles are then recorded for all ionized peptides and fragment ions thereof.

While the raw data acquisition is unbiased, peptide identification requires prior information about the fragment ion patterns and the retention time (RT) of all targeted peptide fragments, which are typically extracted from SWATH assay libraries (Guo et al., 2015). Uncertainties in peptide identification result in inaccurate protein quantification and potential protein mis-identification, especially as only a few peptides per protein are typically detected. Protein isoforms and peptide modifications further complicate computational workflows and exacerbate the variability observed across experiments and platforms. Indeed, while a recent benchmark of different SWATH-MS data processing tools highlighted the convergent identification and reliable quantification performance of all tools (Navarro et al., 2016), careful pre- and post-processing and parameter optimization were needed to achieve robustness in label-free quantitative proteomics. The complexity of current MS data analysis workflows is partly responsible for their slow translation into clinical practice, despite having been long-postulated to enable a huge clinical impact (Aebersold and Mann, 2003).
Contrasting the carefully designed and parameterized workflows commonly used for the analysis of SWATH-MS datasets, we investigate whether state-of-the-art deep learning models could enable the circumvention of protein quantification and the execution of certain predictive tasks directly on raw mass spectrometry data.

Deep learning (DL) has become one of the most active fields in artificial intelligence, with spectacular performances in a broad area of applications such as computer vision, speech recognition and natural language processing. In parallel, recent years have witnessed an exponential increase in the number of DL applications in computational biology (Ching et al., 2018). These works have demonstrated the extraordinary capacity of DL models to automatically learn discriminative features from raw data, thus eliminating the need for intricate feature-engineering. In contrast to targeted proteomic analysis, deep learning is particularly adept at learning abstract features directly from the raw data, with different layers of the network sequentially learning increasingly abstract features in an automatic fashion.

However, the adoption of DL approaches for many applications in computational biology has been slow due to seemingly inescapable data challenges, such as low volume, high sparsity and large heterogeneity associated with the use of different profiling platforms. Regarding the last point, we note that multi-platforms studies are especially frequent in traditionally data-scarce domains such as proteomics. Although the minimum amount of training data depends on many variables, such as the complexity of the task, or the type of noise and data distribution, it is generally accepted that one roughly needs at least 10 times more training samples than parameters. As an example, the 2015 computer vision’s model that beat humans at the task of image classification (Alom et al., 2018) exploited large datasets such as ImageNet (Russakovsky et al., 2015) and iNaturalist (Van Horn et al., 2018), consisting of 1 120 000 and 579 184 images to classify 1001 and 5089 classes respectively. In contrast, the largest proteomic cohorts comprise a few hundred of samples, and each sample requires gigabytes of storage instead of the megabytes typically required for images (Liang et al., 2020). An additional level of complexity is presented by the intrinsic nature of tandem mass spectrometry, where each sample consists of a precursor profile (MS1) and multiple precursor fragment profiles (MS2) and all spectra need to be jointly analyzed to quantitatively characterize a sample.

Despite these challenges, successful applications of deep learning in the field of proteomics have been developed. For instance, DeepNovo-DIA (Tran et al., 2019) captures precursor and fragment ions to identify novel peptides in human antibodies and antigens. However, with DeepNovo-DIA only a handful of spectra close to the investigated feature along the retention-time axis are used, somewhat mitigating the aforementioned restrictions.

Transfer learning for SWATH-MS profile analysis: Transfer learning is the ability to reuse the knowledge gathered from a learning task in an unrelated and oftentimes completely different task (Pan and Yang, 2010). Humans are extremely good at transfer learning, and, for instance, an English speaker will learn Spanish much faster if she already speaks Italian. In the context of machine learning, transfer learning has been applied by repurposing a model for different tasks than their original target task. The underlying assumption is that if two datasets share a common latent space, a model trained on one dataset can export the data relationships learned to the second dataset (Pan et al., 2008).

In this work, we demonstrate an application of transfer learning to automatically classify tumor versus normal samples using raw SWATH-MS profiles. Specifically, we transform raw MS data into an image format, which enables us to reuse pre-trained DL models for image classification, and later transfer the model to a MS-related classification task. In our implementation, the first transferred layers, which have learned to recognize basic image features, are left unchanged and only their output is coupled to different classifier algorithms. Even with such a naive implementation, we can achieve a remarkable classification performance of 0.876 AUC. Our results suggest that applying deep learning and transfer learning techniques might pave the way to a broader usage of raw mass spectrometry data in real-time diagnosis.

### 2 Materials and methods

The goal of this work is to demonstrate that it is possible to process raw liquid chromatography/mass spectrometry data acquired by SWATH-MS as images, encode them as feature vectors and use them for classification purposes using standard machine learning approaches. In this section, we describe the main components of our approach.

#### 2.1 Data

From the Prostate Cancer Outcomes Cohort (ProCOC, ‘PPP1 project’) (Umbehr et al., 2008), 554 tissue biopsies, including both benign and tumor regions for each patient, were sampled from 277 prostate cancer patients. The inclusion of technical replicates resulted in a total of 913 samples considered in this work. Of these, 455 samples are from healthy prostatic tissue and 458 samples are from different types of malignant tumors. Each individual sample of the raw data consists of one precursor profile (MS1) and 100 multiple precursor fragment profiles (MS2) obtained with PCT-SWATH (Guo et al., 2015). A summary of data acquisition and processing to obtain the protein expression vectors can be found in Supplementary Information, Supplementary Section S1. Data acquisition protocols are described in Charmpi et al. (2020).

#### 2.2 Gold standards

To quantify the accuracy of the tested models, we use protein expression vectors where peptides were quantified using targeted data analysis with OpenSWATH (Röst et al., 2014) as gold standard. Peptide quantification exploits prior knowledge about the chromographic and mass spectrometric properties using curated and annotated collections of peptide spectra (Ludwig et al., 2018).

We note that the gold standards are not exempt from biases themselves. For instance, while high performance and accuracy are typically observed for high-abundance proteins, systematic deviations from the expected values are observed for low-intensity signals. The deviations depend on the different physicochemical properties of the peptides and are ubiquitous among the different label-free quantification proteomic software tools. Similarly, all software tools depend on the reliable identification of specific peptides. For this, either tandem MS (MS/MS) libraries coupled with statistical methods to separate true from false matches, or ‘pseudo’-MS/MS spectra that do not require an assay library can be used. In both cases, incorrect peptide identification results in inaccurate protein identification and quantification. Despite these shortcomings, reliable and accurate protein quantification is typically achieved with (SWATH)-MS software methods (Navarro et al., 2016).

In a typical MS-experiment, many peptides cannot be quantified in a run for technical or biological reasons, which results in a large proportion of imputed values. We use linear regression to impute missing values and exclude samples with missing values.

### Table 1. Summary of peptide vector post-processing

| Name    | Processing steps                                |
|---------|-------------------------------------------------|
| peptides2 | Log2 transformation and quantile normalization of samples. |
| peptides3 | Imputation of missing values on peptides2 using technical replicates. |
| peptides4 | Batch normalization over different machine runs performed on peptides3 using ComBat Stein et al. (2015). |
| proteins | Selection of only the top 3 peptides per protein (over all samples). Imputation of missing values with a linear regression. The strongest intensity of proteotypic peptides are adopted as protein intensity. |

Note: During peptide processing, four different intermediate datasets are generated. We test the accuracy of our model on the peptides3, peptides4 and proteins datasets.
number of missing values. We denote this initial dataset after some minimal processing peptides2 (Table 1). To partially overcome this challenge, missing values can be imputed using technical replicates, resulting in the peptides3 dataset. Peptides that still present missing values in some samples putation, or display constant values across all samples are excluded from further analysis. Finally, the most informative peptides are selected, resulting in the peptides4 dataset. Table 1 describes the different processing steps applied to each dataset. A more detailed description of the different processing steps can be found in Zhu et al. (2021).

Each processing step described in Table 1 results in a different number of retained features, as detailed in Table 2. Due to the elimination of samples with missing values, the size of the peptides3 and peptides4 datasets is significantly decreased, from around sixteen thousand peptides features to 1207. The last row of Table 1 resulted in 265 quantified proteins, which we use throughout this work as gold standard.

### 2.3 Mass spectra profiles as images

The output format of different mass spectrometers is vendor-specific, however, most formats can be converted to mzXML format (Pedrioli et al., 2004) with the ProteoWizard software (Chambers et al., 2012). We further modified the Proteowizard software to transform the mzXML input into images of predefined size.

### Table 2. Peptide and proteoform feature vectors

| Dataset     | Number of features | Number of retained features |
|-------------|--------------------|----------------------------|
| peptides2   | 16 644             | 0                          |
| peptides3   | 16 644             | 1207                       |
| peptides4   | 16 104             | 1207                       |
| proteins    | 2103               | 265                        |

**Note:** Summary of the number of initial features and retained features after preprocessing as described in Table 1. As there are no features without any missing value in at least one sample before imputation in the original dataset (peptides2), all features are eliminated, resulting in zero retained features. We investigate the influence of the different post-processing pipelines in the model's classification accuracy in Section 3.2.

### Table 3. Vector encodings overview

| Encoder name [ref] | Input | Output | Retained features (for 512 x 512) in |
|--------------------|-------|--------|------------------------------------|
| resnet_v2_101 (He et al., 2016) | 224 x 224 x 3 | 2048 | 942 (45%) 120 577 (58%) |
| resnet_v2_50 (He et al., 2016) | 224 x 224 x 3 | 2048 | 1145 (55%) 132 459 (64%) |
| resnet_v2_152 (He et al., 2016) | 224 x 224 x 3 | 2048 | 1570 (76%) 164 260 (79%) |
| resnet_large (Zoph et al., 2018) | 331 x 331 x 3 | 4032 | 3671 (91%) 365 296 (89%) |
| inception_resnet_v2 (Szegedy et al., 2017) | 299 x 299 x 3 | 1536 | 1536 (100%) 155 107 (99%) |
| inception_v3_imagenet (Szegedy et al., 2016) | 299 x 299 x 3 | 2048 | 2045 (99%) 206 835 (99%) |
| inception_v2 (Szegedy et al., 2016) | 224 x 224 x 3 | 1024 | 1018 (99%) 103 418 (99%) |
| inception_v3_naturalist (Cai et al., 2018) | 299 x 299 x 3 | 2048 | 2044 (99%) 206 725 (99%) |
| amoebanet_a_a18_f486 (Real et al., 2019) | 331 x 331 x 3 | 7168 | 5114 (71%) 594 543 (82%) |
| nasnet_mobile (Zoph et al., 2018) | 224 x 224 x 3 | 1056 | 618 (58%) 88 492 (82%) |
| inception_v1 (Szegedy et al., 2015) | 224 x 224 x 3 | 1024 | 922 (90%) 102 641 (99%) |
| penset_large (Liu et al., 2018) | 331 x 331 x 3 | 4320 | 4050 (93%) 427 948 (98%) |
| mobilenet_v2_050_224 (Sandler et al., 2018) | 224 x 224 x 3 | 1280 | 1178 (92%) 118 715 (91%) |
| mobilenet_v2_075_224 (Sandler et al., 2018) | 224 x 224 x 3 | 1280 | 1181 (92%) 116 838 (90%) |
| mobilenet_v1_050_224 (Howard et al., 2017) | 224 x 224 x 3 | 512 | 491 (95%) 50 680 (98%) |
| mobilenet_v2_100_128 (Sandler et al., 2018) | 128 x 128 x 3 | 1280 | 988 (77%) 102 634 (79%) |
| mobilenet_v2_075_96 (Sandler et al., 2018) | 96 x 96 x 3 | 1280 | 787 (61%) 92 169 (71%) |
| mobilenet_v1_025_224 (Howard et al., 2017) | 224 x 224 x 3 | 256 | 249 (97%) 25 026 (96%) |
| mobilenet_v1_050_128 (Howard et al., 2017) | 128 x 128 x 3 | 512 | 446 (87%) 46 829 (90%) |

**Note:** Characteristics of image to feature vector encoders available from https://thub.dev/, i.e. image input resolution and output vector size. For any given dataset, constant features over all samples were removed. Feature retention is reported for grid size 512 x 512 and is virtually the same for grid size 2048 x 2048.
on the extracted features due to the small number of MS samples available for analysis, which precludes extensive retraining or fine-tuning.

We use modules a selection of publicly available models from TensorFlowHub as encoder, see Table 3. The table also presents an overview of the vector encodings for all considered models. We briefly describe the architectural families and respective naming conventions of trained variants included in our study:

- **NASNets** are model architectures found with the Neural Architecture Search (NAS), an automated machine learning structure for training new neural networks. NASNet begins with an overall predefined architecture, but optimizes blocks by a reinforcement learning search method (Zoph et al., 2018). Also exploiting NASNet, AmoebaNet-A (Real et al., 2019) is a convolutional neural network, where the architecture of its convolutional cells (or layers) has been found by an evolutionary architecture search in the NASNet search space. For pnasnet-, large (Liu et al., 2018), sequential model-based optimization strategies were used to search for structures in order of increasing complexity, while simultaneously learning a surrogate model to guide the search through structure space. The mobile variant is designed for a constrained computational setting and has a reduced number of parameters and multiply accumulate operations.

- **ResNet** is based on deep residual networks, a family of extremely deep architectures that utilize skip connections, i.e. shortcuts to jump over some layers. Residual networks have shown compelling accuracy and good convergence behavior (He et al., 2016). The last digit in the encoder names in Table 3 represents the number of layers.

- **Inception** is a family of deep convolutional neural network architectures with improved utilization of the computing resources achieved through careful design to enable increased network depth and width, while keeping the computational budget constant (Szegedy et al., 2015, 2016, 2017). Inception-v2 (Szegedy et al., 2016) uses batch normalization at each mini-batch training (Ioffe and Szegedy, 2015), allowing the use of a much higher learning rate and making the network more robust to initialization choices. Inception_v3_inaturalist (Cui et al., 2018) exploits a training scheme that uses higher image resolution and deals with the long-tailed distribution of training data. The knowledge learned from large scale datasets is transferred via fine-tuning to smaller, domain-specific datasets.

- **MobileNets** are designed to run on mobile devices and primarily use depth-wise separable convolutions to reduce the computational burden (Howard et al., 2017). MobileNetV2 is based on an inverted residual structure where the input and output of the residual block are thin linear bottleneck layers (Sandler et al., 2018). Model variant names include, as percentage, a multiplier for the depth in the convolutional layers to control model size and lastly the image input size that affects inference speed.

All the feature encoders were trained on ImageNet (Russakovsky et al., 2015), an extensive image database where images are organized according to word concepts, e.g. cat, bird, flower, etc. The only exception is inception_v3_inaturalist which was trained on the iNaturalist dataset (Van Horn et al., 2018), a dataset of animal pictures. The encoders were developed to ingest color images of predefined sizes. To apply the encoders to the MS images, the MS images are processed in two ways. First, we triplicate the gray–scale channel as rgb channels. Secondly, we resize the images to fit the required encoder input size (often 224×224, see Table 3) using bilinear scaling, Tensorflow’s default resize method. No further pre-processing is performed on the raw data.

Encoding raw MS images as vectors allows us to concatenate the one MS1 and 100 MS2 images (spectra) associated with the same sample into a single vector, which enables us to compare the classification performance of models trained uniquely using MS1 spectra (ms1_only) against models exploiting all spectra (ms1_and_ms2).

However, a downside of including both modalities is that the number of features is significantly increased. To keep the number of features manageable, considering the number of samples available, we eliminate constant features, i.e. features exhibiting a zero standard deviation across all samples. Almost all off-the-shelf feature representations result in some constant features, with the single exception of inception_resnet_v2, which retains all 1536 features. On the other extreme, resnet_v2_101 retains the smallest percentage of features. The percentage of retained features for each encoder is shown in Table 3.

### 2.5 Evaluation through classification performance

Each one of the tested encoders described in Section 2.4 transforms LC-MS/MS data acquired from a single sample into a numerical vector. At this point, we can define various options for MS image resolution, type of encoder and type of spectral data included, resulting in several tabular datasets from the same raw MS data. Each dataset, including the peptide and proteomic gold standards defined in Section 2.2, can be further processed by a downstream machine learning algorithm, hence allowing us to compare the predictive power of the datasets and to investigate the impact of the different options considered when creating the dataset. As we do not wish to limit our analysis to a particular predictive model, we extend our comparison to several of the most frequently used classifiers based on different theoretical foundations, including logistic regression, support vector classification (SVC), random forest and gradient boosted trees (XGBoost), see Table 4.

For all derived datasets, we apply the following pipeline. First, all random seeds are fixed for comparability and reproducibility. Next, a random stratified (meaning the ratio of the classes is kept constant) test set comprising 30% of samples is excluded from training. The retained features are scaled to the range [0, 1] (per feature) on the training set, and the learned transformation reapplied to the test set. To optimize the hyperparameters, we perform a shallow grid search of hyperparameters (see Table 4) via internal six-fold cross-validation and two repeats. The optimization results in an independent set of hyperparameters for each combination of MS image resolution, encoder, ms1_only versus ms1_and_ms2 and classification algorithm. The classifier is trained on the full training set by using the hyperparameters with the best mean test performance measured by AUC. Finally, the trained classifier is evaluated on the test set. We reiterate that, as we implemented a random stratified data split, the ratio of the classes is kept constant in the training and test datasets. The Python code used for the entire pipeline (including data splits, hyperparameter optimization and analysis) can be accessed at https://ibm.biz/mstransc.

| Table 4. Classification algorithms and hyperparameter values tested during optimization |
|---------------------------------|-----------------|------------------|
| Classifier                      | Parameter       | Values           |
| Logistic regression (LG)        | C               | 0.1, 1, 10, 100  |
| Support vector machine (SVC)    | C               | 0.1, 1, 10, 100  |
|                                 | kernel          | 'linear', 'poly', 'rbf' |
| Random forest (RF)              | n_estimators    | 100, 500         |
| Gradient boosted trees (XGBoost)| n_estimators    | 100, 500         |

**Note:** C is a regularization parameter of inverse strength, ‘linear’, ‘poly’ and ‘rbf’ kernel functions refer to the linear, polynomial and radial basis function, respectively. n_estimators is the number of trees in the forest. Classifiers are implemented using scikit-learn (Pedregosa et al., 2011), with the exception of XGBoost (Chen and Guestrin, 2016).
3 Results

We present a comparison of the phenotype classification performances obtained with different proteomic feature vectors (see Section 2.2) and off-the-shelf features (see Section 2.4). We investigate different metrics to quantify the classification performance, including Brier loss, Log loss, Accuracy, F1 score, Youden’s index, Recall, Precision and Specificity. Here, we report only the area under the receiver operating characteristic curve (AUC), while performances based on the additional metrics are available in Supplementary Information.

3.1 Encoders performance

Figure 1 shows the evaluation score of all representations combining both MS1 and MS2 spectra (ms1_and_ms2) and the gold standards. To facilitate comparisons, for each encoder, we average across classifiers and image resolutions and report only the median AUC achieved using both M1 and M2 spectra (ms1_and_ms2).

The ResNet architectures achieve superior performance with both best median (0.849 AUC by resnet_v2_101) and best single result (0.876 AUC by resnet_v2_50), followed by Inception and NASNet architectures that range from 0.827 to 0.777 median AUC. The worst performance is achieved by mobilenet encoders, with the lowest median AUC of 0.623 by mobilenet_v1_050_128. However, even in this case, the AUC is significantly above 0.5 (random prediction). See Table 5 for statistics on all encoders and gold standards. As a group, MobileNet models exhibit the weakest performance and show large variability for different choices of classifier and image resolution.

Similar patterns in terms of the ranked encoder family’s performance are obtained for the off-the-shelf encoders when only the MS1 image is used, see Figure 2. ResNet encoders still perform best, closely followed by NASNet and Inception encoders. Reflecting this similarity, the Spearman’s rank correlation between performances achieved with MS1 and MS2 or only MS1 is 0.808, see Figure 3.

Similar results are obtained if we average across classifiers and resolutions for each encoder by computing the median, although the Spearman’s rank correlation is now slightly higher at 0.875 (see Table 5). Overall, it is quite remarkable that we were able to create high-quality features on a vastly different domain and downstream task without any fine-tuning.

3.2 Gold standards performance

As expected, the classification performance using the gold standards, as defined in Section 2.4, is very good. The best individual result was achieved for the peptides4 dataset (AUC 0.959 achieved with XGBoost). When the results across classifiers are averaged, all gold standard datasets achieve a very high AUC. The proteins and peptides3 representations are virtually indistinguishable, see Table 5, despite the proteins dataset including a much smaller number of features—265 features versus 1207 features in the peptides3 and peptides4 datasets, see Table 1. This suggests that the removal of non-proteotypic peptides and selection of top 3 peptides per protein does indeed preserve most of the biological information contained in the peptides4 dataset.

Interestingly, there are only very minor differences between the performances achieved by the three gold standards. All three evaluated gold standards achieve very good performances, with only a small decrease in standard deviation on the proteins dataset, see Table 5 and Figure 1. One might have expected that the proteins gold standard performs better than the peptides-based gold standards, as it integrates biological knowledge about proteotypic peptides and penalizes peptides less highly expressed and, hence, more likely to be randomly profiled. However, there are two additional considerations to keep in mind. First, peptides3 and peptides4 incorporate a larger number of features, some of which might not be very informative. This might make the classifiers harder to train and result in a larger variability across hyperparameters. Second, as stated in Section 2.2, peptides that still present missing values after imputation are eliminated from the peptides3 dataset. These peptides are not likely to be missing because of technical reasons, but might quite possibly be biologically meaningful peptides that differentiate samples. By removing them, one might be deleting the more processed datasets, i.e., peptides4 and proteins, of important biological signals. For the most processed dataset, i.e., the proteins dataset, this factor might counteract the gain originating from the integration of biological knowledge and reduction of the number of features, resulting in a similar performance to the peptides3 and peptides4 datasets, and only a slightly reduced standard deviation.

3.3 Classifier agreement

Classifiers trained on the gold standard datasets perform very similarly, see Figure 1 and Supplementary Table S1. Logistic regression, SVC and XGBoost models achieve almost indistinguishable performances, only the random forest classifiers showed a weaker performance.

Regarding the classifiers trained on the off-the-shelf encodings, SVC and logistic regression consistently achieve better performances, while random forest performs equally well as XGBoost. However, when using ms1_and_ms2 (very long vectors), random forest performs worse than XGBoost, with a mean difference of 0.053 AUC across all encodings and resolutions. As random forest is methodically close to XGBoost—both methods are based on decision tree algorithms, with XGBoost benefiting from a more advanced training—the observed difference between both methods is expected to be alleviated by further hyperparameter optimization.

3.4 Classification performance when using only MS1 compared to using both MS1 and MS2 spectra

We also investigate whether the inclusion of MS2 images improves the classification performance, and if so, by how much. To investigate this question, as described in Section 2.4, we created two...
Table 5. Summary of classification performances for encoders and gold standards

| Available input | median AUC | mean AUC | σ AUC | architecture |
|----------------|------------|----------|-------|--------------|
| Encoder         | MS1/2      | MS1      |       |              |
| Proteins        | 0.951      | NaN      | 0.010 | NaN          | Proteomics |
| peptides3       | 0.951      | NaN      | 0.012 | NaN          | Proteomics |
| peptides4       | 0.947      | NaN      | 0.012 | NaN          | Proteomics |
| resnet_v2_101   | 0.849      | 0.759    | 0.036 | 0.029        | ResNet     |
| resnet_v2_50    | 0.834      | 0.784    | 0.029 | 0.013        | ResNet     |
| resnet_v2_152   | 0.832      | 0.746    | 0.045 | 0.025        | ResNet     |
| nasnet_large    | 0.827      | 0.749    | 0.029 | 0.025        | NASNet     |
| inception_resnet_v2 | 0.820 | 0.737 | 0.043 | 0.025 | Inception, ResNet |
| inception_v3_imagenet | 0.811 | 0.770 | 0.029 | 0.018 | Inception   |
| inception_v2    | 0.806      | 0.745    | 0.030 | 0.022        | Inception  |
| inception_v3_inaturalist | 0.795 | 0.732 | 0.023 | 0.022        | Inception  |
| nasnet_mobile   | 0.792      | 0.714    | 0.028 | 0.015        | NASNet     |
| inception_v1    | 0.789      | 0.717    | 0.035 | 0.021        | Inception  |
| pnsnet_large    | 0.777      | 0.748    | 0.023 | 0.017        | NASNet     |
| mobilenet_v2_050_224 | 0.765 | 0.622 | 0.039 | 0.031        | MobileNet  |
| mobilenet_v2_075_224 | 0.737 | 0.524 | 0.055 | 0.032        | MobileNet  |
| mobilenet_v1_050_224 | 0.704 | 0.582 | 0.084 | 0.044        | MobileNet  |
| mobilenet_v2_100_128 | 0.687 | 0.485 | 0.050 | 0.018        | MobileNet  |
| mobilenet_v2_075_96  | 0.666 | 0.530 | 0.057 | 0.027        | MobileNet  |
| mobilenet_v1_025_224 | 0.656 | 0.636 | 0.043 | 0.040        | MobileNet  |
| mobilenet_v1_050_128 | 0.623 | 0.512 | 0.036 | 0.030        | MobileNet  |

Note: For each feature encoding module, median, mean and standard deviation (σ) of the classification performance AUC values over the different classifiers are reported. For each statistic, the input of MS image features concatenated (ms1_and_ms2, in the table MS1/2) is compared to MS1 image features only (ms1_only, in the table MS1) for performance comparison.

Fig. 2. Encoding module and choice of classifier drive classification performance. Publicly available modules—trained to classify natural images—were used to encode off-the-shelf feature vectors. Exceptions to this are the gold standard datasets proteins, peptides3 and peptides4, which were obtained using a curated proteomics analysis pipeline. Classification performance, measured by AUC, is reported in order of descending median AUC for different classifiers and two resolutions of MS images (rasterized spectra). Here, we only report results obtained using concatenated feature vectors encoded from MS1 and all MS2 images (ms1_and_ms2). As observed in the figure, the main driver of performance is the encoding of features. Different off-the-shelf features achieve results ranging from 0.623 up to 0.849 median AUC, while gold standard features reached 0.951 median AUC. The variance over results from different classifiers is much larger for off-the-shelf features compared to the gold standard features.

Fig. 3. Classification performance of ms1_only off-the-shelf features. Depicted is the same plot as in Figure 2, but with ms1_only encodings instead of ms1_and_ms2. The order of encoders is identical, with the peptide and protein datasets missing as these cannot be compared to a case where MS2 information is excluded. While the classification performance of ms1_only encodings is generally lower compared to ms1_and_ms2, there is a pronounced drop in performance for mobilenets, with some models performing even worse than random (AUC below 0.5). Different off-the-shelf features achieve results ranging from 0.485 up to 0.784 median AUC.
Indeed, a one-sided paired t-test with the null hypothesis that stems solely from results of mobilenets.

A possible downside of including all MS modalities is a sharp increase in the number of features—a single MS1 image might require hundreds or thousands of features depending on the model, while the concatenation of all images results in hundreds of thousands of features. The significantly larger number of features might enable the encoding of more detailed information about m/z ranges in MS1 spectra. This might boost performance, although the large number of features might increase the need of having larger datasets or lead to overfitting when small datasets are used. Appropriate regularization techniques, or feature pre-selection by, for instance, removing features with low signal-to-noise ratios, might alleviate overfitting. To enhance applicability in a clinical setting, however, it would be desirable to limit data pre-processing and feature selection and/or engineering to an absolute minimum. Hence, while waiting for the next generation of larger proteomics datasets to come, smaller models might be preferable.

3.5 MS image resolution

The resolution of the MS images (as described in Section 2.3) also influences the performances of the different classifiers. We find a small but significant difference in the evaluation scores that favors the 512×512 resolution versus 2048×2048, as shown in Figure 4C. Indeed, a one-sided paired t-test with the null hypothesis AUC_{512×512} ≤ AUC_{2048×2048} was rejected with a level of significance α = 0.001. For these settings, the mean difference in AUCs is 0.016, which leads to rejection of the null hypothesis (P-value of 9.22 × 10^{-11}). Note that images, regardless of the original resolutions, are resized according to each encoder’s input size requirements (see Table 3), most often to 224×224.

4 Discussion

Quantitative proteomics enable the unbiased and faithful characterization of molecular phenotypes. A recent benchmark of several computational workflow tools for the analysis of SWATH-MS data has highlighted the convergent identification and reliable quantification performance of all tools Navarro et al. (2016). However, these workflows require laborious and carefully fine-tuned pre- and post-data processing, and various challenges hinder their broad application in a clinical setting. For instance, SWATH-MS data analysis relies on targeted data extraction strategies, which query the acquired fragment ion maps using a priori information obtained from spectral libraries to identify and quantify peptides Ludwig et al. (2018). While methods have been developed that do not require spectral libraries Ting et al. (2017), their analysis is limited to peptides of known sequence. Furthermore, although it achieves good performance, targeted analysis discards a lot of information, e.g., by ignoring proteins not represented in the spectral library or by limiting quantification to a selection of expected peaks. In doing so, subtle differences between related proteoforms, which might be informative to correctly classify a sample, might be discarded early on in the processing pipeline. It is therefore highly desirable to develop alternative approaches that can exploit the whole proteome information and require minimum or no feature selection.

Deep learning approaches have an extraordinary capacity to automatically learn abstract discriminative features directly from raw data. At the same time, transfer learning is known to be able to achieve very good performances in complex tasks where little data is available, as is often the case in biological data cohorts. Combining the two, we have investigated whether deep learning models for natural image classification (off-the-shelf encoders) can be exploited to produce informative feature vectors directly from raw SWATH-MS spectral profiles. Although raw MS images (Fig. 5) are extremely different from natural images, we were able to achieve very good accuracies for the task of classifying tumor versus normal prostate tissue biopsies.

As gold standard for comparison, we used protein quantification derived from the standard SWATH analysis pipeline tuned by domain experts. While the gold standards achieve better classification results than the off-the-shelf encoders, the generation of peptide and protein datasets from MS raw images requires highly supervised pipelines supplemented with prior knowledge from peptide libraries. Contrary to the careful data processing and model fine-tuning of MS-workflows, our approach does not use any prior knowledge nor does it require any fine-tuning.

As one MS1 and 100 MS2 profiles are obtained from a single sample, we investigated two alternative ways of ingesting raw SWATH-MS data, one based on the sole analysis of MS1 images, and another one that jointly exploits MS1 and MS2 images. Our results show that the inclusion of both MS1 and MS2 information boosts the classification performance of all models, although at the...
price of significantly increasing the number of features. Therefore, when smaller MS-cohorts are used to train the classifiers, the use of only MS1 spectra might be a preferred strategy to prevent overfitting. However, our cross-validation analysis highlighted how regularization and the removal of features with low signal-to-noise ratio results in extremely reliable and robust performance, confirming that overfitting can be mitigated even when dealing with a limited number of samples.

Several improvements might further increase the performance of our model and will be investigated in future work. In terms of biological variability, multiple run alignments might help account for retention time variation between runs, and noise filtering and batch correction might lead to additional accuracy gains in the models exploiting off-the-shelf features. Regarding model improvements, our current model does not reflect the relationship of the different MS2 swathes and MS1. Alternative ways of encoding raw MS data to reflect their dependencies will be investigated in future work. More importantly, from the engineering point of view, our method drastically downsizes the original raw SWATH-MS images to relatively small image sizes. This process is likely to result in accuracy loss, e.g. by binning together peaks that could otherwise be informative for classifying samples. Ingesting the raw data in an uncompressed fashion would be very promising, although this would increase the required number of training samples drastically. However, with the rising popularity of mass spectrometry, larger size cohorts are expected to become available in the near future. Larger cohorts will undoubtedly enable training new or fine-tuning the transferred models, resulting in increased model performance.

Another interesting avenue to explore would be leveraging existing unlabeled spectra to learn MS-specific features. Namely, instead of using pretrained models for natural image classification, autoencoders (Kramer, 1991; Vincent et al., 2010) could be trained on existing raw MS datasets from databases such as PRIDE (Perez-Riverol et al., 2019) and Peptide Atlas (Dessiere et al., 2006). Using MS-specific architectures, this strategy would allow the transformation of raw MS images into feature vectors using unsupervised deep learning techniques. The most fascinating aspect of this approach is the possibility of addressing two challenges at once: data scarcity, since we can learn from spectra generated with different instruments and species; and the need of learning features that are more specific to the MS images. The results obtained in this work using models pretrained on natural images testifies how promising such methods can be.

As an application example, consider the scenario where the cancerous tissue expresses a proteoform with N-terminal truncation compared to the healthy tissue, i.e. some peptides are missing in the cancer proteoform due to the pathophysiology of the disease. Such peptides are expected to be highly discriminative and are expected to be captured on MS images. However, in the conventional SWATH analysis, they are likely to be eliminated somewhere along the processing path unless specifically searched for. By contrast, learning from MS images does not pre-select features, and hence, can identify subtle differences across samples. Furthermore, one can easily envision enhanced models that search for such small differences, e.g. by exploiting interpretability methods (Dhurandhar et al., 2018) to identify the key features that underlie the biological similarities and differences. This is in stark contrast to conventional analysis that can only reveal peptide differences once they have been hypothesized.

To conclude, our results show that networks trained on natural images can generalize to the analysis of MS images surprisingly well. This opens the door to the development of future models trained on larger cohorts of MS images, potentially accelerating the development of deep learning models for proteomics applications in both research and clinical settings.

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